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Focused Ion Beam Microscope (FEI 800x) at SCIF, UC Merced: SOP

Focused ion beam microscope at SCIF • Sample needs to be conductive • Copper tape or Carbon conductive tape can be used • Silver solution should be used only if it is absolutely necessary as it can mess up the parts in the system • Sample holder (Load lock) • Ion column: 30KV; Gallium ion source; Negative extraction voltage (EV) to suck Ga⁺² down through the column • Black knobs around the Ion column are to align the source. Do not touch them unless changing the source. • Automatic variable aperture (AVA) • High current is for rapid milling • Low current is mostly for imaging • Javelin system: Optical microscope; Good for rough navigation • IGP – Ion getter pump • Pre-amplifier for detector • Raster for the beam controller • GIS: Gas injection system; pneumatically controlled chamber; A crucible in the chamber contains Platinum. It defines a pattern. • Common metals: Pt, W, Mo, C • Thickness of deposition: 20 μ • Milling: The least is about a few nanometers but not too deep • Deposition: Usually good to have primary ions and not have secondary electrons as they can widen the deposition area • Ion beam can be used to clean up most of the overspray; Overspray can be milled away as it is usually pretty thin • Sample holder: (*200 mm wafer (8in_notch): Default setting • Check the box which says “move stage to default eucentric height” Key: Start up page ; Swiss knife: Work page: Stage position can be saved • Eucentric Height: ‘Z’ height in the chamber. When you tilt the holder, the sample we are looking at is directly under the ion beam • Touch alarm goes off if the wafer is too close to the source • Ion column: Source (Just 2.2kb to start off the Ga source. Emission current coming off the source is 2.3 μ A) • Extractor: Negative extraction voltage • Suppressor: Positive voltage to push the ions down • Extractor current: Usually not to be changed as it changes other settings. So, if the source doesn’t start up play adjust the suppressor voltage only • Over 14 μ :

*** : These three asterisk mean that the system is ready • Leave and maintain preset option checked • At the end of the day: Turn the source off and shut the high tension button off • High voltage: 30KV • Click column isolation valve • Ion source lifetime: $\mu\text{A}\cdot\text{hr}$ (units); Usually lasts for $3000\mu\text{A}$ as we use $2.2\mu\text{A}$ as a standard preset. • Beam menu: Has the aperture settings/ options • ON option Turns on heat to the Pt • Chip/ Dye that the FAB, works on is called a dead chip/ dye as they kill it just so that there is no Ga contamination • Joystick icon: For imaging • If the image is not to be seen, change the aperture • $70\text{-}350\text{ pA}$ is usually good enough for a good image. Generally 150pA for our system • MUI: Manual user interface • Turbo button on the joystick is for faster movement of the stage • Eucentric height can be set using a tilt test • F5: To toggle between Quad mode and single screen mode • Magnification is related to the visualization window size • We have to be aware of the fact that there is a constant damage being done to the sample on the FIB • Eucentric height: 5000 quad or $10,000$ single mag is ideal • Image: $5\text{-}7\text{nm}$; $10\text{-}20\text{nm}$: More realistic and we have to be at 1pA for this. • Eucentric height set-up: Select a point Tilt stage: and make sure that you are looking at the same point Use only 'Z' to move it up or down when you are at tilted position. Do not use 'X' or 'Y' Move it very slowly to have the sample closer Once the above step is done-- move to another position (eg: 5° tilt) Total- 45° tilt Do another test as a sanity check Select a point- go all the way to 45° • To get a good picture: Scan Continuous scan: DO NOT CHANGE ANY OF THE SETTINGS Single scan: Use high resolution Better signal to noise: If it takes a long time to scan then there is always a risk of burning the sample Align 'X and 'Y' under stage menu to get a straight vertical line if you plan on milling a straight line Stop scanning whenever you can We have various option to select milling patterns on the sample Arrow key is to be selected when moving around a pattern Smallest size pattern is usually the general rule whatever the pattern • For Pt deposition: Select the right beam current. Too much current mills away the sample. Too little current will take a long time and is not good for the sample • Ideally: $2\text{-}6\text{pA}/\mu\text{m}^2$ • Ideal depth of milling is about $0.5\mu\text{m}$ • X; Y; $30/4$ is a reasonable size to mill • Realistically 10nm nanostructures can be imaged • Multiple patterns and different sizes can be deposited at the same time but this can be tricky. Beam is split in this case • Serial or Parallel deposition can be done • Serial: Finishes one pattern completely before moving on to the next pattern

• Parallel: One pace at a time at each pattern Work page: • Material files: Dwell time: Time the beam spends at each point Overlap Normally, dwell time and overlap need not be changed and are usually determined by the material file selected General milling: Si_mtr Deposition: Pt_mtr These settings are optimized and we do not have to change the settings Yellow box (Milling pattern) : Silicon Green box (Deposition pattern) : Platinum • Before selecting the IN button just do a sanity check if the eucentric height is good • Slight shift is normal after inserting the needle • After inserting the needle, grab an image and re-center the pattern • One eye beam frame. Stops deposition and grabs an image • Auto FIN: Automated. Stage movement can be automated for deposition, milling. • Pt- Bulk cleaning • If there is topography on the top surface and Pt is not deposited then you see what is called curtains/ waterfalls • If it is a deep channel then Pt deposition wouldn't be necessary • After deposition hit the GIS out • Filled box; Empty box: Just a pattern box; Thick lines: Milling tool; Thin lines: Clean up tool • There is no correct beam current that is pre-determined. It depends on the sample • 'Y' dimension needs to be about the same as the 'Z' • Small box with the green screen is a real time monitor • Decent polish of a cross section can be obtained at 1000-27000pA. Ideally 350pA can be used for most cross sections • Usually depends on the cross sections • Wear the edges while cleaning up • Usually if the edges are rounded charging might occur • Cleaning • Once done- delete patter- decrease beam current- tilt • Fore shortened: 'Y' as it is tilted. So, the depth has to be calculated/ determined using a different formula when tilted. • During break: Back to 0° (till). HV off. • Auto FIB • Program- Auto FIB-- Run script; 7nm: Smallest diameter • Auto FIB: Very good for repetitive Nano patterning • HFW: Horizontal field width- 1.26nm, 30mm, 800mm • Bitmap program: Milling file • For extended time based application • It is good to set the eucentric height and let it sit for an hour or two avoiding any stage movements. This allows for the stabilization and helps stay stable for longer periods of milling Detection • Detector: Needs to be in CDM ion mode= lot less 2° ions • In 2° ion mode the contrast is not high but a lot of surface (topography) features can be determined. It is also very helpful to determine contrast of metals and other conducting metals. • FEI Annotate: Reads the Tiff file. So, it reads the tilt and gives accurate measurements • Right click on the object- Object properties- Check section and measure (This program can be put on

a laptop) • PSC files when saving the script files • Under the auto FIB category there is a TEM wizard which helps use thin membranes for TEM imaging • Image recognition: Determining the rate of deposition always goes back to the deposition curve 30KV Ga+2 – Typical damage layer- 25nm. If the X, Y stigmation is perfect then when the focus is changed the stigmation changes symmetrically.
Venu Polineni | SCIF

#focused ion beam #Microscopy #Electron microscopy #tem #fib #ions #scif #UC #ucmerced #Polineni #Venu

✕ Close notes



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Intro to Focused Ion Beam Microscopy and its applications in Bio-imaging

Light microscopy techniques in most cases cannot resolve components whose diameters are substantially below the wavelength of light. Electron microscopy provides the resolution required to visualize and characterize such components.

Transmission electron microscopy (TEM) has a limited resolution range in the Z- direction and as a result, limitation of reliability of reconstruction from serial sectioning. Scanning electron microscopy (SEM) coupled with focused ion beam microscopy (FIB) provides a unique mechanism wherein each tissue slice is imaged before sectioning. This decreases the distortion that accompanies TEM serial sectioning. Conventionally, FIB has been in use in the semiconductor and material sciences industry. However, FIB-SEM use in pathology, developmental biology, microstructural anatomy and regenerative medicine has been increasing lately. One of the advantages that FIB-SEM has over TEM is that it is not as labor-intensive. Also, slices removed in the Z-direction are comparable in size with the spatial resolution in the X-Y image face, resulting in the same image data quality in all three axes thus enhancing the accuracy of feature recognition within the data set. 'Slice and view' concept of FIB-SEM facilitates gathering of a large image sequence for processing into a 3D rendering of specimen structures. An ion column, vacuum system, work chamber, a gas system and a

workstation constitute the key components of a FIB system. The ion beam is generated from a liquid metal ion source (LMIS) by the application of a strong electric field. A variable aperture mechanism allows for a fine beam for high-resolution imaging on sensitive samples and a heavy beam for fast and rough milling. Milling takes place as a result of physical sputtering of the target which might lead to damage of the sample. This surface damage of the sample can be minimized by sputter coating the sample surface with gold or platinum coating or platinum deposition. Sputtering mechanism in a FIB usually occurs when recoil atoms receive enough energy to generate a cascade, but the density of moving atoms disregards multiple collisions and collisions between moving atoms. A few scattering events can result in the production of secondary electrons (SE) and detection of SE is the standard mode of imaging in the FIB. A finely focused ion beam is raster scanned over a substrate, and secondary particles are generated in the sample. Electrons or ions are collected on a multiple channel plate (detector) as they leave the sample. Most of the operations on an FIB system are carried out via software. FIB is a powerful tool to prepare cross-sectional specimens for TEM and has a high positioning accuracy of milling at the specific point of interest. FIB offers a unique technique to visualize surface topography and internal architecture of biological tissue. FIB-SEM system helps in the study of microanatomy of model transgenic organisms and also, data can be mined at a later date for features that were not the focus of the original investigation as extensive sample damage doesn't occur as in a TEM. It has been observed that FIB image shows the multi-layered cuticle structure of cross-sectioned hair more clearly than SEM image. The Stem Cell Instrumentation Foundry (SCIF) at UC, Merced has a FIB (FEI 800x) which has Gallium (Ga⁺) as the LMIS source. There is a Hummer VI sputter coating unit and an atomic layer deposition unit for use in sample preparation for the FIB. The next post will feature a detailed explanation of the working mechanism of the FIB and will also include images imaged using the FIB system at SCIF.

The FIB system at SCIF **References:** Bhavsar, S. N., S. Aravindan, et al. (2009). A critical review on microtools fabrication by focused ion beam (FIB) technology. Bushby, A. J., K. M. Y. P'ng, et al. (2011). "Imaging three-dimensional tissue architectures by focused ion beam scanning electron microscopy." *Nature Protocols* 6(6): 845-858. Giannuzzi, L. A. and F. A. Stevie (2005). *Introduction to focused ion beams: instrumentation, theory, techniques, and practice*, Springer Verlag. Ishitani, T., H. Hirose, et al. (1995). "Focused-ion-beam digging of biological specimens." *Journal of electron microscopy* 44(2): 110. Reyntjens, S. and R. Puers (2001). "A review of focused

ion beam applications in microsystem technology." Journal of Micromechanics and Microengineering 11: 287. Vanhecke, D., S. Asano, et al. (2011). "Cryo-electron tomography: methodology, developments and biological applications." Journal of Microscopy. Volkert, C. and A. Minor (2007). "Focused ion beam microscopy and micromachining." MRS bulletin 32(05): 389-399. This post is very much influenced by the above cited articles and additional references for further reading will be provided upon request. Venu Polineni SCIF, UC Merced

#Focused ion beam #microscopy #electron microscopy #stem cell #instrumentation #University of California #Merced

11 notes



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The SkyLight

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#Microscopy #imaging #smartphone #easy

4 notes



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Image Bit Depth, and why your pictures don't look good in Photoshop

Hi All,

For anyone who has trouble with Image depth and such please check out Austin Blanco's Blog for a detailed explanation. Also, he has

explained ways in which you might be able to get around the problem.

<http://www.austinblanco.com/blog/?p=1340>

#Image depth #Photoshop #Bioimaging #Science #Confocal

7 notes



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"It takes heaps of sense to write good nonsense."

--- Mark Twain

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Confocal Microscopy at SCIF

Confocal Microscopy at SCIF

This post is a follow up to the earlier post (Confocal Microscopy: The Basics) and I describe the system we have at SCIF and the SOP for the same. Please feel free to contact me for more information about the system and also if you need any help setting up appointments with the SCIF staff or for a tour of the SCIF facility.

The Stem Cell Instrumentation Foundry (SCIF) provides stem cell researchers at UC Merced and throughout California access to advanced instruments, techniques and collaborators for single cell analysis. The SCIF is housed in a 4260 asf facility which includes Class 1000 and 100 clean rooms for micro/nano fabrication, facilities

for human and mouse stem cell culture, quantitative cell imaging, and workstations.

The SCIF is particularly unique because it will provide a range of microfluidic-based systems enabling researchers, with no prior knowledge of micro/nano techniques, to custom design devices online to their specific needs, rapidly adopting cutting edge research technologies. The SCIF is equipped with advanced "collaboratory" technology to connect researchers to online support, workshops and collaborators. Ancillary core services include materials characterization, vivarium, and computational biology.

The foundry will enable innovations in biotechnologies that will lead to new discoveries about stem cells; discoveries that will enable researchers to increase our understanding about the molecular signals that influence the properties and behavior of stem cells.

The foundry will also provide new opportunities to develop molecular tools and approaches that will be broadly applicable for basic and applied science, and regenerative medicine.

Please visit our website <http://scif.ucmerced.edu/> for more information about our facility.

Nikon Eclipse Ti C1® Confocal system

Laser launch

Ø 3 individual lasers

Ø Manual turn on/off (405 laser: key only [Melles Griot®]) 488 and 561 lasers [Coherent®] : Switch and a key)

Ø Do not unplug the power source after turning off lasers

- Allow the lasers to warm up for 15-20 minutes to reach their maximum efficiency
- Leave the attenuation on maximum transmission as attenuation can be controlled using the software
- All the lasers are connected to the scan head unit
- The angle of the mirrors in the galvos determines path of the laser
- Field zoom (referred to as pixel dwell) is a change in pixel resolution, and not an optical zoom
- The image gets blurry/unclear when the resolution exceeds the power of the lens
- There is a correction collar on the 20x and 40x objectives
- A multimode fiber collects the emitted light and directs it to the PMT which sends light through the dichroic filters for display.
- Emission filters available: 480.25; 515/30; 605/75
- NEVER launch the software without turning on the scan head

The Microscope

- Condenser assembly is manual
- Perfect focus system (PFS) uses an IR laser. ND slider has a filter that blocks IR light emitted from the transmitted light
- Field stop

Ø C1- Does not talk to Sutter instrument

Ø Elements- Talks to the Sutter instrument

- Confocal and wide field are never to be used together

§ Make sure the turret is in the open position for Confocal microscopy

- Red light on the scan head indicates that the interlock is disengaged and the system is ready for use
- The coarse/fine knob on the microscope becomes dysfunctional automatically when PFS is maintaining the focus
- The knob on the PFS adjusts the optical offset lens. Push the green button for accuracy.
- PFS doesn't help finding the best focal plane but it helps you maintain the best focal plane once you find it using the coarse/fine knob
- The LED by the "ON" button will not turn on if a signal is not detected
- Tube lens has to be turned all the way for additional 1x or 1.5x magnification
- Escape button is very helpful when changing magnification or changing objective to prevent any contact between the objective and the slide
- Refocus helps refocus on the specimen after changing objective
- If the new specimen is in a different container DO NOT hit the refocus button. If a different container is used, turn off PFS; lower the objectives, load the specimen container, then refocus.
- Other buttons on the microscope: Transmitted light intensity controlling knob and transmitted light on/off button
- Memory button locks only for PFS
- Elements will remember offset position for each lens whereas C1 will not
- Make sure to turn dichroic filters into place before switching to wide field fluorescence

Software

- Live: Scans the specimen continuously
- Frame lambda: Very useful for multichannel scanning
- To detect auto fluorescence at a particular wavelength turn laser off and turn detector on
- When you uncheck frame lambda and adjust all the parameters (like laser power/gain/offset) and check frame lambda, the last settings will be remembered and applied when you start acquisition
- Gain and Offset:

∅ As you increase the gain, decrease the offset. Gain increases voltage of the detector (PMT). Increases sensitivity but doesn't give a good dark to bright range

∅ Go out of focus and adjust offset until you see static on all the 4 boxes on the screen

∅ Best and Bright options scale display and does not affect the actual fluorescence

∅ Detectors on the SCIF Confocal microscope are 12-bit. Ideal range 1000-2000. Should never exceed 4095.

∅ If the detection intensity is too low increase the gain

∅ General rules for pinhole setting:

- Open or Large for 10x
- 20x: If higher scanning speed is required especially in live cell imaging use large option. For better resolution use Medium
- 40x: Medium
- 60x and 100x: For pictures use small; To scan use open or large
- Dwell time (Pixel dwell): Keep increasing pixel dwell to get good emission intensity numbers. However, pixel dwell has a high potential for killing live cells so, it is better to start off with a large pinhole. It is always better if the laser is turned down and pixel dwell is increased. But, it is not a good option for moving cells
- The info/color/view options determine how the image is displayed on the monitor

- For good pictures on the 'Z' axis adjust step size to half what the 'N' size is displayed as
 - 'Z' should not be adjusted when the PFS is on
 - Index : Allows the user to see each stack and also displays the total number of frames
 - The system is designed not to allow time delay to be set at a shorter time than needed for the image to be captured
- Additional information/references will be provided on request

Venu Polineni | SCIF. UC, Merced

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The Stem Cell Instrumentation Foundry

SCIF.UCMERCED.EDU

The Stem Cell Instrumentation Foundry (SCIF) is a core facility at the University of California (UC), Merced. The core is divided into a wet lab facility and a clean room facility. The wet lab includes a Nikon Eclipse Ti Confocal system, A Nikon Eclipse inverted microscope and a flow Cytometry core that includes BD LSR II Flow Analyzer, BD FACS ARIA II and BD FACS ARIA III cell sorters.

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Confocal Microscopy: The Basics

Hi All,

I've tried to generalize the basic parts of a Confocal system in addition to the common features in a software package for Confocal

Microscopy. Hope it helps give you an idea of the general working of a Confocal system. Feedback is welcome.

Confocal Microscopy: The Basics

Lasers: Gas/ Solid state: Confocal microscopes these days are usually equipped with solid state lasers

- Takes about 15 minutes for the lasers to warm up
- Very stable for extended imaging
- Intensity can be controlled by the software
- It is better to have the laser intensity knob (if there is any) at maximum transmission as it can be controlled by the software
- Wavelength of lasers commonly used range between 405nm – 633nm. A white laser is capable of emitting light over a broad range

Scan head

- Key component to transmit lasers to the objectives on the microscope
- Have mirrors in it to efficiently direct lasers. The angle of mirrors in the galvos determines the path of the laser
- All the lasers are connected to the scan head by an optical cable
- Care must be taken not to touch or change position of the scan head unit as it is calibrated to highest precision which will be lost if moved around

Detector

- Photomultiplier tube (PMT) is the detector used in most Confocal systems
- Emitted light is usually collected by multimode fibers which send it to the PMT. PMT directs it to the dichroic filters for display

Microscope

- A basic microscope with capabilities to hold multiple filters/filter cubes and slots for a scan head and a camera can be upgraded to a Confocal system

- Filters: A slot holds a turret that has various cubes that have dichroic filters. These are instrumental in the emission of a particular wavelength of light on laser exposure of the specimen
- Field Diaphragm: Used to control the amount of light exposure on the specimen
- Condenser: In conjunction with the field diaphragm helps set Kohler Illumination
- Most Confocal microscopes have the ability to perform wide field functions also. Wide field should never be used in conjunction with Confocal functions
- Stage: A motorized stage with a Z-axis movement capability is commonly used to accommodate for time lapse functions
- Ocular lens: Have an adjustable knob to account for papillary distance of the user. Ocular Diopter knob helps obtain a clear view without the aid of spectacles if an user has far/near sightedness
- Objectives: A wide range of objectives can be obtained from various manufacturers. 4X to 100X oil/water immersion objectives are available based on the user's requirement
- Focus knob: Knobs for fine/coarse focusing. A few systems also have the capacity for extra fine focus
- On/Off control for halogen lamp
- A knob to control intensity of transmitted light
- A few systems have controls to change objectives integrated on the microscope or there is a separate controller connected to the system
- There is an open position on the turret for transmitted light/UV light to pass through for wide field/ Differential interference contrast/Nomarski imaging
- The microscope is usually set up on a floating table to avoid loss of focus during extended time lapse applications
- Stage controller: Usually an external control with options for XY and Z axes movement control

Software

- This section includes explanation of the common features seen in most software packages used for Confocal imaging
- Live: Scans the specimen
- Laser bar: Aids in controlling laser intensity
- Gain: To control detector voltage (PMT)
- Offset: Helps increase contrast and decrease background noise
- Frame lambda: Useful for multichannel imaging. Turns on/off a particular channel for each scan
- Average: Scans a set number of times and gives out the best possible image
- Dwell time (Pixel dwell): Increasing pixel dwell will give good emission intensity numbers. However, pixel dwell has a high potential for killing live cells so, it is better to start off with a large pinhole. It is always better if the laser is turned down and pixel dwell is increased. But, it is not a good option for moving cells
- Pinhole: Helps change pinhole size for an imaging process
- o General rules for pinhole setting:
 - Open or Large for 10x
 - 20x: If higher scanning speed is required especially in live cell imaging use large option. For better resolution use Medium
 - 40x: Medium
 - 60x and 100x: For pictures use small; To scan use open or large
- Settings for time lapse and Z stack imaging processes are also displayed and differ based on the software
- Options to set best color range after acquiring an image is included
- Ruler is included
- Image can be viewed in 2D/2D graph/3D volume modes

The Basics Briefly

- **Kohler Illumination:** Illumination of the specimen is the most important variable in achieving high-quality images in microscopy and critical photomicrography. **Köhler** Illumination is as a method of providing the optimum specimen illumination. This method guarantees that the condenser is providing the best amount of light possible for the combination of lenses.

§ **Step 1:** Focus your sample in bright field.

§ **Step 2:** Close the field diaphragm.

§ **Step 3:** Focus the edge of the diaphragm by adjusting the condenser height.

§ **Step 4:** Center the image using the two centering screws.

§ **Step 5:** Open the field diaphragm until it is at the edge of the field view.

- **Raster Scanning:** Confocal microscopy employs a raster scanning mechanism for the sample area to be scanned

- **Pinhole:** Light passes through a pinhole as opposed to the conventional systems. This eliminates the possibility of excess light reaching the sample which in turn decreases background and results in a sharper image

- **Dichroic mirrors:** Responsible for the transmission of excitation and emission light

∅ Feedback is appreciated

∅ Please feel free to let me know if any other feature is to be added

∅ Please send me an email if you need more details regarding any feature listed above or unlisted feature related to the Confocal system

Venu Polineni | SCIF, UC Merced

#Confocal #Microscopy #Basics

20 notes



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Super Resolution Microscopic Imaging

Hi All,

The Stem Cell Instrumentation Foundry (SCIF) at UC, Merced is organizing a talk on Super Resolution Microscopic Imaging on the 1st of December (1-2:30PM), in Kolligian library (room KL159). For questions and RSVP please contact

Ray Williams at rwilliams@techinst.com or Brian Craze at bcraze@nikon.net.

Please see the attached flyer and the document for additional details.

Hope to see you there!

To Whom It May Concern:

As we all know, there is a universal desire among top-end life science researchers to observe tissues and cells more clearly. Optical microscopes are essential for this purpose. However, if multiple objects such as protein molecules cluster at distances of less than 200nm apart, conventional optical microscopes cannot identify them as single objects. In this case, other instrumentation such as electron microscopes previously had to be used.

Over the last several years, engineers and mathematicians have made vast improvements in the ability of scientist to realize resolution higher than ever before achieved by conventional optical microscopes with creation of **Super Resolution Microscopy**. Two of the aforementioned super resolution technologies that have moved the resolution of the optical system beyond the diffraction limit of the optics are **Stoichastic Optical Reconstruction Microscopy (STORM)** and **Structured Illumination Microscopy (SIM)**.

The **STORM** technology is a novel advanced form of optical microscopy and provides a solution for the universal desire among life science researchers to observe tissues and cells more clearly. The technology uses photo-switchable fluorescent probes to temporally separate the otherwise spatially overlapping images of individual molecules, allowing the construction of super resolution images. This new technology reconstructs high resolution fluorescence images **(2D and 3D)** from localization information of the fluorophores detected with high accuracy and calculated from multiple exposures. The amazing aspect of technological improvement is that it generates much more information from detection of single molecule fluorescence emissions by going one step further, from structural to molecular understanding of the specimen. Using this concept, two- and three-dimensional, multicolor fluorescence images of molecular complexes, cells and tissues with a few tens of nanometers resolution has been achieved. N-STORM provides dramatically enhanced resolution of that is 10 times or better than that of conventional optical microscopes, with lateral resolution to approximately 20nm and axial resolution to approximately 50nm, extending the role of the optical microscope to near molecular level resolution.

The **SIM** technique takes advantage of moiré patterns, which are produced by overlaying one pattern with another. The sample under the lens is observed while it is illuminated by a special grid pattern of light. Several different light patterns are applied, and the resulting moiré patterns are captured each time by a digital camera. Computer software algorithms then extract the information in the moiré images and translate it into two and three-dimensional, high-resolution reconstructions. N-SIM provides the fastest imaging capability of super resolution techniques which is effective for live-cell imaging. The newly developed **TIRF-SIM** illumination technique enables Total

Internal Reflection Fluorescence (TIRF) observation with higher resolution than conventional TIRF microscopes and gives more detailed structural information near the cell membrane. In addition, another new 3D-SIM illumination technique has the capability of optical sectioning of specimens, enabling the visualization of more detailed cell spatial structures with lateral resolution of approximately 85nm and axial resolution of approximately 200nm.

We would like to cordially invite you to join us for an educational seminar on theory and implementation of Super Resolution Microscopy **Thursday December 1st from 1-2:30pm**. Please see the attached flyer for details. See you soon!

Best regards,

Raymond Williams

Brian Craze

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Intro

Hello everyone, this is my first attempt at writing a blog. I would like to dedicate this mostly to scientific discussions and focus on scientific imaging, flow Cytometry and clean room usage for biological applications. Emphasis will be laid upon discussing various options for imaging and image analysis; also various imaging platforms will be compared. The stem cell instrumentation foundry (SCIF) at University of California (UC), Merced will feature in most of the posts as it is the place of my work and most of my imaging and clean room experiences that I share will be using the instruments at SCIF. Feedback is welcome, spam not. Images and experiences from my previous work place; Dr. Alejandro Calderon Urrea's laboratory at California State University, Fresno will also be featured in the blog. Without further ado let us delve into the first topic of interest which

includes fluorescence microscopy, confocal imaging and why it is important for most of the bright field imaging applications.

Microscopy has evolved in leaps and bounds since the reconfirmation of Anton Von Leeuwenhoek's observations using a modified version of the then existing model by Robert Hooke. Light microscopy in particular has advanced to a stage where microscopes are put into use to image nanoscale particles. Bright-field microscopes are widely employed for fluorescence studies but the major drawback of a conventional microscope is the excessive background that makes the specimen of interest look blurry. Confocal microscope gives sharper images as it eliminates most of the background as light passes through a pin-hole which blocks excess light and allows for the light to pass only through the pin-hole. Galvanized mirrors help focus light on the sample and in turn eliminate any unwanted exposure. Lasers are used for fluorescence applications as they provide a more stable source of light and also are very specific of the wavelength of light being focused on the specimen. Solid state lasers are used for Confocal applications nowadays and this provides a marked advantage as the intensity of laser remains stable for extended periods of time which makes Confocal imaging suitable even for extended time lapse applications. The beauty of a Confocal system is that it can be built around a simple microscope and customized based on the application of the user. Lasers, objectives, software modules and filters can be added based on the user's needs. The resolution of a confocal microscope is greater by a factor of 1.4 and a confocal microscope is optimal for high speed scanning and ruggedness which makes it an ideal source for 3D imaging.

Most of the software used for confocal imaging renders 3D volume view and analysis possible and this has led for the increased usage of Confocal in developmental and neurobiological studies. Addition of temperature and growth controlled chambers to Confocal microscopes has improved live-cell imaging; in addition, advanced shutter systems and software help control laser exposure and intensity making it easy to monitor developmental processes for an extended period of time. Personally, I have worked with various live and fixed specimens for confocal imaging. A few examples include Algae, Nematodes, Zebra fish, Fungi, Drosophila, mouse and human stem cells. I will try to blog regularly and will start posting on the

various techniques/modifications used for imaging different kinds of samples. Please post your views on using microscopy as a tool for your research needs and also any useful information that might be helpful for others in their research.

#confocal #microscopy #flow cytometry #stem cells #research
#biology #imaging #UC #scientific

34 notes

