Spatially Variable Deconvolution for Lightsheet Microscopy

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Lightsheet Microscopy

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.

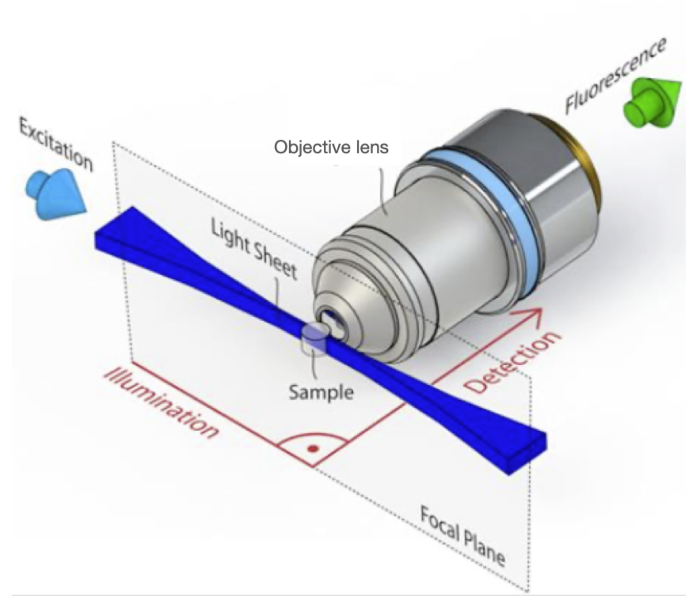
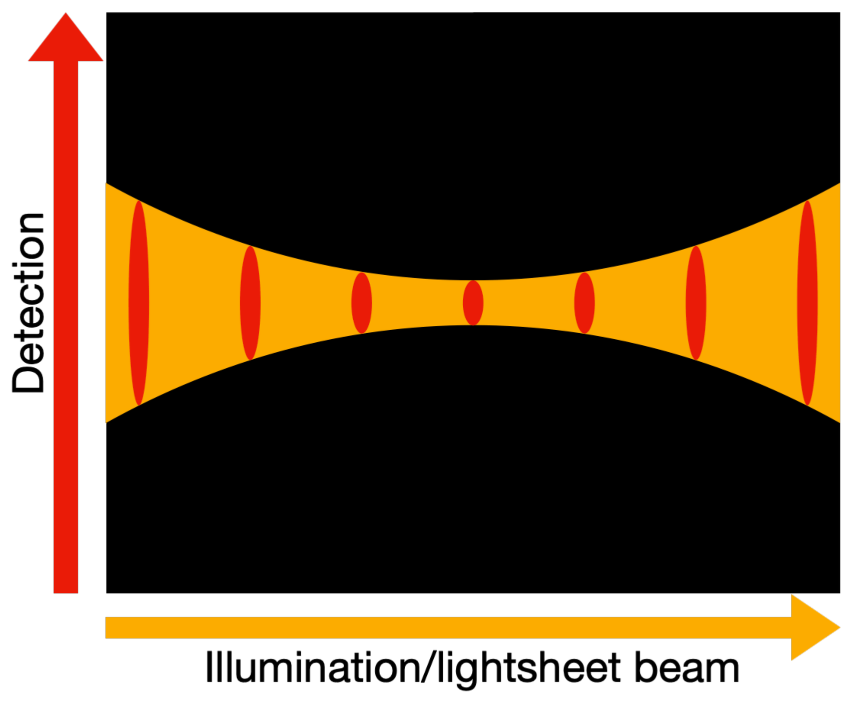


Figure credit: Jörg Ritter, PhD thesis (2011)

Spatially varying PSF



Problem

Due to the interaction of the lightsheet beam and the detection/objective point spread function (PSF), the effective PSF of the system is spatially varying, and therefore standard deconvolution approaches are not applicable.

Goal

In this work, we propose a model for image formation that describes the interaction between the illumination PSF and the detection PSF which replicates the physics of the microscope while leading to a tractable inverse problem.

PSF Model

The detection PSF h is modelled as the Fourier transform of the pupil function multiplied by defocus [3]:

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

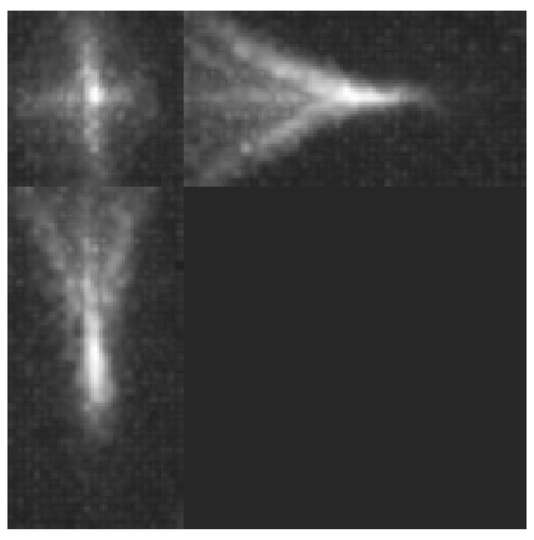
where p is the pupil function, defined as

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

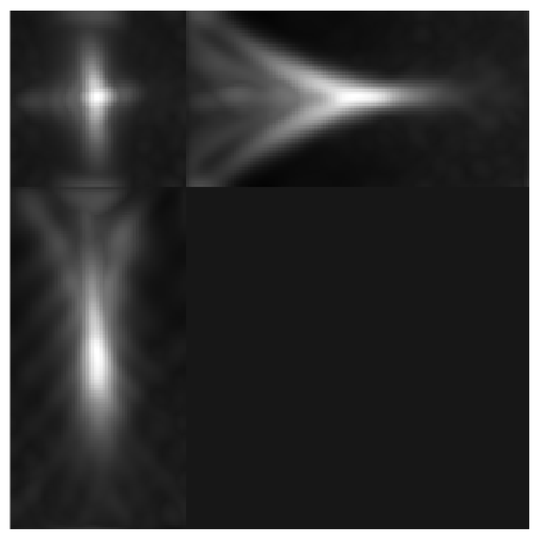
where the phase of the pupil function is computed by least-squares fitting of the coefficients of the first 15 Zernike polynomials using an image of a bead.

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

- n - refractive index
- λ - wave length
- NA - numerical aperture
- g_σ Gaussian blur to take into account other properties not accounted for in our model



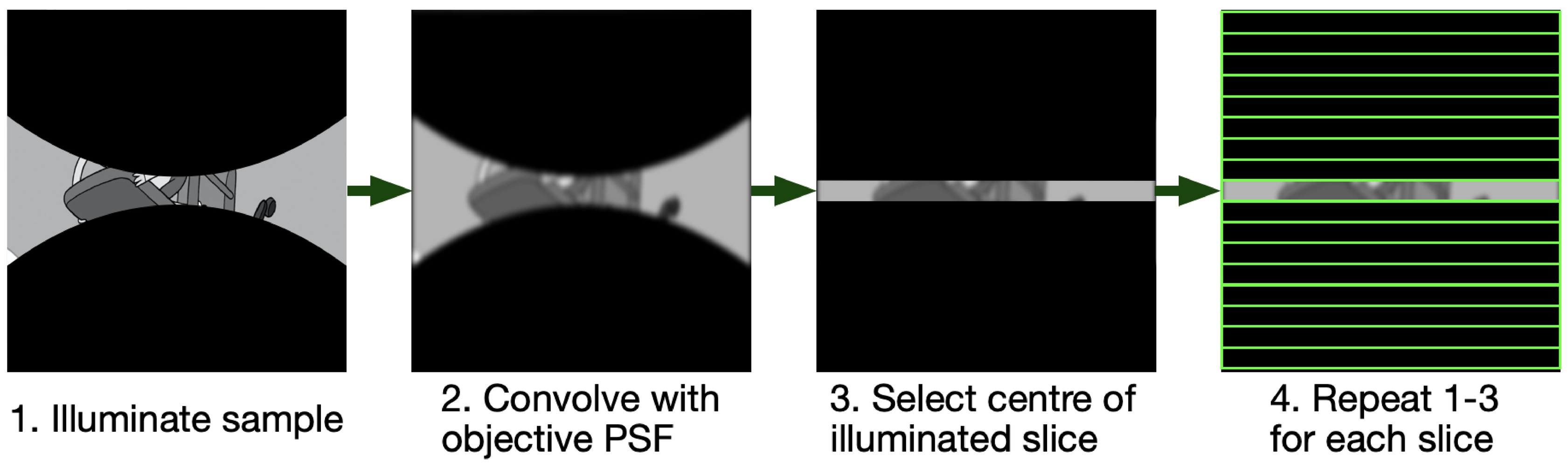
Bead image (MIP)



Estimated detection PSF (MIP)

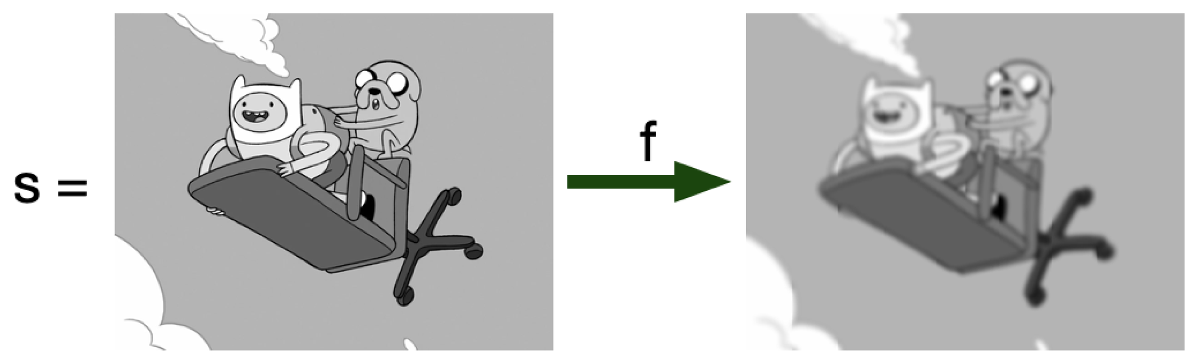
Image Formation Model

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



$$f(x,y,z_0) = \iiint l_{avg_y}(u,v,w) s(u,v,w-z_0) h(x-u,y-v,w) \, du \, dv \, dw \qquad (3)$$

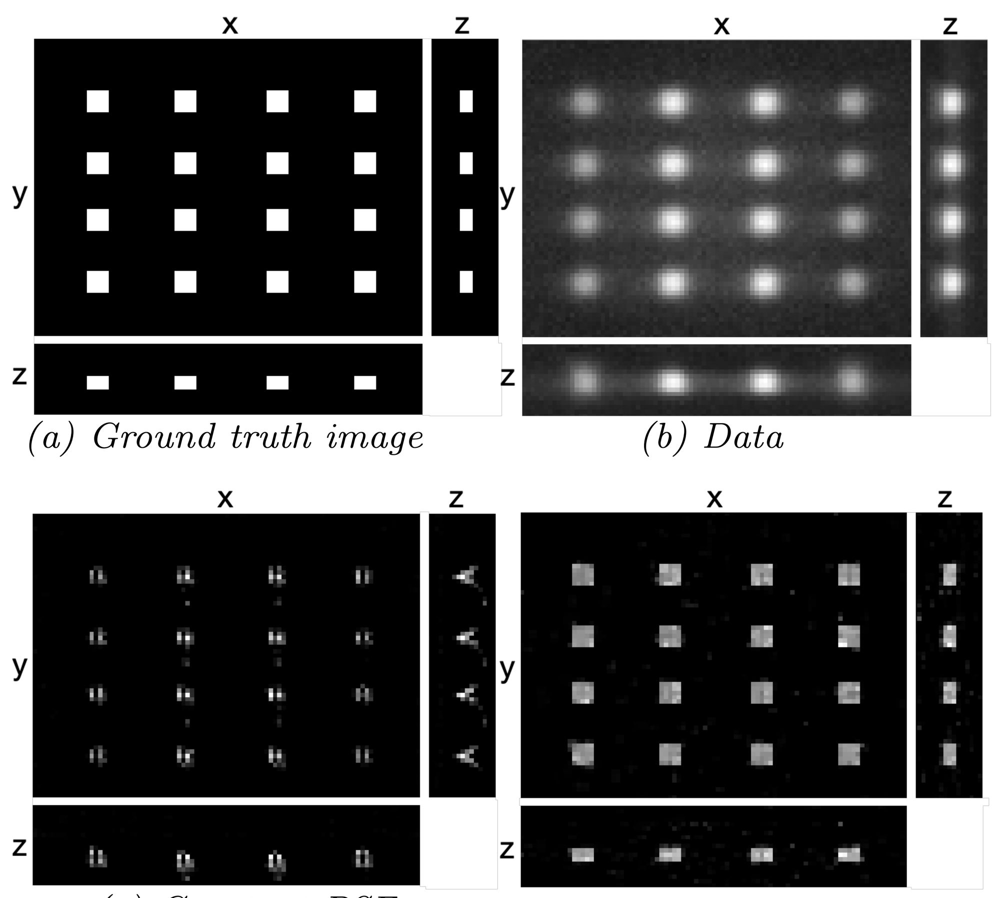
where h is the detection PSF, calculated using (1) and l_{avg_y} is the light-sheet, calculated by averaging the beam PSF obtained in a similar way to h, without Zernike polynomials.



Reconstruction

Let $\hat{f}$ be the image data with Gaussian noise and $f(s)$ the result of applying the forward model (3) to the sample s. To recover s, we solve:

$$\text{Find } \hat{s} \in \text{argmin}_s \left\{ \|\hat{f} - f(s)\|_{L_2} + \lambda TV(s) \right\}$$



(a) Ground truth image *(b) Data* *(c) Constant PSF deconvolution* *(d) Model deconvolution*

- We use the total variation regulariser $TV(s)$ and ℓ_2 fidelity term due to the Gaussian noise in the data.
- To solve the optimisation problem, we apply a version of the Primal Dual Hybrid Gradient (PDHG) algorithm from [1, 2].
- 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).
- The images are shown using maximum intensity projection.

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.
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Marchantia

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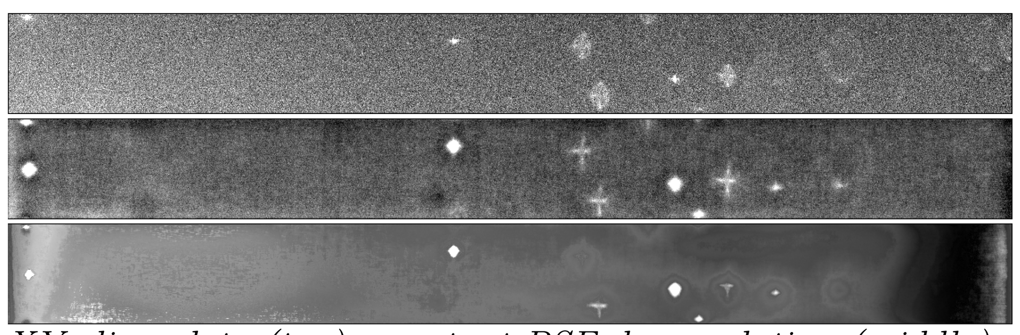
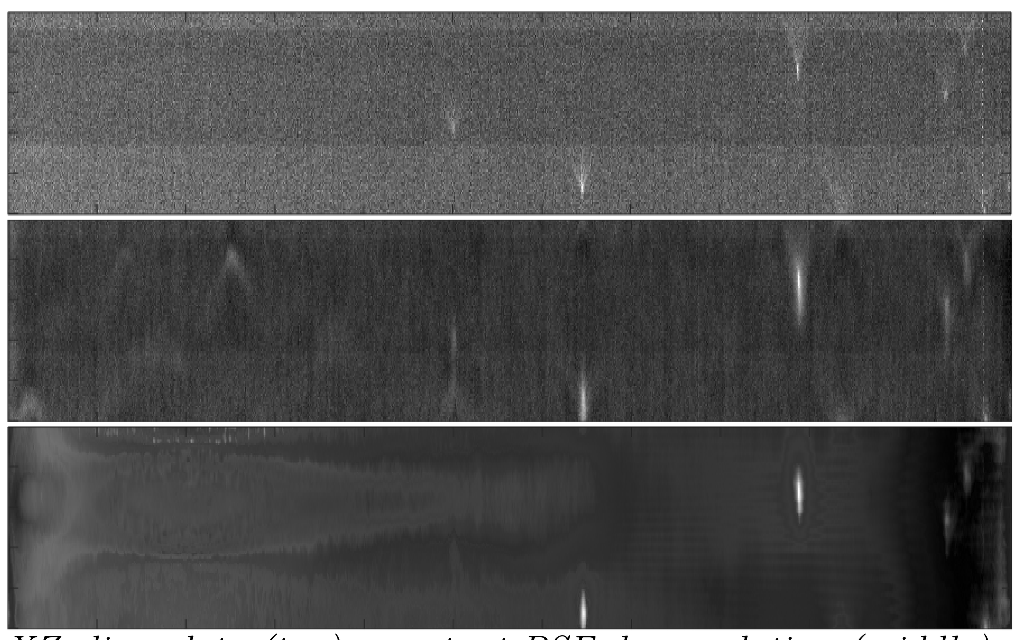
Beads

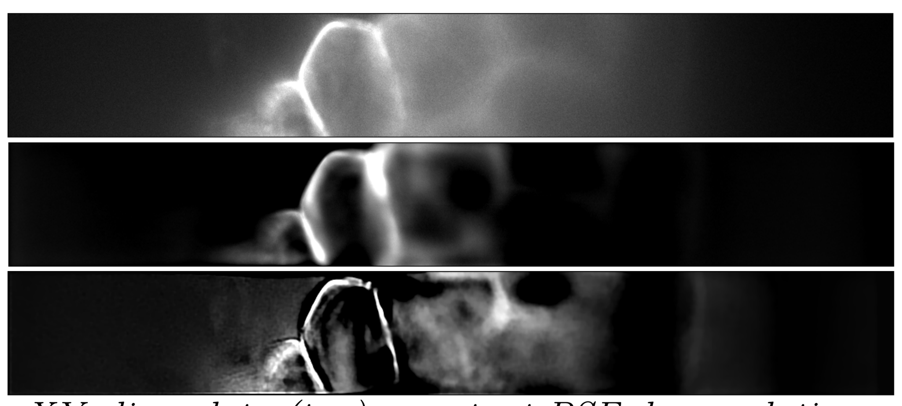


XY slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

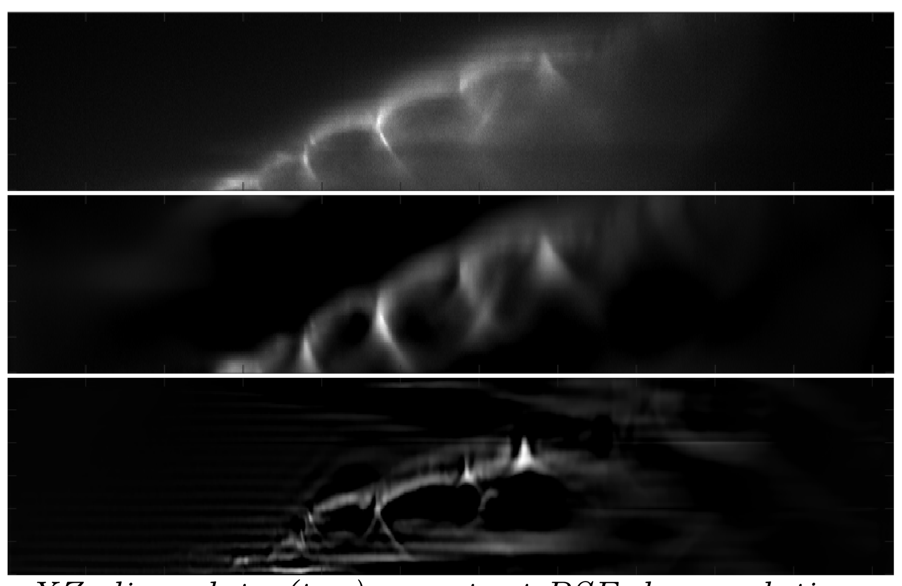


XZ slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

Marchantia



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



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

References

[1] J. Boulanger, N. Pustelnik, L. Condat, L. Sengmanivong, and T. Piolot. Nonsmooth convex optimization for structured illumination microscopy image reconstruction. *Inverse Problems*, 34(9):0–28, 2018.

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Acknowledgments

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