DIAMOND Workshop 2011

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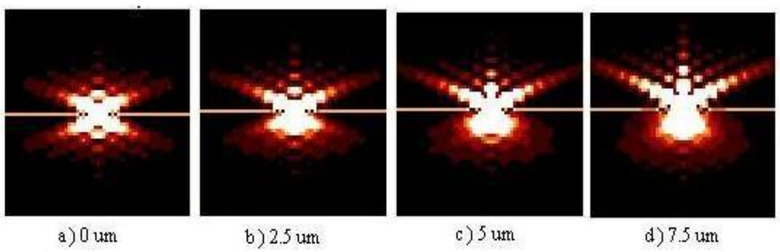
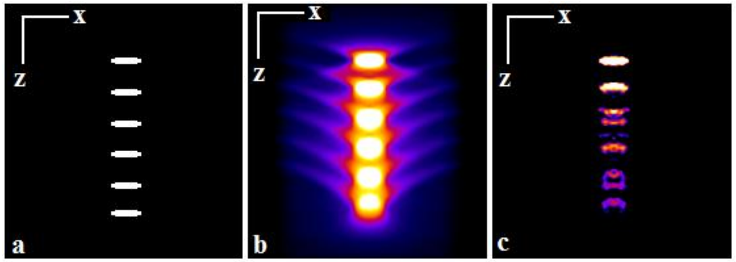
Outline

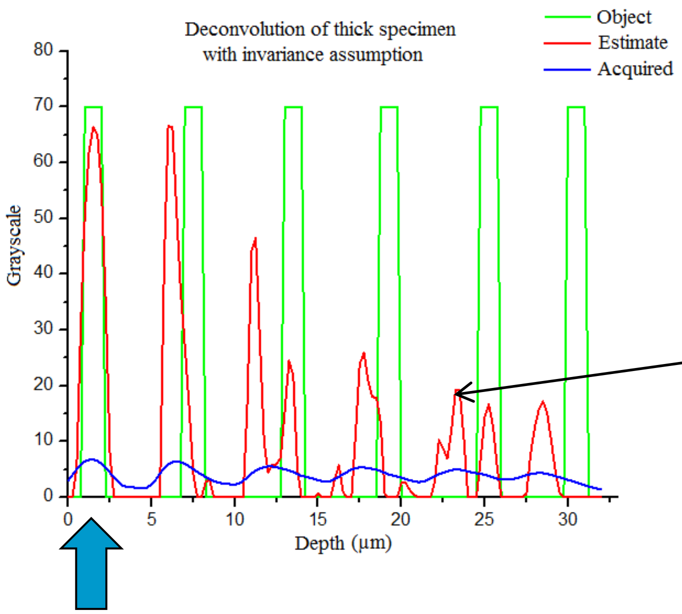
- Work overview
  - Deconvolution with non-invariant PSF
  - PSF Interpolation Z*MIA
- Porting to Macroscope
  - Optical center
  - Z*MIA & EMMA
- Conclusions…

PSF spatial non-invariance – Microscope





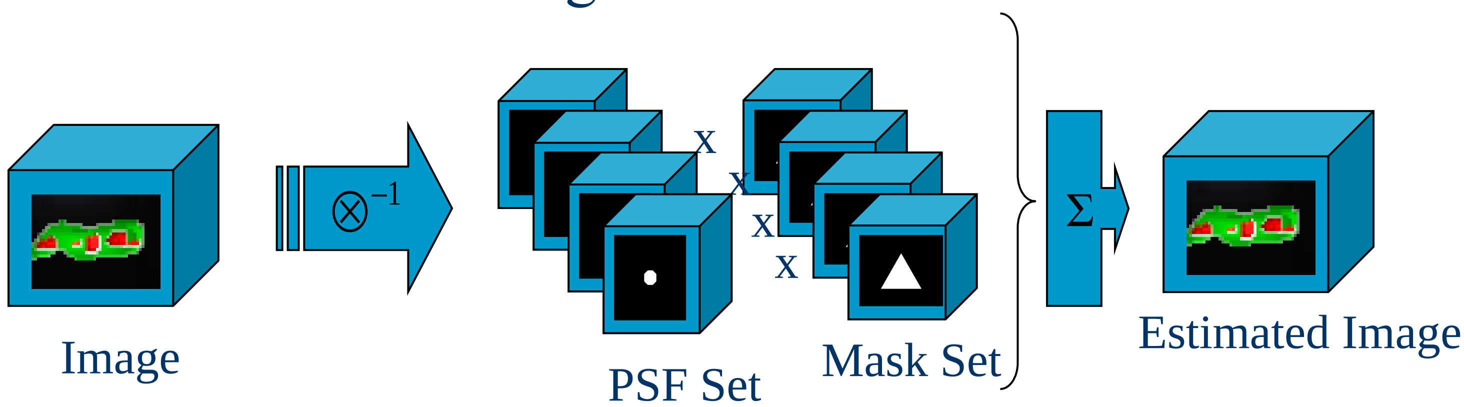
- PSF variations
  - Along the Z axis due to the refractive index mismatch between the specimen and the immersion medium
    - axial asymmetry in the shape of the point spread function
    - increase in size, particularly along the z-axis.
  - Specimen induced aberrations



The estimation errors become larger as we moves away from the used PSF position.

The EMMA solution

Knowing “k” PSF in the system (measured or simulated) , one should do “k” deconvolution each with a different PSF then combine the results using “k” Masks .



For i = 1,2,...,k

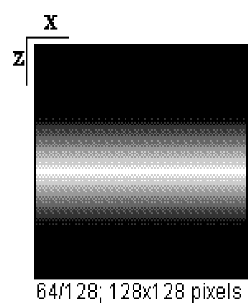
Get PSF number i

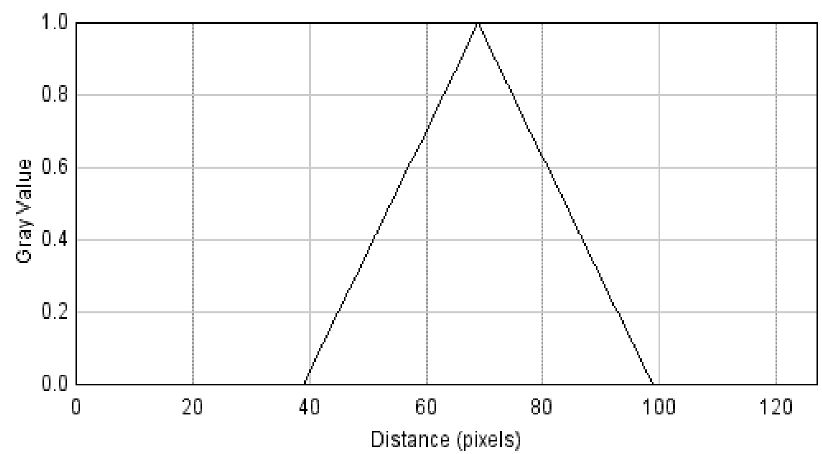
Deconvolve the image

Apply the mask

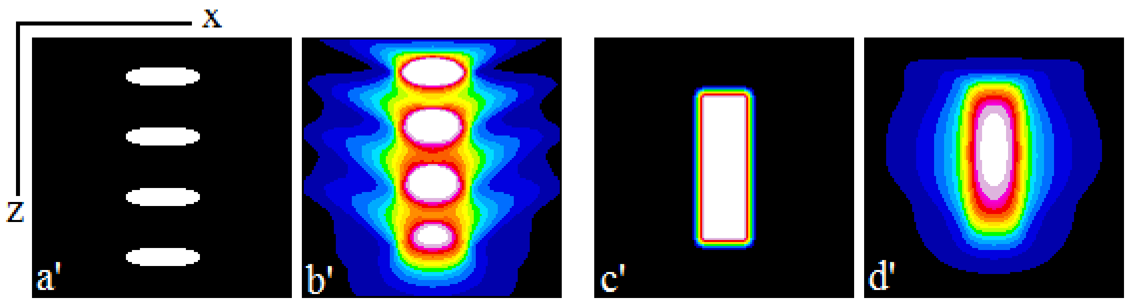
Accumulate the sum

End

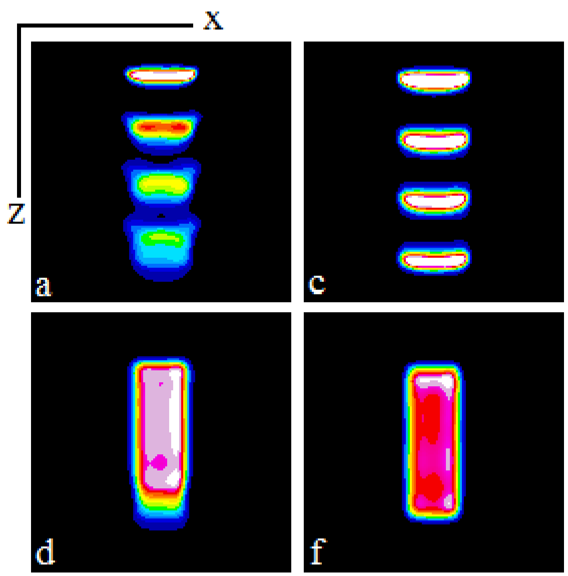




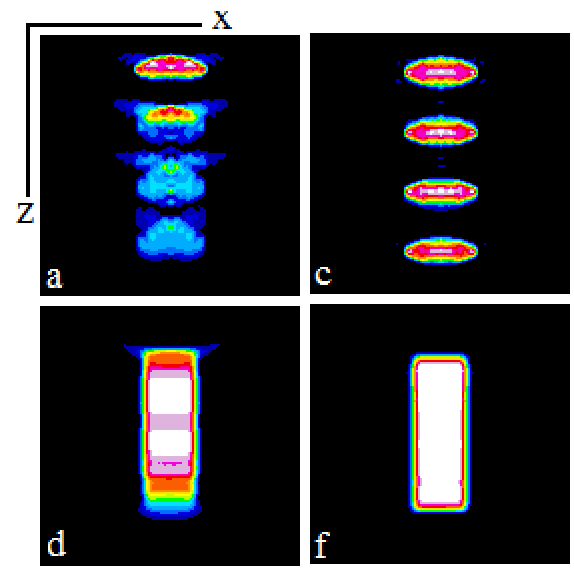
EMMA results



4 beads along the Z axis with 2,25µm of diameter and spaced by 5µm (a')
20,75µm long Parallelepiped along the Z axis with 2,25µm of width (c')
Acquisition simulations (b', c') are done using spatial convolution (PSF for each slice)



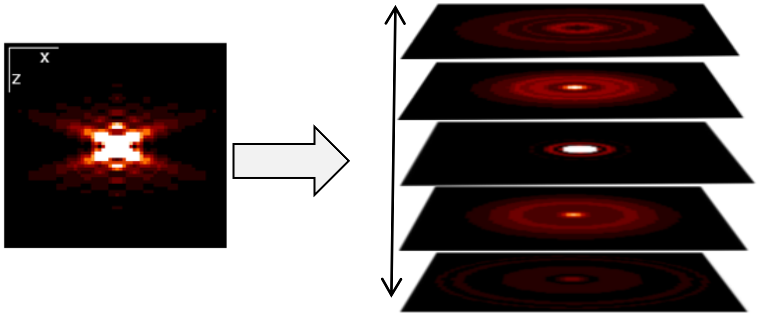
Lucy-R EMMA + LR



LLS EMMA + LLS

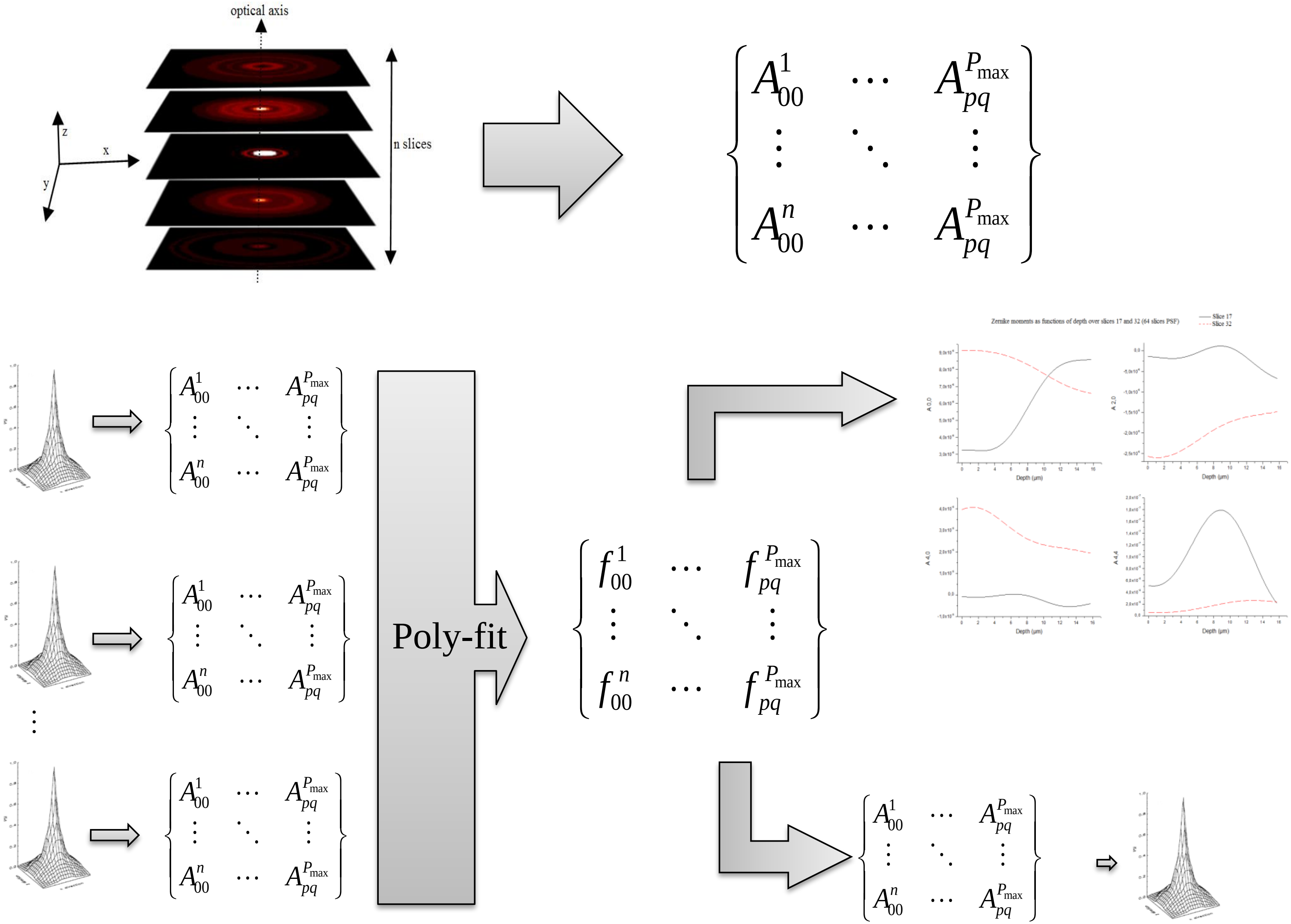
PSF Interpolation – Z*MIA

- Zernike-Moments Interpolation Algorithm
- Gives the possibility to obtain a fairly accurate PSF estimation at any position using only a few measured one.
- Zernike moments are the result of the projection of an image over a set of Zernike polynomials defined over a unit circle. (2D images)
- 3D PSF are treated as a collection of 2D slices



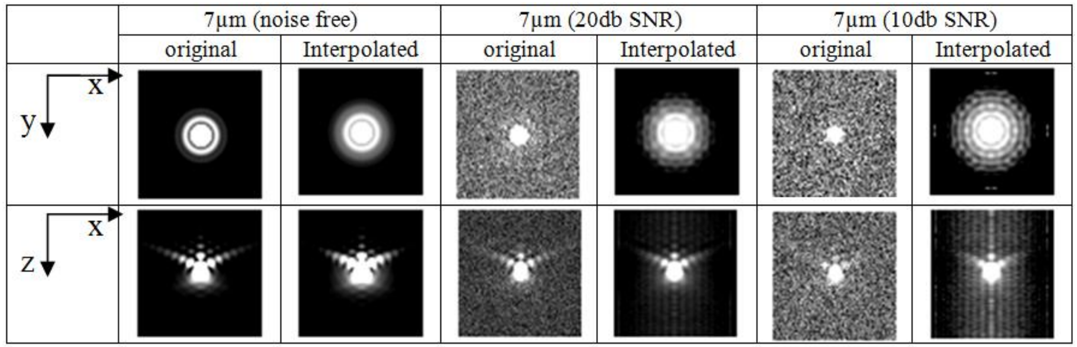
- Paper in JOSA (J. Opt. Soc. Am. A / Vol. 28, No. 9 / September 2011)

PSF Interpolation – Z*MIA

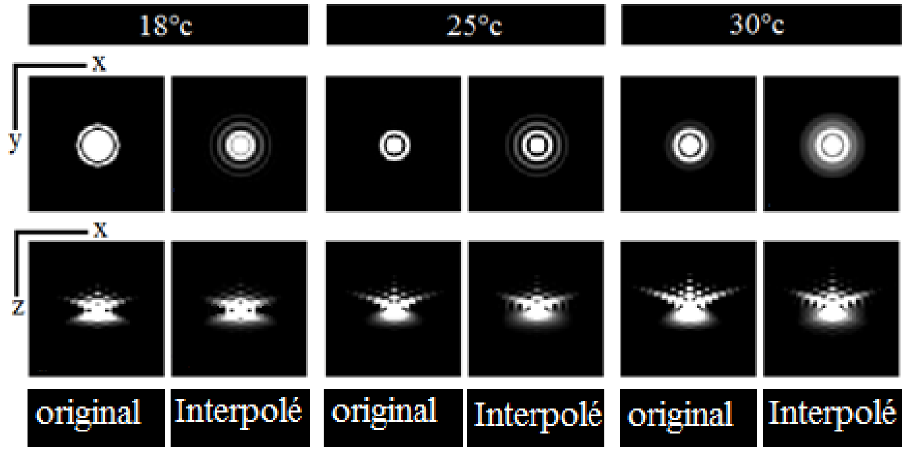


Zernike Moments Interpolation - results

- 6 known PSF are used at positions 0, 3, 6, 9, 12 and 15,75µm

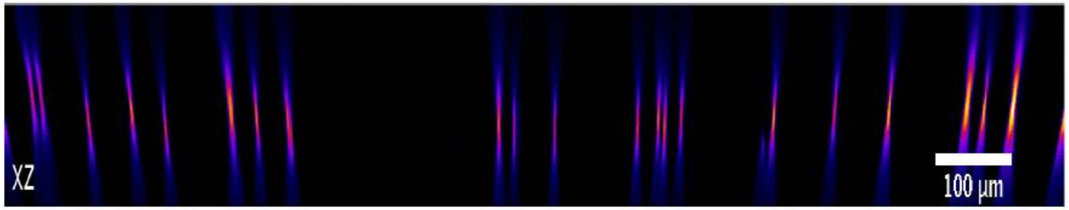


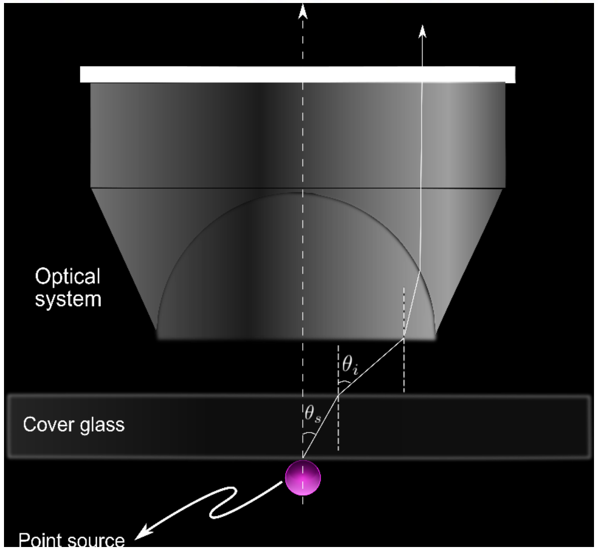
- Z-MIA can be applied on other cases, like immersion oil refractive index change due to ambient temperature



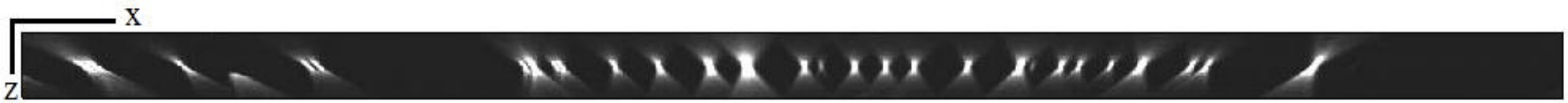
NA: 1,4
Coverslip RI: 1,515
Oil RI: 1,515 +/- 0,0005 (23°c)
Known PSF at: 10, 15, 23, 28 and 33°c

Macroscope case – Lateral variation



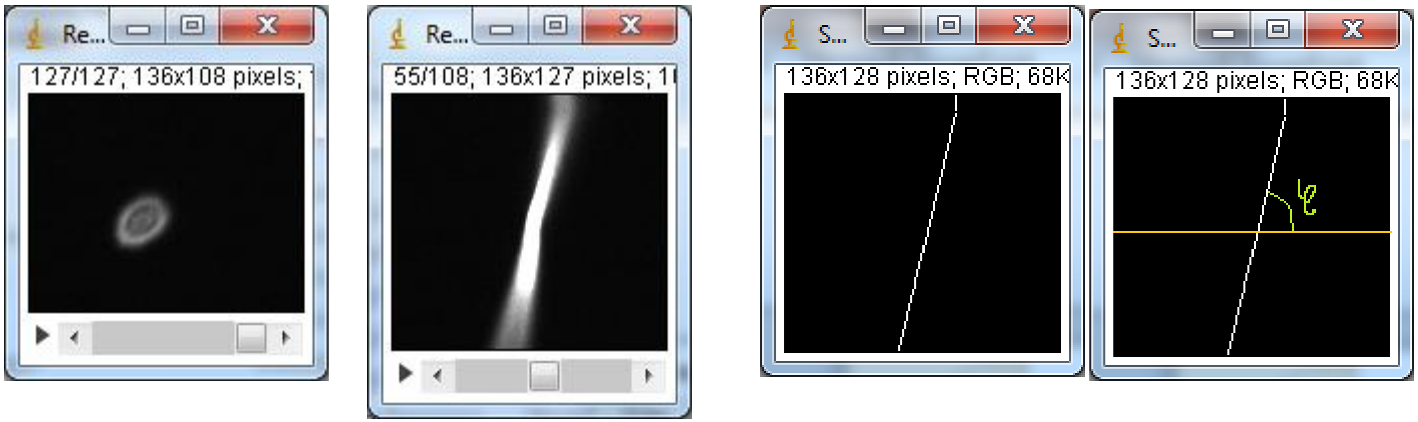


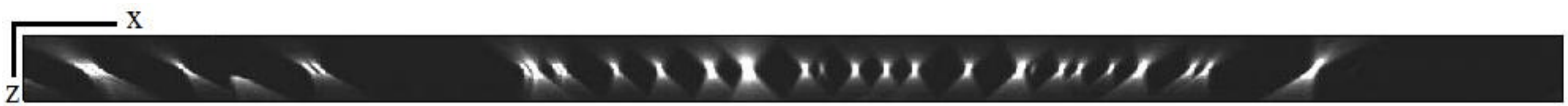
- ❖ The wide field macroscope suffers from sever lateral spatial PSF variations due to the zoom/objective combination

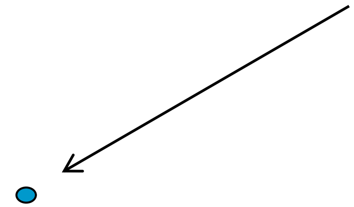


Macroscope Case – Optical centre

- Calculate the tilt in a Macroscope PSF



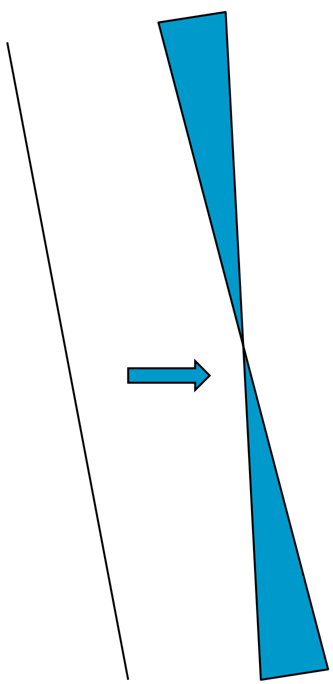


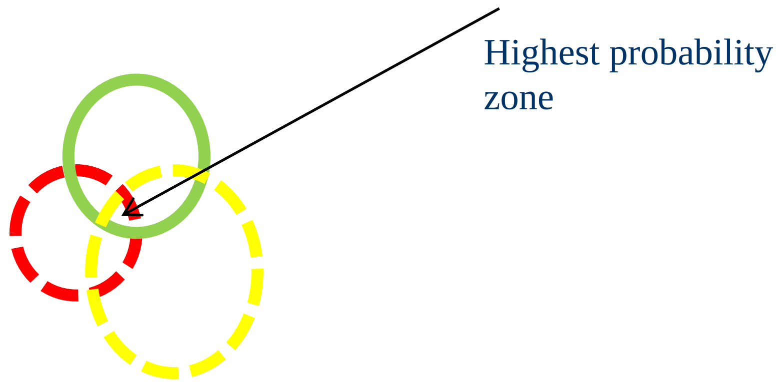


Optical center

Optical Center (Measured data error)

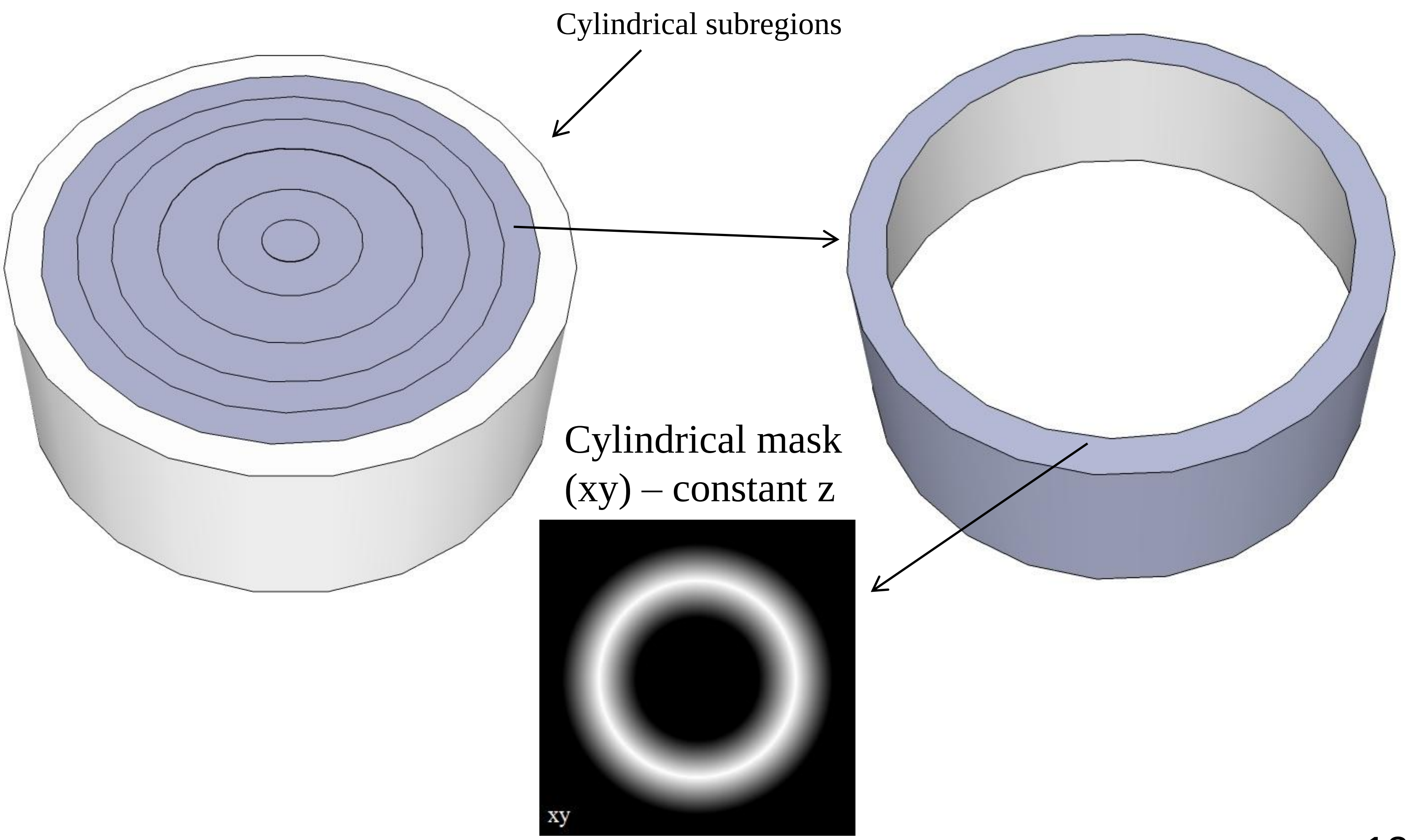
- Applying the method on measured data will not necessarily produce lines that converge to a single point:
  - Errors in the measurements
  - Errors in the method itself
- Introduce a freedom factor:
  - Line → collection of probable lines with a degree of incertitude
  - Zone on probable intersection
  - Define a criterion as center of mass …





Macroscope – Porting EMMA

- A geometric transformation need to be done
  - The PSF variations would be only depending on the radius in a cylindrical system



Macroscope case – Porting MIA

- Optical center is calculated
- PSF are recentred
- MIA is applied along the lateral axis
- Tilt is introduced using the information from the Optical center

Conclusion and Perspectives

- Ways to port EMMA and MIA to the Macroscope case are being investigated
- Optical center estimation method is being developed
- Side project:
  - Developing a hardware synchronized wide field 3D fluorescence microscope:
    - Automated XYZ stage
    - Hardware synchronized illumination-acquisition time (cam-shutter)
    - Full control software build as IJ plugin (Java) with automated acquisition list.
  - CUDA-OpenCl implementations (Jcuda under ImageJ – Jocl)
  - Investigating other image moments to be used with MIA