SIMULTANEOUS AFM AND FLUORESCENCE IMAGING – A METHOD FOR ALIGNING AN AFM PROBE WITH AN EXCITATION LASER

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When a sharp metal or dielectric tip is illuminated, the electric fields within the vicinity of the tip are enhanced [1]. This phenomenon has previously been applied to achieve super-resolution images, in a technique known as tip-enhanced fluorescence microscopy or apertureless near field scanning optical microscopy [2-4].

Whilst a resolution of sub-10nm has been achieved using tip-enhanced fluorescence [5], using the technique to image biological samples is not straightforward. Samples usually have high densities of fluorophores, highlighting the need for a particularly small excitation area to keep background signal to a minimum. This in turn makes aligning the AFM probe with the excitation laser more difficult.

We report the development of a technique which aligns the AFM probe with an incident focussed excitation beam. Our set-up is based on an AFM mounted on top of an inverted microscope, with the excitation laser being delivered by a series of optical components including a 2D Galvo (motorised mirror) system. The technique takes simultaneous height and photon count measurements of a sample and calculates the offset between the two images, giving the distance between the AFM probe and the incident laser. Based on calibration data, a program has been created which then calculates the voltages required by the Galvo system to move the incident laser spot to the location of the AFM probe.

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