Published in final edited form as:

Conf Proc IEEE Eng Med Biol Soc. 2019 July ; 2019: 4791–4794. doi:10.1109/EMBC.2019.8857365.

A Method for Multi-day Tracking of Gastrointestinal Smooth Muscle Contractile Patterns in Organotypic Culture

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Abstract

Gastrointestinal (GI) motility is a key component of digestive health, and it is a complex both rhythmic and arrhythmic process governed by many physiological factors. The ability to accurately track the movements of the GI tissues *in vitro* over multiple days would provide valuable insights into GI tract physiology. A correlational analysis tracking algorithm was developed and applied to intestinal smooth muscle tissues that were maintained in organotypic culture. Physiologically relevant metrics, such as frequency, amplitude and motility index were independently assayed to quantify smooth muscle contractions. The results were validated by manually detected frequency determined using a standard software package. The algorithm was capable of tracking the changes in contractions of the same tissues over six days. This proof-of-concept study demonstrates the feasibility of long-term tracking of GI motility in organotypic cultures over multiple days. The approach allows study of the effects on GI smooth muscle contractility of direct controlled targeting of the cells and molecules in the GI *tunica muscularis*.

I. Introduction

The gastrointestinal (GI) tract displays a number of complex motility patterns, which are governed by an interplay between neuronal, myoelectrical, and hormonal factors [1]. Abnormal motility, also known as dysmotility, can lead to important but poorly understood digestive disorders, such as pseudo-obstruction and slow transit constipation [2-4].

Aspects of GI motility can be assessed using several techniques, each with its advantages and limitations. Clinically, quantification of GI transit by scintigraphy or radiopaque markers, such as gastric emptying and colonic transit, provide a general guide of the amount of content the stomach is capable of emptying into the duodenum and movement in the colon without significant detail. Recent studies have also utilized radioactive measures and MRI for short-term tracking of motility [5]. On the other hand, *ex-vivo* investigations of GI motility involve either one-dimensional read-outs using force transducers, or tracking of landmarks on the tissue either from markers placed on the serosal surface of the GI tract or

from the natural features in tissues [6]. Most of the tracking investigations were done over the short-term or provided a limited spatiotemporal resolution of the GI tract. These studies do not allow longitudinal studies on a time-scale that permits interventions such as gene knockdown.

The main objective of this study was to develop a method for tracking of the movements of intestinal tissue preparations using a camera over multiple days. The techniques will be used to investigate the changes in motility in tissues where specific cell types or molecules targeted for intervention.

II. Methods

A. Video Recording

A section of full thickness jejunum *tunica muscularis* was excised from a wildtype rat and maintained in organotypic culture over six days [7]. Recordings were taken of the tissue using a DP22 camera (Olympus) placed perpendicularly to the tissue on day 0, 3 and 5 following removal. The sampling frequency was 14 frames-per-second (FPS) and the dimensions of each frame were 1920x1440 pixels. Each recording session was typically 300 seconds long.

B. Pre-processing

Signal processing was performed in MATLAB (R2017a, MathWorks). Each frame from the video data was extracted and converted to grayscale. The frame image was then enhanced using histogram equalization and saved in the PNG format. As the camera was fixed in position relative to the tissue, no movement correction technique was required.

For this study, several regions of interest (ROIs) were determined from an initial reference frame. Each ROI consisted of 100 by 100 pixels without any overlap in the reference frame, which gave the best tradeoff between spatial resolution and computation times. Care was taken to ensure that all ROIs contained at least partial coverage of the tissue across all of the frames, as shown in Figure 1.

C. Motion Tracking

The motion analysis technique was based on previously described correlational analysis algorithm for tracking of GI motility [6]. A normalized 2D correlational analysis technique was used to detect the displacements of the features across subsequent frames. The equation of the correlational matrix at the new location ($C_{u,v}$) is

$$C_{u,v} = \frac{\sum_{x,y} [f_{x,y} - \bar{f}_{u,v}][t_{x-u,y-u} - \bar{t}]}{\sqrt{\sum_{x,y} [f_{x,y} - \bar{f}_{u,v}]^2 \sum_{x,y} [t_{x-u,y-u} - \bar{t}]^2}} \quad (1)$$

where f is the image from the next frame, $\bar{t}$ is the mean of the template image from the current frame, $\bar{f}_{u,v}$ is the mean of $f(x,y)$ in the region under the template image. The x and y directions were defined relative to the field-of-view of the camera, not relative to the axis of

the organ. The orientation of the tissue relative to the camera was kept consistent over the multiple days of recording.

The location of the maximum in the C was identified in the new frame as the new position of the ROI. In the case multiple maximums were identified, the centroid of the maximums was identified using the following equation as the displacements,

$$C_x = \frac{\sum_{i=1}^N x_i}{N} \quad (2.1)$$

$$C_y = \frac{\sum_{i=1}^N y_i}{N} \quad (2.2)$$

where x_i and y_i denote the coordinates of the maximums (N) identified in the correlational matrix. Each ROI was assumed to shift no more than double its size in between frames. Therefore, a limit of 200 pixels in each direction around each ROI was imposed in order to eliminate unrealistic jumps during the search between frames.

The search was repeated for every ROI across all the frames. The data was not down-sampled in order to preserve the temporal resolution of the data. Computations were performed on a 4x Xeon 6136 4 Ghz processor of an HPC with 48 cores and 1.18 TB of memory.

An example of how the correlational analysis works is illustrated in Figure 2. A single ROI from Figure 1 was selected from the reference frame (Figure 2A) and compared to another frame two seconds later. The algorithm searched the location in the vicinity of the ROI and calculates the C in the new frame, defined by the inset in Figure 2B. The amount of shift was determined by the difference in the coordinates of the peak in the correlational matrix compared to the reference frame. Tracking was more effective in regions with bigger differences in intensities than regions where the intensities were uniform.

D. Analysis Techniques and Validation

Displacement (d) in pixels was calculated as the Euclidean distance between the reference frame and the current frame at the center of each patch, using the following equation,

$$d_{xy} = \sqrt{(x^0 - x^n)^2 + (y^0 - y^n)^2} \quad (3)$$

where x^0 and y^0 are the center of the ROI in the reference frame and x^n and y^n are the center of the same ROI in the current frame. The average of the displacements was taken then filtered using a median filter.

The frequency of the contractions in the field-of-view was characterized by the mode of the frequencies of the tracked ROIs, calculated using FFT. The additional metrics were characterized from the ROIs within ± 2 cycles-per-minute (cpm) of the mode frequency, which was calculated using the Fast Fourier Transform. This step was implemented to

eliminate ROIs that produced outlier values. Typically, most of the ROIs would be within the range of frequencies suitable for analysis.

The accuracy of the analysis was validated by comparing the frequency calculated using the proposed pipeline to values calculated using ImageJ by selecting a horizontal line across the tissue using the “re-slice” tool. The validation frequency was calculated by independently and the result was blinded to the current analysis.

Three different types of motility index (MI) were quantified to compare the level of contractions of the same tissue over multiple days. The first MI was frequency multiplied by amplitude (FA) [8]. The second MI was the total area under the curve (AUC) in the contractions in the x and y directions [9]. The third MI was a coordination index (CI), calculated as a measure of the ratio of vectors pointing in the same direction using the following equation,

$$CI = \frac{\sqrt{\bar{x}^2 + \bar{y}^2}}{\sqrt{x^2 + y^2}} \quad (4)$$

where x and y are the normalized displacements relative to the reference frame in x and y directions, respectively.

III. Results and Discussion

Movements in the x and y directions of the FOV were successfully tracked on day 0, 3 and 5 days of organotypic culture. An example trace from the movements detected in each direction on day 3 is demonstrated in Figure 3. In this instance, there was an approximately 1.9 s difference in the phase of the displacements in the x and y directions, indicating that the tissue contracted independently in each direction.

The net displacement demonstrated sustained tonic contractions between 7-10 s in duration as shown in Figure 3C, during which the section of the tissue had a non-zero net displacement from the reference position. A limitation during tissue preparation was to determine the exact orientation of the muscle fibers relative to the camera, so the x and y directions did not necessarily align with the longitudinal and circumferential directions of the intestine.

The frequency detected computationally matched the frequency detected manually on all three days within ± 2 cpm on each day. The detected values were also in agreement with previously reported values from mouse small intestine [10].

The tissue maintained motility patterns from day 0 until day 5, as demonstrated in Figure 4 from a single ROI tracked from approximately the same location on day 0, 3, and 5. The contractions in days 3 and 5 (Figure 4B & C) appeared more rhythmic than in day 0 (Figure 4A). This could potentially be due to tissue damage caused by dissection on day 0 and the recovery in the following days. A “waxing and waning” motility pattern could also be observed over time (Figure 4B).

When tracked over all ROIs, there was a slight reduction in frequency, from approximately 28 to 24 cpm over 5 days, or 0.9 cpm per day (Figure 5A). There was also a general reduction in the amplitude contractions by 24% on day 3 and 74% on day 5 compared to day 0 (Figure 5B). While both FA and AUC reduced over time, CI did not decrease, as shown in Figure 5C.

The reductions in FA and AUC were expected as amplitude of contracts decreased over time, likely due to the gradual degradation in the condition of the tissues. On the other hand, the contractions remained coordinated, demonstrating that the communications between cells in the tissue remained in contact.

The method described in this manuscript provides quantitative analysis for demonstrating preservation of contractile activity in several tissue specimens from the same animal for up to 5 days *in vitro*, and the ability to repeatedly measure this activity from same regions of the tissue. This culture technique and analysis pipeline permits the investigator to modify gene expression in targeted cell types and then follow the effects on contractility in a carefully controlled environment. For example, antisense mediated knockdown of gene transcripts, such as by short hairpin lentiviral constructs, can require more than 3 days of treatment before there is effective knockdown of protein expression and a functional response to the change [11].

IV. Conclusion

We present here a unique automated approach monitoring of GI contractility over several days. This approach will allow study of the effects on GI smooth muscle contractility of direct, controlled targeting of the cells and molecules in the GI *tunica muscularis*. Quantitative results demonstrated that the tissue was active until 5 day of recording, with a slight reduction in frequency and a more significant reduction in amplitude. Standard motility indices such as FA and AUC demonstrated a corresponding reduction, but the coordination of the contractions remained consistent throughout, indicating the preservation of the mechanisms responsible for GI motility. In future the displacement should be calibrated to report actual physical units. Improved techniques will be applied to track more complicated nonlinear movement patterns across multiple samples.

Acknowledgment

Support was also provided by the Rutherford Foundation Trust and NIH RO1 DK52766.

Research supported by the Rutherford Foundation, Royal Society of New Zealand.

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Figure 1.

Distribution of regions-of-interest (ROIs) over a frame containing the smooth muscle tissue culture. Each ROI has a dimension of 100 by 100 pixels, highlighted in blue. The center of each ROI is represented by a blue dot. The gray color in the frame represents the tissue and black represents the background.

Figure 2. Movement tracking of a selected region. A. A selected ROI from the reference frame (blue) on day 0. B. The ROI was correlated to the frame two seconds later, with the correlational matrix showing maximum correlation at the displacement between the two frames (inset). The shifted location of the ROI in the next frame is highlighted by the red square.

Figure 3.

Displacement information from a single region-of-interest tracked over 60 s. A.

Displacement in the x-direction. B. Displacement in the y-direction. C. Net displacement

from the reference position.

Figure 4.

Displacement in the x-direction from the same region-of-interest as in Figure 3 tracked over 5 days. A. Day 0. B. Day 3. C. Day 5.

Figure 5.

Characteristics of motility quantified over six days, on day 0, 3, and 5 quantified using all of the regions-of-interest. A. Frequency. B. Amplitude. C. Motility indices.