



ImageXpress Taskbar User Guide

Version 6.7

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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:

💷 Mai 🗖 🗖 💌
Run a Plate
Slide Scanning
Analyze Images
System Maintenance
Help
-
Run IX Taskbar Installer

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
Tum off Transmitted Lamp
<i></i>
Load Protocol
Plate Acquisition Setup
Adjust Correction Collar
Save Protocol
Acquire Plate
*
Set up Slide Dimensions
Slide Autofocus Wizard
9 (A)
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.

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Open	Door - Eject I	Plate
Close	Door - Load I	Plate
Turn of	f Transmitted	Lamp
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1	oad Protocol	
Plate	Acquisition S	etup
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S	ave Protocol	
1	Acquire Plate	
	<i></i>	
Set up	Slide Dimen	sions
Slide	Autofocus Wi	izard
Re	eset Interlock	s
	Help	
A	nalyze Images	S
	Main Menu	

🕦 An... 📼 🗉 🔜 Select Current Plate **Review Plate Data** Close Data Log Scale Images to Full Range AutoScale Images Overlay Images Correct Shading/Background Clear All Regions Region Tools Calipers Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu

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).

- Press F1 for MetaXpress Help Files	^
Call 1-800-635-5577 (North America)	
or +44-118-944-8000 (UK)	
or 00800 665 32860 (Free, parts of EU)	
X Taskbar version 6.7.1, System ID:	
Profile: MetaXpress	v

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

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Open Door - Eject Plate
Close Door - Load Plate
Turn off Transmitted Lamp
a
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

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.

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Open Door - Eject Plate
Close Door - Load Plate
Tum off Transmitted Lamp
1
Load Protocol
Plate Acquisition Setup
Adjust Correction Collar
Save Protocol
Acquire Plate
Set up Slide Dimensions
Slide Autofocus Wizard
÷ (1
Reset Interlocks
Help
Analyze Images
Main Menu

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.

🐠 Ru 📼 🗉 💌
Open Door - Eject Plate
Close Door - Load Plate
Tum 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

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.

🐠 Ru 📼 🔳 🛋
Open Door - Eject Plate
Close Door - Load Plate
Tum off Transmitted Lamp
<i>t</i>
Load Protocol
Plate Acquisition Setup
Adjust Correction Collar
Save Protocol
Acquire Plate
1
Set up Slide Dimensions
Slide Autofocus Wizard
÷ 1
Reset Interlocks
Help
Analyze Images
Main Menu

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

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Open Door	- Eject Slide
Close Door	- Load Slide
Compare Sli	de Workflows
Perform Pr	review Scan
Create S	ican Areas
Acqui	ire Slide
Set up Slid	e Dimensions
Slide Autof	ocus Wizard
Plate Acqu	iisition Setup
тс)OLS
Center	on Click
Laser Autof	ocus on Slide
Find 2	Z Offset
Adjust Con	rection Collar
	2
Reset I	nterlocks
H	lelp
Main	Menu

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.

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Open Door - E	ject Slide
Close Door - L	.oad Slide
Compare Slide	Workflows
Perform Previ	ew Scan
Create Sca	n Areas
Acquire	Slide
Set up Slide D	limensions
Slide Autofoci	us Wizard
Plate Acquisit	ion Setup
TOOL	_S
Center on	Click
Laser Autofocu	us on Slide
Find Z O	ffset
Adjust Correct	tion Collar
22	
Reset Inte	nlocks
Help	
Main M	enu

c. Compare Slide Workflows

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



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Open Doo	r - Eject Slide
Close Doo	r - Load Slide
Compare S	lide Workflows
Perform P	review Scan
Create S	Scan Areas
Acqu	iire Slide
Set up Slid	le Dimensions
Slide Auto	focus Wizard
Plate Acq	uisition Setup
T(00LS
Cente	r on Click
Laser Auto	focus on Slide
Find	Z Offset
Adjust Cor	rection Collar
	2
Reset	Interlocks
ł	Help
Mair	n Menu

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.

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Open Door - E	Eject Slide
Close Door - L	.oad Slide
Compare Slide	Workflows
Perform Prev	iew Scan
Create Sca	n Areas
Acquire	Slide
Set up Slide D	imensions
Slide Autofoc	us Wizard
Plate Acquisit	ion Setup
TOOI	_S
Center on	Click
Laser Autofoci	us on Slide
Find Z O	ffset
Adjust Correct	tion Collar
Reset Inte	nocks
Help)
Main M	enu

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Single slide holder (provided with all systems):



3-slide holder (optional purchase):



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

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Ope	en Door - Eject Slide
Clo	se Door - Load Slide
Com	pare Slide Workflows
Pe	form Preview Scan
C	Create Scan Areas
	Acquire Slide
Set	up Slide Dimensions
Slic	le Autofocus Wizard
Pla	te Acquisition Setup
	TOOLS
	Center on Click
Lase	er Autofocus on Slide
	Find Z Offset
Adj	ust Correction Collar
	2
	Reset Interlocks
	Help
	Main Menu

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.

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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
2
Reset Interlocks
Help
Main Menu

m. Reset Interlocks

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

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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

🕦 Ana 🗖 🗉 🗾
Select Current Plate
Review Plate Data
Close Data Log
· · · ·
Scale Images to Full Range
AutoScale Images
Overlay Images
Correct Shading
· .
Clear All Regions
Region Tools
Calipers
Linescan
· ·
Estimate Module Settings
Load Stack - Current Site
Load Montage - Current Well
· .
Copy Mask and Add to Stack
· .
Help
Run a Plate
Main Menu

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.

Select Current Plate
Review Plate Data
Close Data Log
· ·
Scale Images to Full Range
AutoScale Images
Overlay Images
Correct Shading
· ·
Clear All Regions
Region Tools
Calipers
Linescan
· .
Estimate Module Settings
Load Stack - Current Site
Load Montage - Current Well
· .
Copy Mask and Add to Stack
· .
Help
Run a Plate
Main Menu

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 presentationquality 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.

Ana Select Current Plate Review Plate Data Close Data Log Scale Images to Full Range Auto Scale Images Overlay Images Overlay Images Correct Shading Clear All Regions Region Tools Calipers Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	
Select Current Plate Review Plate Data Close Data Log Scale Images to Full Range Auto Scale Images Overlay Images Overlay Images Correct Shading Clear All Regions Region Tools Calipers Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	🕦 Ana 🗖 🗉 🗾
Review Plate Data Close Data Log . Scale Images to Full Range Auto Scale Images Overlay Images Correct Shading . Clear All Regions Region Tools Calipers Linescan . Estimate Module Settings Load Stack - Current Site Load Montage - Current Well . Help Run a Plate Main Menu	Select Current Plate
Close Data Log Close Data Log Scale Images to Full Range AutoScale Images Overlay Images Correct Shading Correct Shading Clear All Regions Region Tools Calipers Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	Review Plate Data
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Scale Images to Full Range Auto Scale Images Overlay Images Correct Shading Clear All Regions Region Tools Calipers Linescan - Estimate Module Settings Load Stack - Current Site Load Montage - Current Well - Help Run a Plate Main Menu	· ·)
AutoScale Images Overlay Images Correct Shading Clear All Regions Region Tools Calipers Linescan - Estimate Module Settings Load Stack - Current Site Load Montage - Current Well - Help Run a Plate Main Menu	Scale Images to Full Range
Overlay Images Correct Shading Clear All Regions Region Tools Calipers Linescan Linescan Estimate Module Settings Load Stack - Current Site Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	AutoScale Images
Correct Shading Clear All Regions Region Tools Calipers Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	Overlay Images
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Clear All Regions Region Tools Calipers Linescan - Estimate Module Settings Load Stack - Current Site Load Montage - Current Well - Copy Mask and Add to Stack - Help Run a Plate Main Menu	·
Region Tools Calipers Linescan - Estimate Module Settings Load Stack - Current Site Load Montage - Current Well - Copy Mask and Add to Stack - Help Run a Plate Main Menu	Clear All Regions
Calipers Linescan - Estimate Module Settings Load Stack - Current Site Load Montage - Current Well - Copy Mask and Add to Stack - Help Run a Plate Main Menu	Region Tools
Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	Calipers
Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack	Linescan
Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	-
Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack	Estimate Module Settings
Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu	Load Stack - Current Site
Copy Mask and Add to Stack - Help Run a Plate Main Menu	Load Montage - Current Well
Copy Mask and Add to Stack - Help Run a Plate Main Menu	· .
- Help Run a Plate Main Menu	Copy Mask and Add to Stack
Help Run a Plate Main Menu	· · ·
Run a Plate Main Menu	Help
Main Menu	Run a Plate
	Main Menu

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.

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Select Current Plate
Review Plate Data
Close Data Log
-
Scale Images to Full Range
AutoScale Images
Overlay Images
Correct Shading
Clear All Regions
Region Tools
Calipers
Linescan
-
Estimate Module Settings
Load Stack - Current Site
Load Montage - Current Well
-
Copy Mask and Add to Stack
· · ·
Help
Run a Plate
Main Menu

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.



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.

- Shift-Click on objects in the image to select them and similar objects
- **Double-click** on specific objects to add or remove them to/from the selection
- Close the Integrated Morphometry Analysis window when selection is complete
- Module settings will then be calculated based on the selection of objects in the image



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 fullresolution 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 fullresolution images open. Images must have the stage label and stage position information, so images imported from another microscope might not work.

 Ana Select Current Plate Review Plate Data Close Data Log Scale Images to Full Range Auto Scale Images Overlay Images Correct Shading Clear All Regions Region Tools Calipers Linescan Estimate Module Settings Load Stack - Current Site Load Montage - Current Well Copy Mask and Add to Stack Help Run a Plate Main Menu 	
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Copy Mask and Add to Stack	Load Montage - Current Well
Copy Mask and Add to Stack - Help Run a Plate Main Menu	· .
- Help Run a Plate Main Menu	Copy Mask and Add to Stack
Help Run a Plate Main Menu	-
Run a Plate Main Menu	Help
Main Menu	Run a Plate
	Main Menu

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".

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7.6	12.7	13.3	16.8	16.5	19.2	18.8	10.1	20.0	
7.6	12.7	13.3	16.8	16.5	19.2	18.8	10.1	20.0	
7.6	12.7	13.3	16.8	16.5	19.2	19.9	101	20.0	
7.6	12.7	13.3	16.8	16.5	19.2	18.8	10.1	20.0	
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Repeat for other time points / sites / Z planes as desired. The resulting masks will be in a stack, in the order they were copied.



6. System Maintenance taskbar

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Open Door
Close Door
Adjust Correction Collar
Measure Pixel Sizes
Parfocality and XY Offsets
Eject Filters
Set up Shading Correction
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Verify A1 Center
2
Reset Camera Settings
Backup Hardware Settings
Laser Autofocus on Slide
Adjust Stage Position
2
Memorize Current Position
Move To Memorized Position
Reset Interlocks
Help
Main Menu

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.

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Open Door
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Eject Filters
Set up Shading Correction
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Reset Camera Settings
Backup Hardware Settings
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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.



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f. Eject Filters

If this is a widefield system (ImageXpress Micro 4, ImageXpress Nano, ImageXpress Micro XLS, ImageXpress Micro XLS, 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 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.

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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.

Drilled hole for A1 Center calibration



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.

		- call Hardware Settings:	
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•	· ·		Create New Setting
Pressing Set File Association re launched when .stk and marked by *)	n will set the default group and the group to tif images are double-clicked in Exporer	Se Install System Devices	OK

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

Available components Included components ImageXpress Micro Shutter ImageXpress Micro Shutter ImageXpress Micro Fluitors ImageXpress Micro Fluitors ImageXpress Micro Fluitors ImageXpress Micro Fluitors ImageXpress Micro Z Lamp, ImageXpress Micro Trans Shutter ImaneXpress Micro Tra	1. Select ImageXpress Micro, then click Settings
Operate device in emulation mode Parameter Group #1 Parameter Group #2 Parameter Group #3 Anintenance Mode Configure Environment Control Configure Transmitted Light Configure Fluidics Show Version Information Repet Davison	Apply OK Cancel

- 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.

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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.

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Verify A1 Center
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Backup Hardware Settings
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Move To Memorized Position
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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).



Hold down space bar to move faste Hold down B to move slower

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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.

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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**.

Create or Update Plate File.JNL	11/4/2015 6
Create Preview Scan File.JNL	11/4/2015 2
Determine MX Folder.JNL	11/28/2015
Determine Objective Calibration.JNL	7/17/2016 4
Enable Custom Slides.JNL	11/28/2015
Enter Shading Combinations.JNL	9/25/2017 4
Fill Gaps.JNL	9/28/2017 4
Find Wavelengths.JNL	9/28/2017 1
Get Date Stamp.JNL	5/28/2015 2
IXM Taskbar LAF Parameters.INI	7/17/2016 4
Parfocality and PlateBottomReference.JNL	9/26/2017 1
plate_position_next_random.JNL	5/28/2015 2
Remove Special Characters.JNL	6/20/2017 1
Retrieve LAF Parameters for Objective.JNL	7/17/2016 7
Retrieve Plate File Parameters.JNL	5/28/2015 2

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.

IXM Taskbar LAF Parameters.INI - Notepad	
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StartLaserExposure=10	
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StartLaserPower=10	
WorkingDistance=15.5	
[4x 0.13NA]	
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StartLaserExposure=10	
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[4X] StartLaserBower-10	т
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LZUX U.4JNAJ	

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).

🐠 Journal Editor		
File Edit		
Builtin Functions Recorded Journals Actions	Journal:	
Builtin Functions Recorded Journals Actions Assign To Variable Comment Field Delete Variable Exit Playback For/Next If/Then/Else Pause Playback Prompt User Trace While	Journal: C:MXXENTASKBARS\Taskbar_Journals\Utility Journals\Enable Custom Slides.JNL Functions Descriptions Enable Custom Slides Paula Gedraits - MX6 - 2015-11-03 Enable custom slide option Define a Z offset (in um) for custom slide holders Placed in a separate journal for easier access to make adjustments Set this flag to 1 to use custom slides, 0 to disable custom slide option I: EnableCustomSlides = 0 I: EnableCustomSlides = 0 I: 2: CustomZOffset = 3000 I: 3: CustomPlateHeight = 5 I: 4: CustomWellDepth = 2000 Custom slide image must have the dimensions matching a full microplate I: 5: CustomInageName = "Two slides in 48 x 60 slide holder" Enter default scan area positions for custom slide here I: EnableCustomSlides = 0 Edit Function Settings Select Settings To Override	Undo
	Save Run Journal	Exit

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.

🐠 Journal Editor		- • •
File Edit		
Builtin Functions Recorded Journals Actions	Journal:	
Assign To Variable	C:\MX6\TASKBARS\Taskbar_Journals\Utility Journals\Enable Custom Slides.JNL	•
Delete Variable	Functions Descriptions	
Exit Playback For/Next	t: CustomWellDepth = 2000	^
If/Then/Else Pause Plauback	Custom slide image must have the dimensions matching a full microplate Ke 5: Custom/mageName = "Two slides in 48 x 60 slide holder"	
Prompt User	Enter default scan area positions for custom slide here	
While	K = 6: CustomRegionTop = 61 K = 7: CustomBeninnl eft = 50	
	8: CustomRegionWidth = 243	
	X 9: CustomRegionHeight = 302	
	IF EnableCustomSlides = 0 THEN	=
	Karal ID: CustomZOffset = 0 ELSE	
	END IF	
	**** End of Journal ****	
	A Duillin function: Assign (Asignal)	F
	1: EnableCustomSlides = 0	
	Edit Eurotion Settings	
	Colort Cathorne To Quantida	
	Seev Sewings to overlide	
	Save Run Journal	Exit

- **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:

			Image Info			
			Image:	Slide in single slide holder		
Slide in single slide	holder (100%)			Property Name		Property
Q± K			Location	on Disk	C:\MX6\TA9	KBARS\Taskbar_Jo
	Example Slide		File Type	9	MetaSeries S	ingle/Multi-plane TIF
			Creation	Timestamp	Thu Jan 122	22:52:44:954 2012
			Last Sav	red Timestamp	Thu Jan 122	23:03:27:558 2012
	Draw region	Draw region Lookup Table Model for scapping Storage Requirement(Megabytes)	Monochrome	•		
for sea	for scanning		Storage	Requirement(Megabytes)	94.51 KB	
-	ioi sealling		Image W	/idth	381	
16			Image H	eight	127	
- Martin			Image D	epth (bits)	16	
		Image X	Calibration (µm/pixel)	200		
			Image Y	Calibration (µm/pixel)	200	
Well: · Illum: N/A · Ma	a: N/A : 7:0 um		Number	of Planes	1	
Trent, marti N/A, Ma	griga, ziv an	-22	Plane St.	age Label		
		an ann an	Plane St.	age Position X		

Example custom slide holder:

Two slides in 48 x 60 slide holder (100%)		🖤 Image Info	
Q [±] N	E	Image: Two slides in 48 x 60 slide holder	
A1	Example Slide	Property Name	
		Location on Disk	C:\MX6\TASKBAR
● Slide 1		File Type	MetaSeries Single.
		Creation Timestamp	Thu Aug 13 18:47
1		Last Saved Timestamp	Thu Aug 13 19:13
S		Lookup Table Model	N/A
		Storage Requirement(Megabytes)	801.25 KB
<u></u>		Image Width	639
		Image Height	428
		Image Depth (bits)	24
		Image X Calibration (µm/pixel)	200
		Image Y Calibration (µm/pixel)	200
		Number of Planes	1
		Plane Stage Label	
		Plane Stage Position X	
	lide only	Plane Stage Position Y	
		Plane Camera Offset X	-
		Plane Camera Offset Y	
Draw regions on this slide o		Plane Camera Horizontal Bins	1
		Plane Camera Vertical Bins	1
		Plane Z Distance	
Wells - Illum: N/A - Mag: N/A - 7: 0 up		Plane Number: 1	
Went, Martin WA, Mag. WA, 2:0 um		Open Log Configure Log	Image Status Bar

Template blank image:

Blank Slide Holder Template (100%)	🕕 Image Info	
Q :	Image: Blank Slide Holder Template	
	Property Name	
	Location on Disk	C:\MX6\TASKBARS\
	File Type	MetaSeries Single/Mu
	Creation Timestamp	Wed Jun 1 22:06:28:0
	Last Saved Timestamp	Wed Jun 1 22:07:28:6
9	Lookup Table Model	N/A
	Storage Requirement(Megabytes)	801.25 KB
	Image Width	639
	Image Height	428
	Image Depth (bits)	24
	Image X Calibration (pixel/pixel)	200
	Image Y Calibration (pixel/pixel)	200
	Number of Planes	1
	Plane Stage Label	
	Plane Stage Position X	
	Plane Stage Position Y	
	Plane Camera Offset X	
	Plane Camera Offset Y	

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.