

Is light-induced fluorescence better than the endoscopist's eye?

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NE Marcon. Is light-induced fluorescence better than the endoscopist's eye? *Can J Gastroenterol* 1999;13(5):417-421. While recognizing advanced tumours with endoscopy does not generally pose a challenge, cure rates are relatively low, depending on the size and stage of the tumour. Screening tests for cancer are advantageous for diagnosing cancers before the date after which a cure is no longer an option. Many gastrointestinal cancers are diagnosed after the date on which a cure is possible. The present article discusses some of the limitations of conventional white light endoscopy in screening and presents some of the fluorescent-based diagnostics that are being investigated as complements to white light endoscopy. Autofluorescence and fluorescence due to exogenous photosensitizers or precursors are two sources of fluorescence that are being studied. Preliminary results of current investigations are presented, and future research directions are described.

Key Words: Endoscopy; Fluorescence; Gastrointestinal cancer

La fluorescence induite par la lumière est-elle meilleure que l'œil de l'endoscopiste

RÉSUMÉ : Alors que la reconnaissance de tumeurs avancées par endoscopie ne pose généralement pas de problème, les taux de guérison sont relativement bas et dépendent de la taille et du stade de la tumeur. Les tests de dépistage du cancer se révèlent avantageux pour diagnostiquer ces cancers avant qu'il ne soit plus possible de les guérir. De nombreux cancers gastro-intestinaux sont diagnostiqués après toute possibilité de guérison. Le présent article discute certaines des limitations de l'endoscopie traditionnelle à la lumière blanche pour le dépistage et présente quelques diagnostics basés sur l'utilisation de la fluorescence qui sont actuellement à l'étude comme compléments à l'endoscopie à la lumière blanche. L'autofluorescence et la fluorescence dues à des photosensibilisateurs exogènes ou des précurseurs constituent deux sources de fluorescence qui sont actuellement à l'étude. Les résultats préliminaires des recherches actuelles sont présentés et les directions de la recherche future sont décrites.

Recognizing advanced tumours does not generally pose an endoscopic challenge, but the cure rates are relatively low depending on the tumour size and stage. Although the ideal approach is prevention, it is not likely to be immediately achieved. The next best option is to diagnose malignancy at an early stage while it is still confined to the mucosa.

There has been an increasing interest in screening algorithms. The benefit of a screening test in cancer is to advance the date of diagnosis to before the date of escape from cure. For many common gastrointestinal cancers, escape

from curability occurs before the date of customary diagnosis. The questions are, how early is 'early' and how can one decide which lesions in which populations should be screened?

In cancer of the esophagus, the overall five-year survival rate is a dismal 5%. However, if the disease is diagnosed when it is limited to the mucosa, the five-year survival rate is over 90% (1). Endoscopic screening with white light depends on visualizing the mucosa, and targeting for biopsy topographic abnormalities, subtle or obvious nodules, and irregularities. In situations where these are not recognized, a protocol of multiple random samples is undertaken. Will

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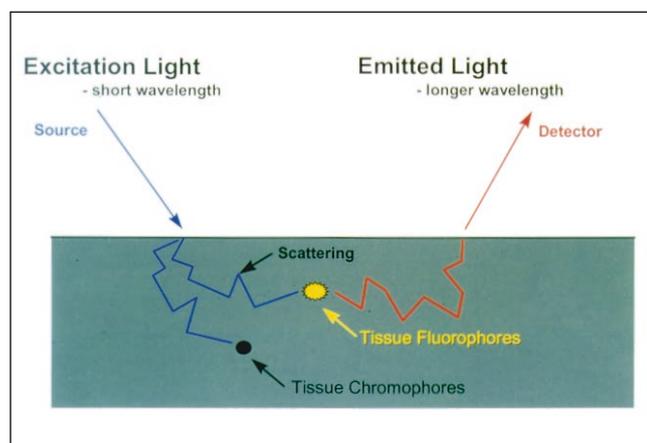


Figure 1) The principle of tissue autofluorescence. Fluorescent molecules (fluorophores) occurring naturally (endogenously) in the tissue are excited by short wavelength light and subsequently produce light at longer wavelengths. Tissue chromophores absorb the excitation light and do not produce fluorescence

new endoscopic technologies such as fluorescence turn out to be better than the endoscopist's eye and allow the identification of more subtle lesions that would be missed with conventional white light endoscopy (WLE)?

A major clinical advance would be an endoscopic system to complement WLE – a system that could identify and locate dysplasias with a high degree of sensitivity and specificity. Lesions not seen with conventional WLE would then be targeted for biopsy. Patients with lesions limited to the mucosa could then be considered for cure with endoscopic techniques, with an excellent chance for cure without the morbidity associated with traditional surgery, or radiation and chemotherapy.

WLE IN DETECTION OF DYSPLASIA

Surprisingly few comparative studies have been published on the ability of white light to detect dysplasia. Shiozaki et al (2) screened patients with head and neck cancer for associated esophageal cancer by using conventional WLE, followed by Lugol's iodine staining. Of 178 patients screened, 13 superficial cancers were detected in nine patients. Nine were not detected with WLE but only with the adjuvant use of Lugol's iodine. All patients were asymptomatic, and eight were node-negative at resection. The results of this study support a role for Lugol chromoendoscopy as an adjuvant to WLE in the squamous esophagus. It should be noted that there was a high incidence of non-neoplastic unstained lesions (a false-positive rate of 55%). Suvakovic et al (3) reported that, of a group of patients diagnosed with cancer of the stomach, one-fifth had had a 'normal' gastroscopy within the previous three years. A dysplastic lesion was not recognized. Assuming that there is an incubation period of years from dysplasia to a mass, perhaps a more sensitive endoscopic imaging system would have identified these lesions at an earlier and, therefore, curable state. Examination of the colon for polyps has its miss rate in back-to-back colonoscopies. Rex et al (4) reported that an experienced endoscopist could

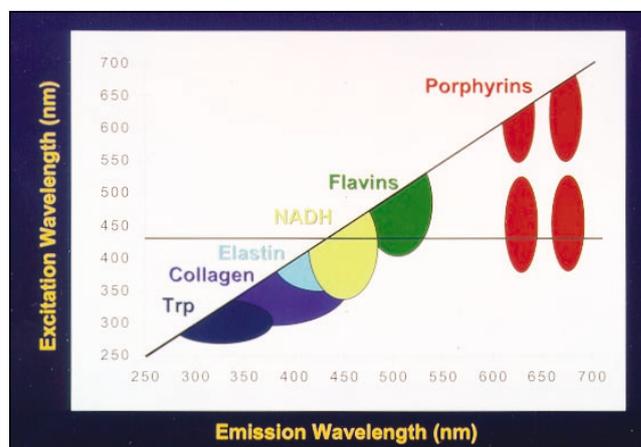


Figure 2) Tissue fluorophores

miss 15% to 24% of neoplastic polyps less than 1 cm, and up to 6% of larger polyps were overlooked at the time of colonoscopy. Up to 45% of patients with ulcerative colitis found to have high grade dysplasia on initial screening colonoscopy have been reported to have a coincidental colorectal cancer that was not endoscopically evident before resection.

FLUORESCENCE-BASED DIAGNOSTICS

Fluorescence-based diagnostics are being investigated as a complementary tool for WLE to improve the detection of dysplasia (5). The two sources of fluorescence are a native, or autofluorescence, and fluorescence due to exogenous photosensitizers or precursors. All tissue, when exposed to either ultraviolet or blue light, emits fluorescent light of a longer wavelength (Figure 1). This light is spread over a range of longer visible wavelengths from green to red. The source of these emitted spectra is naturally occurring molecules (fluorophores) such as collagen, nicotinamide adenine dinucleotide, flavins and porphyrins (Figure 2). Chromophores such as hemoglobin absorb but do not emit fluorescent signals.

Autofluorescence detects changes in the concentration, distribution, and depth of one or more of these components of tissue microarchitecture. As normal tissue transforms to dysplasia through to cancer, it develops a neovasculature where hemoglobin (chromophore) interferes with light penetration as well as emission. As normal tissue becomes dysplastic, its fluorescent signal decreases. Changes are wavelength dependent. Differences in these spectral wavelengths correlate with changes in histology.

Because of technical problems with some detection systems, attempts have been made to enhance the fluorescent signal and detection of dysplasia by the use of exogenous photosensitizers or precursors. A promising compound is 5-aminolevulinic acid, which is a precursor to protoporphyrin IX, which has fluorescent properties (6). The diagnostic efficacy is dependent on a degree of selective localization of the drug in the premalignant tissue. This occurs mainly in the mucosal epithelium. 5-aminolevulinic acid has allowed the

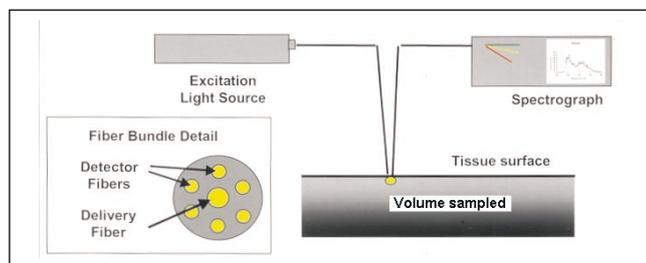


Figure 3) Schematic diagram depicting light-induced fluorescence (LIF) spectroscopy. LIF spectroscopy only samples a small region of the tissue surface layers but measures the full spectrum of fluorescence wavelengths

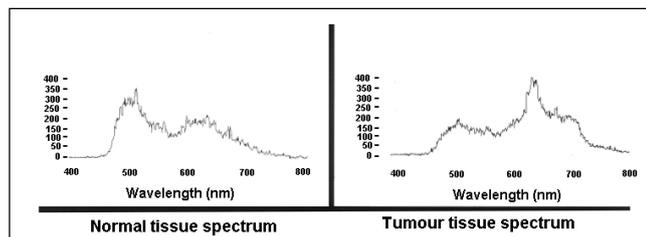


Figure 4) Examples of a normal tissue spectrum and a tumour tissue spectrum

detection of dysplasia in Barrett's esophagus and colitis in some cases (7). The addition of a drug to a screening program adds extra expense, subjects the patient to sunlight sensitivity, albeit for only two to three days, and results in potential liver toxicity and problems with regulatory approval.

HOW TO DETECT FLUORESCENCE ENDOSCOPICALLY

There are two endoscopic methods of detecting autofluorescence and fingerprinting the histological diagnosis of a tissue (normal, hyperplastic, dysplastic or carcinoma) – light-induced fluorescence (LIF) spectroscopy and LIF endoscopy. LIF spectroscopy uses a small diameter optical probe composed of several collecting sensor fibres surrounding a central excitation fibre (Figure 3). The probe is passed through the instrument channel of the endoscope and triggered by a foot pedal. Placed in contact with the tissue, the probe excites and detects the fluorescence in a small volume of tissue around the probe tip. This is recorded as a line spectral curve on a monitor (Figure 4).

Most point spectroscopy devices use steady-state fluorescent measurements. Panjehpour et al (8) used 410 nm blue light and a specific algorithm. Another recent study (9) used time-resolved fluorescent spectroscopy, which measures the delay of fluorescence intensity at a given emission wavelength as a fraction of time after a brief pulse of excitation light (~ 10 ns) at a wavelength of 385 nm. Clinical trials are in progress.

In comparison, real time LIF endoscopy is capable of evaluating the entire mucosal surface. The excitation light (at a single wavelength from a laser or narrow wavelength band from a filtered lamp) serves as the endoscopic light source. The illuminated area produces autofluorescence

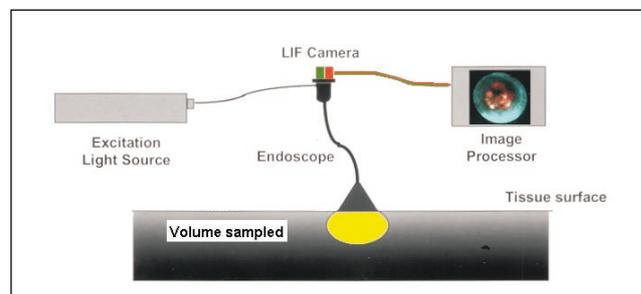


Figure 5) Schematic diagram depicting light-induced fluorescence (LIF) endoscopy. LIF endoscopy is capable of examining the entire mucosal surface by producing pseudocolour images based on spectral ratios

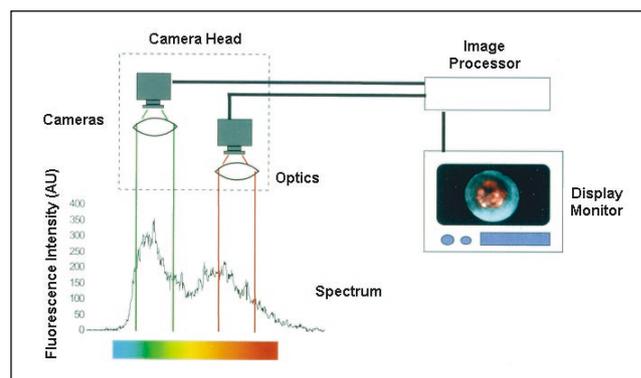


Figure 6) Schematic diagram of the principle of fluorescence imaging. The fluorescence is collected in green and red wavelength bands via two charge-coupled device cameras and processed to produce an image that contrasts normal green mucosa to abnormal red mucosa

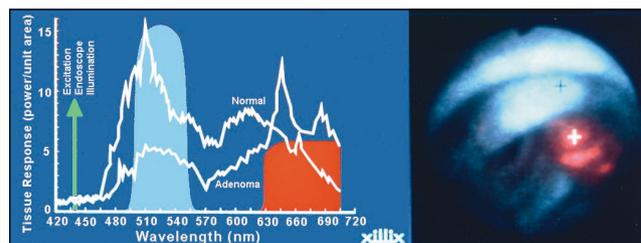


Figure 7) Autofluorescence image of high grade adenoma lesion in the colon with corresponding spectra, using the Xillix LIFE-GI Fluorescence Endoscopy System (Xillix Technologies Corp, Richmond, British Columbia)

when excited by the blue light. A dual spectrum intensified camera captures the resulting autofluorescence image (Figures 5,6). The spectral differences, which contain information about pathology, are used to form a real time pseudocolour image of the tissue, where the normal tissue is green and abnormal tissue is red. The ratio of red to green in the imaging system is standardized over normal mucosa. An image is transmitted in real time to a monitor for the endoscopist to view (Figure 7). The current system can switch back and forth from the white (conventional) endoscopic image to the fluorescent image almost simultaneously, thus rapidly observing wide areas of the mucosa. Two images are formed in real time under blue light excitation (437 nm) – one cor-

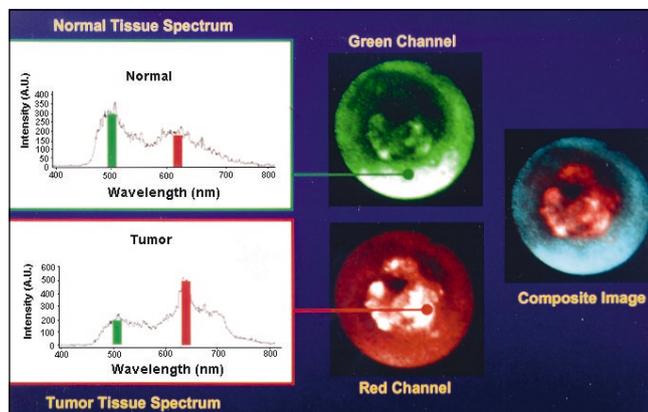


Figure 8) Composition of a light-induced fluorescence spectroscopy image

responds to the green region of the spectrum and the other to the red region. These images are then computer combined so that the endoscopist sees a single real time image in false colour, which depends on the relative red and green fluorescent intensities (Figure 8).

The field of autofluorescence in gastrointestinal endoscopy is less than 10 years old, and in vivo imaging has only begun to appear in the literature in the past two years. The bulk of work has been done using point spectroscopy. In the first in vitro studies, excised colonic polyps were examined with ultraviolet excitation (325 nm) (10). A multivariate analysis of the fluorescent emission spectrum (350 to 600 nm) distinguished normal colon from adenomatous and hyperplastic polyps with accuracies of 100% and 94%, respectively. In the first blind study carried out in vivo using point spectroscopy (370 nm excitation) and a probability-based algorithm to detect colonic dysplasia, Cothren et al (11) determined the correct tissue type in 88% of cases, with a sensitivity of 90% and a specificity of 95%. Using point spectroscopy with 410 nm excitation, Panjehpour (8) applied a stepwise discriminative analysis to selected emission wavelengths to attempt to differentiate between normal squamous tissue and malignant esophageal tissue with a sensitivity of 100% and a specificity of 98%. This group (12) also studied selected patients with Barrett's esophagus and dysplasia. There was a high false positive rate (around 30%); this system was unable to distinguish low-grade dysplasia. It was the first study to identify endoscopically occult premalignant lesions based on tissue autofluorescence.

Studies of LIF spectroscopy (point spectroscopy) have clearly established that tissue autofluorescence can be used to determine accurately the pathological status of gastrointestinal tissue, both in vitro and in vivo. However, the clinical application of such a diagnostic 'point' system has some of the same limitations as traditional endoscopic surveillance for occult dysplasia, including the random process of tissue sampling, the small fraction of mucosa examined and the added question of the generalization of the various analytical techniques. Thus, there is a great advantage to a system that examines all of the surface and allows for target

biopsy. An analogous bronchoscopic system has been developed (Xillix LIFE-GI Fluorescence Endoscopy System, Xillix Technologies Corp, Richmond, British Columbia), which is now commercially available. This system demonstrated a 171% increase in the detection of moderate to severe dysplasia compared with white light bronchoscopy alone, with only a 22% reduction in diagnostic specificity (14). We have had the opportunity to test this system in a large number of gastrointestinal patients. The sensitivity and positivity were calculated comparing the positive (bright red fluorescence) and negative (green fluorescence) clinical endoscopic interpretation by the endoscopy with the histological diagnosis of the corresponding biopsy samples. The sensitivity and positivity for atypia or higher grade lesions were 87% and 79%, respectively (13). All lesions seen with WLE were also seen with the Xillix system. In addition, a number of dysplastic cases not recognized by WLE were 'seen' by LIF endoscopy and were targeted for biopsy in Barrett's esophagus and the colon.

Fluorescent endoscopy imaging has great potential to identify dysplasia, which is seldom identified endoscopically. It can distinguish hyperplastic from adenomatous polyps, survey the scar of previous polypectomies for persistence or recurrence and is useful in monitoring photodynamic therapy photo bleaching.

There are, however, some problems that require further refinement. Inflammatory conditions, both acute (ie, infectious colitis) and chronic (Barrett's esophagus and long standing inflammatory bowel disease) also may give positive (red) images. Excluding the group with Barrett's esophagus, the sensitivity and specificity were 83% and 84%, respectively. All systems that rely on spectroscopy can also record false positive images related to noninflammatory factors, such as adherent food or stool. Thus, in the case of colonoscopy, vigorous bowel preparation and a clean colon are necessary.

A number of questions remain unsolved in relation to autofluorescence diagnostics. What is the optimal choice of the excitation wavelength (below 400 nm or visible light)? Is fluorescence imaging required or can adequate diagnostic information be obtained simply by measuring the complete fluorescence spectra at individual points (point spectroscopy)? What is the best way to analyze the measured spectra or images?

The imaging systems of the future will have simultaneous viewing of white light and fluorescence real time imaging. These may also be coupled with other optical sensing parameters, such as Raman confocal spectroscopy and optical coherent tomography.

Fluorescent endoscopy has great promise to improve the diagnosis of dysplasia. The next millennium will begin the era of molecular endoscopy.

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