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Dragonfly High Speed Confocal:

Why Dragonfly is an ideal imaging system for you

美嘉儀器客服技術部 劉思嫺
www.major.com.tw

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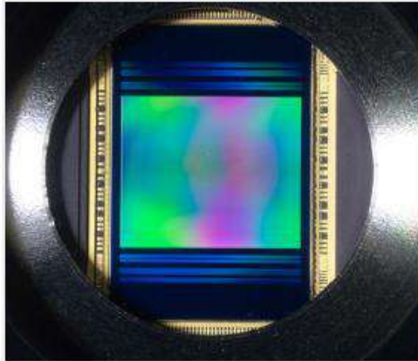
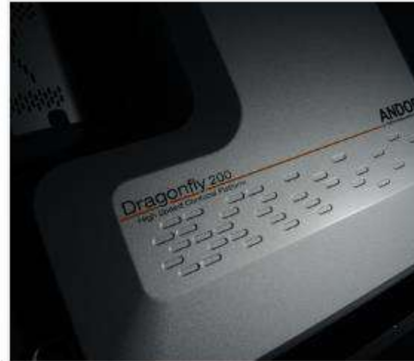
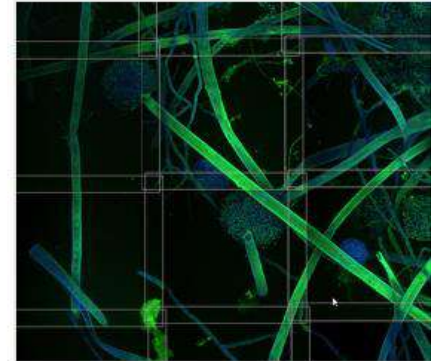


Scientific Cameras

Spectroscopy



Microscopy Systems



Software

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What is an ideal (confocal) imaging system?

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Fast imaging speed

- Dynamic events/large sample
- Save time

High image quality

- Keep original signals
- More details

Low photo-bleaching

- Less photo-toxicity
- More images (time/Z)

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Dragonfly High Speed Confocal

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Faster

20x faster than point-scanners

Deeper

3x SNR improvement in thick samples vs other multi-point scanners

Longer

10x less photo-bleaching vs point scanners

Better

Variety of technics for better image quality (SNR/Dynamic range/Uniformity/Resolution)

© Oxford Instruments 2016

Faster

20x faster than point-scanners

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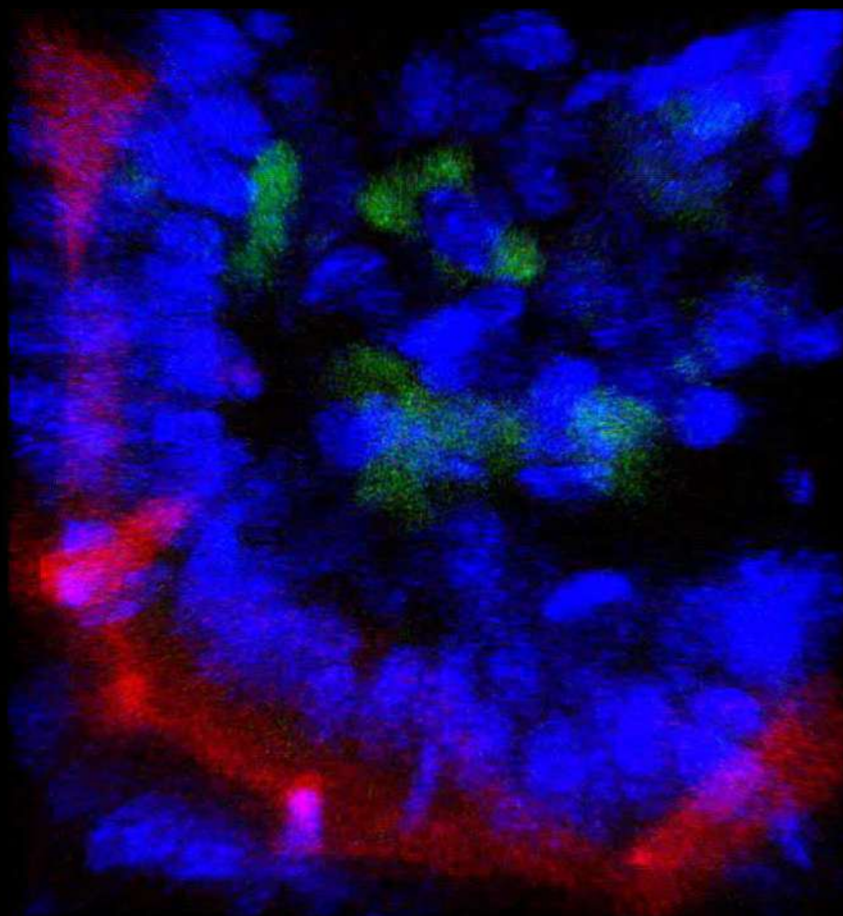
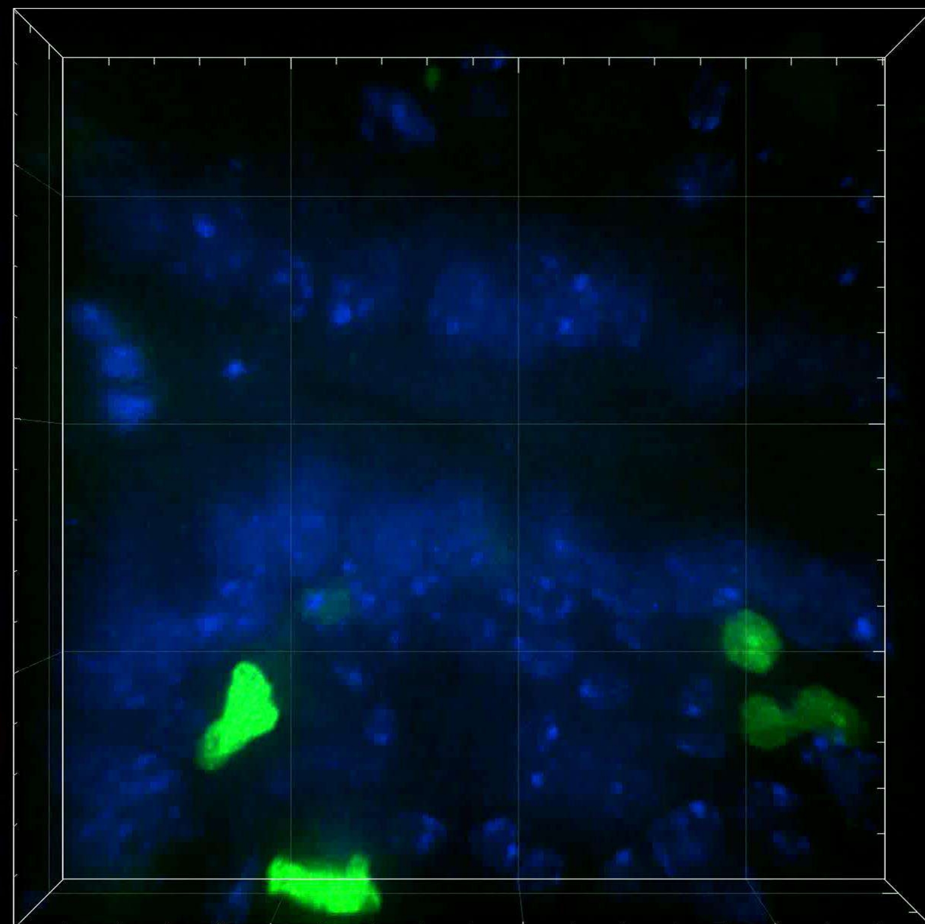
Point scan confocal



Dragonfly



Dynamic migration of Intraepithelial Lymphocytes in Mouse Intestine

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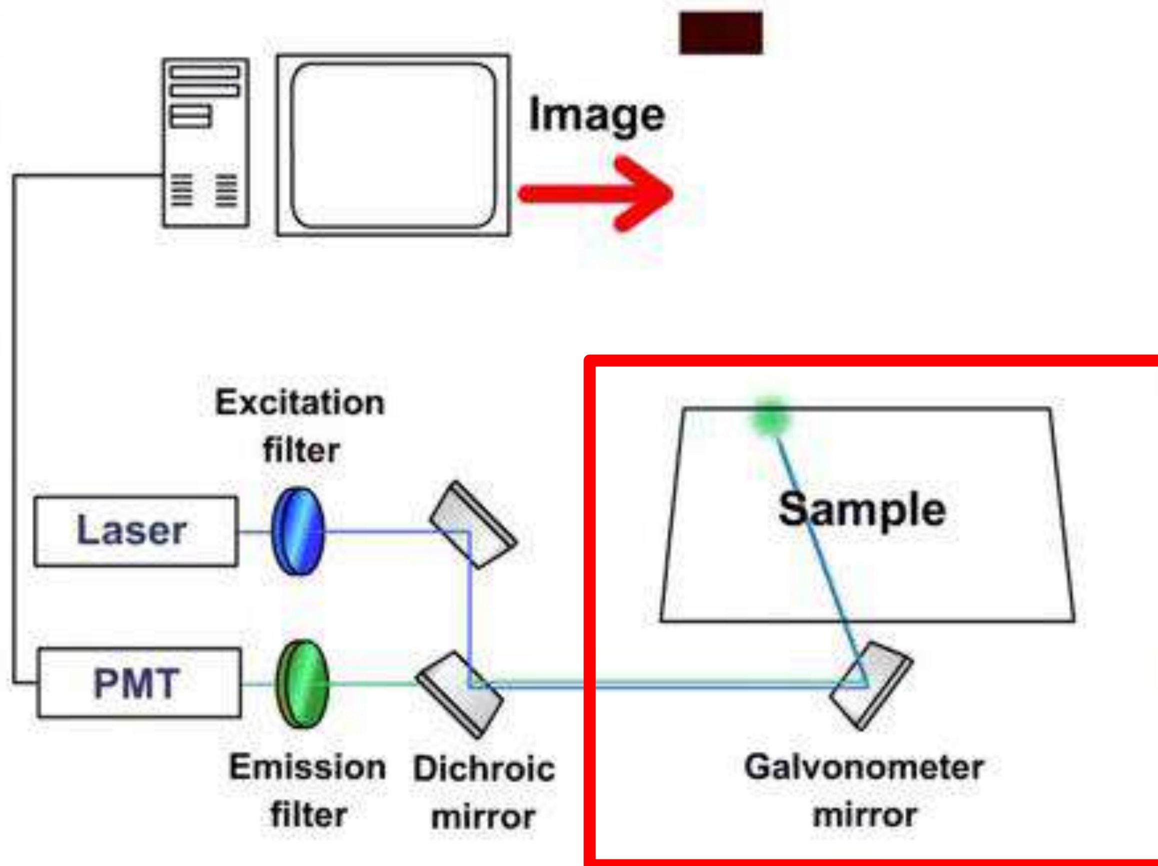
Limitation of Point-scanner



Point by point scanning

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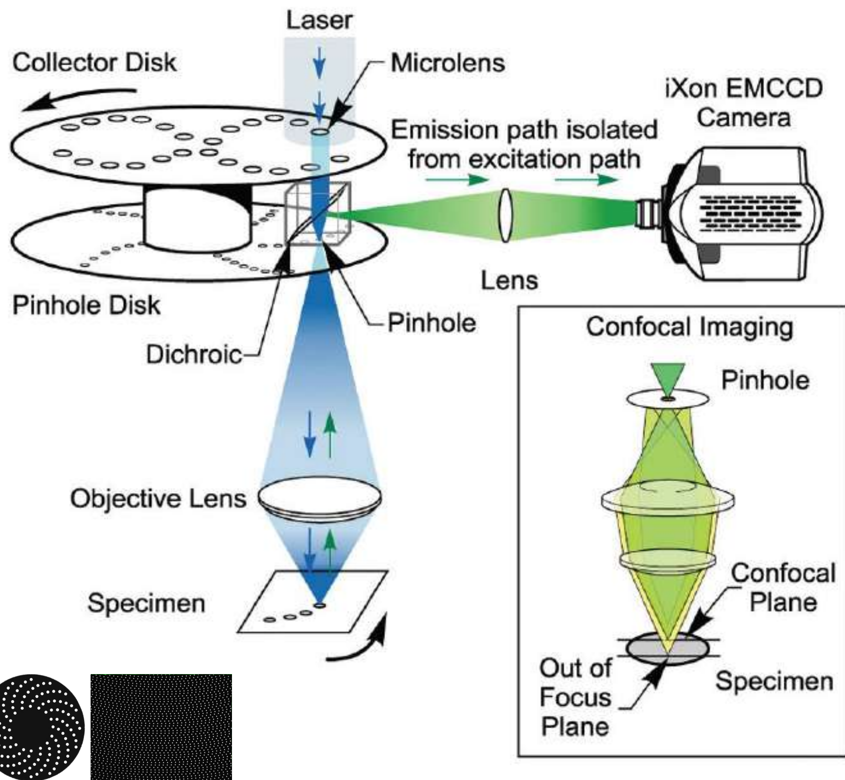
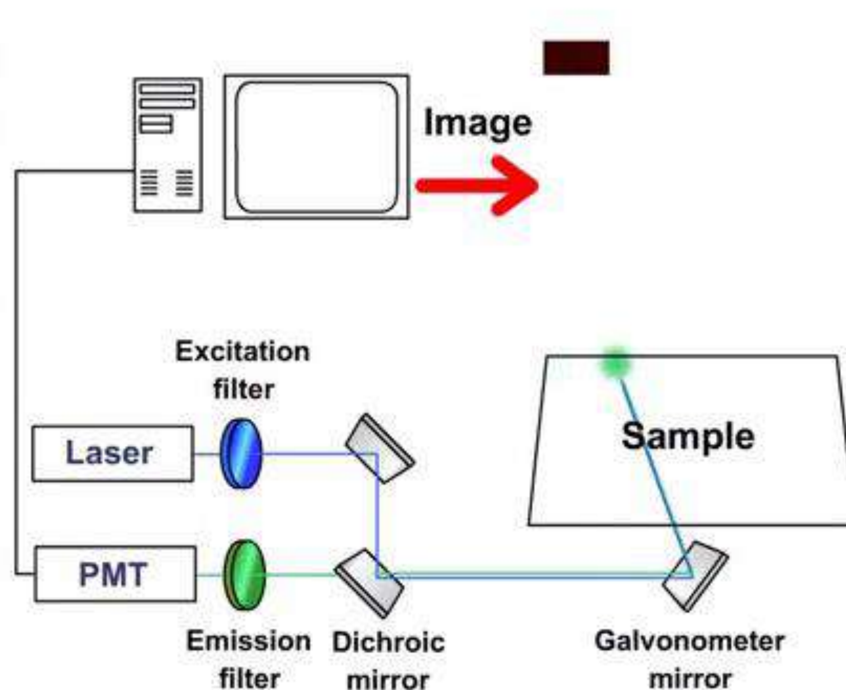
FASTER vs point-scanner

Multi-point scanning instead of single point scanning





Dragonfly

Point scanner

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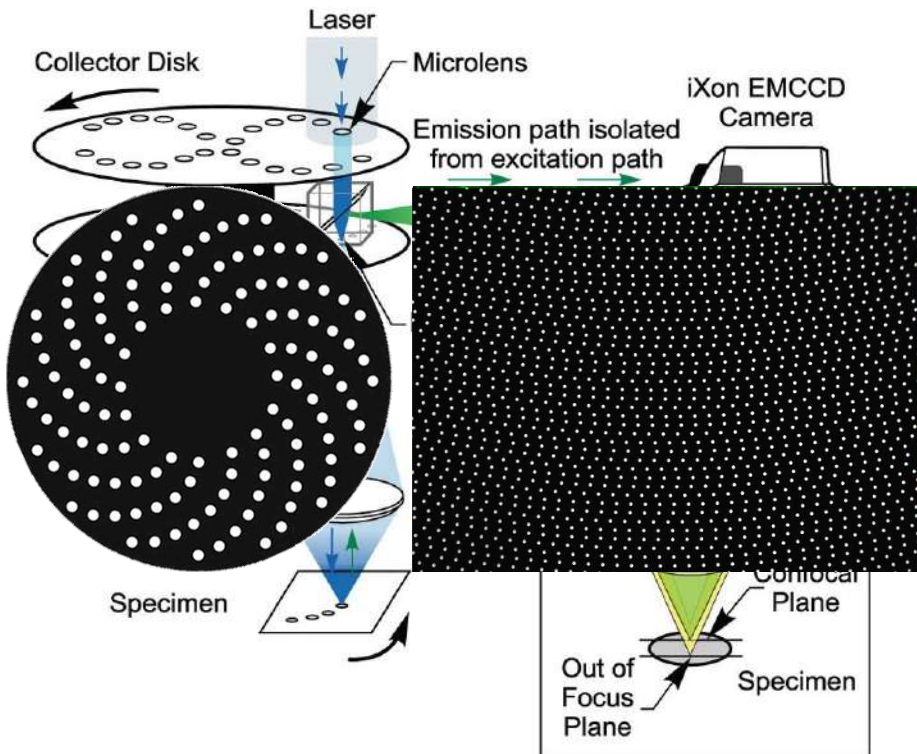
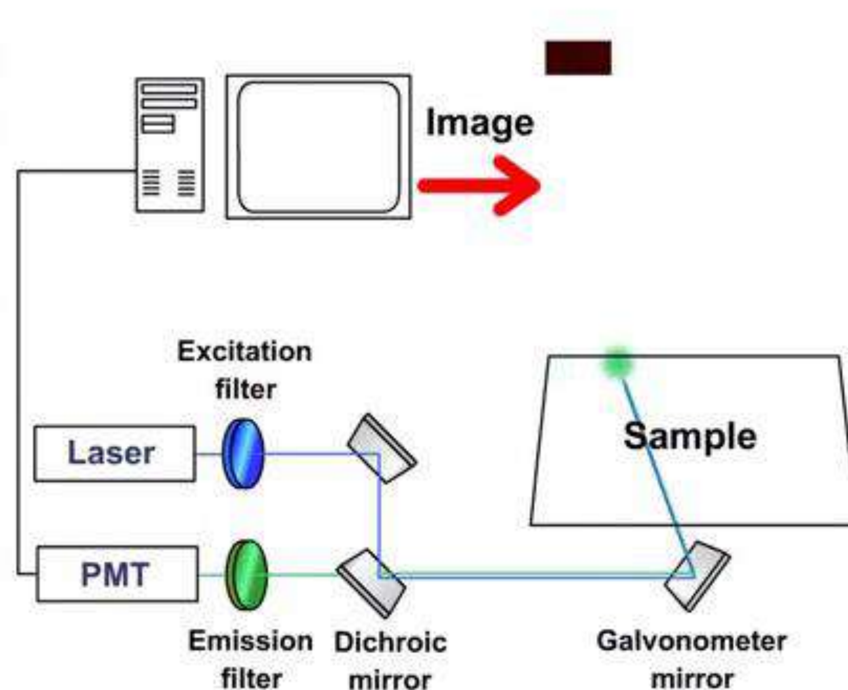
FASTER vs point-scanner

Multi-point scanning instead of single point scanning



Dragonfly



Point scanner

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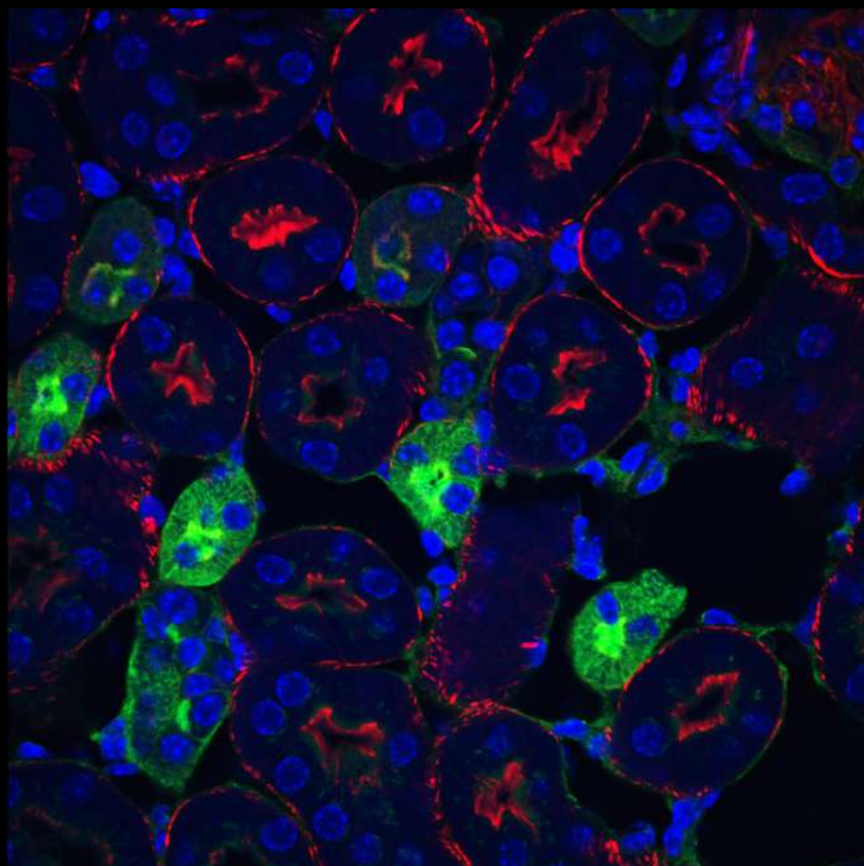
Point scanning Confocal



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Dragonfly is an INSTANT confocal – real-time 3D display

Fusion

Acquisition Control

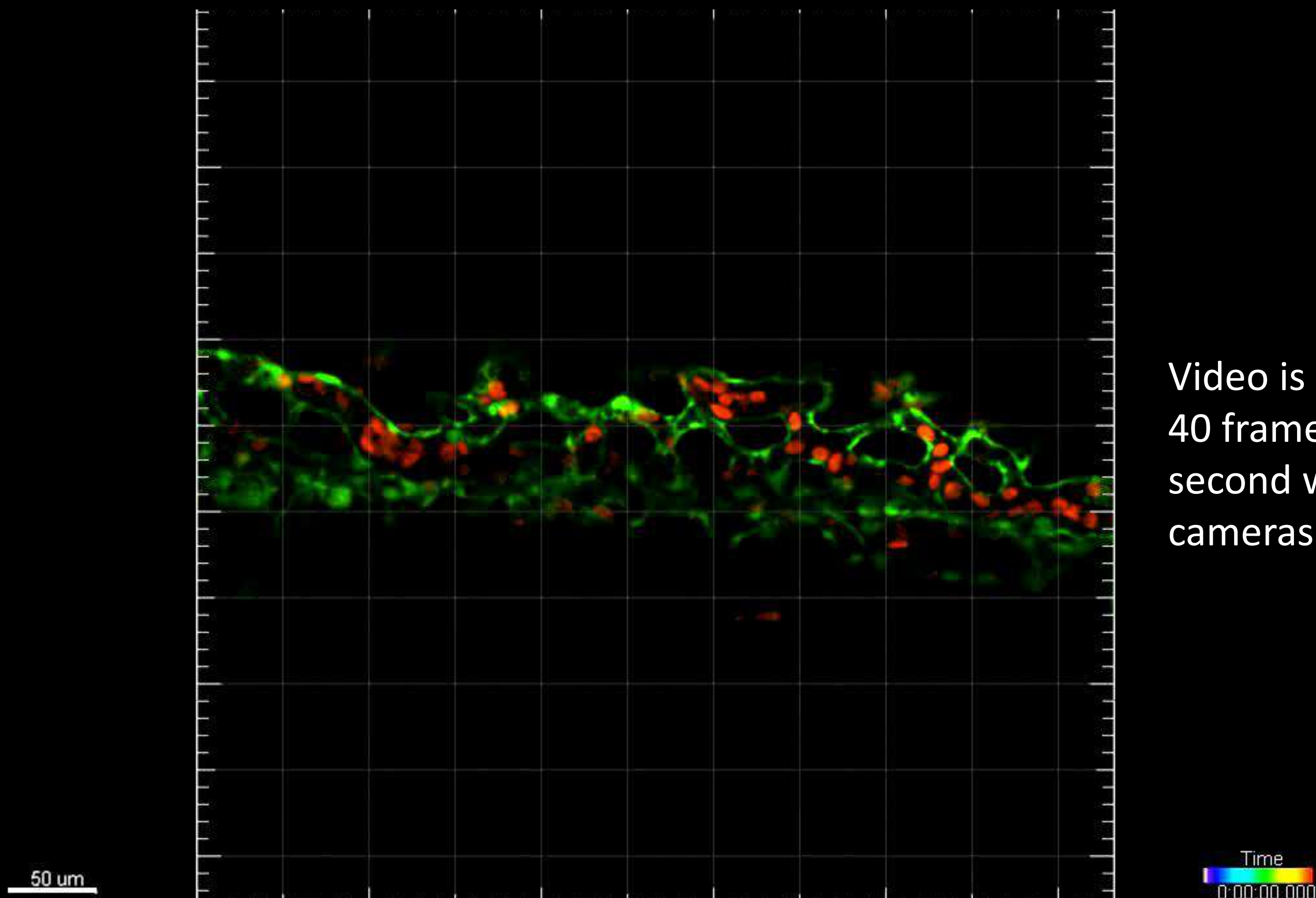
Live

Snap

Acquire

Blood flow in transgenic zebrafish

eGFP – endothelium, DsRed - erythrocytes



Video is real-time 40 frame-pairs per second with two cameras

Image courtesy of Mylène Lancino; Herbomel Lab, Institut Pasteur

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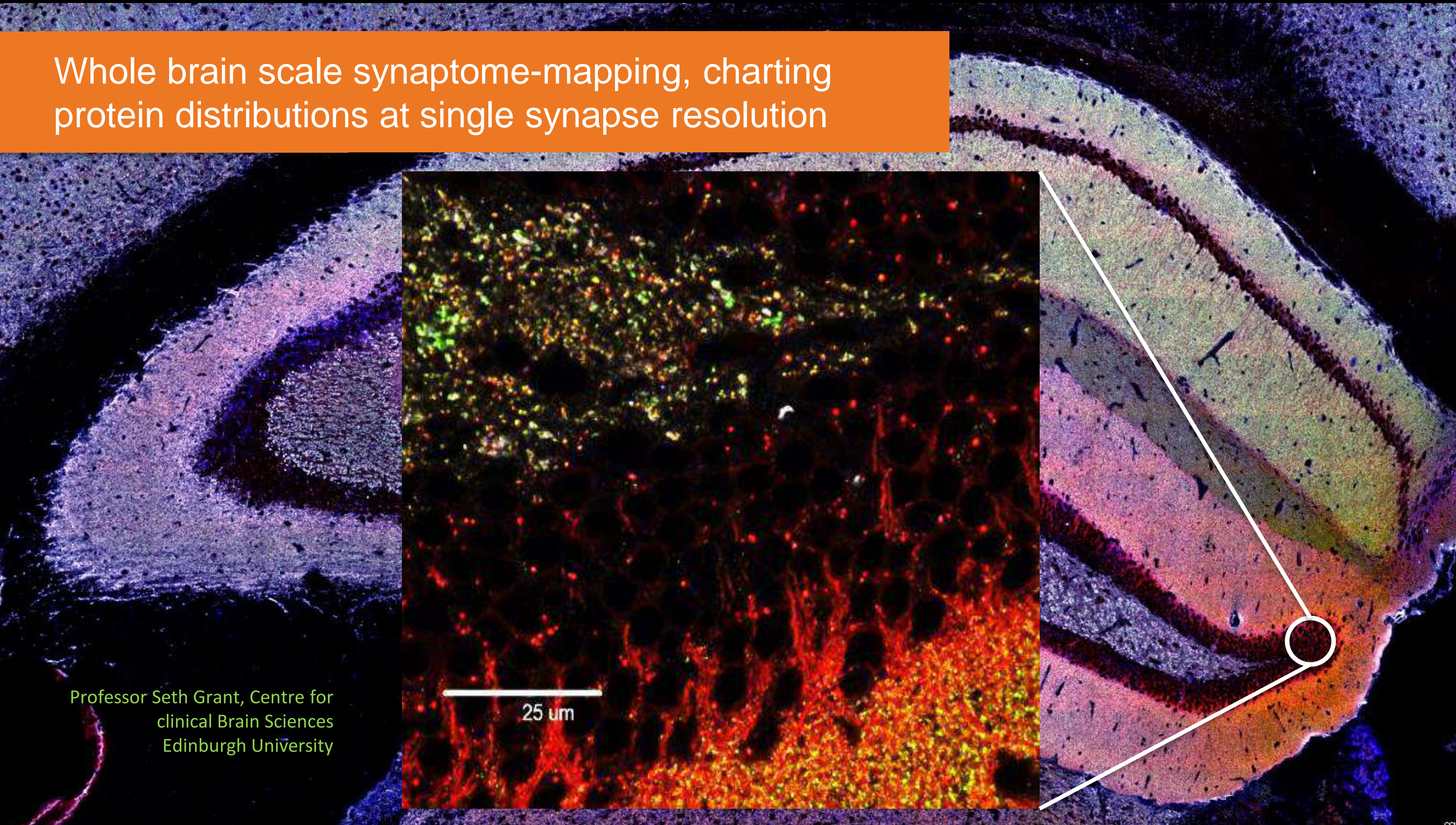
368 tile montage – 4 colours captured in **50 minutes.**
Each individual image of **1024x1024 at 100x magnification.**



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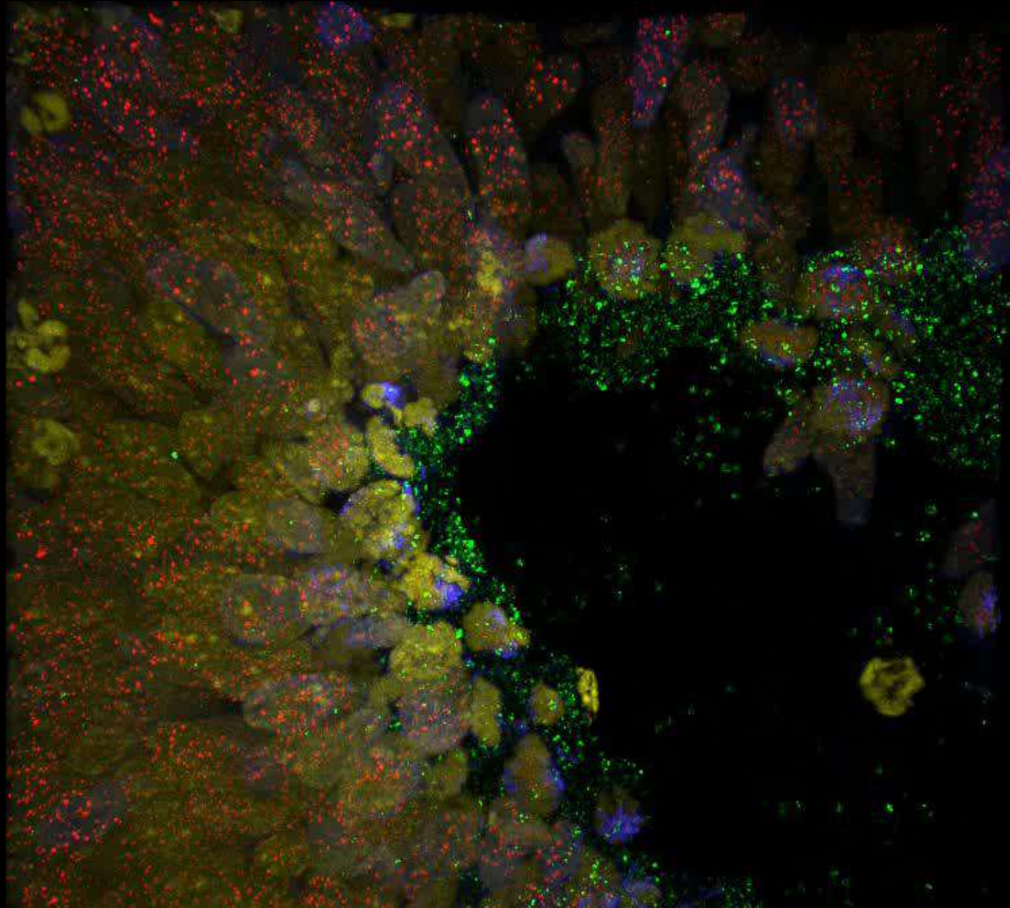
Determining the stage of mitosis by the centromere number.



"Dragonfly enabled me to capture >100 high resolution images for quantification of centromere number (which are 0.2-0.8 microns in size) in at least 1\10th of the time it would have taken on a point scanning confocal. I saved 40 hours of time in preparing a publication"

Dr Carol-Ann Martin, Andrew Jackson Lab, Institute of Genetics and Molecular Medicine, University of Edinburgh

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Skin

405/488/561/640 four colors
35 Z slices per FOV, total 42 FOVs
10 mins vs >2 hrs on point scanner

Image courtesy of John CONNOLLY Lab, IMCB, A*STAR

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Mouse pancreas

X – 5 (6mm)
Y – 11 (13mm)
Z – 50 (500um)
10x obj.

Imaging time

Dragonfly	40mins
Point scanner	13hrs

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Deeper

3x SNR improvement in thick samples vs other multi-point scanners

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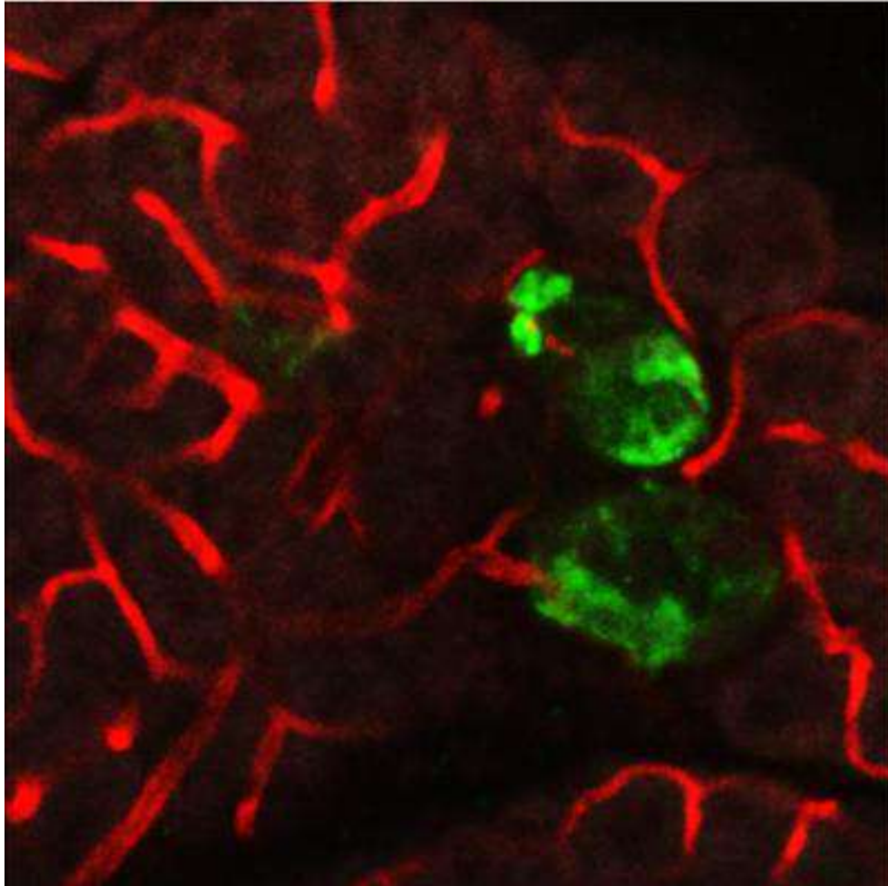
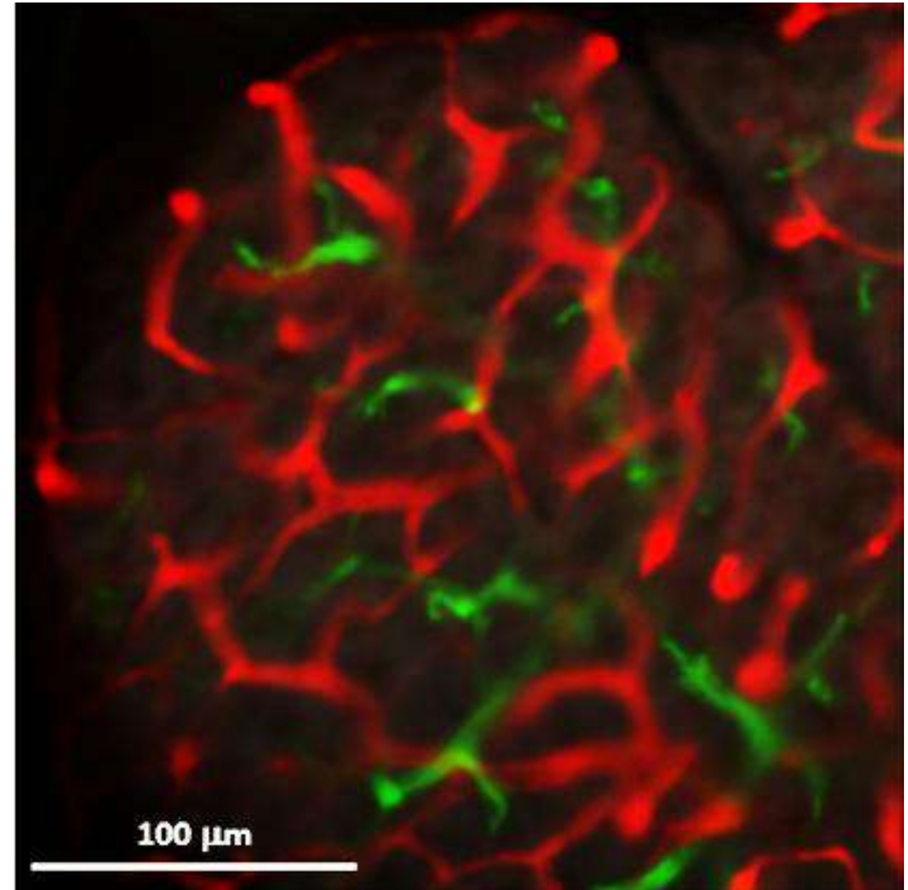
DEEPER vs other multi-point scanner

Optical section from mouse pancreas at 150um depth



Dragonfly



Yokogawa CSU-X

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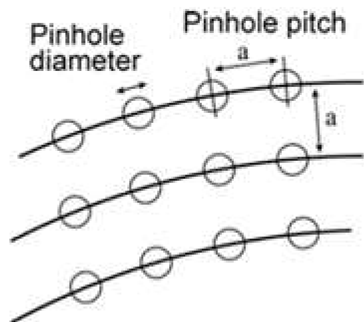
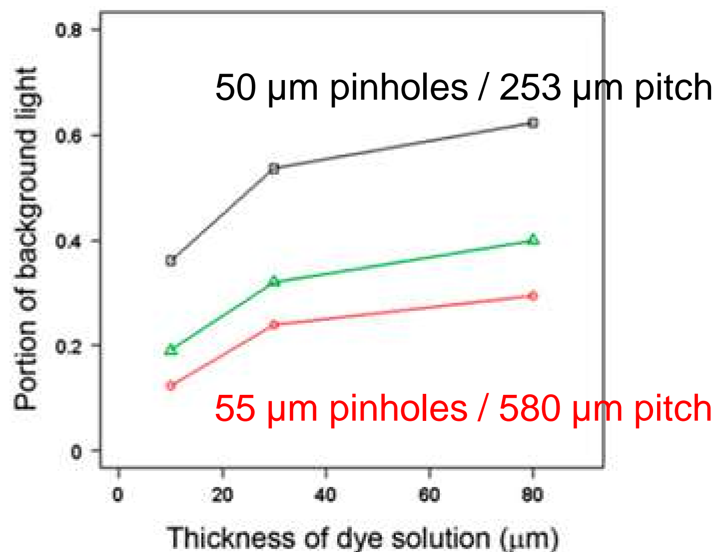
DEEPER vs other multi-point scanner

Traditionally multi-beam starts to suffer with samples over 30um thickness





Traditional multi-point

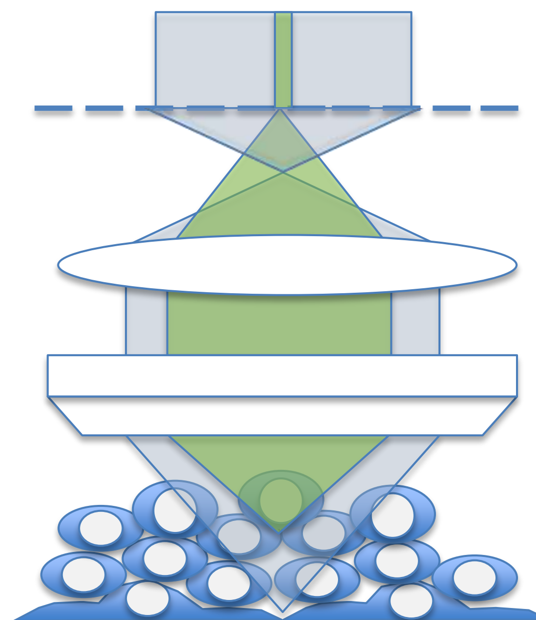


Image adapted from “Improving Spinning disk confocal microscopy by preventing pinhole cross-talk for intravital imaging” PNAS Feb. 2013; vol. 110

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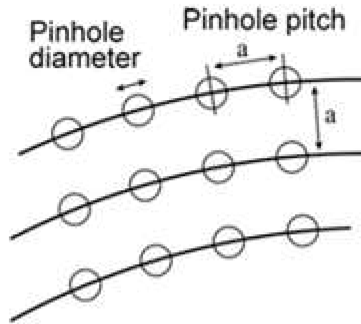
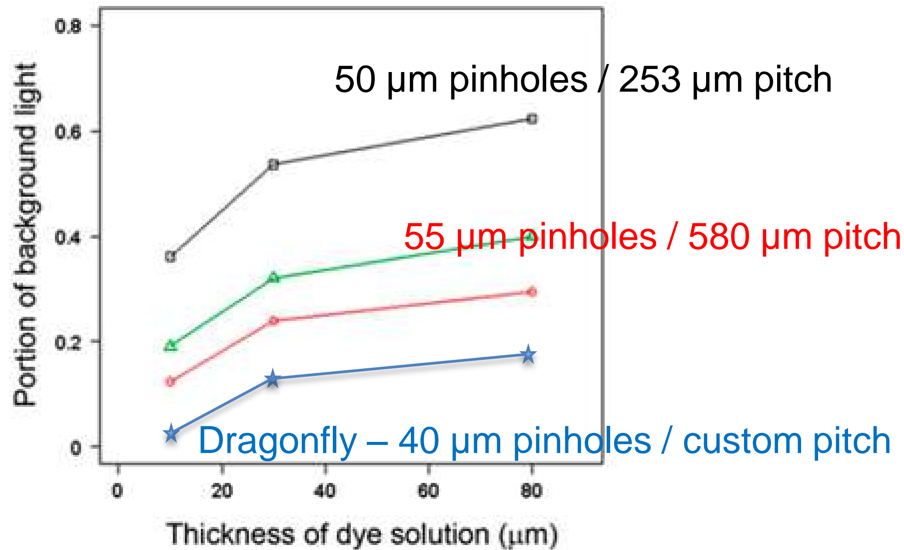
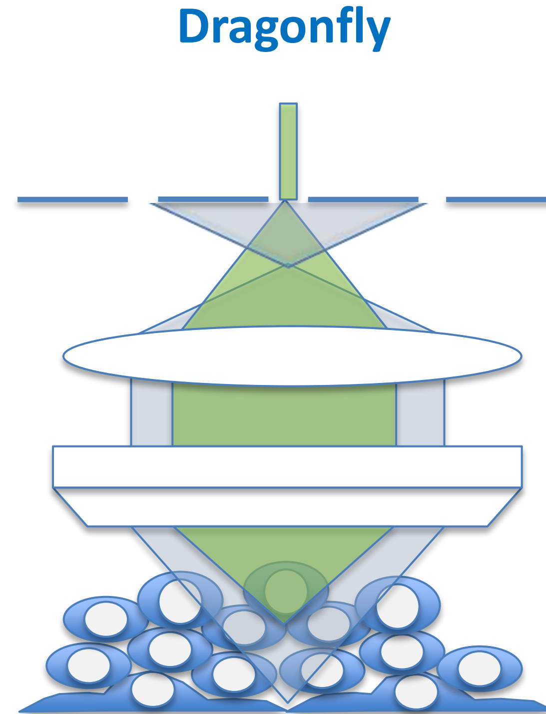
DEEPER vs other multi-point scanner

On Dragonfly the limiting factor in deep specimens is the transparency and refractive effects of the specimen itself







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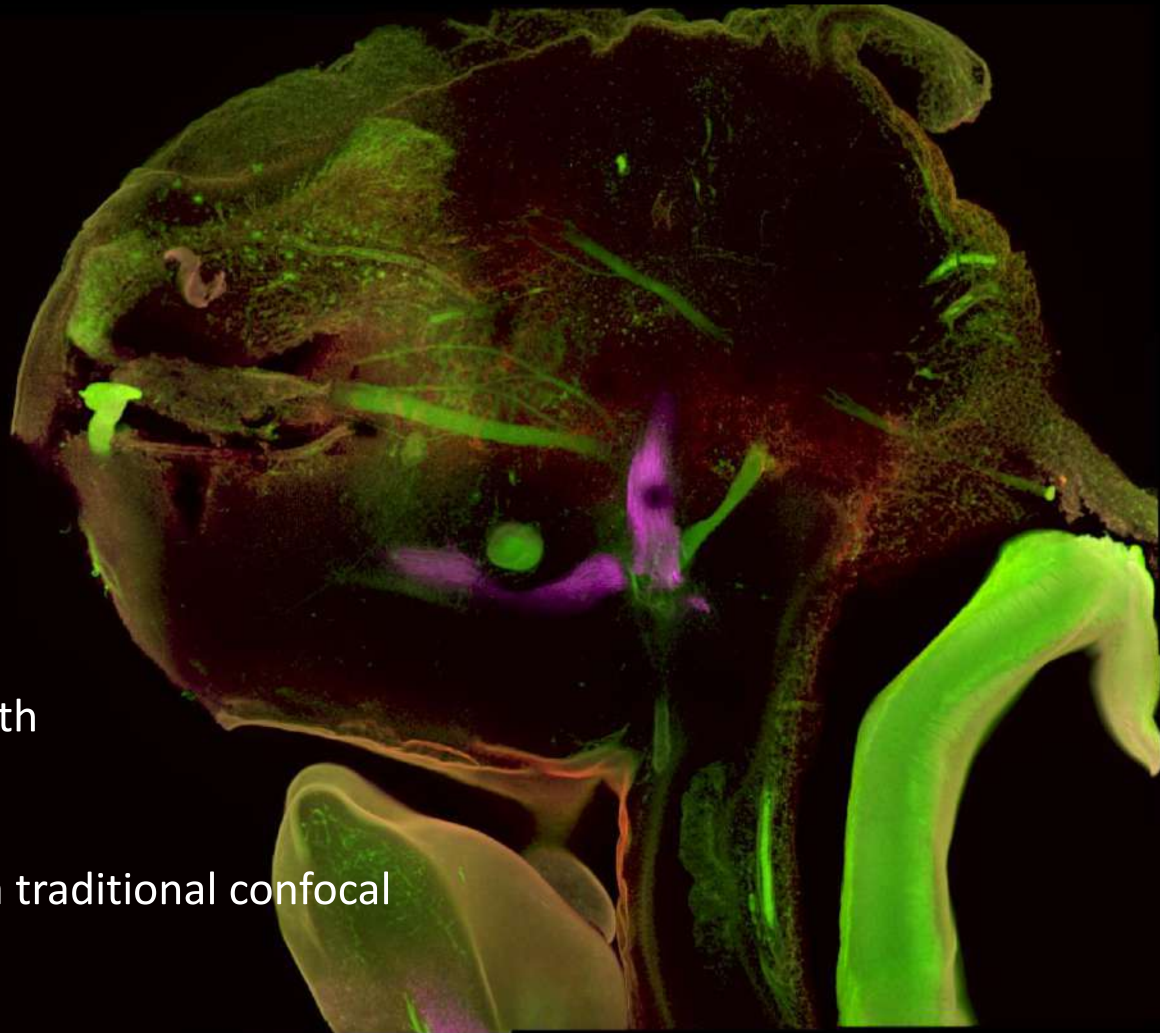
Chick Embryo Head (HH28)



10x
Tiled 7x5
Imaged to 1.2mm depth
220 Z planes
3 colours.
1hrs (vs 15 hours on a traditional confocal

500 um

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Drosophila Brain Neuron

40x water
Tiled 3x2
600 Z planes total 200um
15mins (vs 2hrs on point scanner with less Z slices)

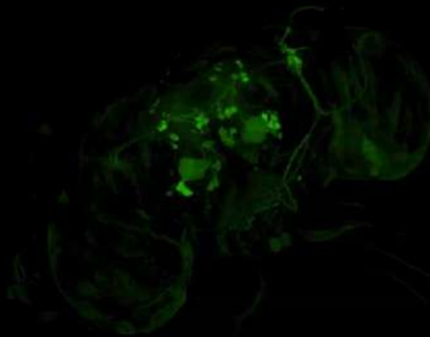

Image courtesy of Brain Research Center, NTHU, Taiwan

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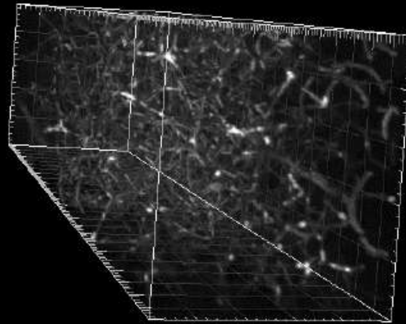
Cleared brain tissue



2.4 mm deep
3DISCO cleared
Perfused with red beads
8000 optical sections
20x/0.95 WI objective

400 um

Courtesy Alan Watson, Uni Pittsburgh

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Longer

10x less photo-bleaching vs point scanners

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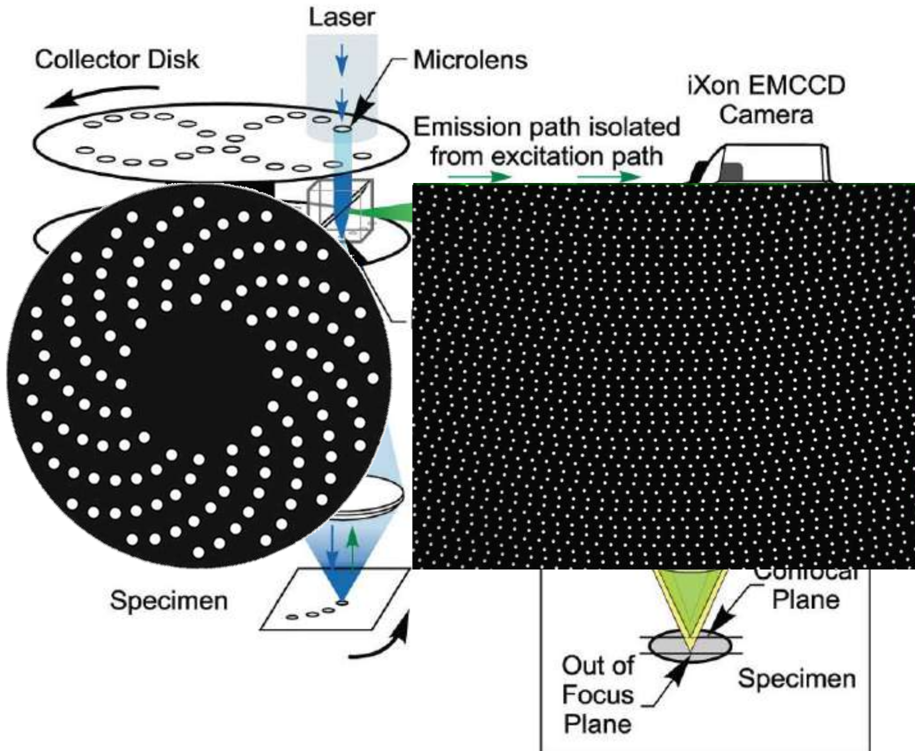
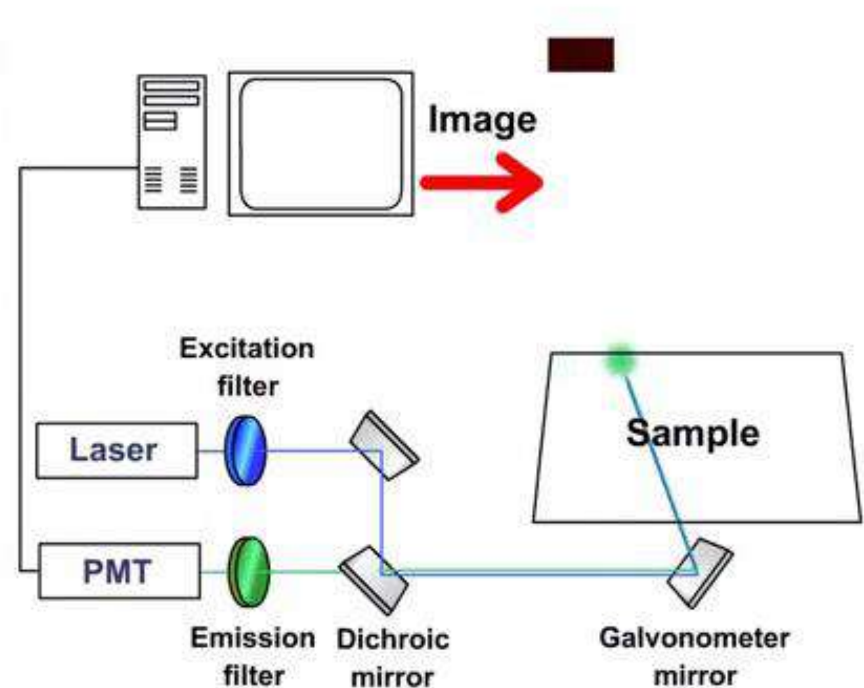
Multi-point scanning instead of single point scanning





Dragonfly

Point scanner

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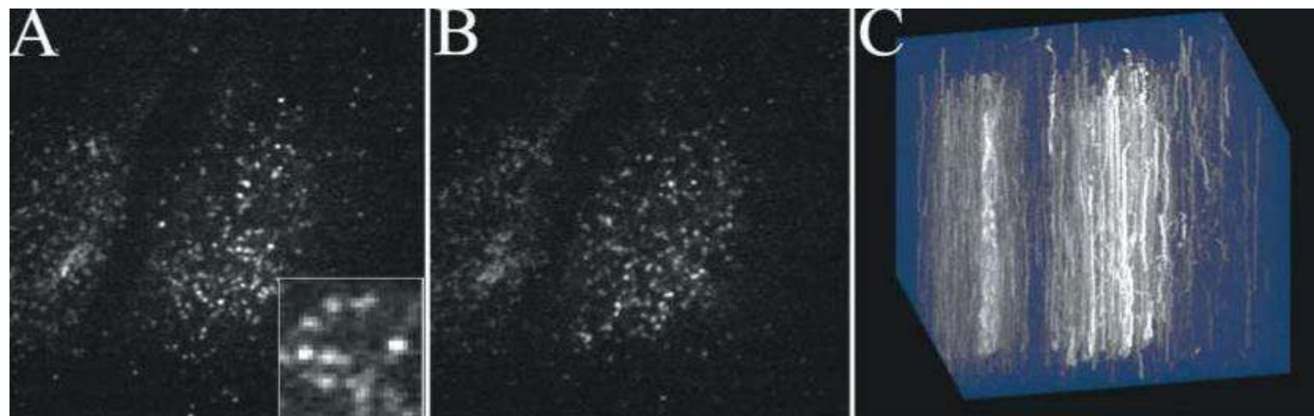
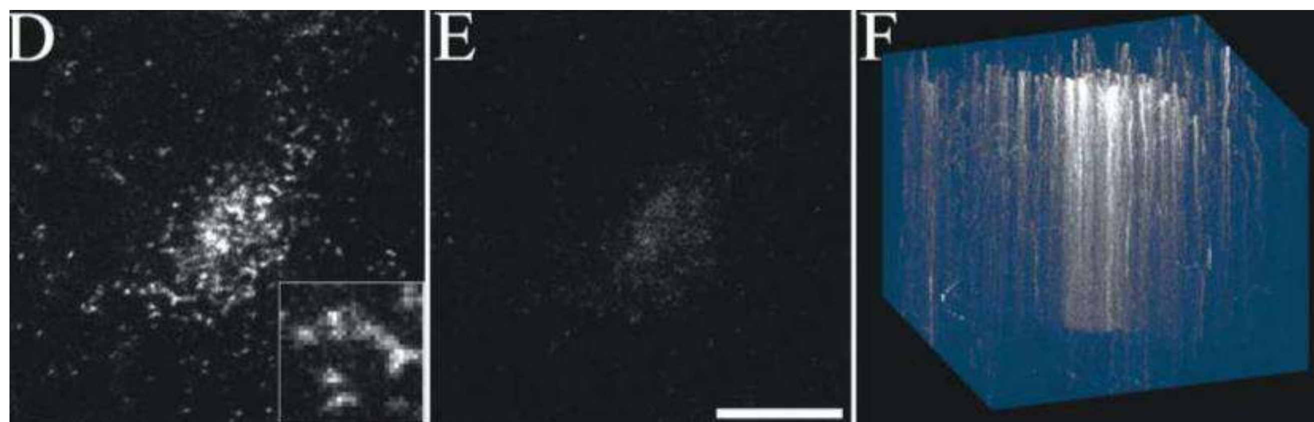
DEEPER & LONGER vs point-scanner

Low photo-bleaching for longer timelapse and more Z slices

Multi-point scanner



Point scanner



Data from Wang et al, Journal of Microscopy, May 2005

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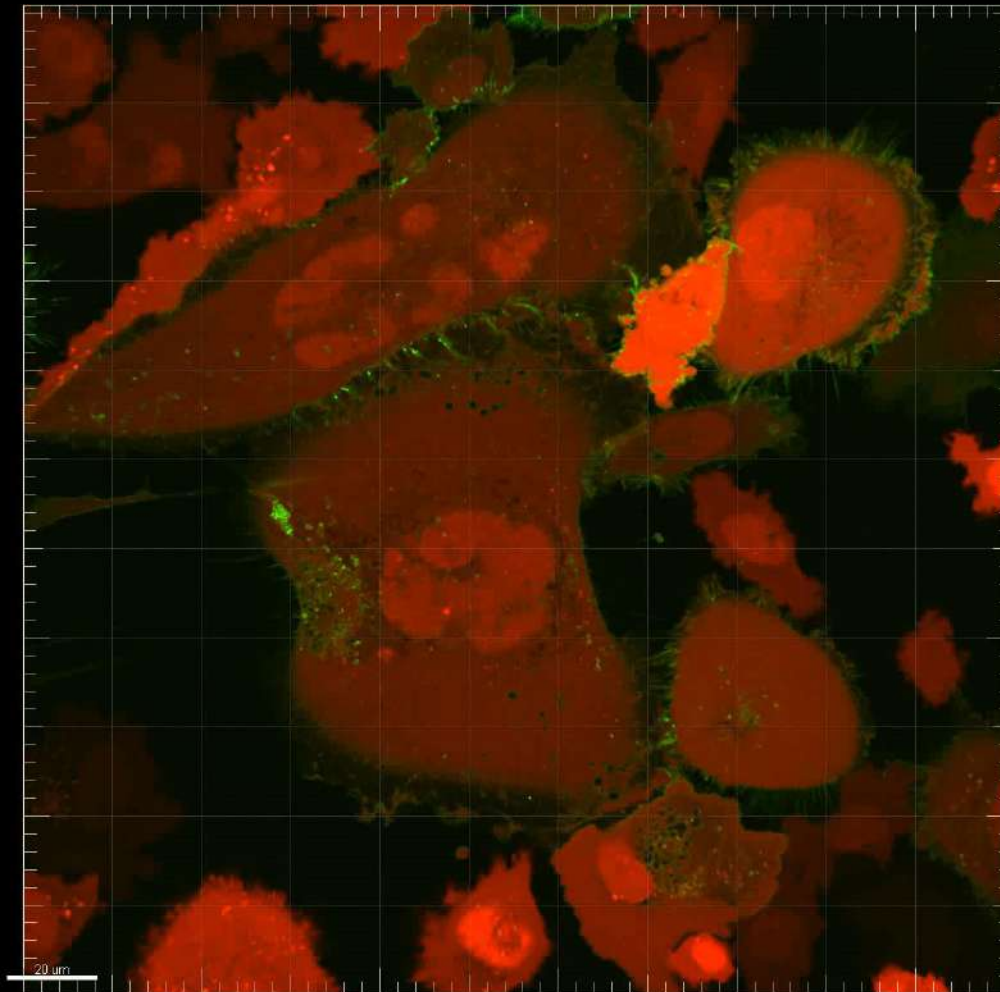
Live Cell Imaging overnight of Z stacks. MIP displayed.

Prostate Cancer Cells. Green: EphB4GFP and Red: SUMO1mKO2



Queensland University of Technology

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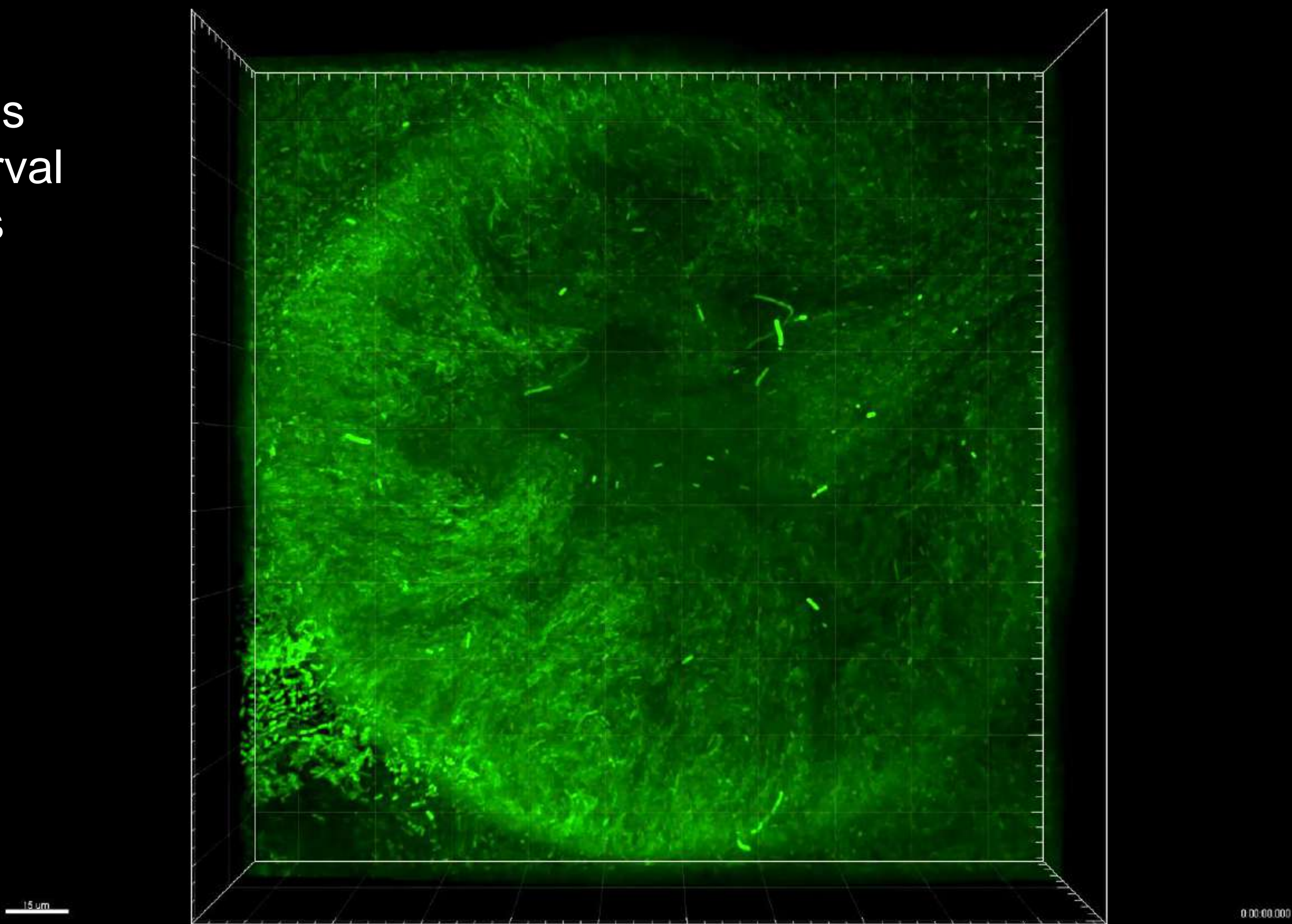
E.coli

60x oil
101 Z slices
4mins interval
Total 10hrs



Institute of Physics, Academia Sinica, TW

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Better

Variety of technics for better image quality (SNR/Dynamic range/Uniformity/Resolution)

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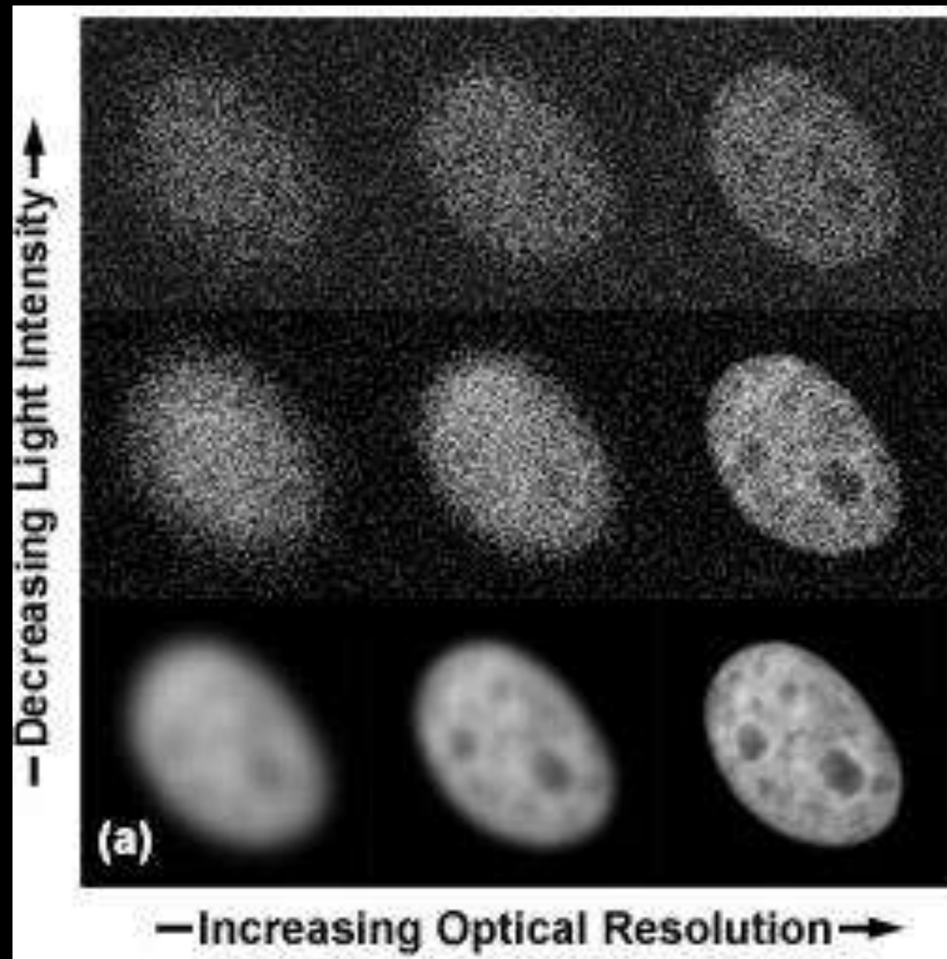
The Importance of SNR



Low SNR

Mid SNR

High SNR

Photobleach, phototoxicity and sample motion all influence SNR and hence resolution

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Image Quality (SNR)

Camera is 2x more sensitive than PMT

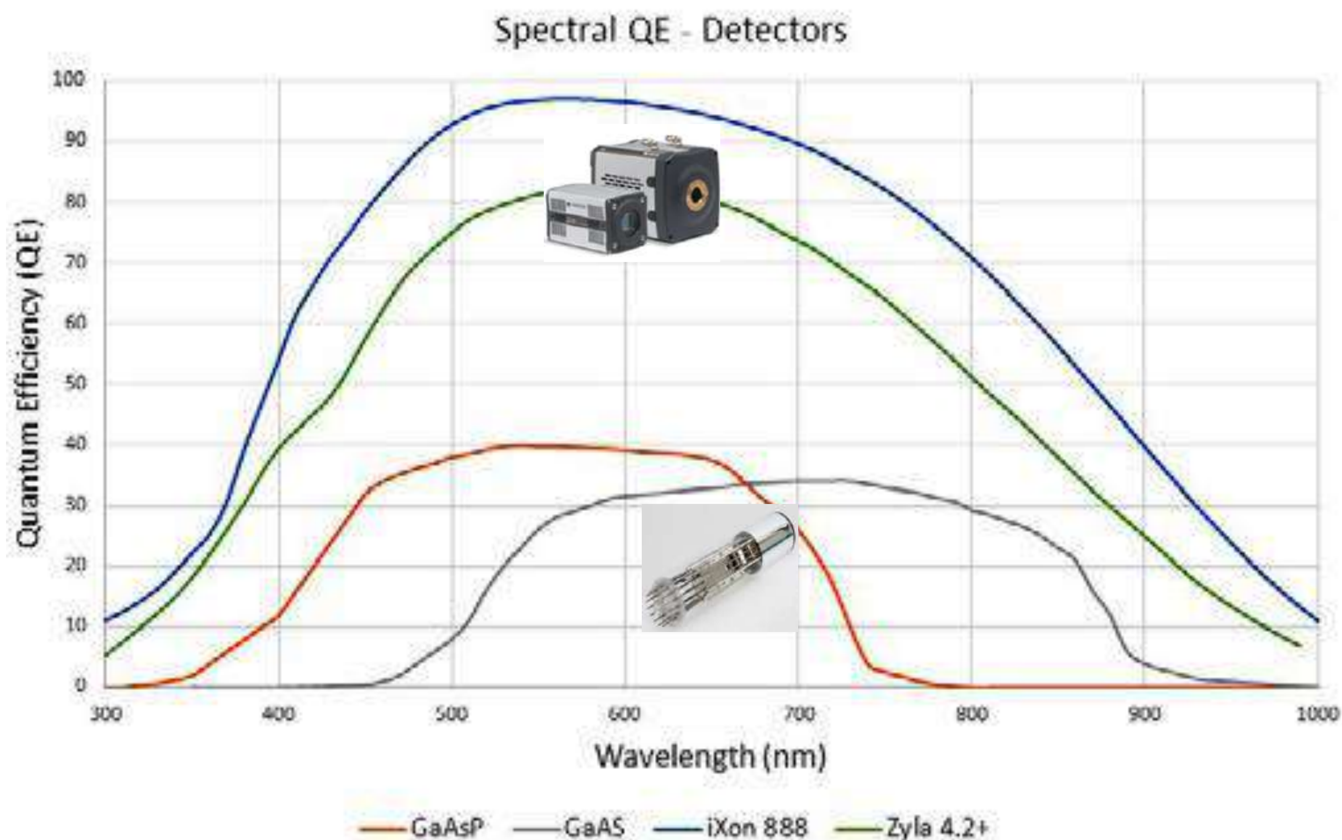

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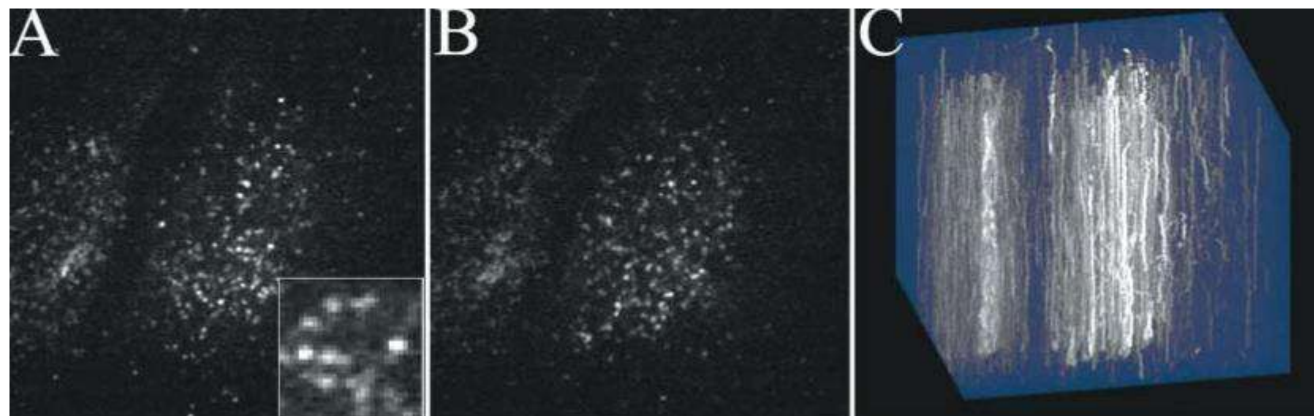
Image Quality (SNR)

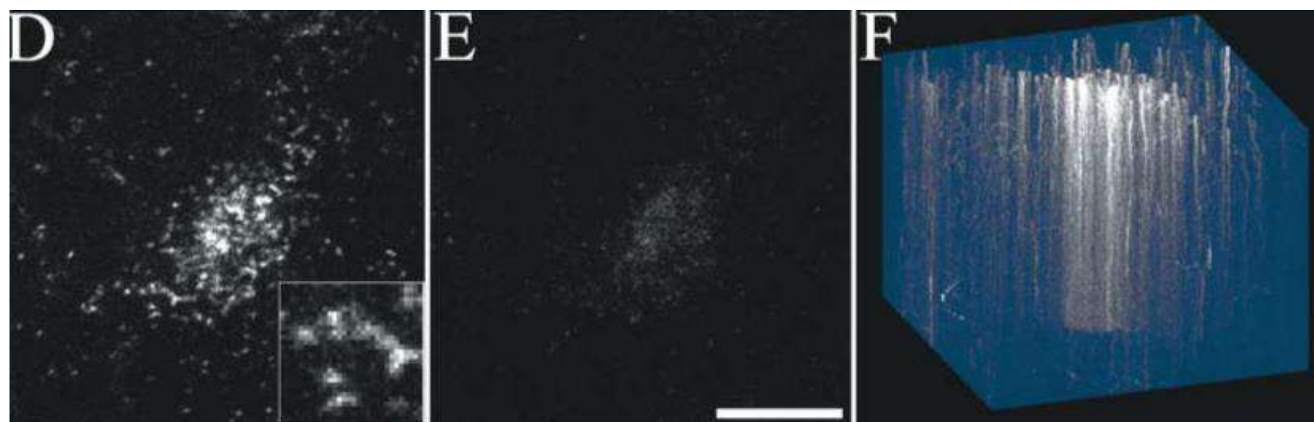
Low photo-bleaching rate to keep high SNR

1st timepoint | **200st timepoint** | **XY-T**

Multi-point scanning



Point scanner



Data from Wang et al, Journal of Microscopy, May 2005

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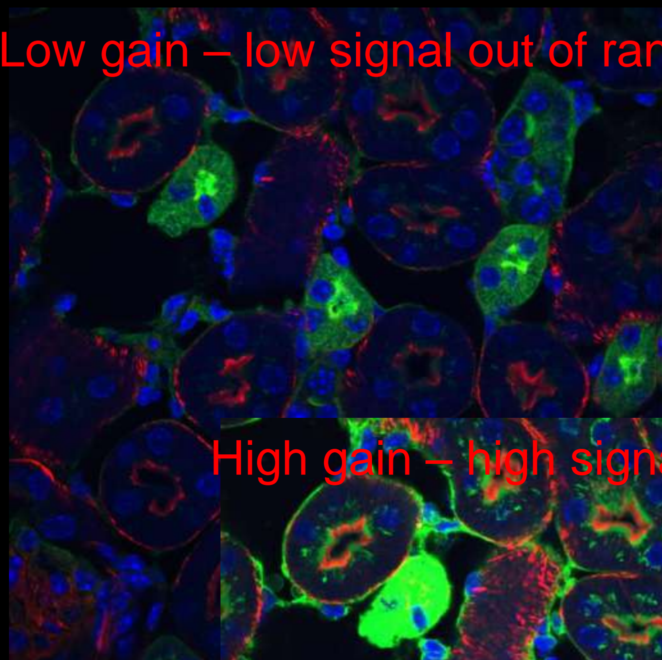
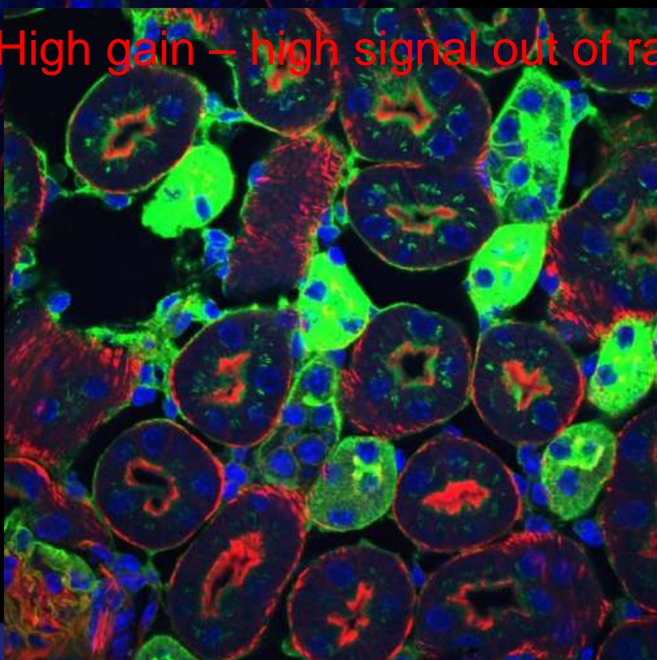
Dynamic Range

PMT-based Confocal

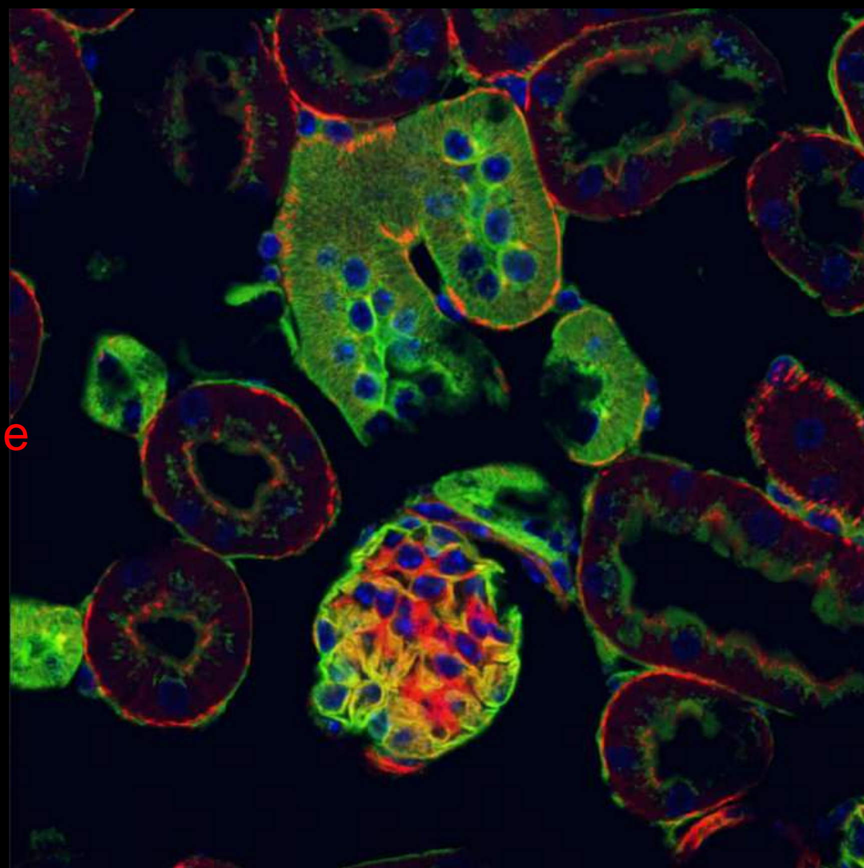
Camera-based Dragonfly Confocal



Low gain – low signal out of range



High gain – high signal out of range



High ***and*** low signal in range

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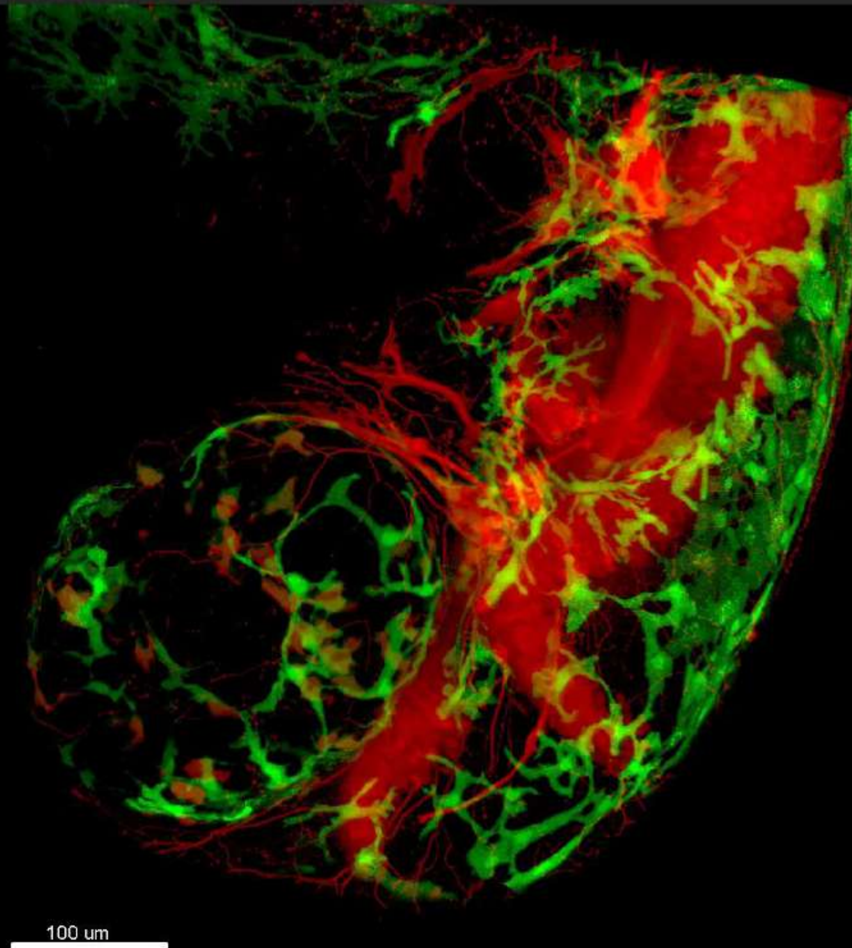
Zebrafish with red neural tube staining and MITFA-GFP cytoplasmic stain for melanocytes.

331 slices captured over **110um depth** at **25x** magnification with **25um pinhole**



Alessandro Brombin (Patton Group), University of Edinburgh.

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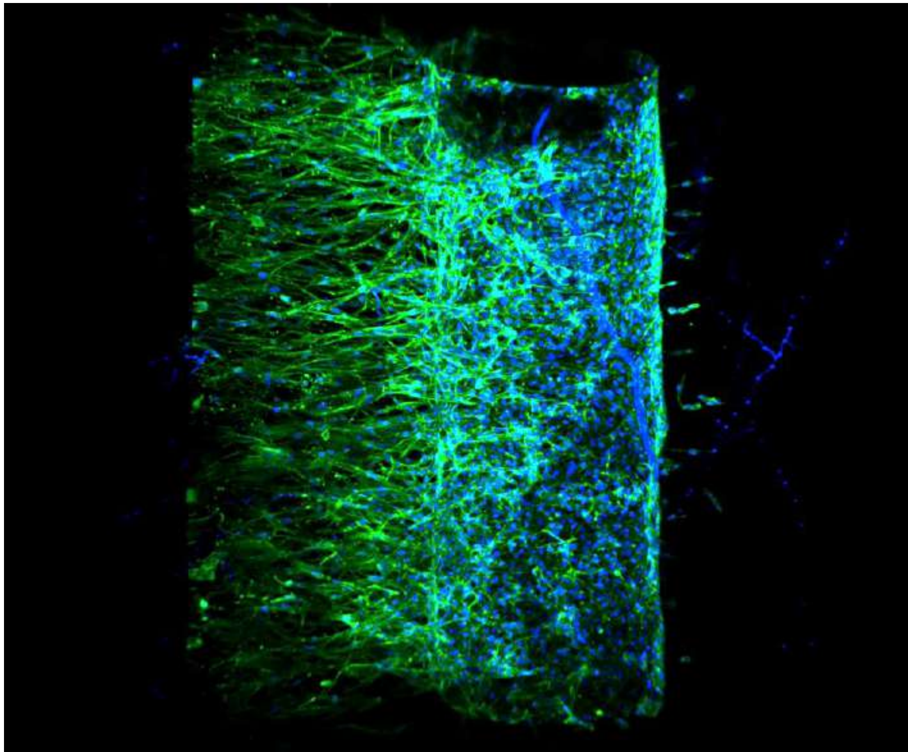
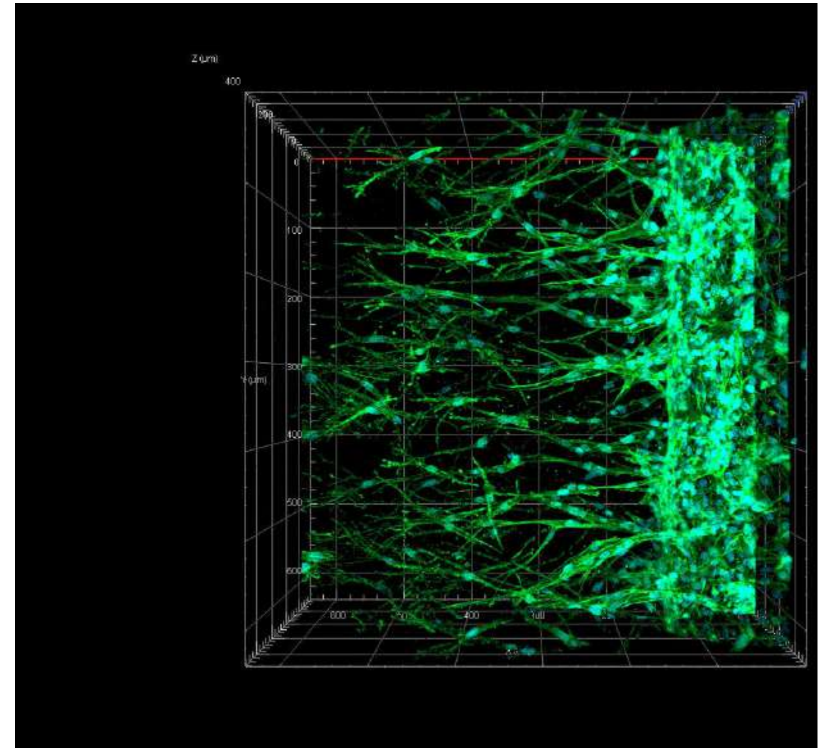
Image Quality (Dynamic Range)

Higher dynamic range means more details



Dragonfly



Point-scanner

Images appear "grainy or binary";
frame averaging often required; details missed

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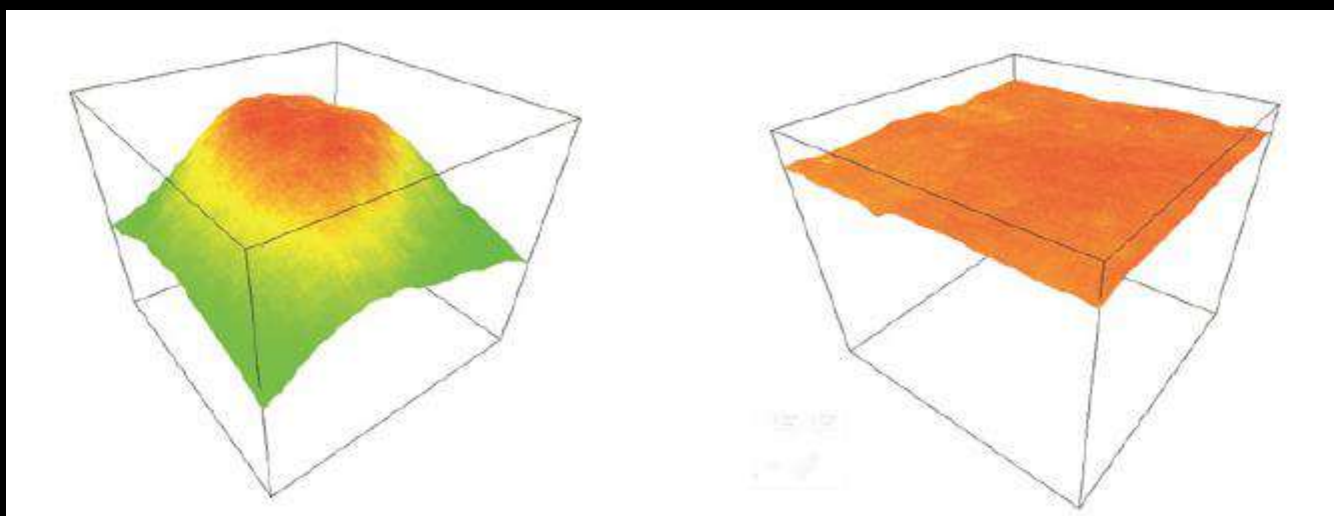
Borealis Perfect Illumination Delivery

Broad Spectrum Uniformity

Standard Illumination

Borealis enhaced Illumination



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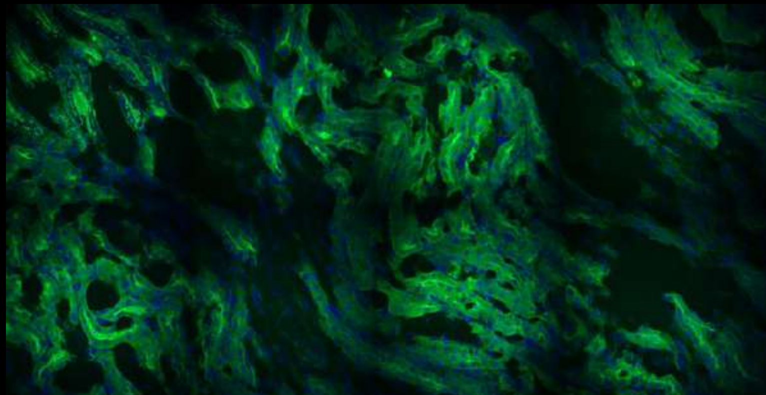
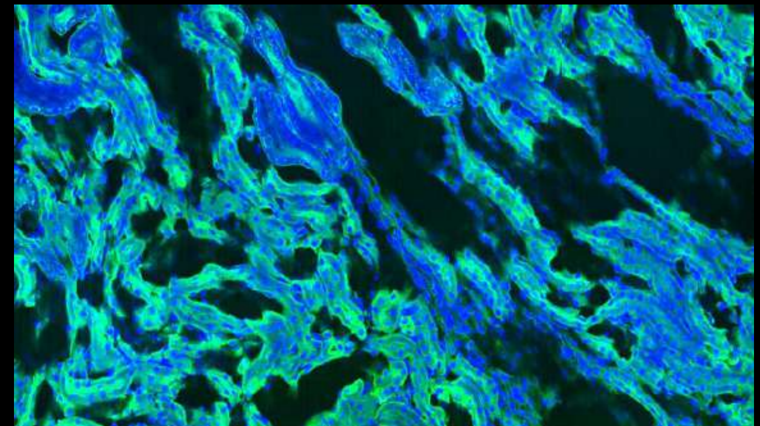
Borealis Perfect Illumination Delivery

Broad Spectrum Uniformity





3x Signal - Higher contrast

Standard CSUW1: exposure = 0.2s

Borealis Illumination: exposure = 0.2s

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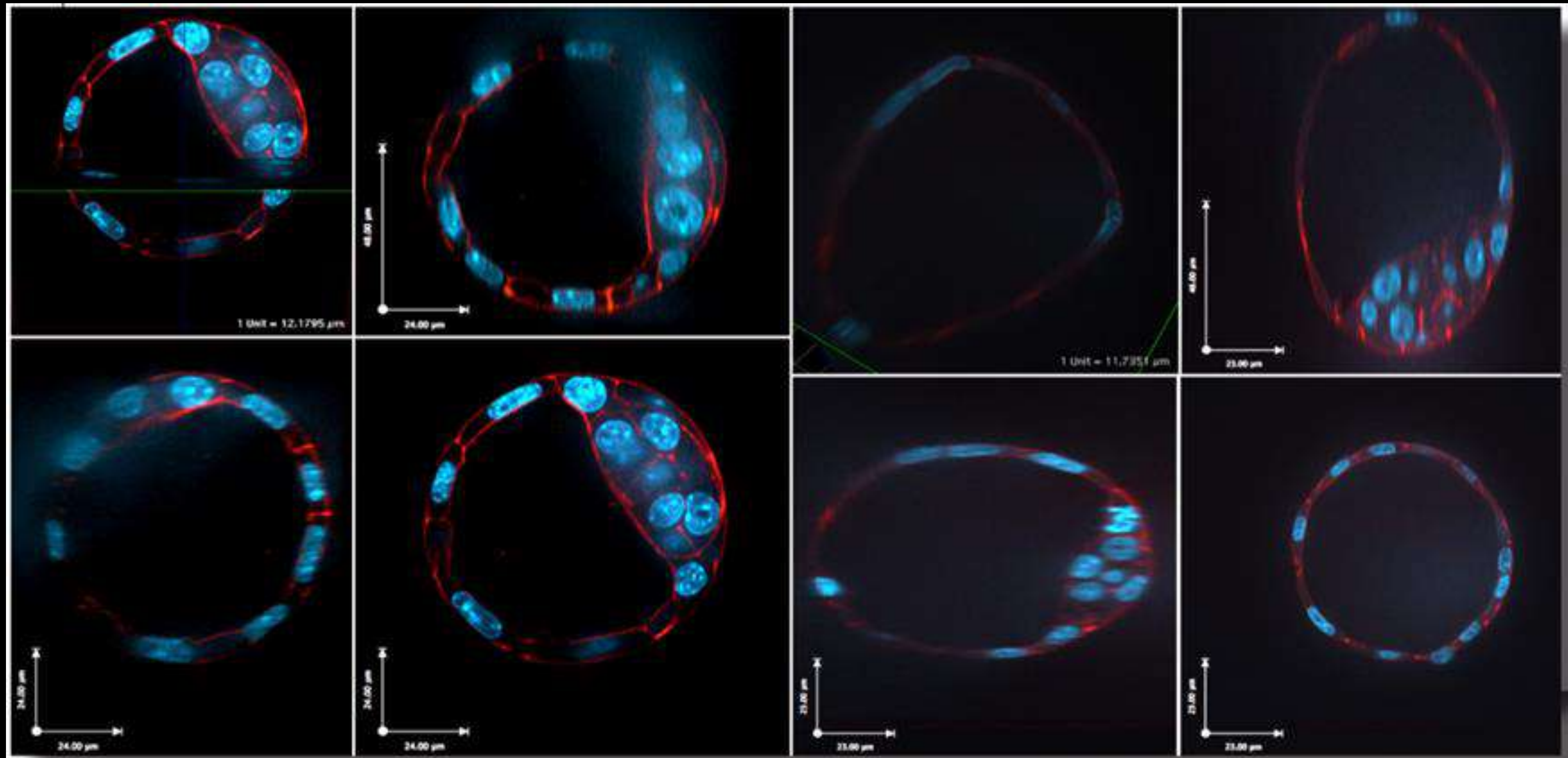
Minimizing Imaging Artefacts and Improving Image Quality



- With Borealis
- No Borealis

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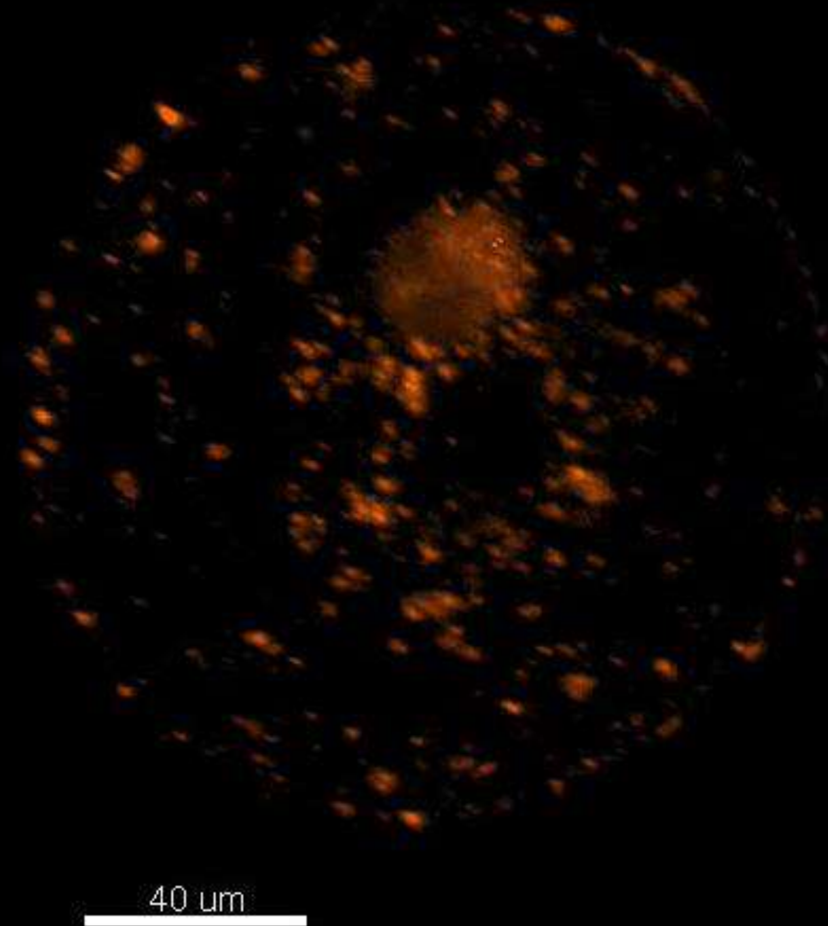
3D visualisation of a mouse egg stained for a plasma membrane protein. 600 focal planes over 80 microns



Curtesy of Katerina Dvorakova-Hortova, Laboratory of Reproduction, Institute of Biotechnology, CAS, v.v.i., BIOCEV, Czech Republic.

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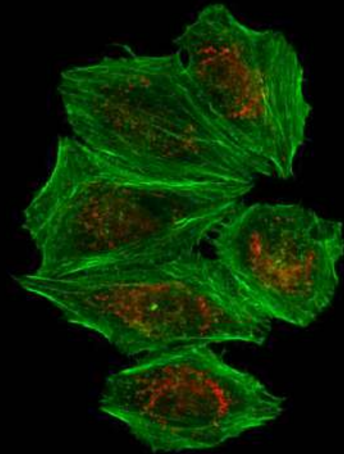
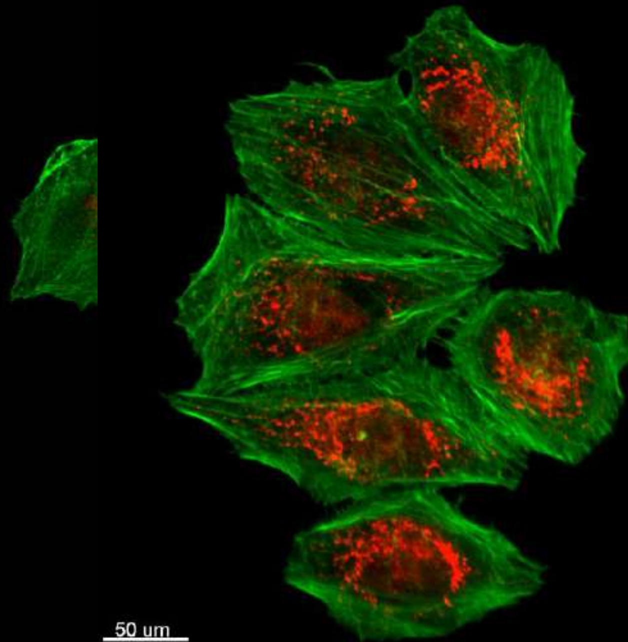
3 position zoom in camera port

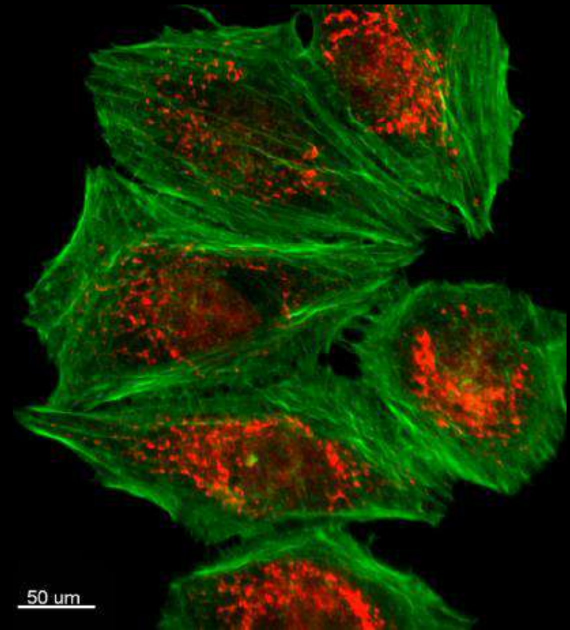
1.0x



1.5x



2.0x



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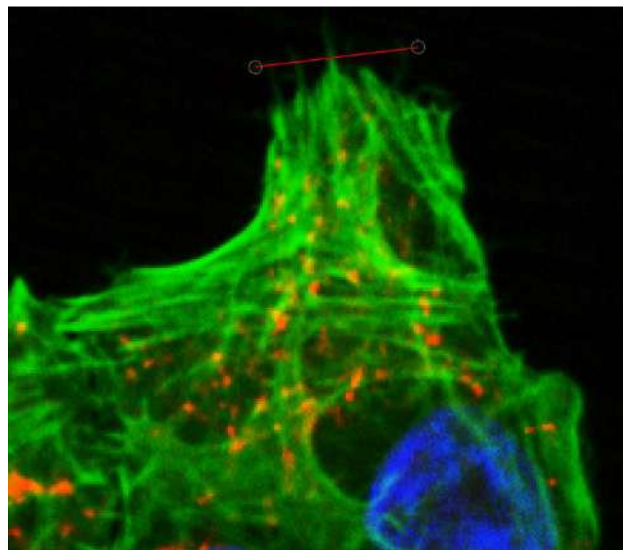
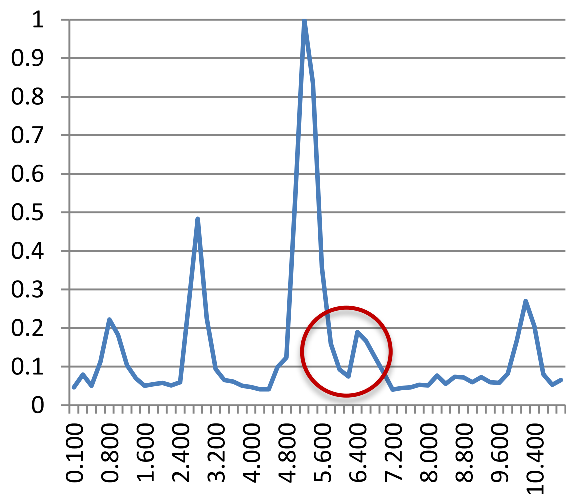
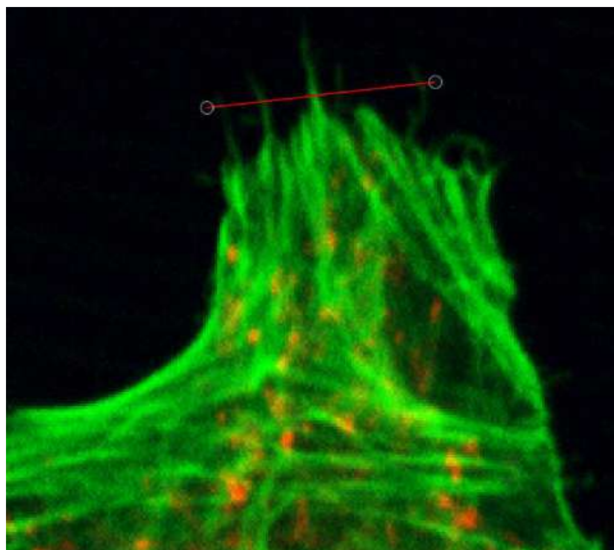
iXon Ultra 888. Actin filaments captured with 60x objective with varying additional camera magnification

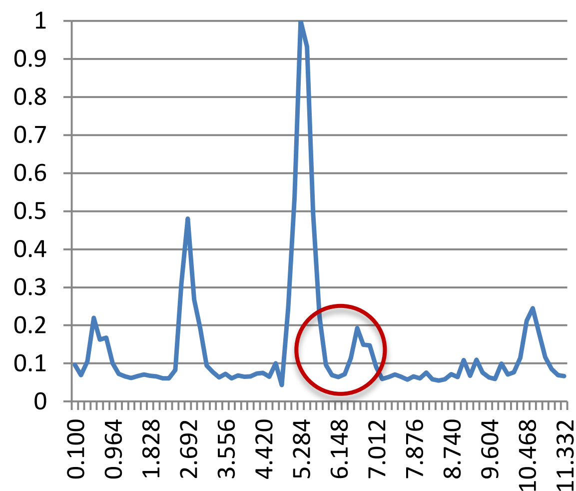


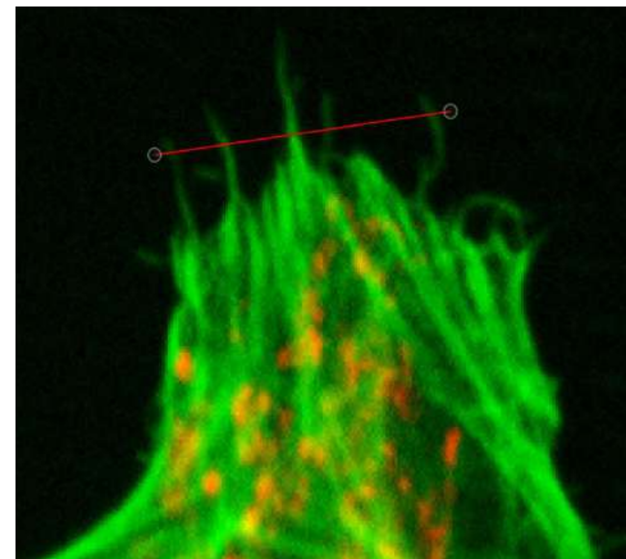
Actin 1.0x



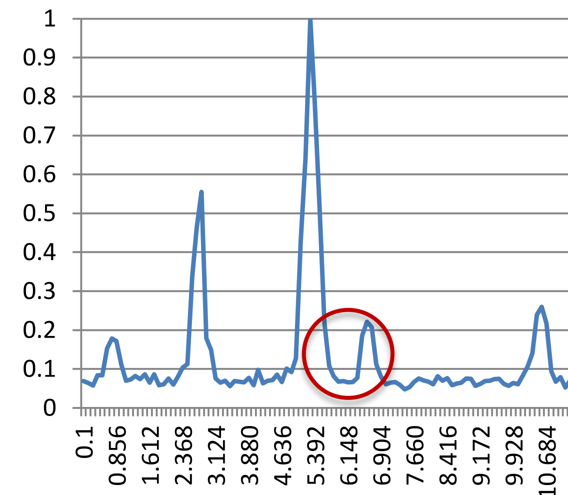


Actin 1.5x





Actin 2.0x



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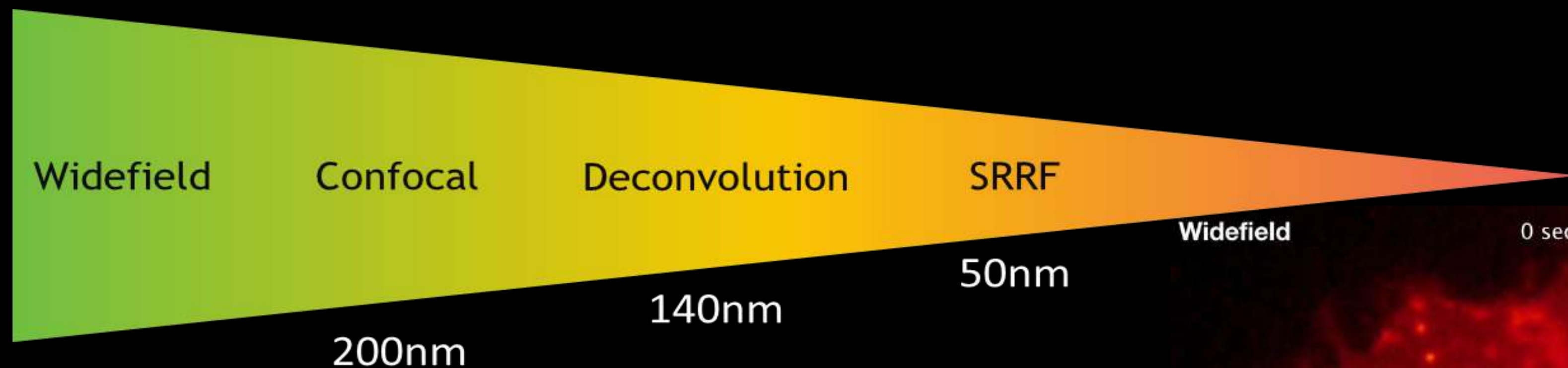
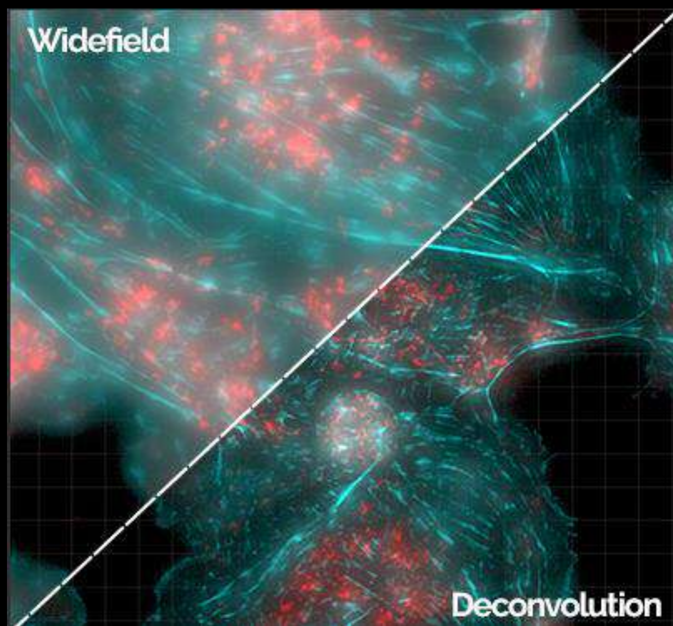
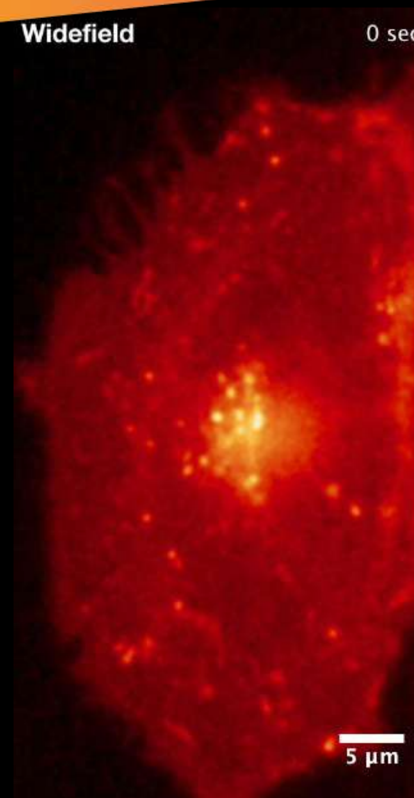
Resolution

Multiple imaging technics offer resolution down to 50nm (super-resolution)







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GPU accelerated deconvolution

Fusion

Welcome back, WILDE Geraint | Imaging

Acquisition Control

Live | Snap

Active Channel | Protocol Channels

ACTIVE CHANNEL

RFP | Confocal

Laser 488nm 0.0 %

EM Gain 0

Exposure Time 200 ms

NAVIGATION

Specimen

New Specimen | Esc. Z

PROTOCOL

Protocol 5

Protocol Channels

RUN PROTOCOL

Run Time: --:--:-- Remaining: --:--:--

Acquire

Image Processing

Hide ClearView™ Deconvolution

CLEARVIEW™ DECONVOLUTION

GPU (CUDA) Compatibility:

Deconvolving

Run Time: 00:04:33 Remaining: 00:07:28

ADVANCED PROCESSING OPTIONS

Hide

Setting	Value	Setting	Value
Edge Artefact Reduction	All (XYZ)	Minimum Intensity Removal	ON
Denoising Filter Size	0.7	Initial Denoising	ON
Denoising Frequency	4	Normalisation	OFF
Number of Iterations	24	Iteration Acceleration	OFF

Restore Defaults for 'Robust (Iterative)' algorithm

more

POINT SPREAD FUNCTION SETTINGS

Hide

Setting	Value	Setting	Value
Imaging Modality	Spinning Disk Confocal	Specimen Refractive Index	1.365
Pinhole Radius (µm)	40.0	Distance From Coverslip (µm)	30
Magnification	100	Immersion Medium	Oil (1.52)
Objective Lens NA	1.4		

Auto-Fill | Reset All | Unlock PSF Settings OFF

Current Calibration Information | Current Channel Information

ALC-1111 | X-1383 | CSU-666 | Left | 0 mm, 0 mm | 133.69 µm | Camera

Start | Multi | ZOOM: Fit (45%) | Pos | x 1024 | y 1024

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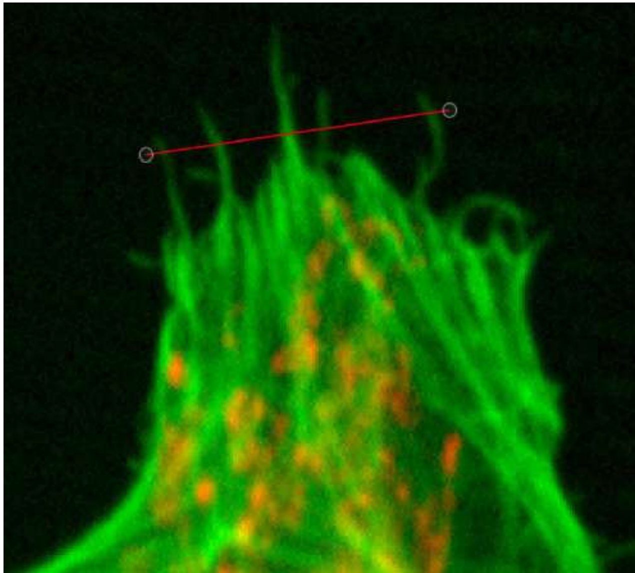
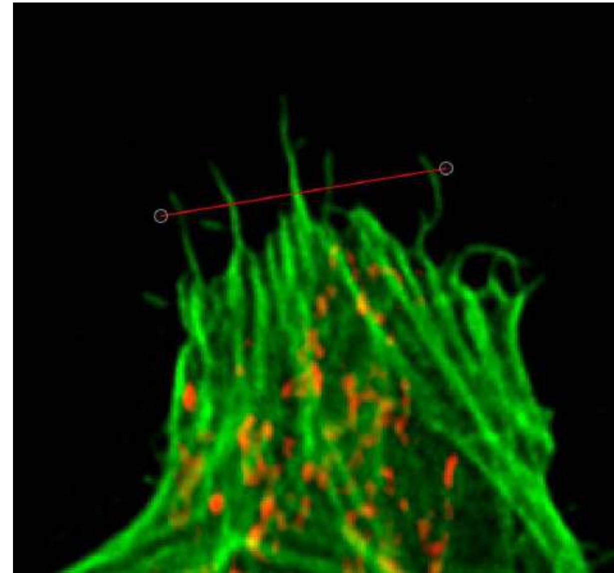
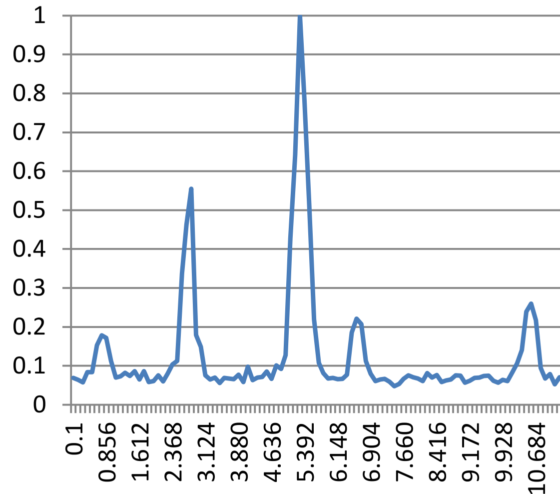
iXon Ultra 888. Actin filaments captured with 60x objective with 2.0x camera magnification. Deconvolution comparison.

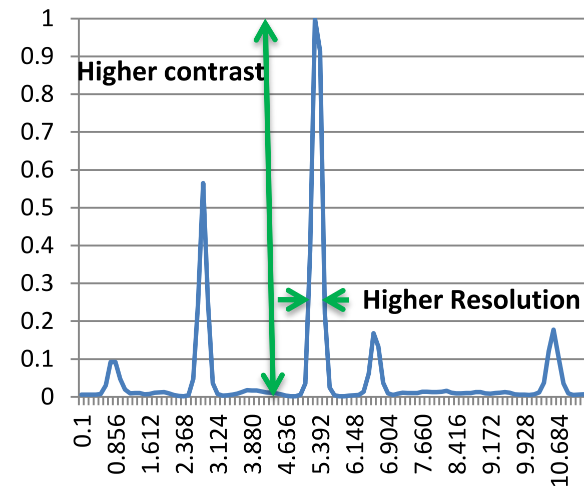




Actin 2.0x



Actin 2.0x Deconvolved



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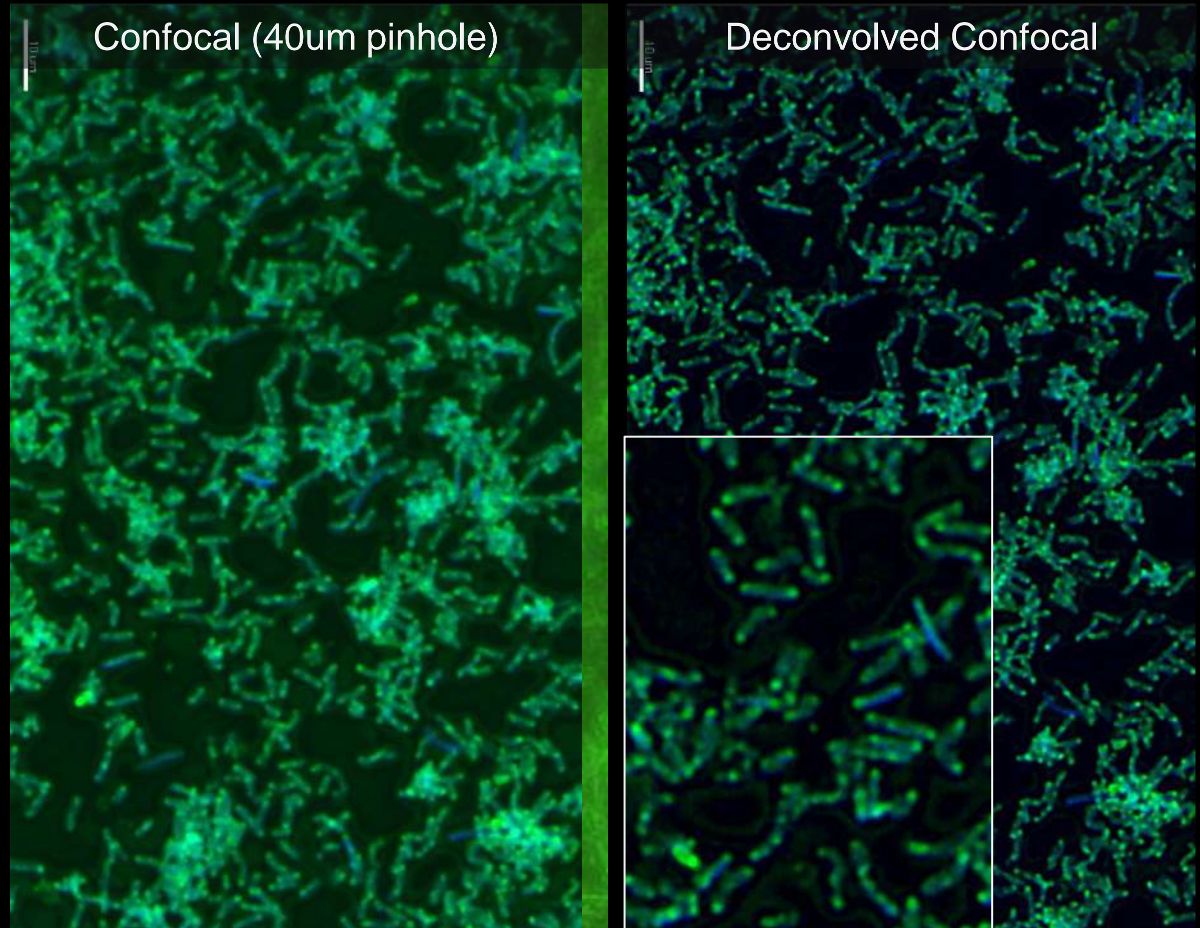
Bacteria on biofilm

40x (0.75 NA) objective. Zyla 4.2



Dr Nigel Ternan & Dr Barry O'Hagan, University of Ulster

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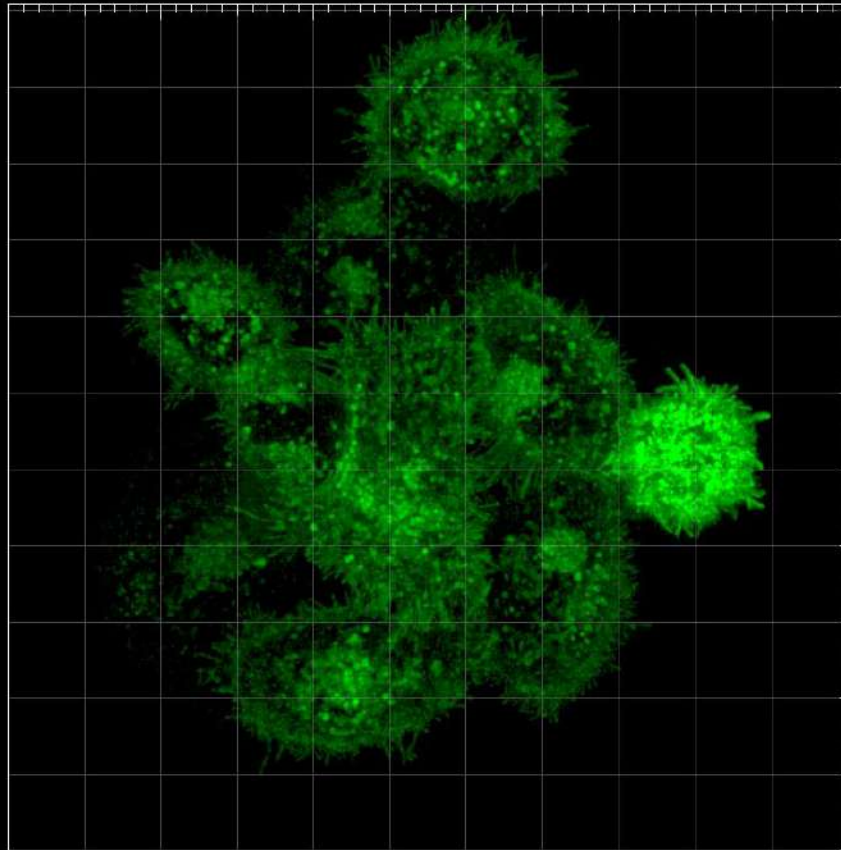
Confocal and Deconvolution

Hela cells expressing GFP fusion protein that is present in focal structures and vesicles. Deconvolution shows improved optical sectioning and enhancement in contrast. Images from same field with Dragonfly – 288 sections , 0.1 um Z step,



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Super-resolution with SRRF

A living sample solution

Combine with confocal

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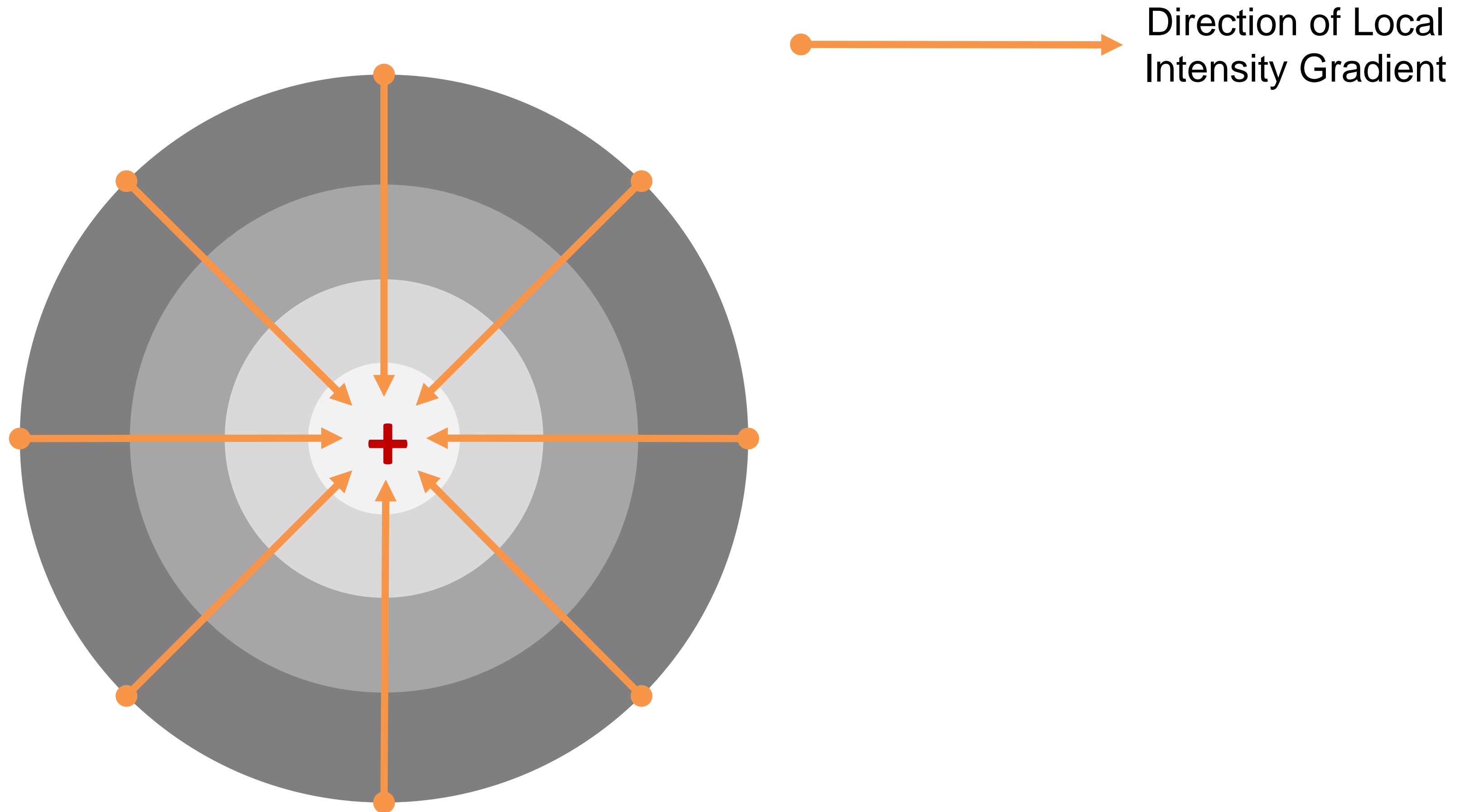
Super Resolution Radial Fluctuations



Fluorescent Marker is at the centre of intensity gradient field
aka **Point of maximum radial symmetry**

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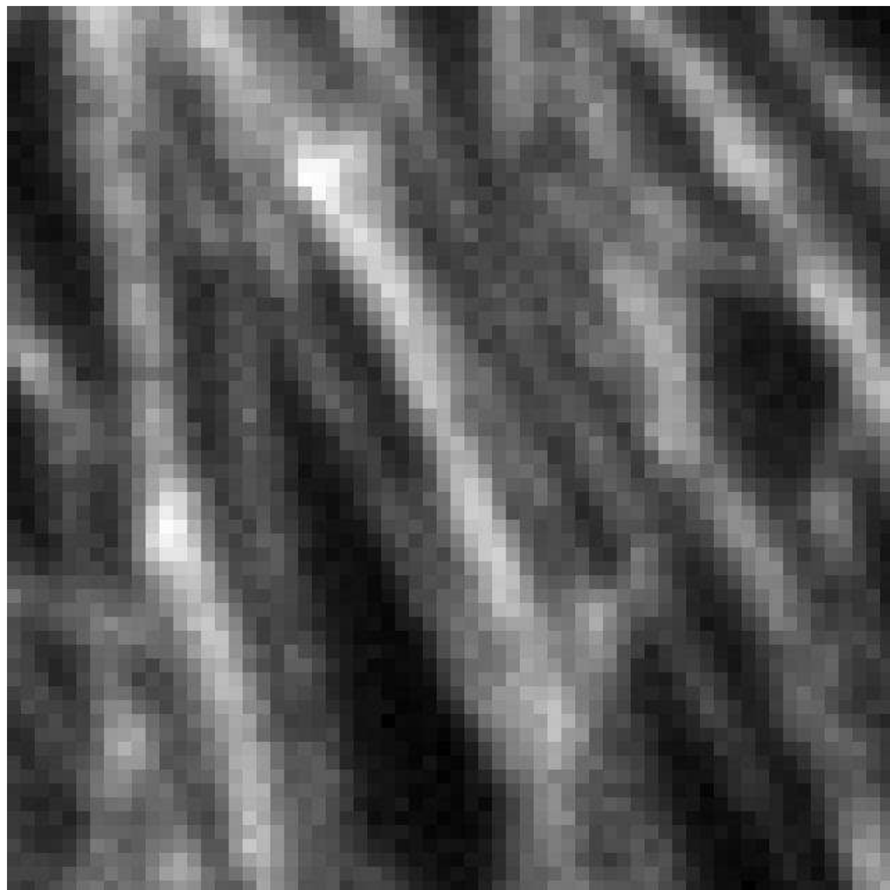
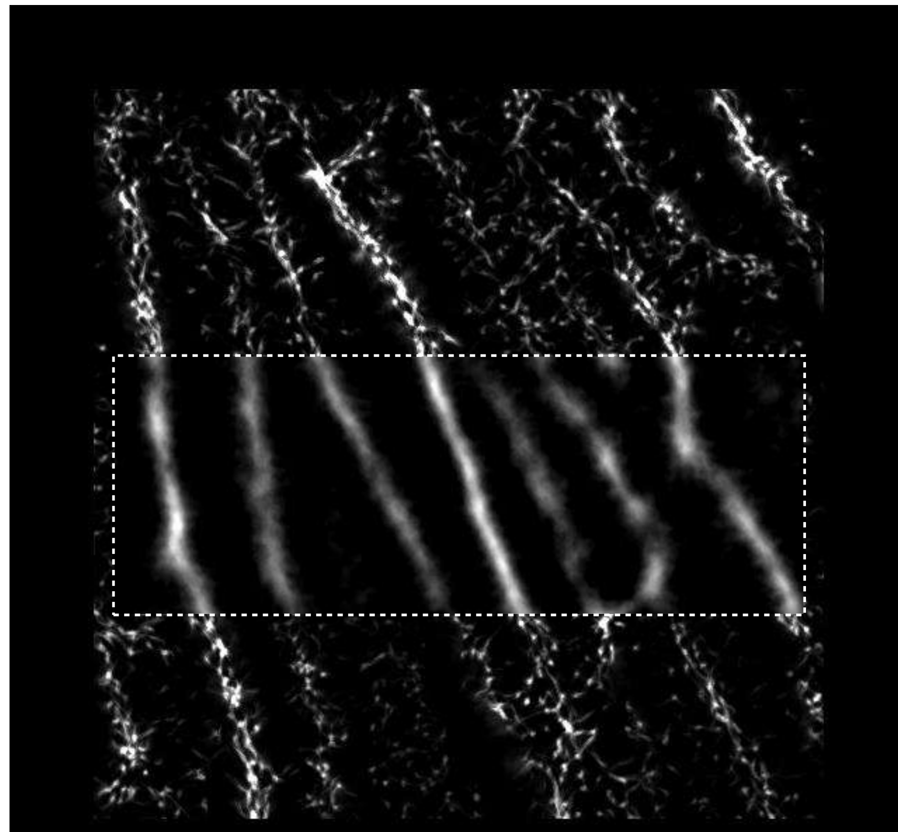
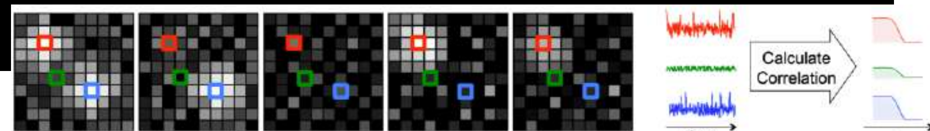
Super Resolution Radial Fluctuations



100 frames at 40mW/cm2





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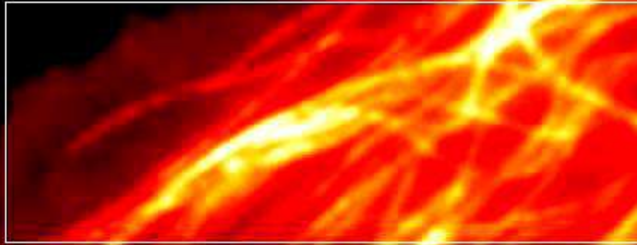
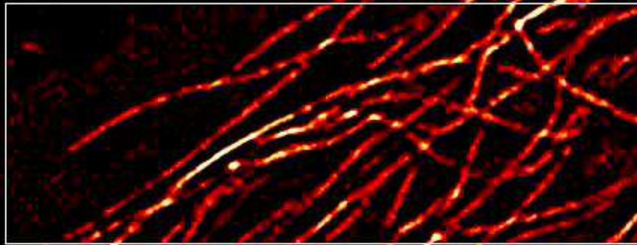
Live-cell, labeled with GFP





(8.5W/cm2) and no special optics

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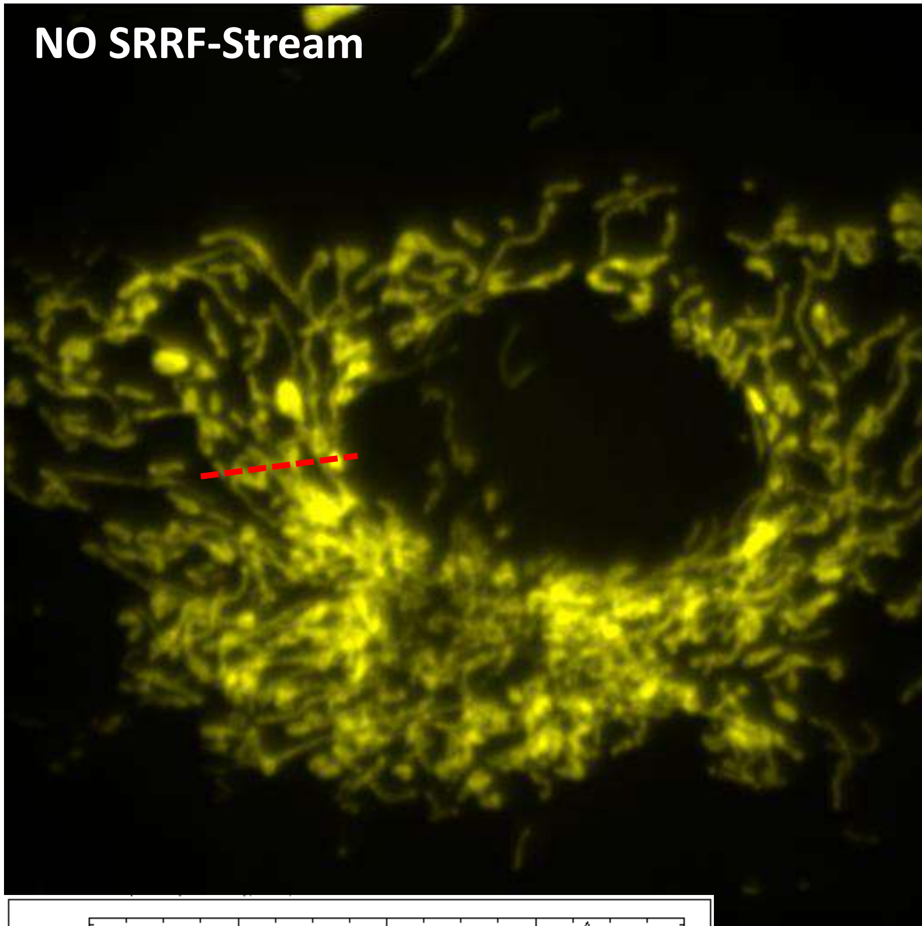
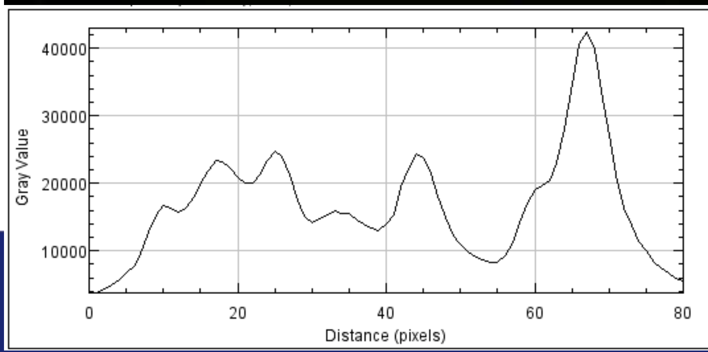
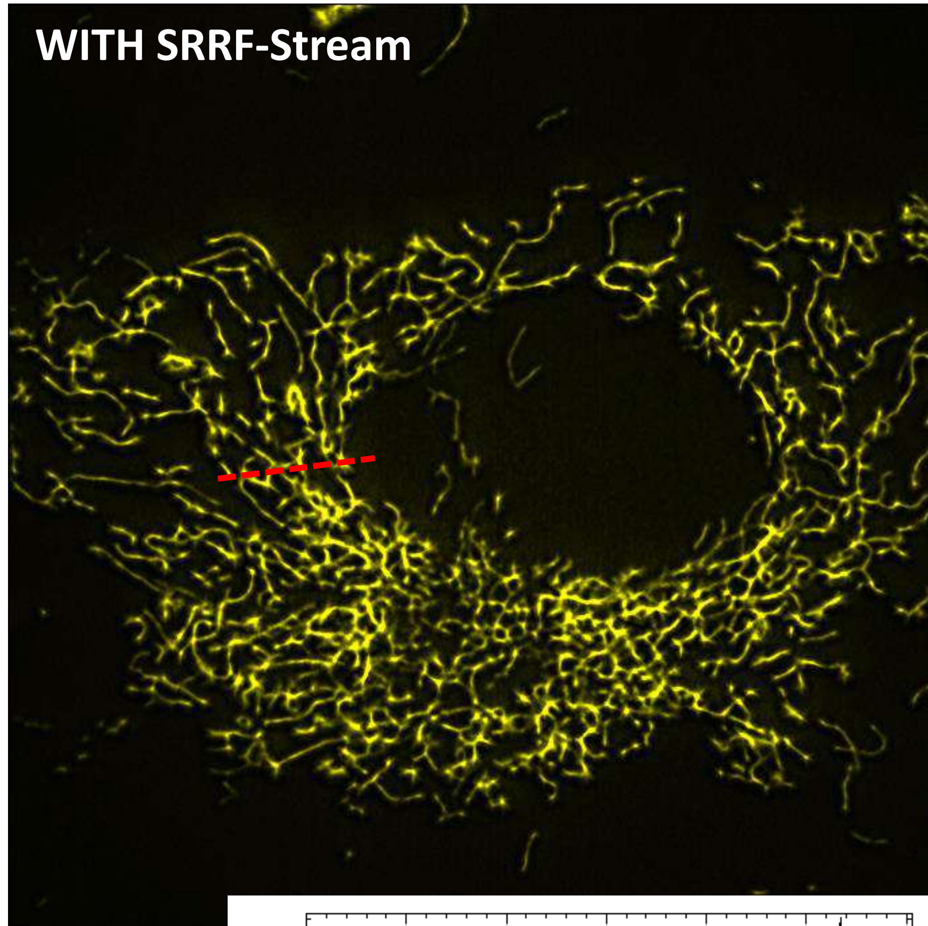
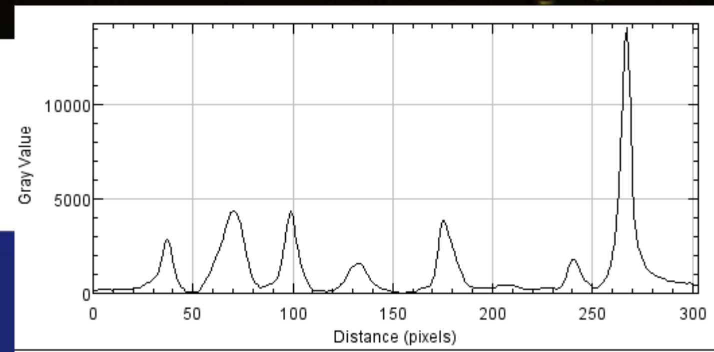
WideField SRRF – 560nm
Mitochondria

iXon Ultra 888
60x TIRF objective
2x camera magnifier
100 images – Average vs SRRF









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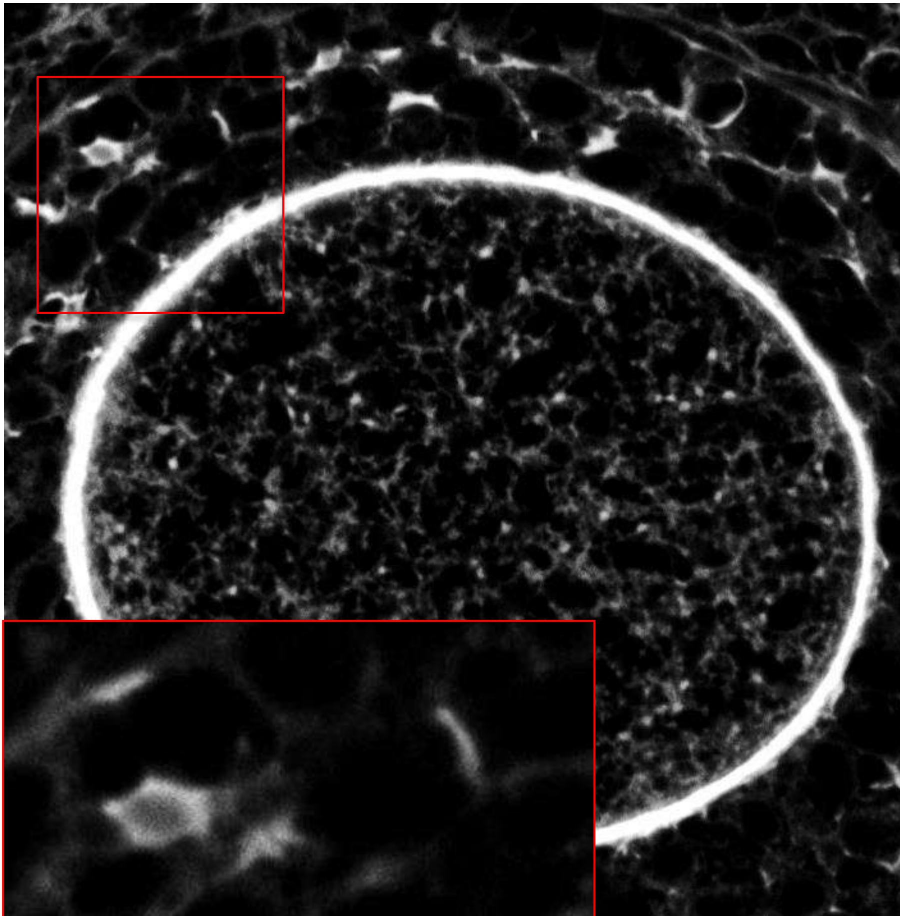

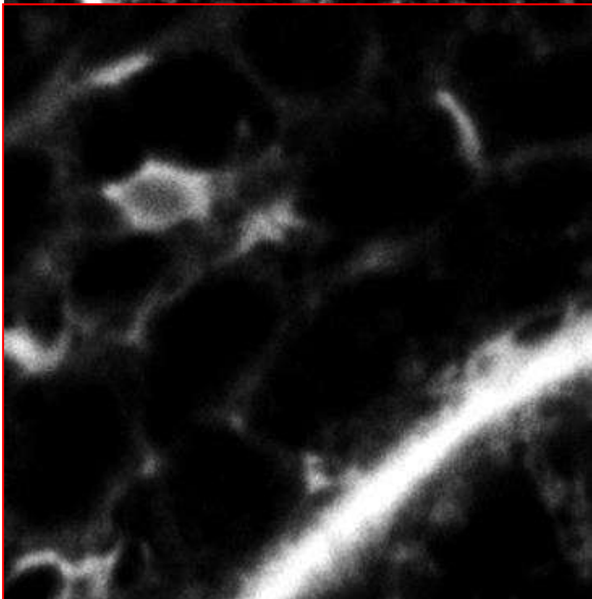

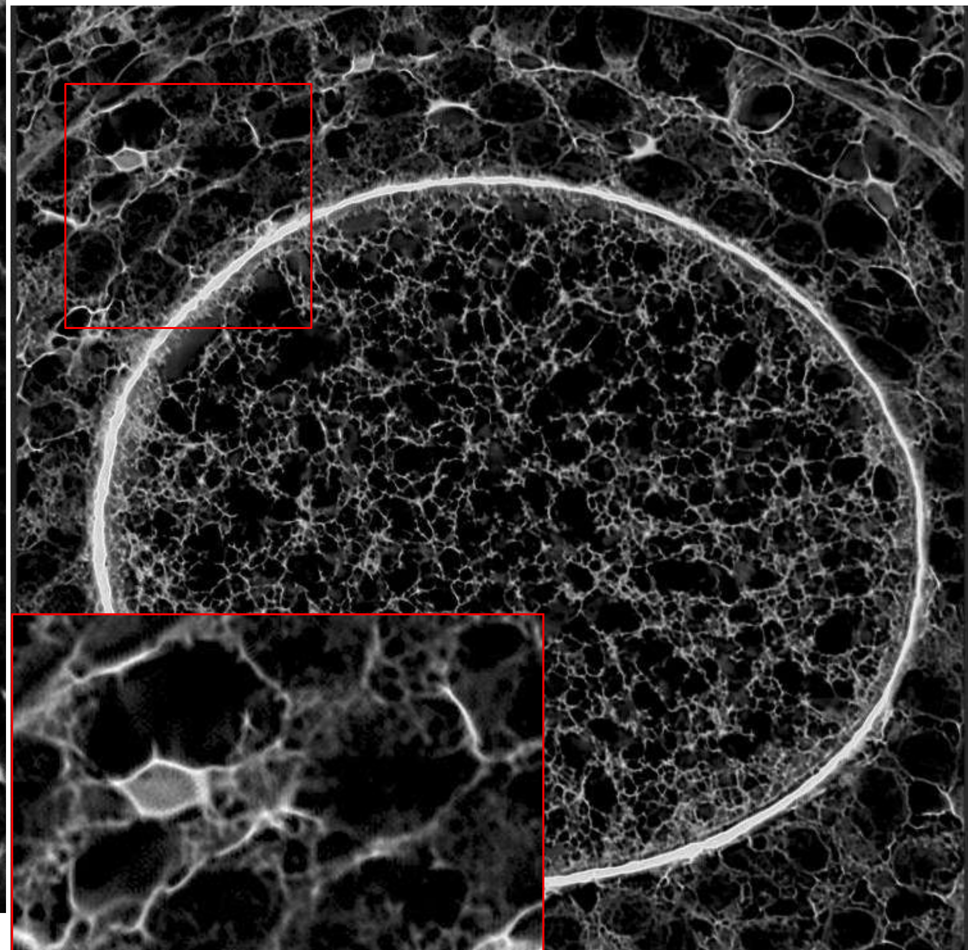

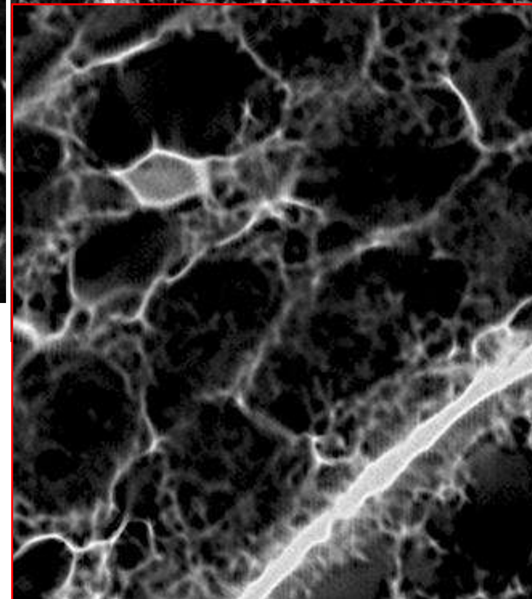

Ovary cross section
100 frames, 20 ms
Radial mag: 4X

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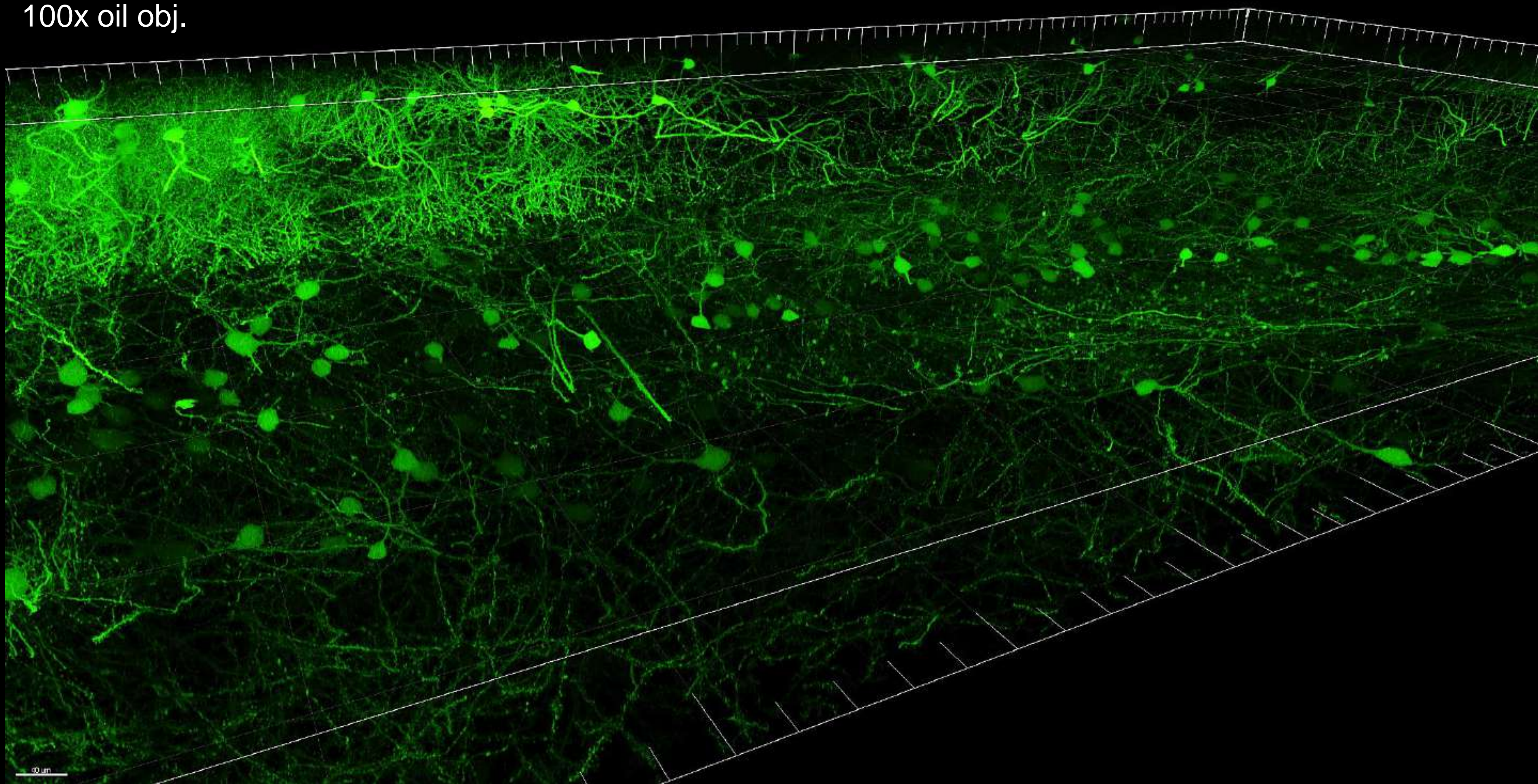
Confocal with SRRF – Brain Neuron

Confocal + SRRF
16x12 stitch
50um depth
100x oil obj.



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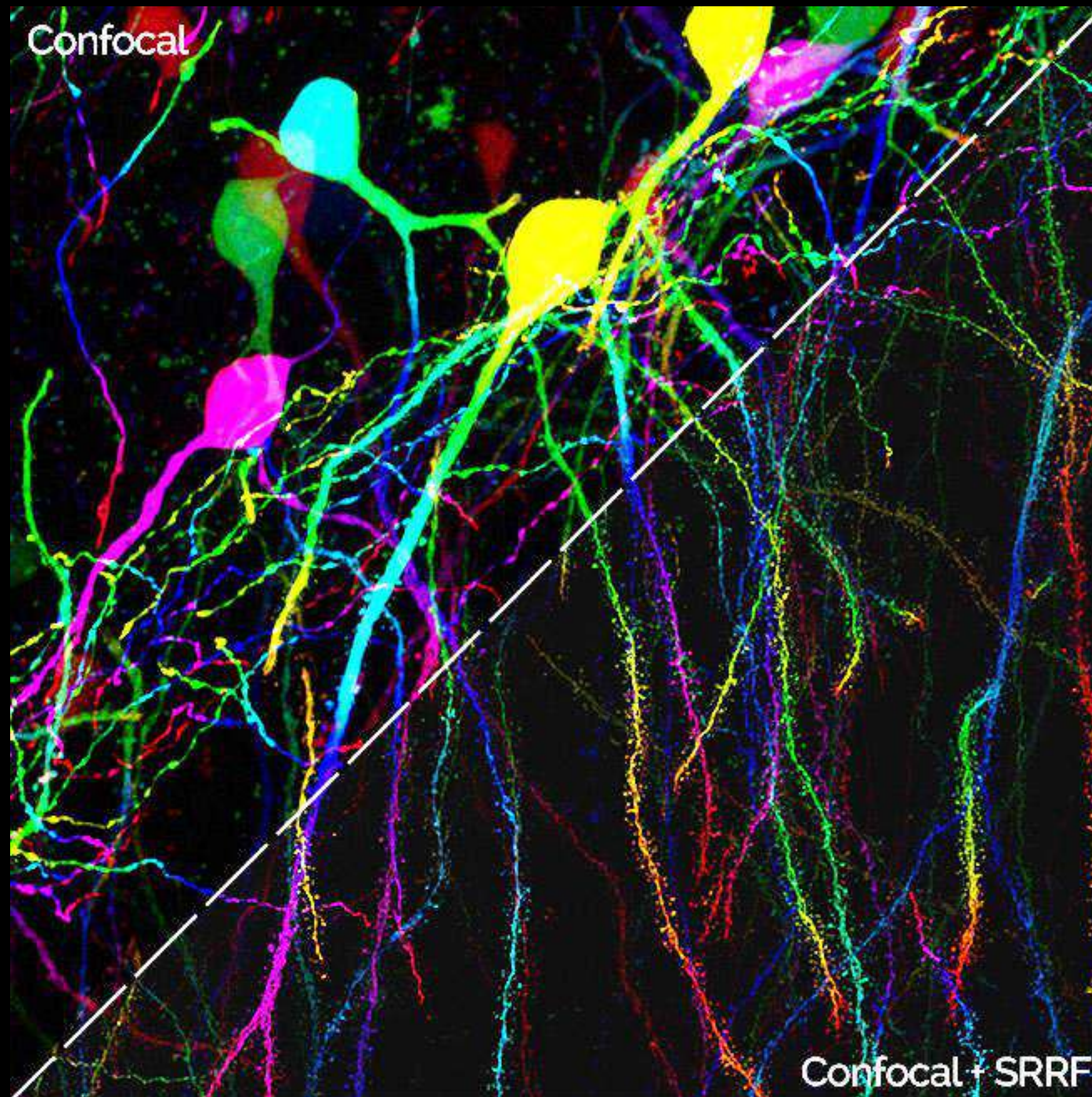
Confocal with SRRF – Brain Neuron



RapiClear YFP labelled neurons

Courtesy of Sunjin Lab

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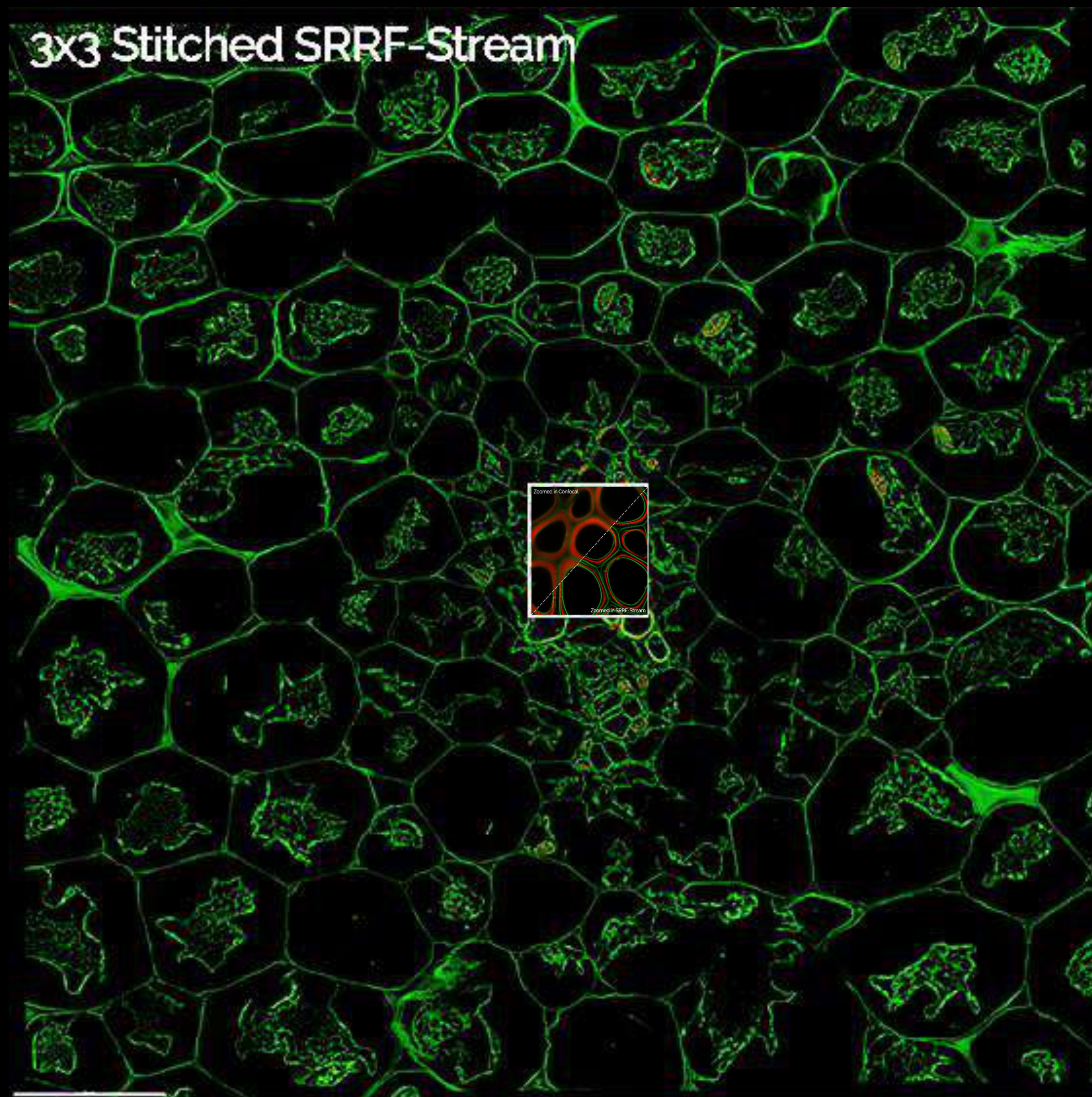
From nanometer to millimeter…



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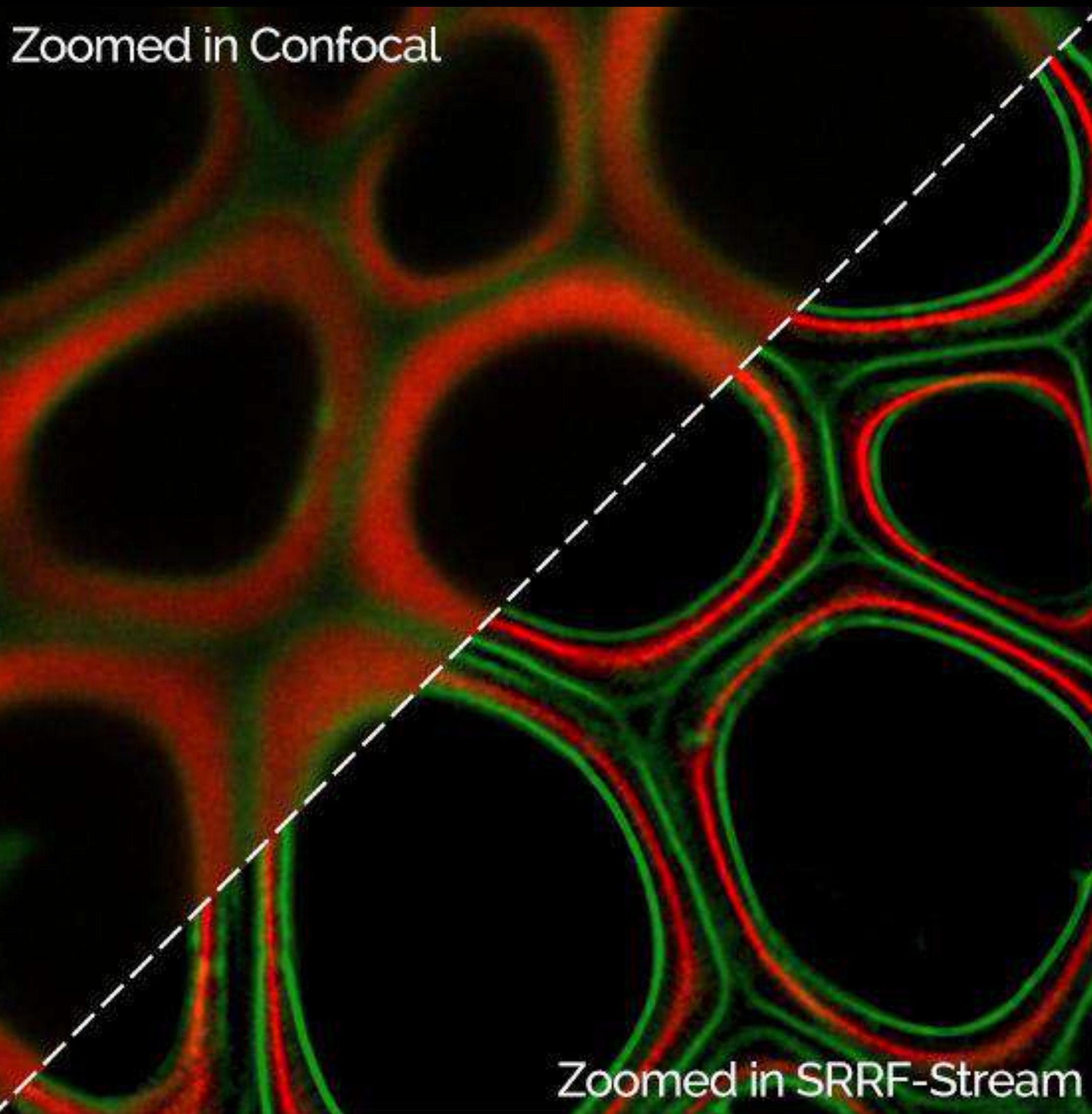
From nanometer to millimeter…



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