![](_page_0_Picture_0.jpeg)

# **Shading Correction**

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![](_page_0_Picture_4.jpeg)

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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

![](_page_1_Picture_9.jpeg)

- 3

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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

![](_page_2_Picture_7.jpeg)

![](_page_2_Picture_8.jpeg)

# Shading (flat-field) correction

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

![](_page_3_Picture_2.jpeg)

![](_page_3_Figure_3.jpeg)

![](_page_3_Picture_5.jpeg)

### Before and after shading correction

![](_page_4_Figure_1.jpeg)

#### Uncorrected: Image is slightly brighter in center

#### Corrected: Only natural variation between cells

![](_page_4_Picture_4.jpeg)

![](_page_4_Picture_6.jpeg)

### Before and after shading correction - pseudocolor

![](_page_5_Figure_1.jpeg)

#### Uncorrected Image is slightly brighter in center

#### Corrected: Only natural variation between cells

![](_page_5_Picture_4.jpeg)

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![](_page_5_Picture_6.jpeg)

## Before and after shading correction

![](_page_6_Figure_1.jpeg)

#### Draw line across images to compare linescans

![](_page_6_Picture_3.jpeg)

![](_page_6_Picture_4.jpeg)

![](_page_6_Picture_5.jpeg)

### Before and after shading correction- linescan

![](_page_7_Figure_1.jpeg)

![](_page_7_Picture_2.jpeg)

![](_page_7_Picture_4.jpeg)

## 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

![](_page_8_Picture_6.jpeg)

![](_page_8_Picture_7.jpeg)

![](_page_8_Picture_8.jpeg)

## 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

![](_page_9_Picture_8.jpeg)

![](_page_9_Picture_9.jpeg)

![](_page_9_Picture_10.jpeg)

### 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

![](_page_10_Picture_9.jpeg)

![](_page_10_Picture_10.jpeg)

![](_page_10_Picture_11.jpeg)

![](_page_10_Picture_13.jpeg)

### 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

![](_page_11_Picture_10.jpeg)

![](_page_11_Picture_11.jpeg)

![](_page_12_Picture_0.jpeg)

### Thank You

![](_page_12_Picture_2.jpeg)

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