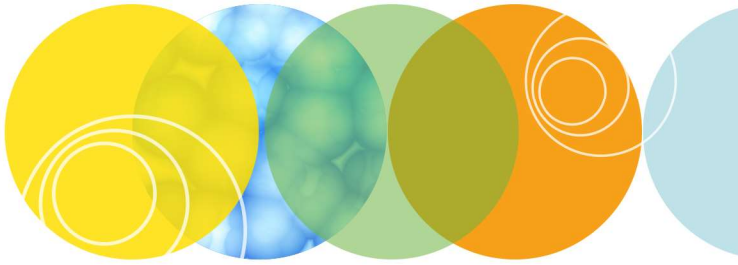


ImageXpress Taskbar User Guide

Version 6.7

Contents

1. Introduction

This guide provides instructions on how to use the optional ImageXpress Taskbar ("IX Taskbar"). The IX Taskbar is an organized collection of tools intended to enhance and/or streamline common tasks and user workflows for an ImageXpress high-content screening system with MetaXpress software.

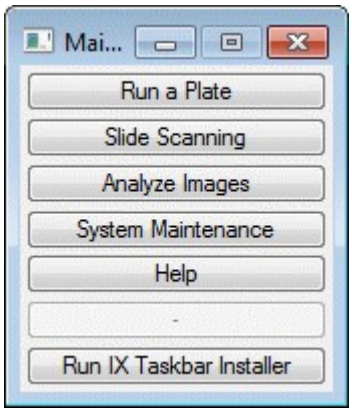
2. Main Menu and Navigation

a. Viewing the taskbar

After installation, the main taskbar should be visible in the MetaXpress window. If you don't see it, press **F4 (Show Taskbar)** on the keyboard. If it still doesn't appear, go to **Journal > Taskbars > Load Taskbar** (standard menu structure) or **Control > Journal > Taskbars > Load Taskbars** (simplified menu structure). Select the **Main Taskbar.JTB** file in the Taskbars directory (e.g. **C:\MX6\Taskbars**).

If desired, you can set the taskbar to always appear on top of other windows by going to **Journal > Taskbar Always on Top** (standard menu) or to **Control > Journal > Taskbar Always on Top** (simplified menu).

The main taskbar looks like this:



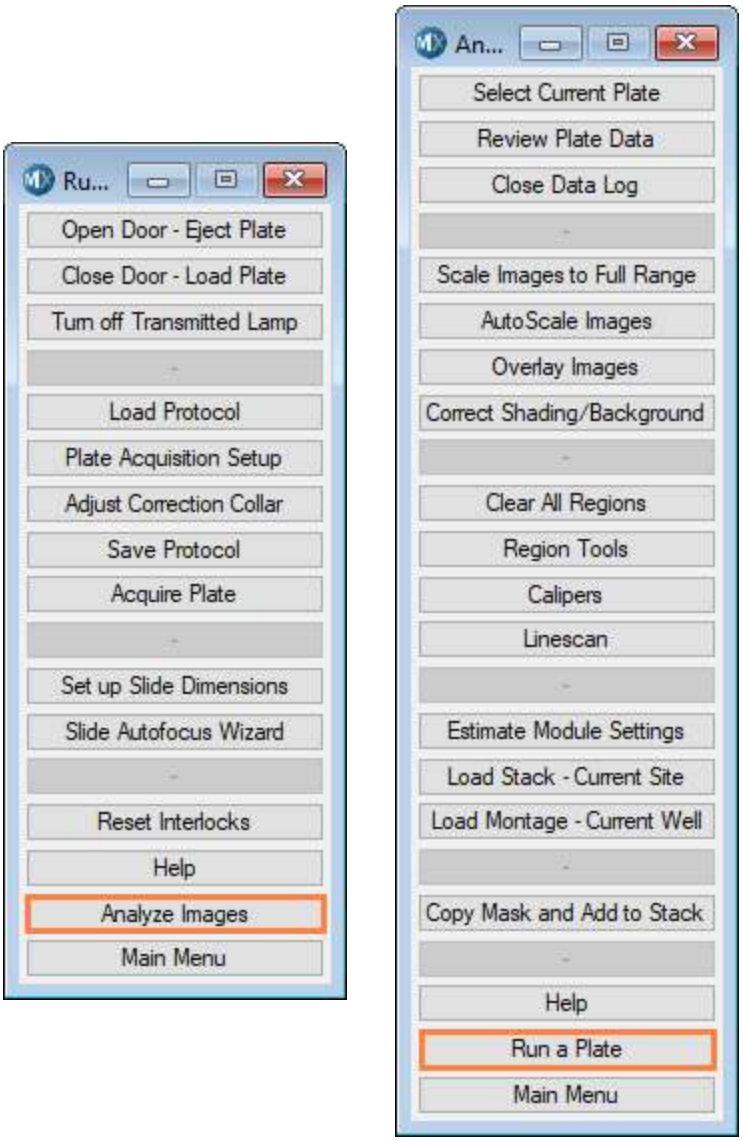
b. Links between taskbars

Clicking on **Run a Plate**, **Slide Scanning**, **Analyze Images**, or **System Maintenance** will take you to the appropriate linked taskbar.

From the other taskbars, click **Main Menu** at the bottom to return to the main taskbar.

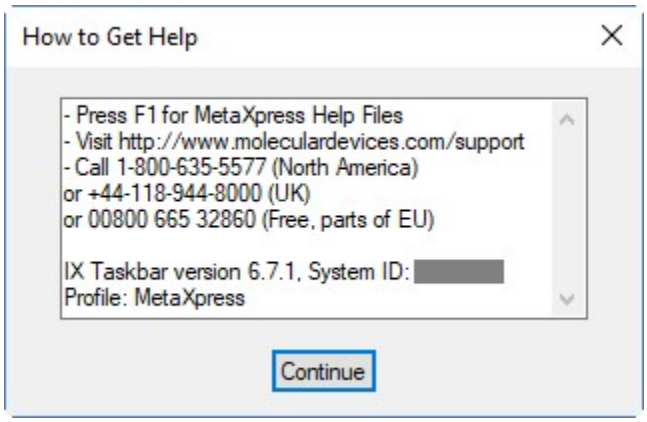

Ru...
Open Door - Eject Plate
Close Door - Load Plate
Turn off Transmitted Lamp
Load Protocol
Plate Acquisition Setup
Adjust Correction Collar
Save Protocol
Acquire Plate
Set up Slide Dimensions
Slide Autofocus Wizard
Reset Interlocks
Help
Analyze Images
Main Menu

Since it is common to frequently switch between acquiring plates and reviewing images, there are also direct links between the Run a Plate taskbar and the Analyze Images taskbar.



c. Help

All taskbars have a **Help** button. Clicking **Help** opens a dialog with basic help information, the taskbar version number, the system ID, and the current MetaXpress group (profile).



d. Run IX Taskbar Installer

Typically, the installer only needs to be run when the taskbar is initially installed. For more information, consult the **IX taskbar v6-7 installation guide**.

3. Run a Plate taskbar

a. Open Door – Eject Plate

Click to open the door so that you can remove the sample plate.
If the door is already open but the plate clamp is closed, this will open the plate clamp.

b. Close Door – Load Plate

Closes the door and loads the sample plate.

c. Turn off Transmitted Lamp

Only appears if your instrument has the Transmitted Light (TL) option. Click to turn off the TL lamp.

d. Load Protocol

Load a saved protocol for Plate Acquisition

e. Plate Acquisition Setup

Open the Plate Acquisition Setup dialog for configuring or testing acquisition settings.

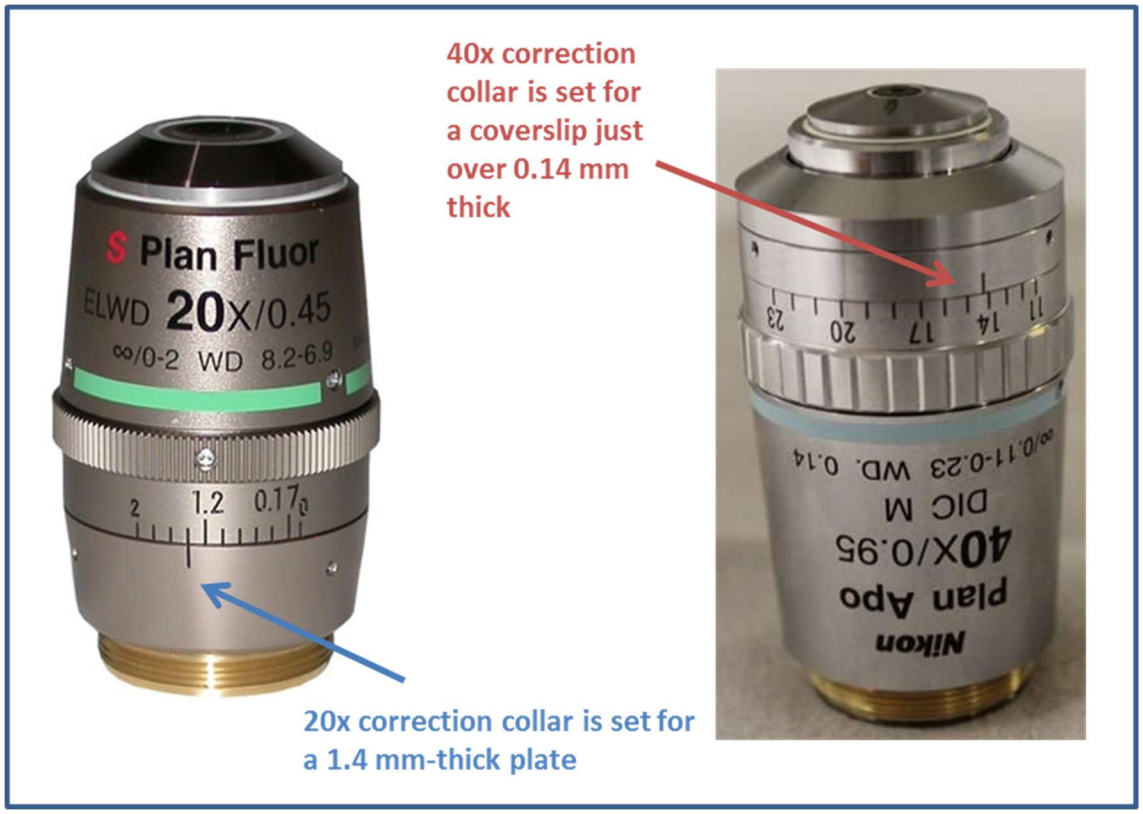
f. Adjust Correction Collar

Certain higher-magnification objectives have a correction collar which can be adjusted to compensate for the thickness of the specific coverslip or plate bottom that you are imaging through. This function allows you to access the objectives for adjusting them, either from the side (if objective is in an end position) or from the top (objective in any position). Water Immersion objectives (if installed) can only be adjusted from the side.

If the correction collar is not adjusted appropriately, the image quality will suffer from spherical aberration, and the system may have difficulty focusing on the sample. It is recommended to check the correction collar setting every time you use one of these objectives when you are switching plate types, or if the system has multiple users.

The correction collar should be set to the physical thickness of the coverslip or plate bottom that you are imaging through. If you are not sure of the thickness of a plate bottom, either consult the plate manufacturer, or use the laser autofocus wizard to measure the plate bottom parameters, then multiply the optical bottom thickness (displayed in the MetaXpress software > Plate Acquisition Setup > Plates tab > Plate Bottom Settings) by the refractive index of the material (RI = 1.52 for glass, 1.59 for polystyrene). The **Adjust Correction Collar** function lists the thickness for common plates. Most coverslips have a thickness of 0.17 mm. In newer versions of the MetaXpress system, the correction collar setting will be calculated and displayed for the plate type currently selected in Plate Acquisition Setup.

When adjusting the objective, it is recommended to use gloves to protect the lens from skin oils. Most objectives display the setting in mm, but a few will display it using units of 100 * mm (set to 17 for a 0.17 mm coverslip). If you need to remove the objective to make the adjustment, be careful not to bump the dial when replacing it in the system.



The **Adjust Correction Collar** function can also be used to apply oil to oil immersion objectives. In this case, always use the option to access the objectives from the top.

g. Save Protocol

Save the current Plate Acquisition settings.

h. Acquire Plate

Run the current plate with the current Plate Acquisition settings.

i. Set up Slide Dimensions

This button, available on the **Run a Plate** and **Slide Scanning** taskbars, launches a wizard for creating a plate file for a slide with regular features. There are two ways to measure the slide dimensions: one is with a ruler showing mm, and the other is visually inside the system. The ruler method is recommended because it is quicker and more accurate.
To use this function, place the slide into the provided slide holder but do not load it in the system yet. Click **Set up Slide Dimensions**. It will step you through measuring the necessary dimensions of the slide in the slide holder.

Single slide holder (provided with all systems):

3-slide holder (optional purchase):

j. Slide Autofocus Wizard

The built-in plate laser autofocus wizard available from Plate Acquisition Setup may give inaccurate results with slides. Instead, you can use the Slide Autofocus Wizard, available on the **Run a Plate** and **Slide Scanning** taskbars.

If you use the "Set up Slide Dimensions" or the "Create Scan Areas" workflow, you will be automatically linked to the Slide Autofocus Wizard.

If you want to set up a new plate file, or add new objectives to an existing slide plate file, you can launch the Slide Autofocus Wizard from the taskbar. In the latter case, you will need the exact name of the plate file, which is available by looking at the list in **Plate Acquisition Setup > Plates** tab, or by finding the file in the Plates subfolder of the MetaXpress installation folder (e.g. **C:\MX6\Plates**).

After running the Slide Autofocus wizard, test the laser autofocus settings on your slide and manually adjust if needed.

k. Reset Interlocks

The ImageXpress instruments have safety interlocks for the following components:

- Laser autofocus
- Laser light source (optional)
- Fluidics (optional)

When the safety interlock is engaged (typically because of an open door or panel), the instrument will not allow the protected function to operate. Usually when the door is closed again, the interlock will reset on its own and the instrument will function normally. Occasionally, if MetaXpress still displays an interlock warning, it may be necessary to use the **Reset Interlocks** button to try to clear the errors.

If **Reset Interlocks** does not resolve the errors, verify that all doors and panels are fully closed and securely, then try again. If necessary, restart the instrument and software.

4. Slide Scanning taskbar

a. Open Door – Eject Slide

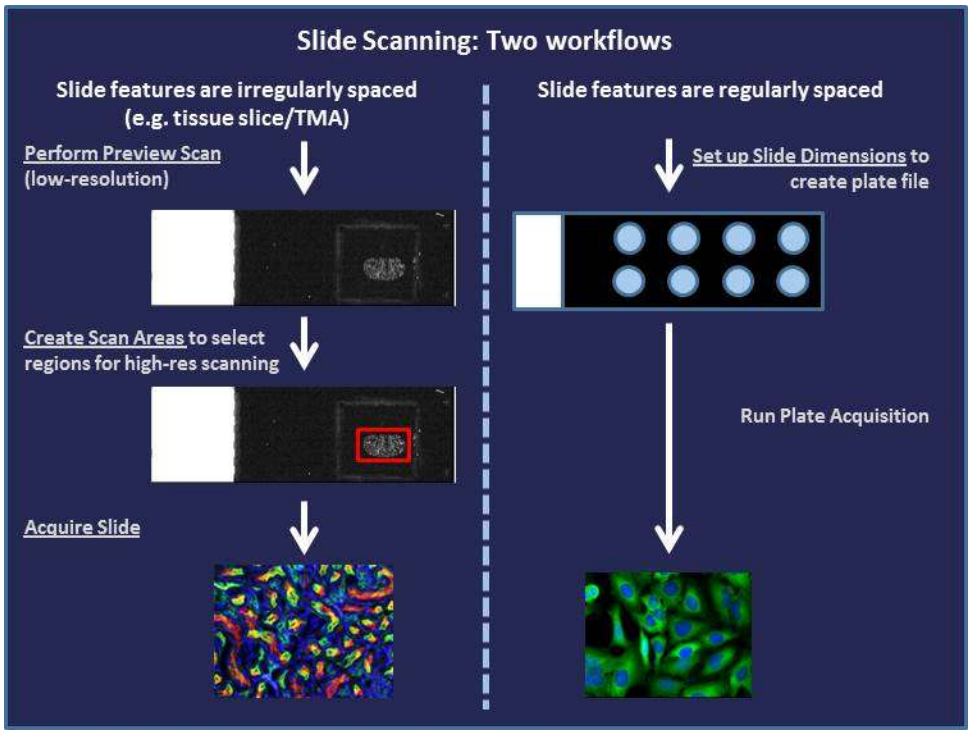
Click to open the door so that you can remove the slide holder.
If the door is already open but the plate clamp is closed, this will open the plate clamp.

b. Close Door – Load Slide

Closes the door and loads the slide holder.

c. Compare Slide Workflows

Click to open an image illustrating the two different slide workflows available from the taskbar.


Slide Scanning: Two workflows
Slide features are irregularly spaced
(e.g. tissue slice/TMA)
Perform Preview Scan
(low-resolution)
Create Scan Areas to select
regions for high-res scanning
Acquire Slide
Slide features are regularly spaced
Set up Slide Dimensions to
create plate file
Run Plate Acquisition

d. Perform Preview Scan

This wizard sets up and runs a low-magnification scan in one wavelength of the whole slide or user-designated portion of the slide. Use this function to quickly identify where on the slide the tissue section or coverslip or other region of interest is located. Transmitted Light or DAPI are most commonly used for the preview scan. A 2x objective is recommended if available; otherwise, use a 4x objective.

e. Create Scan Areas

This function steps user through drawing regions on a low-magnification preview scan, then translates these regions into plate acquisition settings. It creates a new protocol and/or plate file as needed, automatically

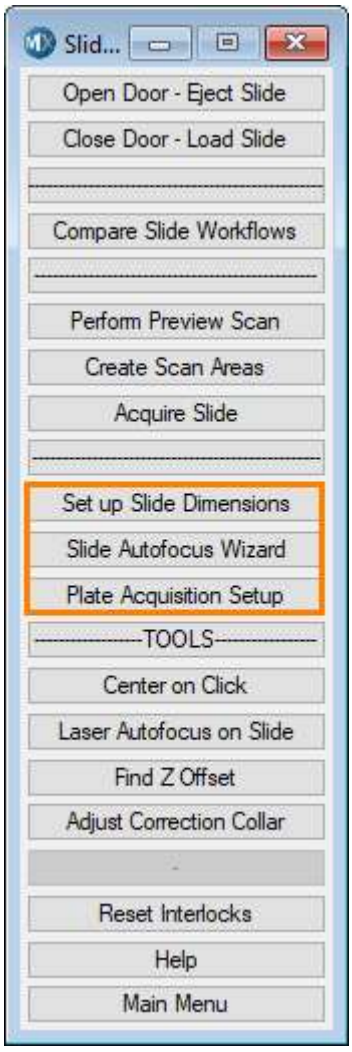
linking to the **Slide Autofocus Wizard** after the rest of the workflow is complete.

Each region selected on the preview scan is translated to a new "well" in the plate acquisition setup. The regions are divided into multiple sites as needed, depending on the region size and the objective selected. Each region must be the same size.

This workflow replaces the "**Slide Region Acquisition**" journals that were made available to MetaXpress 5 users.

f. Acquire Slide

Run the current slide with the current Plate Acquisition settings.



g. Set up Slide Dimensions

This button, available on the **Run a Plate** and **Slide Scanning** taskbars, launches a wizard for creating a plate file for a slide with regular features. There are two ways to measure the slide dimensions: one is with a ruler showing mm, and the other is visually inside the system. The ruler method is recommended because it is quicker and more accurate.

To use this function, place the slide into the provided slide holder but do not load it in the system yet. Click **Set up Slide Dimensions**. It will step you through measuring the necessary dimensions of the slide in the slide holder.

Single slide holder (provided with all systems):

3-slide holder (optional purchase):

h. Slide Autofocus Wizard

The built-in plate laser autofocus wizard available from Plate Acquisition Setup may give inaccurate results with slides. Instead, you can use the Slide Autofocus Wizard, available on the **Run a Plate** and **Slide Scanning** taskbars.

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After running the Slide Autofocus wizard, test the laser autofocus settings on your slide and manually adjust if needed.

i. Center on Click

Use the Center on Click function to move the stage to an area of interest on an image. This is particularly helpful when testing settings on a slide. You can click on the Preview Scan image to jump to a specific location on the slide as needed, instead of using the well/site navigation tools in Plate Acquisition Setup, which may move the sample somewhere unexpected.

This tool works even if you need to click in an area covered by a region. When you use the Center on Click function, it will temporarily hide the region(s) so that you can click in the desired area, then after clicking it will restore the region(s).

j. Laser Autofocus on Slide

Click to perform a laser autofocus on the slide in the current location with the current objective, helpful to quickly find focus. Note: this function uses slightly different settings than the laser autofocus used by Plate Acquisition. If using Plate Acquisition to image your slide, you should verify that the settings in Plate Acquisition work reliably.

k. Find Z Offset

You can use this tool to determine the Z offset from the current position. It offers a choice of image-based autofocus, image-based autofocus with expanded range, or interactive review of Z-stack around current position, with user-defined range and step size. This is similar to the Calculate Offset function available in Plate Acquisition Setup, and may be helpful for users who prefer using the Scan Slide interface for slide imaging. Note: The Scan Slide interface is not recommended for high-magnification imaging.

l. Adjust Correction Collar

Certain higher-magnification objectives have a correction collar which can be adjusted to compensate for the thickness of the specific coverslip or plate bottom that you are imaging through. This function allows you to access the objectives for adjusting them, either from the side (if objective is in an end position) or from the top (objective in any position). Water Immersion objectives (if installed) can only be adjusted from the side.

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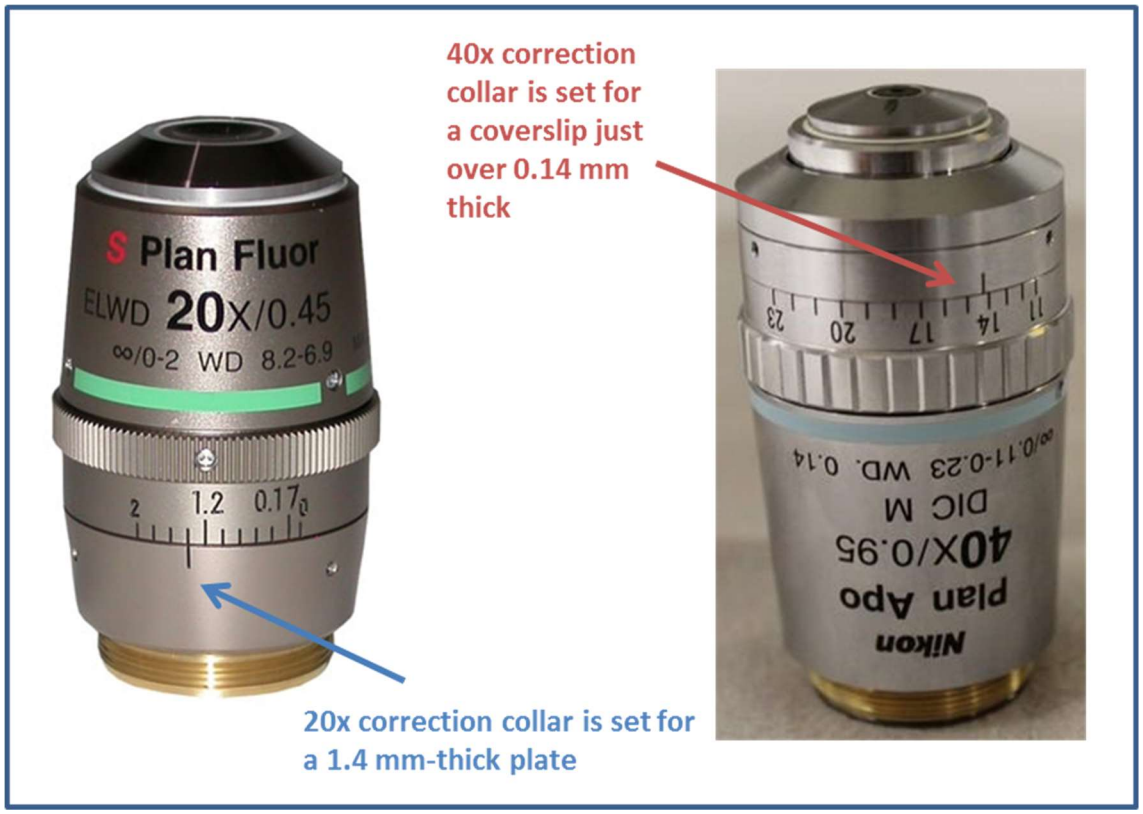


The **Adjust Correction Collar** function can also be used to apply oil to oil immersion objectives. In this case, always use the option to access the objectives from the top.


Slid...
Open Door - Eject Slide
Close Door - Load Slide
Compare Slide Workflows
Perform Preview Scan
Create Scan Areas
Acquire Slide
Set up Slide Dimensions
Slide Autofocus Wizard
Plate Acquisition Setup
----------TOOLS----------
Center on Click
Laser Autofocus on Slide
Find Z Offset
Adjust Correction Collar
Reset Interlocks
Help
Main Menu

m. Reset Interlocks

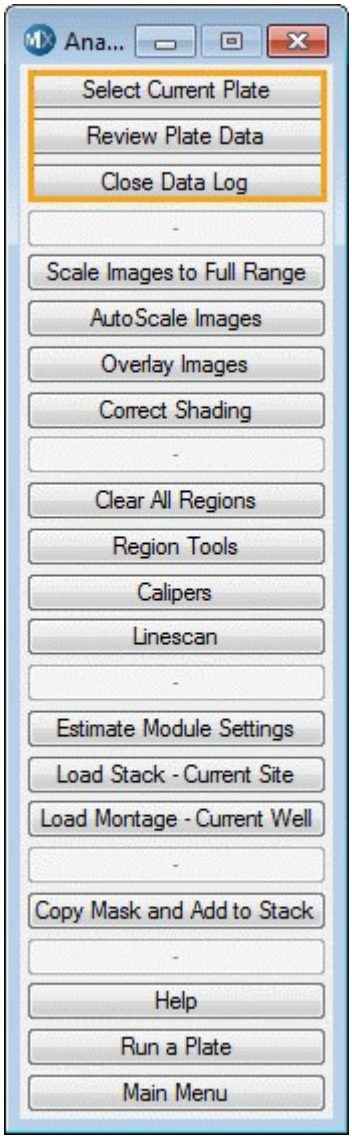
The ImageXpress instruments have safety interlocks for the following components:

- Laser autofocus
- Laser light source (optional)
- Fluidics (optional)

When the safety interlock is engaged (typically because of an open door or panel), the instrument will not allow the protected function to operate. Usually when the door is closed again, the interlock will reset on its own and the instrument will function normally. Occasionally, if MetaXpress still displays an interlock warning, it may be necessary to use the **Reset Interlocks** button to try to clear the errors.

If **Reset Interlocks** does not resolve the errors, verify that all doors and panels are fully closed and securely, then try again. If necessary, restart the instrument and software.

5. Analyze Images taskbar



a. Select Current Plate

Click to select for review the most recent plate acquired in this session of MetaXpress. Note: If no plate was acquired in this session of MetaXpress, this button will not do anything.

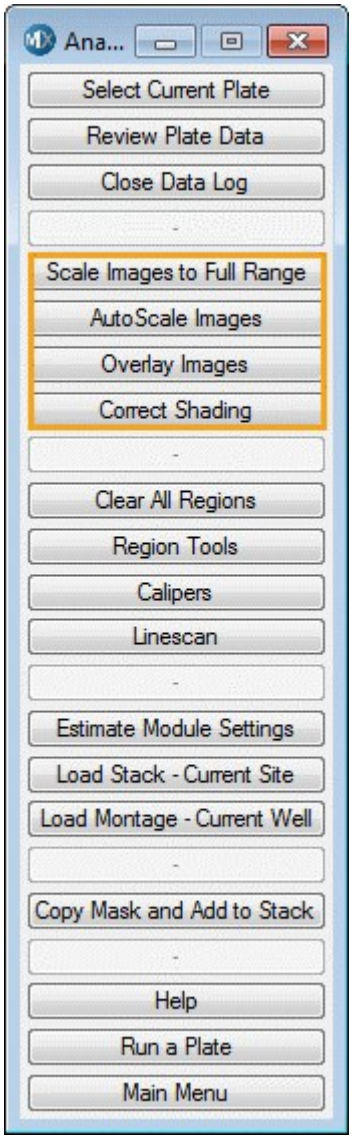
b. Review Plate Data

Opens the Review Plate Data dialog, which allows you to review plate and slide images.

c. Close Data Log

If you have clicked on the **Open Log** button in Review Plate Data to quickly export data to Excel or a text file, the data log will remain open until you close it. This may cause you to accidentally write extraneous data to the log as you test analysis settings. The **Close Data Log** button is a quick way to close the connection to the log.

Note: After closing, if you then reopen the data log and choose to write to the same sheet name or same text file, you may overwrite the existing data.



d. Scale Images to Full Range

Click this to set all open images to full scaling (brightness/contrast), which will be based on a 0-4095 scale for 12-bit cameras (IX Nano) or 0-65535 scale for 16-bit cameras (IX Confocal HT.ai, Micro Confocal, IX Micro 4, IX Micro XLS).

e. AutoScale Images

Click this to set all open images to auto scaling (brightness/contrast), which will be based on the content of the

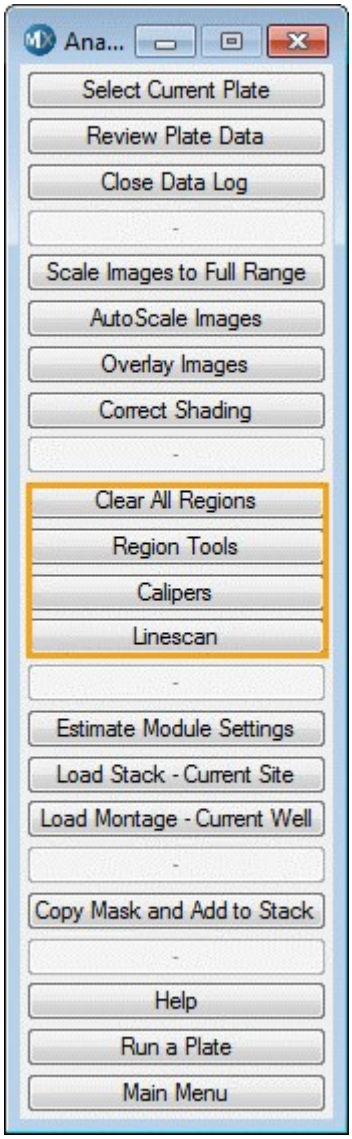
image. Auto scaling enhances the appearance of the objects in the image but makes it more difficult to compare intensity of one image to another.

f. Overlay Images

If there are images open named “Red”, “Green”, and “Blue”, this will automatically overlay them into an RGB image. Otherwise, it will open the Overlay Images dialog, which is recommended for creating presentation-quality color overlays.

g. Correct Shading

This function performs legacy shading correction on an image post-acquisition. You must have both the image to be corrected and the shading correction image open in MetaXpress.



h. Clear All Regions

This clears all regions from the current image. This is the same function found in the Regions menu.

i. Region Tools

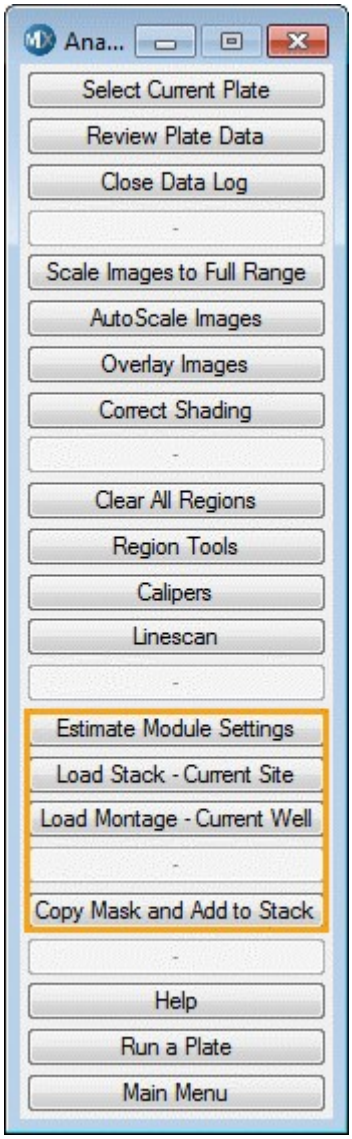
This opens the Region Tools toolbar for manually creating regions, which is helpful if the toolbar is not currently visible.

j. Calipers

This opens the Calipers tool for manually measuring the size of an object, which may be helpful when configuring analysis settings.

k. LineScan

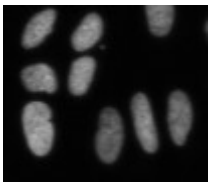
This opens the LineScan graph to quickly view the intensities along a line drawn on the current image. Use the line region tool to draw a new line. This is helpful for quickly estimating the intensity of an object and its local background, which may be helpful when configuring analysis settings.



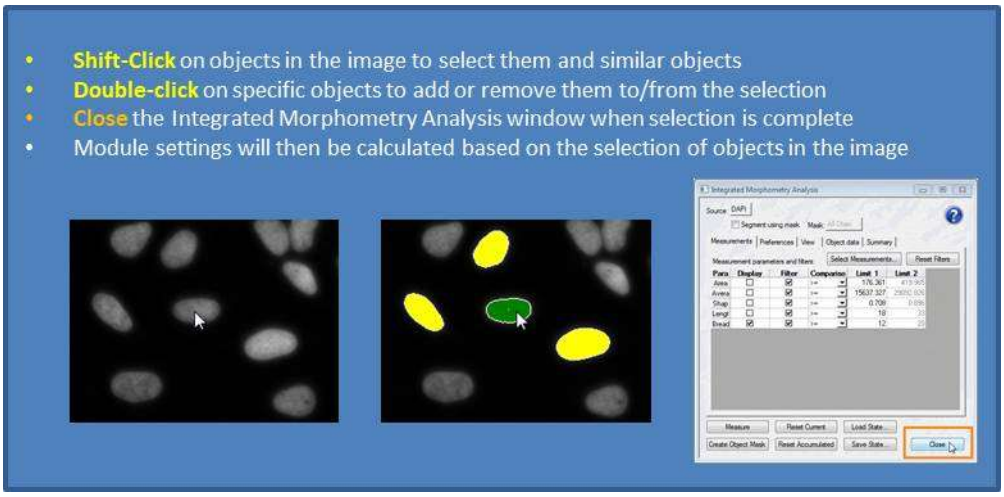
I. Estimate Module Settings

Use this tool to help estimate analysis settings to use in the application modules. It measures width, area, and intensity of objects in the selected image. There are two options: interactive mode (similar to the "Click to Find" tool in the Custom Module Editor) and the automatic mode (only recommended for very simple images such as bright beads on a dark background).

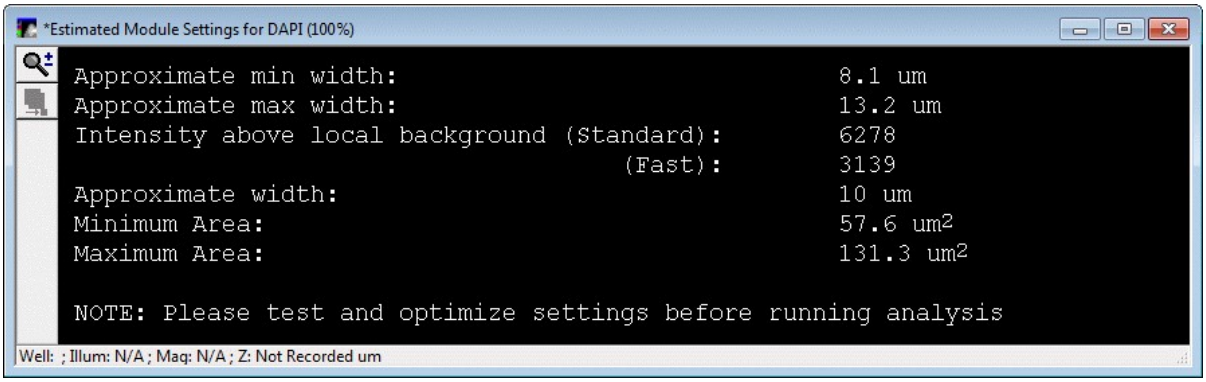
In interactive mode, after selecting the image to measure, adjust the threshold so that objects are distinct.

Original raw image	Good threshold setting: objects are distinct	Poor threshold setting: objects are joined together
		

In the next step, shift-click on individual objects to select them and objects similar to them. You do not need to select all of the objects, just a representative sample (e.g large and small cells, bright and dim cells). When you have selected enough cells, close the Integrated Morphometry Analysis window.



A window will now appear with recommended settings to start with for your application module. Test the settings on several example images and optimize as needed.



m. Load Stack – Current Site

This button will load image stacks for a timelapse or Z series plate for the current well/site/wavelengths being reviewed. With this option, you can stay in the "Well arrangement" view and still load the stacks.

To use this, you must have full-resolution single-wavelength images open from the currently selected plate in Review Plate Data. This tool will detect which well/site and wavelengths you are currently viewing, and load the appropriate stack for each wavelength.

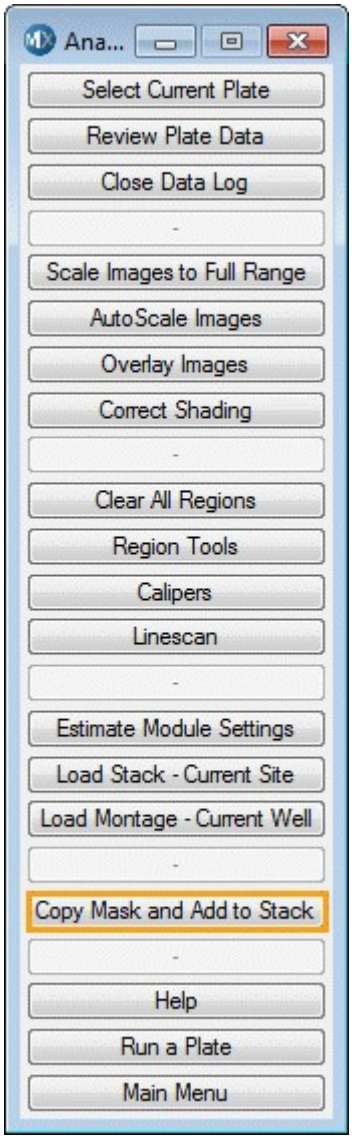
It will not work if the Color Composite option is enabled in Review Plate Data > Display, or if there are no full-resolution images open. Images must have the stage label information, so images imported from another microscope might not work.

n. Load Montage – Current Well

This button will create a montage(s) for a timelapse or Z series plate for the current well/wavelengths being reviewed.

To use this, you must have full-resolution images open from the currently selected plate in Review Plate Data. This tool will detect which well and wavelengths you are currently viewing, and create the appropriate montage for each wavelength, tiling or stitching as appropriate. If the images are from a timelapse or Z series experiment, the montage created will be from the current time point or Z plane being viewed.

It will not work if the Color Composite option is enabled in Review Plate Data > Display, or if there are no full-resolution images open. Images must have the stage label and stage position information, so images imported from another microscope might not work.



o. Copy Mask and Add to Stack

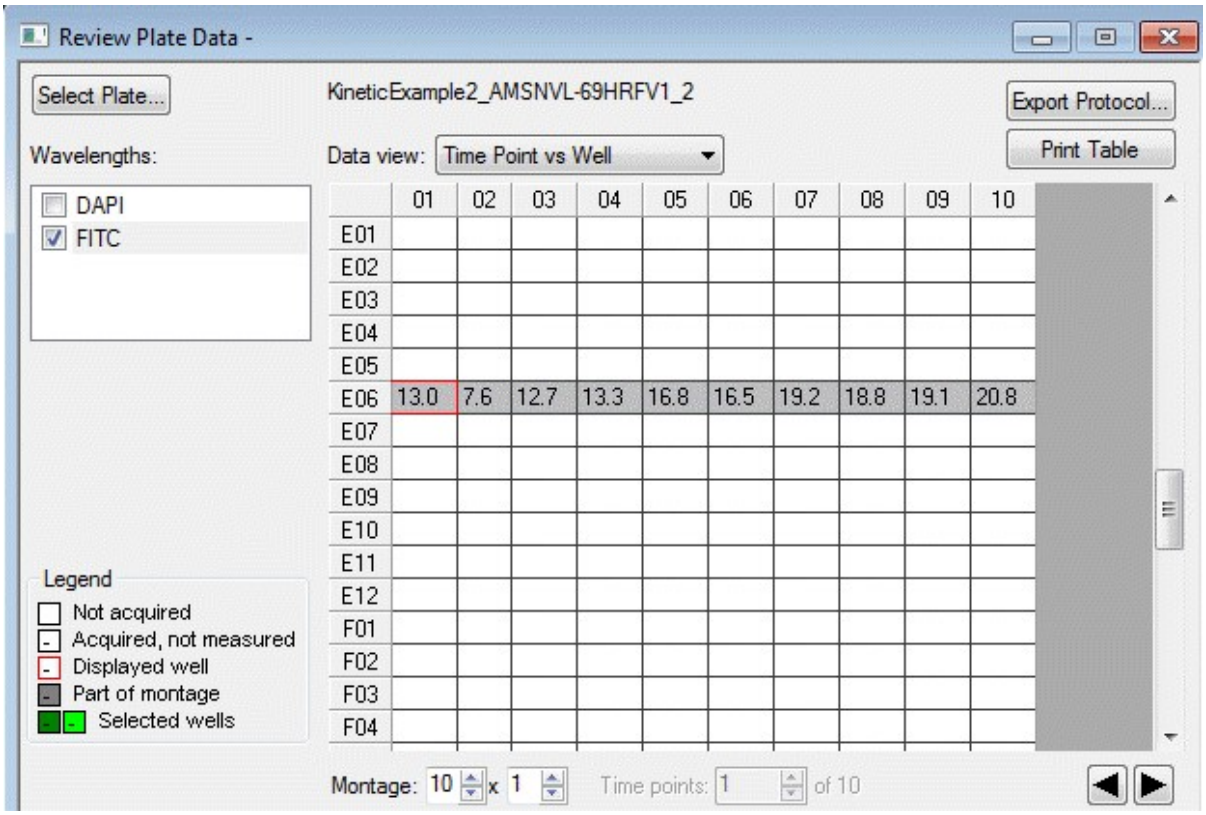
Use this tool to quickly extract segmentation masks saved to the database from a standard or custom module analysis.

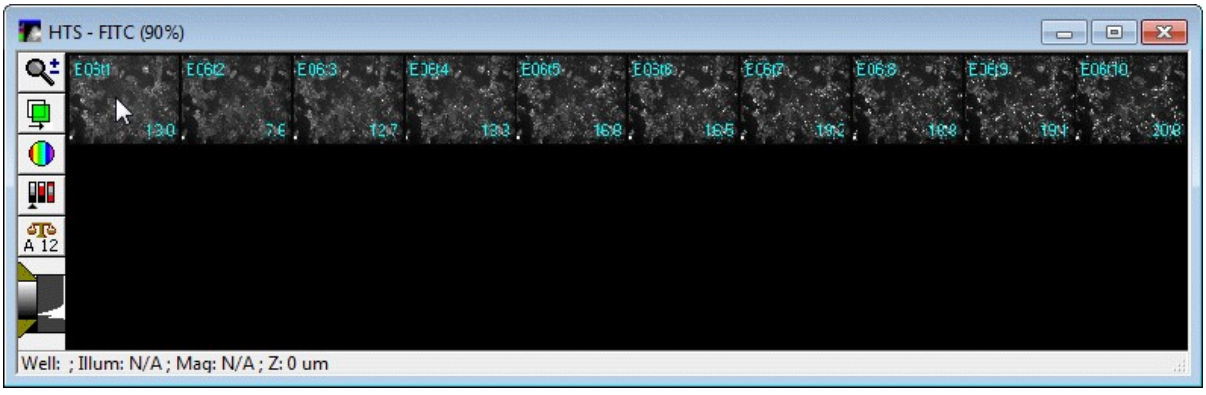
In Review Plate Data, select your plate of interest. On the Measurements tab, select the Measurement Set of interest (if plate was analyzed multiple times).

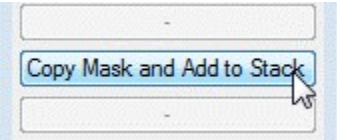
Set up the montage appropriately. If you want to retrieve masks from multiple time points, use the "Time vs Well" view, or use the "Z vs Well" view to retrieve masks from multiple Z planes.

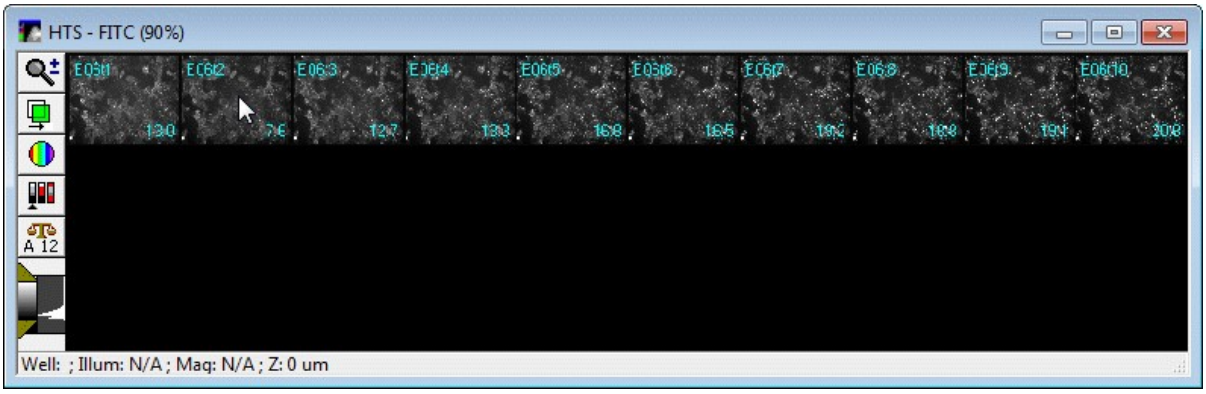
Identify a wavelength to save the overlays from. It is recommended to temporarily close the other

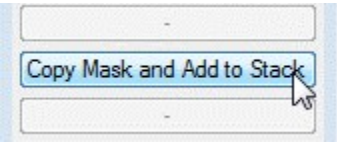
wavelengths to reduce the number of windows open in MetaXpress.
In the desired order, click on the thumbnail, then click on the Copy Mask and Add to Stack button. Repeat for all images that you want to retrieve masks from.
When you are done, review the resulting “Mask Stack”.
Save it with a different name if you want to create another set; otherwise, the next set will keep adding to the same “Mask Stack”.

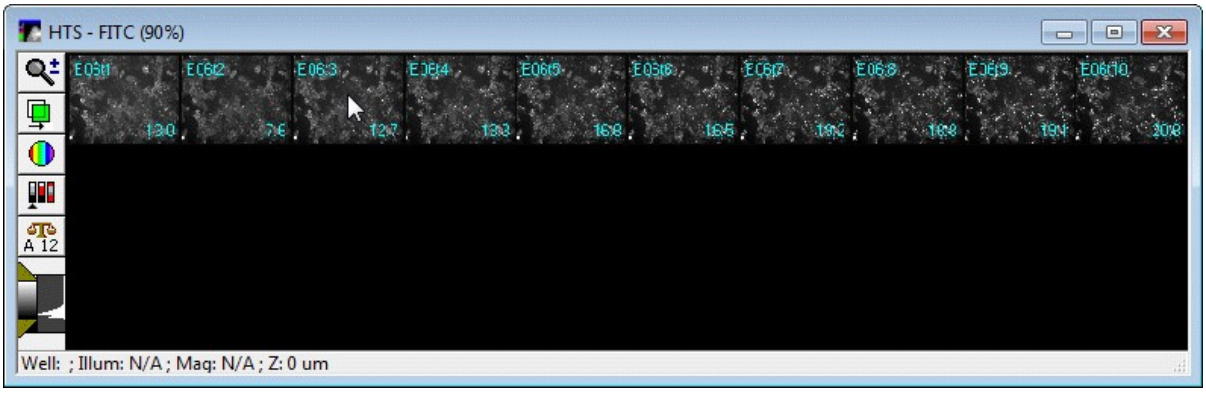


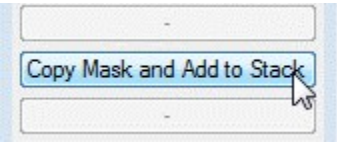




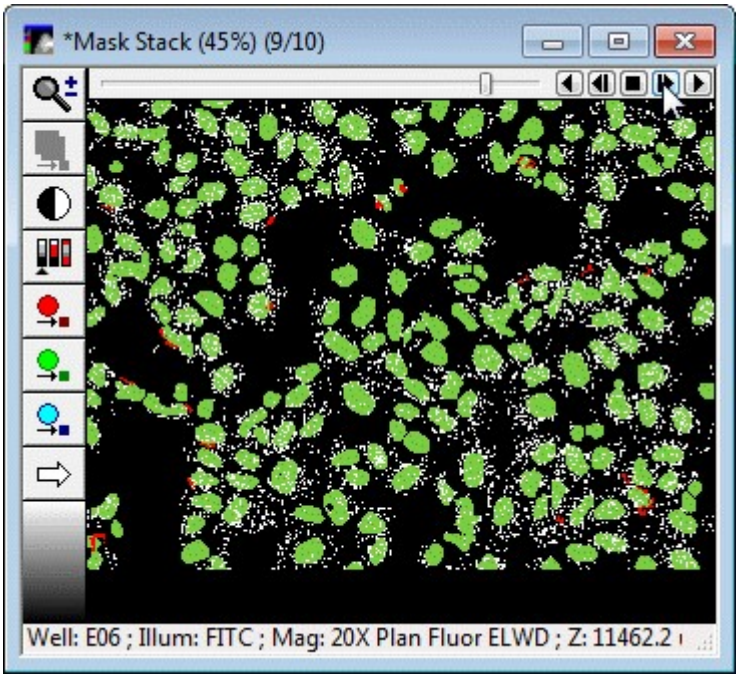




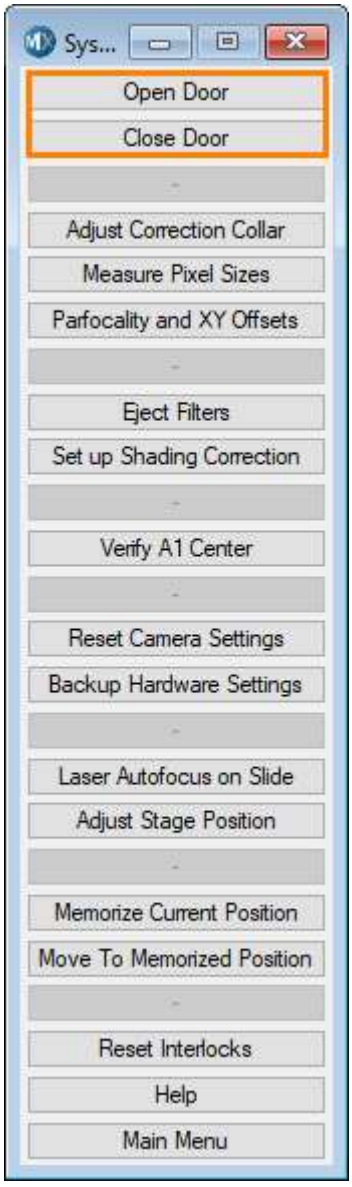




Repeat for other time points / sites / Z planes as desired. The resulting masks will be in a stack, in the order they were copied.


*Mask Stack (45%) (9/10)
Well: E06 ; Illum: FITC ; Mag: 20X Plan Fluor ELWD ; Z: 11462.2

6. System Maintenance taskbar

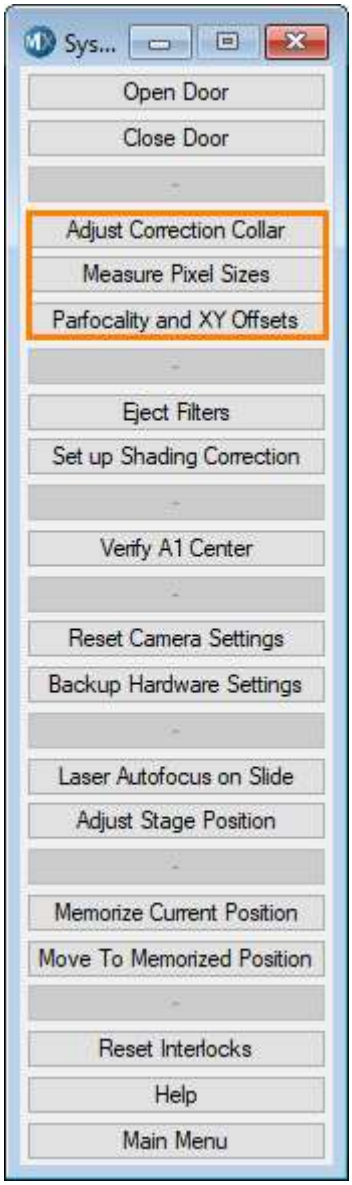


a. Open Door

Click, then select **Yes** to open the door without moving the stage and plate clamp.
Click, then select **No** to open the door and eject the plate normally.

b. Close Door

Click, then select **Yes** to close the door without moving the stage and plate clamp.
Click, then select **No** to close the door and load the plate normally.



c. Adjust Correction Collar

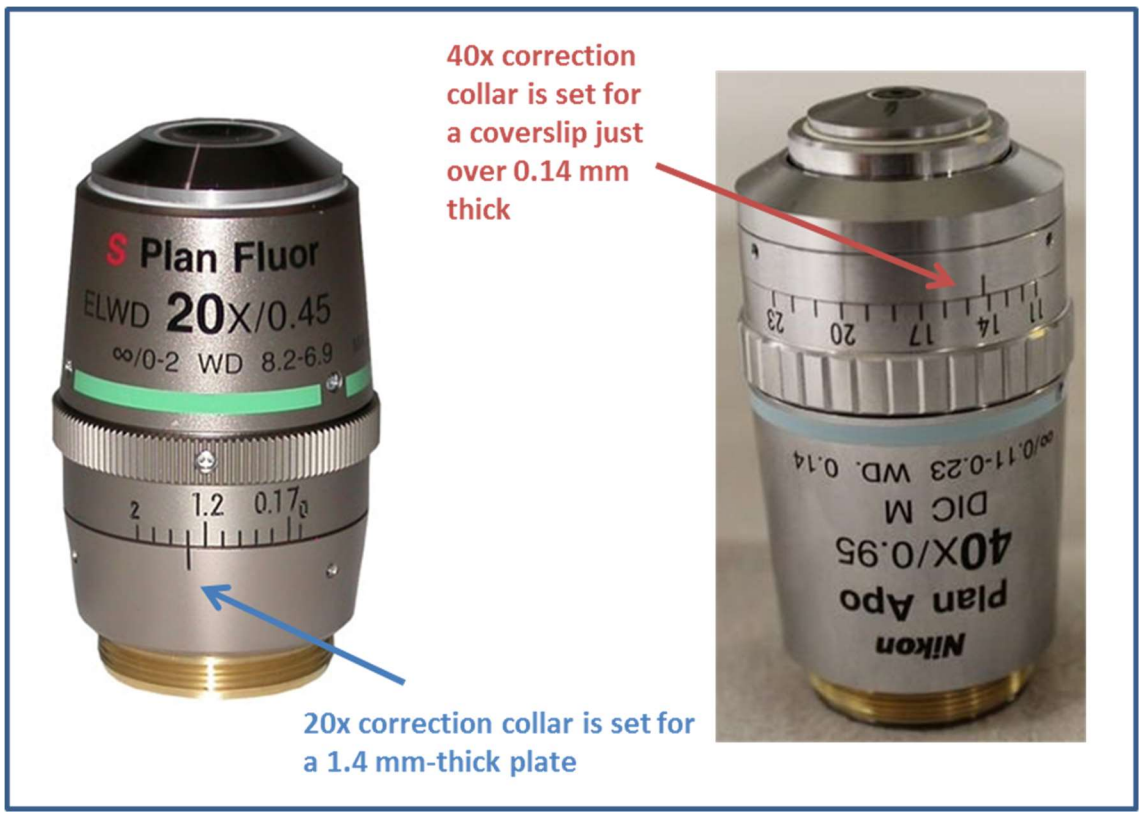
Certain higher-magnification objectives have a correction collar which can be adjusted to compensate for the

thickness of the specific coverslip or plate bottom that you are imaging through. This function allows you to access the objectives for adjusting them, either from the side (if objective is in an end position) or from the top (objective in any position). Water Immersion objectives (if installed) can only be adjusted from the side.

If the correction collar is not adjusted appropriately, the image quality will suffer from spherical aberration, and the system may have difficulty focusing on the sample. It is recommended to check the correction collar setting every time you use one of these objectives when you are switching plate types, or if the system has multiple users.

The correction collar should be set to the physical thickness of the coverslip or plate bottom that you are imaging through. If you are not sure of the thickness of a plate bottom, either consult the plate manufacturer, or use the laser autofocus wizard to measure the plate bottom parameters, then multiply the optical bottom thickness (displayed in the MetaXpress software > Plate Acquisition Setup > Plates tab > Plate Bottom Settings) by the refractive index of the material (RI = 1.52 for glass, 1.59 for polystyrene). The **Adjust Correction Collar** function lists the thickness for common plates. Most coverslips have a thickness of 0.17 mm. In newer versions of the MetaXpress system, the correction collar setting will be calculated and displayed for the plate type currently selected in Plate Acquisition Setup.

When adjusting the objective, it is recommended to use gloves to protect the lens from skin oils. Most objectives display the setting in mm, but a few will display it using units of 100 * mm (set to 17 for a 0.17 mm coverslip). If you need to remove the objective to make the adjustment, be careful not to bump the dial when replacing it in the system.

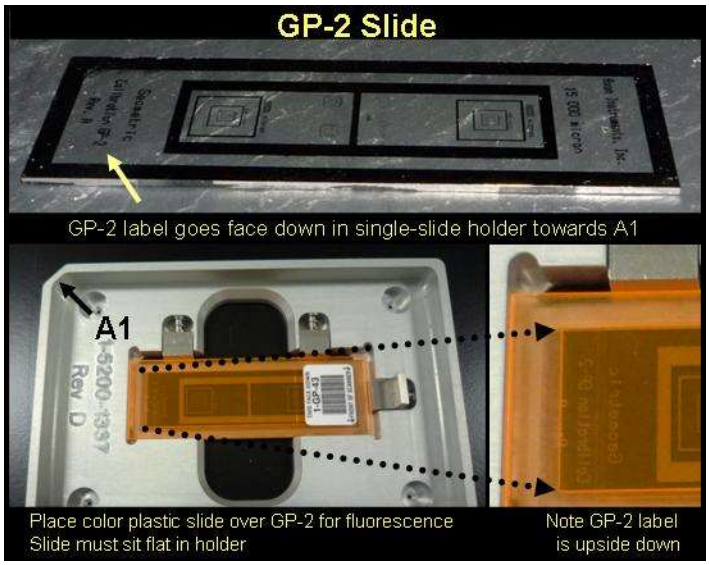


The **Adjust Correction Collar** function can also be used to apply oil to oil immersion objectives. In this case, always use the option to access the objectives from the top.

d. Measure Pixel Sizes

Each objective should have a pixel size calibration associated with it. This is important both for image acquisition (e.g. appropriate positioning of sites for montaging) and for image analysis (accurate size measurements of objects).

If you are configuring a new objective, or if you suspect that the pixel size calibration is incorrect for an existing objective, you can measure it using the provided GP-2 slide in the single slide holder, or by running a test montage on your current sample.

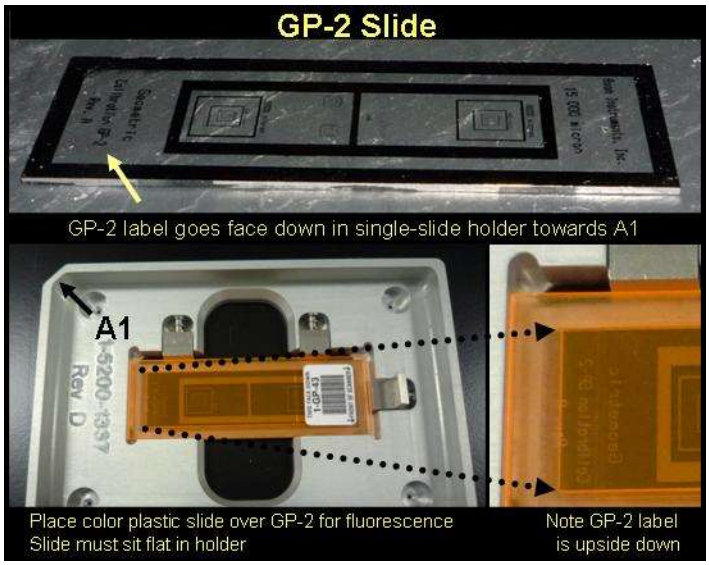


The **Measure Pixel Sizes** wizard will step you through measuring and updating the calibration values. It is recommended to test the new pixel size calibration before running critical experiments.

e. Parfocality and XY Offsets

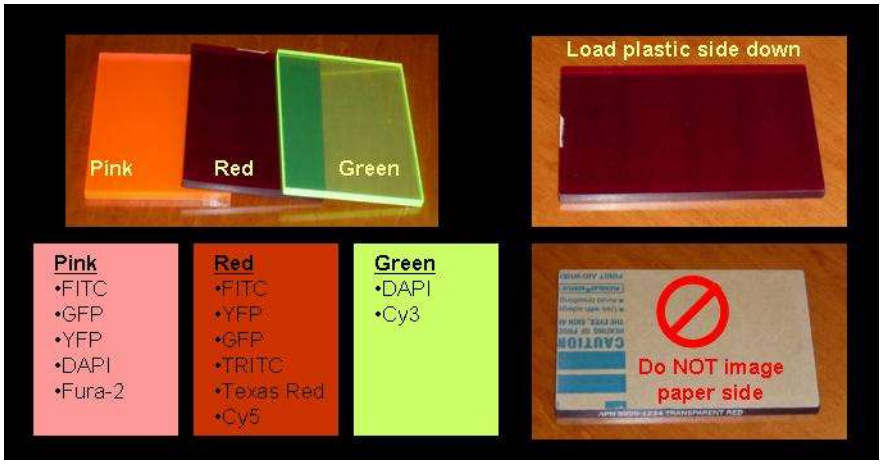
When switching between objectives, there is an offset in Z (parfocality) and an offset in XY (parcentricity). While these offsets are generally small, they may be enough to affect accurate image acquisition. Accurate parfocality measurements are important for focusing on the sample, and accurate XY offsets are important when using positions on a low-magnification image to determine where to image at high magnification (e.g. in the Create Scan Areas workflow for slide scanning).

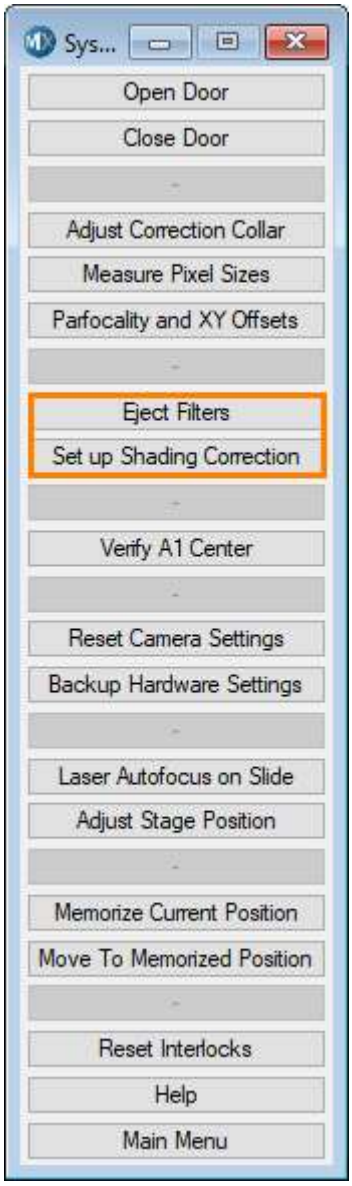
If you are configuring a new objective, or if you suspect that the parfocality or XY offsets are incorrect for the existing objectives, you can measure them using the provided GP-2 slide in the single slide holder.



The **Parfocality and XY Offsets** wizard will step you through measuring and updating the offset values.

If the XY offsets are not critical for your assays, and you want a quicker method to check the parfocality offsets, this wizard offers an alternative routine using a shading plate instead. Any of these colored plastic plates can be used.





f. Eject Filters

If this is a widefield system (ImageXpress Micro 4, ImageXpress Nano, ImageXpress Micro XLS, ImageXpress Micro XL, or ImageXpress Micro Standard), this button will move the filter cubes out into an accessible position for servicing (e.g. swapping filter cubes as needed). Once you have completed your maintenance, it will load the filter cubes into the system.

If this is a confocal system (ImageXpress Confocal HT.ai, ImageXpress Micro Confocal), this button will give you

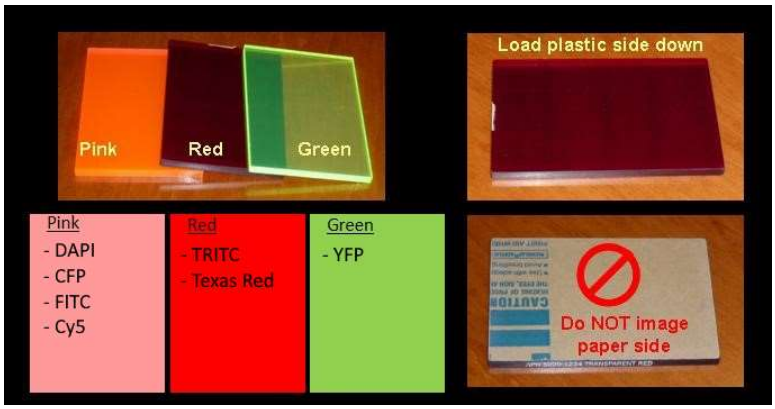
instructions on how to access the filter wheels using the Meta Imaging Series Administrator program.

g. Set up Shading Correction

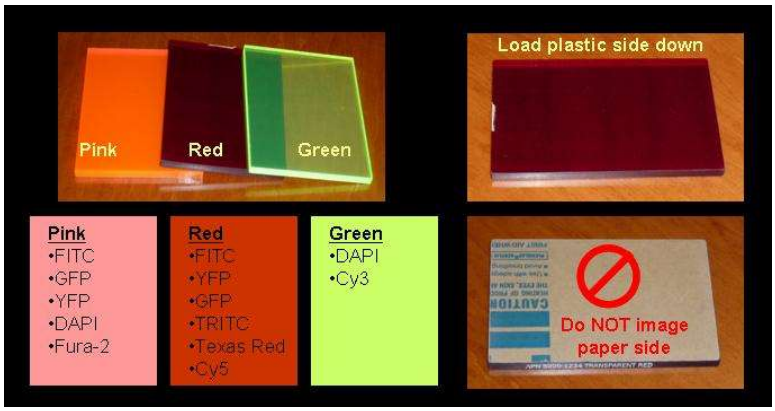
If you want to use legacy shading correction during plate or slide acquisition, you will need to have appropriate shading correction images available. The images should reflect the shading patterns of the optics in the system. Note that MetaXpress 6.1 and above provides automatic shading correction options, which should be tested before deciding to use legacy shading correction.

For a widefield system, fluorescent shading is dependent on the filter set and objective. For a confocal system, fluorescent shading is dependent on the disk selection, filter set, and objective.
Transmitted light shading is also dependent on the plate type, well size, volume used, position in the well, or the slide/coverslip combination used.

In most cases, the shading correction for fluorescence created using the provided plastic shading plates is appropriate. Select the option to set up shading correction for plates.



Shading plate compatibility for IX Confocal HT.ai



Shading plate compatibility for IXM-C, IXM4, IXM-XLS, IXN

If the normal shading correction images are not working well for fluorescent imaging of slides, you can use the option to set up shading correction for slides, and load one of the provided plastic GP slides into the slide holder.

GP slide compatibility for IX Confocal HT.ai

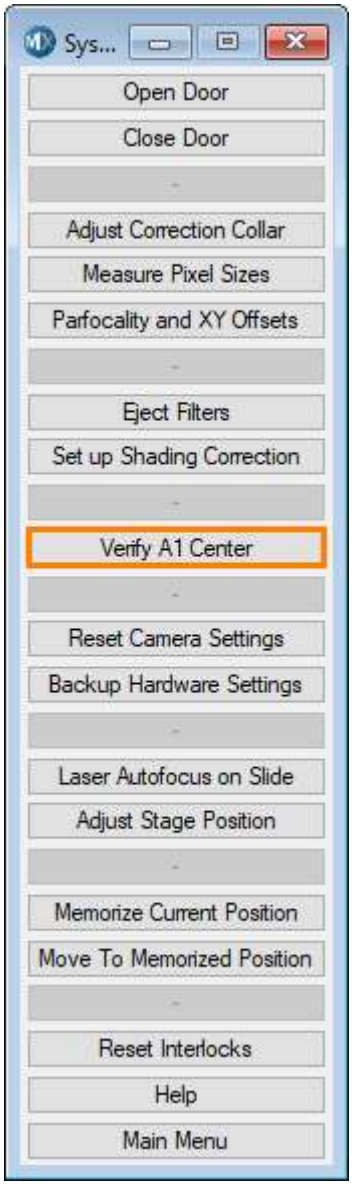
GP slide compatibility for IXM-C, IXM4, IXM-XLS, IXN

For transmitted light (brightfield, phase contrast, or RGB) shading correction on slides, select the option to set up shading correction for slides, and use a blank area on one of your normal slides/coverslips.

For the setup routine using the shading plates, you can select multiple filter sets and/or objectives to run in a single round automatically. The program will suggest a target intensity ~50% of the camera range. For optimal correction, use a target intensity comparable to the images you will be acquiring. Dim images from low-light samples may be overcorrected using bright shading correction images.

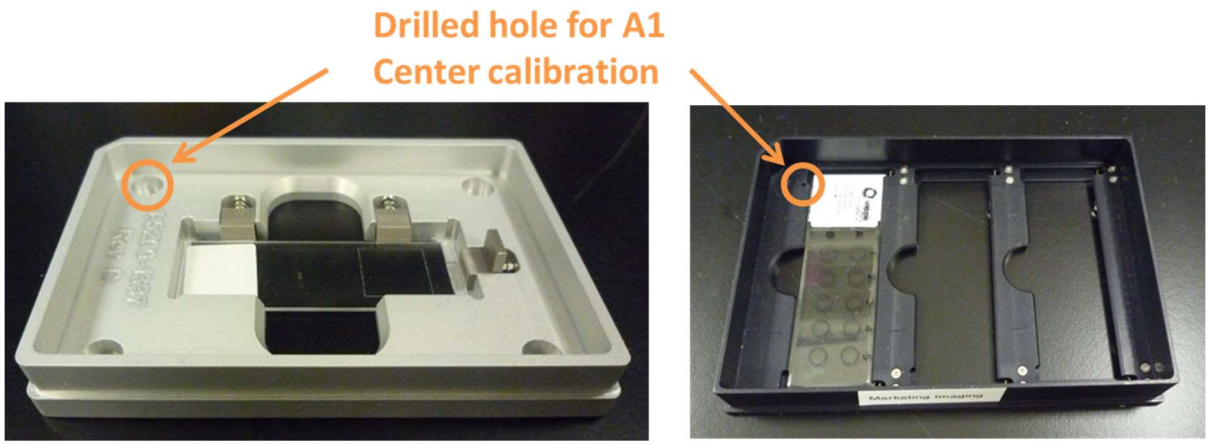
For the setup routine using the slides, you can select multiple filter sets and/or objectives to run, but you will be prompted to adjust each combination manually. You will have the opportunity to adjust the starting stage position, focus, intensity, and exposure time as needed.

The program will collect multiple images from different locations on the shading plate or slide, then calculate the median and save the result images to the selected folder using the required naming scheme.



h. Verify A1 Center

This function provides a quick way to test the A1 Center (XY) Calibration, using the provided slide holder. Both the single slide holder and the 3-slide holder have a drilled hole at the A1 Center position, corresponding to the center of A1 on a standard 96-well plate.

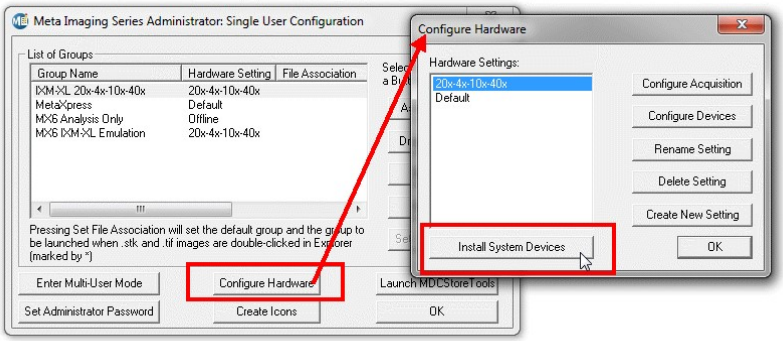


Follow the wizard to focus on the hole and verify if it is centered appropriately.

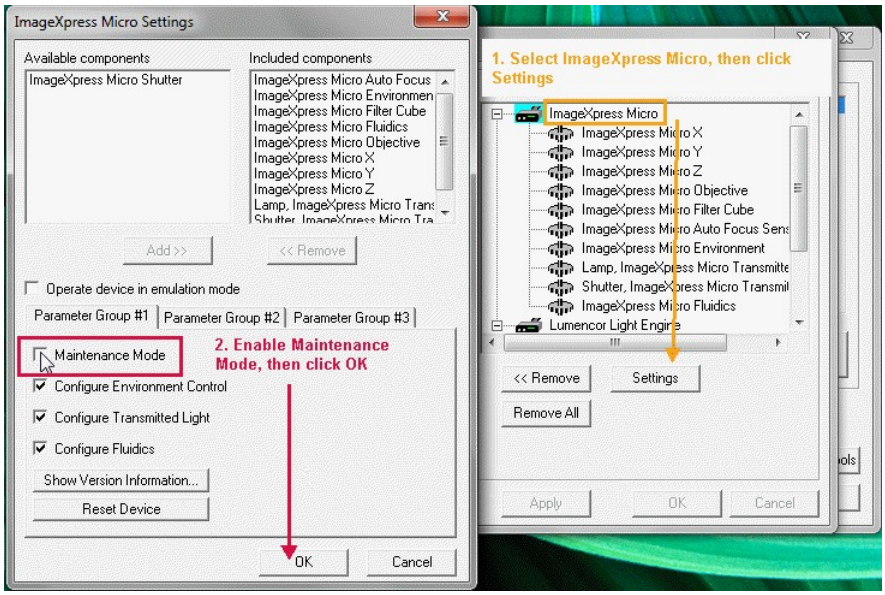
If it is not, you may use the same tool to recalibrate the A1 Center position. To do so, MetaXpress must be running in Maintenance Mode.

To enter Maintenance Mode:

i. Exit MetaXpress.
ii. Start the Meta Imaging Series Administrator program.
iii. Click **Configure Hardware**.
iv. Click **Install System Devices**.



v. On the right, select **ImageXpress Micro** and click **Settings**.
vi. Enable Maintenance Mode.

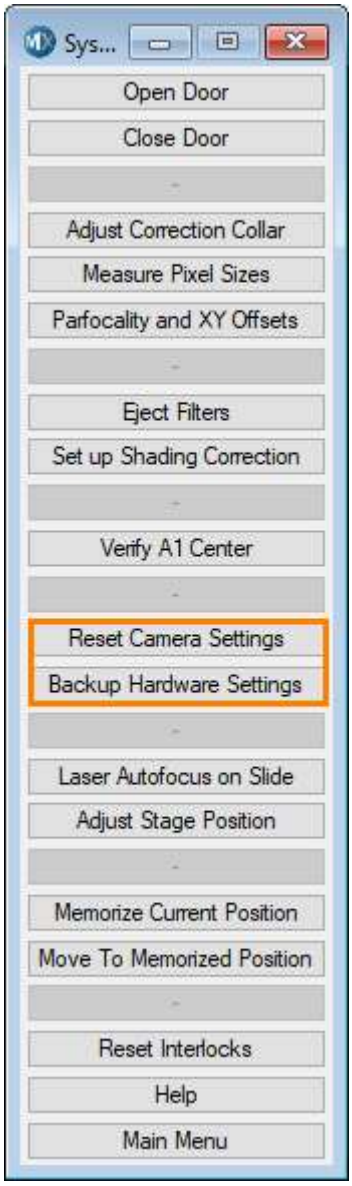


vii. Click OK four times to exit the Meta Imaging Series Administrator program

viii. Start MetaXpress. The top of the window should indicate that it is in Maintenance Mode.

When in Maintenance Mode, click **Verify A1 Center** on the System Maintenance taskbar. You will first be prompted to locate the drilled hole using the lowest magnification objective available. Then you will switch to the reference objective (typically the 10x) and fine-tune the position.

The **Verify A1 Center** tool will then provide instructions on how to enter the new calibration values in the Meta Imaging Series Administrator program. You should also disable the Maintenance Mode at this time.

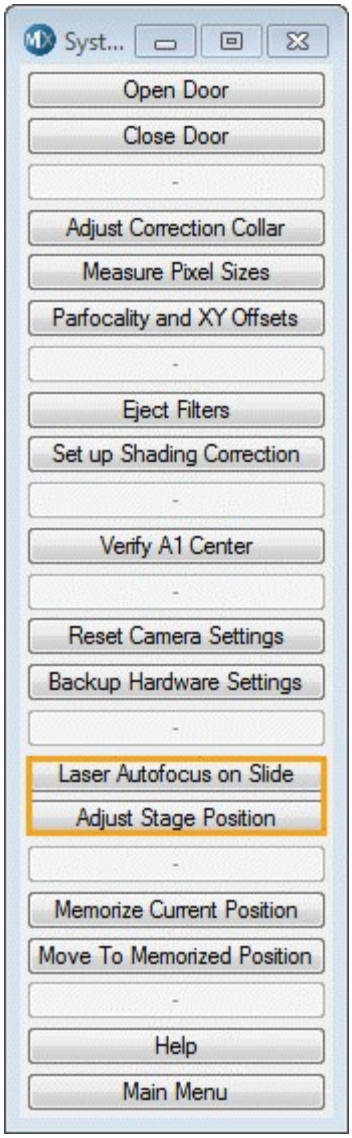


i. Reset Camera Settings

This function resets the camera settings (Acquire dialog) to the default for your instrument type. This might be helpful if you are experiencing unusual behavior from your camera.

j. Backup Hardware Settings

This function gives you a quick way to back up the critical hardware settings in MetaXpress: the illumination settings, magnification settings, and objective calibration settings.

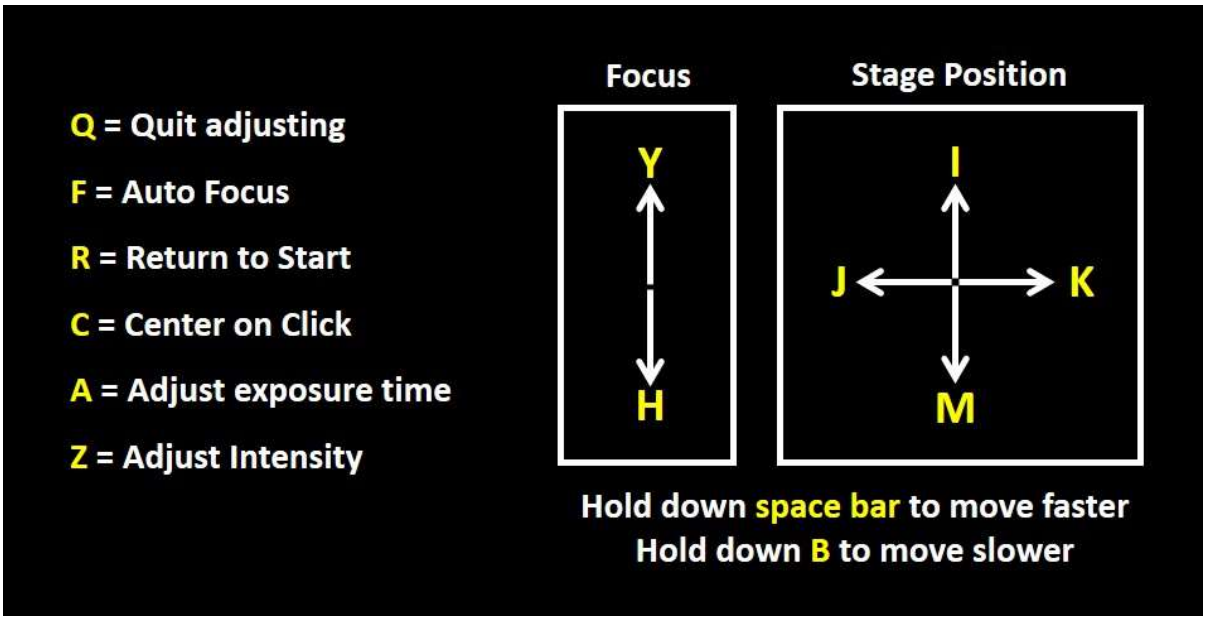


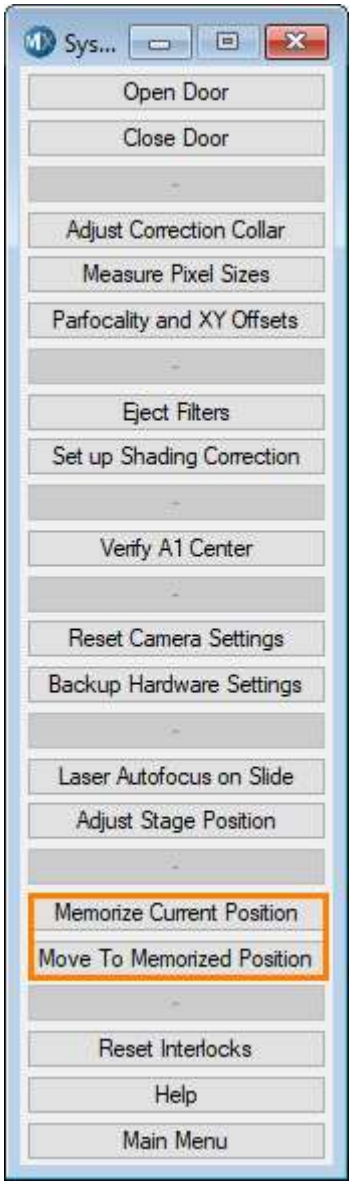
k. Laser Autofocus on Slide

Click to perform a laser autofocus on the slide in the current location with the current objective, helpful to quickly find focus. Note: this function uses slightly different settings than the laser autofocus used by Plate Acquisition. If using Plate Acquisition to image your slide, you should verify that the settings in Plate Acquisition work reliably.

I. Adjust Stage Position

This is an interactive tool for adjusting the stage position of the system, which may be helpful in locating a region of interest on your sample. Instead of using live mode, a new image is snapped after every move. Use your keyboard keys to move the stage as indicated in the instructional image. This tool is also used in some of the maintenance wizards. Note that keyboard must be local to that computer (might not work from remote desktop or webex session).





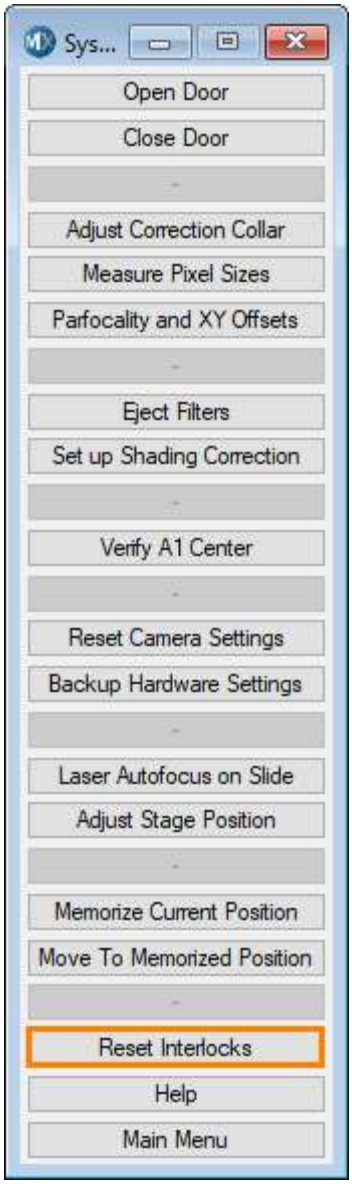
m. Memorize Current Position

Memorize the current X, Y, and Z position so that you can quickly return to it. Click on this, then assign an easy-to-remember name to this position. Note: Positions are only stored within this session of MetaXpress.

n. Move to Memorized Position

Move to a named position stored using the **Memorize Current Position** function.

These tools may be used to verify plate dimensions in the system. With the door closed, use the tools in Plate Acquisition Setup to move to the center of various wells and memorize them (e.g. “a1”). Open the door, then move to memorized position to verify that the well positions are located as expected for your plate.



o. Reset Interlocks

The ImageXpress instruments have safety interlocks for the following components:

- Laser autofocus
- Laser light source (optional)
- Fluidics (optional)

When the safety interlock is engaged (typically because of an open door or panel), the instrument will not allow the protected function to operate. Usually when the door is closed again, the interlock will reset on its own and the instrument will function normally. Occasionally, if MetaXpress still displays an interlock warning, it may be necessary to use the **Reset Interlocks** button to try to clear the errors.

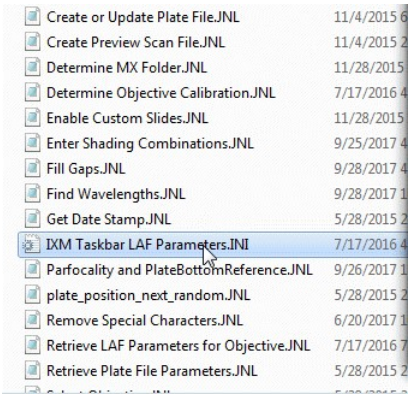
If **Reset Interlocks** does not resolve the errors, verify that all doors and panels are fully closed and securely, then try again. If necessary, restart the instrument and software.

Appendix A – Customize the default Laser Autofocus settings

This procedure is for advanced users only. If you are not familiar with manually adjusting laser autofocus parameters, do not change the settings described here.

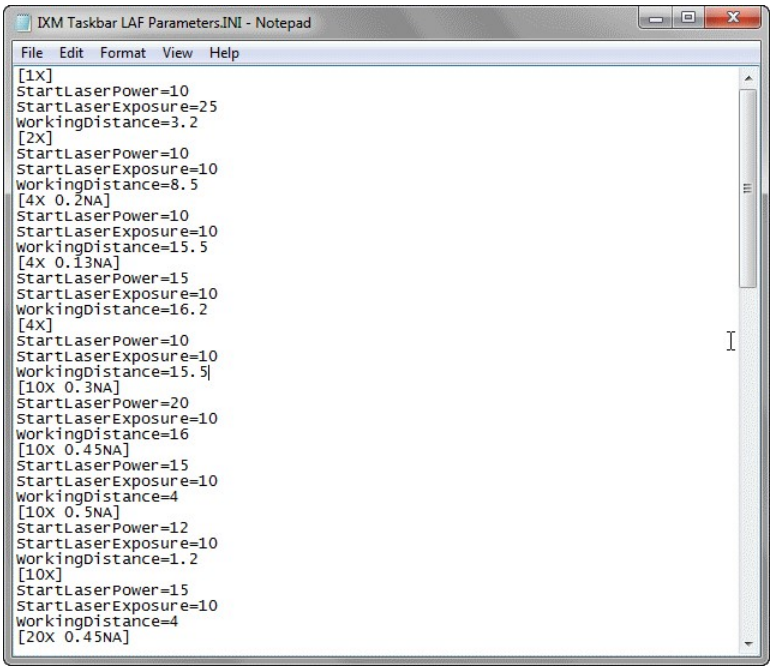
In the IX Taskbar version 6.2 and above, the laser autofocus on slide journal, used in the function **Laser Autofocus on Slide** as well as in tools such as **Set up Shading Correction** or **Perform Preview Scan**, looks up default starting values for the laser autofocus in an .INI file. This makes it easier to adjust the settings if a particular objective is not focusing on slides using the taskbar.

To make adjustments, look in the **\Taskbars\Taskbar_Journals\Utility Journals** subfolder in the MetaXpress installation directory (e.g. **C:\MX6\TASKBARS\Taskbar_Journals\Utility Journals**) and locate the file **IXM Taskbar LAF Parameters.INI**.



Open this file using Notepad or equivalent text editor.

Settings for each objective are organized by the magnification and numerical aperture (NA). If settings do not exist for a particular objective, then the settings under only the magnification are used.



Locate the section for your specific objective, then modify the settings as needed. If you cannot find a section that corresponds to your particular objective, you may add a new section, following the format in the file. Unlike the laser autofocus settings found in Plate Acquisition Setup, the exposure time used here cannot go below 10 ms. If the laser signal is too bright at 10 ms, the laser power must be lowered. Full power is 100. A laser power setting of 25 indicates that 25% full power will be used.

Once you are done modifying the file, save it without changing the file name. Test to verify the new settings are working as expected.

Note that the settings listed here are starting settings, used in the first attempt for laser autofocus. If focus is not found in the first attempt, then it will be retried with longer exposure/higher laser power. If it fails the second attempt, then the search range will be expanded for the 3rd attempt. If it still fails, then either an error is shown to the user, or the user is provided the option to find the focus manually (depending on the specific taskbar function used).

Appendix B – Enable use of Custom Slide holders

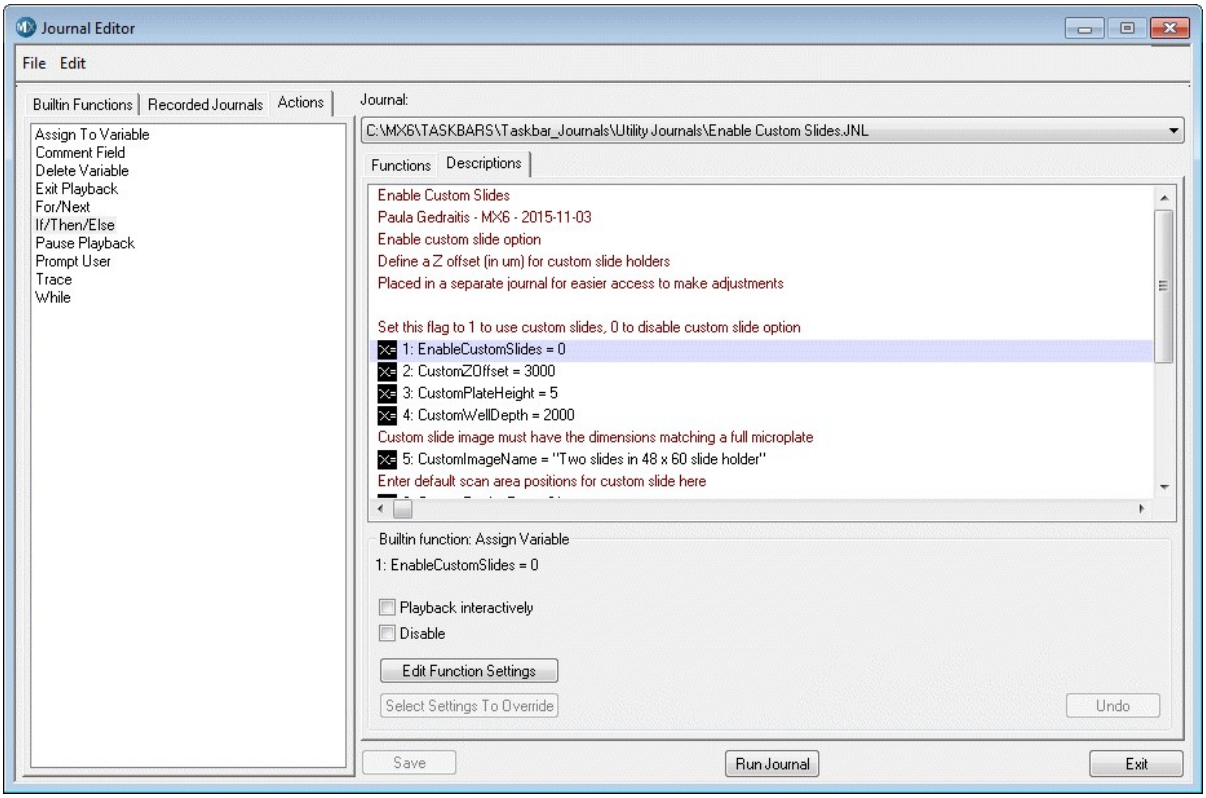
This procedure is for advanced users only. If you are not familiar with image calibration, editing journals or configuring plate files, do not change the settings described here.

The slide tools available on the taskbar (Perform Preview Scan, Create Scan Areas, Set up Slide Dimensions) normally only work with the single slide holder or the 3-slide holder available from Molecular Devices.

However, some users have created their own custom slide holders with unique dimensions and would like to use the same slide tools.

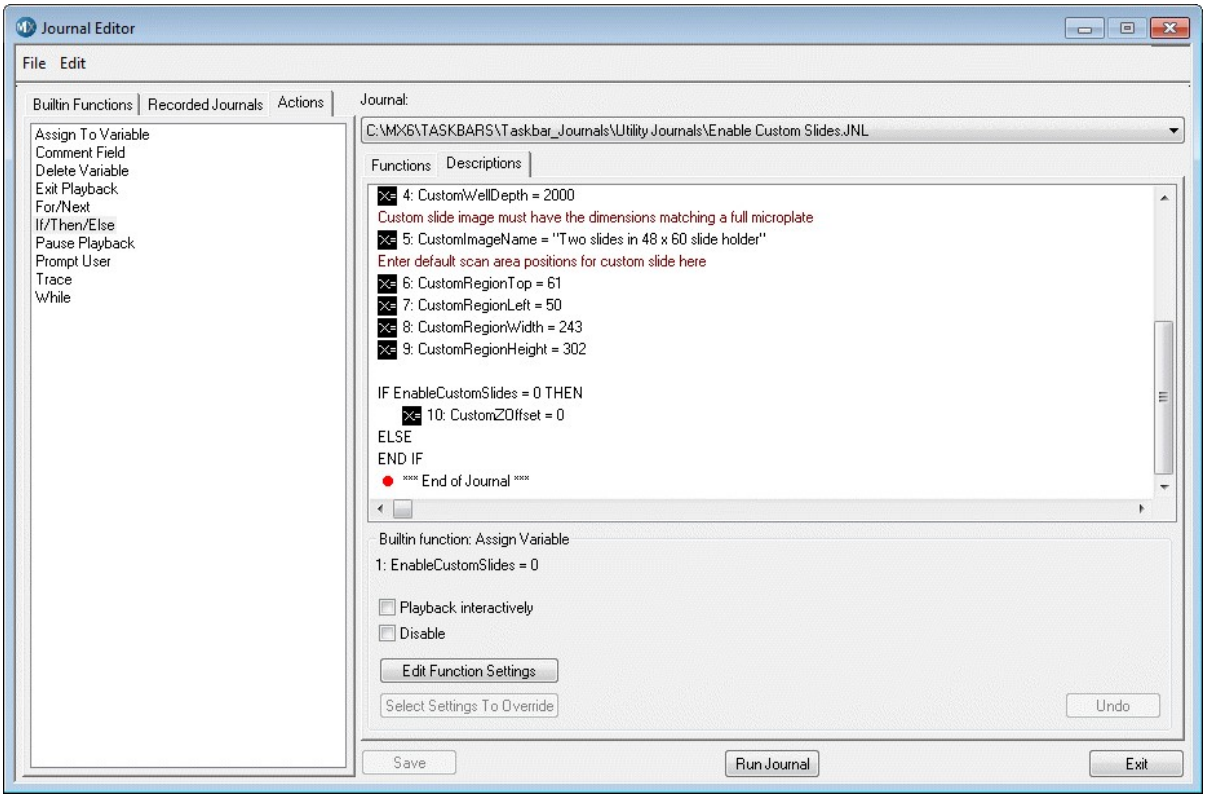
In the IX Taskbar version 6.2 and above, there is a journal provided for enabling the use of a custom slide holder. Note that only one custom slide holder type may be used per installation of the taskbar.

To enable the custom slide holder, go to **Journal > Edit Journal** (standard menu structure) or **Control > Journal > Edit Journal** (simplified menu structure) and select the **Enable Custom Slides.JNL** file in the **C:\MX6\TASKBARS\Taskbar_Journals\Utility Journals** folder (or equivalent).



Change the custom slide parameters (variables) as appropriate.

- **EnableCustomSlides**: Set to 1 to allow use of custom slides, 0 to only allow the single slide holder or 3-slide holder.
- **CustomZOffset**: Alters the start position for the laser autofocus search range used by the taskbar tools. This value is defined in um. Standard slide holders hold the slides in a very low position and use an offset of 0 um. If your slide holder holds slides higher than normal, increase this value accordingly.
- **CustomPlateHeight**: Use to define the plate height in mm which should be used for the custom slide holder. This value, together with the **CustomWellDepth**, will be included in any plate files updated or created using the custom slide option, and will affect the autofocus search range used in Plate Acquisition. It does not affect laser autofocus search range used by the taskbar tools.
- **CustomWellDepth**: Use to define the well depth in um which should be used for the custom slide holder. This value, together with the **CustomPlateHeight**, will be included in any plate files updated or created using the custom slide option, and will affect the autofocus search range used in Plate Acquisition. It does not affect laser autofocus search range used by the taskbar tools.



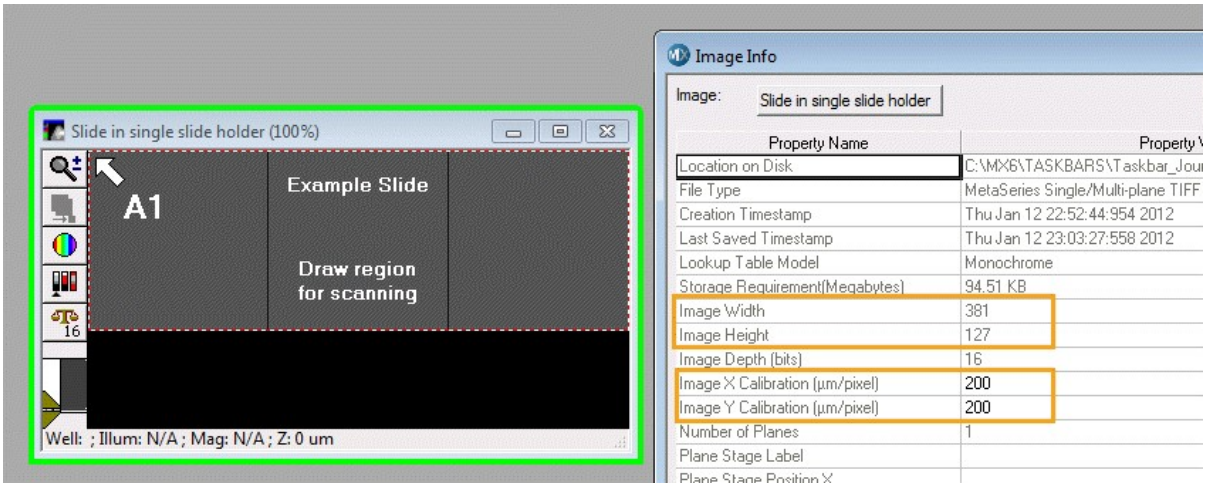
- **CustomImageName**: An image is used for the user to select the general scanning region. This image must be calibrated to match the slide holder dimensions. This variable must be set to the filename of that image. See below for more information.
- **CustomRegionTop**: This value, in pixels, defines the top edge of the starting region for preview

scanning (adjustable by user when setting up Preview Scan).

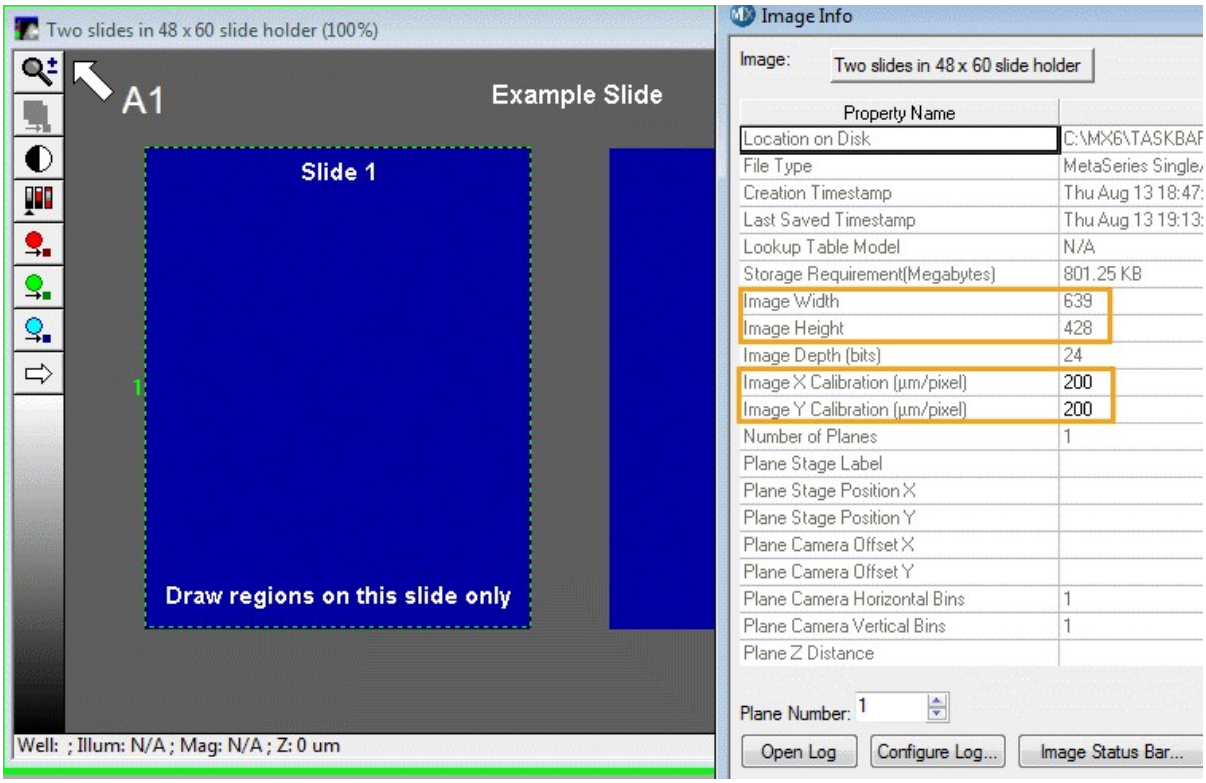
- **CustomRegionLeft**: This value, in pixels, defines the left edge of the starting region for preview scanning (adjustable by user when setting up Preview Scan).
- **CustomRegionWidth**: This value, in pixels, defines the width of the starting region for preview scanning (adjustable by user when setting up Preview Scan).
- **CustomRegionHeight**: This value, in pixels, defines the width of the starting region for preview scanning (adjustable by user when setting up Preview Scan).

You must also create a custom image with appropriate calibration to represent the slide holder. Both a template image, “Blank Slide Holder Template.tif”, and an example image "Two slides in 48 x 60 slide holder" are provided with the taskbar files. Unlike the standard single slide and 3-slide holder images, the custom image must map to a full-sized plate (127.8 mm x 85.5 mm), using the image calibration. The image must be saved to **C:\MX6\TASKBARS\Taskbar_Journals\Images** or equivalent folder, depending on your MetaXpress and taskbar installation directories.

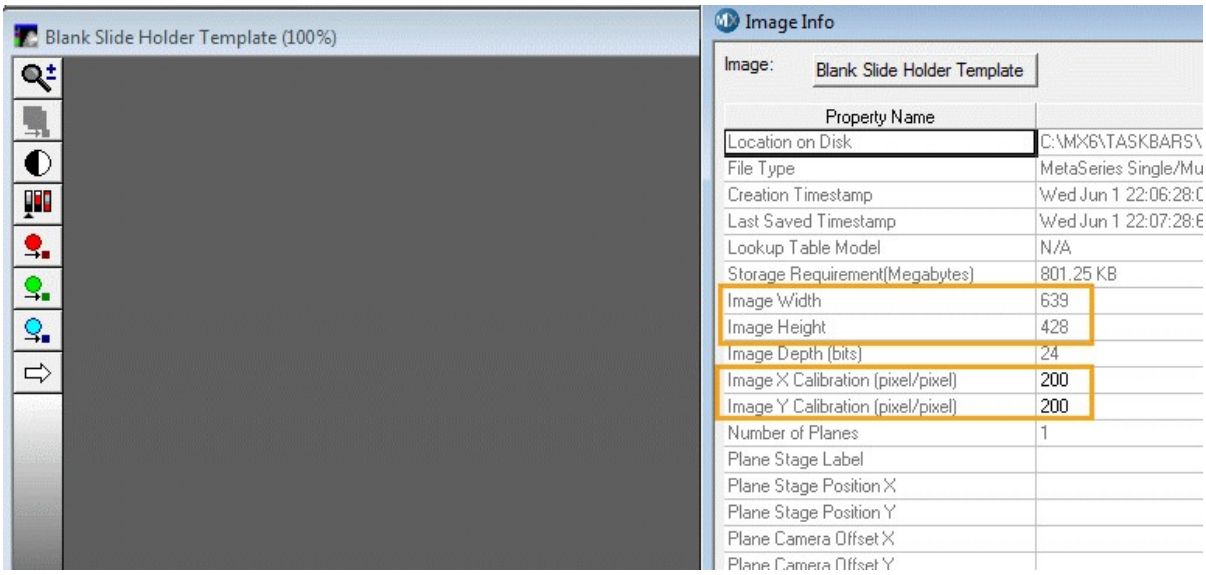
Single slide holder:



Example custom slide holder:



Template blank image:



The performance of the slide scanning tools with a custom slide holder depends on the accuracy of the slide image and the values entered in the journal. It is important to test these settings on example slides before using them for critical experiments.