Spatially Variable Deconvolution for Light-sheet 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.

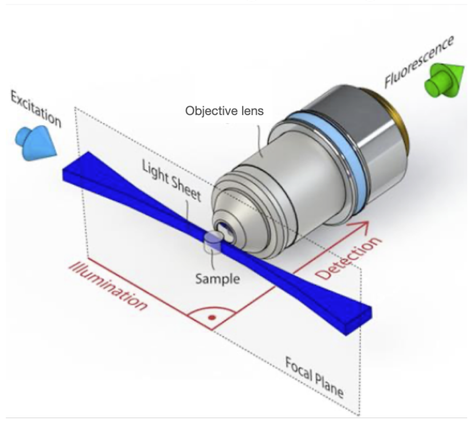
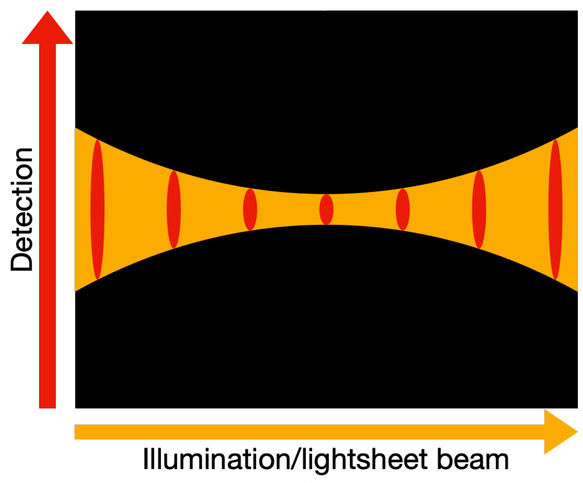


Figure credit: Jörg Ritter, PhD thesis (2011)

SPATIALLY VARYING PSF



PROBLEM

Due to the interaction of the light-sheet 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]:

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

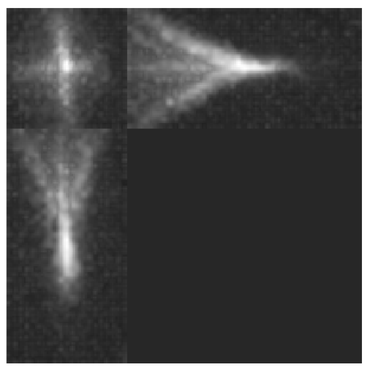
where p is the pupil function, defined as

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

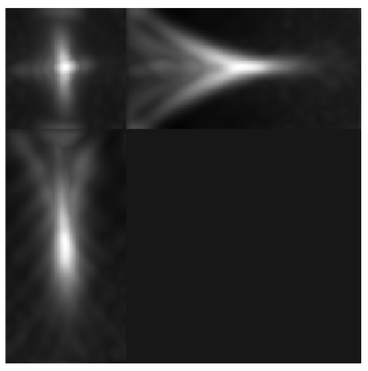
where the phase of the pupil function is computed by least-squares fitting of the coefficients of the first 15 Zernike polynomials using an image of a bead.

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



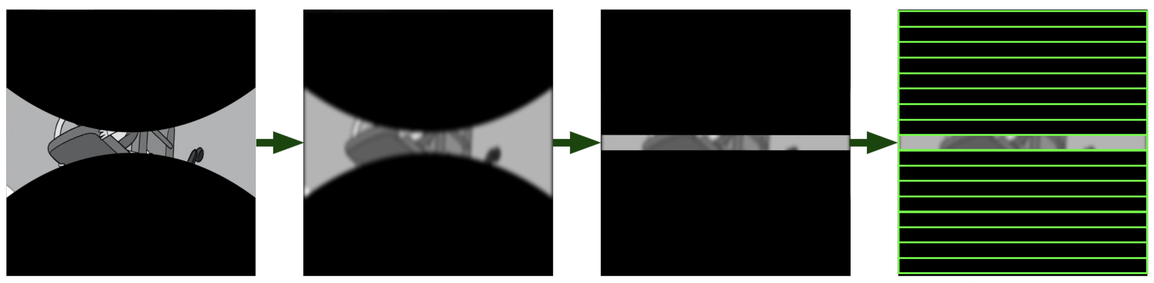
Bead image (MIP)



Estimated detection PSF (MIP)

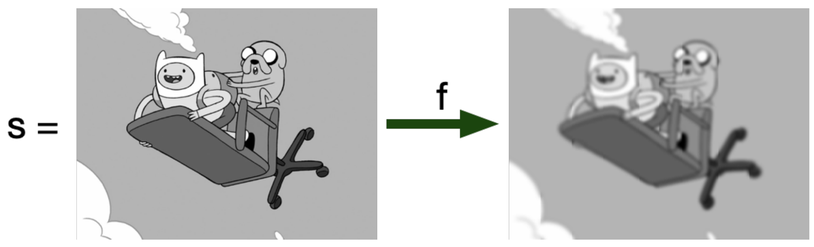
Image Formation Model

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



$$f(x,y,z_0) = \iiint l_{avg_y}(u,v,w) s(u,v,w-z_0) h(x-u,y-v,w) \,\mathrm{d}u \,\mathrm{d}v \,\mathrm{d}w \quad (3)$$

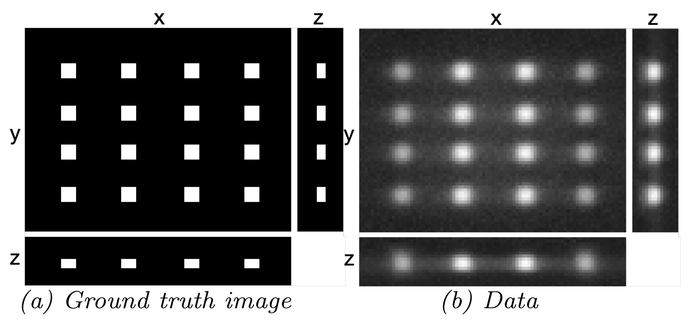
where h is the detection PSF, calculated using (1) and l_{avg_y} is the light-sheet, calculated by averaging the beam PSF obtained in a similar way to h, without Zernike polynomials.



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:

$$\text{Find } \hat{s} \in \operatorname{argmin}_s \left\{ \|\hat{f} - f(s)\|_{L_2} + \lambda TV(s) \right\} \quad (4)$$



(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

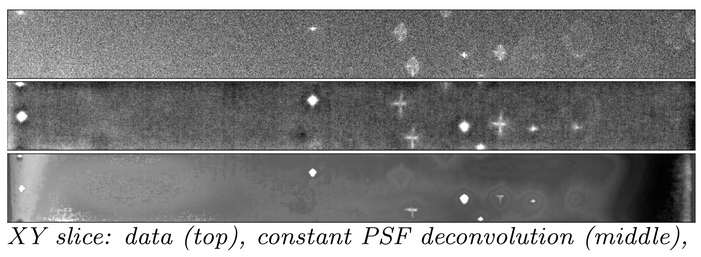
We solve the minimisation problem (4) with the forward model given by (3) and the PSF model in (1) to two 3D image stacks:

1. Beads in agarose (1127 x 111 x 100 pixels).
2. Marchantia plant (1127 x 155 x 100 pixels).

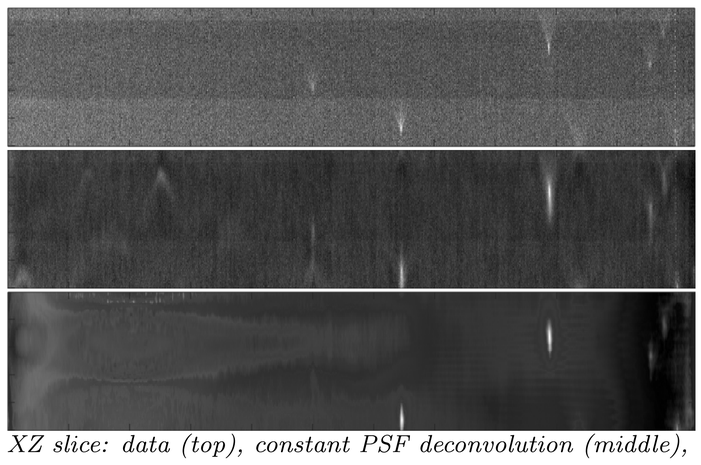
We compare our proposed method with deconvolution using constant PSF, namely the detection PSF h estimated in (1). In this case, we solve the same minimisation problem (4), except that the forward model is only a convolution operator.

- In the bead images, the lobes of the beads due to spherical aberration are minimised, as seen in the XY slice, while in the XZ slice we see that the beads become symmetric and less elongated.
- In the Marchantia image, the contrast is enhanced, the cell walls becoming sharper using our proposed approach compared to the constant PSF deconvolution.

1. Beads

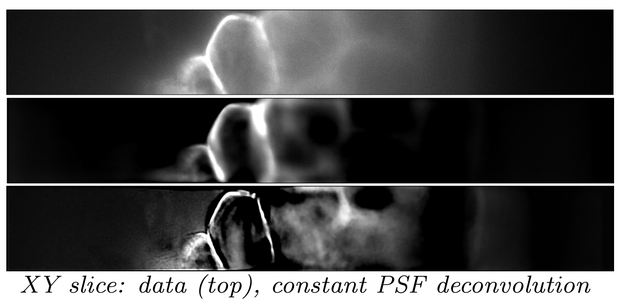


XY slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

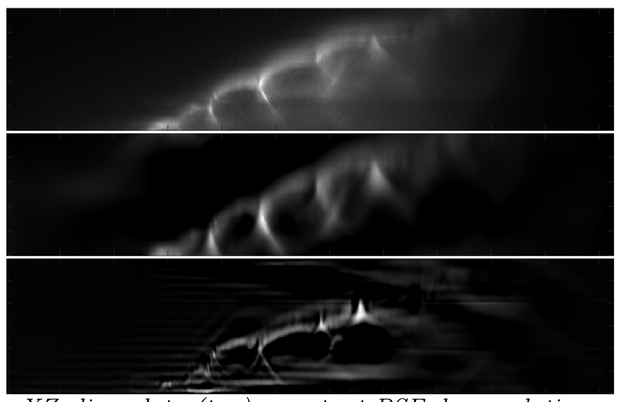


XZ slice: data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

2. 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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