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POSITION PAPER

The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction

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Abstract | Environmental enteric dysfunction (EED) is a disease of the small intestine affecting children and adults in low and middle income countries. Arising as a consequence of repeated infections, gut inflammation results in impaired intestinal absorptive and barrier function, leading to poor nutrient uptake and ultimately to stunting and other developmental limitations. Progress towards new biomarkers and interventions for EED is hampered by the practical and ethical difficulties of cross-validation with the gold standard of biopsy and histology. Optical biopsy techniques — which can provide minimally invasive or noninvasive alternatives to biopsy — could offer other routes to validation and could potentially be used as point-of-care tests among the general population. This Consensus Statement identifies and reviews the most promising candidate optical biopsy technologies for applications in EED, critically assesses them against criteria identified for successful deployment in developing world settings, and proposes further lines of enquiry. Importantly, many of the techniques discussed could also be adapted to monitor the impaired intestinal barrier in other settings such as IBD, autoimmune enteropathies, coeliac disease, graft-versus-host disease, small intestinal transplantation or critical care.

Environmental enteric dysfunction (EED) is a syndrome of the small intestine that is endemic in children and adults across the developing world¹. Although it is by definition asymptomatic, EED in children is strongly associated with growth stunting and poor developmental outcomes^{1–4}. The syndrome manifests as a global disturbance of gut function, leading to impaired intestinal barrier function and poor uptake of nutrients^{2–4}. Environmental origins are suspected, but the aetiology is still not well understood and the most effective route to prevention and treatment remains unclear^{5,6}.

The huge global burden of EED and our poor understanding of the disease motivate the discovery of new biomarkers for diagnosis and monitoring, and particularly for evaluating the efficacy of interventions. Validating these biomarkers, however, is challenging, as the gold standard of endoscopic biopsy and histology is too invasive to be used in large-scale research studies in children. A potential alternative route is via ‘optical biopsy’ techniques, which enable diagnostic information

to be obtained less invasively. Here, rather than excising tissue for subsequent analysis in a laboratory, optical probes are used to make measurements on tissue *in situ*.

In this Consensus Statement, we summarize the current state of knowledge of EED and identify the characteristics that can be measurable by optical biopsy. We then review the most relevant optical biopsy techniques, including a description of the underlying technologies and their potential role in studying EED. We conclude with a set of recommendations for further research and development in this area, with a view to enabling clinical studies.

Methods

This Consensus Statement arose from a horizon scan project funded by the Bill and Melinda Gates Foundation to investigate potential applications of optical biopsy in tackling gut disease in developing countries. The horizon scan was undertaken at Imperial College London, UK, and most of the co-authors were invited to join the project

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advisory board on the basis of their extensive experience in relevant fields (T.T., W.A.F., T.J.M., P.D., S.L., A.M., R.R.-K., G.J.T. and P.K.). Other co-authors were invited to contribute as additional experts (L.S.C., C.L. and F.L.), based on the recommendations of the advisory board. Members of the board and experts met to discuss the horizon scan in London, UK, in November 2015 and San Francisco, USA, in February 2016. Literature searches were performed using Web of Science, as well as through the advice of the advisory board and experts. The results of the horizon scan are presented in this Consensus Statement, which entails a review of EED, a discussion of technologies deemed relevant to EED (based on literature searches and the opinions of the advisory board and experts), and a series of recommendations for the future application of the relevant technologies to the study and diagnosis of EED. In the Recommendations section, each technology has been assessed according to its suitability for application to EED and this assessment includes an evaluation of the cost, invasiveness and appropriateness for developing countries of each device and/or technique. This assessment was performed in a qualitative manner based on currently available information (for example, the present cost and size of the devices) and the opinions of the authors as to feasible future development. Technique suitability for use in developing countries was determined in a qualitative manner after consideration of the need for advanced infrastructure and high levels of training for either system use or data analysis. Probable deployment timescales have also been assigned to each technique based on the current states of development and the degree of validation work that will be required before application to EED. These timescales have been tentatively defined as short-term (suitable for deployment immediately or within ~1 year), medium-term (suitable for deployment within ~1–3 years)

and long-term (suitable for deployment in 3 years or more). A technological readiness level (TRL) is also reported for each technology and this score is discussed further in the Recommendations section.

EED

Before the 1960s, understanding of small intestinal disorders such as coeliac disease had been hindered by the difficulty of obtaining biopsy samples. Newly developed techniques, such as the Crosby capsule, then began to reveal differences between the intestinal villous morphology of residents of developed and developing countries, particularly individuals in India and sub-Saharan Africa. These changes were to some extent reversible if an individual moved between regions⁷. This 'tropical enteropathy' was later understood to be more closely linked to economic conditions than geographical location, and was re-termed 'environmental enteropathy' or EED, with the latter term emphasizing that it is characterized by a global disturbance of gut function⁷.

EED is associated with a number of undesirable developmental outcomes, including poor response to vaccines and increased susceptibility to infection⁸, and most strongly with impaired growth velocity and stunting^{9,10}. It is now thought that EED is an important component of the link between poor environmental conditions and reduced height, and a cause of the limited success of nutritional interventions in overcoming this growth deficit in many populations¹¹. Although stunting in itself is not particularly harmful to the individual, it is a highly visible manifestation of the effect of EED, and correlates with a much wider range of poor socioeconomic outcomes, including poor performance in education, reduced income in adulthood and increased fertility^{11–13}. Earlier diagnosis of EED, even in infants or very young children, could enable targeted interventions.

Diagnosis of EED. Definitive diagnosis of EED is currently by endoscopic biopsy and histology, and the histopathological features indicative of EED include: blunting of villi; increased crypt depth (although some cases show a decrease); reduced villus compartment surface area; increased cellular infiltrate, predominantly lymphocytes and plasma cells; epithelial monolayer fragility and breaks; tight junction and cell adhesion abnormalities; and increased epithelial cell shedding¹⁴. These features are shown in FIG. 1.

Given the practical and ethical difficulties associated with the use of endoscopic biopsy in infants and young children, there has been a considerable drive towards identification of biomarkers of EED that can be monitored less invasively. The 2015 *Technical Meeting on EED*^{15,16} established several descriptive domains of EED (BOX 1) in addition to the structural changes seen in histology, and any practical approach involving optical biopsy is likely to target one or more of these features.

Biomarkers for EED. The most commonly employed EED biomarker is the lactulose:mannitol (L:M) test, used to measure gut absorptive and barrier function¹⁷. Lactulose and mannitol are administered orally, and

urine samples are subsequently collected for up to 6 h. Lactulose — a large disaccharide molecule — is not normally absorbed by the healthy small intestine. Mannitol, on the other hand, is a smaller monosaccharide that passes the healthy intestinal barrier and is found in urine¹⁷. An increased ratio of lactulose to mannitol in the urine, determined by liquid chromatography and/or mass spectrometry, therefore indicates one or both of reduced absorption or impaired intestinal barrier function. However, in practice the results depend strongly on the proficiency of those carrying out the test and the level of diligence observed in infant dietary fasting and regular urine collections¹⁷, and the test does not provide the same direct information as histology.

Many other biomarkers have been proposed as candidates to monitor or diagnose EED (comprehensive reviews are available elsewhere^{14,18}) and new biomarkers are constantly being sought, but there is difficulty in obtaining a 'gold standard' measurement for comparison. Two large-scale trials are also currently investigating the effect of community-level water and sanitary

health (WASH)¹⁹ improvements on factors including child-length for age, child development scores and stunting prevalence (the SHINE trial in Zimbabwe²⁰ and the WASH Benefits Trial in Kenya and Bangladesh²¹). These studies will indicate the degree to which sanitary conditions drive EED and stunting, and hence the level of prevention that could be expected from widespread WASH improvements. Nevertheless, it is not possible to directly link sanitation to specific histological features of EED given the ethical and practical difficulties associated with performing longitudinal studies requiring repeated invasive procedures (under sedation) on asymptomatic infants and children in a developing world setting. Conversely, correlation with long-term developmental outcomes is inherently difficult, time-consuming and subject to confounding influences.

The potential benefit of optical biopsy therefore lies in providing comparable (or superior) information to histology in a less invasive manner. In the following section we highlight technologies with the potential to provide real-time diagnostic information in the small intestine. We then propose specific applications in the measurement of the domains of EED, with consideration of the practical issues related to deploying these techniques in the affected geographical regions. The principal focus is on techniques suitable for limited use within research studies aiming to identify noninvasive biomarkers or treatments, although we also suggest some potential point-of-care tests suitable for more widespread use.

Optical biopsy for the small bowel

Numerous optical biopsy techniques have been reported in the literature, but relatively few have been employed in the small intestine, and hardly any specifically for EED. The optical imaging and spectroscopic techniques included below are therefore only those which have demonstrated potential for use in this setting, either because they can perform measurements relevant to the diagnosis of EED or because there is a history of clinical use related to other small bowel conditions, such as coeliac disease. For each technology we summarize the underlying principles, assess the technological readiness for clinical applications, and identify potential applications in EED.

Endoscopy and capsule endoscopy. Endoscopy is widely used to remotely visualize regions of the gastrointestinal tract, including the small intestine. Most modern endoscopes use incoherent fibre-optic bundles or distal light emitting diodes (LEDs) to illuminate the tissue and miniature cameras to capture live video. Alternatively, wireless capsule endoscopy²², which provides live imaging via an untethered ingested capsule transiting the gastrointestinal tract, has been available since the early 2000s. This approach is considerably less invasive than standard endoscopy, but offers poorer imaging resolution and is non-steerable.

Although biopsies are required for definitive histopathological diagnoses, endoscopy can resolve mucosal structures such as duodenal villi, suggesting that it could identify features of EED. Indeed, endoscopic imaging

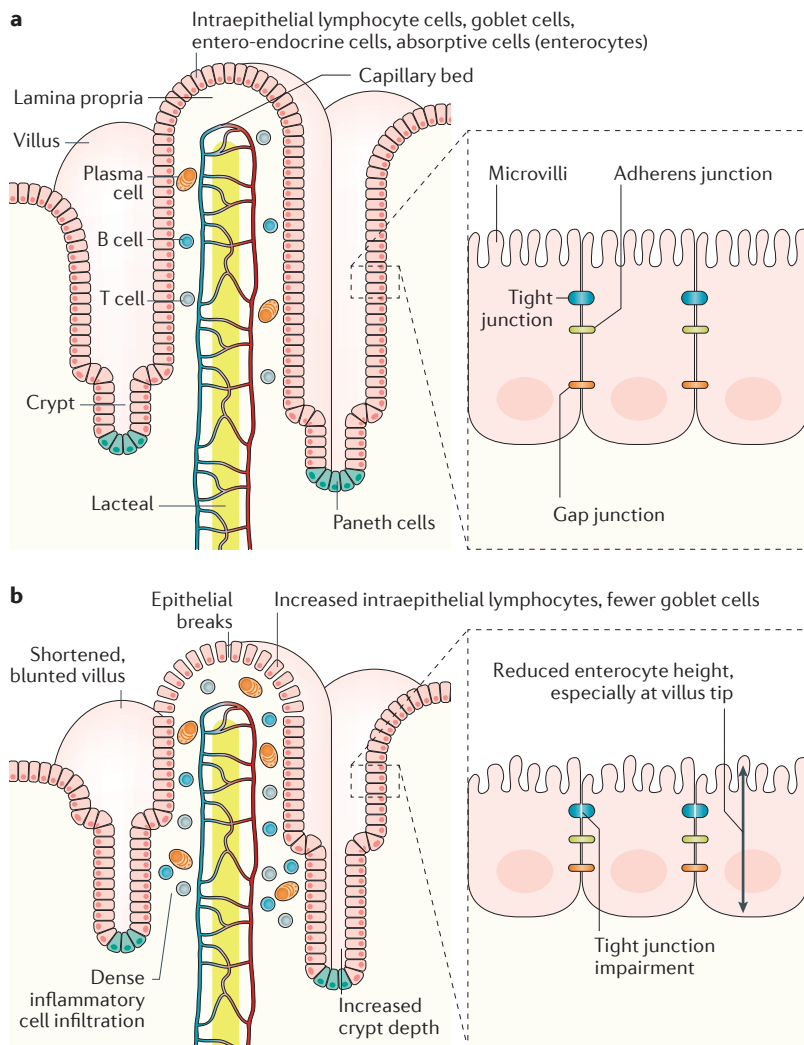


Figure 1 | Features of environmental enteric dysfunction. **a** | Healthy intestine. **b** | Changes to intestinal tissue associated with environmental enteric dysfunction.

has been applied to numerous studies of coeliac disease, which exhibits similar but more extreme changes in intestinal structure to EED, including scalloped duodenal folds, grooves, fissurations and loss of the finger-shaped villi that cover the healthy mucosal surface²³.

The contrast of the villous structure can be enhanced through techniques including water immersion²⁴, high-definition (HD) endoscopy²⁵, chromoendoscopy²⁶, narrow band imaging (NBI)²⁷, and other forms of optical or digital chromoendoscopy such as optimal band imaging (OBI)²⁸, providing sensitivities and specificities of up to 100% for villous atrophy²⁵. FIGURE 2a–d shows the contrast improvement offered by optical chromoendoscopy, revealing clear differences between healthy and unhealthy villous mucosa. Furthermore, HD endoscopy has been applied to preliminary studies of EED in adults (FIG. 2e,f), in which it has been shown capable of identifying changes in the villous architecture that are associated with EED.

Although endoscopic examinations can be performed safely in infants²⁹ and even neonates³⁰, this procedure is considerably more challenging — and has higher associated risks — than when undertaken in adults or older children³¹, even when using the thinnest (<6 mm diameter) paediatric endoscopes. For this reason, endoscopy in children is almost always performed using either deep sedation or anaesthesia (which have their own associated risks³²). It would therefore be ethically difficult to undertake such an invasive procedure for research purposes in asymptomatic infants who would derive no immediate benefit. This issue limits the role of endoscopy to studies in adults or in very small groups of infants (for example, in children who are undergoing endoscopy and/or sedation for other reasons).

Capsule endoscopy has previously been deployed in children to investigate gastrointestinal bleeding, Crohn's disease, coeliac disease and small bowel polyps³³. The technique is approved by the FDA for use in children ≥ 2 years of age³⁴ and safe use has been demonstrated in infants as young as 10 months³⁵. Capsules are inherently less invasive than standard endoscopes as they move through the body via natural motion induced by peristalsis rather than being manually manoeuvred by the clinician. Thus, they are less likely to cause damage to the intestinal epithelium during use²². Importantly, endoscopic placement of the capsule can enable use of capsule endoscopy in children who are unable to swallow the device³⁶, although this method would obviate the benefit of the non-endoscopic approach to some degree.

A review by Ianiri *et al.*²⁵ reported the sensitivity and specificity of capsule endoscopy for the diagnosis of villous damage in patients with coeliac disease as 70.0–95.2% and 63.6–100% respectively, lower values than typical for HD or NBI endoscopy, but nevertheless representing good diagnostic accuracy. Villous atrophy was indirectly measured via assessment of image mosaicism²⁵. Although this approach could be considered a limitation, it has the benefit of permitting automated image analysis³⁷ for use in low-resource settings, and these quantitative results can be matched to the Marsh criteria (a classification based on the presence of intraepithelial lymphocytes, crypt hyperplasia and villous atrophy³⁸) that are used in conventional histological diagnosis of coeliac disease³⁷. However, the risk of capsule retention is $\sim 1\%$ ^{33,39}, a potential complication that might need to be mitigated with tethered capsules or ingestible radio frequency identification tags for device localization⁴⁰. Additionally, preliminary studies in adults would be required to compare capsule measurements to standard endoscopic assessments of villous atrophy.

Box 1 | Descriptive domains of environmental enteric dysfunction

Domains as established by the Technical Meeting on environmental enteric dysfunction (EED), held on 28–30 October 2015 in Vienna, Austria^{15,16}

Increased small intestinal permeability

EED is associated with impaired function of the small intestinal barrier

Nutrient malabsorption

Following from intestinal inflammation and enterocyte immaturity, malabsorption of nutrients (particularly zinc) is associated with stunting

Intestinal inflammation

Infection-induced inflammation of the small intestine represents an important part of EED and is currently poorly understood

Systemic inflammation

While factors such as malaria and upper respiratory infections may contribute to systemic inflammation, it can also be a consequence of gut inflammation and microbial translocation

Bacterial translocation

Translocation of bacteria from the lumen into the lymphatics and blood stream correlates well with stunting, and has the potential to drive severe short term consequences such as septic shock

Microbiota changes

While accurate assessment of the gut microbiota composition is challenging, and studies are rare in children with EED, profound changes are suspected that could induce alterations in the immune system, colonic fermentation, nervous system signalling and metabolic activity

Fluorescence endomicroscopy. Fluorescence endomicroscopy provides high resolution images of tissue *in vivo* via flexible probes^{41,42} and is one of the most widely used and clinically validated optical biopsy techniques (FIG. 3). Unlike standard or even HD endoscopy, endomicroscopy enables direct identification of morphological changes in the tissue microstructure following application of topical or intravenous fluorescent stains. Most clinical studies use optically-sectioning 'confocal' endomicroscopy, whereby a focused laser spot is scanned across the tissue and an image is built up point-by-point. Focusing the collected light through a pinhole before the detector results in rejection of out-of-focus light and a high contrast image⁴¹.

Optical fibres are used for light delivery and collection, and laser scanning is achieved using either a distal^{43,44} or proximal⁴⁵ mechanism. Miniaturized distal scanning mechanisms involve rapidly scanning the tip of a single fibre in two dimensions, using miniaturised magnetic or piezo actuators (FIG. 3a). By contrast, in proximal scanning, conventional 2D scanning mechanisms, such as a pair of galvanometer scanners, are used externally (FIG. 3b). The scanning pattern is then relayed to the tissue via a flexible bundle containing up

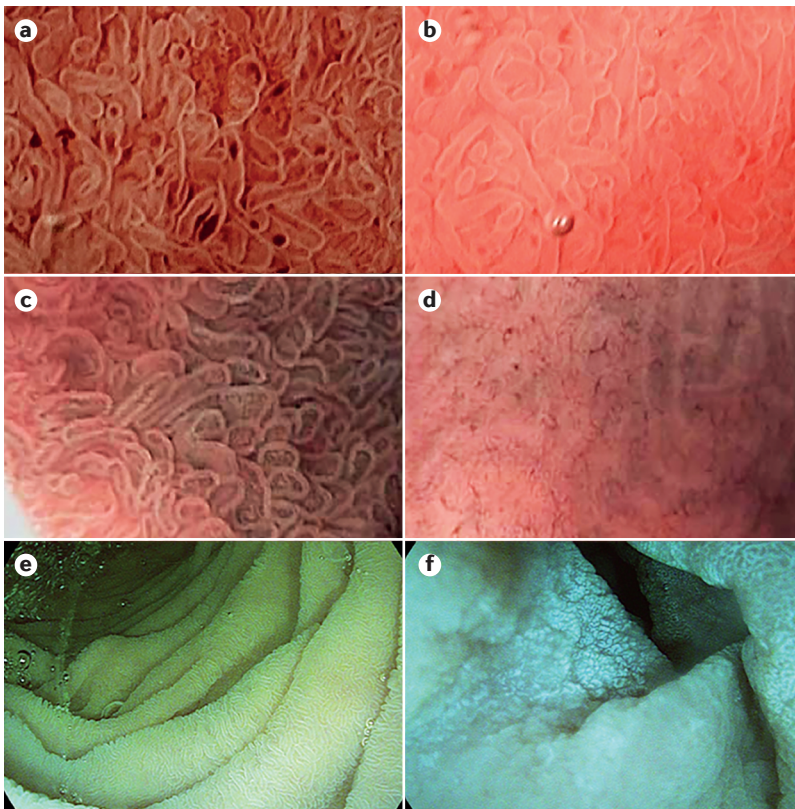


Figure 2 | **Endoscopic imaging of intestinal villi.** **a** | Image illustrating the improved villous contrast provided by optimal band imaging (OBI) and high definition (HD) endoscopy. **b** | An equivalent image utilizing HD endoscopy alone. **c** | Narrow band imaging (NBI) of regions of tissue exhibiting healthy villi. **d** | NBI images showing regions of tissue with absent villi. **e** | HD endoscopy image recorded in an adult with EED exhibiting partial blunting of villi. **f** | HD endoscopy image in an adult with EED showing subtotal villous atrophy. Parts **a,b** reprinted from *Gastrointestinal Endoscopy*, 68, Cammarota, G. *et al.*²⁷, Optimal band imaging system: a new tool for enhancing the duodenal villous pattern in coeliac disease, 352–357, Copyright 2008, with permission from Elsevier. Parts **c,d** © Georg Thieme Verlag KG, reprinted from REF. 26. Parts **e, f** provided by P.K.

to 30,000 fibres, which acts as an image conduit. Distal scanning can provide high resolution images, generally at low frame rates, whereas proximal scanning (using the fibre imaging bundle to relay the scanning pattern from an external system) enables for sub-mm diameter probes at the expense of resolution. Importantly, these sub-mm proximal scanning probes are compatible with paediatric endoscopes, as they can be inserted through the 2 mm diameter working channel. The distal scanning approach is used by the ISC-1000 (Pentax, Japan), a 5 mm diameter endomicroscope integrated into a Pentax endoscope that offers an adjustable working distance for 3D imaging, although this device is no longer commercially available. The proximal scanning method is used in the Cellvizio system (Mauna Kea Technologies, France) and several variations of this system are now available^{46–48}, with the thinnest probe offered by Mauna Kea Technologies having an outer diameter of 0.85 mm (REF. 49). Simple non-scanning endomicroscopes, using a fibre-bundle to flood illuminate the sample and then collect images, have also been applied to the gastrointestinal tract^{50,51} but not commercialized.

Studies in the small intestine, both for coeliac disease and EED itself, have shown that both the ISC-1000 (REF. 52) and the Cellvizio^{53,54} systems can resolve villous structures, the capillary network and both goblet and columnar epithelial cells (FIG. 3d,e) when using intravenous fluorescein as a contrast agent. Other studies have shown that endomicroscopy can diagnose coeliac disease^{55–58} based on recognition of features relevant to EED, such as villous atrophy, crypt hyperplasia and lymphocyte infiltration. In particular, Venkatesh *et al.*⁵⁸ reported successful diagnosis of coeliac disease (100% sensitivity, 80% specificity) in children as young as 1.8 years of age. Semi-automated methods for the detection and segmentation of villi⁵⁶ and for the measurement of goblet cell density⁵⁷ have also been presented.

Fluorescence endomicroscopy also enables observation of gaps or breaks in the intestinal epithelial lining^{59–61}. For patients with IBS^{60,61} the leakage of intravenous fluorescein into the lumen has been visualized in real time⁶¹, indicating that endomicroscopy can be used to monitor dynamic, cellular level changes in gut function and permeability. In a study of EED in 41 Zambian adults using the Pentax ISC-1000 endomicroscope⁴, only 8 (20%) exhibited no luminal leakage of fluorescein and only 2 (5%) showed no fluorescein plumes. By comparison, in a healthy population, 80% showed no fluorescein leakage or plumes ($P < 0.0001$)⁶². Endomicroscopy was also used to quantify single and multiple cell defects, cell shedding events, apoptotic cells and epithelial breakdown lesions in patients with severe and minimal EED, and these features were correlated to the expression of 23 genes that were found to be differentially expressed in intestinal epithelium⁴.

Fluorescence endomicroscopy therefore has the potential to provide information relevant to EED, including structural changes in the small intestine and measurements of gut permeability. Although advanced systems with 3D imaging capabilities could be particularly useful in early studies, such experiments might be restricted by the lack of a commercially available instrument. Thus, smaller diameter fibre-bundle-based systems will probably be more convenient and will certainly be most suitable for use in children (due to their small size and compatibility with paediatric endoscopes). However, endomicroscopy requires use of an endoscope and so is likely to be limited to research studies in adults or small groups of children (possibly those undergoing endoscopy and/or sedation for alternative reasons). Less invasive imaging could be possible using a tethered fluorescence endomicroscopy capsule (similar to the reflectance confocal microscopy capsule⁶³), but a practical system is yet to be demonstrated.

Endoscopic optical coherence tomography. Endoscopic optical coherence tomography (OCT)⁶⁴ provides high resolution, high speed, cross-sectional images to a tissue depth of 1–2 mm. It is predominantly a structural imaging tool, able to visualize the morphology of both villi and crypts in the small intestine⁶⁵. The advantages over endomicroscopy are its high speed (allowing rapid

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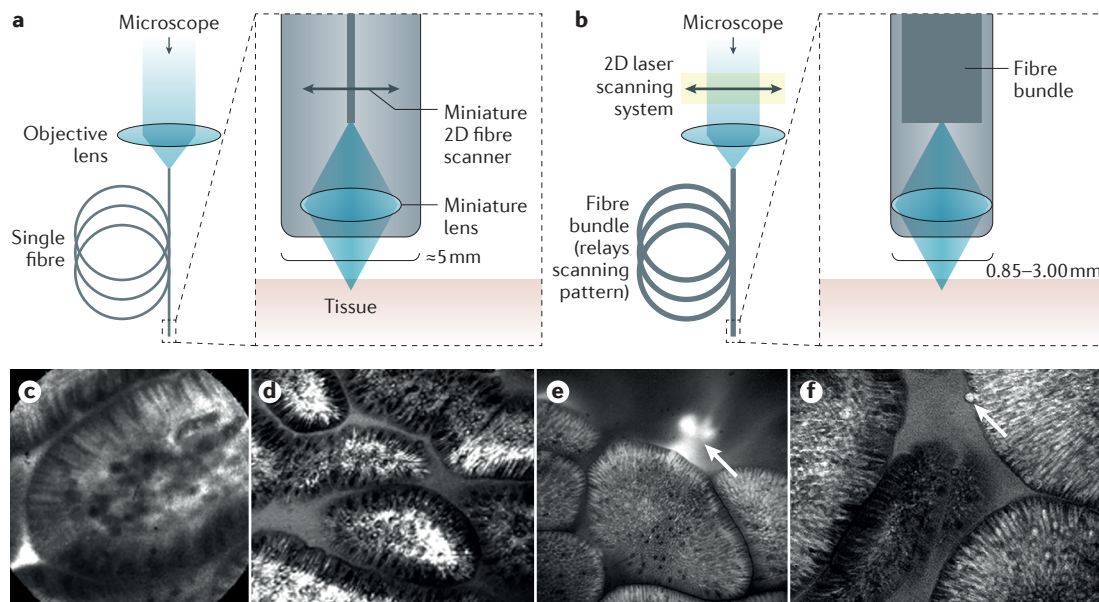


Figure 3 | Endomicroscopy for optical intestinal biopsy. a | Distal scanning endomicroscopy. **b** | Proximal scanning endomicroscopy. The proximal scanning approach results in a smaller probe tip that can be used with paediatric endoscopes. **c** | Cellvizio image of the terminal ileum acquired using intravenous fluorescein staining. **d** | ISC-1000 image of the duodenal mucosa acquired using intravenous fluorescein staining. **e** | ISC-1000 image of the small intestinal mucosa acquired using intravenous fluorescein staining in an adult with EED, showing leakage of fluorescein through the epithelium (arrow). **f** | A cell shedding event (arrow) in the same patient, also visualized using the ISC-1000 system. Part **c** reprinted from *Gastrointestinal Endoscopy*, 77, Turcotte, J.-F. *et al.*⁶⁰, Breaks in the wall: increased gaps in the intestinal epithelium of irritable bowel syndrome patients identified by confocal laser endomicroscopy (with videos), 624–630, Copyright 2013, with permission from Elsevier. Part **d** reprinted from Baishideng Publishing Group Inc, Venkatesh, K. *et al.*⁵⁸ *World J. Gastroenterol.* 15, 2214–2219 (2009). Parts **e, f** provided by P.K.

interrogation of large areas of tissue) and the ability to obtain cross-sectional images, although it is unable to visualize fluorescent dyes.

Detailed descriptions of the principles of OCT are available elsewhere (for example REF. 66), but, briefly, it involves illuminating tissue with a low coherence light source and using interferometry to select (coherence gate) light returning from a chosen depth layer. By scanning the position of the coherence gate it is then possible to generate a depth scan along the z-axis into tissue (also known as an A-scan). Axial resolutions as low as approximately 1 μm are achievable, although 10–20 μm is more common for endoscopic systems. Volumetric imaging is enabled by lateral scanning in two dimensions (with lateral resolutions typically on the order of 10–20 μm)⁶⁶. Modern systems use Fourier domain techniques to achieve A-scans at hundreds of kHz or even MHz (REF. 67) rates by acquiring data from all depths simultaneously.

OCT is inherently fibre compatible and a wide range of endoscopic systems have been developed. The main challenge is the fabrication of a high speed lateral scanning mechanism at the distal tip. A number of forward viewing probes have been reported (for example REF. 68) using similar approaches to distal scanning endomicroscopes, and although these have been used in the gastrointestinal tract (for example REF. 69), side viewing devices are more suited to imaging large areas of lumina. Side viewing probes are readily fabricated by incorporating a right angled prism and one or more micro-lenses at

the distal tip of a fibre. Proximally driven rotation of the fibre probe, or rotation of the prism using a micro-motor, generates circular cross-sectional images, while pull-back of the probe enables collection of volumetric datasets⁷⁰ over large regions of tissue. Probes with diameters <1 mm are achievable — allowing deployment through either standard or paediatric endoscopes and catheters — and have been applied extensively to vascular imaging (for example REF. 71) and within the gastrointestinal tract (for example REF. 72).

Tethered capsule OCT (also referred to as tethered capsule endomicroscopy, TCE) was reported by Gora *et al.*⁷³ in 2013. The capsule (which measures 24.8 mm by 12.8 mm) is swallowed by the patient, rather than being introduced by endoscope, and provides 3D imaging via a pull-back manoeuvre (FIG. 4). This technology is considerably less invasive than endoscopy, as the capsule relies on natural peristalsis to pass through the gastrointestinal tract rather than being externally manoeuvred, and can be undertaken in unsedated (adult) patients⁷³. TCE has been applied to the assessment of Barrett oesophagus, with volumetric images of the entire oesophagus collected in as little as 60 s (REF. 74). There have also been initial reports of extending TCE to rapid, minimally invasive imaging of the small intestine⁷⁵.

Although there have been few *in vivo* OCT investigations of the small intestine, a 2005 study⁶⁵ using an endoscopic probe-based system demonstrated clear visualization of villi. In addition, a subsequent attempt

to diagnose coeliac disease using this technique by assessing villous atrophy⁷⁶ reported a sensitivity and specificity of 100% and 82%, respectively, in a group of 67 children aged 1–14 years. Images from this study are shown in FIG. 4c,d.

OCT therefore seems to offer sufficient resolution for the assessment of changes in the tissue microstructure that are relevant to EED. Moreover, tethered capsule OCT systems provide minimally invasive, high speed imaging and could be applied to large cohorts of adults to obtain an unprecedented level of information from large sections of the small intestine. This approach could also be extended to children and infants, with the risk of capsule retention mitigated by the tether. However, endoscopic introducers are probably necessary in young children to aid swallowing of the capsule, which might be challenging for large-scale implementation. Nonetheless, the deployment of TCE in small

numbers of children could be feasible and could help to provide vital information on the changes that occur in intestinal tissue microstructure during the early stages of development. Interestingly, if smaller capsules are desirable for paediatric use then their development should be feasible, as the combined diameter of all optical and mechanical components contained within the capsule is already considerably smaller than that of the capsule itself (FIG. 4a). For example, 1 mm external diameters have been achieved in the side-viewing vascular or endoscopic OCT probes discussed earlier in this section, which use near-identical technology to that used in capsule OCT.

Fluorescence and reflectance spectroscopy. Optical spectroscopy entails illuminating tissue with light of a known wavelength and recording the intensity of the returning signal, such as fluorescence or scattered

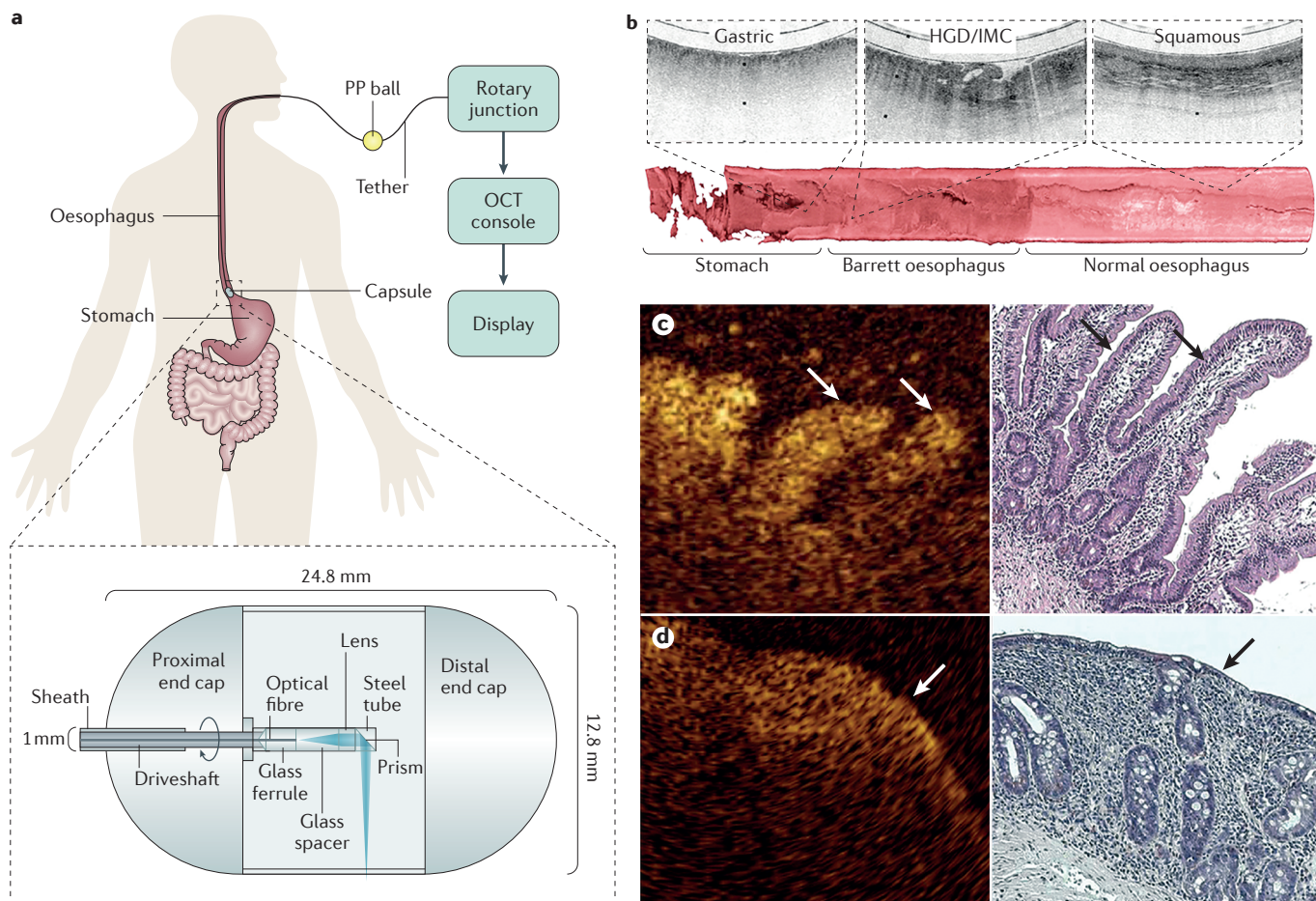


Figure 4 | Tethered capsule OCT and OCT-based visualisation of intestinal villi. **a** | A tethered capsule optical coherence tomography (OCT) system. PP, polypropylene ball handle. **b** | 3D view of the entire oesophagus in a patient with Barrett oesophagus, obtained via 3D rendering of data collected as the OCT capsule is withdrawn from the stomach. Distinct features are revealed by OCT including normal gastric mucosa (left cross-sectional image), high-grade dysplasia (HGD) and intramucosal carcinoma (IMC) (centre), and normal squamous mucosa (right). **c** | Probe-based OCT image collected in the duodenum (left) and corresponding histological image (right) indicating visualisation of the

villous structure in healthy tissue. Finger-like villi are clearly resolved (arrows). **d** | Probe-based OCT image (left) showing areas of villous atrophy (arrows) exhibiting a flattened mucosal surface, and the corresponding histological image (right). Parts **a,b** adapted with permission from Macmillan Publishers Ltd, part of Springer Nature © Gora, M. J. *et al.*⁷³, *Nat. Med.* **19**, 238–240 (2013). Parts **c,d** reprinted from *Digestive and Liver Disease*, 41, Masci, E. *et al.*⁷⁶. Optical coherence tomography in pediatric patients: A feasible technique for diagnosing coeliac disease in children with villous atrophy, 639–643, Copyright 2009, with permission from Elsevier.

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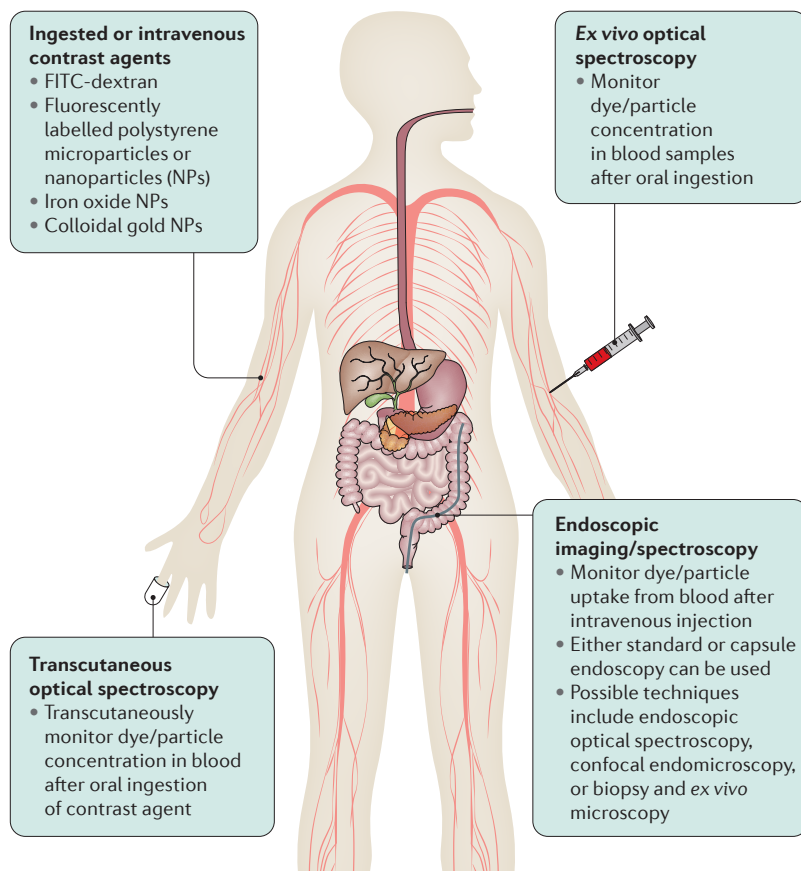


Figure 5 | Overview of possible methods for the assessment of gut permeability using fluorescence spectroscopy or microscopy of exogenous dyes and microparticles. FITC, fluorescein isothiocyanate.

light, as a function of wavelength (these principles are described in detail elsewhere⁷⁷). Although 2D spectral imaging is possible, spectroscopic clinical studies often involve single point measurements only, sacrificing spatial resolution to achieve acceptable acquisition times and to minimise probe complexity.

Although fluorescence and reflectance spectroscopy (along with various further spectroscopic techniques) have been applied to disease studies in the gastrointestinal tract using fibre-based endoscopic probes, this is unlikely to represent the most promising application for EED. Endoscopic spectroscopy could certainly be used to monitor the metabolic status of the gut via fluorescence measurements (for example, of the relative fluorescence intensities or the fluorescence lifetimes of the enzymatic cofactors NADH and FAD)^{78–80}. In addition, a variety of reflectance-based modalities (including diffuse reflectance spectroscopy^{81,82} and elastic scattering spectroscopy⁸³) could enable assessment of the tissue oxygen saturation and the haemoglobin concentration.

It is possible that both metabolic measurements (for example of the tissue redox ratio^{79,80}) and quantification of the haemoglobin concentration and/or tissue oxygen saturation could be relevant to EED, but this applicability is yet to be confirmed experimentally. Another potential use of reflectance spectroscopy in EED could lie in determining some of the morphological changes

relevant to the disease, such as the degree of cellular infiltration or the prevalence of breaks in the epithelial monolayer. Light scattering spectroscopy^{84–86} and low-coherence enhanced backscattering spectroscopy⁸⁷ have both been used to assess aspects of cellular and tissue morphology (*in vivo* in the human oesophagus, colon and duodenum) and, as such, it might be possible to extend these techniques to EED. Fibre-optic fluorescence spectroscopy could also be used to monitor the leakage of intravenous fluorescent dye into the gut lumen in a similar manner to that described for fluorescence endomicroscopy. In all cases, however, these spectroscopic techniques will not provide spatial resolution and, for that reason, would be inferior to their competitors. Furthermore, although the reduced complexity of single point spectroscopy probes means that their outer diameters can often be smaller than those of fluorescence endomicroscopes, they still require an endoscope for deployment and, hence, can be considered equally invasive. In addition, endomicroscopy and OCT systems are (in some cases) commercially available and currently have a higher level of clinical penetration than endoscopic spectroscopy devices, implying that their deployment for research studies will be easier. Thus, it is likely that endomicroscopy and OCT will prove more useful for investigating changes in the morphology and the permeability of intestinal tissue in EED than *in vivo* endoscopic spectroscopy.

An alternative, more promising application of fluorescence spectroscopy in EED could be to monitor gut permeability in a minimally invasive or noninvasive manner from outside of the body, without the use of an endoscope. A number of studies have reported the use of microscopy or spectroscopy combined with fluorescent or reflective microparticles to monitor intestinal closure, transport and translocation in animals^{88–93}. In general, the protocols used in these investigations first involved feeding animals a dose of a contrast agent such as fluorescein isothiocyanate (FITC)-labelled dextran^{88,89}, fluorescently tagged polystyrene microspheres^{90,91}, colloidal gold nanoparticles⁹² or iron oxide nanoparticles⁹³. The uptake of contrast agent from the intestine into the blood, or into specific cells or tissues, was then assessed using spectroscopy or microscopy. Importantly, whereas many of these studies relied upon microscopic examination of *ex vivo* tissue samples to determine the degree of uptake, the work by Weström *et al.*⁸⁸ and Ekström *et al.*⁸⁹ demonstrated direct monitoring of the blood serum concentration of fluorescein (in *ex vivo* blood samples) using fluorescence spectroscopy.

Translating this approach into *in vivo* human use could be possible using a number of methodologies (FIG. 5), the most promising of which would involve the use of fluorescence spectroscopy to make noninvasive measurements of gut leakiness. In this scenario, the patient would receive an oral dose of contrast agent and the concentration of the agent in the blood would be measured some time later using a transcutaneous spectrometer. Such a device could entail a fingerclip system (similar to many commercially available pulse oximeters) or a skin patch. Both could be fabricated at low cost

and would be almost entirely noninvasive. In addition, measurements of fluorescent dyes conjugated to particles of varying sizes would permit semi-automatic quantification of gut permeability. Validation for application to EED could be achieved by combining spectral measurements with L:M tests and/or morphological imaging. Importantly, after successful validation, transcutaneous fluorescence spectroscopy will be suitable for noninvasive monitoring of gut permeability in large cohorts of infants for studies such as the assessment of nutritional or WASH interventions.

Photoacoustic imaging. Photoacoustic imaging (PAI) — which relies upon the photo-acoustic effect, whereby a material absorbing light emits acoustic waves⁹⁴ — involves exciting tissue with nonionizing laser pulses. When absorbed by chromophores in the tissue, this excitation generates heat, expansion of material and subsequent emission of ultrasound that can be detected with an ultrasonic transducer^{95,96}. Both endogenous chromophores, such as oxyhaemoglobin and deoxyhaemoglobin, and exogenous contrast agents can be used, enabling PAI to provide multifaceted data containing both structural and functional information.

As this technology is based upon the detection of ultrasonic pressure waves, PAI affords considerably higher tissue penetration depths than other optical imaging modalities. There is a trade-off between penetration depth and resolution, but sub-mm resolution is achievable at depths of up to a few cm (REF. 96). Penetration of a few cm would be sufficient to image the intestine of an infant from outside of the body, and so PAI might provide another route towards noninvasive investigation of EED, although this application remains highly speculative.

Although numerous chromophores (endogenous or exogenous) can be imaged using PAI, a probable application to EED is in imaging of gut permeability. This procedure would require the use of exogenous contrast agents and would entail protocols similar to those described earlier for fluorescence spectroscopy. One approach would involve imaging the leakage of an intravenously delivered photoacoustic contrast agent from the blood stream into the gut using endoscopic probes. Several endoscopic PAI probes have been reported⁹⁷ and a number have been applied to gastrointestinal studies, both in live animals⁹⁸ and in *ex vivo* samples of human tissue⁹⁹, suggesting that this technique would be entirely feasible. Alternatively, noninvasive PAI could be achieved by using an orally ingested contrast agent along with an external imaging probe (likely to consist of a pulsed excitation laser combined with a commercially available ultrasound transducer similar to the devices reported in REF. 100 and REF. 101). Unlike transcutaneous fluorescence spectroscopy, PAI could permit spatial mapping of the leakiness of the intestine, hence offering a more detailed assessment of the permeability of the gut. Although PAI using an external imaging probe could potentially be applicable to large scale studies in children (due to its noninvasive nature), it is an immature technology and substantial

developmental work will be necessary, for example in combining PAI with other measurement protocols for validation. Nonetheless, due to the potential utility for EED, this development and validation is likely to be worthwhile.

Recommendations

Although each of the techniques described above has potential applications in EED, they differ not only in terms of their format, the information they provide, and the type and scale of study they will be most appropriate for, but also in their technological maturity. The TRLs of each technology are listed in TABLE 1, along with additional information including the expected development timescales. The TRL scale (adapted from a scale originally developed by NASA) ranges from 1 to 9, with ‘1’ indicating an initial concept and ‘9’ representing a mature, commercialized device¹⁰². Other factors relevant to the adoption of these techniques for EED are also listed, including cost, invasiveness and suitability for developing world use. These additional factors are particularly important for large scale studies (given issues that can be expected at scale, such as the need for widespread deployment and the analysis of large datasets), although less-so for early aetiological investigations in small groups of patients. The likelihood of a technology finding other uses in more developed healthcare systems is also a consideration, as this affects the chances of subsequent commercial investment, as well as providing additional routes for testing and validation.

As can be seen from the TRLs in TABLE 1, the time-scale over which techniques could be applied to EED varies markedly. HD and NBI endoscopy and endomicroscopy both have high TRLs, being fully commercialized and widely deployed, and could be adopted for use in EED immediately. Both have the potential to provide vital new information on the structural changes that occur in the gut as a result of EED, and endomicroscopy has already been applied to the study of both gut structure and permeability in adults with EED⁴. Endoscopy and endomicroscopy could be applied in the short term to small-scale aetiological investigations, most practically in cases where children are already undergoing sedation for other procedures (given the invasiveness of the proposed measurements).

Capsule endoscopy could also be used immediately, although some validation (for example, comparison to endoscopy or endomicroscopy) will be required in order to confirm the sensitivity and specificity of the technique in detecting villous atrophy. Subsequently, capsule endoscopy could be applied to studies in large cohorts as the procedure is considerably less invasive than standard endoscopy. Detection of villous atrophy can be achieved using automated image processing algorithms, removing the requirement for manual analysis by a specially trained pathologist. Although it is only approved for children older than 2 years of age, endoscopic introduction would enable capsule imaging in younger children and the development of smaller devices could allow this restriction to be challenged.

Table 1 | Summary of optical biopsy techniques in terms of their suitability for use in EED

Optical biopsy technique	TRL	Cost (approx.)	Invasiveness	Suitability for developing countries	Other applications of technology	Information provided	Deployment timescale
HD and NBI endoscopy	9	\$\$\$	High	Low-medium	High	Villous morphology (plus biopsy collection)	Short-term
Capsule endoscopy	8	\$\$	Medium	Medium	High	Villous morphology	Short-term
Fluorescence confocal endomicroscopy	8	\$\$\$	High	Low	Medium-high	Villous morphology, gut leakiness, bacterial translocation	Short-term
Optical coherence tomography capsule	7	\$\$\$	Medium	Medium	Medium	Villous morphology	Medium-term
External fluorescence spectroscopy	5	\$	Low	High	Low-medium	Gut leakiness, bacterial translocation	Medium-term
External photo-acoustic probe	4	\$\$	Low	Medium	Low-medium	Gut structure, gut leakiness, bacterial translocation	Long-term

Technological readiness level (TRL) is a scale indicating the maturity of a technology. A score of '1' represents an initial concept, whereas a TRL of '9' indicates a mature, commercialised technology. The remaining factors have been determined in a qualitative manner as described in the Methods section. Deployment timescales are tentatively defined as follows: short term – suitable for deployment immediately or within approximately 1 year; medium term – suitable for deployment in 1–3 years; long term – suitable for deployment in >3 years. HD, high-definition; NBI, narrow band imaging.

Transcutaneous fluorescence spectroscopy and tethered capsule OCT are both suitable for medium-term use (within ~1–3 years). Tethered capsule OCT has already been shown to enable imaging of the entire oesophageal mucosa in a matter of minutes using a protocol that is considerably less invasive than endoscopy. Moreover, OCT is capable of resolving intestinal villi, making it a very promising tool for the investigation of EED due to its capability to evaluate many villi in one procedure. Further development will be required to enable intestinal imaging but this should not be a limiting factor. As with capsule endoscopy, use of OCT in children is feasible but will be limited to those aged 2 years and above. Again, this constraint can be mitigated by further miniaturization and the incorporation of endoscopic introducers into experimental protocols.

Fluorescence spectroscopy is also a promising technology due to its potential to be applied almost non-invasively. Fingerclip-style spectrometers could be used to monitor the leakage of orally administered contrast agents from the gut to the blood stream, providing functional information pertinent to EED. This approach will require validation, either using some of the high-TRL imaging instruments discussed earlier or L:M tests. Once this validation is complete, fluorescence spectroscopy will be applicable to large-scale studies in remote locations due to its practically noninvasive methodology, low cost, simplicity of data analysis and ease of deployment. Interestingly, there might be scope to extend this approach for use with multiple molecular probes to evaluate further aspects of intestinal pathophysiology.

Finally, PAI could in the future also offer a non-invasive experimental protocol for the assessment of gut permeability (similar to that described for fluorescence spectroscopy), with an external PAI probe used to image the small intestine from outside of the body. An advantage over spectroscopy could be the ability to obtain low-resolution spatial maps of gut permeability,

although more advanced data analysis will be required (possibly necessitating a pathologist). Substantial research and development work is needed, both in device design and validation, meaning that PAI would only be deployable in the longer term (>3 years).

The list of optical biopsy techniques presented herein is not exhaustive and other approaches might be useful in the study of EED. One example is Raman spectroscopy, which has been used for the detection and identification of bacteria in previous investigations^{103,104} and could prove useful in establishing the make-up of the intestinal microbiota (for example, via measurements of tissue or faecal samples, or through *in vivo* endoscopic studies). Endocytoscopy, a form of white light endomicroscopy, can also visualize normal villi¹⁰⁵, but cannot detect the functional changes visualized by fluorescence endomicroscopy. There is also a range of both optical and nonoptical (typically electrochemical) sensing techniques that enable simultaneous and selective measurements of metabolite (for example, lactate, glucose), electrolyte (for example, pH, sodium, potassium) or other biomarker concentrations in a variety of samples (such as blood, tissue, urine, saliva and sweat, for example in REF. 106). These approaches could have uses, for example, in the detection of cytokines or other markers of inflammation that are relevant to EED. Indeed, some of these approaches (in particular electrochemical sensing and Raman spectroscopy) might complement the optical biopsy techniques discussed in this article, as they have the potential to provide measurements that are not possible with the methods highlighted here. For example, investigating the intestinal microbiome using Raman spectroscopy and assessing gene expression through electrochemical detection of specific biomarkers could both hold substantial merit in the study of EED. As such, all of the above techniques could be worthy of further investigation.

Conclusions

EED is a widespread condition in developing countries, in some cases affecting almost entire regional populations, and has severe negative impacts on the physical and cognitive development of children. Given that EED remains poorly understood, it is vital that new technologies and approaches are investigated and implemented to improve understanding of the disease and to help guide the development of new therapies. We have identified and assessed optical biopsy techniques with potential to assist in this area. Although many optical methodologies could potentially be applied, we have

highlighted endoscopy (including HD, NBI and capsule endoscopy), fluorescence endomicroscopy, OCT, fluorescence spectroscopy and PAI as the most promising. These will provide data not only on structural changes in the intestine, but also on the degree of intestinal permeability. We have identified how each approach could be advanced towards this goal, and we expect each technique to offer important and unique information. The further investigation and development of all of these approaches could result in a pipeline of studies offering rich and varied information on this poorly understood disease.

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Author contributions

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Competing interests statement

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