SELF-INTERFERENCE 3D LOCALIZATION MICROSCOPY IN THE NEAR-INFRARED FOR DEEP TISSUE SINGLE-PARTICLE TRACKING

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Fluorescence microscopy has succeeded in attaining super-resolution localization of single emitters in cellular biology. However, 3D localization deep inside tissue is still challenging . A couple of years ago, our team developed SELFI: self-interference 3D super-resolution microscopy, a framework for 3D single-molecule localization within multicellular specimens and tissues [1, 2]. In this project, we extend the capability of SELFI to the near-infrared (NIR) region where carbon nanotubes (CNTs) are strong emitters. CNTs are indeed now used as fluorescent probes of biological tissue at depth, as they allow excellent tissue penetration, low light scattering, and reduced absorption by the tissue. The aim of this work is to develop NIR SELFI for single-particle tracking applications of CNTs in live brain tissues [3,4].

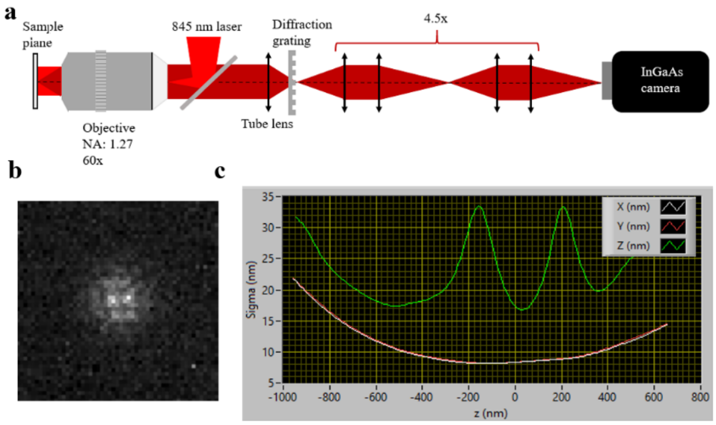

Fig. 1. (a) Optical setup for NIR SELFI. (b) Carbon nanotube imaged with NIR SELFI. (c) Cramèr–Rao lower bound localization precision.

SELFI uses a diffraction grating placed on the optical path of the sample image, generating an interference pattern within diffraction limited images of point emitters (Fig.1.b). A single image obtained with NIR SELFI contains two independent variables: the intensity distribution to extract the intensity centroid to determine the lateral localization, and the wavefront curvature (provided by the interfringes) to get the axial super-localization. SELFI was first developed to localize red emitting dyes and quantum dots [1, 2]. For adaptation with CNTs, we have redesigned the optical system (Fig.1.a) to make it suitable for emission around 1000 nm. The performance of the system is examined by means of the Cramèr–Rao lower bound. The experiments performed show that the 3D-precision achieved with NIR SELFI is below 50 nm (Fig.1.c) for emission around 1000 nm. Therefore, we can now achieve 3D localization in the NIR, permitting 3D single-particle tracking of CNTs at video rate in complex environments.

References:

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