Improving Resolution in Wide-field Fluorescence Microscopy Using Deconvolution Techniques

Charlene Cuellar[1], Nikhil Chacko[2], Dr. Michael Liebling[2]
[1]Contra Costa Community College, San Pablo, CA
[2]Systems Bioimaging Lab, Department of Electrical and Computer Engineering, University of California, Santa Barbara

A. Motivation

- Fluorescence microscopy enables the study of specific regions of interest in live cell imaging.

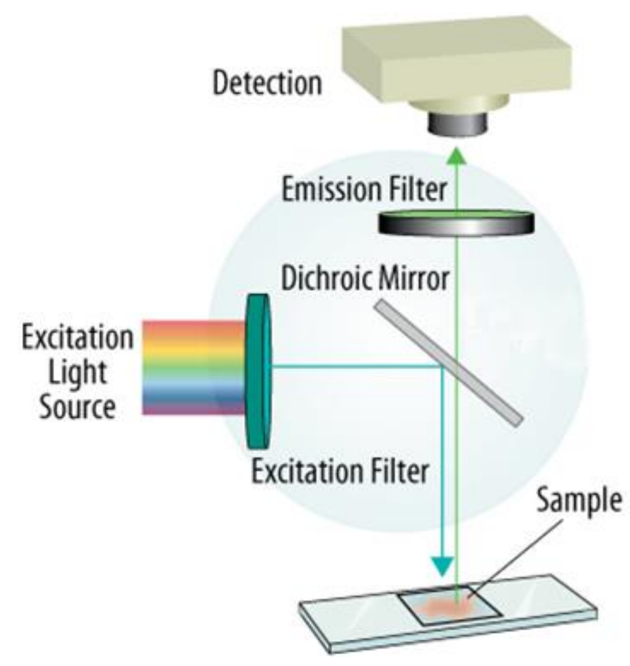


FIG. 1: Fluorescence imaging schematic

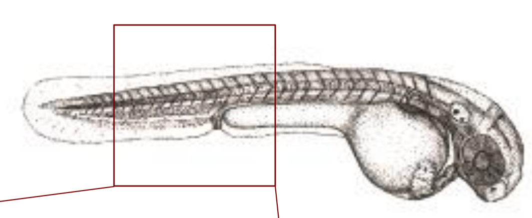
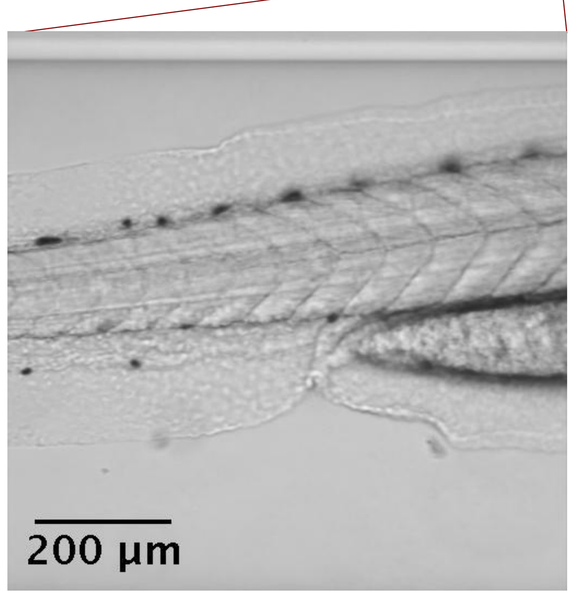
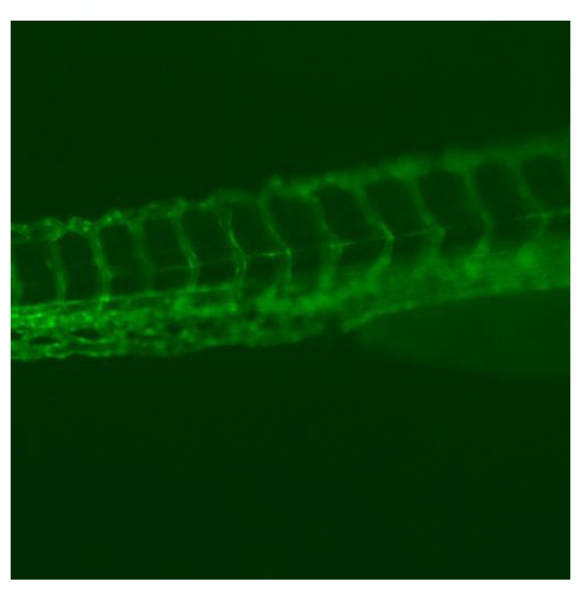


FIG. 2: (a) Zebrafish embryo



(b) Bright-field Microscopy



(c) Fluorescence Microscopy

- Optical Sectioning
3D data stacks are obtained by acquiring multiple 2D images (xy) at different focal planes along the optical axis (z).

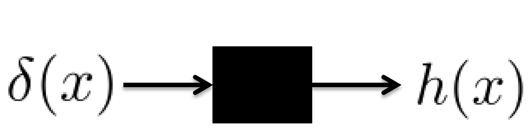
- Drawback of optical sectioning
Objects outside each focal plane being imaged also receive illumination and become fluorescent, undesirably interfering with the imaging of all focal planes. This manifests as blurring along the z-axis during 3D data reconstruction.

- Pursued solution
Use a computational technique (deconvolution) to reverse the blurring process.

B. Deconvolution: problem statement

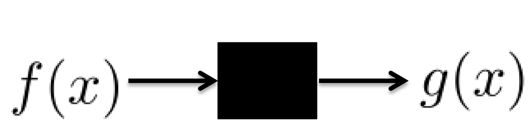
- A linear shift-invariant system is completely characterized by its response to a point source.

Point source



Point-spread function (PSF)

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



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

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

- Fourier Transforms simplify convolutions to multiplications

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

- Given *g(x)* [blurred output] and *h(x)* [PSF], find *f(x)* [original object]: **deconvolution** problem

C. Project Outline

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 observations
Acquire data from multiple angles and perform deblurring using a multi-channel deconvolution algorithm.

C1. PSF Measurement

1. PSF Measurement
- 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.

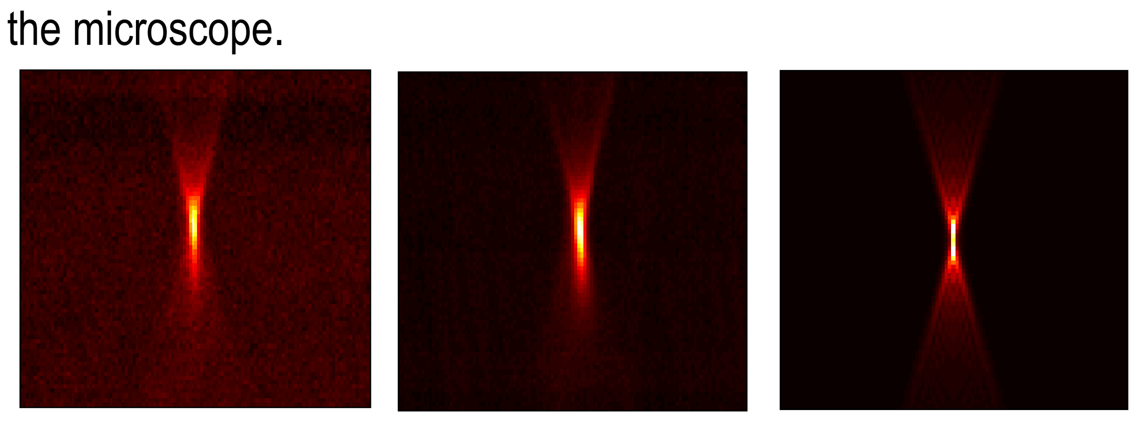


FIG. 3: The xz-plane views of (a) the measured PSF, (b) the average of 10 measured PSFs with less noise content and (c) the Richards and Wolf PSF model generated.

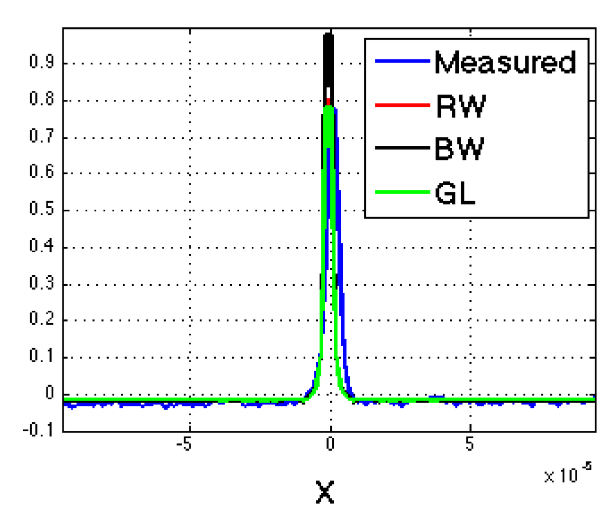
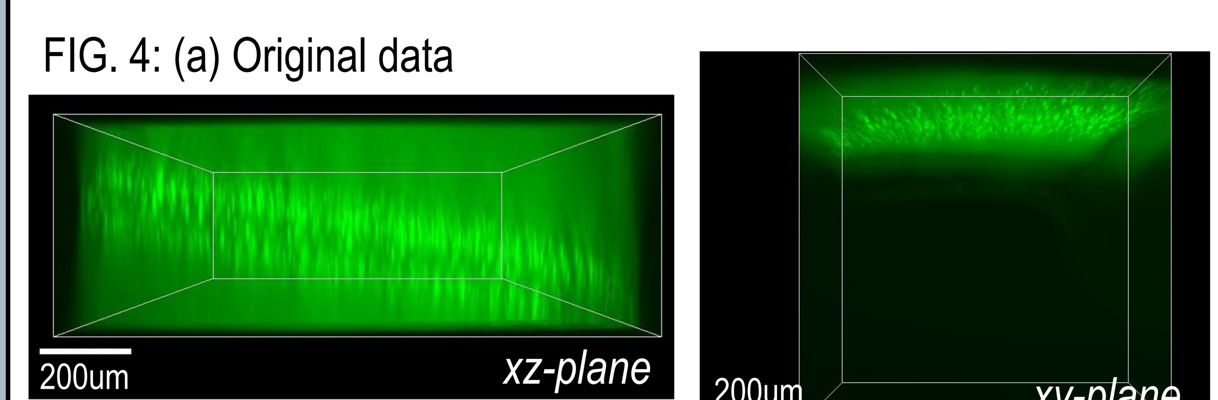


FIG. 4: The PSF intensity plots at the cross section y=0, z=0 of (a) measured, (b) Richards and Wolf (RW), (c) Born & Wolf (BW) model and (d) Gibson and Lanni (GL) PSF models

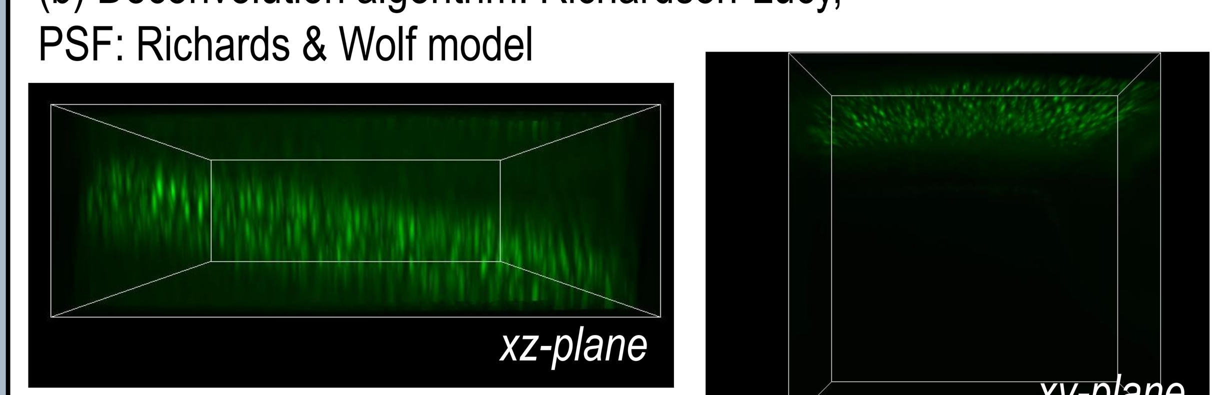
- The measured PSF exhibit a close proximity to the theoretical models generated, except for their amplitude.
- Their similarity advocates the use of theoretical PSF models, which are free of noise, for deconvolution algorithms.

C2. Single-view Deconvolution

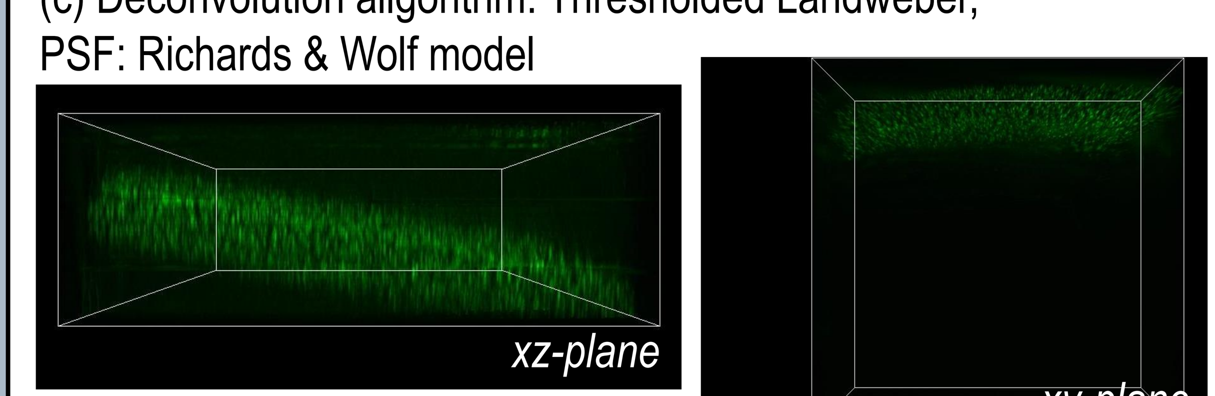
FIG. 4: (a) Original data



(b) Deconvolution algorithm: Richardson-Lucy, PSF: Richards & Wolf model



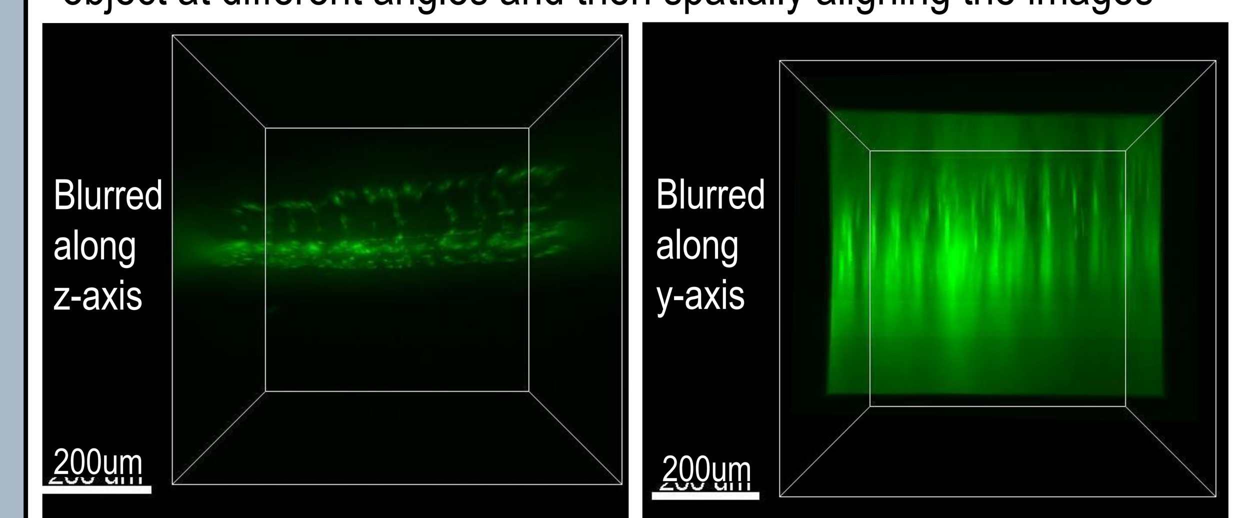
(c) Deconvolution allgorithm: Thresholded Landweber, PSF: Richards & Wolf model



The deconvolution algorithm reduces the blur both in the lateral planes (xy) and the axial direction (z) in our model system, the zebrafish.

C3. Multi-view Deconvolution

FIG .5: Two observations (xy-plane) of the zebrafish blurred along different directions. Since a single-view observation loses information along an axis, it can be compensated by imaging the object at different angles and then spatially aligning the images



- Though spatial registration of observations blurred at different angles is an unsolved problem, simulated results prove to be superior than single-view deconvolution results.

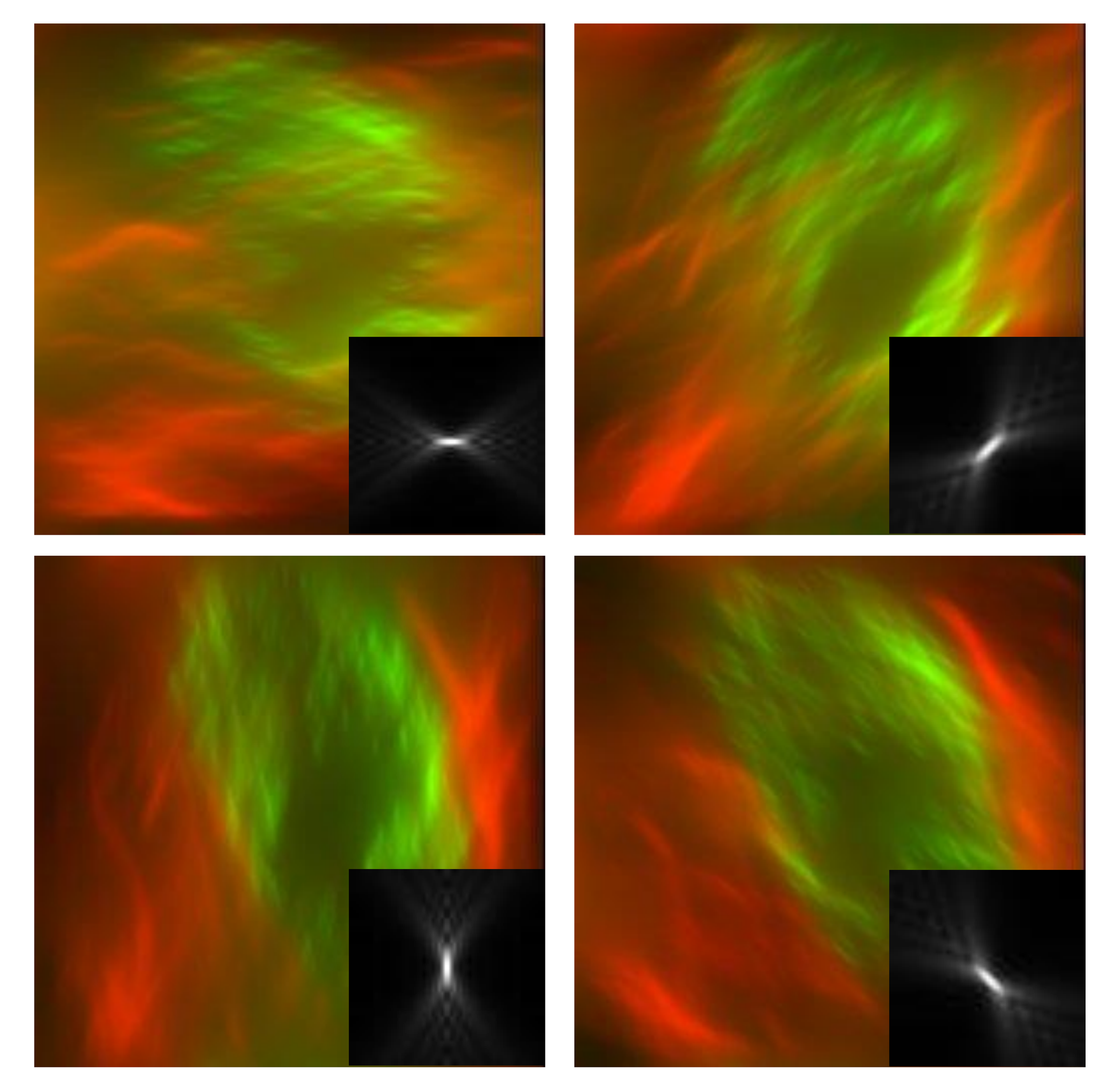
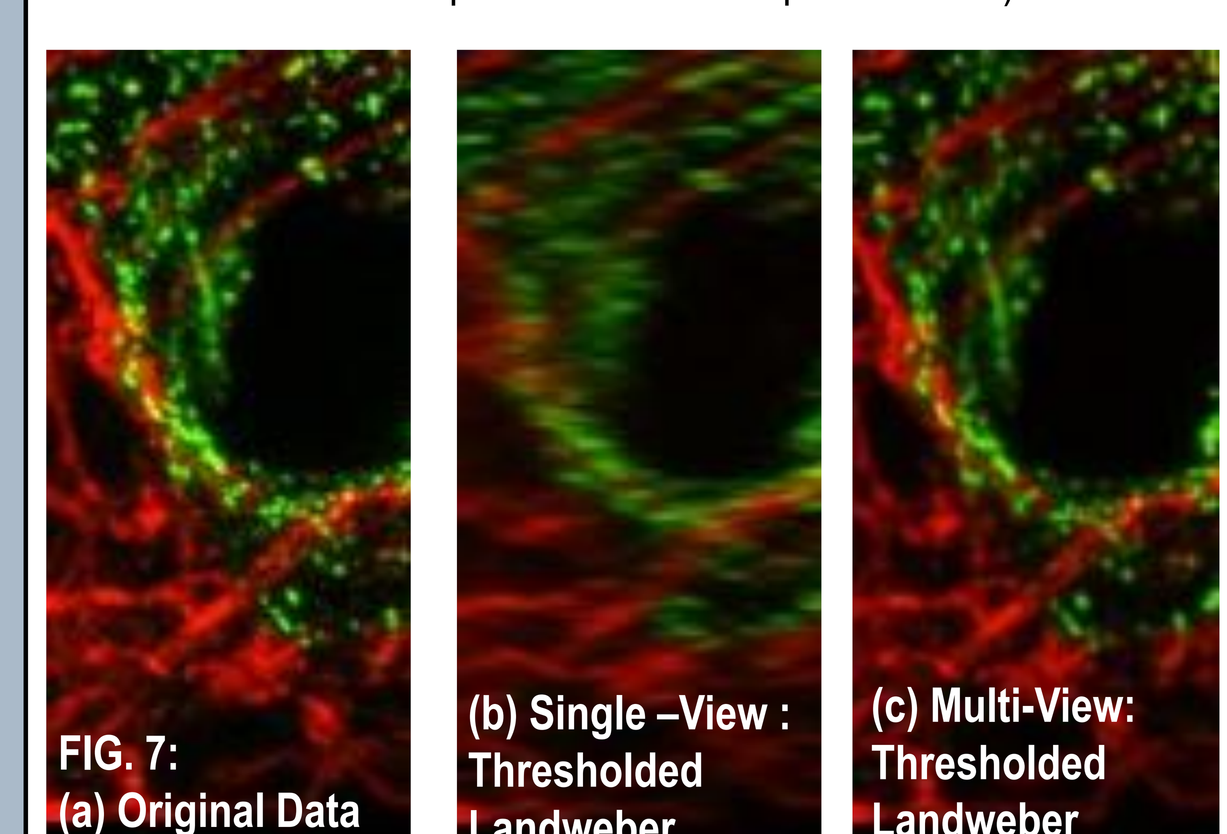


FIG. 6: Four observations (yz-plane) of a mouse blood-brain barrier, blurred (simulated) along different directions (shown inlet is the PSF responsible for the respective blurs)



D. Conclusion and Future Perspective

- The theoretical PSF models generated, according to the instrument and experimental parameters, were close approximations to the actual measured data and produced less noisier deconvolved results
- 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.

Acknowledgements

- *Internships in Nanosystems Science, Engineering and Technology (INSET), UCSB*
- *Systems Bioimaging Lab, UCSB:* Prof. Michael Liebling, Nikhil Chacko, Kevin Chan
- *Center for Science Excellence, CCC:* Seti Sidharta
- *METAS:* Mayra Padilla