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Multicolor Nanoscopy using a New Class of Low-Power Probes

Tilman Rosales[1], Daniel Sackett[2], Jianhua Xu[1], Zhen-Dan Shi[3], Biying Xu[3], Haitao Li[3], Gurpreet Kaur[3], Nalini Shenoy[3], Sarah Cheal[3], Haitao Wu[3], Andres Dulcey[3], Changhui Li[3], Kelly Lane[3], Gary L. Griffiths[3], Jay R. Knutson[1].
[1]NIH/NHLBI, Bethesda, MD, USA, [2]NIH/NICHD, Bethesda, MD, USA, [3]NIH/IPDC, Bethesda, MD, USA.

STED/RESOLFT approaches provide the most promising technology for real-time video nanoscopy. Unfortunately, STED has sometimes been hobbled by two practical problems: large laser powers that (especially in the visible) damage cells and Stokes' shift similarities among dyes that inhibit multicolor imaging. The former occurs because emission must be separated from the powerful STED beam, pushing deactivation into a redder portion of the emission where the cross section is reduced. Our new probes, in contrast, all have very strong cross sections, and they all deactivate at the same NIR wavelength. Hence, we are able to obtain simultaneous superresolved multicolor imaging at the convenient (and less damaging) wavelength of 780nm.

We constructed a home-built microscope to test our probes .Our system generates a green excitation pulse with Gaussian profile in space of just 4-8μW and a more powerful "donut" beam at ~780nm, using several picosecond wide pulses at 80MHz repetition.

We achieve resolutions of ~50nm when coating 20nm aliphatic-amine beads with NHS-ester versions of our dyes. In fixed cells, microtubules coated with a secondary dye-anti mouse antibody bound to a primary mouse antibody specific to α-tubulin demonstrated ~90nm resolution with only a few mW applied to the donut beam. We also directly labeled microtubules with orange and red versions of our dye; in this case, we achieve ~100nm resolution with a single depleting beam.

These dyes are essentially normal fluorophores modified to contain "quenching antennae" at 780nm. Unlike STED, these antennae are best with many ps to hundreds of ps wide laser pulses; thus, inexpensive ns-pulsed 780nm diodes may be helpful in disseminating the technology. We are currently pursuing alternative antennae and additional dye colors appropriate to in vivo nanoscopy.

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Visualizing Tctn2 in the Transition Zone of Primary Cilia using STED Nanoscopy

Bhavik Nathwani[1], Jamie Solimano[2], Tony Yang[1], Nupur Sutaria[1], Jung-Chi Liao[1].
[1]Columbia University, New York, NY, USA, [2]Stuyvesant High School, New York, NY, USA.

Primary cilia are hair-like solitary projections found across most mammalian cell types. They perform essential mechanical and chemical signaling roles, often times through tissue-specific pathways. An absence of translation machinery within the cilium underscores the need for cargo transport in and out of the cilium to maintain the ciliary architecture. Separation of the internal architecture of the cilium from the rest of the cytosolic cell materials is achieved by a "gate structure" at the base of the cilium called the "transition zone". Various molecular complexes within the transition zone have been shown to play significant role in differentiating between cargo that can and cannot enter the ciliary compartment. Despite rapid progress deciphering the biochemistry of these molecular complexes, visualization of transition zone architecture in-situ has been challenging. Utilizing a custom-built STED nanoscope, affording a resolution of ~50 nm at the sample plane, we studied the spatial distribution of molecular complexes within the transition zone. Specifically, we imaged a transmembrane protein tectonic-2 (Tctn-2), important in disorders associated with structural integrity of primary cilia such as Meckel Gerber Syndrome (MKS). A super-resolved view of Tctn-2 distribution indicates a "ring-like" distribution of Tctn-2 within the transition zone. Comparative analysis of images collected using STED and conventional confocal microscopy underscores the advantages of STED, as the structural details visualized by STED is not afforded by confocal. Taken together, our study identifies STED as a potential tool to generate a spatial map of transition zone architecture.

Acknowledgement
This work is supported by NSF grant CMMI-1125760

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Potential Nanotoxicity of Pegylated Gold Nanorods having Different Sizes and Shapes as a Biocompatible Contrast Agent for 2PE Imaging

Gaser N. Abdel Rasoul[1,2], Raffaella Magrassi[3], Marco Scotto D' Abbusco[4], Alberto Diaspro[1,5].
[1]Italian Institute of Technology, Department of Nanophysics - Nanobiotechnology Facilities, Genova, Italy, [2]Department of Physics University of Genova, Via Balbi 5, 16126 Genova, Italy, [3]National Research Council (CNR) - Institute of Marine Sciencces, Genova, Italy, [4]Italian Institute of Technology, Department of Nanophysics, Genova, Italy, [5]Department of Physics, University of Genova, Via Balbi 5, 16126 Genova, Italy.

Gold nanoparticles (AuNPs) entices the attention last years in nanobiotechnology. Different methodologies were designed to produce AuNPs with tunable sizes and shapes. Gold nanorods (AuNRs) are candidates for different applications including cell imaging, smart drug delivery and photothermal therapy. AuNRs dispersion is characterized by two surface plasmonic resonances referring to the interaction of incident light with the free electrons of the transversal and longitudinal axes atoms. The absorption wavelength of the longitudinal surface plasmonic resonance is highly sensitive to the aspect ratio of the NRs and the medium dielectric resonance. Regarding to these spectacular properties and by tuning the aspect ratio of the NRs, AuNRs can be useful as contrast agent for cell imaging through controlling the wavelength of the excitation light. Here we report about AuNRs with two different aspect ratios, photochemically synthesized using CTAB as cationic surfactant. Functionalization was performed on the NRs surface to exchange cytotoxic CTAB molecules with thio methoxypolyethylene glycol (mPEG-SH). In order to evaluate the cellular uptake of the AuNRs and the potential nanotoxicity we used cell line, namely: HeLa. The viability of the cells after nanorods uptake was evaluated by colorimetric assay to reveal the percentage of dead and apoptotic cells after exposure. Furthermore, due to the fact that oxidative stress is one of the mechanisms responsible of toxicity of nanoparticles, we investigated the levels of reduced gluthation (GSH) of treated versus control cells by using a second colorimetric assay. 2PE microscopy at different laser wavelengths has been used to estimate the effectiveness of AuNRs as contrasting agent for cell imaging. Occasionally, TEM images were acquired for cells after exposure to NRs to quantify NRs inside the cells and in which specific regions.

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Near-Interface Brownian Motion of Anisotropic Particles

Sadao Ota, Tongcang Li, Yimin Li, Ziliang Ye, Mohammad-Reza Alam, Xiaobo Yin, Xiang Zhang.
UC Berkeley, Berkeley, CA, USA.

Anisotropic microscopic objects are ubiquitous such as polymers, biological cells and filamentous macromolecules. Near interfaces, the thermal motion of these objects is strongly constrained due to the hydrodynamic interactions, often impacting the overall behavior of the biophysical systems. Thus, understanding this wall-effect is a key to describe many surface-related problems. Unlike the well-studied case of spheres, however, both the experimental and theoretical studies of the near-wall Brownian motion of anisotropic bodies have been elusive due to the lack of ideal imaging techniques and its intrinsically complex system. Here we present the experimental and computational study of the rotational Brownian motion of silicon nanowires tethered on a substrate. A uniquely developed interference method enables the direct visualization of the microscopic rotations of nanowires in three dimensions with high angular (< 0.000005 rad) and temporal resolutions (> 200Hz) with normal CCD camera. The quantitative measurement at short time scales revealed the anisotropic reduction in their rotational diffusivities as a function of the inclined angles. At long time scales, the rotational diffusivity of their free ends into the inclined direction decreased more than 40-80 % in total. We then developed an implicit hydrodynamic model from a string-of-beads idealization, which is equivalent to the infinite-order Stokes superposition of solvent flow around the beads and its implicit solution for their hydrodynamic velocities. The calculation showed excellent agreement with the experimental observations. The demonstrated interferometric method, together with the versatile numerical simulation, provides a systematic approach for studying a variety of colloidal rheology as model biophysical systems. Our observation provides insights into the fundamental diffusive processes, useful for understanding the near-interface behavior of anisotropic microorganisms and macromolecules as well as property of the surfaces themselves.

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A Photochromic Bacterial Photoreceptor with Potential for Super-Resolution Microscopy

Aba Losi[1], Wolfgang Gärtner[2], Sarah Raffelberg[2], Francesca Cella Zanacchi[3], Paolo Bianchini[3], Alberto Diaspro[3], Carmen Mandalari[1], Stefania Abbruzzetti[1], **Cristiano Viappiani**[1].
[1]Università di Parma, Parma, Italy, [2]Max-Planck-Institute for Chemical Energy Conversion (CEC), Mülheim a.d. Ruhr, Germany, [3]Fondazione Istituto Italiano di Tecnologia, Genova, Italy.

We introduce a novel fluorescent reporter with potential for super-resolution microscopy, based on the bacterial photoreceptor YtvA. YtvA (from Bacillus subtilis) comprises a photosensitive flavin based LOV domain, efficiently photo switchable between fluorescent and non fluorescent states using blue and violet light. We have exploited this property to perform sub-diffraction localization of individual YtvA molecules deposited on a coverslip. We also demonstrate Fluorescence PhotoActivation Localization Microscopy (FPALM) studies of live Escherichia coli cells, expressing YtvA molecules.