Published in final edited form as:

Neurogastroenterol Motil. 2012 May ; 24(5): 401–404. doi:10.1111/j.1365-2982.2012.01903.x.

Red chlorophyll: the new barium?

G.W. Hennig

Department of Physiology and Cell Biology, University of Nevada, Reno, NV, USA

Abstract

Imaging of gastrointestinal (GI) motility remotely through the abdominal wall has always been a tradeoff between resolution and invasiveness. Skin reflects and/or absorbs wavelengths of radiation in the ultraviolet and visible ranges but is largely transparent to both high energy radiation (Gamma to X rays; <0.1–10nm) and low energy radiation (infrared to radio waves; 700nm – 10m). Imaging using short wavelength radiation such as X-ray cinematography has excellent spatial and temporal resolution but ionization can produce acute and long term deleterious effects to the patient or animal. Other “slice-based” imaging techniques such as ultrasound/MRI/CT minimize tissue damage, but are also limited in the planar area that can be imaged in a timely fashion. This Viewpoint article will summarize and explore the implications of recent advances in infrared imaging of the GI tract, in particular, an article published in this issue of NGM entitled “In vivo dynamic imaging of intestinal motions using diet-related autofluorescence” in which the authors have used infrared imaging in combination with that most elusive ingredient, standard mouse chow, to capture the motions of the mouse GI tract.

Introduction

Remote imaging is one of the oldest techniques used to record GI motility and was pioneered by Walter Cannon¹ at the turn of the 20th century. Many of Cannon’s investigations of the movement of the GI tract were published before Bayliss and Starling’s² seminal paper on peristalsis was completed and precedes electrical recordings of the GI tract by Alvarez³ by at least two decades. Cannon’s rapid adoption Edison’s fluoroscope revealed not only the coordination of motility in large regions of the gut, but also complicated patterns of contractions such as segmenting activity that could not be appreciated by sampling mechanical or electrical activity at only a few points. The combination of X-rays with cinematography (see⁴) and later video⁵ allowed thorough documentation GI motility and disorders on which the field is based. Since then, X-ray cinematography has limited use in remote GI imaging due to the long term effects of radiation and acute effects on GI motility⁶. Still, the resolution and quality of images captured using fluoroscopic means^{7, 8} remains a standard by which other forms of remote imaging are compared to.

Lowering the dose of high-energy radiation to levels that can still be detected through the abdominal wall has been partially achieved using scintigraphy⁹. Gamma rays from the

radioactive decay of Tc-99m mixed into a meal pass through the abdomen and can be detected using a gamma camera. However, the long collection times (current best of 1 frames every 10 s; see¹⁰) make this technique suitable only for detecting the transit of relatively large volumes, such as during gastric emptying, but the dynamic movements of the gut responsible for transit cannot be resolved.

Penetration of the abdominal wall with low energy ultrasound waves has been employed successfully to image the GI tract¹¹. While ultrasound has excellent temporal resolution, the ultrasound beam is narrow and is normally oriented perpendicular to the abdominal surface so that only a slice of the GI tract can be visualized. This allows for cross and longitudinal sections to be imaged¹², but it is difficult to generate planar images of the gut. 3D ultrasound has been useful to investigate structural changes in the gut¹³, but the acquisition rate is too slow to measure dynamic motions. 4D ultrasound has the potential to be an excellent tool in imaging the GI tract, however the slow frame rates (> 1 s) and limited spatial resolution limit its adoption. Radial ultrasound, while useful to examine the upper and lower extremities of the GI tract in larger animals, is more invasive and is likely to induce mucosal/stretch changes in motility¹⁴. The use of Doppler ultrasonography to examine flow of intraluminal contents has not been successful in the gut as the movement of food particles is too slow.

Similarly, MRI/CT and equivalent “sliced based” imaging systems hold great potential for imaging of GI motility¹⁵ however the speed at which a volume (series of 2D slices) can be acquired is an order of magnitude too slow to properly resolve dynamic wall movements along a region of gut with fidelity.

The issue of visualizing the GI tract through the intact abdominal wall can be bypassed to some extent by externalizing a small region of gut outside of the abdomen in an anesthetized animal. This allows high resolution cameras to be used, and gives the researcher a great deal of flexibility in terms of manipulation of the organ, application of drugs and other dyes/stains that can be used to reveal the structure and activity of specific cell types (e.g. Ca²⁺-sensitive dyes; see¹⁶). However, the sympathetic response and ensuing inflammatory reaction to surgery and handling of the gut are likely to significantly alter “normal” motility patterns.

Infrared imaging

The advent of sensitive imaging cameras (iCCD, emCCD) for applications such as Ca²⁺ imaging in the last two decades has made it feasible to collect and record extremely faint signals at high spatial and temporal resolutions (e.g. 512×512 @ 30 fps, see¹⁷). The cooled chips on these cameras typically have the highest efficiency toward the infrared range and are capable of detecting single photons. One of the first uses of infrared imaging used ligands that fluoresced in response to excitation by far-red light¹⁸. A bevy of near-infrared fluorescent ligands and tailored proteins now exist¹⁹ that can be visualized through the abdomen of the mouse (600–650nm excitation, emission 700–900nm). Far-red light permits deeper penetration into tissue; a property leveraged by 2-photon confocal systems.

A number of innovative and elegant techniques have emerged in the last few years that utilize infrared to image GI movements. One such technique used bacteria that expressed red-emitting luciferase as a bioluminescent contrast agent²⁰. The advantage of this technique is that it required no specialized excitation lighting, or emission filtering, thereby increasing the number of photons that could be captured. The authors were able visualize the stomach and upper GI tract through the abdominal wall of anesthetized mice quite easily even though ~80% of the light was absorbed or scattered. Calculation of gastric volume was performed every 3 mins, which, despite the slow frame rates, compares well to measurements made using scintigraphy. The main disadvantage of this method was that enzyme had a limited half-life (3.5 hrs) and the bioluminescent signal was not particularly bright in comparison to other methods (see below). There was also the question of whether the *E.coli* used to produce the bioluminescence may affect the mucosa and/or motility, which may limit its usage in situations where there is intestinal inflammation.

A different approach by Kwon et al²¹ was to use indocyanine green (ICG), a molecule which when injected into the bloodstream is secreted into the bile from the liver. ICG is fluorescent in the near-infrared range (780nm) and was used to visualize the outline of the small intestine and colon downstream from the biliary duct. The authors included some quite beautiful examples of short peristaltic and segmental activity using this method, suitable for calculating most parameters of motion. Unlike the bioluminescence technique, ICG is photostable and does not alter GI motility. The only drawback of this technique was that the stomach was not labeled, being upstream of the bile duct.

One can only speculate on the events that led the same group to utilize the near infrared autofluorescence properties of normal mouse chow (that contained alfalfa) as a contrast agent²², but it was a most insightful and fruitful application. For not only is the red chlorophyll in alfalfa non-toxic and photostable, it also has to be the one of the least expensive contrast agents costing only ~ 20 \$USD per 15 kg bag! Red chlorophyll is excited at 660nm and fluorescence can be collected >710nm, suitable for imaging through the skin. The article shows this agent clearly outlines in the lumen of the entire GI tract of the mouse (provided enough time was allowed for food to progress to the colon) and both peristaltic and segmental contractions are easily resolved (see supplementary movies; temporal resolution 5 fps). The effectiveness of this technique is likely to usher in an exciting phase in gastrointestinal motility research, where, for the first time, it may be possible to study dynamic wall movements, flow and transit in vivo in a truly physiological setting.

Challenges of in vivo imaging

One of the last remaining hurdles to overcome with in vivo imaging is the need to anesthetize the animal to stabilize them for imaging. All anaesthetics have some effect on GI motility, some more severe than others²³, and therefore use of these agents may alter our concept of physiological behavior. Small rodents used for in vivo imaging are usually secured with limbs outstretched on a heating stage; a position they are unlikely to assume in the course of daily life. Positional restraint may be another factor to consider when interpreting “normal” motility patterns. Progress has been made to develop a system that can detect infrared bioluminescence in freely moving mice²⁴, and with continually improving

camera technologies, such imaging systems are likely to attain sufficient temporal and spatial resolution to accurately measure dynamic movements of the GI tract in freely moving animals.

The most formidable hurdle (that is likely to get more complicated in freely moving mice) is how to deal with the plethora of motions that a live animal generates that are not related to the GI tract. In animals secured to a recording stage, the largest source of movement is from the diaphragm that compresses the upper GI tract, particularly the stomach, at a rate of ~30–40 cycles per minute (cpm). Without correction, this movement is sufficient to distort relative fluorescence intensity and produce erroneous images. Similarly, it is not possible to use fixed regions of interests to calculate diameters²⁵ as the separation between adjacent loops of intestine are likely to be smaller than the magnitude of the respiratory movements. While movement tracking algorithms are effective in stabilizing tissue around a single point²⁶, the distortion produced by respiratory movements is non-linear, such that the stomach moves considerably more than the colon. Correction for respiratory movements requires sophisticated algorithms, involving vector-based distortion matrices. Additionally, rotation, tilt and focus movements will need to be corrected in freely-moving animals. Some progress has been made using spatio-temporal objects (GW Hennig in collaboration with R Delgado, University Rey Juan Carlos, Madrid, Spain; unpublished data.).

The future of in vivo imaging...

The GI tract, particularly the small intestine, is unique compared to most other organ systems in that it coils and loops upon itself in a dynamic fashion in 3 dimensions. The only way to accurately image the small intestine is to scan its width, height and depth simultaneously at suitable time intervals (e.g. 5 volumes per second; vps) so that its full structure can be resolved. Both ultrasound and MRI are capable of 4D imaging, but the acquisition rate is currently too slow to be used to study dynamic motility patterns. The modification of hardware to achieve the most minimal resolution parameters suitable for imaging the GI tract (e.g. 256×256×64 @ 5 vps) is not trivial and will require manufacturers to move away from mechanical stepping of single scan beams to some form of solid-state multiple beam arrangement.

One can only hope such technologies will be developed in our lifetimes, but until then, the autofluorescence imaging method developed by Kwon et al. will allow researchers, and possibly clinicians, to visualize dynamic GI movements with high spatial and temporal resolution and minimal invasiveness.

Acknowledgments

GW is supported by grants (Project 1 & Imaging Core D) from the National Center for Research Resources (5P20RR018751-09) and the National Institute of General Medical Sciences (8 P20 GM103513-09) from the National Institutes of Health. The author would like to thank Dr Kent Sanders and Dr Terence Smith for critical reading of this Viewpoint article.

References

1. Cannon, WB. The mechanical factors of digestion. Hill, L.; Bullock, W., editors. Longmans Green & Co; New York: 1911.
2. Bayliss WM, Starling EH. The movements and innervation of the small intestine. *J Physiol.* 1899; 24:99–143.
3. Alvarez WC. The electrogastrogram and what it shows. *JAMA.* 1922; 278:1116–9.
4. Mitchell RF, Cole LG. Historical notes on X-ray cinematography. *SMPTE Mot Imag J.* 1935; 24:333–45.
5. Ehrlein HJ. A new technique for simultaneous radiography and recording of gastrointestinal motility in unanesthetized dogs. *Lab Anim Sci.* 1980; 30:879–84. [PubMed: 7431874]
6. Picard C, Wysocki J, Fioramonti J, Griffiths NM. Intestinal and colonic motor alterations associated with irradiation-induced diarrhoea in rats. *Neurogastroenterol Motil.* 2001; 13:19–26. [PubMed: 11169122]
7. 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; 114:724–36. [PubMed: 9516393]
8. Abalo R, Cabezas 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; 23:457–e177. [PubMed: 21303434]
9. Griffith GH, Owen GH, Kirkman S, Shields R. Measurement of rate of gastric emptying using Chromium-51. *Lancet.* 1966; 1:1244–1245. [PubMed: 4161213]
10. Krishnamurthy GT, Krishnamurthy S. Extended application of 99mTc- mebrofenin cholescintigraphy with cholecystokinin in the evaluation of abdominal pain of hepatobiliary and gastrointestinal origin. *Nucl Med Commun.* 2010; 31:346–54. [PubMed: 20147863]
11. Holt S, Samuel E. Grey scale ultrasound in Crohn's disease. *Gut.* 1979; 20:590–5. [PubMed: 488756]
12. Jones KL, Doran SM, Hveem K, Bartholomeusz FD, Morley JE, Sun WM, Chatterton BE, Horowitz M. Relation between postprandial satiation and antral area in normal subjects. *Am J Clin Nutr.* 1997; 66:127–32. [PubMed: 9209180]
13. Stevens JE, Gilja OH, Gentilcore D, Hausken T, Horowitz M, Jones KL. Measurement of gastric emptying of a high-nutrient liquid by 3D ultrasonography in diabetic gastroparesis. *Neurogastroenterol Motil.* 2011; 23:220–5. [PubMed: 21087356]
14. Mittal RK, Liu J, Puckett JL, Bhalla V, Bhargava V, Tipnis N, Kassab G. Sensory and motor function of the esophagus: lessons from ultrasound imaging. *Gastroenterology.* 2005; 128:487–97. [PubMed: 15685559]
15. Schwizer W, Fox M, Steingötter A. Non-invasive investigation of gastrointestinal functions with magnetic resonance imaging: towards an “ideal” investigation of gastrointestinal function. *Gut.* 2003; 52:34–39. [PubMed: 12477756]
16. Hennig GW, Spencer NJ, Jokela-Willis S, Bayguinov PO, Lee HT, Ritchie LA, Ward SM, Smith TK, Sanders KM. ICC-MY coordinate smooth muscle electrical and mechanical activity in the murine small intestine. *Neurogastroenterol Motil.* 2010; 22:e138–51. [PubMed: 20059699]
17. Broadhead MJ, Bayguinov PO, Okamoto T, Heredia DJ, Smith TK. Ca²⁺ transients in myenteric glial cells during the colonic migrating motor complex in the isolated murine large intestine. *J Physiol.* 2012; 590:335–50. [PubMed: 22063626]
18. Becker A, Hassenius C, Licha K, Ebert B, Sukowski U, Semmler W, Weidenmann B, Grötzinger C. Receptor-targeted optical imaging of tumors with near-infrared fluorescent ligands. *Nat Biotechnol.* 2001; 19:327–31. [PubMed: 11283589]
19. Shcherbo D, Merzlyak EM, Chepurnykh TV, Fradkov AF, Ermakova GV, Solovieva EA, Lukyanov KA, Bogdanova EA, Zaisky AG, Lukyanov S, Chudakov DM. Bright far-red fluorescent protein for whole-body imaging. *Nat Methods.* 2007; 4:741–6. [PubMed: 17721542]
20. 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. [PubMed: 20546505]

21. Kwon S, Sevick-Muraca EM. Non-invasive, dynamic imaging of murine intestinal motility. *Neurogastroenterol Motil.* 2011; 23:881–e344. [PubMed: 21624010]
22. Kwon S, Davies-Venn C, Sevick-Muraca EM. In vivo dynamic imaging of intestinal motions using diet-related autofluorescence. *Neurogastroenterol Motil.* this issue.
23. Torjman MC, Joseph JJ, Munsick C, Morishita M, Grunwald Z. Effects of isoflurane on gastrointestinal motility after brief exposure in rats. *Int J Pharm.* 2005; 294:65–71. [PubMed: 15814231]
24. Roncali E, Savinaud M, Levrey O, Rogers KL, Maitrejean S, Tavitian B. New device for real-time bioluminescence imaging in moving rodents. *J Biomed Opt.* 2008; 13:054035. [PubMed: 19021415]
25. 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; 517:575–90. [PubMed: 10332103]
26. Bayguinov O, Hennig GW, Sanders KM. Movement based artifacts may contaminate extracellular electrical recordings from GI muscles. *Neurogastroenterol Motil.* 2011; 23:1029–42. [PubMed: 21951699]