DEVELOPING A TIP-ENHANCED FLUORESCENCE MICROSCOPE FOR IMAGING PROTEIN-LINE SAMPLES

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It has been known for several years that a metal tip has the ability to enhance electric fields when illuminated with light of a particular polarization\(^1\). Over the last couple of decades, this phenomenon has been exploited by many groups\(^2-4\) in order to achieve sub-diffraction optical resolution in a technique known as tip-enhanced fluorescence microscopy or ANSOM (apertureless near-field scanning optical microscopy). Using a sharp metallic or dielectric tip to probe a fluorescently-labelled sample increases the near-field of any emitting fluorophores that happen to be located within the vicinity of the tip, effectively increasing the number of photons detected in the far-field. Increasing the number of photons from the fluorophores of interest enhances the signal-to-noise ratio and provides more accurate localization of fluorophores in the sample, allowing images with higher resolution to be attained.

Whilst a resolution of sub-10nm has previously been achieved using tip-enhanced fluorescence\(^5\), the technique still remains largely unsuitable for imaging biological samples with a high density of fluorophores. This is because the laser spot which is being used to excite the fluorophores is much larger than the area over which the tip is able to enhance emissions, meaning a large number of fluorophores will be excited by the laser spot and will contribute to a large background signal. We are currently constructing a tip-enhanced fluorescence microscope using an AFM mounted on top of a series of basic optical components, with the AFM tip acting as the near-field probe for enhancement. We hope to use our microscope to eventually image a line-sample of fluorophores. Having fluorophores in a line will reduce the number of emitters within the laser spot and therefore reduce the background signal, which will hopefully show that this technique is instead suitable for imaging thin slices of biological samples.