EVALUATION OF INTESTINAL MOTILITY WITH MAGNETIC RESONANCE ENTEROGRAPHY AND COMPUTER POST-PROCESSING

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Author declaration

I, Alexander Menys confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Small bowel motility is an essential, physiological process central to the processing of ingested food. The small bowel is however anatomically and functionally complex, varying greatly between individuals and located deep within the abdomen making it extremely difficult to access with instrumentation. As a consequence, and in spite of its known or suspected role in a range of diseases, there remain little in the way of objective tests to evaluate or even observe this process in vivo.

This thesis details the validation and application of a novel computer post-processing technique that allows the quantification of Magnetic Resonance Enterography derived time-series image data. A background to small bowel physiology and existing techniques is first provided along with an introduction to the registration algorithm used throughout this thesis to quantify small bowel motility. The technique is then applied retrospectively to two Crohn's disease patient cohorts to explore how this inflammatory bowel disease influences contractility. A prospective evaluation of segmental motility analysis is then presented drawing attention to large within subject variation, in a cohort of healthy volunteers, as a limitation for this technique. As an alternative, a global motility analysis approach is described and validated. Although global measures of motility appeared robust, factors influencing clinical application are further addressed by expanding the technique to allow motility analysis in free-breathing data. In the final piece of research presented, the application of the global technique to a cohort of Chronic Intestinal Pseudo-Obstruction patients is detailed. The thesis is

concluded with a reflection of the results and a chapter dedicated to the commercial exploitation of the research to address the ongoing need for a robust test to quantise intestinal motility.

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First, I would like to thank Stuart Taylor who has tirelessly supported my PhD, reading the thousands of emails, editing the myriad versions of applications and papers and building, in me, both the confidence and ability to drive my research interests forward. I would similarly like to thank David Atkinson who has demonstrated the utmost patience, providing essential technical input as well as balanced and practical advice throughout this PhD. I was an exceptionally demanding student with little in the way of technical 'ability,' at least early on, and both Stuart and David have gone beyond the call of duty to direct and refine my efforts that now culminate with this thesis. I could not have asked for two finer mentors and cannot thank them enough.

A special mention must go to Dr Freddy Odille who developed the optic-flow registration algorithm for motility analysis. Undoubtedly, without this work the PhD could not exist. I would like to thank Dr Valentin Hamy both for his friendship, collaboration and good times throughout the PhD. I would like to thank all the other people who have helped me throughout my PhD including, Anton Emmanuel, Penny Gowland, Charles Knowles, Caroline Hoad, Shamaila Butt, Andrew Plumb, Asia Ahmed, Emma Helbren & Steve Halligan.

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Preface

The research described in this thesis was carried out at the Centre for Medical Imaging, University College London (UCL) and University College Hospital London (UCLH).

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A full list of publications, abstracts and funding awards can be seen in Appendix 1.

Ethical Approval Statement

Research Ethics Committee approval was sought and obtained for all research detailed in this thesis. All patients provided written, informed consent unless a waiver was in place. Chapters 2-4 used ethical approval entitled 'Data sharing with collaborative partners to develop computer aided detection for the assessment of small bowel using MRI,' REC reference: 10/H0720/91. Chapters 5-8 used the ethical approval entitled 'Post-hoc analysis of dynamic MRI sequences to establish descriptive metrics for small bowel motility in vivo,' REC reference: 11/LO/1634.

Abbreviations

ANOVA:	Analysis of Variance
AU:	Arbitrary unit
BA:	Bland-Altman
BH:	Breath Hold
BTFE:	Balanced Turbo Field Echo
CD:	Crohn's Disease
CI:	Confidence Interval
CIPO:	Chronic Intestinal Pseudo Obstruction
CMI:	Centre for Medical Imaging
CPM:	Contractions per minute
CRP:	C-Reactive Protein

CTI:	Colon Transit Index
DRAM:	Dual Registration of Abdominal Motion
eAIS:	Endoscopic Acute Inflammation Score
ESR:	Erythrocyte Sedimentation Rate
FB:	Free Breathing
FOV:	Field of View
LoA:	Limits of Agreement
MMC:	Migrating Motor Complex
MR:	Magnetic Resonance
MRE:	Magnetic Resonance Enterography
MRI:	Magnetic Resonance Imaging
OF:	Optical Flow
P:	Probability value
PACS:	Picture Archiving and Communication System
REC:	Research Ethics Committee
ROI:	Region of Interest
SB:	Small Bowel
SD:	Standard Deviation
SME:	Small to Medium Enterprise
TE:	Echo Time
TI:	Terminal Ileum
TPN:	Total parenteral nutrition
TR:	Repetition Time
TRE:	Target Registration Error

Thesis Overview

This thesis comprises of ten chapters grouped into four sections outlined below. All of the work is that of the author unless otherwise stated. The beginning of each chapter (with the exclusion of Chapter 1 that is literature review) will consist of a *study question*, *rational*, *hypothesis and aim(s)* along with a declaration of published work.

Section A summarises the literature and background underlying this PhD thesis and introduces the core registration technique used throughout. In **Chapter 1**, an overview is provided of the basic physiology alongside a literature review of the available methods for small bowel motility analysis with a specific focus on emerging techniques using MRI and the landmark papers in this field. **Chapter 2** introduces the registration algorithm developed by Dr Freddy Odille and explains some of the key concepts underlying the analysis technique used through this thesis. The focus of this chapter lies primarily in the validation of the registration technique accuracy and ability to quantitatively grade motility.

Section B introduces the first clinical application of quantified motility assessment using Magnetic Resonance Enterography in two retrospective Crohn's disease cohorts. **Chapter 3** investigates the relationship between terminal ileal motility and inflammatory activity assessed against histopathological and anatomical measures and specifically explores motility as an independent marker of inflammation. **Chapter 4** evaluates more broadly motility changes in stricturing Crohn's disease

and the relation between bowel diameter and motility potential in obstructive episodes.

Section C focuses on prospective investigation in healthy individuals to establish normal ranges and address inherent methodological limitations identified in section B. **Chapter 5** presents data to assess the impact of intra-subject variability on segmental bowel motility assessment. Here it is shown that motility, even in healthy controls, is heterogeneous and a potential source of confirmation bias in the assessment of this form of analysis. **Chapter 6** directly addresses this limitation by presenting a novel method for global bowel motility assessment that removes the requirement to subjectively pick specific bowel loops for analysis. The global method is assessed for intra-reader variability, intra-subject variability and provides evidence for sensitivity using pro-kinetic and paralytic agents to provoke changes in motility.

Building on the results from section C, ***Section D*** addresses the clinical implementation of motility analysis. **Chapter 7** details an extension of the registration methodology to evaluate free-breathing acquisition series of not just the small bowel but also the colon broadening the applicability of the technique. **Chapter 8** goes on to recruit a cohort of Chronic Intestinal Pseudo-Obstruction patients and for the first time draws a direct comparison between healthy controls and a disease group in terms of their small bowel motility.

Section E Concludes this thesis with **Chapter 9** summarising key findings and discussing potential future objectives for the research. Finally **Appendix 4** presents the commercialisation strategy for the exploitation of this thesis.

SECTION A: INTRODUCTION TO SMALL BOWEL MOTILITY AND REGISTRATION AS METHODS OF QUANTITATIVE ASSESSMENT

Small bowel motility is a collection of contractile actions coordinated by the myenteric plexus or 'gut brain' in response to intrinsic and extrinsic factors. Behind this statement is a nervous system with more neurons that the spinal cord, a range of specific hormones that modulate the nature of the contractile actions and an inherent sensitivity to lumen contents and biophysical properties including stretch, that fundamentally influence the way in which the gut behaves. Conditions affecting motility are common and characterised clinically by pain, bloating and altered bowel habit and include both primary diseases of the gut like Crohn's Disease and also secondary involvement in a range of conditions including Parkinson's, diabetes and amyloidosis etc. Collectively, treating the GI symptoms of these diseases has a dramatic effect on the patients' quality of life but attracts a significant healthcare cost, estimated to be at £5.6 billion per year in the UK alone. A range of investigative techniques including manometry and scintigraphy have been developed and used over the past several decades to investigate motility in disease and health. Despite this, clinical uptake remains low and basic understanding of normality is largely absent. In addition, cost and low patient tolerability of existing techniques further inhibits widespread use of motility investigation as part of a clinical work up. Magnetic resonance enterography has become an increasingly popular method for

non-invasively evaluating small bowel motility. As scanner hardware and post-processing has improved, a range of novel methods that directly address existing limitations have emerged with the potential to fundamentally drive scientific research in this field.

Chapter 1 presents a literature review and up to date perspective on the techniques and background surrounding the assessment of gastrointestinal motility and highlights the key methodological limitations within this niche but expanding field. **Chapter 2** introduces a novel method for the objective evaluation of small bowel motility through the registration of 2D time-series images from Magnetic Resonance Enterography. The focuses lies specifically with the validation of the optic-flow algorithm developed by Freddy Odille and an explanation of the key methods used throughout this thesis to quantify small bowel motility.

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW OF SMALL BOWEL PHYSIOLOGY, MOTILITY AND METHODS OF ASSESSMENT

Rationale:

Small bowel motility has long been an active but limited area of research within human investigations complicated by the functional complexity of the bowel and its deep anatomical location. Invasive techniques like manometry can only reach the very distal or proximal regions of the small bowel and remain largely limited to use as a research tool. More recently, MRE has allowed the real-time 'cine' or dynamic imaging of the bowel with the high-resolution images then used to perform non-invasive, quantitative analysis. MRE therefore represents an important and clinically practical advance for the investigation of dysmotility in disease.

Aim(s):

i) Provide an overview of small bowel physiology and the clinical circumstances where motility assessment might be required.

ii) Summarise history and background of small bowel motility assessment and introduce MRI.

iii) Discuss the notable methods for motility assessment using MRE with reference to the current literature.

Physiology Background

The purpose of the small bowel is to absorb the nutrients from our food. This process occurs largely at the micro-level with digestive enzymes being used to break up and translocate the prerequisite components of our meal across the lumen into the mesenteric vasculature. Facilitating this process is the physiological action of the bowel itself, mechanically churning and homogenising intestinal contents to disperse digestive enzymes and propel the chyme through approximately 7m of bowel from the stomach towards the large intestine[1].

This motile action is broadly referred to as 'motility,' produced by the contraction of radial and longitudinal layers of smooth muscle cells within muscularis propria in the bowel wall (Figure 1.1)[1]–[4]. Smooth muscle, controlled by the pacemaking interstitial cells of cajal, will contract at a steady rate in the absence of stimulation; analogous in many ways to the myocardium[5]. Control comes through the operation of a 'neural break' by the myenteric plexus which can entirely inhibit contraction or generate an array of actions to manipulate the bowel contents specific to fasted and fed states summarised in **table 1.1** [6]–[10].

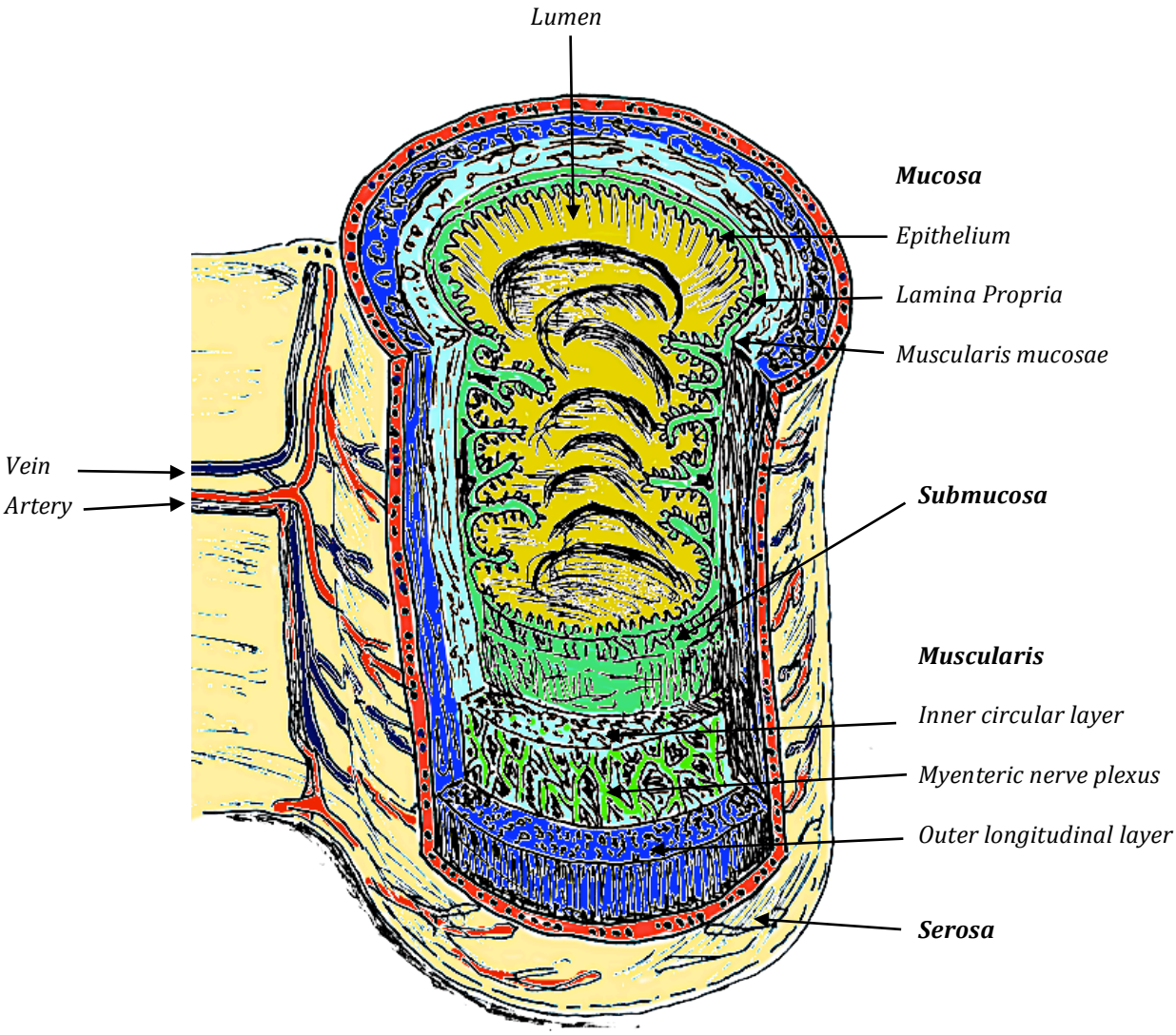

Figure 1.1 Cross section of the small intestine (authors own diagram).

Bowel state	*Contractile pattern*	*Duration*	*Length of Bowel Segment*
Postprandial	Stationary contraction	short	short
Postprandial	Peristaltic waves	short	long
Inter-digestive	Stationary cluster of contractions (Phase I)	long	short
Inter-digestive	Migrating cluster of contractions (Phase II)	long	short, migrating aborally
Inter-digestive	Migrating motor complex (Phase III)	long	long, migrating aborally

Table 1.1 *Summary of contractile actions present in the small bowel.*

A range of influences acting predominantly via the myenteric plexus function to modulate the frequency or pattern of motile actions, split largely into three groups; hormonal, dietary and neurological. These factors may act intrinsically (acetylcholine, tachykinin nitric oxide, glucagon-like protein, vasoactive intestinal peptide (VIP)), as well as extrinsically (motilin, insulin, adrenalin)[2], [3]. Food content further influences motility; glucose for example increases peristaltic rate and pH affects transit time. However much of the evidence surrounding direct dietary modulation of motility remains speculative[1], [11]. The third effector of motility, and perhaps the most obscure and difficult to study is the enteric nervous system itself, together with its interaction with the central nervous system[1], [3], [12], [13]. The basic doctrines of physiology dictate that the actions of parasympathetic nervous system stimulate activity along the GI tract via the vagus nerve while the sympathetic nervous system precipitates the contrary response.

Key point: *Intestinal motility summarises a collection of contractile actions initiated by the myenteric plexus 'gut brain' in response to a range of intrinsic and extrinsic factors.*

Normal values for virtually any part of the GI tract, especially the small intestine, are either non-existent or occupy a large range. Earlier, the length of the small bowel was noted to be on average 7m although this can range from 3m to 9m in adults. With respect to function, the bowel contracts at 8-11 contractions per minute unequally distributed as periods of rapid contraction and silence. Beyond this, normal transit time for the contents of the small intestine take between 30-60 minutes for liquids and between 4-5h for solids depending on meal composition[2]–[4].

Small bowel motility in disease

Numerous studies have documented the involvement of small bowel motility in many disease processes, although the mechanistic process in many remains unclear. In inflammatory bowel diseases such as Crohn's Disease, inflammation, and the subsequent fibrotic process that occurs directly results in varying decreases in small bowel motility[14]–[17]. Conversely in bacterial overgrowth, the absence of motility is postulated as the cause of the disease symptoms characterized mainly by a reduced frequency of the migrating myoelectric complex (MMC) – a motile action regarded as being fundamental to keeping the intestine free from bacteria[12], [18],

[19]. In a number of diseases, including the somewhat controversial irritable bowel syndrome where dysmotility has never been empirically confirmed, it is the proposed absence of these motile actions that are believed to precipitate a response that manifests as pain, change in bowel habit or altered sense of 'well-being' in the patient [20], [21]. In some cases the disease is largely idiopathic for example, chronic intestinal pseudo-obstruction (CIPO) where patients present with abdominal pain and the symptoms of obstructed bowel in the absence of causative lesion, with only full thickness biopsies of the bowel wall able to characterise the origins of the disease [22]–[26]. Several studies have documented the presence of altered gut motility and symptom improvement following administration of motility effecting drugs (neostigmine) in CIPO that further reinforce role of abnormal motility in this disease. However it is entirely unclear as to whether motility is a cause or effect in the disease process[27].

***Key point**: Symptoms suggestive of dysmotility are extremely common in diseases that primarily affect the gut and secondary to assorted neurological conditions and medication regimens, leading to a marked decrease in quality of life for the patient and increased healthcare costs.*

Beyond conditions that appear to originate in the small bowel, motility derangement may often be the systemic effect of a central neurological disorder, indirectly affecting the small bowel via the enteric nervous system, with Parkinson's disease and diabetic neuropathy being some of the more prominent examples[28]–[31]. The data, detailing the impact on motility in these disorders is scant although a wealth of anecdotal, indirect and circumstantial evidence exists to implicate small bowel dysfunction. Specifically in Parkinson's disease, dysmotility has been

described in the oesophagus, stomach and colon, leading in the case of the latter to constipation, one the of most common symptoms of the disease. Interestingly constipation is a disease risk factor associated with Parkinson's in males over the age of 50[31], [32].

Due to its relative accessibility, the colon has been researched relatively thoroughly with respect to its physiology but the small intestine remains enigmatic. In Rats, the use of MPTP (a compound that induces Parkinson like changes) was demonstrated to disrupt the MMC, a process that could be reversed through administration of levodopa[33]. Radiology has demonstrated a dilation of small bowel loops suggesting a loss of tone and manometry has suggested changes in basal motor patterns and auscultation of the abdomen has shown marked decrease in bowel sounds compared to healthy controls. Symptomologically, patients complain often of bloating and experience weight loss as the disease progresses implicating small bowel function[28]. Again, very little is known and the field, as it is stands, is in equipoise with respect to the role of the small bowel motility be it causal, or, more likely, a result of disease pathology. In any case, dysfunction stands to generate additional problems and substantial further cost in healthcare and is therefore of interest to researchers and clinicians alike[28].

Part of the reason for our collective ignorance of small bowel function and variable findings with respect to normality, let alone disease, is the extraordinary complexity of the system and its deep location within the abdomen, largely prohibiting

mechanistic investigation. Nevertheless, numerous techniques have been tried and tested with varying degrees of success. The aim of this first section has been to partially familiarize the reader with the nature of the bowel and in the next section the key investigative techniques will be explored together with their limitations.

Non-MRI based techniques for investigating small bowel motility

Manometry

Manometry has been used extensively to study motion patterns suggestive of myopathy (abnormal muscular function), neuropathy (nerve damage) or obstruction with varying degrees of success and has subsequently obtained the status as the 'gold standard' for motility assessment in the small bowel[21], [34]. Under endoscopic guidance, a catheter is inserted via the oral or anal route into the upper or lower segments of the small bowel. Regular apertures along the catheters length detect pressure changes associated with segmental contraction in the bowel and allow graphical depiction of the peristaltic wave as it propagates along the small bowel (Figure 1.2). In this figure, pressure apertures in the antrum (A), duodenum (D) and jejunum (J) provide contractile activity profiles across the fasted (Figure 1.2a) and fed (Figure 1.2b) states (diagram from Hansen et al.[34])

Key point: *Manometry presents contractile activity as transient changes in luminal pressure offering high temporal resolution at the cost of patient comfort, limited intra-luminal field of view and difficulty in interpreting results.*

Motility quantitation using manometry is extremely difficult with results being variable, difficult to interpret and extremely sensitive to artefact including changes in interstitial pressure (coughing, sneezing, laughing), meals, body position, breathing and confounding pulsatile actions (eg. contraction in adjacent bowel). In addition to this, it is uncomfortable for the subject, invasive and therefore likely to bias the state of activity one intends to observe[34].

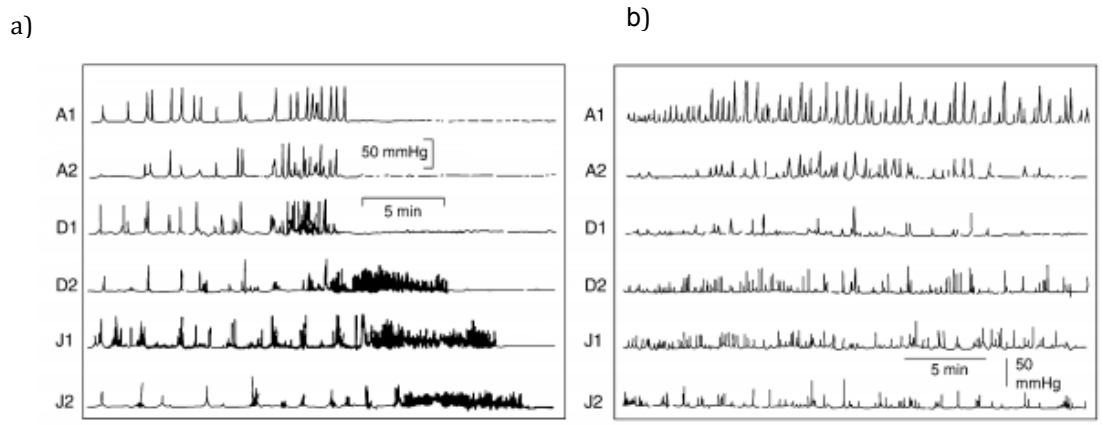

Figure 1.2 *Small intestinal manometry readings for an individual in fasting (a) and postprandial state (b). Diagram from Hansen et al 2002.*

Manometry is also one of the few techniques to deliver 'real' and physiologically intuitive data based on contractile action within the bowel as opposed to a surrogate for motile action eg. transit time. Furthermore, it allows discrimination

between different phases of the contractile cycle and relational information between the sites of contraction based on the known distance between the apertures on the catheter[9], [19], [21], [34], [35]. As sensor technology has improved, new 'high resolution' techniques have been implemented that, along with improved signal processing, has allowed greater diagnostic yield and a continued place in the specialised physiology clinic[36].

Scintigraphy

Scintigraphy addresses some of the limitations of manometry and provides a simple and non-invasive method to track and measure small bowel transit velocity using radio-labelled solid and/or liquid foods[37]. For small bowel and colon transit studies, 3.7MBq of In-111 (half-life: 2.8 days) is administered along with a meal[37]. The movement of the radiation-emitting bowel content is tracked using repeat gamma camera measurements over a 6h period for the small bowel and between 24-48h for the colon. Transit times are defined by the time it takes for the tip of the radiolabelled meal to reach various biological land marks e.g. the terminal ileum for the small bowel. As this is observationally intensive, semi-quantitative reader dependent approximate measurements are recorded at standardised time points after ingestion of the tracer. A normal small bowel would be expected to clear more than 41% of the radiolabelled meal after 6h into the large bowel. Scintigraphy has shown clinical value particularly in the large bowel where the ascending, traverse, descending and recto-sigmoid colon may be easily identified and used in

conjunction with the Colon Transit Index (CTI) to provide semi-quantitative observations [35], [37]–[39].

Key point: *Scintigraphy uses radioisotopes to look at net movement of a tagged meal through the GI tract. The transit time of the meal serves as a surrogate for motility and is relatively inexpensive although lacking in detailed physiological information.*

The CTI offers somewhat transferable methodology to experimental small bowel MR studies were MRI compatible tracers have been used in an analogous manner to the radioisotopes further enhanced by the anatomical information that this modality provides[40]. Despite this, owing to the variability in small bowel shape and the lack of distinct anatomical features by which one can subdivide the bowel, more specific transit times through discrete regions of the bowel are inaccurate even using MR guidance and unfortunately provide little in the way of quantitative information relating to even gross motility patterns in the intestine.

Electrogastrography

Electrogastrography (EGG) was initially developed to record electrical signals in the contractile tissue of the stomach. Subsequent research investigated its application to the small bowel whereby surface electrodes could be placed in regions proximal to sections of the small intestine[41]. The large amount of noise produced from other organs however makes readings ostensibly unusable and in the absence of standard techniques or electrophysiological parameters is likely to be of little use in the clinical setting. Despite these fundamental limitations, this work is important as

it offers the only direct observation of the physiological control mechanisms behind the observed motility and is therefore worthy of mention. In addition, the excellent temporal resolution and potential for ambulatory studies being completely non-intrusive (analogous to a 24-Hour ECG) make this approach appealing[41]. Subcutaneous insertion of electrodes (during surgery) has been reported in an experiment designed to record direct measurements of small bowel electrical activity and could go some distance to validating observations made from cutaneous studies but are unlikely to make a clinical impact in the short term[41], [42].

Capsule endoscopy

Wireless capsule endoscopy (WCE) permits visualisation of the whole intestine as the capsule moves along the tract under the power of largely peristaltic action. WCE has become increasingly popular with gastroenterologists for visualising the lumen of the bowel with features such as ulceration, largely invisible radiologically, being identifiable in the mid portions of the intestine beyond the reaches of the endoscope[43]. As the technology has grown increasingly sophisticated and compact, additional instrumentation has been designed to assess features including pH and pressure, the latter bearing relevance, as seen in manometry, to peristaltic action[43]–[46]. However unlike manometry, the focal nature of this technique, that is, all observations being taken from a single moving location over time, makes assessment of the data from WCE insensitive to specific motile actions like Phase III migrating contractions. Nevertheless, assessment of pressure curves from the

capsule or image processing of shear from the video series has revealed some intriguing data on motile actions through the bowel and this certainly has an application for detecting the absence or presence of activity[44].

The precise utility of such analysis remains elusive although there is a clear advantage to wrapping information of anatomical features, transit time, transient pressure changes into a single well-tolerated modality. As sensor technology improves, it is likely that an increase in the use of WCE for physiological studies will be seen.

Other techniques

A wide range of interesting and increasingly sophisticated techniques has been developed to offer yet more information on the physiology of the small bowel. Ultrasound can be used to obtain visual data on segments of the small bowel and 3D ultrasound of the intestine could be of interest the future[47]. Gadolinium capsules in MR have been used to explore transit times through the small bowel but suffers the same problems observed in scintigraphy. However it may be coupled with a standard MR protocol to gain additional information[48].

Key point: *Each technique has strengths and weaknesses but collectively represent a push towards developing a better understanding of how small bowel motility works through extraction of meaningful quantitative parameters to improve disease diagnosis in the clinic.*

For the remainder of this introduction MR enterography will be considered along with the necessary post acquisition analysis required to derive quantitative information. This emerging field has offered some exciting new avenues of exploration over the last several years that address a number of the issues and limitations addressed above and introduce new opportunities for investigations into small bowel motility.

Motility analysis using MRI

Magnetic Resonance Imaging is perhaps the most advanced imaging tool at the clinicians' disposal. The technique uses a strong, uniform magnetic field to excite hydrogen atoms present in water and fat. By then applying an oscillating magnetic field at the appropriate resonant frequency, a radio frequency signal can be received when the excited protons return to equilibrium that together with gradient encoding allows the construction of the MRI image[49]. The contrast between different tissues is determined by the rate at which excited atoms dephase and return to equilibrium, that in turn depends on their local chemical environment. It is MRI's sensitivity to hydrogen that makes it an excellent modality to image soft tissue. In addition, the use of non-ionising radiation makes it safe and well suited for the investigation of chronic conditions and those that manifest at a young age. In Crohn's disease for example, MRI can deliver high-resolution images of the bowel wall together with extra-luminal changes taking place that are essential for the accurate grading of disease activity and timely administration of medical therapy.

The ability of non-invasive and safe tests to inform medical management is beneficial for the patient in that it helps to mitigate side effects and increase their return to health. It is further economically valuable where the use of expensive medications can be better targeted. As such, the role of MRI in Crohn's disease and the high level of anatomical information it provides is now indicated by international consensus statements and the role of this modality is expanding[50], [51]. Alongside the anatomical information there is emerging value in the capacity for functional imaging. Dynamic or 'cine' imaging allows a uniquely physiological view of the bowel and is increasingly being used alongside structural imaging to investigate bowel pathology. This thesis explores the value of MR beyond its anatomical value looking specifically at the functional potential of this increasingly popular and available imaging modality.

There are however a number of methodological considerations including subject preparation, scanner sequences and image processing that have a large impact on assertions made surrounding a physiological process such as motility.

Subject preparation

All imaging strategies targeting the small bowel are dependent on adequate distension of the small intestine alongside sufficient luminal contrast so that the bowel wall can be suitably discriminated and the intra-intestinal space observed. MR enterolysis, where liquid is rapidly injected directly into the intestine via naso-

duodenal catheter, was developed to ensure adequate distension along the length of the intestine. While enterolysis provided exceptional distension and contrast, the invasive nature of the procedure was less well tolerated by patients and more time consuming. Because of this, the oral administration of contrast, MR enterography, became popular. To this end the simplest contrast agent one might use is water yielding a bright signal on T2 weighted imaging and dark on T1. However administration of water results in rapid absorption along the small bowel and poor, inconsistent bowel distension (Figure 1.3). A solution to this problem is to mix the water with various additives to inhibit absorption and various formulations exist today with choice dictated by availability, patient compliance, cost and experience [52], [53]. Two of the most popular preparations found in the literature are the mannitol solution and locust bean gum with mannitol solution.

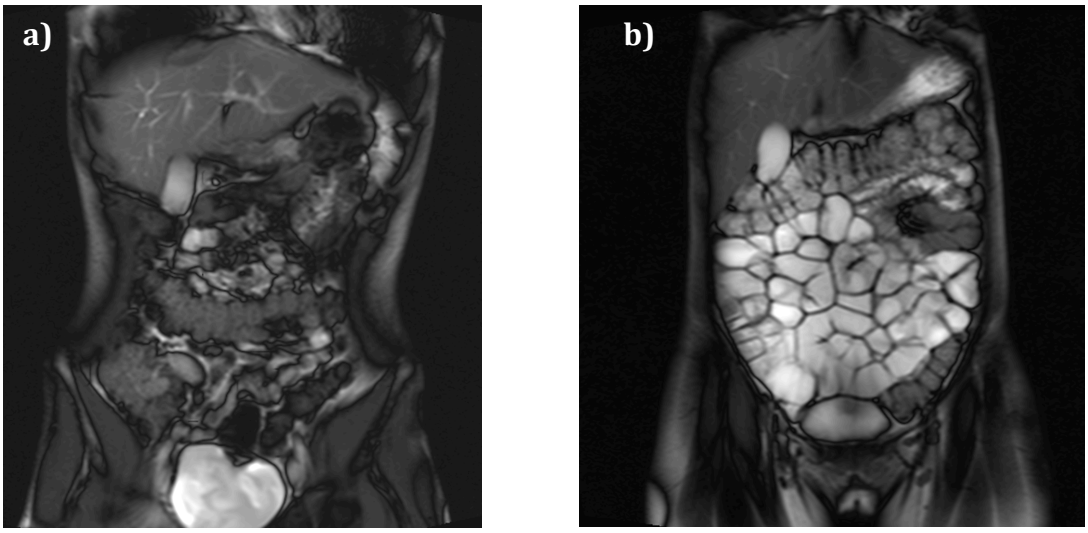

Figure 1.3 *Small bowel prepared by MR enterography with water (a) and with mannitol/ Locust bean gum solution (b).*

Mannitol is a carbohydrate classed as a sugar-alcohol with its underlying chemical structure preventing intestinal absorption[54]. Its inherent osmotic properties help reduce the amount of water reabsorbed along the gut and also add a slightly sweet taste that appears to improve the patient experience. Locust bean gum is extracted from the seeds of the Mediterranean Carob tree (*Ceratonia siliqua*) and is used in a range of foodstuffs principally as a thickening agent. Whilst on its own does not taste particularly pleasant, it may be sweetened but more importantly provide better bowel distension and fewer diarrhoeal instances when compared to mannitol alone[52]. Additional paramagnetic compounds such as gadolinium-based contrast agents might also be added to provide high luminal signal on T1-weighted studies. However, oral gadolinium is expensive and together with the associated risk of toxicity represents an unnecessary further step towards imaging the lumen at this time.

Key point: *Oral contrast induces a stretch in the lumen that likely has a stimulatory effect on motility characterised by strong 'postprandial' segmental contractile activity.*

In the clinical setting a typical contrast solution would involve around 2% mannitol in 1000ml H_2O and 0.2% locust bean gum but may vary across institutions. A patient would be required to fast from solids for around 4h prior to their scan, drinking the entirety of the contrast solution at regular intervals anywhere between 30mins and 3h before their scan until just before they enter the scanner. The amount drunk over time directly influences how much of the bowel becomes distended and can have an

influence on how long the patient can tolerate being in the scanner. The patient is usually required to lie prone on the scanner bed as this helps to reduce displacement of the bowel out of the slice of interest during the scan. This is particularly important for improving intra-patient study reproducibility. Wherever possible, a breath hold will be used during the scan to reduce respiratory motion with this time running from between 15-30s depending on the number of frames in the sequence and the patients capabilities. Pre breath-hold hyperventilation can help with respect to the latter along with a good communication interface between scanner operator and patient.

Scan sequences

Even at rest, the rhythmical contractile activity of the small intestine is sufficient to create motion artefacts on the majority of small bowel sequences used routinely in the clinic today. As a consequence, antispasmodic medication is routinely given to paralyse the bowel in an attempt to improve study quality. For obvious reasons this is not the case when performing small bowel motility studies where instead a high temporal resolution is used to mitigate and indeed capture the effects of peristaltic movement at the cost of spatial resolution. Fast scans such as T2-weighted single-shot fast-spin echo (FSE) sequences allow planning of the dynamic sequence stacks in the coronal plane through the abdomen to cover the entire small bowel in a short

time. It is essential to develop a rough understanding of the individual's bowel anatomy as there is a large degree of heterogeneity between patients with landmarks, including the terminal ileum, potentially being lost between scans slices. A typical dynamic sequence for motility imaging is usually a fast T1-weighted gradient echo or fast T2 weighted single shot free precession (SSFP) or any other sequence with a acquisition time of less than 1s which provides a satisfactory impression of small bowel peristalsis. The selected sequence is repeated every 500-1000ms to gather around 20 frames during a single breath hold with a 1.5T scanner. A slice thickness of around 10mm is most frequently used as this provides good bowel wall contrast and good spatial coverage of the small intestine. The number of dynamic sequences in a stack is invariably dependent on the patient size. If a patient is slim 10 slices should be sufficient, where the patient is morbidly obese the intestine may become sufficiently separated through the abdomen so that complete coverage would require in the region of 25 slices, although this is rarely performed. Potentially, the dynamic stacks can be collected in any plane however visual motility interpretation is less intuitive from the axial or sagittal planes. The total scan time allocated in most centres for dynamic acquisition is around 10-15 minutes prior to spasmolytic administration and additional sequences diagnostic sequences (anatomical T2, diffusion, dynamic contrast enhancement etc.).

Motility Metric	Unit	Description	Strengths	Weakness
Contraction rate	Frequency (Hz) expressed as contractions per minute (CPM)	A diameter change of more than 10% of the mean luminal diameter for the time series	Physiologically intuitive, simple	Long time periods required for analysis of contraction frequency
Lumen Diameter	mm	The mean luminal diameter	Rapid analysis, simple	Does not describe contractility
Contraction Amplitude	mm	Max diameter - minimum diameter	Rapid analysis, simple, describes bowel contractile potential	Blind to time domain
Amplitude/ diameter ratio	Unitless	Amplitude/diameter	Rapid analysis, simple, less susceptible to bias from large calibre bowel	Blind to time domain
Jacobian Standard Deviation	Unitless	Registration derived motility surrogate based on quantification of the fractional change in a pixels area summarised by the SD of its Jacobian determinant through time	Application in global and segmental analysis, rapid, powerful parametric analysis tool, validated prospectively	Complex calculation, unitless metric, surrogate measure
Image Tagging	Hz	Tagline voxel deformation	High temporal resolution, suitable for global analysis	non-specific measurement, un-characterised physiological meaning
Chyme flow rate measurement	Mm/min	Flow velocity	Direct physiological measure	Uncharacterised relationship between segmental flow and peristalsis

Table 1.2 *Summary of quantitative techniques to evaluate small bowel motility.*

Temporal resolution

There has been a tradition of scanning the small bowel at a <1s temporal resolution and there is a certain wisdom for this where one's analysis is dependent on real observations of the bowel (ie. diameter change of the luminal space over time)[14], [17], [55]–[59]. Where possible, the maxim of 'the faster the better' has been applied. However, certainly on the 1.5T platforms, it has been difficult to improve on 2 images per second whilst retaining adequate spatial resolution. Segmental, diameter changes of the bowel lumen have proved the mainstay of quantitative MR motility measurement in the peer-reviewed literature to date. However, with advanced post-processing techniques being developed and increasing scanner field strength there has been a general move away from these direct measurements of local luminal calibre into more global parametric forms of analysis with temporal resolution being sacrificed for spatial resolution in terms of complete volume coverage.

Scanning at different field strengths

A brief note on field strength. Scanning at 1.5T allows the acquisition of images at sufficient spatial and temporal resolution to observe peristalsis in vivo. From these quantitative measurements can be made. The fundamental limitation at this field strength, and largely surpassed by scanning at 3T, is the limitation of analysis to a single 2D slice of bowel through time. At 3T, using specialised sequences, one can acquire volumetric data in much the same way one might acquire single slice data at

1.5T permitting analysis of the bowel as a system with temporal coherence between anatomical positions.

MRI - Motility quantitation

Motility might be assessed through a number of approaches summarised in table 1.2. One might simply 'eyeball' the cine series and comment on the presence of static areas of the bowel (stricture etc). Beyond this, one might utilise a quantitative approach to count the number of contractions in a given section of bowel. Finally, more advanced registration and post-processing techniques might be implemented to extract information based on deformation fields permitting observations across the bowel as a system. The implementation of quantitative metrics for small bowel motility observations will be the subject of this next section and these take a variety forms that move away from the limitations of subjective analysis. As this field is fairly new, some select publications have been used to explore the most popular techniques for motility analysis.

Physical measurement

By far the most common technique for motility analysis in the small bowel is through the use of manually adjusted ROIs, usually taking the form of a line drawn across the lumen perpendicular to the axis of the bowel in the coronal plane[60]. The length of this line can be adjusted on each image and the series of lengths

plotted against time to give a peristaltic wave. Where the subject is scanned in the sagittal plane, polygonal ROIs placed over the cross section of the bowel and adjusted also provide a good impression of peristaltic action[55]. The reason for the popularity of this metric is due largely to the simplicity of analysis, together with the observation of a 'real' process - that is, a contraction in the bowel as opposed to abstract surrogates. There are two key limitations with this approach with the first being through plane motion and the second in its time consuming nature[60].

Key point: Direct measurement of bowel diameter is a simple and descriptive way of quantitatively evaluating bowel wall motion. It is the most commonly used motility metric reported in the literature.

Through plane motion occurs where an individual's anatomy moves perpendicular to the imaging plane during acquisition caused largely, in MRE, by respiration removing a given bowel loop from the acquired image. In theory, one could move to a different slice to make the measurement. However, as most of these studies are performed at 1.5T there is as much as a 60s temporal gap between anatomical positions through the abdomen and thus temporal continuity with respect to small bowel physiology is lost. As a result a 'good' bowel loop is subjectively picked that is well distended and present though the sequence. Even then, it is rare that a good sinusoidal curve is produced. Consequently peristalsis is rarely reported in terms of cycles per minute and many groups instead take the standard deviation of change in area size or line length over time and use this as their motility metric[60]. Peak counting may be performed where the number of 'peaks' in luminal diameter change is counted. There remains a great deal of ambiguity in how one might interpret such data, however, with a contraction generally considered to be a

diameter change greater than 10% of the mean luminal diameter through that time series [59] [61]. The second limitation is the fairly common issue of labour intensiveness, where manual measurements need to be made, limiting the use of this technique exclusively to research. In an early paper by Froehlich et al 70,000 measurements were made in ten subjects with this level of user input being impractical in most scenarios [60]. Since then, an automated technique has been developed and validated by this group however, the technical description of these has not yet been published[59]. In spite of these limitations, the technique is popular in that it does not interfere with standard clinical work up, is more objective than visual assessment by the examining radiologist, and is more methodologically transparent in the sense that it is an automation of a task that could otherwise be performed manually.

Flow rate measurement

Peristaltic contraction serves to mix and drive the food bolus through the bowel. Luminal transit may be used as a surrogate for the degree to which peristalsis is taking place and measurements of 'flux' or flow of the intestinal contents through a segment of bowel can be quantified using phase-contrast (PC) pulse sequences originally developed for angiographic techniques in large vessels[62]. In their publication, Gutzeit et al. (2010) validated this approach in a synthetic phantom and applied it to 10 healthy volunteers with a modest 400ml preparation of gadolinium spiked water preparation. Volunteers were scanned in the sagittal plane for phase

contrast flow quantitation as well as traditional cine imaging for a period of 30s in each case. The technique was found to be well tolerated in all volunteers however distension was poor, limiting the number of places an observation might be made. In spite of this, PC revealed rhythmical velocity changes within the lumen and this was also observed using a manually adjusted ROI at the same location, with area change being plotted instead of velocity over time. In both instances, velocity and area change were demonstrated to be suppressed following the administration of butylscopolamine bromide (Buscopan) suggesting both observations were linked to physiological activity related to peristalsis. The fact that chyme flow and radial contractions in the bowel appear linked, although sounding obvious, does not necessarily indicate a causal relationship - the rigid acrylic phantom used in this publication was not exhibiting peristalsis for example. Upstream motile action would be sufficient to effect pulsatile movements of intestinal contents in the absence of local peristaltic action. However, the two appeared to correlate well in this publication. Despite this, sensitivity to Buscopan strongly supported the process of flow being related to peristaltic action and thus qualifies this as a surrogate for motility that further serves as a quantitative outcome. A further consideration for this technique is the neglect of segmental contraction to 'churn' bowel contents that constitutes a proportion of total motile action, especially in the presence of a perceived meal. Again, although correlation with manually delineated area changes over time appears high, this may serve to confound an observation designed to detect net flow through a singular loop of bowel.

Spin labelling

In an *n=1* proof of concept study, Sprengers et al. used a motion coding technique also referred to as SPAMM (spatial modulation of the magnetization) originally developed for cardiac motion imaging to develop a technique to quantize intestinal motion[63], [64]. In SPAMM, a short pre-pulse sequence periodically saturates the magnetization that will eventually appear as a line or tag pattern in the image. As the tissue deforms, the tagged spins deform accordingly and this motion can then be analysed (often in the frequency domain). From here, assertions with respect to peristalsis might be made in a quantitative manner.

In this study, Sprengers et al. demonstrate that application of this technique could be expanded beyond cardiac imaging to the abdomen. The healthy volunteer was prepared with 1000ml mannitol solution after fasting and scanned continuously for 8 minutes. Following a scout scan the tagged dynamic sequence was run (FOV = 40x40x3.6cm) for two minutes before glucagon was injected to paralyse the bowel. By splitting the frequency spectra into discrete ranges, loosely representative of physiological motion (0.0-0.2Hz = low, 0.3 - 0.5Hz = Breathing, 0.6-0.8Hz = Intermediate and 1.0-1.2Hz = cardiac), the spectral power was then assessed at the different frequencies to quantitatively describe on small bowel motility and the induced change following glucagon injection. Sprengers at al. postulated the frequency band between 0.0-0.2Hz corresponded to what one might expect in terms of contractile activity in the small bowel (8-10 contractions per minute based on previous studies using MRI and the existing physiology literature)[56], [60]. Accordingly a linear reduction in spectral power was observed in this frequency

band across the quadrants over the four time points (2,4,6 and 8 minutes) following drug administration.

This is an interesting technique and good example of the varied ways one might extract metrics from magnetic resonance. However, there are a number of methodological and conceptual issues that are important to consider when analysing small bowel motility using this or similar techniques. Firstly, the tag pattern does not discriminate between structures in the abdomen (blood, bowel, intestinal contents, liver, muscle) making the technique non-specific. As a result, analysis, as performed here, by quadrant leads to a grouping of low frequency, static structures in with the motile bowel. By then collapsing these frequencies together in a range between 0 and 0.2Hz, the potential to negatively bias any observation made in a quadrant where significant proportion of the signal is derived from static structure. A clear partial resolution of this issue is through specific ROI placement, for example around the small intestine while (mostly) precluding fixed structures.

Key Point: Spin labelling offers a direct means of evaluating motion arising from global bowel motility with a high temporal resolution. Tagging is however nonspecific and the low anatomical resolution of anatomical structure makes further annotation difficult.

However, expanding on the theme of non-specificity and focusing only on the intestine, the tag lines run through both the bowel wall and its contents indiscriminately. Therefore, deformation due to flow from the intestinal contents is inseparable from the contractile activity of the bowel wall as far as the tagged deformation is concerned and presumably spans a broad frequency bandwidth. Although flow and peristalsis are largely concomitant (as demonstrated by Gutzeit

et al.) one can make, at best, limited inferences on one from the other, especially with respect to pathology in the bowel[62]. This is an issue where, for example, a narrowed, stenotic section of bowel experiences flow due to upstream peristaltic effects but does not in itself peristalse as it is often seen in inflammatory bowel disease.

This technique is nevertheless promising and offers a more direct, global impression of motion within the small bowel with a high temporal resolution that shows some sensitivity to pharmaceutical intervention.

Registration

The final section of this chapter introduces image registration and provides a brief summary of how this approach can be applied to assist the quantitation of small bowel motility. This topic is the specific focus of the next chapter and the central tool used in experiments described in the remainder of the thesis. The key fundamental concepts that underpin image registration are addressed here with a view to providing a high-level overview of what is, in itself, a vast area of active research. The book, 'Medical Image Registration' by Hajnal, Hill and Hawkes [65] provides an excellent introduction to this topic for further reading. Further, more recent and comprehensive reviews can be found in[66]–[70].

Registration algorithms are driven by pixel (or voxel) intensity or feature correspondence between images; in other words, corresponding intensity patterns or features (for example, anatomical features) in each input image are matched to bring the images into spatial alignment. So-called image-intensity-based algorithms are widely-used as they provide a general-purpose solution for many registration problems where different images from different imaging modalities are to be registered. Such intensity-based algorithms canonically contain three key elements: a similarity measure, a transformation model, and an optimization scheme. In the simplest case, when two images are registered, one image is commonly referred to as the 'source' image, whereas the other is referred to as a 'target' or 'reference' image. The source image is then transformed by applying a mathematical transformation that displaces individual pixel (voxel) locations of the source image so that the resulting transformed source image is aligned with the target image. For the case where there are multiple input images, multiple source images may be registered to a single target. In recent years, however, a number of so-called groupwise registration approaches have emerged which aim to determine anatomical correspondence within a set of images so that these can be registered together without the potential bias introduced by selecting any particular image as the target image. The similarity measure, as the name suggests, is a numerical metric that quantifies the alignment input images with the assumption that these images are aligned once the similarity measure reaches its maximum value (commonly referred to as the 'global maximum' value). Notable examples include the sum of squares difference (found by subtracting overlapping pixel (voxel) intensity values of transformed input images to find the intensity difference and then summing the square of these values to arrive a single number), correlation

coefficient, and so-called information theoretic measures such as mutual information [71][72] and normalized mutual information[73], which are based on image entropy. Similarity measures vary in their complexity and computational requirements, which leads to variations in processing speed, in some cases leading to significant computational burden for more complicated measures. The choice of similarity measure needs to broadly suit the intended application and the nature of and variation between the input images.

The transformation model computes the displacement vector that transforms the co-ordinates of each pixel (voxel) from the source image to the corresponding co-ordinates in the target image. Common transformation models are rigid-body transformations in which the entire image is translated and/or rotated, an affine, which combines a rigid-body transformation with a sheer and scaling transformation, and nonrigid where each pixel undergoes a potentially unique displacement to its target such that the source image is warped to fit the target. The pixel (voxel) displacements across the image are collectively referred to as the deformation or displacement field. Constraining the transformation model is crucial to maintain biological plausibility in an algorithm; for example, one is measuring bowel wall motion, the contraction is unlikely to exceed 2cm and one would therefore constrain the algorithm accordingly. Introducing constraints also prevent non-physical aberrations such as image folding and tearing. The similarity measure and transformation model are linked by the optimization scheme, the goal of which is the numerical maximisation of the similarity function (or equivalently the minimisation of the inverse of the similarity function) that signifies the alignment of two images. Classical numerical optimisation schemes to

perform this maximisation (minimisation) include gradient descent, conjugate gradient, and Gauss-Newton schemes, but many others have been developed for particular image registration problems. Typically, the cost function – i.e. the function to be maximised/minimised – employed by the optimisation scheme is closely related to the similarity measure, but may be modified to incorporate constraints (for example, an increasing penalty for increasing displacements that would relate to physically implausible deformations) and/or mathematical regularisation to improve the stability of the optimisation scheme.

The rationale for using image registration in this thesis lies in the availability of dynamic data sets acquired as part of clinical routine at University College Hospital without the need for new scanner hardware or protocol changes. Beyond this, a dual benefit with respect to analysis can be found in the generation of deformation fields to not only automatically propagate user placed ROIs but also to generate quantitative parametric maps which could serve as a further means of quantifying motility.

The only paper published at present concerning the use of registration in small bowel motility analysis is that written by Odille et al. (2012), which is discussed in detail in the next chapter[57]. When image registration is referred to in a more general sense, the desirable end point is a set of new images without a particular physical perturbation to permit faster or more accurate clinical measurements – a classic case being the removal of respiratory motion from a

dynamic contrast enhanced MRI image series. Conversely, where one is interested in motion analysis, the registered images cease to be of interest with the emphasis now lying on the degree to which the original images had to be deformed to reach a given target image.

Odille et al. applied an optical flow technique to small bowel dynamic image data to 'correct' local deformation caused by peristalsis between consecutive MRI images. The accuracy of the registration was assessed by comparing an automatically propagated region of interest through the time series to a manually corrected gold standard, consisting of the mean score of two independent reviewers in 10 subjects. The high levels of agreement supported the accuracy of this registration approach and also provided a convenient way to perform frequency based segmental small bowel motility analysis in a timely fashion. Beyond this, direct analysis of the deformation field itself could be performed, and in this paper it was demonstrated that deformation-field-generated metrics of motility are potentially more powerful than physical measures when scoring intestinal motility compared with clinical grading.

Key point: Image registration can be used to automatically quantify physical measurements of bowel motility and can provide parametric information based on analysis of the deformation fields.

Conclusion

Small bowel motility is an enigmatic and exciting area for research. Over the course of this introduction, it is clear that not only is there an array of investigational techniques at the researchers disposal, but excitingly, new and increasingly versatile imaging based techniques are being developed with the potential to explore as yet uncharted areas of this fundamental component of human physiology. In the next chapter, the use of image registration, introduced here, will be expanded and the initial validation work that took place presented.

CHAPTER 2: INTRODUCTION AND VALIDATION OF THE OPTIC FLOW REGISTRATION TECHNIQUE TO EVALUATE SMALL BOWEL MOTILITY

Research Question:

Can image post-processing be used to objectively evaluate MRE-derived small bowel motility data?

Rationale:

Manual assessment of small bowel motility through manual adjustment of ROIs between time points in dynamic series is time-consuming with the potential for user error. Although valuable as a research tool, an automated approach would broaden the appeal of the technique to a clinical audience where motility analysis might be used to objectively grade disease, measure response to medication or help in forming a diagnosis.

Hypotheses:

1) Medical image registration can be used to automate ROI placement and 2) provide surrogate measures of motility based on deformation fields.

Aim(s):

i) Validate the accuracy of the optic-flow image registration technique for correcting bowel wall movement.

ii) Evaluate the ability of the optic-flow technique to objectively grade clinical scoring of bowel motility.

Author Declaration

Research presented in this chapter was published in: Odille, F., Menys, A., Ahmed, A., Punwani, S., Taylor, S. A., & Atkinson, D. (2012). Quantitative assessment of small bowel motility by nonrigid registration of dynamic MR images. Magnetic Resonance in Medicine : 68(3), 783–93. doi:10.1002. Dr Freddy Odille wrote the registration algorithm and MATLAB GUI used in this study under the supervision of Dr D. Atkinson. The author's contribution involved organising the clinical validation, collecting data, performing statistical analysis of the data and acting as one of the two observers to assess registration accuracy.

2.1 Introduction

In the previous chapter key physiological themes were covered relating to small bowel physiology and how MR has been used to evaluate motility through evaluating the dynamic images as a 'cine' loop. Beyond this, the quantitative assessment of lumen diameter or area change through time is possible but is time consuming where performed manually and impractical for clinical use. The final section of Chapter 1 introduced medical image registration as a method for both automating the analysis of small bowel motility data and also providing a number of additional methods, based on the analysis of deformation fields, to assess motility.

In this chapter a nonrigid image registration technique developed by Odille et al. is validated to estimate 2D time-varying displacement fields in a time series of dynamic MRI images[57]. However, nonrigid registration is technically challenging with the small bowel time series data presenting some particular difficulties: First, in addition to in-plane local displacement cause by bowel wall motion, through-plane motion is observed either from movement of the diaphragm or peristalsis itself contributing additional local intensity changes to the images. Second, another source of intensity changes in the bowel arises from the flow of chyme (intestinal contents) both within and through plane. This large variation in consecutive image intensities is often problematic for image registration algorithms because they can find it difficult to distinguish an apparent intensity change due structures moving from a change in the underlying intensity. Where the assumptions of the three cardinal similarity measures (sum of squared difference, correlation coefficient and normalised mutual information) are poorly satisfied or violated altogether resulting in poor image registration.

A solution is presented in Odille et al. through jointly incorporating an explicit model of intensity change that is optimised simultaneously with the displacement field based on an assumption that local intensity changes between two images can be described locally by a smooth function. Simultaneous nonrigid registration and modelling of time intensity changes takes the form of a sequence of linear least-squares problems which are easer to solve individually than the optimisation of generalised similarity measures and constitutes the novelty of this approach. The image registration produces a dense displacement field over the whole field of view that serves the dual purpose of generating parametric maps that may be further

analysed to produce surrogate measures of motility and to propagate user-placed ROIs through the time series.

This chapter describes specifically the validation of the optic flow technique assessing both the 1) accuracy and 2) the ability of the registration derived metric to reflect a radiologist's motility evaluation. An overview of the registration theory is provided with associated equations provided in Odille et al. alongside this validation[57].

2.2 Methods

2.2.1 Theory: Joint nonrigid registration and modelling of intensity changes

The goal of image registration is to place the same anatomy at the same image position in all frames within a series of images. After successful registration, the frames played as a movie should look static. To perform this re-alignment of anatomical structures, a different deformation field is applied to each frame to warp it to a reference frame and subsequently these deformation fields are used here to quantify motility.

As discussed in the previous chapter, registration algorithms have three basic components; the transformations that describe the deformation fields, the cost function that measures the similarity of the reference and warped frames, and the

mechanism for optimising the deformation field. For small bowel motility, local nonrigid deformations (i.e. small anatomical changes in position caused by bowel contractility) needed to be permitted and so a parametric representation (e.g. using B-splines) on the deformation was not used. The changes in deformation during the registration optimisation are driven by the local image intensity gradients in a similar way to the classic optic flow technique[74]. By using a sum of squared difference cost function, these deformation changes can be computed directly from the image gradients. This provides for a rapid means to perform the optimisation of the deformation field and permits non-linear deformations. A constraint on the deformation fields is added to prevent artificial collapse or tearing in the images. To cope with underlying intensity changes (rather than motion), Odille added an intensity change map that is also optimised by the algorithm. Although developed independently, it turned out this extension to the classic optic flow had been previously published[74]. In common with most registration algorithms there are tuning parameters that require setting. These parameters were set based on observations from trial data but were not changed for any of the studies in this thesis. Also in common with other algorithms, visual inspection sometimes revealed some inaccuracies in the deformation fields. The purpose of this chapter was first to establish the extent of said errors in the registration result and secondly to demonstrate that the metrics derived from the deformation fields provide clinically meaningful and useful correlations.

2.2.2 Patient population:

Ten patients (7 female, mean age 31) with histopathologically confirmed Crohn's disease were recruited from the hospital database for this study based on the assumption that such patients would exhibit greater variability in terms of bowel motility. Each subject underwent MRI enterography (MRE) with a view to evaluating disease recurrence and there were no specific exclusion criteria.

2.2.3 MRI Protocol

MRE was performed on a 1.5T Siemens Avanto scanner (Siemens, Erlangen, Germany). All patients fasted for 4h prior to the ingestion of 1L of 2% mannitol, 0.2% locust bean gum oral solution drunk at regular intervals over 40 minutes prior to the scan. All patients lay prone in the scanner. No antispasmodic agents were administered until after the acquisition of the dynamic images.

A balanced steady-state free precession sequence was used for dynamic scanning of the small bowel with the following parameters: flip angle 60, TR = 3.8ms, TE = 1.9ms, 256x200 matrix filling, zero-filling to 512x512, 1x1mm in plane resolution, 10mm slice thickness and 0.8 seconds temporal resolution. Between 7 and 16 dynamic sequences were acquired for each patient depending on the patients' body habitus. The slice position of each dynamic block was selected by the attending radiographer to cover the entirety of the small bowel. Each dynamic block was acquired on inspiration breath-hold lasting around 20 seconds.

2.2.4 Registration Implementation

Each patient's dataset was downloaded and anonymised from the hospital PACS system (Agfa HealthCare, Middlesex, United Kingdom) before being loaded into a MATLAB (The MathWorks, Natick, MA) function, written by F. Odille to set several key parameters necessary for the registration. A reference image from within the time series was selected based on its closeness to the median image of the series as defined by the minimal Euclidian distance between images. All the other images are registered to this median image. Images were down sampled to 256x256 for registration as the previous zero-filling to 512x512 is only useful for visualisation purposes. Zero-filling was not used and no cropping of the original images took place. Registration was implemented in a multi-resolution manner and the displacement fields and maps for intensity changes were initialised to zero for the first 'course' scale of registration. A total of four scales were used corresponding to multi-resolution factors 1/8, 1/4, 1/2 and 1. Images were down-sampled to each of these resolution levels after Gaussian filtering to avoid aliasing. After registration convergence had been reached at a given resolution level, the displacement fields are interpolated to the next (finer) resolution and the process is repeated until convergence at the final scale has been achieved.

2.2.5 Assessment of registration accuracy

A graphical user interface and region of interest (ROI) tools native to MATLAB were used to draw linear and polygonal regions of interest in the small bowel by two observers (Asia Ahmed 2 years experience and Alex Menys 6 months experience).

Specific experience in ROI placement was gained by adjusting line and area ROIs in consensus under supervision of a consultant radiologist (Prof. S. A. Taylor). Lines were drawn perpendicular to the central axis of the bowel running lumen to lumen and polygons were placed where the bowel presented as an ellipse in cross section (Figure 2.1). Users placed, in consensus, a set of 5 line and 5 polygon ROIs at random positions on the median registration reference images (each median image represented a different anatomical position in the coronal plane) in each of the 10 patient data sets. All ROIs were propagated by the registration deformation fields through the time series. In theory, the length of the line ROIs and area of the polygonal ROIs would change according to the dynamic movement of the small bowel. Each of the two observers, independently, proceeded to manually adjust the line length and polygon areas for each time point. Modification was allowed by dragging the ends of the line or the vertices of the polygon ROIs to a new position believed to be better representative of the bowel wall location.

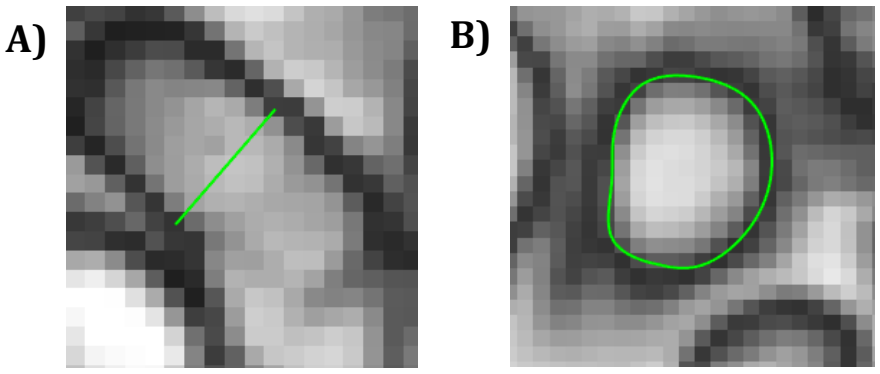

Figure 2.1. Figure 2.1. *Region of interest (ROI) placement in the small bowel. A linear region of interest running to the interior of the bowel wall, across the central axis of the bowel (a) and a polygonal region of interest with a spline fit where the bowel is seen in longitudinal cross section (b). In both cases, ROI's were placed around the outer-lumen boundary, in the bowel wall.*

The average of the two observers ROI lengths and areas was used to construct a ground-truth score for each ROI position. To assess the accuracy of the registration this ground truth was compared to the automatically propagated registration result.

2.2.6 Assessment of automated motility quantitation

The application of the registration deformation fields to generate quantitative measures describing motility was assessed by comparing a set of metrics to a subjective gold-standard constituting the consensus evaluation by two consultant Radiologists (6 and 5 years in Magnetic Resonance Enterography). Five Crohn's Disease patient data sets were used with a total of 83 ROIs (39 linear and 44 polygonal). The radiologists subjectively graded the bowel motility at each of the ROIs on a four-point scale from 1 (normal) to 4 (static). Scores 2 and 3 represented bowel that was peristalsing more slowly than normal bowel and bowel that was essentially static with some movement respectively. This grading scale was developed especially for this study in the absence of previous, validated methodology.

This study used four quantitative methods to assess the motility at each of the 83 ROIs consisting of standard deviation of length/area change, standard deviation of the Jacobian determinant, standard deviation of the intensity change and combined measure of the Jacobian determinant and intensity change. An overview of the selected metrics is presented in Table 2.1.

Line and area change ($\sigma_{Line/Area}$) metrics are commonly used in the literature and detailed in the previous chapter[56], [59], [76], [77]. Their use here represents the role the algorithm can play in acquiring 'real' measurements expressed in distance as opposed to abstract surrogate metrics. The standard deviation of area and length is taken to summarise these data and expected to increase where motion arising from peristalsis is large.

Metric name	***Symbol***	***Measure***	***Measure summary***
Line/Area SD	$\sigma_{Line/Area}$	distance (mm)	Standard Deviation
Line/Area Jacobian	σ_J	fractional change in area	Standard Deviation
Line/Area Intensity	σ_C	intensity	Standard Deviation
Line/Area combined	σ_{JC}	fractional change in area and intensity	Standard Deviation

Table 2.1. *Summary table of the four proposed metrics to evaluate motility*

The standard deviation of the Jacobian determinant (σ_J) was used as a quantitative metric. In theory, the Jacobian determinant should be robust to rigid movement as seen in liver that should produce a score of 1 (Figure 2.2). Conversely, deformations that represent either a fractional increase or decrease in area should produce a score either smaller or greater than 1. We would expect to see such a process in the local deformation fields generated during the registration of small bowel motility data. An illustrative example and additional

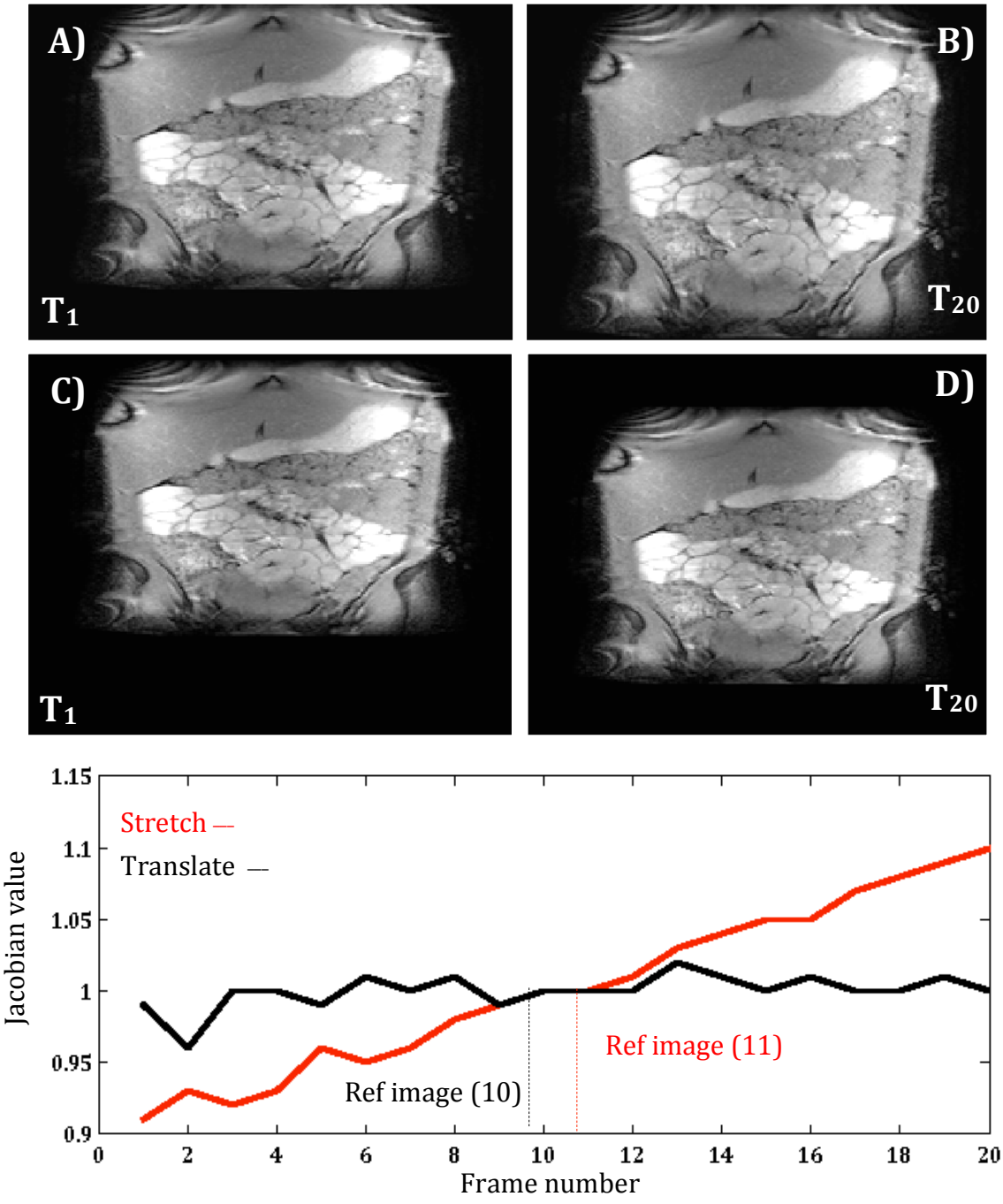

Figure 2.2 – Figure text over page

Figure 2.2 *The effect of image stretch and translation on the Jacobian determinant. For illustrative purposes, two types of transformations were applied to a target image and the Jacobian for each pixel calculated. The first was an iterative stretch transformation repeated for 20 time points beginning with A and ending with B. The Second transformation was a translation, repeated again from 20 time points from C to D. These images were registered using the optic flow code and a series of deformation fields produced which were assessed by taking a fixed pixels value through time and plotting its Jacobian (E). For both the stretch and translate series, the Jacobian was 1 for the reference image confirming there was no change in area taking place. For the stretch images, the Jacobian value decreases as compression occurs in the image away from the reference image and an increase in the Jacobian as the image stretches away from the reference. Where the image is translated in a rigid fashion a value of approximately 1 is maintained throughout the time series. Broadly this demonstrates the Jacobian measure is representative of fractional area change but not rigid-motion and should therefore be valuable in interrogating bowel wall motion.*

explanation is provided in Figure 2.2. The standard deviation is used to summarise the Jacobian score through time producing a SD Jacobian motility map with 0 where no motion is present and increasing where nonrigid deformation is present (Figure 2.2).

To expand on this further as this metric is central to this thesis, imagine the *mean* Jacobian was taken (instead of the standard deviation), where symmetrical changes took place in bowel wall motion (as is likely due to the cyclical contraction of the bowel) there would be little 'net' change in area with a fractional increase followed by a proportional fractional decrease. The Jacobian in this instance of symmetrical increase/decrease, regardless of its magnitude would have a value close to 1, suggestive of no motility. By taking the SD instead, 1 standard deviation (±67.5%) is observed from the mean Jacobian value with a larger standard deviation describing greater *variation* in area increase/decrease from 1 in the time series. For example, a 0.34AU score would suggest that ±67.5% of that pixels area change through time will be within ±0.34AU from 1. The lower the area change through time, the smaller the SD Jacobian value. The SD Jacobian metric is therefore a summary measure, blind to the number of time points, with dimensionless units.

A measure to represent intensity change (σ_C) was investigated as flow, caused by the movement of intestinal contents (chyme) is postulated to correlate with peristaltic activity. An intensity map was generated by taking the standard deviation of the intensity field, generated from the registration with the score being directly related to the variation in intensity through time (Figure 2.3).

A final parametric map was generated by multiplying the SD Jacobian and SD Intensity maps (σ_{JC}) to capture changes from both deformation and intensity through time (Figure 2.3).

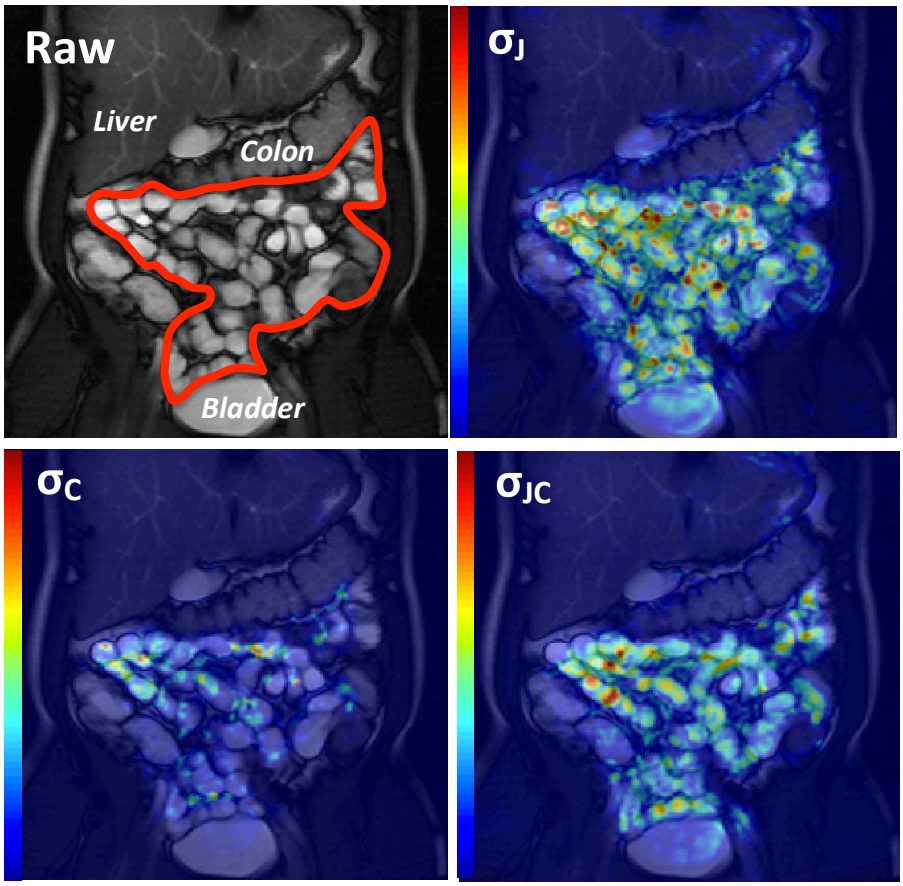

Figure 2.3. *Parametric motility map overview. The reference frame from an individual (raw) with the three parametric motility map overlays (red= high motility and blue = low motility). Small intestine outlined in red with key anatomy labelled in top-left 'Raw' image. All three maps appear similar with structures including the liver and colon attracting low motility scores although differences can be seen in the maps across the small bowel.*

2.2.7 Statistical analysis

Accuracy of the registration was assessed by comparing the ground-truth ROI length and areas, produced by the observers, to the algorithm-propagated lengths and areas. Bland-Altman limits of agreement were used to assess agreement both between observers and between ground truth and the registration algorithm. Evaluation of the quantitative assessment of motility was performed using ANOVA to detect significant differences between mean of metrics according to the 4 categories of bowel motility assigned by the radiologists (significance at $P < 0.05$) with Tukey-Kramer post-hoc testing to categorise groups. Pearson's correlation coefficient was additionally calculated to further characterise the metrics.

2.3 Results

2.3.1 Inter-reader agreement

The ground-truth was calculated by averaging the two observers line length and polygon area measurements. Analysis of line length ROIs with Bland Altman Limits of Agreement revealed a mean difference between readers linear ROI measurements of 0.2mm with a range of observed values between 6 and 25mm. 95% LoA were ±2.3mm (Figure 2.4a). Similar analysis of polygon areas between the two readers demonstrated a mean difference between readers of 5mm^2 with a range of observed values between 70 and 780mm^2 95% LoA were ±46mm^2 (Figure 2.4b).

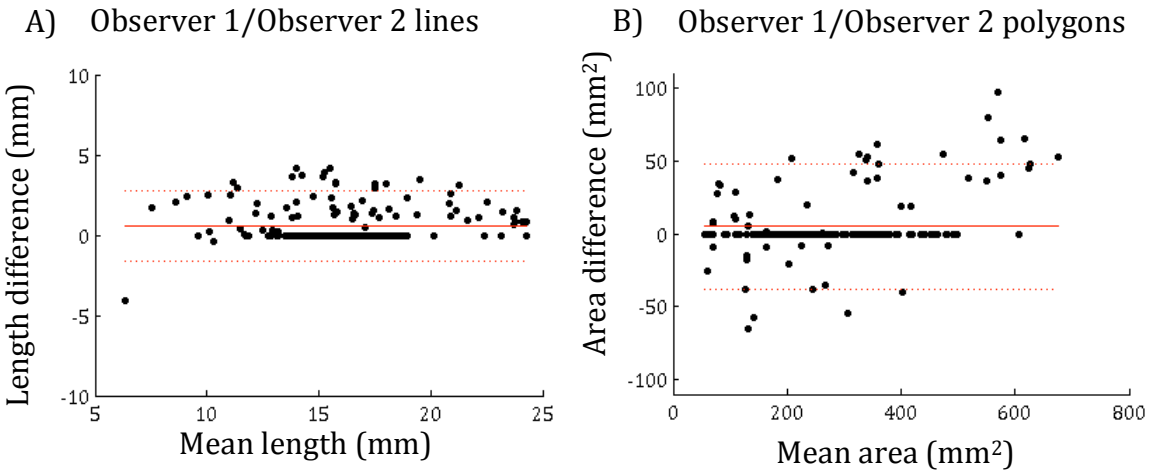

Figure 2.4. *Bland-Altman Limits of Agreement between observers for line (A) and polygon (B) ROIs.*

2.3.2 Assessment of registration accuracy

Comparing ground-truth to registration propagated line length ROIs demonstrated a mean difference of 0.5mm across the range of values 5 to 25mm (Figure 2.5a). 95% LoA was ± 2mm. Analysis of polygon ROIs showed a mean difference between the algorithm and ground truth of 5mm^2 with a range of observed values between 70 and 705mm^2 95% LoA were ±20mm^2 (Figure 2.5b).

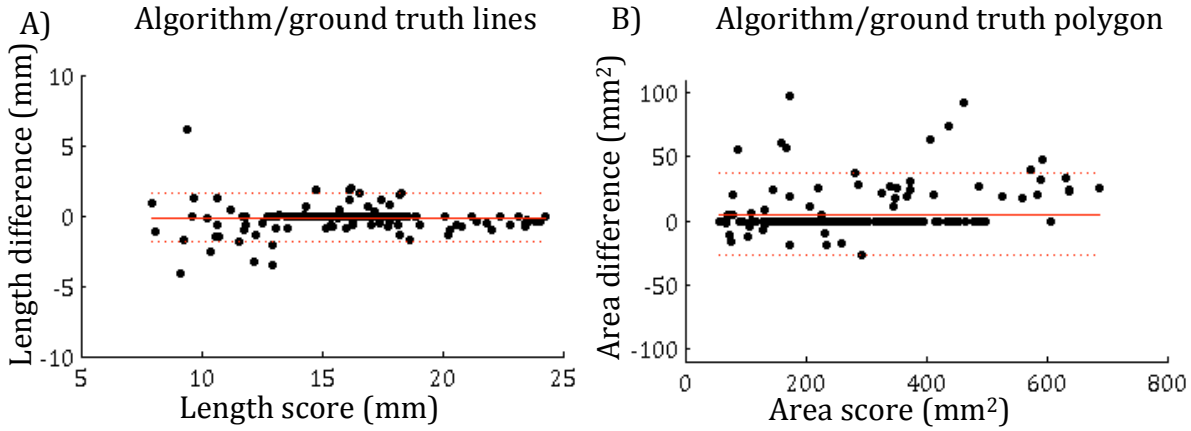

Figure 2.5. *Bland-Altman Limits of Agreement between ground-truth and algorithm propagated line (A) and polygon (B) ROIs.*

2.3.3 Assessment of automated motility quantitation

A total of 83 line (39) and polygon (44) radiologist drawn observations were made to which the metrics were compared. Correlation between motility metrics and clinical grading are summarised in table 2.2 and in Figure 2.6 with each metric demonstrating a strong negative correlation that was statistically significant.

Metric	Correlation (Line)	Correlation (Polygon)	ANOVA (Line)	ANOVA (Polygon)
	R (P-Value)	***R (P-Value)***	***F Statistic/P value***	***F Statistic/P value***
$\sigma_{Line/Area}$	-0.70 (<0.001)	-0.57(<0.001)	3.2 (<0.033)	7.1 (<0.001)
σ_{J}	-0.78 (<0.001)	-0.83(<0.001)	23.8 (<0.001)	35 (<0.001)
σ_{C}	-0.71(<0.001)	-0.55 (<0.001)	12.5 (<0.001)	8.3 (<0.001)
σ_{JC}	-0.74(<0.001)	-0.50 (<0.001)	15 (<0.001)	5.5 (0.003)

Table 2.2 *Pearson correlation coefficients and ANOVA test statistics for the assessment of clinical grading against metric generated motility scores.*

Analysis with ANOVA demonstrated an extremely significant F statistic in all cases suggesting the mean of at least one of the four groups was significant from the rest. This was confirmed with visual assessment of the data where there was a clear difference in the 'normal' motile bowel and the hypo-motile, category 4 bowel.

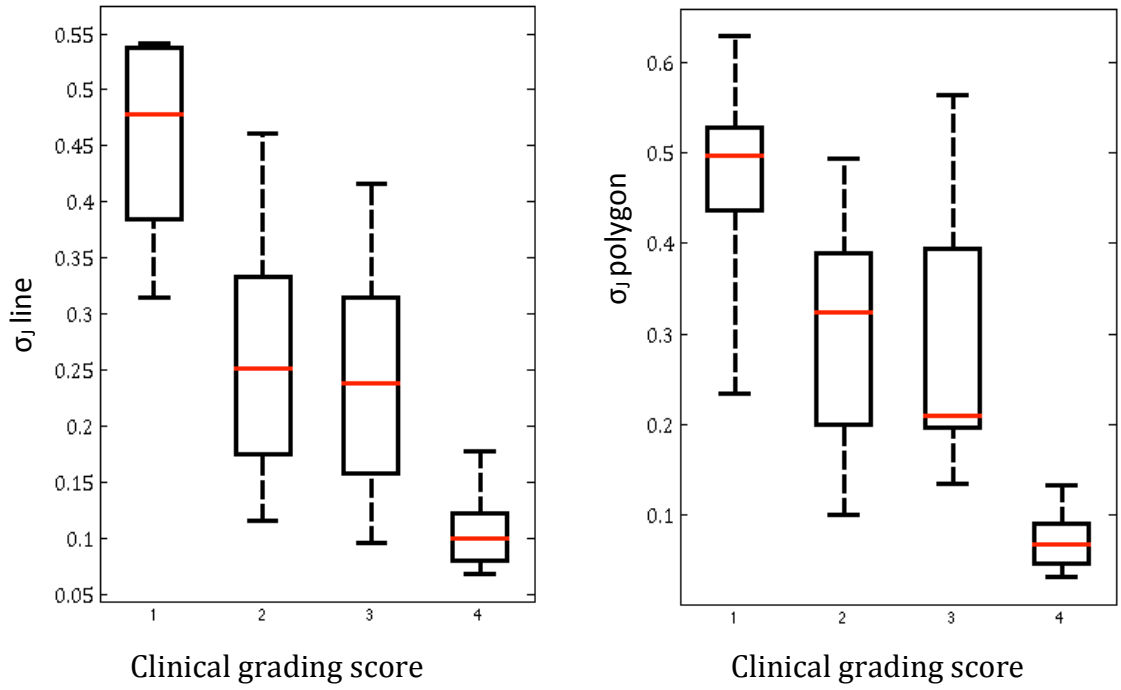

Figure 2.6*. Boxplots for SD Jacobian (σ_J) Line and polygon motility metrics across the four clinical grading's 1 (normal) to 4 (slow)*

Motion Index	Group Tested	Sig diff groups Line	Poly
$\sigma_{line} / \sigma_{polygon}$	1	2,3,4	3,4
	2	1,3	n
	3	1	1
	4	1,2	1
σ_J	1	2,3,4	2,3,4
	2	1,4	1,4
	3	1,4	1,4
	4	1,2,3	1,2,3
σ_C	1	3,4	4
	2	1	1
	3	1	1
	4	1,2	1,2
σ_{JC}	1	2,3,4	4
	2	1,2	n
	3	1	n
	4	1,2	1,2

Table 2.3 *Tukey-Kramer post-hoc testing of motility metrics. This test helps to demonstrate which groups are significantly different within line and polygon ROIs. The numbers in significant groups represent which groups are significantly different from each other.*

Post-hoc analysis to identify which groups could be separated using objective scoring and in all cases, the intermediate motility (groups 2 and 3) appeared challenging with data summarised in table 2.3. Broadly the SD Jacobian was capable of stratifying the greatest number of groups although all metrics struggled to differentiate intermediate motility scores.

2.4 Discussion

The purpose of this chapter has been to introduce the optic flow registration algorithm, demonstrate its accuracy and finally present an explanation and justification for how small bowel motility might be objectively evaluated.

In this study, the manual ground truth was the mean of two observers corrected line lengths or polygon areas. Bland-Altman (BA) Limits of Agreement (LoA) was employed to estimate the mean difference between the observers for both ROI types. BA LoA can be used to estimate the between (inter)-reader variation, an essential component of assessing the quality of our ground-truth. In this study 95% of the estimated inter-reader variation was expected to fall within ±1.5mm in lines or ±15mm^2 in polygon range. The question then becomes, is this level of variation acceptable between readers? To answer this, variance within the context of the range of values observed in the study was examined which, for lines, was 17mm and polygons 700mm^2. For both ROI types, the level of inter-reader variation is nearly an order of magnitude smaller than the range of observed values, meaning variation between readers will be likely to have little effect on the measurement and confirms the suitability of this ground truth for comparative purposes.

Confidence in the gold standard allowed its comparison to the algorithm propagated ROIs. When observing the algorithm propagated ROI, it appears ‘to “track” the bowel wall. This is the result of the registration finding the true deformation field of the bowel with the algorithm making no differentiation between abdominal

structures. The ROI position, determined by the deformation field, was analysed in terms of length or area and compared to the ground truth. Again using BA LoA, both ROI types demonstrated a small mean difference of 0.5mm and 2mm^2 for the respective ROI types, effectively at the resolution of the images themselves at 1x1mm. Examining once more the interplay within measures with respect to the range of the data, a negligible variance is seen between the techniques in relation to the range of values measured. Approximately 5x and 12x the amount of variation occurred across the cohort compared to within the observations for line and polygon ROIs respectively. Thus it can be reasonably concluded from these data that the registration algorithm is capable of accurately correcting the time-series data.

There are nevertheless some limitations here with the first being that line length alone is not a sufficient indicator of registration accuracy. Where respiration or through plane motion removes a loop of bowel from the image, the algorithm might translocate the ROI to a adjacent bowel loop for example maintaining a similar length but being fundamentally wrong in terms of its location. Using a breath-hold protocol helped to mitigate this as a problem. Here the ability to automatically propagate a ROI faithfully is not just a useful tool to evaluate registration accuracy but can be used as a primary endpoint for quantitative investigation of the bowel and, even now, the most common metric used takes less than a second with the help of the registration code[16], [56], [76]–[78].

When comparing the algorithm to subjective radiologist assessment of motility, the parametric motility metrics performed well, each providing a novel approach to quantitatively evaluate motility. The best performing metric was the SD Jacobian

that produced a high correlation coefficient against clinical grading and an ability to categorise motility into groups. Importantly, this metric exclusively assesses deformation as opposed to intensity change that represents a key physiological point of consideration. Following MRE preparation, the lumen of the bowel is filled with fluid yielding a bright signal; this signal produces a high signal intensity that in turn leads to a high intensity metric score. In many cases, this filling of the bowel lumen will trigger type 2 contractile actions and so will undoubtedly correlate with motility. Where the bowel is collapsed however, peristalsis can and often does continue which is poorly reflected in an intensity driven metric but representative of important physiological activity. This bias of the metric towards filling with oral preparation may mitigate the usefulness of the technique especially where patients cannot tolerate large volumes of liquid. An additional consideration is the generalizability of the finding where different clinical protocols might provide different contrast (eg. sequence variations) that would need to be first corrected for motility quantitation[62]. A partial solution provided here was to combine the intensity map with the deformation map to produce an additional motility metric and this indeed correlated well with clinical grading (Table 2.2). However, until the relationship between intestinal content and motility is better resolved, the inclusion of intensity in a metric to describe motility is a potential source of error to an already complex method of analysis. One key limitation in this part of the study was the gold standard grading. A semi-quantitative 4-point scale was used here in the absence of a validated method for objectively evaluating dynamic MRE data. Despite the experience of the examining radiologists, this grading remained subjective and therefore subject to bias. It was exceptionally difficult to identify category 2 and 3 motility scores consistently and in many ways demonstrates the purpose and

necessity of this research. When grouping category 2 and 3 together, the intermediate motility could be resolved by several of the metrics but this might be too stringent a test for the radiologist and should not necessarily be seen as a limitation of the registration. In practice, radiologists will simply state whether peristalsis is present or not rather than commenting on the rate of contractile activity and so there is certainly room for the development of a more robust gold-standard.

A second limitation of all of these metrics lay in the fact that they did not have a temporal component as the SD of deformation or intensity variation over the number of time points was taken. The larger the SD Jacobian the more fractional area change there is and hence more elastic movement of the pixels. The implication being that if one were to increase the number of time points, it would increase the 'height' of the histogram without changing overall spread of values. In the case of breath-hold studies running over 20s this is acceptable and even advantageous as a step to standardise the data. However the potential problem arises when the bowel is examined from a physiological perspective. The bowel, as discussed in the previous chapter, is a heterogeneous organ with varied contraction cycles and quite a unique behaviour in response to various stimuli. The SD Jacobian measure in effect removes much of this information and undoubtedly, when moving to longer time series may conceal otherwise valuable data contained within the time series. A solution might be found in the first instance by, instead of taking the standard deviation through time, recording a cumulative score per minute to give the metric a temporal component, bringing it in line with other metrics including 'contractions per minute' of diameter and making the metric more accessible for research uses.

Beyond this, the texture and heterogeneity of the values beneath an ROI might be investigated with scores using kurtosis and other forms of histogram analysis potentially representing an exciting new area of research.

Summary

This chapter has introduced the core technology that underpins this PhD and some of the key methodological concepts that run throughout this thesis. In the next two chapters the registration algorithm is applied in two proof-of-concept studies to demonstrate both the role of registration in motility analysis and also the power of MRI to gain insight into an extremely challenging aspect of human physiology.

SECTION B: RETROSPECTIVE INVESTIGATION OF SMALL BOWEL MOTILITY CHANGES IN CROHN'S DISEASE

Section B explores the practical application of quantitative motility analysis in patient datasets. The Centre for Medical Imaging was granted ethics for the retrospectively use of clinical small bowel imaging data the University College Hospital providing a large cohort of over 1300 patient scans with dynamic MRI imaging. Most patients were referred for known or suspected Crohn's disease and this provided the starting point for clinical investigations detailed in this thesis. Application of the registration algorithm to a cohort of 28 Crohn's Disease (CD) patients is described in **Chapter 3** where the relationship between inflammation and motility at the terminal ileum is explored. The hypothesis being tested is that motility is affected by the level of inflammation (ie the more the bowel is inflamed, the lower the motility). The inflammatory process that characterises Crohn's disease often leads to a narrowing of the bowel lumen and in severe cases may severely restrict or completely obstruct the passage of contents along the GI tract. This functional obstruction leads to dilatation of the bowel immediately upstream of the obstructed bowel and is extremely common in Crohn's disease, but data relating to the functional motility changes are poorly represented in the literature and remain largely anecdotal. In **Chapter 4,** data is presented investigating stricturing disease in 81 CD patients where it is hypothesised that that quantitative changes in

motility occur both at the site of the stricture and in the preceding dilated bowel in relation to the magnitude of the calibre change.

CHAPTER 3: QUANTITATIVE ASSESSMENT OF TERMINAL ILEAL MOTILITY IN CROHN'S DISEASE PATIENTS

Research Question:

What is the relationship between small bowel motility and inflammatory activity at the terminal ileum in Crohn's disease (CD) patients?

Rationale:

Inflammatory activity in Crohn's diseases leads to thickening of the bowel wall and an array of discrete structural changes in bowel appearance. Contrast enhancement, mural thickening and T2 signal have all been correlated with an increased inflammatory score assessed through biopsy and serve as a non-invasive means of grading CD. Additional features predictive of inflammatory activity would be valuable in the clinic to inform therapeutic intervention.

Hypothesis:

A decrease in small bowel motility correlates with an increase in inflammatory activity at the terminal ileum in CD patients.

Aim(s):

i) Correlate small bowel motility against a histopathological (eAIS) reference at the terminal ileum.

ii) Correlate motility against anatomical MRI markers of inflammation and establish whether motility might serve as an independent predictor of inflammation.

Author Declaration

The work presented in this chapter was lead by the author including obtaining ethical permission, performing the literature review, data collection and analysis and manuscript preparation under the supervision of Dr D Atkinson and Prof. S. A Taylor. This research has been published in: Menys, A., Atkinson, D., Odille, F., Ahmed, A., Novelli, M., Rodriguez-Justo, M., … Taylor, S. A. (2012). Quantified terminal ileal motility during MR enterography as a potential biomarker of Crohn's disease activity: a preliminary study. European Radiology, 22(11), 2494–501. doi:10.1007

3.1 Introduction

Crohn's disease is a chronic, relapsing inflammatory bowel disease predominantly affecting the gastrointestinal tract. Patients typically present with abdominal pain, diarrhoea and symptoms suggestive of bowel obstruction. Across Western countries, the prevalence of Crohn's disease (CD) is increasing with approximately 157/100,000 diagnosed with CD in the UK[79]. Despite it's high prevalence, the aetiology of the disease is poorly defined with numerous genetic and environmental factors being postulated. Familial aggregation has been described for over 70 years with twin concordance studies across Northern European countries demonstrating a clear genetic component to the disease[80]. Genome wide association studies have identified 70 susceptibility loci for CD across 17 chromosomes, although none have

revealed a ‘smoking gun’ from a genetic perspective[81]–[83]. These investigations have however uncovered important clues as to the implicated pathways, the majority of which suggest an aberrant intestinal immune system[84]–[87]. Despite a growing understanding of the genetic basis for the disease, this component still explains only 20% of the heritability of the disease with much of the research in this area now focusing on epigenetic and environmental factors[88], [89].

The significance of environmental factors is illustrated by the increasing incidence in previously less affected ethnic groups after moving to high incidence areas[89]. Myriad studies have investigated the effects of air pollution, sedentary lifestyle, Western diet for example. Smoking in particular has compelling evidence linking it to CD severity. [90]–[94]. CD frequently occurs after infections gastroenteritis and the search for a causative infectious agent has been sought with several adhesive bacterial types being proposed candidates although little compelling evidence has been provided thus far for a single microbial origin[95].

Despite the severity of this disease, those diagnosed with Crohn’s have only a slightly reduced life expectancy even though 50% of patients require surgery within 10 years of their diagnosis[96], [97]. Current treatment of CD aims to achieve sustained remission with the goal of disrupting the destructive course of the disease that would otherwise lead to intestinal failure and associated complications[98]. A growing number of increasingly expensive therapeutic agents are now available for the management of patient symptoms with fast acting steroids or anti-TNF agents routinely used to provide rapid symptom relief [50]. Rational use of immunosuppressive therapies in Crohn’s disease relies on accurate identification of

those patients with acute inflammation – so-called “active disease” – who are most likely to respond to the treatment. Assessing disease activity is difficult and a number of approaches are employed, ranging from clinical assessments based on patient symptomatology (such as the Crohn’s disease activity index and Harvey Bradshaw index), biochemical markers such as ESR, CRP and stool calprotectin, and endoscopic and histopathological grading[50], [98].

Advances in medical imaging, initially using CT, have facilitated high resolution cross sectional imaging of the bowel wall to evaluate intra and importantly extra-luminal disease activity[99]–[103]. More recently Magnetic Resonance Enterography (MRE) has further added to the diagnostic toolset available in the modern clinic with the additional advantage over CT of not using ionising radiation. MRI is increasingly used in the diagnosis of CD to assess disease activity and objectively inform therapeutic strategies. Features of inflammatory disease including increased bowel wall T2 signal, bowel wall thickness and enhancement have been strongly correlated with disease activity on endoscopy and histopathology and increasingly present a safe and non-invasive method of grading and observing disease[104]–[109]. Beyond imaging, a diagnosis of CD of course includes patient history and objective data from a range of blood, histopathological, endoscopic and imaging investigations with no single test, at present, being sufficient to make a diagnosis in isolation. However, some investigations such as MRI have the capability to offer multiple insights on disease physiology beyond structural changes in the bowel non-invasively and without the use of ionising radiation.

One might reasonably assume that motility will be reduced in Crohn's disease based on the appearance of the bowel wall alone. Surgical resection of severely diseased bowel reveals marked hypertrophy of the wall and this is routinely described clinically based on cross sectional imaging (Figure 3.1 A-D)[105]–[107], [109]. This thickening is likely to have a marked impact on the ability of the bowel to contract with both inflammatory and fibrotic infiltrate likely playing a role. Fibrosis undoubtedly has a direct and irreversible impact on motility, quite literally stiffening the wall such that any contractile potential is lost (Figure 3.1 E&F). More complex is the effect of inflammation with both a direct impact of swelling and oedema impairing contraction but also the potential for indirect, cytokine mediated suppression of motility[110]–[113]. Although anecdotal evidence and a single preliminary study have suggested motility is altered in active CD, there were no quantitative investigations at the time of this work to draw a relationship between specifically inflammatory activity and motility[14]. In this chapter it was hypothesised that motility will be negatively impacted by inflammation. However, of further interest will be the correlation with routine anatomical markers of activity such as bowel wall thickness with which motility is potentially inseparably related.

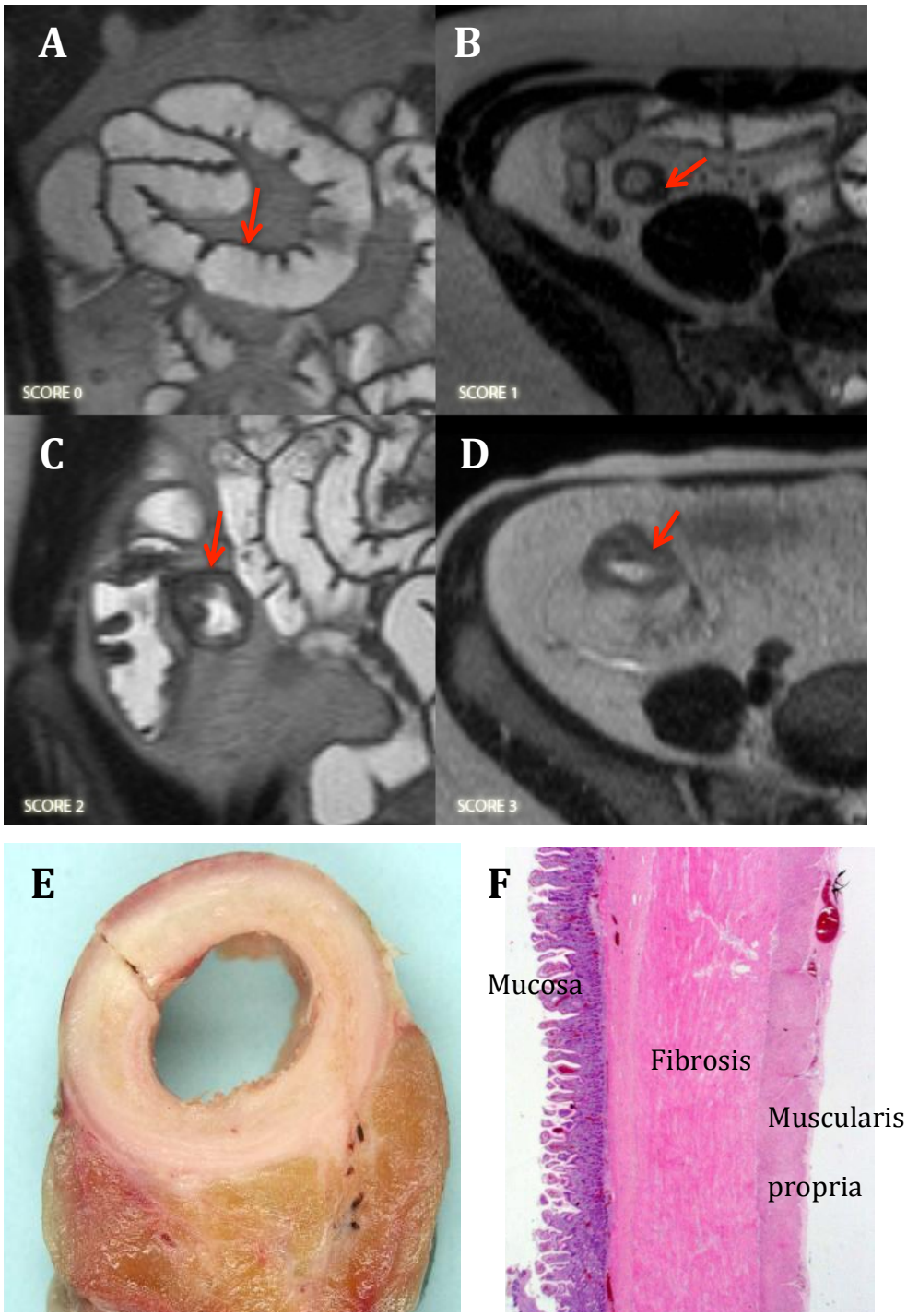

Figure 3.1 *Anatomical overview of changes in small bowel structure in CD. Axial MRE slices of four progressively thickened (A-D) small bowel loops. Bowel wall thickness has been negatively correlated with both luminal and trans-luminal histopathological markers of inflammation [95]. E shows a resected bowel loop with extensive fibrotic process (red bracket) and haematoxylin-eosin staining F. Such extensive fibrotic changes are likely to irreversibly induce motility changes in bowel wall (E & F provided by Dr. M. Rodriguez Justo, Histopathology, UCLH)*

3.2 Materials and methods

3.2.1 Patient cohort

A review of the UCLH institutional database (2008–2011) was undertaken to identify patients fulfilling the study eligibility criteria of (i) prior histologically proven diagnosis of Crohn's disease, (ii) undergoing clinically indicated disease assessment with MRE (including cine MRI motility sequences – see below) and (iii) endoscopic terminal ileal biopsy within 4 weeks of MRI.

Twenty-eight patients (mean age 34 range 16–71, 18 female, 10 male) fulfilled all eligibility criteria. The demographics of this cohort are represented in Table 3.1. Most (n=18) had ileocolonic disease. The median time between MRE and endoscopy was 4 days (range 0-30), and in 25 of the cohort, the time difference was 15 days or less seven of the cohort had undergone terminal ileal resection and 21 had no previous surgical intervention (table 3.1).

3.2.2 MRI Protocol

Patients underwent the same MR protocol for the coronal motility sequences as per chapter 2.2 using the same 1.5T Siemens MRI scanner. Following dynamic data acquisition, 20 mg of IV spasmolytic (Buscopan, Boehringer Ingelheim, Ingelheim, Germany) was administered and the routine clinical protocol was run to acquire anatomical images including: axial and coronal fat/non-fat-saturated HASTE images. Coronal volumetric interpolated breath-hold examination (VIBE) acquisitions were

performed at 30 and 70 s post-injection of 10 mL gadopentetate dimeglumine (Magnevist, Berlex Laboratories, Montville, NJ, USA). Detailed parameters of all MR sequences are provided in Table 3.2.

Parameter	Number of Patients
Disease Duration	
Less than 1 year	8
Between 1 and 5	4
Between 5 and 10 years	5
Over 10 years	6
Disease Distribution	
Colonic	2
Ilecolic	13
Isolated Terminal ileum	5
Small bowel beyond terminal ileum	3
Current Immunosuppressive medication	
none	2
1 agent	13
2 agents	8
Surgical History	
No previous surgery	17
One Operation	5
Two previous operations	1

Table 3.1*. Patient demographics illustrating the distribution of disease duration and location, medication status and surgical history of the recruited cohort.*

	Coronal balanced SSFP	**Coronal/ axial HASTE**	**Coronal/ axial TrueFISP**	**Coronal Baseline VIBE**	**Coronal 30s and 70s VIBE**
Field of view (mm)	Variable	Variable	Variable	Variable	Variable
NO. Slices	20	20/26	25/34	48	48
Stacks	6-16	1/4	1/2	1	1
TR (ms)	3.85	1200/800	4/4.2	17.2	7.2
TE (ms)	1.93	86/86	1.7/2.1	2.4	2.4
Image matrix	256 x 184	256x195	256 x 205	256 x 135	256 x 135
Slice thickness (mm)	10	4/4	4/4	3	3
Slice gap	< 10	5.2/5.4	5.2/5.4	0	0
Averages	1	1	1	1	1
Turbo factor		195	-	-	-
iPAT		Grappa x2	n/a	n/a	n/a
Flip Angle	61	50	46	10	10

Table 3.2 MR protocol sequence parameters using a 1.5T Siemens Avanto scanner (Siemens, Erlangen, Germany)

3.2.3 Motility assessment

Anonymised datasets were downloaded from the hospital PACS system and processed with the optical flow registration software detailed in the previous chapter section 2.2.4. For this study, the standard deviation of the Jacobian (SD Jacobian) determinant was used as a surrogate for motility as this was most strongly correlated with clinical grading of motility as detailed in the previous chapter validating the registration software (see 2.3.3). In summary, the SD Jacobian

metric evaluates the fractional change in area produced by the deformation fields generated by registration of the time series images.

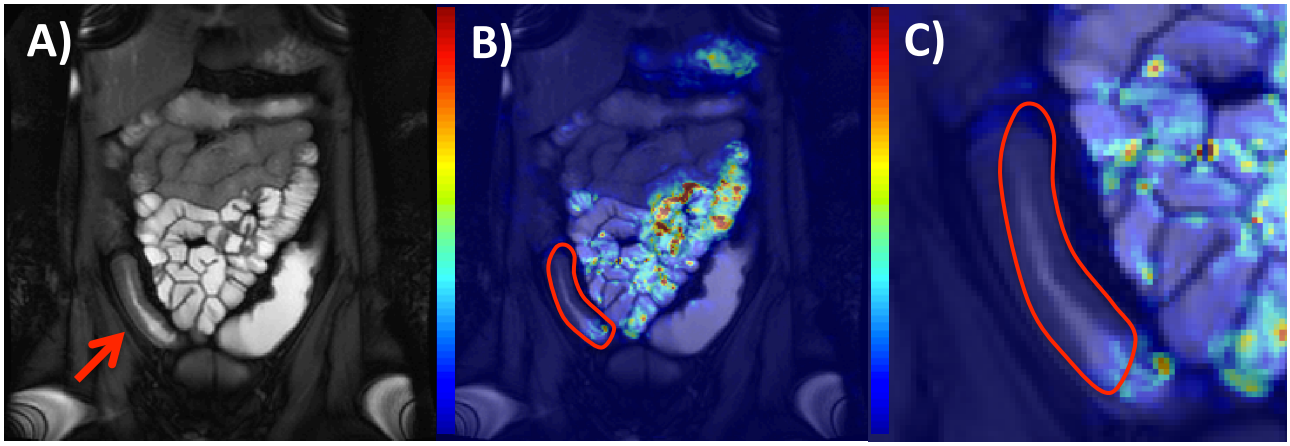

Figure 3.2 *ROI Placement at the Terminal Ileum. a) A single image from a dynamic set of 20 with a ROI placed on a diseased terminal Ileum. Red arrow points to diseased, thickened terminal ilem. b) Summary image of the dynamic sequence with a the Jacobian standard deviation parametric map overlay demonstrating areas associated with larger degrees of motion (red) and decreased motion (blue). Motion is assessed by assigning each pixel in the image with an associated displacement value, expressed as the standard deviation of its Jacobian. c) Magnified view of the terminal ileum where a user defined ROI produces the mean value for the pixel displacement within the ROI and hence the motility value.*

A single observer (Prof Stuart Taylor), who was blinded to all clinical data, manually drew a polygonal ROI within the last 3 cm of the terminal ileum within a frame of the cine stack best depicting this section of small bowel and the motility score (SD Jac) recorded (Fig. 3.2).

3.2.4 Anatomical MRI grading of disease activity

Two consultant radiologists (Dr S. Punwani and Prof. SA Taylor) reviewed all 28 datasets using a picture archiving and communication system (PACS) viewing system, unaware of the motility scores or clinical information. In particular they reviewed all MRI sequences acquired (Table 3.3) but specifically not the motility sequences. In consensus the radiologists applied a qualitative score of MRI disease activity to the last 3 cm of terminal ileum based on that described by Steward et al (Table 3.3)[107]. In this work qualitative scoring (0–3) of mural thickness, mural T2 signal, mural enhancement and perimural T2 signal were all correlated with disease activity based on a transmural histopathological reference. The sum of the scores of these four parameters for each patient constituted the anatomical MR activity index (aMRI).

Score	0	1	2	3
Mural thickness	1-3mm	>3-5mm	>5-7mm	>7mm
Mural T2 Signal	Equivalent to normal bowel wall	Minor increase in signal-bowel wall appears dark grey on fat saturated images	Signal-bowel wall appears light grey on fat saturated images	Signal-bowel wall contains areas of white high signal approaching that of luminal content
Perimural T2 signal	Equivalent to normal mesentery	Increase in mesenteric signal but no fluid	Small fluid rim (≤2mm)	Larger fluid filled rim (>2mm)
Enhancem -ent	Equivalent to normal bowel wall	Minor enhancement- bowel wall signal greater than normal small bowel but significantly less than nearby vascular structures	Moderate enhancement- bowel wall signal increased by somewhat less than nearby vascular structures	Marked enhancement - bowel wall signal approaches that of nearby vascular structures

Table 3.3 *MRE anatomical activity grading algorithm based on Steward et el*[12]

3.2.5 Histopathological reference

In each patient the terminal ileal biopsy obtained during colonoscopy was stained with haematoxylin-eosin and reviewed in consensus by two experienced pathologists (Dr. M. Rodriguez-Justo & Prof. Marco Novelli, 3 and 10 years' experience respectively), who were unaware of clinical details or MRI findings. The histopathologists applied an endoscopic biopsy acute inflammatory score (eAIS) based on the typical morphological features of Crohn's disease described in guidelines published by the European Crohn's and Colitis Organization[51], and first proposed by Steward et al[107]. The numerical system includes variables associated with epithelial damage and neutrophilic infiltration (i.e. epithelial damage, architectural changes, epithelial neutrophils and lamina propria, erosion/ulceration, and presence of granulomas; Table 3.4). At least three samples of terminal ileum biopsy were collected for each patient and the highest score for each was used for that patient, in accordance with the standard procedure at UCLH.

Histological variable	Grade
Erosion or ulceration	0 = No, 1 = Yes
Polymorphs in the lamina propria	0 = No, 1 = Yes
Cryptitis	0 = No, 1 = Yes
Crypt and abscess formation	0 = No, 1 = Yes
Inflammatory exudates	0 = No, 1 = Yes
Granulomas	0 = No, 1 = Yes

Table 3.4 *Histopathology grading scheme for eAIS*

3.2.6 Statistical analysis

The Shapiro–Wilk test ($\alpha = 0.5$) was used to examine whether motility, anatomical MR activity index and histopathological grading were normally distributed.

Patients were also divided into those with histopathological evidence of inflammation on terminal ileal biopsy (eAIS ≥1) and those without (eAIS=0). The motility index was compared between the two groups using the Wilcoxon rank sum test.

The association (if any) between the derived MRI motility index and the histopathological eAIS score, and was assessed by Spearman's rank correlation. Spearman's rank was also used to assess the strength of association between the anatomical MRI grading of activity and both the eAIS score and motility index.

A linear regression model was built to test if the addition of motility beyond anatomical MR grading could improve the prediction of inflammation. The first stage of the data analysis examined the ability of the anatomical MRI grade to predict inflammation in a univariate analysis. In the second stage of analysis, the benefit of including a motility score with the anatomical MRI grade was examined. Statistical significance was assessed at $P < 0.05$.

3.3 Results

3.3.1 Motility score and histopathological correlation

Across the 28 patients, the mean motility score was 0.27 (range 0.06–0.55) and the mean histopathology score (eAIS) was 1.5 (range 0–5). One of the cohort had dilatation of the terminal ileum.

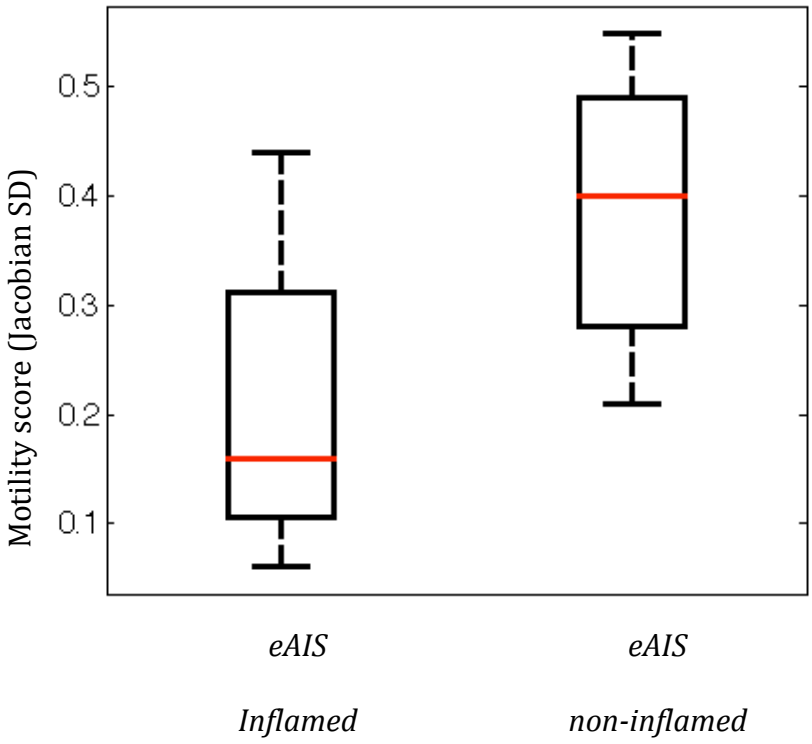

Figure 3.3 *Boxplot of inflamed and non-inflamed study subjects assessed by eAIS revealed a statistically significant difference in motility scores between groups.*

The motility index for non-inflamed terminal ileum (eAIS score 0; n = 12, mean 0.37, range 0.13 to 0.55) was significantly greater than bowel exhibiting active disease

(eAIS ≥ 1; *n* = 16, mean 0.19, range 0.06 to 0.44; *P* = 0.002; T-stat 3.4 [df27]; CI, 0.07–0.28; Fig. 3.3).

There was a significant negative correlation between the motility index and histopathological grading of activity (eAIS; Rho = -0.52, *P* = 0.005; Fig. 3.4). This correlation remained when subjects with a history of surgical resection were excluded (*n* = 21, Rho -0.49, *P* = 0.002).

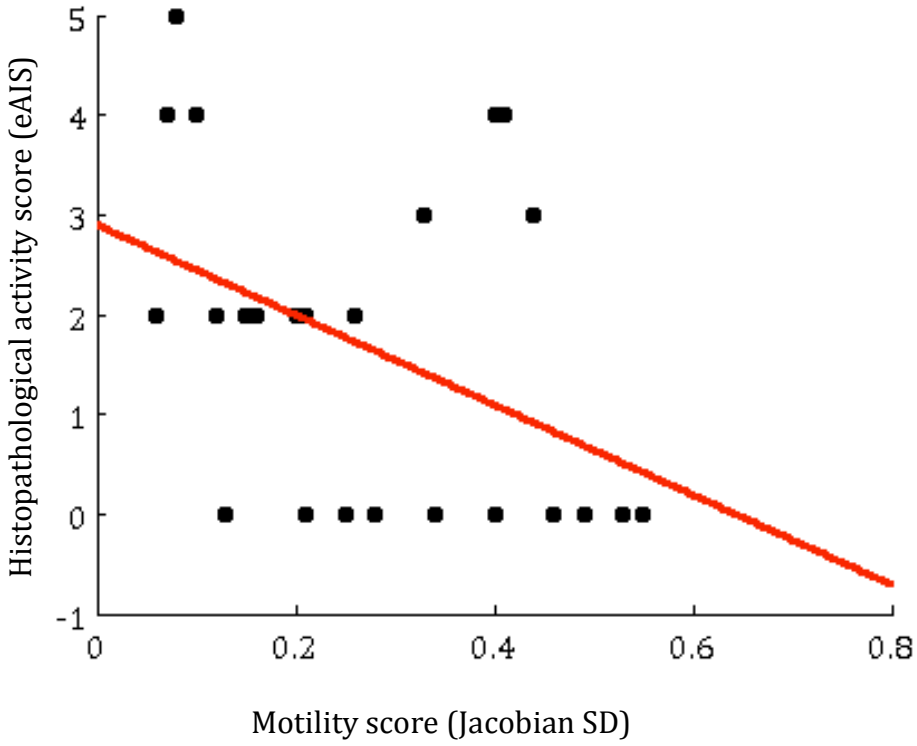

Figure 3.4 *Scatter plot of histopathological activity score (eAIS) of inflammation and motility score Jacobian SD.*

3.3.2 Anatomical MRI grade and Histopathological Reference

The mean anatomical MRI grade (sum of T2 signal, mural thickness, mural enhancement and perimural oedema scores) was 3.8 (range 0 to 10). There was a statistically significant positive correlation between anatomical MRI grade and histopathology (eAIS) score (Rho = 0.67, *P* <0.001).

3.3.3 Motility score and Anatomical MRI grade

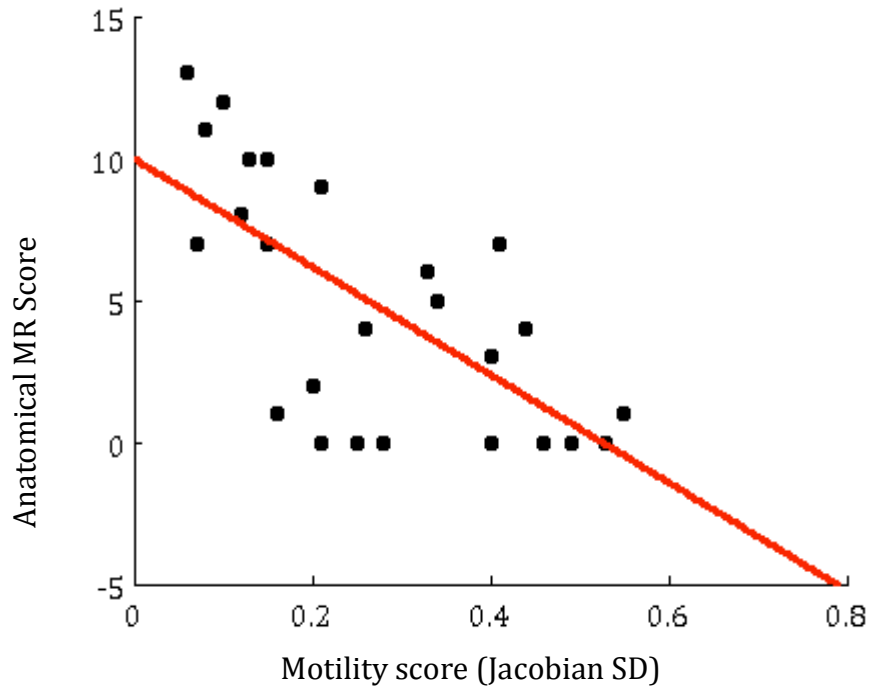

Figure 3.5 *Scatter plot of anatomical MRI scores activity and motility score (Jacobian SD).*

3.3.4 Regression analysis

The regression model confirmed anatomical grading was a statistically significant predictor of histopathological eAIS score with a regression coefficient 0.29 (adjusted R^2 = 0.42; P <0.001). However, addition of the motility index to the model did not confer any statistically significant benefit (regression coefficient -0.94) with a significance value of P = 0.65. Singularly, motility produced a model with an adjusted R^2 value of 0.23 (P = 0.005).

	Rho	**p**
Mural thickness	-0.52	0.005
T2 Signal	-0.69	0.002
Perimural signal	-0.45	0.016
Enhancement	-0.67	<0.001
Total anatomical MRI grade	-0.7	<0.001

Table 3.5 *Spearman's Rank correlation between anatomical MR parameters and SD Jacobian motility score.*

3.4 Discussion

The evidence to support the use of medical imaging to predict disease activity in Crohn's disease is increasingly apparent. Whilst there remains some debate as to which technique and parameters are optimal, the role of MRI in this context is expanding. The potential of motility assessment to aid the classification of disease activity over conventional MRI parameters has however received little attention.

Chapter 2 presented the validation of a registration-based method for quantitatively evaluating motility, and here applied it in a clinical cohort to investigate a disease process that is very likely to influence small bowel motility. A statistically significant negative correlation between objectively measured terminal ileal motility and an independent histopathological score of disease activity was found.

It is acknowledged that the histopathological standard of reference utilised in the current study was based on an endoscopic biopsy which by definition cannot encompass the entire thickness of the bowel wall. However the eAIS score did include factors known to be indicative of inflammatory activity. Furthermore, histopathological grading of biopsies is a recognised reference for Crohn's disease activity and has continued to appear as the gold-standard in numerous studies[51]. It is very likely that inflammatory activity on biopsy is closely correlated with activity throughout the bowel wall. Indeed Steward et al. found that an MRI model of activity derived using transmural histopathological sections from surgical specimens could equally predict the eAIS score based on endoscopic biopsy [107].

In this study motility was also correlated with independently assessed anatomical MRI grading of activity using mural thickness, T2 signal, contrast enhancement and perimural oedema. This anatomical scoring system has been proposed and validated previously by Steward et al. study[107]. Using regression modelling, although the predictive ability of the anatomical MRI grading for histopathological inflammation was confirmed, the strength of this prediction was not improved by adding the motility index. This suggests that motility itself may not be an independent marker of activity beyond existing anatomical parameters. Indeed there was a very strong negative correlation between motility and wall thickness alone. It appears intuitive that diseased bowel wall thickness and motility should be inversely correlated but this relationship could be exploited when looking for reversible versus irreversible changes following treatment in future studies. It is unclear for example if as wall thickness reduces, motility recovers although this reversibility could be an important parameter for differentiating inflammatory from fibrotic disease where the latter is irreversible. Data presented in the next chapter (chapter 4) begins to explore this concept in a study of patients with Crohn's disease related strictures.

As described in Chapter 1, small bowel motility is a complex and only partly understood process even in healthy individuals. Although the motility index was significantly different between those with and those without histopathological inflammation, there was clearly an overlap and a relatively wide range of motility in "normal" (eg. no inflammation) individuals. Although the routine UCLH clinical MRE protocol kept the period of starvation and composition of the oral contrast agent

constant in all subjects, other factors that may influence motility such as smoking, caffeine intake, time of day and hormonal fluctuation associated with the menstrual cycle were not controlled for[114], [115]. In disease states, and particularly in Crohn's disease, additional factors over and above acute inflammation may affect motility, including drug regimens, disease duration, location, coexistence of colitis, previous surgery and mural fibrosis. Such parameters require detailed consideration in prospective studies. The study population was relatively heterogeneous although perhaps this is a strength as the data may be more generalisable. However, equally it means that the various parameters that may have confounded the analysis were fully controlled for and may be responsible for some of the outlying data points. Of note, the statistically significant correlations between motility and eAIS were preserved when 7 patients with prior ileal resection were excluded. Perhaps of most interest is the effect of fibrosis – intuitively, fibrotic bowel will peristalse differently from normal bowel and the additional contribution of acute inflammation is unknown. It would seem reasonable to further hypothesise that both inactive, fibrotic disease and acutely inflamed bowel would decrease motility and both would attract very different eAIS scores, and this could certainly explain why such variation was observed within the 'no inflammation' participants in out cohort. Conversely, two of the patients recorded high motility and inflammatory scores which might perhaps be explained by acute mucosal inflammation at the TI. Alternatively, motility might be more variable than first assumed and indeed this will form the subject of chapter 5. Nevertheless, fibrosis and acute inflammation usually co-exist in Crohn's disease, with one predominating over the other making any assertions with respect to the fibrotic nature of the bowel difficult to demonstrate empirically [116]. Whether it will ever be possible to

use motility to differentiate between fibrosis and acute inflammation remains speculative. Certainly it is plausible that upon follow up, a patient, after receiving anti-inflammatory treatment, might demonstrate a change in motility at the site of their lesion which could in turn be used to estimate their fibrotic load. Although in our cohort only one of the terminal ileum segments was dilated, it seems likely that bowel dilatation, for example upstream of a stricture, could influence motility and indeed this will be investigated more thoroughly in the next chapter.

This study does have limitations. Using only he highest biopsy score to assign the per patient eAIS score, although standard practice at UCLH, could have introduced sampling bias. However the alternative of using an average of all biopsy scores could have skewed the data, for example if there was a large number of near normal biopsies in an otherwise highly inflamed segment. An endoscopic severity score was not included here although it might have been useful. However scoring systems are rarely used in routine clinical practice and the cohort was retrospective. A prospective study design would have been preferable, although because of the invasiveness and risks of colonoscopy, even prospective studies will likely be limited to including only those undergoing colonoscopy for clinical indications, and thus will also be open to similar spectrum bias. As with all studies attempting to validate MR markers of disease activity against endoscopic or histological standards of reference, it was not possible to be certain the endoscopic biopsy site exactly matched the site from which imaging scores were obtained. This was partially mitigated by using the last 3 cm of terminal ileum.

Summary

This chapter detailed the first application of a post-processing technique to assess motility in a clinical cohort. The results showed that motility could be used as an independent quantitative marker of inflammatory diseases activity and correlated with existing, well validated measures. In the next chapter, Crohn's disease will remain the subject of interest but this time, exploring a long held but largely un-investigated feature of the diseases, strictures, and the functional changes that take place in relation to this process.

CHAPTER 4: SMALL BOWEL STRICTURES IN CROHN'S DISEASE: A QUANTITATIVE INVESTIGATION OF INTESTINAL MOTILITY USING MR ENTEROGRAPHY.

Research Question:

How do Crohn's disease strictures influence small bowel motility?

Rationale:

It is anecdotally well known that stricturing disease has an adverse effect on small bowel motility both at the site of the stricture as well as in the proximal dilated bowel. Although several investigations have demonstrated motility changes in animal models, there is little to no quantitative data to explore this process either in humans or in Crohn's disease. It is furthermore unknown whether motility changes in the case of the pre-stricture bowel are reversible following treatment that in turn has an impact on surgical management of the disease.

Hypothesis:

A negative correlation exists between the calibre of the pre-stenotic bowel and its motility but this is reversible where the obstruction is treated.

Aim(s):

i) Quantitate small bowel motility in patients with stricturing CD both within and upstream of the obstructive lesion.

ii) Investigate changes in pre-stricture motility between scans where a follow up investigation is available.

Author declaration

The work presented in this chapter was lead by the author including obtaining ethical permission, performing the literature review, data collection and analysis and manuscript preparation. Dr E. Helbren provided radiological input for the identification and annotation of small bowel strictures. The project was conducted under the supervision of Prof. S. A Taylor. This research has been published in: Menys, A., Helbren, E., Makanyanga, J., Emmanuel, A., Forbes, A., Windsor, A., … Taylor, S. A. (2013). Small bowel strictures in Crohn's disease: a quantitative investigation of intestinal motility using MR enterography. Neurogastroenterology and Motility: 25(12), 967–e775. doi:10.1111/nmo. 12229.

4.1 Introduction

The previous chapter described and investigated the inflammatory process that takes place in Crohn's disease. In many cases this process leads to progressive wall thickening and narrowing of the bowel lumen resulting in stricturing disease. Inflammatory strictures can themselves cause obstruction of the bowel. Furthermore, following repeat flares of the disease, the bowel wall may become fibrotic and permanently thickened, gradually occluding bowel lumen. In many cases, the bowel becomes chronically obstructed due to the irreversible nature of the fibrotic stricture, which is one of the leading causes for surgical intervention including resection and balloon dilatation (carried out in over 80% of patients with

long term Crohn's disease) [97]. Obstructed patients will often present clinically with severe abdominal pain and vomiting and radiological imaging will reveal often striking dilatation in a region of bowel (Figure 4.1) upstream of a stricture caused by the functional hold-up of intestinal contents. Most small bowel strictures are beyond the reach of the endoscope and therefore imaging is pivotal to diagnosis and long-term assessment.

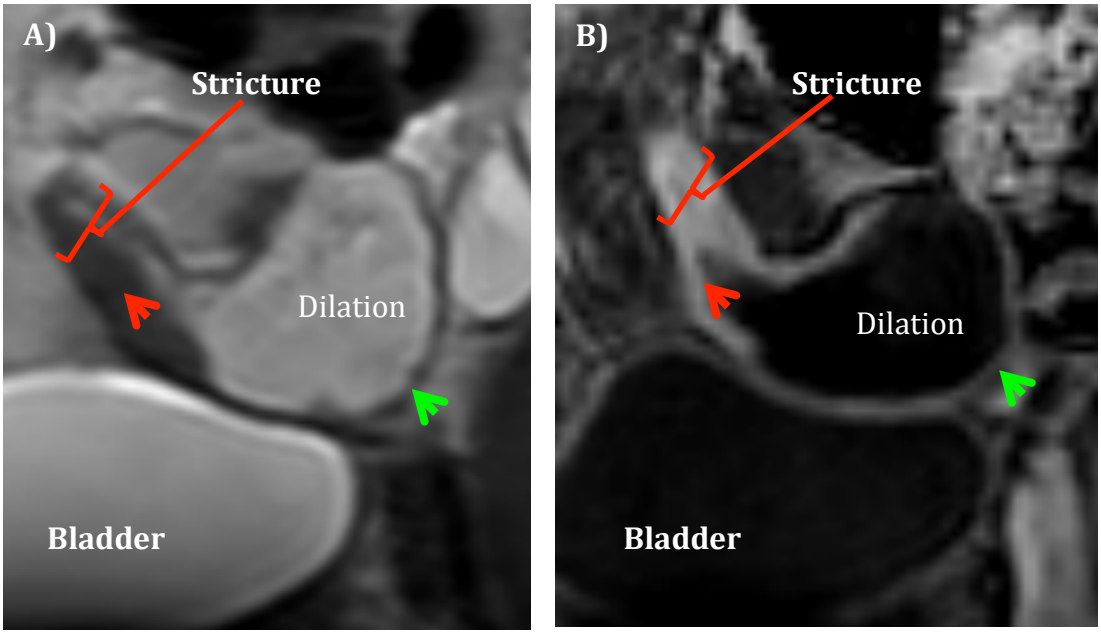

Figure 4.1 *Depiction of a small bowel stricture imaged with a T2 weighted (A) and post-contrast THRIVE (B) sequences. A narrowing of the bowel lumen can be seen in both images characterised by a thickening of the bowel wall (red arrow). The proximal dilation is characteristic of such luminal narrowing and while the bowel wall itself remains 'normal' (green arrow) the calibre of the bowel increases to many times that of unaffected intestine.*

The data in this chapter examines functional motility aspects alongside the well described anatomical abnormalities associated with stricturing Crohn's disease, which to date has received relatively little attention. Since the advent of the barium

follow-through, where contrast is ingested to view the bowel lumen, abnormalities in how the bowel peristalsis related to strictures has been described, although little in the way of published data exists on the topic [117]. Anecdotally, the thickened strictured bowel was found to be hypo-peristaltic, but pre-stricture the (often) dilated bowel demonstrated variable altered motility being either greater or reduced compared to adjacent normal bowel[117]. This perception has been further reinforced by pre-clinical studies in animals which have quantitatively demonstrated in both transient and longer-term changes in motility(Prihoda, Flatt, & Summers, 1984). Prihoda demonstrated in a canine model an acute increase in proximal small bowel motility when an obstruction was induced by inflating an intraluminal balloon, with an associated decrease in distal motility[118]. Quantitative studies in humans have however remained essentially absent from the literature for a number of reasons. Barium follow though, whilst an excellent technique for viewing the bowel lumen, is difficult to quantitate with a further reliance on ionising radiation that has increasingly mitigated its use in the modern clinic[51], [120]. Traditional methods for evaluating motility including manometry are contraindicated due to the presence of obstruction and thus the investigation of this aspect of CD has remained largely neglected.

This chapter investigates the functional dynamic motility data from a cohort of CD patients with sticturing disease and specifically to evaluate motility alteration in obstructed bowel. In the previous chapter, the data suggests that motility was inversely correlated with bowel wall thickness and therefore it is reasonable to hypothesise that the thickened bowel in strictures will almost certainly also be hypo-motile. Of greater interest however is what happens immediately upstream of

strictures in the dilated pre-stenotic bowel and in regions of anatomically 'normal' bowel to look for the first time at using MRI to investigate the impact of stricturing Crohns on small bowel motility. This retrospective investigation again had access to UCLH's small bowel MR database and therefore also had the opportunity to investigate instances where patients returned to the clinic for follow-up scans. Additionally, the opportunity to investigate temporal changes between individuals undergoing small bowel enterography was exploited.

4.2 Methods

Patient cohort

A primary review of UCLH database (2008–2012) was undertaken to identify a list of patients fulfilling the study eligibility criteria of: i) previous clinical diagnosis of Crohn's disease, ii) undergoing MR enterography (MRE) as part of routine clinical practice, including motility sequences (see below) and iii) the term 'stricture' reported by the reviewing radiologist at the time of scan.

The MRE examinations from 350 identified patients were reviewed by a radiologist with 4 years experience of reporting MRE (Dr Emma Helbren) to identify those with a true small bowel stricture. For the purposes of the current study, a stricture was defined as a focal reduction in small bowel luminal calibre with associated bowel wall thickening and an appreciable increase in diameter of the calibre of the pre-

stricture bowel compared to normal bowel diameter. This review identified a cohort of 130 potentially eligible patients.

A further 25 were excluded where the dynamic sequence did not adequately include the stricture site, or the breath-hold was inadequate for the assessment of stricture motility. Finally a further 14 were excluded after review of clinical records if they were taking medication known to affect bowel motility (notably corticosteroids, butylscopolamine and opioids). The final cohort consisted of 91 patients (median age 36, range 18 to 88, 37 male). The median disease duration was 11 years (range 0 to 42), 40 had undergone surgical resection; 72 where on one or more disease-specific medication. The specifics of these data are summarised in Table 4.1.

Forty-one of the 91 patients had 2 or more MRE examinations during the period of data collection. Where patients had undergone multiple MRE examinations over the time period the examination that best-displayed stricture on the motility sequences was used for the primary analysis. However, MRE from chronologically later scans in the same patient were analysed if the same stricture could be identified on the follow up MRE (eg had not been surgically removed or inadequately covered by the motility sequence). Overall, the follow-up MRE was analysed in a total of 21 patients (median age 34, 9 male). The mean between the initial and follow up scan was 14 months.

Parameter	Number of Patients
Disease Diagnosis	
Less than 1 year	12
Between 1 and 5 years	10
Between 5 and 10 years	18
Over 10 years	51
Medication at time of scan	
None	22
1 agent	31
2-4 agents	21
more than 4 agents	17
Surgical history	
No surgical history	52
1 operation	26
2 Operation	13
Disease Distribution	
Ileo-colonic (2)	55
Ileal(3)	33
Other small bowel(4)	1

Table 4.1 *Patient demographics for primary cohort of 91 subjects.*

MRI Protocol

During the 4 year time period, UCLH introduced a 3T MRI system alongside its existing 1.5T MRI scanner. Consequently, of the 91-subject cohort, 84 were imaged

on a 1.5T MRI (Siemens Avanto system (Siemens, Erlangen, Germany)), and 7 patients on a Philips Achieva 3T MRI scanner (Philips Healthcare, Netherlands). Patients were prepared using the same protocol across both scanners as per chapter 2.2.2. The dynamic sequences acquired at 3T used the following parameters flip angle 45, TR = 1.96ms, TE = 0.98ms, 200x167 matrix filling, zero-filling to 512x512, 1x1mm in plane resolution, 10mm slice thickness and 1 second temporal resolution.

Motility assessment

All images were registered as per chapter 2.2.4 with the standard deviation of the Jacobian determinant, averaged under a ROI again used as the motility metric for this study.

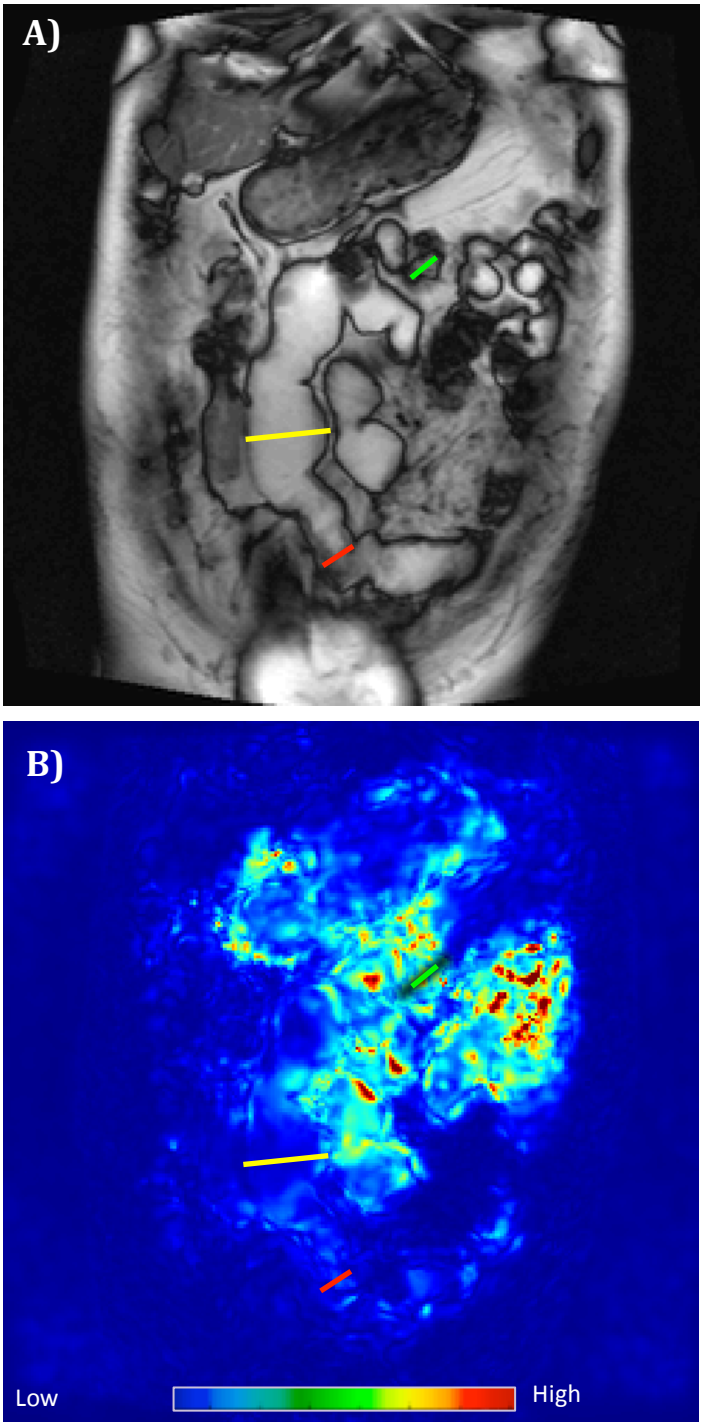

Figure 4.2 *A) ROI placement in the normal, unaffected pre-stricture bowel (green line), in the pre-stricture, dilated bowel (yellow line) and at the stricture (red line). All ROIs were placed serosa to serosa as opposed to lumen to lumen in contrast to the other chapters where line ROIs were placed within the bowel wall. B) The motility score for each ROI was calculated by sampling the corresponding parametric SD Jacobian map for the dynamic series.*

A single observer (radiologist with 5 years experience of MRE E. Herlbren) placed a total of three regions of interest (ROIs) in order to quantify motility and small bowel diameter using the motility analysis software. All ROIs were linear and drawn perpendicular to the central axis of the bowel from serosa to serosa on the coronal image. Specifically, serosa-serosa measurements were made as this made annotation of strictured bowel (where no lumen is present) possible and measurements methodologically consistent across the three ROIs. This differs from chapter 2 where line ROIs were placed lumen to lumen in morphologically normal bowel. The first region of interest was placed in a normal segment of bowel, proximal to the stricture and in the same anatomical region (eg. where the stricture was in the ileum) as the normal reference bowel was also drawn in the ileum. The second ROI was drawn across the immediate pre-stricture bowel at the point of maximum dilatation across the cine-loop. The third ROI was placed halfway along the total length of the strictured bowel (Figure 4.2 A). ROIs were automatically propagated through the 20 frame time series by the software algorithm.

From each ROI, two numerical metrics were derived:

1. Motility score in arbitrary units describing the motility of the underlying bowel beneath the region of interest (standard deviation of the determinant of the pixel's Jacobian and expressed as, a motility score (SD Jacobian) (Figure 4.2B).

2. The maximum diameter of the bowel based on the length of the ROI.

Patients with follow up scans

Where individuals had a follow up MRE fulfilling the eligibility criteria described above, the datasets were arranged into chronological order and ROIs duplicated as described above so that the same sections of bowel were examined across the two time points. Side by side comparison of all sequences from the initial and follow up MRI were utilised by the observer to facilitate this matching of ROI placement.

Statistical analysis

The Shapiro-Wilk test ($\alpha = 0.5$) was used to examine whether motility, and diameters adhered to a Gaussian distribution. Where non-normality was observed, data was log-transformed or non-parametric tests were used.

Quantitative differences in bowel diameter was assessed using repeat measures analysis of variance (ANOVA) with Tukey-Kramer post-hoc analysis as was the difference in bowel motility across the three locations with significance measured at $P<0.05$).

Motility scores across the demographics (including disease duration, medication use and surgical history) were assessed for normality and evaluated using Kruskal Wallis testing.

Relationships between the diameter and motility of the normal bowel, pre-stricture dilatation and stricture itself was examined using Pearson's correlation coefficient at a significance of $P<0.05$.

For patients with follow up studies, the percentage change in motility and percentage change in diameter of the dilated bowel were calculated.

4.3 Results

4.3.1 Size and motility differences across the stricture, dilatation and normal bowel

The mean diameter of normal bowel, pre-stricture dilation and stricture site was 20mm (range 12 to 38mm), 30mm (range 14 to 104mm) and 15mm (range 5 to 34mm) respectively. Analysis with repeat measures ANOVA revealed an F-statistic of 112.91 with $P<0.001$. Post hoc analysis suggested the mean diameter of the pre-stricture bowel was significantly different to that of the normal and strictured bowel in this cohort (Figure 4.3A).

Mean small bowel motility in the normal bowel was 0.43A.U (range 0.17 to 0.75), mean pre-stricture bowel motility was 0.28A.U (range 0.3 to 0.95A.U) and mean stricture motility was 0.15A.U (range 0.02 to 0.71). Analysis with repeat measures ANOVA revealed an F-statistic of 101.6 with $P<0.0001$. Post hoc analysis suggested mean motility in the three regions of bowel were statistically significantly different.

Motility scores across the cohort were stratified by disease duration, drug regimen and surgical history (grouped as per table 4.1) and analysed using Kruskal-Wallis testing after failing Shapiro-Wilk test ($\alpha = 0.5$) normality test. No significant differences were found when stratifying the cohort into groups based on disease duration, medication or surgical history for motility score at the three bowel regions (Table 4.3).

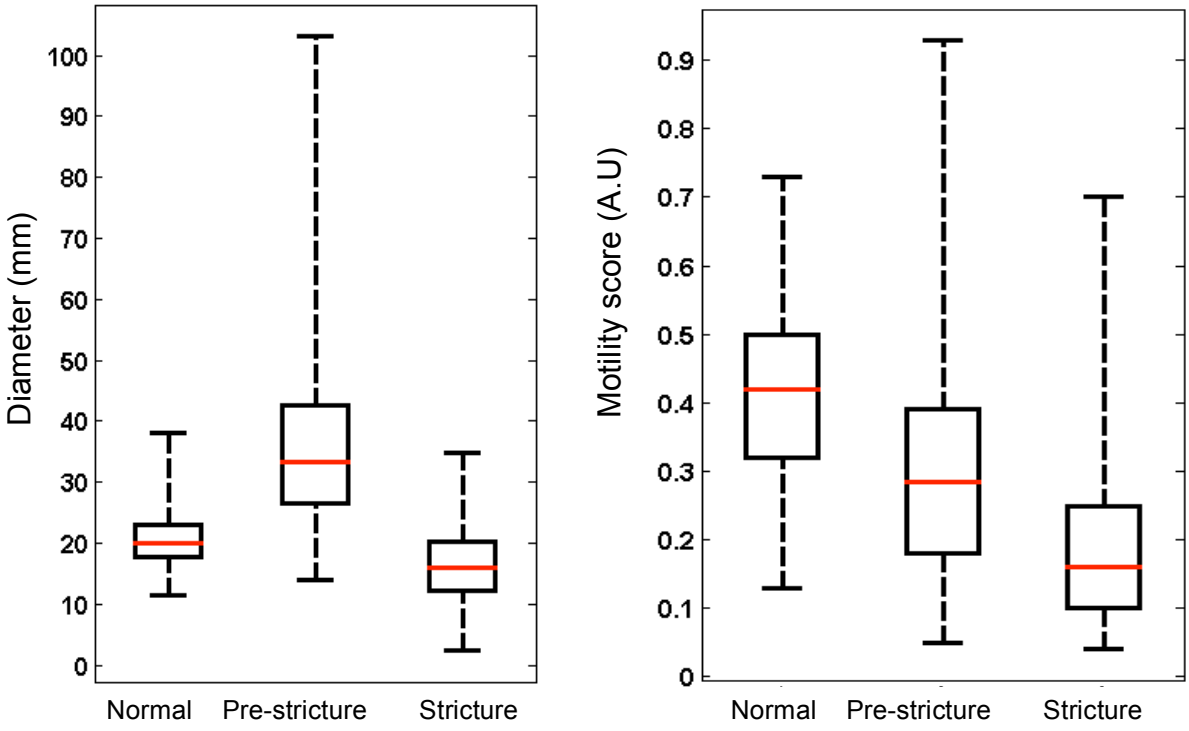

Figure 4.3. *A) The serosa-serosa diameter (mm) for the 91 subjects small bowel loops across the normal, pre-stricture and strictured bowel. B) Motility scores at the same locations. Black box represents inter-quartile range, red horizontal lines show data median and dotted lines show data min/max limits.*

	Kruskal-Wallis *P* value		
Motility (AU)	***Normal***	***Dilated***	***Stricture***
Disease			
Duration	0.71	0.6	0.46
Medication	0.18	0.51	0.95
Surgical History	0.32	0.96	0.62

Table 4.2. *Patient motility stratified by demographics. Values represent P vale of Kruskal Wallis testing*

4.3.2 Relationship between pre-stricture small bowel diameter and motility

There was a strong negative correlation between pre-stricture small bowel diameter size and motility with a Pearson's correlation coefficient at -0.47, P = <0.001. Raw data was non-normal ($\alpha > 0.05$) and a log-transformation was applied to normalise the distribution (i.e. make the data normally distributed) to make use of the more powerful parametric test. Thus in general the more dilated the bowel, the lower its motility (Figure 4.4). A significant relationship was also found between stricture diameter and motility (R = -0.42, P<0.001). No significant relationships were found within the normal bowel between motility and diameter.

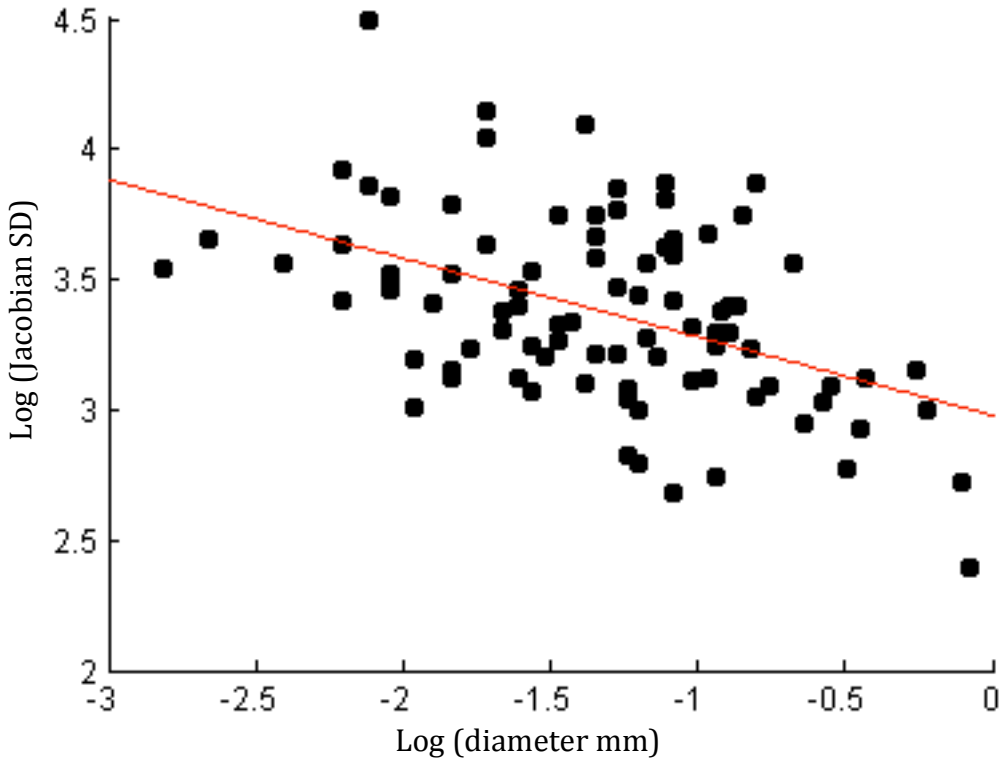

Figure 4.4 *A negative correlation was observed between normalised log-transformed pre-stricture bowel diameter and motility*

4.3.3 Changes in motility over time

21 subjects underwent two scans (mean scan time between scans 14 months (range 4 to 26 months)). The mean percentage change in pre-stricture bowel diameter across this cohort showed a decrease of 7.8% (range -56.6% to +62.5%) with a mean percentage motility change of -58% (range -625.0% to 88.7%). There was again a negative correlation between the percentage change in diameter and motility (Spearman's Rho coefficient of -0.6, P = 0.007). This suggests that as the pre-stricture bowel diameter decreases, motility increases and vice versa suggesting reversibility.

Analysis of the normal segment of bowel over the two time points as a comparison demonstrated a much smaller mean change and range in diameter change compared to the pre-stricture bowel (mean decrease in motility of 3.3% (range -14.7% to +13.6%) (Figure 4.5 – green vs. red dots). Similarly the mean and range of motility change was smaller than in the pre-stricture bowel (mean motility change 11% (range -47.2% to 36.8%) (Figure 4.5 – green dots). There was no significant correlation between percentage change in diameter and motility in normal bowel (Spearman's Rho of 0.08 P = 0.94).

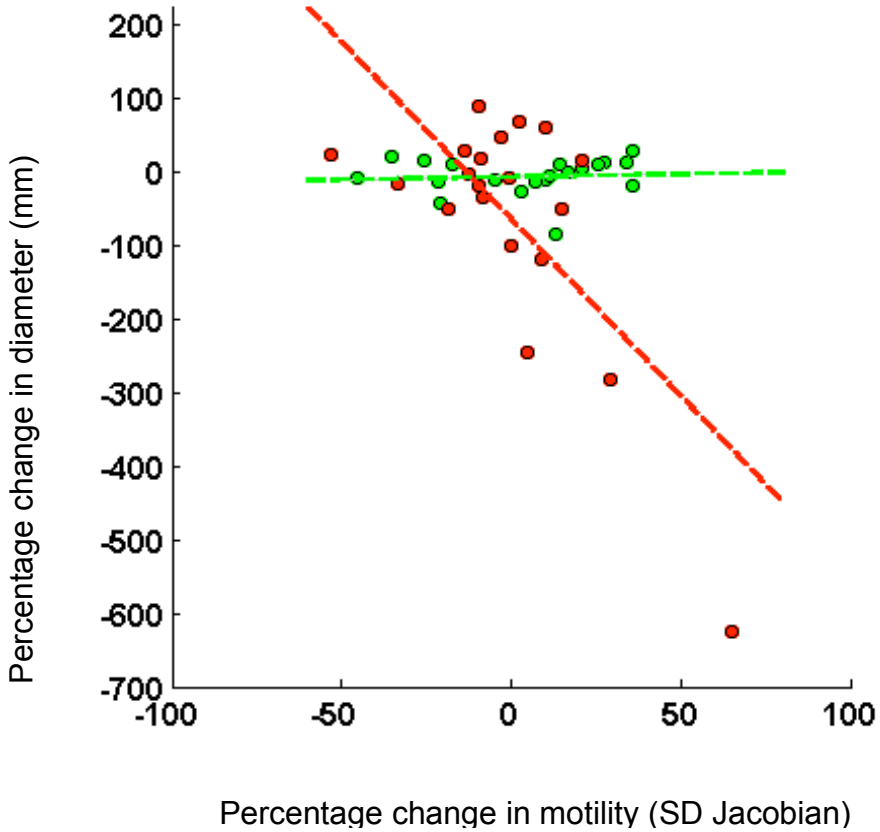

Figure 4.5 *Percentage change in motility plotted against percentage change in diameter for normal bowel (green) and pre-stricture bowel (red)*

4.4 Discussion

The observation that intestinal obstruction leads to changes in contractile activity is well known. The vast majority of research into the effects of obstruction on bowel function has been conducted in animal models with relatively little data in patient cohorts, particularly those with Crohn's disease related strictures. To our knowledge, this was the first study to systematically quantify regional small bowel motility using MRE in patients with stricturing Crohn's disease.

Data presented in this study suggested that motility within a stricture is significantly decreased in comparison to normal and the immediate pre-stricture bowel. Crohn's disease results in inflammatory and fibrotic infiltrate causing bowel wall thickening and it is intuitive that this should reduce local peristaltic activity and indeed evidence was presented in the previous chapter to support this (table 3.5). Perhaps of more interest are the observations made on the pre-stricture bowel. Such bowel is theoretically not directly involved with the Crohn's disease, yet any disturbance in motility will add to the patient symptom load, and potentially influence the size of surgical resection if performed[50], [51]. Here it was demonstrated that pre-stricture small bowel has reduced motility compared to normal bowel and that this reduction is inversely correlated to its diameter for example the more dilated the bowel, the lower its contractibility. This raises the concept of bowel "failure", where obstructed bowel undergoes compensatory dilation, which then produces ineffective and reduced motility if the obstruction is not relieved. A crucial question is whether this change is reversible? In the present

cohort evidence was found supporting the potential reversibility of this phenomenon. In the 21 patients with follow up examinations, the diameter of the pre-stricture bowel changed, and, importantly, as the bowel diameter reduced, motility increased.

These data may have a physiological explanation based on the current literature. In a canine model Prihoda et al. induced acute small bowel obstruction[118] and observed an acute surge in intestinal motility in the immediate proximal bowel following balloon obstruction, followed by a decrease and stasis in the longer term. This reduction of motility mirrors our observations. However, stricture-related obstruction in CD is an insidious process and a transient surge in motility is unlikely to be observed by chance in clinical practice. Thus, Prihoda's observations could not be reproduced here. Shi et al. reported induction of COX-2 in response to stretch receptor activation in rats and that this acts as a direct inhibitor of bowel motility[113]. This stretch-receptor-induced arbitration of motility could potentially serve as a protective measure where an obstruction is encountered, i.e. to decrease peristalsis in response to an obstruction rather than increasing pressure to the point that a rupture in the bowel wall might occur. It also could explain our finding that as pre-stricture diameter decreases, motility increases. This dynamism in the motility at the pre-obstructive bowel in relation to calibre may be of wider importance within the surgical setting. For example, dilated bowel resected at surgery due to presumed loss of function, may in fact be salvageable. Potentially valuable future studies might be conducted investigating this reversibility following therapeutic intervention using motility as a biomarker of response.

The study does have limitations. By definition, the cohort likely included a range of inflammatory and fibrotic strictures and without histopathological correlation it was not possible to further investigate how the stricture type influences our motility changes. It does however raise the intriguing possibility that disease response to medication can be monitored by quantitative MRI motility measurements rather than subjective patient symptomatology. A range of disease chronicity, as well as patients with and without a surgical history, were included in this study, complicating the interpretation of the results. However, age, medications, surgical history and disease duration had a negligible effect on the results. Clearly, prospective studies are now indicated to investigate the use of MRI to predict the natural history of Crohn's strictures and to evaluate follow up following medical interventions in comparison to conventional, endoscopic clinical and biological markers. The length of the stricture was not assessed in this study as the spatial and temporal gaps between the motility data sets made meaningful analysis of the data difficult especially where the stricture ran perpendicular to the imaging plane. In future studies, the length of the stricture and its global motility might be of further interest especially in conjunction alongside the concept of disease reversibility to allow more conservative and targeted resection of bowel. In this study, ROIs were placed serosa-serosa across the three regions of bowel mainly to standardise measurements at the stricture, where the lumen could often not be seen. In practice, it was difficult to measure the bowel serosa of the morphologically normal bowel with high accuracy due to the difficulty of distinguishing the boundaries of the bowel wall (often 1-2 pixels in thickness) due to the limited spatial resolution of the MR images. A final methodological point that is of key importance to this thesis is the identification of a 'normal' loop of small bowel; several studies have suggested

indirect effects of an inflammatory state on bowel motility and it would be naïve to assume anatomically normal bowel on MRI is entirely comparable to healthy controls. However, a clear mechanism with robust empirical evidence in humans has yet to appear in the literature [121].

Summary

The data presented in this chapter demonstrates clear differences between normal, strictured and pre-stricture bowel in Crohn's disease. Motility reduces with increasing small bowel dilatation but this process seems potentially reversible in some. As touched on in this chapter, one of the crucial issues that have made interpretation of the clinical findings challenging thus far is the absence of well-characterised healthy individuals to which disease states can be compared.

SECTION C: IDENTIFYING AND ADDRESSING LIMITATIONS IN SMALL BOWEL MOTILITY ASSESSMENT WITH MRE.

Section B demonstrated the way in which registration derived measures of small bowel motility could be used to investigate dysmotility in Crohn's disease, a condition often characterised by a morphological change in the bowel wall anatomy driven by an inflammatory process. Importantly, this structural change is identifiable on imaging and the lesion was used in Chapters 3 and 4 to inform ROI based analysis. From a broader clinical perspective, many of the conditions that would potentially benefit from a diagnostic technique to evaluate motility are not defined by a discrete segmental abnormality, but rather affect global bowel motility as a whole. Indeed, a range of neuropathic and myopathic diseases (e.g. Parkinson's), as well as idiopathic conditions such as irritable bowel syndrome (IBS), have apparently structurally normal bowel but are also likely to exhibit aberrant motility underlying at least some of their clinical manifestations. Following ethical approval for prospective investigation into healthy individuals, **Section C** aims to build on the previous chapters and investigate the concept of 'normal motility' in healthy, well-controlled subjects**. Chapter 5** specifically addresses limitations in segmental forms of motility analysis with the central hypothesis being that variability, even within a healthy subject, is too high to allow inferences from a single bowel segment to be made in the absence of a discreet lesion**. Chapter 6**

introduces and details the validation of a novel, global method for the analysis of small bowel motility. By assessing global over segmental motility, it is hypothesised that inter-scan variability within individuals is reduced and that this approach can be used to more robustly detect changes in motility following the administration of motility altering drugs against placebo.

CHAPTER 5: VARIABILITY OF SEGMENTAL SMALL BOWEL MOTILITY QUANTITATION USING MAGNETIC RESONANCE ENTEROGRAPHY

Research Question:

What are the potential methodological issues arising from segmental (local bowel loop) ROI placement in small bowel motility analysis?

Rationale:

Analysis of 'cine' MRI using segmental ROIs has become increasingly popular for investigating bowel motility with a growing number of techniques now using metrics such as 'contractions per minute' to describe small bowel motility in both health and disease. However, small bowel motility variation in normal subjects both within and between scans remains poorly described creating an important methodological consideration for quantitative investigation using gerneralsiations from segmental analysis as a study outcome.

Hypothesis:

Small bowel motility is sufficiently heterogeneous in healthy subjects both within-scan and between-scan that segmental ROI, driven observations are not suitable in the absence of an overt lesion or anatomical landmark.

Aim(s):

i) Examine intra-scan variability of small bowel motility in healthy subjects

ii) Examine inter-scan variability of small bowel motility in healthy subjects

iii) Compare two validated segmental motility metrics (Jacobian SD and CPM).

Author declaration

The work presented in this chapter was lead by the author including obtaining ethical permission, performing the literature review, data collection and analysis and manuscript preparation. Dr. A. Plumb placed and adjusted ROIs. The project was conducted under the supervision of Prof. S. A Taylor. New motility imaging sequences were developed by Dr David Atkinson to optimise spatio-temporal resolution at 3T. Candidate sequences were reviewed and selected by the author and Prof. S. A. Taylor based on image quality and added information (e.g. volumetric coverage). This research has been published in: Menys, A., Plumb, A., Atkinson, D., & Taylor, S. A. (2014). The challenge of segmental small bowel motility quantitation using MR enterography. The British Journal of Radiology, 87(1040), 20140330. doi:10.1259/bjr.20140330

5.1 Introduction

Section B presented data exploring motility changes in the small bowel at specific locations along the bowel, the TI in Chapter 3 and surrounding a stricture in chapter 4. This segmental form of analysis has become popular with other research groups with various examples of quantitative assessment of dynamic sets in a range of conditions, most notably inflammatory bowel disease and enteric dysmotility syndromes[15], [16], [76], [78], [121], [122].

Despite a growing literature, consensus has yet to be reached or even suggested as to the best method of quantitatively analysing small bowel data and indeed a range of motility metrics are proposed summarised in table 1.2 [15], [16], [56]–[59], [123]. The most commonly used metric is the change in luminal diameter at a fixed position through the time series. By plotting diameter against time, a characteristic curve can be produced with the number of contractions expressed per minute (CPM) giving an intuitive and broadly accepted metric for small bowel motility[15], [16], [56], [59], [76], [78], [121], [124]. Indeed, several studies (including those detailed in section B) have reported a relationship between CPM and dysmotility in disease, either compared to a histopathological standard or 'normal' reference bowel loops [15], [16], [58], [76]. An array of additional metrics derived both from bowel diameter measures and more abstract processing techniques have further been implemented with varying degrees of effectiveness in disease and health [15], [55], [57], [64], [76], [121], [123].

Although intuitively attractive, the robustness of assessing overall enteric motility using only an isolated loop of bowel has received relatively little attention to date irrespective of the precise metric applied. It is unclear how representative the selected bowel loops are of overall small bowel motility and if normal motility intrinsically differs between bowel segments, for example between the jejunum and ileum. Furthermore, the repeatability of single loop metrics, even in normal individuals, is not well described, knowledge of which is vital if segmental analysis are to be used to diagnose, guide treatment and monitor enteric pathology and make inferences on disease.

The purpose of this study is to explore segmental variation in small bowel motility in healthy volunteers measured using two commonly reported small bowel metrics (Contractions per minute and Jacobian Standard Deviation (SD)) looking at 1) within scan motility variation between different segments, 2) between scan variation (repeatability) across two time points.

5.2 Methods

5.2.1 Patients

20 healthy volunteers (mean age 28, range 22 to 48, 14 Male) were recruited over an 18-month period. Each subject underwent a specific small bowel motility protocol. Inclusion criteria included ability to give informed consent, non-smokers and abstinence from caffeinated and alcoholic drinks on the day of the scan. Exclusion criteria were any known chronic intestinal disease, any long–term medication excluding the oral contraceptive, self reported GI symptoms or history of GI surgery. Volunteers were recruited prospectively by advertisement and interview.

5.2.2 Protocol

Volunteers fasted for 4h prior to ingesting 1L of 2% mannitol solution over the 50 minutes prior to the MRI scan. Locust bean gum was not used in this study nor in clinical practice at 3T following a change in UCLH MRE protocols with no appreciable difference seen in scan quality, an improvement in patient acceptance and an operational cost saving. Subjects ingested the mannitol at regular intervals such that the last of the solution was consumed immediately before entering the scanner room. Subjects lay in the prone position and were scanned using a Philips Achieva 3T Multi-transmit MRI scanner (Philips Healthcare, Netherlands) using the manufacturer's torso coil (XL-TORSO). Each subject underwent planning sequences followed by a multi-slice balanced turbo field echo (BTFE) motility sequence

(coronal, 2.5x2.5x5mm voxel size, FOV 420x420x30mm, FA 20 degrees, TE=1.85ms, TR=3.7ms dual channel RF transmit with adaptive RF shimming), no slice gap with 6 slices in a volume. Each 3cm block volume was acquired during a 20 second breath hold with temporal resolution of 1 volume per second (20 time points acquired per breath-hold). Blocks were acquired sequentially through the abdomen during repeat breath-holds to cover the whole small bowel volume. The author selected the volume that best displayed the terminal ileum and data acquisition was extended to a 1-minute period of free breathing. The total scan duration was 20 mins with other anatomical and motility scans performed detailed in chapter 6.2.

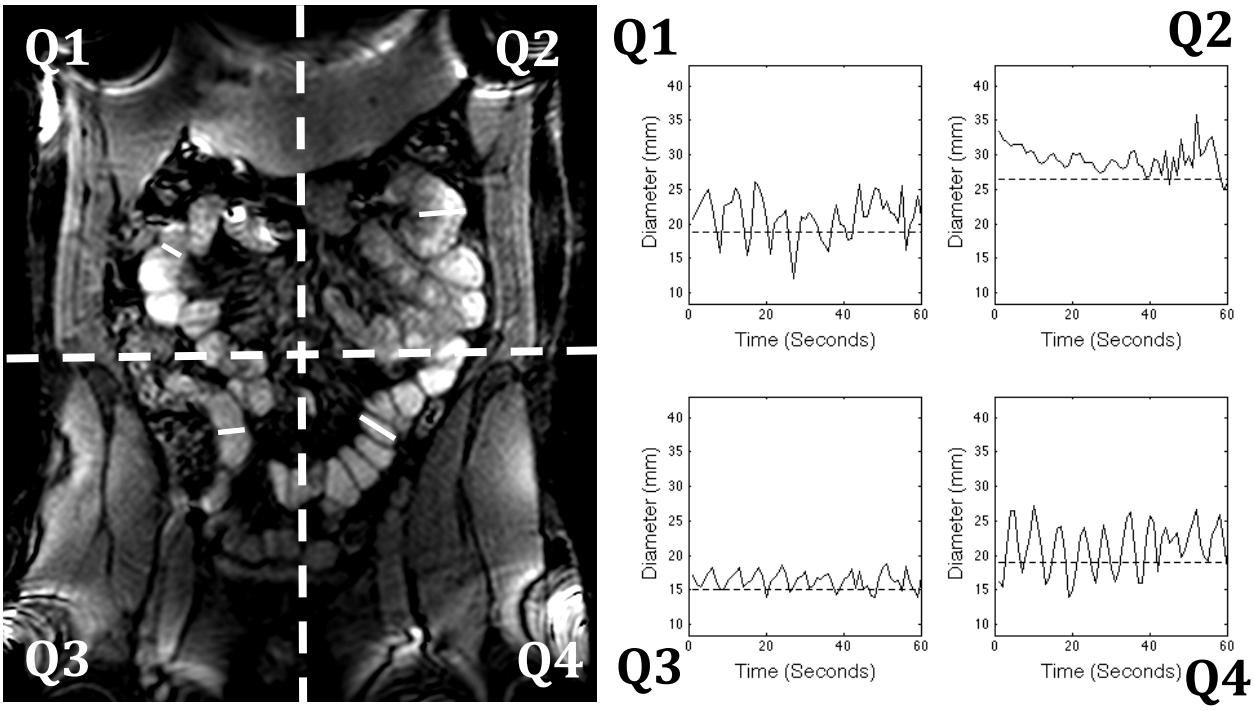

Figure 5.1 *Positions of the four ROIs in quadrants 1-4 with the TI in Q3 along with time series plots of line lengths for the respective quadrants*

Each volunteer underwent a second MR examination after a mean gap of 4 weeks (range 2 to 7) using an identical preparation and MRI protocol. The time of day at

which time the second scan was undertaken was within 1 hour of the first in all cases.

5.2.3 ROI placement

The coronal cine blocks were divided into 4 quadrants (upper right (Q1), upper left (Q2), lower right (Q3), lower left (Q4) by placing 2 intersecting lines located at the mid point in the x and y direction respectively-Figure 5.1).

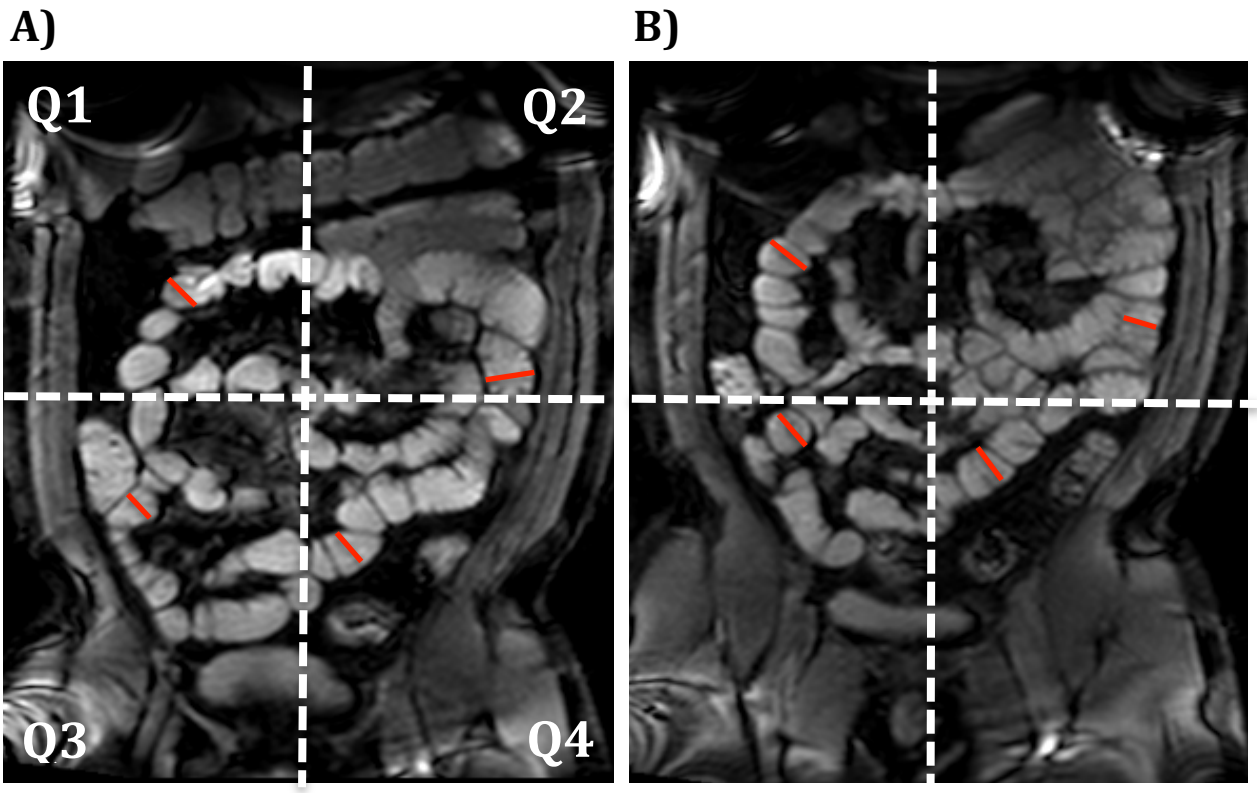

Figure 5.2 *Repeat scans across two time points (A & B) with ROIs in each quadrant indicated by red lines. Anatomical markers including the ileo-caecal valve, duodenojejunal flexure were used to aid ROI duplication.*

A radiologist with 5 years' experience of MR enterography (Dr. A. Plumb) reviewed all the cine blocks encompassing the small bowel volume and chose the single best block which included the terminal ileum in Q3, but also ensured a small bowel loop in the remaining 3 quadrants could be followed in its entirety throughout the 20 second time series (ie the loop was well distended and remained in plane on at least one of 6 slices within in the block). For each subject, the observer then placed a linear ROI across the bowel lumen, from serosa to serosa in a well-distended loop in each of the 4 quadrants on the most appropriate slice in the block (Figure 5.1). The Q3 ROI was specifically placed within the last 5cm of the terminal ileum (Figure 5.1). The ROI was automatically propagated using the registration deformation fields across the 20-second time series. The observer then manually adjusted the length and exact position of each copied ROI to ensure it closely followed the lumen of the bowel loop over the 20 seconds of data acquisition. The process was repeated in the free breathing data with ROIs in each quadrant as described previously.

The whole process was repeated for the second scan for each volunteer. The position of each of the 4 ROIs was replicated as far as possible by the observer (Dr. A Plumb) using knowledge of the location in the z-axis of the first block together with visible anatomical landmarks (eg ileo-caecal valve, DJ flexure etc) (Figure 5.2)

5.2.4 Motility analysis

Contraction per minute

The line length of each ROI was plotted against time to create a motility curve (Figure 5.1). The mean ROI length was calculated and < 10% mean length horizontal lines were indicated on the motility curve and small bowel contractions manually counted by the author. A small bowel contraction was recorded where there was a decrease in luminal diameter greater than 10% of the mean small bowel diameter for the given time series[77]. The contraction rate was expressed in contractions per minute (CPM) (scaled appropriately for the 20 second BH data). An example time series plot for each quadrant is provided in Figure 5.1. Contractions were rounded to the nearest integer.

Registration derived motility score

Again the SD Jacobian score was used as per chapter 2.2.4

Statistical analysis

All data was assessed for normality using Shapiro-Wilk testing (alpha P<0.5). Correlation between the two motility metrics was assessed using Pearson's correlation in breath-hold and free-breathing data. The level of agreement between the two motility metrics in each of the four quadrants within a single scan was assessed using intra-class correlation.

For assessment of intra-subject repeatability across the two scan times, the CPM and Jacobian SD for all 4 ROIs were assessed with Bland-Altman limits of agreement adjusted for multiple observations per individual[125]. This was performed for breath-hold data and then repeated for the free breathing data.

All data collection and statistical analysis was performed using MATLAB.

5.3 Results

All subjects achieved adequate distension of the small bowel to allow ROI placement in each of the 4 quadrants for both scans.

5.3.1 Correlation between motility metrics

CPM and the Jacobian SD motility metric showed moderate positive correlation in both breath-hold (Pearson R = 0.42, p <0.001, Figure 5.3A) and during free-breathing (Pearson R = 0.58, p <0.001, Figure 5.3B).

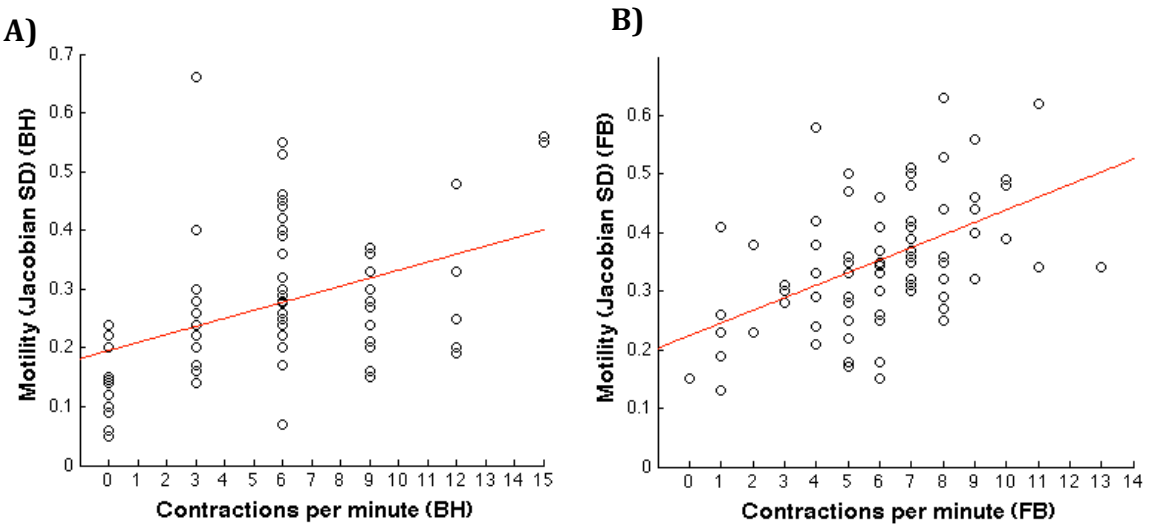

Figure 5.3 *Correlation between contraction rate and SD Jacobian metric in breath hold A) and free breathing B).*

5.3.2 Within-scan intra-subject variation between different bowel segments

BREATH HOLD

	Contractions Per Minute (CPM)		
	Mean	***Range***	***SD***
Q1	6	0 to 15	1
Q2	5	0 to 12	1
Q3 (TI)	3	0 to 9	2
Q4	6	0 to 12	1

FREE BREATHING

	Contractions Per Minute (CPM)		
	Mean	***Range***	***SD***
Q1	6	1 to 13	3
Q2	6	1 to 11	2
Q3 (TI)	6	0 to 10	3
Q4	6	1 to 10	3

BREATH HOLD

	Motility AU (Jacobian SD)		
	Mean	***Range***	***SD***
Q1	0.29	0.09 to 0.66	0.1
Q2	0.32	0.20 to 0.53	0.15
Q3 (TI)	0.21	0.05 to 0.51	0.12
Q4	0.27	0.06 to 0.47	0.1

FREE BREATHING

	Motility AU (Jacobian SD)		
	Mean	***Range***	***SD***
Q1	0.38	0.13 to 0.53	0.1
Q2	0.37	0.24 to 0.62	0.1
Q3 (TI)	0.29	0.15 to 0.49	0.1
Q4	0.37	0.18 to 0.63	0.13

Table 5.1 *Summary data for intra-scan variation across breath hold and free breathing protocols (left and right) using the two metrics (top = contractions, bottom = Jacobian SD)*

Breath hold results:

The CPM and Jacobian SD mean, range and standard deviation for each of the 4 quadrants is summarised in table 1. Assessment with intra-class correlation in

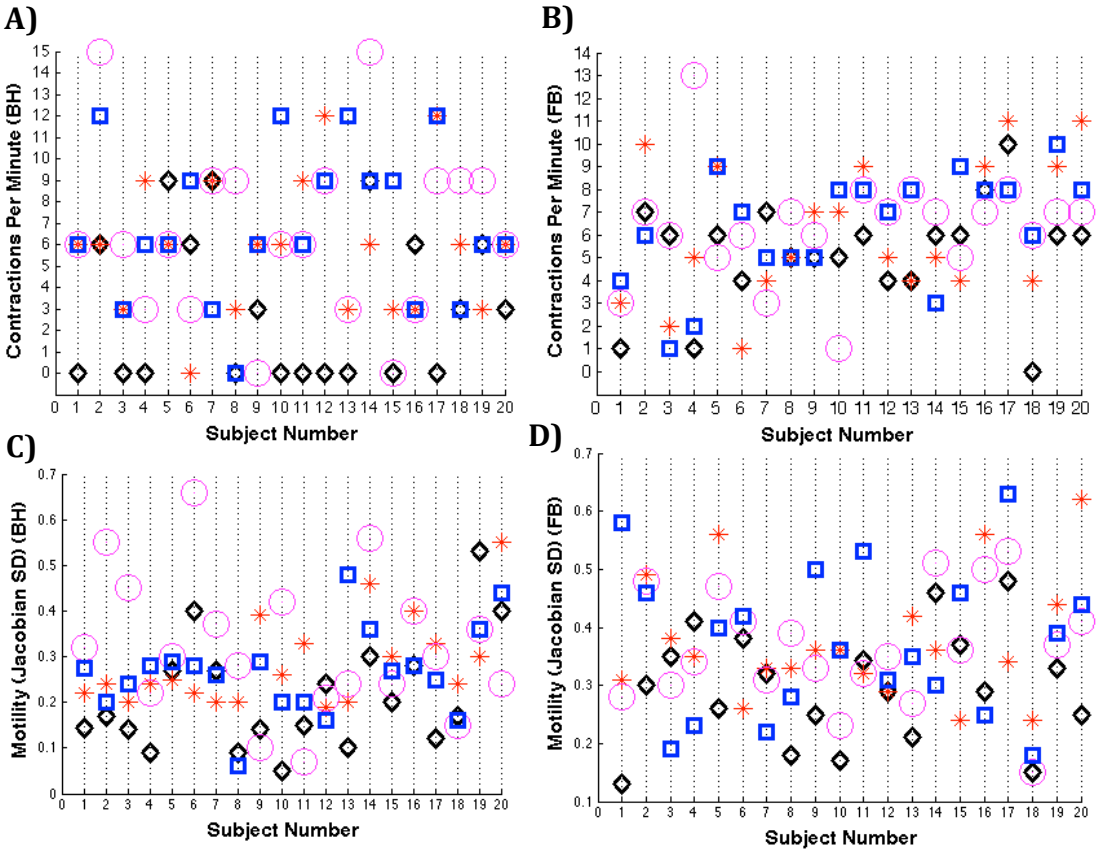

Figure 5.4 *Raw data plots for each subject where 'purple circles' represent Q1, 'red stars' Q2, 'black diamonds' Q3 (TI) and 'blue squares' Q4. Contraction rates in breath-hold and free-breathing are shown in A & B with respective plots for Jacobian SD in C & D.*

breath hold data demonstrated an R-coefficient of 0.06, p = 0.1. Individual data points for each subject are presented in Figure 5.4A & C. Similar assessment of the

SD Jacobian motility metric demonstrated a significant but weak ICC R-coefficient = 0.2, p = 0.027 (Figure 5.4C). These data broadly suggests high variation in both contraction rate and motility score between quadrants in the same individual.

For example the CPM variation within volunteer 13 was 0 CPM in Q2 and 12CPM in C4 while the total range across the cohort in CPM was 0 to 15CPM. Similarly for the Jacobian SD metric, subject 2 had motility values in Q2 of 0.15AU and 0.55AU in Q1 while the range across the cohort was 0.05 to 0.66.

Free breathing results

The mean, range and standard deviation of each quadrants contraction rate over the 60s free breathing period is summarised in table 1. Individual data points for each subject are presented in Figure 5.4 (B&D).

In general the magnitude of CPM was similar to that acquired during a 20 second breath-hold (table 1), although the variation within individuals was reduced (Figure 5.4 B&D). Notably with the intra-class correlation demonstrated a marginally increased and significant R-coefficient of -0.26, $p = 0.05$. The result for the Jacobian motility metric remained similar with a weakly significant R co-efficient of 0.19, p = 0.03. In the breath hold data, there was high variation between quadrants in individual volunteers. For example subject one had Q3 and Q4 motility of 0.13 and 0.58AU while the max and min motility across the cohort ranged between 0.13 to 0.62AU.

5.3.3 Within-subject repeatability between different time points

Breath hold results

Across all ROIs in all subjects, the mean CPM at time point 1 was 6 contractions (range 0 to 15) and mean CPM at time point 2 was 6 contractions (range 0 to 13). Bland-Altman limits of agreement for repeat measures demonstrated a mean difference of 0 contractions with a LoA of ± 8 contractions (Figure 5.5a) suggesting relatively poor repeatability of CPM across time for matched segments.

Using the Jacobian SD metric, mean motility score at time point 1 was 0.28AU (range 0.05 to 0.66) and mean motility at time point 2 was 0.26AU contractions (range 0.07 to 0.63). Bland-Altman limits of agreement for repeat measures demonstrated a mean difference of 0.01 contractions with a LoA of ± 0.32 contractions (Figure 5.5c) suggesting again a large source of intra-subject variability.

Free breathing results

Mean contraction number at time point 1 was 6 contractions (range 0 to 13) and mean contraction at time point 2 was 6 contractions (range 0 to 11). Bland-Altman limits of agreement for repeat measures demonstrated a mean difference of 1 contraction with a LoA of ±6 contractions. (Figure 5.5b), suggesting relatively poor repeatability of CPM across time.

Using the Jacobian (SD) metric, mean motility score at time point 1 was 0.35AU (range 0.05 to 0.63AU) and mean CPM at time point 2 was 0.34AU contractions (range 0.13 to 0.69AU). Bland-Altman limits of agreement for repeat measures demonstrated a mean difference of 0.01 AU with a LoA of ± 0.32 contractions (Figure 5.5d) suggesting a large source of intra-subject variability.

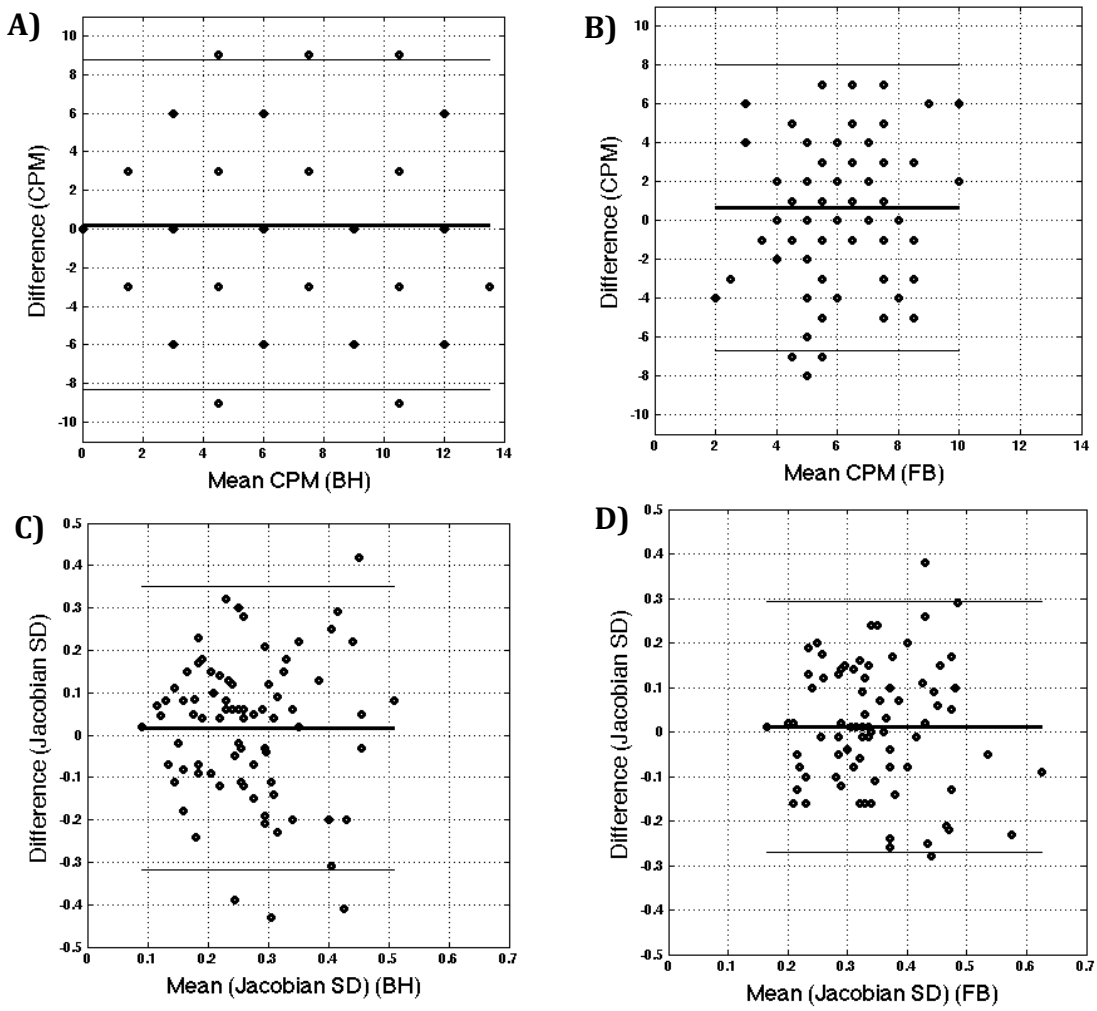

Figure 5.5 *Bland-Altman limits of agreement (LoA) adjusted for repeat observations per individual, for contraction rate in breath-hold and free breathing (A & B). Respective motility scores with Jacobian SD metric (C & D).*

5.4 Discussion

The purpose of this chapter was to examine the variation of segmental small bowel motility in a cohort of 20, well-controlled, healthy volunteers. These data show that large variation in segmental motility within the same individual was demonstrated both at different small bowel locations and at the same location at different times. This raises important methodological limitations when performing segmental analysis of bowel motility, regardless of the metric used.

Through employing both breath-hold and free-breathing protocols this investigation replicates the majority of study protocols described in the literature. Intuitively it might be presumed that free-breathing data is better suited for CPM analysis as the longer period of data collection makes it more robust to potential transient surges in contractile activity[6], [34]. Indeed there was evidence for this with longer acquisition times where there was decreased intra-subject variance and an increased ICC. Nevertheless, even with 60 seconds of free breathing there remained large variation in the segmental motility across small bowel quadrants and between different scan times.

With FB appearing superior for the CPM metric, BH studies might appear redundant. However there are several potential advantages. First, a 20s breath hold is rapid and, if the aim is to sample the whole small bowel volume (requiring multiple acquisitions), a smaller scan time is preferable, particularly if implemented in clinical practice. Again from a practical perspective, the breath-hold protocol

reduces the data volume generated and allows more feasible analysis with manual quantitation or post-processing. Furthermore, the anterior- posterior displacement caused by the respiratory cycle can "remove" sampled small bowel loops for periods within the time series. Breath-holds reduce/eliminate displacement caused by respiration which ensures any bowel loop can be sampled and not just those well seen throughout the time period, which may reduce sampling bias. Many MRI motility metrics have been developed to work over these shorter time periods for example contraction amplitude where only a single, well formed contraction is sampled independent of time assuming the scan is long enough to fully resolve one contraction [55], [76], [121]. This study saw a negligible reduction in variance for the Jacobian SD metric between BH and FB protocols. For longer time periods, the Jacobian metric could be standardised, as the Contraction rate has been, to a period of one minute for example and is likely to represent the direction of future work.

This study further aimed to examine the range of within-subject motility during a single scan by placing a series of ROIs, one in each quadrant. The data showed that there was in fact large variation within the different segments of bowel. Further examination of the data graphically and visually confirmed the apparent heterogeneity of contractions through the bowel, raising an important point for further consideration. In several recent studies, subjective selection of bowel loop based on good distension has been performed and assumed to be representative of global motility, for example higher motility in this loop being attributed to a non-diseased state[76], [78], [121]. Across both BH and FB the results suggested that many of the subjects possessed both relatively 'hypo-motile' and 'hyper-motile' segments based on both their CPM and Jacobian SD scores. This within-subject

heterogeneity presents a serious challenge to the persevering notions of hyper/hypo motile bowel derived from an analysis of limited small bowel loops. The fact that both metrics provided comparable ICC scores and spread of data values implies this reflects genuine physiological variability and is not particular to any specific metric. These results might further explain why relatively low motility was seen in CD patients in chapter 3 with no evidence of inflammatory activity. Although principally descriptive, these results may serve a role in determining study power in future investigations examining segmental dysmotility in health and disease.

The final result presented in this study describes poor intra-subject repeatability of the CPM and Jacobian SD metrics at time points on average 4 weeks apart. This held true for both breath-hold and free-breathing datasets. Indeed intra-subject variation over time appeared similar in magnitude to between subject-variation for several participants in the cohort. The study cohort was standardised for a number of factors that might affect small bowel motility with particular attention paid to preparation with oral contrast agent, caffeine and nicotine intake, time of day and ingestion of medication. One of the principal influencing factors for the variation might be variation in ROI placement between scans. Although care was taken to duplicate precisely the ROI position across the quadrants it was in fact difficult in the absence of *in-situ* markers, to be certain of the exact location of the previous ROI. In this respect it was ensured that one ROI for each scan was placed within 5cm of the terminal ileum where a high degree of certainty could be achieved with respect to the re-positioning of the ROI later on. Even then, at the TI high variation was again observed between scans both during breath-hold and free-breathing across the two metrics.

With respect to limitations, 60 seconds of free breathing might still be argued to be too short a time to evaluate motility with respect to contractions per minute and extended free-breathing studies should be conducted to evaluate the impact on repeatability and reproducibility of analysis. Another limitation lies in the assessment of only two metrics in this study with still no clear consensus for the usage of certain metrics, including mean-diameter and contraction amplitude, established. Additionally, the cohort described in this study is relatively small and homogeneous in terms of age. A larger sample size would assist in the better characterisation of motility in healthy subjects using MRI. In this study, we used a 3T MRI machine that provided a significant advantage over 1.5T scanners in terms of spatial and temporal resolution. The registration algorithm is nevertheless designed for 2D time series and therefore can still be performed at lower field strength so long as images of sufficient spatial resolution are obtained. The added value of volumetric sequences was convenient for this study as it allowed better bowel loop matching but remain of indeterminate value in wider practice.

Whether or not segmental metrics are useful for the investigation of small bowel motility is unclear. Undoubtedly, where ROI placement can be guided by the existence of pathology (such as a stricture), segmental methods of analysis are valid and, as scanner hardware and software improves, this form of analysis will likely become increasingly valuable for the investigation of diseases affecting specific bowel segments. However, due to the apparent heterogeneity within normal bowel, great caution must be placed on inferences based on analysing subjectively placed segmental ROIs.

Summary

MRI quantified normal segmental small bowel motility demonstrates wide variation across different locations within the small bowel, and in the same location at different time points. This presents an important limitation in the application of segmental analysis in the investigation of global dysmotility.

CHAPTER 6: A TECHNIQUE FOR GLOBAL SMALL BOWEL MOTILITY ASSESSMENT USING DYNAMIC MRI

Research Question:

Can global small bowel assessment be used to provide robust, repeatable and sensitive measures of small bowel motility?

Rationale:

The data in chapter 5 demonstrated the high variability in ROI based analysis of bowel loops regardless of the metric used largely due to the complexity of the gut and absence of anatomical landmarks to inform ROI placement. Diseases that feature dysmotility (Parkinson's, diabetes, scleroderma, amyloidosis, IBS etc) often feature an anatomically normal bowel making segmental forms of analysis difficult to use in an unbiased fashion. The deformation fields generated through image registration have thus far been explored segmentally however this method can be expanded by placing larger ROIs to segment the entire small bowel, averaging the motility score to create a global metric describing motility.

Hypotheses:

Global small bowel motility analysis can be used to provide quantitative measures of motility that are 1) repeatable and 2) sensitive to pharmacological stimuli.

Aim(s):

i) Demonstrate global measures of SBM are reproducible between observers.

ii) Global measures demonstrate good intra-subject repeatability across two time points.

iii) Demonstrate sensitivity of global measures to the drug provocation against placebo.

Author declaration

The work presented in this chapter was led the author who designed and managed the study, acquired the ethics, performed the data collection, statistical analysis and produced the manuscript. The radiologists Dr A. Plumb and Dr A. Ahmed served as the study radiologists. The gastroenterologist Dr. A Alam administered neostigmine/placebo to subjects. Dr. D Atkinson and A. Menys developed the MRI sequence used in this study for volumetric motility acquisition. The research was supervised by Drs A Emmanuel and D Atkinson and Prof. S Taylor and published in: Menys, A., Taylor, S. A., Emmanuel, A., Ahmed, A., Plumb, A. A., Odille, F., … Atkinson, D. (2013). Global Small Bowel Motility: Assessment with Dynamic MR Imaging. Radiology DOI: 13130151

6.1 Introduction

Section B explored the practical application of image registration to quantify motion at fixed points along the bowel. This 'segmental' form of analysis is useful for interrogating a specific pathology at a definable position and valuable for deriving real, physiological information in terms of contraction rates or amplitude to evaluate local motility. However, data from the previous chapter, demonstrated large variability in motility of anatomically normal small bowel scans making this selection of 'healthy' bowel loops a key a major source of potential observer & conformation bias. Importantly from a clinical perspective, many of the conditions

that would potentially benefit from a diagnostic technique to evaluate motility are not defined by focal enteric abnormality such as strictures. Neuropathic and myopathic diseases (e.g. Parkinson's, diabetes, scleroderma, amyloidosis) as well as idiopathic conditions (IBS) have anatomically normal bowels making segmental forms of analysis likely limited in such disorders [24], [29]–[32].

This chapter specifically focuses on the development of a quantitative technique to evaluate global small bowel motility with a view to the clinical investigation of pan-gut dysmotility disorders. Chapter 2 introduced the analysis of deformation fields can be used to produce parametric motility maps that were used in chapters 3 & 4 to investigate individual bowel loops of interest. Here, this concept is expanded to delineate the whole small bowel and taking the average of the SD Jacobian score under the ROI(s). This study will also test the hypothesis that inter reader agreement will deteriorate when tasked with delineating the whole bowel volume when compared to interrogating a discrete loop only [59].

The use of deformation fields as a surrogate for motility represents a novel method for describing bowel motion and as such, a direct comparison to a gold-standard is very challenging. Manometry looks only at small regions of the bowel and scinitgiraphy examines transit time only as a surrogate for motility. A study protocol that could investigate the key attributes of the proposed method including sensitivity to pharmacological stimuli reproducibility and inter-observer reproducibility of metrics was implemented. This study took the form of a placebo-controlled crossover investigation in healthy individuals. The use of a crossover study allowed the examination of the magnitude of within-subject variation in

motility, one of the central tenets of a robust technique. Where all controllable environmental factors are maintained, a healthy individuals physiology be it heart rate, blood pressure or, in this case, bowel motility should stay the same. The study format also allowed administration of placebo-controlled pro-kinetic (neostigmine) and spasmolytic (butyscopolamine) agents to evaluate the techniques ability to quantitatively evaluate the effect of known motility influencing agents[126].

6.2 Materials and Methods

6.2.1 Volunteer selection

This study used the same healthy control cohort described in chapter 5.2.1. Patients in the neostigmine arm of the study were checked for contraindications to the drug including asthma and cardiac history.

6.2.2 Summary of Study design

The design was a randomised subject and reader blinded, placebo-controlled, cross-over study that investigated, 1) intra-subject repeatability of motility measures in visits separated by a mean of four weeks (described in chapter 5.2.1) and, 2) sensitivity of the technique to pharmacologically induced changes in small bowel motility. Two parallel arms were conducted. In the first arm, volunteers were randomised between IV placebo and IV butylscopolamine (inhibitor of bowel motility). In the second arm, volunteers were randomised between IV placebo and IV neostigmine (pro-kinetic agent). Drug/placebo order was given according to a randomisation block generated by the author.

6.2.3 Sample size and power calculation

A sample size calculation was performed based on examining the agreement between repeated measurements of baseline small bowel motility. Preliminary

motility measurements on four patients undergoing MRE, as part of routine clinical care at our institution, were used to develop an understanding of the expected variation (Appendix 2.1). It was postulated that baseline resting bowel motility should not vary by more than 20% between MRI visits and that the difference between measurements would have a standard deviation of 10%. A sample size of 20 volunteers was required to estimate subject variance to within 7.5% of the true population value.

In 3 patients, again with normal MRE findings, repeat motility scans before and after butylscopolamine revealed a mean decrease of 50% in small bowel motility (Appendix 2.2). From this, it was determined a sample size of 11 volunteers gave 90% power at alpha 0.05 to detect a 10% change in motility following drug administration, assuming a within-subject standard deviation of 10%, would be sufficient for this study.

6.2.4 MR Protocol

All subjects were prepared as per chapter 5.2. For the purposes of drug administration, an intravenous cannula (22G, Introcan Safety, Braun) was inserted into an antecubital vein by the attending radiographer approximately 15 minutes before the scan start time.

The motility scan protocol was designed to "cine capture" small bowel motility during a 20 second breath hold (temporal resolution 1 volume per second) and to

encompass the majority of the small bowel volume within this single breath hold. Specifically, a 3D Balanced Turbo Field Echo (BTFE) sequence was used to acquire, every second, a 15cm (15 x 1cm slices) coronal volume through the abdomen and pelvis, repeated for 20 seconds (2.5x2.5x10mm voxel size, FOV 420x420mm, FA 20, TE=1.7ms, TR=3.5ms, SENSE (3LR, 1.5AP), no slice gap, dual channel RF transmit with adaptive RF shimming). The cranio-caudal location of the 15cm volume acquisition was selected by the author using the sagittal, coronal and transverse axial planner scans to best cover small bowel volume. The most anterior portion of the block was positioned along the abdominal musculature on the sagittal view. Note, this sequence is different to that used in 5.2.2.

6.2.5 Drug administration

Following acquisition of the baseline motility scan, volunteers in the Butylscopolamine arm (n=10) randomly received an IV 10ml bolus of saline or 10ml bolus of Butylscopolamine (containing a 20mg dose, Boehringer, Ingelheim, administered by attending radiographer) through their venous catheter. An identical procedure was followed for those in the Neostigmine arm (n=11), with volunteer randomised between a 10ml saline bolus or 10ml bolus of Neostigmine (containing 0.5mg Neostigmine Methylsulphate, Mercury Pharma, UK). Within 2 minutes of administration of the placebo or active drug, the cine motility sequence was repeated as described above.

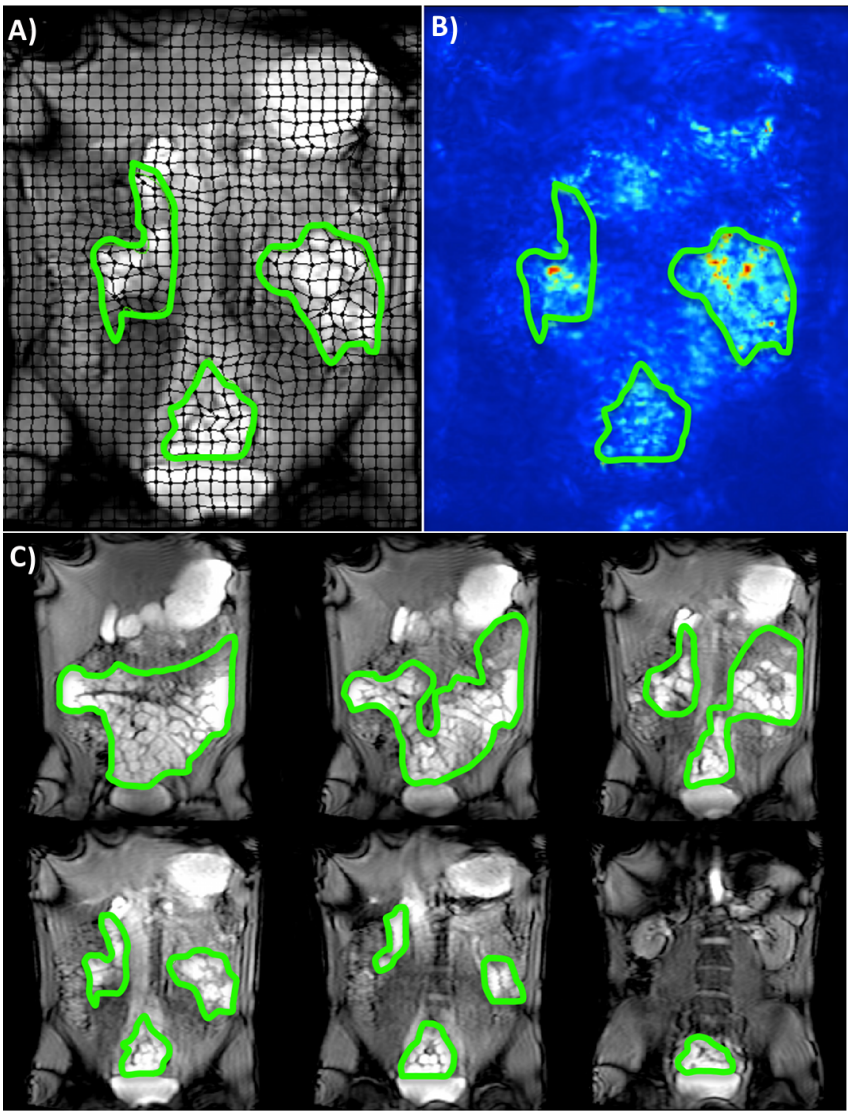

Figure 6.1 *ROI placement and motility score extraction. A) The motility sequence captures a 15-slice volume (six slices shown here) at a 1 second per volume temporal resolution. This is repeated for 20s in a breath hold. The small bowel is initially manually segmented. B) Visualisation of the deformation field, produced by the registration algorithm, as a deformation grid. As an illustration, where greater movement of the underlying small bowel takes place, greater distortion occurs in the grid. C) Analyses of the per-pixel deformation grid as a parametric map with the motility score, extracted as the mean pixel (SD Jacobian) value under the ROI.*

Each volunteer then re-attended for a second MRI scan after a mean gap of 4 weeks (range 2 weeks to 7 weeks), no individual's scan start time varied by more than 1h between the two visits. Volunteers were prepared and examined in exactly the same way as previously except that the IV injection was crossed-over so that placebo was given when they had previously received the drug and vice-versa.

6.2.7 ROI placement

Two radiologists (Drs A. Plumb and A. Ahmed, each with a minimum four year's experience of small bowel MRI) independently placed regions of interest (ROIs) around the small bowel included in each coronal slice over the 15-slice volume, using a MATLAB GUI. The author randomised presentation of the data sets to the readers, both between individual volunteer visits, and between baseline measurement and post drug/placebo measurement.

The two radiologists drew a large region of interest on each coronal slice to encompass as much of the visualised small bowel volume as possible (Figure 6.1A). Solid organs and the colon and stomach were excluded as were major blood vessels. Mesenteric structures between bowel loops, where present, were excluded also. Where necessary, more than one ROI was placed per slice to ensure the whole small bowel was included (Figure 6.1). For each anatomical slice position, the ROI was automatically propagated through the 20-second time series by the software without the need for further adjustment. ROI placement was repeated for each of the 15 coronal slices where bowel was visualised. The motility score (AU) was

derived and recorded for each ROI, and represented the mean of all the included pixels within the ROI (as this quantity is a mean over the ROI, it is relatively independent of ROI size). A global AU score of motility across the small bowel volume was then calculated by averaging all ROIs.

6.2.8 Statistical analysis

Shapiro-Wilk testing was used to determine normality of data for each stage of the analysis. Agreement between the two radiologists for measurement of volunteer mean motility score across all datasets was evaluated using the Bland-Altman (BA) limits of agreement and intra-class correlation. As multiple measurements were made in the same individual, limits of agreement were adjusted to take into consideration multiple within-subject observations(1). The mean of the two readers' score was thereafter used as the 'ground truth' global motility value for each individual volunteer scan.

Intra-subject agreement between baseline motility measurements was determined by calculating the mean difference and BA limits of agreement[125]. The within-subject coefficient of variation (wCV) was also calculated: wCV is computed via dividing within subject standard deviation (from one-way ANOVA) by the group mean, multiplied by 100. This gives a percentage that reflects repeatability, smaller scores reflecting greater repeatability.

For each volunteer, the mean AU following active drug and placebo was compared using a paired t-test. Baseline motility was compared to that following placebo for

each volunteer using a paired t-test to investigate any “placebo” response change in motility.

6.3 Results

6.3.1 Inter-observer agreement

Inter-observer agreement was assessed in the neostigmine and Butylscopolamine group separately with four motility scores made per volunteer (two baseline, one post drug and one post placebo). In the Butylscopolamine group, the mean difference for the motility AU between the 2 observers was -0.005 with adjusted LoA ±0.02 across motility AU ranging from 0.06 to 0.395AU (Figure 6.2A). The intra-class correlation coefficient was 0.98. The mean difference in the motility score (AU) between the two observers in the neostigmine group was -0.005AU and the BA 95% adjusted limits of agreement (LoA) were ±0.02 across a range of values from 0.245 to 0.435 (Figure 6.2B).

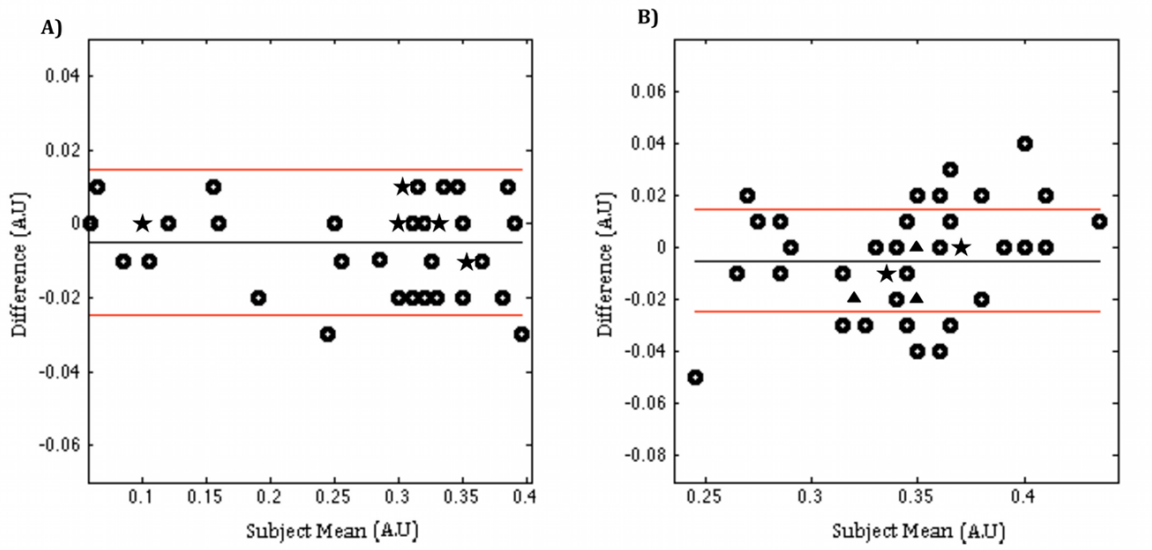

Figure. 6.2 *Bland-Altman limits of agreement between observer 1 and observer 2 motility scores in the Butylscopolamine (A) and Neostigmine (B) groups.*

6.3.2 Intra-subject variability

One of the 21 volunteers (in the Butylscopolamine arm) failed to perform an adequate breath hold after drug administration affecting the motility score interpretation, and was excluded from the analysis.

Baseline motility scores from the first scan attendance (mean score 0.34, range 0.28-0.39) and second scan attendance (mean score 0.34, range 0.30-0.40) is shown in 6.3A. The mean difference between scans was 0.0025AU with BA 95% limits of agreement at ±0.044 (Figure 6.3B). Plotting the observed difference against the observation means revealed no trends, signifying measurement error was unlikely related to motility AU value. Within subject coefficient of variation was calculated at 4.9%.

6.3.3 Motility changes following Neostigmine

11 subjects (9 male, mean age 27.6y) received Neostigmine and saline placebo (Figure 6.4A). The mean motility following the administration of placebo was 0.32AU (range 0.25 to 0.35) and Neostigmine was 0.39AU (0.32 to 0.44) with a mean group difference of 0.07 (95% CI = 0.038 to 0.098, $p < 0.001$) representing a 22% increase in small bowel motility. Ten of 11 subjects showed an increase in motility after blinded Neostigmine administration.

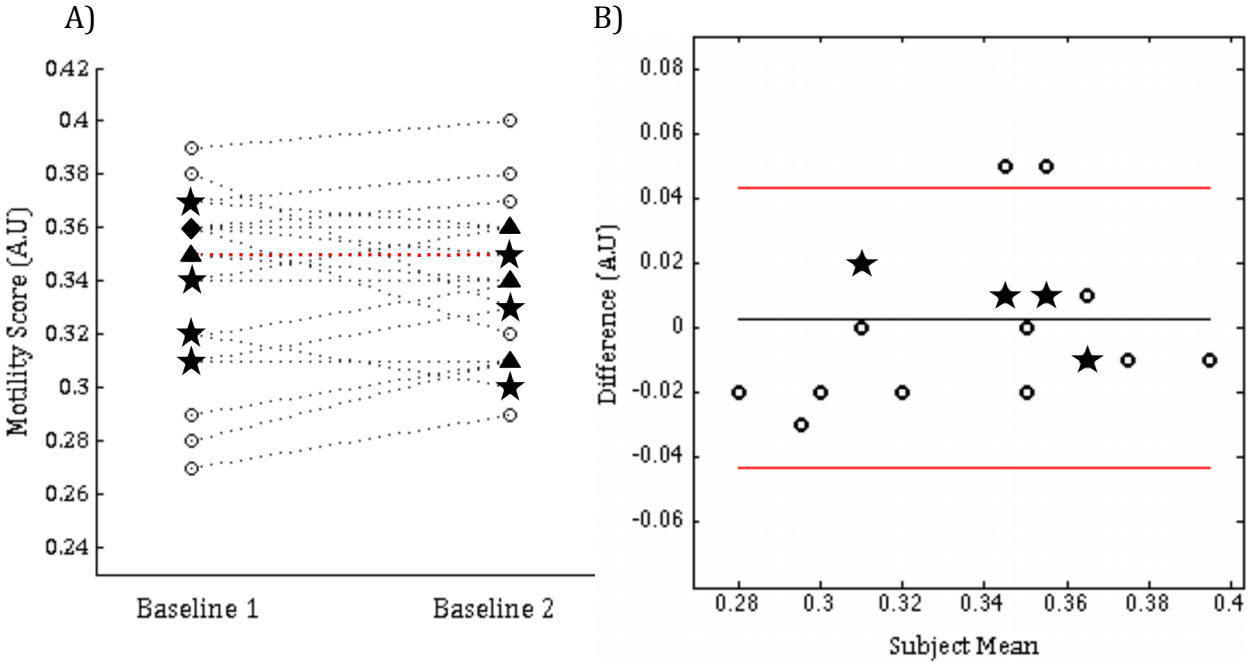

Figure 6.3 *Baseline variability across two time points. A) Line plots for each of the 20 volunteers showing global motility at baseline scan 1 and baseline scan 2. B) Bland-Altman limits of agreement between baseline scans. Stars represent co-incidental observations, dots single observations.*

6.3.4 Motility changes following Butylscopolamine

Nine subjects (4 male, average age 31.5y) were analysed (Fig. 6.4B). The mean motility scores following administration of placebo and Butylscopolamine were 0.30AU (0.20-0.39) and 0.13AU (0.06-0.34) respectively, with a mean difference of 0.17 (95% CI from 0.10 to 0.23, P <0.001) and representing a 57% decrease in motility. All individuals showed a reduction in motility with blinded butylscopolamine administration.

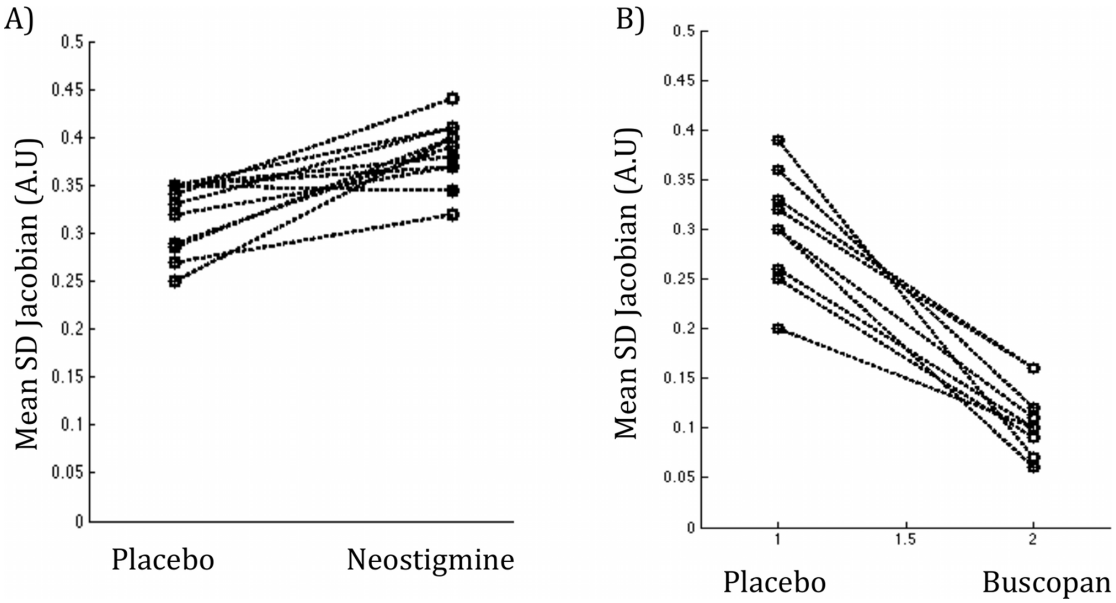

Figure 6.4 *Response to drug provocation. A) Line plot showing the change in motility for 11 volunteers on Neostigmine arm of the trial and B) for the 9 volunteers on the Butylscopolamine against placebo.*

6.2.5 Changes in small bowel motility from baseline following placebo

Across the 20 volunteers mean baseline motility was 0.33 AU (0.26-0.40) and following placebo decreased to 0.30AU (0.19 to 0.39) with a mean difference of -0.03 AU (95% CI 0.01 to 0.04), *P*=0.002. A significant increase in mean global motility persisted [0.39 (0.32 to 0.44)] following Neostigmine even when compared to the pre-neostigmine baseline scan [0.34AU (0.28 to 0.37)] for those individuals on that arm, mean difference +0.04AU (95% CI 0.02 to 0.07 p = 0.0026).

6.4 Discussion

This study showed that a registration derived imaging surrogate for global small bowel motility based on MRI is both repeatable in normal subjects and sensitive to changes induced by medication that exerts known pharmacological effects on gut function.

Assessment of within-subject repeatability using Bland-Altman plot demonstrated good within-subject repeatability between time points with the mean difference between scans close to zero with narrow limits of agreement. Similarly, the coefficient of variation was low, at less than 5%, suggesting low intra-subject variation and simple subjective evaluation of the line plots revealed consistent motility scores for most participants across the two scan dates. Given the many factors that influence small bowel function, it was reassuring that similarity between subjects scanned at least 2 weeks apart could be seen using the standardised acquisition methodology and software. Standardising the acquisition methodology and software were likely key to this, with confounding factors including dietary (fasted state, limiting caffeine and nicotine intake) and diurnal ones (same afternoon time slot) well controlled here. A uniform oral preparation of mannitol was also used, both to control oral intake and to distend the small bowel in a predictable fashion. The technique was well tolerated, and is practical in clinical practice.

Variation in baseline motility for three of the volunteers was relatively large compared to the rest of the cohort. One of these volunteers failed to perform an adequate breath hold and was excluded from the analysis presented. In the other two volunteers there was an apparent difference in mannitol distribution between the two scan attendances, with selective retention of contrast within the stomach (with an associated higher motility score, Figure 6.5). This could be related to difference in gastric emptying times between the two visits, or on differing ingestion rates. Volunteers were instructed to ingest the contrast steadily over the 50 minutes before the scan but this may need to be standardised more thoroughly in future investigations.

An interesting observation was that of a small but significant decrease in motility following administration of the saline placebo. Assuming this observation generalises from our relatively small cohort, it is possible that slowing was related to a combination of the volunteers' anticipation of receiving the injection, increasing familiarity to lying in the scanner or to the mannitol moving though the bowel. Alternatively, the placebo (saline) might precipitate a genuine parasympathetic response in small bowel motility and be, in itself, a method for provoking the autonomic nervous system with a potential value in function testing in a range of conditions featuring autonomic/enteric dysfunction. In any case, it should be considered in the design of future studies and a placebo should be used when investigating the effects of motility altering drugs using MR based techniques or where repeat measurements are made during a single scanning session.

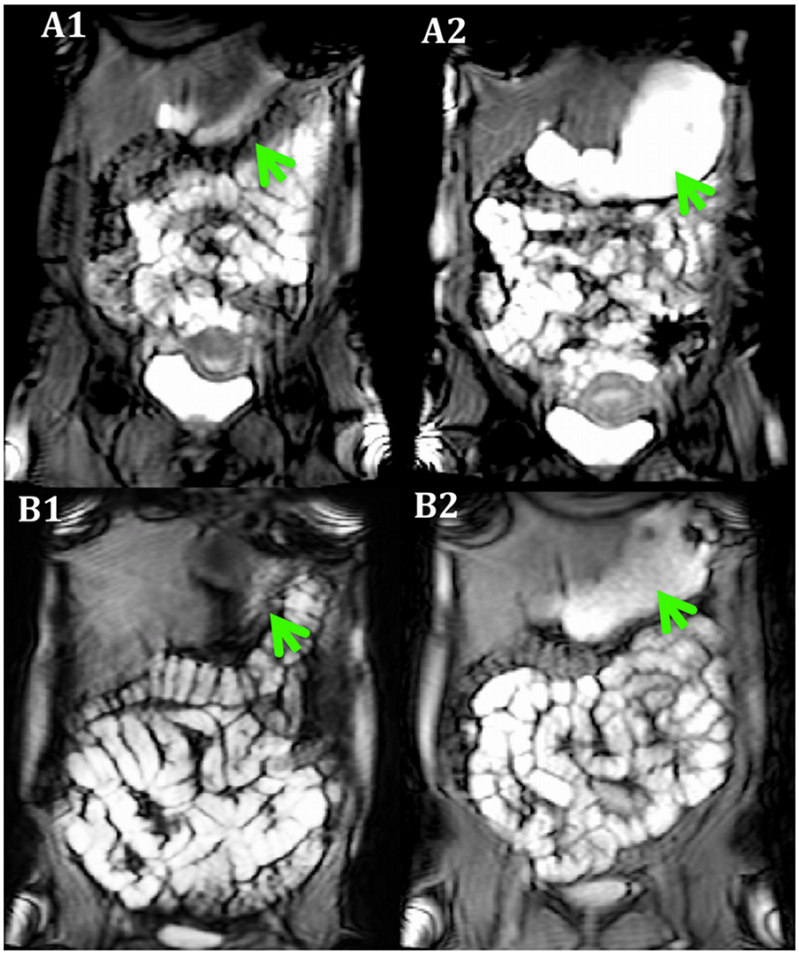

Figure 6.5 *Two volunteers (A & B) with higher score variability between baseline scans and large differences in mannitol distribution through the bowel (stomach indicated with green arrow). Lower scores were observed where there was a low stomach content of mannitol (A1 & B1) compared to high mannitol content (A2 & B2).*

The second aim of the study was to test the ability to detect changes in bowel motility induced by medication with known pharmacological effects on bowel function. Butylscopolamine relaxes enteric smooth muscle due to its antagonism of muscarinic receptors – it is routinely used in clinical practice to improve image quality during abdominal MRI. Gutzeit et al used segmental quantification of

motility to show at least a 50% decrease in segmental bowel motility following 40mg in six volunteers. A single well-displayed loop of small bowel was used to quantify motility by measuring cross sectional area change over time. In this study, 20mg produced a strong and predictable anti-kinetic effect, reducing motility by approximately 56%. This is comparable to the effects described by Gutzeit, although my assessment reflected motility across the whole small bowel volume against a placebo control.

Neostigmine was used to provoke motility, which, although not usually administered within the MRI scanning environment, has a demonstrated stimulatory effect in vivo (2-5). Although 2mg has previously been shown to exert a prokinetic effect in chronic intestinal pseudo-obstruction, the chance of adverse effects were minimised by administering just 0.5mg concordant with that used by Serrea et al. (2, 6). At this dose a significant, detectible change in motility was observed with a 22% increase compared to placebo. The stimulatory effect remained significant even when compared to baseline. Detection of such a change with a relatively small dose of Neostigmine suggests it could form a sensitive biomarker, although translation into clinical practice may need a larger dose to detect an abnormal or attenuated response in disease states. An interesting future research objective could be to develop alternative ways of provoking bowel motility. In this study, although no adverse events were seen in response to either drug, the safety requirements to administer neostigmine required a detailed history check and scan attendance by a gastroenterologist making this potentially challenging as part of clinical routine. In cardiac MR, numerous physical and pharmacological

protocols have been developed and this might constitute an exciting future research project.

A limitation is the movement of a bowel loops in the through-slice direction (i.e. perpendicular to the imaging plane). The technique is relatively robust to this process because it is performed in breath hold using a 1cm slice thickness with no slice gaps. Thus, bowel that transition between slices produces deformation field changes in each slice and these contribute to the score for the volume. A further limitation of the approach is the time required for analysis. Drawing the ROIs is time consuming, taking at least 5mins per volume cumulatively up 45 minutes per patient when anatomy was complex. Variation due to differences in ROI placement may be another disadvantage, but in the study the two independent assessors showed narrow limits of agreement and a mean difference of just 0.005AU. An additional limitation of the work relates to the fact that our study population were young, but there is no specific reason to expect that different results would be obtained in a more senior cohort adhering to the same protocol.

The registration algorithm and analysis generated a unitless global motility score that served as a surrogate for small bowel motility. As seen in chapter 2, the algorithm is “blind” to the source of physical deformation in the region of interest and careful segmentation helped exclude motion from other moving structures such as the aorta. The requirement for a 20s breath-hold acquisition protocol represents an additional limitation in this experiment. A breath-hold protocol was nevertheless used principally due to the registration algorithm being unable to separate respiratory motion from that of the bowel wall with the potential to bias the motility

score. This reliance on breath-hold data might prove to be problematic where patients are unable to hold their breath or where longer time series are required to evaluate the varied facets of small bowel motility and indeed this will be explored in the next section.

Summary

In conclusion, registration quantified global small bowel motility captured using MRI in human volunteers shows good repeatability and can detect changes in motility induced by pharmacological intervention. In the next chapter, the reliance on breath-hold protocols is addressed with a view to increasing the generalisability of this technique both to the small bowel and beyond.

SECTION D: IMPLEMENTING NEW TECHNIQUES FOR SMALL BOWEL MOTILITY ASSESSMENT IN THE CLINIC

Section B presented data demonstrating a clear application for quantitative motility analysis using MRE and post processing. In Section C, limitations were highlighted in segmental methods of analysis along with a solution in the form of global analysis - all of which was conducted in healthy well-controlled volunteers. The subject of **Section D** is a further extension to the image post-processing to maximise clinical viability and explore the role of global motility in the assessment of motility in a prospective disease cohort. The healthy subjects used in section C tolerated the protocol well and were able to perform the breath-hold required for global analysis. However, a practical limitation exists here where often severely ill patients cannot comply with this requirement potentially impacting on clinical value. Further, from a physiological perspective, many of the slower contractile actions in the small bowel, of interest to a clinical diagnosis, take place over minutes, clearly outside the scope of a breath-hold protocol.

In **chapter 7**, a technique called Dual Registration of abdominal motion is validated in free-breathing data sets as an additional means of investigating dynamic series in both small bowel and colonic datasets. Finally in **Chapter 8** the global technique is applied to a cohort of Chronic Intestinal Pesudo-Obstruction patients, a cohort characterised by dilated bowel and reduced motility. This patient cohort provides an ideal substrate for this work as a reduction in motility has been previously been

demonstrated allowing here the demonstration of this techniques clinical applicability. Novel information will be further added to this area of research through administering neostigmine against placebo control to see what, if any, affect it has on small bowel motility, using the healthy cohort from chapter 5 as a comparison.

CHAPTER 7: DUAL REGISTRATION OF ABDOMINAL MOTION TO QUANTIFY BOWEL MOTILITY IN FREE-BREATHING DATA

Research Question:

Can a pre-registration respiratory motion correction step be used to extend the application of the optic-flow technique to free breathing bowel motility data?

Rationale:

A range of contractile actions take place in the bowel beyond local segmentation. In the colon especially, a single contraction might take place every several minutes making breath-hold protocols unsuitable for the investigation of these studies. In addition, many patients are not capable of performing a breath-hold and therefore overcoming the reliance on this protocol would represent an important advance for clinical application.

Hypothesis:

Respiratory motion correction can be used prior to motility assessment to broaden the application of the optic-flow registration technique in FB data.

Aim(s):

i) Demonstrate registration accuracy against gold-standard in FB small bowel and colon data.

ii) Demonstrate comparability of parametric maps in BH and FB corrected data sets.

Author Declaration

Research presented in this chapter was the result of the collaborative efforts of the thesis author and Dr. V Hamy. The author led the study and was responsible for ethical approval, data collection, study management and manuscript preparation in: Menys, A., Hamy, V., Makanyanga, J., Hoad, C., Gowland, P., Odille, F., Atkinson, D. (2014). Dual registration of abdominal motion for motility assessment in free-breathing data sets acquired using dynamic MRI. Physics in Medicine and Biology, 59(16), 4603–19. doi:10.1088. Dr. V. Hamy was responsible for algorithm development, combining his RPCA registration technique with the Odille optic-flow code. The technical specifics can be found in: Hamy, V. et al., 2013. Respiratory Motion Correction in Dynamic-MRI : Application to Small Bowel Motility Quantification during Free Breathing. In Proceedings of Medical Image Computing and Computer-Assisted Intervention. pp. 132–140. ROI validation of the algorithm was performed by the author and Dr. J Makanyanga. Supervision of the project was provided by Dr. D Atkinson. Additional dynamic colon data was provided by Dr C. Hoard and Prof. P Gowland at the University of Nottingham.

7.1 Introduction

This thesis has so far explored image registration as a means of generating surrogate measures of bowel motility. In each of the studies presented, the data has been acquired in breath hold principally to remove the affects of respiratory motion that would otherwise bias motility quantitation. Although the results have so far

supported optic-flow accuracy in correcting local deformation, it is of course blind to the source of deformation with organs such as the liver attracting a motility score. Optic-flows restriction to breath-hold limits application for important groups of pathological conditions where aberrant small bowel motility patterns take place over minutes or conditions which predominantly affect the colon where the period between peristaltic waves is longer than a breath hold duration.

The purpose of this chapter is to explore a pre-processing step to remove the effects of respiratory motion from the data before performing registration with optic-flow. Although there are several potential methods to do this, the Centre for Medical Imaging at the time, exploring a new technique called Robust Principle Component Analysis (RPCA) that provided some interesting possibilities with respect to the processing time series data[127]. RPCA is a modification of the widely used statistical procedure Principle Component Analysis (PCA) that addresses PCAs limitations with respect to grossly corrupted data. RPCA is a fascinating statistical tool and while an in depth discussion is outside the scope of this chapter, an introduction will be made here to illustrate how this chapter arrived at the technique as a means of differentially correcting respiratory motion.

Let M be a matrix with each column constituting all the pixels from a 2D matrix representing a single time-point of a dynamic series. RPCA splits M into a low-rank matrix (L) and a corresponding sparse matrix (S) with the sum of L and S being equal to the original matrix M. A regularization parameter lambda (λ) controls the weighting of the perturbation in the observed 'corrupted' data and, in effect, serves

as a trade-off between the L and S components. For high lambda values all data will appear in L and conversely for low lambda values data will appear in S (Figure 7.1).

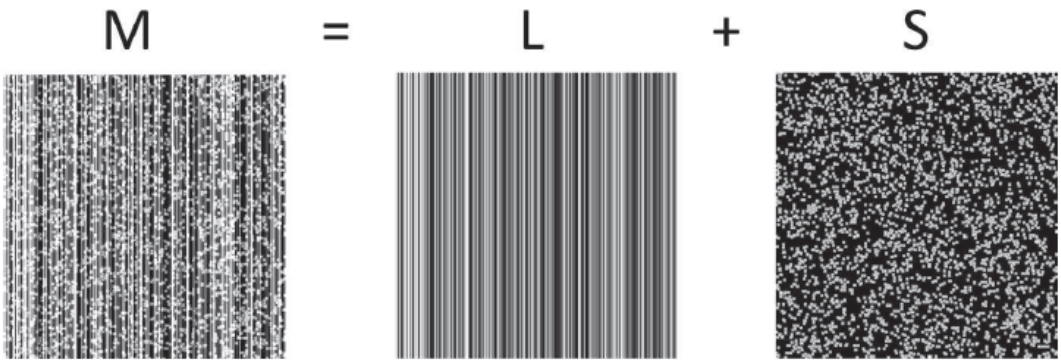

Figure 7.1 *A corrupted matrix (M) is separated into low rank (L) and sparse (S) components through selection of a suitable trade-off parameter called lambda (λ).*

The RPCA technique finds applications across computer science and one of best examples can be seen in CCTV footage of people standing in a foyer (Figure 7.2A-C). The decomposition of the time series results in the static features of the room (floor, desk etc) appearing in the low-rank matrix and the people (dynamic content) in the sparse. This technique originally presented itself as a potential method to quantify bowel motility and application of the code to the motility series provided an interesting result, isolating bowel motility (sparse) from the static structure (low-rank) after some empiric tuning of lambda (figure 7.2D-I). A more in depth investigation of this premise formed the MSc project for Pauline Ferry and an abstract can be found in the Appendix 3.

Although an interesting project, a number of limitations existed over its applicability as a competing technique to optic flow[57]. First it did not provide deformation

fields and so the ability to propagate ROIs through time series is lost. Second, although the motility maps RPCA produced looked reasonable and indeed corresponded to optic-flow driven metrics, it was unclear as to exactly what was driving high motility scores (e.g. bowel wall motion, intensity from chime flow, some combination). Additionally, RPCA was not as sensitive to drug-induced motility changes when compared to the Jacobian SD score and for these reasons, RPCA as a motility surrogate, was abandoned. While RPCA did not appear to add particular value for direct motility quantitation, it played a central role in another project taking place in the department at that time. Valentin Hamy used RPCA in combination with registration to address the problem of spatial misalignments in dynamic contrast enhanced (DCE) time series. Hamy's assumption was that RPCA could be used to separate low-rank motion components from sparse contrast enhancement within an iterative framework to allow progressive re-alignment of the imaged features[128]. One of the key challenges for registration of DCE data is the dual effect of continual intensity change with motion from respiration. Without intensity changes in the data, the residual complexity algorithm could be applied to much greater effect with the resultant deformation fields finally being then used to correct the original time series data. This technique was robust and worked well across organs in a number of different DCE data series and was named Robust Data Decomposition Registration (RDDR)[128].

It is hypothesised in this study that this technique could be extended to correct the respiratory motion in dynamic small bowel motility data, which unlike peristalsis was fairly periodic. In this schema, the intensity changes filling sparse matrix would be replaced the local deformation caused by the small bowel motility. As before,

respiratory motion appearing in the low rank could be registered to correct the original data. The corrected RDDR could then be registered with optic-flow algorithm as per chapter 2.2.4 to finally correct local deformation caused by peristalsis and from this, generate the motility maps.

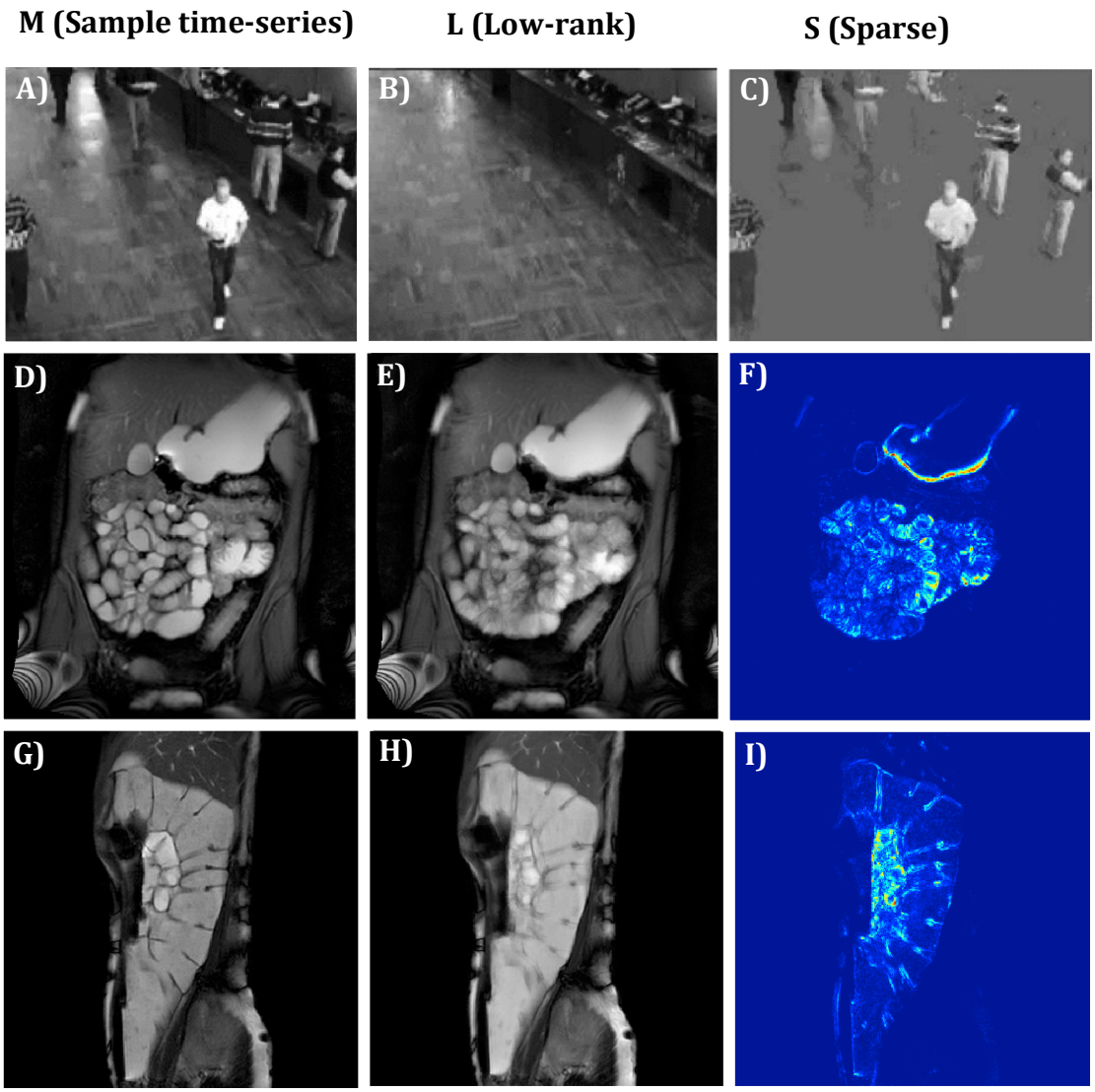

Figure 7.2 *Three examples of RPCA decomposition with each image representing a single time point from a dynamic series. A is the original Candes (2009) CCTV footage with the low rank (B) static and sparse (C) dynamic components separated by a variable lambda. D and G represent dynamic series of the small bowel and colon respectively. Low rank components from decomposition are shown in E and H with the sparse motility data in F and I. These motility maps can be analysed in the same way as the SD Jacobian maps seen throughout this thesis, providing an alternate surrogate for motility.*

7.2 Methods

7.2.1 Dual Registration of Abdominal Motion

Robust Data Decomposition Registration.

Our aim was to use Robust Data Decomposition Registration (RDDR) as a pre-processing step to register and remove the respiratory component of motion, whilst preserving peristaltic motion in the data. This method uses RPCA to decompose the cine data into low rank (L) and sparse (S) components [127]. The low-rank component tends to contain the slowly varying respiratory motion and the sparse component the local rapid changes due to peristalsis. This decomposition does not perfectly separate the two physiological motions and to remove only the respiratory motion, RDDR applies an iterative registration scheme. Within RDDR, RPCA performs the decomposition in (1):

$$\begin{aligned} &\text{minimize } \|L\|_* + \lambda\|S\|_1 \\ &\text{subject to } L + S = M \end{aligned} \tag{1}$$

where $||.||_*$ and $||.||_1$ respectively represent the nuclear norm (i.e. the sum of the matrix singular values) and the l1-norm (i.e. the sum of the absolute values of the matrix elements). M, L, and S respectively correspond to the input data, the low rank, and the sparse component. The parameter lambda (λ) appearing in (1) is a trade-off parameter that determines the relative amount of information in L or S[127]. For the purpose of separating and correcting respiratory motion from peristaltic motion, lambda is varied in an iterative scheme that includes successive registrations of the low rank frames as shown in Figure 7.3. For lower values of lambda, only elements of respiratory motion appear in L. As lambda increases, more

respiratory motion is present and peristalsis gradually appears as shown in Figure 7.3. At each iteration, the frames contained in the low-rank component are all registered to the frame that minimizes the difference to the pixelwise statistical median over time. The resulting deformation fields are applied to the initial time-series so that a part of the motion can be removed. This process (decomposition + registration) is repeated for increasing values of the trade-off parameter. The deformation fields generated at each registration stage are added to a single global deformation field applied to the initial time series after the last iteration to avoid loss of information caused by multiple resampling.

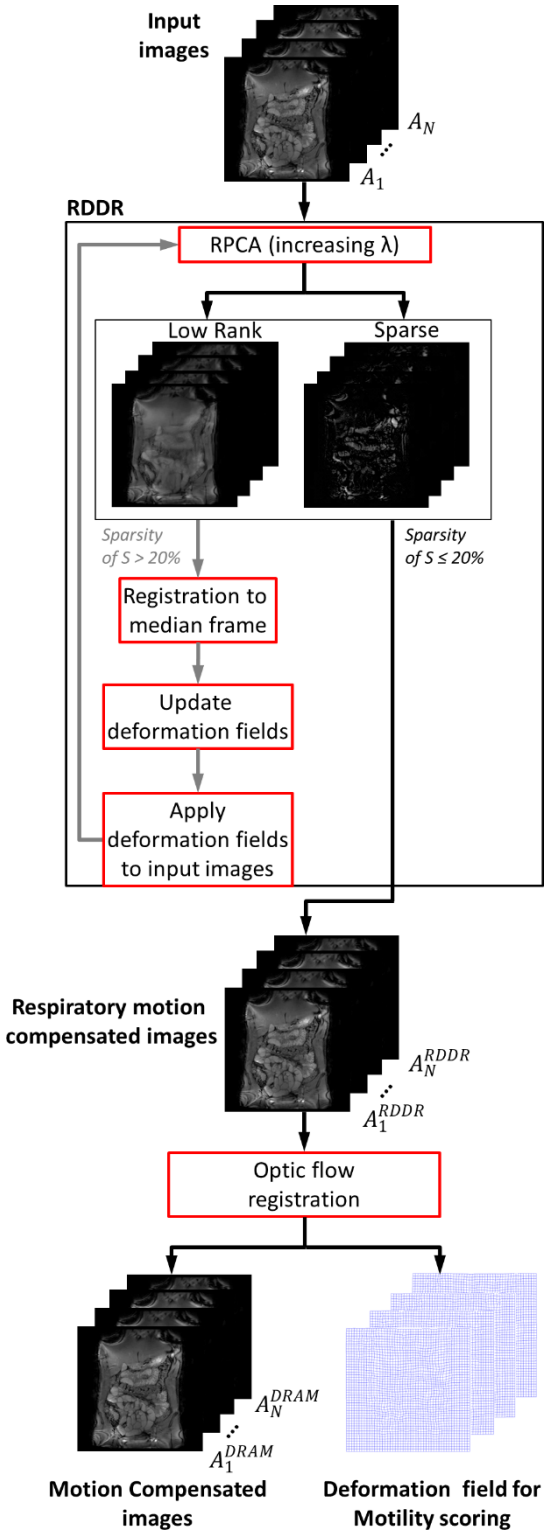

Figure 7.3 *Flow chart illustrating the process of DRAM. The parameter lambda is gradually increased in RDDR to let more information appear in the Low rank component over iterations.*

The registration steps within RDDR use the residual complexity similarity metric [129] and transformation fields are described using B-spline based free form deformations. The 2D control point grid used here has a relatively large spacing (10 pixels) aimed at capturing the large-scale deformations due to respiratory motion.

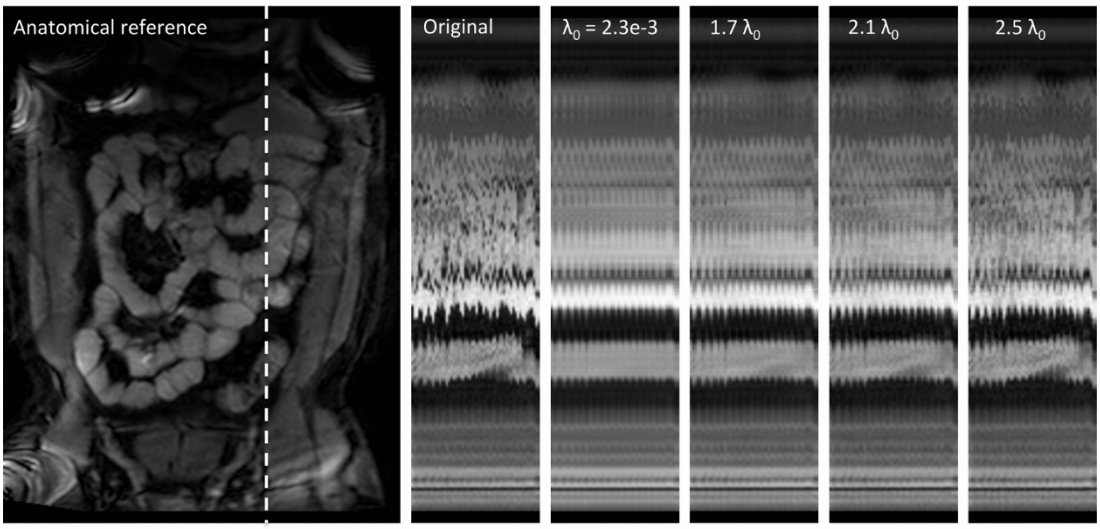

Figure 7.4 *Effect of increasing lambda on RPCA low rank component in an example data set. Time cuts for the original time series and various low rank components are shown. The location of these time cuts is indicated by a white dashed line in the anatomical reference.*

RPCA settings

The starting value of lambda is chosen such that the rank of L in the first iteration of RDDR is the number of time frames divided by four. This initial value of lambda was empirically found to be high enough to include some elements of respiratory motion in L and low enough to keep peristalsis in the sparse component [128]. For lower values of lambda, respiratory motion might entirely appear in the sparse component making the first iteration useless. The starting value of lambda is

logarithmically incremented in subsequent iterations similar to the original version of the algorithm. The same settings were used for all the datasets (both small bowel and colon) analysed in this study.

This stopping criterion is designed so that no peristalsis appears in the registered low rank components. A threshold on the sparsity of the RPCA sparse component was used to terminate the iterations. Given the pseudo-periodical characteristic of respiratory motion and peristalsis, the optimum threshold for lambda was chosen using an analysis of test data in the frequency domain, inspired by previous work from Sprengers et.al. [130]. The frequency of peristalsis is expected to be the same in both breath hold and free breathing. Thus the difference between breath-hold and free breathing data in the Fourier domain should show only the contribution of respiratory motion. Such a difference is used as an indicator of the effect of each iteration in RDDR. Spectral powers were computed by summing the Fourier transform of time-intensity variations for every pixel over the entire field of view. Figure 7.4 presents the evolution of the spectral power difference with respect to the sparsity of RPCA sparse component. A minimum difference appears clearly when the sparsity is equal to 20%. The stopping criterion for RDDR is then chosen to be when the sparsity of S falls below a threshold of 20% (as indicated in Figure 7.5).

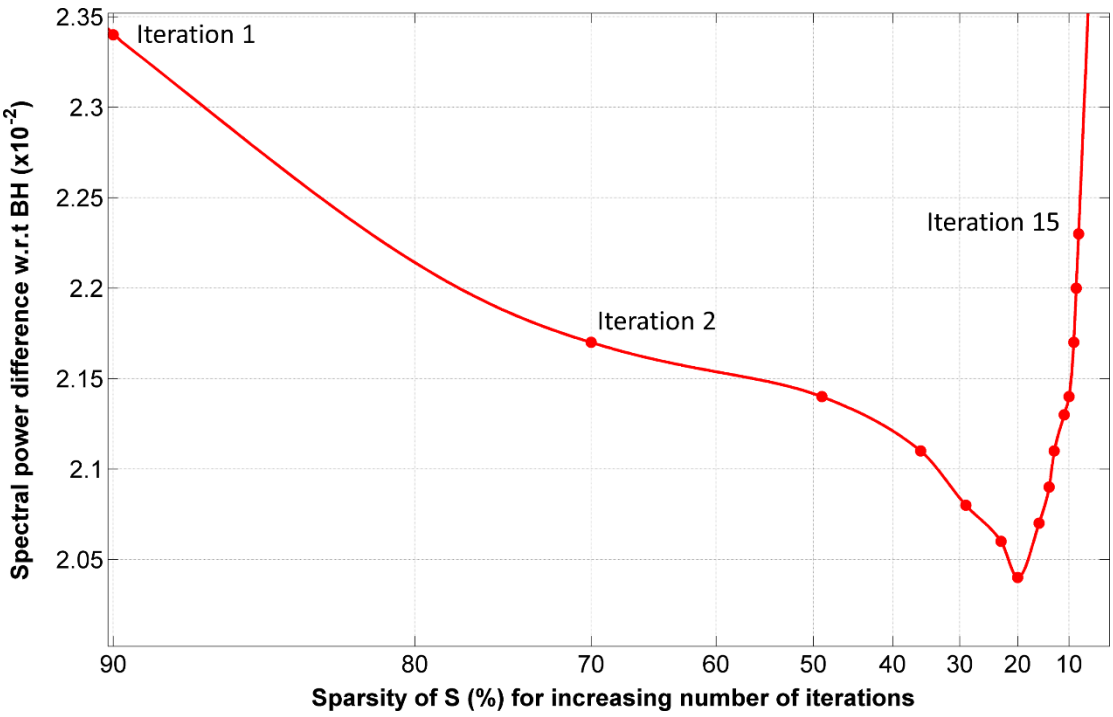

Figure 7.5 *Spectral analysis of a subject for tuning of RDDR stopping criterion. Spectral differences between gradually corrected data and breath-hold are progressively reduced until a minimum is reached. The sparsity of S at that minimum value (20%) is chosen as lower threshold to stop the iterative registration and avoid deterioration of the information on motility.*

Motility Quantification

Optic flow was applied to the RDDR corrected data as per chapter 2.2.4 without additional adjustments.

7.2.2 Study Overview

In this study motility is quantified in dynamic small bowel and colonic data with the OF registration algorithm. The purpose of this study was to evaluate the ability of

the pre-processing registration step, RDDR, to correct free breathing motion before OF processing. Two main results are provided that focus on 1) the ability of OF-alone and DRAM to faithfully propagate a line ROI (i.e. a 1D line drawn across the bowel lumen, perpendicular to the central axis of the bowel) through processed small bowel and colonic time series data using the average of two independent manually propagated ROIs as a gold standard. 2) Free breathing parametric motility maps in small bowel data sets registered with OF-alone and DRAM, using breath hold OF data as a gold standard. A summary is provided in Figure 7.6.

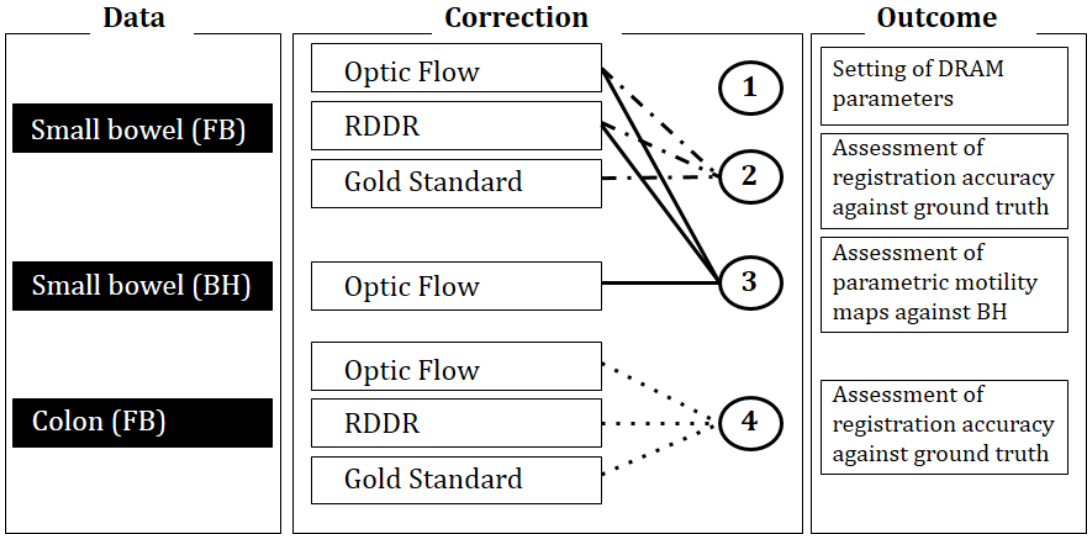

Figure 7.6 *DRAM Study overview. 1) Setting of DRAM parameters. 2) Registration accuracy of the two methods was investigated by comparing the ability of the two algorithms to propagate a ROI against a manually adjusted 'gold-standard.' 3) Registration accuracy was assessed as per (2) in the colonic data sets. 4) Parametric motility maps derived from free breathing data sets were compared to a breath-hold pseudo ground-truth.*

7.2.3 Subject population

Two data sources were used for the validation of this technique. The first was from the prospective study of small bowel motility in 20 volunteers, and the second in a study of colonic motility in 6 volunteers provided by Nottingham University.

Small bowel

The same 20 healthy subjects were used as described in Chapter 5.2.1.

Colon

Colon data sets for 6 healthy volunteers were used in this study with subject demographics as follows, mean age 27, range 19-43 years, 1 Male. Volunteers were included in the study where they were able to give informed written consent, were non-smokers and had abstained from alcohol for 24 hours prior to the study day. Volunteers were excluded if they had any history of serious acute or chronic illness, especially gastro-intestinal, if they regularly used medication which interfered with GI function or had previous GI surgery (excluding appendectomy). Volunteers were recruited prospectively by advertisement.

MRI protocol

7.2.4 Scan Protocol

Small bowel

Volunteers were prepared and scanned as per chapter 5 using the 6-slice BTFE sequence, with the key difference between this study being that both breath-hold and free-breathing series were collected.

The motility sequence was run first on inspiration breath-hold to collect a total of 20 images of the same anatomical slice. This process was repeated following a 10 s recovery period with the subject this time instructed to 'gently free-breathe' whilst a total of 60 images were acquired in the same anatomical position unchanged from the breath-hold scan.

Colon

The volunteers arrived after an overnight fast. Scans were carried out at baseline and at hourly intervals following consumption of either 1 L or 2 L Polyethlene glycol (PEG) formulation. A total of 9 data sets from 6 subjects are included in this work to highlight differing amounts of colonic motility and breathing effects, selected subjectively based on visual inspection of the time series by the study scientist (Dr. Caroline Hoad). All volunteers underwent a baseline scan and then hourly scans after ingestion of PEG. The data in this study comprises of 2 baseline scans and 7 scans at various time points post ingestion. Subjects lay in the supine position and were scanned using a Philips Achieva 1.5 T MRI scanner using the XL-Torso receiver coil.

The colon motility scan consisted of a single slice BTFE sequence positioned in the sagittal plane through the ascending colon (sagittal plane, voxel size 1.5x1.5x15 mm^3, FOV 330x228x15 mm^3, FA 70 degrees, TE=1.5 ms, TR=3.0 ms). Temporal resolution was 1 slice per second and scans were acquired during two minutes of thoracic free breathing (example time point in 7.2G).

7.2.5 Assessment of the effect of registration

The effect of respiratory motion correction using RDDR was assessed by investigating the fidelity of the optic flow algorithm to propagate a line ROI through the 1) small bowel and 2) colon free-breathing time series data.

Small bowel

One gastroenterology research fellow and one research scientist (Dr. Jessica Makanyanga - 3 years experience MRE, the author - 3 years experience small bowel MR) identified, in consensus, a small bowel loop in the upper left quadrant of each subject, which remained visible through the time series (i.e. did not move out of plane).

Colon

This process was repeated in the colon data sets, where the same two observers placed in consensus two line ROIs in the ascending portion of the colon.

In both types of data, the line ROIs were automatically propagated through the time series by both OF alone and the OF component of DRAM based on the registration deformation fields, and the results saved. The ROI was then manually corrected independently by both JM and AM for each time point. Agreement between readers was assessed using Bland-Altman limits of agreement and Intra-class correlation. The manually corrected line ROIs for the two observers were averaged for each time point and used to create a ground truth for each data set.

Accuracy of the OF-alone and DRAM algorithms to the ground truth was compared by: 1) assessing change in line length over time between the manually corrected and automatically propagated ROIs using Bland-Altman limits of agreement (LoA). 2) Assessing the variance of the displacement of ROIs by computing the target registration error (TRE) i.e. the distance between each line end-point of the manually corrected and automatically propagated ROIs. A threshold for TREs was set to 1e-3 mm. Errors below this value were considered as zero.

Validation of motility scoring

The global small bowel ROI was placed by the author and Dr. J Makanyanga in consensus for each subject: 1) the breath-hold registered with OF 2) the free breathing registered with DRAM data sets; and 3) the free-breathing registered with OF-alone. The 20 s BH data (i.e. without respiratory motion) served as a ground truth. For each data series, the parametric SD Jacobian motility map was calculated.

7.3 Results

7.3.1 Registration assessment

Example images of time cuts obtained after registration are shown in Figure 7.7. The time cut representation shows correction of breathing motion after RDDR with little apparent effect on peristaltic motion.

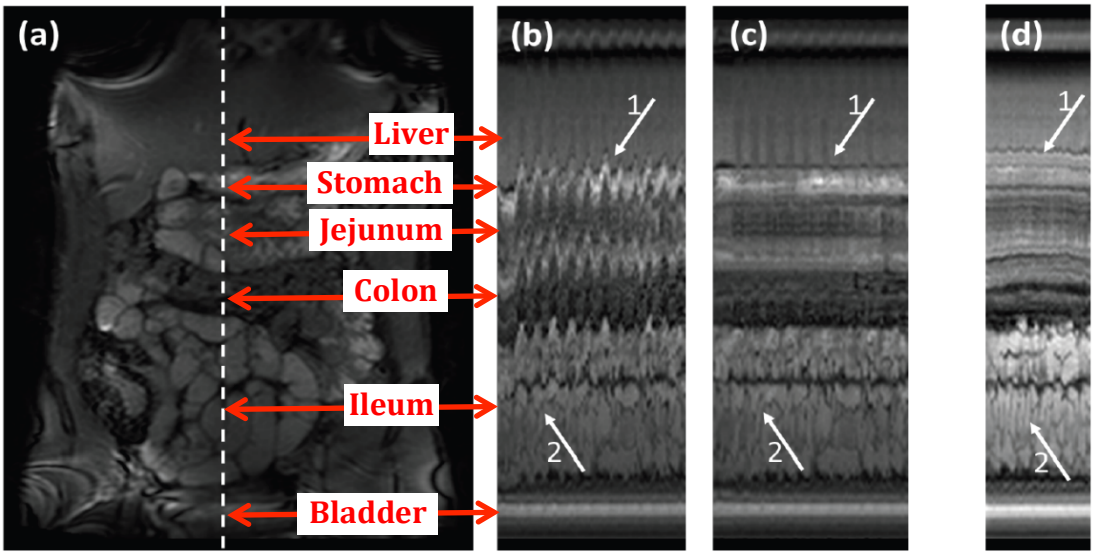

Figure 7.7 *Time-cut representations illustrating organ motion before and after registration of images from a dynamic MR time-series obtained on a healthy volunteer: (a) A coronal MR scan at a particular time-point within the dynamic MR series. The spatial location of the time cuts, shown in (b), (c) and (d), is indicated by a white dashed line, with different organs labelled. Each time-cut image represents the image intensities along this line as a function of time (horizontal axis). (b) Time-cut image before registration with RDDR. (c) Time-cut image before registration with RDDR. (d) Time-cut image based on breath-hold data for comparison; note that the time-cut series is smaller due to the fewer number of available time points (20 images as opposed to 60). It can be seen by comparing images (b), (c), and (d) that*

respiratory motion at the interface between the stomach and liver, indicated by arrow 1, are accurately corrected by RDDR, whilst small bowel motility information, indicated by arrow 2, is preserved.

Small bowel

In the small bowel, two observers manually propagated a linear region of interest through 60 time points in each of the 20 subjects. Inter-reader variability was assessed through Bland-Altman LoA and intra-class correlation (ICC). For the manually corrected OF-alone data, mean difference between readers was 0.4 mm (95% LoA ±7.3 mm). ICC was 0.85. For the manually corrected DRAM data the mean difference between readers was 0.54 mm, LoA ±3.4 mm. ICC was 0.96.

The BA analysis of line length ROIs in OF-alone registered and DRAM registered data with the manual measurements (mean of two observers) is shown in Figure 7.8a and 7.8b. For the OF-alone registered data the mean difference between the manually corrected and automatically propagated ROIs was - 2.0 mm (95% LoA ±9 mm). For the DRAM processed images mean difference was -0.48 mm (95% LoA ± 4.15 mm).

Target Registration Errors were below the threshold in 49% of the cases with OF only and in 70% of the cases after pre-processing with RDDR. For nonzero TREs (Figure 7.8c), OF-alone yielded a median error of 0.5 mm (IQR 2.27 mm) and DRAM yielded a median error of 0.05 mm (IQR 0.1 mm).

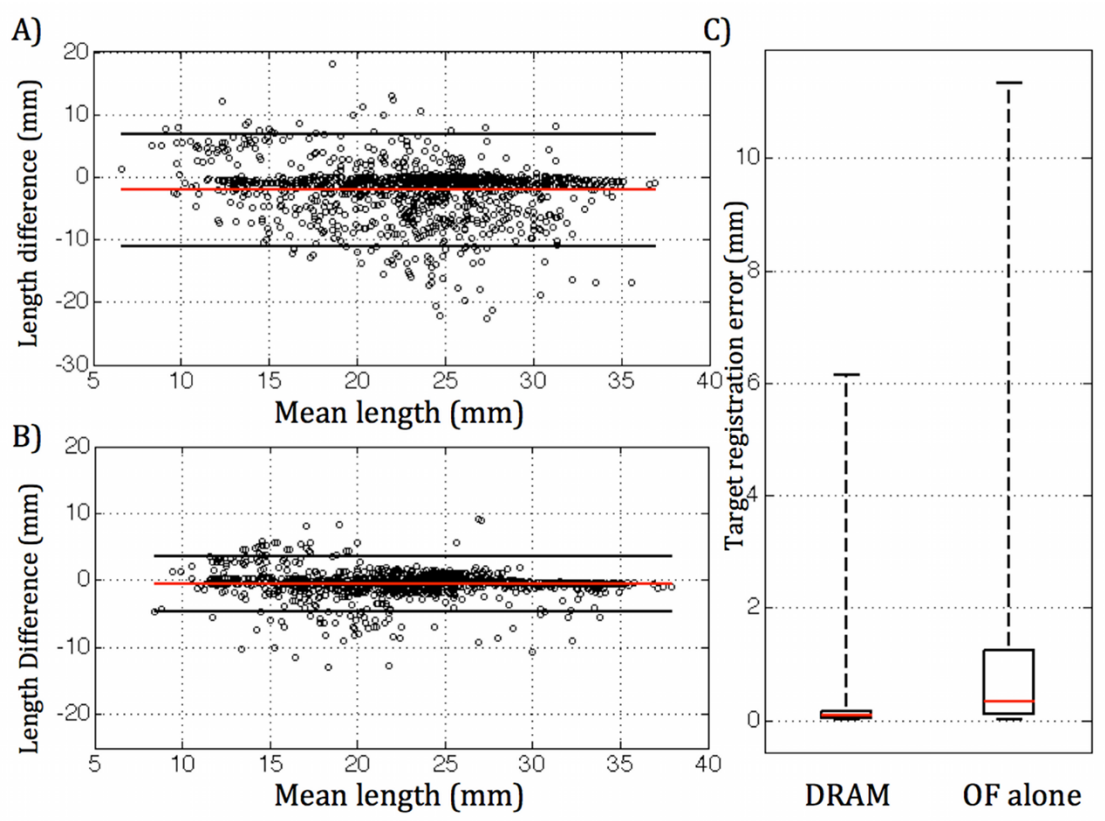

Figure 7.8 *Bland Altman limits of agreement for line ROIs in small bowel data registered with OF against manually corrected ground truth (A) and data registered with DRAM against manually corrected ground truth (B). Target registration error in DRAM and OF alone (C).*

Colon

Inter-reader variability in the colon was examined in exactly the same way as the small bowel result. For the manually corrected OF-alone data, mean difference between readers was 0.2mm (95% LoA ±1.1mm). ICC was 0.98. For the manually corrected DRAM data the mean difference between readers was 0.28mm, LoA ±1.7mm. ICC was 0.99.

The BA analysis of line length ROIs in OF-alone registered and DRAM registered data with the manual measurements (mean of two observers) is shown in Figure 7.9a and 7.9b. For the OF-alone registered data the mean difference between the manually corrected and automatically propagated ROIs was -1.25 mm (95% LoA ±7.57 mm). For the DRAM processed images mean difference was -0.13 mm (95% LoA ± 1.96 mm).

Target Registration Errors were below the threshold in 37% of the cases with OF-alone and in 70% of the cases after pre-processing with RDDR. For nonzero TREs (Figure 7.9c), OF alone yielded a median error of 4.9 mm (IQR 8 mm) and DRAM yielded a median error of 2.2 mm (IQR 2.1 mm).

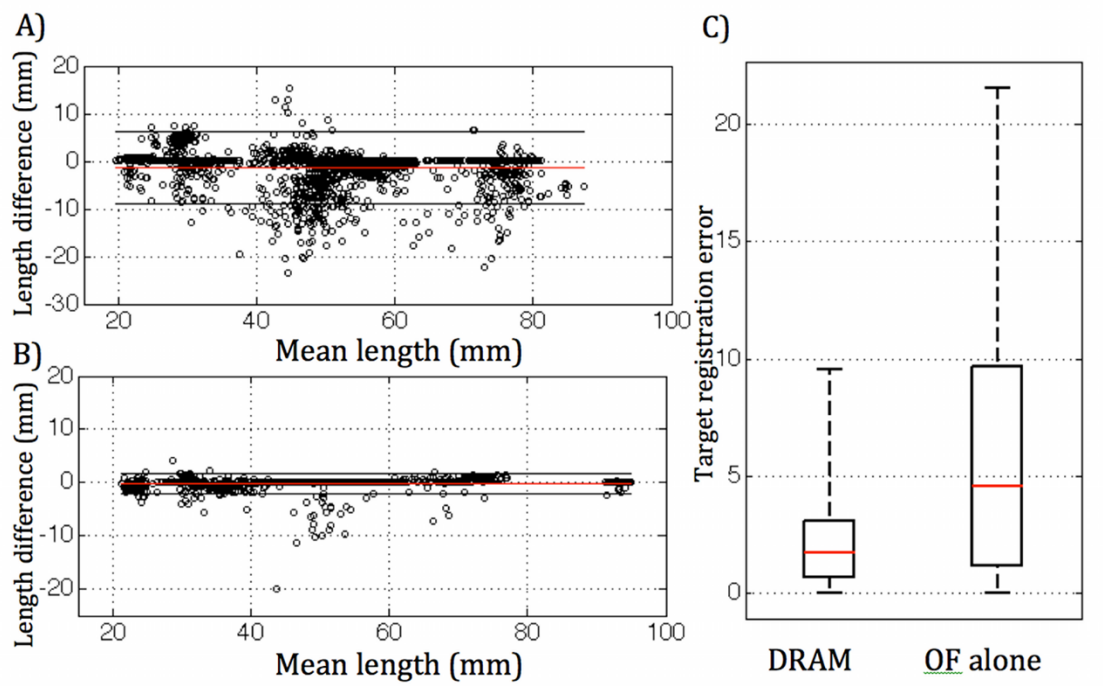

Figure 7.9. Bland Altman limits of agreement for line ROIs in colon data registered with OF against manually corrected ground truth (A) and data registered with DRAM against manually corrected ground truth (B). Target registration error in DRAM and OF alone (C).

An example plot of the line lengths through time, propagated by OF and DRAM, is shown in Figure 7.10.

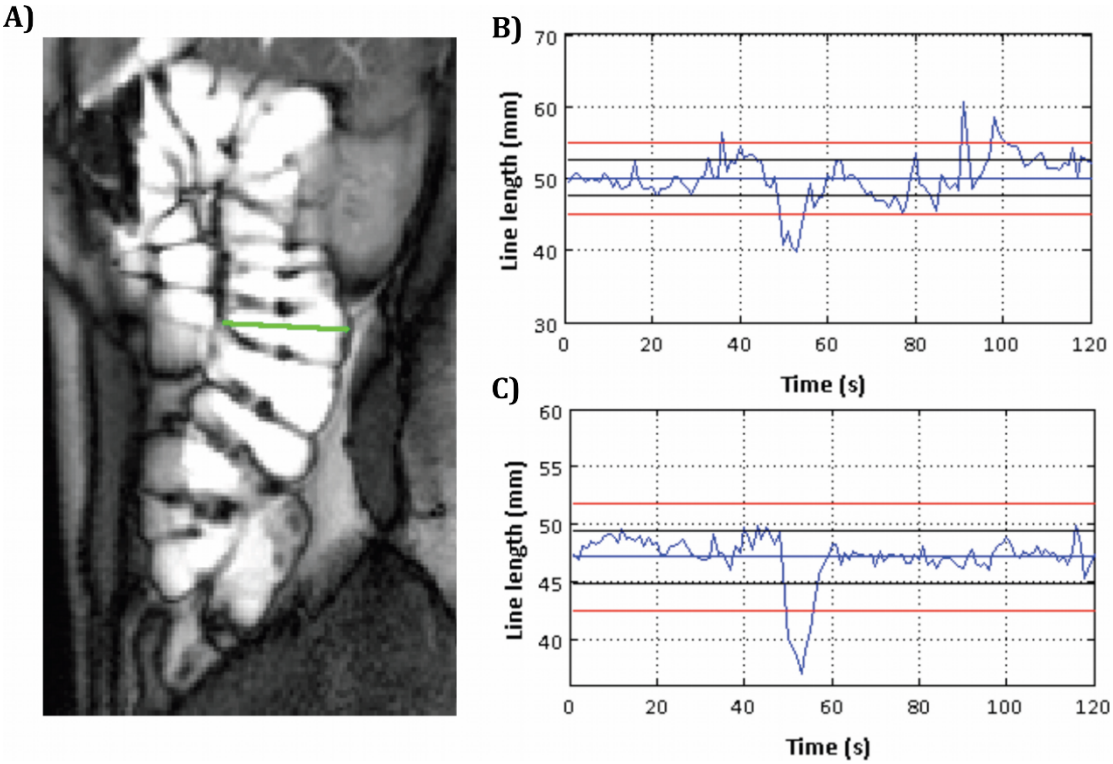

Figure 7.10 *Sagittal view of the ascending colon with line ROI across colon diameter (a). Line ROI propagated in data registered with OF (b) and DRAM (c). Both ROIs remained manually unadjusted. Blue line represents mean line length, black lines represent ±5% and red lines show ±10% of mean diameter.*

7.3.2 Validation of motility scoring

The mean global motility score within the manually placed ROIs for the BH data sets across the cohort was 0.340 (range 0.181 to 0.422). Mean global motility score for DRAM registered data was 0.335 (range 0.189 to 0.430) and OF alone free-

breathing data sets was 0.365 (range 0.268 to 0.458). Subjective visualisation of motility colormaps is shown in Figure 7.11 with data summarised in Figure 7.12.

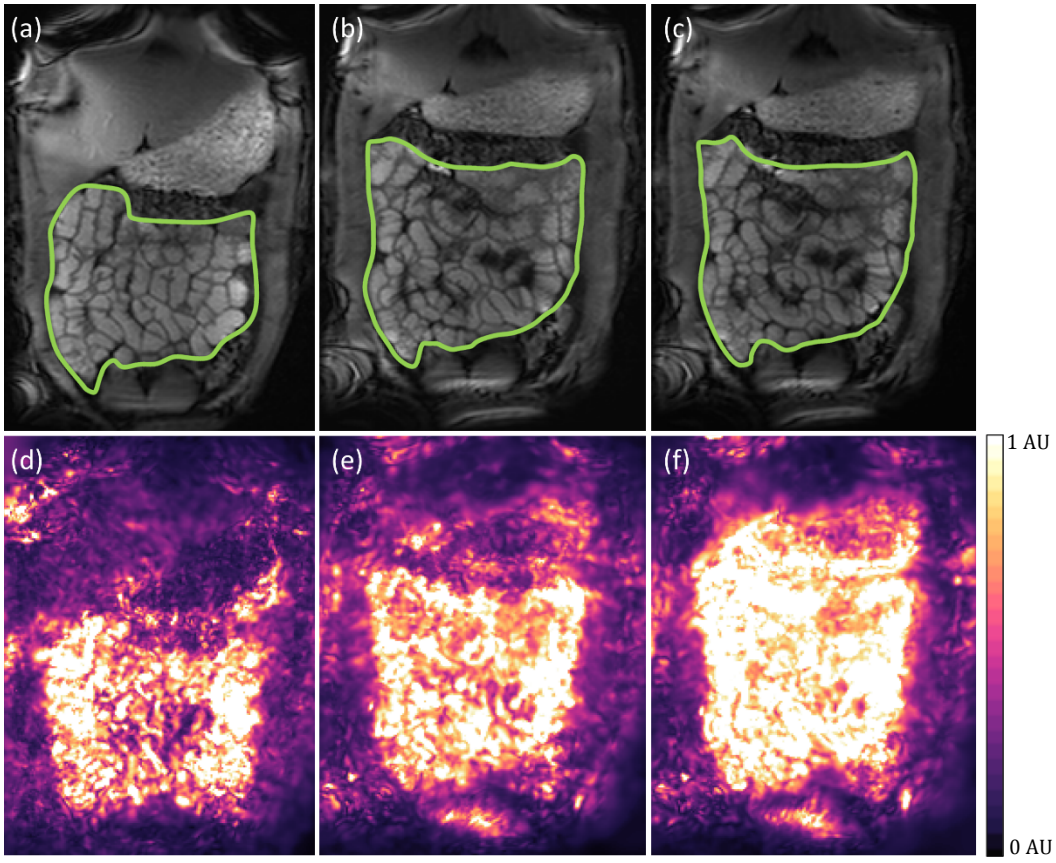

Figure 7.11 *Parametric motility maps in BH and FB data. AU denotes absolute units, normalised between 0 and 1. Example data with contoured small bowel region and motility maps for breath-hold ground truth (a, d), DRAM (FB) (b, e) and free breathing optical flow registration alone (c, f) respectively. Respiratory motion compensation is visible as reduced motility in the transverse colon closest to the diaphragm and systemically over the small bowel. The effect of RDDR is less apparent in the lower bowel further from the diaphragm where the effects of free breathing are less pronounced. Motility map shows black as lower motility and white as higher.*

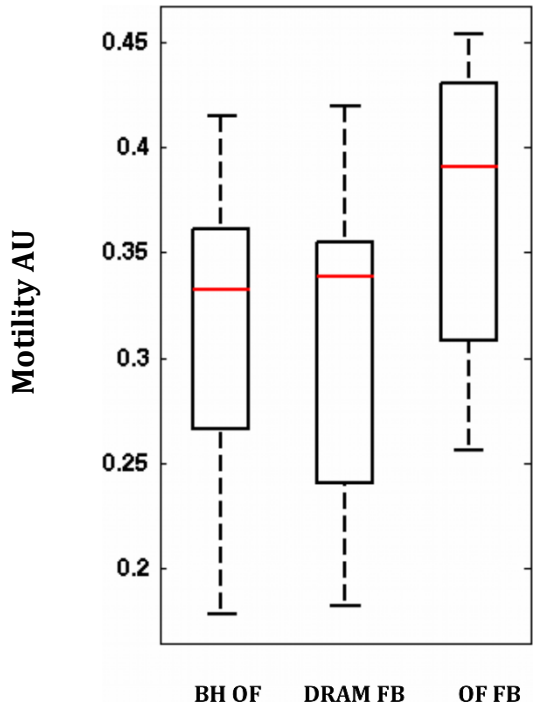

Figure 7.12 *Box plots for OF derived motility scores in the 20 subjects with range (dotted line), interquartile range (box) and median (red horizontal line) for Breath hold optic flow registered data (BH OF), free breathing DRAM (DRAM FB) and free breathing optic flow alone (OF FB) registered data.*

7.4 Discussion

This chapter aimed to validate a two-stage technique that first corrects respiratory motion before applying an existing OF method to register local deformation generated by peristalsis. Such an approach would allow rapid and robust data analysis from longer datasets acquired in free breathing.

Within the scheme parameters were selected empirically once, with the same values used thereafter for all datasets providing results that appear generalisable to both the colon and small bowel. An iterative scheme was used within RDDR to remove the respiratory component of motion. However, there was the potential of losing physiological information related to peristalsis by over-estimating lambda in this iterative process. This risk was reduced by modifying the original version of the algorithm to impose a specific stopping criterion. To evaluate the effect of this, the motility metric was compared between the pseudo-ground truth breath-hold and the free breathing DRAM data over global small bowel ROIs. The analysis demonstrated comparable results using free breathing DRAM data and the pseudo-ground truth of the BH. Specifically the breath-hold OF registration gave comparable global scores to DRAM and a positive bias in OF-alone registered global motility scores in free breathing datasets was observed. This supports the conclusion that DRAM removes respiratory motion whilst leaving peristaltic motion largely intact. The breath-hold data was not a perfect ground truth as the data was temporally separated from the subsequent free breathing data collection. However the 30s time difference from the commencement of the breath-hold to the

commencement of the free-breathing series is unlikely to impact significantly on bowel motion especially when assessed in a global manner.

The accuracy of the registration technique was assessed by comparing algorithm-propagated ROIs through the time series data and comparing their size and position to a manually adjusted ground truth. Assessment of the DRAM corrected data demonstrated greater registration accuracy with a mean error comparable to previous values from breath-hold data by Odille and Bikelhaupt [57], [59]. The DRAM data did however show a slightly larger variance in the BA LoA when compared to the original Odille data using breath-hold data. This is likely due to several factors, principally the choice of ROI position which in the current study was the upper left quadrant (i.e. proximal bowel close to the diaphragm) with the specific intention of challenging the capabilities of the respective algorithms with the effects of respiration. Assessment of the displacement distance of the adjusted ROIs compared to the manual gold standard was performed to identify ROIs that may have been mis-registered to adjacent bowel loops. This comparison is a good test for registration, as it is based directly on displacements reflecting registration accuracy and has not previously been performed in other small bowel motility validation studies. On average, less manual correction was necessary in the DRAM data and when ROIs were adjusted. The median distance and variance was several times lower than that without RDDR pre-processing. By collectively assessing these two components of registration fidelity in a challenging region of bowel, both DRAM and OF algorithms were subjected to a robust test and in both cases DRAM was found to perform better in comparison to the ground truth and comparable to existing literature values derived using BH OF.

An important component of the investigation was the application of the methodology to colonic data sets. Physiologically, the colon is quite different to the small bowel with a less frequent contraction rate [131]. Using the same parameters employed for the small bowel registration the data demonstrated again that DRAM performed well, largely correcting the effects of free breathing and permitting the accurate registration of colon wall deformation. Automated propagation of a linear ROI through the time series was possible with seemingly accurate assessment of contraction compared to our ground truth with manual adjustment. Due to the slow contraction rate of the colon, this increase in registration fidelity is important, as manual measurements are exceptionally time consuming and not practicable in clinical practice. In this study the technique was applied to two colonic regions per data set in a total of nine data sets, which is relatively small. Furthermore, with only two contractions expected to occur over the two minute scan, future work might extend the data acquisition time to around 10 minutes to more fully explore colonic physiology with MR. However the aim in this preliminary work was to demonstrate the broad applicability of the unaltered DRAM technique to the colon, an organ with different physiological characteristics to the small bowel and using data acquired at a different MRI field strength and over a different number of time frames.

Summary

This chapter validated a new post-processing methodology for extracting quantitative metrics to assess small bowel and colonic motility during free-breathing. Improvement was demonstrated both in segmental and global analyses when using DRAM that has gone on to be used for evaluation of data at the University of Nottingham and largely broadens the applicability of optic flow to other areas of the GI tract.

CHAPTER 8: GLOBAL SMALL BOWEL MOTILITY IN CHRONIC INTESTINAL PSEUDO-OBSTRUCTION ASSESSED USING MRE AND IMAGE POST-PROCESSING

Research Question:

Can global small bowel motility analysis be used to quantitatively demonstrate physiological differences between healthy controls and Chronic Intestinal Pseudo Obstruction?

Rationale:

CIPO patients are broadly acknowledged as having altered, decreased bowel motility and a number of research studies have found neuropathic and myopathic origins for this disease that both result in decreased intestinal motility. By quantifying altered motility in this cohort against controls, this study aims to provide additional confirmatory evidence for its sensitivity and more broadly applicability in a clinical cohort within the hospital setting.

Hypothesis:

CIPO patients will have a lower motility score at baseline compared to controls and an altered response to neostigmine.

Aim(s):

i) Compare baseline motility in CIPO and controls.

ii) Compare CIPO and control response to IV neostigmine versus placebo.

Author declaration

The work presented in this study was led by the author who obtained the ethics at both UCLH and QML for recruitment, collected and analysed data. Dr Shamaila Butt was responsible for patient recruitment at UCLH and administration of Neostigmine. The study was supervised by Dr Anton Emmanuel and Prof. Stuart Taylor. This work has not yet been published although preliminary work has been presented at: Menys, A., Butt S., Plumb., A., Atkinson D., Emmanuel A., Taylor S. (2014) Quantitative assessment of global small bowel motility in Chronic Intestinal Pseudo-Obstruction and controls: A Preliminary Study. 2114, ISMRM.

8.1 Introduction

Chronic Intestinal Pseudo-Obstruction is a rare but severe disease characterised by the failure of the intestinal tract to propel its contents, clinically mimicking mechanical intestinal obstruction (Figure 8.1). CIPO represents an important cause of intestinal failure in both paediatric (15%) and adult (20%) patients. Sufferers typically have a marked, chronic decrease in the quality of life through disabling digestive symptoms (nausea, diarrhoea, pain etc.) and malnutrition through loss of functional capacity[24], [132]–[134]. Therapy is difficult and often provides unsatisfactory results. Patients invariably require nutritional support, pharmacological treatment targeted at symptom relief and in some cases undergo multiple ineffective surgical interventions for misdiagnosed mechanical obstruction.

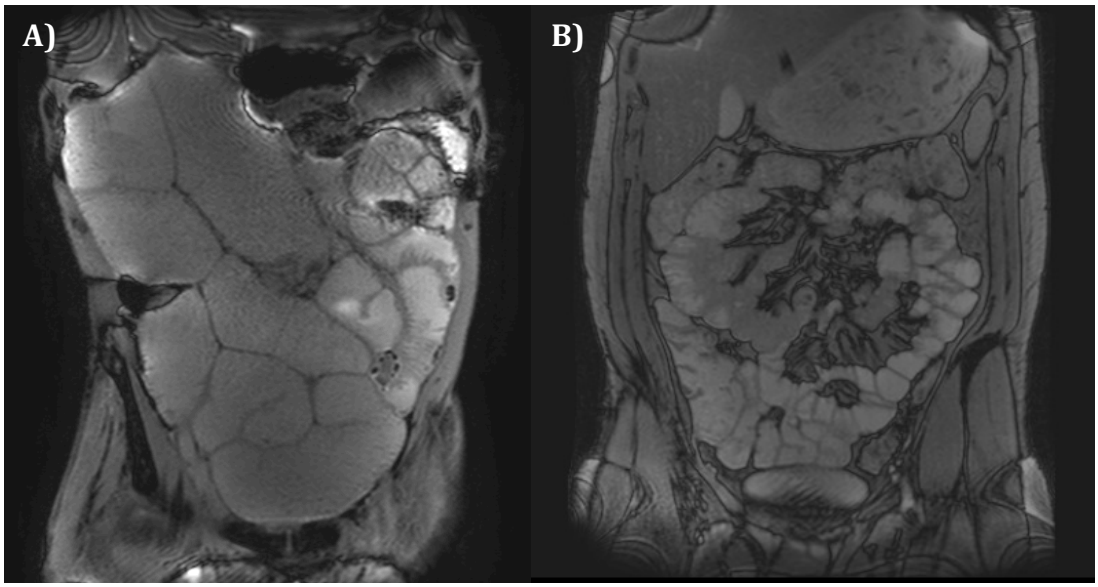

Figure 8.1 *An example BTFE coronal slice from a CIPO (A) and healthy (B) subject. Note the extensive bowel loop dilatation in A.*

CIPO can be primary or arise secondary to a range of diseases that affect the intrinsic and extrinsic GI tract nerve supply; some may be central (Parkinson's), peripheral (diabetic neuropathy, scleroderma) or the result of an infective episode (Chagas disease) summarised in Table 8.1. Primary forms of the disease are poorly understood although a number of gene loci have been identified implicating connective tissue deregulation[135], [136]. The secondary, myopathic classification of the disease is generally considered more insidious and confirmable only through biopsy of the affected bowel. A controlled multi-centre study suggested an alpha-actin deficit in the smooth muscle of the bowel might be responsible for the loss of function [23]. Myopathic CIPO generally attracts a worsened prognosis due to a lack of therapeutic targets[24].

Underlying Disease classification	***Main causes***
Diseases of the central, autonomic and enteric nervous system	Stroke, Parkinson's, encephalitis, calcification of basal ganglia, orthostatic hypotension, Von Recklinghausen, Hirschsprungs
Immune-mediated and collagen disease	Scleroderma, amyloidosis Ehlers-Danlos, LES, Malignancy
Endocrine and metabolic diseases	Diabetes, hypothyroidism, hypoparathyroidism, pheochromocytoma
Other	Radiation enteritis, clonidine, phenothiazines, antidepressants, antiparkinsonians, chemotherapy, bronchodilators, anthraquinones, jejunal diverticulosis, Chagas disease.

Table 8.1 *Main causes of secondary CIPO adapted from Antonucci et al. 2008.*

CIPO can be diagnosed through evaluation of a plain film x-ray where the dilated bowel loops with a high volume of gas are often self-evident. Cross sectional imaging using MRI and CT allows more thorough evaluation of the bowel wall and the exclusion of mechanical obstruction as a cause of the disease, [133]. Beyond the marked increase in luminal diameter, the bowel wall appears essentially normal and anatomical imaging is less informative in borderline or transient cases. In addition to dilatation, decreased motility is recognised as one of the cardinal changes present in CIPO with numerous studies conducted using manometry, reporting changes in the coordination and amplitude of bowel contraction in both neuro- and myopathic forms of the disease[132], [137]–[139].

The purpose of this study is to apply the global motility analysis technique, validated in chapter 6, to a CIPO cohort to demonstrate a difference in motility between healthy controls and the cohort of patients. In preparation, a detailed

discussion took place around disorders of dysmotility with gastroenterologists specialising in neurogastroenterology and reviewed the literature for quantitative information pertaining to low motility in disease. A range of candidate conditions including general 'dysmotility,' IBS-C, Parkinson's, amyloidosis, diabetes and all featured as candidate conditions for the first proof of principal study based on speculated dysmotility as assessed by transit studies or other indirect measures. However none appeared to have the same body of evidence that exists for CIPO.

This study hypothesises that CIPO patients display reduced bowel motility compared to that of controls and is accepted *a priori* that quantitative demonstration of this will not represent a novel finding. It will however demonstrate a clinical application of the MRI technique within the hospital setting and provide important confirmatory evidence for the MRI motility assessment paradigm. Of a more broad scientific interest is the administration of 0.5mg neostigmine against placebo control to investigate the effects of an anticholinesterase inhibitor on motility. Neostigmine is well recognised to relieve patient symptoms in CIPO, likely due to its direct action on the gut smooth muscle and it is further hypothesised here that a response will be seen in cases of neuropathic CIPO but that the response may be different to that of healthy controls [25]–[27].

8.2 Methods

8.2.1 Summary of Study design

This prospective study took the form of patient and reader blinded placebo-controlled cross-over study in both CIPO patients and healthy controls. This study investigated small bowel motility differences at baseline between CIPO and controls and in response to 0.5mg IV neostigmine. Drug placebo was administered according to a randomisation block generated by the author.

8.2.2 Healthy control selection

The same 11 healthy subjects who received neostigmine/placebo previously described in chapter 6.2.3 were used again in this study.

8.2.3 CIPO patient selection

7 CIPO patients (4 male, mean age 57, range 35 to 90) were identified through a specialist GI clinic. All patients had a prior diagnosis of CIPO and visibly dilated small bowel loops consistent with CIPO. Full patient demographics are presented in table 8.2 – In summary, four of the cohort had a primary diagnosis of CIPO, two of the cohort were receiving nutritional support through TPN. Two patients had a stoma and two had undergone previous surgery. All patients stopped taking any medication that might influence motility including antiemetics, opioids, anti-diarrhoeals, SSRIs for example, for one week prior to their scan.

Ref	*Age*	*Sex*	*Time between scans (days)*	*Underlying diagnosis*	*Treatment*	*Primary/ secondary*	*TPN*	*Surgery*
CIPO-01	70	F	2	Scleroderma	prokinetiks/ antibiotics	Secondary (Scleroderma)	N	N
CIPO-02	35	M	2	Dysmotility	prokinetics	Primary	N	Y (Colonic)
CIPO-03	54	M	19	Scleroderma	Cardiac for AF, antibiotics	Secondary (Scleroderma)	Y	N
CIPO-04	68	F	19	Dysmotility	antibiotics	Primary (Constipation)	N	N
CIPO-05	90	M	8	Visceral myopathy,	omeprazole, losartan	Primary	N	N
CIPO-06	48	F	19	Scleroderma	antibiotics,	Secondary (Scleroderma)	Y	N
CIPO-07	36	M	7	Dysmotility	motility	Primary	N	Y (Colonic)

Table 8.2 *CIPO patient demographics*

8.2.4 MRI Protocol

The previous cohort of healthy subjects used in the neostigmine arm of the validation study described in 6.2.5 are again used here as controls. The CIPO patients underwent the exact same preparation. Two of the CIPO participants were unable to finish the mannitol drink and the total volume consumed was recorded and the same volume presented to them at follow up to ensure comparability. All CIPO patients abstained from motility influencing medication for 1 week prior to their first scan appointment.

8.2.5 Drug Administration

Neostimgine was administered as previously described in chapter 6.2.5. Each subject then re-attended for a second MRI scan after a mean gap of 3 weeks (range 2 days to 7 weeks), no individual's scan start time varied by more than 1h between the two visits. Subjects were prepared and examined in exactly the same way as previously except that the IV injection was crossed-over so that placebo was given when they had previously received the drug and vice-versa. For the two patients who could not complete the whole mannitol volume the first time, the same volume as preciously consumed was provided at the second visit.

8.2.6 ROI placement

The author randomised the presentation of the data sets to the reader. A single consultant radiologist (Dr. A Plumb, 6 years experience) placed ROIs around the small bowel in each coronal slice where it was present as per figure 6.1.

8.2.8 Statistical Analysis

Normality was assessed in all data series using Shapiro-Wilk testing (alpha 0.05).

1. Baseline variation in global small bowel motility was assessed in CIPO and healthy controls using Bland-Altman Limits of agreement.
2. The mean baseline global motility score for each study participant was calculated and the CIPO and control group compared using T-testing.

3. The difference between neostigmine and placebo induced motility was calculated and tested for significance across both cohorts using paired T-testing.
4. The percentage difference neostigmine and placebo induced motility from their respective baseline was calculated and compared across the cohorts ie. Neostigmine (CIPO) versus Neostigmine (Control) and Placebo (CIPO) versus Placebo (Control) and tested using T-test.

8.3 Results

8.3.1 Baseline variation

Across the CIPO cohort, mean baseline motility at the first attendance was 0.2AU (range 0.12 to 0.3) and at the second was 0.21 (range 0.16 to 0.29). The mean difference between scans was -0.019AU with BA limits of agreement at ±0.061AU (figure 8.2A). In the control cohort mean baseline motility at the first attendance was 0.34 (range 0.275 to 0.37) and with a mean score at the second of 0.34 (range 0.315 to 0.38). The mean difference between the cohort was 0 units with BA limits of agreement at ±0.042 (figure 8.2B).

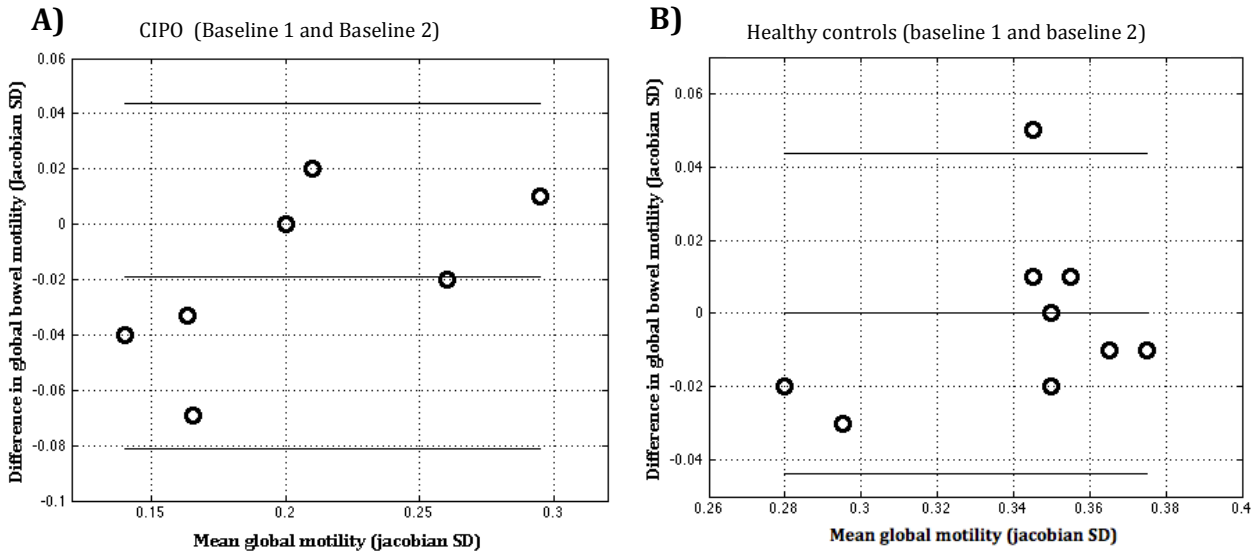

Figure 8.2 *Bland Altman Limits of Agreement for CIPO patients (A) and healthy controls (B) of global small bowel motility between visits.*

8.3.2 Difference in baseline motility between groups

The median baseline global motility score across the two scan visits for the CIPO cohort was 0.2AU (range 0.14 to 0.295) and for the control subjects it was 0.34 (range 0.28 to 0.375) (Figure 8.3). There was a statistically significant mean difference between groups of 0.14AU, P = <0.001 (CI 0.09 to 0.18). The two lowest scores in the CIPO cohort were attracted by patients with an underlying diagnosis of scleroderma.

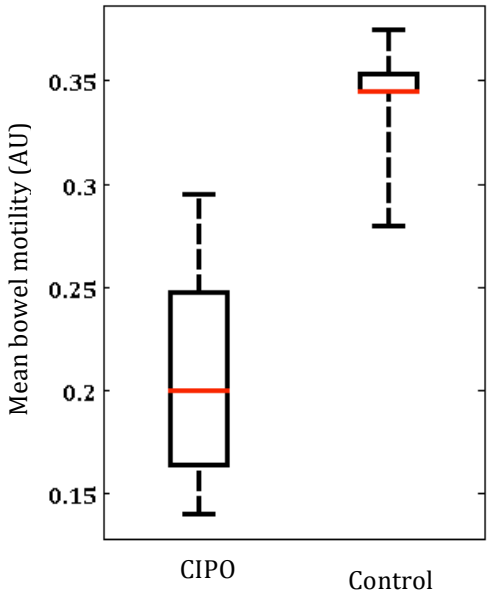

Figure 8.3 *Mean baseline global motility scores across the two visits for CIPO and control subjects.*

8.3.3 Response to neostigmine

Each subject's response to neostigmine was assessed first against placebo. In the CIPO group, mean global motility score following neostigmine was 0.27AU (range

0.16 to 0.42) and placebo was 0.20AU (range 0.14 to 0.31). There was a statistically significant mean difference of 0.07AU between neostigmine and placebo, P = 0.013 (CI 0.02 to 0.11) (Figure 8.4A).

In the healthy controls, mean global motility following neostigmine was 0.39AU (range 0.32 to 0.44) and placebo was 0.32AU (range 0.25 and 0.35). As reported in chapter 6 section 5.2.3, there was a statistically significant mean difference between mean motility after neostigmine and placebo of 0.07AU, P = 0.005 (CI = 0.038 to 0.100) (Figure 8.4B).

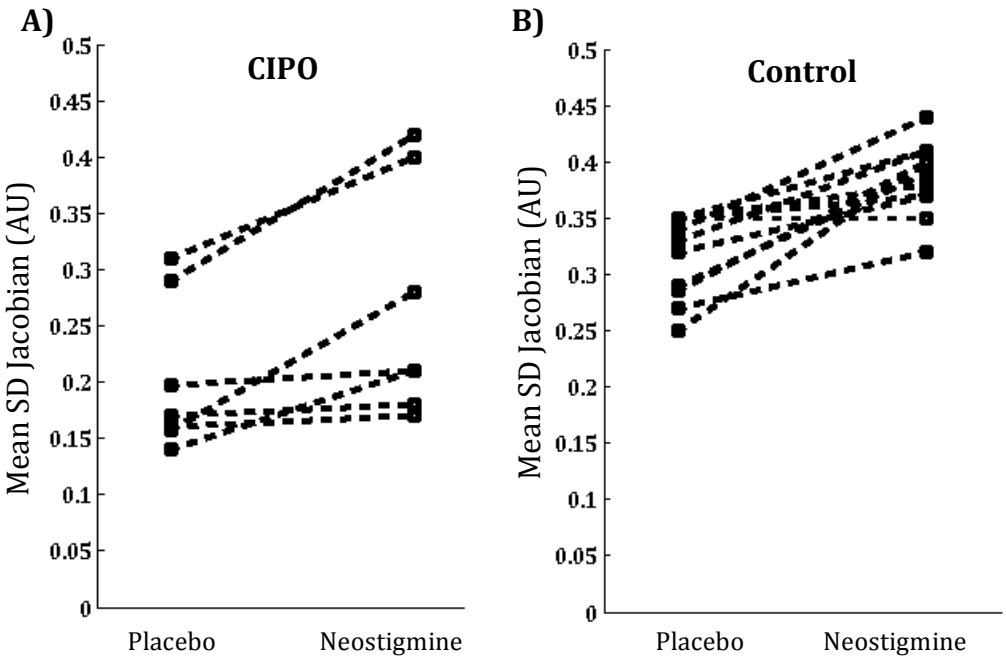

Figure 8.4 *Placebo versus Neostigmine line plots for CIPO patients (A) and controls (B).*

8.3.4 Relative response to Neostigmine challenge

The relative percentage change in motility following neostigmine and placebo from the respective baseline is presented in Figure 8.5. This additional comparison was performed to explore differences in motility response in CIPO and controls. Such subtle response variation could be potentially concealed by large baseline variability across the two time points.

Comparison of percentage change in motility following neostigmine/placebo against baseline demonstrated a significant difference between CIPO (mean percent increase 38 percent, (range 4.5 to 60) and controls (mean percent increase 13.5, range 0 to 39) of 24.6 percent, P = 0.0015 (CI 10.9 to 38.2). There was however no statistically significant difference in response compared to baseline following administration of placebo (P = 0.89).

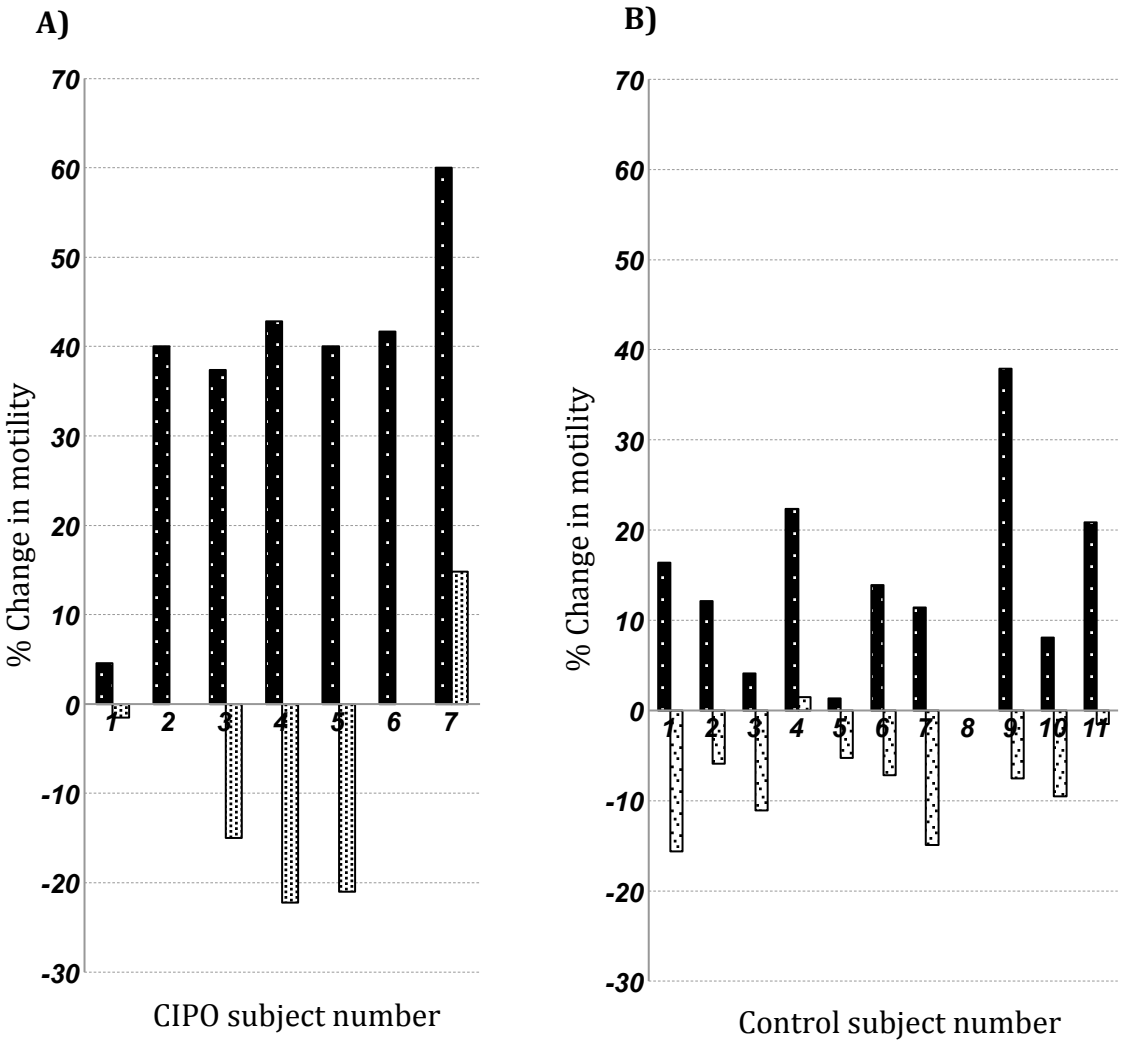

Figure 8.5 *The percentage change from the respective baseline for neostigmine (black dotted) and placebo (white dotted) for CIPO (A) and control (B).*

8.4 Discussion

Data collected for this chapter confirms proof of concept for motility analysis using image registration in a clinical cohort demonstrating a difference both in baseline motility against controls and also response to the pro-kinetic agent neostigmine.

In chapter 6, good repeatability between scans was demonstrated for healthy, well controlled volunteers. In this study, the data again showed reasonable repeatability although the LoA were larger at ±0.061 in the CIPO cohort compared to the controls where the LoA ±0.042. The LoA were partly exaggerated by the small number of subjects but nevertheless, it was perhaps unexpected that this variability should be higher as each patient had established chronic disease and the time interval between scan dates was small. Although the CIPO cohort was well controlled in terms of medication and preparation it is likely that the underlying disease could lead to more irregular bowel contractility and indeed these patients anecdotally reported a large variation in the bowel symptoms. Two of the patients struggled to consume the entirety of the 1L Manitol solution which may have influenced bowel motility. However, the volume consumed in the follow up visit was carefully matched and their motility scores did not appear to be outliers.

It is broadly understood that small bowel motility in CIPO is reduced and this was investigated here by comparing the average small bowel score for each visit in the CIPO and control cohorts [24], [132]. There was a large, statistically significant difference between group means with only a marginal overlap between cohorts

confirming the hypothesis that motility is lower in this disease. This result further agrees with other research using segmental methods of small bowel motility quantification of MRI published recently by Ohkubo et al [76]. Interestingly, there was however higher variance in motility across the CIPO cohort with the three patients who had an underlying diagnosis of scleroderma attracting the lowest motility scores.

As presented in chapter 6.3.3, a statistically significant increase in motility was observed in healthy controls following neostigmine compared to placebo. This study demonstrates that a significant difference is also observable in the CIPO cohort in response to the same dose of neostigmine, although again a larger variance was observed in the response with three patients in particular (two of whom had scleroderma) demonstrating only a marginal increase in motility. Due to the relatively high variability in baseline motility seen in 7.3.1 it was decided to further investigate the relative response to neostigmine and placebo. The reason being that where a patient recorded a baseline motility for scan 1 of 0.32AU followed by 0.35 following neostigmine and then a baseline score at scan 2 of 0.37 and placebo change at 0.36 – the large difference in baseline would conceal the proceeding positive and negative changes from neostigmine and placebo. By examining the percentage change from baseline, it was possible to remove some of the potential bias introduced by physiological resting variability. In both the CIPO and control cohorts a positive response to neostigmine could be observed which was in fact significantly greater in the CIPO cohort. There was no significant difference between cohorts following placebo challenge supporting this observation as a real phenomenon. A response to neostigmine raises an interesting clinical consideration

in that it signifies that, in-spite of an overall decreases motility, the potential for contractile actions is still present. Neostigmine is a powerful acetycolinesterase inhibitor that acts at the level of the smooth muscle and is a well established method for symptom relief [25]–[27]. One might reasonably hypothesise that none of the above cohort have a myopathic form of the disease.

One of the main limitations of this study is the relatively small number of controls and in particular CIPO patients. Further complicating this problem is that CIPO is often a secondary process to a range of other diseases. Three of our cohort had scleroderma with the remaining 4 having a range of poorly characterised dysmotilities precipitating their CIPO diagnosis. The heterogeneity of the cohort makes the findings difficult to generalise, although valuable for hypothesis generation. One of the main challenges faced in this study was recruitment where even at a leading neurophysiology centre, the number of patients with CIPO and eligible for study participation, were low (1 patient per 3 months). An interesting addition to this or future studies would be biopsy information on the myopathic versus neuropathic forms of CIPO where one might anticipate an altered response to neostigmine or other pro-kinetic agents. A further limitation is the difference in age between controls (who were mainly young) and patients whose ages ranged between 35 and 90. Many factors that are believed to influence motility were well controlled including time of day, bowel preparation, fasting, caffeine intake etc. In particular patients temporarily stopped motility altering medications (including painkillers, laxatives, SSRIs etc) 7 days before their scan that was greater than any of the medication half-lives. In an attempt to minimise the patient time off medication the minimum follow-up time between scans was decreased to 2 days. All

of the healthy controls had a minimum delay between scans of 2 weeks and the difference in time between scans might impact on baseline repeatability. However, as the healthy subjects displayed a lower intra-scan variability this was unlikely to be of importance in this study.

The main purpose of this investigation was to demonstrate proof of concept for an MRI with post-processing technique to evaluate motility in a clinical cohort. Although feasibility was demonstrated in healthy controls, this is the first time that a prospective investigation has been carried out in a patient group using this technique. CIPO were used as a target group for this study as they are widely recognised by the neurogastroenterology community as having low motility with a visible and clearly identifiable phenotype characterised by the bowel dilatation[24], [133]. The mean baseline motility of the CIPO cohort was greater than that of the healthy controls presented in chapter 6.3.4 who received Buscopan (0.13AU). This suggests that even though CIPO baseline motility was low, it was still higher than healthy controls receiving a spasmolytic. This result is interesting in that it highlights the potential value of this technique in quantify relatively small differences in motility. One might imagine an application for assessing the efficacy of a drug that acts on the smooth muscle might help differentiate between the forms of the disease potentially avoiding the need for biopsy. Whether this is useful in the diagnosis and management of CIPO remains the subject of future work. Conversely, the technique might be extremely useful for other diseases of suspected dysmotility including IBS of whom a subselection may have a genuine, mechanical dysmotile component. From a practical perspective, all patients conformed to the protocol which took 30min of scanner time (half that of a routine MRE investigation as well

as being non-invasive and widely available and compatible with most clinical MRI scanners). No adverse events were encountered in response to the administration of neostigmine.

Not addressed in this study is assessment of the free-breathing data or colonic motility. Chapter 7 introduced the DRAM technique to evaluate longer time series. However it was not used here for reasons of consistency with normal control data presented in chapter 5. It will be interesting to see whether intra-scan variability decreases when extended time series are used. Due to its superficial, distal location, the colon has been more thoroughly investigated in terms of motility in CIPO and again, using DRAM, there is the potential to perform detailed assessment of the colon in addition to small bowel.

Summary

This chapter has explored the clinical applicability of small bowel motility assessment with MRI. A number of new projects and research questions exist from these data alone and over the course of this PhD, a number of exciting new opportunities for in vivo motility assessment have arisen.

SECTION E: CONCLUSIONS, FUTURE WORK AND EXPLOITATION

Section E concludes this thesis with Chapter 9 summarising the key findings providing a more personal reflection on the results and their broader interpretation within the context of the recent literature. In the futures section of this chapter, some of the important but less tangible challenges emerging from the work will be discussed, specifically regarding how the validated technology might be used to further research outside of the Centre for Medical Imaging and ultimately translate into the clinic.

By way of a follow on to the core themes of Chapter 9, Appendix 4 deviates from the science altogether and focuses on the business and commercialisation potential of the research. This section may be of interest to readers with a commercial background, those looking to develop their technology beyond the research setting or perhaps those yet to start a translational project, who are trying to better determine their research question.

CHAPTER 9: DISCUSSION OF RESULTS AND FUTURE PERSPECTIVES

Discussion of results

In this final section I conclude the thesis by discussing the research questions asked at the beginning of each chapter and presenting my perspective of the future of this research. I will also move into the first person to hopefully inject some interest into what is a somewhat subjective reflection of the key findings emerging from this project. Each of the following sections is presented as the original research question running from chapters 2 to 8 followed by a response written within the context of the thesis findings as a whole.

Can image post-processing be used to objectively evaluate MRE derived small bowel motility data?

In this Chapter, I detailed the validation process for the registration algorithm developed by Freddy Odille to analyse dynamic time-series MRI data. For a long time, image registration has been an extremely popular topic of research in medical imaging with numerous applications in oncology and neurology, for example measuring the change in size of various structures. Application to the bowel was, however, novel and an excellent demonstration of collaborative research, matching a clinical problem with a latent technical solution. The opportunity was also timely given the increasing implementation of Magnetic Resonance Enterography at UCLH as a routine diagnostic technique for evaluating Crohn's disease, thereby providing a

large amount of data including dynamic motility series. Chapter 1 provided the background to the thesis and stressed the major opportunities to researchers in this field. In Chapter 2 my validation experiments demonstrated that the algorithm could accurately propagate a user placed ROI through a time series, demonstrating that the local deformation caused by bowel wall movement was corrected well. This allowed a significant practical step in terms of ROI propagation (e.g. minimising the labour intensity of plotting of lumen diameter over time) but also a new possibility in the form of surrogate measures for motility based on the deformation fields themselves. This concept indeed proved to be a powerful measure to summarise motility both segmentally and ultimately globally, being sensitive to pharmacologically induced changes in motility and demonstrating good intra-subject repeatability. An ongoing challenge has however been the meaning of the selected metric, the standard deviation of the Jacobian determinant and other surrogate measures of motility. This has been especially pertinent when presenting research data to clinically focused audiences. The notion of deformation fields quantified in this way seems detached from the underlying physiological processes and therefore troubling to many interested in the nature of the physiological action. Potential solutions exist in the form of providing entirely new metrics, for example pixel movement in space (mm) per unit of time (min) which would perhaps more directly present the data from the registration process and be broadly transferable should new registration schema be presented. As was demonstrated in Chapter 2, several other candidate metrics were developed alongside the SD Jacobian and hundreds more exist that might be of value. However, the SD Jacobian measure was used throughout this PhD first because it correlated best against radiologist grading and secondly for the boarder reason establishing a consistent, if somewhat abstract,

measurement in the published literature. A temptation is to measure an experimental finding using a range of motility metrics to see which produces the best result against the hypothesis. Indeed we have seen this in other studies, described in chapter 1, where contraction rate, max diameter and diameter standard deviation are all used to measure bowel motility in the same paper. The implication of using multiple measures being a higher chance of a type 1 (false positive) error being observed and published where a spurious correlation or result is found. It has been my goal to provide consistency and transparency in my results and this has prohibited my developing and implementing additional measures of motility. In future work, I hope to go back and re-validate a range of metrics based on feedback from the community and a better understanding of GI physiology however such research was outside the scope of this PhD thesis.

What is the relationship between small bowel motility and inflammatory activity at the terminal ileum in Crohn's disease patients?

Bowel wall thickness has long been associated with inflammatory activity in CD and it remains intuitive that as the bowel becomes thickened, the ability of the bowel to contract decreases. The hypothesis in chapter 3 may appear in retrospect relatively obvious. However it was unclear if there was any relationship between actual inflammatory activity and motility, not just with wall thickness per se. Furthermore at the time, only one small, qualitative paper using motility to assist with lesion detection in CD had been published[14]. Within the broader context of the field, new

biomarkers for inflammation including diffusion, enhancement, magnetization transfer etc., were all being explored to try and more accurately categorise disease using MRE [104], [107], [109], [140]–[142]. Quantified motility was therefore an obvious target to explore with the validated technique. Motility was in fact negatively correlated with inflammatory activity and, based on the regression analysis presented, performed approximately as well as the other MR based features. One interesting observation from our data was the large number of non-inflamed subjects with "low" motility. Following the publication of this study and, while presenting the results at conferences, many postulated or at least found it feasible that the motility was influenced by humoral factors mediated by inflammation[110]–[113], [121][16], [121][16], [121][16], [121][16], [120][16], [119][16], [118][16],. That is to say, as yet unknown factors were introducing high variation into histopathologically un-inflamed subjects to indirectly suppress motility. At this time, there were no reference ranges for MRE assessed healthy small bowel motility so it was difficult to comment either way.

How do Crohn's disease strictures influence small bowel motility?

Chapter 4 investigated the impact of stricturing CD on small bowel motility, and this time I expanded the analysis to the stricture itself, the upstream dilation and a morphologically and functionally normal reference loop of bowel. Robust differences were demonstrated between the thickened bowel at the stricture and the normal bowel confirming the observations described in chapter 3 – thickened bowel does not peristalse. Less variation was seen this time in the motility of the normal reference loops, likely because we specifically selected a bowel loop that

was visibility peristalsing. This study demonstrated that the dilated bowel appeared to lose its capacity to contract as its calibre increased. Interestingly however the data suggested that the phenomena might be reversible with time. Although retrospective, the large number of subjects in this study, together with consistency of the findings with previous animal data, helped demonstrate the potential of quantified motility analysis to be a useful surrogate of bowel dynamics in health and disease. Poor motility in dilated bowel upstream of a stricture is well recognised by radiologists and surgeons in clinical practice, yet the literature is surprisingly sparse of any direct support for the widely held notions regarding motility dynamics around a stricture[118]. It was therefore an altogether satisfying study to complete, not least on account of the huge amount of work Emma Helbren and I had to perform (examining virtually every MRE scan at UCLH over a three-year period) but quantitation was provided essentially for the first time to support a well described clinical phenomena.

What are the potential methodological issues arising from segmental (local bowel loop) ROI placement in small bowel motility analysis?

While the retrospective studies in Section B were conducted, I recruited and scanned a cohort of 20 healthy subjects with a view to developing a better understanding of normality of bowel motility. Chapter 5 was a descriptive study that presents in as unbiased-a-fashion as possible, healthy reference ranges using the two techniques commonly used to investigate motility, contractions per minute and our SD Jacobian metric[12], [16], [76]–[78]. A systematic approach was adopted to look at intra-scan variability (i.e. between bowel segments) and variability at two

time points in our healthy subjects across both breath-hold and free-breathing protocols. While this study is perhaps heavy on data, it is important for several reasons. First, it showed that intra-scan variability, that is variability within someone's own bowel segments, is surprisingly high and almost as high as the variability across the study population as a whole. The majority of the subjects had at least one 'hypo-motile' bowel loop, with motility comfortably within the range of the most 'inflamed subjects' identified in chapter 3, regardless of the metric used (also see Cullmann et al., 2013;). Second, the variability observed in inter-scan reproducibility of the metrics was even higher. Even where the TI was used as a landmark and relatively high certainty could be achieved in accurate ROI placement across the scans, variability was very high. In this study I endeavoured to make it a fair reflection of the methodologies used both by us and by the other groups and was somewhat surprised by just how variable the data was despite the stringent study control and also by the relatively poor correlation between the SD Jacobian and CPM metrics. Although the paper was eventually published in BJR, it was first rejected by Radiology with almost no criticism other than, it not being directly relevant to a general radiological journal and then by NGM where they felt that such heterogeneity was "obvious". Despite the latter comment, it had not prevented a rush of papers being published in 2013 using segmental methodologies to make assertions regarding motility using MRE based approaches and a collective lack of appreciation in the methodological pitfalls in this type of quantitative research[16], [76], [78], [121].

Can global small bowel assessment be used to provide robust, repeatable and sensitive measures of small bowel motility?

Global motility assessment based on the evaluation of SD Jacobian score averaged across the entire bowel was an significant underlying concept behind my PhD and thus data presented present in Chapter 6 was a major advance over segmental analysis. Many of the conditions that originally interested me in this form of research, including IBS, Parkinson's, Diabetes etc require a global method to evaluate motility. Despite severe gastrointestinal symptoms, there is often no causative lesion found and patients appear "radiologically normal". It is in this form of global assessment that the registration technique was able to truly demonstrate its value allowing largely unbiased evaluation of the whole abdominal gut as a system. The one problem from a scientific point of view was the lack of any kind of gold standard; manometry is invasive and provides segmental data only, whilst scintigraphy looks at transit, not bowel wall motion. I attempted to address the issue of technique validation by performing a blinded, placebo controlled cross-over study enabling the evaluation of both technique sensitivity to drugs with known pharmacological effects, and intra-subject repeatability. I demonstrated high inter-observer agreement between two radiologists who individually segmented the bowel for analysis.. Quantified motility also demonstrated good intra-subject repeatability over two time points, superior than that demonstrated using segmental analysis, likely helped by removing any variability in ROI placement and negating intersegment differences. Potentially the most interesting result arising from this study was the ability of the technique to detect a change in motility driven by a pharmacological stimulus[21], [27]. Neostigmine was capable of increasing motility and Buscopan conversely by decreasing motility against a placebo control in one of the first studies of its kind using MRI.

Can a pre-registration respiratory motion correction step be used to extend the application of the optic-flow technique to free breathing bowel motility data?

In Chapter 6 I helped develop and validate the DRAM technique to correct respiratory motion and allow motility quantitation in free breathing data using optic flow as described before. The basis for chapter 6 evolved through intra-departmental collaboration and partly through an emerging need from my collaborators outside of UCL looking to measure bowel wall motion in colonic data sets of 120+ images acquired using free-breathing protocols. I was also becoming increasingly interested in extended protocols designed for the small bowel to examine other types of contractile actions such as the colon that, again, would require extended free-breathing protocols. Registration using OF alone would result in reasonable motion correction especially in the pelvis where diaphragmatic movement was reduced but there was a large bias in the SD Jacobian score where respiratory motion was conflated with that of local deformation caused by the bowel. The RDDR pre-processing step was effective at removing the respiratory motion and the subsequent application of optic-flow resulted in good motion correction and similar results as those seen in chapter 2. The ability to automatically propagate ROIs through the time series did not end up featuring particularity prominently in this thesis, with a preference instead for the SD Jacboian measure. Conversely, for our collaborators at UoN, contraction measurement was one of the primary means of analysing motility and therefore this technique has become very popular in subsequent studies at that institution with over 300 data sets analysed to date.

Can global small bowel motility analysis be used to quantitatively demonstrate physiological differences between healthy controls and Chronic Intestinal Pseudo Obstruction?

Finally in Chapter 7 I explore the application of the global motility analysis technique to a cohort of CIPO patients. The data in this study demonstrated a clear difference in motility compared to healthy controls. The DRAM technique was not applied to this cohort for the sake of comparability with Chapter 6. At the time this chapter was written, only 7 patients had been scanned owing to the extraordinary difficulty in recruitment. One of the key concepts emerging from this thesis is the measurement of the bowel as a system and ability to monitor change induced in this case through the use of medication. Neostigmine in particular acts at the level of the smooth muscle in the GI tract and therefore has a fundamental effect on contraction. The ability to non-invasively probe the bowel opens up exciting opportunities with other pharmacological agents with a view to better exploring enteric pathology.

Future perspectives

A 'gut feeling' is an almost daily occurrence for most of us in some respect and a cross-cultural reference that demonstrates the intimate relationship we share with our viscera. The rich neurological input from our gut into our central nervous system modulates many aspects of our mood and general well being but remains poorly understood. Conditions like Irritable Bowel Syndrome affect as many as 1 in

10 people and radically decreases the quality of life for millions of people around the world, yet no test or biomarker exists outside of subjective and limited clinical scoring based on bowel habit. It may well be that aberrant motility is not responsible for the IBS phenotypes observed, but even if that is the case a test to rule true dysmotility in or out would benefit patients and healthcare providers alike. Few other systems remain as poorly understood as the gut in medical science and this is one of the primary driving factors influencing this PhD and the research themes discussed. This thesis presents some of the earliest validation into advanced motility assessment techniques that will hopefully go on to support further grants on the clinical practicality of the test, making the technique well poised to influence patient investigation. There are however two large limitations for progressing this research beyond the various smaller methodological points raised throughout this thesis.

The first concerns how the technique will be used in the clinic. With the limited data presented here, a gastroenterologist (the potential user) simply does not have enough information to use MRE motility analysis in a meaningful way clinically. Even viewing the raw cine images is insufficient to add any information to the clinical work up, especially where even healthy subjects appear to have completely static segments of bowel. Is this a case of the clinically useful information not being there or the observer simply not knowing what to look for? Until large-scale studies are performed in healthy controls and well characterised patient groups, we unfortunately will not be able to answer this question. Motility assessment has the potential to add value in the absence of structural abnormality (e.g. in autonomic or myopathic conditions affecting bowel). Ultimately the value will come down to not

just the identification of dysmotility but also the practical capacity to do something with that information. What makes this research exciting is that there are already an array of medications for motility that are currently used in a trial-and-error approach to treat a range of bowel conditions and thus, the techniques presented here will be able to fit into a clinical framework in some capacity -even if just to stop unnecessary prescription. The response to the technique has been positive with several grants written to date by other research groups outside of the Centre for Medical Imaging, using the techniques described here as the primary end-point in diseases ranging from obesity, hypermobility disorders, IBS and Crohn's. As the literature using the optic flow and DRAM techniques increases, there will theoretically be a broader move towards using these methods in trials, research and ultimately the clinic however this brings us to a second important point.

A second major limitation I have now encountered has been distribution of the motility analysis techniques. The implementation of the registration code was split across several computer languages and operating systems making distribution of the source code difficult. Beyond this, users did not want to have to invest in new computers to run the relatively demanding algorithms nor troubleshoot the innumerable problems emerging from prototyped code. As a short-term solution, I have been providing the registration result and returning key data as DICOM files (e.g. the SD Jacobian map or the key figures so that they might perform the further analysis in their DICOM viewer). A growing number of data sets have been now been processed from a range of institutions around the world. The demand for the techniques led me to founding the business Motilent Ltd. The goal of Motilent was to raise additional funding through the various investment opportunities for SMEs in

London to re-code all of the registration algorithms used and build, more formally, a product that might go on to not only serve as a research tool but also a clinical diagnostic tool. Funding could also be used to secure patent protection for emerging technology and establish a consultancy to drive technique use and development of the technique. In appendix 4 of this thesis I present a complete move away from the science all together and look at the commercial opportunities and route to market for a technique for motility evaluation to address the two big questions raised in this last section.

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APPENDIX 1: PUBLICATIONS, CONFERENCES AND FUNDING

A1.1 Publications

Menys, A., Hamy, V., Makanyanga, J., Hoad, C., Gowland, P., Odille, F., … Atkinson, D. (2014). Dual registration of abdominal motion for motility assessment in free-breathing data sets acquired using dynamic MRI. Physics in Medicine and Biology, 59(16), 4603–19. doi:10.1088/0031-9155/59/16/4603

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G. Bhatnagar, Menys, A., Makanyanga, J., Pendse, D., Atkinson, D., Taylor, S. (2014). Changes in Global Small Bowel Motility in Response to Inflammation in Crohn's Disease. *ESGAR*

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Kamalanathan E. A., Menys A., G. Bhatnagar, S. Butt, N. Zarate, A. Emmanuel, D. Atkinson, S. Taylor; (2014) Quantitative assessment of global small bowel motility in chronic intestinal pseudo-obstruction and controls: a preliminary study ESGAR

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Dikaios, N., Tremoulheac, B., Menys, A., Arridge, S., Atkinson, D. (2014). Discrete shearlets as a sparsifying transform in a split Bregman reconstruction of low-rank plus sparse component from undersampled (k, t)-space small bowel data. *ISMRM*. (pp.4386-).

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A1.3 Grants & Funding

£10,000, (2014, 36 months) Bright Ideas award for business development. UCL Advances. Lead Applicant

£4000 (2014, 3 months) UCL Advances PhD Commercialisation plan. UCL Advances. Lead Applicant

£12532.50, (2014 -18 Months) Novel Bowel Motility Quantification with Magnetic Resonance Enterography. Enteric HTC. Lead Applicant

£4700, (2013 – 12 months) Quantified small bowel motility in Chronic Intestinal Pseudo-Obstruction. Radiological Research Trust. Lead Applicant

£5000, (2012 – 12 months) MR Quantified global small bowel motility analysis. Royal College of Radiologists – Kodak award. Co-Applicant

APPENDIX 2: POWER CALCULATION FOR GLOBAL MOTILITY ASSESSMENT

The technique for global motility assessment proposed in chapter 6 had no existing counterpart or gold-standard at the time of the study to which it could be compared. In the absence of literature values or external data to guide a power calculation a small cohort of Crohn's disease patients were used both to help identify a suitable sample size for the study and to demonstrate proof of principle to the Research Ethics committee. Selection of a suitable sample size is important both in terms of reducing the chances for adverse events (especially when administering drugs) and effectively managing the study cost. Two small studies are summarised here in abstract form detailing that helped to determine our study numbers.

A2.1 Baseline intra-subject repeatability

A2.1.1 Purpose

Determine mean baseline motility values and mean within-subject standard deviation for sample size-calculation within the context of estimating the accuracy of the Bland-Altman 95% Limits of Agreement with the SD Jacobian metric.

A2.2.1 Methods

Four patients (2 male) were identified from our institutional database who had received two scans over a two year period and had not undergone any surgery. In all cases, radiology was reported as 'unchanged' between scans. Patients had undergone institutional small bowel preparation as per chapter 2.2.4 with dynamic scanning. The entire small bowel was segmented and a global motility value calculated by averaging the mean SD Jacobian motility score. A sample size calculation was performed with a 90% power (possibility of correctly rejecting the null hypothesis) with an alpha of 0.05.

A2.3.1 Results

The mean global motility score for scan 1 was 0.29AU (range 0.25 to 0.34) and scan 2 was 0.27AU (range 0.24 to 29). The mean within-subject standard deviation was 10.04%. A sample size of 20 subjects based on these data would provide 95% BA LoA to within 7.5% of the true population value.

A2.4.1 Conclusion

20 subjects represented a reasonable compromise with negligible increase in accuracy up to 5% and 3% requiring 46 and 128 subjects respectively assuming the data is generalisable. These data were from a small, poorly characterised cohort of patients with small bowel disease and can therefore only serve to inform study design in a very general capacity.

A2.2 Subject response to drug administration

A2.1.2 Purpose

Determine the number of subjects necessary to demonstrate a significant change in motility following administration of IV drugs against placebo.

A2.2.2 Methods

Three patients (1 male) received an additional dynamic scan immediately after their IV Buscopan injection during routine clinical practice with the institutional MRE peroration as per chapter 2.2.4. The entire small bowel was segmented and a global motility value calculated by averaging the mean SD Jacobian motility score for both pre and post-Buscopan dynamic series. A sample size calculation was performed with a 90% power with an alpha of 0.05.

A2.3.2 Results

Mean global motility score pre-Buscopan was 0.26AU (range 0.24 to 0.31) and following Buscopan was 0.17AU (range 0.09 to 0.23). There was a mean difference of 0.09AU (35%) between groups. Based on these data, we would require a sample size of just 3 subjects to determine a significant difference. 10 subjects should have sufficient power to detect a difference of 10% between groups.

A2.4.2 Conclusion

Although Buscopan appeared to have a potent effect on motility in these subjects, the global effect of neostigmine is relatively unknown and 10% sensitivity was felt to be sufficient to detect a motility change against placebo whilst making use of all patients recruited for the baseline study.

APPENDIX 3: THE USE OF RPCA TO QUANTIFY MOTILITY

Small bowel motility assessment of 3D MR time series data via Robust Principal Component Analysis

[1]P. Ferry, [1]A. Menys, [2]F. Odille, [3]A. Emmanuel, [1]S. A. Taylor and [1]D. Atkinson

[1]Centre for Medical Imaging and Centre for Medical Image Computing, UCL, [2]INSERM U94, Nancy University, France, [3]Department of Gastroenterology and Nutrition, UCH

A3.1 Introduction

The small bowel spontaneously contracts to mix and move the intestinal contents - this motion is referred to as small bowel motility. Motility is affected in many pathologies e.g. Crohn's disease [1] and therefore might be used as a disease biomarker. Robust Principal Component Analysis (RPCA) has recently shown its ability to detect motion in dynamic image sequences in a variety of fields e.g. face recognition [2]. Such a method applied to cine 3D MR small bowel data can potentially provide a rapid assessment of motion and therefore provide functional information on disease presence, extent and response to treatment.

A3.2 Methods

According to RPCA theory, a given matrix M can be decomposed into the sum of two components: L, a low rank matrix and S a sparse matrix. It is possible to exactly recover L and S by solving the following convex optimisation problem [1]: $\min_{L,S} \|L\|_* + \lambda\|S\|_1 \quad s.t. \quad M = L + S$ where $\|.\|_*$ denotes the nuclear norm of a matrix i.e. the sum of its singular values, $\|.\|_1$ denotes the l1-norm i.e. the sum of the modulus of each element and lambda (λ) is a trade-off parameter between the sparse and the low-rank components. This parameter plays a key role in this matrix decomposition.

Image acquisition: 3T Philips Achieva scanner, coronal bTFE 3D sequence, TR/TE 3.51/1.66ms, slice thickness=10 mm. 15 slices per volume and 20 temporal positions with temporal resolution 1 volume per second. Subjects fasted for 4h prior to drinking 1L 2% mannitol. A prospective study was carried out in 6 normal volunteers who received a baseline scan, followed by the spasmolytic agent Buscopan.

Image processing: To reduce intensity variations, Contrast Limited Adaptive Histogram Equalization (CLAHE) [3] was applied. The reference decomposition trade-off parameter was chosen as λ_0=1/$\sqrt{}$ (max (dim (M))) where M is the data arranged with the volume for each frame as a column. Subsequently, δ RPCA decompositions were performed on the data set with λ varying by δ steps over the range [0.5 λ_0; 9 λ_0]. The sparse volumes obtained were converted to binary values

and the motility metric generated from a 3D average sparse overlay as follows: $S_{avg}(:,:,:) = \sum_{t=1}^{end} \sum_{\lambda=\lambda min+s}^{\lambda max-s} S(:,:,:,t,\lambda)$ with $s = (9 - 0.5)\,\lambda_0/\delta$. ROIs were drawn around the whole small bowel or part of it in order to assess mean S_{avg} value inside. 3D RPCA results were correlated against the current gold standard 2D method i.e. Optic flow registration technique assessed by standard deviation of Jacobian determinant [4] using linear regression model and Pearson's correlation coefficient. Mean Pre and Post Buscopan injection motility measurements for both methods were compared using a paired T-test.

A3.3 Results

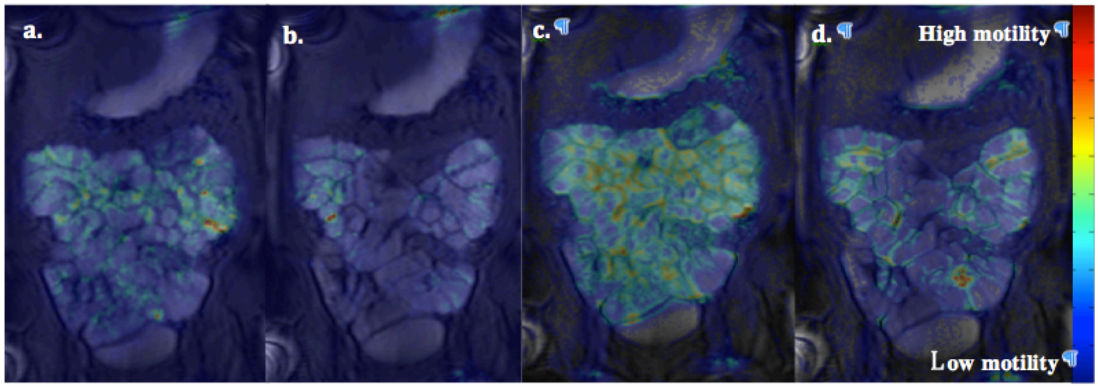

Figure A3.1: *Motility overlays in a normal volunteer pre Buscopan (a, c) and post Buscopan (b, d). STD Jacobian determinant method (a,b) and 3D RPCA with δ=25 (c,d).*

The method is fast (~ 1min/ RPCA, ~ 10 min for complete map). CLAHE pre-processing significantly reduces the sensitivity of the method to spatial variation of intensity - emphasising bowel wall motion. Similar regions of bowel are highlighted

by both methods (RPCA and STD Jacobian determinant). Moreover the small bowel wall is well detected. RPCA was able to detect Buscopan induced

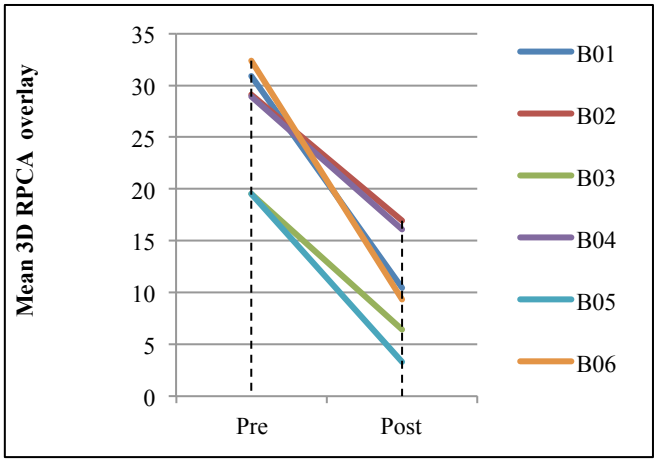

Figure A3.2: *Motility scores in 6 volunteers using global small bowel ROIs, (a) mean STD Jacobian determinant Pre and Post-Buscopan injection, (b) mean 3D RPCA value (δ=10).*

changes in motility (***Fig. A3.1*** and ***A3.2***). The Pearson correlation coefficient between 3D RPCA measurements and gold standard metrics on ROIs was 0.87 and the regression model R^2 was 0.76. According to both methods Buscopan provokes a significant decrease in mean global small bowel motility of normal volunteers ($P < 0.001$).

A3.4 Conclusion

We implemented 3D RPCA with intensity flattening to objectively evaluate global small bowel motility. RPCA maps permit both rapid visual and quantitative assessment of global small bowel motility. Future work: development of a method to

normalize maps between patients and prospective application to assessment of bowel disease and treatment response.

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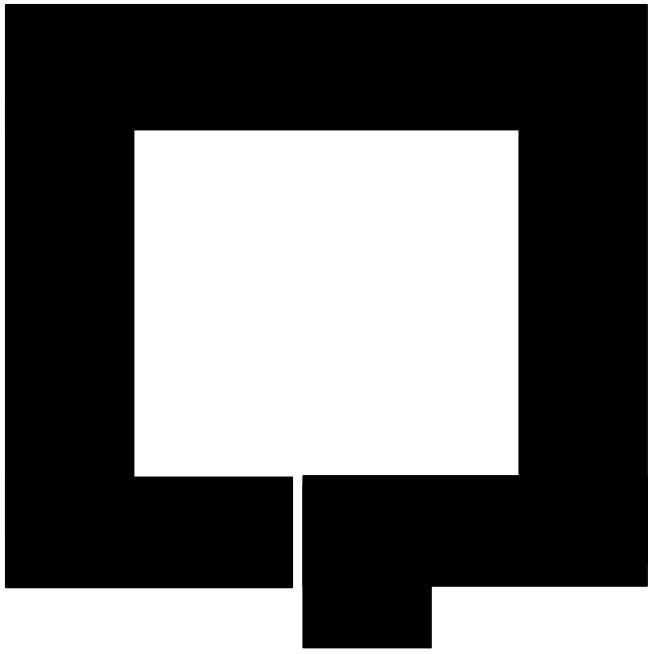
APPENDIX 4: THESIS COMMERCIALISATION PLAN FOR THE EXPLOITATION OF THE RESULTING ADVANCES

Overview

This extended appendix has been included in the Thesis as part of the UCL Advances Enterprise Scholarship that funded an extension to the PhD to explore commercial opportunities arising from the research. The company Motilent Ltd was founded by the Thesis author and won the UCL Enterprise Award 2014 for best business idea along with a £10,000 convertible loan for business development. For the sake of ease of communication (especially to a relatively lay audience), the 'optic-flow' registration algorithm was re-branded to GIQuant. For this chapter, a modified version of an application to the Technology Strategy Boards: Biomedical Catalyst funding call has been used as the most comprehensive and thorough piece of business focused writing to date requesting £1,132,658 to develop both business and academic objectives. This chapter has omitted scientific detail as it has already been discussed extensively leading up to this point. Instead, how the research can be commercialised is discussed in terms of market, need, opportunities and return on investment. Whilst not routinely the subject of a PhD, the construction of a business plan and this application has been incredibly valuable for distilling the value of research within the wider, social and business context and also in bringing together a larger group of researchers and individuals in an attempt to drive this technology beyond its singular application in research.



MOTILENT Ltd.

Alex Menys – Founder

Project Brief: Technology Strategy Board: Biomedical Catalyst – Late Stage Award

Published by TSB:

Across the world healthcare models are facing greater challenges, both physically and financially, in providing for a growing, ageing population with an increasing burden of disease. The long-term sustainability of current models of provision is increasingly questioned with the upward trend in healthcare spending becoming a significant portion of a nation's GDP. The drive therefore to deliver efficient and effective healthcare has never been more pertinent.

In order to meet these challenges, both companies and academics must recognise the drivers behind them and work together towards developing innovative technologies and processes which provide solutions for:

- Disease prevention and proactive management of health and chronic conditions
- Earlier and better detection and diagnosis of disease leading to marked improvements in patient outcomes
- Highly effective treatments that are tailored to patients' needs and either modify the underlying disease or offer potential cures

The Medical Research Council and the Technology Strategy Board are working together to deliver the Biomedical Catalyst scheme providing responsive and effective support for the best life science opportunities arising in the UK.

Grant funding through the Biomedical Catalyst is available to UK commercial businesses (who are SME's) and researchers looking to develop innovative solutions to healthcare challenges either individually or in collaboration.

Late-Stage award - Key features

Projects can range from 1 to 3 years' duration, the maximum grant available is £2.4 million and the minimum project size is £250k. Businesses (who are SME's) will be funded at up to 60% although elements of projects judged to be 'experimental development' will be funded at the lower intervention rate of 35%. Late–stage awards enable applicants to take a well developed concept and demonstrate its effectiveness in a relevant environment. The award is designed to support applicants seeking to evaluate the clinical utility of their new product, process or service.

The work required in these projects will be dependent upon the stage of maturation and the proposed application of the technology. We do expect that all projects will be based on significant prior research where the feasibility has been demonstrated in a model system. Examples of project work in this late-stage award category may include:

drug development projects looking to achieve clinical demonstration of safety and efficacy including the initial demonstration of drug safety (phase I) through to human proof of concept (phase II). Only where appropriate would we consider supporting a phase III trial, in this case the project work would be considered 'experimental development' and attract the lower rate of intervention (it is assumed

that in the majority of cases the funding offered through this scheme would be insufficient to conduct a phase III trial)

Project title: Gastrointestinal motility assessment with medical imaging analysis

Summary of proposed Project

Motilent (the Micro SME) has, alongside academic partners, developed and validated software technology called GIQuant that for the first time allows non-invasive assessment of gastrointestinal tract function based on routinely acquired medical images using Magnetic Resonance Imaging (MRI). Bowel dysfunction is extremely common and debilitating, affecting as many as 1 in 10 people in the UK. This project has two key objectives that enable:

1) The development and subsequent commercialisation of the GIQuant technology for the clinical quantitative evaluation of gastrointestinal motility in disease.

2) The crucial clinical testing in several of the country's leading bowel disease centres to establish the effectiveness and utility of this form of imaging analysis in the clinical setting at the point of direct patient care for key conditions including Parkinson's, Constipation and Irritable Bowel syndrome (IBS).

Academic Collaborators

Lead Academic Partner: Professor Stuart A Taylor, Centre for Medical Imaging, UCL. 3rd Floor East, 250 Euston Road, London, NW1 2PG

Relevant Funding comes from NIHR and Biomedical Research Centre, UCLH who funded Motilent CEO's IMPACT PhD. Motility analysis work supported by Royal College of Radiologists Kodak grant (£4900), Radiological Research Trust charities proof of concept grant (£4995) and most recently enteric HTC pump prime funding (£12,540). SA Taylor was awarded an NIHR HTA grant (11/23/01) 'Diagnostic accuracy for the extent and activity of newly diagnosed and relapsing Crohn's disease: Multicentre prospective comparison of MR imaging with small bowel ultrasound' (£908k) which will collect additional motility datasets on over 200 patients from sites around the UK.

Summary of key collaborators:

>>UCL: Centre for Medical Imaging – Prof. Stuart Taylor (Academic lead), Dr Anton Emmanuel (Gastroenterologist) and Dr David Atkinson (Physicist).

>>University of Nottingham: Sir Peter Mansfield MR Centre - Prof. Penny Gowland (Physicist), Prof. Robin Spiller (Gastroenterologist), Dr Luca Marciani (GI Phys) & Dr Caroline Hoad (physicist).

>>Queen Mary's University: Neurogastroenterology group – Prof. Charles Knowles (Surgeon) and Prof. Qasim Aziz (Gastroenterologist) & Dr Mark Scott (Physiologist).

Section 1: The business proposition

Question 1: What is the Healthcare need that this project intends to address and with what solution?

Healthcare need: Patients with long-term bowel symptoms, including constipation, diarrhoea, abdominal pain and nausea suffer a profound and often lifelong reduction in quality of life. These symptoms are broadly collapsed into Functional gastrointestinal disorders (FGID) affecting 10-25% of the UK population with 20% of these patients referred to a consultant contributing to between 20-50% of the gastroenterology workload. Functional gastrointestinal disorders are often chronic, mis-diagnosed and under-reported resulting in multiple physician outpatient visits, numerous medication trials and often unnecessary diagnostic testing in pursuit of a diagnosis. This results in large, ongoing direct costs to the healthcare provider ($10bn per year in US) and societal costs ($20bn) where sufferers take twice the number of sick days per year against controls.

Motility and its dysfunction (dysmotility) is one of the cardinal patho-aetiological determinants of FGID. While the definition of FGID requires that an organic diagnosis cannot be made using standard tests, it is acknowledged that more complex tests of structure and function can yield findings of pathophysiological significance. The healthcare need we are aiming to address in this proposal is the lack of non-invasive and quantitative tests to investigate gastrointestinal motility in FGID. The clinical value of such a test would lie in its ability to sub-categorise the FGID patient group, decrease time to diagnosis and phenotype (an important

component of their disease) to direct existing and emerging new therapies. Further, rapid and accurate diagnosis would lead to large cost savings in health resource utilisation.

Proposed Solution: Motilent (the SME) in collaboration with the Centre for Medical Imaging (UCL) has developed and validated an image registration software tool (GIQuant) to non-invasively evaluate intestinal motility using data acquired as part of existing clinical routine (detailed section 5). GIQuant is an increasingly widely used research tool however, the large amount of low-level user input (bowel segmentation, registration etc.) makes it impractical as a clinical tool. In this project we aim to package GIQuant into a streamlined, validated and clinically integrated service. This service would be comparable to many other routine diagnostic tests and thus follow well validated clinical pathways as follows: 1) Clinician requests MRI scan with GIQuant analysis at their institution. 2) Patient is scanned and data transmitted to Motilent for analysis. 3) Motilent technicians perform analysis to generate a paper/digital bowel physiology report (Fig A4.1). 4) Report returned to requesting clinician. Through collaboration with our academic partners, we will have the opportunity to incorporate other measures of bowel function available from MRI to produce a comprehensive report of bowel motility. Ultimately, we envision a customisable report of evidence-based quantitative features describing bowel function.

Magnetic Resonance Gastrointestinal Physiology Report

Patient Name: John Smith
Hospital Number: 4032781
Scan quality poor/moderate/good

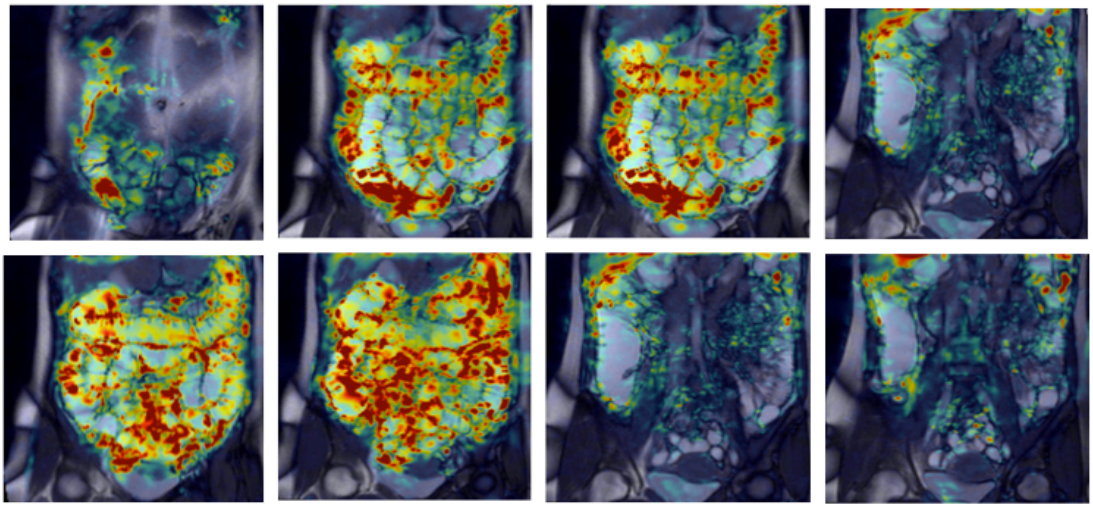
DOB: 25/08/1972
Address: 250 Euston Road, NW1
Hospital: UCLH (Prof Gastro)

Gastric Physiology

Volume oral contrast consumed:	1000Ml
Time to scan:	50 mins
Volume at scan:	44Ml
Gastric emptying rate:	18ml/min
Gastric motility:	0.12 AU, 1 contraction min^{-1}

Small bowel Physiology

Volume oral contrast consumed:	1000Ml
Time to scan:	50 mins
SB motility (mean):	0.32 AU (normal range 0.2 to 0.45)
SB variance (variance):	0.02 (normal range 0.001 to 0.032



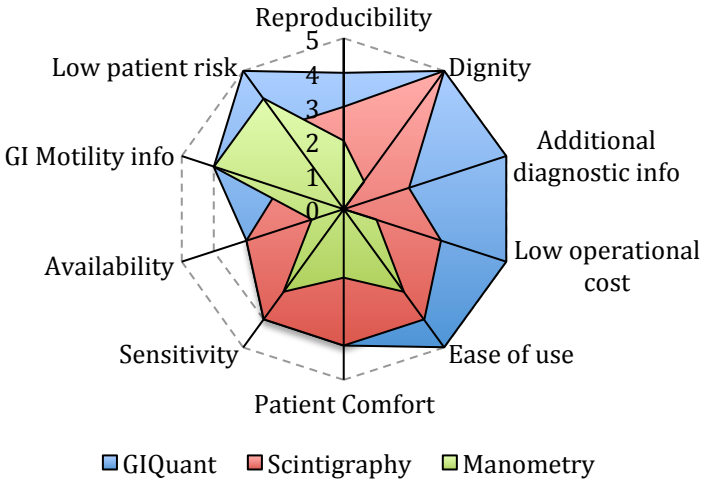
Colon Physiology

Distension:	AC: yes/no	TC: yes/no	DC: yes/no
Contractions:	AC: yes/no	TC: yes/no	DC: yes/no
Colon motility (mean):	AC: 0.21AU	TC: 0.22AU	DC: 0.02AU
Colon motility (contractions min^{-1}):	AC: 1	TC: 2	DC: 0

Analysis by Motilent Technician on 20/6/2014: Signed off by Radiologist 21/6/2014

Figure A4.1 Motilent small bowel physiology report

The healthcare challenge: There is no uniform cure for FGID. Patients have a similar life expectancy to healthy individuals and it is often quoted for IBS that "it may not kill you but it ruins your life." Treatment focuses on targeted management of specific symptoms over a long-term e.g. analgesics, laxatives or anti-diarrhoea agents. Treatments should begin with accurate diagnosis, however in the case of the FGID, diagnostics either do not exist or are limited to research investigations with limited clinical applicability and issues of interpretation (particularly quantitative diagnosis in the individual). The anatomical and functional complexity of the gastrointestinal tract makes the development of effective diagnostics very bowel challenging with the small bowel alone containing 10^8 neurons (similar to the spinal chord). Its deep anatomical location further makes it largely inaccessible to conventional invasive instrumentation. It is widely accepted that the current diagnosis of Irritable Bowel Syndrome for example (part of the FGID spectrum) is likely a heterogeneous collection of as yet un-characterised diseases. In the absence of diagnostic tests to identify these phenotypes, the quality of care for these patients remains unsatisfactory and societally expensive. The outputs of this project will provide clinicians with a new investigative option that can be used across hospitals for negligible setup costs or staff training. Our proposal will focus specifically on contractility in these patients for which there are a number of existing and new drugs currently in late phases of clinical development. The outputs of this project will provide robust data to validate the proposed service which in turn will be used to indicate which patients will likely respond to such medication and serve to phenotype this heterogeneous patient cohort and expedite patient diagnosis.



1. *Reproducibility - scan findings consistent by reader*
2. *Dignity – Provide dignity for patient*
3. *Additional diagnostic info – opportunity for extra investigational findings to aid diagnosis*
4. *Low operation cost – cost to maintain equipment/staff*
5. *Ease of use – level of staff training required*
6. *Patient comfort – how well test is tolerated*
7. *Sensitivity – quantitative capabilities of test to detect changes in motility*
8. *Availability – number of centres capable of performing test*
9. *GI Motility info – unambiguous information to assess GI motility*
10. *Low patient risk – likelihood of patient no experiencing harm*

***Figure A4.2**. Radar plot to demonstrate area for opportunity with GIQuant analysis, Scintigraphy transit study and Manometry.*

Comparison with existing technology: A radar plot analysis summarising existing technologies benefits can be found in Figure A4.2. Manometry is the current standard for motility assessment but, as an intraluminal test, is necessarily invasive. Investigation is largely restricted to the proximal and distal 1 meter of GI tract. Where superficial motility is examined (e.g. oesophagus or ano-rectum) manometry costs around £500 per scan in the NHS with approximately 7000+ procedures per year. Full colonic investigation costs over £2000 and represents a major

undertaking for physician and patient alike (full bowel preparation and colonoscopy + 24hr recording and analysis). High resolution manometry is now being marketed costing between £1000 and £3000 per investigation. The test remains associated with existing limitations with respect to invasiveness, dignity, patient discomfort and limited range. Crude measures of whole gut transit can be obtained using radio-opaque marker studies. These however yield little in the way of segmental functional data required to either understand disease pathophysiology or direct specific therapy in FGID. More complex techniques including isotope scintigraphy can give a measure of segmental transit time (e.g. small and large bowel but cost approximately £400 per investigation). Both methods require ionising radiation making them unsuitable for chronic conditions or those that manifest in childhood. Further, colonic scintigraphy requires twice-daily departmental visits for scans each day for 5 days. Emerging technologies including the ingestion of a 'smart-pill' to measure pH, pressure and transit along the GI tract and thus provides some information on motility costing £700 per investigation. Cross-sectional imaging and endoscopy are usually performed prior to capsule endoscopy and coherent information on motility is limited and it remains largely a research tool at present. The capsule must be recovered from the stool.

In comparison with the above methods, the advantages of GIQuant are:

1. Most patients will at some stage undergo cross-sectional imaging (CT/MRI) even if they are having the above specialist motility tests and so many will already have available data;
2. Widespread availability of GIQuant i.e. wherever an MRI scanner is installed. Little need for staff training or dependence on specialist GI facilities;

3. GIQuant performs global (whole GI tract) and segmental assessment of motility not just in areas of easy accessibility;

4. GIQuant also yields anatomical information from the standard MRI report;

5. GIQuant assessment is non-invasive, dignified and comfortable for the patient: an additional 15 minutes of scanning even if added to existing scan.

6. GIQuant provides well-validated direct high resolution images and metrics of global motility.

7. GIQuant is considerably cheaper even where MRI is included (See also section 4).

Healthcare Impact: Societally, £2.9bn per year is lost through loss in productivity in the UK arising from FGID, attributing to 20% of all work absenteeism. Better diagnostics and targeted medical management will be central to reducing this this cost. For the first time, patients will have access to a safe radiation free non-invasive quantitative test to examine global and segmental intestinal motility.

Question 2: What is innovative about your idea?

Unique Selling Point: There is no clinical test to evaluate global contractile activity in the bowel. We have developed and validated a computational approach to assessing both global and segmental GI tract motility using routinely available MRI data allowing quantitative, objective assessment of motility. In this proposal we

package this technological advance into a convenient clinical test ready for wide scale clinical adoption.

Commercial Innovation: Hospitals pay-per scan for medical imaging analysis allowing straight-forward costing into clinical care pathway. Low operating costs can be maintained by Motilent where data analysis takes place on-site without the need for hardware manufacture, distribution and associated regulatory approval. The model has a precedent in the NHS with MRI quantification of liver iron. Service has 'vertical' scalability where increased demand can be met quickly by increasing numbers of technicians, server space etc. at Motilent. Service has 'lateral' scalability where novel technologies for image analysis can be incorporated into business to increase revenue. Proposed service compatible with all MRI data allowing international expansion of business without the need for new equipment or specialised personnel.

Scientific Innovation: In vivo validation work has demonstrated that GIQuant is sensitive to pharmaceutical stimulation of the gut, repeatable and robust to user error making it an excellent clinical tool for the evaluation of motility. Proof of principal has been shown in disease states including Crohns disease and chronic intestinal pseudo obstruction. This portfolio of validation data does not exist for any other competing software solution. A current total of 8 full publications and 32 abstracts and presentations have been generated over 3 years providing initial validation of the solution. In addition to GIQuant other analysis methodologies developed by the academic partners will broaden the scope of information provided

by the service including gastric emptying rate, transit time, gastric accommodation all of which can be calculated from routine MRI images.

Technical Innovation: MRI images are sent to Motilent and processed using GIQuant to produce a data-file. This file is a key exploitable product generated by Motilent where it can be: 1) Assessed onsite by a Motilent technician to produce the Motilent report (Figure A4.1). In this route to Market, Motilent will deal directly with the hospital, abstracting all time consuming analysis away from the clinic. 2) Returned to the user in its raw format where the academic or pharmaceutical company, will be able to perform analysis themselves directly using the Motilent file reader. The exact combination of the above is likely to vary based on the users needs and flexibility in this respect is a core service image that Motilent is keen to adopt. Our business model places Motilent as a central hub to process MRI data returning either the raw data as a file to the user for further analysis or as a report. The key strength of this model is that data can be sent globally to the Motilent servers for analysis allowing high volume analysis. Use of the Motilent reader would further add important branding for the service. This process could be fully automated at minimal operating cost generating early stage revenue.

Timeliness: Patients with GI disease increasingly undergo MRI scans as part of their clinical evaluation with MRI advocated as the primary imaging modality for assessing the bowel by many international consensus committees. Where the number of MRI scanners across Europe is set to increase by 15% over the next 10 years, the role of this modality is going to become increasingly prominent especially in the diagnosis and grading of chronic conditions and less expensive as the

hardware technology improves. For example, multi-parametric MRI has recently been indicated as first line diagnostic/surveillance tool for prostate cancer by NICE and as the leading biomarker to identify and grade dementias. Imaging with MRI is becoming increasingly versatile with the reporting of the digital images now decentralised from the point of acquisition and the value of secondary analysis adding even more value to the modality.

Over 20 new image processing technology companies have appeared collectively demonstrating a growing trend towards this form of investigation although there is no med-tech company that specialises in the analysis of the GI tract. This proposal gathers together UK leaders in this field to provide a service that would be scalable and generalisable across all healthcare providers where imaging is used. Analysis of GI function is arguably the most complex of imaging targets with minimal research at present. At UCL, UoN and QML we have contributed to some of the leading work in this field and importantly validated it in clinical cohorts. Beyond this, the collaborative partners have excellent working relations with the FGID community presenting an excellent opportunity to disseminate findings and technology during the project. GIQuant is now primed for commercialisation and the expansion of similar companies, albeit in other fields, makes this a crucial and timely step for a UK company to establish itself in this area. In the UK, the NHS is required to deliver £20bn of efficient savings by 2015. In an independent report, Deloitte identified fast, accurate and widely available diagnostics as an essential long-term contributor to cost savings as well as essential for improving the quality of life for the patient.

Competitors: Competition comes from manufacturers of instrumentation designed to measure motility and software solutions. As discussed above, high resolution manometry and smart pills are now being marketed by companies (GIVEN etc) and financial reports from last year suggest uptake that is mainly limited to private healthcare providers in the US. A number of medical imaging software companies exist that have commercialised other existing technology focusing on mainly dementia (IXICO, Iconometrix), Cancer (Mirada, Texrad), musculoskeletal/body (Image Analaysis, AMRA, ResonanceHealth), inflammation/respiratory (Bioxydyn) or some combination of the above. In all of these fields the technology is more developed on account of its popularity as a research topic and largely open access however commercial viability has been established through applying the techniques to meet the needs of the Pharmaceutical industry users seeking regulatory approval. For many of these companies, advanced image analysis does not currently represent clinical benefit and therefore penetration into clinical practice has been limited. ResonacneHealth conversely have identified a clinical problem without a satisfactory clinical solution (liver iron overload). Despite the market size being small, they have established an international user base through having their service indicated in clinical guidelines, charging £250-1000 per scan for a single numerical measurement. The GI tract is less popular as a research topic but an expensive and important clinical discipline. We have developed the leading technology in this field to directly address a clinical need and are looking to commercialise it directly.

There is only one direct software competitor that at present does not appear to be trading. Motasso by Sohard AG has been used by only one research group who helped to validate the tool. No attempt was made to develop Motasso beyond its

research application. Motasso is not currently available and is of limited use for motility analysis providing assessment of segmental bowel motility only. GIQuant is conversely robustly validated with 7 papers in well-respected journals. A further six papers are currently in development or in press with a collaborative partners across Europe all demonstrating clinical application. The technical barriers to duplicating our software are significant especially with respect to implementation. Robust validation would require time and money alongside clinical input to scan patients. All members of the proposal team hold senior positions at leading conferences in the field of gastrointestinal disease and regularly peer review the published literature. Although the number of papers on intestinal motility is increasing, assessment remains largely manual and impractical for clinical use. Two other research groups have proposed techniques to evaluate motility using MRI although neither are as well validated as GIQuant nor tested clinically.

Question 3: How do you intend to exploit the opportunity?

Exploitable outputs from this project include:

1) A clinical service to provide analysis of bowel motility. Exploit by integrating into clinical workflow and charging for the service.

2) A data analysis service to provide motility analysis for academic and research users. Exploit by providing as a service, producing a GIQuant 'motility file' and

providing a reader tool for academics or pharmaceutical users to analyse their own data.

Market 1:

Academic users. Approximately £5.04bn is spent globally per year on research into digestive diseases. As per the clinic, there are significant limitations in existing motility assessment techniques impeding current research. MRI is an emerging technique for motility analysis with a rapidly growing literature (+5% per year). ***Why:*** 1) provides scientific evidence base to support technique 2) access academic clinicians who would bring into clinical practice where utility is seen 3) generate early revenue at low running cost to Motilent. 4) Few regulatory barriers. ***How:*** Design and implement a service that accepts research MRI data and returns the processed result for further analysis by the academic user.

Route to Market: Option 1 (Direct): We charge academics per data set returning to them a GIQuant data file containing the motility data. They then use a free 'reader' to analyse their own data. This is, in principle, the same as Adobe's PDF file, which Adobe charges to create but makes free to read. Without the need for Motilent 'hands-on' analysis, we can largely automate the computational processing and generate crucial early revenue. Option 2 (Indirect): We license the software to PACS providers eg Biotronics 3D, Feedback PLC who have and interest in image post-processing. Although we sacrifice revenue we achieve greater market penetration through accessing existing users with relevant provider subscriptions.

Market 2:

Clinical users. In the UK 1.05m patients are seen in secondary care with FGID symptoms. Over half of these patients receive some form of medical imaging as part of their diagnosis (X-ray, CT, MRI, US), £130m is spent on other clinical tests and £240m is spent on medications specifically for their symptoms. The cost of FGID symptoms comes from the failure to diagnose and identify an effective treatment strategy. Prevalence of FGID is similar across Europe and the US resulting in between 21.19m to 105.95m secondary care referrals per year in the US. Inpatient costs of motility disorders have been estimated at $1bn per year in the US for inpatients alone. ***Why:*** Large revenue potential if even a small uptake is achieved. ***How:*** Provide clinically integrated service that abstracts all analysis away from the clinic running analysis as a service compatible with clinical pathways.

Route to market: Option 1 (Direct): Receive clinical data direct from hospitals and process to generate a motility report (Figure A4.1). Use in-house technicians to provide time-intensive analysis and cost into service. This represents the greatest opportunity for Motilent revenue. Option 2 (Direct): Return un-analysed data as per part I. Hospital technician provides analysis. Option 3 (Indirect): License service to specialist clinics/centres who run GIQuant data and analyse themselves.

Market dynamics: Although several markets exist (academic, clinical, pharma etc) it is often the same individual acting in a different capacity in each. For example, an academic clinician may spend 20% of their time running their research group, 55% of their time working in their NHS clinic, 20% in their private clinic and the remainder acting as a advisor to pharmaceutical companies, hospital commissioning boards, charities and other initiatives central to the success and clinical uptake of

emerging diagnostic techniques. Appreciation of this interplay is central to Motilent's route to market approach and our goal at this early stage is to identify and support these senior and high profile individuals in the GI motility community. Their initial research will provide them with product understanding and bring the potential for them to include it in the private setting where cost is transferred onto the patient. Motilent's primary commercial barrier for entering the clinical market will be the existing clinical culture for gastrointestinal motility investigation.

Potential barriers: GIQuant has no direct commercial equivalent and clinicians have never had access to a quantitative tool to evaluate and detail global bowel motility. Many clinicians will not know how or when to request such tests in the absence of clinical guidelines and therefore we anticipate a substantial but not insurmountable barrier to short-term acceptance of the technique. While publications using GIQuant have attracted citations and interest the technique itself is poorly understood along with its potential for clinical impact. Therefore in this project we need not only build up a commercially viable service but also the corresponding evidence base for technique use. This evidence will in turn be used to create clinical guidelines indicating the techniques use and result in clinical uptake especially in the NHS. As a result, our route to the ultimate, clinical market is multi-layered commencing with the academic users and working through the less financially restricted private clinics until wide-spread and evidence-based service utility is achieved.

Section 2: The project details

Question 4: Describe the Return on Investment

Market: Academic/Pharma. Units p/a. 3000. Cost per unit: £50. When: year 2. Growth p/a: 10%. Revenue p/a: £150,000. Gross Profit p/a: £135,000. Justification: Based on existing contracts and grants where GIQuant has been costed. Use as an endpoint for drug studies is currently driving estimated unit volume.

Market: Consulting/setup fees. Units p/a 5. Cost per unit: £10,000. When: Immediate. Growth p/a: 25%. Revenue p/a: £50,000. Gross profit p/a: £40,000. Justification: Many projects currently require intensive help to set up (eg. protocol development). Motilent will develop a consulting service largely to assist pharma projects.

Market: Clinical (private healthcare). Units p/a 1200. Cost per unit: £500. When: year 3. Growth: 10%. Revenue p/a: £600,000. Gross profit: £420,000. Justification: The private healthcare sector in the UK is the primary consumer of GI techniques. We anticipate higher uptake driven by patient demand. We will offer a specialised GIQuant report with customisable analytics to meet clinical demand and charge more for this option.

Market: Clinical (NHS specialist tertiary). Units p/a: 1900. Cost per unit: £250: When: Year 3: Growth p/a: 5%. Revenue p/a: £475,000. Gross profit: £190,000. Justification: Without clinical guidelines, uptake will only be seen in specialist centres as run by proposal applicants generating the stock GIQUant report (Figure

A4.1). Value challenging to predict until clinical data becomes available, where value is demonstrated revenue in 1% of FGID workload, revenue p/a could be as high as £25m.

ROI based on the proposed, conservative values we anticipate becoming profitable by the end of year 2. We have used UK values for clinical markets for financial analysis where we can base numbers on existing clinical experience. We anticipate that ROI on TSB funding will be seen by years 6-7 based on UK revenue alone. This figure is however very diffiuclt to estimate at present.

>> Building market share: The early stage of our business development is centred around stimulating interest in key, industry leading clinical users to both raise awareness through research and bring into their clinical practice. This is not however a long term market strategy and towards the end of the project we will be looking to actively engage with new users through:

1) Direct engagement with GI community with a formal product launch at Digestive Diseases Week (15,000 attendee conference), emphasising Motilent's connection with academic partners and hosting educational sessions on the technology.

2) Continued engagement with clinicians providing free trial data analysis, scanner setup assistance and 1-on-1 webcast demos on our website for training and educational purposes.

3) Develop relationships with charities (eg. Bowel and Cancer Research, Crohn's & Colitis) to engage with patients and build patient awareness of service. We consider patient awareness to be key to accessing the private market. Further Patient engagement will be achieved by directed advertising through information in private clinic, web questionnaires and patient forums.

4) Dissemination of research generated by academic partners through popular media outlets, press releases, our website and social media in layman-friendly terms to further increase awareness.

5) Appoint specialised sales staff to develop and maintain existing relationships while engaging in non-UK markets. This will involve deployment to specialist clinics and major conferences worldwide, targeting countries identified by OECD and IFFGD as having a high MRI machine per capita (eg. Germany & USA) and flexible user-centric healthcare pricing.

6) We will constantly review the efficiency of our systems and infrastructure, scaling to meet demand and evaluating quality management. For example, where a form of analysis is not used, we will not spend resources performing that analysis and use saving to either decrease service price to attract new customers or increase revenue.

7) We will drive and fund research using Motilent technologies to build the volume of literature supporting efficacy and exploiting the potential for the technology to be applied in the diagnosis and management of other GI illnesses.

Further development: Building on this project, we will look to add additional software techniques as 'modules' to the report increasing the customisability of the service. TSB funding will enable us to set up a pipeline to translate the large amount of high quality research taking place in the UK into a suite of products to directly benefit the patient. Our close ties through Prof. David Hawkes at the Centre for Medical Image Computing will be central to this process. We plan to grow the business organically where possible, feeding profit back into research, both strengthening our business position and helping to maintain the UK's position as leaders in this field. Penetrating markets outside of Europe will require further regulatory approval. Specifically, the ISO:13485 certification will be required to enter clinical diagnostics however, we are aware of the requirements and have planned accordingly in our project to ensure equipment (eg servers) are in line with regulatory standards to facilitate this process at year 3. By year 3 we hope to have established a robust product and at this stage, the business will undergo a marketing transition with respect to the GIQuant exploitation strategy placing an emphasis on sales to clinical users in order drive revenue. We will at this time appoint the appropriate staff and seek external input from investors to fully exploit the project deliverables.

Question 5: What technical approach will be adopted and how will the project be managed?

The project has been broken down into a number of objective-focused Work Packages (WPs) that are deemed suitable to target main deliverables. The project will ensure that its objectives are met within the desired timescale and to budget by employing reliable project management and risk management system e.g. a tailored version of PRINCE2. WPs will be based on delivering predefined outcomes.

The project will be overseen by a study management group (SMG) with responsibility to monitor the milestones. The SMG will meet every 4 months for the duration of the project (9 meetings in total). The SMG will comprise the 4 applicants, 1 patient interests representative Deborah Gilbert (bowel and cancer Research), the lead technical developer to the project (Laurence Bourn) and the Motilent project manager. The group will thus include senior representatives from each partner to ensure that the views of those providing clinical, design, technical and marketing services are represented. Within the SMG, the WPs will be managed by the Clinical (CM: Stuart A Taylor) and Technical Manager (TM: Laurence Bourne) in consultation with the Project manager (PM: Katherine Prescott) to ensure efficient delivery.

Technical Rationale: Our techniques must work across institutions regardless of PACS provider to be commercially viable. Providing bespoke add-ons to existing solutions is slow in terms of customised engineering, version turnaround, access to end-users and the danger of commercial lock-in limiting product dynamism essential to an SME. An alternative is to provide either turn-key systems or bespoke end-user software which still requires substantial SME support and generate integration challenges for clinical IT administrators. Increasingly, SMEs are using

the web to offer their imaging services. Deploying services co-located with the growing number of affordable certified (ISO:27001) secure data centres. Advantages include robust physical security, best-in-class web access, multiple redundant power backup and 24/7 secure access. Clinical institutions have readily adopted these services mainly to allow outsourcing of the reporting effort (e.g. 3dnetmedical.com, pacsmail etc.) whilst decreasing risk to patient confidentiality. Furthermore, web-based services can drastically reduce hardware and software maintenance costs on-site. This will be essential for Motilent where business will be drawn through high volume processing from global data sites. Within academic environments data is anonymised and downloaded from PACS to allow customised and independent assessment with the most common platform Osirix (free, excellent UI) where an emphasis is placed on functionality. Our first project deliverable will be to create a plug-in for Osirix for academic data analysis. This will 'read' the Motilent data file and allow academic near-immediate access to the service from project commencement. Replacing of our own UI will be necessary however secondary to IT infrastructure. (Key: A = academic, B = business, WP = work package)

Deliverable B1: Develop and validate data annotation toolset

Objective: All processed data must be visualised for annotation either by a technician or an academic user.

>WP B1.1 (month 0-6) An Osirix ROI annotation plug-in and MATLAB code for the analysis of GIQuant output data. Made available to the academic partners.

>WP B1.2 (Month 8-32) On-line ROI tool for the (anonymised) analysis of MRI + GIQuant data. Enhancements to the online portal to support private clinicial institutions and personnel.

Deliverable B2: Develop and validate network back end of data handling

Objective: Secure, robust and scalable network with web-interface development alongside appropriate safety steps to insure patient data integrity.

- WP B2.1 (Month 6-24) An automated online service for storage and processing of (anonymised) institution data delivering GIQuant segmentation fields compatible with the Osirix annotation plug-in.
- WP B2.2 (Month 18-36) Secure end-to-end data pathway suitable for handling and reporting patient data to and from our servers. Clinicians drive the uptake of new tech into their PACS environment.
- WP B2.3 (Month 36) Internal audit for ISO:13485 certification.

Deliverable B3: Establish minimum requirements for Motilent's GIQuant report

Objective: Together with academic partners to identify key features for the Motilent report and establish training protocol.

- WP B3.1 (Month 28-30) Test a monitored and audited in-house bespoke report creation and delivery through the on-line service.
- WP B3.2 (Month 30-32) Feedback from academic partners into the report generation process for specific diseases.
- WP B3.3 (Month 34) Report handover from academic partners.

Clinical Deliverables:

Eight clinical deliverables will develop scientific background to support GIQuant service. Each deliverable represents a unique data set. Scanning will take place at UCL and UoN where robust MR protocols and infrastructure already exist. Clinical investigations (manometry) will take place at Barts Health NHS Trust. Primary ethics application will take place through UCL with SSIs issued to UoN and Barts Health. All data will be anonymised immediately following collection.

Deliverable A1

Healthy control scanning. Objective: Scan 120 healthy controls to establish normative data. Recruitment will be clustered by demographic categories to ensure community matching and generalisability for A2. Controls will be recruited through advertisement with key partners (charities, HEIs, enteric HTC) and by invitation of WP2 patient spouses participation. All will be screened to exclude relevant GI disease and FGID, the latter by validated questionnaires. Control scans will last 30min.

- WP A1.1 (month 8-24) 60 healthy volunteers scanned/reported UCL
- WP A1.2 (month 8-24) 60 health volunteers scanned/reported UoN

Deliverable A2

Patient scanning. Objective: Clinically evaluate the GIQUant service in patient cohorts comparing GI motility to normal controls. Each centre has selected an important disease area in which they have a strong referral base and international academic reputation. Patients will temporarily stop relevant medication prior to their scan. Recruitment will take place through existing databases, outpatient clinics

and advertisment. Patients will be scanned as per established MRE protocols to identify differences in gastric, small bowel and colonic motility compared to controls in regions relevant to the disease.

- WP A2.1 (month 8-32) Scan/report 30 mid-late stage Parkinson's disease patients (UCL).
- WP A2.2 (month 8-32) Scan/report 30 IBS-Diarrhoea patients (UoN)
- WP A2.3 (month 8-32) Barts Health / QMUL will perform high resolution pan-colonic manometry in a cohort of 15 patients and 15 new controls all of whom will be sent to UCLH imaging department for MRI and thence GIQuant analysis.

Deliverable A3

MRManometry probe development Objective: Validate the MRManometry probe. This technique prototyped by Motilent is promising method to produce high-resolution spatiotemporal maps of bowel wall contraction powered by GIQuant. MRManometry would provide a direct alternative to invasive manometry.

- WP A3.1 (0-6months) Assessment of the MRManometry tool using a computer simulation will be performed to assess accuracy. Data visualisation methods will be developed for interpretation (UCL).
- WP A3.2 (4-8 months) Using an MR compatible manometry catheter, invasive manometry will be performed simultaneously with MR to establish technique con-concordance in 2 volunteers (UoN).
- WP A3.3 (10-32 months) Using the MRI data from WP2.3, MRManometry will be performed to demonstrate the clinical application of the technique to a patient cohort.

Alternative technical solutions might involve packaging the core technology as a stand-alone software solution to individual hospitals. We are cautious of this approach as it would require extensive system maintenance. The core processing is computationally expensive and lends itself well to parallelised network processing and the necessary hardware to perform locally would likely make the time requirements to perform analysis unviable. Alternatively, this system could be packaged as part of existing PACS or cloud based systems with processing capabilities (Biotronics 3d). However, the need for manual annotation would again preclude from clinical applications. Employment of a technician for annotation purposes may be feasible and cost effective for service providers where high volumes of patients are scanned, in which case an on-site licencing strategy might be used.

Question 6: What are the risks (technical, commercial and environmental) to project success? What is the project's risk management strategy?

SWOT Analysis of the project plan resulted in the compilation of the Risk register below with RISK (low/medium/high) and IMPACT (low/medium/high) with MITIGATION strategy for key project risks.

1. Technical Risks:

1.1 RISK: MRI data variability is high (HIGH) IMPACT: Results from different scanners cannot be compared (High). MITIGATE: Introducing standardised scaling and noise estimation checks into the registration pathway. Such standardisation issues have been overcome in other areas quantitative MRI imaging (eg. Ferriscan by Resonance Health) and proposal team has extensive experience in this field.

1.2 RISK: Data transfer to Motilent not convenient or compatible with trust-setup (Medium) IMPACT: Technical issues including data format, access to trust network, internet accessibility from PACS computers etc prevent Motilent accessing data (High). MITIGATE: Appoint experienced PACS systems engineer and ensuring data transfer can occur through multiple channels ranging from burning to DVD/ web-based transfer/ PACS e-transfer.

1.3 RISK: Motilent does not obtain regulatory approval (Medium) IMPACT: Cannot service clinical market (High). MITIGATE: Use team experience of ISO:13485 approval and upkeep to design a robust process ready for approval during/upon project completion.

1.4 RISK: MRI scan protocol compliance is low (Medium). IMPACT: Bias introduced into data affecting quantitative result (Medium). MITIGATE: Auditing scanning through this study and publishing guidelines where user error affects results. Where necessary provide staff training.

1.5 RISK: Motilent cannot match demand for service (Low). IMPACT: Service quality is reduced and revenue decreased (High). MITIGATE: through implementing scalable systems. Use cloud-processing paying per hour to match computing demand.

2. Commercial Risk:

2.1 RISK: Clinical uptake/demand is low (High). IMPACT: Business is not economically viable (HIGH). MITIGATE: by servicing a patient demographic where there is a large clinical need and few existing diagnostic options. Allocate sufficient funding across leading academic groups in the UK to conduct high impact research to gather evidence for service efficacy. Use evidence base and influence of academic team where relevant to incorporate service into clinical guidelines. Provide service to academic and pharmaceutical users to generate revenue and increase evidence base for service. Engage with users to identify key service needs adjusting analysis where necessary to match said needs. Invest in marketing and user communication (2.2).

2.2 RISK: Service visibility is low (High). IMPACT: Low revenue lose first to market advantage (HIGH). MITIGATE: by advertising direct to users at gastroenterology meetings and conferences. Ensure website has high visibility using search engines. Approach specialist clinics and offer discounted trials and financing options to encourage uptake. Continue to work with charities to increase company and service exposure. Make sales appointment a priority by project end.

2.3 RISK: Competitive services emerge (Medium) IMPACT: Lose market share and negative impact on revenue (Medium). MITIGATE: by planning for regulatory approval (1.3). Maximise visibility (2.2). Ensure data analysis occurs on time and to a high standard. Where necessary adjust cost or refine service at a reduced cost to meet exact user needs. Build product portfolio where new techniques become available. Service academic and pharmaceutical market segments.

3. Managerial Risk:

3.1 RISK: Project cashflow (Medium). IMPACT: Motilent cannot pay staff or other direct/indirect costs (HIGH). MITIGATE: taking out low interest business loan. Use Motilent equity to attract external investment. Seek equity investment (eg. Feedback Plc). Quickly access research markets with have low barrier to entry with low running costs. Investigate multiple routes to maket.

3.2 RISK: Project is undercosted (Medium) IMPACT: Fail to deliver project (High). MITIGATE: using team experience of operating a technology SME to identify and factor key costs. Raise additional capital as per 3.1. Academic costing for scanning and projects are well established and capacity for patient drop-out.

3.3 RISK: Conflicts regarding IP research ownership (Medium) IMPACT: Delayed service development, additional cost to service and project (Medium). Not usually problematic where industrial funding is provided -common across institutions (Medium). MITIGATE: Sign terms of service contracts with host institutions that stipulate IP arrangements. Where IP belongs to host institution negotiate licencing and cost into service.

4. Environmental

4.1 RISK: Concerns of patient confidentiality prohibit uptake (High). IMPACT: Motilent cannot interact with hospitals or clinicians are dissuaded from using service (HIGH). Acquire ISO regulatory approval to provide institutional assurances (1.3). Develop robust processes to safeguard patient information (eg. anonymise patient details for analysis). Require data anonymisation prior to Motilent upload for non-clinical studies. Ensure Motilent servers have security.

4.2 RISK: There is not sufficient MRI capacity to support service (Medium). IMPACT: Motilent cannot extract revenue potential from market (HIGH). MITIGATE: by

providing cost-benefit for service (2.1). Establish partnerships with private scanning companies to increase capacity. Support proposals to invest in additional scanners.

5. Other

5.1 RISK: Patient recruitment is low/slow (Medium). IMPACT: Study deliverables are not met and evidence for service efficacy not provided (HIGH). MITIGATE: by appointing full-time clinical fellow specifically for recruitment. Utilising existing experience operating large studies. Utilise full available project length of 3 years.

5.2 RISK: Academic studies cannot take place without ethical approval (Medium) IMPACT: Study deliverables are not met and evidence for service efficacy not provided (HIGH). MITIGATE: Acquire all ethical approval for multi-site investigations in advice of study commencement. Audit regularly to avoid breaches that might lead to study being closed down at one of the sites.

To ensure risks are mitigated and managed a project manager will be appointed with the specific purpose with prior experience working on TSB and similar grants. Using a "light-touch" PRINCE2 methodology, the Motilent Project Manager will monitor the time, cost, quality, scope, risk and benefit performance goals of the project through monthly/weekly contact with investigators and the developer/UCLH fellow respectively. A mitigation strategy and re-assessment of budget will be produced for any identified risks. Through performing detailed forecasting, Motilent is confident it can accommodate the cash-flow required at project start with sufficient flexibility to allow for any delays in claims being paid.

Academic institutions will invoice Motilent quarterly for equipment costs further helping cashflow.

Question 7: Does the team have the right skills and experience and access to facilities to deliver the project and exploit it?

Motilent: Responsible for exploiting the business deliverables for this project and addressing practical and regulatory barriers for the implementation of the proposed service. Alex Menys developed and validated the GIQuant technique for his PhD publishing 6 papers in the last 3 years on the topic and over 30 conference presentations. He has formed a collaborative network of leading GI specialists across the UK and Europe and is an invited speaker at GI meetings. He has driven the commercial exploitation of his scientific research underlying this project, founding Motilent and won the UCL Bright Ideas Enterprise award. Laurence Borne is Motilent head of technology and has expertise in software engineering and web-based DICOM viewing and has formally worked for Biotronics 3D where he developed the 3DNet medical platform, currently the only web-based PACS system. He has experience with ISO:13485 approval and upkeep. Katherine Prescott is head of Motilent Operations and has extensive project management experience in technology SME's. She has previously worked on both early and late stage projects funded by TSB. Prof. David Hawkes is Motilents lead advisor providing key business input for commercialsiation of medical imaging technology. He has 40 years experience in medical imaging research and a significant track record in bringing innovative imaging technology to the clinic and commercial exploitation. He has

refereed 350+ publications in medical imaging. He was co-founder of IXICO plc, that was listed on Aim in 2013 as well as scientific advisor to several other successful start-ups (e.g. Dexela, recently bought by Perkin Elmer, and VisionRT). CMIC is one of the largest academic groups internationally applying novel computational imaging technology to provide healthcare solutions and has a significant track record with support from EPSRC, Wellcome Trust, DoH, NIHR, EU and industrial funding.

University College London: UCL already provides imaging for a number of large trials many with 1000+ patients. UCLH has a world-leading gastroenterology service and access to National Hospital for Neurosurgery and Neuroscience (NHNN) for patient recruitment and a publication and grant award track record to demonstrate research delivery. Prof Stuart Taylor is a NIHR senior investigator and currently CI of two large multicenter HTA grants (£2M+) investigating the use of MRI in cancer staging and Crohn's disease respectively. He has expertise in MRI enterography and has published widely on the topic. He sits on the European Society of Gastrointestinal and abdominal radiology/European Crohn's and colitis organization joint guidelines committee for imaging in IBD and NICE advisor. Dr Anton Emmanuel is a specialist in neurogastroenterology and has published 200+ articles on functional bowel disease been awarded over 1M in grants. He has a local and tertiary referral practice for patients for gut motility disorders, especially IBS and chronic constipation. The Unit attracts 450 such new referrals per year. In addition he has an appointment in the NHNN seeing 300 new patients per year. Dr David Atkinson provides technical expertise for clinical MR scanning and within-

hospital data transfer. He has a background in MR physics, and PI on numerous EPSRC grants totaling over £1M, author on over 150 peer reviewed publications.

Queen Mary's University London: Prof Charles Knowles is a Clinical professor of Surgical Research at QMUL and a consultant colorectal surgeon at Bart's Health NHS Trust (the largest new hospital in Europe). He has a strong track record of bowel disease research as briefly evidenced by 90 peer reviewed publications, and over £1M grant funding in last 24 months (inc. 4 NIHR grants for clinical trials). He is deputy director of the Bowel Function Health technology Cooperative (Enteric), one of 2 pilot DOH funded HTCs that aim to find innovative ways in which to bring together patients, carers, doctors, scientists and industry to develop new UK technologies, treatments and devices in the field of bowel disorders.

Within this role, he has secured funding to pioneer the development and validation of several new technologies in the bowel disease field and developed a national clinical network of over 20 trial centres. He is joint director of the new proposed NIHR HTC. He is on 2 grant award panels of NIHR. Prof Quasim Aziz Currently he is an executive committee member of the European Society of Neurogastroenterology and Motility and also a member of the United European Gastroenterology Federation Education Committee. He has published numerous original articles in reputed medical journals such as Nature Medicine, Nature Neuroscience, Lancet and Gastroenterology on the interaction of the brain and bowel is recognised globally as a leading authority in bowel motility. Dr Mark Scott: is the author of over 90 peer-reviewed publications, 8 book chapters, and has recently been Guest Editor of 2 journal supplements dedicated to the subject of chronic constipation. Together with

Professor Charles Knowles and Mr Peter Lunniss, he leads a flourishing research programme centered on clinical GI (principally colorectal) physiological investigation, involving the supervision of several research fellows.

University of Nottingham: The Nottingham group have worked together over two decades to develop novel methods of imaging the function of the gastrointestinal tract in health and disease. Penny Gowland is a Professor of Physics with more than 25 years of experience of working on developing quantitative MRI methods to answer biomedical questions. She has published over 180 papers on application of MRI, held grants of >£16M and is a Fellow of ISMRM. Dr Luca Marciani is a physicist by background and specialist of MRI of gastrointestinal function for gastroenterology and food science. He translated to the NDDC and is now Assistant Professor in GI MRI and sits on the NIHR NDDBRU Strategy Board. He has coordinated many successful multi-disciplinary research projects as CoI and PI with over 50 peer reviewed publications and over £4m funding. Prof Robin Spiller is an academic gastroenterologist and Co-Director of the NIHR NDDBRU, Elected Senior NIHR Investigator and member of NIHR EME board. He has longstanding experience of interdisciplinary projects studying abnormalities of gastrointestinal function and sensation in functional bowel disorders. Dr Caroline Hoad is a physicist who specialises in developing MR methods for studying the GI tract. She authored over 20 peer-reviewed publications on the development and applications of quantitative MRI in vivo.

Question 8: Justify the project funding and describe the added value?

We have modified the scope and aims of the proposed project following the feedback from our previous expression of interest.

Motilent costs

Salary costs: (incl. NI, superannuation):

Project Manager	for 3 years @ 42,500/year =	127,500
Senior web developer	for 3 years @ 62,500/year =	187,500
Senior medical imaging developer	for 3 years @ 62,500/year =	187,500
50% FTE Principle Investigator	for 3 years @ 30,000/year =	90,000
Senior Sales/Marketing Manager	for 6 months @ 45,500/year =	22,750
>Total Salary for 3 years:		£622,250

>>Equipment/Consumable

Apple Macbook Pro:	1400 x 2 = 2800
Development workstation:	1000 x 2 = 2000
Microsoft Visual Studio 2012:	850 x 2 = 1700
Windows 8.1 Enterprise:	270 x 2 = 540
Dell PowerEdge R320:	500
Windows Server2012r2:	600
SQLExpress:	Free
>Total Equipment costs	£8140

>>Other costs:

Server/Infrastructure (incl. DNS reg.&ISO:27001 colocation at Telehouse East with 4A power&100Mb 2-way connection):	5460 /year = 16,380
Utilities:	30,000/year = 90,000
Travel:	15,000
Patient Outreach & Marketing:	10,000/year = 30,000
Market Analysis Consultant (one off)	7,000

IP Protection: 25,000

>Total Other Costs for 3 years: £176,380

__Total SME project costs over 3 years: £ 821755__

__TSB SME funding over 3 years @ 60%: £493053__

Academic costs (80% FeC)

Clinical Fellow UCL (100% FTE)	for 3 years @ 36,402/year =	109,207
Post-Doc UoN (50% FTE)	for 3 years @ 19,471/year =	58,413
Research nurse UoN (10% FTE)	for 3 years @ 2668/year =	8,004
7.5%FTE UCL Prof Stuart Taylor	for 3 years @ 10,201/year =	24,483
2.5%FTE UoN Prof Penny Gowland	for 3 years @ 5,000/year =	15,000
2.5%FTE QMuL Prof Charles Knowles	for 3 years @ 5,000/year =	15,000
>Total staff costs:		£230,107

>>Equipment/consumable cost

Laptop computer UCL	1,000
MRI Scanner time UCL (60h @450/h):	27,000
MRI Scanner time UoN (50h @400/h):	20,000
Manometry costs QMuL (1000 per investigation):	30,000
Subject recruitment (£20 gift voucher for 140 controls):	2,800
Transport (patient and academic):	12,000
Total Equipment/Consumables	92,800

>>Indirect Costs

Estates – Labs UoN	for 3 years @ 10,989/year =	32,967
Infrastructure – Technicians UoN	for 3 years @ 958/ year =	2,874
Indirect Cost Rate UoN	for 3 years @ 33,657/year =	100,972
Estates - UCL	for 3 years @ 18,921/year =	56,765
Infrastructure - Lab Technicians UCL	for 3 years @ 2255/year =	6,765
Indirect Cost Rate UCL	for 3 years @ 38,785/year=	116,355
>>Total Indirect		316,698

Total UCL	£ 348,103
Total UoN	£ 244,502
Total QMuL	£ 47,000

__Total academic costs =_____£ 639,605

Technical justification: We looked at a variety of hosting models and locations for this application. Due to the sensitivity of patient data, it is essential that Motilent manages the server infrastucture co-located with an ISO:27001 data centre. Hands on experience will also facilitate deployment of server nodes in other locations as demand increases. This infrastucture provides sufficient resources for 1000 studies to be uploaded and processed per week sufficient to cover the academic and commercial goals for the first years. Development costs are largely to fund 2 expert level software engineers that can deliver the technical deliverables following a rigorous quality standard. A wide variety of technical expertise is required to deliver this project. The project manager will liaise with the academic partners, manager development deliverables and be responsible for timely delivery of project milestones. We have appointed a project manager through Motilent as this avoids HEI overheads and is more in line with the business objectives of the project. We have costed marketing consulting towards the end of the project as we feel this will be crucial to deliverable exploitation as well as the first 6 months of a sales appointment to drive uptake post completion.

Clinical justification: Recruitment is invariably the rate limiting step for academic projects and we have proposed cautious numbers based on previous experience that optimise cost and study power. We have allocated the majority of the HEI moneys to the appointment of a clinical fellow who will be able to both recruit, scan and assess the data. UoN has a specialist GI research facility with experienced post-doctoral scientists and a research nurse to address recruitment. Our academic partners have secured full research rates for scanning that is 50% lower than usual commercial rates. Co-investigators have taken lower %FTE to reduce project cost. We have costed patient travel based on previous experience averaging out at £20 per study participant. Additional money is included as gift vouchers to incentivise healthy control recruitment.

Question 9: How does financial support from the Technology Strategy Board and its funding collaborators add value?

The proposal applicants and their respective research groups represent a UK based consortia of world leading academics specialising in gastrointestinal disease with an outstanding track record in academic research. Together with their institutional colleagues, they have published over 400 papers in the top peer reviewed journals (Lancet, Nature Medicine, Gastroenterology, Gut etc) and generated in excess of £20m in grants as lead investigators and co-PIs. GIQuant as been used as a research tool at UCL and UoN for the past 18 months and has demonstrated clear utility as a research tool and for clinical investigations. Dr Caroline Hoad, Prof Penny Gowland & Prof. Robin Spiller have recently been awarded a MRC 'Confidence in Care,' grant

for GIQuant analysis of colonic motility worth £55,000. Dr Asma Fikree and Prof Qasm Aziz are currently leading an application to investigate GI function in hyper-mobility disorders worth £750,000. A number of smaller projects for pilot studies in Parkinson's, Chronic Pseudo-Obstruction, diabetes have also been awarded to a value of £50,000, all using GIQUant that will provide the preliminary data to support larger studies. This interest in GIQunat has emerged purely from the very preliminary studies published by Menys et al and as the literature using the technique grows, we expect to see an uptake in other areas of R&D. A number of other studies into motility in a range of diseases are planned and the academic partners have the track-record to maximise the likelihood of success.

From a strategic perspective the TSB Biomedical Catalyst funding provides an essential way for Motilent to raise initial funding. The status associated with TSB funding is such that it represents an excellent indicator to investors as to the quality of the USP, team and business stratergy. The TSB investment further drives down the amount of money needing to be raised, allowing Motilent to maintain a higher level of equity in the company and have better control over its direction. Motilent has already been approached by Feedback Plc. a company actively acquiring medical imaging technology companies (eg. TexRAD, Cambridge Computed Imaging) to develop a portfolio of services to exploit this burgeoning area of research. Feedback would be willing to assist Motilents growth in exchange for equity, however TSB funding would be central to Motilent's negotiating position. At present, Motilent is early stage micro-SME in a precarious position with respect to its IP, infrastructure and marketing experience. In order to maintain its technological advantage and USP, it is crucial that Motilent be 'first-to-market' in GI

function testing and this will involve early partnership. Feedback have extensive sales experience and knowledge of entering global markets with medical technology and therefore represent an excellent early-stage candidate partnership for Motilent. We however are extremely keen to maintain as much control in the company at this stage and TSB funding will be essential to enabling us to do this.

TSBs provision of 100% FTE academic funding allows Motilent to bring in the countries leading academics and invests them early in the project. As stated throughout this application, leading clinical-academics serve across research, clinical, regulatory and commercial disciplines making their input on this project crucial both in the early development and later stages for clinical uptake. By providing 100% FTE (especially considering the current high HEI overheads) this permits a valuable reciprocal arrangement between Motilent and the partners allowing them to make additional staff appointments and generate new research and grants. TSB support of our parallel workflow between SME and HEIs further allows dynamic exchange to quickly produce a high quality user solution. Often, a commercial beta product is tested by its users and then reiterated taking time and impacting on user confidence. We believe that this proposal maximises the value of the Biomedical Catalyst using clinical feedback to direct product development at the earliest possible stage and as a team. It is well recognised that medical diagnostics take a long time to develop [Deloitte] and do not represent the ideal short-term investment valued by venture capitalists despite often high eventual returns.

The accrued interest on business loans makes financing of such projects by a small SME challenging. Biomedical Catalyst is extremely suited to Motilent's current

needs where an evidence base, product and service need to be created from new. The three-year duration of the project is additionally of value where time can be invested in developing user-interface components of the service that would otherwise be categorised as low-priority. For example the user-interface is essential, especially where we see the technique being used outside of radiology by users largely unfamiliar with medical imaging in research. This is widely accepted as one of the primary reasons medical imaging research is not translated to the clinic.

Motilent will provide value to the UK economy through several ways both in the short and long term. First, it will provide jobs both directly by employing software engineers and technicians. As the business grows, marketing and client engagement will become increasingly important and result in further appointments. Jobs will also be provided indirectly through research applications using the technique, UCL is currently advertising a PhD and a post-doctoral position focused around advanced GI quantitation techniques inspired by the success with GIQuant and this proposal will employ another 1.5 people to conduct research using this tool. Motilent will add value through stimulating research in the UK. GIQuant is compatible with a widely used imaging modality and therefore the ability for UK institutions to conduct research using the technique will be greatly increased following the development of a commercial product. The indirect value of research comes through the award of high value grants which help consolidate the UKs position as a global research centre and attract high-calibre students and academics from around the world.

During the course of the project Motilent will generate revenue through trading with academic users. Even if no clinical value in GIQuant exists, the academic findings from the project will have a major impact on our current understanding of the bowel dysmotility. If GIQuant never becomes more than a research tool, its commercialisation will be valuable to the research community and still capable of generating revenue through licensing to other PACS providers (Biotronics 3D, Feedback, Osirix MD etc) at almost no running cost to Motilent. Motilent plans to generate additional revenue in exchange for equity where necessary as discussed above. Where clinical value is demonstrated, the GIQuant reporting service will be rolled out to specialist clinics and medical centres. Motilents business model means that all data will be processed in the UK and by UK based technicians maximising UK generated revenue. At 5 years following the conclusion of this project, we anticipate our inclusion into clinical guidelines which will significantly strengthen our market position. The infrastructure and paradigm developed during the project will allow the development of new tools both within and outside of the GI tract and expansion of the service. Motilent's lead advisor (Prof. D. Hawkes) is the director of the Centre for Medical Image Computing (CMIC) one of the worlds leading medical imaging science groups. Key projects of relevance to GIQuant is the newly developed and validated Colon CTC registration algorithm for polyp detection in the bowel cancer screening program. The close connection between UCL and Motilent will enable translation of this work and produce competitive products to rival companies offerings.