Adaptive optics for microscopy

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Outline

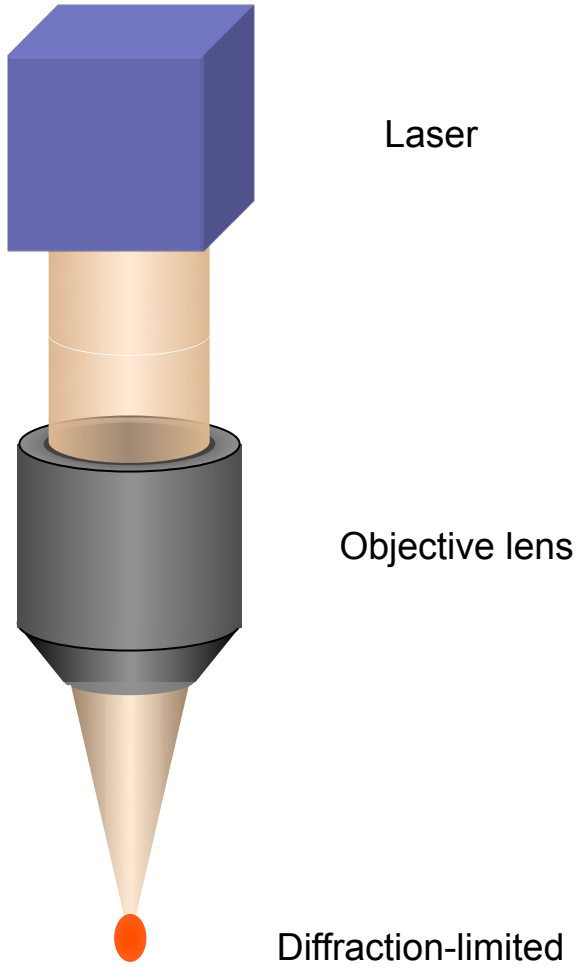
- Aberrations in microscopy and the need for adaptive optics
- Methods of aberration measurement and correction in microscopy
- Applications of adaptive optics in high-resolution 3D microscopy
- Challenges in adaptive microscopy

High resolution photonic engineering

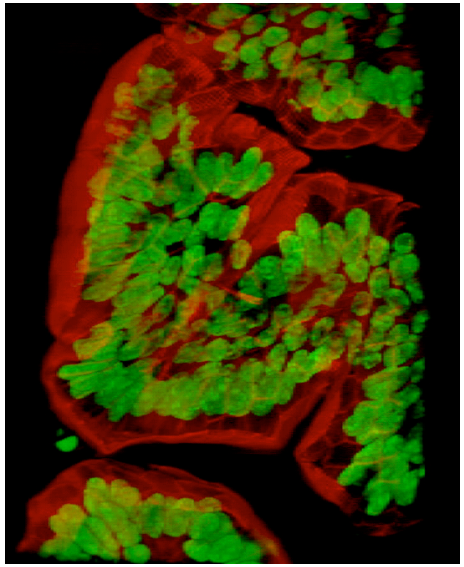
- Focus size/resolution of system
  - Wavelength
  - Numerical aperture (NA) of objective
  - 3D resolution
  - ~250nm lateral, ~500nm axial

$$\Delta x \approx \frac{\lambda}{2NA} \qquad \Delta z \approx \frac{n\lambda}{NA^2}$$

- Applications
  - Observation: microscopy
  - Modification: micro/nano fabrication
  - Manipulation: optical tweezers
  - Stimulation: biology

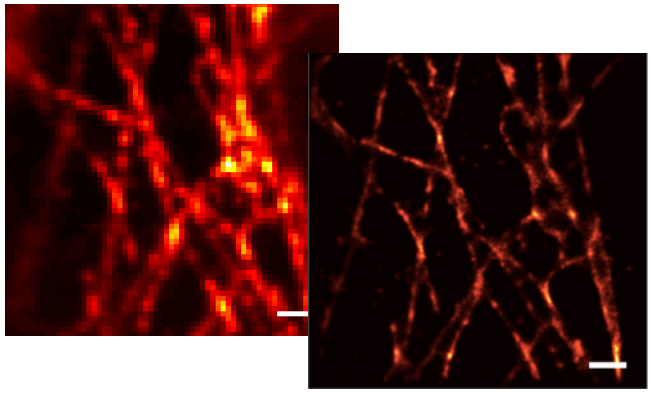


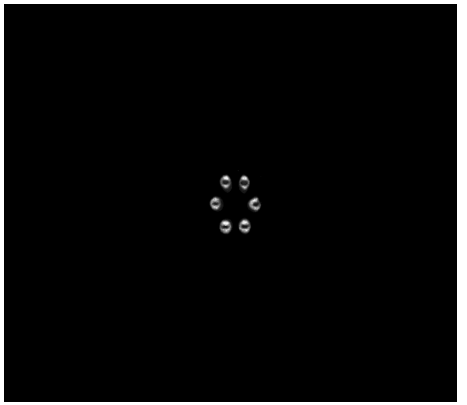
Dynamic optics for microscopy



High resolution biomedical microscopy

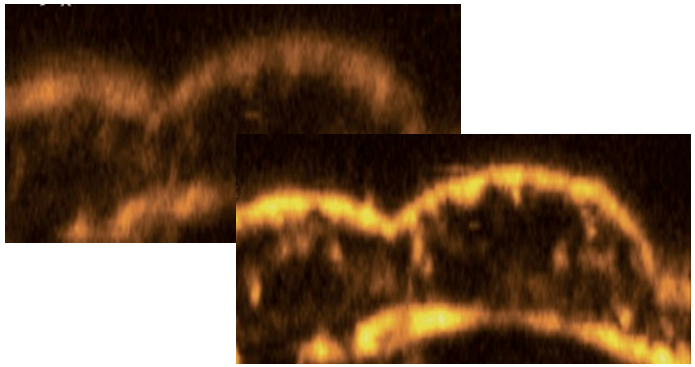
Super resolution microscopy



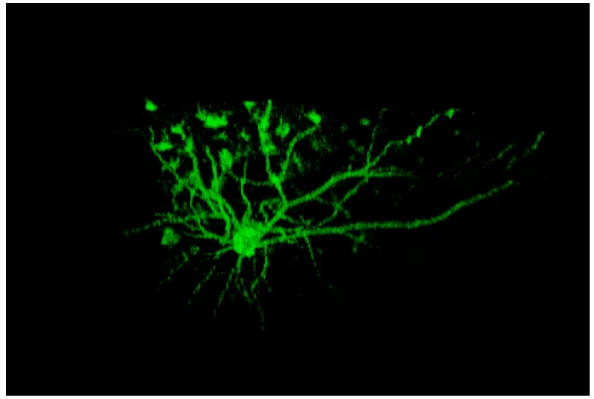


Microscopy for photonic characterisation

Adaptive optics for aberration correction

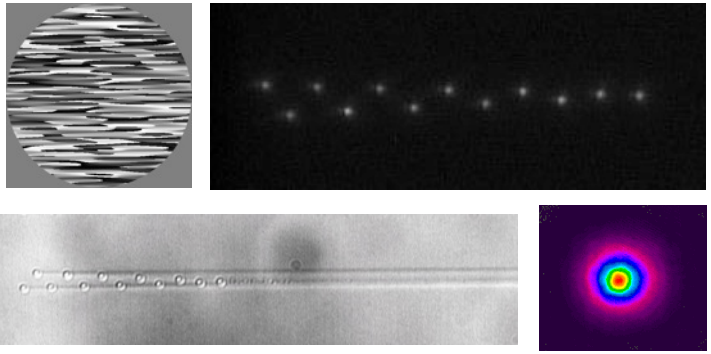


Rapid 3D scanning microscopy

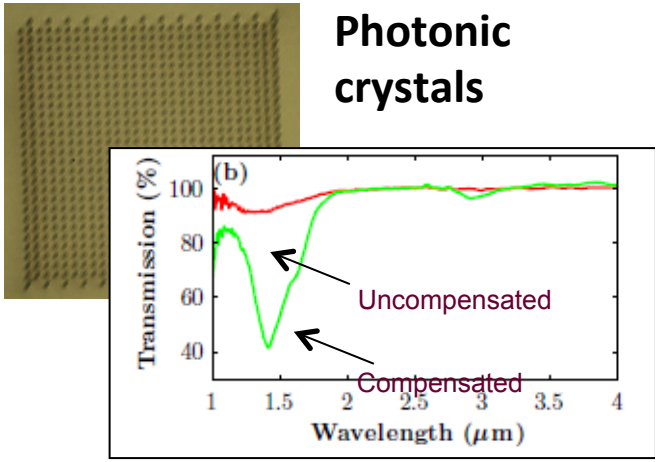


Dynamic optics for photonic fabrication

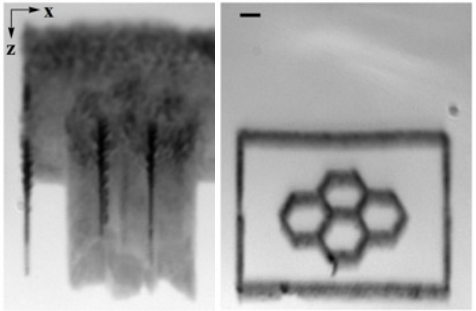
Waveguide circuits



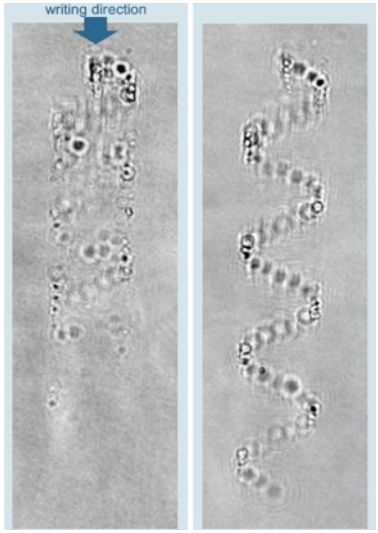
Photonic crystals

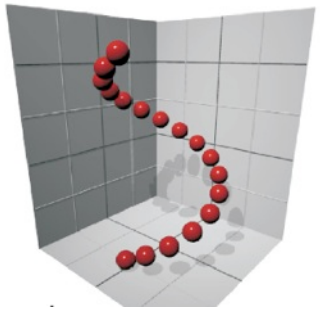


Laser fabrication in diamond

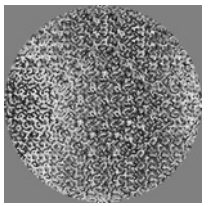


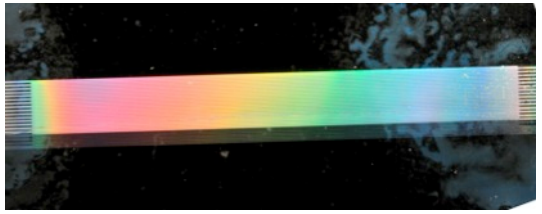
Parallel 3D laser fabrication





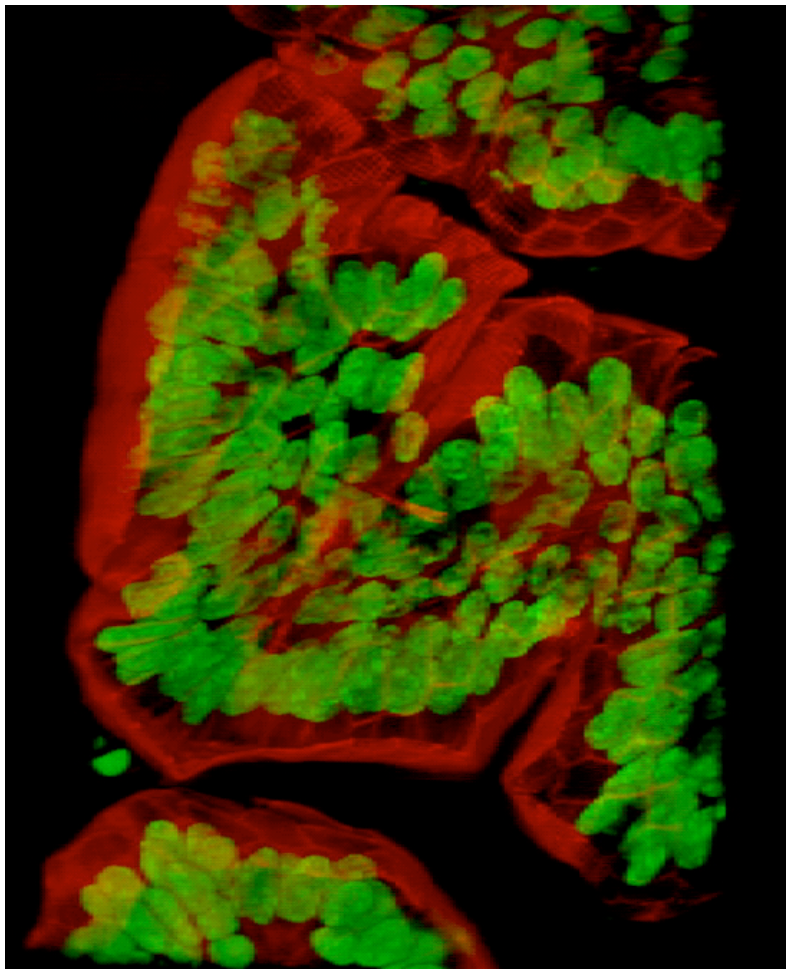
Holographic volume optics





Three-dimensional optical microscopy

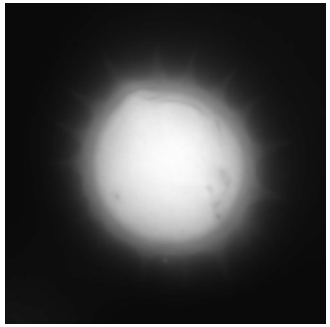
- High resolution optical microscopy
  - 3D sub-cellular resolution (~100nm)
  - Specific marking of structures
  - Physical and biochemical information
  - In situ imaging of live specimens
- Applications
  - Biological research
  - Medical imaging
  - Materials
- Methods
  - Laser scanning confocal, multiphoton
  - Widefield sectioning



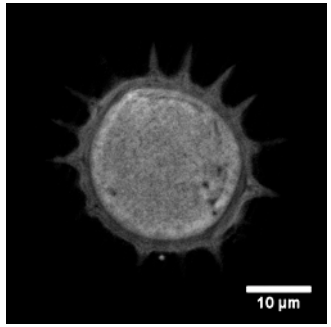
Scanning optical microscopy

- Three dimensional resolution through optical sectioning

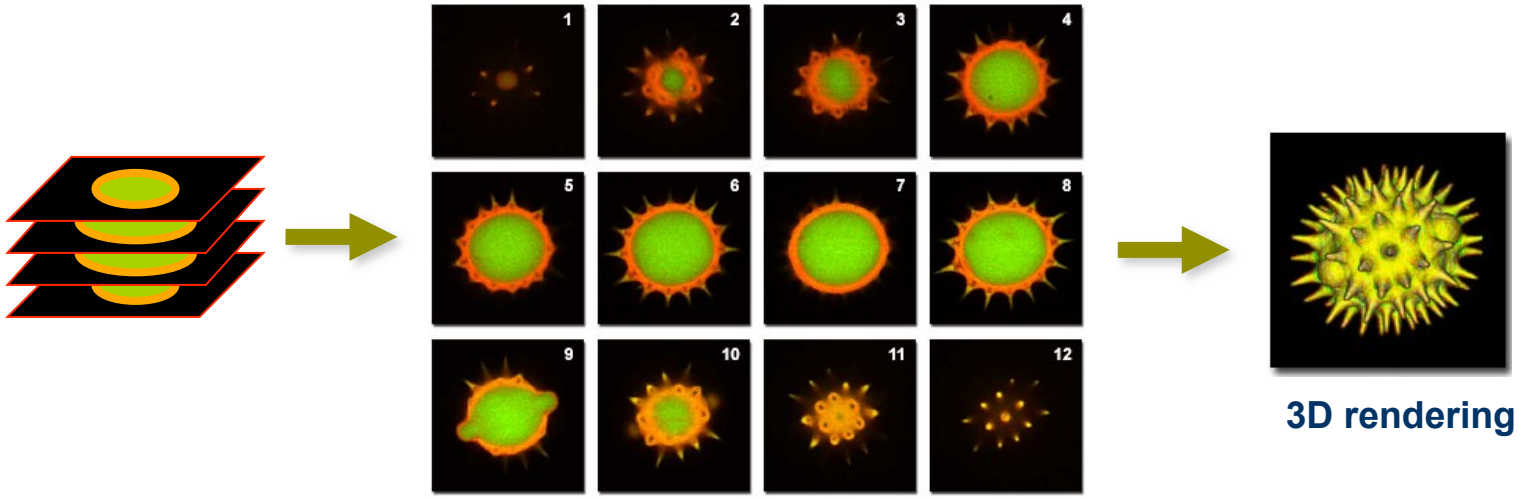
Conventional image



Optically sectioned image

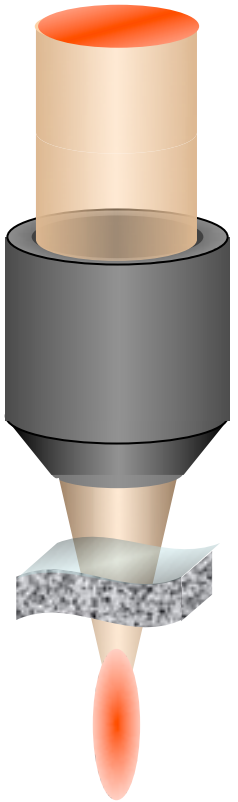


- Reconstruction of three-dimensional structure of thick specimens



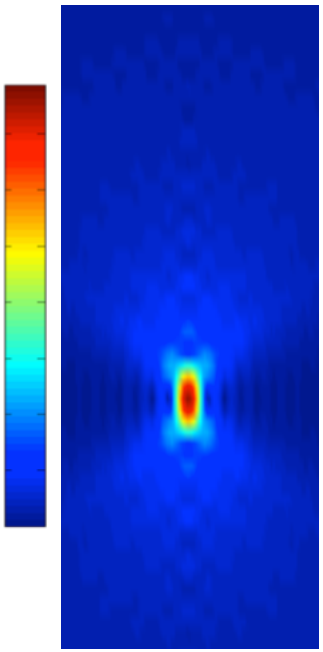
Aberrations in microscopes

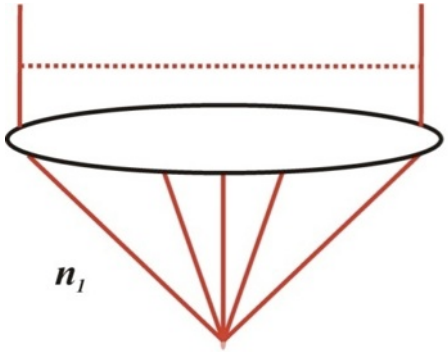
- Sources of aberrations
  - Optical system imperfections
  - Specimen refractive index
- Effects of aberrations
  - Enlarged focal spot
  - Loss of resolution
  - Decrease in image quality and contrast

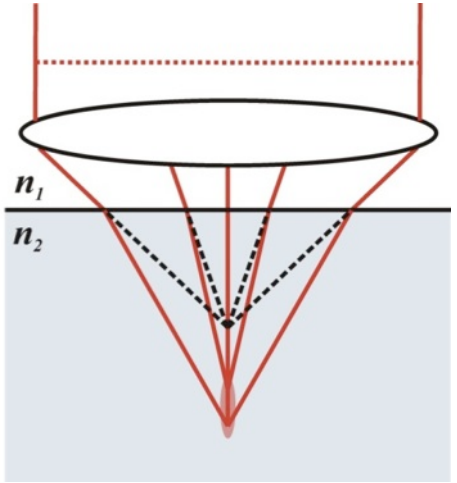


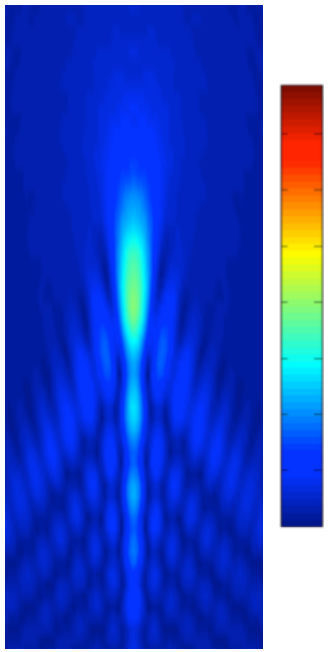
Aberrations from index mismatch

- Depth dependent spherical aberration when focussed through a refractive index mismatch (e.g. immersion/mounting medium)
- Aberrations increase with depth, numerical aperture and magnitude of refractive index mismatch







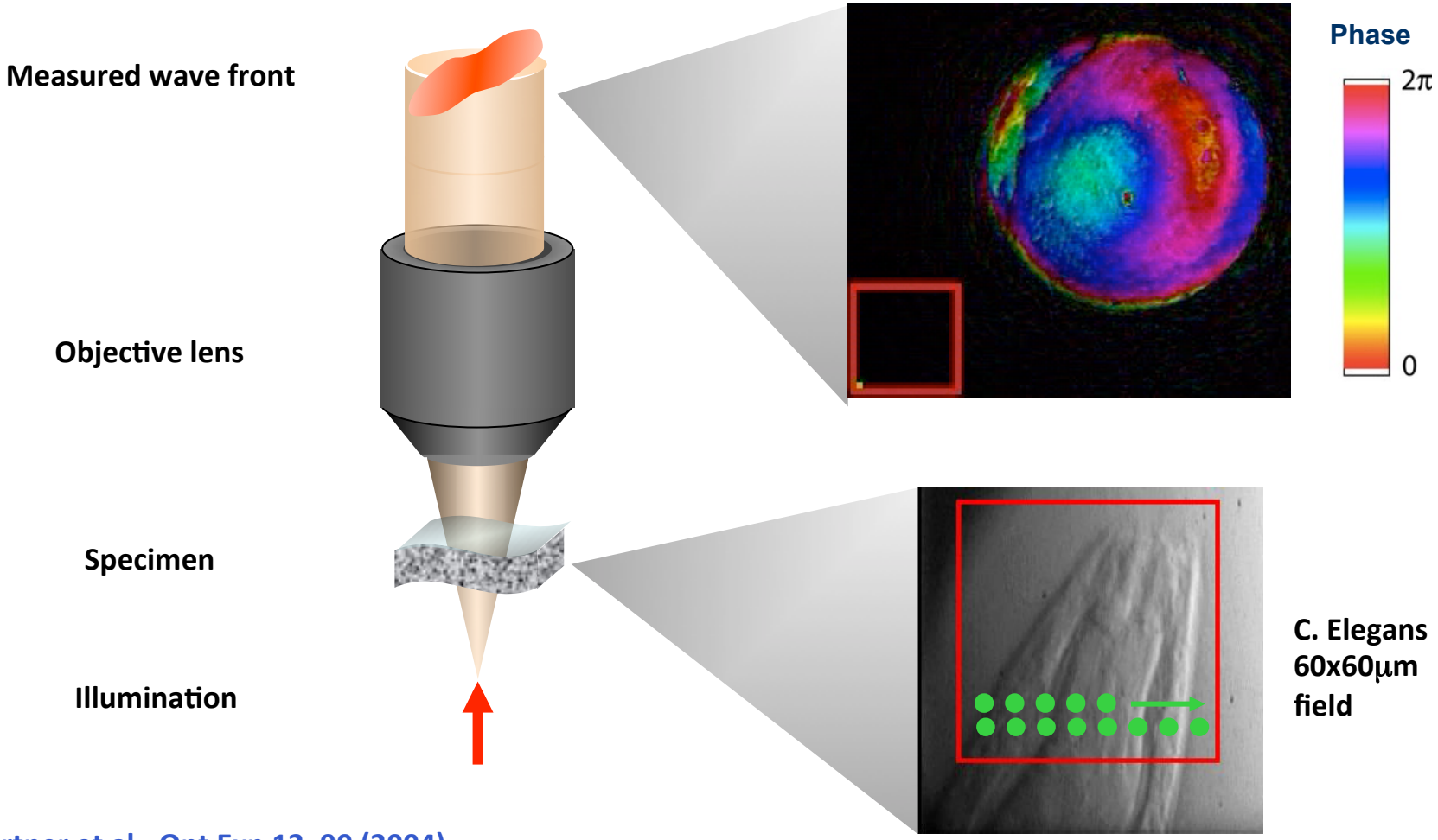


Effects of aberrations

- Enlarged focal spot
- Loss of resolution
- Decrease in contrast

Specimen-induced aberrations

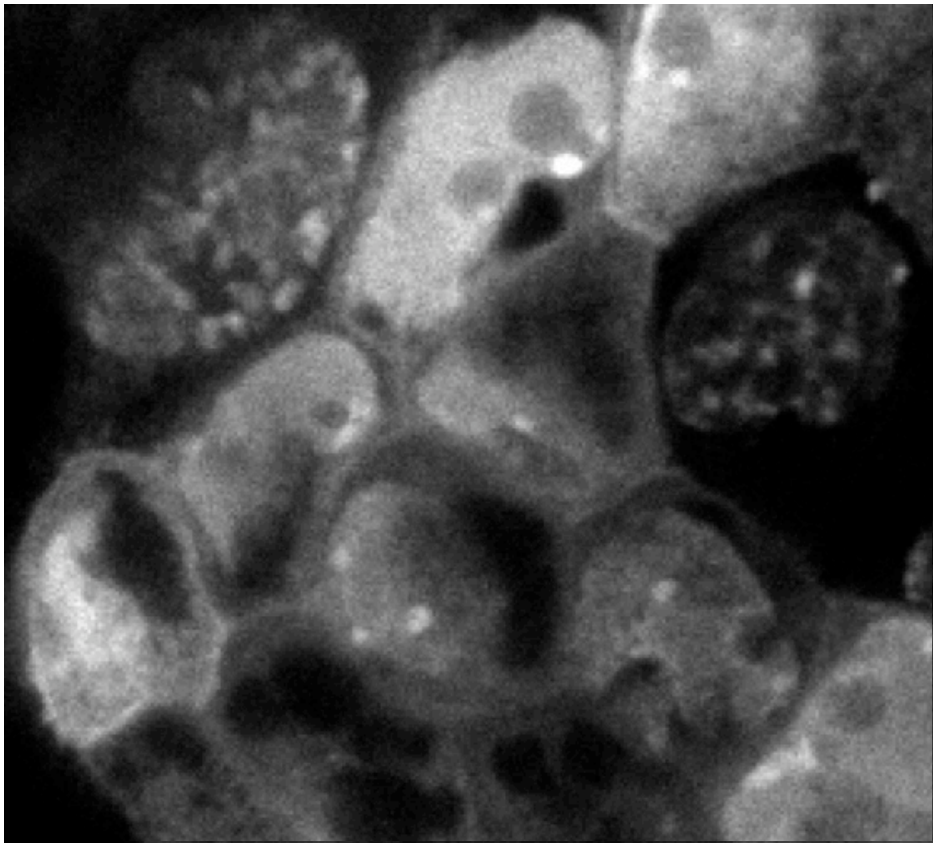
- Variations of refractive index throughout specimen structure
- Measurement of phase aberrations through interferometry at λ = 633nm



Schwertner et al., Opt Exp 12, 90 (2004)

Effects of aberrations in microscopy

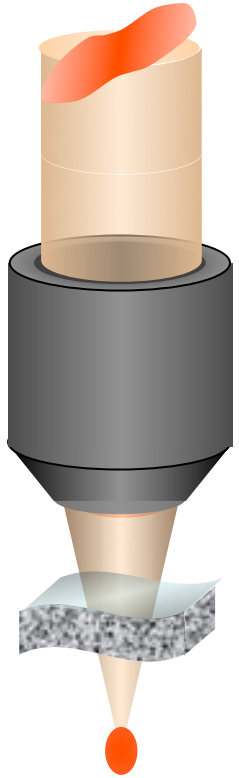
- Two-photon excitation fluorescence microscope: DAPI/GFP labelled mouse embryo
- Images show correction of specimen induced aberrations
- Aberrations cause loss of resolution and contrast



Debarre et al., Opt Lett 34, 2495 (2009)

Aberration correction in microscopy

- Correction of aberrations
  - Generate input wave front with conjugate phase
  - Cancelled by specimen aberrations
  - Diffraction limited focus restored
  - Lower laser powers, lower marker concentration, reduced toxicity



Pre-aberrated wave front

$-\Psi$

Objective lens

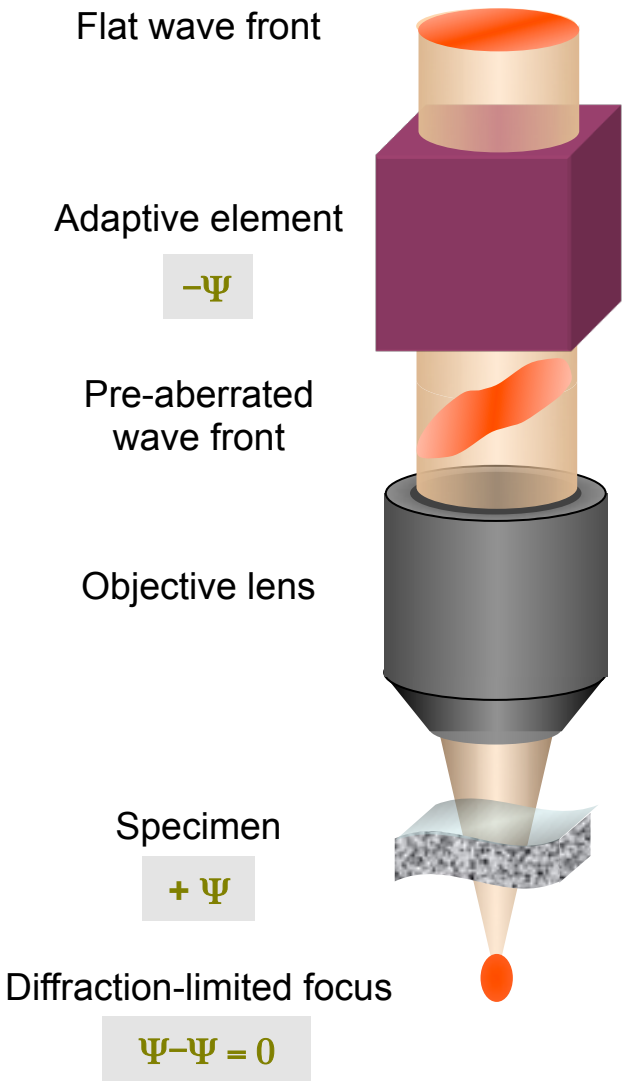
Specimen

Ψ

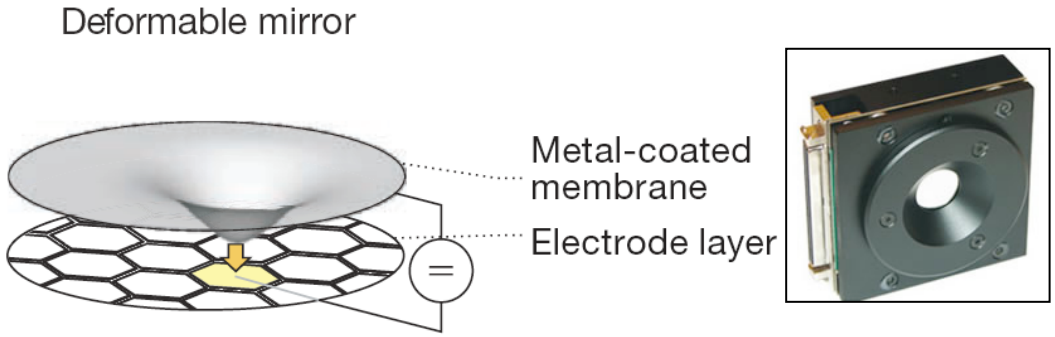
Diffraction-limited focus

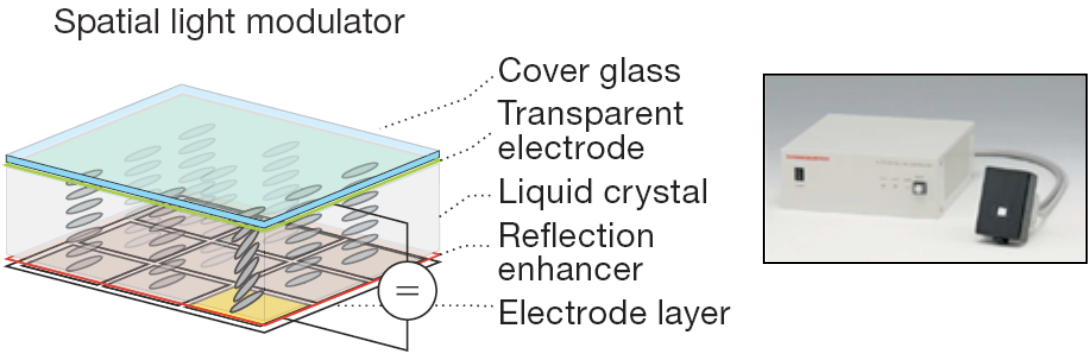
$\Psi - \Psi = 0$

Aberration correction



- Pre-aberrate and correct wave fronts using adaptive element – a deformable mirror or SLM

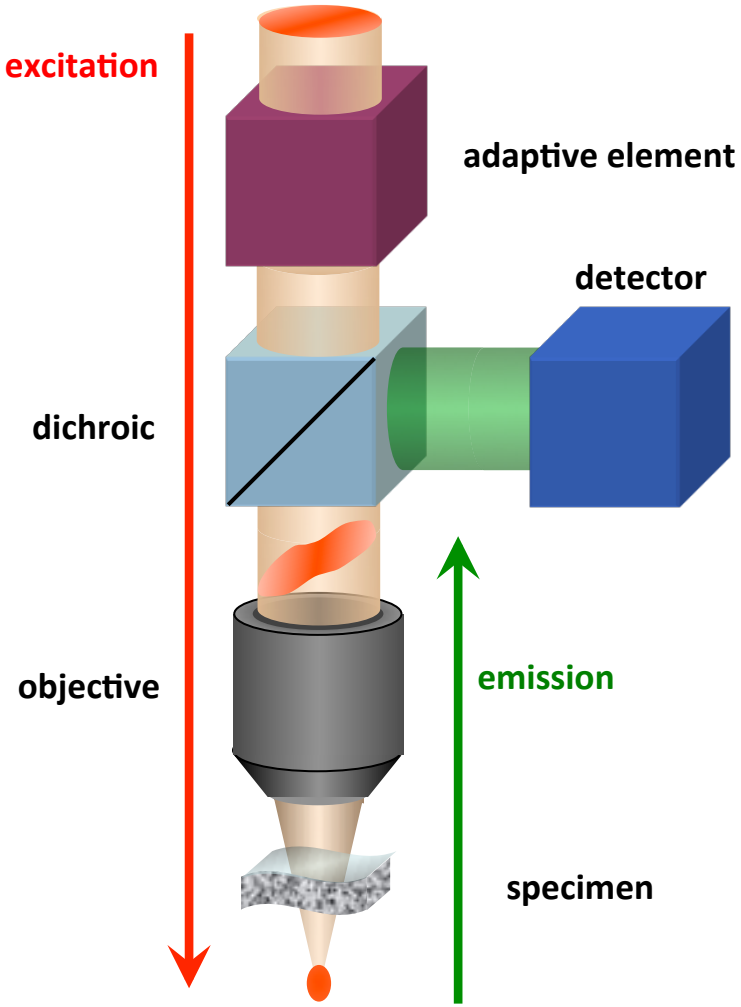




Adaptive microscope configuration

- Placement of adaptive elements depends on type of microscope

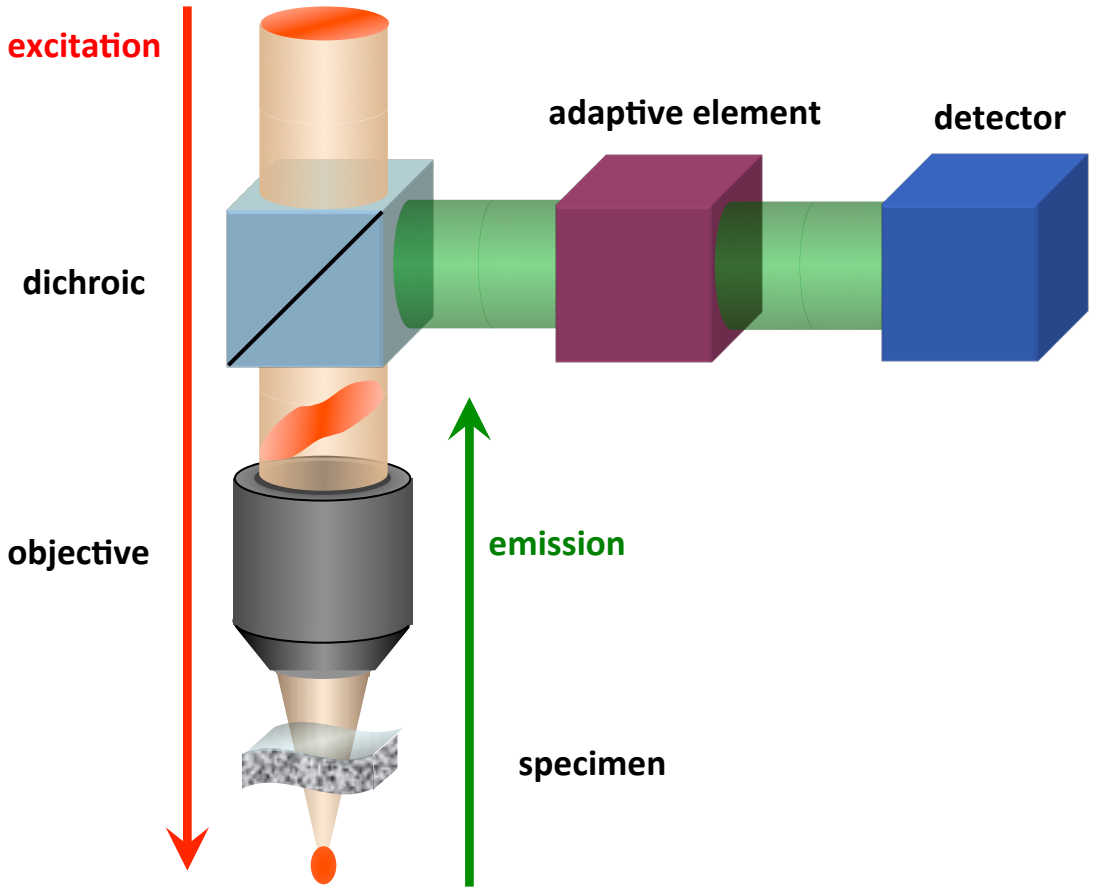
  - Correction required **only in illumination** path
  - E.g. non-linear microscopies (two-photon, harmonic generation)
  - Aberrations have no effect in detection path



Adaptive microscope configuration

- Placement of adaptive elements depends on type of microscope

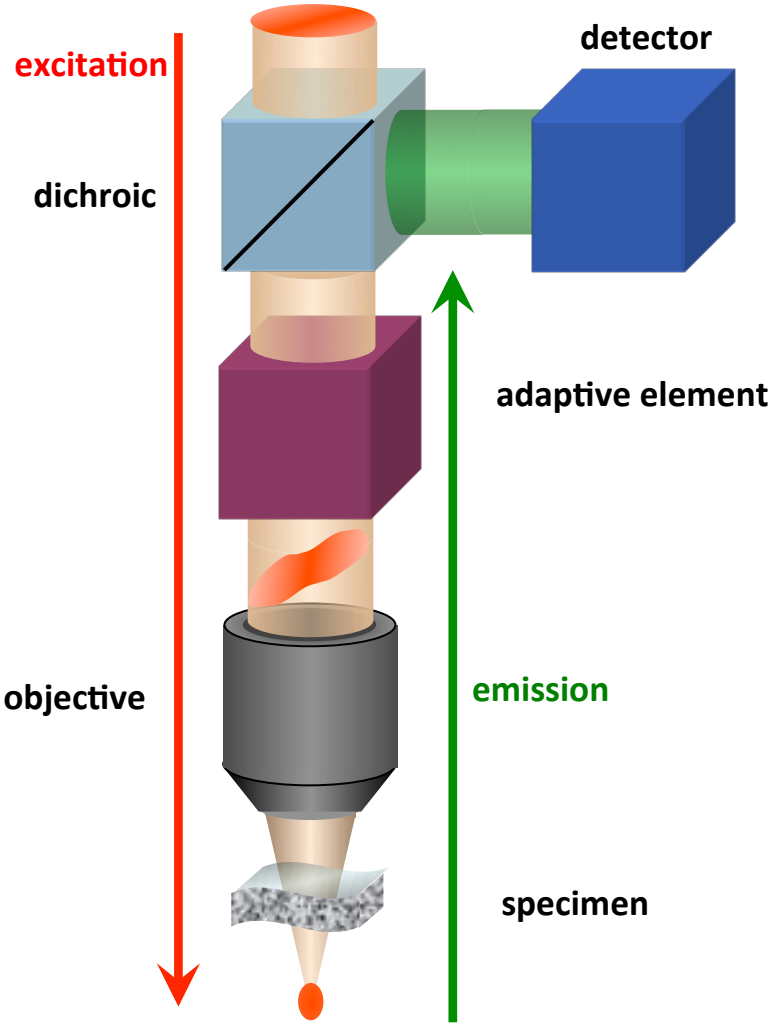
  - Correction required **only in detection** path
  - E.g. conventional widefield microscopy
  - Aberrations have no effect in illumination path



Adaptive microscope configuration

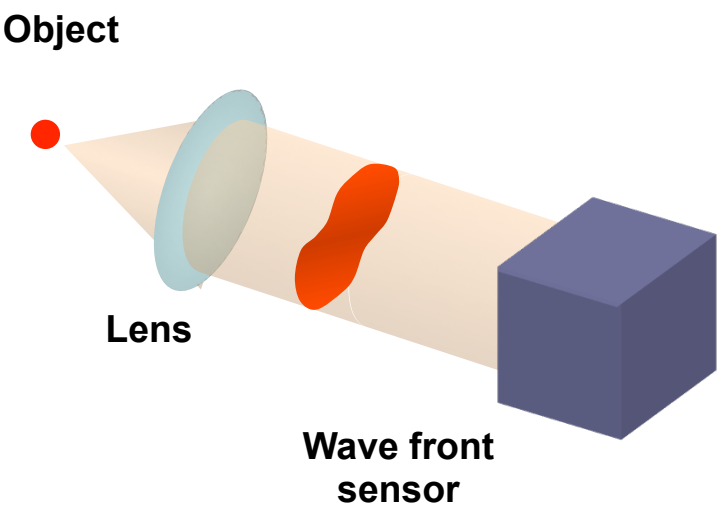
- Placement of adaptive elements depends on type of microscope

  - Correction required in **both illumination and detection** paths
  - E.g. confocal microscopy, spinning disk microscopies
  - Aberrations in both paths have effects on image quality

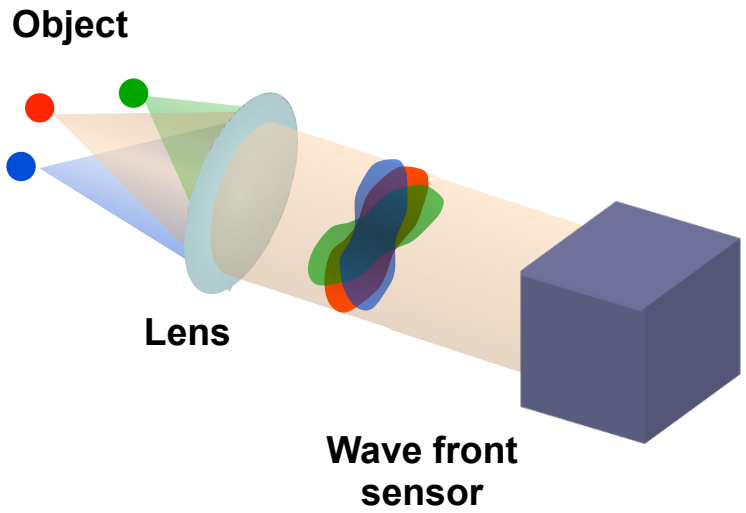


Wave front sensing

- Wave front sensing in traditional adaptive optics
  - Point-like object
  - Well defined wave front



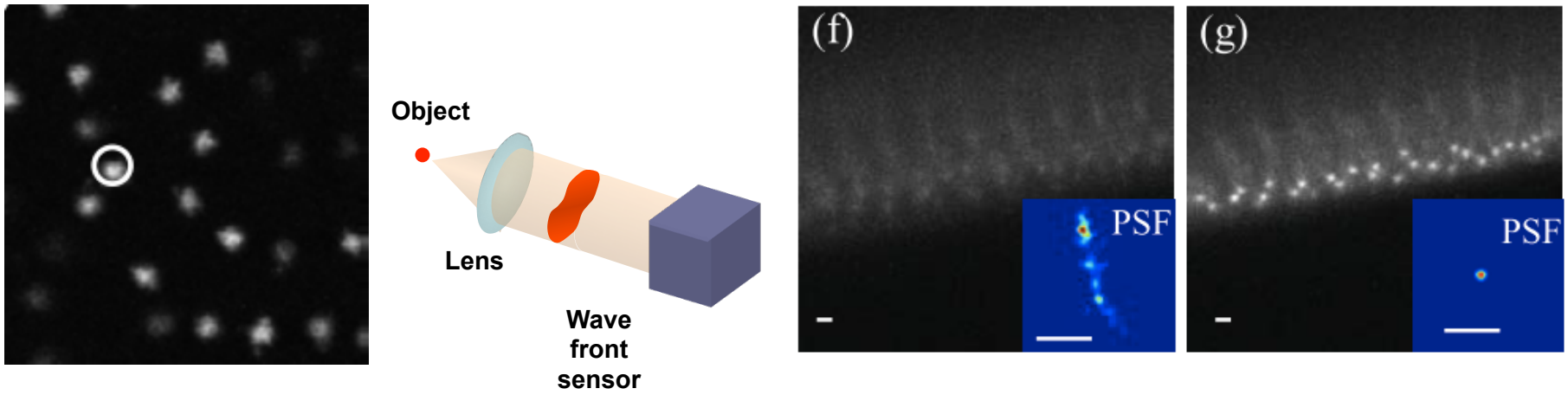
- Wave front sensing in general imaging systems
  - 2D or 3D object
  - Superposition of wave fronts
  - Out-of-focus light



- Wave front sensing in 3D microscopy needs method to exclude out-of-focus light

Direct wave front sensing in optical microscopy

- Methods for direct sensing
  - Use isolated point-like objects as “guide-stars” for Shack-Hartmann sensor



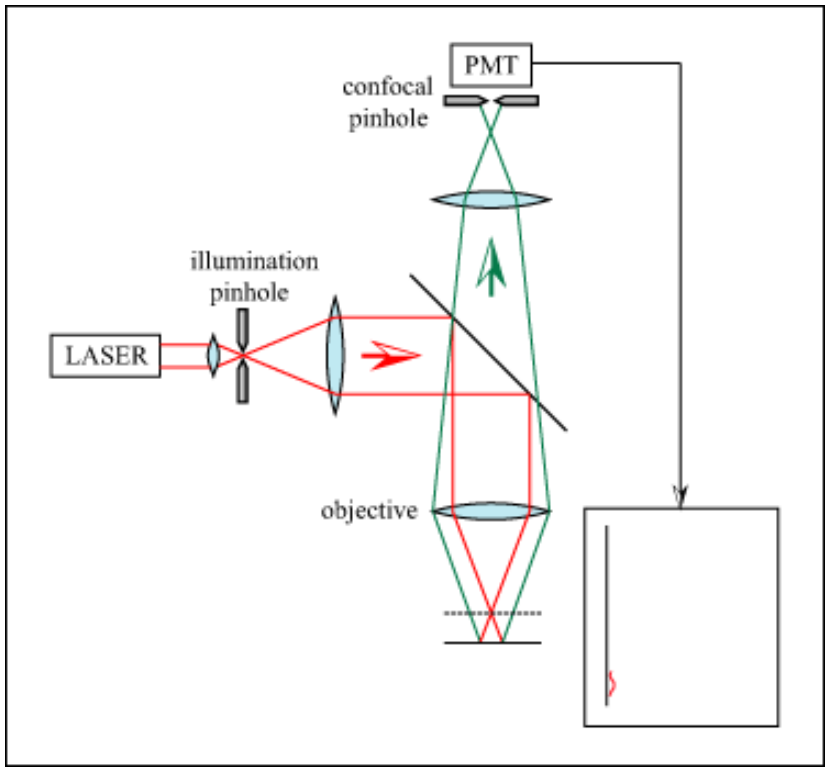
Live imaging using adaptive optics with fluorescent protein guide-stars

Xiaodong Tao,[1,*] Justin Crest,[2] Shaila Kotadia,[2] Oscar Azucena,[1] Diana C. Chen,[3] William Sullivan,[2] and Joel Kubby[1]

2 July 2012 / Vol. 20, No. 14 / OPTICS EXPRESS 15969

Direct wave front sensing in optical microscopy

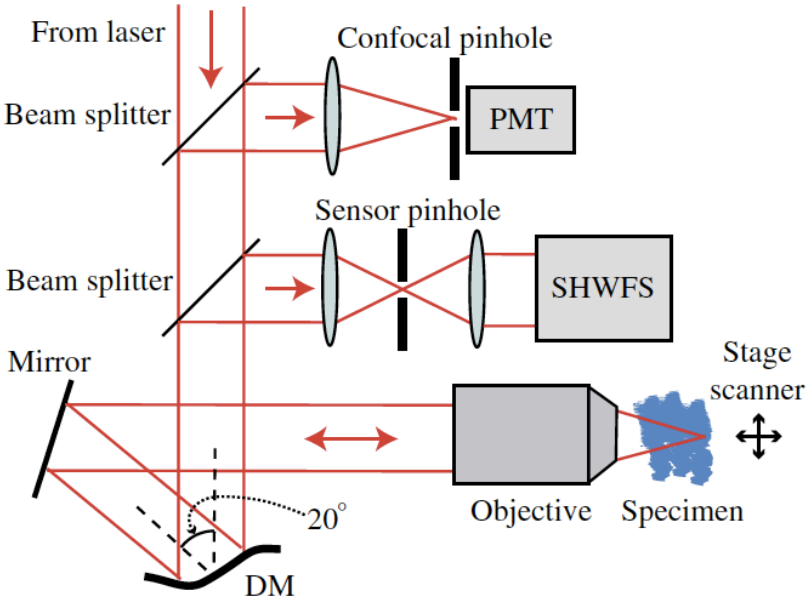
- Confocal microscopy
  - Reflection, phase contrast, fluorescence, polarisation

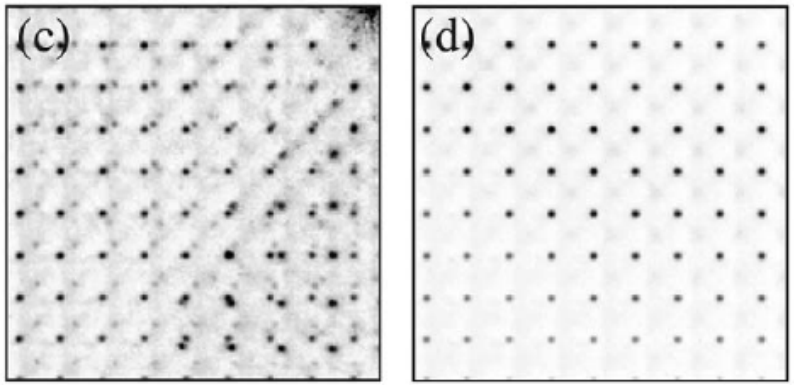


- Direct wave front sensor could use spatial filter pinhole like the confocal microscope to exclude out of focus light

Direct wave front sensing in optical microscopy

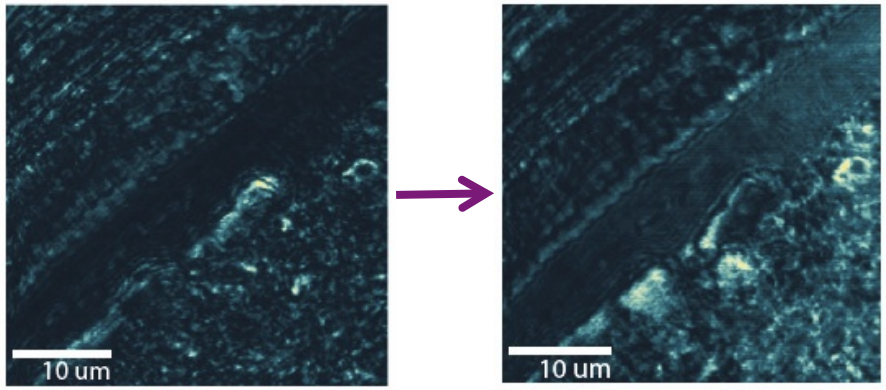
- Methods for direct sensing
  - Use pinhole to filter light from specimen before it reaches the sensor
  - Example using illumination back scatter from specimen
  - (Also shown using fluorescence emission)





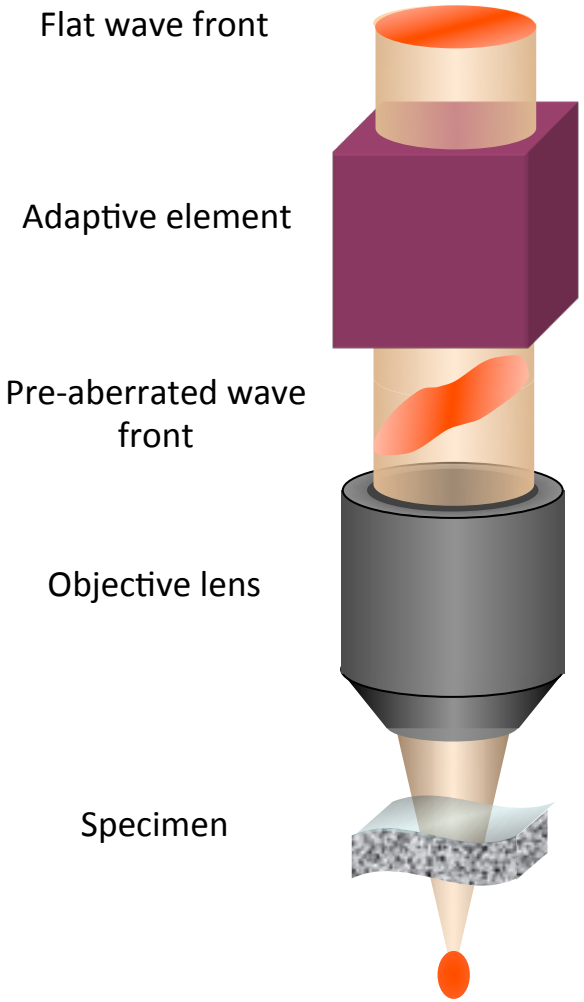
No pinhole

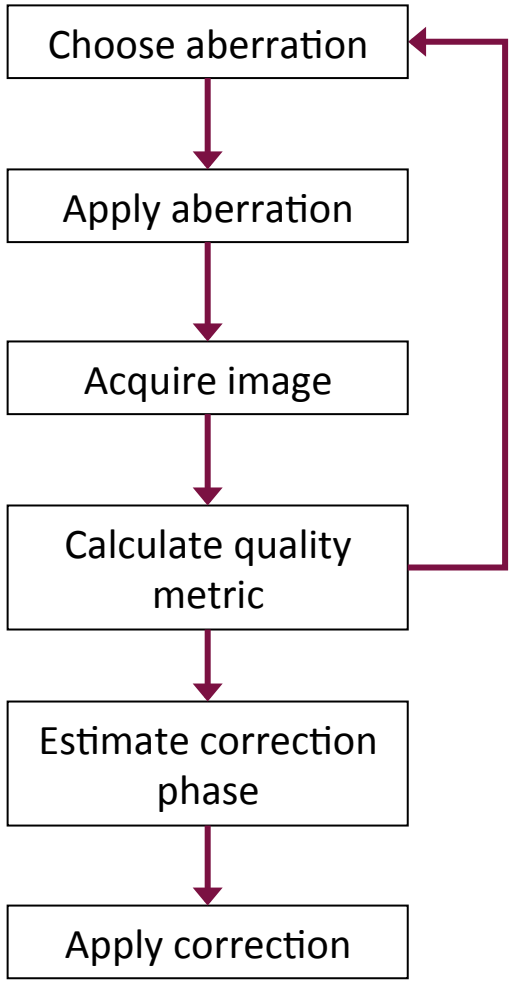
With pinhole



Rahman & Booth, Appl Opt 52, 5523 (2013)

Indirect aberration measurement





Aberration representation

Optimisation metric

Estimator

Efficient correction procedure

Image based wave front sensing

- How can one measure aberrations induced in optical path to the focus?
- Images are formed through 3D discrimination of in focus light – source for sensing
- Image formation modelled as convolution:

$$I(x,y,z) = f(x,y,z) \otimes h(x,y,z)$$

Image — **Aberration and structure dependent**

Object — **Structure dependent**

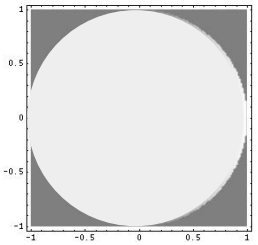
Point spread function — **Aberration dependent**

- We need a scheme that separates effects of object structure and PSF aberrations
- Achieved through choice of aberration modes, feedback metric and estimator

Aberration mode effects

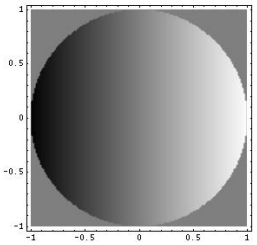
- Zernike polynomials as example modal basis set
- Certain modes have no effect on image quality – should be removed from scheme

No effect

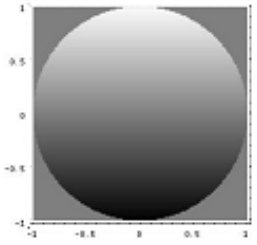


$Z_1(r, \theta)$ – piston

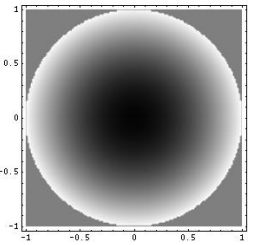
Geometric distortion



$Z_2(r, \theta)$ – tip

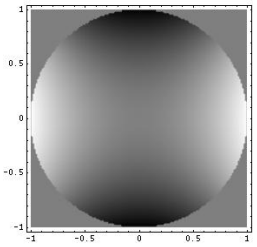


$Z_3(r, \theta)$ – tilt

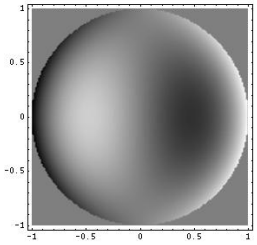


$Z_4(r, \theta)$ – defocus

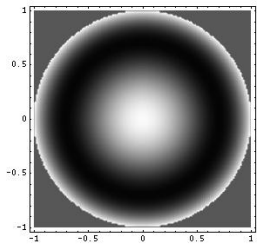
Effect on signal intensity / resolution



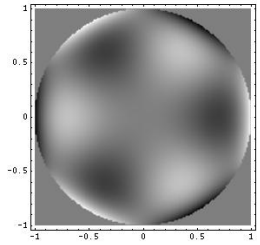
$Z_5(r, \theta)$ – astigmatism



$Z_7(r, \theta)$ – coma



$Z_{11}(r, \theta)$ – spherical



$Z_{18}(r, \theta)$ – trefoil

Choice of modes for adaptive microscopy

- Choice influenced by adaptive element, sensing method, specimen properties

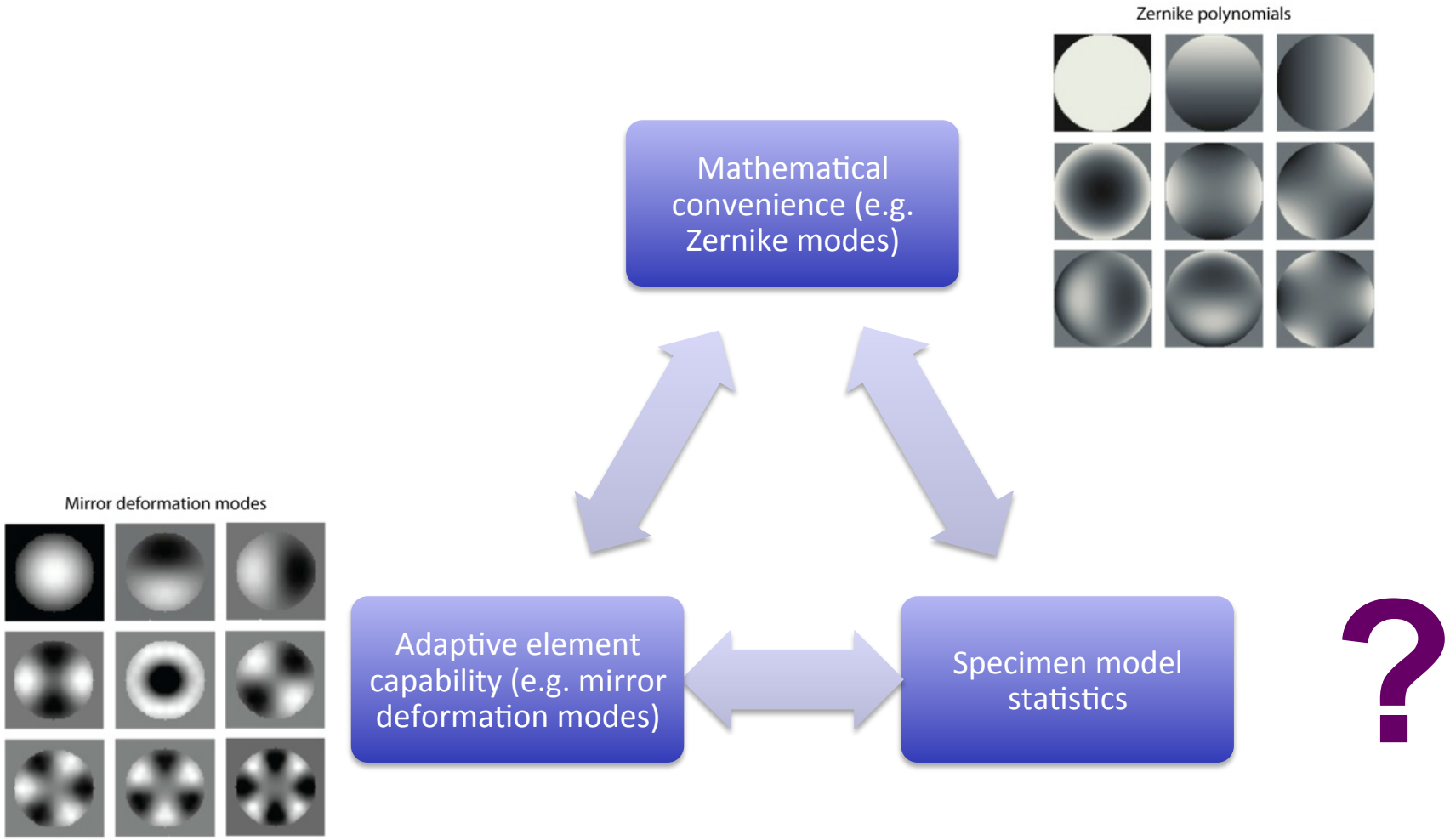


Image based adaptive optics

- Example: transmission microscope - Correction of a single aberration mode (astigmatism)
- Quadratic maximisation using three image measurements with applied aberrations
- Low spatial frequency magnitude as quality metric

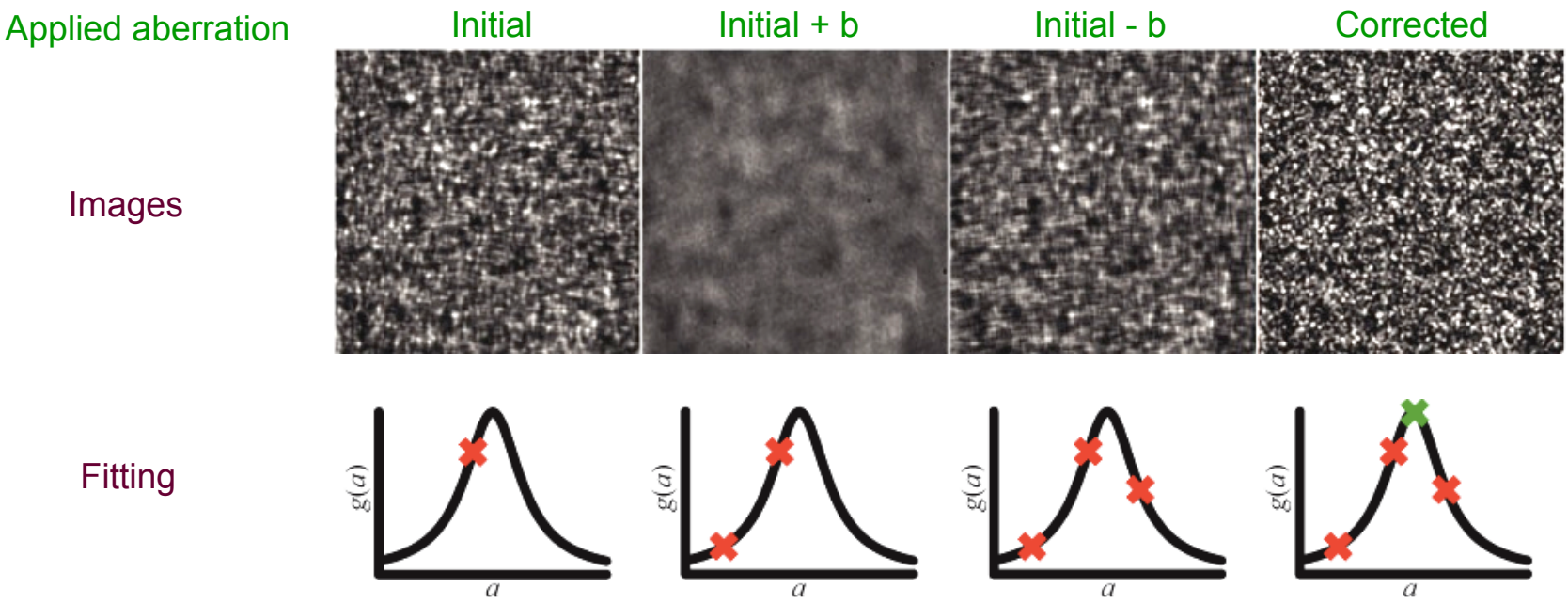
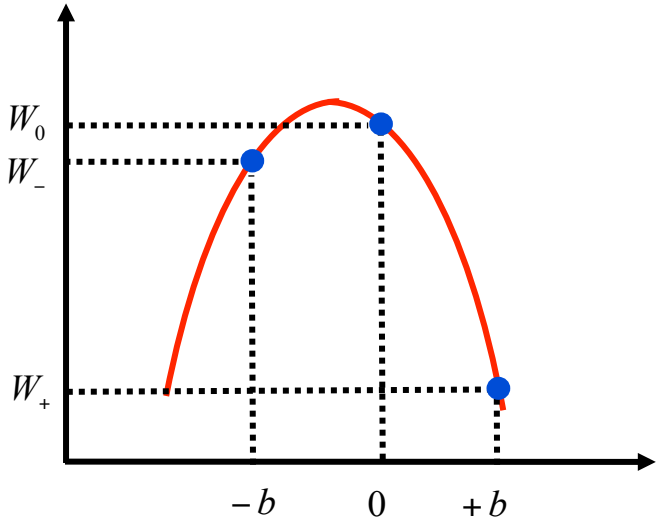


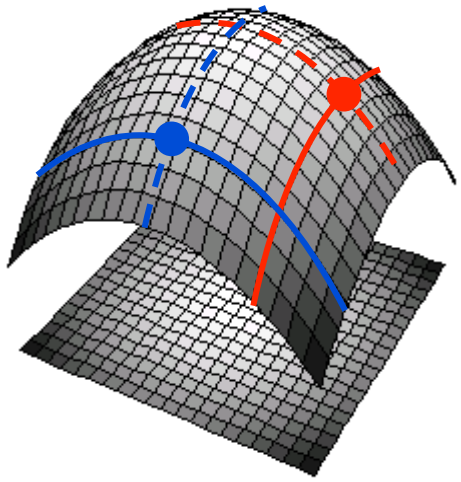
Image based adaptive optics

- Find a mathematical representation with suitable optimisation metric
- One variable parabolic maximisation – simple algorithms

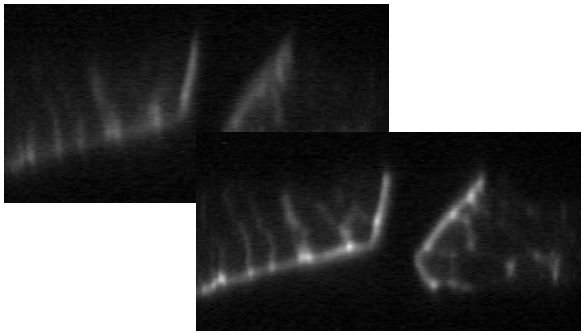


$$a_{corr} = -\frac{b}{2}\frac{(W_+ - W_-)}{(W_+ - 2W_0 + W_-)}$$

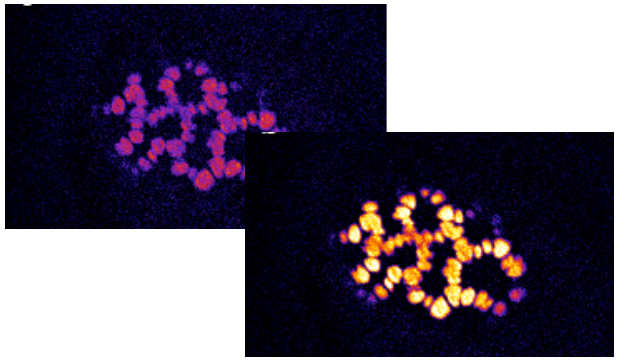
- Take three measurements per mode
- Multi-variable parabolic maximisation – separable maximisation in each variable
- 2N+1 measurements for N modes



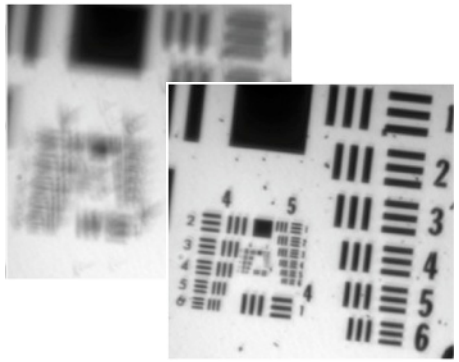
Demonstrations of AO in microscopy



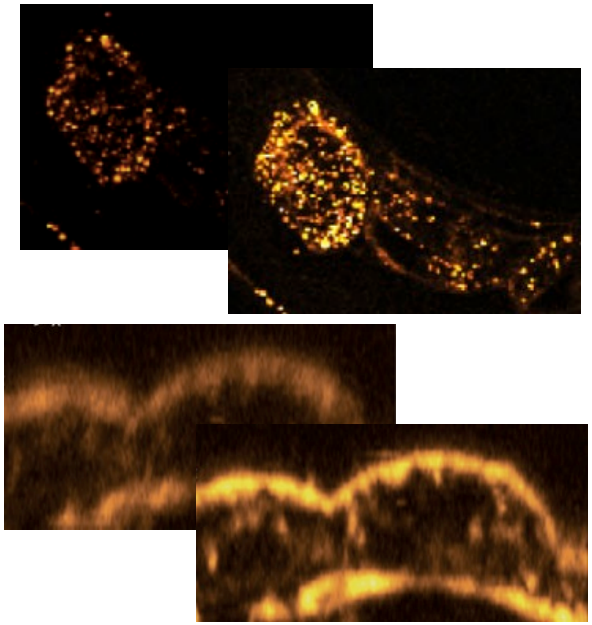
Confocal fluorescence microscopy
Booth et al., PNAS 99, 5788 (2002)



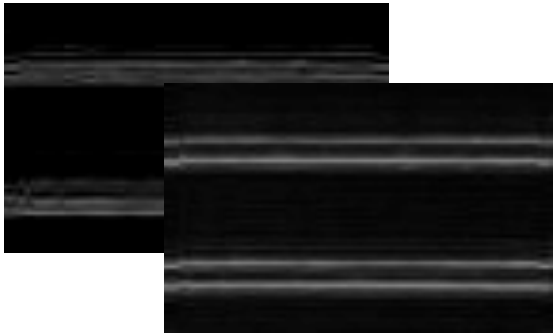
Structured illumination microscopy
Debarre et al., Opt Expr 16, 9290 (2008)



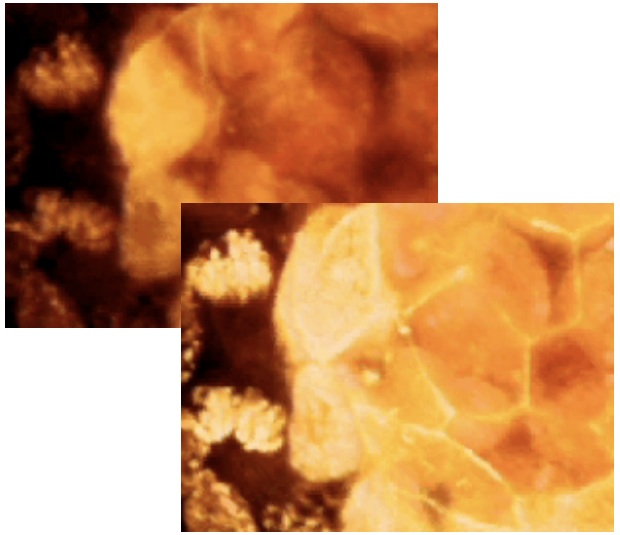
Transmission microscope
Debarre et al., Opt Expr 5, 8176 (2007)



Third harmonic microscopy
Jesacher et al., Opt Lett 34, 3154 (2009)

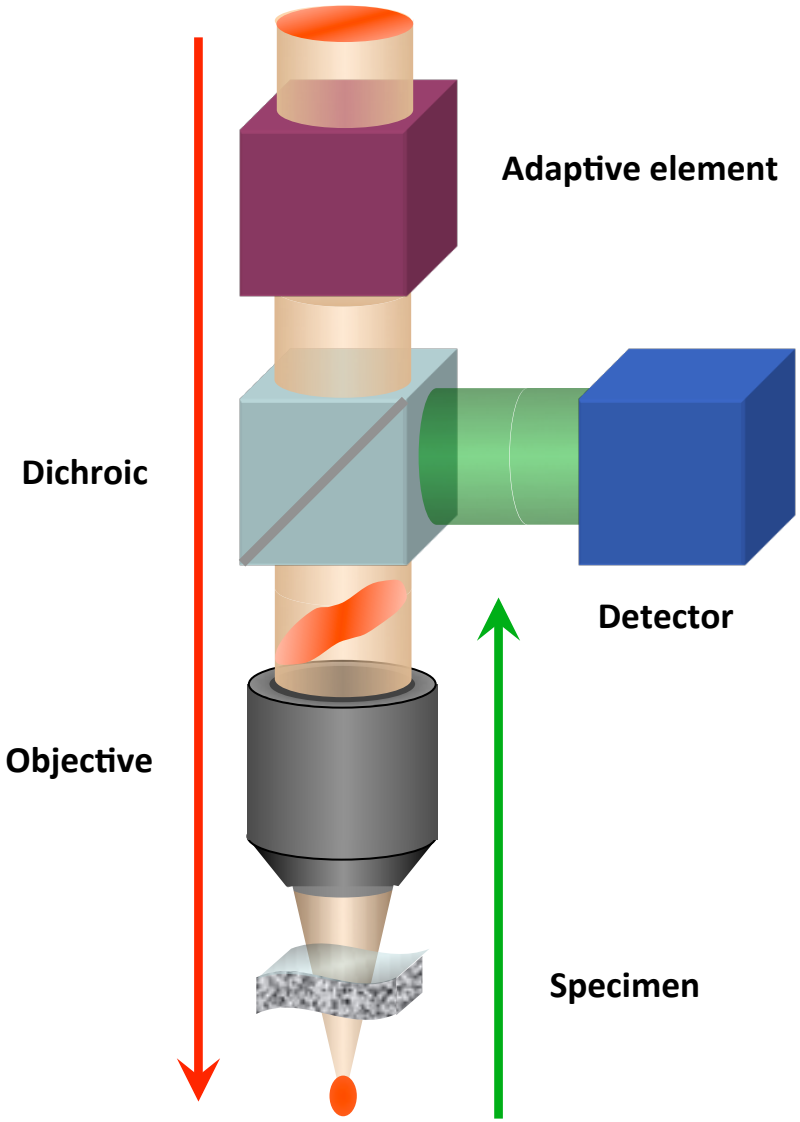


Confocal reflection microscope
Booth et al., Appl Phys Lett 88, 31109 (2006)



Two-photon microscopy
Debarre et al., Opt Lett 34, 2495 (2009)

Two-photon microscopy

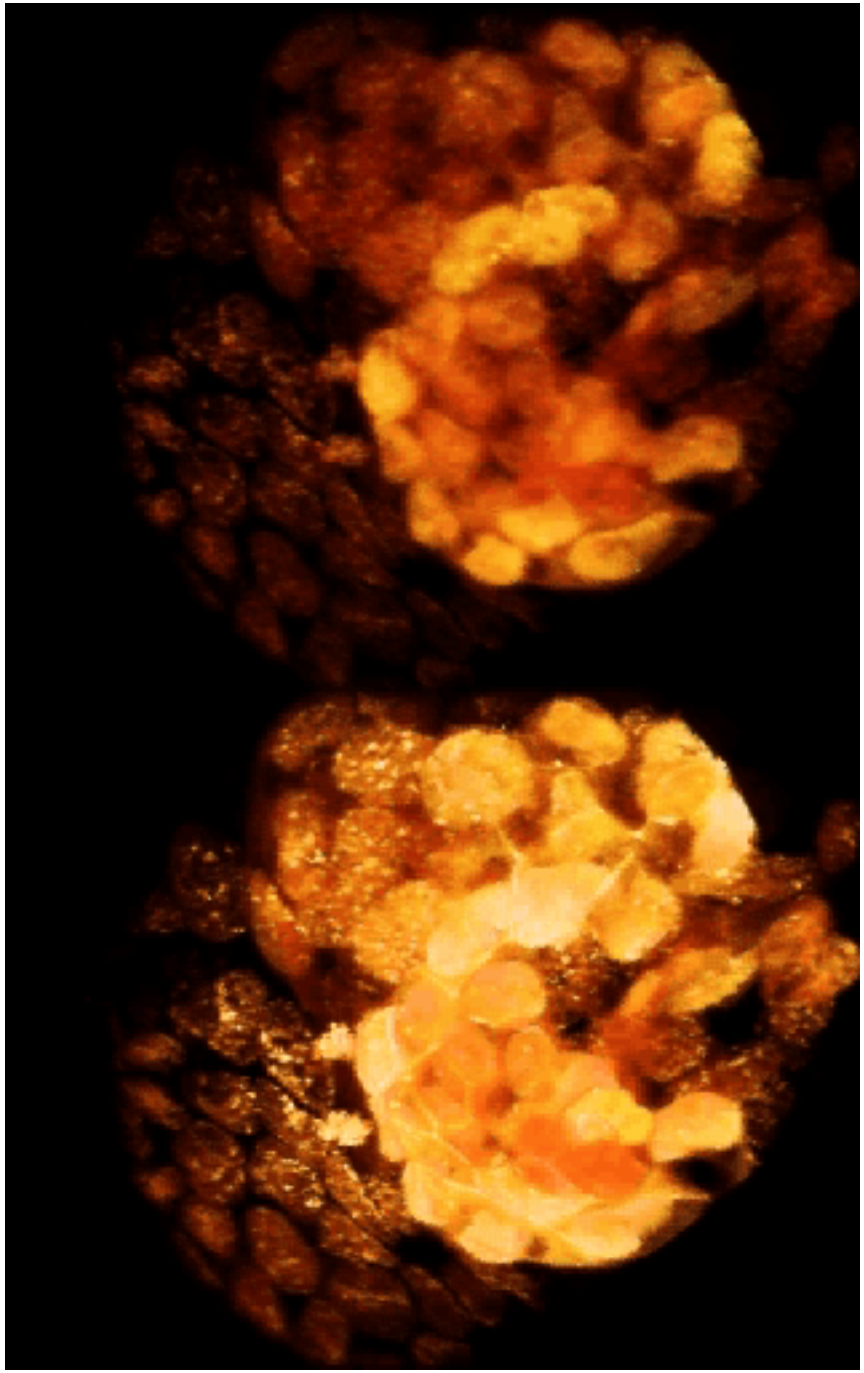


- Correction required only on illumination path
- Large area detector - aberrations in emission path have no effect.
- Metric - total image intensity: $\Sigma_i I(x,y)$
- Modes - optimum two-photon
- Laser: Ti-Sapphire 100fs, 850nm, 76MHz, Spectra Physics Tsunami
- Deformable mirror: Boston Micromachines μDM, 140 element, 4x4mm, 2.5μm range.
- Objective: 1.2 NA water immersion, Olympus UPlanApo 60x WPSF

Adaptive optics for two-photon microscopy

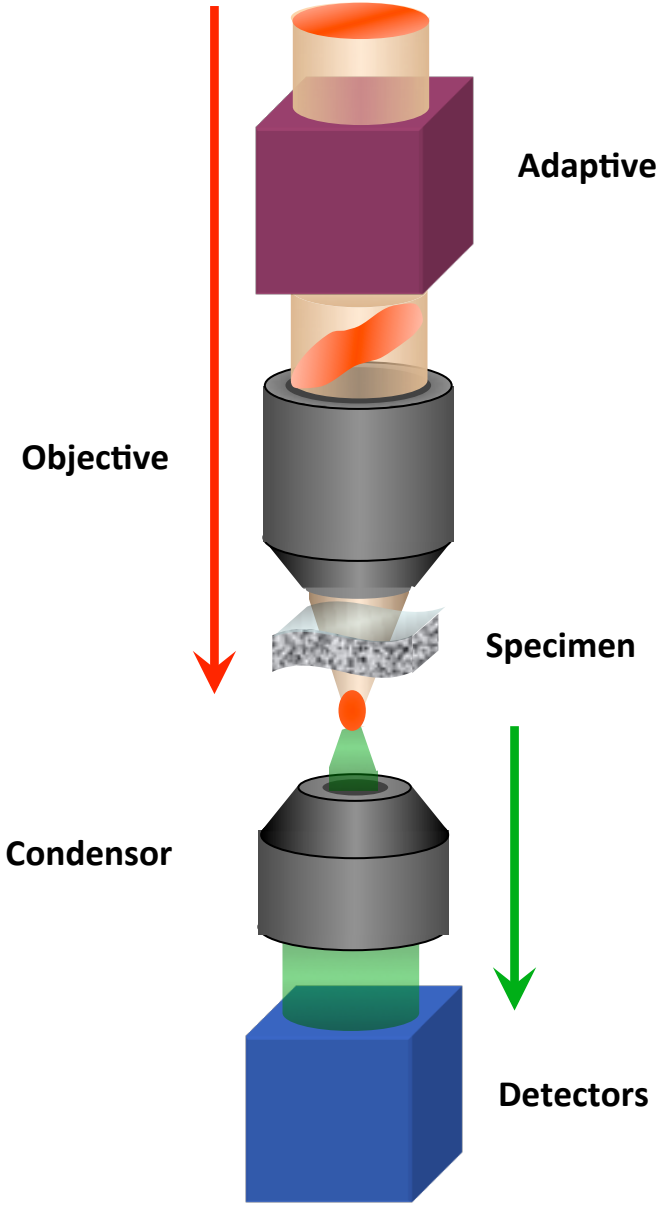
Correction of specimen induced aberrations in 3D imaging of a fluorescently labelled mouse embryo using a two-photon laser scanning microscope.

Original - Top
Corrected - Bottom



Debarre et al., Opt Lett 34, 2495 (2009)

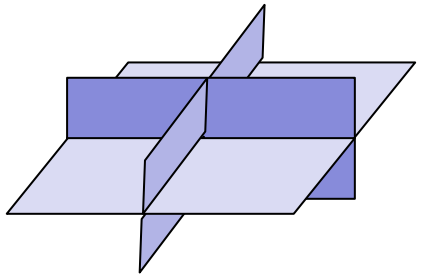
Harmonic generation microscopy

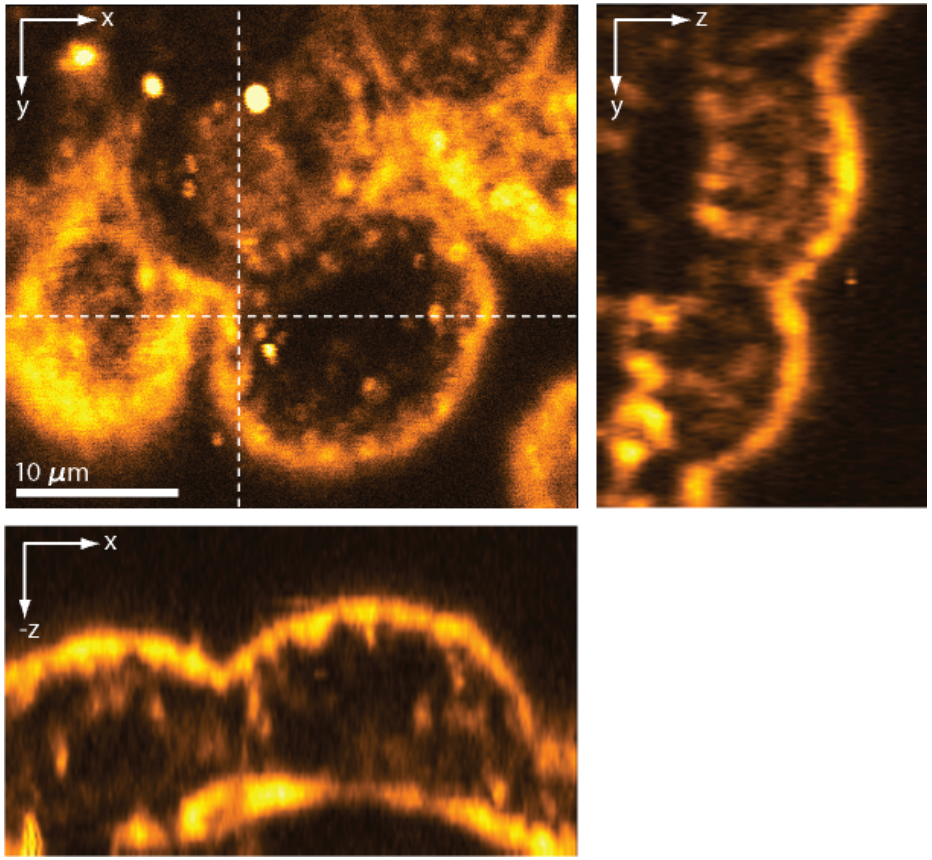


- Intrinsic contrast from optical properties of specimen (3rd order nonlinear susceptibility $\chi^{(3)}$)
- Second and third harmonic emission detected in trans configuration
- Large area detector - aberrations in emission path have no effect – correct illumination only
- Metric - total image intensity: $\Sigma_i I(x,y)$
- Modes - Zernike polynomials
- Laser: Cr-Forsterite, 1230nm, 65fs, 100MHz, Del Mar Mavericks
- Deformable mirror: Imagine Optics Mirao, 56 actuators, large range
- Objective: 1.15NA water immersion, Olympus UApo/340, 40x.

Adaptive THG microscopy of embryos

- xyz stack of THG images of unlabelled mouse embryo – contrast from intrinsic optical properties

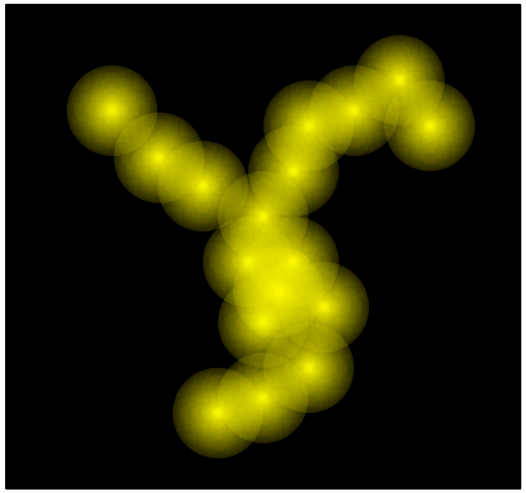




Jesacher et al., Opt Lett 34, 3154 (2009)

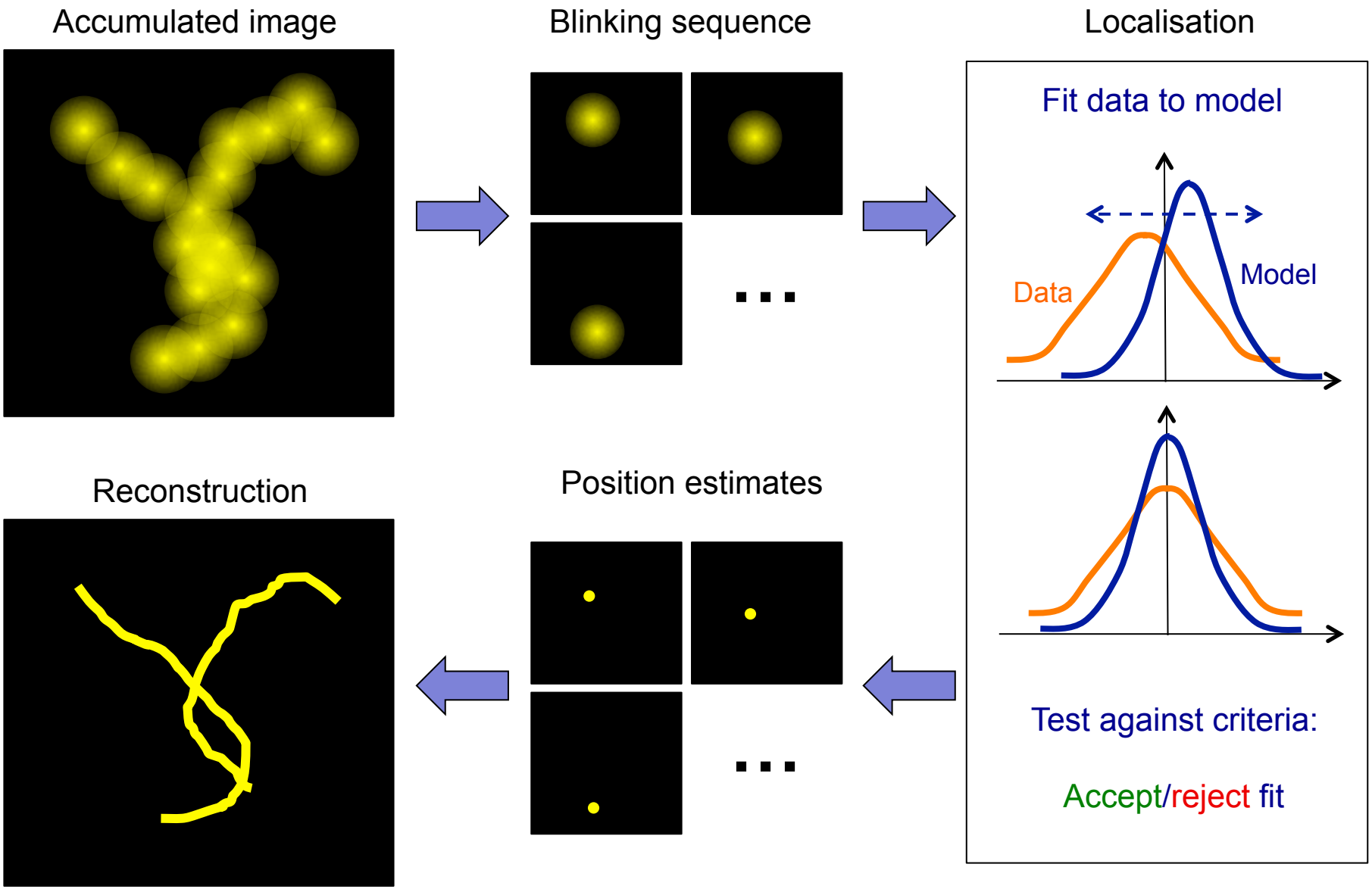
Principles of superresolution localisation microscopy

- Localisation methods: PALM, STORM, GSDIM and variants
- Isolated emitters localised with higher precision than microscope diffraction limit



- Stochastic activation of fluorophores
- Acquire sequence of blinking images
- Localise each emitter
- Represent data in an image

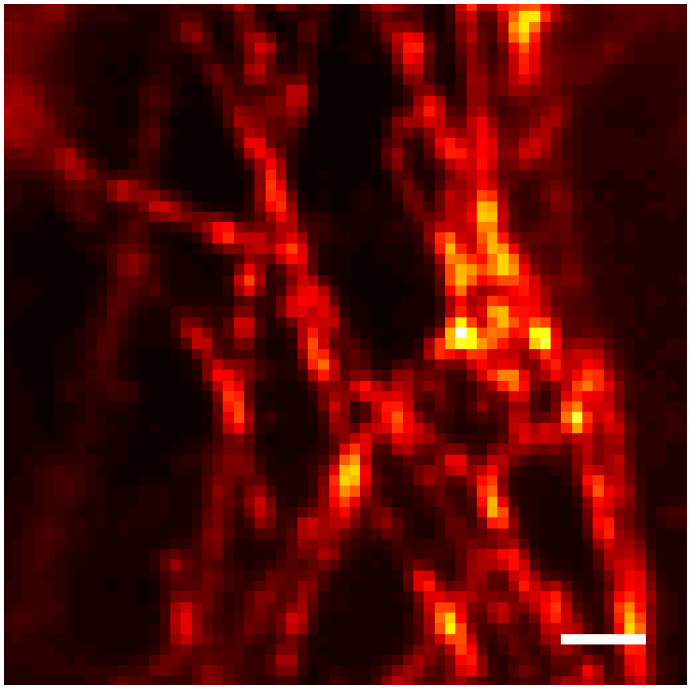
Principles of localisation microscopy



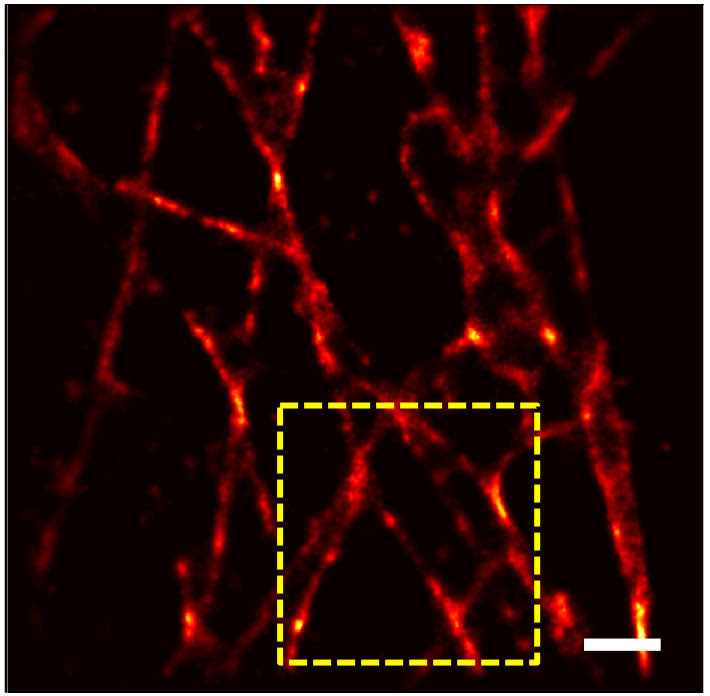
Aberration correction for STORM

- STORM images taken in multilayer culture of mouse embryonic stem cells with Alexa 647 labelled microtubules in Vectashield
- Illumination wavelength 655nm. Objective 1.4 NA oil immersion. Depth approx 15 μm. Scale bar 1 μm.

SOFI variance images



STORM images



Specimens provided by J. Demmerle and L. Schermelleh, Oxford

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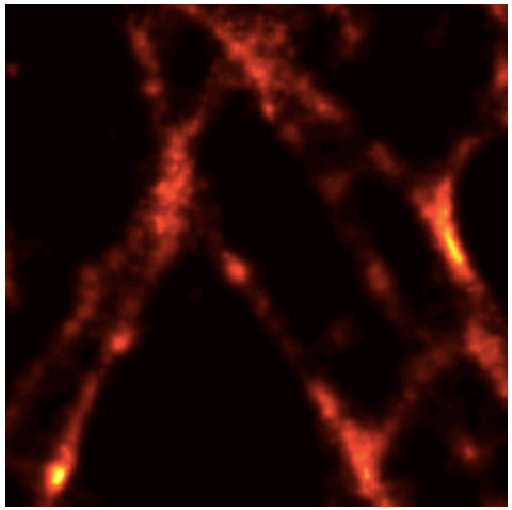
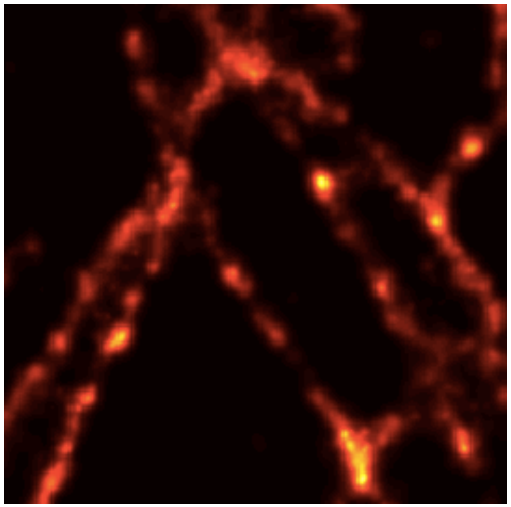
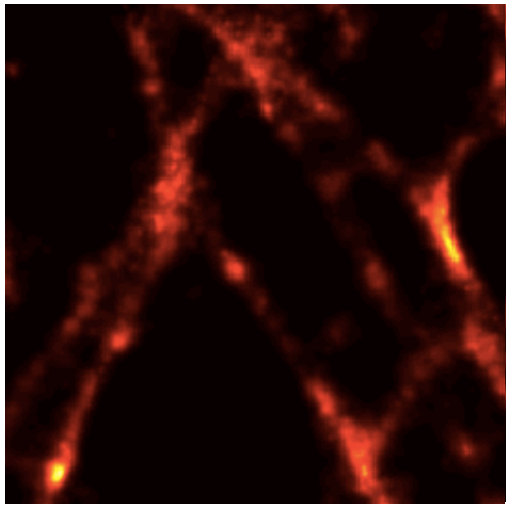
Before correction



After correction



Comparison



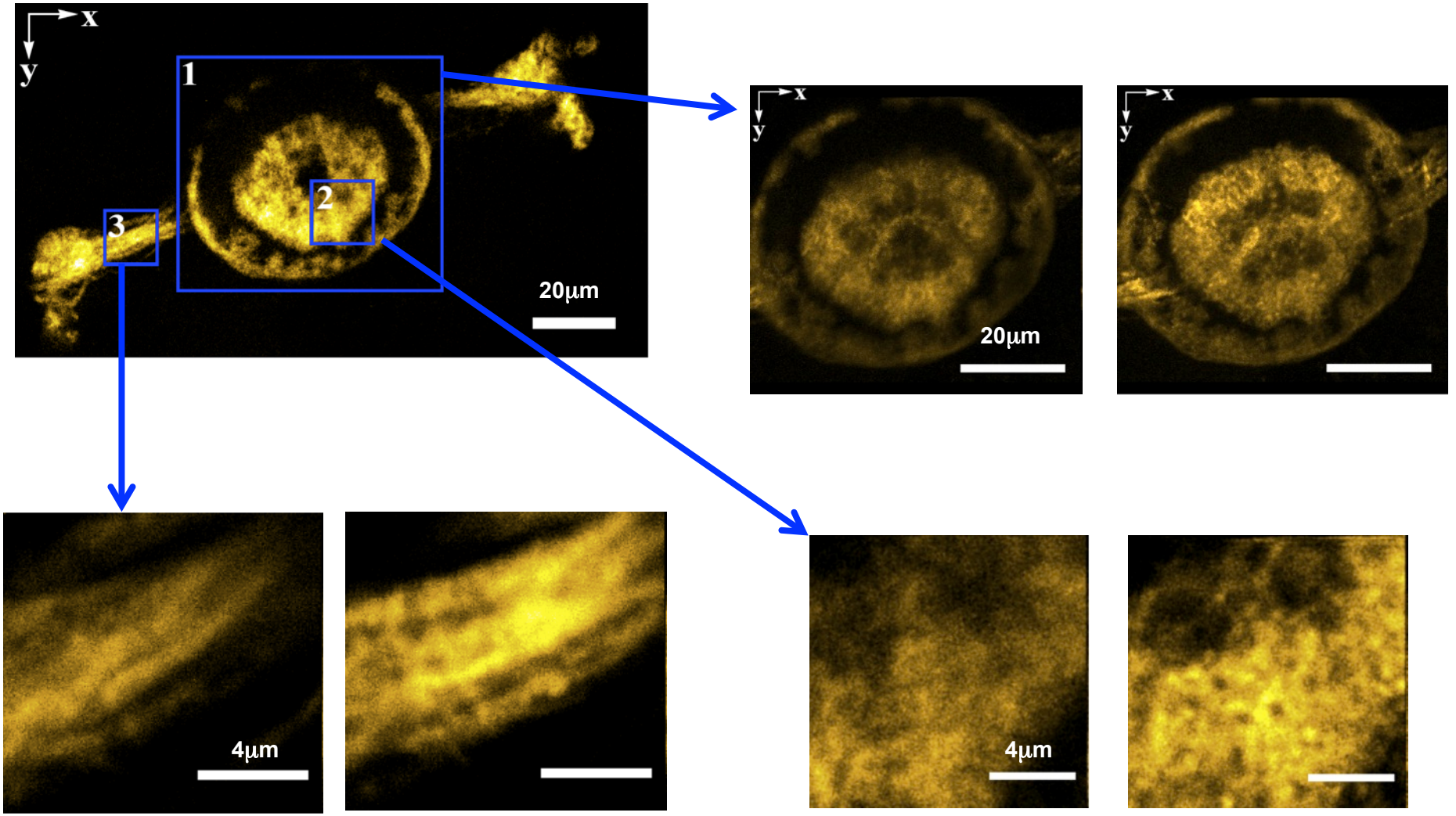
Specimens provided by J. Demmerle and L. Schermelleh, Oxford

Challenges in adaptive microscopy

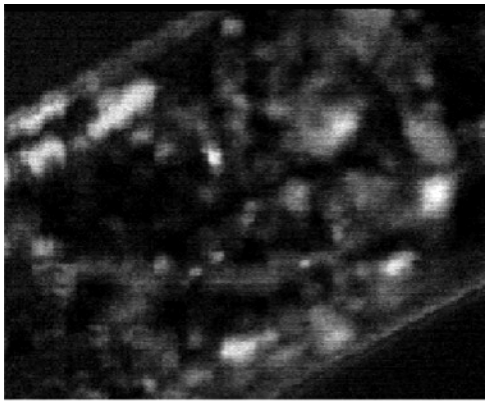
- Wave front measurement (indirect or direct)
  - Amplitude range
  - Speed
  - Accuracy

- Spatial aberration variations
  - Aberrations not generally constant across field of view (anisoplanatism)
  - Speed of correction elements insufficient for scanning systems
  - Imaging systems need correction in parallel

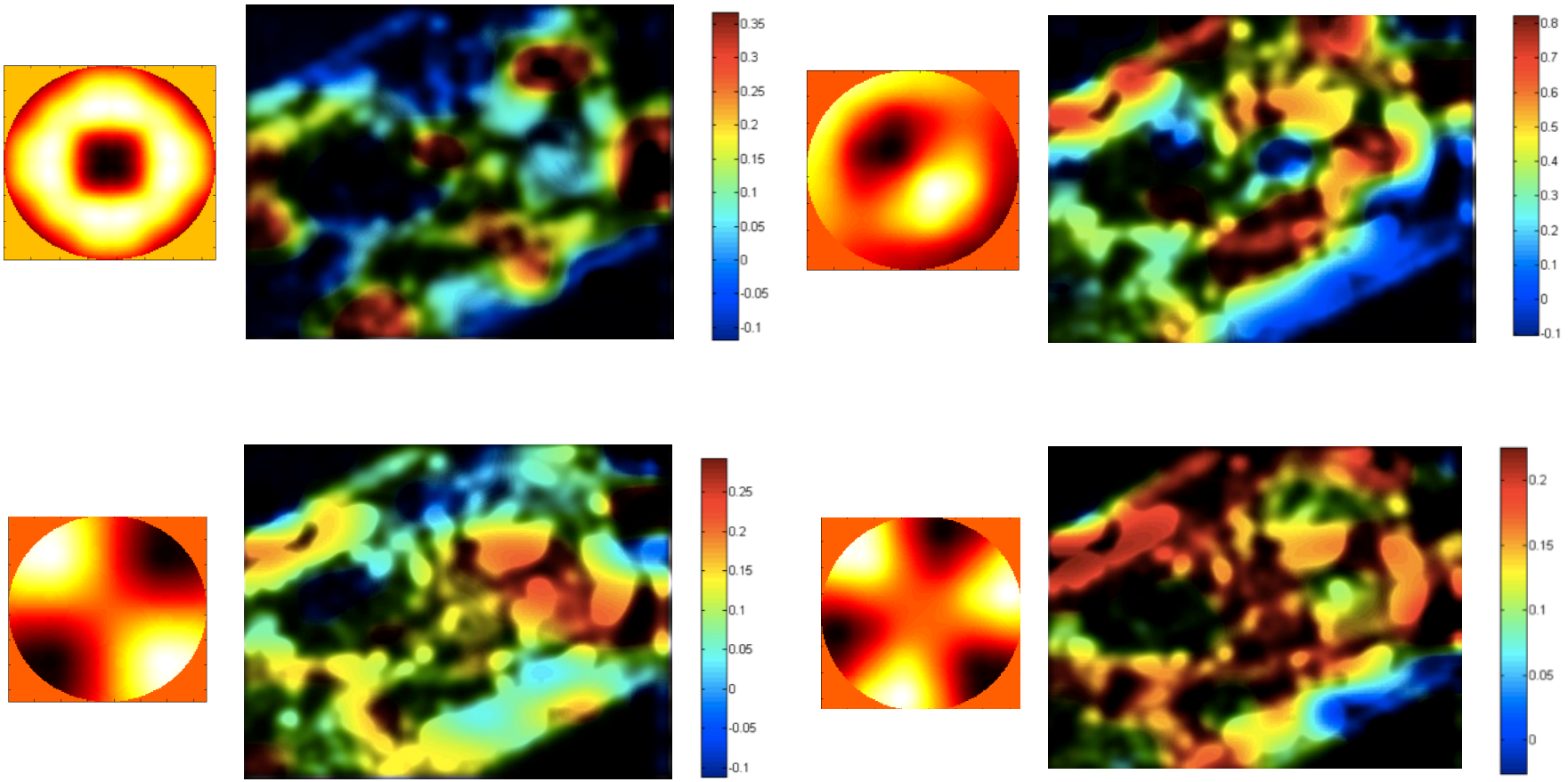
- Adaptive two-photon fluorescence microscope images of central complex in intact Drosophila (fruit fly) brain, GFP labelling
- Correction of different aberrations in different specimen regions



Aberration maps

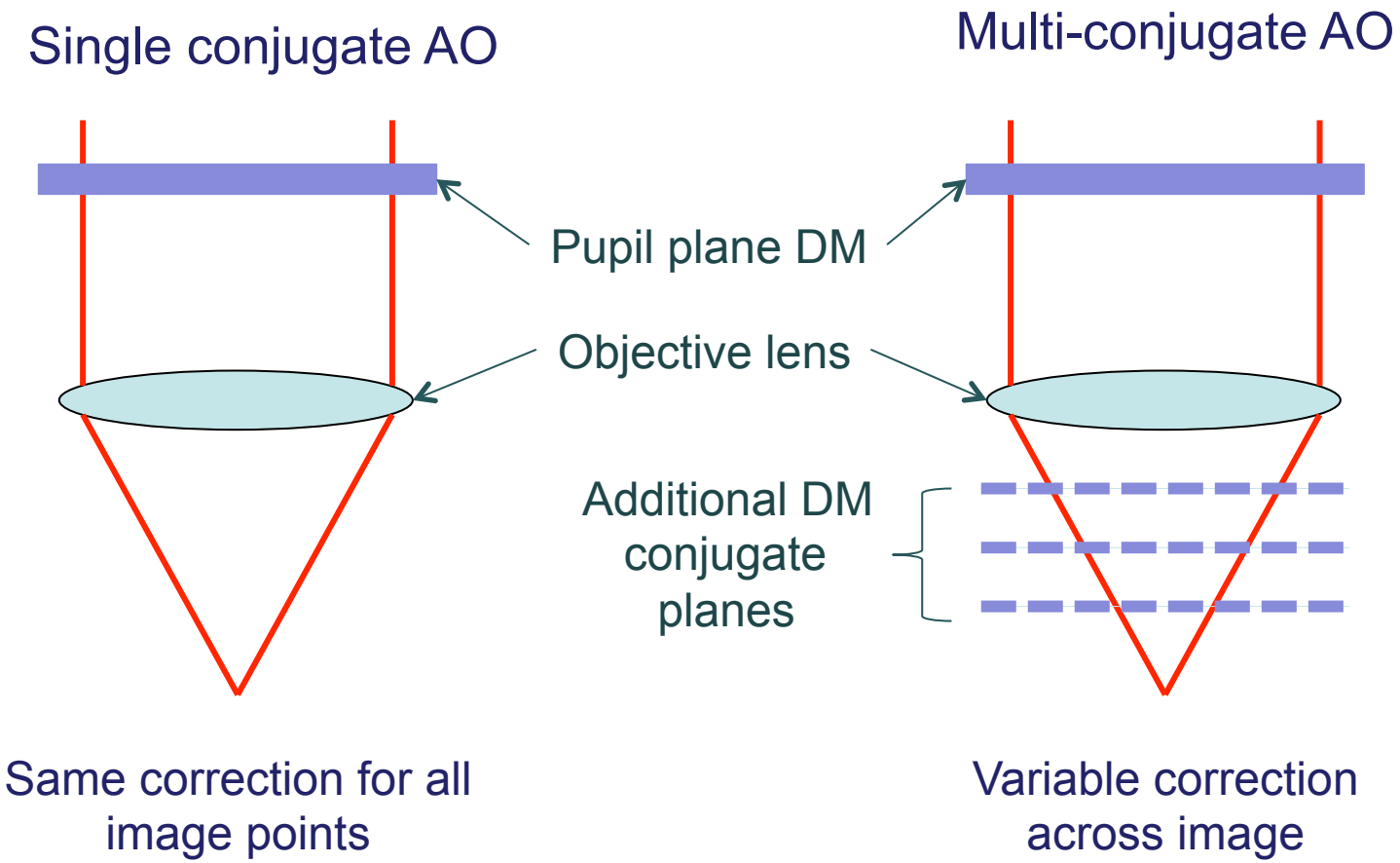


- C.Elegans images - various aberration modes
- Third harmonic generation (THG) microscope



Correction of spatially varying aberrations

- Modelling of multi-conjugate adaptive optics (MCAO) in microscopes



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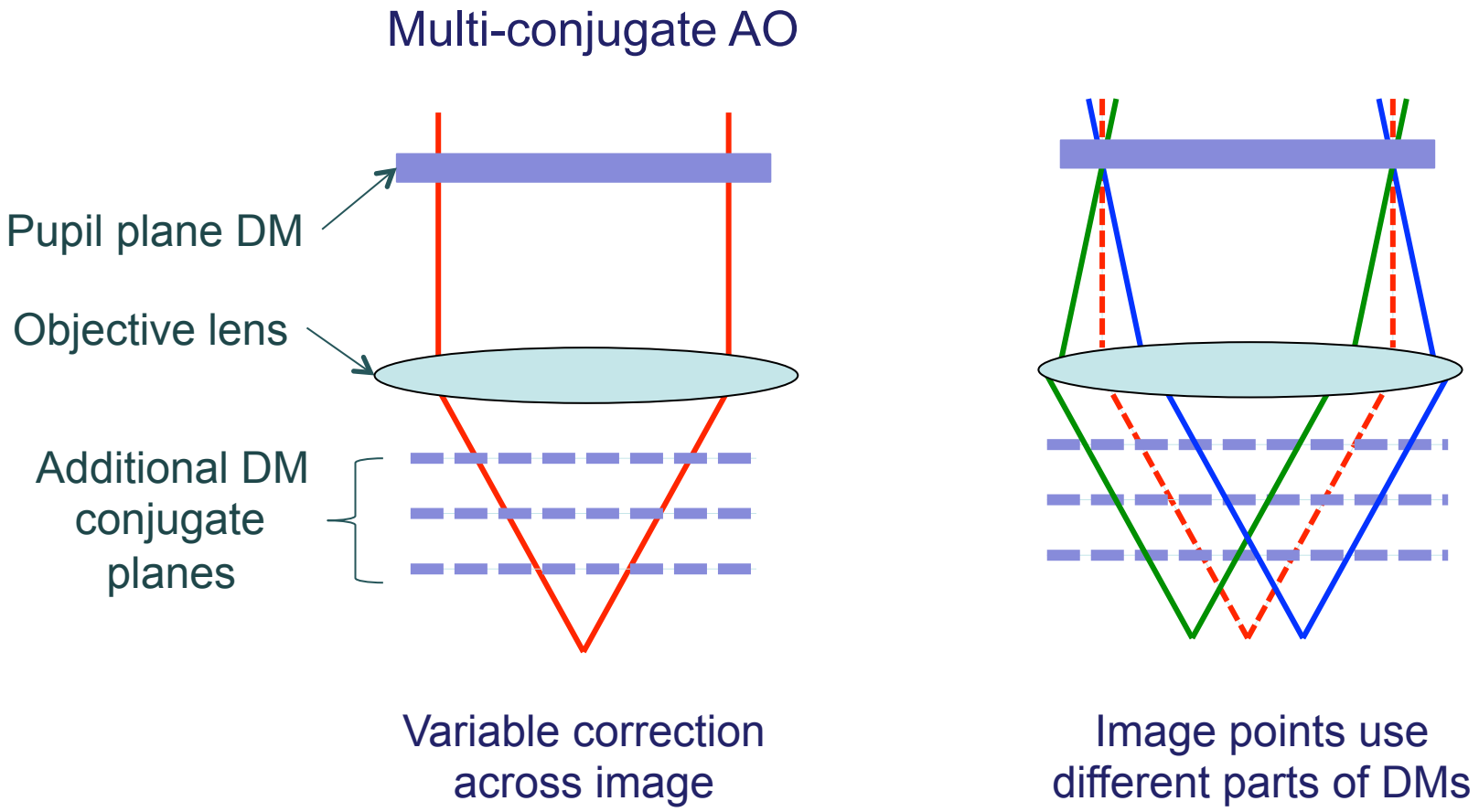
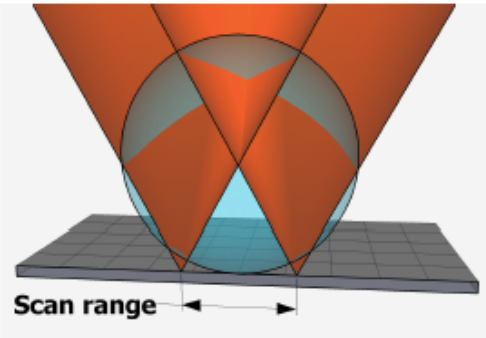
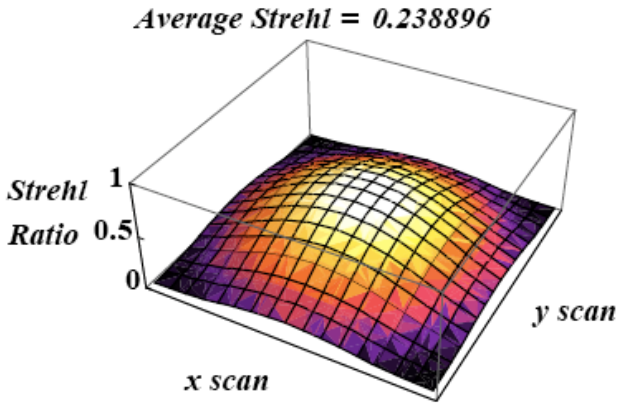


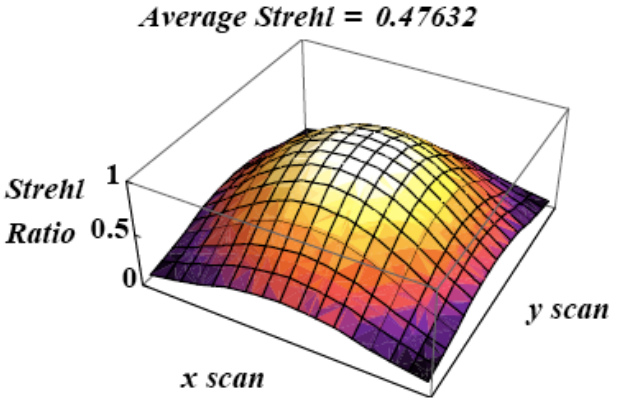
Correction of spatially varying aberrations

- MCAO model – spherical specimen before imaged plane
- DMs set by minimisation of mean square phase error across field

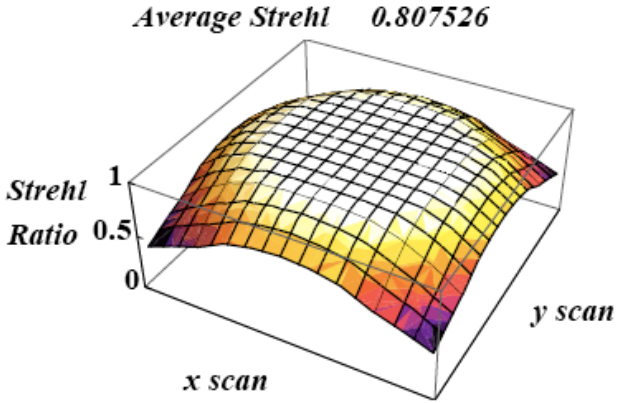




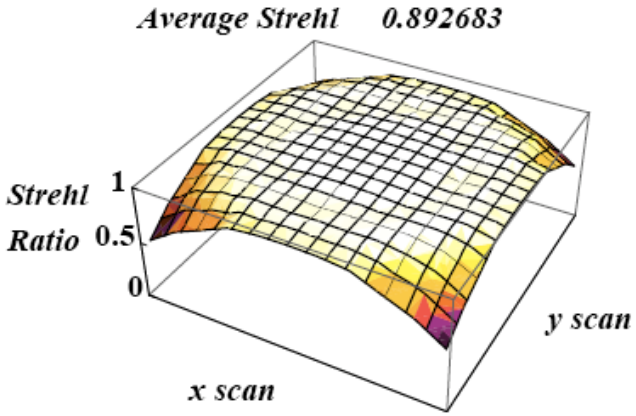
No correction



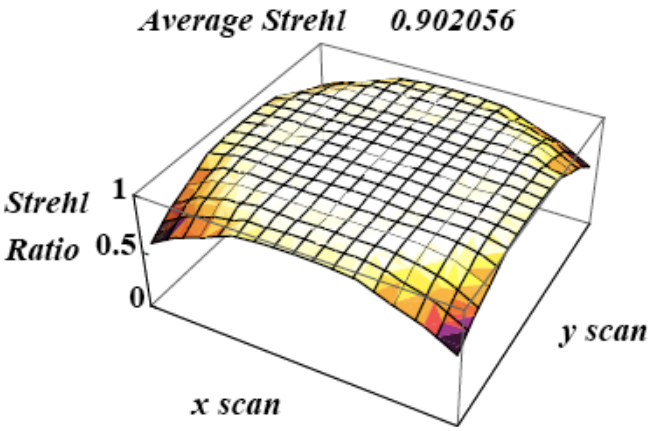
Pupil DM



Two DMs



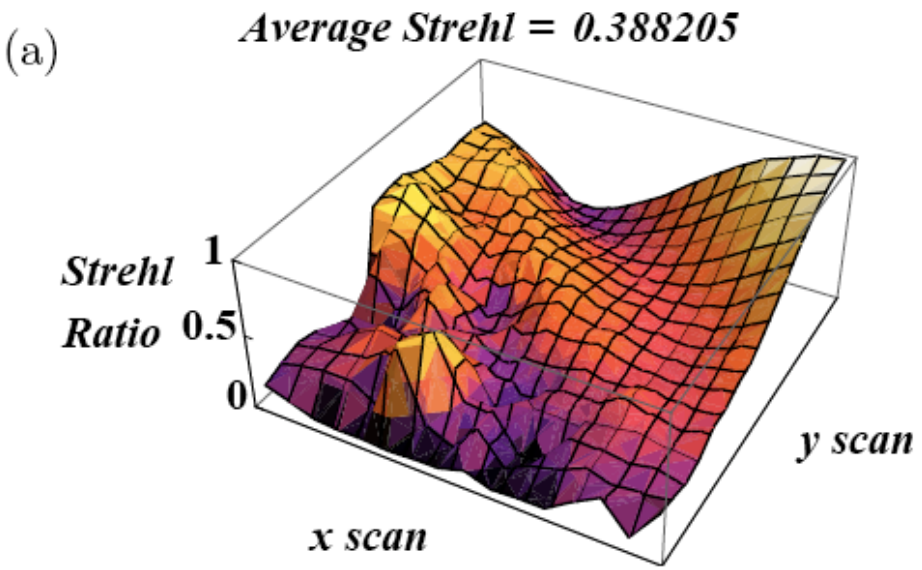
Three DMs



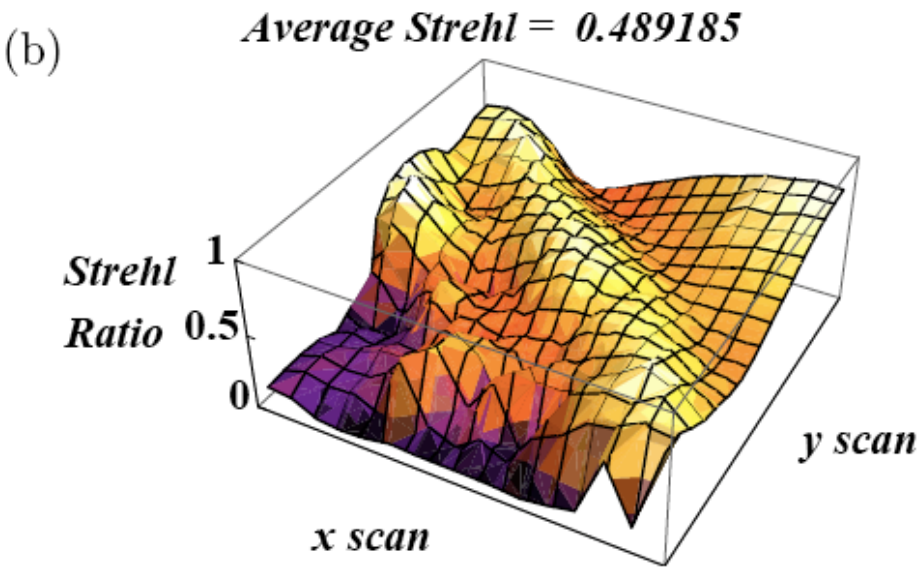
Four DMs

Correction of spatially varying aberrations

- 2D MCAO modelling – Real aberration data from C. Elegans specimen
- Equivalent imaged region 16 x 16 μm



No correction



Four DMs

Conclusion

- Adaptive optics is capable of correction complex aberrations of thick specimens in a wide range of microscope modalities
- Wave front sensor based systems / indirect methods using image feedback
- Sensorless AO methods for various forms of microscopy can be made efficient with careful choice of modes and metrics
- AO challenges are arising in each new application – size, complexity, speed, spatial variation

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Matthias Clausen, Christian Eggeling, Weatherall Institute of Molecular Medicine, Oxford

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- SAOT, FAU Erlangen-Nürnberg

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