

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.

LIGHT-SHEET MICROSCOPE

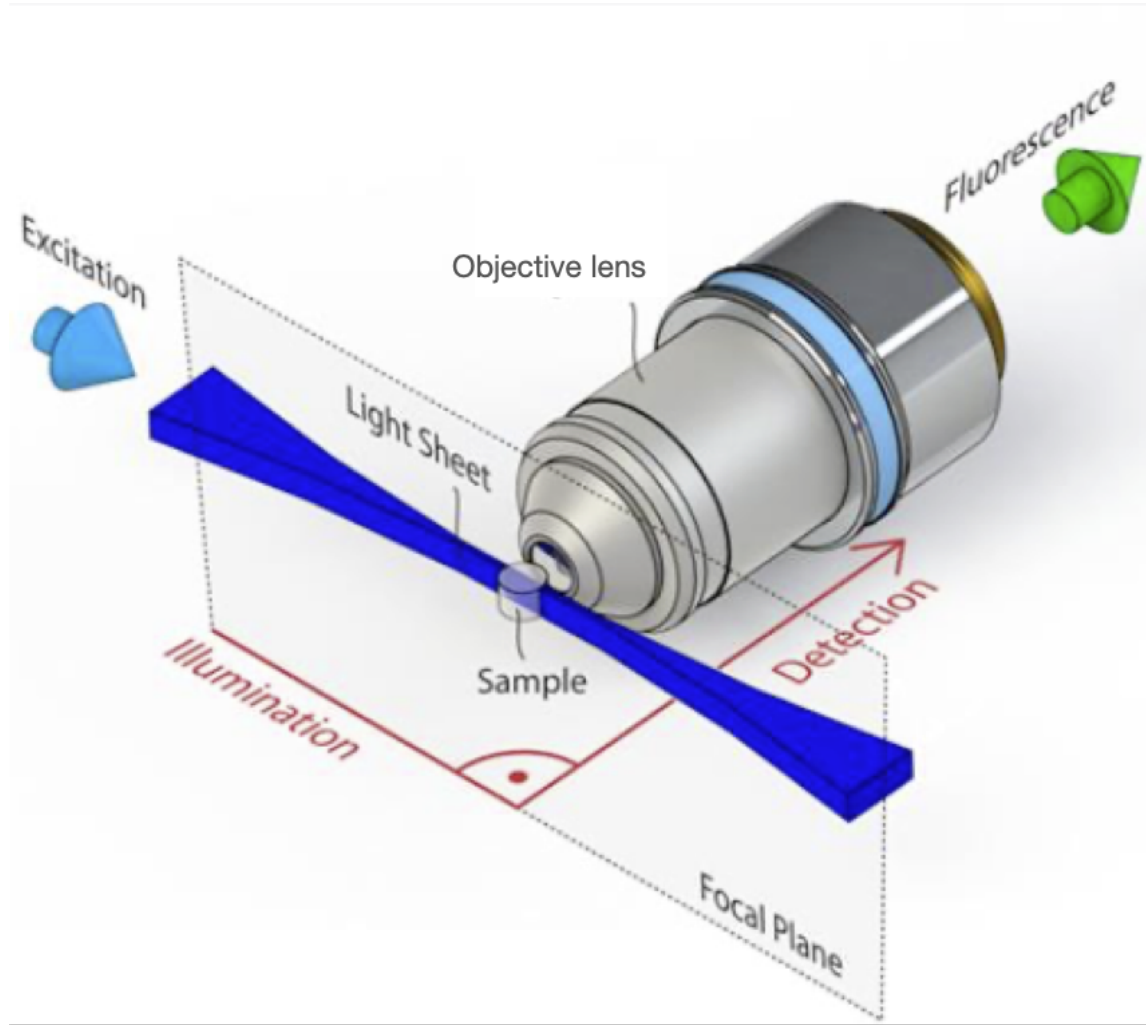
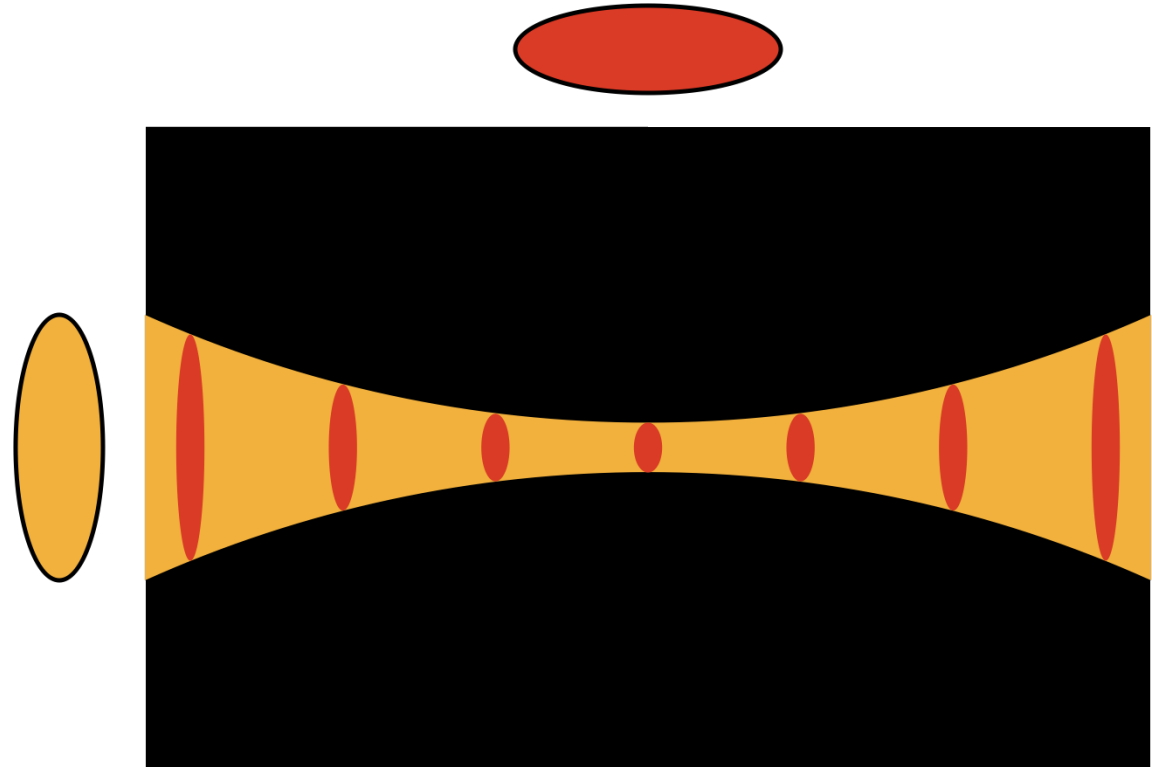


Figure credit: Jörg Rütter, PhD thesis (2011)

A light-sheet microscope consists of two objectives: TODO etc

SPATIALLY VARYING PSF



PROBLEM

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

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 given in (1) and  $l$  is calculated similarly (TODO explain)

TODO: insert diagram of the image formation to explain the forward model

PSF MODEL

TODO We calculate the objective PSF  $h$  by using a model that includes defocus etc etc.

TODO: reference to paper

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

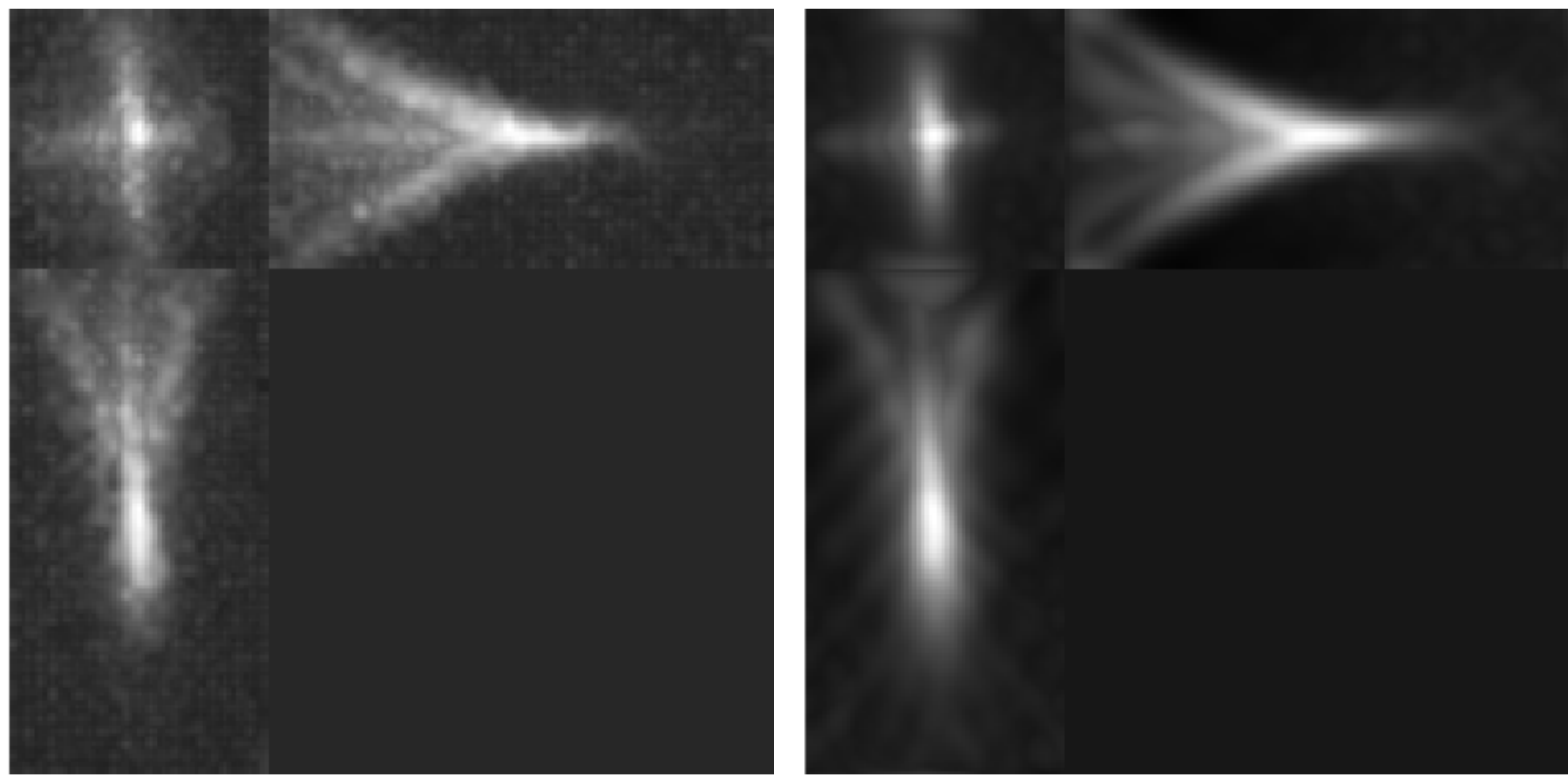
with the pupil function  $p$  defined

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

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:

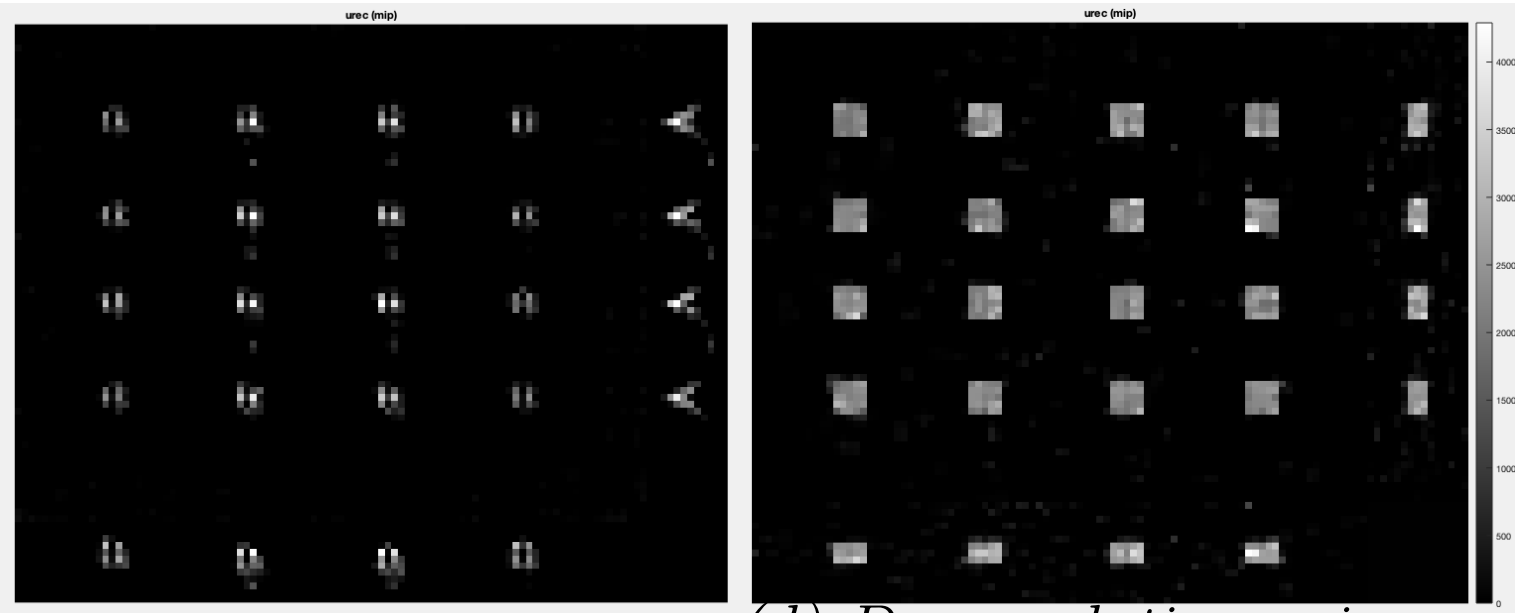
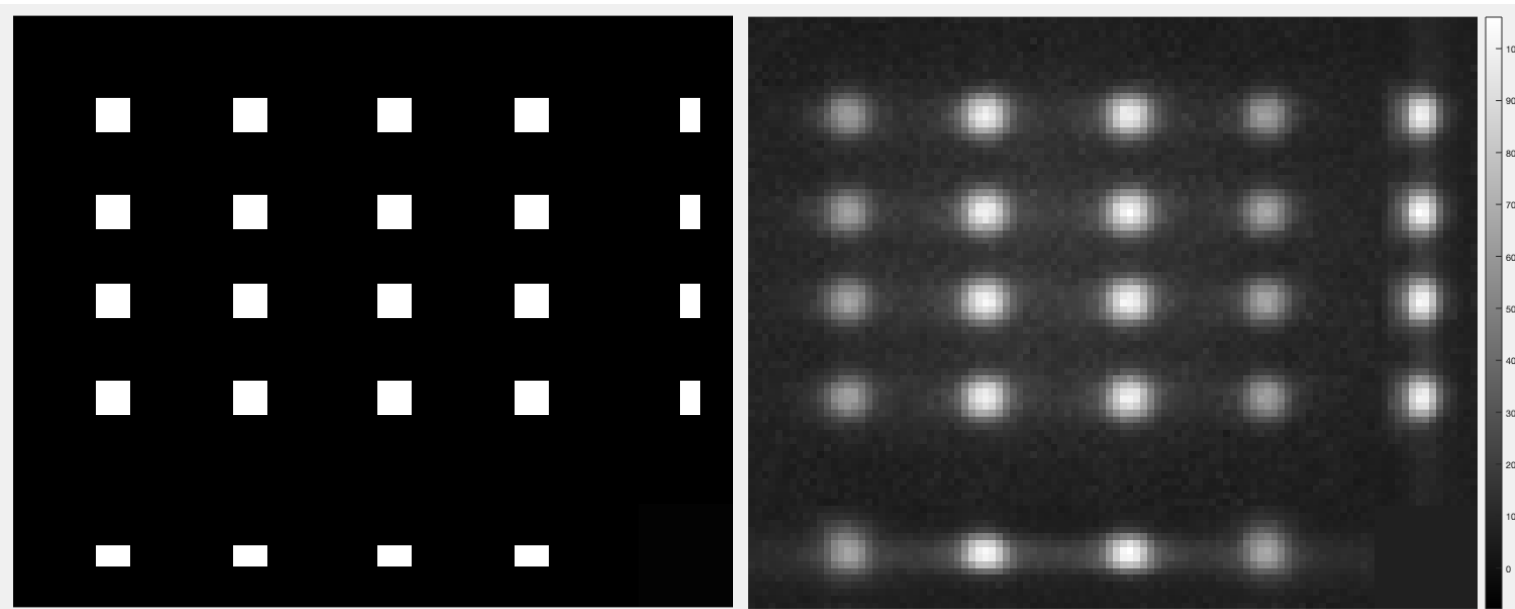
- $n$  - refractive index
- $\lambda$  - wave length
- NA - numerical aperture
- $g_\sigma$  Gaussian blur to take into account other properties not accounted for in our model



RECONSTRUCTION

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

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

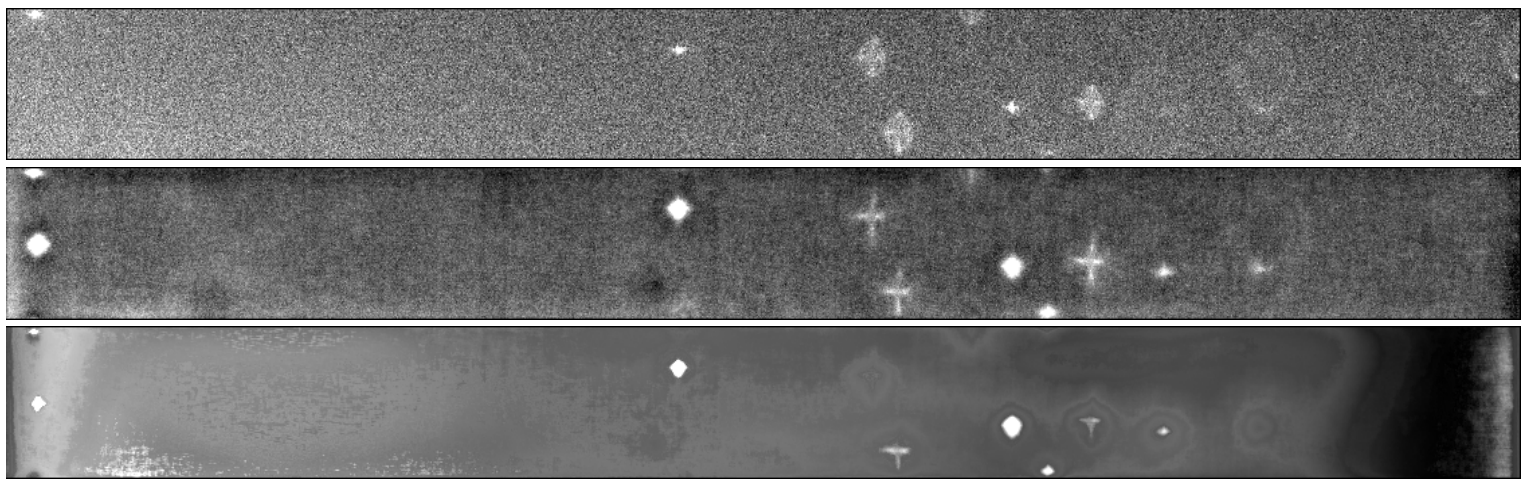


- To solve the optimisation problem, we apply a version of the Primal Dual Hybrid Gradient (PDHG) algorithm TODO:reference
- In the figure on the left, we show an example of a simulated sample (a) to which we apply the forward model with Gaussian noise (b) and the result of deconvolving using only a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

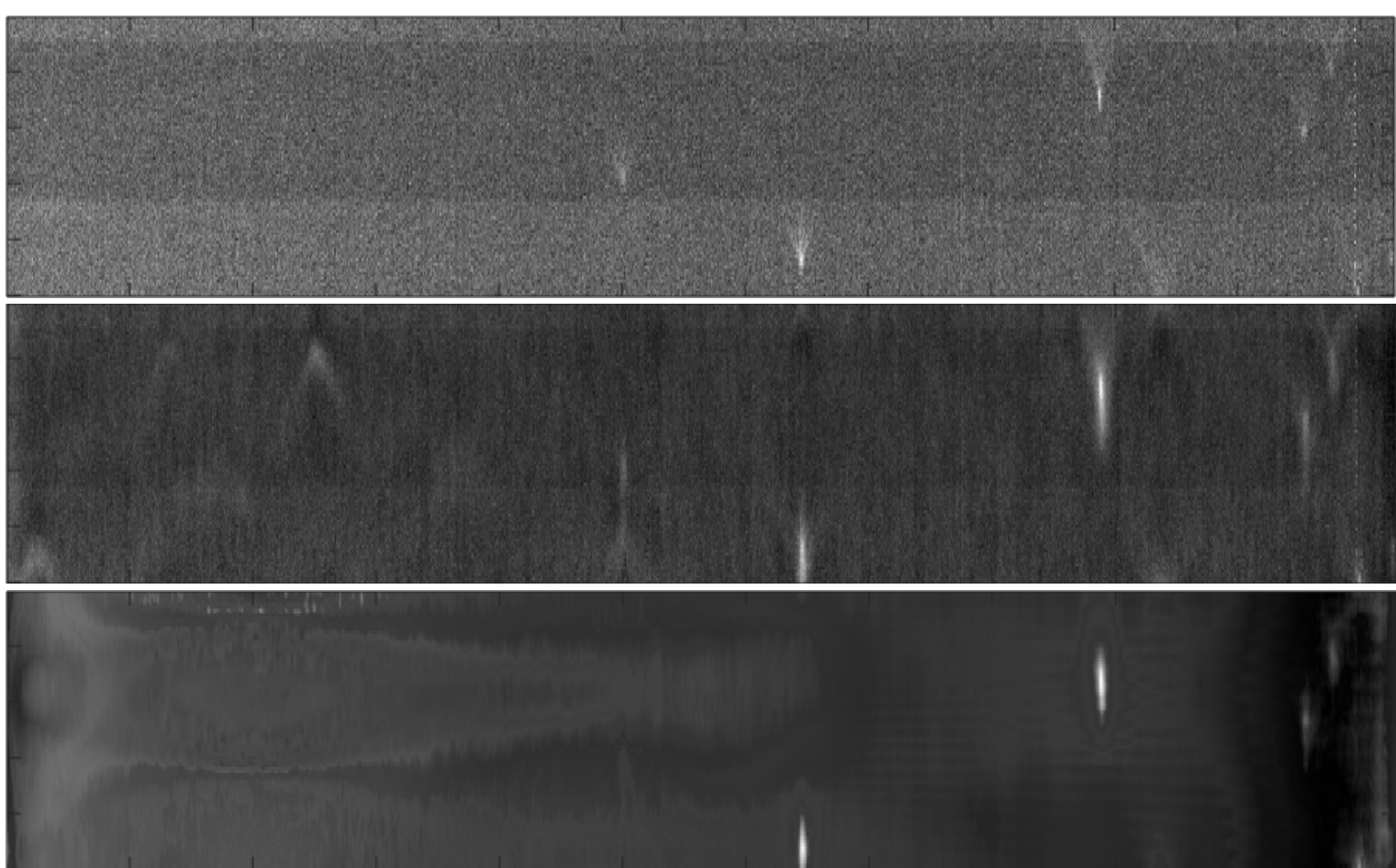
RESULTS

BEADS

Full resolution image: 1127 x 111 x 100



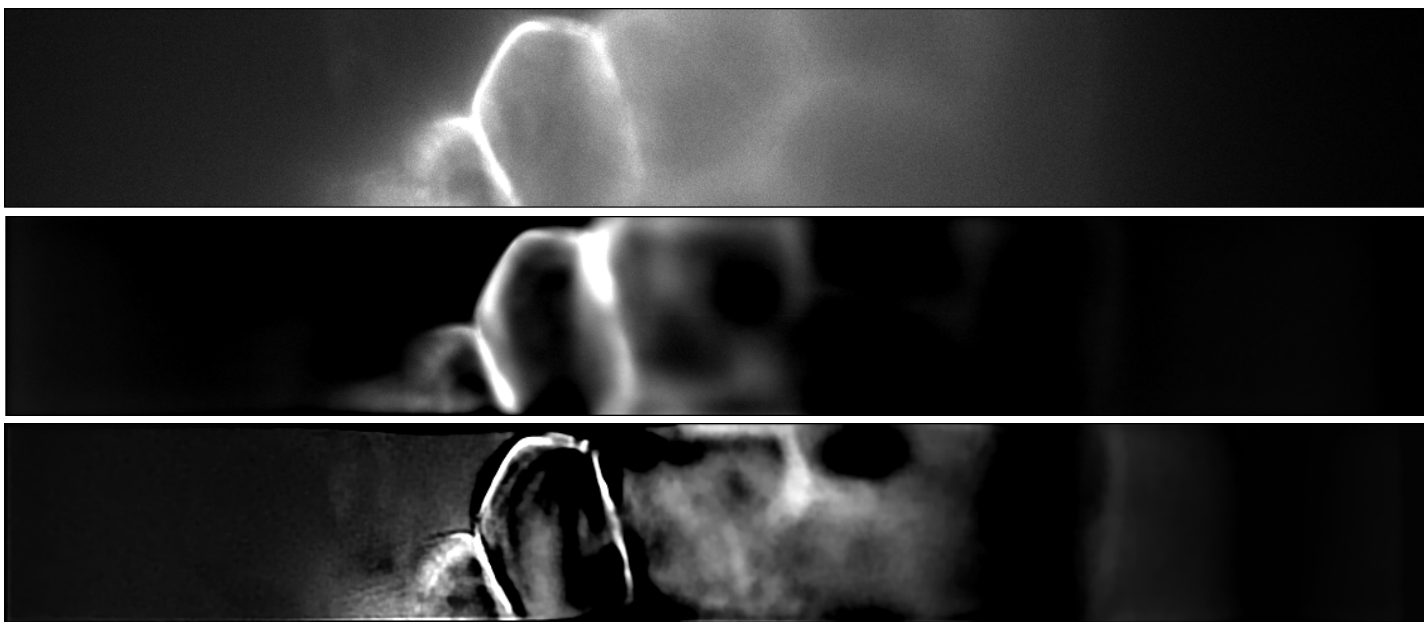
Data (top), constant PSF deconvolution (middle), model deconvolution (bottom)



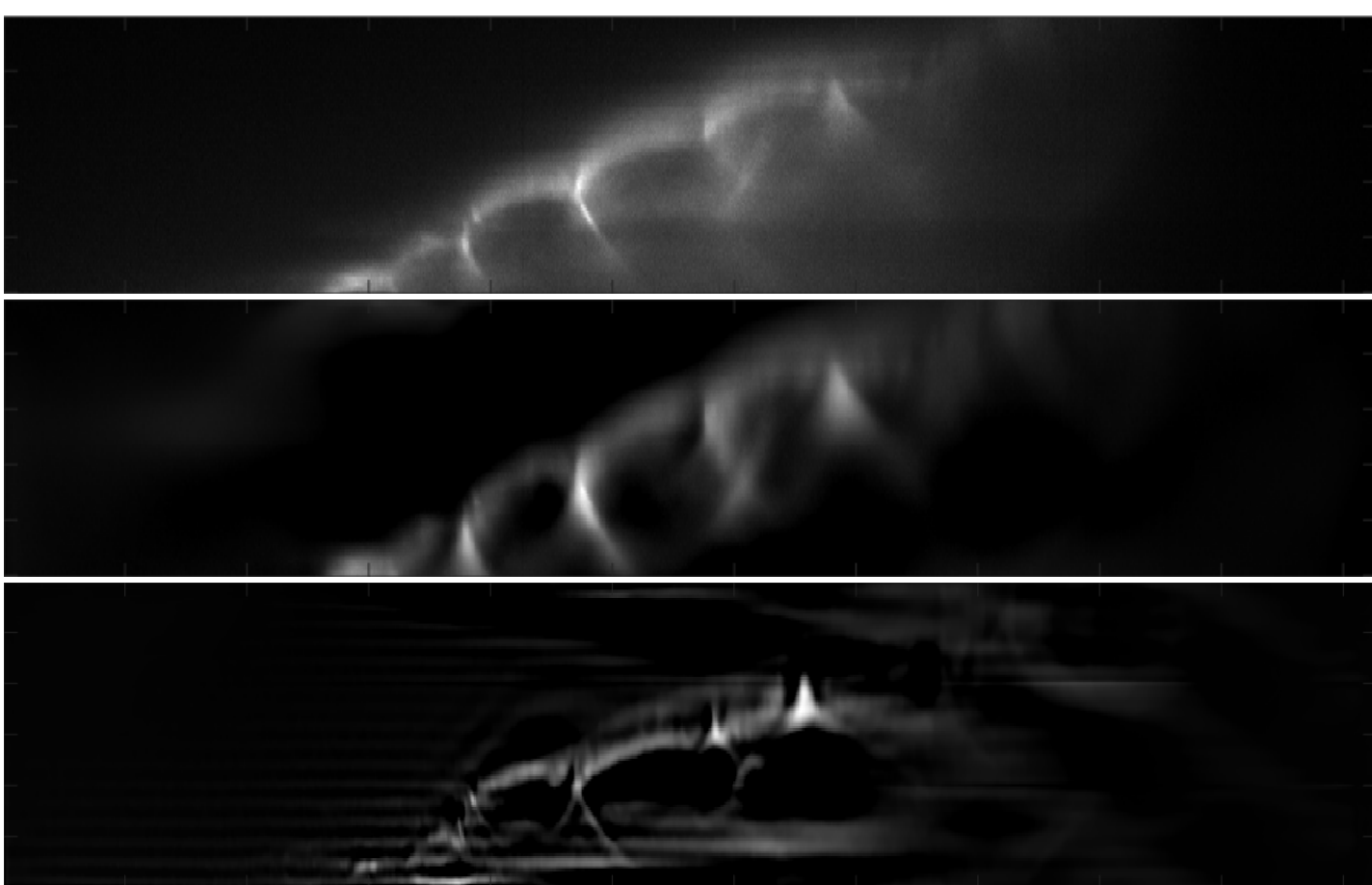
Data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

MARCHANTIA

Full resolution image: 1127 x 155 x 100



Data (top), constant PSF deconvolution (middle), model deconvolution (bottom)



Data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

REFERENCES

ACKNOWLEDGMENTS