Non-invasive transcutaneous spectroscopy for the assessment of gut permeability

Abbreviated title: GutPerm

Protocol version 3.0: 26-January-2018

REC Reference:
IRAS Project ID: 242462

MAIN SPONSOR: Imperial College London

FUNDER: Imperial College London

STUDY COORDINATION CENTRE: Surgical Innovation Centre (Paterson Building), St. Mary's Hospital, Imperial College London & Imperial College Healthcare NHS Trust, South Wharf Road, London, W2 1NY

REC reference:

Protocol authorised by:

<table>
<thead>
<tr>
<th>Name &amp; Role</th>
<th>Date:</th>
<th>Signature:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Alex Thompson</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
STUDY MANAGEMENT GROUP

Chief Investigator & Principal Investigator: Dr Alex Thompson
Imperial College Research Fellow
Surgical Innovation Centre (Paterson Building)
Department of Medicine
Imperial College London
St Mary’s Hospital
London
W2 1NY
alex.thompson08@imperial.ac.uk

Co-Investigators:
Professor Robert Goldin
Professor of Gastro-intestinal and Liver Pathology
Department of Cellular Pathology
Imperial College London
Clarence Wing
St Mary's Hospital
London
W2 1NY
r.goldin@imperial.ac.uk

Professor Mark Thursz
Professor of Hepatology
Department of Surgery and Cancer
Imperial College London
Imperial College Healthcare NHS Trust
St Mary’s Hospital
London
W2 1NY
m.thursz@imperial.ac.uk

Dr James Maurice
Clinical Research Fellow
Department of Surgery and Cancer
Imperial College London
Imperial College Healthcare NHS Trust
St Mary’s Hospital
London
W2 1NY
james.maurice@imperial.ac.uk

Professor Francesca Cordeiro
Professor of Ophthalmology
Imperial College Ophthalmic Research Group (ICORG)
Imperial College London
Western Eye Hospital
London
NW1 5QH
francesca.cordeiro@nhs.net

Study Management: Dr Alex Thompson
Imperial College Research Fellow
Surgical Innovation Centre (Paterson Building)
Department of Medicine
Imperial College London
St Mary’s Hospital
London
W2 1NY
alex.thompson08@imperial.ac.uk

SPONSOR
Imperial College London is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office
Imperial College London
Room 221
Medical School Building
St. Mary’s Campus
Norfolk Place
London
W2 1PG
Tel: 020 7594 1872

FUNDER
This study is funded by the Imperial College Research Fellowship scheme.

GLOSSARY OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>EED</td>
<td>Environmental enteric dysfunction</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro-intestinal</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>ICG</td>
<td>Indocyanine green</td>
</tr>
<tr>
<td>IMP</td>
<td>Investigational medicinal product</td>
</tr>
<tr>
<td>L:M</td>
<td>Lactulose:Mannitol</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and Healthcare products Regulatory Agency</td>
</tr>
<tr>
<td>NRES</td>
<td>National Research Ethics Service</td>
</tr>
</tbody>
</table>
PD  Photodiode
PEG  Polyethylene glycol
REC  Research Ethics Committee
SAE  Serious adverse event

KEYWORDS

Fluorescence, spectroscopy, gut permeability, non-invasive, low-cost
STUDY SUMMARY

Title
Non-invasive transcutaneous spectroscopy for the assessment of gut permeability

Design
Basic science study involving procedures with human participants

Research questions
Is it possible to use transcutaneous optical spectroscopy to non-invasively assess the permeability of the intestine via measurements of the concentration of orally administered contrast agents in the blood?

Outcome measures
Spectroscopic measurements of gut permeability will be assessed via comparison against a range of traditional markers:

1. Where the blood concentration is known – for example, in ophthalmology patients who have received a direct intravenous dose of contrast agent – a direct comparison will be made without the need for further measurements.
2. In subjects receiving an oral dose of contrast agent, blood samples will be taken alongside the spectroscopic measurements in order to permit accurate ex vivo quantification of the serum concentration in the laboratory.
3. In patients who are also undergoing polyethylene glycol (PEG)-based permeability assays, spectroscopic permeability measurements will be compared to the results of the more traditional approach.
4. Finally, in patients for whom intestinal biopsy and histology data is available, spectroscopic permeability measurements will be compared against histological measures of epithelial damage and permeability.

Investigational medical device
The device under investigation in this study is an optical spectrometer designed for the transcutaneous measurement of the concentration of a contrast agent in the blood. Two versions of the same device will be tested – a bench-top system that provides high level spectral information (‘mark I’) and a miniaturised wearable system suitable for wider clinical application (‘mark II’).

Contrast agents
A variety of contrast agents will be tested in this study in order to find the most suitable agent(s) for transcutaneous quantification of gut permeability. The agents tested will include fluorescein, indocyanine green (ICG), fluorescein isothiocyanate (FITC) conjugated dextran (FITC-dextran), and FITC-conjugated polyethylene glycol (FITC-PEG). For the FITC-dextran and FITC-PEG studies, contrast agents with varying molecular weights will be investigated.

Population
Three groups will be studied:

Group 1 Ophthalmology patients who are receiving an intravenous dose of either fluorescein or ICG as part of their routine ophthalmic care (e.g. as part of a fluorescence angiography examination). These patients will take part in
preliminary studies aimed at determining whether it is possible to detect fluorescein and ICG in the blood using transcutaneous fluorescence measurements.

Group 2  **Healthy subjects** with no known issues of increased gut permeability. These subjects will act as negative controls in all gut permeability studies.

Group 3  **Gastro-intestinal (GI) and non-GI patients** who are expected to exhibit increased gut permeability (e.g. patients with coeliac disease, inflammatory bowel disease (IBD), liver disease, HIV or another condition in which increased intestinal permeability is common). The more extreme cases in this group will act as positive controls.

**Eligibility**
Aged 18 and over; able to give informed consent; no prior adverse reactions to fluorescein, ICG, dextran or PEG.
BACKGROUND

Increased gut permeability – often referred to as “leaky gut” – occurs when the intestinal barrier becomes compromised and involves the leakage of bacteria and other pathogens from the intestine into lymph and blood vessels [1, 2]. This results in a cycle of infection and inflammation that can drive additional consequences and further exacerbate the issue of increased permeability. The condition is associated with many widespread diseases including coeliac disease [1, 3], inflammatory bowel disease (IBD) [3-5], human immunodeficiency virus (HIV) [6], fatty liver disease [7-9] and environmental enteric dysfunction (EED) [10, 11]. Importantly, in many of these diseases, the role and impact of intestinal barrier function is not yet completely understood and the methods used to assess permeability are cumbersome. As a result, increased permeability of the intestine has not yet been conclusively linked to outcomes in these illnesses [1].

One disorder that is particularly relevant to this project is EED, a chronic inflammatory condition of the small intestine that impairs both nutrient absorption and barrier function [10, 11]. It is endemic in many developing countries – where it manifests in children at a very early age (< 6 months) – and can lead to malnutrition, poor response to vaccines, and severely restricted physical and cognitive development [12-17]. As such, it is strongly associated with poor developmental and socio-economic outcomes including growth stunting, poor performance in education, reduced income in adulthood, and increased fertility [18-20].

Increased gut permeability plays a pivotal role in EED [21, 22], where the leakage of pathogens from the intestine causes repeated infections, which lead to additional inflammation in the gut. In turn, this further damages the absorptive and barrier functions of the intestinal epithelium, trapping those who suffer with this condition in a cycle of infection, inflammation and malnutrition.

At present, both EED itself and the role of increased gut permeability in EED (and in many of the other diseases highlighted above) are not well understood [1, 23, 24]. For this reason, the development of tools that can accurately monitor gut permeability and provide further information on its role in all of the above conditions is vitally important. Ideally, such devices should be minimally invasive and suitable for widespread deployment to allow large scale studies. Furthermore, if they are to be applicable to ailments that are prevalent in the developing world such as HIV and EED, these tools will also need to be low in cost. Importantly, systems of this sort could be useful not only in the investigation of the diseases above but also in providing a method for diagnosis, screening, and assessment of the efficacy of interventions.

The most common technique that is currently used to directly assess gut permeability is the lactulose:mannitol (L:M) test [25]. Using this approach, patients receive an oral dose of the two sugars lactulose and mannitol, and urine samples are subsequently collected for up to six hours. The concentrations of the two sugars in urine are determined via liquid chromatography or mass spectrometry, and the L:M ratio provides an indication of the permeability of the intestine. A low L:M ratio indicates normal permeability, as lactulose is a large disaccharide molecule that is not absorbed by the healthy intestine (and, hence, is not found in urine) while mannitol is a smaller monosaccharide that passes the healthy intestinal barrier. An increase in the L:M ratio then indicates either increased permeability, impaired absorption, or a combination of the two. While the L:M test has been widely used to measure permeability, results are dependent on the proficiency of those carrying out the protocol as well as the level of diligence observed in dietary fasting (which is required prior to the test) and regular urine
collection [25]. These problems are particularly acute when performing tests in infants, which is desirable in the case of EED (and, in some cases, coeliac disease). Moreover, in all cases the protocol is time consuming for both the patient and the care provider, and post-processing of urine samples is required in a laboratory, which can be challenging in developing world settings (where suitable infrastructure may not be available).

Some alternative approaches based on similar methodologies are also available (see [2]), with one example involving the use of an orally administered polyethylene glycol (PEG) solution containing PEG molecules of varying molecular weights [1, 26]. Concentrations in urine are assessed in the same manner as described above, but more detailed permeability information is provided due to the wider range of molecular sizes being investigated (compared to just two in the L:M test). Nonetheless, this approach is prone to the same problems as L:M tests, suggesting that improved protocols will still be desirable.

Endoscopic Biopsy and histology can also be used to assess the fragility of the intestinal epithelial barrier. However, this approach only provides an indirect, highly localised measure of the permeability and also represents an invasive procedure that is particularly undesirable in children and infants.

Fluorescence spectroscopy may offer an alternative approach for the assessment of gut permeability, as it has previously been used in animal studies to quantify both intestinal closure and barrier function [27, 28]. In these investigations, intestinal permeability was assessed by feeding animals (rats and pigs) doses of fluorescent dextrans of varying molecular weights and then measuring the relative concentrations of these markers in the blood some time later. This was achieved using ex vivo measurements of blood samples and demonstrated that the passage of the fluorescent dextrans from the digestive system into the blood stream was dependent on both the state of intestinal closure and the size of the markers [27, 28].

Interestingly, it may be possible to translate this approach to non-invasive, in vivo use in humans through the use of transcutaneous detection of fluorescence (i.e. measurement of the fluorescence of the blood-borne markers through the skin). This method would entail a patient receiving an oral dose of a fluorescent contrast agent, with the concentration of that contrast agent in the bloodstream being measured in a transcutaneous arrangement using fluorescence spectroscopy. This approach – which would not require collection of urine samples – would provide a direct measure of permeability and would be considerably less time consuming and labour intensive than the traditional L:M and PEG tests. Thus, it could be expected to offer considerable improvements in terms of both the reliability of the test and the experience of the patients. In addition, the devices used for fluorescence detection could be both low in cost and small in size, and only minimal post-processing would be required, making it possible to more quickly return test results to patients.

In the experiments proposed here we aim to test and validate transcutaneous fluorescence spectroscopy as a tool for the non-invasive quantification of gut permeability. This will entail testing two devices – a ‘mark I’ bench-top system and a miniaturised ‘mark II’ device suitable for larger scale deployment – along with four fluorescent contrast agents (fluorescein; indocyanine green, ICG; fluorescein isothiocyanate conjugated dextran, FITC-dextran; and FITC-conjugated PEG, FITC-PEG). These devices and contrast agents will be applied to patient studies with the key aims of validating transcutaneous spectroscopy, developing and optimising a fluorescence-based gut permeability assay (in terms of both the device and the protocol), and applying the optimised system to wide-ranging disease studies. Importantly, the
successful development of this novel gut permeability sensor is likely to have a considerable impact in this field due to the opportunities it will offer for both non-invasive diagnostics and large-scale investigational studies.

AIMS & OBJECTIVES

This study has three overarching aims:

1. to determine whether it is possible to monitor the permeability of the human gut using transcutaneous fluorescence spectroscopy of fluorescein, ICG, FITC-dextran and/or FITC-PEG;
2. to develop, optimise and validate fluorescence-based permeability sensing via comparison to traditional gut permeability measurements (including PEG-based permeability assays and histological assessments) in healthy volunteers and positive controls (i.e. subjects with highly increased permeability such as untreated coeliac or IBD patients);
3. to investigate a wide spectrum of gut permeabilities and to correlate transcutaneous permeability measurements to disease state in some or all of the conditions studied (which will include IBD, coeliac disease, HIV and fatty liver disease).

MATERIALS & METHODS

Instrumentation

Two separate optical systems will be developed and used in this study. The first is a bench-top device consisting of two laser sources for excitation of fluorescence, a spectrometer for detection, and a bifurcated optical fibre probe to allow interrogation of the fluorescence signal at the subjects' skin (see Figure 1). This system is described in detail in Appendix A and is similar in design to the spectrometers reported in [29, 30]. Briefly, it entails two laser sources – at wavelengths of 488 nm and 785 nm – which permit excitation of fluorescence from fluorescein/FITC and ICG respectively. The fibre probe acts to deliver laser light to the subjects' skin and to direct the excited fluorescence signal to the spectrometer. This allows for detection of the fluorescent dyes in the blood stream of the subjects. In order to ensure laser safety, the laser power at the distal end of the fibre probe will be limited. The allowable power levels will vary with the skin-probe distance, which will be tested in early experiments to determine the optimum probe position. Thus, the optical powers will range from 63 µW (skin contact) to 565 µW (maximum skin-probe separation of 2 mm – see Appendix A) at 488 nm and from 93 µW (skin contact) to 836 µW (2 mm) at 785 nm. At these power levels the light output will be below the maximum permissible exposure for the skin (at the appropriate probe-skin separation) and will be eye-safe when the probe is held at a distance of >10 cm [31-33]. As the optical system is also contained within an interlocked, light-tight container, it can therefore be used by non-expert users in a clinical environment without the use of laser safety goggles and without risk of injury.

The second device used in this study will be a miniaturised version of the system described above. This device will use light emitting diodes (LEDs) for excitation and photodiodes (PDs) for detection of fluorescence (and for detection of the directly scattered laser light). It will entail a wireless, wearable tool that is secured in contact with the subjects' skin during use. As above, the optical excitation powers will be limited in order that the system is eye-safe and skin-safe...
during use. The exact design of the ‘mark II’ device will be guided by the results collected with the bench-top (‘mark I’) system and the first stage of experimentation with the miniaturised system will entail validation against the bench-top version. All optical and electrical safety concerns will be factored into the design process, which is discussed in more detail in Appendix B.

![Schematic diagram of the 'Mark I' bench-top optical system.](image)

**Figure 1. Schematic diagram of the 'Mark I' bench-top optical system.** All optical components are contained within a light-tight container that is interlocked to ensure the laser beam can only be manipulated by expert users. The optical power is attenuated using neutral density (ND) filters such that the output at the distal tip is below the maximum permissible exposure for skin at all times and is eye-safe when held at a distance of >10 cm. In this configuration the device can be classified as a Class 1 laser system and, hence, can be used by non-expert laser users in a clinical environment. The fibre probe consists of seven 200 µm diameter optical fibres, one for excitation (blue) and six for collection of fluorescence (yellow) – see insets at bottom right. At the distal tip of the probe the central excitation fibre is surrounded by the six collection fibres such that the ‘active area’ of the probe spans a diameter of approximately 600 µm.

**Contrast agents**

As discussed above, four contrast agents will be used in these studies: fluorescein, ICG, FITC-dextran and FITC-PEG. In Stage 1 of the experiments (see ‘Experimental protocols’ section below), either fluorescein or ICG will be administered to patients via intravenous injection. In Stages 2 and 3, combinations of fluorescein, ICG, FITC-dextran and/or FITC-PEG will be administered to patients orally (as a solution) with a variety of combinations tested (e.g. each dye individually, fluorescein + ICG, FITC-dextran/FITC-PEG + ICG, and fluorescein followed by FITC-dextran/FITC-PEG). In all cases, the dyes will be administered to subjects by a trained healthcare professional and all measurements will be carried out within a hospital environment so that patients/volunteers are monitored for signs of side effects at all times. Doses will be limited to minimise the risk of complications, and the safety issues relating to each of the individual contrast agents are described below.

**Fluorescein** Fluorescein is approved for medical use with intravenous delivery for ophthalmic angiography [34-36]. It is also often used ‘off-label’ with oral delivery
– either as a solution or in the form of a tablet/capsule – for the same purpose [34, 35, 37-39], and this has been shown to be both safe and effective [37, 40-44]. Indeed, it has been observed that both serious and minor adverse effects are less common when administering fluorescein orally rather than intravenously [41]. In addition, fluorescein has been further used ‘off-label’ for a variety of surgical and endoscopic imaging applications (e.g. [45, 46]). In the experiments proposed here, subjects will receive either an intravenous or oral dose of fluorescein depending on the experiment in which they are taking part. The maximal intravenous dose will not exceed 500 mg (typically administered as a 5 ml solution with a concentration of 100 mg/ml) as per the Medicines and Healthcare products Regulatory Agency (MHRA) guidelines [34, 35]. In the case of oral delivery, a maximal dose of 5 g will be used. This is approximately double the maximal dose typically used in oral fluorescein angiography, however, it can be considered safe as complications are extremely rare with oral fluorescein delivery [37, 40, 41, 44]. Furthermore, it will be necessary to test higher doses than those used for retinal imaging in order to determine the minimum oral doses that are detectable using a transcutaneous measurement protocol (i.e. lower doses will be detectable in the eye – due to its transparent nature – than through the skin). Once suitable minimum concentrations have been established, these will be used in all further experiments.

**ICG**

ICG is approved for medical use in solution using intravenous delivery for a variety of applications [47-50] and safe human use with oral delivery has also been reported (e.g. [51]). Subjects will receive either an intravenous or oral dose depending on the experiment in which they are taking part. The total dose delivered to a given patient will not exceed 5 mg of ICG per kg of body weight (mg/kg) for both intravenous and oral delivery. No significant toxic effects have been observed at this dose [49, 52] and it corresponds to the MHRA guidelines for intravenous use in adults [47]. Hence, it can also be expected to be a safe dose when administered orally. In addition, individual intravenous doses (i.e. those administered for a single experiment) will not typically exceed 0.5 mg/kg body weight, as per the MHRA guidelines [47]. Importantly, these dosage levels are also in agreement with doses used in investigational studies with oral dye delivery, in which this method of ICG administration was found to be safe (e.g. [51]).

**FITC-dextran**

FITC-dextran is not yet approved for medical use, however, it has been applied to a limited number of in vivo clinical studies in humans using both intravenous [53] and oral [54] delivery. In addition, oral delivery of FITC-dextran has been used widely in animal experiments (e.g. [27, 28]). In human studies, total oral and intravenous doses of up to 1 g have been used (delivered as a 100 ml solution at 10 mg/ml orally and as a 10 ml solution at 100 mg/ml intravenously) [53, 54], and FITC-Dextran has been shown to be non-toxic at up to 18 times this dose in rats [53]. Comparable oral doses have been used in animals, corresponding to 20-250 mg/kg in piglets [27, 28] and up to 2.5 g/kg in rats [28]. FITC-Dextran is stable in solution and shows no evidence of degradation into smaller molecular weight species [53, 55]. This has been demonstrated both in vitro in solution [53, 55] and in vivo in rabbits after intravenous injection [55]. Furthermore, when applied orally in animals, FITC-dextran is detected in tact in the blood stream and the uptake into blood is dependent on both the molecular
size and the state of intestinal closure [27, 28]. Similarly, after oral ingestion in humans, FITC-dextran is observed in tact in urine, with negligible free fluorescein detected [54]. Together, these results indicate that any degradation of FITC-dextran in the stomach and intestine is minimal. In the experiments proposed here FITC-dextran will be administered orally. In order to mitigate the risk, maximal doses of 1 g will be used. This corresponds to a dose that has previously been used safely in humans (using both intravenous and oral delivery) [53, 54] and is below the levels set out for oral delivery of fluorescein above. This dose is also well below the maximum allowable dose of dextran, which is approved for use at considerably higher concentrations under certain circumstances [56]. Prior to administration, FITC-dextran solutions will be sterilised for clinical use via filtration through 0.2 µm filters, in accordance with the preclinical protocol reported in reference [53]. This will ensure that any heavy metal constituents or endotoxins are only present in very low concentrations that are suitable for human use [53].

**FITC-PEG**

At present, FITC-PEG is not approved for clinical use and it has not yet been used in vivo in humans. Despite this, it has been used widely in research studies for both cellular (e.g. [57-62]) and animal imaging (e.g. [63]) experiments, where the PEG molecule is often used as a linker such that fluorescein-based fluorescence imaging can be combined with an additional functionality (e.g. thermal tumour ablation, selective binding, etc.) using a single molecule. In these studies, FITC-PEG has been shown to have a low toxicity in cells (in cases where it is not linked to an additional toxic moiety for therapeutic or other purposes) [57, 61-63]. Furthermore, PEG itself is used clinically [64] – for both intestinal permeability measurements [65, 66] and as a laxative [67-69] – and is known to be inert, non-toxic and stable (i.e. it is not broken down by gut bacteria, nor it is metabolised by GI tissues) [66]. In FITC-PEG, the binding between the FITC moiety and the PEG moiety is almost identical to the binding between FITC and dextran in FITC-dextran (see Figure 2). Thus, it can be expected that FITC-PEG will be stable within the GI tract in the same manner that FITC-dextran is (see above). As such, FITC-PEG can be considered safe for in vivo human use assuming that the concentrations of FITC and PEG are respectively kept below their maximum allowable doses. For this reason, we will use a maximum FITC-PEG dose of 1 g, and this will be administered orally as a solution. This corresponds to the level set out for FITC-dextran above – which has previously been used safely in humans with oral administration – and is also below the maximum allowable dose of fluorescein. In addition, it is well below the maximum allowable dose of PEG, which is approved for use at considerably higher levels [65-69]. As with FITC-dextran, prior to administration FITC-PEG solutions will be sterilised via filtration through 0.2 µm filters to allow safe clinical use.

In experiments where two dyes (ICG + fluorescein or ICG + FITC-dextran/FITC-PEG) are administered simultaneously, the doses will be limited as described above. The aim of these experiments will be to determine whether both dyes can be detected simultaneously (through their differing excitation and emission profiles) and whether ratios of the fluorescence intensities of the two dyes can be used to provide a readout of permeability.
In experiments where fluorescein and FITC-dextran/FITC-PEG are administered in sequence, the FITC-dextran/FITC-PEG dose will be taken into account when calculating the maximum fluorescein dose. In this way, both the total fluorescein dose and the FITC-dextran/FITC-PEG dose will be kept within the limits described above. These experiments will serve to investigate whether ratios of the fluorescein and FITC-dextran/FITC-PEG fluorescence intensities can provide a readout of permeability. In this case, administration of the dyes will be performed in sequence (rather than simultaneously) as the spectral properties of the contrast agents are very similar.

Figure 2. Molecular structures of FITC-dextran and FITC-PEG. (A) FITC-dextran. Asterisk (*) indicates that the location of the FITC binding is random and can occur at any hydroxyl group. (B) FITC-PEG. Dotted red circles highlight the binding between the FITC moiety and the dextran/PEG moiety. The binding is similar in both cases indicating that the stability of the two molecules (at the highlighted binding site) in any given environment is likely to be comparable.

Patient/subject selection
We aim to recruit patients/subjects for this study from three groups as detailed below.

Group 1  **Ophthalmology patients** who are receiving an intravenous dose of either fluorescein or ICG as part of their routine ophthalmic care (e.g. as part of a fluorescence angiography examination). These patients will take part in early tests (Stage 1) determining whether it is possible to detect intravenous fluorescent dyes at known concentrations using a transcutaneous measurement protocol.

Group 2  **Healthy subjects** with no known issues of increased gut permeability (negative controls).

Group 3  **GI and non-GI patients** who are expected to exhibit increased gut permeability (including, but not limited to: coeliac, IBD, HIV and liver disease patients). This group will represent the cases for the gut permeability study. Participants in this group who exhibit especially high intestinal permeabilities (as measured using
control techniques such as PEG permeability assays or histology) will serve as positive controls.

Eligible participants will be identified by the regular clinical teams in the relevant departments (HIV and Hepatology outpatient clinic, GI outpatient clinic, ophthalmology clinic). If a patient agrees to participate in the study their details will be passed on to the research fellow directly involved in the study. Participants will be asked to provide written informed consent. Healthy volunteers will be recruited from Imperial and hospital staff.

**Experimental protocols**
The experimental protocols will entail giving patients a dose of contrast agent as described above and then making transcutaneous fluorescence measurements for up to three hours by placing the optical probe in contact with the skin. In the case of the ‘mark I’ bench-top spectrometer, the tip of the fibre probe will be secured using a 3D-printed clamp such that it is either in contact with the skin or held up to 2 mm above the skin's surface. A variety of measurement locations will be tested with this system in order to determine the most suitable for widespread deployment. The data from these studies will be used to guide the development of the casing for the miniaturised 'mark II' device (as well as the optical/mechanical/electrical design) such that it can be secured in the most appropriate body location. When using the miniaturised device, the entire system will be secured in place on the subject’s skin using the custom-designed 3D-printed casing (e.g. on the fingertip, arm, earlobe or other location, as determined by the bench-top measurements). The exact experimental protocols will vary depending on the phase of the investigation. Thus, the protocols are broken down into three stages (each with its own specific aims) as described below.

**Stage 1**
In the first stage of the experiments we will recruit ophthalmology patients who are due to receive intravenous doses of fluorescein or ICG as part of scheduled angiography examinations. Measurements in these patients will serve to test whether it is possible to detect fluorescein/ICG in the blood using transcutaneous fluorescence spectroscopy at the known concentrations used for ophthalmic angiography. Experiments will be performed at the same time as the patient’s angiography meaning that they will require no additional hospital visits and no additional doses of fluorescent contrast agents (beyond that used for their eye examination). Before the fluorescent dye used for the angiography is administered (in accordance with the procedure recommended by the patient’s ophthalmologist), the spectrometer will be attached to the patient’s skin as described above. Background fluorescence data will be collected for five minutes before the patient receives their dose of fluorescein/ICG. Measurements will then continue for the duration of the angiography and for some time afterwards (up to a maximum total experimental time of three hours, depending on the availability of the patient). Throughout the experiment fluorescence spectra will be recorded at regular intervals (e.g. every 30 s). These measurements will be carried out using the ‘mark I’ device in the first instance and they may also be repeated using the ‘mark II’ device as part of its validation. With the ‘mark I’ device, the probe-skin distance will be varied and a range of body locations will be tested as measurement sites in order to determine the optimum probe position/orientation. These preliminary tests will serve to optimise the protocols used in Stages 2 and 3. In the event that it is not possible to recruit a sufficient number of ophthalmology patients, we will also
consider recruiting healthy volunteers for the Stage 1 intravenous injection protocol.

Stage 2

The aim of the second phase of the experiments will be to validate transcutaneous fluorescence spectroscopy as a tool for the assessment of gut permeability. To this end, we will recruit healthy volunteers as negative controls and patients with GI (or other) conditions who are expected to exhibit particularly high intestinal permeabilities (e.g. untreated coeliac or IBD patients) as positive controls. These subjects will be given oral doses of the fluorescent contrast agents and transcutaneous fluorescence measurements will be made using the ‘mark I’ bench-top device.

Once they have agreed to take part in the study, a time will be arranged for the subjects to come into the hospital for measurements to be taken. On the day of their study visit, the subjects will first be asked a series of questions regarding their general details, medical history and current diet (including details of any medication they are taking and their current alcohol intake). Measurements will also be made of the subjects’ height, weight and waist circumference, and we will ask for consent to access their medical records. Once the preliminary questions and measurements are complete, the optical probe will be secured in contact with the skin and the subject will receive an oral dose of fluorescein, ICG, FITC-dextran, FITC-PEG or a combination thereof (with the doses limited as described in the ‘Contrast agents’ section). Transcutaneous fluorescence measurements will be recorded at regular intervals (e.g. every 30 seconds) for up to 3 hours, beginning five minutes before the contrast agent is administered in order to allow collection of a baseline. During the experiment, two 5 ml blood samples will also be taken – one prior to administration of the contrast agent and one at the point at which the peak fluorescence intensity is detected – in order to allow correlation of the fluorescence measurements with the blood concentration of the fluorophores. This concentration will be measured using a laboratory-based spectrofluorometer (as described in [27]) after the transcutaneous fluorescence procedure is complete. Some of the collected blood may also be used to assess the degree of bacterial translocation.

Following the spectroscopic gut permeability tests, subjects will be asked to make two further contributions to the study. Firstly, they will be asked to provide a fresh stool sample within approximately 48 hours of their study visit. This sample will be analysed to determine the concentration of fluorescent dye in the stool (which will provide an indication of intestinal transit time) and may also be assessed with the aim of identifying and quantifying the microbiota. Secondly, subjects will be asked to take a PEG permeability test, which will act as a ‘gold standard’ measurement against which we will compare our spectroscopic permeability readings. Subjects will be given a specific collection kit that will allow them to take the PEG permeability test at home at a convenient time. The test entails drinking a 250 ml aqueous solution of PEG molecules of varying molecular masses and then collecting urine for the following six hours. The urine sample is then shipped to a laboratory for analysis using prepaid packaging provided by the research team.
In patients who have had or are due to have an intestinal biopsy as part of their clinical care, we will ask for consent to use any tissue from the biopsy that is surplus to diagnostic requirements for further analysis in our study. This will allow correlation of the spectroscopic gut permeability test with histological measurements of epithelial damage or permeability (e.g. quantification of the efficacy of the tight junctions via Ussing Chamber measurements).

Finally, in a subset of the healthy subjects, we will ask the volunteers to take the spectroscopic gut permeability test twice. In the first instance, this will take place as described above. In the second case, however, the volunteers will receive a dose of the chosen fluorescent contrast agent(s) in combination with a hyperosmotic solution that will act to transiently increase their gut permeability (i.e. in a similar manner to that described in references [54, 70]). Thus, by comparing the results in these subjects with and without the hyperosmotic solution, we will be able to validate the capability of the spectroscopic test to monitor changes in intestinal permeability. Interestingly, this validation will be possible without the requirement for additional ‘gold standard’ measurements (such as PEG permeability tests or histopathology, as discussed above) as each subject will act as their own internal control. A range of hyperosmotic solutions will be investigated, including sucrose, glucose, glycerol and sodium chloride, with maximum doses of 60 g, 30 g, 15 g and 5 g (in 100 ml of water) respectively. Importantly, at these concentrations, all solutions are safe for human consumption (all are widely used in the food industry – for example, a 500 ml bottle of Coke contains approximately 50 g of sugar) and can be expected to instigate temporary alterations in intestinal permeability [54, 70].

Overall, the aim of Stage 2 is to develop, optimise and validate the spectroscopic assay proposed in this protocol as a readout of gut permeability. This will involve determining the optimum combination and dose of contrast agents and investigating data analysis/processing approaches to ascertain which offer the optimal permeability readouts. This will be achieved by testing a range of contrast agent combinations and analysis protocols, and comparing the acquired data to one or more of the ‘gold standard’ measurements discussed above.

Stage 3

Based on the data collected in Stage 2, we will design and develop an optimised, wearable device (‘mark II’) – along with an optimised experimental protocol – suitable for larger scale studies. This device and protocol will then be applied to disease studies aimed at: (i) demonstrating the capability of the optimised spectroscopic gut permeability assay to accurately monitor a wide range of permeabilities; and (ii) correlating spectroscopic measures of permeability to disease state (via comparison to the ‘gold standard’ markers discussed above). The experimental protocol used will be identical to that described for Stage 2, but the use of the ‘mark II’ device and the optimised procedure (which will most likely entail a much shorter measurement duration) will allow for measurements in considerably larger cohorts. We will aim to recruit patients expected to exhibit increased gut permeability with a variety of diseases. This will include (but will not necessarily be limited to) inflammatory bowel diseases (such as Crohn’s disease and colitis), coeliac disease, fatty liver disease, and HIV. In cases where patients receive a treatment/therapy that
begins after their planned spectroscopic gut permeability test, we will also seek consent to make multiple longitudinal measurements to allow us to determine whether this approach can be used to monitor the impacts of interventions. Overall, the experiments performed in Stage 3 will serve to demonstrate the utility of the spectroscopic gut permeability assay in a wide array of prevalent disorders.

**Schedule of procedures**

As described above, participants will take part in a series of procedures as part of this study, with some carried out in the hospital by the study team and others undertaken at home by the subjects/patients themselves. The procedures will vary depending on the stage of the investigation and the patient/subject group. Thus, the table below shows and describes the procedures that will be performed for each patient group.

<table>
<thead>
<tr>
<th>PROCEDURE</th>
<th>DESCRIPTION</th>
<th>VISIT</th>
<th>GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>The Study Doctor will discuss the Patient Information Sheet and Informed Consent Form with the patient and will answer any questions. Once the patient feels satisfied that their questions have been answered and feels certain that they want to join the study, the doctor will ask them to sign the Informed Consent Form. At this point we will also arrange a time for the patient to come in and participate in the study (the study visit).</td>
<td>Consultation visit (prior to study)</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Inclusion/exclusion criteria</td>
<td>The Study Doctor will go through the inclusion and exclusion criteria to ensure that the patient is eligible to join the study by asking questions regarding medical history.</td>
<td>Consultation visit (prior to study)</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>General, medical and dietary questions; measurements of height, weight, etc.</td>
<td>Subjects will be asked a few questions about their general details and medical history, including any medication they are taking. Height, weight and waist circumference will be measured. Subjects will also be asked some questions about their diet, including current alcohol intake.</td>
<td>Study visit</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Consent to use tissue from intestinal biopsy</td>
<td>If patients in group 3 have had or are due to have an intestinal biopsy as part of their clinical care, we will ask for their consent to use any surplus tissue from the biopsy in our study. This will allow us to correlate the results of the spectroscopic gut permeability test with histological markers of tissue damage or increased intestinal permeability.</td>
<td>Study visit</td>
<td>3</td>
</tr>
<tr>
<td>Intravenous injection of contrast agent: fluorescein or ICG</td>
<td>Ophthalmology patients will receive an injection of either fluorescein or ICG, as prescribed by their ophthalmologist. The dye and dose used will be exactly the same as for their normal angiography examination.</td>
<td>Study visit</td>
<td>1</td>
</tr>
<tr>
<td>Oral administration of contrast agent: fluorescein, ICG</td>
<td>All participants other than ophthalmology patients will receive an oral dose of one or more fluorescent dyes (fluorescein, ICG, FITC-dextran and/or FITC-PEG). This will</td>
<td>Study visit</td>
<td>2, 3</td>
</tr>
<tr>
<td>PROCEDURE</td>
<td>DESCRIPTION</td>
<td>VISIT</td>
<td>GROUP</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>FITC-dextran and/or FITC-PEG</td>
<td>be administered as a single solution of approximately 300 ml (similar to the size of a can of soft drink) for the subject to drink.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spectroscopic gut permeability test</td>
<td>We will attach a small device (spectrometer) to the arm or fingertip of the subject. This device will be used to make measurements of the amount of fluorescein/ICG/FITC-dextran/FITC-PEG in the bloodstream, through the skin. The device is comfortable to wear and will not break, damage or hurt the skin in any way.</td>
<td>Study visit</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Oral dose of hyperosmotic solution</td>
<td>A subset of the healthy volunteers will receive an oral dose of a hyperosmotic solution (which will act to temporarily increase their gut permeability) as part of a second spectroscopic gut permeability test. This will take place at a second study visit. Other than the dose of the hyperosmotic solution (which will consist of sucrose, glucose, glycerol or sodium chloride), the procedure will be identical to the volunteers’ first spectroscopic gut permeability test.</td>
<td>Second study visit</td>
<td>2</td>
</tr>
<tr>
<td>Blood samples</td>
<td>We will take two 5 ml blood samples during the tests (equivalent to about one teaspoon of blood for each sample). The first will be taken before the measurements begin and the second will be taken during the measurements.</td>
<td>Study visit</td>
<td>2, 3</td>
</tr>
<tr>
<td>Adverse events</td>
<td>Participants will be asked if they are undergoing or have experienced any adverse events (such as any problems, symptoms or discomforts).</td>
<td>During and after study visit</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Stool sample</td>
<td>Within 48 hours of the Spectroscopic gut permeability test we will ask subjects to provide a fresh stool sample, which will be used for further analysis.</td>
<td>2 days after study visit</td>
<td>2, 3</td>
</tr>
<tr>
<td>PEG gut permeability test</td>
<td>Finally, subjects will be asked to take a PEG permeability test sometime in the week following their study visit. This can be carried out at home at the convenience of the participant, and they can administer the test themselves. We will provide a collection kit to perform the PEG permeability test, which will involve drinking a solution and then collecting urine for the following 6 hours. Subjects will then post the collected urine sample to a laboratory for analysis using prepaid packaging. We will ask that subjects try to perform the PEG permeability test within 1 week of their study visit.</td>
<td>2+ days after study visit</td>
<td>2, 3</td>
</tr>
</tbody>
</table>

**Patient recruitment**

Potential participants will be approached by members of their healthcare/clinical team. This will either be in the outpatient department or prior to their appointment by telephone. For those patients approached on the phone, patient information sheets will be sent to them in the post prior to their outpatient appointment. All participants who agree to see the study team will then be provided with an information sheet (if they were not already sent one) and given an opportunity
to discuss their involvement in the study after their clinic appointment. Informed consent will be taken by trained (GCP accredited) clinical members of the study team after a minimum of 24 hours has elapsed from the time at which the study information was provided.

Healthy volunteers will be recruited from Imperial College and St. Mary’s Hospital staff. Potential volunteers will be approached in person by members of the research team. Study information will then be provided as described above and informed consent will be taken by trained (GCP accredited) clinical members of the study team (a minimum of 24 hours after the study information was provided).

INCLUSION CRITERIA

- Ability to give informed consent
- Aged 18 years or above
- No evidence of prior adverse reactions to fluorescein, ICG, dextran or PEG
- No evidence of prior adverse reactions to iodine (for ICG experiments only)
- **For healthy volunteers:** healthy with no active GI/liver disease (or other condition in which increased gut permeability is expected, e.g. HIV) and no antibiotics taken within the previous four weeks.
- **For cases:** exhibiting symptoms of GI, liver or other diseases (e.g. HIV) in which increased intestinal permeability is expected.
- **For ophthalmology patients recruited in Stage 1:** healthy (i.e. as described above for healthy volunteers) and prescribed to have an ophthalmic angiography with an intravenous injection of either fluorescein or ICG.

EXCLUSION CRITERIA

- Unable to give informed consent
- Aged <18 years
- Previous adverse reaction to fluorescein, ICG, dextran or PEG
- Known allergy to iodine (for ICG experiments only)
- Pregnancy

WITHDRAWAL OF SUBJECTS

Subjects are free to withdraw consent for inclusion in the study at any time and, where requested, their samples/data will be removed from further study and destroyed.

ADVERSE EVENTS

Definitions

**Adverse event (AE):** any untoward medical occurrence in a patient or clinical study subject.
Serious adverse event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of existing inpatients’ hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Reporting procedures
All AEs should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non-serious AEs
All such events, whether expected or not, should be recorded.

Serious AEs
An SAE form should be completed and sent to the Chief Investigator within 24 hours. However, relapse and death due to a pre-existing condition, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the [name of REC] where in the opinion of the Chief Investigator, the event was:
- ‘related’, i.e. resulted from the administration of any of the research procedures; and
- ‘unexpected’, i.e. an event that is not listed in the protocol as an expected occurrence.

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES (National Research Ethics Service) SAE form for non-IMP (investigational medicinal product) studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
Sponsor
Imperial College Joint Research Compliance Office – jrco@imperial.ac.uk.
SAMPLE SIZE & STATISTICAL ANALYSIS

We aim to recruit 50 patients.

This will include a total of 20 subjects with no suspected intestinal health issues. This subset will be made up of ophthalmology patients undergoing fluorescein or ICG angiography and healthy volunteers. We will aim for 5 of these patients to receive intravenous doses of fluorescent contrast agents (ophthalmology patients) and for the remaining 15 (healthy volunteers) to be given oral doses. The measurements on subjects receiving intravenous dyes will serve to allow a simple test as to whether the fluorescence signal can be detected in a transcutaneous arrangement. The measurements on subjects receiving oral doses will act as the healthy control measurements in which intestinal permeability is expected to be normal.

A further 30 patients will be recruited who are expected to show increased intestinal permeability. These patients will have either GI conditions or non-GI conditions such as HIV and liver disease for which increased intestinal permeability is expected.

The study will serve to answer a number of clinical questions including whether transcutaneous fluorescent signals can be detected after intravenous and oral administration of contrast agents and whether these signals correlate to traditional (either direct or indirect) measures of gut permeability (i.e. PEG-based permeability assays or histological assessments). The most important clinical question, however, is whether the fluorescence-based permeability assay can be used to differentiate between subjects with normal intestinal permeability and those with increased permeability. Therefore, we estimated the number of patients required to answer this question with a statistical power of over 80% and an alpha value of below 0.05. This calculation requires knowledge of the mean values and standard deviations that will be obtained using the fluorescence permeability assay. As these are currently unknown (because this is a new test), we used previously reported L:M (lactulose:mannitol) ratios to estimate the power and alpha values. L:M ratios are likely to provide a similar readout to the fluorescence permeability assay as we will be investigating differences (ratios) between the fluorescence intensities of small (fluorescein/ICG) and large (FITC-dextran/FITC-PEG) fluorophores. Thus, the L:M ratio (which measures the relative urinary recovery of one large and one small sugar molecule) can be considered analogous to the fluorescence assay, and previously reported L:M ratios should serve as a suitable substitute in these calculations.

In healthy patients with normal intestinal permeability, L:M ratios are typically on the order of 0.01-0.02 [71, 72]. In patients with coeliac disease and Crohn’s disease, increased L:M ratios have been reported, corresponding to 0.105 [72] and 0.085 [71] respectively. Considerable intra- and inter-patient variation is observed in both healthy volunteers and patients with increased intestinal permeability, with standard deviations of up to approximately 100% of the mean values having been reported [71, 72].

Using the patient numbers and L:M ratios above, we can calculate the statistical power for a comparison of the means of two samples (i.e. healthy volunteers vs. patients with increased permeability). With 45 patients (15 healthy, 30 increased permeability), following the calculation described at http://powerandsamplesize.com-Calculators/Compare-2-Means/2-
Sample-Equality, we find that an alpha value of 0.05 and a statistical power of above approximately 80% can be obtained even when using the highest observed standard deviations. For example, using a standard deviation of 0.085 for both the normal permeability and increased permeability cohorts, with L:M ratios of 0.021 and 0.085 respectively (i.e. as reported by Andre et al. [71], with standard deviations for both cohorts estimated as 100% of the increased permeability mean), we obtain an alpha value of 0.05 and a statistical power of 79.2%. Using lower standard deviations or greater differences between the two means provides even higher statistical powers. This represents acceptable statistical significance and indicates that with 45 patients (assuming the means and standard deviations above) we would be able to confidently reject the null hypothesis that the mean permeabilities of the healthy and increased permeability cohorts are in agreement (H0: \( m_h - m_b = 0 \)) – i.e. we would be able to confirm that the fluorescence-based gut permeability assay was capable of detecting changes in intestinal permeability.

Of course, the actual mean values and standard deviations observed when using the fluorescence-based permeability assay will only be determined once the experiments commence. However, the above estimates represent acceptable statistical parameters, suggesting that the study will be capable of producing statistically significant results based on the proposed level of recruitment (assuming that the mean values and standard deviations obtained with the fluorescence-based assay are comparable to those reported in L:M tests).

Statistical analysis will be performed using Matlab, and professional statistical advice will be sought where necessary.

DATA COLLECTION, DATA HANDLING & RECORD KEEPING

Explicit consent for access to medical records by members of the research team will be gained. Transfer on magnetic/optical media or networks will only be in encrypted form, according to local NHS ICT protocols. Where data is stored on NHS or Imperial College computers, appropriate access controls will be in place to ensure that access to confidential research information is restricted to those who need it. Paper records (consent forms etc.) will be stored securely on NHS premises. This will be within a locked filing cabinet or cupboard in a locked office to which only the senior research team has access.

The Data Protection Act and Caldicott principles will be adhered to at all times.

Data will be pseudo-anonymised as soon as possible. Data recorded on the case record form will be identified by a unique reference number. This will only be linked to the individual patient separately, in a secure database held by the recruiting clinicians. Samples, case record forms and other trial documentation will be labelled only with this unique identifier.

Identifiable patient data will only be stored on secure computers which may only be accessed by the clinicians involved in the patients’ clinical care. A unique identifying numerical code – which is distinct from the NHS number or hospital record number – will be assigned to each record. This unique identifier will be used for all research data stored on investigators’ computers. This pseudo-anonymised data will be kept on NHS and University computers. Such data will be encrypted to the local ICT requirements.
Only members of the research team who hold a relevant NHS Trust contract will have access to the medical records of those who agree to participate. Explicit consent for this access will be sought from each participant on the consent form. Information gleaned from such access will remain entirely confidential, and will only be recorded anonymously in study records.

It is Imperial College policy that all data relating to research, including consent forms, are kept for 10 years.

**SAMPLE STORAGE**

Any samples collected from patients will be handled and processed in line with relevant biosafety regulations. In the case of samples collected from HIV-infected patients, all processing will be undertaken within a category 3 laboratory.

The samples will be registered and stored as a sub-collection of Imperial’s Tissue Bank. This Tissue Bank fully conforms to HTA regulation. All freezers in the unit are constantly monitored by T-Scan alarm systems and are fully secured. Access to samples is by permission of the study CI. Freezers are locked and are kept within rooms with pin code entry systems.

At the end of the research, samples where consent allows for future research use will be transferred into the Biobank, which operates under the licence of Imperial College.

**STOPPING/DISCONTINUATION RULES**

The study is due to continue for four years. It is anticipated that healthy subject and ophthalmology patient measurements will be undertaken in the first year, while measurements on GI patients and other non-GI patients with increased gut permeability will continue for the duration of the study. There are no specific stopping rules as this is primarily a feasibility study investigating the novel gut permeability assay.

**RESEARCH GOVERNANCE, MONITORING, ETHICS AND R&D APPROVAL**

The study will be conducted in compliance with the Research Governance Framework for Health and Social Care and Good Clinical Practice. The study will be conducted in accordance with the approvals of the Research Ethics Committee and the Joint Research & Compliance Office of Imperial College London & Imperial College Healthcare NHS Trust.

**Indemnity**

Imperial College London holds negligent harm and non-negligent harm insurance policies, which apply to the experiments outlined in this protocol.

**Sponsor**

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.
Funding
Imperial College London is funding this study through the Imperial College Research Fellowship scheme. This funding does not cover participant payments, therefore, participants will not be reimbursed for taking part in the study.

PUBLICATION POLICY
Results of the study will be disseminated by conference presentation and peer reviewed journal publication. This will be in open access formats wherever possible.
APPENDIX A

Optical setup and laser safety considerations for bench-top fluorescence spectrometer (‘mark I’)

The optical setup for the bench-top fluorescence spectrometer (‘mark I’) used in the first stage of these experiments is shown in Figure 1. The two laser sources have wavelengths of 488 nm and 785 nm, which permit excitation of fluorescence from fluorescein/FITC and ICG respectively. The output from the two lasers are combined using a dichroic beamsplitter and coupled into the excitation channel of the fibre probe. This delivers the excitation light to the measurement site and the fluorescence is then collected by the detection channel of the probe and routed back to the spectrometer via an emission filter (in order to reject any directly scattered excitation light). The fibre probe is electrically insulating to avoid the risk of electric shock to the subject or user. All optical components are securely mounted on an optical breadboard and are contained within a light-tight box such that the only point at which laser radiation is emitted is at the tip of the fibre probe. Neutral density filters are positioned directly in front of the two lasers to limit the optical power to a level at which the maximum intensity that can be achieved at the distal end of the probe (i.e. when light is optimally coupled into the optical fibre) is below the maximum permissible exposure for the skin [31-33]. For both excitation wavelengths, this exposure limit will vary depending on the distance of the probe from the skin. A range of probe-skin distances will be tested in initial validation experiments to determine the optimal distance for spectral permeability measurements, and for each known distance the power will be limited accordingly. When the probe is in contact with the skin the power will be limited most stringently. In this case, the optical powers at the fibre probe output will be restricted to approximately 63 µW at 488 nm and 93 µW at 785 nm. The maximum distance from the skin at which the fibre probe will be secured will be approximately 2 mm. At this distance the excitation light will illuminate a circle of 600 µm diameter – i.e. it will fully illuminate the surface area from which the detection fibres collect light (see Figure 1). In this arrangement, as the light is spread over a larger area, the maximum allowable power at the distal tip of the probe increases to approximately 565 µW at 488 nm and 836 µW at 785 nm. For other fibre-skin separations the maximum permissible power will be calculated in the same manner and the laser outputs limited accordingly. Importantly, even with the highest power levels discussed above (i.e. at the maximum probe-skin separation), the light that is output from the fibre probe will also be below the maximum permissible exposure for the eye when the tip of the probe is held at a distance of >10 cm [31-33] (assuming a blink reflex of <0.5 s).

Thus, in this configuration (with the light-tight box closed), the laser system is eye-safe and can be operated without the use of laser safety goggles. In addition, the system has an interlock that cuts out the laser emission if the lid is removed. This ensures that the system can be safely operated by non-expert users and allows it to be classified as a Class 1 laser device. Any re-alignment work is performed by expert laser users only and is carried out in a separate locked room with blacked out windows. In this case, the interlock is overridden by the expert user and alignment is performed while wearing appropriate laser safety goggles, in accordance with local risk assessments. For all clinical measurements, the interlock is engaged and the lid is closed such that the laser system is eye-safe, can be classified as a Class 1 laser product, and can be operated by non-expert laser users without risk of injury.
APPENDIX B

Discussion of design and safety considerations for the miniaturised (‘mark II’) fluorescence spectrometer

A miniaturised, wearable fluorescence spectrometer will be developed and validated as part of this study, with the aim of deploying this ‘mark II’ system to larger scale studies of gut permeability. This wearable monitor will use LEDs and PDs for excitation and detection of fluorescence. While the exact optical design of the device will be guided by the information collected with the ‘mark I’ bench-top system, a likely approach will involve the use of two LEDs for excitation of fluorescence (centre wavelengths of approximately 488 nm and 785 nm) and four PDs for detection. In this arrangement, optical filters will be placed in front of each of the PDs such that they specifically detect light over the following wavelength ranges: 485-495 nm (for measurement of the 488 nm excitation power); 505-600 nm (for detection of fluorescein fluorescence); 780-790 nm (for measurement of the 785 nm excitation power); and 800-900 nm (for detection of ICG fluorescence). This would allow detection of fluorescence signals from both fluorescein and ICG, as with the ‘mark I’ system.

The optical excitation power will be limited to ensure laser safety, and the exact power limits will be determined once the final device has been designed/manufactured and the relevant dimensions are known (i.e. the illuminated area, the LED-skin distance, etc.). All components will be contained within a 3D-printed plastic case, which will be sealed to ensure that no electrical components come into contact with the skin of the subjects or the researchers. As such, it will be possible for the device to be safely operated by non-expert users. The case will also act to secure the device such that it is in contact with the patients’ skin at all times during the measurements. The exact embodiment will be designed after data has been collected with the bench-top system that reveals the most suitable measurement location on the subject’s body. This is likely to entail a ‘fingerclip’ type device, however skin patches, earlobe clips or other embodiments may also be considered, and this will be dependent on the results of measurements made with the ‘mark I’ device. Overall, the miniaturised device will be designed such that it is suitable for safe and widespread clinical deployment by non-expert users.
REFERENCES

1. E. M. M. Quigley, "Leaky gut - concept or clinical entity?," Current Opinion in Gastroenterology, 32(2), 74-79 (2016)


5. S. Y. Salim and J. D. Soderholm, "Importance of disrupted intestinal barrier in inflammatory bowel diseases," Inflammatory Bowel Diseases, 17(1), 362-381 (2011)


7. B. Schnabl and D. A. Brenner, "Interactions between the intestinal microbiome and liver diseases," Gastroenterology, 146(6), 1513-1524 (2014)


17. The MAL-ED Network Investigators, "The MAL-ED study: A multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and..."
children up to 2 years of age in resource-poor environments," Clinical Infectious Diseases, 59(suppl 4), S193-S206 (2014)
34. Medicines and Healthcare products Regulatory Agency (MHRA), Public assessment report - fluorescein sodium 100 mg/ml, solution for injection, in PL 41349/0001, Medicines and Healthcare products Regulatory Agency (MHRA), Editor, 2015

35. Medicines and Healthcare products Regulatory Agency (MHRA), Summary of product characteristics - fluorescein sodium 100 mg/ml, solution for injection, in PL 43956/0002, Medicines and Healthcare products Regulatory Agency (MHRA), Editor, 2015


47. Medicines and Healthcare products Regulatory Agency (MHRA), Summary of product characteristics - verdye (ICG) 5 mg/ml injection, in PL 44791/0001, Medicines and Healthcare products Regulatory Agency (MHRA), Editor, 2016


51. S. Sou, T. Matsui, T. Yao, M. Naito, M. Yorioka, T. Beppu, T. Nagahama, and K. Futami, "Differentiating enterocutaneous fistulae from suture abscesses complicating crohn's disease


69. Medicines and Healthcare products Regulatory Agency (MHRA), *Public assessment report - dulcosoft 10 g powder for oral solution in sachet; macrogol 4000 10 g powder for oral solution in sachet (macrogol 4000)*, in PL 23022/0112-0113, Medicines and Healthcare products Regulatory Agency (MHRA), Editor, 2015