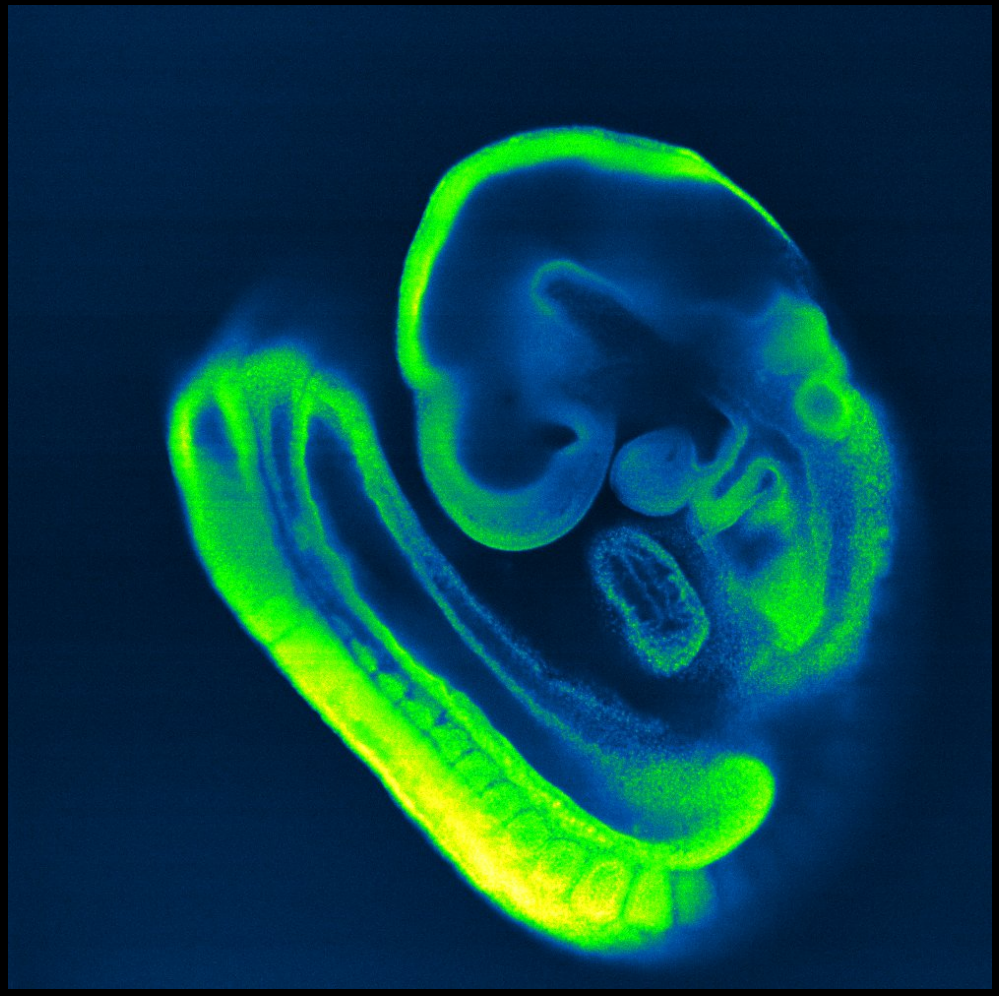
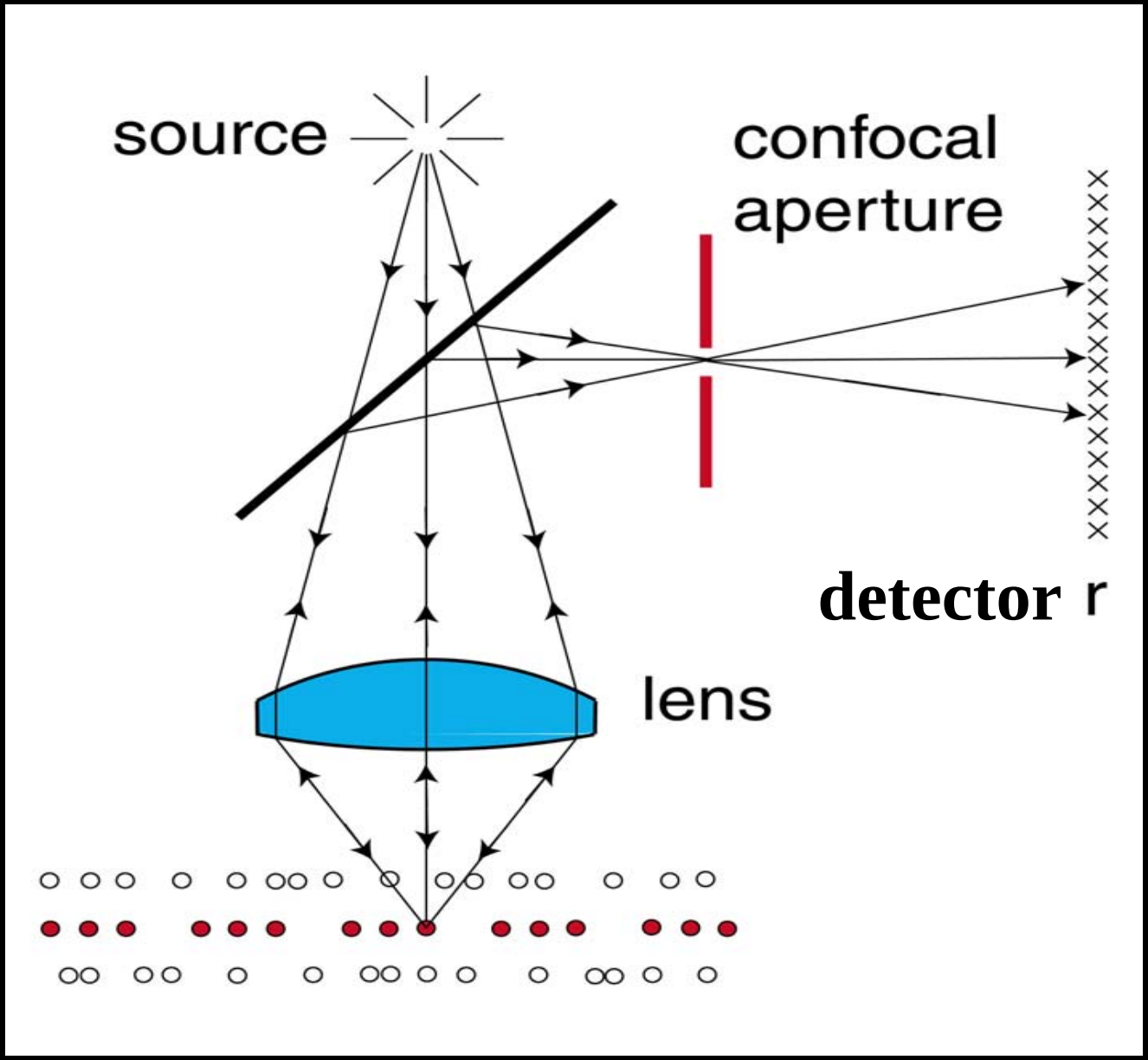
Optical mesoscopy with a new giant lens



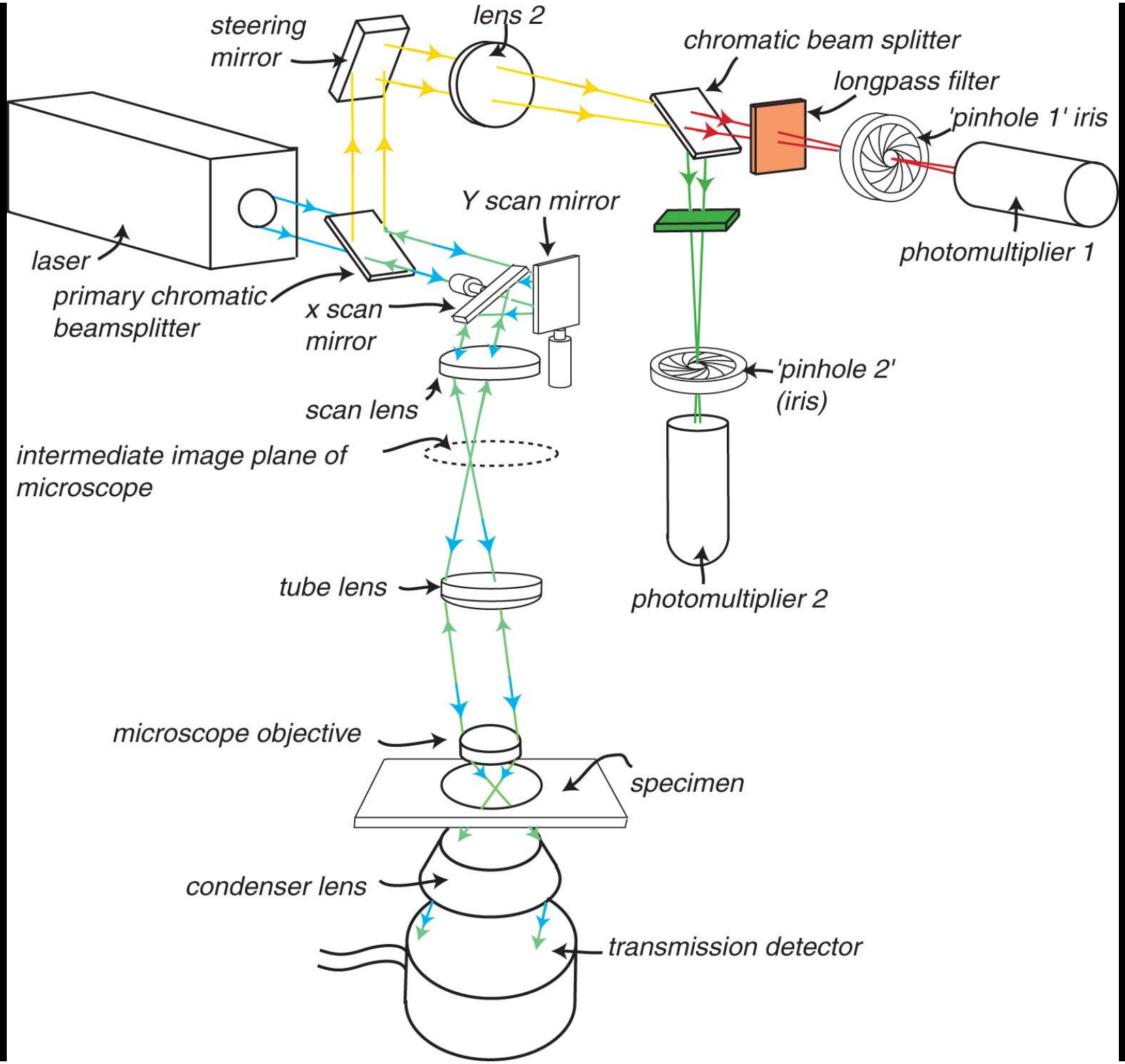
Gail McConnell, Johanna Tragardh, John Dempster & Brad Amos



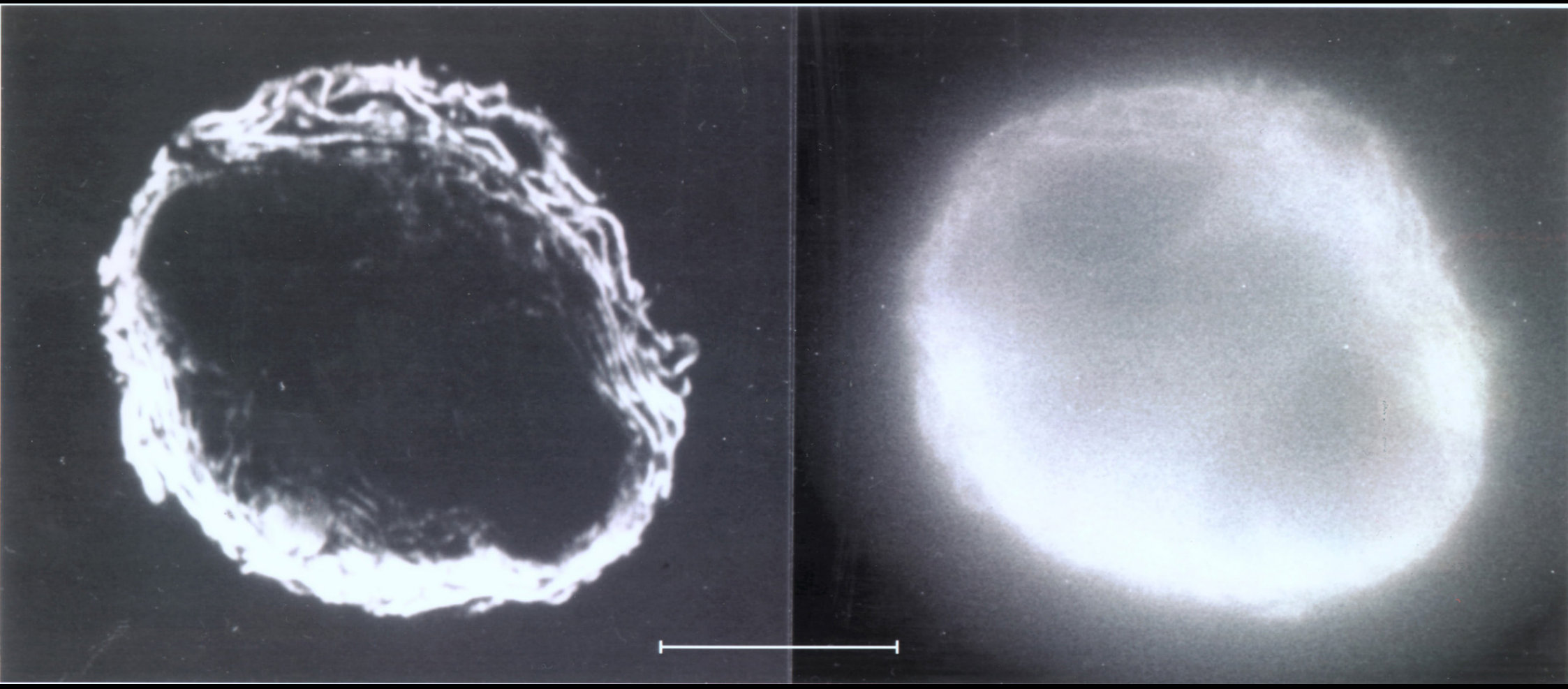
The spot
on specimen, source
and detector aperture
are at conjugate
optical foci

Advantage:
Small depth of focus

WB Amos, LMB


steering mirror
lens 2
chromatic beam splitter
longpass filter
'pinhole 1' iris
photomultiplier 1
Y scan mirror
laser
primary chromatic beamsplitter
x scan mirror
'pinhole 2' (iris)
scan lens
intermediate image plane of microscope
tube lens
photomultiplier 2
microscope objective
specimen
condenser lens
transmission detector

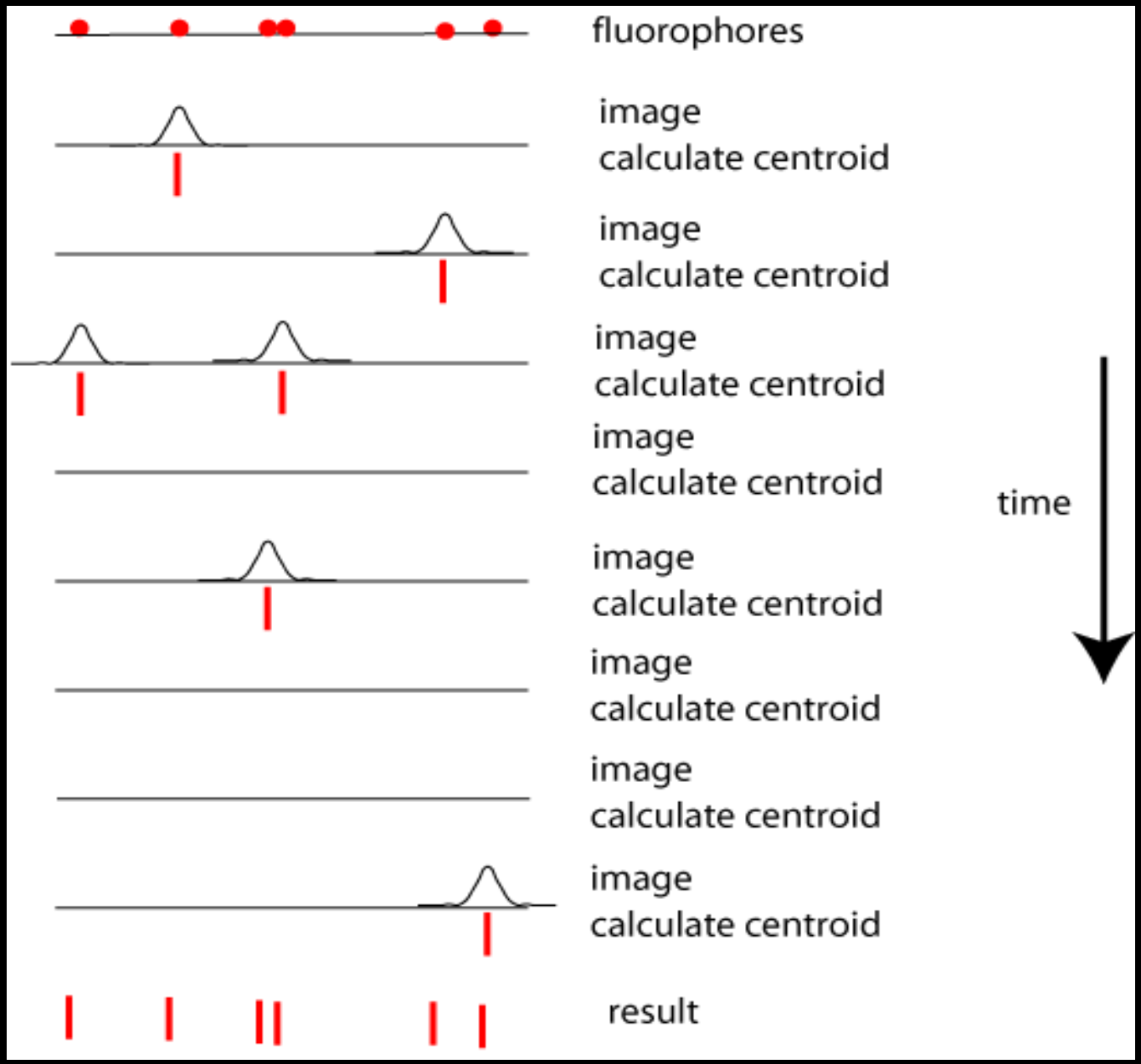
Plasmacytoma cell stained for the Golgi cisternae by Gordon Koch (LMB, Cambridge) shows optical sections of the cisternae (left)

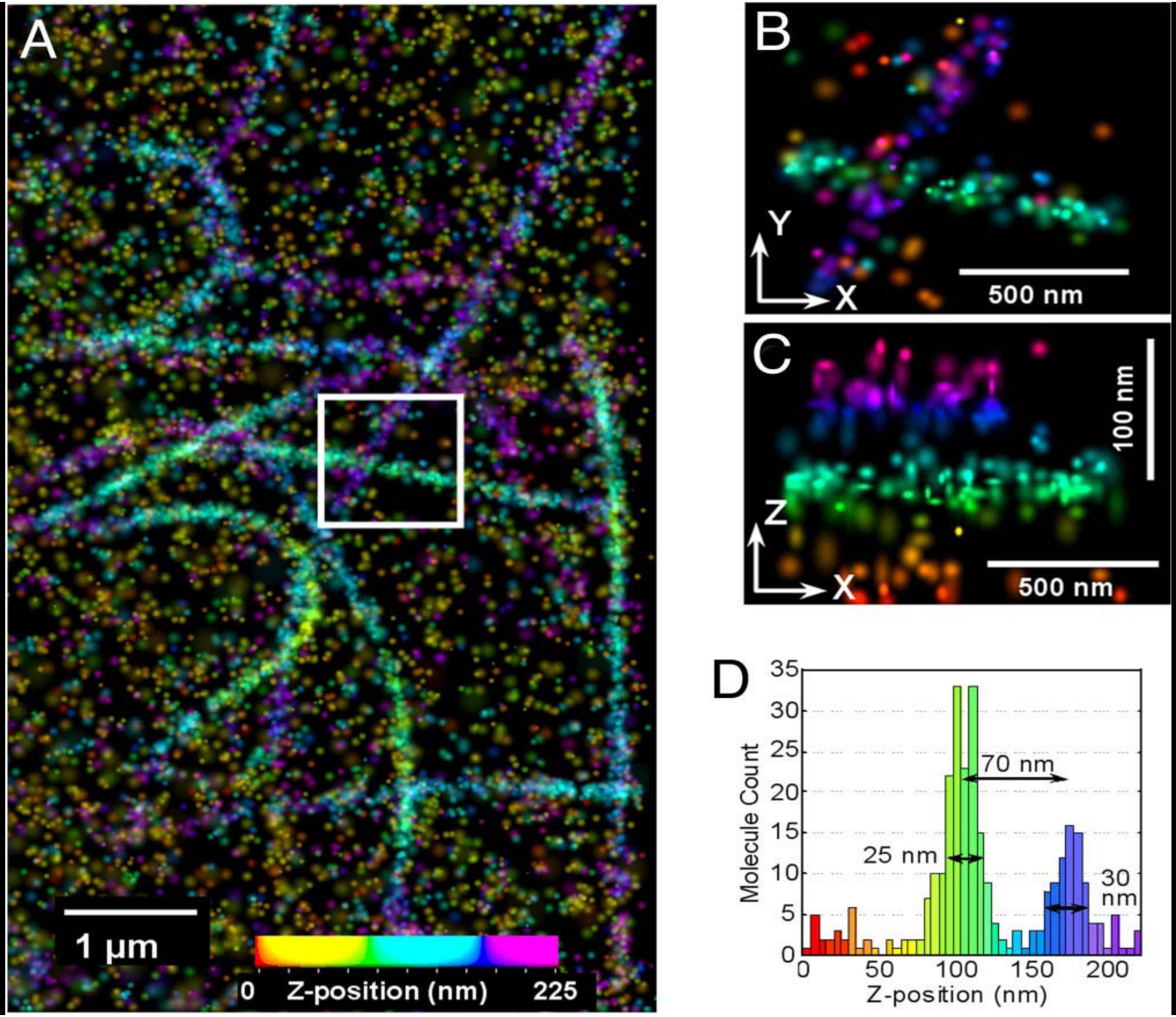


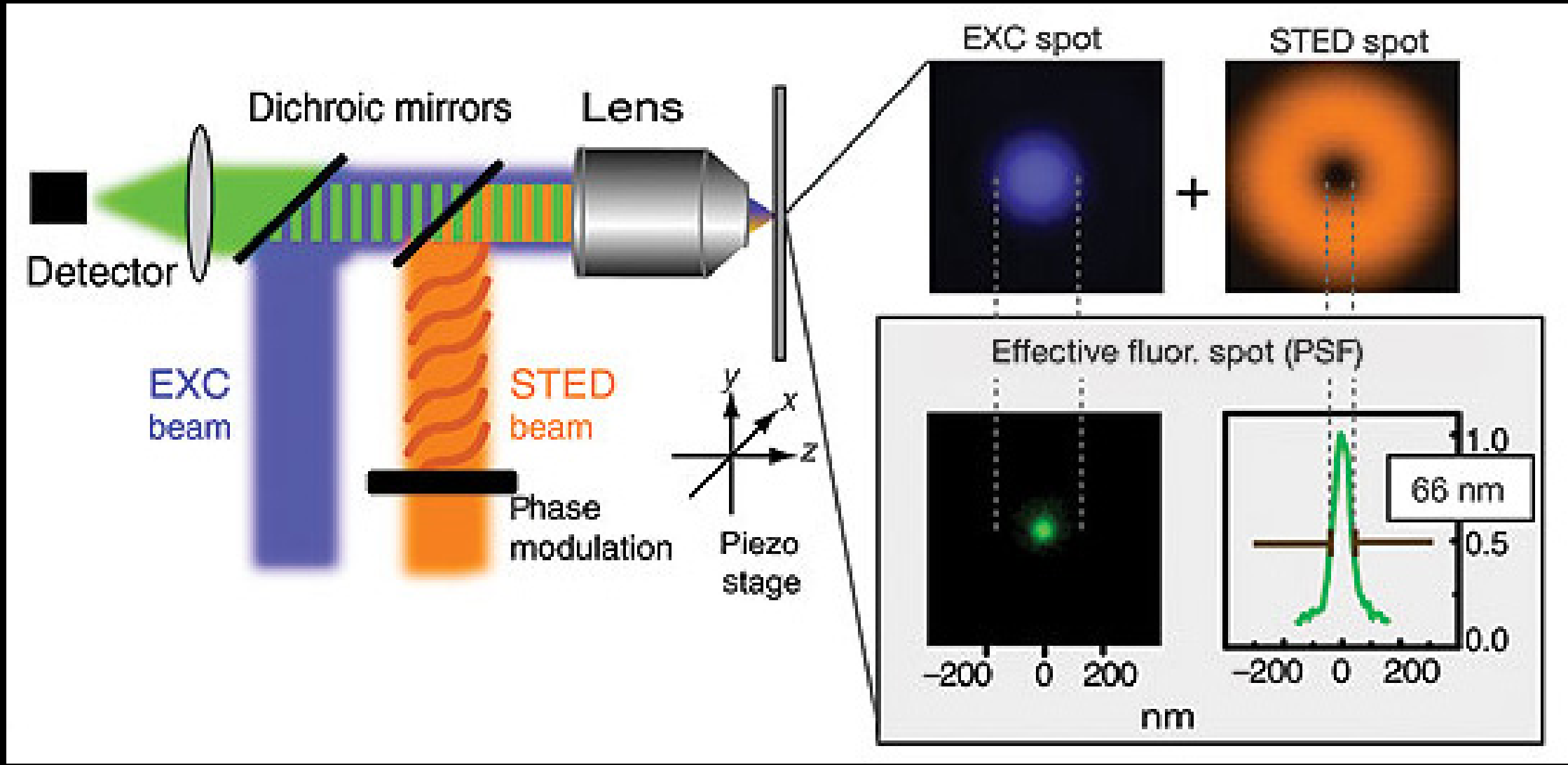
An example of sparse imaging:

PALM
(photoactivated localisation microscopy):
Betzig et al. (2006) Science 313 1642-1645

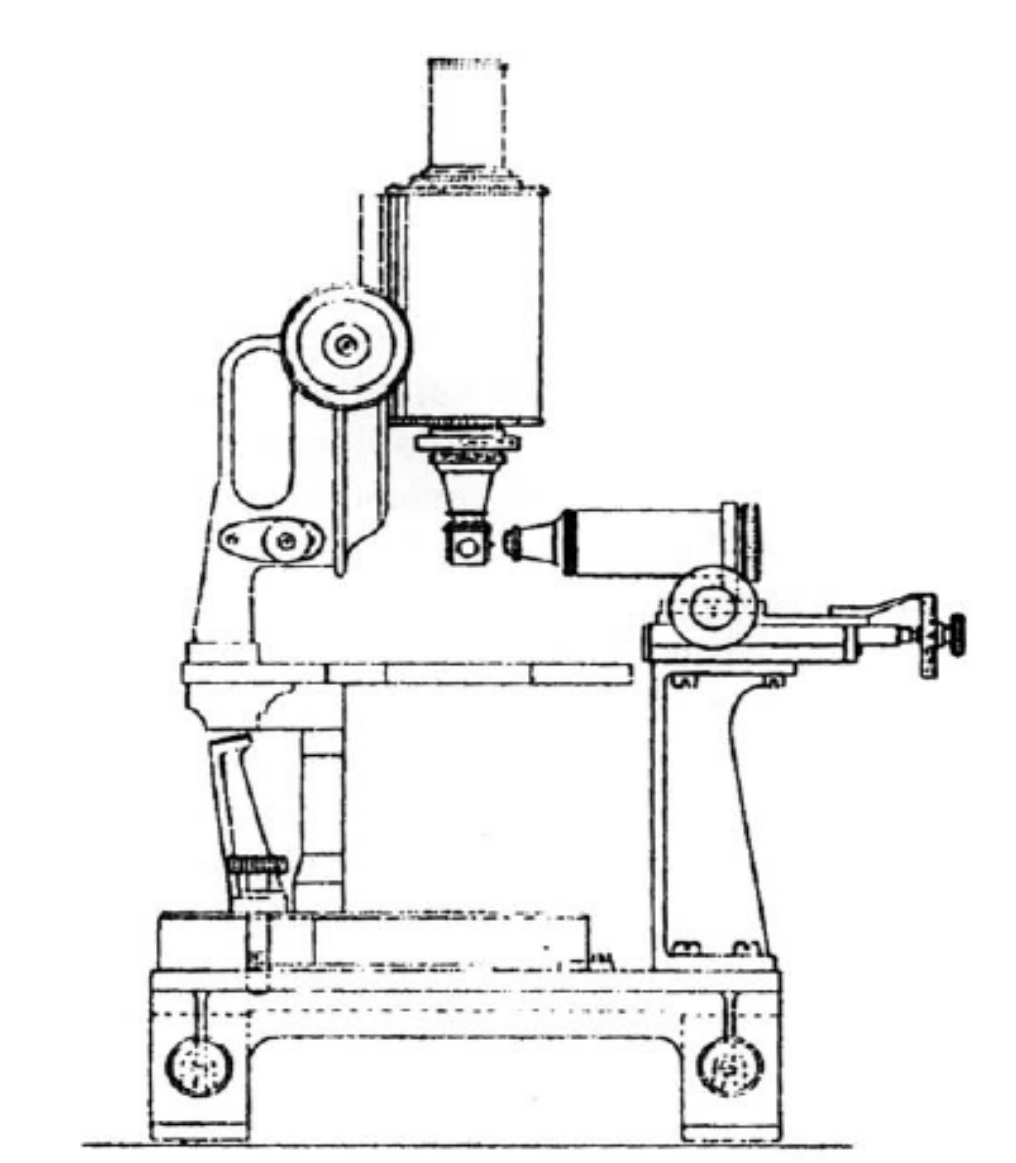
Repeated imaging of a sparse specimen




A
1 µm
0
Z-position (nm)
225
B
Y
X
500 nm
C
Z
X
100 nm
500 nm
D
Molecule Count
35
30
25
20
15
10
5
0
0
50
100
150
200
Z-position (nm)
70 nm
25 nm
30 nm


Dichroic mirrors
Lens
Detector
EXC
beam
STED
beam
Phase
modulation
y
x
z
Piezo
stage
EXC spot
STED spot
+
Effective fluor. spot (PSF)
1.0
66 nm
0.5
0.0
–200
0
200
–200
0
200
nm

Ultramicroscopy (Siedentopf and Zsigmondy, 1903)



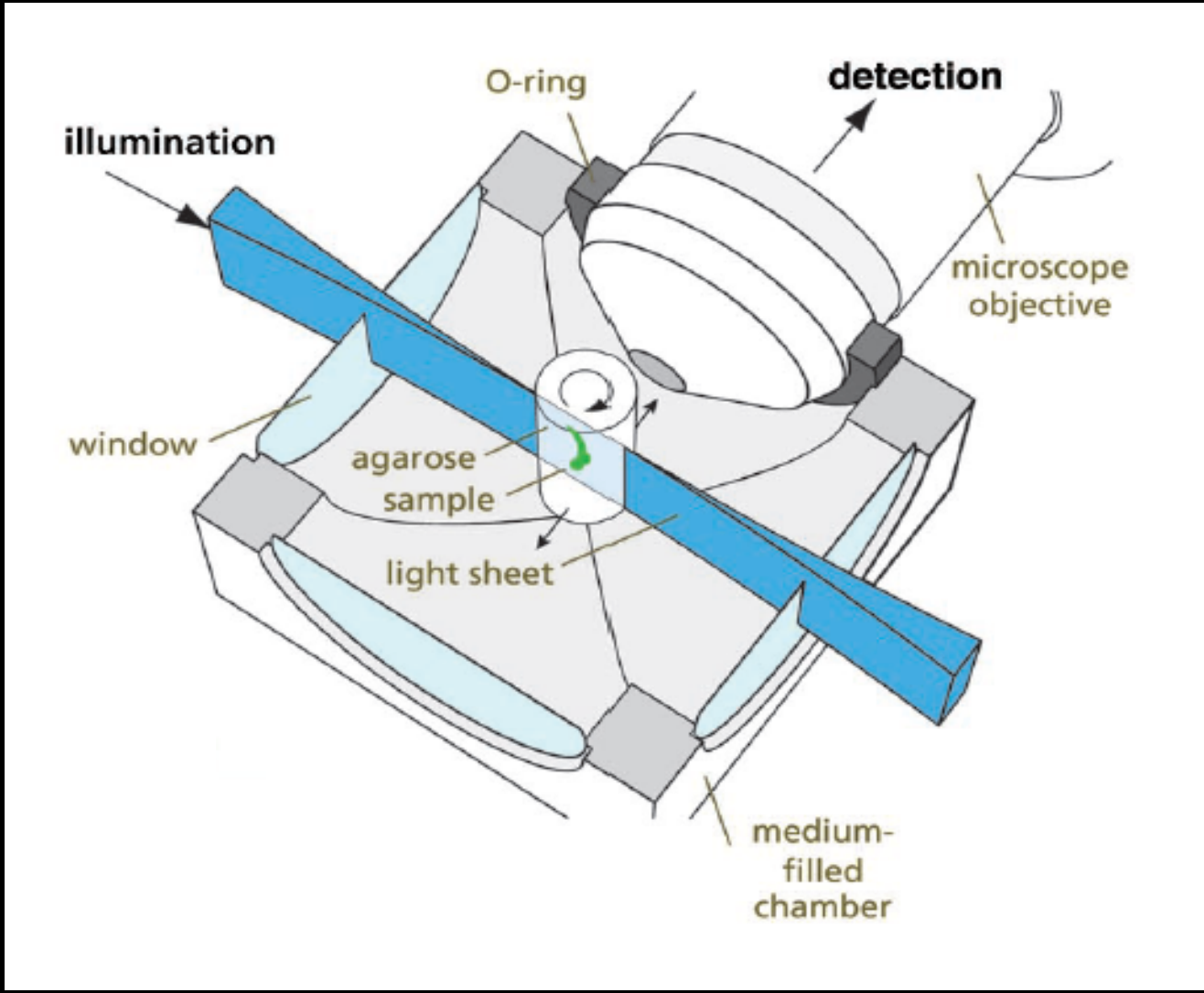
Used sunlight through a slit aperture to observe gold particles

Specimen holder fixed to objective lens

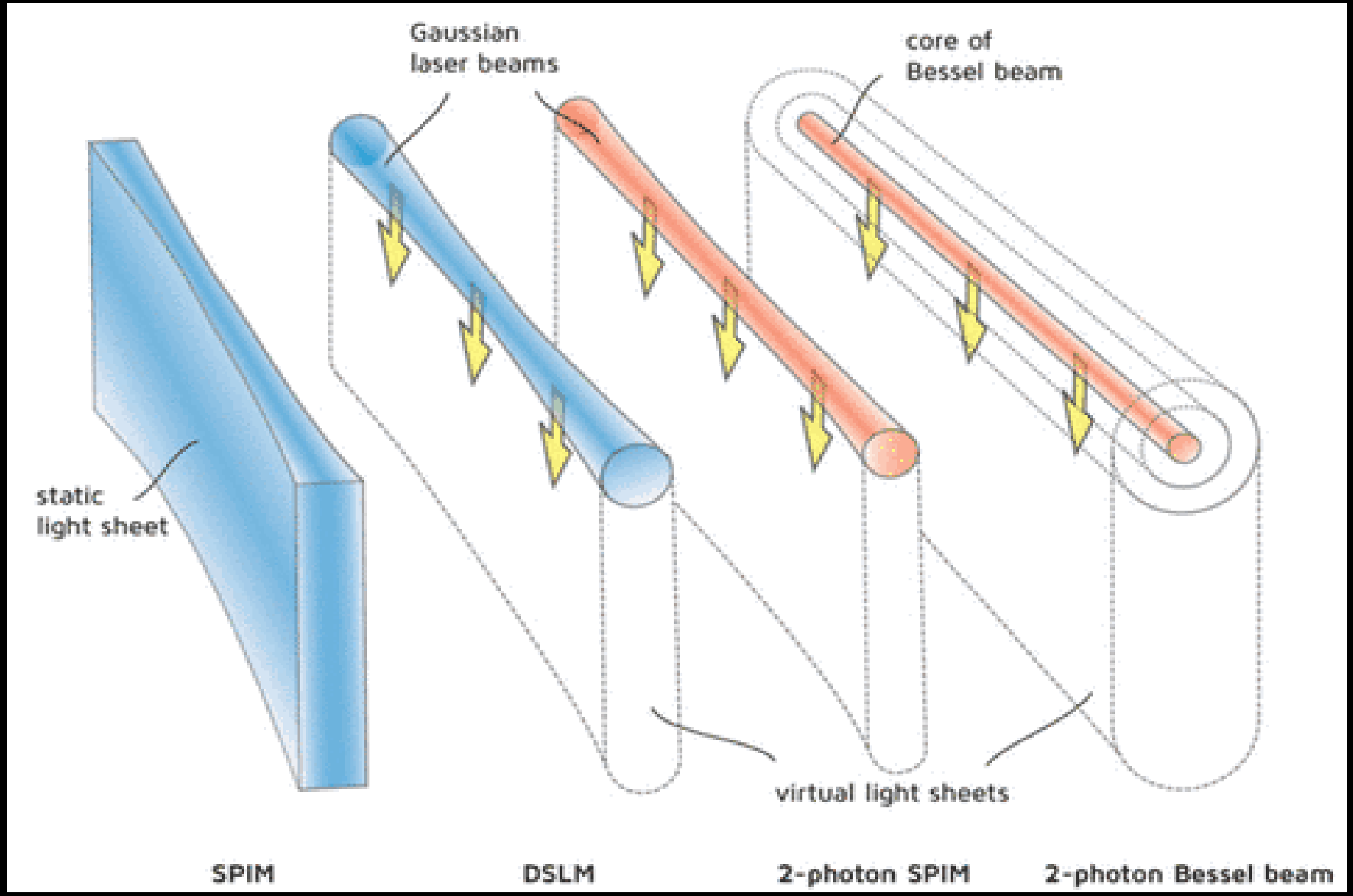
(Magnifications of the lenses were not given in the 1903 paper).

SPIM (Huisken, 2004)

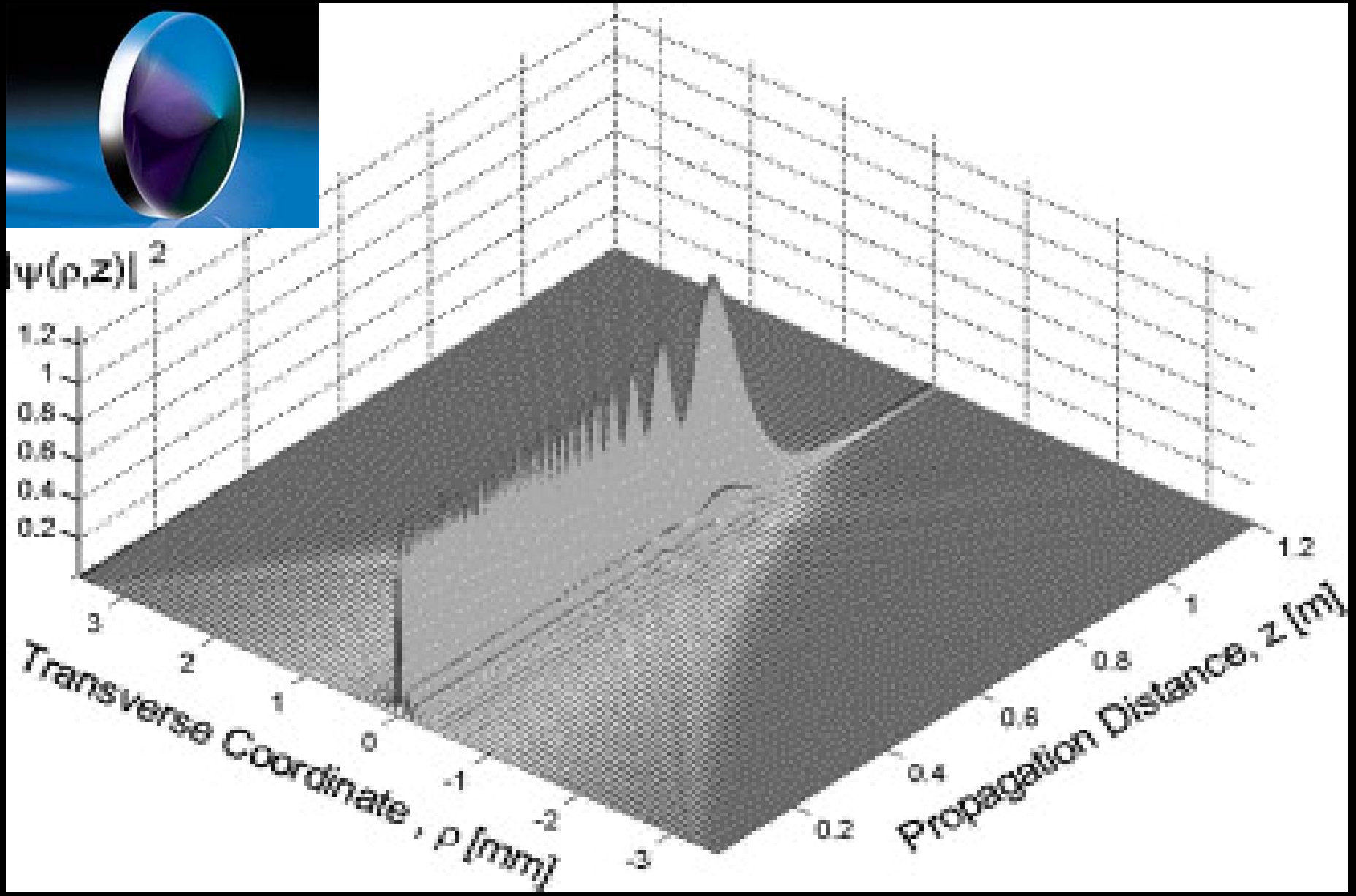
Selective plane imaging



Name/Acronym	Date	Light Source	Light Sheet	Detection Angle, °	Illumination	Specimen Size	Citation
Ultramicroscopy	1903	Sunlight	Slit aperture	90	Single	Gold beads	Siedentoph and Zsigmondy 1903
OPFOS	1993	532-nm laser	Cylindrical lens	90	Single	Cochlea >1 cm	Voie et al. 1993; Voie and Spelman 1995; Voie 2002
Theta confocal	1995	450-, 550-nm laser	Pinhole, not light sheet	102	Single	<1 cm	Stelzer et al. 1995
TSLM	2002	540-nm laser	Cylindrical lens	90	Single	>1 cm	Fuchs et al. 2002
SPIM	2004	488-nm laser	Cylindrical lens	90	Single	<1 cm	Huisken et al. 2004
mSPIM	2007	488-nm laser	Pivoting cylindrical lens	90	Dual	<1 cm	Huisken and Stainier 2007
HROPFOS	2007	532-nm laser	Cylindrical lens	90	Single	>1 cm	Buytaert and Dirckx 2007
Ultramicroscopy	2007	488-nm laser	Cylindrical lens	90	Dual	>1 cm	Dodt et al. 2007
OPM	2008	532-nm laser	Cylindrical lens	60	Single	<1 cm	Dunsby 2008
OCPI	2008	488-nm laser	Cylindrical or gradient index lens	90	Single	<1 cm	Holekamp et al. 2008
DSLIM	2008	400- to 650-nm laser	f-theta lens	90	Dual	<1 cm	Keller et al. 2008; Keller and Stelzer 2008; Keller et al. 2010
TSLIM	2009	532-, 488-nm laser	Cylindrical lens	90	Dual	>1 cm	Santi et al. 2009
HiLo	2010	491-nm laser	Scanned line	0	Single	>1 cm	Mertz and Kim 2010

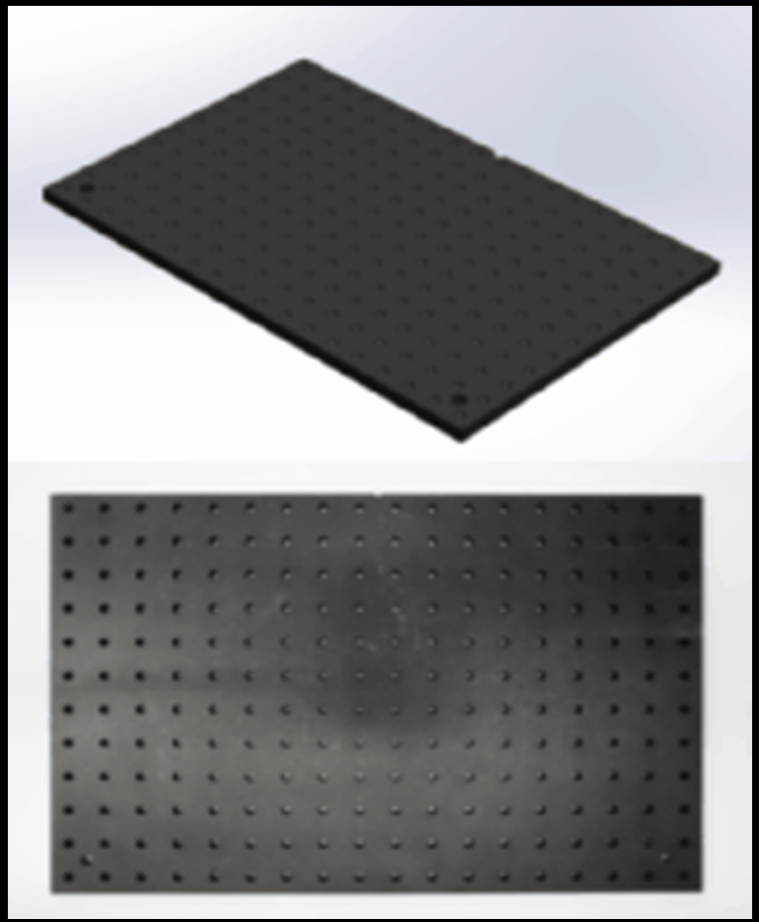

Gaussian
laser beams
core of
Bessel beam
static
light sheet
virtual light sheets
SPIM
DSLM
2-photon SPIM
2-photon Bessel beam

Creating a Bessel beam



Open SPIM

http://openspim.org



Digital Light Sheet Microscopy (=scanned light sheet microscopy)

Example:

Drosophila melanogaster development (20 hours) using two-photon DLSM (Caltech):

http://www.youtube.com/watch?v=6C11c-03ihc

Light sheet advantages

Light sheet microscopy offers

- multi-colour fluorescence imaging
- optical sectioning
- low photo toxicity
- fast and sensitive detection with the latest camera technology (EMCCD, sCMOS)
- good penetration in scattering tissues
- multi-view acquisition by rotation of the sample or scanning of excitation beam
- BUT specimen must be very transparent.

Objective design and resolution constraints

The Mesolens

- Widefield mesoscopy
- Confocal mesoscopy

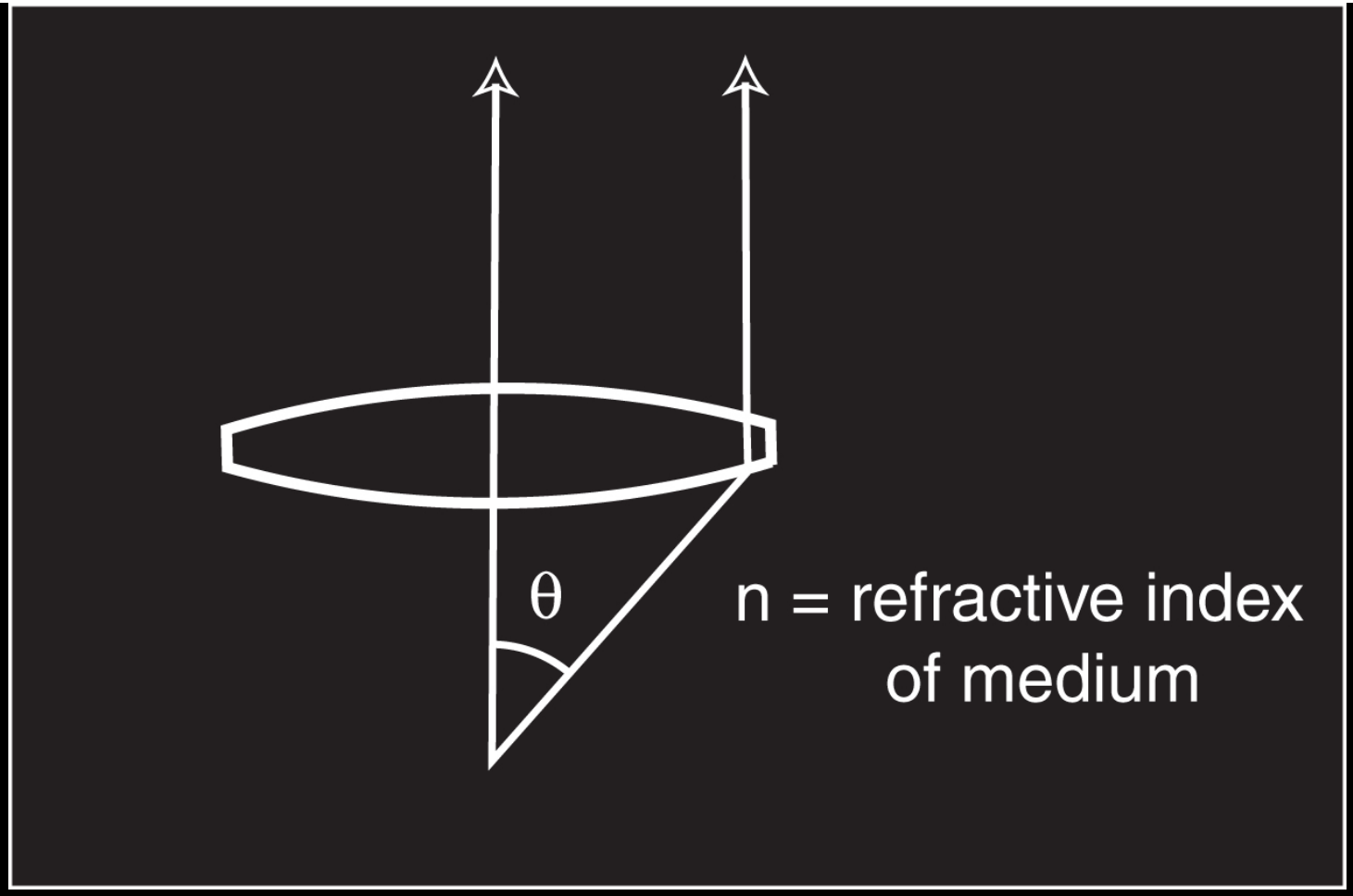
Future work

Objective design and resolution constraints

The Mesolens

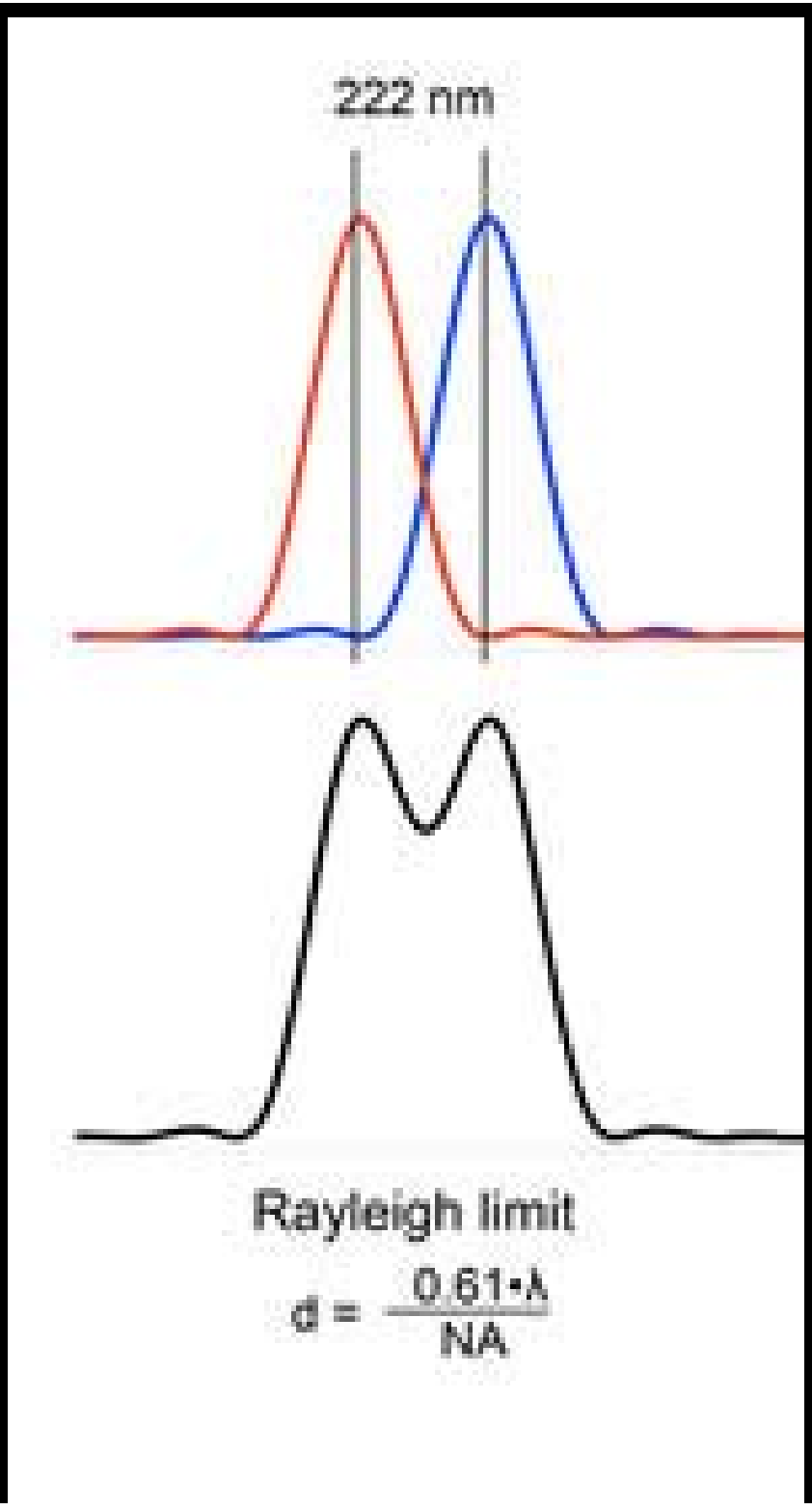
- Widefield mesoscopy
- Confocal mesoscopy

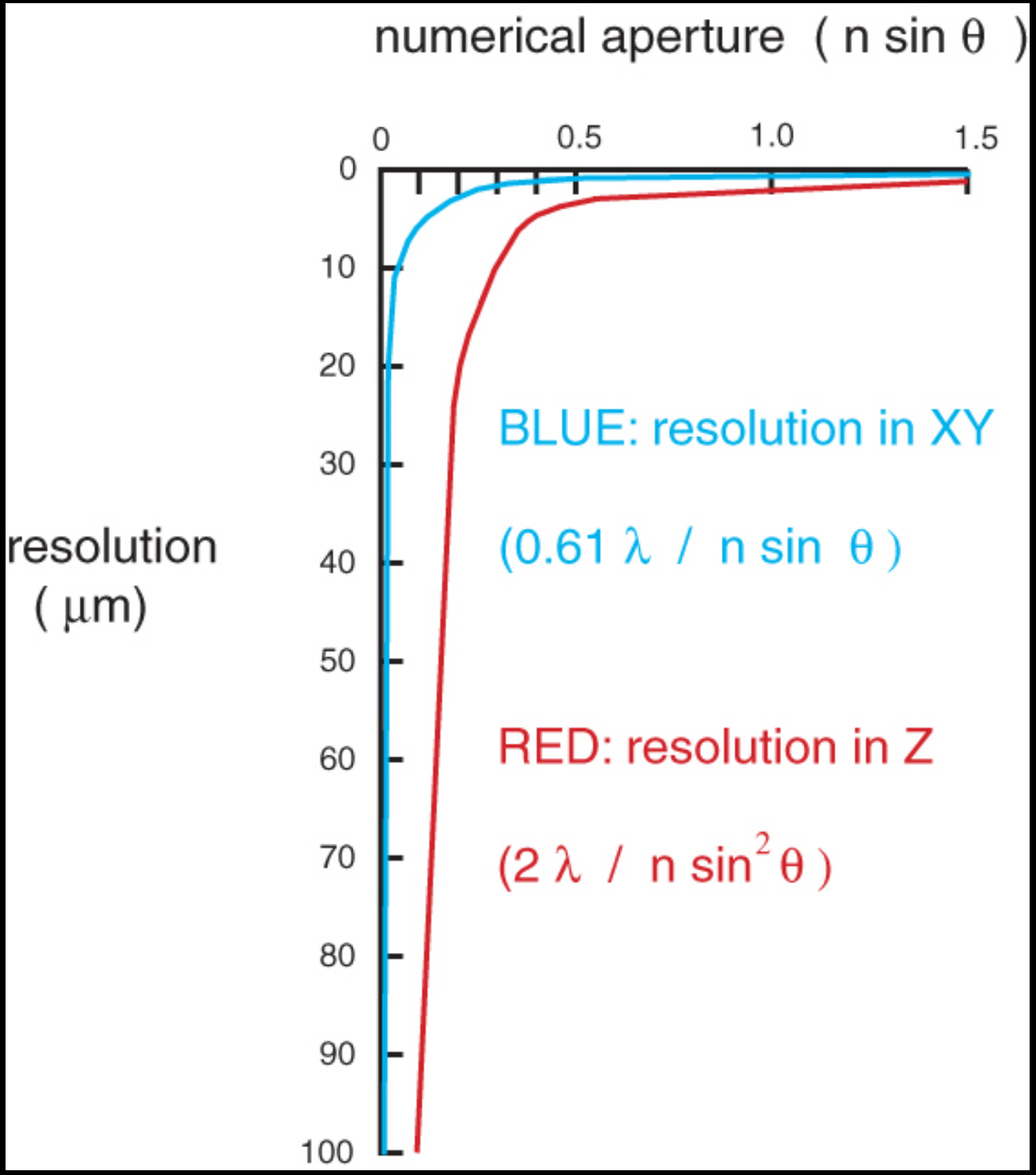
Future work

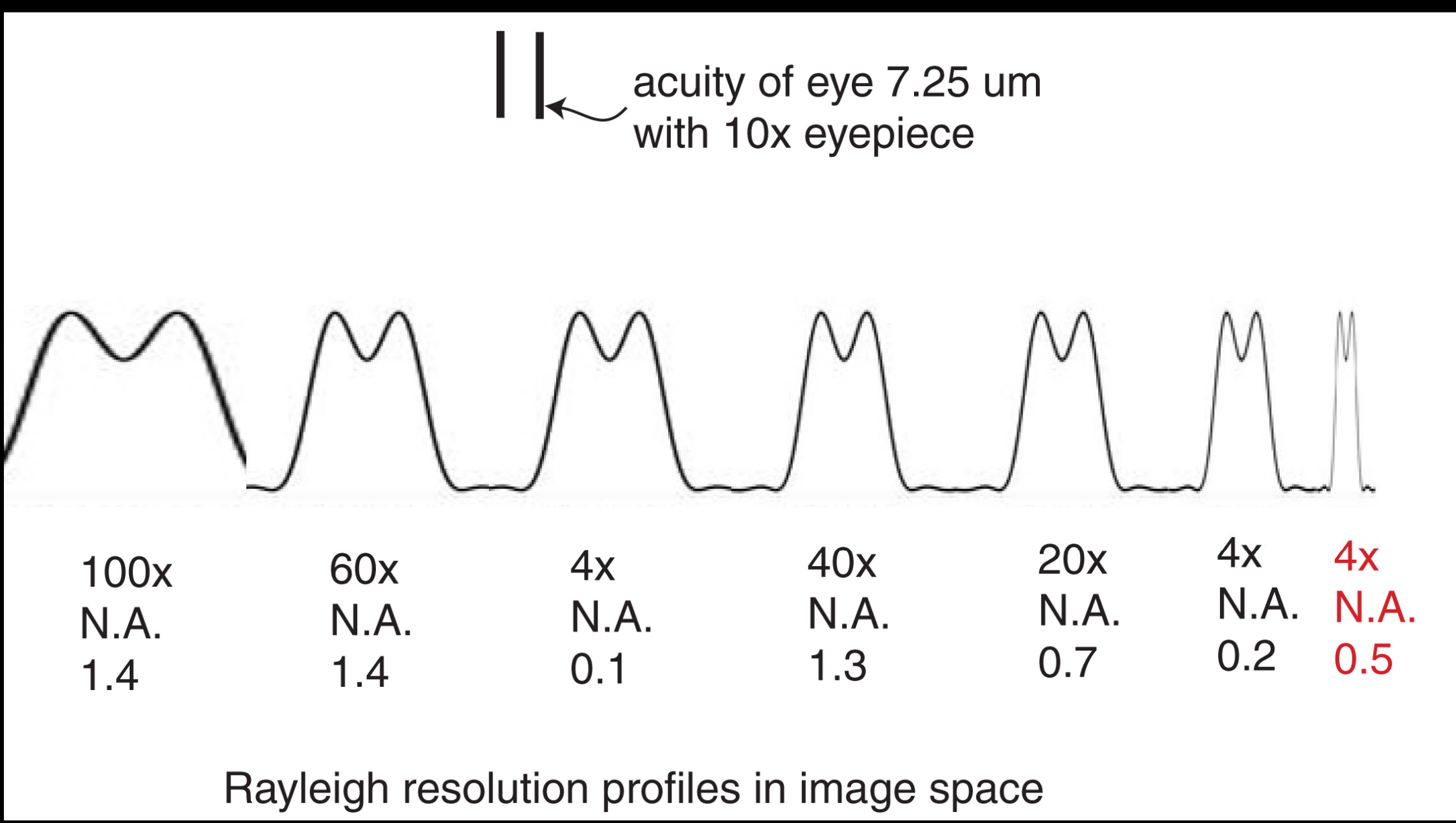


Numerical Aperture (NA) = $n \sin \theta$

resolution in xy plane, $r = 0.61 \lambda / NA$


222 nm
Rayleigh limit
$d = \frac{0.61 \cdot \lambda}{NA}$


numerical aperture (n sin θ)
0
0.5
1.0
1.5
0
10
20
30
40
50
60
70
80
90
100
resolution
(μm)
BLUE: resolution in XY
(0.61 λ / n sin θ)
RED: resolution in Z
(2 λ / n sin² θ)



Rayleigh resolution profiles in image space

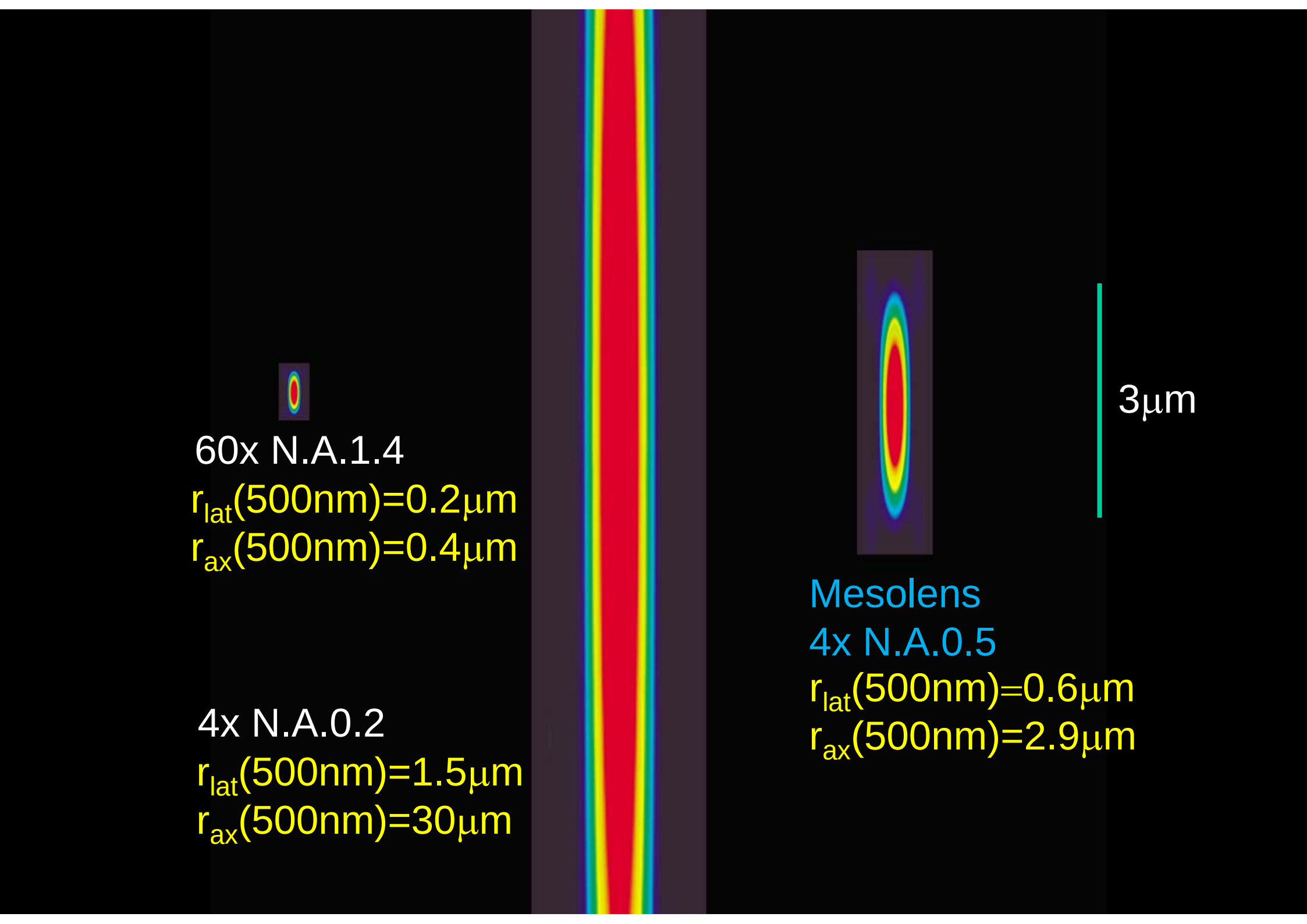
Mesolens: large volumetric imaging, sub-cellular resolution

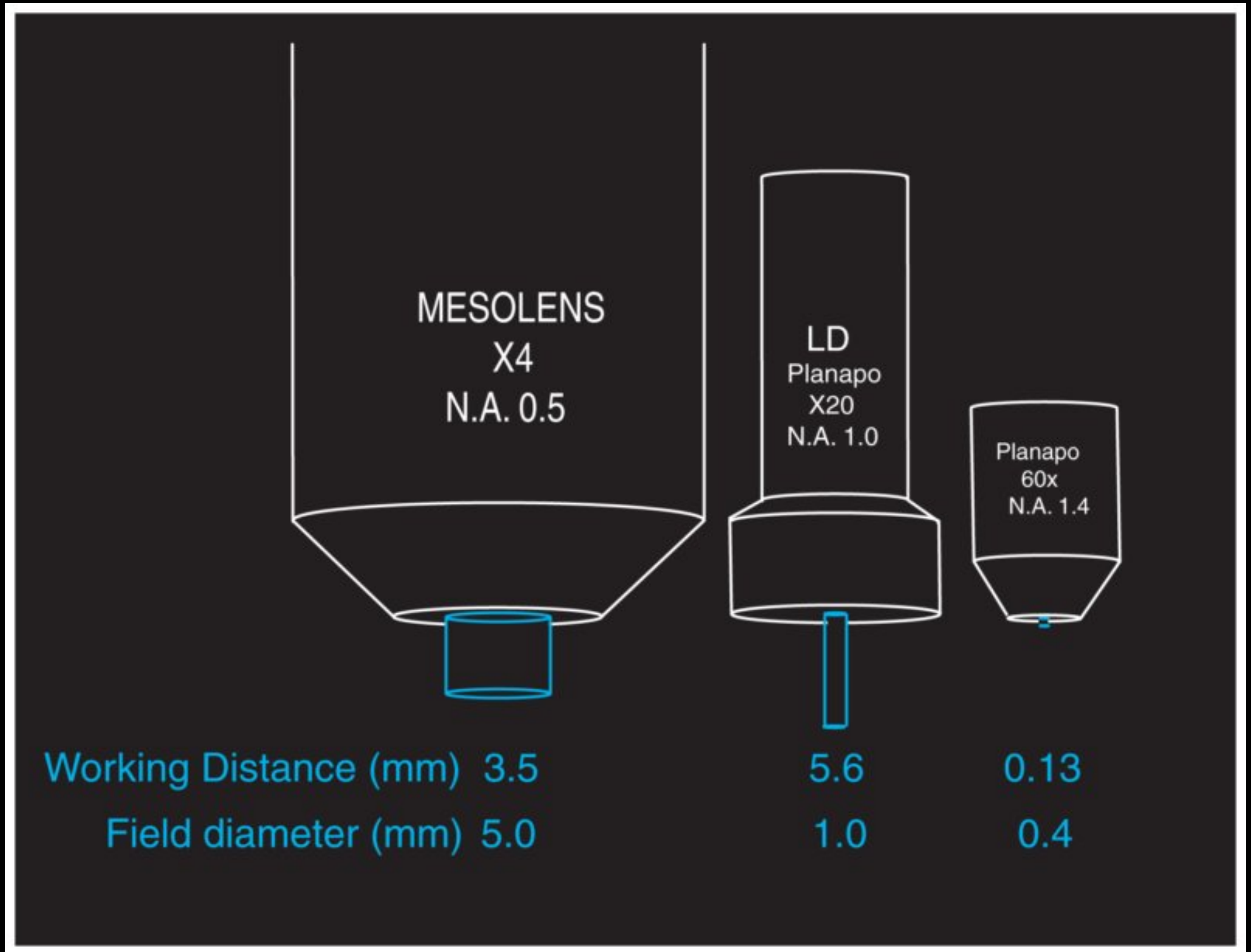
Objective design and resolution constraints

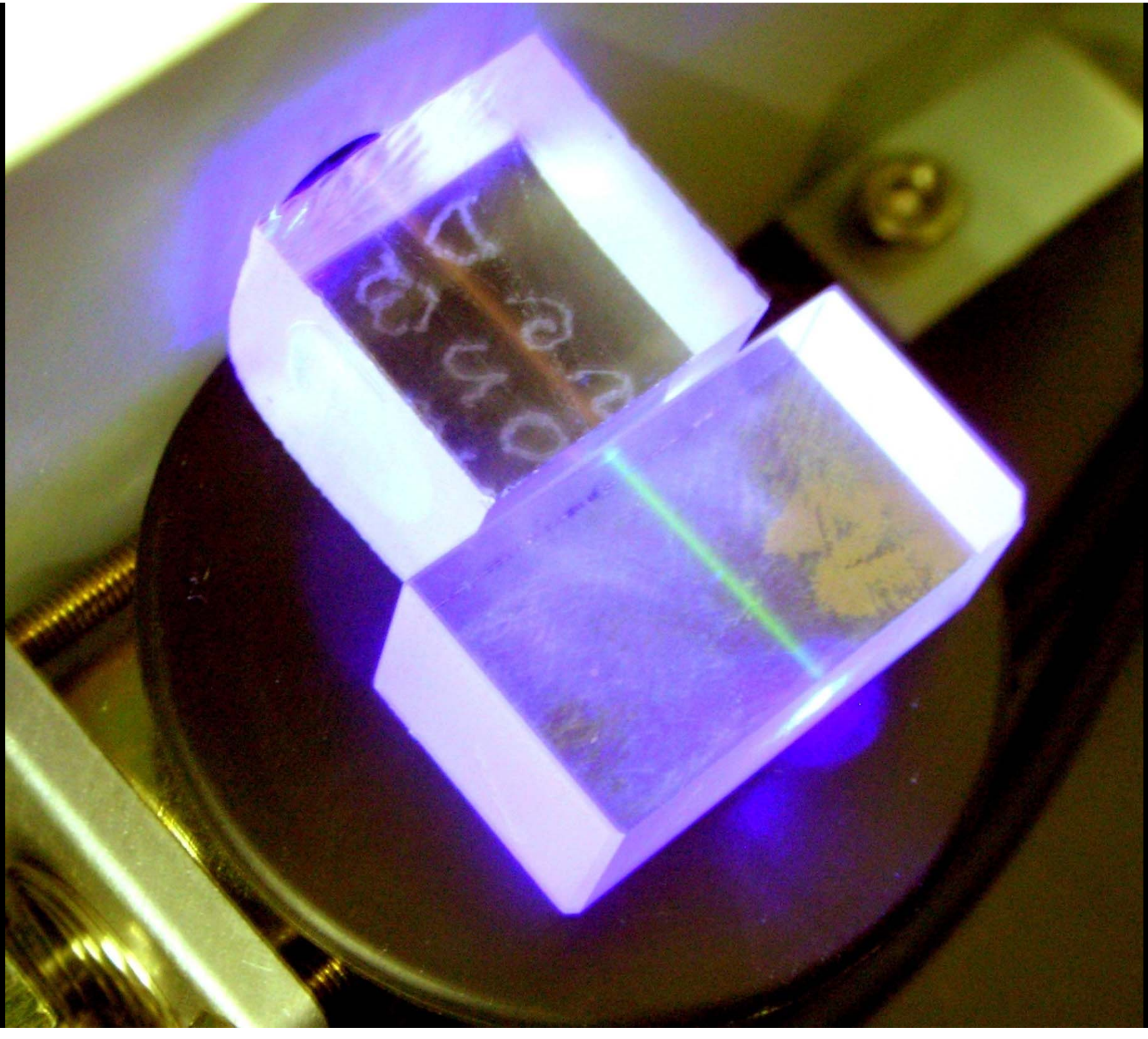
The Mesolens

- Widefield mesoscopy
- Confocal mesoscopy

Future work


60x N.A.1.4
r_{lat}(500nm)=0.2μm
r_{ax}(500nm)=0.4μm
4x N.A.0.2
r_{lat}(500nm)=1.5μm
r_{ax}(500nm)=30μm
Mesolens
4x N.A.0.5
r_{lat}(500nm)=0.6μm
r_{ax}(500nm)=2.9μm
3μm


MESOLENS
X4
N.A. 0.5
LD
Planapo
X20
N.A. 1.0
Planapo
60x
N.A. 1.4
Working Distance (mm) 3.5 5.6 0.13
Field diameter (mm) 5.0 1.0 0.4



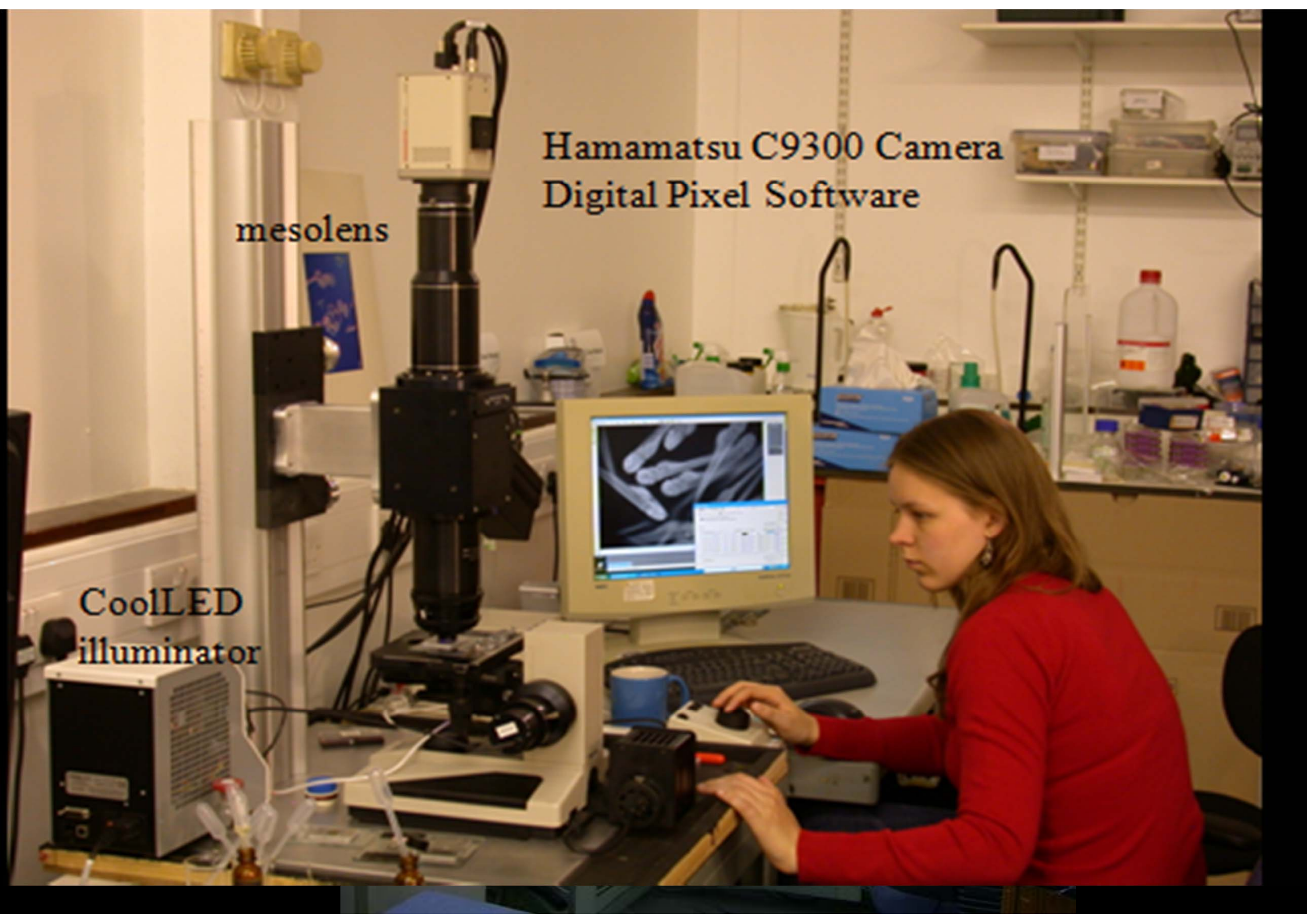
Objective design and resolution constraints

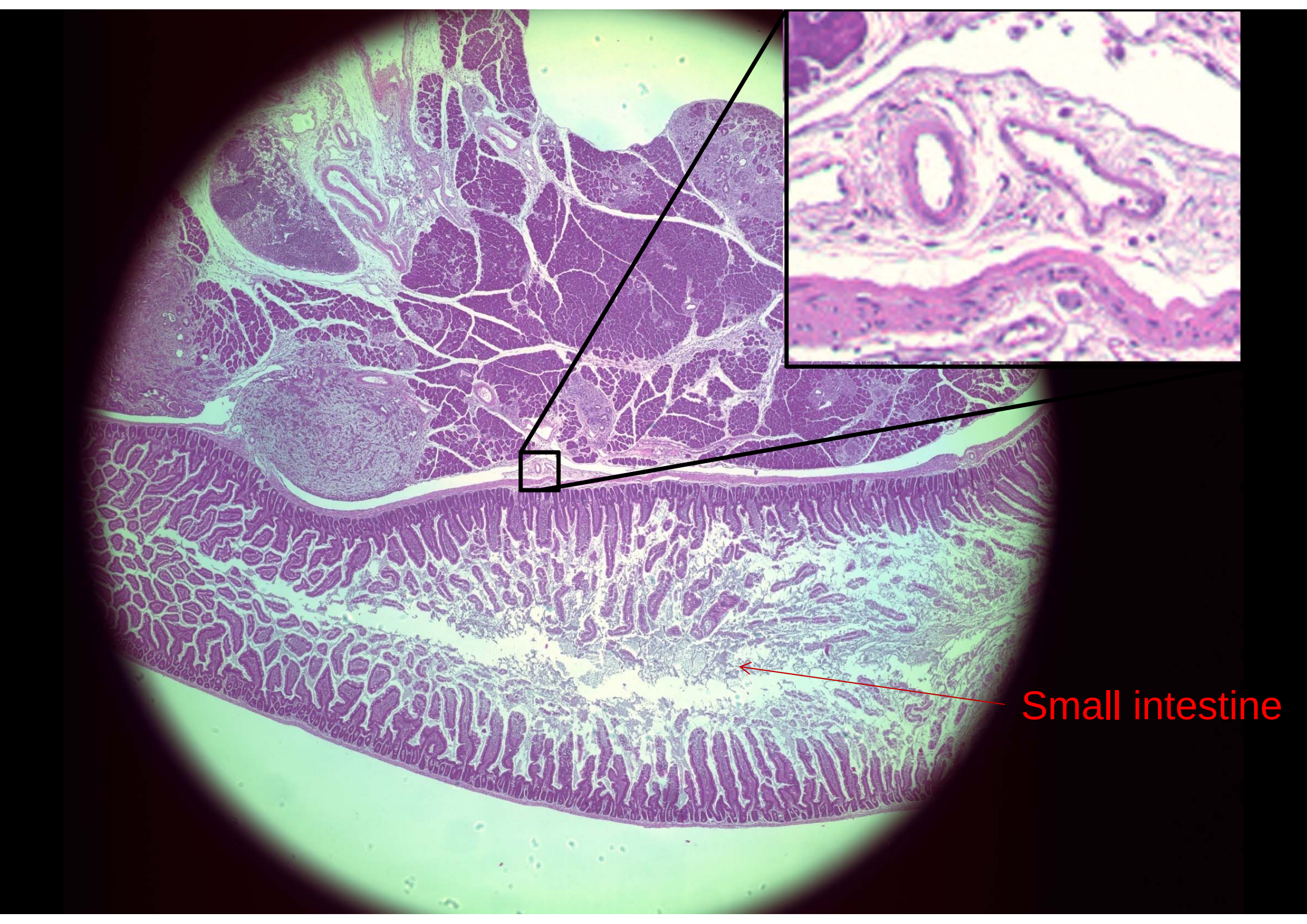
The Mesolens

Widefield mesoscopy

Confocal mesoscopy

Future work


Hamamatsu C9300 Camera
Digital Pixel Software
mesolens
CoolLED
illuminator


Small intestine

Movie:

embryonic rat brain explant showing

Neurones (green)
Glial cells (red)
Nuclei (blue)

Section of 15 day old mouse embryo head (montage derived from a single Mesolens image by moving the camera)

4mm diameter

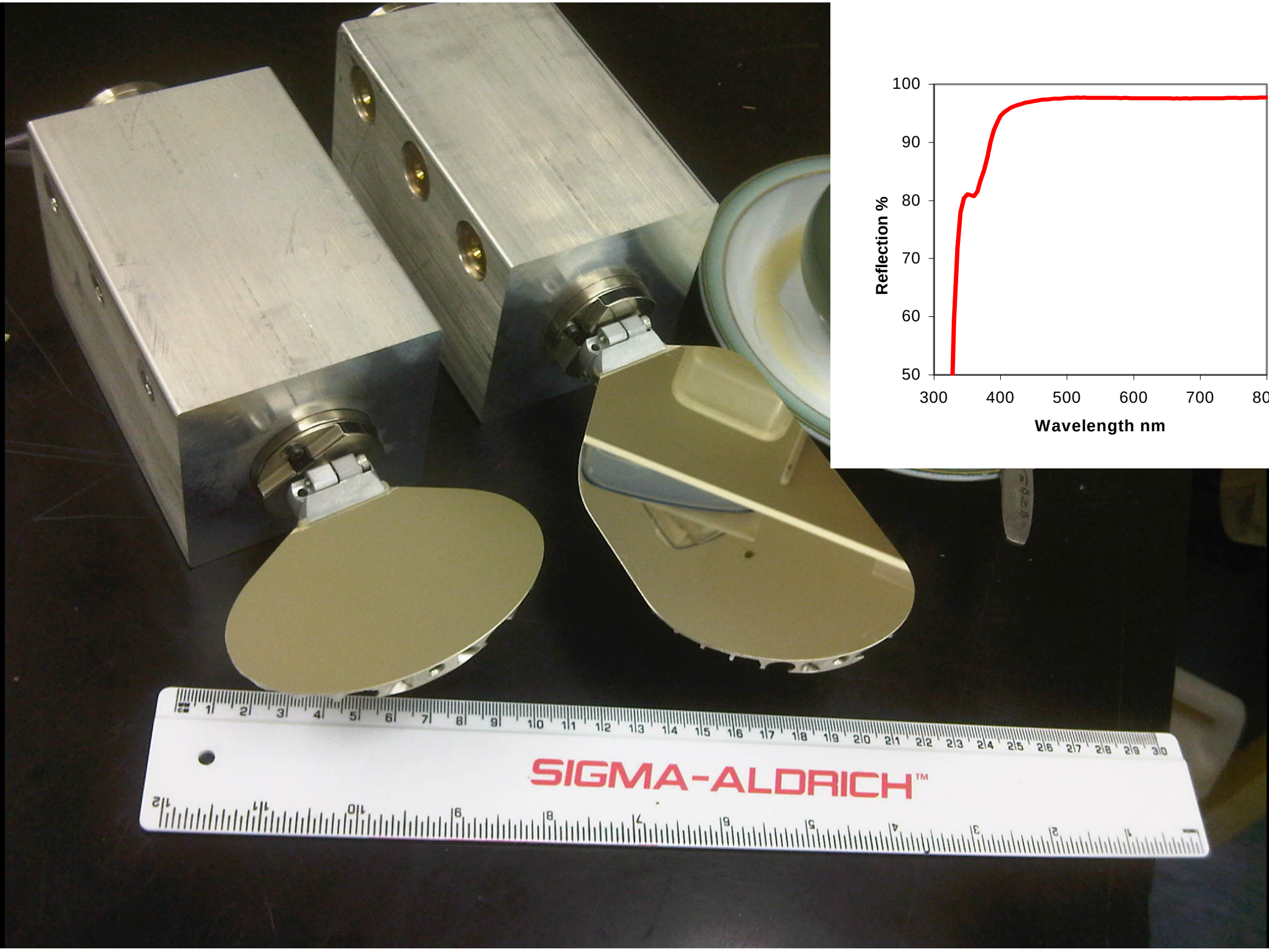
Objective design and resolution constraints

The Mesolens

Widefield mesoscopy

Confocal mesoscopy

Future work

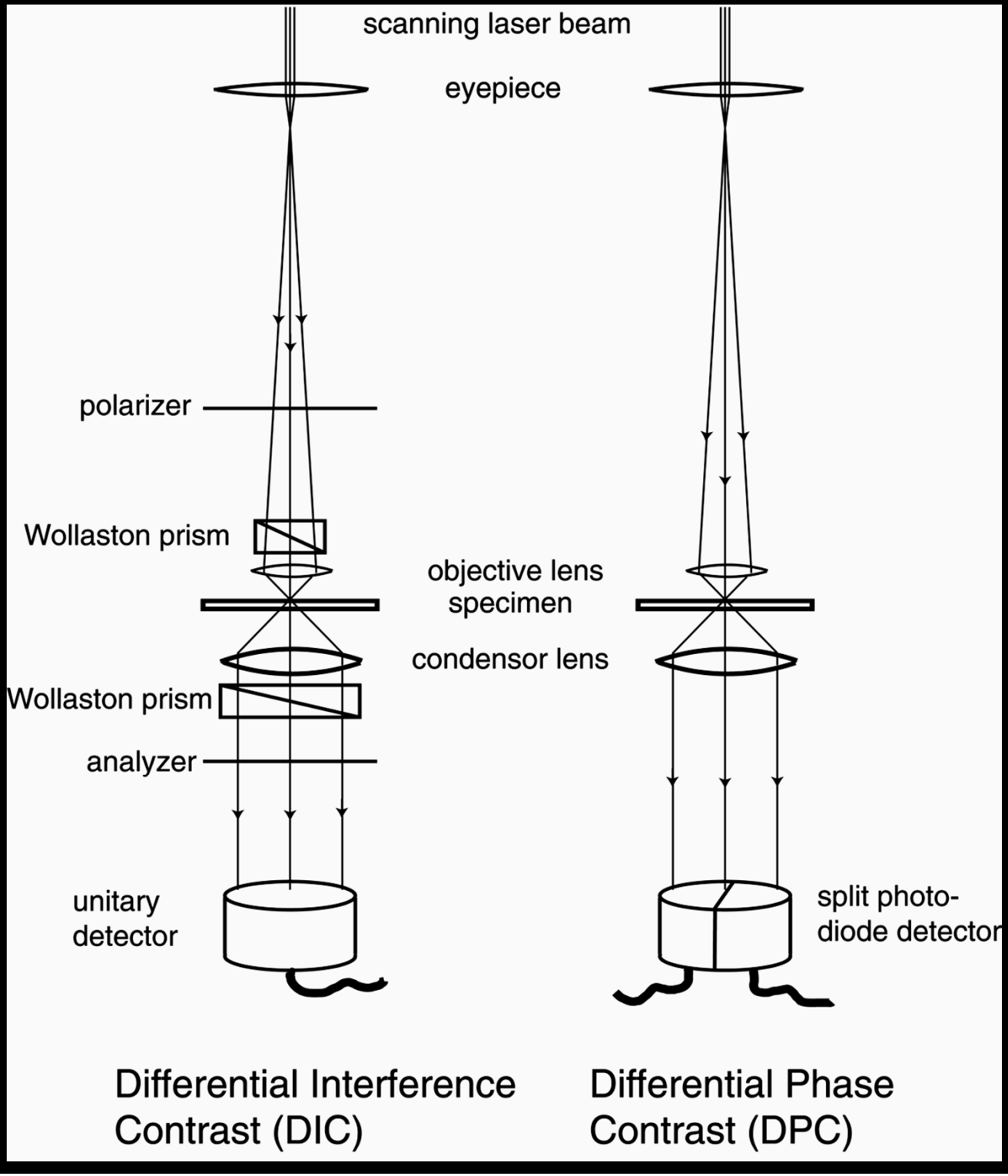

SIGMA-ALDRICH™

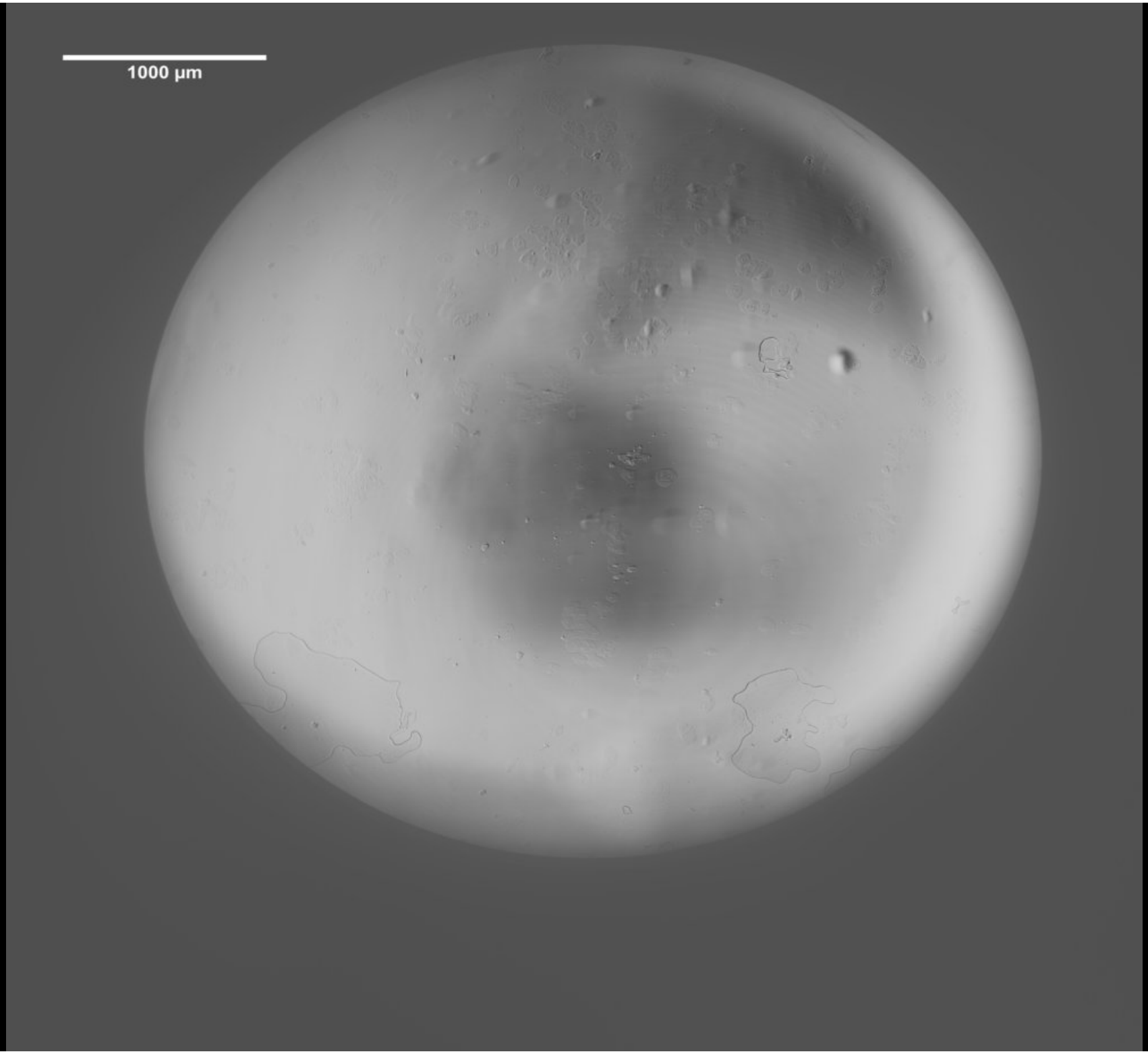
100
90
80
70
60
50
Reflection %
300
400
500
600
700
800
Wavelength nm

Confocal mesoscopy of 10-day mouse embryos

Whole mounts, fixed and cleared in glycerol after staining with a nuclear marker (Acridine Orange): *ImageJ*

1. *Whole mouse*
2. *Region of interest (cardiovascular system)*


scanning laser beam
eyepiece
polarizer
Wollaston prism
objective lens
specimen
condensor lens
Wollaston prism
analyzer
unitary
detector
split photo-
diode detector
Differential Interference
Contrast (DIC)
Differential Phase
Contrast (DPC)


1000 µm

Objective design and resolution constraints

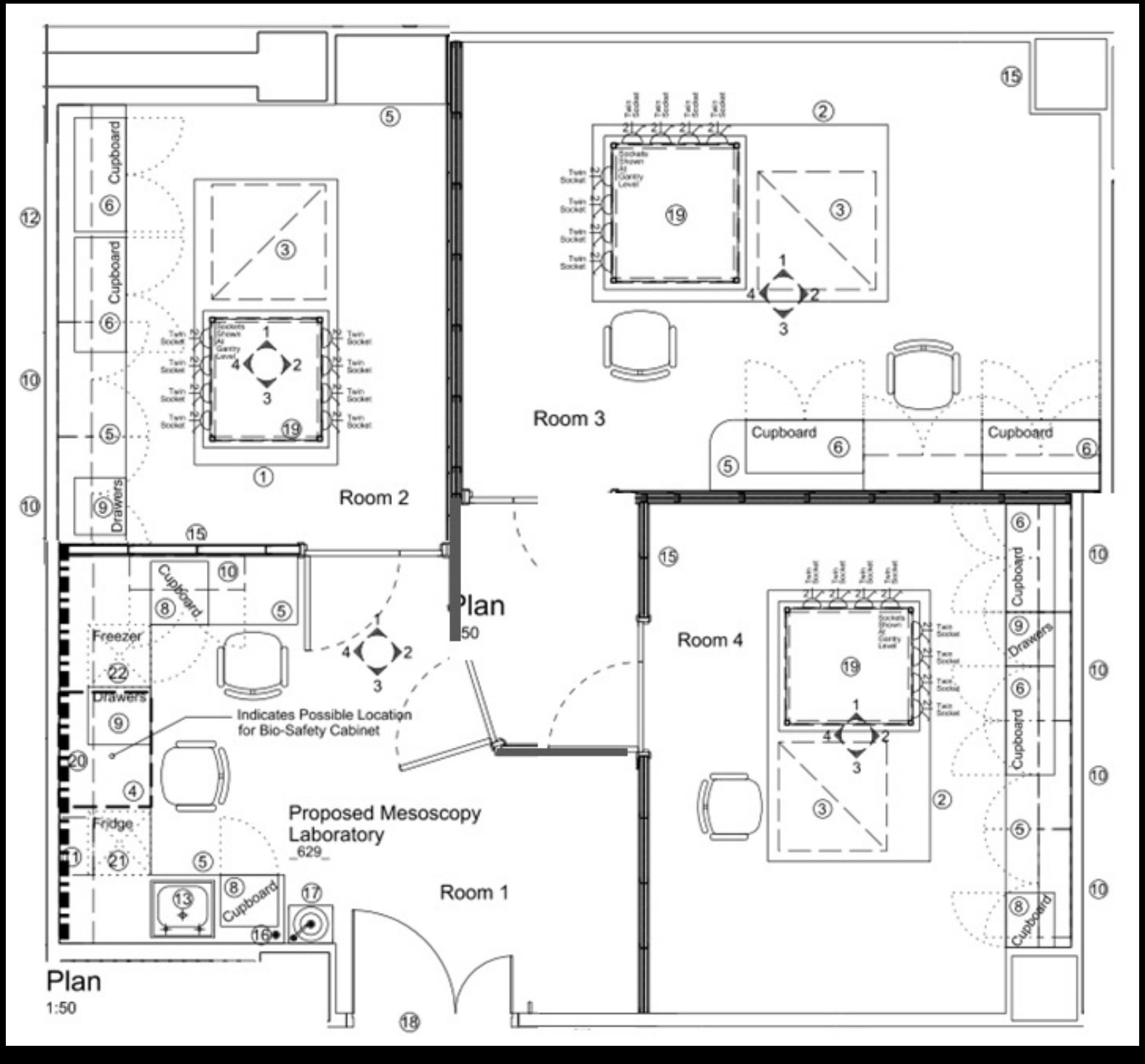
The Mesolens

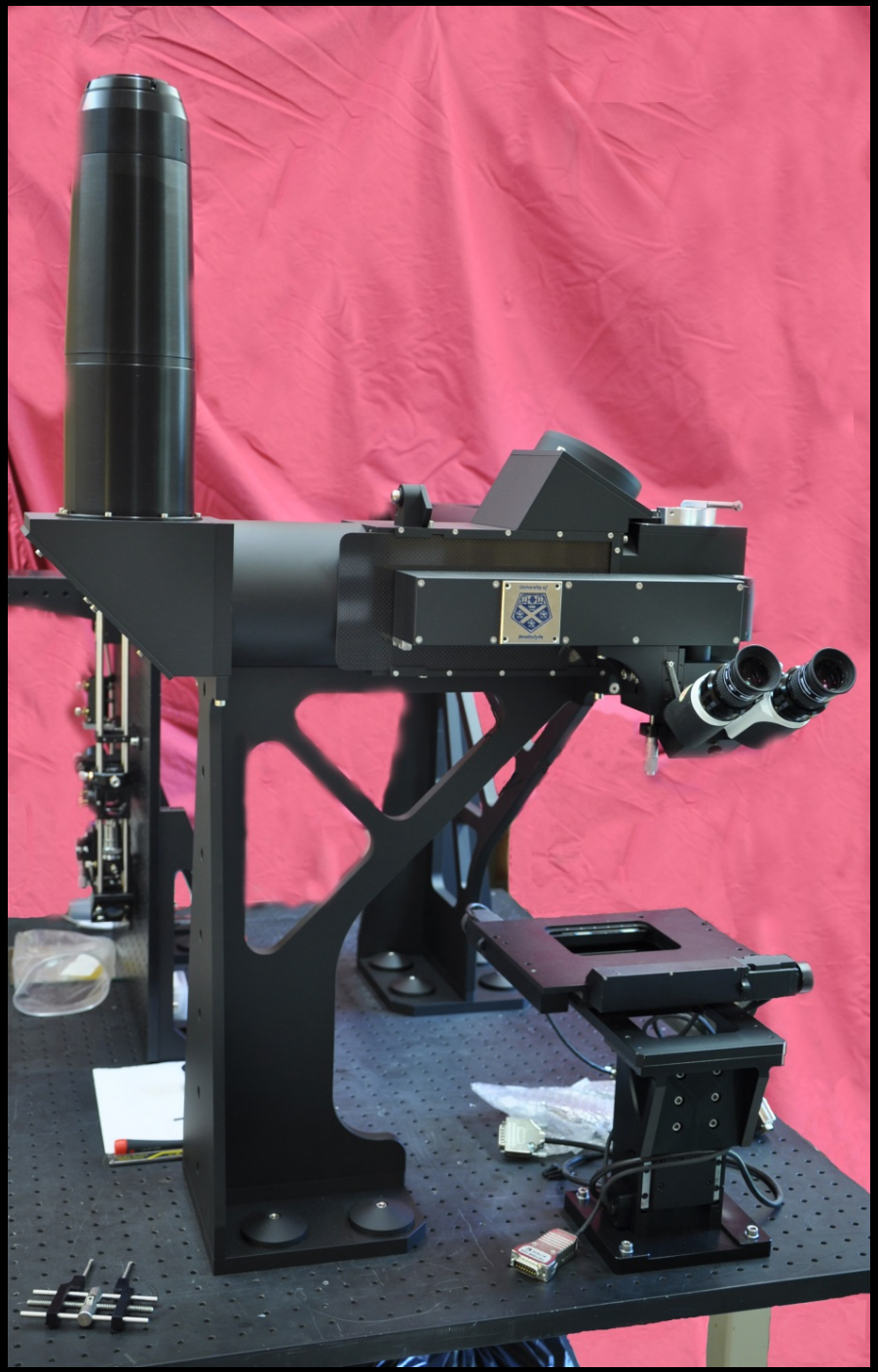
- Widefield mesoscopy
- Confocal mesoscopy

Future work

Mesolab: An Evaluation Centre for Optical Mesoscopy for Biomedical Research at the University of Strathclyde

http://mesolab.wordpress.com


Room 2
Room 3
Room 4
Room 1
Cupboard
Drawers
Freezer
Fridge
Indicates Possible Location for Bio-Safety Cabinet
Proposed Mesoscopy Laboratory
_629_
Plan
1:50



Mesolab projects and collaborators

MesoDev WP1: Fast wide-field mesoscopy, J. Dempster (Strathclyde)
MesoDev WP2: Multi-photon mesoscopy, G. McConnell (Strathclyde)
MesoDev WP3: Digital scanning lightsheet mesoscopy, M. MacDonald (Dundee)
MesoDev WP4: Fluorescence lifetime mesoscopy, B. Vojnovic (Oxford)

MesoApp WP1: Mesoscopic bioluminescence recording to understand circadian timing, M. Hastings (Cambridge)
MesoApp WP2: 'Super-wide-field' single cell gel electrophoresis assays, M. Boyd (Strathclyde)
MesoApp WP3: Three-dimensional and four-dimensional optical mesoscopy of developing mouse embryos, T. Weaver (MRC Harwell)
MesoApp WP4: Three-dimensional and four-dimensional image analysis of developing mouse and human embryos datasets acquired using optical mesoscopy R. Baldock (Edinburgh) and S. Lindsay (Newcastle)
MesoApp WP5: Visualising infection and immunity at the mesoscopic scale *in vivo*, O. Millington (Strathclyde)
MesoApp WP6: Simultaneous mesoscopy and electrophysiological recording of large neural networks, K. Mathieson (Strathclyde)

Stemmer Imaging…

6576 x 4384 pixel + pixel shift technology = 260 Megapixel @ 0.6fps

Acknowledgements

Brad Amos & Es Reid (Mesolens Ltd)
Johanna Tragardh (DPC & dispersion calculations)
John Dempster (Mesoscan software)
Ged Drinkwater (Control electronics)
Rumelo Amor (Optical mesoscopy)
Yvonne Vallis (Rat brain explant specimens)
Gavin Chapman (Mouse embryo specimens)
Mike Hastings (Bioluminescent tumour cells)
Marie Boyd (Comet assay specimen)