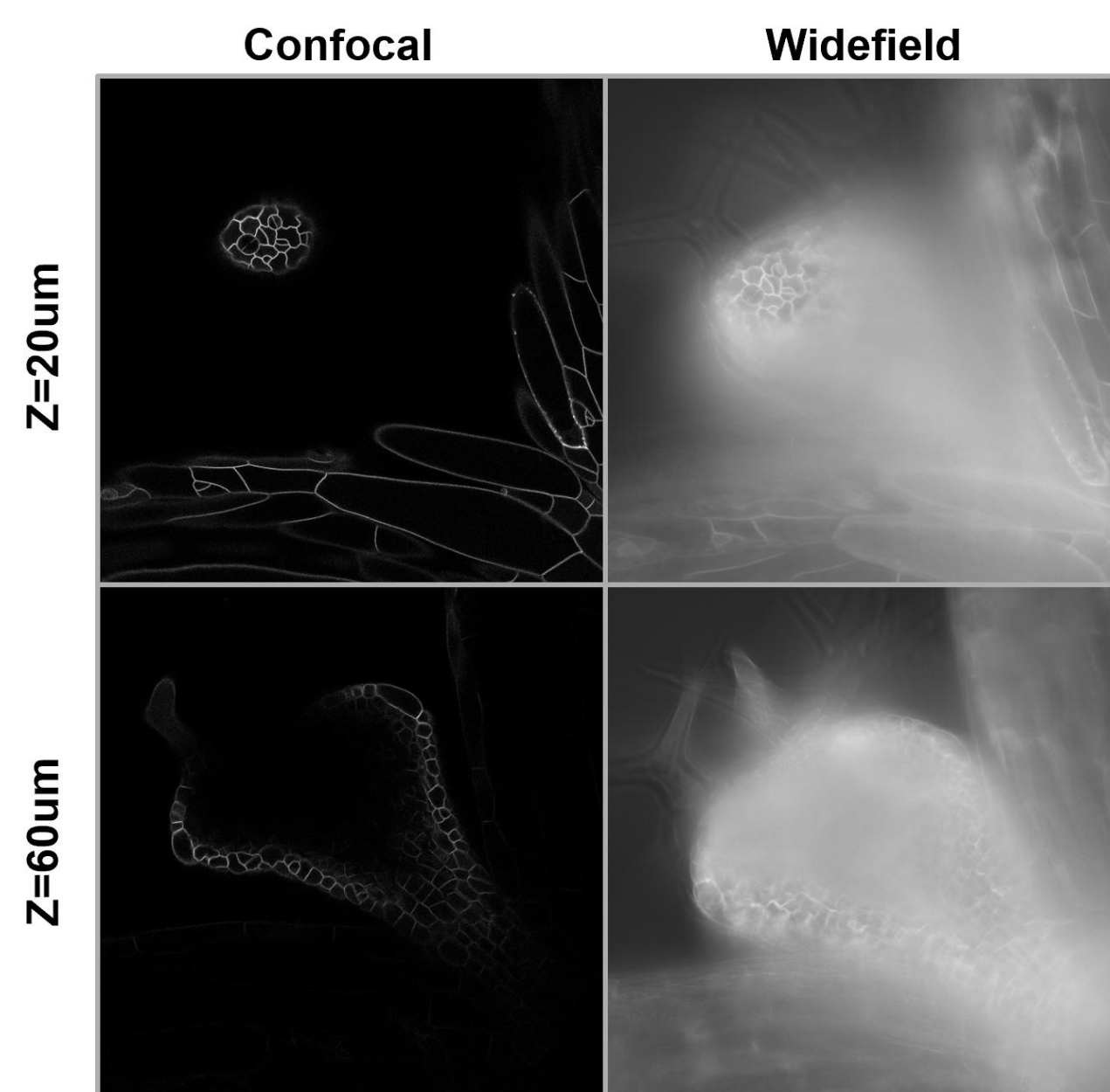


Exploring Widefield Fluorescence Image Formation

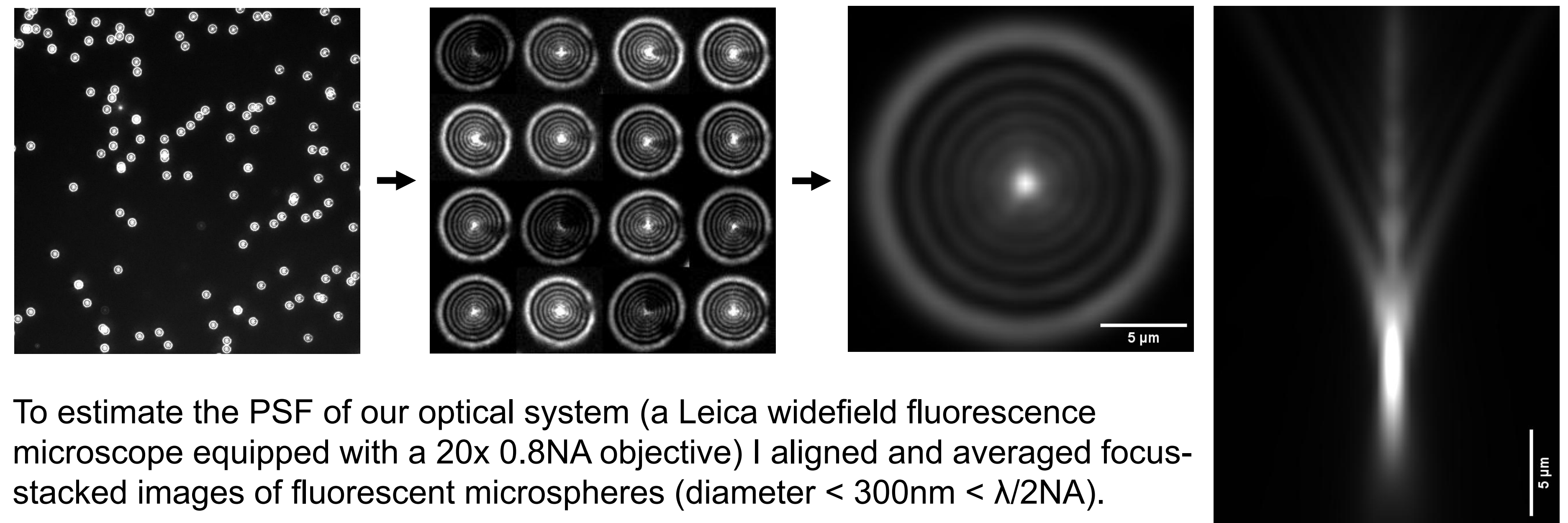
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Motivation



Confocal microscopes yield high 3D imaging clarity through elimination of out of focus light with a spatial pinhole, but they present certain disadvantages in terms of illumination requirements, imaging time, and equipment cost. Here I explore various aspects of widefield fluorescence microscopy image formation and deconvolution as I consider this modality as an alternative.

Technique / PSF estimation



To estimate the PSF of our optical system (a Leica widefield fluorescence microscope equipped with a 20x 0.8NA objective) I aligned and averaged focus-stacked images of fluorescent microspheres (diameter $< 300\text{nm} < \lambda/2\text{NA}$).

Related Work

Deconvolution of widefield fluorescence microscopy focus-stacks is a well studied approach for 3D imaging.¹ An essential first step is an estimation of the point spread function (PSF) of the optical system, which can be done theoretically or experimentally.^{1, 2} With an image formation model in hand various inverse methods can be employed to achieve deconvolution. Noise in the image formation process presents a difficulty and must be properly accounted for to avoid reconstruction artifacts.³

References

- [1] McNally *et al.*, *Three-Dimensional Imaging by Deconvolution Microscopy*, Methods 1999
- [2] Kirshner *et al.*, *3-D PSF fitting for fluorescence microscopy: implementation and localization application*, Journal of Microscopy, 2012
- [3] Ikoma *et al.*, *A convex 3D deconvolution algorithm for low photon count fluorescence imaging*, Scientific Reports, 2018

Results / Initial Deconvolution Attempts



Above we see the result of convolving a simulated 10um hollow sphere with our PSF. As an initial attempt at deconvolution, I tried deconvolving with a wiener filter. It doesn't take much noise to throw it off.

