MetaXpress® 6 FAQ

Creating Shading Correction Images

Date Revised 07/09/15 Version B

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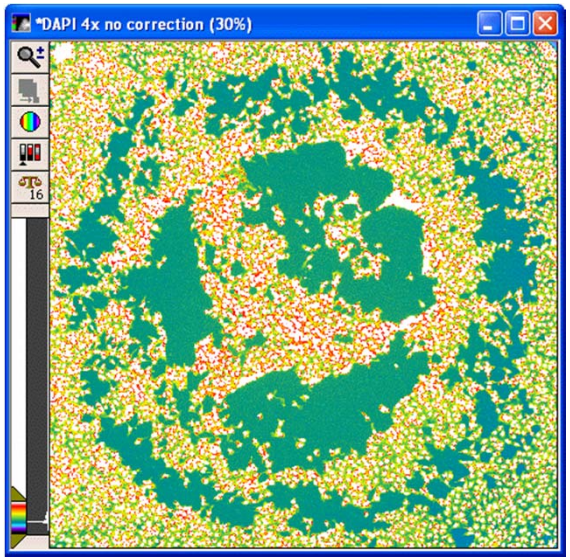
Frequently **A**sked **Q**uestions Overview

The purpose of this FAQ guide is to explain when and how to create shading correction images using the **IXM Taskbar**.

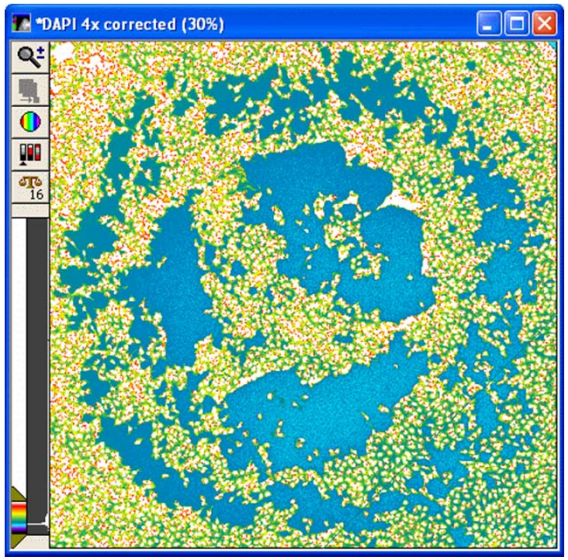
Refer to corresponding chapters on installing the latest Taskbar available on User Forum: http://metamorph.moleculardevices.com/forum/

Why Correct for Shading in Images?

Uneven shading in images can be a function of specific objective, filter set, and other optics. In general, uneven shading is more apparent at lower magnification. In the example below, the image on the left shows brighter pixel intensity in the middle of the image. Shading correction images can be applied to reduce these effects as shown in the image on the right.



Uncorrected image (pseudocolor):
Image is slightly brighter in center



Corrected image (pseudocolor):
Only natural variation between cells

Why and When to Create Shading Correction Images

Shading correction images can be applied to aesthetically enhance the image for display purposes. Additionally, corrected images have more accurate cell-by-cell intensity measurements.

New shading correction images should be created when:

- New light source / lamp installed
- New liquid light guide installed
- New filter set installed
- New objective installed
- Current images are not correcting appropriately

Creating Shading Images

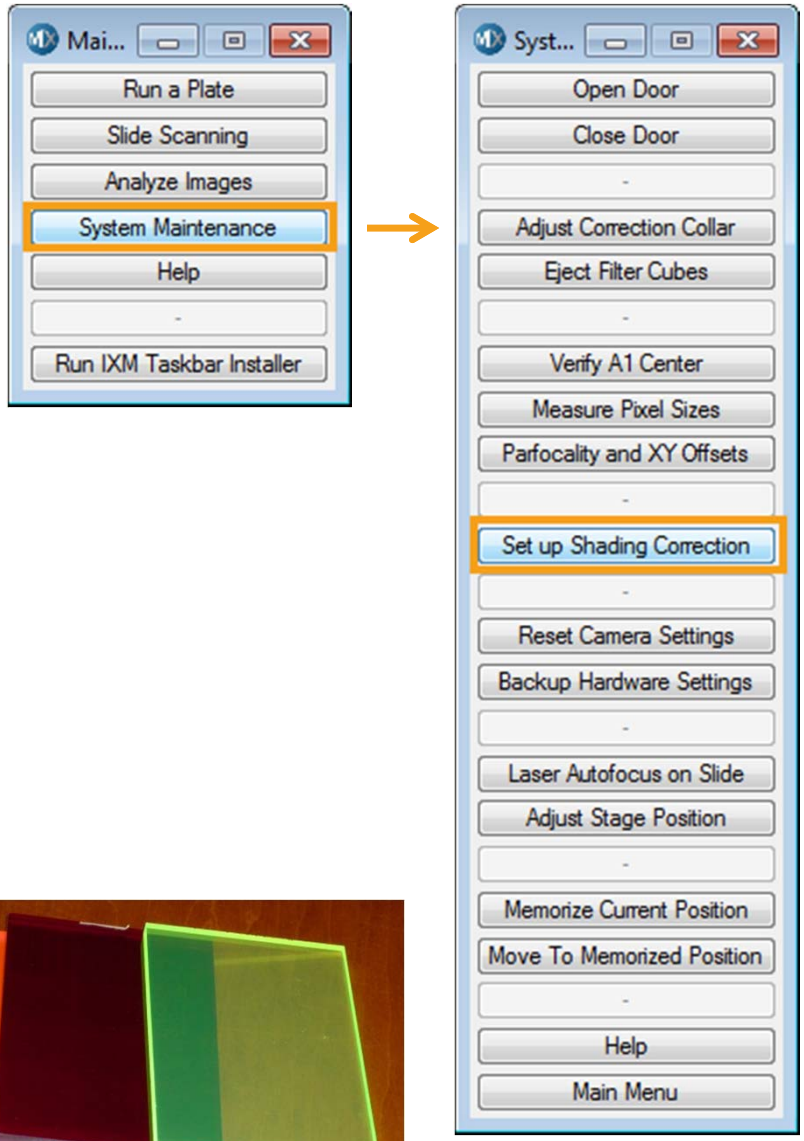
1. Identify the filters and objective combinations that need shading images

2. If the objective(s) have correction collars, set to 0

3. Locate the shading plates
   - Usually stored in the IXM accessory kit
   - Remove paper from one side if needed

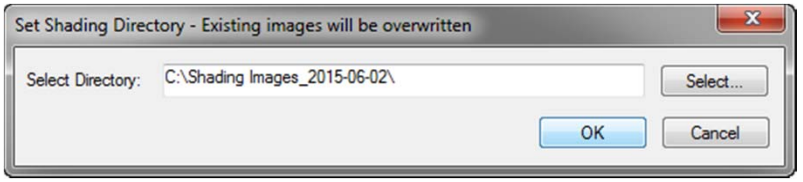
4. In the MetaXpress software, make sure the **IXM Taskbar** is loaded. Click on **System Maintenance**

5. Click **Set up Shading Correction**

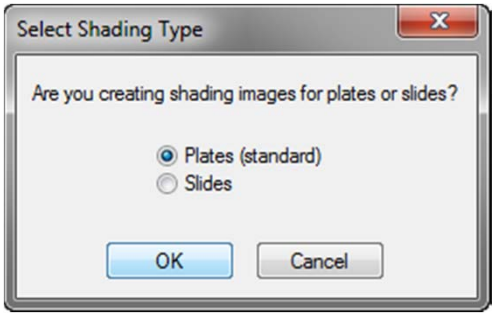


Creating Shading Images

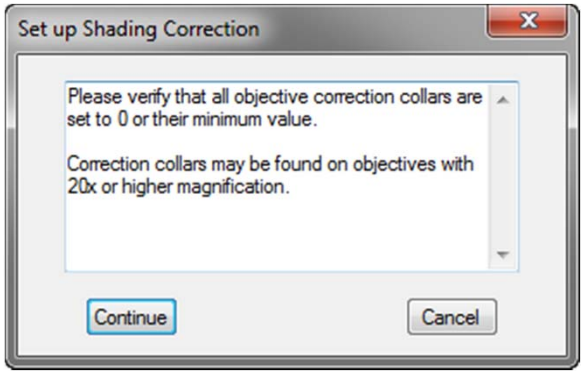
6. Select a directory for saving the new shading images
   - A directory with today's date is suggested
   - Existing images in the directory may be overwritten



7. Select **Plates** for standard shading images and click **OK**



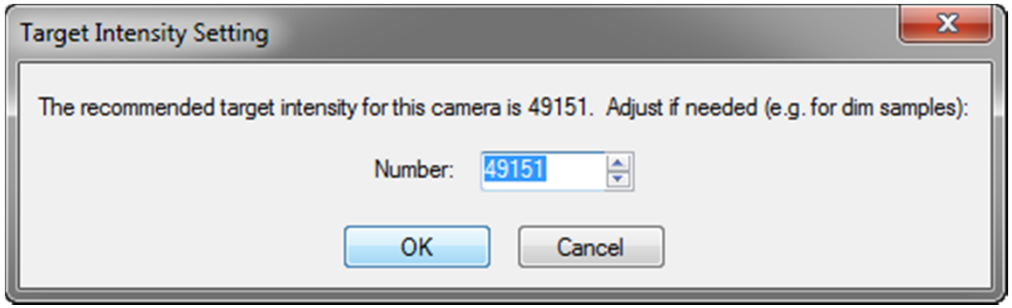
8. Click **Continue** to confirm that objective correction collars are set to 0



Creating Shading Images

9. The **Target Intensity Setting** dialog will appear. A suggested intensity value is displayed based on the system type. However, the value may need to be adjusted:
   - Standard images may overcorrect dim samples
     - In this case, set the intensity to the maximum intensity expected in a typical sample
   - Shading images with different intensity settings may be stored in different folders

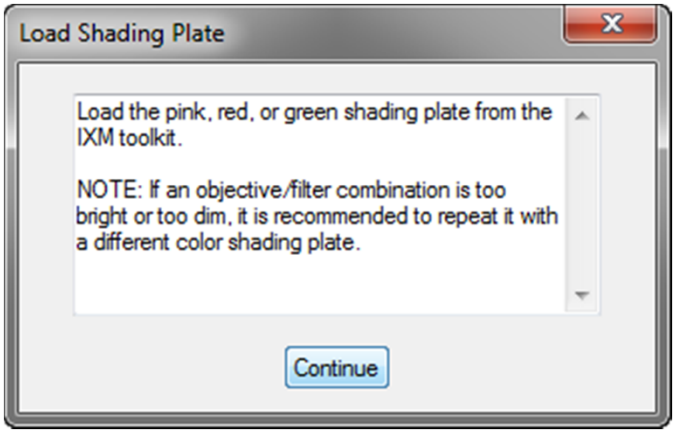
10. Click **OK**

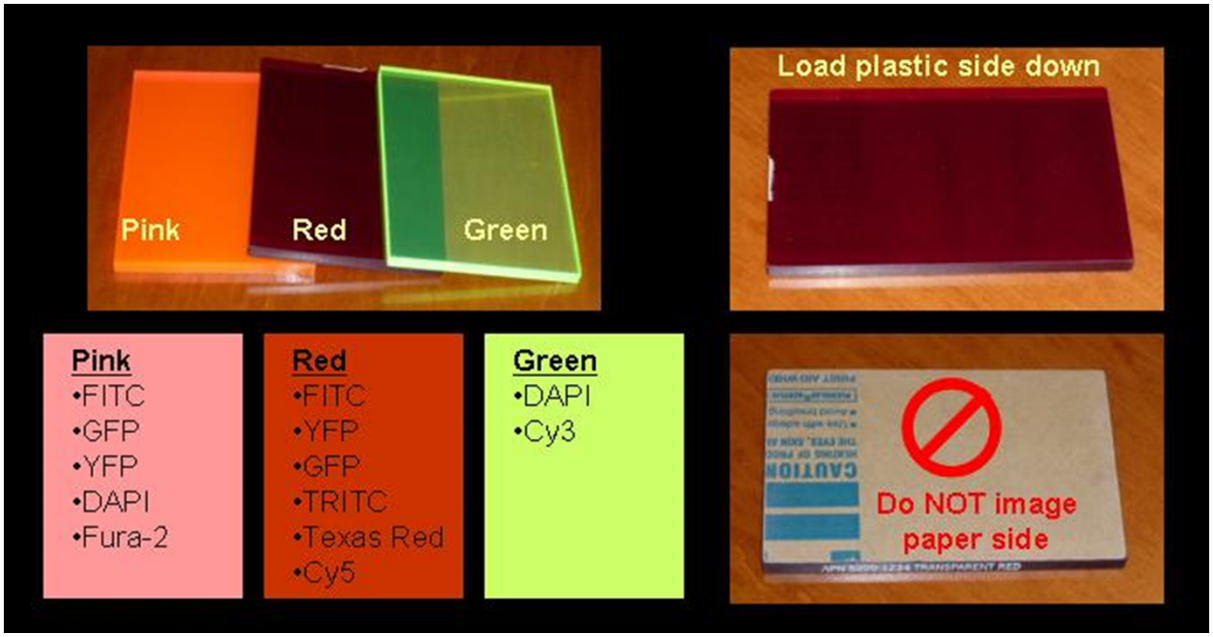


Creating Shading Images

11. Load the appropriate flat field shading plate into the system
    - Refer to the diagram below for suggestions on which color plate to use

12. Click **Continue** when done



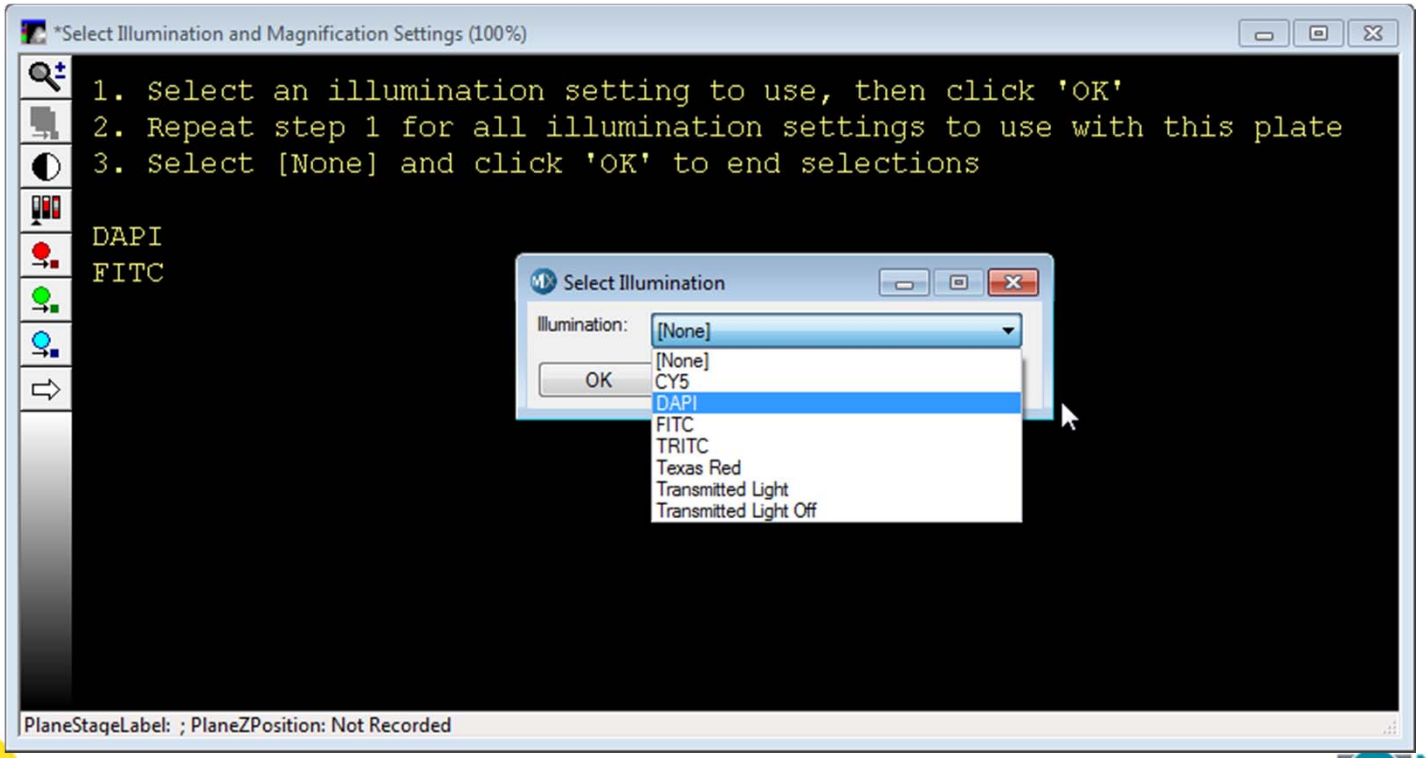


Creating Shading Images

13. Select the desired **Illumination** setting from the drop-down menu and click **OK**

14. Repeat step 13 for all illumination selections for this plate. The list will be updated in the **Select Illumination and Magnification Settings** dialog

15. Select **[None]** when done and then click **OK**

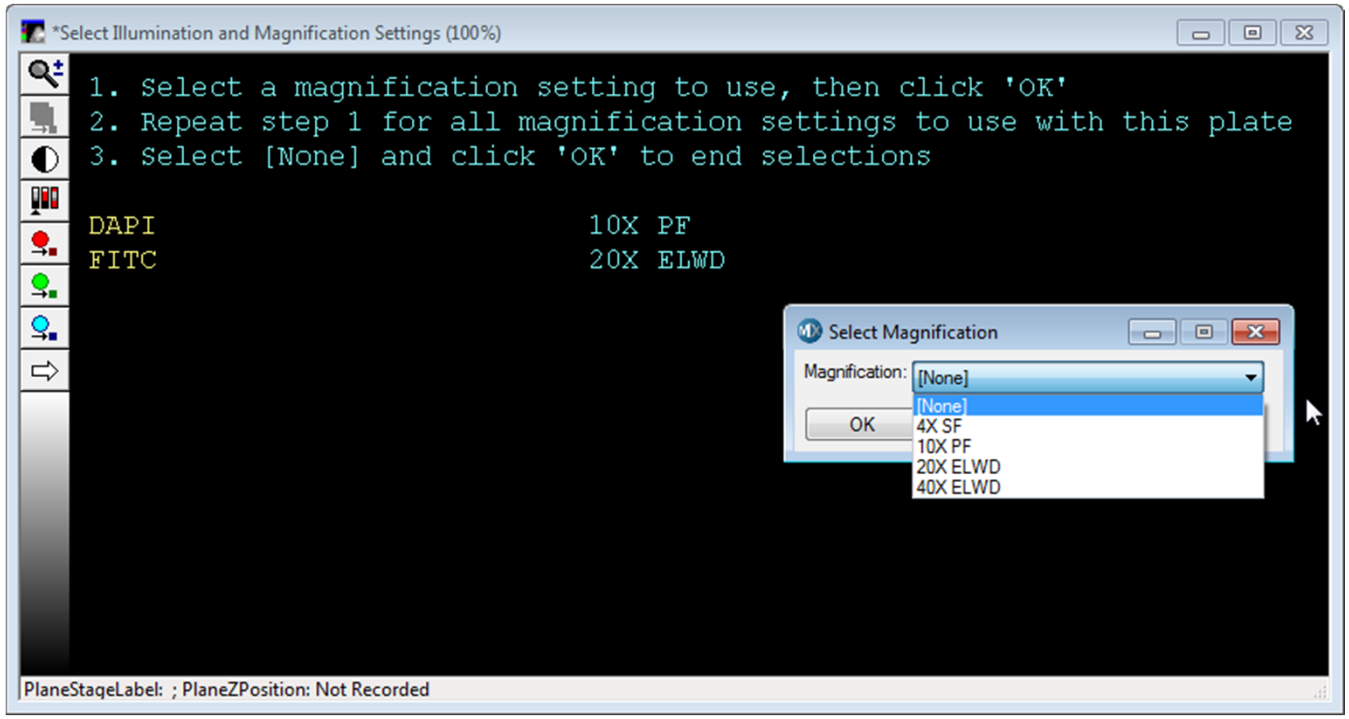


Creating Shading Images

16. Select the desired **Magnification** setting from the drop-down menu and click **OK**

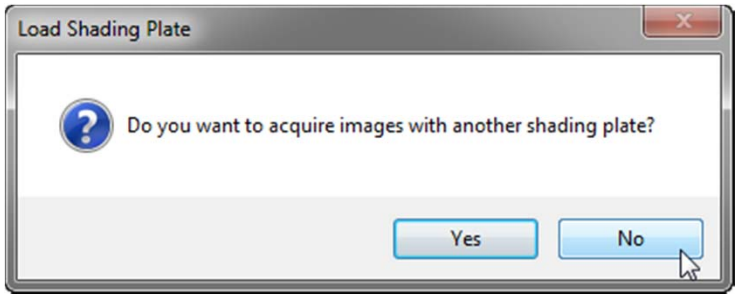
17. Repeat step 16 for all objective selections for this plate. The list will be updated in the **Select Illumination and Magnification Settings** dialog

18. Select **[None]** when done and then click **OK**

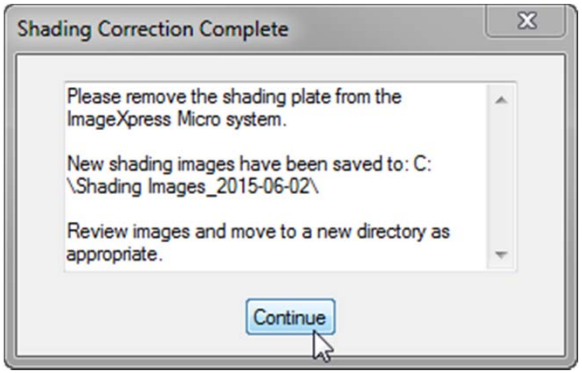


Creating Shading Images

20. The system will now automatically acquire, create, and save the shading correction images. No other functions are possible during this time.



21. When complete, you will be given the option to continue with another shading plate. Click **Yes** if desired and follow the prompts. Otherwise, click **No**.



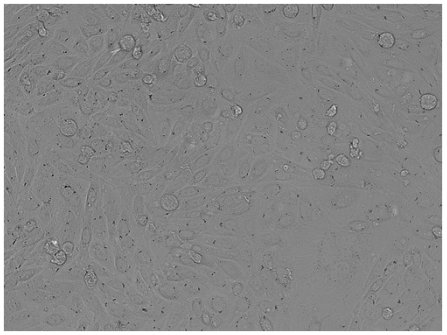
22. A log of the completed images and location will appear. Click **Continue** to complete the process.

Viewer - C:\Shading Images_2015-06-02\Shading.Log

	A	B	C	D
1	Shading Image Name	Timestamp	Exposure Time	Status
2	Shading_4X S Fluor_DAPI	Tue Jun 2 14:08:11:302 2015	41	Success
3	Shading_4X S Fluor_FITC	Tue Jun 2 14:09:31:146 2015	25	Success
4	Shading_10X Plan Fluor_DAPI	Tue Jun 2 14:10:22:401 2015	62	Success
5	Shading_10X Plan Fluor_FITC	Tue Jun 2 14:11:14:124 2015	45	Success
6	Shading_20X S Plan Fluor ELWD_DAPI	Tue Jun 2 14:12:13:186 2015	120	Success
7	Shading_20X S Plan Fluor ELWD_FITC	Tue Jun 2 14:13:04:532 2015	83	Success
8	Shading_40X S Plan Fluor ELWD_DAPI	Tue Jun 2 14:14:46:072 2015	203	Success
9	Shading_40X S Plan Fluor ELWD_FITC	Tue Jun 2 14:15:38:332 2015	137	Success

Special Case: Transmitted Light with Plates

Standard shading correction images may not be suitable for transmitted light (brightfield) images taken on a plate. Variation in images can be result of instrument optics, plate optics, and the meniscus effect in the wells.



Do not use the **Setup Shading Correction Image** in the main IXM taskbar.

Prior to acquiring the sample plate:

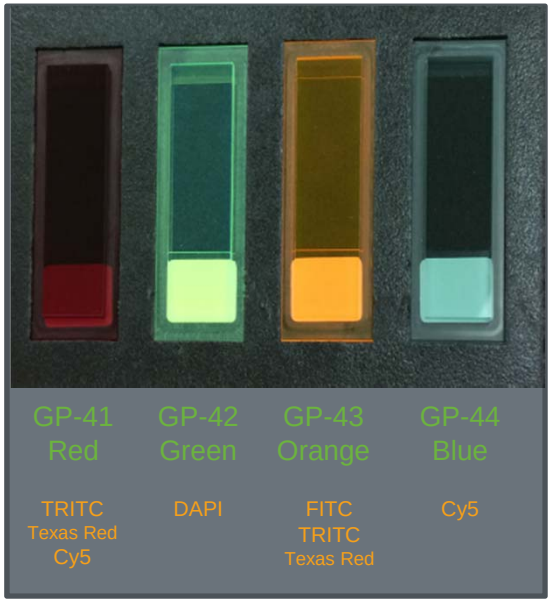
- Set up extra wells on the plate with the same volume of buffer as you will use in the assay (it is suggested to setup up at least 4 wells)
  - Shading can also vary from site-to-site. If you plan to acquire multiple sites, you may need to generate different shading correction images for each site.
- Use **Plate Acquisition Setup** to acquire the extra wells with identical settings as the sample wells
- In **Review Plate Data**, right-click to select the wells
  - If shading varies from site to site, make sure to select only one site at a time
- Click on the **Load Image** button to create a stack of the images
- In the main menu, select **Process > Stack Arithmetic > Median** to reduce artifacts
- Save with appropriate naming convention:
  Shading_[Mag Setting]_[Illum Setting].tif
- When acquiring your sample and single site, perform shading correction during the acquisition. Otherwise, shading correction will need to be applied after acquisition.

Special Case: Slides

Standard shading correction images may not be suitable for microscope slides.

Fluorescence - if the standard shading images do not work well:

- Use the **Set Up Shading Correction > Slides** option
- Use the **GP-41**, **GP-42**, **GP-43**, **GP-44** slides from the IXM accessory kit
- Follow the prompts

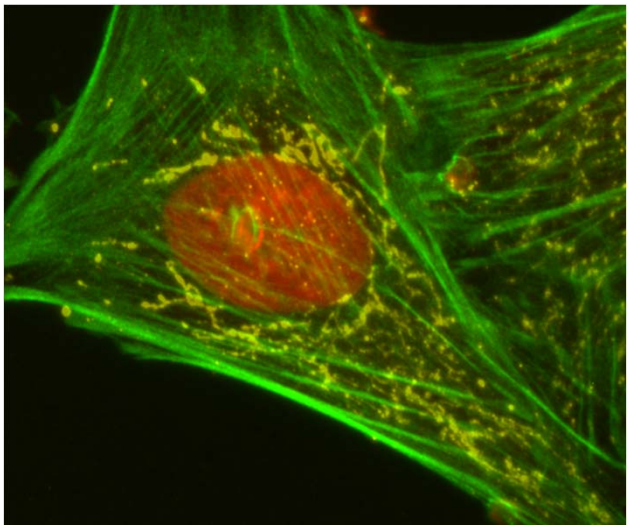


Transmitted Light or Colorimetric (RGB) imaging

- Do not use special characters in the illumination setting name
- Use the **Set Up Shading Correction > Slides** option and follow the prompts
- Use blank area of sample slide or a matching blank slide/coverslip
- Brightfield and phase contrast should be done separately

Special Case: Oil-Immersion Objectives

Standard shading correction images may not be suitable for images acquired using oil-immersion objectives



Do not use the **Setup Shading Correction Image** in the main IXM taskbar.

- Do not use the plastic shading plates or GP slides
- Use a plate or slide with the same (or similar) fluorophore free in solution
- Use **Plate Acquisition Setup** to acquire the wells with identical settings as the sample wells
- In **Review Plate Data**, right-click to select the wells
- Click on the **Load Image** button to create a stack of the images
- In the main menu, select **Process > Stack Arithmetic > Median** to reduce artifacts
- Save with appropriate naming convention:
  Shading_[Mag Setting]_[Illum Setting].tif

Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments