# PSF model for fluorescence MACROscopy imaging


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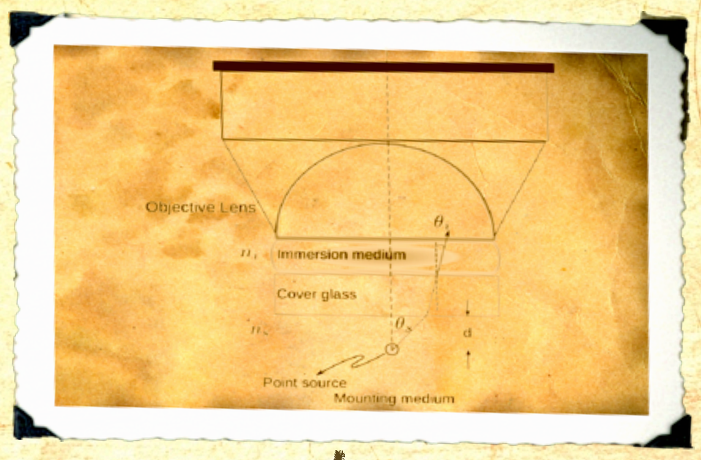
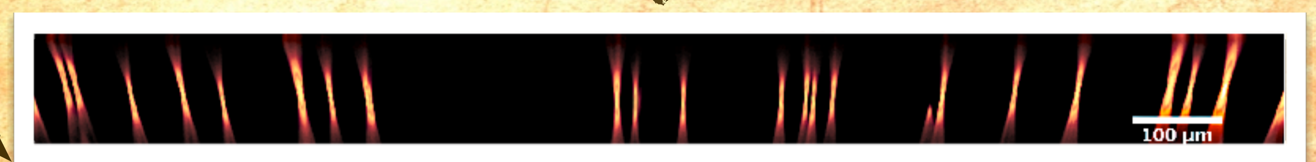
## INTRODUCTION

Fluorescent MACROscope is useful for observing large samples (of the order of a few centimeters) and has the following advantages:

- large object fields,
- large working distances, and
- parallax-free imaging.

### IMAGING FIELD ABERRATIONS USING POINT SOURCE

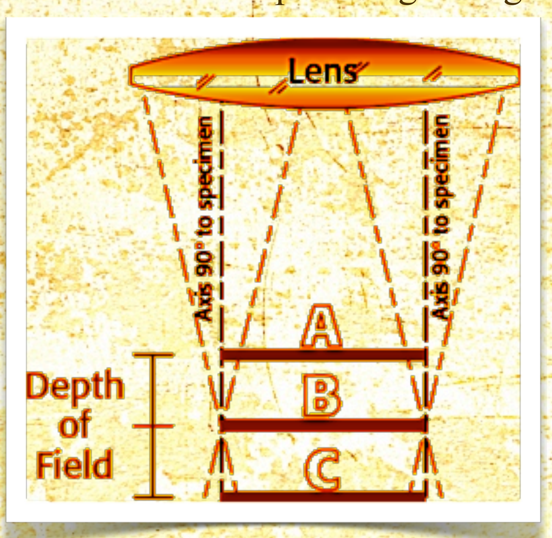




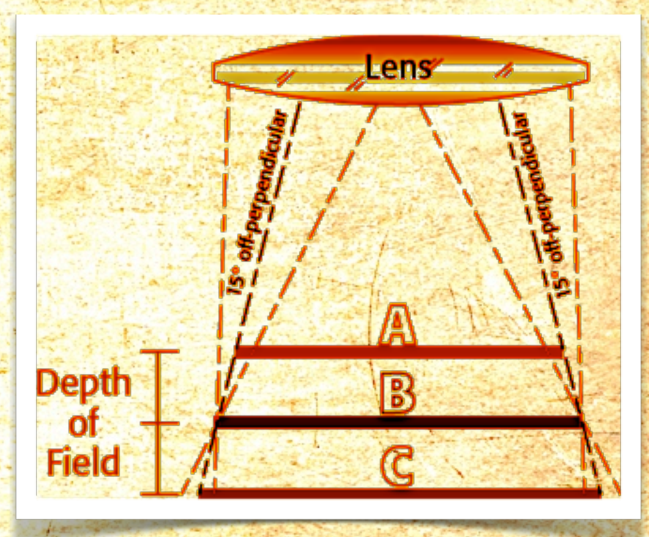

Point-spread function (PSF) -> Image of ideal point source (4 µm). Total magnification 6.25x.

## OBJECTIVE

To model the point-spread function (PSF) of a MACROscope operating with field aberrations due to optical vignetting.




Telecentric lens assembly




MACROscope lens assembly

### WHY A PSF MODEL IS IMPORTANT?

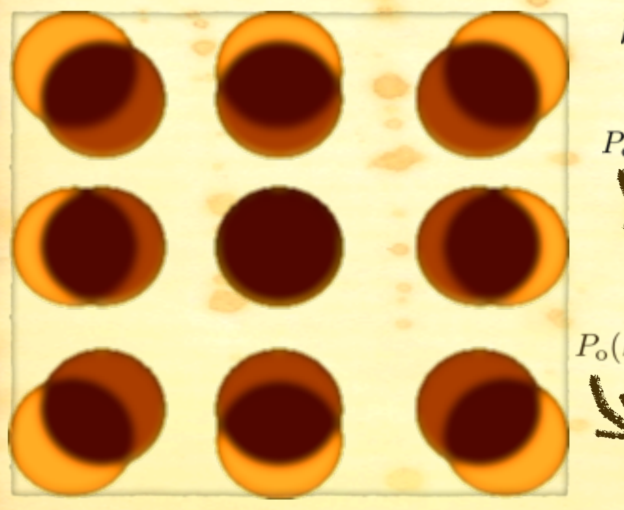
$$i(\mathbf{x}) = \mathcal{P}(h(\mathbf{x}) * o(\mathbf{x}) + b((\mathbf{x})))$$

Observed volume — PSF — Unknown synthetic object

## METHODOLOGY

Stokseth's PSF model — Excitation PSF — Emission PSF

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



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

$$P_o(k_x, k_y, z) = \begin{cases}\exp(jk_0\phi(\theta_i, \theta_s, z)), & \text{if } (\sqrt{k_x^2 + k_y^2}) < \frac{2\pi}{\lambda_{ex}}\text{NA}\\ 0, & \text{otherwise}\end{cases}$$

Pupil function for a MICROscope

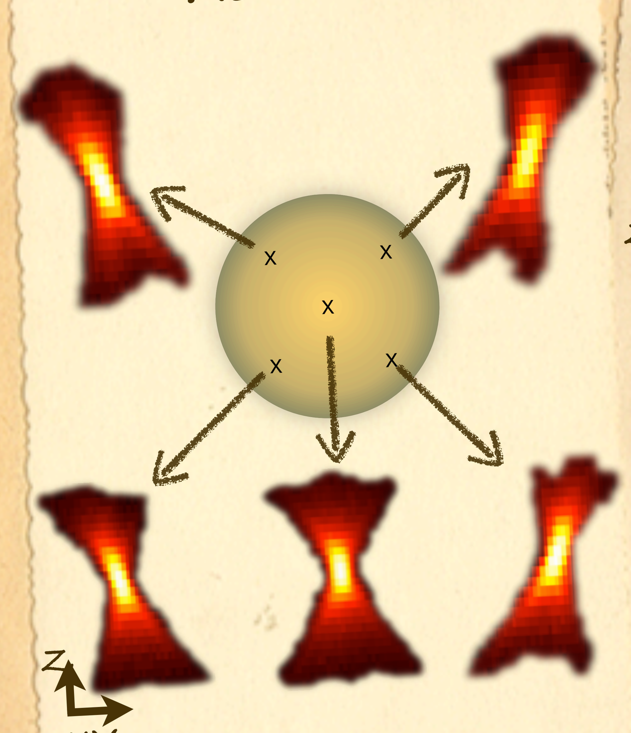
$$P_o(k_x, k_y, z) = P_o(k_x, k_y, z) \times \begin{cases}\exp(jk_0\phi(\theta_i, \theta_s, z)), & \text{if } (\sqrt{(k_x - r_x)^2 + (k_y - r_y)^2}) < \frac{2\pi}{\lambda_{ex}}\text{NA}\\ 0, & \text{otherwise}\end{cases}$$

Pupil function for a MACROscope

Lens displacement y-direction ($r_y$)

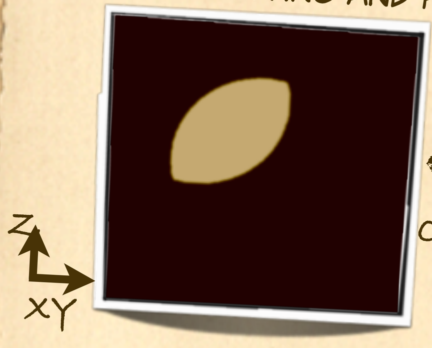
Lens displacement x-direction ($r_x$)

## RESULTS

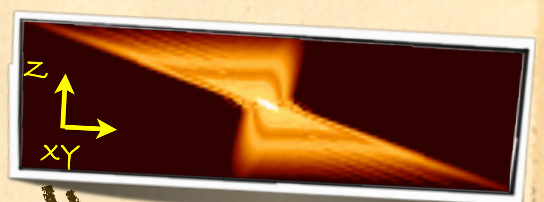


Experimentally obtained bead images for different lateral position in the field

### SIMULATE PUPIL OPTICAL VIGNETTING AND PSF



Cat's eye effect



Simulated PSF

Measured bead image

## CONCLUSIONS

1. MACROscope PSF varies as a function of the lateral position.
2. Vignetting was observed for small zooms (large FOV).
3. This initial PSF model will be enhanced with newer acquisitions on different systems.


## ACKNOWLEDGEMENTS

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