Waves everywhere:
Modeling microscopy images using Fourier optic

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Introduction

Understanding the propagation of the light from the sample to the camera

Why

- to estimate the performance of setups
- to recognize the degradation induced by to the microscope (and possibly correct it)

How

- traveling from light propagation to PSF modeling
- traveling through the equations
- illustrating many phenomena using PropagationLab *icy plugin*

https://github.com/FerreolS/PropagationLab

Helmholtz equation

In **homogeneous** medium the **scalar** electric field is such that:

$$\nabla^2 E(\boldsymbol{r}) + k^2 E(\boldsymbol{r}) = 0 \text{ with } \begin{cases} k &= \frac{2\,\pi}{\lambda} \\ \boldsymbol{r} &= (x, y, z) \end{cases}$$

- **Plane wave as solution of Helmholtz equation**

$$P(\boldsymbol{r}) = \exp\left(\pm \jmath\, \boldsymbol{k}^\top \boldsymbol{r}\right) \text{ s.t. } \|\boldsymbol{k}\|_2^2 = k^2$$

General solution:
3D wavefield as a linear combination of plane waves

Complex representation of plane waves

Electric field of a plane wave going in the direction **k**

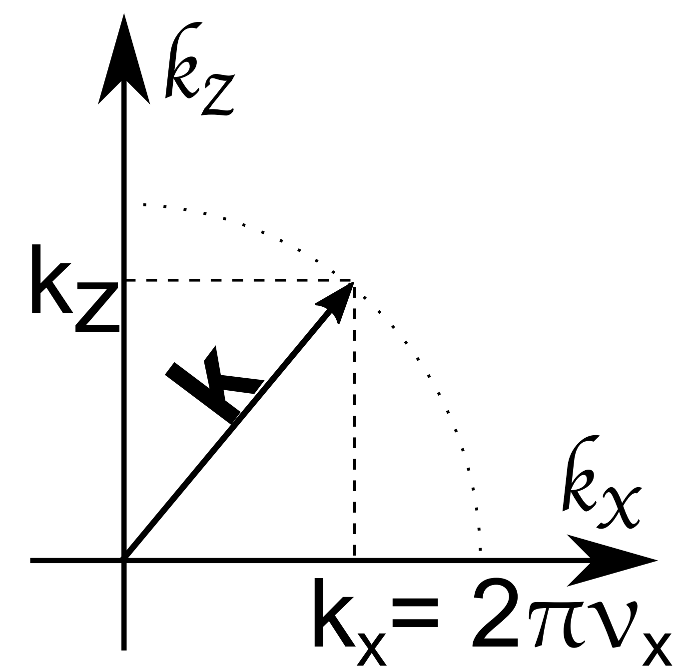
$$P_{\boldsymbol{k}}(\boldsymbol{r},t) = \cos\left(\boldsymbol{k}^\top \boldsymbol{r} - \omega\, t\right)$$
$$= \mathrm{Re}\left(\underbrace{\mathrm{e}^{\jmath\, \boldsymbol{k}^\top \boldsymbol{r}}}_{P_{\boldsymbol{k}}(\boldsymbol{r})} \mathrm{e}^{-\jmath\, \omega\, t}\right)$$

wavenumber

$$k = \frac{2\,\pi}{\lambda} = \frac{\omega}{c}$$
$$= \|\boldsymbol{k}\|_2$$
$$= \sqrt{k_x^2 + k_y^2 + k_z^2}$$

Complex amplitude

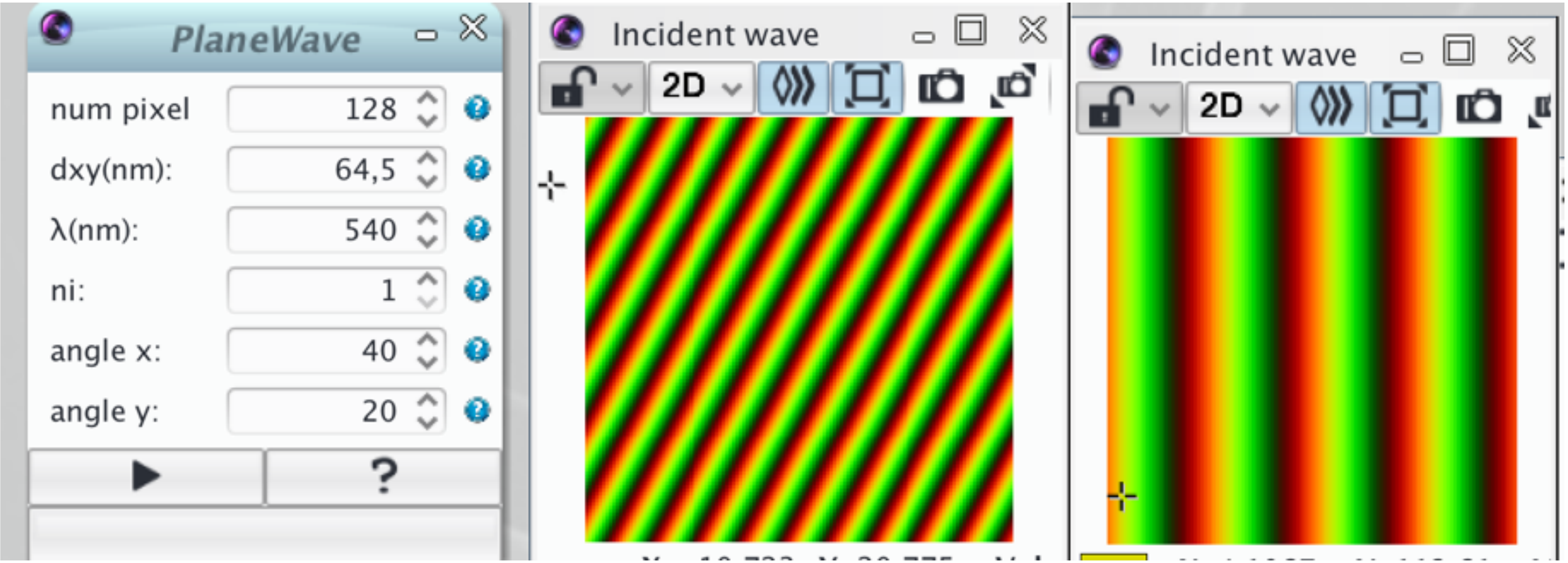
$$P_{\boldsymbol{k}}(\boldsymbol{r}) = \exp\left(\jmath\, \boldsymbol{k}^\top \boldsymbol{r}\right)$$



with $\boldsymbol{k}^\top \boldsymbol{r} = k_x\, x + k_y\, y + k_z\, z$

The vector $\boldsymbol{k} = \begin{pmatrix} k_x \\ k_y \\ k_z \end{pmatrix}$ pointing in the direction of propagation

Complex representation of plane waves



3D wavefield

- **3D wavefield is a sum of plane waves**

$$E(\boldsymbol{r}) = \iiint_{-\infty}^{+\infty} \hat{E}(\boldsymbol{k}) \exp\left(\jmath\, \boldsymbol{k}\, \boldsymbol{r}\right) \mathrm{d}\boldsymbol{k}$$

A Fourier transform!

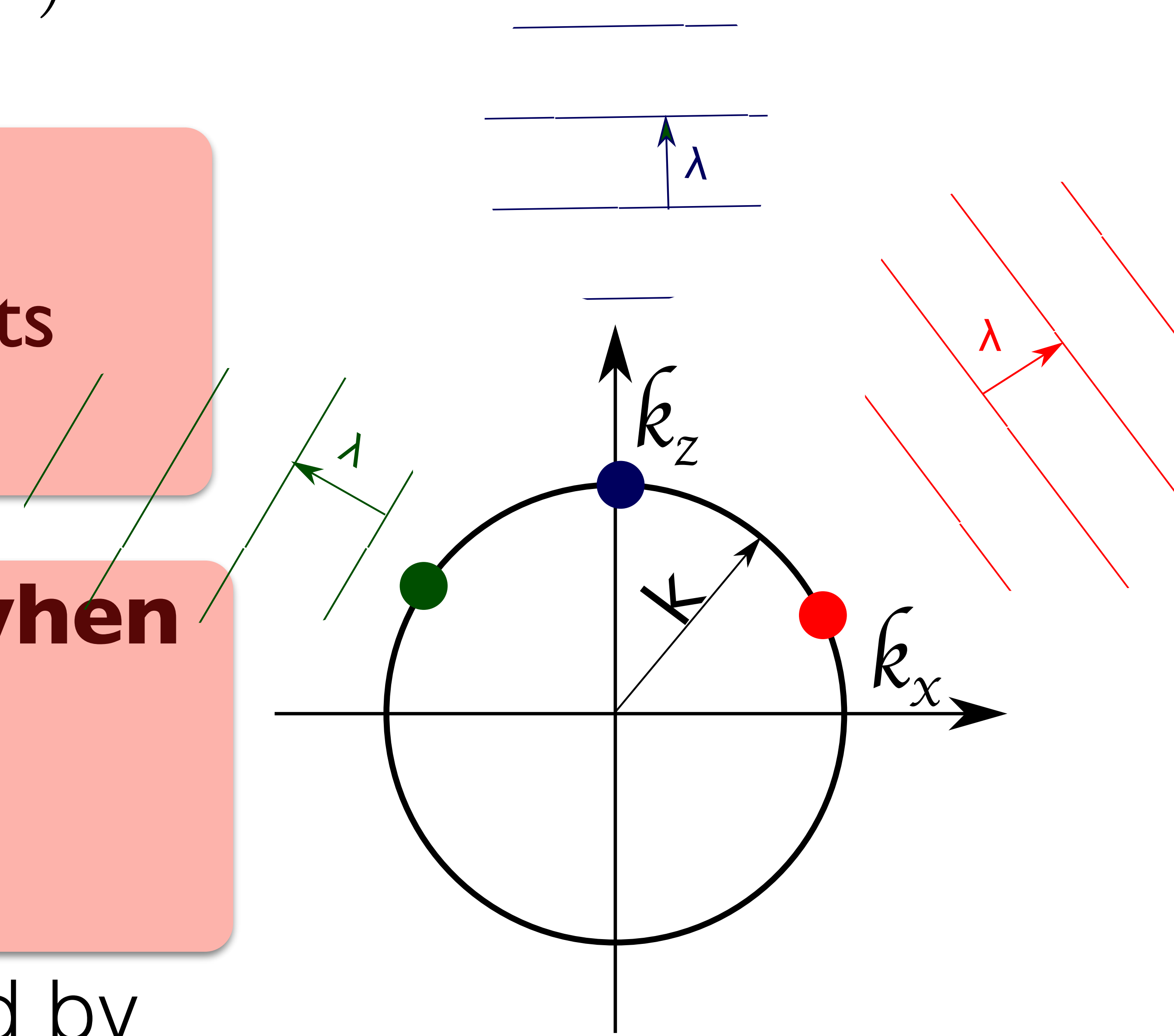
Easy computation of coefficients

$$\hat{E}(\boldsymbol{k}) = \rho\, \mathrm{e}^{\jmath\, \phi}$$

Waves propagates only when

$$\|\boldsymbol{k}\|_2 = \frac{2\,\pi}{\lambda}$$

The 3D field is fully described by the 2D surface of the Ewald sphere



Ewald sphere

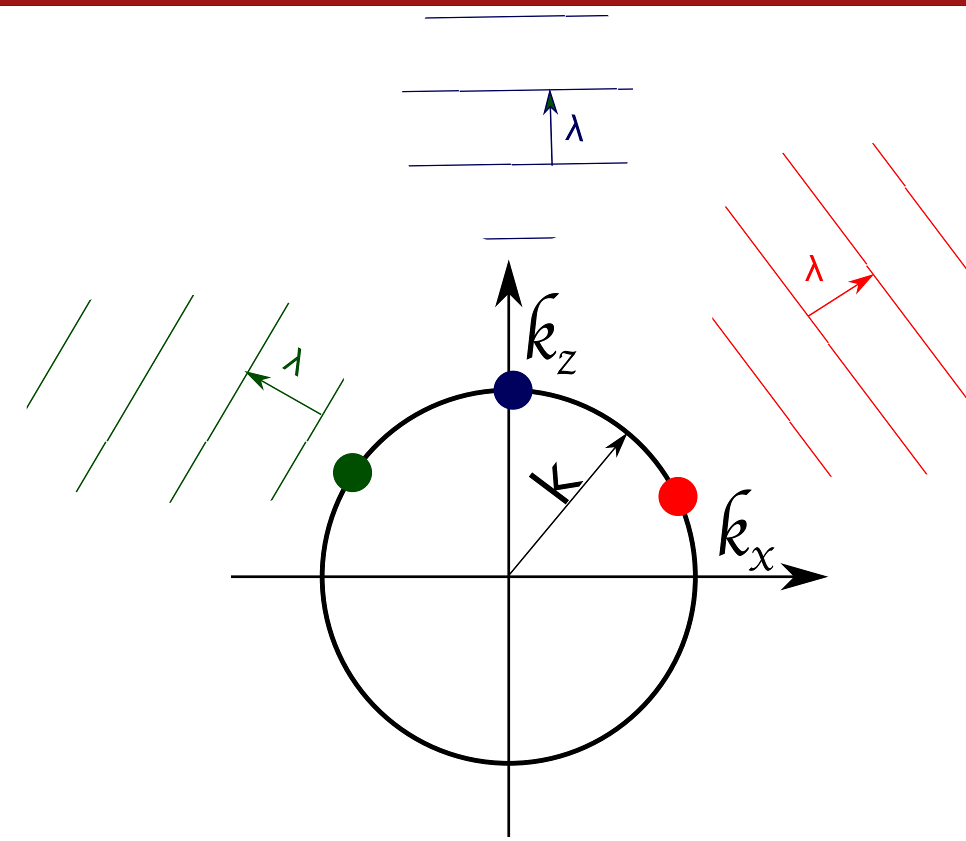
Angular spectrum representation

2D formulation

Plane wave E can be re-written as:

$$k_x^2 + k_y^2 + k_z^2 = k^2 \Longleftrightarrow k_z = \pm\sqrt{k^2 - k_x^2 - k_y^2}$$

$$E(x,y,z) = \mathrm{e}^{\jmath\,(k_x\,x + k_y\,y + z\,k_z)}$$
$$= \mathrm{e}^{\jmath\,(k_x\,x + k_y\,y)}\,\mathrm{e}^{\pm\jmath\,z\,\sqrt{k^2 - k_x^2 - k_y^2}}$$



Properties

$$k_z = -\sqrt{k^2 - k_x^2 - k_y^2} \longrightarrow \text{wave propagating in the half-space } z < 0$$

$$k_z = +\sqrt{k^2 - k_x^2 - k_y^2} \longrightarrow \text{wave propagating in the half-space } z > 0$$

$$k_x^2 + k_y^2 \leq k^2 \longrightarrow \text{propagating wave}$$

$$k_x^2 + k_y^2 > k^2 \longrightarrow \text{non-propagating wave: } \textbf{evanescent} \text{ wave}$$

Angular spectrum representation

Forward propagating plane wave:

$$P(x,y,z) = \mathrm{e}^{\jmath\,(k_x\,x+k_y\,y)}\,\mathrm{e}^{+\jmath\,z\,\sqrt{k^2-k_x^2-k_y^2}}$$

The Fourier transform becomes 2D

Wave-field at z=0 : $E(x,y,0) = \iint_{-\infty}^{+\infty} \hat{E}(k_x,k_y;0)\,\mathrm{e}^{\jmath\,(k_x\,x+k_y\,y)}\mathrm{d}k_x\,\mathrm{d}k_y$

Wave-field at z : $E(x,y,z) = \iint_{-\infty}^{+\infty} \hat{E}(k_x,k_y;0)\,\mathrm{e}^{\jmath\,z\,k_z}\,\mathrm{e}^{\jmath\,(k_x\,x+k_y\,y)}\mathrm{d}k_x\,\mathrm{d}k_y$

The 3D wave-field is totally described by the 2D wave-field at z=0

$$\hat{E}(k_x,k_y;z) = \hat{h}_z(k_x,k_y)\,\hat{E}(k_x,k_y;0)$$

$$\hat{h}_z(k_x,k_y) = \mathrm{e}^{\jmath\,z\sqrt{k^2-k_x^2-k_y^2}}$$

Angular spectrum propagator

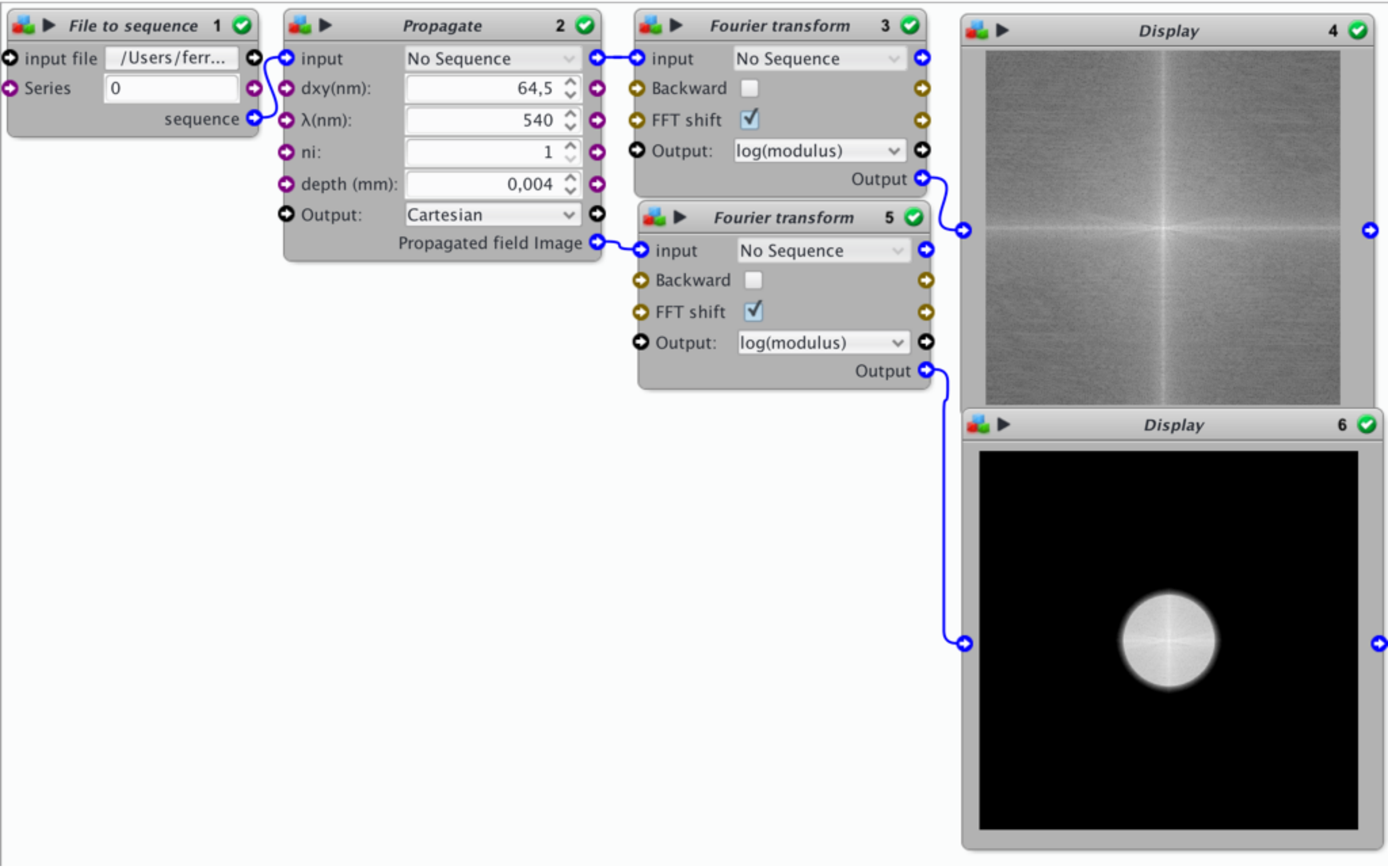
Propagation of plane wave

$$\hat{h}_z(k_x, k_y) = \mathrm{e}^{\jmath\, z \sqrt{k^2 - k_x^2 - k_y^2}}$$

Angular spectrum propagator

- Each plane wave propagates independently
- propagation is just a phase shift
- phase shifting is more important for high frequencies
- frequencies higher than k are cut

Propagation cutoff



Interaction with a planar sample

Complex transmission of the sample

$$o_r = \mathrm{e}^{(\jmath\, k\, n_r - \mu_r)s_r}$$

thickness

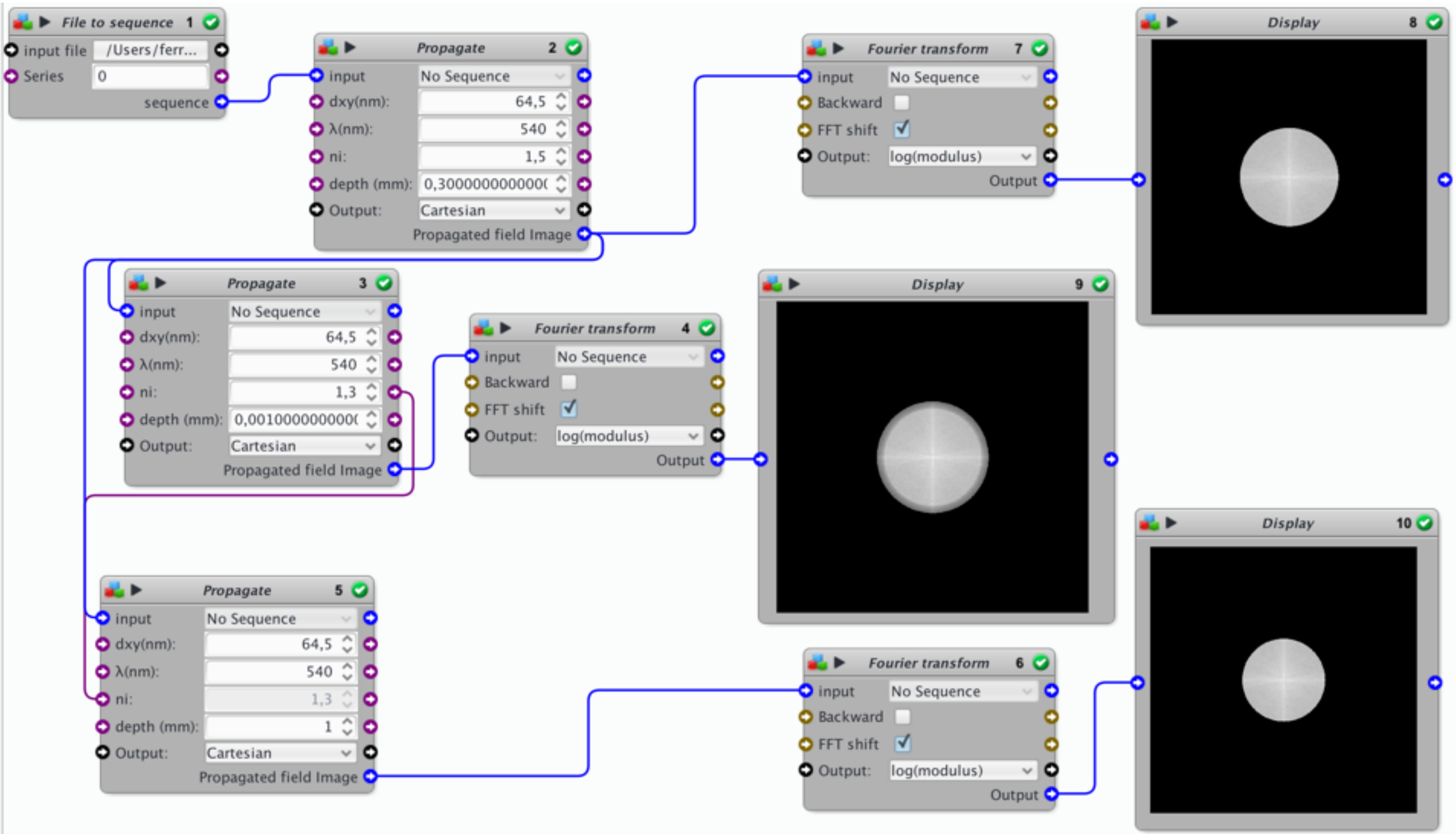
refractive index

attenuation

Complex amplitude after interaction

$$E_t(\boldsymbol{r}) = E_i(\boldsymbol{r})\, o(\boldsymbol{r})$$

Evanescent waves



Fresnel approximation

$$\hat{h}_z(k_x, k_y) = \mathrm{e}^{\jmath\, z \sqrt{k^2 - k_x^2 - k_y^2}}$$

Angular spectrum propagator

$$\sqrt{k^2 - k_x^2 - k_y^2} = k\,\sqrt{1 - \frac{k_x^2 + k_y^2}{k^2}} \approx k\left(1 - \frac{k_x^2 + k_y^2}{2\,k^2}\right)$$

Amounts to paraxial approximation:
the field varies slowly in the transverse direction.

$$\hat{h}_z(k_x, k_y) = \mathrm{e}^{-\jmath\, \frac{k_x^2 + k_y^2}{2\,k}}$$

Fresnel propagator

Fresnel in space domain

$$\hat{h}_z(k_x, k_y) = \mathrm{e}^{-\jmath \, \frac{k_x^2 + k_y^2}{2\,k}}$$

Fresnel propagator

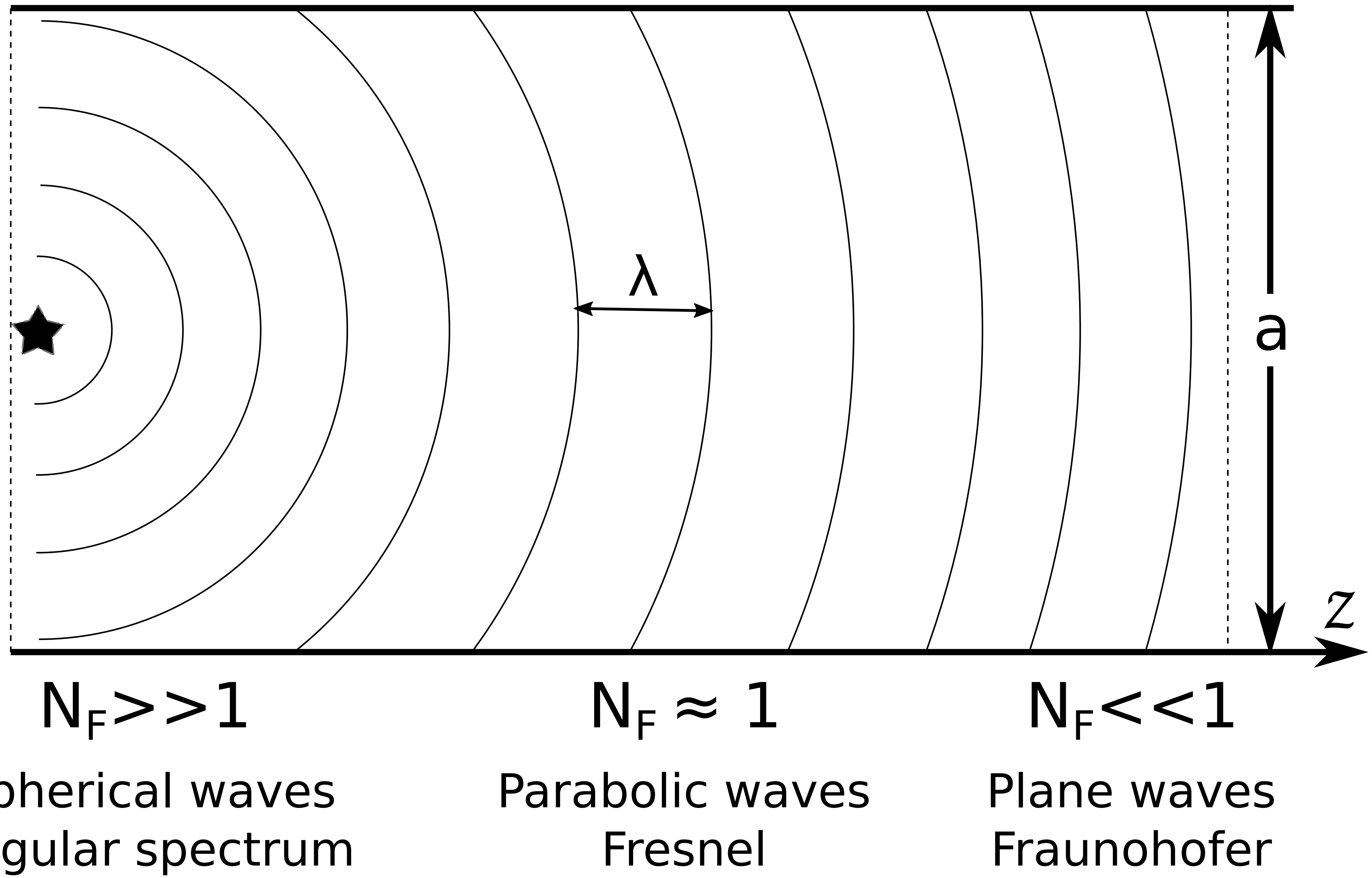
Multiplication in Fourier = convolution in space

- **Fresnel propagator in space domain (convolution)**

$$h_z(x, y) = \frac{1}{\jmath\,\lambda\,z} \mathrm{e}^{\jmath\,\pi\,\frac{x^2 + y^2}{\lambda\,z}}$$

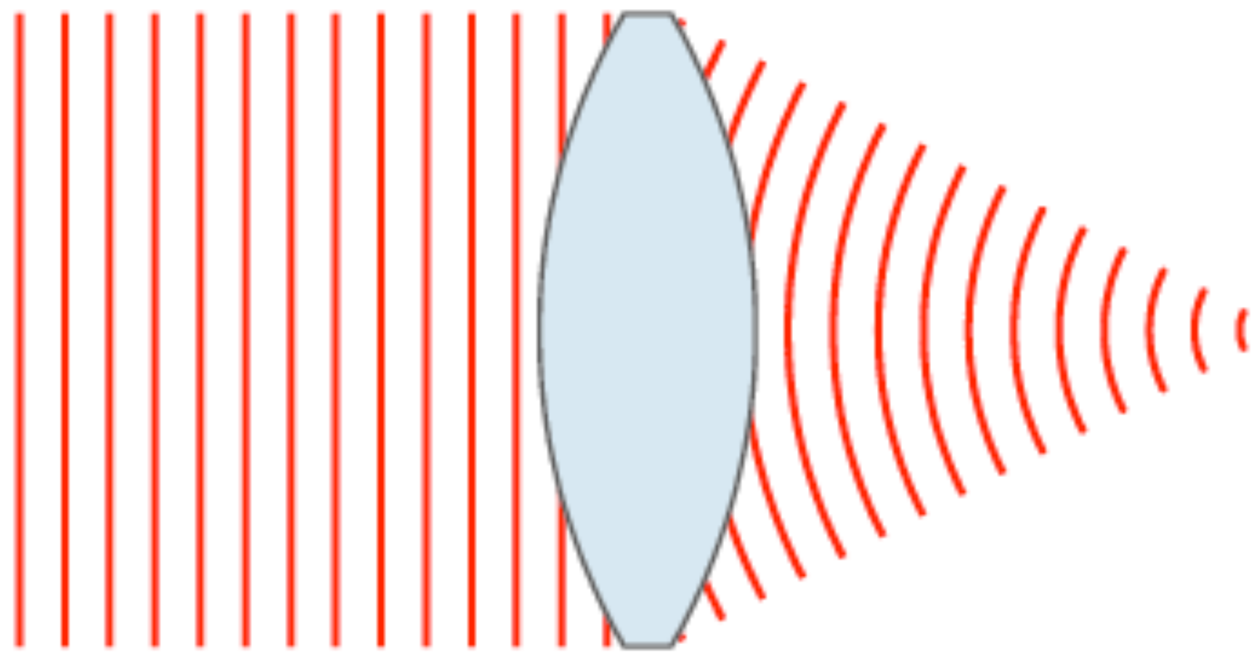
Fresnel number

Number of period across the half aperture $N_F = \frac{a^2}{\lambda z}$



Diffraction by a lens

A lens curves the wavefront



A planar wave becomes a parabolic wave

A lens of focal distance f has a complex transmittance with a parabolic phase shift:

$$T(x, y) = \exp\left(\jmath \frac{k}{2\,f}\left(x^2 + y^2\right)\right)$$

Diffraction by a lens

A lens of focal distance f has a complex transmittance

$$T(x,y) = \exp\left(\jmath \frac{k}{2\,f}\left(x^2+y^2\right)\right)$$

Right after the lens:

$$E(x,y,0^+) = T(x,y)\,E(x,y,0)$$

Propagation to the focal plane = convolution by the Fresnel function

$$E(x,y,f) = E(x,y,0^+) * h_f(x,y)$$
$$= \iint T(x',y')\,E(x',y',0)\,h_f(x-x',y-y')\,\mathrm{d}x'\,\mathrm{d}y'$$

with $h_f(x,y) = \dfrac{1}{\jmath\,\lambda\,f}\mathrm{e}^{\jmath\,\pi\,\frac{x^2+y^2}{\lambda\,f}}$

Diffraction by a lens

A lens of focal distance f has a complex transmittance

$$T(x,y) = \exp\left(\jmath \frac{k}{2\,f}\left(x^2+y^2\right)\right)$$

Right after the lens:

$$E(x,y,0^+) = T(x,y)\,E(x,y,0)$$

Propagation to the focal plane = convolution by the Fresnel function

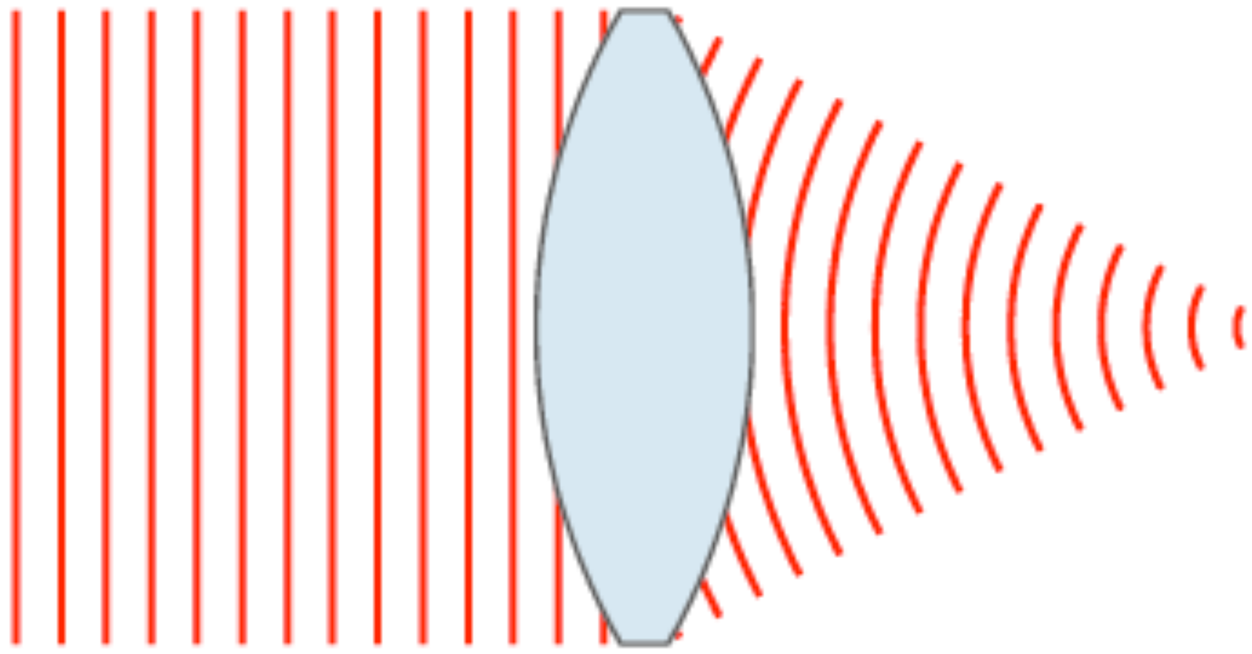
$$E(x,y,f) = \iint_{-\infty}^{+\infty} E(x',y',0)\,\mathrm{e}^{\jmath\frac{\pi}{\lambda f}\left(x'^2+y'^2\right)}\,\mathrm{e}^{-\jmath\frac{\pi}{\lambda f}\left((x-x')^2+(y-y')^2\right)}\mathrm{d}x'\,\mathrm{d}y'$$

$$E(x,y,f) = \mathrm{e}^{\jmath\frac{\pi}{\lambda f}\left(x^2+y^2\right)}\iint_{-\infty}^{+\infty} E(x',y',0)\,\mathrm{e}^{\jmath\frac{2\pi}{\lambda f}\left(x'x+y'y\right)}\,\mathrm{d}x'\,\mathrm{d}y'$$

$$= \mathrm{e}^{\jmath\frac{\pi}{\lambda f}\left(x^2+y^2\right)}\,\hat{E}\left(\frac{x}{\lambda f},\frac{y}{\lambda f},0\right)$$

Fourier transform

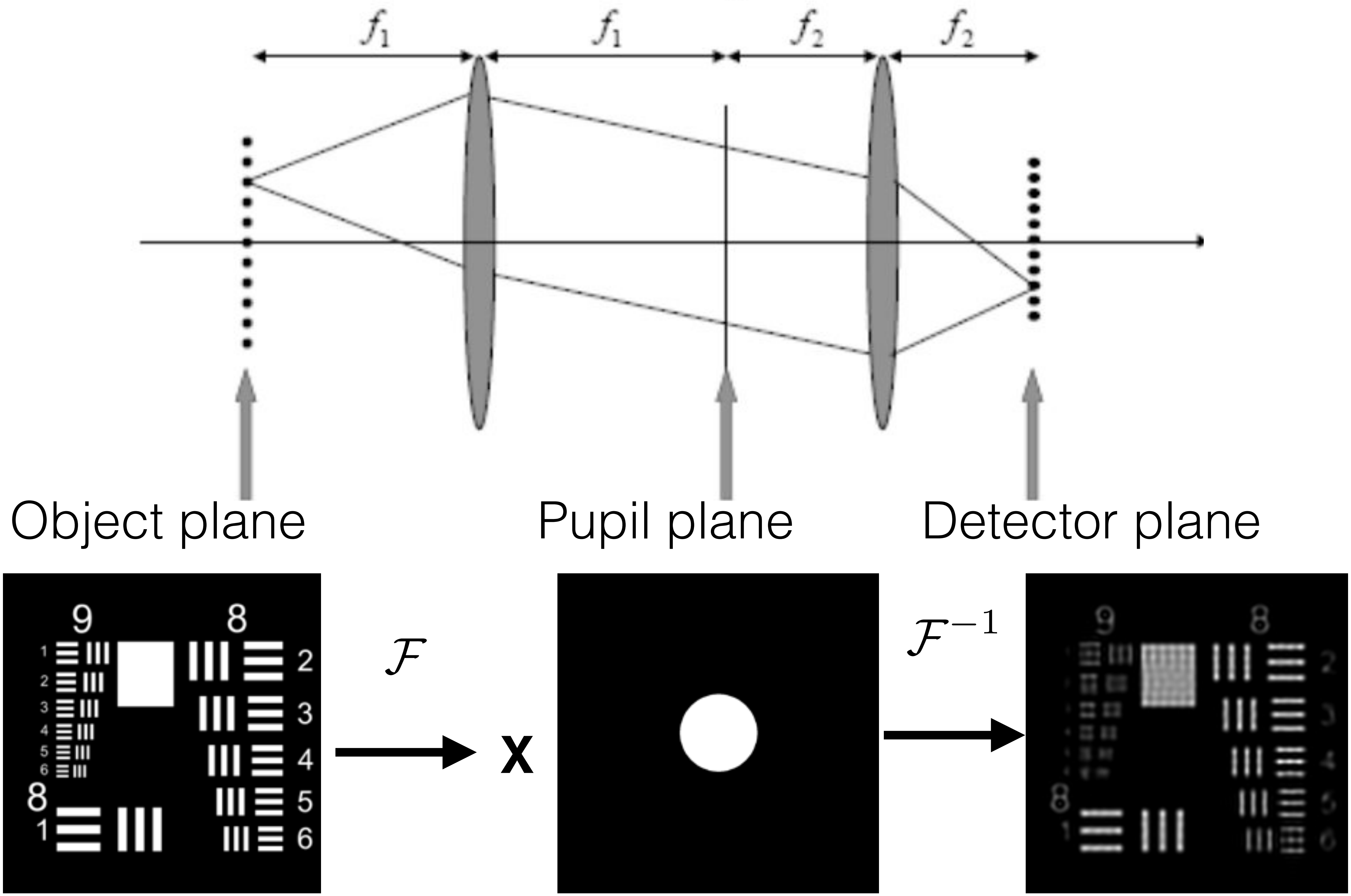
Pupil function



Lenses have finite extension -> high angles (ie frequencies) are cut.

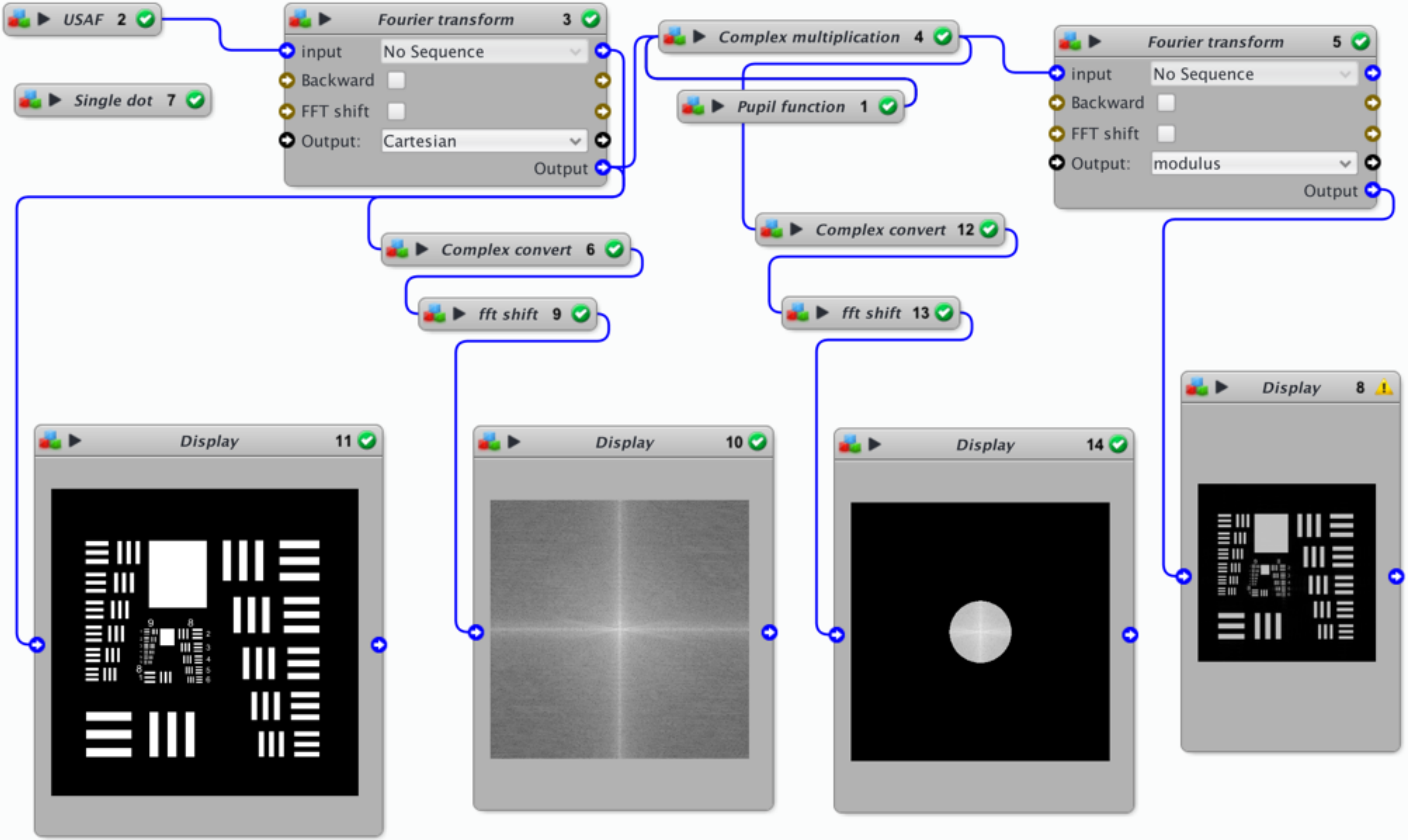
The pupil function cut frequencies higher than NA/λ

4f setup



f_1
f_1
f_2
f_2
Object plane
Pupil plane
Detector plane
$\mathcal{F}$
x
$\mathcal{F}^{-1}$

4F system


USAF 2
Single dot 7
Fourier transform 3
input
No Sequence
Backward
FFT shift
Output:
Cartesian
Output
Complex multiplication 4
Pupil function 1
Fourier transform 5
input
No Sequence
Backward
FFT shift
Output:
modulus
Output
Complex convert 6
Complex convert 12
fft shift 9
fft shift 13
Display 8
Display 11
Display 10
Display 14

Fourier ptychography

Illumination with tilted incident waves

Multiplication in space is convolution in Fourier domain:
- illumination by a plane wave shifts the spectrum of the object

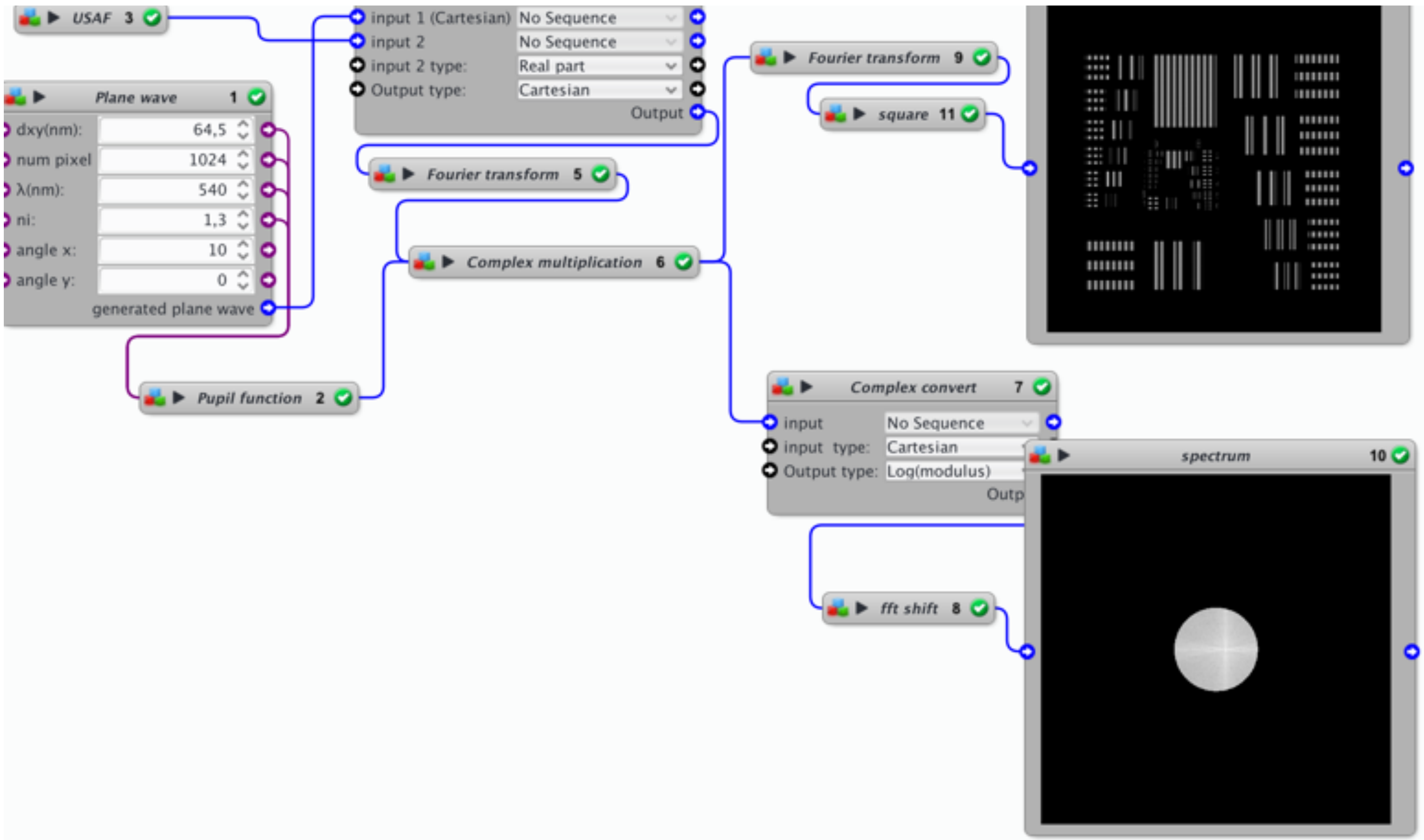
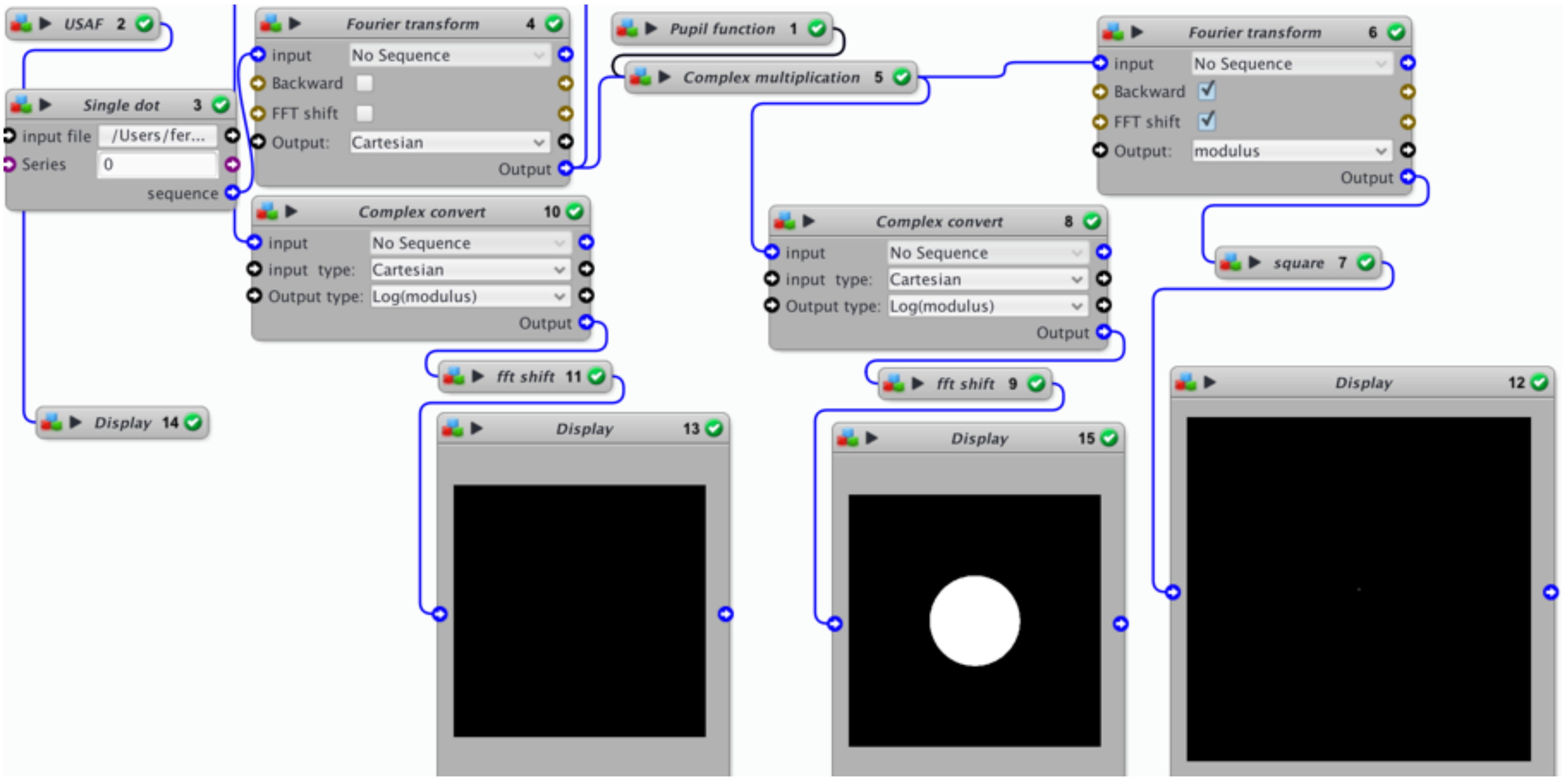


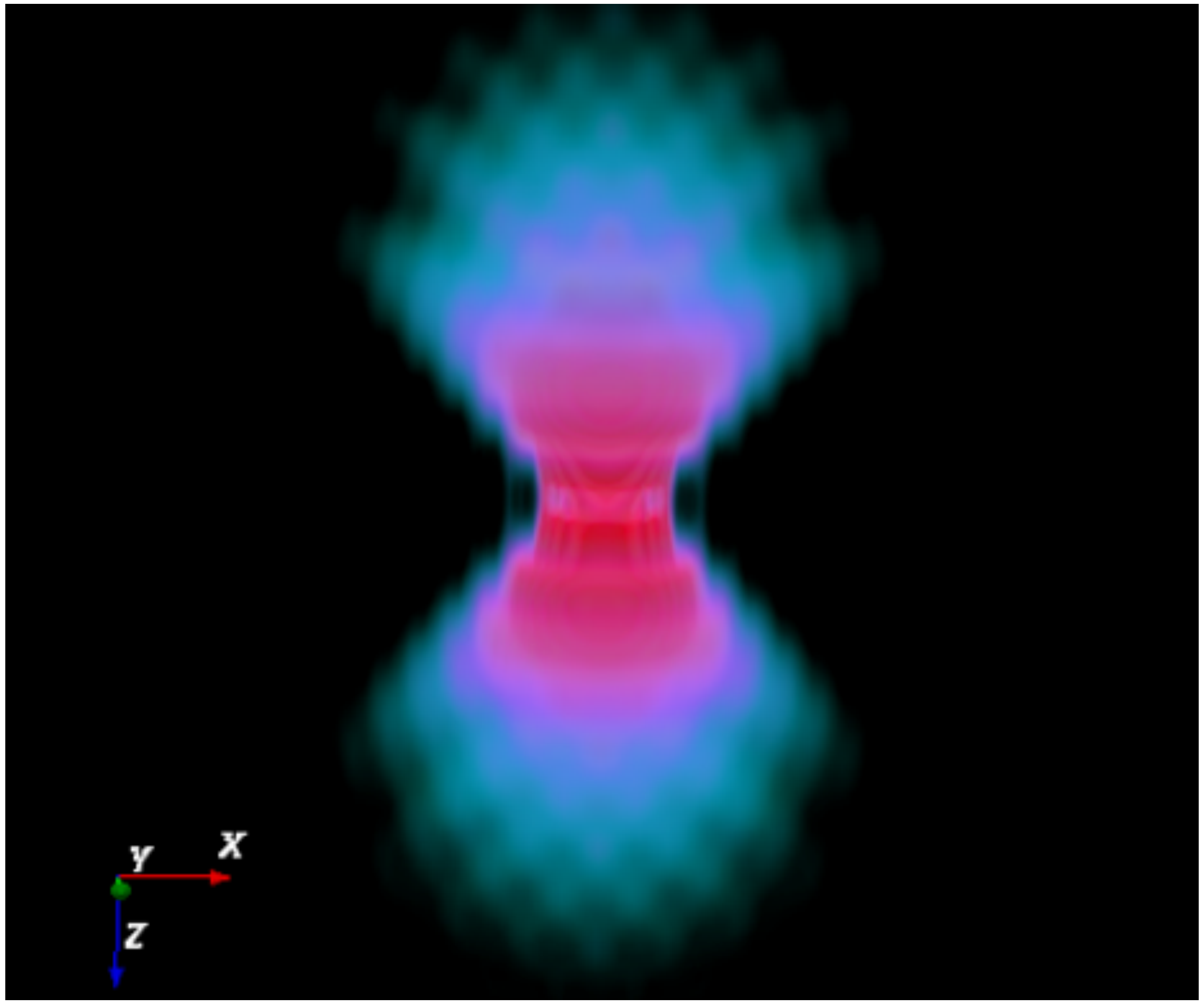
Image of a single emitter



Going 3D: the widefield PSF

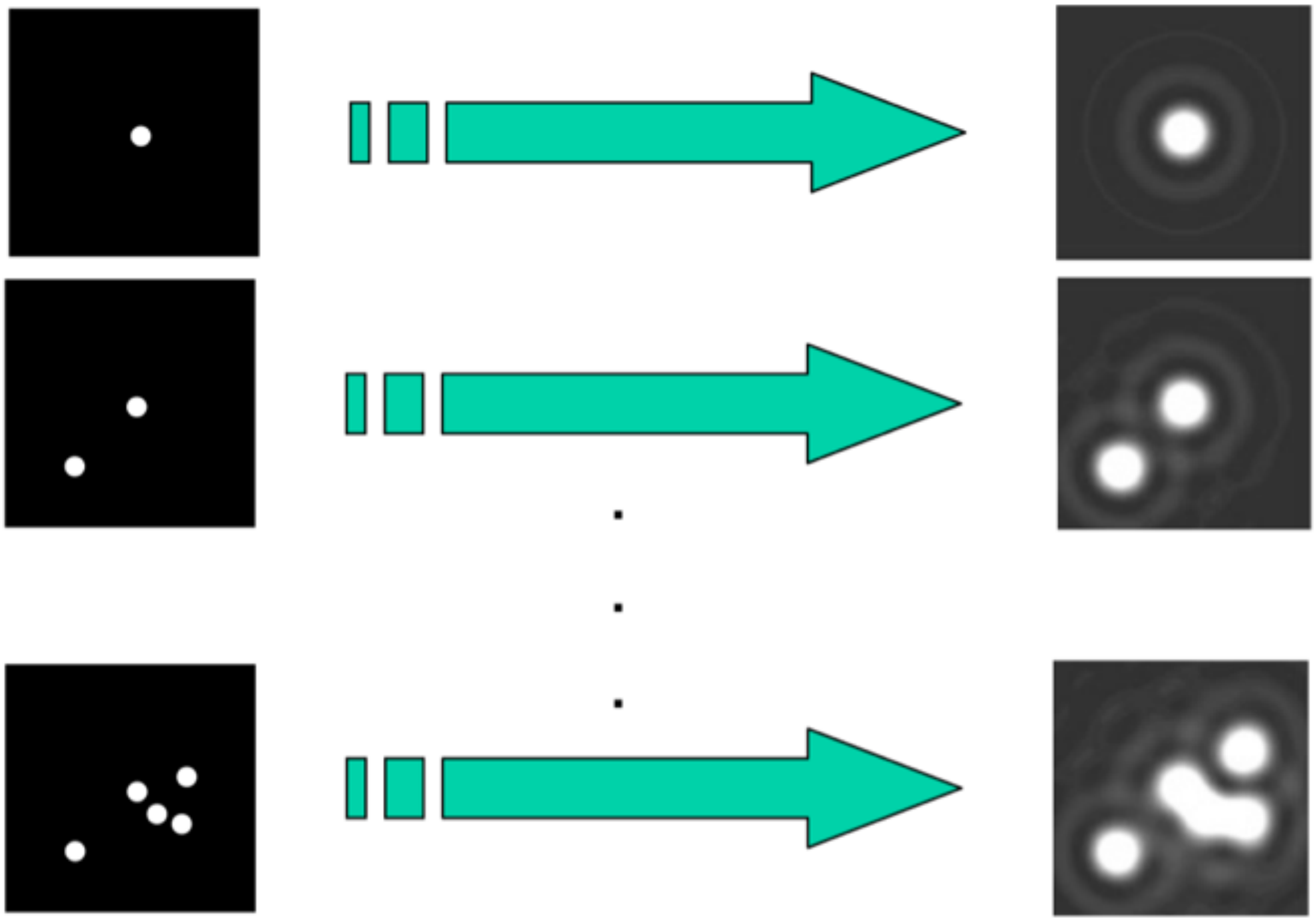
For a single emitter, the flux in each z plane is fully described by the pupil function.

3D PSF generated by propagating the wave to each plane in depth around the focal plane.



Convolution

Emitters are incoherent: the image is the sum of the image of emitters



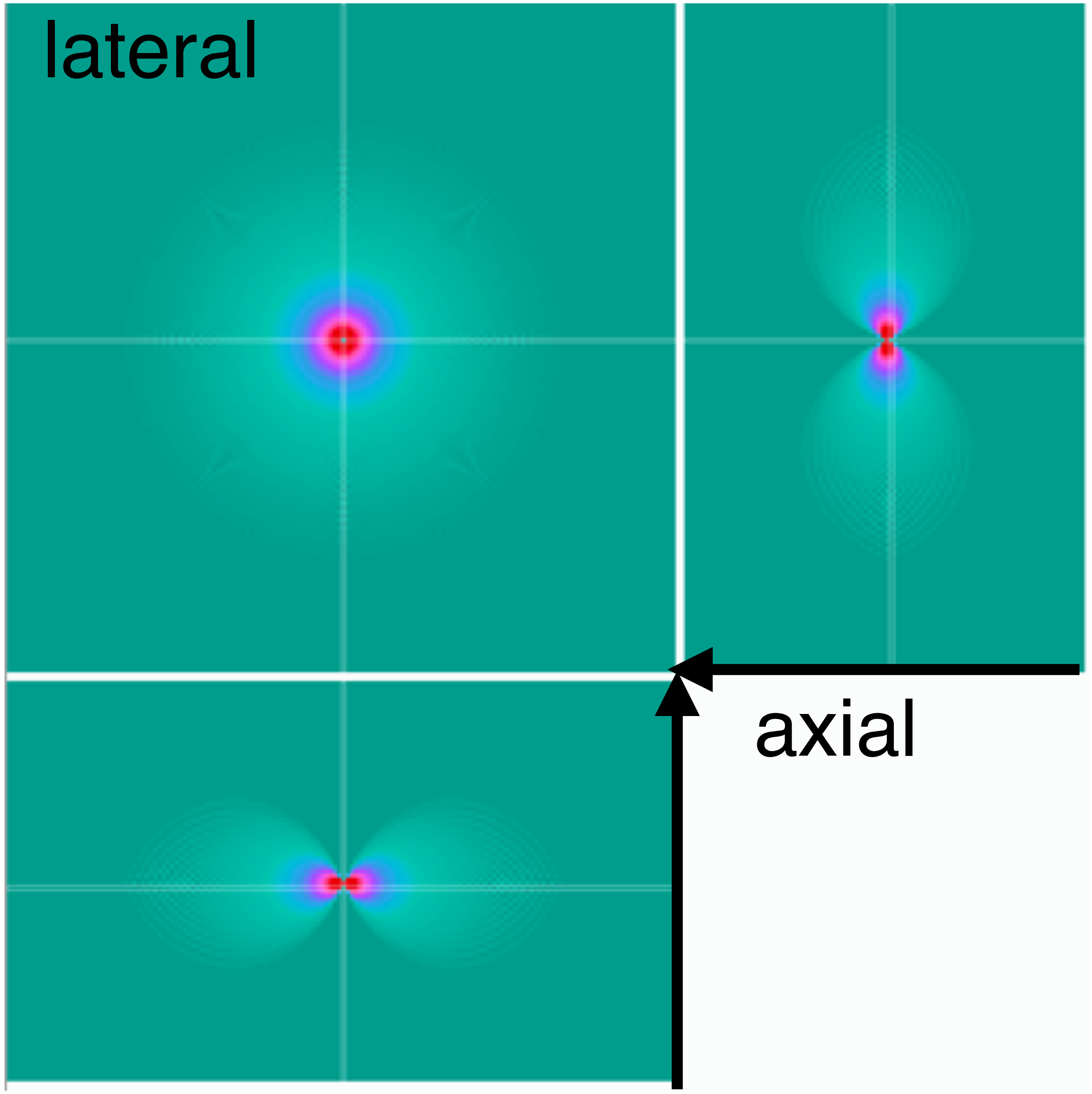
Convolution

The missing cone

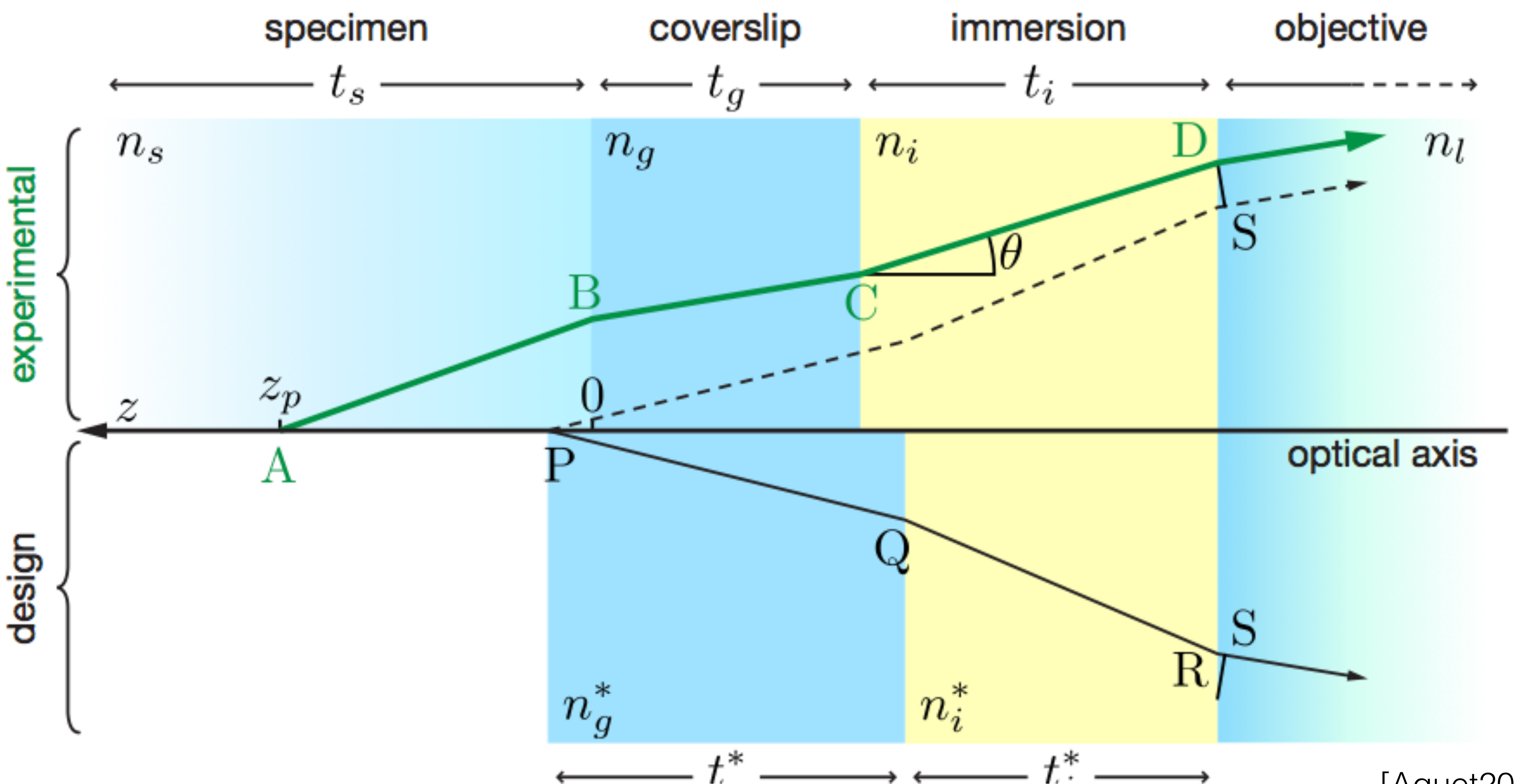
Donut shaped OTF

OFT cut most frequency along the depth axis:
Very bad optical sectioning!

PSF spectrum (OTF)



Aberrations: refractive index mismatch



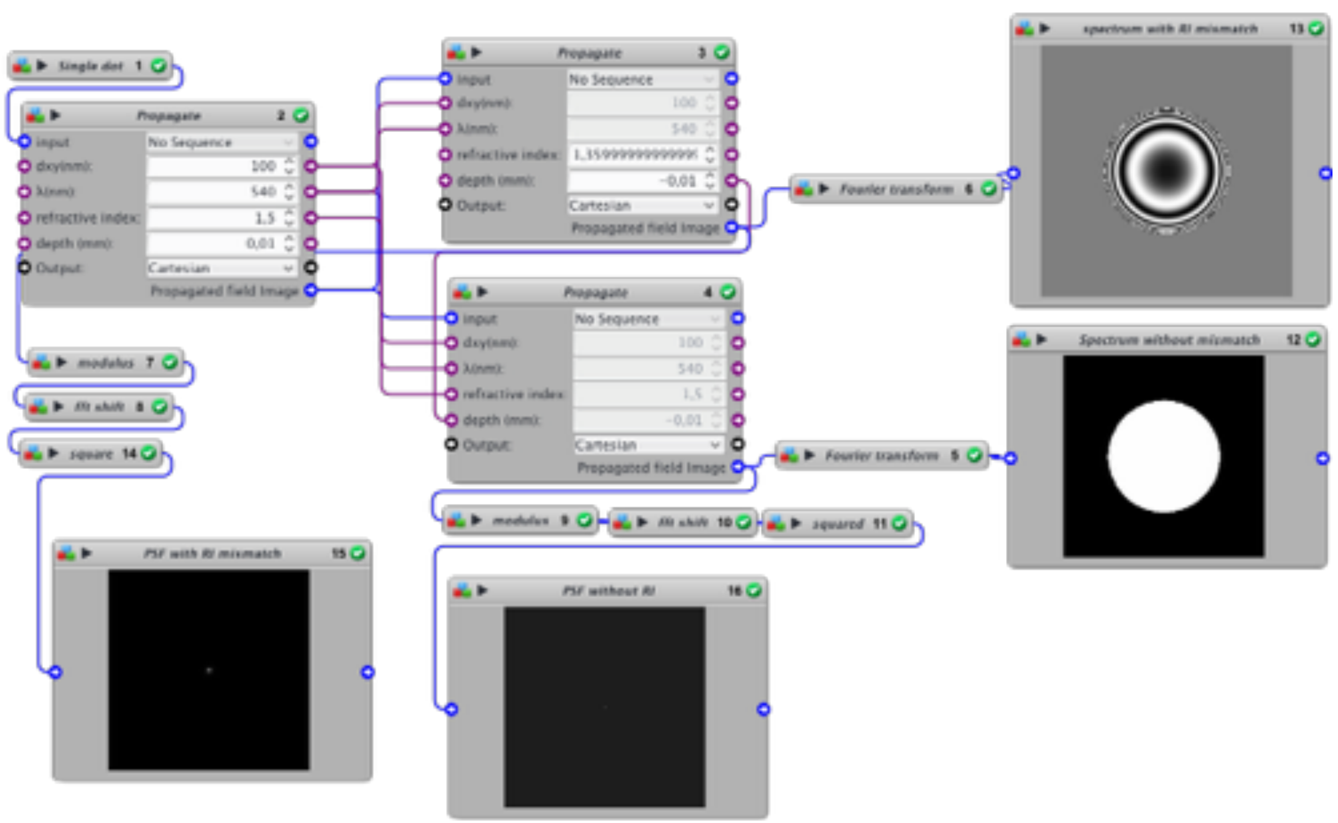
[Aguet2010]

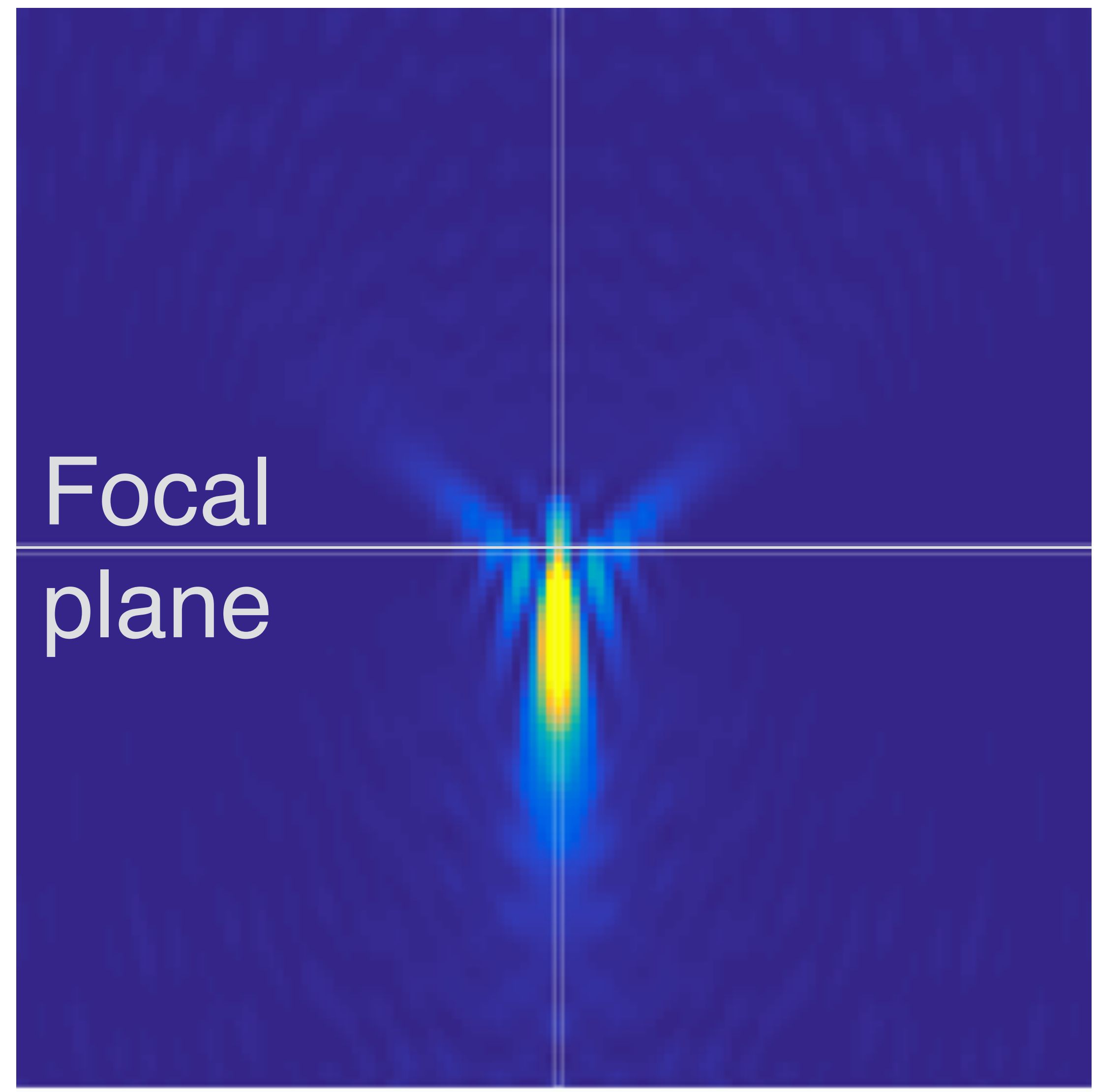
Aberrations: refractive index mismatch

RI mismatch induces aberrations:

➡ strong as we focus deeper

➡ changes the focalisation depth



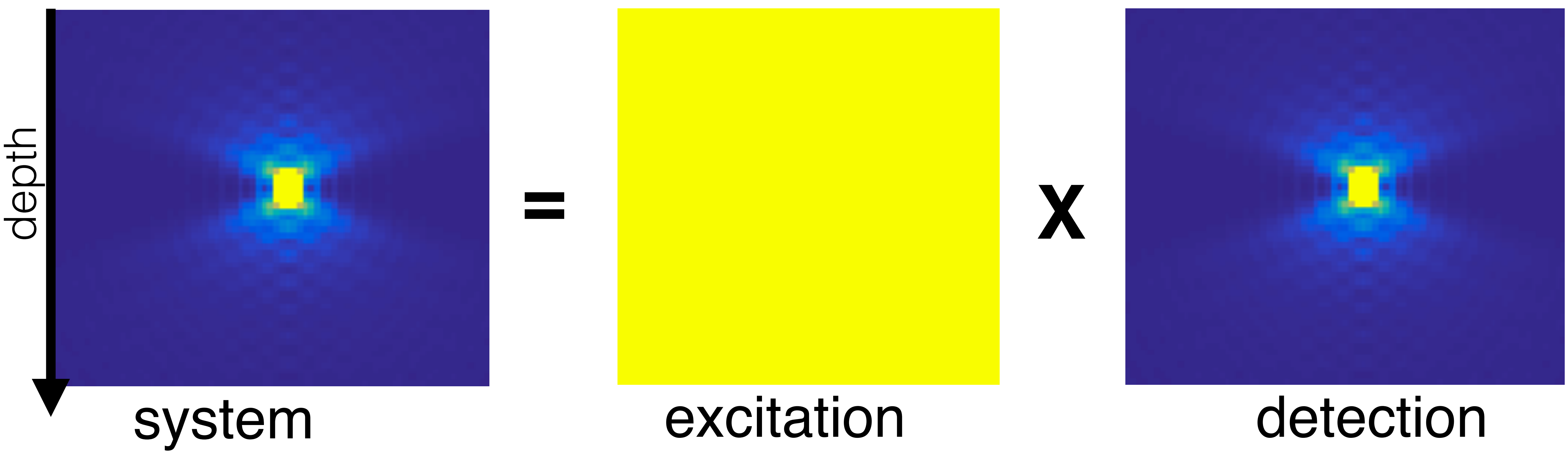


Increasing optical sectioning and resolution by crafting the PSF

$$\mathrm{PSF}_{\mathrm{sys}} = \mathrm{PSF}_{\mathrm{ex}} \times \mathrm{PSF}_{\mathrm{det}}$$

system excitation detection

- **Widefield**



2 photons

The PSF is the 3D probability density $P_{1\mathrm{p}}(\boldsymbol{r})$ of the photons distribution

Probability density of 2 photons interaction:

$$P_{2\mathrm{p}}(\boldsymbol{r}) = P_{1\mathrm{p}}(\boldsymbol{r}) \times P_{1\mathrm{p}}(\boldsymbol{r})$$

$$\mathrm{PSF}_{2\mathrm{P}} = \mathrm{PSF}_{1\mathrm{P}} \times \mathrm{PSF}_{1\mathrm{P}}$$

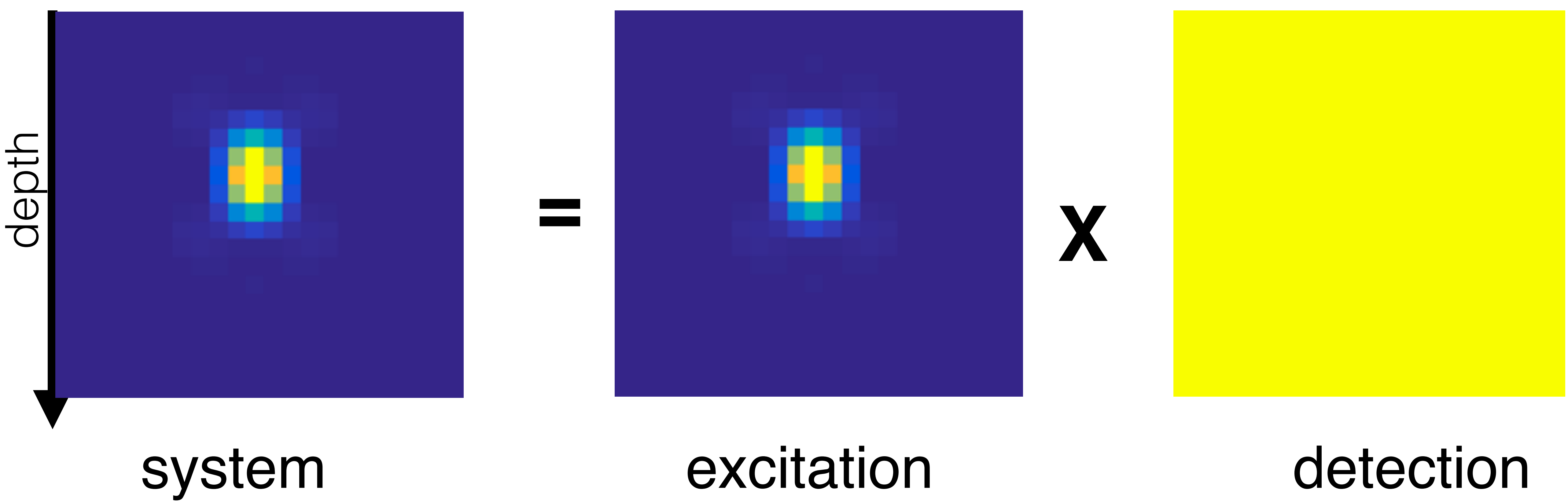
Collecting all the emitted light: $\mathrm{PSF}_{\mathrm{det}} = 1$

2 photons

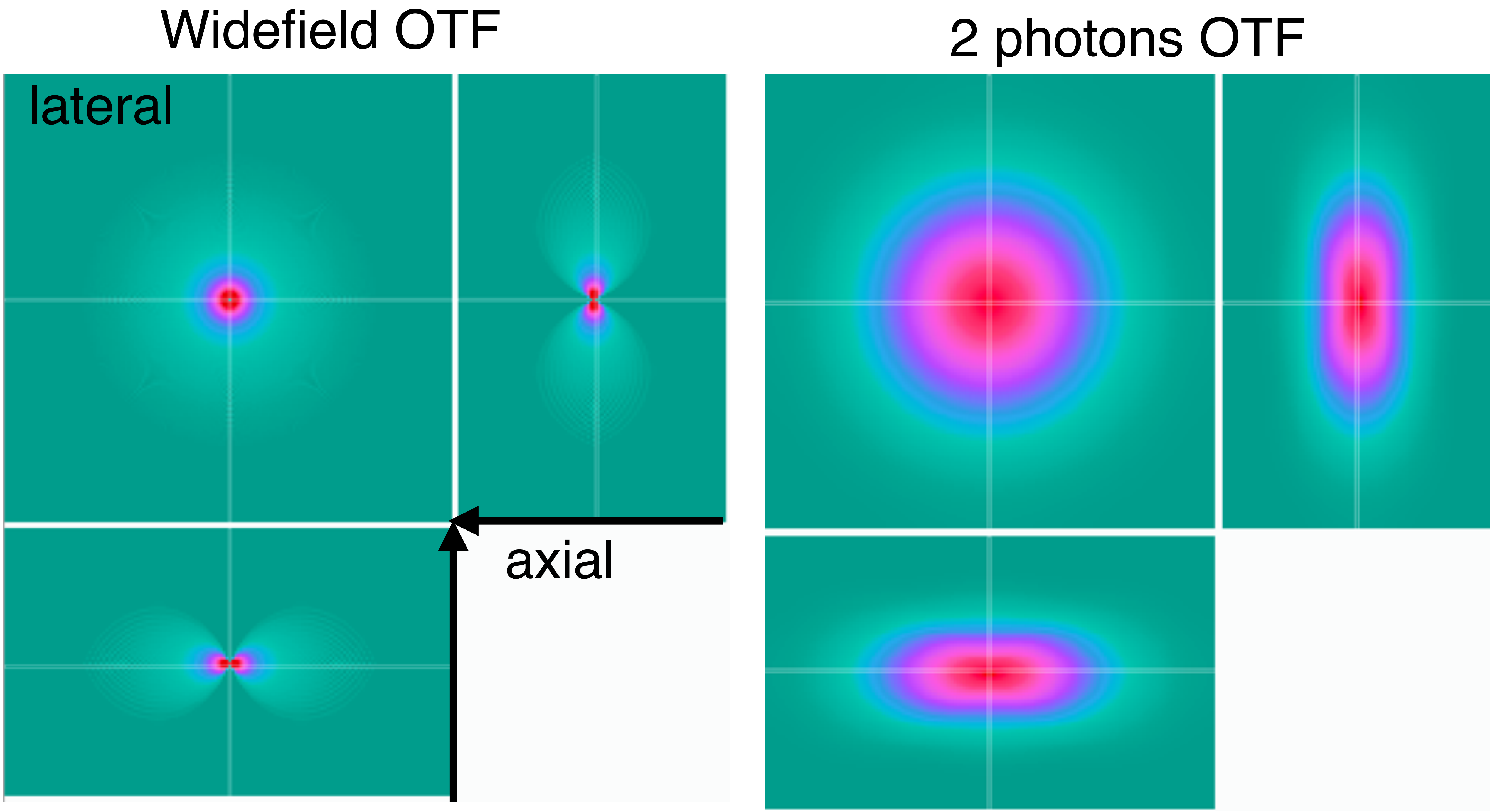
excitation PSF $\mathrm{PSF}_{2\mathrm{P}} = \mathrm{PSF}_{1\mathrm{P}} \times \mathrm{PSF}_{1\mathrm{P}}$

Collecting all the emitted light: $\mathrm{PSF}_{\mathrm{det}} = 1$

2 Photons PSF

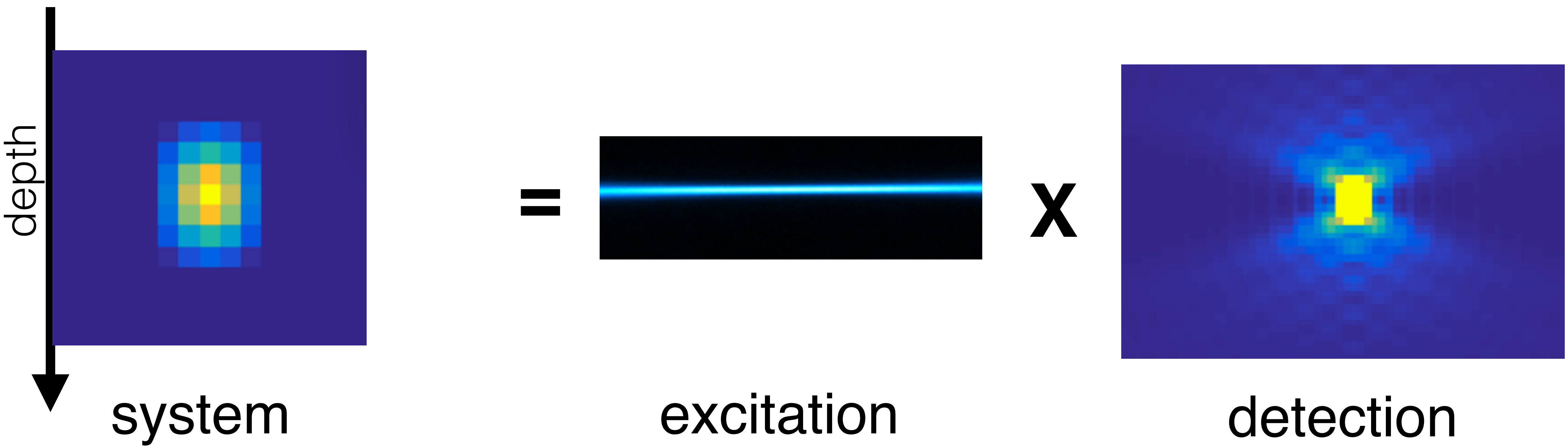


2-photons



$$\mathrm{PSF}_{\mathrm{sys}} = \mathrm{PSF}_{\mathrm{ex}} \times \mathrm{PSF}_{\mathrm{det}}$$

system excitation detection



Question?