

Axial localisation of luminophores in a modified 4π -microscope with partial coherence interferometry

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Increasing the spatial resolution of optical microscopes is of overriding importance for many applications. Many solutions have been proposed to achieve sub-wavelength resolution. In particular, for localising luminescent species for cell biology applications, the use of high numerical aperture objectives in confocal mounts has shown giving exciting volumes elongated like rice grains whose diameter do not exceed 450 nm in the transverse plane. However, along the optical axis, their minimum length is about 1 μm . Reducing this length is a challenging question.

A solution for increasing the axial resolution is to produce interference phenomena with a 4π -microscope in order to “structure” both the illumination beam around the focal plane of the microscope objective and the fluorescent signal in the confocal volume [1-3]. In addition to an increased axial resolution, 4π -microscopes have shown to give stronger signal/noise ratio than conventional confocal microscopes. However, due to the length of the two arms of the interferometer, 4π -microscopes need accurate stabilisation and calibration procedures. In order to overcome this drawback, we propose a different configuration in which a modified 4π -microscope is coupled to a Michelson interferometer (see figure). The axial position of the fluorophore can be determined with sub-wavelength accuracy by using partial coherence interferometry [4]. The two interfering waves travel across almost the same path, thus the sensitivity of the set-up to thermal and mechanical drifts is reduced. In addition to the position of the fluorophore, its emission spectrum can be determined from the interferogram.

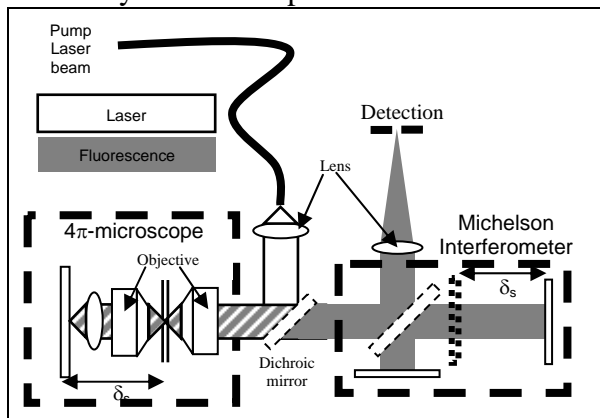


Figure 1: Modified 4π -microscope coupled to a Michelson interferometer

In this presentation, the properties of the device are investigated both numerically and experimentally. In particular, the variation of the signal due to a displacement of the object along the optical axis and in the transverse plane is studied.

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