Point-spread function model for fluorescence MACROscopy imaging

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Road map

MACROscope-Best of the two worlds

Figure 1: Leica MACROFLuo^{TM}

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MACROscope-Best of the two worlds

Figure 1: Leica MACROFLuo^{TM}

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- large working distances (up to 97mm),
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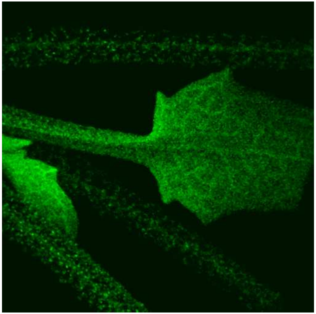

Figure 2: Confocal MACROscope image of *Arabidopsis Thaliana* seedlings ©INRA Sophia Antipolis, France.

Numerical aperture and resolution

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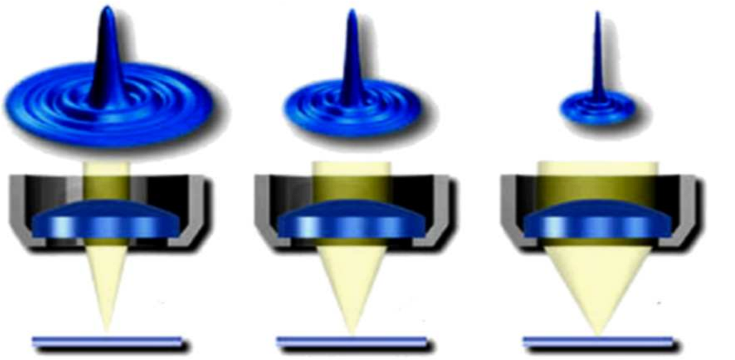


Figure 3: Numerical aperture and resolution for microscope.

Numerical aperture and working distance

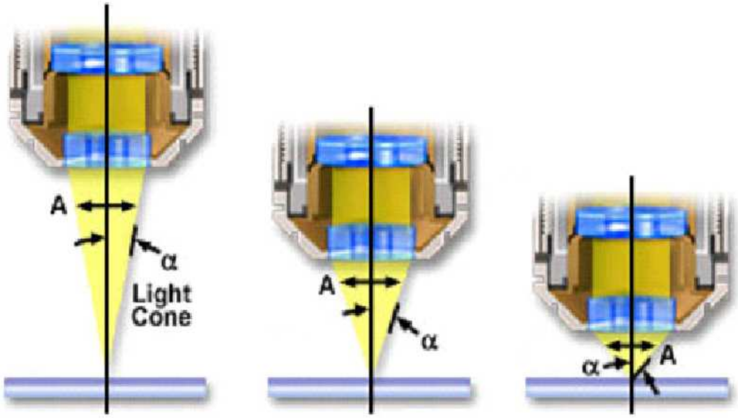


Figure 4: Schema of numerical aperture influencing working distance.

Why do we need a point-spread function (PSF)?

Figure 5: Confocal MACROscope image of *Arabidopsis Thaliana* seedlings

Experiments on the MACROscope

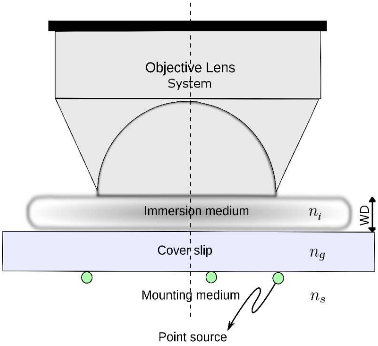
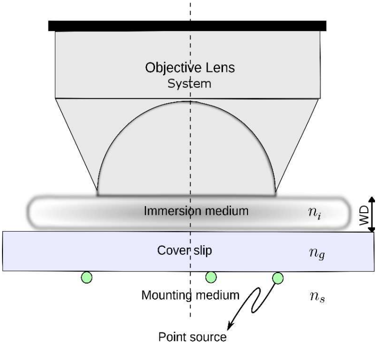


Figure 6: Experimental determination of the impulse response of the MACROscope by imaging point sources.



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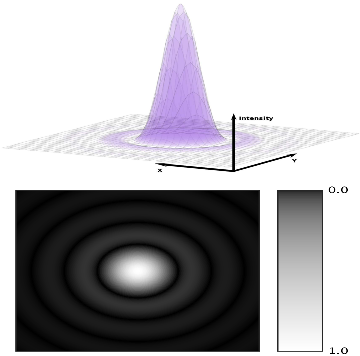

Figure 7: 2D Airy disk function.

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

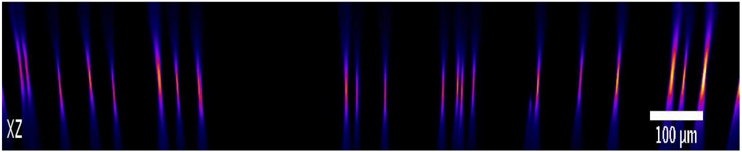

Figure 8: Radial projection of 4μm beads as imaged from a Leica Widefield MacroFluo^{TM}Z16 APO. The objective has a 5X magnification and a zoom of up to 16X. ©Herbomel lab, Pasteur Institute.

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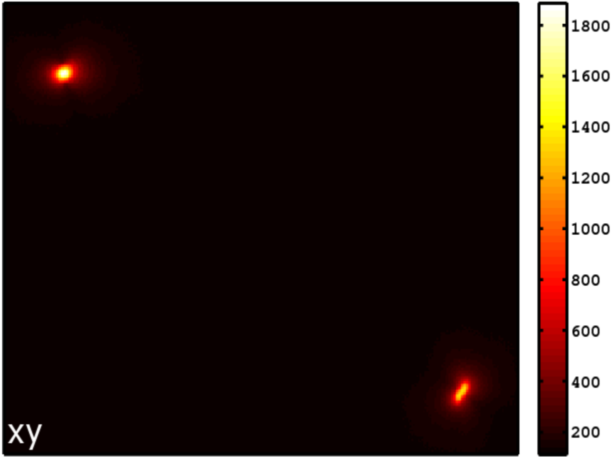

Figure 9: Axial projection of 4μm beads as imaged from a Leica Widefield MacroFluo^{TM}Z16 APO. The objective has a 5X magnification and a zoom of 1.6X. ©Herbomel lab, Pasteur Institute.

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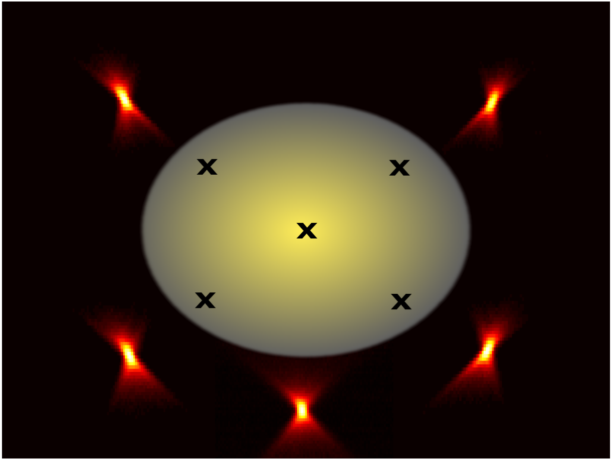

Figure 10: Bead images taken at different lateral positions of the field (as marked by the cross).

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- 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

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

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- and the incoherent PSF is

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

Asilomar coffee cup

Figure 11: Out-of-focus highlights (OOFH) for the Asilomar coffee cup. (Photograph by Praveen Pankajakshan.)

Cat'e eye effect

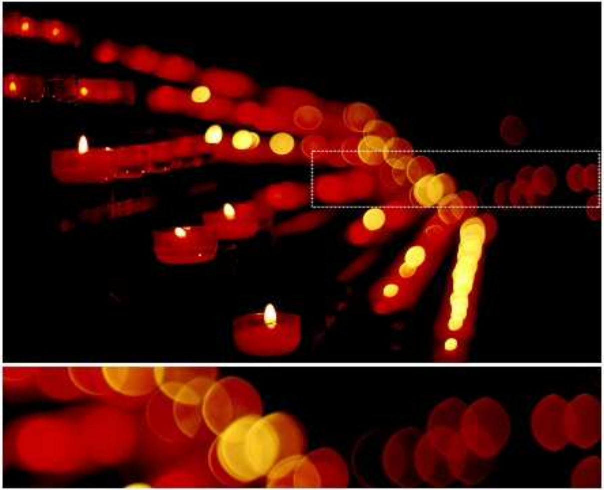

Figure 12: The Cat's eye effect is seen in the out-of-focus highlights (OOFH). (Photograph by Peter Boehmer.)

Optical vignetting

Figure 13: Lens viewed from the front. (Photograph by Peter Boehmer.)

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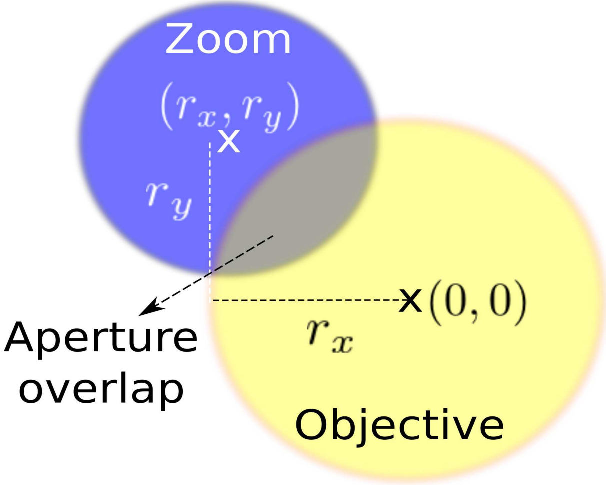
Figure 13: Lens viewed from the front. (Photograph by Peter Boehmer.)

Figure 14: Lens viewed from the side. (Photograph by Peter Boehmer.)

Limiting apertures overlapping



Pacific grove beach at no zoom

Figure 15: Optical vignetting example at minimum zoom. (Photograph by Praveen Pankajakshan.)

Pacific grove beach zoom position $1\times$

Figure 16: Optical vignetting disappears as zoom is increased. (Photograph by Praveen Pankajakshan.)

PSF modeling by using scalar diffraction theory

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- parameters of the PSF: $\omega_h = \{\mathsf{NA}_{\mathsf{Obj}}, \mathsf{NA}_{\mathsf{Zo}}, r_x, r_y\}$

Compare experimental PSF and theoretical PSF

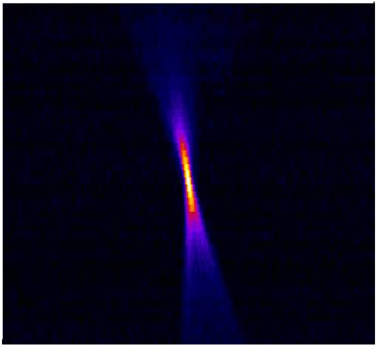

Figure 17: Experimental image of point source. Bead diameter 4μm and zoom 1X. ©Imaging Center, IGBMC, France.

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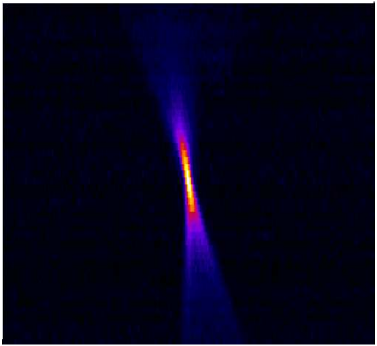

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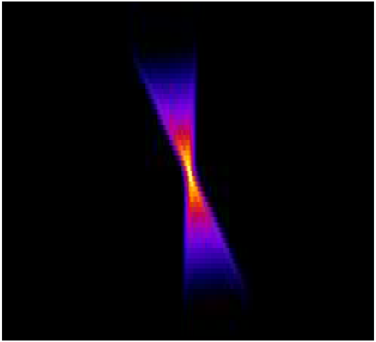

Figure 18: Simulated image of point source assuming the maximum NA of 0.5.

Optically vignetting the pupil

Figure 19: Simulating the vignetting.

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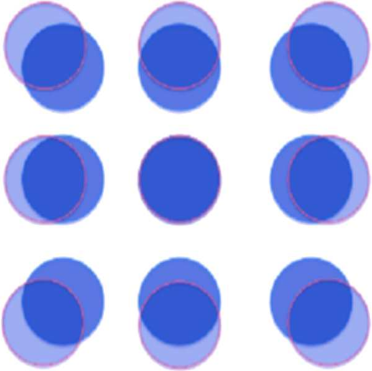

Figure 20: Schematic of overlapping apertures.

Degree of vignetting

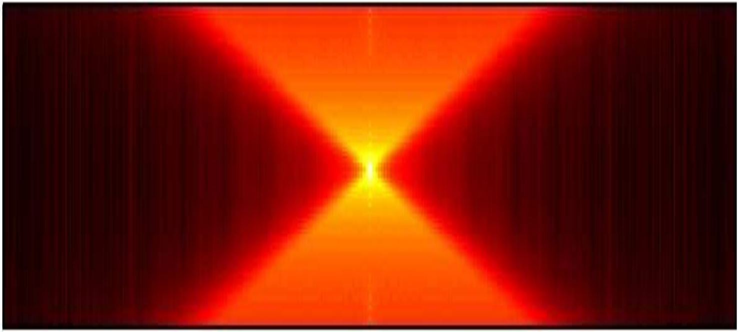

Figure 21: Simulated image of point source assuming 100% overlap of the apertures.

Degree of vignetting

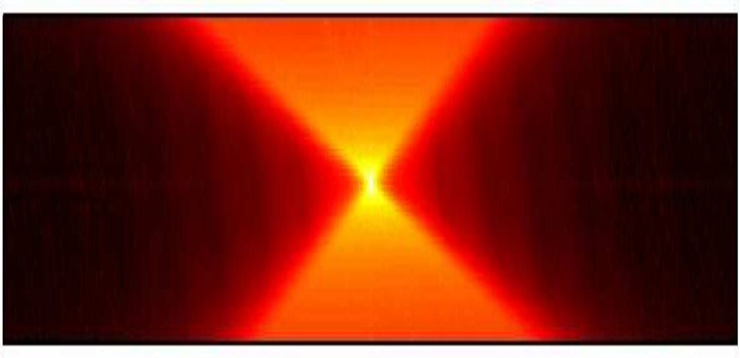

Figure 22: Simulated image of point source assuming an overlap of 75% of the apertures.

Degree of vignetting

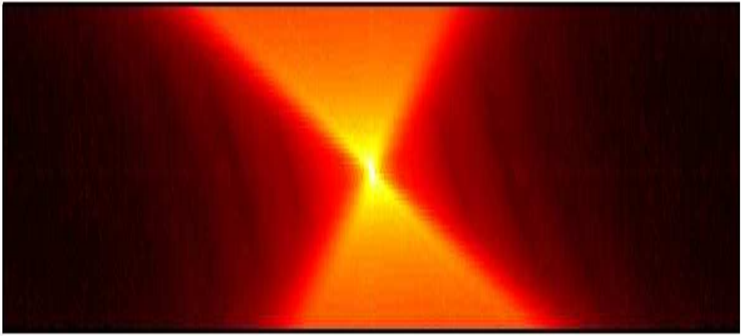

Figure 23: Simulated image of point source assuming an overlap of 50% of the apertures.

Degree of vignetting

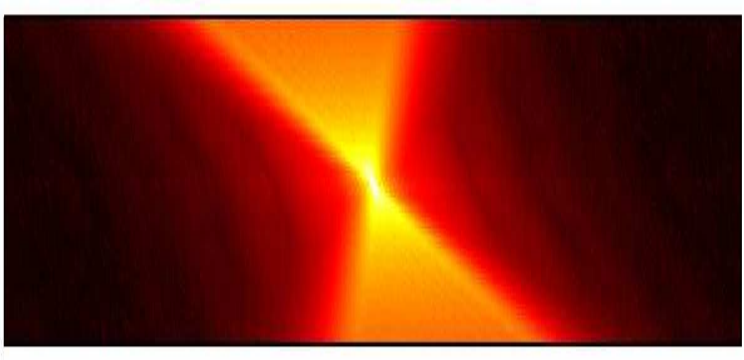

Figure 24: Simulated image of point source assuming an overlap of 25% of the apertures.

Degree of vignetting

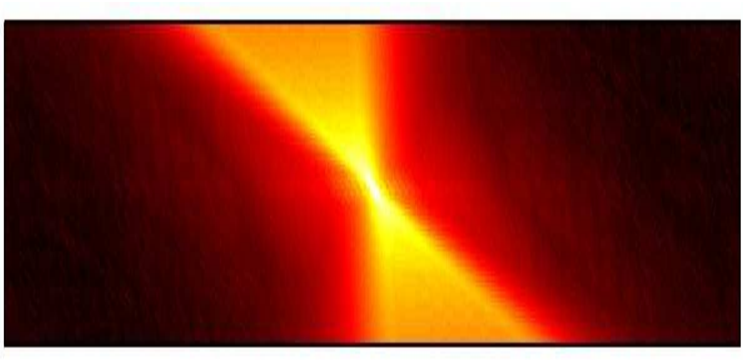

Figure 25: Simulated image of point source assuming an overlap of 10% of the apertures.

Conclusions and perspectives

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- restoration of images can be achieved with the complete or partial knowledge of the theoretical PSF.

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- the authors are grateful to Dr. Philippe Herbomel from the Institut Pasteur, France and Dr. Didier Hentsch from IGBMC, France for the images and the discussions,
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- we acknowledge our colleagues for their support.

For more information see:
http://www-syscom.univ-mlv.fr/ANRDIAMOND/.