Calibration of Imaging System Is the First Step toward Quantitative Image Analysis

Abstract:
Immunofluorescence image provides important spatial information for biomarkers and is an essential tool for cell biologists, especially with the development of digital imaging technology. However, it is still regarded as a semi-quantitative method because there are so many factors affecting your final image. Here we are working to fill the gap to make immunofluorescence imaging a quantitative method. The first step toward this goal is to calibrate the imaging system that includes light source, optical path (filter cube) and CCD camera. Dark current is an important parameter to gauge the quality of your camera and can be estimated by capturing an image with no light condition. Knowing the dark current of your camera could help you to compare two images with different exposure times. Next we found that you can use colored plastics bars to calibrate the intensity of the light source, attention has to be paid on the focal plane on the plastic bar and the uniformity of the plastic bar in order to compare day to day variation coming from the light source. Finally flat field correction needs to be performed for each filter cube because even with the best alignment, you could still get 20% difference in pixel intensity at the field of view. We will use antibody validation as an example to show how we quantitatively assess the quality of antibodies. All images were acquired using Zeiss Imager Z1 and processed using ImageJ (version 1.40J).

Keywords:
calibration, quantitative, flat field correction, dark current, light source

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