

LIGHTSHEET MICROSCOPY

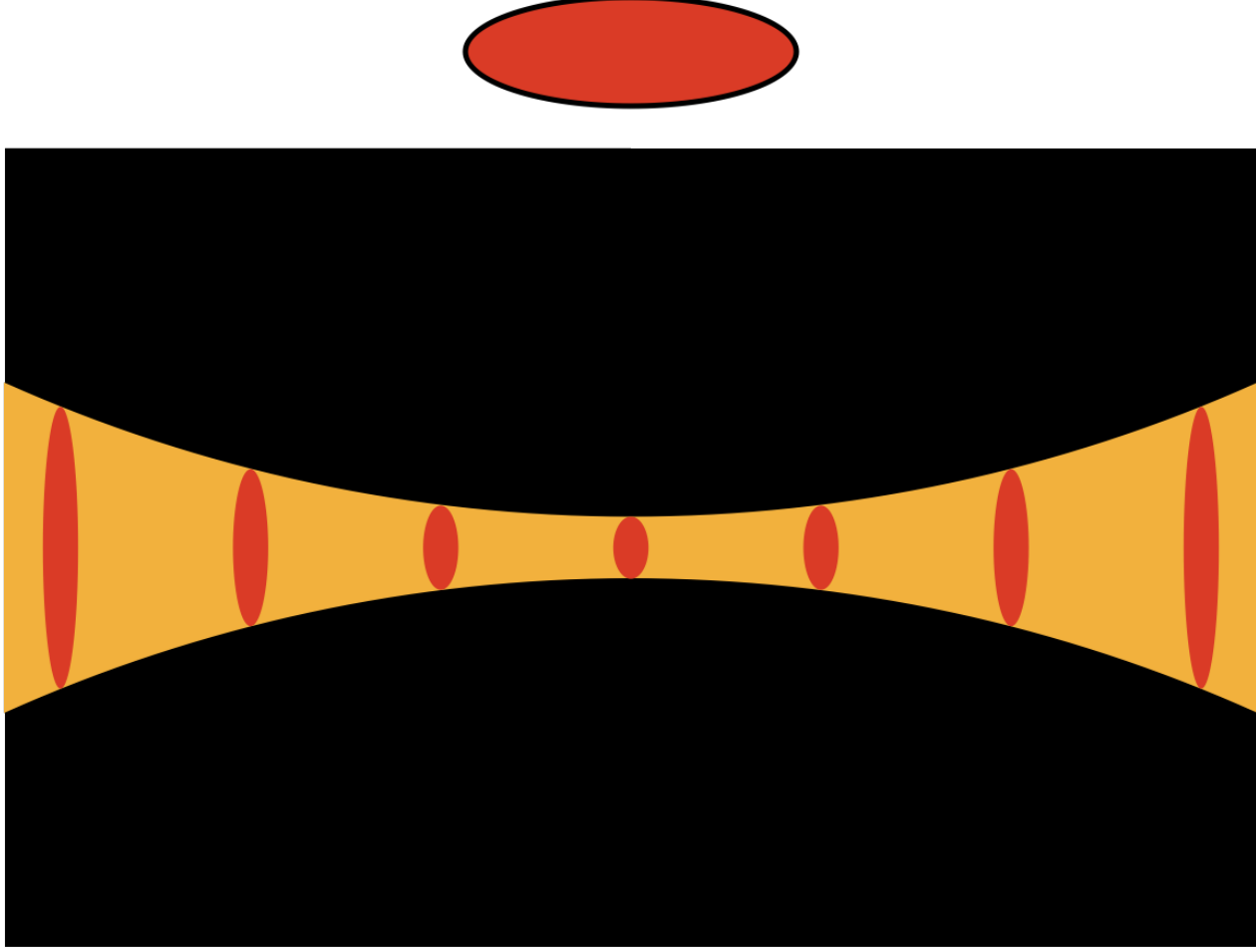
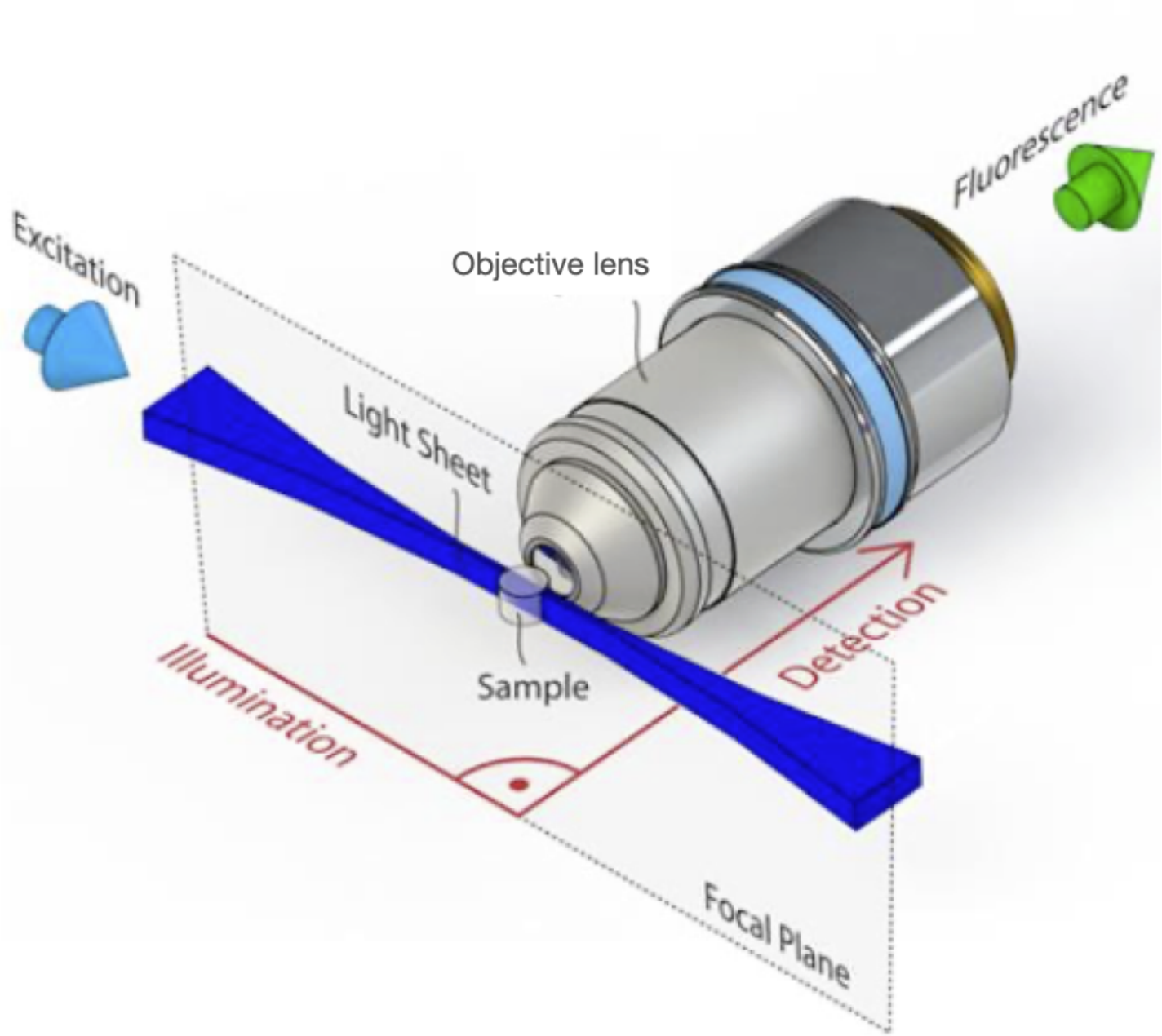
TODO: a few words about why lightsheet is good

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

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.

Figure credit: Jörg Ritter, PhD thesis (2011)

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

PSF MODEL

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

TODO: reference to paper

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

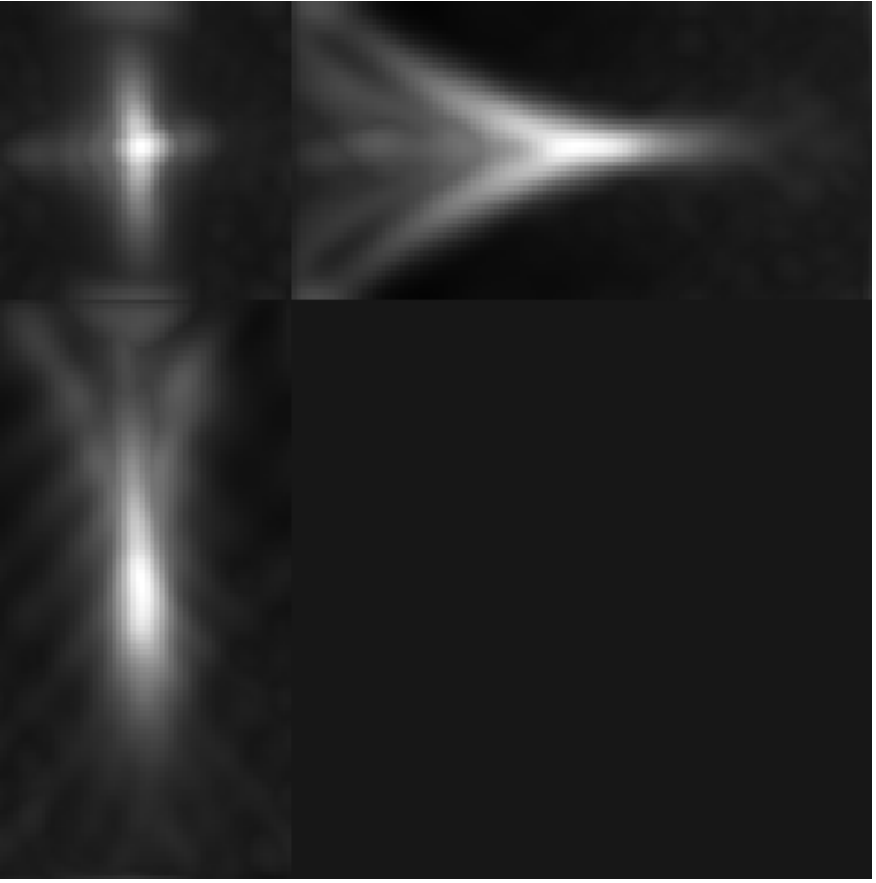
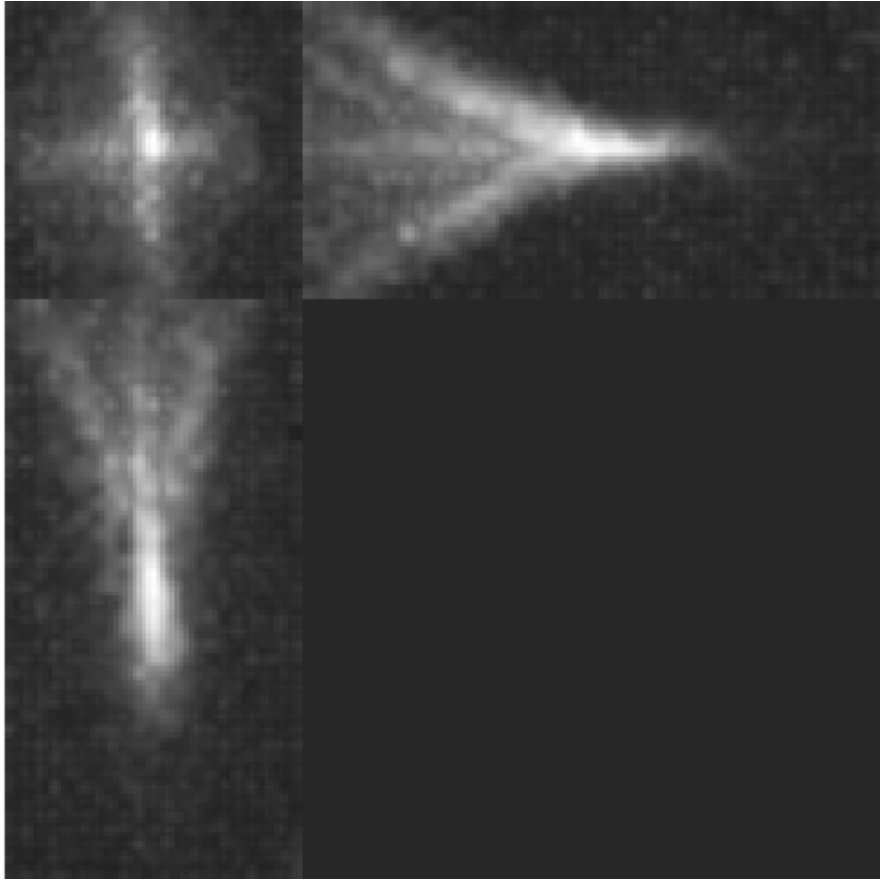
with the pupil function  $p$  defined

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

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



Bead image (MIP)

Estimated objective PSF (MIP)

IMAGE FORMATION MODEL

FORWARD 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

RECONSTRUCTION

TODO: refine this text, also make it take into account that we don't always use L2 for fidelity or TV for regularization. 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:

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

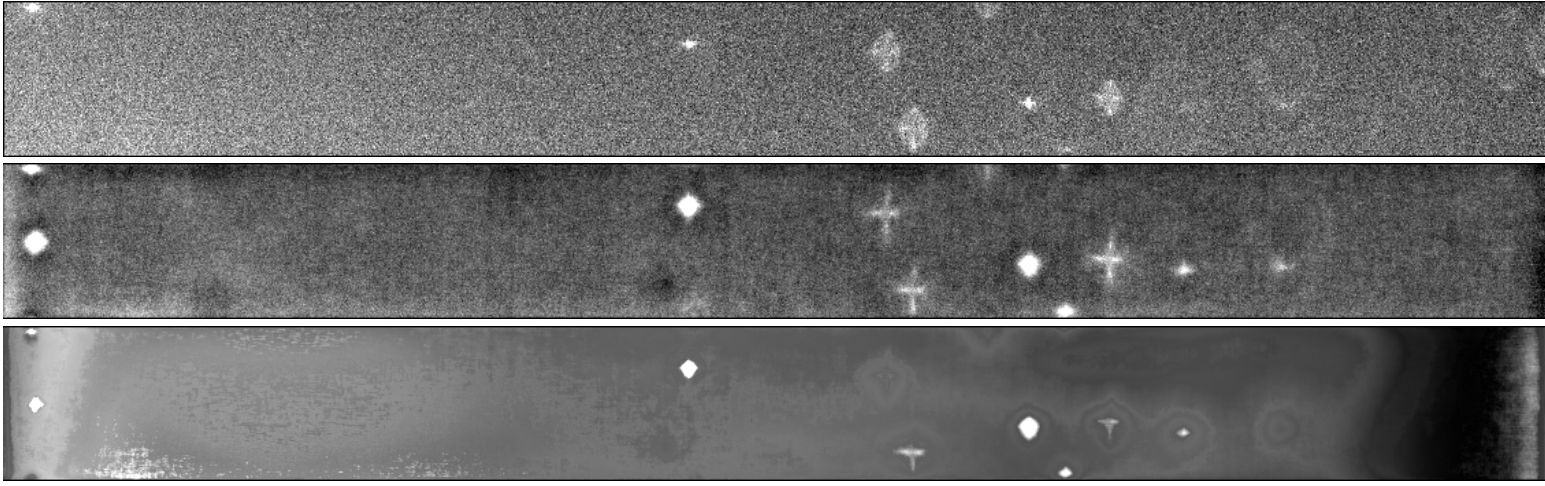
using a version of the Primal Dual Hybrid Gradient (PDHG) algorithm. TODO: reference.

RESULTS – SIMULATED DATA

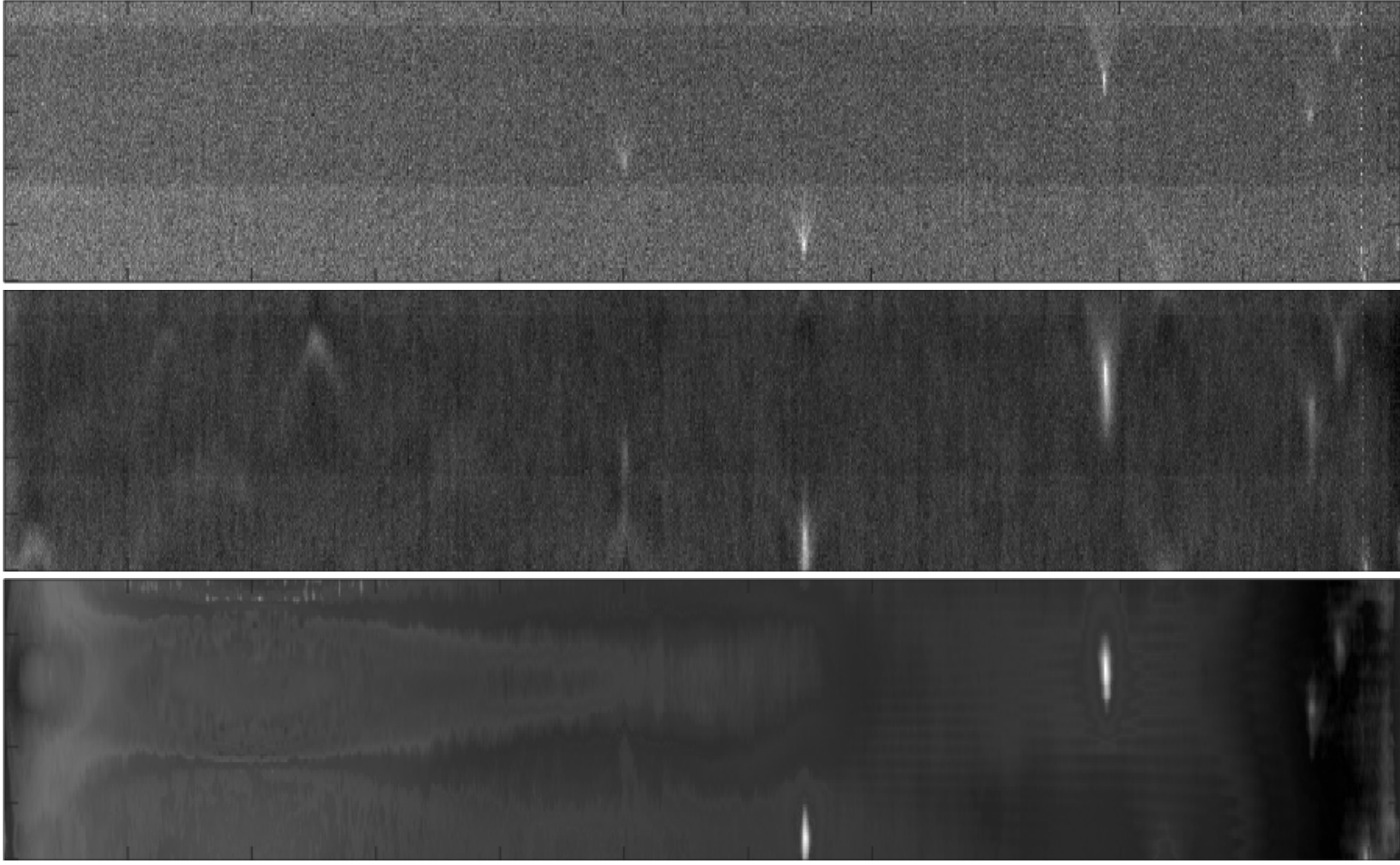
RESULTS – REAL DATA

BEADS

Full resolution image: 1127 x 111 x 100



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

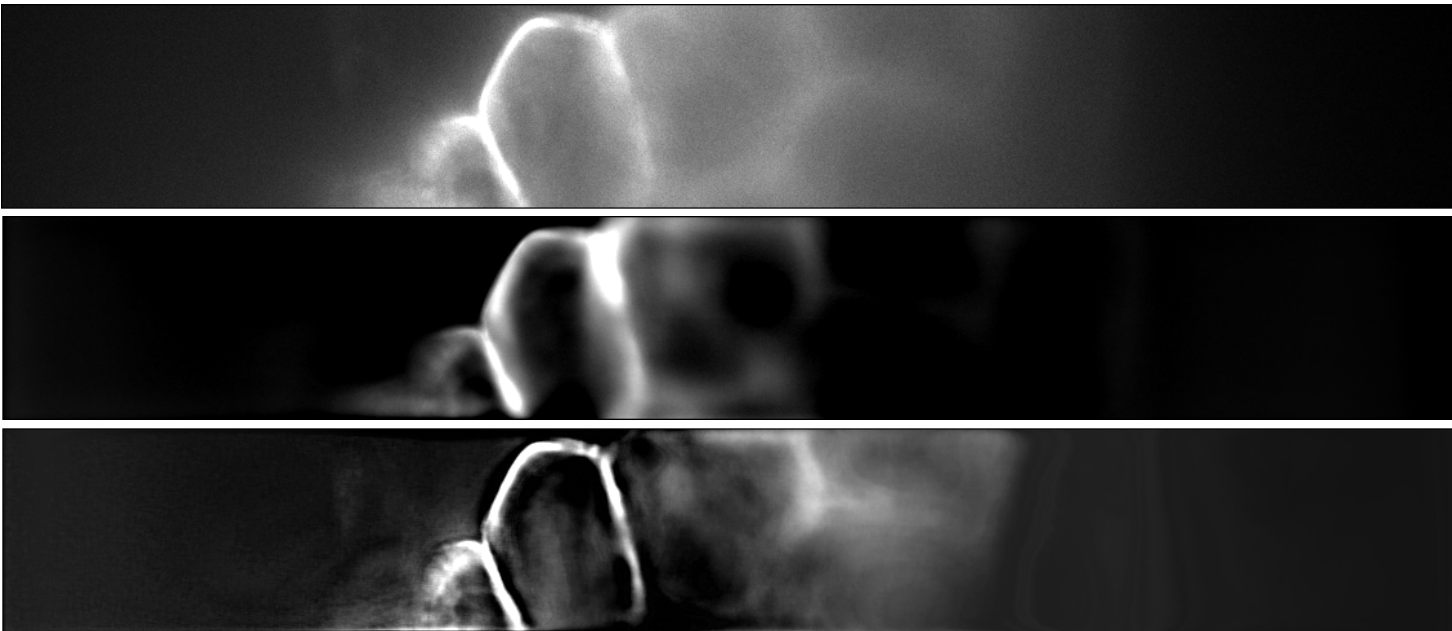


Data (top), constant PSF deconvolution (middle), model deconvolution (bottom)

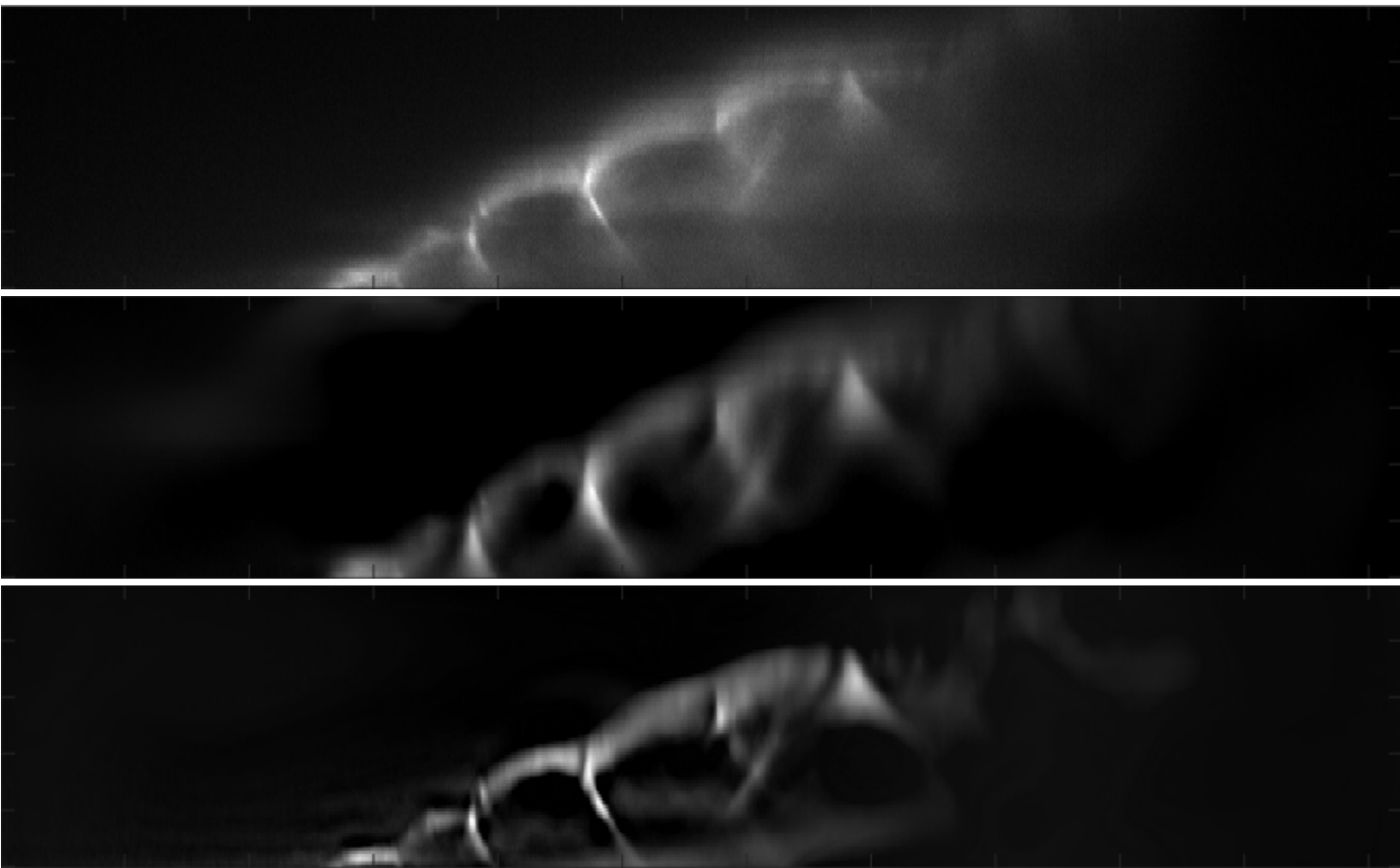
MARCHANTIA

TODO: include the latest results here because better

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)