

Shading Correction

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Summary presentation

- This presentation covers the following topics:
 - What is shading correction?
 - Shading correction images4
 - Example images before and after
 - Example linescans before and after
 - How to apply shading correction
 - How to create shading correction
 - Special cases



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Shading (flat-field) correction

- Corrects for uneven illumination in images
- Shading is a function of specific objective, filter set, and other optics
 - Generally more correction is needed at lower magnifications
- Use when cell-by-cell intensity measurements are critical
 - Protein expression
 - Cell cycle





Shading (flat-field) correction

• 10x vs. 4x illumination (pseudocolor for intensity)







Before and after shading correction



Uncorrected: Image is slightly brighter in center

Corrected: Only natural variation between cells





Before and after shading correction - pseudocolor



Uncorrected Image is slightly brighter in center

Corrected: Only natural variation between cells



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Before and after shading correction



Draw line across images to compare linescans







Before and after shading correction- linescan







How to apply shading correction

- Option to correct images during acquisition:
 - Plate Acquisition Setup > Acquisition Loop tab
 - Select folder with shading images
 - Corrected images are automatically saved in database

	Number of wavelengths: 2
	Autofocus options
	Enable image-based focusing (for acquisition or laser recovery)
Acquisition Loop	
	Perform shading correction Directory C:\MX4\Shading







How to apply shading correction

- If correction not done at acquisition, images can be corrected before analysis either manually or with a journal
 - Process > Background and Shading Correction
 - Use "Correct Shading" button on Analysis taskbar for individual images
 - Incorporate into an analysis journal for a full plate
- How Shading Correction is performed
 - Sample image is divided by the correction image to generate flat field ratio
 - Result is scaled to original intensity range







Creating shading images

- Create new images if:
 - New light source / lamp installed
 - New light guide installed
 - New filter set installed
 - New objective installed
 - Current images are not correcting appropriately
- Use plastic plates provided in ImageXpress toolkit
- Use "Set up Shading Correction" button on System Maintenance taskbar









Creating shading images – special cases

- Oil-immersion objectives or 640 laser on ImageXpress Ultra
 - Do NOT use plastic plates
 - Use a well or slide with the fluorophore free in solution
 - Focus and acquire an image and save with appropriate naming (Shading_Objective_Filter.tif)
 - Make sure shading image is same size or larger than image to be corrected
- Transmitted Light
 - For plates, set up an extra well with the same volume of buffer as in cell samples
 - Since illumination will vary in different parts of the well, correction must be done after acquisition
 - For slides, collect shading images using a blank slide







Thank You



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