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 microscopyMicroscopy: 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.

Super Resolution Fluorescence Microscopy Department Of

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 pro fi le developments have been in super-resolution fl uorescence 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 Provides video accompaniment for certain techniques on a companion website for a multi-dimensional perspective: http://booksite.elsevier.com/9780124095137

This book describes developments in the field of super-resolution fluorescence microscopy or nanoscopy. In 11 chapters, distinguished scientists and leaders in their respective fields describe different nanoscopy approaches, various labeling technologies, and concrete applications. The topics covered include the principles and applications of the most popular nanoscopy techniques STED and (f)PALM/STORM, along with advances brought about by fluorescent proteins and organic dyes optimized for fluorescence nanoscopy. Furthermore, the photophysics of fluorescent labels is addressed, specifically for improving their photoswitching capabilities. Important applications are also discussed, such as the tracking and counting of molecules to determine acting forces in cells, and quantitative cellular imaging, respectively, as well as the mapping of chemical reaction centers at the nano-scale. The 2014 Chemistry Nobel Prize ® was awarded for the ground-breaking developments of super-resolved fluorescence microscopy. In this book, which was co-edited by one of the prize winners, readers will find the most recent developments in this field.

This book presents a comprehensive and coherent summary of techniques for enhancing the resolution and image contrast provided by far-field optical microscopes. It takes a critical look at the body of knowledge that comprises optical microscopy, compares and contrasts the various instruments, provides a clear discussion of the physical principles that underpin these techniques, and describes advances in science and medicine for which superresolution microscopes are required and are making major contributions. The text fills significant gaps that exist in other works on superresolution imaging, firstly by placing a new emphasis on the specimen, a critical component of the microscope setup, giving equal importance to the enhancement of both resolution and contrast. Secondly, it covers several topics not typically discussed in depth, such as Bessel and Airy beams, the physics of the spiral phase plate, vortex beams and singular optics, photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), structured illumination microscopy (SIM), and light-sheet fluorescence microscopy (LSFM). Several variants of these techniques are critically discussed. Noise, optical aberrations, specimen damage, and artifacts in microscopy are also covered. The importance of validation of superresolution images with electron microscopy is stressed. Additionally, the book includes translations and discussion of seminal papers by Abbe and Helmholtz that proved to be pedagogically relevant as well as historically significant. This book is written for students, researchers, and engineers in the life sciences, medicine, biological engineering, and materials science who plan to work with or already are working with superresolution light microscopes. The volume can serve as a reference for these areas while a selected set of individual chapters can be used as a textbook for a one-semester undergraduate or first-year graduate course on superresolution microscopy. Moreover, the text provides a captivating account of curiosity, skepticism, risk-taking, innovation, and creativity in science and technology. Good scientific practice is emphasized throughout, and the author ' s lecture slides on responsible conduct of research are included as an online resource which will be of interest to students, course instructors, and scientists alike.

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.

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.

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

Fundamentals of Light Microscopy and Electronic Imaging, Second Edition provides a coherent introduction to the principles and applications of the integrated optical microscope system, covering both theoretical and practical considerations. It expands and updates discussions of multi-spectral imaging, intensified digital cameras, signal colocalization, and uses of objectives, and offers guidance in the selection of microscopes and electronic cameras, as well as appropriate auxiliary optical systems and fluorescent tags. The book is divided into three sections covering optical principles in diffraction and image formation, basic modes of light microscopy, and components of modern electronic imaging systems and image processing operations. Each chapter introduces relevant theory, followed by descriptions of instrument alignment and image interpretation. This revision includes new chapters on live cell imaging, measurement of protein dynamics, deconvolution microscopy, and interference microscopy. PowerPoint slides of the figures as well as other supplementary materials for instructors are available at a companion website: www.wiley.com/go/murphy/lightmicroscopy

Cell Membrane Nanodomains: From Biochemistry to Nanoscopy describes recent advances in our understanding of membrane organization, with a particular focus on the cutting-edge imaging techniques that are making these new discoveries possible. With contributions from pioneers in the field, the book explores areas where the application of these novel techniques reveals new concepts in biology. It assembles a collection of works where the integration of membrane biology and microscopy emphasizes the interdisciplinary nature of this exciting field. Beginning with a broad description of membrane organization, including seminal work on lipid partitioning in model systems and the roles of proteins in membrane organization, the book examines how lipids and membrane compartmentalization can regulate protein function and signal transduction. It then focuses on recent advances in imaging techniques and tools that foster further advances in our understanding of signaling nanoplatforms. The coverage includes several diffraction-limited imaging techniques that allow for measurements of protein distribution/clustering and membrane curvature in living cells, new fluorescent proteins, novel Laurdan analyses, and the toolbox of labeling possibilities with organic dyes. Since superresolution optical techniques have been crucial to advancing our understanding of cellular structure and protein behavior, the book concludes with a discussion of technologies that are enabling the visualization of lipids, proteins, and other molecular components at unprecedented spatiotemporal resolution. It also explains the ins and outs of the rapidly developing high- or superresolution microscopy field, including new methods and data analysis tools that exclusively pertain to these techniques. This integration of membrane biology and advanced imaging techniques emphasizes the interdisciplinary nature of this exciting field. The array of contributions from leading world experts makes this book a valuable tool for the visualization of signaling nanoplatforms by means of cutting-edge optical microscopy tools.

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.

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