Mesoscale optical imaging

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Focus on Microscopy 2026
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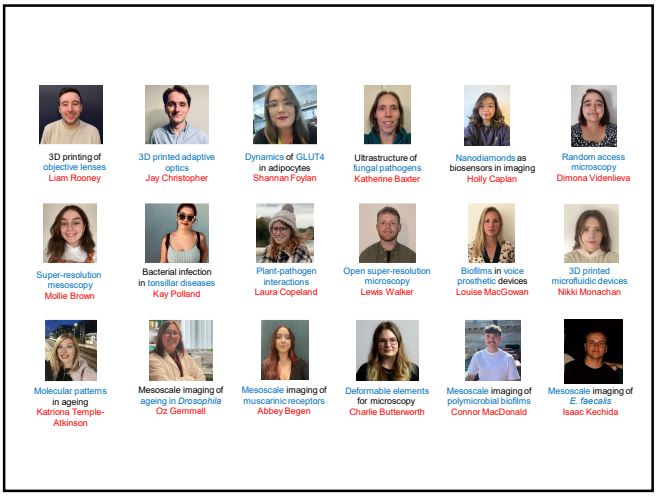

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Learning objectives

- Define mesoscale imaging and its importance
- Understand physical and optical constraints
- Compare major imaging hardware architectures for mesoscale imaging
- Explore computational reconstruction methods
- Connect systems to biological applications

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Tutorial structure

1. Introduction to mesoscale imaging
2. Fundamentals
3. Current methods and solutions
   a) Simple/surface
   b) Volumetric
   c) High-speed
   d) Computational
4. Data in mesoscale imaging
5. Outlook

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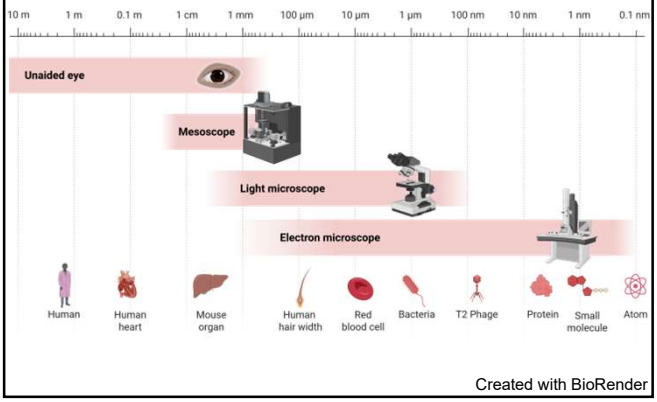
Introduction to mesoscale imaging



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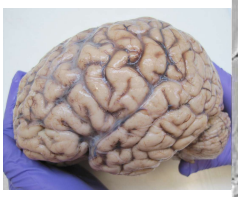
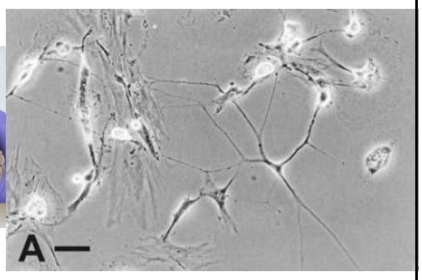
Motivation for mesoscale imaging





Macikova *et al*, Biologia, 2009

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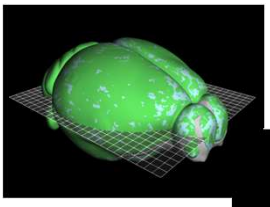
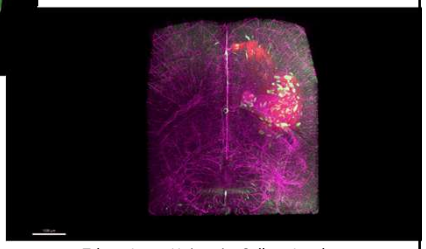
Motivation for mesoscale imaging



Allen Brain Explorer® beta

Fluorescent staining/reporters for molecular specificity
Technologies for whole organ/large volume imaging & image data processing and analysis



Tchern Lenn, University College London

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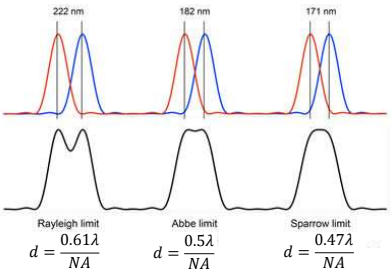
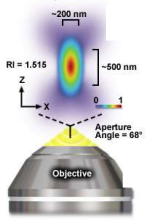
Key parameters - resolution

$\lambda = 510$ nm
NA= 1.4



$d = \frac{0.61\lambda}{NA}$ $d = \frac{0.5\lambda}{NA}$ $d = \frac{0.47\lambda}{NA}$



$d = \frac{2\lambda n}{NA^2}$

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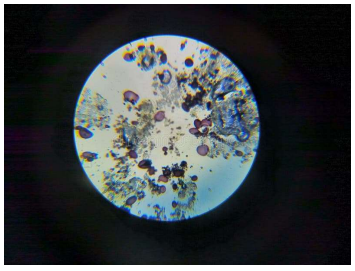
Key parameters – field of view



Field of view = FN/Mag

For camera systems:
FOV = sensor size / system magnification

Magnification is the magnification of the object at the intermediate image plane. The image is then further magnified by the eyepiece or sensor system.

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Key parameters – space bandwidth product

Product of the field of view & spatial frequency range:

$$\mathrm{SBP} = \pi\left(\frac{\mathrm{FN}}{2\mathrm{Mag}}\right)^2 \cdot \pi\left(\frac{2}{\lambda} \cdot \mathrm{NA}\right)^2 = \frac{\pi^2 \mathrm{FN}^2}{\lambda^2}\left(\frac{\mathrm{NA}^2}{\mathrm{Mag}^2}\right).$$

Determines the number of resolvable pixels in the image field

SBP ≈ number of resolvable pixels → drives data size

For 3D imaging, multiply by z but consider whether resolution is isotropic

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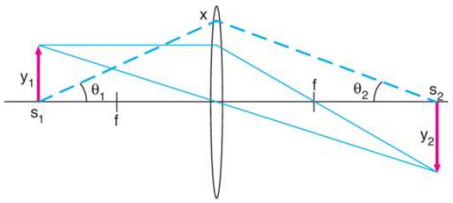
Lagrange invariance / Smith-Helmholtz invariance



$$H_i = n_i \bar{y}_i u_i$$

$$n_1 sin\theta_1 = n_2 sin\theta_2$$

Larger FOV -> lower NA
Higher NA -> smaller FOV

Cannot (easily) increase NA and FOV simultaneously. This applies to conventional single-objective systems: modern methods trade time, computation, or multiplexing to bypass it.

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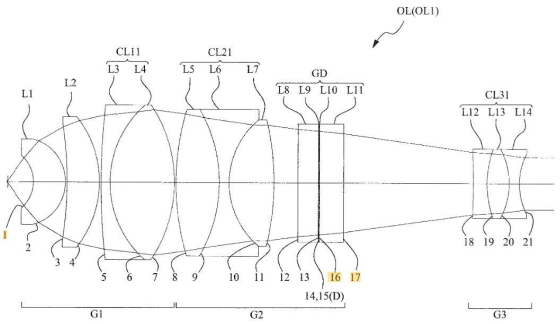
Objective lenses



Designed to fit on existing microscopes (mostly)

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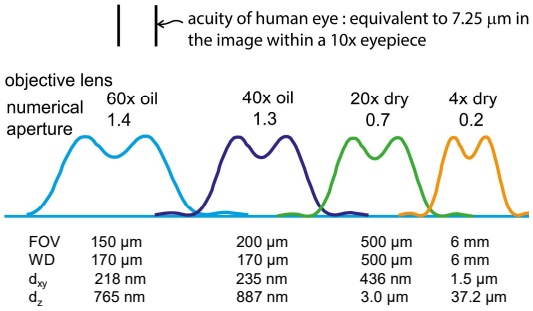
Performance of objective lenses



FOV	150 µm	200 µm	500 µm	6 mm
WD	170 µm	170 µm	500 µm	6 mm
d_{xy}	218 nm	235 nm	436 nm	1.5 µm
d_z	765 nm	887 nm	3.0 µm	37.2 µm

High NA = high resolution, short working distance, small FOV, high optical throughput
Low NA = poor resolution, long working distance, large FOV, low optical throughput

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Performance of objective lenses

Mag.	NA	FOV (dia.)	WD	r_{xy}	r_z
2x	0.05	12 mm	100 mm	8 µm	440 µm
4x	0.1	6 mm	10 mm	4 µm	110 µm
10	0.4	1 mm	3 mm	840 nm	6.9 µm
20	0.7	500 µm	1.5 mm	480 nm	2.3 µm
40	0.7	250 µm	700 µm	480 nm	2.3 µm
60	1.3	125 µm	170 µm	260 nm	650 nm
100	1.4	90 µm	170 µm	240 nm	560 nm

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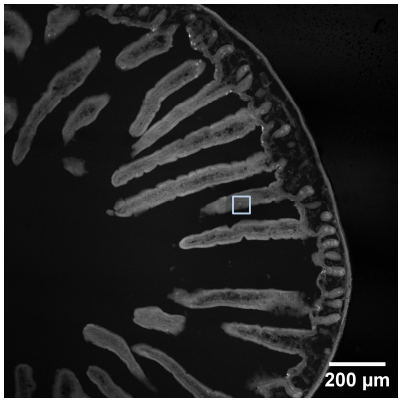
Field of view and resolution



Mouse intestine section

Nuclei stained with Sytox Green

Imaged with 10x/0.4 NA lens on a confocal microscope

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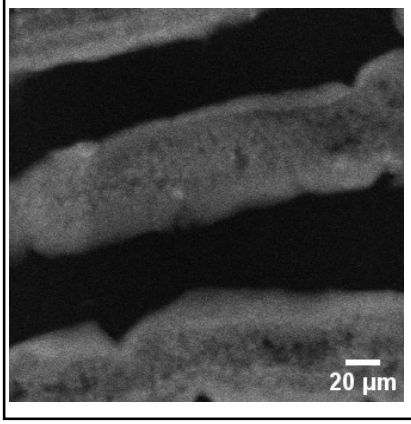
Field of view and resolution



Mouse intestine section

Nuclei stained with Sytox Green

Imaged with 10x/0.4 NA lens on a confocal microscope

Digital zoom

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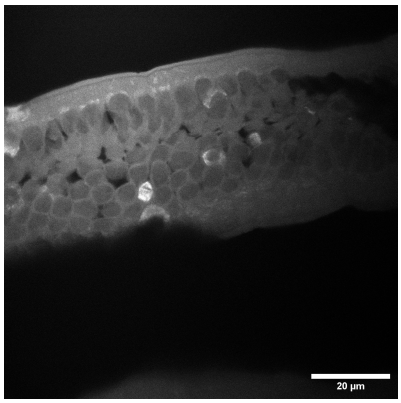
Field of view and resolution



Mouse intestine section
Nuclei stained with Sytox Green

Imaged with 100x/1.3 NA lens on a confocal microscope

Higher resolution image, but can image only 1/200th of the area of the 10x lens

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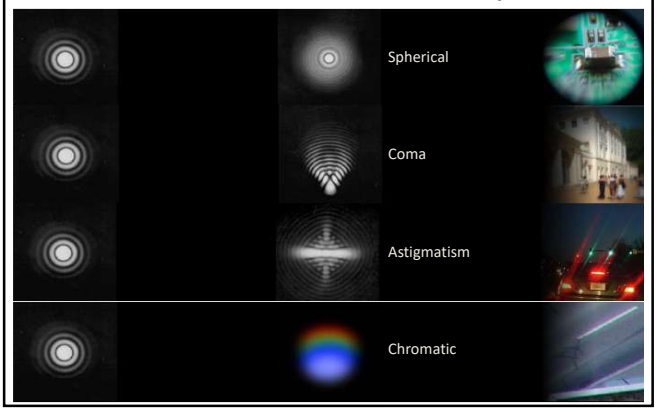
Common aberrations in optics



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Dependence of aberrations on varying aperture stop

Aberration	Scaling with aperture diameter (D)	Notes
Spherical	$\propto D^2$ (longitudinal), $\propto D^3$ (transverse)	Dominant on-axis aberration
Coma	$\propto D^2$	Off-axis; also depends on field angle
Astigmatism	$\propto D$	Stronger dependence on field than aperture
Chromatic	weak dependence on D	Primarily set by material dispersion

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Dependence of aberrations on varying lens diameter

Effect	Scaling with lens diameter
Ray geometry	invariant (similarity transform)
All transverse aberrations	$\propto D$
All longitudinal aberrations	$\propto D$
Chromatic aberration (absolute)	$\propto D$

Opening an aperture introduces higher-order rays while making a larger lens scales all aberrations linearly

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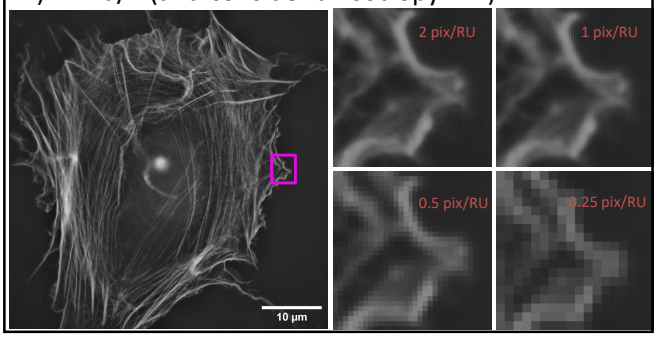
Shannon-Nyquist sampling criterion

Between 2 & 4 pixels per resolution unit (in x, y, z). $\Delta x \leq d/2$ (and consider anisotropy in z)



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Method choice is driven by research question

- Do you need specialized sample preparation?
- Must it be compatible with live-cell imaging?
- Do you have access to a fancy mesoscope?
- How easy is it to use?
- What lateral resolution can you achieve?
- What axial resolution can you achieve?
- What temporal resolution can you achieve?
- Can you do multi-colour imaging?
- How susceptible is it to image artefacts?
- Once you have the data, **what will you do with it**?

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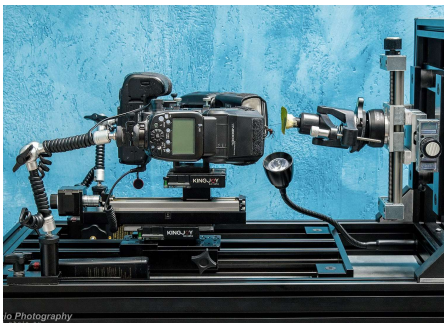
Macrophotography

Mm-scale objects
~0.1x to 1x mag



Low NA, long WD, short depth of field

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Advantages & disadvantages of macrophotography over microscopy

✅	❌
Simpler, cheaper	Limited magnification (usually 1:1)
No specific sample prep	Poor resolution (>> cellular, usually organism level)
Long working distance	Only surface details visible, no internal structure visible
Natural colour and aesthetics	Shallow depth of field
Non-contact, non-disruptive, compatible with life	No molecular specificity
Easier lighting control	

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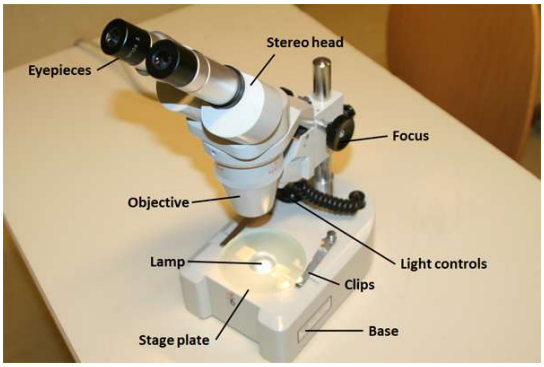
Stereomicroscopy



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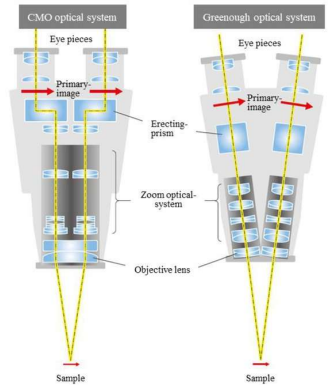
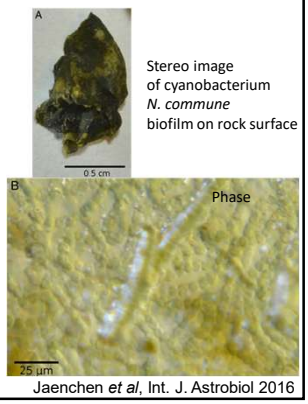
Stereomicroscopy



Miyake et al, SPIE OE&A, 2019



Stereo image of cyanobacterium *N. commune* biofilm on rock surface

Jaenchen *et al*, Int. J. Astrobiol 2016

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Magnification and NA change simultaneously in the stereomicroscope

TABLE 1. Magnification and NA specifications for the Olympus MVX-10 zoom microscope with the MVPLAPO 2× objective and the corrected dipping cap (CDC).

Zoom	Total magnification	Detection NA	Optical resolution at 600 nm (0.6/2NA) in µm	Image pixel size (Andor Neo) in µm (6.5 µm/mag)
0.63	1.36	0.07	4.29	4.78
0.8	1.72	0.09	3.33	3.78
1.0	2.15	0.11	2.73	3.02
1.25	2.69	0.14	2.14	2.42
1.6	3.44	0.19	1.58	1.89
2.0	4.30	0.23	1.30	1.51
2.5	5.38	0.30	1.00	1.21
3.2	6.88	0.38	0.789	0.945
4.0	8.61	0.48	0.625	0.755
5.0	10.8	0.50	0.600	0.602

Williams *et al*, JoM 2023

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Advantages & disadvantages of stereomicroscopes for mesoscale imaging

✅

- Simpler, cheaper than bespoke optics
- No/minimal specific sample prep
- Large magnification range
- Long working distance
- Non-contact, non-disruptive
- Versatile illumination options
- Molecular specificity with fluorescence
- Binocular view, great for topology

✖

- Poor resolution (> cellular level)
- Magnification and resolution change simultaneously
- Only surface details visible, no internal structure visible
- Shallow depth of field
- Best suited to high contrast specimens
- Surface reflections can cause glare

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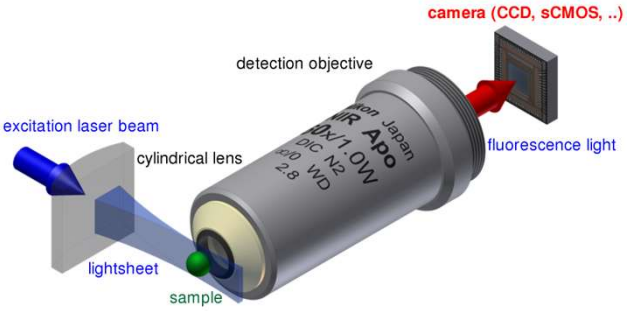
Lightsheet microscopy



https://en.wikipedia.org/wiki/Light_sheet_fluorescence_microscopy#/media/File:Spim_prinziple_en.svg

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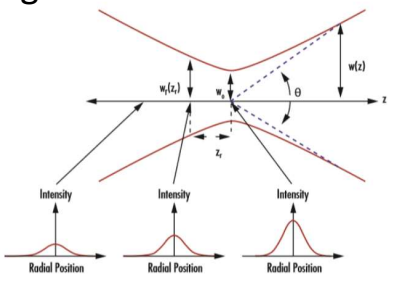
Lightsheet considerations



$$z_R = \frac{\pi w_0^2}{\lambda} \qquad w(z) = w_0\sqrt{1+\left(\frac{\lambda z}{\pi w_0^2}\right)^2} = w_0\sqrt{1+\left(\frac{z}{z_R}\right)^2}$$

https://www.edmundoptics.co.uk/knowledge-center/application-notes/lasers/gaussian-beam-propagation/

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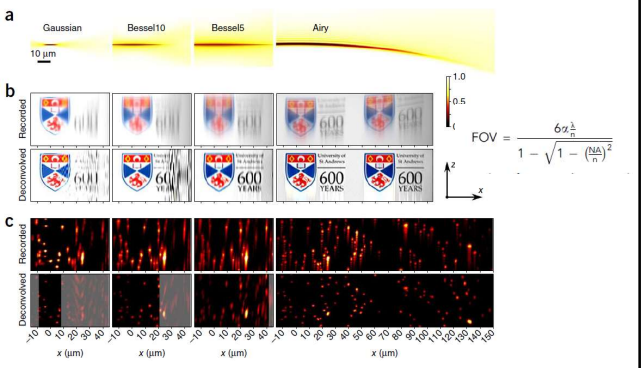
Propagation-invariant beams



$$\mathrm{FOV} = \frac{6\sigma\frac{\lambda}{n}}{1-\sqrt{1-\left(\frac{\mathrm{NA}}{n}\right)^2}}$$

Vettenburg *et al*, Nature Methods 2014

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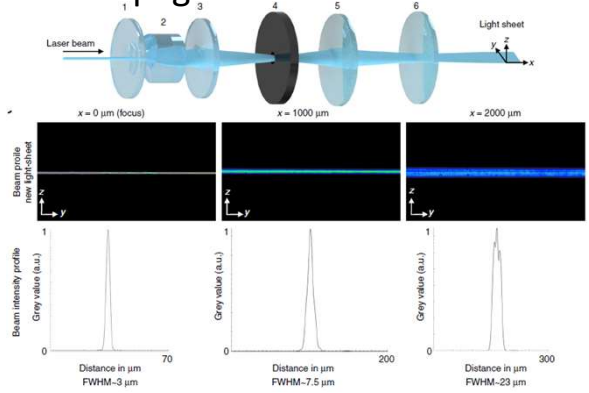
Propagation-invariant beams



Pende *et al*, Nature Comms 2018

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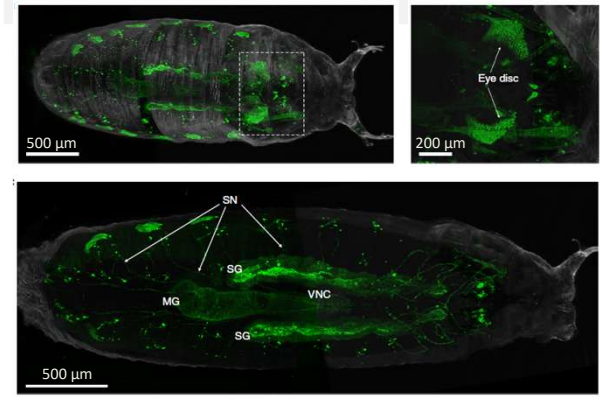
Propagation-invariant beams



Pende *et al*, Nature Comms 2018

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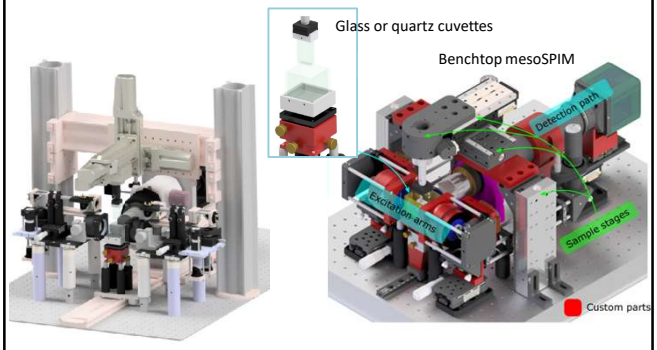
mesoSPIM



Vladimirov *et al*, Nat Meth 2024

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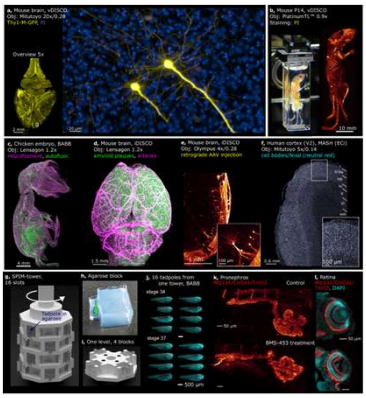
mesoSPIM



Vladimirov *et al*, Nat Meth 2024

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mesoSPIM community slide

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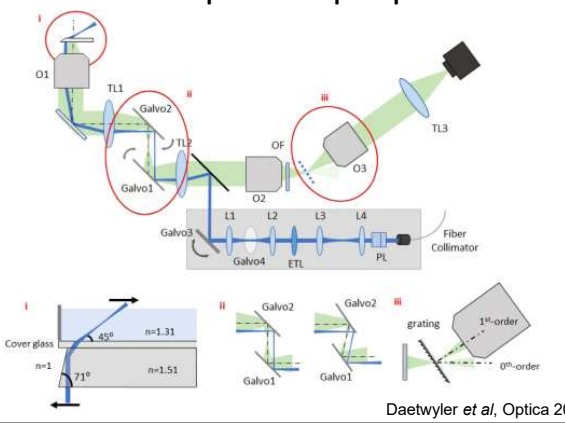
Mesoscopic oblique plane



Daetwyler *et al*, Optica 2023

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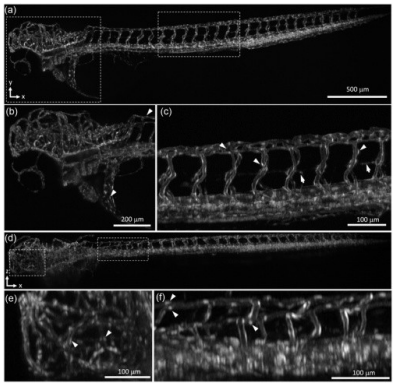
Mesoscopic oblique plane

3.7 mm x 1.5 mm x 1 mm
2.3 µm lateral, 9.2 µm axial
12 Hz per volume



Daetwyler *et al*, Optica 2023

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Advantages & disadvantages of lightsheet mesoscale imaging

✅

- Large FOV, large volume of capture
- Good optical sectioning, high SNR
- Molecular specificity
- Compatible with live cell recording
- Cleared and scattering tissue
- Reduced photobleaching and photodamage
- Multi-colour imaging

❌

- Usually expensive
- Sample prep and mounting can be challenging
- Shadows and artefacts
- Data volume
- Complex alignment
- Limited penetration depth without clearing

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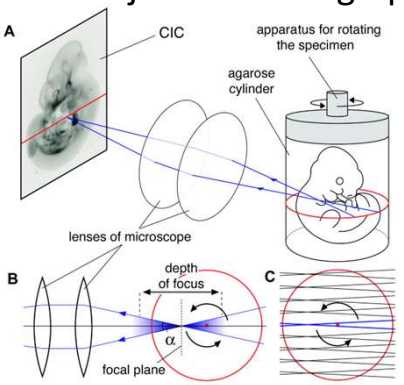
Optical Projection Tomography



Sharpe *et al*, Science 2002

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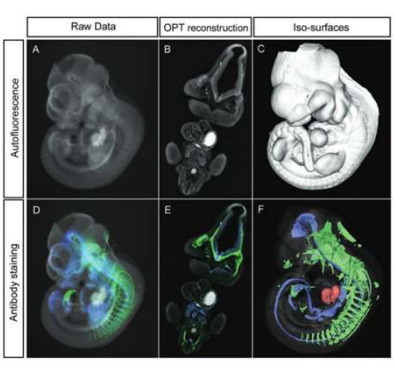
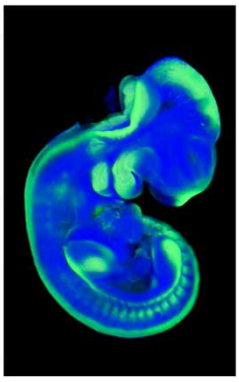
Optical Projection Tomography





Sharpe *et al*, Science 2002

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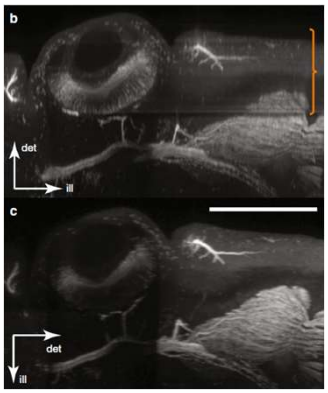
Stripe artefacts and OptiSPIM



Mayer *et al*, Light: S&A 2018

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Advantages & disadvantages of optical projection tomography

✅

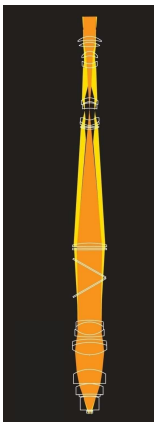
- Large FOV, large volume of capture
- Relatively simple optical setup
- Molecular specificity with fluorescence
- Multimodal
- Multi-colour imaging possible

❌

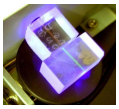
- Samples must be transparent or cleared
- Not suitable with live cell recording, long acquisition times
- Limited spatial resolution
- Sensitive to sample misalignment during rotation

48

Mesolens



Magnification:	4x
Numerical aperture:	0.47
Image field:	6 mm x 6 mm
Working distance:	3 mm
Resolution (xy):	600 nm
Resolution (z):	4 µm
Immersion:	oil, water, glycerol
Chromatic correction:	400-750 nm
Flat field:	5.5 mm
Nyquist sampling:	400 megapixels
Image size (16-bit):	500 MB (per image)

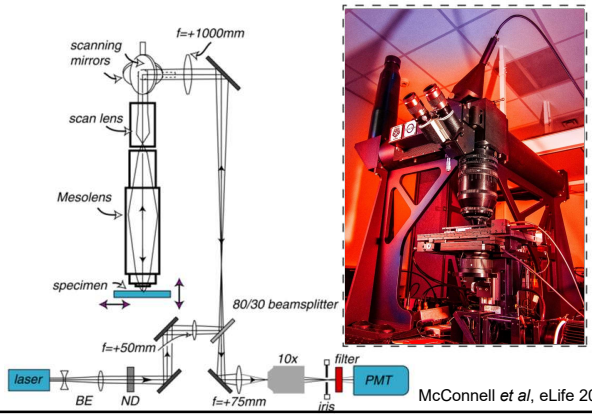


Time taken to build an image = variable…

McConnell *et al*, eLife 2016

49

Confocal Mesolens



McConnell *et al*, eLife 2016

50

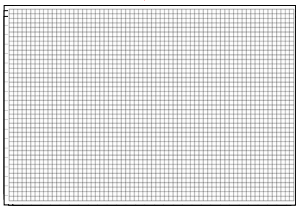
A camera for the Mesolens

28 Megapixel sensor
3 x 3 pixel shift

PZT

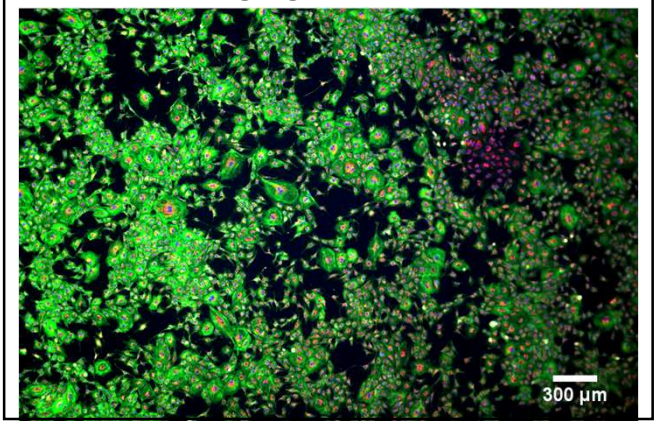
~250 Megapixel image

PZT



51

Camera imaging with the Mesolens



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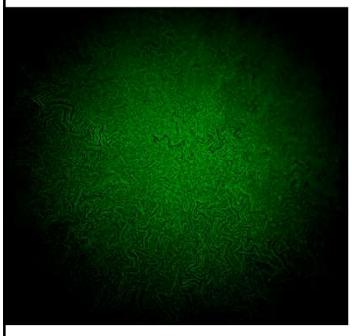
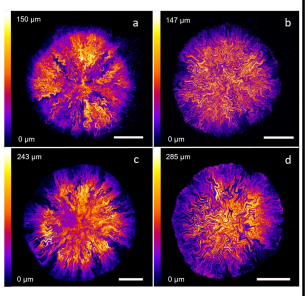
Biofilm imaging with the Mesolens



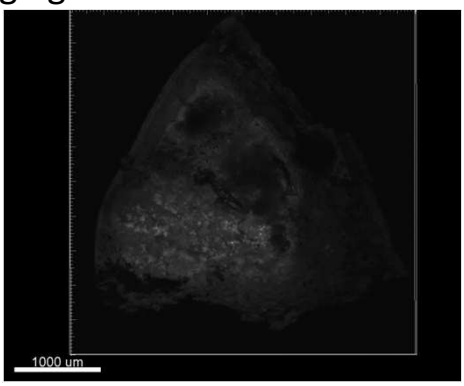


Rooney *et al*, ISME 2020

Bottura *et al*, Biofilm 2022

53

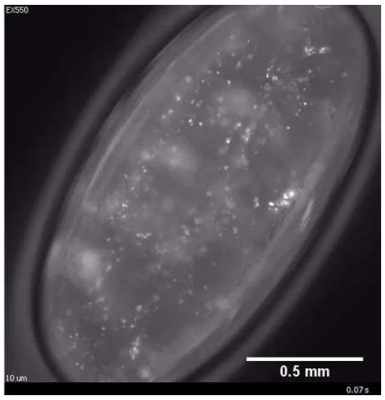
Imaging infection with the Mesolens



Francis *et al*, Sci Rep 2021

54

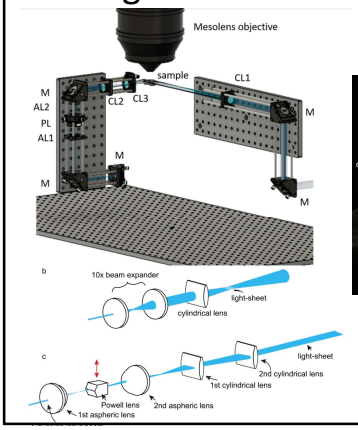
Cell dynamics with the Mesolens

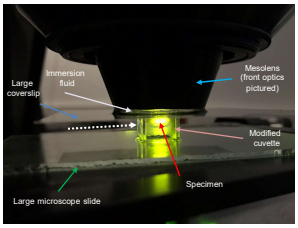


Regan *et al* (unpublished)

55

Lightsheet for the Mesolens





Battistella *et al*, iScience 2022

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Advantages & disadvantages of the Mesolens for mesoscale imaging

✅

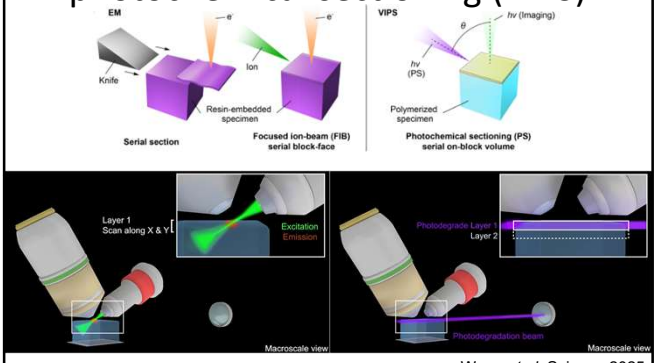
- Large FOV, high-resolution
- Good optical sectioning, high SNR, with confocal or lightsheet
- High optical throughput
- No special sample prep
- Compatible with some super-res methods

❌

- Limited availability, expensive to manufacture
- Confocal is slow, camera is slow for a camera
- Large physical size
- Limited penetration depth without clearing

57

Volumetric imaging via photochemical sectioning (VIPS)



Wang *et al*, Science 2025

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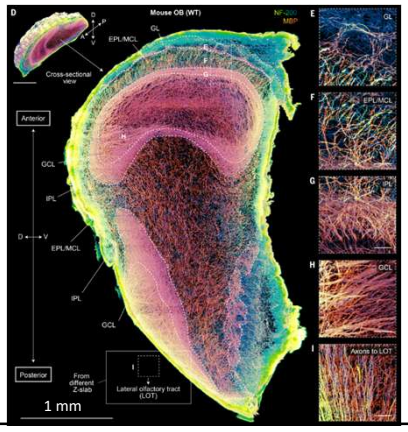
VIPS



Wang *et al*, Science 2025

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Advantages & disadvantages of VIPS for mesoscale imaging

✅

- Photochemical sectioning – less distortion
- High SNR
- Good depth penetration
- Compatible with existing microscopes
- Molecular specificity

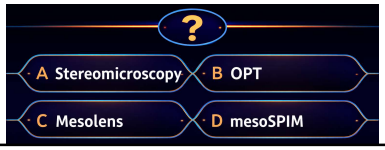
❌

- New so protocols still emerging – but exciting!
- Requires specialized photochemistry
- Very limited live cell compatibility

60

Mid-tutorial question (optional short break)

You're tasked with imaging a 1 cm^3 cleared mouse brain, with sub-cellular resolution throughout. Resolution is important: ideally you want to see cell nuclei which are fluorescently stained with DAPI and are around 5 μm in diameter. Which method do you choose (and why)?



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Tutorial structure

1. Introduction to mesoscale imaging
2. Fundamentals
3. Current methods and solutions
   a) Simple/surface
   b) Volumetric
   c) High-speed
   d) Computational
4. Data in mesoscale imaging
5. Outlook

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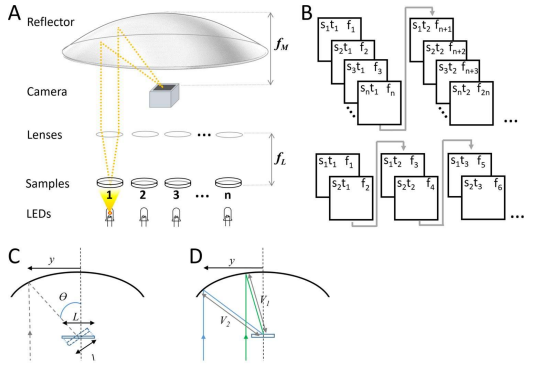
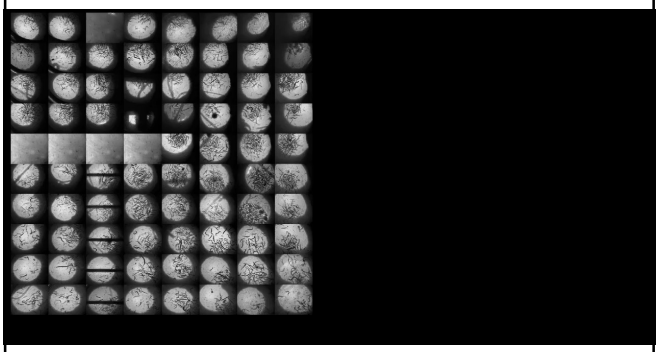
Random Access Parallel microscopy



Ashraf *et al*, eLife 2021

63

Random access parallel microscopy



Ashraf *et al*, eLife 2021

64

Advantages & disadvantages of RAP for mesoscale imaging

✅

- Ultra-fast imaging of selected regions
- Parallelized excitation/ detection
- Reduced photobleaching and photodamage
- Ideal for sparse or dynamic samples
- Flexible illumination patterns

❌

- Complex optical setup
- Not suited for full-volume imaging
- Requires prior knowledge of ROI
- Precise timing needed for parallel scanning
- Potential resolution trade-offs

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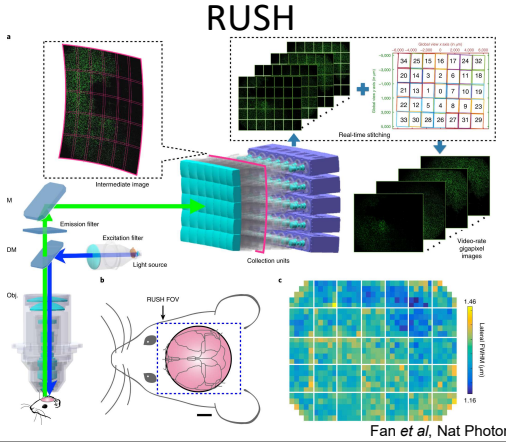
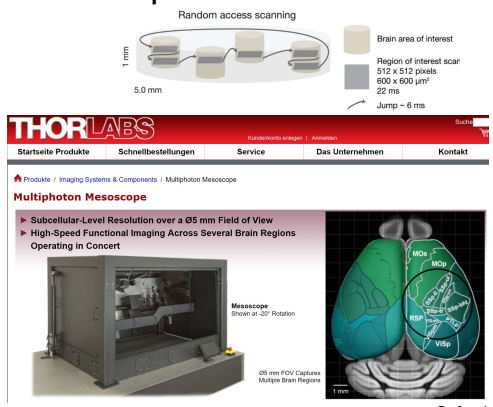
RUSH



Fan *et al*, Nat Photonics 2019

66

Multiphoton mesoscale imaging



Sofroniew *et al*, eLife (2016)

67

Advantages & disadvantages of random access scanning

✅

- Ultra-fast temporal resolution
- Targeted imaging of regions of interest (ROIs)
- Reduced photobleaching and photodamage
- Ideal for functional imaging
- Flexible scanning patterns
- Efficient use of laser power
- Compatible with multiphoton microscopy

❌

- No full-field image by default
- Requires prior knowledge of ROIs
- Challenging data interpretation
- Alignment and calibration sensitive
- Limited for dense samples

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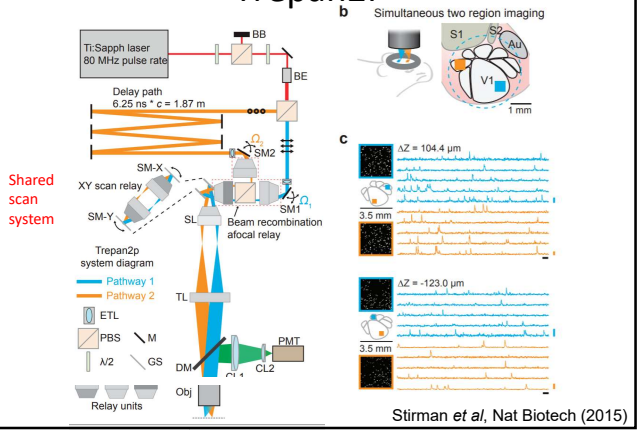
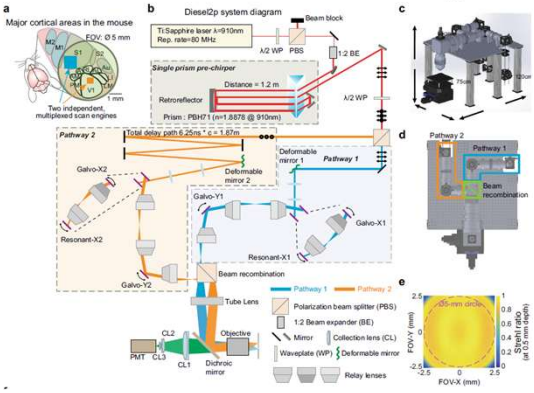
Trepan2P



Stirman *et al*, Nat Biotech (2015)

69

Diesel2P – dual scanning



Yu *et al*, Nat Comms 2021

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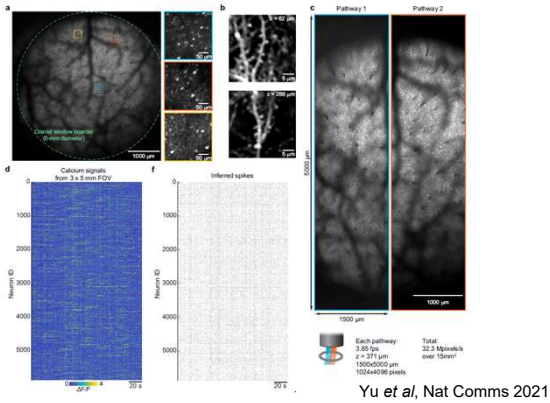
Diesel2P



Yu *et al*, Nat Comms 2021

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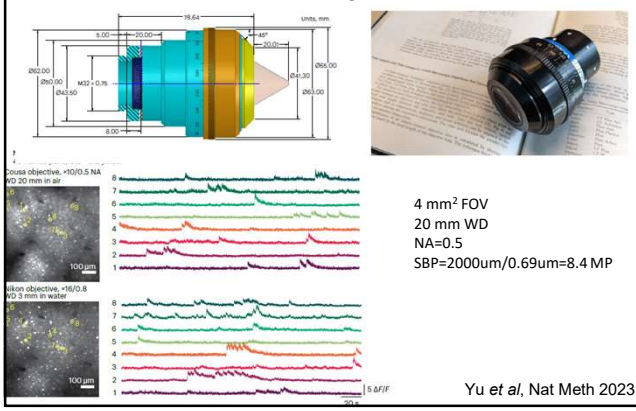
Cousa objective



4 mm² FOV
20 mm WD
NA=0.5
SBP=2000um/0.69um=8.4 MP

Yu *et al*, Nat Meth 2023

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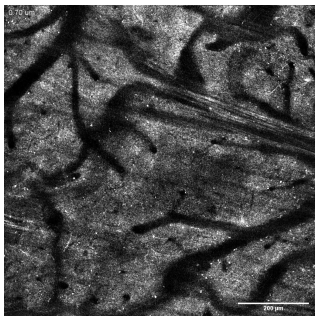
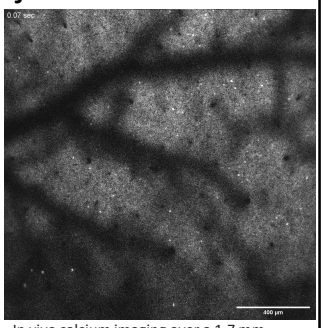
Cousa objective



Z stack of in vivo calcium imaging. The *z* plane range spans from the brain surface to the depth of 500 µm. Frame size is 1,024 × 1,024 pixels.



In vivo calcium imaging over a 1.7 mm diameter FOV. Frame rate is 15.4 frames per s. Frame size is 1,536 × 1,536 pixels. Imaging depth is 250 µm. Yu *et al*, Nat Meth 2023

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Advantages & disadvantages of Cousa objective for mesoscale imaging

✅

- Ultra-long working distance
- Compatible with *in vivo* imaging
- Air immersion (no water needed)
- Large field of view (>4 mm²)
- Compatible with 2P and 3P microscopy
- Subcellular resolution

❌

- Large physical size
- Optimization for specific wavelengths
- Still limited by tissue scattering

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Tutorial structure

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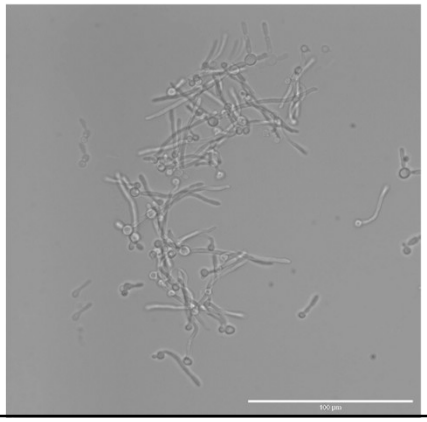
Stitching and tiling



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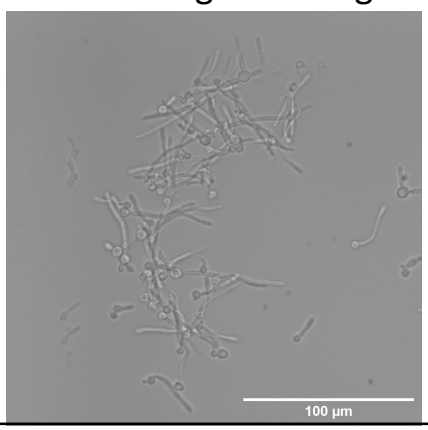
Stitching and tiling



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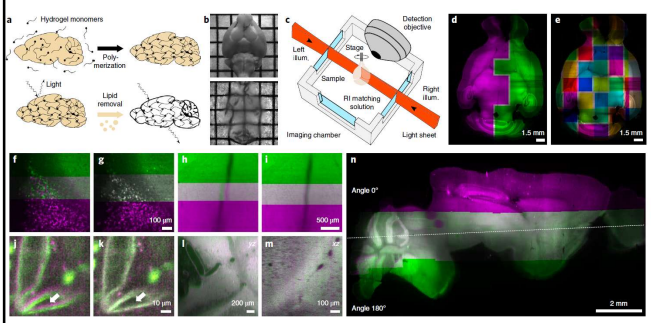
BigStitcher



Hoerl *et al*, Nature Methods 2019

78

Advantages & disadvantages of tiling

✅

- Optical set-up can be the same as an ordinary microscope
- No specific sample prep
- Multi-modal imaging possible (e.g. brightfield + fluorescence)
- Multi-dimensional imaging is possible

❌

- Prone to introducing registration artefacts
- Specimen must not be tilted relative to the optical axis of the microscope
- Large number of algorithms, blending methods etc, and best results are often determined empirically
- Not easily compatible with oil immersion
- End image quality can depend on precision of microscope stage position

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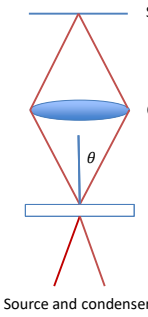
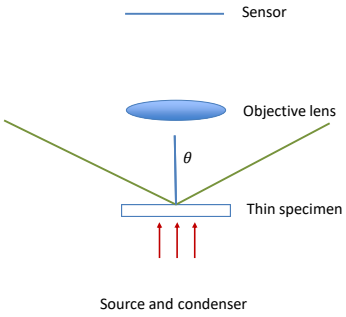
Limit to NA in light microscopy



$$d = \frac{0.61\lambda}{NA}$$

80

Limit to NA in light microscopy

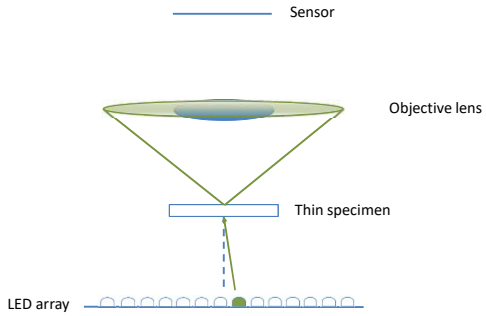


Still

$$d = \frac{0.61\lambda}{NA} = \frac{0.61\lambda}{n\sin\theta}$$

81

Fourier Ptychographic Microscopy



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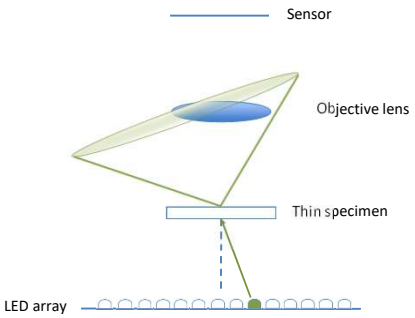
Fourier Ptychographic Microscopy



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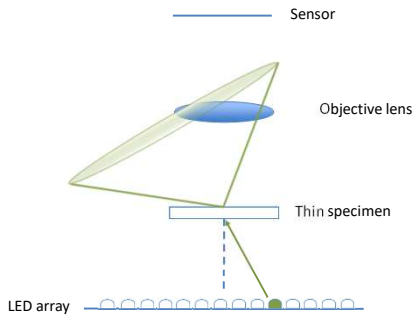
Fourier Ptychographic Microscopy



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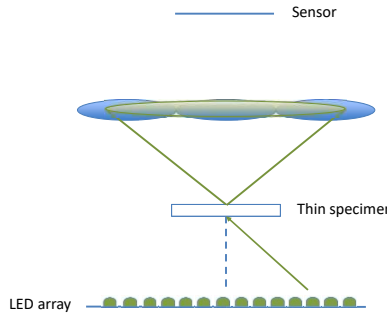
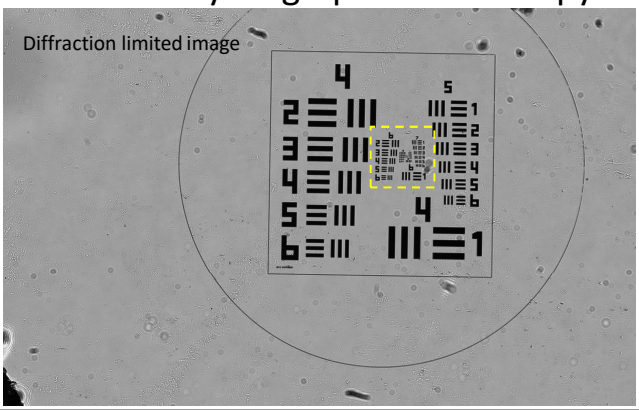
Fourier Ptychographic Microscopy



Effectively create a 'synthetic' objective lens
With a higher NA by reconstructing multiple images into a single image

85

Fourier Ptychographic Microscopy



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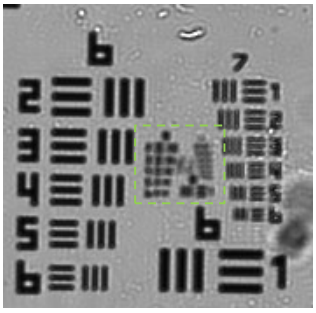
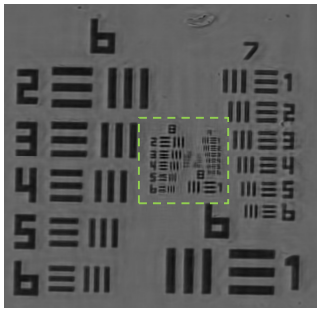
Fourier Ptychographic Microscopy

Diffraction limited image



Reconstructed FPM image



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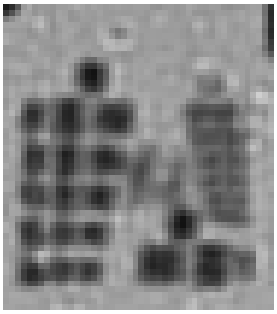
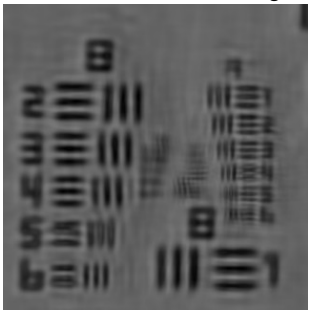
Fourier Ptychographic Microscopy

Diffraction limited image



Reconstructed FPM image



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Advantages & disadvantages of FPM

✅

- Optical set-up is comparably simple, just need high power LED array, no condenser lens
- High resolution imaging possible (5-8-fold improvement)
- Multi-colour imaging is possible and phase information is free
- No specific sample prep

❌

- Largely incompatible with live cell imaging because…
- Acquisitions take minutes or longer
- Computationally intensive and often requires a lot of troubleshooting
- Computation is very prone to artefacts
- Datasets can be very large

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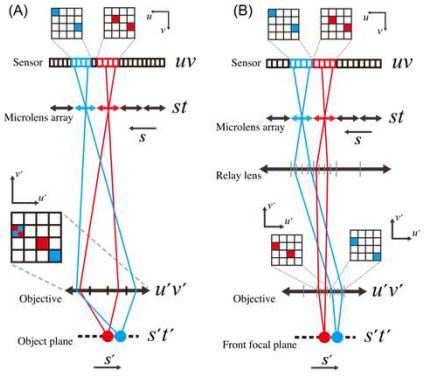
Lightfield microscopy



https://en.wikipedia.org/wiki/Light_field_microscopy

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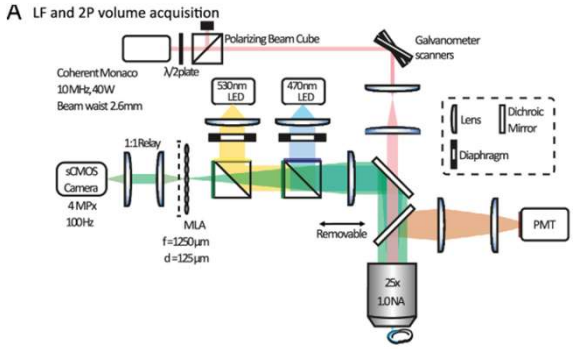
Lightfield microscopy



Howe *et al*, PNAS 2025

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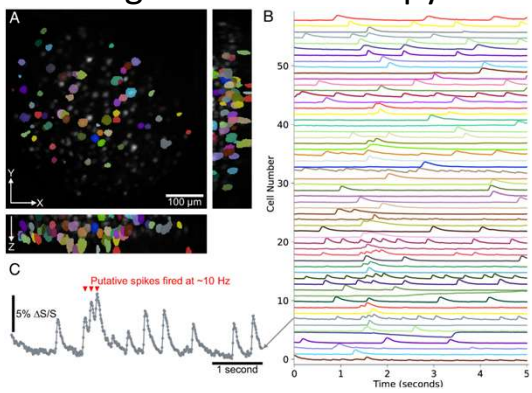
Lightfield microscopy



Howe *et al*, PNAS 2025

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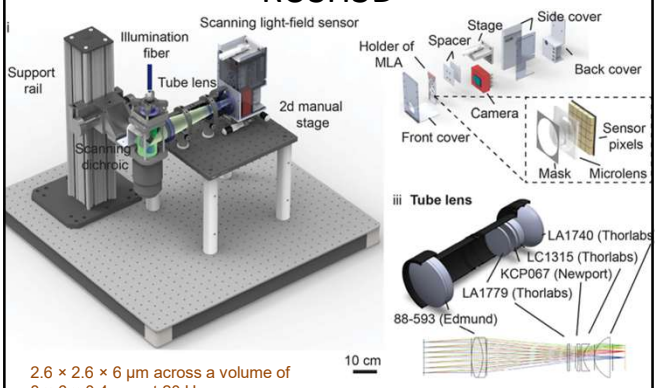
RUSH3D



2.6 × 2.6 × 6 µm across a volume of 8 × 6 × 0.4 mm at 20 Hz

Zhang *et al*, Cell 2024

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RUSH3D

Cortex-wide 3D neural recording

Zhang *et al*, Cell 2024

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Advantages & disadvantages of lightfield microscopy

✅

- Instant volumetric imaging (single shot 3D)
- Very high temporal resolution
- No mechanical scanning required
- Good for live imaging
- Relatively simple modification of widefield systems
- Large field of view

❌

- Lower spatial resolution
- Heavy computational reconstruction
- Reconstruction artefacts
- Limited depth resolution
- Reduced signal efficiency
- Limited performance in thick/scattering tissue
- Requires careful calibration

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Common pitfalls in mesoscale imaging

- Sample-induced aberrations
- Refractive index mismatch
- Specimen movement
- Specimen flatness
- Specimen mounting challenges
- Mechanical (i.e. hardware) instabilities, e.g. drift

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Tutorial structure

1. Introduction to mesoscale imaging
2. Fundamentals
3. Current methods and solutions
   a) Simple/surface
   b) Volumetric
   c) High-speed
   d) Computational
4. Data in mesoscale imaging
5. Outlook

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Data

Data size ~ resolved volume *x* channels *x* time *x* bit depth

E.g. Nyquist sampled single-colour Mesolens 2D image, 16-bit
4.4 mm x 3.0 mm FOV: 19643 x 13393 pixels = 2.6 x 10^8 pixels
16-bit = 2 bytes

2.6 x 10^8 pixels x 2 bytes = 5.2 x 10^8 bytes = 520 MB

Acquisition, processing, tiling/fusion, analysis, storage, sharing…

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Data file sizes (TIFF)

Stereomicroscopy: MB
Tiling: MB to GB
FPM: 0.5 GB - 5 GB
Lightsheet: 10 GB to PB
RUSH3D: 2.5 GB/s
OPT: 10 GB to 500 GB
Lightfield: 50 GB to 500 GB/dataset
Mesolens: 639 GB (single colour, full resolution)
mesoSPIM: 100 GB to 2 TB

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Data file formats

OME TIFF – simple, huge, slow, good compatibility with software

HDF5/BigDataViewer – chunked, scaleable

Zarr/N5 – scaleable, increasingly popular

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Data bottlenecks

Storage – local disks fill immediately, TB infrastructure needed

I/O – reading TB-scale data is slow. SSD v HDD can matter more than CPU

Memory – cannot load full dataset into RAM, hence chunking

Transfer and sharing – Moving 1 TB over a network can take hours…

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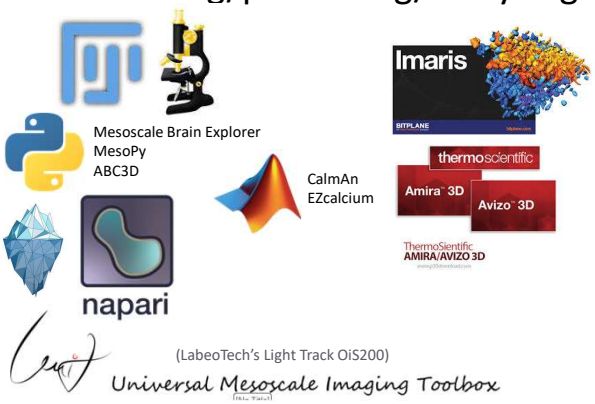
Data viewing/processing/analysing



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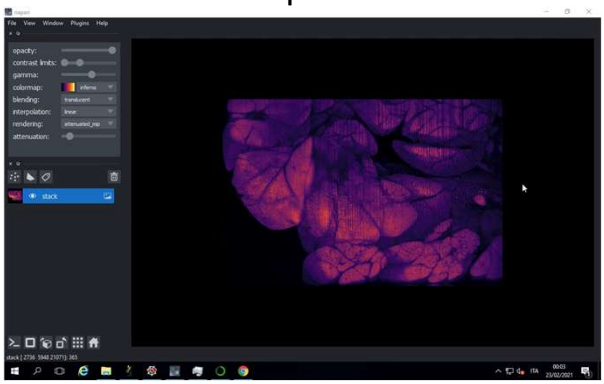
napari



Battistella, Wesencraft *et al* (unpublished)

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Data advice

Only acquire at the data you need: determined by the biology, not the physics. Resolution choices can cost TB (and time and money!)

Organise data: use clean file structures etc

Consider GPU/cloud workflows

Use compression safely

Consider using cluster/high performance facilities

FIJI/ImageJ/napari wherever possible

Sharing: Zenodo is free (up to 50 GB per dataset, max 100 per record)

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Tutorial structure

1. Introduction to mesoscale imaging
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   a) Simple/surface
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   d) Computational
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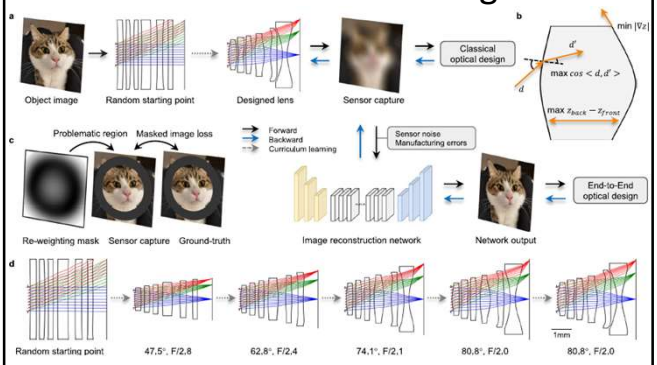
AI-informed lens design



Yang *et al*, Nat Comms 2024

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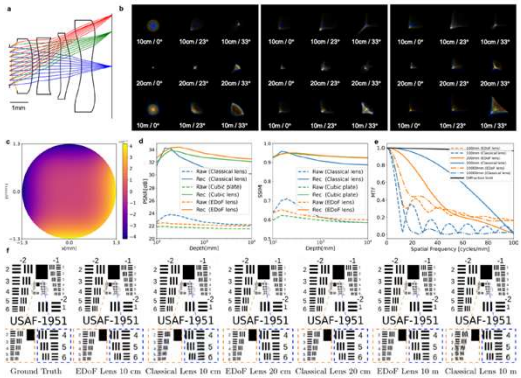
AI-informed lens design



Yang *et al*, Nat Comms 2024

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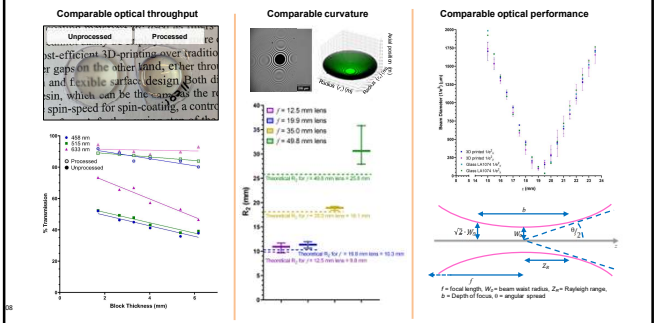
Next generation lenses



Christopher *et al*, BOE 2024
Rooney *et al*, AMT 2024

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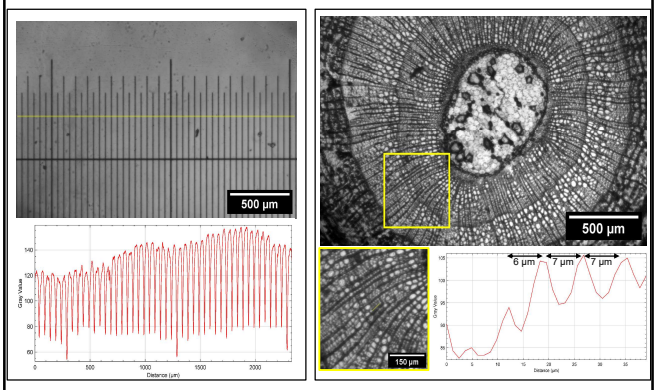
Next generation lenses



Rooney *et al*, AMT 2024

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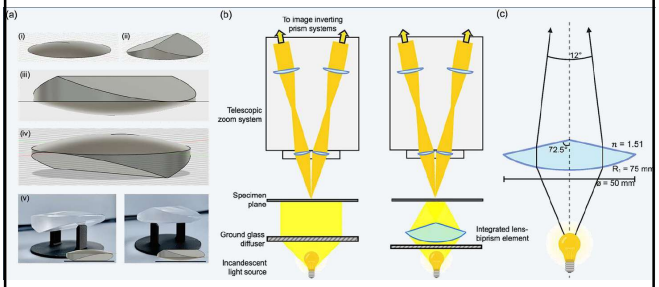
Next generation lenses



Rooney *et al*, Microscopy & Microanalysis (2025)

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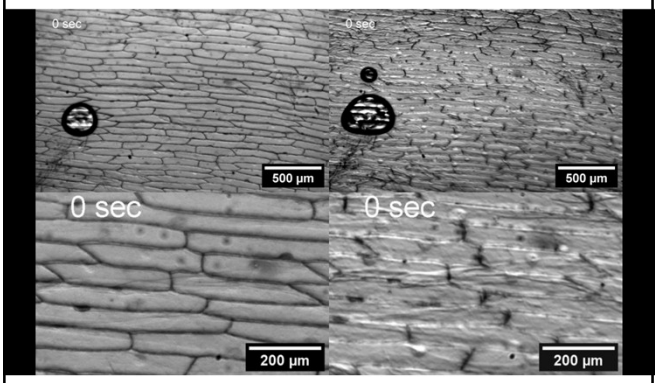
Next generation lenses



Rooney *et al*, Microscopy & Microanalysis 2025

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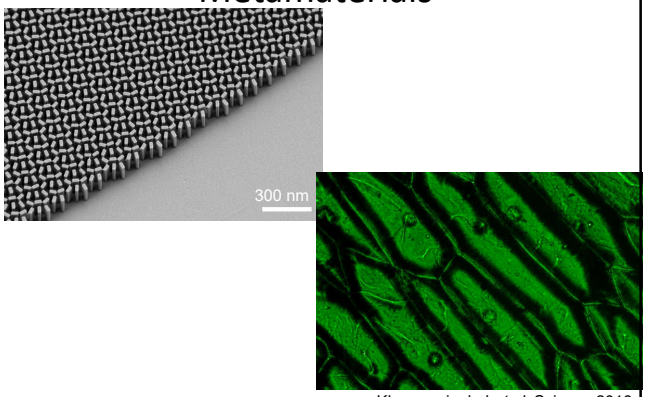
Metamaterials



Khorasaninejad *et al*, Science 2016

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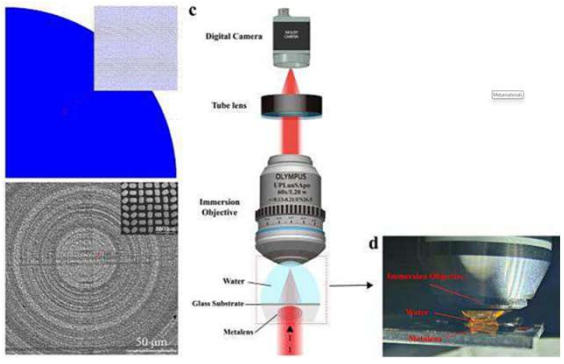
Metamaterials



Zhou *et al*, OLT 2023

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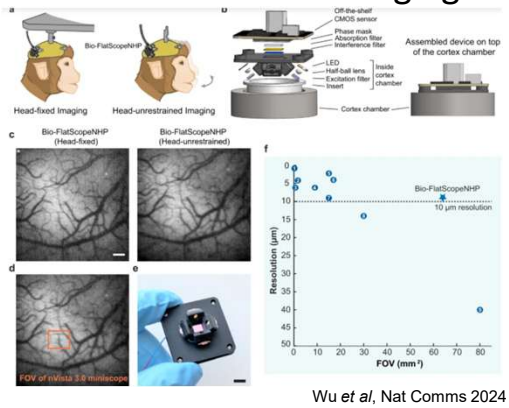
In vivo mesoscale imaging



Wu *et al*, Nat Comms 2024

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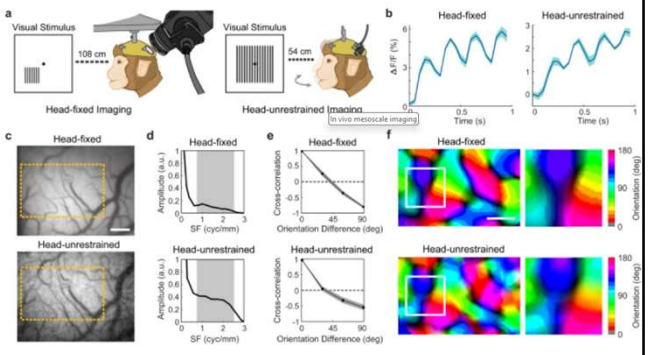
In vivo mesoscale imaging (mini-2P)



Wu *et al*, Nat Comms 2024

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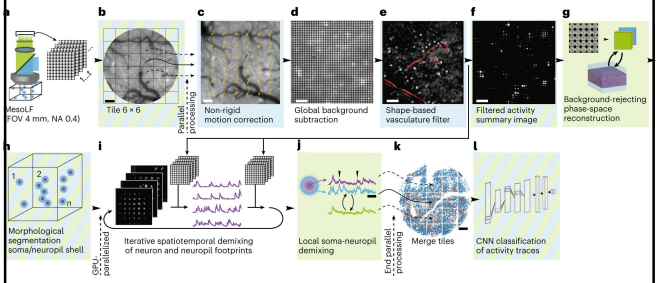
Quantitative imaging



Noebauer *et al*, Nat Meth 2023

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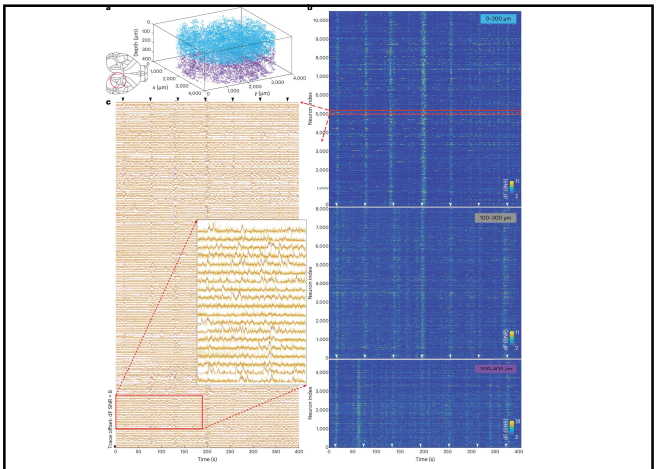

Noebauer *et al*, Nat Meth 2023

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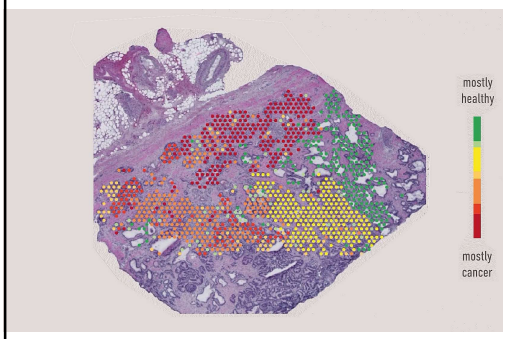
Spatial transcriptomics



10x Genomics
6.5 mm FoV
55 µm

Slide-seq v2
Similar FoV
10 µm

Erickson *et al*, Nature 2022

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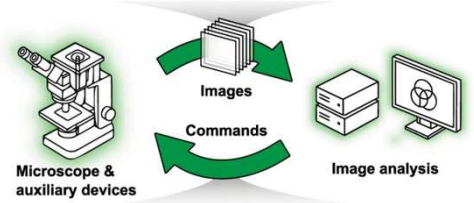
Information-efficient imaging



- Multiplexing → (light-field, VIPS, Mesolens, Cousa…)
- Sparse sampling, avoid redundancy → (random access scanning)
- Parallelization → (random access parallelization)

https://smartmicroscopy.github.io/

Smart Microscopy Working Group

Smart or Adaptive Feedback Microscopy aims at connecting bioimage analysis (and other algorithms) with fully motorized and computer-controlled microscopes to generate automated and adaptive imaging workflows. This strategy can also be used to manage changes in the sample's environment (e.g. fluidic devices) and directly link the microscope to image data management systems.

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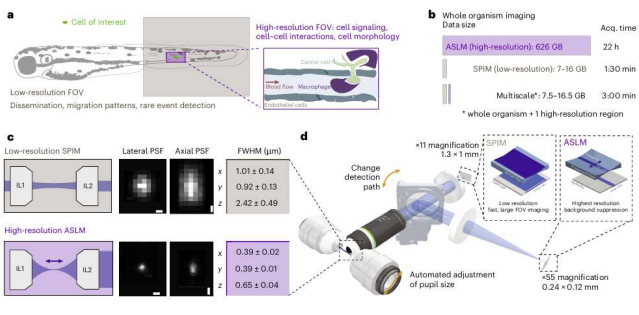
Self-supervised, self-driving



Daetwyler *et al*, Nat Meth 2025

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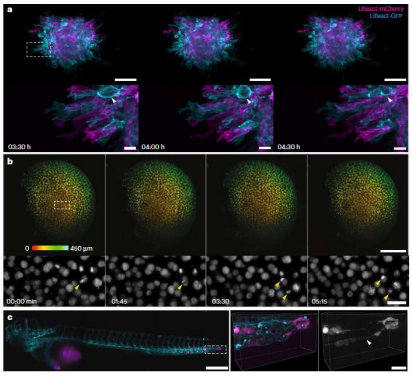
Self-supervised, self-driving mesoscope



Daetwyler *et al*, Nat Meth 2025

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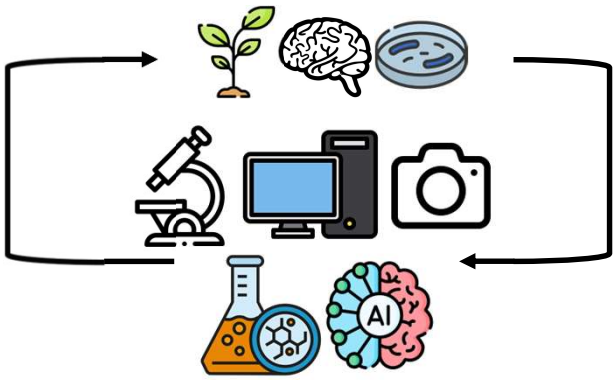
Final remarks



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Two PhD studentships available for 2026 start: speak to me or email me for more details

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