

Revolutionary Resolution

- the OMX microscope platform

Markus Posch, Dundee

Overview of OMX

temporal

super-resolution on the OMX platform

spatial

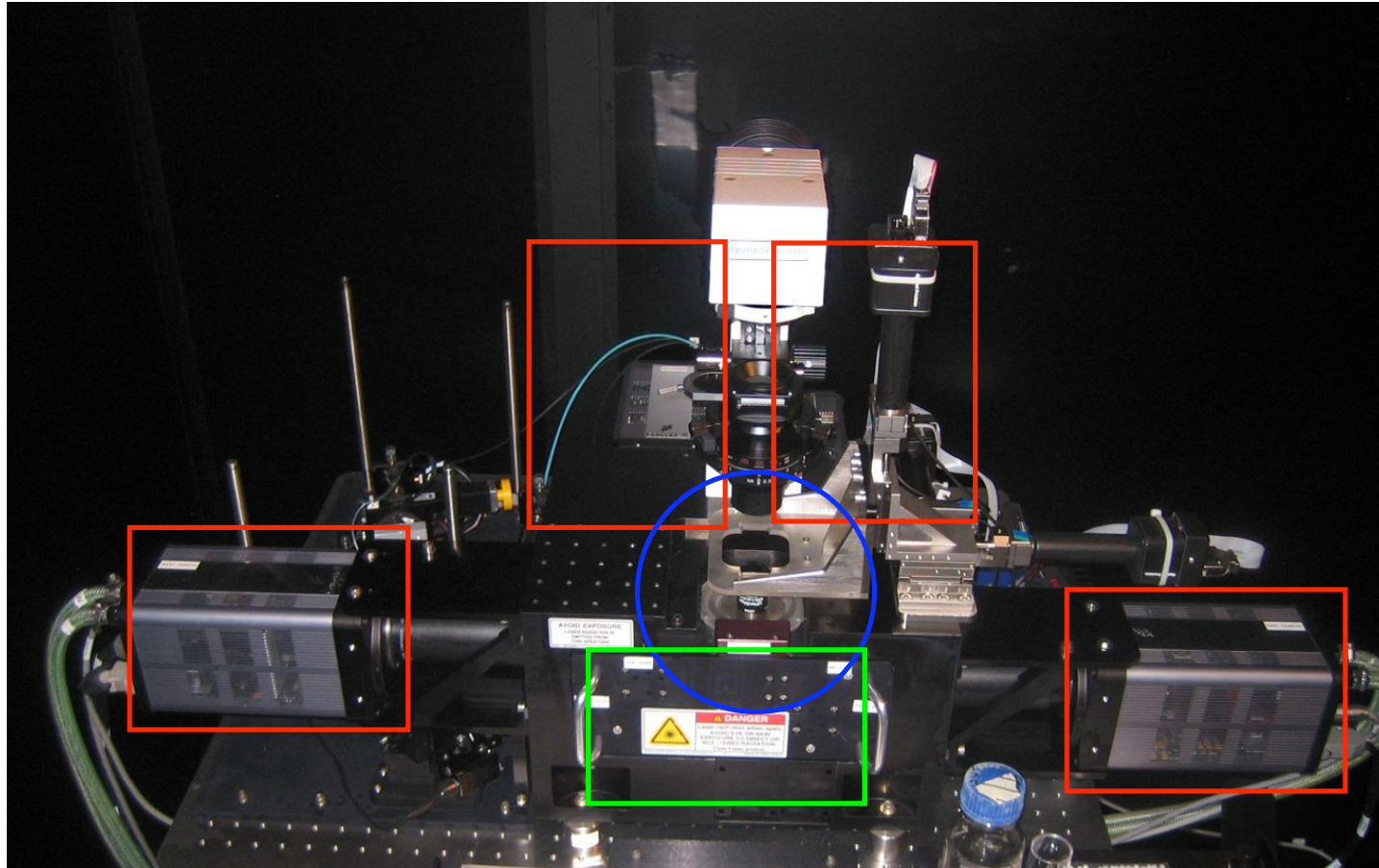
inverted fluorescent light microscope

4 laser lines for wide field illumination

4 EMCCD cameras

special design: *no scattered light (“dark”)*
mounting (thermal stability)
pizeo controlled stage

The OMX platform

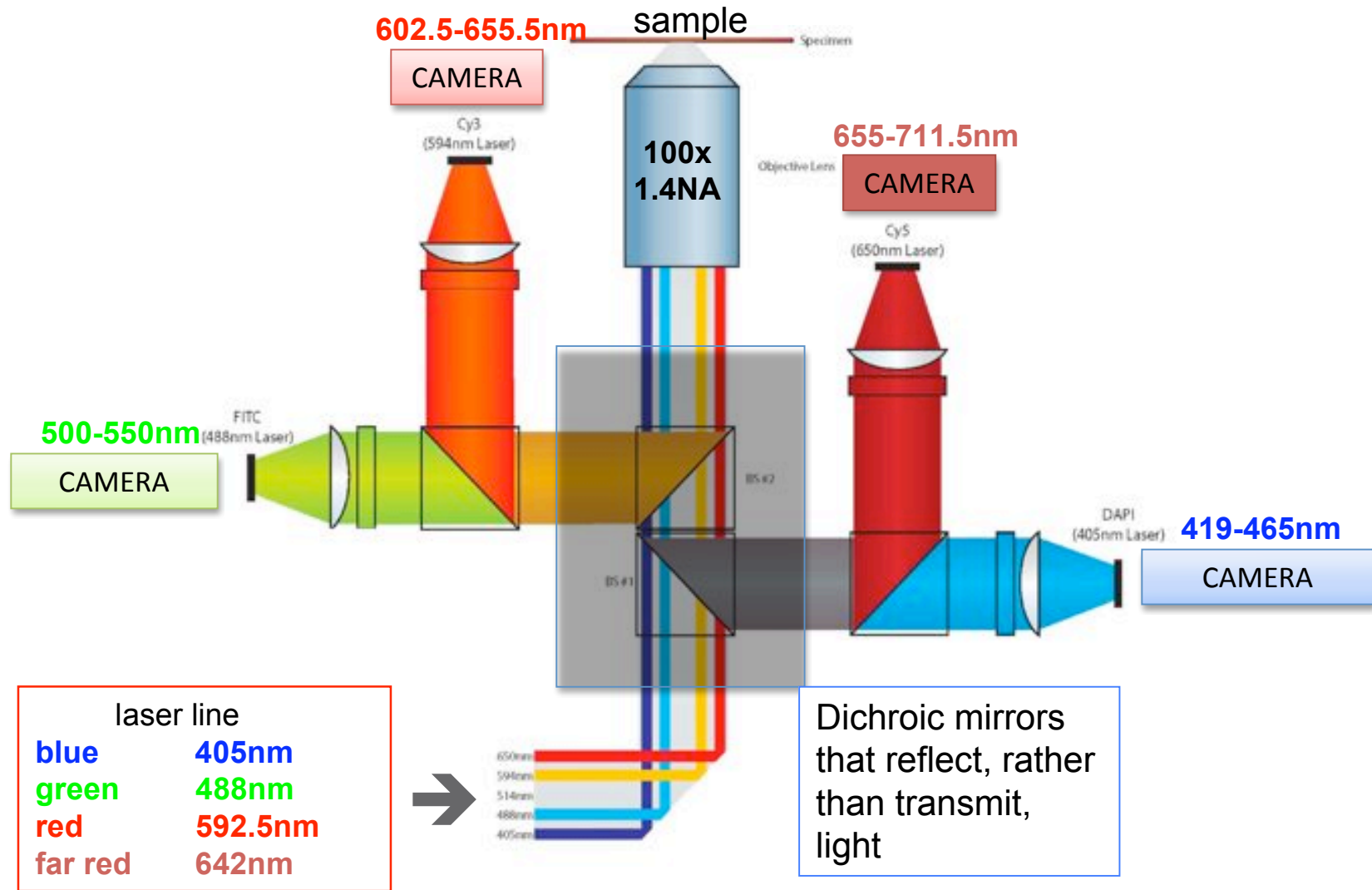


ceramic mounting
of lens and stage

4 EMCCD 16bit cameras

fixed dichroic mirror element
eliminating stray light

OMX optical setup

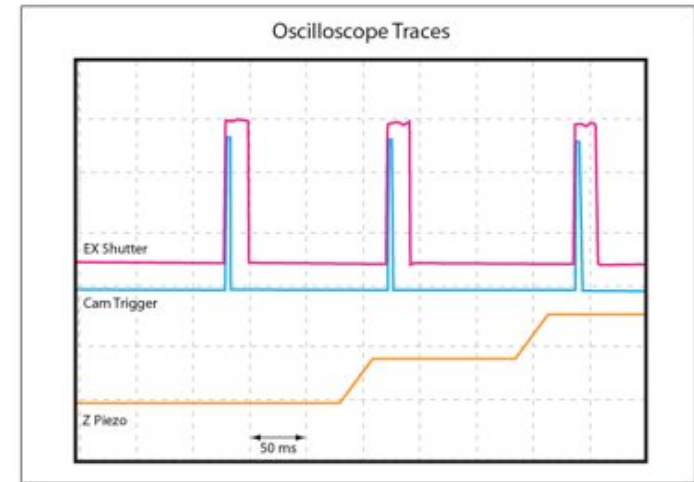


Fast-Live Imaging

4 cameras: simultaneous imaging in 4 channels

piezo driven z-stacking - highest speed

optimised shutter-camera trigger times

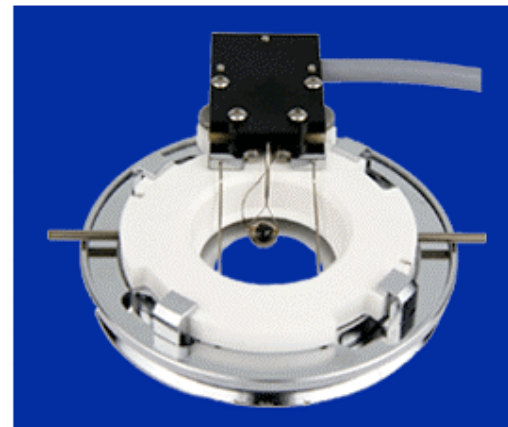


Applied Precision LLC

environmental control:

Biopetechs FCS2 chamber:

closed chamber
heated
perfusable
no CO₂ supply



Biopetechs

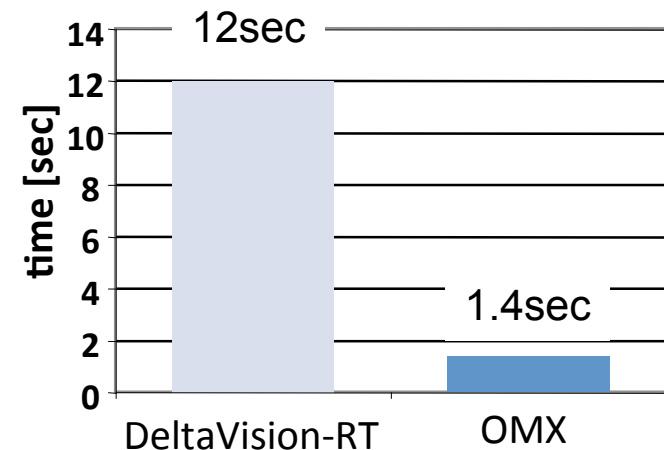
How fast can we image?

2 channels
1ms exposure
0.2 μ m steps through z
10Hz camera readout

image size	Simultaneous wavelength acquisition		Sequential wavelength acquisition	
	Frames/s	Timelapse (ms)	Frames/s	Timelapse (ms)
128x128	95	21	51	39
256x256	66	31	52	39
512x512	44	46	42	48

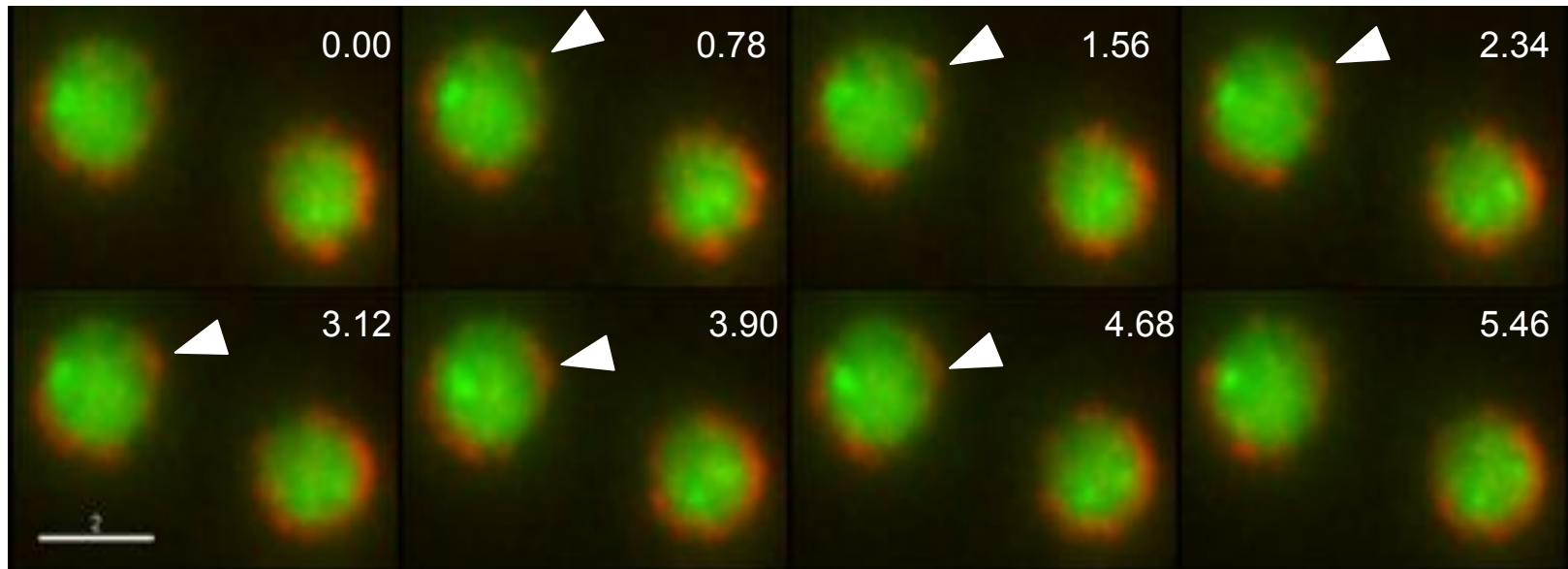
How long for acquiring one stack?

50ms exposure
3 channels (acquired sequentially)
7 z-sections (5 μ m total)
0.7 μ m spaced



S. cerevisiae

relative dynamics of chromosome loci and nuclear pores



EGFP=TetO Chrom 4
mCherry=Nuclear Pore

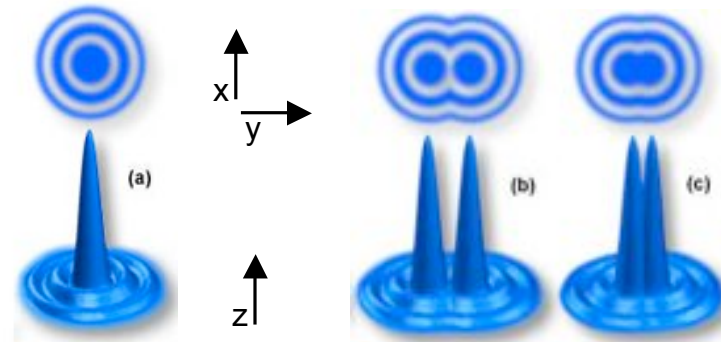
14 z stacks
3.9 μ m total
0.3 μ m step size
Simultaneous acquisition
5 ms exp.
780 ms. timelapse

Spatial Super-Resolution

Rayleigh:

limit at which 2 intensity maxima
can be resolved as separate entities

$$D = \frac{0.61 \times \lambda}{NA}$$



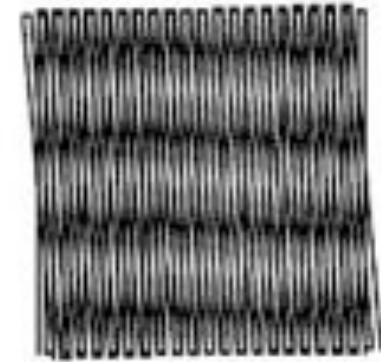
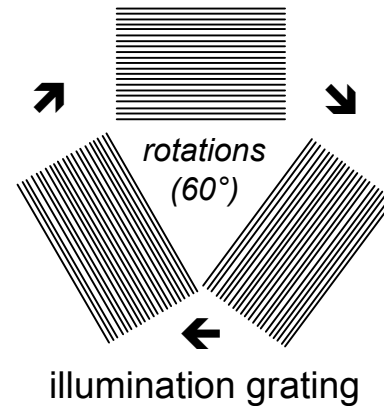
Point Spread Function
pattern of light from
single 'point' source

resolved unresolved

	Resolution in xy (nm)	Resolution in z (nm)
3D-SIM	100	200
WF	230	1000
CLSM	180	500
2-photon	200	400
TIRF	230	100

Structured Illumination (SI)

patterned light illumination
shifted along 5 phases
rotated in 3 angles



Moiré pattern

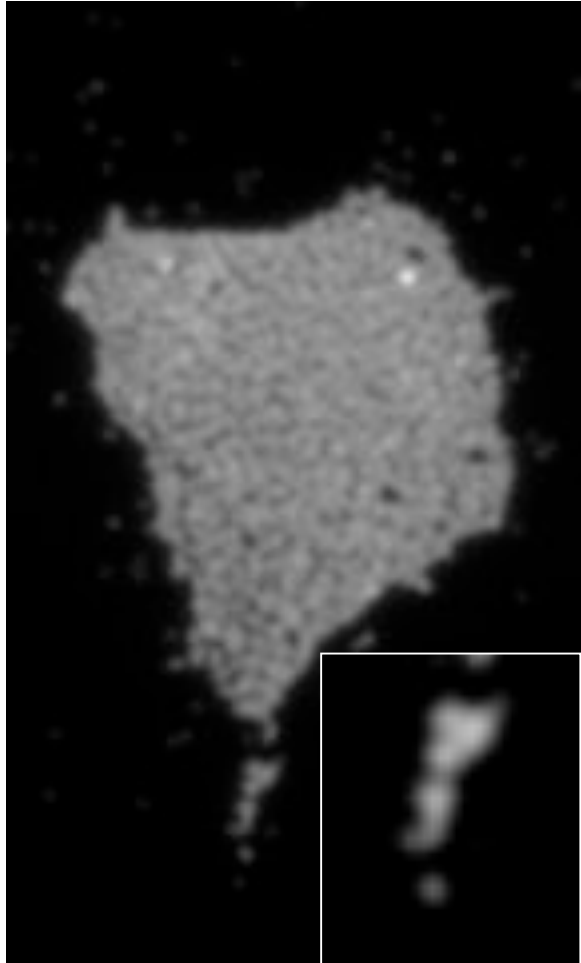
patterned light generates new Moiré pattern

computer reconstruction: reverse calculation of identities that
had given rise to these Moiré patterns
(via Fourier-transforms in higher frequency space)

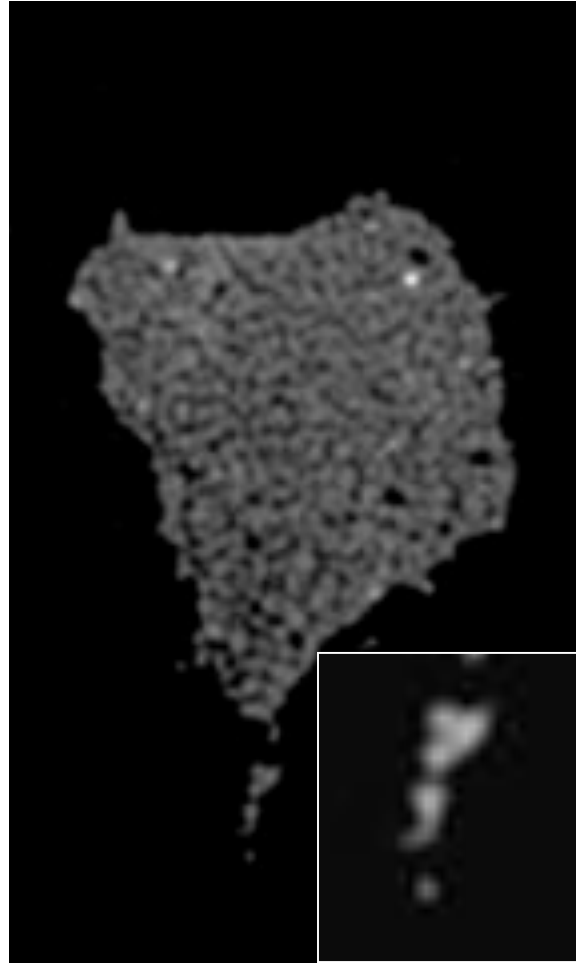
- image with highly improved spatial resolution
- combine with 125nm z-sectioning: **3D-SIM** (axial resolution)

Comparison of Microscopy Techniques

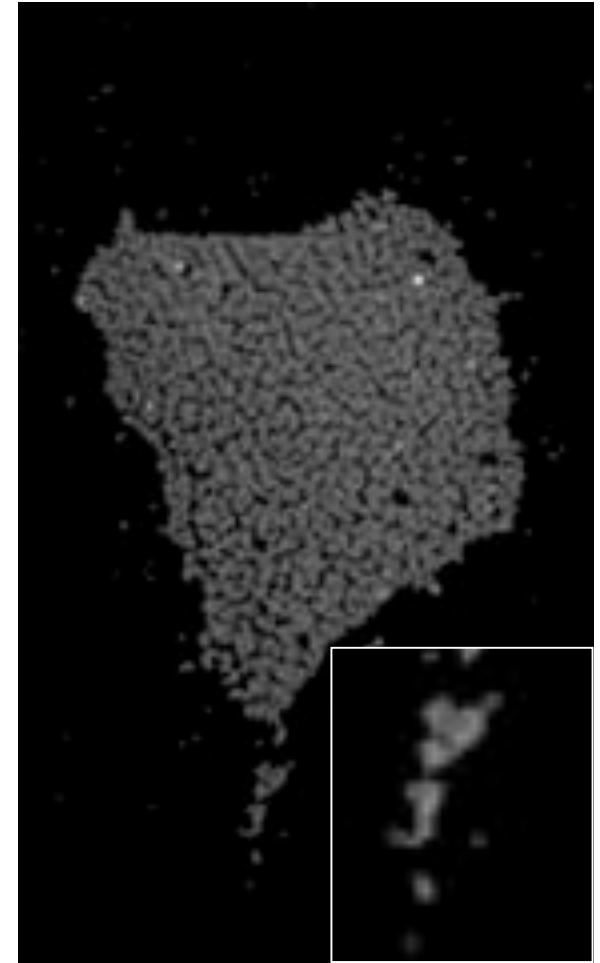
Conventional



Deconvolved

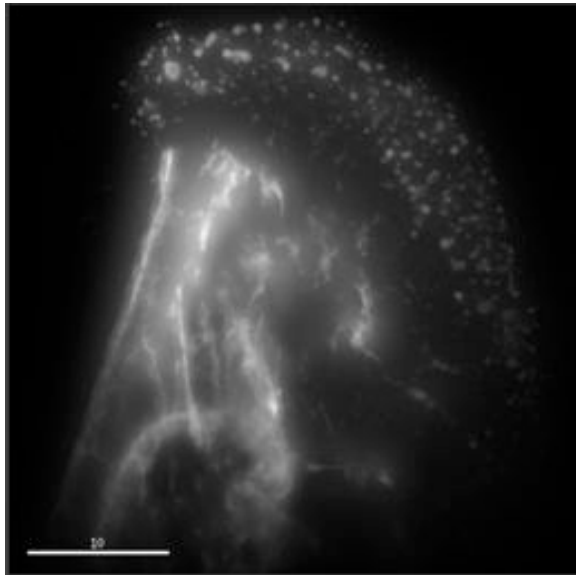


OMX

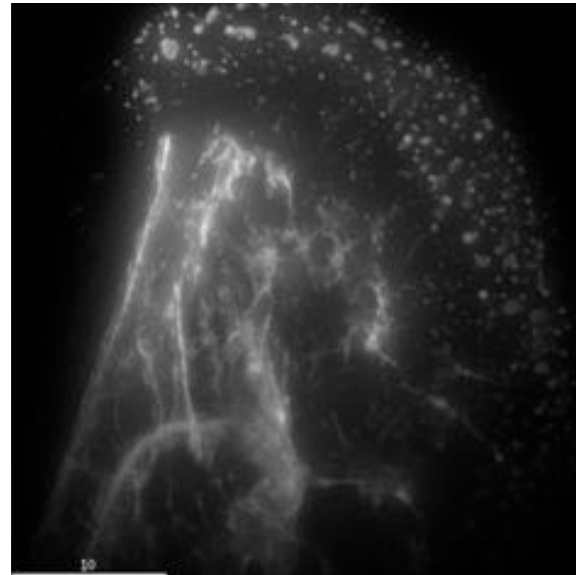


Intermediate filaments

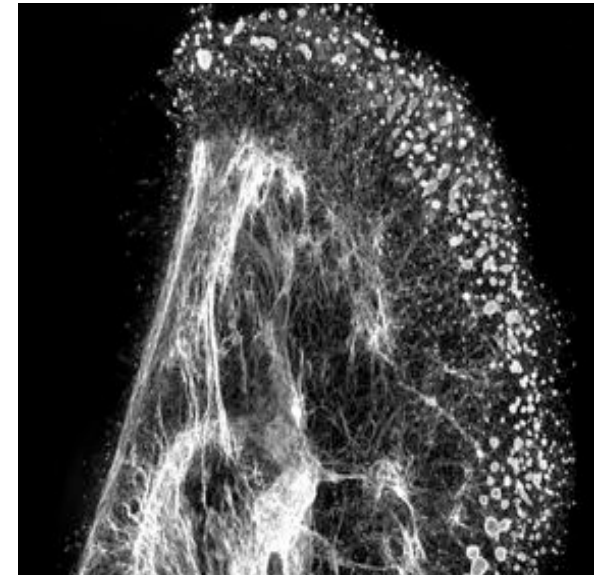
Keratin-14



Conventional

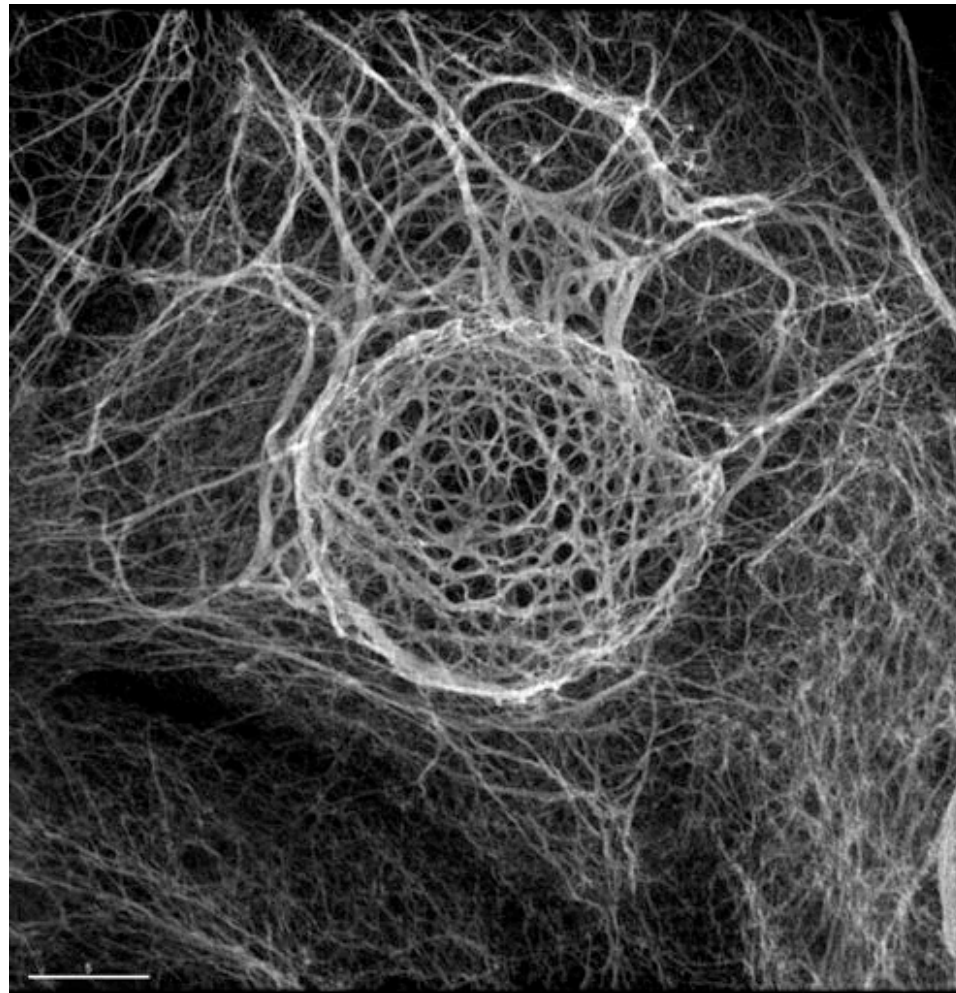


Deconvolved

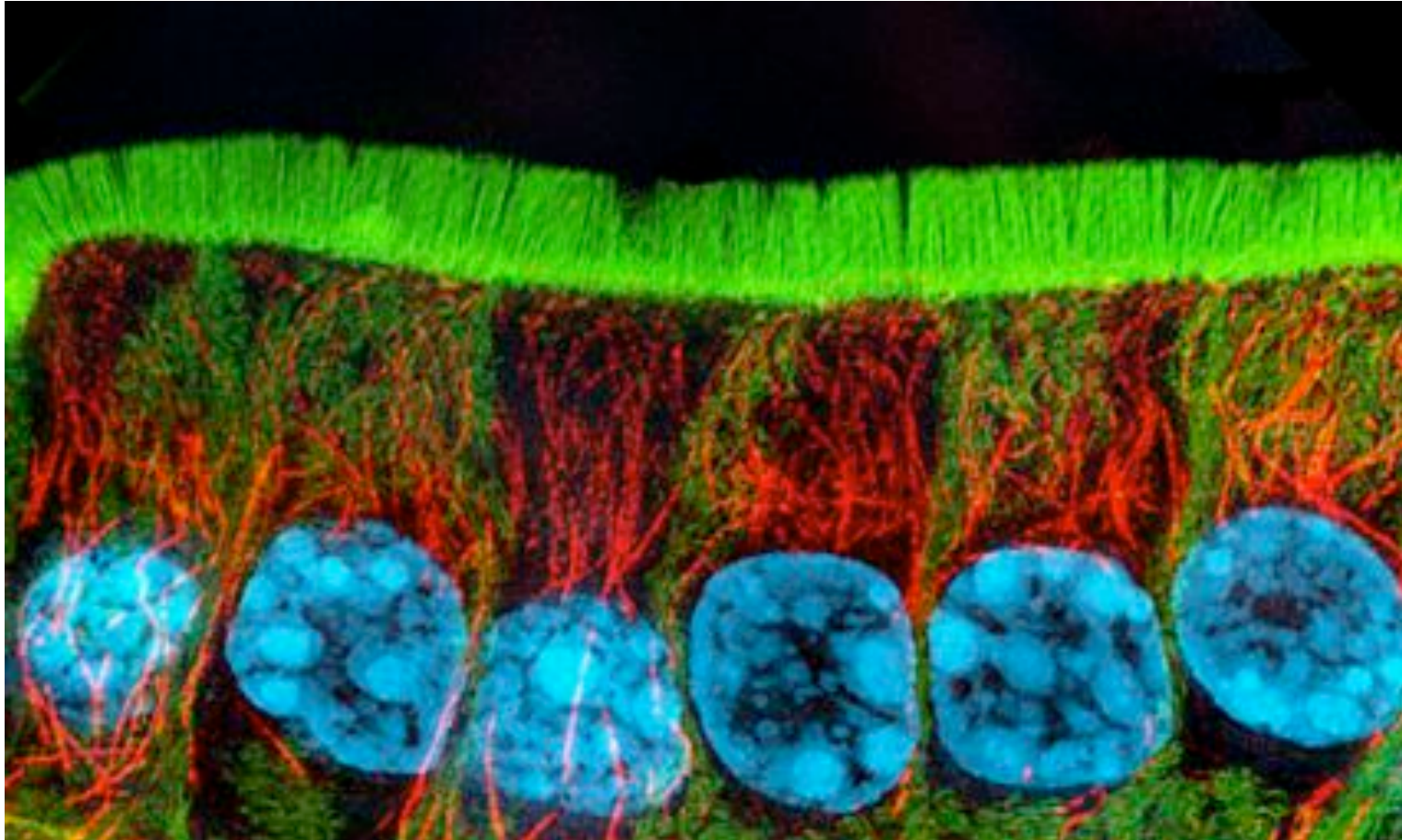


OMX 3D-SIM

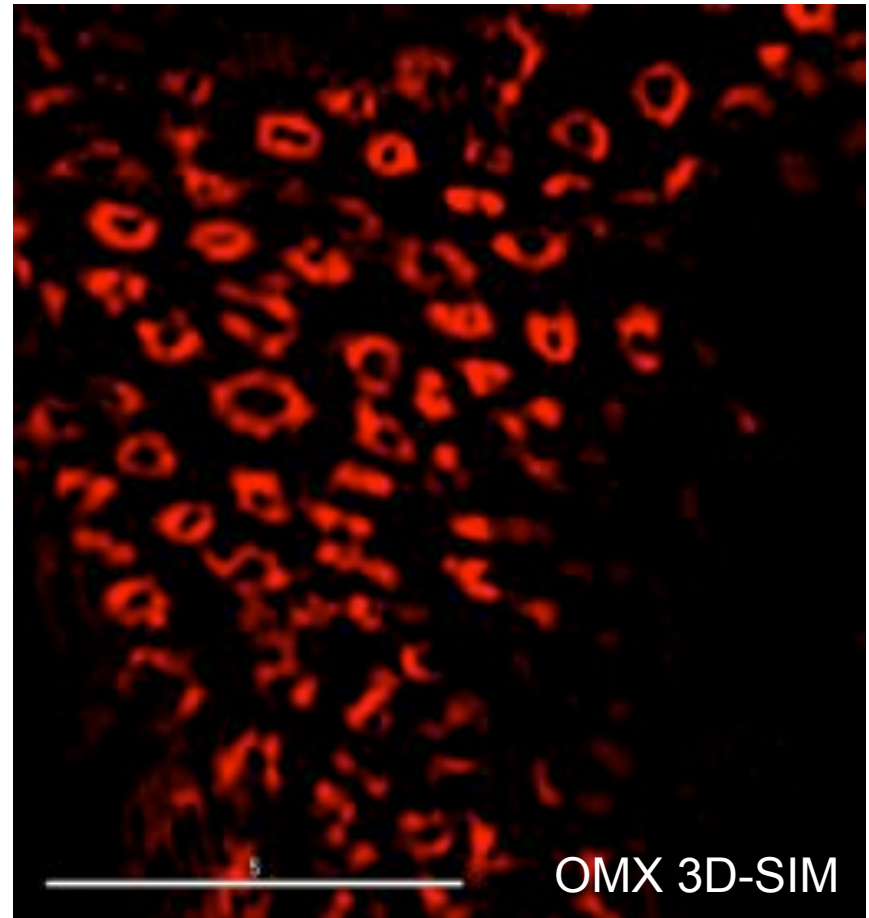
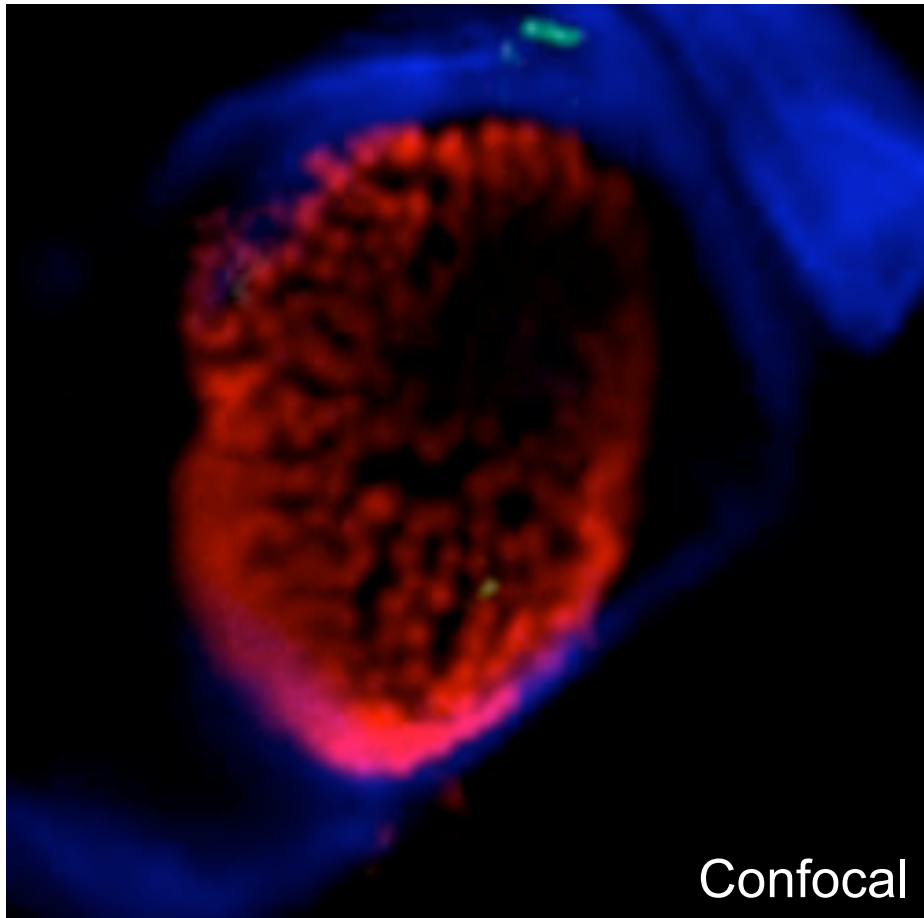
Intermediate filaments



Microvilli in the mouse gut epithelium

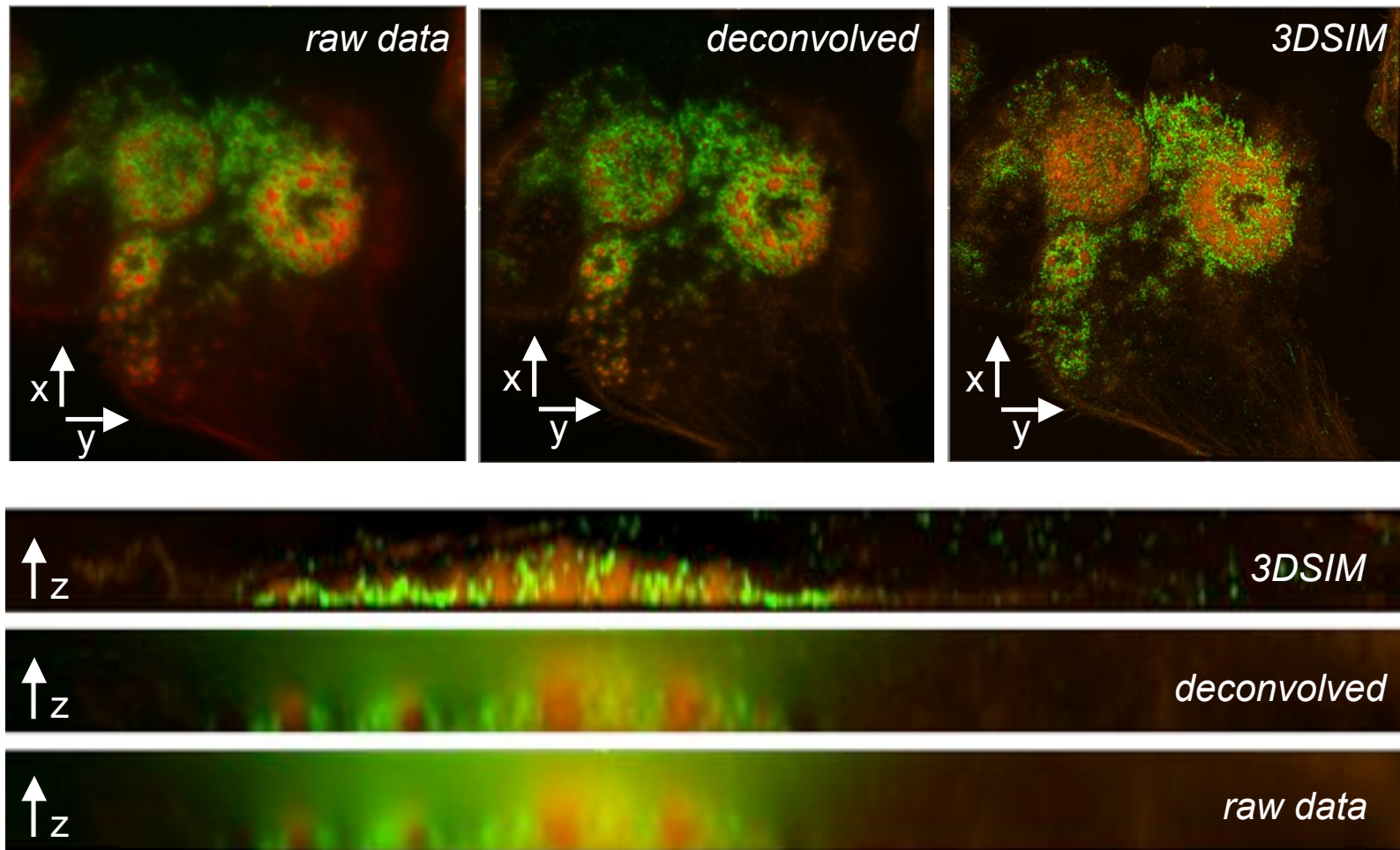


Tobacco sieve plate plasmodesmata



Karl Oparka and Emma King

Dendritic cell podosomes



Gawden-Bone C, Zhou Z, King E, Prescott A, Watts C, Lucocq J

Samples tried and tested

The list is long and ever-growing...

- Mitotic spindles, podocytes, actin and intermediate filaments in human cells
- Plasmodesmata pores in epidermal peels from plant leaves
- FISH in mouse embryos
- Actin and microtubules in mouse gut cryosections
- *Drosophila* synapses
- Budding yeast spindles
- Protein accumulation in *Staphylococcus aureus*

How difficult is it?

multicolour fluorescence imaging with of fixed samples -

no specific sample prep required

however a few considerations:

photo bleaching: *fluorophores:* Alexa 488, Alexa 594, PacificBlue

fluorescent proteins: EGFP, TagBFP

antifade: p-phenylenediamine/glycerol, prolong etc.

use no. 1.5 cover glass

imaging to a depth of max. 16µm from cover slip

What OMX can do for you:

temporal super resolution:

4 channels simultaneously

up to 50 frames per second

z-stacking

spatial super resolution:

resolution down to 100nm in xy and 200nm in z

3 channels (blue, green and red)

classic IF sample preps

How difficult is it?

acquisition control via single screen

individual channel/camera control

The screenshot displays a microscopy software interface with several key components:

- Acquisition Controls (Top Left):** Includes settings for Imaging Mode (Sequential), Light Path (Conventional), Channel (FITC, DAPI, Cy5, A594), Mode (EMCCD 5MHz), EMCCD Gain (3000), Exposure (30, 40, 5, 10), Excitation (468, 495, 642, 593), and %T (10.0%, 1.0%, 1.0%, 10.0%).
- Stage Positioning (Middle Left):** Shows a 2D stage map with coordinates (x=14073.0, y=14604.0, z=12935.2) and movement controls for dx, dy, dz, and Z safety limit.
- Stitched Mosaic Image (Bottom Left):** A large image showing a grid of individual channel images, with a zoomed-in view of a specific region.
- 4 Channel/Camera Image Display (Right):** A 2x2 grid of live images from different channels, each with its own statistics (Min, Max, Mean) and temperature (Temp: -20.0 C).

stage control in x, y and z

4 channel/camera image display

stitched mosaic image

The SULSA OMX homepage

<http://microscopy.lifeci.dundee.ac.uk/omx>



The screenshot shows the homepage for the OMX super-resolution microscope. At the top right, it says "microscopy home". On the left, there is a grayscale image of a biological specimen. The main heading is "structured illumination mx fast live-cell microscopy". Below this is a navigation menu with links: "home", "omx", "staff", "news & events", "images", "links & resources", and "contact us". The main content area is titled "Introduction to OMX" and contains the following text:

In October 2008, the College of Life Sciences at the University of Dundee took delivery of one of only seven OMX super-resolution microscopes in the world.

The technology of OMX consists of two main imaging protocols, Structured illumination and fast, multi-spectral, live-cell 3D imaging. These functions will allow users to explore the spatial and temporal elements of biological processes with greater resolution, using light microscopy, than ever before in Scotland.

OMX has been brought to Scotland by the Scottish Universities Life Sciences Alliance (SULSA), a strategic partnership between the Universities of Aberdeen, Dundee, Edinburgh, Glasgow, St Andrews and Strathclyde, and the Scottish Funding Council (SFC).

By linking researchers together and pooling resources, SULSA provides Scottish life scientists with the state-of-the-art technologies they need to retain their competitive edge. OMX is a perfect example of this.

At the bottom of the page, there are logos for "university of dundee" and "SULSA".

Acknowledgements

All OMX users

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