Jack Riley, February 2026, EE367

Computational Imaging Winter 2026 Course Project Proposal

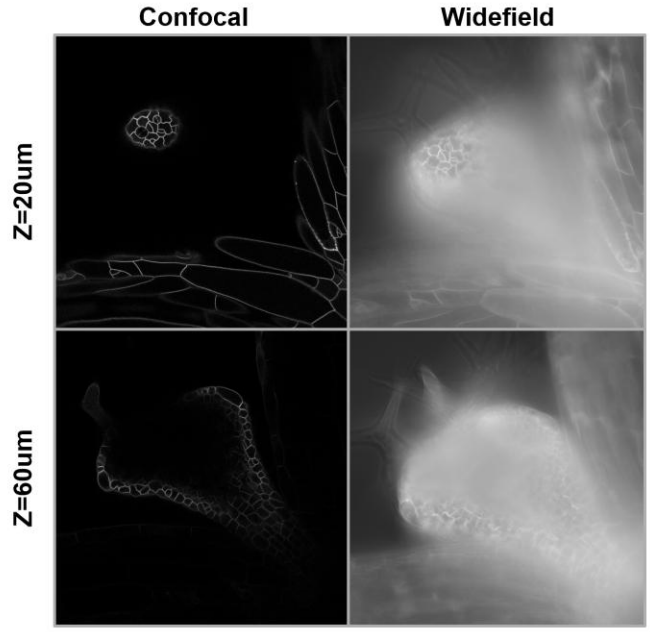

Figure. Images of a young, developing *Arabidopsis* leaf expressing a membrane-localized red fluorescent protein. The images on the left were collected with a laser scanning confocal microscope, the images on the right with a widefield epifluorescence microscope.

The laser-scanning confocal microscope is a workhorse of modern biological imaging. By removing out of focus light with a spatial pinhole, confocal microscopes confer a high degree of axial clarity, enabling robust three-dimensional reconstruction of biological structures. But these microscopes do have their disadvantages. High sample illumination requirements (leading to phototoxicity), slow scanning speeds, and high equipment costs all present contexts in which an alternative imaging modality might be preferable. The relatively simpler widefield fluorescent microscope can yield fast imaging times with lower illumination requirements and represents a potential alternative. With fine stage control, one can collect a focal stack using either modality. The dispersed illumination and lack of pinhole on a widefield scope, however, often leads to an unclear image contaminated with a great deal of out-of-focus light (figure). Deconvolution can help resolve this issue and produce a more accurate representation of the 3D distribution of fluorophores of the sample.[1,2] For my EE367 course project I intend to explore different strategies for deconvolution of widefield fluorescence images.

In my research, I spend a great deal of time analyzing cells on the surface of developing leaves using images collected on our confocal microscope (figure). This involves extraction of 3D surfaces and cell boundaries to explore cell behaviors over time.[3] We also have access to a widefield epifluorescence microscope that can collect focal stacks and I would like to see if I can, using some of the deconvolution approaches we've learned about in class, obtain images of sufficient quality for my usual downstream analysis. This will first involve probing the optics of the microscope to get a good estimation of the PSF. Then I will implement some of the strategies we've learned about for solving inverse problems. Likely the estimation of the PSF will represent one of the main hurdles to getting a high-quality reconstruction because of the nature of these samples, they are bright, but thick and of variable distance from the coverslip.

(1) McNally, J. G.; Karpova, T.; Cooper, J.; Conchello, J. A. Three-Dimensional Imaging by Deconvolution Microscopy. *Methods* **1999**, *19* (3), 373–385. https://doi.org/10.1006/meth.1999.0873.
(2) Ikoma, H.; Broxton, M.; Kudo, T.; Wetzstein, G. A Convex 3D Deconvolution Algorithm for Low Photon Count Fluorescence Imaging. *Sci. Rep.* **2018**, *8* (1), 11489. https://doi.org/10.1038/s41598-018-29768-x.
(3) Barbier De Reuille, P.; Routier-Kierzkowska, A.-L.; Kierzkowski, D.; Bassel, G. W.; Schüpbach, T.; Tauriello, G.; Bajpai, N.; Strauss, S.; Weber, A.; Kiss, A.; Burian, A.; Hofhuis, H.; Sapala, A.; Lipowczan, M.; Heimlicher, M. B.; Robinson, S.; Bayer, E. M.; Basler, K.; Koumoutsakos, P.; Roeder, A. H.; Aegerter-Wilmsen, T.; Nakayama, N.; Tsiantis, M.; Hay, A.; Kwiatkowska, D.; Xenarios, I.; Kuhlemeier, C.; Smith, R. S. MorphoGraphX: A Platform for Quantifying Morphogenesis in 4D. *eLife* **2015**, *4*, e05864. https://doi.org/10.7554/eLife.05864.