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Functional imaging of colonic mucosa with a fibered confocal microscope for real time *in vivo* pathology

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Abstract

Background & Aims—Histological interpretation of disease is currently performed with static images of excised tissues, and is limited by processing artifact, sampling error, and interpretive variability. To demonstrate use of functional optical imaging of viable mucosa for quantitative evaluation of colonic neoplasia in real time.

Methods—Fluorescein (5 mg/ml) was topically administered in (n=54) human subjects undergoing screening colonoscopy. Fluorescence images were collected with 488 nm excitation at 12 frames/second with the confocal microendoscopy system. Movement of fluorescein in the transient period (<5 sec) and the lamina propria: crypt contrast ratio in the steady state phase (>5 sec) were quantified.

Results—Normal mucosa showed circular crypts with uniform size, hyperplasia revealed proliferative glands with serrated lumens, and adenomas displayed distorted, elongated glands. For t<5 sec, fluorescein passed through normal epithelium with a peak speed of $1.14 \pm 0.09 \mu\text{m}/\text{sec}$ at t=0.5 sec, and accumulated into lamina propria as points-of-fluorescence that moved through the interglandular space with an average speed of $41.7 \pm 3.4 \mu\text{m}/\text{sec}$. Passage of fluorescein through adenomatous mucosa was substantially delayed. For t>5 sec, high sensitivity, specificity, and accuracy was achieved using a discriminant function to evaluate the contrast ratio to distinguish normal from lesional mucosa (91%, 87%, and 89%, respectively; p<0.001), hyperplasia from adenoma (97%, 96%, and 96%, respectively; p<0.001), and tubular from villous adenoma (100%, 92%, and 93%, respectively; p<0.001).

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Conclusion—Confocal imaging can be performed *in vivo* to assess the functional behavior of tissue in real time for providing pathological interpretation, representing a new method for histological evaluation.

INTRODUCTION

Confocal microscopy provides clear images from thick layers of tissue using the principle of optical sectioning, and can achieve sufficient resolution to provide fine details about cells and sub-cellular structures.¹ Recent advances in instrument miniaturization^{2–5} have allowed for the development of flexible, fibered confocal microscopes that have a size compatible with medical endoscopes and speed to image without motion artifact, enabling *in vivo* imaging in hollow organs to detect disease.^{6–10} These instruments can collect either backscattered light to provide details about tissue micro-architecture, or fluorescence images to provide insights into mechanisms of mucosal function, and thus provide an unprecedented opportunity to assess viable tissue in real time. Previous studies with fibered confocal microscopes demonstrated static images of mucosal microanatomy for comparison to histology.^{11–13} This investigation uses a novel confocal instrument to acquire dynamic images of tissue function for assessment of pathology.

We aim to observe functional differences in the uptake and distribution of topically applied fluorescein by colonic crypts to distinguish among normal mucosa, hyperplasia, and adenoma. Crypts are collections of epithelial cells organized into glands, whose lumens are exposed to the mucosal surface.¹⁴ The tight junctions in between neighboring cells form a barrier to luminal contents. Passage of fluid and solutes from the lumen into the lamina propria, a loose matrix of connective tissue that contains vasculature and lymphatics, is regulated by the epithelial layer. Specifically, crypt transport is mediated by transport proteins located on the apical (luminal) and basolateral (lamina propria) surfaces of the epithelial cell membrane. Solute also can pass along a ‘paracellular’ pathway through regulated permeability of the intercellular tight junctions. Here, we assess the uptake of topically administered fluorescein, an FDA approved drug used clinically to detect epithelial defects in cornea,¹⁵ as a substrate for organic anion transport (OATP)^{16,17} and multidrug resistance (MRP) proteins,^{18,19} and as a marker of solute movement via the paracellular pathway.^{20,21} Effort was made to distinguish normal colonic mucosa from that exhibiting hyperplasia or adenomatous changes. Hyperplasia is a non-neoplastic proliferation of colonic mucosa that results from reduced exfoliation of normal epithelium, and adenoma is a pre-malignant condition that arises from unregulated epithelial growth. These lesions are commonly found on routine screening colonoscopy.

METHODS

A. Confocal imaging system

The confocal imaging system, Cellvizio®-GI (Mauna Kea Technologies, Paris, France), consists of a flexible (1.5 mm diameter) miniprobe, control unit, and processing software. A 488 nm (peak absorption of fluorescein) semiconductor laser (Coherent, Inc, Santa Clara, CA) delivers the excitation beam to a 4 kHz oscillating mirror for horizontal scanning (lines) and then to a 12 Hz galvo mirror for vertical scanning (frames). The mirrors raster scan the beam across the proximal face of an imaging bundle (Fujikura Ltd, Tokyo, Japan) that contains over ten thousand optical fibers (1.9 μ m core diameter, 3.3 μ m average intercore spacing). A gradient index (GRIN) microlens located at the distal end focuses the beam into the tissue (0 μ m working distance) with a lateral and depth resolution of 3.5 and 15 μ m, respectively, measured by a standard imaging target. Images are collected in a horizontal plane (*en face*) at 12 frames per second with a field of view of 600 $\times$ 500 μ m². Fluorescence is collected by the same lens, and refocused back into the illumination fiber. The cores of the fiber act as collection pinholes for

rejecting out of focus light to perform optical sectioning. A long pass filter rejects the excitation light, and fluorescence is detected with an avalanche photodiode. Image processing performed includes subtraction of fiber autofluorescence and calibration of individual fiber transmission efficiencies.

B. Imaging Protocol

IRB approval was granted by Stanford University Medical Center and the Veterans Administration Palo Alto Health Care Systems. Patients undergoing routine screening colonoscopy were recruited into the study, and informed consent was obtained. Those with coagulation abnormalities, poor preparation, or low tolerance for colonoscopy were excluded. A total of 54 subjects with mean age of 65 ± 10 years were included. When lesional mucosa, including hyperplasia and adenomatous lesions, was seen on the conventional white light image, a standard spray catheter was inserted through the instrument channel, and 3 ml of 5% acetic acid was topically applied to the mucosa to remove mucus followed by 3 ml of 5 mg/ml fluorescein sodium (Akorn, Inc, Buffalo Grove, IL). No rinse was applied. As shown in Fig. 1a, the confocal microscope (blue light from laser) was passed through the instrument channel (3.7 mm diameter) of the colonoscope (Olympus CFQ-160), whose objective lens collects the standard video image produced by reflection of white light. The distal end of the confocal miniprobe was then placed gently and directly onto the lesional mucosa and the immediately adjacent normal mucosa using the endoscopic image for guidance, until the contrast reached the focal plane of the instrument, as shown in Fig. 1b. The site imaged was centered in the field of view of the endoscope to avoid sites of fluorescein accumulation. The total amount of time needed to obtain the measurements per site varied from 2 to 3 minutes, including passing the spray catheter and miniprobe. A pinch biopsy was obtained from all sites imaged, and processed for routine histology which was used as the standard to distinguish 'normal' from 'hyperplastic' and 'adenomatous' mucosa (stained with hematoxylin and eosin, H&E).

C. Image Analysis

The captured video sequences were exported into individual images for evaluation. Images of normal and neoplastic mucosa from the same subject were grouped together. Those that displayed discrete glands with centrally located lumens and well-defined space in between the glands (lamina propria) were included in the analysis. The image immediately preceding the appearance of fluorescence signal in the running video record determined the start time ($t=0$). The transient ($t < 5$ sec) behavior of fluorescein uptake and movement was measured using Volocity software ver 4.0.1 (Improvision, Coventry, England) by identifying regions of interest (ROI) using an intensity threshold of 35% of the maximum in each image, a level sufficient to reject spurious ROI's that arise from noise. Image functionality was quantified by measuring the steady state fluorescence contrast ratio (CR) defined by a ratio of the mean intensity from the lamina propria to that of a crypt using an average of 3 sites chosen randomly from each region with an area of $35 \times 35 \mu\text{m}^2$, sufficient to span several cells and to account for image variability. The identity of the imaged structures were established by rigorous validation of image scale and image plane, so as to ensure satisfactory mapping of the image to the histologic samples obtained from the same site. The intensity measurements were made without knowledge of the histology results; likewise, histology was evaluated in a blinded fashion, without knowledge of either the endoscopic white light or fluorescent image findings.

D. Statistical Analysis

All results are expressed as mean $\pm$ standard deviation, and significance is determined using a paired t-test for 'normal versus lesional' and two-sided t-test with unequal variance for the others. The performance of the fluorescence contrast ratio to predict mucosal pathology was

evaluated using a leave-one-out cross-validation method where one data point was removed from the set, and the remaining data were used to generate a linear discriminant function with STATA, ver 9.2 (College Station, TX).²² The data point removed was then evaluated by this discriminant function, and mucosal sites were designated as positive for disease if the fluorescence contrast ratio was less than the discriminant function (negative if above). This process was repeated for all of the data points in each set, and the results were compared to histology as the standard. Bootstrapping was performed to increase the number of data points in each set by random sampling with replacement. Diagnostic performance is defined by sensitivity = $TP/(TP + FN)$, specificity = $TN/(TN + FP)$, positive predictive value = $TP/(TP + FP)$, negative predictive value = $TN/(TN + FN)$, and total accuracy = $(TP + TN)/(TP + TN + FP + FN)$, given the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN).²³

RESULTS

Real time confocal fluorescence images were collected *in vivo* from colonic mucosa following topical administration of fluorescein in $n = 54$ human subjects, and no toxicity associated with contrast administration was reported. Movement of fluorescein in the direction from the crypt lumen to the lamina propria was observed, and is shown in Fig. 2, scale bar 20 μm . Contrast can first be appreciated in the crypt at $t = 0.08$ sec, shown in Fig. 2a, where it then moves in a radial direction from the lumen toward basolateral aspect of the epithelial layer, and accumulates into focused intracellular points-of-fluorescence (dotted red circle) at $t = 0.67$ sec, shown in Fig. 2b, presumably intracellular vesicles. The fluorescence then enters the lamina propria at $t = 0.75$ sec, and remains as a point-of-fluorescence with travels with an average speed of $41.7 \pm 3.4 \mu\text{m/s}$, shown in Fig. 2c. The fluorescence intensity in the crypt decreases, concurrent with the appearance of multiple points-of-fluorescence (mean surface area of $0.54 \times 10^3 \pm 0.02 \times 10^3 \mu\text{m}^2$) in the lamina propria at $t = 4.5$ sec, shown in Fig. 2d. The radial speed of fluorescein travel within the crypt is shown in Fig. 2e for the transient phase ($t < 5$ sec), and a peak speed of $1.14 \pm 0.09 \mu\text{m/s}$ is achieved at $t = 0.5$ sec. The movement of two separate points-of-fluorescence within the lamina propria is shown in Fig. 2f, where the center of each is plotted in the horizontal plane at 0.083 sec intervals.

Steady state ($t > 5$ sec) images of normal colonic mucosa, hyperplasia, tubular adenoma and villous adenoma, along with the corresponding histology (H&E) are shown in Fig. 3, scale bar 40 μm . For normal mucosa, shown in Fig. 3a, individual colonocytes (c) can be seen surrounding the lumen (l) of the crypt, and the apical (ap) and basolateral (bl) epithelial cell membranes can be clearly distinguished. Contrast enhances the lamina propria (lp) in between the glands. The crypts are circular in shape, approximately uniform in size, and have oval lumens. For hyperplasia, shown in Fig. 3b, proliferative colonocytes (c) line an irregularly shaped lumen (l) that displays inward buckling in a serrated or saw-toothed appearance. The crypts are circular in shape but significantly larger in size than that of normal mucosa. Contrast can be seen in the lamina propria (lp) beyond the border of the gland. For the tubular adenoma, shown in Fig. 3c, distorted colonocytes (c) surround an elongated lumen (l), and the crypts appear eccentric in shape and are slightly larger in size than that of normal mucosa. Contrast appears to accumulate along the basolateral (bl) border of the epithelial cells rather than the apical (ap) surface. Moreover, the fluorescence intensity in the lamina propria (lp) appears reduced. For the villous adenoma, shown in Fig. 3d, enlarged colonocytes (c) surround a significantly elongated lumen (l). Contrast accumulates within the colonocytes in a punctuate fashion with multiple filling defects. The adenomatous mucosa exhibits a papillary appearance with elliptical mucosal profiles which are significantly larger in size than that of normal mucosa. Moreover, the fluorescence intensity in the lamina propria (lp) of these papillary profiles is significantly reduced.

The lamina propria: crypt fluorescence contrast ratio for normal mucosa (n=54), hyperplasia (n=24), tubular adenoma (n=25), and villous adenoma (n=5) are shown in the scatter plot in Fig. 4. For all subjects, a greater value was found for normal than for lesional mucosa, results shown in separate columns. On average, the fluorescence contrast ratios (mean $\pm$ std dev) for normal, hyperplasia, tubular adenoma, and villous adenoma are 1.29 $\pm$ 0.24 (range 0.85 to 1.74), 0.92 $\pm$ 0.10 (range 0.69 to 1.08), 0.60 $\pm$ 0.10 (range 0.43 to 0.81), and 0.41 $\pm$ 0.03 (range 0.38 to 0.44), respectively, and are represented by the horizontal lines. The type I error rate (p-value) was < 0.001 for 1) normal versus lesional mucosa (including hyperplasia, tubular adenoma, and villous adenoma), 2) hyperplasia versus adenoma (including tubular and villous), and 3) tubular versus villous adenoma.

By defining contrast ratios below the discriminant function as positive for lesional mucosa (above as negative) the tradeoffs among sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for distinguishing normal from neoplastic mucosa can be determined, shown in Fig. 5a. The optimal tradeoff between sensitivity and specificity is found from the receiver-operator characteristic (ROC) curve, shown in Fig. 5b, where the discriminant function of 0.0 results in a sensitivity, specificity and accuracy of 91%, 87% and 89%, respectively. Moreover, the area-under-the-curve (AUC) of 0.97 reflects the quality of this test (AUC = 1 is ideal). Similarly, use of the contrast ratio to distinguish hyperplasia from adenoma (dysplasia) is shown in Fig. 5c, where values below the discriminant function are defined as positive for dysplasia (above as negative). The corresponding ROC curve is shown in Fig. 5d and has an AUC = 0.99. A discriminant function of 0.5 corresponds to a sensitivity, specificity and accuracy of 97%, 96%, and 96%, respectively. Finally, the ability to distinguish tubular from villous adenomas is shown in Fig. 5e, where values below the discriminant function are defined as positive for villous (above as negative). The corresponding ROC curve is shown in Fig. 5f and has an AUC = 0.98. A discriminant function of -1.0 achieves a sensitivity, specificity and accuracy of 100%, 92%, and 93%, respectively. Bootstrapping the data with 200 points in each group was performed, but did not reveal any significant differences in the results.

DISCUSSION

Here, we demonstrate a novel method of functional optical imaging to perform accurate pathological evaluation of living tissue, using a fibered confocal microscope by observing crypt uptake and distribution of topically applied fluorescein. In the transient period ($t < 5$ sec), we observed contrast enter through the crypt lumen, travel across the epithelium, and accumulate in basolateral intracellular vesicles that dissipate in the lamina propria. Subsequently, the lamina propria: crypt fluorescence intensity ratio was used to assess pathology during steady state ($t > 5$ sec). This approach is quantitative, thus may improve our ability to make a definitive evaluation of disease and improve patient outcomes in situations where conventional pathology has shown limitations. For example, although pathologists are readily able to distinguish ‘adenomatous’ (dysplastic) polyps obtained during screening colonoscopy from normal and hyperplastic mucosa in the colon, the assessment of dysplasia in the setting of ulcerative colitis²⁴ and Barrett’s esophagus²⁵ is plagued by lack of diagnostic consistency, as evidenced by human interpretive differences, and significant inter- and intra-observer variability.^{26,27}

In this study, dynamic observation of the behavior of topical fluorescein provides immediate ability to distinguish dysplasia from hyperplastic and normal mucosa. Indeed, the result found for normal mucosa (CR > 1) suggests that crypt transport mechanisms play a more significant role than paracellular permeability for maintaining fluorescein homeostasis, and the finding for lesional mucosa (CR < 1) implies that transport is impaired in transformed mucosa, resulting in a potentially very accurate method for discriminating between normal and lesional mucosa *in situ*. Furthermore, the results for distinguishing hyperplasia from adenoma and tubular from

villous adenoma are promising, and suggest that this method may be a useful clinical tool in that regard as well. While the diagnostic performance of this method was quite good in this study, the results may differ for a new validation sample or in clinical practice. This study does not permit anatomic identification of the points-of-fluorescence that accumulate in the lamina propria, but it seems reasonable to note that the lamina propria is a heterogeneous tissue compartment so that sequestration of the fluorescent solute may well be occurring.

The fibered confocal microscope used in this study has the size and flexibility to pass through the instrument channel, and to be accurately placed onto the mucosa with guidance from the white light image. Furthermore, topical administration of contrast is safe and adequate for visualizing epithelial function. Optical sections (working distance 0 μm , depth resolution 15 μm) were collected near the mucosal surface to best visualize luminal uptake of contrast. Previous studies of endoscopic confocal images used intravenously administered fluorescein to demonstrate instantaneous snapshots of tissue that exhibit a striking resemblance to subsequent histology.^{11–13} However, observation of functional mucosal physiology, such as contrast uptake, would be challenging at the imaging speeds (0.8 frames per second) used. Furthermore, while separate optics are used for collecting the white light and confocal images, the components are packaged together in the distal end of the endoscope, a feature that limits use of the white light image to guide placement of the confocal window.

This study represents a first demonstration of the use of physiological properties of viable tissue to perform assessment of pathology. Future applications may include targeted detection with topically administered peptides,²⁸ antibodies,²⁹ and small molecules.³⁰ Functional imaging is commonly performed in clinical radiology for the study of solid organs. For example, positron emission tomography (PET) assesses tissue metabolism of intravenously administered ¹⁸fluorodeoxyglucose (FDG) to distinguish benign from malignant tumors.³¹ The continued development of functional methods with optical imaging has great importance for the detection and evaluation of pre-malignant lesions in the epithelium of hollow organs, where greater than 80% all cancer originate and early detection may be preventative.³² Furthermore, with use of medical endoscopes, fibered instruments can be placed in close proximity to tissue and can collect images with sub-cellular resolution in real time, a level of performance that far exceeds that of other imaging modalities. Finally, methods of functional optical imaging can be generalized to evaluate pathology in other hollow organs, such as bladder, breast, cervix, colon, lung, oropharynx, pancreas, skin and stomach.

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References

1. Pawley, JB., editor. Handbook of Biological Confocal Microscopy. 3. Springer Verlag; New York: 2006.
2. Yelin D, Rizvi I, White WM, Motz JT, Hasan T, Bouma BE, Tearney GJ. Three-dimensional miniature endoscopy. *Nature* 2006;443:765. [PubMed: 17051200]
3. Rouse AR, Kano A, Udovich JA, Kroto SM, Gmitro AF. Design and demonstration of a miniature catheter for a confocal microendoscope. *Appl Opt* 2004;43:5763–71. [PubMed: 15540433]
4. Laemmel E, Genet M, Le Goualher G, Perchant A, Le Gargasson JF, Vicaut E. Fibered confocal fluorescence microscopy (Cell-viZio) facilitates extended imaging in the field of microcirculation. A comparison with intravital microscopy. *J Vasc Res* 2004;41:400–11. [PubMed: 15467299]

5. Kiesslich R, Burg J, Vieth M, Gnaendiger J, Enders M, Delaney P, Polglase A, McLaren W, Janell D, Thomas S, Nafe B, Galle PR, Neurath MF. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer *in vivo*. *Gastroenterology* 2004;127:706–13. [PubMed: 15362025]
6. Thiberville L, Moreno-Swirc S, Vercauteren T, Peltier E, Cave C, Bourg Heckly G. *In Vivo* Imaging of the Bronchial Wall Microstructure Using Fibered Confocal Fluorescence Microscopy. *Am J Respir Crit Care Med* 2007;175:22–31. [PubMed: 17023733]
7. Kitabatake S, Niwa Y, Miyahara R, Ohashi A, Matsuura T, Iguchi Y, Shimoyama Y, Nagasaka T, Maeda O, Ando T, Ohmiya N, Itoh A, Hirooka Y, Goto H. Confocal endomicroscopy for the diagnosis of gastric cancer *in vivo*. *Endoscopy* 2006;38:1110–4. [PubMed: 17111332]
8. Kiesslich R, Goetz M, Burg J, Stolte M, Siegel E, Maeurer MJ, Thomas S, Strand D, Galle PR, Neurath MF. Diagnosing *Helicobacter pylori* *in vivo* by confocal laser endoscopy. *Gastroenterology* 2005;128:2119–23. [PubMed: 15940642]
9. Meining A, Bajbouj M, Schmid RM. Confocal Fluorescence Microscopy for Detection of Gastric Angiodysplasia. *Endoscopy*. 2007in press
10. Meining A, Schwendy S. In-vivo Histopathology of Lymphocytic Colitis. *GI Endo*. 2007in press
11. Kiesslich R, Gossner L, Goetz M, Dahlmann A, Vieth M, Stolte M, Hoffman A, Jung M, Nafe B, Galle PR, Neurath MF. *In vivo* histology of Barrett's esophagus and associated neoplasia by confocal laser endomicroscopy. *Clin Gastroenterol Hepatol* 2006;4:979–87. [PubMed: 16843068]
12. Kakeji Y, Yamaguchi S, Yoshida D, Tanoue K, Ueda M, Masunari A, Utsunomiya T, Imamura M, Honda H, Maehara Y, Hashizume M. Development and assessment of morphologic criteria for diagnosing gastric cancer using confocal endomicroscopy: an ex vivo and in vivo study. *Endoscopy* 2006;38:886–90. [PubMed: 16981104]
13. Polglase AL, McLaren WJ, Skinner SA, Kiesslich R, Neurath MF, Delaney PM. A fluorescence confocal endomicroscope for in vivo microscopy of the upper- and the lower-GI tract. *Gastrointest Endosc* 2005;62:686–95. [PubMed: 16246680]
14. Kumar, V.; Fausto, N.; Abbas, A., editors. Robbins and Cotran: Pathologic Basis of Disease. 7. WB Saunders Co; Philadelphia, PA: 2005.
15. Physicians' Desk Reference. 60. Thomson PDR; Montvale, NJ: 2006.
16. Ballesterio MR, Monte MJ, Briz O, Jimenez F, Gonzalez-San Martin F, Marin JJ. Expression of transporters potentially involved in the targeting of cytostatic bile acid derivatives to colon cancer and polyps. *Biochem Pharmacol* 2006;72:729–38. [PubMed: 16844096]
17. Hawkins BT, Ocheltree SM, Norwood KM, Eggleton RD. Decreased blood-brain barrier permeability to fluorescein in streptozotocin-treated rats. *Neuroscience Letters* 2007;411:1–5. [PubMed: 17110033]
18. Endter S, Becker U, Daum N, Huwer H, Lehr CM, Gumbleton M, Ehrhardt C. P-glycoprotein (MDR1) functional activity in human alveolar epithelial cell monolayers. *Cell Tissue Res*. 2006
19. Kuwayama K, Miyauchi S, Tateoka R, Abe H, Kamo N. Fluorescein uptake by a monocarboxylic acid transporter in human intestinal Caco-2 cells. *Biochem Pharmacol* 2002;63:81–8. [PubMed: 11754877]
20. Seki T, Kanbayashi H, Nagao T, Chono S, Tabata Y, Morimoto K. Effect of cationized gelatins on the paracellular transport of drugs through caco-2 cell monolayers. *J Pharm Sci* 2006;95:1393–401. [PubMed: 16625653]
21. Jewell C, Cashman KD. The effect of conjugated linoleic acid and medium-chain fatty acids on transepithelial calcium transport in human intestinal-like Caco-2 cells. *Br J Nutr* 2003;89:639–47. [PubMed: 12720584]
22. Mehrubeoglu M, Kehtarnavaz N, Marquez G, Duvic M, Wang LV. Skin lesion classification using oblique-incidence diffuse reflectance spectroscopic imaging. *Applied Optic* 2002;41:182–92.
23. Rosner, B. Fundamentals of Biostatistics. 6. Belmont, CA: Duxbury Press; 2005.
24. Rubin DT, Turner JR. Surveillance of dysplasia in inflammatory bowel disease: The gastroenterologist-pathologist partnership. *Clin Gastroenterol Hepatol* 2006;4:1309–13. [PubMed: 17110299]
25. Sharma P, Falk GW, Weston AP, Reker D, Johnston M, Sampliner RE. Dysplasia and cancer in a large multicenter cohort of patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2006;4:566–72. [PubMed: 16630761]

26. Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, Ahren C, Correa P, Hamilton SR, Morson BC, et al. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum Pathol* 1983;14:931–68. [PubMed: 6629368]
27. Reid BJ, Haggitt RC, Rubin CE, Roth G, Surawicz CM, Van Belle G, Lewin K, Weinstein WM, Antonioli DA, Goldman H, et al. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 1988;19:166–78. [PubMed: 3343032]
28. Kelly K, et al. Detection of invasive colon cancer using a novel, targeted, library-derived fluorescent peptide. *Cancer Res* 2004;64:6247–51. [PubMed: 15342411]
29. Koppe MJ, Oyen WJ, Bleichrodt RP, Hendriks T, Verhofstad AA, Goldenberg DM, Boerman OC. Combination therapy using the cyclooxygenase-2 inhibitor Parecoxib and radioimmunotherapy in nude mice with small peritoneal metastases of colonic origin. *Cancer Immunol Immunother* 2006;55:47–55. [PubMed: 15868166]
30. Wang W, Kim SH, El-Deiry WS. Small-molecule modulators of p53 family signaling and antitumor effects in p53-deficient human colon tumor xenografts. *Proc Natl Acad Sci U S A* 2006;103:11003–8. [PubMed: 16835297]
31. von Schulthess GK, Steinert HC, Hany TF. Integrated PET/CT: current applications and future directions. *Radiology* 2006;238:405–22. [PubMed: 16436809]
32. American Cancer Society. *Cancer Facts and Figures 2006*. American Cancer Society; Atlanta: 2006.

Abbreviations

l	lumen
c	crypt
lp	lamina propria
ap	apical
bl	basolateral
CR	contrast ratio

Fig. 1.

a) The fiberoptic confocal microscope (blue light from laser) passes through the instrument channel of a standard colonoscope, whose objective collects a wide field image under white light illumination, b) guiding placement for *in vivo* imaging of polyp and normal mucosa.

Fig. 2.

Movement of fluorescein in colonic mucosa. a) initial uptake by crypt, $t = 0.08$ sec; b) expansion from lumen toward basolateral aspect of epithelium and accumulation into focused intracellular points (dotted red circle), $t = 0.67$ sec; c) point of fluorescence enters the lamina propria, $t = 0.75$ sec; d) concurrent appearance of multiple points in the lamina propria, $t = 4.5$ sec, scale bar $20 \mu\text{m}$. e) Instantaneous radial speed of *en face* fluorescein movement through epithelium in direction from lumen to lamina propria, peak occurring at $t = 0.5$ sec. f) Movement of two separate points of fluorescence within the lamina propria at 0.083 sec time intervals.

Fig. 3. Steady state ($t > 5$ sec) distribution of fluorescence in colon with corresponding histology (H&E) for a) normal mucosa, b) hyperplasia, c) tubular adenoma, and d) villous adenoma, scale bar 40 μ m. The crypt lumens (l), apical (ap) and basolateral (bl) borders of epithelial cells (c), and lamina propria (lp) can be distinguished.

Fig. 4.

Lamina propria: crypt contrast ratios of fluorescence intensities from neighboring sites of normal (n=54) and lesional (n=24 hyperplastic, n=25 tubular adenoma, and n=5 villous adenomas) mucosa from the same patient are shown in separate columns.

Fig. 5. Quantitative performance of functional parameter (lamina propria: crypt fluorescence contrast ratio) used to assess pathology and ROC curve of tradeoffs for distinguishing a,b) normal from lesional; c,d) hyperplasia from adenoma; e,f) tubular from villous adenoma.