Bi/BE 227 Winter 2020

Assignment #5

Super Resolution Microscopy

Schedule:
Feb 19: Assignment
Feb 19 to Mar 4: Work on assignment
Mar 4: Assignment due. Presentations 10:00 am

Goals for this week

A. Comparison of Optical sectioning methods: Confocal versus Deconvolution.
B. Comparison of Deconvolution to AiryScan super resolution acquisition

Samples

A. BPAE cell slide from Invitrogen.
B. Same sample.

INSTRUCTIONS:

A. Comparison of Optical sectioning methods: Confocal versus Deconvolution
Using the LSM 410s/310 in the teaching lab you will collect a series of two-color images using the prepared BPAE cell slides. These slides are stained with with MitoTracker™ Red CMXRos, Alexa Fluor™ 488 Phalloidin, and DAPI. You will not look at DAPI for these images (Do you know why?).

Data sets to acquire:

1. Standard confocal with pinhole wide open.
2. Standard confocal with pinhole set to 1 airy unit.

Taking everything you have learned so far, collect images using each imaging parameter above with optimal settings (such as pixel size, scan speed, averaging) using the 63x 1.4 NA objective. The Z step sized used should be the optimal determined before (50% overlap) but you should also take Z-stacks that are oversampling (at least doubling the number of slices or more) which is particularly important for deconvolution (4 Z-stacks total).

Once you have these images, perform deconvolution using the Huygens deconvolution software on each of the four data sets.

B. Comparison of Optical sectioning methods: Confocal versus Deconvolution
Using the LSM 880 you will collect a series of three-color images using the prepared BPAE cell slides. These slides are stained with with MitoTracker™ Red CMXRos, Alexa Fluor™ 488 Phalloidin, and DAPI.

Data sets to acquire:

3. AiryScan with detector set to optimal resolution.
4. AiryScan with detector set to resolution vs sensitivity.

You will again collect images with optimal settings (such as pixel size, scan speed, averaging) using the 63x 1.4 NA objective. Airyscan has specific requirements for settings. It is important that your image histogram spread across less than half the range. The reason for this is that the later Airyscan processing will use the full range and you don't want saturated pixels. For the Airyscan images there are three parameters you have to watch for. (1) You have to use the Optimal frame size; (2) You will have to zoom in slightly until the Zoom is not red; (3) The pinhole has to be opened up for Airyscan (again beyond the red range).

For both parts A and B you will then need to present the comparison between image collection and pre- and post-deconvolution images and comment on the differences. What are the pros and cons between the techniques?

Analysis Ideas:
Present graphs of line profiles of the same part of an image, such as actin filaments, between the techniques before and after deconvolution. Which technique/processing produces the "best" representation of the filaments?

Are there more artifacts with one technique over another?