

# Imaging gastric emptying with IVISense Gastrointestinal 750.

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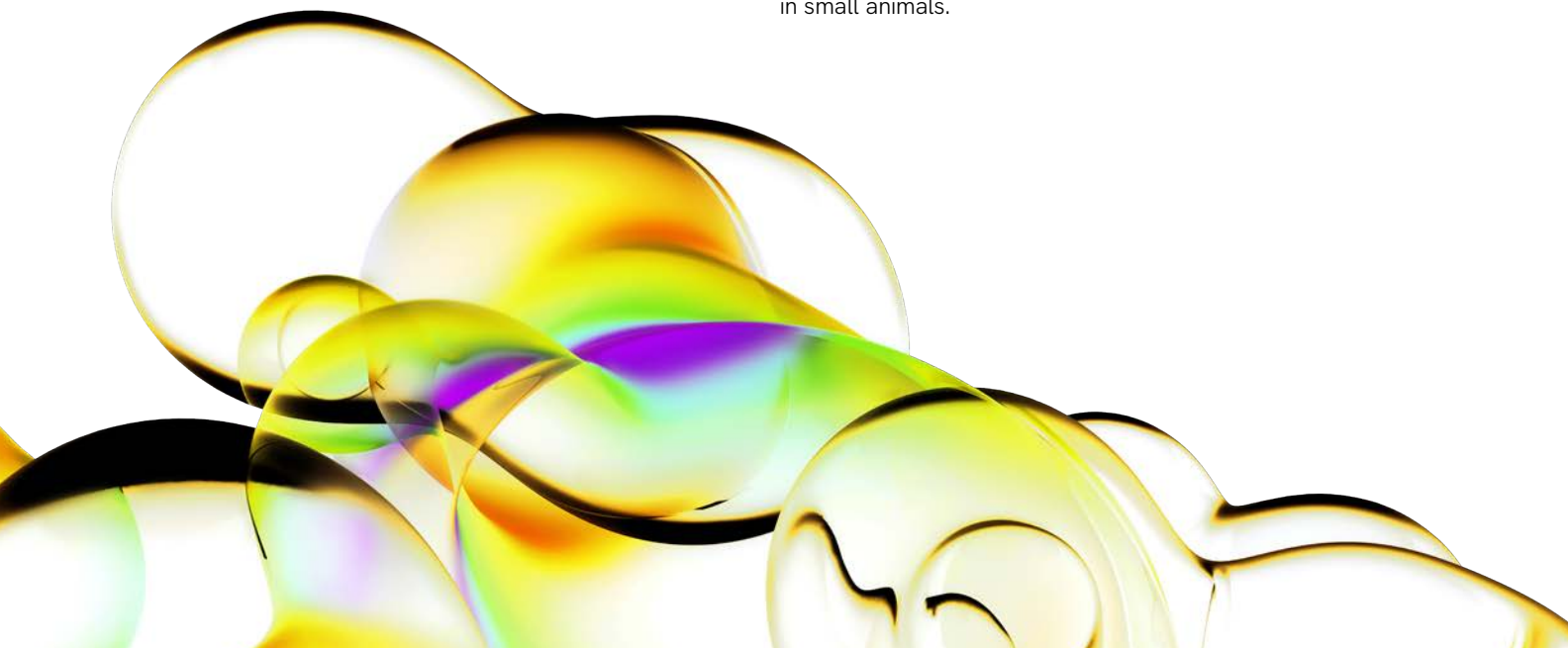
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## Abstract

The accurate quantitation of gastric emptying is crucial for understanding the physiological, pathological and pharmacological mechanisms underlying gastric motility both clinically and in animal studies. Current methods used for determining gastric emptying rates in small animals are either terminal or involve radioactive tracers, incurring high financial and time cost. We have developed a novel, near-infrared (NIR) fluorescent imaging agent, IVISense™ Gastrointestinal 750, to monitor and quantitate gastric emptying rates in murine models *in vivo*, non-invasively, and in real time. The imaging agent is comprised of an acid-stable NIR fluorophore conjugated to a non-absorbable pharmacokinetic modifier designed to be formulated in liquid or incorporated into a solid meal. Using female 8 week old BALB/c mice recipients, 0.25 nmol IVISense Gastrointestinal 750 was orally gavaged in liquid formulation (PBS) or fed in egg yolk, an experimental approach designed to detect changes in gastric emptying. *In vivo* 3D fluorescent imaging and quantitation using fluorescence molecular tomography system at multiple time points revealed a gastric half-life ( $t_{1/2}$ ) of  $36 \pm 16$  minutes for the liquid formulation and  $63 \pm 18$  minutes for the egg formulation. Clonidine (an  $\alpha_2$  adrenergic agonist, 1 mg/kg) further inhibited gastric emptying time for the egg formulation, halting the process completely between 2 and 4 h and ultimately delaying the completion of gastric emptying to greater than 6-7 h. IVISense Gastrointestinal 750 signal can also be quantitated as it transits into the intestines, ultimately decreasing in intensity and volume as it is excreted. These findings reveal that IVISense Gastrointestinal 750 and 3D fluorescence imaging *in vivo* can be used successfully to quantitate gastrointestinal transit of both liquid and solid diets in small animals.



## Materials and methods

### Fluorescent agents for the quantitation of gastric emptying

IVISense Gastrointestinal 750, non-targeted physiologic agent, was used to image the rate of gastric emptying. The imaging doses were 0.25-1.0 nmoles in 150 mL saline administered via oral gavage in all imaging studies.

### Gastric emptying model

Eight week-old female BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA) and maintained in a pathogen-free animal facility with water and low-fluorescence mouse chow (Harlan Tekland, Madison, WI). Handling of mice and experimental procedures were in accordance with Revvity IACUC guidelines and approved veterinarian requirements for animal care and use. Mice were depilated (Nair® depilatory cream, Church & Dwight Co., Inc., Princeton, NJ) 24 h prior to initiation of studies. BALB/c mice were gavaged with agent (0.25 nmole) in PBS, final volume of 200  $\mu$ L. The effect of solid food on gastric emptying rates was assessed by feeding some mice cooked egg yolk into which 25  $\mu$ L of IVISense Gastrointestinal 750 (0.25 nmole) was pipetted.

### *In vivo* fluorescence tomographic imaging and analysis

Mice were anesthetized by isoflurane inhalation. Depilatory lotion was applied thickly on skin over the lower torso (front, back, and sides) of each mouse, rinsed off with warm water, and re-applied until all fur had been removed. Mice were then imaged using fluorescence molecular tomography which collected both 2D surface images as well as 3D tomographic imaging datasets.

### Fluorescence tomography reconstruction and analysis

The collected fluorescence data was reconstructed for the quantitation of three-dimensional fluorescence signal within the stomach and intestines. Three-dimensional regions of interest (ROI) were drawn encompassing the relevant biology. A variety of background thresholding levels were applied systematically, and results were compared to other independent readouts for validation.

## Results

### IVISense Gastrointestinal validation

IVISense Gastrointestinal 750 NIR fluorescent imaging agent designed to monitor and quantitate gastric and intestinal emptying non-invasively in murine models *in vivo* as represented in Figure 1. The fluorophore of this agent is acid-stable and conjugated to a non-absorbable pharmacokinetic modifier designed to be formulated in liquid or incorporated into a solid meal.

As a validation of IVISense Gastrointestinal transit through the gastro-intestinal system, the agent was orally gavaged in multiple mice, and tissue was excised for *ex vivo* fluorescence imaging. Figure 2 shows the kinetics of localization of the PBS-formulated agent in different gastrointestinal regions over 4 h. *Ex vivo* imaging (Figure 1C) shows that IVISense Gastrointestinal empties from the stomach over 1 h, rapidly moves through the duodenum and jejunum, and more slowly transits through the ileum, cecum, and colon.

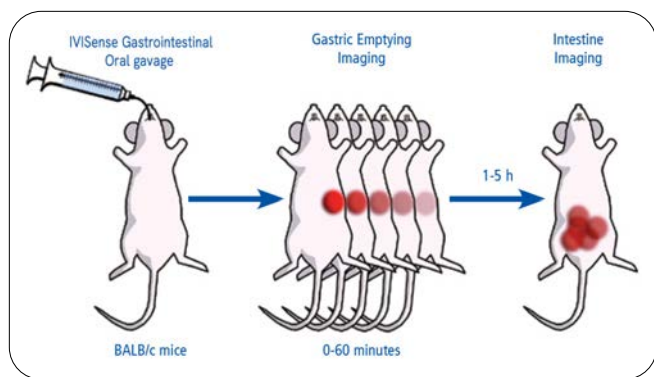


Figure 1: Gastric emptying and intestinal imaging.

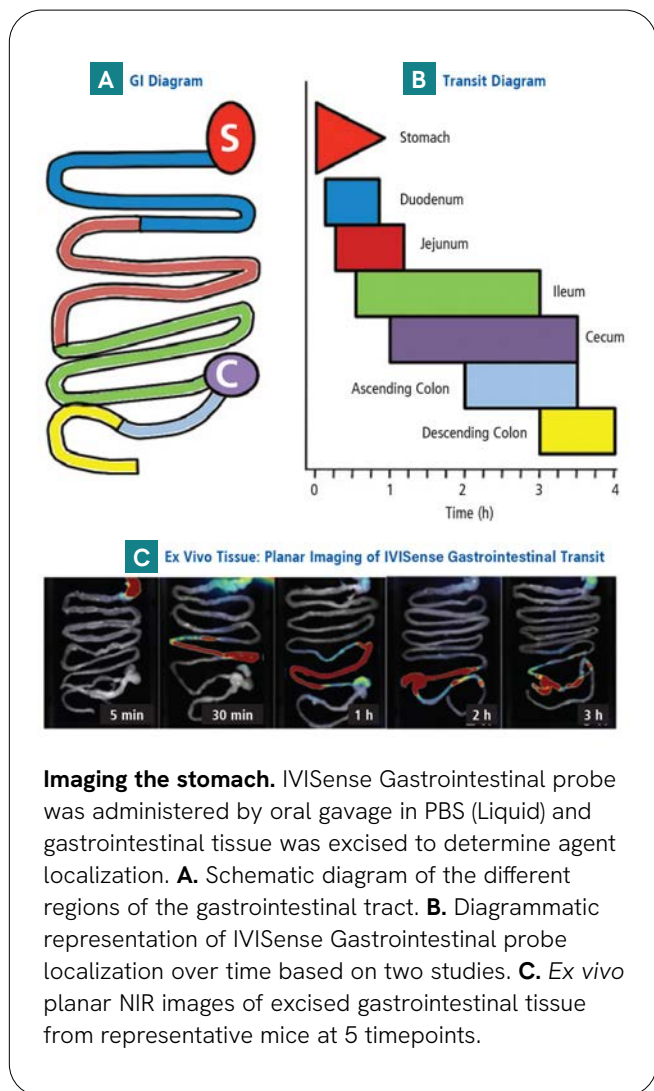


Figure 2: Gastrointestinal transit kinetics.

### In vivo imaging of gastric emptying

Gastric emptying can be assessed non-invasively by imaging the stomach region over time, from immediately after oral gavage of IVISense Gastrointestinal through 1.5 h. Figure 3 (left panels) shows 3D fluorescence tomography images of representative mice to illustrate the difference in gastric emptying rates (GER) of fluorescence depending on whether the agent is administered with solid food (egg yolk) ( $GER = 47 \pm 11\%/h$ ) or as a liquid formulation ( $GER = 167 \pm 28\%/h$ ). Allowing access to solid chow after liquid formulation gavage has an appreciable effect on the emptying rate ( $GER = 78 \pm 9\%/h$ ). It is interesting to note that planar imaging (Figure 3, right panels), which only qualitatively detects fluorescence at the surface of the animal, was relatively ineffective in monitoring gastric emptying. Because of the depth of the stomach, there was a dramatic loss of sensitivity of detection with only 50-70% decrease in stomach fluorescence.

Fluorescence molecular tomography quantitation of the kinetics of change in these small groups of mice (Figure 4A) show the low variability and altered kinetics with the different IVISense Gastrointestinal imaging approaches. Comparable delays were achieved with the addition of solid food substances either admixed in egg or by allowing access to mouse chow, and this was a measurable and statistically-significant change (Figure 4B).

It is important to note, however, that the gastrointestinal tract contains significant pockets and regions of air throughout the lumen and that this air can cause an artifactual enhancement of fluorescent signal during *in vivo* imaging. Furthermore, the formulation of IVISense Gastrointestinal agent and/or the volume of agent administered can lead to introduction of additional air artifacts. Indeed, the admixture of IVISense Gastrointestinal agent in egg yolk appeared to increase the amount of gastrointestinal air. Similarly, gavaging of small volumes of the agent (*i.e.* 100  $\mu$ L or less) permits significant retention of air in the stomach, enhancing the apparent stomach fluorescence. However, the pattern of changes of fluorescence will not be altered, and normalization to starting stomach signal allows comparison of pattern changes between different formulations or changes that occur with drug treatment.

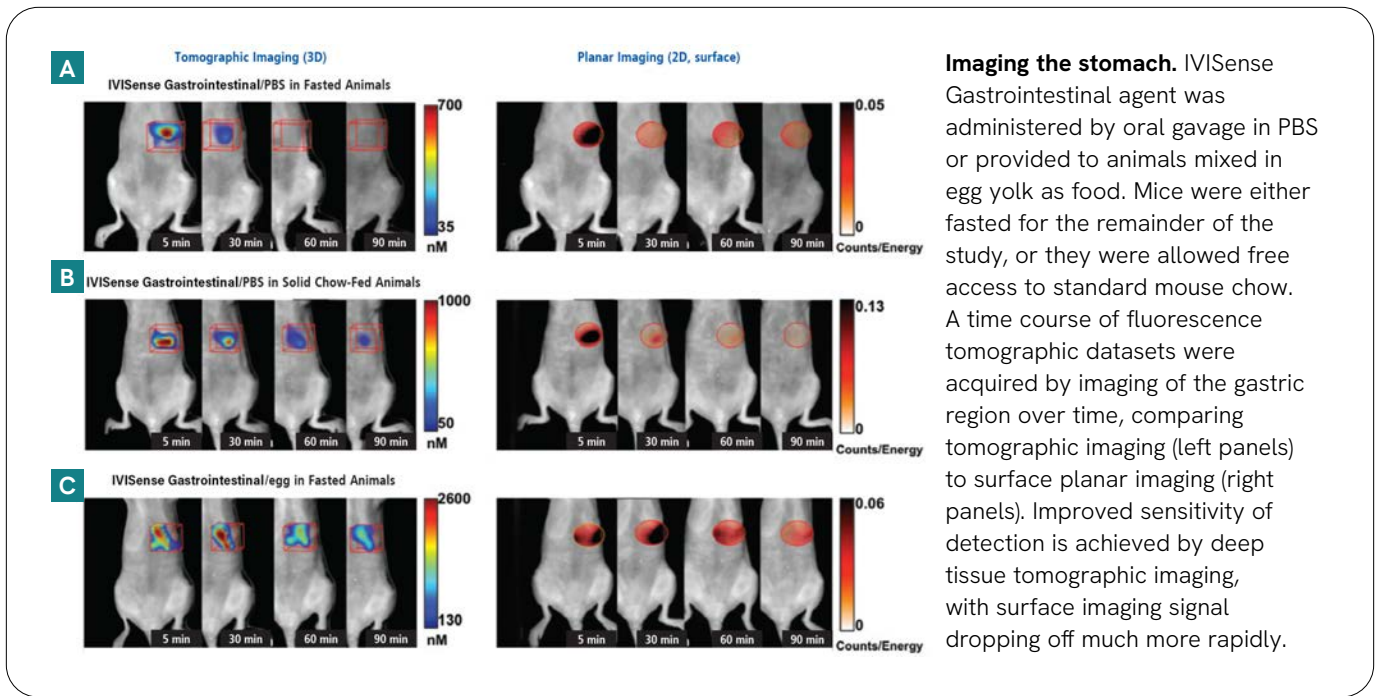


Figure 3: Imaging gastric emptying.

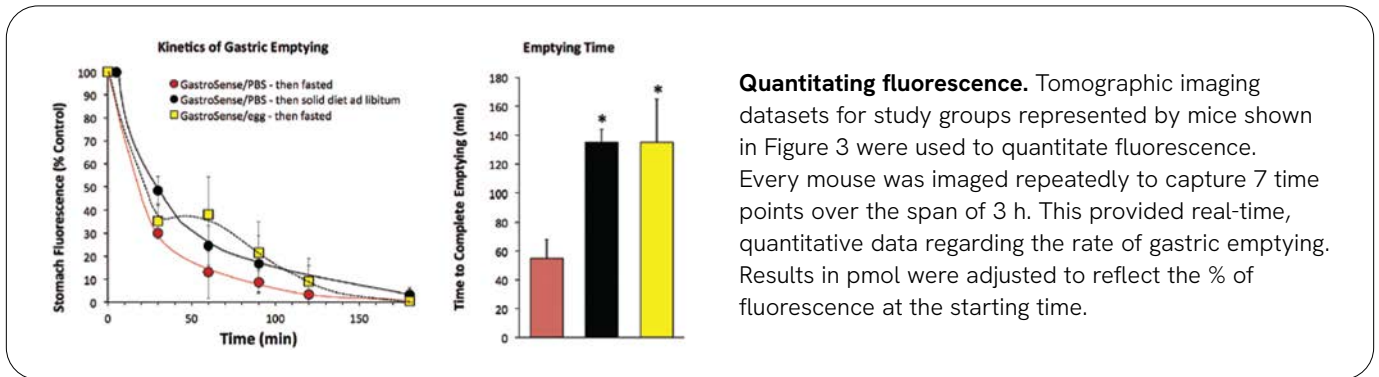


Figure 4: Fluorescence molecular tomography quantitation of gastric emptying.

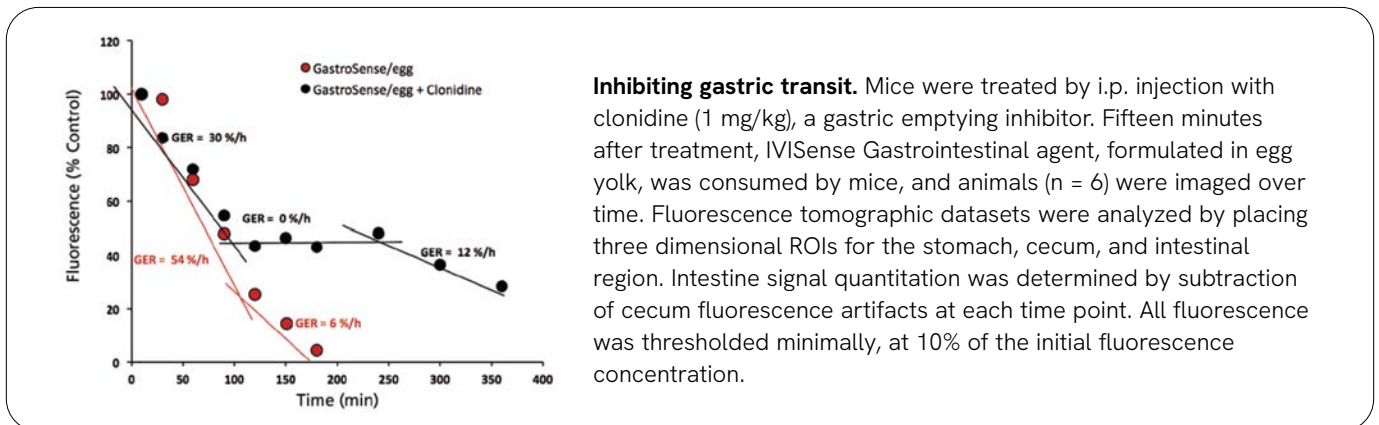


Figure 5: Inhibiting gastric motility with clonidine.

To assess the ability of IVISense Gastrointestinal agent to detect drug-induced alterations in gastric motility, we used clonidine, an  $\alpha_2$  adrenergic agonist that affects gastric fundus contractility and delays stomach emptying. Mice were treated with clonidine 15 minutes prior to feeding with a IVISense Gastrointestinal/egg formulation (Figure 5). Mice were imaged repeatedly over a 3 h period to quantitate stomach fluorescence. A subtle decrease in the rate of gastric emptying was evident over the first 90 minutes, after which gastric emptying nearly halted for 2 h. Gastric clearance increased as drug effects appeared to decline 4 h post dosing.

### In vivo imaging of gastrointestinal transit

IVISense Gastrointestinal agent, upon emptying from the stomach, enters the intestines and provides a tool for quantitating intestinal transit. Figure 6 shows the quantitative results of non-invasive tracking of stomach emptying in the context of intestinal transit kinetics. Measurement of signal in the cecum, as compared to the intestines (minus the cecum), show that the cecum contributes approximately 50% of the signal generated in the entire intestine. Interestingly, the volume of fluorescence

in the cecum is substantially lower, suggesting that the signal is approximately 3-4X brighter than signal in the rest of the intestines. Based on *ex vivo* tissue studies showing an even transit through the tissue, we believe that the cecum signal is overestimated by 3-4X, presumably due to air or gas within the cecum.

Gastrointestinal transit can also be assessed using the IVISense Gastrointestinal/egg yolk formulation (Figure 7). As shown in Figure 4A, stomach emptying is delayed as compared to formulation of the agent in PBS. It is interesting to note that the total intestine and cecum signal is approximately 4-fold reduced (compare Figure 7A to Figure 6A), although it is unclear whether the longer residence time in the stomach increases degradation of this agent. However, clearly the intestinal transit rate (ITR) after 2 h is still approximately 13-fold slower for egg-formulate IVISense Gastrointestinal (ITR = 4.2%/h) than that of IVISense Gastrointestinal/ PBS (ITR = 54%/h). Even representing rates as a percentage of the signal seen at 120 minutes, there is still a 3-fold difference in ITR (24%/h for egg yolk formulation vs. 72%/h for the PBS formulation).

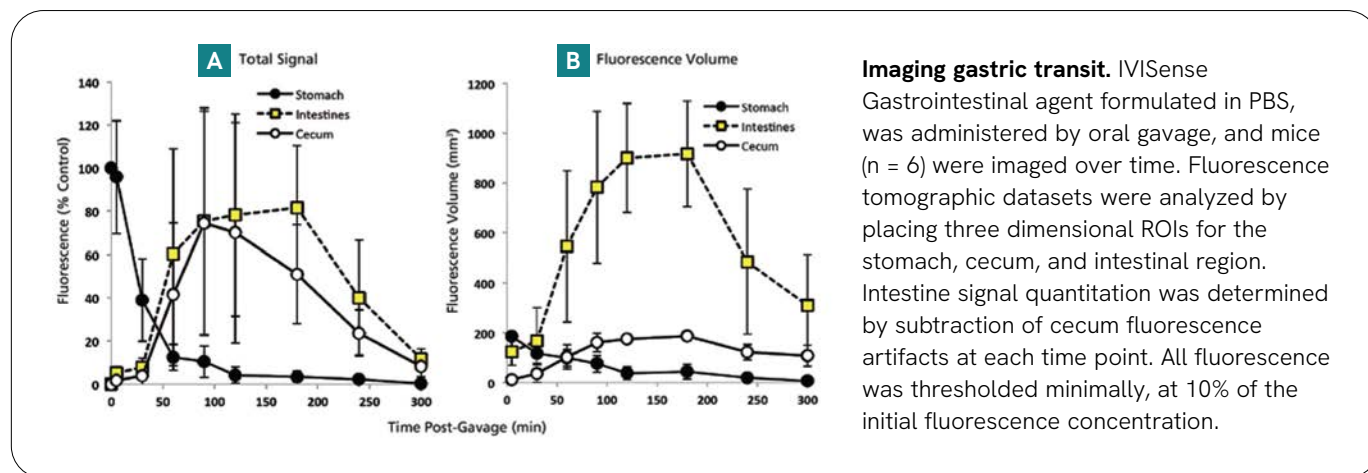
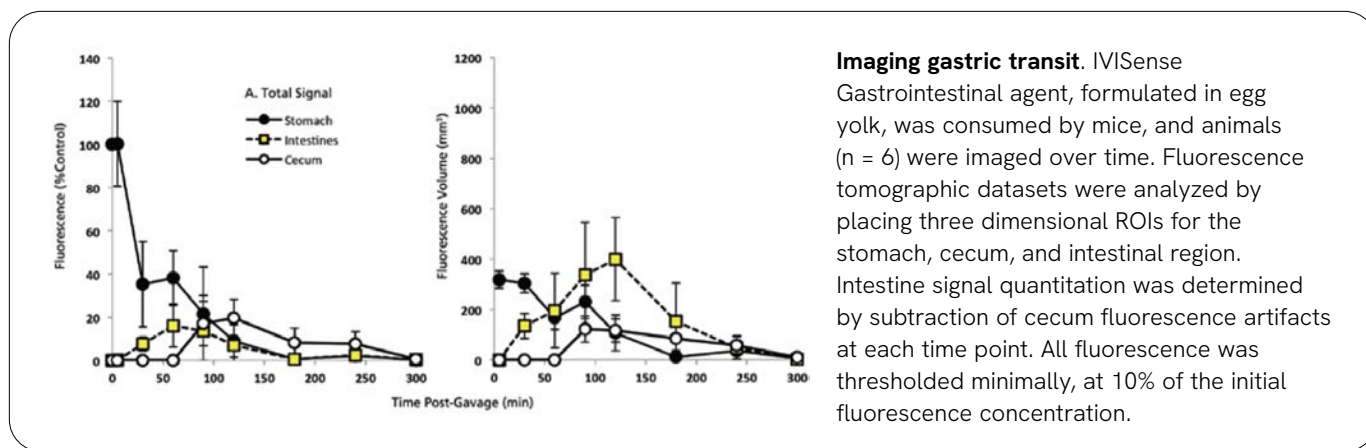


Figure 6: Gastric transit (IVISense Gastrointestinal/PBS).

**Imaging gastric transit.** IVISense Gastrointestinal agent formulated in PBS, was administered by oral gavage, and mice (n = 6) were imaged over time. Fluorescence tomographic datasets were analyzed by placing three dimensional ROIs for the stomach, cecum, and intestinal region. Intestine signal quantitation was determined by subtraction of cecum fluorescence artifacts at each time point. All fluorescence was thresholded minimally, at 10% of the initial fluorescence concentration.



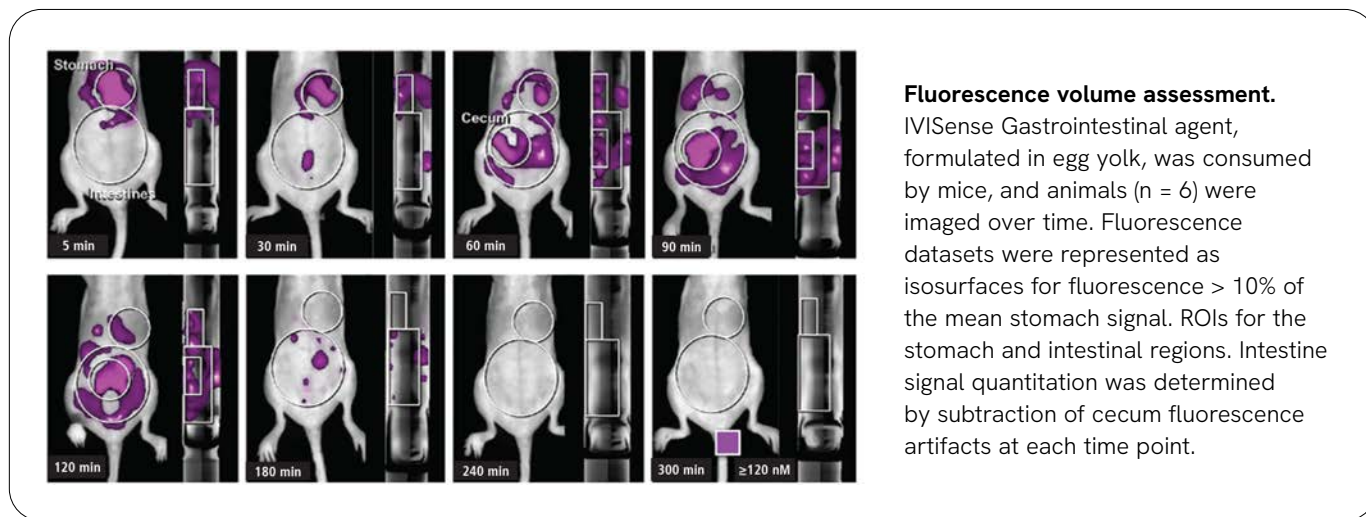


**Imaging gastric transit.** IVISense Gastrointestinal agent, formulated in egg yolk, was consumed by mice, and animals (n = 6) were imaged over time. Fluorescence tomographic datasets were analyzed by placing three dimensional ROIs for the stomach, cecum, and intestinal region. Intestine signal quantitation was determined by subtraction of cecum fluorescence artifacts at each time point. All fluorescence was thresholded minimally, at 10% of the initial fluorescence concentration.

Figure 7: Gastric transit (IVISense Gastrointestinal/egg yolk).

Fluorescence volume can be assessed quantitatively, but also represented as fluorescence isosurfaces (i.e. 3-dimensional images of all fluorescence greater than a given cut-off threshold) as shown in Figure 8. Although the physics of light propagation through tissue place limitations on the generation of images with high anatomical accuracy (particularly in large regions of continuous, overlapping signal), you can see clear regional differences in fluorescence across the intestines. We are currently validating an approach for using such data as a

tool for intestinal co-registration with other agents that detect inflammatory pathology within the intestines, as this may be a useful tool for improving the sensitivity of disease detection by limiting the analysis to GI tissue. Based on very preliminary studies, intra-rectal administration may be an alternative for the specific localization of the descending colon (which shows poor retention of IVISense Gastrointestinal probe signal when given orally).



**Fluorescence volume assessment.** IVISense Gastrointestinal agent, formulated in egg yolk, was consumed by mice, and animals (n = 6) were imaged over time. Fluorescence datasets were represented as isosurfaces for fluorescence > 10% of the mean stomach signal. ROIs for the stomach and intestinal regions. Intestine signal quantitation was determined by subtraction of cecum fluorescence artifacts at each time point.

Figure 8: Fluorescence volume images (IVISense Gastrointestinal/egg yolk).

Table 1: Summary guidelines for IVISense Gastrointestinal application.

	Imaging time
<b>Oral agent (PBS formulation)</b>	
Gastric emptying	0-2 h
Intestinal transit	1-4 h
Effect of drug treatment	0-5 h
Intestinal contrast agent	~2 h
Inflammatory bowel disease	0-5 h decreased signal, faster transit
<b>Oral agent (egg yolk formulation)</b>	
Gastric emptying	0-2 h
Intestinal transit	1-4 h
Effect of drug treatment on food transit	0-5 h
<b>Intrarectal agent (PBS formulation)</b>	
Descending colon contrast agent, combined with disease-imaging agents	Immediate Imaging

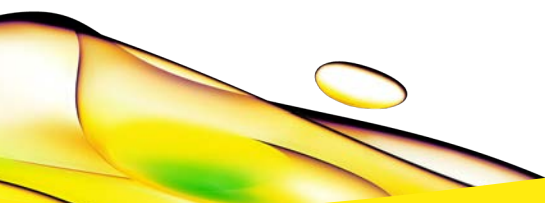
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