Characterizing the aberrations in a fluorescence MACROscope

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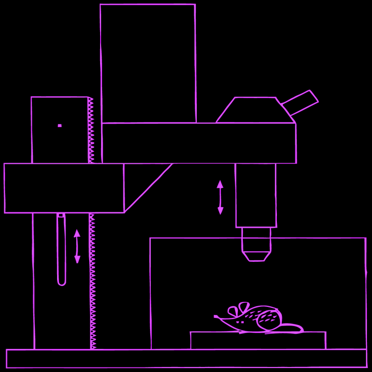
Widefield fluorescence MACROscope



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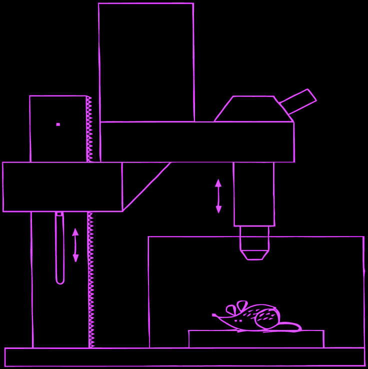

Central points

- Low magnification objective lens is combined with apochromatic zoom lens,

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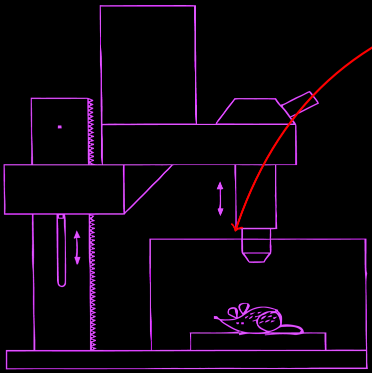
Widefield fluorescence MACROscope



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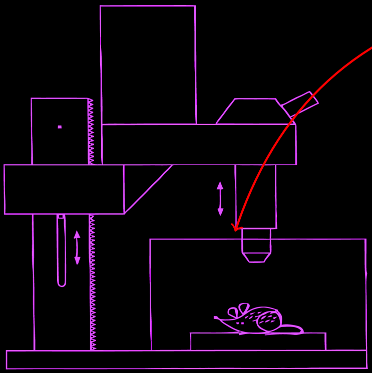

Central points

- Low magnification objective lens is combined with apochromatic zoom lens,
- permits large object fields (up to 35mm) and large working distances (up to 97mm),
- parallax-free and precise imaging,
- multi-color fluorescence.

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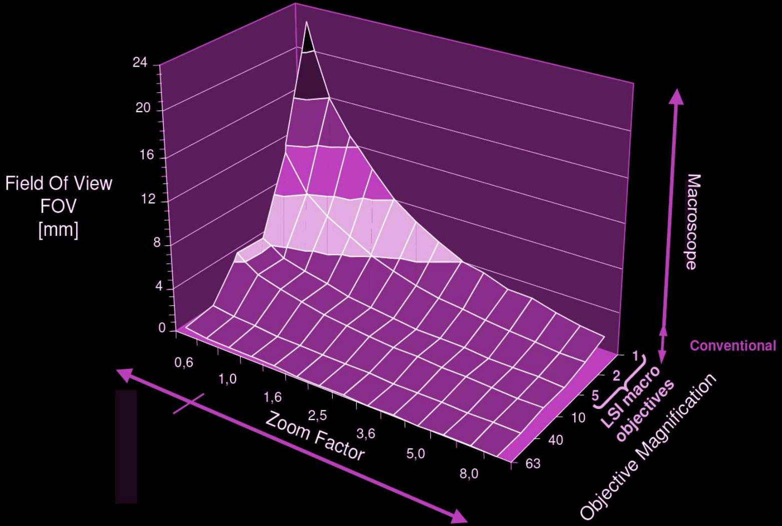
Field Of View
FOV
[mm]
24
20
16
12
8
4
0
0,6
1,0
1,6
2,5
3,6
5,0
8,0
Zoom Factor
1
2
5
10
40
63
LSI macro objectives
Objective Magnification
Macroscope
Conventional

Best of two worlds: Convallaria sample

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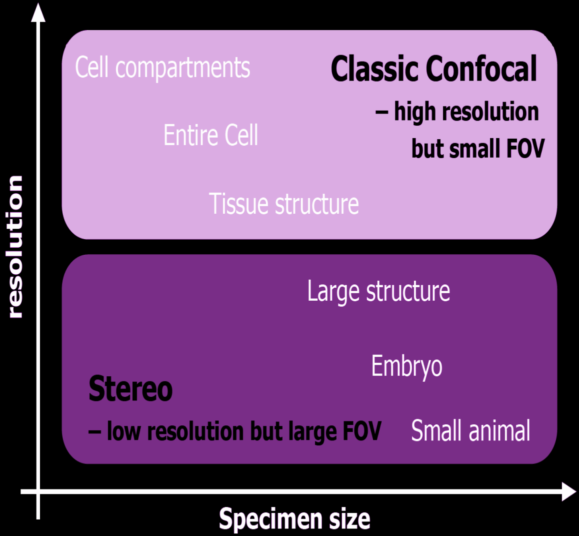
Best of two worlds: Convallaria sample

Best of two worlds



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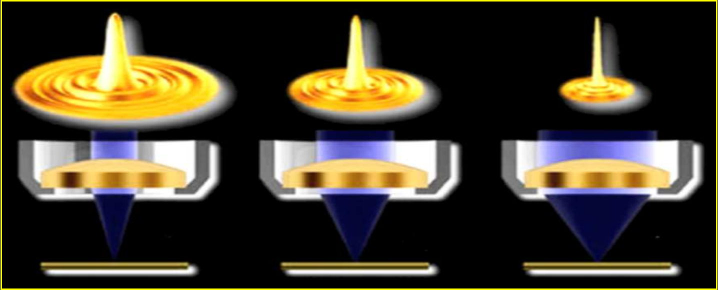
Numerical aperture and resolution

$$\mathrm{NA} = n_i \times \sin\alpha$$

Numerical aperture and resolution

$$
\begin{aligned}
\text{NA} &= n_i \times \sin\alpha \\
&= 1.00 \times \sin\alpha
\end{aligned}
$$



Numerical aperture decreases

Numerical aperture and resolution

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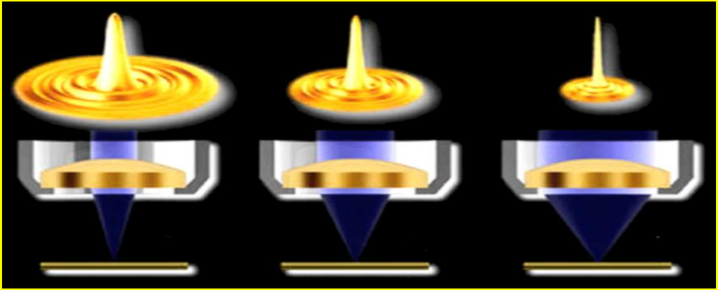

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Resolution decreases

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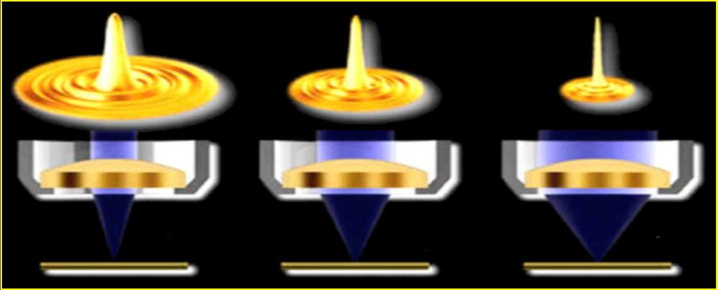

Can we increase the resolution?

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Numerical aperture and resolution

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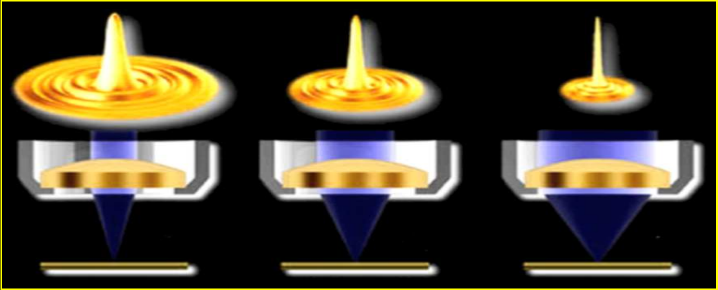

Yes, we can!

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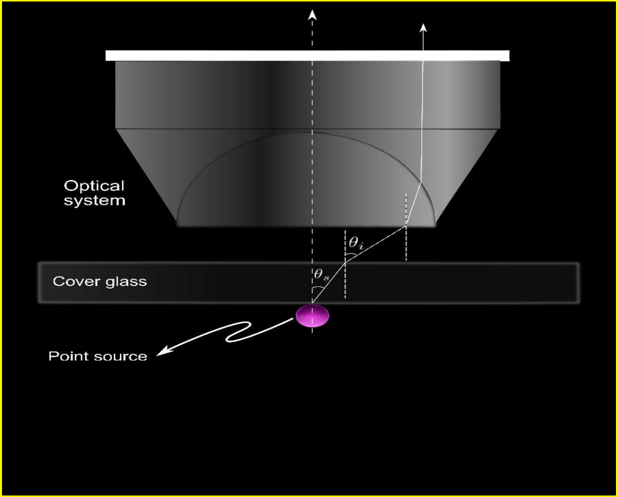
Experimental impulse response



Experimental impulse response

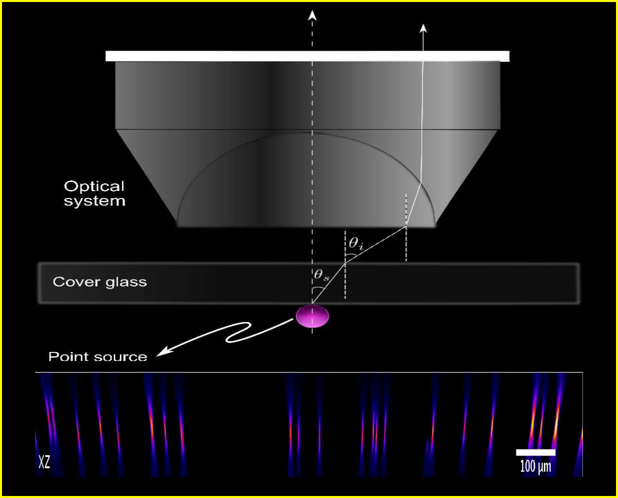


Figure 1: 2.5μm beads imaged using a Leica Widefield MacroFluo^{TM} Z16 APO fit with $5\times$ objective and the $1.6\times$ zoom. ©Herbomel lab, Pasteur Institute.

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MACROscopes-Are they really the best of the two worlds?

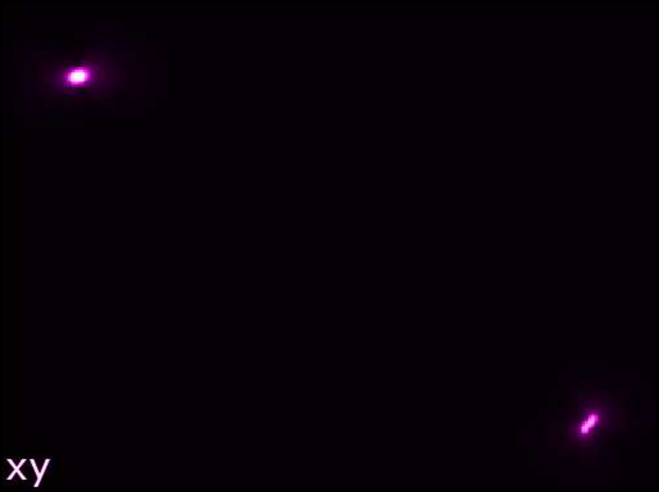

Figure 2: Axial projection of the beads. ©Herbomel lab, Pasteur Institute.

Forward problem: Characterizing the MACROscope

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Incoherent scalar PSF model

- If $P(k_x, k_y, z)$ is the 2D complex pupil function and λ is the wavelength, the amplitude PSF can be calculated by just $2N_z$ number of 2D FFTs as

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

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- and the incoherent PSF is

$$h_{Th}(\mathbf{x}; \lambda_{\text{ex}}, \lambda_{\text{em}}) = C |h_A(\mathbf{x}; \lambda_{\text{ex}})| \times |h_A(\mathbf{x}; \lambda_{\text{em}})|$$

- λ_{ex} and λ_{em} are the excitation and emission peak wavelengths.

Incoherent scalar PSF calculation

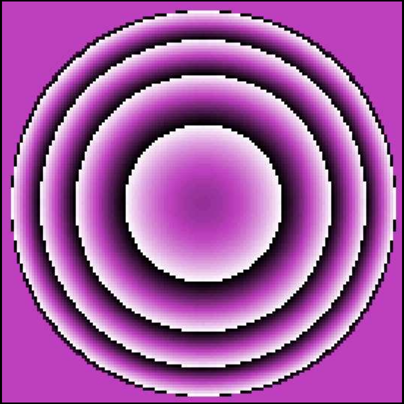

Figure 3: Defocus phase

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Incoherent scalar PSF calculation

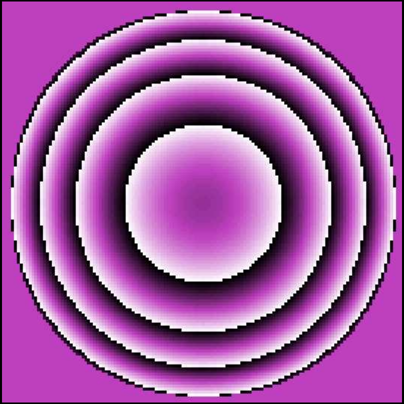

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Figure 4: Defocus PSF

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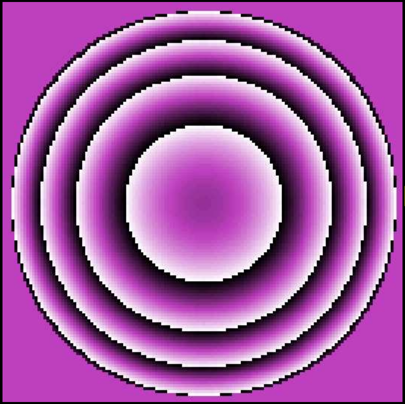

Figure 3: Defocus phase

Fourier transform $\longrightarrow$

Figure 4: Defocus PSF

Hypothesis

Figure 5: Lens viewed from the front. (Photograph by Peter Boehmer.)

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Hypothesis

Figure 5: Lens viewed from the front. (Photograph by Peter Boehmer.)

Figure 6: Lens viewed from the side. (Photograph by Peter Boehmer.)

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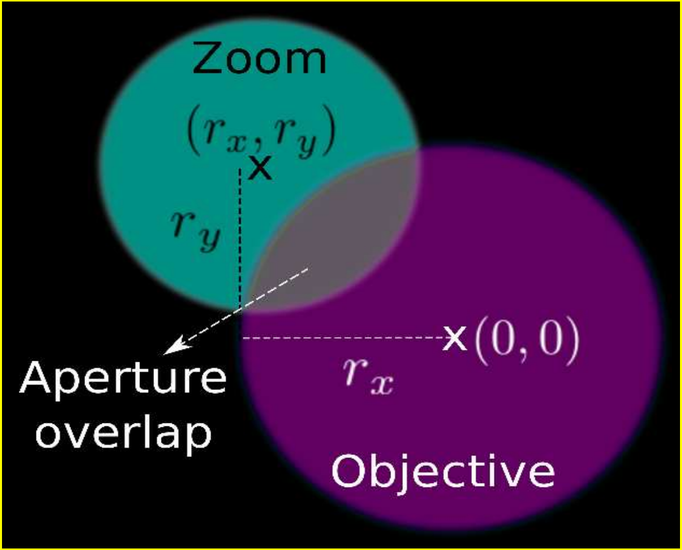
Optical vignetting and aperture overlap



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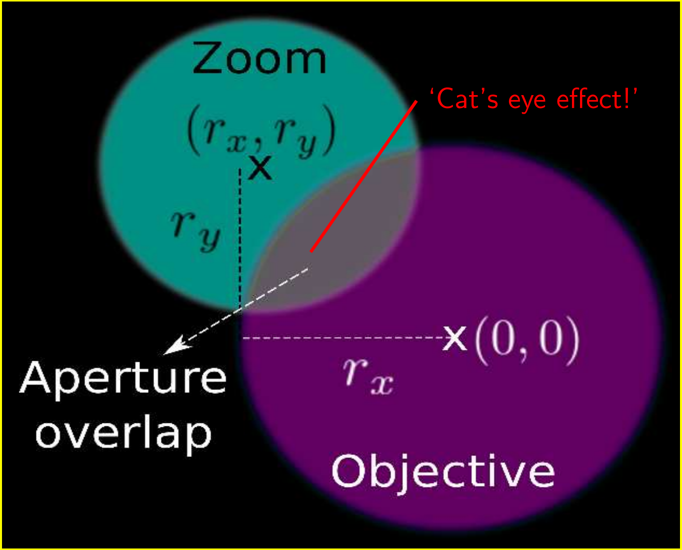

Macro PSF calculation by pupil vignetting

- For a MICROscope, the pupil function is

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$$P_m(k_x, k_y, z; \lambda) = \begin{cases} e^{jk_0\phi(\theta_i,\theta_s,z)}, & \text{if } \sqrt{k_x^2 + k_y^2} < \frac{2\pi}{\lambda}\mathrm{NA}_{\mathrm{Obj}} \\ 0, & \text{otherwise.} \end{cases}$$

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- Modification for a MACROscope

$$P(k_x, k_y, z; \lambda) = \begin{cases} P_m, & \text{if } \sqrt{(k_x - r_x)^2 + (k_y - r_y)^2} < \frac{2\pi}{\lambda}\mathrm{NA_{Zo}} \\ 0, & \text{otherwise.} \end{cases}$$

- $\mathrm{NA_{Obj}}$ and $\mathrm{NA_{Zo}}$ are the objective and zoom lens NA; (r_x, r_y) are the relative displacements.

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Calculations in the lateral field

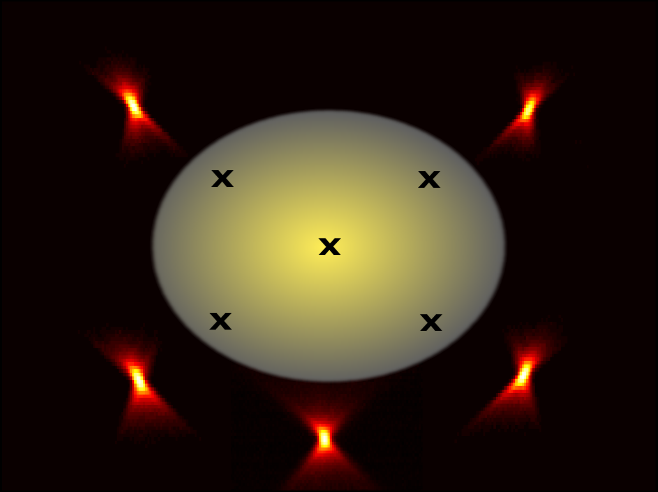

Figure 7: We can characterize the behavior at any position in the lateral field.

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Out-of-focus highlights (OOFH)

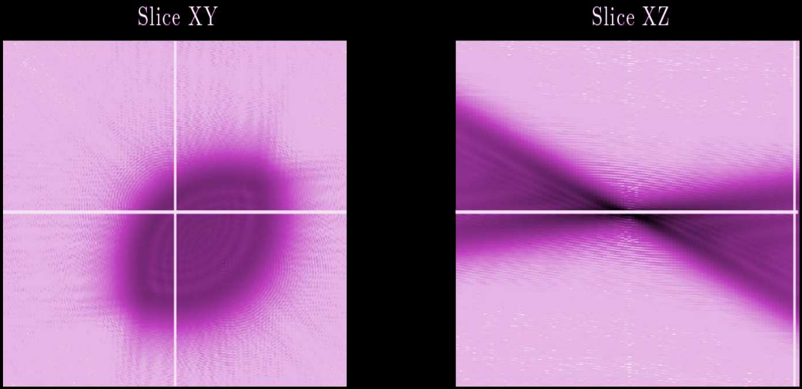

Figure 8: Theoretically calculated MACROscope PSF in log scale. NA$= 0.5$, lateral sampling 178.33nm, axial sampling 1000nm.

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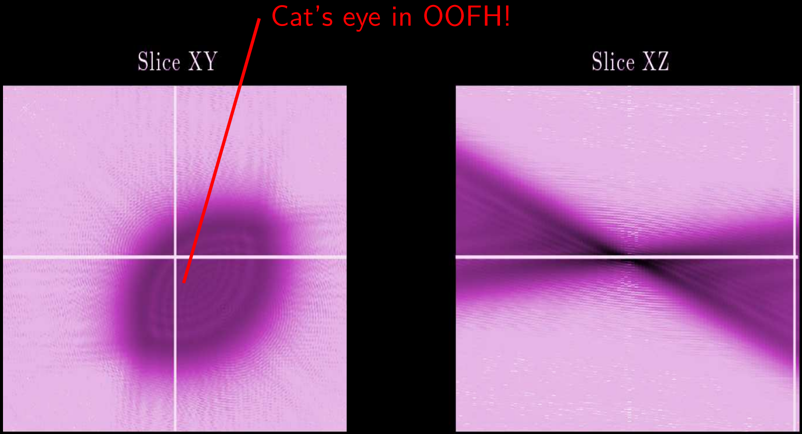

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- The PSF can vary experimentally,
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- the pupil function is not often available,
  - the wavefront can be sensed by using a Shack-Hartmann wavefront sensor,
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  - some information is available at the OOFH,

- Can the aberrations in the optics of the objective be determined from the OOFH?
- Can the estimated wavefront be useful for correcting the distortions?

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Wavefront sensing from intensity data

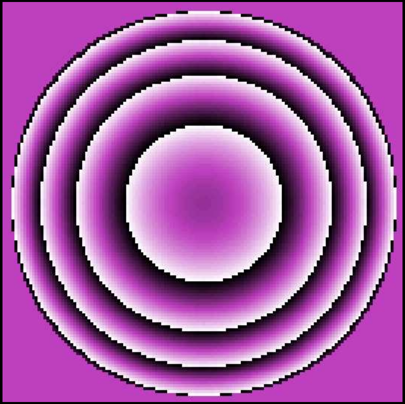

Figure 9: Defocus phase

Wavefront sensing from intensity data

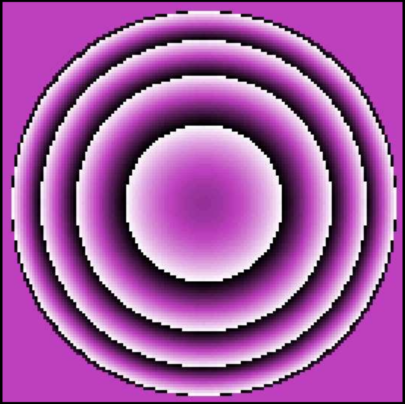

Figure 9: Defocus phase

Figure 10: Defocus PSF

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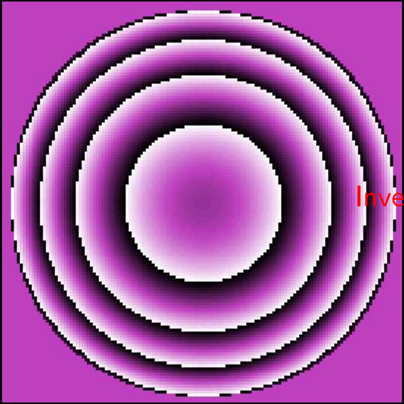

Figure 9: Defocus phase

Inverse Fourier transform ←

Figure 10: Defocus PSF

Inverse problem: Wavefront sensing from intensity data

Wavefront sensing-a Bayesian interpretation

- For uncorrelated low photon count data the observation is:

$$i(\mathbf{x}) = \mathcal{P}\{|h_{\mathsf{A}}(\mathbf{x})|^2 + b(\mathbf{x})\}, \forall \mathbf{x} \in \Omega_s$$

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- Considering Poissonian photon counting statistics, the likelihood of obtaining image $i(x)$ from a diffraction-limited point source:

$$\Pr(i|h_{\mathsf{A}}) = \prod_{\mathbf{x}\in\Omega_s} \frac{(h_{\mathsf{A}} + b)(\mathbf{x})^{i(\mathbf{x})} \exp(-(h_{\mathsf{A}} + b)(\mathbf{x})}{i(\mathbf{x})!}$$

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- From the Bayes' theorem, the *a posteriori* is

$$\Pr(h_{\mathsf{A}}|i) = \frac{\Pr(i|h_{\mathsf{A}})\Pr(h_{\mathsf{A}})}{\Pr(i)}$$

Global idea for sensing

- Aberration invariance to defocus

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- Estimate the near-focus amplitude distribution, $\hat{h}_{\text{A}}$, by maximizing the *a posteriori* (MAP) or minimizing the cologarithm of the *a posteriori*

$$\hat{h}_{\text{A}}(\mathbf{x}; \varphi_{\text{aberr}}) = \arg\min_{h_{\text{A}}(\mathbf{x})} -\log[\Pr(h_{\text{A}}|i)], \text{s. t. } k_{\text{MAX}} < \frac{2\pi\text{NA}}{\lambda_{\text{ex}}}$$

k_{MAX} is the pupil support,

- this can be solved by using a fixed-point iterative algorithm.

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Experiment on intensity data

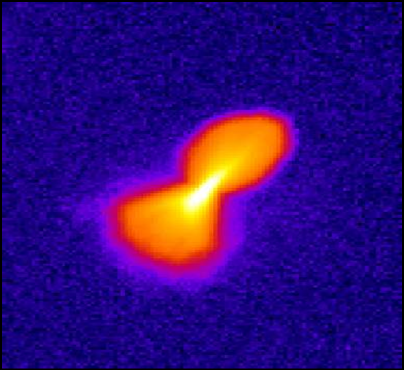

Figure 11: Radially projected 2.5μm observed intensity volume. ©Imaging Center, IGBMC, France.

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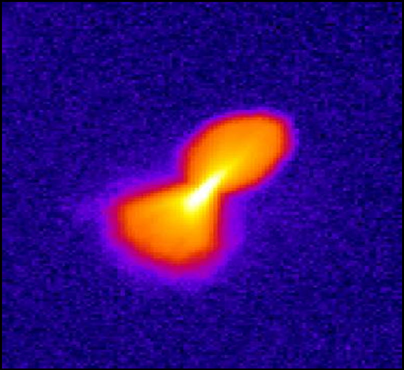
Experiment on intensity data



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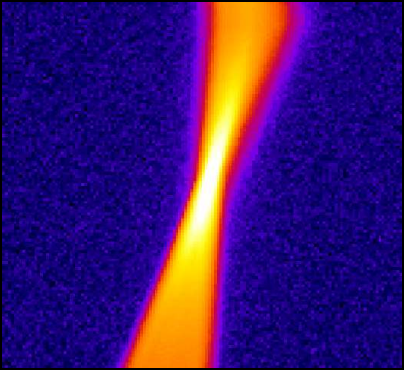
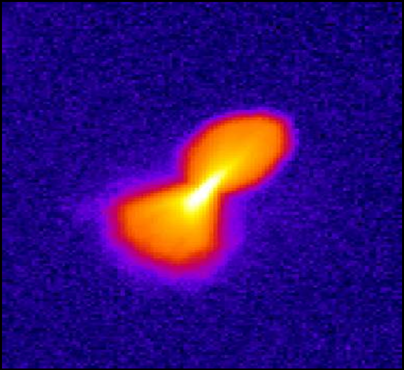


Figure 12: Axially projected 2.5μm observed intensity volume. ©Imaging Center, IGBMC, France.

Experiment on intensity data



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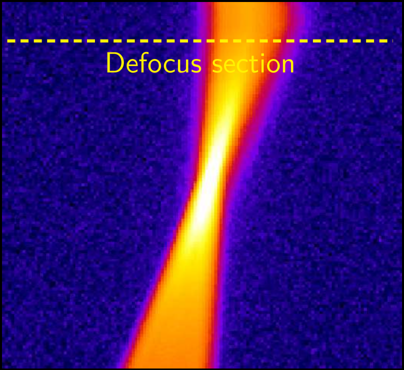

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Results: Wavefront sensed from intensity data

Figure 13: OOFH radial section of the observed volume, z $= -57\mu$m. ©Imaging Center, IGBMC, France.

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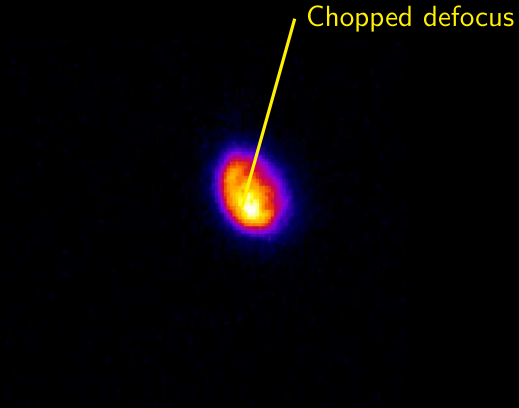

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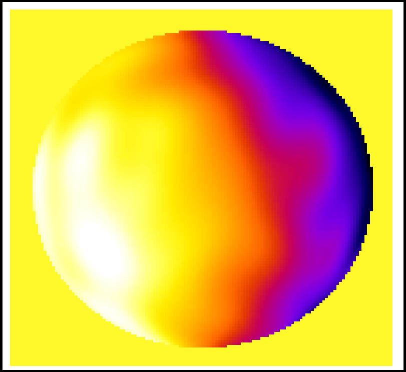

Figure 14: Retrieved unwrapped pupil phase from the intensity images $\tau = 0.9$ and the maximum number of iteration is 40.

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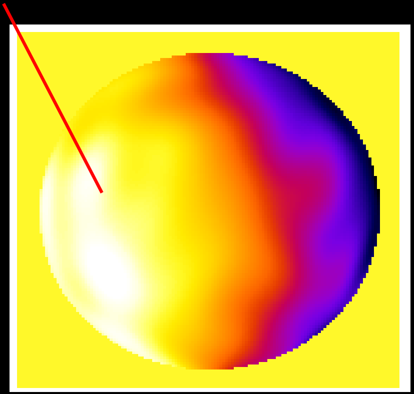

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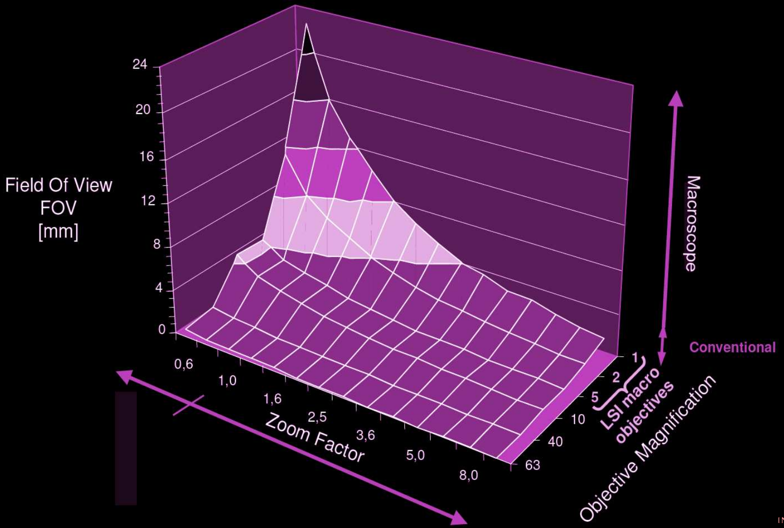
Implications: define workable zoom



Where to from here?

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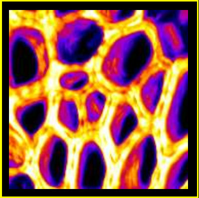

Figure 15: MIP of original

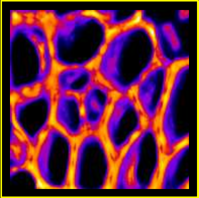

Figure 16: MIP of deconvolved.

Acknowledgements

- We thank ANR DIAMOND for funding this project,
- we are grateful to the scientific contribution from Dr. Philippe Herbomel, Institut Pasteur, and Dr. Didier Hentsch, IGBMC,
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Thank you!

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