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Computer vision-based diameter maps to study fluoroscopic recordings of small intestinal motility from conscious experimental animals

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Abstract

Background: When available, fluoroscopic recordings are a relatively cheap, non-invasive and technically straightforward way to study gastrointestinal motility. Spatiotemporal maps have been used to characterize motility of intestinal preparations in vitro, or in anesthetized animals in vivo. Here, a new automated computer-based method was used to construct spatiotemporal motility maps from fluoroscopic recordings obtained in conscious rats.

Methods: Conscious, non-fasted, adult, male Wistar rats (n=8) received intragastric administration of barium contrast, and 1-2 hours later, when several loops of the small intestine were well-defined, a 2 minutes-fluoroscopic recording was obtained. Spatiotemporal diameter maps (Dmaps) were automatically calculated from the recordings. Three recordings were also manually analyzed for comparison. Frequency analysis was performed in order to calculate relevant motility parameters.

Key Results: In each conscious rat, a stable recording (17-20 seconds) was analyzed. The Dmaps manually and automatically obtained from the same recording were comparable, but the automated process was faster and provided higher resolution. Two frequencies of motor activity dominated; lower frequency contractions (15.2±0.9 cpm) had an amplitude approximately five times greater than higher frequency events (32.8±0.7 cpm).

Conclusions & Inferences: The automated method developed here needed little investigator input, provided high-resolution results with short computing times, and automatically compensated for breathing and other small movements, allowing recordings to be made without anesthesia. Although slow and/or infrequent events could not be detected in the short recording periods analyzed to date (17-20 seconds), this novel system enhances the analysis of in vivo motility in conscious animals.

KEYWORDS
Computer vision-based methods, Dmaps, fluoroscopy, frequency, rat

1 | INTRODUCTION

Many methods have been developed to analyze gastrointestinal motility,[1] each with its own advantages and limitations. Non-invasive imaging methods are important for studies of motility in conscious animals and humans. Magnetic resonance (MR) has demonstrated utility for motility studies in humans,[2] but in animals, it requires anesthesia, which interferes with motor function.[3,4] In addition, it uses complex equipment, which is often not easily accessible. Scintigraphy and single photon emission computed tomography (SPECT) can detect gavaged technetium-label [5,6] allowing non-invasive evaluation of gastrointestinal transit. However, these are costly, involving radionuclides and anesthetics for laboratory animals, and have limited resolution. Infrared/fluorescence reflectance imaging can be applied to mice but produce low morphological resolution.[7–10]

Radiographic techniques are relatively inexpensive and X-rays have been used for a reproducible semi-quantitative analysis, to study acute and chronic effects of drugs in conscious rats [1,11–20] and mice.[1] These methods may also be applied in pathologic models.[21,22] However, X-rays were not as useful to study motor function of the small intestine due to its complex movements, and a more invasive method (charcoal) has typically been used to evaluate upper gastrointestinal transit.[23]

Propagation of small intestinal contractions is difficult to study with still image X-rays but can be measured with endoscopic capsules [24] in the absence of anesthetics. However, these devices are costly, require considerable computing power, are too large for laboratory animals and only measure motility at a single, moving site. Fluoroscopic recordings have been applied to evaluate dynamic gastrointestinal motility in a few studies,[25–29] but have rarely been used to analyze small intestinal contractility.

Spatiotemporal diameter maps (Dmaps) are bi-dimensional representations of changes in diameter along a gut segment over time. They have been applied extensively to study motility of intestinal segments in vitro, recorded by video cameras.[30–33] Dmaps have also been used in exteriorized intestinal preparations,[34] but rarely to analyze small intestinal activity in conscious animals in vivo.[27] Dmaps provide a powerful way to display and analyze quantitatively complex motor patterns. The aim of this project was to develop an automated method to combine fluoroscopic recordings of small intestinal motility in conscious rats with spatiotemporal maps displaying the results.

Key Points

- Small intestinal motility is difficult to analyze in vivo in non-anesthetized experimental animals.
- Fluoroscopy was used to record small intestinal motility in conscious rats. A dedicated software displayed temporal changes in diameter along a segment of the small intestine as spatiotemporal maps (Dmaps).
- The new method for calculating and building the Dmaps is much faster and provides higher resolution than manual methods. It is also faster than previous automatic procedures, while providing results that are suitable for spatiotemporal analysis.

2 | MATERIALS AND METHODS

The in vivo experiments were designed and performed in accordance with the European and Spanish legislation on care and use of experimental animals (EU Directive 2010/63/EU for animal experiments; R.D. 53/2013), and were approved by the Ethics Committees at both Universidad Rey Juan Carlos (URJC) and Hospital General Universitario Gregorio Marañón (HGUGM), where the fluoroscopic recordings were performed.

2.1 | Animals

Male Wistar rats (200-315 g, n=8) were obtained from the Veterinary Unit of HGUGM (Madrid, Spain). Animals were housed in groups (3-4/cage) in standard transparent cages (60 cm×40 cm×20 cm), under environmentally controlled conditions (temperature=20°C; humidity=60%), with a 12 hours light/12 hours dark cycle. Animals had free access to standard laboratory rat chow (Harlan Laboratories Inc., Barcelona, Spain) and sterile tap water, and were not fasted prior to loading with contrast medium or recording (see below).

2.2 | Fluoroscopic recordings of gastrointestinal motor function

For these experiments, 2.5 mL of a suspension of barium sulfate (Barigraph AD, Juste SAQF, Madrid, Spain; 2 g/mL, *t*=22°C) in tap water was administered per os. Plain facial radiographs and fluoroscopic videos of the gastrointestinal tract were obtained using a Digital X-Ray apparatus (Siemens, Siremobil Compact L, Erlangen, Germany; 60 kV, 7 mA) and captured with NPG Real DVD Studio II (pilot study) or with Elgato Video Capture software (all other experiments). X-rays (still photographs) were used to determine the right moment to start fluoroscopic recordings. Exposure time was 0.06 seconds for X-ray shots, and 1-2 minutes for fluoroscopic recordings. Rats were immobilized in a prone position by placing them inside adjustable hand-made transparent plastic tubes. To reduce stress, rats were habituated to stay within the plastic device for the recording period and were released immediately after each X-ray shot or video recording (i.e., immobilization lasted less than 2.5 minutes). X-rays were recorded at various times after administration of the contrast medium. Fluoroscopic videos were recorded at 25 frames/s, after several loops of the small intestine had filled with contrast medium but before the cecum was substantially filled. This normally occurred 1-2 hours after contrast administration, according to X-ray images. The investigator was protected by lead shields for thyroid and abdomino-pelvic viscera, and remained

behind a lead screen, at least 2 m from the X-ray source, during image capture.

2.3 | Manual analysis of fluoroscopic recordings

One representative video (Video S1, ID 1, pilot study) was analyzed both manually and with the new computerized method described in the following sections. The manual analysis was performed to validate the new method.

For the manual analysis, Quick Time Pro was used to extract sequences at one frame/second from the fluoroscopic recording. A small intestinal segment approximately 2.5 cm long was chosen for high contrast and analyzed, frame-by-frame, using Image J 1.38 for Windows (National Institute of Health, USA, free software: http://rsb.info.nih.gov/ij/). For this, a piece of transparent tubing with 50 dots with 0.5 cm spacing was fixed to the computer monitor screen with plasticine, running along the midline of the gut segment (using zoom as needed), this served as a ruler. Using Image J, lines perpendicular to the ruler at each dot were drawn to measure diameter. Data from all frames were combined in Microsoft Excel and represented as a Dmap, in which distance along the intestinal segment (in mm) was represented on the *Y* axis, time in seconds (frames) was represented on the *X* axis, and diameter was represented by a gray scale (contractions white, distension black). Figure 1 shows the steps of this manual process.

2.4 | Automatic analysis of fluoroscopic recordings

Figure 2 depicts the control flow diagram with the main stages of the computerized method. The first stage was acquisition of the video sequence using the digital X-Ray apparatus. Despite being semi-immobilized in a plastic sleeve, breathing and other small movements caused some instability in the image sequence. A global stabilization of the intestinal region of interest (ROI) was defined by an expert observer. This was then used by the automated

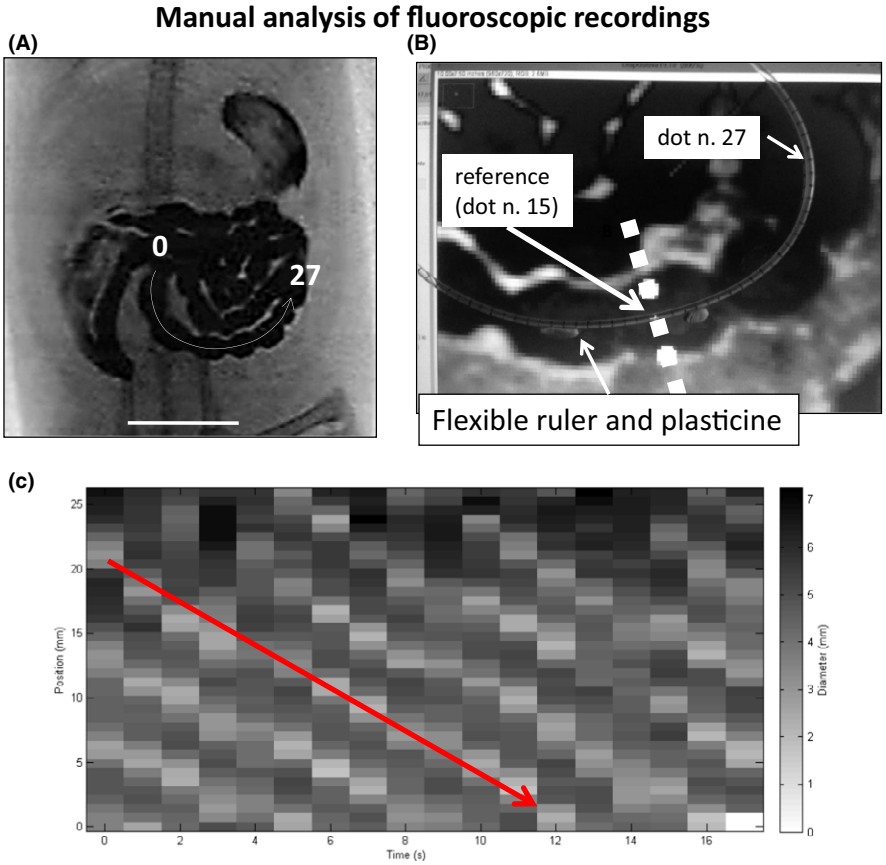

FIGURE 1 Manual analysis of rat small intestinal motor function from fluoroscopic recordings. Small intestinal contractility was recorded in vivo in non-anesthetized rats using fluoroscopy. Videos were recorded after intragastric administration of barium sulfate (2.5 mL, 2 g/mL). The change with time in diameter along a well-contrasted segment of the small intestine was measured with the aid of Image J and a calibrated flexible ruler placed on the computer screen and represented by spatiotemporal (diameter) maps (Dmaps) (see text for further details). Videos were recorded at 25 frames/s, but sequences of only one frame/s (extracted with the aid of Quick Time Pro) were used for the manual analysis. The first frame (A) of one representative video (pilot experiment, Supplementary material) for one rat is shown; in this frame, the arrow shows the direction of the measurement of diameter along the segment chosen for the analysis (0 represents the first position along it). Each frame was opened in Image J and zoomed in to show the intestinal segment of interest, upon which the flexible ruler was placed; the changes in diameter along the segment and over time were measured using the dots on the ruler as reference. The Dmap obtained in this analysis is shown in (C); contractions are shown as sequences of light gray squares moving in a particular direction over time (propagation of one contraction is represented by the red arrow). Scale bar (A): 23 mm

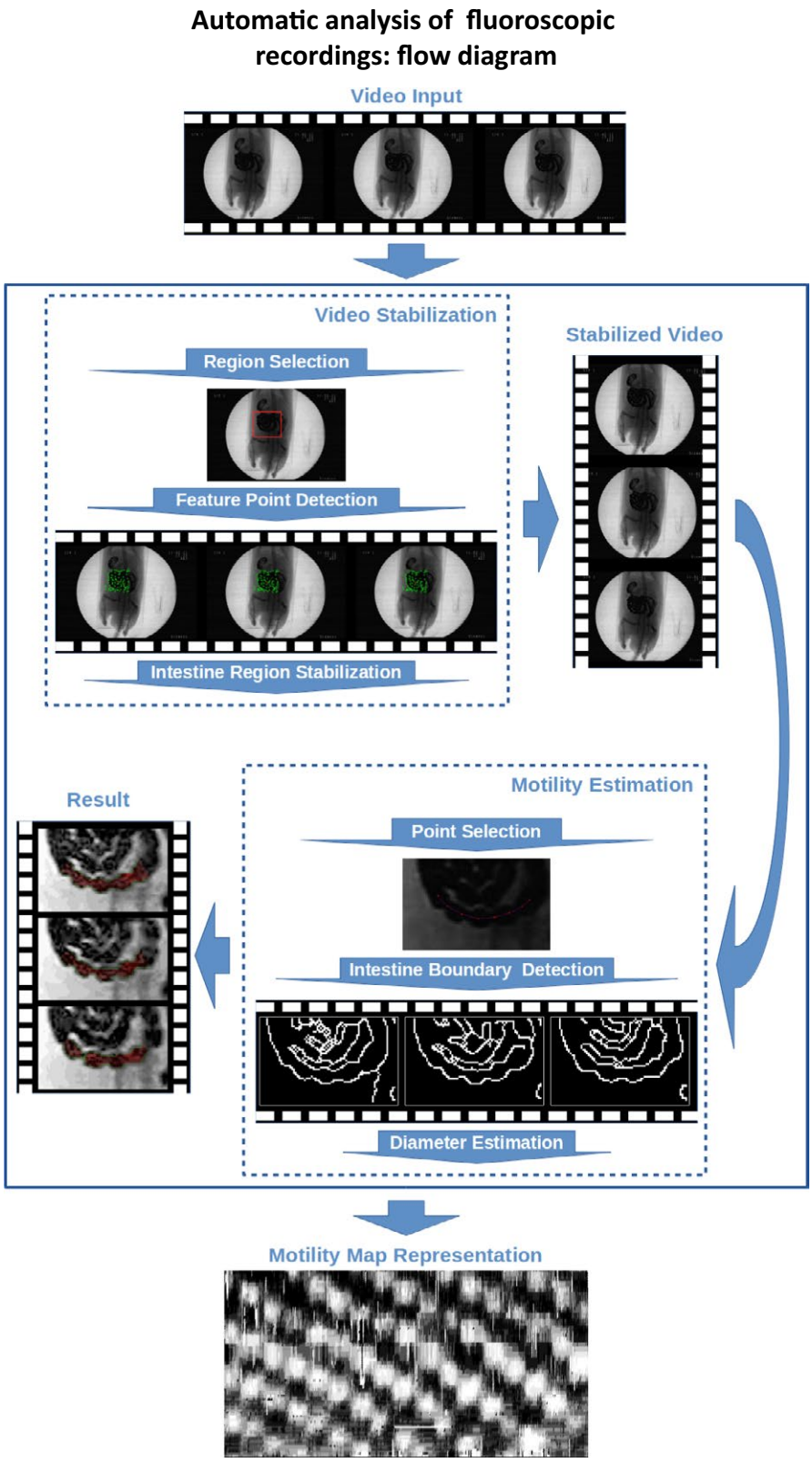

FIGURE 2 Method overview. An image sequence is received by the application as input. The trained investigator selects a region of interest, and the application stabilizes the video sequence by computing its global motion. The user marks some points of interest inside the intestine in the first video frame and the gastrointestinal diameter along the interesting trajectory is estimated automatically. This automatic measurement process is then repeated for the whole sequence in a closed loop, correcting and updating the longitudinal axis at each time step. Finally, the spatiotemporal motility map (Dmap) is returned by the application

stabilization algorithm. Once stabilized, the trained investigator selected control points of the intestine and the automatic motility analysis was performed. Finally, results were postprocessed and presented as a Dmap. In the following sections, details of this process are described.

2.5 | Automatic video stabilization

Our video stabilization method consisted of three main stages: (i) automated selection of sharp features to track, (ii) feature point tracking, and (iii) displacement correction (motion compensation).

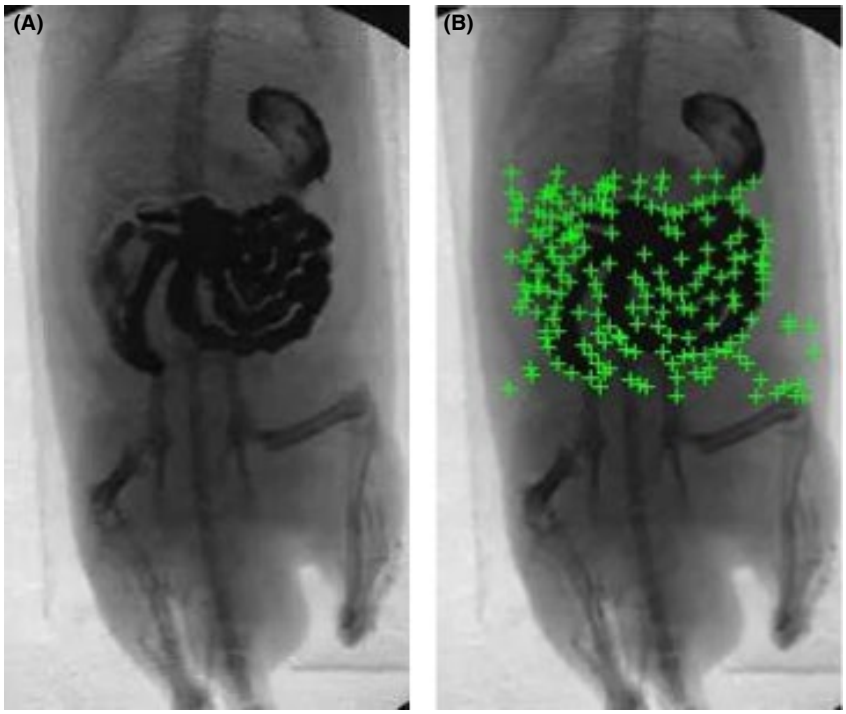

FIGURE 3 Selection of feature points for automatic video stabilization. The method automatically selects optimal points to be tracked in the considered region. Optimality is based on the invariant features of the pixels under translation, rotation or scale changes in the image. A, Initial frame of the video recording. B, Selected featured points (green) on the initial frame shown in (A). Scale bar: 23 cm

The selection of sharp features was based on the work of Shi and Tomasi,[35] which automatically selects optimal points, based on invariance in terms of translation, rotation, or scale changes. Figure 3 shows an example of automatic feature selection where several optimal points were identified.

These points were then tracked in order to compensate for their global motion, assuming that movement was mainly due to rat breathing or external movements. Tracking routines were then used to establish the correspondence between feature points in successive frames. For this, we used the Kanade-Lucas-Tomasi (KLT) method,[36,37] in which two tracked points in consecutive frames are deemed to correspond if the bidirectional error does not exceed a predefined threshold (1 pixel) when performing backward tracking.

Finally, we performed global motion compensation as follows: Let $\acute{p}(t-1)$ and $\acute{p}(t)$ be the geometrical center of the feature points cloud at time $t-1$ and t, respectively. We computed a displacement estimation of the whole region between two successive time steps as follows:

$$\Delta\acute{p} = \acute{p}(t) - \acute{p}(t-1)$$

where $\Delta\acute{p} = (\Delta\acute{x}, \Delta\acute{y})$ is a vector representing the displacement of the region. We then applied an inverse displacement to the selected image region at time t to stabilize the whole video sequence with respect to the first frame.

2.6 | Automatic motility study

Once the image sequence had been stabilized, an expert observer manually selected several points located in the midline of an appropriate region of the intestinal segment (red connected points in Figure 4A); this was a simple and rapid procedure. The rest of the process was then fully automated.

Firstly, a smooth continuous curve, representing the longitudinal axis of the region of interest (red points and trajectory in Figure 4A) was generated, using a spline method. Secondly, the images in the video sequence were equalized and the edges of the intestine were detected.[38] Figure 4B shows a typical example. Thirdly, the method computed the perpendicular distance from the spline curve to the two edges, thus obtaining the diameter at each point for each frame. Figure 4C shows an example. The system then ran automatically, correcting the position of the midline (longitudinal axis) at each time step by dividing the calculated diameter by two. Results are represented as a diameter motility map (Dmap) as shown in Figure 5A. For easier understanding, figure 5B shows the temporal changes in diameter (contractions and distensions) at a defined position of the Dmap shown in Figure 5A.

2.7 | Frequency analysis of the spatiotemporal motility maps

Finally, for each Dmap, a spectral frequency analysis was performed using Fast Fourier Transform (FFT), filtering the DC component. This allowed comparison of results with other previous studies. Specifically, the magnitude of the FFT was computed for each row of the Dmap, which corresponded to the gut diameter over time at every point along the segment (Figure 5B). An average along all sections provided the frequency response of the entire map. Figure 5C shows the result obtained for the Dmap shown in Figure 5A.

As the signals obtained were short (about 20 seconds), the effect of the boundary conditions assumed by the FFT could be exaggerated, degrading the frequency estimation. To counter this, periodograms (the Fourier Transform magnitude of the autocorrelation of the signals) were also computed. This technique, complemented by prewindowing

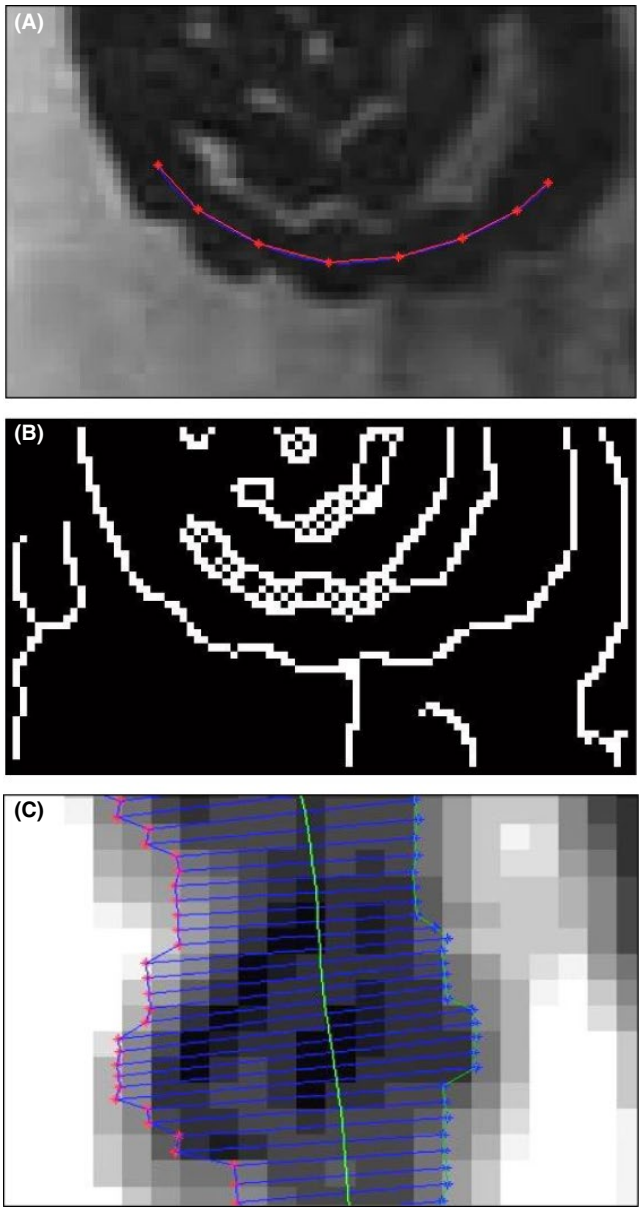

FIGURE 4 Automatic motility study. Images illustrate the 3-step process for automatic analysis of small intestine motility. A, Detail of the intestine region selection and spline generation. B, Edge detection. C, Diameter estimation

the signal with a Gaussian window, alleviates the effects of boundary discontinuities due to the lack of stationarity (short signals). Figure 5D shows an example, obtained from the Dmap in Figure 5A.

From the Dmap, several other measures were computed, including the distance between contractions and velocity of propagating contractions, the average cycle (constriction+relaxation) period (T) (which corresponds to the inverse of the main characteristic frequency (T (s) = 1/F(Hz)), and the diameter at maximum (Dmax) and minimum (Dmin) dilatation. The maximum amplitude of a complete cycle was defined as A = 0.5*(Dmax−Dmin), i.e., the difference between the maximum radius value (0.5*Dmax) and the minimum one (0.5*Dmin) during a period. The average contraction diameter (ACD) and average relaxation diameter (ARD) were computed as:

$$ACD = \frac{1}{N}\sum_{i=1}^{N} Dmin_i \qquad ARD = \frac{1}{N}\sum_{i=1}^{N} Dmax_i$$

where $Dmin_i$ is the i-th value of the contraction diameter and $Dmax_i$ is the i-th value of the relaxation diameter in the sequence, and N represents the total number of radius values available in the sequence.

3 | RESULTS

3.1 | Pilot study: manual vs automated analysis of small intestinal contractility

Manual analysis of 18 seconds of video trace took 3-4 hours to create a low resolution Dmap (Figure 1). The proximal and distal ends of the intestinal segment (at the top and bottom of the Dmap, respectively) had poorer contrast than the rest of the segment. Nevertheless, in the 18 frames (corresponding to 18 seconds) analyzed, 6-7 contractions could be identified. The distance between simultaneous contractions was approximately 6.7 mm, and the frequency of contractions was 0.22 Hz (13.3 cycles/min). The diameter of the segment ranged from a minimum of 1.5 to a maximum of 7.5 mm. The average contraction was 3.2 mm in amplitude, and the average relaxation was 5.4 mm. Contractions propagated at 1.3 mm/s and they appeared to run consistently in the same direction.

For the same video, the automated system took approximately 5 minutes to build the Dmap (shown in Figure 5 and 6A), at 25 frames/s and with higher spatial resolution. Some artifacts were visible (vertical pale lines) but 7-8 propagating contractions were clearly identified in the 20.76 seconds duration of the automatically measured video. The distance between simultaneous contractions was 7.2 mm, and contractions occurred at a frequency of 0.26 Hz (15.5 cycles/min). The diameter of the segment ranged from a minimum of 0.05 mm to a maximum of 7.2 mm. Average contraction diameter (ACD) was 1.5 mm and average relaxation one (ARD) was 5.2 mm. The velocity of the propagating contractions was 1.4 mm/s and they all ran in the same direction.

Descriptively, the Dmaps showed a series of contractile complexes that progressed along the intestinal segment at about 1.4 mm/s (diagonal, beaded bands). These recurred at approximately 4-5 seconds intervals at any point along the segment. Each of these complexes was made up of a contraction that varied in amplitude as it progressed, with an approximate cycle duration of 1.3 seconds. It should be noted that it was not possible to determine the direction of propagation from these recordings.

3.2 | Automated analysis of small intestinal contractility in a group of animals

As manual and automated analysis yielded similar results for the pilot experiment, we applied automated analysis in a further seven animals, using Elgato Video Capture, with the same fluoroscopy camera. Acquisition was at 25 frames per second and all the frames were included for the analysis after stabilization. Figures S1-S7 show the Dmaps of the seven additional animals used for the study. The basic pattern was similar to that described above, with complexes visible in all seven preparations.

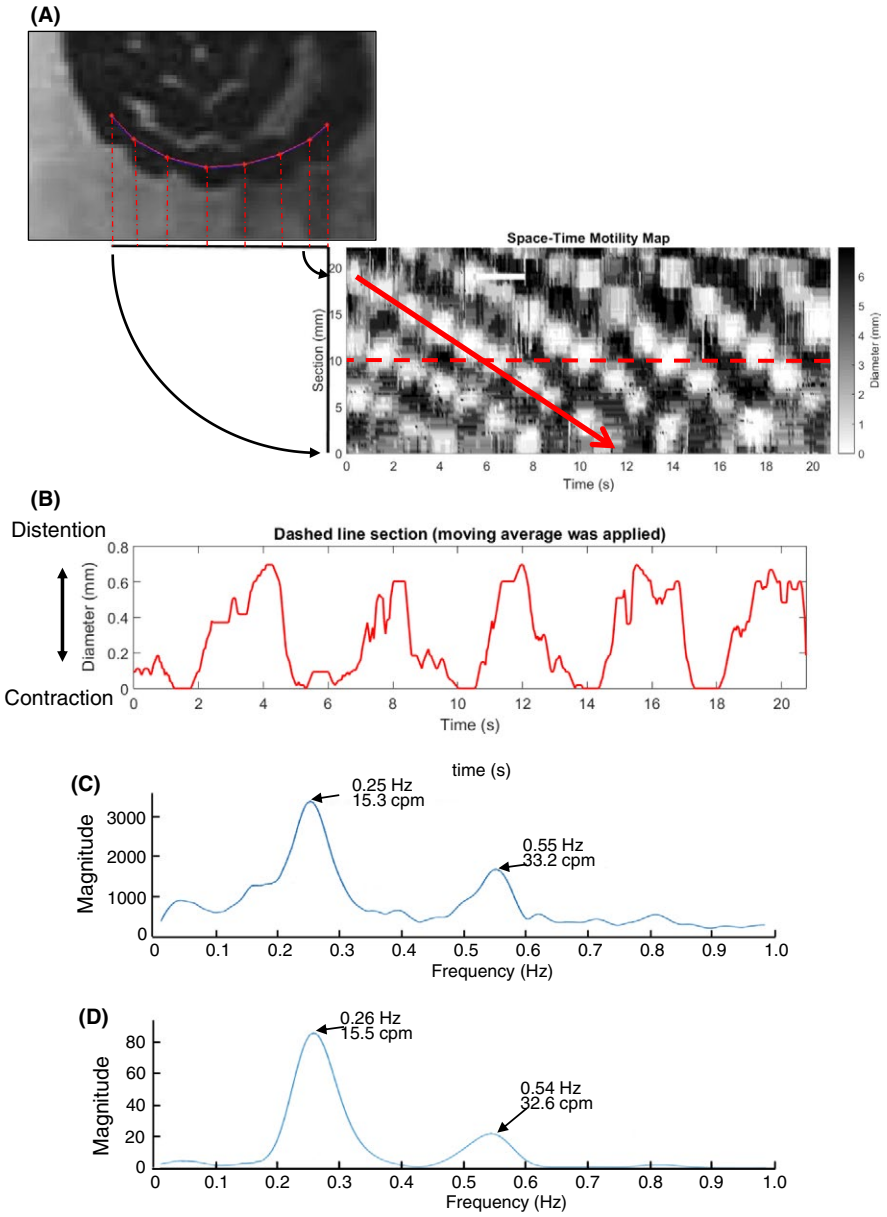

FIGURE 5 Example of spatiotemporal Dmap obtained automatically from a fluoroscopic recording and its frequency analysis. A, A particular frame of the fluoroscopic recording is shown in which the longitudinal axis of a small intestinal segment is defined. This longitudinal axis is represented as the *Y* axis of the Dmap. The *X* axis of the Dmap corresponds to the duration of the video analyzed. As in Figure 1, contractions are shown as sequences of light gray squares moving in a particular direction over time (propagation of one contraction is represented by the red arrow on the Dmap). B, example of temporal changes in diameter (contractions and distensions) at a defined point of the intestinal segment, shown as a dotted red line in (A). C, Frequency response from raw data acquired in (A) using FFT (Fast Fourier Transform). DC component was filtered. D, Power Spectral Density response (periodogram) from raw data acquired in (A). Windowing was applied with a gaussian window. DC component was filtered

Numerical results calculated for this group were also similar to those described above (see Table S1), with small differences in the average distance between contractions, average relaxations, and average amplitudes. Figures 6A and B show FFT and periodogram analysis averaged for the seven additional preparations. The FFT (Figure 6A) showed two main peaks at 0.25 Hz and 0.54 Hz. In the periodogram (Figure 6B), the same peaks occurred also at 0.25 Hz and 0.54 Hz.

Finally, two additional recordings (#6 and #8) were also manually analyzed as described before. The corresponding maps and the results of the frequency analysis are shown for manual and automatic analysis in Figures S8-S9. Results were comparable to those obtained automatically.

4 | DISCUSSION

In the present study, we have developed computerized methods to analyze small intestinal contractility recorded fluoroscopically in conscious rats, and display the results as spatiotemporal (diameter) maps. This automated analysis provided similar results to trial manual analyses performed by us on the same recordings, but with higher spatial and temporal resolution and in a much shorter timeframe (5 minutes vs 3-4 hours per recording).

To date, there have been few reports on the contractility of the rat small intestine in vivo using imaging techniques and, as far as we know, none carried out without anesthesia. Fluoroscopy is a radiological technique that is suitable for the non-invasive study of gastrointestinal motility in small animals in vivo.[39] It is also frequently used in clinics and hospitals to assess the structures and function of the gastrointestinal tract in adults and children. It has also been used to evaluate motility at a single time point in adult mice and rats.[26,39–41] Fluoroscopic imaging reveals how gastrointestinal contractions move luminal contents and does not require invasive surgery or procedures. Thus, it can be applied several times to the same animal, providing the benefits of repeated-measures designs.

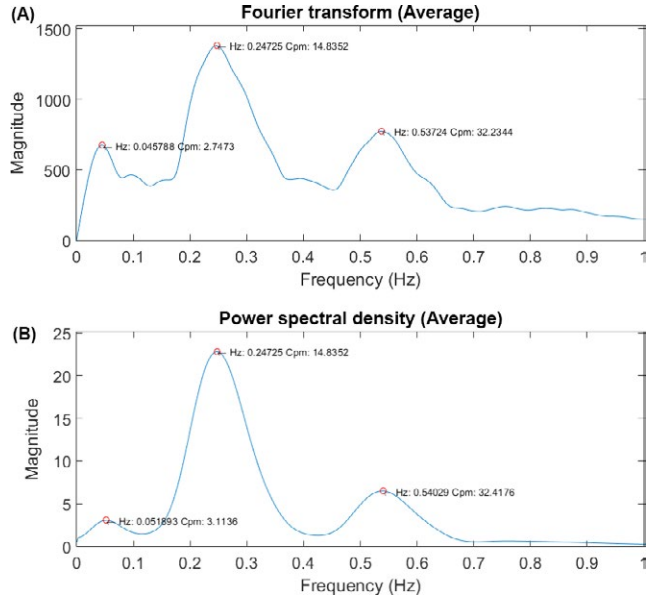

FIGURE 6 Average histograms obtained from the spatiotemporal Dmaps (#2-8 from Table S1). A, Average of frequency response using FFT (Fast Fourier Transform). B, Average of power spectral density response (periodogram). N=7

One to two hours after administering contrast medium, the distal small intestine (probably jejunum, 41) was well-loaded (meaning that it was homogeneously filled with barium, providing clear contrast against the background) and displayed robust contractility. In previous studies [26,41] of fasted anesthetized female rats (49-112 days old), jejunal contractions measured at 3 points occurred at 14±2.1 per minute (range: 11±1.5-20±4.6, n=7). In the present study, in non-fasted male rats, a frequency of 17.3±0.9 contractions per minute (ranging from 14 to 20, n=7) was recorded (non-significant difference: P=.18, unpaired t test with Welch's correction). The Dmaps generated in the present study effectively sampled many more points along the specimen, allowing for more accurate measurement of the timing and spread of contractions.

A recent elegant study analyzed rat small intestinal contractility in vivo, using magnetic resonance imaging (MRI), under anesthesia, with an automated computer-based method for 3-dimensional MRI (conceptually comparable to the present report).[3,4] In that MRI study, images were put in registration but frames with large respiratory excursions (>1-2 mm) were removed.[3] In the current study, video stabilization ensured that no frames were removed and there were no gaps in the Dmaps. Studies of MRI in humans have used algorithms similar to ours to compensate for breathing movements.[42,43] In the study of Ailiani et al.,[3,4] two segmentation algorithms were applied sequentially: a 3D live wire (3D LW) and directional-dynamic-gradient-vector-flow-snakes routine (DDGVFS), based on work by Liang et al.[44] They indicated that their method obtained adequate segmentation for 97% of frames (from qualitative evaluation of their segmentation results), but the remainder had to be segmented manually. In the current study, the segmentation procedure was fully automated. Ailiani et al. used a thinning algorithm [45] to identify the gut midline, using distance transform maps and applied an advanced Principal Component Analysis (PCA) of the segmented data of rat jejunum, using the method of Cootes et al. [46] to compute dominant eigenvectors and eigenmodes of motility data. This sophisticated analysis produced Dmaps of longer recording durations and thus allowed detection of slow and/or infrequent events, but was highly computation-intensive. The method developed in the present study used all video frames, revealing a very homogeneous motility pattern in conscious animals, and was much faster. While Ailiani et al. needed ~4 hours per data set, including 1000 images,[3] our system provided a thorough analysis of ~500 images in about 5 minutes.

Video stabilization was a key aspect of our study; indeed the latter stages of analysis depended heavily on it. The system described here was robust, but not perfect. If the rat moved suddenly (as occurred occasionally in the absence of anesthesia), the computed intestinal midline was displaced, producing a subsequent miscalculation of intestinal diameter. These occasional occurrences were readily identified and excluded, allowing reliable Dmaps to be generated for conscious animals.

Occasionally, the chosen segment would move so that it overlapped with other intestinal loops (due to folding and motility of the gut within the abdominal cavity), leading to obvious miscalculations of intestinal diameter. This was very apparent in some Dmaps, which were then discarded. In each recording, we were able to identify at least one continuous period of about 20 seconds to build an informative Dmap, which yielded a characteristic, homogeneous motility pattern. Future work will ascertain if this pattern may be specifically altered by pathology or drug treatments.

Ailiani et al. compared isofluorane, which reduced overall gut motility,[47] to thiobutabarbital.[4] Their rats were in the fed state and had similar weight range to the present study but their sex was not stated. Their use of anesthetics made longer recordings feasible, revealing slower and intermittent motor patterns. Peristaltic, segmental contractions and also periods of quiescence were reported. In the present study, movements and overlapping of intestinal profiles made it difficult to obtain Dmaps longer than 20 seconds; unsurprisingly, only segmental contractions were clearly identified. These have been reported to be the most frequent small intestinal motility pattern,[4] and it is not surprising that they dominated our short recordings. Ailiani reported peristaltic sequences in 90% of their recordings, but quiescent periods (17-180 seconds) only accounted for 10%-16% recordings in either isoflurane or thiobutabarbital. The lack of quiescent periods in our recordings may be due to their short durations, or may reflect the lack of anesthesia.

Ailiani et al. [4] reported that segmental contractions had two main frequency components, an observation that we confirmed in the present study. Our low frequency component (~0.25 Hz both in the FFT and periodogram) was similar to that described by Ailiani (0.27 in isoflurane- and 0.30 in thiobutabarbital-anesthetized rats). We also recorded a higher frequency component (0.54 Hz) comparable to the frequencies reported previously (0.42 and 0.49 Hz in isoflurane and thiobutabarbital, respectively). More importantly, in Ailiani et al.,[4] segmental contractions did not appear to propagate in either direction; a velocity of propagation was only reported for peristaltic contractions. We speculate that anesthesia may disrupt the propagation of segmenting contractions via a mechanism that is currently unidentified.

Comparing our Dmaps to previous in vivo and in vitro studies of rat small intestine, it should be noted that in the current study, animals were not fasted and the small intestine still had residual content. Under these circumstances, migrating motor complexes (MMCs) were expected to be suppressed, but continuous segmenting contractions were seen, characterized by rythmic contractions that propagated. These may reflect interactions between neurogenic and myogenic mechanisms in the intestinal wall. Ferens et al. [48] described propagating peristaltic contractions in exteriorized segments of small intestine in anesthetized rats, evoked by raised intraluminal pressure. These contractions propagated aborally slightly faster (2.8-4.0 mm/s), but did not show the cyclic rhythmicity that was so prominent in the Dmaps (e.g: in Figure 5) of the present study.

The higher frequency events may reflect the localized spike patches caused by propagation of smooth muscle action potentials [49] or to interactions between multiple oscillators in the gut wall.[50] Further study is needed to characterize the underlying mechanisms.

5 | CONCLUDING REMARKS

A new method has been developed to characterize some aspects of small intestinal motility based on video fluoroscopy recordings (patented: P201531687), which provide good morphological images, from laboratory animals in vivo without the need for anesthetics. The ability to produce spatiotemporal (diameter) maps (Dmaps) from intact, conscious animals is invaluable for analysis of some regular rhythmic patterns of motility. Currently, this new approach is limited by technical issues (the short duration of recordings, the inability to define direction of propagation), and by factors related to the complexity of gut motor function (variation in gut motor patterns between regions; differences in motor function between the fed and the fasted state). It must be acknowledged that, in its present configuration, our method does not allow us yet to define all known gut motility patterns, especially those that occur intermittently. However, the method is fast and relatively easy to implement and gives a reliable quantitative snapshot of some aspects of ongoing intestinal motility. The current limitations will be specifically addressed in future studies. Furthermore, the software developed here has the potential to be adapted for video recordings obtained with other imaging modalities, including dynamic magnetic resonance, which does not involve exposure to ionizing radiation, and could thus be applicable to human studies.

Although the present results have many limitations, we anticipate that this technique will make it possible to identify changes in some aspects of motility in conscious animals in pathology or in the presence of drugs.

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DISCLOSURE

No competing interests declared.

AUTHOR CONTRIBUTION

AEL-P and RA performed the in vivo experiments; RA performed the manual analysis of the fluoroscopic recordings; IR developed and performed the experimental validation of the computer vision-based method under the guidance of JJP and ASM. RA, JJP and ASM designed the study and wrote the paper; SB contributed essential intellectual input to the design of the study and reviewed the manuscript; MIM-F obtained financial support and reviewed the manuscript.

REFERENCES

1. Girón R, Pérez-García I, Abalo R. X-ray analysis of gastrointestinal motility in conscious mice. Effects of morphine and comparison with rats. *Neurogastroenterol Motil*. 2016;28:74–84.
2. Marciani L. Assessment of gastrointestinal motor functions by MRI: a comprehensive review. *Neurogastroenterol Motil*. 2011;23:399–407.
3. Ailiani AC, Neuberger T, Brasseur JG, et al. Quantitative analysis of peristaltic and segmental motion in vivo in the rat small intestine using dynamic MRI. *Magn Reson Med*. 2009;62:116–126.
4. Ailiani AC, Neuberger T, Brasseur JG, et al. Quantifying the effects of inactin vs Isoflurane anesthesia on gastrointestinal motility in rats using dynamic magnetic resonance imaging and spatio-temporal maps. *Neurogastroenterol Motil*. 2014;26:1477–1486.
5. Tsopelas C, Adam B, Liebregts T, Holtmann G, Bartholomeusz FD. Colonic transit studies with 99mTc-rhenium sulfide colloid in live rats. a preliminary study. *Hell J Nucl Med*. 2008;11:96–100.
6. Padmanabhan P, Grosse J, Asad AB, Radda GK, Golay X. Gastrointestinal transit measurements in mice with 99mTc-DTPA-labeled activated charcoal using NanoSPECT-CT. *EJNMMI Res*. 2013;3:60.
7. Zhao M, Yang M, Baranov E, et al. Spatial–temporal imaging of bacterial infection and antibiotic response in intact animals. *Proc Natl Acad Sci U S A*. 2001;98:9814–9818.
8. Gremlich HU, Martínez V, Kneuer R, et al. Noninvasive assessment of gastric emptying by near-infrared fluorescence reflectance imaging in mice: pharmacological validation with tegaserod, cisapride, and clonidine. *Mol Imaging*. 2004;3:303–311.
9. de Backer O, Blanckaert B, Leybaert L, Lefebvre RA. A novel method for the evaluation of intestinal transit and contractility in mice using fluorescence imaging and spatiotemporal motility mapping. *Neurogastroenterol Motil*. 2008;20:700–707.
10. Roda A, Mezzanotte L, Aldini R, Michelini E, Cevenini L. A new gastric-emptying mouse model based on in vivo non-invasive bioluminescence imaging. *Neurogastroenterol Motil*. 2010;22:1117–e288.
11. Cabezos PA, Vera G, Castillo M, Fernández-Pujol R, Martín MI, Abalo R. Radiological study of gastrointestinal motor activity after acute cisplatin in the rat temporal relationship with pica. *Auton Neurosci*. 2008;141:54–65.
12. Cabezos PA, Vera G, Martín-Fontelles MI, Fernández-Pujol R, Abalo R. Cisplatin-induced gastrointestinal dysmotility is aggravated after chronic administration in the rat comparison with pica. *Neurogastroenterol Motil*. 2010;22:e224–e225.
13. Abalo R, Cabezos PA, López-Miranda V, et al. Selective lack of tolerance to delayed gastric emptying after daily administration of WIN 55,212-2 in the rat. *Neurogastroenterol Motil*. 2009;21:1002–e80.
14. Abalo R, Cabezos PA, Vera G, Fernández-Pujol R, Martín MI. The cannabinoid antagonist SR144528 enhances the acute effect of WIN 55,212-2 on gastrointestinal motility in the rat. *Neurogastroenterol Motil*. 2010;22:694–e206.

15. Abalo R, Cabezos PA, Vera G, López-Miranda V, Herradón E, Martín-Fontelles MI. Cannabinoid-induced delayed gastric emptying is selectively increased upon intermittent administration in the rat: role of CB1 receptors. *Neurogastroenterol Motil*. 2011;23:e177.
16. Abalo R, Cabezos PA, Vera G, López-Pérez AE, Martín MI. Cannabinoids may worsen gastric dysmotility induced by chronic cisplatin in the rat. *Neurogastroenterol Motil*. 2013;25:e292.
17. Abalo R, Chen C, Vera G, et al. In vitro and non-invasive in vivo effects of the cannabinoid-1 receptor agonist AM841 on gastrointestinal motor function in the rat. *Neurogastroenterol Motil*. 2015;27:1721–1735.
18. López-Miranda V, Soto-Montenegro ML, Uranga-Ocio JA, et al. Effects of chronic dietary exposure to monosodium glutamate on feeding behavior, adiposity, gastrointestinal motility, and cardiovascular function in healthy adult rats. *Neurogastroenterol Motil*. 2015;27:1559–1570.
19. Vera G, López-Pérez AE, Uranga JA, Girón R, Martín-Fontelles MI, Abalo R. Involvement of Cannabinoid Signaling in Vincristine-Induced Gastrointestinal Dysmotility in the Rat. *Front. Pharmacol*. 2017;8:37. doi: 10.3389/fphar.2017.00037.
20. Vera G, López-Pérez AE, Martínez-Villaluenga M, Cabezos PA, Abalo R. X-ray analysis of the effect of the 5-HT3 receptor antagonist granisetron on gastrointestinal motility in rats repeatedly treated with the antitumoral drug cisplatin. *Exp Brain Res*. 2014;232:2601–2612.
21. Abalo R, Cabezos PA, Vera G, López-Miranda V, Herradón E, Martín MI. Radiographic analysis of gastrointestinal motor function in type 1 and type 2 diabetic rats. *Neurogastroenterol Motil*. 2010;22:61.
22. Vegezzi G, Al Harraq Z, Levandis G, et al. Radiological analysis of gastrointestinal dysmotility in a model of central nervous dopaminergic degeneration: comparative study with conventional in vivo techniques in the rat. *J Pharmacol Toxicol Methods*. 2014;70:163–169.
23. Vera G, Castillo M, Cabezos PA, et al. Enteric neuropathy evoked by repeated cisplatin in the rat. *Neurogastroenterol Motil*. 2011;23(370–378):e162–e163.
24. Drozdzal M, Seguí S, Radeva P, Malagelada C, Azpiroz F, Vitrià J. Motility bar: A new tool for motility analysis of endoluminal videos. *Comput Biol Med*. 2015;65:320–330.
25. Abalo R, Vera G, López-Pérez AE, Martínez-Villaluenga M, Martín-Fontelles MI. The gastrointestinal pharmacology of cannabinoids: focus on motility. *Pharmacology*. 2012;90:1–10.
26. Perry RL, Carrig CB, Williams JF, Johnson CA, Kaneene JB. Anatomic features and radiographic observations of gastric emptying and small intestinal motility in the rat. *Lab Anim Sci*. 1993;43:586–593.
27. Der T, Bercik P, Donnelly G, et al. Interstitial cells of cajal and inflammation-induced motor dysfunction in the mouse small intestine. *Gastroenterology*. 2000;119:1590–1599.
28. Wang XY, Lammers WJ, Bercik P, Huizinga JD. Lack of pyloric interstitial cells of Cajal explains distinct peristaltic motor patterns in stomach and small intestine. *Am J Physiol Gastrointest Liver Physiol*. 2005;289:G539–G549.
29. Williams K, Koyama T, Schulz D, et al. Use of fluoroscopy to study in vivo motility in mouse pups. *J Pediatr Gastroenterol Nutr*. 2011;52:679–685.
30. Hennig GW, Costa M, Chen BN, Brookes SJ. Quantitative analysis of peristalsis in the guinea-pig small intestine using spatio-temporal maps. *J Physiol*. 1999;517:575–590.
31. Bercík P, Bouley L, Dutoit P, Blum AL, Kucera P. Quantitative Analysis of Intestinal Motor Patterns: Spatiotemporal Organization of Nonneural Pacemaker Sites in the Rat Ileum. *Gastroenterology*. 2000;119:386–394.
32. Dinning PG, Arkwright JW, Costa M, et al. Temporal relationships between wall motion, intraluminal pressure, and flow in the isolated rabbit small intestine. *Am J Physiol Gastrointest Liver Physiol*. 2011;300:G577–G585.
33. Kuizenga MH, Sia TC, Dodds KN, et al. Neurally mediated propagating discrete clustered contractions superimposed on myogenic ripples in ex vivo segments of human ileum. *Am J Physiol Gastrointest Liver Physiol*. 2015;308(1):G1–G11.
34. Janssen PW, Lentle RG, Chambers P, Reynolds GW, De Loubens C, Hulls CM. Spatiotemporal organization of standing postprandial contractions in the distal ileum of the anesthetized pig. *Neurogastroenterol Motil*. 2014;26:1651–1662.
35. Shi J, Tomasi C. Good features to track. In Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR, 1994). Seattle, Washington, USA: IEEE Computer Society Press; 1994:593–600.
36. Lucas BD, Kanade T. An iterative image registration technique with an application to stereo vision. In Proceedings of the 7th International Joint Conference on Artificial Intelligence. Vol. 2. San Francisco, CA, USA: Morgan Kaufmann Publishers Inc.; 1981:674–679.
37. Tomasi C, Kanade T. Detection and tracking of point featuresImage Rochester NY, Technical Report CMU; 1991:91–132.
38. Canny J. A computational approach to edge detection. *IEEE Trans Pattern Anal Mach Intell*. 1986;6:679–698.
39. Der-Silaphet T, Malysz J, Hagel S, Larry Arsenault A, Huizinga JD. Interstitial cells of cajal direct normal propulsive contractile activity in the mouse small intestine. *Gastroenterology*. 1998;114:724–736.
40. Mashimo H, Kjellin A, Goyal RK. Gastric stasis in neuronal nitric oxide synthase-deficient knockout mice. *Gastroenterology*. 2000;119:766–773.
41. Perry RL, Williams JF, Carrig CB, Kaneene JB, Van Schillhorn Veen TW. Radiologic evaluation of the liver and gastrointestinal tract in rats infected with Taenia taeniaeformis. *Am J Vet Res*. 1994;55:1120–1126.
42. Hamy V, Menys A, Helbren E, et al. Respiratory motion correction in dynamic-MRI: application to small bowel motility quantification during free breathin. *Med Image Comput Comput Assist Interv*. 2013;16:132–140.
43. Hamy V, Dikaios N, Punwani S, et al. Respiratory motion correction in dynamic MRI using robust data decomposition registration - application to DCE-MRI. *Med Image Anal*. 2014;18:301–313.
44. Liang J, McInerney T, Terzopoulos D. United snakes. *Med Image Anal*. 2006;10:215–233.
45. Saha P, Chanda B, Majumder D. A single scan boundary removal thinning algorithm for 2-D binary object. *Pattern Recognit Lett*. 1993;14:173–179.
46. Cootes TF, Taylor CJ, Cooper DH, Graham JA. Active shape models - their training and application. *Comput Vis Image Underst*. 1995;61:38–59.
47. Torjman MC, Joseph JI, Munsick C, Morishita M, Grunwald Z. Effects of Isoflurane on gastrointestinal motility after brief exposure in rats. *Int J Pharm*. 2005;294:65–71.
48. Ferens DM, Chang EC, Bogeski G, Shafton AD, Kitchener PD, Furness JB. Motor patterns and propulsion in the rat intestine in vivo recorded by spatio-temporal maps. *Neurogastroenterol Motil*. 2005;17:714–720.
49. Lammers WJEP. Propagation of individual spikes as "patches" of activation in isolated feline duodenum. *Am J Physiol Gastrointest Liver Physiol*. 2000; 278:G297–G307.
50. Huizinga JD, Lammers WJ. Gut peristalsis is governed by a multitude of cooperating mechanisms. *Am J Physiol Gastrointest Liver Physiol*. 2009;296:G1–G8.

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