Introduction to Modern Confocal Microscopy and its Applications (Lecture in English)

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Collaborator: Andor Technology, Oxford Instruments

One of the drivers of novel research breakthroughs in modern Life Sciences is the use of microscopy. Therefore, it is not surprising that researchers use a wide range of microscopy techniques to understand fundamental concepts in Life Sciences and biomedical research. In fact, novel technologies and experimental methodologies are essential to push the boundaries of research.

In this talk, I will explain the principles of fluorescence microscopy and the differences between widefield and confocal microscopy. In addition, an overview of the differences between multipoint confocal imaging systems (also known as spinning disks) and point scanner confocal imaging systems (also known as laser scanning confocal microscopes) will also be given. Finally, I will introduce Dragonfly, Andor's High-end multipoint confocal. Dragonfly.

Dragonfly Multimodal Confocal was designed to integrate biological imaging from single cells to a tissue or organism context. Key to this is exceptionally high background rejection in thick samples, a very low noise floor to retain detection of low signal fluorescence as well as high-intensity labelling, and live volume rendering for instant sample exploration.

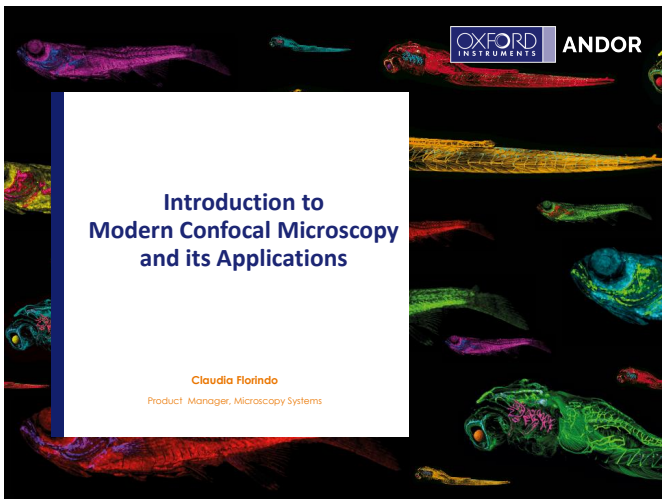
Researchers using the Dragonfly confocal platform publish outstanding science in high-profile journals. In this webinar, I will show examples of published data acquired with the Dragonfly multimodal system. In addition, I will present data from different research areas in biomedical science and show different applications/techniques of up-to-date microscopy.

現代のライフサイエンスにおいて、顕微鏡は新しい研究のブレークスルーをもたらす原動力のひとつとなっています。研究者が生命科学や生物医学研究の基本的な概念を理解するために、さまざまな顕微鏡技術を使用しており、研究の限界を押し広げるためには、新規の技術や実験方法論が不可欠です。

本講義では、蛍光顕微鏡の原理と蛍光顕微鏡と共焦点顕微鏡の違いについて説明します。さらに、マルチポイント共焦点イメージングシステム（スピニングディスク共焦点）とポイントスキャン共焦点イメージングシステム（レーザー走査型共焦点）の違いについても説明します。最後に、Andor のハイエンドマルチポイント共焦点である Dragonfly を紹介します。

Dragonfly マルチモーダル共焦点は、単一細胞から組織や個体の状況まで、生物学的なイメージングのために設計されました。その鍵となるのは、厚いサンプルにおける非常に高いバックグラウンド除去、高輝度シグナルと同様に低シグナル蛍光を検出するための非常に低いノイズ、サンプルを瞬時に探索するためのライブボリュームレンダリングです。

Dragonfly の共焦点プラットフォームを使用する研究者は著名なジャーナルに優れた科学を発表しています。本講義では、Dragonfly マルチモーダルシステムで取得された公開データの例を紹介します。また、バイオメディカルサイエンスの様々な研究分野のデータを紹介し、最新の顕微鏡の様々なアプリケーション技術を紹介する予定です。



Todays´Talk

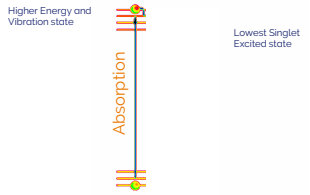
- I - Introduction to Fluorescence microscopy
- II – Principles in Confocal Microscopy
- III - Overview of Dragonfly system and applications

I - Introduction to Fluorescence microscopy

Today´s Webinar

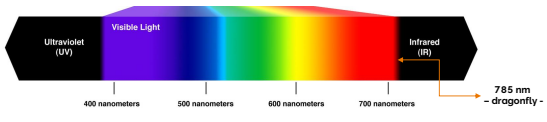
- How does Fluorescence works
- What is the hardware required
- Caveats in Fluorescence microscopy
- Set up and Design

1. How does Fluorescence works
Physics of Fluorescence

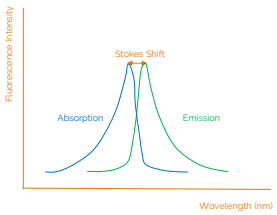


- Fluorescence is the emission of photons by atoms or molecules whose electrons are transiently stimulated to a higher excitation state by radiant energy from an outside source.
- Fluorescence ceases in the moment that incident excitation light terminates.

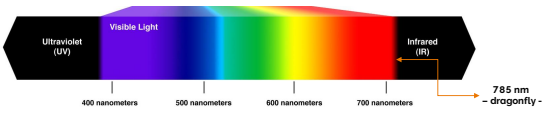
1. How does Fluorescence works
Fluorochromes



- Molecules that fluoresce are called fluorochromes.
- Fluorochromes have maximum absorption and emission peaks
- Stokes shift is the difference between the absorption an emission peaks



1. How does Fluorescence works
Fluorochromes

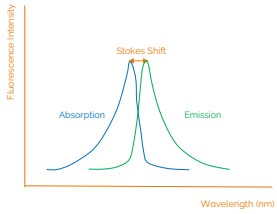


- The Features of the fluorochromes are dependent on the chemical environment:

pH, redox potential, ionic strength….

- Three desirable features of any fluorochrome:

1) High quantum efficiency,
2) Resistance to quenching
3) Resistance to photobleaching



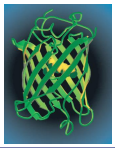
1. How does Fluorescence works
Fluorochromes

Chemical dyes

- Alexa dyes
- Hylite dyes
- cyanine dyes
- DAPI
- Acridine Orange
- Oregon green

Biological dyes

GFP



1. How does Fluorescence works
Fluorescent Proteins

Fluorescent proteins

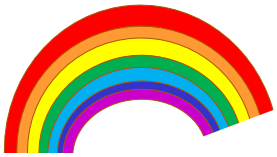


Photo-convertible – fluorescent proteins

Photo-switchable – fluorescent proteins

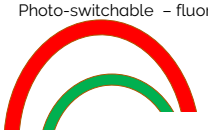
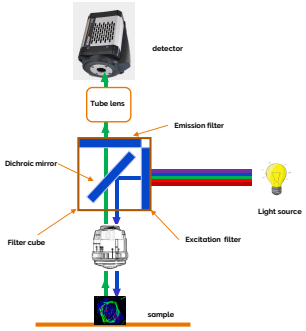


Photo activatable

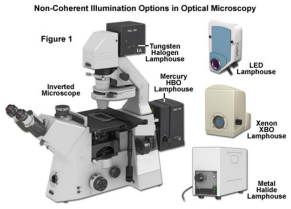
2. hardware

The epi-fluorescence microscope



- Light source
- Fluorescent filter set/cube
- Objective lenses
- Digital acquisition cameras

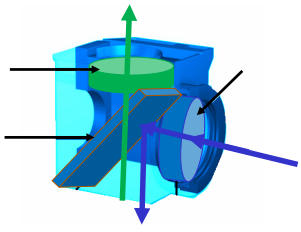
2. Hardware
Light source for fluorescent microscopy



- 10-100x brighter than halogen lamps
- Mercury arc lamp
- Xenon arc lamp
- Metal Halide
- LED

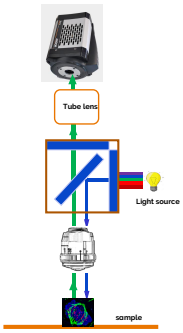
•Integrated Laser Engine (ILE)
•for Widefield laser illumination

2. Hardware
Fluorescent filter set

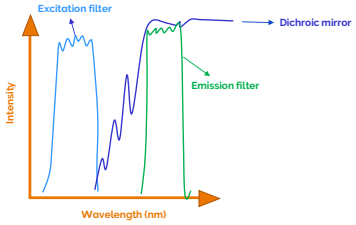


- Fluorescent filter set are arranged in a filter cube
- 3 main components: excitation filter, dichroic mirror, emission filter

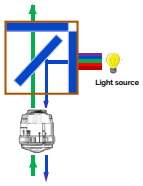
2. Hardware
Fluorescent filter set

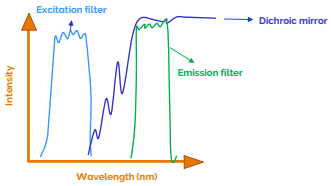


• **Fluorescent filter set enable illumination of the sample with specific excitation wavelength while registering only specific emission light to the observer**



2. Hardware
Fluorescent filter set



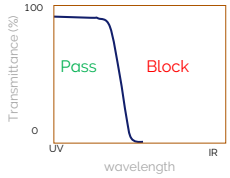


- excitation filter specifically allows the passage of excitatory wavelength light
- Dichroic mirror directs excitation light to the sample and enables specific passage of emission light to the emission filter
- Emission filter further stringe the passage of specific emitted wavelength from the specimen

2. Hardware
Fluorescent filter set

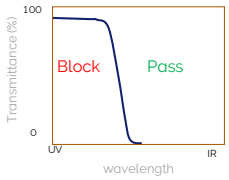
• different types of filters named after their capacity to discriminate between different wavelengths

• excitation filter are in general short pass

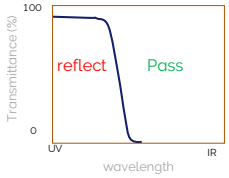


2. Hardware
Fluorescent filter set

- emission filters are in general long pass

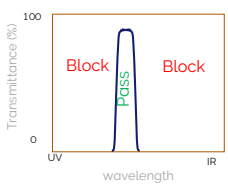


- Dichroic splitters reflect some wavelengths and allow others to pass.



2. Hardware
Fluorescent filter set

- Narrow band pass filters
  - Only allow a stringent set of wavelengths to pass through



2. Hardware
Fluorescent filter set

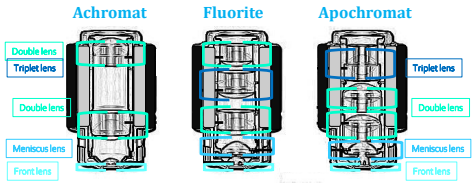
• **By using the characteristics of different filters and dichroic mirrors we can create specific filter set adequate to the type of fluorescent imaging experiments**

• **Two basic types of filter cube sets: 1) single and 2) multiple filter sets**

• **Single fluorescent filter set enable high signal to noise ratio but slower imaging**

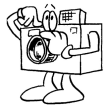
- **multiple fluorescent filter set enable faster imaging but lower signal to noise ratio**

2. Hardware
Objectives



- Higher NA → Higher resolution
- transparent to UV light (Use UV dyes)
- made out low-fluorescent glass
- plan-fluorite and plan apochromatic objective lenses are ideal
- Lenses have to correct for chromatic plane shift and act as a condenser

2. Hardware
Cameras

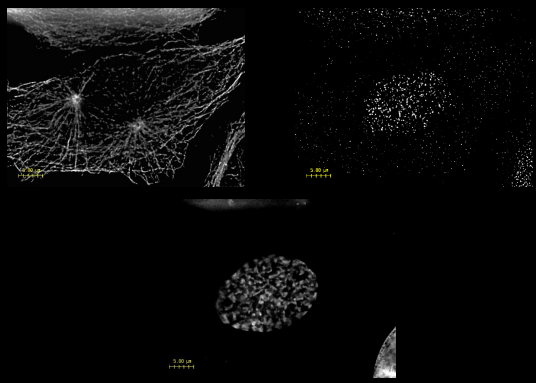


How about cameras?
Are they colour cameras or Black and white?

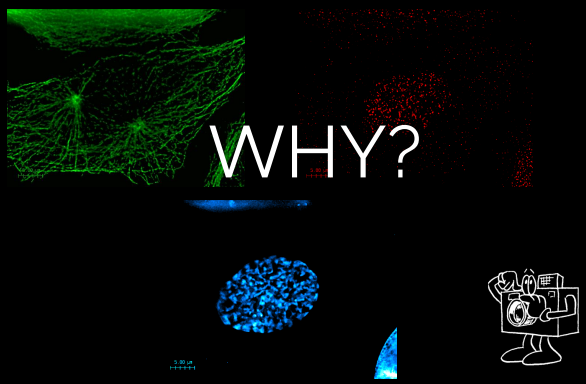
In Fluorescence Microscopy most common cameras are Black and White

2. Hardware
Cameras

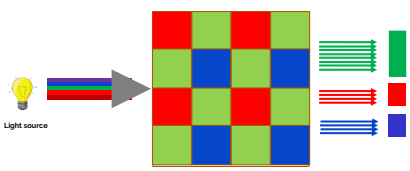
2. Hardware
Cameras



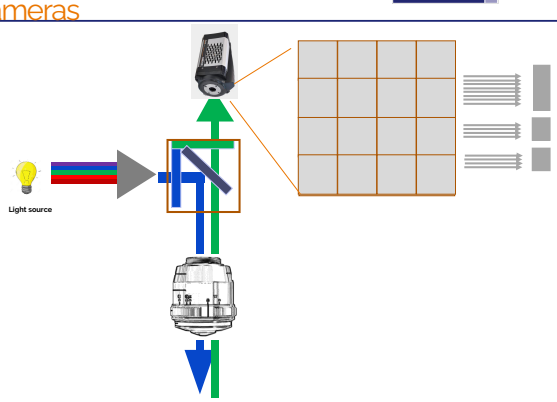
2. Hardware
Cameras



2. Hardware
Cameras



2. Hardware
Cameras



3. Caveats in Fluorescence microscopy

Auto-fluorescence

Bleed-though effect of fluorescent filter set available

dye photobleaching

live cell phototoxicity

3. Caveats in Fluorescence microscopy
Autofluorescence

The causes of auto-fluorescence:

Autofluorescence of endogenous molecules

Less than ideal filter set

reactivity of to the fixative used

Reflections and scattering of light in the optical pathway

3. Caveats in Fluorescence microscopy
bleed trough effect

Extremely relevant when imaging: multiple fluorochromes or fluorescent proteins simultaneously.

Causes of Bleed-though:

- non ideal filter set that in which the band pass wavelengths are quite close.
- non ideal fluorochrome choice for the experiment / microscope set up.

3. Caveats in Fluorescence microscopy
bleed trough effect

Possible solutions:

Reduced exposure times minimize the effect

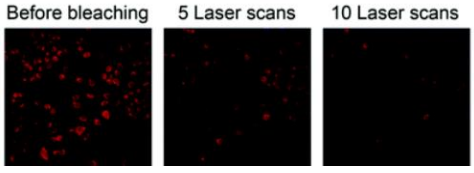
Use high specific filter set with narrow bandpass.

Nevertheless there might always be some signal cross-over.

3. Caveats in Fluorescence microscopy
Dye Photobleaching

Photobleaching or fading is the chemical alteration of a dye or a in a way that will be irreversibly damaged.

When a dye suffers photobleaching it will not Fluoresce anymore.



Orit Redy-Keisar, et al., 2015.
Organic & Biomolecular Chemistry

3. Caveats in Fluorescence microscopy
Dye Photobleaching

Caused by increased exposure of fluorochromes to light

Intensified energy exposure leads to formation of radicals, which will cause modifications in the covalent bounds of the fluorochrome.

The result is transition from singlet state to the triplet state.

Photobleaching is irreversible

3. Caveats in Fluorescence microscopy
Dye Photobleaching

Avoiding photobleaching:

Use the most photostable dye possible.

Reduce the O2 in the sample
- use N2
- Use oxygen scavengers

use of anti-fading reagents in the embedding media

Reduce exposure time

3. Dye Photobleaching Applications
FRAP

Applications of photobleaching in microscopy:

FRAP - Fluorescence Recovery After Photobleaching

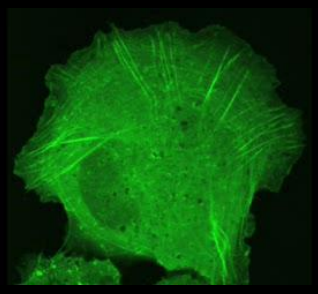
Diffusion of molecules

Vesicles transport

Transport along the microtubules

3. Dye Photobleaching Applications
FRAP

Protein diffusion and compartmentalisation



FRAP done using ANDOR Mosaic (photo stimulation applications)

3. Caveats in Fluorescence microscopy
Live cell phototoxicity

The Light sources used in are highly energetic and can also transmit UV light.

Filters and dichroic mirrors are not totally efficient in blocking those wavelengths

This causes:
Damage in cell wall lipids and proteins leading to rapid cell death

3. Caveats in Fluorescence microscopy
Live cell phototoxicity

Solutions:

Reduce effect with additional UV filters, exposure times and balanced redox environment (when using metal halide light sources)

Use laser widefield illumination. This will selectively illuminate the sample only with the chosen laser lines

Use longer wavelengths for imaging in live cells

If possible use NIR wavelengths (avoid UV)

Choose an imaging system compatible with live imaging experiments, such as a dual micro lens spinning disk system.

3. Set up and design

What are the microscopes available?

What filter sets do those microscopes have?

What are the lasers available?

What do I want to do?

3. Set up and design

What do I want to do?

Live or fixed sample

Multicolour or single colour experiment?

Fluorescent antibodies
Fluorescent proteins
Quantum dots

} Select the appropriate fluorochromes or fluorescent proteins

Design the sample preparation protocol

Before going to the microscope – design the image acquisition protocol

SUMMARY

- How does Fluorescence works
- What is the hardware required
- Caveats in Fluorescence microscopy
- Set up and Design

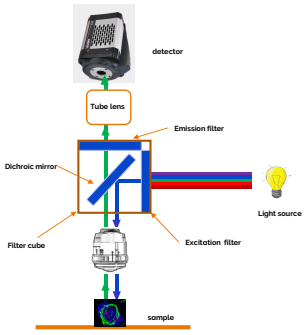
II – Principles in Confocal Microscopy

Summary of today

- Review of Fluorescence Microscopy
- What is a confocal microscope
- Point scanner confocal Microscopes
- The Pinhole
- Spinning disk confocal Microscope
- Which Microscope to choose depending on application

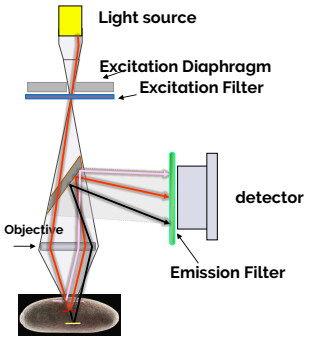
Quick review of epi-fluorescence Microscope

The epi-fluorescence microscope

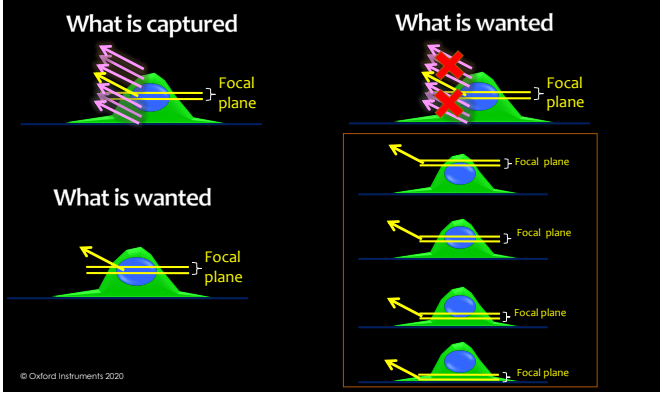


- Light source
- Fluorescent filter set/cube
- Objective lenses
- Digital acquisition cameras

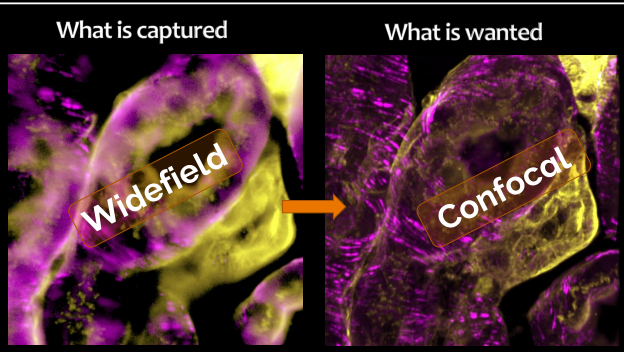
Detection In an epifluorescence microscope



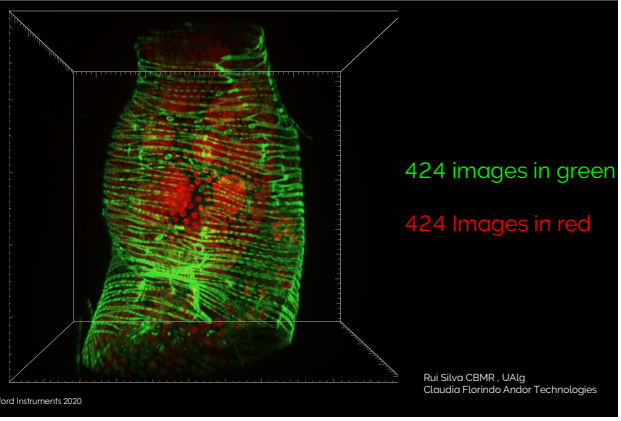
Detection In an epifluorescence microscope



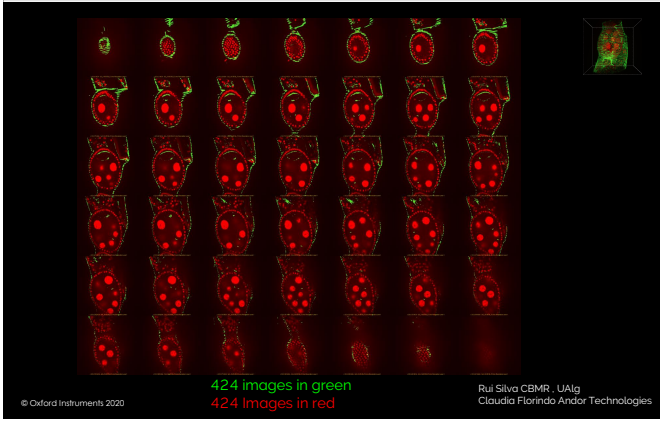
Detection In an epifluorescence microscope



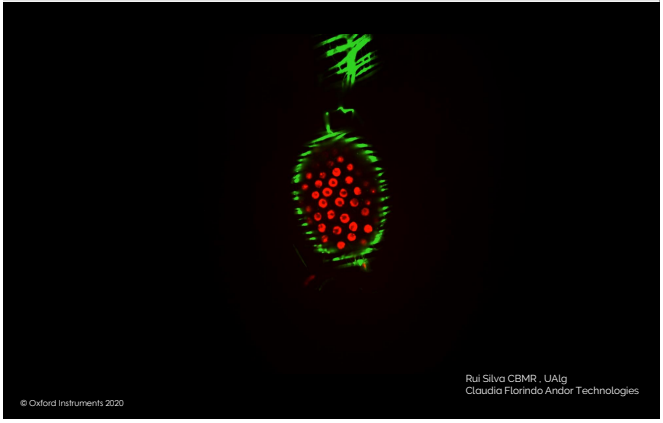
How to acquire a 3D image with a 2Dimaging system?



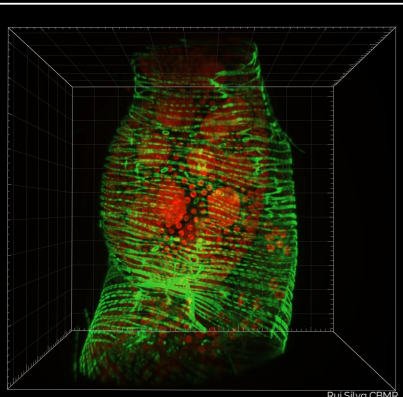
Acquire all the planes you need



Acquire all the planes you need

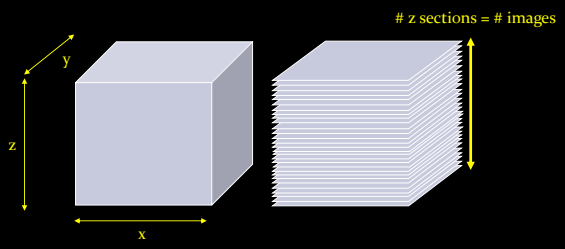


Visualize you sample in 3D



Rui Silva CBMR , UAlg
Claudia Florindo Andor Technologies

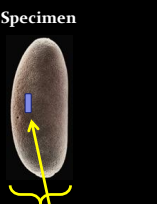
3D Image Reconstruction



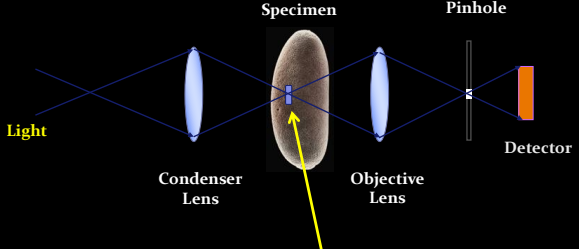
A confocal data set is similar to a book. A book has many pages, and each page shows information only available if you move down to that page and ready it. Reading a page in a book, is just like scanning with a confocal microscope – you remove all of the other pages!

How a confocal image is formed?

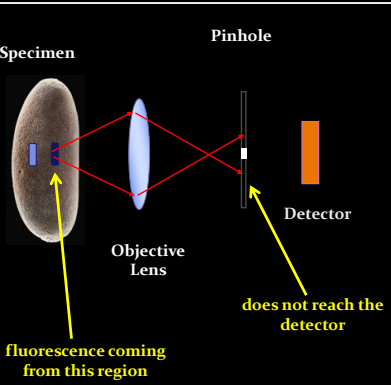
Acquiring a confocal image



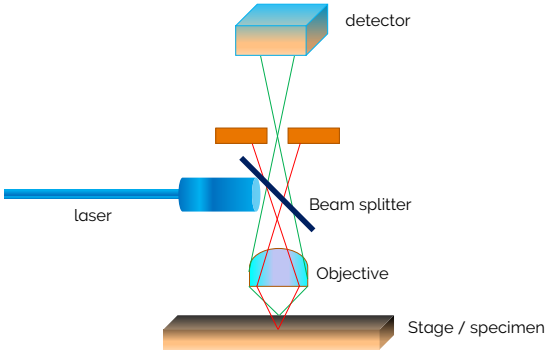
Acquiring a confocal image



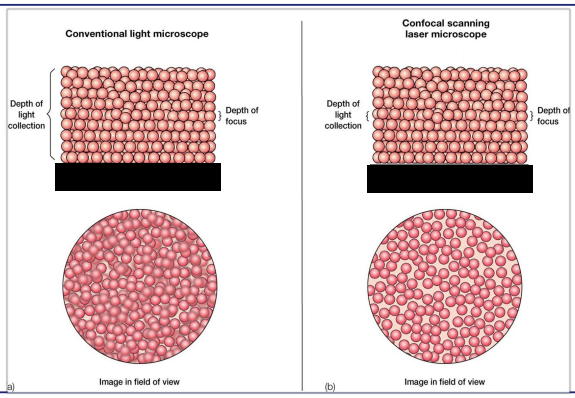
Acquiring a confocal image



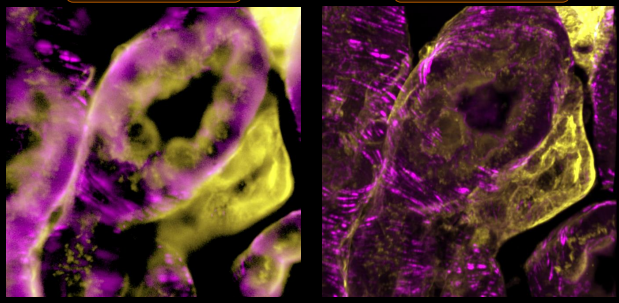
Principles of confocal microscope



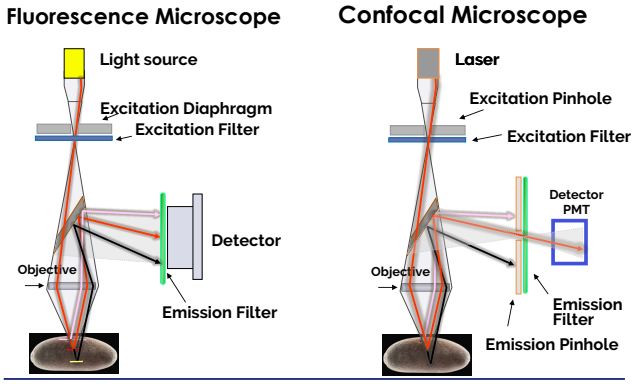
Acquiring a confocal image



Acquiring a confocal image



Epifluorescence vs Confocal



Confocal PMT detectors

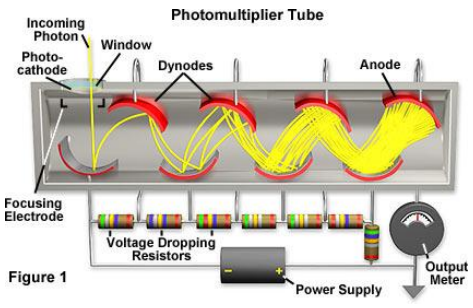
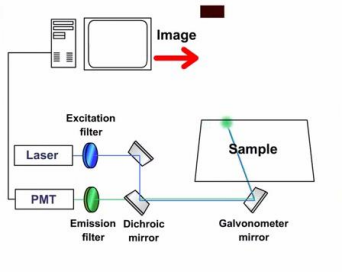


Figure 1

The Confocal Pinhole

Size	Result on the **Intensity** of the Image	Result on the **Resolution** of the Image
Increase opening	Increase Intensity	Decrease resolution
Decrease opening	Decrease intensity	Increase resolution

Confocal Point Scanner acquisition



Scan speed is a limiting factor

Confocal Point Scanner Summary

The laser beam excites a point on the specimen.
It also inadvertently excites other points on the specimen.

↓

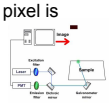
Only the in-focus emission light is allowed to be detected by the PMT.

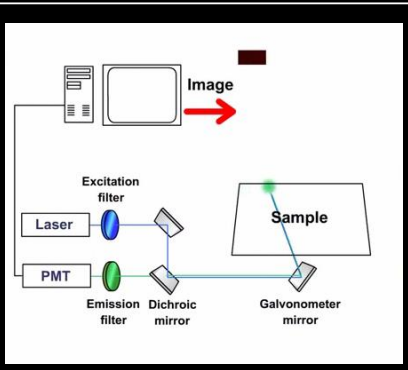
↓

The light detected by the PMT is associated to a pixel (picture element) on the monitor.

↓

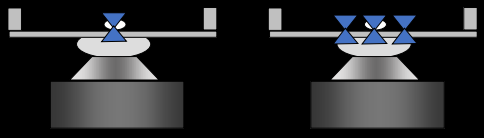
The laser beam then moves to the next point and another pixel is collected.



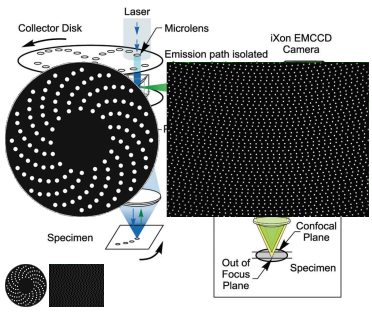


Spinning disk confocal microscopes

Point scanners and Spinning disks



Spinning disks



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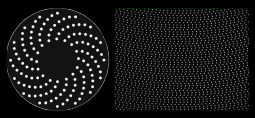
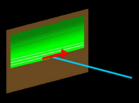
Spinning disks confocal



Single Point Scanning Confocal



sCMOS & EMCCD Camera Technology

Up to 90% QE

Photo-multiplier tube

Up to 45% QE

Challenges in spinning disk confocals

OXFORD INSTRUMENTS ANDOR

- Practical limit of SNR is often set by the non-specific background of the specimen.
- This is the biggest challenge for multi-beam owing to the lack of a single discrete pinhole.
- Traditionally multi-beam starts to suffer with samples over 30um thickness

Importance of Pinhole size and spacing

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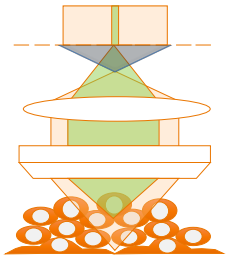
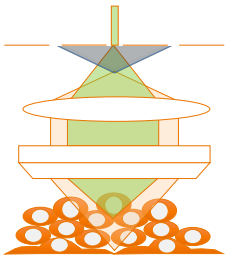
Traditional spinning disk

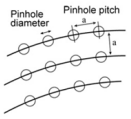


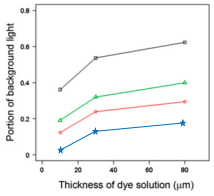
New generation
e.g. Dragonfly



Importance of Pinhole size and spacing

OXFORD INSTRUMENTS ANDOR





50 µm pinholes / 253 µm pitch

100 µm pinholes / 580 µm pitch

55 µm pinholes / 580 µm pitch

40 µm pinholes / custom pitch (Dragonfly)

Image adapted from "Improving Spinning disk confocal microscopy by preventing pinhole cross-talk for intravital imaging" PNAS Feb. 2013; vol. 110

Examples spinning disk confocal images

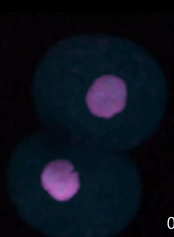
OXFORD INSTRUMENTS ANDOR

Mouse heart Lymphatic vessels

Mouse heart lymphatic vessels, showing Lyve-1 in cyan and SMA in yellow.
The image is a stitch of 9 tiles and was imaged over a 720 µm Z range.
Sample courtesy of Claire Bouvard,Laboratoire BioSanté U. Grenoble, France

Examples spinning disk confocal images

OXFORD INSTRUMENTS ANDOR



Mouse early embryonic development

Movie 1- Mouse Fertilized eggs, imaged with dragonfly & Camera: Sona4BV-11.
Sample courtesy of Dr. Eiichi Okamura, Shiga University of Medical Science

Movie2 - Mus musculus neuronal staining. Sample from the " BRAIN Initiative® (nih.gov)". Image courtesy of Dr Hong Wei Dong, Department of Neurobiology, UCLA

Which Microscope should you choose?

Selecting the best match to your sample and experiment
Fixed sample

	Fixed image	Large multi tile image
Thin <30 um	Widefield	Spinning disk
Thick > 30 um	Spinning disk Point scanner	Spinning disk Point scanner

Spectral unmixing – **Point scanner**

Low light imaging – **Spinning disk**

Selecting the best match to your sample and experiment
Live sample

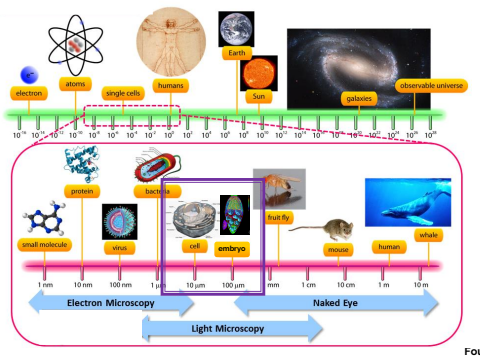
	Low temporal Resolution (Up to 1 fps – full FOV)*	High temporal resolution (>100 fps - full FOV)*
Thin <30 um	widefield Spinning disk	Spinning disk Widefield
Thick > 30 um	Spinning disk Point scanner	Spinning disk

Spectral unmixing – **Point scanner**
Low light imaging – **Spinning disk**

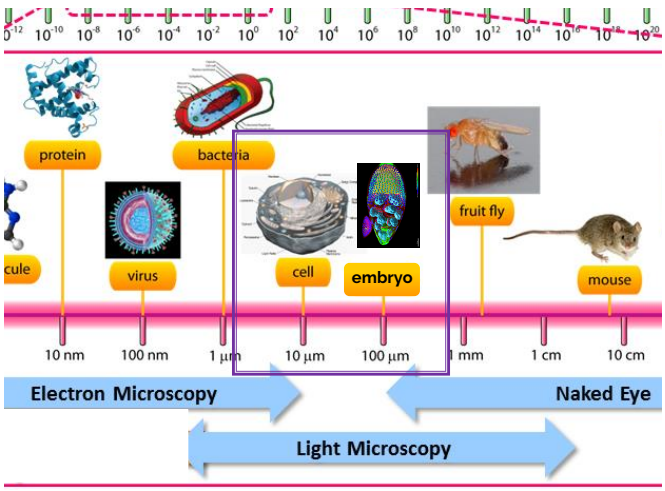
* Higher frame rates are possible if the FOV is reduced

Overview of Dragonfly system and applications

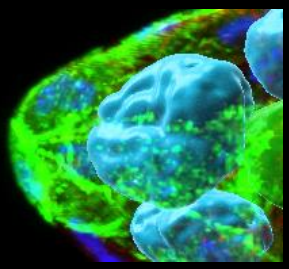
The scales of life
What can you see with the light microscope?



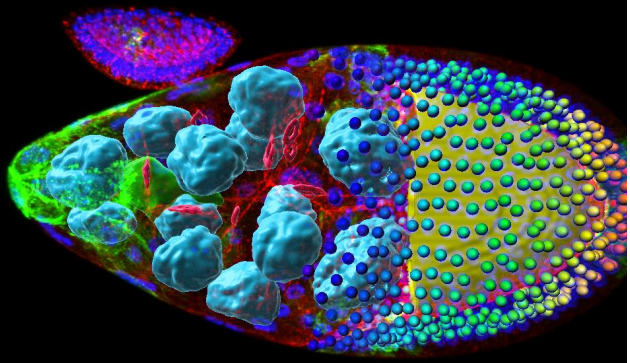
Foundation of Biology
https://socratic.org/biology/introduction-to-biology/foundation-of-biology



The Cells



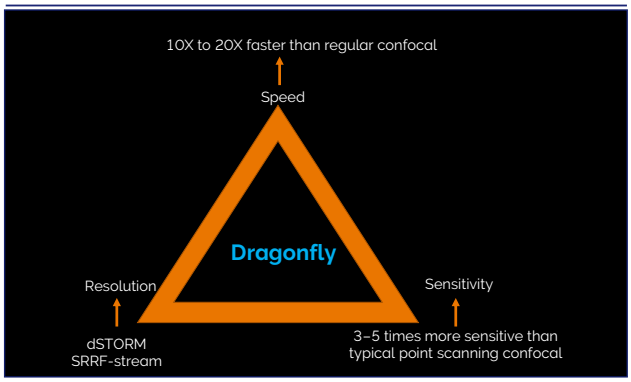
The context



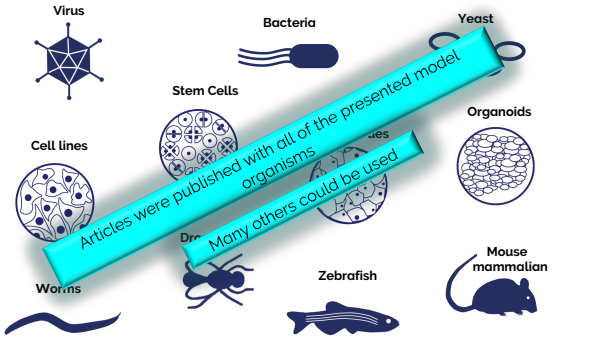
How?

Overview of Microscopes and illumination strategies

Dragonfly vs Point scanners



MODEL ORGANIMS that can be used in Dragonfly



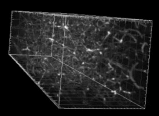
Imaging Applications I

Dragonfly for Deep imaging applications

- Image deep in to tissues (mm depth)
- Image deep in to tissues and simultaneously acquire live data
- Acquire deep imaging data with simultaneously double colours labelling
- Combining SRRF with confocal mode allows Super Resolution far beyond the edge of the coverslip.

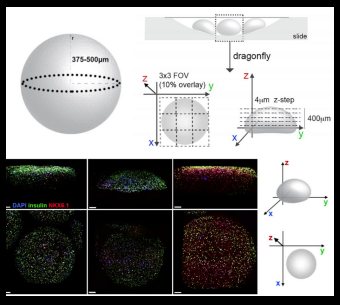
Imaging deep
Confocal spinning disk mode

2.4 mm deep | 3DISCO cleared | Perfused with red beads
8000 optical sections | 20x objective



Movie courtesy of Alan Watson, Uni Pittsburgh

Visualising stem cell differentiation
Mode: Confocal Spinning Disk
Deep Imaging



High magnification, and in-depth imaging (400 µm) revealed the the positive effects of confinement on stem cells differentiation

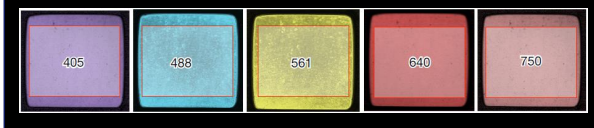
Whole mount beads were imaged using the Andor Dragonfly 505 with an 20x dry objective . Each bead was imaged with 3 × 3 fields of view, which covered the entire bead

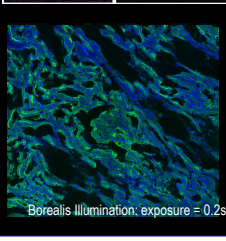
Legøy T.A. et al., Scientific Reports *(2020) 10:414 (figure 51)*
Corresponding author Simona Chera

Imaging Applications II
MONTAGE &
Montage with deep imaging

Borealis Perfect Illumination Delivery

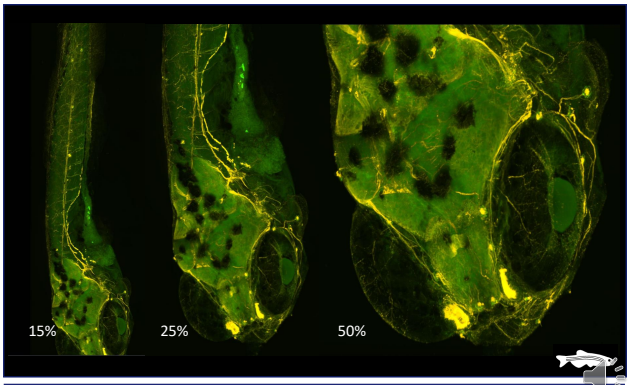
Broad Spectrum Uniformity



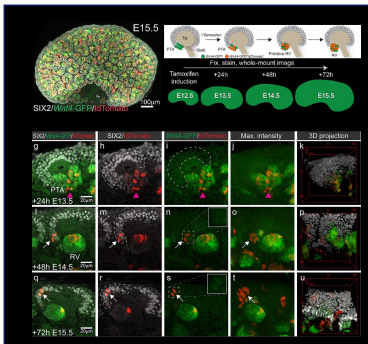


3x signal
Higher contrast

Montage acquisition & integrated stitching



Cell Migration & cell commitment
Mode: Confocal Spinning Disk
Deep Imaging
Tile imaging



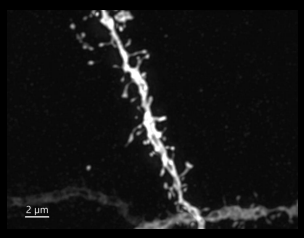
Visualising cells and Tissues (fixed and live)

Understanding the regulation of nephrogenesis and its relevance for grow during organogenesis.

Lawlor K.T., et al., **eLIFE *(2019)*** *10:414 (figure 1)*
Corresponding author Alexander N Combes

Visualising Neurons
Mode: confocal spinning disk

Visualise neurons with extraordinary detail and, in their own environment



Mouse Brain tissue (237stack x 24 tiles) = 5 688 Images in 25 min

Sample courtesy of Dr. Dan Ohtan Wangat Kyoto University & Dr. Hidenorilto at Aichi Developmental Disability Center, Japan

Developmental Biology (genetic disorders)
Mode: Confocal Spinning Disk
Deep Imaging
Tile imaging

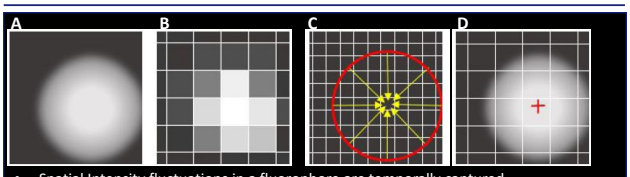
Embryo Head (HH28)
captured at 10x, tiled (7x5) and Stitched , Imaged to 1.2mm depth, 220 planes, 3 colours.
2hrs (vs 15 hours on a point scanner)

Imaging Applications III

Live cell compatible Super Resolution
&
Super Resolved deep imaging

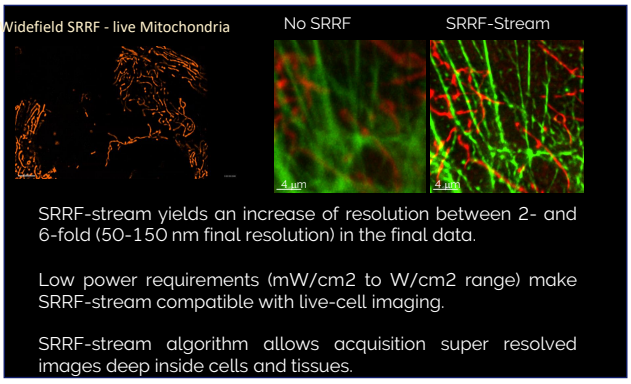
Super-Resolution Radial Fluctuations (SRRF, reads as *surf*)

What is SRRF?
Super Resolution Radial Fluctuations



- Spatial Intensity fluctuations in a fluorophore are temporally captured
  - Typically, short exposures for fast sampling
- Intensity gradient from a single fluorophore is spread over a number of pixels (B).
  - The radiality symmetry is indicative of the locale of fluorescence emitters
- A super-sampled grid (C) is used to provide a weighted super-resolution estimate of the point of origin (D)
- In SRRF the number of images per sequence can be varied to trade spatial and temporal resolution

SRRF-Stream



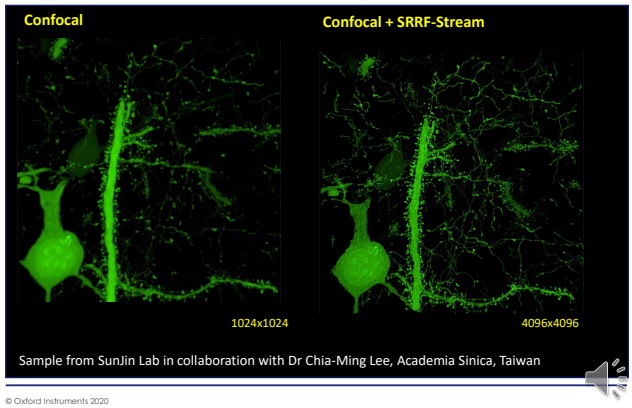
SRRF-stream yields an increase of resolution between 2- and 6-fold (50-150 nm final resolution) in the final data.

Low power requirements (mW/cm2 to W/cm2 range) make SRRF-stream compatible with live-cell imaging.

SRRF-stream algorithm allows acquisition super resolved images deep inside cells and tissues.

Visualising Neurons away from the coverslip

Mode: Confocal Spinning Disk + SRRF-stream
Deep Super Resolution Imaging



Sample from SunJin Lab in collaboration with Dr Chia-Ming Lee, Academia Sinica, Taiwan

Imaging Applications IV

LIVE Imaging

&

LIVE Imaging deep inside the tissues

Challenges in Live cell Imaging

Live imaging deep in the tissues

- Photobleaching
- Phototoxicity
- Speed (high temporal resolution)
- High spatial resolution
  (breaking the diffraction limit- 200 nm)

Visualising Blood flow (ultra fast dynamic events)

Mode: confocal spinning disk

Live inside the intestine:
Deep imaging of the blood flow

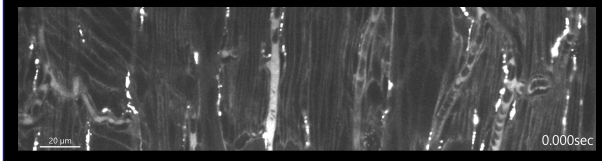
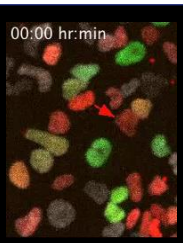


Image at 200 fps with Andor sCMOS

Sample courtesy of Dr. Takahiro Kuchimaru.
Jichi Medical University, Japan

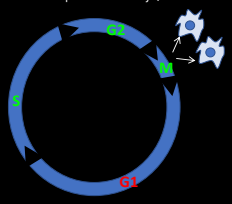
Visualising cell cycle dynamics

Long time lapse –
No photobleaching or phototoxicity



Live imaging with Andor Dragonfly spinning disk confocal microscope and Andor FUSION software.

Images were captured every 7 minutes for 24–48hrs.



Fairchild C.L.A. et al., Scientific Reports *(2019) 9:15336*
Corresponding author: Anna La Torre

RESULT:
Very long time lapse imaging without photobleaching or phototoxicity.

Imaging Applications V

In Situ Multiplex Imaging

Expansion Microscopy & Spatial Transcriptomics

Science

Expansion sequencing: Spatially precise in situ transcriptomics in intact biological systems

Shahar Alon[1,2,3,*], Daniel R. Goodwin[1,2,*], Anubhav Sinha[1,2,4,*], Asmamaw T. Wassie[1,2,5,*], Fei Chen[1,6,*], Evan R. Daugharthy[7,8,†,‡], Yosuke Bando[1,9], Atsushi Kajita[10], Andrew G. Xue[1], Karl Marrett[10], Robert Prior[10], Yi Cui[1,2], Andrew C. Payne[1,2], Chun-Chen Yao[1,2], Ho-Jun Suk[1,2,4], Ru Wang[1,2], Chih-Chieh (Jay) Yu[1,2,5], Paul Tillberg[1,6], Paul Reginato[1,5,6,7,8], Nikita Pak[1,2,11], Songlei Liu[7,8], Sukanya Punthambaker[7,8], Eswar P. R. Iyer[6], Richie E. Kohman[7,8], Jeremy A. Miller[12], Ed S. Lein[12], Ana Lako[13], Nicole Cullen[13], Scott Rodig[13], Karla Helvie[14], Daniel L. Abravanel[15,16], Nikhil Wagle[14], Bruce E. Johnson[14], Johanna Klughammer[6], Michal Slyper[6], Julia Waldman[6], Judit Jané-Valbuena[6], Orit Rozenblatt-Rosen[6], Aviv Regev[6,17,18], IMAXT Consortium[19,¶], George M. Church[7,8,#,**], Adam H. Marblestone[1,**,††], Edward S. Boyden[1,2,5,17,18,20,#,**]

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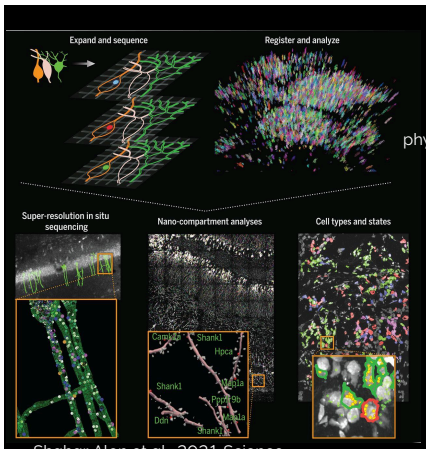
DOI: 10.1126/science.aax2656



In situ sequencing of physically expanded specimens enables multiplexed mapping of RNAs at nanoscale, subcellular resolution throughout intact tissues

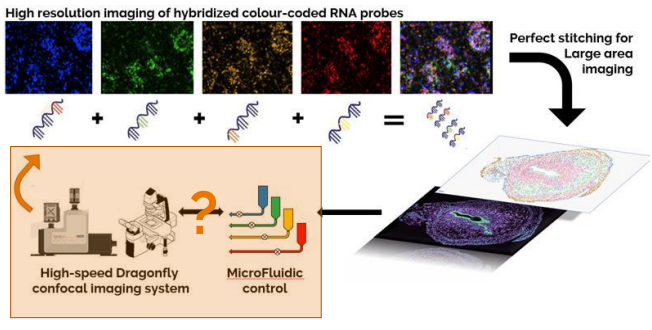
Shahar Alon et al., 2021 Science
Expansion sequencing: Spatially precise in situ transcriptomics in intact biological systems

How?

Overview of Fusion interaction with external devices

In Situ Multiplex imaging with Dragonfly

7 Analysis pipeline is now used to register, labelled RNA nucleotides, evaluate short sequences and then match to longer sequence reads for gene expresssion profiling.

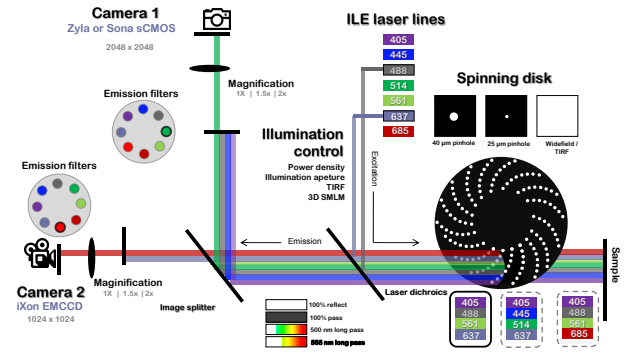


Through REST-API

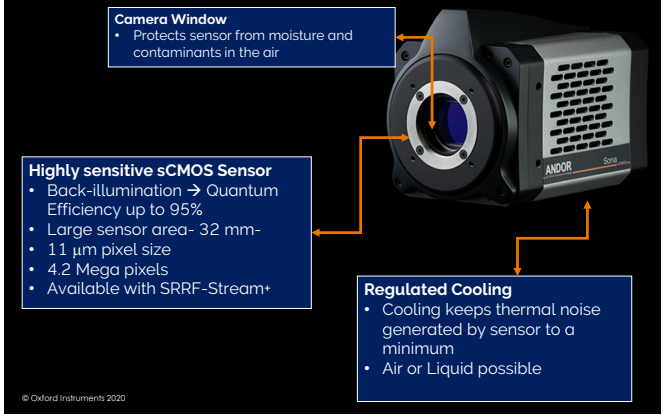
Dragonfly

Overview & Imaging modalities

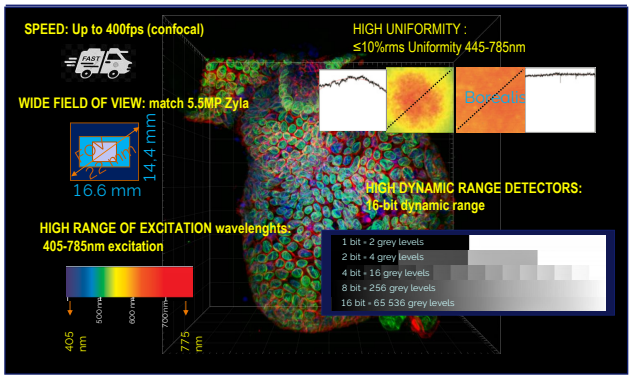
Dragonfly
Multimode spinning disk Overview



Quick look into Sona 4.2B-11 Camera

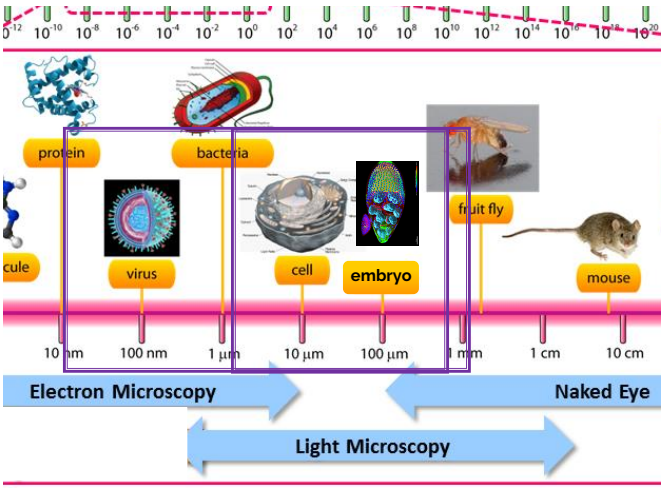


Key Specifications of the Dragonfly



Can we cross more scales with the dragonfly?

DRAGONFY 600

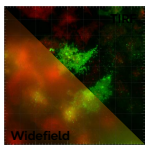


Three innovations in the Dragonfly 600

High-Powered Laser Engine (HLE)

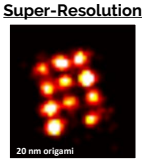
more power
shorter exposure times
deeper imaging

Borealis-TIRF



better, artefact-free TIRF imaging
easy to set-up & reproducible
wider array of TIRF compatible probes

Streamlined Super-Resolution



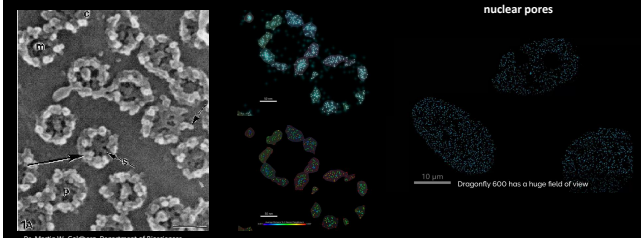
fast and accurate
workflow for SMLM
(dSTORM, DNA-paint & more)

Dragonfly images Nuclear Pore Complex

EM – of the nuclear pores

Dragonfly – SMLM of the Nuclear Pores

From the nucleus to the nuclear pores



Dr. Martin W. Goldberg, Department of Biosciences, Durham University, UK
Image from Journal of Cell Science 106, 261-274 (1993)

NUP96 structures in nuclear pore complex imaged by DNA-PAINT in BTIRF mode, reconstructed with Picasso and visualized in Imaris. Image courtesy of Dr Florian Schueder, Yale University.

Dragonfly's multi-point scanning is based on microlens spinning disk (MSD) technology

From acquisition to analysis

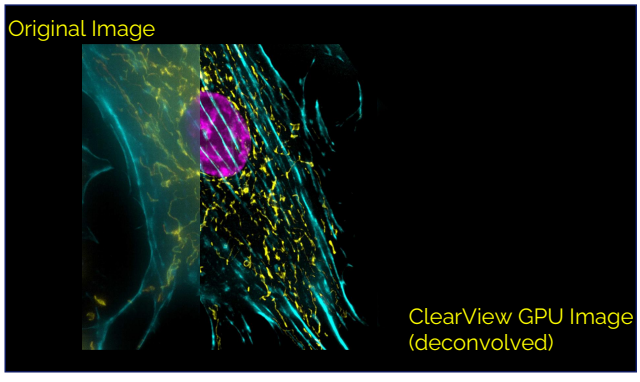
Complete Workflow with Dragonfly and Imaris

Clear View GPU Deconvolution

"the application of deconvolution methods can always increase image quality, regardless of the source of the image...Deconvolve everything!"

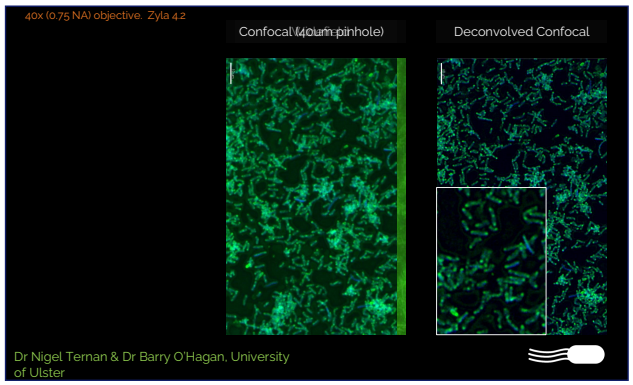
Mark B. Cannell, Angus McMorland, and Christian Soeller, Handbook of Biological Confocal Microscopy, Chapter 25

Enhancing your image for analysis (see the unseen)



Deconvolution Examples: Bacteria on Biofilm

OXFORD INSTRUMENTS ANDOR



Deep Imaging and 3D analysis Dragonfly & Imaris

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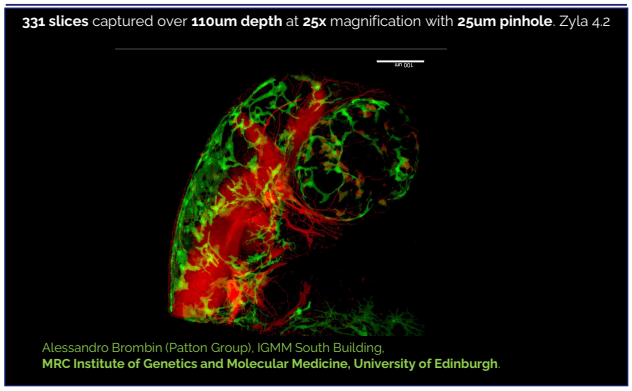

Deep Imaging and 3D analysis Dragonfly & Imaris

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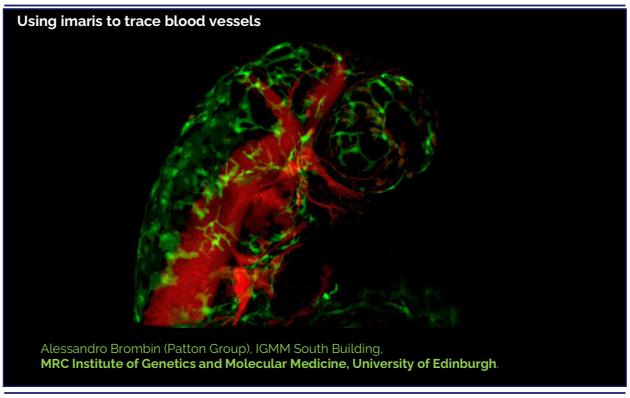

Dragonfly Publications High Impact science

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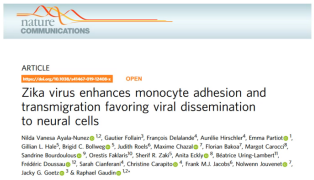

The Cells

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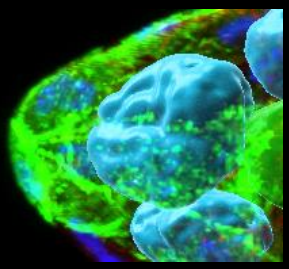

The context

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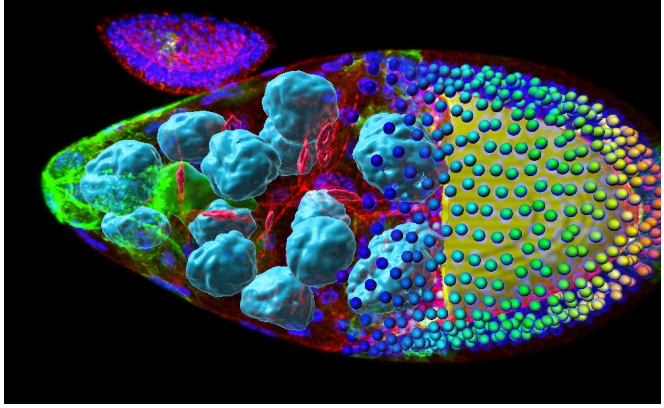

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Thank you for your attention!

Any Questions?
Please contact me: Claudia Florindo – c.florindo@andor.com

Inclusive • Trusted • Innovative & Progressive • Wholehearted