Endomicroscopy

- Endomicroscopy is a technique for obtaining histology-like images from inside the human body in real-time, a process known as 'optical biopsy'.
- It generally refers to fluorescence confocal microscopy, although multi-photon microscopy and optical coherence tomography have also been adapted for endoscopic use.
- Commercially available clinical and pre-clinical endomicroscopes can achieve a resolution on the order of a micrometre, have a field-of-view of several hundred μm, and are compatible with fluorophores which are excitable using 488 nm laser light.
- he main clinical applications are currently in imaging of the tumour margins of the brain and gastro-intestinal tract, particularly for the diagnosis and characterisation of Barrett's Esophagus, pancreatic cysts and colorectal lesions.
- A number of pre-clinical and transnational applications have been developed for endomicroscopy as it enables researchers to perform live animal imaging.
- Major pre-clinical applications are in gastro-intestinal tract, toumous margin detection, uterine complications, is chaemia, live imaging of cartilage and tendon, organoid imaging etc.
- Conventional, widefield microscopy is generally unsuitable for imaging thick tissue because the images are corrupted by a blurred, out-of-focus background signal.
- Endomicroscopes achieve optical sectioning (removal of the background intensity) using the confocal principle - each image frame is assembled in a point-by-point fashion by scanning a laser spot rapidly over the tissue.

- In table-top confocal microscopes the scanning is usually performed using bulky galvanometer or resonant scanning mirrors.

- Endomicroscopes either have a miniaturised scanning head at the distal tip of the imaging probe, or perform the scanning outside of the patient and use an imaging fibre bundle to transfer the scan pattern to the tissue.

Types of Endomicroscopy :

- **Single Fibre Endomicroscopes**
- **Fibre Bundle Endomicroscopes**
- **Distal Scanning Endomicroscopes**
- **Non-Confocal Endomicroscopes**
- **Confocal Endomicroscopes**

Single Fibre Endomicroscopes:

- Single Fibre Endomicroscopes use the tip of an optical fibre as a spatial filter, enabling miniaturisation of the microscope.
- 488nm blue laser passes from the source through an optical fibre to a flexible hand-held probe.
- Optics in the probe focus the laser to a spot in the tissue, exciting fluorescence.
- Emitted light is captured into the optical fibre and passed through an optical filter to a detector.
- An image is generated by scanning the focused spot throughout the image plane and compiling the point intensity measurements.
- The image plane can be translated up and down in the sample, allowing generation of 3D image stacks.
- Single fibre endomicroscopes have similar resolution of a conventional confocal microscope.

Fibre Bundle Endomicroscopes

- Fibre bundles were originally developed for use in flexible endoscopes. and have since been adapted for use in endomicroscopy.
- They consist of a large number (up to tens of thousands) of fibre cores inside a single shared cladding, are flexible, and have diameters on the order of a millimetre.
- In a coherent fibre bundle the relative positions of the cores are maintained along the fibre, meaning that an image projected onto one end of the bundle will be transferred to the other end without scrambling.
- Therefore, if one end of the bundle is placed at the focus of a table-top confocal microscope, the bundle will act as a flexible extension and allow endoscopic operation.
- Since only the cores, and not the cladding, transmit light, image processing must be applied to remove the resulting honey comb-like appearance of the images.
- Each core essentially acts as an image pixel, and so the spacing between fibre cores limits the resolution.
- The addition of micro-optics at the distal tip of the bundle allows for magnification and hence higher resolution imaging, but at the cost of reducing the field-of-view.

Digtal Scanning Endomicroscopes

- Digtal scanning endomicroscopes incorporate a miniature 2D scanning apparatus into the imaging probe.
- The laser excitation and returning fluorescent emission are sent to and received from the scanning head using an optical fibre.
- Most experimental devices have either used MEMS scanning mirrors, or direct translation of the fibre using electromagnetic actuation.

Non-Confocal Endomicroscopes

- Widefield endomicroscopes (i.e. non-depth sectioning microscopes) have been developed for select applications, including the imaging of cells.
- Optical coherence tomography and multi-photon microscopy have both been demonstrated endoscopically.
- Successful implementations have used distal scanning rather than fibre bundles due to problems with dispersion and light loss.

Applications:

- Endomicroscopy has been used to study many gastrointestinal disorders
- Small bowel disorders investigated with Endomicroscopy include celiac disease and graft versus host disease.
- Gastric cancer and helicobacter pyloric gastric cancer have been imaged with Endomicroscopes
- Endomicroscope has been used to help target biopsies in all these disorders as well and may also reduce the number of biopsies needed to achieve diagnosis.

Confocal Endomicroscopes

- Confocal Endomicroscopes is recently developed endoscopic technology that allows for histological analysis of tissue in vivo. Conventional endoscopy involves identifying lesions grossly followed by biopsy for histological analysis.
- Confocal Endomicroscopes allows for the performance of real time biopsy during endoscopy by observation of the mucosal layer of the gastrointestinal tract at the cellular level. Images are displayed in real time during the examinations.
- Two systems are currently available and have been approved by the FDA,
- Tip-integrated confocal laser endoscope and
- A flexible fibre-based confocal miniprobe.

- The technique of Confocal Endomicroscopes has been used for diagnosis of upper gastrointes-tinal disorders such as Barrett's esophagus, gastric carcinoma, Cerliac disease.
- It also has application in diagnosis of lower gastrointestinal and biliary tract disorders such as colon polyps, Ulcerative colitis and pancreaticobiliary strictures.
- Confocal laser endomicroscopy (CLE) is an endoscopic modality developed to obtain very high magnification and resolution images of the mucosal layer of the GI tract. CLE is based on tissue illumination with a low-power laser with subsequent detection of the fluorescence of light reflected from the tissue through a pinhole (Fig. 1).1 The term confocal refers to the alignment of both illumination and collection systems in the same focal plane.
- The laser light is focused at a selected depth in the tissue of interest and reflected light is then refocused onto the detection system by the same lens. Only returning light refocused through the pinhole is detected. The light reflected and scattered at other geometric angles from the illuminated object or refocused out of plane with the pinhole is excluded from detection. This dramatically increases the spatial resolution of CLE allowing cellular imaging and evaluation of tissue architecture at the focal plane during endoscopy

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- Confocal imaging can be based on tissue reflectance or fluorescence.
- Confocal devices based on tissue reflectance do not require any contrast agents, but current prototypes using 2-photon strategies have relatively low resolution, which significantly compromise in vivo imaging and clinical utility. CLE by using topical and/or intravenous fluorescence contrast agents generates images with resolution similar to traditional histological examination.
- CLE systems have included through-the-scope probes or dedicated endoscopes with integrated CLE systems.

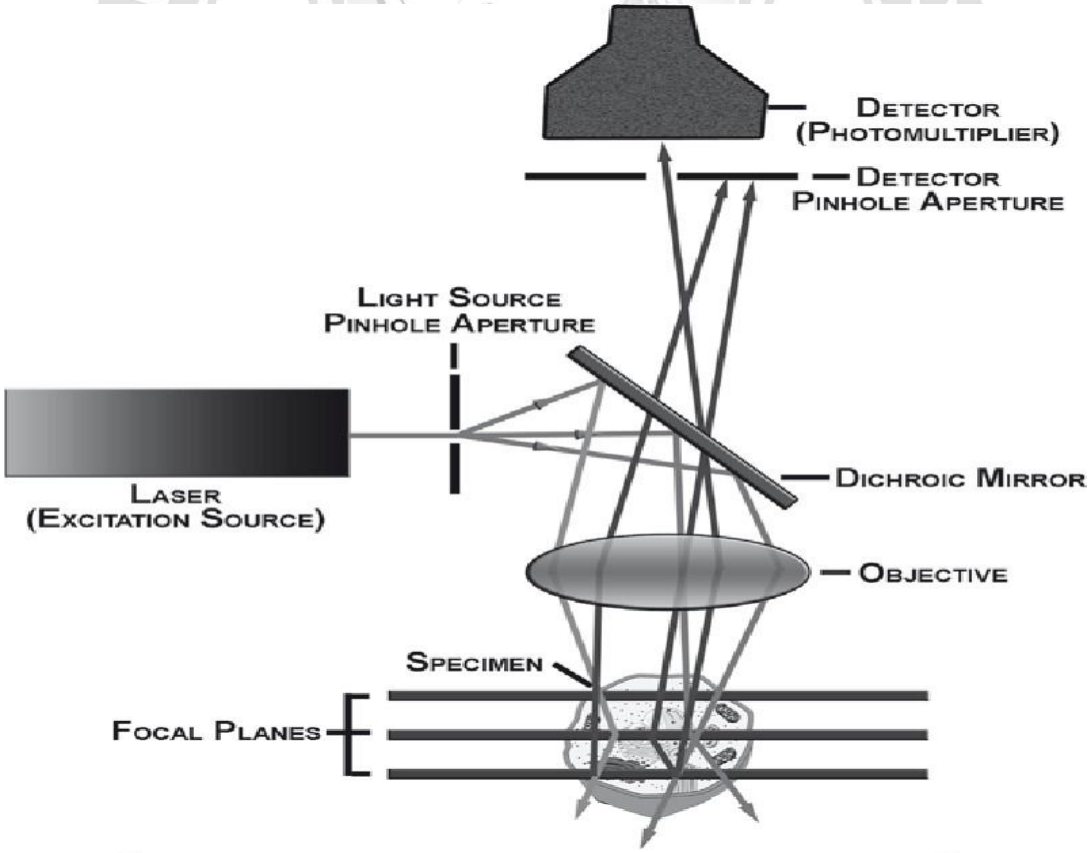

Fig:5.4.1. Schematic of confocal laser endomicroscopy principles.-Source-Web

Probe-based CLE(Confocal laser endomicroscopy)

- Probe-based CLE The probe-based CLE (pCLE) system comprises a fiberoptic bundle with an integrated distal lens that is connected to a laser scanning unit (Fig. 2).
- The probe-based system to date has a fixed focal length and so it can only scan in a single plane unlike current microscope systems that can create cross-sectional images at different depths.
- In pCLE systems, the individual optical fibers function as the pinhole
- CholangioFlex probes, designed for use during ERCP, require an endoscope accessory channel of at least 1.0 mm, whereas the other probes designed for use in EGD and colonoscopy require a channel of at least 2.8 mm. All probes generate dynamic (9-12 frames/s) images.
- The depth of imaging from the surface of the confocal lens is 40 to 70 mm for CholangioFlex probes and 55 to 65 mm for both GastroFlex UHD and ColoFlex UHD probes.
- The maximal field of view for CholangioFlex probes is 325 mm and 240 mm for Gastroflex UHD and ColoFlex UHD probes
- The resolution of the CholangioFlex probe is 3.5 mm, whereas for GastroFlex UHD and ColoFlex UHD probes, it is 1 mm (Mauna Kea Technologies).
- probes can be reused after disinfection for as many as 10 to 20 examinations.

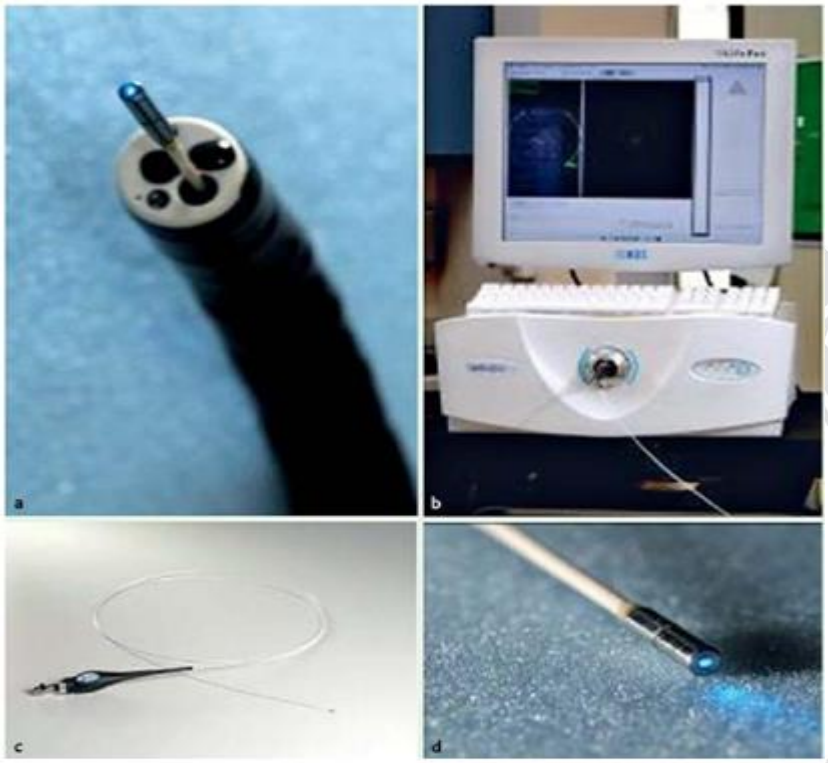

Figure 5.4.2. Probe-based confocal laser endomicroscopy (pCLE) system (Cellvizio; Mauna Kea Technologies, Paris, France) showing endoscope with a probe via an accessory channel (A), laser scanning unit (B), pCLE probe (C), and pCLE with laser illumination (D)- Source-Web

Endoscope-based CLE

- Endoscope-based CLE (eCLE) uses a confocal microscope (Optiscan, Victoria, Australia) integrated into the distal tip of a conventional endoscope
- The diameter of the eCLE endoscope is 12.8 mm, and the tip length is increased to accommodate the laser microscope so that there is a 5-cm rigid portion

It can be used for upper and lower GI tract examinations, but is too large for pancreaticobiliary imaging.

- With this setup, white-light endoscopy and eCLE can be performed simultaneously with images displayed on dual monitors
- Images are collected at a scan rate of 1.6 frames/s (1024 512 pixels) or 0.8 frames/s (1024 1024 pixels) with an adjustable depth of scanning ranging from 0 to 250 mm, a field of view of 475 475 mm.

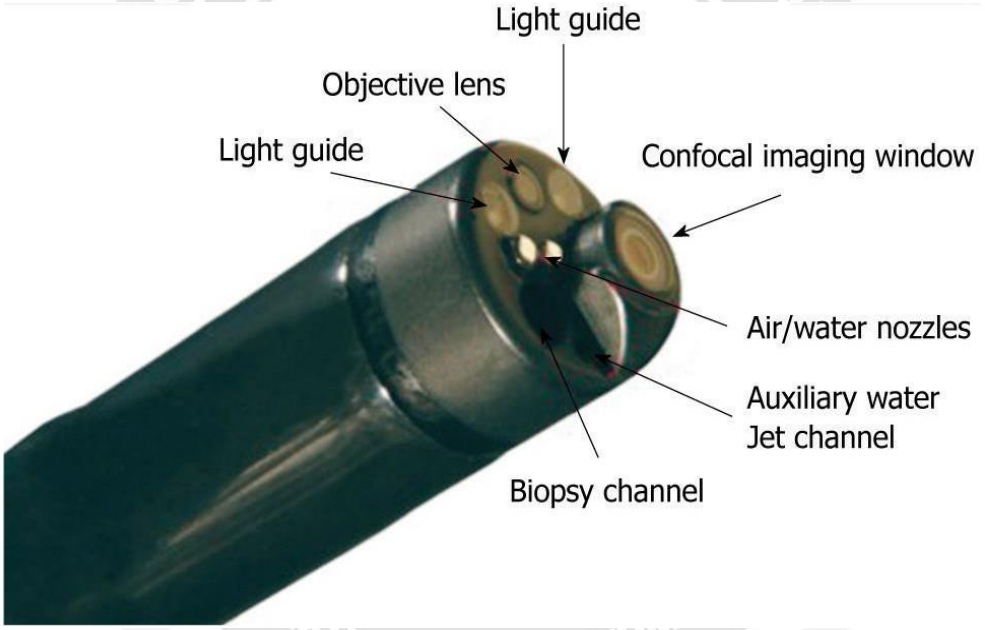

Fig:5.4.3 Endoscope-based confocal laser endomicroscope

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