Investigation Into The Relationship Between Intestinal Slow Waves, Spike Bursts, and Motility

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Bioengineering, Auckland Bioengineering Institute, The University of Auckland, 2022.
Abstract

The small intestine musculature consists of longitudinal and circular muscle layers, whose coordinated contractions and relaxations facilitate the breakdown and digestion of food via motility. Intestinal motility is governed by a multitude of regulatory mechanisms including neuronal, hormonal, myogenic means, and is associated with two bioelectrical events: (i) slow waves generated and propagated by interstitial cells of Cajal, and (ii) spike bursts which are believed to be calcium currents in the smooth muscle cells. The relationship between slow waves, spike bursts, and motility is of critical interest to the intestinal function, but is not clearly defined at the organ level. Recent high-resolution electrical mapping has significantly improved the experimental and clinical understanding of slow wave activity in normal and dysrhythmic states. However, a similar level of understanding has not been translated to spike bursts. Furthermore, electrophysiological studies are generally performed separately from motility measurements, and there is a need to integrate these two modalities to understand the electrophysiological basis of motility. A limited number of simultaneous electrophysiological and motility studies exist, but are typically performed in-vitro, and have utilised 1-dimensional electrode arrays and motility measurements. This thesis aims to investigate the spatiotemporal relationships between slow waves, spike bursts, and motility, via simultaneous high-resolution mapping of bioelectrical activity and motility in the in-vivo intestine.

First, an experimental setup was developed to simultaneously measure the bioelectrical and contractile activity of exposed intestinal segments from anaesthetised pigs and rabbits in spatiotemporal detail. The bioelectrical activity was recorded using high-resolution flexible electrode arrays (16 × 8 configuration, 4 mm inter-electrode spacing). The contractile activity was simultaneously recorded using a cross-polarised camera setup (5-megapixel camera BFS-U3-50S5M-C, Blackfly S, FLIR, USA; fitted with lens HF25SA-1, Fujifilm, Japan; 70 × 59 mm field of view) at 20 frames-per-second, and was synchronised with the bioelectrical recordings. Slow waves were detected, and frequency, amplitude, velocity were quantified using existing methods. A new automated framework was developed for spike burst analysis. The framework included detection of spike bursts and clustering them into patches, validated by manual review. The spike burst patches were segmented into longitudinal, circumferential, and propagating circumferential patches based on the activation patterns of spike bursts. Spike bursts and spike burst patches were quantified by calculating frequency, amplitude, duration, patch size, energy, and velocity.

A method based on free-form deformation was developed to map and quantify in-vivo motility patterns in high spatiotemporal detail. The deforming geometry of the intestine in video sequences was modelled by a biquadratic B-spline mesh. Then, Green-Lagrange strain fields
were computed based on the change in geometry to quantify the surface deformations from motility. A nonlinear optimisation scheme was applied to mitigate the accumulation of tracking error associated with image registration. The strain error was maintained under 1 % and the optimisation scheme was able to reduce the rate of strain error by 97 % during synthetic tests. The algorithm was able to generate 2D strain fields to quantify the contractile activity across the surface of the in-vivo intestine including anisotropic contractions, and were able to simultaneously map the coordinated activity of the circular and longitudinal muscle layers during motility patterns. The level of contraction (strain), rate of contraction (strain-rate), frequency, and velocity of propagation were calculated to quantify motility patterns.

The experimental setup and analytical methods were applied to investigate the spatiotemporal dynamics between slow waves, spike bursts, and motility in the in-vivo jejunum of 6 anaesthetised pigs and 3 rabbits. Two types of spike bursts were observed: (i) smaller morphology slow wave associated spike bursts that activated periodically with slow waves (0.1 ± 0.1 mV, 0.8 ± 0.3 s, 10.8 ± 4.0 cpm in pigs; 0.1 ± 0.1 mV, 0.4 ± 0.2 s, 10.2 ± 3.2 cpm in rabbits), and (ii) larger aperiodic independent spike bursts that were not associated with slow waves were observed in pigs (1.4 ± 0.8 mV, 1.8 ± 1.4 s, 3.2 ± 1.8 cpm). Spike bursts activated as longitudinal or circumferential patches with associated contractions in the respective directions. The level of contraction correlated with the amplitude, size, and energy of spike burst patches. The rate of contraction correlated with the amplitude, duration, size, and energy of spike burst patches. Segmental contractions of 16 ± 9 % in pigs were spatially correlated with circumferential patches of independent spike bursts. Pendular longitudinal contractions of 19 ± 6 % in pigs, 12 ± 4 % in rabbits were spatially correlated with longitudinal patches of slow waves associated spike bursts. Propagating circumferential patches of independent spike bursts led to spontaneous peristaltic contractions of 36 ± 4 % in pigs, which propagated slower than slow waves (3.7 ± 0.5 mm/s vs 10.1 ± 4.7 mm/s slow-wave velocity, \( p = 0.007 \)). Propagating circumferential patches of slow waves associated spike bursts led to cyclic peristaltic contractions of 17 ± 2 % in rabbits, which occurred at a similar frequency to slow waves (11.0 ± 0.6 cpm vs 10.8 ± 0.6 cpm slow wave frequency, \( p = 0.97 \)), and propagated at a similar velocity to slow waves (14.2 ± 2.3 mm/s vs 11.5 ± 4.6 mm/s slow wave velocity, \( p = 0.162 \)). The results demonstrated that spike burst propagation patterns dictated the resultant contractions. Spike bursts occurred coupled to slow wave activations, but also operated independent of slow wave activations, demonstrating that in the jejunum, slow waves were not always correlated to the contractile response.

The experimental setup and analysis methods were also applied to investigate the electrophysiological and contractile changes during mesenteric ischaemia, in spatiotemporal detail. Experiments were performed on 5 anaesthetised pigs with the bioelectrical and video mapping techniques as described previously. First, the baseline activity was recorded, then the mesenteric vessels supplying to the intestinal segment were clamped to induce ischaemia (for 18.2 ± 9.0 minutes), and again unclamped to record the activity during reperfusion (for 3.5 ± 1.0 minutes). Slow wave entrainment within the ischaemic region diminished, resulting in sporadic slow wave activations and a reduction in frequency from 12.4 ± 3.0 cpm to 2.5 ± 2.7 cpm (\( p = 0.0006 \)). The deterioration of slow waves blocked the slow wave propagation across the ischaemic intestinal segment, and decoupled the distal slow wave activity from the proximal slow waves. During
reperfusion, slow waves regained the normal rhythmic nature, increased to 11.5 ± 2.9 cpm, and propagated throughout the previously ischaemic segment. Spike burst frequency increased during ischaemia from 1.1 ± 1.4 cpm to 8.7 ± 3.3 cpm ($p = 0.0003$), activated as propagating and non-propagating circumferential patches, and caused a spasm of circumferential contractions. During reperfusion, the frequency of spike bursts decreased to 2.7 ± 1.4 cpm, and contractions subsided. The intestine also underwent tonal contraction during ischaemia, with the diameter decreasing from 29.3 ± 2.6 mm to 21.2 ± 6.2 mm ($p = 0.0020$). During reperfusion, the intestinal diameter increased to 27.3 ± 3.9 mm. The intestinal slow wave, spike burst, and diameter measurements were not statistically different for baseline and reperfusion ($p > 0.05$). The decrease in slow waves, increase in spike bursts, and the tonal contraction can objectively identify ischaemic segments in the intestine and could also be used to verify successful revascularisation during surgery.

The work presented in this thesis improves the understanding of the relationship between bioelectrical slow waves, spike bursts, and motility in the intestine. In conclusion, spike burst propagation primarily dictates the motility patterns, and slow waves play a key role in initiating cyclic motility patterns by coordinating spike burst activations. This thesis also demonstrates that abnormal slow waves, spike burst activity, and abnormal contractile patterns could be used to diagnose gastrointestinal conditions such as mesenteric ischaemia. In addition, the methods developed in this thesis can be translated into other areas of the gut to investigate motility patterns and electrophysiological control.
To my parents Athula & Chandrani Kuruppu,
and to my brother Supun.
Acknowledgements

First and foremost, I am deeply grateful to my supervisors Dr. Niranchan (Nira) Paskaranandadavadel and Prof. Leo K. Cheng for providing me this opportunity, and for introducing me to this fascinating field of gastrointestinal (GI) electrophysiology and motility. I would like to thank Nira for his excellent supervision, supportiveness, encouragement, endless dedication, and guidance throughout the course of my PhD. I would also like to thank Leo for his excellent supervision, patience, guidance, and constructive feedback, that contributed to a rewarding research experience for me. I have greatly benefited from their keen research insight and scientific rigour, and the work provided in this thesis would not have been possible without their support.

Secondly, I would like to thank all those who have generously contributed their time and effort to the work presented in this thesis. I would like to thank Dr. Timothy R. Angeli-Gordon for his assistance with in-vivo experimental mapping studies and for his constructive feedback throughout the course of this PhD. I would like to thank Dr. Recep Avci for his assistance with statistical methods and for his advice. I would also like to express my sincere gratitude to Mrs. Linley Nisbet for her technical expertise during the in-vivo experimental mapping studies. Furthermore, I thank Prof. Poul Nielsen and Dr. Prasad Babarenda Gamage for their assistance while developing the motility mapping algorithm and the camera setup, for their many innovative ideas, and for the constructive feedback. I would also like to thank Dr. Alex Dixon, Dr. Sam Richardson, and Dr. Amir Hajirassouliha for their advice while designing the camera setup.

I thank all the members of the GI group (past and present) for being a wonderful team to work with. I would like to specially thank Jaime Lara, Alexander Chan, Henry Han, and Zahra Aghababaie – the Symonds Street side of the GI group, for their collegiality and for the lively discussions during our many coffee breaks. Thirdly, I would like to thank Health Research Council of New Zealand and Auckland Bioengineering Institute for funding the work presented in this thesis.

Last, but not least, I would like to thank my parents for encouraging me to pursue postgraduate studies, recommending University of Auckland, and for supporting me throughout my journey. I would also like to thank my brother for his encouragement, and without him constantly questioning when I am going to finish, this thesis would still not be complete.
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<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>Arachidonic Acid</td>
</tr>
<tr>
<td>AC</td>
<td>Adenylate Cyclase</td>
</tr>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>ANO1</td>
<td>Anoctamin-1</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>Ca/CaM</td>
<td>Calcium-Calmodulin</td>
</tr>
<tr>
<td>CaM</td>
<td>Calmodulin</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic Guanosine Monophosphate</td>
</tr>
<tr>
<td>CICR</td>
<td>Calcium Induced Calcium Release</td>
</tr>
<tr>
<td>cpm</td>
<td>Cycles Per Minute</td>
</tr>
<tr>
<td>CPU</td>
<td>Central Processing Unit</td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DMap</td>
<td>Diameter Map</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiography</td>
</tr>
<tr>
<td>EGG</td>
<td>Electrogastrography</td>
</tr>
<tr>
<td>ENS</td>
<td>Enteric Nervous System</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
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<tr>
<td>FEVT</td>
<td>Falling-Edge Variable Threshold</td>
</tr>
<tr>
<td>FFD</td>
<td>Free-Form Deformation</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
</tr>
<tr>
<td>FGID</td>
<td>Functional Gastrointestinal Disorder</td>
</tr>
<tr>
<td>GC</td>
<td>Guanylate Cyclase</td>
</tr>
<tr>
<td>GEMS</td>
<td>Gastrointestinal Electrical Mapping Suite</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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xxvii
<table>
<thead>
<tr>
<th>Acronyms</th>
<th>Description</th>
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<tbody>
<tr>
<td>GPCR</td>
<td>G-Protein Coupled Receptor</td>
</tr>
<tr>
<td>GPU</td>
<td>Graphics Processing Unit</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable Bowel Syndrome</td>
</tr>
<tr>
<td>ICC</td>
<td>Interstitial Cells Of Cajal</td>
</tr>
<tr>
<td>IP&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Inositol 1,4,5-Trisphosphate</td>
</tr>
<tr>
<td>ISB</td>
<td>Independent Spike Burst</td>
</tr>
<tr>
<td>MAD</td>
<td>Median Absolute Deviation</td>
</tr>
<tr>
<td>MENG</td>
<td>Magnetoenterography</td>
</tr>
<tr>
<td>MLC</td>
<td>Myosin Light Chain</td>
</tr>
<tr>
<td>MLCK</td>
<td>Myosin Light Chain Kinase</td>
</tr>
<tr>
<td>MLCP</td>
<td>Myosin Phosphatase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>Pi</td>
<td>Inorganic Phosphate</td>
</tr>
<tr>
<td>PIP&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phosphatidylinositol 4,5-Bisphosphate</td>
</tr>
<tr>
<td>PKA</td>
<td>CAMP-Dependent Protein Kinase</td>
</tr>
<tr>
<td>PKG</td>
<td>CGMP-Dependent Protein Kinase</td>
</tr>
<tr>
<td>PLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phospholipase A2</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>PMU</td>
<td>Pacemaker Unit</td>
</tr>
<tr>
<td>RMS</td>
<td>Root Mean Square</td>
</tr>
<tr>
<td>ROI</td>
<td>Region Of Interest</td>
</tr>
<tr>
<td>SALPA</td>
<td>Suppression Of Artefacts By Local Polynomial Approximation</td>
</tr>
<tr>
<td>SERCA</td>
<td>Sarco/endoplasmic Reticulum Calcium ATPase</td>
</tr>
<tr>
<td>SMC</td>
<td>Smooth Muscle Cell</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal To Noise Ratio</td>
</tr>
<tr>
<td>SQUID</td>
<td>Superconducting Quantum Interference Device</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic Reticulum</td>
</tr>
<tr>
<td>SSD</td>
<td>Sum Of Squared Difference</td>
</tr>
<tr>
<td>SWASB</td>
<td>Slow Wave Associated Spike Burst</td>
</tr>
<tr>
<td>TRPC</td>
<td>Transient Receptor Potential Cation Channel</td>
</tr>
<tr>
<td>TTL</td>
<td>Transistor-Transistor Logic</td>
</tr>
<tr>
<td>UP</td>
<td>Unitary Potential</td>
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VIP  Vasoactive Intestinal Peptides
Chapter 1

Motivation

The gastrointestinal (GI) tract plays a pivotal role in sustaining life by providing a constant supply of nutrients required for our daily tasks, including repair and growth of tissues. Nutrients are extracted and absorbed primarily in the intestine through digestion of ingested food, aided by the coordinated contractions of the musculature known as motility. GI disorders are highly prevalent in today’s society. In particular, functional gastrointestinal disorders (FGIDs) are a major challenge, which are characterised by chronic symptoms (abnormal motility, altered gut sensation, gut-brain dysfunction) in the absence of demonstrable pathology on conventional testing [1]. A recent epidemiological study performed on 33 countries reported that more than 40% of people worldwide suffer from FGIDs [2]. Of all FGIDs, functional intestinal disorders were the most prevalent, with 33.4% of internet participants suffering from at least one of the six disorders considered, including debilitating conditions such as functional diarrhoea, functional constipation, functional distension, and irritable bowel syndrome (IBS) with symptoms such as cramping, abdominal pain, bloating, gas, and diarrhoea. In New Zealand, dyspepsia is reported by 30 – 34.2%, constipation by 9.9%, diarrhoea by 2.5% and IBS by 21% of adults [3]. FGIDs also pose a significant economic burden on the society, where for instance, in the United Kingdom, treatment costs had amounted to £72.3 million in the year 2014 to 2015 [1]. In New Zealand, acute gastrointestinal illnesses results in 1 million visits to the general medical practitioner per year, more than 300,000 antibiotics being dispensed, and more than 4.5 million days of paid work lost [4]. GI disorders also contribute to a substantial burden on the health care system, where for example in Canada 12% of the hospital admissions were related to GI disorders (excluding cancer), compared to 9% for circulatory diseases, and 23% for respiratory diseases [5].

GI motility is governed by co-regulatory mechanisms including neural, hormonal, and myogenic means [6]. GI motility is electrophysiologically underpinned by two bioelectrical events: (i) an omnipresent rhythmic change in potential called slow waves, which are generated and propagated by interstitial cells of Cajal (ICC), and (ii) fast fluctuations known as spike bursts which are believed to be calcium currents in the smooth muscle cells (SMCs) that trigger contractions [7]. Dysrhythmic slow waves and ICC defects have been observed with functional motility disorders, linking disordered electrophysiological pathways as a mechanism for perpetuating motility disorders [8]–[10]. In addition, ICC defects have been observed in conditions such as intestinal pseudo-obstruction [9], slow-transit constipation [11], and IBS [10]. Abnormal slow wave activities
have also been observed in GI disorders, such as with diabetes [12], post-surgical dysmotility [13], mesenteric ischaemia [14], and mitochondrial neurogastrointestinal encephalomyopathy [15].

The relationship between bioelectrical events and motility, and the abnormal electrophysiology observed with GI disorders had resulted in a crucial interest to utilise bioelectrical activity as clinical biomarkers. Therapeutic techniques derived from the cardiac field to correct arrhythmias are being investigated to correct dysrhythmic slow wave propagations, such as with pacing [16] and ablation of ectopic slow wave initiations [17], as potential treatments for GI disorders. Slow waves are also being investigated as a potential biomarker for functional motility disorders [8], [18]. The diagnostic utility of slow waves also stems from the cardiac field, where electrocardiography (ECG) is used to detect cardiac abnormalities such as arrhythmias, coronary heart disease, and myocardial infarction [19]. Electrogastrography (EGG) and surface electroenterograms, where slow wave activity is measured using electrodes placed on the abdomen, and magnetogastrography and magnetoenterography (MENG), where slow wave activity is measured using superconducting quantum interference device (SQUID) magnetometers, are being investigated as non-invasive techniques to assess dysrhythmias in the stomach and the intestine [20]–[23]. For instance, Richards et al. and Somarajan et al. have shown promising results on detecting mesenteric ischaemia using the reduction of slow wave frequency measured with MENG recordings [23], [24]. However, diagnosing intestinal disorders still remains a major challenge in clinical pathology. For instance, acute mesenteric ischaemia has a mortality rate of 60 - 80 %, which has not improved over the last 70 years [25]. The use of functional bioelectrical markers such as slow waves and spikes could offer novel diagnostic techniques to support clinical decision making.

1.1 Challenges

Organ level GI electrophysiology was pioneered by Alvarez, with his paper on the discovery of slow waves in the stomach in 1922, where he states “we have, then, at our disposal a new method with which to study the activities of the digestive tract” [26]. The statement had become true over the last century and had opened new avenues for therapies and diagnostic tools to combat GI disorders. However, GI electrophysiology as a clinical utility is derived from its relationship to motility, and yet functional and spatial implications of slow waves and spike bursts on motility are not well understood.

Spike bursts display a stronger association with contractions [27], [28], while slow waves can be observed even without contractions [29]. However, electrophysiological investigations with high-resolution electrical mapping techniques have been largely limited to slow waves except for a limited number of studies focused on the spatial patterns of spike bursts published by Lammers et al. [12], [29], [30]. Previous studies have shown different spike burst activation patterns and interactions with slow waves with different motility patterns [12], [31], [32], which indicates that the spatial activation patterns of spike bursts and their affinity to slow waves play a key role in determining the motility patterns. However, existing studies do not show a clear spatiotemporal correlation between the spike burst activation patterns and the motility patterns due to technological limitations. Some studies have predominantly focused on the bioelectrical activity
and have not mapped or quantified the motility patterns [12], [33]. Other studies have utilised
1-dimensional electrode arrays and motility measurements, and therefore, have not captured or
correlated the 2-dimensional spike burst, slow wave activations on the tissue surface with surface
deformations [31], [32]. In addition, existing studies have predominantly been performed in-vitro
in tissue baths [12], [32]–[34], and may not represent the integrative electrophysiological state of
the gut. As a result, the spatiotemporal correlation of electrophysiology and motility patterns
has not been conclusively shown. Simultaneous in-vivo high-resolution electrical and motility
mapping studies are needed to define the relationship between slow waves, spike bursts, and
motility.

Another contributing reason for the lack of simultaneous spatiotemporal investigations of
slow waves, spike bursts, and motility, is the 1-dimensional nature of the existing motility de-
formation measurement techniques. Although extracellular electrical mapping techniques have
improved over the years to measure the bioelectrical activity across the 2D surface of the tis-
sue with high-resolution electrical mapping, motility contractile patterns in the intestine are
still quantified based on the diameter along the length of the intestine (circumferential con-
tractions) [35]–[38], or the distance between markers placed along the intestine (longitudinal
contractions) [39]. Methods have been developed to map motility patterns based on strain-rate
with higher spatial resolution, but they do not measure the level of contraction of the tissue, but
rather the rate of contraction [40], [41]. A motility mapping technique that can quantify the con-
tractions and relaxations across the 2D surface of the tissue could be paired with high-resolution
electrical mapping to greatly enhance our understanding of motility.

Simultaneous high-resolution electrical and motility mapping can also be applied to inves-
tigate intestinal disorders where dysrhythmic activity can be quantitatively and qualitatively
assessed. For instance, during mesenteric ischaemia previous studies have reported a decrease in
slow wave events, increase in spike bursts, dysrhythmic slow wave propagations, and spasms of
contractions [14], [18], [42]–[45]. However, high-resolution electrical mapping has only been ap-
plicated to mesenteric ischaemia in two studies: one study exclusively on slow wave propagation [14],
and another solely on spike bursts [45]. A simultaneous spatiotemporal analysis of slow waves and
spike bursts has not been performed during mesenteric ischaemia. Furthermore, the abnormal
contractile activities during mesenteric ischaemia have not been quantified, or analysed in rela-
tion to the bioelectrical activity. Therefore, simultaneous high-resolution electrical and motility
mapping will enable a better understanding of bioelectrical pathways and contractile function
during mesenteric ischaemia.

1.2 Thesis objectives

The research presented in this thesis aims to achieve the following objectives to address the
challenges outlined in the previous section.

1. Develop methods to simultaneously map and quantify bioelectrical slow waves, spike bursts,
and motility, in high-resolution across the surface of the in-vivo intestine (Chapters 3–4).
2. Utilise the new simultaneous bioelectrical and motility mapping technique to investigate the spatiotemporal relationship between slow waves, spike bursts, and motility of the in-vivo intestine (Chapter 5).

3. Utilise the new simultaneous bioelectrical and motility mapping technique to investigate the electrophysiological and contractile changes during mesenteric ischaemia (Chapter 6).
Chapter 2

Background

GI motility plays a key role in sustaining life, and is governed by co-regulatory mechanisms of neural, hormonal, and myogenic means. This chapter outlines the various mechanisms involved in coordinating GI motility, particularly focusing on the small intestine. The chapter reviews the current understanding of the intestinal electrophysiology and its relationship to motility in health and during mesenteric ischaemia. The chapter also reviews the existing recording modalities for intestinal bioelectrical and mechanical activities.

2.1 The digestive system

Digestion is the process by which ingested food is broken down into small water-soluble molecules for absorption. These molecules are subsequently used in the function, repair, and growth of tissues in the body. To that end, the digestive system plays a critical role for sustaining life, by providing the body with a continual supply of water, electrolytes and nutrients. The digestive system consists of the GI tract (Fig. 2.1), salivary glands, pancreas, liver, and gallbladder; with distinctive roles to facilitate digestion, absorption, excretion and protection.

The GI tract is a continuous tube comprising of several organs extending from the mouth to the anus, where active processes of digestion takes place. The key parts of the GI tract from proximal to distal are: mouth, oesophagus, stomach, small intestine, colon, and rectum as shown in Fig. 2.1. Digestion begins in the mouth where the ingested food is masticated i.e., chewed, and mixed with the digestive enzymes secreted by the salivary glands. The resulting bolus of food, known as chyme, is transferred through the oesophagus to the stomach where it is stored and mixed with gastric acid. The contents of the stomach empties into the small intestine, where most of the absorption of nutrients take place. Finally, the remaining content is transferred to the colon (large intestine), which is primarily responsible for desiccation and compaction of waste, with storage in the rectum prior to elimination via the anus.

2.2 Intestinal anatomy

The small intestine is a tubular structure, about 7–8 m in length and 3–4 cm in diameter in adult humans. It is attached to the posterior abdominal wall and suspended by a thin membranous
Background

Figure 2.1: The GI tract runs from the mouth to the anus, and contains the primary organs of the digestive system. The main organs of the GI tract are labelled with arrows, and the direction of propagation towards the mouth is known as retrograde and towards the anus is antegrade. Adapted from [46].

material called the mesentery, which gives it greater mobility within the abdomen. The mesentery contains blood vessels, lymphatics, nerves to supply the intestine, and areas of fat to retain heat in the organs. The intestine plays several key roles such as breakdown, mixing, transport of chyme, chemical digestion with enzymes, and absorption of nutrients.

The anatomy of the intestine is shown in Fig. 2.2. It consists of three sections: duodenum, jejunum, and ileum. The duodenum is the most proximal part of the intestine and also the shortest. It is a C-shaped structure of about 25 – 38 cm in length, which connects to the stomach through the pylorus, and ends at the ligament of Treitz. It receives chyme mixed with gastric acid from the stomach in a controlled manner regulated by the pyloric sphincter. It also receives digestive enzymes and bile from the liver and the pancreas, which are combined to chemically break down chyme. The duodenum is connected to the jejunum through the duodenojejunal flexure. The end of the duodenum and the start of the jejunum can be identified by the ligament of Treitz, which is a suspensory muscle that tethers the junction between them [47]. The jejunum is the longest section of the intestine, approximately 2.5 m in length, and specialises in the absorption of nutrients from chyme. The ileum is the final section of the intestine. The start of the ileum is unclear as it does not share a clear division with the jejunum, but it can be identified by the smaller diameter compared to the jejunum [48]. Its primary function is to absorb vitamin B12, bile salts, and other products of digestion not absorbed by the jejunum. The ileum ends with the ileocecal valve through which the small intestine connects to the cecum of the large intestine.

The intestinal wall is made up of several layers as seen in the cross-section in Fig. 2.3. The outermost layer is called the serosa. It is made of simple squamous epithelium tissue and secretes serous fluid to lubricate the sliding movements of the intestine against other surfaces.
The next layer is muscularis externa, which contains longitudinal and circular muscle layers. The muscle layers are made up of smooth muscle cells that are arranged longitudinally in the longitudinal muscle layer and along the diameter in the circular muscle layer, respectively. The longitudinal muscle cells deform along the length, shortening and lengthening the intestinal segment, and circular muscle cells deform along the circumference, increasing or decreasing the diameter of the intestine. The coordinated contractions and relaxations of these muscle layers help breakdown, mix, and transport chyme, through a process known as motility. Between the
two muscle layers lies the myenteric plexus, also known as the Auerbach’s plexus. The myenteric plexus contains enteric neurons with ascending and descending nerve fibres. These nerve fibres, when triggered by events such as distension, stimulate the muscle layers by releasing neurotransmitters such as acetylcholine (ACh) and substance P, or inhibit them by releasing vasoactive intestinal peptides (VIP) and nitric oxide (NO) at different locations to facilitate motility [52], [53].

Next to the muscularis externa lies the submucosal layer. The submucosa is a dense irregular layer of connective tissues that contains large blood vessels, lymphatic vessels, and neurons that support the mucosa. The blood vessels in the submucosa transport the nutrients absorbed by the mucosal layer to other regions of the body. It also contains a network of neurons that form the submucosal plexus, also known as the Meissner’s plexus. These neurons can trigger ganglionic secretions in the presence of certain chemicals in chyme, such as acids, which aids in the digestion and protection of the intestine [54].

The mucosal layer is the final layer of the intestinal wall, and is in contact with chyme. The mucosa is made of three layers. The topmost layer is the epithelium, where most of the digestive, absorptive, and secretory processes occur. Below that lies a layer of connective tissue called lamina propria. The bottom-most layer of the mucosa is the muscularis mucosae, which is a thin layer of smooth muscle. The mucosal layer of the intestine specialises in absorption. It contains circular folds that protrude into the lumen to increase the surface area available for absorption. On top of that, it contains small protrusions called villi and even smaller protrusions on the villi called microvilli to further increase the surface area. The mucosal layer also secretes electrolytes and other enzymes to aid in digestion and to protect the GI system from infections.

2.3 Smooth muscle cells (SMC)

The mechanical contractions of the intestine are mediated by SMCs. There are two major types of smooth muscles: single-unit and multi-unit, of which the intestinal smooth muscle are single-unit or unitary smooth muscle. Unitary smooth muscle consists of thousands of SMCs (180,000 cells/mm³ [55]) arranged into sheets or bundles. In the case of intestinal smooth muscle, they are arranged into circular and longitudinal layers, where the cells are oriented along the circumference or the length of the intestine, respectively. The SMCs are electrically and mechanically coupled to their neighbours to form a functional syncytium such that multiple SMCs undergo contraction when activated.

Intestinal SMCs are mononucleate spindle-shaped cells, about 500 µm in length and 5 µm in diameter in the relaxed state. An illustration of SMCs is given in Fig. 2.4. As much as 80 % of the SMC volume is composed of contractile filaments and dense bodies [56]. They also contain ion channels, various receptors, second messenger generators, and intracellular calcium stores called sarcoplasmic reticulum (SR) required for contractions, and control of muscles through endocrine, neurocrine, paracrine, myogenic means [57]–[59]. There are three types of filaments found in SMC: thin actin filaments, thick myosin filaments, and intermediate filaments. Actin and myosin filaments generate contractile force in the muscles through a sliding mechanism known as
Smooth muscle cells (SMC) cross-bridge cycling [55], [56]. The actin filaments are attached to the cell through electron rich regions known as dense bodies. Dense bodies cover about 50% of the cell membrane surface area and are also present within the cell. They are attached to the cell membrane by the intermediate filaments. This arrangement ensures that during cross-bridge cycling of actin and myosin, the cell membrane is pulled inwards, contracting the cell [55].

![Figure 2.4](image.png)

Figure 2.4: The physical structure of smooth muscle cells. The upper illustration shows actin filaments radiating from the dense bodies. The bottom illustration shows the relation of myosin filaments to the actin filaments in the smooth muscle cells known as the sidepolar cross-bridge arrangement that generate contractile forces. Adapted from [60].

The cell membranes of SMCs have small invaginations called caveolae, which play a crucial role in the deformability of SMCs [55]. Caveolae increase the surface area of the SMCs by about 70%. When contracting, these invaginations fold, giving the cells a corrugated appearance. These folds of adjacent cells can interdigitate, allowing SMCs to shorten considerably more than other muscle cells, such as skeletal muscle. When distending, the caveolae open up, further stretching the membrane, and gives the muscle cells a smooth appearance. Caveolae also play a role in the electrical signalling of the SMCs [61]. Caveolae contain ion channels, receptors, and second messenger generators, that aid in signalling in SMCs. These signalling mechanisms can trigger the release of calcium and muscle contraction.

Neighbouring SMCs are electrically and mechanically coupled to each other through various surface junctions [55]. Gap junctions form in places where adjacent cells come into proximity. They allow ions and certain molecules to pass between cells and enable action potentials to travel from one cell to another. Gap junctions act as points of electrical coupling in SMCs and allow multiple cells to cooperate. Dense bodies of adjacent cells in close proximity form attachments with the same electron-dense material as the dense bodies, known as intermediate junctions. Dense bodies that fall outside of intermediate junctions are anchored to collagen fibrils abundant in the intercellular space. Intermediate junctions function as the mechanical coupling between SMCs. Intermediate junctions and collagen fibrils anchor the SMCs and allow movement of the cells as a whole [55].
2.3.1 Contraction of SMC

The contraction of smooth muscles occurs in response to the increase in cytosolic free calcium concentration within the cells \([\text{Ca}^{2+}]_i\). The free calcium in the cell binds to a molecule called calmodulin (CaM) to form calcium-calmodulin (Ca/CaM). Ca/CaM activates an enzyme called myosin light chain kinase (MLCK), which phosphorylates the myosin heads (i.e., phosphorylation of myosin light chain (MLC)) and starts the cross-bridge cycle. Cross-bridge cycling is inhibited by myosin phosphatase (MLCP), which detaches the phosphates from the myosin and deactivates them. Therefore, in order for the muscle to contract, \([\text{Ca}^{2+}]_i\) should be high enough to activate MLCK at greater levels than MLCP.

The activated myosin and actin undergo cross-bridge cycling as shown in Fig. 2.5. This process contracts the cell by converting chemical energy into mechanical energy through hydrolysis of adenosine triphosphate (ATP). The myosin heads are initially detached from the actin filament and have ATPs attached. After MLCK activates a myosin head, it can hydrolyse the ATP to form adenosine diphosphate (ADP) and inorganic phosphate (Pi). ATP hydrolysis puts the myosin head in the high energy cocked position, where it is able to attach to the actin filament. Once the myosin head is attached to actin, it releases the Pi, which causes the myosin head to bend forward, pulling the actin filament. This action is called the power stroke. Finally, the ADP molecule is exchanged for an ATP, which puts the myosin head back in the detached position as in step one. This cycle continues until the myosin filaments are deactivated.

![Cross-bridge cycling of actin and myosin filaments that generate the contractile force in SMC.](image)

Smooth muscle contraction can be regulated through a multitude of mechanisms. Some of the major pathways that could contract or relax SMC are shown in Fig. 2.6. SMC contraction can occur through electromechanical coupling due to the depolarisation of the membrane, or
through pharmacological coupling due to the action of various agonists. Depolarisation of SMCs can occur through slow waves generated in ICC or due to stretch through mechanosensitive ion channels [63], [64]. If the depolarisation reaches a certain threshold level, it could open voltage-gated calcium channels increasing $[\text{Ca}^{2+}]_i$. The increased $[\text{Ca}^{2+}]_i$ activate ryanodine receptors and release calcium from internal stores – SR, known as calcium induced calcium release (CICR) [59].

Figure 2.6: Major cellular mechanisms that regulate contraction and relaxation of SMC. Pathways leading to enhanced contraction are indicated in red, and relaxation pathways are indicated with blue. The arrows indicate activation/stimulation of compounds, while lines ending with a bar indicate inhibition. Membrane depolarisation can cause contractions through the opening of voltage-gated calcium ($\text{Ca}^{2+}$) channels. Neurotransmitters and hormones can affect contraction by binding to G-protein coupled receptors (GPCR). Neurotransmitters such as acetylcholine (ACh) depolarise the membrane by activating non-selective cation channels such as TRPC. ACh through Gq protein release calcium from intracellular stores (sarcoplasmic reticulum). Hormones such as cholecystokinin (CCK) through Gi/Go proteins activate chloride channels. Muscle relaxation is mainly mediated by vasoactive intestinal peptide (VIP) through Gs protein and nitric oxide (NO) by activating guanylate cyclase (GC). They hyperpolarise the membrane by opening $\text{K}^+$ channels and Ca$^{2+}$ ATPase pumps. They also enhance myosin phosphatase (MLCP) activity, which in turn inhibit cross-bridge cycling and contraction. AA: arachidonic acid, AC: adenylate cyclase, CaM: calmodulin, cAMP: cyclic adenosine monophosphate, cGMP: cyclic guanosine monophosphate, DAG: diacylglycerol, Gs, Gi, Go, Gq: types of G proteins, IP$_3$: inositol 1,4,5-trisphosphate, IP$_3$R: inositol 1,4,5-trisphosphate receptors, MLCP: phospholylated myosin light chain, MLCK: myosin light chain kinase, MLC: myosin light chain, MLCP: myosin light chain phosphatase, PKA: cAMP-dependent protein kinase, PKG: cGMP-dependent protein kinase, PLA$_2$: phospholipase A2, PLC: phospholipase C, RyR: ryanodine receptors, SERCA: sarco/endoplasmic reticulum calcium ATPase.

SMC contractions are also regulated through neurotransmitters emitted by the enteric neu-
rons. The neurotransmitter ACh binds to a family of G-protein coupled receptors (GPCRs) called muscarinic receptors [57], which can activate non-selective cation channels in the transient receptor potential cation channel (TRPC) family, such as TRPC4 and TRCP6 [65]. The TRPC6 channel can also activate from stretch and cause depolarisation leading to contraction [59]. ACh can also activate phospholipase C (PLC) through Gq protein [57], which break down phosphatidylinositol 4,5-bisphosphate (PIP$_2$) into inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG). IP$_3$ activate IP$_3$ receptors in the SR and release calcium from the internal stores. On the other hand, DAG inhibits the action of MLCP and thereby enhance the contraction without affecting [Ca$^{2+}$]$_i$.

Hormones can also affect SMC contraction through GPCR. For instance, cholecystokinin (CCK) can bind to GPCR coupled to Gi/Go proteins and activate phospholipase A2 (PLA$_2$) [58]. PLA$_2$ generate arachidonic acid (AA), which in turn activate chloride channels. The chloride channels release Cl$^-$ out of the cell and thereby depolarise the membrane.

### 2.3.2 Relaxation of SMC

Muscle relaxation occurs due to the depletion of [Ca$^{2+}$]$_i$. When the membrane is repolarised through slow waves, voltage-gated calcium channels will close, and calcium will be eventually pumped out through other channels. Depletion of [Ca$^{2+}$]$_i$ dissociates Ca/CaM, resulting in decreased MLCK activity and decreased phosphorylation of myosin heads, leading to relaxation.

The main relaxant peptides in the gut are VIP and NO [56]. Hormones such as the fight or flight hormone norepinephrine can also relax GI SMC [59]. VIP and norepinephrine bind to GPCR coupled to Gs proteins and stimulate adenylate cyclase (AC). AC generate cyclic adenosine monophosphate (cAMP), which activate cAMP-dependent protein kinase (PKA). NO can diffuse into the cells and stimulate guanylate cyclase (GC). GC, in turn, generate cyclic guanosine monophosphate (cGMP), which activate cGMP-dependent protein kinase (PKG). PKA andPKG inhibit IP$_3$ receptors and stimulate sarco/endoplasmic reticulum calcium ATPase (SERCA) pumps leading to a net uptake of calcium by the SR. They also activate calcium ATPase and K$^+$ pumps, which repolarise the membrane and inhibit voltage-gated calcium channels. PKA andPKG also enhance the action of MLCP, which leads to increased dephosphorylation of myosin heads, and thus, inhibit contraction for a given amount of [Ca$^{2+}$]$_i$.

### 2.3.3 Calcium sensitisation

The force generated by SMC at a given [Ca$^{2+}$]$_i$ can be varied. Certain agonists could increase force development even when [Ca$^{2+}$]$_i$ is fixed [66]. Furthermore, different agonists can generate different levels of contractions with the same [Ca$^{2+}$]$_i$ [67]. This variation in contraction strength is achieved by a calcium-independent process known as calcium sensitisation, where the muscle constriction is tuned by varying the activity of MLCP. Calcium sensitisation help with tonic contractions [56] and enables the muscle to sustain a contraction once the initial calcium influx had dissipated [59].

Calcium sensitivity is increased by inhibiting the action of MLCP. Neurotransmitters such as ACh bound to GPCR inhibit MLCP activity through DAG [57]. GPCR can also inhibit MLCP
through AA [68] and G protein RhoA [69]. The inhibition of MLCP causes more MLC to be phosphorylated and activated, with a concomitant increase in contraction. Calcium sensitivity is reduced by cAMP and cGMP by enhancing the activity of MLCP [70].

### 2.4 Enteric nervous system (ENS)

The GI tract has its own nervous system called the enteric nervous system (ENS), which can function independently of the central nervous system (i.e., brain and the spinal cord). The ENS consists of more than 5 times the neurons than in the spinal cord, and is also known as the second brain or the ‘gut brain’ [71]. In the intestine, these neurons are mainly collected into two types of ganglia: myenteric and submucosal plexuses, and forms a highly connected mesh like system along the intestinal wall (Fig. 2.7). The autonomous nature of the ENS is evident by the fact that the intestine is capable of generating complex neurogenic motility patterns, even when it is removed and isolated from the body [72].

![Figure 2.7: A histochemically stained intestinal loop from a chick. The myenteric plexus is visible as a mesh on the intestinal wall. Reproduced from [55].](image)

The ENS consists of three types of neurons: (i) intrinsic sensory neurons, (ii) interneurons, and (iii) motor neurons. Intrinsic sensory neurons monitor the muscle and mucosal activity and transmit this information for processing in the ENS and the central nervous system [73]. These neurons are responsive to distension, luminal chemistry, mechanical stimulation of the mucosa, and are broadly divided into two types: (i) mechanosensory and (ii) chemosensory neurons [74]. Most of the neurons in the ENS are interneurons. These neurons assimilate information from sensory neurons and compute coordinated inputs to motor neurons to generate complex motility patterns [75]. Finally, motor neurons innervate the circular and longitudinal smooth muscle layers, muscularis mucosae, and secretomotor neurons innervate secretion glands [76]. The smooth muscle layers are innervated by two types of motor neurons: (i) excitatory neurons and (ii) inhibitory neurons, which contracts or relaxes the muscle, respectively [77]. Excitatory neurons innervate the muscle adjacent and just proximal to the nerve cell, and inhibitory neurons innervate the muscle just distal to the nerve cell [76]. In the circular muscle layer, each motor neuron innervate a 0.5 to 2 mm wide band that runs around the circumference, and in the longitudinal
muscle layer, each neuron innervates a patch of 2 mm long and 1 mm wide [78]. Circular and longitudinal muscle layers are innervated by neurons in the myenteric plexus, while the muscularis mucosae is innervated by neurons in the submucosal plexus [79].

Motility patterns driven by the ENS can occur through mechanotransduction (in response to mechanical stimuli) or chemotransduction (in response to chemical stimuli). For instance, one of the primary motility patterns driven by the ENS is distension induced peristalsis or the peristaltic reflex. Distension induced peristaltic contractions are triggered by stretch receptors which form a contraction proximal to the stimulated site and a relaxation distal to the site, moving the intraluminal content (that caused the stretch) forward [72]. This stretch induced peristaltic reflex is called the ‘law of the intestine’, and facilitate movement of contents. On the other hand, ENS can also induce motility patterns based on the intraluminal content, triggered by chemosensory neurons present in the mucosa, such as nutrient-induced segmental contractions [80]. The combined action of sensory neurons, interneurons, and motor neurons enable the ENS to perform intricate contractile patterns in response to the current state of the intestine.

2.5 Interstitial cells of Cajal (ICC)

In 1911, the Spanish Nobel prize laureate Santiago Ramon y Cajal observed a nerve like branching network of cells that we know today as ICC [81]. The earliest observations of stained images and drawing of ICC cells made by Cajal [82], [83] are shown in Fig. 2.8B–C. With the development of tissue preparations and electron microscopy in the latter half of the 20th century, it was discovered that ICC are closely associated with SMC and neurons. The interest in ICC was renewed in 1982 when Thuneberg proposed ICC as intestinal pacemaker cells [84]. This hypothesis was supported by the observation that selective injury of ICC stained with methyl blue abolished slow wave activity [85]. Conclusive breakthrough came when it was discovered that injecting mice with a neutralising antibody to c-kit (a type of protein receptor in ICC) impeded the development of ICC and caused GI abnormalities [86]. Later, studies showed that W/W<sup>v</sup> mice with spontaneous c-kit mutations had significantly reduced ICC and resulted in no slow wave activity [87], [88]. Recordings in rat stomachs that lack neural control confirmed ICC’s ability to generate slow waves independently [89].

ICC cells are found throughout the GI tract. They are classified into several subpopulations based on the anatomical location [90], as shown in Fig. 2.9. ICC located in the myenteric plexus are called ICC-MY or ICC-MP. Intramuscular ICC (ICC-IM) are found in the muscle layers and are further subdivided into ICC-CM in the circular muscle layer and ICC-LM in the longitudinal muscle layer. Another subgroup of ICC-IM called ICC-SEP are found in the connective tissue between muscle bundles in large animals [91], [92], where they help to conduct slow waves deep into the muscle layers [93]. ICC-DMP are located in the deep muscular plexus of the intestine, between the inner and outer sublayers of the circular muscle. Submucosal ICC (ICC-SM) and ICC in the submucosal plexus (ICC-SMP) are found in the stomach and the colon, respectively. ICC-SS, called subserosal ICC, are found in the intestine [84] and the colon [94]. The main ICC populations found in the intestine are ICC-MY, ICC-IM, and ICC-DMP [6]. ICC-MY form
Interstitial cells of Cajal (ICC)

Figure 2.8: (A) Santiago Ramon y Cajal in his lab [83]. (B) A drawing of ICC cells made by Cajal, and (C) slides of ICC cells stained with methyl blue by Cajal. Reproduced from [82].

a cellular network around the myenteric plexus and are the primary pacemaker cells of the intestine [95]. On the other hand, ICC-IM of the intestine are sparsely distributed. They do not appear to make cell networks and are oriented along the long axis of the SMCs [90]. ICC-DMP has a similar arrangement to ICC-CM and run parallel to circular muscle fibres. They are closely incorporated into the deep muscular plexus and are involved in neurotransmission [96].

Figure 2.9: ICC populations found in the GI tract. ICC-SM and ICC-SMP are the inner most ICC found in the border between submucosa and circular muscle. ICC-DMP occur between the circular muscle sublayers in the intestine, ICC-LM and ICC-CM occur in the longitudinal and circular muscle layers, respectively. ICC-SEP occur within the connective tissue between muscle bundles in large animals, and ICC-MY are located between circular and longitudinal muscle layers. Finally, ICC-SS are found within the subserosa. Reproduced from [97].
ICCs perform several key roles in the GI tract. The primary function of ICC is to act as the pacemaker cells of the gut, and to create a propagation pathway for slow waves. ICC contain gap junctions between individual cells, which facilitate the propagation of slow waves along the intestine. They also passively conduct slow waves to the SMCs through sparse gap junctions [98]. Apart from that, ICC also mediate cholinergic and nitrergic neurotransmission from the ENS [96], [99]. In addition, ICCs act as myogenic stretch receptors mediated through mechanosensitive ion channels [100] and can affect the slow wave frequency [101] and muscle excitability in response to stretch. Finally, they also play a role in setting the resting membrane potential of SMCs, through gas signalling, particularly via the influence of carbon monoxide [102].

2.5.1 Generation of slow waves

The primary function of ICC is the generation and propagation of slow waves. Several theories have been proposed on how ICC generate slow waves [103]. The following is an up-to-date description of the processes involved in the generation of slow waves as described in [92]. The mechanisms involved are summarised in Fig. 2.10.

ICC cells contain subcellular structures called pacemaker units (PMUs) formed by apposition of endoplasmic reticulum (ER) (type of SR but with additional capabilities to synthesise molecules), mitochondria, and plasma membrane containing ion channels, covering a volume of the cytoplasm. The pacemaker cycle is initiated by a stochastic release of calcium to the PMU cytoplasm from the ER via IP$_3$ receptors [104], [105]. This increases the local calcium concentration, which lead to transient activation of Anoctamin-1 (ANO1) voltage-gated calcium-activated Cl$^-$ channels, giving rise to spontaneous transient inward currents or pacemaker currents [106], and concomitant spontaneous transient depolarisations or unitary potentials (UPs) [107]. When the ICC cell reaches the threshold depolarisation due to the summation of UPs from multiple PMUs, voltage-gated dihydropyridine-resistant calcium channels are activated. The ensuing calcium entry causes additional calcium release from IP$_3$-dependent calcium channels in the ER, which rapidly depolarises the cell, initiating the slow wave. The ANO1 channels require a lower calcium concentration to activate at more positive potentials [108]. Therefore, the depolarisation could activate ANO1 in the neighbouring cells leading to regeneration of the slow wave or entrainment by the region with the highest frequency, and propagation. The increased calcium concentration activates outward potassium channels such as ERG K$^+$ channels [109] and large-conductance calcium activated K$^+$ channels (BKchannels) [110], which causes repolarisation. The plateau phase of the slow wave is maintained by the balance between inward and outward conductances (Ca$^{2+}$ influx, Cl$^-$ efflux, and K$^+$ efflux). Repolarisation occurs due to the inactivation of inward currents, activation of outward K$^+$ channels such as the delayed-rectifier K$_v$1.1 channel [111], and the uptake of calcium through SERCA. Apart from the channels discussed here, other channels such as non-selective cation channels [112] and Na$^+$ channels [113] also contribute to the generation of slow waves.
2.6 Organ level electrophysiology of the intestine

Some of the cellular processes that lead to contraction described above changes the membrane potential and can be registered via extracellular electrical recordings. This was first recorded by Walter C Alvarez in 1900s when he attached a galvanometer to the stomach and the intestine using silver plated wire electrodes [26], [114]. In his experiments he observed a wave like bioelectrical activity that we know today as slow waves. He also noticed that these bioelectrical waves resemble the mechanical tension generated by the muscles, thereby demonstrating a relationship between bioelectrical slow waves and motility, effectively pioneering organ-level GI electrophysiology.
2.6.1 Slow waves

One of the first recordings of slow waves made in the cat stomach antrum by Alvarez [26] is shown in Fig. 2.11A. The morphology of slow waves in extracellular recordings performed by placing electrodes on the serosal surface of the GI organs are biphasic, while those directly recorded across the cell membrane via intracellular electrical recordings are typically monophasic (Fig. 2.10E). The biphasic extracellular slow waves are characterised by an upstroke and a negative deflection, that can be approximated by the second derivative of the intracellular slow wave morphology [115], [116]. A more recent biphasic extracellular recording performed in the pig intestine jejunum by Angeli et al. [117] is shown in Fig. 2.11B.

![Extracellular slow wave recordings](image)

Figure 2.11: Extracellular slow wave recordings. (A) Historical slow wave recording published by Alvarez in 1922 from the in vitro cat stomach. (B) A more recent slow wave recording from the in vivo pig intestine jejunum. Adapted from [26] and [117].

The understanding of intestinal electrophysiology had continued to grow throughout the 20th century through experimental and methodological advances. Sparsely distributed electrode recordings formed the foundation of our understanding of intestinal bioelectrical activity [26], [118], [119]. However, these low-resolution recordings were forced to make assumptions about the activity occurring between and around electrodes which precluded accurate analysis of spatial slow wave characteristics like velocity profile and propagation patterns. In 1996, high-resolution GI mapping was translated from the cardiac field by Lammers et al. [120]. High-resolution electrode arrays utilise 100s of electrodes densely packed in a 2D grid format, separated by a few millimetres. High-resolution extracellular recordings can capture the bioelectrical activity of the 2D tissue surface, and has improved our spatial understanding of slow wave propagation dynamics [34], [121].

Extracellular recordings conducted throughout the intestine ranging from duodenum to ileum have revealed the existence of a slow wave frequency gradient in the intestine. The frequency gradient has been demonstrated both in-vivo and in-vitro, in several species, including humans [119], [122], [123]. In humans, slow waves activate at 12 cycles per minute (cpm) in the duodenum, and the slow wave frequency decreases to 8 cpm at the ileum [122], [124], [125]. Similar slow wave frequency gradients have been observed in the species utilised in this thesis. For instance, in pigs, slow waves activate at 16.6 ± 0.4 cpm in the duodenum and the frequency decreases to
8.4 ± 0.7 cpm in the terminal ileum [121]. In rabbits, slow waves activate at 20.0 ± 1.2 cpm in the duodenum and the slow wave frequency decreases to 10.5 ± 0.9 cpm in the ileum [126]. The most popular theory suggests that ICC have intrinsic frequencies along the length of the intestine at which they generate slow waves. Proximal regions with higher intrinsic frequencies entrain distal regions, until the disparity between the entrained frequency and the intrinsic frequency is too large for the distal ICC to follow [76]. New pacemakers (i.e., sites of slow wave initiation) originate at such sites to entrain further distal regions, leading to the frequency gradient. The theory of proximal entrainment of distal segments is further supported by transectional studies of the intestine, where it had been observed that the slow wave frequency drops immediately distal to the site of transection [123], [127]. However, a cat in-vitro study had reported that slow waves can propagate the full length of the in-vitro intestine, and the frequency gradient is caused by occasional conduction blocks, which stops some of the waves along the intestine [128].

The amplitude of slow waves also display a general decrease from duodenum to ileum in most species [34]. For instance, in pigs, slow wave amplitudes decrease from 0.15 ± 0.02 mV in the duodenum to 0.04 ± 0.01 mV in the ileum [121]. However, in rabbits, an increasing trend has been observed for slow wave amplitudes, from 0.10 ± 0.02 mV in the duodenum to 0.29 ± 0.05 mV in the ileum [126]. In humans, slow wave amplitudes were reported as 0.3 mV in the jejunum, however, sufficient studies have not been performed to verify the amplitude gradient [129]. Apart from the amplitude gradient along the intestine, slow waves also exhibit a smooth rise and fall in the amplitude over time, known as ‘waxing and waning’ (or ‘spindling’), as shown in Fig. 2.12. Waxing and waning of slow waves is believed to be caused by low frequency transient depolarisations occurring in the ICC-DMP, which in turn modulates the slow wave amplitude [130]. However, slow wave amplitudes in-vitro have been reported to be highly regular, with waxing and waning rarely being observed, suggesting that the competing frequencies may mainly manifest in-vivo.

![Figure 2.12: Waxing and waning of slow waves (spindles) recorded in-vivo from a conscious rabbit and an anaesthetised cat. Adapted from [131].](image)

The velocity of slow waves also declines from duodenum to ileum. For instance, in pigs, slow waves propagate at 14.9 ± 1.2 mm/s in duodenum, and the velocity decreases to 8.8 ± 0.7 mm/s in ileum [121]. Similarly, in rabbits, slow waves propagate at 14.4 ± 3.4 mm/s in duodenum,
and decreases to $12.3 \pm 3.4$ mm/s in ileum [126]. In humans, slow waves propagate at 10.1 mm/s in the jejunum, but sufficient studies have not been performed to verify the velocity gradient [129]. Spatially, slow waves propagate in an anisotropic manner. The slow wave velocity is faster circumferentially than longitudinally (e.g., 1.3:1 in the cat intestine) [132]. This anisotropic conduction causes the slow waves to rapidly orient circumferentially, and thereafter propagate longitudinally like a ring of excitation.

Classically, slow waves were believed to propagate along the length of the intestine in antegrade (aboral) and retrograde (oral) directions [119]. With the advent of high-resolution bioelectrical recordings, it was apparent that the slow wave propagation in the intestine is much more dynamic and transient [34], [117]. Slow waves can originate from multiple locations along the length of the intestine (i.e., sites of initiation, typically termed ‘pacemakers’) [34]. These pacemaker locations in the small intestine are dynamic and transient in time, with new pacemakers emerging and established pacemakers becoming entrained by activation from neighbouring pacemakers, creating a complex and variable pattern of slow wave activation. Some of the slow wave propagation patterns captured with high-resolution recordings are shown in Fig. 2.13. Apart from the typical slow wave propagation patterns such as, antegrade (Fig. 2.13A), retrograde (Fig. 2.13B), and pacemaker propagation (Fig. 2.13D), patterns that are generally recognised as abnormal such as, dissociated slow wave activity due to conduction blocks (Fig. 2.13E), re-entry (Fig. 2.13F), circumferential propagation (Fig. 2.13F), have also been observed in the healthy intestine [117], [126]. For instance, in the in-vivo canine intestine, antegrade slow wave propagation has been observed in 84 % of the recordings, retrograde 11 %, conduction blocks 2 %, pacemakers 3 % [34]. In the in-vivo pig intestine, pacemakers have been observed in 31 % of the recordings, re-entry 23 %, and circumferential propagation in 35 % [117], which further highlights the dynamic nature of slow waves.

Figure 2.13: Slow wave propagation patterns observed in-vivo in the pig intestine. Slow wave propagation is depicted by plotting the slow wave activation times across the electrode array as isochrones. Circumferential propagation of slow waves can be seen in (D) pacemakers, and (F) re-entry.
2.6.2 Spike bursts

A confusion first arose when it was found that slow waves do not always lead to contractions, which was soon resolved with the discovery of spikes or action potentials [29]. Spikes were discovered by Richter et al. in the in-vivo canine stomach with suction electrodes [133]. Similar to intracellular recordings, in monophasic extracellular suction electrode recordings, these action potentials appear as one or more fast fluctuations that look like individual spikes. However, in typical biphasic extracellular recordings, these action potentials appear as intervals of fast spike like fluctuations, and are commonly referred to as spike bursts [28], [134]. Spike bursts were discovered in the intestine by Puestow et al., where they were observed as fast fluctuations appearing along with the slow waves [135]. Although spike bursts were not thoroughly investigated, Puestow et al. noted that the amplitude and the number of these fluctuations were fairly proportional to the visible activity of the intestine [135]. Three examples of spike burst recordings are shown in Fig. 2.14. A monophasic spike burst recording made by Richter et al. is shown in Fig. 2.14A, spike burst recording by Puestow et al. in Fig. 2.14B, and a more recent recording of spike bursts in the pig intestine in Fig. 2.14C.

Figure 2.14: Extracellular spike burst recordings. (A) Historical monophasic spike recording published by Richter et al. from the in-vivo canine stomach. (B) An early spike burst recording from the isolated canine intestine. (C) A more recent spike burst recording from the in-vivo pig intestine jejunum. Adapted from [133], [135].

Soon after the discovery, it was found that spike bursts are associated with contractions [136]. Spike bursts are believed to be caused by calcium currents [7], [137], such as due to the opening of voltage-dependent calcium channels [138], [139]. Simultaneous measurements in isolated SMCs taken from the guinea-pig antrum had shown that slow waves superimposed with spikes considerably increases the cytosolic calcium concentration, and increase muscle tension [63]. The
organ level relationship between spike bursts and contractions was analysed in the human intestine by Øigaard et al. [27]. The authors observed that 80% of the contractions, quantified with intraluminal pressure, spatially correlated with spike bursts, and the spike burst amplitude and duration (time length) positively correlated with the intraluminal pressure. The spike bursts also correlated with the level of outflow from the intestine, and further illustrated its association to contractions [28].

Slow waves and spike bursts display distinctly different propagation characteristics [29]. As described in Section 2.6.1, slow waves propagate throughout the intestine until they are blocked by a collision with another slow wave or a conduction block. In contrast, spike bursts only propagate for short distances and spontaneously terminate [125],[134]. These contrasting propagation dynamics of spike bursts have been clearly captured with high-resolution extracellular recordings. Lammers et al. first analysed spike activity with high-resolution recordings in the isolated duodenum of cats [140]. It was found that spike bursts propagate much faster than slow waves[140], and activate localised regions known as patches [30],[141]. For example, spike bursts propagated six times faster than slow waves in the duodenum of cats (14 ± 5 mm/s slow wave velocity vs 85 ± 33 mm/s spike velocity) [140]. Spike burst propagation is also anisotropic, and based on the primary propagation direction, two types of spike patches can be identified as shown in Fig. 2.15. Spikes that propagate predominantly in the circumferential direction activate circumferential spike patches (Fig. 2.15A), while those that propagate predominantly longitudinally activate longitudinal patches (Fig. 2.15B) [30],[141]. Circumferential and longitudinal spike patches are believed to be caused by calcium currents in the circular and longitudinal muscle layers, respectively [30]. In comparison, circumferential spikes propagate much faster than longitudinal spikes, and activates a wider region, as a ring around the intestine, while longitudinal spikes activate a much thinner longitudinal region [30].

In early studies, spike bursts were believed to be initiated exclusively by slow waves [142]. If the membrane potential caused by the slow wave depolarisation is above a certain threshold, voltage-dependent calcium channels open and initiate spike bursts [143],[144]. The relation between slow waves and spike bursts is perfectly described by Daniel et al., who stated that “slow wave is an advancing zone of enhanced excitability, which, when further enhanced by local factors, lead to action potentials and contractions” [145]. These slow wave associated spike bursts would activate with the slow wave cycles in a periodic manner [32],[34]. Even these slow wave associated spike bursts can be recorded due to two processes. When the spike bursts are initiated by slow waves, they appear in phase with the slow wave activations, while spike bursts that have conducted along the muscle layers from the surrounding regions may appear out of phase to the slow waves due to the difference in conduction velocity [144].

As described in Section 2.3.1, calcium currents can also occur without the mediation of slow waves, and in such cases spike bursts can occur independently. For example, spike bursts have been observed in W/Wv knockout mice that lack slow waves [33]. Furthermore, peristaltic waves are a type of spike burst that is independent of slow waves and have been observed during segmentation and peristaltic contractions [146] (Figs. 2.15C–D). These peristaltic waves spontaneously activate ring like segments, and propagate in aboral or oral directions, before terminating spontaneously [12], much like a propagating circumferential spike burst patch (Fig. 2.15C). Peristaltic
waves are larger than slow wave associated spike bursts ($0.05 - 5.0$ mV slow wave associated spikes vs $0.1 - 1.0$ mV peristaltic waves in the cat intestine), and propagate slower than slow waves ($12.9 \pm 2.8$ mm/s slow wave vs $9.8 \pm 2.5$ mm/s peristaltic waves), unconstrained by slow wave propagation spanning over multiple slow wave cycles [12]. In the colon, two types of spike bursts have been identified based on the origin [147]. Smaller spike bursts have been found to be resistant to neural inhibitors, and are recognised as myogenic spike bursts. Larger spike bursts have been found to be eliminated by neural inhibitors, and are recognised as neurogenic spike bursts [147], [148]. However, a similar investigation of the types of spike bursts in the intestine has not been performed.

### 2.7 Organ level electrophysiology – motility relationship

The coordinated activations of regulatory mechanisms described in Section 2.3.1 leads to various motility patterns that accomplish different roles. Non-propagating circumferential contractions, known as segmental contractions, are the most common type of contractions in the intestine [149]. Segmental contractions help break down intraluminal content, and rhythmic segmental contractions, known as the segmentation motility pattern help with both mixing and breaking down [150]. Propagating circumferential contractions, defined as peristaltic contractions, transport intraluminal content along the intestine [33], [39], [151]. Rhythmic longitudinal contractions, known as pendular contractions, help with mixing [152].
Motility patterns are underpinned by different slow wave and spike burst activations depending on the regulatory mechanisms involved. Peristaltic contractions are driven by spike bursts propagating along the length of the intestine as a sheath or a ring [12]. Two types of peristaltic contractions have been reported: (i) peristaltic contractions driven by slow waves and spike bursts, (ii) peristaltic contractions driven solely by spike bursts. Peristaltic contractions driven by spike bursts associated with slow waves that cause outflow in synchrony with slow waves have been observed in healthy animal models [32], [33]. These peristaltic contractions occur in a cyclic manner, and have also been identified separately as oscillating propagations as they can propagate in both oral and aboral directions [32]. However, peristaltic contractions can propagate in the oral direction, where they are commonly referred to as anti-peristalsis, anstalsis, or retrograde peristaltic contractions, while aboral propagation is referred to as peristalsis, katasalsis, or antegrade peristaltic contractions [33], [153], [154]. Cyclic peristaltic contractions in the intestine are driven by the coordination of both neurogenic and myogenic mechanisms [32], [33], while a similar type of cyclic circumferential contractions called myogenic ripples in the colon are reported to be myogenically mediated [36].

Peristaltic contractions solely driven by spike bursts independent of slow waves have been observed in the intestines of both healthy animal models and \(W/W^v\) knockout mice that lack ICC [12], [33]. These peristaltic contractions spontaneously originate in response to distension, and are believed to be neurally mediated [12], [33], [95]. Distension induces a reflex action in the intestine called the myenteric reflex, also known as the ‘law of the intestine’ [155]. Myenteric reflex causes a contraction proximal to the distended site and a relaxation distal to distended site, which moves the intraluminal content in the aboral direction. Spontaneous peristaltic contractions may have been mediated by the myenteric reflex, however, oral propagation of these peristaltic contractions have also been observed [12].

Longitudinal propagation of spike bursts associated with slow waves have been observed with pendular contractions [34]. However, spike bursts have been recorded with collinearly placed electrodes, therefore, the predominant propagation direction or the 2D spatial activation pattern of spike bursts has not been verified as longitudinal patches. Furthermore, pendular contractions in the intestine are anisotropic, and cause dissimilar contractile activities across the diameter [156]. For instance, larger longitudinal contractions have been observed in the mesenteric side compared to the anti-mesenteric side of the in-vitro rat duodenum [156], however, the electrophysiological basis of this anisotropic behaviour has not been investigated.

2.8 Spatiotemporal mapping of intestinal motility

X-rays had been used to observe GI wall motion as early as 1890s [150], [157] and cinematography had been used to investigate intestinal wall motion as early as 1927 [158]. In these early studies, motility patterns were observed rather than quantified. Later, video recordings were used to quantitatively analyse GI motility. In [35], the change in diameter was tracked in video recordings to detect propagating contractions and periodic wall motion in the in-vitro rat intestine (Fig. 2.16A). Hennig et al. improved upon the above method by utilising markers placed
along the length of the intestine to measure longitudinal muscle contractions in the guinea-pig small intestine [39]. Lammers et al. used markers placed in a 2D grid format (9 dots per cm) to record both longitudinal and circumferential displacements with higher resolution [7]. Bercik et al. used a new approach, where a semi-transparent liquid was injected into the intestine, and the change in illumination was used to identify contracted and relaxed regions [159].

Figure 2.16: (A) One of the earliest diameter maps (DMap) by Benard et al., where the arrow indicates a propagating contraction. (B) A more recent DMap by Kendig et al., where lighter regions indicate smaller diameter, darker regions indicate larger diameter. White arrows indicate imaging artefacts and black arrows indicate propagating contractions. Adapted from [35] and [160].

Displacements measure the wall motion, but do not quantify the deformations caused by the motility patterns, and are susceptible to rigid body motion such as breathing. At present, the most commonly used method to assess intestinal motility is by mapping the changes in diameter from a video sequence. Motility patterns are commonly represented by generating spatiotemporal maps, also known as diameter maps (DMaps), by colour coding the size of diameter along the length of the intestine (horizontal axis) at different times (vertical axis) (Fig. 2.16). DMaps have been used to analyse colonic peristalsis [36], inhibitory effects of somatostatin [37], and effects of microflora on motility [38]. DMaps have also been used to analyse the interplay between ICC and the ENS [161], and to identify different states of contractions in the colon [162].

However, diameter and marker-based methods provide 1-dimensional strain measurements (i.e., diameter along the intestine, or distance between markers placed in a row) [39]. Therefore, these traditional methods are not ideal to simultaneously map the deformations of motility patterns with the latest high-resolution extracellular electrical mapping techniques, which can record the bioelectrical activity across the 2D tissue surface. The intestine undergo localised and anisotropic motility patterns, which are difficult to capture with these traditional motility mapping methods. A typical example is that the degree of contraction on the mesenteric and anti-mesenteric side can be different [156]. Spike bursts in the intestine could also initiate localised circumferential and longitudinal contractions [7]. These localised contractile activities may not be captured by the traditional methods due to the accumulation of contractions with passive distension across the diameter and across markers.
Strain-rate fields have been used to overcome the limited resolution in diameter and marker-based measurements [40]. In this strain-rate method, the displacements of a grid of virtual points covering the region of interest of the organ were determined using normalised cross-correlation, and spatially differentiated to obtain the strain-rate field (change in strain with respect to time). Therefore, unlike diameter and marker-based methods, strain-rate fields can be used to map the activity of the 2D tissue surface [163]. Strain-rate fields have been used to analyse motility in the ileum and the stomach [40], [41], [163]. Although strain-rate fields (and strain-rate maps) have been useful in investigating GI motility, they have inherent limitations. First, strain-rate cannot be used to quantify the deformation caused by the motility patterns, but rather computes the rate of deformations. As a result, it is also difficult to map slow deformations with strain-rate. Motility patterns such as slow tonal changes in the cecum have a low time rate of deformation (strain-rate), and can be concealed by noise [163].

An alternative technique of mapping motility patterns is manometry, where contractions are quantified using intraluminal pressure sensors [164]. High-resolution manometric catheters with closely placed pressure sensors have recently been developed, and provide an excellent non-invasive method of quantifying motility patterns in the colon [164]. However, high-resolution manometric catheters can be difficult to position into the intestine, and therefore are not widely used [165]. Furthermore, due to the relative sparseness of pressure sensors (placed at 1 to 5 cm intervals) manometry cannot provide comparable spatial resolution to video mapping (where a pixel length typically corresponds to micrometres), but can span much greater distances. Video mapping appears to be the best option considering cost, complexity and availability. However, accurate algorithms that can measure the intestinal deformations with comparable spatiotemporal resolution to high-resolution electrical mappings need to be developed.

2.9 Electrophysiology and motility in mesenteric ischaemia

Mesenteric ischaemia is a condition caused by inadequate blood flow to the intestine, and characterised by ‘pain out of proportion to physical examination’ [166]. Other symptoms of mesenteric ischaemia includes nausea (44 % of cases), vomiting (35 %), diarrhea (35 %), heart rate over 100 beats per minute (33 %), rectal bleeding (16 %), and constipation (7 %) [167]. Mesenteric ischaemia can occur suddenly in the acute form with sudden severe abdominal pain, or gradually in the chronic form, with the cardinal symptom being abdominal pain that sets in 1 to 2 hours after a meal [168]. Chronic mesenteric ischaemia affects about 1 per 100,000 people per year [169]. Out of the highly collateralised three main branches that supply the intestine (celiac axis, superior mesenteric artery, and inferior mesenteric artery), compromise of at least two are required to cause chronic mesenteric ischaemia [170].

Acute mesenteric ischaemia affects about 5 per 100,000 people per year [171], and is associated with poor outcomes with mortality rates between 60 % to 80 % [25]. There are four main pathophysiology associated with acute mesenteric ischaemia: arterial embolism, arterial thrombosis, non-occlusive pathologies, and mesenteric venous thrombosis [172]. Arterial embolism is the cause for about 50 % of the acute mesenteric ischaemia cases [168]. It occurs due to a blood
clot from elsewhere, usually from a cardiac source (such as due to the formation of a blood clot from atrial fibrillation), getting lodged in a mesenteric artery. Arterial embolism mostly affects the superior mesenteric artery due to the high basal flow rate and the anatomic angle [170]. Arterial thrombosis, also known as acute-on-chronic mesenteric ischaemia, is the cause for about 20% of the acute mesenteric ischaemia cases, and typically results from delayed diagnosis of chronic mesenteric ischaemia [168]. Arterial thrombosis typically results from slow build up of fat in the mesenteric blood vessels until critically narrowing the vessel, where due to the low flow, blood clots occur on the fat layer. However, any scenario leading to low flow or hypotension can lead to arterial thrombosis, including myocardial infarction, acute viral illness, and clinical interventions such as cardiac surgery [173]. Non-occlusive pathologies are observed in 20% of the acute mesenteric ischaemia cases, and has the worst in-hospital mortality rates [168]. Non-occlusive mesenteric ischaemia has many causes, but ultimately results from prolonged intestinal vasoconstriction and reduced intestinal blood flow. The most common causes include myocardial failure [174], septic shock [174], prolonged vasopressor infusion [174], and cocaine ingestion [175]. Finally, mesenteric venous thrombosis is the least common form of acute mesenteric ischaemia, and is found in about 10% of the cases [168]. It is caused by the impairment of veins due to blood clots, ultimately resulting in the infarction of the affected intestinal segment. The most commonly involved vessels include the superior mesenteric vein (70% of the cases), portal vein, and the inferior mesenteric vein [176].

Mesenteric ischaemia leads to bioelectrical and contractile changes in the intestine. Hypoxia caused by mesenteric ischaemia leads to a reduction in frequency of slow wave events, and in some instances with prolonged ischaemia [14], [43], are completely eliminated [18]. In other instances, slow wave activity has remained irregular during mesenteric ischaemia [177]. Mesenteric ischaemia also affect the propagation patterns of slow waves, and abnormal slow wave propagation patterns such as transient conduction blocks (Fig. 2.17B) and circumferential propagations (Fig. 2.17C) have been observed. In contrast, mesenteric ischaemia increases the spike burst activity [45], [178]. In certain instances, spike bursts have been eliminated with prolonged ischaemia [177], but in others, they have remained at a lower frequency (compared to the initial increase) [178]. Spatial activation patterns of spike bursts during mesenteric ischaemia has not been thoroughly investigated, however, some evidence suggests that propagating spike bursts patches are initiated [45].

Mesenteric ischaemia also leads to contractile changes in the intestine, the most prominent being the transient increase in circular contractions called a spasm [177], [178]. This spasm of circular contractions may be a direct result of the increased spike burst activity [45], [177], [178], however, a simultaneous analysis of electrophysiological and contractile changes during ischaemia has not yet been conducted. In addition to the spasm, tonal contractions have also been observed with prolonged hypoxia, and is hypothesised to be a result of the damage to inhibitory neurons [42]. Apart from the above observed contractile changes, alterations to electrolyte homeostasis [44] and increased activity of calcium sensitisation agents [179] have also been reported during ischaemia, and could also alter the intestinal contractile activity.
2.10 Summary

Intestinal motility is governed by a multitude of regulatory mechanisms, and is associated with two bioelectrical events: (i) slow waves generated and propagated by ICC, and (ii) spike bursts which are believed to be calcium currents in the smooth muscle cells that trigger contractions. Various interactions between slow waves and spike bursts have been observed with different motility patterns. GI disorders such as mesenteric ischaemia are associated with abnormal bioelectrical activity, and result in abnormal contractile patterns. However, a simultaneous spatiotemporal analysis of slow waves, spike bursts, and motility has not been performed. High-resolution extracellular recordings are the current state of the art method of assessing the intestinal bioelectrical activity, and are capable of capturing the spatiotemporal dynamics of slow waves and spike bursts. Intestinal motility has been assessed using displacement measurements, deformation measurements based on diameter and marker-based longitudinal length, and deformation rate measurements. Deformation is the ideal metric to map intestinal motility, however, currently available motility mapping techniques only provide 1-dimensional deformation measurements of motility patterns, and therefore are not suitable for pairing with high-resolution bioelectrical recordings for simultaneous analysis. Advanced motility mapping techniques, which can be paired with high-resolution bioelectrical measurements could significantly improve our understanding of the electrophysiological basis of intestinal motility in health and disease.
Chapter 3

High-Resolution Mapping of Bioelectrical Slow waves and Spike Bursts

The methods presented in this chapter have been published as a conference proceeding titled “A Framework for Spatiotemporal Analysis of Gastrointestinal Spike Burst Propagation”, at the 2019 international conference of the IEEE Engineering in Medicine and Biology Society [180].

The chapter describes the experimental setup and the algorithms used for the acquisition and analysis of intestinal extracellular bioelectrical signals. High-resolution extracellular slow wave analysis methods have been well established with previous studies in pigs [121], rabbits [126], and humans [181]. However, high-resolution spike burst analysis techniques are lacking, and therefore, new methods are required for the detection, clustering, and quantification of spike burst patches.

3.1 Animal preparation

In-vivo physiological data acquisition was from white cross-breed weaner pigs and New Zealand white rabbits, under the ethical approval of the University of Auckland Animal Ethics Committee. Animal care and preparation were performed as described in previous studies on each species [121], [126], [182]. Pigs were fasted prior to surgery, then subjected to general anaesthesia with Zoletil (Tiletamine HCl 50 mg/mL and Zolazepam HCl 50 mg/mL), and maintained with Isoflurane (2.5 – 5 % with an oxygen flow of 400 mL within a closed-circuit anaesthetic system). Rabbits were not fasted prior to surgery as they are prone to stasis [183]. Rabbits were subjected to anaesthesia with Propofol 1 % at a dosage of 10 mg/kg, and maintained with Isoflurane similar to the pigs. Vital signs such as heart-rate, blood pressure, and core temperature were continuously monitored throughout the experiments in both species, and were maintained within the normal physiological limits. At the end of the experiments, pigs were euthanised with a bolus injection of 50 mL of magnesium sulfate while still under anaesthesia. Rabbits were euthanised
with intravenous administration of pentobarbital 300 mg at a dosage of 60 mg/kg, while also under anaesthesia.

In both species, a midline laparotomy was performed to expose the small intestine for recording. Handling of the viscera was kept to a minimum to not affect the bioelectrical activity.

3.2 Experimental setup

Extracellular electrical activity of the intestine was recorded using flexible high-resolution electrode arrays developed by Du et al. [184]. The electrode array was constructed by vertically stacking four such $16 \times 2$ high-resolution electrodes to form a $16 \times 8$ configuration (Fig. 3.1A). The electrodes were spaced 4 mm apart, which had previously been validated to be sufficient to capture GI bioelectrical activity using the spatial variant of the Nyquist theorem [121], [185]. These flexible high-resolution electrode arrays have previously been applied in both the stomach and the intestine of pigs [121], [184], rabbits [126], and humans [129], [186]. Similarly, in this thesis, the extracellular bioelectrical activity was recorded by placing an exposed segment of the intestine on top of the electrode array (Fig. 3.1C), and was recorded as unipolar signals with reference to a common reference electrode placed on the shaved hindquarter thigh of the animal.

Figure 3.1: Experimental setup used to map the extracellular bioelectrical activity of the intestine. (A) $16 \times 8$ flexible high-resolution electrode array. (B) An exposed segment of the intestine placed over the electrode array. (C) BioSemi ActiveTwo signal acquisition system.

The bioelectrical signal was acquired using a BioSemi ActiveTwo system (BioSemi, Amsterdam, Netherlands), which can simultaneously record from up to a maximum of 256 electrode channels (Fig. 3.1C). BioSemi ActiveTwo system can also record up to 16 independent trigger signals which can be used to synchronise the bioelectrical recordings with other data acquisition systems. The amplifier was powered by a DC battery, and was electrically isolated from the computer (and main power connection to the computer) through an optical fibre connection. The bioelectrical signals were recorded at a sampling frequency of 512 Hz, and the signals were displayed real-time through the ActiveTwo acquisition software (Fig. 3.1C) for verification during the experiments. The experimental setup utilised in thesis to record the bioelectrical activity of the intestine is an established method, that has been previously applied to record GI bioelectrical activity in humans [8], [129], [186], pigs [121], [181], [184], and rabbits [126].
3.3 Filtering

Bioelectrical data analysis was performed using the Gastrointestinal Electrical Mapping Suite (GEMS) [187], with new functions programmed for tasks as specified. Data processing was performed in three stages: filtering, event detection, and visualisation. The filtering stage entailed baseline drift correction and noise removal, as shown in Fig. 3.2. Fig. 3.2A shows raw signal traces containing baseline drift recorded during experiments. The baseline drift in each channel was estimated by first applying a median filter (window size = 1.5 s) and then a Savitzky-Golay filter (window size = 1.5, polynomial order = 2) to the raw signals (Fig. 3.2B). Then, the estimated baseline drifts were removed from the raw signals (Fig. 3.2C). Next, the drift corrected signals were filtered using a two step process. First, the common noise was estimated as the median signal across all electrode channels, and was subtracted from each channel [188]. Then, the high-frequency noise was further filtered by applying a Savitzky-Golay filter (window size = 0.1 s, polynomial order = 9), which resulted in the final filtered signals with visible slow wave and spike burst events, as shown in Fig. 3.2D.

3.4 Slow wave detection and quantification

Slow wave detection was performed in a quasi-automated manner with manual intervention when necessary. Slow wave activation times were identified as the negative deflections of the events (Fig. 3.3A), and were detected using a well established algorithm called the falling-edge
variable threshold (FEVT) method (detection threshold = 4, refractory period = 3 s) [189]. FEVT algorithm has been previously applied to detect slow wave events in stomach and intestine recordings for over 10 years [8], [121], [129]. However, FEVT can result in erroneous markers when spike bursts are present. Therefore, detected events were reviewed and manually marked when the markers were not on the slow wave activation phase.

The detected slow wave events were clustered into propagating slow wave cycles using the REGROUPS (Region Growing Using Polynomial-surface-estimate Stabilization) algorithm [190], which had also been previously established in stomach and intestine slow wave recordings [8], [121], [129]. Fig. 3.3A shows clustered slow wave events marked with different colours and Figs. 3.3B–D show the propagation patterns of three clustered cycles, with the activation times plotted as isochrones in slow wave activation time maps. However, REGROUPS can result in incorrect clustering during complex slow wave propagation patterns, such as with conduction blocks and re-entry. The slow wave clusters were manually reviewed by looking at activation time videos (described in Section 3.6) and the incorrectly clustered events were manually clustered.

### 3.4.1 Quantified slow wave metrics

Slow waves were quantified by computing the following three metrics:

- **Amplitude of slow waves:** The amplitude of a slow-wave event was taken as the difference between the maximum and minimum voltage levels within a 1 s window centred at its activation time [191].
- **Frequency of slow waves**: The slow wave period was computed as the time difference between adjacent slow wave events in each electrode. The frequency was computed as the mean reciprocal of slow wave period across all electrodes and reported in cpm.

- **Velocity of slow waves**: The velocity of slow wave cycles was computed using a finite difference approach with Gaussian smoothing to reduce artefacts, implemented in GEMS [192].

### 3.5 Spike burst detection and quantification

Spike bursts were detected using a method derived from [45]. First, slow waves were removed from the filtered signals by performing suppression of artefacts by local polynomial approximation (SALPA) (window size 0.3 s, black electrical traces in Fig. 3.4) [193]. The high-frequency, high-amplitude wave forms of spike bursts were accentuated by computing the 4th order differential energy operator transforms of the resulting signals as given in Eqn (3.1) [194],

\[
\]  

(3.1)

Figure 3.4: Electrical traces show the slow wave removed signals with spike bursts detected and clustered into patches shown with coloured crosses. The red trace shows the energy transform of electrode 2. The blue trace shows the threshold level (\(T_i\)) for the energy transform used to detect the spike bursts.

where \(n\) is the discrete time, \(X\) is the signal, and \(E\) is the energy transformed signal. Then, smoothing was applied to the energy transformed signals using a moving mean filter (window size 1 s). The resulting energy transforms were used as the detection signals for the spike bursts (red trace in Fig. 3.4). The threshold level (\(T_i\)) for each electrode channel was empirically determined as given in Eqn (3.2),
\[ T_i = 20 \times \sigma_i \]
\[ \sigma_i = 1.4286 \times \text{MAD}(E_i) \]  

where \( i \) is the electrode number and \( \sigma_i \) is the standard deviation of its energy transform. The standard deviations were estimated using the median absolute deviation (MAD) of the respective energy transforms, where 1.4286 is the scaling factor for normal distributions [195]. The calculated threshold levels (blue trace in Fig. 3.4) were applied to the energy transforms (red trace in Fig. 3.4) to identify start-times and end-times of the spike burst events (crosses on spike bursts in Fig. 3.4). The detected events were reviewed, and undetected spike bursts were manually marked.

The detected spike burst events were clustered into spatial propagation regions known as patches using a region growing method derived from [196]. The detected spike burst events in neighbouring electrodes were grouped together into patches based on the time differences between them (start-time difference < 1 s). New spike burst events were iteratively added to the patches as given in the pseudocode in Fig. 3.5 until all the events were clustered. Fig. 3.4 shows clustered spike burst events in temporal form marked with coloured crosses. Fig. 3.6 shows the spatiotemporal activations of three clustered spike burst patches with their spike burst start-times plotted as isochronal maps (spike burst activation time maps). A circumferential spike burst patch that activated predominantly in the circumferential direction is shown in Fig. 3.6A, a longitudinal spike burst patch that activated predominantly in the longitudinal direction in Fig. 3.6B, and a circumferential spike burst patch that propagated in the longitudinal direction as a sheath or a ring in Fig. 3.6C. The clustered spike bursts were reviewed, and errors were manually re-clustered prior to further analysis.

### 3.5.1 Quantified spike burst metrics

Spike bursts and spike burst patches were quantified by computing the following metrics:

- **Amplitude of spike bursts**: Amplitude of spike burst events was calculated as the difference between the maximum and minimum voltage levels during the spike burst signal.

- **Duration of spike bursts**: The duration of a spike burst was taken as the difference between its start and end-time.

- **Amplitude of spike burst patches**: The amplitude of a spike burst patch was defined as the mean amplitude of all its spike bursts.

- **Duration of spike burst patches**: The duration of a spike burst patch was defined as the mean duration of all its spike bursts.

- **Size of spike burst patches**: The size of a spike-burst patch was computed as the area of the electrode array activated by its spike bursts and reported in mm\(^2\).
Input: List of spike burst events \[\text{[start-time, electrode number, patch number]} = \text{master\_list}\]

1. Sort \text{master\_list} according to start-time
2. \text{current\_patch} = 0
3. \textbf{for} \(i \leftarrow 1\) \textbf{to} end of \text{master\_list} \textbf{do}
4. \(E(i) = i\)-th event in \text{master\_list} without a patch number
5. Increment \text{current\_patch}
6. Set \(E(i)\)'s patch number as \text{current\_patch}
7. \(Q = [E(i)]\)
8. \textbf{until} \(Q\) is empty \textbf{do}
9. \(q = \text{Next element in } Q\)
10. \(\text{valid\_events} = \{\text{Subsequent events in } \text{master\_list} \text{ in neighbouring electrodes to } q \mid \text{patch number} = 0, dt < 1\ s\}\)
11. /* where, \(dt = \text{start-time difference between } q\) and that event */
12. Set \text{valid\_events}' patch number as \text{current\_patch}
13. Add \text{valid\_events} to the end of \(Q\)
14. Remove \(q\) from \(Q\)
15. \textbf{end}

Output: \text{master\_list} with patch numbers filled

Figure 3.5: Pseudocode of the spike burst clustering algorithm.

Figure 3.6: Isochronal activation time maps for 3 types of spike burst patches. (A) Circumferential spike burst patch. (B) Longitudinal spike burst patch. (C) Propagating circumferential spike burst patch, where the longitudinal propagation is much slower, and could be a result of coordinated activation of circumferential patches.

- **Energy of spike burst patches**: The energy of a spike burst patch was defined as the product of its amplitude, duration, and patch size \((\text{mV s mm}^2)\).

- **Velocity of propagating spike burst patches**: The velocity of a propagating spike burst patch (such as the one shown in Fig. 3.6D) was computed with the same finite difference approach used for slow waves (Section 3.4.1), using the start-times of its spike bursts.

### 3.6 Activation time videos

Activation time videos were generated for slow waves and spike bursts to visualise the activations across the electrode array. In each video frame, electrode locations with slow wave or spike burst activations within the last 1 s were indicated with coloured squares. The opacity of the coloured squares were linearly scaled based on the time difference between the frame and the event, where
shorter the time difference, closer the opacity to 1. The activations were colour-coded according
to the slow wave cycles or spike burst patches, respectively. Fig. 3.7 shows four snapshots from a
slow wave activation time video, where the propagation of two slow wave cycles are shown with
blue and orange, along with unclustered events shown in grey. These activation time videos were
also used to validate the clustering of slow wave and spike burst events.

Figure 3.7: Four snapshots from a slow wave activation time video. Slow wave cycles are colour-coded
according to the clusters, and unclustered events are shown in grey.

3.7 Summary

Slow wave and spike burst detection and analysis were performed in a semi-automated manner
with software algorithms to improve efficiency, and to increase accuracy. The experimental setup
for extracellular bioelectrical recordings, and slow wave analysis methods were largely adapted
from previous studies with minor changes to filtering and parameters. A new framework was de-
veloped for the detection, clustering, and quantification of spike burst patches. The simultaneous
slow wave and spike burst mapping techniques developed in this chapter can now be paired with
high-resolution motility mapping to obtain a cohesive understanding of intestinal motility.
Chapter 4

High-Resolution Mapping of Intestinal Motility

The work presented in this chapter has been published in IEEE Transactions on Biomedical Engineering, in a paper titled “High-resolution spatiotemporal quantification of intestinal motility with free-form deformation” [197].

Our understanding of the GI electrophysiology has greatly improved with the advent of high-resolution bioelectrical mapping techniques. The progress of GI electrophysiology over the last few decades is a testament to the importance of spatial resolution in recording techniques for understanding biological systems. However, GI motility mapping techniques have not experienced a similar improvement in spatial resolution over the years. A high-resolution motility mapping technique that can quantify the contractions and relaxations across the tissue surface can be paired with high-resolution bioelectrical mapping methods to obtain a unified understanding of motility. This chapter describes a novel framework that can attain the above goals.

Currently available diameter and marker based motility mapping methods are limited in resolution as they provide 1-dimensional strain measures and depict the cumulative effect across the diameter or between markers [39]. As a result, these methods are not ideal for analysing nonhomogeneous contractions occurring in the intestine, such as the difference in degree of contractions in the mesenteric and anti-mesenteric sides of the intestine [156], and are also not suitable for analysing the effect of localised bioelectrical activities such as spike patches [30] on motility. More recently, strain-rate fields computed with spatial differentiation of displacements have been used to quantify contractions at a higher spatial resolution [40]. However, strain-rate cannot be used to quantify the level of deformation caused by the motility patterns, but rather computes the rate of deformations. As a result, it is also difficult to map slow deformations with strain-rate, and video recordings have to be downsampled to capture the low time-rate contractions [163]. Motility patterns such as slow tonal changes in the cecum have a low time rate of deformation (strain-rate), and can be concealed by noise due to differentiation [163].

Free-form deformation (FFD) based methods can also be used to map motility patterns from video recordings, and overcome the above limitations. FFD has been used to quantify
deformations in the sheep diaphragm [198], to estimate material properties of the skin [199], and to quantify wall motion during heart beats [200]. This approach allows more rigorous definitions of strain to be used rather than length based strain measurements, and therefore, can quantify deformations independent of rigid body motion and shear. Furthermore, since strain is measured relative to a reference frame, it can be used to quantify both phasic and tonal contractions regardless of their time-rates.

This chapter proposes a novel framework and a mathematical algorithm that uses FFD to map intestinal motility patterns from video recordings. The steps taken to quantify motility patterns are shown in Fig. 4.1. First, displacement fields were calculated to quantify the motion between video frames (Section 4.1). Then, the geometry of the intestine was modelled using a biquadratic B-spline mesh on the first frame, which was deformed based on the displacement fields to track the contractile deformations across time (Section 4.2). The mesh was iteratively adjusted to mitigate the propagation of tracking error (Section 4.2.4), and finally, the contractile deformations were quantified by calculating the Green-Lagrange strain based on the change in geometry. Section 4.3 describes the experimental setup used to apply the FFD algorithm to in-vivo experiments. The algorithm was first verified using synthetic tests, and then validated by performing controlled translational tests on excised tissue. Finally, the algorithm was applied to analyse in-vivo intestinal motility, and the feasibility is demonstrated with experimental results in Section 4.5.

![Figure 4.1: Step-by-step diagram of the FFD motility mapping algorithm.](image)

4.1 Estimating the displacements of the tissue

Displacement fields were generated to obtain the $X,Y$ displacements of pixels from one frame (template) to a subsequent target frame. Displacement fields were generated by interpolating the displacements at an evenly distributed set of pixel positions (virtual points) spaced at 20 pixels covering the entire frame. The displacements of the virtual points were identified using a phase-based subpixel image registration technique [201]. The selected image registration algorithm offers better accuracy with low-textured images compared to existing published algorithms [201], therefore, was deemed the best approach to register displacements using the intrinsic texture of the tissue. To enhance the level of detail, a speckle pattern was applied by lightly spraying Indian ink on the surface.
First, the $X$ and $Y$ gradients of the frames were computed by applying the 7 point cubic first derivative Savitzky-Golay convolutional kernel given in Eqn (4.1), along the rows ($SGD_X$) and the columns ($SGD_Y$) of the frame, respectively. The convolutional weights of the kernel were calculated using Gram polynomials as described in [202]. The gradient (total gradient, $tSGD$) of the frame was formed by combining the results in the complex form, as $SGD_X + iSGD_Y$, where $i$ is the imaginary unit.

$$SGD(\text{Kernel}) = [22, -67, -58, 0, 58, 67, -22] \cdot \frac{1}{252} \quad (4.1)$$

The displacement of a virtual point from the template frame (frame $\gamma$) to the target frame (frame $\beta$) was determined by comparing the surrounding gradients in a $129 \times 129$ pixel window in the two frames ($tSGD_\gamma$ and $tSGD_\beta$). To compute the integer displacement, $tSGD_\gamma$ and $tSGD_\beta$ were normalised, and a Hamming window was applied to reduce boundary effects. Then, the integer displacement was determined through normalised cross-correlation of $tSGD_\gamma$ and $tSGD_\beta$.

The subpixel displacements were identified using the fourier-domain shift property. First, the virtual point in the target frame was moved to account for the integer displacement, and $tSGD_\gamma$, $tSGD_\beta$ were re-computed. They were normalised as before, and a Hann window was applied (instead of the Hamming window) to attenuate high-frequency noise while preserving low-frequency information. The frequency domain representations of $tSGD_\gamma$ and $tSGD_\beta$ were obtained through the fast fourier transform (FFT), and the phase shift was found by taking the argument of $tSGD_\gamma(\omega) \cdot tSGD_\beta(\omega)$, where $\omega$ is the angular frequency. The subpixel displacement was calculated as the gradient of the phase at the centre of the window. This subpixel displacement was added to the integer displacement to obtain the total displacement of the virtual point.

The quality of the displacement values was assessed by calculating the Shannon entropy of their template windows, and the spread of their normalised cross-correlation peaks. Displacements with entropy less than 3 and a peak spread more than a quarter of the window size (i.e., 32 pixel for this application) were disregarded when computing the displacement fields. The $X, Y$ displacement fields that map frame $\gamma$ to frame $\beta$ are denoted as $\Delta_{\gamma,\beta}^{(X)}$, $\Delta_{\gamma,\beta}^{(Y)}$ in the following sections. The quality of the displacement field was measured as the reciprocal of the root mean square (RMS) peak spread of the displacement values, and is denoted as $W_{\gamma,\beta}$.

### 4.1.1 Verification of the displacement measure

The displacement measure was verified by performing synthetic translational shifts on an image of the intestine. First, a spline surface was fitted to the image, such that the image is mathematically described as $I(x, y)$, where $x, y$ are the pixel locations. Then, a subpixel shifted image can be obtained by $I(x-x_{\text{shift}}, y-y_{\text{shift}})$, where $x_{\text{shift}}, y_{\text{shift}}$ are the amounts in pixels, by which the image is shifted. The image was shifted from 0.5 pixels to 20 pixels in 1.5 pixel increments, and the RMS error was recorded as 0 pixels for integer shifts, and $0.00270 \pm 0.00008$ pixels for subpixel shifts.
4.2 Quantifying intestinal deformations

The contractions and relaxations of the intestine were quantified by strain based on the change in geometry across frames. The block diagram in Fig. 4.2 shows the overall motility mapping procedure. The strain tensor was calculated in reference to a convected coordinate system called the material coordinates, which was embedded onto the intestinal tissue surface in the reference/undeformed state such that the coordinate system deforms with contractions. The longitudinal axis ($\xi_1$) and the transverse axis ($\xi_2$) of the material coordinates were aligned to be in the longitudinal and circular muscle fibre directions respectively (see Fig. 4.2). The geometry of the intestine was modelled as a 2-dimensional surface using a biquadratic B-spline mesh, such that it mapped the material coordinates to their cartesian global coordinates ($X, Y$) as shown in Eqn (4.2).

$$
X(\xi_1, \xi_2) = \sum_{i=0}^{n} \sum_{j=0}^{n} N_{i,k}(\xi_1)N_{j,k}(\xi_2)P_{i,j}^{(X)}
$$

$$
Y(\xi_1, \xi_2) = \sum_{i=0}^{n} \sum_{j=0}^{n} N_{i,k}(\xi_1)N_{j,k}(\xi_2)P_{i,j}^{(Y)}
$$

where $P^{(X)}$, $P^{(Y)}$ are the control point matrices (parameters) of dimensions $(n+1) \times (n+1)$, $k$ is the degree of the B-spline (here $k = 2$), $n$ is the order of the B-splines, $N_{i,k}$ are the basis functions.
functions as defined in Eqn (4.3).

\[
N_{i,\kappa}(\xi) = \frac{\xi - t_i}{t_{i+\kappa} - t_i} N_{i,\kappa-1}(\xi) + \frac{t_{i+\kappa+1} - \xi}{t_{i+\kappa+1} - t_{i+1}} N_{i+1,\kappa-1}(\xi)
\]

\[
N_{i,0}(\xi) = \begin{cases} 
1, & \text{if } t_i \leq \xi < t_{i+1} \\
0, & \text{otherwise}
\end{cases}
\]

(4.3)

and \(t_i\) is the knot vector given by:

\[
t_i = \begin{cases} 
0, & k > i \\
\nu - k, & k \leq i \leq n \\
n - k + 1, & n < i
\end{cases}
\]

(4.4)

The material coordinates were defined in the range: \(\xi \in [0, m]; m = n - k + 1\). The number of elements (or knots in the terminology of B-splines) of the B-spline mesh \((n + 1)\) was determined based on the size of the region of interest modelled.

The biquadratic B-spline provides \(C^1\) continuity (therefore, continuous strains) with fewer parameters (one parameter per knot interval) than other higher order interpolation schemes such as bicubic-Hermite interpolation. B-splines with unequal orders can be selected to obtain a rectangular B-spline mesh.

### 4.2.1 Modelling the geometry of the intestine

The region of interest (ROI) of the intestine was modelled as a 2-dimensional surface by fitting a biquadratic B-spline mesh with number of elements (or knots in terminology of B-splines) determined based on the size of the ROI. The boundary of the ROI was manually specified in the first frame, and the material coordinates were determined. One of the material axes of the boundary is always zero or \(m\) depending on the side (e.g., for top/anti-mesenteric boundary \(\xi_2 = 0\), bottom boundary \(\xi_2 = m\)), and the other axis goes from 0 to \(m\) along the boundary. Equidistant \((n + 1)\) points were selected from each boundary as boundary knots. Internal knot locations were interpolated from the boundary knots. The geometry was modelled by finding the optimum control points in the matrices \(P(X)\) and \(P(Y)\) that fit the biquadratic B-spline mesh to the boundary points, boundary knots, and the interpolated internal knots.

The mesh was fitted to the set of \(D\) points chosen above by minimising the sum of squared difference (SSD) between their known global coordinates \((X^d, Y^d)\) and the global coordinates mapped through their material coordinates \((\xi_1^d, \xi_2^d)\) according to Eqn (4.2), as shown in Eqn (4.5).

\[
SSD_X = \sum_{d=1}^{D} \left[ X(\xi_1^d, \xi_2^d) - X^d \right]^2
\]

\[
SSD_Y = \sum_{d=1}^{D} \left[ Y(\xi_1^d, \xi_2^d) - Y^d \right]^2
\]

(4.5)

The nonlinear functional form of mesh fitting, which had been used in finite element modelling in other contexts [198], was adapted to solve Eqn (4.5).
Minimising Eqn (4.5) with respect to an arbitrary control point $P_{a,c}^{(X)}$ in $P^{(X)}$ (i.e., $a, c$ are indices between 0 and $n$) gives:

$$\frac{\partial SSD_X}{\partial P_{a,c}^{(X)}} = 0 \quad \text{(Minima are stationary points)}$$

$$\sum_{d=1}^{D} X(\xi_1^d, \xi_2^d) \frac{\partial X(\xi_1^d, \xi_2^d)}{\partial P_{a,c}^{(X)}} \bigg|_{(\xi_1^d, \xi_2^d)} = \sum_{d=1}^{D} X^d \frac{\partial X(\xi_1^d, \xi_2^d)}{\partial P_{a,c}^{(X)}} \bigg|_{(\xi_1^d, \xi_2^d)}$$

where,

$$\frac{\partial X(\xi_1^d, \xi_2^d)}{\partial P_{a,c}^{(X)}} \bigg|_{(\xi_1^d, \xi_2^d)} = N_{a,k}(\xi_1^d)N_{c,k}(\xi_2^d)$$

Substituting the definition for $X(\xi_1^d, \xi_2^d)$ in Eqn (4.2) gives:

$$\sum_{d=1}^{D} \sum_{i=0}^{n} \sum_{j=0}^{n} N_{i,k}(\xi_1^d)N_{j,k}(\xi_2^d)N_{a,c}(\xi_1^d)N_{c,k}(\xi_2^d)P_{a,c}^{(X)} = \sum_{d=1}^{D} N_{a,k}(\xi_1^d)N_{c,k}(\xi_2^d)X^d$$

For all possible points $P_{a,c}^{(X)}$, Eqn (4.7) gives $(n+1)^2$ linear equations for $(n+1)^2$ unknowns in $P^{(X)}$. Then, the mesh can be described by a linear set of equations in the matrix form as shown in Eqn (4.8), where $p^{(X)}, p^{(Y)}$ are the vectorized forms of $P^{(X)}, P^{(Y)}$, of size $(n+1)^2$. $A$ is a matrix of size $((n+1)^2 \times (n+1)^2)$, and $f^{(X)}, f^{(Y)}$ are vectors of size $(n+1)^2$.

$$A \cdot p^{(X)} = f^{(X)}$$

$$A \cdot p^{(Y)} = f^{(Y)}$$

The optimum set of control points that fit the mesh to the boundary was obtained using the least-squares method as:

$$p^{(X)*} = (A^T A)^{-1} \cdot A^T \cdot f^{(X)}$$

$$p^{(Y)*} = (A^T A)^{-1} \cdot A^T \cdot f^{(Y)}$$

4.2.2 Tracking the geometry of the intestine

The mesh was fitted from the current frame $\gamma$ to the next based on the displacement field between the two frames. The current B-spline mesh was used to resolve the global coordinates of an evenly distributed set of material points $(\xi_1^d, \xi_2^d)$ using Eqn (4.2). Here, $5n$ material points were chosen as that was able to faithfully capture the deformations due to intestinal contractions, however, a higher number of points will be able to provide a higher definition of contractions. The global coordinates of these points in the next frame $(\gamma + 1)$ were computed using the displacement fields as shown in Eqn (4.10)

$$X_{\gamma+1}^d = X_{\gamma}(\xi_1^d, \xi_2^d) + \Delta_{\gamma,\gamma+1}^{(X)} \left[ X_{\gamma}(\xi_1^d, \xi_2^d), Y_{\gamma}(\xi_1^d, \xi_2^d) \right]$$

$$Y_{\gamma+1}^d = Y_{\gamma}(\xi_1^d, \xi_2^d) + \Delta_{\gamma,\gamma+1}^{(Y)} \left[ X_{\gamma}(\xi_1^d, \xi_2^d), Y_{\gamma}(\xi_1^d, \xi_2^d) \right]$$

where $\Delta_{\gamma,\gamma+1}^{(X)}, \Delta_{\gamma,\gamma+1}^{(Y)}$ are the X and Y displacement fields.

Once the new global coordinates were found, the optimum control points that fit the mesh to the new frame were computed using the least-squares method as shown in Eqn (4.5)–(4.9).
4.2.3 Computation of Green-Lagrange strain

The Green-Lagrange strain was evaluated based on the change in geometry of the intestine. The mesh fitted to the first frame \((X_1, Y_1)\) was defined as the undeformed state, and the meshes of subsequent frames were defined as deformed states. The Green-Lagrange strain tensor \((E_{IJ})\) for a deformed mesh \((X_\gamma, Y_\gamma)\) fitted to frame \(\gamma\) was calculated in reference to the material coordinate system as shown in Eqn (4.11) [198],

\[
E_{IJ} = \frac{1}{2} (g_{IJ} - G_{IJ}) \left( G^I \otimes G^J \right) ; I, J \in \{1, 2\} \tag{4.11}
\]

where,

\[
G_I = \frac{\partial X_1(\xi_1, \xi_2)}{\partial \xi_I} \hat{E}_1 + \frac{\partial Y_1(\xi_1, \xi_2)}{\partial \xi_I} \hat{E}_2
\tag{4.12}
\]

\[
g_I = \frac{\partial X_\gamma(\xi_1, \xi_2)}{\partial \xi_I} \hat{E}_1 + \frac{\partial Y_\gamma(\xi_1, \xi_2)}{\partial \xi_I} \hat{E}_2
\tag{4.13}
\]

\[
G_{IJ} = G_I G_J = \begin{bmatrix} G_{11} & G_{12} \\ G_{21} & G_{22} \end{bmatrix} \quad g_{IJ} = g_1 g_2
\tag{4.14}
\]

\(\hat{E}_1, \hat{E}_2\) are the basis of the spatial coordinate system (i.e., of the video frame); \(G_1, G_2, g_1, g_2\) are basis vectors tangential to the undeformed and deformed mesh lines (Eqn 4.12–4.13); \(G_{IJ}\) and \(g_{IJ}\) are the second order tensor fields of the undeformed and deformed bases (Eqn 4.14); \(G^1, G^2, g^1, g^2\) are the undeformed and deformed contravariant bases such that \(G_I G^J = \delta_I^J, g_I g^J = \delta_I^J; \delta\) is the Kronecker delta, and \(\otimes\) is the tensor product.

The derivatives were calculated by applying the differentiation of B-splines given in [203]. The derivative of a \(k\) degree B-spline in the variable \(\phi\) defined with the knot vector \(t\) and control points vector \(p\), is given by the \((k - 1)\) degree B-spline as shown in Eqn (4.15).

\[
S(\phi) = \sum_{i=0}^{n} N_i,k(\phi) \cdot P_i
\]

\[
\frac{\partial S(\phi)}{\partial \phi} = \sum_{i=0}^{n-1} N_i+1,k-1(\phi) \cdot Q_i
\tag{4.15}
\]

where,

\[
Q_i = \frac{k}{t_{i+k+1} - t_{i+1}} \cdot (P_{i+1} - P_i)
\]

Applying this same technique to calculate \(\frac{\partial X(\xi_1, \xi_2)}{\partial \xi_1}\) from Eqn (4.2) yields:

\[
\frac{\partial X(\xi_1, \xi_2)}{\partial \xi_1} = \frac{\partial}{\partial \xi_1} \sum_{i=0}^{n} \sum_{j=0}^{n} N_i,k(\xi_1) N_j,k(\xi_2) P_{ij}^{(X)}
\]

\[
\frac{\partial X(\xi_1, \xi_2)}{\partial \xi_1} = \sum_{i=0}^{n-1} \sum_{j=0}^{n} N_{i+1,k-1}(\xi_1) N_j,k(\xi_2) \frac{k}{t_{i+k+1} - t_{i+1}} \left( P_{i+1,j}^{(X)} - P_{ij}^{(X)} \right)
\tag{4.16}
\]

Similarly, other derivative terms in Eqn (4.11) can be calculated using the above approach (Eqn 4.16).

It should be noted that errors could arise in the edge elements due to not being constrained by surrounding elements from all sides. These errors, known as edge effects were avoided by not using the edge rows and columns of the mesh in the analysis.
4.2.4 Accounting for propagation of tracking error

A problem that arises with FFD, and other methods that utilise template-based tracking, is the template update problem [204]. The template used for tracking has to be updated to reflect the current appearance of the object (texture of the portion of tissue in the context of this paper). When the template is updated based on a previously tracked location, the tracking error associated with the location propagates through the new template to the next location measurement, causing the error to accumulate over time. In FFD, the image registration error associated with the displacement fields results in error in the mesh fit. When the mesh is fitted from the current frame to the next, this error propagates resulting in an increase in the error with time. The template update problem has been addressed using adaptive template update strategies [205], probabilistic methods [206], and by correcting the template drift using the first frame [204], to name a few. These strategies have been developed primarily for real-time applications, and therefore, do not take advantage of the complete video sequence available to offline applications.

In this application, the propagating error can be mitigated by taking advantage of all the frames in the video. The propagating error was mitigated using a novel approach by iteratively adjusting the fitted (deformed) mesh to all the displacement fields through a quasi-newton non-linear optimisation scheme. The optimisation scheme minimised the objective function as in Eqn (4.17),

\[
\min_{p(x),p(y)} W_{\gamma\beta} \left( X_\gamma(\xi^d_1, \xi^d_2) - X_\gamma(\xi^d_1, \xi^d_2) - \Delta_{\gamma,\beta}^{(X)} \left[ X_\gamma(\xi^d_1, \xi^d_2), Y_\gamma(\xi^d_1, \xi^d_2) \right] \right)^2 + W_{\gamma\beta} \left( Y_\beta(\xi^d_1, \xi^d_2) - Y_\gamma(\xi^d_1, \xi^d_2) - \Delta_{\gamma,\beta}^{(Y)} \left[ X_\gamma(\xi^d_1, \xi^d_2), Y_\gamma(\xi^d_1, \xi^d_2) \right] \right)^2; \forall d, (\gamma < \beta)
\]

(4.17)

where \( W_{\gamma\beta} \) is the reciprocal of the RMS peak spread of the displacement fields \( \Delta_{\gamma,\beta} \); \( X_\gamma, Y_\gamma, X_\beta, Y_\beta \) are global coordinates of an evenly distributed set of material points \( (\xi^d_1, \xi^d_2) \) resolved from the meshes fitted to the corresponding frames \( \gamma, \beta \) using Eqn (4.2).

4.2.5 Verification of the deformation measure

The strain measure was verified by evaluating strains in synthetically generated displacement fields. Synthetic displacement fields were generated to avoid errors from image registration, and to add known amounts of noise to test the robustness of the fitting procedure. Linear stretching along \( X \) and \( Y \) axes can be simulated with displacements as shown in Fig. 4.3. By generalising this concept, the displacement fields that simulate stretching from frame \( i \) with strain \( S[i] \) to frame \( j \) with strain \( S[j] \) were calculated as given in Eqn (4.18). However, it should be noted that Eqn (4.18) simulates pure stretching and is not representative of shear or rotation.

\[
\Delta_{ij}^{(X)}(x, y) = \frac{S[j] - S[i]}{S[i]} \times x
\]

\[
\Delta_{ij}^{(Y)}(x, y) = \frac{S[j] - S[i]}{S[i]} \times y
\]

where,

\[
1 \leq i, j \leq 10
\]

\[
1 \leq S[i], S[j] \leq 1.09
\]

(4.18)
Quantifying intestinal deformations

\[ x_0 = \frac{x_1}{1.01} \]

\[ x_1 \]

\[ 1.02x_0 \]

\[ t = 0 \]

101 \% length

\[ t = 1 \]

102 \% length

\[ dx \]

Figure 4.3: Calculation of synthetic displacement field at \( x_1 \) that simulates linear stretching from 101 \% length to 102 \% length. Stretching to 101 \% causes the point at \( x_0 \) in the undeformed state to move to \( x_1 \). In order to achieve a 102 \% stretch in the next time step, \( x_0 \) in the undeformed state should be moved to 1.02\( x_0 \). The displacement at \( x_1 \) is the amount the point should travel from \( x_1 \) to 1.02\( x_0 \).

The simulated strains can be extracted by deforming a mesh according to the simulated displacement fields whose material axes are aligned along the spatial axes of the frame. The strain measurement error associated with simulated stretching of a 2D sheet from 100 \% length to 109 \% in both X and Y axes in 10 frames, under different noise levels, is shown in Fig. 4.4. At zero noise (SNR = \( \infty \)), the measurement error remained at zero, since the mesh was able to conform to the stretch without error verifying the operation of the algorithm. However, when the noise level was greater than zero, the measurement error increased with time due to the propagation of tracking error.

In the simulated stretching test, the nonlinear optimisation step (Section 4.2.4) was able to reduce the accumulation of error, as indicated by the relatively constant rms error curves in Fig. 4.4. Even at the highest tested noise level, it was able to reduce the rate of change in strain error from 0.007 to 0.0002 per frame (97 \% decrease).

However, the optimisation step adds a significant computational burden to the overall analysis, and the synthetic test (for SNR = 84 dB, repeated for 5 times each) took 3637.8 ± 77.4 s to run with the optimisation step as opposed to 36.6 ± 4.8 s without the optimisation step. Furthermore, the optimisation step requires displacement fields to be generated between each frame ((Number of Frames)^2) as opposed to only between adjacent frames, which require further computational steps. However, this FFD based method can be used without the optimisation step to achieve the same benefits with faster execution, but at the cost of accuracy, and similar methods have been used in other fields [198], [207]. The implementation of the algorithm could also be parallelised, such as with simultaneous registration of multiple frames, to harness the performance of graphics processing unit (GPU) and multi-core central processing unit (CPU) to improve execution time.
High-Resolution Mapping of Intestinal Motility

4.2.6 Comparison of FFD base strain measure and strain-rate from spatial differentiation of displacements

A comparison of this FFD based method (without optimisation) and strain-rate from spatial differentiation of displacements for a synthetic test case (described Section in 4.2.5) with noise of 84 dB is presented in Fig. 4.5. Strain was measured from a row of material points, and strain-rate was measured from a row of fixed points. Compared to the strain-rate map generated with spatial differentiation of displacements (Fig. 4.5C), the trend of the deformation was recognisable in the FFD based strain map even under a noise level of 84 dB SNR (Fig. 4.5A). The results highlight the ability of this FFD based method to map deformations under noise, regardless of the rate.

4.3 Experimental setup

A photo of the experimental setup is shown in Fig. 4.6. The motion of the intestine was recorded using a monochrome 5-megapixel machine vision camera (BFS-U3-50S5M-C, Blackfly S, FLIR, USA) fitted with a 25 mm lens (HF25SA-1, Fujifilm, Japan). It was positioned perpendicularly above the surface of the intestine, to obtain a field of view of 70 × 59 mm. Video recordings were carried out at 20 frames-per-second, which was sufficient to capture intestinal contractions [41]. Frames were saved in 16-bit RAW format to retain all of the captured information without compression.
**Experimental setup**

4.3.1 Specular reflection and cross-polarised lighting

The intestine is inherently wet, and it must be kept without drying out to retain physiologically realistic activity. The wet intestinal tissue reflects directional light into the camera causing regions of bright patches in the image known as specular reflection (Fig. 4.7). Regions with specular reflection do not contain information of the tissue texture, and therefore, cannot be tracked (Fig. 4.8A). Furthermore, these bright spots could move in unrealistic directions due to the change in angle between the surface and the light source during deformations corrupting the displacement fields. Diffused reflection (Fig. 4.7) on the other hand only contain the wavelengths that were not absorbed by the tissue, and therefore contain information of the texture.

Specialised lighting setups can be used to reduce specular reflection. Figure 4.8 shows images taken under diffused and cross-polarised lighting setups compared to an image taken under room lighting. The subject can be illuminated from all sides with diffused lighting by reflecting the point light sources (LEDs) off of large surfaces. When the subject is illuminated from all directions, majority of the light rays captured by the camera would be via diffused reflection (Fig. 4.8B). Another approach is to use a cross-polarised lighting setup to block specular reflected light. By perpendicularly polarising the light source and the camera, specular reflected light can be blocked while letting in diffuse reflected light (Fig. 4.8C). The cross-polarised lighting setup provided the best quality images with least specular reflection and most detailed textures, therefore, was used in the experimental setup to capture intestinal contractions.

---

**Figure 4.5:** Comparison of FFD based strain measurements with strain-rate from spatial differentiation of displacements. Optimisation of the FFD based method was performed for 100 iterations. Maps were generated for a synthetic test case where a 2D sheet was stretched from 100 % to 109 % in 10 frames. (A) Strain maps from FFD based method, (B) strain-rate maps from FFD based method, and (C) strain-rate maps from spatial differentiation of displacements.

<table>
<thead>
<tr>
<th>Without noise (Ground truth)</th>
<th>SNR = 84 dB</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>(A)</td>
</tr>
<tr>
<td>(B)</td>
<td>(B)</td>
</tr>
<tr>
<td>(C)</td>
<td>(C)</td>
</tr>
</tbody>
</table>
Figure 4.6: Experimental setup used to record intestinal motility. A segment of the intestine was exposed through a midline incision in the abdominal area. The intestine was illuminated with a cross-polarised lighting setup, and the motility patterns were recorded with a camera perpendicularly positioned above the intestine.

4.3.2 Camera parameters

The camera parameters must be adjusted to get a well exposed, sharp image. The image exposure depends on the exposure interval, aperture (f-stop), and the gain of the camera, while the sharpness of the image depends on the exposure interval, focus, and the f-stop. Whether an image is properly exposed can be identified by looking at its histogram. For a well-exposed image, the histogram should spread out over the complete range of pixel intensities supported by the camera sensor (Fig. 4.9).

The exposure interval is the amount of time the camera sensor is exposed for before capturing an image. The exposure interval should be high enough to adequately expose the image (let enough light in), but should be low enough to avoid motion blur. In this experimental setup, the sensor was exposed for 1/100 s or 10 ms.

There are two considerations when selecting the aperture size, or the f-stop value of the lens. First, the f-stop value dictates the depth of field (DOF) of the camera or the distance between
Experimental setup

Figure 4.8: Images of the intestine taken under different lighting setups. (A) Under room lighting, (B) diffused lighting, and (C) cross-polarised lighting. Image taken under cross-polarised lighting has more detail and minimal specular reflection.

The depth of field (DOF) can be calculated according to Eqn (4.19) [208]:

\[ DOF \approx \frac{2 \times C \times F_n \times f_d \times (f_d - f)}{f^2} \]  

(4.19)

where, \( C \) is the diameter of the circle of confusion (smallest part of the image with acceptable sharpness), \( F_n \) is the f-stop value, \( f_d \) is the focused distance, and \( f \) is the focal length of the lens (here 25 mm). If the f-stop value is too low (e.g., f/2.0), the depth of field will be too low, and a slight out of plane movement of the intestine (e.g. during breathing motion) would cause it to go out of focus. If the f-stop is too high (e.g., f/16), the depth of field would be high, however, the overly narrow aperture would cause the light rays to diffract, which would soften the image. General guidelines suggest that the mid-range of the f-stop values supported by the lens (e.g. around f/8) gives the sharpest image [209]. Secondly, the f-stop determines the amount of light let into the camera sensor. A high f-stop value would let in less light, therefore, a brighter lighting setup would be required to properly expose the image. In here, f/5.6 was selected as it provided sufficient exposure and depth of field.
High-Resolution Mapping of Intestinal Motility

Figure 4.9: Histograms of a poorly exposed image and a well exposed image. Histogram of the well exposed image is more spread out, and contains pixels in the complete range of intensities supported by the camera sensor.

The amount of noise in the image increases with the camera gain, therefore, the gain must be kept as low as possible. The intensity of the light source should be increased to obtain a properly exposed image at the selected f-stop. However, if the intensity of the light source cannot be practically increased, the gain can be increased to adequately expose the image. The noise level of the camera at different gain settings were estimated by comparing a photo taken with the lens cap to a synthetic black image (pixel intensity = 0). The signal to noise ratio (SNR) of the images at different gain setting were calculated, and are given in Table 4.1. The results show that the selected machine vision camera was capable of producing high quality images with less noise (SNR > 100) even with increased gain. In here, gain of 20 dB, and SNR = 114 dB was deemed an acceptable quality for images as our algorithm was able to estimate strain with under 1% error at SNR = 98 dB (refer Section 4.2.5).

Table 4.1: Comparison of camera noise level under different gain settings. Images were captured with the lens cap on, under different gain settings, and their noise levels were calculated with reference to a synthetically generated black image.

<table>
<thead>
<tr>
<th>Gain (dB)</th>
<th>Signal-to-noise ratio (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>131</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
</tr>
<tr>
<td>10</td>
<td>124</td>
</tr>
<tr>
<td>20</td>
<td>114</td>
</tr>
<tr>
<td>30</td>
<td>96</td>
</tr>
</tbody>
</table>

Finally, the lens must be properly focused on to the intestine to acquire a sharp image. Although the sharpness can be evaluated visually, here, it was quantified as described in [210] to help obtain the sharpest image possible. The captured image was decomposed into high-
Experimental setup

frequency \( (h_{w}(f)) \) and low-frequency \( (l_{w}(f)) \) bands using the MATLAB wavelet decomposition function ‘wavedec2’, with level = 2, and mother wavelet ‘db2’. Then, the sharpness of the image was quantified as Eqn (4.20), where a higher value of \( W \) indicate greater sharpness as shown in Fig. 4.10. The camera focus was manually tuned to maximise \( W \) in the region of interest.

\[
W = \frac{\|h_{w}(f)\|}{\|l_{w}(f)\|} \quad (4.20)
\]

where \( \| . \| \) denotes the Euclidean norm.

4.3.3 Validation of the experimental setup

The experimental setup was validated by performing translational shifts on an excised portion of the intestine under experimental conditions. The RMS error between the known translational shift and the estimated translational shift, along with the RMS error in the estimated strain (which should be zero) are provided in Table 4.2. The RMS strain error was under 1 %.

Table 4.2: Translational shifts and RMS strain errors detected when translating an excised segment of intestine.

<table>
<thead>
<tr>
<th>Known translation (mm)</th>
<th>Calculated translation(^1) (mm)</th>
<th>RMS strain error(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.5256 ± 0.0057</td>
<td>0.0010</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0517 ± 0.0036</td>
<td>0.0014</td>
</tr>
<tr>
<td>1.5</td>
<td>1.5383 ± 0.0043</td>
<td>0.0016</td>
</tr>
<tr>
<td>2.0</td>
<td>2.0299 ± 0.0071</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

\(^1\) Calibration error = 0.39 pixels
\(^2\) Strain should be 0 during translation.
4.4 Strain fields and spatiotemporal strain maps

Strain fields were generated to quantify the contractile activity across the 2D surface of the intestine. Fig. 4.11 shows three frames where strain fields have been overlaid on a video sequence of longitudinal contractions. Since the strain across the surface was quantified, localised, anisotropic, contractions and relaxations occurring in the intestine can be detected. Longitudinal strain fields ($E_{11}$) were used to quantify longitudinal contractile activity, and transverse strain fields ($E_{22}$) were used to quantify circumferential contractile activity.

![Longitudinal strain fields overlaid on a video sequence of longitudinal contractions. The strain fields were able to quantify anisotropic, localised longitudinal contractions of the intestine. The blue regions indicate contracted areas while red indicate elongated areas.](image)

Strain maps were generated to represent the spatiotemporal dynamics of the motility patterns. The contractions were mapped to the spatiotemporal maps as depicted in Fig. 4.12, where the horizontal axis of the map corresponds to the longitudinal axis of the intestine ($\xi_1$), and the vertical axis corresponds to time. The colour map indicates the mean longitudinal strain ($E_{11}$) in longitudinal strain maps, and mean transverse strain ($E_{22}$) in transverse strain maps (Fig. 4.2), along the length of the intestine, at a particular time. The mean strain was taken within a subset of $\xi_2$ values depending on which region (mesenteric border, anti-mesenteric border, middle) the contractions occurred. Negative strain indicate shortening of the muscles ($-0.1$ strain $= 10\%$ contraction), while positive strain indicate elongation of the muscles.

The strain maps were used to calculate the velocity and frequency of motility patterns. Non propagating contractions appeared as horizontal bands of negative strain and propagating contractions appeared as diagonal bands (Fig. 4.12). The slope of the diagonal band denotes the velocity of the contraction. The frequency was calculated as the number of contractile events in the map within a time interval. The mean deformation caused by each motility pattern was quantified with mean strain.

4.5 Motility in the in-vivo intestine

The feasibility of the FFD framework was validated by performing in-vivo experiments on five female, cross-breed, weaner pigs ($41 \pm 3$ kg), and a female white rabbit (4 kg). The experiments
Figure 4.12: Transverse strain map depicting an antegrade peristaltic contraction. The spatiotemporal map shows the variation of mean transverse strain along the length of the intestine with time. The peristaltic contraction appeared as a diagonal band of negative strain angled towards the direction of propagation, and the gradient of the diagonal band gives the velocity of propagation. Three snapshots of the intestine at 4 s, 8 s and 12 s, are displayed to show the propagating contraction, and their relationship to the spatiotemporal strain map.

were performed under the ethical approvals of the University of Auckland Animal Ethics committee. Animal preparation and anaesthesia were performed as previously described in Section 3.1. In summary, a midline laparotomy was performed, and a segment of the small intestine was exposed and placed on top of the abdomen. The intestine was distended by injecting warm saline (10 – 15 mL) to elicit motility patterns. The experimental setup and the algorithms described in the previous sections were used to record and quantify observed motility patterns within a total duration of 39 minutes (437 GB of video data).

4.5.1 **Longitudinal contractions**

We observed 12 longitudinal contractions in total, in 2 pig studies. The algorithm was used to quantify the longitudinal and circumferential activity of the 2D surface of the intestine, and the high-resolution strain fields were able to clearly capture the anisotropic behaviour of the longitudinal muscle (Fig. 4.13). The results much more clearly show that longitudinal contractions can be different across the diameter [156], and in the given instance of Fig. 4.13, most of the contractions occurred at the mesenteric side of the intestine, while the anti-mesenteric side was relatively inactive. These longitudinal contractions caused $-0.21 \pm 0.6$ strain ($21 \pm 6\%$ contraction) along the longitudinal axis, and did not display corresponding circumferential activity, as shown in the transverse strain field in Fig. 4.13.

Further analysis of the difference in contractions on the mesenteric and anti-mesenteric sides is provided in Chapter 5.
4.5.2 Segmental contractions

Segmental contractions were the most commonly observed motility pattern, and 64 segmental contractions were observed in total, in the 5 pig studies, alone and in combination with other patterns (Fig. 4.14 & 4.15). These were localised ring-like contractions that did not propagate along the intestine, and therefore, registered as horizontal bands of negative strain in the transverse strain maps. The width of the blue bands in Fig. 4.14 denotes the length of the intestine that underwent contraction, while height represents the duration it was in the contracted state. Segmental contractions caused $-0.17 \pm 0.10$ strain ($17 \pm 10\%$ contraction) along the transverse axis.

4.5.3 Spontaneous peristaltic contractions

Peristaltic contractions are propagating circumferential contractions, that transfer intraluminal content along the intestine [39]. Two types of peristaltic contractions were observed in these experiments. In two pig studies, 7 antegrade and 4 retrograde spontaneous peristaltic contractions were observed. These peristaltic contractions did not occur periodically, but spontaneously originated in different parts of the distended section and propagated distally (antegrade), and orally (retrograde), at a velocity of $3 \pm 1$ mm/s, as indicated by the arrows in Fig. 4.15. Most of these contractions propagated short distances ($\leq 12$ mm) in our field of view, and caused $-0.36 \pm 0.04$ strain ($36 \pm 4\%$ contraction) along the transverse axis. The longest antegrade
spontaneous propagating contraction observed during the study is shown in the transverse strain map in Fig. 4.16A. As indicated by the diagonal band of negative strain, the peristaltic contraction propagated distally for about 16 mm before terminating with a segmental contraction at the end (indicated by the horizontal band of negative strain). There was synchronous longitudinal activity with the spontaneous peristaltic contraction as shown in the longitudinal strain map in Fig. 4.16E. The intestinal segment contracted longitudinally before the beginning of the peristaltic contraction (Fig. 4.16E, light blue region after 0 s) and expanded following the contraction (Fig. 4.16E, red region). The longitudinal contraction is further evident by the width of the elements in the right side of the mesh in Fig. 4.16B prior to the peristaltic contraction being smaller than the width of those in the left side of Fig. 4.16D after the contraction. This initial longitudinal contraction is referred to as the preparatory phase of peristaltic contractions [39], and is now presented in high spatiotemporal resolution.

4.5.4 Cyclic peristaltic contractions

Cyclic peristaltic contractions were mapped for 12 cycles in the rabbit study. Unlike spontaneous peristaltic contractions, cyclic peristaltic contractions occurred at regular intervals as can be seen by the parallel bands of negative strain in Fig. 4.17, and caused $-0.17 \pm 0.02$ strain ($17 \pm 2 \%$ contraction) along the transverse axis. The pattern and direction of travel of these contractions became defined after differentiating the strain map with respect to time (i.e., in the strain-rate map). Cyclic peristaltic contractions occurred at $11.0 \pm 0.6$ cpm and propagated faster than spontaneous peristaltic contractions, at $16 \pm 4$ mm/s. Back and forth propagation of these cyclic
Transverse strain map presenting spontaneous peristaltic contractions and segmental contractions observed during a pig study. Peristaltic contractions appeared as diagonal bands indicated by arrows, which occurred as continuous waves (solid arrows) or in multiple stages (dashed arrow). The segmental contractions appeared as columns (or rectangles). These peristaltic contractions were not periodic, but spontaneously originated in the distended region, and propagated varying lengths. Video frames after saline injection with the deforming mesh colour-coded according to transverse strain fields. Contractions were also observed 2 times in this rabbit study, and could be a mechanism for mixing intraluminal content. One such instance is indicated by the dashed arrows in the strain-rate map given in Fig. 4.17.

4.6 Advantages of FFD motility mapping algorithm

The experimental results show that this FFD algorithm can be successfully applied to in-vivo experiments to map a wide range of motility patterns, at much higher resolution. The active deformations in the intestine typically involve coordinated contractions and relaxations of circular and longitudinal muscles around the diameter (circumferential contractions) and along the length (longitudinal contractions) of the intestine, respectively. As we have aligned our material coordinate system along these directions, the circular and longitudinal contractile activity was simultaneously measured by transverse ($E_{22}$) and longitudinal ($E_{11}$) strains, respectively. For example in Fig. 4.13, pure longitudinal contractions and relaxations are reflected in the longitudinal strain, but not in the transverse strain. Currently available diameter-based and marker-based methods measure only circumferential or longitudinal contractions, and the two methods have to be combined to measure both activities. Some motility patterns, such as peristaltic contractions, are caused by the coordinated contraction and relaxation of both circular and longitudinal...
Advantages of FFD motility mapping algorithm

Figure 4.16: The circumferential and longitudinal activity recorded during a spontaneous peristaltic contraction observed in pig. The peristaltic contraction appeared as a diagonal band of negative strain in the transverse strain map. The intestine contracted longitudinally before the beginning of the peristaltic contraction and expanded once the peristaltic contraction passed. The peristaltic contraction terminated with a segmental contraction during which the intestine contracted both circumferentially and longitudinally. Three snapshots of the intestine at 9.6 s, 13.6 s and 17.6 s show the propagating contraction. The mesh fitted to the above spontaneous peristaltic contraction and the generated strain fields are shown in video form in Supplementary Video 1 (https://doi.org/10.17608/k6.auckland.17711528).

muscles [39]. Therefore, simultaneous analysis of both circumferential and longitudinal contractile activity is important to obtain a more detailed understanding of motility patterns, and to identify interactions between the two muscle layers (Fig. 4.16).

The FFD algorithm can overcome several drawbacks in existing spatiotemporal mapping techniques outlined in [163]. The diameter and marker-based methods measure the cumulative effect across the diameter or between markers along the length of the intestine [39]. The FFD algorithm can generate longitudinal and transverse strain maps that mimic DMaps (Fig. 4.12–4.17). In addition, it can also generate area strain maps [163] that show the continuous 2D strain field of the surface of the intestine (Fig. 4.13), which can identify areas of contractions and anisotropic behaviour of the muscle. Furthermore, the area strain maps can be overlaid onto the video sequences to provide a real-time record of changes in sites of contraction. Previously, strain-rate had been used to overcome the above limitations [40], [41]. However, strain-rate does not quantify the level of contraction. This FFD algorithm was able to quantify the level of contraction during the observed motility patterns. Furthermore, strain-rate may not be optimum for tracking tonal contractions, as video recordings needed to be downsampled to capture slow deformations [163]. With the FFD algorithm, strain can be applied to map both phasic and tonal motility patterns, and strain-rate can also be calculated and utilised when necessary.
Figure 4.17: A transverse strain map and its strain-rate map (differentiated with respect to time) depicting cyclic peristaltic contractions observed in the rabbit study. Top two arrows indicate periodic contractions propagating distally. The two dashed arrows indicate back and forth propagation of a cyclic peristaltic contraction, presumably mixing the intraluminal content. In this situation, differentiating the strain map (strain-rate map) makes the underlying mechanical activity easier to interpret. The strain map indicates the contracted or relaxed state of the intestine, while the strain-rate map shows the contracting spatiotemporal regions in blue and relaxing (expanding) regions in red. The mesh fitted to cyclic peristaltic contractions and the generated strain fields are shown in video form in Supplementary Video 2 (https://doi.org/10.17608/k6.auckland.17711528).

4.7 Limitations

Although the developed method was able to accurately capture deformations, there are four key limitations. First, the optimisation step added a significant computational burden to the overall analysis. Secondly, the strain measurements computed here were 2-dimensional approximations of the 3-dimensional strains in the tissue, and are affected by the convexity of the intestine. Thirdly, the mapped area of the intestine shown here did not extend to the complete width of the diameter as the outermost elements of the mesh were discarded. However, the mesh can be further extended towards the edges by increasing the number of elements to reduce the area covered by the outermost elements. Finally, since motility patterns were mapped based on visible deformation of the intestine, it limits the analysis of isometric contractions [162].

The developed experimental setup also imposes two key limitations. First, to record motility with the current experimental setup, the intestine should be exposed. The abdominal incision can cause reflex inhibition and affect the recorded motility patterns [211]. Secondly, the exposed intestinal segment’s temperature and moisture were not strictly controlled in the experiments. The heat generated from the lighting setup kept the intestinal segment relatively warm, and warm saline was applied to the intestinal surface during long recordings to maintain the temperature and moisture. However, loss of temperature and moisture during the course of the studies could
have affected the recorded motility patterns [212].

4.8 Summary

The objective of this chapter was to develop a method to quantify strain fields with high spatial resolution from in-vivo intestinal motility recordings that mitigate accumulation of tracking error. During synthetic tests, the FFD algorithm had an RMS strain error under 1 % and the optimisation step reduced the rate of strain error by 97 %. The algorithm was applied to map 64 segmental, 12 longitudinal, and 23 peristaltic contractions in the jejunums of 5 pigs and a rabbit. Coordinated activity of the two muscle layers could be identified and the strain fields were able to map and quantify the anisotropic contractions of the intestine. Longitudinal contractions of $21 \pm 6 \%$ and segmental contraction of $17 \pm 10 \%$ were observed in pigs. Frequency and velocity were also quantified, from which two types of peristaltic contractions were identified: (i) spontaneous peristaltic contractions of $36 \pm 4 \%$ occurred and propagated at $3 \pm 1 \text{ mm/s}$ in two pigs, and (ii) cyclic peristaltic contractions of $17 \pm 2 \%$ occurred at $11.0 \pm 0.6 \text{ cpm}$ and propagated at $16 \pm 4 \text{ mm/s}$ in a rabbit. The experimental results demonstrated the feasibility and the advantages of FFD based motility mapping. The validated motility mapping method can now be applied with simultaneous slow wave and spike burst mapping to drive physiological investigations into the underlying control mechanisms of GI motility.
Chapter 5

The Relationship Between Slow waves, Spike bursts, and Motility

Preliminary results have been published as a conference proceeding titled “High-Resolution Mapping of Intestinal Spike Bursts and Motility”, at the 2020 international conference of the IEEE Engineering in Medicine and Biology Society [213].

The work presented in this chapter has been published in the Journal of Neurogastroenterology and Motility, in a paper titled “The relationship between slow waves, spike bursts, and motility, as defined through high-resolution electrical and video mapping.” [214].

The spatiotemporal correlation of intestinal bioelectrical activity and motility patterns is poorly understood. A limited number of studies have identified different activation patterns of bioelectrical slow waves and spike bursts during peristaltic [12], [32] and pendular [34] contractions. These previous studies indicate that spike burst activation patterns and their affinity to slow waves play a key role in determining the motility patterns. However, existing studies have not shown a clear spatiotemporal relationship between slow waves, spike bursts, and motility due to technological limitations. Some studies have predominantly focused on the bioelectrical activity and have not mapped or quantified the motility patterns [12], [33]. Other studies have utilised 1-dimensional electrode arrays and motility measurements, therefore, have not clearly captured or correlated the 2-dimensional spike burst, slow wave activations on the tissue surface, and the surface deformations. In addition, existing studies have predominantly been performed in-vitro in tissue baths [12], [32]–[34] and may not represent the integrative electrophysiological state of the gut.

The objective of this chapter is to apply high-resolution bioelectrical and motility mapping techniques developed in the Chapters 3 and 4 to identify how spatiotemporal activations of slow waves and spike bursts are related to differing motility patterns in the in-vivo intestine.
5.1 Experimental setup

Experiments were performed in-vivo on 6 female cross-breed, weaner pigs (41.7 ± 2.5 kg), and 3 female white rabbits (4.0 ± 0.7 kg), under the ethical approval of the University of Auckland Animal Ethics Committee. Animal care, preparation and anaesthesia were performed as previously described in Section 3.1. The experimental setup is shown in Fig. 5.1. In summary, a midline laparotomy was performed, and a section of the jejunum was exteriorised and placed on top of an electrode array to record the bioelectrical activity using the BioSemi ActiveTwo system (Figs. 5.1C–D, see Section 3.2 for a detailed description). The mechanical contractions were simultaneously recorded from a machine vision camera positioned perpendicularly above (Figs. 5.1A–B, see Section 4.3 for a detailed description). The bioelectrical and video recordings were synchronised by registering a transistor-transistor logic (TTL) pulse generated from the camera during exposure with the BioSemi ActiveTwo system. After recording the baseline activity (duration 5 ± 4 minutes), the jejunum section was distended by injecting warm saline (10—15 mL) to induce contractions (duration 4 ± 2 minutes).

Bioelectrical slow wave and spike burst mapping and analysis were performed as described in Chapter 3. Slow wave frequency and velocity were quantified as given in Section 3.4.1. Spike burst frequency, amplitude, duration, and spike burst patch amplitude, duration, energy were quantified as detailed in Section 3.5.1. Spike bursts that activated in a periodic manner with the slow wave cycles in the signal traces were identified as slow wave associated spike bursts, and those that activated independently in an aperiodic manner were identified as independent spike bursts. Video mapping and quantification of motility patterns were performed as described in Chapter 4. Level of deformation, frequency, and velocity of motility patterns were quantified as given in Section 4.4.

Results are reported as mean ± standard deviation. Wilcoxon rank sum test was used as the statistical test to identify significant changes in the electrical parameters (amplitudes and durations) of slow wave associated and independent spike bursts. Pearson correlation was used to identify the trends between the level of contraction and the spike burst patch amplitude, duration, size, energy. \( p < 0.05 \) was considered significant.

5.2 Bioelectrical activity and contractions

Slow waves in the jejunum occurred at 12.6 ± 2.3 cpm in the pig studies, and 10.8 ± 1.5 cpm in the rabbit studies. Spike bursts occurred in conjunction with contractions. Spatially, slow waves propagated along the intestine and spike burst activated as localised patches, as shown in Chapter 3. Circumferential spike burst patches that predominantly activated around the lumen, and longitudinal spike burst patches that activated along the length of the intestine, were associated with contractions in the respective muscle orientations, as shown in Sections 5.4–5.7. Both slow wave associated and independent spike bursts were observed in the baseline and distended experiments as shown in Table 5.1, and were associated with different motility patterns.

The results demonstrated that spike burst propagation patterns ultimately dictated the resultant contractile response. Fig. 5.2 shows a representative example, where an activation of a
Figure 5.1: Simultaneous bioelectrical and video mapping setup. (A) Schematic arrangement of the cross-polarised camera setup described in Chapter 4 and the electrode array described in Chapter 3 on the intestine. The camera exposure active signal was recorded along with the bioelectrical signals by the BioSemi ActiveTwo system for synchronisation with the video recording. (B) A photo of the actual in-vivo experimental setup with an intestinal segment exteriorised. (C) Intestine as visible from the camera, placed over the flexible electrode array. (D) Flexible electrode array used for high-resolution bioelectrical mapping (16×8 configuration; 4 mm spacing).
The Relationship Between Slow waves, Spike bursts, and Motility

Table 5.1: Motility patterns and the relationship between slow waves and spike bursts observed during experiments. Spike bursts either occurred with slow wave cycles (slow wave associated spike burst (SWASB)) or independently to the slow waves (independent spike burst (ISB)). N/A indicate not performed and dash indicate no activity.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Motility patterns</th>
<th>Baseline Spike burst type</th>
<th>Motility patterns</th>
<th>Distended Spike burst type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td>Segmental, Pendular</td>
<td>ISB, SWASB</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pig 2</td>
<td>—</td>
<td>—</td>
<td>Segmental, Spontaneous peristalsis</td>
<td>ISB</td>
</tr>
<tr>
<td>Pig 3</td>
<td>Pendular</td>
<td>SWASB</td>
<td>Segmental</td>
<td>ISB</td>
</tr>
<tr>
<td>Pig 4</td>
<td>—</td>
<td>—</td>
<td>Segmental, Spontaneous peristalsis</td>
<td>ISB</td>
</tr>
<tr>
<td>Pig 5</td>
<td>—</td>
<td>—</td>
<td>Segmental</td>
<td>ISB</td>
</tr>
<tr>
<td>Pig 6</td>
<td>—</td>
<td>—</td>
<td>Segmental</td>
<td>ISB</td>
</tr>
<tr>
<td>Rabbit 1</td>
<td>Cyclic peristalsis</td>
<td>SWASB</td>
<td>Cyclic peristalsis</td>
<td>SWASB</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>Pendular</td>
<td>SWASB</td>
<td>—</td>
<td>SWASB</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>Pendular</td>
<td>SWASB</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

A circumferential spike burst patch led to a circumferential contraction. The relationship between the electrical parameters of spike burst patches and the level of deformation was analysed using 48 segmental contractions as shown in Fig. 5.3. There were significant correlations between the level of contraction indicated by negative strain and the amplitude ($r = -0.47, p < 0.0001$, Fig. 5.3A), patch size ($r = -0.39, p = 0.006$, Fig. 5.3C), and the energy ($r = -0.51, p < 0.0001$, Fig. 5.3D) of the spike burst patches. Stronger correlations were found between the amplitude, duration, patch size, and energy of spike burst patches with the rate of contraction, as shown in Table 5.2.

Table 5.2: Correlations between spike burst patch parameters and the rate of contraction quantified by strain-rate.

<table>
<thead>
<tr>
<th>Spike burst patch parameter</th>
<th>Correlation coefficient (r)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude</td>
<td>-0.44</td>
<td>$p = 0.004$</td>
</tr>
<tr>
<td>Duration</td>
<td>-0.39</td>
<td>$p = 0.010$</td>
</tr>
<tr>
<td>Patch size</td>
<td>-0.61</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Patch energy</td>
<td>-0.74</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>

Previous studies have shown that spike bursts increase intraluminal pressure and outflow [27], [28]. Here we showed the spatiotemporal correlation between spike burst patches and the sites of contraction. Longitudinal spike burst patches are likely calcium currents that trigger contractions in the longitudinal muscle layer, while circumferential spike burst patches are likely calcium...
currents in the circular muscle layer [30]. As a result, these patches caused contractions in the respective directions. The electrical parameters of spike bursts likely indicate the extent of the inward calcium currents [215]. A stronger calcium current may likely lead to a faster increase in the intracellular calcium concentration and therefore, led to an increased rate of contraction. It may also lead to a higher overall intracellular calcium concentration and therefore, caused a larger contraction [63]. A larger spike burst patch size may result in the activation of a large portion of the muscle layer, therefore, caused a larger area of the tissue to undergo contraction. However, it should be noted that the calcium sensitivity of the SMCs can be altered by the presence of various enzymes, resulting in a varied contractile response for similar calcium concentrations [56], [68]. The presence of such enzymes may have contributed to the high variance of the correlations presented in Fig. 5.3.

5.3 Slow wave associated vs independent spike bursts

Spike bursts occurred coupled to slow wave activation at times, but also operated independent of slow wave activation at other times, demonstrating that in the jejunum, slow waves were not always correlated to the contractile response. In 2 pigs and 3 rabbits, spike bursts occurred with slow waves at 10 ± 0.5 cpm in pigs (slow wave frequency for this subset was 11.8 ± 0.8 cpm), 10.2 ± 3.2 cpm in rabbits (slow wave frequency for this subset was 10.8 ± 1.4 cpm), and are referred to as slow wave associated spike bursts. Slow wave associated spike bursts are shown in Fig. 5.4A, where fast fluctuations of spike bursts can be seen with the slow wave cycles marked with red pluses. In 6 pigs, another type of spike bursts occurred independently to the slow waves as shown in Fig. 5.4B in an aperiodic manner, less frequently (3.2 ± 1.8 cpm spike burst frequency vs 12.0 ± 2.0 cpm slow wave frequency for this subset in pigs), and could be
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Figure 5.3: Correlation between spike burst patch parameters and the level of contraction quantified by mean strain. (A) Spike burst patch amplitude, (C) spike burst patch size, and (D) spike burst patch energy displayed significant correlations with the level of contraction.

distinctly identified from slow wave associated spike bursts. Distending the intestine caused an increase in independent spike burst activity in pigs, but rabbits did not exhibit independent spike bursts. The independent spike bursts and slow wave associated spike bursts displayed different electrical characteristics as shown in Fig. 5.5. Independent spike bursts observed with segmental and spontaneous peristaltic contractions had a larger morphology than the slow wave associated spike bursts observed with cyclic peristalsis and pendular contractions. The independent spike bursts had a higher mean amplitude (1.4±0.8 mV vs 0.1±0.1 mV, \( p < 0.001 \) in pigs; 0.1±0.1 mV slow wave associated spike bursts in rabbits, Fig. 5.5B) and a higher mean duration (1.8±1.4 s vs 0.8±0.3 s, \( p < 0.001 \) in pigs; 0.4±0.2 s slow wave associated spike bursts in rabbits, Fig. 5.5D).

Slow wave associated spike bursts and independent spike bursts displayed similar characteristics to myogenic vs neurogenic spike bursts observed in the colon [147], and slow wave associated spike bursts vs peristaltic waves defined in the cat small intestine [12]. Slow wave associated spike bursts had lower amplitudes and shorter durations, similar to myogenic spike bursts and slow wave associated spike bursts in cats. Independent spike bursts had higher amplitudes and longer durations, similar to neurogenic spike bursts and peristaltic waves. The affinity to slow waves suggests that slow wave associated spike bursts are at least partly myogenically mediated. They occurred periodically with slow waves and led to cyclic motor patterns modulated by slow waves. The independent spike bursts are likely activated by the firing of enteric neurons [148] unrelated to the underlying slow waves. However, since neural and myogenic inhibitory agents were not used in this study, the regulatory or co-regulatory mechanisms of these two types of
spike bursts could not be conclusively verified.

The higher amplitude and duration of independent spike bursts suggest that they may be attributed to larger calcium currents than slow wave associated spike bursts. Slow wave dependent calcium currents occur through voltage-dependent calcium channels [63], [216]. As given in Section 2.3.1, neurogenic mechanisms can cause calcium entry through multiple pathways [216]. For instance, the neurotransmitter ACh can activate both transient receptor potential cation channels and intracellular calcium release [65], [216]. The resulting rapid depolarisation could also activate voltage-dependent calcium channels, which could lead to a larger inward calcium current compared to only voltage-dependent calcium channels in slow wave associated spike bursts, and accords with our results where independent spike bursts correlated with larger deformations.

Although slow wave associated spike bursts occurred with the slow wave cycles, their propagation patterns were not always identical to the slow waves (Fig. 5.6). There may be two ways spike bursts can propagate along the intestine. (i) The propagating slow wave may initiate spike bursts in SMCs in its path [63] where they would appear to propagate coupled to the slow waves in the bioelectrical recordings [144], and (ii) the initiated spike bursts may also independently propagate along the smooth muscle layers [137], [144] as circumferential and longitudinal spike burst patches [30]. The two modes of propagation are further evident by the fact that slow wave associated spike bursts deviated from the slow wave propagation more along the rows of electrodes in longitudinal spike burst patches (Fig. 5.10D), but deviated less along the rows in circumferential patches (Fig. 5.9D). The resulting spike burst propagation would be through a combination of both: initiation by propagating slow waves and independent propagation along the SMCs, as shown in Fig. 5.6, and therefore, could deviate from the slow waves.
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Figure 5.5: Electrical parameters of independent spike bursts (ISB, white box plots) and slow wave associated spike bursts (SWASB, grey box plots). (A) Amplitude of spike bursts observed during motility patterns in each species. (B) Amplitudes of ISBs and SWASBs in each species. ISBs had a significantly higher amplitude than SWASBs (*p < 0.001 in pigs). (C) Duration of spike bursts observed during motility patterns in each species. (D) Durations of ISBs and SWASBs in each species. ISBs had a significantly higher duration than SWASBs (*p < 0.001 in pigs).

Furthermore, our results indicate there may be an inhibitory relationship between independent spike bursts and slow wave associated spike bursts. In one pig experiment, slow wave associated spike bursts were observed during baseline but were replaced by independent spike bursts after distension. The mechanism behind the replacement of slow wave associated spike bursts with independent spike bursts is not clear. A previous report in the colon has identified that neurogenic spike burst activations inhibit myogenic spike bursts and suggests that there may be some enteric neural control over the mechanisms underlying myogenic spike bursts [147]. The results in this thesis indicate that a similar relationship between independent spike bursts and
5.4 Segmental contractions

Segmental contractions are non-propagating circumferential contractions, and are the most common type of contractions in the intestine [149]. They help to break down intraluminal content, and rhythmic segmental contractions, known as the segmentation motor pattern, help with both mixing and breaking down [150].

Segmental contractions (64 segmental contractions were observed in pigs) were driven by independent spike bursts with a mean amplitude of $1.3 \pm 0.7$ mV and a mean duration of $1.8 \pm 1.5$ s. A representative example of the underlying bioelectrical activity during segmental contractions is shown in Fig. 5.7. These non-propagating circumferential contractions caused the intestine to contract by $16 \pm 9\%$ and were registered as horizontal bands of negative strain in the transverse strain maps (Fig. 5.7A). During segmental contractions spike bursts activated as circumferential spike burst patches as shown in Fig. 5.7B. The spike bursts were not coupled to the slow waves as seen in the slow wave activation time map in Fig. 5.7C, and the electrical traces in Fig. 5.7D.

5.5 Spontaneous peristaltic contractions

Peristaltic contractions are propagating circumferential contractions that help transport intraluminal content along the intestine. These contractions can propagate in either direction. Peristaltic contractions that propagate distally are called antegrade peristaltic contractions or peristalsis [153], while ones that propagate orally are called retrograde peristaltic contractions [162] or anti-peristalsis [153]. In addition, variations of peristaltic contractions have been reported under different names, a foremost classification being spontaneous or irregular peristaltic contractions versus cyclic or regular peristaltic contractions [12], [32], [33], [36].

Spontaneous peristaltic contractions (11 peristaltic contractions were observed in pigs) were driven by independent spike bursts with a mean amplitude of $2.3 \pm 0.6$ mV and a mean du-
Figure 5.7: Slow wave and spike burst activity recorded during segmental contractions. (A) Spatiotemporal transverse strain map which shows the segmental contractions and relaxations observed in the intestine. (B, C) Activation maps of the spike burst and slow wave activity just prior to the segmental contraction marked by the white square in A. The shaded area represents the position of the intestine on the electrode array. (D) The electrical signal traces from a row of electrodes with slow wave events marked as red plus symbols. The region activated by the circumferential spike burst patch in B underwent contraction as shown by the white square in A. These spike bursts and the corresponding contractions did not show a connection to the underlying slow waves as seen in D. The above activity is presented in video form in Supplementary Video 3 (https://doi.org/10.17608/k6.auckland.17711528).
Cyclic peristaltic contractions

Cyclic peristaltic contractions were observed in 1 out of 3 rabbit studies, and continuously occurred throughout the total recording duration of 17 minutes. These contractions were driven by slow wave associated spike bursts with a mean amplitude of 0.2 ± 0.1 mV and a mean duration of 0.2 ± 0.1 s. The underlying bioelectrical activity during cyclic peristalsis is shown in Fig. 5.9. These longitudinally propagating circumferential contractions occurred periodically at a similar frequency to the underlying slow waves (11.0 ± 0.6 cpm contraction frequency vs 10.8 ± 0.6 cpm slow wave frequency, \(p = 0.97\)), and were registered as periodic diagonal bands of negative strain in the transverse strain map (Fig. 5.9A). During these regularly propagating circumferential contractions, spike bursts activated as longitudinally propagating circumferential spike burst patches (Fig. 5.9B). The spike bursts activated periodically with slow wave cycles, which resulted in the continuous cyclic peristaltic contractions, as shown in the signal traces in Fig. 5.9D. Back and forth propagation of cyclic peristaltic contractions were also observed, and were caused by spike bursts from adjacent competing slow wave pacemakers (dashed arrows in Fig. 5.9A & 5.9D). However, the propagation patterns of slow waves and the spike bursts deviated at times
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Figure 5.8: Slow wave and spike burst activity during spontaneous peristaltic contractions. (A) Spatiotemporal transverse strain map recorded during spontaneous peristaltic contractions. The spontaneous activation and propagation of these peristaltic contractions are indicated with arrows. Segmental contractions are indicated with rectangles. (B, C) Activation maps of spike burst and slow wave activity during the peristaltic contraction indicated by the white arrow in A. The shaded area represents the position of the intestine on the electrode array. The black rectangle in B indicates the field of view of the camera. (D) Electrical signal traces from a row of electrodes with slow wave events marked as red plus symbols. The white arrows indicate the propagating spike bursts shown in B and the black arrows indicate the slow wave propagation shown in C. The spontaneous peristaltic contractions were caused by longitudinally propagating circumferential spike burst patches as indicated by the white arrows. These peristaltic contractions and the spike bursts did not show any connection to the slow wave activity as seen in D. The above activity is presented in video form in Supplementary Video 4 (https://doi.org/10.17608/k6.auckland.17711528).
(Fig. 5.9B vs Fig. 5.9C). At the instance shown in Fig. 6, slow waves and the spike bursts both originated at similar positions in the electrode array and propagated in either direction. The distal propagation of slow waves were blocked by a conduction block at the centre of the array, which did not affect the spike bursts. Cyclic peristalsis caused shallower circumferential contractions compared to spontaneous peristalsis (17 ± 2 % cyclic peristaltic contraction vs 36 ± 4 % spontaneous peristaltic contraction, p < 0.001). In addition, cyclic peristaltic contractions propagated much faster than spontaneous peristalsis (14.2 ± 2.3 mm/s cyclic peristalsis velocity vs 3.7 ± 0.5 mm/s spontaneous peristalsis velocity, p < 0.001), which was closer to the underlying slow wave propagation velocity (14.2 ± 2.3 mm/s contraction velocity vs 11.5 ± 4.6 mm/s slow wave velocity, p = 0.162).

Cyclic peristaltic contractions displayed similar characteristics to partly or fully myogenic peristalsis in the literature [32], [33], [36]. The periodicity, propagation velocity in relation to slow waves, level of deformation caused, and the underlying bioelectrical activity of cyclic peristaltic contractions were similar to propagating oscillations in mouse intestine [32], myogenic ripples in guinea-pig colon [36], and peristalsis in mouse intestine [33]. The similarities shown to myogenic motility patterns in the literature and the affinity to slow waves observed here suggest that cyclic peristalsis is likely at least partly driven by myogenic slow waves.

5.7 Pendular contractions

Longitudinal contractions, also known as pendular contractions help with mixing intraluminal content [152]. Pendular contractions were observed throughout the recording duration in 3 studies as shown in Table 5.1. Pendular contractions were driven by slow wave associated spike bursts with mean amplitudes of 0.13 ± 0.06 mV in pigs, 0.09 ± 0.05 mV in rabbits, and mean durations of 0.8 ± 0.3 s in pigs, 0.5 ± 0.2 s in rabbits. The underlying bioelectrical activity during pendular contractions is shown in Fig. 5.10. Pendular contractions caused the intestine to longitudinally contract by 19 ± 6 % in pigs, 12 ± 4 % in rabbits, and registered as horizontal bands in the longitudinal strain map (Fig. 5.10A). During these longitudinal contractions, spike bursts activated as longitudinal patches (Fig. 5.10B). The spike bursts periodically activated with the slow wave cycles as seen in the activation maps in Fig. 5.10B–C and the electrical traces in Fig. 5.10D. However, the propagation patterns of slow waves and spike bursts deviated at times. In the instance indicated by the dashed arrows in Fig. 5.10D, a spike burst from a distal slow wave propagated beyond the slow wave collision, deviating from the slow wave activity. Furthermore, the spike burst activity in the mesenteric and anti mesenteric sides were not uniform, therefore, the contractions were also not uniform across the diameter. In the given instance, mesenteric side of the intestine displayed more activity than the anti-mesenteric side.

The underlying slow wave and spike burst activity during pendular contractions have been previously analysed using 1-dimensional electrode arrays [31]. The 2-dimensional high-resolution electrode arrays utilised here adds validation that the predominant spatial activation of spike bursts was in the longitudinal direction. In addition, it was identified that dissimilar contractions in the anti-mesenteric and mesenteric borders commonly observed during pendular contractions [156] were due to different spike burst activities in the respective borders. Furthermore,
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Figure 5.9: Slow wave and spike burst activity during cyclic peristalsis. (A) Spatiotemporal transverse strain map which shows the regularly occurring propagating contractions of cyclic peristalsis. (B, C) Activation maps of spike burst and slow wave activity during the peristaltic contraction indicated by the solid arrow in A. The shaded area represents the position of the intestine on the electrode array. The black rectangle in B indicate the field of view of the camera. (D) Electrical signal trances from a row of electrodes in B, C, where red plus symbols indicate slow wave events. The solid arrow indicate the spike burst propagation shown in B. The dash arrows show propagating spike bursts from two slow wave pacemakers that gave rise to the back and forth propagating contraction indicated with the dashed arrows in A. Cyclic peristaltic contractions were caused by longitudinally propagating circumferential spike burst patches that were associated with slow waves as seen in D. The spike bursts activated periodically with the slow waves causing propagating circumferential contractions at the frequency of the slow waves. The above activity is presented in video form in Supplementary Video 5 (https://doi.org/10.17608/k6.auckland.17711528).
Figure 5.10: Slow wave and spike burst activity during pendular contractions. (A) Spatiotemporal longitudinal strain maps that show the longitudinal muscle activity in the mesenteric and anti-mesenteric side of the intestine. (B, C) Activation maps that show the propagation of slow waves and spike bursts during the time interval indicated by the rectangle in A. The shaded area represents the position of the intestine on the electrode array. Spike bursts activated as longitudinal patches. The spike burst activity was different in the mesenteric and anti-mesenteric sides, and the contractile activity was also different as seen in A. (D) Electrical signal traces from a row of electrodes with the slow wave events marked as red plus symbols. The solid white arrow indicate the longitudinal propagating spike burst shown in B, and the solid black arrows indicate the propagating slow wave shown in C. The dashed arrows indicate an instance where the spike bursts of a distal slow wave propagated beyond a slow wave collision. The above activity is presented in video form in Supplementary Video 6 (https://doi.org/10.17608/k6.auckland.17711528).
the slow wave associated spike bursts indicate that myogenic mechanisms could be involved with pendular contractions. A previous study has reported that pendular contractions were not affected by the neural inhibitory agent tetrodotoxin, suggesting they are myogenically driven [156]. However, it should be noted that since the spike bursts and concomitant pendular contractions were not uniform across the intestinal diameter, contractions quantified from the top surface of the intestine could not be accurately spatially correlated with the electrical events recorded from the bottom surface.

5.8 Inter-species differences

There were differences between the observed motility patterns in pigs and rabbits. Pigs displayed both slow wave associated and independent spike bursts along with corresponding contractions. In the rabbit studies, only slow wave associated spike bursts were observed along with corresponding contractions. As a result, spontaneous peristaltic contractions were observed only in pigs, while cyclic peristaltic contractions were observed only in the rabbit. However, it is possible that cyclic peristaltic contractions could occur in pigs, and slow wave independent motility patterns could occur in rabbits [216], although they were not observed in our experiments. It is plausible that differences in animal care procedures could have contributed to the observed discrepancy in the bioelectrical activity between pigs and rabbits (See Section 3.1). Pigs were fasted prior to the experiments, however, rabbits were not fasted prior to surgery, as it can lead to hypomotility and compromise gut function [183]. Studies have shown that slow wave associated spike bursts increase in the postprandial period, although the key pathways regulating this difference are yet to be elucidated [217]. There are also physiological differences between pigs and rabbits. The transit time of content through the rabbit small intestine is faster compared to other animals [218], and therefore, cyclic peristalsis might be more prominent in rabbits than other species. In rabbits, small intestine motility is regulated in part by motilin, similar to humans and is in contrast to most other animal species [218]. However, motilin does not play a prominent role in pigs [219]. Therefore, observed bioelectrical and contraction patterns could have been influenced by animal care procedures and physiological variance.

5.9 Limitations

Several limitations in the current study design can be recognised. First, anaesthesia and abdominal incision for open surgery could influence the electrical and mechanical activities of the gut, and could cause reflex inhibition [211]. Furthermore, the temperature and moisture were also not strictly regulated in these experiments, and could influence the bioelectrical and contractile activities [212], [220]. However, steps were taken to maintain the intestinal temperature and moisture, as detailed in Section 4.7. Secondly, the motility patterns were quantified using visible deformations, and not the true mechanical state of the muscle. Thirdly, the bioelectrical activity was recorded from the bottom surface of the intestine (the intestine was placed over the electrode array), while the contractions were recorded from the top surface of the intestine (by the camera
positioned perpendicularly above). The bioelectrical and contractile activities were assumed to be uniform across the circumference of the intestine. Finally, neural and myogenic inhibitory agents, ion channel blockers were not used to conclusively verify the regulatory mechanisms and ionic pathways of bioelectrical and contractile events. It should also be noted that since motility patterns were not modulated in this study, cyclic peristaltic contractions were only observed in one rabbit study. An extended discussion of limitations and further perspectives are provided in Chapter 7.

5.10 Summary

In this chapter, high-resolution bioelectrical and motility mapping techniques were used to analyse the relationship between slow waves, spike bursts, and motility. This chapter serves as the first study to define in-vivo intestinal motility based on slow waves and spike bursts in spatiotemporal detail. The results demonstrated that spike burst propagation patterns ultimately dictated the resultant contractile response. Two types of activity were present, where (i) spike bursts were coupled to slow wave activations (slow wave associated spike bursts), and (ii) independent of slow wave activations (independent spike bursts). This demonstrates that in the jejunum, slow waves were not always correlated to the contractile response. Slow wave associated spike bursts had a smaller morphology and activated more frequently with slow waves. Independent spike bursts had a larger morphology and activated less frequently. Spike bursts activated as longitudinal or circumferential patches with associated contractions in the respective directions. The level of contraction and the rate of contraction depended on the amplitude, duration, size, and energy of the spike burst patches. Propagating circumferential patches of independent spike bursts led to spontaneous peristaltic contractions independent of slow waves in pigs. Propagating circumferential patches of slow wave associated spike bursts led to cyclic peristaltic contractions modulated by slow waves in rabbits.
Chapter 6

Electrophysiological and Contractile Nature of Mesenteric Ischaemia

The work presented in this chapter has been selected as a finalist for the Mary Bullivant award at MedSci NZ conference, 2021, and as a finalist for Falling Walls Lab – New Zealand, 2021.

Intestinal electrophysiology is currently being investigated as a potential diagnostic tool for mesenteric ischaemia [23], [24]. A detailed spatiotemporal understanding of the electrophysiological changes during mesenteric ischaemia, and their implications on contractile activity is imperative to lay the basis for electrophysiology as a diagnostic tool. Bioelectrical investigations of mesenteric ischaemia has typically been performed with sparse recordings, with only two studies in high-resolution [18], [43], [177], [221]. The high-resolution studies focused exclusively on (i) slow waves [14] and (ii) an algorithm for spike burst detection [45]. A simultaneous high-resolution analysis of slow waves, spike bursts, and contractile activity during mesenteric ischaemia has not been performed. This chapter applies the high-resolution bioelectrical and motility mapping techniques developed in Chapter 3 and Chapter 4 to perform a simultaneous analysis on electrophysiological and contractile changes during mesenteric ischaemia, and to identify new biomarkers for diagnosis.

6.1 Experimental setup and data analysis

Ethical approval was provided by the University of Auckland Animal Ethics Committee. All experiments were performed in-vivo on 5 female cross-breed, weaner pigs (40.4 ± 1.7 kg). Animal care, preparation, and anaesthesia were performed as previously described in Section 3.1. The experimental arrangement is shown in Fig. 6.1. In summary, a midline laparotomy was performed, and a section of the small intestine jejunum was exteriorised and placed on top of an electrode array to record the bioelectrical activity (Figs. 6.1B–C, see Section 3.2 for a detailed description). The mechanical contractions were simultaneously recorded from a machine vision camera positioned perpendicularly above (Fig. 6.1A, see Section 4.3 for a detailed description). The bioelectrical and video recordings were synchronised by registering a TTL pulse generated from
the camera during exposure with the BioSemi ActiveTwo system used to record the bioelectrical signals. First, the baseline activity of the intestine was recorded for $5.0 \pm 2.8$ minutes. Then, the vessels from the vascular arcade supplying blood to the intestinal segment were clamped with arterial forceps or ligated using cross-woven strings to induce localised ischaemia (Fig. 6.1C), and the activity was recorded for $18.2 \pm 9.0$ minutes. Finally, the mesentery was unclamped to allow revascularisation of the intestinal segment, and the reperfusion activity was record for $2.5 \pm 1.0$ minutes.

Figure 6.1: Mesenteric ischaemia bioelectrical and video mapping method. (A) Arrangement of the cross-polarised camera setup and the electrode array on the intestine. The camera exposure active signal was recorded along with the bioelectrical signals by the BioSemi ActiveTwo for synchronisation. (B) Flexible array used for high-resolution bioelectrical mapping ($16 \times 8$ configuration; $4$ mm spacing). (C) Intestine as visible from the camera, placed over the flexible electrode array, and the blood vessels were clamped to induce ischaemia.

### 6.1.1 Data analysis

Slow wave and spike burst detection, activation map generation, and amplitude computation were performed as previously described in Chapter 3. The slow wave amplitude was calculated as the mean amplitude of all the events in each 60 s interval. The slow wave and spike burst frequency for each electrode was computed as the number of events within a 60 s interval, as cpm. The slow wave and spike burst frequency for a time interval was calculated as shown in Fig. 6.2, as the mean of the maximum frequencies of each column of electrodes, and reported in cpm. The maximum frequency was taken along each column to address undetected events on the edge electrodes due to loss of contact during circumferential contractions. Slow wave and spike burst frequency heatmaps were generated by colour-coding the frequency in each electrode for
Experimental setup and data analysis

qualitative assessment. The period based slow wave frequency calculation method in Section 3.4.1 was not used as the slow wave activity was sporadic during ischaemia.

Figure 6.2: Calculation of mean frequency of slow wave and spike burst events during ischaemia. The grid indicates the electrode array, and the illustration indicates the position of the contracted intestinal segment over the array. The number of events in each electrode in the 60 s interval is colour-coded and indicated by the intensity from white (no events) to red (maximum number of events). Mean frequency was calculated by taking the maximum number of events in the 60 s interval, in each column. The maximum was taken to avoid loss of contact due to circumferential contractions from affecting the results.

Mapping and quantification of motility patterns were performed as described in Chapter 4. The tonal contractions were quantified by calculating the diameter of the intestine from the video frames as shown in Fig. 6.3. The mesenteric and anti-mesenteric borders of the interested intestinal segment were manually marked in the video frames, after which they were fitted with splines. The mean distance between the corresponding points of the mesenteric and anti-mesenteric splines (yellow lines in Fig. 6.3B) was computed as the diameter in millimetres.

Figure 6.3: Calculation of intestinal diameter from video frames. (A) The video frame with the interested segment of the intestine manually specified. The red and green dots indicate the corresponding start and end points of the mesenteric and anti-mesenteric borders. (B) The diameter of the intestine at an evenly distributed set of points indicated by yellow lines. The lines connect the mesenteric border to the corresponding points in the anti-mesenteric border. The camera calibration was used to map these pixel distances to mm.
All values are reported as mean ± standard deviation. Trends in the frequencies and amplitudes were analysed using the linear mixed effect model given in Eqn (6.1), and quantified using the fixed effect coefficients (r) with significance given in p-values (where X is the measurement). Repeated measures ANOVA and Tukey post-hoc test were used to compare the frequencies, amplitudes, and intestinal diameters at the end of ischaemia and reperfusion to baseline. A p-value of <0.05 was considered significant.

\[ X \sim 1 + \text{Time} + (1 + \text{Time}|\text{Experiment}) \]  \hspace{1cm} (6.1)

The bioelectrical and contractile activities during baseline, ischaemia, and reperfusion are shown qualitatively in video form in Supplementary Video 7 (https://doi.org/10.17608/k6.auckland.17711528).

6.2 Deterioration of slow waves during ischaemia

High-resolution bioelectrical recordings illustrated that the ability of the ischaemic region to entrain slow waves diminished, and led to sporadic, uncoordinated activation of slow waves in the ischaemic region. The deterioration of slow waves was analysed by quantifying and comparing the slow wave amplitude and frequency over time. The trends in slow wave frequencies and amplitudes during ischaemia and reperfusion are shown in Fig. 6.4. Comparisons of the slow wave frequencies and amplitudes during baseline, at the end of ischaemia, and at the end of reperfusion are shown in Fig. 6.5. During baseline, slow waves activated at a mean frequency of 12.4±2.9 cpm. During ischaemia, the frequency of slow wave events decreased with time, and with extended ischaemia in two of these experiments (more than 20 minutes) they completely disappeared (\( r = -0.0067 \, \text{cpm/s}, \ p < 0.0001 \), Fig. 6.4A). The mean slow wave frequency at the end of the ischaemia recordings was significantly lower than baseline (2.5 ± 2.7 cpm vs 12.4 ± 2.9 cpm, \( p = 0.0006 \); Fig. 6.5A). The slow wave frequency increased during reperfusion when the blood supply was restored (\( r = 0.0248 \, \text{cpm/s}, \ p = 0.0035 \), Fig. 6.4A). The mean slow wave frequency at the end of reperfusion was significantly higher than at the end of ischaemia, and was not significantly different to baseline (11.5 ± 2.9 cpm vs 2.5 ± 2.7 cpm, \( p = 0.0019 \); vs 12.4 ± 2.9 cpm, \( p = 0.6350 \); Fig. 6.5A). The amplitude of slow waves were not significantly different between baseline, at the end of ischaemia, and at the end of reperfusion (0.15±0.09 mV vs 0.18±0.04 mV vs 0.14±0.05 mV, RANOVA \( p = 0.6642 \); Fig. 6.5B).

During baseline, consistent cyclic propagation of slow waves across the intestinal segment was observed (Fig. 6.6A & 6.6D). As shown in the frequency heatmap in Fig. 6.6G, an even distribution of slow wave events was present throughout the intestinal segment. However, after the blood flow was restricted, slow wave activations became progressively spatially and temporally irregular in the ischaemic region, which propagated short distances, but not throughout the segment as was typical during baseline recordings. As shown in Fig. 6.6B, cyclic slow wave activations were observed in the surrounding region, but they did not entrain the ischaemic region, which acted as a conduction block. The slow waves did not propagate through the ischaemic region, which decoupled the slow wave activity in the distal intestinal segment. Therefore, as
Figure 6.4: Slow wave activity during baseline, ischaemia, and reperfusion, with time. The fixed effect linear trends and the confidence intervals are shown in black. The fixed effect coefficient (r) gives the slope. (A) The frequency of slow wave events decreased during ischaemia and in prolonged experiments, completely disappeared. The slow wave frequency increased during reperfusion when the blood vessels were unclamped. (B) The amplitude of slow waves did not show a significant change during baseline, ischaemia, and reperfusion.

Figure 6.5: Comparison of (A) Slow wave frequency (RANOVA p <= 0.0005) and (B) slow wave amplitude (RANOVA p = 0.6642, groups statistically similar) during baseline, at the end of ischaemia, and at the end of reperfusion.
shown in Fig. 6.6E, the opposing edges of the ischaemic region were activated by antegrade propagating slow waves, and retrograde slow waves, respectively. As shown in Fig. 6.6G, the frequency of slow wave activations progressively decreased in the ischaemic region compared to the surrounding regions. When the blood supply was restored during reperfusion, slow waves regained their normal cyclic nature (Fig. 6.6C), and again propagated throughout the previously-ischaemic segment, as shown in the activation map in Fig. 6.6F. As shown in Fig. 6.6G, the frequency of slow wave events increased in the ischaemic region, and returned to an even spatial distribution similar to baseline.

Similar slow wave deteriorations have been observed in previous studies during ischaemia, albeit in low resolution [18], [23], [44], [221]. Temporal analysis of slow waves had registered this deterioration as a reduction in frequency [18], [23], [44], [221]. The results presented in this thesis indicate loss of entrainment as a contributing reason for the reduced slow wave frequency. Szurszewski et al. noted the sporadic nature of slow wave activations by utilising four electrodes, where they identified that the slow wave events in the ischaemic region are independent from the surrounding regions, and that their conduction to the rest of the electrodes could not be determined [43]. Similar evidence for reduced slow wave coupling and irregular slow wave activity during ischaemia have been observed by Kyi et al. [44] and Cabot et al. [177]. The sporadic nature of slow waves in the ischaemic region had also been observed in a previous high-resolution bioelectrical mapping study, however, the sporadic slow wave activity had been transient [14]. The current results show that ischaemia leads to progressively irregular slow wave activity, until finally they are completely suppressed.

In these experiments, the ischaemic region acted as a conduction block to the propagating slow waves, preventing entrainment across the intestine. Similar slow wave conduction blocks were observed in the ischaemic region by Lammers et al., but they were unstable and slow waves had returned to antegrade propagation in some cycles [14]. In this study, stable conduction blocks were observed with ischaemia, and decoupled the slow wave activity in the proximal and distal regions adjacent to the ischaemic region. Prior research has conclusively demonstrated that isolating the dominant slow wave pacemaker by transection give rise to new distal pacemaker regions [222]. It is likely that the conduction block caused by the ischaemic region gave rise to a separate pacemaker region distal to the mapped site. As a result, in cases where the centre of the electrode array was ischaemic, the proximal edge of the ischaemic region (supplied by surrounding blood vessels) was activated by antegrade propagating slow waves from a proximal pacemaker, while the distal edge was activated by retrograde propagating slow waves from a distal pacemaker. These observations confirm the presence of slow wave conduction observed during ischaemia with sparsely distributed electrodes [43], and now provide improved spatiotemporal definition.

Extended ischaemia completely suppressed slow waves in this present study. It has been widely established that hypoxia caused by restricted blood flow leads to the elimination of slow waves [18], [43], [44]. In certain studies, it had taken 7 to 90 minutes for the slow waves to be eliminated [18], [178], [221]. However, in some studies slow waves had persisted as irregular activations [44], [177]. Therefore, it is likely that the elimination or persistence of slow waves
Deterioration of slow waves during ischaemia

Figure 6.6: The slow wave activity during baseline, ischaemia, and reperfusion. (A–C) Bioelectrical signals from a row of electrodes. Slow waves are marked with red crosses. Cyclic slow wave activity was observed throughout the intestinal segment during baseline. During ischaemia, surrounding slow wave activity could not entrain the ischaemic region (red shaded area). During reperfusion, the surrounding slow waves could again entrain the complete intestinal segment and slow waves regain the normal cyclic nature. (D–F) Slow wave activation time maps that show the spatial propagation. During baseline, slow waves propagated throughout the intestinal segment. During ischaemia, slow wave propagation was blocked due to the inability to entrain the ischaemic region (red shaded area). After reperfusion, slow wave propagation returned to normal. (G) Spatial distribution of slow wave frequency which shows the deterioration of slow wave activity during ischaemia, and the improvement during reperfusion.
Electrophysiological and Contractile Nature of Mesenteric Ischaemia depends on the level of blood flow available to the intestinal segment, or the level of hypoxia caused by the restricted blood flow. The relationship between slow waves and blood flow is further evident by the fact that slow wave frequency decreases with the level of occlusion of the blood vessels [23]. Furthermore, during ambient and stagnant hypoxia, a 50% reduction in inhaled oxygen or partial occlusion of the superior mesenteric artery but with sufficient blood flow had not affected the slow wave activity. However, a 75% reduction in inhaled oxygen had reduced the slow wave frequency, and severe restriction to blood flow by thrombosis had completely eliminated the slow waves [178]. Since the blood flow was not measured in this present study, it is possible that collateral flow may have led to higher variability in the measured slow wave activity.

It was found that the slow wave deterioration from short duration ischaemia was temporary, and slow wave activation readily recovered after reperfusion with consistent frequency and amplitude compared to the baseline recordings. Slow waves can return to regular activations during reperfusion after less than 3 hours of ischaemia [177], before the onset of pathological changes [223]. The temporary inhibition is likely due to the impairment of ATPase cellular pumps and exchangers [224], [225] required for the generation of slow wave potentials [97]. The operation of these pumps require ATP produced by mitochondria. Hypoxia caused by the restricted blood supply decreases the mitochondrial membrane potential [226], which in turn drastically decrease ATP synthesis [227]. Korogod et al. used an ICC model [228] to simulate the effect of hypoxia on slow waves [229]. The simulation results showed that the hypoxic suppression of ATPase pumps, and the reduction of mitochondrial membrane potential eliminated slow wave activity. In this thesis, irregular slow waves persisted even when the ischaemic region could no longer entrain surrounding slow waves to facilitate propagation. This sporadic nature maybe due to the heterogeneity of oxygen available to cells. Cells contain oxygen stores that are depleted during ischaemia [225]. ICC in tissue regions that possess enough residual oxygen may still be able to generate slow waves under restricted blood flow, but if the surrounding cells have depleted oxygen stores, they may not be able to entrain. When all the oxygen stores have depleted, slow wave activity would be eliminated, which also accords with the results here of complete cessation of slow waves after extended ischaemia.

6.3 Spike bursts and circumferential contractions during ischaemia

Restriction to the blood flow or oxygen level causes a transient increase in contractions in the intestine called a spasm [177], [178]. This thesis found that these circumferential contractions spatiotemporally correlated with the activation of circumferential spike burst patches. The increase in spike bursts was quantified by calculating the mean spike burst amplitudes and frequencies with time. The trends in spike burst frequencies and amplitudes during ischaemia and reperfusion are shown in Fig. 6.7. Comparisons of the spike burst frequencies and amplitudes during baseline, at the end of ischaemia, and at the end of reperfusion are shown in Fig. 6.8. During baseline, spike bursts were only observed in two experiments, and they occurred at a mean frequency
of 1.1 ± 1.4 cpm. During ischaemia, the frequency of spike bursts increased ($r = 0.0043$ cpm/s, $p = 0.0205$, Fig. 6.7A), and the mean frequency of spike bursts at the end of ischaemia recordings was significantly higher than at baseline (8.7 ± 3.3 cpm vs 1.1 ± 1.4 cpm, $p = 0.0003$; Fig. 6.8A). During reperfusion, the frequency of spike bursts decreased ($r = -0.0217$ cpm/s, $p = 0.0035$, Fig. 6.7A). The mean frequency of spike bursts at the end of reperfusion was significantly lower than in ischaemia, and was not significantly different to that of baseline recordings (2.7 ± 1.4 cpm vs 8.7 ± 3.3 cpm, $p = 0.0029$; vs 1.1 ± 1.4 cpm, $p = 0.1621$; Fig. 6.8A). The mean spike burst amplitude at the end of ischaemia was also significantly higher compared to baseline (1.1 ± 0.3 mV vs 0.3 ± 0.3 mV, $p = 0.0001$; Fig. 6.8B), but the spike burst amplitude did not display a significant increasing trend during ischaemia ($r = 0.0005$ mV/s, $p = 0.0647$, Fig. 6.7B). The mean spike burst amplitude at the end of reperfusion was significantly lower compared to ischaemia, and was not significantly different to baseline (0.4 ± 0.3 vs 1.1 ± 0.3 mV, $p = 0.0001$; vs 0.3 ± 0.3 mV, $p = 0.1736$; Fig. 6.8B).

Figure 6.7: Spike burst activity during baseline, ischaemia, and reperfusion, with time. The fixed effect linear trends and the confidence intervals are shown in black. The fixed effect coefficient ($r$) gives the slope. (A) The frequency of spike bursts increased during ischaemia and decreased during reperfusion. (B) The amplitude of spike bursts did not show a significant trend.
During ischaemia, spike bursts activated as circumferential patches and was associated with circumferential contractions. Both non-propagating circumferential contractions (i.e., ‘segmental contractions’) and propagating circumferential contractions were observed, as depicted in the strain maps in Fig. 6.9A with a square and an arrow. Segmental contractions correlated with non-propagating circumferential spike burst patches as shown in the spike burst activation map in Fig. 6.9D along with the corresponding electrical signal traces in Fig. 6.9B. Propagating circumferential contractions correlated with propagating circumferential spike burst patches as shown in the spike burst activation map in Fig. 6.9C, and the electrical signal traces in Fig. 6.9B. Circumferential contractions that propagated in both antegrade and retrograde directions were observed in video recordings. Once the blood flow was restored, contractions subsided with a corresponding decrease in spike burst events, as shown in Fig. 6.9A at 1197 s. Spatially, spike bursts activated more frequently in the region where the blood vessels were clamped and decreased towards the surrounding non-ischemic regions (Fig. 6.9E).

Previous studies have also found that restriction to the blood flow or oxygen level causes a transient increase in contractions in the intestine called a spasm [177], [178]. This thesis found that these circumferential contractions spatiotemporally correlated with the activation of circumferential spike burst patches. Circumferential spike burst patches are attributed to calcium currents occurring in the circular muscle layer [30], and this could have triggered the circumferential contractions. Spike burst activations during ischaemia are thought to be neurally mediated as they are abolished by tetrodotoxin [230]. The observed spike bursts were similar to ones that occur independently to slow waves described in Section 5.3, and mediate non-myogenic motility patterns, which suggest that they are indeed neurogenic. In some studies, spike bursts were suppressed after an initial increase with the restriction to blood flow [177], [178]. However, in this study, spike bursts persisted throughout the ischaemic recordings. In other studies, spike bursts had been completely eliminated by prolonged ischaemia [177], which may be due to damage to enteric nerves [43], [44]. Spike bursts and contractile activity can persist after the transient increase at a diminished level when the blood flow is not fully restricted [178], and full occlusion is required for complete elimination of spike bursts [45]. The persistence of spike bursts in the present study may have been due to the relatively short duration of ischaemia recordings and incomplete restriction of blood vessels.
Spike bursts and circumferential contractions during ischaemia

Figure 6.9: Contractile and spike burst activity of the intestine observed during baseline, ischaemia, and reperfusion. (A) Transverse strain maps depicting the circumferential contractions of the intestine. The intestine was quiescent during baseline, but went into a spasm of circumferential contractions when the blood supply was restricted. The arrow indicates a propagating contraction and the box indicates a segmental contraction. The circumferential contractions subsided when the blood supply was restored. (B) Electrical traces from a row of electrodes that show the spike burst activity during ischaemia. The propagating contraction in A was caused by the propagating circumferential spike burst patch (arrow). The segmental contraction was caused by the non-propagating circumferential spike burst patch (box). (C–D) Activation time maps which show the spatial activations of the spike bursts shown in B. Red shaded area indicates the ischaemic region. (E) Spatial distribution of spike burst frequency which shows the increase in spike bursts during ischaemia, and the decrease during reperfusion. Spike burst frequency was higher in the distal ischaemic portion.
6.4 Tonal contraction of the intestine during ischaemia

The intestine also underwent tonal contraction during ischaemia. Comparisons of the intestinal diameter during baseline, at the end of ischaemia, and at the end of reperfusion, are shown in Fig. 6.10. The intestine had a mean diameter of 29.3 ± 2.6 mm during baseline. With the tonal contraction, the mean diameter at the end of ischaemia recordings was significantly lower than at baseline (21.2 ± 6.2 mm vs 29.3 ± 2.6 mm, \( p = 0.0020 \); Fig. 6.10). During reperfusion, the tonal contraction subsided. The mean diameter at the end of reperfusion was significantly higher than in ischaemia, and was not significantly different to baseline (27.3 ± 3.9 mm vs 21.2 ± 6.2 mm, \( p = 0.041 \); vs 29.3 ± 2.6 mm, \( p = 0.1539 \); Fig. 6.10).

Figure 6.10: Tonal contraction of the intestine during ischaemia. (A) The diameter of the intestine during baseline, ischaemia, and reperfusion recorded in the five experiments. The diameter decreased during ischaemia due to the tonal contraction, and increased after reperfusion. (B) A photo of the intestinal segment during baseline. (C) The same segment during ischaemia with tonal contraction.

Szurszewski et al. also noted a reduction in lumen diameter in hypoxic segments, which was believed to be due to damage to inhibitory neurons in the myenteric plexus [42]. The results in this thesis indicate that the tonal contraction may have been caused by a less permanent condition, and could be a result of the alterations to ionic homeostasis caused by ischaemia [224], [225]. Simulation of ischaemia has shown that suppression of ATPase pumps lead to increased intraluminal calcium concentrations. In fact, electrolyte analysis of intestinal muscle have revealed an increased concentration of \( \text{Ca}^{2+} \), \( \text{Na}^+ \), \( \text{H}_2\text{O} \), and \( \text{Cl}^- \) in the ischaemic state [44]. The tonal contraction during ischaemia may have resulted from the increased calcium concentration. Ischaemia also enhances the activity of enzymes such as PLA\(_2\) [179], which increases the calcium sensitivity of SMCs [68], and could also contribute to the tonal contraction [56]. The fact that the tonal contraction subsided once the blood flow was re-established suggest that it occurred due to a temporary condition, such as the increase in calcium concentration or calcium sensitisation.
6.5 Limitations

It should be noted that the results presented here are from acute localised ischaemia. The indicators discussed here may vary during whole organ ischaemia, such as with the occlusion of the superior mesenteric artery. The abdominal incision, temperature, and moisture changes could also have affected the bioelectrical and contractile activities of the intestine. However, steps were taken to maintain the temperature and moisture of the intestine, as detailed in Section 4.7. Furthermore, the blood flow was not monitored during these experiments, and therefore, collateral flow may also have affected the results. The identified biomarkers were not analysed during progressive levels of ischaemia, but is required to assess the viability in clinical settings.

6.6 Summary

This chapter serves as the first study to simultaneously analyse the spatiotemporal dynamics of slow waves, spike bursts, and contractions in high-resolution during mesenteric ischaemia. Slow wave entrainment within the ischaemic region diminished, resulting in sporadic slow wave activations and a reduction in the frequency from $12.4 \pm 3.0$ cpm to $2.5 \pm 2.7$ cpm. During reperfusion, slow waves regained the normal rhythmic nature, propagated throughout the previously ischaemic segment, and the frequency increased to baseline levels. The amplitude of slow waves did not display significant trends during ischaemia and reperfusion, and the slow wave amplitudes during baseline, at the end of ischaemia, at the end of reperfusion were similar. Conversely, spike burst frequency increased during ischaemia from $1.1 \pm 1.4$ cpm to $8.7 \pm 3.3$ cpm, activated as circumferential patches, and caused a spasm of circumferential contractions. After reperfusion, the frequency of spike bursts decreased to $2.7 \pm 1.4$ cpm, and contractions subsided. The amplitude of spike bursts also did not display significant trends during ischaemia and reperfusion. However, the spike burst amplitude at the end of ischaemia was significantly higher than baseline, and after reperfusion, spike burst amplitude was again lower, similar to baseline. The intestine also underwent tonal contraction during ischaemia, with the diameter decreasing from $29.3 \pm 2.6$ mm to $21.2 \pm 6.2$ mm. During reperfusion, the intestinal diameter increased to baseline levels. The intestinal slow wave, spike burst, and diameter measurements were similar for baseline and reperfusion. The results of this study identified several indicators for mesenteric ischaemia: (i) loss of entrainment of slow waves in the ischaemic region, (ii) sporadic activation of slow waves, (iii) decrease in slow wave frequency, (iv) distal and proximal propagation of slow waves in the proximal and distal edges of the ischaemic region, (v) increased spike burst activity in the ischaemic region compared to surrounding regions, and (vi) tonal contraction of the intestinal diameter in the ischaemic region. The biomarkers presented in this thesis could objectively identify ischaemic segments of the intestine and could also be used to verify successful revascularisation, such as during bowel resection surgery.
Chapter 7

Conclusions and Future Directions

The work presented in this thesis advances our understanding of the relationship between bio-
electrical slow waves, spike bursts, and motility in the intestine. A key contribution of this
thesis towards motility mapping is the development of a novel framework for simultaneous high-
resolution mapping of bioelectrical and contractile activities from across the 2D surface of the
in-vivo intestine. The framework was applied to map in-vivo intestinal motility patterns, which
serves as the first simultaneous spatiotemporal analysis of slow waves, spike bursts, and motility.
The method was also translated to analyse the electrophysiological and contractile changes in
high-resolution for the first time during mesenteric ischaemia. The following sections summarise
the key findings and suggest important areas for future research.

7.1 Objective 1: Development of methods to simultaneously
map and quantify in-vivo intestinal slow waves, spike bursts,
and motility.

The first objective of this thesis was to develop methods to simultaneously map and quantify
slow waves and spike bursts, along with the motility patterns from the in-vivo intestine. The
experimental setup for extracellular bioelectrical recording, and slow wave analysis were based
on previously established methods [121]. Slow waves were detected and clustered into propa-
gating cycles with manual intervention. The propagation patterns of slow waves were visualised
using slow wave activation time maps and slow wave activation time videos. Slow waves were
quantified by calculating frequency, amplitude, and velocity. A new framework was developed for
the detection, clustering, and quantification of spike burst patches. The framework along with
manual validation was used to cluster detected spike bursts into longitudinal, circumferential,
and propagating circumferential patches, based on the spatial activation patterns. The activa-
tion patterns of the spike burst patches were visualised using spike burst activation time maps
and spike burst activation time videos. Spike bursts were quantified by calculating frequency,
amplitude, and duration. Spike burst patches were quantified by calculating the amplitude, du-
ration, size, and energy. The velocity was also calculated for propagating circumferential spike
burst patches. These methods were able to measure the bioelectrical activity at a much higher
spatial resolution from across the surface of the intestine compared to previous methods used in simultaneous bioelectrical and motility mapping studies [31], [32].

The motility patterns were simultaneously recorded using a cross-polarised camera setup positioned perpendicularly above the intestine. The motility recordings were synchronised to the bioelectrical data by recording the camera exposure active signal along with the bioelectrical signals. A novel FFD based algorithm was developed to quantify the contractile activity across the 2D surface of the intestine, at a much higher spatial resolution than previously available methods [7], [35], [39], [40]. The algorithm was first verified using synthetic tests, and then validated by performing controlled translational tests on excised tissue. The FFD algorithm had an RMS strain error under 1 %, and a quasi-Newton optimisation step was able to reduce the rate of strain error by 97 % during synthetic tests. The FFD algorithm was applied to in-vivo experiments, where it was able to map a wide range of motility patterns, such as segmental contractions, peristaltic contractions, and longitudinal contractions. Strain fields were calculated, and was able to quantify localised, anisotropic contractions from across the 2D surface of the in-vivo intestine, which was not possible with previous methods [7], [39], [41], [163]. Strain maps were generated to present the spatiotemporal dynamics of motility patterns, and metrics such as frequency and velocity were quantified. Another advantage over previous methods was that longitudinal and circumferential contractions could be simultaneously measured using longitudinal and transverse strain, and the interactions between the two muscle layers could be analysed during motility patterns such as peristaltic contractions.

The simultaneous bioelectrical and motility mapping methods developed in this thesis can be further improved in several ways. The experimental setup developed in this thesis recorded the bioelectrical activity from the bottom surface of the intestine by placing the intestine over a high-resolution electrode array, while the motility was recorded from the top surface of the intestine via a camera positioned above the intestine. It was assumed that the activities around the circumference are uniform. The assumption of uniform activity was valid for circumferential spike burst patches, which activated circumferential segments of the intestine. However, the current setup limited the analysis on the effects of longitudinal spike burst patches, which activated longitudinal strips and were not uniform around the circumference. Optical mapping techniques with voltage sensitive dyes could be used to measure the bioelectrical activity from the top surface of the intestine, same surface as the resulting contractions [231], and could be used to analyse nuanced relationships between the two. However, GI optical mapping techniques are currently in the early stages, and produce weaker signals with low SNR. Further research with improved camera setups, better filtering and detection techniques could develop optical mapping into a viable alternative for high-resolution electrodes.

The FFD motility mapping algorithm can also be further improved. First, the quasi-Newton optimisation adds a significant computational burden to the overall analysis (100× the execution time without optimisation). FFD algorithm can be used without the optimisation step to achieve the same benefits outlined above with faster execution, but at the cost of accuracy, and similar methods have been used in other fields [198], [207]. The implementation of the algorithm could also be parallelised, such as with simultaneous registration of multiple frames, to harness the
Spatiotemporal relationship between slow waves, spike bursts, and motility

performance of GPUs and multi-core CPUs to improve execution time. Secondly, the strain measurements computed here were 2D approximations of the strains in the tissue in 3D space, and are affected by the convexity of the intestine. The algorithm can be extended to 3D measurement of true strain by incorporating a stereo camera setup. Finally, since motility patterns were mapped based on visible deformation of the intestine, they can be affected by passive deformations such as due to the elasticity of the tissue [163]. For instance, modelling studies have estimated the elastic stiffness of guinea-pig small intestine tissue to be between 3.25 GPa to 4.27 GPa [232]. However, the visible contractions may not represent the modelled estimates of elasticity due to the passive distension from surrounding tissue. Furthermore, when using an imaging-based approach, isometric contractions that are not visible cannot be investigated [162]. A possible solution would be to integrate the deformation measurements along with simultaneous high-resolution manometry recordings [164] to estimate the true mechanical state of the muscle [162].

7.2 Objective 2: Investigation into the spatiotemporal relationship between in-vivo intestinal slow waves, spike bursts, and motility.

The second objective of this thesis was to analyse the spatiotemporal relationship between slow waves, spike bursts, and motility in the in-vivo intestine. The findings of this thesis demonstrate that spike burst propagation patterns ultimately dictated the resultant contractile response. However, slow waves coordinated the spike burst activations and provided a platform for cyclic motility patterns. Spike bursts occurred coupled to slow wave activations at times (slow wave associated spike bursts), but also operated independent of slow wave activations at other times (independent spike bursts), demonstrating that in the jejunum, slow waves were not always correlated to the contractile response. Slow wave associated spike bursts had a smaller morphology compared to independent spike bursts with a lower amplitude (0.1 ± 0.1 mV vs 1.4 ± 0.8 mV in pigs, $p < 0.001$) and a lower duration (0.8 ± 0.3 s vs 1.8 ± 1.4 s in pigs, $p < 0.001$). The spike bursts activated as longitudinal or circumferential patches with associated contractions in the respective directions. The level of contraction correlated with the amplitude, size, and energy of spike burst patches. Stronger correlations were observed between the rate of contraction and the amplitude, duration, size, energy of spike burst patches. Segmental contractions of 16 ± 9 % in pigs spatially correlated with circumferential patches of independent spike bursts, and occurred independently to slow wave activations. Pendular longitudinal contractions of 19 ± 6 % in pigs, 12 ± 4 % in rabbits correlated with longitudinal patches of slow wave associated spike bursts. Propagating circumferential patches of independent spike bursts led to spontaneous peristaltic contractions of 36 ± 4 % in pigs, which propagated slower than slow waves (3.7 ± 0.5 mm/s vs 10.1±4.7 mm/s slow wave velocity, $p = 0.007$). Propagating circumferential patches of slow wave associated spike bursts led to cyclic peristaltic contractions of 17 ± 2 % in rabbits, which propagated at similar velocities to slow waves (14.2 ± 2.3 mm/s vs 11.5 ± 4.6 mm/s slow wave velocity, $p = 0.162$), and occurred in regular intervals at similar frequencies to slow waves (11.0 ± 0.6 cpm vs 10.8 ± 0.6 cpm slow wave frequency, $p = 0.97$).
Based on the findings, several avenues for future research were identified. In this thesis, a range of motility patterns were governed by distinct spatiotemporal dynamics of slow waves and spike bursts. Two notable motility patterns were the spontaneous peristaltic contractions driven solely by spike bursts, and the cyclic peristaltic contractions driven by slow wave associated spike bursts. Although both these motility patterns are propagating contractions, the functional aspects of their different characteristics (spontaneous vs cyclic, lower velocity vs higher velocity, stronger contraction vs lesser contraction) are unknown. In the future, fluid dynamic simulations can be used to identify the functional differences between different motility patterns, and to identify the functional importance of slow waves in producing cyclic motility patterns. Current fluid dynamic simulations typically utilise simulated wall motions and may not be realistic [152], [233], [234]. The motility mapping techniques developed in this thesis can be extended to 3-dimensional measurements with volumetric constraints and could be adapted to provide a pathway for including anatomically specific motility patterns for fluid dynamic simulations. Such a method can be used to analyse fluid flow characteristics in depth, for instance, in regards to multi-phase fluid flow and fluid-structure interactions pertaining to slow wave dependent and slow wave independent contractile patterns.

A main difficulty faced during these experiments was collecting data on a range of motility patterns, as the types of motility patterns observed in-vivo were not regulated. In the future, pharmacological prokinetic agents such as domperidone [235], metoclopramide [236], and prucalopride [237] can be used to elicit specific motility patterns, to verify the findings on a much larger dataset. The same approach can also be used to evaluate the effectiveness of pharmacological agents in improving motility in the intestine. Furthermore, neural inhibitory agents such as tetrodotoxin can be used to investigate the myogenic and ENS control of specific motility patterns. Inhibitory agents will also be useful to verify the co-regulatory mechanisms involved in the spatiotemporal dynamics between slow waves and spike bursts, such as in slow wave associated spike bursts vs independent spike bursts. Furthermore, the methods used in the thesis can also be applied in-vitro to gain a controlled understanding of the mechanisms of motility. Finally, the same methods can be applied to analyse the motility in other GI organs such as in the stomach and colon.

7.3 Objective 3: Investigation into the electrophysiological and contractile changes during mesenteric ischaemia.

The final objective of this thesis was to define the spatiotemporal dynamics of slow waves, spike bursts, and contractions in high-resolution during mesenteric ischaemia. The results demonstrated that slow wave entrainment within the ischaemic region diminished, resulting in sporadic slow wave activations, and a reduction in slow wave frequency from $12.4 \pm 3.0$ cpm in baseline to $2.5 \pm 2.7$ cpm at the end of ischaemia ($p = 0.0006$). The lack of entrainment blocked the propagation of slow waves into the ischaemic region, and decoupled the slow waves across the region. However, the deterioration of slow waves was temporary for the short duration and localised ischaemia simulated in this thesis. During reperfusion, the slow waves regained the nor-
Electrophysiological and contractile changes during mesenteric ischaemia

mal rhythmic nature and propagated throughout the previously ischaemic segment. As a result, the frequency of slow waves increased to $11.5 \pm 2.9$ cpm, which was not statistically different to baseline ($p = 0.6350$). The slow wave amplitude did not significantly change during baseline, ischaemia, and reperfusion. Conversely, the frequency of spike bursts increased during ischaemia from $1.1 \pm 1.4$ cpm in baseline to $8.7 \pm 3.3$ cpm at the end of ischaemia ($p = 0.0003$). The spike bursts activated as circumferential patches during ischaemia and were associated with a spasm of circumferential contractions. Non-propagating circumferential spike burst patches correlated with segmental contractions, and propagating circumferential spike burst patches correlated with propagating circumferential contractions in proximal and distal directions. During reperfusion, the frequency of spike bursts again decreased to $2.7 \pm 1.4$ cpm, which was not statistically different to baseline ($p = 0.1621$), and the contractions subsided. The amplitude of spike bursts did not display a significant increasing trend during ischaemia, or a significant decreasing trend during reperfusion. However, the amplitude of spike bursts at the end of ischaemia was significantly higher than baseline ($1.1 \pm 0.3$ mV vs $0.3 \pm 0.3$ mV, $p = 0.0001$), and the amplitude at the end of reperfusion was not different to baseline ($0.4 \pm 0.3$ mV, $p = 0.1736$). Apart from the spasm of circumferential contractions, the ischaemic region also underwent tonal contraction. The diameter of the intestine at the end of ischaemia was significantly smaller than during baseline ($21.2 \pm 6.2$ mm vs $27 \pm 2.6$ mm, $p = 0.0020$). During reperfusion, the tonal contraction subsided, and the diameter increased to $27.3 \pm 3.9$ mm, which was not statistically different to baseline ($p = 1539$).

The electrophysiological and contractile changes during mesenteric ischaemia further validate three aspects of the relationship between intestinal electrophysiology and motility. The spasm of circumferential contractions mediated by circumferential spike burst patches, amidst the deterioration of slow waves validates that in the intestine, slow waves do not always correlate with the contractile response. However, deterioration of slow waves will likely inhibit slow wave mediated motility patterns, such as cyclic peristaltic contractions and pendular contractions, in the ischaemic region. The results further indicate that tonal contractions can occur without corresponding bioelectrical activity, possibly resulting from increased calcium or calcium sensitisation, through mechanisms that do not affect the membrane potential. More importantly, the results of this study identified several biomarkers for mesenteric ischaemia: (i) loss of entrainment of slow waves in the ischaemic region, (ii) sporadic activation of slow waves, (iii) decrease in slow wave frequency, (iv) distal and proximal propagation of slow waves in the proximal and distal edges of the ischaemic region, (v) increased spike burst activity in the ischaemic region compared to the surrounding regions, (vi) and the tonal contraction of the ischaemic region.

In the future, non-invasive methods to detect these biomarkers can be investigated. The reduction in the slow wave frequency can be detected with SQUID magnetometers, and have been identified as a promising indicator to detect ischaemia [23], [24], [238]. Non-invasive slow wave measurements such as cutaneous electrode arrays placed on the abdomen, and magnetometers have shown promising results on detecting gastric slow wave propagation patterns [239], [240]. At present, it is unclear whether the same techniques could be applied to detect intestinal slow wave propagations, which occur in a much more complex, multi-pacemaker conduction system.
Conclusions and Future Directions

compared to their gastric counterparts [34], [117]. It is anticipated that with utilisation of newer advancements, such as with active electrodes [241] and new registration methods [242], non-invasive slow wave mapping may potentially be applied to identify abnormal propagation in the intestine, such as the loss of entrainment in the ischaemic region. Conversely, spike burst activity can be captured using magnetometers [243] and could be used as an additional non-invasive indicator for ischaemia. Furthermore, magnetic resonance imaging and other non-invasive imaging techniques could be used to detect the reduced diameter to further validate ischaemic segments.

It should also be noted that the results in this thesis are from acute localised ischaemia. In the future, the same methods used in this thesis can be applied to assess the viability of these biomarkers during whole organ ischaemia such as with the occlusion of the superior mesenteric artery. Furthermore, the current experimental setup can be supplemented with pressure-driven occlusion cuffs and ultrasonic flow probes to regulate and monitor blood flow to assess the spatiotemporal changes during progressive levels of ischaemia [45].

The biomarkers of mesenteric ischaemia presented in this thesis could objectively identify ischaemic segments of the intestine within the confines of the current experimental arrangement. Future research with non-invasive bioelectrical and contractile mapping techniques on the whole organ along with progressive levels of ischaemia could develop these biomarkers into a feasible diagnostic tool to screen mesenteric ischaemia. The results from this thesis further indicate that high-resolution bioelectrical recordings could be a useful tool in surgical settings. Previous research has emphasised that criterion such as return of colour and arterial pulsations after revascularisation are unreliable predictors for the viability of the intestine [244], [245]. The biomarkers presented in this thesis based on high-resolution bioelectrical mapping could also be used to verify successful revascularisation such as during bowel resection surgery.

7.4 Concluding remarks

The work presented provides a new platform for investigating the implications of slow wave and spike burst dynamics on intestinal motility in spatiotemporal detail. The experimental results of this thesis presents a significant step forward in understanding the bioelectrical basis of intestinal motility, and provide a foundation for incorporating bioelectrical information into clinical utility. This thesis found that the spike burst propagation patterns ultimately dictated the resultant contractile response, but slow waves play a vital role by coordinating cyclic motility patterns such as cyclic peristalsis and pendular contractions. The results also identified spatiotemporal changes in electrophysiological and contractile activities during mesenteric ischaemia, where detection biomarkers were recognised. The results presented in this thesis highlight the importance of future research in this area, especially in the area of fluid dynamic simulations, which can be used to identify the functional aspects of motility patterns, and to recognise the functional importance of slow waves and cyclic motility patterns. Furthermore, advancements in electro-mechanical mathematical models incorporating calcium dynamics with overall contractile patterns could characterise complex multiscale nonlinear behaviours. In addition, further improvement or utilisation of non-invasive techniques could be investigated to better diagnose mesenteric ischaemia
based on the identified spatiotemporal biomarkers. Such investigations could further improve our understanding of GI electro-mechanical relationship, and could propel GI bioelectrical information to be used for clinical evaluations.
Appendix A

Achievements and publications

A.1 Journal publications


A.2 Conference publications


A.3 Other achievements


• Finalist for Mary Bullivant award at MedSci NZ conference, “Electrophysiological and contractile nature of mesenteric ischemia and reperfusion, as defined through simultaneous high-resolution electrical and video mapping”, Virtual, 2021.
References


References


References


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References


