# SPATIALLY VARIABLE DECONVOLUTION FOR LIGHTSHEET MICROSCOPY

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#### LIGHTSHEET MICROSCOPY

TODO: a few words about why lightsheet is good

Light sheet microscopy is a type of fluorescence microscopy used in cell biology due to its fast acquisition times and low photo-damage to the sample. This is achieved by selectively illuminating a slice of the sample using a sheet of light and detecting the emitted fluorescence signal using a dedicated objective orthogonal to the plane of the sheet.

• Project aim: computationally improve the quality of light sheet microscopy images.

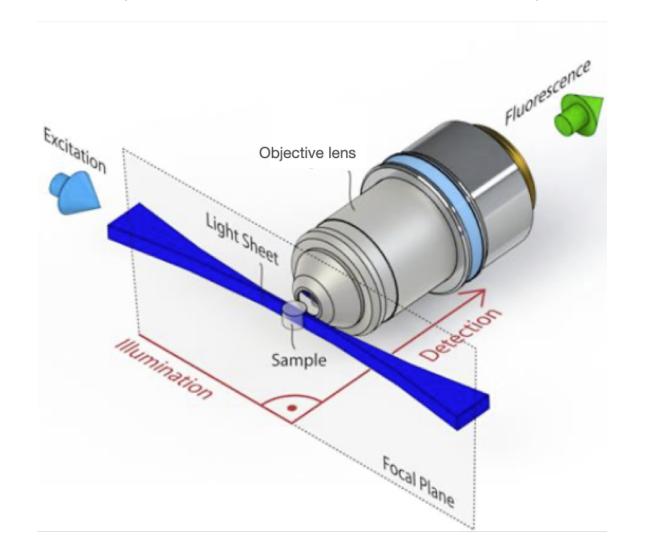
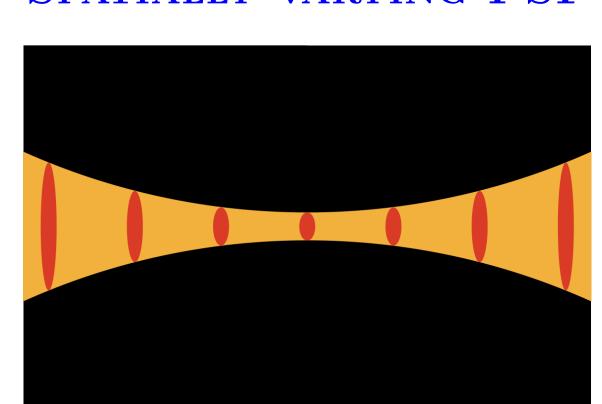


Figure credit: Jörg Ritter, PhD thesis (2011) A light-sheet microscope consists of two objectives: TODO etc

#### SPATIALLY VARYING PSF



PROBLEM

Due to the combination of light-sheet beam (yellow) and the objective PSF (red), the effective PSF of the system is spatially varying, and therefore standard deconvolution approaches are not applicable.

# PSF MODEL

TODO We calculate the objective PSF h by using a model that includes defocus etc etc. TODO: reference to paper

$$h(x,y,z) = \left| \iint g_{\sigma} * p(\kappa_x, \kappa_y) e^{2i\pi z} \sqrt{(n/\lambda)^2 - \kappa_x^2 - \kappa_y^2} e^{2i\pi(\kappa_x x + \kappa_y y)} \, d\kappa_x \, d\kappa_y \right|^2$$
 (1)

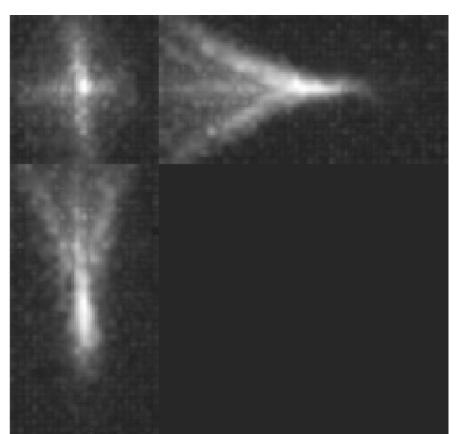
with the pupil function p defined

$$p(\kappa_x, \kappa_y) = \begin{cases} e^{2i\pi \sum_{j=1}^{15} c_i Z_j(\kappa_x, \kappa_y)} & \text{for } \rho = \sqrt{\kappa_x^2 + \kappa_y^2} \le NA/\lambda, \\ 0, & \text{otherwise.} \end{cases}$$
(2)

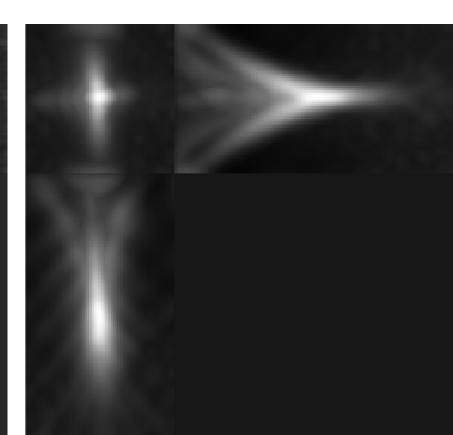
where the phase of the pupil function is computed by least-squares fitting of the coefficients of the first 15 Zernike polynomials using an image of a bead.

The parameters of the model are given by the experimental setup used to acquire the image:

- *n* refractive index
- $\lambda$  wave length
- NA numerical aperture
- $g_{\sigma}$  Gaussian blur to take into account other properties not accounted for in our model



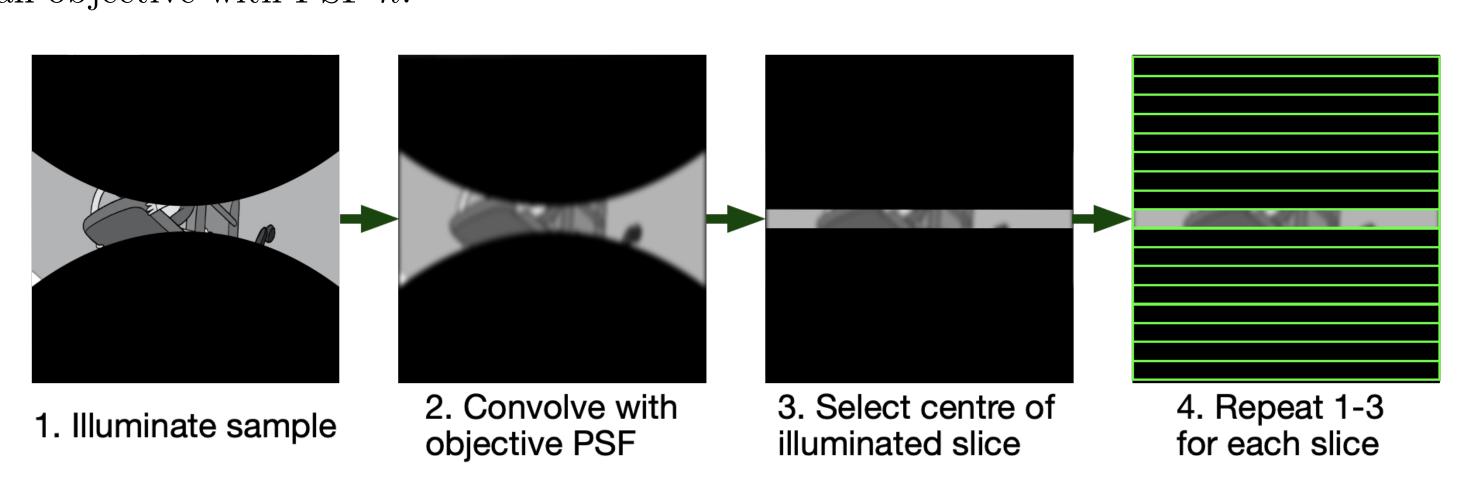
Bead image (MIP)



Estimated objective PSF (MIP)

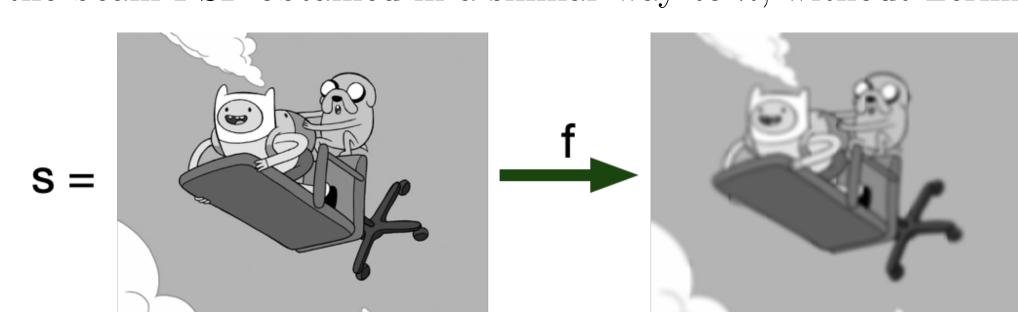
#### IMAGE FORMATION MODEL

The sample s illuminated at  $z = z_0$  by the light-sheet l and the photons are collected by an objective with PSF h:



$$f(x, y, z_0) = \iiint l_{avg_y}(u, v, w) s(u, v, w - z_0) h(x - u, y - v, w) du dv dw$$

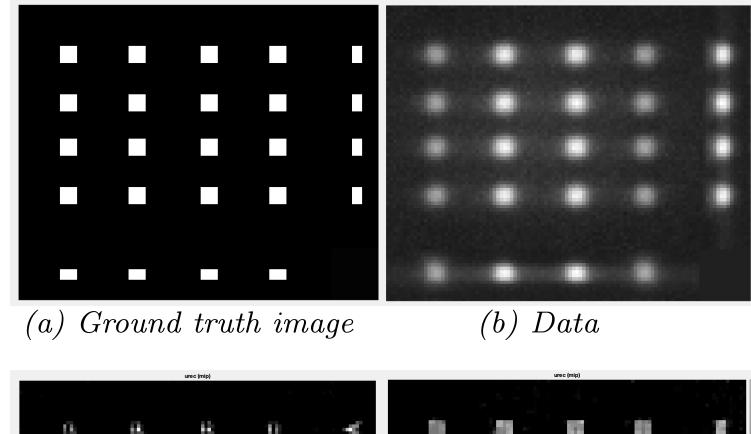
where h is the objective PSF, calculated using (1) and  $l_{avg_y}$  is the light-sheet, calculated by averaging the beam PSF obtained in a similar way to h, without Zernike polynomials.



#### RECONSTRUCTION

Let f be the image data and f(s) the result of applying the forward model to the sample s. To recover s, we solve:

Find 
$$\hat{s} \in \operatorname{argmin}_{s} \left\{ \|\hat{f} - f(s)\|_{L_{2}} + \lambda TV(s) \right\}$$



(d) Model deconvolution

(c) Constant PSF de convolution

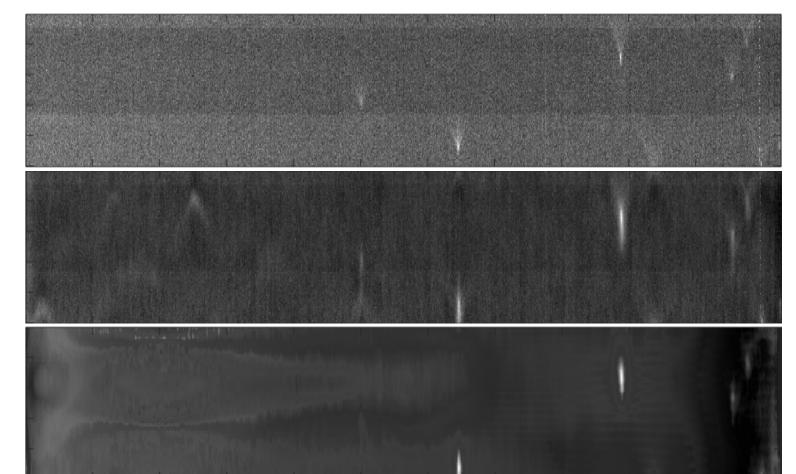
- To solve the optimisation problem, we apply a version of the Primal Dual Hybrid Gradient (PDHG) algorithm TODO:reference
- In the figure on the left, we show an example of a simulated sample (a) to which we apply the forward model with Gaussian noise (b) and the result of deconvolving using only a spatially varying PSF (c) and the full forward model (d).

#### RESULTS

#### BEADS

- We apply the proposed method to a sample of beads in agarose.
- The dimensions of the sample are 1127 x 111 x 100 pixels.
- XY slice: data (top), constant PSF deconvolution

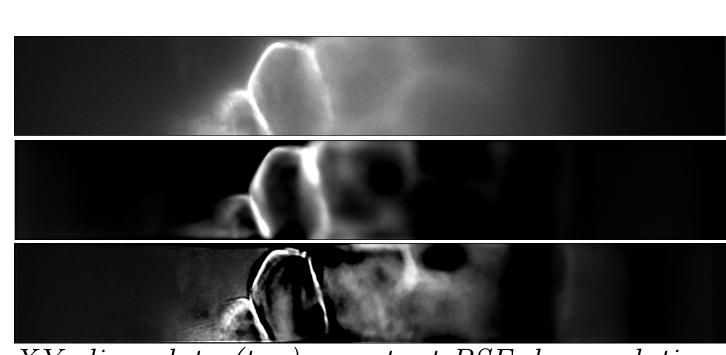
(middle), model deconvolution (bottom)



XY slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

#### MARCHANTIA

- We apply the proposed method to a sample of Marchantia plant.
- The dimensions of the sample are 1127 x 155 x 100 pixels.



XY slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)



XZ slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

## ACKNOWLEDGMENTS

### REFERENCES