

MBItion 2013 Workshop Schedule

	1st Week							2nd Week					
	13-May-13	14-May-13	15-May-13	16-May-13	17-May-13	18-May-13	19-May-13	20-May-13	21-May-13	22-May-13	23-May-13		
	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday		
9.30am-11am	Theory #: T1 Light Sensors and Cameras [PC Chen]	-	Theory #: T2 Clearing Agents and Refractive Index Match [PC Chen]	Theory #: T3 STORM [Clement]	Theory #: T4 PALM [Samuel]	Theory #: T6 Optical Single Molecule Tracking [Felix]	No Workshop	Theory #: T7 Superresolution Microscopy at Work [Hu Xian]	-	Theory #: T8 Confocal Principles [Steve]	Theory #: T9 Human Perceptions, Seeing is Believing [Glen]		
11am-12noon	Hands-on #: 1 Discussion	Hands-on #: 4 Discussion	Hands-on #: 7 Discussion	Hands-on #: 10 Discussion	Hands-on #: 13 Discussion	Hands-on #: 15 Discussion		Hands-on #: 16 Discussion	Hands-on #: 19 Discussion	Hands-on #: 22 Discussion	Hands-on #: 25 Discussion		
12noon-12.30pm	iLAS2 [Emma] STORM [JC Soo] SpinningDisk[Jasmine] Co-localization [Steve] Cell Staining [Glen]	NSIM [Liu Jun] PALM [Kah Jun] Delta Vision [Emma]	iLAS2 [Emma] STORM [JC Soo] SpinningDisk[Jasmine] Cell Staining [Glen]	NSIM [Clement] PALM [Kah Jun] Delta Vision [Emma]	FCS [Xianke] PALM [Samuel] Zeiss Upright [Liu Jun]	STORM [Hu Xian] Frapping [Emma] Baths/Chambers [Steve]		NSIM [Liu Jun] PALM [Kah Jun] STORM [Clement] SpinningDisk[Jasmine] Co-localization [Steve]	STORM [Hu Xian] Cell Staining [Glen] Baths/Chambers [Steve]	NSIM [Liu Jun] PALM [Kah Jun] STORM [Hu Xian] SpinningDisk[Jasmine]	Cell Staining [Glen]		
12.30pm-1.45pm	Lunch (Not Provided)							No Workshop	Lunch (Not Provided)				
2pm-3pm	Hands-on #: 2 Discussion	Hands-on #: 5 Discussion	Hands-on #: 8 Discussion	Hands-on #: 11 Discussion	Friday Workshop (with refreshmt) FCS [Xianke]				Hands-on #: 17 Discussion	Hands-on #: 20 Discussion	Hands-on #: 23 Discussion	Hands-on #: 26 Discussion	
3pm-3.30pm	NSIM [Liu Jun] PALM [Kah Jun] Delta Vision [Emma] SpinningDisk[Jasmine] Co-localization [Steve]	iLAS2 [Emma] STORM [JC Soo] Ca2+ Imaging [Glen] Fast Resonant [Steve]	NSIM [Liu Jun] PALM [Kah Jun] Delta Vision [Emma] SpinningDisk[Jasmine]	iLAS2 [Emma] STORM [Clement] SpinningDisk[Jasmine] Baths/Chambers [Steve] PS & Movies [Glen]					NSIM [Clement] STORM [Hu Xian] Zeiss 710 [Liu Jun] SpinningDisk[Jasmine] Co-localization [Steve]	NSIM [Liu Jun] PALM [Kah Jun] Ca2+ Imaging [Glen] Fast Resonant [Steve]	SpinningDisk[Jasmine]	NSIM [Liu Jun] Baths/Chambers [Steve] PS & Movies [Glen]	
3.30pm-4pm	Tea Break (Provided)								No Workshop	Tea Break (Provided)			
4pm-5pm	Hands-on #: 3 Discussion	Hands-on #: 6 Discussion	Hands-on #: 9 Discussion	Hands-on #: 12 Discussion	Hands-on #: 14 Discussion					Hands-on #: 18 Discussion	Hands-on #: 21 Discussion	Hands-on #: 24 Discussion	Hands-on #: 27 Discussion
5pm-5.30pm	NSIM [Liu Jun] PALM [Kah Jun] Delta Vision [Emma] Zebrafish/SD [Glen] 4D Zebrafish [Steve]	iLAS2 [Emma] STORM [JC Soo] Ca2+ Imaging [Glen] Fast Resonant [Steve]	NSIM [Liu Jun] PALM [Kah Jun] Delta Vision [Emma] Zebrafish/SD [Glen] 4D Zebrafish [Steve]	iLAS2 [Emma] STORM [Clement] SpinningDisk[Jasmine] Baths/Chambers [Steve] PS & Movies [Glen]	FCS [Xianke] PALM [Samuel] Zeiss Upright [Liu Jun]					NSIM [Clement] STORM [Hu Xian] Zeiss 710 [Liu Jun] Zebrafish/SD [Glen] 4D Zebrafish [Steve]	NSIM [Liu Jun] PALM [Kah Jun] Ca2+ Imaging [Glen] Fast Resonant [Steve]	Zebrafish/SD [Glen] 4D Zebrafish [Steve]	NSIM [Liu Jun] Baths/Chambers [Steve] PS & Movies [Glen]

Abstracts for Theory Sessions & Friday Workshop

"Light Sensors and Cameras"

Speaker: Ping Chin CHENG

This is an in depth overview of the scanning and camera system's in use in modern high end microscopes. It offers a cursory overview of the working principles of light to electric charge conversion, the read out, and the conversion of charge into digital numbers.

I addresses specific advantages and inherent limitations of today's cameras and sensors and how they should best be made use of.

It also includes some aspects of emerging technologies and some microscopy images of the cameras used for microscopy itself.

"Clearing Agents and Refractive Index Match"

Speaker: Ping Chin CHENG

Working with fixed samples brings the convenience that one has more time at hand to record a high quality static image of the specimen. For larger specimen, clearing agents have been available for some time now and they help to overcome the inevitable light loss inside thicker structures. The agents can be fine tuned to more than one property and hence can help to achieve dramatically better microscopy.

The talk addresses the dangers and limitations of clearing agents but also explores the topic of index matching to overcome spherical aberration. We try to have some agents ready for the audience to work on their own samples after the talk.

"Super Resolution Microscope: STORM - Imaging Beyond the Diffraction Limit"

Speaker: Clement KHAW

Nikon N-STORM is a groundbreaking new Super Resolution imaging systems based on Nikon's Ti-E inverted microscope technology which is capable of multi-spectral two and three-dimensional nanoscopy. The Nikon N-STORM super-resolution microscope system combines "STochastic Optical Reconstruction Microscopy" technology, licensed from Harvard University. With lateral resolution of approximately 20nm and axial resolution of approximately 50nm, N-STORM reconstructs high resolution fluorescence images (2D or 3D) from localization information of fluorophores detected with high accuracy and calculated from multiple exposures. Because of this, the technique generates much more detailed information and goes from producing an understanding of mere structural data to a nearly molecular understanding of the specimen. N-STORM differs from conventional fluorescence microscopy in that it does not observe all the fluorescently labelled molecules in the sample at the same time, but activates only a very low percentage at any one given time. Repeating this process and acquiring multiple frames allows statistical localization of molecules with nanometer accuracy resulting in a final Super-Resolution image.

N-STORM reconstructs nanoscale resolution fluorescence images in three dimensions from localization information derived from fluorophores detected in the z-axis with high accuracy and calculated from multiple exposures. Just as single molecules can be localized in the x-y dimension, the N-STORM 3D component can localize the same single molecule along the z-axis providing high accuracy, three-dimensional, super-resolution imaging.

Abstracts for Theory Sessions & Friday Workshop

"Disclosing the Secrets of Your Fluorescence-Labelled Biological Sample with PALM"

Speaker: Samuel KO

With the development of fluorescent indicators and recombinant proteins, the technique of fluorescence microscopy has become widely established as a research instrument for investigating biological specimens. Historically, to get a high resolution microscopic image is an issue because of the diffraction limit (the resolving power of a light microscope is limited to approximately 200nm in the lateral (XY) and 500nm in the axial (Z) direction that was first described by Ernst Abbe in 1873). Nowadays, superresolution techniques disclose the details with overcoming the diffraction limit and provide ultra-structural studies. **PhotoActivated Localization Microscopy (PALM)** uses photoswitchable dyes and enables an outstanding localization accuracy down to 10 nm for single fluorescent signals in cells and tissues. As a single molecule imaging method, it opens up completely new dimensions for a quantitative analysis of complex biological specimens.

Friday Workshop:

"Fluorescence Correlation Spectroscopy for Bioscience Research"

Speaker: Xianke SHI

Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS) are widely used biophysical techniques to determine biomolecular concentration, photophysical dynamics of fluorophores, diffusion coefficients of NAs and proteins, and dissociation constants of interacting particles. Nowadays, the application of FCS and FCCS has been extended from prepared solutions to living cells and even living organisms. In this presentation, we will discuss the fundamental of FCS/FCCS: What is FCS/FCCS? What it can do for biologist? How we can use it for our research work?

Abstracts for Theory Sessions & Friday Workshop

"Optical Single Molecule Tracking"

Speaker: Felix MARGADANT

The optical tracking is a subset of the field of superresolution microscopy. It replaces the mere imaging and then following proteins which carry a fluorescent molecule - a fluorochrome - with detecting and then localizing the position of the fluorochrome better than the diffraction limit does permit. For this to be possible, the speckles created by individual fluorophores must be visually separated so that they do generally not overlap.

This talk is about how to follow single molecules over time, calculate stretch and orientation but also on the attempts to overcome the signal density limit and how to fight the large percentage of erroneous measurements and how to recognize artifacts.

"Superresolution Microscopy at Work - a Guide to Our Systems"

Speaker: Xian HU

The field of superresolution is THE fashionable field of microscopy of at least the decade. While the limitations are still high and the experiments are more tedious but the reason for the gold rush is easy to explain: many crucial answers how proteins fold, how and where they adhere, and how the conformation evolves can all be observed within one order of magnitude of the optical diffraction limit of about 200nm. The biggest limitation for the user is that none of the techniques which has evolved to this day covers all the experiments of interest of today. The same specimen might even have to be viewed with entirely different and mutually exclusive methods.

The field also underwent a schism into three entirely different fundamental methods. The ones where the measurement is taken at a very small, controlled spot (STED and surface soliton imaging), the ones where the measurement is at random locations separated over time (PALM, STORM, GSD), and finally single molecule tracking where the signals are isolated.

We hear speakers on both the latter two methods which are summarized as localization microscopy. And I will focus on the experimental side, what can be done with each method quickly, safely, and with a high accuracy. I will also focus on what cannot yet be done and what is needed for the field.

Abstracts for Theory Sessions & Friday Workshop

"Confocal Principles - Applied Aberrations & A Brief Introduction to Multiphoton"

Speaker: Steve CODY

This is a summary talk of confocal principles and the evolution that led to it. It addresses quantitative aspects of confocal performance and quality and the limitations of confocal imaging. The different technologies in use are addressed, including the spinning disk and the multiphoton microscope, and an overview of the role of the confocal instruments in the age of superresolution microscopy is given. Dyes and scan protocols will be addressed.

The talk is complementary to the practical session on confocal microscopy.

"Human Perceptions, Seeing is Believing"

Speaker: Glen McDONALD

The human eye is not a very suitable sensor to process the large homogeneous field and the very high dynamic range of the microscopy cameras in use today. 4000 to 250,000 levels of grey cannot be perceived at once nor can the high spatial accuracy be grasped by the human eye. To make matters worse, the spectral emissions of our samples do not correspond favorably with the three channel detector cones of the human eye. And finally displays and printers have a dynamic range and color space which is even narrower than the one of our visual system.

This talk focuses on how to reduce photometric data in a way that it is quantitatively conveyed to the user. That is reports the correct differences in brightness, maintains spatial truth, and does not further degrade the spectral quality of the sample.

It will also include a short section on how to navigate large, 3D, spectral datasets.