Nikon AX with NSPARC Demonstration Report

Imperial College London

15th - 24th May

Shedding New Light On **MICROSCOPY**

Dear Imperial College London research staff

Many thanks to all those who attended our demonstration at the Facility Imaging and Light Microscopy and bought samples to image on our new NSPARC detector for AX confocal.

Please find within this report a snapshot of the images collected from the various sessions. It was a great pleasure to image such a variety of samples and I hope that this was a useful opportunity for you to experience our systems hardware and software's capabilities and gain an idea of what tools may be available to generate new kinds of data with greater speed and clarity.

If anyone would like to discuss any of the images presented, or other microscopy needs please do get in touch.

We ask that you please do not redistribute any of the content or images within this report.

Best wishes, Stefan

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Demonstration Hardware Overview

1. **Nikon Eclipse Ti2-E** – Inverted microscope base. Fully motorised to allow automated acquisition and control. Perfect focus system (PFS), hardware based and instantaneous to allow focus stability over long timelapses. Market leading FOV (25mm) to allow more data in a given image, or vastly increased tile scan speeds. With 2 side ports, up to 5 light source inputs, multiple modalities can be combined in one Ti2-E base.

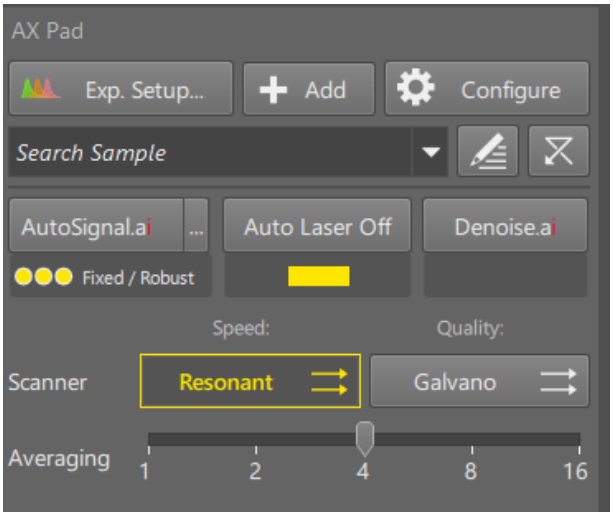
2. **AX Confocal with NSPARC detector** – Nikon Spatial Array Confocal (NSPARC) detector utilizes an ultra-low noise single photon detector array to collect a two-dimensional image at each scanned point. This method based on image scanning microscopy (ISM) principle improves signal-to-noise ratio by increasing the available signal level while simultaneously allowing imaging at increased resolution due to pixel reassignment. Single-photon sensitivity and array detection extend the capabilities of the AX system by revealing unseen details in every image, while array detection pushes the boundaries of resolution beyond the theoretical limits.

3. **OKOLab Stage top incubator** – Compact solution for maintaining temperature and gas environments for live cell imaging. Can also be quoted as a full cage setup.

4. **Plan Apochromat Lambda D Objectives** – High-performance objectives with a large FOV of 25 mm at all magnifications, with high NA and superlative chromatic corrections

5. **Silicon Immersion Objectives** - Objective lenses, perfect for imaging 3D cell cultures and thick tissue samples, provide superior depth and resolution performance for biological imaging by more closely matching the refractive index of cells and tissues.

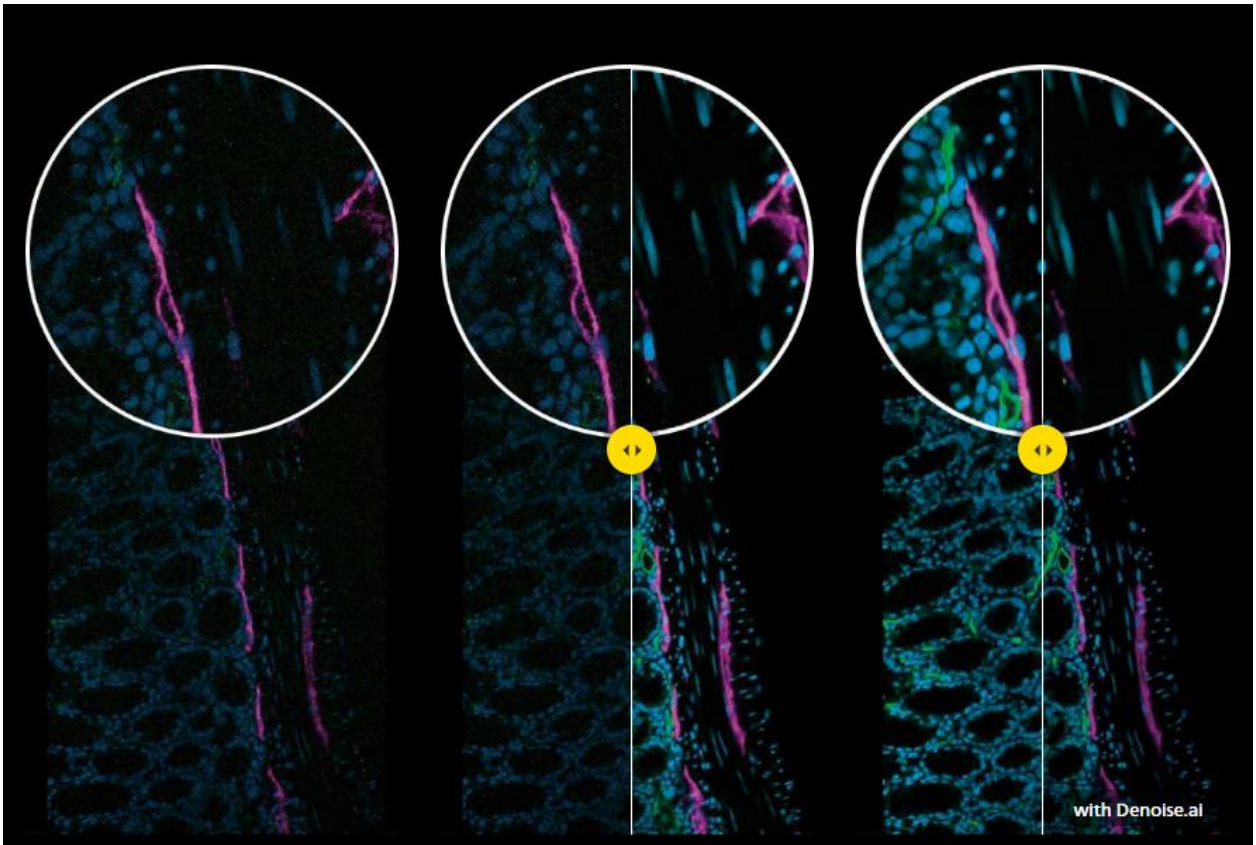
Software Highlights



NIS-C (Confocal Package) is inclusive of all the tools required to acquire, process and analyse your data in one sitting.

AX operation has been refined to make acquisition as quick and easy as possible, whilst still offering full control and repeatability.

Tools such as Autosignal.Ai, Auto Laser Off, and Denoise.Ai allow users of all experience levels to get high signal to noise images, whilst enhancing sample viability and minimising bleaching .



Starting Point

Confocal imaging has multiple variables that must be fine-tuned for the best image quality, a statistically valid signal-to-noise ratio, and long-term sample stability. NIS-Elements AI tools are designed to assist in achieving these targets.

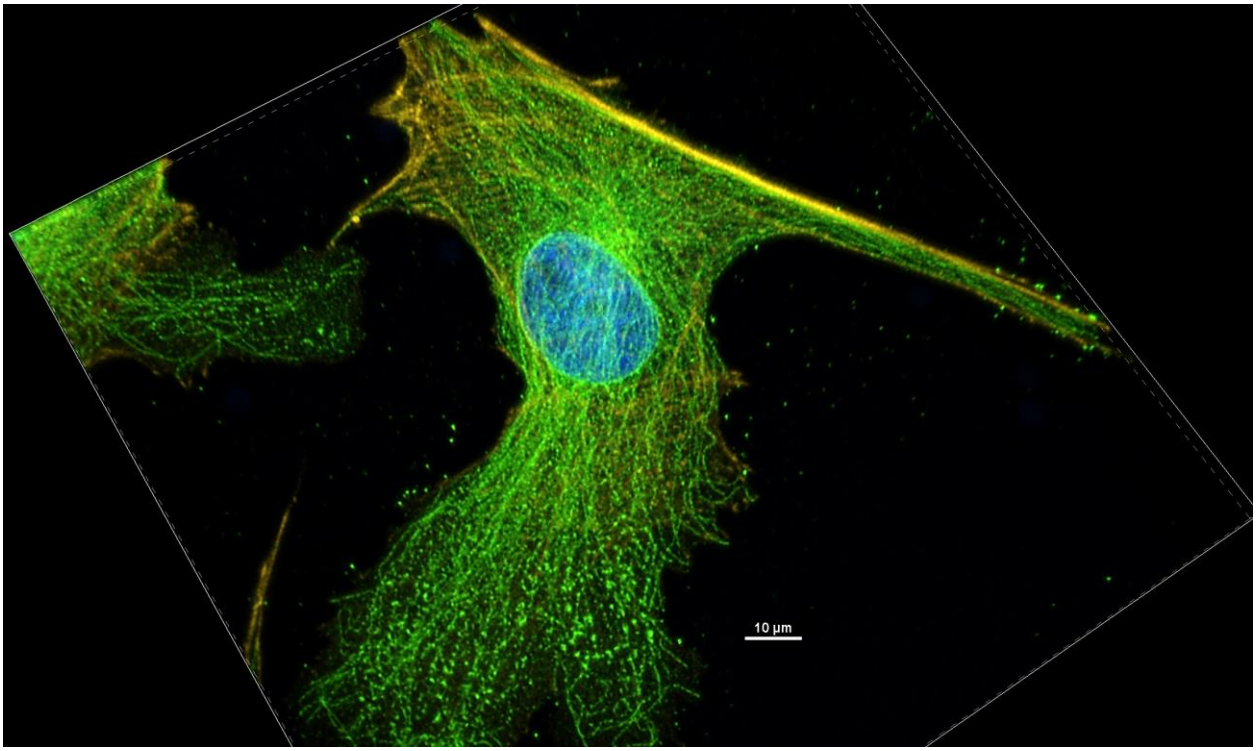
Autosignal.ai

New for AX/AX R: Autosignal.ai can suggest the best illumination and detection settings automatically, instead of users manually attempting to find the best settings by trial and error, or while scanning live and exposing the sample unnecessarily.

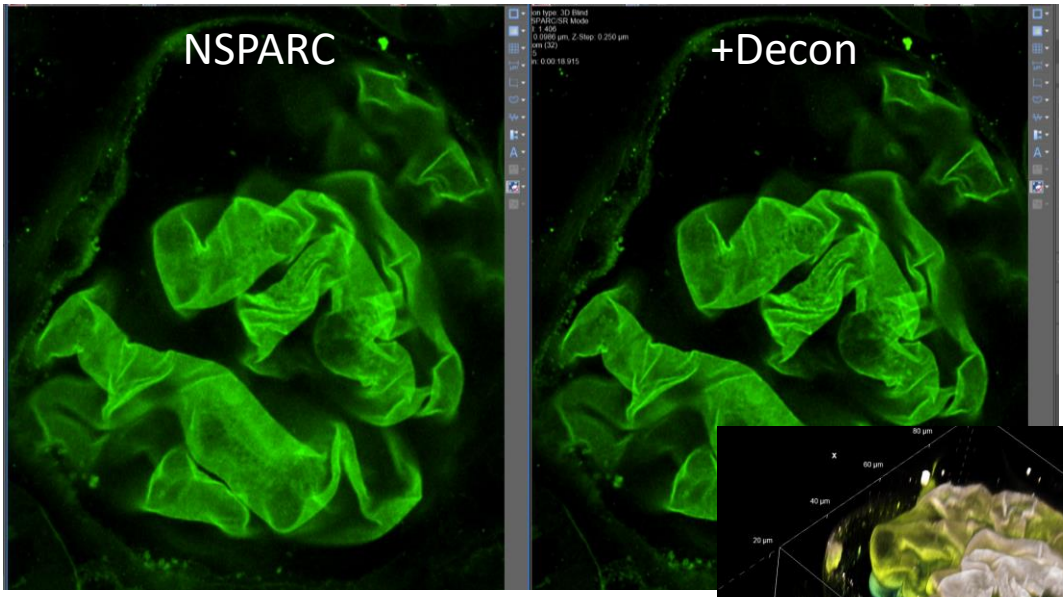
Denoise.ai

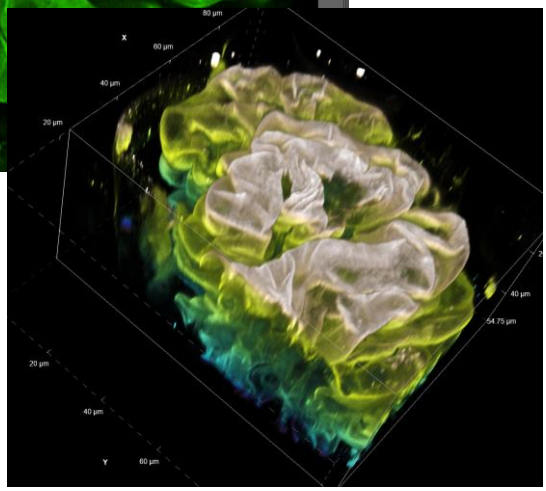
Shot noise is the main noise source in confocal imaging. Denoise.ai can remove the shot noise component from confocal images, improving the image quality and assisting in downstream segmentation.

Test Samples



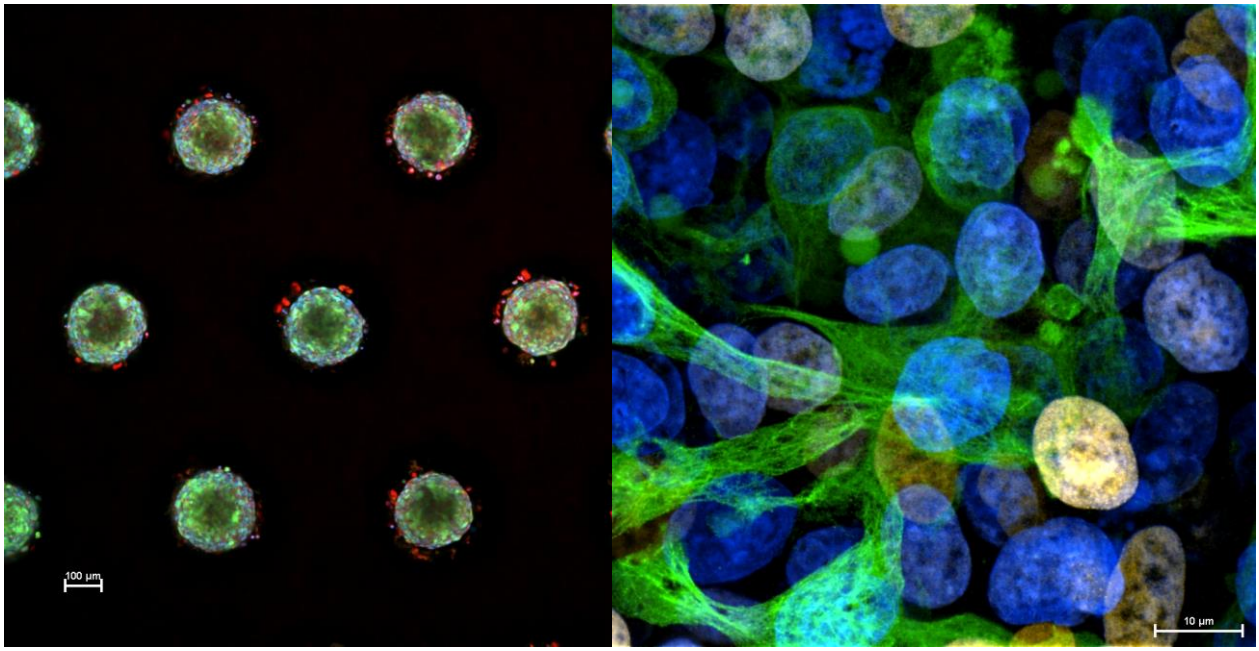
BPAE Cells
Objective: 20x 0.8 NA
NSPARC + Deconvolution

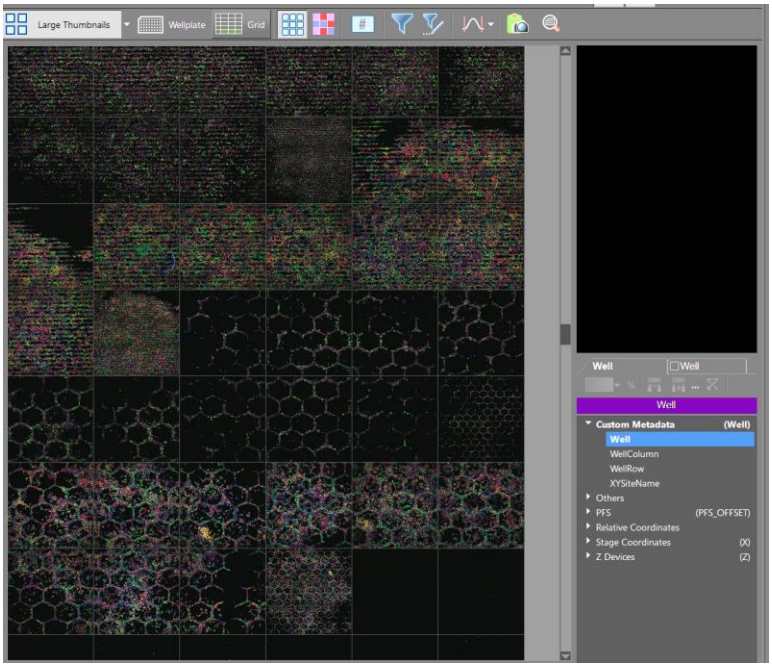




Mouse Kidney Section (Glomerulus)
Objective: 25x 1.1 NA Silicon Immersion
Notes: Excellent transmission and resolution at depth

Session 1&2





JOBS
Acquisition & Analysis Designer

NIS.ai

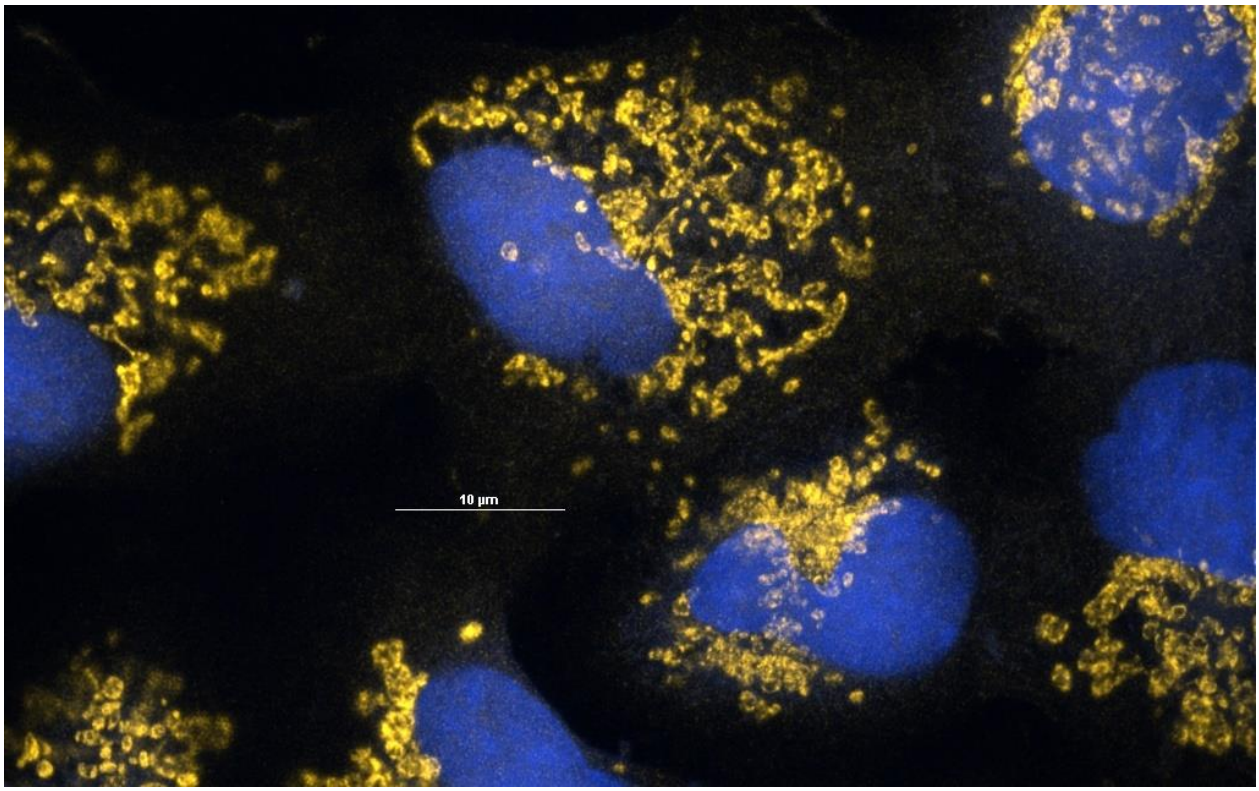
GA
General Analysis

High Content Acquisition with JOBS, GA3 and NIS.Ai

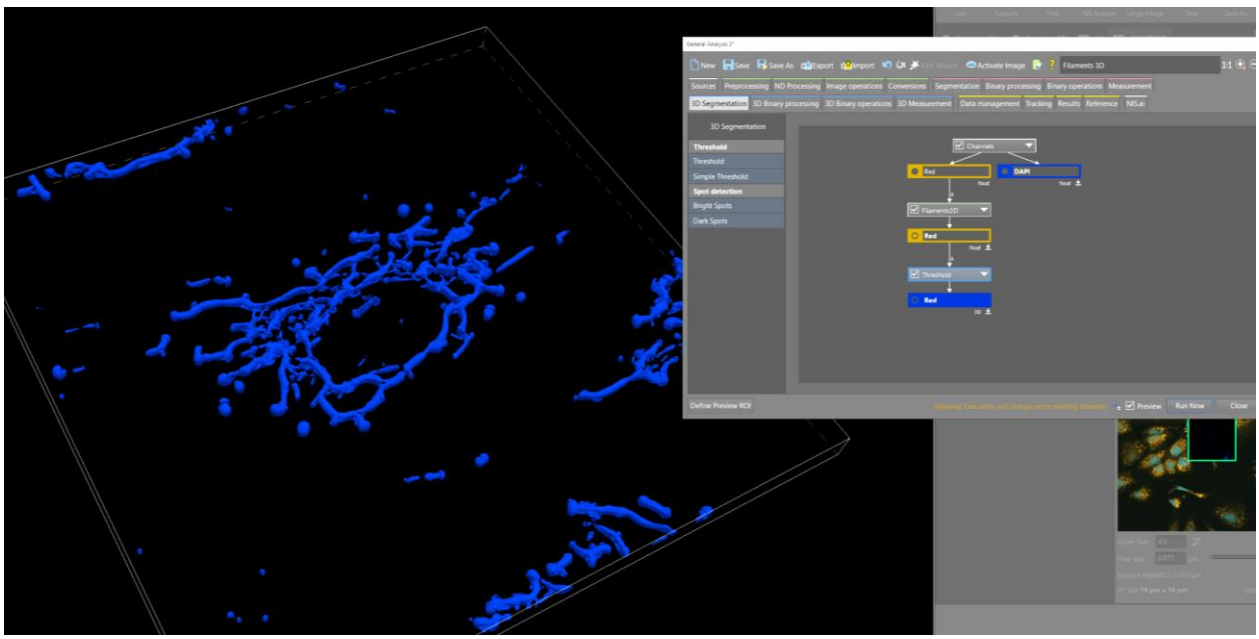
Design high content automated acquisition pipelines with JOBS. A simple graphical interface allows users of all levels to develop acquisition pipelines involving a multitude of parameters, ideal for high content applications or tissue stitching. JOBS can also be paired with our General Analysis 3 module, which similarly allows the development of analysis pipelines via a graphical interface. On the fly analysis can be incorporated into JOBS pipelines, allowing the user to acquire, analyse, and add new acquisition parameters with one button. Our artificial intelligence (NIS.Ai) modules can also be included in GA3.

For example: Take low magnification image of all wells > Threshold and find highest signal cell > Switch to high magnification and acquire 10 brightest cells

Session 3

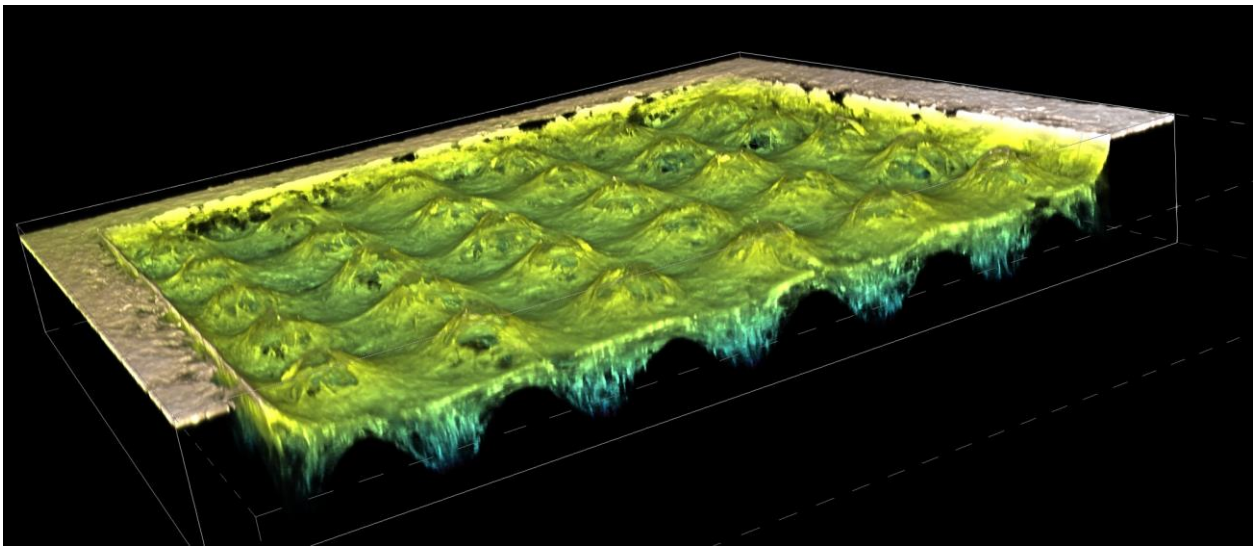


Fragmented Mitochondria
Objective: 60x Lambda D, 1.42 NA Oil
NSPARC + Deconvolution



Increased resolution and signal to noise ideal for effective threshold and automated analysis of networks via GA3.

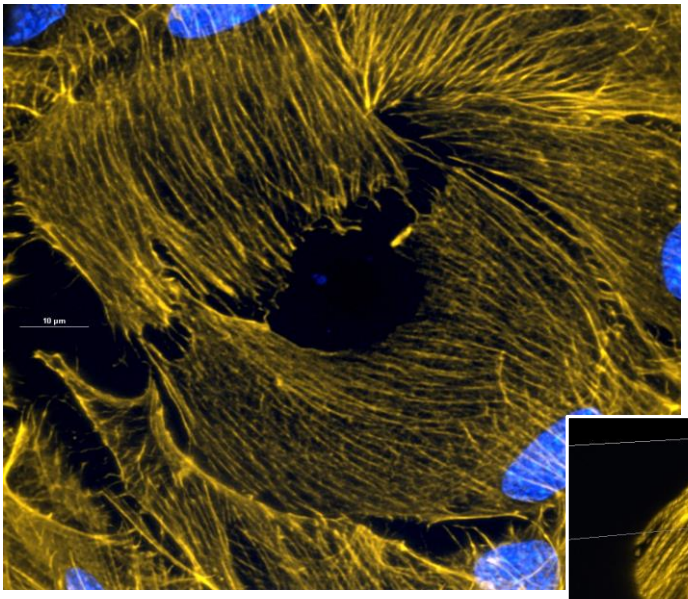
Session 4



Cells on 3D Gel

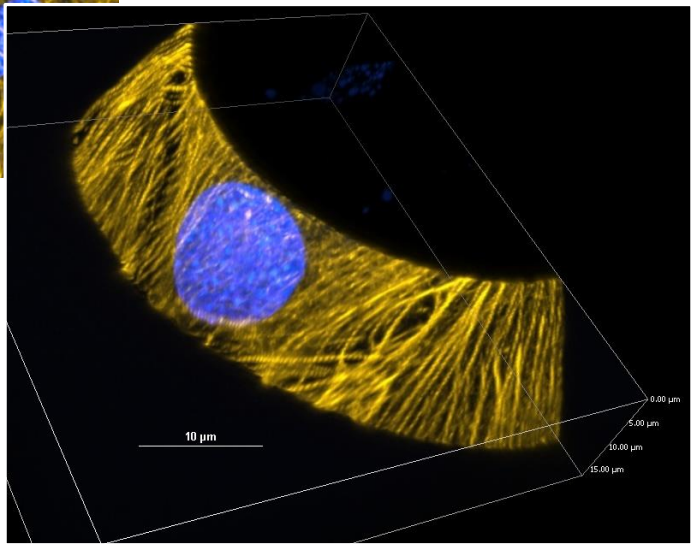
Objective: 10x Lambda D, 0.3 NA

Notes: AX Detector, overview tile scan, 5x quicker than usual due to large FOV and high signal to noise resonant scanning

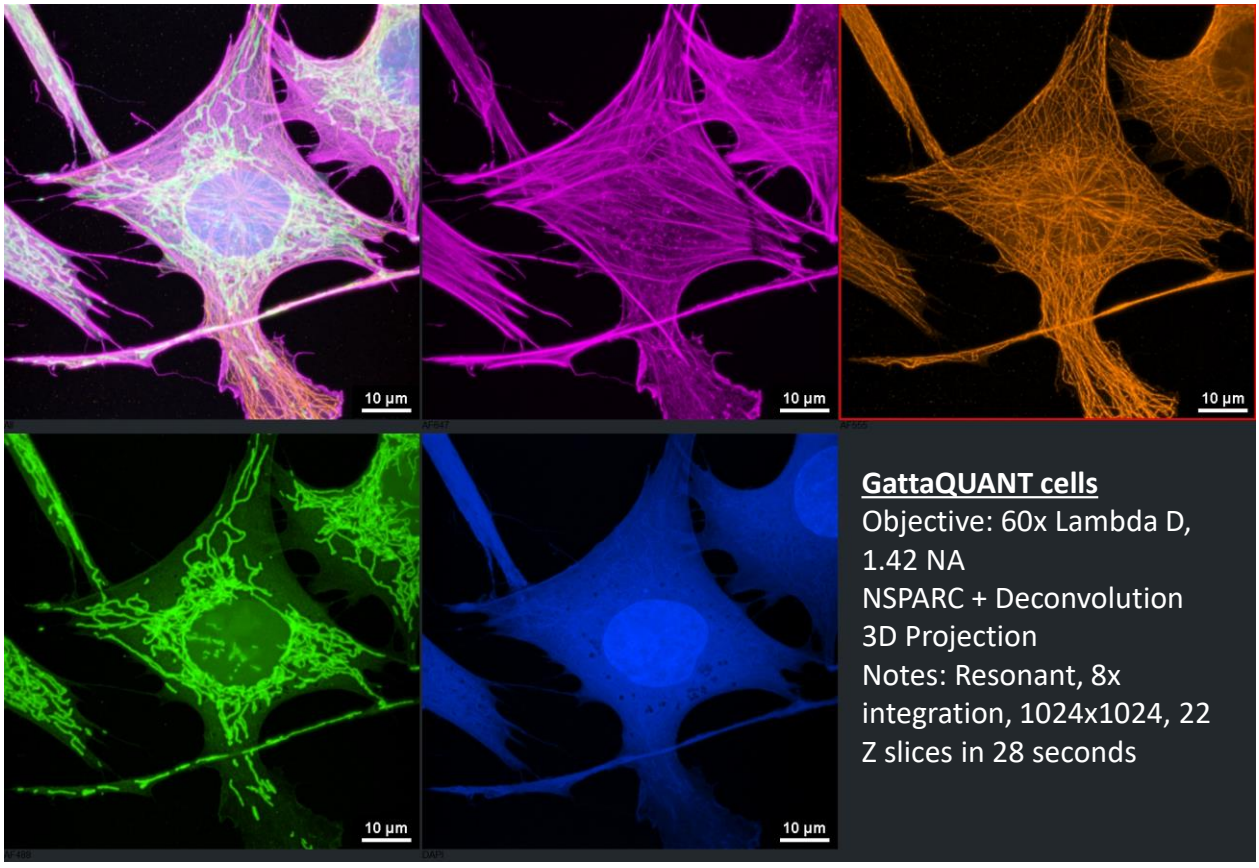


Objective: 20x Lambda D, 0.8 NA
NSPARC + Deconvolution
Maximum Intensity Projection

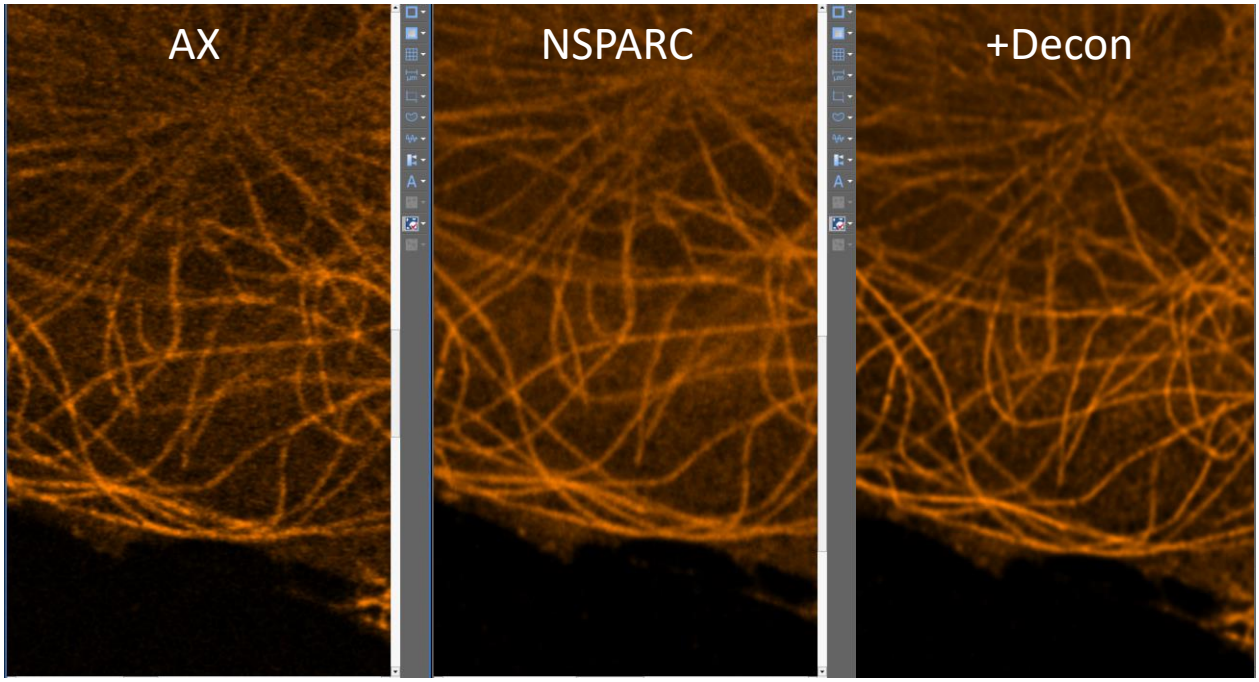
Objective: 100x SIlicon, 1.33 NA
NSPARC + Deconvolution
3D Projection
Notes: Resonant, 8x integration, 1024x1024



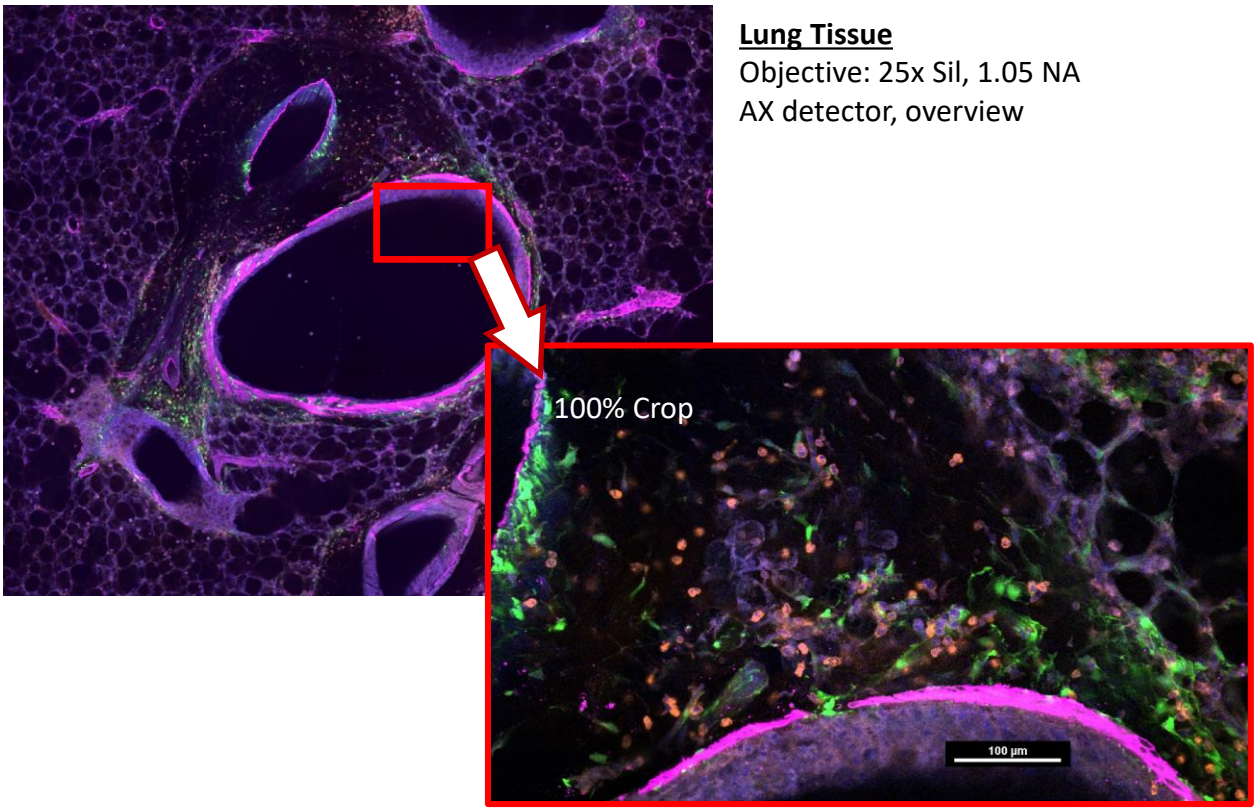
Session 5



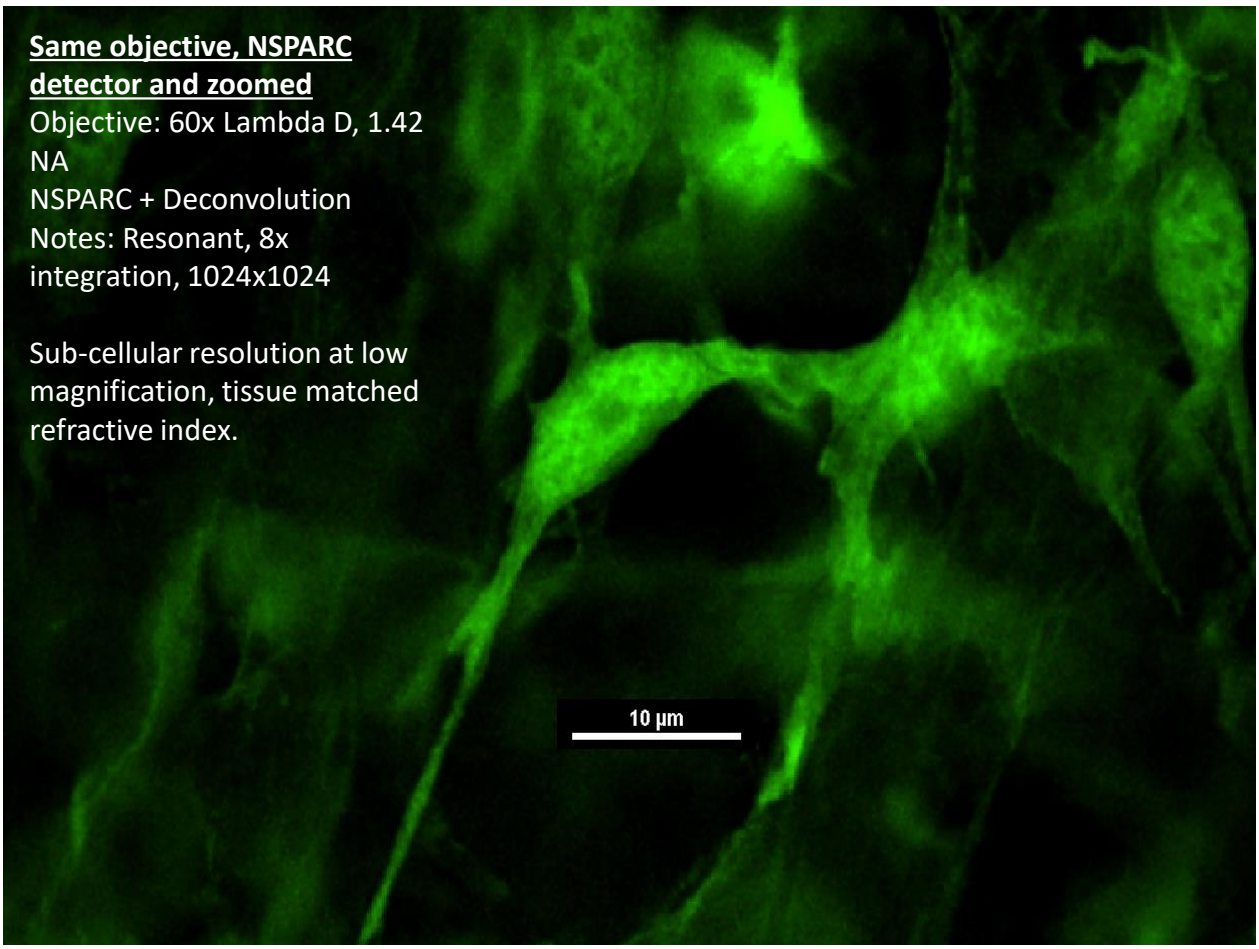
GattaQUANT cells
Objective: 60x Lambda D, 1.42 NA
NSPARC + Deconvolution
3D Projection
Notes: Resonant, 8x integration, 1024x1024, 22 Z slices in 28 seconds



Session 6



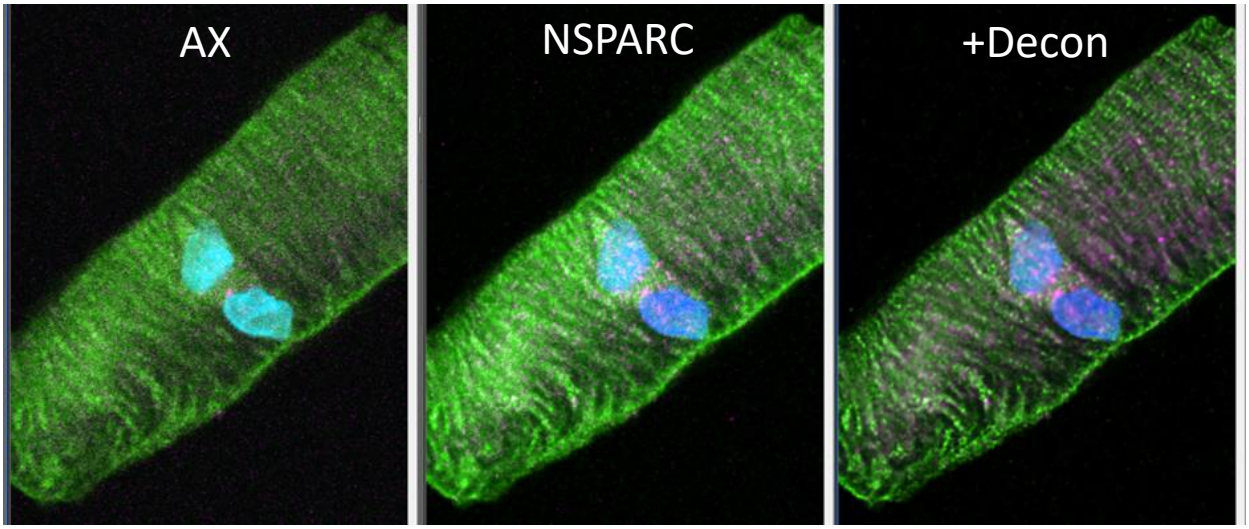
Lung Tissue
Objective: 25x Sil, 1.05 NA
AX detector, overview



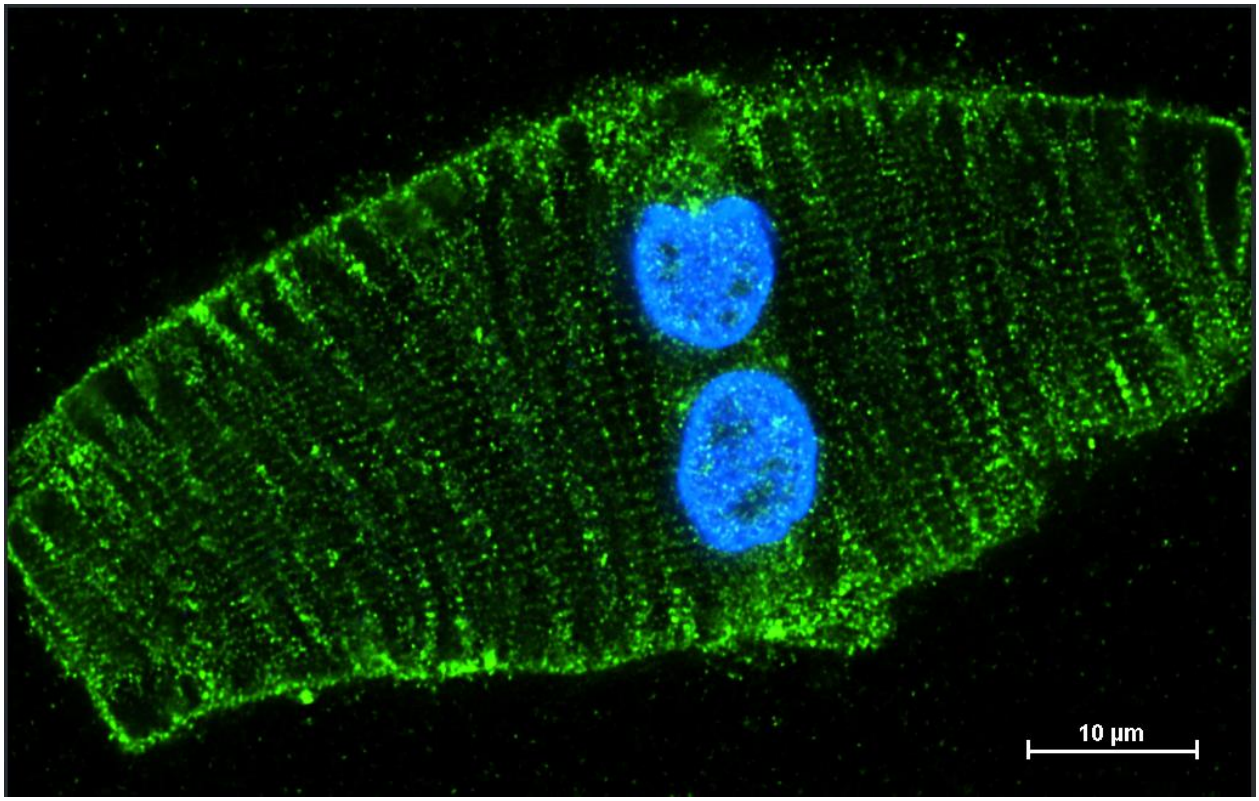
Same objective, NSPARC detector and zoomed
Objective: 60x Lambda D, 1.42 NA
NSPARC + Deconvolution
Notes: Resonant, 8x integration, 1024x1024

Sub-cellular resolution at low magnification, tissue matched refractive index.

Session 7



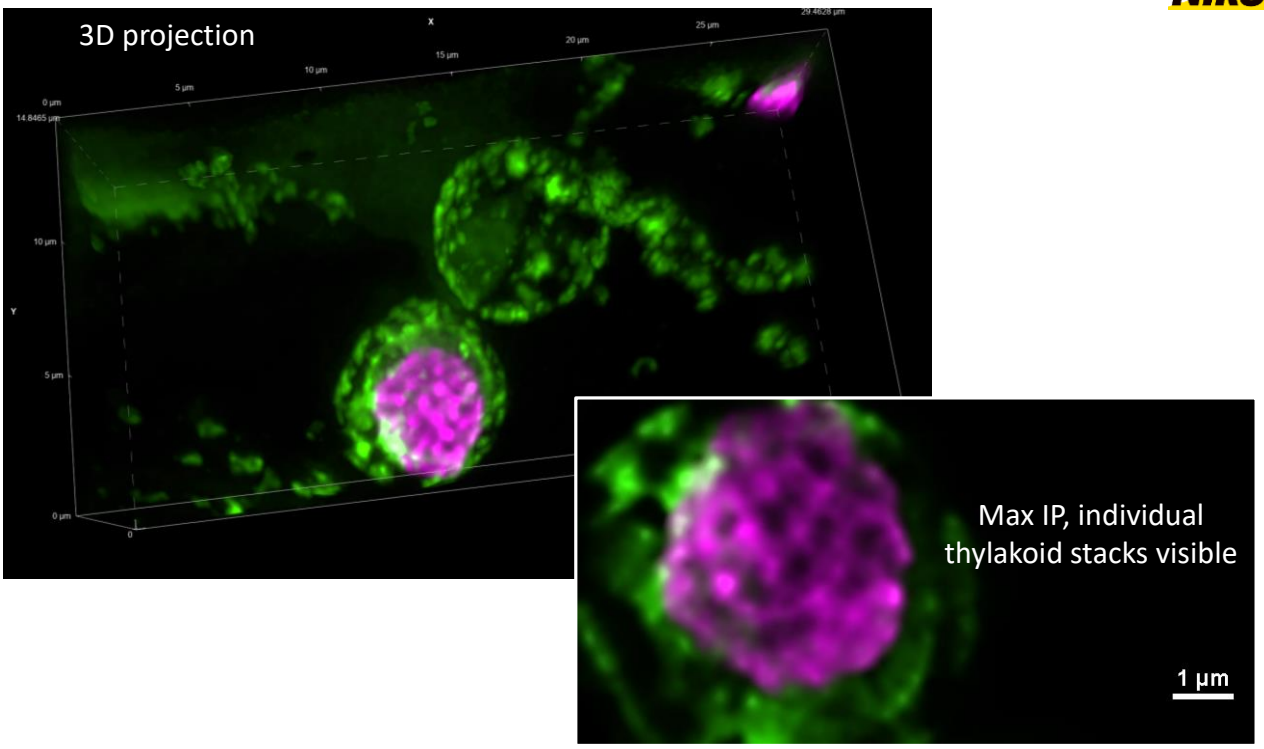
Cardiomyocytes
Objective: 20x Lambda D, 0.8 NA
Notes: Resonant, 8x integration, 1024x1024, 3 colours, 73 Z slices in 1min 18s



Objective: 60x Lambda D, 1.42 NA
NSPARC + Deconvolution
Notes: Resonant, 8x integration, 1024x652, 3 passes, 142 Z slices in under 5 minutes.

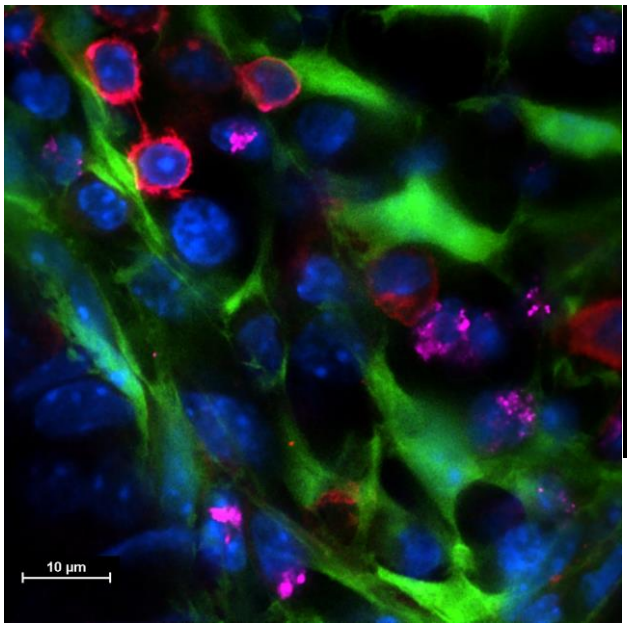
Exceptional resolving power even at lower magnifications allowing full cell to be captured in one FOV at super resolution, at high frame rates

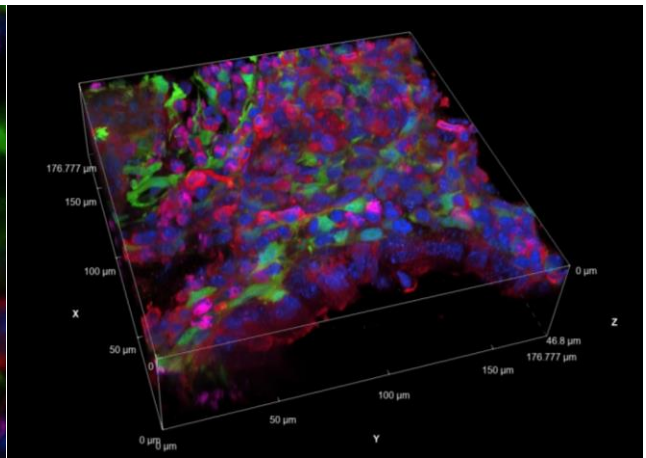
Session 8



Chloroplasts, autofluorescence (magenta) & surface protein (green)
Objective: 100x Silicon, 1.35 NA
NSPARC + Deconvolution
Notes: Resonant, 8x integration, 512x258, 2 colours, 56 Z slices in 30 seconds. 0.3 second frame times allow super-resolution acquisition in live cells, without movement of sample affecting imaging.

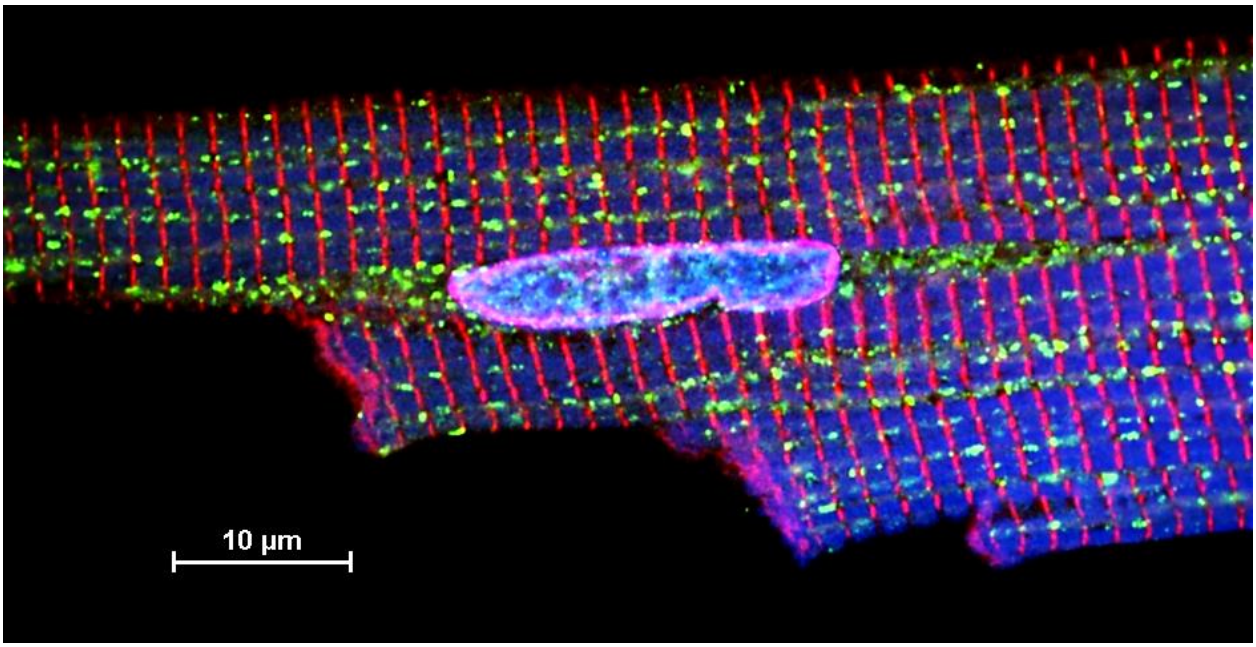
Session 9





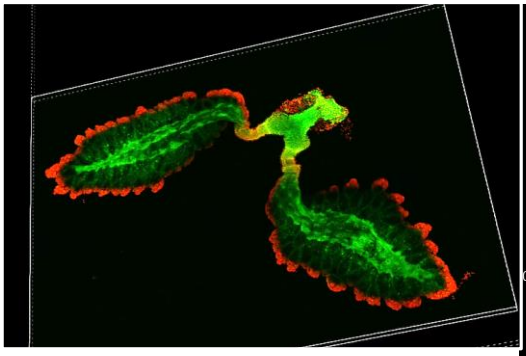
Lung Tissue
Objective: 100x Silicon, 1.35 NA
NSPARC + Deconvolution (Left)
Notes: Resonant, 8x integration. Ideal resolution and signal at depth

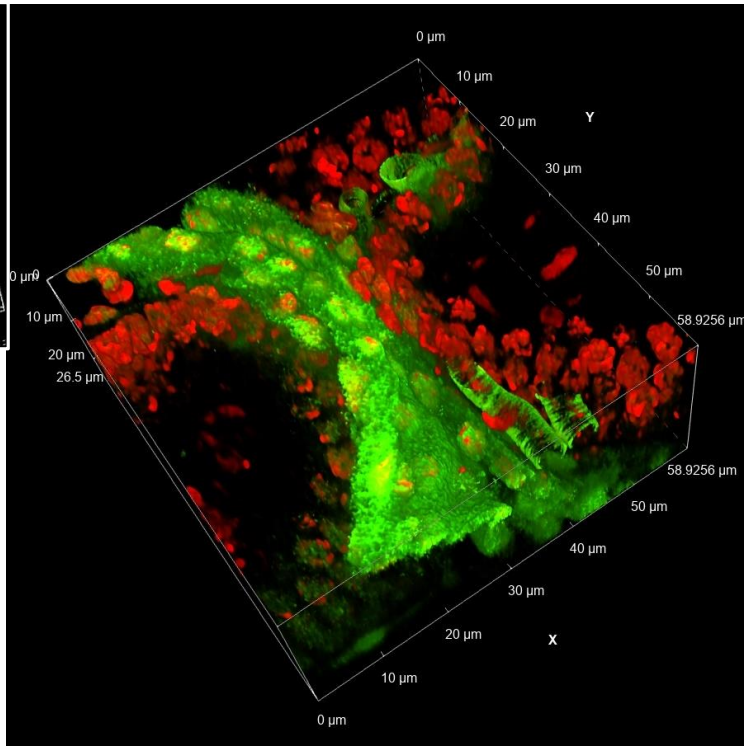
Session 10



Cardiomyocytes
Objective: 100x Oil, 1.42 NA
NSPARC + Deconvolution
Notes: Resonant, 8x integration.

Session 11





Mosquito Ovaries
Objective: 100x Sil, 1.35 NA (Right)
AX overview (Left), NSPARC + Deconvolution (Right)

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