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Widely Accessible Method for Superresolution Fluorescence Imaging of Living Systems

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Superresolution fluorescence microscopy overcomes the diffraction resolution barrier and allows the molecular intricacies of life to be revealed with greatly enhanced detail. However, many current superresolution techniques still face limitations and their implementation is typically associated with a steep learning curve. Patterned illumination-based superresolution techniques [e.g., stimulated emission depletion (STED), reversible optically-linear fluorescence transitions (RESOLFT), and saturated structured illumination microscopy (SSIM)] require specialized equipment, whereas single-molecule-based approaches [e.g., stochastic optical reconstruction microscopy (STORM), photo-activation localization microscopy (PALM), and fluorescence-PALM (F-PALM)] involve repetitive single-molecule localization, which requires its own set of expertise and is also temporally demanding. Here we present a superresolution fluorescence imaging method, photochromic stochastic optical fluctuation imaging (pcSOFI). In this method, irradiating a reversibly photoswitching fluorescent protein at an appropriate wavelength produces robust single-molecule intensity fluctuations, from which a superresolution picture can be extracted by a statistical analysis of the fluctuations in each pixel as a function of time, as previously demonstrated in SOFI. This method, which uses off-the-shelf equipment, genetically encodable labels, and simple and rapid data acquisition, is capable of providing two- to threefold-enhanced spatial resolution, significant background rejection, markedly improved contrast, and favorable temporal resolution in living cells. Furthermore, both 3D and multicolor imaging are readily achievable. Because of its ease of use and high performance, we anticipate that pcSOFI will prove an attractive approach for superresolution imaging.
P. Dedecker et al, PNAS 109:10909 2012.

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Axial Super Resolution Topography of Focal Adhesion by Confocal Microscopy

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Focal adhesions mediate cell-ECM interaction through a complex protein network and have been well studied in terms of protein functions and interactions. The protein organization within focal adhesions was a subject of state of the art super resolution methods due to its thin structure well below diffraction limit. However, most of the current super resolution approaches rely on either sophisticated optics or photoactivable compounds, limiting their application.
In this work we present a phasor-based method to determine the precise axial position of focal adhesion proteins using conventional confocal microscope. 3D image stacks with small axial step size (50 nm or lower) were acquired. For each pixel, the intensity along the axial axis was Fourier transformed. The first harmonic of the transform was represented as phasor, and the angle difference was used for calculating the relative axial center-of-mass position of the fluorescence compound. The resolution of the axial position depends on the photons collected and the pixel size along the axial axis. For a flat fluorescence surface, the pixel standard deviation of axial position is between 60 to 100 nm, depending on the signal level. After averaging and filtering, the standard deviation of the mean position can be in the 10 nm range.
The phasor method was applied to paxillin-GFP expression and F-actin at focal adhesions. The standard deviation of paxillin axial location is similar to the flat fluorescence surface tested, while F-actin shows a sharp increase in height towards cell center. With proper reference, accurate fluorescent protein axial position comparison can be made across samples. This method also provides an alternative way to present 3D image based on center-of-mass topography rather than intensity.
With the advantage of simple data acquisition, this phasor method could have wide dissemination and application potentials.

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Fast 3D Single Molecule Tracking with Multifocal Plane Microscopy in Polarized Epithelia Reveals a Novel Cellular Process of Intercellular Transfer

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The study of intracellular trafficking processes represents a fundamental problem in many areas of biomedical research. Single molecule imaging approaches are well suited to study heterogeneous processes in live cells. However, 3D single molecule imaging of intracellular trafficking events in a thick sample such as an epithelial-cell monolayer poses several technical challenges. Specifically, we require a methodology that not only enables fast 3D tracking of single molecules across a cell monolayer, but also enables the imaging of the cellular environment with which the single molecule interacts. Current approaches that are widely used for 3D single molecule imaging/tracking are not well suited for studying the intracellular trafficking pathways due to restricted imaging depth, poor temporal resolution, and the ability to track only a few molecules at one time. Here we show that multifocal plane microscopy (MUM), a 3D imaging modality developed by our group, provides the much needed solution to this longstanding problem by demonstrating fast 3D tracking of quantum dot labeled transferrin molecules in a ~10 micron thick epithelial cell monolayer. The use of MUM led to the unexpected discovery of a novel cellular process, intercellular transfer, that involves the rapid exchange of Tf molecules between two adjacent cells in the monolayer. We also report 3D single molecule tracking of endocytosis and exocytosis at the lateral plasma membrane of cells in the monolayer. This lateral membrane has been notoriously difficult to image with other cellular imaging modalities. A detailed characterization of these events based on the temporal and 3D intracellular spatial behavior of Tf molecules has been made. The methods and approaches used in this study have broad applicability to investigate 3D trafficking pathways in other cell systems and models.

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Understanding Switching Variability in dSTORM Type Microscopy

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Single molecule localization based on switching of conventional dyes (dSTORM/RPM/GSDIM) is one of the simplest super-resolution methods. dSTORM relies on a buffer containing thiol and an oxygen scavenging system to facilitate switching with one limitation of the technique being variability in switching behavior. Careful preparation helps, but mountant failures occur sufficiently often that differentiating between poor labeling and non-functioning mountant can be hard. Combining computational modeling with simple assays of both [thiol] and [O_2] has yielded insight into the causes of this variability.
Our numerical model predicts that switching will not occur at nominal [thiol] and [O_2] because both bright and dark states are too short lived. It also predicts dye-mediated consumption of oxygen and thiol, allowing switching after an initial depletion period. In experimental measurements, [thiol] decays rapidly with a concomitant reduction in dissolved oxygen. In the absence of either dye or enzymatic scavengers, this decay has a half life ranging from ~20 mins to 2 hrs. The reaction is significantly accelerated by trace amounts of redox-active dye. We find these thiol-dye-oxygen reactions considerably more effective at scavenging oxygen than enzymatic scavangers, with the thiol-dye-oxygen system exhibiting both a faster rate and a much lower final [O_2]. Literature on thiol use in radiation therapy suggests that thiol decay results from radical induced chain reactions with dissolved oxygen. Radical induction and chain propagation offer a plausible explanation for the high variability in half life and the acceleration conferred by trace amounts of dye. The ability of thiol based scavenging to achieve a lower [O_2] than enzymatic scavangers, combined with the observation that many dyes blink in the absence of enzymes, raises the interesting possibility that thiol might set end [O_2] with enzymes acting to preserve [thiol] during initial stages of depletion.

Platform: Protein-Lipid Interactions II

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Membrane Physical Properties Influence Transmembrane Helix Formation

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The pHLIP peptide has three states: (I) soluble in aqueous buffer, (II) bound to the bilayer surface at neutral pH, and (III) inserted as a transmembrane (TM) helix at acidic pH. The membrane insertion of pHLIP at low pH can be used to target the acidic tissues characteristic of different diseases, such as cancer. We find that the α-helix content of state II depends on lipid acyl chain length but not cholesterol, suggesting the helicity of the bound state may be controlled by the bilayer elastic bending modulus. Experiments with the P20G variant show the proline residue in pHLIP reduces the α-helix content of both states II and III. We also observe that the membrane insertion pKa is influenced by membrane physical properties, following a biphasic pattern similar to the membrane thickness optima observed for the function of eukaryotic membrane