Supplementary Figures

Supplementary Figure 1. Current standard of care for endoscopic surveillance of patients with Barrett’s oesophagus. High definition white-light endoscopy is combined with random biopsies, an approach that is limited by poor sensitivity (as low as 40%\(^1\)) as well as being time consuming, costly and prone to sampling error\(^2\). These limitations have motivated research into advanced optical methods for endoscopic surveillance.

Supplementary Figure 2. Advanced optical methods in clinical use for endoscopic BO surveillance: typical images.

Typical images for the advanced optical methods listed in Table 2.

A: Histopathology: Left: Squamous-lined epithelium. Middle: Columnar lined oesophagus with absence of goblet cells (original magnification, 200X). Right: High-grade dysplasia in Barrett’s oesophagus showing both architectural and cytologic atypia as well as crowded crypts with branching and papillary formation, cytologic pleomorphism and loss of polarity (original magnification, 100X). Reproduced with permission\(^3\).

B: Chromoendoscopy, AA: Left: Barrett’s with HD-WLE. Right: Same patient note dysplasia only visible post AA with early loss of acetowhiteness (Olympus Lucera ELITE processor, GIF-HQ290 gastroscope). Reprinted with permission\(^4\).

C: Hardware-based Virtual Chromoendoscopy, NBI: Left: High-resolution images of NDBE using NBI. Note the presence of circular (solid black arrow) and ridge/villous (red arrow) mucosal patterns arranged in an orderly fashion and blood vessels that follow the mucosal ridge architecture (dashed arrows). Reproduced with permission\(^5\). Right: High-resolution images of dysplastic BO using NBI. Irregular mucosal and vascular patterns in BO patient using NBI. Note the irregular mucosal (black arrow) and vascular patterns (red arrow). Reproduced with permission\(^5\).

D: Software-based Virtual Chromoendoscopy, FICE: Left: Conventional white-light image of the gastroesophageal junction. (From FICE ATLAS Fujinon.) Right: Flexible spectral imaging color enhancement image of the gastroesophageal junction. (From FICE ATLAS Fujinon.) Reproduced with permission\(^6\).

E: Autofluorescence Imaging: Left: Image of a true positive area: the flat lesion is hardly visible on the white light image and showed high-grade intraepithelial neoplasia in biopsies. Reproduced with permission\(^7\). Right: Image of a true positive area: the flat lesion is hardly visible on the white light image and showed high-grade intraepithelial neoplasia in biopsies. Reproduced with permission\(^7\).

F: Endoscopic Trimodal Imaging: See HD-WLE, AFI, NBI.

G: pCLE: Left: Probe-based confocal laser endomicroscopy images showing non-dysplastic Barrett’s oesophagus. Right: and BO with early oesophageal adenocarcinoma. Images captured in-vivo using GastroFlex UHD (Cellvizio; Mauna Kea Technologies, Paris, France) after injection of sodium fluorescein (2.5 mL, 10%). Scale bar = 20µm. Reproduced with permission\(^8\).

H: eCLE: Confocal images of the upper part of the mucosa layer (about 30–50µm vertical depth). Left: Barrett’s epithelium. Typical villiform shape and presence of goblet cells (yellow arrows) can be identified. Right: Barrett’s-associated neoplasia. At
confocal images black cells with irregular borders and shapes with high dark contrast to surrounding tissue were present. Images captured in-vivo with confocal laser endoscope (EC-3870iFK; Pentax, Tokyo, Japan) after injection of 10% fluorescein sodium (5–10 ml IV). Reproduced with permission®.
Supplementary Figure 3. Emerging optical methods for endoscopic BO surveillance: typical images/data.

Typical images/data for the emerging optical methods listed in Table 3.

A: OMI: **Left:** White-light image showing several areas of Barrett’s oesophagus (labelled BO in the diagram), identified by salmon-red mucosa, surrounded by squamous epithelium but no macroscopically visible structural abnormalities to suggest the presence of neoplasia. **Right:** Targeted fluorescence image showing enhancement of the signal from HGD. Reproduced with permission10.

B: OCT/OFDI/VLE: Features consistent with dysplasia, including irregular glandular architecture (arrowheads). Tissue histopathology results confirmed diagnoses of dysplasia. Captured with Nvision VLE Imaging System. Scale bar = 1 mm. Reproduced with permission11.

C: ESS/DRS/LSS: Representative spectra obtained with elastic scattering spectroscopy in-vivo. AU: arbitrary units. Reproduced with permission12.

D: a/LCI: **Left:** Angle-resolved depth scan of light scattered from tissue captured using a/LCI probe in vivo. Lighter shades of grey indicate increased amount of scattered light. **Right:** Scatter plot with each biopsy plotted as a function of its nuclear size and density, as measured by the a/LCI system, and categorized by its pathological diagnosis. Reproduced with permission13.

E: Polarimetry: Not used in Barrett’s.


G: FLIM: Not used in Barrett’s.

H: MPM: Not used in Barrett’s.

I: ERS: The mean in vivo confocal Raman spectra of columnar-lined epithelium, non-dysplastic BO, and high-grade dysplastic BO acquired from BO patients during clinical endoscopic examination. Each Raman spectrum is acquired within 0.1–0.5 s. The spectra have been normalized to the Raman peak at 1445 cm⁻¹ for comparison purposes. Reproduced with permission3.

J: CARS: Not used in Barrett’s.

K: MSI/HSI: Not used in Barrett’s.
References
