

L.7: Spatially-offset fluorescence spectroscopy using ring illumination and point collection for sub-surface measurements in layered tissue

There is a growing current interest in investigating source-detector separation based fluorescence spectroscopic techniques for automated non-invasive and real-time measurement of characteristic emission bands of sub-surface layers in highly turbid layered samples like tissues. Despite having the ability to probe sub-surface signals, a general limitation of all such techniques is an increasingly poorer signal-to-noise (SNR) ratio with increasing separation between the illumination and the collection points. We, at LBAS, have developed spatially-offset fluorescence spectroscopy (SOFS), a novel technique of depth-sensitive fluorescence spectroscopy for non-destructive sub-surface interrogation of layered turbid samples.

The technique is based on the configuration of point collection fixed at the centre of an illumination ring on the surface of a target sample. In the developed SOFS system, by making use of an axicon lens, the collimated fluorescence excitation beam is incident onto the sample surface in the form of concentric illumination rings of varying radii and the emitted fluorescence exiting the sample surface at the centre of the concentric rings is collected for detection. The radii of the rings onto the sample surface are varied by giving displacement to the axicon lens along the axis of the illumination beam.

The SOFS system utilizes a home-built N_2 laser ($\lambda_{ex} = 337 \text{ nm}$) as the fluorescence excitation source. The laser emits 10 ns pulses with a repetition rate of 10 Hz at pulse energy of 300 μJ . The output of the laser beam through a multi-mode fiber is collimated by an achromatic doublet lens. An axicon lens, AXL of apex angle 170° in combination with a plano-convex lens converts the collimated laser beam into a ring shaped beam. A dichroic filter kept at an angle of 45° with respect to the axis of the axicon steers the ring-shaped collimated beam on to the sample surface. The fluorescence emission is transmitted through the same dichroic filter, collimated by another achromatic doublet and then passed through a long-pass filter. The measurements of the fluorescence emission at different spatial-offsets are performed by a chip based spectrometer (USB 4000, Ocean Optics). The zero-offset refers to the position of the lens-axicon assembly which condenses the ring-shaped illumination beam into a point illumination on the sample surface and the non-zero offsets refer to the positions of the assembly which form concentric rings of illumination on the surface. The non-zero offsets can be obtained by displacing the assembly away from the dichroic filter (in the direction of arrow in Fig. L.7.1).

The ability of the system to measure fluorescence spectra of the subsurface layer was demonstrated using a non-biological phantom made of a green dye card of thickness $\sim 2.0 \text{ mm}$ with a layer of tissue paper of thickness 0.15 mm pasted on its top and a biological tissue sample consisting of a thin epithelial membrane (thickness $\sim 0.25 \text{ mm}$) on the top of a much thicker muscle tissue (thickness $\sim 10 \text{ mm}$).

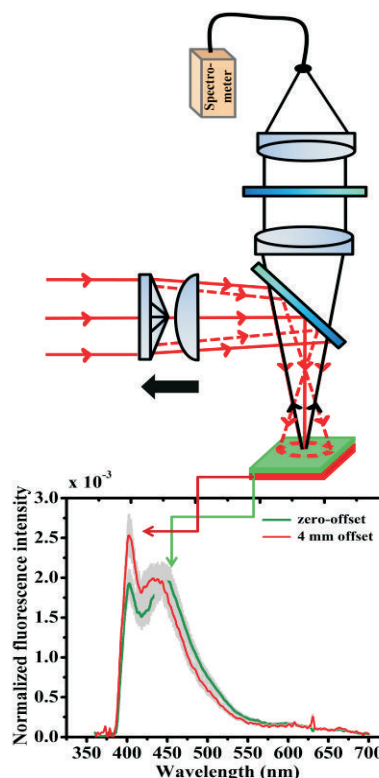


Fig. L.7.1: Experimental setup for spatially-offset fluorescence spectroscopy. Fluorescence spectra measured from a two-layered biological tissue at zero and non-zero spatial offset

The fluorescence spectra measured at zero and 4 mm spatial-offset respectively from the two-layered biological tissue are shown in Fig. L.7.1. In the spectrum corresponding to the 4 mm offset the intensity of the emission maxima at $\sim 395 \text{ nm}$, known to be characteristic of the structural protein collagen, is seen to be considerably higher than the intensity of the peak at $\sim 450 \text{ nm}$ believed to be due to the coenzyme NADH (reduced nicotinamide adenine dinucleotide). In contrast, the intensity of the maxima at $\sim 450 \text{ nm}$ is found to be substantially enhanced in the spectrum corresponding to the zero spatial offset. The detailed description of the study and its results will be found in *Biomedical Engineering Letters* 6(4) 265, 2016 (doi: 10.1007/s13534-016-0238-y).

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