Improving Resolution in Wide-field Fluorescence Microscopy Using Deconvolution Techniques

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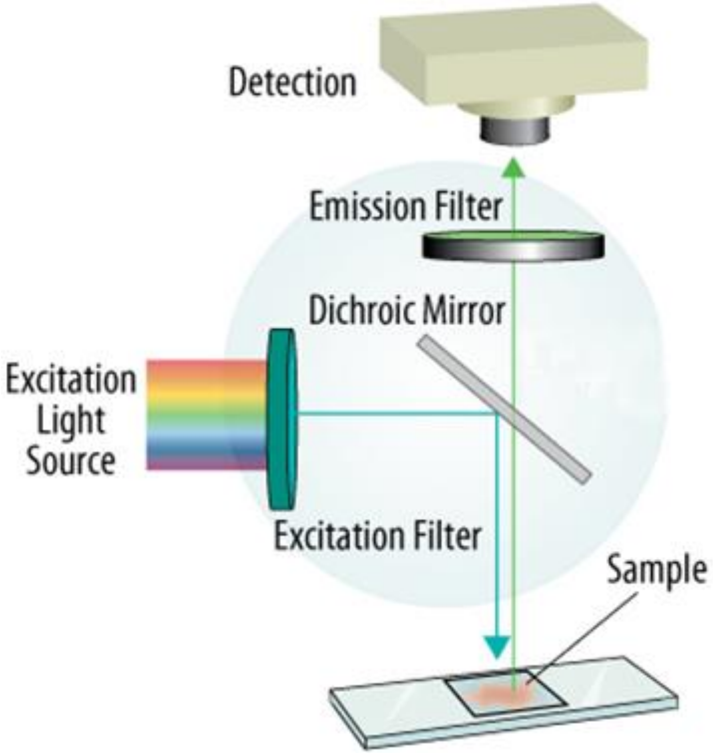
UCSB

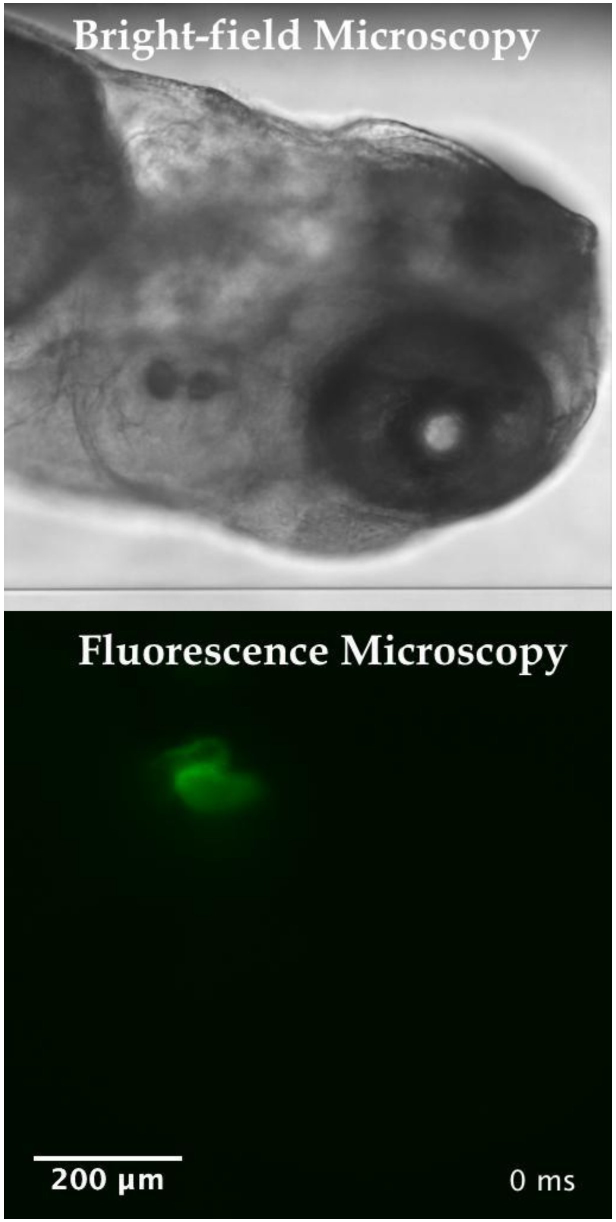
Systems Bioimaging Lab
Development

Fluorescence Imaging

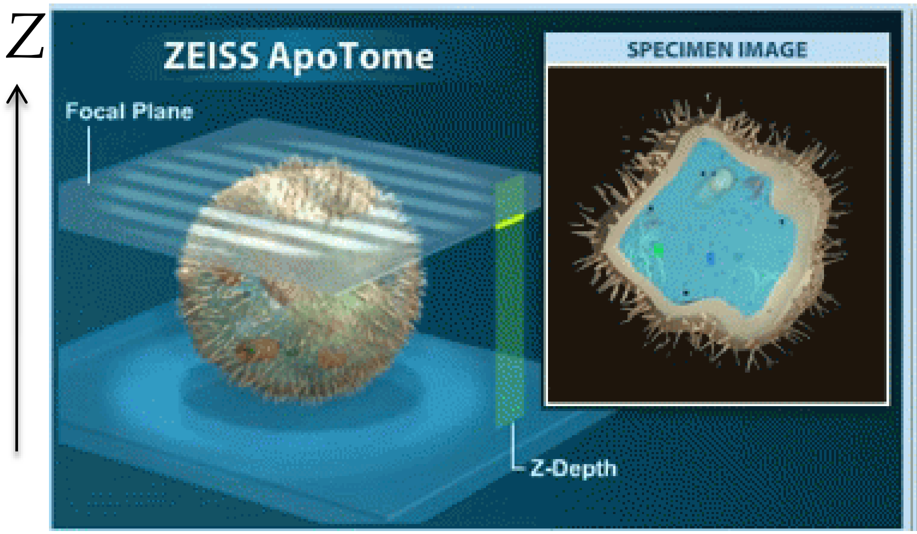
Simplified Model of Fluorescence Imaging



Fluorescence is exploited in Fluorescence Microscopy



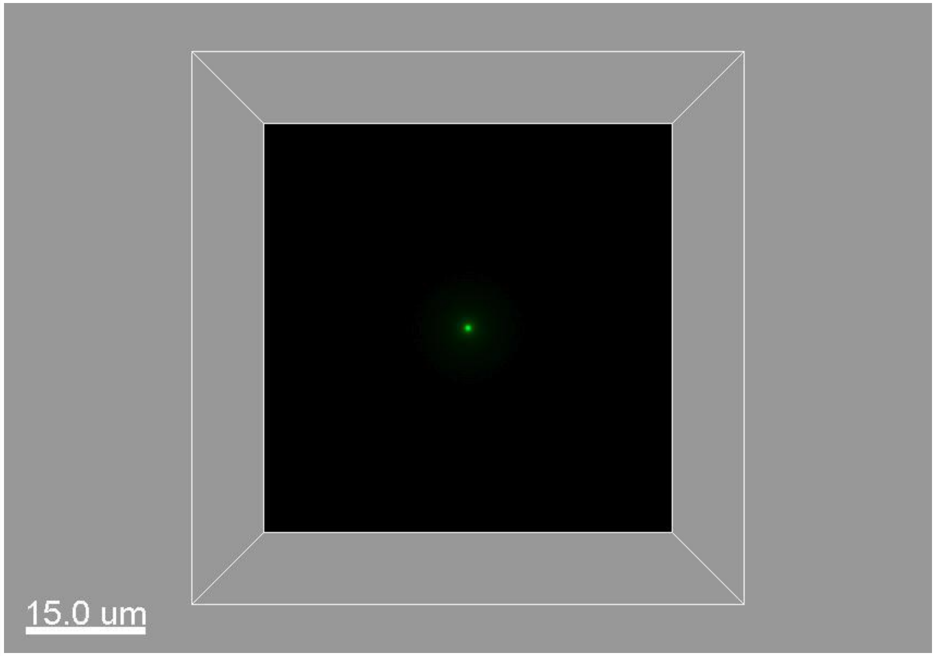
We acquire 3D datasets by **Optical Sectioning Microscopy**



Drawbacks

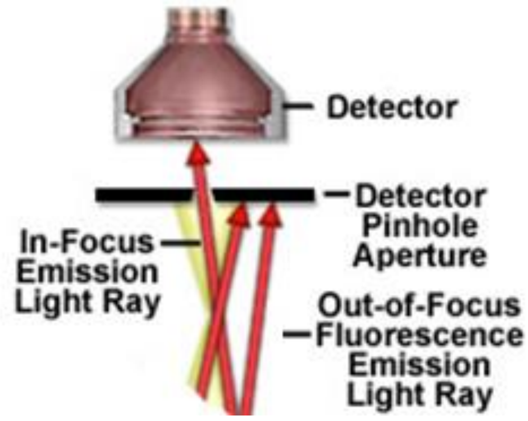
- Out-of-focus adjacent planes contaminate image.
- Bad axial (z) resolution.

A point source that exists in only one focal plane along z spreads to other focal planes: **Point Spread Function (PSF)**

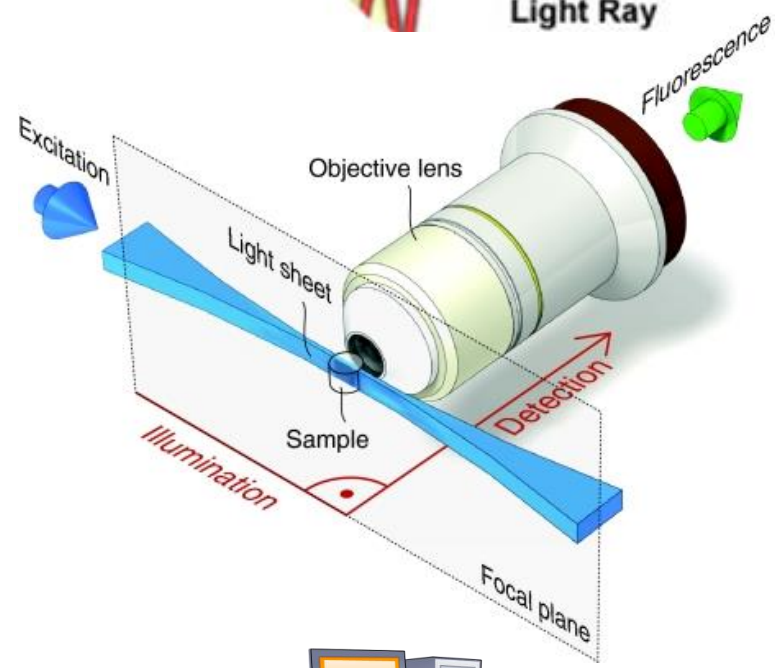


We can alleviate the blur by:

(a) Illuminating the whole sample and using a physical barrier to block rogue light rays. (*Confocal Microscopy*)



(b) Restricting the excitation to the plane of interest alone. (*Light-sheet Microscopy*)



(c) Image processing (*Deconvolution*)

Deconvolution methods assume a shift-invariant model

A shift-invariant system is completely characterized by its response to a point source:

Point source $\delta(x) \longrightarrow \blacksquare \longrightarrow h(x)$ *Point-spread function (PSF)*

Any signal can be represented as a linear combination of many points.

Any signal response can be represented as a linear combination of many PSFs.

$$f(x) \longrightarrow \blacksquare \longrightarrow g(x)$$

$$f(x) = \int f(\xi) \cdot \delta(x - \xi)\mathrm{d}\xi \qquad g(x) = \int f(\xi) \cdot h(x - \xi)\mathrm{d}\xi$$

Fourier Transforms simplify convolutions to multiplications

$$F(\omega) \stackrel{\text{def}}{=} \int_{-\infty}^{\infty} f(\xi) \cdot e^{-j\omega x}\mathrm{d}x \qquad G(\omega) = F(\omega) \cdot H(\omega)$$

Given *f(x)* and *h(x)*, find *g(x)*: convolution problem

Given *g(x)* and *h(x)*, find *f(x)*: **deconvolution** problem

Project Steps

1. **Model the available microscope (Leica DMI 6000B)**
   Determine the PSF characteristic to the microscope in the lab.

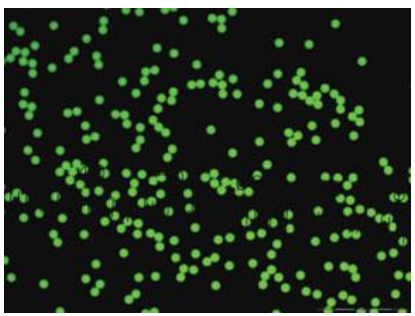
2. **Deconvolve data from Single-View observation**
   Use PSF to deblur 3-D data acquired by the microscope.

3. **Deconvolve data from Multi-View observation**
   Acquire data from multiple angles and perform deblurring using a multi-channel deconvolution algorithm.

1. PSF determination

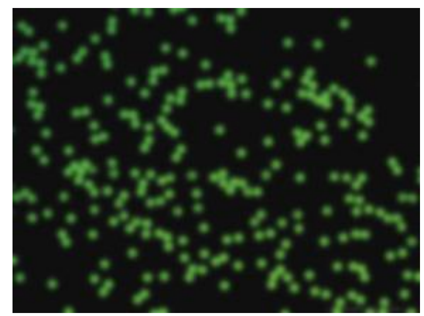
Fluorescent beads having a diameter less than the spatial resolution of the device approximate point sources.

The blurred observation of any single bead hints to the PSF of the microscope.



fluorescent beads

Leica DMI6000b

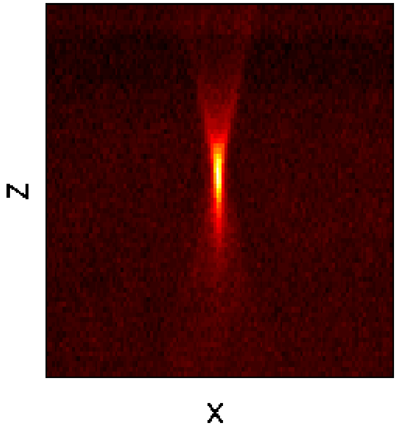


blurred observation

3D PSF Intensity Graphs (*x-z plane*)

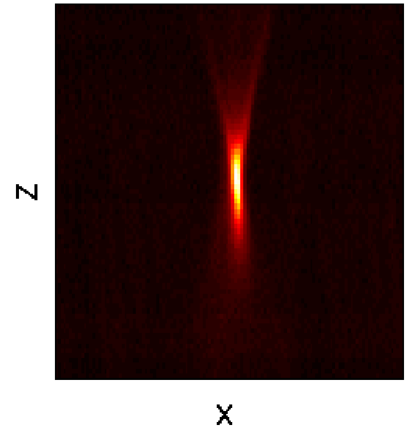
Comparison of measured PSF to theoretical models

A single measured PSF exhibits significant amount of noise.



Measured PSF

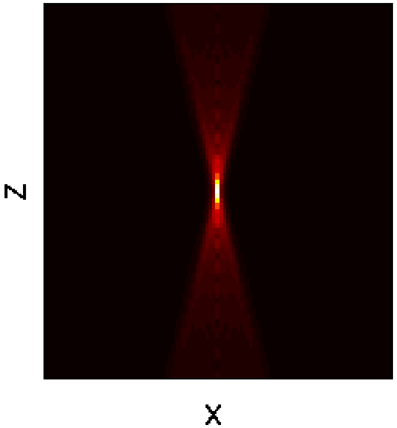
Average over multiple similar data →



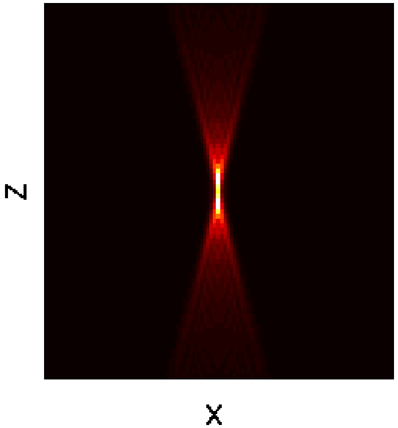
Averaged PSF

Averaging reduces the standard deviation of the noise component, improving signal quality.

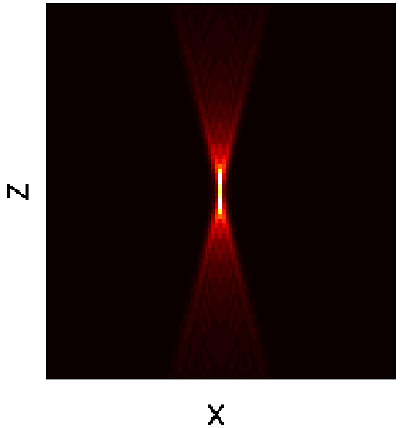
Theoretical models generated according to parameters in experimental setup:



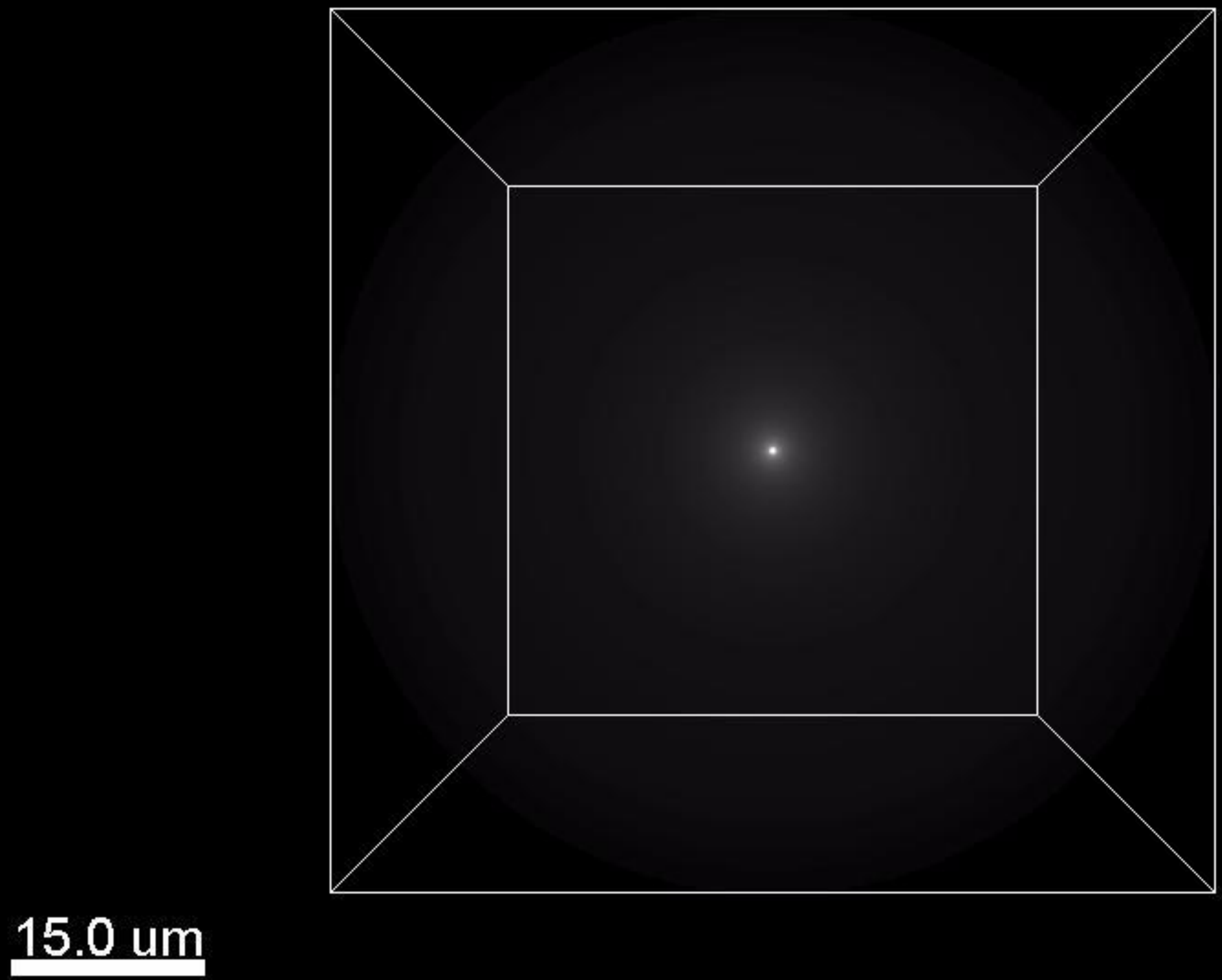
Born & Wolf PSF



Gibson & Lanni PSF

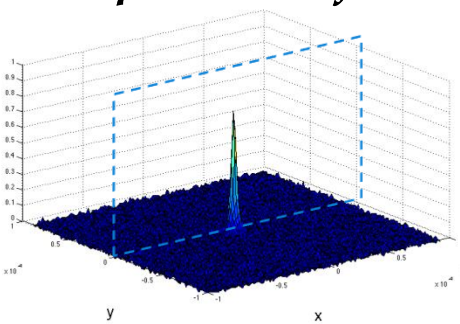


Richards and Wolf PSF

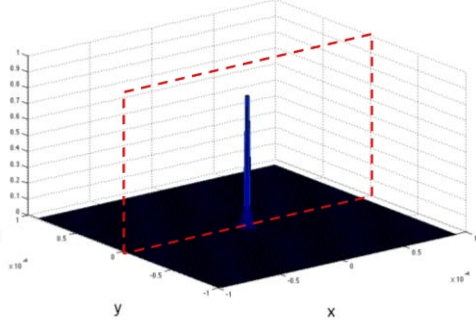

15.0 um

3D PSF Intensity Graphs (*x-y plane)*

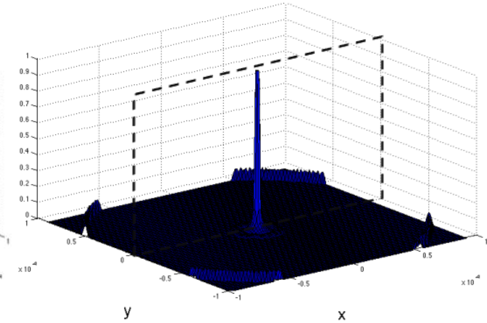
Comparison of measured PSF to theoretical models



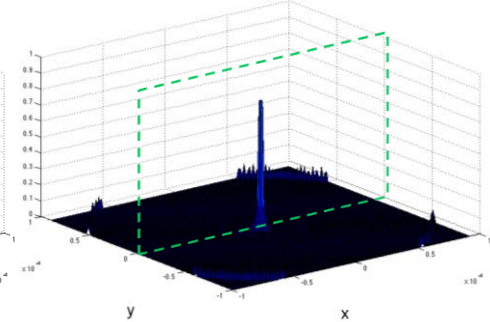
Measured PSF (averaged) in *x*-*y* plane at *z* = 0 μm



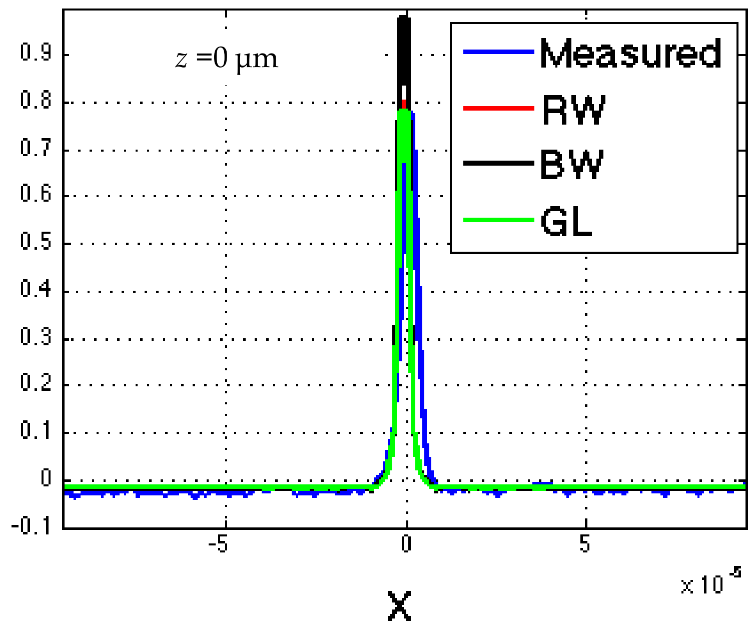
Richards-Wolf (RW) PSF Model in *x*-*y* plane at *z* = 0 μm



Born-Wolf (BW) PSF Model in *x*-*y* plane at *z* = 0 μm

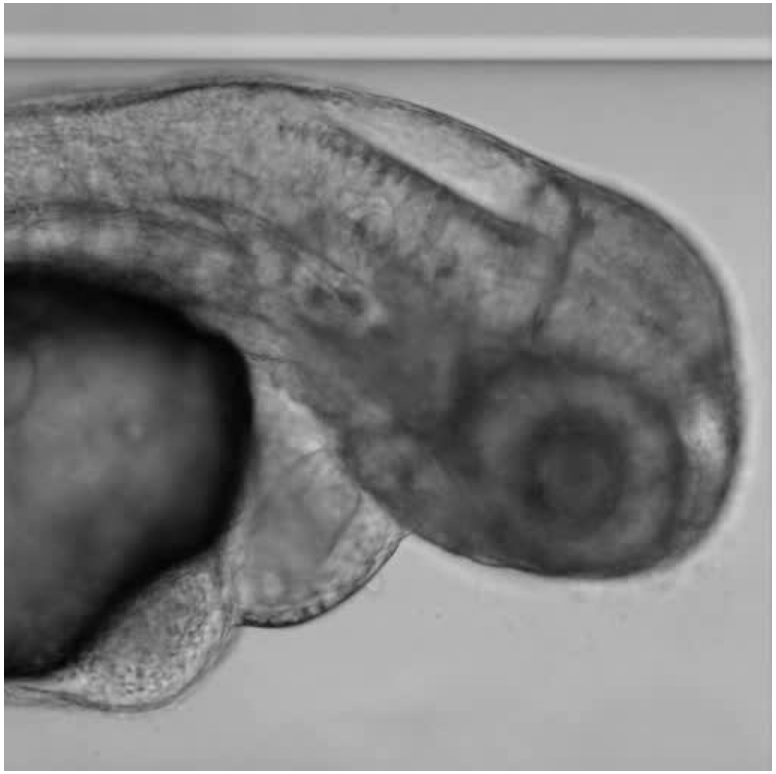


Gibson-Lanni (GL) PSF Model in *x*-*y* plane at *z* = 0 μm

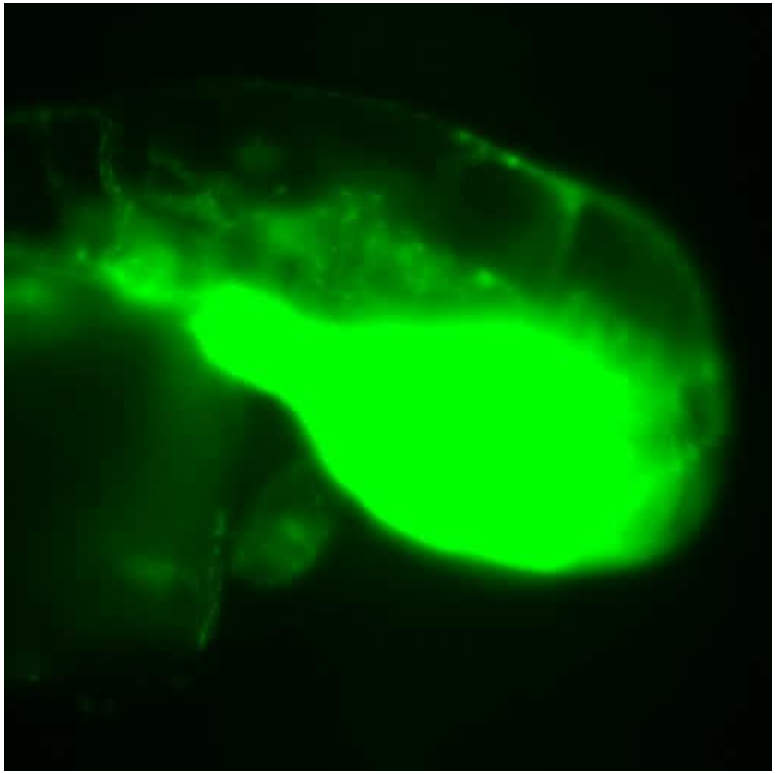


2. Deconvolve data from Single-View observation

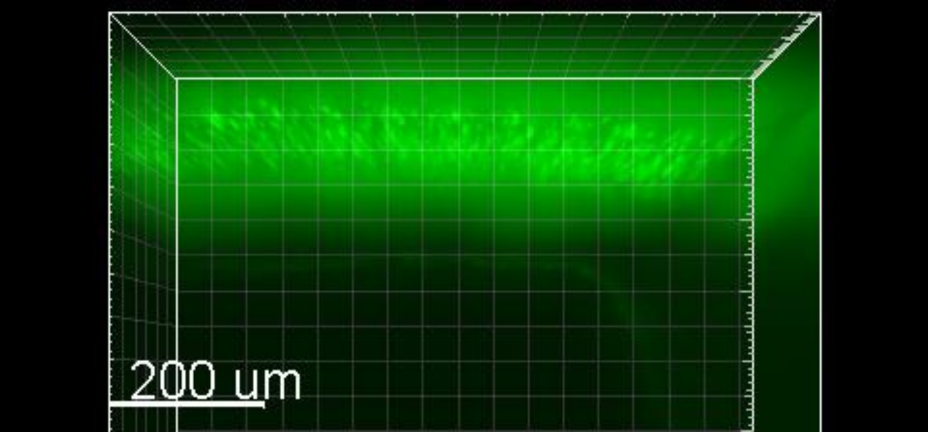
Zebrafish Tg(f1ia:EGFP), fluorescent along the vetebral column, was used in the experimental setup for imaging.



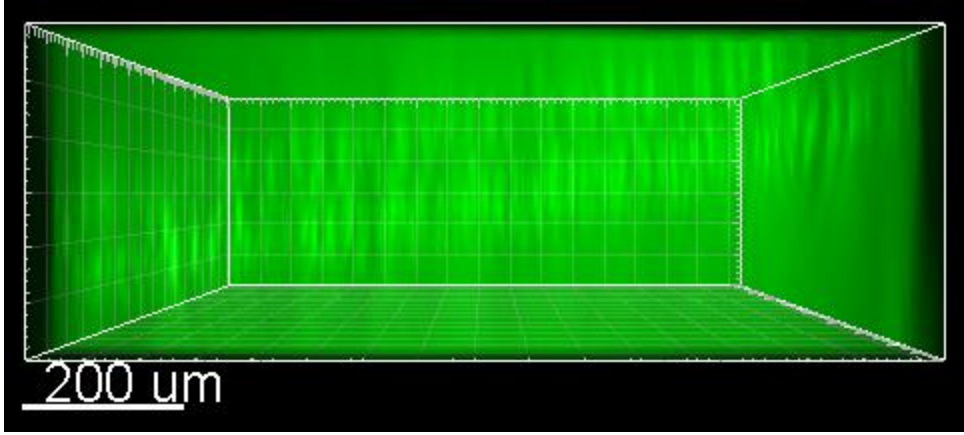
Bright-field Microscopy



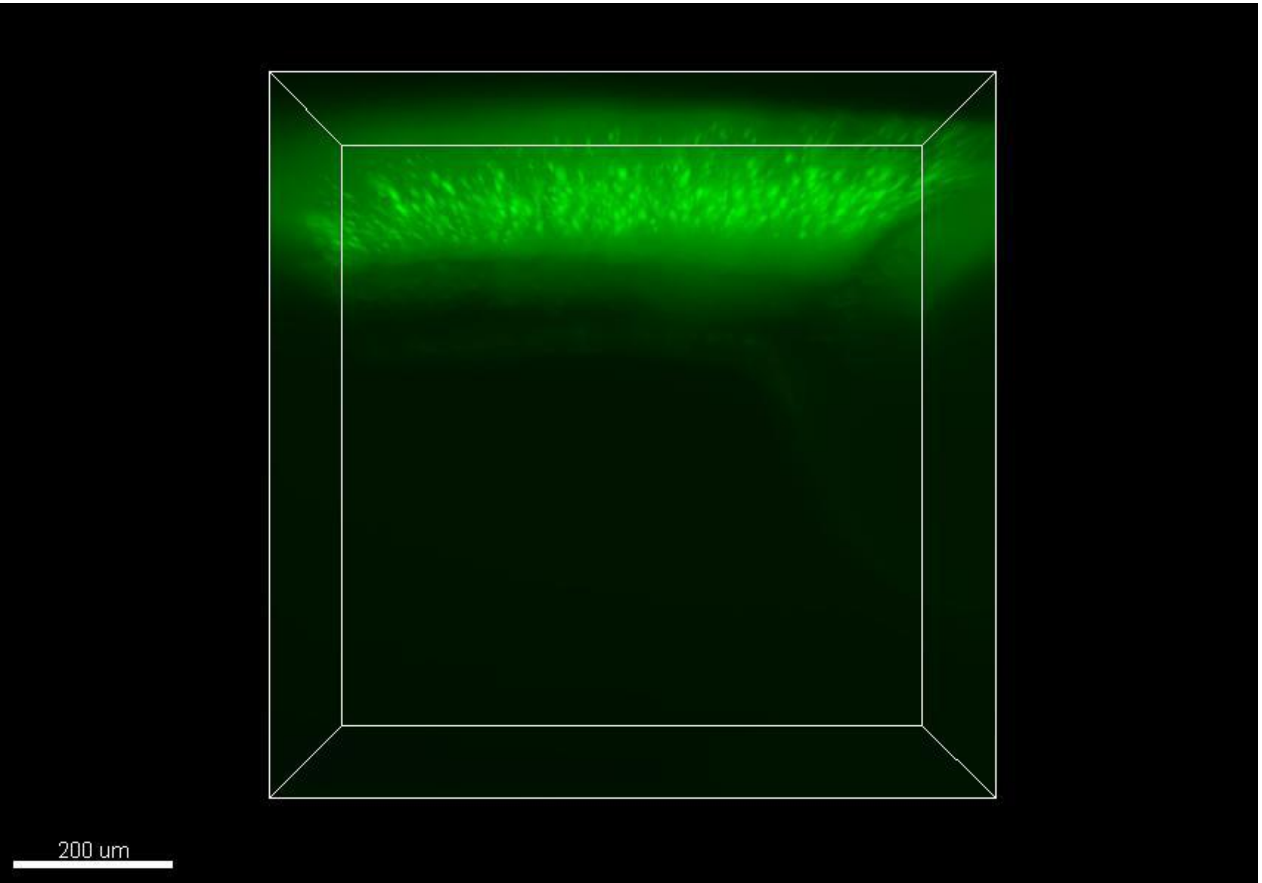
Fluorescence Microscopy



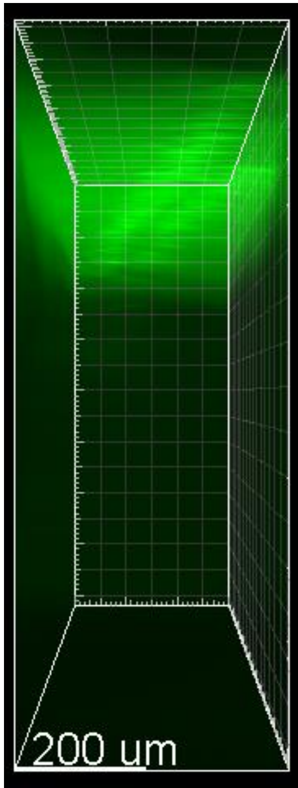
xy-plane



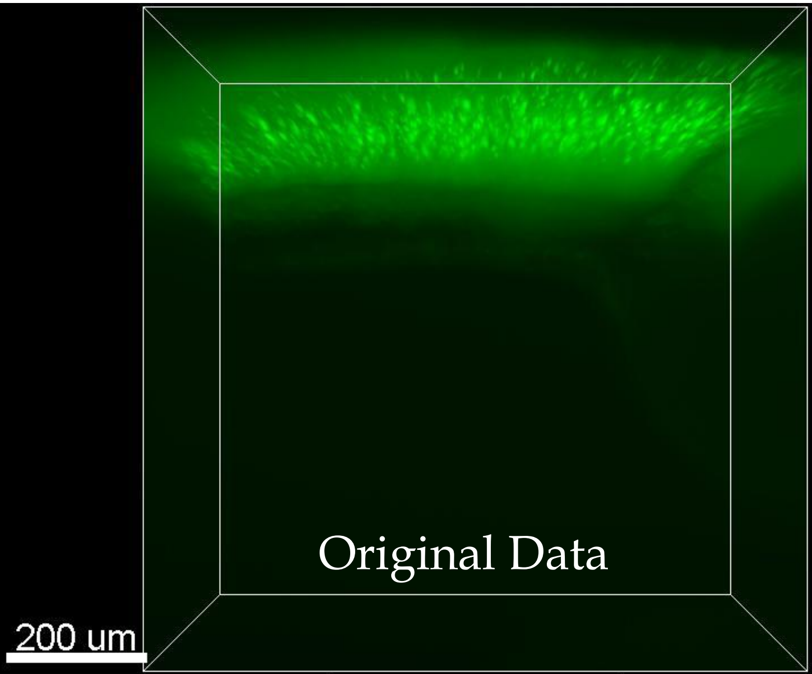
xz-plane

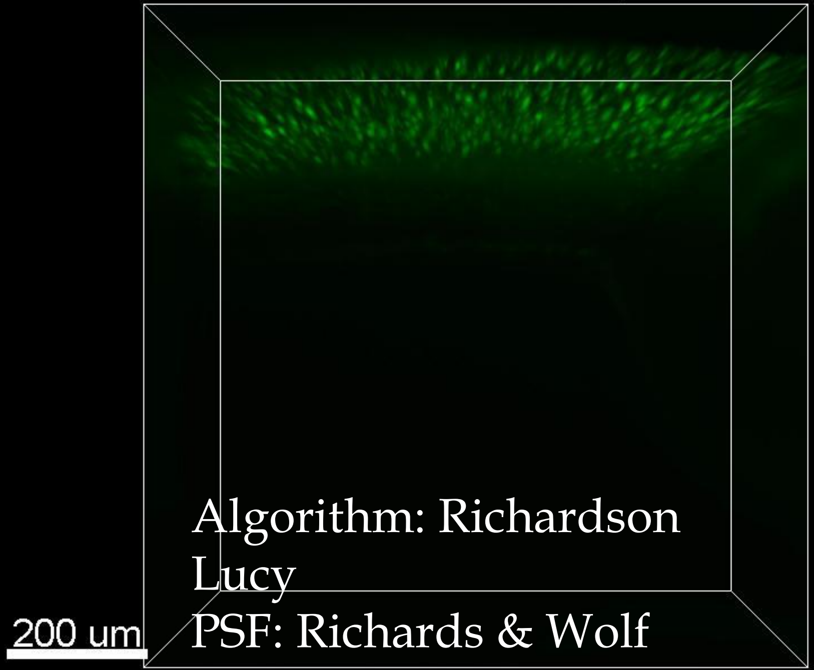


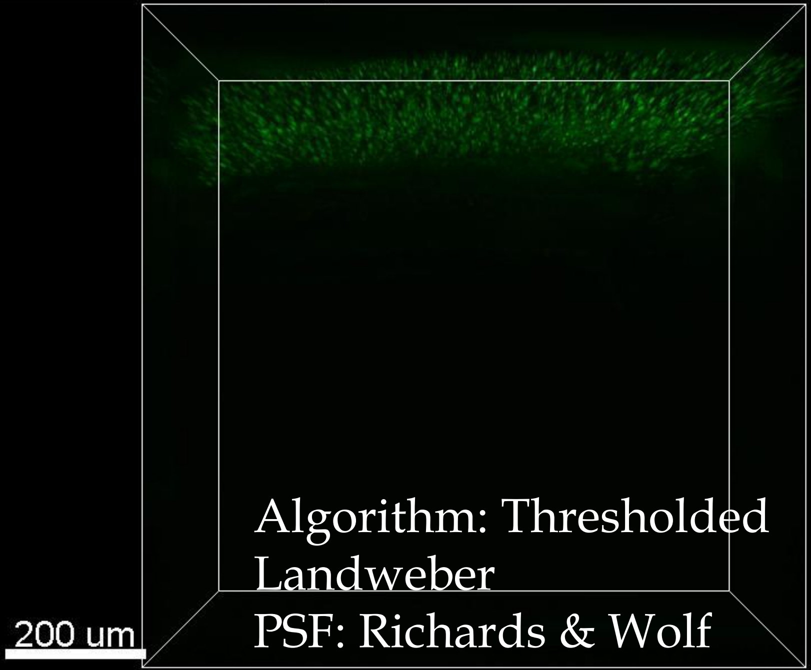
yz-plane



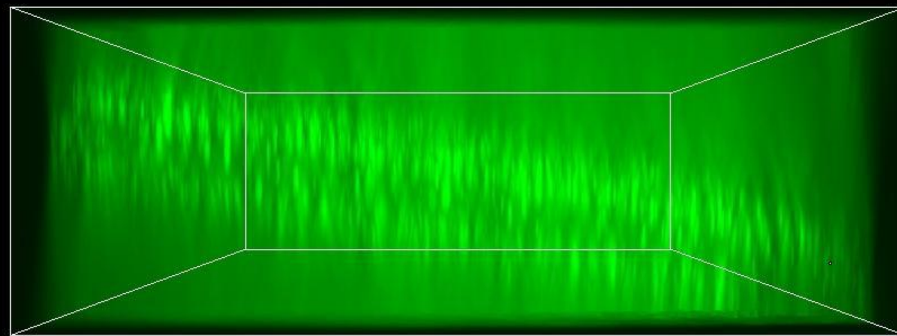
Deconvolution Results – xy-plane





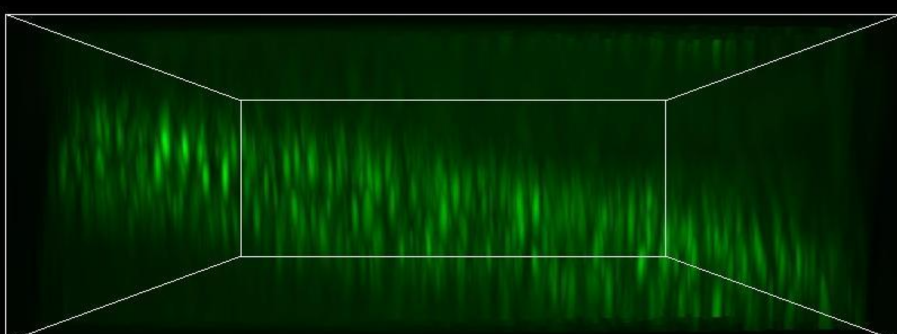


Deconvolution Results- xz-plane



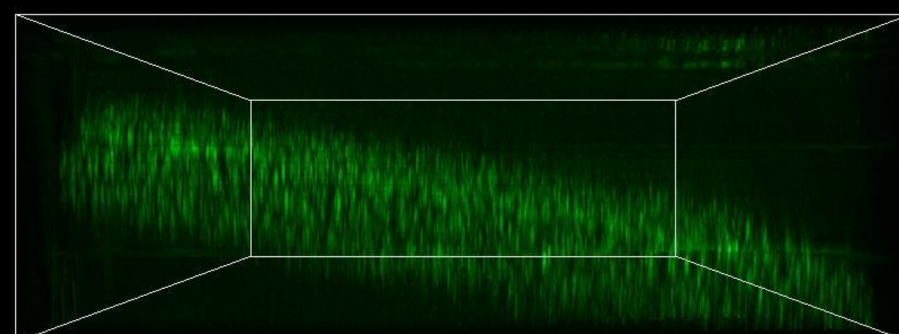
Original Data

200 um



Algorithm: Richardson Lucy
PSF: Richards & Wolf

200 um



Algorithm: Thresholded Landweber
PSF: Richards & Wolf

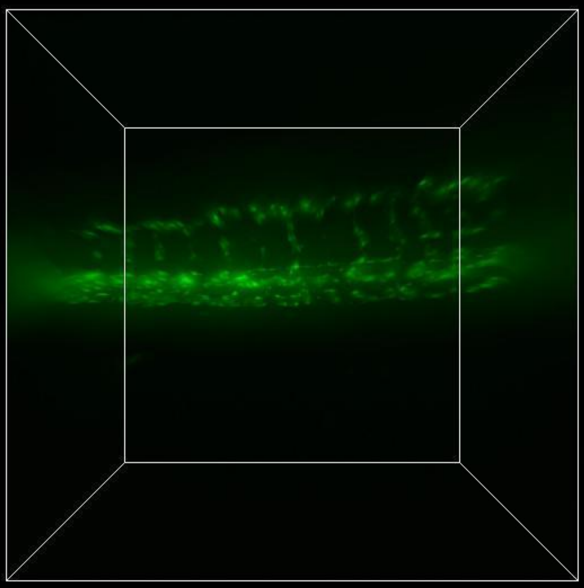
200 um

3. Deconvolve data from Multi-View observation

We place the specimen within a tube that is connected to a stepper motor. The stepper motor is controlled by an Arduino programming board, which is interfaced to Matlab.

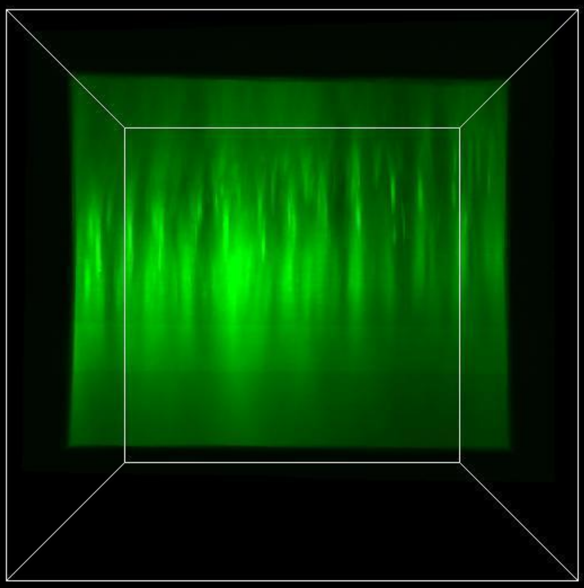
Zebrafish Multi-View - xy-plane

Angle 0
(data is
blurred
along the
z-axis)



200 um

Angle 90
(data is
blurred
along the
y-axis)

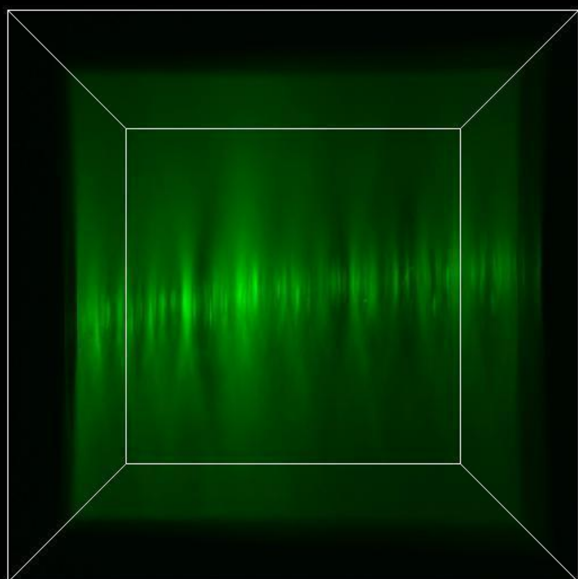


200 um

Zebrafish Multi-View - xz-plane

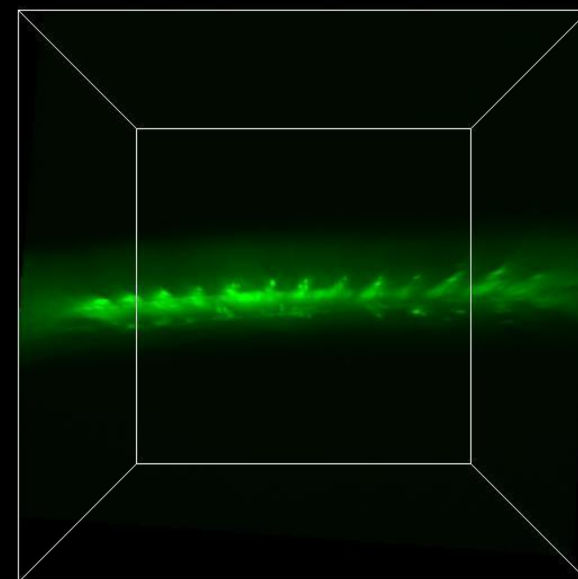
Angle 0
(data is
blurred
along the
z-axis)

200 um



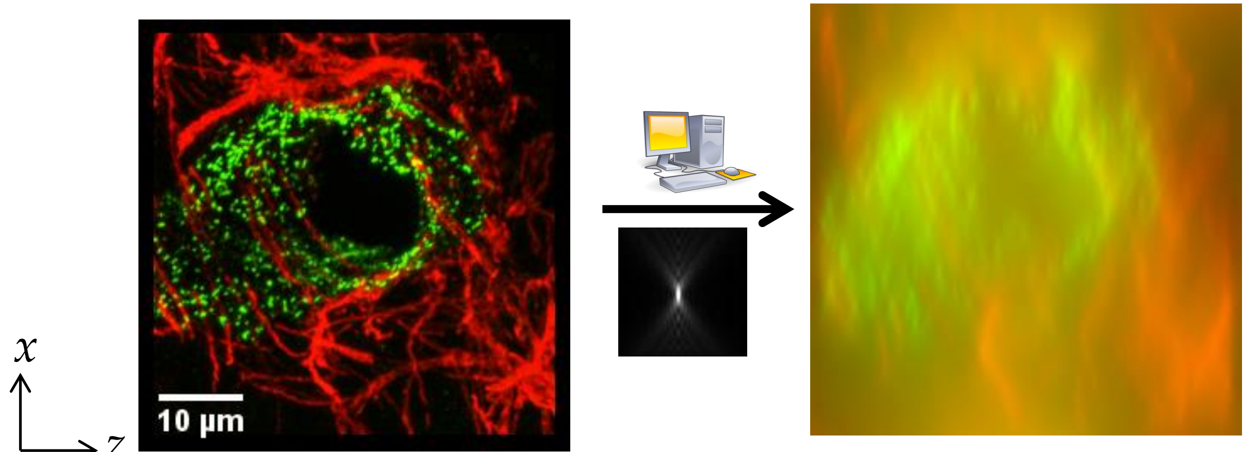
Angle 90
(data is
blurred
along the
y-axis)

200 um



The blur along different directions makes it difficult to spatially register two data sets, making any registration algorithms based on spatial landmarks difficult to use

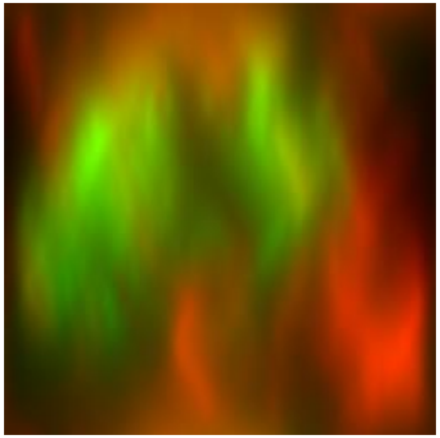
3. Deblur data from Multi-View observation (Simulation Results)



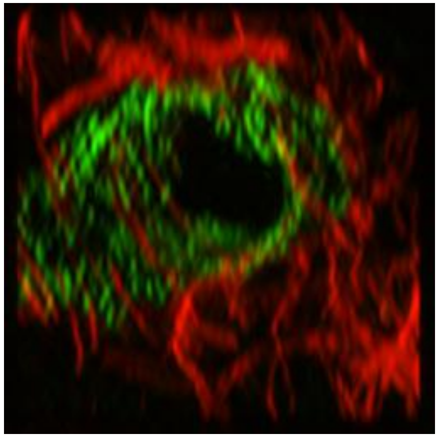
Original Object

Blurred "Observation"

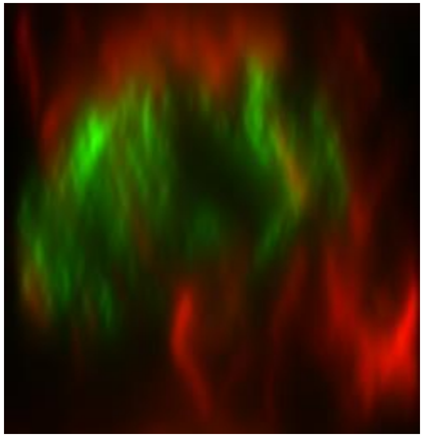
Single-View Deconvolution Results



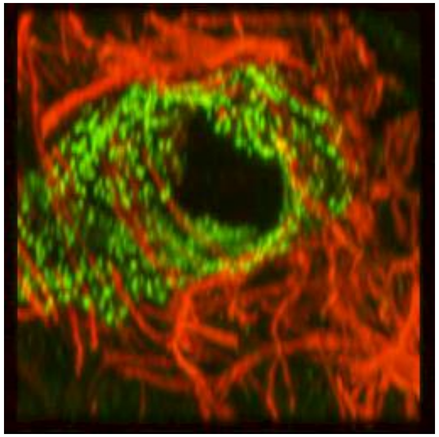
Landweber



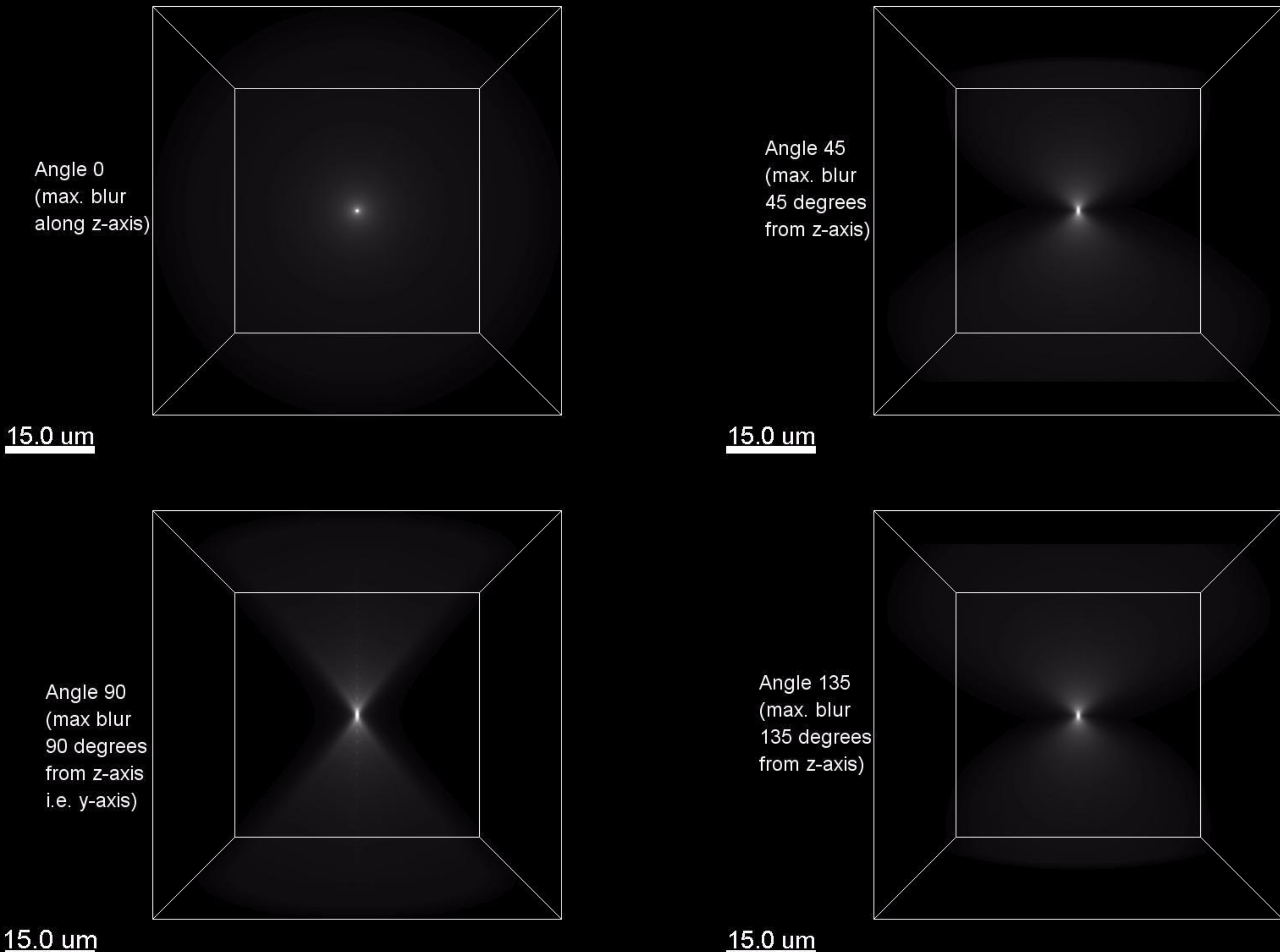
Regularized Inverse Filtering

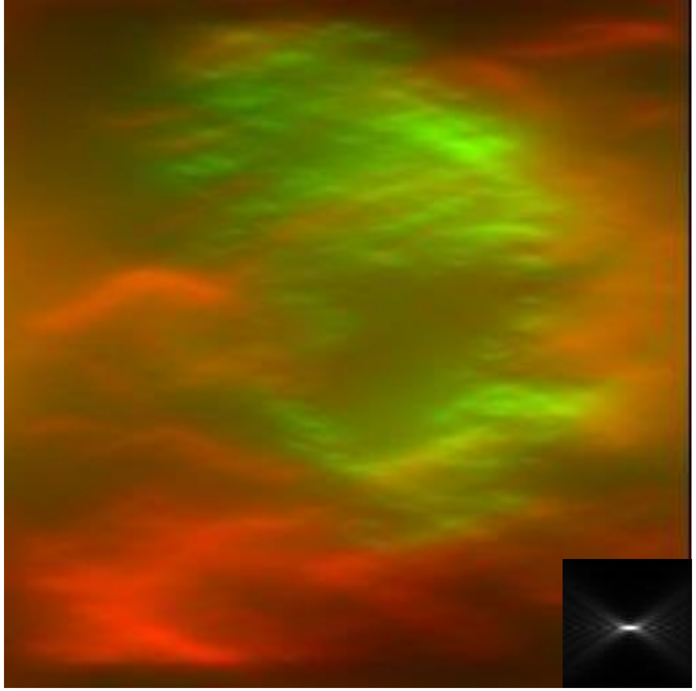
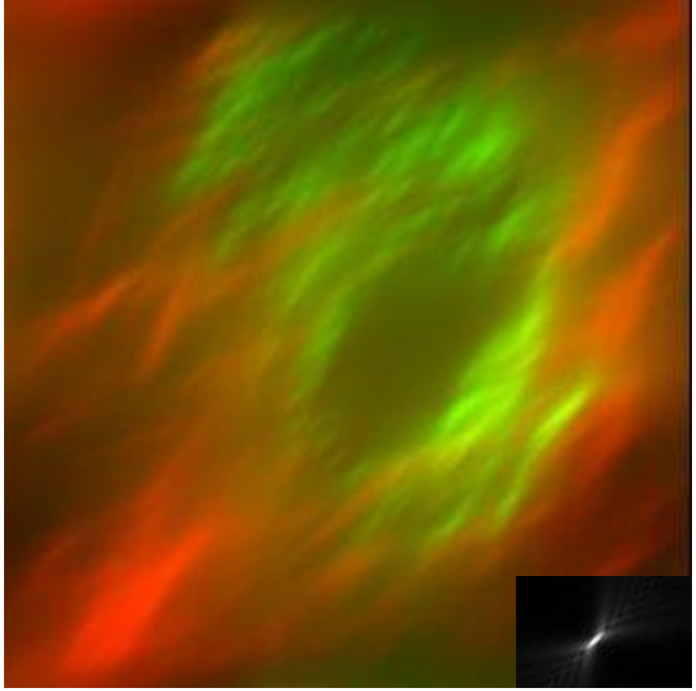
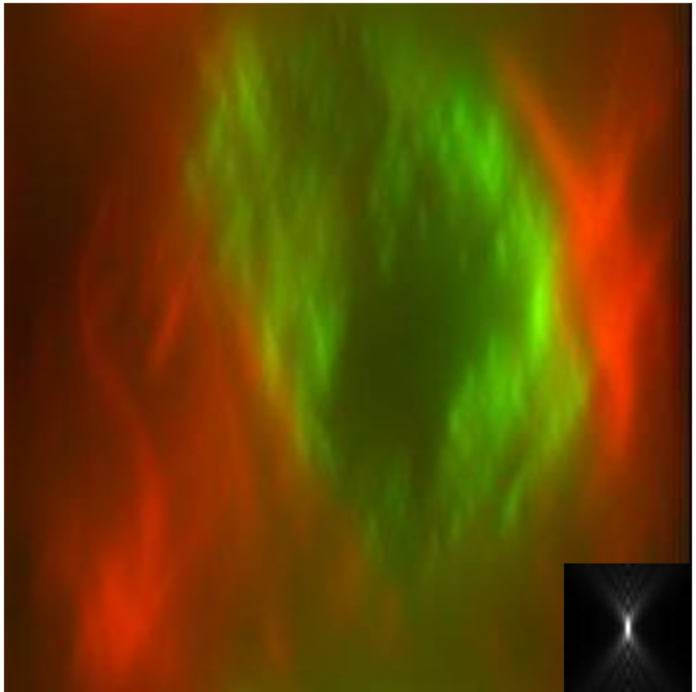
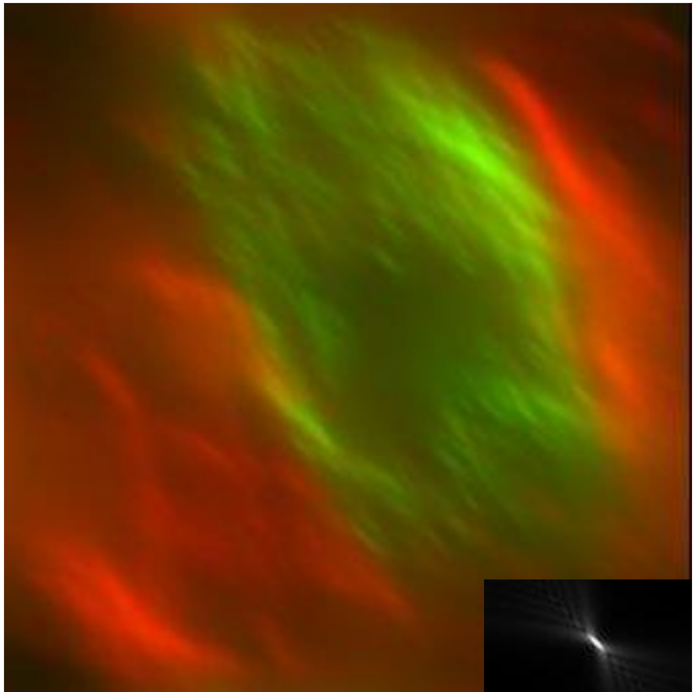


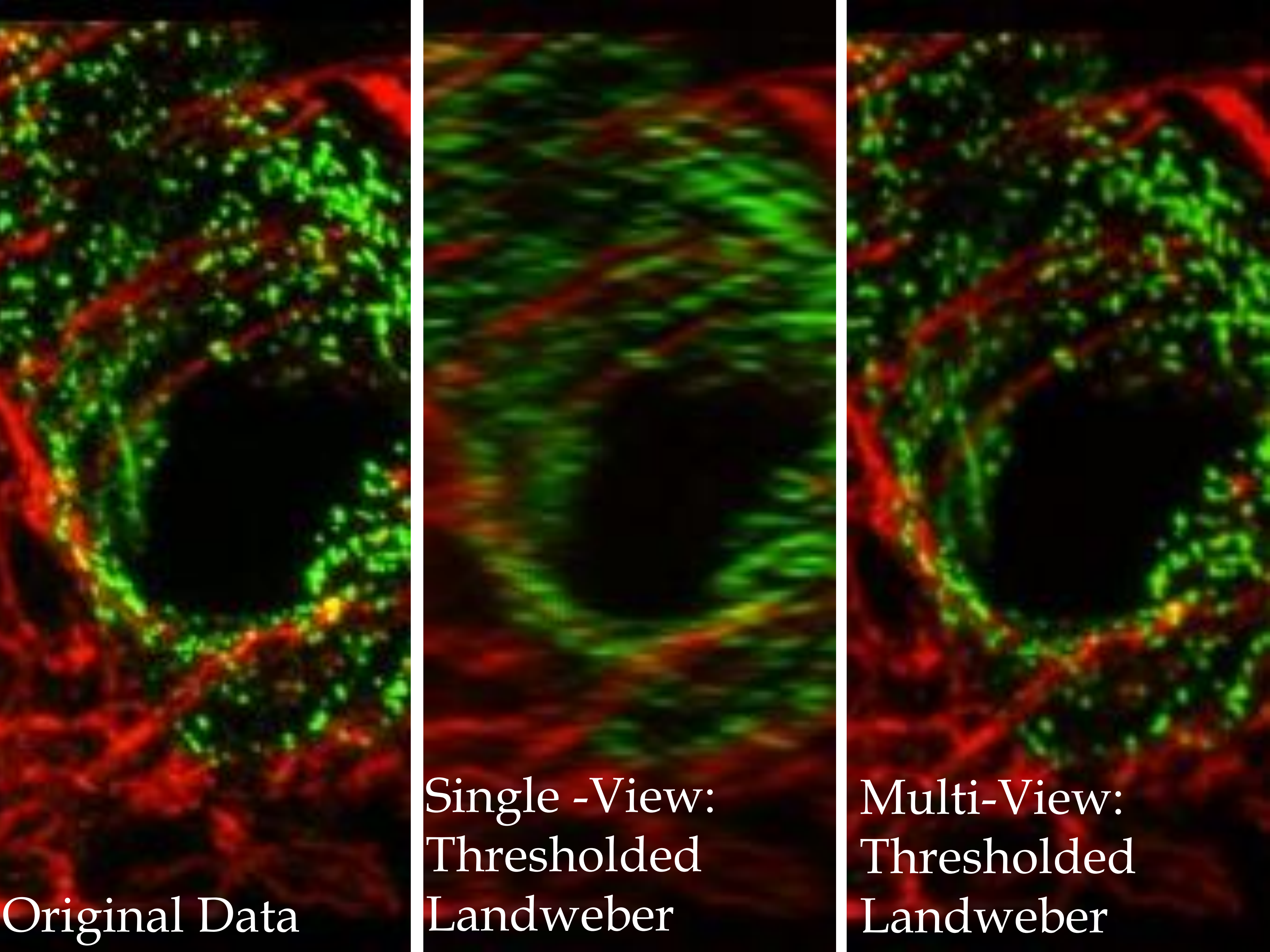
Richardson-Lucy



Thresholded Landweber


Angle 0
(max. blur
along z-axis)
15.0 um
Angle 45
(max. blur
45 degrees
from z-axis)
15.0 um
Angle 90
(max blur
90 degrees
from z-axis
i.e. y-axis)
15.0 um
Angle 135
(max. blur
135 degrees
from z-axis)
15.0 um


Original Data
Single -View:
Thresholded
Landweber
Multi-View:
Thresholded
Landweber

Conclusions and Future Prospective

(a) The theoretical PSF models generated according to the instrument and experimental parameters were close approximations to the actual measured data.

(b) Deconvolved results using the theoretical PSF models were observed to be superior (less noisier) than that with the measured PSF.

(c) The faithful registration of actual 3D datasets blurred along different angles still remains a problem that is unsolved, though the simulation results for the multi-angle deconvolution look promising.

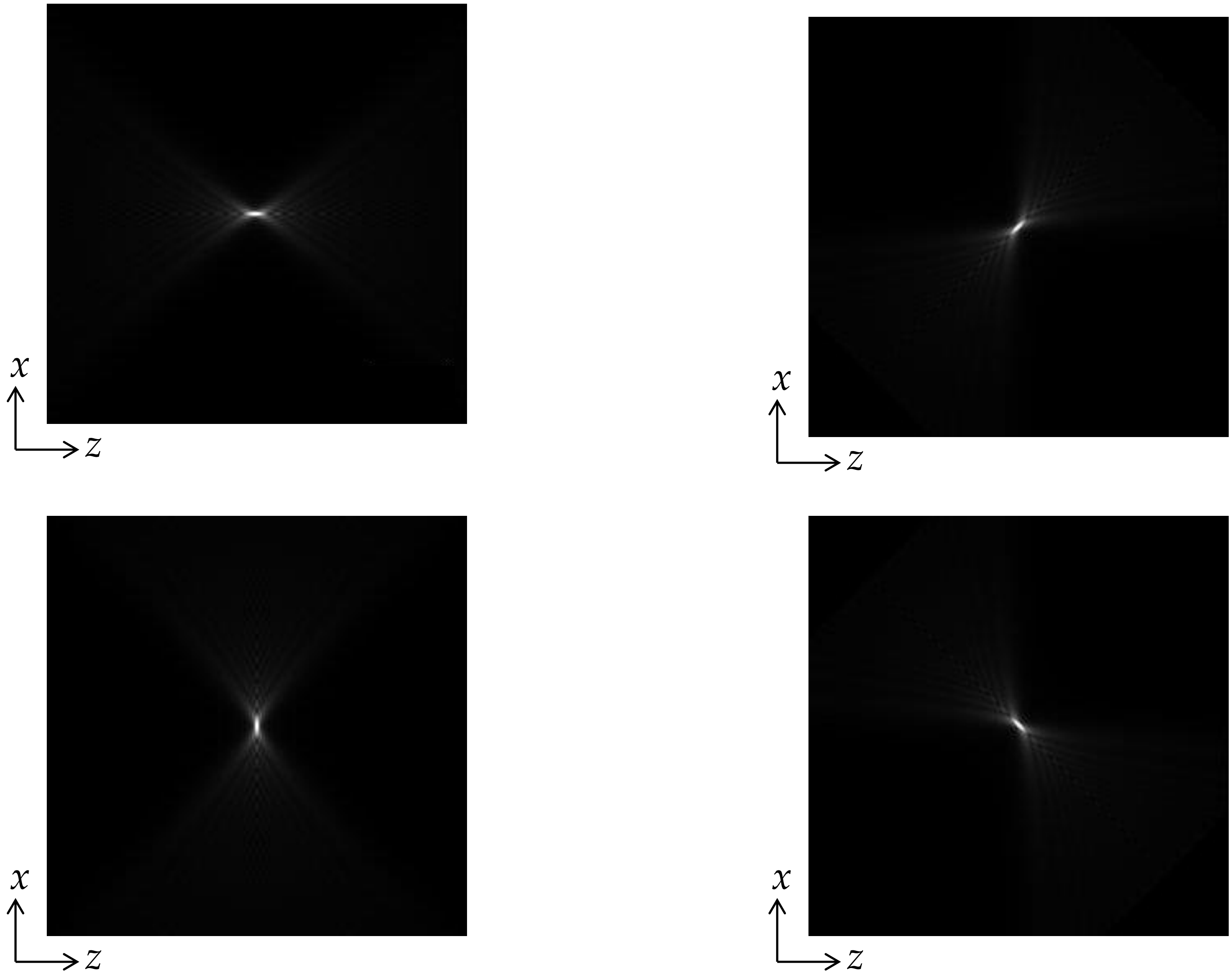
Thank You

•INSET - Internships in Nanosystems Science, Engineering and Technology

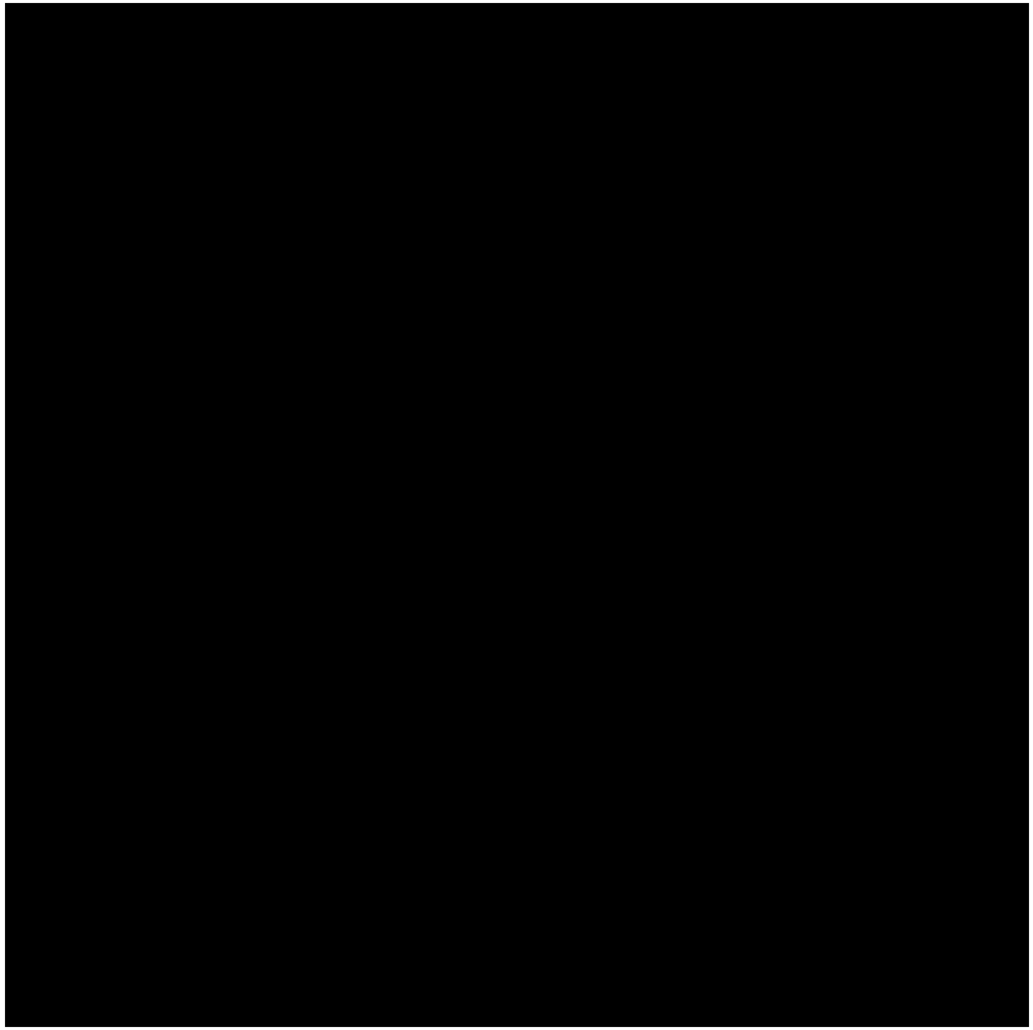
•Systems Bioimaging Lab

Dr. Michael Liebling
Nikhil Chacko
Kevin Chan
Michael Lee

Appendix

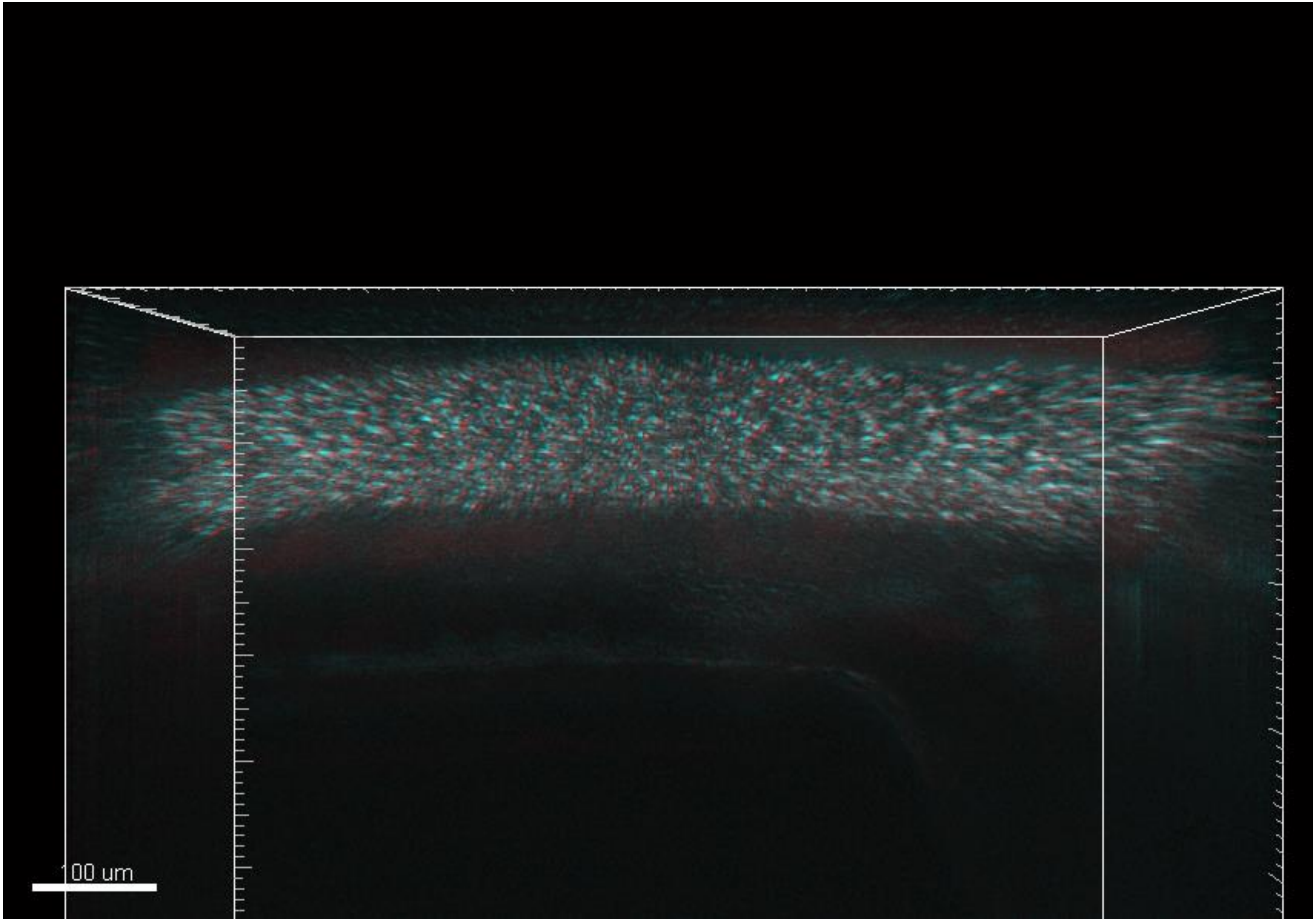

x
z
x
z
x
z
x
z

Zebrafish – Z-Stack



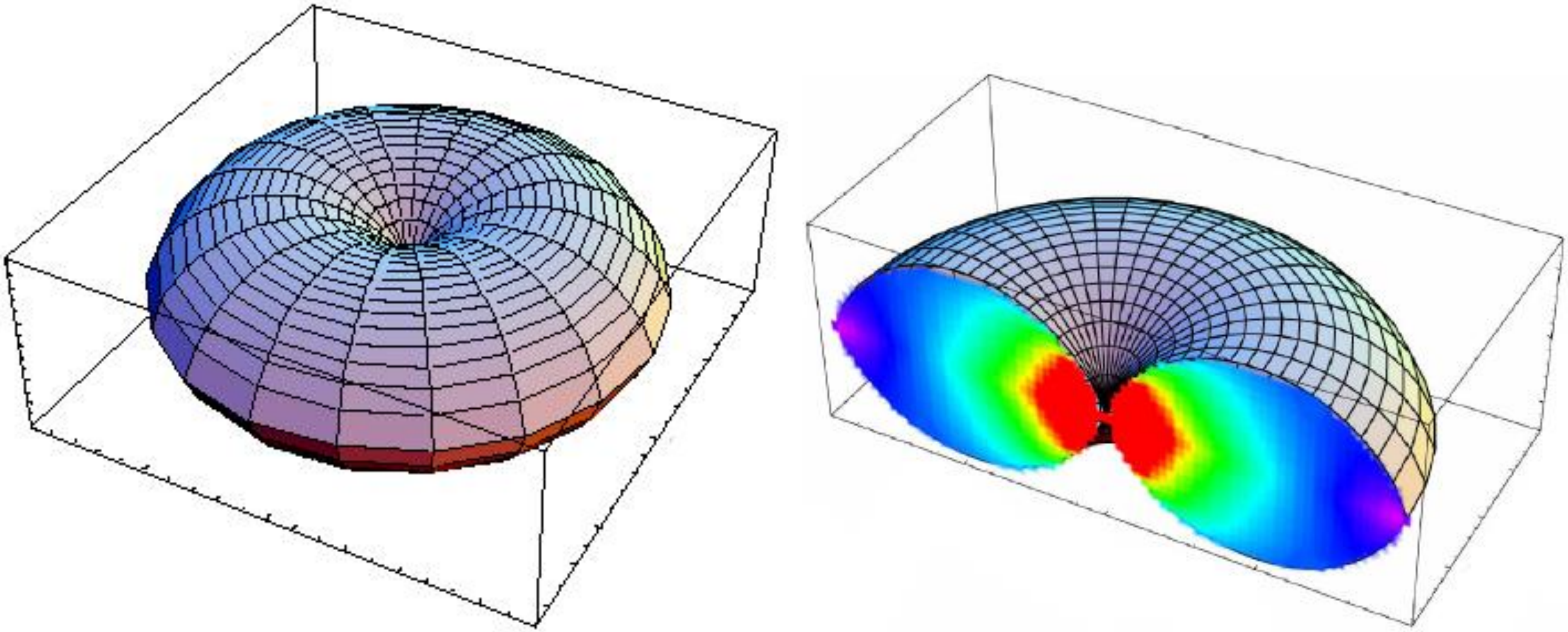
Zebrafish Single-View Deconvolution Results

Measured VS Theoretical PSF

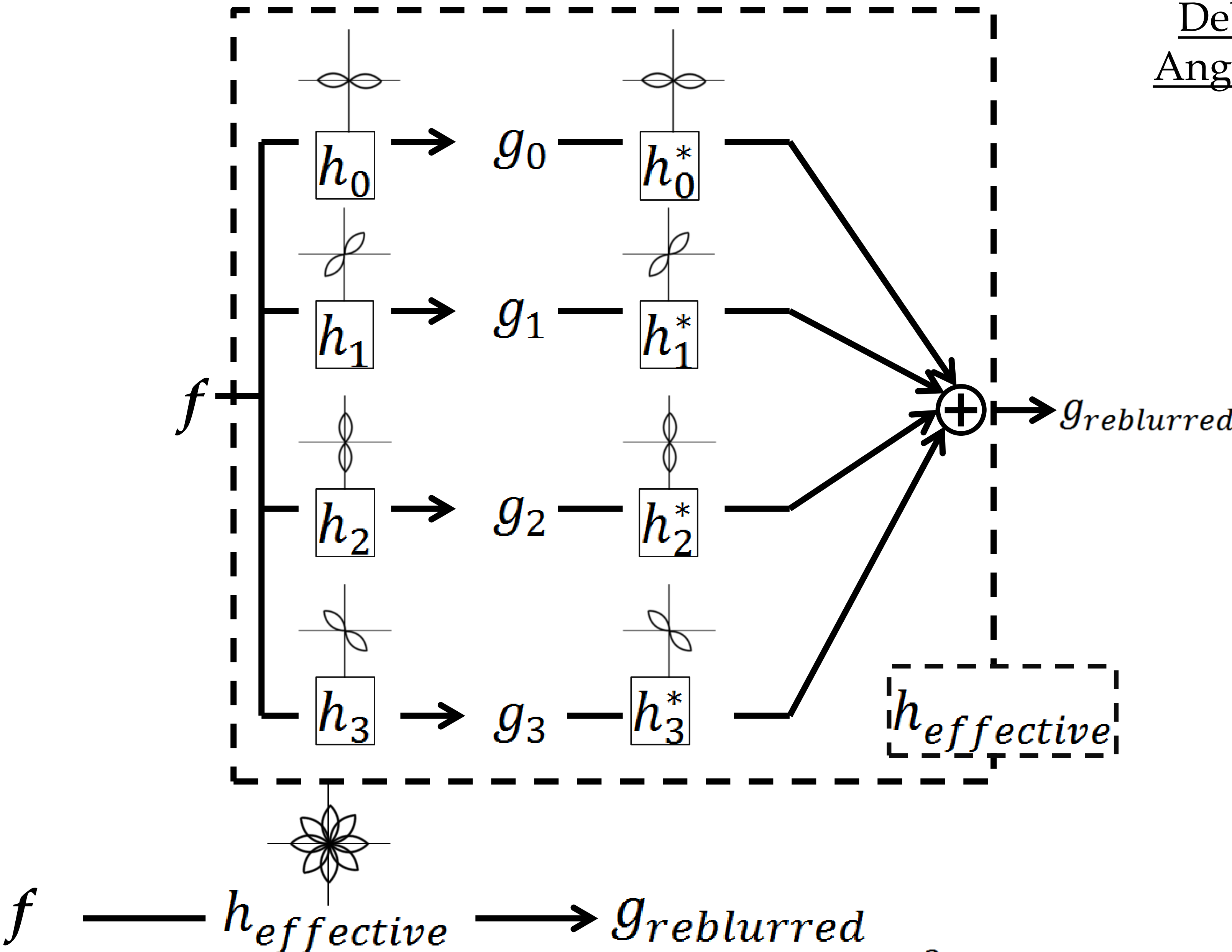


Method	Signal Estimate	Comments
Inverse Filtering	$\underset{\sim}{f}_e = \arg\min \| \underset{\sim}{g} - \underset{\sim}{H} \cdot \underset{\sim}{f} \|_2^2$	• Linear filtering operation • Amplifies noise when H(ω)⟶ 0
Landweber	$\underset{\sim}{f}_e = \arg\min \| \underset{\sim}{g} - \underset{\sim}{H} \cdot \underset{\sim}{f} \|_2^2$	• Iterative • Doesn't amplify noise
Regularized Inverse Filtering	$f_e = \arg\min \| \underset{\sim}{g} - \underset{\sim}{H} \cdot \underset{\sim}{f} \|_2^2 + \lambda \| \gamma \cdot f \|_2^2$	• Tries to smoothen image in addition to inverse filtering
Wiener Filtering	$f_e = \arg\min E \mid F(\omega) - F_e(\omega) \mid^2$	• Linear filtering operation • Used in noisy cases
Richardson-Lucy	$f_e = \arg\max$ $P(f \mid g) = \dfrac{P(g \mid f) \cdot P(f)}{\int P(g \mid f) \cdot P(f) \cdot df}$	• Assumes that input is Poisson distributed (appropriate for photon noise in data) • Developed from Bayes' Theorem
Thresholded Landweber (TL)	$\underset{\sim}{f}_e = \arg\min \| \underset{\sim}{g} - \underset{\sim}{H} \cdot f \|_2^2 + \lambda \| \underset{\sim}{W} \cdot \underset{\sim}{f} \|_1^1$	• Assumes wavelet coefficients of the data to be estimated are sparse.
Multi-Channel Thresholded Landweber	$\underset{\sim}{f}_e = \arg\min \sum_{i=0}^{M-1} \| \underset{\sim}{g}_i - \underset{\sim}{H}_i \cdot f \|_2^2 + \lambda \| \underset{\sim}{W} \cdot \underset{\sim}{f} \|_1^1$	• Extension of TL to multi-channel framework.

PSF- Fourier Transform Representation



Deblur Multi-Angle(Channel) Image



$$C(f) = \sum_{i=0}^{3} ||g_i - H_i \cdot f||_2^2 + \lambda ||W \cdot f||_1^1$$