Novel endoscopic techniques in early gastrointestinal neoplasia

MEDICAL DOCTORATE

M.D.

Dr. Gaius Longcroft-Wheaton

MB,BS, MRCP(U.K.)

The thesis is submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Medicine (M.D.) of the University of Portsmouth

School of Pharmacy and Biomedical sciences

Registration period

01/09/2009-01/02/2012
Abstract

Advances in endoscopic practice are challenging traditional views on how neoplasia should be managed in the gastrointestinal system. Identifying pre-malignant changes in the gut is central to successful early management of most cancers. There has been rapid growth in the equipment and technology available for evaluating potentially neoplastic lesions in the gastrointestinal tract. However, the evidence base for how they should be used is limited. This thesis investigates the use of chromoendoscopy and ‘virtual computed chromoendoscopy’ in the gastrointestinal tract for the identification, assessment and management of early gastrointestinal neoplasia.

The first part of the thesis reviews data on the health burden of gastrointestinal neoplasia. It reviews the background research into advanced endoscopic techniques in the colon and oesophagus and outlines the deficiencies in the published literature.

Chapters 7-9 describe the development and validation of a new tool (N.A.C.) for in-vivo histology prediction of colonic polyps <10mm in size using Flexible Spectral Imaging Colour Enhancement (FICE) and indigo carmine (IC) chromoendoscopy without optical magnification. Chapter 7 describes a study to determine the optimum FICE setting for assessment of polyps. Setting 4 came out as the best setting for this purpose. Chapters 8 and 9 describe two studies to define the key surface features associated with neoplastic and non-neoplastic polyps using first a picture based study and then in an in-vivo study. N.A.C. was then used in a prospective cohort
study of polyp assessment in a Bowel cancer Screening Population, described in Chapter 10. A prospective study of 232 polyps <10mm in size was performed. FICE and IC significantly improved the accuracy of the in-vivo diagnosis of polyps as compared to WLI endoscopy. In-vivo prediction of polyp histology allowed the endoscopist to set the correct surveillance interval in 83% of cases using WLI alone and in 97% of cases using either FICE or IC based on BSG guidelines. Chapter 11 describes a study which attempts to identify the role of high resolution (HD) endoscopes in lesion characterization. HD endoscopes significantly improved the ability of the endoscopist to make an in vivo diagnosis using FICE compared to standard resolution endoscopes with FICE but had no effect on the WLI or IC predicted diagnosis.

Chapter 12 describes the use of acetic acid in a cohort study for the identification of neoplasia within Barrett’s oesophagus to establish the sensitivity and specificity of the technique. Acetic acid chromoendoscopy had a sensitivity of 95.5% and specificity of 80% for the detection of neoplasia. There was a correlation between lesions predicted to be neoplastic by acetic acid and those predicted by histological analysis ($r=0.98$). There was a significant improvement in the detection of neoplasia using acetic acid compared with white light endoscopy ($P=0.001$). This method is then refined in a second study by attempting to exploit the aceto-whitening reaction. This is described in Chapter 13. Data from 146 areas of Barrett’s was collected from 121 patients. 84% were male. Mean age 69. In total 72/86 metaplasia, 6/14 LGD, 26/27 HGD, 15/15 IMC and 12/12 areas of invasive cancer were recognised correctly by the endoscopist. A ROC curve was produced for the identification of high risk neoplasia (HGD, IMC and invasive cancer) using the aceto-whitening timings.
The area under the curve was 0.93 (95% C.I.0.89-0.97), demonstrating a low probability that a randomly chosen positive case will exceed the value for a randomly chosen negative case. Using a cut off threshold of 142 seconds a sensitivity for neoplasia of 98% (95% C.I. 89-100) and specificity of 84% (95% C.I. 74-91) was achieved.

The final chapter of the thesis discusses the clinical impact of the research and how it could be introduced to clinical practice. It reflects on where deficiencies in the published literature still exist where the future research needs are.
Dedicated to my Mother and Father. Without their tireless love and support this work would not have been possible.
Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

This thesis represents original work carried out in the endoscopy department of Queen Alexandra Hospital and in the department of Pharmacy and Biomedical Sciences, University of Portsmouth, United Kingdom. Where use has been made of the work of others it is acknowledged.

The work was carried out under the supervision of Dr. Pradeep Bhandari and Dr. James Brown, University of Portsmouth. Co-supervisor was Professor Ian Cree (University of Portsmouth).
Acknowledgements

This thesis would not have been possible without the contribution of many individuals. Dr. Pradeep Bhandari, my chief supervisor has been unstinting in his passion and drive towards understanding how technological advancements in endoscopy can be used to improve patient care. Without his tireless enthusiasm and support this thesis would not have been possible. Also James Brown, my University co-supervisor provided an invaluable scientific insight from a unique perspective outside of the field of advanced endoscopy which was immensely valuable in developing a balanced and structured project.

Special thanks should be extended to Dr. David Poller and Dr. David Cowlishaw, who reviewed and assessed all of the histological specimens in this work. Without their specialist skills and expertise in delivering a gold standard of histological diagnosis it simply would not have been possible to develop any validated endoscopic assessment tools at all.

I would like to acknowledge the efforts of Mr. Bernard Higgins in developing my skills in Medical Statistics. His support powering the study and review of my analysis of the results has been invaluable.

This work was conducted in Queen Alexandra Hospital in Portsmouth. I would like to thank all of the endoscopy unit staff in being so supportive in ensuring that I have had the right equipment at the right times and supporting my needs throughout the studies. Research is only possible through such support and I am deeply grateful for
all the times people have stayed late and gone the extra mile to ensure the work is a success.

I would like to thank Professor Anoop Chauhan. His efforts in developing and supporting research fellowships within Queen Alexandra hospital have provided the economic means for this work to be completed.

Finally I would like to thank all of the patients who have taken part in these studies. Without such support clinical research is not possible and it is only through such kind acts of generosity that endoscopic research can be conducted.

**Funding:** Queen Alexandra Hospital research fellowship post.

**Competing interests:** None
Publications arising from this work

FULL PAPERS


CONFERENCE ABSTRACTS

DDW ABSTRACTS


BSG ABSTRACTS


Longcroft-Wheaton G, Bhandari P. Acetic acid enhanced chromoendoscopy is more cost effective than protocol guided biopsies in a high risk Barrett’s population; results from a large prospective series. Gut. 2011 Apr; 60(Suppl1): A32 doi:10.1136/gut.2011.239301.65


Mead R, Longcroft-Wheaton G, Bhandari P. Diagnostic white light endoscopy – not as simple as it looks *Endoscopy* 2010 Oct; 42 (Suppl I) A262


BOOK CHAPTERS


<table>
<thead>
<tr>
<th>Part</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>2</td>
</tr>
<tr>
<td>Declaration</td>
<td>6</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>7</td>
</tr>
<tr>
<td>Publications arising from this work</td>
<td>9</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>14</td>
</tr>
<tr>
<td>List of tables</td>
<td>20</td>
</tr>
<tr>
<td>List of figures</td>
<td>22</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>24</td>
</tr>
</tbody>
</table>

**PART A: Background to advanced endoscopic techniques**

| Chapter 1: Introduction                    | 27   |
| Chapter 2: Colonic neoplasia              | 38   |
| 2.1 Chromoendoscopy                       | 43   |
| 2.2 Crystal Violet                        | 44   |
| 2.3 Indigocarmine                         | 50   |
| 2.4 Methylene Blue                         | 58   |
| 2.5 Acetic acid                           | 59   |
| 2.6 Vascular Enhancement techniques       | 60   |
| 2.7 Narrow Band Imaging                    | 61   |
| 2.8 FICE                                   | 66   |
| 2.9 i-scan                                 | 72   |
| 2.10 Confocal Endomicroscopy              | 73   |
| 2.11 High resolution colonoscopy          | 77   |
| 2.12 Summary                              | 80   |

| Chapter 3: Barrett’s oesophagus           | 84   |
| 3.1 Chromoendoscopy                       | 87   |
| 3.2 Methylene Blue                         | 88   |
| 3.3 Indigocarmine                         | 89   |
| 3.4 Acetic acid                            | 90   |
8.2 Aims

8.3 Methods

8.4 Results

8.5 Discussion

Chapter 9: Validation of the N.A.C. classification system for FICE

9.1 Introduction

9.2 Methods

9.3 Results

9.4 Conclusions

Chapter 10: FICE and Indigocarmine in Neoplasia diagnosis

10.1 Introduction

10.2 Aims

10.3 Methods

10.4 Statistical analysis

10.5 Rescope intervals

10.6 Financial analysis

10.7 Results

10.8 True histological diagnosis

10.9 In-vivo diagnosis

10.10 Accuracy by polyp size <5mm

10.11 Cost effectiveness of in-vivo diagnosis

10.12 Implications for the study cohort

10.13 Implications for the National BCSP

10.14 Surveillance intervals

10.15 Discussion

10.16 Implications for clinical practice

10.17 Implications for research

10.16 Conclusions

Chapter 11: Standard definition vs high definition

11.1 Introduction
11.2 Methods ............................................................. 200  
11.3 Endoscopic assessment tool ...................................... 202  
11.4 Statistical analysis .................................................. 203  
11.5 Study population .................................................... 204  
11.6 True Histological diagnosis ....................................... 206  
11.7 Accuracy for neoplasia ............................................. 206  
11.8 Additional gain from indigocarmine ............................ 207  
11.9 Accuracy for non-neoplastic lesions ............................ 207  
11.10 Accuracy for neoplasia by polyp size ......................... 208  
11.11 Rescope intervals .................................................. 210  
11.12 Discussion .......................................................... 211  
11.13 Implications for clinical practice .............................. 213  
11.14 Implications for future research ............................... 214  
11.13 Conclusions ....................................................... 215  

Chapter 12: Barrett’s neoplasia study ............................... 216  
12.1 Introduction .......................................................... 217  
12.2 Questions to be addressed ........................................ 220  
12.3 Justification for the acetic acid studies ....................... 220  
12.4 Hypothesis ............................................................ 222  
12.5 Primary aim .......................................................... 222  
12.6 Secondary aim ........................................................ 222  
12.7 Ethics ....................................................................... 223  
12.8 Statistical analysis ................................................... 223  
12.9 Feasibility ............................................................... 224  
12.10 Methods ............................................................... 224  
12.11 Patient population ................................................... 225  
12.11.1 Inclusion criteria ............................................... 225  
12.11.2 Exclusion criteria ............................................... 225  
12.12 Endoscopic protocol ............................................... 226  
12.13 Mucosal cleansing ................................................... 226  
12.14 Standardisation of technique .................................... 226  
12.15 Assessment tool .................................................... 229
12.16 Study design ................................................................. 230
12.17.1 Demographics .................................................................. 232
12.17.2 True neoplasia ................................................................. 235
12.17.3 Visible abnormalities ......................................................... 235
12.17.4 Targeted Histology ........................................................... 236
12.17.5 Sensitivity and Specificity of targeted histology ................. 236
12.17.6 Missed neoplasia ............................................................... 237
12.18 Discussion ................................................................. 239
12.19 Strengths ........................................................................... 241
12.20 Limitations ................................................................. 242
12.21 Implications for clinical practice ........................................ 244
12.22 Implications for future research ........................................... 244
12.23 Conclusions ................................................................. 245

Chapter 13: Role of acetowhitening time in the diagnosis of neoplasia 246
13.1 Introduction ........................................................................ 247
13.2 Hypothesis ........................................................................ 248
13.2.1 Primary aim .................................................................... 248
13.2.2 Secondary aim ............................................................... 248
13.3 Methods ............................................................................ 249
13.3.1 Inclusion criteria ............................................................ 249
13.3.2 Exclusion criteria ............................................................ 249
13.4 Ethics committee approval .................................................. 250
13.5 Statistical analysis ............................................................. 251
13.6 Endoscopic protocol ........................................................... 251
13.7 Assessment tool ............................................................... 253
13.8 Results ............................................................................ 254
13.8.1 True histology ............................................................... 254
13.9 Acetowhitening timings; metaplasia vs high risk neoplasia ...... 256
13.10 Acetowhitening timings; cases misdiagnosed ....................... 256
13.11 ROC curve; Metaplasia versus high risk neoplasia ............... 257
13.12 ROC curve: HGD versus cancer ........................................... 258
13.13 ROC curve: HGD+IMC versus invasive cancer ................. 260
13.14 Discussion 265
13.15 Strengths of the study 266
13.16 Limitations 267
13.17 Implications for clinical practice 268
13.17 Future research needs 269
13.18 Conclusions 269

PART C: Summary of the work

Chapter 14: The potential clinical impact of the findings 271
14.1 Significance of the results 272
14.2 Colonic neoplasia studies 273
14.3 The challenge 275
14.3.1. Medico-legal issues 275
14.3.2 Training 276
14.3.3 Tools 277
14.3.4 Culture 278
14.4 Future research needs 279
14.5 Barrett’s neoplasia studies 281
14.6 The challenge 281
14.6.1 Training in chromoendoscopy for lesion recognition 283
14.6.2 Mass application of the techniques 284
14.7 Future research needs 284
14.7.1 Acetic acid in a surveillance population 285
14.7.2 Risk stratification of patients with Barrett’s 286
14.7.3 Acetic acid chromoendoscopy versus mapping biopsies 286
14.7.4 Acetic acid versus trimodal imaging and confocal endomicroscopy 287
14.7.5 Acetic acid and electronic imaging combined 287
14.7.6 Low grade dysplasia 288
14.8 Conclusions 289

Chapter 15: References 290
List of tables

Table 1: Breakdown of pit pattern by true histology ........................................ 49
Table 2: Accuracy of indigo carmine by lesion type ........................................... 51
Table 3: comparison of accuracy of in-vivo diagnosis with white light and indigo carmine with and without optical magnification ........................................ 52
Table 4: Summary of publications stating accuracy, sensitivity and specificity of chromoendoscopy with optical magnification ........................................ 55
Table 5 Summary of publications stating accuracy, sensitivity and specificity of chromoendoscopy with indigo carmine on polyps<10mm without optical magnification ........................................ 58
Table 6: Comparison of studies using acetic acid chromoendoscopy ................. 60
Table 7: Accuracy, sensitivity and specificity for NBI and trimodal imaging ........ 66
Table 8: FICE pre set bandwidths (nm) ............................................................... 67
Table 9: Summary of papers published using FICE for in-vivo diagnosis ............ 72
Table 10: Summary of papers published using confocal endomicroscopy for in-vivo diagnosis .......................................................... 76
Table 11: Chromoendoscopy studies in Barrett's oesophagus .............................. 105
Table 12: Electronic imaging studies in Barrett's oesophagus .............................. 106
Table 13: Pre-programmed Frequencies for FICE ............................................. 120
Table 14: Pre-programmed settings for FICE .................................................. 140
Table 15: Optimun FICE settings ................................................................. 144
Table 16: N.A.C. criteria for FICE assessment ................................................ 154
Table 17: In-Vivo validation of N.A.C. criteria ................................................ 167
Table 18: Demographics of patient population ................................................. 180
Table 19: in-vivo diagnosis of neoplasia by endoscopic modality (WLI vs FICE vs IC) 182
Table 20: Accuracy of in-vivo diagnosis by true histology of polyp 183
Table 21: Accuracy with polyps<5mm WLI, FICE, IC diagnosis by polyp histology 184
Table 22: Evaluation of histopathology costs within the study population 186
Table 23: Patient demographics for study population 205
Table 24: Accuracy for neoplasia SD vs HD 206
Table 25: Accuracy non-neoplastic lesions SD vs HD 207
Table 26: Accuracy by polyp size 209
Table 27: Surveillance intervals 210
Table 28: Visible abnormalities WLI vs AA 235
Table 29: Targeted vs true histology 236
Table 30: Sensitivity and specificity by patient group 237
Table 31: True histology of cohort 254
Table 32: Mean aceto-whitening times by lesion histology 256
Table 33: Sensitivity and specificity of aceto-whitening for neoplasia 257
Table 34: Sensitivity and specificity for aceto-whitening HGD vs Cancer 259
Table 35: Sensitivity and specificity for aceto-whitening HGD+IMC vs invasive cancer 260
Table 36: Coordinates of the Curve 262
List of figures

Figure 1: Diagrammatic representation of Kudo pit patterns

Figure 2: Flow chart for examination of polyps with cresyl violet

Figure 3: Sessile 3mm tubular adenoma showing the Kudo type III pit pattern and strong vascular pattern intensity

Figure 4: 3mm hyperplastic polyp showing the kudo type II pit pattern and weak vascular pattern intensity

Figure 5: Oesophagus: A: routine endoscopic appearance B: post acetic acid chromoendoscopy

Figure 6: Barrett’s high grade dysplasia visualized with the Cellvizio miniprobe

Figure 7: Key components of the spectral imaging system

Figure 8: Spectral reflectance as a function of wavelength

Figure 9: Three principle components (a) and cumulative contributions (b) of spectral reflectance of the rectal membrane

Figure 10: Hyperplastic polyp WLI

Figure 11: Hyperplastic polyp FICE setting 4

Figure 12: Hyperplastic polyp IC

Figure 13: Adenomatous polyp WLI

Figure 14: Adenomatous polyp FICE setting 4

Figure 15: Adenomatous polyp IC

Figure 16: Hyperplastic and adenomatous polyp viewed with WLI and all 10 FICE settings

Figure 17: Hyperplastic polyp demonstrating pallor (hypovascularity) with white light
Figure 18: Hyperplastic polyp: Note this is hypervascular.

Figure 19: Hyperplastic polyp FICE setting 8

Figure 20: Adenoma on white light

Figure 21: Adenoma demonstrating a well organised pericryptal vascular pattern

Figure 22: Adenoma on FICE setting 4. The lesion is hypervascular. The vascular pattern is less clear but is ordered and crosses the entire surface of the lesion.

Figure 23: Cancer on white light

Figure 24: Cancer viewed with FICE setting 4

Figure 25: Comparison of polyp SD vs HD

Figure 26: Flow chart for patient recruitment

Figure 27: Study design flow chart

Figure 28: Barrett's metaplasia without any evidence of neoplasia

Figure 29: Neoplasia within Barrett's

Figure 30: Traumatized mucosa after 5% acetic acid

Figure 31: Pathway for AA Chromoendoscopy

Figure 32: Pathway for recruitment

Figure 33: Pathway for aceto-whitening study

Figure 34: Pathway for recruitment

Figure 35: ROC Curve for metaplasia Vs neoplasia

Figure 36: ROC curve HGD vs Cancer

Figure 37: ROC curve HGD and IMC vs invasive cancer.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>AFI</td>
<td>Auto-florescence imaging</td>
</tr>
<tr>
<td>ASGE</td>
<td>American Society of Gastrointestinal Endoscopy</td>
</tr>
<tr>
<td>BADCAT</td>
<td>Barrett’s Dysplasia and Cancer taskforce Consensus Group</td>
</tr>
<tr>
<td>BCSP</td>
<td>Bowel Cancer Screening Programme</td>
</tr>
<tr>
<td>BSG</td>
<td>British Society of Gastroenterology</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge Coupled Device</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>EMR</td>
<td>Endoscopic mucosal resection</td>
</tr>
<tr>
<td>ESD</td>
<td>Endoscopic sub-mucosal dissection</td>
</tr>
<tr>
<td>FICE</td>
<td>Flexible Spectral Imaging colour enhancement</td>
</tr>
<tr>
<td>GORD</td>
<td>Gastro Oesophageal Reflux Disease</td>
</tr>
<tr>
<td>HD</td>
<td>High Definition</td>
</tr>
<tr>
<td>HGD</td>
<td>High grade dysplasia</td>
</tr>
<tr>
<td>HP</td>
<td>Hyperplastic Polyp</td>
</tr>
<tr>
<td>IC</td>
<td>Indigo carmine</td>
</tr>
<tr>
<td>IMC</td>
<td>Intra-mucosal cancer</td>
</tr>
<tr>
<td>LGD</td>
<td>Low grade dysplasia</td>
</tr>
<tr>
<td>MB</td>
<td>Methylene blue</td>
</tr>
<tr>
<td>NBI</td>
<td>Narrow Band Imaging</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
</tr>
<tr>
<td>PIVI</td>
<td>Preservation and Incorporation of Valuable Endoscopic Innovations</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operator Curve</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Definition</td>
</tr>
<tr>
<td>Sm</td>
<td>Sub-mucosal</td>
</tr>
<tr>
<td>WLI</td>
<td>White light imaging</td>
</tr>
</tbody>
</table>
PART A:

Background to advanced endoscopic techniques
Chapter 1

Introduction
Introduction

Gastrointestinal malignancies represent a significant health burden, with colonic, oesophageal and gastric cancer being the 3rd, 9th and 10th most common malignancies respectively in the UK (1). With the exception of pancreatic cancer, survival has improved significantly over the last 3 decades, with survival rates doubling for oesophageal and colonic malignancies. Unfortunately despite these advances there is significant world wide variation in both the prevalence and survival from these conditions.

Oesophageal cancer is the 9th most common cancer in the UK, with 7800 people diagnosed every year, accounting for 5% of all cancer deaths in the UK. Rates have increased by 50% over the last 30 years (2). It is more common in men than women, with an incidence of 8.8-14.1 per 100,000 in men, and 4.8-5.7 per 100,000 in women. (3). The 5 year survival remains low at 9% (4), which is primarily due to cancer being diagnosed at a late stage. There is therefore a need for early diagnosis so that the disease can be managed at an early and treatable stage.

A significant risk factor for adenocarcinoma of the oesophagus is Barrett’s epithelium, an acquired pre-malignant condition, caused by reflux of gastric contents into the oesophagus. The gastric acid damages the normal squamous epithelium, becoming replaced by a columnar epithelium. The newly updated definition by the British society of Gastroenterology defines Barrett’s as ‘an endoscopically apparent..."
area above the oesophagogastric junction that is suggestive of Barrett's which is supported by the finding of columnar lined oesophagus on histology’ (5) (6).

Barrett’s oesophagus affects up to 1.6% of the general population (7). It is found in 15-20% of gastrointestinal endoscopies performed for symptoms of reflux and the incidence is increasing in the West (2) (8). It has the potential to progress into adenocarcinoma. Risk factors for this include male gender, age >45, long segment (>8cm) disease, a history of longstanding reflux, early age of onset of gastro-oesophageal reflux disease (GORD), duodeno-gastrooesophageal reflux, mucosal damage and family history (6).

Colorectal cancer represents a major health burden in the West, accounting for 550,000 deaths per year worldwide (1). Whilst the adenoma to carcinoma pathway is not fully understood, the vast majority of cases arise on a background of adenomatous colonic polyps. This is important as removal of these lesions provides a potential method of cancer prevention which has led to the development of colonoscopy based screening programmes to detect these pre-malignant changes. Early evidence has suggested that this is an effective method of reducing cancer incidence (9).

Advances in endoscopic practice are challenging traditional views on how we should be managing cancer in the gastrointestinal system. Over the last 10 years there has been a period of rapid growth in the equipment and technology available for evaluating potentially malignant lesions in the gastrointestinal tract. These include
advanced endoscopes with high definition screens, image processors and magnification facilities, endomicroscopes and dye sprays for enhancing mucosal patterns. Many of these are being used in daily practice. However, the evidence base for how they should be used, and their effectiveness in predicting histology and changing patient management is limited. It is widely accepted that correctly identifying high risk pre-malignant changes in the gut is central to successful early management of most cancers. This includes both the detection of dysplastic areas within Barrett’s oesophagus and the correct classification of polyps as either hyperplastic, adenomas or polyp cancers. The development of these new technologies; chromoendoscopy, ‘virtual chromoendoscopy’ using digital image enhancement techniques built into the endoscopes (Flexible Spectral Imaging Colour Enhancement (FICE), Narrow Band Imaging and i-scan) and high resolution endoscopes have opened up a wealth of new possibilities which have yet to be fully explored.

At the same time as new technologies have been emerging the development of the Bowel Cancer Screening Programme (BCSP) within the UK has increased the number of colonoscopies being performed in the age group 59-70 yrs. It is known that patients in this age group have a very high incidence of polyps. Targets in colonoscopy have driven up standards in lesion detection, with compulsory reporting of adenoma detection rates in the U.K. However, not all polyps are adenomas as 50% of small lesions (<10mm in size) are hyperplastic, which have negligible malignant potential. Due to a lack of in-vivo diagnostic techniques it is current policy that all polyps are removed and sent for histological examination, exposing patients
to the risks of polypectomy, including bleeding and colonic perforation, whilst providing already stretched health service providers with a significant expense in terms of histological assessment of these polyps. In the oesophagus it is current policy to offer regular surveillance gastroscopy to patients with Barrett’s in an attempt to identify the development of dysplasia. However, this is still being done with protocol driven, non targeted biopsies which is time consuming, expensive and still misses dysplastic lesions. If we are to be able to offer appropriate targeted endoscopic treatments in both of these areas it is important that the lesions can be assessed accurately.

In recent years there has been considerable development in techniques for examining both the upper and lower gastrointestinal tract. This has included the introduction of numerous dye sprays, including acetic acid in the oesophagus and indigocarmine in the colon, and the development of electronic imaging techniques, including Narrow Band Imaging and FICE. However, the evidence base is incomplete. Much of the published data comes from the Far East in different patient populations to those seen by the Western endoscopist. Disease incidence and prevalence for most gastrointestinal malignancies show considerable worldwide variation. In particular there is very little Barrett’s in Japan and most of Asia, resulting in a paucity of publications into this condition. The problem facing the Western endoscopist is therefore a lack of understanding of how these emerging techniques fit together and how to apply advanced endoscopic imaging techniques to the management of Western patients.

The American Society of Gastrointestinal Endoscopy (ASGE) has recently launched a new initiative to identify important clinical questions related to endoscopy and to establish the diagnostic and therapeutic threshold for the endoscopic techniques to
resolve these clinical questions (10). This is known as PIVI (preservation and incorporation of valuable endoscopic innovations) and has been developed to direct endoscopic technology development towards resolving these important clinical issues in endoscopy. A concern has been that measures need to be taken to avoid the widespread use of an endoscopic technology before clinical studies demonstrating effectiveness have been performed. Likewise, potentially valuable innovations are being prematurely abandoned due to a lack of utilization. The first remit of the PIVI has been to investigate real time endoscopic assessment of the histology of diminutive colonic polyps. The ASGE has issued the following statements:

1) In order for colorectal polyps <5mm in size to be resected and discarded without pathologic assessment, endoscopic technology (when used with high confidence) used to determine histology of polyps <5mm in size, when combined with the histopathologic assessment of polyps >5mm in size, should provide a 90% agreement in assignment of post-polypectomy surveillance intervals when compared to decisions based on pathology assessment of all identified polyps.

2) In order for a technology to be used to guide the decision to leave suspected rectosigmoid hyperplastic polyps <5mm in size in place (without resection), the technology should provide greater than 90% negative predictive value (when used with high confidence) for adenomatous histology.

These are fundamental questions which need to be answered in regards to the evaluation of colonic neoplasia. It also needs to be shown whether this can be
applied to colonic polyps in a Western screening population. The optimal method for examination of these polyps needs to be established. This includes the type of endoscope which should be used (standard definition versus high definition) and whether a diagnosis can be made without optical magnification. The features of the polyps which can be exploited to make an accurate diagnosis needs further study. This essentially centres around the lesion classification system that should be used and whether the complex systems pioneered in Japan are the only way to establish an *in-vivo* diagnosis. The specific roles for chromoendoscopy and electronic imaging in the characterization of colonic neoplasia needs to be identified. In particular when to choose a certain technique needs to be investigated as does the benefit of using dye spray in addition to electronic imaging. Furthermore, how *in-vivo* diagnosis fits into an assessment protocol needs to be established. In particular whether there is a benefit in terms of cost saving, should be examined. Finally the adverse effects arising from an incorrect *in-vivo* diagnosis should be studied.

The ASGE has not yet released a PIVI for the assessment of Barrett’s. However, a group of 60 clinicians and scientists from 14 different countries working in 10 separate disciplines have developed the Barrett’s Dysplasia and Cancer Taskforce Consensus Group (BADCAT). The aims of this group is to produce evidence based guidelines for best clinical and cost effective management of high grade dysplasia and early mucosal cancer in Barrett’s oesophagus, which are likely to form the basis of all future national guidelines around the world. One of the areas covered by this review is advanced endoscopic imaging and some of the studies from this thesis have contributed towards this document, and will be discussed later in Chapter 12.
The most important question that needs to be answered in Barrett’s oesophagus is whether neoplasia can be routinely observed during endoscopy. If so, how frequently this is possible with white light needs to be established, before the benefits of any enhancement technique can be demonstrated. The role of acetic acid chromoendoscopy in improving the visualization of neoplasia within Barrett’s needs to be addressed, and in particular how the acetic acid technique should be used and the results interpreted.

The purpose of the studies described in this thesis is to answer most of the questions raised above. Specifically, the studies aim to evaluate the concept of in-vivo diagnosis and the role of advanced endoscopic techniques in facilitating an accurate in-vivo diagnosis. The work will focus on detecting early neoplasia in both Barrett’s and colonic polyps. These are common problems facing the Western endoscopist and are increasing in prevalence (3) (1).
Hypothesis

Modern endoscopic techniques of chromoendoscopy and vascular enhancement can be used to localise and characterize neoplasia within the gastrointestinal tract.

Aims of this thesis

1) To establish whether the vascular enhancement technique FICE and indigo carmine chromoendoscopy can be used to characterize polyps in the colon without optical magnification

2) To establish whether acetic acid chromoendoscopy can be used to localise and characterise neoplasia within Barrett's oesophagus
Objectives of the thesis

3) To establish the optimum FICE setting for the assessment of the surface and vascular patterns and structures of colonic neoplasia.

4) To develop and validate a lesion assessment tool for use with the FICE electronic imaging system.

5) To apply these tools to a Western bowel cancer screening population and establish whether in-vivo diagnosis is possible in this patient group.

6) To establish whether there is additional benefit from using indigocarmine after assessment with the electronic imaging modality FICE.

7) To establish whether neoplasia within Barrett’s oesophagus is visible endoscopically using white light gastroscopy.

8) To assess the accuracy of acetic acid dye spray in the detection of Barrett’s neoplasia during gastroscopy.

9) To explore and analyse the aceto-whitening reaction seen after acetic acid dye spray, and to develop this as an objective assessment tool for the differentiation of different stages of neoplasia.
Chapters 2 and 3 consider the current evidence base for the emerging imaging technologies in both the lower and upper gastrointestinal tract respectively. This includes an extensive review of the literature describing the use of indigocarmine, methylene blue and acetic acid chromoendoscopy, the role of vascular enhancement techniques, including narrow band imaging (NBI), Flexible Spectral Imaging Colour Enhancement (FICE), i-scan, and confocal endomicroscopy. They discuss the limitations of the current published literature and investigate the current research needs in detail. Chapter 4 is a technical overview of how FICE works to enhance the vasculature of the gastrointestinal system.

Chapters 5-11 describe the experimental work conducted into the characterization of colonic neoplasia. This begins with the development and validation of a novel classification system for colonic polyps using FICE with standard endoscopes followed by an in-vivo study using this tool on polyps <10mm in a bowel cancer screening population. The specific role for the technique will be examined, and whether there is additional gain from dye spray with indigo carmine after a diagnosis with FICE has been made is also evaluated. The effect on clinical outcome from an incorrect diagnosis is examined and a cost evaluation for replacing traditional histological examination with an in-vivo diagnosis within the Bowel Cancer Screening Programme is reviewed. The data is then subdivided into procedures performed with standard definition and high definition endoscopes and the effects of this on diagnostic accuracy calculated.
Chapter 12 describes the experimental work conducted into the evaluation of Barrett’s neoplasia using acetic acid chromoendoscopy. It begins with the development of an assessment protocol which is then used in both a screening and high risk population to detect neoplasia within Barrett’s. The accuracy for white light examination is established and the additional gain from acetic acid calculated. The technique is then refined in Chapter 13 by a further study exploring whether the differential loss of acetowhiteness between Barrett’s metaplasia and dysplasia can be exploited to further improve the accuracy of neoplasia detection.

Finally the results from all of the experimental work are drawn together in Chapter 14. In particular the clinical implications of the findings are discussed, and how they can be applied to U.K. endoscopic practice. Areas where uncertainty still remains are identified along with the need for future research.
Chapter 2

Colonic neoplasia
Colonic neoplasia

Colorectal cancer is a significant health concern. There are 1.4 million new cases each year, accounting for an estimated 550,000 deaths worldwide \(^1\). Although much has been learnt about the molecular events leading to colorectal cancer, there is still no cure for advanced disease. There is therefore an urgent need to develop better disease prevention strategies. Colorectal cancer develops in a series of well-defined steps; from normal mucosa to adenomatous polyp through varying degrees of dysplasia and finally adenocarcinoma \(^1\). Because of this adenoma-carcinoma sequence of events there has been a drive in recent years in most Western countries to develop bowel cancer screening programmes. The concept is to find adenomas before they turn into cancers and remove them, or to find early cancers which can be surgically treated with good results.

Numerous approaches have been taken to bowel cancer screening. One approach is to offer everyone over a cut off age (usually between 55-60) a colonoscopy. This is the method used in the United States. In the United Kingdom a faecal occult blood test has been used. This is a non invasive test which involves the patient returning 3 stool samples to be examined for the presence of occult blood (FOB). It is about 60% sensitive, meaning that approximately 6 out of 10 patients who undergo colonoscopy with positive FOBs have some form of pathology, not necessarily cancer). The positive predictive value of faecal occult bloods for cancer is around 11% and between 18-35% for adenomas. \(^{11}\) \(^{12}\). Colonoscopy findings are normal in around 50% of patients with positive FOBs.
Whilst polyps are a common finding during colonoscopy, not all polyps are the same. Hyperplastic polyps have negligible potential to turn into cancer, especially when small (<10mm) and located in the left colon (a common site). These account for one third of all small polyps (13). In contrast adenomas can turn into cancer (14). Finally polyps can already be cancers.

The management of polyps >10mm is simple as they are either adenomas which need removal or cancers which require surgical resection. There is a possibility of big polyps being hyperplastic serrated adenomas. Given the risk of malignant transformation, they need removal as well. It has been traditionally felt that hyperplastic polyps cannot be separated clinically from adenomas or polyp cancers. For this reason all polyps are removed. However, polypectomy is associated with significant risks (15) and results in an immediate cost in processing the samples, equipment and most importantly, increases the procedure time.

Because of the difference in risk between hyperplastic and adenomatous polyps, there is a need for in-vivo diagnostic techniques. If the endoscopist could make a confident in-vivo diagnosis then hyperplastic polyps <10mm could be left in-situ or removed but not retrieved thus reducing pathology costs. This has been recognised as important by the ASGE, who have released guidelines defining the standards which have to be met for any technology used for in-vivo diagnosis that is intended to replace conventional histological examination (10). These guidelines have stated that for a resect and discard policy to be adopted for polyps <5mm in size, when endoscopic technology is combined with the histopathologic assessment of polyps >5mm in size, this should provide a 90% agreement in assignment of post-
polypectomy surveillance intervals, when compared to decisions based on pathology assessment of all identified polyps. In order to be able to leave small (<5mm) left sided hyperplastic polyps in place the requirements are more stringent, and the technology should provide greater than 90% negative predictive value for adenomatous histology, if used for this purpose.

There is also a need for diagnostic skills to differentiate adenomas from cancers. Whilst polyp cancers <10mm are rare, endoscopists are increasingly attempting to remove large flat polyps, rather than referring them for traditional surgical resection with the increased risks of associated morbidity and mortality. It is dangerous to attempt endoscopic resection of polyp cancers, with an increased risk of perforation and a high probability of lymph node metastasis. As the risk of cancer increases with the size of polyp, endoscopists who resect large polyps need to have very good skills in in-vivo diagnosis so as to avoid resecting polyps containing cancer.

Unfortunately white light examination is not adequate to make an effective in-vivo diagnosis, with a diagnostic accuracy of around 70%. This has led to research into methods for enhancing the lesion surface or vascular pattern to enable a more detailed and discriminatory examination to be performed. There have been several methods that have been proposed to achieve this:

1) Chromoendoscopy
2) Electronic imaging techniques
3) Confocal endomicroscopy
Unfortunately the evidence base for all of these techniques is incomplete. As a result it is still the accepted standard of care to resect and send all polyps regardless of size for histological assessment.
2.1: Chromoendoscopy

Chromoendoscopy involves the application of a dye to the gastrointestinal tract. The intention is to both identify and characterize lesions. The techniques were pioneered in Japan where initial experience was in the use of the vital stain crystal violet to characterise colonic neoplasia.
2.2: Crystal violet chromoendoscopy

Crystal violet is a vital stain that irreversibly binds to cellular structures, highlighting surface patterns in great detail. It was with this dye that the first attempts were made at in-vivo histology prediction for colonic polyps. The early work was conducted by Professor Kudo in Japan, where the surface patterns of 14,023 colonic lesions was correlated to pathology (16). An association was found between individual surface pits and crypts when pit patterns were examined in-vivo with magnifying colonoscopy (x60). The lesions were then resected, stained with haematoxylin and examined using stereo microscopy (x60). The following observations were made on the properties of the surface pits seen with magnifying colonoscopy (x 60):

1) **Normal round pit**: The crypts were all straight and non-branching. The cells looked histopathologically normal. These were diagnosed as normal (100%).

2) **Small asteroid pits**: The crypts were all straight and non-branching.

   Histopathologically, the cells showed slight swelling but no atypia. These glands were diagnosed as hyperplastic (100%).

3) **Large asteroid pits**: Branching crypts. This occurred repeatedly, becoming smaller each time. Histopathologically, the cells were slightly swollen and mildly atypical. These crypts were diagnosed as hyperplastic or as serrated adenoma.

4) **Small round pits**: The crypts were all straight and nonbranching.

   Histopathologically the cells showed borderline malignant or carcinomatous change. These crypts were diagnosed as borderline malignant (72%) or adenocarcinoma (28%). It was noted that macroscopically this pattern was seen most often in depressed lesions.
5) **Oval pit:** These crypts were all branching repeatedly with several round crypts. Histopathologically, the cells looked moderately atypical, and these glands were diagnosed as adenoma (100%).

6) **Gyrus-like pit:** The giant crypts were all branching repeatedly with several round or oval shaped crypts. Histopathologically, the cells were moderately atypical, and again these crypts were diagnosed as adenoma (100%), and were found in almost all the tubulovillous adenomas.

7) **Non-pit:** Non-structural glands. Histopathology these glands were diagnosed as adenocarcinoma (100%) which had invaded the submucosal or deeper layers.

The patterns were classified into categories:

Type I: normal round pit

Type II: small and large asteroid pits

Type III: small round pit

Type IIII: oval pit

Type IV: gyrus-like pit

Type V: non-pit pattern

This became known as Kudo's classification and macroscopically they were seen on both protruding and depressed lesions. The findings were highly significant (16) and this has formed the basis for all future work into in-vivo diagnostic techniques. See Figure 1.
Professor Kudo continued the work by using magnifying endoscopes and crystal violet to observe and predict the histology of colonic lesions in vivo (17). The pathway he used for examination is shown in figure 2.
In total 2050 lesions were examined and the correlation of pit pattern to pathology is shown below. The authors concluded that there was an association between pit pattern and underlying histology, and that an *in-vivo* diagnosis could be made. Cresyl violet (0.2%) staining was used because it actually stains the margins of the pits, providing a clear definition of the pattern, which unlike indigo carmine is merely retained in the pit orifices. See table 1.
Pit pattern with magnifying electric videoscope

<table>
<thead>
<tr>
<th></th>
<th>1, 2</th>
<th>3S</th>
<th>3L</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histopathologic diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma</td>
<td>46</td>
<td>29</td>
<td>1145</td>
<td>150</td>
<td>11</td>
<td>1381</td>
</tr>
<tr>
<td>Villous adenoma</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>47</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>Cancer</td>
<td>0</td>
<td>3</td>
<td>71</td>
<td>72</td>
<td>22</td>
<td>168</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>46</td>
<td>32</td>
<td>1233</td>
<td>269</td>
<td>33</td>
<td>1613</td>
</tr>
</tbody>
</table>

Table 1: Breakdown of pit pattern by true histology (Reproduced from Kudo et al 1996 (17))

Overall it was felt that neoplastic lesions could be differentiated from non-neoplastic lesions with an accuracy of 81.5%. Subsequent studies showed this to be an underestimate of the accuracy which could in practice be obtained, and sensitivities of up to 98% have since been reported, which will be discussed later. It should be noted that a IIIa pattern was shown to represent adenoma in the majority of cases rather than cancer. This has been repeatedly shown in all of the subsequent studies.

Kudo pit pattern analysis has been accepted as the gold standard method for the examination of colonic neoplasia. However, it poses a number of problems. Vital stains such as crystal violet are inconvenient to use. They have to be dripped onto the surface of the lesion and allowed to fix for several minutes, followed by adequate
washing prior to evaluation. This can be very time consuming. Furthermore, there are concerns surrounding DNA toxicity (18). For this reason it is generally accepted that vital stains are not practical for daily use outside of Japan or a research setting. This led to a search for alternative dyes.
2.3: Indigo carmine

Whilst crystal violet is a vital stain, indigo carmine is a blue dye which does not bond to or react with human tissue in any way. It simply sits on the surface of tissues, highlighting surface patterns. For this reason it is a very safe dye. Furthermore, it is easier to use than crystal violet as the results are instant. As it does not bind to tissues, excess dye can be sucked away if necessary.

Chromoendoscopy can be used for two purposes; to find polyps or to characterise neoplasia. A Cochrane meta-analysis of clinical trials has concluded that chromoendoscopy with indigocarmine enhances the detection of neoplastic polyps.

The initial work with indigo carmine for in-vivo diagnosis was conducted in Japan by Kato et al. who retrospectively analysed 4445 lesions using magnifying endoscopy with indigo carmine dye spray. All of the lesions were less than 5mm in size. Lesions were assessed in vivo and correlated with histopathological analysis using a similar methodology to that employed by Kudo et al in the previously described study. The findings suggested that the diagnostic accuracy for hyperplastic polyps with a type I or II pattern was 75%, adenomas with a type III or IV pattern 94% and invasive cancer with a type V pattern in 85% of cases. The sensitivities and specificities are shown in table 2.

<table>
<thead>
<tr>
<th></th>
<th>Hyperplastic</th>
<th>Adenoma</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>42%</td>
<td>98%</td>
<td>82%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99%</td>
<td>52%</td>
<td>99%</td>
</tr>
</tbody>
</table>

Table 2: Accuracy of indigo carmine by lesion type
The results here show marked difference between the sensitivity and specificity. This data suggests that high sensitivity was achieved by compromising the specificity resulting in a large proportion of hyperplastic polyps being overcalled as adenomas. Furthermore, the data was dependent on 100x magnification with a zoom endoscope.

Further work was conducted in Japan into indigo carmine (21) which investigated the differences between chromoendoscopy with and without magnification in the examination of small (<10mm) polyps. The results were encouraging, with a sensitivity for neoplasia of 93.1% and specificity of 76.1% being achieved. However, there was an improvement with magnification. This suggested that in appropriately skilled hands, in-vivo diagnosis was possible without the need for magnification endoscopy or vital stains. See table 3.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional endoscopy</td>
<td>84%</td>
<td>88.8%</td>
<td>67.4%</td>
</tr>
<tr>
<td>Chromoendoscopy without magnification</td>
<td>89.3%</td>
<td>93.1%</td>
<td>76.1%</td>
</tr>
<tr>
<td>Chromoendoscopy with magnification</td>
<td>95.6%</td>
<td>96.3%</td>
<td>93.5%</td>
</tr>
</tbody>
</table>

Table 3: comparison of accuracy of in-vivo diagnosis with white light and indigo carmine with and without optical magnification.

There were further studies looking at magnification endoscopy with indigo carmine for in-vivo histology prediction which showed similar results. (22) (23) (24)

There has been some work done outside of Japan using magnifying chromoendoscopy with indigo carmine. Tischendorf et al in Germany conducted a
prospective cohort study of neoplastic vs non neoplastic polyps using both narrow band imaging and indigo carmine with zoom endoscopy. A sensitivity of 91.7% and specificity of 90% was achieved for indigo carmine using Kudo pit pattern analysis (25). A large study from the Weisbaden group in Germany compared indigocarmine and the electronic imaging modality FICE in the assessment of polyps <10mm. The primary aims of this study were lesion detection, with the intention of evaluating FICE for this purpose. However a sub-group of 280 lesions were assessed using indigocarmine for histology prediction. A sensitivity for neoplasia of 87.6% and specificity of 62.0% was achieved (26). High definition endoscopes were used without optical magnification. There was a further German study by a different group examining indigocarmine chromoendoscopy using high resolution endoscopes. This study investigated 273 lesions <5mm, with a sensitivity of 94%, specificity of 64% and accuracy of 83% (27). This study differed from the other studies described in that it only examined rectosigmoid polyps. Therefore the results could not be applied to right sided lesions. An American study showed similar results using standard resolution (410,000 pixel) endoscopy with indigo carmine, where 500 polyps <10mm in size found anywhere in the colon were evaluated, achieving a sensitivity of 82% and specificity of 82% (28). A limitation of this study was that it was a multi-centre multi-endoscopist study where most of the investigators were not equally experienced in in-vivo diagnosis. Training was via a videotape of 30 test cases with a separate answer sheet for the endoscopist to review prior to starting the study. This was not a validated training regimen and therefore this study may be underestimating the potential for indigocarmine as a diagnostic tool.

There was a prospective Italian cohort study examining 240 polyps <5mm with magnifying chromoendoscopy. This study reported an accuracy of 95.4%, a
sensitivity of 97.5% and specificity of 94.3%. These results were better than those seen in most of the other series (29).

There was a United Kingdom cohort study where 1008 flat lesions of any size were examined using zoom endoscopy and indigocarmine. A sensitivity for neoplasia of 98% and specificity of 92% was achieved. However, it should be noted that the endoscopist was permitted to use crystal violet when the pit patterns could not be adequately visualised with indigo carmine. Therefore this should be regarded as an in-vivo diagnosis study rather than a specific evaluation of indigo-carmine (30). Furthermore, the authors found it difficult to differentiate non invasive from invasive lesions, with a sensitivity of 50% and specificity of 98%.

The strength of all of these studies is that all of the assessments were made by Western endoscopists, suggesting that the findings seen in the Japanese papers could be translated to Western practice. All but one of the studies were single centre, single endoscopist experiences.

A notable point that can be observed in most of the described studies is that there is a trade off between adenoma sensitivity and specificity. Many of the studies with the highest sensitivity have a low specificity, typically between 60-70%. Whilst this is probably the safest approach to in-vivo diagnosis, it is not ideal. The ultimate goal for diminuitive polyps would be to have the ability to confidently leave small hyperplastic polyps, reducing the risks posed by polypectomy. To achieve this, the sensitivity and specificity both need to be very high.

All of the studies described so far have required magnifying zoom endoscopes. These are summarised in table 4. A problem with this strategy is that this is not standard practice in the west. Magnifying endoscopes are larger and more difficult to
manoeuvre. They also have a narrower field of view. Furthermore, magnified assessment of lesions is time consuming. In the west, endoscopy lists are traditionally busy, making time constraints an unavoidable issue. This has discouraged many people from seriously considering in-vivo histology prediction as a realistic option.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Journal</th>
<th>Year</th>
<th>Size</th>
<th>Dye</th>
<th>No polyps</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axelrad</td>
<td>USA</td>
<td>Endoscopy</td>
<td>1996</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>24</td>
<td>93%</td>
<td>95%</td>
<td>NR</td>
</tr>
<tr>
<td>Togashi</td>
<td>Japan</td>
<td>Dis Colon Rectum</td>
<td>1999</td>
<td>All size</td>
<td>Indigo carmine</td>
<td>923</td>
<td>92%</td>
<td>73.3%</td>
<td>88.4%</td>
</tr>
<tr>
<td>Kato</td>
<td>Japan</td>
<td>Endoscopy</td>
<td>2001</td>
<td>&lt;5mm</td>
<td>Indigo carmine</td>
<td>4445</td>
<td>94%</td>
<td>75%</td>
<td>NR</td>
</tr>
<tr>
<td>Tung</td>
<td>Taiwan</td>
<td>Am J Gastro</td>
<td>2002</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>175</td>
<td>93.8%</td>
<td>64.6%</td>
<td>80.1%</td>
</tr>
<tr>
<td>Fu</td>
<td>Japan</td>
<td>Endoscopy</td>
<td>2003</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>206</td>
<td>96.3%</td>
<td>93.5%</td>
<td>95.6%</td>
</tr>
<tr>
<td>Konishi</td>
<td>Japan</td>
<td>Gastrointestinal</td>
<td>2003</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>405</td>
<td>97%</td>
<td>100%</td>
<td>93%</td>
</tr>
<tr>
<td>Su</td>
<td>Taiwan</td>
<td>Dig Dis Sci</td>
<td>2004</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>270</td>
<td>95.1%</td>
<td>86.8%</td>
<td>91.9%</td>
</tr>
<tr>
<td>Hurlstone</td>
<td>UK</td>
<td>Gut</td>
<td>2004</td>
<td>Any size</td>
<td>Indigo carmine and crystal violet</td>
<td>1008</td>
<td>98%</td>
<td>92%</td>
<td>NR</td>
</tr>
<tr>
<td>Palma</td>
<td>Italy</td>
<td>World Gastroenterology</td>
<td>2006</td>
<td>&lt;5mm</td>
<td>Indigo carmine</td>
<td>240</td>
<td>97.5%</td>
<td>94.3%</td>
<td>95.4%</td>
</tr>
<tr>
<td>Sonwalker</td>
<td>UK with</td>
<td>Endoscopy</td>
<td>2007</td>
<td>Any size</td>
<td>Indigo carmine</td>
<td>709 (513&lt;10mm)</td>
<td>91%</td>
<td>87%</td>
<td>90%</td>
</tr>
<tr>
<td>Tiscendorf</td>
<td>Germany</td>
<td>Endoscopy</td>
<td>2007</td>
<td>Any size</td>
<td>Indigo carmine</td>
<td>200 (100 with IC)</td>
<td>91.7%</td>
<td>90%</td>
<td>NR</td>
</tr>
<tr>
<td>Ince</td>
<td>Turkey</td>
<td>Hepatogastroenterology</td>
<td>2007</td>
<td>Any size</td>
<td>Indigo carmine</td>
<td>80%</td>
<td>89%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>Pohl</td>
<td>Germany</td>
<td>American Journal of Gastroenterolog</td>
<td>2008</td>
<td>&lt;20mm</td>
<td>Indigo carmine (picture)</td>
<td>150</td>
<td>95.5%</td>
<td>73.8%</td>
<td>87.7%</td>
</tr>
<tr>
<td>Togashi</td>
<td>Japan</td>
<td>Gastrointestinal</td>
<td>2009</td>
<td>&lt;5mm</td>
<td>Indigo carmine</td>
<td>107</td>
<td>90%</td>
<td>74%</td>
<td>86%</td>
</tr>
</tbody>
</table>

Table 4: Summary of publications stating accuracy, sensitivity and specificity of chromoendoscopy with optical magnification (NR=not reported).
Indigo carmine without optical magnification

There has been a lack of studies published into the use of non magnifying endoscopes for in vivo histology prediction using indigo carmine. Whilst this may sound like a small difference in practice it is critical. Examining polyps with a magnifying endoscope allows up to 120x optical magnification. This enables tiny details on the surface of lesions to be seen in great detail. In the west, there has been so little experience with endoscopes offering optical magnification that in-vivo diagnosis has been dismissed by many as either impossible, or a Japanese fairy tale that cannot be used in the West.

The first study comparing optical magnification versus standard non magnification for making an in-vivo diagnosis was from Japan. (31). It looked at 660 patients, who were allocated into two groups of 330 patients each. Polyps <10mm were included, with 812 lesions in total. The results were disappointing for the non-magnifying limb, with an accuracy of 68% for non zoom assessment compared to 92% using zoom endoscopes. However there was a subsequent UK study looking at indigo carmine with non magnifying endoscopes (32). This prospective study looked at 709 colonic polyps of any size. A sensitivity of 91% and a specificity of 82% was achieved. A sub group analysis of lesions <10mm was performed which found that size made very little difference to the diagnostic accuracy, with a sensitivity for neoplasia of 91% and specificity of 87% when examining small polyps. However, there are a number of important issues surrounding this study. Although it was conducted in the United Kingdom and the procedures were performed by a UK trained endoscopist, the lesion assessments were all carried out by a visiting Japanese expert. Therefore,
although it was of value to demonstrate that such assessments could be performed in a western setting, and that an in-vivo assessment may be possible without magnifying zoom endoscopy, it should be recognised that the assessments were not by western trained clinicians. As such it should be seen as a Western Japanese collaboration, and therefore it is difficult to be confident that the results could be directly applied to Western practice. A summary of the studies conducted on small polyps<10mm where magnification has not been used are shown in table 5. Currently only six studies have been published investigating this question, with variable results. It is therefore an area where further research is needed. A potential problem is that all of the studies have attempted to assess the polyps using Kudo pit patterns. It is important to understand that this system was designed for use with optical magnification. As described previously, this allows very subtle changes to be visualised. Whilst there certainly are elements of the Kudo patterns which can be seen without magnification, it is poorly understood what the subtle differences are. Therefore misclassification is inevitable in some cases.

If a technique is to be applied in a wider sense it is critical that the assessment tool is robust and that it can be universally applied without need for modification by the individual using it.
<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Journal</th>
<th>Year</th>
<th>Size</th>
<th>Dye</th>
<th>No polyps</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axelrad 0AM</td>
<td>USA</td>
<td>Endoscopy</td>
<td>1996</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>24</td>
<td>93%</td>
<td>95%</td>
<td>NR</td>
</tr>
<tr>
<td>Eisem</td>
<td>USA</td>
<td>Gastrointestinal Endoscopy</td>
<td>2002</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>520</td>
<td>82%</td>
<td>82%</td>
<td>82%</td>
</tr>
<tr>
<td>Fu</td>
<td>Japan</td>
<td>Endoscopy</td>
<td>2003</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>206</td>
<td>96.3%</td>
<td>93.5%</td>
<td>95.6%</td>
</tr>
<tr>
<td>Konishi</td>
<td>Japan</td>
<td>Gastrointestinal endoscopy</td>
<td>2003</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>405</td>
<td>97%</td>
<td>86%</td>
<td>100%</td>
</tr>
<tr>
<td>Apel</td>
<td>Germany</td>
<td>Gastrointestinal endoscopy</td>
<td>2006</td>
<td>&lt;5mm</td>
<td>Indigo carmine</td>
<td>273</td>
<td>94%</td>
<td>64%</td>
<td>83%</td>
</tr>
<tr>
<td>Pohl</td>
<td>Germany</td>
<td>Gut</td>
<td>2009</td>
<td>&lt;10mm</td>
<td>Indigo carmine</td>
<td>280</td>
<td>87.6%</td>
<td>62.0%</td>
<td>NR</td>
</tr>
</tbody>
</table>

Table 5: Summary of publications stating accuracy, sensitivity and specificity of chromoendoscopy with indigo carmine on polyps<10mm without optical magnification (NR=not reported)
2.4: Methylene blue

To date there has been very little published on methylene blue in the evaluation of polyps in the colon for *in-vivo* diagnosis. Unlike indigo carmine, methylene blue is a vital stain which is taken up into cells. Dysplastic epithelium takes up this dye less readily than normal gastrointestinal epithelium. There is a theoretical risk of DNA toxicity (33). As such there has only been one study published investigating its use in both lesion detection and *in-vivo* diagnosis in the last 30cm of the colon. The results were very good, claiming 100% accuracy (34). However, this study was predominately interested in evaluating i-scan, and more studies are needed to confirm whether these results can be repeated in a different setting.
2.5: Acetic acid

Acetic acid is a vital stain which leads to reversible acetylation of nuclear proteins. This leads to vascular congestion which improves the visualisation of surface patterns and the vasculature. It also acts as a very effective mucolytic. Despite this there have been very few publications on its use in the evaluation of colonic polyps. A small Australian study, in collaboration with a Japanese expert, prospectively evaluated 73 polyps <10mm with both acetic acid and acetic acid followed by indigo carmine (35). Magnifying zoom endoscopes were used. The results were encouraging, with an accuracy of 96%, sensitivity of 95% and specificity of 95% with acetic acid alone. When indigo carmine was subsequently added the accuracy rose to 98%, with a sensitivity of 100% and specificity of 97%. The authors postulated that this was because of both the mucolytic effect and the aceto-whitening reaction accentuating the pit pattern. It is important to note that the study was very small. There has been a further study conducted (36). However, it was even smaller, with just 54 polyps examined. It was based on pictures shown to 16 different assessors (6 gastroenterologists, 5 residents and 5 medical students) none of whom had prior experience in pit pattern interpretation. Perhaps, rather unsurprisingly, the results were poor, with a reported accuracy of 62.4%, sensitivity of 81.8% and specificity of 41.2%. See Table 6.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Journal</th>
<th>Date</th>
<th>Dye</th>
<th>Zoom</th>
<th>No polyps</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Togashi</td>
<td>Australia</td>
<td>Endoscopy</td>
<td>2005</td>
<td>Acetic acid</td>
<td>Zoom</td>
<td>73</td>
<td>95%</td>
<td>95%</td>
<td>96%</td>
</tr>
<tr>
<td>Hwan Kim</td>
<td>Korea</td>
<td>World Journal Gastroenterology</td>
<td>2008</td>
<td>Acetic acid</td>
<td>Zoom</td>
<td>54</td>
<td>81.8%</td>
<td>41.2%</td>
<td>62.4%</td>
</tr>
</tbody>
</table>

Table 6: Comparison of studies using acetic acid chromoendoscopy
2.6: Vascular enhancement techniques and electronic imaging

As chromoendoscopy became established as a standard of care, endoscope manufacturers started looking into ways of improving the endoscopes to simulate such techniques without the need for dyes. A criticism of chromoendoscopy by some endoscopists has been that it is messy and time consuming. Furthermore, it physically colours the mucosa, requiring extensive washing if the endoscopist decides that he or she wants an unstained view. There was therefore a desire for a push button technology to create a 'virtual chromoendoscopy'.
2.7: Narrow band imaging

The first commercially available system came from Olympus, known as narrow band imaging (NBI). The concept of NBI was designed to improve the visualisation of mucosal surface patterns. It is bound on the principle of variable penetration of light depending on its wavelength. Red light penetrates deep into the submucosa but doesn’t help with surface pattern assessment. When blue and green light is at a wavelength range of 415-540nm it does not penetrate deep but enhances mucosal surface patterns. Blue light displays superficial capillary networks whilst green light highlights subepithelial vessels. The result is a high contrast image which makes the interpretation of surface vascular patterns possible. NBI uses a physical filter to block red light and to narrow the bandwidth of the blue and green light, hence improving visualisation of surface patterns.

A lot of the early studies into narrow band imaging centred around lesion detection. Early studies suggested that there was some improvement (37) (38). This was not however repeated in later investigations (39) (40) (41) (38). The overall conclusions were that the gains seen in the preliminary studies were largely due to inexperienced endoscopists, still on a steep section of the polyp detection learning curve improving their polyp detection skills by using the technique, whereas when used by experts, who already had high adenoma detection rates, there was no gain (40).

There have been numerous publications investigating the potential for In vivo histology prediction using narrow band imaging (42) (43) (44) (45) (46) (47) (38) (48)
Similar results have been achieved to those seen with indigocarmine. There have been several publications which have concentrated on non-magnifying endoscopes, the most notable being the DISCARD study from St. Marks hospital. It should be noted however that this was predominately a study of in-vivo diagnosis, and the use of indigocarmine was allowed. Whilst the authors argued that this was only needed in a minority of cases, it is difficult from the study to ascertain the efficacy of NBI on its own. Another study compared the accuracy of NBI with and without magnification (50). This showed no statistically significant difference with or without magnification. However, the polyps were not assessed in-vivo. Pictures were taken and then reviewed after the procedure by two endoscopists. Therefore the results have to be interpreted with caution.

The surface patterns seen with NBI are similar but not identical to Kudo’s pit patterns (as previously described). NBI enhances vessels around the pits, whereas Kudo’s patterns are the actual pattern of distribution of pits on the surface of the polyp. It has been commented that in practice these are very similar to pit patterns. However, it has led to the development of new classification systems specifically for NBI, based around meshed capillary vessels (51) (52) and vascular pattern intensity, which have been validated against Kudo pit pattern analysis (53). To visualise capillary patterns it is necessary to have optical magnification and both of these classification systems were validated using HD magnifying endoscopes. In practice however, these classification systems have been applied both with and without optical magnification. See Figures 3 and 4.
Unlike the studies into indigocarmine dye spray, where Japanese research predominates, the work into NBI have come from a larger range of countries. This perhaps reflects the reluctance of western endoscopists to embrace dye spray. It should be noted however that the largest NBI study (1473 polyps) comes from Japan (48). See table 7.
Of all of the vascular enhancement techniques the biggest evidence base exists for NBI. Furthermore it is a tool where classification systems and assessment techniques have been developed specifically for use with it. Unlike all of the other vascular enhancement techniques data has been produced from more than one centre, suggesting that the techniques are reproducible in expert hands. Unfortunately all of the data has been produced using high definition equipment and it is necessary to assume, at present, that this is a prerequisite for in-vivo diagnosis. This may however not actually be the case and a study is justified to review the requirement for high definition equipment. As most studies have come from expert tertiary referral centres, it is important to explore how applicable these findings are to a wider group of endoscopists by performing multicentre studies.
Table 7: Accuracy, sensitivity and specificity for narrow band imaging (NBI), auto fluorescence imaging (AFI) and trimodal imaging. IC= indigo carmine, HD= high definition NR= not reported

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Journal</th>
<th>Year</th>
<th>Modality</th>
<th>Endoscope</th>
<th>No</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Machida</td>
<td>Japan</td>
<td>Endosc</td>
<td>2004</td>
<td>NBI</td>
<td>Zoom</td>
<td>43</td>
<td>100%</td>
<td>75%</td>
<td>NR</td>
</tr>
<tr>
<td>Chiu</td>
<td>Taiwan</td>
<td>Gut</td>
<td>2007</td>
<td>NBI (pictures)</td>
<td>Zoom non zoom</td>
<td>180</td>
<td>82.86%</td>
<td>87.95%</td>
<td>71-89%</td>
</tr>
<tr>
<td>Rastogi</td>
<td>USA</td>
<td>GIE</td>
<td>2008</td>
<td>NBI</td>
<td>HD (38)</td>
<td>123</td>
<td>86.92%</td>
<td>86.92%</td>
<td>NR</td>
</tr>
<tr>
<td>Sikka</td>
<td>USA</td>
<td>Endosc</td>
<td>2008</td>
<td>NBI</td>
<td>HD without magnification</td>
<td>80</td>
<td>95%</td>
<td>90%</td>
<td>NR</td>
</tr>
<tr>
<td>Rogart</td>
<td>USA</td>
<td>GIE</td>
<td>2008</td>
<td>NBI</td>
<td>Zoom</td>
<td>265</td>
<td>80%</td>
<td>81%</td>
<td>80%</td>
</tr>
<tr>
<td>East</td>
<td>UK</td>
<td>Endosc</td>
<td>2008</td>
<td>NBI</td>
<td>HD+zoom</td>
<td>116</td>
<td>88%</td>
<td>91%</td>
<td>89.6%</td>
</tr>
<tr>
<td>Ragstogi</td>
<td>USA</td>
<td>Am J Gastro</td>
<td>2009</td>
<td>NBI</td>
<td>HD without magnification</td>
<td>236</td>
<td>96%</td>
<td>NR</td>
<td>93%</td>
</tr>
<tr>
<td>Ignjatovic</td>
<td>UK</td>
<td>Lancet Onc</td>
<td>2009</td>
<td>NBI with IC</td>
<td>HD</td>
<td>278</td>
<td>94%</td>
<td>89%</td>
<td>93%</td>
</tr>
<tr>
<td>Sano</td>
<td>Japan</td>
<td>GIE</td>
<td>2009</td>
<td>NBI</td>
<td>HD+zoom</td>
<td>150</td>
<td>96.4%</td>
<td>92.3%</td>
<td>95.3%</td>
</tr>
<tr>
<td>Wada</td>
<td>Japan</td>
<td>GIE</td>
<td>2009</td>
<td>NBI</td>
<td>HD+zoom</td>
<td>617</td>
<td>90.9%</td>
<td>97.1%</td>
<td>NR</td>
</tr>
<tr>
<td>Henry</td>
<td>USA</td>
<td>GIE</td>
<td>2010</td>
<td>NBI</td>
<td>Uncertain</td>
<td>126</td>
<td>93%</td>
<td>88%</td>
<td>91%</td>
</tr>
<tr>
<td>Wada</td>
<td>Japan</td>
<td>Dig Endosc</td>
<td>2010</td>
<td>NBI</td>
<td>Uncertain</td>
<td>147</td>
<td>88.9%</td>
<td>98.9%</td>
<td>98.2%</td>
</tr>
<tr>
<td>Wang</td>
<td>USA</td>
<td>GIE</td>
<td>1999</td>
<td>AFI</td>
<td>Prototype system</td>
<td>18</td>
<td>83%</td>
<td>100%</td>
<td>NR</td>
</tr>
<tr>
<td>Tischenendorf</td>
<td>Germany</td>
<td>Endosc</td>
<td>2010</td>
<td>NBI (pictures)</td>
<td>HD with and without zoom</td>
<td>200</td>
<td>87.9%</td>
<td>92.1%</td>
<td>90.5%</td>
</tr>
<tr>
<td>Buchner</td>
<td>USA</td>
<td>GIE</td>
<td>2010</td>
<td>NBI</td>
<td>HD non zoom</td>
<td>41</td>
<td>84%</td>
<td>75%</td>
<td>80.5%</td>
</tr>
<tr>
<td>Eker</td>
<td>Sweden</td>
<td>Gut</td>
<td>1999</td>
<td>AFI</td>
<td>Prototype</td>
<td>46</td>
<td>100%</td>
<td>96%</td>
<td>NR</td>
</tr>
<tr>
<td>McCallum</td>
<td>UK</td>
<td>GIE</td>
<td>2008</td>
<td>AFI</td>
<td>Prototype system</td>
<td>75</td>
<td>85%</td>
<td>81%</td>
<td>NR</td>
</tr>
<tr>
<td>Van Den Broek</td>
<td>NZ</td>
<td>Clinical Gastro and Hepat</td>
<td>2009</td>
<td>Trimodal imaging</td>
<td>HD + zoom</td>
<td>208</td>
<td>99%</td>
<td>35%</td>
<td>63%</td>
</tr>
</tbody>
</table>
2.8: Flexible spectral imaging colour enhancement (FICE)

After the introduction of narrow band imaging by Olympus other endoscope manufactures followed suit with their own systems for vascular enhancement. Unlike NBI, which utilises a physical filter, FICE is a post processor technology which captures spectral reflectance by a colour CCD video endoscope. This is sent to a spectral estimation matrix processing circuit contained in the video processor. The reflectance spectra of corresponding pixels that make up the conventional image are mathematically estimated. From these spectra, it is feasible to reconstruct a virtual image of a single wavelength. Three such single-wavelength images can be selected and assigned to the red, green, and blue monitor inputs, respectively, to display a composite colour-enhanced multi band image in real time. In practice this can be used like narrow band imaging to remove data from the red part of the waveband and narrow the green and blue spectra. However, the system is flexible. It has 10 pre set digital filter settings with the ability to program more. See table 8.

FICE: pre set bandwidths (nm)

<table>
<thead>
<tr>
<th>Preset</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>500</td>
<td>500</td>
<td>550</td>
<td>540</td>
<td>520</td>
<td>500</td>
<td>580</td>
<td>520</td>
<td>540</td>
<td>550</td>
</tr>
<tr>
<td>G</td>
<td>445</td>
<td>470</td>
<td>500</td>
<td>490</td>
<td>500</td>
<td>480</td>
<td>520</td>
<td>450</td>
<td>415</td>
<td>500</td>
</tr>
<tr>
<td>B</td>
<td>415</td>
<td>420</td>
<td>470</td>
<td>420</td>
<td>405</td>
<td>420</td>
<td>460</td>
<td>400</td>
<td>415</td>
<td>400</td>
</tr>
</tbody>
</table>

Table 8: FICE pre set bandwidths (nm)

Whilst FICE is a technically more complex technology than NBI, and therefore potentially more flexible, this can prove offputting to clinicians who can find the multitude of different settings confusing. This has not been helped by the relatively small amount of research conducted into the system.
The largest study into the *in-vivo* histology prediction of colonic polyps comes from the Weisbaden Group in Germany. They conducted a prospective study of 150 polyps <2cm and compared to indigocarmine dye spray with low (50x) and high (100x) magnification using high resolution endoscopes (650,000 pixel CCD). The study was performed by taking static pictures of each polyp and reviewing them by 3 different readers after the procedure (54). They found that an accuracy of 83% and 90%, sensitivity of 89.9% and 96.6% and specificity of 73.8% and 80.3% could be achieved with low and high magnification, respectively. The results were essentially the same with Indigo carmine with no statistically significant difference observed between the two modalities. There are some important criticisms to note about this study. As it is based on static images it is unclear whether the results are directly transferrable to *in-vivo* diagnosis on a busy list. Furthermore, as lesions over 1cm were allowed, it is unclear whether these results could be achieved with smaller diminuitive polyps which are arguably harder to assess. Furthermore, no attempt was made to assess lesions without any magnification and only high definition (650,000 pixel CCD) endoscopes were used. Given that in most of the units around the world which use Fujnon equipment, 410,000 pixel standard definition scopes are the norm and this is an important consideration. Many people who have used FICE comment that the image quality is very poor and that is mostly due to the limitations of the SD endoscope and not due to FICE.

The same team went on to conduct a further prospective randomised study with the primary aim to investigate the impact of FICE on adenoma detection rates (ADR) (26). Again high definition endoscopes were used but the optical magnification function was not utilised. It showed that FICE did not improve ADR. FICE and indigo carmine were both able to differentiate adenomas from hyperplastic polyps<10mm in
size. There was a sensitivity of 93% and specificity of 61.2% in differentiating adenomas from hyperplastic polyps with FICE, comparable but not superior to that of indigocarmine (90.4%), with no statistically significant difference between the two techniques observed ($p=0.44$). Again it can be seen that specificity has been sacrificed to achieve an adequate sensitivity. Whilst safe, this approach does limit the cost benefit position of in-vivo diagnosis, with an overall accuracy of 84.7%.

There are further problems with the methodology of this study. The authors did not develop a specific tool for assessing polyps with FICE, instead relied on Kudo’s pit patterns which are not validated for FICE. It is generally accepted that pit patterns are not well visualised with any form of optical enhancement. Therefore although the results of the study are encouraging they may not represent what can truly be achieved with the technology. Furthermore, it is important to recognise that the primary end point of the study was not lesion differentiation, but lesion detection. Therefore whilst this study is a step towards understanding FICE, it should be seen more as a proof of concept than a final definitive answer.

There has been a Japanese study looking at histology prediction using FICE. This study was quite small, looking at 107 polyps <5mm in size and utilised optical magnification with high definition scopes. With high magnification (100x) a sensitivity of 93% specificity of 70% and accuracy of 87% was achieved. There was a small drop in accuracy with low (50x) magnification (87%) (23). This was an in-vivo study and is essentially supportive of the results from the Weisbaden group. Again it does not answer the question of whether similar results are achievable with standard definition or non-magnifying endoscopes. Again Kudo’s patterns were used for the assessments and the study was also quite small and performed by Japanese experts.
Not all studies support these findings. There was a recent study in which five endoscopists assessed 144 pictures of 19 polyps to establish the diagnostic accuracy of WLI, FICE and indigo carmine in making a histology prediction for diminutive polyps <10mm in size. The results were disappointing, with a mean diagnostic accuracy for WLI of 57%, FICE without zoom of 58.9% and IC without zoom of 70.5% (55). It should be noted that the methodology of this study could be criticised in many ways. The number of lesions was extremely small and it was picture based. Furthermore, it is unclear how experienced the endoscopists were in making an in-vivo diagnosis. They achieved similar (poor) results with indigocarmine which is out of keeping with previous studies. Furthermore, the Sano classification was used to assess the lesions with FICE. This is a system designed for Narrow band imaging (51). Practically, the appearances are different with FICE to NBI, and the Sano classification has never been validated for use with FICE. It is therefore hard to say that this study is a true reflection of what can actually be achieved with FICE.

Similar to narrow band imaging, FICE enhances vascular patterns. As a result it is not possible to directly describe Kudo’s pit patterns. Unfortunately there is no widely adopted classification system which can be used with FICE. A study from Brazil has described a surface pattern system which is not dissimilar to Kudo pit pattern classification but describing vascular patterns seen with FICE (56). The study enrolled 309 lesions ranging in size from 1-50mm, with 242 lesions <5mm in size. Again only high definition endoscopes were used and no attempt to examine without magnification was made. The authors commented that they felt optical magnification was essential for analysis of vascular patterns. An accuracy of 98.3% sensitivity of
99.2% and specificity of 94.9% was achieved. The advantage of including larger lesions was that 22 cases of colorectal cancer could be examined, enabling a classification system to be validated. However, it does mean that the very high sensitivity and specificity cannot be directly compared to the other studies looking at much smaller lesions. The authors did not attempt to analyse accuracy on the basis of lesion size.

There has been a study which has compared confocal endomicroscopy, narrow band imaging and FICE against each other. (57) This reported a sensitivity of 73% and specificity of 68% for FICE, sensitivity of 84% and specificity of 75% for NBI and a sensitivity of 91% and specificity of 76% for probe based confocal endomicroscopy. Optical high definition scopes were used but optical zoom was not. Because the number of polyps in either the NBI (41) or FICE (78) assessments was small the confidence intervals were very wide. The results for NBI were worse than other studies have reported. The FICE results were also much lower than expected but confocal was surprisingly much better than anticipated.

FICE has been examined for the detection of colorectal neoplasia. As with narrow band imaging, results have been disappointing, with several randomised controlled trials showing no improvement in lesion detection (58) (59) (26).

Subjectively many endoscopists are confused with FICE. Literature is sparse, with most of the studies being either small, picture based assessments, or assessing large lesions (which are arguably easy to call). Due to the complexity of the system many have been left confused regarding the application and practical usefulness of the various FICE settings.
FICE is a technology which shows promise in *in-vivo* histology prediction. Whilst early results are encouraging published literature remains sparse and has come from only three groups using high definition magnifying endoscopes, so its widespread use and application remains questionable. There is also a lack of validated tools for use with FICE and it is unclear which setting is optimal for the examination of colonic lesions. Further work is required to determine whether the experiences of the Weisbaden Group can be repeated in other centres, whether the technique is viable using standard definition scopes and whether optical magnification is required. Although the negative studies are either picture based or are a sub-group analysis of a study into another technology, they should not be dismissed. It could not at present be recommended as a replacement to histological analysis and the evidence base is not strong enough to call for a multi-centre study of reproducibility until it is established whether *in-vivo* diagnosis is possible at all using this system. See table 9 for a summary of the studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Journal</th>
<th>Year</th>
<th>Modality</th>
<th>Endoscope</th>
<th>No</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pohl</td>
<td>German</td>
<td>Am J Gastro</td>
<td>2008</td>
<td>FICE (picture)</td>
<td>HD low and high magnification n&lt;20mm</td>
<td>150</td>
<td>89.9%</td>
<td>73.8%</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96.6%</td>
<td>80.3%</td>
<td>90%</td>
</tr>
<tr>
<td>Pohl</td>
<td>German</td>
<td>Gut</td>
<td>2009</td>
<td>FICE (subgroup analysis)</td>
<td>HD non zoom &lt;10mm</td>
<td>321</td>
<td>93%</td>
<td>61.2%</td>
<td>84.7%</td>
</tr>
<tr>
<td>Togashi</td>
<td>Japan</td>
<td>GIE</td>
<td>2009</td>
<td>FICE</td>
<td>HD low (50x) and high (100x) magnification n&lt;5mm</td>
<td>107</td>
<td>93%</td>
<td>70%</td>
<td>87%</td>
</tr>
<tr>
<td>Teixiera</td>
<td>Brazil</td>
<td>GIE</td>
<td>2009</td>
<td>FICE</td>
<td>HD with zoom polyps up to 50mm</td>
<td>309</td>
<td>99.2%</td>
<td>94.9%</td>
<td>98.3%</td>
</tr>
<tr>
<td>Parra-Blanco</td>
<td>Spain</td>
<td>World Journal Gastro</td>
<td>2009</td>
<td>FICE (picture)</td>
<td>HD with and without zoom &lt;5mm (picture)</td>
<td>19</td>
<td>NR</td>
<td>NR</td>
<td>58.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70.5%</td>
</tr>
<tr>
<td>Buchner</td>
<td>USA</td>
<td>Gastro-enterology</td>
<td>2010</td>
<td>FICE (sub-group analysis)</td>
<td>HD non zoom any size</td>
<td>78</td>
<td>73%</td>
<td>68%</td>
<td>72%</td>
</tr>
</tbody>
</table>

Table 9: Summary of papers published using FICE for *in-vivo* diagnosis. NR=not reported.
2.9: i-scan

The most recent introduction to vascular enhancement has come from Pentax. In many ways i-scan is a similar technology to FICE. It is a post processor reconstruction from spectral reflectance data. At present high definition 1.3 million pixel CCD endoscopes are available which have been marketed for use with this system. However, these are not equipped with optical magnification.

Early studies using i-scan have suggested that increased lesion detection can be achieved using the surface pattern enhancement setting (60). This study is surprising given the opposite findings with NBI and FICE. These results could be explained by the endoscopist improving during the course of the study and it is unclear whether these same benefits would be seen by a larger cohort of experienced endoscopists. The study also made an in-vivo histology prediction on 145 polyps <10mm with a sensitivity of 98%, specificity of 100% and accuracy of 98.6%, using Kudo pit patterns. There has been a further study by the same group which claims that i-scan can identify more small lesions than white light. Again this study also looked at in-vivo diagnosis, claiming 100% accuracy for both chromoendoscopy and methylene blue in the examination of the last 30cm of the colon (34). However, there is a lack of research into this technique. Again, although the technique enhances vascular structures, the image is very different to that achieved with NBI or FICE. Therefore it would be incorrect to assume that the results seen with these techniques are transferrable. Further research is needed urgently with this technology.
2.10: Confocal endomicroscopy

All of the techniques so far described have revolved around enhancement of either surface patterns or surface details. Confocal microscopy attempts to go a stage further and provide the endoscopist with a dynamic microscopic image during endoscopy. A low powered laser is focused onto the tissues and the lens used to focus the beam is both the condenser and objective folding optical path. As a result, the illuminated point coincides with the point of deflection within the specimen. Light emanating from this point is focused onto a detector, with light from outside of the illuminated point rejected. The term ‘confocal’ is derived from the illumination and detection systems existing in the same focal plane. The image is created by measuring light from successive points by scanning in a raster pattern. The result is a greyscale cross section of the specimen. Planes at multiple depths can be obtained, up to a maximum depth of 250 µm (61).

There are currently two manufacturers of CE marked confocal microscopes for use in the gastrointestinal tract. Pentax have a dedicated endoscope with a confocal microscope built into the tip (EC3870CIKF). This utilises an argon laser delivering an excitation wavelength of 488nm at a power output of <1 mW. It can capture at up to 1.6 frames/second at 1024x1024 pixels. Slice thickness is 0.7µm with a 475x475µm field of view. The endoscope also provides a conventional view, enabling standard white light endoscopy to be performed until a lesion requiring examination is found. Focal distance can be adjusted in real time from the controls of the endoscope. Cellvizio have created a probe based system. This system consists of a small 2.5mm probe which is passed down the biopsy channel in a standard endoscope. This has a field of view of 600x500 µm at 12 frames per second. Both of the systems require
intravenous administration of an enhancement marker, fluorescein dextran (10% w/v) to enhance the image.

A study of 115 polyps using the Pentax endomicroscope reported a sensitivity 90.3%, specificity 95.7% and accuracy 93.9% for polyps <10mm in size. For polyps >10mm a sensitivity of 97.3%, specificity of 100% and accuracy 97.3% was reported (62). A study performed in the United Kingdom on 162 lesions showed similar results, with a sensitivity of 97.4%, specificity of 99.3% and overall accuracy of 99.1% (63). A very small study using the Cellvizio miniprobe looked at 36 colorectal lesions and reported an accuracy of 91.7%, sensitivity of 92.3% and specificity of 91.3% (64). A further small study of 32 lesions using the miniprobe reported a sensitivity of 100%, specificity of 84.6% and accuracy of 92.3%. A much larger study compared miniprobe confocal endomicroscopy against NBI and FICE, reporting a sensitivity of 91% (57) and specificity of 76%. It claimed that the results demonstrated a statistically significant benefit from the probe based confocal endomicroscope over NBI or FICE. However, the results seen with vascular enhancement were unimpressive and out of keeping with previous NBI studies. Furthermore, although the results with vascular enhancement were analysed separately for NBI and FICE, it is quite difficult to comment whether the differences between these two different vascular techniques are significant, as the numbers in each group were very small and the confidence intervals wide. If the results with the confocal probe are compared to the other studies conducted with NBI they are not particularly impressive. It should be noted that probe based endomicroscopy is marketed on a per-use per patient basis. Each probe can only be activated a set number of times, so whilst it can be used for any number of polyps in a single patient it has a cost for every patient it is used in. This is around £250 per use.
difficult to justify when such good results have been reported with magnifying endoscopes and chromoendoscopy with indigocarmine. This is not an issue for the Pentax endomicroscope. However, the scopes and equipment are expensive. To run an average list requires at least 3 colonoscopes. Therefore there would be a significant initial financial outlay with this setup. Another problem with confocal endomicroscopy is that it requires the endoscopist to learn how to interpret what are essentially histopathological sections. It is currently unclear what the learning curve is for this. Given the reluctance amongst Western endoscopists to adopt optically magnifying endoscopes into routine practice, it is reasonable to question whether there would be issues regarding uptake of confocal endomicroscopy into daily use. It is likely that if such techniques become established, it is more likely to be in the assessment of challenging high risk lesions where the questions are is a lesion cancer or an adenoma with atypical pit pattern / appearance, and whether it is suitable for endoscopic resection or not. Unfortunately the technical limitations of confocal endoscopy limit the depth of assessment to 250µm. This means that it cannot be used to establish whether a lesion penetrates beyond SM-1 invasion, which is of prognostic significance. See table 10.

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Journal</th>
<th>Year</th>
<th>Modality</th>
<th>Endoscope</th>
<th>No</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hurlstone</td>
<td>UK</td>
<td>Br J Surg</td>
<td>2008</td>
<td>Confocal</td>
<td>Pentax</td>
<td>162</td>
<td>97.4%</td>
<td>99.3%</td>
<td>99.1%</td>
</tr>
<tr>
<td>Meining</td>
<td>Germany</td>
<td>Clinical gastro and hepatol</td>
<td>2007</td>
<td>Confocal</td>
<td>Cellvizio</td>
<td>36</td>
<td>92.3%</td>
<td>91.3</td>
<td>91.7%</td>
</tr>
<tr>
<td>Xie</td>
<td>China</td>
<td>Endoscopy</td>
<td>2010</td>
<td>Confocal</td>
<td>Pentax</td>
<td>115</td>
<td>90.3%</td>
<td>95.7%</td>
<td>93.9%</td>
</tr>
<tr>
<td>Depalma</td>
<td>Italy</td>
<td>Dig Liver Dis</td>
<td>2010</td>
<td>Confocal</td>
<td>Cellvizio</td>
<td>32</td>
<td>100%</td>
<td>84.6%</td>
<td>92.3%</td>
</tr>
<tr>
<td>Buchner</td>
<td>USA</td>
<td>Gastro-enterology</td>
<td>2010</td>
<td>Confocal</td>
<td>Cellvizio</td>
<td>119</td>
<td>91%</td>
<td>76%</td>
<td>87%</td>
</tr>
</tbody>
</table>

Table 10: Summary of papers published using confocal endomicroscopy for in-vivo diagnosis
2.12 High definition endoscopy

Recent years have seen a dramatic change in the resolution of endoscopy equipment. In a similar manner to the introduction of high definition television and computer equipment into the home, all of the main endoscopy equipment being actively marketed is making claims to be ‘high definition’. It is important to appreciate that when describing the resolution of a setup, there are three independent aspects of importance; the capture resolution of the charge coupled device (CCD) in the endoscope itself, the output resolution of the endoscopy stack and the display resolution of the visual display unit. In addition to absolute resolution of the screen, the refresh rate and temporal resolution (progressive scan or interlaced picture) can also affect image quality, particularly if the picture is rapidly changing. It is important how these are connected together, as a significant degradation in picture quality can occur if a digital signal has to be converted into an analogue one for transfer from stack to screen.

Studies into high definition endoscopy have proved problematic for a number of reasons. Endoscope manufacturers are defensive regarding the resolution of components of their equipment, and often use confusing terminology for marketing. Olympus have declined to reveal what the resolution of their ‘high definition’ and ‘high resolution’ endoscopes actually is. Therefore it is very difficult to define what these terms, other than a marketing phrase, mean for their equipment. Fujinon have officially provided this study with the absolute resolution of their standard definition and high definition (Super) CCD chips as 410,000 and 650,000 pixels respectively, although this information has not been available in the public literature until now. Pentax on the other hand have publically declared the resolution of their CCD as 1.3 million pixels. All of the stacks output 1.3 million pixels via digital outputs to the
visual display unit. Fujinon HD endoscopes therefore utilize a 2:1 interpolation factor. In contrast no interpolation occurs for Pentax endoscopes. It is unknown what this factor is for Olympus systems.

It should be noted that in all of the studies performed examining the role of electronic imaging in making an in-vivo diagnosis high definition endoscopes have been used. A similar picture is seen for conventional chromoendoscopy. It is therefore difficult to ascertain whether this is a pre-requisite for the use of these techniques. However, at present most of the endoscopes in use across the Western world are standard definition. Therefore if in-vivo diagnosis is to be widely adopted, endoscopists need to feel confident that the published evidence is applicable to their equipment. Furthermore, if endoscopy units are to make effective business cases for upgrading their equipment to perform in-vivo diagnosis, robust evidence needs to be produced to support that it is actually superior to existing equipment.

There has been very little published directly comparing standard definition to high definition endoscopy. A retrospective study performed by the Mayo Clinic in the United States of America compared adenoma detection rates between standard and high definition Olympus endoscopes (65). It found that HD endoscopy resulted in a significantly higher detection rate (28.8% vs 24.3%). A study looking at the Pentax system compared HD endoscopy with i-scan to standard definition endoscopy without i-scan (60). It looked at 220 patients and found a significant benefit in adenoma detection (38% vs 13%) using high definition endoscopes. It should be noted that this is not a pure study of high definition endoscopy but also utilized the
surface enhancement capabilities of i-scan, and it did not attempt to define what contribution i-scan made to the improved detection rate.

Not all of the published literature supports a benefit from high definition endoscopes in lesion detection. A study of 130 patients randomized to standard or high definition endoscopy reported that there was no significant gain from HD colonoscopies (66). A meta-analysis of the published literature concluded that there were only marginal differences between standard and high definition colonoscopy in lesion detection and that there was no benefit in the identification of high risk adenomas (67).

To date there has been no published literature comparing high definition to standard definition endoscopy in lesion characterization.

In summary the role of high definition endoscopy in clinical practice has not yet been adequately defined. It could be argued that eventually all endoscopes will be high definition as departments slowly replace older equipment. However, this is likely to take time and there is currently a lack of clarity as to whether the results published using high definition equipment can be applied using older standard definition endoscopes. In the current era of austerity, it is difficult to justify the economic outlay for replacing functional older equipment without clear evidence of benefit.

It is again important to be cautious before extrapolating the findings from one manufacturer to the others. To define benefit, studies will need to be conducted separately for each technology as the potential gains may be greater for some systems than others. It is important not to confuse screen resolution with the resolution of the CCD within the endoscope itself.
2.12: Summary

There have been some studies which have shown that *in-vivo* diagnosis is possible. The best evidence is for indigocarmine using high definition endoscopes with optical magnification. Of the vascular enhancement techniques narrow band imaging is the best described and it is probably possible to achieve similar results to those seen with indigocarmine, again using high definition magnifying endoscopes. Other novel technologies such as confocal endomicroscopy have shown promise but are hampered by an unknown learning curve and significant capital outlay. It is unclear how all of these technologies fit together in routine clinical practice.

There are key deficiencies in the current knowledge base. Most of the large studies have been conducted in Japan, with a significantly different patient population and disease incidence. The training and job pattern of Japanese endoscopists is different to that practiced in the West (dedicated colonoscopists in Japan versus general gastroenterologists in Northern America and Europe), which makes it easier for Japanese doctors to attain and maintain skills in magnifying endoscopy and *in-vivo* diagnosis. Furthermore, the research has not been uniform across the different techniques available. Much of the available literature has established what can be achieved in a tertiary referral centre environment with magnifying endoscopes and dyes, which do not reflect standard practice in a Western setting. Even the studies into vascular enhancement have all been largely conducted on Narrow Band Imaging, with limited applicability to the other electronic imaging systems.

As a result, there is still significant scepticism in the West as to whether *in-vivo* diagnosis of colonic polyps is possible in Western hands on routine lists. As a result,
standard practice is still to remove all polyps. If a clear evidence base is not established in tightly defined clinically relevant patient groups with appropriately validated tools for assessment, this position will not change.

The biggest potential area for in-vivo diagnosis in a Western setting is on Bowel Cancer Screening lists. It has been recognised that polyp detection in this group is very high, so the cost savings of in-vivo diagnosis will be equally high (68). However, very few studies have been conducted on this cohort of patients.

In order to achieve these goals it will be necessary for robust tools to be developed for in-vivo diagnosis. Whilst the Pit Pattern Assessments pioneered by Professor Kudo have formed a solid base for further work, they ideally require magnifying endoscopes to work at their best. They were essentially developed for differentiating adenoma from cancer for endoscopic mucosal resection work and are impractical to apply on a high volume list. Furthermore, it is generally accepted that they are sub-optimal for the assessment of the vascular patterns seen with the commonly available electronic imaging techniques (NBI, FICE and i-scan). Whilst criteria have been developed for Narrow Band Imaging, there are very few tools available for FICE or i-scan. Of note, whilst NBI is a single setting technology, FICE and i-scan can be used with a wide range of different settings and there has been very little work conducted with these technologies to demonstrate which setting should be used for any given task.
It is important that in the development of these tools, attention is paid to Western endoscopic practice. Assessments have to be both accurate and straight forward to perform. Any assessment criteria which relies on subtle differences in complex patterns using optical magnification, are likely to be of use in a research setting and in highly specialised, low volume centres only. There needs to be different tools for a high volume screening population looking at low risk lesions, to those used for tertiary centre assessment of high risk large lesions. This is a point which has been consistently overlooked.

It is critical that the tool being used to assess the polyps is carefully defined. In many ways the growth of new technologies has outstripped the rate of research backing up or disproving their usefulness. It is often tempting to try and extrapolate findings from one, conceptually similar device, to another. If misdiagnosis is to be prevented, then this approach must be avoided. In particular, terminology must be defined so that endoscopists understand the strengths and limitations of a particular setup. It is also important to understand the implications of standard definition and high definition endoscopes in in-vivo diagnosis, especially in conjunction with electronic enhancement techniques like FICE. It is necessary to be precise regarding whether results are being achieved using standard definition or high definition setups, and what is actually meant by high definition such as the CCD resolution, screen resolution and connections between stack and screen.

New technologies will not be adopted if practising clinicians cannot find a robust evidence base for their use. In particular in-vivo diagnosis will never be considered a safe alternative to conventional histological analysis (the current standard of care) if the case for it is hazy, imprecise or based on slim poorly researched evidence.
In summary, if in-vivo diagnosis is to become a reality, there are a number of deficiencies in current knowledge which need to be addressed:

1) Validated lesion assessment tools need to be developed to facilitate in-vivo diagnosis using the various different imaging modalities

2) Whether in-vivo diagnosis of colonic polyps can be performed by Western endoscopists on Western patients

3) Whether the experiences of a few, well trained endoscopists, can be expanded to a larger population model

4) What populations are most suitable for in-vivo diagnosis?

5) Whether acceptable results can be achieved with indigocarmine without magnifying endoscopes

6) Whether FICE and i-scan can achieve similar results to those seen with Narrow Band Imaging and what settings should be used to achieve this

7) Whether acceptable results can be achieved using Narrow Band Imaging, FICE or i-scan without magnifying endoscopes

8) What effect high definition endoscopes have on the above imaging modalities (needs repeating on all systems with and without magnification)

9) The cost impact of in-vivo diagnosis

10) The effect of a missed diagnosis

11) Potential roles for technology such as confocal endomicroscopy
Chapter 3

Barrett’s Oesophagus
Barrett’s oesophagus

Oesophageal cancer is the 9th most common cancer in the UK, accounting for 5% of all cancer deaths in the UK. Unfortunately, rates have continued to rise over the last 30 years. For reasons which are unclear, it is more common in men than women, with an incidence of 8.8-14.1 per 100,000 in men, compared to 4.8-5.7 per 100,000 in women. (3). Patients typically present with vague early symptoms. This makes early diagnosis very difficult, with most cancers identified at a late stage where the only treatment options are palliative. This is reflected with a 5 year survival of 9% (4).

There are known risk factors for oesophageal cancer. Sadly these include many factors which cannot be changed, such as male gender, age >45, early age of onset of GORD, duodeno-gastrooesophageal reflux, mucosal damage and family history (6).

It is known that one of the most significant risk factors for adenocarcinoma of the oesophagus is Barrett’s epithelium. This is an acquired pre-malignant condition which effects up to 1.6% of the general population (7). The incidence is increasing in the West, mirroring that of oesophageal cancer (2) (8). It is caused by the reflux of gastric contents into the oesophagus, and is found in 15-20% of gastrointestinal endoscopies performed for symptoms of reflux. The gastric acid damages the normal squamous epithelium, which becomes replaced by a columnar epithelium as a protective measure. The newly updated definition by the British Society of Gastroenterology defines Barrett’s as ‘an endoscopically apparent area above the
oesophagogastric junction that is suggestive of Barrett's which is supported by the finding of columnar lined oesophagus on histology' (5) (6).

There is not a national screening policy for the detection of upper gastrointestinal malignancy or for Barrett's. However, once Barrett's is detected patients are entered into a surveillance programme. The benefits of this are controversial, as the absolute risk of malignant transformation is low, 0.8-1.5% per annum. Some studies have concluded that because of this there is no benefit to surveillance (6). Computer modelling has been used to predict an effective balance point for surveillance intervals, and a widely used interval for surveillance endoscopy is 2 years (69). The two yearly gastroscopy involves quadrantic random biopsies every 2cm as a part of the standard protocol. The cost per life year saved is around £19,000.
3.1: Chromoendoscopy in Barrett’s oesophagus

Dysplasia in Barrett's is very subtle and difficult to see. As a result of this, conventional protocols are based on multiple biopsies in an attempt to capture this dysplasia, based on the conventional wisdom that suggests that the more biopsies we take, the higher the chance of diagnosing neoplasia. However, random biopsies are not ideal for identifying neoplasia within Barrett's. There are a range of techniques available for examining the oesophagus in detail, and can help improve the neoplasia pick up rate. Chromoendoscopy can be used to identify areas of Barrett’s metaplasia and dysplasia. Several dyes have been used, with most of the research examining methylene blue (MB), indigo carmine (IC) and acetic acid (AA).
3.2: Methylene blue

Methylene blue 0.5% (MB) is an absorptive stain which highlights areas of specialised columnar epithelium. It stains both the nucleus and cytoplasm of cells, with the nucleus typically staining a deeper colour. It has predominately been used for identifying specialized columnar epithelium (70) (71) (72) (73). In some small studies it has been suggested that dysplasia and cancer are detected more frequently than with random 4 quadrant biopsies (74). Unfortunately MB is inconvenient to use. It must be left in contact with the mucosa for 3 minutes followed by vigorous washing to clear away excess dye. As a result the endoscopic appearances are unpredictable, subjective and not reproducible (75). There have also been concerns raised about DNA toxicity with MB so it is falling out of favour. A meta-analysis of studies found no benefit in either the detection of intestinal metaplasia or neoplasia (76) and therefore its use cannot be recommended at present.
3.3: Indigo carmine

Whilst Indigo carmine has been used in many studies looking at colonic neoplasia, it has not been studied to such a degree in the oesophagus. As in the colon, it is not absorbed by the oesophageal and Barrett’s mucosa, but accumulates in the pits and valleys between cells, highlighting the architecture (77). It is a contrast agent which can highlight mucosal irregularities and has been very helpful in the colon. However, results have been less encouraging in the oesophagus. There have been two studies which have suggested that it may help in the detection of Barrett’s dysplasia (78) (79). However, these trials have been hampered by small numbers of patients with dysplasia or cancer. Therefore results should be interpreted with caution. The mechanism of action and poor evidence base makes this an unlikely dye to be of use in the diagnosis of dysplasia.
3.4: Acetic acid

Acetic acid 2.5% (AA) when sprayed onto Barrett’s mucosa causes a reversible acetylation of nuclear proteins to occur. This leads to an acetowhitening reaction, with increased opacity of the mucosal surface (80). It also causes vascular congestion and improves surface pattern evaluation (80). This potentially enables the early recognition of neoplasia. See figure 5. It has been used successfully in the oesophagus for the identification of Barrett’s metaplasia.

Figure 5: Oesophagus: A: routine endoscopic appearances. B: post acetic acid chromoendoscopy (Obtained using an Olympus standard definition gastroscope)

One of the earliest studies used acetic acid to identify small islands of residual Barrett’s metaplasia after endoscopic treatment of Barrett’s (81). It examined a small
population of 21 patients, reporting positive results. The same team went on to conduct a study of 49 patients with Barrett’s metaplasia and defined the mucosal surface patterns associated with specialised intestinal metaplasia (82). Using magnifying endoscopes, 129 areas were examined and four different mucosal patterns defined; round pits (I), reticular (II), villous (III) and ridged (IV). It concluded that the yield for intestinal metaplasia was 0% for pattern I, 11% for pattern II, 87% for pattern III and 100% for pattern IV. Overall, a sensitivity for intestinal metaplasia of 96.5% a specificity of 88.2% and accuracy of 92.2% was achieved. This study did not have any dysplasia cases in it. A further small study examined 28 patients where 72 biopsies of Barrett’s metaplasia were taken. A sensitivity for metaplasia of 95.5% and specificity of 24.9% was achieved (83). Whilst this was a very small study six biopsies did show high grade dysplasia. Three of these had been suspected at the time of the endoscopy due to two particular mucosal patterns, a local loss of the normal ridged cribriform pattern and hypervascularisation of the mucosa.

A further randomised crossover study was undertaken using acetic acid for the detection of Barrett’s metaplasia (84). This was again very small, consisting of just 32 patients. Patients were randomized to either standard video endoscopy with quadrantic biopsies or to magnifying endoscopy with acetic acid. All patients were re-examined after 14 days post initial endoscopy with the corresponding procedure. It found that magnifying endoscopy enabled the prediction of Barrett’s epithelium with a sensitivity of 100% and specificity of 66% and accuracy of 83.8%. The biopsies obtained following exposure to acetic acid yielded a significantly higher percentage of tissues containing Barrett’s metaplasia (78%) compared to random biopsies (57%). Again this study had no dysplasia cases and the authors recognised this as a limitation of the study. A further small study of 20 patients again demonstrated the
effectiveness of acetic acid in the identification of Barrett’s metaplasia, with a sensitivity of 100%, specificity of 82% and accuracy of 90% (85).

Not all of the studies published using acetic acid for the identification of intestinal metaplasia gave positive results. A prospective randomized study of 137 patients concluded that random biopsies of endoscopically apparent Barrett’s oesophagus yielded specialized intestinal metaplasia at the same rate with random biopsies as with acetic acid targeted biopsies (86).

An important question is whether acetic acid could be used to detect and localise neoplasia. It has been successfully used in the detection of squamous neoplasia of the cervix during colposcopy (87). Whilst no randomised controlled trials for its use for this purpose in the oesophagus exist, early cohort studies have demonstrated effectiveness in the identification of dysplasia. The sensitivity for the identification of neoplasia has been suggested to be 71-100%, with a specificity between 80-99%.

An early cohort study of 100 patients attempted to investigate whether neoplasia could be localised using acetic acid (88). It classified the mucosal patterns as either normal (uniform reticulum along the entire columnar lined oesophagus) or abnormal (reticulum presenting areas of rough or irregular appearance). Dysplasia was found in 86.7% of rough or irregular areas compared to 0% in areas with a normal pattern. This corresponded to a sensitivity for neoplasia of 100% and a specificity of 97.7%. The study was limited by the low number of dysplasia / cancer cases in the series of just 15 patients. A further study examined 394 pictures taken from 96 patients with Barrett’s epithelium after dye spray with 1.5% acetic acid. It characterized the mucosa into three pit patterns, I-dotted round pits, II-villous and ridged, III-irregular and distorted, with the latter representing neoplasia. The images were shown to 6 blinded assessors. High grade intraepithelial neoplasia or cancer was correctly
identified in 89% cases, with intestinal metaplasia correctly called in 92% cases (89). The study showed very little intraobserver variability, with a kappa score of 0.959. It was however only a study of static images and therefore how applicable it is to live endoscopy is unclear. A further prospective cohort study supported these findings, again using mucosal pit patterns to predict histology (90). The primary end point was detection of intestinal metaplasia, dysplasia and cancer. All of the procedures were recorded and viewed by three endoscopists. Still images were taken from the videos and shown to six different endoscopists. The authors found that there was a high yield of metaplasia and dysplasia in the biopsies taken, with columnar lined epithelium in 6 patients, specialized intestinal metaplasia in 49 patients, low grade dysplasia in 5 patients, high grade dysplasia in one patient and adenocarcinoma in three patients. 24% of patients had a histological upgrade when compared to their previous surveillance endoscopy. There was good inter and intra-observer agreement in assessing pit-patterns with Kappa values of 0.571 and 0.709 respectively. The study was limited by the very small volume of dysplasia in the study. Furthermore, it was not an in-vivo localization study and as such the results have to be interpreted with caution. A study of 57 patients looked at a high risk population with both acetic acid and FICE. There were 30 patients with high grade dysplasia or early cancer in the cohort. On a per lesion analysis it found a sensitivity of 87% for dysplasia. This did not change significantly when an analysis was performed on a per patient basis, with a sensitivity of 83%. Similar results were obtained using FICE (91).

Review of the literature suggests that to date there has been no single, well powered study to conclusively support the role of acetic acid in the diagnosis of neoplasia in Barrett’s. Most of the studies have been trying to identify Barrett’s epithelium or
intestinal metaplasia with the help of acetic acid. However, some of the small series have shown a potential for acetic acid to be used as a tool for the diagnosis of neoplasia in Barrett’s. There are still no objective criteria related to acetic acid assisted diagnosis of neoplasia in Barrett’s.

This thesis will aim to evaluate the role of acetic acid in the diagnosis of Barrett’s neoplasia and will also aim to establish some objective criterion for acetic acid assisted diagnosis of Barrett’s neoplasia.
3.5: Electronic imaging in Barrett’s oesophagus

Electronic imaging techniques are widely available and have been used in the evaluation of Barrett’s oesophagus. Narrow band imaging has been used more commonly than the other two techniques, FICE and i-scan.
3.6: Narrow Band imaging

Narrow band imaging (NBI) has been used in the examination of Barrett's oesophagus, utilising the principles described in the last chapter. It carries the potential advantage over chromoendoscopy techniques that it can be activated at the push of a button on the endoscope. It highlights mucosal vascular patterns to enhance abnormal neoplastic areas. The earliest study was conducted in Japan and was published in 2004 (92). It looked at images from 11 patients viewed with both conventional white light and NBI and compared the quality of the images for visualization of the oesophagogastric junction, capillary vessels and columnar lined oesophagus, on a four point scale. The authors found that in all cases, visualization was superior with NBI and that endoscopic and histological diagnoses correlated more closely with NBI than with white light. This led to teams across the world conducting studies aimed at defining the endoscopic features of Barrett’s metaplasia and neoplasia when viewed with the system.

An open label study from North America used images of Barrett’s metaplasia viewed with Narrow Band Imaging to define the patterns in Barrett’s mucosa (93). Of the 51 patients examined, 28 had intestinal metaplasia, 8 had low grade dysplasia, 7 had high grade dysplasia and 8 had cardia-type mucosa. A ridge or villous pattern was predictive of intestinal metaplasia with a sensitivity of 93.5% and specificity of 86.7%. High grade dysplasia was associated with an irregular distorted pattern with a sensitivity of 100% and specificity of 98.7%. The authors commented that NBI was unable to distinguish intestinal metaplasia from low grade dysplasia. It should be recognised that with just 7 high grade dysplasia cases seen in this series, the predictive value for neoplasia should be interpreted with caution. A similar picture study from the Netherlands (94) reported similar results in 63 patients with 198
areas. Intestinal metaplasia was characterized by villous/gyrus-forming vessels (80%) which had regular vascular patterns, or a flat mucosa with regular normal appearing long branching vessels (20%). In contrast high grade dysplasia was characterized by three abnormalities; an irregular or disrupted mucosal pattern, irregular vascular pattern or abnormal blood vessels. They found that all areas of neoplasia had at least one of these findings, with 85% having 2 or more. The study reported a sensitivity of 94% and specificity of 76% for neoplasia. However, the small size of the study (48 areas of high risk neoplasia) makes interpretation of these results difficult. A Japanese group also published a study in 2007 looking at 217 sites from 58 patients with Barrett’s oesophagus (95). Of these cases, 6 had superficial adenocarcinoma. This study concentrated on examining the role of fine mucosal patterns and the additional gain from the examination of capillary patterns. In this study the results were not quite so impressive. Intestinal metaplasia could be identified by the presence of a cerebriform mucosal pattern with a sensitivity of 56%, specificity of 79%, or by a deoxyribonucleic acid (DNA) pattern (sensitivity 77%, specificity 94%). All 6 cancers were correctly identified. The additional examination of capillary patterns improved this further. The authors concluded that this was important for detecting metaplasia and neoplasia in Barrett’s.

A study from Nottingham in the United Kingdom attempted to define Barrett’s oesophagus in terms of microstructural and microvascular patterns (96). A total of 344 areas from 50 patients with Barrett’s oesophagus were examined. It was found that a regular microstructural pattern with tubular/linear or villous patterns was associated with intestinal metaplasia in 90.6% of lesions. An absent microvascular pattern was associated with metaplasia in 98.5% of cases. The combination of a
regular microstructural pattern (tubular / villous or linear) with an absent microvascular pattern could detect intestinal metaplasia with 100% sensitivity and 78.8% specificity. It was also noted that the presence of an irregular microvascular / microstructural pattern could help predict the presence of high grade dysplasia with a sensitivity of 90% and specificity of 100%. Unfortunately there were only 10 cases of dysplasia in the study, four of which were low grade, making it difficult to draw any firm conclusions. Furthermore, the system was complex. In a larger prospective cohort study from the same group, 109 patients were examined in an attempt to develop and validate a simple classification system based on the same concepts (97). In total 1021 distinct areas were examined. Four patterns were identified: Round pits with regular microvasculature (A), villous/ridge pits with regular microvasculature (B), absent pits with regular microvasculature (C) and distorted pits with irregular microvasculature (D). Columnar mucosa without intestinal metaplasia was associated with the type A pattern. The positive predictive value (PPV) and negative predictive value (NPV) for this was 100% and 97%. Types B and C were associated with intestinal metaplasia, with a PPV of 88% and NPV of 91%. Type D was associated with high grade dysplasia with a PPV of 81% and NPV of 99%. On the basis of the positive results obtained in this study, the same authors attempted to use this classification system to predict histology from pictures taken from 75 areas in 21 patients using both NBI and white light (98). Five expert endoscopists reviewed the images. A sensitivity of 88.9% was achieved with NBI compared to 71.9% with white light. NBI was significantly superior to white light for the prediction of dysplasia.

Based on the positive findings from these validation exercises, work began on evaluating whether NBI could be used prospectively to detect advanced dysplasia.
more efficiently than with mapping biopsies. A tandem endoscopy study involving 65 patients compared standard resolution endoscopy to NBI. It found that NBI directed biopsies detected dysplasia in more patients (57%) compared to biopsies taken using standard resolution endoscopy (43%) (99).

A potential drawback with narrow band imaging has been the lack of intraobserver agreement between endoscopists when using the classification systems described above. A recent study compared the three most commonly used classification systems by showing 84 high quality videos from 32 patients to nine independent endoscopists, who had not been involved in the development of the classification systems (100). The global accuracy was 46% and 47% using the systems described by the British and American studies, and 51% with the classification system described by the Amsterdam group. Accuracy for dysplasia was 75% using all three systems, with an accuracy for intestinal metaplasia ranging from 57%-63%. The intraobserver agreement was fair to moderate, with a kappa of 0.34 for the Nottingham system, 0.47 for the Amsterdam system and 0.44 for the North American classification system. The authors concluded that all three systems revealed substantial limitations when assessed externally and that as a result, NBI could not replace random biopsies for histopathological analysis.

A systemic review of the evidence for Narrow Band Imaging has concluded that whilst the body of literature for this technique is growing it is still unclear whether it is adequate for the detection of Barrett’s (101).
3.7: Auto-fluorescence

At around the same time as the introduction of Narrow Band Imaging another technology was under development known as Auto-fluorescence imaging (AFI). This is a novel technique which is based on the principle of variable fluorescence between tissues. Normal mucosa, when exposed to light, emits a green fluorescence as compared to magneta / pink fluorescence in neoplastic areas within the mucosa. This principle is exploited by the technique of AFI to detect early neoplasia. Initial studies used this technique on its own as a single imaging modality. They suggested that whilst neoplasia detection rates were very good (91%), with a 2.8 fold increase in dysplasia detection (102), a 51% false positive rate limited its use. (103).

3.8: Tri-modal imaging

It was becoming clear that there were inherent problems associated with the subjective classification systems developed for use with NBI. The poor intraobserver agreement seen when these tools were used by clinicians independent from the research teams who had developed them, suggested that on its own, NBI had an inadequate and variable sensitivity for the identification of neoplasia within Barrett’s that was very operator dependent. This led to the concept of tri-modal imaging being developed. Whilst the false negative rate of autoflourescence would prevent it from being used as a solo imaging modality, it could be used to identify abnormal areas. These could then be examined in detail with high resolution white light (HRE) and narrow band imaging. The concept is that HRE and AFI act as a red flag, highlighting potential abnormal areas which may have been overlooked during NBI assessment and improving the sensitivity over that of NBI alone. The focused assessment of
these higher risk areas flagged up by AFI using Narrow Band Imaging could then identify those which are actually normal, improving the specificity of the test above that normally achieved with AFI. Therefore the best features of both AFI and NBI would be exploited in the form of an integrated test. The initial work into this concept was led from the Academic Medical Centre in Amsterdam, where a multi-centre prospective cohort study of high risk patients was conducted (104). Five centres were involved and a total of 84 patients were examined. The initial results were excellent. AFI identified all 16 patients with neoplasia previously seen with high resolution white light but also identified an additional 11 patients with early neoplasia that had not been previously seen. Whilst the false positive rate for AFI was high at 81%, NBI reduced this to 26%. This led the same team to investigate tri-modal imaging further with a tandem endoscopy study. This produced interesting results. It examined 87 high risk patients with suspected neoplasia who underwent tri-modal imaging guided assessment (with targeted and random biopsies), followed by white light assessment (targeted and random biopsies) 8-12 weeks later (105). It found that whilst tri-modal imaging improved the targeted detection of neoplasia, there was no significant difference in the overall yield of neoplasia for tri-modal imaging, compared to standard video endoscopy and mapping biopsies. It was essentially a negative study with the authors concluding that at present, this imaging modality cannot be seen as a replacement for mapping biopsies, even in a high risk population. See table 12.
3.9: FICE

Fujinon has developed a technology for vascular enhancement, known as FICE. As described in the previous chapter rather than using filters it utilises a post processor technology to digitally reconstruct spectral data to enhance particular wavelengths. A prospective cohort study of 72 patients demonstrated that the identification of Pallisade vessels using FICE, provided clear demarcation between Barrett’s mucosa and the gastric mucosa which was superior to standard white light endoscopy (106). This study did not attempt to diagnose dysplasia and used transnasal Fujinon endoscopes. These are very small with a more limited field of view and no optical magnification. As such they may not be the optimum way of using FICE. A further prospective cohort study of 57 patients compared FICE with random biopsy in patients with suspected high grade intraepithelial neoplasia or early cancer. A sensitivity of 92% and specificity of 97% for FICE was achieved (91). There was high grade dysplasia or early cancer in 24/57 patients. Therefore, whilst small, this study is encouraging. Due to its nature as a post processor technology, FICE can utilise a wide range of different frequencies for lesion enhancement.

All of the ‘virtual chromoendoscopy’ techniques (NBI, FICE and i-scan) produce different appearances. Although some of the skills are transferrable, additional training is normally required to transfer between systems.
3.10: i-scan

i-scan is the most recent form of electronic imaging introduced on the Pentax endoscopes. From a technical standpoint it is similar to FICE in that it is a post-processor technology that takes spectral reflectance data to reconstruct an image of specific frequencies. Unfortunately, there have been no papers published at all using i-scan for the detection of neoplasia within Barrett's. As such, one can only speculate at present as to whether it will prove useful for this application.
3.11: Confocal endomicroscopy

Confocal endomicroscopy is a new technology for obtaining true *in vivo* histology. Studies in Barrett’s surveillance have suggested that associated neoplasia could be predicted with a sensitivity of 96.4% (107). It has been shown that confocal endomicroscopy improves the yield of neoplasia in apparent Barrett’s oesophagus, compared to a 4 quadrant random biopsy protocol (108). There is limited evidence that it is also effective in the identification of gastric cancers (109). This technique only works once neoplasia has been identified by the endoscopist using other endoscopic techniques, so it does not help improve detection but can improve the confidence for diagnosis. This is an excellent experimental technique which has yet to find a major clinical role. See figure 6.

![Figure 6: Barrett's high grade dysplasia visualized with the Cellvizio miniprobe](image-url)
<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Year</th>
<th>Design</th>
<th>No patients</th>
<th>Modality</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharma</td>
<td>Gut</td>
<td>2003</td>
<td>Prospective cohort</td>
<td>80 patients</td>
<td>Indigo carmine</td>
<td>Surface patterns in Barrett’s 97% IM ridged / villous 17% circular LGD all ridged / villous HGD all irregular / distorted</td>
</tr>
<tr>
<td>Canto</td>
<td>GIE</td>
<td>2000</td>
<td>Cohort study</td>
<td>43 patients</td>
<td>Methylene blue</td>
<td>Neoplasia was diagnosed in significantly more MB targeted specimens (12% vs. 6%) and in significantly more patients (44% vs. 28%) than by random biopsy technique.</td>
</tr>
<tr>
<td>Canto</td>
<td>Endoscopy</td>
<td>2001</td>
<td>Ex and en vivo cohort study</td>
<td>47 patients</td>
<td>Methylene blue</td>
<td>Ex vivo accuracy for IM=87% in vivo accuracy for IM=90% Light to absent staining and moderate to marked heterogeneity were significantly associated with high grade dysplasia or cancer</td>
</tr>
<tr>
<td>Kouklakis</td>
<td>Endoscopy</td>
<td>2003</td>
<td>Cohort study</td>
<td>975 patients 3900 biopsy specimens</td>
<td>Methylene blue</td>
<td>Methylene blue improved the sampling of IM compared to random biopsy (88% vs 1.4%)</td>
</tr>
<tr>
<td>Ngamruengphon</td>
<td>GIE</td>
<td>2009</td>
<td>meta-analysis</td>
<td>450 patients 9 studies</td>
<td>Methylene blue</td>
<td>There was no significant improvement in detection of IM or neoplasia</td>
</tr>
<tr>
<td>Guelrud</td>
<td>GIE</td>
<td>1996</td>
<td>Cohort study</td>
<td>21 patients post endo therapy</td>
<td>Acetic acid</td>
<td>Acetic acid enhanced the ability to identify remnant islands of Barrett’s metaplasia</td>
</tr>
<tr>
<td>Guelrud</td>
<td>GIE</td>
<td>2001</td>
<td>Cohort study</td>
<td>49 patients</td>
<td>Acetic acid with HRE</td>
<td>Standard endoscopy identified an endoscopic pattern in 1.5% of the areas, standard endoscopy and acetic acid in 8.5%, magnification endoscopy alone in 38%, enhanced magnification endoscopy in all 128 endoscopic areas. Enhanced magnification endoscopy was effective in identifying IM in BE.</td>
</tr>
<tr>
<td>Meining</td>
<td>Endoscopy</td>
<td>2004</td>
<td>Cohort study video sequences</td>
<td>51 patients</td>
<td>acetic acid and methylene blue</td>
<td>No differences observed before and after instillation of acetic acid or methylene blue staining for the detection of IM</td>
</tr>
<tr>
<td>Reaud</td>
<td>Gastroentrol Clin Biol</td>
<td>2006</td>
<td>Cohort study</td>
<td>28 patients</td>
<td>Acetic acid IM zoom endoscopy</td>
<td>sensitivity and specificity for metaplasia of 95.5% and 42.9%</td>
</tr>
<tr>
<td>Hoffman</td>
<td>GIE</td>
<td>2006</td>
<td>RCT with crossover</td>
<td>31 patients</td>
<td>Acetic acid metaplasia No dysplasia</td>
<td>sensitivity 100% specificity 66% accuracy 83.8%</td>
</tr>
<tr>
<td>Fortun</td>
<td>Aliment Pharmaco &amp; Therapeut</td>
<td>2006</td>
<td>Cohort study with historical controls</td>
<td>64 patients compared to 62 historical controls 9 neoplasia</td>
<td>Acetic acid + zoom</td>
<td>24% had a histological upgrade with AA enhanced magnification endoscopy. Detection of IM of 74% and there were two additional cancers found</td>
</tr>
<tr>
<td>Ferguson</td>
<td>Am J Gastroent Drology</td>
<td>2008</td>
<td>RCT</td>
<td>137 patients</td>
<td>Acetic acid intestinal metaplasia</td>
<td>No difference between AA targeted and random biopsies in yield of IM.</td>
</tr>
<tr>
<td>Pech</td>
<td>Acta Gastroent Drology Belg.</td>
<td>2008</td>
<td>Cohort study</td>
<td>20 patients</td>
<td>Acetic acid metaplasia</td>
<td>Accuracy 90% Sensitivity 100% Specificity 82%</td>
</tr>
<tr>
<td>Longcroft-Wheaton</td>
<td>Clin Gastroent Drology Hepatol</td>
<td>2010</td>
<td>Cohort study</td>
<td>190 procedures</td>
<td>Acetic acid non zoom</td>
<td>Sensitivity 95.5% Specificity 80%</td>
</tr>
<tr>
<td>Pohl</td>
<td>Am J gastroenterology</td>
<td>2010</td>
<td>Cohort study</td>
<td>701 patients</td>
<td>Acetic acid</td>
<td>Sensitivity 96.7% Specificity 66.5%</td>
</tr>
</tbody>
</table>

Table 11: Chromoendoscopy studies in Barrett’s oesophagus. AA= acetic acid, MB= methylene blue,BE= Barrett’s oesophagus, IM=intestinal metaplasia, LGD=low grade dysplasia, HGD= High grade dysplasia, HRE= high resolution endoscopy
<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Year</th>
<th>Design</th>
<th>No patients</th>
<th>Modality</th>
<th>Results</th>
</tr>
</thead>
</table>
| Pohl                   | Endoscopy    | 2007 | RCT cross over          | 57 patients | FICE and acetic acid | sensitivity 83% AA               Sensitivity 92% FICE  
|                        |              |      |                         | 24 HGIN early cancer |                      Specificity 97% AA and FICE |
| Osawa                  | J Gastro     | 2009 | Prospective cohort      | 72 patients | FICE           | Identification palisade vessels                                        |
| Hamamoto               | J gastro     |      | Picture study           | 11 patients | NBI            | Surface characteristics seen with NBI and WLI                          |
| Sharma                 | GIE          | 2006 | prospective cohort study.| 51 patients | NBI            | IM: Sensitivity 93.5%, specificity 86.7%.  
|                        |              |      |                         | 15 dysplasia |                | HGD The sensitivity 100%, specificity 98.7%.                           |
| Anagnostopoulos        | Alimentary pharmac ol ther | 2006 | Cohort study            | 50 patients | NBI            | Identification patterns IM Sensitivity 100%, Specificity 78.8%        |
| Kara                   | GIE          | 2006 | Picture study           | 63 patients | NBI            | Identification patterns. SIM Villous/gyrus 80%. Flat long branching vessels 20% HGIN Irregular mucosal vascular patterns  
|                        |              |      |                         | 198 areas    |                | Sensitivity neoplasia 94% Specificity 76%                              |
| Goda                   | GIE          | 2007 | Picture study           | 217 areas from 58 patients | NBI            | Identification of surface patterns. All intramucosal cancer correctly identified |
| Curvers                | GIE          | 2009 | Systemic review         | 40 publications | NBI            | Whether NBI improves the detection of early neoplastic lesions in BE is, unclear |
| Wolfsen                | Gastro-enterol | 2008 | Tandem endoscopy study  | 65 patients, all with previously detected dysplasia | NBI            | More dysplasia and higher grades of dysplasia were found by NBI than with standard resolution white light endoscopy |
| Singh                  | Scand J Gastro | 2009 | Prospective series      | 21 patients | NBI            | Accuracy 88.9%, superior to WLI for detection of dysplasia             |
| Singh                  | Endoscopy    | 2008 | Prospective cohort study.| 109 patients | NBI            | NBI-Z grading corresponded to the histological diagnosis. The PPV and NPV for type A pattern (columnar mucosa without intestinal metaplasia) were 100% and 97% respectively; for types B and C (intestinal metaplasia) they were 88% and 91%, and for type D (high-grade dysplasia) 81% and 99% |
| Ortner                 | Gut          | 2003 | Prospective cohort study.| 53 patients | AFI            | Dysplasia was detected at a rate 2.8-fold higher with AFI. Compared to random biopsy |
| Curvers                | Gut          | 2008 | Prospective multi-centre study. | 84 patients, 30 HGIN / EC | AFI + NBI | AFI detected an additional 11 patients with early neoplasia that were not identified with HRE. In three patients no abnormalities were seen but random biopsies revealed HGIN. AFI detected an additional 102 lesions; 19 contained HGIN/EC. False positive rate of AFI after HRE: 81%. NBI false positive rate 26%. |
| Curvers                | Gastro-enterol | 2010 | Randomised cross over   | 87 patients | AFI+NBI        | Tri-modal imaging superior to WLI in the targeted detection of HGD/cancer. However, yield of tri-modal imaging biopsies significantly inferior to overall yield of white light protocol guided biopsies |

Table 12: Electronic imaging studies in Barrett’s oesophagus, WLI= white light endoscopy, NBI=Narrow Band Imaging, AFI=auto-fluorescence, AA=acetic acid, PPV=positive predictive value, NPV=negative predictive value, IM=intestinal metaplasia, HGD=high grade dysplasia, HGIN=High grade intraepithelial neoplasia, EC= epithelial cancer, Ca=cancer
3.12: Treatment

In order to appreciate why localising neoplasia within Barrett’s is important it is necessary to understand how the treatment of Barrett’s associated neoplasia has changed in recent years. Traditionally the only treatment for high grade dysplasia and intramucosal adenocarcinoma was an oesophagectomy. This is a highly invasive intervention, associated with significant mortality and morbidity, variable according to centre, with high volume units producing better results (110). Post operative morbidity is accepted to be significant, with rates between 30% and 50%, with a mortality of 2-10% (111).

Endoscopic resection and ablation techniques are becoming increasingly popular due to low morbidity and mortality. Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) involve removing the mucosal and submucosal layers of the oesophagus. EMR uses either a cap and snare kit from Olympus or Duette banding ligator from Cook, to remove the abnormal tissue. This can be taken in one piece (112) (113) (114) or, for larger areas, piecemeal excision can be performed. EMR gives a better histological diagnosis as compared to biopsy. However, if a lesion is greater than 1.5cm, then piecemeal resection can make it hard to determine completeness of the lateral resection margins. ESD uses a specialised endoscopic knife to dissect out neoplastic areas of any size in an en-block fashion. It can provide a clear resection margin but carries increased risks, including that of perforation. This can be combined with argon plasma coagulation (APC) or multipolar electrocautery (MPEC), which both aim to destroy any residual abnormal tissue through either ionised argon gas or an electric current.
Ablative photodynamic therapy (PDT) involves the use of a photosensitizing agent which is preferentially taken up by tumour tissue (115). After a suitable time period an endoscopy is performed where the abnormal area is exposed to light at an appropriate wavelength. The neoplasia which has preferentially taken up the drug then undergoes cell death. Using this technique a randomised controlled trial (RCT) has shown 98% efficacy at eliminating low grade dysplasia (116). Success has also been demonstrated with HGD and superficial T1 cancers, with successful ablation of HGD as high as 93% in one prospective series (117), although a more recent RCT by the same author has suggested that complete HGD ablation is achieved in 77% of cases over a mean follow up period of 24 months (118). However, it can cause stricture formation and photosensitivity reactions. Radiofrequency ablation (RFA) is similar in concept. Radiofrequency electrodes deliver thermal energy through a focal device or balloon inflated to make contact with the oesophageal wall. This induces mucosal destruction. The depth of burn is less than with photodynamic therapy which improves the safety profile of RFA over that of PDT, with a low oesophageal stricture rate of 6%, no deaths and no perforations were seen in a large sham-controlled trial (119). RFA is a useful technique for multifocal dysplasia (120). However, it is not suitable for raised nodular areas which should first be removed by EMR. There have been no trials conducted to date which show whether RFA combined with EMR is any better than EMR alone.

In order to offer any localised treatment, it is essential that the lesion can be seen and accurately staged. It would clearly be impossible to perform EMR without some form of lesion localisation, and ablative therapies could be very dangerous if applied
to the wrong lesions. Both photodynamic therapy and radiofrequency ablation (HALO) do not provide the endoscopist with a tissue sample for examination. Therefore if an invasive cancer was inadvertently ablated, in time the patient may develop avoidable lymph node metastasis. Furthermore, after treatment it is crucial that the patient can be safely followed up. A problem with all endoscopic treatments is that they can leave behind residual Barrett’s metaplasia. Even with HALO ablation this is not uncommon. As a result the potential for metachronous lesions exists. The field will often be structurally abnormal in these patients with the potential for buried neoplasia, making assessment challenging.
3.13: Summary

Advanced imaging modalities are already challenging the way we manage high grade dysplasia and early cancer within Barrett’s oesophagus. However, the evidence base is incomplete and many of the new technologies are being applied based on limited evidence of effectiveness. Whilst certain techniques have demonstrated benefit in prospective cohort studies there are a lack of randomised controlled trials, particularly in a surveillance population. Many of the techniques, such as acetic acid and FICE are incompletely understood, and there are likely to be ways of improving the sensitivity and specificity which can be achieved with them.

It is very important to appreciate just how important developments in this field are. Oesophageal cancer carries a very poor prognosis. Early diagnosis and treatment of pre-malignant or early malignant changes are, at present, the only real way to reduce mortality and morbidity in this field. Advances in endoscopic treatments (EMR and HALO ablation) have raised the game significantly. Whereas previously patients diagnosed with HGD faced the unpleasant prospect of oesophagectomy (if fit for this major operation), it is now possible to endoscopically remove visible lesions. It is therefore important not just to find neoplasia but to localise it.

To help the clinician in the management of Barrett’s, a task force has been set up called BADCAT, to review the evidence for all aspects of its management. This is currently in the final stages of publication. One of the noted features however was the lack of evidence available for all of the advanced imaging modalities. At present there is the greatest evidence for the use of acetic acid (due in large part to the study
forming a part of this thesis) however, it is accepted that the case is not yet closed and there is an urgent need for further studies in this field.

In particular the key areas in need of further research are:

a) Establishing the effectiveness of acetic acid in the detection of neoplasia in a low risk surveillance population

b) Understanding the role of the aceto-whitening reaction in the examination of neoplasia within Barrett’s

c) Further larger studies into the effectiveness of vascular enhancement techniques in the examination of Barrett’s in both low and high risk populations. In particular studies with FICE and i-scan are needed

d) Studies to examine whether acetic acid and vascular enhancement techniques can be used together to accurately localise neoplasia within Barrett’s and whether this is more effective than either technique on its own

e) Randomised controlled trials of all of the above mentioned techniques
Chapter 4

Flexible Spectral Imaging Colour Enhancement

(FICE)
This chapter aims to describe the principles behind FICE. To understand how this technology enhances vascular structures, it is necessary to outline the key steps in the image processing that it performs.

Visible white light consists of electromagnetic waves with a wavelength of 400 to 700nm. When white light is used to illuminate an object some light is reflected which is perceived by the L, M or S cones in the retina that are sensitive to red (R) green (G) or blue (B) light respectively. This is then perceived as colour by the occipital lobe of the cerebrum. Traditional charge coupled device (CCD) image capture systems are based on the trichromatic theory of image reproduction, characterized by the additive colour mixing of the primary colours red, green and blue. Each of these three primary colours has a wide bandwidth. The human eye is thought to be able to distinguish 16.7 million colours, or 256 levels for each primary colour.

Traditional CCD systems have limitations. The quality of colour images is influenced by the spectral characteristics of the imaging device, illumination and the visual environment. Therefore recording and reproduction of spectral information on the object rather than just RGB information can result in a better and more faithful reproduction of the original image as it adjusts for these factors. This is the system used for electronic museums and digital archiving. It is now being applied to endoscopy. The key design concept is that a standard colour reproduction is inadequate to diagnose early neoplasia, and that colour reproduction can be improved by principal component analysis.
All image reproduction aims to create a two dimensional representation of a three dimensional structure. To achieve this, three dimensional information \((x,y,z)\) is projected onto a two dimensional plane for recording. In traditional image capture three bands (RGB) are subsequently captured and mixed to achieve colour reproduction. The characteristics of an object can therefore be expressed as the function \(O(x,y,z,t,\lambda)\), where \(t\)=time and \(\lambda\) is the wavelength of visible light. To simplify this for further discussion time, special coordinates and angle of deviation will henceforth be disregarded to allow focus on the wavelength information of object \(O(\lambda)\). Light sources have a specific spectral emissivity, \(E(\lambda)\). Any filters can be described as having a spectral transmittance of \(f(\lambda)\). Using these factors the image can be described as follows:

Spectral emissivity of light source: \(E(\lambda)\)
Spectral reflectance of object: \(O(\lambda)\)
Spectral sensitivity of CCD camera: \(S(\lambda)\)
Spectral transmittance of the lens and fibre: \(L(\lambda)\)
Spectral transmittance of filters: \(f(\lambda)\) (i=R,G,B)
camera output: \(V_i\) (i=R,G,B)

Camera output can be expressed with the following equation:

**Equation 1:**

\[
V_i(x,y) = \int E(\lambda) f(\lambda) L(\lambda) S(\lambda) O(\lambda) \, d\lambda
\]

\(i=r,g,b\)

\(x,y=\)coordinate of the object
This can also be expressed as a vector equation

**Equation 2:**

\[ V_i = H_i O \]

where \( H_i \) is the operator that transforms the vector \( O \) (spectral reflectivity characteristics of the original object) into vector \( V_i \) the ‘camera’ output in red, green and blue.

Therefore the final colour reproduction seen on the visual display device (CRT or LCD screen) is determined from the input of value \( V_i \) after it has undergone a mathematical transformation which takes into account the characteristics of the display, the visual environment and light source.

The aim of the exercise is to convert the input data into a visual screen output which captures and enhances the key characteristics and clinical features of the original object. The resultant image therefore takes into account:

a) Spectral reflectance of the tissue being examined
b) Spectral characteristics of the illuminating light source
c) Spectral characteristics of the imaging system
However, more can be achieved than simply creating an accurate reproduction of the original image. The output image can be calculated with different spectral transmittance by the addition of digital colour ‘filters’. See figure 7.

To understand how this works it is necessary to look at the spectral reflectance data captured from multiple samples of colorectal mucosa. Providing the spectral reflectance of the tissues to be examined is known, the colour reproduction can be predicted by the system. This has been quantitatively measured using spectroscopy systems built into specialised spectral endoscopes. These devices consist of a light source, optical endoscope, spectroscope and optical multichannel analyzer. Reflected light is measured by the spectroscope and analysed electronically. Wavelength calibration can be performed with a mercury spectrum and white plate. This is a very processor intensive process which has to be calculated for every point on an object. When this was initially attempted in the late 1980s computers were not
fast enough to perform this effectively. As a result attempts were made to estimate spectral reflectance from camera output by solving the integral equations described previously. The key problem is that compared with the camera output, spectral reflectance has a greater number of dimensions. The measurement of white light between 400-700nm at 5nm intervals is associated with 61 dimensions. In the graph below (figure 8) the spectral reflectance is plotted as a function of wavelength for these samples.

![Spectral reflectance as a function of wavelength](image)

*Figure 8: Spectral reflectance as a function of wavelength (Reproduced from Mayake et al 2005 (121))*

However, whilst calculating 61 dimensions would be impractical, it can be seen from this graph that there are three peaks corresponding to the most important wavelengths. Using principle component analysis of this data it has been shown that three components can give a good representation of spectral reflectance for
colorectal mucosa, with 99.7% of the reflection spectra expressed by these components. See figure 9.

![Figure 9: Three principle components (a) and cumulative contributions (b) of spectral reflectance of the rectal membrane 1=green, 2=red, 3=blue (Reproduced from Mayake et al 2005 (121))](image)

To obtain a reconstructed and enhanced image of the original object \( (O') \) based on spectral reflectance data an inverse transformation matrix is required \( H_i^{-1} \). This can be populated using the Wiener estimation method.

\[
O' = H_i^{-1} V_i
\]

(\( \text{where } i = \text{R,G & B} \))

An endoscope was initially used to capture sample RGBs from Macbeth Colour checker charts corresponding to spectral reflectance \( O \) where camera output \( V_i \) could be measured. Illumination came completely from the light source of the
endoscope. This was then used to populate a ‘lookup’ table from which the matrix $H_i^{-1}$ can be filled.

$$
\begin{bmatrix}
\lambda_1 \\
\lambda_2 \\
\lambda_3
\end{bmatrix} =
\begin{bmatrix}
K_{1r} & K_{1g} & K_{1b} \\
K_{2r} & K_{2g} & K_{2b} \\
K_{3r} & K_{3g} & K_{3b}
\end{bmatrix}
\begin{bmatrix}
R \\
G \\
B
\end{bmatrix}
$$

Using this matrix, it is then possible to choose any set of wavelengths that captures and enhances the key characteristics and clinical features of the colorectal mucosa, based on the three principle components discussed above. Put simply whereas a conventional television image is made up by mixing red, green and blue light it is now possible to mix any frequencies to enhance particular structures. As the system is based on a look up table it is not processor intensive and can be achieved in real time. FICE assigns the estimated spectral images to each RGB component sent to the display device to produce the final colour image. This final image can be constructed from many combinations of spectral image at different wavelengths to enhance the visualisation of pathology, with the optimum selection depending on the kind of tissue and disease being examined. In this manner, various combinations of wavelengths can be used to form the final displayed image. By the same logic, wavelengths that are not desirable can be left out. This achieves a similar function to a physical filter. However, it is much more flexible, as potentially a large combination of images can be produced and switched between rapidly. The EPX 4400 comes with ten frequencies pre-programmed. These are shown in table 13.
It is currently unclear which settings are most suitable for a given task. There have been very few studies performed in the colon, and each study has used a different setting. A small picture study has suggested that setting 4 yields the clearest image of the colorectal mucosa \(55\). However, this did not examine any pathology, and has not been backed up by any cohort observational studies demonstrating superiority of one setting over another. There has been so little work conducted outside of the colon, it is simply not possible to comment whether one setting is superior to another.

<table>
<thead>
<tr>
<th>Preset</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>500nm</td>
<td>500nm</td>
<td>550nm</td>
<td>540nm</td>
<td>520nm</td>
<td>500nm</td>
<td>580nm</td>
<td>520nm</td>
<td>540nm</td>
<td>550nm</td>
</tr>
<tr>
<td>G</td>
<td>445nm</td>
<td>470nm</td>
<td>500nm</td>
<td>490nm</td>
<td>500nm</td>
<td>480nm</td>
<td>520nm</td>
<td>450nm</td>
<td>415nm</td>
<td>500nm</td>
</tr>
<tr>
<td>B</td>
<td>415nm</td>
<td>420nm</td>
<td>470nm</td>
<td>420nm</td>
<td>405nm</td>
<td>420nm</td>
<td>460nm</td>
<td>400nm</td>
<td>415nm</td>
<td>400nm</td>
</tr>
</tbody>
</table>

Table 13: Pre-programmed Frequencies for FICE
PART B

Experimental work
Chapter 5

Colonic polyp studies

Background to investigations
5.1 In-vivo diagnosis

Polyps are commonly found during colonoscopy and it is current practice to remove all of these lesions, as some have the potential to develop into cancer. As described in the previous chapters, polyps are not all the same. It is established that hyperplastic polyps have negligible malignant potential, especially when small (<10mm) and in the left colon. These polyps account for one third of all small polyps (13). In contrast adenomas can progress to cancer and therefore require resection (14). Polyp cancers should be biopsied and referred for specialist intervention.

The management pathway for polyps >10mm is clear and well described. Most large polyps are either likely to be adenomas which need removal or cancers, which need careful consideration for endoscopic or surgical resection. Even large hyperplastic polyps have to be removed as there is the possibility that they are serrated adenomas which carry malignant potential. However, the majority of polyps found during screening endoscopy are small, representing over 90% of the polyps encountered (122). The optimum management of polyps <10mm in size is not so well established. Because it has been traditionally felt that it is difficult for the endoscopist to differentiate hyperplastic polyps from adenomas, it is current practice for all polyps to be removed. However, polypectomy is associated with significant risks (15). It results in a prolongation of the procedure time which has an immediate impact on endoscopy output and has histology related cost implications. If an endoscopist could make a confident in-vivo diagnosis, then hyperplastic polyps <10mm could be left in-situ or removed but not retrieved to reduce pathology costs.
The previous chapters have described Japanese studies of \textit{in-vivo} diagnosis. These have involved examination of polyp surface patterns (Kudo’s pit patterns) and have achieved excellent results (16) (17) (20). Unfortunately this work initially involved vital staining with crystal violet and optical magnifying endoscopes which are cumbersome and time consuming to use. This is only used in the assessment of probable cancer in Japan and is not routinely available on Western screening lists. There have been numerous studies into the use of indigocarmine as a diagnostic tool, which is easier to use than crystal violet. However, most of these studies have still required the use of magnifying endoscopes and analysis of Kudo pit patterns. Whilst this is considered acceptable in Japan, the inconvenience of dye spray and magnification endoscopy coupled with the perceived complexity of Kudo pit pattern recognition, has resulted in Western endoscopists questioning its role in Europe and North America.

There has been interest in electronic imaging for the \textit{in-vivo} diagnosis of colonic polyps. Olympus, Fujinon and Pentax have introduced vascular and surface enhancement features into their endoscopes. A potential advantage of these technologies over chromoendoscopy is that they are activated by the press of a button and therefore straightforward to apply on busy lists. Narrow band imaging (NBI) from Olympus is an optical filter technology which enhances the visibility of vessels on the mucosal surface (123). Whilst this does not directly replicate the dye spray appearances which highlight surface patterns, it was proposed that these could be used for the purposes of \textit{in-vivo} diagnosis. A recent study has shown the utility of NBI in differentiating neoplastic from non neoplastic lesions <10mm in size (47) with an accuracy of 93%. There are also numerous assessment tools for use with the technology. The details of these studies were reviewed in Chapter 2.
Fujinon have developed a post processor technology called Flexible Spectral Imaging Colour Enhancement (FICE). White light endoscopy captures reflected light in a wide spectrum (400nm-700nm) with a CCD device. FICE processes this conventional image into a spectral image composed of specific wavelengths and displays them in real time. This was described in detail in chapter 4. Whilst the method of processing the image is quite different, the concept is similar to Narrow Band imaging. The benefits of digital processing however mean that multiple wavelengths can be used in image reconstruction and the endoscopist can manually select the best mode to assess the polyp. This enables subtle structural and vascular patterns on the surface of polyps to be assessed in greater detail than can be achieved with white light alone.

It is important to recognise that whilst the concept of what FICE is attempting to achieve is similar to NBI, its implementation is fundamentally different. The image produced looks different to what is seen with NBI and therefore it would be potentially dangerous to assume that the assessment tools developed for NBI could be used with FICE. A further problem with FICE has been its complexity. To date there have been very few studies conducted into its effectiveness in making an in-vivo diagnosis. The results from the published studies have been variable, with some claiming good results, others disagreeing. Most of the small volume of literature that is available has not invested in developing the appropriate tools for use with FICE, instead using classifications such as KUDO pit patterns which have never been validated for use with electronic imaging. The only published classification system for polyps using FICE utilised optical magnification and the authors specifically stated that they felt FICE could not be used without optical magnification. As a result,
endoscopists have been left with a lack of information on how to use this technology, and indeed whether it is of any value at all. Subsequently many endoscopists have been left dissatisfied with FICE. Common complaints are that picture quality is very variable, for no apparent reason, and that it is confusing to know what setting to use and how to interpret the images.

There are therefore four key issues which need to be addressed:

1) A tool needs to be developed for assessing colonic polyps using FICE without optical magnification

2) The optimum FICE setting for examining colonic polyps needs to be established

3) The impact of high definition (HD) endoscopes on FICE assessment

4) The role of FICE in performing in-vivo diagnosis of colonic polyps

Without addressing these issues FICE cannot be used in clinical practice for the in-vivo diagnosis of neoplasia. Misdiagnosis of colonic polyps is a potentially very serious issue, as it can result in mismanagement of the patient. Therefore a robust assessment tool which is reliable and demonstrated to work in a well powered study is an essential requirement.
5.2 High definition versus standard definition endoscopy

High definition endoscopy is now being offered from all of the major endoscope manufacturers, and it has been predicted that the uptake of high definition equipment will be a major growth area over the next 5 years (124). Modern colonoscopes utilise charge coupled devices (CCD) with pixel densities of up to 1.3 million pixels. In contrast, standard definition endoscopes have CCD resolutions of around 410,000 pixels. To define a system as high definition requires a high resolution charge coupled device (CCD) and a processor capable of outputting a digital high resolution signal to a display of at least 1024x768 pixels. However, there is remarkably little known regarding the potential value of high definition over standard definition equipment.

Some work has been performed into *in-vivo* diagnosis of colonic polyps. However, all of these studies have been performed using high definition equipment, usually with optical magnification (20) (26) (47) (49). Unfortunately not all endoscopy units are equipped with enough high definition equipment to regularly run complete lists without resorting to the use of older, standard definition (SD) equipment.

If an in-vivo diagnosis of small polyps<10mm in size could be made this would result in a significant cost saving (68). However, endoscopists need to feel confident that the equipment available is fit for this purpose. Acquiring high definition (HD) endoscopes represents a significant capital investment and their clinical value remains uncertain. In the current era of austerity it is unclear whether the expense of upgrading existing equipment can be justified.
Again the Fujinon endoscopes are under researched in this area. There are currently no studies to answer whether HD makes any difference to diagnostic capabilities. In clinical practice, the newer HD scopes are more expensive than older SD endoscopes and clinicians need to know the clinical advantages of these endoscopes to justify the extra cost.
5.3 Study aims

1) To develop a new classification system for colonic polyps using FICE without optical magnification

2) To establish the optimum FICE setting to use for assessing colonic polyps

3) To assess the accuracy of *in-vivo* diagnosis of colorectal polyps<10mm using FICE as an electronic imaging tool using this new classification system

4) To establish whether there is any additional gain from indigocarmine dye spray as a chromoendoscopic imaging tool.

5) To compare the accuracy of standard and high definition Fujnon colonoscopes in the diagnosis of neoplastic polyps<10mm using white light, FICE and indigocarmine chromoendoscopy
Chapter 6

Methods

Development of a new polyp classification system using FICE
6.1: Introduction

The main assessment tools available for in-vivo histology prediction are white light examination, electronic imaging (NBI, FICE, i-scan) and dye spray (indigocarmine). Whilst the established Kudo classification system for colonic neoplasia is accepted to be an effective tool for the in-vivo histology prediction of colonic polyps using indigocarmine with optical magnification, it is often perceived as being difficult and impractical to apply in a Western setting outside of a research programme. Furthermore, it is generally accepted that many of the features described in Kudo’s pit patterns are not always visible without optical magnification. For this reason a new classification system, known as N.A.C., was developed by this department for assessing colonic polyps with indigocarmine (125) without optical magnification.

N.A.C. stands for Non adenomatous (hyperplastic), Adenomatous and Cancer. It involves the assessment of two key structural elements; vascularity when viewing the polyp with white light, and surface patterns after indigocarmine dye spray. The key appearances of polyps using the N.A.C. classification are as follows:

N = Non adenomatous: Relative pallor (hypovascular) on white light with the surface pattern after indigocarmine appearing similar to the adjoining normal mucosa with large, non compact crypts
A = Adenomas: Hypervascular appearance on white light with the surface pattern after indigocarmine appearing regular but different from the surrounding mucosa (round, tubular round or gyriform patterns)

C = Cancer: Hypervascular appearance on white light with an irregular surface pattern after indigocarmine

The situation for the classification of colonic polyps using electronic imaging with FICE is less clear. The only previous classification system for FICE was defined by Texiera et al. using optical magnification (56). This is a complex system. Furthermore, the authors stated that they felt it was not applicable to use this without optical magnification.

Therefore a new classification system for colonic polyps using FICE without optical magnification was developed based on the criteria defined in the N.A.C classification system. This system would be based on assessment of the key structural components described in the N.A.C. classification system. It was felt that vascular patterns and surface patterns would not be visible with white light. Therefore the assessment of vascularity would be performed using white light, with the assessment of vascular patterns and mucosal patterns performed with FICE. The intention would be for this system to be simple to apply on a routine basis and suitable for use with standard (non magnifying) endoscopes. Figures 10 to 15 demonstrate the appearances of hyperplastic polyps and adenomas seen with white light, FICE and after indigocarmine dye spray when viewed with a non magnifying high resolution endoscope.
Figure 10: Hyperplastic polyp White light

Figure 11: Hyperplastic polyp FICE setting 4

Figure 12: Hyperplastic polyp Indigo carmine
Figure 13: Adenomatous polyp White light

Figure 14: Adenomatous polyp FICE setting 4

Figure 15: Adenomatous polyp Indigo carmine
It was unclear which FICE setting would be optimal for the examination of colonic polyps, or which of the previously defined features of the N.A.C. criteria would be visible using FICE. Therefore three pilot studies were conducted:

1) Defining the optimum FICE setting (Chapter 6)
2) Defining the N.A.C. criteria using FICE (Chapter 7)
3) Validation of the N.A.C. criteria for FICE by making *in vivo* assessments (Chapter 8)

### 6.2: Location of the studies

All work was conducted at Queen Alexandra Hospital in Portsmouth. This is a major district general hospital on the South Coast of England covering a catchment population of 600,000 people. It offers a tertiary advanced endoscopy service to the entire south coast, for the diagnosis and treatment of early cancer in the oesophagus, stomach, duodenum and colon with three lists a week dedicated to this, including two lists a month with anaesthetic support. There is an established Bowel Cancer Screening Programme running from the endoscopy department, with two screening colonoscopy lists a week dedicated to colonoscopy screening.
5.3: Ethics approval

The Portsmouth research and ethics committee was approached for permission to conduct the study. It was assessed at the December 2009 meeting and was felt by the panel that the studies were principally of the endoscopist and their ability to make an in-vivo diagnosis. As it did not involve any additional procedures to the patient (FICE and indigocarmine are routinely used in the assessment of polyps for reasons other than in-vivo diagnosis, principally to delineate borders for resection), it did not require permission. A waiver for permission to conduct the study was granted by the committee REC No.:09/H0501/94.

The studies were registered with the European Clinical Trials Database (EudraCT 2009-016742-10) and with the American Clinical Trials Database (ClinicalTrials.gov, NCT01182623).
Chapter 7

Defining the optimum FICE setting
7.1: Introduction

There has been very little work performed which has examined which FICE setting is best suited for examining colonic polyps. The previous studies which have used FICE as an in-vivo diagnostic tool have all used different settings. As described in chapter 4, FICE is capable of reconstructing the image based on many different wavelengths, with 10 pre set combinations available as standard. The settings vary considerably in terms of their appearance, with some appearing dark and others much lighter. Whilst this provides considerable flexibility, it is not practical to examine every lesion with all 10 settings. There is currently confusion amongst endoscopists as to which setting should be used for examining colonic polyps. This is unfortunate, as it is difficult to develop or validate any classification system without first establishing which FICE setting should be used for the assessments.

A single study from Spain attempted to use FICE in the examination of colonic mucosa, and concluded that setting 4 (520,500,405nm) was superior to the other settings for the examination of vascular and surface patterns (55). However, that study only examined normal colonic mucosa, which is not necessarily representative of the best setting for the examination of colonic polyps.
7.2: Aims

This study aims to determine the optimum FICE setting for the examination of the key structural components of colonic polyps, based on the pre-programmed reconstructions built into the EPX4400 processor.
**7.3: Methods**

The optimum settings for FICE was determined in a preliminary picture study. Images were prospectively collected from patients undergoing colonoscopy on Bowel Cancer Screening Lists. Exclusion criteria were: poor bowel preparation, active inflammation or the presence of melanosis coli. All procedures were performed using Fujinon EC530 and EC 590 colonoscopes and the EPX 4400 processor. Optical magnification, where available, was not used. Patients had standard bowel preparation with three sachets of sodium picosulphate. For morning lists this was given at breakfast, lunchtime and in the evening on the day before the procedure. For afternoon lists two sachets of sodium picosulphate were administered on the morning of the procedure, given at 6:00 and midday. Polyps were identified using white light endoscopy and examined on withdrawal. Prior to examination and image capture, the polyps were cleaned by flushing 10-20ml of water to remove any visible debris, with care taken not to traumatisé the mucosa. The polyp was then brought around to the 6 o’clock position for detailed examination. Lesions where a good, stable view could not be obtained were excluded. Digital photographs were then taken of the polyps with white light and all 10 FICE settings. The wavelengths used in these reconstructions are shown below in table 14 and illustrated in figure 16.

<table>
<thead>
<tr>
<th>Preset</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>500nm</td>
<td>500nm</td>
<td>550nm</td>
<td>540nm</td>
<td>520nm</td>
<td>500nm</td>
<td>580nm</td>
<td>520nm</td>
<td>540nm</td>
<td>550nm</td>
</tr>
<tr>
<td>G</td>
<td>445nm</td>
<td>470nm</td>
<td>500nm</td>
<td>490nm</td>
<td>500nm</td>
<td>480nm</td>
<td>520nm</td>
<td>450nm</td>
<td>415nm</td>
<td>500nm</td>
</tr>
<tr>
<td>B</td>
<td>415nm</td>
<td>420nm</td>
<td>470nm</td>
<td>420nm</td>
<td>405nm</td>
<td>420nm</td>
<td>460nm</td>
<td>400nm</td>
<td>415nm</td>
<td>400nm</td>
</tr>
</tbody>
</table>

*Table 14: Wavelengths used in each FICE setting*
Figure 16: A hyperplastic and adenomatous polyp visualised with white light and all 10 FICE settings
Images were saved directly from the digital source to the SD memory card built into the processor to avoid artefacts associated with analogue image capture from the other outputs from the stack. These included hyperplastic polyps, adenomas and cancers. Two pictures were taken with each setting for each polyp, with the lesser quality image discarded. The images were collated into an album using Microsoft Powerpoint 2007. Images were stored in uncompressed TIFF format at 1026x770 resolution.

The library was reviewed independently by two endoscopists (GLW+PZB) who scored each image for the clarity of:

1) Mucosal surface patterns
2) Surface vascular patterns
3) Vascularity
4) Visibility of artefacts

This was rated on a four point scale: 0=poor, 1=fair, 2=good, 3=excellent. In the case of artefacts a low score meant that more artefacts were seen. These included stool debris and mucous which had the potential to obscure views of mucosal surface details.

No sample sizes were calculated as this was a pilot study and there was no data available regarding the examination of colonic polyps with FICE. The mean scores and standard deviation for each assessor using white light and all 10 FICE settings was calculated. Mean scores from each assessor were compared using an unpaired Students t test and standard deviations were reported. The mean score for any setting which was more than two standard deviations from the overall mean score for a given assessor was noted.
7.4: Results

Images of 30 polyps with white light and all 10 FICE settings were examined; 13 hyperplastic polyps, 15 adenomatous polyps and 2 polyp cancers. The results are shown in table 15. Setting 4 was found by both assessors to be superior, with an overall score 2 standard deviations above the mean score for each assessor. The key benefits were in terms of clarity of mucosal and vascular patterns. There was no significant difference in the scores for the other settings. White light scored poorly for the assessment of surface and vascular patterns by both assessors. However, a good assessment of overall vascularity could be made with white light. Both assessors felt that there were less artifacts seen with white light which helped to bring the overall score for white light up.
<table>
<thead>
<tr>
<th></th>
<th>Mucosal surface</th>
<th>Vascular pattern</th>
<th>Vascularity</th>
<th>Artifacts</th>
<th>Overall</th>
<th>Mucosal surface</th>
<th>Vascular pattern</th>
<th>Vascularity</th>
<th>Artifacts</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>44</td>
<td>46</td>
<td>68</td>
<td>63</td>
<td>221</td>
<td>42</td>
<td>37</td>
<td>69</td>
<td>77</td>
<td>225</td>
</tr>
<tr>
<td>0</td>
<td>55</td>
<td>61</td>
<td>71</td>
<td>49</td>
<td>236</td>
<td>47</td>
<td>48</td>
<td>55</td>
<td>64</td>
<td>214</td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>62</td>
<td>71</td>
<td>50</td>
<td>238</td>
<td>52</td>
<td>51</td>
<td>53</td>
<td>61</td>
<td>217</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>66</td>
<td>68</td>
<td>51</td>
<td>241</td>
<td>62</td>
<td>56</td>
<td>59</td>
<td>60</td>
<td>237</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>66</td>
<td>70</td>
<td>56</td>
<td>246</td>
<td>61</td>
<td>54</td>
<td>58</td>
<td>59</td>
<td>232</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>70</td>
<td>70</td>
<td>56</td>
<td>256</td>
<td>83</td>
<td>80</td>
<td>80</td>
<td>79</td>
<td>322</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>57</td>
<td>66</td>
<td>53</td>
<td>226</td>
<td>65</td>
<td>60</td>
<td>62</td>
<td>62</td>
<td>249</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>57</td>
<td>62</td>
<td>52</td>
<td>217</td>
<td>68</td>
<td>59</td>
<td>61</td>
<td>63</td>
<td>251</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>58</td>
<td>71</td>
<td>49</td>
<td>225</td>
<td>60</td>
<td>56</td>
<td>56</td>
<td>59</td>
<td>231</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>59</td>
<td>66</td>
<td>50</td>
<td>224</td>
<td>62</td>
<td>59</td>
<td>57</td>
<td>60</td>
<td>238</td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td>61</td>
<td>63</td>
<td>49</td>
<td>225</td>
<td>71</td>
<td>59</td>
<td>58</td>
<td>59</td>
<td>247</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>52 (4.9)</td>
<td>62 (4.6)</td>
<td>68 (3.2)</td>
<td>53 (4.3)</td>
<td>232 (12)</td>
<td>61 (11)</td>
<td>56 (10)</td>
<td>61 (7.7)</td>
<td>64 (7.2)</td>
<td>242 (29)</td>
</tr>
</tbody>
</table>

Table 15: Numerical scores of images obtained using Fujinon EC530 and EC590 colonoscopes and EPX 4400 processor using either white light (W) or each of the 10 pre-programmed FICE settings (0-9). Data are presented as mean ± SD from 30 individual polyps (Hyperplastic, adenoma and cancers)
7.5: Discussion

Overall, vascularity could be seen clearly using white light. However, vascular and surface patterns could not be clearly identified using this alone. The optimum setting for using FICE to assess vascular and surface patterns is setting 4 (520,500,405nm). All of the FICE digital filters unfortunately enhance artefacts, including poor bowel preparation and other debris. These findings support those from the previous study examining the optimum FICE setting for the examination of colonic mucosa (55) and suggest that for the examination of colonic polyps, setting 4 provides optimum enhancement.

The default settings provided by the manufacturer were used. Whilst it is possible to program the EPX4400 processor to use any combination of wavelengths, this would not have answered the question which most endoscopists ask, which is; how to rapidly set up the system for use in making an in-vivo diagnosis of colonic polyps. If further work is to be conducted in developing a simple and easy to apply classification system, it would be unlikely to gain widespread acceptance if this were a custom setting. This is not to say that it would not be possible to develop a setting which visualises surface structures in more detail. However, the range of settings provided as standard is quite comprehensive and one of the criticisms of FICE is that it is already overly complex, and there does not appear to be a need to make this situation any worse.
It should be noted that neither of the assessors felt that setting 8 provided an optimum image for the assessment of vascular or surface structures. The wavelengths utilised by setting 8 corresponds to those utilised by the physical filter of the Narrow Band Imaging System from Olympus. This highlights the point that NBI and FICE are different technologies, and that results obtained from one of the systems cannot be extrapolated to the other.

It is important to note that surface patterns could be visualised using FICE. The original design concept behind all of the electronic imaging systems (NBI, FICE and i-scan) is that they enhance surface vasculature. However, traditional methods of in-vivo diagnosis have relied upon surface pattern assessment using Kudo pit patterns. It was suggested in a previous study that FICE may be able to enhance surface patterns (26) and this data would appear to support this. This would suggest that FICE could be applied to assessment using the N.A.C criteria previously described. It also introduces an additional factor which can be exploited, the assessment of vascular structures, which cannot be done with indigocarmine. Further work is required in this area.

The study was conducted using both standard definition and high definition colonoscopies. Whilst it was possible to identify the vascular and surface patterns using both standard and high definition equipment it was subjectively harder to obtain a high quality image using the standard definition colonoscopies. This is not reflected well in a picture study, where all of the images reviewed are of a high quality, but would have an impact when making an in-vivo assessment. A similar point was raised in a previous study (55) where the authors concluded that their
results only applied to high definition equipment. Optical magnification was not used in the generation of the library, and assessment of surface and vascular patterns could be made without magnification. This contradicts the findings of Teixeira et al. (56) who suggested what FICE assessments were only possible when optical magnification is used.

In summary FICE setting 4 appears to be the optimum setting for the examination of colonic polyps. Surface and vascular patterns can be identified without optical magnification. This calls for further work into development of a classification system for the assessment of colonic polyps without optical magnification.
Chapter 8

Development of N.A.C. for FICE
8.1: Introduction

Whilst there is considerable interest in *in-vivo* diagnosis, it is not possible to recommend any technique for widespread use without the development of a robust assessment tool. The Kudo pit pattern classification is established for use with magnifying endoscopes and indigocarmine (126), and the Portsmouth research group has proposed the N.A.C. classification system for use with standard endoscopes (125). There have been numerous polyp classification systems proposed for use with Narrow Band Imaging (51) (53) (38). However, there is only one classification system proposed for use with FICE, which is only validated for use with optical magnification (56). This is unfortunate as most screening colonoscopy is performed with standard endoscopes which lack optical magnification. Furthermore, many endoscopists complain that magnification colonoscopes are bulky and challenging to use on a routine basis. Whilst it is possible that elements of these classification systems may be applicable to FICE without magnification, this has never been proven, and Teixeira et al. specifically stated in their discussion that they believed their classification system only applied to high resolution endoscopes with optical magnification (56).

It was demonstrated in the previous study that FICE is capable of enhancing surface and vascular patterns when used without optical magnification. These could potentially be used in a similar way to the surface pattern assessments used in the N.A.C. classification system for indigocarmine, with the additional advantage that vascular patterns could also be exploited. However, it is uncertain how reliably these patterns can be seen *in-vivo* when magnification is not used. How these can be used
in making an *in-vivo* diagnosis is unclear. This study aims to define the key surface and vascular patterns seen on hyperplastic polyps, adenomas and cancers when viewed with FICE, and to modify the existing N.A.C. classification system to use these patterns in place of conventional indigocarmine based assessment.

### 8.2 Aims

To define the vascular patterns and surface patterns visible on colonic polyps in a picture based study using FICE as the assessment tool, and to modify the previously defined N.A.C. classification system to be used with these patterns.

### 8.3 Methods

Images were prospectively collected from patients referred for Screening Colonoscopy. All procedures were performed using Fujinon EC530 and EC 590 colonoscopes and the EPX 4400 processor. Optical magnification, where available, was not used. Exclusion criteria: were poor bowel preparation, active inflammation or the presence of melanosis coli. Patients had standard bowel preparation with three sachets of sodium picosulphate. For morning lists this was given at breakfast, lunchtime and in the evening on the day before the procedure. For afternoon lists two sachets of sodium picosulphate were administered on the morning of the procedure, given at 6:00 and midday. Polyps were identified using white light endoscopy and examined on withdrawal. Prior to examination and image capture, the polyps were cleaned by flushing 10-20ml of water to remove any visible debris,
with care taken not to traumatise the mucosa. The polyp was then brought around to the 6 o’clock position for detailed examination. Lesions where a good, stable view could not be obtained were excluded. An image library of polyps was created using white light and the clearest FICE settings. In each case the true pathology reported by an expert gastrointestinal pathologist was known for each polyp image. Images were saved directly from the digital source to the SD memory card built into the processor to avoid artifacts associated with analogue image capture from the other outputs from the stack. A minimum of two pictures were taken with each setting for each polyp, with the lesser quality image being discarded.

A library of Images was created using Microsoft Powerpoint 2007 for Windows and stored in an uncompressed TIFF format at 1026x770 resolution. The library was examined by two blinded endoscopists expert in lesion recognition (GLW+PZB) to score each polyp picture on its vascularity, vascular and surface patterns. These were then compared with the true histopathology.

No sample sizes were calculated, as this was a pilot study, and there was no previous data available to base such calculations upon. Categorical data are presented as frequencies and percentages.
8.4: Results

An image library of 661 images of 67 polyps was examined. In each case the true histology was known. These images were examined and key structural elements noted. From this the N.A.C. classification system was adapted for use with FICE.

It was found that one structural element could be examined with white light and three different structural elements could be identified using FICE:

1) Vascularity on white light
2) Vascularity with FICE
3) Vascular patterns with FICE
4) Surface patterns with FICE

It was noted that more adenomas appeared hypervascular with FICE than with white light (94% vs 78%). Therefore whilst N.A.C. for indigocarmine was based purely on assessment of vascularity with white light and assessment of surface patterns with indigo carmine the N.A.C classification for FICE would be based on all of these features.

The frequency of each of these changes is shown in table 16. Based on these features polyps would be be graded as N (non-neoplastic) A (adenoma) C (cancer).
**Hyperplastic polyps:** The white light appearances are Pale (hypoaemic). With FICE they appear hypovascular in the majority of cases with a low density, sparse and thin vascular pattern which looks similar to the surrounding mucosa. There may be the occasional vessel but this does not follow a pericryptal pit pattern. The surface pattern consists of large non compact crypts or no visible crypts.

**Adenomatous polyps:** Dark (Hyperaemic) on white light. With FICE they appear hypervascular in the majority of cases, with a dense, well organised regular pericryptal vascular pattern which is different to the surrounding mucosa. The surface pattern consists of small compact and regular crypts.

**Cancers:** Very dark (hyperaemic) on white light. With FICE there is a disorganised and irregular vascular pattern that is different to surrounding mucosa. The pattern may be lost completely. The surface pattern is distorted, with irregular, disorganised crypts.

Figures 17-24 demonstrate these appearances. It was noted that whilst there were only very subtle differences in the picture quality between SD and HD endoscopes when viewing the mucosa with white light, when using FICE it was easier to examine the surface vessel structures with an HD endoscope. See figure 25.
<table>
<thead>
<tr>
<th></th>
<th>Hyperplastic (%)</th>
<th>Adenomas (%)</th>
<th>Cancers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White light</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale (Hypoaemic)</td>
<td>25/30</td>
<td>7/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>83%</td>
<td>22%</td>
<td>0%</td>
</tr>
<tr>
<td>Dark (hyperaemic)</td>
<td>5/30</td>
<td>25/32</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>17%</td>
<td>78%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>FICE Vascularity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale</td>
<td>24/30</td>
<td>2/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>Dark</td>
<td>6/30</td>
<td>30/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>94%</td>
<td>0%</td>
</tr>
<tr>
<td>Very dark</td>
<td>0/30</td>
<td>0/32</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>FICE vascular pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent vascular pattern</td>
<td>15/30</td>
<td>0/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Faint vessels not following crypts</td>
<td>15/30</td>
<td>0/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Regular Pericryptal pattern</td>
<td>0/30</td>
<td>30/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>94%</td>
<td>0%</td>
</tr>
<tr>
<td>Dense, irregular pattern</td>
<td>0/30</td>
<td>0/32</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>FICE Surface pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No surface pattern</td>
<td>19/30</td>
<td>0/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>63%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Large, non compact crypt pattern</td>
<td>11/30</td>
<td>0/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>37%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Small, compact, regular pattern</td>
<td>0/30</td>
<td>28/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>88%</td>
<td>0%</td>
</tr>
<tr>
<td>Disorganised, irregular pattern</td>
<td>0/30</td>
<td>0/32</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Cannot assess</td>
<td>0/30</td>
<td>4/32</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>12%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 16: N.A.C. criteria for FICE assessment. Data presented as frequency of occurrence of a particular characteristic for hyperplastic polyps, adenomas and cancers
Figure 1: Hyperplastic polyp demonstrating pallor (hypovascularity) with white light and high definition colonoscope.

Figure 2: Hyperplastic polyp with white light and a standard definition colonoscope: Note this is hypervascular.

Figure 3: Hyperplastic polyp FICE setting 8 and a standard definition colonoscope. Note it is hypovascular and has a sparse vascular pattern. The only visible vessel shows no organisation or pattern and is not following a crypt.
Figure 20: Adenoma on white light with a standard definition colonoscope. Whilst the surface and vascular patterns are unclear in this image it is clearly hypervascular.

Figure 21: Adenoma with FICE on setting 4 viewed with a high definition colonoscope: It demonstrates a well organised pericryptal vascular pattern.

Figure 22: Adenoma on FICE setting 4 viewed with a high definition colonoscope. The lesion is hypervascular. The vascular pattern is less clear but is ordered and crosses the entire surface of the lesion.
Figure 23: Cancer on white light viewed with a high definition colonscope. Note how the lesion is hypervascular with a disorganised area in the centre. Whilst the pattern is unclear it is clearly abnormal.

Figure 24: When viewed with FICE setting 4 the centre has a disorganised vascular pattern. The edge is ordered and is adenoma. If this area was biopsied it would come back as high grade dysplasia only and a misdiagnosis could be made.
Figure 25: Adenomatous polyp viewed with an HD endoscope (A) and SD endoscope (B). Note how the capillary pattern is clearer in A than B.
The key structural elements that have been used in the N.A.C. classification system for indigo carmine can be identified using FICE. In addition, vascular patterns can also be seen. There is a difference in the distribution of these patterns between hyperplastic polyps, adenomas and cancers, which could be utilised for the purposes of \textit{in-vivo} diagnosis.

It was reassuring to observe that surface patterns could be examined in the majority of cases. However, it should be noted that in 12\% of adenomas it was not possible to assess these patterns. In contrast vascular patterns could be determined in all of the lesions. Therefore although FICE can identify surface pits, it is better at identifying vascular patterns. It is therefore necessary to assess both surface and vascular patterns in the classification of colonic polyps. It was also noted that a greater percentage of adenomas had increased vascularity when viewed with FICE than when assessed with white light alone (78 vs 94\%). This would suggest that an additional advantage of using FICE in place of indigo carmine is that a more accurate assessment of overall vascularity can be made as indigo carmine cannot be used to enhance this, therefore when N.A.C. is used with indigo carmine, the assessment of vascularity is completely based on the white light assessment when using dye spray.
Patterns could be assessed without difficulty without optical magnification. However, it is important to stress that this is a picture based study and that this may prove more difficult in-vivo. It is generally accepted that picture studies need to be treated with caution in this respect. Whilst it may be acceptable to spend some time looking at a static image for subtle changes this is not generally possible during a procedure with a lightly sedated patient. It is therefore not possible from this to dismiss the idea that optical magnification may be of benefit in rapidly assessing surface or vascular patterns. It is however reasonable to propose that surface structures can be assessed without optical magnification, which contradicts the conclusions reached by Teixeira et al. in their study (56).

Whilst assessments could be made with both standard definition and high definition images, it was noted that images captured with a high definition colonoscope were easier to interpret. It was not possible from the data collected to sub-analyse the effects of high definition images on the clarity of surface patterns, as the number of images was not adequate and the differences not large enough to draw any meaningful conclusions. However, it may be an issue for in-vivo diagnosis.

A limitation of this study is that it is picture based. Whether these assessments can be made rapidly in-vivo is unclear. However, it provides the framework for the development of an in-vivo study to investigate this further.
In summary there are key vascular and structural patterns associated with hyperplastic polyps, adenomas and cancers which can be identified without optical magnification. These could potentially be exploited for the purposes of *in-vivo* diagnosis using the FICE system.
Chapter 9

Validation of the N.A.C. classification system for FICE
9.1: Introduction

It has been established that there is a clinical need for a new polyp classification system based around FICE without optical magnification, as there is currently only one validated system available for use with FICE which requires high resolution magnifying endoscopes (56). The last two chapters have described the development of provisional tools for the in-vivo classification of colonic polyps using the FICE system without optical magnification, based on the previously described N.A.C. classification system for indigocarmine.

The proposed N.A.C. classification system is based on an assessment of vascularity with white light, followed by an assessment of vascularity, vascular and surface patterns using FICE. However, up to this point the development of this assessment tool has all been based on picture based assessments. Before this system can be utilised in a prospective study of in-vivo diagnosis it is necessary to demonstrate that the characteristics described can be reliably identified in-vivo.

Making an in-vivo assessment is fundamentally different to the examination of a picture of a lesion. During live colonoscopy it is necessary to make a rapid diagnosis. Polyps cannot always be positioned perfectly, and movement artifacts can distort assessments. This can be a problem for complex systems which rely on the examination of subtle changes.

This study aims to validate the N.A.C. classification developed in the last two chapters in-vivo on screening colonoscopy lists.
9.2: Methods

A pilot prospective study was performed to test the accuracy of the previously identified characteristics for the N.A.C. classification system for FICE in vivo. Patients were recruited from screening colonoscopy lists. Fujinon equipment was used. The endoscopes were EC-530 and EC-590 colonoscopes with an EPX 4400 processor. Optical magnification was not used. Patients had standard bowel preparation with three sachets of sodium picosulphate. For morning lists this was given at breakfast, lunchtime and in the evening on the day before the procedure. For afternoon lists two sachets of sodium picosulphate were administered on the morning of the procedure, given at 6:00 and midday.

Polyps were identified and assessed during withdrawal. The morphology and size was noted and the polyps were cleaned with 10-20ml water prior to examination. To assess the characteristics of the polyps with both white light and FICE, the image was frozen and the key structural elements previously identified in the picture study recorded prospectively on a dedicated proforma. This included vascularity with white light, and vascularity, vascular pattern and surface pattern assessment with FICE. Assessments were performed by a single endoscopist (GLW). All FICE assessments were performed using setting 4 (520nm, 500nm, 405nm). The lesions were then removed and sent for histopathological examination by an expert gastrointestinal pathologist.
No sample sizes were calculated, as this was a pilot study, and there was no previous data available to base such calculations upon. Categorical data was presented as frequencies and percentages.
9.3: Results from the in-vivo validation of N.A.C. for FICE

In total 111 polyps were assessed with 71 polyps from male patients and 40 from female patients. The median age of the patients was 65 (range 44-83) and the median size of the polyps was 5mm (range 1-20). There were 46 hyperplastic polyps, 64 adenomas and 1 cancer.

The vascularity with white light, and the vascularity, vascular patterns and surface patterns observed with FICE are shown in table 17. The results were similar to those obtained in the picture study, although the assessment of vascularity using white light appeared to be a less reliable predictor of histology than previously predicted. Increased vascularity seen with FICE was more predictive of adenomatous histology than the hyperaemia seen with white light (83% vs 63%).

The presence of faint vessels not following the crypts and an absent surface pattern were most predictive that a lesion was non-neoplastic. The presence of large non compact crypts was less predictive of hyperplastic (non-neoplastic) pathology, with 13% of adenomas having large non-compact crypts and 18% having an absent vascular pattern. It is possible that a very pale pericryptal pattern surrounding large crypts could be incorrectly identified as an absence of vascular pattern, which would explain these observations.
<table>
<thead>
<tr>
<th>Table 17</th>
<th>Hyperplastic (%)</th>
<th>Adenomas (%)</th>
<th>Cancers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WLI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale (Hypoaemic)</td>
<td>27/46</td>
<td>11/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>59%</td>
<td>17%</td>
<td>0%</td>
</tr>
<tr>
<td>Normal (Normoemic)</td>
<td>2/46</td>
<td>8/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>13%</td>
<td>0%</td>
</tr>
<tr>
<td>Dark (hyperaemic)</td>
<td>17/46</td>
<td>45/64</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>37%</td>
<td>70%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>FICE Vascularity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale pattern</td>
<td>31/46</td>
<td>11/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>67%</td>
<td>17%</td>
<td>0%</td>
</tr>
<tr>
<td>dark pattern</td>
<td>15/46</td>
<td>53/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>33%</td>
<td>83%</td>
<td>0%</td>
</tr>
<tr>
<td>Very dark pattern</td>
<td>0/46</td>
<td>0/64</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>FICE vascular patterns</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent vascular pattern</td>
<td>22/46</td>
<td>8/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>48%</td>
<td>13%</td>
<td>0%</td>
</tr>
<tr>
<td>Faint vessels not following crypts</td>
<td>16/46</td>
<td>1/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>35%</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>Regular Pericryptal pattern</td>
<td>8/46</td>
<td>55/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>17%</td>
<td>86%</td>
<td>0%</td>
</tr>
<tr>
<td>Dense, irregular pattern</td>
<td>0/46</td>
<td>0/64</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>FICE Surface pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No surface pattern</td>
<td>17/46</td>
<td>0/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>37%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Large, non compact crypt pattern</td>
<td>18/46</td>
<td>6/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>39%</td>
<td>9%</td>
<td>0%</td>
</tr>
<tr>
<td>Small, compact, regular pattern</td>
<td>8/46</td>
<td>56/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>17%</td>
<td>88%</td>
<td>0%</td>
</tr>
<tr>
<td>Disorganised, irregular pattern</td>
<td>0/46</td>
<td>0/64</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Cannot assess</td>
<td>3/46</td>
<td>2/64</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>7%</td>
<td>3%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Table 17: N.A.C. criteria for in-vivo FICE assessment. Data presented as frequency of occurrence of a particular characteristic for hyperplastic polyps, adenomas and cancers*
9.4: Conclusions

The proposed N.A.C. criteria for FICE can be visualized *in-vivo*. The distribution of patterns correlates closely with those found in the previous picture based study. Patterns were visible without optical magnification and are therefore suitable for use in a prospective cohort study of FICE as a diagnostic tool.

The last three studies have described the development and validation of a new polyp classification system, N.A.C. for FICE. This system is unique in that it is designed for use without optical magnification and does not necessitate a high resolution endoscope. This carries the advantage that it is applicable to standard Western screening colonoscopy lists where magnifying high definition endoscopes are not routinely available. By demonstrating that the classification system can be *utilised in-vivo* the evidence has been provided to justify an *in-vivo* diagnostic cohort study of the predictive value of FICE as a diagnostic tool for colonic polyps.

A limitation of this validation exercise is that it has not been able to subdivide the effects of using standard definition and high definition endoscopes. However, it is reassuring that whilst the images were noted to be clearer with a high definition endoscope the assessments were still possible with standard definition equipment. The number of cancers in the *in-vivo* assessment exercise was very low, with just one case found. However, the appearances were identical to those observed in the
picture study. For hyperplastic polyps and adenomas the results were very similar between the picture study and in-vivo study and therefore it is reasonable to expect that the true appearances for polyp cancers are those described in this study. The incidence of cancer in small polyps is very low and it is generally accepted that these are not the most difficult aspect of in-vivo diagnosis. It is in small lesions that *in-vivo* diagnosis is likely to have the greatest potential impact. Therefore whilst this is a limitation the validation, is still robust enough to justify an *in-vivo* diagnostic trial.

The findings of this study also validate the use of a picture study in the development and validation of future studies of endoscopic devices for lesion characterisation. The findings from the picture study described in chapter 8 were a good surrogate marker of the findings seen *in-vivo*, which opens up many possibilities for future research.
Chapter 10

FICE and Indigo carmine in Neoplasia Diagnosis During Colonoscopy

Prospective in-vivo study
Polyps are a common finding during colonoscopy. It is current practice to remove these lesions, as some have the potential to develop into cancer. However, not all polyps are the same. Hyperplastic polyps have negligible malignant potential, especially when small (<10mm) and in the left colon. These polyps account for one third of all small polyps (13). Adenomas can progress to cancer and require resection (14).

The management pathway for polyps >10mm is simple as they are either likely to be adenomas which need removal, or cancers which need careful consideration for endoscopic or surgical resection. There is a possibility of large polyps being hyperplastic or serrated adenomas which need removal as well. The management of polyps <10mm in size is not well established. It has been traditionally felt that it is difficult to differentiate hyperplastic polyps from adenomas by the endoscopist. For this reason all polyps are removed. However, polypectomy is associated with significant risks (15) and results in an immediate cost in processing the samples, polypectomy equipment and time. If an endoscopist could make a confident in-vivo diagnosis then hyperplastic polyps <10mm could be left in-situ or removed but not retrieved to reduce pathology costs, as suggested in the DISCARD study (47). This has been recognised as important by the American Society for Gastrointestinal Endoscopy (ASGE), who have recently launched an initiative to define the standards which have to be met for any technology to be used for in-vivo diagnosis as a replacement for conventional histological examination (10). This PIVI programme (preservation and incorporation of valuable endoscopic innovations) has developed
new paradigms for colonoscopic management of diminutive polyps and have set mandatory standards for these paradigms.

The standard for a ‘resect and discard’ strategy for polyps<5mm in size states that an endoscopic technology can be accepted if it can set the endoscopic surveillance interval with \( \geq 90\% \) accuracy, as compared to standard histology for polyps of any size. A second standard has been set for a technology to be used to ‘leave’ suspected rectosigmoid hyperplastic polyps <5mm in place without resecting them. This standard demands that technology should provide \( \geq 90\% \) negative predictive value (NPV) for adenomas. These standards are very important and timely as most of the available technology will now be tested against them.

Japanese studies have involved assessment by examination of polyp surface patterns (Kudo’s pit patterns) and have achieved excellent results (126). This was originally described using vital staining with crystal violet and magnifying endoscopes which is cumbersome and time consuming. This has not been routinely adopted in Western practice and is now only used in Japan for the diagnosis of cancer invasion depth. There is also concern about the safety of crystal violet (18). Indigo carmine has been used with optical magnification for \textit{in-vivo} diagnosis of colonic polyps (20) (21). This does not bond to or react with human tissue in any way, as it simply sits on the surface of tissues, highlighting surface patterns, and has no safety concerns.

There has been interest in electronic imaging for the \textit{in-vivo} diagnosis of colonic polyps. Olympus, Fujinon and Pentax have introduced vascular and surface enhancement features into their endoscopes. Narrow band imaging (NBI) from Olympus is an optical filter technology which enhances the visibility of vessels on the
mucosal surface (123). A recent study has shown the utility of NBI in differentiating neoplastic from non neoplastic lesions <10mm in size, with an accuracy of 93% (47).

Fujinon has developed a post processor technology called Flexible Spectral Imaging Colour Enhancement (FICE). White light endoscopy captures reflected light in a wide spectrum (400nm-700nm) with a CCD device. FICE processes this conventional image into a spectral image composed of rays of specific wavelengths and displays them in real time. The system has ten preset wavelength patterns and the endoscopist can manually select the best mode to assess the polyp. This enables an endoscopist to assess the subtle structural and vascular patterns on the surface of polyps in greater detail than can be achieved with white light alone. This could have implications for bowel cancer screening programmes, where in-vivo diagnosis could be used to reduce histopathology related costs (68). However, most of the studies on FICE have included large polyps or been based on the assessment of pictures (26) (54) (35) (56). There have not been any studies investigating the in-vivo diagnostic capabilities of FICE in a Bowel Cancer Screening population, and only one study which has involved an in-vivo assessment without optical magnification on lesions<10mm in size (26).

10.2: Aims

This study aims to assess the accuracy of in-vivo diagnosis of colorectal polyps<10mm in the U.K. Bowel Cancer Screening Programme (BCSP), using FICE as an electronic imaging tool without optical magnification. It also aims to establish
the advantage of IC dye spray (without magnification) when used as an additional technique after evaluation of polyps with FICE.

10.3: Methods

The study has ethical approval (REC No. 09/H0501/94) and was registered with the European Clinical Trials Database Eudra CT 2009-016742-10 and with Clinical trials.gov NCT01182623.

This was a prospective single blinded observational study performed on consecutive asymptomatic patients within the U.K. Bowel Cancer Screening Programme (BCSP). All patients had a positive faecal occult blood test prior to colonoscopy. Exclusion criteria were: a diagnosis of a familial polyp syndrome, a diagnosis of inflammatory bowel disease, poor bowel preparation or melanosis coli, which alters surface pattern visualisation and could influence polyp assessment. No other exclusion criteria were permitted to preclude selection bias. All consecutive polyps were prospectively assessed. As the aim of the study was to establish the effectiveness of FICE and IC as diagnostic tools, it was important to avoid the potential confounding factor of multiple endoscopists with differing skills in in-vivo diagnosis. Therefore all assessments were performed by a single endoscopist (PB) with expertise in in-vivo diagnosis of polyps for over 8 years. The endoscopies were performed using EC-530 and EC-590 Fujinon colonoscopes and EPX 4400 processor without optical magnification. A flat screen Sony 24 inch WUXGA LCD display was used (LMD-2450 MD) with a 1125 x 1080 resolution. Connections between the EPX4400 processor and monitor were via a digital video interface (DVI) connector. Polyps were cleaned of any debris using 10-20ml of water with 2ml of 10% simethicone before
assessment. Care was taken not to traumatise the surface of the lesions. Size of polyps was determined endoscopically using the biopsy forceps (measuring 8mm with open Jaws). Polyps <10mm in size were assessed using white light (WLI) followed by FICE. Indigocarmine dye spray was then applied to the polyp. Before each change in imaging modality, the predicted diagnosis was recorded by a member of the research team for that modality, with no possibility for revisiting the prediction once made. Based on the pilot work performed prior to this study the FICE settings used for this work was preset 4 (R:520nm G:500nm B: 405nm). Indigo carmine (IC) dye spray was made up in a concentration of 0.2% and 5ml was passed down the biopsy channel via a 20ml syringe.

WLI, FICE and chromoendoscopy were used to assess the polyp morphology, polyp colour, density of vessels, vessel pattern and surface pattern using the N.A.C. criteria developed and described in the previous chapters.

Finally the polyps were removed and sent for histological analysis by a consultant histopathologist, who was blinded to the diagnosis made by the endoscopist. All pathology reporting was performed by an accredited Colon Cancer Screening pathologist. Serrated adenomas were treated as neoplastic for the purpose of calculating accuracy of in-vivo histology prediction (i.e. the in-vivo diagnosis was considered to be incorrect if the endoscopist called a serrated adenoma hyperplastic).
10.4: Statistical analysis

The study was prospectively powered. The assumptions were made that 40% of polyps found are hyperplastic, that the true sensitivity for neoplasia with both FICE and indigocarmine would lie between 85-95%, and that the true specificity with FICE and indigocarmine lies between 75-90%. It was felt that analysis should be performed on a per lesion basis. With 80% power, assuming a 5% significance level and phi coefficient of 0.2, 150 polyps would need to be assessed to achieve statistical significance. To demonstrate a 10% difference in the accuracy between FICE and indigo carmine, 200 polyps would need to be assessed to produce significant results. IBM SPSS-18 for Windows was used for statistical calculations. Accuracy, sensitivity and specificity of in-vivo diagnoses using WLI, FICE and indigo carmine was compared to histology and calculated with 95% confidence limits. The comparisons between white light, FICE and IC are ‘within subject’ on a per lesion basis. Therefore the McNemar’s test for repeated measurements was used.
10.5: Rescope intervals

A prediction of rescope interval was made using FICE and IC dye spray assisted diagnosis, according to British Society of Gastroenterology (BSG) guidelines (127) and the American Society of Gastrointestinal Endoscopy (ASGE) guidelines (128). This was compared to the actual surveillance interval based on the true histological diagnosis.

10.6: Financial analysis

Financial costs were identified for tissue fixation, processing, staining and pathology reporting. Costs were then calculated per polyp for the whole cohort. Cost estimates were then calculated for various clinical approaches, on a per patient and per cohort basis.
10.7: Results

In total 138 patients underwent colonoscopy for bowel cancer screening from September 2009 to September 2010, of which 124 patients met the inclusion criteria and consented to enter the study. 89 were found to have polyps <10mm in size. Either standard definition (SD) or high definition (HD) endoscopes were randomly allocated by nursing staff on a basis of availability, with the endoscopist blind to the resolution of endoscope allocated. In total 232 consecutive polyps <10mm were assessed. See figure 26. The size, location and morphology of polyps are shown in table 18.
Enrollment

Assessed for eligibility (n=138)
- Excluded (n=14)
  - Not meeting inclusion criteria (n=6)
  - Declined to participate (n=8)
  - Other reasons (n=0)

Randomized allocation of endoscope (SD or HD)

Allocation

Allocated to SD endoscopy (n=58)
- Patients found to have polyps<10mm (n=40)
- Number of polyps<10mm found (n=89)

Allocated to HD intervention (n=66)
- Patients found to have polyps<10mm (n=49)
- No. of polyps<10mm found (n=143)

Follow-Up

Lost to follow-up (n=0)

No. polyps<10mm analysed (n=89)
- Polyps<10mm excluded from analysis (n=0)

Analysis

Lost to follow-up (n=0)

No. polyps<10mm analysed (n=143)
- Polyps<10mm excluded from analysis (n=0)

Total No. polyps analysed (n=232)
Total No. of polyps excluded (n=0)

Figure 26: Flow chart for patient recruitment
<table>
<thead>
<tr>
<th>Table 18: Demographics of patient population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Patients</strong></td>
</tr>
<tr>
<td>Total patients</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Mean age</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>Polyps</td>
</tr>
<tr>
<td>Mean size (mm)</td>
</tr>
<tr>
<td>(range)</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>Right sided</td>
</tr>
<tr>
<td>Left sided</td>
</tr>
<tr>
<td>Morphology</td>
</tr>
<tr>
<td>a) Pedunculated</td>
</tr>
<tr>
<td>b) Non pedunculated (flat)</td>
</tr>
<tr>
<td>i) is</td>
</tr>
<tr>
<td>ii) iiia</td>
</tr>
<tr>
<td>iii) iiib</td>
</tr>
<tr>
<td>iv) iiia+iib</td>
</tr>
</tbody>
</table>
10.8: True histological diagnosis

The breakdown of the histology of the cohort showed that 77/232 (33%) were hyperplastic and 155/232 (67%) were neoplastic. We defined neoplasia as low risk as adenomas with no villous component and no high grade dysplasia. There were 121 tubular adenomas with low grade dysplasia. High risk neoplasia was defined as polyps with a villous component, HGD or cancer. There were 34 high risk lesions (31 TVA+LGD, 1 TVA+HGD and 2 cancers).

10.9: In-vivo diagnosis

The accuracy, sensitivity and specificities are shown in table 19. There was a significant difference in the accuracy and sensitivity between WLI, FICE and IC. FICE significantly (P<0.002) improved the sensitivity to 88% as compared to 75% with WLI. IC improved the sensitivity even further to 94% (P<0.0001). The additional use of IC showed a numerical improvement over FICE alone, but this difference failed to reach statistical significance (P=0.07).
<table>
<thead>
<tr>
<th></th>
<th>Image modality</th>
<th>P- Values, pair-wise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WLI</td>
<td>FICE</td>
</tr>
<tr>
<td>Accuracy (95% C.I.)</td>
<td>165/232</td>
<td>200/232</td>
</tr>
<tr>
<td>Sensitivity (95% C.I.)</td>
<td>116/155</td>
<td>137/155</td>
</tr>
<tr>
<td>Specificity (95% C.I.)</td>
<td>49/77</td>
<td>63/77</td>
</tr>
<tr>
<td>PPV (95% C.I.)</td>
<td>116/144</td>
<td>137/151</td>
</tr>
<tr>
<td>NPV (95% C.I.)</td>
<td>49/88</td>
<td>63/81</td>
</tr>
</tbody>
</table>

Table 19: in-vivo diagnosis of neoplasia in polyps<10mm by endoscopic modality. WLI=white light, IC=indigo carmine, PPV=Positive predictive value, NPV=negative predictive value.

There were two cancers in this cohort of polyps and both of these were correctly diagnosed by FICE and IC dye spray. One was diagnosed as an adenoma under white light. See table 20.
<table>
<thead>
<tr>
<th></th>
<th>WLI</th>
<th>FICE</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplastic</td>
<td>49/77</td>
<td>63/77</td>
<td>65/77</td>
</tr>
<tr>
<td></td>
<td>64%</td>
<td>82%</td>
<td>84%</td>
</tr>
<tr>
<td>Low risk Adenoma (tubular adenoma)</td>
<td>89/121</td>
<td>106/121</td>
<td>114/121</td>
</tr>
<tr>
<td></td>
<td>74%</td>
<td>88%</td>
<td>94%</td>
</tr>
<tr>
<td>High risk neoplasia (TVA+LGD, TVA+HGD + Cancer)</td>
<td>27/34</td>
<td>31/34</td>
<td>32/34</td>
</tr>
<tr>
<td></td>
<td>79%</td>
<td>91%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Table 20: Accuracy of in-vivo diagnosis by true histology of polyp. WLI= white light, IC=indigo carmine, TVA=tubule villous adenoma, LGD= Low grade dysplasia, HGD=high grade dysplasia

10.10: Accuracy by polyp size<5mm

In this cohort there were 155/232 polyps <5mm in size (diminuitive polyps). A sub-group analysis of accuracy was performed for 155 lesions <5mm using WLI, FICE and IC. This is shown in table 21. Indigocarmine after FICE was significantly more sensitive for neoplasia than FICE alone (P=0.037). The negative predictive value for indigocarmine after FICE was numerically superior to FICE assessment (90% vs 78%). However, this difference just failed to reach statistical significance (P=0.066).
<table>
<thead>
<tr>
<th></th>
<th>WLI</th>
<th>FICE</th>
<th>IC</th>
<th>WLI vs FICE</th>
<th>WLI vs IC</th>
<th>FICE vs IC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>104/155 (66.5%)</td>
<td>129/155 (83%)</td>
<td>139/155 (90%)</td>
<td>0.001</td>
<td>P=0.000</td>
<td>P=0.097</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>59/90 (66%)</td>
<td>75/90 (83%)</td>
<td>84/90 (93%)</td>
<td>P=0.066</td>
<td>P=0.000</td>
<td>P=0.037</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>45/65 (69%)</td>
<td>54/65 (83%)</td>
<td>55/65 (85%)</td>
<td>P=0.064</td>
<td>P=0.061</td>
<td>P=0.812</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>59/79 (75%)</td>
<td>75/86 (87%)</td>
<td>84/94 (89%)</td>
<td>P=0.04</td>
<td>P=0.011</td>
<td>P=0.653</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>45/76 (59%)</td>
<td>54/69 (78%)</td>
<td>55/61 (90%)</td>
<td>P=0.014</td>
<td>P=0.000</td>
<td>P=0.066</td>
</tr>
</tbody>
</table>

Table 21: Accuracy of diagnosis polyps<5mm by endoscopic modality. WLI=white light, IC=indigo carmine, PPV=Positive predictive value, NPV=negative predictive value.

### 10.11: Cost effectiveness of *in-vivo* diagnosis

The UK (NHS) costs for pathology was utilised, which equates to a cost of £58.90 ($94.30) per cassette of tissue. This is in keeping with the standards laid out in the Lord Carter independent review into pathology services and is competitive with other similar laboratories across Europe and North America (9). The cost for histological assessment of the cohort using three different protocols was calculated.

A) **Traditional protocol:** Retrieve and send all polyps<10mm for histological assessment

B) **Portsmouth protocol:** Retrieve and send suspected adenomas and cancers<10mm but not hyperplastic polyps<10mm for histological assessment
C) **Futuristic protocol:** Discard all adenomas and hyperplastic polyps <10mm and only send suspected cancers for histological examination

**10.12: Implications for the study cohort**

The potential costs for each of these strategies are shown in table 6. Based on our data a potential cost saving of £109 ($174.51) per person undergoing screening colonoscopy could be made.

**10.13: Implications for the National Bowel Cancer Screening Programme**

Within the national BCSP 12153 colonoscopies are performed per annum with 11,619 benign polyps <10mm removed per year. Using this data and taking the same approaches outlined above we have calculated that *in-vivo* diagnosis could represent a potential saving of £678,252.81 ($1,085,900) per annum for histology related costs, or £55 ($88.03) per person. This is shown in table 22.
<table>
<thead>
<tr>
<th></th>
<th>Traditional protocol (A)</th>
<th>Portsmouth Protocol (B)</th>
<th>Futuristic protocol (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cohort</td>
<td>£13,644.80 ($21,845.66)</td>
<td>£9247.30 ($14,805.60)</td>
<td>£176.70 ($282.90)</td>
</tr>
<tr>
<td>Cost saving over</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>traditional protocol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A)</td>
<td>NA</td>
<td>A-B</td>
<td>A-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>£4397.5 ($7040.51)</td>
<td>£13,468.1 ($21,552.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.5 fold)</td>
<td>(77 fold)</td>
</tr>
<tr>
<td>Bowel Cancer screening</td>
<td>£684,319.51 ($1,095,090)</td>
<td>£318,766.8 ($510,766.80)</td>
<td>£6066.7 ($9711.38)</td>
</tr>
<tr>
<td>programme U.K.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost saving over</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>traditional protocol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A)</td>
<td>NA</td>
<td>A-B</td>
<td>A-C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>£365,552.71 ($585,165.20)</td>
<td>£678,252.81 ($1,085,900)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.2 fold)</td>
<td>(113 fold)</td>
</tr>
</tbody>
</table>

Table 22: Evaluation of histopathology costs within the study population

There were 39 adenomatous polyps incorrectly diagnosed by the endoscopist using white light, 18 using FICE and 9 with IC. Taking the cost of histological examination of all polyps in the cohort as £13,644.80 ($21,845.66) the additional expense incurred to correctly diagnose these by sending all polyps for histological examination in place of endoscopic assessment was calculated. For white light this cost was £350 ($560.27). When using FICE this increased to £758 ($1213.38) and if IC assessments were used the additional pathology cost increased to £1516 ($2,426.77)
A predicted rescope interval was estimated for the cohort. 20/89 patients had additional larger polyps which would have influenced the rescope interval and were excluded. In the remaining 69 patients WLI correctly predicted the rescope interval for 57/69 (83% CI: 72%-90%) of patients using BSG guidelines and 58/69 (84%, CI: 74%-91%) using ASGE guidelines.

FICE correctly predicted rescope intervals for 67/69 (97%, CI: 89% - 100%) of patients using BSG and ASGE guidelines.

IC correctly predicted rescope intervals for 67/69 (97%) of patients (CI: 89% - 100%) using BSG guidelines and 68/69 (99%, CI: 91% - 100%) using ASGE guidelines.
10.15: Discussion

This is the first study reporting outcomes of FICE followed by IC in the assessment of polyps<10mm in the BCSP. It shows that WLI endoscopy can accurately predict *in-vivo* histology in 71% of cases. FICE improves this to 86% and the additional use of IC increases this further to 91%. With FICE it would be possible to set the rescope interval accurately in 97% of patients and with IC in 98% of cases. This demonstrates that both FICE and indigo carmine dye spray, when used after FICE, are excellent tools for making an *in-vivo* histological diagnosis for small polyps<10mm and fulfil the standards outlined in the ASGE PIVI for adopting a resect and discard policy (10). It is important to diagnose all adenomas accurately and this study has demonstrated that FICE and IC are significantly superior in making that diagnosis, compared to WLI. There may be some additional benefit from dye spray when assessing difficult to call lesions. These results are comparable to those reported in a large Japanese series. In the Japanese series magnification endoscopy was used to achieve a 98% sensitivity for adenomas but the specificity was poor at 52% (20).

No statistically significant difference was seen between FICE and IC when assessing lesions<10mm. However, for lesions <5mm, the additional use of indigo carmine resulted in an improvement in the sensitivity for neoplasia (93% vs 83%) which was significant (P=0.037). This resulted in the negative predictive value improving from 78% to 90%. This is important as it is one of the key standards outlined in the ASGE PIVI for *in-vivo* diagnosis (10). Whilst FICE is adequate for a resect and discard policy, it is inadequate for use as a technology to guide the decision to leave
suspected rectosigmoid hyperplastic polyps <5mm in size in place (without resection). The technology would need to provide a negative predictive value greater than 90% for adenomatous histology when used for this purpose, which was not demonstrated in this study. In contrast indigocarmine dye spray demonstrated a 90% NPV and is probably adequate. This information provides important guidance for the endoscopist in how to use both of these approaches for in-vivo diagnosis.

Colorectal cancer is a major cause of mortality worldwide, and removal of adenomas has been shown to reduce mortality from the disease (9). This has led to the development of colorectal cancer screening programmes. These need to be delivered to a mass population, and as such are expensive. In a period of economic austerity it is necessary to control costs if such programmes are to be sustainable.

The U.K. national BCSP has shown that the majority of polyps detected in the programme are <10mm (122). Accurate in-vivo diagnosis, as shown in this study, can reduce the need for resection and pathological analysis of all of these polyps. The data has shown that this could lead to a potential cost saving of £678,253 ($1,085,900) within the U.K. BCSP.

A recent study based on Markov modelling suggested that adoption of a resect and discard policy within a U.S. screening population (using Narrow Band Imaging for in-vivo assessments) could result in savings of $25 per person, resulting in an annual undiscounted saving of $33 million, without any meaningful impact on screening
efficacy (68). The results from our study support this. Using both this data and the United Kingdom Bowel Cancer Screening data would suggest that the actual savings could be greater than those predicted by the Markov model. However, it should be noted that the U.K. programme employs faecal occult blood screening prior to colonoscopy, and therefore the incidence of polyps found at colonoscopy is high, potentially further increasing the pathology costs, and potential cost saving from in-vivo diagnosis, for each procedure.

Some work has been performed with indigo carmine in predicting polyp histology, with sensitivities and specificities for neoplasia between 82% to 98% and 64% to 95%, respectively (20) (22) (28) (129). In all of these studies magnifying endoscopes were used, which is impractical in daily practice in Western countries. It is therefore difficult to draw any useful conclusions from these studies about the role of IC in the management of polyps<10mm in daily Western practice with non magnifying scopes. One study compared indigo carmine with magnification to standard colonoscopy and found that magnification increased the accuracy for polyp differentiation from 84% to 96% (21). This data shows a very high accuracy and sensitivity for in-vivo diagnosis of polyps using indigo carmine dye spray after evaluation of polyps with FICE.

In a recent study five endoscopists assessed 144 pictures of 19 polyps to establish the diagnostic accuracy of WLI, FICE and indigo carmine in making a histology prediction for polyps <10mm in size. The results were disappointing, with a mean diagnostic accuracy without magnification for WLI of 57%, FICE of 58.9% and IC of 70.5% (55). However, there was considerable variation in the results obtained between the five endoscopists, and the number of lesions from which the pictures
were taken was small. These results were different to an earlier large cohort study of 150 flat lesions under 20mm in size (54). This prospective series showed that using FICE, with low and high magnification, a sensitivity for neoplasia of 89.9% and 96.6%, specificity of 73.8% and 80.3%, and diagnostic accuracy of 83% and 90% was achieved. Results were comparable to that of conventional chromoendoscopy. However, this study was based on the assessment of pictures rather than a true in-vivo assessment and polyps of up to 20mm in size were included. In practice polyps >10mm always need removal, and differentiation of adenomas from hyperplastic lesions in this group is merely an academic exercise. One can also argue that in-vivo diagnosis is a lot easier for polyps >10mm, although the sensitivity in this study is similar to the results presented in this chapter. A further prospective randomised study was conducted by the same group (26), with the primary aim to investigate the impact of FICE on adenoma detection rates (ADR). It showed that FICE did not improve ADR. However, FICE and indigocarmine were both able to differentiate adenomas from hyperplastic polyps<10mm in size. The findings demonstrated a sensitivity of 93% and specificity of 61.2% in differentiating adenomas from hyperplastic polyps with FICE, comparable but not superior to indigocarmine (90.4%), with no statistically significant difference between the two techniques (p=0.44). The study described in this chapter compliments the published literature by demonstrating a high clinical accuracy of FICE in the assessment of small polyps <10mm in a Bowel Cancer Screening population. We have also shown a small insignificant benefit of IC dye spray when used after FICE assessment. This is a very pragmatic design and clearly identifies the role of FICE and IC in daily practice.

The estimated rescope interval is important in the management of patients found to have adenomatous polyps. Current practice involves resection or biopsy of all
polyps. Surveillance intervals are predicted on the basis of number of polyps and the interval is then re-adjusted after the true histology becomes available after 1-2 weeks. This results in extra work and a delay in setting the accurate surveillance interval. This study has shown that this can be correctly set in 97% of cases with FICE and IC at the time of endoscopy before the patient leaves the department.

The UK Bowel Cancer Screening Programme is generating a large volume of samples for already overstretched pathology departments. A small study has suggested that community pathologists correctly identified 91% polyp cancers, 94% adenomas and 75% of hyperplastic polyps (130). As a result of this, all polyps within the programme have to be reported on by an expert gastrointestinal pathologist. This is in many ways unfortunate as it diverts a significant proportion of their time away from performing other tasks, despite these lesions having very low malignant potential. During the same time period there has been a growth in the technique of endoscopic mucosal resection, resulting in the generation of complex multi piece specimens for expert pathologists to report. These are complex reports requiring a lot of expertise and time. It is not unreasonable to question whether the practice of sending all small polyps for histological assessment can be justified, when there are now accurate methods for endoscopists to use to assess polyps in-vivo with minimal impact on clinical care, especially when the pathologist’s time is needed so desperately for more complex tasks.

One of the limitations of this study could be the use of a single centre and single endoscopist. However, the aim was to demonstrate the potential accuracy of FICE
as an assessment tool and therefore it was necessary to exclude the potentially confounding influence of multiple endoscopists with differing levels of ability in \textit{in-vivo} diagnosis. However, similar results have been published by other groups using multiple endoscopists and other techniques such as Narrow Band Imaging. As such there is no reason to believe that the technique could not be learnt and applied by all screening endoscopists. There will always be a need for training in these techniques but the learning curve is relatively short (131). One of the other limitations of this study is that the design does not allow the direct comparison of indigo carmine with FICE, as one could argue that the IC assessment was positively biased by the WLI and FICE assessment performed prior to it. Whilst this remains a possibility, the data addresses the more important question of what IC adds after FICE assessment. From a practical point of view this is the question that most endoscopists want answered. The study has a pragmatic design which is applicable to daily clinical practice when an endoscopist will use WLI followed by FICE in an attempt to make an \textit{in-vivo} diagnosis. Most endoscopists will resort to IC spray following electronic imaging if they are uncertain of the diagnosis and this data shows a small but statistically insignificant incremental benefit of IC used after FICE for polyps<10mm, but a significant benefit when assessing diminuitive polyps<5mm.

Surveillance intervals can now be determined based on \textit{in-vivo} diagnosis without waiting for histology, and the practice of routinely sending all polyps for histological examination is difficult to justify. This data proves the feasibility and applicability of \textit{in-vivo} diagnosis of polyps<10mm in size and that it is cost effective in a screening population. It calls for a multicentre study to prove that it can be generalised on a national basis.
10.16: Implications for clinical practice

Surveillance intervals can now be determined based on *in-vivo* diagnosis without waiting for histology, and the practice of routinely sending all polyps for histological examination is difficult to justify. This data proves the feasibility and applicability of *in-vivo* diagnosis of polyps<10mm in size and it is cost effective in a screening population. Bowel cancer screening in the United Kingdom is performed by endoscopists who undergo specialist training and examination prior to practice. This is therefore an ideal environment for such techniques to be implemented safely. It would also provide an ideal cohort of endoscopists to test and validate training tools on as there would be mandatory assessment involved. If this proves successful then it could potentially be expanded to a wider population.

*In-vivo* diagnosis is of relevance beyond simple cost savings. As discussed previously lesions can be poorly managed if an inappropriate assumption is made by the examining endoscopist. In particular small cancers can be wrongly removed (and location not marked) if no attempt at diagnosis by the endoscopist is made. Likewise, some small polyps can be located in very challenging positions, and removal can prove risky and challenging. For hyperplastic polyps a point can be reached where risk outweighs benefits. In these cases a high degree of certainty regarding lesion histology *in-vivo* would be beneficial in guiding the endoscopists decision in whether to proceed with resection. Therefore all endoscopists could benefit from examining lesions with these techniques even if they do not feel confident in adopting a resect and discard policy on a routine basis.
This study provides the evidence base to justify a multicentre study to determine whether *in-vivo* diagnosis could be generalised on a national basis. This is required to address the key limitation of this study that it is single centre and single endoscopist based. For the data to be generalisable it should ideally be expanded to incorporate *in-vivo* diagnosis with techniques utilising Narrow Band Imaging, i-scan and indigo carmine chromoendoscopy as a single modality of assessment. This would incorporate a much wider range of equipment scenarios where *in-vivo* diagnosis would be possible making *in-vivo* diagnosis a more attractive option. Such a study will require the training of many endoscopists in the technique of *in-vivo* diagnosis. Research is therefore needed into the development of effective training tools before such a study can be conducted. Ideally these tools should be easy to use with a short learning curve.
10.18: Conclusions

This study demonstrates that FICE is an excellent tool for the evaluation of colonic polyps<10mm. There is a trend towards a further improvement in accuracy from indigo carmine chromoendoscopy after FICE assessment for polyps<10mm in size, and a significant improvement in sensitivity for neoplasia in polyps<5mm in size. Our data suggests that an in-vivo diagnosis, in place of conventional histological analysis, would have a negligible impact on the rescope interval of the patients involved and could reduce costs and the pathology workload incurred from screening colonoscopy considerably. It has the potential to replace histological assessment of polyps<10mm in size.
Chapter 11

High definition vs Standard definition endoscopy in the assessment of colonic neoplasia
11.1: Introduction

There have been considerable developments in endoscopic technology in recent years. High definition endoscopy is now being offered by all of the major endoscope manufacturers, and it has been predicted that the uptake of high definition equipment will be a major growth area over the next 5 years (124). Modern colonoscopes have charge coupled device (CCD) pixel densities of up to 1.3 million pixels, compared to standard definition endoscopes which have CCD resolutions of around 410,000 pixels. Whilst precise definitions vary between manufacturers, it is generally accepted that to define a system as high definition requires a high resolution charge coupled device (CCD) connected to a processor capable of outputting a digital high resolution signal to a display of at least 1024x768 pixels. However, there is remarkably little known regarding the potential value of high definition over standard definition equipment.

Polyps are a common finding during colonoscopy, and there have been numerous publications examining the role of the endoscopist in performing an in-vivo diagnostic assessment of these lesions in place of traditional histological analysis (47) (26). However, all of these studies have been performed using high definition equipment (26) (54) (56) and usually with optical magnification (54) (56). Unfortunately, not all endoscopy units are equipped with enough high definition endoscopes or with magnifying endoscopes.

If endoscopists start making decisions based on in-vivo diagnosis of polyps<10mm in size then a substantial cost saving could be achieved related to histopathology (68).
However, for this strategy to be adopted, endoscopists need to feel confident that the equipment available is fit for this purpose. The recent ASGE PIVI (preservation and incorporation of valuable endoscopic innovations) has attempted to address this issue, by defining the standards a device needs to meet if it is to be used for in-vivo diagnosis (10). In order for polyps <5mm in size to be resected and discarded without pathologic assessment, endoscopic technology should provide a 90% agreement in assignment of post-polypectomy surveillance intervals, when compared to decisions based on pathological assessment of all identified polyps. In order to leave rectosigmoid hyperplastic polyps <5mm without resection, the technology should provide a negative predictive value for adenomatous histology greater than 90%. It has been difficult to achieve such high standards with standard endoscopes but one wonders if high definition scopes will help achieve this.

Acquiring high definition (HD) endoscopes represents a significant capital investment and their clinical value remains uncertain. In the current era of austerity, it is unclear whether the expense of upgrading existing equipment can be justified. This study aims to compare the accuracy of standard and high definition Fujnon colonoscopes in the diagnosis of neoplastic polyps<10mm using Flexible Spectral Imaging Colour Enhancement (FICE) without optical magnification and indigocarmine dye spray.
11.2: Methods

The study has ethical approval (REC No. 09/H0501/94) and was registered with the European Clinical Trials Database (Eudra CT 2009-016742-10) and with (Clinical Trials.gov NCT01182623).

This study was a retrospective analysis of the data collected in the study described in the previous chapter, where consecutive polyps<10mm in size were assessed by a single endoscopist (PB) who was trained and experienced in in-vivo diagnostic methods. Patients were all referrals for screening colonoscopy on a standard Bowel Cancer Screening list, where a mix of standard and high definition colonoscopes were routinely used. Exclusion criteria were; a diagnosis of inflammatory bowel disease, familial polyp syndromes or poor bowel preparation, all of which could influence surface pattern assessment. The endoscopies were performed using Fujinon colonoscopes and an EPX 4400 processor. The endoscope was allocated randomly by the nursing staff on a basis of availability and was not influenced by the endoscopist who was blinded to the resolution of the colonoscope being used. This was not therefore a randomised controlled trial, as a prospective randomization was not performed prior to the study commencing. However, allocation was random, with no influence from the research team. Each colonoscope had a unique number from which its resolution could be identified. This was not unblinded until the end of the study.
The colonoscopes were equipped with either a standard definition CCD (SD) EC-530 410,000 pixel, or a high definition Super CCD (HD). This included the EC-530 or EC-590-zw 650,000 pixel colonoscopes. Procedures where polyps were examined with SD colonoscopes were grouped as group A and procedures where polyps were examined with HD colonoscopes were grouped as group B. Optical magnification was not used in either of the groups. For each procedure this was prospectively recorded. A flat screen Sony 24 inch WUXGA LCD display was used (LMD-2450 MD) with a 1125 x 1080 resolution, connected to the EPX4400 processor via a digital video interface (DVI) connector. Therefore, the only component of the system where the resolution was being changed was the CCD in the colonoscope. Polyps were initially identified with white light endoscopy. They were cleaned of debris prior to assessment using 10-20ml of water with 2ml of 10% simethicone. Care was taken not to traumatise the surface of the lesions. The size of the polyps was determined endoscopically using the open jaws of the biopsy forceps measuring 7mm in diameter. Polyps <10mm were assessed using WLI and FICE and the diagnosis made by the endoscopist (neoplastic vs non neoplastic) was recorded for each modality of imaging. Finally the polyp was assessed using Indigo carmine (IC) dye spray, made up in a concentration of 0.2% and 5mls was passed down the biopsy channel via a 20ml syringe. Again, the diagnosis made by the endoscopist was prospectively recorded. The maximum time allocated for assessment with each modality was 30 seconds. See figure 27.
11.3: Endoscopic assessment tool

Assessment of the polyp morphology, polyp colour, density of vessels, vessel pattern and surface pattern was performed. Based on the pilot work described in chapters 7, 8 and 9, FICE setting 4 (R:520nm G:500nm B: 405nm) was used for all of the assessments. Vascular patterns were examined with FICE using the NAC classification system described in the previous chapters. Finally, the polyps were removed and sent for histological analysis by a consultant histopathologist, who was blinded to the diagnosis made by the endoscopist. All pathology reporting was done
by an accredited Colon Cancer Screening pathologist. For the purposes of analysis, serrated adenomas were defined as neoplastic lesions, and therefore, if the endoscopist called such polyps as hyperplastic, this would represent an incorrect diagnosis. Patient data was collected using a dedicated form.

11.4: Statistical analysis

For the comparison of SD versus HD endoscopy a power calculation was performed. This was based on the following assumptions; that 70% of the polyps found were neoplastic, that the true sensitivity for neoplasia with FICE and indigo carmine using HD endoscopes would be between 90-99%, and that the true sensitivity for FICE and indigo carmine using SD endoscopes would be between 80-90%. To demonstrate a 15% absolute difference in the accuracy for neoplasia between HD and SD endoscopy with 80% power (assuming a 5% significance level and phi coefficient of 0.2), a total of 152 neoplastic polyps would need to be assessed, with 76 in each group, requiring a total sample of 218 polyps to produce significant results. IBM SPSS-18 for Windows was used for statistical calculations and analysis was performed on a per lesion basis. McNemar's test was used for the pairwise comparisons. The accuracy for correct diagnosis of neoplasia using WLI, FICE and indigo carmine, for both SD and HD endoscopes, was compared to histology and 95% confidence intervals calculated.
11.5: Study population

In total 124 patients underwent colonoscopy for bowel cancer screening.

Group A (SD): 58 patients, 40 of whom were found to have polyps

Group B (HD): 66 patients, 49 of whom were found to have polyps

In Group A 89 polyps <10mm in size were found. In group B 143 polyps<10mm in size were found. The groups were comparable in terms of age, gender, size, location and morphology of polyps, as shown in table 23.
<table>
<thead>
<tr>
<th></th>
<th>Group A (SD)</th>
<th>Group B (HD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>40</td>
<td>49</td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Mean age</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>14.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Polyps</td>
<td>89</td>
<td>143</td>
</tr>
<tr>
<td>Mean size (mm) (range)</td>
<td>4.95</td>
<td>4.55</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>2.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Right sided</td>
<td>46</td>
<td>33</td>
</tr>
<tr>
<td>Left sided</td>
<td>43</td>
<td>110</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedunculated</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Non pedunculated</td>
<td>85</td>
<td>132</td>
</tr>
<tr>
<td>Ia</td>
<td>38</td>
<td>74</td>
</tr>
<tr>
<td>Iiia</td>
<td>45</td>
<td>56</td>
</tr>
<tr>
<td>Iib</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Iiia+iic</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lesion size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5mm</td>
<td>52</td>
<td>103</td>
</tr>
<tr>
<td>5-10mm</td>
<td>37</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 23: Patient demographics for study population
11.6: True histological diagnosis

In Group A (SD group) 61/89 (69%) lesions were neoplastic and 28/89 (31%) were non neoplastic. In Group B (HD group) 95/143 (66%) were neoplastic and 48/143 (34%) were non neoplastic.

11.7: Accuracy for neoplasia

The accuracy for diagnosis of neoplasia was compared between group A and Group B. There was a significant improvement in the sensitivity of FICE using HD scopes. High definition scopes did not have any significant impact on the sensitivity or accuracy of WLI or IC diagnosis. See table 24.

<table>
<thead>
<tr>
<th>Accuracy for neoplasia</th>
<th>Group A (SD)</th>
<th>Group B (HD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLI</td>
<td>44/61</td>
<td>72/95</td>
<td>0.51</td>
</tr>
<tr>
<td>(95% C.I.)</td>
<td>72%</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(65-78%)</td>
<td>(70-81%)</td>
<td></td>
</tr>
<tr>
<td>FICE</td>
<td>49/61</td>
<td>88/95</td>
<td>0.02</td>
</tr>
<tr>
<td>(95% C.I.)</td>
<td>80%</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(74-84%)</td>
<td>(88-96%)</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>57/61</td>
<td>89/95</td>
<td>0.95</td>
</tr>
<tr>
<td>(95% C.I.)</td>
<td>93%</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(88-96%)</td>
<td>(89-97%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 24: Accuracy for neoplasia Standard definition (SD) versus High definition (HD) colonoscopes 95% confidence intervals are in brackets. WLI= white light. IC= indigo carmine
11.8: Additional gain from indigo carmine

HD colonoscopes did not improve the accuracy of the IC assessment. However, when IC was used after FICE with SD scopes, the assessment was superior to FICE assessment alone with SD scopes (p=0.032). Additional assessment with IC did not provide any additional benefits as compared to FICE with HD scopes (p=0.77).

11.9: Accuracy for non neoplastic lesions

There was no significant difference in the accuracy for hyperplastic polyps between group A (SD) and group B (HD). In both groups FICE and IC assessment was significantly better than white light assessment (P=0.011). See table 25.

<table>
<thead>
<tr>
<th></th>
<th>Group A (SD)</th>
<th>Group B (HD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLI</td>
<td>17/28</td>
<td>32/48</td>
<td>0.6</td>
</tr>
<tr>
<td>(95% C.I.)</td>
<td>61%</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(46-74%)</td>
<td>(56-76%)</td>
<td></td>
</tr>
<tr>
<td>FICE</td>
<td>24/28</td>
<td>39/48</td>
<td>0.62</td>
</tr>
<tr>
<td>(95% C.I.)</td>
<td>86%</td>
<td>81%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(72-94%)</td>
<td>(72-87%)</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>25/28</td>
<td>40/48</td>
<td>0.48</td>
</tr>
<tr>
<td>(95% C.I.)</td>
<td>89%</td>
<td>83%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(78-96%)</td>
<td>(75-89%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 25: Accuracy for non neoplastic lesions standard definition (SD) versus high definition (HD) colonoscopes. 95% confidence intervals are in brackets. WLI= white light. IC= indigo carmine
A sub group analysis was performed on neoplastic lesions based on lesion size. For all modalities of imaging, accuracy for neoplasia was better for lesions >5mm in size, regardless of the resolution of endoscope used. This was statistically significant for WLI and FICE but failed to reach significance for IC. (WLI p=0.003, FICE p=0.045, IC=0.879). Accuracy for neoplasia was significantly better with high definition endoscopes when using FICE in polyps 5-10mm in all sizes (P=0.038). There was a trend towards an improvement when using HD scopes to assess polyps 5-10mm in size when using white light and indigo carmine, although this was not statistically significant (P=0.09). No improvement was seen from HD scopes when assessing polyps <5mm when using white light or indigo carmine. There was a trend towards an improvement when using FICE, although the study was not powered adequately for this test and the difference failed to achieve statistical significance. See table 26.
<table>
<thead>
<tr>
<th>Table 26: Breakdown of accuracy for neoplasia of in-vivo diagnosis by polyp size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (SD)</strong></td>
</tr>
<tr>
<td>&lt;5mm</td>
</tr>
<tr>
<td><strong>WLI</strong></td>
</tr>
<tr>
<td>20/31</td>
</tr>
<tr>
<td>65%</td>
</tr>
<tr>
<td><strong>FICE</strong></td>
</tr>
<tr>
<td>23/31</td>
</tr>
<tr>
<td>74%</td>
</tr>
<tr>
<td><strong>IC</strong></td>
</tr>
<tr>
<td>30/31</td>
</tr>
<tr>
<td>94%</td>
</tr>
<tr>
<td>5-10mm</td>
</tr>
<tr>
<td><strong>WLI</strong></td>
</tr>
<tr>
<td>24/30</td>
</tr>
<tr>
<td>80%</td>
</tr>
<tr>
<td><strong>FICE</strong></td>
</tr>
<tr>
<td>26/30</td>
</tr>
<tr>
<td>87%</td>
</tr>
<tr>
<td><strong>IC</strong></td>
</tr>
<tr>
<td>27/30</td>
</tr>
<tr>
<td>90%</td>
</tr>
</tbody>
</table>
11.11: Rescope intervals

A comparison of rescope intervals using BSG and ASGE guidelines was performed. Whilst a trend was observed towards improved accuracy in the setting of the rescope interval using high definition endoscopes with FICE and indigo carmine, no statistically significant difference was observed. See table 27.

<table>
<thead>
<tr>
<th></th>
<th>BSG guidelines</th>
<th>ASGE guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White Light</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>26/31</td>
<td>27/31</td>
</tr>
<tr>
<td></td>
<td>84%</td>
<td>87%</td>
</tr>
<tr>
<td>HD</td>
<td>31/38</td>
<td>31/38</td>
</tr>
<tr>
<td></td>
<td>82%</td>
<td>82%</td>
</tr>
<tr>
<td><strong>FICE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>29/31</td>
<td>29/31</td>
</tr>
<tr>
<td></td>
<td>94%</td>
<td>94%</td>
</tr>
<tr>
<td>HD</td>
<td>38/38</td>
<td>38/38</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Indigo carmine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>29/31</td>
<td>30/31</td>
</tr>
<tr>
<td></td>
<td>94%</td>
<td>97%</td>
</tr>
<tr>
<td>HD</td>
<td>38/38</td>
<td>38/38</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.8</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.112</td>
<td>0.112</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.112</td>
<td>0.265</td>
</tr>
</tbody>
</table>

Figure 3: Rescope intervals predicted from in-vivo histology prediction with standard definition (SD) or high definition (HD) colonoscopes using BSG and ASGE guidelines
11.12: Discussion

This study shows that when using Fujinon coloscopes a high resolution 650,000 pixel CCD (super CCD) is superior to a standard definition 450,000 pixel CCD colonoscope for the examination of neoplastic polyps<10mm using FICE. On sub-group analysis when assessing diminuitive polyps<5mm in size, FICE with high definition endoscopes showed a trend towards improved accuracy for the diagnosis of neoplasia, but this failed to reach statistical significance. A future trial is needed to investigate this further. For polyps 5-10mm in size FICE was significantly better when using high definition endoscopes for the diagnosis of neoplasia. The other important finding of our study was that high definition scopes do not improve the accuracy of diagnosis with white light or whilst using indigo carmine chromoendoscopy. FICE and indigocarmine dye spray are both excellent tools for making an in-vivo histological diagnosis for small polyps. FICE is significantly better when used with an HD scope, whilst IC performance is very good with standard definition colonoscopes and does not get any better with high definition colonoscopes. It is important for endoscopists to understand the equipment that they are using, and in particular the limitations imposed by a particular setup. This study is of direct clinical significance as it suggests a potential algorithm for the in-vivo assessment of small polyps. When using a high definition Fujinon endoscope, there is no additional gain from indigo carmine dye spray. However, when using a standard definition scope, it may be necessary to dye spray some lesions, as there is a statistically significant gain from indigo carmine after assessment with FICE using a standard definition endoscope.
There has been a significant drive from endoscope manufacturers in recent years to market their latest high definition endoscopes. Worldwide the global endoscopy market is worth $2,385 million, with 75% of the market held by four major players. Olympus is the market leader, controlling 47% of the market share followed by Fujinon corporation with 10%. It is felt that the adoption of high definition technology could drive this up to $3,524 million by 2016 (53). However, there has been a lack of literature demonstrating any benefit from this. In the current era of austerity, it is necessary for all clinicians and health care providers to make difficult decisions as to where money should be spent, and updating endoscopy equipment should be based on evidence as far as possible.

As discussed earlier, there has been a recent study published examining the potential cost savings within a United States of America screening population which could be made from in-vivo diagnosis (68). Using Narrow Band Imaging for in-vivo assessments with a resect and discard policy, the Markov model predicted that an annual undiscounted saving of $33 million could be made, without any meaningful impact on screening efficacy. Whilst this may be the case it is important that the endoscopist feels confident that the technology they have available in their unit is capable of producing the same results. This study provides important information for users of Fujinon equipment and provides a potential argument for justifying the cost of updating to high definition equipment if a resect and discard policy is to become widely adopted. It is important to note that no benefit was seen from high resolution endoscopes in making an indigo carmine based assessment using Fujinon equipment. This is reassuring, as even units with older equipment can make accurate in-vivo assessments by using IC chromoendoscopy.
There has been limited work published on FICE previously, with all of the studies utilising high resolution endoscopes (55) (54) (26), with most of the studies using optical magnification (55) (54). This data suggests that excellent results can be achieved with FICE and indigo carmine without optical magnification, and defines the role of high definition endoscopes for in-vivo diagnosis of small colonic polyps. It is important to be aware that the performance of HD scopes depends on various factors and it is important to have a high resolution screen connected to the stack via a digital video interface (DVI) output, as used in this study.

High definition endoscopes have been on the market for some time but their clinical role remains uncertain. This study demonstrates some of the differences between SD and HD Fujinon endoscopes. HD scopes do not alter the diagnostic accuracy of WLI and IC assessments in the diagnosis of neoplastic polyps<10mm, but significantly improves (p=0.02) their assessment with FICE. This defines a clinical benefit of Fujinon HD scopes above that of the older SD scopes.

11.13: Implications for clinical practice

With considerable talk about in-vivo diagnosis it is important for clinicians to understand the limitations of the equipment they are using. With standard definition endoscopes in regular use this study provides a valuable insight into what their limitations are and how they can be overcome. This could potentially prevent the endoscopist from making an incorrect diagnosis due to over confidence in the
equipment they are using. It is unknown whether similar limitations would be seen for other forms of vascular enhancement (Narrow Band Imaging and i-scan) as all of the studies into these technologies have been performed using high resolution endoscopes. However, it raises an important safety question which, until resolved, would suggest that it may be better to restrict in-vivo diagnosis using vascular enhancement to high resolution endoscopes where the accuracy of diagnosis is more evidence based. The results with indigo carmine however would suggest that a chromoendoscopy assessment is independent to the resolution of the endoscope and would offer a safe alternative to vascular enhancement when high resolution endoscopes are unavailable.

11.14: Implications for research

It is unknown whether similar limitations may be seen with standard definition endoscopes when used with Narrow Band Imaging or i-scan. All of the studies into these technologies have been conducted with high resolution endoscopes and there is therefore an urgent need to ascertain whether they can be used at all for in-vivo diagnosis without high resolution endoscopes. This has critical safety implications and there is therefore an urgent need for research into this.
11.15: Conclusions

This retrospective analysis demonstrates that FICE and indigo carmine dye spray are excellent tools for the evaluation of small colonic polyps <10mm in size. High definition scopes can improve the sensitivity of FICE. Results with indigo carmine are independent of the type of scope (HD or SD). The data suggests that the use of either FICE or IC in place of conventional histological analysis would have a negligible impact on the rescope interval of the patients involved. It has the potential to replace histological assessment of small polyps <10mm.
Chapter 12

Barrett’s Neoplasia Study
12.1: Introduction

The incidence of oesophageal adenocarcinoma is rising in the Western world. It is responsible for 14,500 deaths per year in the United States (132) and 7,000 per year in the UK (3). Barrett’s dysplasia is a well established precursor, which is the basis for endoscopic surveillance of patients with Barrett’s oesophagus. Standard protocols require the collection of large numbers of biopsies which are conventionally taken in a random quadrantic fashion at 2cm intervals. Non targeted multiple biopsies have remained standard practice as most of the dysplastic areas are difficult to see with standard resolution white light endoscopes, and only 13% of early neoplastic lesions appear as macroscopically visible nodules (133). It has been shown that intensive surveillance biopsies as per the Seattle protocol using jumbo forceps does not improve the detection of intramucosal adenocarcinoma any more reliably than less intensive protocols with standard biopsy forceps (134). Endoscopic surveillance of Barrett’s oesophagus makes clinical sense due to its malignant potential, but the poor pick up rate of neoplasia during routine surveillance with white light endoscopy and quadrantic biopsy questions the cost effectiveness of this strategy (135). Recent developments have led to improvements in the optical resolution of endoscopes and new technologies have been developed which enhance mucosal visualisation. Improved methods for identifying in-vivo dysplastic areas use either chromoendoscopy (78) (90) (79) (136), of which acetic acid is one method, or narrow band imaging (99), and spectral imaging techniques like Flexible Spectral Imaging Colour Enhancement (FICE) (137).
Chromoendoscopy for the detection of Barrett’s metaplasia has been available for many years. The three dyes commonly used are methylene blue (MB) (74), indigo carmine (IC) (77) and acetic acid (AA) (84).

Acetic acid dye spray is used routinely for the detection of cervical pre-cancerous lesions, including glandular lesions, at colposcopy (87). Acetic acid was originally used in the oesophagus as an aid to detect small segments of residual Barrett’s metaplasia after ablation therapy.

Acetic acid, when sprayed on Barrett’s mucosa, leads to reversible acetylation of nuclear proteins, leading to an aceto-white area, causing vascular congestion and improving the visualisation of the mucosal surface (80). This allows the mucosal surface patterns to be assessed, improving the diagnosis of dysplasia or cancer. The reaction only lasts a few minutes, with dysplastic tissue losing the aceto-whitening more quickly than background Barrett’s epithelium, further highlighting abnormal areas. See figures 28-29.
Figure 28: Barrett's metaplasia without any evidence of neoplasia

Figure 29: Neoplasia within Barrett's
12.2: Questions to be answered

The key clinical problems in the management of patients with Barrett’s oesophagus are:

1) The detection of neoplasia within Barrett’s epithelium
2) Localisation of the focus of neoplasia

Unfortunately, traditional protocol guided mapping biopsies do not detect all neoplasia (134). Furthermore they are cumbersome and imprecise at localising neoplasia. For this reason there is a clinical need for improved methods for neoplasia detection. There has been interest in the modern advanced electronic imaging technique tri-modal imaging, which combines the techniques of Narrow Band Imaging and autoflourescence. However, a recent large multi-centre randomized tandem endoscopy study has suggested that the technology delivers disappointing results when used for this task, and the authors have concluded that tri-modal imaging cannot be used in place of mapping biopsies, even in a high risk population (105). This is discussed in detail in chapter 3.

12.3: Justification for the acetic acid studies

Prior to this work there has been a lack of studies examining the role of acetic acid in the detection and localisation of neoplasia. This is very important as the identification of HGD within Barrett’s changes management from surveillance to treatment. Given the growth of advanced endoscopic resection techniques now available, the need to localise neoplasia within Barrett’s has never been more acute. Even if a non targeted therapy is to be considered (radiofrequency ablation or photodynamic therapy), it is still important to be able to localise neoplasia. Ablative techniques do not yield any
pathology. Therefore if a misdiagnosis is made and an area of invasive cancer is not seen, it can have disastrous consequences, with potentially curable patients being left undertreated. Therefore there is a massive clinical need for localisation techniques.

The aim of this study will be to use acetic acid to localise neoplasia within Barrett’s oesophagus. Acetic acid has already been used in small pilot studies to examine Barrett’s metaplasia (81) (82), and assessment tools have been developed for identifying patterns associated with metaplastic and neoplastic Barrett’s epithelium using the technique (83) (88) (89). These studies have been very small with limited numbers of neoplasia cases, but demonstrate the potential for acetic acid to be used for in-vivo diagnosis.

Acetic acid is widely available and could potentially be used with any endoscope. This makes the study of its use as a diagnostic tool important as, if successful, it would carry the benefit over other advanced imaging techniques of not being dependent on the availability of a high end endoscope from a particular manufacturer. This would make widespread dissemination of any benefits from the research much easier to apply in the wider endoscopy community.
12.4: Hypothesis

We hypothesise that acetic acid guided chromoendoscopy will improve the detection of neoplastic foci within Barrett’s epithelium by improving the visibility of the neoplastic foci and allowing targeted biopsies of that area, as compared to white light assessment.

12.5: Primary aim

To establish the effect of acetic acid in improving the visualisation of neoplasia in Barrett’s oesophagus as compared to white light examination

12.6: Secondary aim

To establish the sensitivity and specificity of acetic acid assisted neoplasia detection within Barrett’s oesophagus
12.7: Ethics
This study was discussed with the Portsmouth Ethics Committee. Acetic acid is a recognised technique for the identification of Barrett’s metaplasia, and hence could be used in the oesophagus during gastroscopy if desired, as part of the medical consent for the procedure. From a research governance perspective the ethics committee considered this to be a study of the endoscopist’s ability to interpret the findings from endoscopy, and as such did not require ethics committee approval. Each patient had signed an informed consent form for the endoscopy procedure.

12.8: Statistical analysis
Before starting the studies the design was discussed with the department of Mathematics and Biostatistics within the University of Portsmouth. Unfortunately there were no prior studies suggesting what the potential sensitivity or specificity for neoplasia with acetic acid would be. As such power calculations could not be performed. Therefore the advice was to conduct the investigation as a pilot study.

A recruitment target of 100 patients was set. Spearman’s rank correlation coefficient (r) was calculated to assess the correlation between predicted and true histology. The proximity of r to one shows the strength of correlation, with r=1 being a 100% correlation. Chi² test was used to assess the statistical significance of the difference between two limbs (WLI vs AA). P<0.05 was considered to be statistically significant.
It is the accepted standard of care to follow up patients post EMR with 3 monthly gastroscopies to look for metachronous neoplasia. Appearances can change post treatment, and patients do develop dysplasia between endoscopies, which is why they are followed up. Previous endoscopy results should not influence the findings on a given procedure. Therefore the analysis was performed on a per procedure basis rather than per patient. It is important that if targeted treatments are to be performed that it is possible for patients to be safely followed up, and this is reflected in the study design.

12.9: Feasibility

The department is a specialist centre for the assessment and treatment of early gastrointestinal neoplasia, taking referrals from across the South of England for the endoscopic management of dysplasia and intramucosal cancer within Barrett’s oesophagus.

12.10: Methods

All patients undergoing AA dye spray for evaluation of Barrett’s oesophagus were recorded prospectively on a computer database. All procedures were performed by a single experienced endoscopist (PB) with expertise in lesion recognition and endoscopic mucosal resection (EMR).
**12.11: Patient population**

The patient population included Barrett’s cases where dysplasia had never been identified previously (group A), and tertiary referrals for suspected oesophageal neoplasia where dysplasia had been found or suspected on random biopsy and post EMR follow up cases where dysplasia had been treated endoscopically (group B). Patients would be recruited from screening endoscopy lists. They would consist of three distinct populations:

**12.11.1: Inclusion criteria**

1) Symptomatic patients with Barrett’s metaplasia but no previous history of neoplasia referred for close inspection of the Barrett’s

2) Patients with neoplasia detected on random biopsy. It should be invisible to the referring endoscopist.

3) Patients with previous neoplasia, endoscopically treated by either EMR or radiofrequency ablation

**12.11.2: Exclusion criteria**

1) Oesophagitis or other coexisting inflammation

2) Contact bleeding

3) Systemic connective tissue disorder

4) Oesophageal motility disorder

5) Coagulopathy

6) Acute mucosal trauma to the oesophagus during the procedure
12.12: Endoscopic protocol

All patients were endoscoped on a dedicated chromoendoscopy list. 25% of the cases were performed under local anaesthetic with xylocaine (10mg spray), with 75% choosing conscious sedation using i.v. midazolam, starting at 2.5mg and increasing in 1mg intervals as required to a maximum of 5mg. Each procedure was allocated 15-20 minutes. Gastroscopy was performed using Fujinon EG-590zw and EG-590wr gastrosopes with the EPX 4400 processor (Fujinon, Tokyo, Japan). Magnification, if available, was not used for the detection of neoplasia.

12.13: Mucosal cleansing

It was noted than mucous impaired views and made inspection for subtle irregularities difficult. Therefore patients were given 50 mL of a solution containing 5 ml of 10% N-acetyl cysteine and 5 ml of simethicone to drink prior to undertaking the procedure to act as a mucolytic and bubble-bursting agent. Endoscopy was then performed.

12.14: Standardisation of technique

Inflammation can often mimic low grade dysplasia and can confuse the diagnosis of more serious changes. Therefore severe oesophagitis, contact bleeding or acute mucosal trauma were exclusion criteria for the study. An initial pilot assessment was performed on 10 patients with Barrett’s metaplasia.
Acetic acid comes as a 5% solution for medical use. Initially three different dilutions of acetic acid were trailed which had been used in the previous studies reported in the literature:

- 1.25%
- 2.5%
- 5%

It was found that the supplied 5% concentration caused mucosal oozing which, whilst mild, interfered with subtle surface pattern assessment, defeating the point of the exercise. See figure 30. 1.25% and 2.5% did not result in inflammation. However, 1.25% led to patchy aceto-whitening. Whilst adequate for surface pattern assessment, the variable nature meant that aceto-whitening timings may have been unreliable. Therefore 2.5% solution was used for the study. This was made up by mixing 10mls of 5% solution with 10mls of 0.9% normal saline in a 20ml syringe.
There were two potential methods for applying the acetic acid; one method would have been to flush 20mls of 2.5% acetic acid down the biopsy channel of the endoscope, the other was to use a spray catheter. Both approaches were attempted during the pilot study. However, flushing the dye straight down the endoscope sometimes resulted in non uniform coverage of the oesophagus. Spray catheters are inexpensive and readily available in every endoscopy unit. Therefore, it was decided that a spray catheter should be used in every procedure. This allows for targeted application of the acetic acid, facilitating uniform coverage of the Barrett’s epithelium. This is particularly valuable in assessing long segments of Barrett’s. 

Figure 30: Traumatized mucosa after 5% acetic acid
12.15: Assessment tool

To help identify dysplasia after AA spray, an assessment of the following features was made:

- **Surface pattern** (ridged, villous, nodular, round, irregular)
- **Mucosal vascular pattern** (regular or irregular)
- **Acetowhitening reaction**: (normal or abnormal)

Barrett’s mucosa was classified by the endoscopist as **non dysplastic** if the surface pattern was round, tubular, villous or ridged, the vascular pattern was normal and the acetowhitening reaction was normal.

Mucosa was defined as **dysplastic** if the following was observed: irregular surface patterns, increased vascularity, an irregular microvascular pattern or early disappearance of aceto-whitening from a focal area of mucosa as compared to the rest of the Barrett’s mucosa (83).

Mucosa was defined as **invasive cancer** if there was a complete loss of surface patterns, increased vascularity, disorganised and dense microvascular pattern and rapid disappearance of the aceto-whitening. In addition depressed areas (Paris type IIc) were regarded as suspicious for malignancy.
12.16: Study design

Patients were first examined with conventional WLI, with any debris thoroughly removed and any visible abnormality suspicious of neoplasia noted (limb A of figure 31). The length of the Barrett’s segment was measured from the top of the gastric fold to the squamocolumnar junction, with islands of Barrett’s recorded separately. Twenty ml of AA (2.5%) dye spray was then applied using a spray catheter to identify additional dysplastic foci within the Barrett’s segment over and above that which was seen by WLI. (limb B of figure 31).
After AA chromoendoscopy all lesions that became visible were noted and a targeted biopsy was taken and sent in a separate pot to pathology (limb C of figure 31). This was followed by quadrant biopsies at every 2 cm of the remaining Barrett's segment. These were sent separately for pathological review (non targeted histology). If a suspicious lesion was seen on WLI, but appeared normal after AA dye
spray it was biopsied as a part of the quadrantic biopsy strategy. In cases where an abnormality was seen on acetic acid targeted biopsy, four quadrant biopsies were not taken from the same quadrant at the same latitude as the targeted biopsy. The final histological diagnosis was achieved by combining the results of the targeted biopsies (limb C) and non targeted quadrantic biopsies (limb D of figure 31). All histological slides were reviewed by two expert pathologists. Analysis was performed on a per-procedure basis, and not on a per lesion basis.
12.17.1: Demographics

In total 190 procedures were performed on 119 patients with Barrett’s metaplasia and neoplasia. The recruitment pathway is shown in figure 32. 78 procedures were performed in group A (patients with no prior history of dysplasia) and 112 procedures were performed in group B (tertiary referral group). The median age of the patient cohort was 65 (range: 35-87) with 75% male. The median length of Barrett’s segment was 4cm (range: 2-15).
Figure 32: Flow chart for patient recruitment

Enrollment

Assessed for eligibility (n=202)

Excluded (n=7)
- Not meeting inclusion criteria (n=2)
- Declined to participate (n=5)
- Other reasons (n=0)

Intervention

Allocated to intervention (n=195)
- Received allocated intervention (n=190)
- Did not receive allocated intervention (inflamed mucosa prevented examination (n=5)

Follow-Up

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Analysis

Analysed group A (n=78)
Excluded from analysis (n=0)

Analysed group B (n=112)
Excluded from analysis (n=0)
12.17.2: True neoplasia

Histologically confirmed Barrett's neoplasia was found in 46% of the study population (88 out of 190 procedures). There were 21/88 cases of early cancer (T1a and T1b), 51/88 cases of high grade dysplasia and 16/88 cases of low grade dysplasia.

12.17.3: Visible abnormalities

Visible neoplasia was noted by the endoscopist during 43/190 procedures with WLI endoscopy, and during 102/190 following acetic acid dye spray. Use of WLI endoscopy alone, significantly underestimated Barrett's neoplasia. AA dye spray significantly (P=0.001) improved the Barrett's neoplasia detection rate (2.5 fold) compared to WLI alone as shown in table 28.

<table>
<thead>
<tr>
<th>Visible abnormality</th>
<th>Conventional WLI</th>
<th>AA predicted in vivo histology</th>
<th>B-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limb A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>147</td>
<td>88</td>
<td>-59 (1.6 fold)</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>43</td>
<td>102</td>
<td>59 (2.3 fold)</td>
</tr>
</tbody>
</table>

Table 28: WLI vs AA predicted diagnosis (WLI=white light, AA=acetic acid)
12.17.4: Targeted histology

Histology results obtained from AA dye spray targeted biopsies were compared with the final pathological diagnosis (a combination of random and targeted biopsy results). An excellent correlation was identified (r=0.9). See table 29. AA targeted biopsies diagnosed 63 of the 67 dysplasia cases. All 21 of the 21 cancers were diagnosed by AA targeted biopsies compared to 13 of the 21 with WLI. In some patients more than one area of dysplasia was found by targeted biopsy. However, in all cases where dysplasia was found on targeted biopsy, there were no additional abnormalities found on random biopsy.

<table>
<thead>
<tr>
<th></th>
<th>Targeted histology Group C</th>
<th>Actual Histology (random + targeted ) Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplasia (HGD+LGD)</td>
<td>63</td>
<td>67</td>
</tr>
<tr>
<td>Cancer</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td>r=0.99</td>
</tr>
</tbody>
</table>

Table 29: Targeted vs true histology (LGD=Low grade dysplasia, HGD=high grade dysplasia)

12.17.5: Sensitivity and specificity of AA targeted histology

Four out of 88 Barrett’s neoplasia cases were not identified on targeted histology, giving a false negative rate of 4.5% for all neoplasia. If the low grade dysplasia population is excluded then the false negative rate falls to 2.7% (2/72).
20/102 non dysplastic Barrett’s mucosa, suspected to be neoplastic following acetic acid dye spray because of visible abnormalities, turned out to be inflammation, giving a false positive rate of 19.6%.

The overall sensitivity for identification of neoplasia was 95.5% with a specificity of 81%. See table 30. On subgroup analysis we found a sensitivity of 77% and specificity of 85% for Group A and a sensitivity of 98.7% and specificity of 75% for group B.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Prevalence of Neoplasia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cohort</td>
<td>95.5</td>
<td>81</td>
<td>46.3</td>
</tr>
<tr>
<td>Group A</td>
<td>77</td>
<td>85</td>
<td>16.7</td>
</tr>
<tr>
<td>Group B</td>
<td>98.7</td>
<td>73</td>
<td>66.9</td>
</tr>
</tbody>
</table>

Table 30: Sensitivity and specificity by patient group

12.17.6: Missed neoplasia

There were no missed cancers in either group.

There were four missed cases of dysplasia; 2 LGD and 2 HGD.

Three out of the four missed dysplasia were in group A. Two of these cases were classified as low grade dysplasia and one was classified as high grade dysplasia on the initial multiple quadrantic biopsies but were not identified on AA spray. All these
patients have had at least two further gastroscopies at 6 month intervals with multiple further biopsies and no further evidence of dysplasia has been found. One patient had missed high grade dysplasia in group B. This patient had received two previous endoscopic mucosal resections and APC ablation, so had multiple islands of neo-squamous epithelium.
12.18: Discussion

This is a large series of acetic acid chromoendoscopy, with a large number of neoplasia cases being detected by this technique. This series shows that acetic acid dye spray significantly improves the detection of neoplasia in Barrett's oesophagus as compared with white light endoscopy. It illustrates that acetic acid targeted biopsies can diagnose dysplasia in the majority of patients (95.5%), without the need of further multiple non-targeted biopsies. It also illustrates that no cancers are missed by acetic acid targeted biopsies.

Only four out of 88 patients with neoplasia were missed by acetic acid dye spray, two of these were low grade dysplasia, the other two were high grade dysplasia. These mucosal dysplasias were picked up on conventional quadrant biopsy. All of these cases were challenging both for the endoscopist and the pathologist. Low grade dysplasia is a difficult histological diagnosis to make, with an element of subjectivity involved, and at times inflammation can raise a possibility of LGD. There is a departmental policy of repeating endoscopy in patients with LGD after 8 weeks of high dose proton pump inhibitor therapy (omeprazole 40mg o.d.). Both the patients have had two further gastroscopies on high dose acid suppression with multiple biopsies but no further LGD has been discovered, suggesting that the initial finding could be inflammation related.

Another patient from group A had HGD diagnosed on one of the several biopsies taken as a part of the quadrant biopsy strategy. He has had three further gastroscopies and no further dysplasia has been found. This raises the possibility of 1 microscopic monofocal HGD.
The 4th patient with missed dysplasia was from group B and was found to have HGD. This was a challenging case with a history of two previous EMRs and APC ablation resulting in multiple islands of neo-squamous epithelium. He went on to undergo further HALO ablation and has had no further recurrence of disease.

The remaining 84 cases of Barrett’s neoplasia were detected by targeted biopsy. Additional quadrantic biopsies did not alter the overall diagnosis. This is an important finding and it questions the logic of further quadrantic biopsies in patient groups where neoplasia is already detected by acetic acid dye spray. This finding potentially has significant cost and resource implications for the endoscopist and the pathologist.

The findings from this series are similar to another study quoting a surprisingly high sensitivity of 100% and specificity of 97.7% (88). However, only 13/100 of the patients in the study had dysplasia, making it difficult to draw any meaningful conclusions from it with respect to dysplasia detection.

Whilst patients with excessive inflammation were excluded, a significant factor in the false positive rate of 19.6% was the presence of inflammatory changes appearing like dysplasia. This highlights the importance of treatment with proton pump inhibitors prior to endoscopy. When Barrett’s epithelium looks inflamed it is sometimes necessary to arrange further assessment after 8 weeks on a higher dosage of acid suppression, and this intervention should not be overlooked.

There was a high prevalence of neoplasia in group A of 16.7%. In a Barrett’s surveillance population this would be expected to be much lower. However, it should be noted that this population was not a standard surveillance population. It consisted
of Barrett’s surveillance cases and symptomatic patients who had no prior diagnosis of dysplasia who had been listed for assessment on a chromoendoscopy list. Therefore it is not surprising that the prevalence of dysplasia in this cohort is higher than a standard asymptomatic surveillance population.

Group B is a complex group, consisting of all patients with previously diagnosed dysplasia being assessed for EMR and post EMR follow up patients to look for metanchronous neoplasia. Endoscopic treatment in this cohort depends on accurate localisation of neoplasia and we were reassured to find that dysplasia could be targeted with a high degree of accuracy in this cohort.

Electronic imaging techniques like narrow band imaging (NBI), and spectral imaging (FICE) have been reported in the evaluation of Barrett’s neoplasia (93). However, the studies have been very small and it is not possible to draw any firm conclusions from these studies. A problem with all ‘virtual chromoendoscopy’ techniques is that they have cost and resource implications as they require commitment to a particular endoscope manufacturer, and need the most recent image processors and endoscopes. Many units (even in the Western world) do not have the latest models of processor and endoscopes, and therefore cannot use this technology. Acetic acid dye spray can be performed in any unit and is compatible with all makes and models of endoscope, irrespective of the manufacturer.

12.19: Strengths

This is a single centre, single endoscopist study, performed by an experienced endoscopist. Whilst this can be seen as a weakness it can also be regarded as a strength. The design was intended to test the technique, rather than the
endoscopists ability to use it correctly. Therefore the results do represent what can be achieved by using acetic acid, and it avoids the criticism that missed neoplasia could be due to the endoscopists inexperience in applying the technique and in interpreting the results. Furthermore, there were a large number of neoplasia cases in the study. The data is therefore robust and a true reflection of acetic acid as a diagnostic test, and unlikely to be distorted by a single wrong diagnosis. The design reflected how acetic acid is likely to be used in clinical practice and answers the important clinical questions of whether neoplasia can be localised and if high risk patients can be followed up safely. It also demonstrated that the technique could be used with standard endoscopes without optical magnification, which reflects routine Western practice.

12.20: Limitations

This is a single centre study, where all endoscopies have been performed by a single experienced endoscopist (PB). The results are therefore not generalisable to all endoscopists. It is probable that to achieve similar results formalised training would need to be undertaken in lesion recognition. This is an accepted part of endoscopy, and if dye spray is to be used widely in the assessment of Barrett’s it will be necessary to address this issue. However, it is probable that intensive training in a large volume expert centre can help achieve the expertise in a relatively short period of time.

The study is not a randomised controlled trial, and as such it is not possible to say how much of the dysplasia would have been found or missed by random biopsies.
had dye spray not been used. That would be best assessed by a tandem endoscopy at a 6-8 week interval. However the data does show that dysplasia was visible in only 50% of our patients on high resolution WLI. Could protocol guided quadrantic biopsies every 2cm be able to pick up the dysplasia in the remaining 50% of patients with invisible (WLI) dysplasia? This is a difficult question to answer from the design of this study, but it is reasonable to propose that it would be very difficult to diagnose the dysplasia by quadrantic biopsies alone in this group of patients with invisible dysplasia. Even if it does, then it would require a very large number of biopsies with significant resource implications. Alternatively, the endoscopist could elect to use acetic acid spray and that will help better define the dysplasia that is already seen on WLI and also highlight the invisible (WLI) dysplasia as seen in 50% of the dysplastic population.

This data is not enough to completely abandon the strategy of protocol guided biopsies in all patients with Barrett’s, but it does justify the role of acetic acid in patients with invisible dysplasia on WLI and also questions the additional gain of multiple non-targeted biopsies after dysplasia has been seen with acetic acid spray.
12.21 Implications for clinical practice

In a high risk population this study would suggest that acetic acid is a very effective tool for the identification and localisation of neoplasia within Barrett's oesophagus, and is more accurate than white light assessment alone. This has very important clinical implications as it enables targeted treatment for neoplasia to be performed such as endoscopic mucosal resection (EMR). This can potentially avoid the need for oesophagectomy in the majority of patients with high grade dysplasia or intramucosal cancer. It would be a realistic long term goal to abandon non targeted biopsies when no visible neoplasia is seen with acetic acid. However, this is a cohort study and a randomised controlled trial is needed to reproduce these findings before this practice can be adopted. Acetic acid may have a role in a routine surveillance population. However, this data is insufficient to recommend this practice at present.

12.22 Implications for future research

A randomized tandem endoscopy study in a high risk population is the next logical step in evaluating this technique further. This would serve the function of both demonstrating reproducibility of this data in a robust way and would also demonstrate its wide spread applicability in a high risk population. A multi-centre multi endoscopist cohort study is also needed in a surveillance population to establish whether acetic acid could be used to replace random biopsies. This would need to be large as the prevalence of neoplasia would be much lower in this cohort.
The development of training tools in the use of acetic acid are important if the technique is to become widely used, and the duration of the learning curve to competence needs to be established. This may be achievable through picture or video based assessments, and a study will be required to establish whether this form of training is possible.

There is currently no data available concerning the use of acetic acid followed by the additional use of vascular enhancement techniques (NBI, FICE or i-scan), and whether there is any additional gain from this is unclear. Further research into this is needed. However, the studies would need to be large given the high sensitivity demonstrated in this study and it could be argued that even if a statistically significant benefit was seen it may not be clinically significant.

12.21: Conclusions

This study demonstrates that acetic acid targeted biopsies are an excellent tool to localise prevalent neoplasia within Barrett’s esophagus. It is cheap, quick, universally available and effective. It questions the relevance of additional non-targeted biopsy in patients where AA has already identified neoplasia.
Chapter 13:

Use of the Aceto-whitening timing in the diagnosis of Barrett's neoplasia
13.1: Introduction

In the previous study it was demonstrated that neoplasia within Barrett’s could be detected using acetic acid assisted chromoendoscopy (138). It is known that acetic acid leads to reversible acetylation of nuclear proteins and vascular congestion which improves the visualisation of surface and vascular patterns. The detection of neoplasia within Barrett’s has been based on the assessment of the following:

- Mucosal Surface pattern (ridged, villous, nodular, round, irregular)
- Mucosal vascular pattern (regular or irregular)
- Aceto-whitening reaction: (normal or abnormal)

The surface and mucosal vascular patterns have been described in several studies and are relatively well understood (88) (89) (90) (91) and have been demonstrated to be effective in the localization of neoplasia. However, the aceto-whitening reaction is poorly understood.

In the study described in the previous chapter, assessment was predominately on surface patterns. It was noted during the previous study that dysplastic tissue appeared to lose its whitening early compared to metaplastic Barrett’s epithelium. However, the difference in the timings has never been studied. This has left endoscopists uncertain how to interpret these changes. The aim of this study is to explore the clinical significance of the aceto-whitening reaction in the diagnosis of Barrett’s neoplasia.
13.2: Hypothesis

The loss of aceto-whitening can be quantified to accurately distinguish between Barrett’s metaplasia, dysplasia and invasive cancer.

13.2.1: Primary aim

To quantify the aceto-whitening timing thresholds for distinguishing between metaplasia, dysplasia and invasive cancer within Barrett’s oesophagus.

13.2.2: Secondary aim

To determine the sensitivity and specificity of the aceto-whitening reaction in the diagnosis of Barrett’s neoplasia.
13.3: Methods

The investigation was constructed as a prospective case control study. Barrett’s epithelium was examined to identify areas of neoplasia. All procedures were performed by a single experienced endoscopist (PB) with expertise in lesion recognition and endoscopic mucosal resection (EMR).

13.3.1: Inclusion criteria

- Patients with known Barrett’s metaplasia where dysplasia had never been identified previously
- Patients who had undergone endoscopic treatment for neoplasia previously and were under surveillance for metachronous neoplasia
- Patients suspected of neoplasia referred for evaluation for endoscopic mucosal resection or other targeted endoscopic treatment of the lesion

3.3.2: Exclusion criteria

- Oesophagitis
- Contact bleeding
- Acute mucosal trauma
13.4: Ethics committee approval

The study was reviewed by the Oxford ethics committee. Patient consent was required for entry into the study on a per-procedure basis as procedures could take longer than a standard acetic acid enhanced gastroscopy, due to the timing involved. Approval was granted REC reference number 10/H0605/30.

13.5: Statistical analysis

Unfortunately there were no prior studies suggesting what the differential timings in the aceto-whitening reaction may be. As such power calculations could not be performed. Statistical advice was requested from the Department of Mathematics and Biostatistics within the University of Portsmouth. The recommendation was to conduct the investigation as a pilot study. It was suggested that a minimum of 100 lesions should be examined: 50 healthy areas of Barrett’s metaplasia, 30 areas of dysplasia (10 low grade and 20 high grade), 10 intra-mucosal cancer and 10 invasive cancers. The best method of analysis was to use the data to generate a receiver operator curve (ROC) to determine threshold timings for the diagnosis of invasive cancer, intramucosal cancer, high grade dysplasia and metaplasia. In the event that the numbers were not large enough to be statistically significant, the study would still be likely to generate the information required to confidently power a larger study.
13.6: Endoscopic protocol

All patients were endoscoped on a dedicated chromoendoscopy list. 25% of the cases were performed under local anaesthetic with xylocaine (10mg spray), with 75% choosing conscious sedation using i.v. midazolam, starting at 2.5mg and increasing in 1mg intervals as required to a maximum of 5mg. Each procedure was allocated 15-20 minutes. Patients were given 50 mL of a solution containing 5 mL of 10% N-acetyl cysteine and 5 mL of simethicone to drink prior to undertaking the procedure to act as a mucolytic and bubble-bursting agent. Gastroscopy was then performed using Fujinon EG-590zw and EG-590wr gastrosopes with the EPX 4400 processor (Fujinon, Tokyo, Japan). Magnification, if available, was not used for the detection of neoplasia or for the purposes of aceto-whitening timings. Assessment of the mucosal surface and any obvious abnormalities seen with white light examination was recorded prior to dye spray with acetic acid. Acetic acid was applied using a spray catheter to areas of healthy Barrett’s epithelium and the time taken for the aceto-whitening to disappear was recorded.

In each case the time taken for the aceto-whitening to disappear was recorded. The areas were then biopsied to confirm the diagnosis. Histology was correlated to the aceto-whitening disappearance time to establish whether there was a correlation between the degree of neoplasia (cancer, high grade dysplasia or low grade dysplasia) and the aceto-whitening time. Figure 33 shows the process of assessment.
Figure 33: Protocol for acetowhitening study

1. Drink of 10% N-acetyl cysteine & Simethicone
2. High resolution White light examination
3. Acetic acid (2.5%) enhanced endoscopic examination
4. Note the acetowhitening reaction and time taken for it to disappear
5. Wash tissues with water and take targeted biopsies to confirm histology
6. Quadrant biopsies of the rest of the Barrett’s mucosa
7. Correlate the time taken for acetowhitening to disappear to histology
13.7: Assessment tool

The aceto-whitening time was measured using a stop clock in minutes and seconds.

The start time was defined as beginning after the entire length of Barrett’s oesophagus had been completely coated with acetic acid and all excess dye sucked away (Time A).

The stop time for disappearance of aceto-whitening was defined as the moment of first appearance of erythema within the aceto-white Barrett’s epithelium (time B).

\[ \text{Acetowhitening time} = B - A \]

This was trialled in 20 patients prior to introduction into the study to decide the best way of timing (stopwatch versus on-screen clock), the best person to record the timing (endoscopist versus investigator) and the potential implications of it in terms of increased time taken for the procedure. It was found that the endoscopist calling the start and stop times, with a researcher recording these on the data proforma, were most reproducible and worked well. The onscreen clock was more precise as the endoscopist could read it straight off the screen and the researcher transcribe it directly onto the data sheet. The procedure was not found to be delayed by timing, as it would take at least 5 minutes to adequately visually assess the Barrett’s epithelium, record measurements according to Prague criteria and complete the procedure. Washing the mucosa thoroughly prior to quadrantic biopsy was found to be essential as excess acetic acid could cause local oozing which interfered with views.
13.8: Results

13.8. True histology

In total 133 patients were approached to enter the study. 131 met the inclusion criteria and 129 consented to enter the study. At the time of gastroscopy a further 8 cases were subsequently excluded as not meeting the inclusion criteria for acetic acid chromoendoscopy due to the presence of significant visible inflammation on white light. In total data was collected from 146 areas of Barrett’s were collected in 121 patients, of which 84% were male. The pathway for recruitment is shown in figure 34. The breakdown of the true histology is shown in table 31:

<table>
<thead>
<tr>
<th>True histology</th>
<th>Metaplasia</th>
<th>LGD</th>
<th>HGD</th>
<th>IMC</th>
<th>Invasive cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>86</td>
<td>14</td>
<td>27</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 31: True histology of cohort. LGD= low grade dysplasia, HGD=high grade dysplasia, IMC=intra-mucosal cancer

Areas were identified by assessment of surface patterns, surface vasculature and vascularity. In total, 72/86 normal Barrett’s epithelium was correctly identified as normal. HGD was correctly identified in 26/27 cases, 15/15 IMC and 12/12 of the invasive cancer was recognised correctly by the endoscopist. 6/14 LGD was correctly identified. This resulted in a sensitivity for high risk neoplasia of 98% with a specificity of 89%.
Figure 34: Recruitment pathway for the study
13.9: Aceto-whitening timings: Metaplasia versus high risk neoplasia

Amongst cases correctly identified the mean and range of timings for the disappearance of the aceto-whitening time by histology are shown in table 32. The time for disappearance of LGD, HGD, IMC and invasive cancer was significantly less than that of metaplasia, with a mean aceto-whitening time greater than two standard deviations less than that for metaplasia (p<0.05).

<table>
<thead>
<tr>
<th>Histology</th>
<th>Mean time for acetowhitening to disappear (seconds)</th>
<th>Standard deviation</th>
<th>Median time for acetowhitening to disappear (seconds)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaplasia</td>
<td>387</td>
<td>164</td>
<td>350</td>
<td>142-621</td>
</tr>
<tr>
<td>LGD</td>
<td>58</td>
<td>28</td>
<td>58</td>
<td>15-90</td>
</tr>
<tr>
<td>HGD</td>
<td>54</td>
<td>28</td>
<td>53</td>
<td>20-140</td>
</tr>
<tr>
<td>IMC</td>
<td>24</td>
<td>13</td>
<td>20</td>
<td>12-50</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>23</td>
<td>26</td>
<td>10</td>
<td>3-81</td>
</tr>
</tbody>
</table>

Table 32: Mean acetowhitening times by lesion histology (LGD= low grade dysplasia, HGD=high grade dysplasia, IMC= intra-mucosal cancer)

13.10: Aceto-whitening timings: Cases incorrectly diagnosed

There were 14 false positive cases in the series. In 4/14 of these cases the changes were very subtle and it was suspected clinically that the histology may be metaplasia only. In the remaining 10 cases the areas had the appearances of dysplasia with a disordered surface pattern. In all cases there was a mild degree of inflammation present which affected the diagnosis. There were 9 false negative cases, one HGD
and 8 LGD. In 2 of the cases of LGD coexisting areas of HGD had been correctly identified in the same endoscopy.

### 13.11: ROC curve: Differentiation of high risk neoplasia from metaplasia using aceto-whitening timings

A ROC curve was produced for the identification of high risk neoplasia (HGD, IMC and invasive cancer) using the aceto-whitening timings. The area under the curve was 0.93, demonstrating a low probability that a randomly chosen positive case will exceed the value for a randomly chosen negative case. The asymptomatic significance was 0.000. (95% C.I. 0.89-0.97). Using a cut off threshold of 142 seconds a sensitivity for neoplasia of 98% (95% C.I. 89-100) and specificity of 84% (95% C.I. 74-91) could be achieved. If the threshold was increased to 288 seconds a sensitivity of 100% could be achieved but this compromised the specificity by reducing it to 60.5%. If the threshold was reduced to 60 seconds the sensitivity was reduced to 72% with a small improvement in specificity to 86%. See tables 33 and 36 and figure 35.

<table>
<thead>
<tr>
<th></th>
<th>Acetowhitening time of 142 seconds</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>97%</td>
<td>89-100</td>
</tr>
<tr>
<td>Specificity</td>
<td>84%</td>
<td>74-91</td>
</tr>
</tbody>
</table>

*Table 33: Sensitivity and specificity of acetowhitrning for neoplasia*
13.12: ROC curve: Differentiation of HGD from IMC and invasive cancer using aceto-whitening timings

A ROC curve was produced for HGD versus IMC/invasive cancer. The Area under the curve was 0.829. The asymptomatic significance was 0.000 (95% C.I. 0.703-0.95). If the threshold timing is set at 30 seconds a sensitivity of 74% (95% C.I. 49-91) and specificity of 82% (62-94) is seen. If a cut off timing is increased to 51 seconds, a sensitivity for cancer of 90% and specificity of 54% could be achieved. If the threshold is changed to 23 seconds a sensitivity of 63% and specificity of 85% is seen. See table 34 and figure 36.
Table 34: Sensitivity and specificity for acetowhite-ning HGD vs Cancer

<table>
<thead>
<tr>
<th></th>
<th>Acetowhitenig threshold of 30 seconds</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>74%</td>
<td>49-91</td>
</tr>
<tr>
<td>Specificity</td>
<td>82%</td>
<td>62-94</td>
</tr>
</tbody>
</table>

Figure 36: ROC curve HGD vs Cancer

Diagonal segments are produced by ties.
13.13: ROC curve: Differentiation of HGD+IMC from invasive cancer using acetowhitenning timings

A ROC curve was produced for HGD + IMC versus invasive cancer. The asymptomatic significance was 0.004. The area under the curve was 0.786 (61-96). This demonstrated that using a cut off of 20 seconds, a sensitivity for invasive cancer of 67% (95% C.I. 35-90%) and specificity of 85% (69-95%) could be achieved. If the timing threshold was increased to 41 seconds a sensitivity of 75% and specificity of 62% was obtained. If the cut off was decreased to 11 seconds a sensitivity of 58% and specificity of 97% was seen. See table 35 and figure 37.

<table>
<thead>
<tr>
<th>Table 35</th>
<th>Acetowhitenning threshold of 20 seconds</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>67%</td>
<td>35-90</td>
</tr>
<tr>
<td>Specificity</td>
<td>85%</td>
<td>69-95</td>
</tr>
</tbody>
</table>

Table 35: HGD and IMC vs Invasive cancer
Figure 37: ROC curve HGD+IMC vs Invasive Cancer

Diagonal segments are produced by ties.
<table>
<thead>
<tr>
<th>Positive if Less Than or Equal To</th>
<th>Sensitivity</th>
<th>1 – Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td>3.5000</td>
<td>.022</td>
<td>.000</td>
</tr>
<tr>
<td>4.5000</td>
<td>.043</td>
<td>.000</td>
</tr>
<tr>
<td>7.0000</td>
<td>.109</td>
<td>.000</td>
</tr>
<tr>
<td>9.5000</td>
<td>.130</td>
<td>.000</td>
</tr>
<tr>
<td>11.0000</td>
<td>.174</td>
<td>.000</td>
</tr>
<tr>
<td>13.0000</td>
<td>.196</td>
<td>.000</td>
</tr>
<tr>
<td>14.5000</td>
<td>.217</td>
<td>.012</td>
</tr>
<tr>
<td>16.0000</td>
<td>.261</td>
<td>.012</td>
</tr>
<tr>
<td>18.5000</td>
<td>.283</td>
<td>.012</td>
</tr>
<tr>
<td>22.5000</td>
<td>.348</td>
<td>.012</td>
</tr>
<tr>
<td>25.5000</td>
<td>.348</td>
<td>.023</td>
</tr>
<tr>
<td>27.0000</td>
<td>.391</td>
<td>.035</td>
</tr>
<tr>
<td>29.0000</td>
<td>.413</td>
<td>.035</td>
</tr>
<tr>
<td>33.5000</td>
<td>.457</td>
<td>.035</td>
</tr>
<tr>
<td>39.5000</td>
<td>.478</td>
<td>.035</td>
</tr>
<tr>
<td>43.5000</td>
<td>.478</td>
<td>.047</td>
</tr>
<tr>
<td>47.0000</td>
<td>.565</td>
<td>.058</td>
</tr>
<tr>
<td>49.5000</td>
<td>.587</td>
<td>.070</td>
</tr>
<tr>
<td>51.5000</td>
<td>.630</td>
<td>.070</td>
</tr>
<tr>
<td>54.0000</td>
<td>.674</td>
<td>.070</td>
</tr>
<tr>
<td>55.5000</td>
<td>.674</td>
<td>.081</td>
</tr>
<tr>
<td>57.5000</td>
<td>.696</td>
<td>.081</td>
</tr>
<tr>
<td>59.5000</td>
<td>.717</td>
<td>.093</td>
</tr>
<tr>
<td>62.5000</td>
<td>.761</td>
<td>.140</td>
</tr>
<tr>
<td>67.5000</td>
<td>.783</td>
<td>.140</td>
</tr>
<tr>
<td>71.0000</td>
<td>.826</td>
<td>.151</td>
</tr>
<tr>
<td>72.5000</td>
<td>.848</td>
<td>.151</td>
</tr>
<tr>
<td>73.5000</td>
<td>.848</td>
<td>.163</td>
</tr>
<tr>
<td>76.0000</td>
<td>.870</td>
<td>.163</td>
</tr>
<tr>
<td>79.5000</td>
<td>.891</td>
<td>.163</td>
</tr>
<tr>
<td>85.5000</td>
<td>.913</td>
<td>.163</td>
</tr>
<tr>
<td>115.0000</td>
<td>.957</td>
<td>.163</td>
</tr>
<tr>
<td>141.0000</td>
<td>.978</td>
<td>.163</td>
</tr>
<tr>
<td>148.0000</td>
<td>.978</td>
<td>.174</td>
</tr>
<tr>
<td>164.0000</td>
<td>.978</td>
<td>.186</td>
</tr>
<tr>
<td>177.5000</td>
<td>.978</td>
<td>.198</td>
</tr>
<tr>
<td>195.5000</td>
<td>.978</td>
<td>.209</td>
</tr>
<tr>
<td>216.5000</td>
<td>.978</td>
<td>.221</td>
</tr>
<tr>
<td>230.5000</td>
<td>.978</td>
<td>.233</td>
</tr>
<tr>
<td>239.0000</td>
<td>.978</td>
<td>.244</td>
</tr>
<tr>
<td>Positive if Less Than or Equal To</td>
<td>Sensitivity</td>
<td>1 – Specificity</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------</td>
<td>----------------</td>
</tr>
<tr>
<td>244.0000</td>
<td>.978</td>
<td>.279</td>
</tr>
<tr>
<td>251.5000</td>
<td>.978</td>
<td>.291</td>
</tr>
<tr>
<td>257.5000</td>
<td>.978</td>
<td>.302</td>
</tr>
<tr>
<td>263.5000</td>
<td>.978</td>
<td>.314</td>
</tr>
<tr>
<td>268.0000</td>
<td>.978</td>
<td>.326</td>
</tr>
<tr>
<td>269.5000</td>
<td>.978</td>
<td>.337</td>
</tr>
<tr>
<td>274.5000</td>
<td>.978</td>
<td>.372</td>
</tr>
<tr>
<td>282.0000</td>
<td>.978</td>
<td>.384</td>
</tr>
<tr>
<td>286.5000</td>
<td>.978</td>
<td>.395</td>
</tr>
<tr>
<td>288.5000</td>
<td>1.000</td>
<td>.395</td>
</tr>
<tr>
<td>294.5000</td>
<td>1.000</td>
<td>.407</td>
</tr>
<tr>
<td>301.0000</td>
<td>1.000</td>
<td>.477</td>
</tr>
<tr>
<td>306.5000</td>
<td>1.000</td>
<td>.488</td>
</tr>
<tr>
<td>319.5000</td>
<td>1.000</td>
<td>.512</td>
</tr>
<tr>
<td>329.0000</td>
<td>1.000</td>
<td>.523</td>
</tr>
<tr>
<td>331.5000</td>
<td>1.000</td>
<td>.535</td>
</tr>
<tr>
<td>335.5000</td>
<td>1.000</td>
<td>.547</td>
</tr>
<tr>
<td>344.0000</td>
<td>1.000</td>
<td>.570</td>
</tr>
<tr>
<td>350.5000</td>
<td>1.000</td>
<td>.593</td>
</tr>
<tr>
<td>352.5000</td>
<td>1.000</td>
<td>.605</td>
</tr>
<tr>
<td>354.5000</td>
<td>1.000</td>
<td>.616</td>
</tr>
<tr>
<td>357.5000</td>
<td>1.000</td>
<td>.628</td>
</tr>
<tr>
<td>363.0000</td>
<td>1.000</td>
<td>.651</td>
</tr>
<tr>
<td>380.0000</td>
<td>1.000</td>
<td>.663</td>
</tr>
<tr>
<td>395.5000</td>
<td>1.000</td>
<td>.674</td>
</tr>
<tr>
<td>397.5000</td>
<td>1.000</td>
<td>.686</td>
</tr>
<tr>
<td>400.0000</td>
<td>1.000</td>
<td>.698</td>
</tr>
<tr>
<td>406.5000</td>
<td>1.000</td>
<td>.709</td>
</tr>
<tr>
<td>413.5000</td>
<td>1.000</td>
<td>.721</td>
</tr>
<tr>
<td>418.0000</td>
<td>1.000</td>
<td>.733</td>
</tr>
<tr>
<td>425.0000</td>
<td>1.000</td>
<td>.744</td>
</tr>
<tr>
<td>435.5000</td>
<td>1.000</td>
<td>.756</td>
</tr>
<tr>
<td>443.0000</td>
<td>1.000</td>
<td>.779</td>
</tr>
<tr>
<td>449.0000</td>
<td>1.000</td>
<td>.791</td>
</tr>
<tr>
<td>458.5000</td>
<td>1.000</td>
<td>.802</td>
</tr>
<tr>
<td>472.0000</td>
<td>1.000</td>
<td>.814</td>
</tr>
<tr>
<td>488.5000</td>
<td>1.000</td>
<td>.837</td>
</tr>
<tr>
<td>Positive if Less Than or Equal To(^a)</td>
<td>Sensitivity</td>
<td>1 – Specificity</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td>518.5000</td>
<td>1.000</td>
<td>.849</td>
</tr>
<tr>
<td>546.0000</td>
<td>1.000</td>
<td>.860</td>
</tr>
<tr>
<td>564.0000</td>
<td>1.000</td>
<td>.872</td>
</tr>
<tr>
<td>580.0000</td>
<td>1.000</td>
<td>.884</td>
</tr>
<tr>
<td>588.0000</td>
<td>1.000</td>
<td>.895</td>
</tr>
<tr>
<td>596.0000</td>
<td>1.000</td>
<td>.907</td>
</tr>
<tr>
<td>610.5000</td>
<td>1.000</td>
<td>.919</td>
</tr>
<tr>
<td>622.5000</td>
<td>1.000</td>
<td>.930</td>
</tr>
<tr>
<td>630.5000</td>
<td>1.000</td>
<td>.942</td>
</tr>
<tr>
<td>652.5000</td>
<td>1.000</td>
<td>.953</td>
</tr>
<tr>
<td>686.0000</td>
<td>1.000</td>
<td>.965</td>
</tr>
<tr>
<td>834.0000</td>
<td>1.000</td>
<td>.977</td>
</tr>
<tr>
<td>978.0000</td>
<td>1.000</td>
<td>.988</td>
</tr>
<tr>
<td>993.0000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

\(a\). The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.
13.14: Discussion

This study demonstrates that the aceto-whitening time is an important tool for the diagnosis of high risk neoplasia within Barrett's. It has shown that by using a threshold of 141 seconds, a sensitivity for all neoplasia of 97% can be achieved with a specificity of 84%. This makes the aceto-whitening timing an excellent diagnostic tool for the identification of high risk neoplasia. However, it is also possible to use the technique to further characterise the neoplastic area to predict the presence of sub-mucosally invasive disease. By examining the curve with the differentiation of mucosal neoplasia from invasive cancer as the focus, a specificity for invasive cancer of 85% can be achieved by using a threshold timing of 20 seconds. Whilst the possibility that the lesion is not invasive cannot be excluded if the aceto-whitening disappears after this time (sensitivity 67%), the probability that the lesion is invasive (and hence not endoscopically resectable) is very high if the aceto-whitening disappears before 20 seconds. This is more effective than other proposed methods of predicting depth of invasion such as endoscopic ultrasound (139) (140) (141) (142).

The exact cut off point for metaplasia vs neoplasia and mucosal neoplasia vs invasive disease is a trade off between sensitivity and specificity. As demonstrated in the results the values quoted are the optimal cut offs for each assessment. Practically the vast majority of neoplasia becomes apparent within 90 seconds. However, by observing for 141 seconds more subtle but significant findings do occasionally become apparent. Waiting for 288 seconds (nearly 5 minutes) is likely to add so little and result in so many over called areas that this approach cannot be
recommended. Likewise, invasive cancer can take longer than 20 seconds to appear. Disappearance over this threshold therefore does not mean definitively that a lesion is intramucosal (and therefore endoscopically treatable). However, it does provide the endoscopist with additional valuable information to stratify risk. The ideal points will always involve a degree of compromise, which is inherent in this kind of numerical test. It should be noted that surface pattern assessment also involves judgement calls, but these will always be more subjective and therefore difficult to reproduce.

13.15: Strengths of the study

The development of this tool provides an objective tool for examining areas of neoplasia. The traditional assessment of surface patterns is difficult, subjective and ideally requires optical magnification. There is a learning curve associated with this and a question remains regarding reproducibility of such assessments. In contrast, the timing for the disappearance of aceto-whitening is an objective numerical measure with hard end points. This is easy to interpret by any endoscopist and can be translated into clinical practice with minimal training. Furthermore, it can be used without optical magnification.

Acetic acid is an important tool in the assessment of Barrett’s. There have been two large studies published recently which have suggested that acetic acid is an effective tool for localising neoplasia. A German study of 701 patients found a sensitivity of 96.7% and specificity of 66.5% for dysplasia (143). The other published study is described in this thesis (138). This large study conducted by the Portsmouth
research group in the United Kingdom consisted of 190 procedures. Acetic acid identified invisible dysplasia or cancer 2.2x more frequently than WLI, with a sensitivity of 97% and specificity of 80% for the detection of dysplasia or cancer. Both of these studies were conducted without the benefit of this data on how best to exploit the aceto-whitening reaction. It is possible that by combining surface pattern assessment with the differential in the aceto-whitening that the sensitivity and specificity could be improved further. This is an important area for future research.

13.16: Limitations

A limitation of this study is that the moment of disappearance of the acetowhitening is subjective and dependent on a single endoscopist's interpretation. However, it was found that in practice once the area started to lose the whitening it changed over a matter of a few seconds. Therefore this is unlikely to significantly impact on the findings. The study has a pragmatic practical design which reflects how endoscopy is practiced. In order for the acetowhitening reaction to be clinically exploited, a simple measurement has to be used, or it could not be applied on busy lists. A simple timing can easily be performed by every endoscopist in any unit without the need for specialist equipment.

In a similar way to surface pattern assessment the aceto-whitening timing is affected by co-existing inflammation. As such there is a false positive rate associated with the test. It is necessary therefore to completely treat inflammation prior to assessment. Furthermore, it is not effective in the accurate identification of low grade neoplasia, where the accuracy is just 33%. However, this is not such a major issue. It is the
identification of high risk neoplasia which alters management, and it is here that the aceto-whitening timings are very effective. Therefore the focus of the study was on the differentiation of high risk neoplasia from metaplasia, and high grade dysplasia from invasive cancer. The diagnosis of high grade neoplasia changes management from observation to treatment, and the diagnosis of invasive cancer changes management from endoscopic treatment to surgical care.

13.17: Implications for clinical practice

A concern for all endoscopists who treat Barrett’s neoplasia within Barrett’s has been the consequences of failing to correctly identify submucosally invasive cancer. These lesions cannot be cured by endoscopic mucosal resection and if resection is attempted the risk of perforation is high. If ablative techniques are used such as radio-frequency ablation the consequences can be even worse as no tissue sample is obtained and the patient may be denied potentially curative surgery on the mistaken belief that the disease has been cured. Until now there has been no techniques which could predict depth of invasion of a lesion. This study would suggest that the aceto-whitening time is a specific indicator for invasive disease. Whilst a longer time to disappearance of aceto-whitening does not exclude submucosally invasive disease, a rapid disappearance should be considered to be concerning and raise a high index of suspicion that a lesion is advanced and potentially not curable by an endoscopic approach.
13.17: Future research needs

There is a need for a multicentre, multiendoscopist study to verify these findings. If confirmed this could form the basis of the development of an endoscope based technology to automate the test. All of the modern endoscopes capture data digitally using a charge coupled device (CCD). Most can measure in some form the spectral reflectance from tissues. It would not require a significant change in endoscope design to be able to objectively measure the parameters described in this study and automatically alarm when whitening had disappeared in an area. In the case of Fujinon and Pentax endoscopes, which already contain micro-processors designed for image processing, a software update may be all that is required. Whilst this is some way away it provides a starting point for future research. This study does not attempt to address how the aceto-whitening reaction occurs and why there is a differential in the timings. It can be speculated that neoplastic tissue is highly vascular, and that in turn results in early reversal of the changes observed, but this is not proven. This data calls for further research into the mechanism behind the aceto-whitening reaction.

13.19: Conclusions

Understanding of the aceto-whitening reaction has been limited. This study addresses this apparent deficiency in the literature and by exploiting the aceto-whitening reaction, it is possible to both localise and predict the severity of neoplastic change. It is likely that this phenomenon can be exploited to improve the sensitivity and specificity of acetic acid chromoendoscopy in the detection of high risk neoplasia.
PART C

Summary of the work
Chapter 14

The potential clinical impact of the studies
14.1: Significance of the results

The central ethos behind this thesis has been around the concept of clinical judgement during endoscopy. In clinical practice it is a central role of the doctor to be able to interpret clinical symptoms and signs and make a diagnosis based on objective criteria. In the field of endoscopy however this process has become diluted. In the assessment of colonic polyps standard practice has been to just remove lesions and send to histopathology for a diagnosis. Likewise, in the oesophagus, assessment of Barrett’s has been based around mapping biopsy on the theory that the endoscopist cannot make a diagnosis of early neoplasia and requires a pathologist to do this. Historically the reasons for this are clear. Early endoscopes had a poor image quality and only gross abnormalities could be seen. Therefore the best which one could hope to achieve was to take a biopsy. However, this position has changed. In an era of high resolution endoscopy, vascular enhancement, magnifying endoscopes and chromoendoscopy a wealth of diagnostic possibilities have been opened. However, they will not be adopted unless tools for their use can be developed and evidence that they work produced. This is analogous to the work performed into bedside clinical examination by the 19th century physicians which is so central to modern practice today. The studies described in this thesis go some way towards addressing this issue, by examining the emerging roles of advanced diagnostic techniques in the detection and examination of neoplasia in the upper and lower gastrointestinal tract. The central theme has been to empower the endoscopist by providing a set of evidence based tools which can be easily learnt and applied in routine Western practice.
14.2: Colonic neoplasia studies

A new and simple to use polyp classification system has been developed. This has been designed for use by Western endoscopists who are unfamiliar with the complexities of the Japanese polyp classification systems and with the standard non magnifying endoscopes, which are the standard of care in Western practice. It utilises the FICE vascular enhancement system which is currently under researched and until now has lacked any validated tools.

This tool was developed using a picture library and then validated with an in-vivo study. It was then shown to work effectively in a large prospective series in a Bowel Cancer Screening population. This not only demonstrated that the classification system worked, but also validated the concept that picture based studies are a good surrogate marker for in-vivo assessment. This is important as it provides a model for the development of future tools for in-vivo assessment using other devices.

The studies described in this thesis have demonstrated that small colonic polyps<10mm can be examined in more detail than previously thought and that an accurate prediction of histology can be made with a non magnifying endoscope. This has significant cost implications within the National Bowel Cancer Screening Programme. The studies have also demonstrated the role of high definition endoscopes. There is a benefit from using the newer high definition scopes if electronic vascular enhancement techniques are to be used but are of no additional benefit over older standard definition equipment if dye spray assessments with
indigocarmine are to be performed. This has the potential to form the basis of guidelines for endoscopists as to which method should be used to perform an in-vivo diagnosis dependent on equipment available. The research addresses the key standards proposed by the ASGE in their recent PIVI that a technology needs to meet in order to be used for in-vivo histology prediction for colonic polyps (10).

The cost implications should not be underestimated. These studies have demonstrated that just within the U.K. National Bowel Cancer Screening Programme £678,253 could be saved per annum. This figure is consistent with predictions made by previous studies based on mathematical models (68). It is a reality that all health care systems are under financial pressure. If costs can be reduced without harm to patients, then there is a duty for health care providers to do so. What is an acceptable degree of accuracy when assessing polyps<10mm in size? It is important to understand that small adenomas less than 10mm in size are low risk lesions. Every day clinicians are making judgement calls, from the GP who sees a patient with a headache to the surgeon who examines a patient with abdominal pain. For safe and effective management, this is essential. The system would collapse if everyone with a headache was sent for a computed tomography scan to exclude a brain tumour. Indeed, it could be argued that over investigation can actually cause harm, both physical and psychological. Conceptually the situation is no different for the endoscopist, and proposals in this thesis are really no different. Is it not reasonable that an endoscopist, empowered with the appropriate evidence based tools, should be expected to be able to make a diagnosis and a clinical decision on such low risk lesions?
It is important to understand that the benefits in making an in-vivo diagnosis are not simply of economic interest. In the U.K. there is a delay of anything from one to six weeks for biopsy specimens to be reported on, depending on urgency of request. During this period patients can experience considerable anxiety wondering what the lesion is. Likewise, if the endoscopist is uncertain of the significance of a lesion then an inappropriate treatment might be undertaken (e.g. removal of a cancer by an inadequate technique) which could impact on future management. Furthermore, most pathology departments are already overburdened with work. It is questionable whether it is a good use of the histopathologists time to be reporting on small polyps of low malignant potential if an accurate in-vivo diagnosis can be made by the endoscopist. Freeing the pathologist up from this low risk, high volume work would enable more time to be spent on examining high risk lesions such as multipiece endoscopic mucosal resection specimens from large polyps, where the risk of invasive disease is much higher.

14.3: The challenge

14.3.1: Medico-legal issues

A common concern raised by endoscopists is the medico-legal implications of in-vivo diagnosis. These are of course entirely justified. Without appropriate tools for assessment, training in their use, and guidance on how to apply them in routine clinical practice, this kind of change in practice cannot occur. The ASGE PIVI has gone a long way in developing a framework of standards for in-vivo diagnosis. This
will provide the endoscopist with protection by defining what an acceptable level of accuracy is. The work in this thesis addresses some of the issues around validated assessment tools for this purpose and identifies tools which meet the defined standards. Electronic image capture systems will enable high quality images of the discarded lesions to be kept, which could be reviewed at a later date if a query over the diagnosis is raised. A key priority for the national and international endoscopy associations should be to assemble the evidence as it becomes available to guide clinicians in the application of new advanced techniques as they become available.

14.3.2: Training

New innovation brings with it new challenges. A question which can be raised regarding *in-vivo* diagnostic techniques relates to the learning curve for acquiring the skills. The studies described in this thesis cannot answer this question as they have been conducted by a single skilled endoscopist. It is an area where more work is urgently needed. There have however been some preliminary studies which suggests that the techniques for examining colonic neoplasia are not particularly difficult to learn and can be acquired using picture based training programmes in as little as 20 minutes (131). Whilst this position may be optimistic, it has been the case since the early days of modern clinical practice that clinicians have to be prepared to learn new skills and adopt new practices as evidence dictates. In the United Kingdom there have been considerable strides taken in recent years to improve the quality of gastrointestinal endoscopy, with quality standards set for completion rates and polyp detection rates. A similar position is seen in many countries across Europe and in the United States. This is a similar position to that seen in surgical procedures.
and it is accepted that endoscopists must be prepared to audit their completion rates and demonstrate competence to practice. In the U.K. Bowel Cancer Screening Programme endoscopists are even more tightly controlled, with additional examinations required before being appointed as screening practitioners. Therefore the necessity for training should not be seen as a barrier but as an opportunity to provide a better service.

14.3.3: Tools

Training requires effective, validated and easy to use training tools. As previously described, all of the validated classification methods for indigo carmine and FICE have been developed for use with magnification endoscopes. Magnifying endoscopes are not in common practice in the U.K. They are perceived as bulky, cumbersome and difficult to use and most endoscopists do not like their handling characteristics. They are also expensive to purchase. In order for effective training to be delivered it is therefore essential to have robust assessment tools which are validated for use with the tool the endoscopist will actually be using. In the U.K. Western Europe and North America this is realistically a standard endoscope without optical magnification. The development of the N.A.C. system goes some way towards addressing this problem as it is a validated and relatively simple tool which is designed for use without optical magnification. The work in this thesis has demonstrated that these assessments can be performed on standard western lists without increasing procedure time.
14.3.4: Culture

Within Western practice there has been a tendency towards believing that a good examination is a quick one. This is reflected by the densely packed lists seen in general endoscopic practice. This may have been acceptable when the aim was to simply identify gross pathology, but for screening it is necessary to identify subtleties. It is for this very reason that every Bowel Cancer Screening list is limited in terms of the number of procedures which can be put onto it. However, it is not enough to simply reduce the number of procedures on a list and to expect clinicians to change working practices which have been developed over decades. At present, endoscopy is driven by a biopsy dependent culture. In order for management to improve it is necessary to adopt a new philosophy of ‘see more, biopsy less’, where the endoscopist is actively engaging in making a diagnosis. The concept is not intended to render the pathologist redundant. It does however require a mindset where the endoscopist accepts that diagnosis should be seen as a collective responsibility, and intelligently targets and selects what is sent for histopathological examination, thereby making best use of resources and ensuring that the best chance of achieving a correct diagnosis is achieved.

The development of new in-vivo diagnostic techniques can help in overcoming these challenges. They provide the endoscopist with the tools to identify and characterise subtle pre malignant and early cancerous lesions, and also provide a method to reduce expenditure in confirming that a non-neoplastic lesion is indeed harmless. This is a very important concept. Investment in training of clinicians in new techniques has to be justifiable in terms of both benefit to patients and cost.
effectiveness. Sometimes a new innovation only offers an advantage in one of these areas making uptake difficult. In the current challenging economic climate, health economists have to feel confident that introduction of a new test is financially sustainable. Therefore it is a reality of modern practice that in proposing a new diagnostic paradigm, attention is paid to this issue.

14.4: Future research needs

There is a need for multi-centre, multi endoscopist studies to assess the applicability and reproducibility of colonic in-vivo histology prediction using both vascular enhancement techniques and indigocarmine dye spray on a wider basis. It is generally accepted that whilst single centre studies are best suited to prove the effectiveness of a technique, these results can be difficult to achieve with a larger group of clinicians and pose different challenges. Furthermore, additional studies into learning curves for these techniques should be undertaken to establish how best to train endoscopists in the future. In many ways these two areas should be conducted in tandem, as to conduct multi-centre studies will require many more endoscopists performing in-vivo diagnosis and it would be a golden opportunity to demonstrate how long or arduous the learning curve actually is. These studies need to be undertaken on busy lists that are applicable to real world practice if the studies are to be taken seriously, and should use endoscopes from a variety of equipment manufacturers.

It is important to stress that the development of vascular enhancement techniques has opened up a new research challenge in endoscopy. The three main manufacturers (Olympus, Fujinon and Pentax) have all introduced spectral imaging
technology into their equipment, but they all work quite differently. Therefore it is generally accepted that results from one system cannot be applied to the other systems (55). It could be argued further that it is dangerous to extrapolate from one system to another as it could leave the endoscopist with a false reassurance that a particular technique was more effective than it actually is. To date, the vast majority of the research has been published on Olympus Narrow Band Imaging. This thesis helps to address this disparity but there is still some distance to be covered until all of the differences between the systems are completely understood. In particular there is a lack of evidence for the Pentax i-scan technology. This system is unique in having a 1.3 million pixel CCD as standard, and given the difference in the diagnostic capabilities of FICE using high definition endoscopes compared to standard definition endoscopes, it will be of interest to know what capabilities are seen with this very high resolution system. Studies need to be conducted comparing NBI with FICE and i-scan, with and without optical magnification to establish if one system is superior for the purposes of in-vivo diagnosis. It is also necessary to compare vascular enhancement techniques to confocal endomicroscopy. There has been one study performed which has done this (57), but this was underpowered and combined NBI and FICE into a single category. The results using vascular enhancement techniques were disappointing and not in keeping with other studies.
14.5: Barrett's neoplasia studies

The studies in this thesis have demonstrated that acetic acid is an effective tool for diagnosing and localizing neoplasia within a high risk Barrett’s population. This challenges the current paradigm in Barrett’s surveillance which is based around non-targeted mapping biopsies. Furthermore, the acetowhining reaction can be utilised diagnostically to improve the sensitivity and specificity in improving this test. This is an objective numerical assessment tool which is simple to learn and apply to routine practice. Furthermore, it has been shown to have potential for differentiating high grade dysplasia from intramucosal cancer and from invasive cancer. This is very important. Whilst high grade dysplasia and intramucosal cancer are endoscopically treatable invasive cancer is not. There are currently no other tools available which can do this. The acetowhining timings have been shown to be effective for this task. Whilst not 100% accurate they provide the endoscopist with much more information than previously thought possible and so empowers him or her to make a better decision as to how to best treat the patient.

14.6: The challenge

The current culture in Barrett’s surveillance is biopsy driven. If the endoscopist could ‘see more and biopsy less’ then more effective surveillance could potentially be performed. By targeting neoplastic lesions using acetic acid chromoendoscopy it may be possible to improve both screening efficacy and the cost effectiveness of surveillance by reducing the number of biopsy cassettes required for each patient. However, this requires a shift away from a protocol biopsy driven culture.
Changing the current mind set of endoscopists away from the current biopsy driven culture will not be an easy challenge. Even with evidence based tools, there will be a reluctance to move away from the established standard of care which endoscopists feel comfortable using, and it should be recognised that it will take time for such concepts to become fully adopted. It is likely that before endoscopists become confident to adopt a ‘see more and biopsy less’ strategy that a move towards ‘see more and biopsy more’ will occur, where a combination of advanced imaging and biopsies will be used. This should not be discouraged. It is by observing the accuracy and usefulness of advanced imaging with the additional safety of biopsies that the endoscopist will gain confidence in to adopt new techniques. There may also be a generational effect, where newly trained clinicians are trained from the ground up, with a different mind set being more willing to embrace this new philosophy.

It is important to understand that a change in approach is needed. As discussed in chapter 3, there have been concerns raised regarding the efficacy and cost effectiveness of Barrett’s screening, and the question has been raised as to whether it is of any benefit at all (6). Because of this, studies have been commissioned to investigate this issue, including the multicentre Barrett’s oesophagus surveillance study (BOSS) based in the United Kingdom. Biopsy forceps are small compared to the overall surface area of Barrett’s metaplasia and even the most intensive protocols only examine 3% of the total Barrett’s present. It is therefore not surprising that small neoplastic foci can be missed by such methods, making surveillance ineffective. The analogy would be to find a needle in a haystack by randomly sampling the hay. Therefore a localisation method is critically needed. The BOSS study may not answer the question which needs to be answered as it is working
along the principle of protocol driven biopsy based surveillance, and a better question of whether the cost benefit profile of surveillance can be improved by using an enhancement technique such as acetic acid chromoendoscopy is not being addressed. Much of the expenditure in screening endoscopy is on the processing of pathology specimens. It is important to appreciate that reimbursement for pathology costs is on a per cassette basis. To follow Seattle or Cleaveland clinic protocols effectively requires each biopsy to be sent in a separate cassette (so that an abnormality can be localised). This can amount to anything between 8-40 cassettes per patient. For surveillance programmes to be sustainable they need to detect pathology in a cost effective manner. The use of acetic acid targeted biopsies in place of mapping biopsies could reduce the number of biopsies required and so reduce the pathology related costs of screening.

14.6.1: Training in chromoendoscopy techniques for lesion recognition

Training in lesion recognition and assessment is essential if in-vivo diagnosis is to become successful. Barrett’s assessment poses particular challenges in this respect. As described previously, bowel cancer screening is performed by expert consultant colonoscopists who have to complete mandatory additional training and assessment prior to undertaking screening lists. In contrast, Barrett’s surveillance is typically delegated to more junior staff, with the majority now performed by nurse endoscopists. Furthermore, the number of assessments performed per year can be low. For training to be successful and cost effective, it will be necessary to have both validated and simple to use assessment tools, but also to rationalise who and how many endoscopists perform the assessments.
It is important to understand that it is the lesion recognition skills which take time and effort to acquire, not the practical skills of flushing dye down the endoscope. This may seem obvious, but it highlights one of the fundamental challenges in a move towards in-vivo diagnosis. There is a culture amongst clinicians to see endoscopy as a practical skill, and that endoscopy training should involve being taught a practical technique. However, it is a knowledge base which has to be developed to make in-vivo diagnosis safe, and this needs to be based on robust and reproducible evidence.

14.6.2: Mass application of the techniques

If acetic acid enhanced chromoendoscopy is to be applied to the screening population on a national basis, a change in the way screening is approached is necessary. Rather than the current paradigm, it is likely that screening on expert Barrett’s lists in high volume specialised centres, run by a small number of trained individuals, would represent the most cost effective way forward. The clinicians performing these procedures would be exposed to neoplasia on a regular basis, and could maintain the skills in a similar manner to that seen in Bowel Cancer Screening. Furthermore, the lists could then be set up with the appropriate equipment to conduct the procedures effectively and would avoid the hurdle of all endoscopy units needing to invest in this respect. Auditing of outcome would be easier and it would make the identification of potential problems easier.
14.7: Future research needs

14.7.1: Acetic acid in a surveillance population

There is a clinical need for more studies investigating the role of acetic acid in a Barrett’s surveillance population. This poses a particular challenge. The data presented in this thesis comes from a high risk group where the prevalence of neoplasia is high. From a skills perspective this means that the endoscopist assessing these patients is exposed to neoplasia on a regular basis and is therefore constantly seeing the subtle changes associated with high risk lesions. In a low risk surveillance population the prevalence would be less by a factor of 10. It is not known if the results described in this thesis will be equally good in a surveillance population. However, there is an urgent need for research into the use of acetic acid to improve the gain for Barrett’s surveillance. As described previously, Barrett’s surveillance in the United Kingdom is largely performed by junior endoscopists. They are different to the clinicians examining high risk patients who are all consultants with an interest in early cancer diagnosis. Therefore any future studies looking at a surveillance population should be undertaken with this in mind and study design should utilise the kind of endoscopist who will be performing these procedures in mainstream practice. Again learning curves for lesion recognition have not been established and work needs to be done in this area.
14.7.2 Risk stratification of patients with Barrett’s

It is likely that the Barrett's population is a heterogeneous group with varying levels of risk, and what is needed for the future is a better way to risk stratify patients, so that high risk individuals can be offered more intensive surveillance, and low risk patients can be reassured and discharged from follow up. At present basic demographic factors are understood, including male gender, Caucasian origin, smoking history and length of Barrett's segment, but this is a crude measure. The development of molecular markers to flag risk is needed. There has been some work already done investigating nuclear atypia by flow cytometry in Barrett's biopsies (144) (145) and this may enable patients with metaplasia on biopsy to be further sub-divided. This is likely to be the tip of a very large iceberg. Circulating DNA markers and specific molecular markers that could be identified on a simple blood test are badly needed. If these factors could be used in combination, a risk profile could potentially be established. Advanced endoscopic imaging would also be important here. It is possible that acetic acid assisted chromoendoscopy for Barrett's assessment could be another tool in the risk stratification of patients. Future research is needed into the use of a one-off advanced endoscopy with acetic acid combined with biomarkers to risk stratify the patients.

14.7.3: Randomized controlled trial of acetic acid chromoendoscopy versus protocol guided mapping biopsies

There have not been any randomised controlled trials performed comparing acetic acid chromoendoscopy to protocol driven mapping biopsies. This is perhaps not surprising as the studies published had been so small that a randomised controlled
trial could not be clinically justified. The cohort studies presented in this thesis provide a justification for a randomised controlled tandem endoscopy study in a high risk population. This is important as positive results were initially seen using tri-modal imaging for the identification of Barrett’s neoplasia in cohort studies (104) but were not repeated in a multicentre tandem endoscopy study (58).

14.7.4 Acetic acid versus trimodal imaging and confocal endomicroscopy

There are currently several different techniques available for examining Barrett’s for the presence of neoplasia, including trimodal imaging and confocal endomicroscopy. It is currently however unclear which of these techniques is superior. Studies comparing these techniques are therefore needed.

14.7.5: Acetic acid and electronic imaging combined

The combination of acetic acid with electronic imaging has never been studied. Given that NBI, FICE and i-scan all enhance vascular areas, and that the loss of acetowhitening has been shown in this thesis to be central to the use of acetic acid as a diagnostic tool, it is not unreasonable to postulate that it may be possible to make the technique more sensitive by the additional use of electronic imaging after acetic acid dye spray. The concept of combining two techniques has already been proven to be of benefit in tri-modal imaging, and the concept here is not dissimilar. A potential problem with investigating this is that the sensitivity of acetic acid alone is already very good. Therefore a large study would be needed to show a statistically significant gain. However, even a small gain would be of clinical significance when
the consequence could be a missed early treatable cancer, so this kind of study is easy to justify.

14.7.6: Low grade dysplasia

Most of the work described so far has concerned high grade dysplasia within Barrett’s. This is perhaps unsurprising as it is the development of high grade dysplasia which alters management from surveillance to treatment. However, low grade changes should not be dismissed as unimportant. Diagnosis still necessitates increased intensity of surveillance and can cause significant anxiety and distress to the patient. Studies investigating whether chromoendoscopy, electronic imaging or confocal endomicroscopy have any role in the evaluation of low grade dysplasia are needed.
14.8: Conclusions

This set of studies demonstrates the feasibility of *in-vivo* diagnosis of neoplastic lesions in the upper and lower gastrointestinal tract using a combination of dye sprays and vascular enhancement techniques. It demonstrates and quantifies some of the differences between standard definition and high definition endoscopes. It also evaluates the potential financial impact that *in-vivo* diagnosis can make in selected populations of patients. The studies provide validated tools for lesion characterization and assessment and empower the endoscopist to act as a clinician and make a diagnosis, rather than as a technician who just collects biopsies. The tools used could potentially be applied to the assessment of other novel endoscopic devices and validate a method for such studies in the future.
Chapter 15

References
Bibliography


