

## TECHNICAL NOTE

# A novel method for the evaluation of intestinal transit and contractility in mice using fluorescence imaging and spatiotemporal motility mapping

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**Abstract** This study introduces a novel, simplified method for the evaluation of murine intestinal transit and contractility using fluorescence and video imaging. Intestinal transit was measured by evaluating the intestinal distribution of non-absorbable fluorescein-labelled dextran (70 kDa, FD70) along the gastrointestinal (GI) tract. After excision of the GI tract, two full-field images – one in normal illumination mode and another in fluorescent mode – were taken with a charge coupled device (CCD) camera and subsequently matched for calculation of fluorescence distribution along the GI tract. Immediately after, intestinal contractility was evaluated in different regions of the intact intestine by spatiotemporal motility mapping (i.e. video imaging). In control mice, the small intestine showed vigorous oscillatory contractions and FD70 was primarily distributed within the terminal ileum/caecum at 90 min postgavage. As validation step, the effect of intestinal manipulation (IM, surgical procedure) and two pharmacological agents – known to alter GI motility – was tested. At 24 h postoperatively, spontaneous contractile activity of the small intestine was nearly abolished in IM mice, leaving the small intestine distended and resulting in a significantly delayed intestinal transit. In accordance, spontaneous mechanical activity of

circular muscle strips in standard organ baths was significantly reduced in IM mice compared to control mice. Administration of atropine (1–3 mg kg<sup>-1</sup>, i.p.) suppressed spontaneous contractile activity along the entire intestinal tract and induced a dose-related delay in intestinal transit. In contrast, metoclopramide (3–10 mg kg<sup>-1</sup>, i.p.) markedly increased contractile activity – however only in the upper GI tract – and accelerated intestinal transit in a dose-dependent manner.

**Keywords** contractility, gastrointestinal motility, imaging, intestinal transit, mice, spatiotemporal mapping.

**Abbreviations** CCD, charge coupled device; FD70, fluorescein-labelled dextran 70 kiloDalton; GC, geometric centre; GI, gastrointestinal; HNE, 4-hydroxy-2-nonenal; IM, intestinal manipulation; IL, interleukin; MDA, malondialdehyde; MPO, myeloperoxidase; PMSF, phenylmethylsulphonyl fluoride; SEM, standard error of the mean; UV, ultraviolet; 3-D, three-dimensional;  $\bar{\phi}_{mean}$ , mean diameter.

## INTRODUCTION

Intestinal transit and contractility are important parameters in gastrointestinal (GI) motility studies. At present, intestinal transit in rodents is usually measured by gavage of a colorimetric, fluorescent or radioactive marker, and the subsequent determination of this marker in the divided intestinal segments of the GI tract.<sup>1–6</sup> Therefore, these methods do not allow to evaluate intestinal contractility in the intact intestine of the same animal. This study describes a novel

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method to measure both intestinal transit and contractility in the intact intestine quasi-simultaneously using fluorescence and video imaging. To validate this newly developed method, the effect of abdominal surgery and two different pharmacological agents – known to alter GI motility – was tested.<sup>7–11</sup>

## MATERIALS AND METHODS

### Animals

Male C57/Bl6 mice (20–25 g) were purchased from Janvier (Le Genest St-Isle, France) and maintained in standard cages with free access to water and pellets. All experimental procedures were approved by the Ethical Committee for Animal Experiments, Ghent University, Belgium.

### Surgical procedure

Mice were anaesthetized with inhaled isoflurane (induction 5%, maintenance 2%) and the abdomen was opened by midline laparotomy. The small intestine was everted and then compressed for 5 min along its entire length by using sterile moist cotton applicators (intestinal manipulation, IM). The bowel was repositioned in the abdominal cavity and the incision was closed by two layers of continuous sutures. Mice were gavaged at 22 h 30 min postoperatively and killed by cervical dislocation 90 min postgavage.<sup>3,5</sup> Age-matched non-operated mice served as controls.

### Drug administration

In a separate set of experiments, atropine (1–3 mg kg<sup>-1</sup>; cholinergic antagonist) or metoclopramide (3–10 mg kg<sup>-1</sup>; 5-HT<sub>4</sub> receptor agonist) was given intraperitoneally (i.p.) 30 min before gavage and animals were killed by cervical dislocation 30 min postgavage.<sup>10,11</sup>

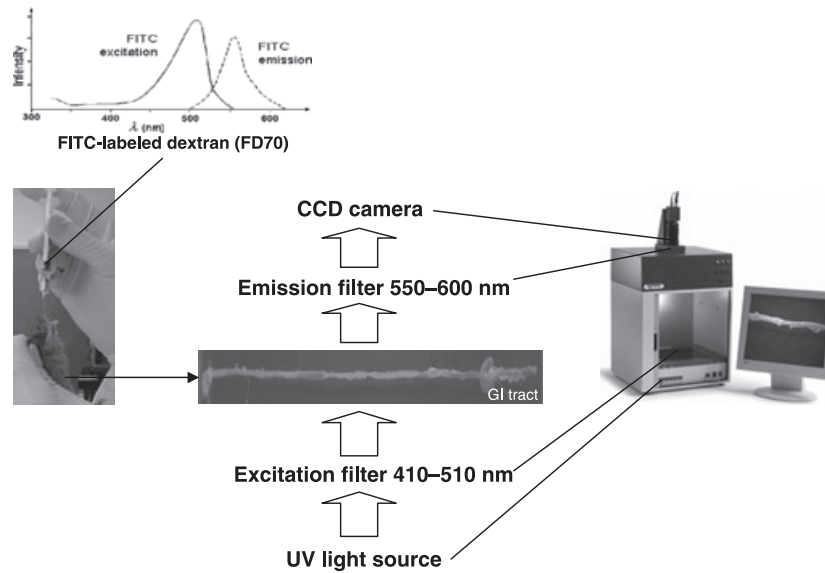
### Intestinal transit

Intestinal transit was measured by evaluating the intestinal distribution of non-absorbable fluorescein-labelled dextran (70 kDa, FD70; Invitrogen, Merelbeke, Belgium) along the GI tract. Mice were killed by cervical dislocation, respectively, 90 min (IM) or 30 min (pharmacological agents) after the oral ingestion of FD70 (200 µL, 25 mg mL<sup>-1</sup>). The abdomen was cut open, a ligature was placed around the lower oesophagus (just above the cardia) and rectum, and

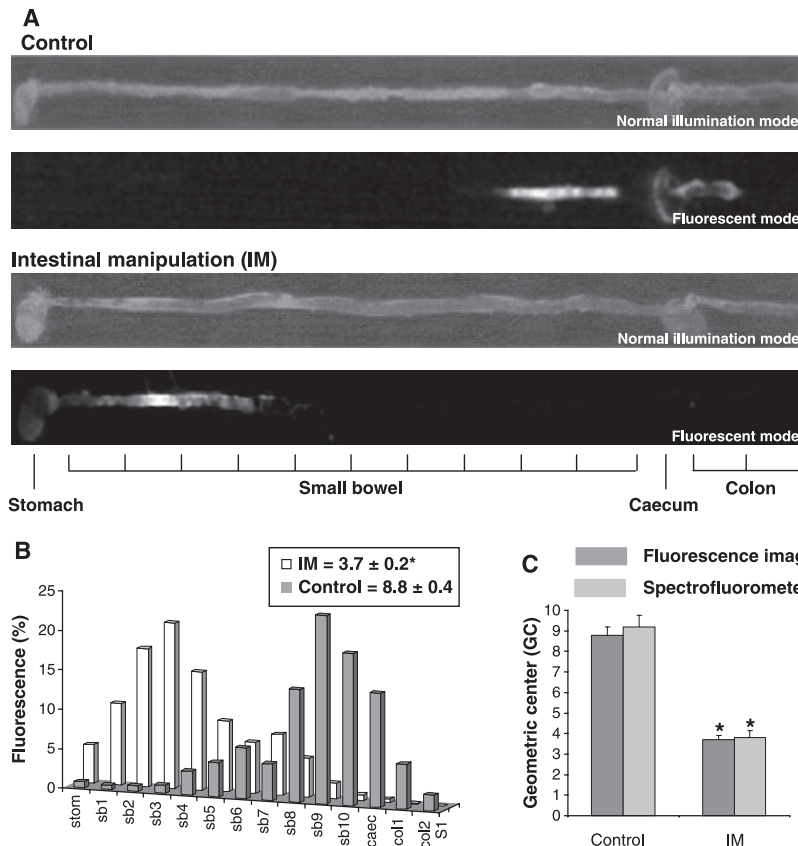
the entire GI tract was excised. Next, the mesentery was removed and the GI tract (length: ±25–30 cm) was pinned down with pins at 7.5-cm intervals in a custom-made Petri dish (5 × 30 cm) filled with Krebs solution (composition in mmol L<sup>-1</sup>: NaCl 188.5, KCl 4.8, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> 1.2, CaCl<sub>2</sub> 1.9, NaHCO<sub>3</sub> 25.0, glucose 10.1), containing 1 mmol L<sup>-1</sup> phenylmethylsulphonyl fluoride (PMSF) and aerated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Immediately after, FD70 was visualized using the Syngene GeneFlash system (Syngene, Cambridge, UK). The system consisted of a ultraviolet (UV)-light source, an excitation filter (410–510 nm conversion screen), a 8-bit monochrome charge coupled device camera equipped with a f/1.2 8–48 mm zoom lens, and an emission bandpass filter to detect fluorescence (550–600 nm emission; Fig. 1). Two full-field images – one in normal illumination mode and another in fluorescent mode – were taken and subsequently matched for analysis (Fig. 2A); the fluorescent intensity throughout the entire GI tract was analysed and calculated using custom-made software (Intestinal Transit software, written as ImageJ plugins which can be downloaded from <http://www.heyman.ugent.be/En/DownloadsEn.htm>). Data were expressed as the percentage of fluorescence intensity per segment (stom, stomach; sb, small bowel segments 1–10; caec, caecum; col, colon segments 1–2) and plotted in a histogram. The geometric centre (GC) was calculated by the formula:  $\Sigma(\% \text{ FD70 per segment} \times \text{segment number})/100$ .<sup>12</sup> To validate this new imaging technique, intestinal transit was also evaluated as described previously.<sup>3</sup> Therefore, the GI tract had to be cut into 14 segments of equal length and the fluorescent signal in each sample was determined by using a spectrofluorometer (Victor, Perkin-Elmer, Waltham, MA, USA).

### Contractility imaging and spatiotemporal motility mapping

Immediately after the evaluation of intestinal transit by fluorescence imaging – which took only 10 s (two full-field images) – the spontaneous contractile activity (i.e. oscillatory contractions) in different regions of the GI tract (duodenum, jejunum, ileum, and colon) was recorded using the same 8-bit monochrome CCD camera, a Pinnacle Dazzle Platinum video acquisition device, a HP Pavillion zd8000 notebook, and a commercially available software package (Pinnacle Studio 10). Recordings were analysed according to a modified method described by Seerden *et al.*<sup>13</sup> Briefly, a 6-cm-long segment was recorded for 30 s and the video files were imported in ImageJ at 25 frames per second



**Figure 1** Experimental set up and schematic protocol. Ninety minutes (surgical procedure) or 30 min (pharmacological agents) after oral administration of fluorescein-labelled dextran (FD70), mice were killed by cervical dislocation and the GI tract was excised. Intestinal transit was evaluated by fluorescence imaging of FD70 along the GI tract, and high-resolution spatiotemporal motility mapping – based on real-time video recordings – was used to study intestinal contractility. The imaging system consisted of a UV-light source, an excitation filter (410–510 nm conversion screen), an 8-bit monochrome CCD camera equipped with a f/1.2 8–48 mm zoom lens, and an emission bandpass filter to detect fluorescence (550–600 nm emission).



**Figure 2** Intestinal transit study by fluorescence imaging. (A) Representative images showing the macroscopic appearance of the excised GI tract (normal illumination mode) and the distribution of FD70 along the GI tract (fluorescent mode) 90 min after oral administration of FD70. In control mice, FD70 is primarily distributed within the terminal ileum and caecum. Surgical manipulation of the intestine (intestinal manipulation, IM) caused significant delay in the transit of FD70. (B) Transit histograms for the distribution of FD70 along the GI tract (stom, stomach; sb, small bowel; caec, caecum; col, colon) 90 min postgavage. IM causes a significant delay in intestinal transit [geometric centre (GC) =  $3.7 \pm 0.2$  vs GC =  $8.8 \pm 0.4$  for control]. (C) GC as measured by fluorescence imaging (dark grey bars) or spectrofluorometer (light grey bars). GCs are given as mean values  $\pm$  SEM ( $n = 6-8$ ). \*Indicates  $P < 0.05$  vs control.

(40-ms interframe interval; 8-bit greyscale images). After the contrast threshold value was set, the images (i.e.  $30 \times 25$  images) were converted to black-and-white and the mean diameter ( $\bar{D}_{\text{mean}}$ ) of the intestinal segment under study was measured in the first frame #1. Next, all 750 frames were sequentially analysed using Amplitude Profiler software – written as ImageJ plugin. The change in intestinal diameter within this 30-s period for every pixel (768 pixels) along this 6-cm-long intestinal segment was calculated by the following equation: [(maximal diameter – minimal diameter)/maximal diameter]\*100 and expressed as % contraction amplitude. The 5th, 25th, 50th, 75th and 95th percentile was determined among these 768 amplitude values and the mean value of these 768 amplitude values was calculated ( $n = 1$ ; Fig. 3A); as the colon was sometimes <6 cm long, the same calculations were carried out for 600–768 amplitude values. Finally, the mean of these respective percentiles and mean values was calculated for each experimental group ( $n = 6-8$ ) and represented in a box-and-whisker plot (Figs 3B and 4B). The oscillatory changes in intestinal diameter were also represented in a three-dimensional (3-D) plot using Spatiotemporal Motility Mapping software – written as ImageJ plugin with a GnuPlot backend – allowing to see contractility in function of time (Fig. 3C). Sample recordings of these oscillatory contractions as well as the ImageJ plugins can be downloaded from <http://www.heyman.ugent.be/En/DownloadsEn.htm>.

### Organ bath muscle contractility

After determination of intestinal transit and contractility in the intact intestine, a mid-jejunal segment was isolated to study spontaneous mechanical activity of circular (mucosa-free) muscle strips in standard organ baths from, respectively, unoperated (control) and operated (IM) mice at 24 h postoperatively. Contractile activity was calculated as gram\*second per square millimetre ( $\text{g}\cdot\text{s}\cdot\text{mm}^{-2}$ ). Cross-section area ( $\text{mm}^2$ ) was determined as tissue wet weight (mg)/[tissue length at optimal load (mm)\*density ( $1.05\text{ mg}\cdot\text{mm}^{-3}$ )].<sup>3</sup>

### Measurement of inflammatory parameters

Tissue myeloperoxidase (MPO) activity – marker of leucocytic infiltration – was measured as described previously.<sup>5</sup> Malondialdehyde/4-hydroxy-2-nonenal (MDA/HNE) levels – markers of oxidative stress – were determined using the Lipid Peroxidation Assay kit (Oxford Biomedical, Oxford, MI, USA). Interleukin (IL)-1 $\beta$  and IL-6 levels were determined by ELISA (Invitrogen, Merelbeke, Belgium).

### Data analysis

Data are expressed as mean values  $\pm$  standard error of (SEM) ( $n = 6-8$ ). Statistical analysis was performed using an unpaired Student's *t*-test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Intestinal transit

In control mice, FD70 was primarily distributed within the terminal ileum and caecum when mice were killed 90 min after ingestion of FD70 (GC =  $8.8 \pm 0.4$ , as measured by fluorescence imaging). After IM, the fluorescent signal was confined to the more proximal segments of the small intestine (GC =  $3.7 \pm 0.2$ ), a finding that is consistent with the presence of postoperative ileus (Fig. 2A,B).

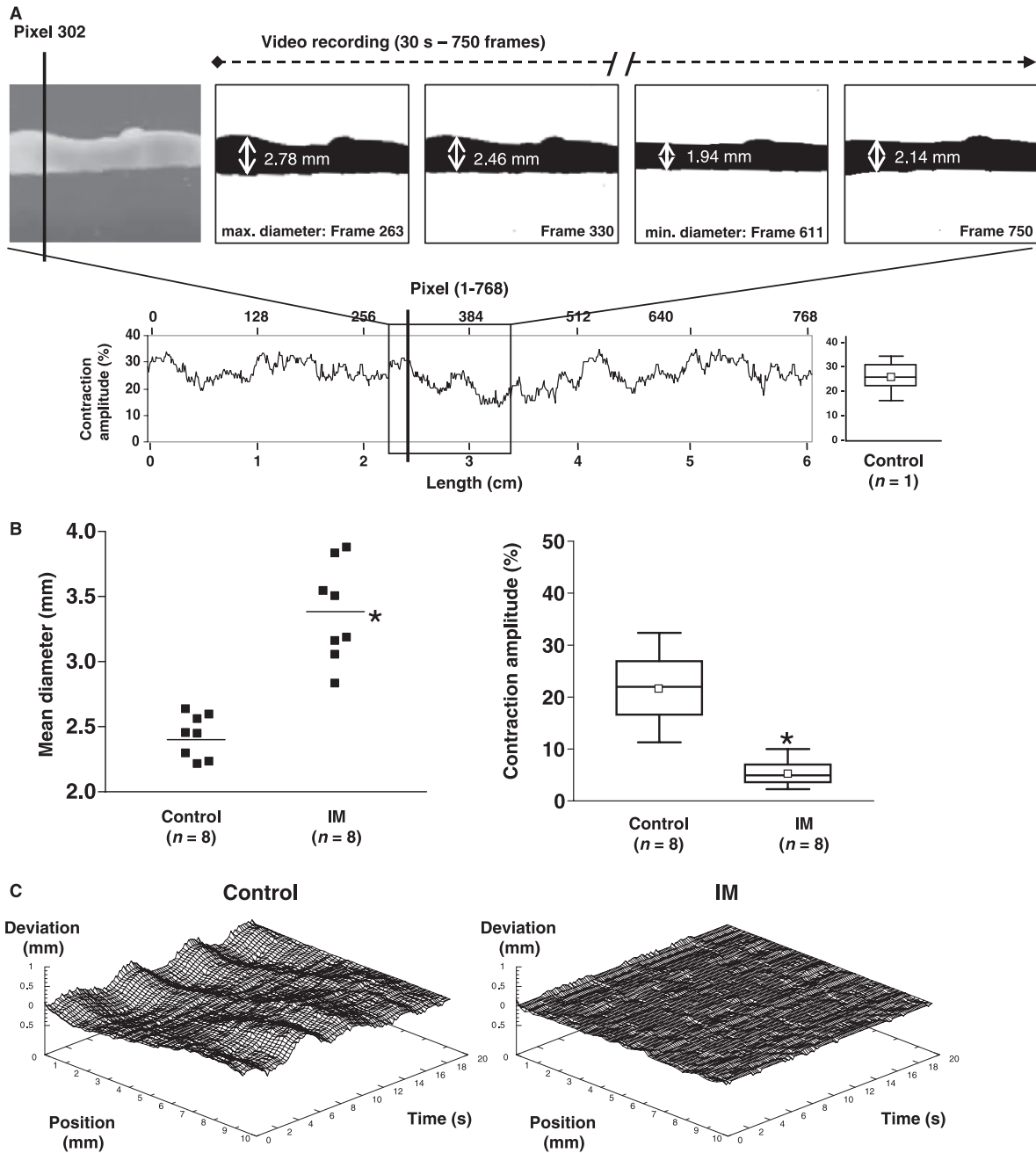
When evaluating the effect of pharmacological agents on intestinal transit, mice were killed 30 min postgavage, which resulted in a GC =  $4.5 \pm 0.3$  for control mice. Administration of atropine ( $1-3\text{ mg}\cdot\text{kg}^{-1}$ , i.p.) induced a dose-related delay in intestinal transit, while metoclopramide ( $3-10\text{ mg}\cdot\text{kg}^{-1}$ , i.p.) significantly accelerated intestinal transit in a dose-dependent manner (Fig. 4A).

Fluorescence imaging and spectrofluorometric determination of intestinal transit yielded comparable results (Figs 2C and 4A).

### Contractility imaging and spatiotemporal motility mapping

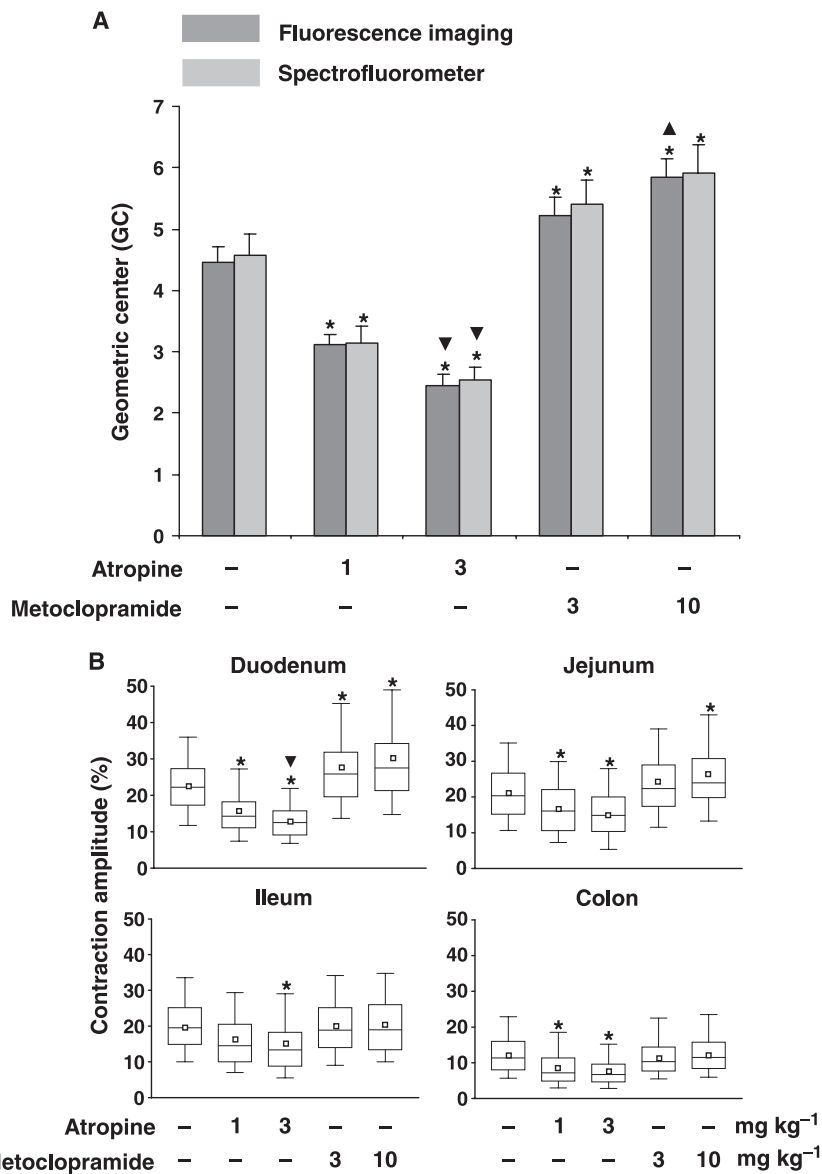
In control mice, the jejunum showed vigorous contractile activity, resulting in a mean contraction amplitude of 21.7% and a mean diameter ( $\bar{D}_{\text{mean}}$ ) of  $2.4 \pm 0.1$  mm. Surgical manipulation of the intestine nearly abolished these oscillatory contractions (mean contraction amplitude = 5.1%), leaving the small intestine distended ( $\bar{D}_{\text{mean}} = 3.4 \pm 0.2$  mm; Fig. 3B,C). In addition, contractile activity of jejunal smooth muscle strips was investigated in standard organ baths. Circular muscle strips from control mice generated spontaneous phasic activity with a mean contractile force of  $57.4 \pm 10.2\text{ g}\cdot\text{s}\cdot\text{mm}^{-2}$ , while IM significantly suppressed contractile activity to a mean contractile force of  $16.7 \pm 2.6\text{ g}\cdot\text{s}\cdot\text{mm}^{-2}$  ( $P < 0.05$ ).

To evaluate the effect of pharmacological agents on intestinal contractility, the contractile activity in different regions along the GI tract (duodenum, jeju-



**Figure 3** Intestinal contractility study by spatiotemporal motility mapping. (A) Schematic diagram showing an example of the oscillatory contractions of the small intestine as recorded in a control animal. The change in intestinal diameter (% contraction amplitude) within a 30-s period was calculated for every pixel along a 6-cm-long intestinal segment (768 pixels). Among these values, the 5th, 25th, 50th, 75th and 95th percentile was determined for the intestinal segment under study ( $n = 1$ ). (B) The left panel shows a scatter plot representing the mean diameter (mm) of the mid-jejunal segment; the solid line represents the mean of all measured values within each experimental group ( $n = 8$ ). The intestine was distended 24 h after intestinal manipulation (IM) compared to control mice. The right panel shows a box-and-whisker plot representing the contraction amplitude (%) of the spontaneous oscillatory contractions in the same mid-jejunal segments; upper and lower ends of boxes represent 75th and 25th percentiles; whiskers represent 95th and 5th percentiles; the median is a solid line within the box; the mean value is represented by the  $\square$  symbol ( $n = 8$ ). The contraction amplitude was markedly suppressed after IM. \*Indicates  $P < 0.05$  vs control (mean values). (C) Representative contractility traces showing the spontaneous oscillatory contractions in a 10-mm mid-jejunal segment (X-axis) as deviations in mm (Y-axis) for a period of 20 s (Z-axis); the intestinal diameter measured at  $t = 20$  s was used as reference value. Oscillatory contractions were vigorous in control mice, but almost completely abolished after IM.

**Figure 4** Effect of atropine and metoclopramide on intestinal transit and contractility. (A) Geometric centre (GC) for the distribution of FD70 along the GI tract 30 min after oral administration of FD70. Atropine 1–3 mg kg<sup>-1</sup> induced a dose-related delay in intestinal transit, while metoclopramide 3–10 mg kg<sup>-1</sup> significantly accelerated intestinal transit in a dose-dependent manner. No difference was observed between fluorescence imaging and spectrofluorometric determination of intestinal transit. GCs are given as mean values ± SEM (*n* = 6–8). \*Indicates *P* < 0.05 vs control; ▼ indicates *P* < 0.05: atropine 3 mg kg<sup>-1</sup> vs atropine 1 mg kg<sup>-1</sup>; ▲ indicates *P* < 0.05: metoclopramide 10 mg kg<sup>-1</sup> vs metoclopramide 3 mg kg<sup>-1</sup>. (B) Box-and-whisker plots representing the contraction amplitude (%) of the spontaneous oscillatory contractions in four different regions of the GI tract (duodenum, jejunum, ileum and colon). Atropine (1–3 mg kg<sup>-1</sup>, i.p.) suppressed spontaneous contractile activity along the entire GI tract, while metoclopramide (3–10 mg kg<sup>-1</sup>, i.p.) increased contractile activity only in the upper GI tract. Upper and lower ends of boxes represent 75th and 25th percentiles; whiskers represent 95th and 5th percentiles; the median is a solid line within the box; the mean value is represented by the □ symbol (*n* = 6–8). \*Indicates *P* < 0.05 vs control; ▼ indicates *P* < 0.05: atropine 3 mg kg<sup>-1</sup> vs atropine 1 mg kg<sup>-1</sup> (mean values).



num, ileum, and colon) was recorded using video-imaging. Fig. 4B shows that atropine (1–3 mg kg<sup>-1</sup>, i.p.) suppressed spontaneous contractile activity along the entire GI tract, while metoclopramide (3–10 mg kg<sup>-1</sup>, i.p.) increased contractile activity only in the upper GI tract.

**Inflammatory parameters**

All measured inflammatory parameters were significantly increased after IM: MPO activity, MDA/HNE, IL-1β and IL-6 levels in intestinal tissue of IM mice were, respectively, 3.6-, 3.1-, 2.8- and 13.3-fold higher compared to control mice (Table 1).

**Table 1** Inflammatory parameters in intestinal tissue of control and surgically manipulated (IM) mice

	MPO <sup>†</sup>	MDA/HNE <sup>‡</sup>	IL-1β <sup>§</sup>	IL-6 <sup>§</sup>
Control	39.2 ± 6.5	13.5 ± 2.4	231.9 ± 28.0	8.3 ± 0.9
IM	141.8 ± 10.8*	41.4 ± 3.9*	641.7 ± 53.9*	110.6 ± 20.3*

IM, intestinal manipulation. <sup>†</sup>Myeloperoxidase (MPO) activity was measured as an index of leucocytic infiltration, and expressed as units per g tissue. <sup>‡</sup>Malondialdehyde (MDA) and 4-hydroxy-2-non-enal (HNE) levels were measured as markers of oxidative stress, and expressed as nmol per mg protein. <sup>§</sup>Protein expression levels of interleukin (IL)-1β and IL-6 were measured by ELISA, and expressed as pg per 100 mg tissue. \*Indicates *P* < 0.05 for IM vs control.

## DISCUSSION

In the present study, we introduce a novel method to measure both intestinal transit and contractility quasi-simultaneously in the murine intestinal tract. Evaluation of intestinal transit by fluorescence imaging avoids the division of the GI tract in separate segments, reducing manipulation and processing time. Intestinal contractility can be measured in different regions of the intact intestine without the necessity of preparing isolated smooth muscle strips.

### Intestinal transit

Intestinal transit was evaluated by fluorescence imaging of FD70 in the intact intestine (i.e. within the intestinal lumen). As this method only required two full-field images – one taken in normal illumination mode and one in fluorescent mode – the processing time for intestinal transit studies was dramatically reduced compared to the traditional methods of measuring intestinal transit in rodents. Thereby, the intestine is typically divided into a specific number of equal segments and the colorimetric (e.g. phenol red, Evans blue), fluorescent (e.g. FD70, rhodamine-dextran) or radioactive (e.g.  $^{99m}\text{Tc}$ ,  $^{51}\text{Cr}$ ) signal in each sample is subsequently determined by using a spectrophoto(fluoro)meter or gamma counter.<sup>1–6</sup> Recently, a novel method monitoring the progression of a small magnetic pill through the entire GI tract (Magnet Tracking) has been proposed for *in vivo* studies of GI motility in the rat.<sup>14</sup> An alternative method to evaluate *in vivo* intestinal transit is the determination of the whole gut transit time using radiopaque markers or carmine red.<sup>15,16</sup>

In the present study, surgical manipulation of the intestine significantly delayed intestinal transit – as measured 24 h postoperatively – and, thus, resulted in a significantly reduced GC value. Administration of atropine (1–3 mg kg<sup>-1</sup>, i.p.) induced a dose-related delay in intestinal transit, while metoclopramide (3–10 mg kg<sup>-1</sup>, i.p.) markedly accelerated intestinal transit in a dose-dependent manner. The results obtained by fluorescence imaging correlated with those obtained by spectrofluorometry (i.e. traditional method – GI tract divided into 14 equal segments) and are consistent with data in literature.<sup>3,5,10,11</sup>

### Intestinal contractility

The most common way to evaluate intestinal contractility in laboratory animals is the analysis of *in vitro* smooth muscle mechanical activity in

standard organ baths.<sup>3,5,8</sup> In recent years, high-resolution spatiotemporal motility mapping has been introduced in GI research.<sup>13,17–19</sup> In this study, we used high-resolution spatiotemporal mapping – based on real-time video recordings – to study spontaneous contractile activity in the murine intestinal tract.

In control mice, the jejunum showed pronounced contractile activity; however, surgical manipulation of the intestine nearly abolished these spontaneous oscillatory contractions; a finding that was confirmed by our contractility studies in standard organ baths. We also demonstrated that the intestine of operated mice was significantly dilated, an observation that has been previously reported in rat small intestine<sup>20</sup> and is commonly used as a diagnostic feature of postoperative ileus in larger animals and humans.<sup>21,22</sup> Moreover, we showed that atropine (1–3 mg kg<sup>-1</sup>, i.p.) suppressed spontaneous contractile activity along the entire GI tract, while metoclopramide (3–10 mg kg<sup>-1</sup>, i.p.) increased contractile activity only in the upper GI tract. These findings are supported by previous studies, reporting the inhibitory effect of atropine on intestinal contractility along the entire GI tract,<sup>10,23,24</sup> whereas metoclopramide has been shown to enhance only upper GI tract motor activity in rats and dogs.<sup>25,26</sup>

### Inflammatory parameters

As our method to evaluate intestinal transit and contractility could record both parameters within a very short time – and, hence, required minimal tissue manipulation – intestinal tissue samples were processed for the determination of leucocytic infiltration (MPO), oxidative stress (MDA/HNE), and protein expression levels of IL-1 $\beta$  and IL-6. In accordance with literature, all of these parameters were markedly increased in tissue samples of the IM group.<sup>3,5,27–30</sup>

## CONCLUSIONS

In the present study, we introduce a novel method to evaluate murine intestinal transit and contractility in a fast, accurate, and easy-to-implement manner. As this method – based on fluorescence imaging and spatiotemporal motility mapping – allows measuring both parameters very rapidly and without major manipulation of the gut, the intestine can still be used to determine other *in vitro* parameters. Therefore, this method may facilitate the study of GI motility under both normal and disease conditions.

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