Making light work in microscopy

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Optically sectioning microscopes

through-focus series of images
three-dimensional imaging

Light efficient implementation

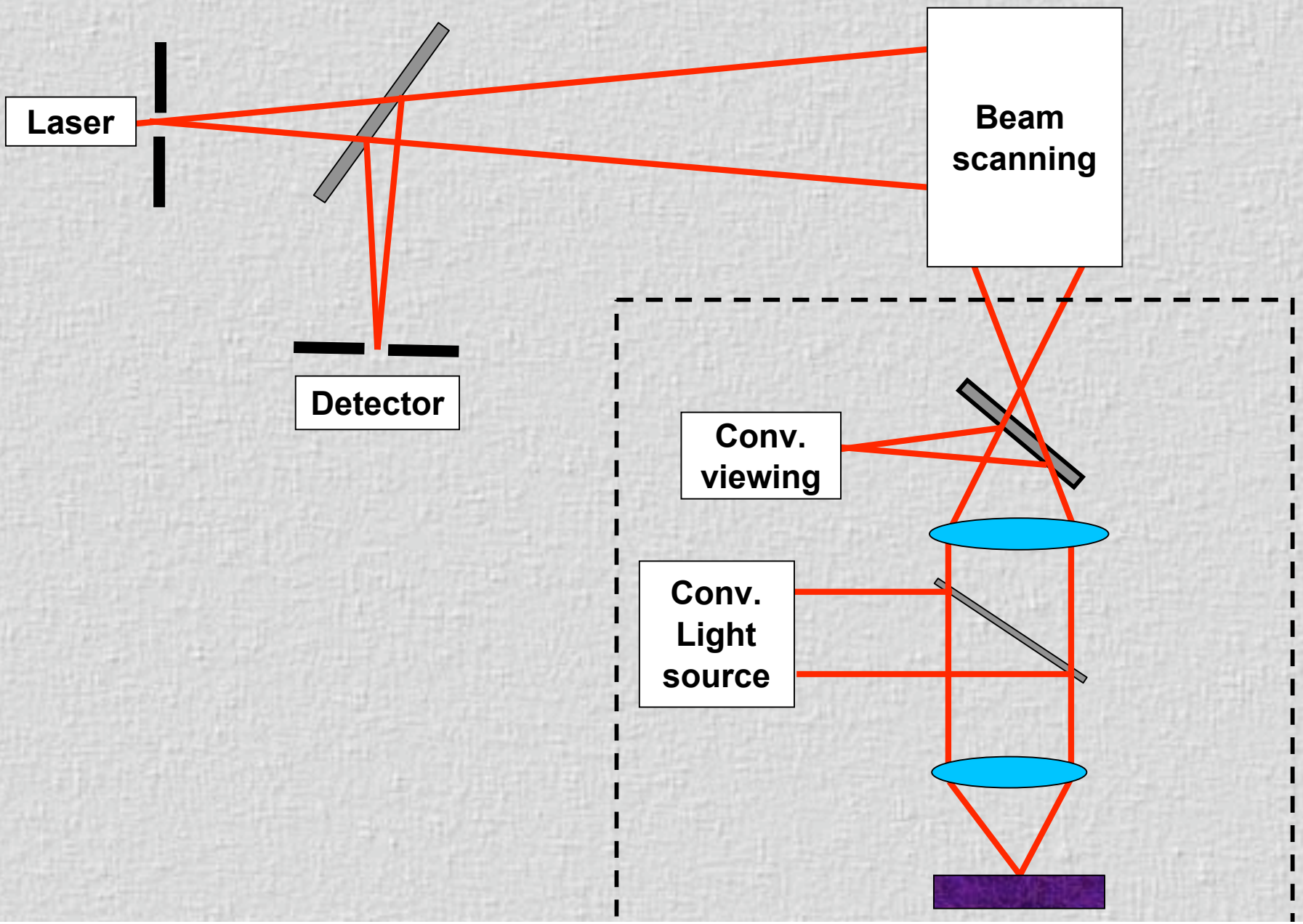
aperture correlation
structured illumination

Fast focussing

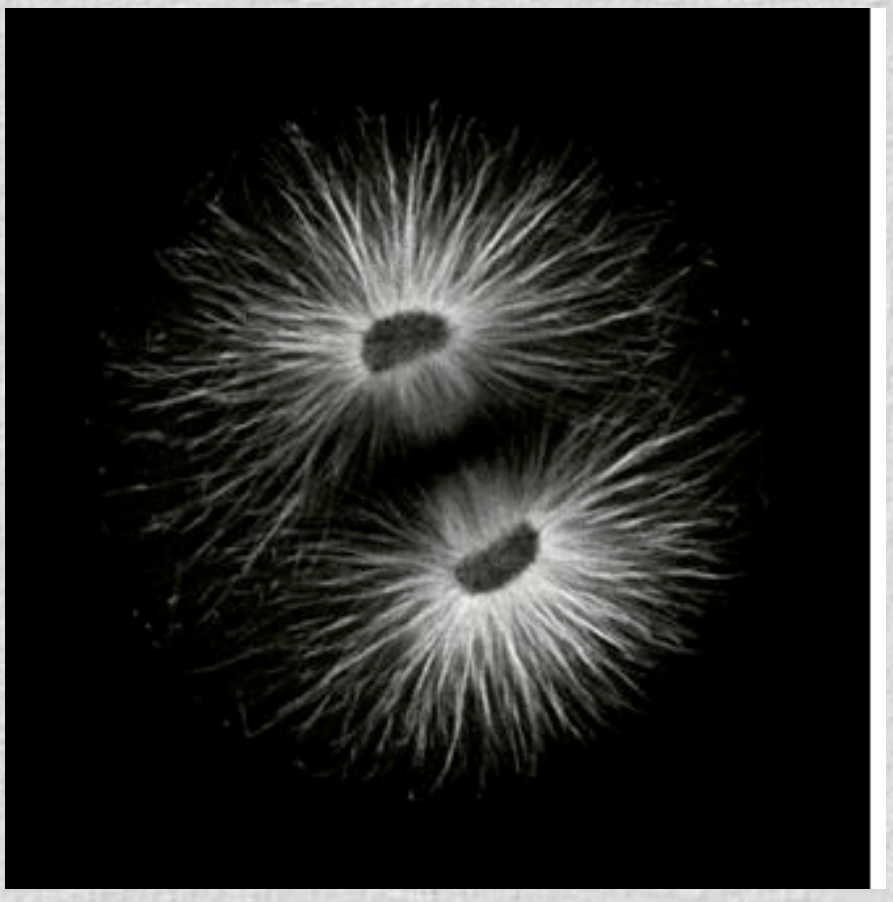
live cell imaging

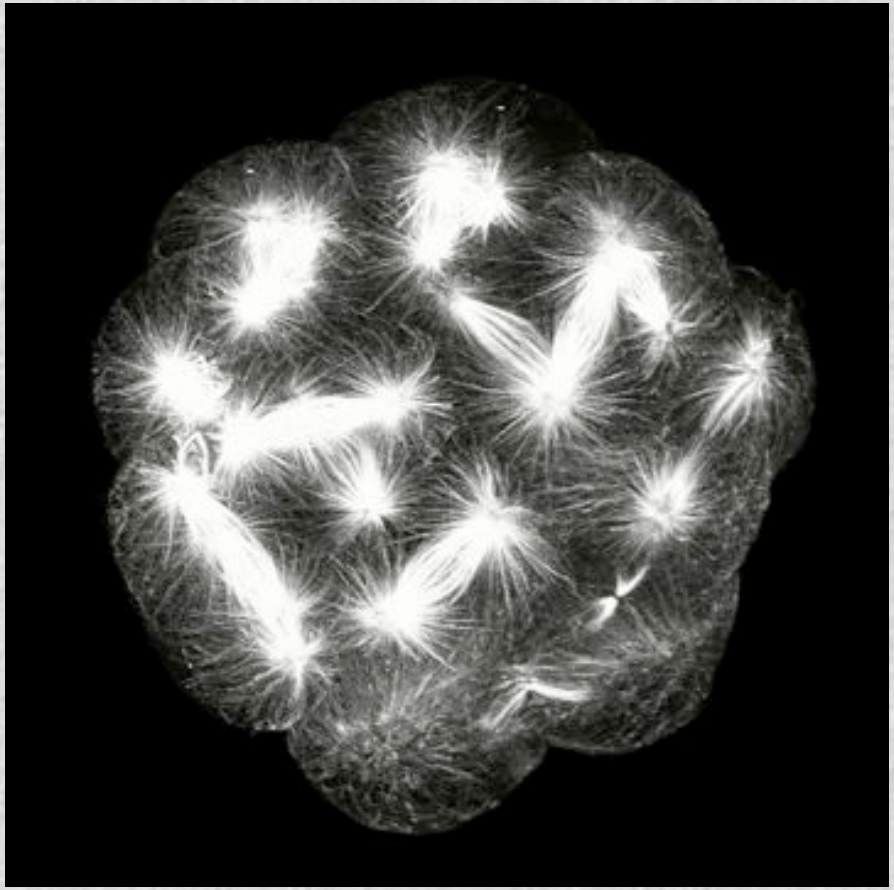
Structured illumination

Confocal Microscope



Embryo





Drawbacks

- Lasers
  - Brightness
  - Limited wavelength choice
- Usually not real time
- Pinhole alignment problems
- Need to scan

Goals

- Lasers
  - Brightness
  - Limited wavelength choice

No laser

- Usually not real time

Real time

- Pinhole alignment problems

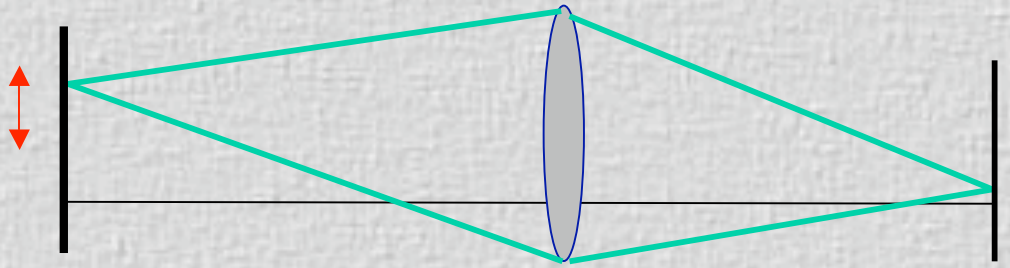
Easy alignment

- Need to scan

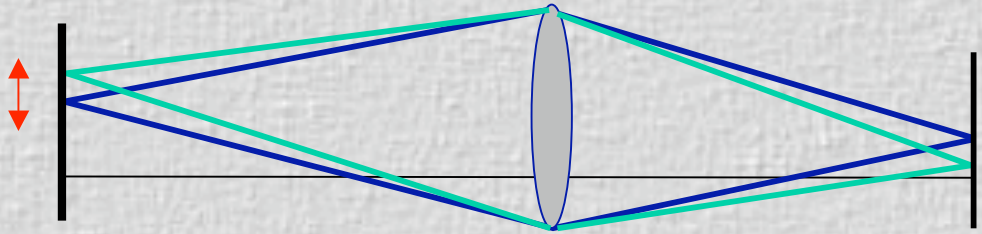
No scanning

The principle

- Single point system



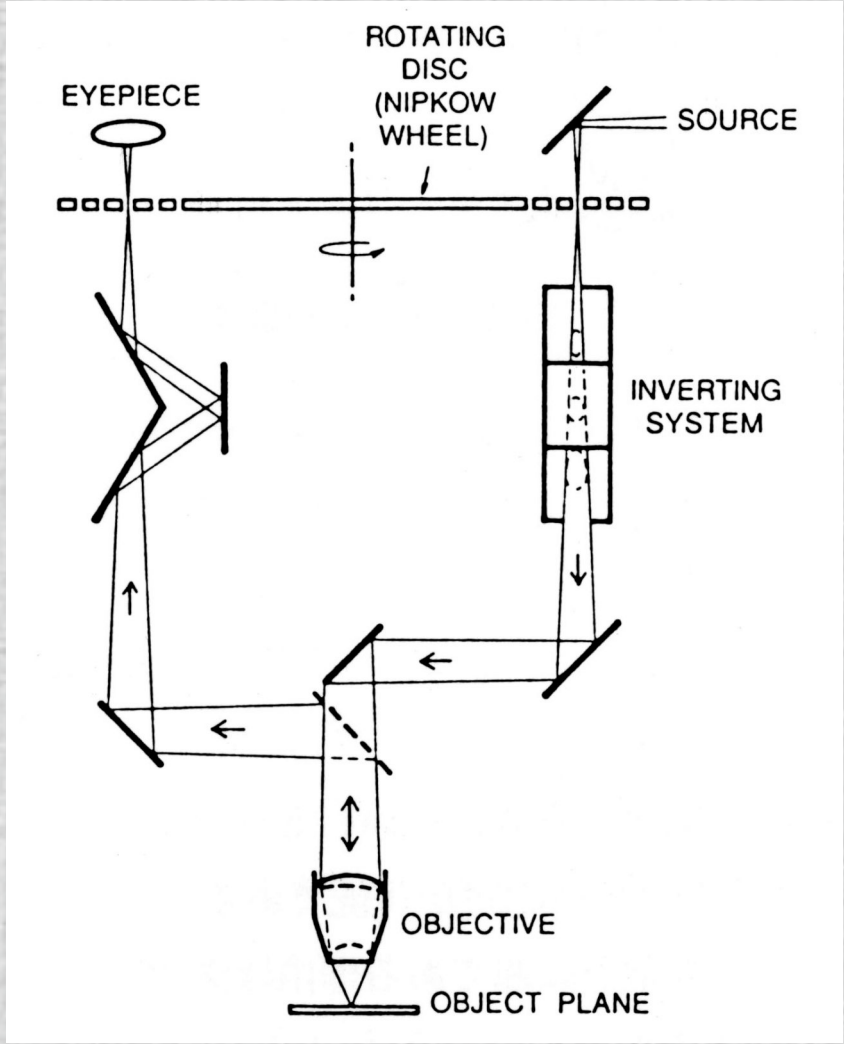
- Two point systems



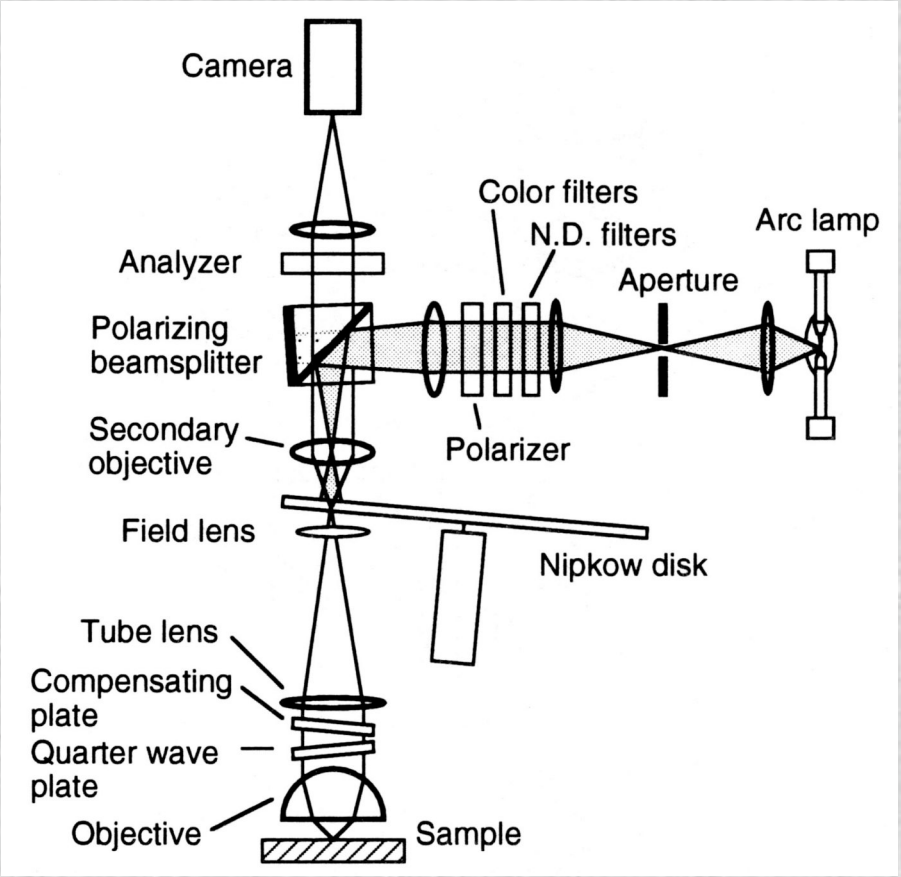
Multiple point --- TSM

space pinhole far apart --- cross talk

TSM schemes

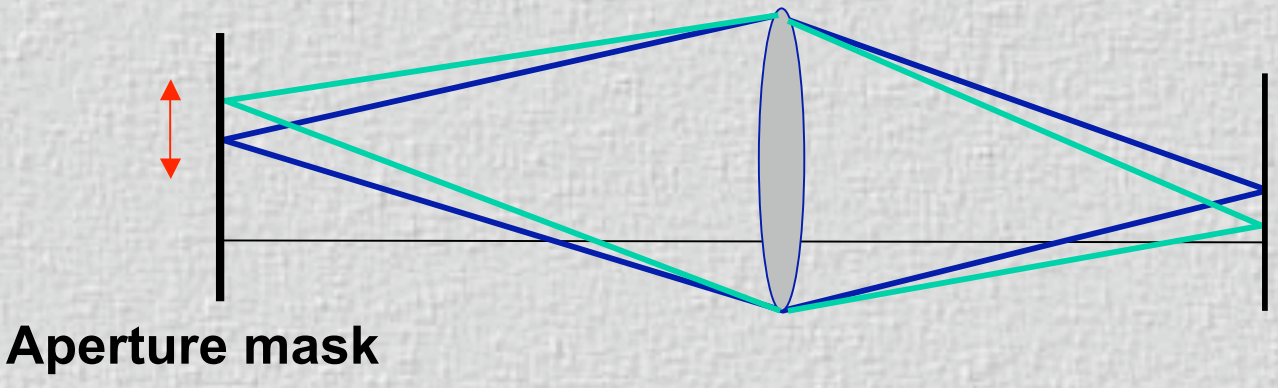


Original Petran scheme



Kino approach

Aperture correlation



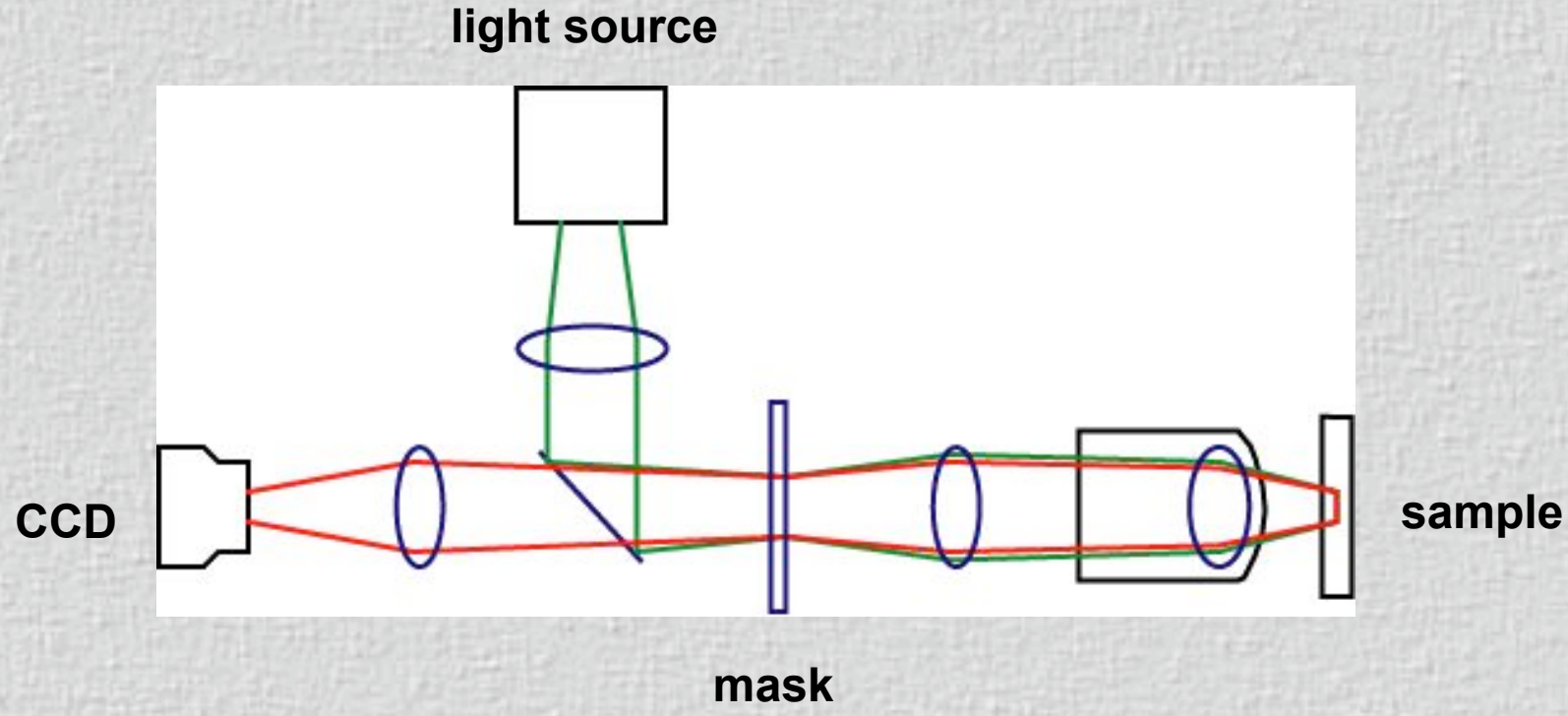
Place pixels close together

Use time sequential pixel transmission, $b_i(t)$

$$\langle b_i(t) b_j(t) \rangle = \delta_{ij} \qquad \langle b_i(t) \rangle = 0$$

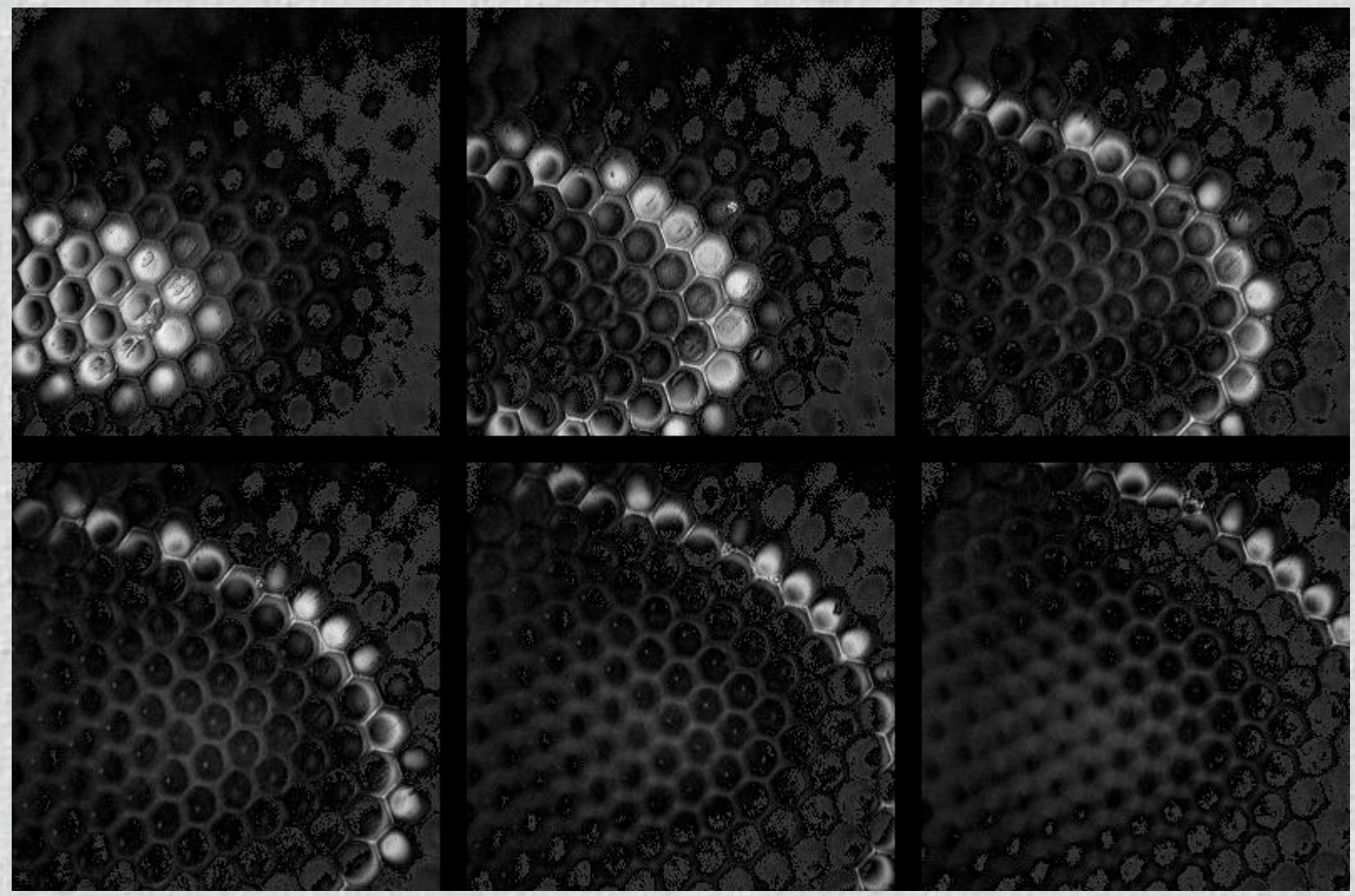
No cross-talk

The system

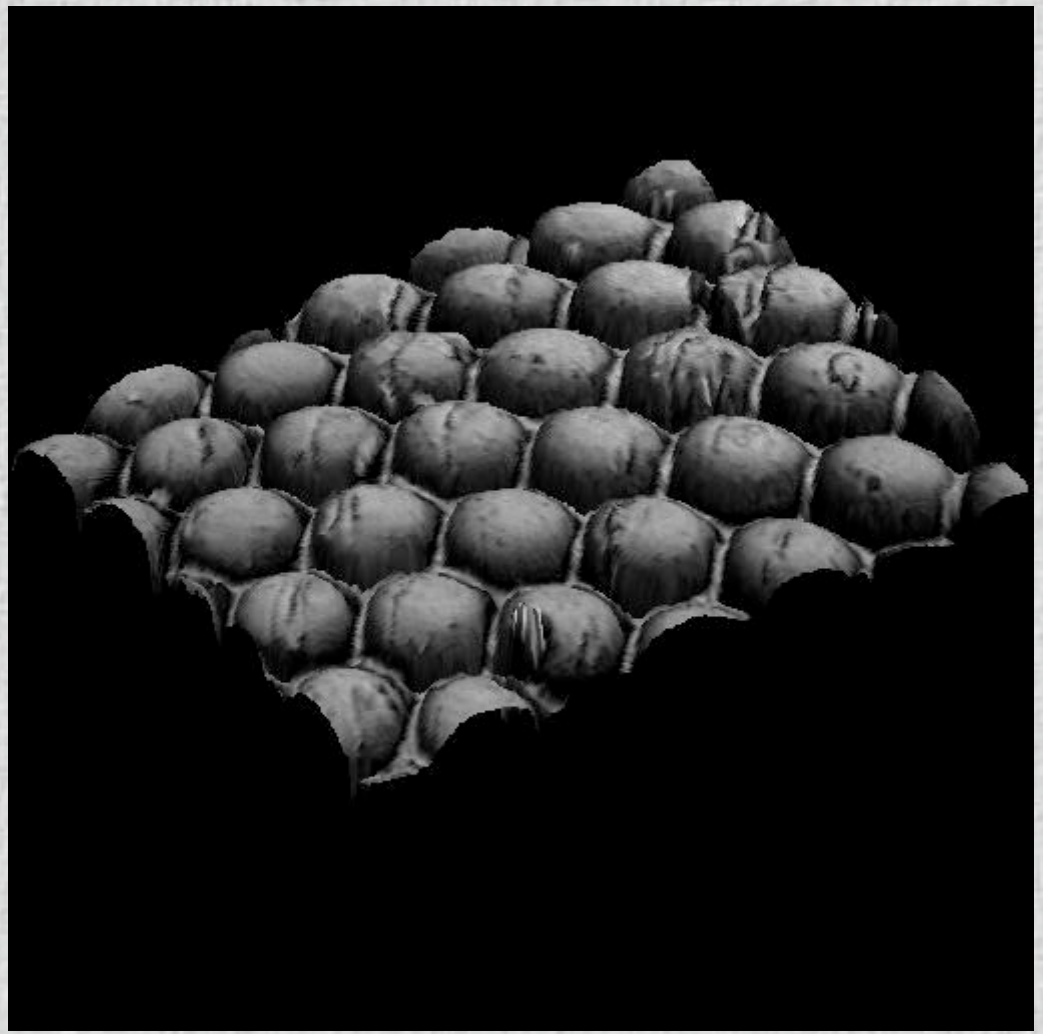


- mask encodes pattern and performs reciprocal filtering
- averaging required to remove pattern
- standard microscope illuminator
- 25 (30) confocal frames/second

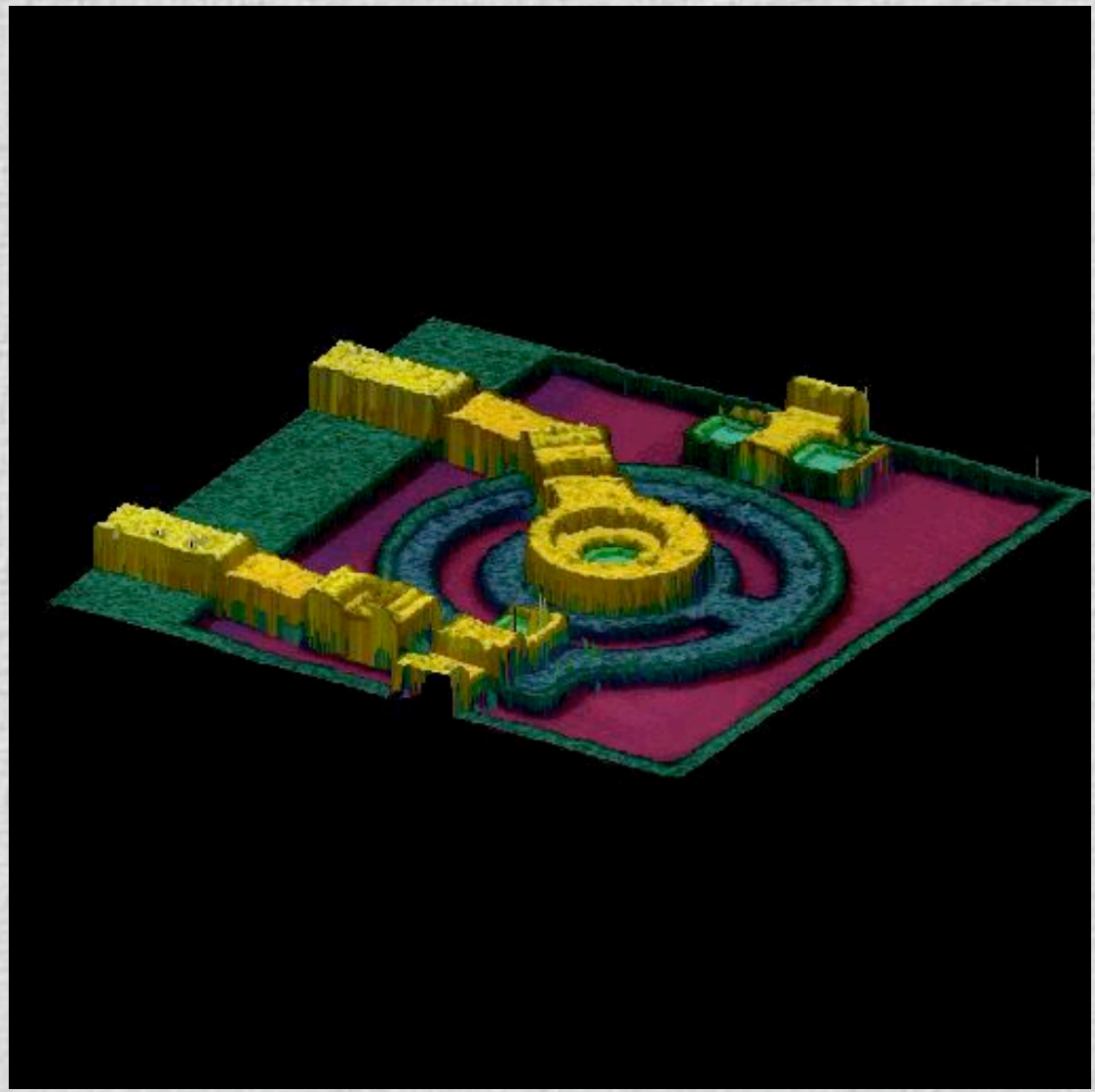
Through focus series – fly’s eye



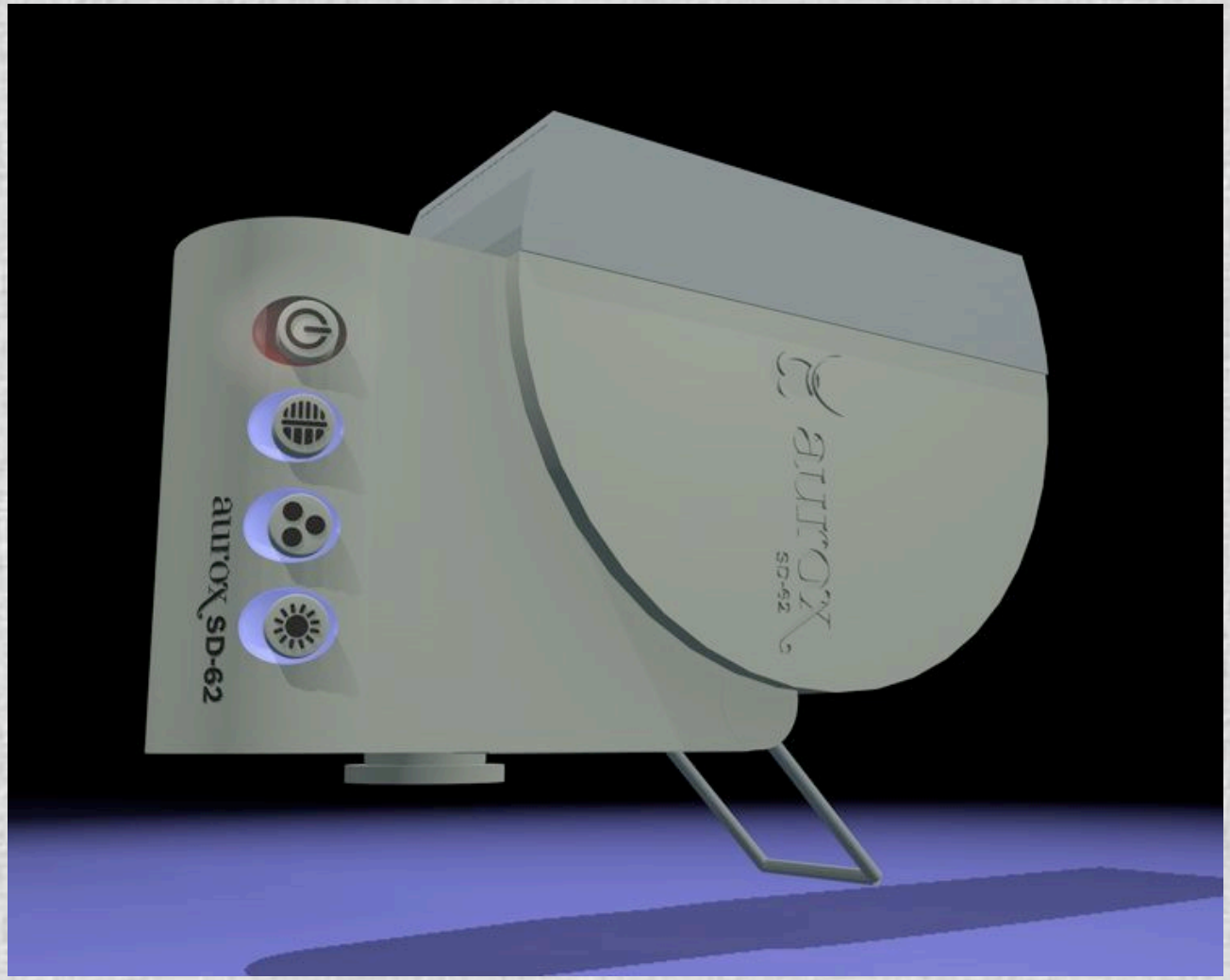
Fly’s eye



Transistor

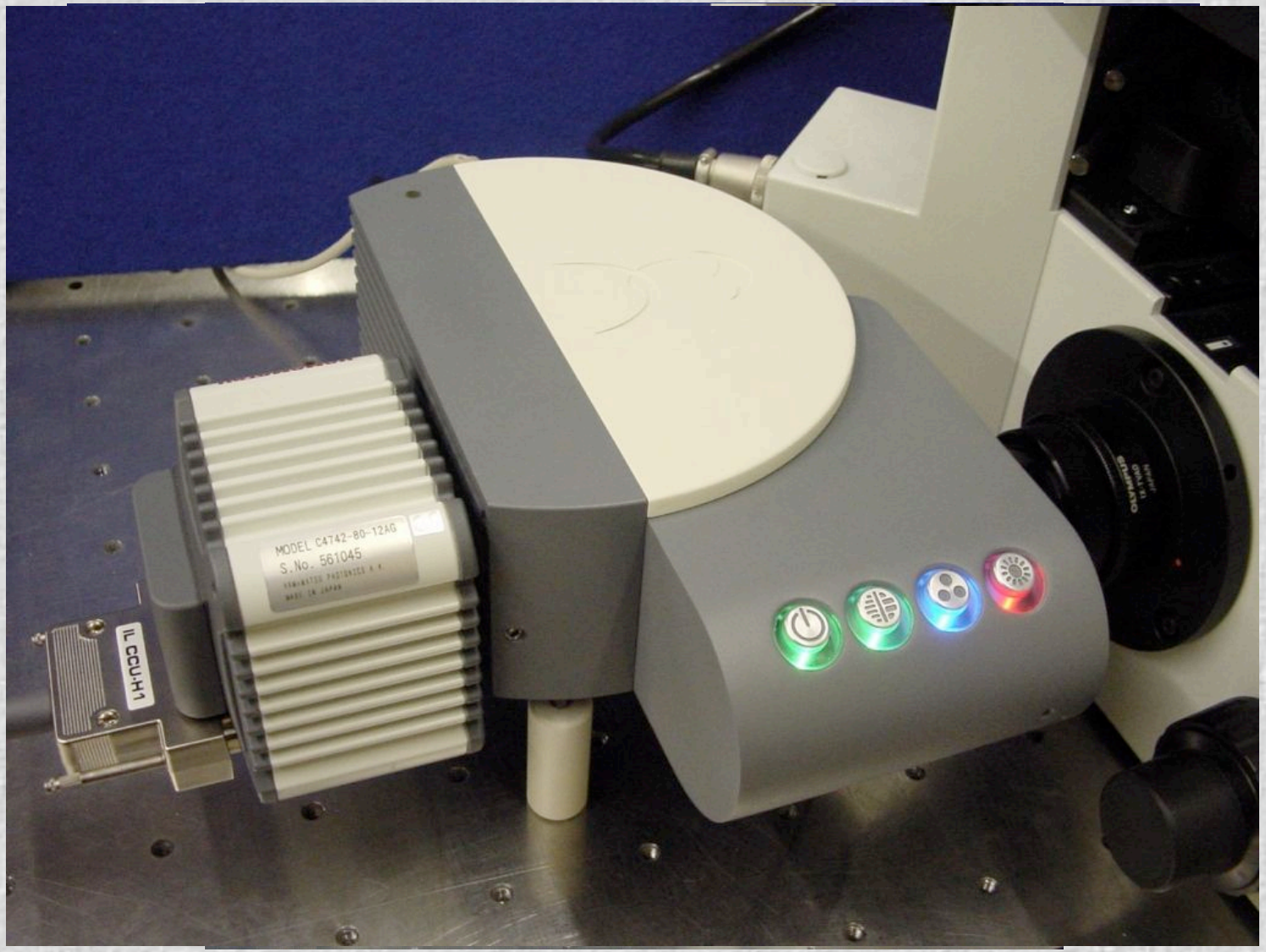


Aurox SD-62



aurox

Aurox SD-62



Goals

- Lasers
  - Brightness
  - Limited wavelength choice

No laser

- Usually not real time

Real time

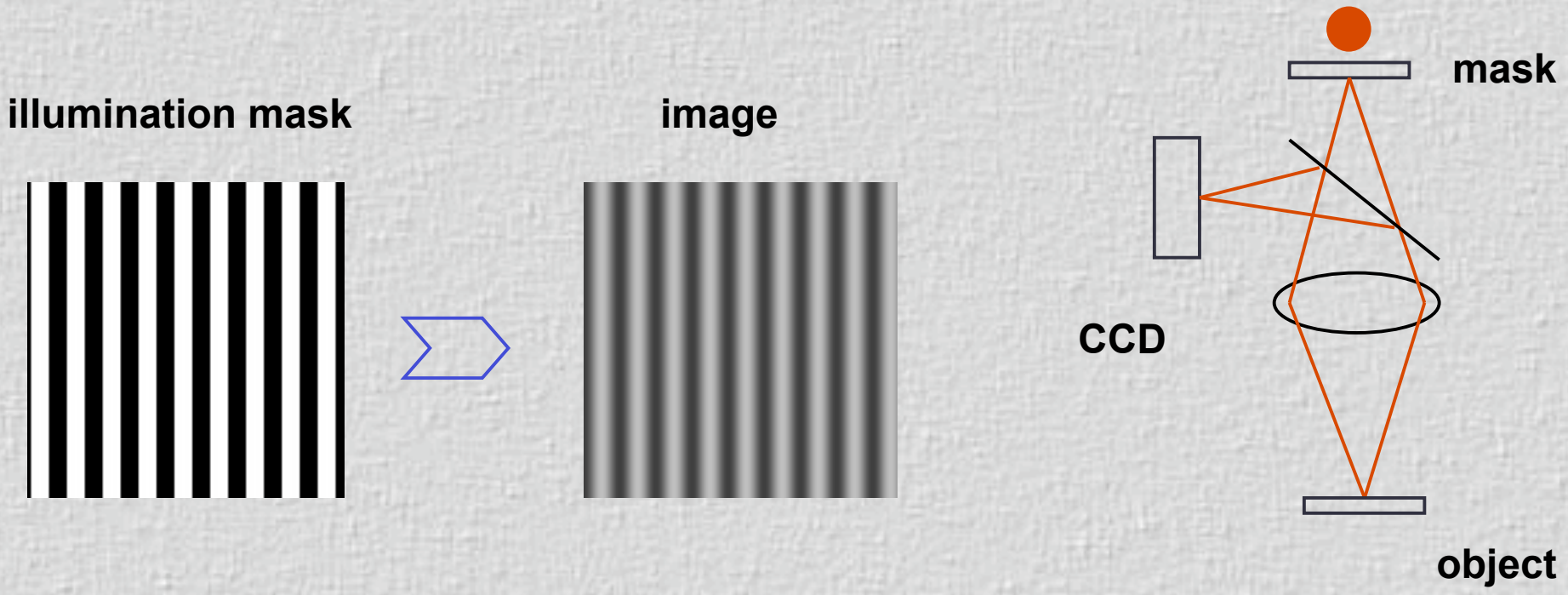
- Pinhole alignment problems

Easy alignment

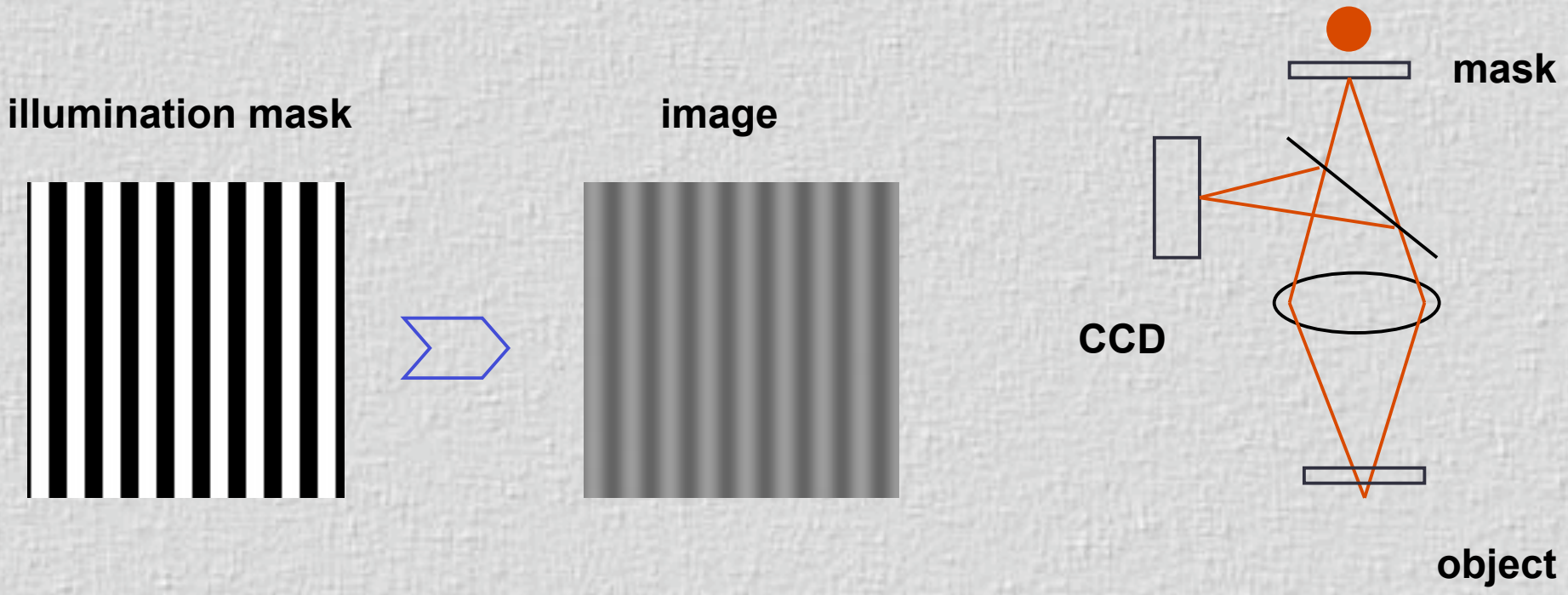
- Need to scan

No scanning

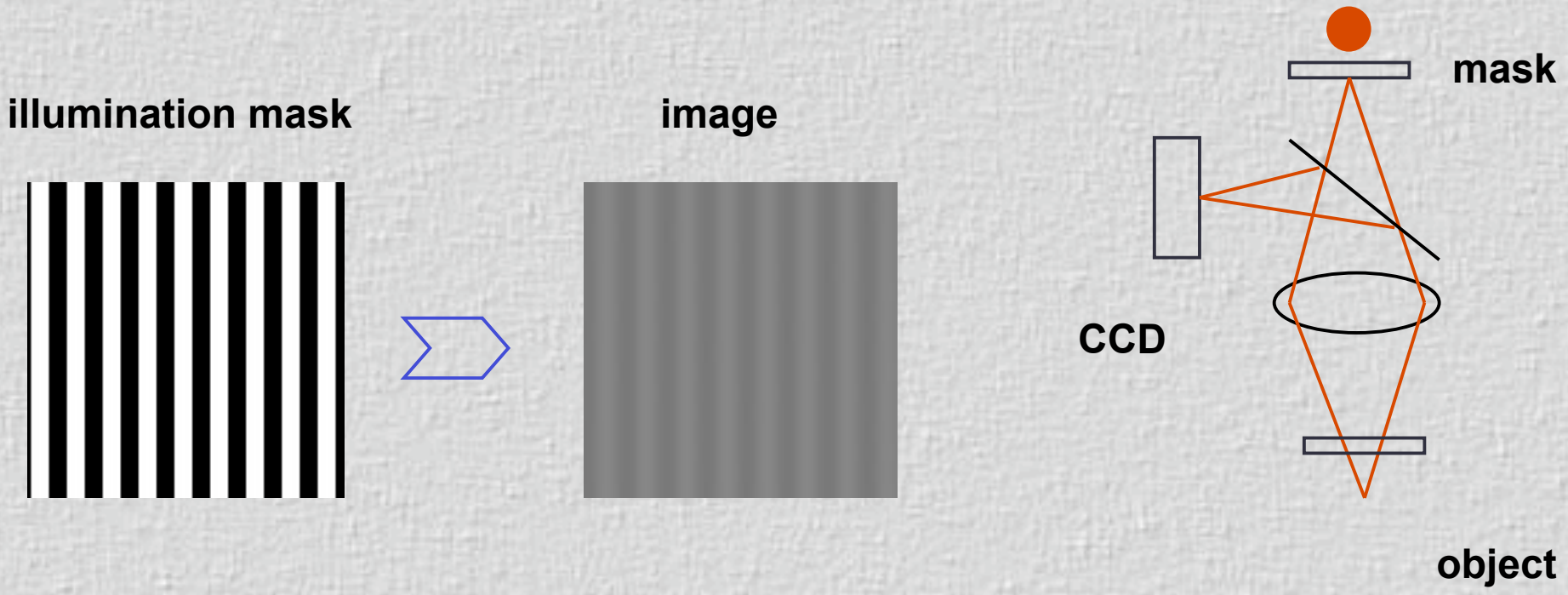
Example: grid illumination



Example: grid illumination



Example: grid illumination



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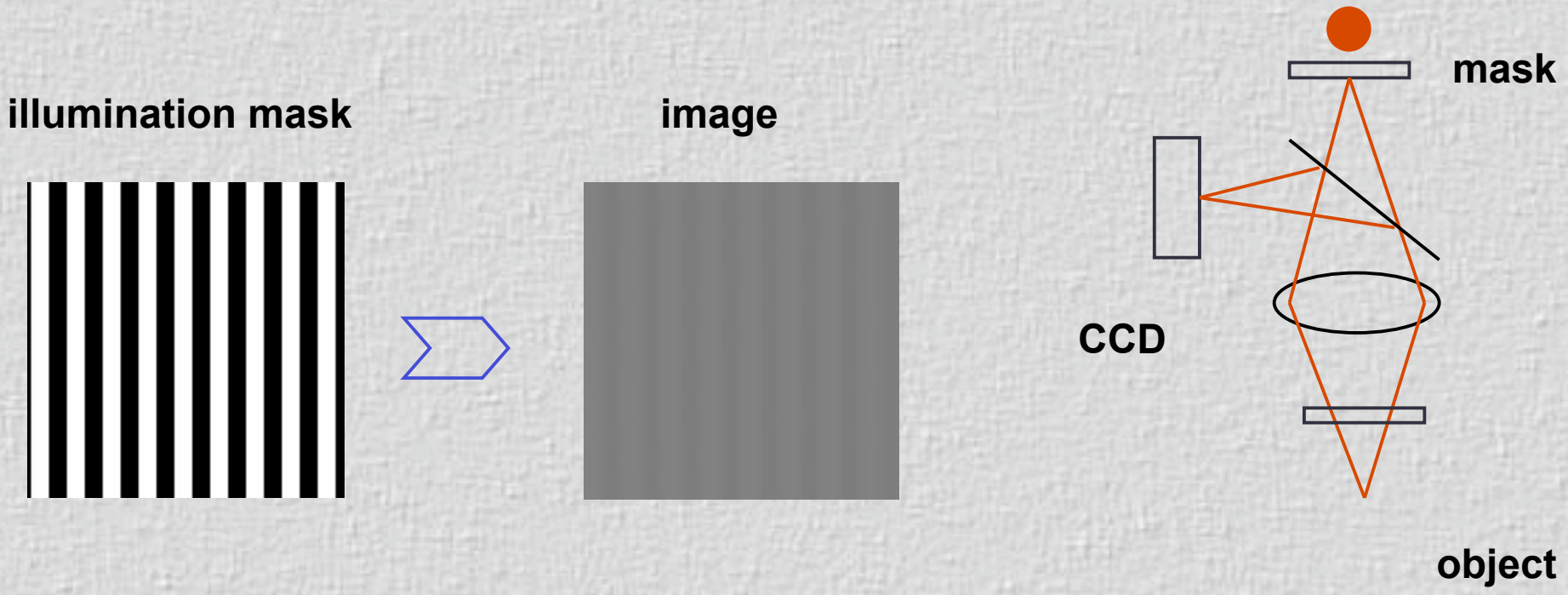
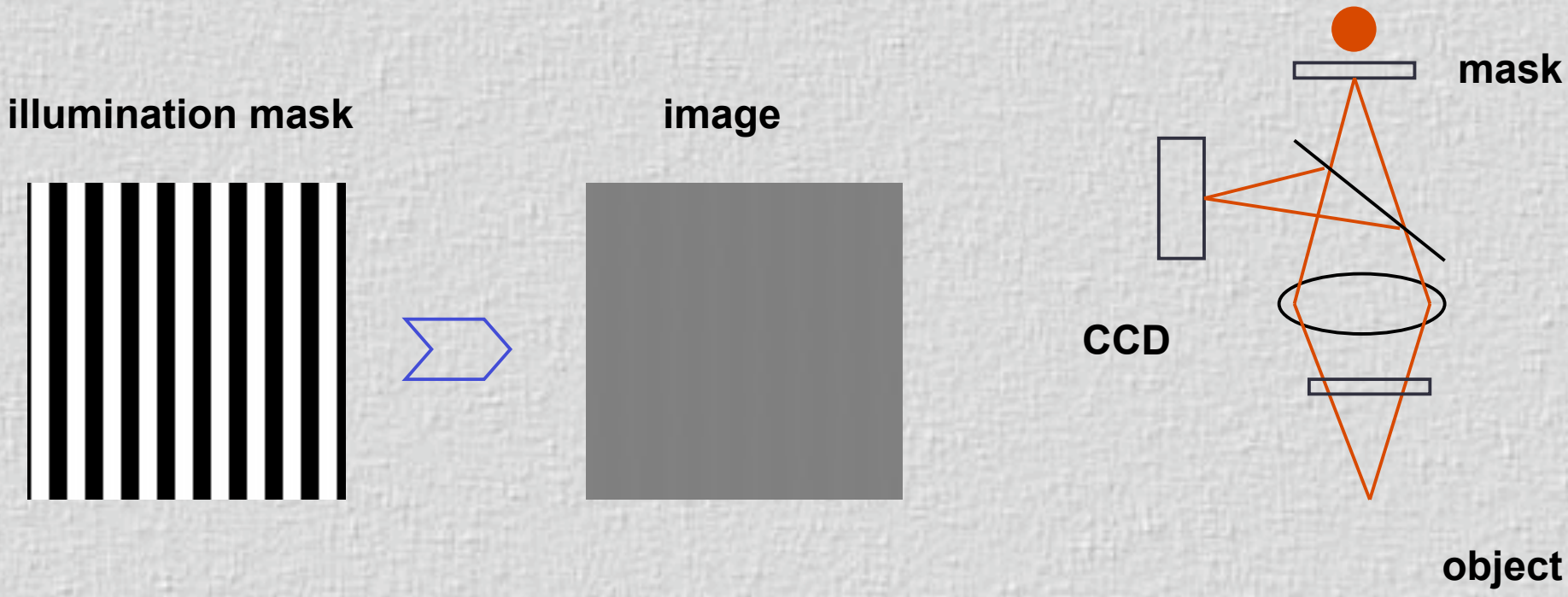


Example: grid illumination



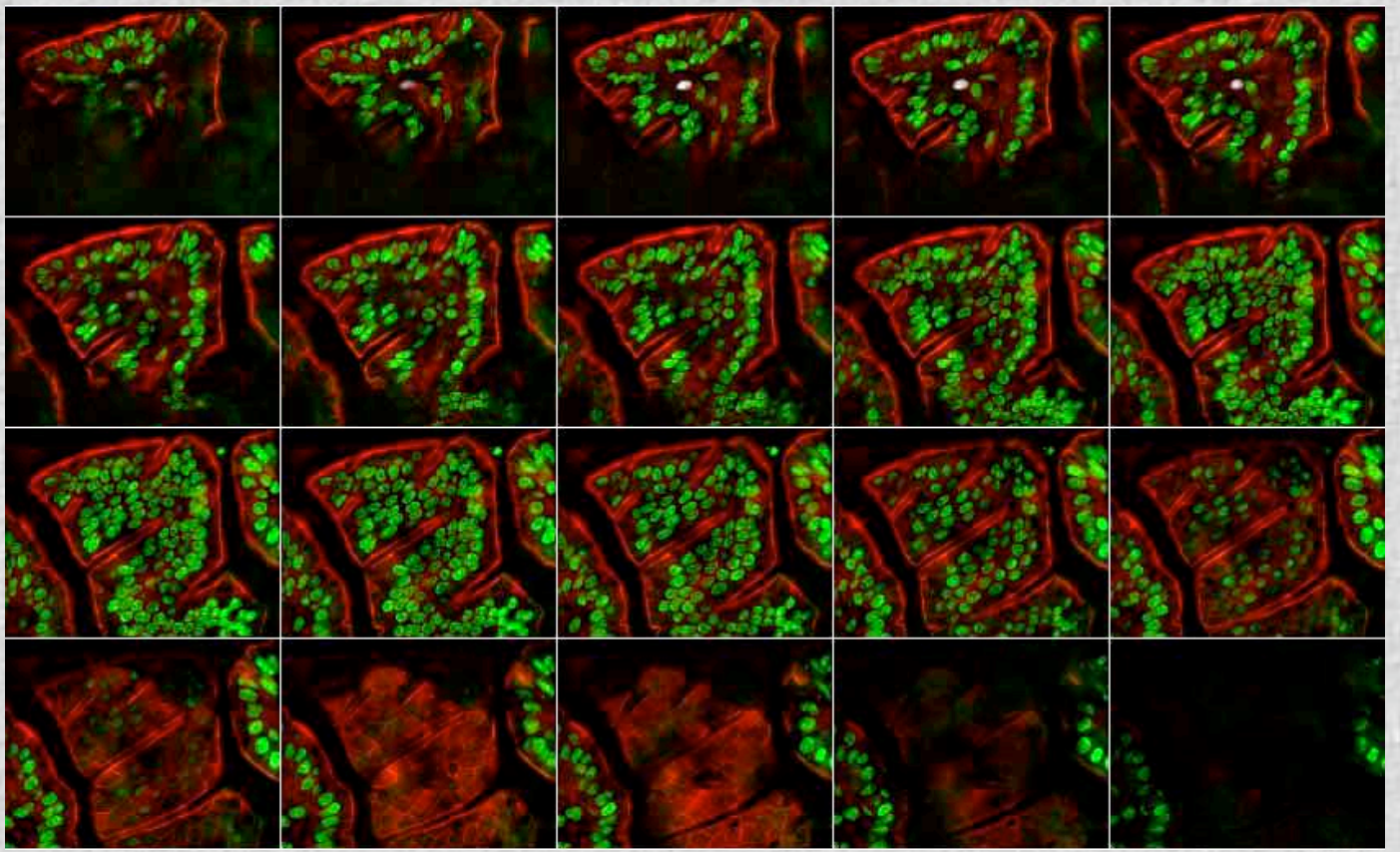
Structured illumination microscopy

impart pattern on the illuminating light beam → extract part of the image containing the pattern → remove the pattern

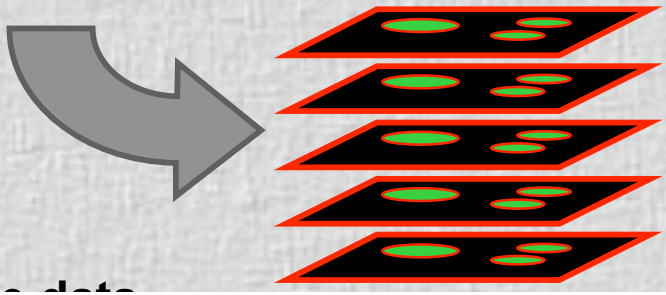
modulation | selective detection | demodulation

Add-on to conventional microscope

Sectioning microscopy

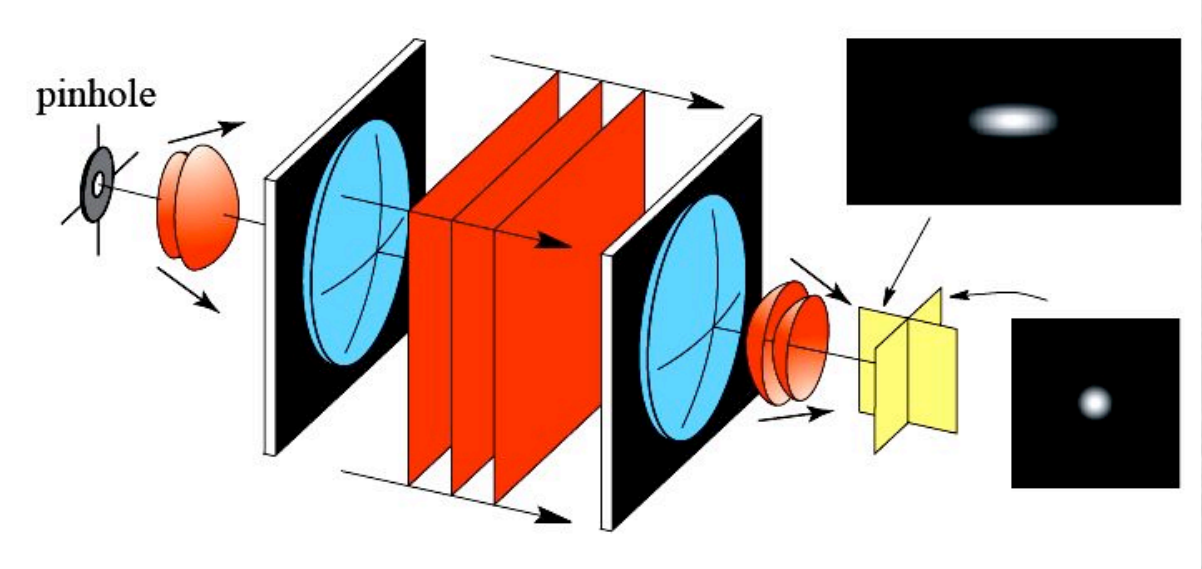


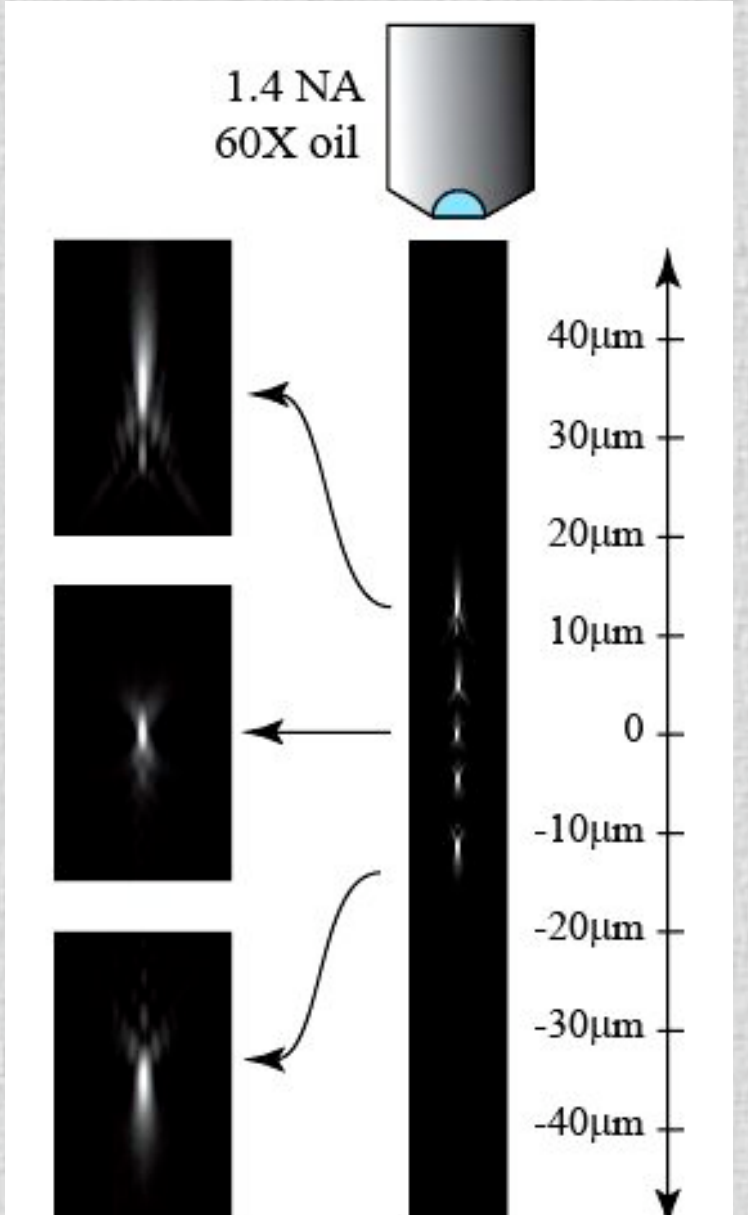
Through-focus
image sections



3D image data

Scanning approaches

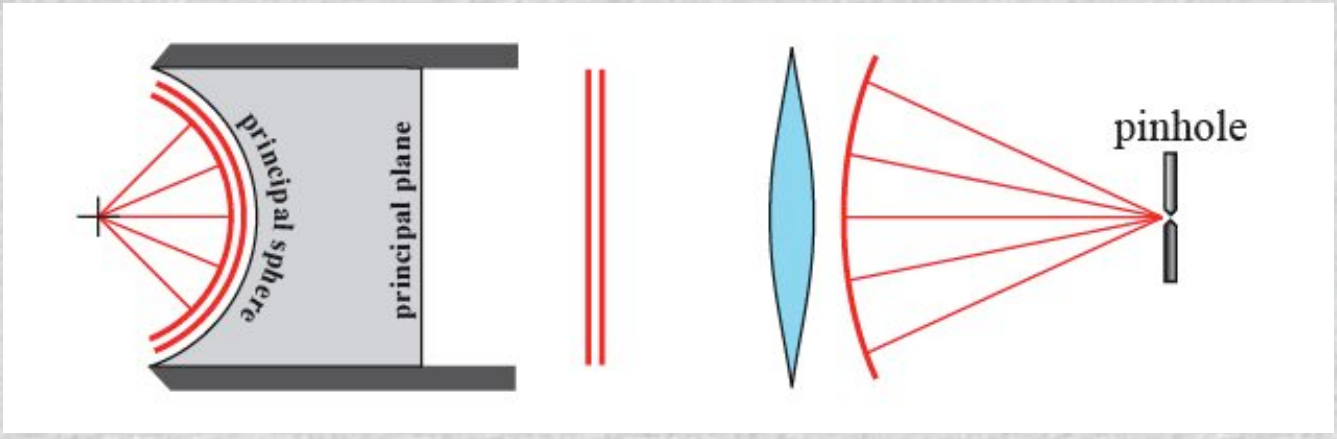




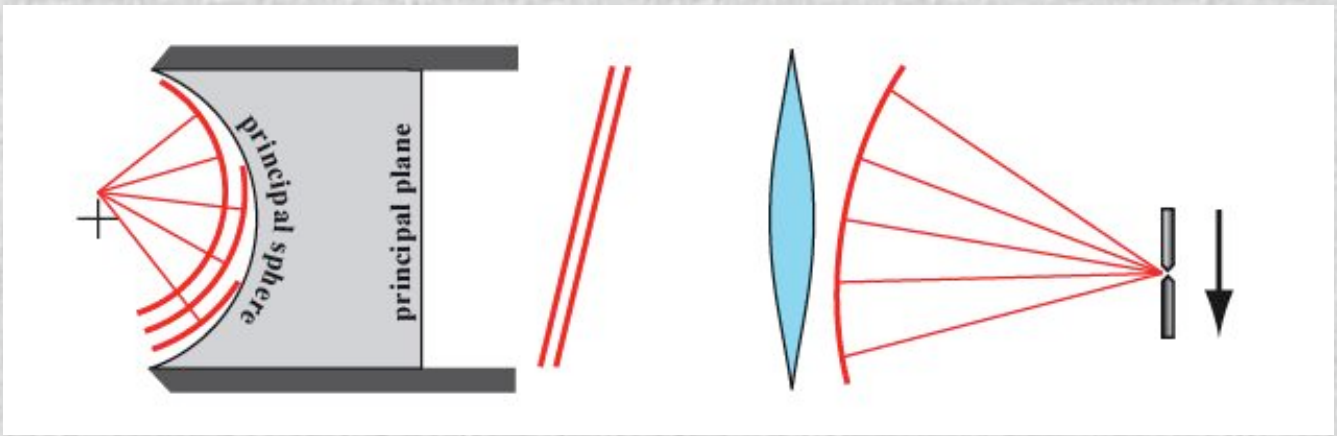
- **Scan sample xyz**
- **xy optically z sample**
  - Galvanometers – single point
  - Nipkow disc – many points
- **xy optically z optically**
  - AOM
  - Vari-focus lenses

The sine condition

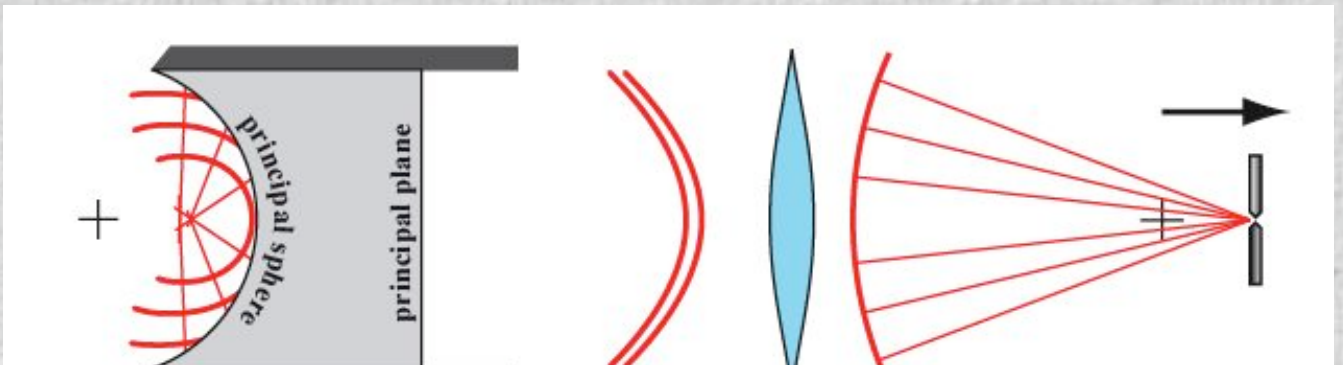
- Focus



- Lateral



- Axial



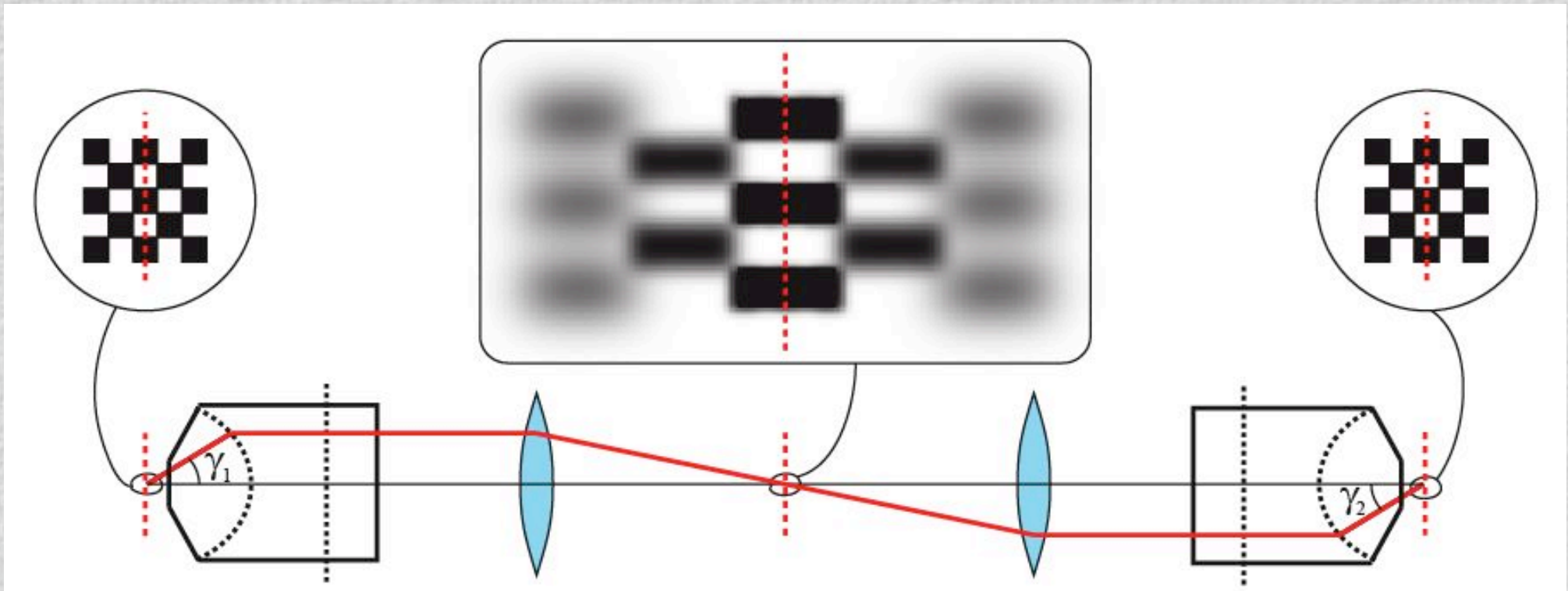
Perfect imaging

$$u'(x',\ y',\ z') \quad = \quad C \ \ u\left(\frac{x'}{M},\ \frac{y'}{M},\ \frac{z'}{M}\right)$$

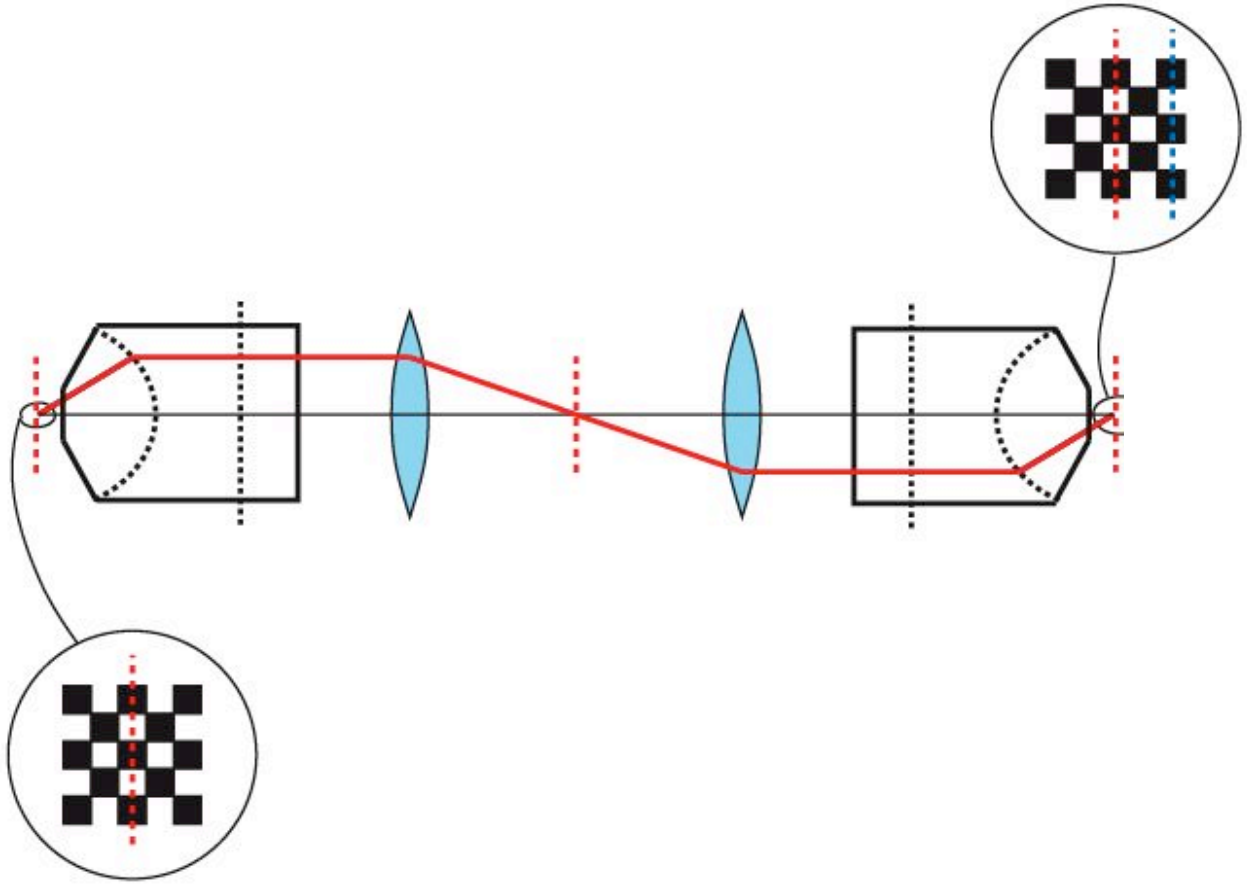
Geometrical optics -- **sine, Herschel, Maxwell conditions**

Wave theory

$$|M| \quad = \quad \frac{n_1}{n_2} \qquad \gamma_1 \quad = \quad \gamma_2$$



Transmission system



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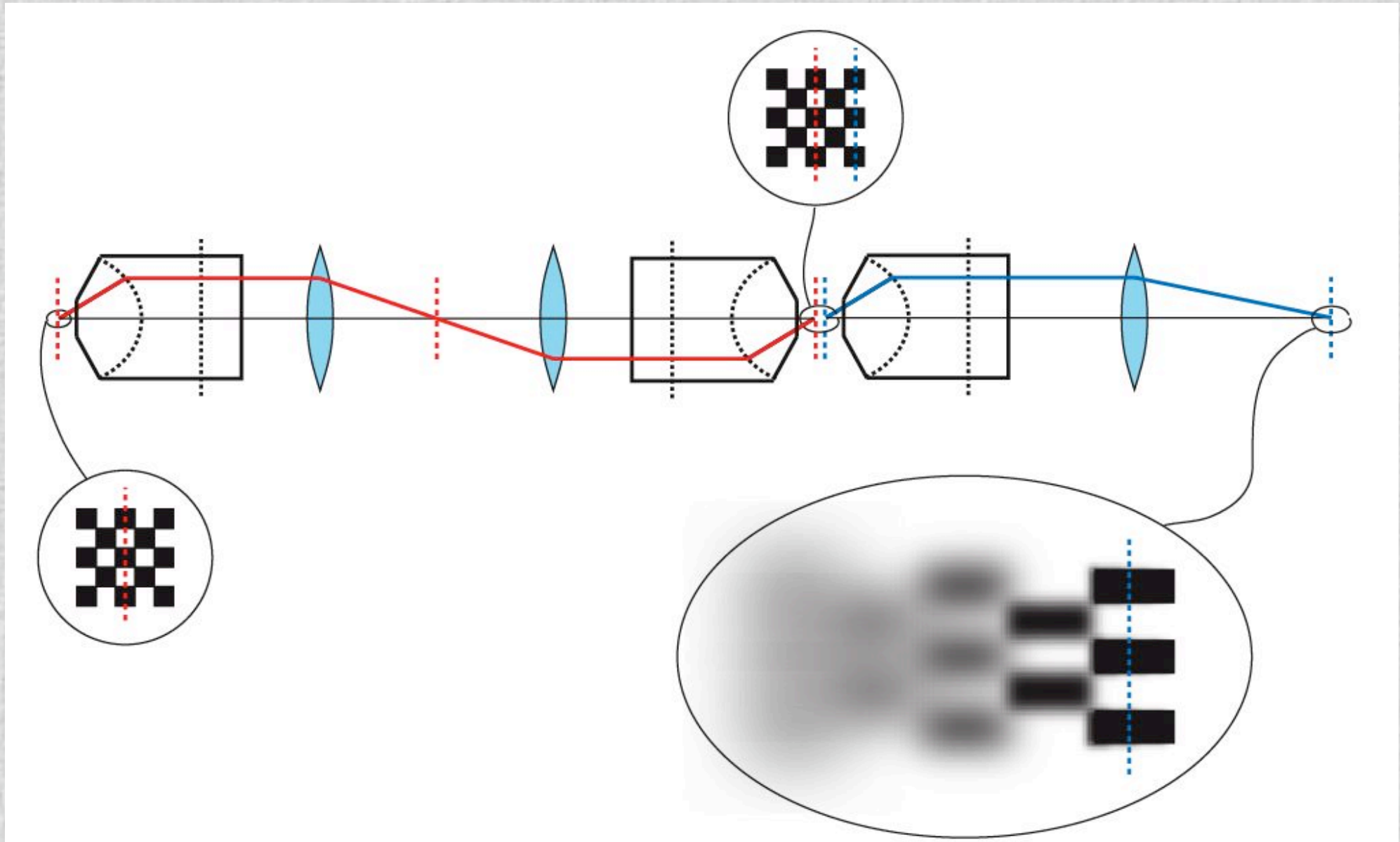
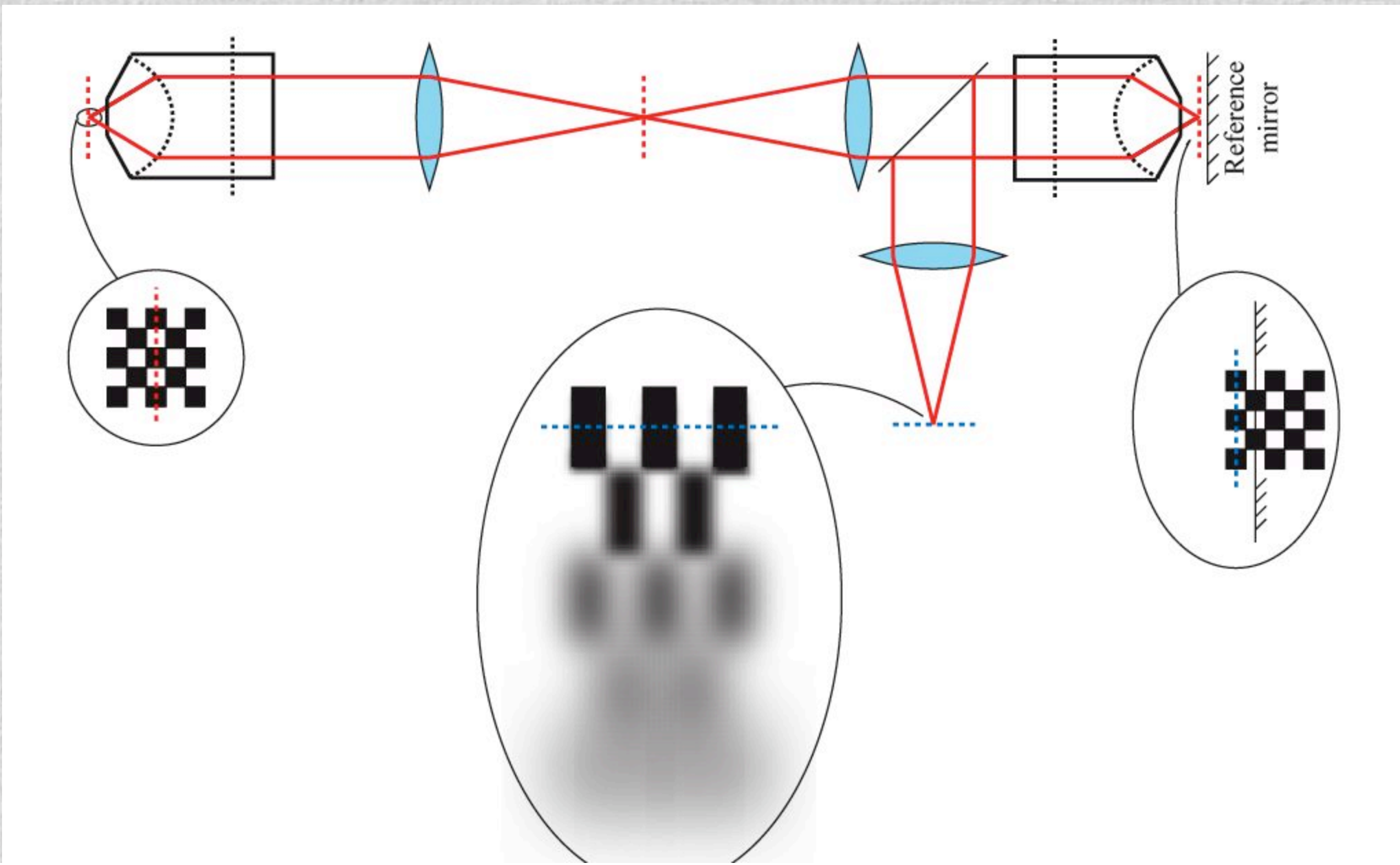
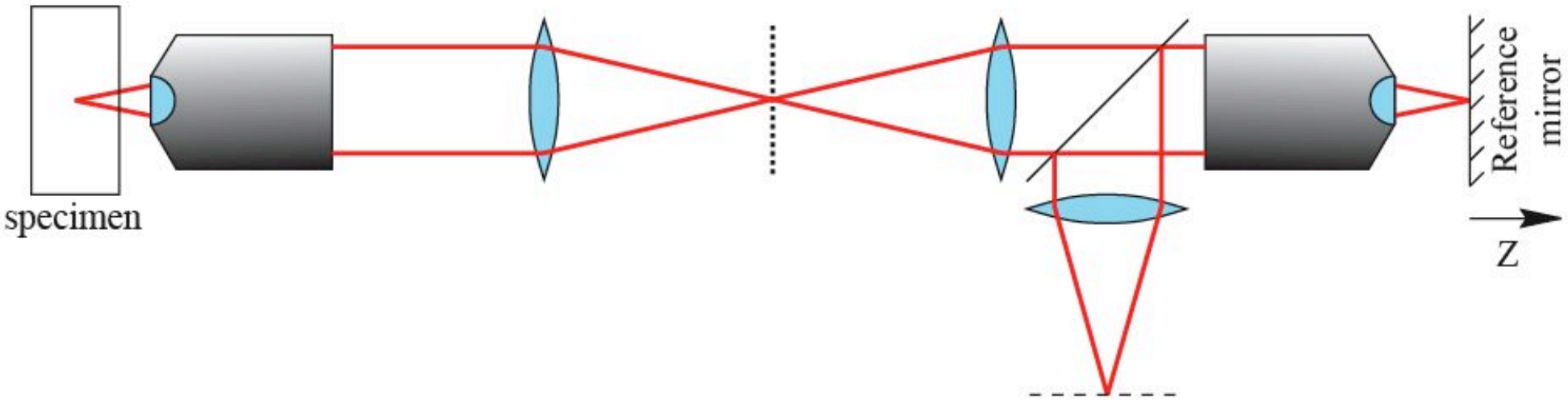


Reflection system

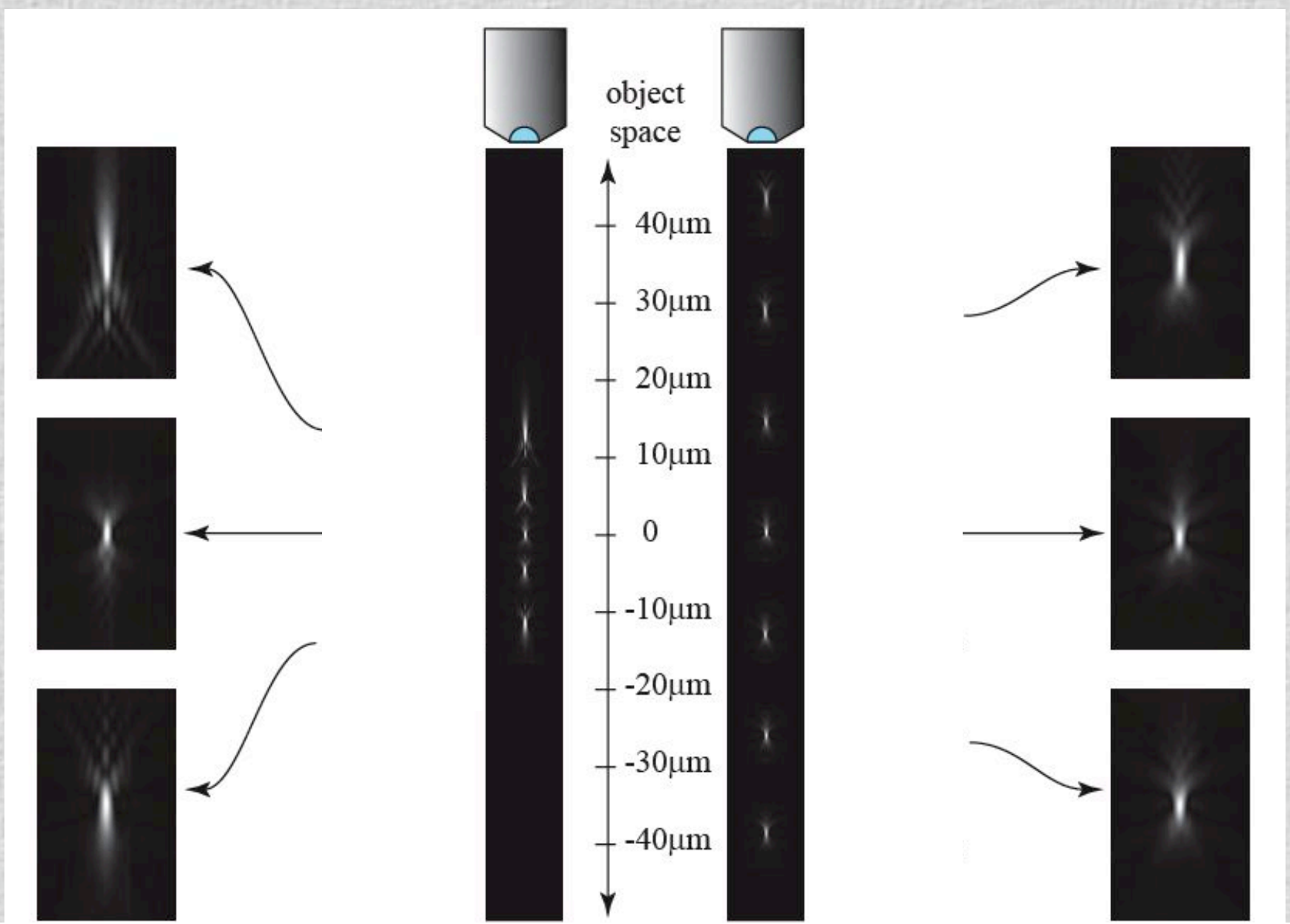


Summary of appproach

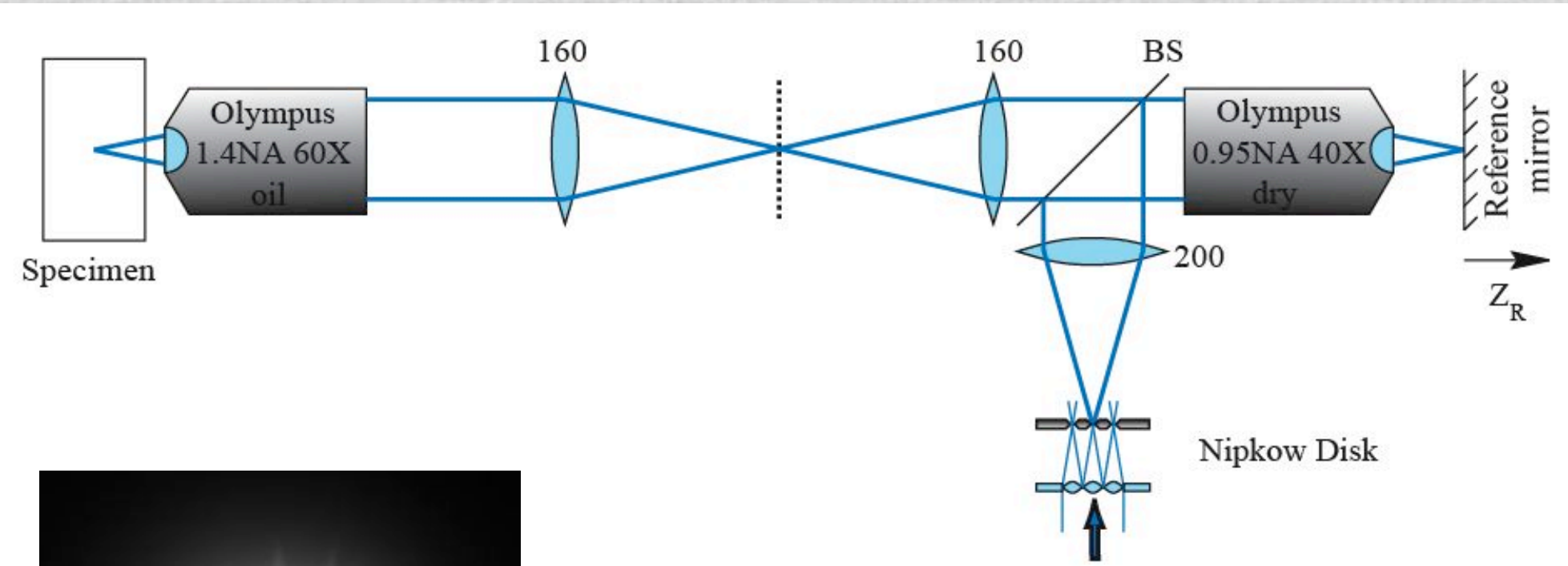


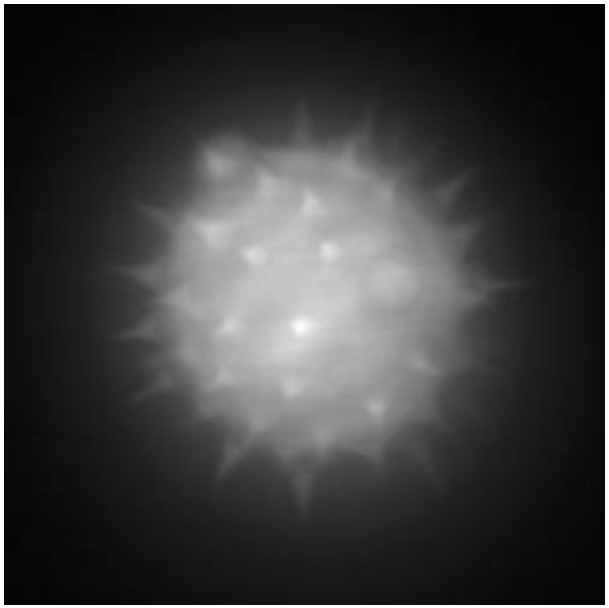
- ‘relay’ specimen to remote space
- Perfect image of object formed
  sine and Herschel conditions both fulfilled
- Fast focussing via mirror
- Specimen remains stationary during imaging

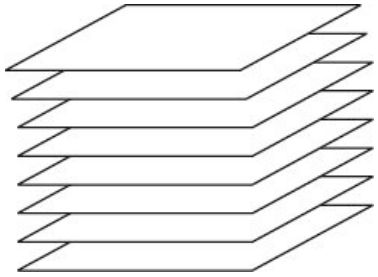
New system

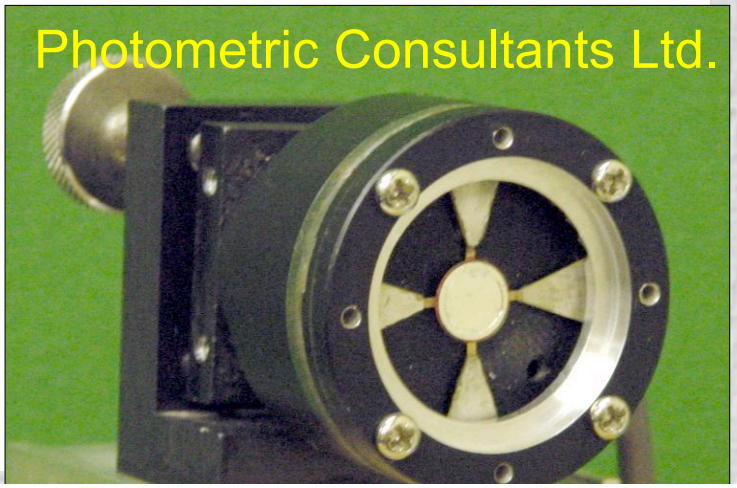


Extended depth of field









Summary

Confocal microscope

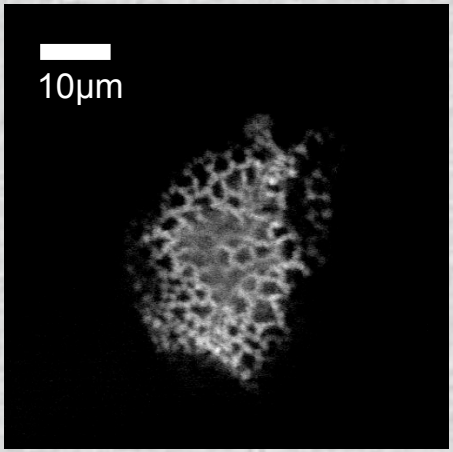
Light efficient implementation

aperture correlation

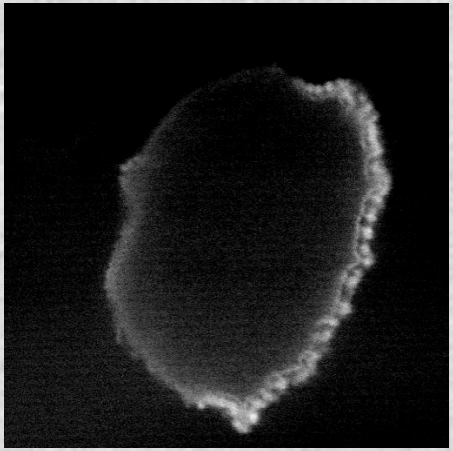
structured illumination

Fast focussing

live cell imaging



In focus



+ 20µm