Spectroscopic Applications in Gastrointestinal Endoscopy

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Recently, a number of reports have heralded endoscopic triumphs in the cancer prevention arena. For instance, colonoscopic polypectomy resulted in ~50% reduction in mortality from colorectal cancer (CRC)[1]. With regards to Barrett's, radio-frequency ablation showed a remarkable ~90% decrease in esophageal adenocarcinoma development in patients harboring high grade dysplasia patients [2]. However, there is considerable work remaining as highlighted by the continued toll of CRC (second leading cause of cancer deaths) and the increasing incidence of esophageal adenocarcinoma. This provides the impetus for development of adjunct endoscopic technologies.

For colonoscopy, one of the vexing obstacles is the marginal protection of colonoscopy against right-sided disease. [3] In addition, the adenoma detection rate varies several fold even among well-trained endoscopists[4] and this appears to mirror CRC protection[5]. Furthermore, modest patient compliance with colonoscopy has meant that much less accurate approaches (e.g. fecal immunohistochemical blood test) have almost comparable rate of CRC diagnosis [6]. Finally, since early stage CRC and advanced adenomas are infrequent in the screening population (~6-7%), the vast majority of colonoscopies have no direct cancer prevention benefit that can be derived from polypectomy of biologically significant precursor lesions. . This is also true for upper endoscopies for esophageal adenocarcinoma given recent reports that Barrett's progression to carcinoma is markedly lower than previous estimates[7].

To address these hurdles, there have been a myriad of optical technologies developed. These span the gamut from improved visualization through modifications incorporated into the endoscope (e.g. high definition, narrow band imaging, autofluorescence) to fiberoptic probes that can yield microscope-like images (including

optical coherence tomography, confocal endomicroscopy) or visualize molecular probe or provides tissue micro-architectural quantification via various spectroscopic approaches[8]. For this review, we will primarily focus on spectroscopy since many non-spectroscopic techniques were covered in a recent Advances in Translational Science.[9]

Principles of Spectroscopy

Spectroscopy characterizes objects based on how it interacts with light. Light scattering can be dichotomized into elastic (no change in energy and hence wavelength with scattering; this is the dominant process of light transport in tissue) and non-elastic (scattering alters wavelength). Spectroscopic analysis typically harnesses light scattered in the backward direction (towards incident light) and can encompass a wide range of wavelengths. Elastic scattering is driven by spatial variations of tissue refractive index (determined by local macromolecular density) and thus provides fundamental insights into the size distribution of tissue structures (organelles, chromatin structure, collagen fibers, etc.) and the spatial correlation of macromolecular density (figure 1). Depending on the technique, length scales as large as a few microns (e.g., cell nuclei) and as small as a few tens of nanometers (e.g., macromolecular complexes) can be assessed. In addition, as light propagates through the tissue, absorption is inevitable and mainly due to hemoglobin. Thus, the absorption spectra can be used to measure tissue blood concentration, oxygen saturation, and sizes of blood vessels. With regards to inelastic light scattering the most commonly employed is Raman spectroscopy which measures vibrational and rotational aspects of molecules and thus gives insights into the molecular composition

of the tissue with its ability to discriminate proteins, nucleic acids, lipids, etc. Given that the majority of clinical trials to date have related to elastic light scattering spectroscopic, we will focus on these technologies.

Spectroscopic techniques can also be classified based on a mode of signal acquisition (figure 1): "point measurement" fiber-optic spectroscopy (the depth of measurements is determined by the source of contrast and probe design), *in situ* spectroscopic microscopy (e.g., low-coherence interferometry (LCI), inverse scattering spectroscopic optical coherence tomography (ISOCT)), and *ex vivo* spectroscopic microscopy (e.g., partial wave spectroscopic nanocytology, quantitative phase microscopy).

Advantages of Spectroscopy:

Spectroscopic output is typically quantitative and not an image which is both a major strength and potential weakness. On the positive side, this does not require particular training in image analysis (not requiring the gastroenterologist to become a pathologist). In addition, tissue characteristics that otherwise are not possible to assess by means of endoscopic visualization can be assessed such as chemical composition and nanoscale tissue structure. Conversely, the lack of image may lead to some reticence (more of a "black box" approach) and may not adequately capture tissue/tumor heterogeneity. Other positive attributes of spectroscopy include no requirements for contrast agents (in contradistinction to confocal or molecular imaging) and several fold better resolution than conventional light microscopy (interrogating structures at submicron length scales).[8] Furthermore, spectroscopy

typically can yield rapid, almost real time assessment which is mandatory for clinical practice.

Clinical Applications/Current State of the Art:

Instead of focusing on technology *per se*, it is more apropos to focus on clinical utility. As summarized in figure 2, for biomedical optics in general, and spectroscopy in particular, these applications can be broadly subdivided into four major categories: 1. Optical biopsy 2. Identification of flat dysplasia 3. Improved polyp detection 4. Risk stratification through field carcinogenesis detection.

Optical biopsy

Clinical Need: Optical biopsy refers to *in situ* determination of the histology. This is attractive since it may allow eschewing polypectomy (if the lesion is non-adenomatous), with its inherent risks and cost (pathology charges etc). Furthermore, occasionally polyp retrieval from the right colon necessitates complete colonoscope withdrawal and re-insertion thereby increasing procedure time, patient discomfort and risk of complications. Deciding which polyps can forego removal ("diagnose and leave behind") or pathological analysis ("resect and discard strategy") would markedly increase the efficiency of endoscopic practice*Technologies and Performance:* There are a number of non-spectroscopic (confocal endomicroscopy, chromo-endoscopy with pit pattern analysis) as well as spectroscopic techniques designed for this indication. With regards to the latter, one example is elastic scattering spectroscopy (ESS). ESS has shown excellent discrimination between hyperplastic versus adenomatous tissue (84% sensitivity, 84% specificity)[10]. Whether this is sufficient for clinical practice is unclear (see "obstacles" section below). ESS uses separated delivery and collection fibers to obtain a signal that arises largely from the first millimeter of tissue (mucosa

and submucosa) and enables a quantitative analysis of tissue micro-morphology and blood supply. Importantly, it has already been coupled with a polypectomy snare fostering translational to clinical practice.

Identification of Polyps

Clinical Needs: Approaches to improve adenoma detection have either targeted endoscopic blind spots (behind folds, flexures) or visualization of subtle lesions in the field of view (e.g. flat and depressed lesions). Much of the efforts to date has been on improvements to the endoscope *per se* (high definition, narrow band imaging, auto-fluorescence etc.) or contrast agents (molecular imaging, chromo-endoscopy) which have been recently reviewed[9].

Technologies and Performance: Since most spectroscopy is typically narrow field (small amount of tissue interrogated), it is not necessarily conducive to this application. However, one approach has been polarization-gated spectroscopy for detection of the increase in blood supply (EIBS), found in the normal mucosa surrounding or in the area of a lesion. This can have a potential applications as a "red flag" (identifying neoplasia-harboring colonic segments that warrants increased endoscopic scrutiny) with a sensitivity for advanced adenomas of 92% and specificity of 78%[11]. Moreover, fine mapping may be possible since the magnitude of EIBS mirrors proximity to the lesion.

Flat Dysplasia Detection

Clinical Need: Flat dysplasia is the hallmark for carcinogenesis in Barrett's and ulcerative colitis. The current state of the art is multiple random biopsies looking for the proverbial "needle in the haystack". However, obtaining a large number of random

biopsies are tedious, expensive, adds potential complications and potential false negatives (since typically <5% of mucosa is sampled).

Technologies and Performance: There are a plethora of techniques that have been developed with applications largely focused on Barrett's esophagus. One of the most widely used techniques appears to be confocal endomicroscope although a recent multi-center study failed to validate this approach[12]. Spectroscopic approaches for dysplasia identification in Barrett's esophagus include:

1. Light Scattering Spectroscopy (LSS)—LSS uses polarization to isolate scattering from the surface layer of the epithelium to provide information predominantly on nuclear size. In a small clinical trial of the first generation system, LSS identified dysplasia with ~90% accuracy[13]. The next generation system (endoscopic polarized scanning spectroscopy) had a sensitivity of 92% and a specificity of 96%[14].

2. Angle-resolved low coherence interferometry (a/LCI). This novel technique evaluates nuclear size with the depth resolution making it particularly powerful. Indeed, a/LCI nuclear measure at 200-300 μm depth separated dysplastic from non-dysplastic tissue with an area under receiver operating characteristic curve (AUROC) of 0.91, (100% sensitivity and 84% specificity)[15].

3. ESS: Initial reports indicate promising diagnostics for identifying high risk mucosa with a 92% sensitivity and 60% specificity[16].

<u>Risk Stratification through Field Carcinogenesis Detection</u>:

Clinical Need: From a population perspective, the goal is to identify and remove all advanced adenomas in order to prevent future CRC. The challenges include the

insufficient resources (funding, endoscopic capacity) and complication rate inherent in performing colonoscopy on the entire "average risk" population (~100 million Americans over age 50) to identify all neoplasia-harboring subjects. This is juxtaposed with the remarkably low yield of advanced adenomas in these screening/surveillance procedures, resulting in >90% of colonoscopies being unproductive from a cancer prevention perspective. Clearly, "personalizing" screening strategies is paramount. Since utilizing demographic factors alone has been suboptimal (age, gender, diet, family history etc. yielded AUROC of ~0.60 for CRC)[17], attention has focused on other approaches especially field carcinogenesis detection.

Field Carcinogenesis-overview:

Field carcinogenesis (also known as field effect, field defect) is the biological concept that the genetic/environmental milieu that results in a permissive environment for a focal adenoma/carcinoma to develop is diffuse and exists throughout the colon. The tumor location is determined by stochastic events (e.g., truncation of APC tumors suppressor gene or epigenetic silencing of hMLH1)[18]. This, in essence, is the rationale for surveillance colonoscopy (higher risk of recurrent adenomas throughout the colon). However, since adenomas are somewhat insensitive for colon carcinogenesis risk, research has focused on earlier events in the predysplastic (microscopically normal) mucosa. Indeed, various biomarkers including genomic, proteomic, cellular (apoptosis/proliferation), epigenetic (methylation/microRNA) from the distal colon can correlate, albeit imperfectly, with risk of neoplasia throughout the colon[19,20]. However, these changes are heterogeneous but may share common micro-architectural aberrations.

Technologies and Performance: Spectroscopic techniques have the power for submicron resolution and thus can detect/quantify the micro- and nano-architectural consequences of these genetic/epigenetic changes.

Low Coherence Enhanced Backscattering Spectroscopy (LEBS): Advent of LEBS represents a major advance for this application through providing quantitative insights into mucosal micro-architecture at length scales ≥40 nm via a novel optical self-interference phenomenon. The proof-of-concept study analyzed endoscopically-normal rectal mucosal biopsies from 273 patients undergoing screening/surveillance colonoscopy. Rectal LEBS was able to discriminate between patients with no neoplasia versus those with advanced adenomas with excellent performance (AUROC 0.89)[21]. A recent *in vivo* fiber-optic LEBS probe study was performed in 574 subjects. Five LEBS readings (each requiring 250 milliseconds) were recorded from the rectal mucosa prior to colonoscopy. In the blinded validation set, *in vivo* LEBS was able to discriminate between patients with no neoplasia versus those with advanced adenomas with 87% sensitivity and 78% specificity[22]. The test was not confounded by patients' demographic and risk factors or benign lesions, and remained accurate regardless of adenoma location (distal versus proximal lesions).[19]

Other Techniques: Several other approaches have corroborated the ability to interrogate rectal mucosa to predict colonic neoplasia such as rectal microvascular analysis with polarization-gated spectroscopy (AUROC of 0.88 for advanced adenomas in a study of 216 patients) [23, 24]. Preliminary reports with ESS also demonstrated strong diagnostics. Finally, nanocytology performed on rectal brushings via partial wave spectroscopic (PWS) microscopy (sensitive to nanoscale cellular architecture) manifested an AUROC for advanced adenomas of 0.85[25]. This pre-screen paradigm

may enable some proportion procedures in neoplasia-free patients to be avoided thereby allowing society to focus the finite endoscopic capacity for therapeutic (potentially cancer-preventing) procedures and include the currently unscreened population These approaches may be translatable to ulcerative colitis dysplasia as indicated by a report using a related technique (spatial-domain low-coherence quantitative phase microscopy) that yielded a sensitivity of 100% with specificity of 75%[26] in a small cohort (n=28).

Current Obstacles/ Challenges for Implementation

1. Optical biopsy

One of the most fundamental issues for optical biopsy is developing the clinical paradigm[27]. The "diagnose and leave behind" approach may be less attractive for small lesions as polypectomy is relatively safe/effective. Large lesions are more likely to be neoplastic and thus have a higher probability of needing removal (both adenomatous or even potentially serrated lesions) and thus optical biopsy may not change management[28]. With the "resect and discard" strategy, small lesions are easy to recoverable (via suction) whereas in larger lesions one would need to be concerned about the presence of high grade dysplasia which might necessitate formal pathological evaluation. On the other hand, there are several scenarios where this information would be clearly very useful. For instance, in the anti-coagulated patients, presence of multiple pseudo-polyps or those undergoing acute GI bleed, optical biopsy would potentially be of considerable value. Thus, a nuanced clinical vision is critical for implementation of these strategies.

Other concerns include the cost-benefit analysis with regards to both equipment (instrumentation/probes) and the associated time to set up/take readings.

Finally, there is the issue of risk management. According to the recently published American Society Gastrointestinal Endoscopy taskforce consensus statement, mandates that these technologies should have > 90% negative predictive value and concurrence with pathology for the strategies[29] [30]. Whether these guidelines will mitigate endoscopist liability for any future neoplasia in these patients need to be determined.

2. Adenoma detection:

Spectroscopic false positive reading may be time consuming and lead to considerable endoscopist frustration. In addition, with better endoscopes (higher definition), improvement in techniques such as right-sided retroflexion and more scrutiny of endoscopist performance (standardization of withdrawal time and adenoma detection rate), the added value of these techniques are less clear. The improvement in patient outcomes will need to be clarified since lesions only detected with the benefit of adjuvant techniques are probably small and unlikely to be clinically significant.

3. Flat dysplasia Detection

Many of the issues center on the near field nature of spectroscopy (evaluating ~1mm^2 spot size). Thus, mapping the mucosa is difficult although technical advances such as endoscopic polarized scanning spectroscopy (EPSS) appears to make this feasible[14]. The cost of instrumentation/probe and extra endoscopic time is a major consideration for application of any of these technologies. The ability to biopsy the precise spot can be an issue although the solution may be to couple the probe with biopsy forceps (such as with ESS). Finally, if more dysplasia is noted than with standard approaches, this may raise a question of an over-diagnosis bias.

4. Field Carcinogenesis Detection

The issues revolve around clinical implementation and rubrics. Will this be limited to the endoscopy suite or able to transition to the primary care setting which has its inherent challenges? These studies will need to be performed in unprepped patients and thus unclear if the gastroenterologist (especially high risk population) or primary care physician (with logistic challenges with the latter). While feasibility has been demonstrated in pilot studies, this needs to be validated in larger scale studies. Another issue that needs to be addressed is the impact of past neoplasia on the potential use for surveillance. From a population perspective, one needs to determine whether to target a high sensitivity and accept a lower specificity (very few false negatives but increased number of false positives requiring colonoscopy) or vice versa. Finally, while there are other potential applications in the GI tract via extended field carcinogenesis (esophageal squamous mucosa→Barrett's dysplasia or peri-ampullary duodenal mucosa→pancreatic cancer)[19] the diagnostic ability of these approaches needs to be determined in large scale studies.

In conclusion, the technological advances have engendered considerable enthusiasm among endoscopists. However, it is critical to focus on the particular clinical issues and ascertain how the technologies will impact upon decision making paradigms. The crux of the matter is patient outcomes and cost-effectiveness. Furthermore, it is paramount to view these technologies in the context of the evolving landscape of clinical endoscopy (need for increased productivity and improved patient outcomes) and the macro-economic health care environment (cost constraints, etc.). These issues are clearly surmountable and several technologies are undergoing the requisite large scale validation necessary to bridge the bench to bedside chasm.

These are exciting times to be an endoscopist at the front lines of this technological revolution in spectroscopy.

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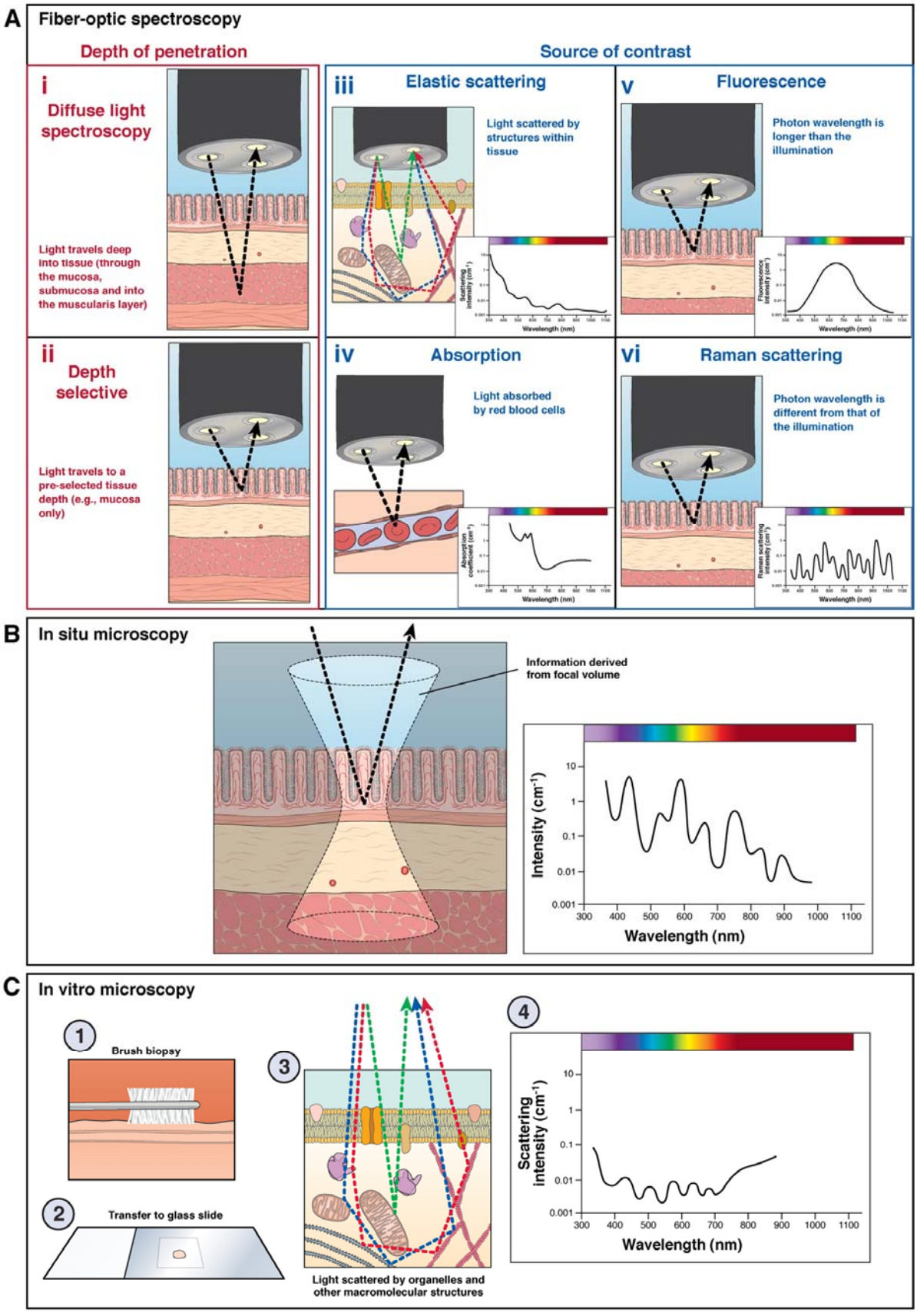
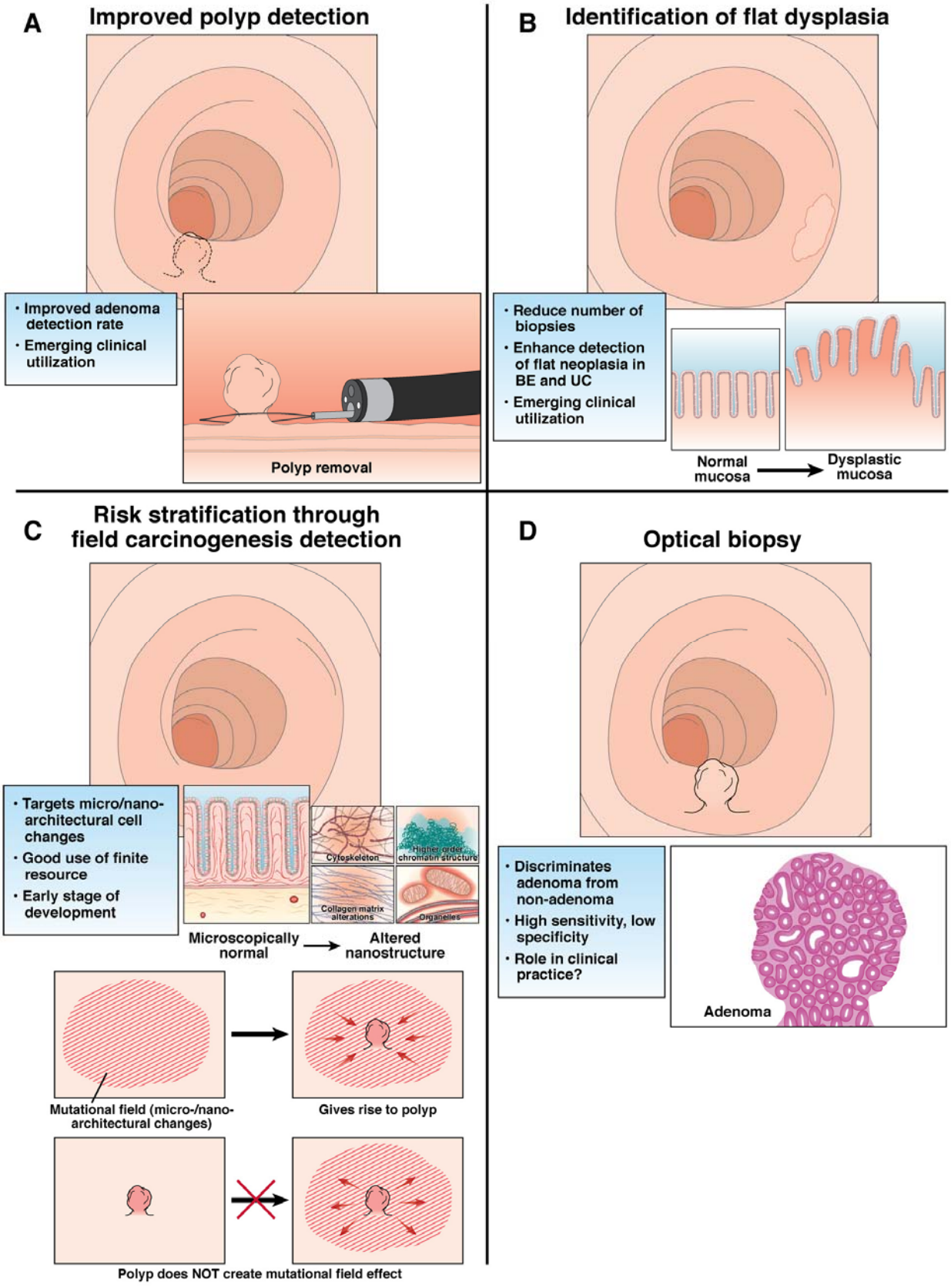

A
Fiber-optic spectroscopy
Depth of penetration
Source of contrast
i
Diffuse light spectroscopy
Light travels deep into tissue (through the mucosa, submucosa and into the muscularis layer)
ii
Depth selective
Light travels to a pre-selected tissue depth (e.g., mucosa only)
iii
Elastic scattering
Light scattered by structures within tissue
iv
Absorption
Light absorbed by red blood cells
v
Fluorescence
Photon wavelength is longer than the illumination
vi
Raman scattering
Photon wavelength is different from that of the illumination
Wavelength (nm)
B
In situ microscopy
Information derived from focal volume
Intensity (cm-1)
10
1
0.1
0.01
0.001
300
400
500
600
700
800
900
1000
1100
Wavelength (nm)
C
In vitro microscopy
1
Brush biopsy
2
Transfer to glass slide
3
Light scattered by organelles and other macromolecular structures
4
Scattering intensity (cm-1)
10
1
0.1
0.01
0.001
300
400
500
600
700
800
900
1000
1100
Wavelength (nm)


A
Improved polyp detection
• Improved adenoma detection rate
• Emerging clinical utilization
Polyp removal
B
Identification of flat dysplasia
• Reduce number of biopsies
• Enhance detection of flat neoplasia in BE and UC
• Emerging clinical utilization
Normal mucosa
Dysplastic mucosa
C
Risk stratification through field carcinogenesis detection
• Targets micro/nano-architectural cell changes
• Good use of finite resource
• Early stage of development
Cytoskeleton
Higher order chromatin structure
Collagen matrix alterations
Organelles
Microscopically normal
Altered nanostructure
Mutational field (micro-/nano-architectural changes)
Gives rise to polyp
Polyp does NOT create mutational field effect
D
Optical biopsy
• Discriminates adenoma from non-adenoma
• High sensitivity, low specificity
• Role in clinical practice?
Adenoma