Aberrations and adaptive optics for biomedical microscopes

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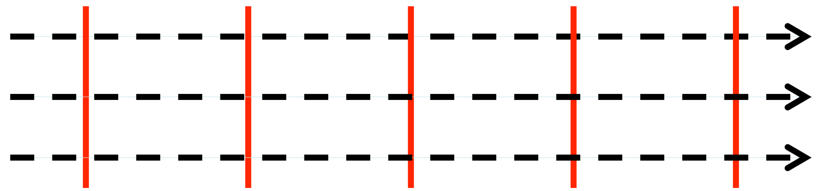
Outline

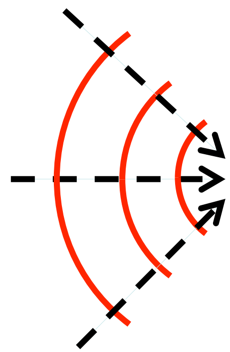
- Rays, wave fronts and aberrations
- Wave front control and aberration correction
- Adaptive optics for microscopes

Rays, wave fronts and aberrations

- Light propagation can be considered in terms of rays or wave fronts



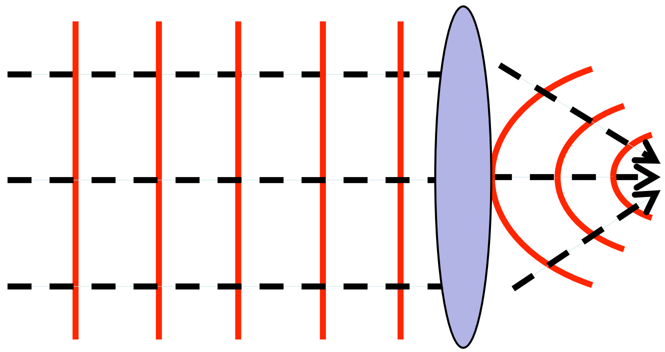
- In the collimated beam, the rays indicate the direction of propagation.
- Plane wave fronts are normal to beam direction and propagate along rays



- In a focussed beam, the rays meet at a point.
- The spherical wave fronts converge on the same point

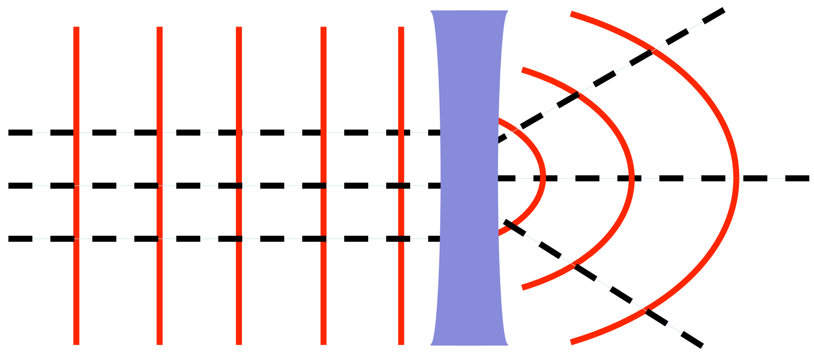
Operation of a lens

- A lens converts a collimated beam into a focussed beam



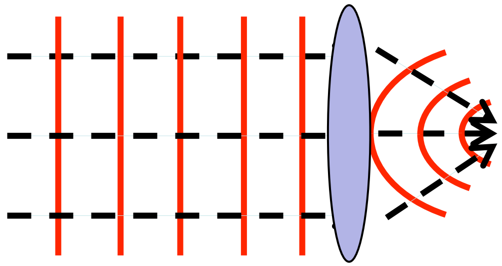
- Rays are refracted by the lens to meet at the focal point

- Wave fronts are delayed by the higher refractive index glass to create a converging spherical form

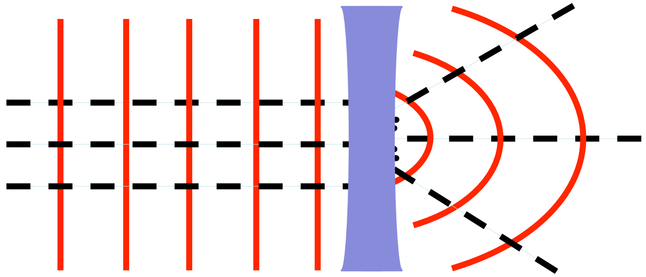


Operation of a lens

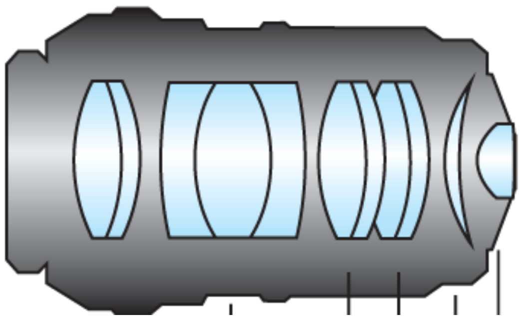
- Positive focal length lens



- Negative focal length lens



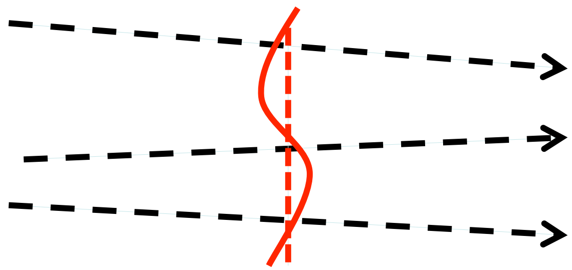
- Compound microscope objective



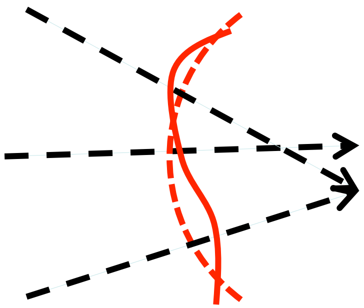
Optical aberrations

- Aberrations are the deviation of rays or wave fronts from their ideal form

- Collimated beam – deviations in local propagation direction



- Focussed beam – rays no longer meet at a point; wave fronts do not perfectly converge

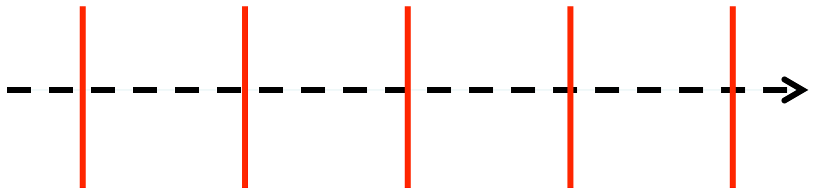


Optical phase

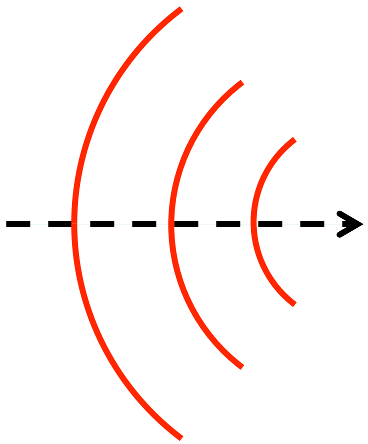
$$U(x,y) = A(x,y)\exp\left[i\phi(x,y)\right]$$

- Variations in phase $\phi(x,y)$ describe the shape of optical wave fronts

- For plane wave (collimated beam) the wave fronts are flat, $\phi(x,y)$ is a planar function



- For a converging wave (focussed beam) the wave fronts are spherical, $\phi(x,y)$ describes a spherical surface

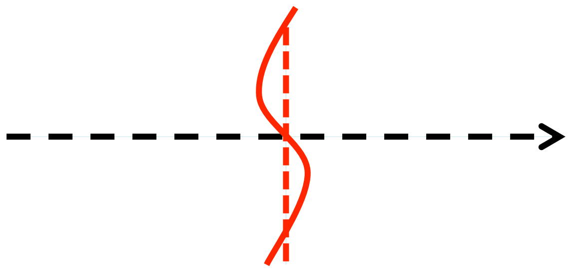


Optical aberrations

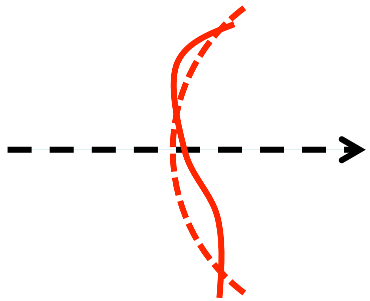
$$U(x,y) = A(x,y)\exp\left[i\phi(x,y)\right]$$

- Phase function $\phi(x,y)$ can also describe the deviation in shape of optical wave fronts from a reference – definition of aberration

- Planar reference surface

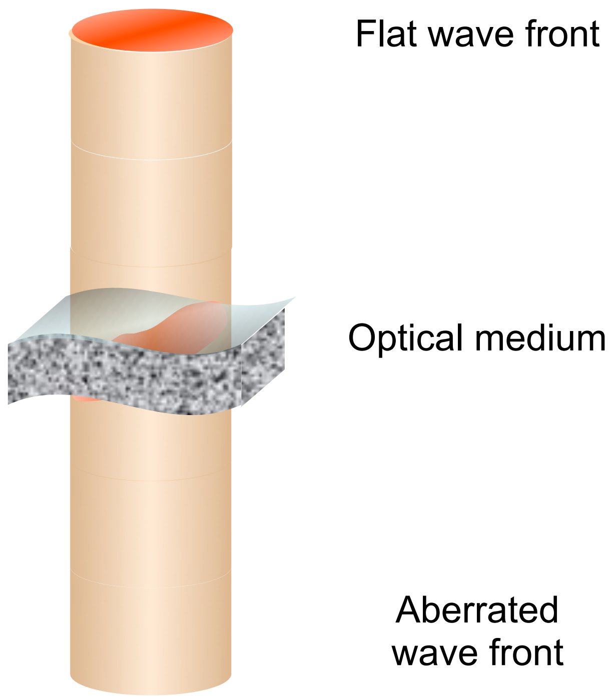


- Spherical reference surface



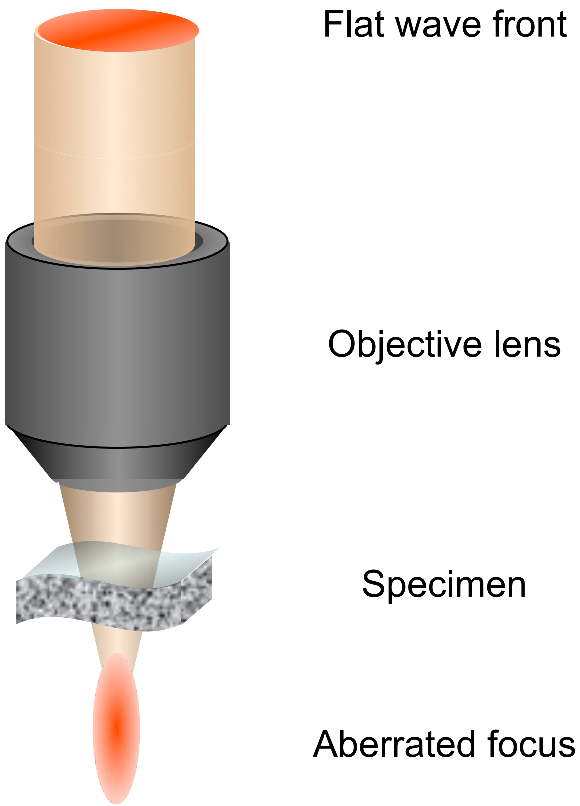
Optical aberrations

- Light propagates as wave fronts
- Speed dependent on refractive index
- Spatial variation in optical properties – distortion of wave front
- Wave front distortion – aberration



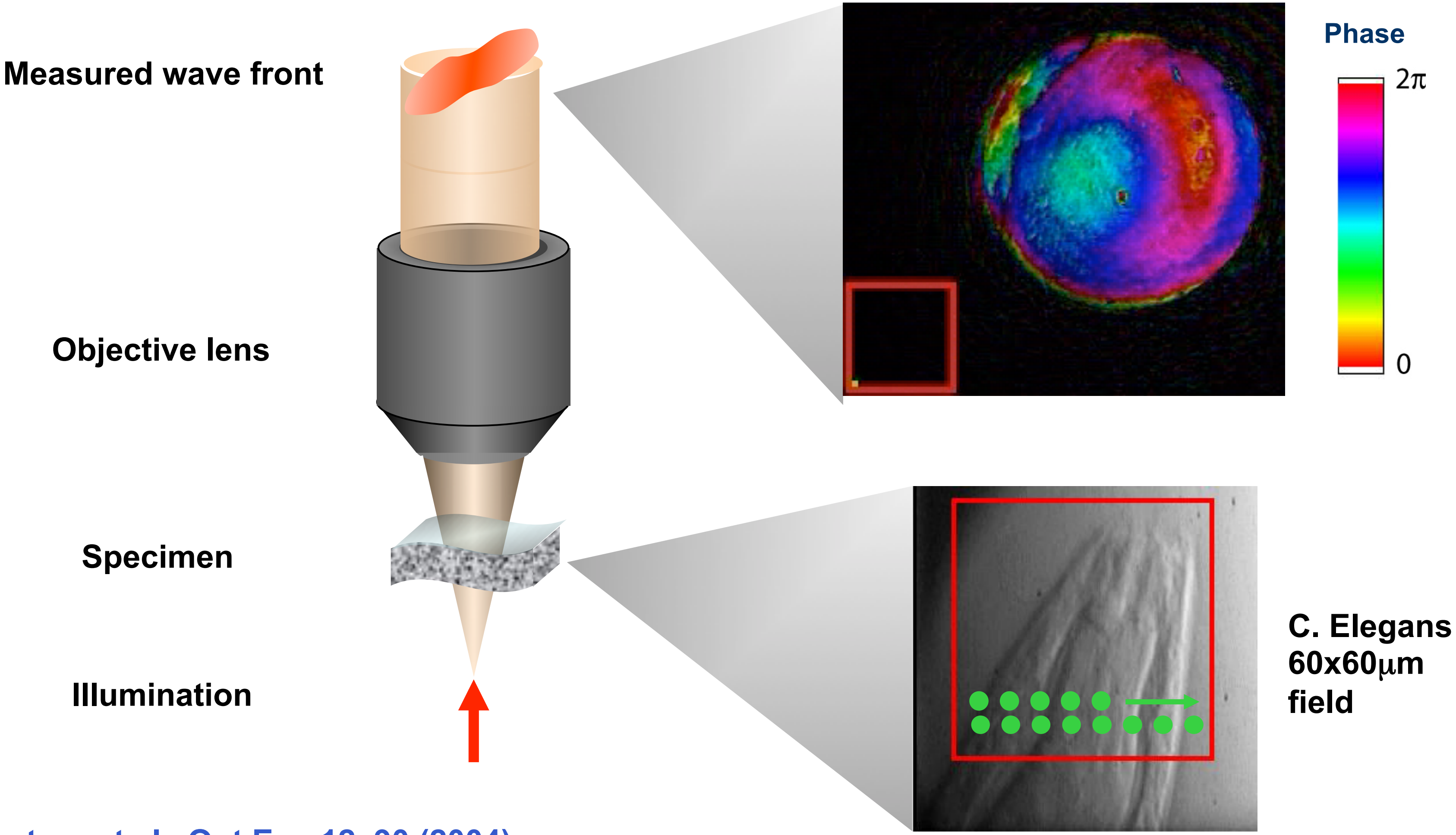
Aberrations in microscopes

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



Specimen-induced aberrations

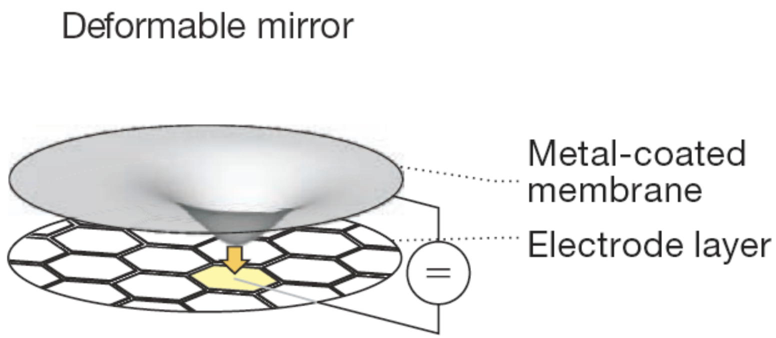
- Variations of refractive index throughout specimen structure
- Measurement of phase aberrations through interferometry at λ = 633nm

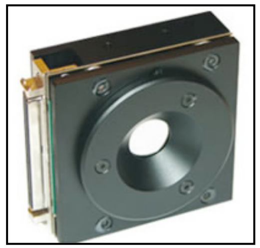


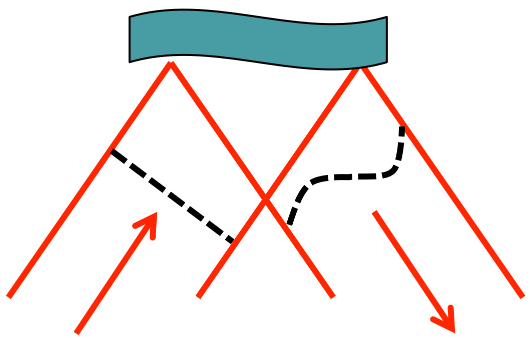
Schwertner et al., Opt Exp 12, 90 (2004)

Correction of optical aberrations

- Introduce an equal but opposite (conjugate) aberration
- Use a dynamic optical element – adaptive optics







Correction of optical aberrations

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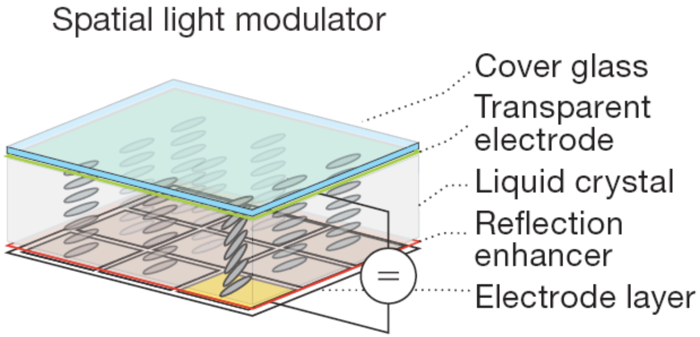
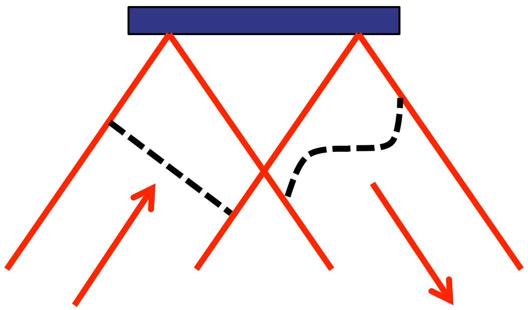
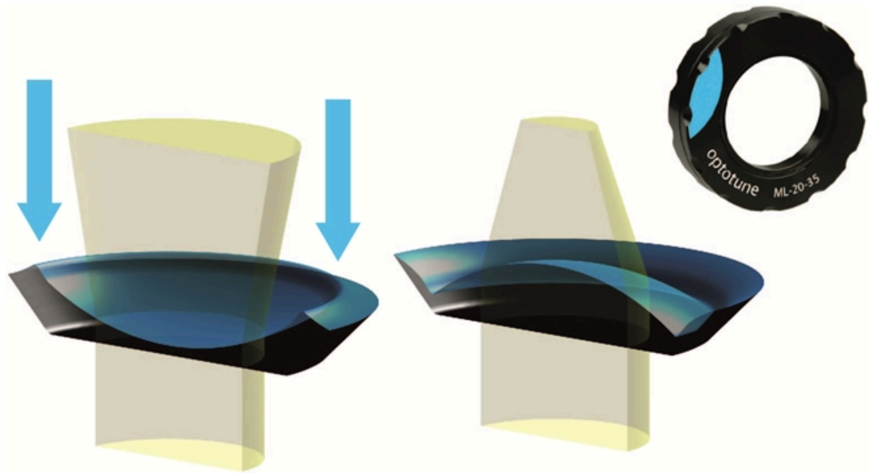


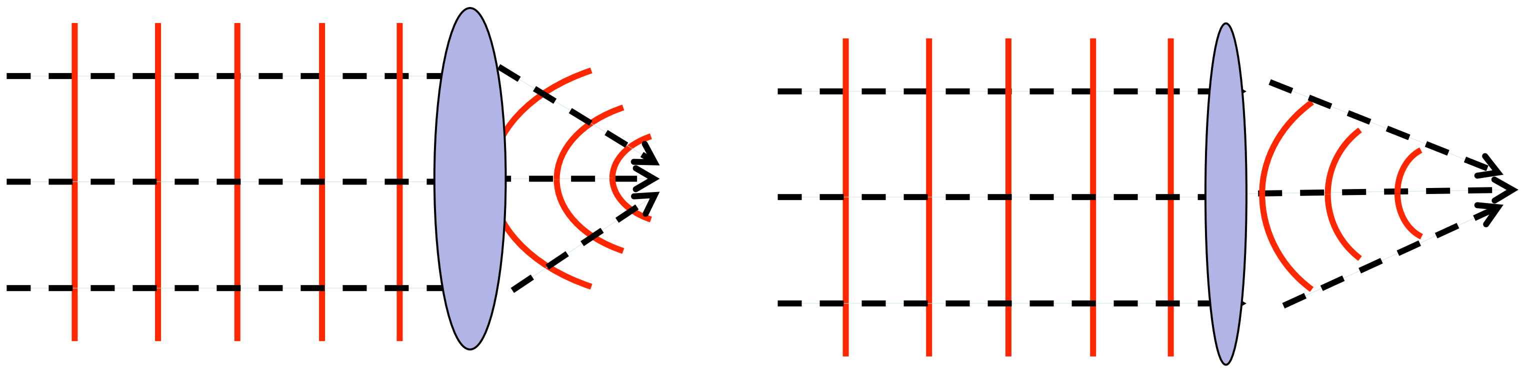


Correction of optical aberrations

- Introduce an equal but opposite (conjugate) aberration
- Use a dynamic optical element – adaptive optics

Tunable lens



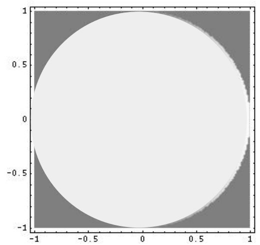


Aberrations as modes

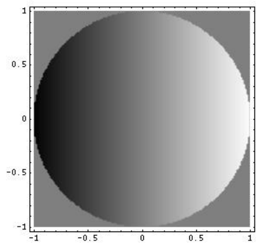
- Aberrations can be complex functions – represent as series of modes
- Zernike polynomials as example modal basis set (for circular beams)

$$\phi(r,\theta) = \sum_{i=1}^{N} a_i Z_i(r,\theta)$$

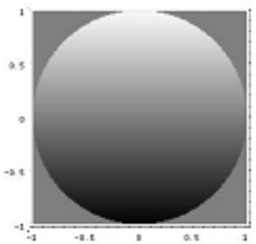
- Aberration described by set of coefficients a_i



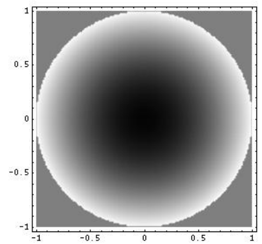
$Z_1(r, \theta)$ – piston



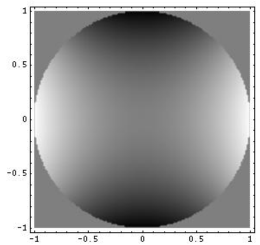
$Z_2(r, \theta)$ – tip



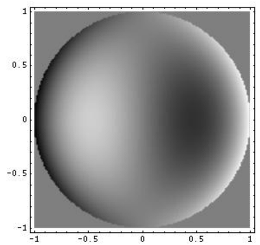
$Z_3(r, \theta)$ – tilt



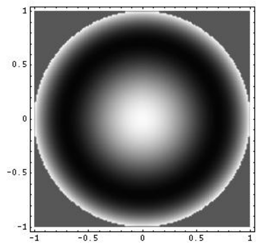
$Z_4(r, \theta)$ – defocus



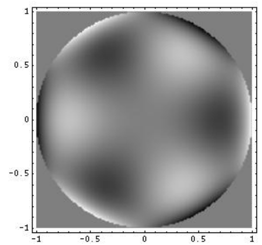
$Z_5(r, \theta)$ – astigmatism



$Z_7(r, \theta)$ – coma



$Z_{11}(r, \theta)$ – spherical



$Z_{18}(r, \theta)$ – trefoil

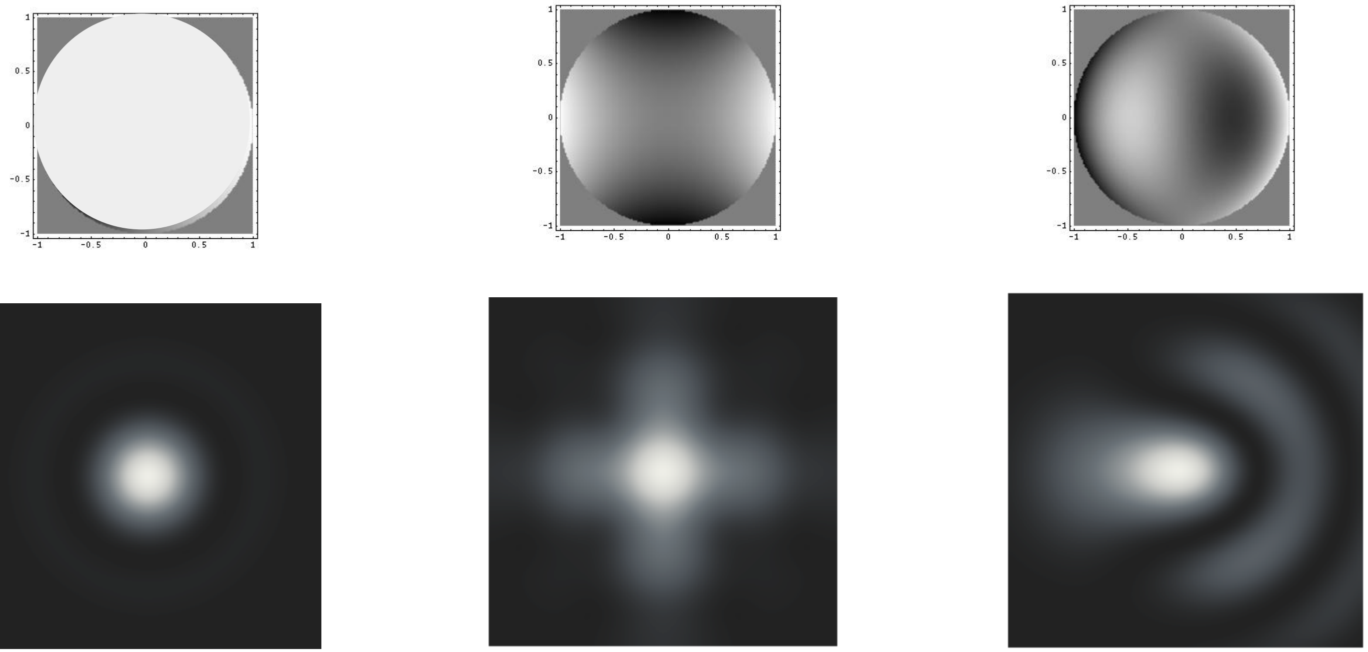
Aberrations in the focus

- Effects of aberrations on the focal spot intensity

No aberration | **Astigmatism** | **Coma**

Pupil phase

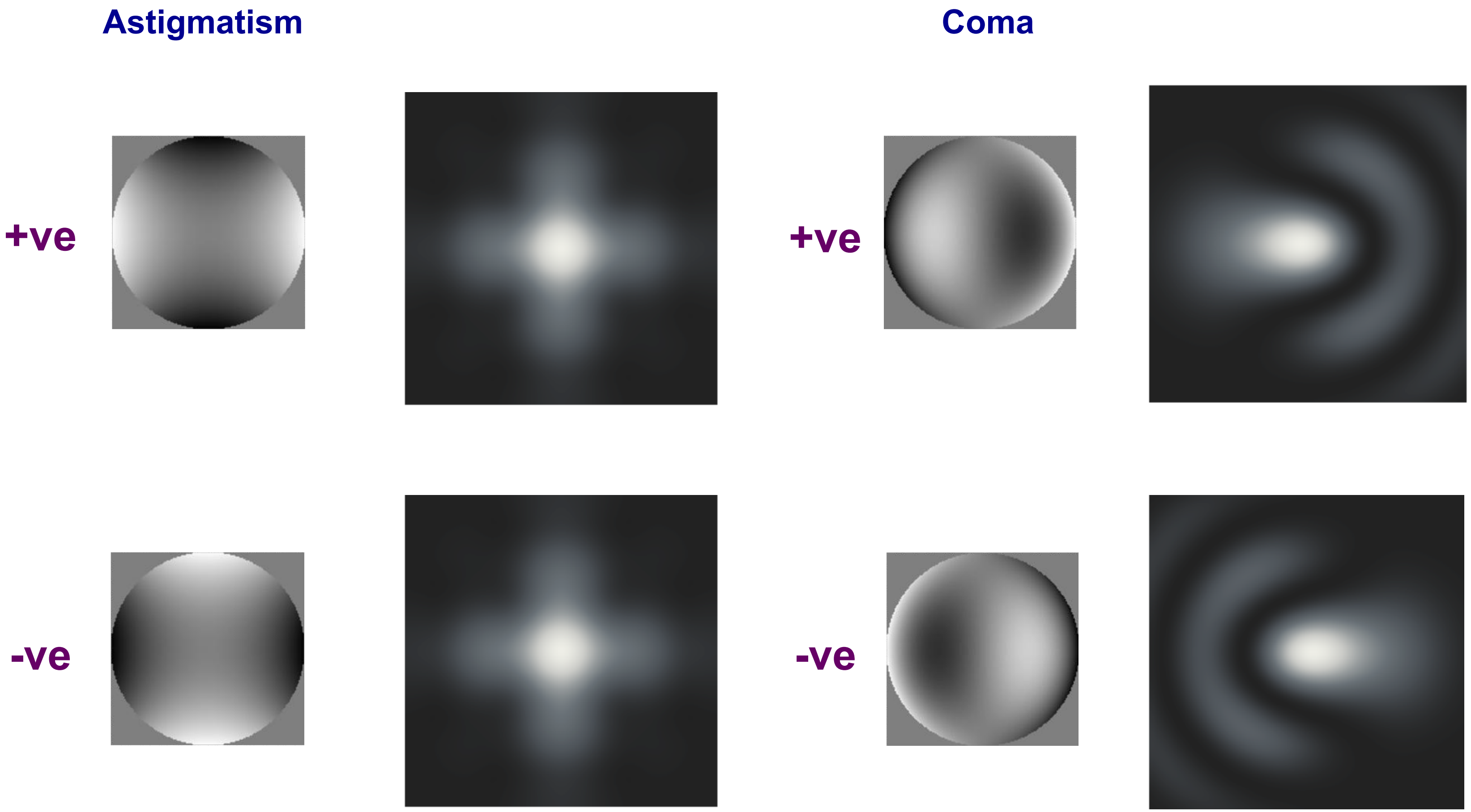
Focal intensity



- Some information about aberrations can be retrieved from focal spot

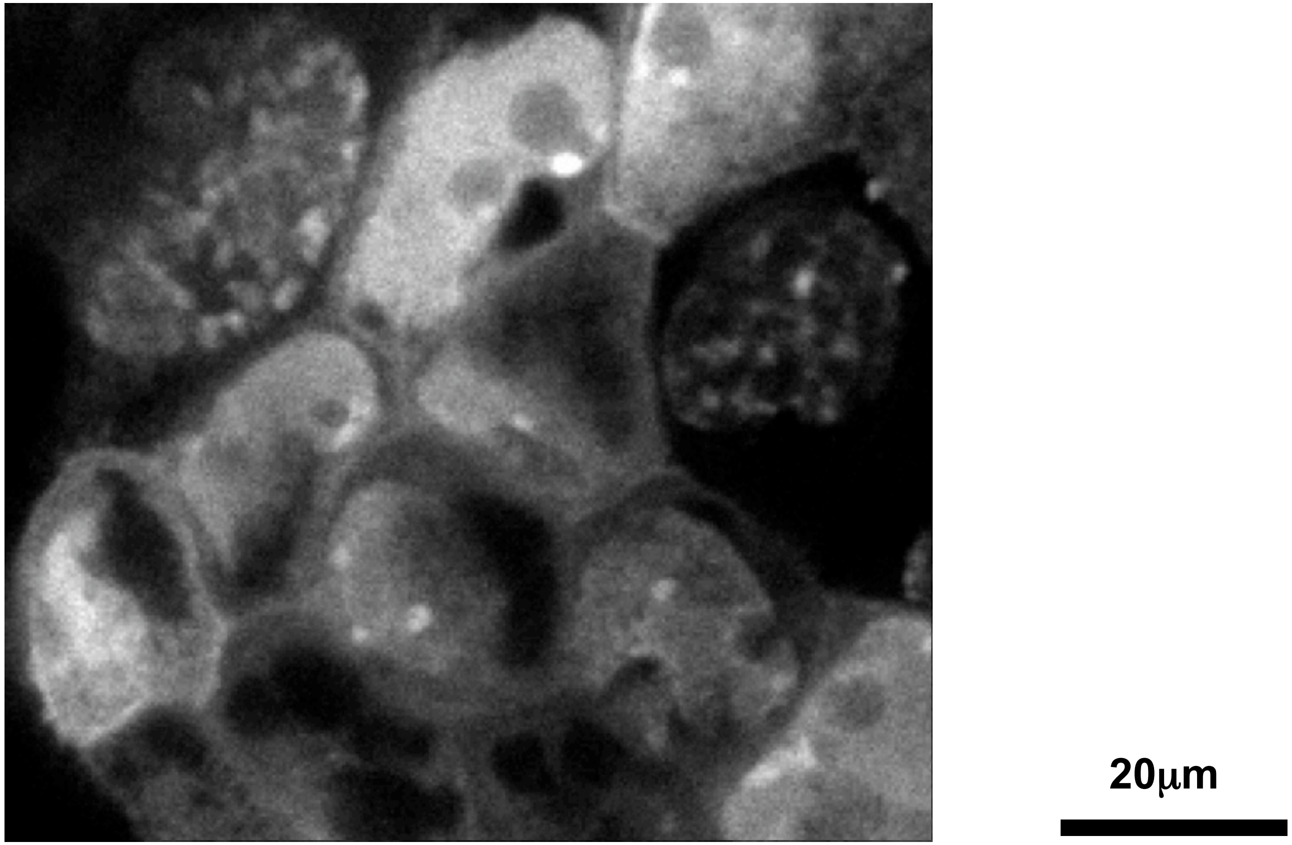
Aberrations in the focus

- Pupil phase cannot be obtained unambiguously from focal intensity



Effects of aberrations in microscopy

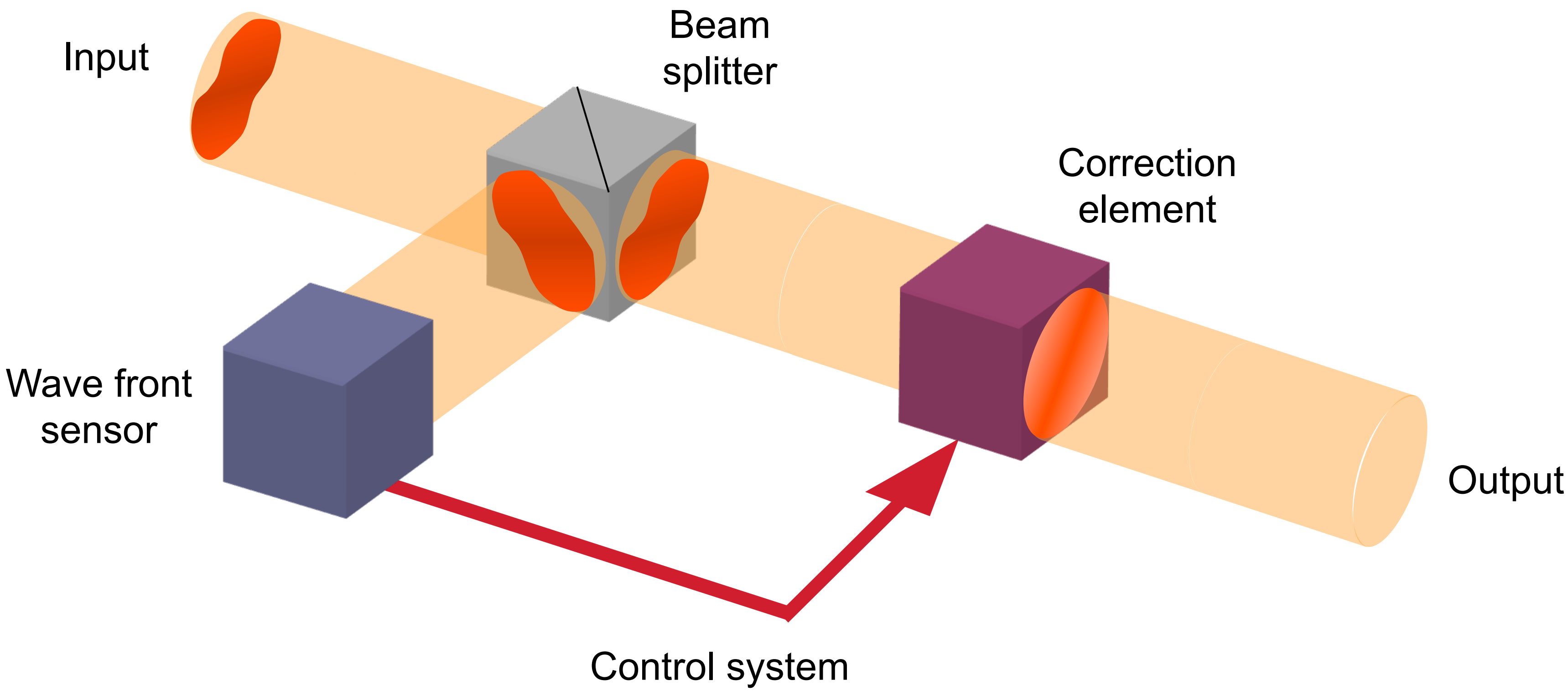
- Two-photon excitation fluorescence microscope: DAPI/GFP labelled mouse embryo
- Images show correction of specimen induced aberrations
- Aberrations cause loss of resolution and contrast



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

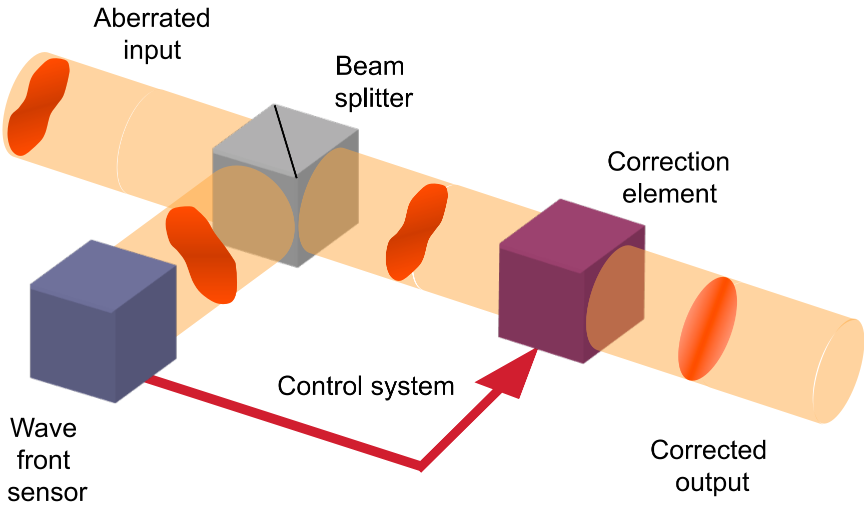
Adaptive optics

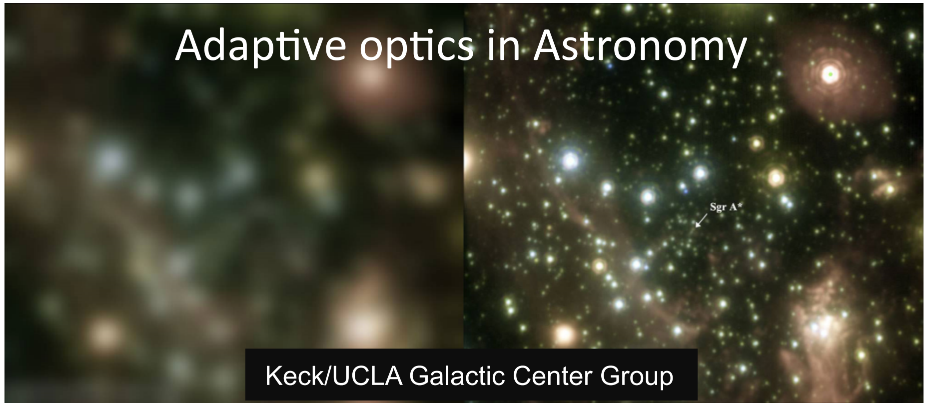
- Principle of a traditional adaptive optics system



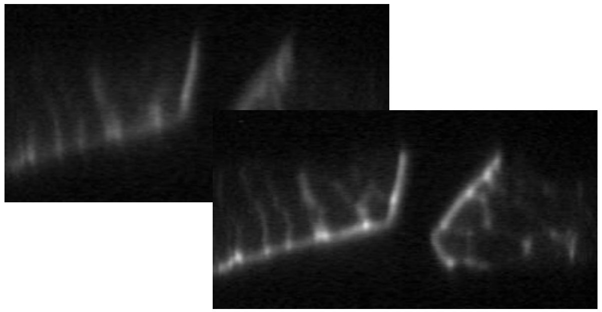
Adaptive optics for high resolution microscopy

Using deformable mirror technology from astronomy to improve microscope images by removing optical aberrations

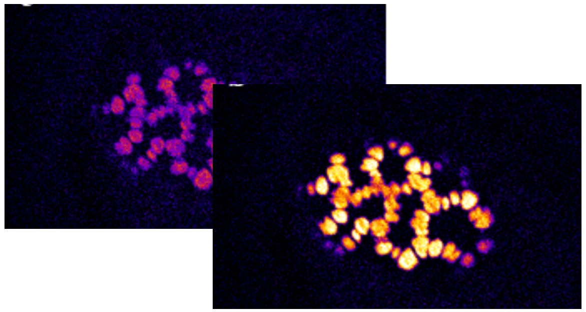




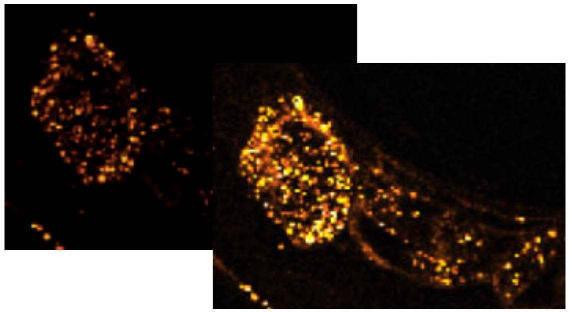
Applications in microscopy



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



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



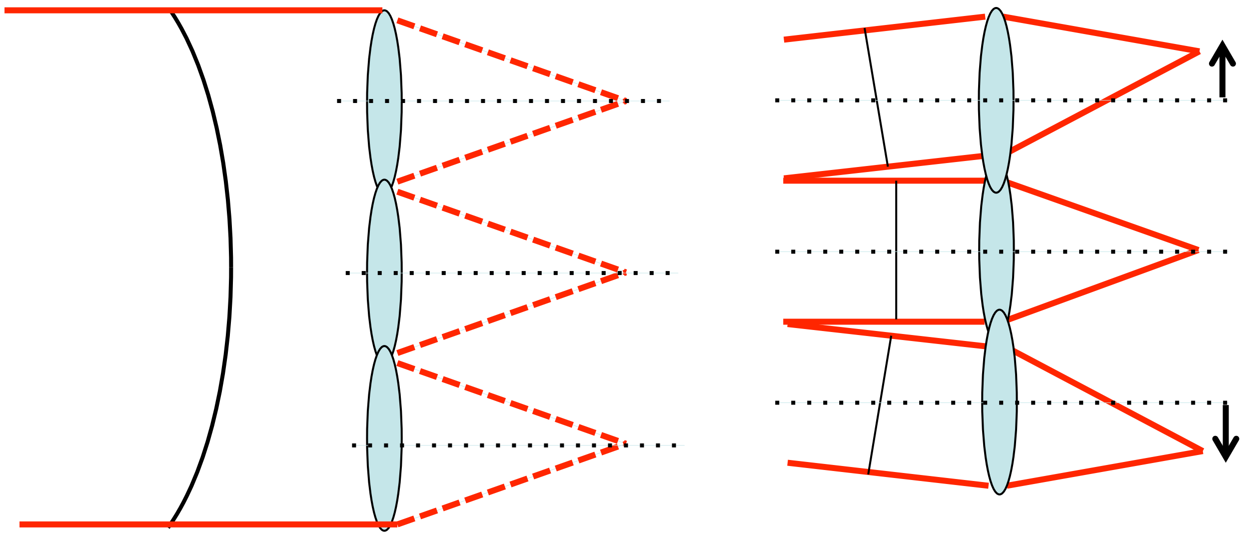
Third harmonic microscopy
Jesacher et al., Opt Lett 34, 3154 (2009)

Wave front sensors

- Most common wave front sensor – Shack Hartmann

$$\Delta x \propto \frac{\partial \phi}{\partial x}$$

- Shift in lenslet focus measured on camera gives local phase gradient



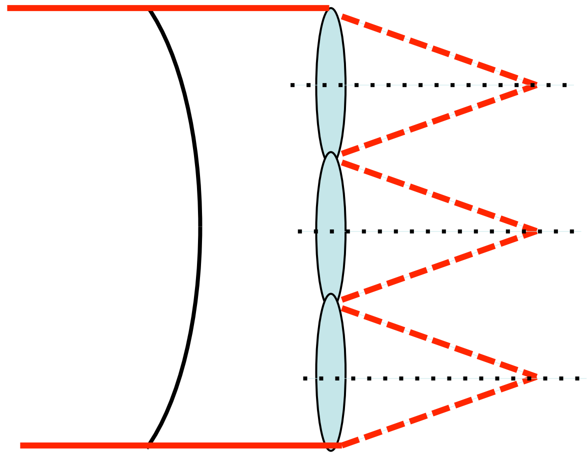
Δx, Δy shifts

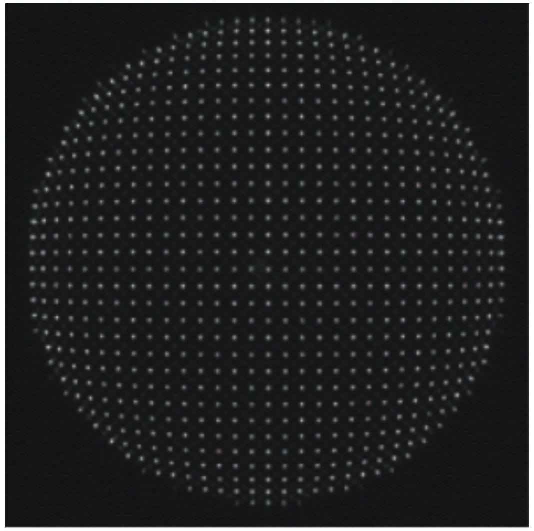
wave front gradients

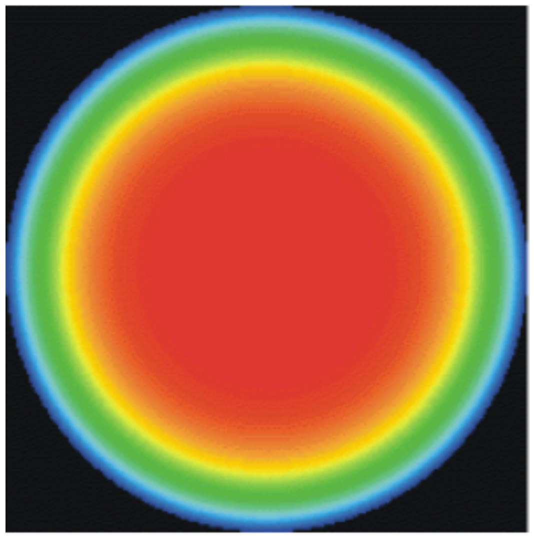
reconstructed wave front

Wave front sensors

- Most common wave front sensor – Shack Hartmann

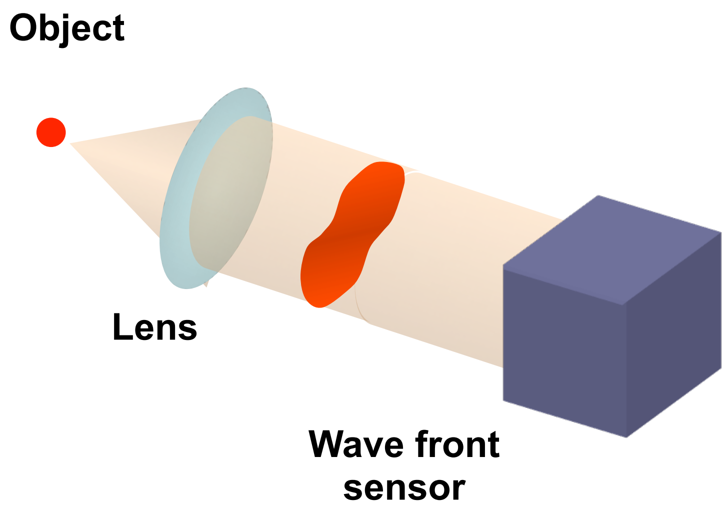




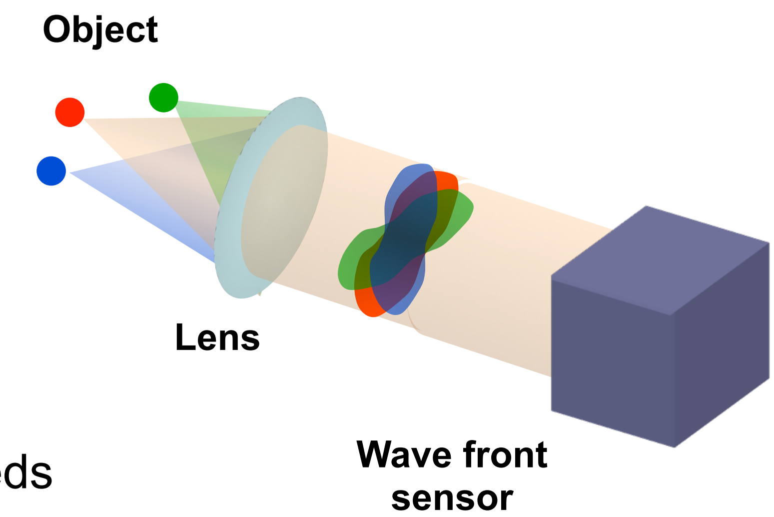


Wave front sensing

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



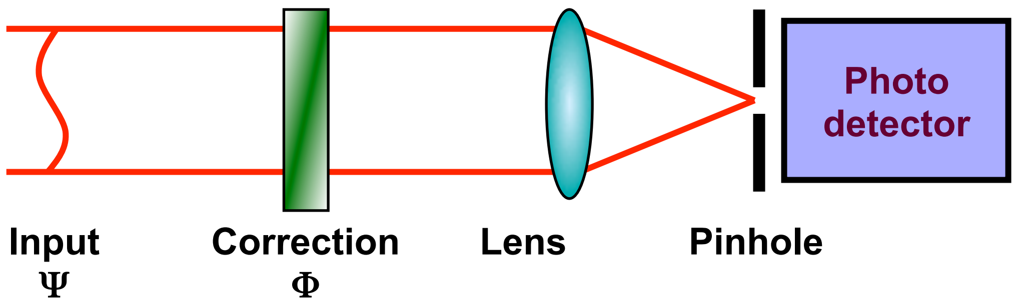
- Wave front sensing in general imaging systems
  - 2D or 3D object
  - Superposition of wave fronts
  - Out-of-focus light



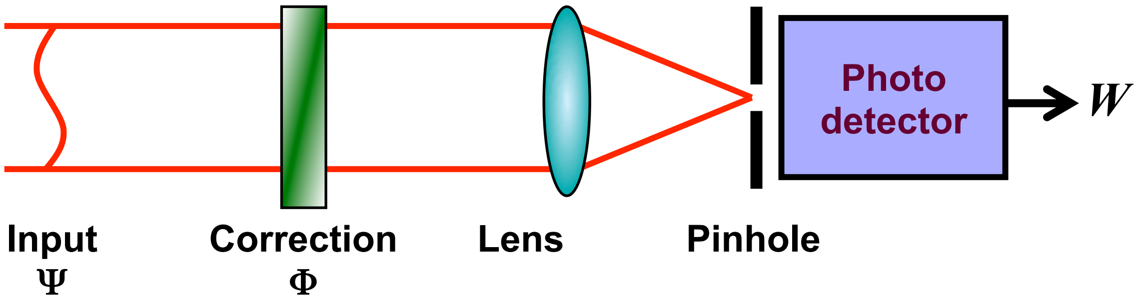
- Wave front sensing in 3D microscopy needs method to exclude out-of-focus light

Sensing without wave front sensor

- Phase information is encoded (somehow) in the intensity of the focal spot
- Phase can be in principle be found from a set of focal intensity images
- Images consist of many intensity measurements (one per pixel)
- Interesting question:
  - Is it possible to measure the phase using a single detector?

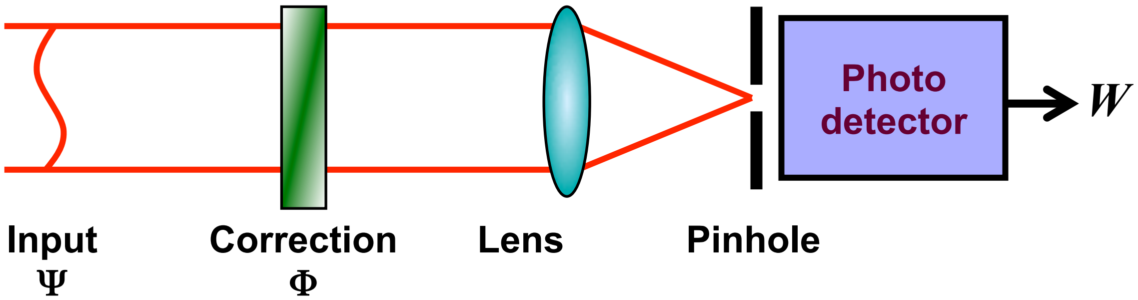


Sensorless system



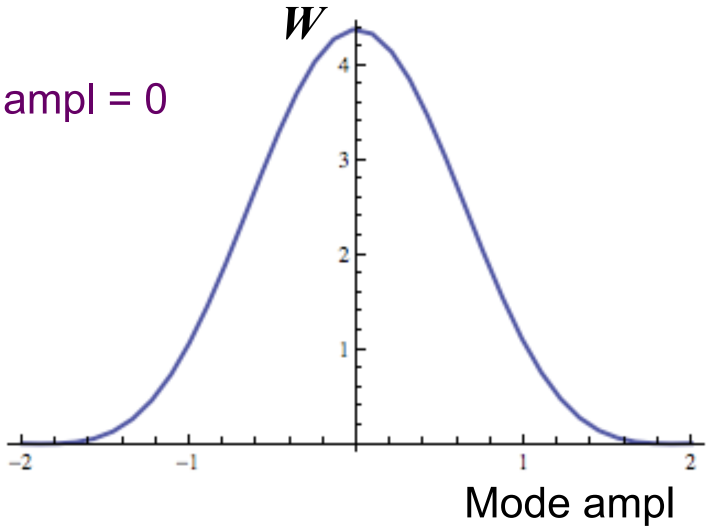
- Photodetector signal W measures on-axis intensity (centre of focal spot)
- We control correction Φ to attempt to compensate input aberration Ψ
- Corrected input wavefront = maximum photo-detector signal
- Example with astigmatism:

Sensorless system

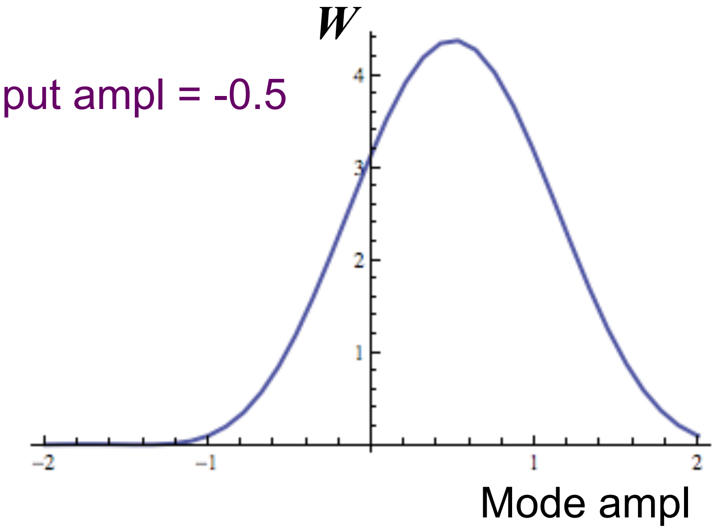


- Example with astigmatism:

Input ampl = 0

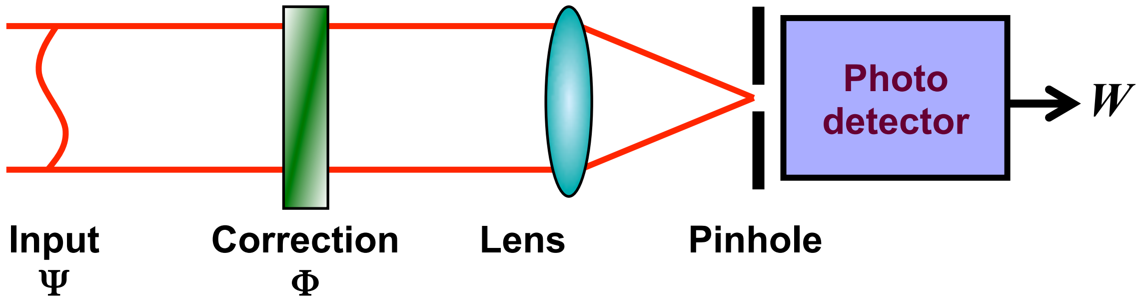


Input ampl = -0.5



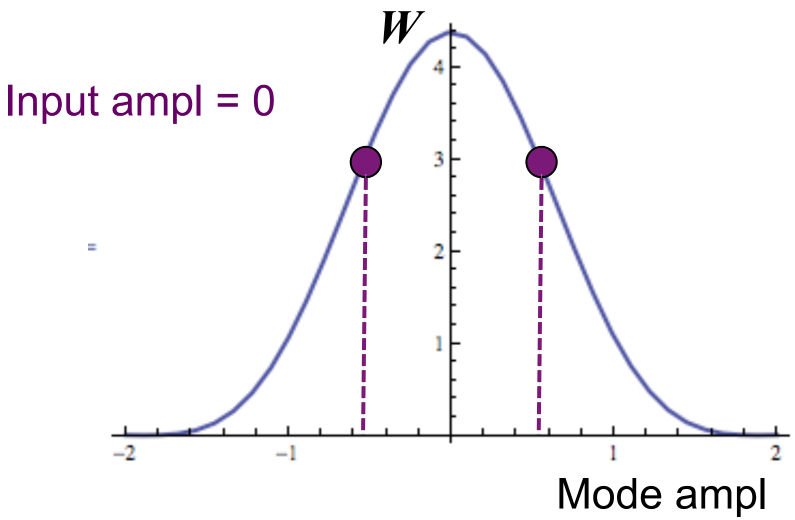
- Possible strategy – cycle through possible correction amplitudes and choose the one with highest signal

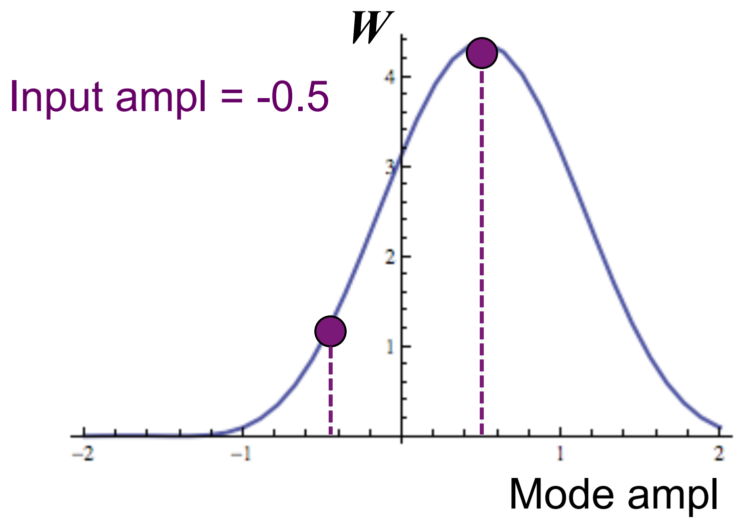
Sensorless system



- Possible strategy – cycle through possible correction amplitudes and choose the one with highest signal
  - Problem – many measurements required

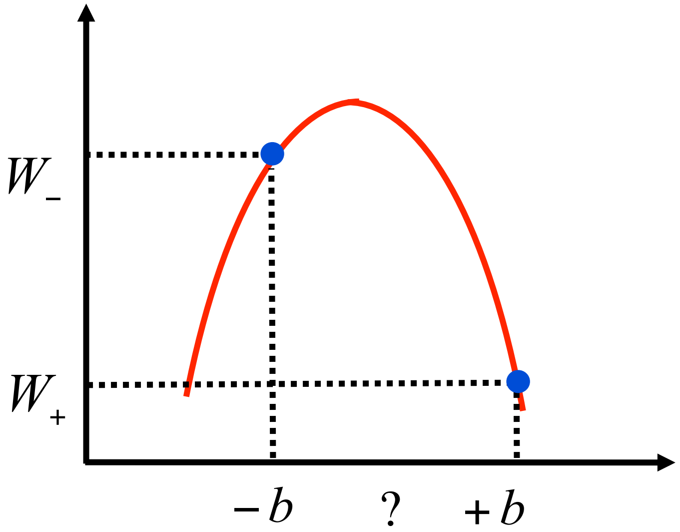
- Shape of function is known in advance – fewer measurements sufficient





Finding the peak

- Find peak of function with two measurements
- Signal W as function of aberration amplitude a is quadratic: $W(a) \approx c\left(1-a^2\right)$
- One variable parabolic maximisation – simple algorithms

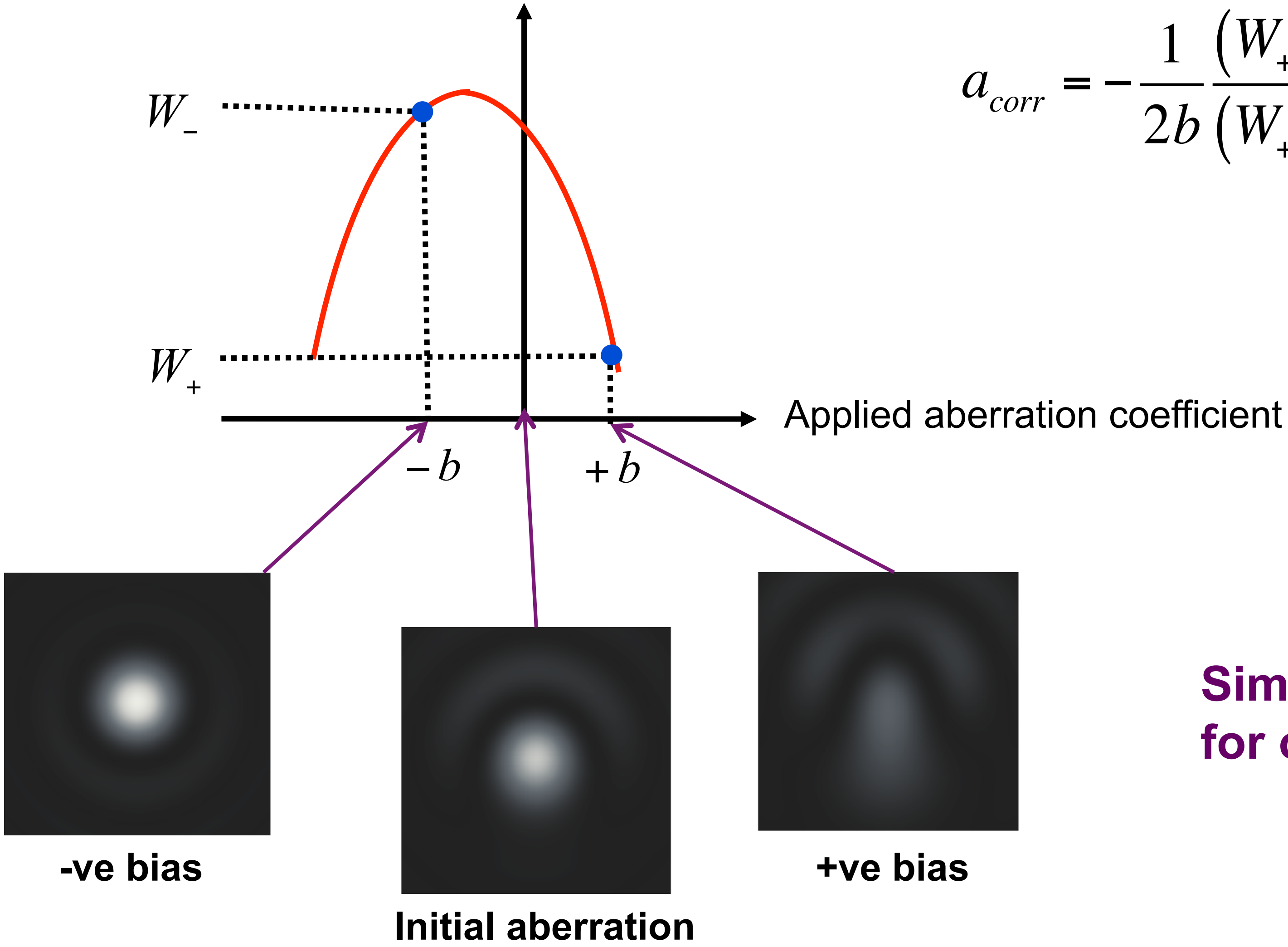


$$a_{corr} = -\frac{1}{2b}\frac{\left(W_+ - W_-\right)}{\left(W_+ + W_-\right)}$$

- Start with input aberration
- Add positive amount of mode $+b\, Z_i$ and measure W_+
- Add negative amount of mode -$b\, Z_i$ and measure W_-
- Calculate correction aberration

Finding the peak

Example using coma mode



$$a_{corr} = -\frac{1}{2b}\frac{(W_+ - W_-)}{(W_+ + W_-)}$$

Simply repeat for other modes?

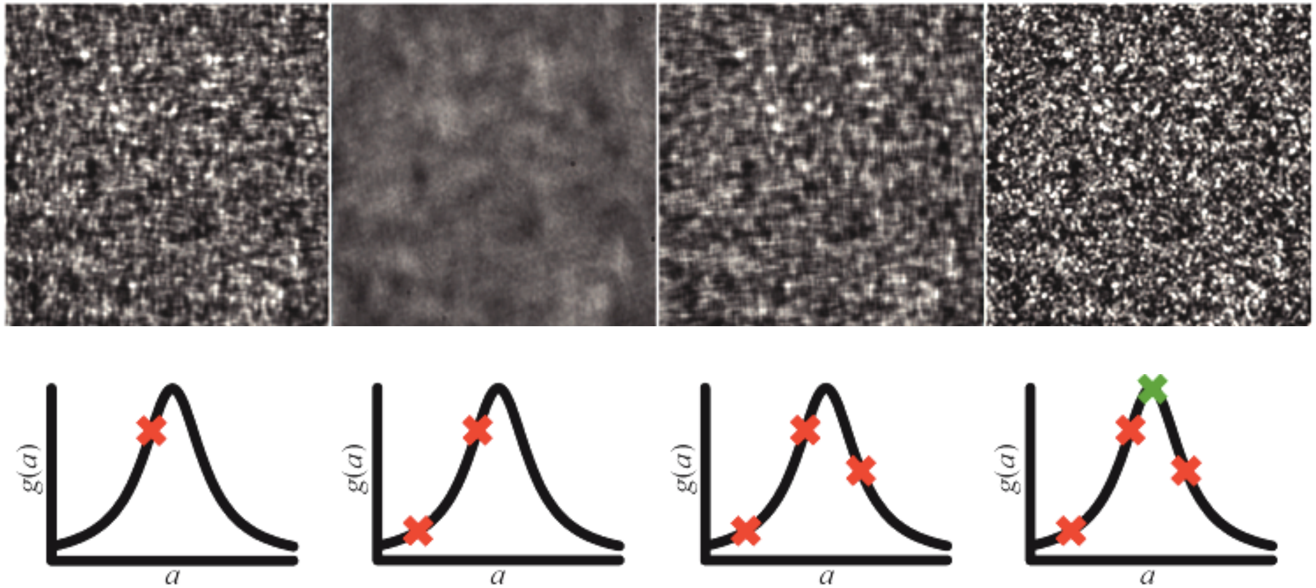
Image based adaptive optics

- 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

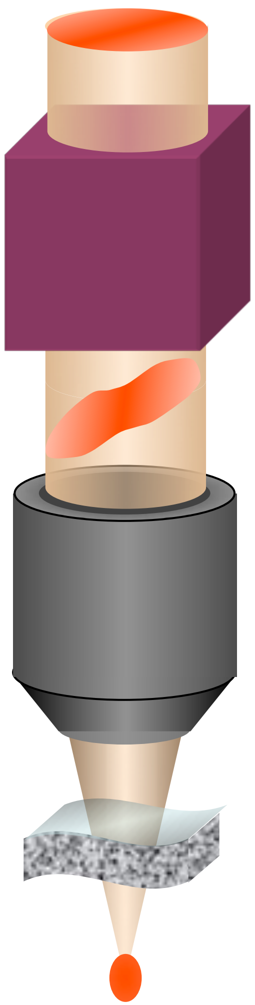
Applied aberration | Initial | Initial + b | Initial - b | Corrected

Images

Fitting



Indirect aberration measurement



Choose aberration
↓
Apply aberration
↓
Acquire image
↓
Calculate quality metric
↓
Estimate correction phase
↓
Apply correction

Aberration representation

Optimisation metric

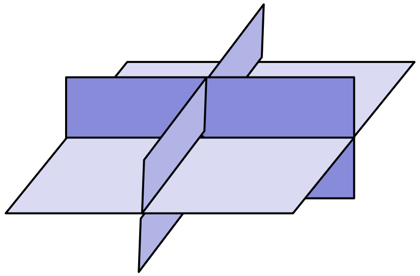
Estimator

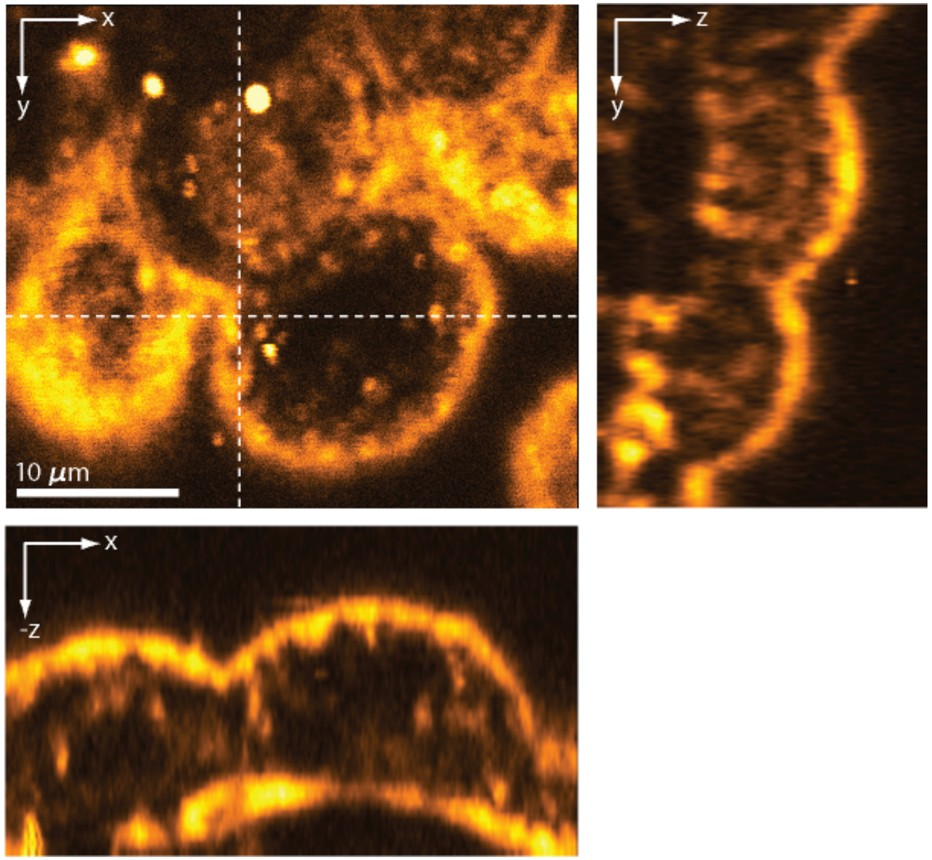
↓

Efficient correction procedure

Adaptive THG microscopy of embryos

- xyz stack of Third Harmonic Generation (THG) images of unlabelled mouse embryo – contrast from intrinsic optical properties



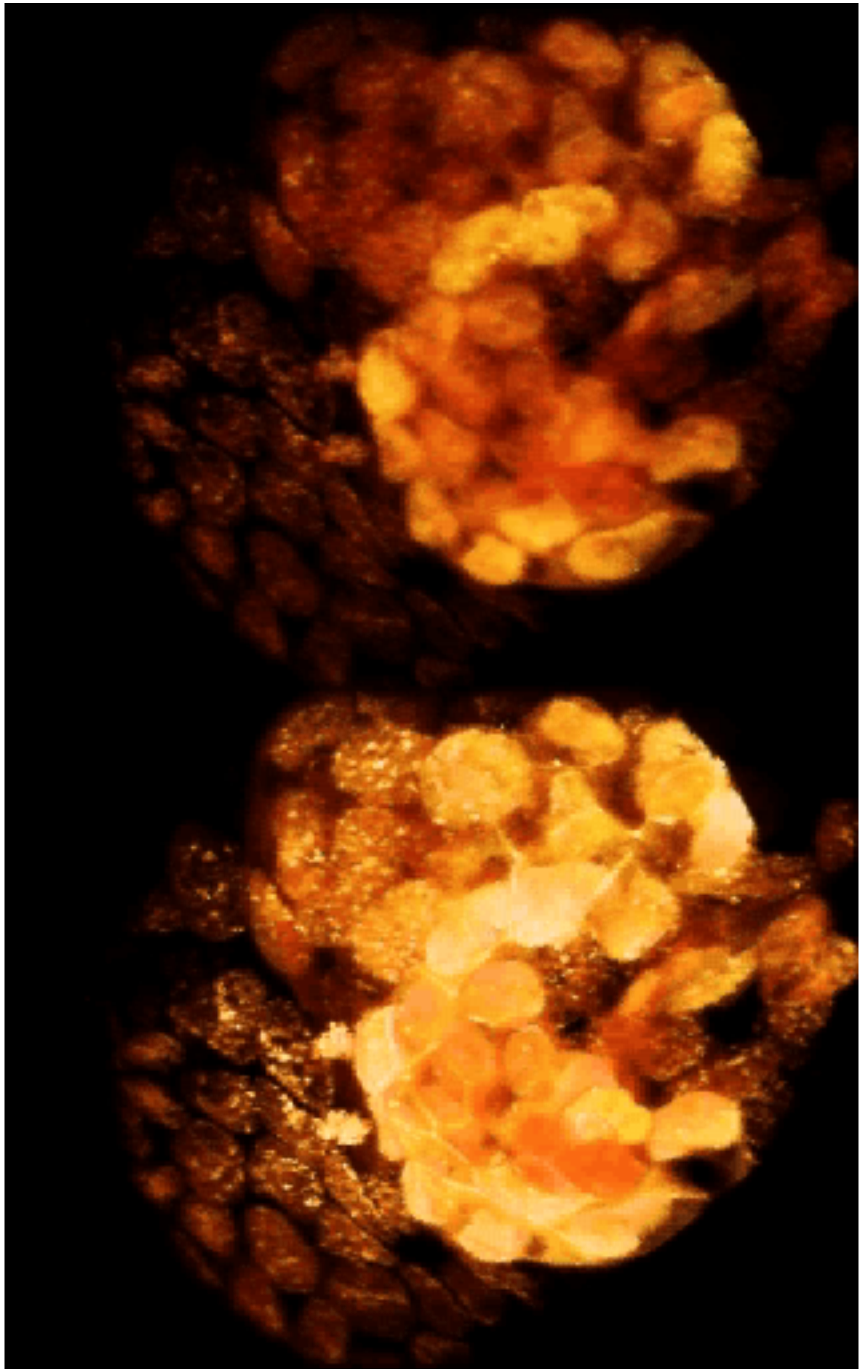


Jesacher et al., Opt Lett 34, 3154 (2009)

Adaptive optics in 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)

Conclusion

- Rays, wave fronts and aberrations
  - Relationship between rays and wave fronts
  - Operation of lenses
  - Aberrations as deviation from ideal wave front
- Wave front control and aberration correction
  - Deformable mirrors
  - Liquid crystal spatial light modulators
  - Adaptive lenses
- Adaptive optics for microscopes
  - Wave front sensors
  - Sensorless, image based adaptive optics