Characterizing intestinal strictures of Crohn’s disease in vivo by endoscopic photoacoustic imaging

Hao Lei,1 Laura A. Johnson,2 Kathryn A. Eaton,3 Shengchun Liu,4 Jun Ni,1 Xueding Wang,5,6 Peter D. R. Higgins,2 and Guan Xu6,*

1Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
2Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA
3Department of Microbiology and Immunology, University of Michigan Medical Center, Ann Arbor, Michigan, MI 48109, USA
4College of Physical Science and Technology, Heilongjiang University, Harbin, 150080, China
5Department of Biomedical Engineering, University of Michigan Medical School, Ann Arbor, MI 48109, USA
6Department of Radiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
*guanx@med.umich.edu

Abstract: Crohn’s disease (CD) is one type of inflammatory bowel disease where both inflammation and fibrosis cause the thickening of the bowel wall and development of the strictures. Accurate assessment of the strictures is critical for the management of CD because the fibrotic strictures must be removed surgically. In this study, a prototype capsule-shaped acoustic resolution photoacoustic (PA) endoscope, which can perform multilength side-view scanning, was developed to characterize the intestinal strictures of CD. The imaging performance of the probe was tested in phantom experiments and a rabbit trinitrobenzene sulfonic acid (TNBS) model with acute (inflammatory only) or chronic (mixed fibrotic and inflammatory) colitis in vivo. The motion artifacts due to intestinal peristalsis and the respiratory motion of the animals were compensated to improve image qualities. Quantitative molecular component images derived from multilength PA measurements of normal, acute and chronic intestinal strictures demonstrated statistically significant differences among the three groups that were confirmed by histopathology. A longitudinal study demonstrated the capability of the system in monitoring the development of fibrosis. The results suggest that the proposed novel, capsule-shaped acoustic resolution PA endoscope can be used to characterize fibrostenotic disease in vivo.

© 2019 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

1. Introduction
Crohn’s disease (CD) is a chronic disease of the bowel affecting 700,000 people in the United States [1–3]. The chronic bowel damage seen in CD is characterized by obstructing intestinal strictures due to a mixture of inflammation, fibrosis and muscular hypertrophy [4,5]. The largely acute inflammatory strictures can be treated medically, while chronic fibromuscular strictures are irreversible and require surgical resection [3]. The current practice in the treatment of CD intestinal strictures is repeated therapeutic trials of anti-inflammatory therapy, usually corticosteroids, until therapeutic failure is confirmed, which involves months of ineffective therapy, hospitalizations, and adverse steroid effects before an inevitable surgery [6]. Therefore, timely CD management would greatly benefit from accurate assessment of the presence and extent of fibrosis in intestinal strictures [7,8].

The current clinical assessment of CD strictures is through endoscopic biopsies [9]. In endoscopy, small samples of tissue are snipped from the superficial layer of the strictures and examined by histopathology for evidence of inflammation. Though structuring disease occurs in the deeper tissue layers, specifically the lamina propria and muscularis [10], conventional
endoscopic biopsies are limited to the superficial epithelial layer, and are unable to detect evidence of fibrosis [11]. Any evidence of inflammation is generally considered an indication for more corticosteroids [6], though this is futile in chronic fibromuscular strictures [12]. In addition, comprehensive evaluation of the bowel is inherently limited due to biopsy size.

Conventional imaging modalities including Ultrasound (US) imaging [13], Magnetic Resonance Imaging (MRI) [1,14], and Computed Tomography (CT) [4,5,15] can be applied to identify and locate the intestinal strictures, and measure bowel wall thickness. These modalities cannot assess intestinal strictures at molecular level, and cannot differentiate inflammatory from fibrotic strictures. This leaves a prognostic gap that is currently filled by repeated empiric courses of corticosteroids [6], which lead to significant adverse events and delays in timely treatment with surgery.

Photoacoustic (PA) imaging is an emerging non-radiative and non-ionizing technology combining optical spectroscopy and ultrasonography [16,17]. During a PA measurement, a pulsed or modulated laser illumination causes targeted molecules to vibrate, generating a tiny thermoexpansion within the targeted tissue. This thermoexpansion causes vibrations that propagate in the form of mechanical waves. The mechanical waves can be measured by US transducers for reconstruction of images representing the optical absorption properties within the illuminated tissue volume. PA imaging with sequential illumination at multiple wavelengths allows for the assessment of each individual molecular component with a tissue volume at ultrasonic resolution [18,19]. PA imaging systems are also capable of generating naturally co-registered ultrasonography images by using the pulse-echo mode of the US transducer [20–22]. In our previous work [23], we have validated the feasibility of characterizing the intestinal inflammation and fibrosis in CD by quantifying the hemoglobin and collagen content in ex vivo human and animal tissue using an acoustic resolution PA microscopy (ARPAM) system. The system has achieved PA imaging at the depth of 7 mm beneath stricture inner surface with a resolution of 50 µm. We have also demonstrated the feasibility of detecting intestinal inflammation and fibrosis in vivo in a trinitrobenzene sulfonic acid (TNBS) rat model [24].

Recently, several PA endoscopy systems have been developed [21,22,25–29]. However, all of these studies focused on imaging the hemoglobin content which is only correlated to the inflammation. In addition, the circular scanning of these probes limited their capability of rapidly assessing a longitudinal segment of the intestine. The tomographic image reconstruction in a circular geometry also provides limited the resolution in deep tissue. In this study, a prototype capsule-shaped ARPAM probe with a longitudinal field of view was developed for characterizing both the inflammation and fibrosis in the intestinal strictures. The probe was compatible with clinical endoscopes and was preliminarily tested intra-rectally using TNBS rabbit models [30,31]. In a proof-of-concept longitudinal study in the chronic TNBS rabbit model, animals were scanned over 8 weeks to monitor disease progression from acute inflammation to chronic fibrosis. The quantitative imaging results were statistically analyzed and confirmed with the histopathology.

2. Methods and materials

2.1 Development of the capsule-shaped ARPAM probe and peripheral systems

Figure 1(a) shows the schematic diagram of the prototype capsule-shaped ARPAM probe including the acquisition system. The probe consists of a capsule shell with a side-viewing window and a translation stage fabricated using a 3D printer with VisiJet M3 Crystal (ProJet 3500, 3D System, Rock Hill, SC, USA). As shown in Fig. 1(b), the movement of the translation stage was guided by two parallel tracks on the inner surface of the capsule shell. A spherically focused transducer (50 MHz central frequency, 80% bandwidth, 3.2 mm focal length, 2 mm aperture, LiNbO3, Medical Ultrasonic Transducer Resource, University of Southern California, CA, USA) and a multimode optical fiber (0.39 NA, 600 µm core, Thorlabs, Newton, NJ, USA) were fixed in the translation stage as shown in Fig. 1(c). As
shown in Fig. 1(d), the tip of the optical fiber was placed in the stage with an 8° tilted angle so that the delivered light reflected by the optical prism (1.5mm×1.5mm×1mm, Tower Optical Corporation, Boynton Beach, FL, USA) and projected through the side-viewing window. Figure 1(e) displays the calculated beam pattern projected onto bowel wall. The translation stage was attached to a compression spring (compression rate 0.13 lbs/mm over 5 mm range, McMaster-Carr, Elmhurst, IL, USA) and driven by an external step motor through a steel pulling string. The side-viewing window was sealed with transparent low density Polyethylene (PE) membrane with a thickness of 12.7 μm and the probe shell was filled with deionized water for optical transmission and acoustic coupling. The probe was tested with and without the membrane when submerged in water. No significant attenuations of the optical and acoustic energy were observed. During the scanning, the side-viewing window contacted the surface of the imaging volume and the PA measurements were acquired over a longitudinal range of 4 mm at the step of 30 μm.

A tunable (680 nm ~1700 nm) Optical Parametric Oscillator (OPO) laser (VibrantB, Optek Inc., Carlsbad, CA, USA) pumped by the second harmonic output of a Nd:YAG pulsed laser (Brilliant B, Quantel, Bozeman, MT, USA) was used as the illumination source. The output of the laser was split by a glass slide, which separated about 6% laser energy off the coupling path towards an optical power meter (PMKIT, Newport Corporation, Newport, NJ, USA). Optical energy of 1.5 mJ per pulse at 10 Hz pulse repetition rate was coupled to the fiber optics in the probe, forming an optical energy density of approximately 7 mJ/cm² at the scanning surface. The energy level is below the safety limit established by American National Standard Institute (ANSI) [32]. The PA signal were amplified by 40dB with a low noise pre-amplifier (AM-1605-BNC, MITEQ, Hauppauge, NY, USA), and recorded by a 14-bit digitizer card (200 MHz, GaGe, DynamicSignals LLC, Lockport, IL, USA). For each scanning step or sampling location, PA signals were averaged 30 times to improve signal-to-noise ratio. Figure 1(f) shows the photograph of the capsule-shaped probe when acquiring measurements.

![Fig. 1.](image)

Fig. 1. (a) Schematics of the prototype capsule-shaped acoustic resolution PA microscopy probe including the acquisition system. (b) Cross-section of the capsule-shaped probe. (c) Bottom view of the translation stage without capsule shell. (d) Illustration of the light path through the optical prism. (e) Calculated beam pattern projected onto bowel wall. (f) Photograph of the capsule-shaped probe.

2.2 Data processing and image reconstruction

Based on our previous work [23], the 720 and 1310 nm wavelengths were selected for characterizing the hemoglobin in inflammation and collagen in fibrosis, respectively Fig. 2(a). Illumination at both wavelengths were coupled to the fiber optic in the probe using the same set of optics. The light diffusion patterns at both wavelengths [Fig. 2(b) and (c)] were calculated using Monte Carlo method [33] used in our previous study. The calculation was
based on the assumption that the pathological accumulation of hemoglobin and collagen are optical perturbations over a homogeneous optical background. The estimated optical properties of background tissues are $\mu_a = 0.4 \text{mm}^{-1}$, $\mu'_a = 1.18 \text{mm}^{-1}$ at 720 nm, and $\mu_s = 1.31 \text{mm}^{-1}$, $\mu'_s = 0.5 \text{mm}^{-1}$ at 1310 nm, where $\mu_a$ and $\mu_s$ are the absorption and the reduced scattering coefficients, respectively. The PA signal intensities were compensated by the non-uniform light fluence distribution along the penetration depth in Fig. 2(d).

![Graph](image)

**Fig. 2.** (a) Relative optical absorption spectra of blood and collagen [34,35]. The vertical dashed lines mark the selected wavelengths at 720 and 1310 nm, respectively. a.u. = arbitrary units. Hb. = Hemoglobin. (b) and (c) are Monte Carlo simulated 2D light fluence distribution in the scanning plane at 720 and 1310 nm, respectively. (d) Light fluence distribution along the transducer focusing axis (red dashed lines in b and c).

Intestinal peristalsis and the respiratory motion of the animals produced slight variations of the distance between the transducer and the tissue surface during the 30 repetitive measurements at each scanning location. Such displacements are reflected as the phase mismatches on the order of 20 ns (corresponding to 30 µm) among the measurements. As shown in Fig. 3(a), no continuous black and white lines can be found. Averaging of the phase mismatched signals in Fig. 3(a) leads to the reduced signal-to-noise ratio shown in Fig. 3(b). A phase correlation based on least-square fitting was applied to the measurements to compensate for the phased mismatch. Figure 3(c) shows the phased compensation measurements, which are more consistent phases in fluctuations, i.e. continuous black and white lines. Figure 3(d) shows the averaged signal with improved signal-to-noise ratio. Using similar phase correlation method, we also aligned the signals at the neighboring data acquisition locations to compensate for bowel and respiratory motion. Figure 3(e) shows the
signals at 80 scanning steps side-by-side, phase discontinuity can be observed as the tissue architectural features are not shown as continuous black and white curves. The phased compensated signals in Fig. 3(f) have shown more continuous black and white curves.

The US beam pattern for the applied fixed spherical focusing transducer was simulated by FOCUS software package (https://www.egr.msu.edu/~fultras-web/). As shown in Fig. 4(a), the lateral focusing decays away from the physical focal point of the transducer. To improve the spatial resolution of B-scan PA images in the out-of-focus region, synthetic aperture focusing technique (SAFT) and coherence factor (CF) weighting [36] were applied during the image reconstruction. Its performance was tested with nichrome wires with a diameter of 40 μm at different distance away from the probe surface. The result of the cross-sectional scans before and after performing SAFT-CF were displayed in Fig. 4(b) and (c), respectively. Axial and lateral resolution were determined to be about 65 and 90 μm based on the averaged full width at half maximum (FWHM) in Fig. 4 (d) and (e), respectively.
The molecular components images showing the distribution of hemoglobin and collagen within the scanned tissue were resolved pixel-wise using Eq. (1) [23]:

\[
\begin{bmatrix}
    c_{\text{Hb}} \\
    c_{\text{cg}}
\end{bmatrix}
= 
\begin{bmatrix}
    \mu_{\text{Hb,720}} & \mu_{\text{cg,720}} \\
    \mu_{\text{Hb,1310}} & \mu_{\text{cg,1310}}
\end{bmatrix}^{-1}
\begin{bmatrix}
    A_{720} \\
    A_{1310}
\end{bmatrix}
\]  

where \(A_{720}\) and \(A_{1310}\) are the reconstructed total optical absorption at 720 and 1310 nm, respectively, which are proportional to PA intensities. \(\mu_{\text{Hb}}\) and \(\mu_{\text{cg}}\) are the absorption coefficient of total hemoglobin (Hb) and collagen (cg) at 720 and 1310 nm, respectively. \(c_{\text{Hb}}\) and \(c_{\text{cg}}\) are the relative concentrations of total hemoglobin and collagen. The optical absorption of total hemoglobin at 720 nm was estimated by averaging the optical absorption coefficients of oxy and deoxygenated hemoglobin.

2.3 In vivo animal experiments

All the animal imaging protocols in this study has been approved by the Institutional Animal Care and Use Committee at the University of Michigan.
2.3.1 Rabbit TNBS model of colitis and fibrosis

The endoscopic probe was tested in vivo using young (75 to 100-day old) New Zealand white rabbits of both sexes (Covance, Kalamazoo, MI, USA or University of Michigan, ULAM Breeding Colony, Ann Arbor, MI, USA). The rabbits were maintained under general anesthesia using 4-5% inhaled isoflurane during all TNBS administration and imaging procedures. Vital signs and anesthesia depths were continuously monitored during anesthesia. To induce acute inflammation, a single intra-rectal dose of 40 mg TNBS in 25% ethanol was delivered 7 cm into the rectum/distal colon using a 10 French size catheter. To induce intestinal fibrosis, multiple escalating intra-rectal doses of TNBS (from 40 to 165 mg) with 2 to 4 week healing intervals were used to replicate the cycles of inflammation and tissue repair that trigger fibrosis as we and others have observed in the rat TNBS model [37–40].

2.3.2 PA scanning procedure

As shown in Fig. 5, the rabbits were scanned in a uniform recumbent left-sided position, under anesthesia. The probe surface was covered by US gel and surgical lubricant before inserting in to the rectum of the rabbits. The probe was positioned at 7 cm from the distal end of the colon, i.e. the location of TNBS delivery, while the side-view window of the probe was oriented toward the ground to ensure revisiting the same imaging plane. The colon was scanned during a forward and reverse cycle of the translation stage. The measurements at the two wavelengths (720 and 1310 nm) were acquired during forward and reverse trips of the translation stage, respectively. The scanning time was 8 minutes, per scanning direction. The pixel intensities within the hemoglobin and collagen images were averaged for later statistical analysis.

![Experiment setup for rabbit intestine imaging in vivo.](image)

Fig. 5. Experiment setup for rabbit intestine imaging in vivo.

2.3.3 Comparison between normal, acute, and chronic TNBS rabbits using PA imaging

8 male and 4 female young New Zealand rabbits were randomly divided into two groups: acute and chronic. Prior to the first TNBS administration, PA imaging of all animals was performed as normal control. The first group of five rabbits received a single acute 40 mg/kg dose of TNBS. The in vivo colonoscopic PA imaging was performed 2 days after TNBS administration during peak acute intestinal inflammation. The second group of seven rabbits received three cycles of escalating TNBS doses at 40 80 and 160 mg/kg over two months, with PA imaging performed 2 - 5 days after the third TNBS treatment. At the end stage of the PA imaging sequence, the rabbits in both groups were euthanized by an intravenous overdose of sodium pentobarbital.
2.3.4 Longitudinal study of fibrotic progression in the chronic TNBS model using PA imaging

To test the utility of the PA probe for tracking disease development from acute inflammation to chronic fibrosis, a longitudinal study was performed with four animals using the chronic TNBS rabbit model. This longitudinal cohort received treatments identical to the chronic group in section 2.3.1, but were PA scanned at baseline, prior to each of the 3 TNBS treatments, and at the termination of the experiment at week 8, as illustrated in Fig. 8(a).

2.3.5 Histology

Proximal (untreated, i.e. normal) and distal (treated) colons of the euthanized rabbits were harvested and processed for histology. Hematoxylin and eosin (H&E) staining and Masson’s trichrome staining were performed by the University of Michigan Cancer Center Histology and Immunoperoxidase Lab (Ann Arbor, MI, USA) and McClinchey Histology Lab (Stockbridge, MI, USA), respectively. Digital photomicrographs of proximal and distal colon sections were captured using an Olympus BX51 microscope at the University of Michigan Microscopy and Image Analysis Laboratory (Ann Arbor, MI, USA). To quantify the amount of collagen content, trichrome-stained slides were digitized at 20X ocular (~200x) magnification on a digital slide scanner (Leica Aperio AT2, Leica Biosystems, Buffalo Grove, IL). Annotated areas of lamina propria were digitally drawn and blue pixels were quantified using a commercial algorithm for color deconvolution (Leica Aperio Color Deconvolution, v.9.1, Leica Biosystems). Algorithm parameters were specifically optimized for the red and blue of the trichrome by selecting small areas with only the indicated colors and defining the RGB values for the stain, then using these values as the target stain input parameters. Threshold input parameters for weak, moderate, and strong positive staining were optimized by visual examination of a test image by a board-certified veterinary pathologist (KAE) in a blinded manner. Three-four random sections of lamina propria were quantified for each slide. Dense fibrosis was defined as the sum of percent strong and percent moderate staining per slide.

2.3.6 Statistics

The statistical analysis for differentiating the normal, acute and chronic conditions were performed using build-in ttest function in MATLAB (2017, Mathworks, Natick, MA, USA). The null hypothesis is that the three conditions cannot be differentiated using PA measurements. The correlation between the TNBS doses and the PA intensities were calculated using corrcoef function in MATLAB (2017, Mathworks, Natick, MA, USA). The null hypothesis is that the two values do not have correlation with each other.

3. Results

3.1 Pathological analysis

The gross pathology and the histology photographs are shown in Fig. 6(a) and (b). In H&E stained sections of acute injury in Fig. 6(a) in the middle column, there is coagulation necrosis of the mucosa (bracket), inflammatory infiltrate (arrow), and hemorrhage (arrowhead). Chronic injury on the right column in Fig. 6(a) is characterized by widespread replacement of the mucosa by fibrous connective tissue (arrows) and increased density of fibrosis in the submucosa (arrowhead), not present in normal or acute sections. Statistical analysis of the fibrosis in the trichrome staining, as described in 2.3.5, has shown that the chronic animals has approximately two and half times collagen content compared to the normal and acute animals at the treated location \(p = 0.032\), Fig. 6(c)].
3.2 Quantitative characterization of inflammatory and fibrotic disease in the TNBS rabbit model

Figure 7(a) illustrates the representative PA molecular component images from normal, acute TNBS, and chronic TNBS rabbit colons, respectively. Hemoglobin tissue distribution was rendered in red and collagen distribution was rendered in green. As illustrated in Fig. 7(b), hemoglobin content significantly increased in both acute and chronic conditions compared to the normal condition. An approximately 2-fold hemoglobin signal intensity was observed in the acute condition compared to the normal condition \( p = 0.0189 \). In the chronic condition, an approximately 3-fold collagen signal intensity was observed compared to the acute condition \( p = 0.0002 \), Fig. 7(c). The quantitative PA results were consistent with the gross pathology and histological quantification.
Fig. 7. (a) Representative PA molecular component images of normal, acute and chronic conditions. Hemoglobin distribution was rendered in red and collagen distribution was rendered in green. Both color scales were normalized to the maximum relative concentration of hemoglobin. (b) The boxplots of averaged intensities of hemoglobin distribution for normal, acute, and chronic images. An approximately two-fold increase in hemoglobin signal intensity was observed in the acute condition compared to the normal condition (p = 0.0189). (c) The boxplots of averaged intensities of collagen distributions for normal, acute, and chronic condition. An approximately three-fold collagen signal intensity was observed in the chronic condition compared to the acute condition (p = 0.0002).

3.3 PA measurement of collagen accumulation over time in the chronic TNBS model

As detailed in the experimental timeline in Fig. 8(a), four in vivo PA scans (blue arrows) were performed prior to each TNBS treatment (red arrows) and at the end of the longitudinal study. As illustrated in Fig. 8(b), the resolved PA molecular component images have shown increasing collagen deposition within individual animal over time. In the entire longitudinal cohort, PA imaging detected increased collagen accumulation in response to cycles of TNBS-induced inflammation and tissue healing, with a dramatic increase in collagen between the 3rd and 4th scans in Fig. 8(c). Overall, the PA resolved collagen content significantly correlated with the number of TNBS treatments (r = 0.798, p = 0.0002).
4. Discussion

The acute and chronic TNBS rabbit models in this study developed gross pathology and histopathology similar to the TNBS rat models in our previous studies [23,24,37–40]. One major difference between the rabbit model and the rat model is the lack of lipid components in the healthy and diseased colon tissues. Therefore, we removed 1220 nm targeting lipid
content in our previous work [23], and used only 720 and 1310 nm targeting hemoglobin and collagen.

As illustrated in this study, the molecular components measured in the PA images in vivo are consistent with the gross pathology and the histopathology. The endoscopic PA imaging approach quantitatively differentiates inflammatory from fibrotic intestinal strictures, and objectively quantifies the development of fibrotic disease. In contrast to our transcutaneous PA imaging with resolution on the order of hundreds of microns [24], the endoscopic PA imaging approach provides an order higher resolution at tens of microns and, therefore, provides more detailed tissue pathology. Another advantage of the endoscopic approach over the transcutaneous one is that the endoscopic delivery of illumination avoids the optical energy attenuation through the skin and subcutaneous tissue.

This study demonstrates the feasibility of endoscopic PA imaging to characterize CD-like intestinal pathology in rabbit models. This is a dramatic improvement over current endoscopic technology, which can only measure inflammation in the surface epithelium, and can provide no data on fibromuscular damage in the deeper layers of the bowel wall. In future development, we will further miniaturize the capsule probe to be compatible with the instrument channel of a standard pediatric colonoscope. This miniaturized design will allow the withdrawal of the PA probe after prognostic assessment, and the insertion of conventional endoscopic instruments for interventional procedures such as balloon dilation without repositioning the colonoscope. The PA imaging speed can be potentially improved by using lasers with higher repetition rate, which will substantially mitigate the motion artifacts observed in Fig. 3. We will also integrate US imaging using a pulser-receiver for extracting structural information of the intestinal strictures, although extra scanning time will be introduced.

5. Conclusion

We have developed a novel, capsule-shaped ARPAM probe with axial resolution of 65 µm and lateral resolution of 90 µm to measure the molecular components characterizing inflammation and fibrosis in intestinal strictures. The in vivo discriminatory capability of the PA probe was validated in rabbit models of intestinal inflammation and fibrosis. In this proof-of-concept longitudinal study in the rabbit TNBS models, PA imaging is feasible for monitoring the development of intestinal fibrosis over time, which could provide valuable information for clinical decision-making during CD management. Patients with strictures that are of the chronic fibromuscular phenotype could go to surgical resection in a timely fashion, avoiding months of intermittent intestinal obstruction, hospitalizations, and the side effects of futile empiric trials of corticosteroids. These results demonstrate that this PA endoscopic probe can be used to characterize fibrostenotic disease in vivo. Future development will be focused on the miniaturization and improvement of scanning speed of the probe.

Funding

National Institute of Allergy and Infectious Diseases (R21AI12209801A1); American Gastroenterological Association Boston Scientific Career Development Technology and Innovation Award; Crohn’s and Colitis Foundation Senior Research Award (581584); National Cancer Institute (1R37CA22282901A1); National Institute of Arthritis and Musculoskeletal and Skin Diseases (5R01AR060350).

Disclosures

The authors declare that there are no conflicts of interest related to this article.

References

1. I. C. Lawrance, C. J. Welman, P. Shipman, and K. Murray, “Correlation of MRI-determined small bowel Crohn’s disease categories with medical response and surgical pathology,” World J. Gastroenterol. 15(27), 3367–3375 (2009).
2. E. V. Loftus, Jr., P. Schoenfeld, and W. J. Sandborn, “The epidemiology and natural history of Crohn’s disease in population-based patient cohorts from North America: a systematic review,” Aliment. Pharmacol. Ther. 16(1), 51–60 (2002).

3. R. D. Cohen, L. R. Larson, J. M. Roth, R. V. Becker, and L. L. Mummert, “The cost of hospitalization in Crohn’s disease,” Am. J. Gastroenterol. 95(2), 524–530 (2000).

4. G. R. Lichtenstein, A. Olson, S. Travers, R. H. Diamond, D. M. Chen, M. L. Pritchard, B. G. Feagan, R. D. Cohen, B. A. Salzberg, S. B. Hanauer, and W. J. Sandborn, “Factors Associated with the Development of Intestinal Strictures or Obstructions in Patients with Crohn’s Disease,” Am. J. Gastroenterol. 101(5), 1030–1038 (2006).

5. O. Oberhuber, P. C. Stangl, H. Vogelsang, E. Schober, F. Herbst, and C. Gasche, “Significant association of strictures and internal fistula formation in Crohn’s disease,” Virchows Arch. 437(3), 293–297 (2000).

6. W. A. Faubion, Jr., E. V. Loftus, Jr., W. S. Harmsen, A. R. Zinsmeister, and W. J. Sandborn, “The natural history of corticosteroid therapy for inflammatory bowel disease: A population-based study,” Gastroenterology 121(2), 255–260 (2001).

7. J. Cosnes, “Can we modulate the clinical course of inflammatory bowel diseases by our current treatment strategies?” Dig. Dis. 27(4), 516–521 (2009).

8. D. W. Jones and S. R. G. Finlayson, “Trends in Surgery for Crohn’s Disease in the Era of Infliximab,” Ann. Surg. 252(2), 307–312 (2010).

9. S. M. Dandanides, W. D. Carey, R. Petras, and E. Achkar, “Endoscopic small bowel mucosal biopsy: a controlled trial evaluating forceps size and biopsy location in the diagnosis of normal and abnormal mucosal architecture,” Gastrointest. Endosc. 35(3), 197–200 (1989).

10. H. Mattthes, H. Herbst, D. Schuppan, A. Stallmach, S. Milani, H. Stein, and E.-O. Riecken, “Cellular localization of procollagen gene transcripts in inflammatory bowel diseases,” Gastroenterology 102(2), 431–442 (1992).

11. G. K. Mukharia, S. G. Singh, M. Tripathi, V. D. Aggarwal, P. P. Tiwari, V. Sreenivas, and S. D. Gupta, “Clinical, Endoscopic, and Histological Differentiations Between Crohn’s Disease and Intestinal Tuberculosis,” Am. J. Gastroenterol. 105(3), 642–651 (2010).

12. J. K. Kelly and T. O. Siu, “The strictures, sinuses, and fissures of Crohn’s disease,” J. Clin. Gastroenterol. 8(5), 594–598 (1986).

13. Z. Tarján, G. Töth, T. Győrke, A. Mester, K. Karlinger, and E. K. Makó, “Ultrasound in Crohn’s disease of the small bowel,” Eur. J. Radiol. 34(3), 176–182 (2000).

14. P. Paolantoni, R. Ferrari, F. Vecchietti, S. Cucchiara, and A. Laghi, “Current status of MR imaging in the evaluation of IBD in a pediatric population of patients,” Eur. J. Radiol. 69(3), 418–424 (2009).

15. M. Boudiaf, P. Soyer, C. Terem, J. P. Pelage, E. Maissiat, and R. Rymer, “CT Evaluation of Small Bowel Obstruction,” Radiographics 21(3), 613–624 (2001).

16. P. Beard, “Biomedical photoacoustic imaging,” Interface Focus 1(4), 602–631 (2011).

17. M. Xu and L. V. Wang, “Photoacoustic imaging in biomedical science,” Rev. Sci. Instrum. 77(4), 041101 (2006).

18. L. V. Wang and S. Hu, “Photoacoustic Tomography: In Vivo Imaging from Organelles To Organs,” Science 335(6075), 1458–1462 (2012).

19. X. Wang, Y. Pang, G. Ku, X. Xie, G. Stoica, and L. V. Wang, “Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain,” Nat. Biotechnol. 21(7), 803–806 (2003).

20. J. Yuan, G. Xu, Y. Yu, Y. Zhou, P. L. Carson, X. Wang, and X. Liu, “Real-time photoacoustic and ultrasound dual-modality imaging system facilitated with graphics processing unit and code parallel optimization,” J. Biomed. Opt. 18(8), 086001 (2013).

21. J.-M. Yang, R. Chen, C. Favazza, J. Yao, C. Li, Z. Hu, Q. Zhou, K. K. Shung, and L. V. Wang, “A 2.5-mm diameter probe for photoacoustic and ultrasonic endoscopy,” Opt. Express 20(21), 23944–23953 (2012).

22. J.-M. Yang, C. Favazza, R. Chen, J. Yao, X. Cai, K. Maslov, Q. Zhou, K. K. Shung, and L. V. Wang, “Simultaneous functional photoacoustic and ultrasonic endoscopy of internal organs in vivo,” Nat. Med. 18(8), 1297–1302 (2012).

23. H. Lei, L. A. Johnson, S. Liu, D. S. Moons, T. Ma, Q. Zhou, M. D. Rice, J. Ni, X. Wang, P. D. Higgins, and G. Xu, “Characterizing intestinal inflammation and fibrosis in Crohn’s disease by photoacoustic imaging: feasibility study,” Biomed. Opt. Express 7(7), 2837–2848 (2016).

24. Y. Zhu, L. A. Johnson, Z. Huang, J. M. Rubin, J. Yuan, H. Lei, J. Ni, X. Wang, P. D. R. Higgins, and G. X. Xu, “Identifying intestinal fibrosis and inflammation by spectroscopic photoacoustic imaging: an animal study in vivo,” Biomed. Opt. Express 9(4), 1590–1600 (2018).

25. C. Li, J.-M. Yang, R. Chen, C.-H. Yeh, L. Zhu, K. Maslov, Q. Zhou, K. K. Shung, and L. V. Wang, “Urogenital photoacoustic endoscope,” Opt. Lett. 39(6), 1473–1476 (2014).

26. J.-M. Yang, C. Li, R. Chen, Q. Zhou, K. K. Shung, and L. V. Wang, “Catheter-based photoacoustic endoscope,” J. Biomed. Opt. 19(6), 066001 (2014).

27. H. He, G. Wissmeyer, S. V. Ovesian, A. Buehler, and V. Ntziachristos, “Hybrid optical and acoustic resolution optoacoustic endoscopy,” Opt. Lett. 41(12), 2708–2710 (2016).

28. Y. Li, R. Lin, C. Liu, J. Chen, H. Liu, R. Zheng, X. Gong, and L. Song, “In vivo photoacoustic/ultrasonic dual-modality endoscopy with a miniaturized full-field-of-view catheter,” J. Biophotonics 11, e201800034 (2018).

29. Y. Li, Z. Zhu, J. C. Jing, J. J. Chen, A. E. Heidari, Y. He, J. Zhu, T. Ma, M. Yu, Q. Zhou, and Z. Chen, “High-Speed Integrated Endoscopic Photoacoustic and Ultrasound Imaging System,” IEEE J. Sel. Top. Quantum Electron. 25(1), 1–5 (2019).
30. I. Depoortere, T. Thijs, G. van Asseche, J. C. Keith, Jr., and T. L. Peeters, “Dose-dependent effects of recombinant human interleukin-11 on contractile properties in rabbit 2,4,6-trinitrobenzene sulfonic acid colitis,” J. Pharmacol. Exp. Ther. 294(3), 983–990 (2000).
31. D. Anthony, F. Savage, V. Sams, and P. Boulos, “The characterization of a rabbit model of inflammatory bowel disease,” Int. J. Exp. Pathol. 76(3), 215–224 (1995).
32. American National Standards Institute, American National Standard for Safe Use of Lasers (Laser Institute of America, 2007).
33. L. Wang, S. L. Jacques, and L. Zheng, “MCML-Monte Carlo modeling of light transport in multi-layered tissues,” Comput. Methods Programs Biomed. 47(2), 131–146 (1995).
34. H.-W. Wang, N. Chai, P. Wang, S. Hu, W. Dou, D. Umulis, L. V. Wang, M. Sturek, R. Lucht, and J.-X. Cheng, “Label-Free Bond-Selective Imaging by Listening to Vibrationally Excited Molecules,” Phys. Rev. Lett. 106(23), 238106 (2011).
35. W. F. Cheong, S. A. Prahl, and A. J. Welch, “A review of the optical properties of biological tissues,” Quantum Electronics, IEEE Journal of 26(12), 2166–2185 (1990).
36. M.-L. Li, H. E. Zhang, K. Maslov, G. Stoica, and L. V. Wang, “Improved in vivo photoacoustic microscopy based on a virtual-detector concept,” Opt. Lett. 31(4), 474–476 (2006).
37. R. W. Stidham, J. Xu, L. A. Johnson, K. Kim, D. S. Moons, B. J. McKenna, J. M. Rubin, and P. D. Higgins, “Ultrasound elasticity imaging for detecting intestinal fibrosis and inflammation in rats and humans with Crohn’s disease,” Gastroenterology 141(3), 819–826 (2011).
38. K. Kim, L. A. Johnson, C. Jia, J. C. Joyce, S. Rangwalla, P. D. R. Higgins, and J. M. Rubin, “Noninvasive Ultrasound Elasticity Imaging (UEI) of Crohn’s Disease: Animal Model,” Ultrasound Med. Biol. 34(6), 902–912 (2008).
39. J. R. Dillman, R. W. Stidham, P. D. Higgins, D. S. Moons, L. A. Johnson, and J. M. Rubin, “US elastography-derived shear wave velocity helps distinguish acutely inflamed from fibrotic bowel in a Crohn disease animal model,” Radiology 267(3), 757–766 (2013).
40. J. R. Dillman, S. D. Swanson, L. A. Johnson, D. S. Moons, J. Adler, R. W. Stidham, and P. D. Higgins, “Comparison of noncontrast MRI magnetization transfer and T -Weighted signal intensity ratios for detection of bowel wall fibrosis in a Crohn’s disease animal model,” J. Magn. Reson. Imaging 42, 801–810 (2014).