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Development of fibre-optic Raman probes for *in vivo* diagnosis of upper gastrointestinal cancers

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Introduction

Cancers of the stomach and oesophagus pose major global public health challenges. In order to improve outcomes for patients with these cancers, early detection and treatment of pre-cancerous lesions is essential. However, currently up to 50% of oesophageal and gastric dysplasia (pre-cancer) is macroscopically invisible and so cannot be detected by clinicians during endoscopy. As a result, upper gastrointestinal (GI) cancers are often detected at a late stage by which time they are incurable. Urgent action is required to develop a clinical test that could allow real-time *in vivo* diagnosis of pre-cancerous dysplasia eliminating the need for biopsy and enabling immediate endoscopic therapy to be targeted at the pre-cancerous lesion resulting in improved prognosis.

Fibre-optic Raman spectroscopy has shown great potential as a diagnostic tool in the upper GI tract, as well as at other tissue sites. Raman spectroscopy generates a unique tissue spectrum which facilitates analysis of the biochemical constituents in a tissue sample and can be used as an adjunct to histological diagnosis. The technique involves excitation of the target tissue with a monochromatic laser. The majority of the excitation laser light is elastically scattered by the tissue (the wavelength is unchanged) but a small proportion, approximately 1 in 10^6 photons, is inelastically scattered (Raman scattering), with an altered wavelength, the Raman shift.

Technological developments have highlighted the feasibility of fibre-optic Raman spectroscopy as an endoscopic diagnostic tool during *in vivo* assessment of patients. However, for Raman spectroscopy to be clinically applicable *in vivo* it must be rapid, cost-effective and highly accurate for pre-cancerous dysplasia. In addition, there must be a mechanism to enable probing of hollow viscera that are 15–40 cm (oesophagus) and 40–60 cm (stomach) away from the oral cavity. This presents the challenge of coupling Raman spectroscopy to a fibre-optic probe whilst maintaining low background signal, with efficient delivery and collection optics, and whilst adhering to the size specifications necessary for endoscopic use.

In this article we review the state of the art development of fibre-optic Raman probes for *in vivo* tissue diagnosis in the upper gastrointestinal tract. We identify the major breakthroughs in probe development, reflect on results of the first *in vivo* trials, and discuss the crucial challenges that must be overcome before Raman spectroscopy can be considered for widespread clinical implementation.

Why Raman spectroscopy?

Raman spectroscopy is a non-invasive, reproducible and cost-effective technique that objectively measures highly specific biochemical information within a clinically practicable timescale (seconds). Raman spectroscopy can also be used in combination with fibre-optic probes enabling assessment within the upper gastrointestinal tract and other hollow organs. In addition, the technique does not require tissue preparation (fixation or staining) prior to use, making it perfect for use as an *in vivo* diagnostic tool.

Fibre-optic probe development

A modern fibre-optic Raman spectroscopy system incorporates a single monochromator, a high quality charged-coupled device (CCD) detector, a series of optical filters, a computer and a specialised fibre-optic probe (Figure 1). Near infrared (NIR) (785–830 nm) excitation wavelengths are utilised to help to minimise tissue fluorescence and spectral disruption from optical components.

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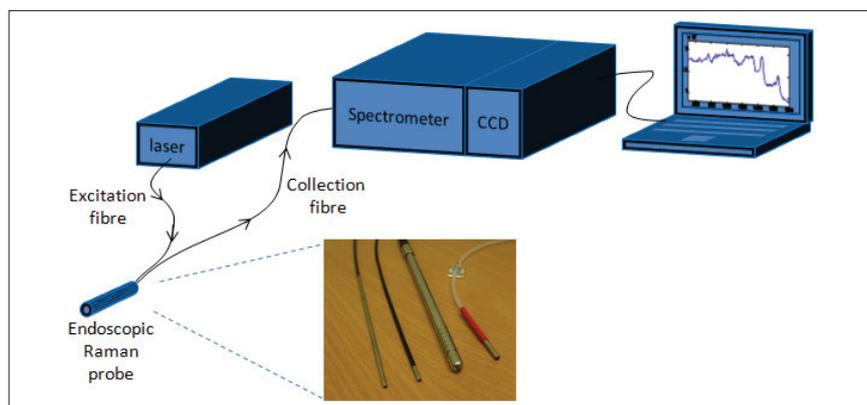
Figure 1. A typical fibre-optic probe-spectrometer interface.

Raman signals are inherently weak, and biochemical changes between normal and invisible dysplastic (pre-cancerous) cells are subtle. When analysing tissue, excitation laser power must be kept low to prevent any chance of thermal injury, meaning signal intensity is limited. As a result, Raman probes must have sufficient collection efficiencies to ensure an adequate signal-to-noise ratio (SNR). In addition their design must comply with a number of dimensional specifications to ensure compatibility with medical endoscopes.

Fibre-optic Raman probes (not endoscope compatible) are well established and some are commercially available. The majority utilise separate illumination and collection fibres to reduce unwanted intrinsic fibre signal and typically include multiple 100–200 µm diameter fibres in conjunction with in-built optical filters.

In 1998, Mahadevan-Jansen *et al.* published *in vivo* results using a fibre-optic Raman probe to analyse dysplastic change in cervical tissue.[1] Silica–silica fibres were shown to cause less signal disruption than sapphire and liquid guide fibres despite their tendency to generate significant background signal at low wavenumbers (below $900\,cm^{-1}$). The probe consisted of a single excitation fibre and 50 collection fibres and had a total diameter of 12 mm. The excitation fibre was positioned at the edge of the probe, and the laser output (at 789 nm) was reflected by a gold mirror at an angle to produce a 900 µm diameter excitation spot on the tissue surface. A bandpass filter on the distal tip of the excitation fibre blocked fluorescence and Raman scattering generated by the fibre, but transmitted the excitation light. Although demonstrating that Raman spectra could feasibly be measured *in vivo* using a fibre-optic, the dimensions of this probe and the time taken for spectral acquisition (90 s) meant this design was impractical for use in the gastrointestinal tract.

The next important challenge was to develop an effective miniature fibre-optic probe that employed state-of-the-art probe technology but which was compatible for use with a medical endoscope. An endoscope is 90 cm in length and has a 2.8 mm diameter instrument channel through which a Raman probe could be delivered. This presents a considerable technical challenge; to package a probe with all its constituent parts (filters, collimating lenses, illumination and collection channels, and external casing) into a durable device with a diameter smaller than 2.8 mm!

In 1999 Shim *et al.* reported on utilising an endoscope compatible, fibre-optic Raman probe consisting of one central delivery fibre (400 µm diameter) surrounded by seven collection fibres each with a 300 µm diameter (Visionex, Inc., Sunnyvale, CA, USA, probe).[2] Filters were placed over the fibres approximately 2.5 cm from the probe tip and the collection fibres were part bevelled so that they predominantly collected signal from a zone in front of the delivery
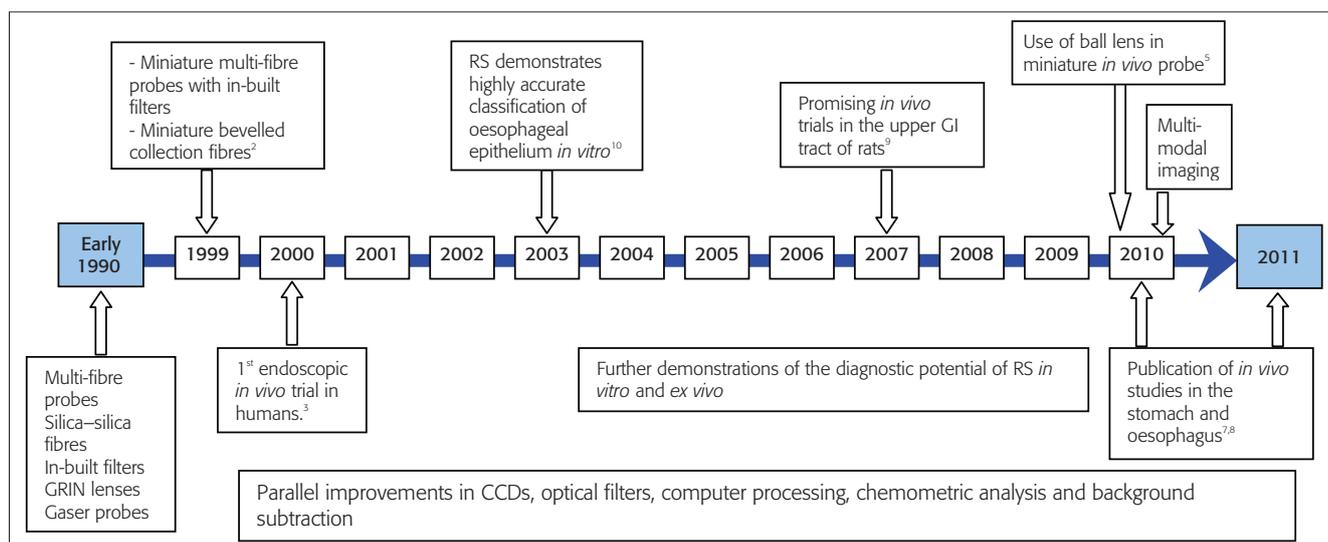

Figure 2. Progress towards *in vivo* Raman spectroscopy in the upper gastrointestinal tract (RS, Raman spectroscopy).

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fibre and at a specified collection depth, determined by the bevelled angle. This design provided a means of controlling the sampling volume whilst optimising collection efficiency.

In 2000, Shim *et al.* reported the first *in vivo* Raman measurement of gastro-intestinal tissue using the Visionex probe described above (Table 1).[3] In this feasibility study the probe was passed through an endoscope into the oesophagus, stomach and colon. Twenty patients successfully underwent the endoscopic procedure following routine sedation and no complications were reported. The probe was positioned to touch an area of mucosa for 5 s during

which time readings were taken. The probed site was then biopsied to allow comparison of Raman spectra with histopathology at that site. Subtle differences were reported in spectra from normal and diseased areas but the data acquired were not sufficient to discriminate between pathological groups. Whilst demonstrating feasibility, Shim *et al.* concluded that more work was needed to enhance the SNR, to minimise acquisition times, and to improve data interpretation before reliable results could be generated. This study illustrated that endoscopic Raman spectroscopy could be performed safely in human patients, but was not large enough to

enable comparison with results generated using laboratory based Raman spectrometers.

Early fibre-optic probes collected significant spectral contributions from tissue that is deeper than 500 μm. More recently a greater emphasis has been placed on targeted illumination of the epithelial lining of the gastrointestinal tract. Pre-cancerous dysplasia develops in epithelial cells and, by definition, has not yet invaded deeper structures. For optimum diagnostic accuracy, probes must collect signal from the epithelial layer and minimise spectral contributions from scattering occurring in deeper tissues.

Table 1. *In vivo* Raman spectroscopy in the upper GI tract (trials are in humans unless stated). All probes incorporated miniature in-built optical fibres. [+]NBI—narrow band imaging, [*]AFI—autofluorescence imaging.

Year	Ref.	Site	Number of patients/spectra	Measurement parameters	Probe design	Accuracy/conclusions
2000	3	Oesophagus, stomach (and colon)	20 patients 400 spectra	Diode laser at 785 nm Acquisition time, 5 s ~500 mW of excitation power	Visionex probe, 7 bevelled collection fibres, 1 excitation fibre Silica fibres Shutter to block white light Tissue contact Collection depth ~500 μm	Feasibility demonstrated Subtle difference in normal and diseased spectra not sufficient to discriminate between pathologies. More work to enhance SNR, minimise acquisition times, improve data analysis Background signal subtraction demonstrated
2005	4	Oesophagus	65 192 spectra	Diode laser at 785 nm Acquisition time, 5 s 70 mW power output	Single delivery fibre, 6 bevelled collection fibres Collection depth 800 μm	Sensitivity 86% and specificity 88% for dysplasia (compared to non-dysplastic oesophagus)
2007	9	Rat oesophagus and stomach		Ti:sapphire laser at 785 nm 80 mW power output	Single central delivery fibre, 8 collection fibres	Feasibility demonstrated Further improvements in SNR necessary
2010	7	Stomach	71 patients 1102 spectra	Diode laser at 785 nm Acquisition time, 0.5 s 1.5 W cm^{-2} power density at probe tip	Single central delivery fibre, 32 collection fibres (diameter 1.8 mm) System used in conjunction with NBI Collection depth 800 μm	Delineation of malignant gastric ulcers (from benign gastric tissue): Sensitivity, 82.1% Specificity, 95.3%
2011	8	Oesophagus	27 patients 75 spectra	Diode laser at 785 nm Acquisition time, 0.5 s 1.5 W cm^{-2} power density at probe tip	Probe as above. Multimodal imaging—NBI+ plus AFI* Collection depth 800 μm	Delineation of oesophageal cancer (from normal oesophageal mucosa): Sensitivity, 97.0% Specificity, 95.2%

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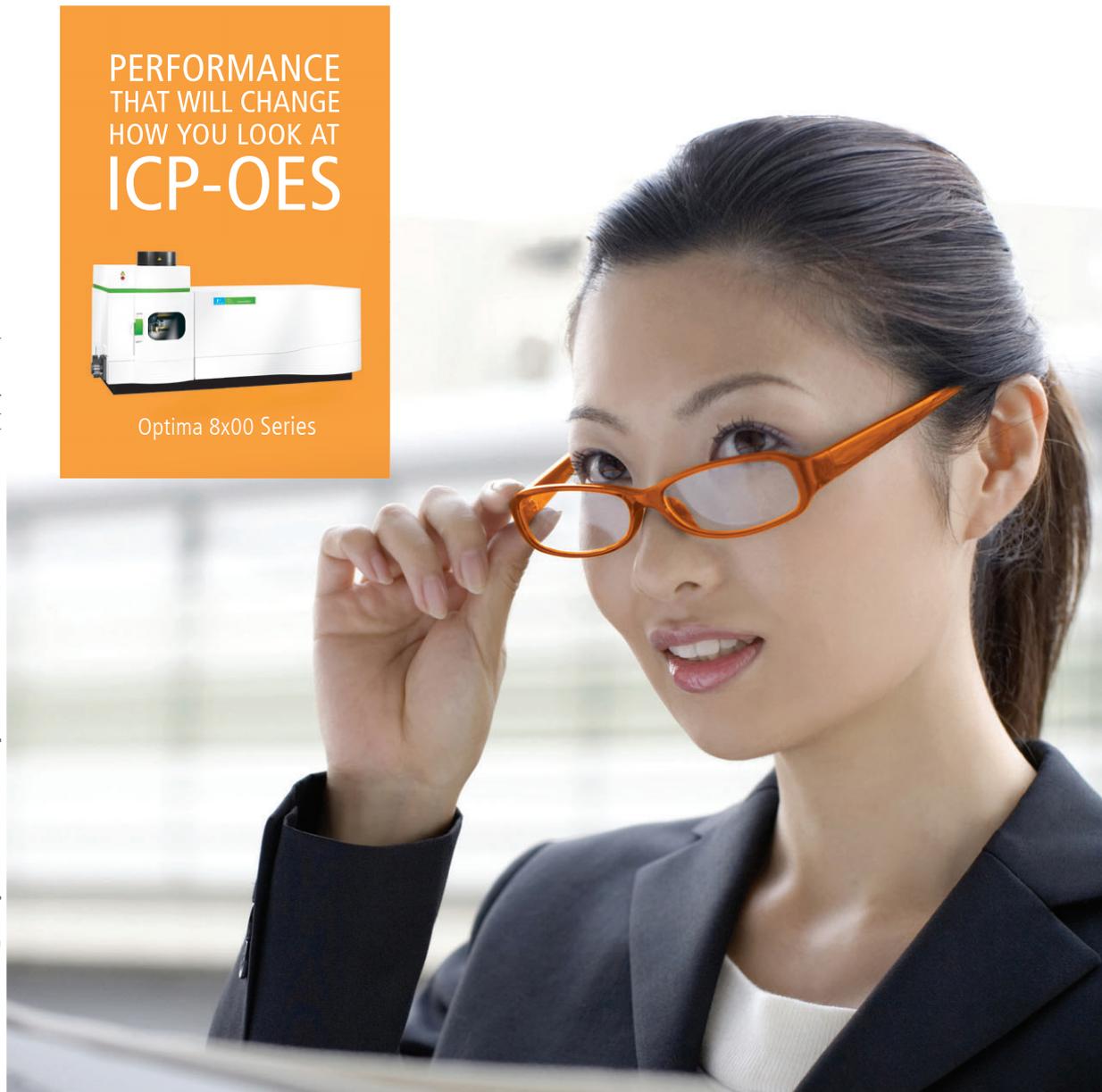
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In 2005, Wilson and co-workers published results of an *in vivo* trial in the upper gastrointestinal tract of humans. They used a probe consisting of a central delivery fibre surrounded by six 300 µm diameter bevelled collection fibres that achieved an estimated sampling depth of 500 µm.[4] In conjunction with a diode laser (emitting at 785 nm) and a modern CCD detector they reported an accuracy of 87% for delineating between normal oesophageal tissue and dysplasia.

Huang and co-workers recently described the use of a ball lens at the distal tip of a novel Raman probe to focus excitation light onto the epithelium and help collimate scattered light into the collection fibres.[5] Day *et al.* have used a graded index (GRIN) lens to the same effect in their novel probe that has been specifically designed for spectral measurement in the oesophagus.[6] This probe consists of a single illumination and a single collection fibre with collimating lenses to ensure optimal optical coupling, and in-built filters to exclude intrinsic signal generated within the optical cables. The single fibre, which has a small aperture, shows a high degree of selectivity for detecting spectral signal arising from the epithelium. This probe was used to interrogate oesophageal biopsy tissue *in vitro* and demonstrated that Barrett's oesophagus, dysplasia and cancer could be successfully separated with 71–88% sensitivity and 77–99% specificity.[6]

In 2010, following a number of preliminary *in vitro* and *in vivo* studies in rats, Huang and co-workers published a series of results from *in vivo* trials of endoscopic Raman spectroscopy for the detection of gastric neoplasia in humans. They used a 1.8 mm diameter Raman probe consisting of 32 collection fibres surrounding a single central delivery fibre. The probe had two stages of optical filters, at its proximal and distal ends, and used 785 nm excitation. Each spectrum was acquired in 0.5 s with a laser light irradiance of 1.5 W cm^{-2}. In December 2010 they reported a diagnostic sensitivity of 82.1% and a specificity of 95.3% for delineating malignant gastric ulcers from benign tissue.[7] In 2011, the same Raman probe system was used in the oesophagus demonstrating a sensitivity of 97.0% and a specificity of 95.2% for detecting oesophageal cancer.[8]

Conclusions

It is now eleven years since the first *in vivo* trial of Raman spectroscopy in the gastrointestinal tract and there has been considerable progress in probe design, NIR diode laser availability, spectrometer capability and data processing during this time. Recent studies have demonstrated the ability to collect clinically applicable results in practical timescales. However, further developments remain essential to reduce background spectral signal, optimise collection efficiency and ensure that signal is derived from the epithelial surface so that the earliest pre-cancerous changes can be detected whilst still at a potentially curable stage.

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