Technical Note on Borealis - Perfect Illumination Delivery™

Why illumination uniformity matters

- Precise imaging measurements start with uniform illumination
- Uniform illumination starts with borealis
- Borealis paves your way to better results

Reliable and accurate quantitative comparisons of fluorescence intensities across an image are impossible when the field illumination profile is not uniform [1]. Uneven illumination of the sample will cause the intensity of a feature in one region of the field of view to register a different magnitude than the intensity of a feature of equal fluorophore concentration in another region of the field of view. We have developed Borealis, a combination of optical techniques resulting in Perfect Illumination Delivery™, which addresses the issue of uneven illumination.

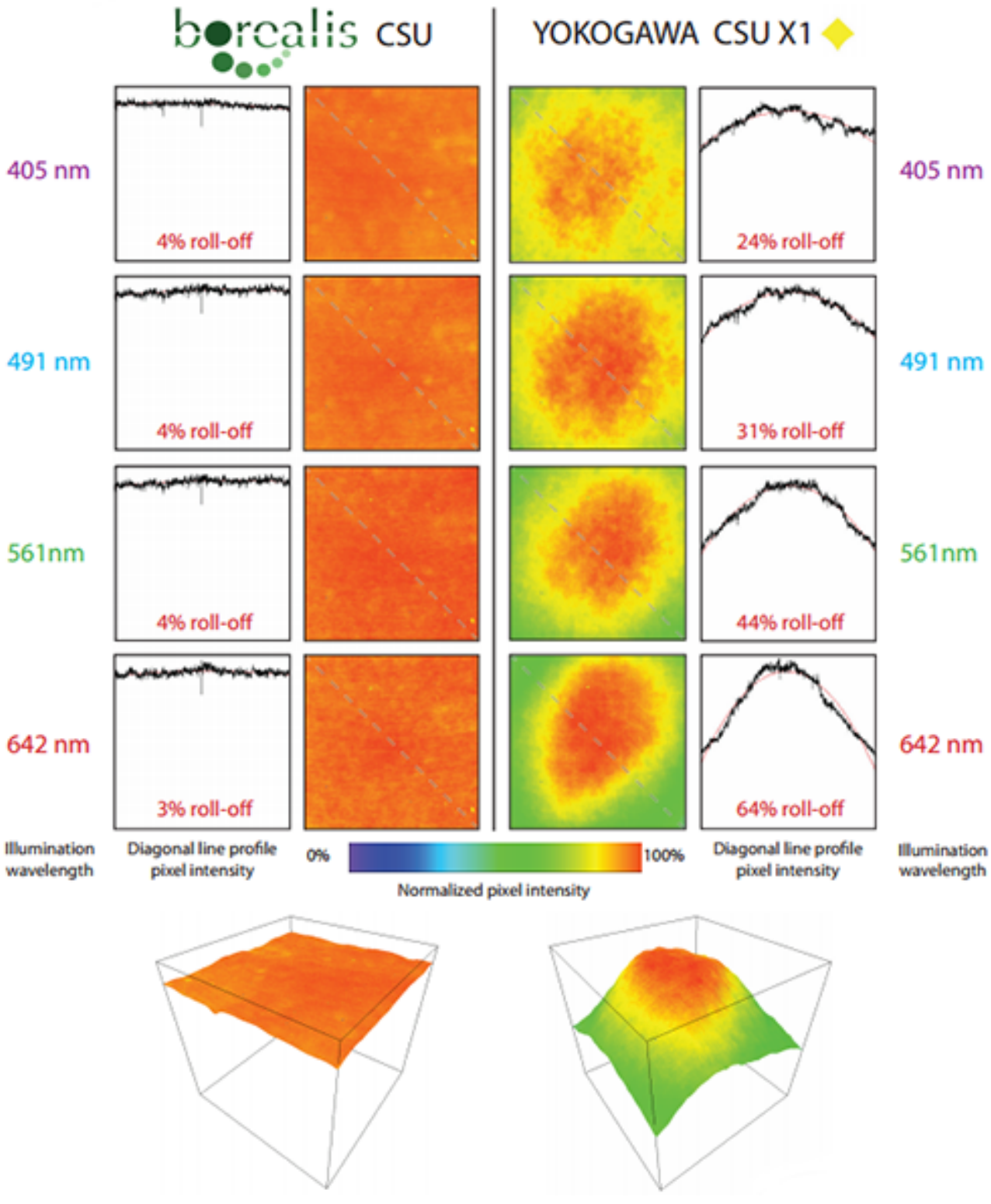
When trying to determine the level of expression of a fluorescently tagged protein within a population of cells with uneven illumination, cells in the center of the image will appear to be brighter than those at the edges of the image. This problem can lead to incorrect interpretation and analysis of the image data, especially when comparisons are made between images captured in different spectral channels (ie: FRET, calcium ratio imaging, colocalization, etc.).

Furthermore, most automated image analysis algorithms assume even illumination across the field of view [2]. Gradients in the illumination profile prevent proper thresholding, segmentation, object counting, 3D reconstruction, morphology and intensity distribution evaluation. The detrimental effects of uneven illumination are even more striking when a mosaic composed of images from a number of adjacent fields of view is analyzed since automatic stitching algorithms may fail to recognize overlapping image regions.

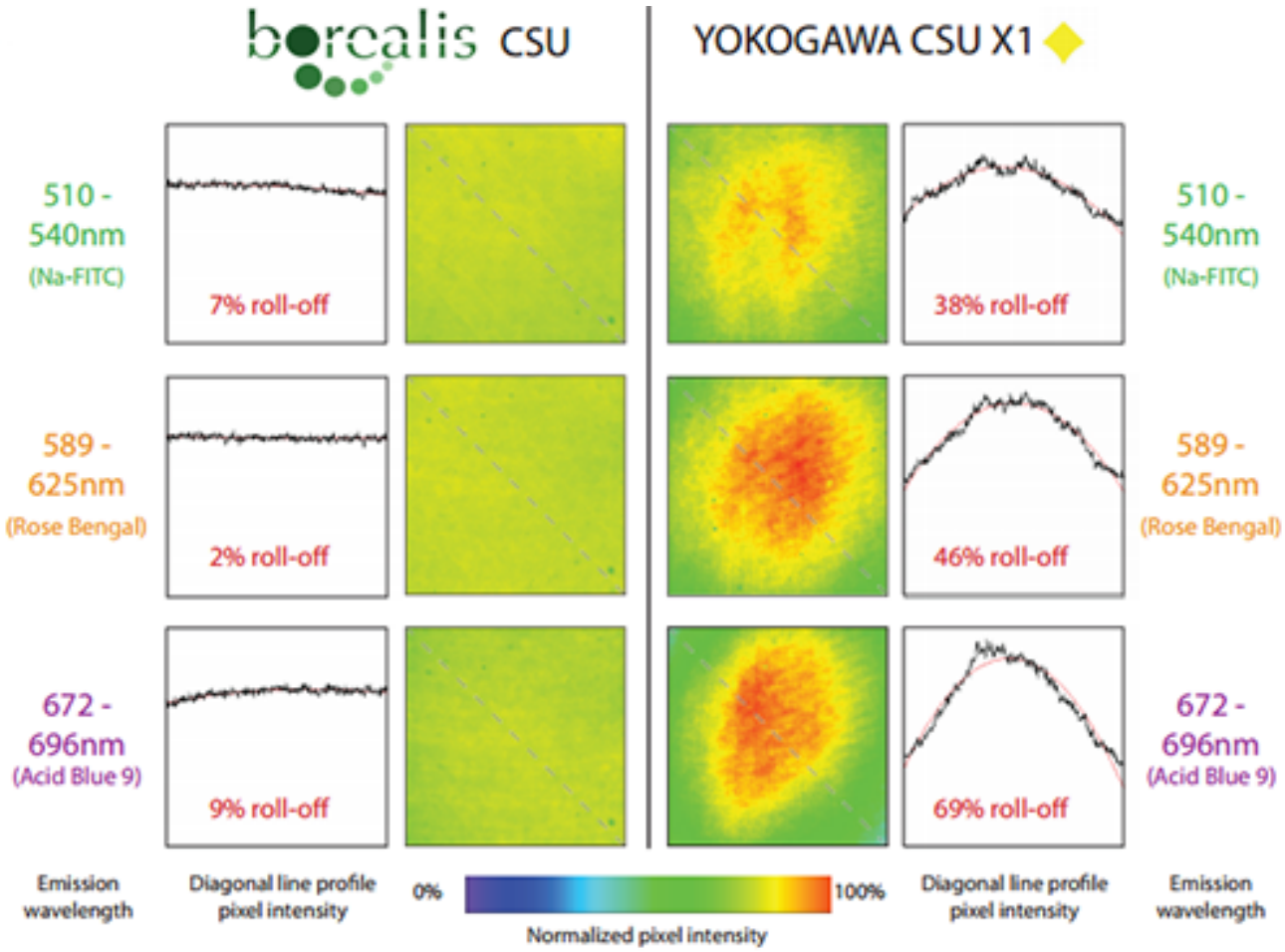
Borealis Perfect Illumination Delivery™ enables:

- quantitative imaging with a CSU
- stable, flat illumination restricted to the imaged field of view reducing stray light artifacts
- near-perfect uniformity across tiled image fields when viewing large specimens and populations

These capabilities are shown in the first figure by measuring the corner-to-corner intensity roll-off of the illumination profile across the CSU disk pinhole plane with a beam profiler camera. Similar information about the illumination uniformity can also be attained by imaging the fluorescence emission of various concentrated dye solutions with the appropriate excitation/emission wavelength combination (see second figure) [3]. Any additional inhomogeneity's in the illumination profile observed in these fluorescence images are likely the result of lens field curvature or spatially non-uniform reflection/transmission through the dichroic mirror and/or emission filters, camera lens vignetting, or a combination thereof. All beam profiler and fluorescence images correspond to the intermediate image plane area viewed by a typical EMCCD camera (8.2 x 8.2 mm). Percent roll-off is determined as 100% minus the ratio between the minimum and maximum intensity values along a fitted parabolic curve to a diagonal line profile.



Illumination uniformity of CSU pinhole plane as shown with a beam profiler camera at different wavelengths.



Illumination uniformity relates to the uniformity in the fluorescence emission as shown in these confocal images of concentrated dye solutions imaged using a back thinned EMCCD Camera.

The results above clearly demonstrate the benefits of Borealis with its Perfect Illumination Delivery technology™. There are a number of additional technical notes which expand further on the application benefits of Borealis.

References:

1. Waters, J.C., Accuracy and precision in quantitative fluorescence microscopy. Journal of Cell Biology, 2009. 185(7): p. 1135-1148.
2. Wolf, D.E., C. Samarasekera, and J.R. Swedlow, Quantitative analysis of digital microscope images, in Digital Microscopy, 3rd Edition. 2007, Elsevier Academic Press Inc: San Diego. p. 365-396.
3. Model, M.A. and J.L. Blank, Concentrated dyes as a source of two-dimensional fluorescent field for characterization of a confocal microscope. Journal of Microscopy-Oxford, 2008. 229(1): p. 12-16.