Characterization and Correction of Field Distortions

Praveen Pankajakshan
AIQ, Pasteur Institute, Paris, France.
29 November 2011

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Large Field Imaging!

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Part 1: Outline

Need a larger working distance?

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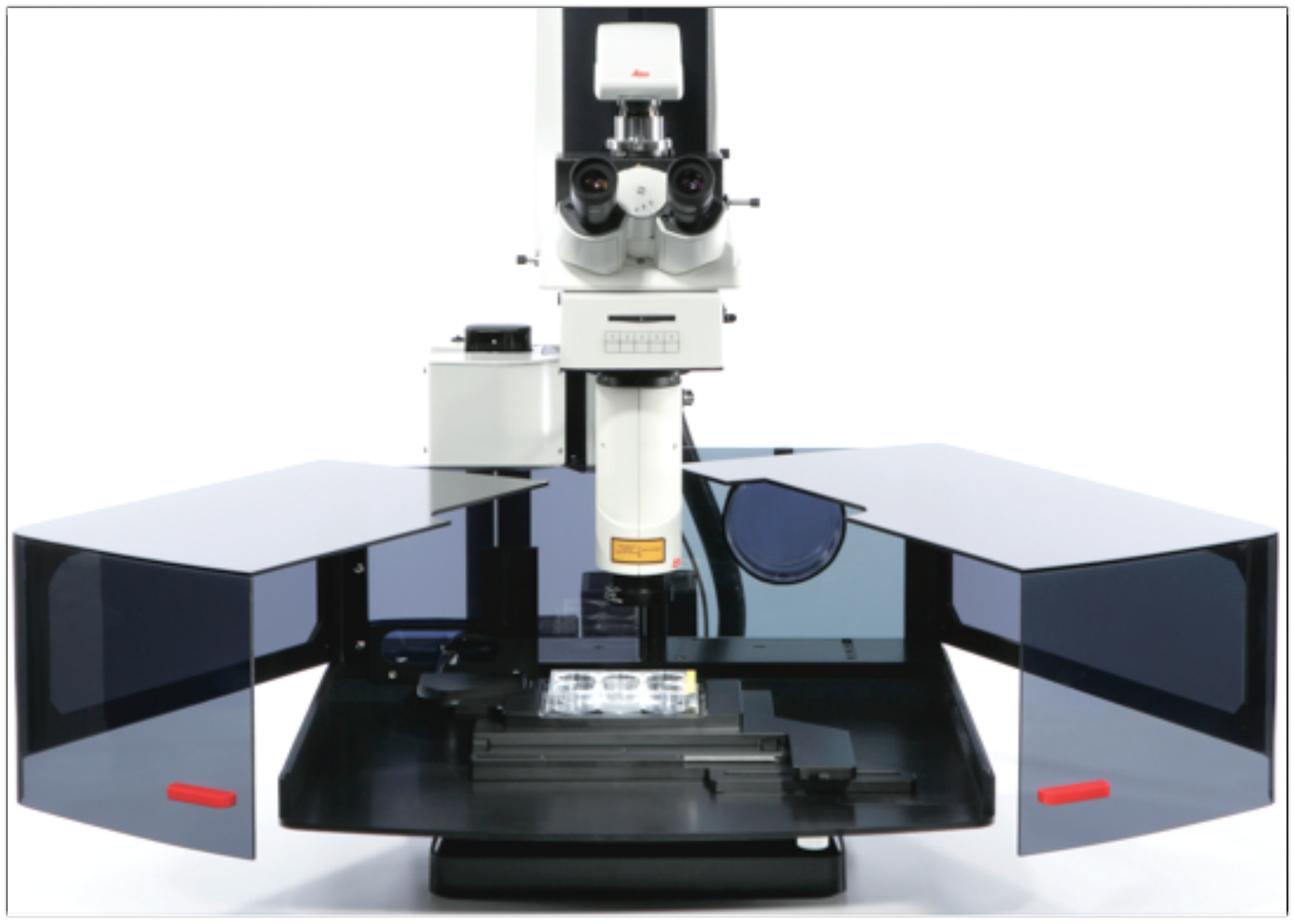
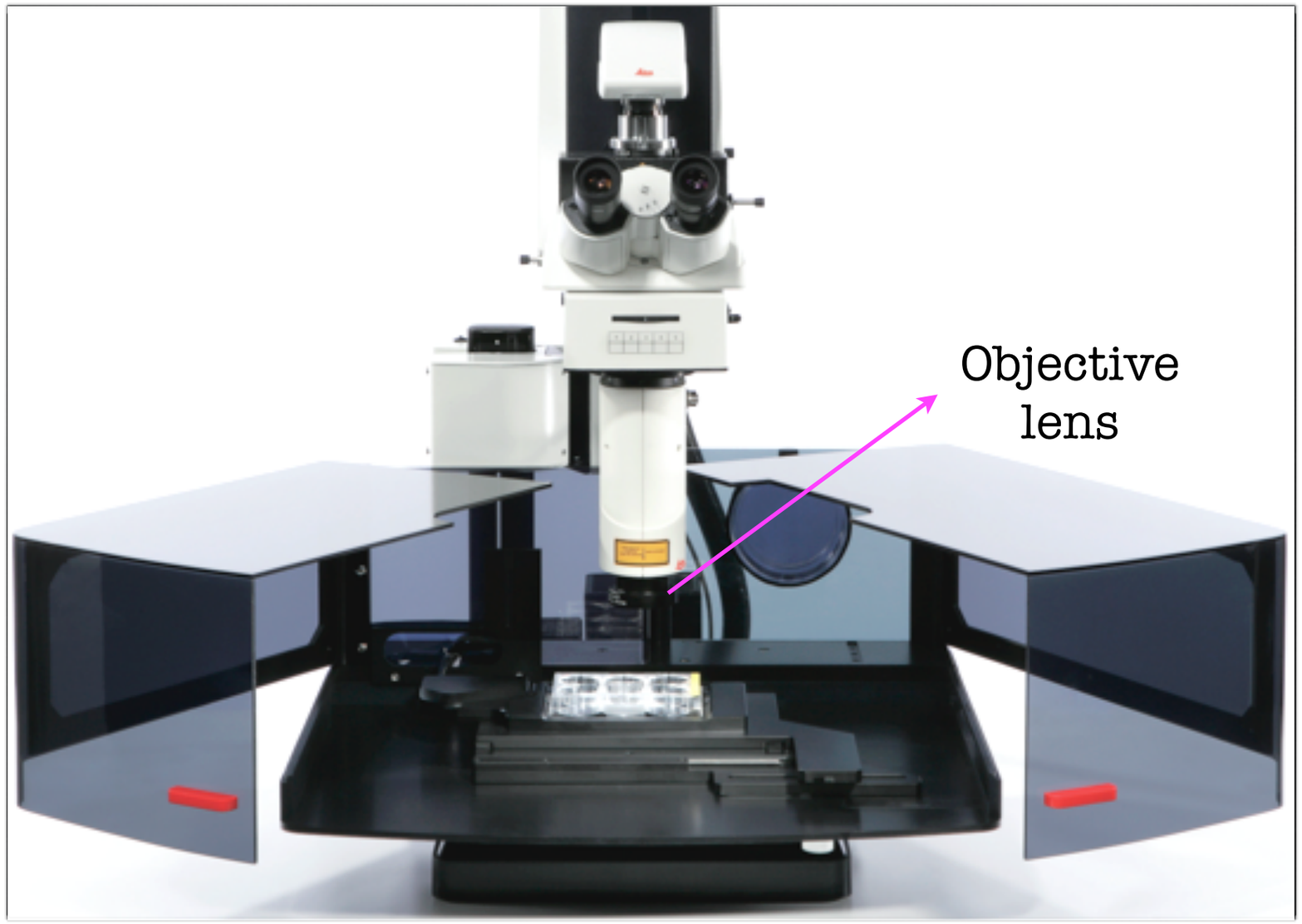
Fluorescence MACROscopes



Leica Wide-Field MacroFluo

P. Sendrowski et al., “Arrangement for analyzing microscopic and macroscopic preparations,” WO 2009/04711, Apr. 2009, PCT/EP2008/062749.

Fluorescence MACROscopes



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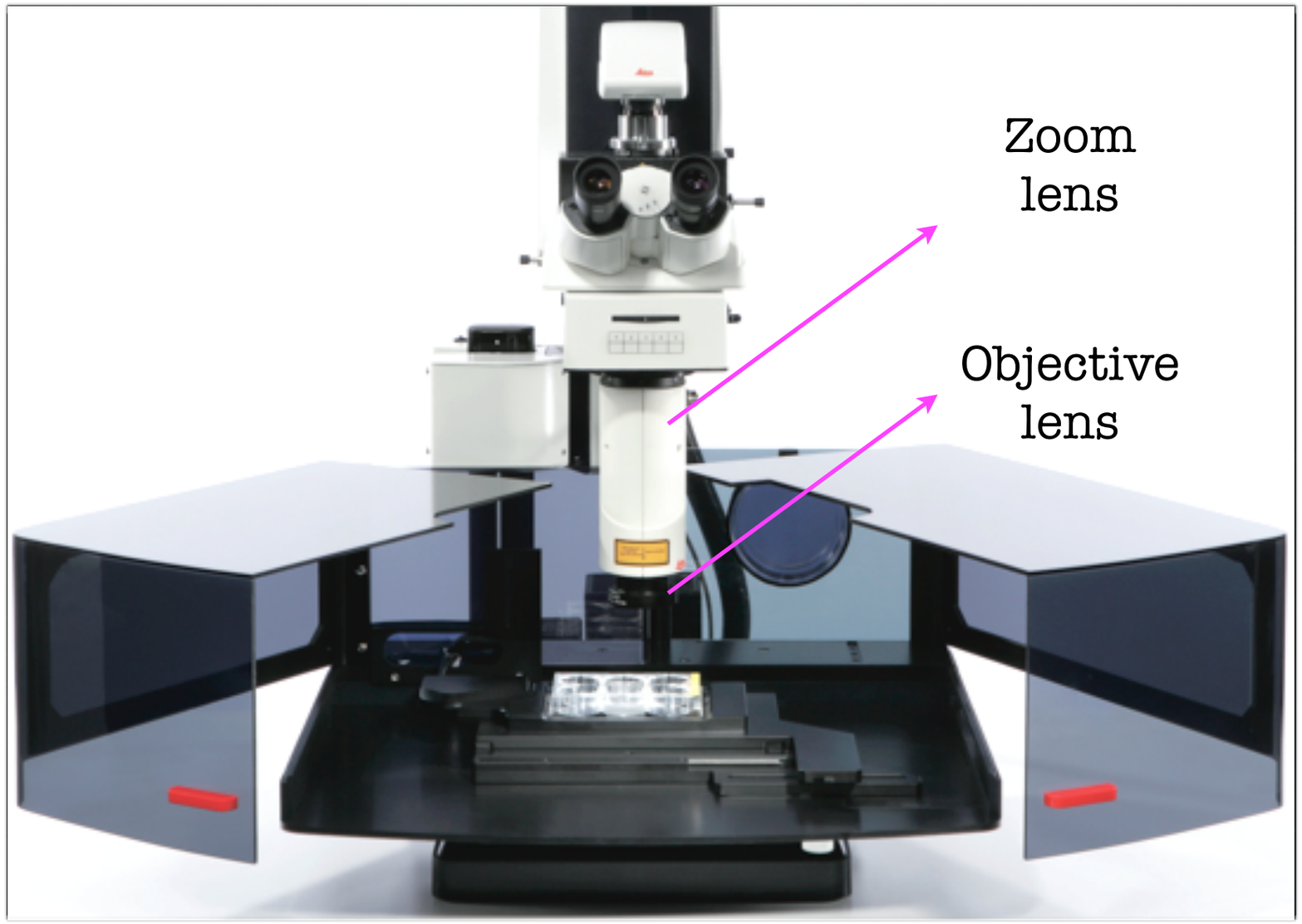

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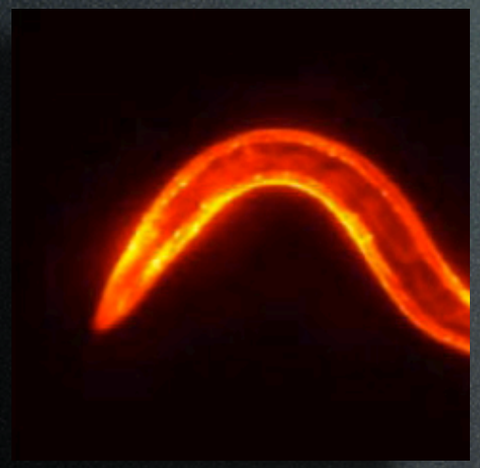
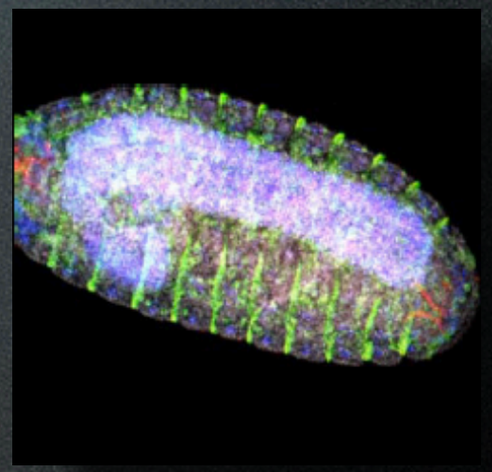
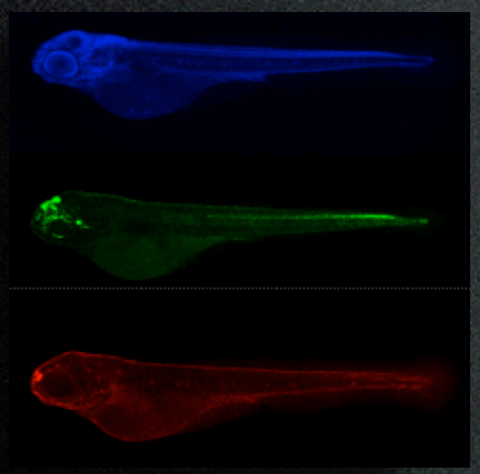
MACROscopic samples







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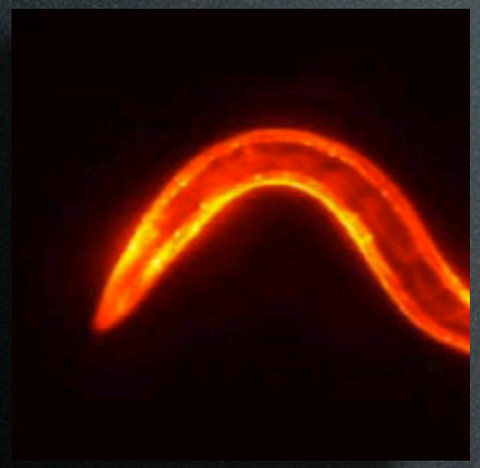

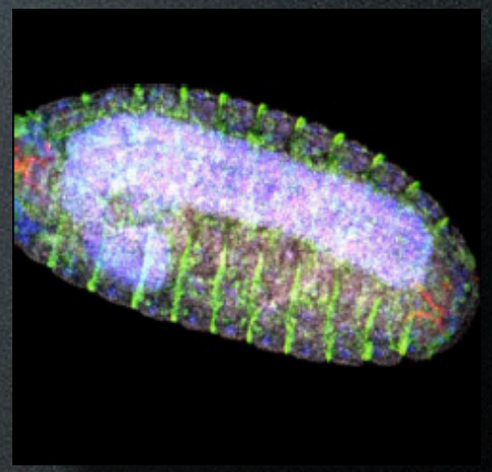

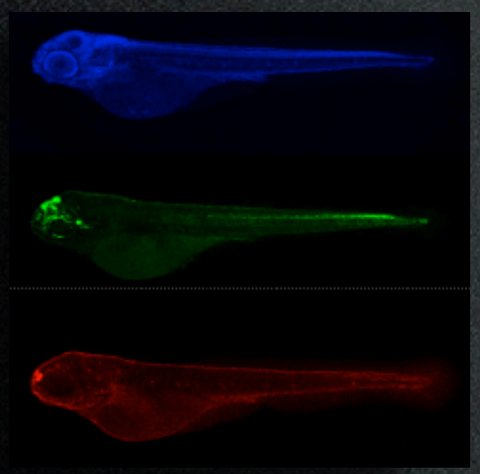

Best of two worlds

Convallaria sample

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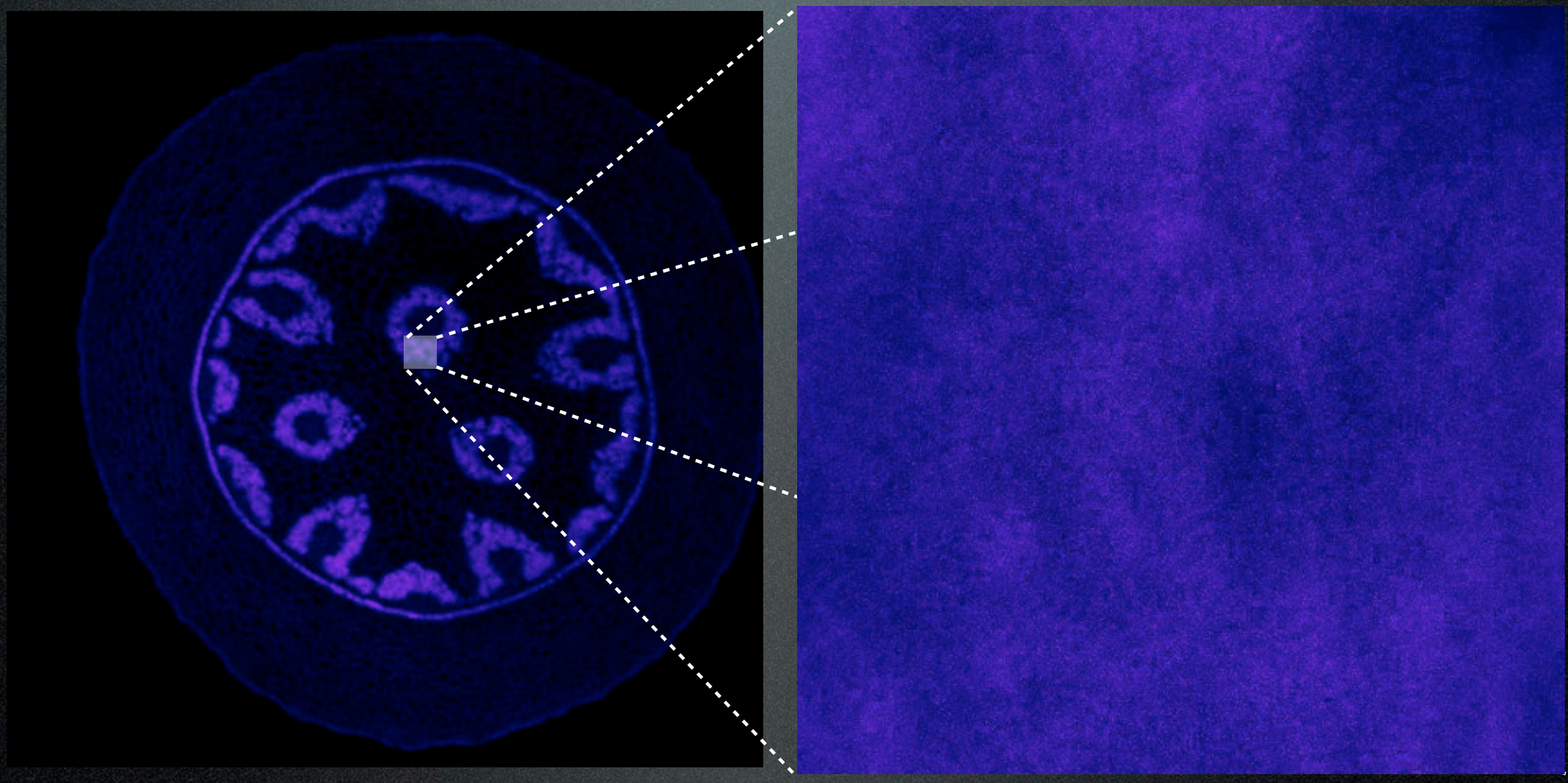

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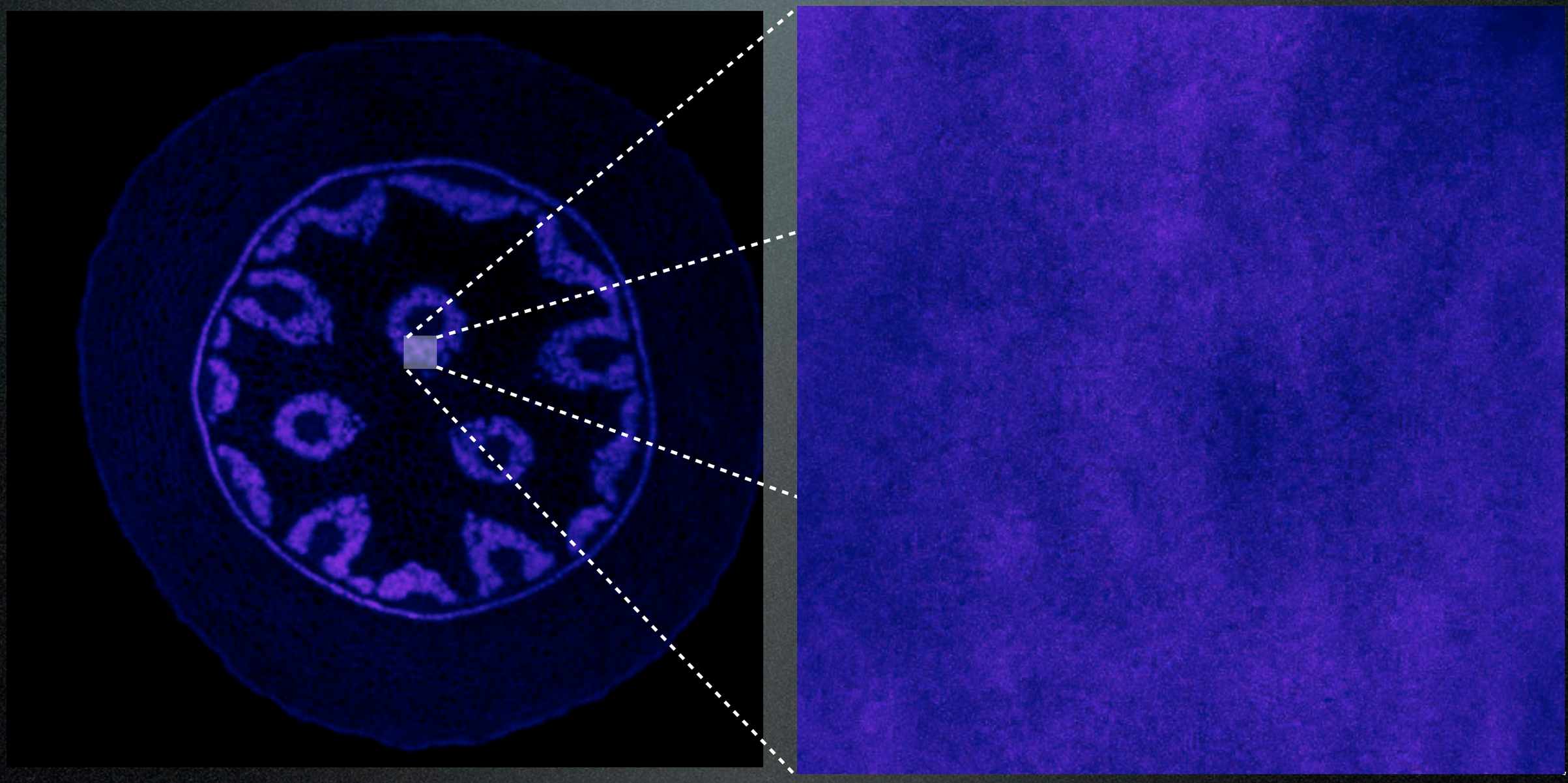


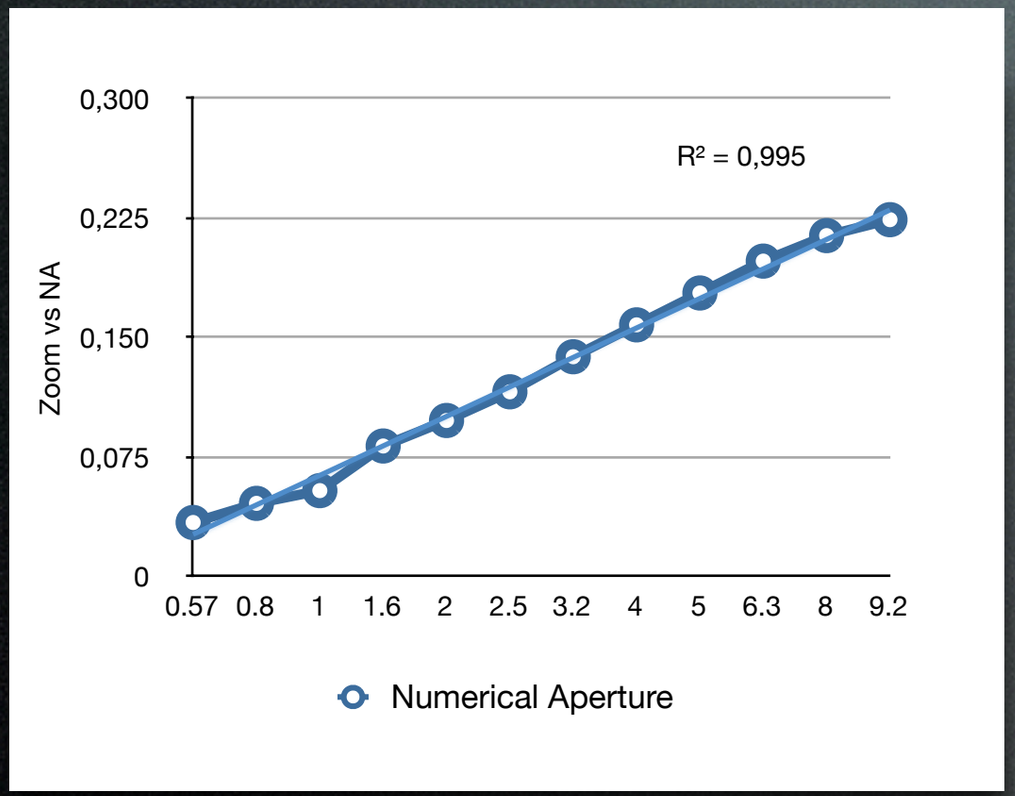
Convallaria sample

Some numbers

Objective	NA	FOV	Working Distance	Resolution
PL APO 1x	0,12	16mm	97mm	1.65um
PL APO 2x	0,24	8mm	39mm	830nm
PL APO 5x	0,50	3.2mm	19.5mm	390nm

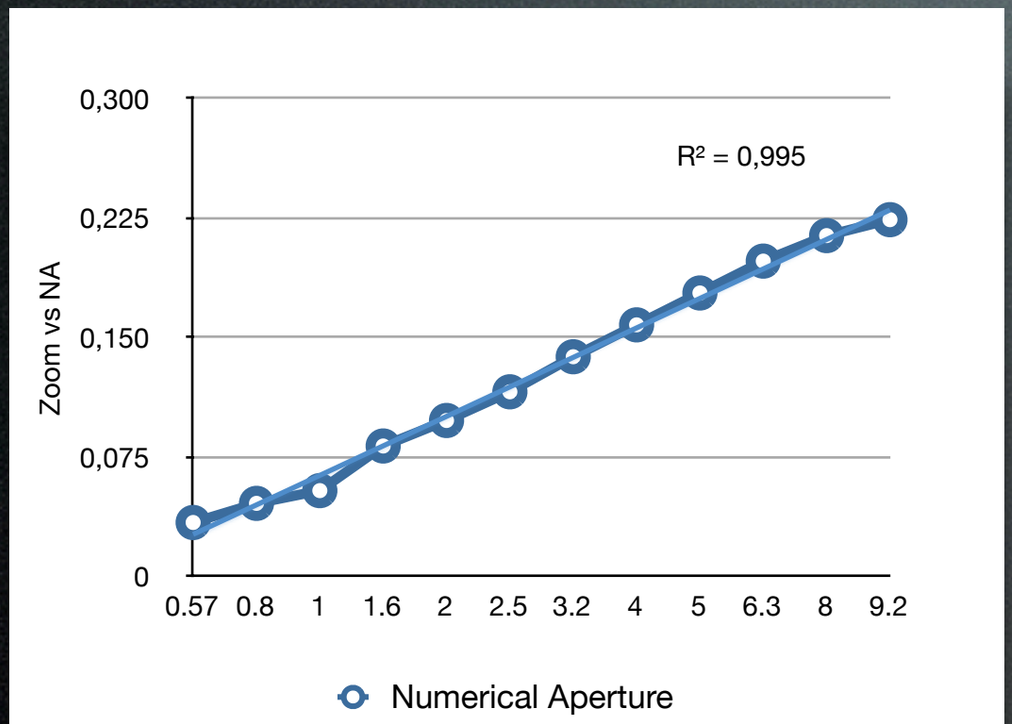
- Minimum resolution: 390nm
- Maximum working distance: 97mm
- Maximum FOV: 16mm

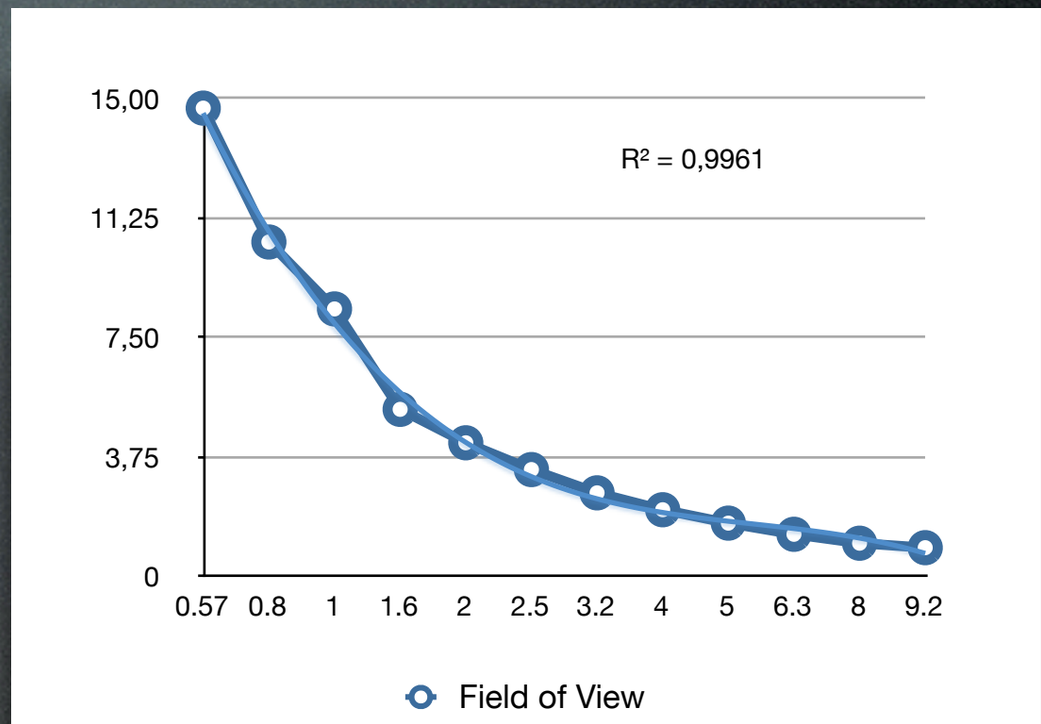
Zoom vs NA/FOV



Planapo 2.0x objective
Z16APO

Zoom vs NA/FOV





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Bridging Optics and Image Processing

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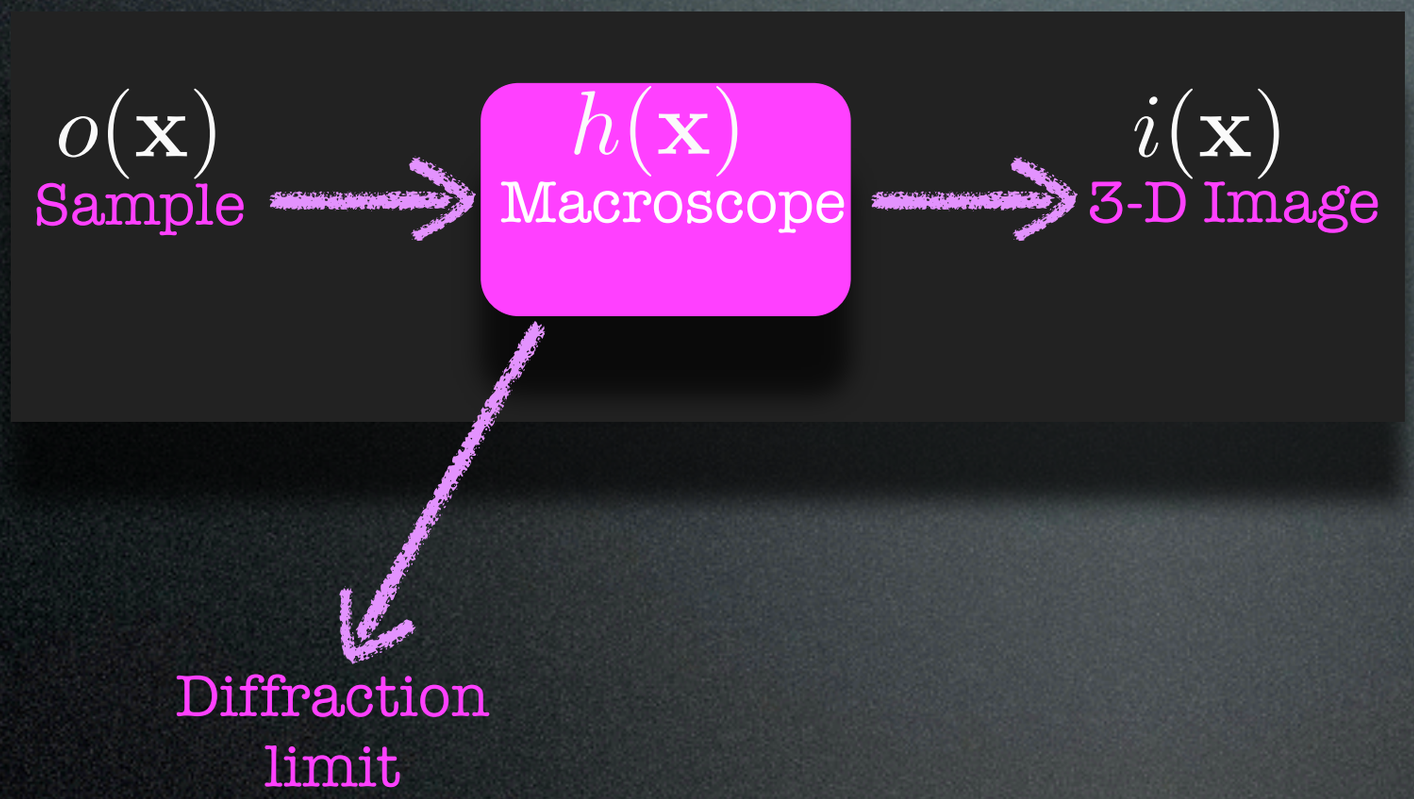

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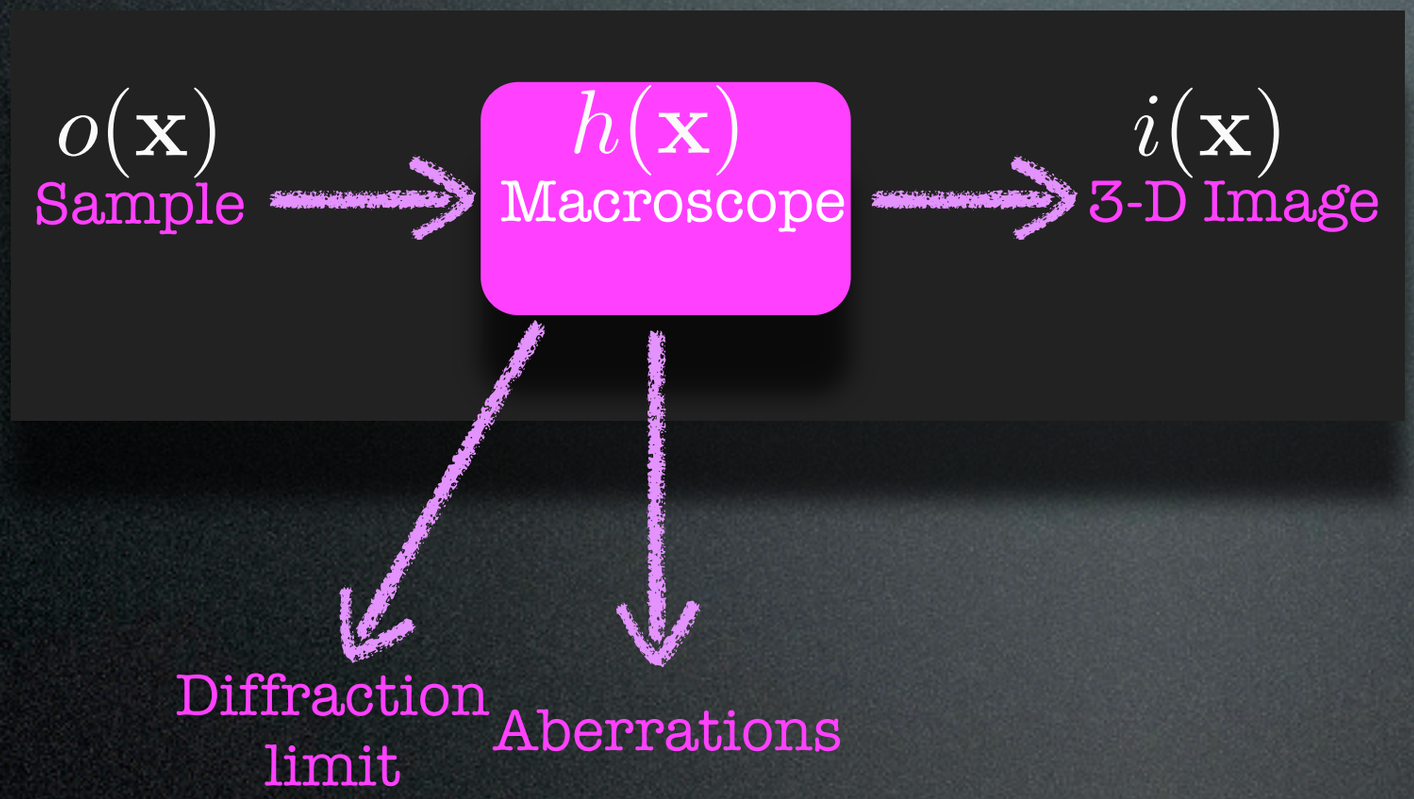

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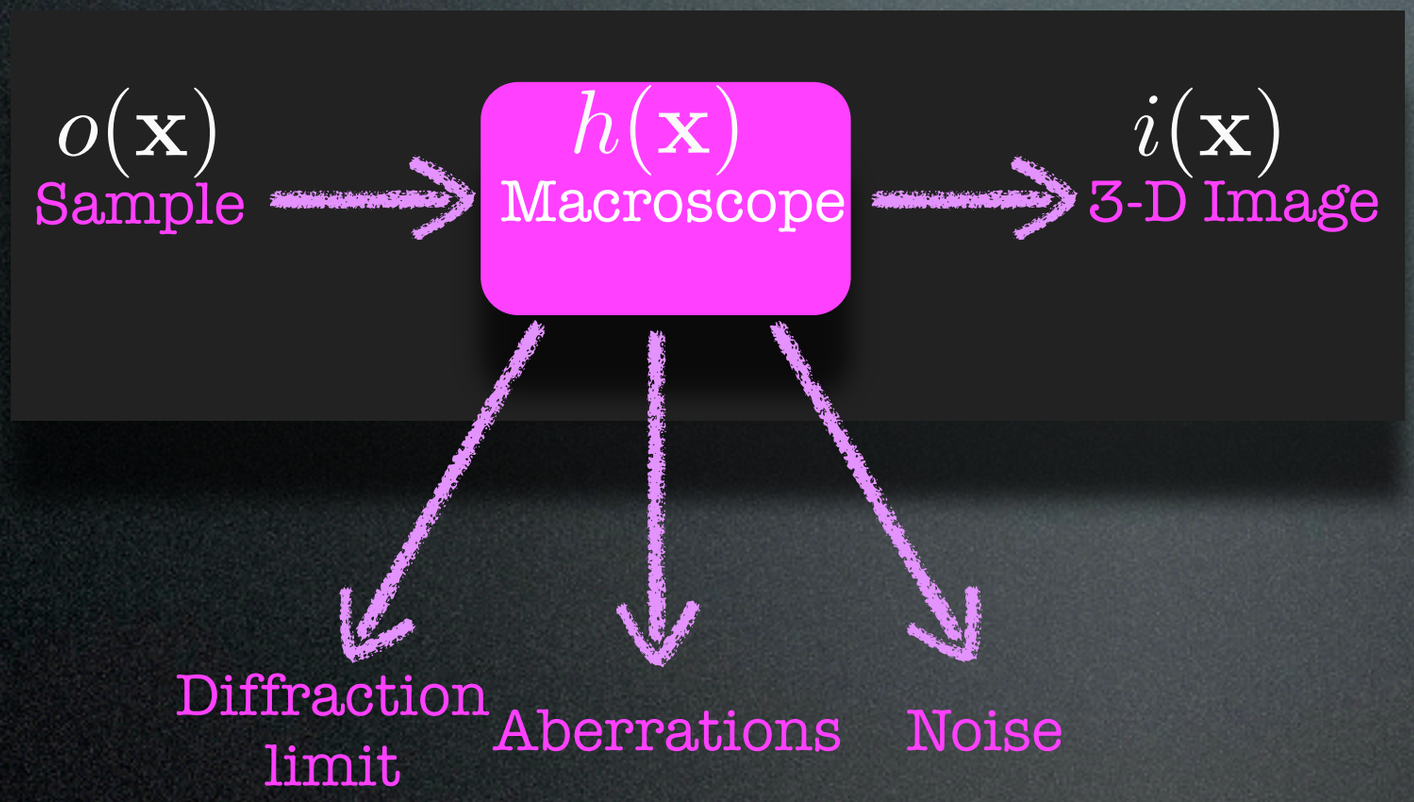

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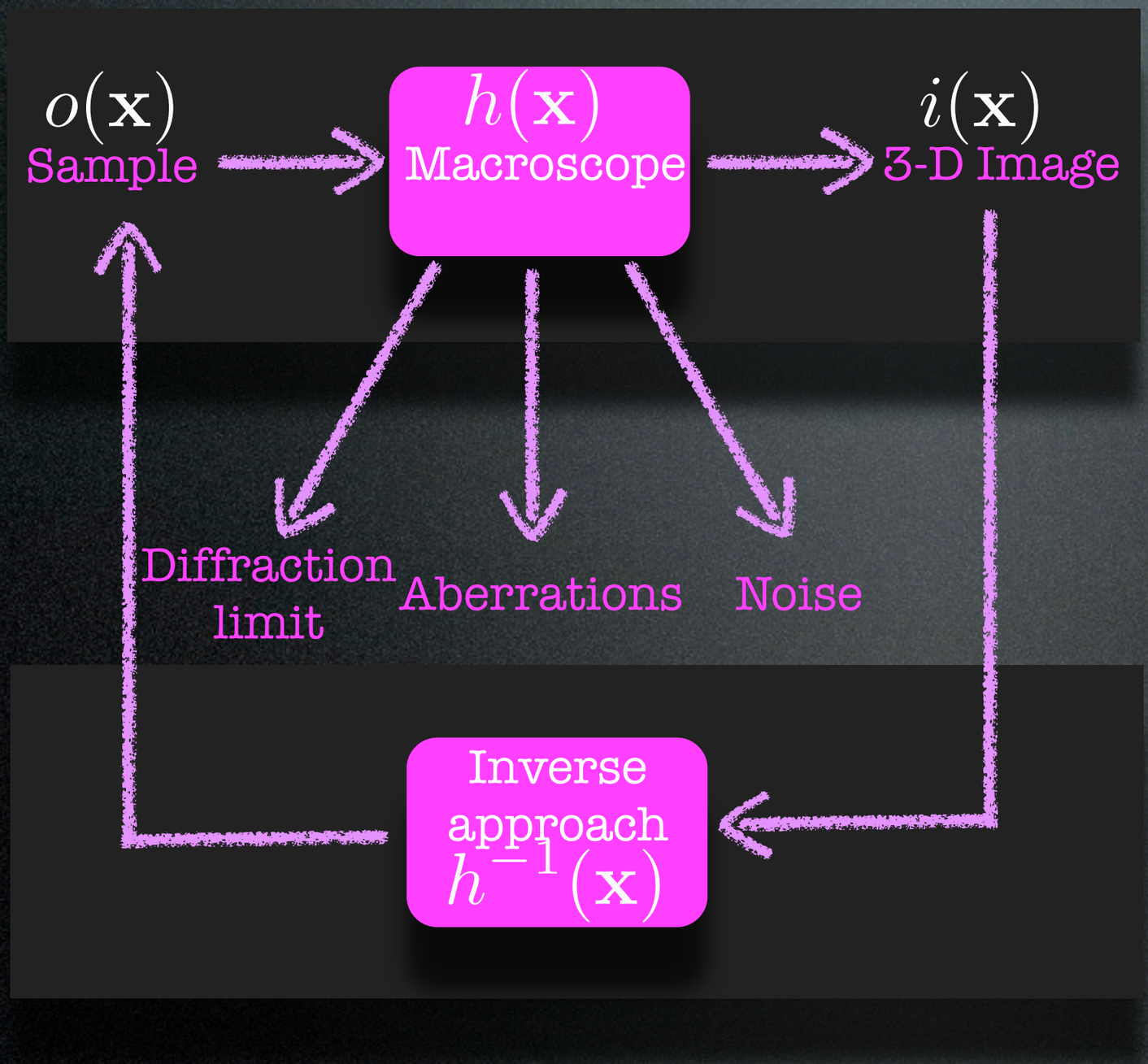

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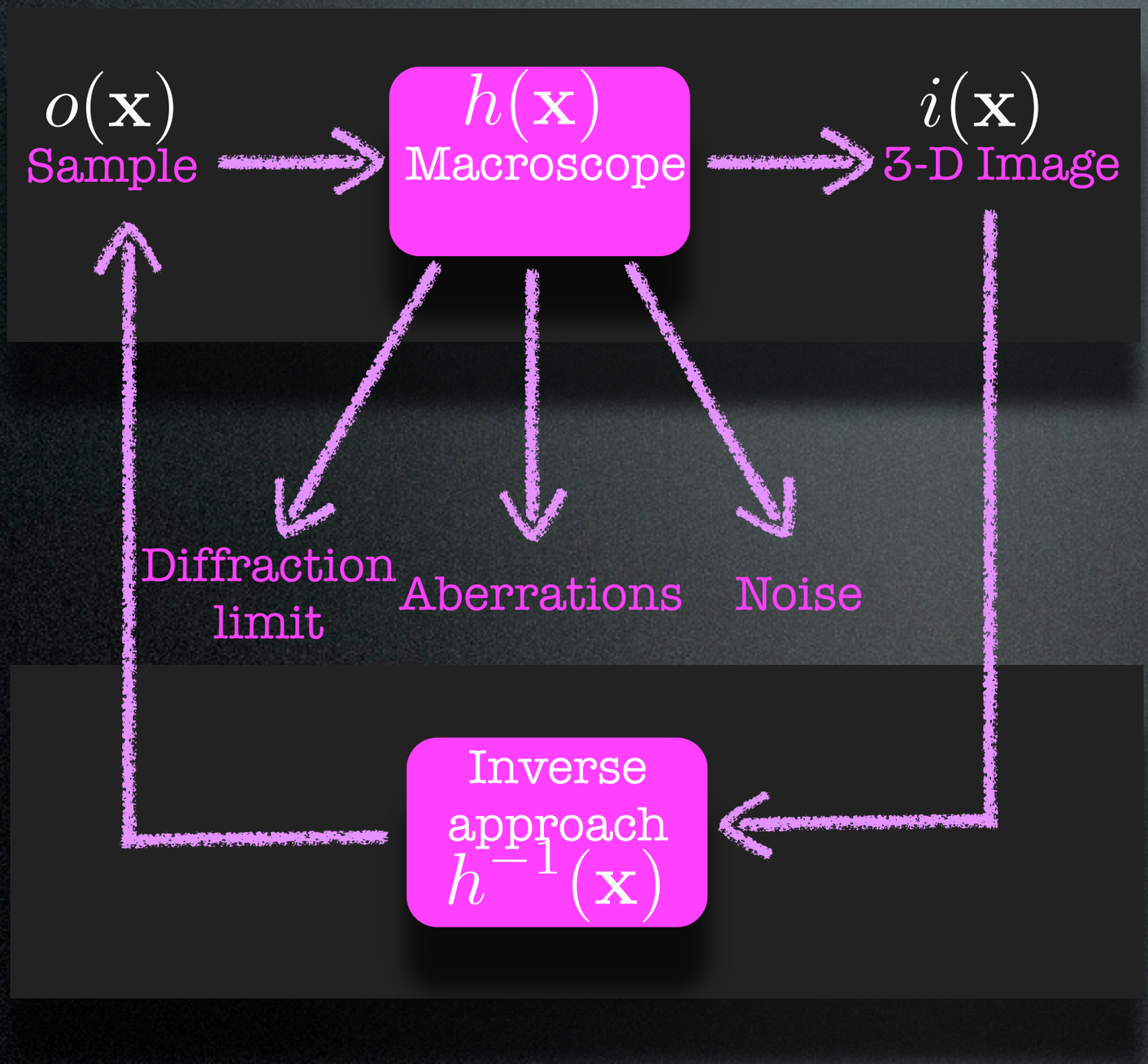
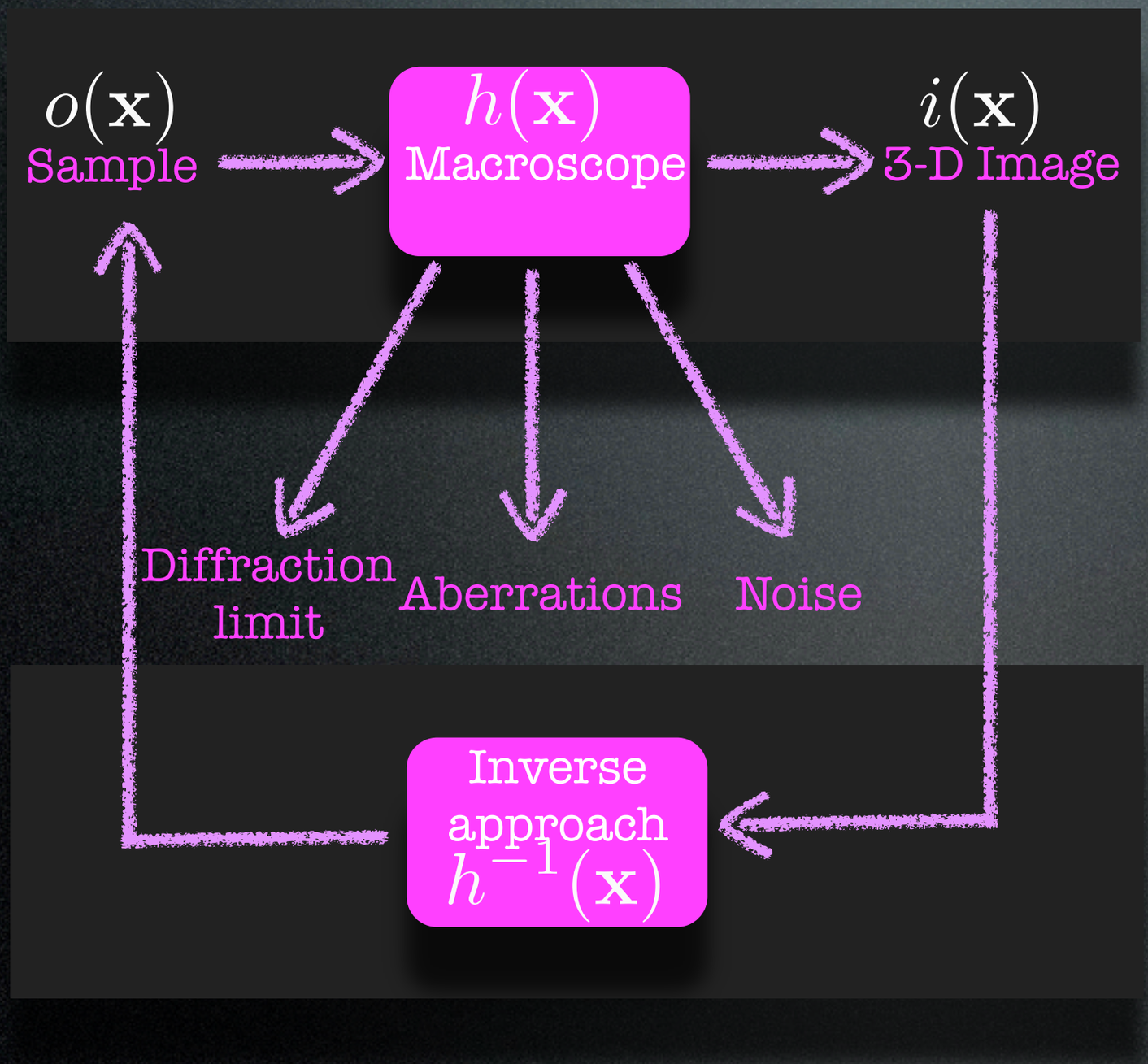


- Improvement in resolution by deconvolution.

Bridging Optics and Image Processing



- Improvement in resolution by deconvolution.
- Aberration correction and noise reduction

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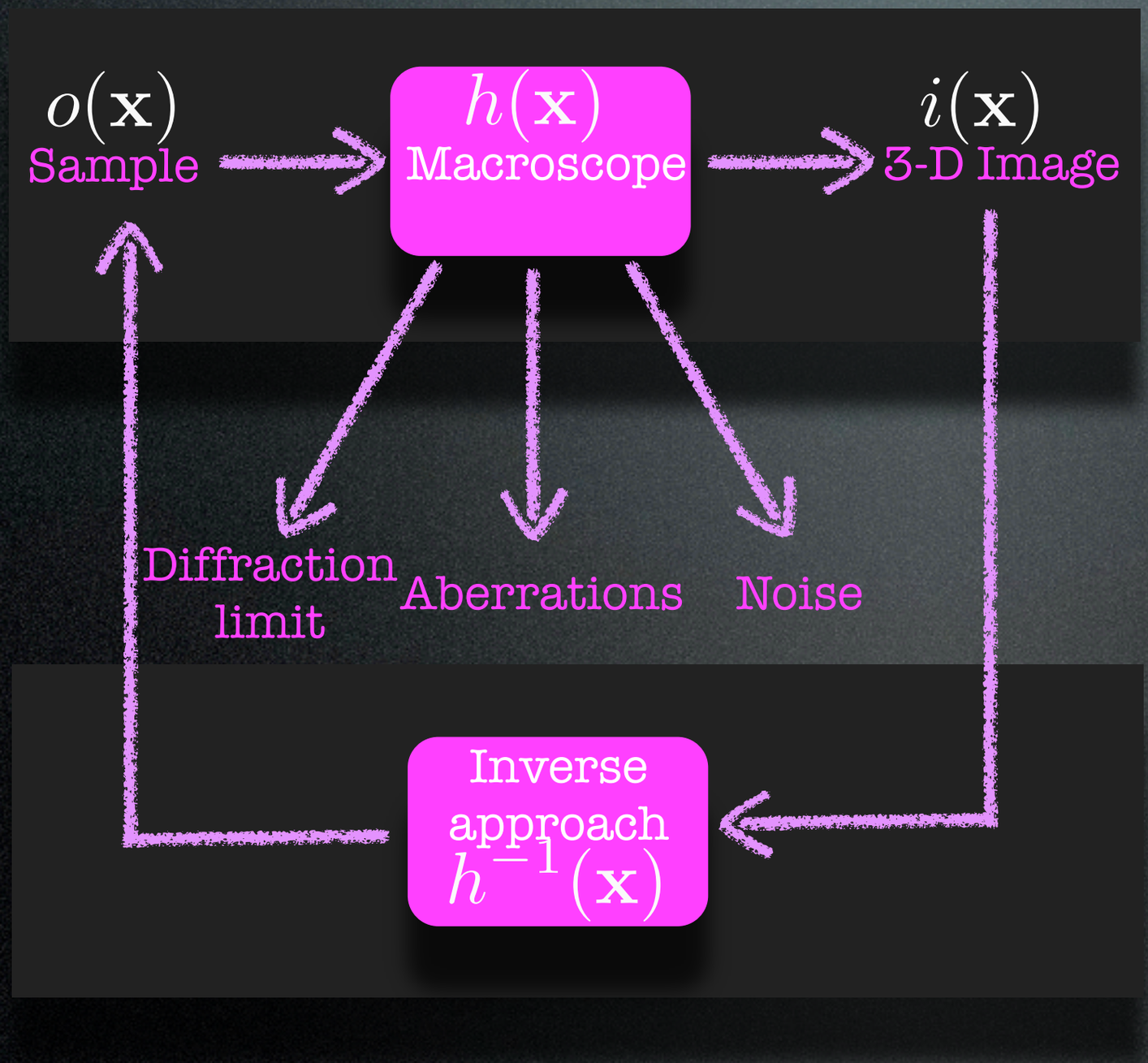

- Improvement in resolution by deconvolution.
- Aberration correction and noise reduction
- Right model of imaging.

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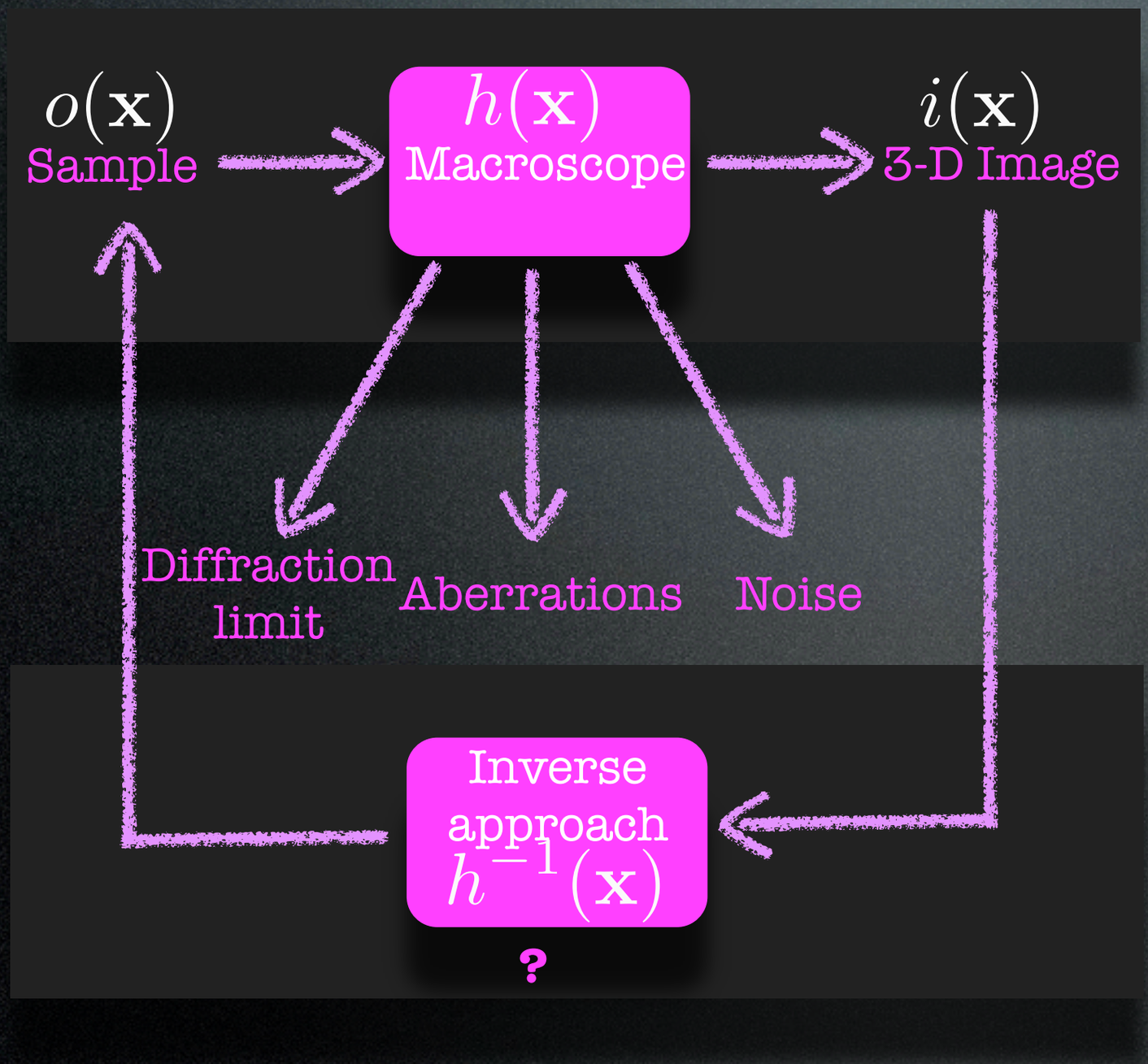

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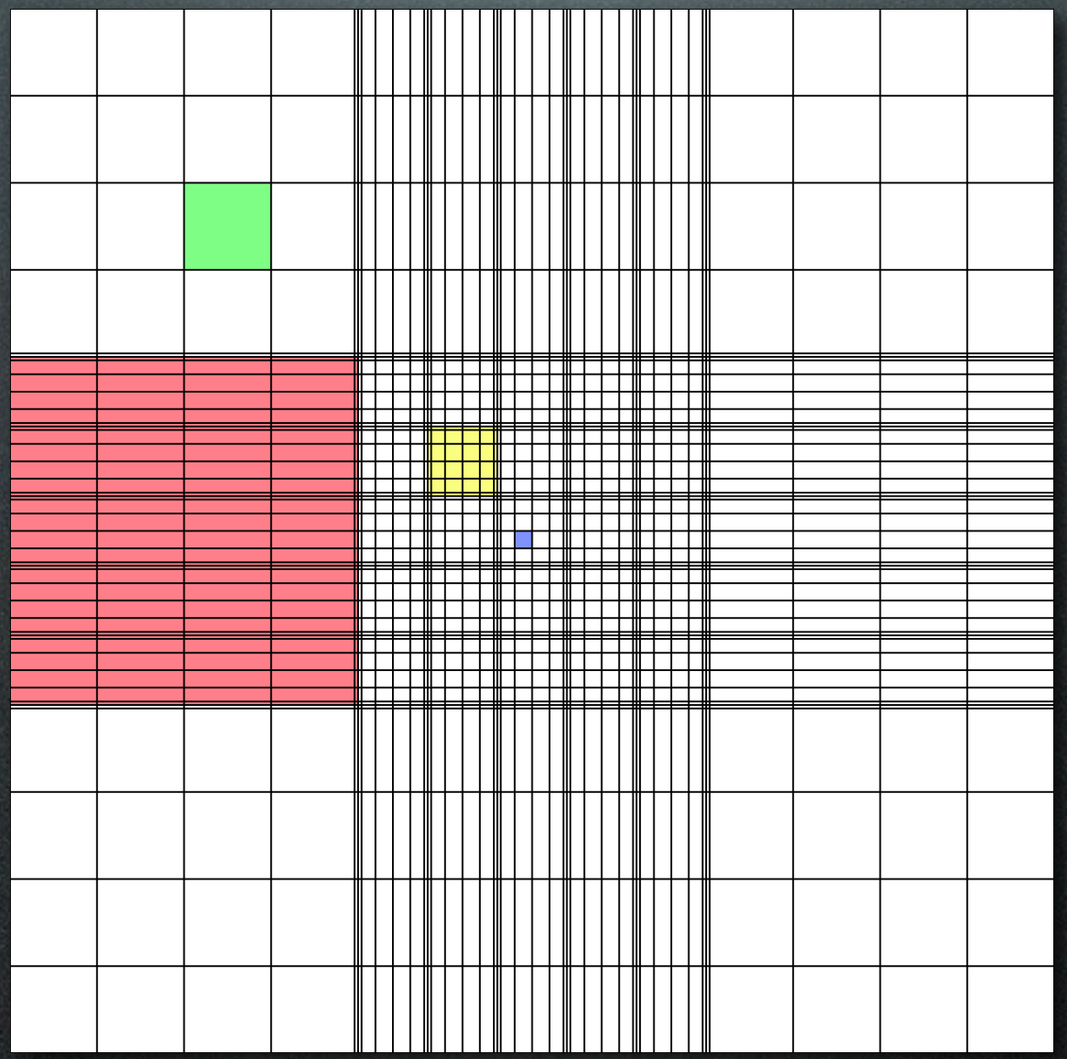
Investigation: Grid slides



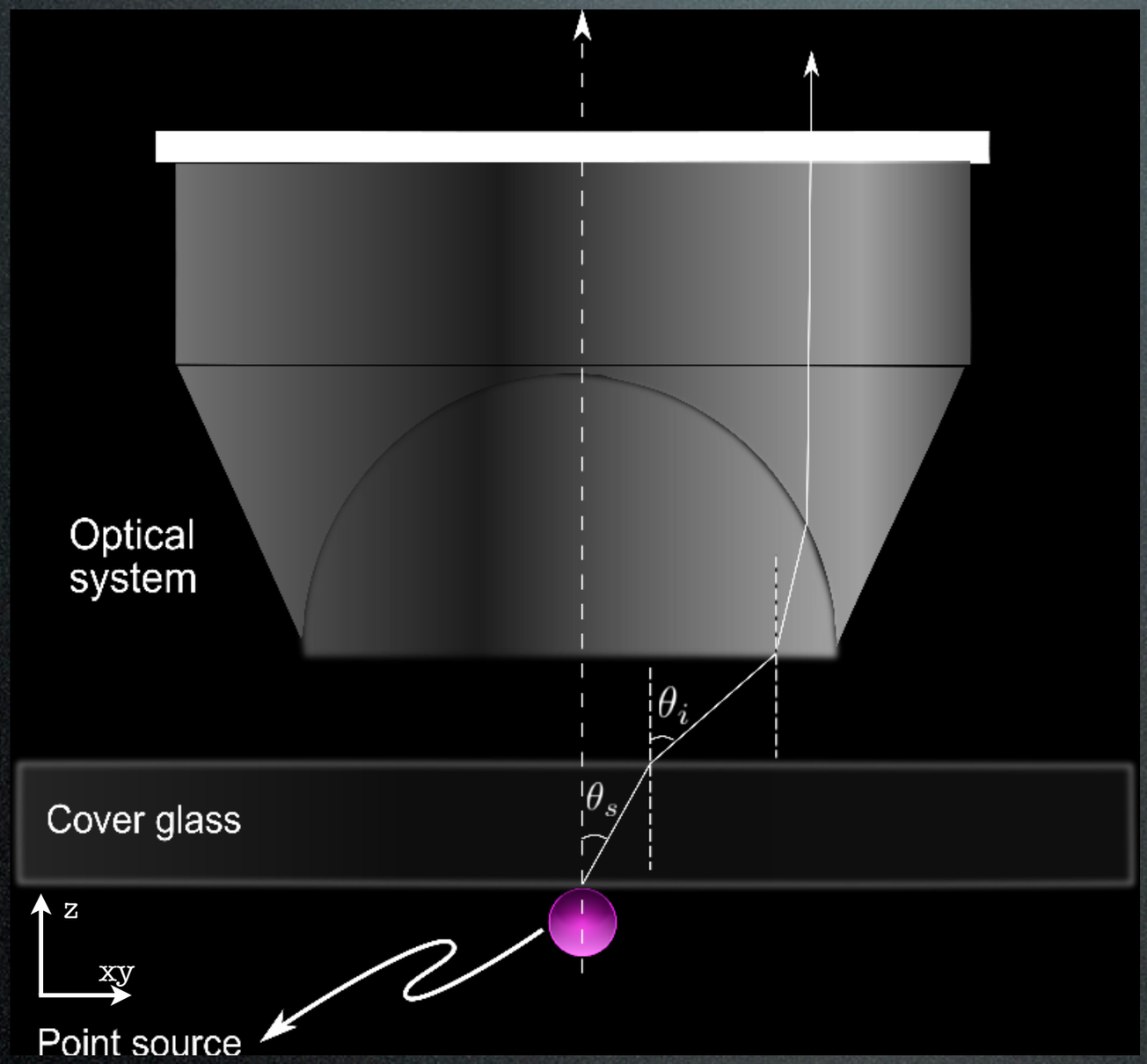
Haemocytometer with known grid dimensions

Grid slides in transmission mode

Focal frame

Objective: 2x,
Zoom: 12.7x
50um z-step

Characterization: Beads as ‘guide stars’



Imaging 2.5um and 4um fluorescent beads

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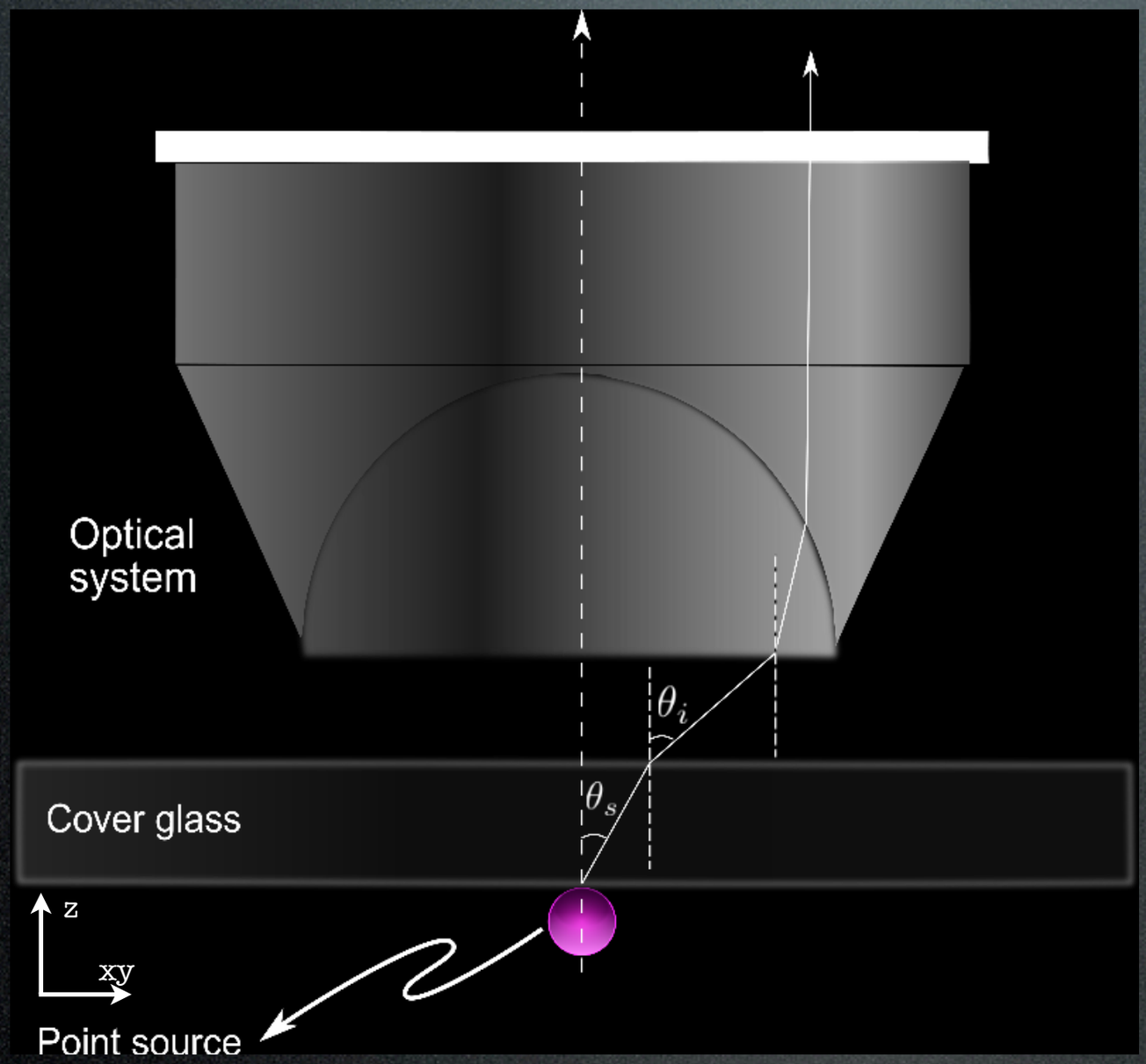

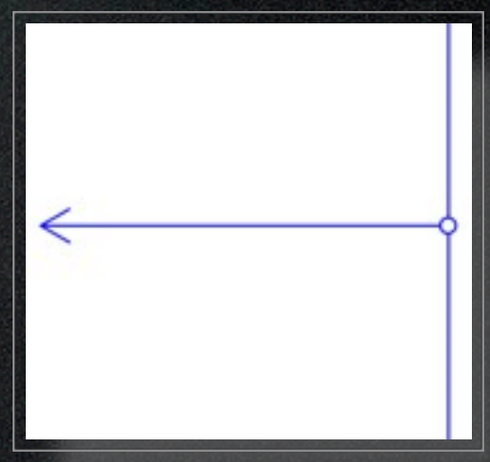

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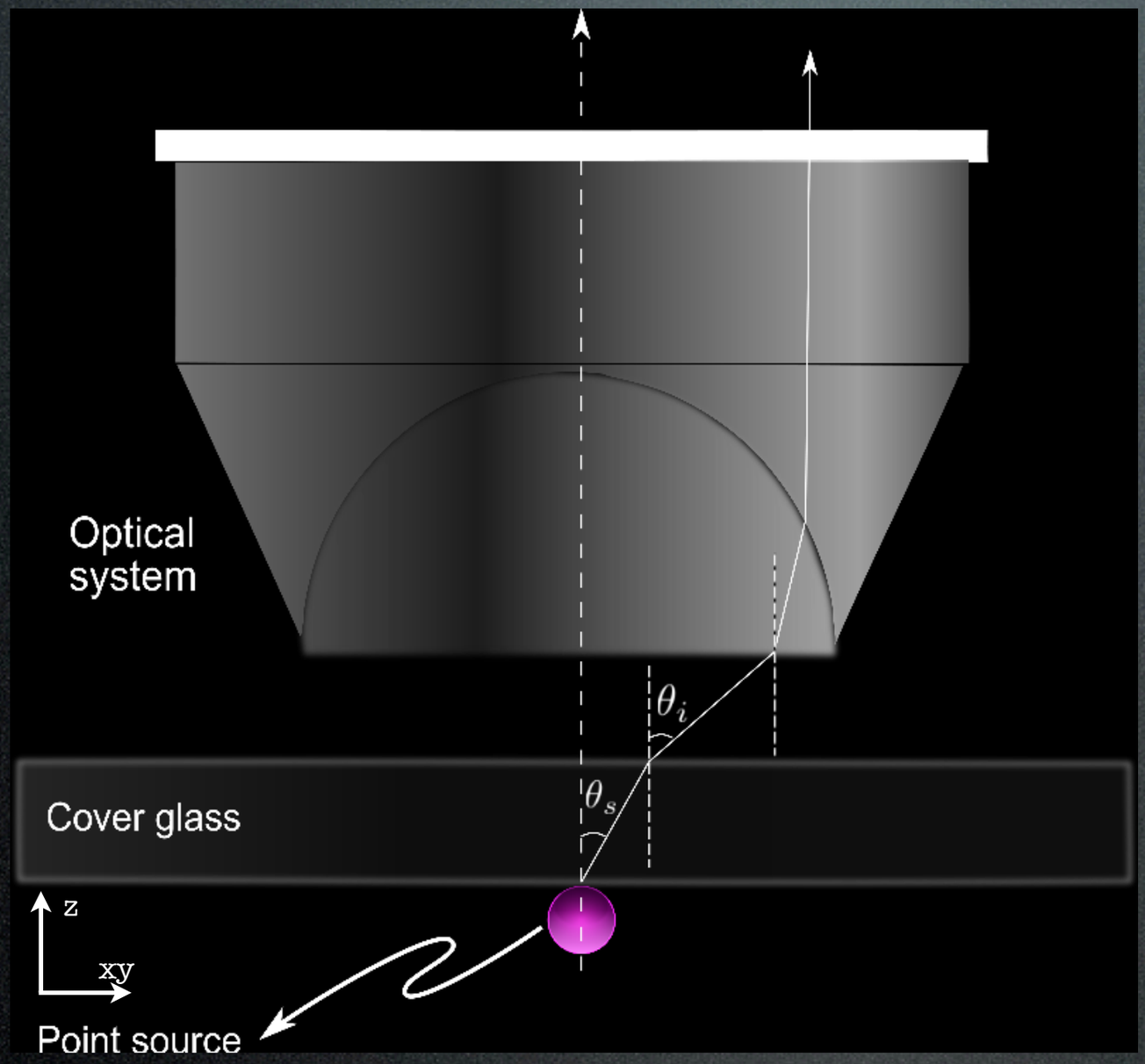

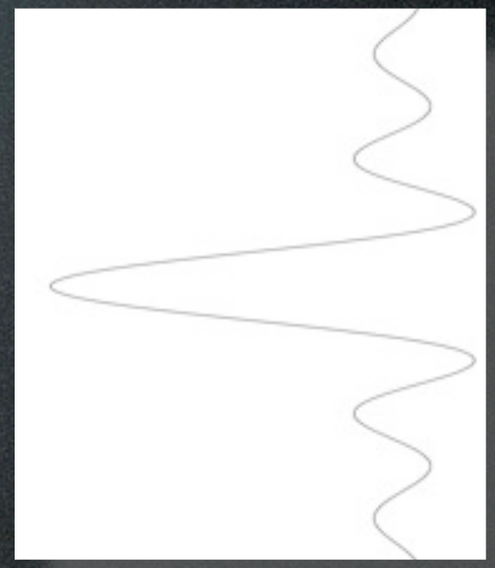

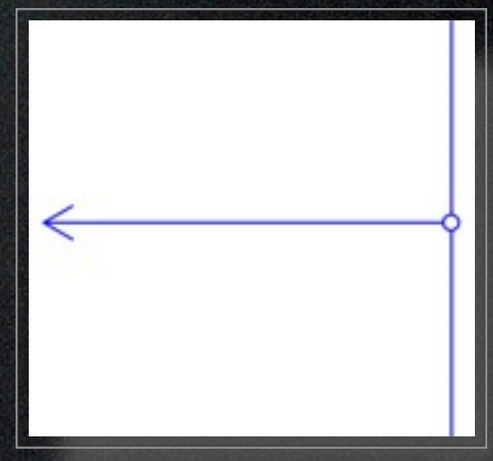

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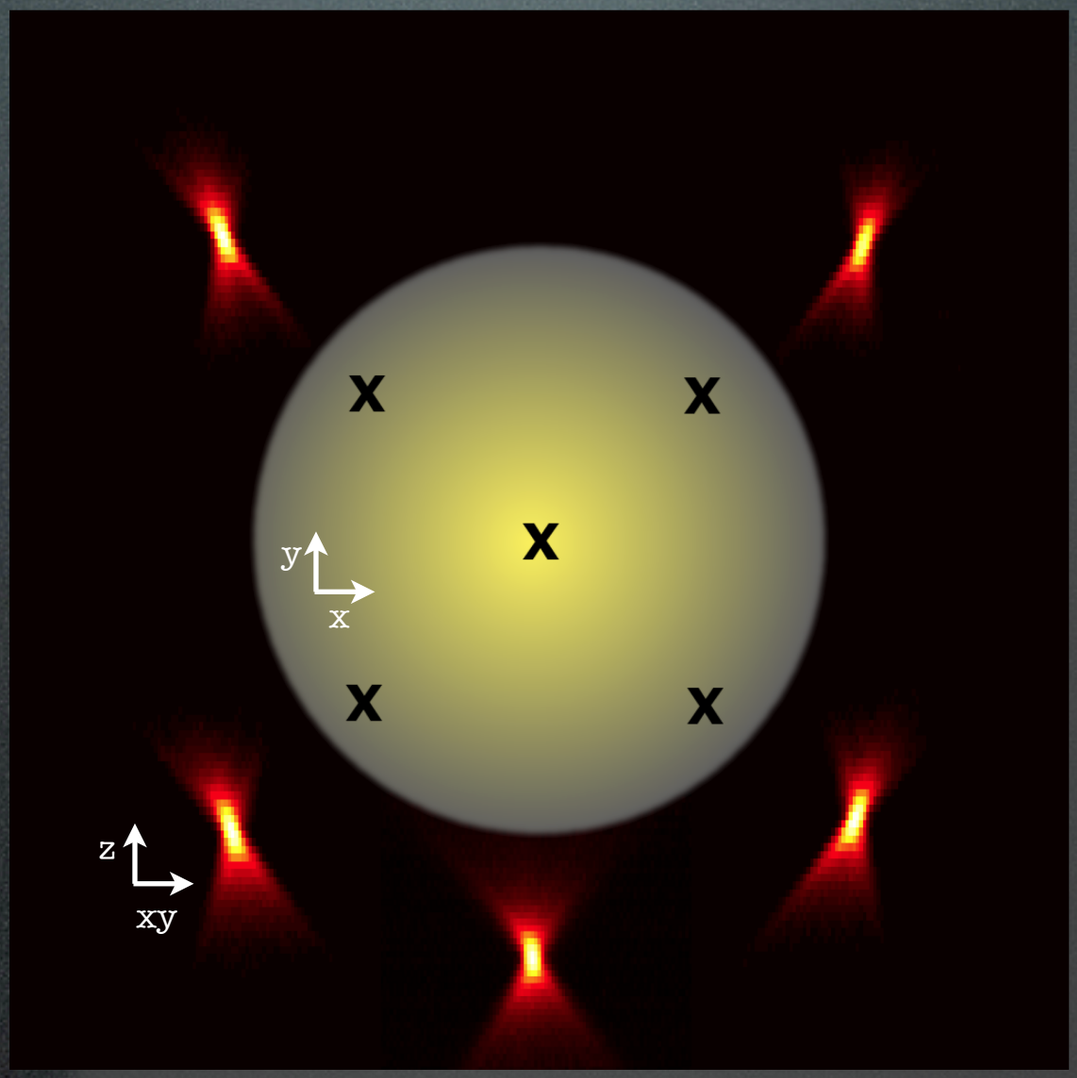
Space-invariance



Laterally varying impulse response

Large Field is great but ...

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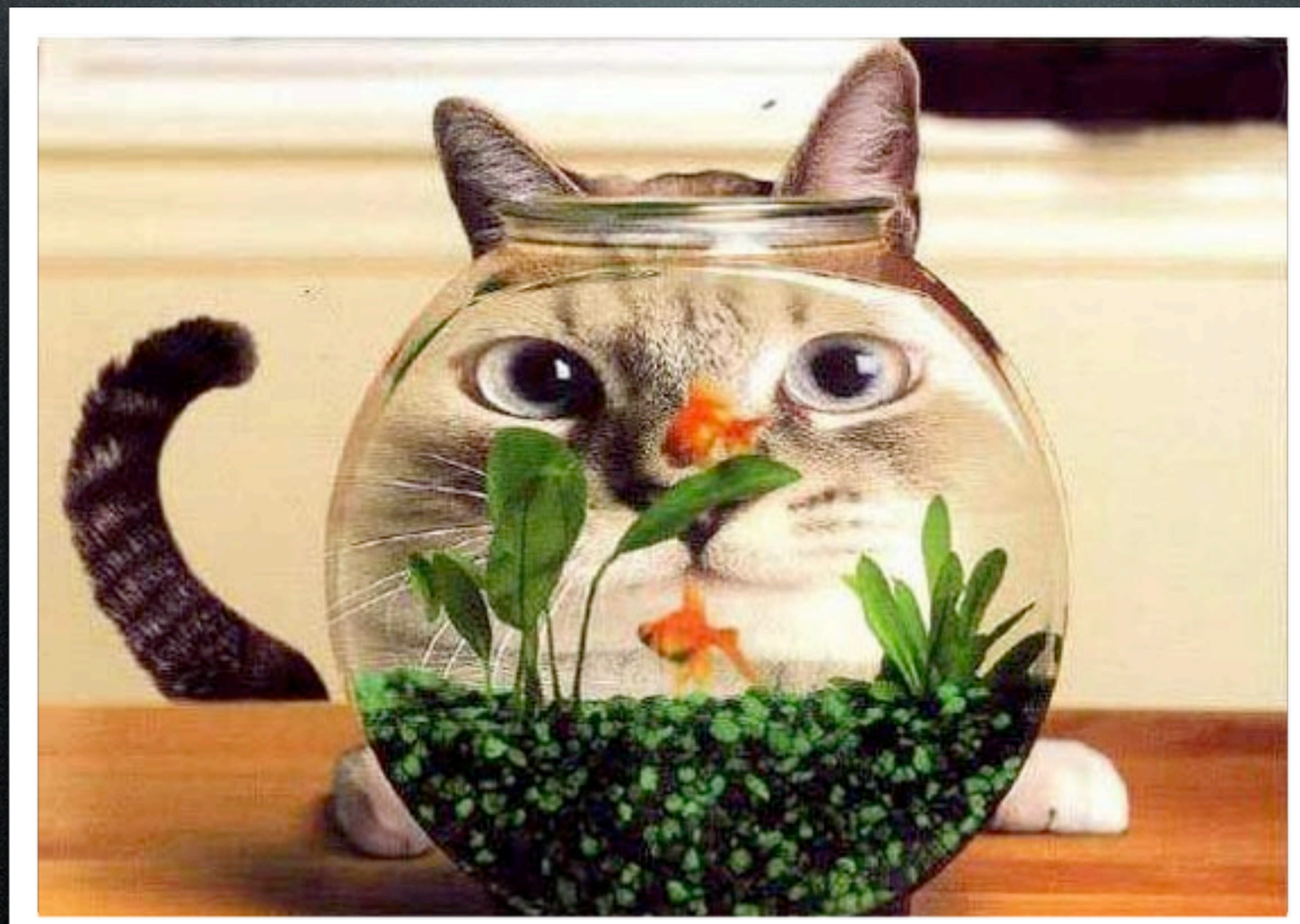
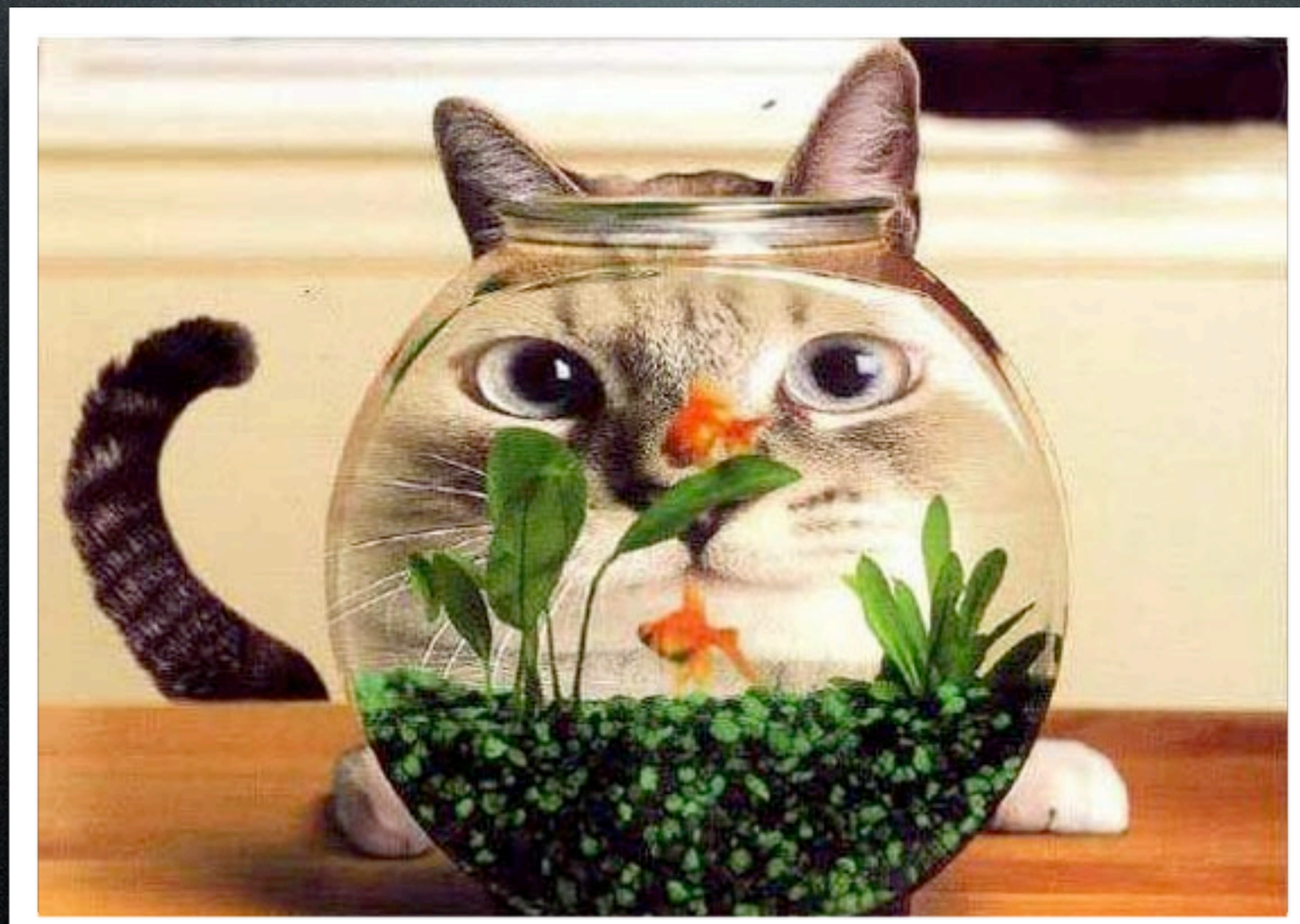


Field Distortions?

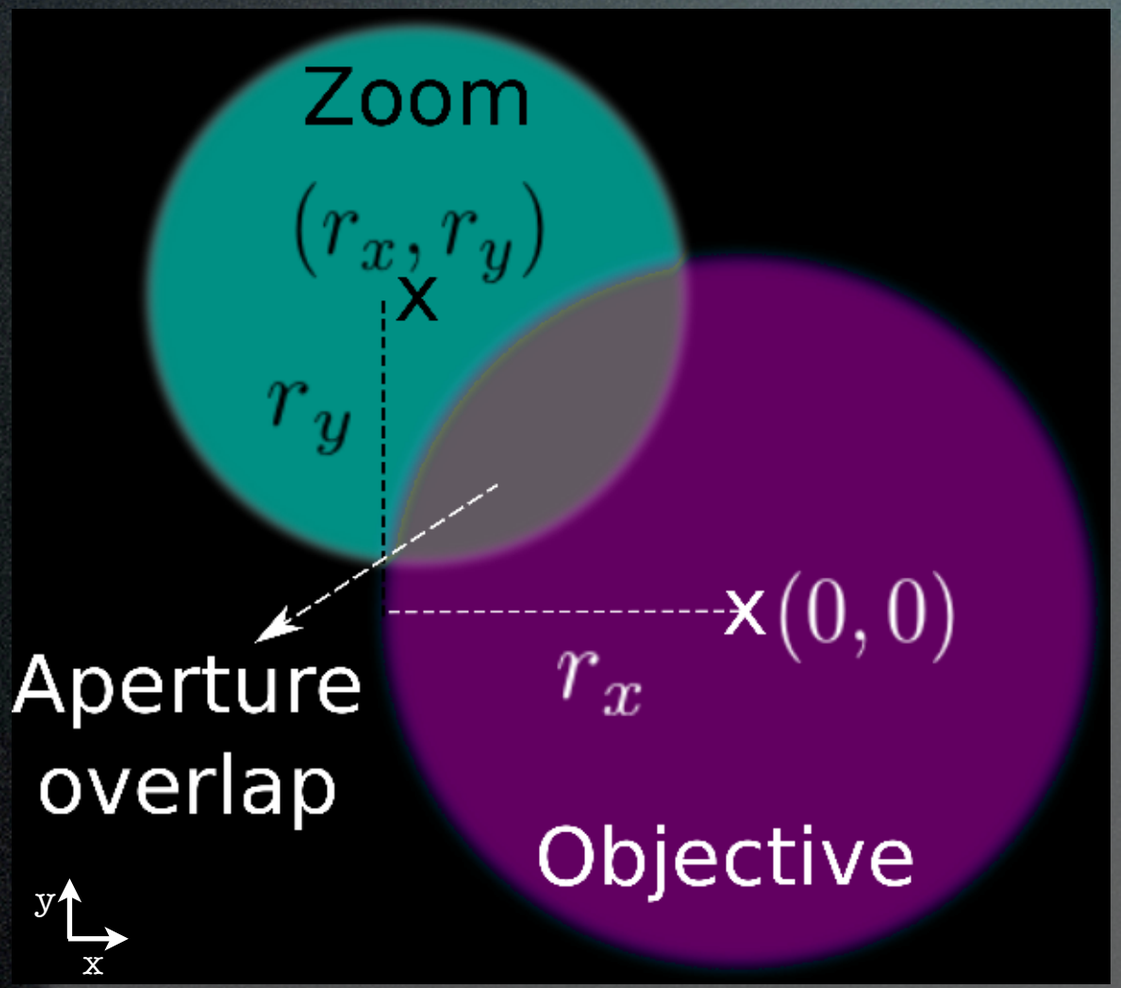
Large Field is great but ...

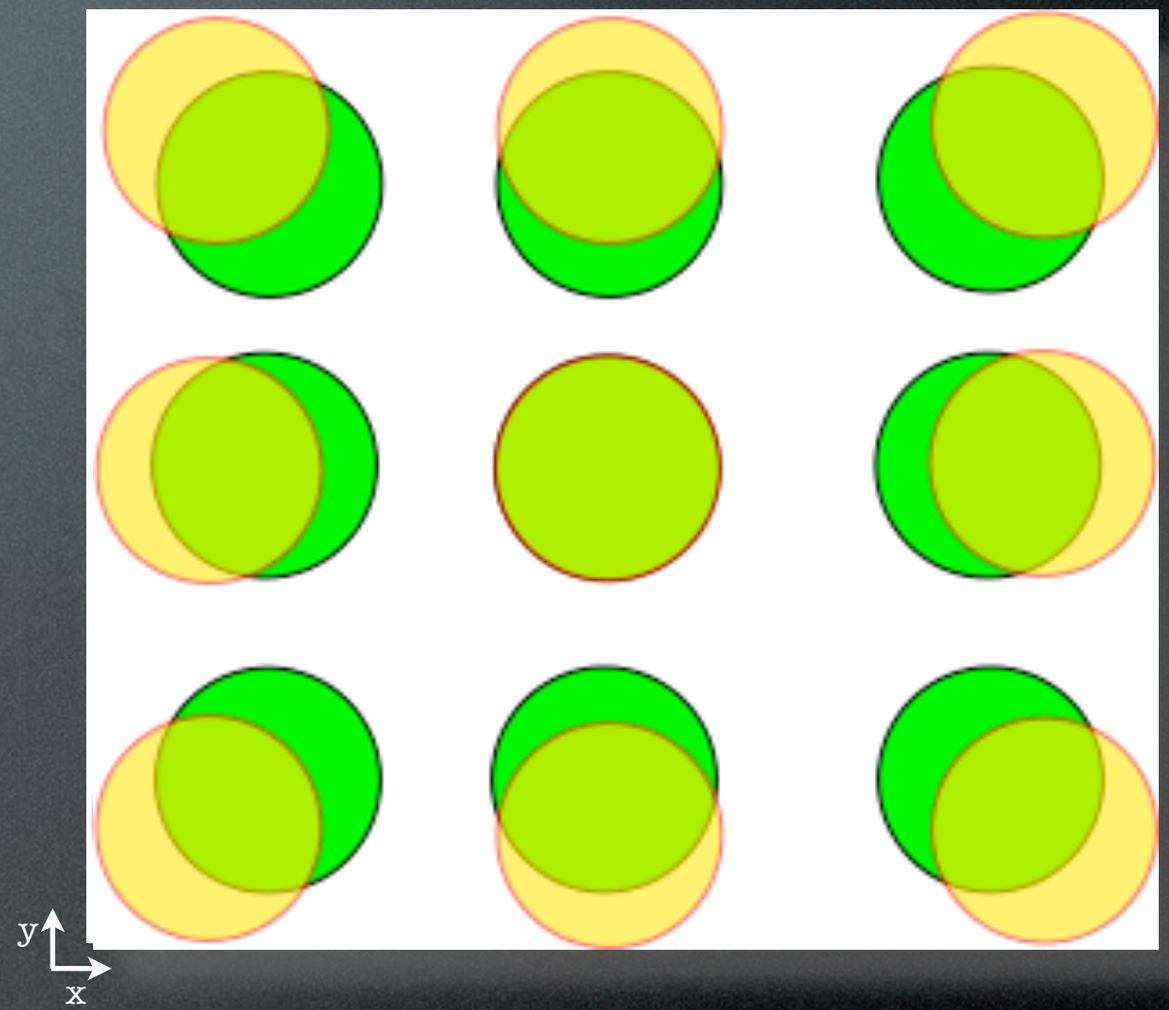


Yes!
... but different

Field Distortions?

Pupil function model





Aperture shape dependence on lateral positions

P. Pankajakshan, et al., “Point-spread function model for fluorescence macroscopy imaging,” in Proc. of Asilomar Conference on Signals, Systems and Computers, Nov. 2010.

Forward model: PSF

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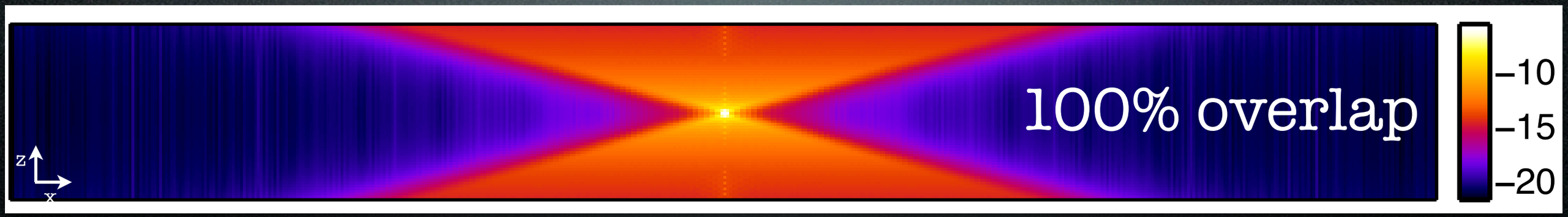

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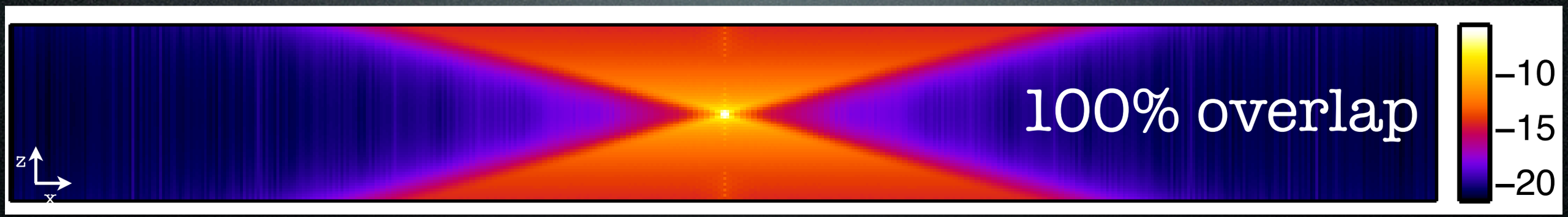

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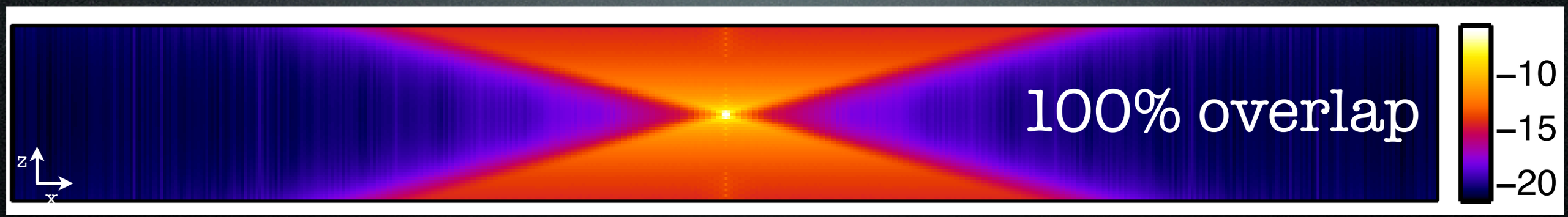

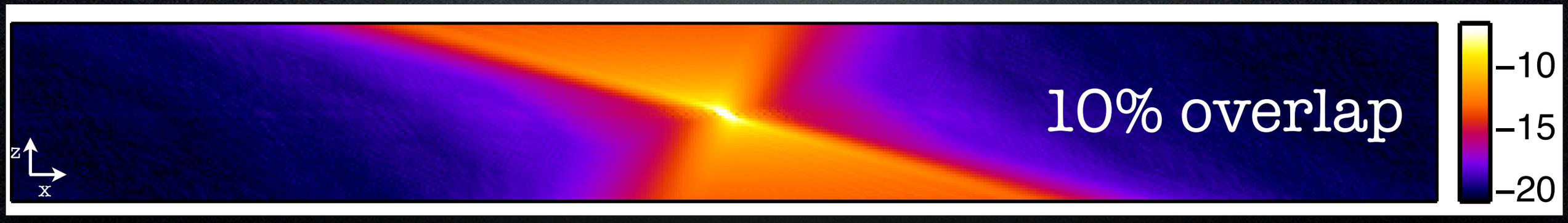

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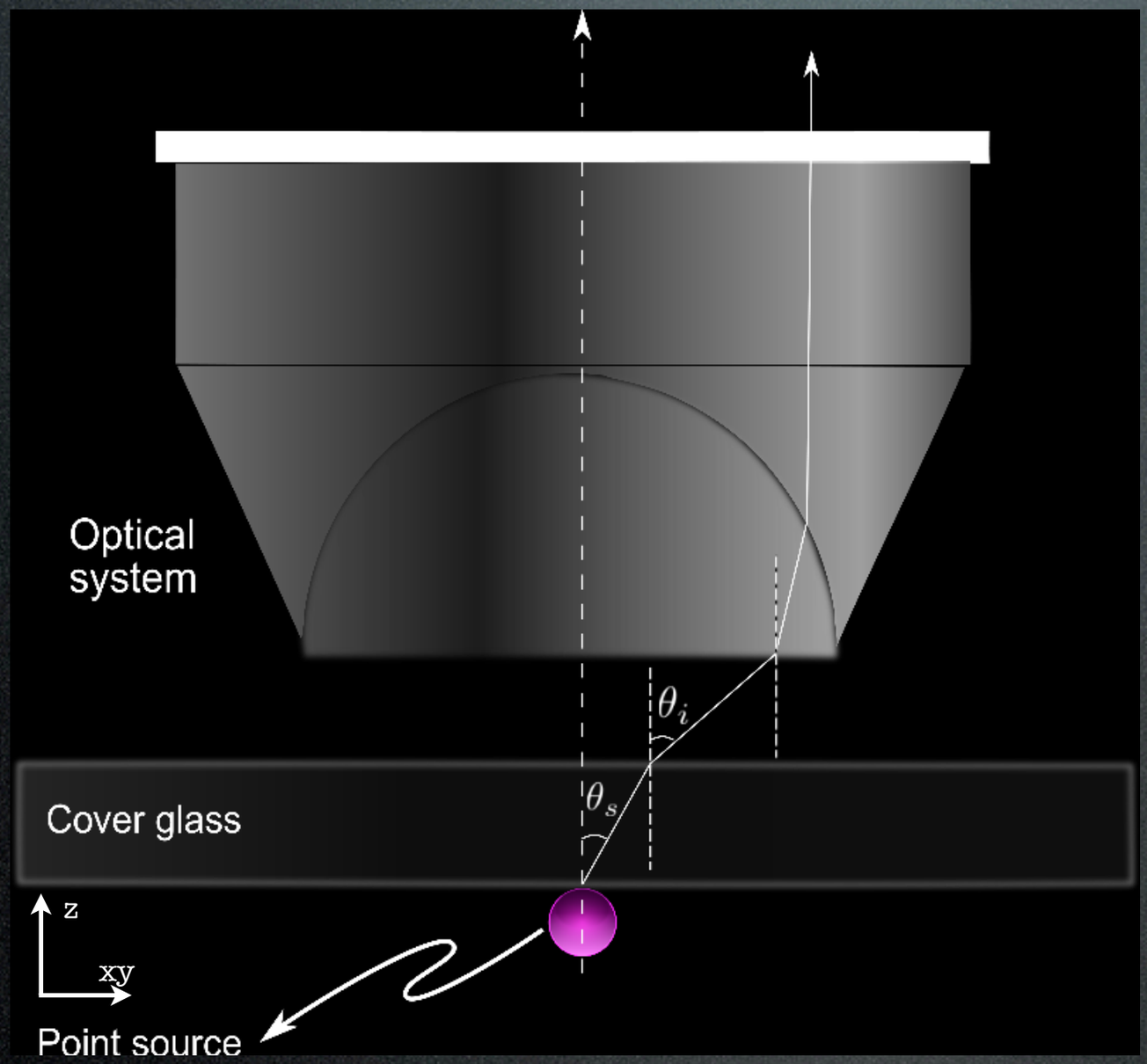
Thats all folks!

Characterization: Beads as ‘guide stars’



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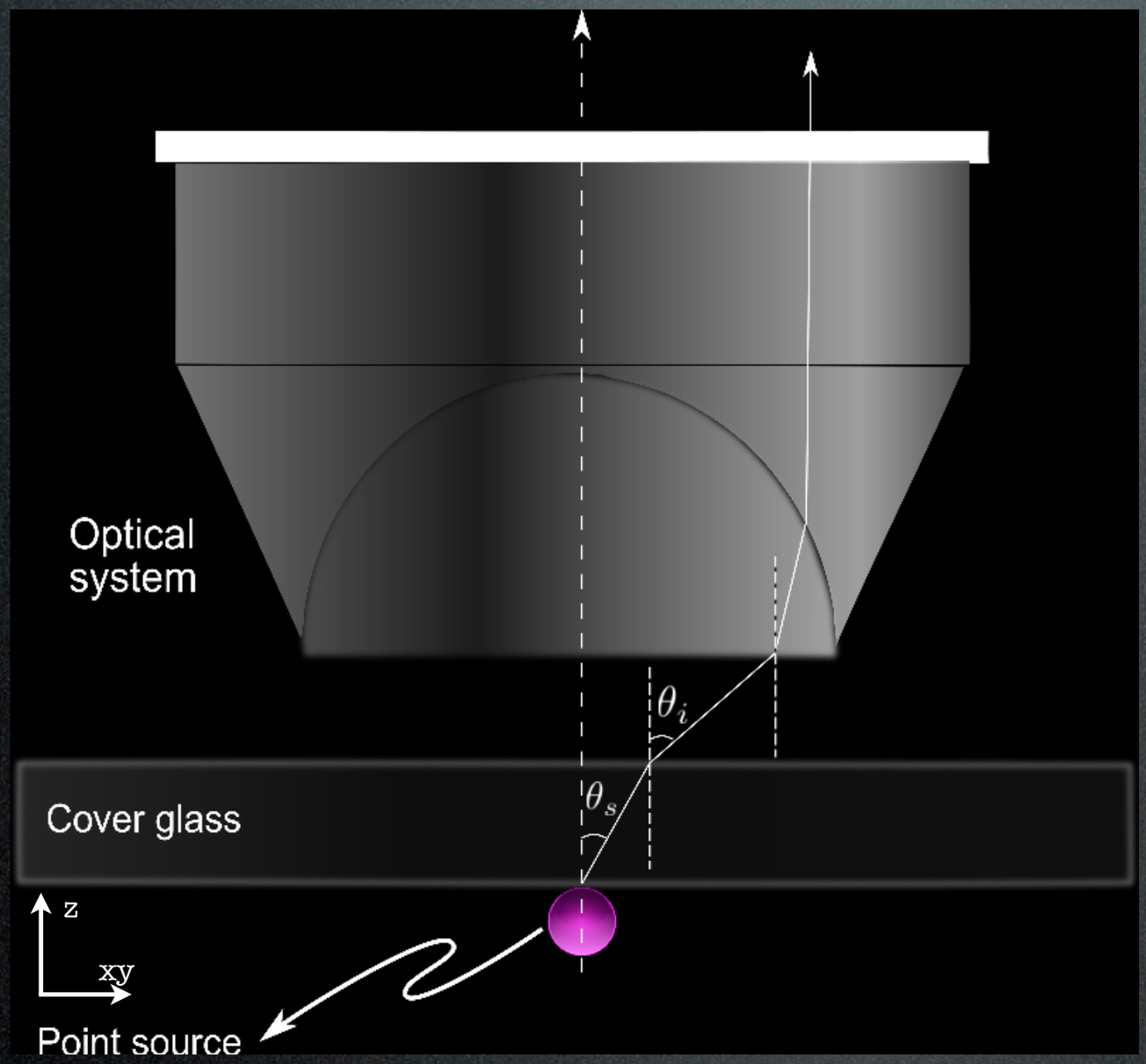

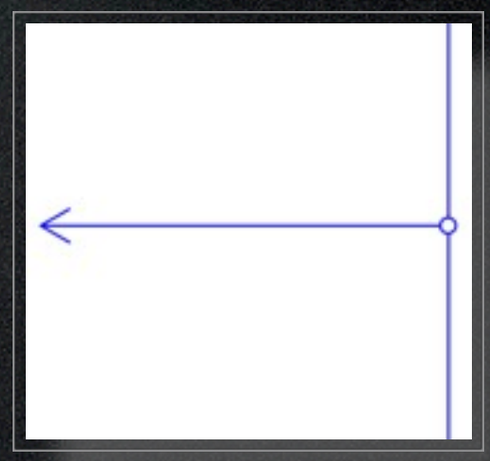

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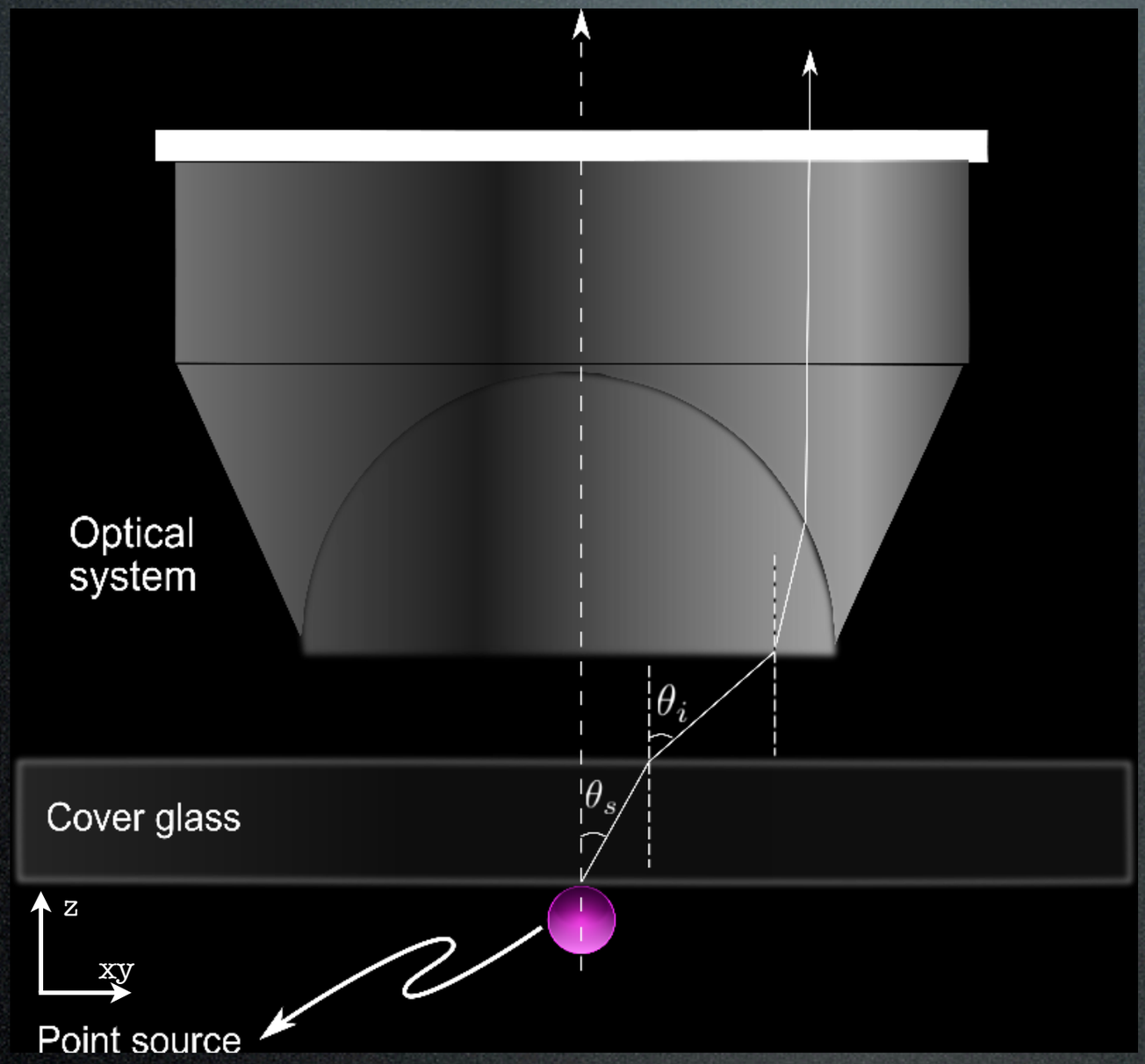

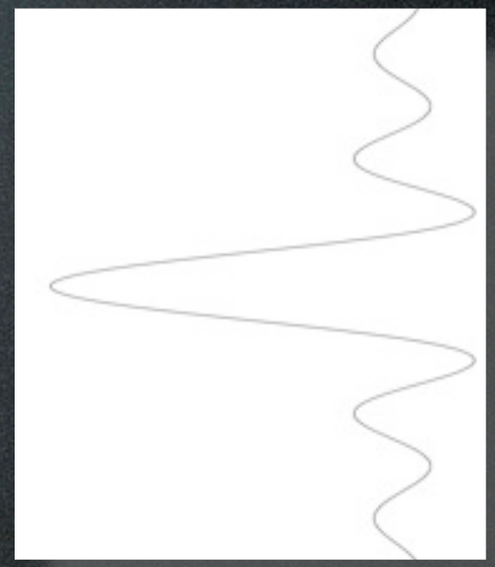

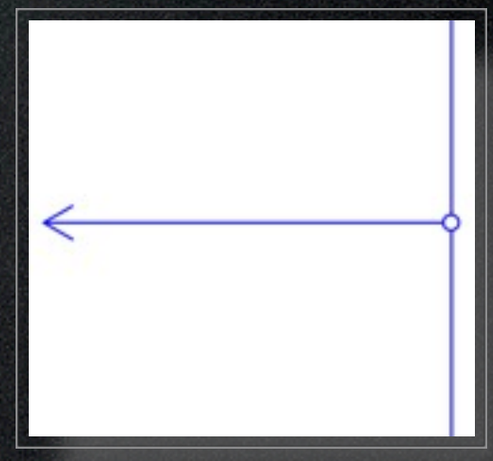

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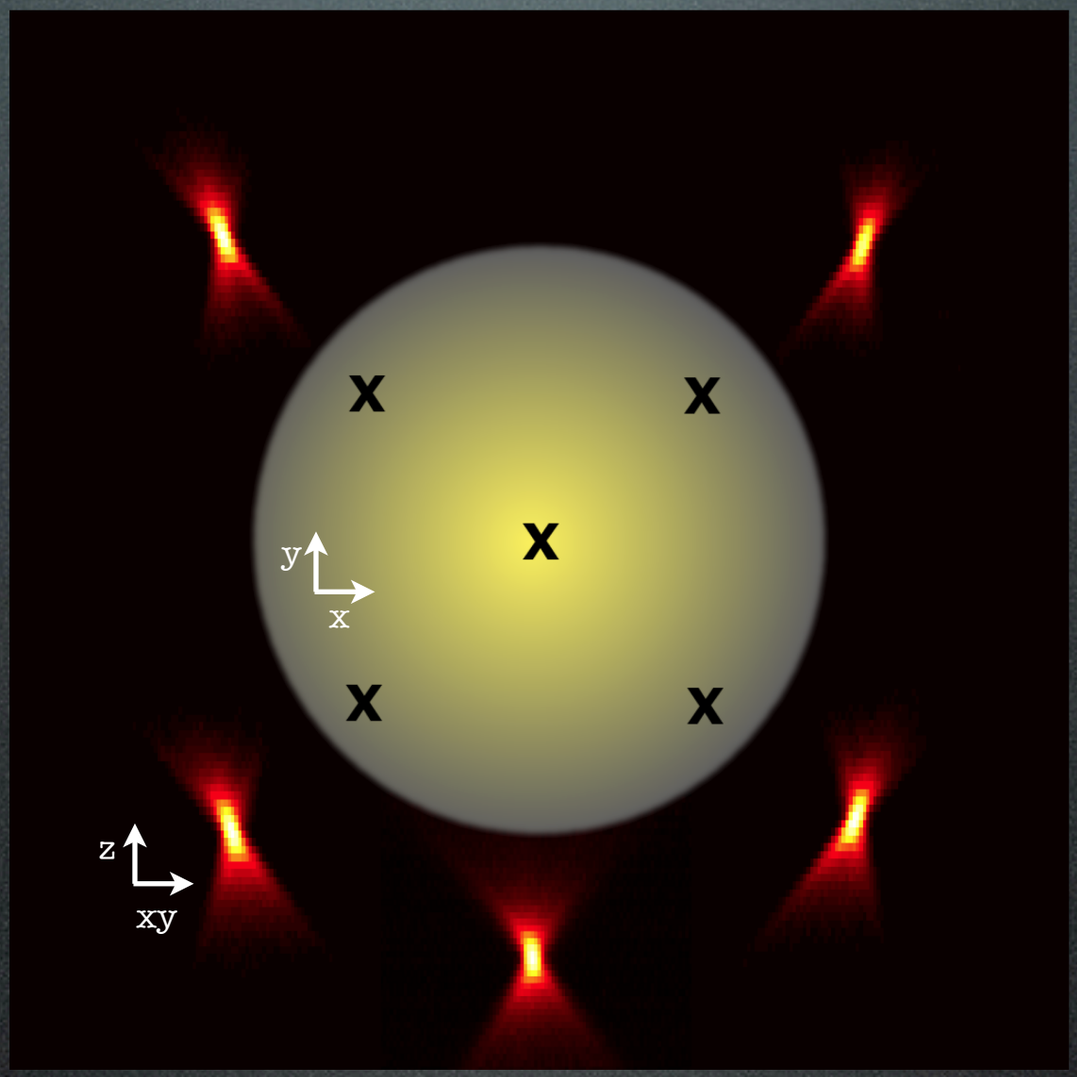

Laterally varying impulse response

Forward PSF model

$$h_A(x, y, z; \lambda) = \int_{k_x} \int_{k_y} P(k_x, k_y, z) \exp(j(k_x x + k_y y)) dk_y dk_x$$

P. A. Stokseth, “Properties of a defocused optical system,” J. Opt. Soc. Am. A, vol. 59, pp. 1314–1321, Oct. 1969.

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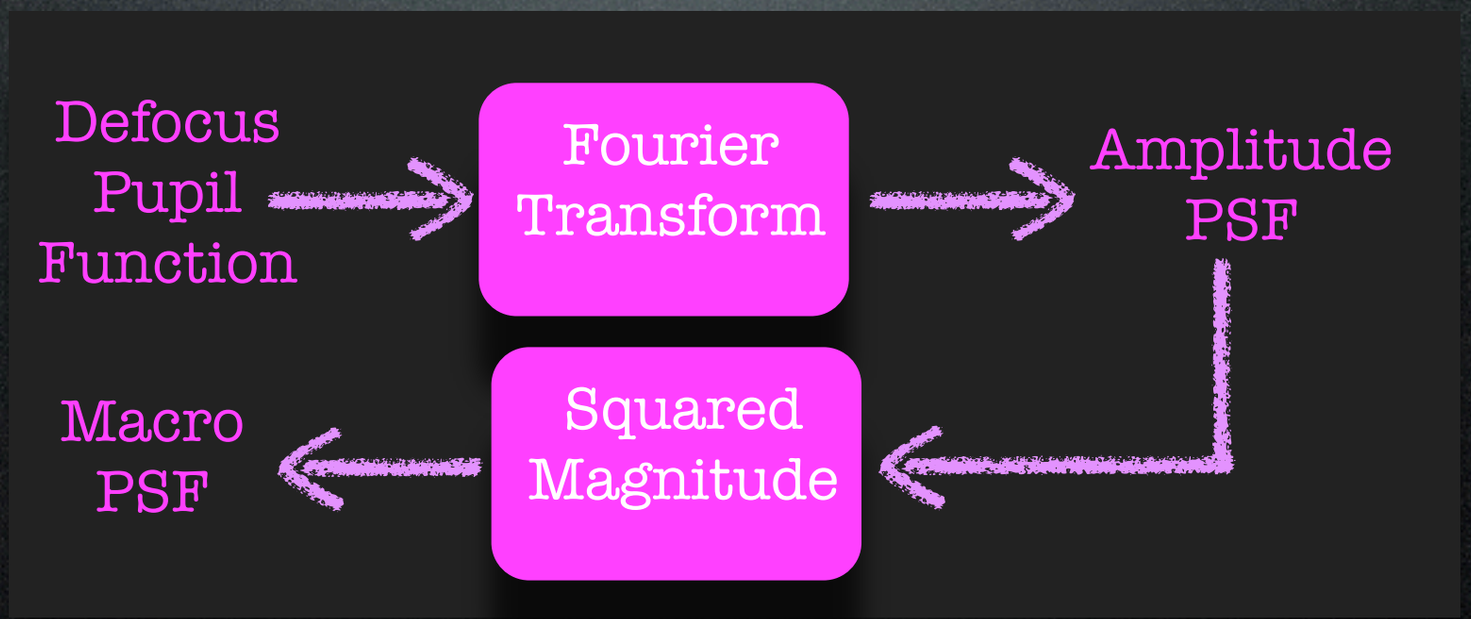

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Forward PSF model

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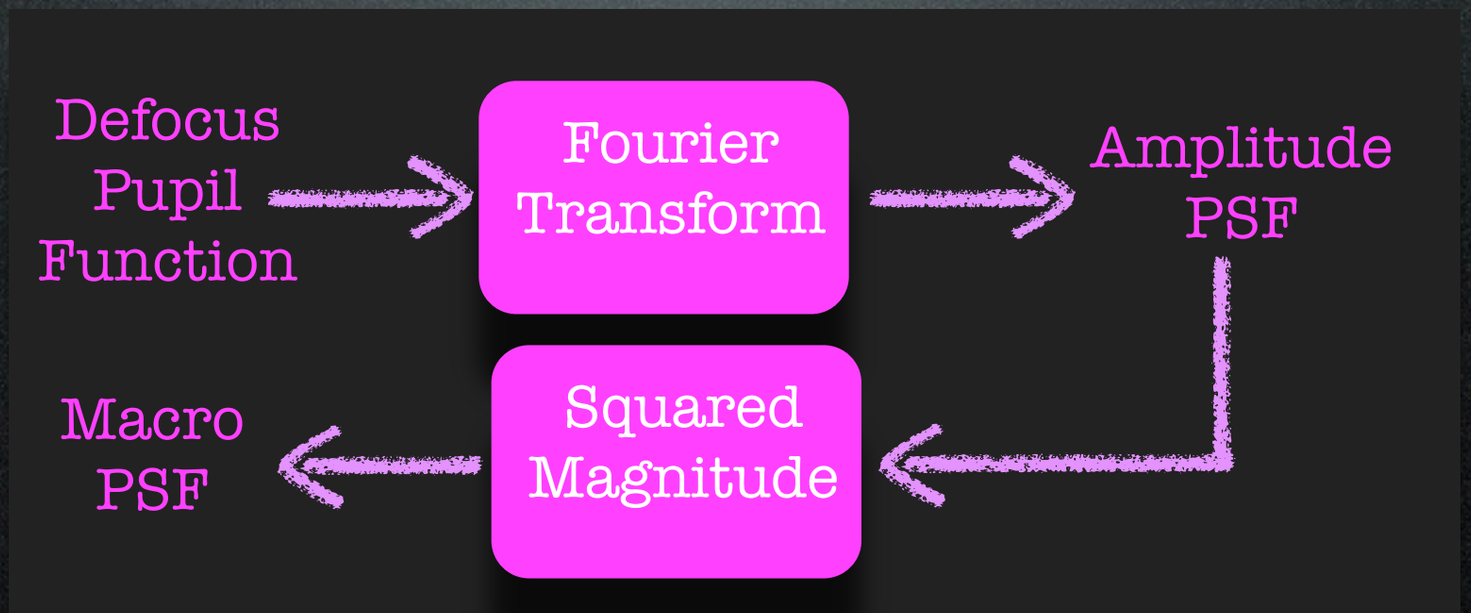

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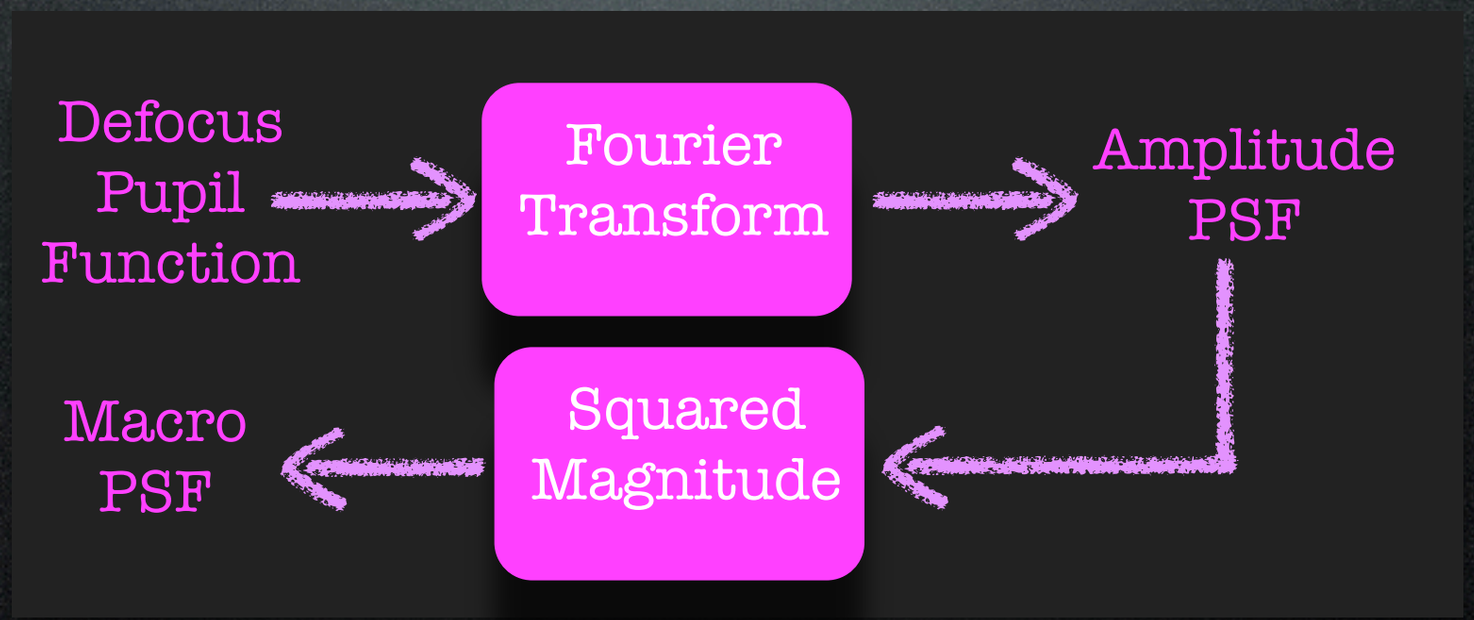


P. A. Stokseth, “Properties of a defocused optical system,” J. Opt. Soc. Am. A, vol. 59, pp. 1314–1321, Oct. 1969.

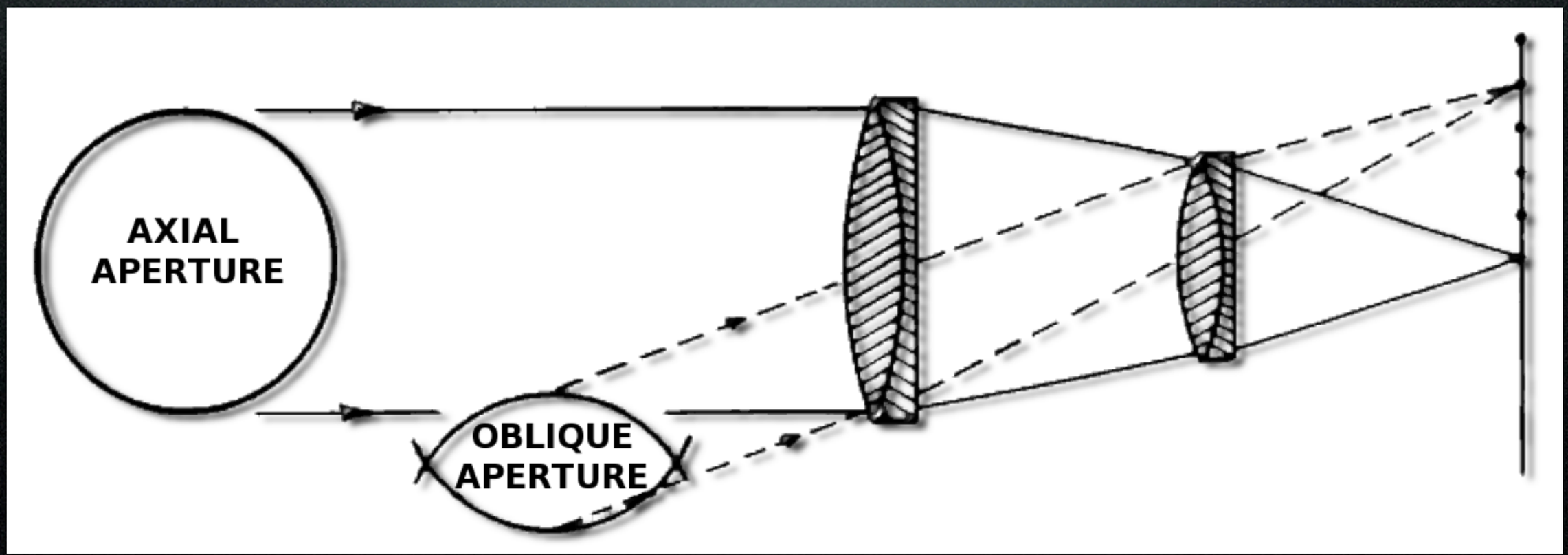
Modification for Large Fields

$$P(k_x,k_y,z) = \begin{cases} P_{\text{obj}}(k_x,k_y,z), & \text{if } \dfrac{\left((k_x-r_x)^2+(k_y-r_y)^2\right)^{\frac{1}{2}}}{k_i} < \dfrac{\text{NA}_z}{n_i} \\ 0, & \text{otherwise .} \end{cases}$$

- When the acquisition parameters are known, theoretically PSF can be calculated.
- The amplitude PSF can be calculated by just $2N_z$ number of 2D FFTs.

P. A. Stokseth, "Properties of a defocused optical system," J. Opt. Soc. Am. A, vol. 59, pp. 1314–1321, Oct. 1969.

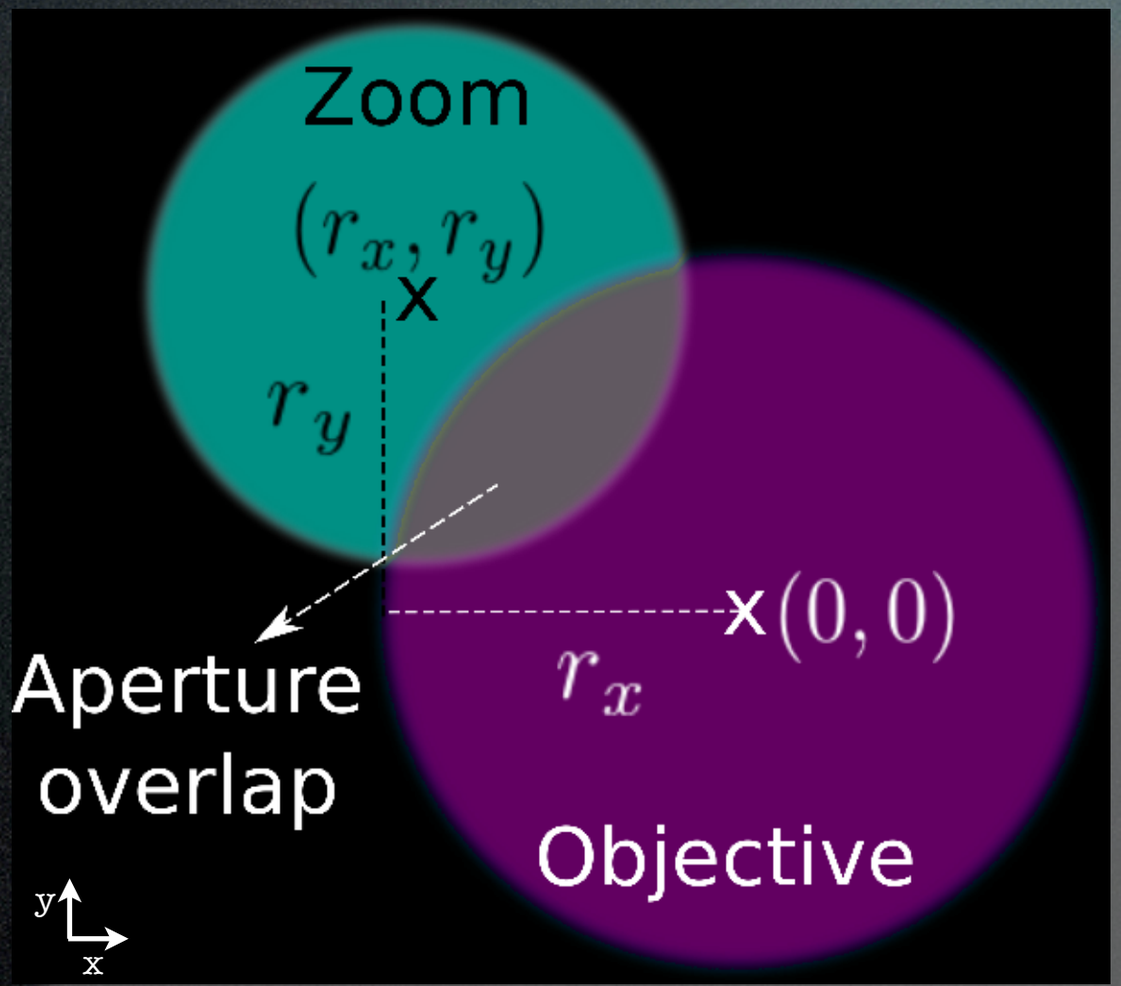
Hypothesis: Off-axis vignetting

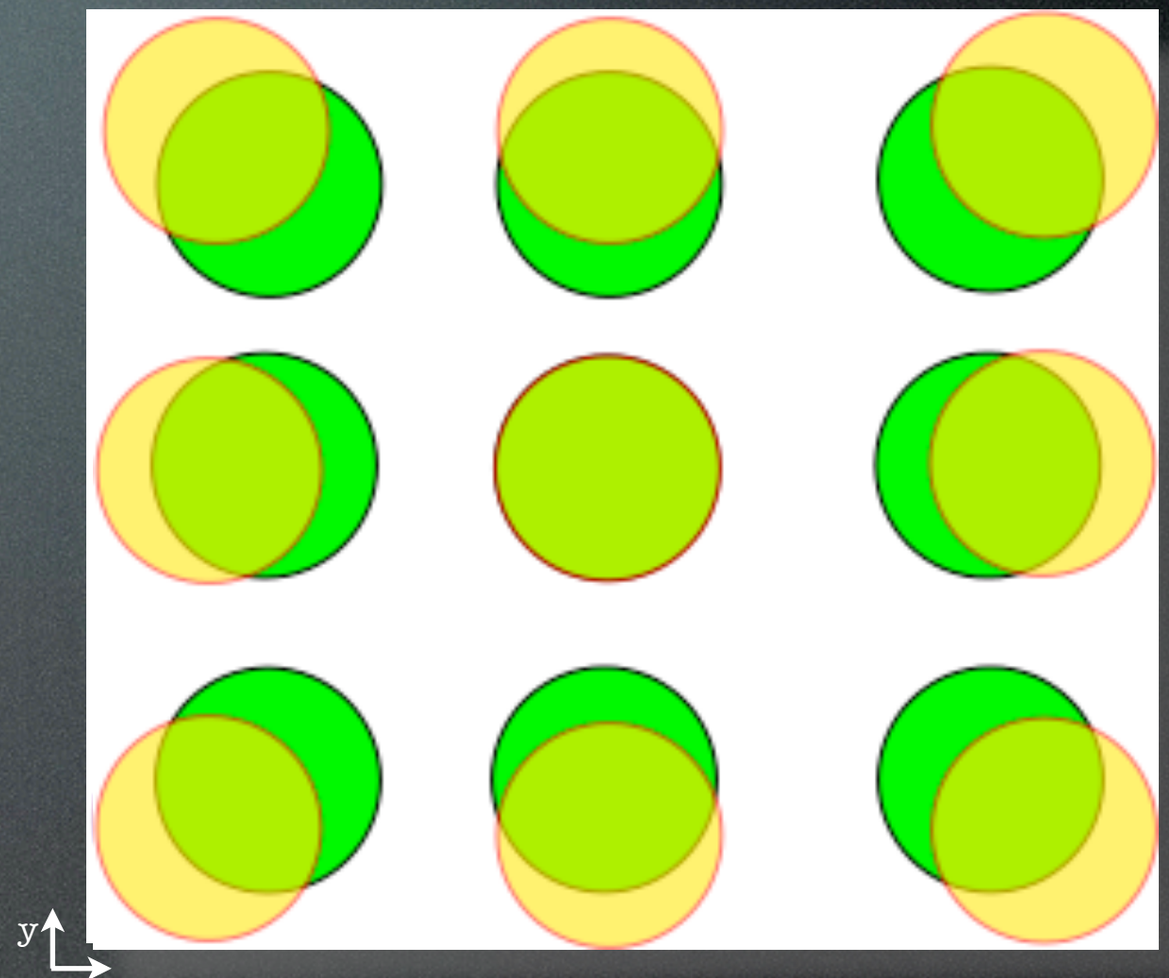


Aperture shape for two different lateral positions

R. Kingslake, Optics in Photography, SPIE Publications, June 1992.

Modeling Hypothesis





Aperture shape dependence on lateral positions

P. Pankajakshan, et al., “Point-spread function model for fluorescence macroscopy imaging,” in Proc. of Asilomar Conference on Signals, Systems and Computers, Nov. 2010.

Forward model: PSF

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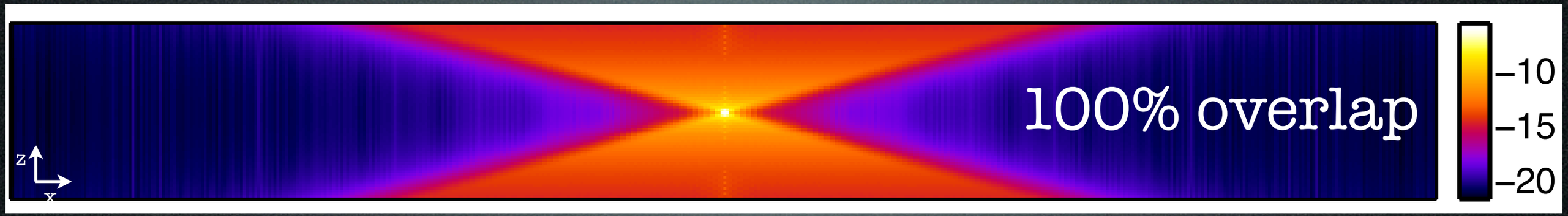
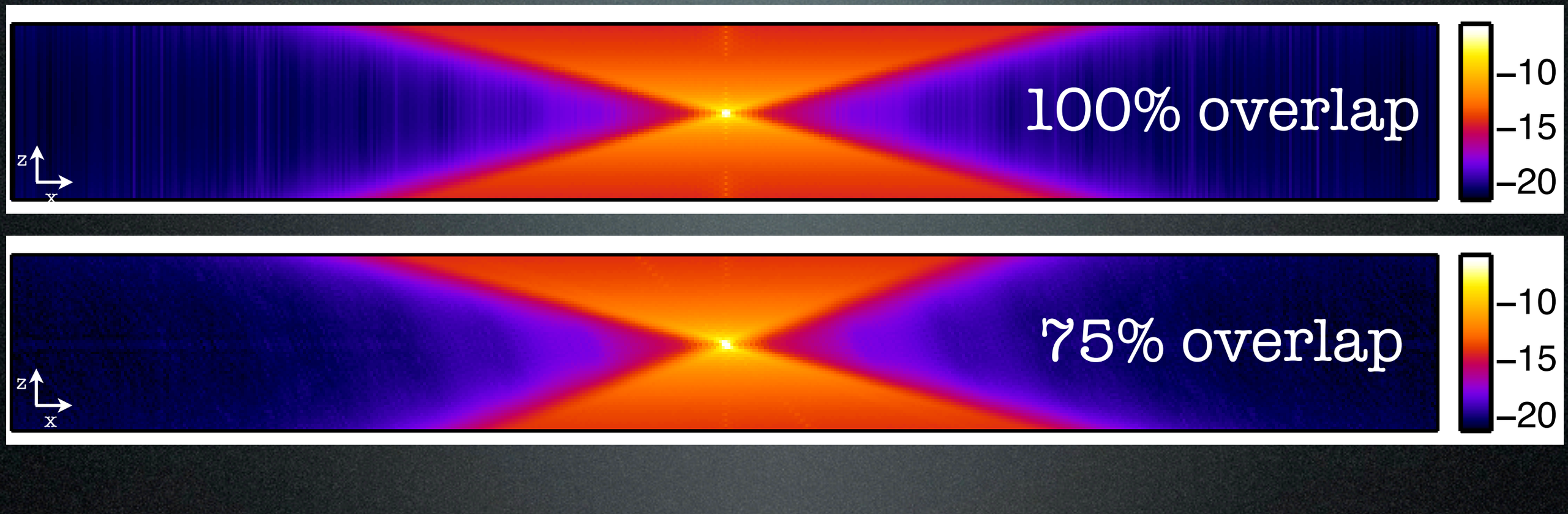
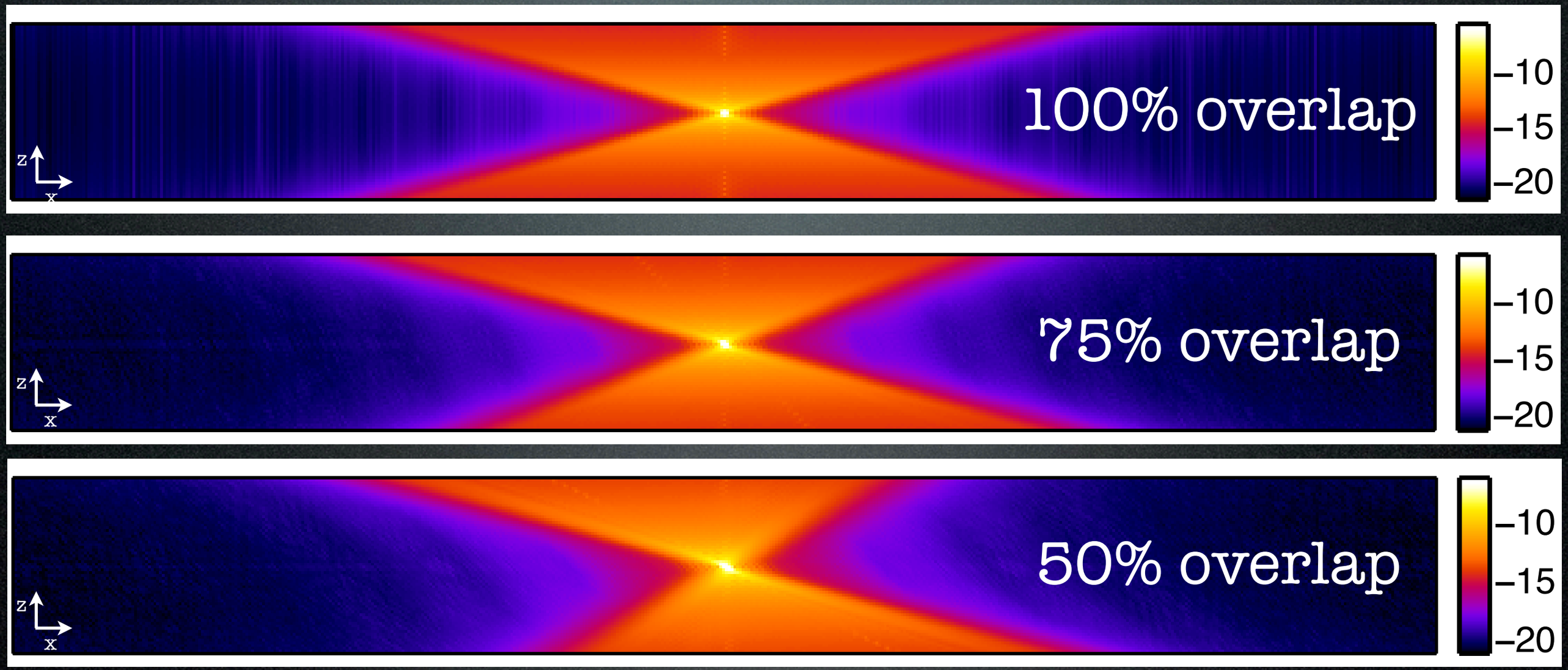


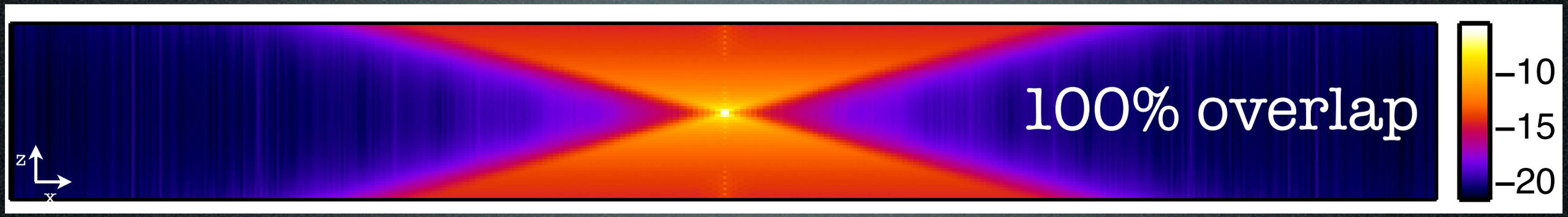
Forward model: PSF

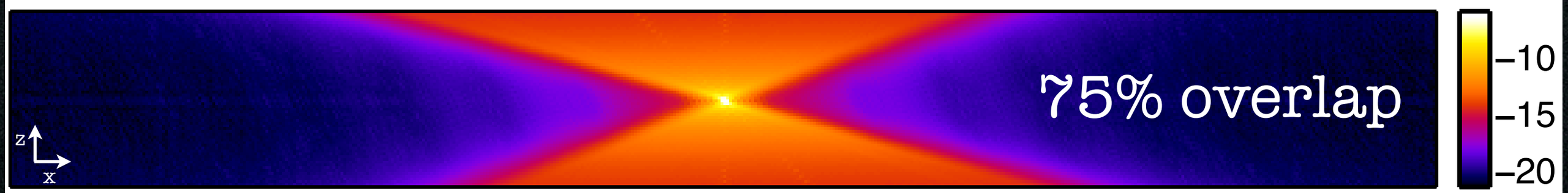


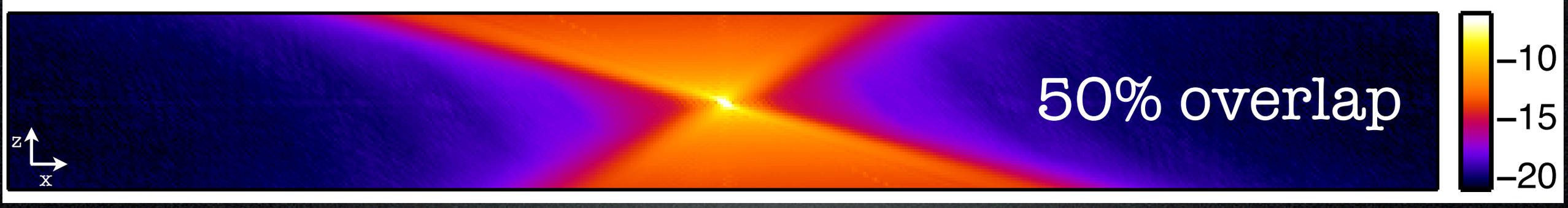
Forward model: PSF

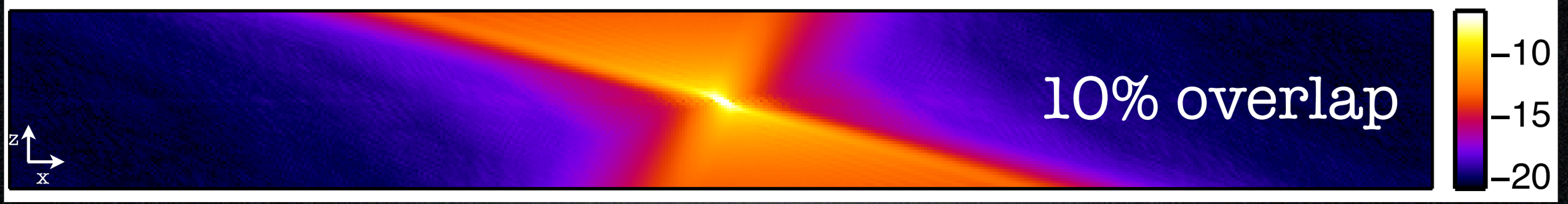


Forward model: PSF

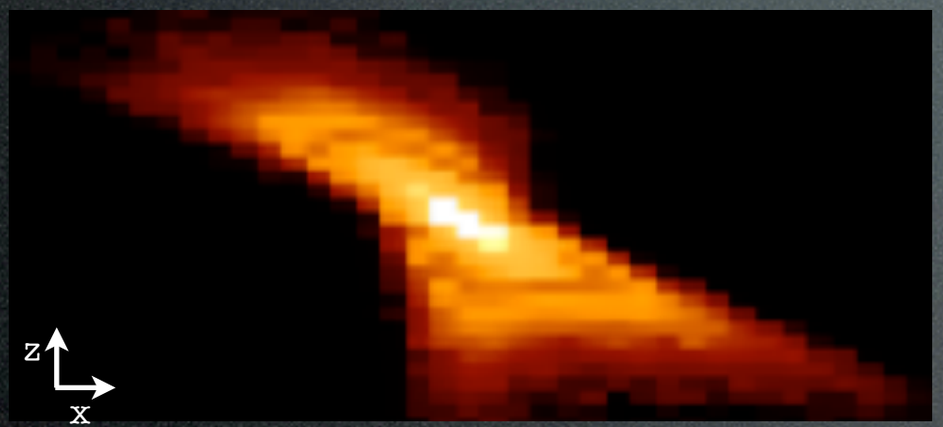




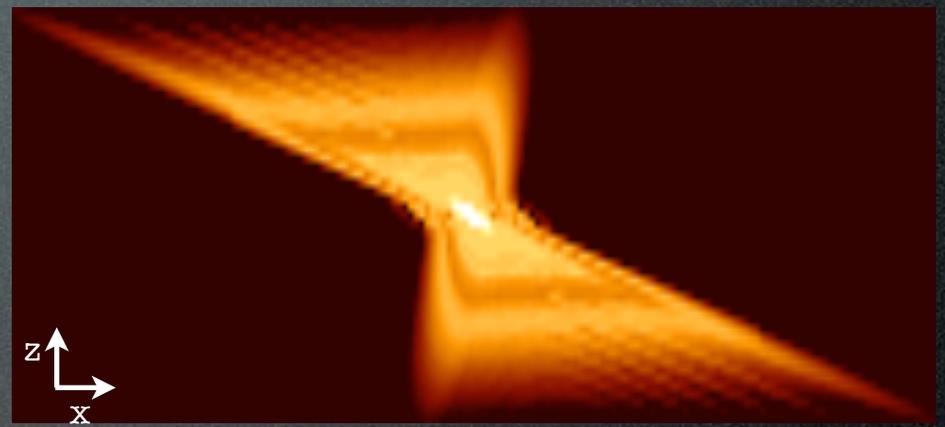




Forward model comparison



Experimental image of point source. (C) Imaging Center IGBMC, France.



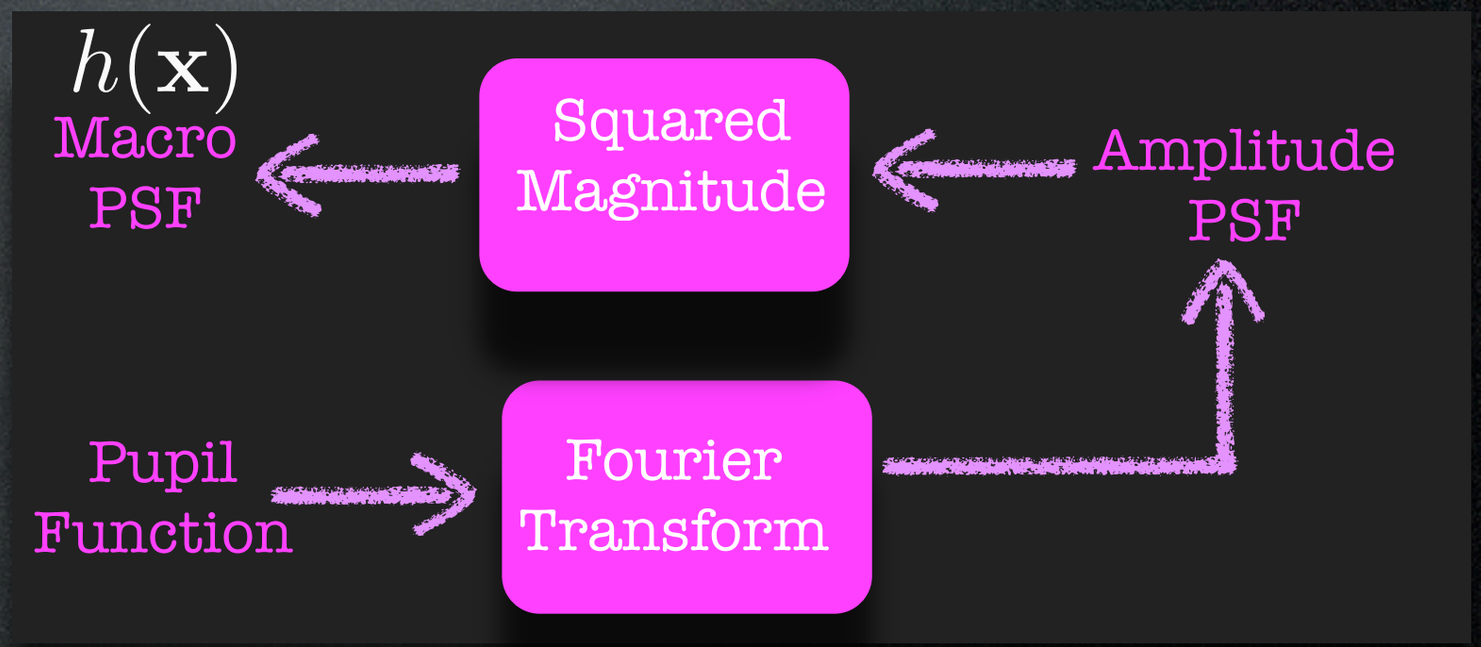
Computationally generated image of point source assuming effective NA of 0.5

P. Pankajakshan et al. 2010

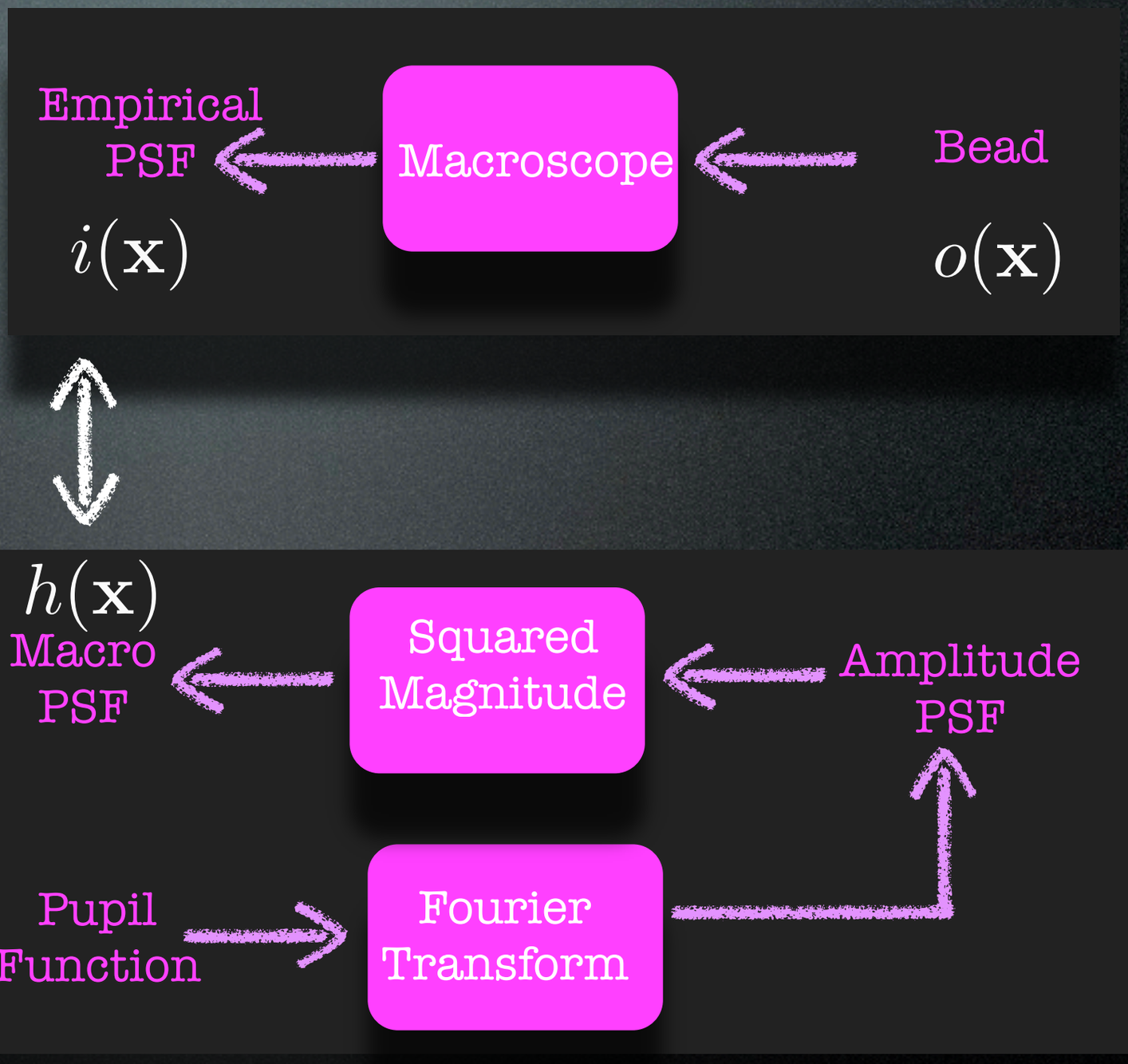
Inverse Problem

Inverse Problem

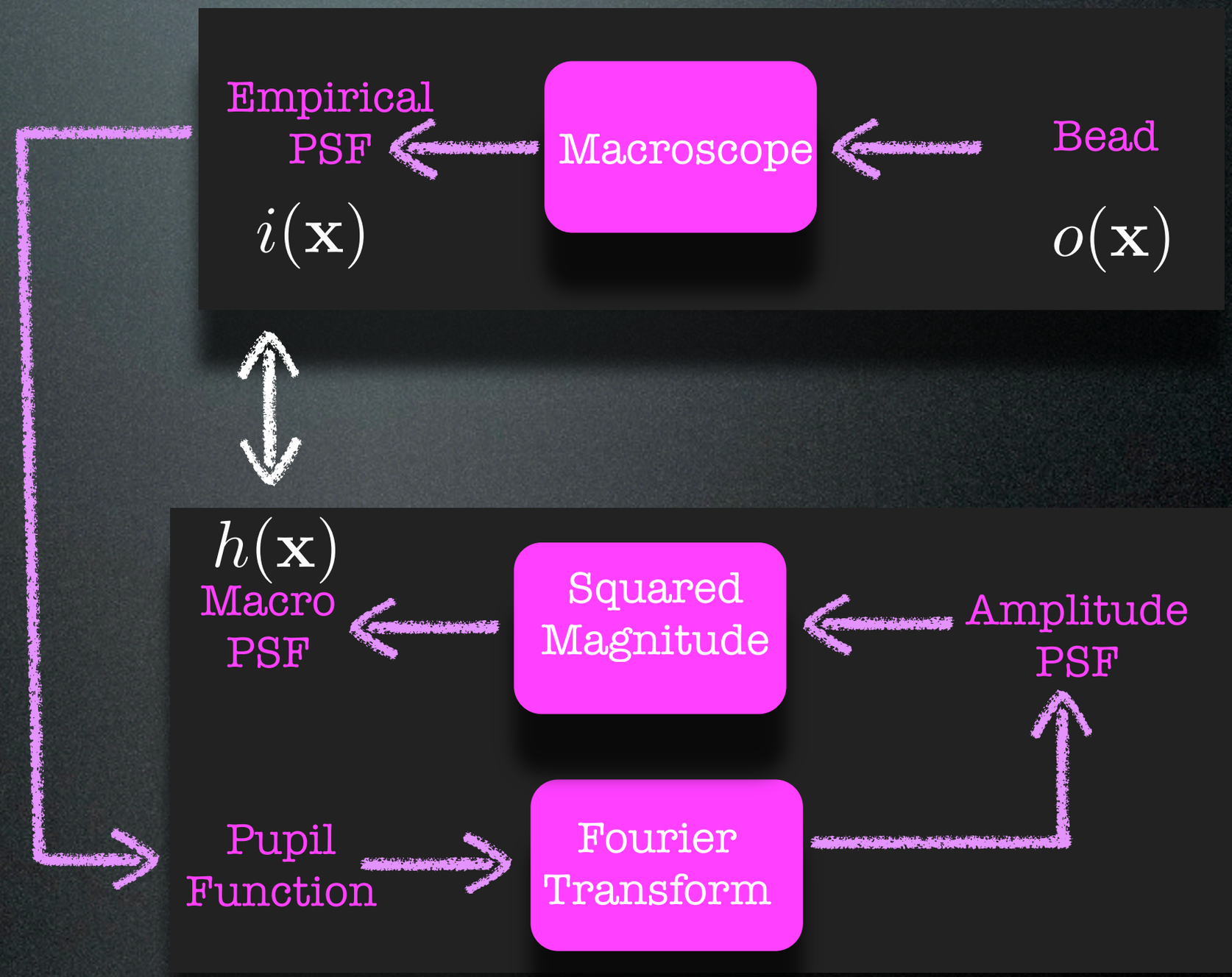
Inverse Problem



Inverse Problem



Inverse Problem



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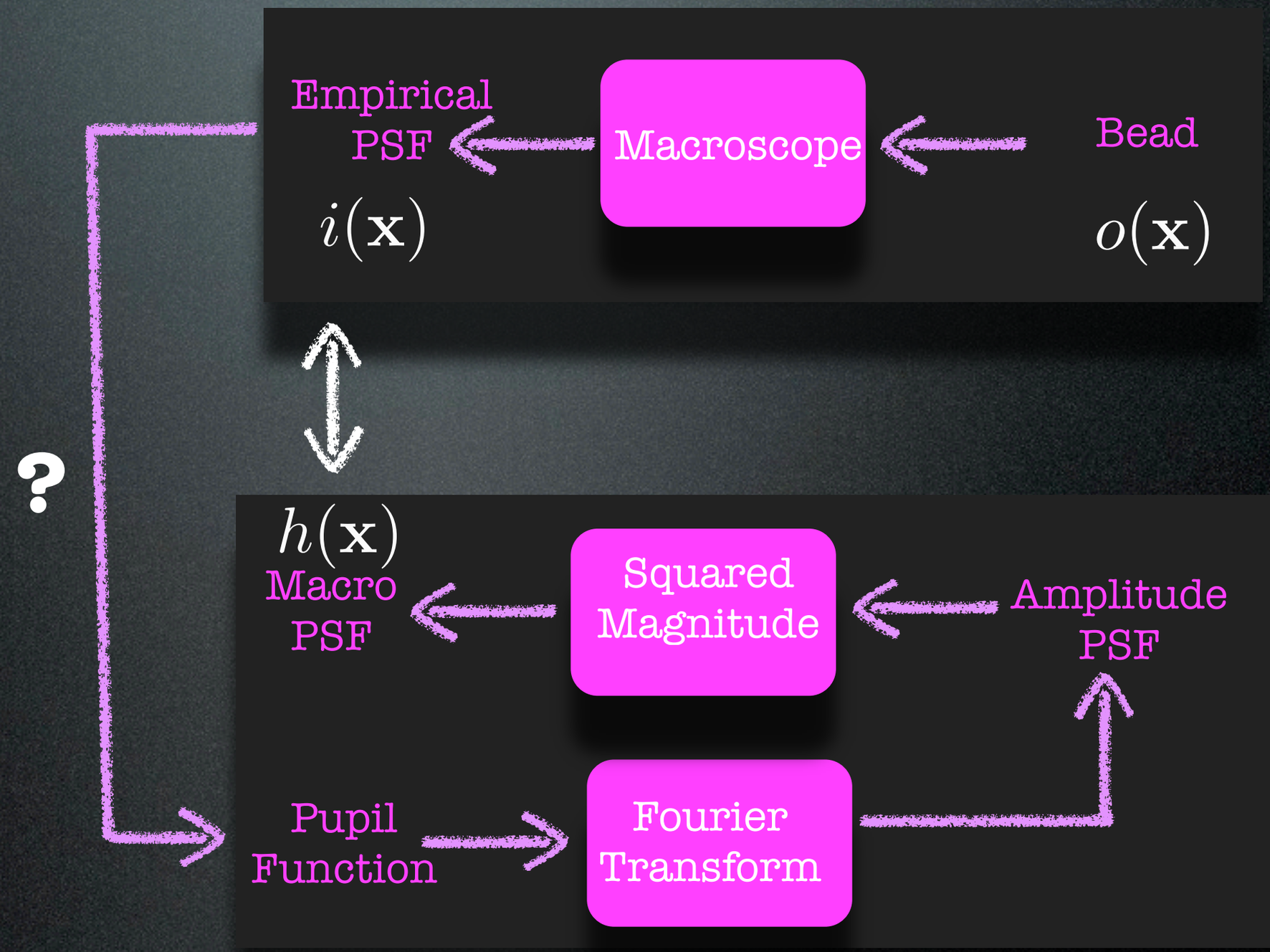
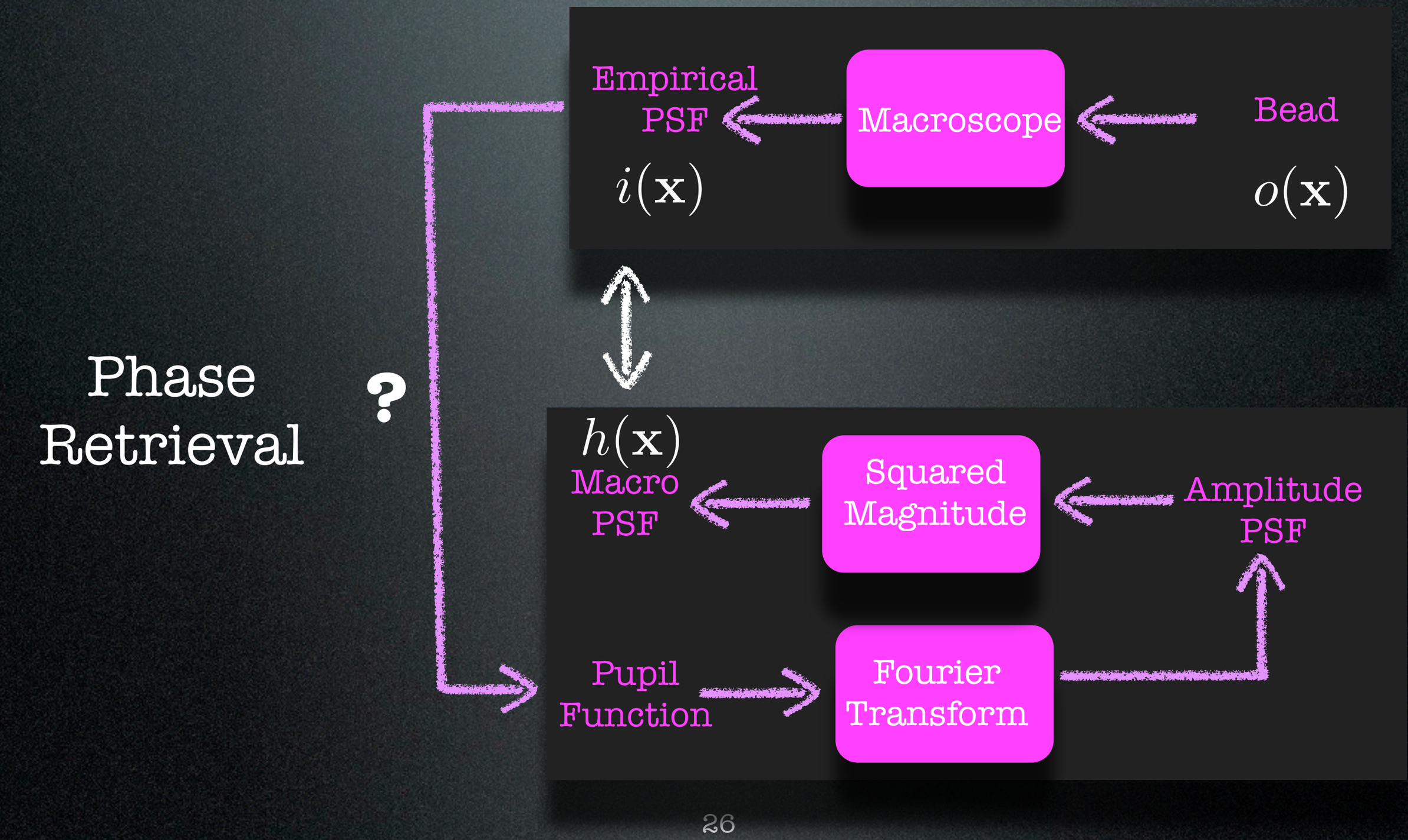
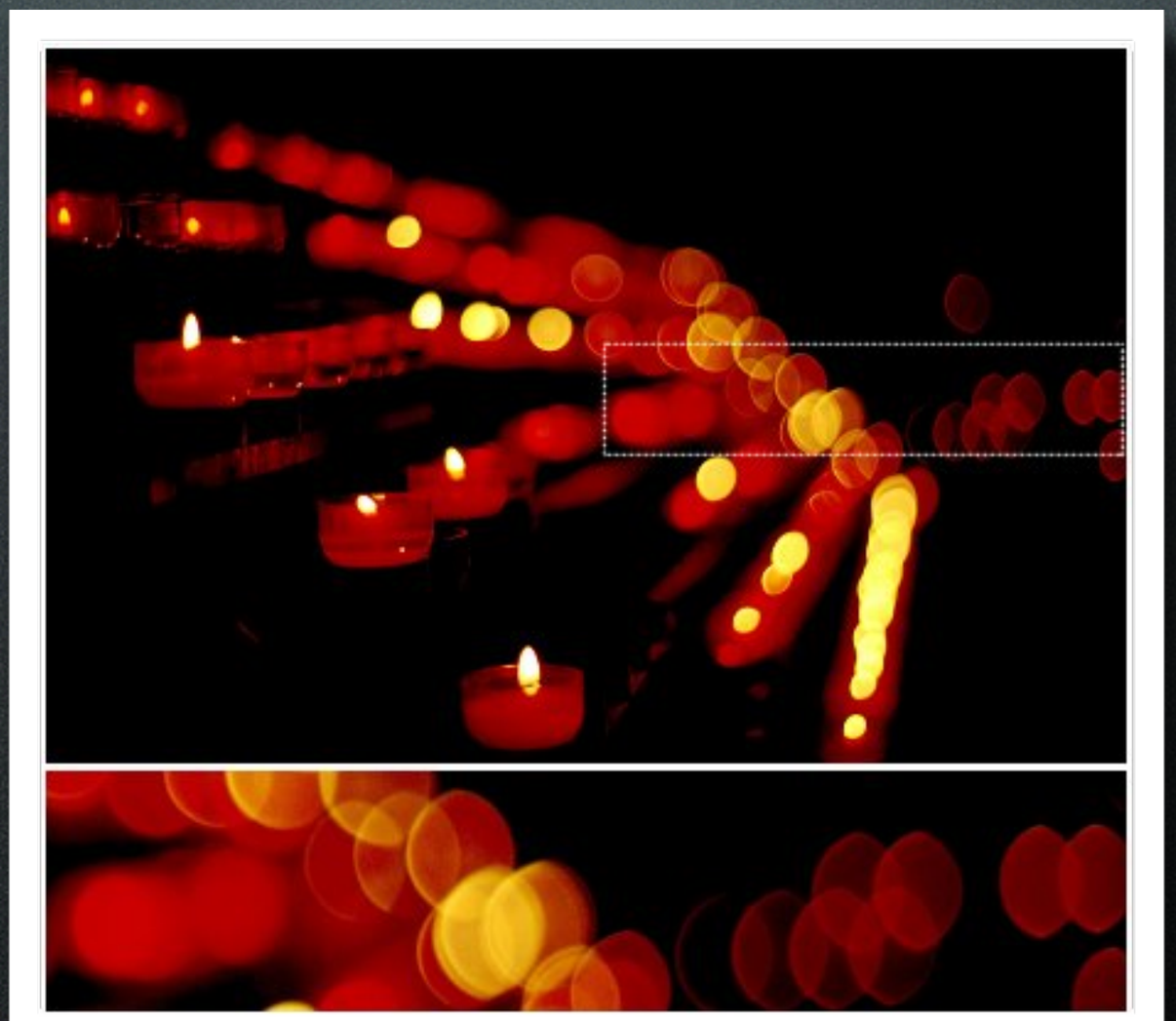


Inverse Problem



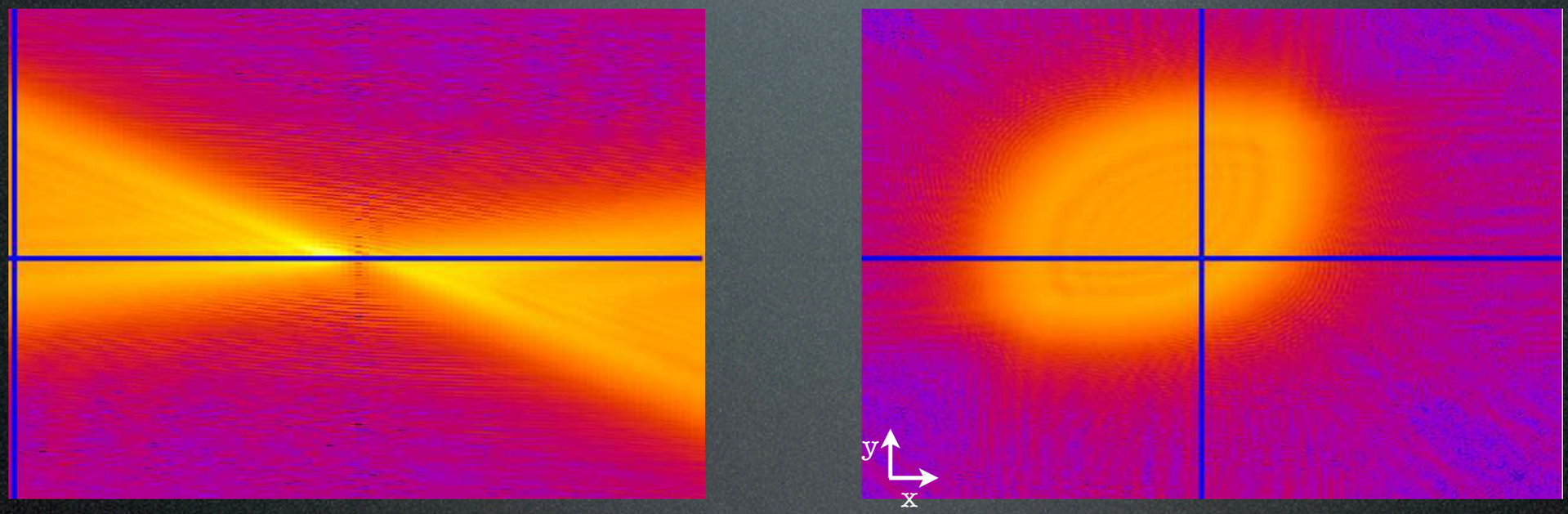
Cat's eye effect



Cat's eye effect as seen in the out-of-focus highlights (OOFH).

(Photograph by Peter Boehmer.)

OOFH

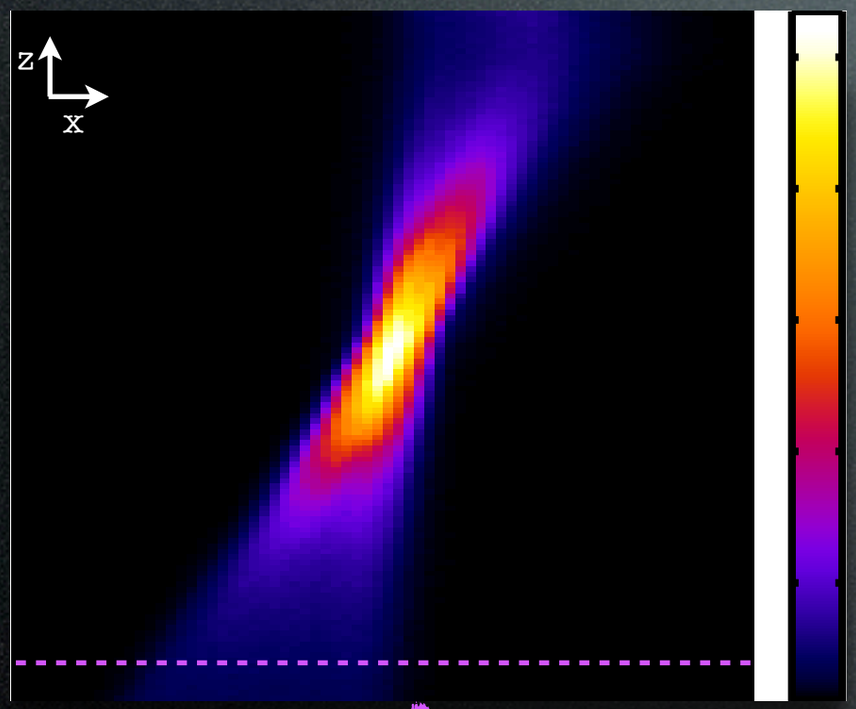


Cat's Eye

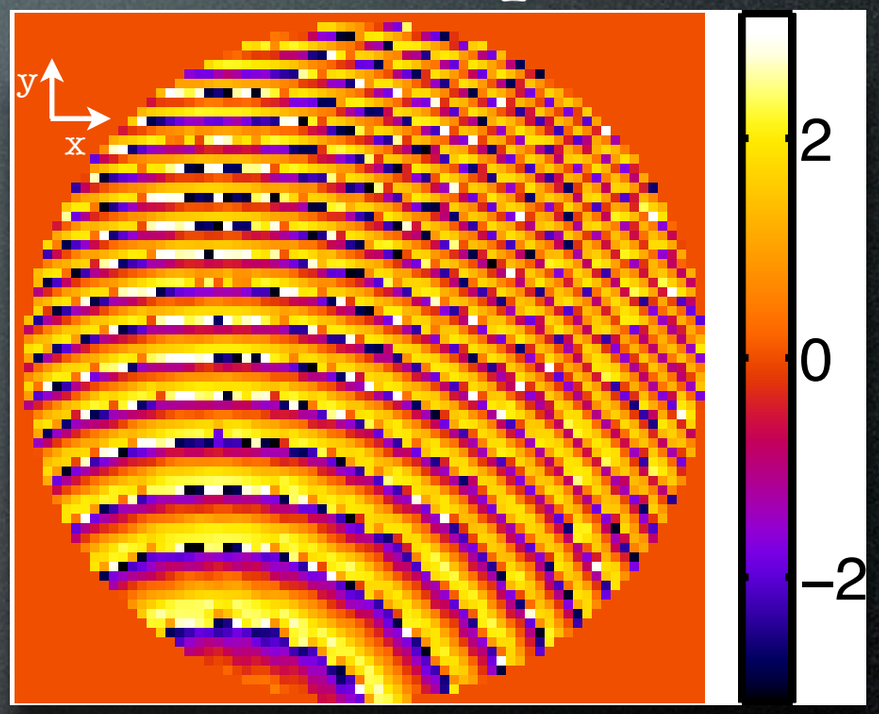
OOFH

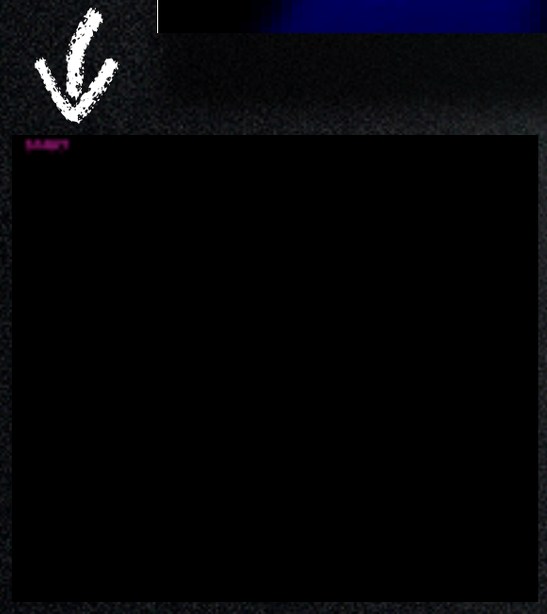
Validation: Phase Retrieval

Empirical PSF



Estimated phase





Iterative phase retrieval

Part 2: Details