Super Resolution Fluorescence Microscopy Department Of

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Xiaowei Zhuang (Harvard/HHMI) Part 1: Super-Resolution Fluorescence Microscopy Microscopy: Super-Resolution Microscopy (Xiaowei Zhuang) **An introduction to super-resolution microscopy of living cells Super resolution microscopy | Stimulated emission depletion (STED) microscopy** Introduction to Super-Resolution Localization Microscopy ~~Microscopy: Super-Resolution: Structured Illumination Microscopy (SIM) (David Agard)~~ *Microscopy: Super-Resolution: Overview and Stimulated Emission Depletion (STED) (Stefan Hell) DeltaVision OMX Flex super-resolution microscope: Product overview*

A. Diaspro - Cutting-edge technology: super-resolution fluorescence microscopy**Microscopy: Super-Resolution: Localization Microscopy (Bo Huang)** ~~Microscopy: Designing a Fluorescence Microscopy Experiment (Kurt Thorn)~~ BioTechniques - Super Resolution Microscopy *3D Microscopes: To boldly go...* Class I Speaker - Xiaowei Zhuang *The benefits of magnification beyond resolution | Amateur Microscopy Inside The World's Most Powerful New Microscopes 20_STORM Microscopy_Paulsen* ~~Microscopy: Phase, Polarization, and DIC (Stephen Ross)~~ *Lattice light-sheet microscopy Microscopy: Resolution (Jeff Lichtman)* Microscopy: Diffraction (Jeff Lichtman) Principles of STED microscopy Viewing super-resolution cells in real time Super resolution fluorescence microscopy's nanometer resolution gain insights into bacterial cells Super-resolution microscopy *Seminar: Localization-Based Super-Resolution Fluorescence Imaging Jennifer Lippincott-Schwartz (NIH) Part 3: Super Resolution Imaging* ~~Single-molecule fluorescence microscopy enables super-resolution imaging of DNA replication and...~~ *Eric Betzig: development of super-resolved fluorescence microscopy* ~~Ghost Imaging Speeds Up Super-Resolution Microscopy~~ *Super Resolution Fluorescence Microscopy Department*

Recently, several new technologies, collectively termed super-resolution microscopy or nanoscopy, have been developed that break or bypass the classical diffraction limit and shift the optical resolution down to macromolecular or even molecular levels . Some of these technologies have now matured from the breadboard stage to commercially available imaging systems, making them increasingly attractive for broad applications and defining a new state of the art.

A guide to super-resolution fluorescence microscopy ...

Achieving a spatial resolution that is not limited by the diffraction of light, recent developments of super-resolution fluorescence microscopy techniques allow the observation of many biological structures not resolvable in conventional fluorescence microscopy. New advances in these techniques now give them the ability to image three-dimensional (3D) structures, measure interactions by ...

Super-Resolution Fluorescence Microscopy | Annual Review ...

Pushing the Limits of Fluorescence Microscopy: Fluorescent Probes for Super Resolution Imaging Technologies (BioProbes ® 64) Beyond light's limits: Fluorescence imaging at the nanoscale—Fluorescent probes for three super-resolution modalities—STORM, SIM, and STED microscopy (BioProbes ® 70) Fluorescence SpectraViewer.

Super-Resolution Microscopy | Thermo Fisher Scientific - UK

This is where fluorescence microscopy steps in, hence the rapid development of super-resolution fluorescence microscopy as a field of physical sciences and the two Nobel Prizes already awarded for...

Microscopy beyond the resolution limit | EurekAlert ...

Super-resolution fluorescence microscopy is an important tool in biomedical research for its ability to discern features smaller than the diffraction limit. However, due to its difficult implementation and high cost, the super-resolution microscopy is not feasible in many applications.

Super-resolution fluorescence microscopy by stepwise ...

Super Resolution Fluorescence Microscopy Department Achieving a spatial resolution that is not limited by the diffraction of light, recent developments of super-resolution fluorescence microscopy techniques allow the observation of many biological structures not resolvable in conventional fluorescence microscopy.

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Huang B, Bates M, Zhuang X. Super-resolution fluorescence microscopy. Annu Rev Biochem. 2009;78:993-1016. Bates M, Huang B, Zhuang X. Super-resolution microscopy by nanoscale localization of photo-switchable fluorescent probes. Curr Opin Chem Biol. 2008 Oct;12(5):505- 14.

Super-Resolution Fluorescence Microscopy

Spectral precision distance microscopy (SPDM) is a family of localizing techniques in fluorescence microscopy which gets around the problem of there being many sources by measuring just a few sources at a time, so that each source is "optically isolated" from the others (i.e., separated by more than the microscope's resolution, typically ~200-250 nm), if the particles under examination have different spectral signatures, so that it is possible to look at light from just a few molecules at a ...

Super-resolution microscopy - Wikipedia

Super-resolution microscopy, whilst it can allow the observer to peer more deeply into nature, cannot be used at the molecular level. For this type of research, there is an even more powerful tool...

Super-Resolution Microscopy vs. Electron Microscopy

Super-resolution imaging is now achievable on a conventional epi-fluorescence microscope with little to no modification to the optical design. The fine structural information embedded in a sequence of fluorescence images can be restored using single particle localization as implemented in STORM and PALM, or intensity fluctuation statistics as in SOFI, ICA and SCORE.

Spatial Covariance Reconstructive (SCORE) Super-Resolution ...

Next, we incorporate optimization into the study of a super-resolution fluorescence microscopy technique, structured illumination microscopy. Super-resolution reconstruction is achieved even with a series of random unknown illumination patterns, which is not possible without proper optimization formulation.

Computational fluorescence and phase super-resolution ...

Imagingmethodsforcellsandtissueshaveprogressedrapidlyinthepastdecade,providing unrivalledopportunitiesfornewinsightsintobiologicalmolecularmechanisms[1–3].While many of the highest proﬁle developments have been in super-resolution ﬂuorescence and singlemoleculemethods,otherinnovationsinelectronorX-raymicroscopyhavefacilitated visualisationofultrastructuralmorphology[4,5].

CryoSIM: super resolution 3D structured illumination ...

The team experimented with the concept of digital holography for fast fluorescence detection by tracking the three-dimensional (3-D) trajectory of individual nanoparticles using an in-plane ...

Holographic fluorescence imaging to 3-D track ...

Here we describe a new method, named LS-SOFI, that combines light-sheet fluorescence microscopy and super-resolution optical fluctuation imaging to achieve fast nanoscale-resolution imaging over large fields of view in native 3D tissues. We demonstrate the use of LS-SOFI in super-resolution analysis of neuronal structures and synaptic proteins,

Super-resolution light-sheet fluorescence microscopy by SOFI

This is where fluorescence microscopy steps in, hence the rapid development of super-resolution fluorescence microscopy as a field of physical sciences and the two Nobel Prizes already awarded for...

Moving microscopy beyond the resolution limit

Super-resolution fluorescence microscopy (nanoscopy) enables imaging with a spatial resolution much higher than the diffraction limit of optical microscopy. However, the methods of fluorescence nanoscopy are still poorly suitable for studying living cells. In this review, we describe some examples of live nanoscopy-based discoveries and focus on the development of methods for nanoscopy and specific fluorescent labeling aimed to decrease the damaging effects of light illumination on live samples.

Live-Cell Super-resolution Fluorescence Microscopy ...

Several methodologies have been developed over the past several years for super-resolution fluorescence microscopy including saturated structured-illumination microscopy (SSIM), stimulated emission depletion microscopy (STED), photoactivated localization microscopy (PALM), fluorescence photoactivation localization microscopy (FPALM), and stochastic optical reconstruction microscopy (STORM).

Review of super-resolution fluorescence microscopy for biology

We improve multiphoton structured illumination microscopy using a nonlinear guide star to determine optical aberrations and a deformable mirror to correct them. We demonstrate our method on bead phantoms, cells in collagen gels, nematode larvae and embryos, Drosophila brain, and zebrafish embryos. Peak intensity is increased (up to 40-fold) and resolution recovered (up to 176 ± 10 nm laterally, 729 ± 39 nm axially) at depths 250 μm from the coverslip surface.

Adaptive optics improves multiphoton super-resolution imaging

Multicolor super‐resolution fluorescence microscopy has been demonstrated by several means, such as employing fluorophores with different fluorescence activation wavelengths, 12 – 15 fluorophores with well‐separated emission spectra, 9, 16 – 19 by ratio‐metric imaging of fluorophores with overlapping emission spectra, 9, 20, 21 or by taking advantage of other spectral properties, such as fluorescence lifetime. 22

Fluorescence Microscopy: Super-Resolution and other Novel Techniques delivers a comprehensive review of current advances in fluorescence microscopy methods as applied to biological and biomedical science. With contributions selected for clarity, utility, and reproducibility, the work provides practical tools for investigating these ground-breaking developments. Emphasizing super-resolution techniques, light sheet microscopy, sample preparation, new labels, and analysis techniques, this work keeps pace with the innovative technical advances that are increasingly vital to biological and biomedical researchers. With its extensive graphics, inter-method comparisons, and tricks and approaches not revealed in primary publications, Fluorescence Microscopy encourages readers to both understand these methods, and to adapt them to other systems. It also offers instruction on the best visualization to derive quantitative information about cell biological structure and function, delivering crucial guidance on best practices in related laboratory research. Presents a timely and comprehensive review of novel techniques in fluorescence imaging as applied to biological and biomedical research Offers insight into common challenges in implementing techniques, as well as effective solutions

Single molecules were first detected at low temperatures twenty-six years ago in the laboratory of W.E. Moerner. Subsequent technological advances have allowed researchers to study single molecules at room temperatures and within living cells, providing novel biological insight about underlying spatial and dynamical heterogeneity. By combining single molecule detection with the ability to control the emissive state of the fluorescent label (also called "active control"), a suite of super-resolution imaging techniques has been developed. These single-molecule-based super-resolution imaging strategies leverage the fluorescence microscope's ability to non-invasively study multiple targets within living cells, while bridging the resolution gap between optical and electron microscopies. In large part, future advances to improve single molecule and super-resolution imaging require better fluorophore and labeling technologies. Utilizing fluorophore with higher photon yields will increase the resolution of super-resolution images and the data acquisition speed. Additionally, a greater library fluorophores with different of colors and sensing capabilities will enable application to more imaging targets. Currently, many single molecule and super-resolution experiments within living systems use fluorescent proteins because the labeling of target proteins is more straightforward. However, the limited photon yield of fluorescent proteins often results in tantalizingly fuzzy super-resolution images. Imaging the same targets, labeled instead with brighter organic emitters, could provide more image detail, but better fluorogenic and genetically encoded labeling schemes must be developed and discovered. The first chapter of this dissertation will introduce and discuss the historical context and basic principles of single molecule and super-resolution imaging. Chapter 2 will then describe the general experimental procedures necessary for quantitative single molecule and super-resolution imaging, including quantifying the number of photons detected (and emitted) from a single molecule, as well as the preparation of bacterial samples for fluorescence microscopy. Later chapters apply these fundamental experimental measurements to study bacterial biology and fluorophore photophysics. Chapters 3 and 4 concern the development and characterization of organic emitters suitable for single molecule or super-resolution imaging, work achieved with the synthetic collaboration of organic chemists in the laboratory of Professor Robert J. Twieg at Kent State University. Chapter 3 discusses the optimization of rhodamine spirolactam photoswitching such that activation could occur at visible wavelengths. The optimized rhodamine spirolactams were then covalently attached to the surface of bacterial cells and imaged with three-dimensional super-resolution. Images of the bacterial cell surface demonstrates a marked improvement in labeling uniformity, specificity, and density compared to previous methods which labeled the surface with the transient binding of a membrane sensitive dye. Chapter 4 introduces a novel enzyme-based strategy to control the fluorescence from nitro-aryl fluorogens. A proof-of-principle experiment demonstrated that endogenous nitroreductase enzymes within bacterial cells could catalyze the fluorescence-activating reaction, thus generating free fluorophores, which were detectable on the single-molecule-level within the cell. Lastly, chapter 5 summarizes three-dimensional imaging experiments (performed in collaboration with the laboratory of Professor Lucy Shapiro in the Department of Developmental Biology at Stanford University) of components of the bacterial gene expression machinery labeled with fluorescent proteins. Super-resolution imaging is ideally suited to the small size scale of bacterial cells, and a wealth of biological insights remains to be discovered. Simultaneously improving fluorophore photon yield, specificity, and active control strategies will have a profound impact on super-resolution precision and speed.

High resolution imaging in three dimension is important for biological research. The RESOLFT (Reversible Saturable Optical Fluorescence Transitions) fluorescent microscopy is one technique which can achieve lateral super-resolution imaging. Two-photon microscopy naturally generate high resolution in the longitudinal direction with less background compared to single photon excitation. We combine these two methods to realize three-dimensional high-resolution imaging. This super-resolution method also is limited in imaging speed. We use a spatial light modulator (SLM) as a flexible phase mask of the microscopy. It is used to compensate the system aberration, as well as increasing the imaging speed. The parallel scanning generates multiple super-resolution focuses as an array or in arbitrary positions by phase retrieval calculation. This microscopy combined with SLM control could applied to high throughput 3D imaging or multiple spots tracking in high-resolution.

While there are many publications on the topic written by experts for experts, this text is specifically designed to allow advanced students and researchers with no background in physics to comprehend novel fluorescence microscopy techniques. This second edition features new chapters and a subsequent focus on super-resolution and single-molecule microscopy as well as an expanded introduction. Each chapter is written by a renowned expert in the field, and has been thoroughly revised to reflect the developments in recent years.

This book encompasses the full breadth of the super-resolution imaging field, representing modern techniques that exceed the traditional diffraction limit, thereby opening up new applications in biomedicine. It shows readers how to use the new tools to increase resolution in sub-nanometer-scale images of living cells and tissue, which leads to new information about molecules, pathways and dynamics. The book highlights the advantages and disadvantages of the techniques, and gives state-of-the-art examples of applications using microscopes currently available on the market. It covers key techniques such as stimulated emission depletion (STED), structured illumination microscopy (SSIM), photoactivated localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM). It will be a useful reference for biomedical researchers who want to work with super-resolution imaging, learn the proper technique for their application, and simultaneously obtain a solid footing in other techniques.

Plasmonics is an emerging field mainly developed within the past two decades. Due to its unique capabilities to manipulate light at deep subwavelength scales, plasmonics has been commonly treated as the most important part of nanophotonics. Plasmonic-assisted optical microscopy techniques, especially super-resolution microscopy, have shown tremendous potential and attracted much attention. This book aims to collect cutting-edge studies in various optical imaging technologies with advanced performances that are enabled or enhanced by plasmonics. The basic working principles, development details, and potential future direction and perspectives are discussed. Edited by Zhaowei Liu, a prominent researcher in the field of super-resolution microscopy, this book will be an excellent reference for anyone in the field of nanophotonics, plasmonics, and optical microscopy.

This volume presents current advanced technologies and methods used in super-resolution microscopy. The chapters in this book cover a wide range of topics such as introducing super-resolution microscopy into a core facility; two-photon STED microscopy for nanoscale imaging of neural morphology in vivo; correlative SIM-STORM microscopy; two-color single-molecule tracking in live cells; and correlative single molecule localization microscopy and confocal microscopy. Written in the highly successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Cutting-edge and comprehensive, Super-Resolution Microscopy: Methods and Protocols is a valuable resource for both established and novel researchers and users in this field.

The imaging of small cellular components requires powerful instruments, and an entire family of equipment and techniques based on the confocal principle has been developed over the past 30 years. Such methods are commonly used by neuroscience researchers, but the majority of these users do not have a microscopy or a cell biology backgrounds and do can encounter difficulties in obtaining and interpreting results. This volume brings experts in high-resolution optical microscopy applications in neuroscience and cell biology together to document the state of the art. Outlining what is currently possible, the volume also discusses promising developments for the future and aids readers in selecting the most scientifically meaningful approach to solve their questions. Each chapter discusses instrumentation and technology in relationship to application in research. All of the common and cutting edge trends are covered - fluorescence / laser electron / nonlinear microscopy, infrared fluorescence, multiphoton imaging, tomography, FRAP, live imaging, STED, PALM/STORM, etc. * The first comprehensive volume on cellular imaging with a focus for its application in neuroscience * Concluding chapter compares the merits of various techniques * Full color throughout, maximizing users comprehension of the results obtainable via various methods * Features outstanding and truly international scholarship, with chapters written by leading experts in neuroscience and cell biology * Discusses cutting edge methods such as STED, PALM/STORM, nonlinear microscopy and more

"Our genetic material, the deoxyribonucleic acid (DNA), is folded in the nucleus in an extremely close-packed manner. Therefore the organization of the nucleic material in eukaryotic cells, the chromatin, affects many cellular properties and processes. Though chromatin has been subject of research for almost 150 years, its exact structure in the sub-chromosome scale is still unknown. The small length scale, below the resolution limit of fluorescent microscopy, forced the scientific community to study chromatin using electron microscopy and X-ray based methods, which are damaging to biological samples, and produce ambiguous results, especially in-vivo. We present a study of the chromatin structure inside the nucleus using a fluorescence based method. Chromatin inside cells is labeled uniformly in a way that causes minimal disruption to its natural behavior. A combination of scanning fluorescent correlation spectroscopy (sFCS) and super-resolution time-gated stimulated emission depletion microscopy (gSTED)can be used to both image the cells and measure the structural properties of the chromatin, to a higher resolution than achieved before in fluorescent in-vivo studies. Our preliminary results show evidence of Gaussian shaped sub-structures on the scale of 300-400 nm, a behavior typical of a "beads-on-a-spring" system." -- abstract.

The book focuses on various detection targets applied in single cell studies, including tumor tissue cells, circulating tumor cells (CTCs), disseminated tumor cells (DTCs), circulating tumor DNA (ctDNA), cell-free DNA (cfDNA) and cancer stem cells (CSCs). It also discusses and compares detection methods using these detection targets in different fields to reveal single cell biomedical functions. The volume focuses not only on the methods already been established and validated, and also the methods newly developed. The book also highlights the importance and potential of single cell biomedicine in the development and validation of precision medicine strategies. It is useful for researchers and students in the field of cell biology, molecular medicine and precision medicine etc.