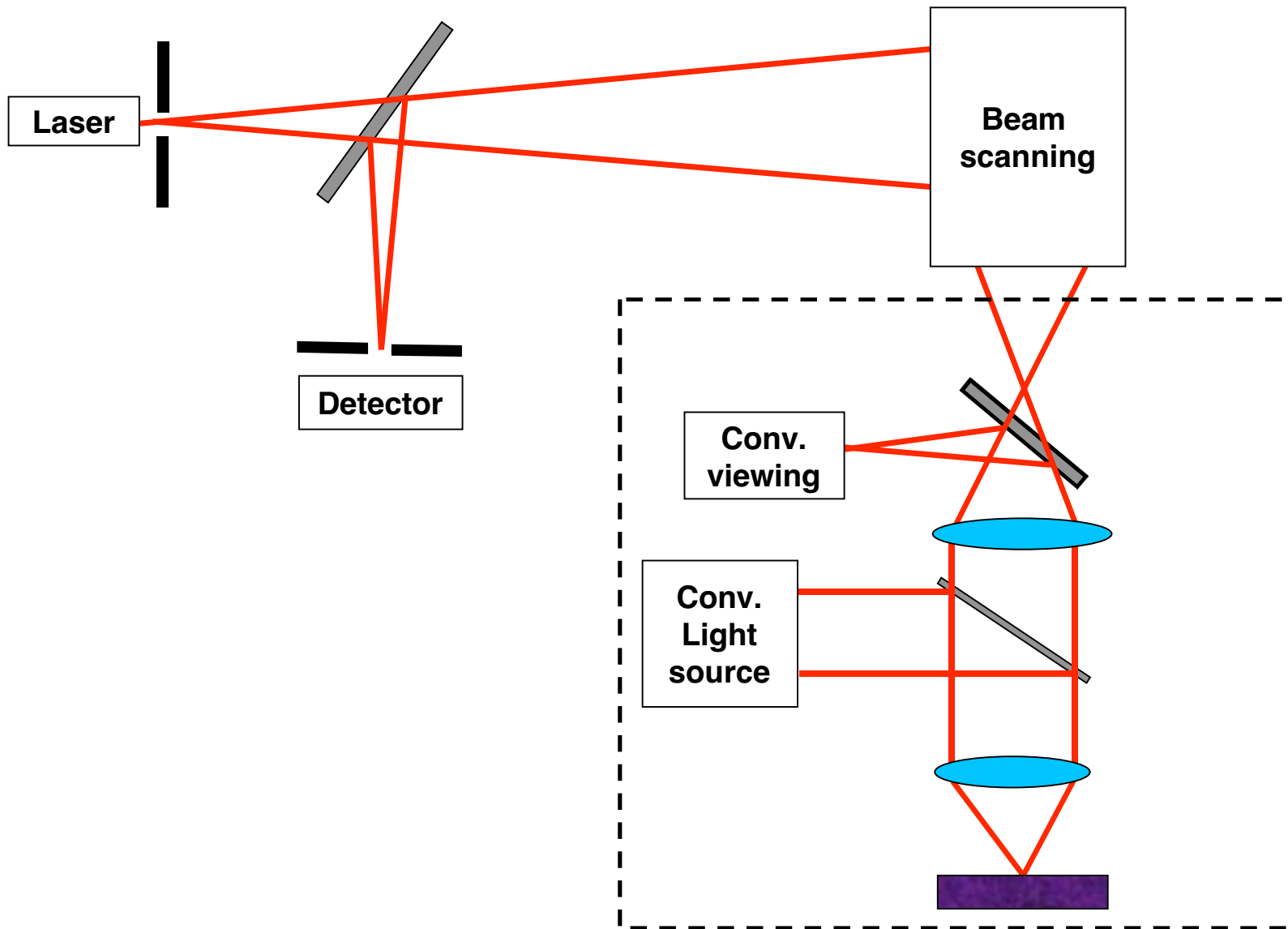


# **Adaptive optics in confocal microscopy**

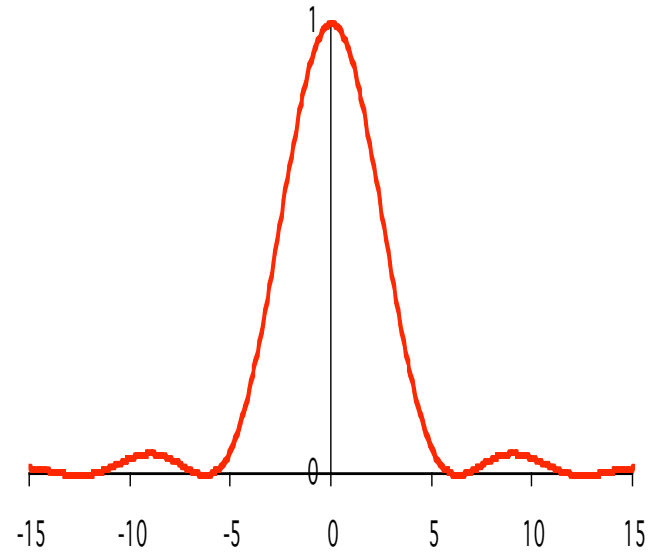
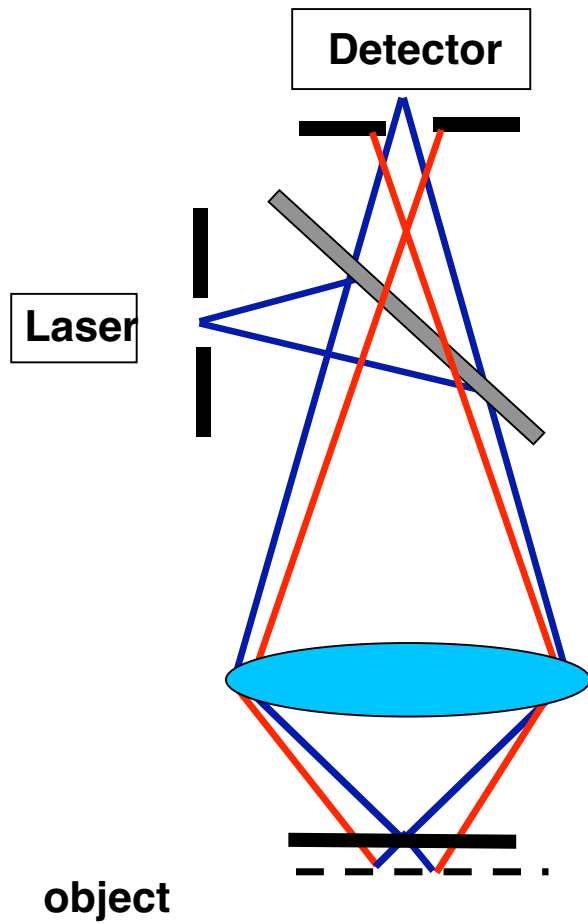
**Tony Wilson**  
**University of Oxford**

- The Confocal microscope**
- The aberration problem**
- Wavefront sensing**
- Wavefront generation**
- Examples**

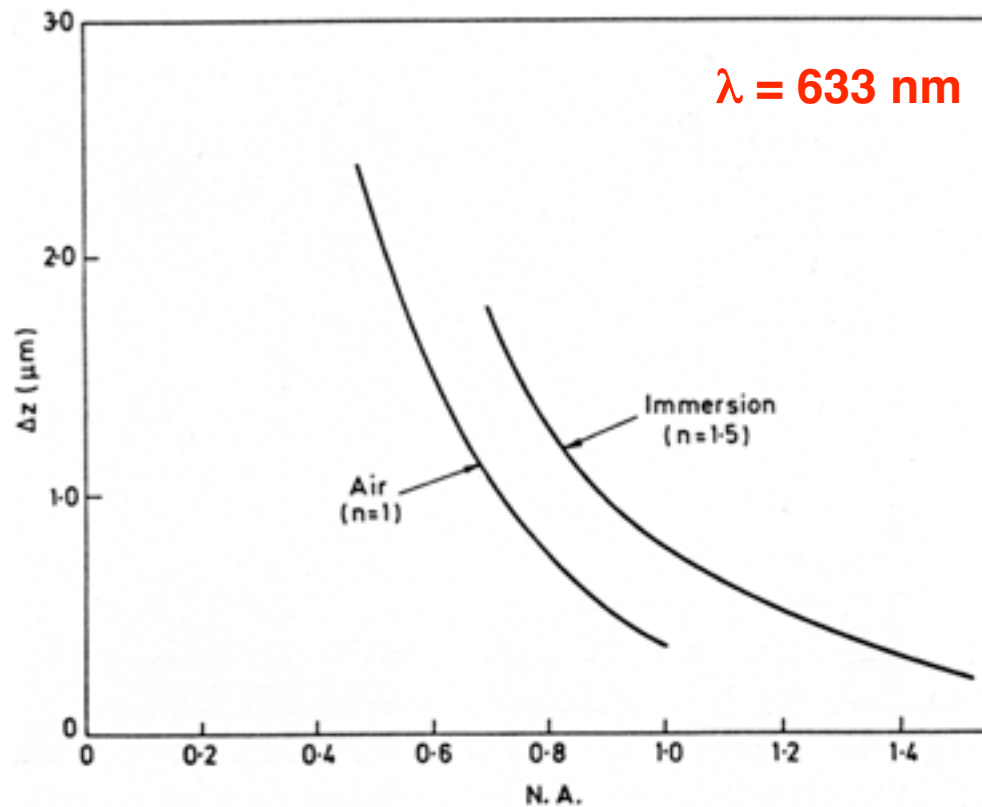
# Confocal Microscope



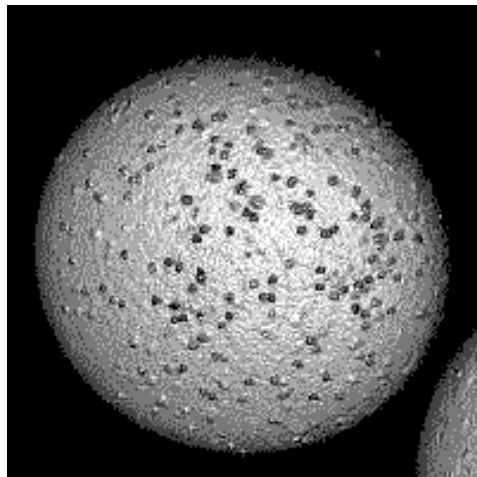
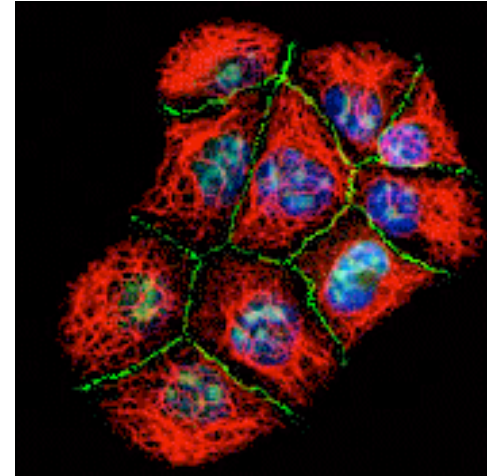
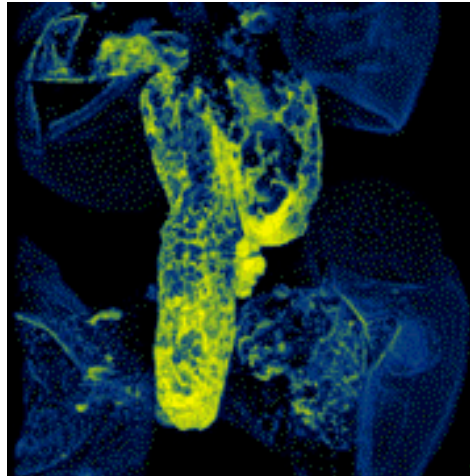
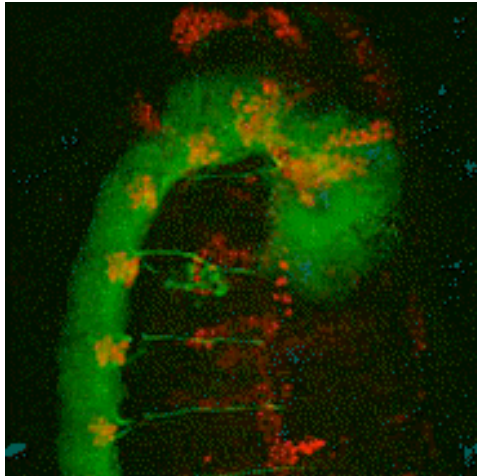
# Optical Sectioning



# Optical Sectioning Strength



# Typical images



**Manufacturers include:**

**Zeiss**

**Olympus**

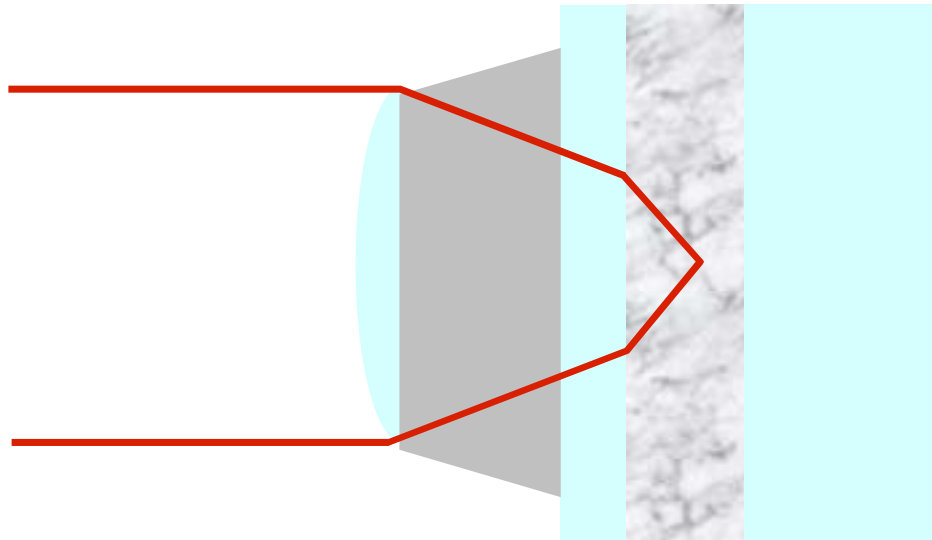
**Leica**

**Nikon**

**...**

**Prices ~100k monies upwards**

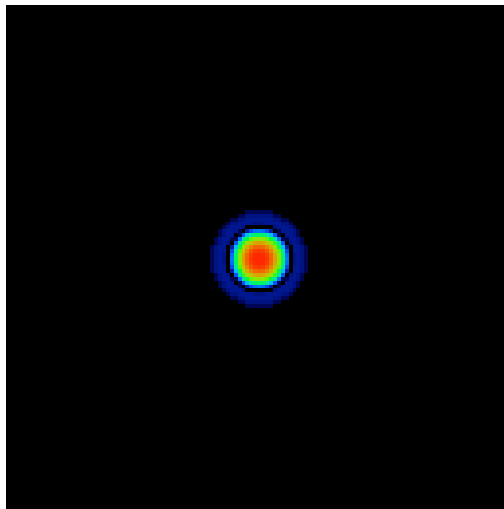
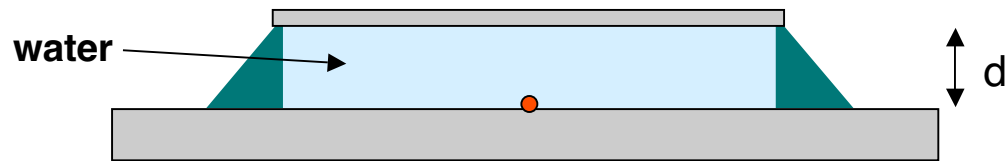
# Why adaptive optics is needed



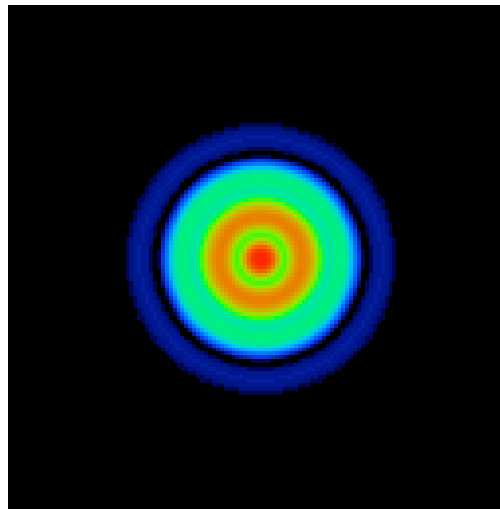
- **For best performance use high NA lenses (up to 1.4)**
- **Aberrations introduced by:**
  - **Index mismatches at boundaries**
  - **Specimen inhomogeneities**
- **Causes loss of both signal and resolution**
- **Penetration depths typically limited to 20 $\mu$ m**

# Aberrations

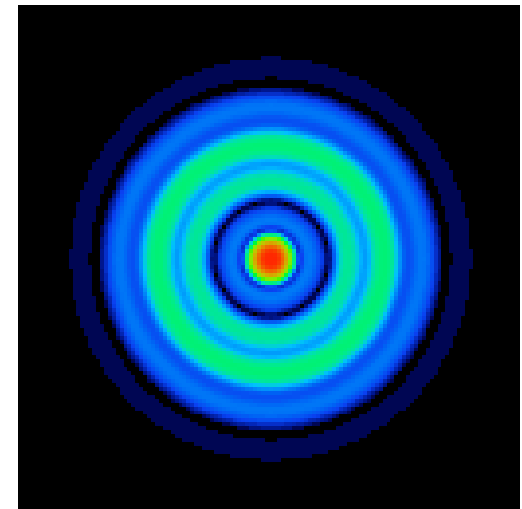
- Point spread functions



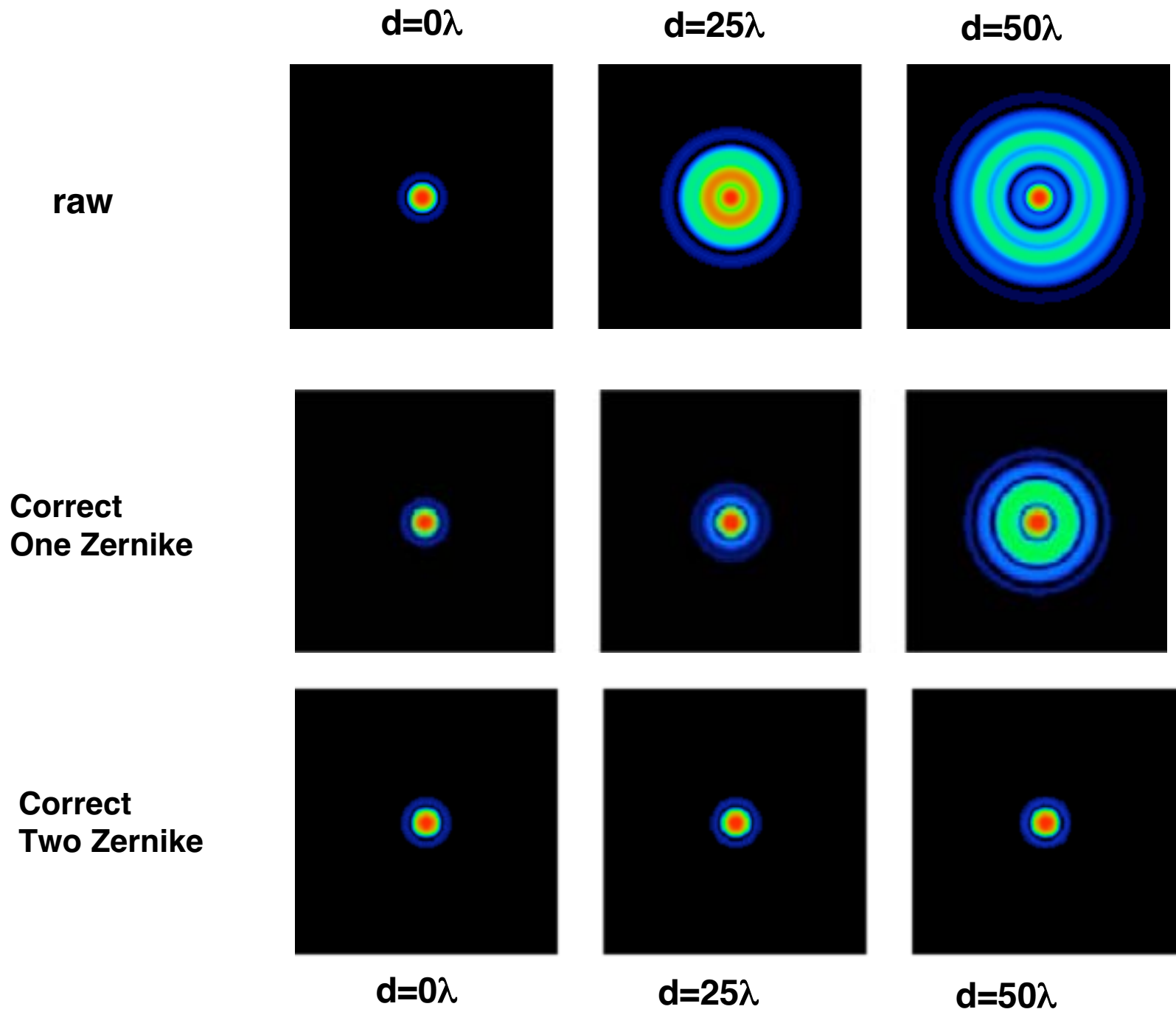
$d=0\lambda$



$d=25\lambda$



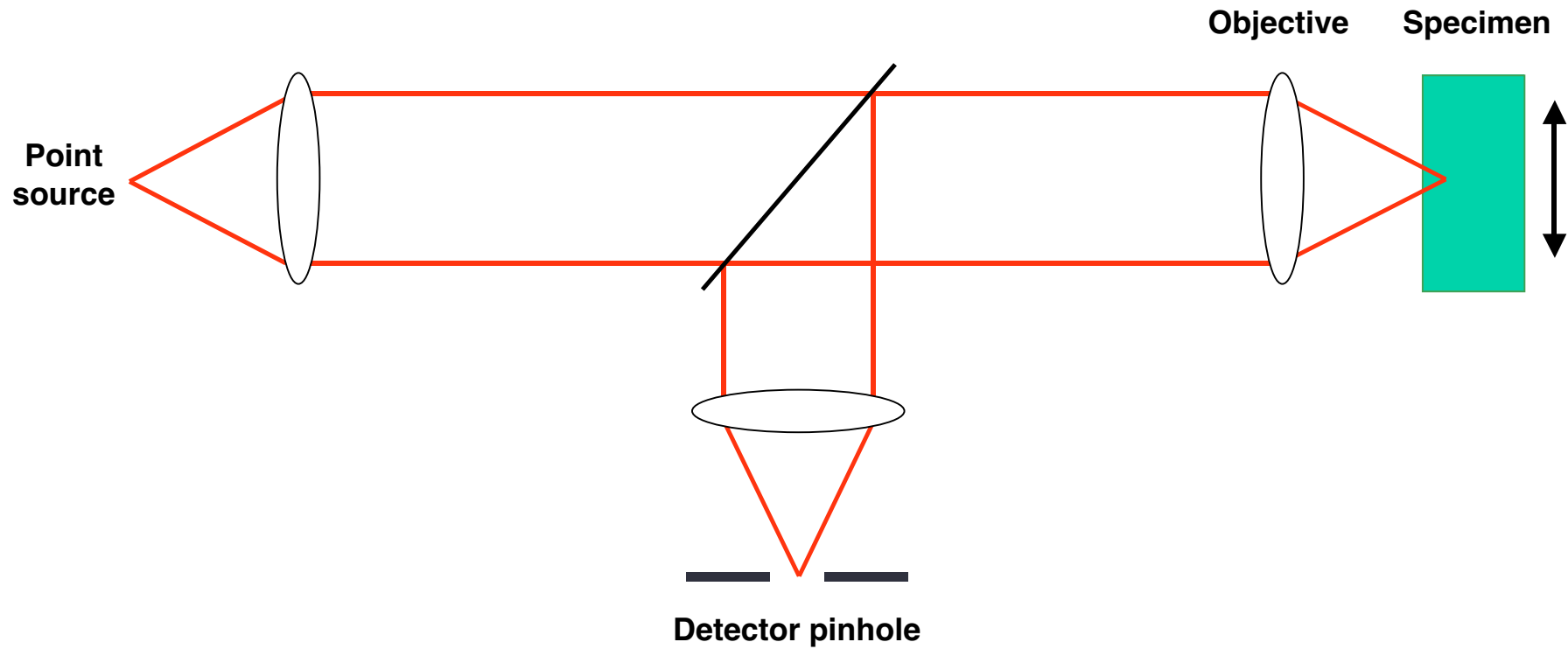
$d=50\lambda$



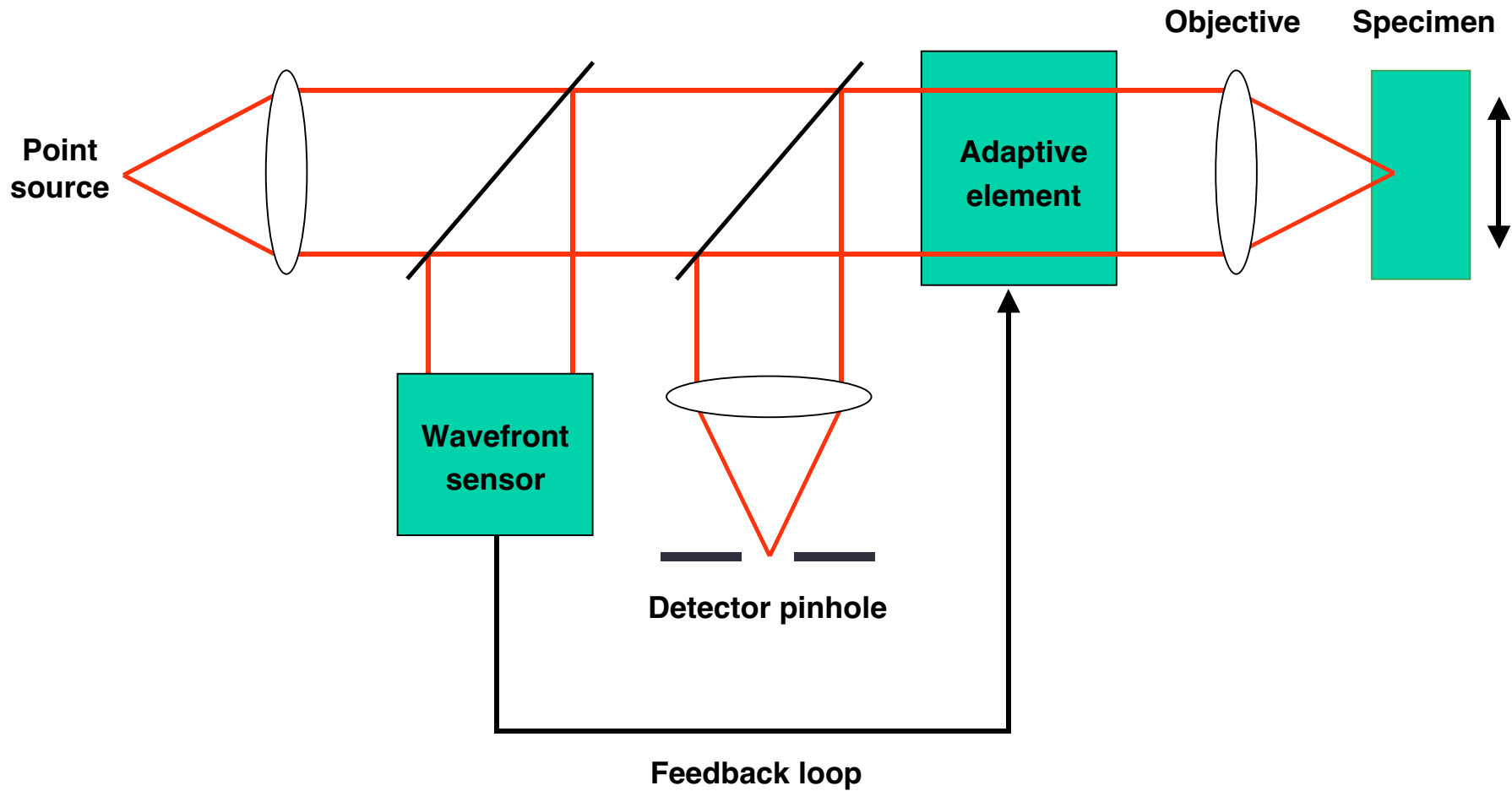
# The problem

- **Optical sectioning -- aberration problem**
- **Adaptive optics solution**
- **Wavefront sensing – modal – optical sectioning**
- **Wavefront generation**
- **Examples**

# An adaptive optics system

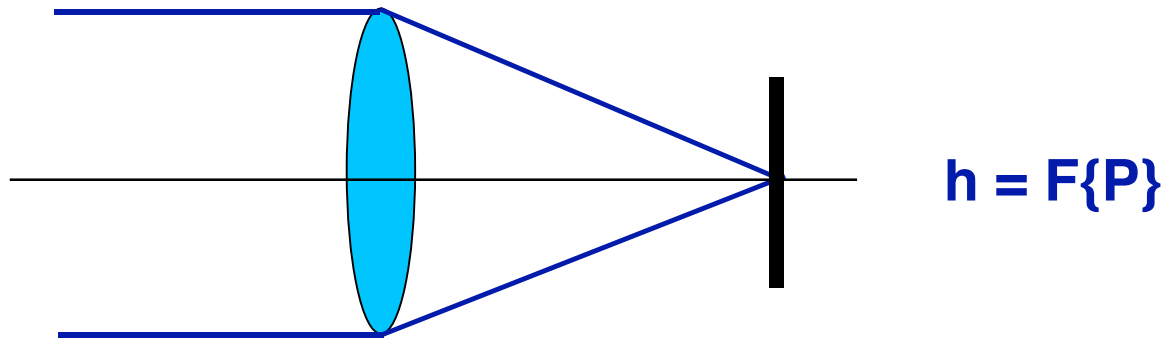


# An adaptive optics system



# Wavefront control

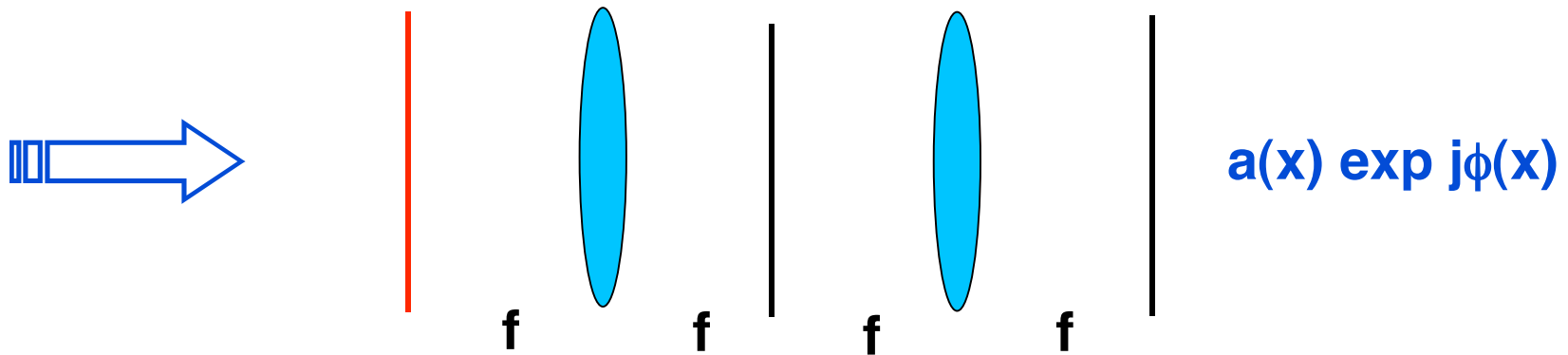
- Remove system/specimen induced aberrations
- Control – **tune** – pupil function



Point spread function **engineering**

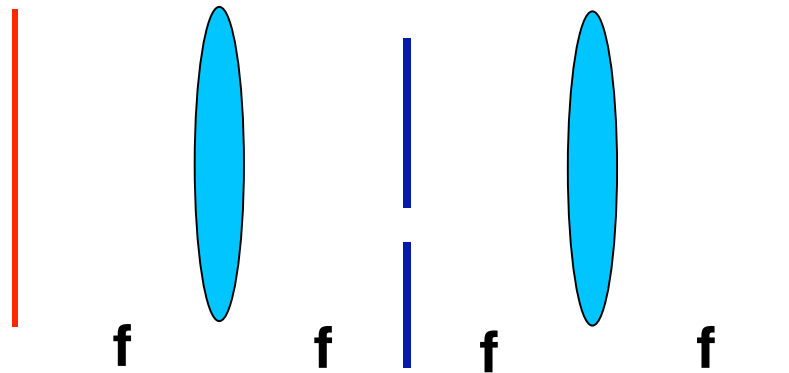
# Wavefront control

- **4- $f$  optical system**



- **Binary optical element, pixellated  $(x,y)$  +1 and -1**
- **Uniform plane wave input**
- **$a(x) \exp j\phi(x)$  output**

# Wavefront generation

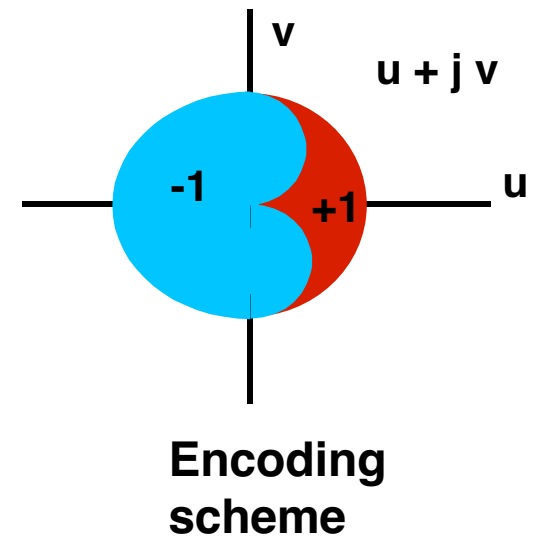


Encode SLM +1 or -1

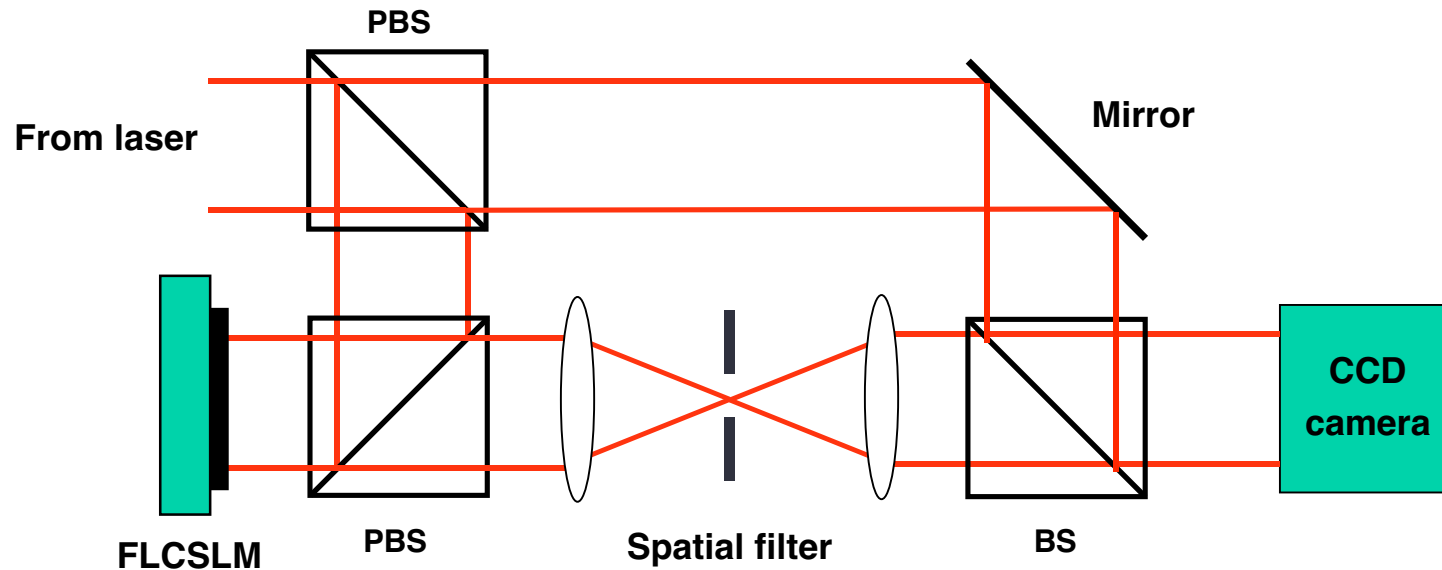
Fourier transform with lens

Spatially filter desired diffracted order

Fourier transform back



# Wavefront generator



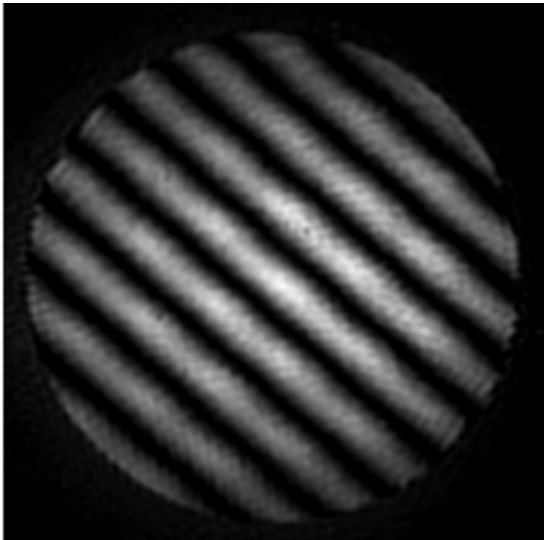
PBS = polarising beam splitter    FLCSLM = ferro-electric liquid crystal spatial light modulator

- **Arbitrary amplitude and phase wavefronts**
- **Reconfigurable at  $\sim 2.5\text{kHz}$  (Displaytech FLCSLM)**
- **Inefficient use of light ( $< 5\%$  throughput)**

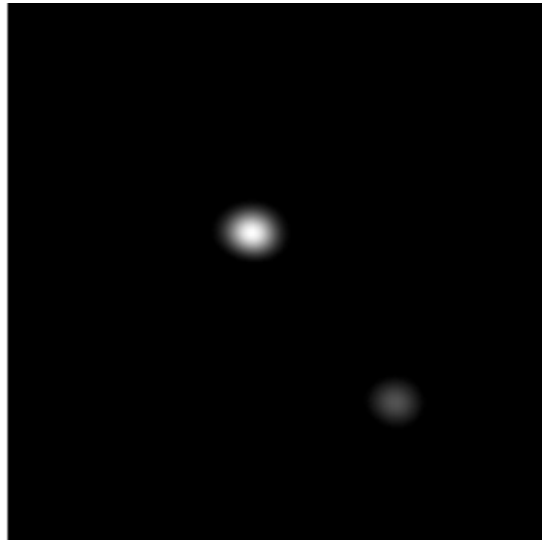
# Point spread function engineering

- **Uniform pupil function**

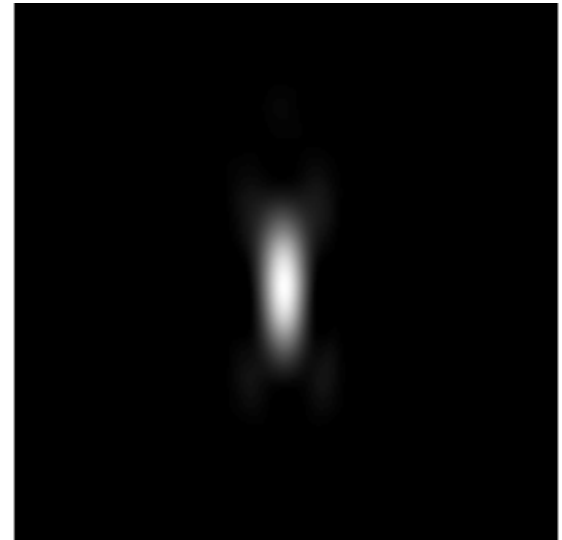
Pupil



x-y



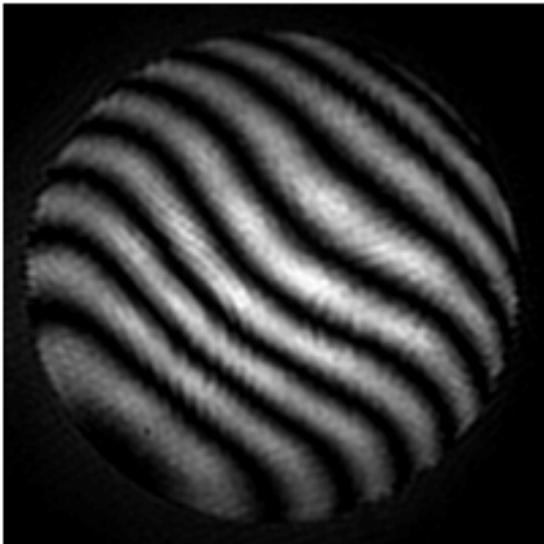
x-z



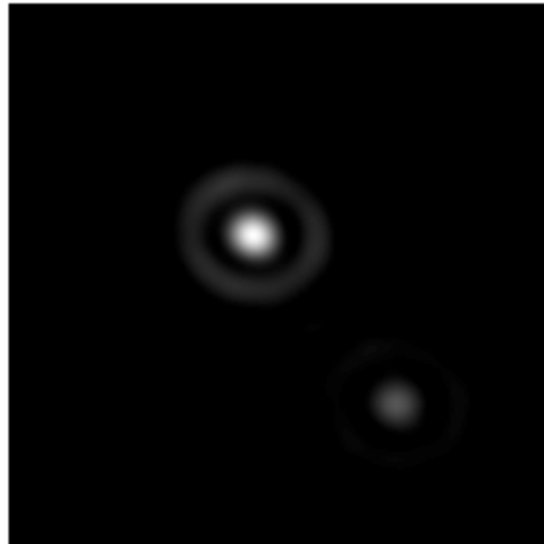
# Point spread function engineering

- Simulate spherical aberration

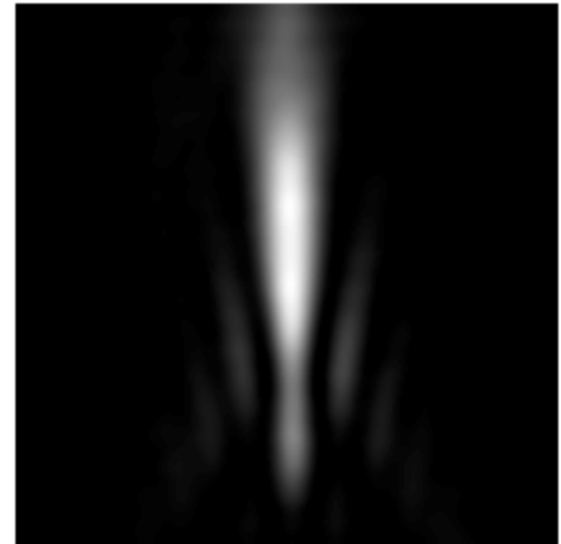
Pupil



x-y



x-z



# Wavefront sensing

- **Conventional methods are not suitable due to out-of-focus light**

- **New wavefront sensor incorporating pinhole detectors**

**Axial selectivity similar to confocal microscope**

**Direct measurement of **Zernike** aberration modes**

- **Why Zernike modes?**

**Convenient mathematical description of aberrations**

**Correspond to traditional aberrations**

**e.g. spherical, coma, astigmatism**

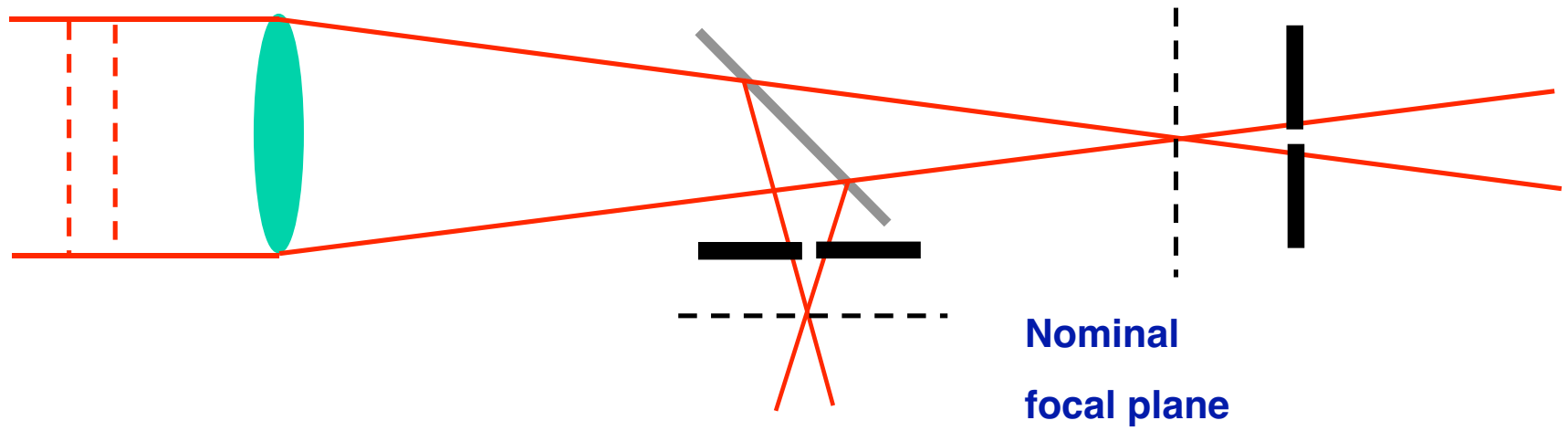
**Only lower order correction needed**

# Wavefront Sensor

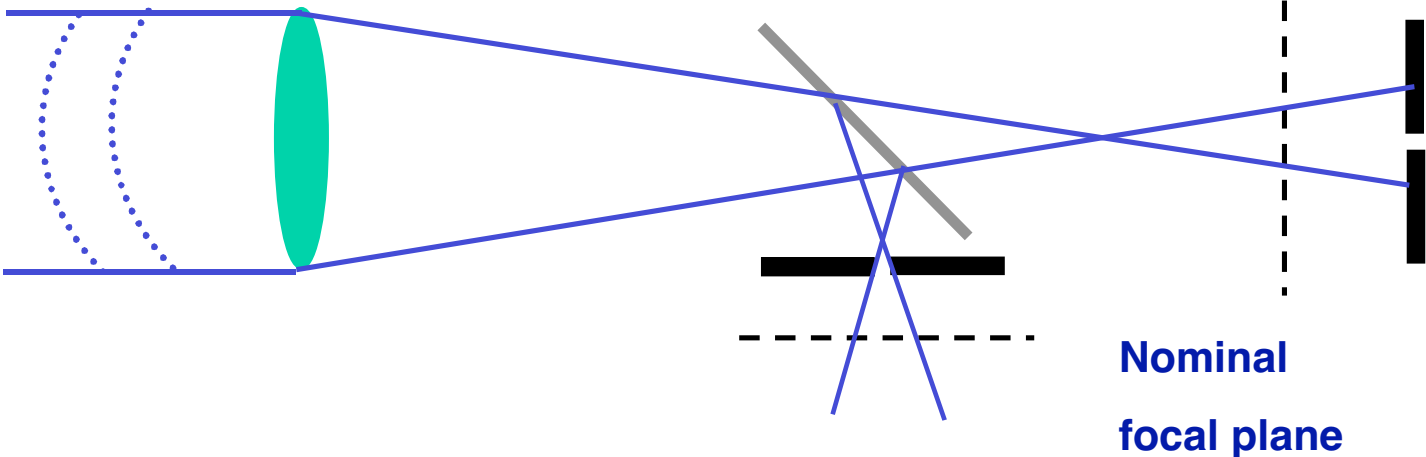
- **Senses amount of Zernike modes present**
- **Inherent optical sectioning**
- **How to detect defocus?**

How to detect other Zernike modes?

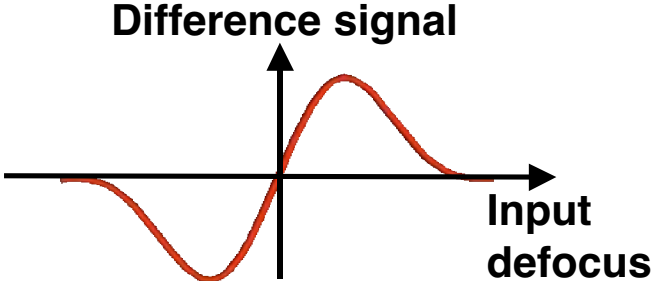
# Defocus sensor

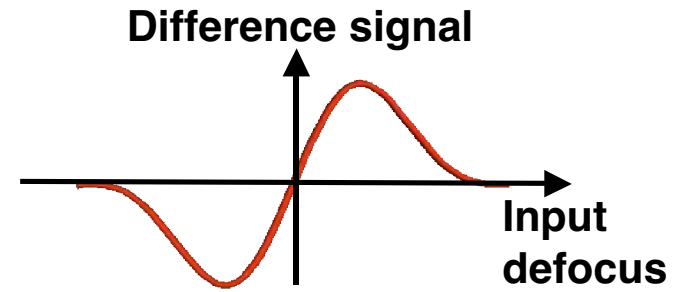
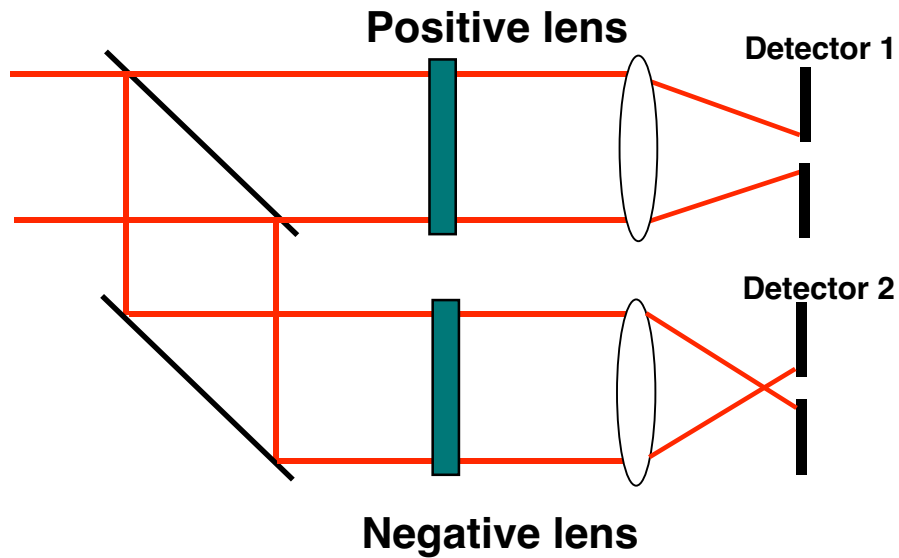


# Defocus sensor



Display difference  
Signals from two  
detectors





**Positive defocus**    **(positive aberration bias)**

**Negative**    **(negative aberration bias)**

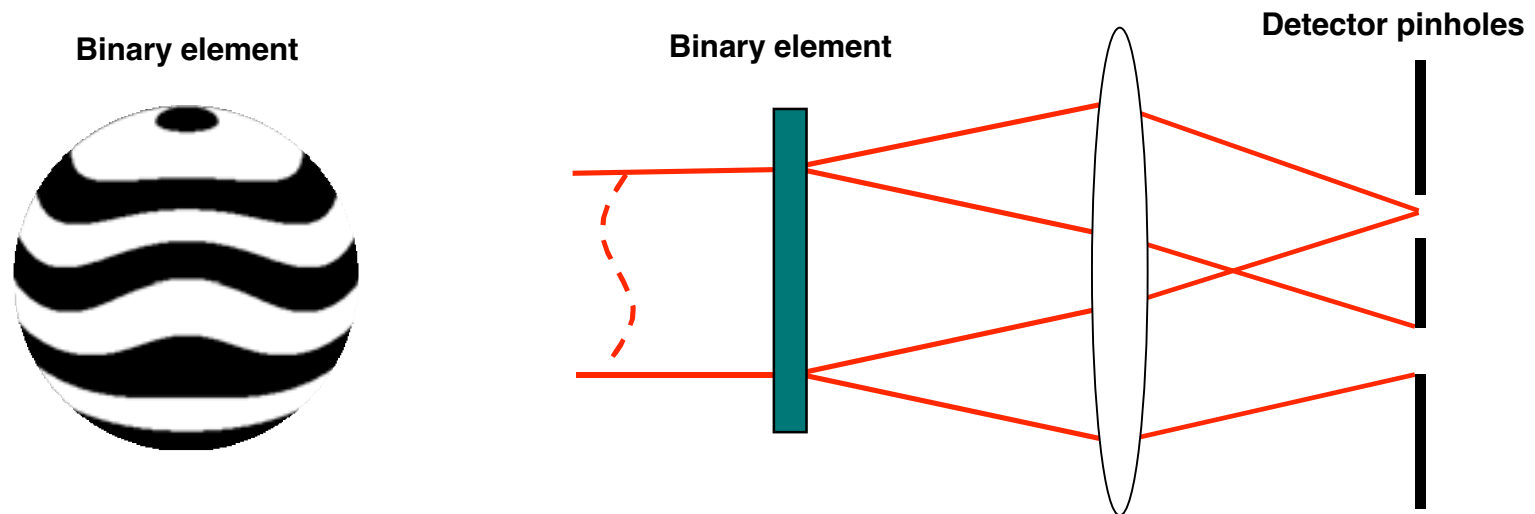
**Form difference signal**

# Wavefront sensing

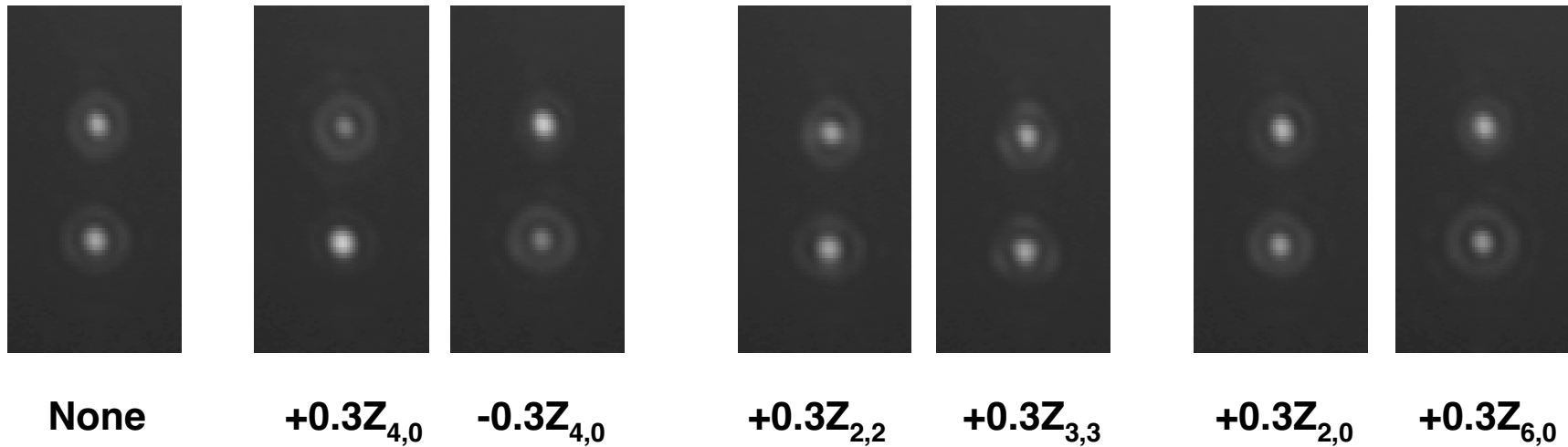
- ☞ Create binary element: 1) add tilt  $\tau$  to aberration mode,  $\phi$   
2) binarise result

$$\exp j(\phi + \tau) + \exp j(-\phi - \tau) + \frac{1}{3} \exp j(3\phi + 3\tau) + \frac{1}{3} \exp j(-3\phi - 3\tau) + \dots$$

Diffraction orders +1 and -1 are separate positively and negatively biased spots

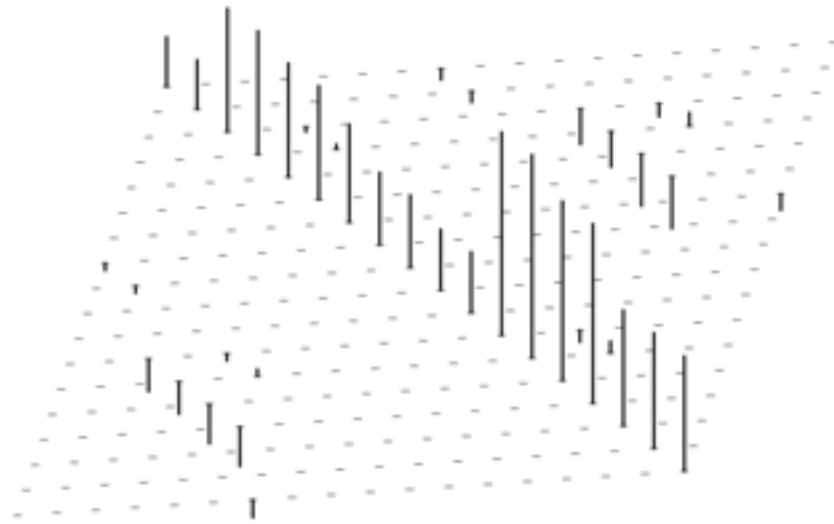


# Spherical aberration sensor



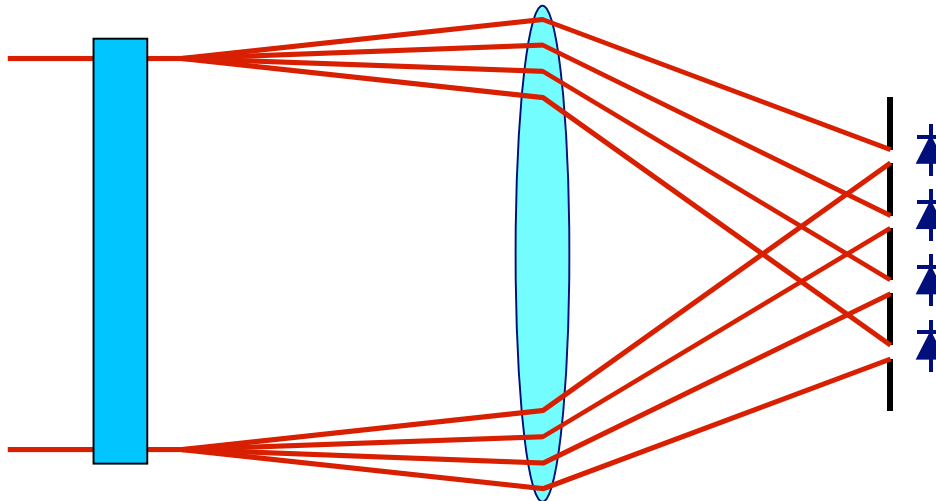
- $Z_{4,0}$  aberration bias – first order spherical
- Strong differential response to  $Z_{4,0}$
- Weak or no differential response to other aberrations

# Cross-talk



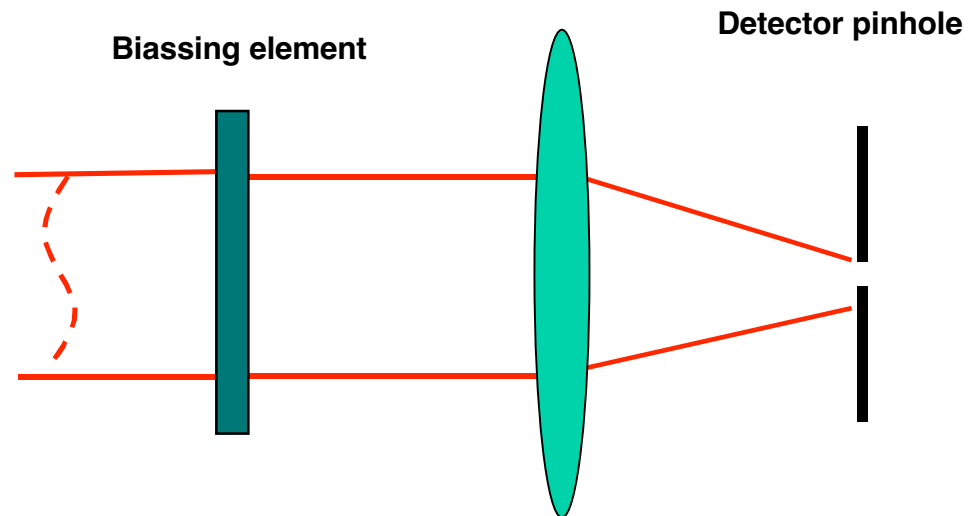
# Wavefront sensing

- Use binary element to create two biased spots simultaneously
- Use multiplexed binary element to sense many modes simultaneously



# Sequential biasing

- Add +ve and -ve bias sequentially with adaptive element

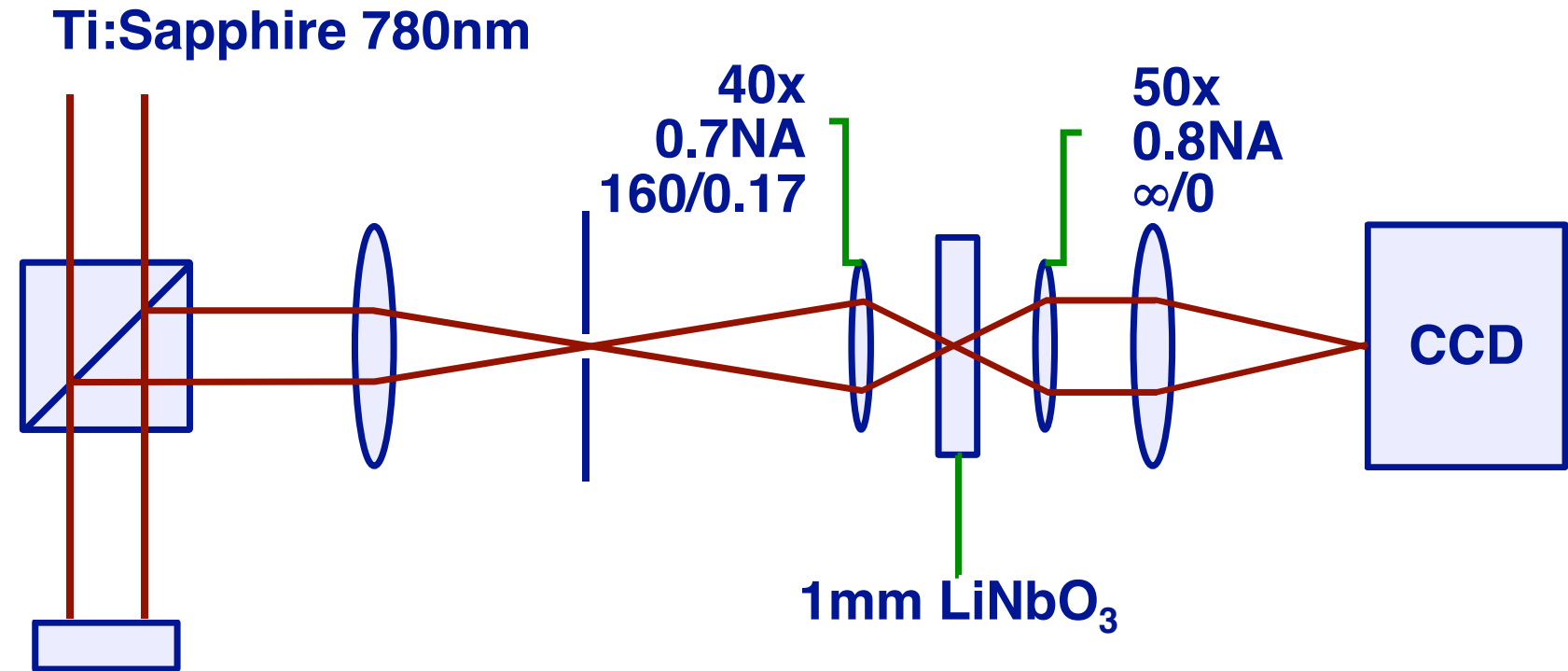


# Aberration correction for 3D optical memory

• Write bit data deep in  $\text{LiNbO}_3$  crystal by two-photon absorption

- Refractive index of  $\text{LiNbO}_3$  is 2.3
- Dry lens necessary
- Spherical aberration limits possible writing depth
- Use FLCSLM wavefront generator to pre-aberrate wavefronts
- Bit data written at depth of 1mm into crystal

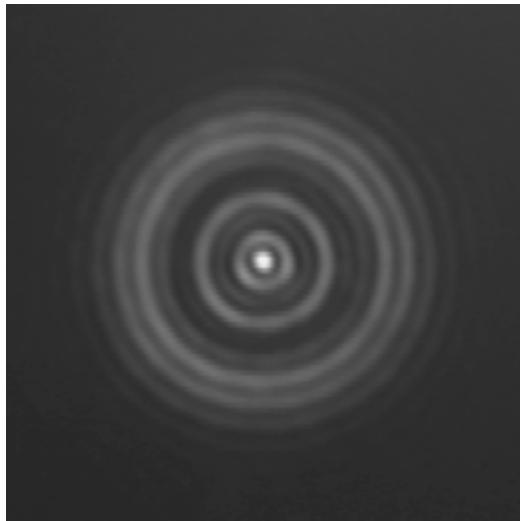
# Our System



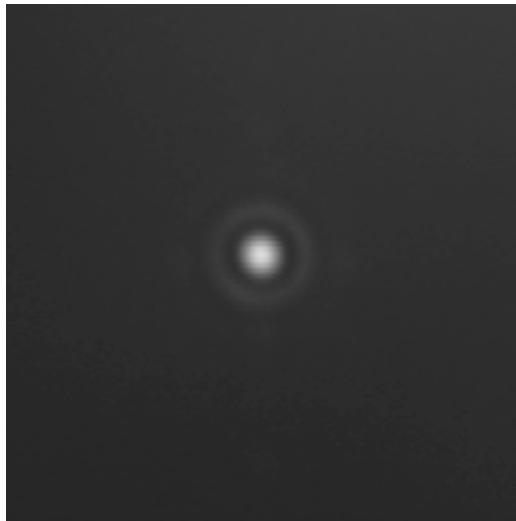
Ferroelectric liquid  
crystal spatial light  
modulator,  
FLCSLM

- Aim to record optical memory data through depth of LiNbO<sub>3</sub> by 2-photon absorption

# Aberrations in 1mm LiNbO3



**"Nominal"  
focus**



**"Best"  
focus**

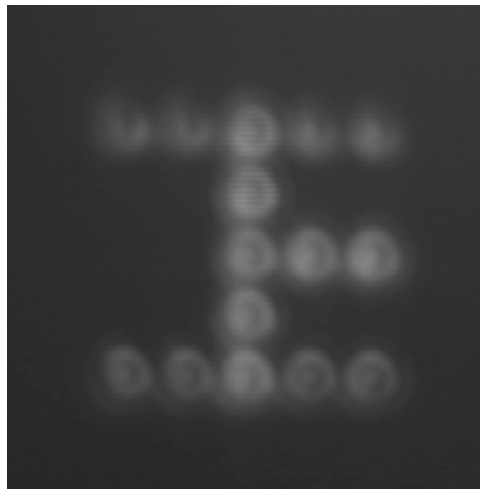


**Corrected**

# Scanning in 3-D



top

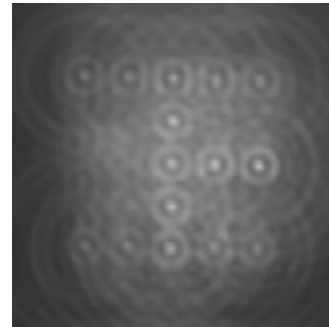
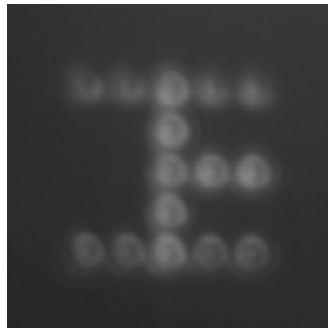
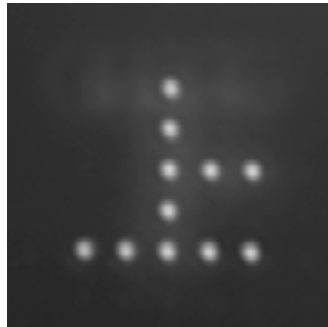


bottom

- Scan in x,y and z using the FLCSLM
- 3.2  $Z_{2,0}$  between planes = 20  $\mu\text{m}$  in  $\text{LiNbO}_3$

# Corrected vs. uncorrected

Nominal  
focus

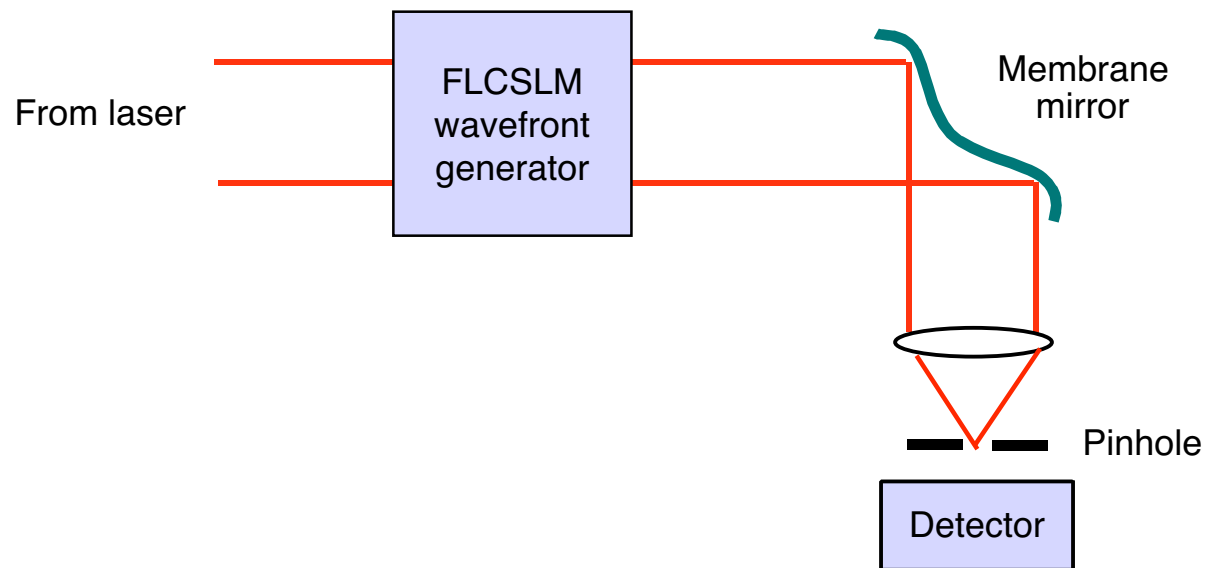


Nominal  
focus



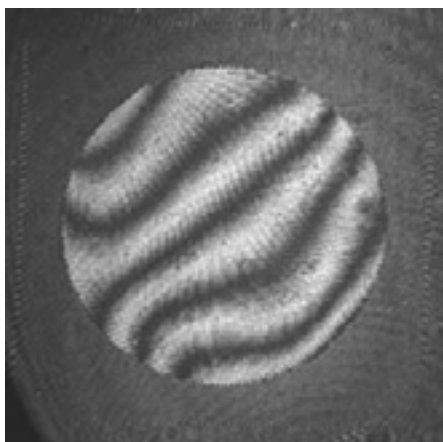
# Sensing and correction with deformable mirror

- **Static aberration introduced by FLCSLM wavefront generator**
- **Membrane mirror used as correction element**
- **Zernike aberration modes measured by sequential MWFS**

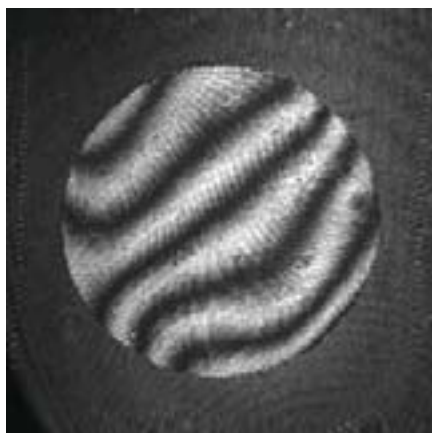


# Sensing and correction with deformable mirror

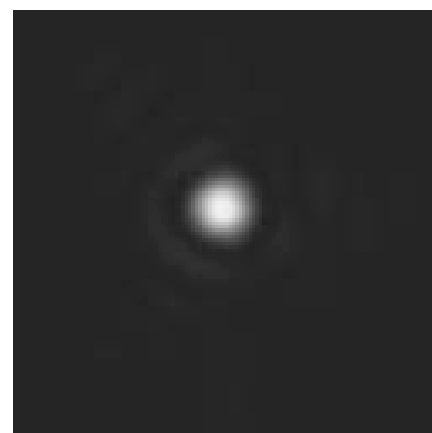
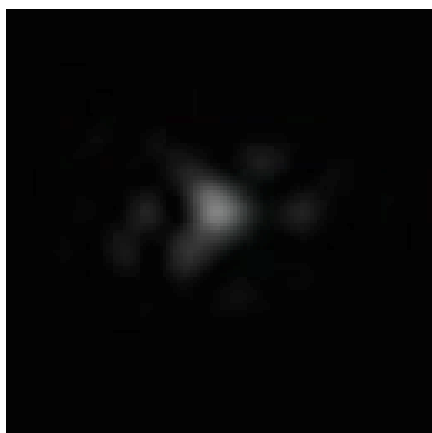
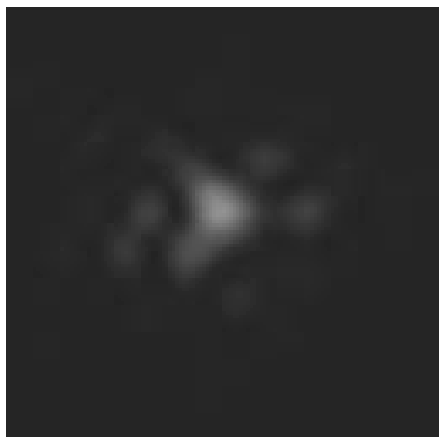
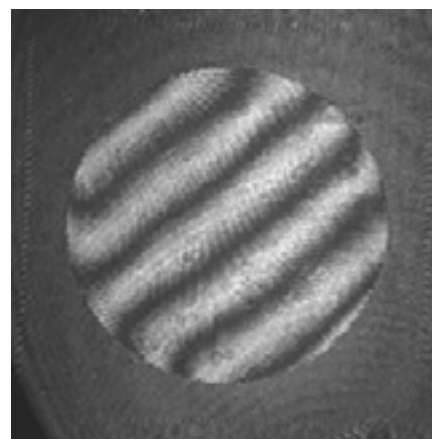
**Before**



**During**

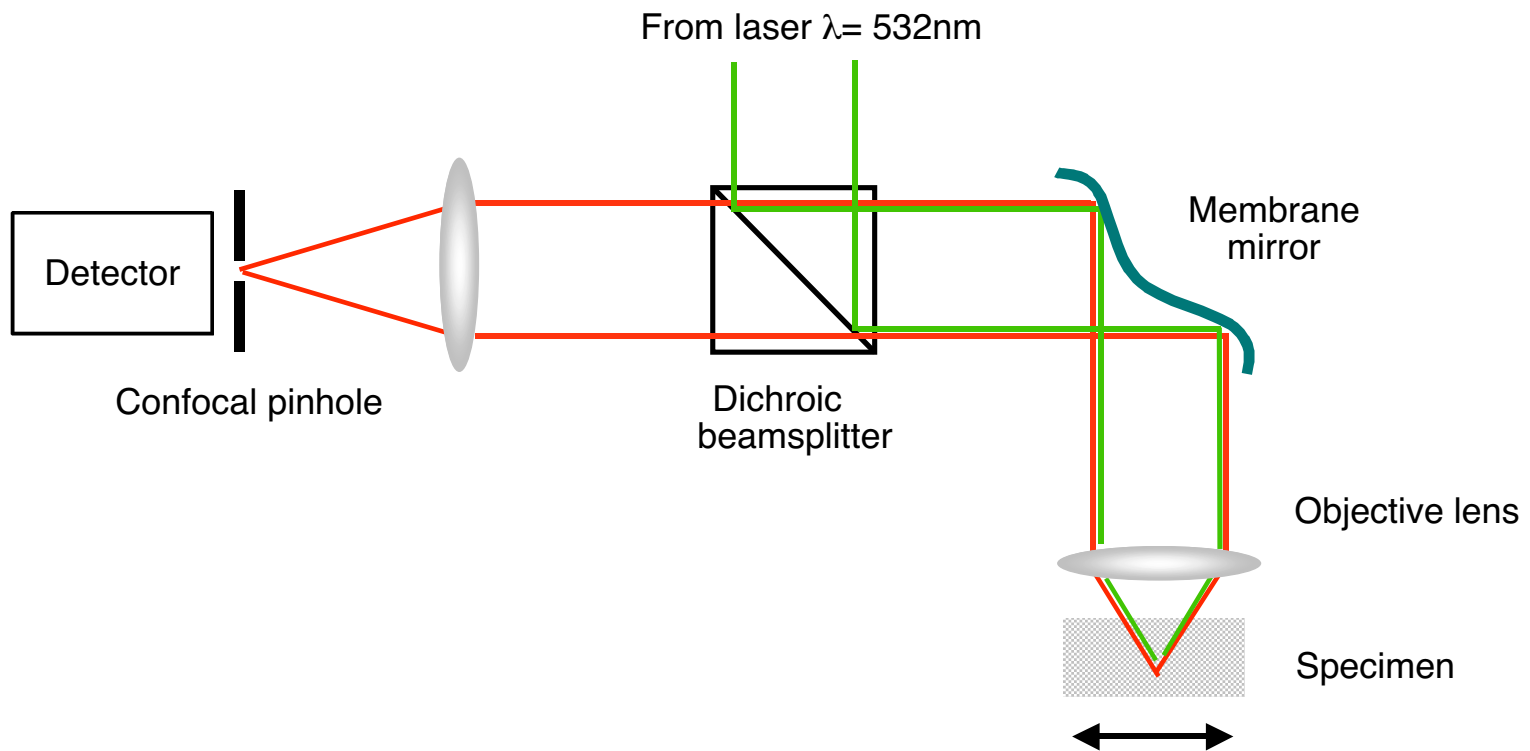


**After**



# Adaptive optics in the confocal microscope

- Membrane mirror used as both correction and MWFS element
- Wavefront measurement calculated from frame averages
- Correction of field independent aberrations



## **(Interim) Conclusions**

- **Wavefront generation and correction**
- **Aberration measurement by wavefront sensing**

## **Demonstrations**

- **Closed loop operation**
- **Aberration correction for two-photon writing of thick optical memory**
- **Adaptive optics for single photon fluorescence microscopy**

# Further developments

- **Fixed element wavefront sensor with pinhole/diode array**
- **Measure aberrations introduced by ‘typical’ biological specimens.**