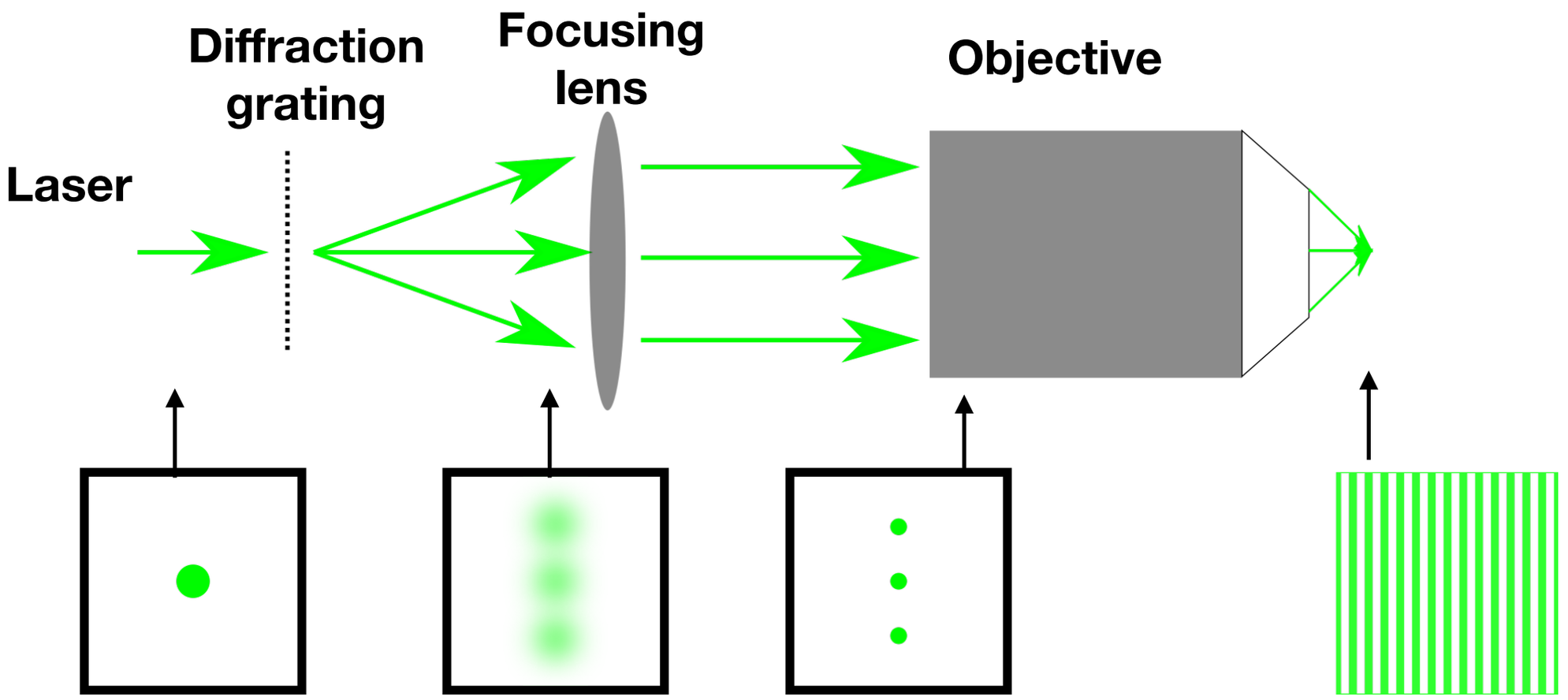
Implementing SIM in practice

How to do SIM

- Generate stripes
- Project into sample
- Image
- Reconstruct

Stripe generation

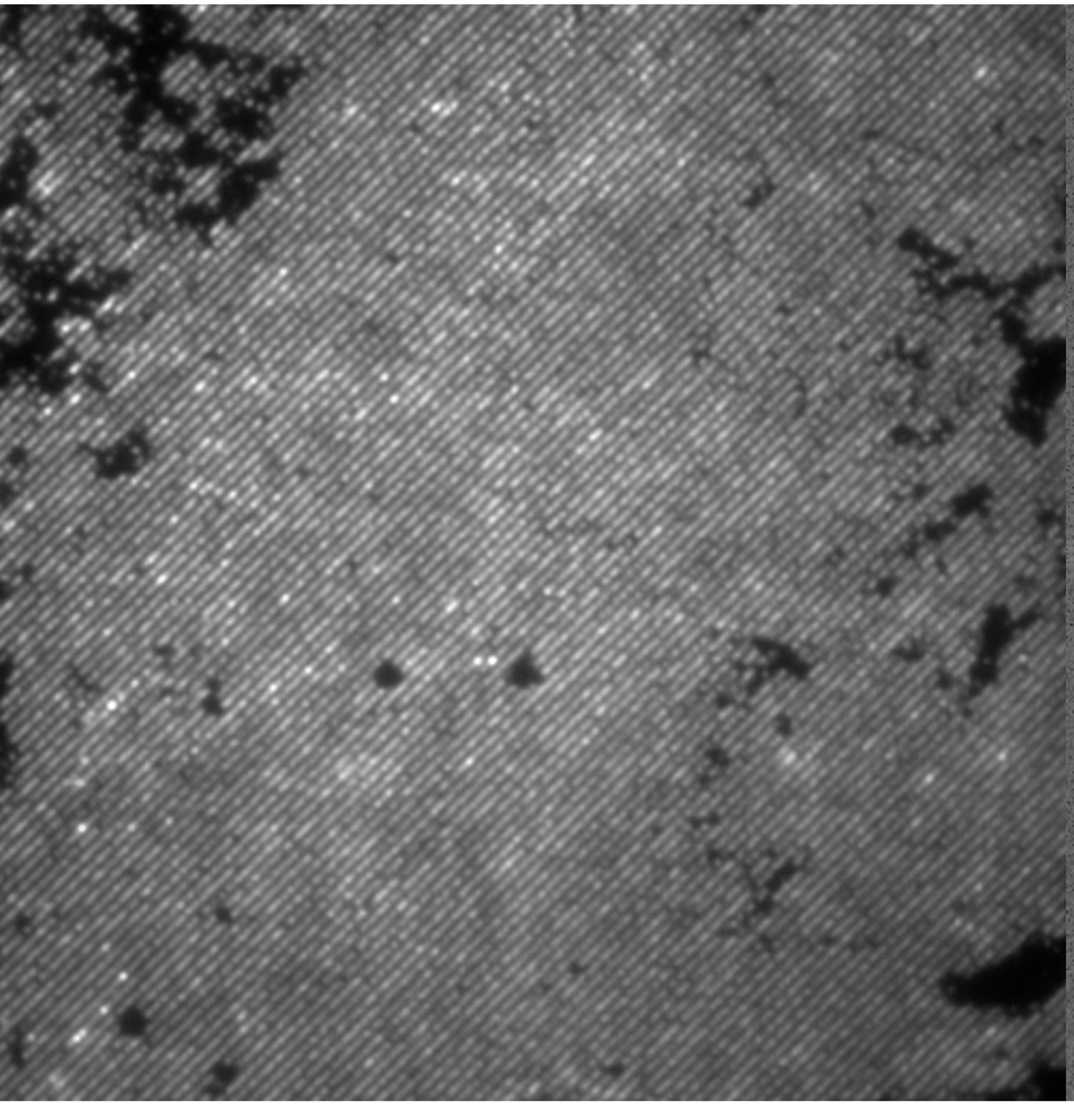


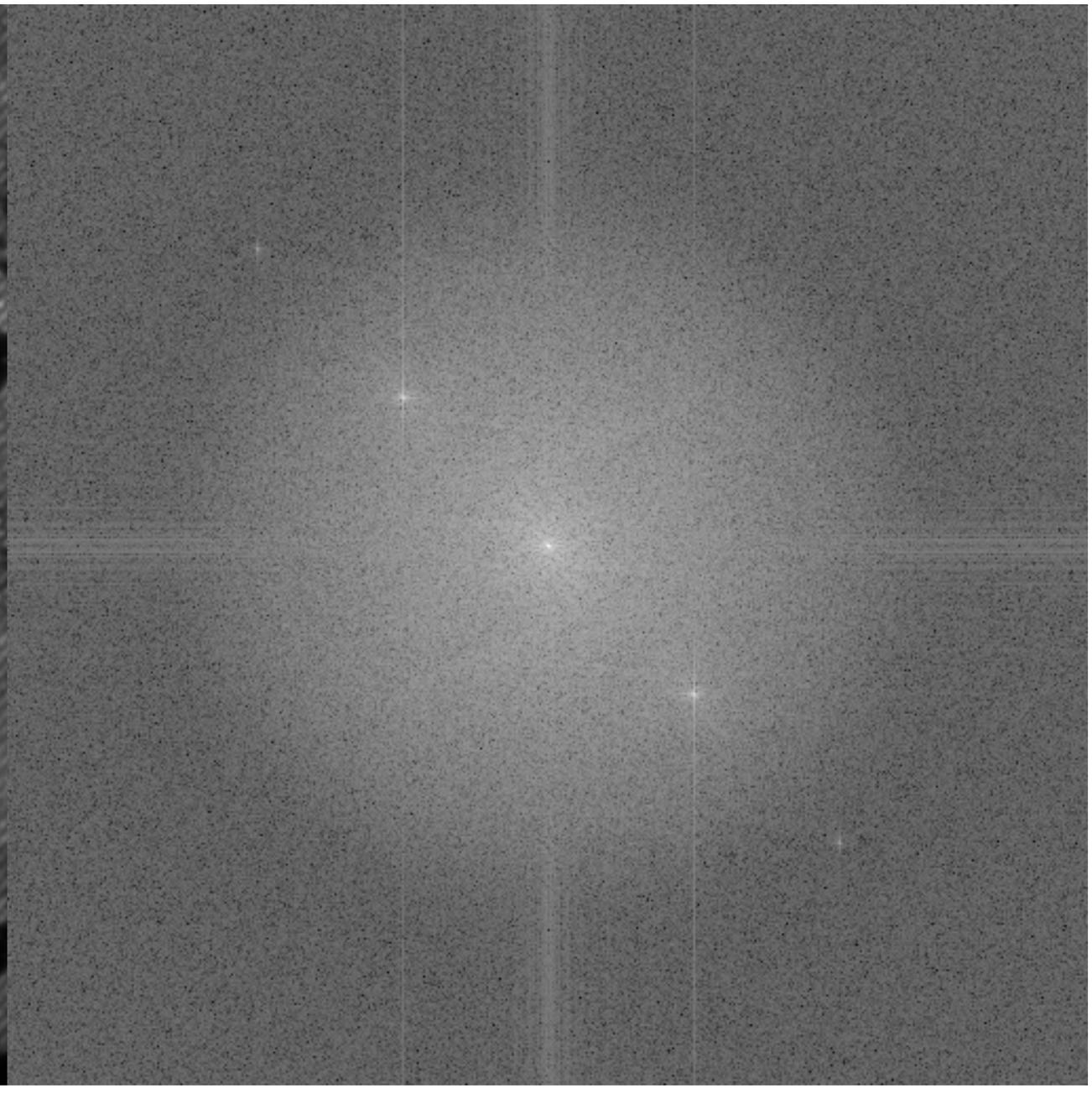
Light distribution in plane. Not to scale!

Stripe complications

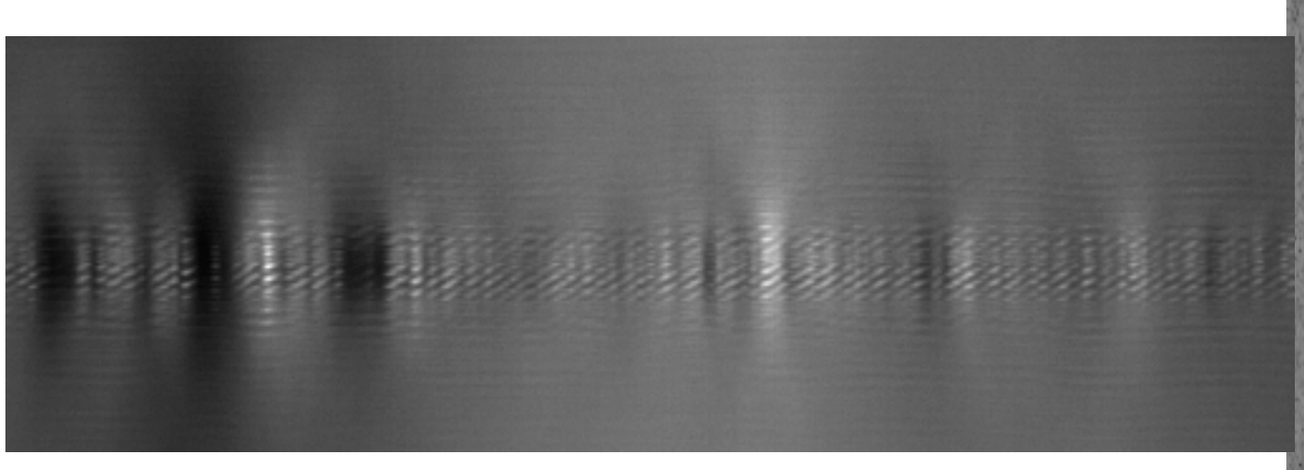
- Try to avoid 0 and 90 deg, pixel artifacts
- Polarisation
- Contrast
- Rotation
- Width change with wavelength

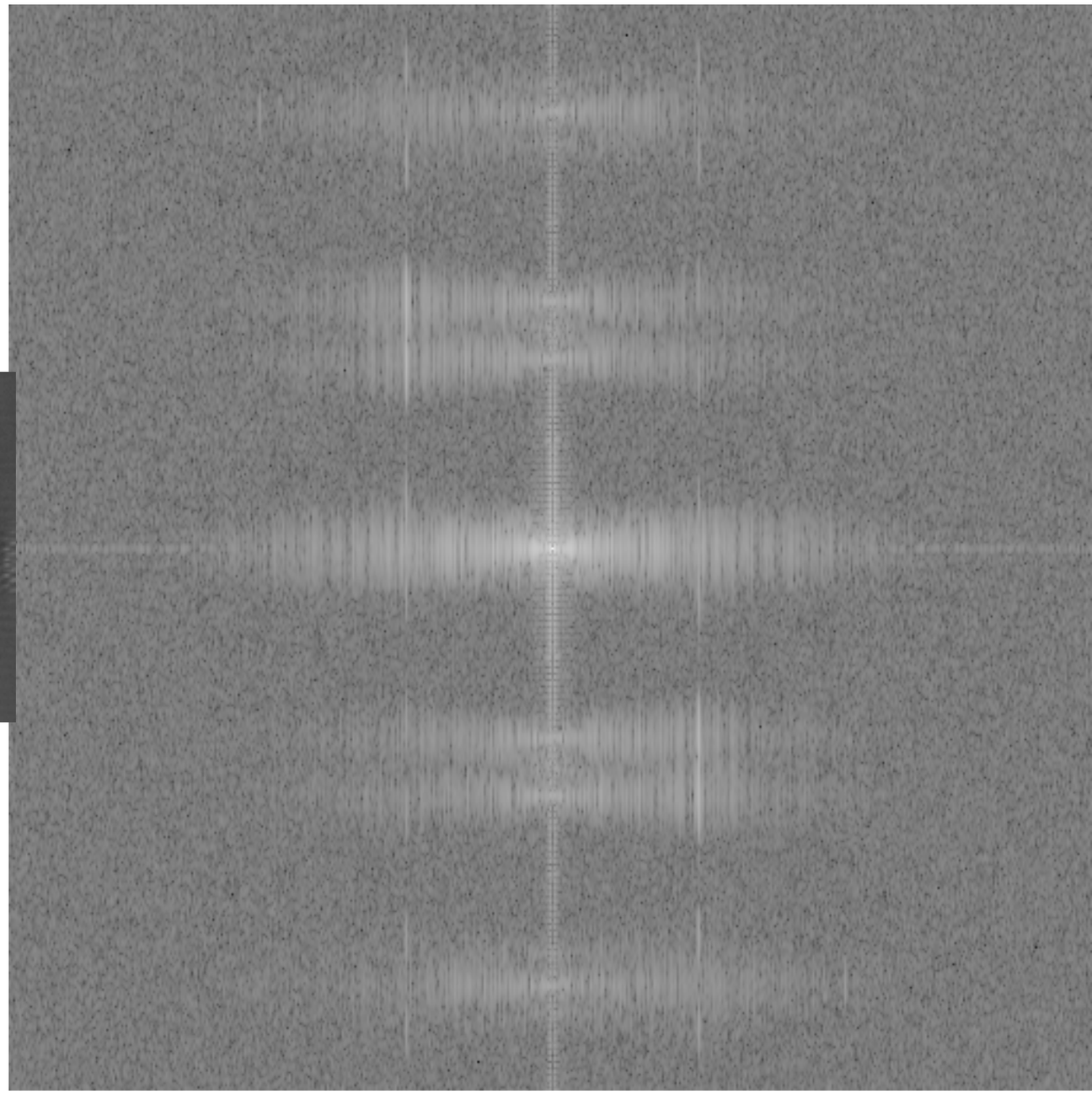
Images and their FFTs

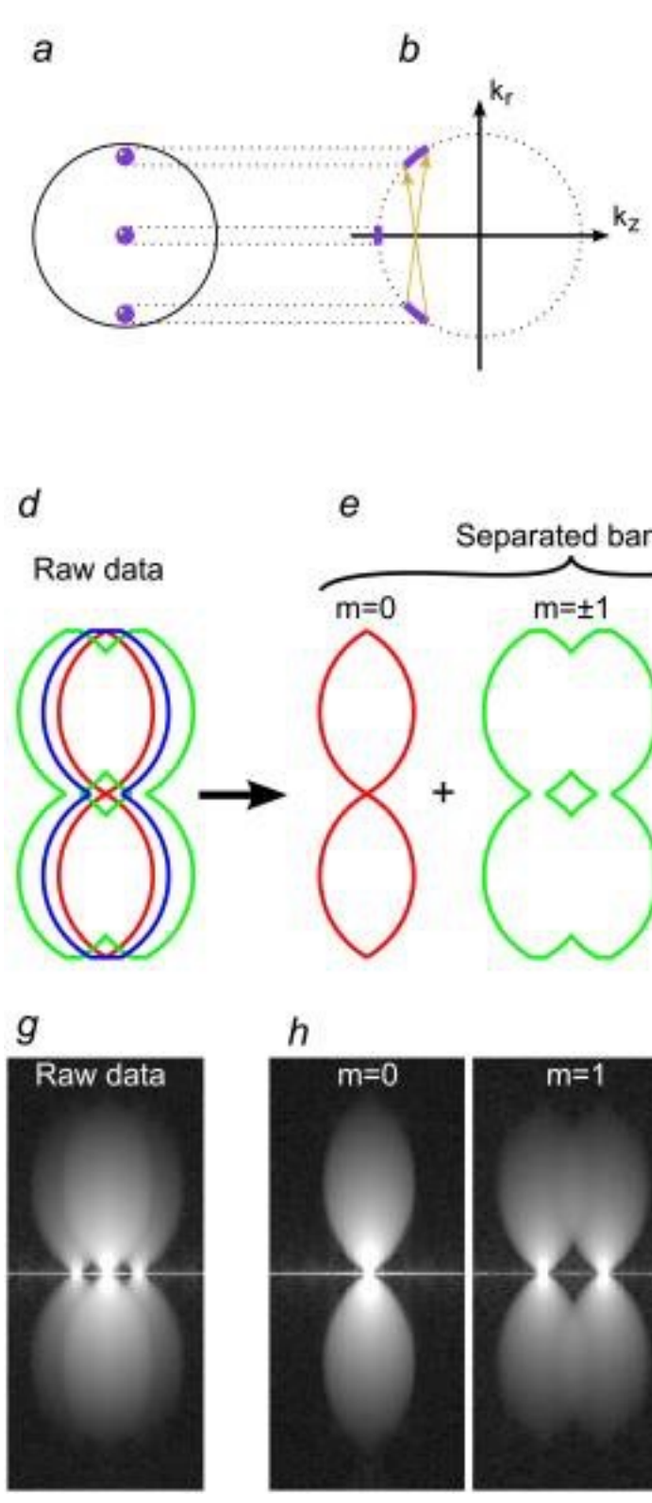


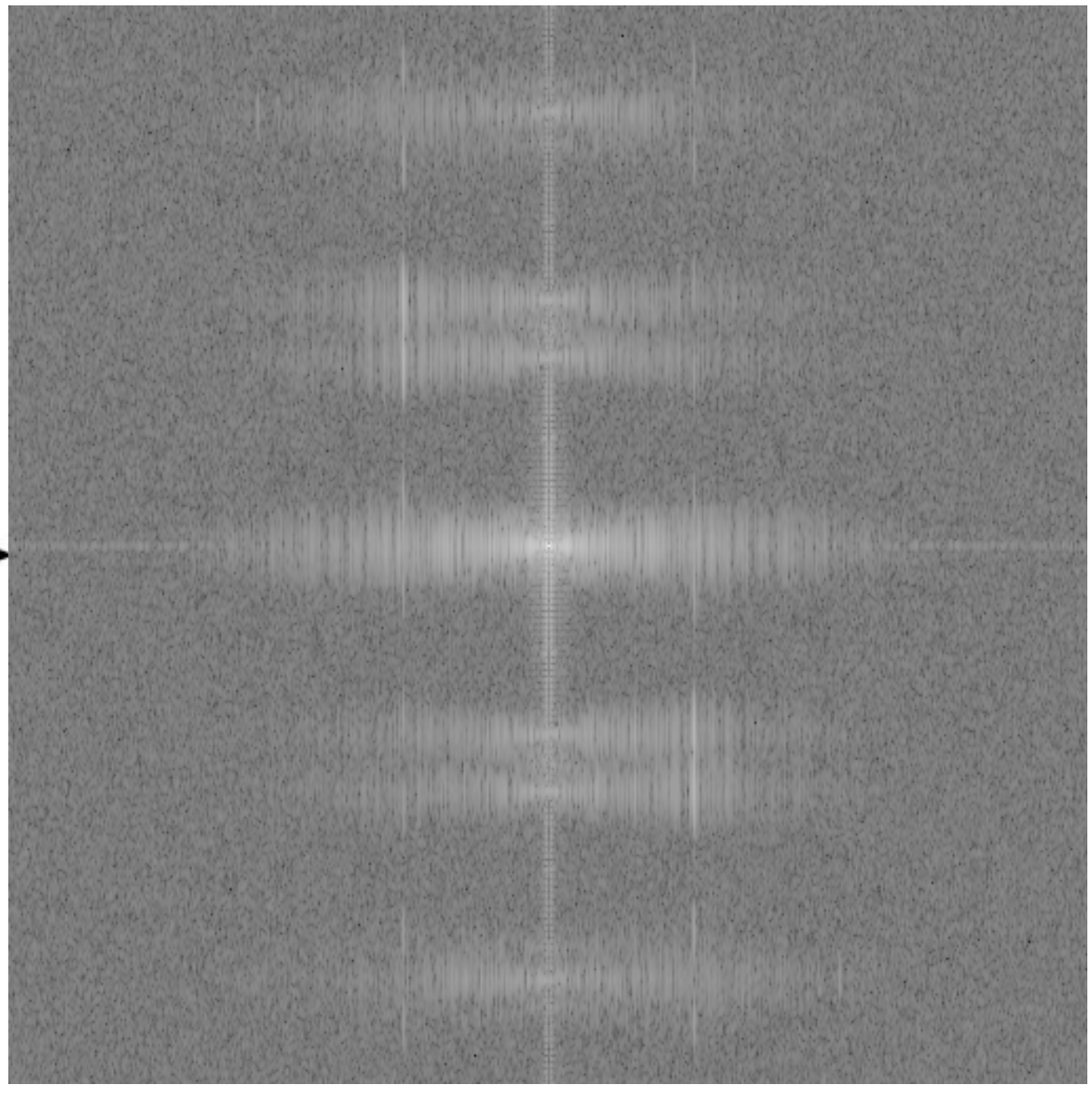


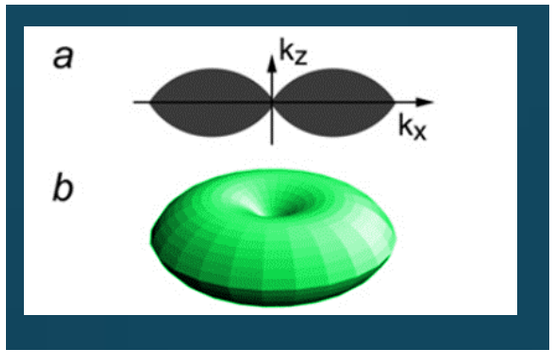
Now look at vertical section

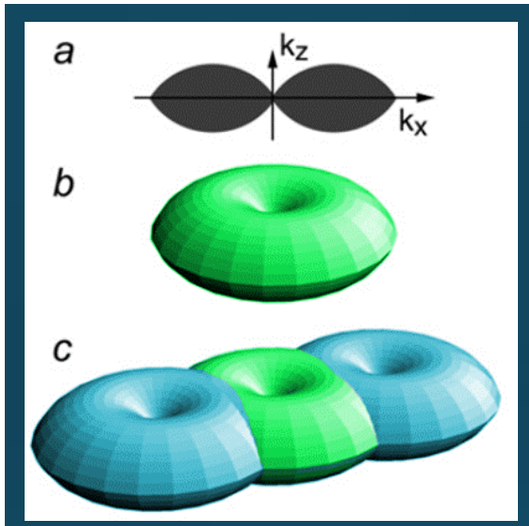





a
b
k_r
k_z
c
k_r
k_z
d
Raw data
e
Separated bands
m=0
m=±1
m=±2
+
+
g
Raw data
h
m=0
m=1
m=2

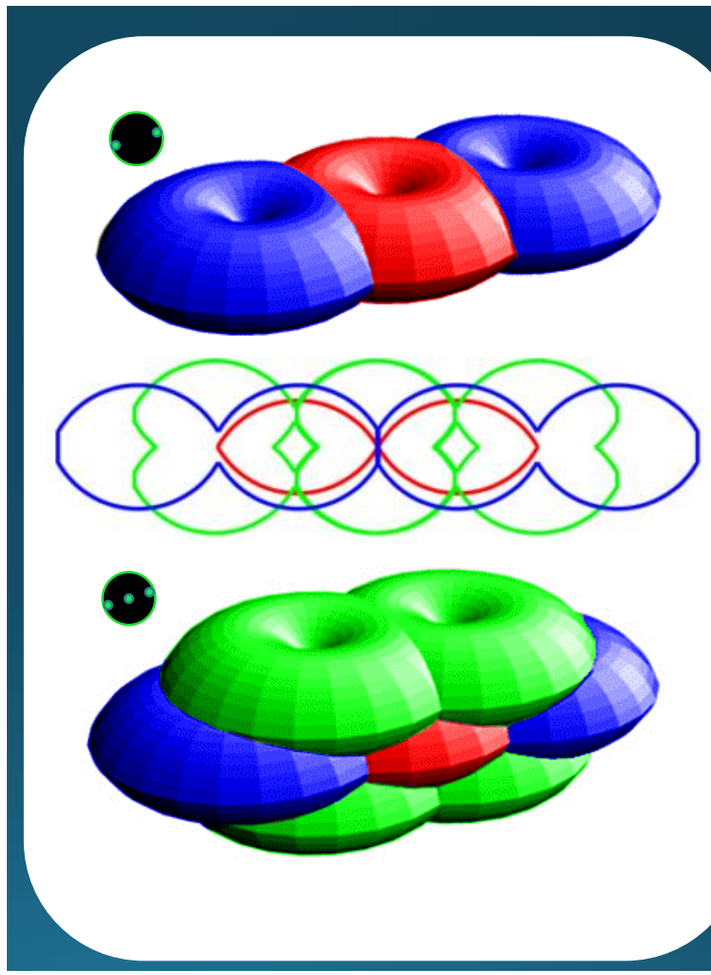


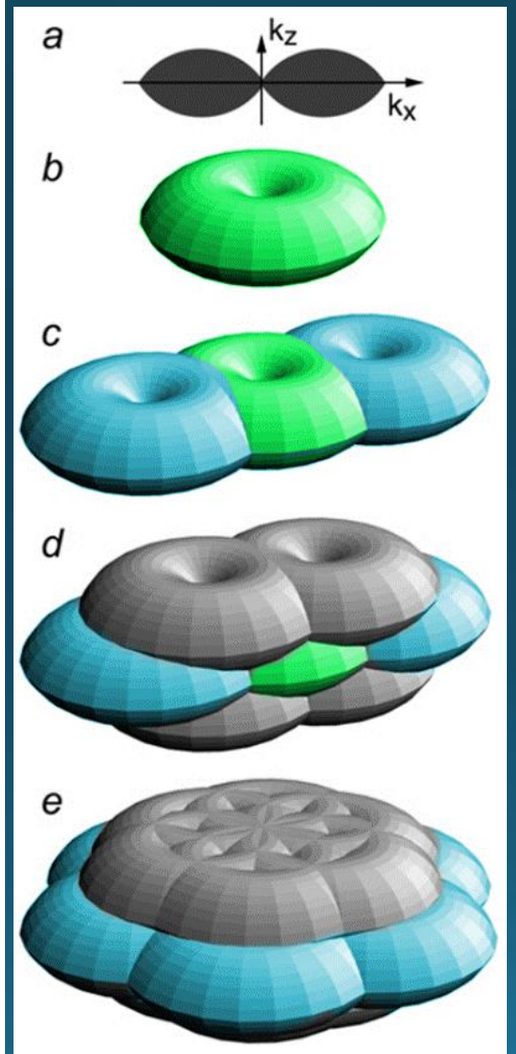




Conventional microscopy:
"missing cone" problem
→
poor axial (*z*) resolution.

1st-order spots only





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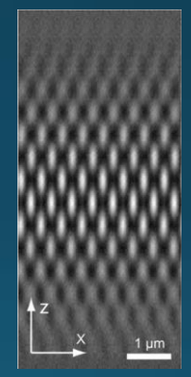
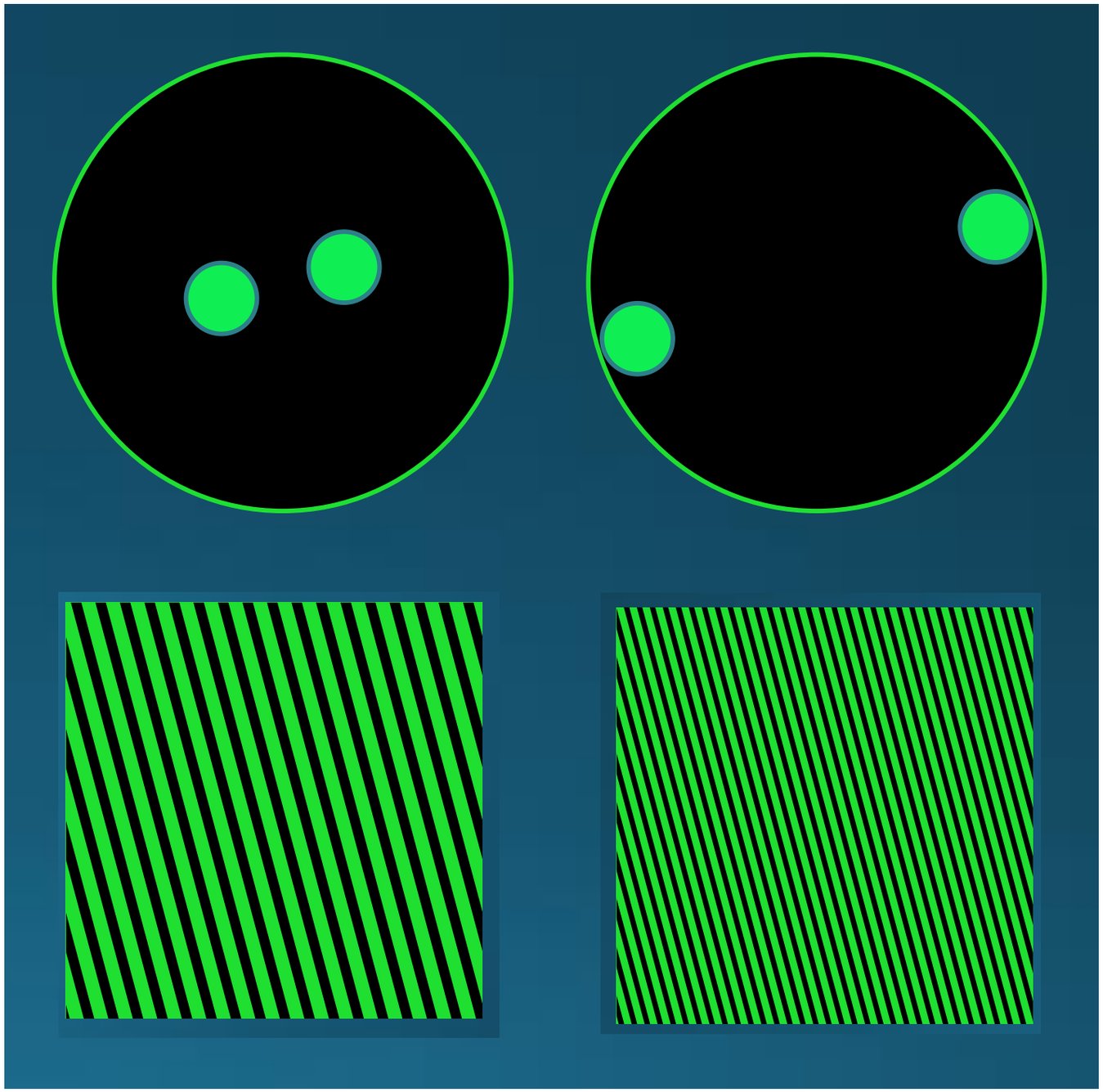
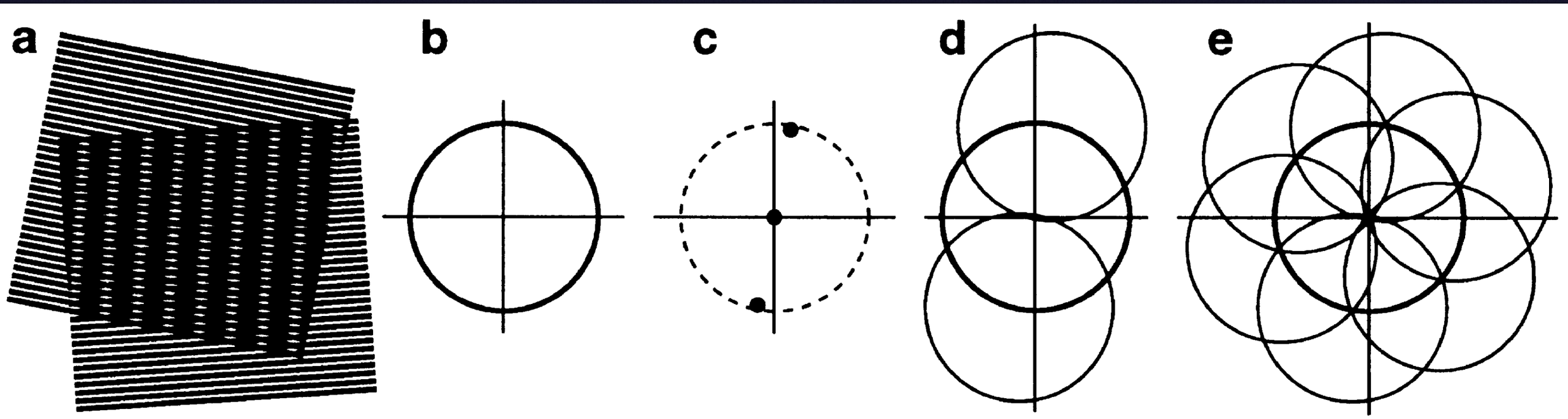


Zero- and 1st-order spots:
"missing cone" is filled

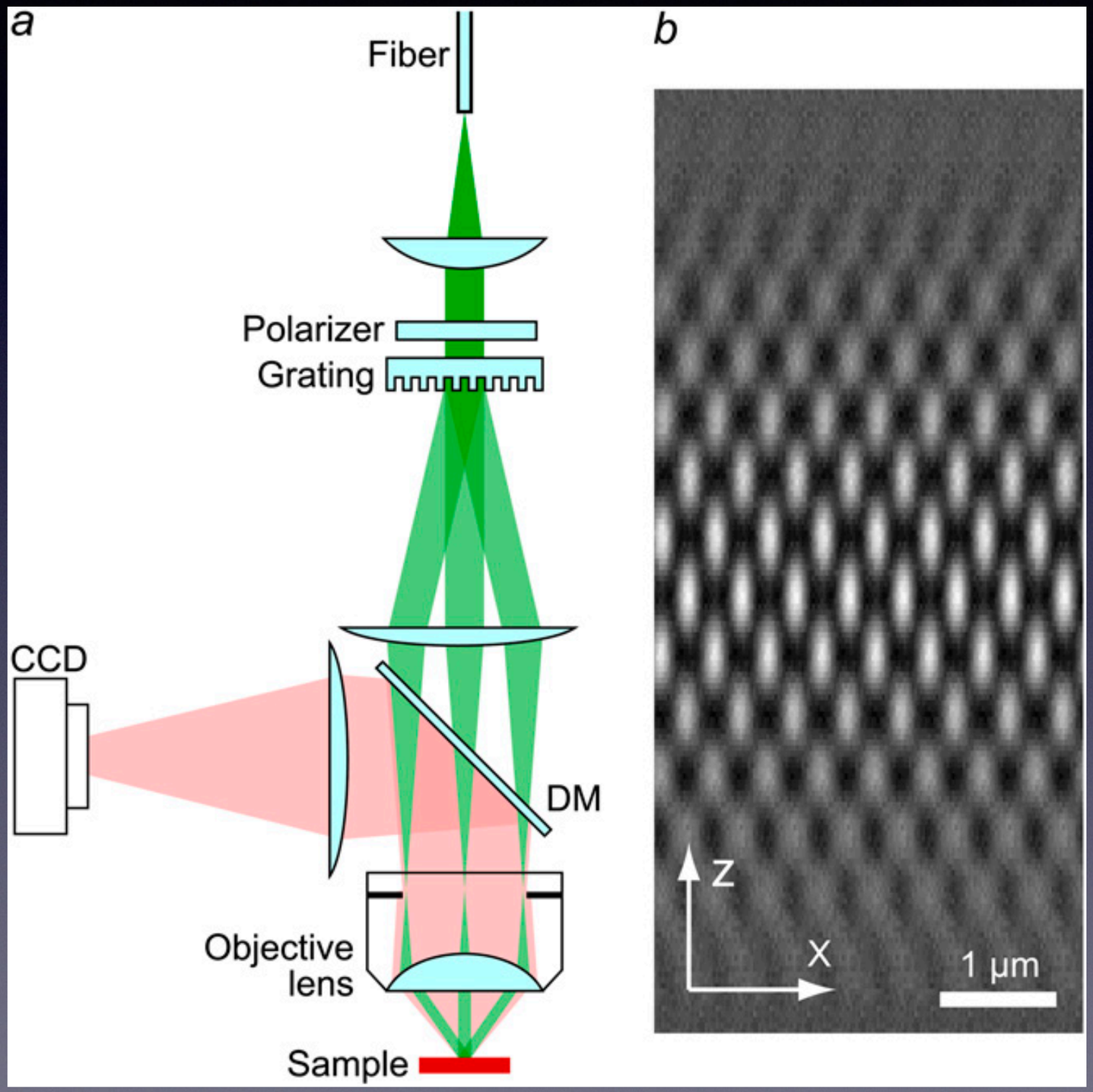
Gustaffson *et al.*, Biophysical Journal 94 (2008)



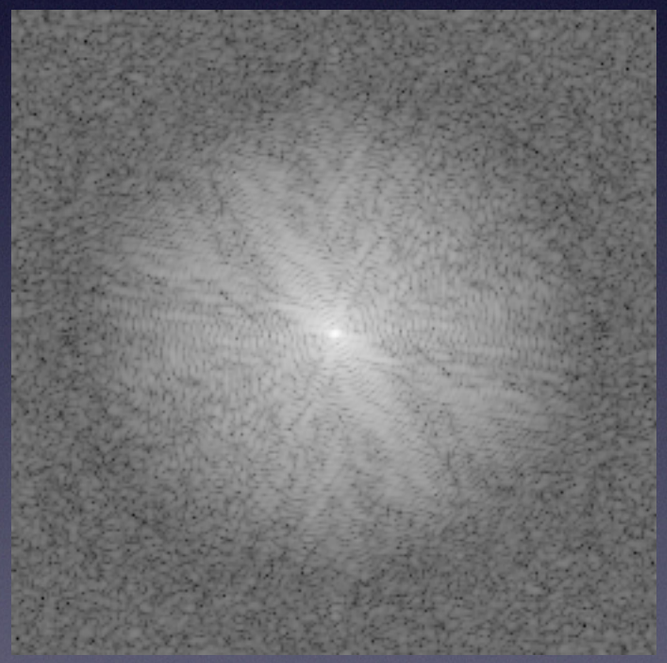
Fourier space explanation



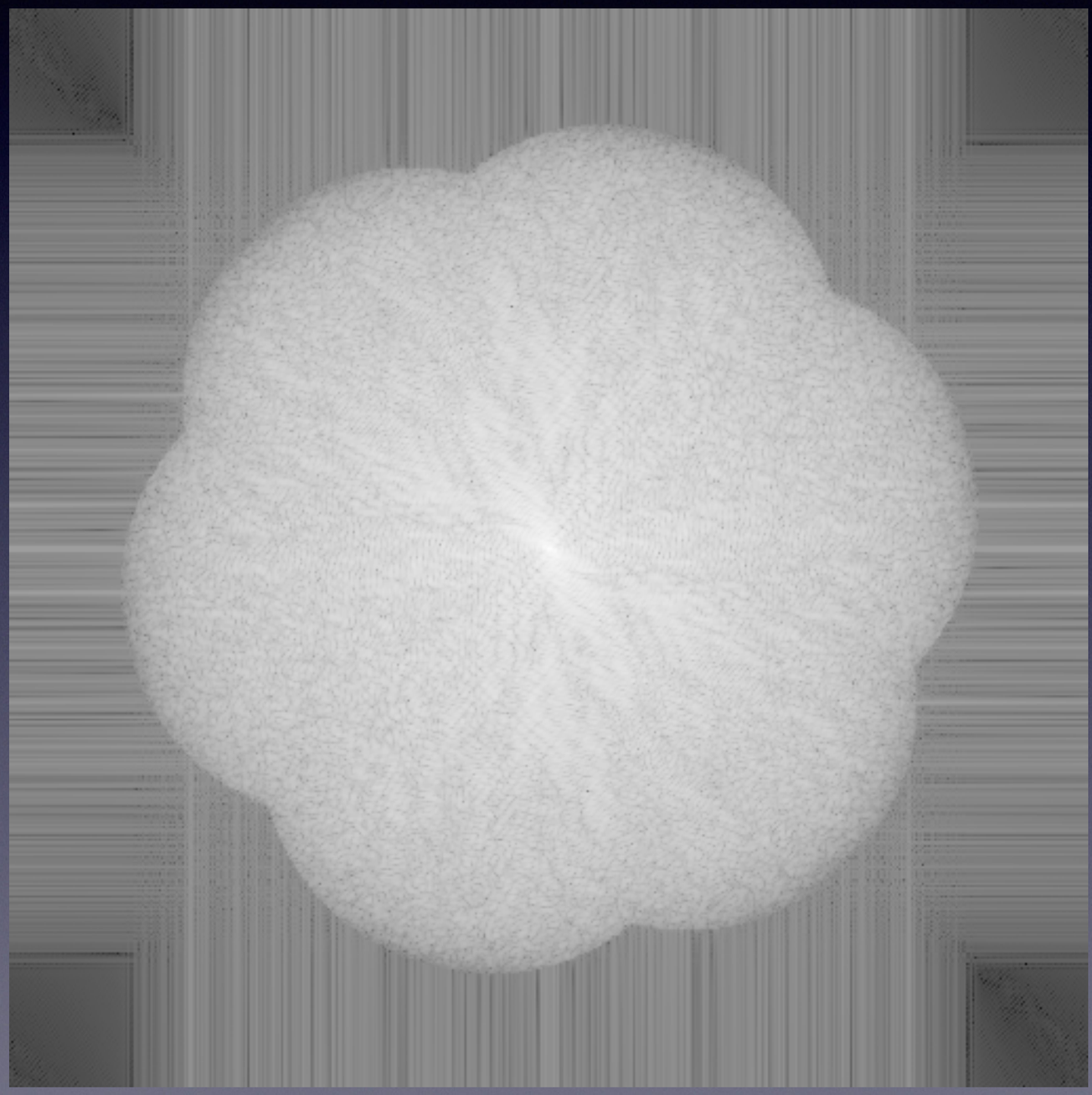
Stripes in Z


a
Fiber
Polarizer
Grating
CCD
DM
Objective lens
Sample
b
Z
X
1 µm

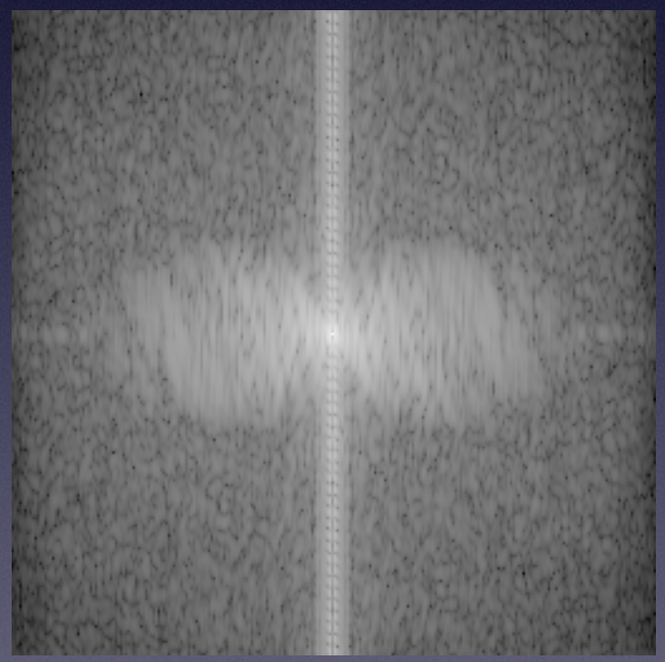
Fourier Transforms



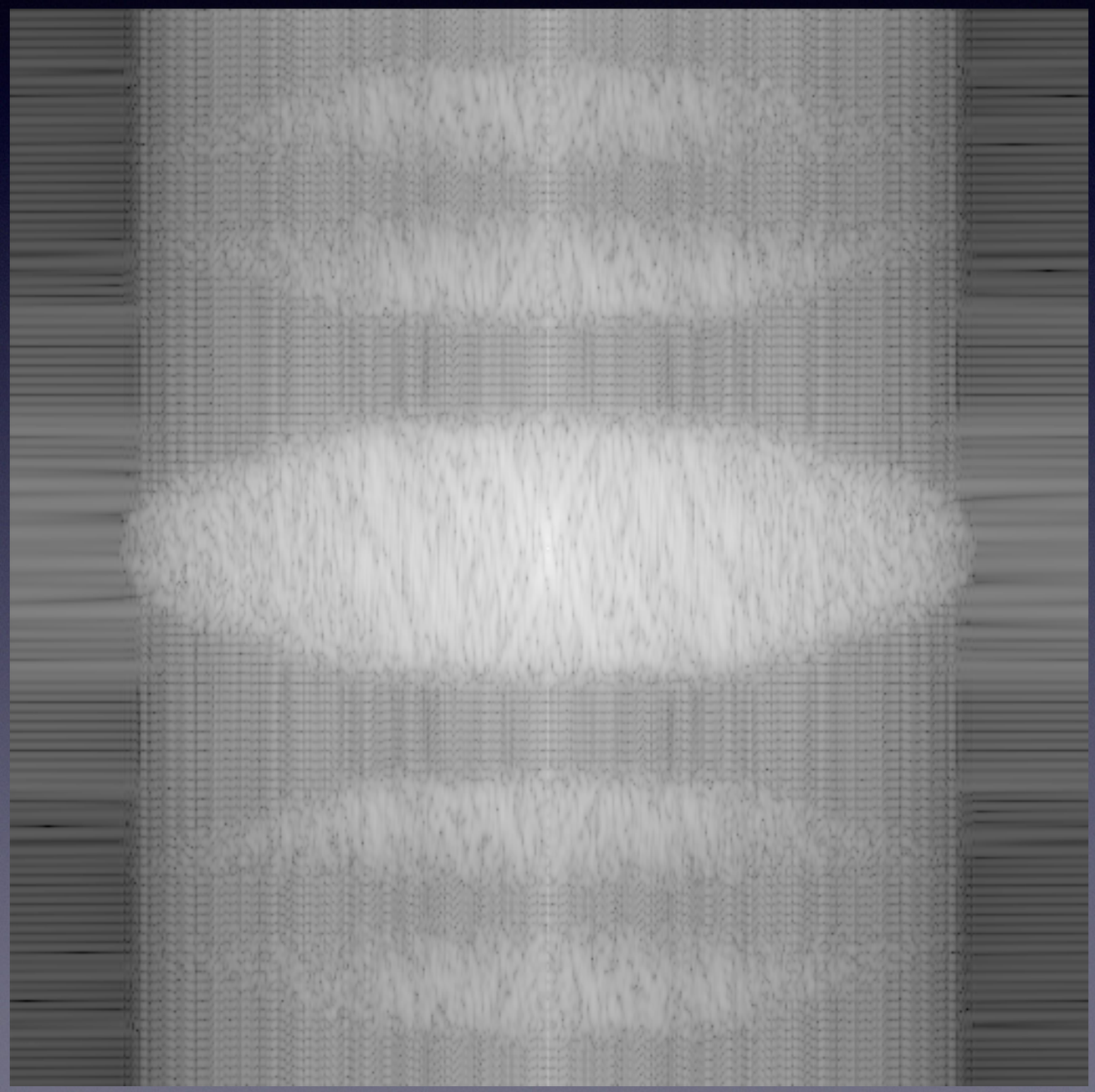
X-Y



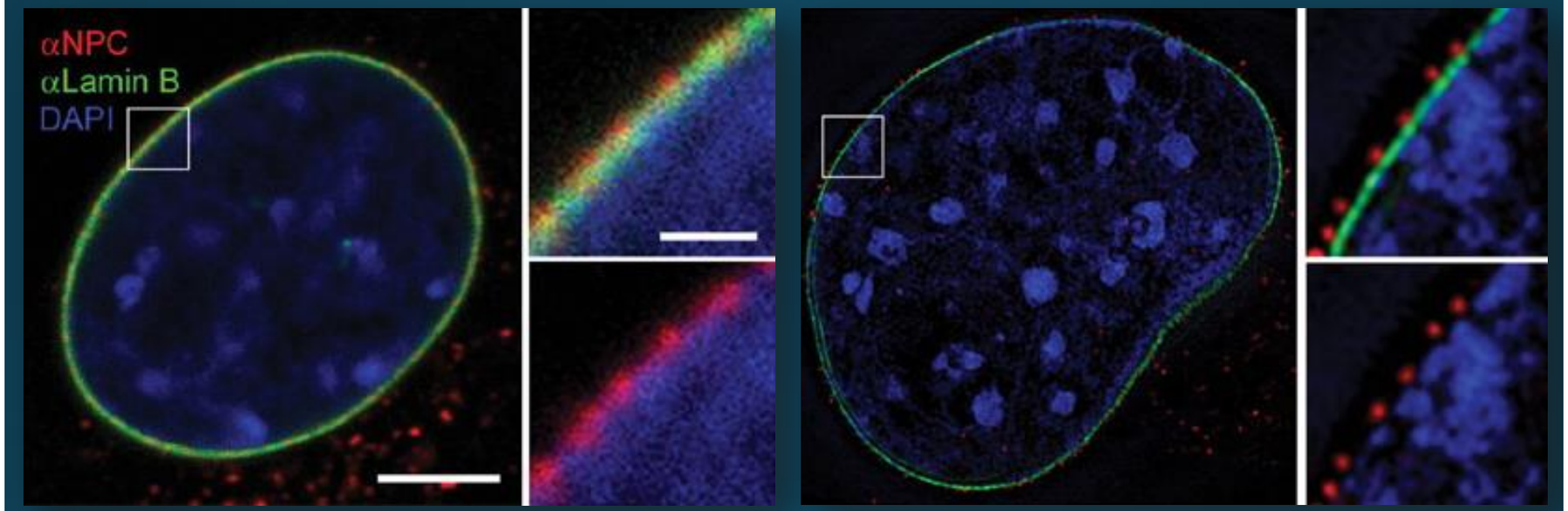
Fourier Transforms



X-Z



Multichannel SIM



DNA
nuclear lamina
nuclear pore complexes

Lothar Schermelleh (Micron), Science (2008)

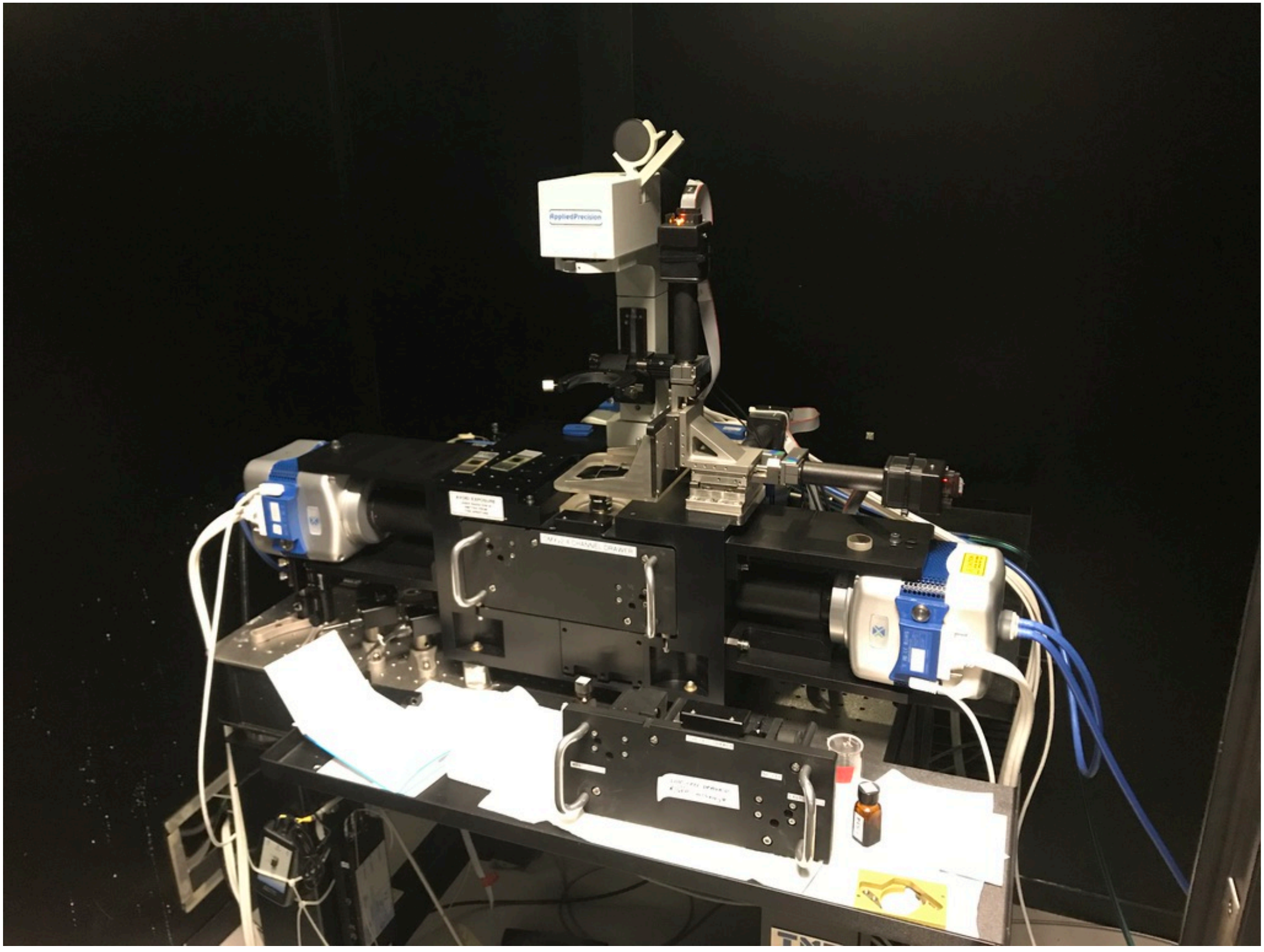
OMX design goals

- SIM
- Maximise emission light throughput
- Extreme stability
- Flexible control
- Speed

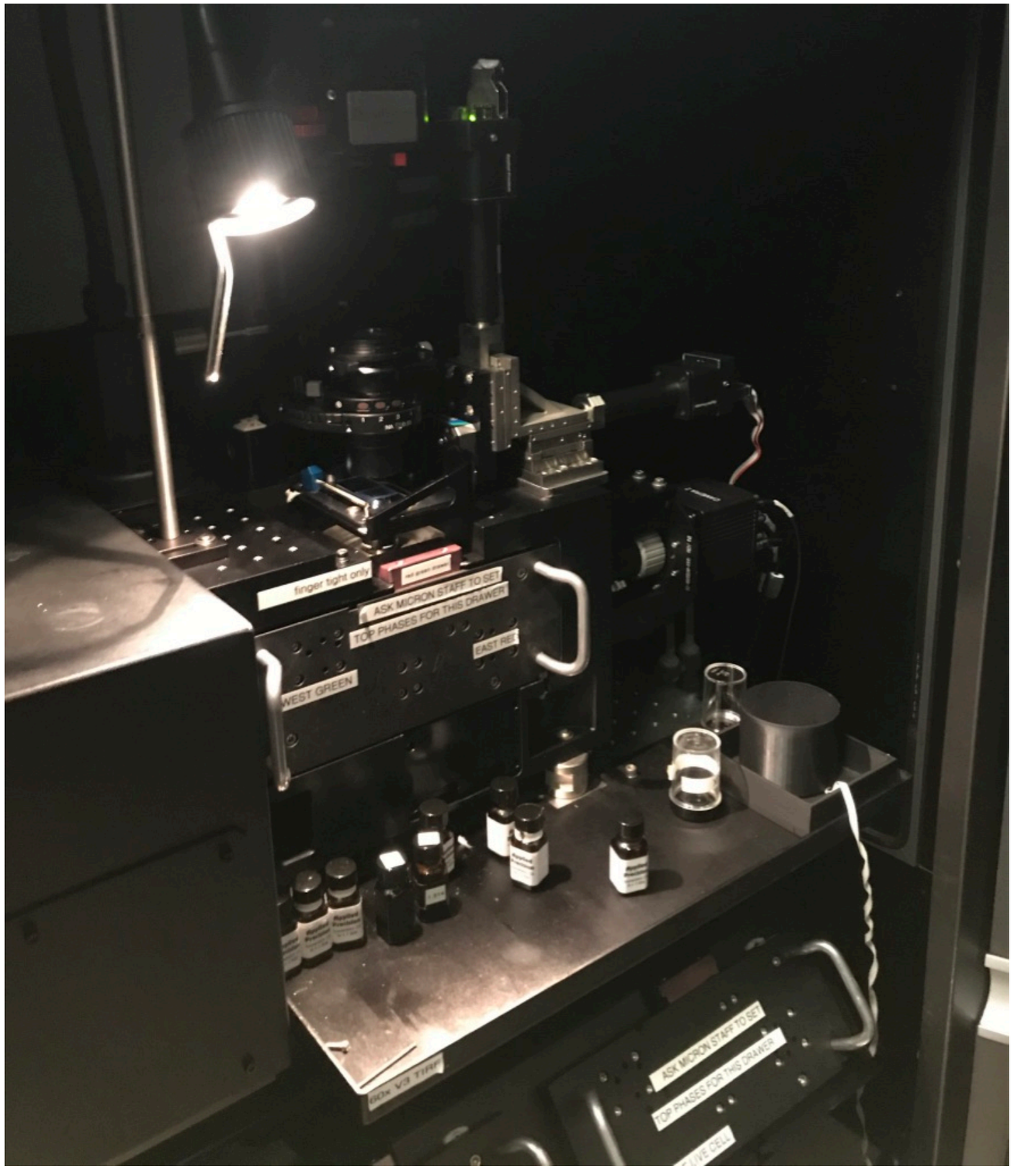
OMX history

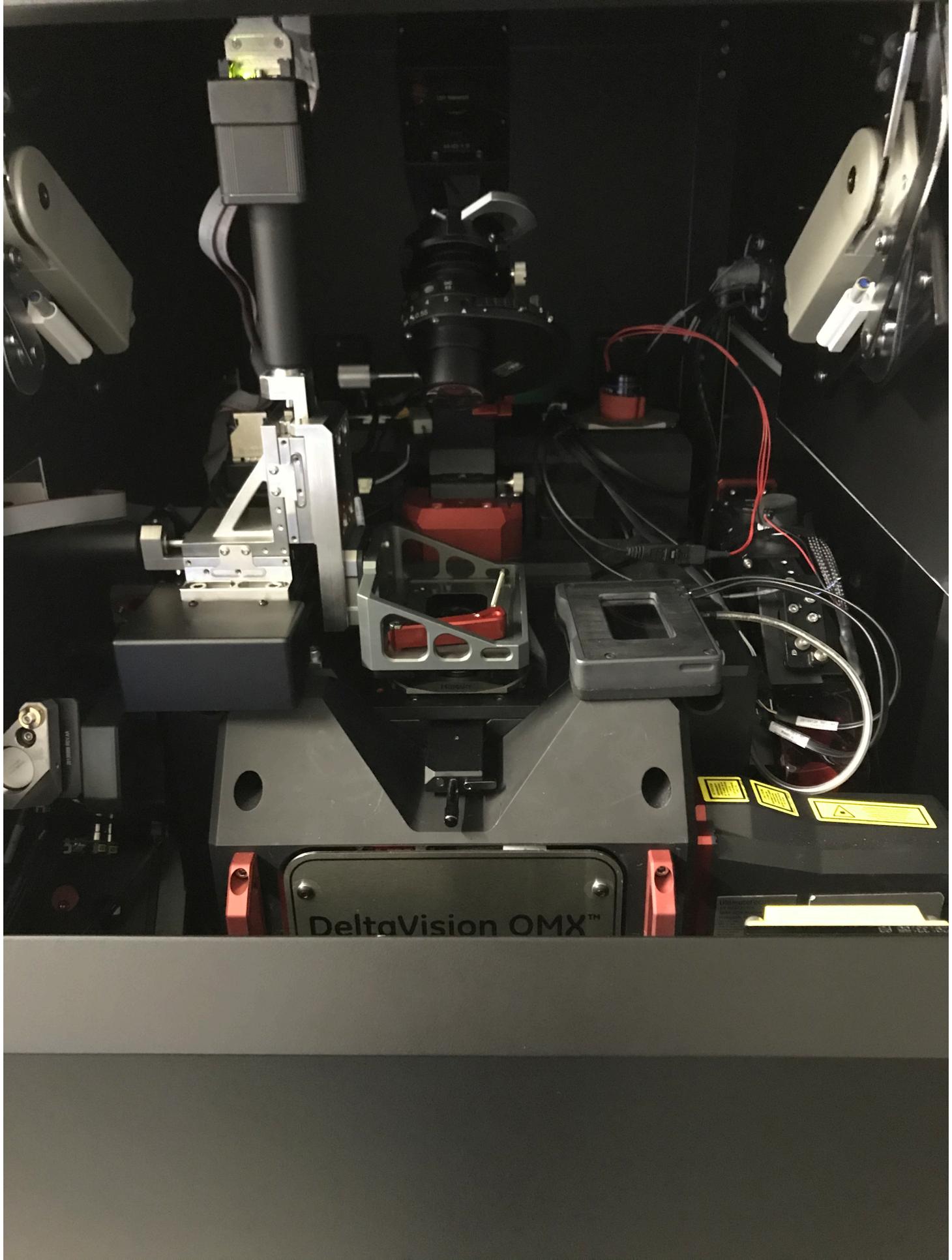
- V1 Sedat lab UCSF early 2000's
- V2 7-8 built about 2007-08. 2 left - almost exact copy of V1
- V3 2008 - 2011 - introduce Blaze, TIRF modules and sCMOS cameras
- V4 2011 - 2018 - new block changed dichroic etc
- SR 2019 onwards - smaller, new SIM light path

OMX V2



OMX V3


finger tight only
ASK MICRON STAFF TO SET
TOP PHASES FOR THIS DRAWER
WEST GREEN
EAST RE
60x V3 TIRF
ASK MICRON STAFF TO SET
TOP PHASES FOR THIS DRAWER


DeltaVision OMX™

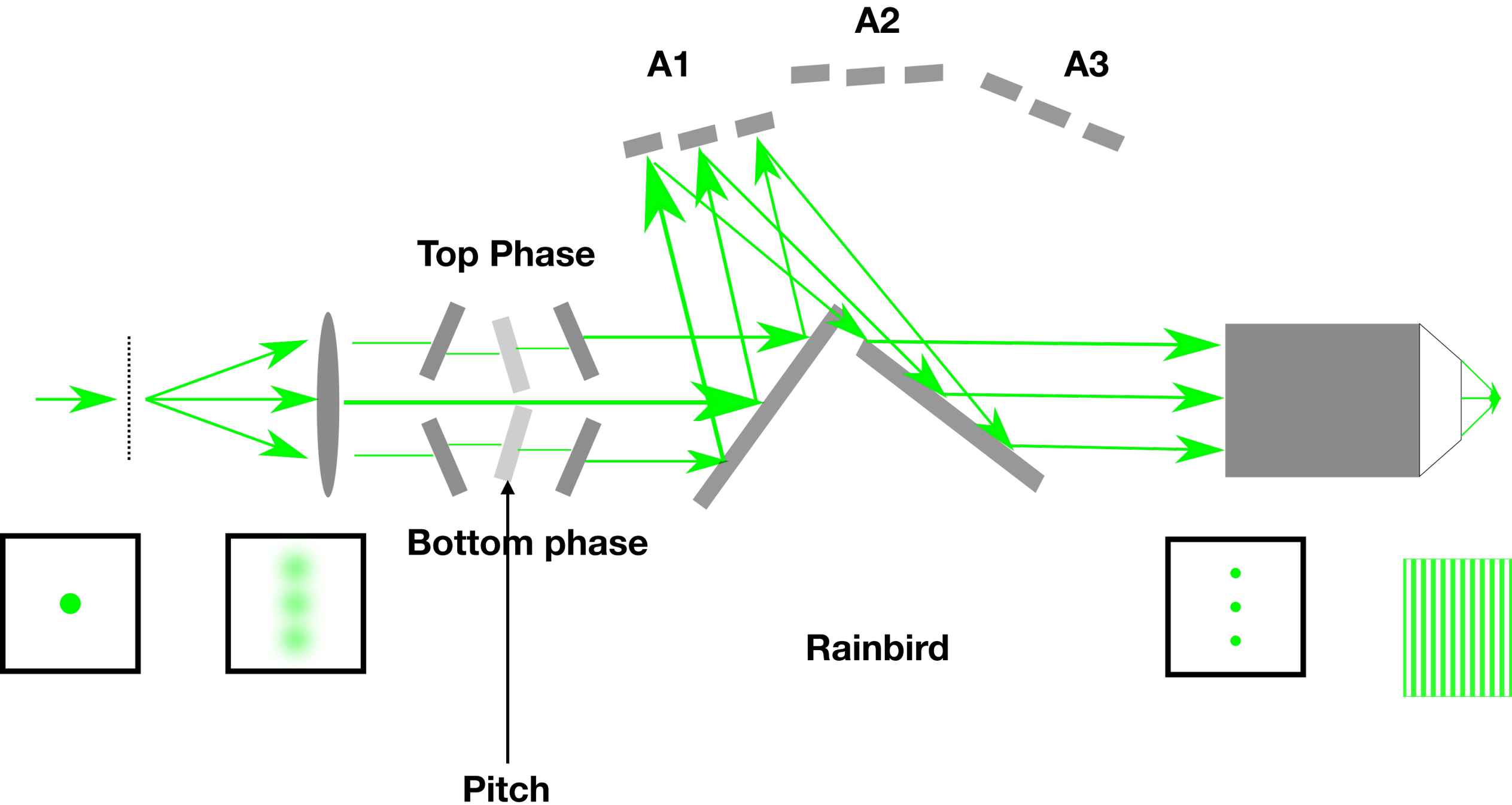
OMX V1&2

- Physically rotated grating
- Phase shifts by moving grating with piezo
- Single stripe width in all colours
- “fast” laser shutters, 12 ms cycle time
- semi-coherent excitation beams
- Python control software (cockpit v1)
- WF and SIM only

Linear Polariser

OMX V3

- Blaze fast SIM
- Fixed grating
- 9 beam paths for 3 beams at 3 rotations
- Galvo phase shifting
- Galvo stripe pitch control
- Galvo laser shutters
- single mode fibre:- coherent excitation beams.
- TIRF & FRAP option
- Java based OMXapp

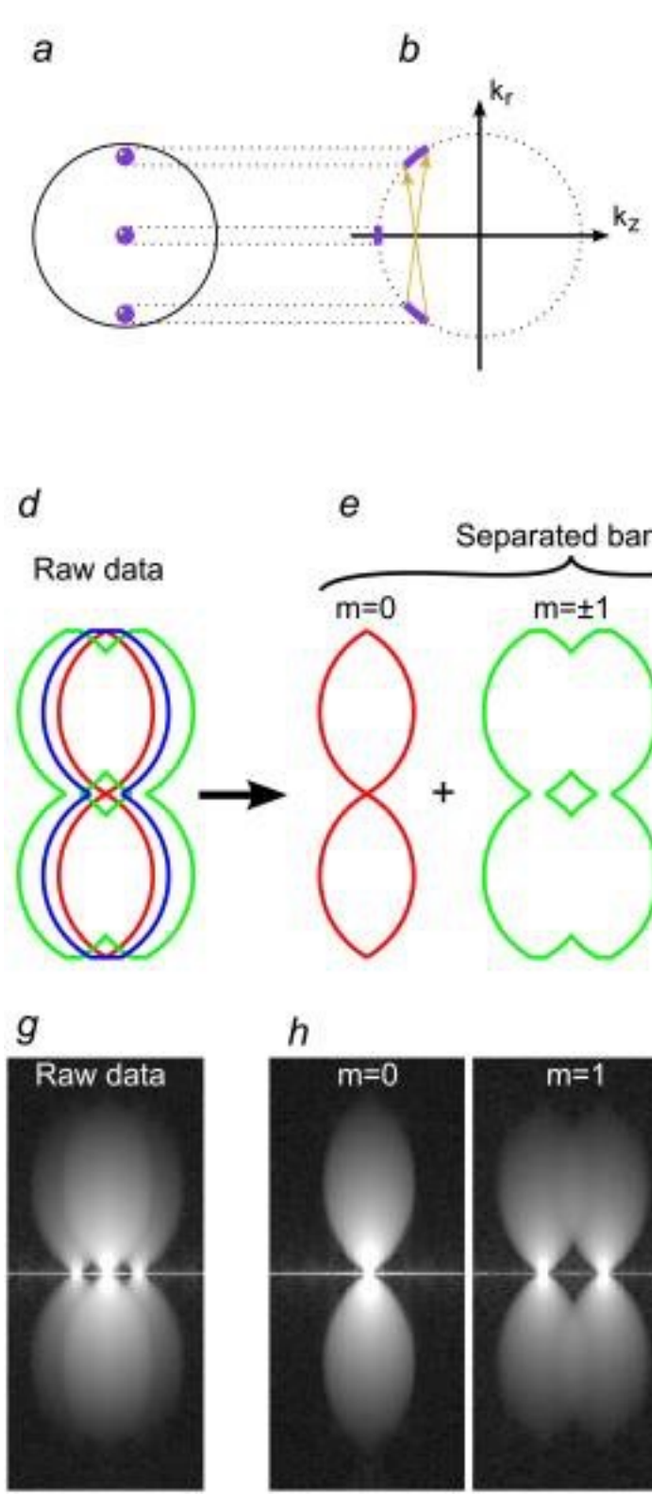

A1
A2
A3
Top Phase
Bottom phase
Pitch
Rainbird

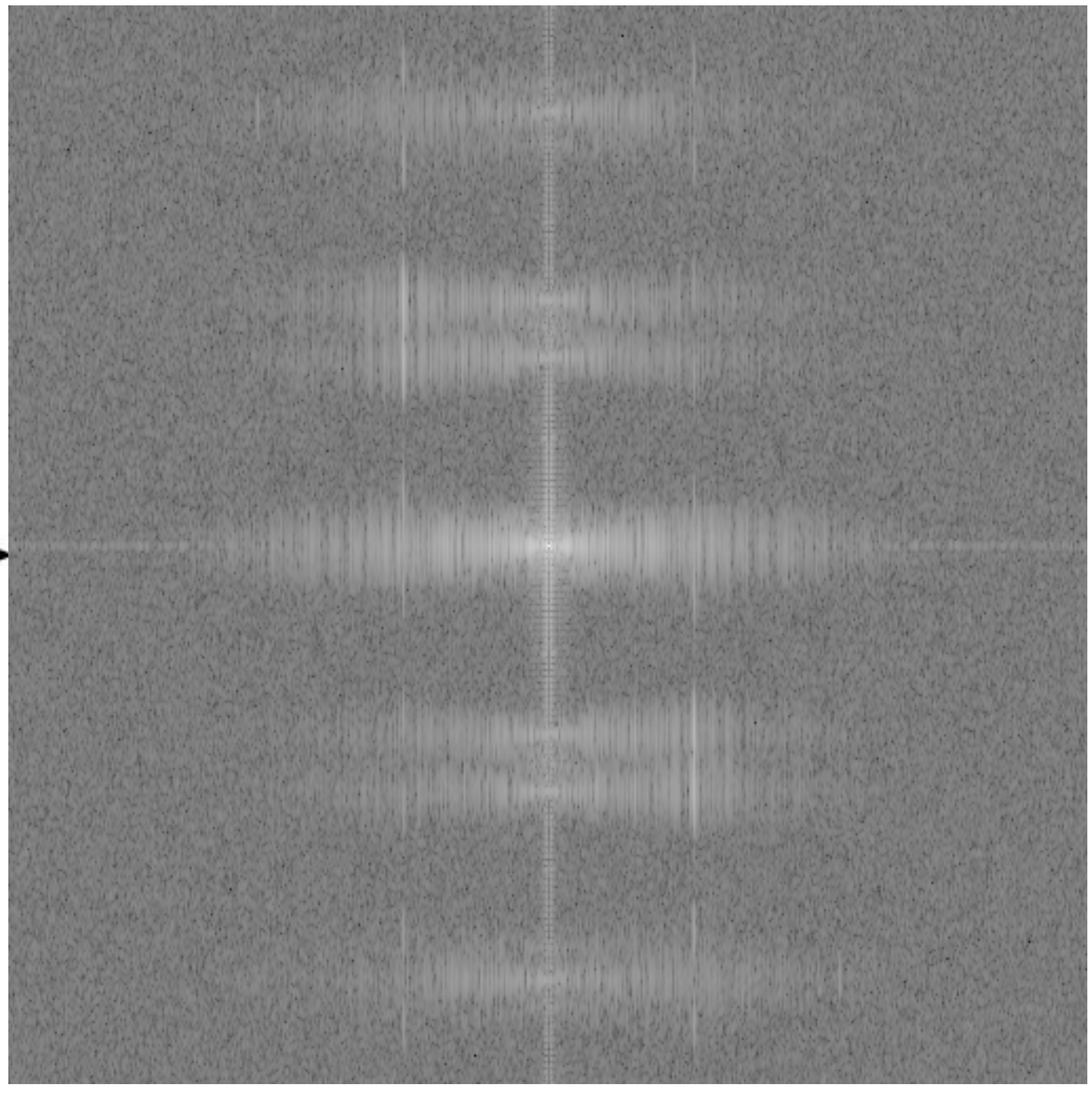
OMX V4

- New optics block
- Motorised dichroic and filter wheels.
- Reversed dichroic orientation
- Separate WF/TIRF and SIM excitation paths
- Lower power lasers
- Addition of concentrator lens to allow higher power density for SMLM imaging

OMX SR

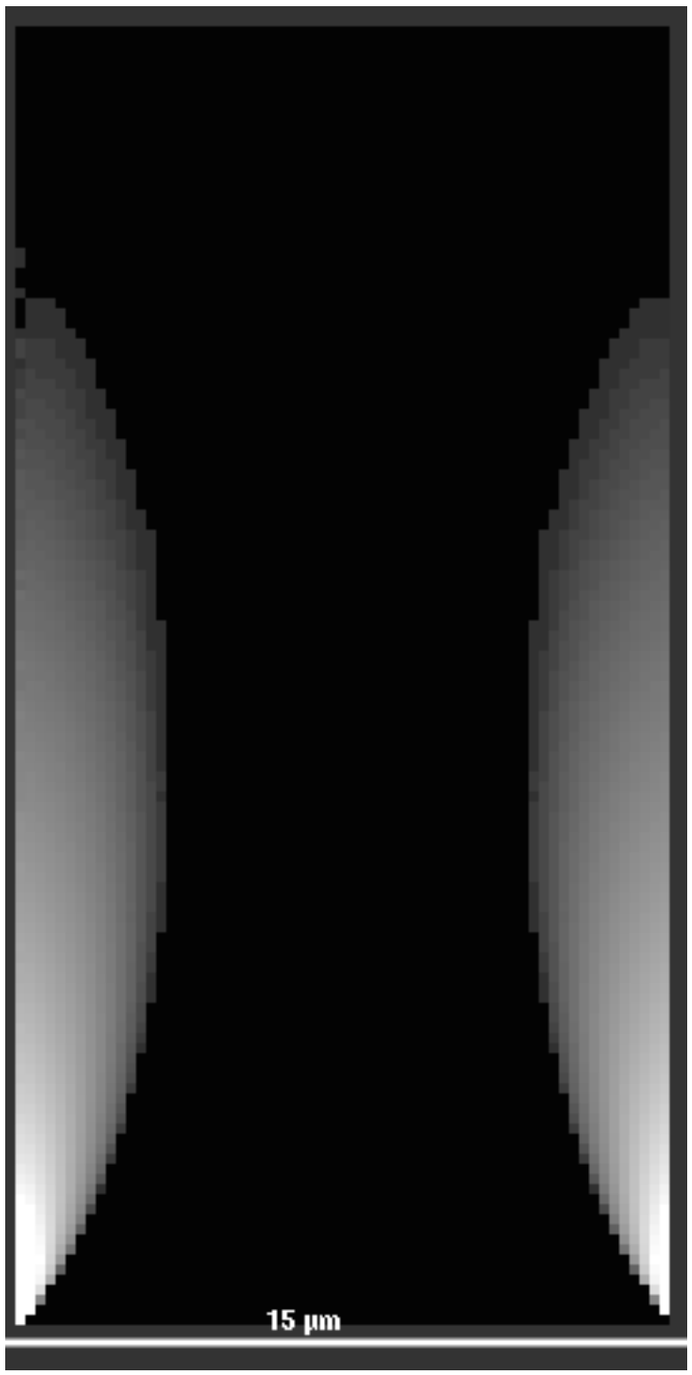
- Miniaturised V4
- Redone Blaze optics, apparently more stable and easier to align
- Commercial laser engine rather than custom laser table


a
b
k_r
k_z
c
k_r
k_z
d
Raw data
e
Separated bands
m=0
m=±1
m=±2
+
+
g
Raw data
h
m=0
m=1
m=2

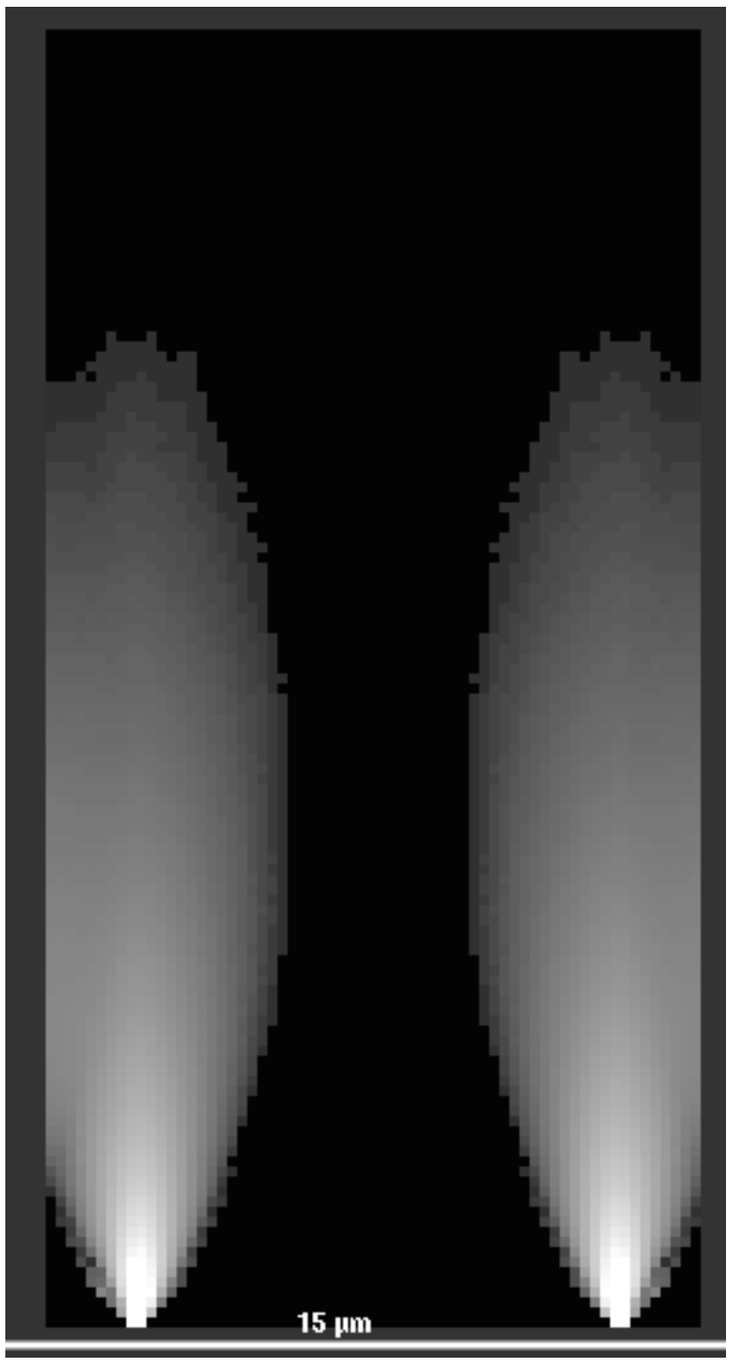


A OMX OTF

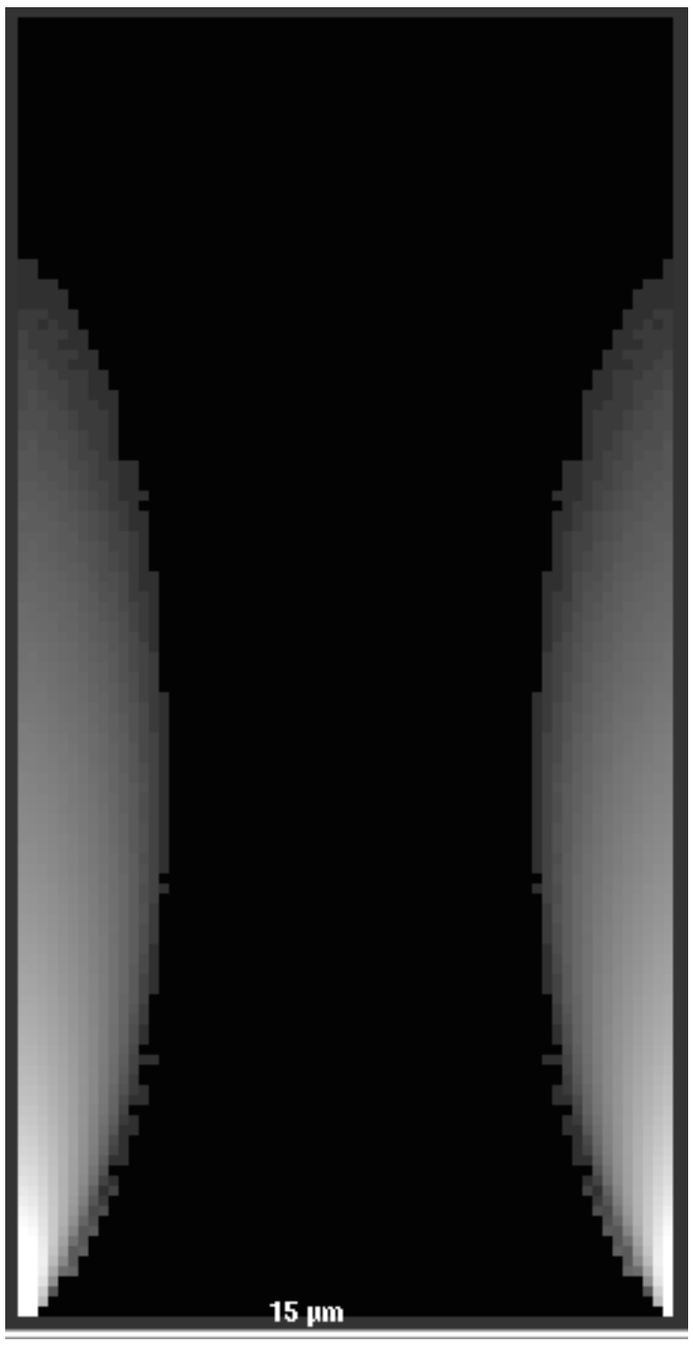
0th order



1st order

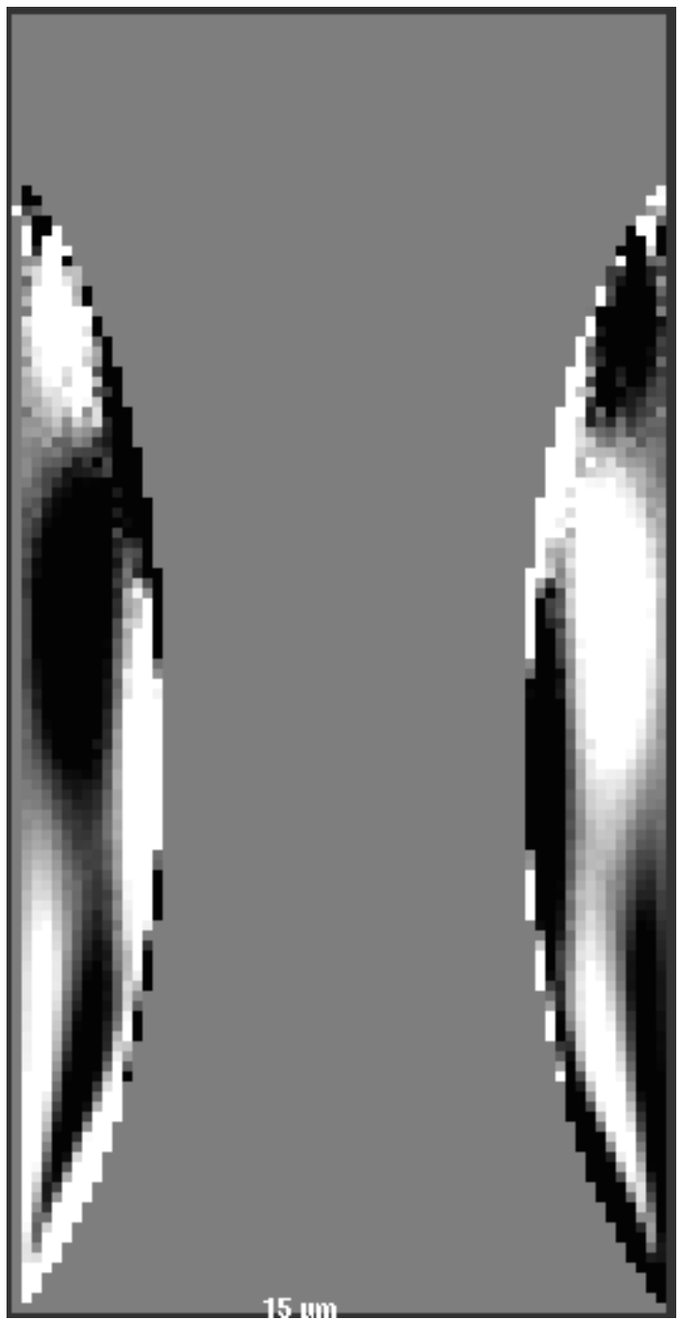


2nd order

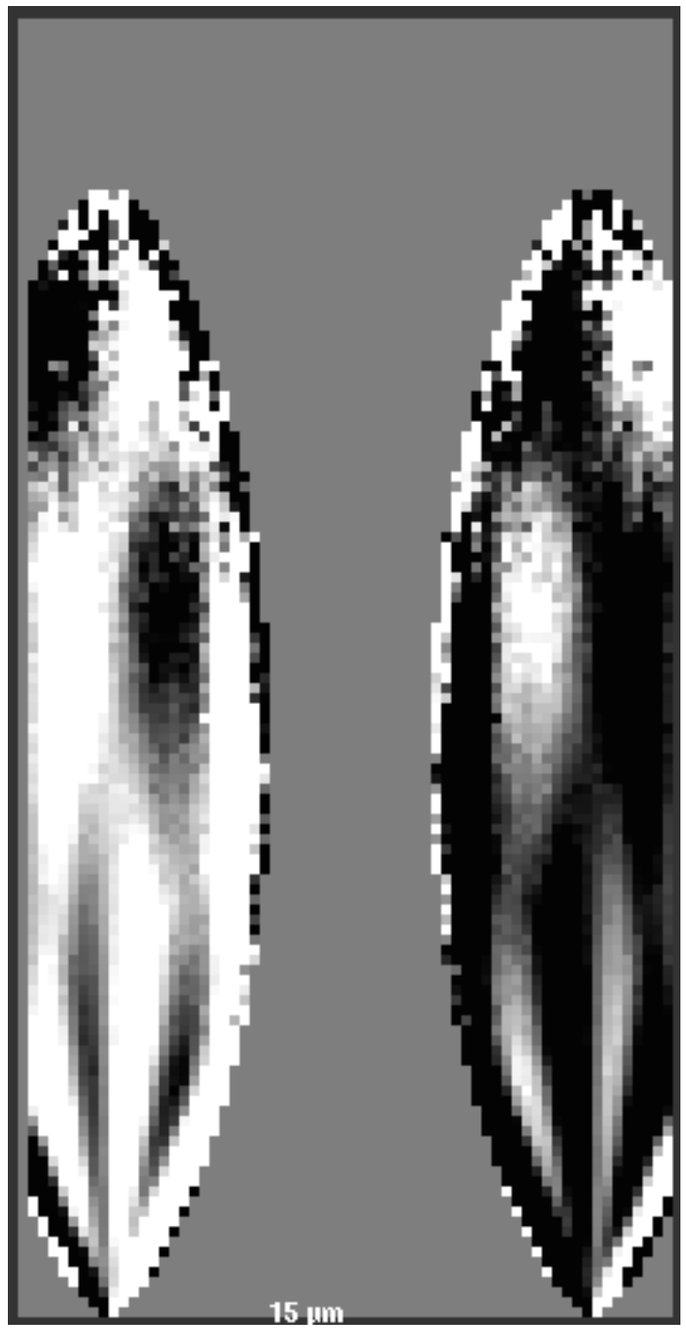


A OMX OTF

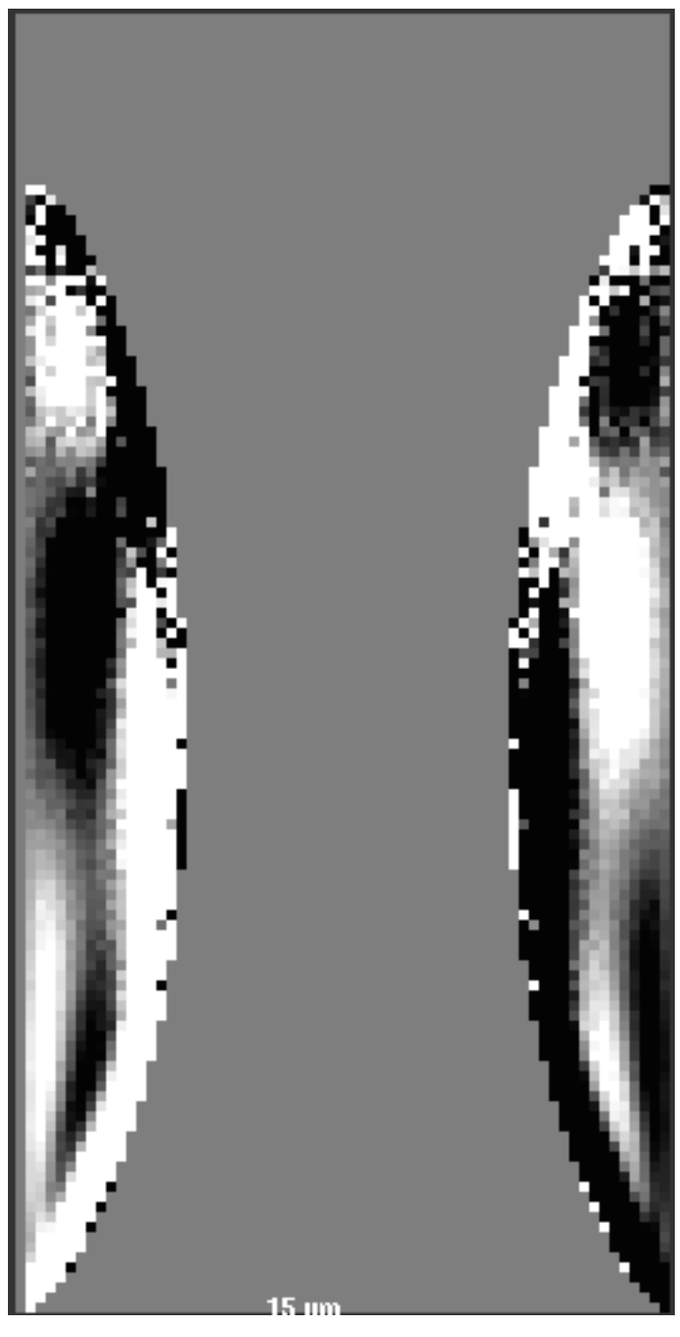
0th order



1st order

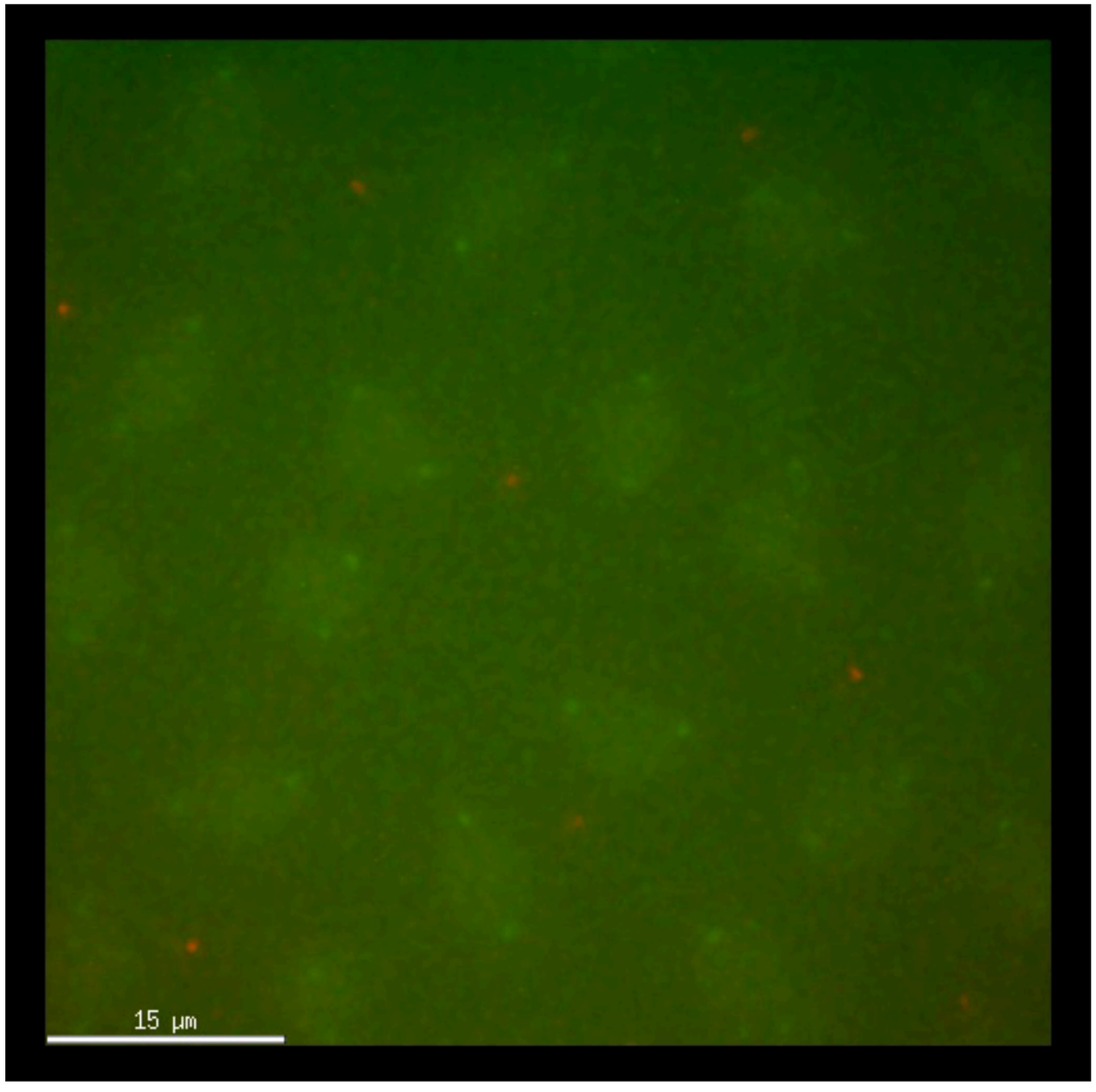


2nd order



Other OMX strengths

- Fast
- Sensitive
- Stable



Drosophila oocyte Polo and AuroraB.
Ian Dobbie & Yaseen Ladak

Data collected at 2 wavelengths, 33 Z-planes, 1 stack per 2.35s, 600 time points (total time ~ 23min, 20GB data file)

Micron SIM systems

- OMX V2
- OMX V3 - with SDK for custom control (eg SIM-FRAP)
- DeepSIM - upright with AO
- CryoSIM - upright cryogenic imaging - at Diamond
- CryoSIM II - adding AO to cryosim.