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High Resolution Non-Linear Spectroscopic Imaging

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Traditional light microscopy suffers from the diffraction limit, which limits the spatial resolution to λ/2. The current trend in optical microscopy is the development of techniques to bypass the diffraction limit. Resolutions below 40 nm will make it possible to probe biological systems by imaging the interactions between single molecules and cell membranes. These resolutions will allow for the development of improved drug delivery mechanisms by increasing our understanding of how chemical communication within a cell occurs. The materials sciences would also benefit from these high resolutions. Nano-materials can be analyzed with Raman spectroscopy for molecular and atomic bond information, or with fluorescence response to determine bulk optical properties with tens of nanometer resolution.

Near-field optical microscopy is one of the current techniques, which allows for imaging at resolutions beyond the diffraction limit. Using a combination of a shear force microscope (SFM) and an inverted optical microscope, spectroscopic resolutions below 20 nm have been demonstrated [1]. This technique has been named tip enhanced near-field optical microscopy (TENOM) [2]. The key to this technique is the use of solid metal tips, which are illuminated in the far field by the excitation wavelength of interest. These tips are custom-designed using finite difference time domain (FDTD) modeling techniques, then fabricated with the use of a focused ion beam microscope. The measure of the quality of tip design is based directly on the field enhancement obtainable. The greater the field enhancement of the tip, the more the ratio of near-field to far-field background contribution will increase. Ultimately, the elimination of the far-field signal by a decrease of illumination (laser) power will provide the best signal-to-noise ratio in the near-field images.

We have developed a system (FIG 1) that employs two-photon non-linear excitation to allow the imaging of the fluorescence from almost any visible fluorophore at resolutions below 20 nm without changing filters or excitation wavelength [3]. The ability of the microscope to image samples at atmospheric pressure, room temperature, and in solution makes it a very promising tool for the biological and materials science communities. The microscope demonstrates the ability to image topographical, optical, and electronic state information for single-molecule identification. A single computer, simple control circuits, FPGA data acquisition, and a simplified optical system control the microscope. This versatility enables the end user to custom-design experiments from confocal far-field single molecule imaging to high resolution scanning probe microscopy imaging.

Presented will be the current capabilities of the microscope, including high-resolution near-field images of J-aggregates with PICI dye (FIG 2). Single molecules of Rhodamine 6G imaged in the far-field at room temperature and atmospheric pressure will be presented to demonstrate the sensitivity of the microscope. A comparison is made with the use of mode-locked 50 fs pulsed laser systems vs. continuous wave systems on single molecules and biological systems. Raman spectroscopy capabilities that are currently under development will also be discussed. Integration of an intensified CCD camera with a high-resolution monochromator will allow for spectral imaging of multiple Raman lines simultaneously. This will eliminate the need for multiple Raman notch filters, making the system more dynamic and universal. We are currently working on the dissemination of the project as an open system design [4].

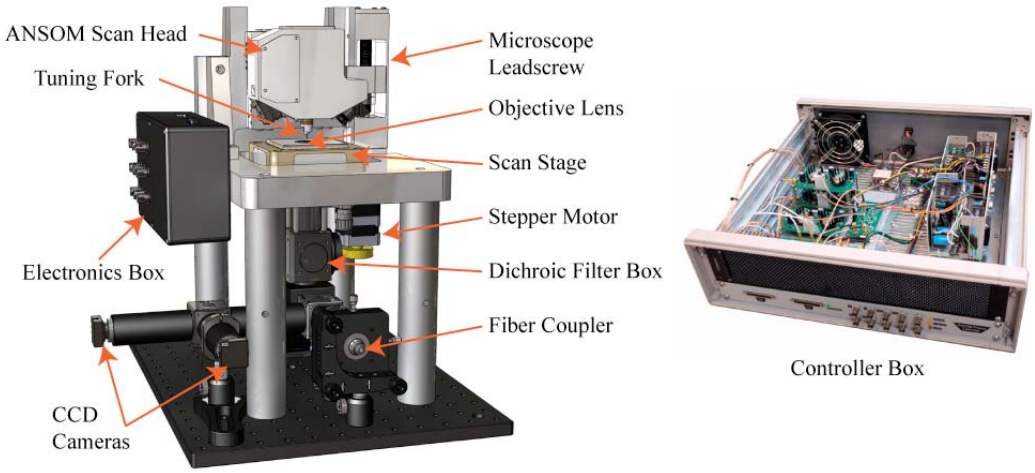

FIG 1: A 3D CAD representation of the TENOM system. The microscope interfaces to a controller box (lid removed to show circuitry), which conditions the analog signals from the microscope to the FPGA data acquisition card. All controls and image data are viewed in real time using Windows™ and programmed in LABView™.

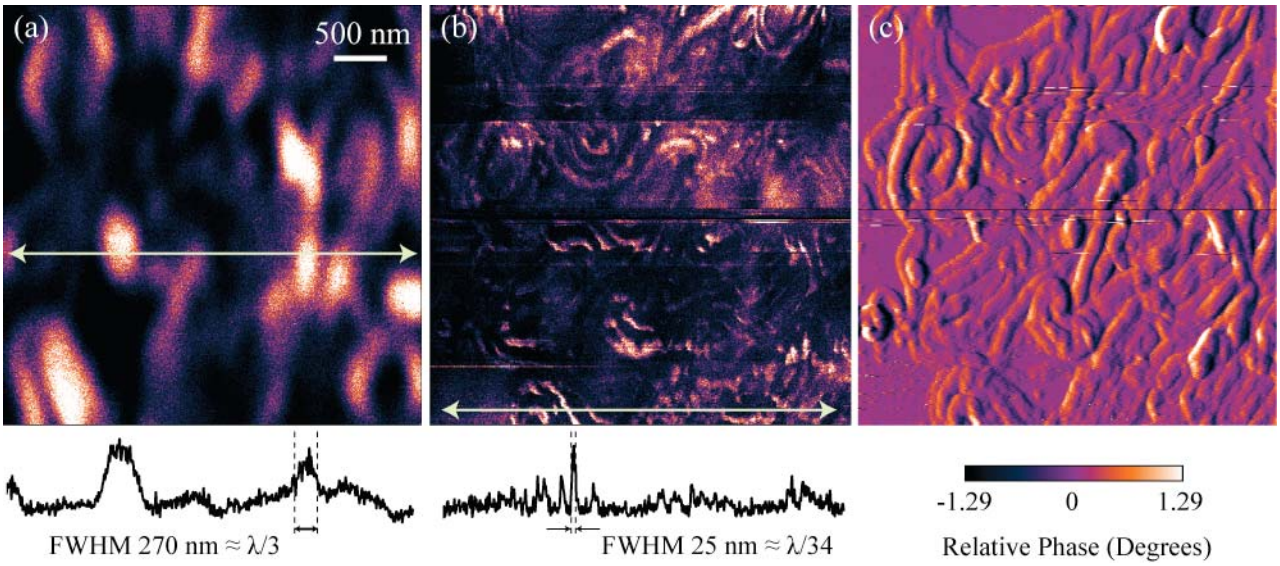

FIG 2: Two-photon imaging of J-aggregates with PICI dye excited at 833 nm with a mode-locked laser operating at 50 fs pulses. (a) Far-field diffraction limited, (b) near-field with average excitation power of 9.8 μW, same ROI as (a), (c) phase feedback image generated by the error signal for the topography scan, simultaneously acquired with (b).

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[2] E. J. Sánchez, L. Novotny, X.S. Xie, *Phys. Rev. Lett.* 82, 20 1999, 4014.
[3] NSF awards DBI-0500812, ECCS-NSF-0520891, NSF-DMR-REU-069280, NSF-DMR-REU-1004737, and NSF-0722660, ONAMI, and ONR awards (#N00014-07-1-0457) (#N00014-08-1-1237) (#N00014-10-1-0082)
[4] www.ansom.physics.pdx.edu for project information.

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