

SETTING UP A SLIDE AS PLATE QUICK START GUIDE FOR METAXPRESS 3.1-5.x

This guide will help you set-up slide scanning as a plate to acquire images through Plate Acquisition Setup

NOTE Shading correction is very important for imaging slides, especially those with colorimetric stains. Correction images should be configured with the same type of slide and coverslip that you are using for your sample. If acquiring images without overlap, click on System Maintenance on the main taskbar, and select Slide Shading Correction. If acquiring images with overlap, on the main toolbar, click on Apps \rightarrow Scan Slide. Use the Acquire Shading Image button on the Acquisition tab to create shading correction images. T is recommended to save slide shading correction images in a folder separate from plate shading correction images. Make sure that Shading Correction is enabled in Plate Acquisition your experiment.

SECTION A: Setup Slide with as a Plate Definition

1. Click on Set up Slide as Plate in the taskbar



2. Enter a name for your slide setup. This name will appear in the drop down menu of the Plate tab in the Plate Acquisition Setup wizard. Click **OK**



3. Select the appropriate slide holder. Click OK

Select Slide Holder	X
Select holder used for this slide:	
Single Slide Holder	
Three-Slide Holder: Slide 1	
Three-Slide Holder: Slide 2	
Three-Slide Holder: Slide 3	
Three-Slide Holder: All Slides	
ОК	Cancel
ide. Click OK	
Set Slide Thickness	X

- 4. Select the orientation of your slide. Click OK
- 5. The next few dialogs will ask you to define your slide in terms of spacing in the slide holder. You will be presented with screenshots giving you instructions with how to set up your slide as a plate. It is recommended to have a metric ruler handy.

Select slide orientation: Coverslip down (0.17 mm) Coverslip up (1.0 mm)

6. You will be asked to set up multiple areas of your slides as wells. You can set up multiple rows and columns. Note that if you are using a 3-slide holder, you can only have one column per slide. Please see screen shot below.

🔽 Multi-well Slid	de Examples (100%)			Multi-well Slide
		1-slide holder 4 columns 2 rows	3-slide holder 2 columns 4 rows	Multiple areas can be set up as multiple wells. Side areas must have regular spacing for multi-well setup (see example image). If in doubt, create a separate plate file for each slide area.
		1-slide holder 2 columns 1 row	<u>3-slide holder</u> 2 columns 1 row	Continue
	000	No regular spaci • Set each covers separate plate file	ng: lip up as a	
E		Rectangular cov • Single coverslip multiple adjacent	erslip: can be set up as square wells	

7. Enter the number of columns and click **OK**

Slide Columns	23
Enter number of columns:	
1	
ОК	Cancel

8. Enter the number of rows and click **OK**

Slide Rows	×
Enter number of rows:	
1	
ОК	Cancel

9. Enter the well shape and then click **OK**

Enter Well Shape	×
Enter well shape:	
Square	
	Cancel

10. Select the method for measuring the position of your sample. It is recommended to choose **Measure** with ruler in mm. Click **OK**. You will need a metric ruler for the next few steps.

Select Method	×
Select method for measuring	positions:
Measure with ruler in mm	
O Visual measurement insid	e ImageXpress system
OK	Cancel

11. The following message will appear defining the column and row offsets. Click **Continue**

Column Offset: Distance from left side of holder to center of A1.	
Row Offset: Distance from top side of holder to center of A1.	
See example image. Measure in mm.	-

12. Use the instructions to calculate column and row offsets

Row and Column Offsets (100%)	
C Distance from top side to A1 center is Row Offset C C C C C C C C C C C C C C C C C C C C C C Distance from left side to A1 center is Column Offset	1
Distances may be easier to measure with slide holder flippe	ad over

13. Enter the column offset in mm and click **OK**

		100	
	Column Offset	J	
	Enter column offset in mm:		
	0		
	OK Cancel		
14. Enter the row offset and click OK			
	Enter Row Offset	J	
	Enter row offset in mm:		
	0		
	OK Cancel		
15. Enter the well diameter in mm	and click OK		
	Enter Well Diameter	J	

Enter Well Diameter	X
Enter Well Diameter in mm:	
ОК	Cancel

16. A message will appear stating that you have successfully created a plate layout for your slide. You will now be prompted to set up laser autofocus settings. Click **Continue.** Warning: use this journal to set up autofocus settings, do not the run laser autofocus wizard from the Plate Acqustion Setup wizard on your slide.



17. Load the slide holder with a sample slide in the instrument and click **OK**

viake sure the slide is loaded into the system in the Single Slide Holder.	*
Press Continue.	

18. A message will appear with directions on selecting an objective to set up laser autofocus settings. Click **Continue**



19. Select an objective from the drop down menu and click **OK**. It is recommended to start with the 10X objective first.

Select Ma	gnification	
Magnification:	10X Plan Fluor	•
ОК]	Close

20. An image will appear called Find Sample along with a message asking you to select an objective. Click Continue and repeat the above process for all objectives.



- 21. If you are working with an objective that has a correction collar, a message dialog box will appear asking you if the correction collar needs to be set.
 - a. Click **YES** if you need to change the correction collar on the objective. If you do not have any objectives with correction collars, click **NO** and skip to step 22.

Adjust Correction C	Collar	x
Does the objectiv	e correction collar ne	ed to be set?
	Yes	No

b. You will be asked how you would like to access the objective. Select the appropriate choice and click **OK**



c. If you selected "From the top" in step b above, you will need to remove the slide holder from the system. When done, click **Continue**

. Remove plate or <mark>s</mark> lide holder	
Click Continue	
	-

d. Remove the objective from the instrument and set it to the appropriate correction collar setting. The dialog box lists correction collar settings for commonly used plates and slides. Set the correction collar to the appropriate number based on the thickness of your slide. If imaging with coverslip down, typical settings are 0.17. If imaging with coverslip up, typical settings are

1.0. Once the correction collar has been set, replace the objective in the instrument and click **Continue**.

- Adjust Objective
- e. Load the slide holder back in the instrument and click Continue

 Replace plate or slide holder 	~
2. Click Continue	

22. Once laser autofocus has been set for all objectives, select [None] from the drop down menu and click **OK**

Select Magnification	- • -
Magnification: [None]	•
ОК	Close

23. The process of setting up your Slide as a Plate is not complete, click Continue



SECTION B: Acquire Slide Using Plate Acquisition Setup

- 1. On the main tool bar, select Screening → Plate Acquisition Setup. On the **Names and Description** tab, enter a name for the *experiment set* and *experiment base name*
- 2. On the **Objective and Camera** tab, select an objective and binning settings



3. Click on the Plate tab and select the plate from the drop down menu that was created in Section A

periment- IAF Test	Plate name:	Singleslideholder	uncelle5	-	Sava Confin	untion
Names and Description	Hate Hame.	Singleanderiolderin	00001130		Jave Coning	
Objective and Camera- 20X S Pla						
Plate- Singleslideholder-fluocel						
Wells to Visit- 1 of 1		Number of rows:		Number of columns	\$C	Well shap
Sites to Visit- multi-site						Square
Timelapse- 1 time point(s)		Well	\square	Column		Plate
Acquisition Loop		diameter (µm):		spacing (µm):		length (mr
Autofocus		13000 🖵	\square	3000		127.0
W1 DAPI		3				
Journals- 0 selected		Column	$(\Box \Box$	Row		Plate
Display Settings		85500		spacing (pm).		85.5
Post Acquisition			11 1 1			00.0
Summary						
		Row offset (μm): 42000 ÷		' Well depth (µm): 13431 🚔		Plate height (mr 14.3
	Edit Plate Bo	ottom Settings	Laser Autofoo	us Wizard		
						(

4. Click the one **Acquisition Loop** tab, and select the **Autofocus** sub tab. Select "*Focus on plate bottom, then offset by bottom thickness*" from the Well to well autofocus drop down menu

periment- IAF Test	Laser-based Focusing		
Names and Description	Configure Laser Settings	.]	
Objective and Camera- 20X S Plan	Welling well ended a sure of		1
Plate- Singleslideholder-fluocells!	well to well autorocus	ocus on plate bottom, then offset b	y bottom thickness
Wells to Visit- 1 of 1	Image-based Focusing		
Sites to Visit- multi-site	Algorithm: Standard	▼ Binning: 1 🚔	Custom exposure times
Timelapse- 1 time point(s)			
Acquisition Loop	Allow image-based focu	using for recovery from laser-based	well bottom failures
Autofocus			
W1 DAPI			
Journals- 0 selected	Initial well for finding sample	First well acquired	• A • 1 +
Display Settings	Number of wells to attempt in	nitial find sample 1	
Post Acquisition	Cha Antafaana		
Summary	Site Autorocus	First site only	•
			View Focusing Details

- 5. On the Acquisition Loop tab, select the correct number of wavelengths you want acquire.
- 6. Set up each wavelength you want to acquire using the W wavelength tabs.
 - a. Select wavelength from the drop down menu
 - b. Enter exposure time
 - c. Find the offset
- 7. Journal tab
 - a. Usually leave everything deselected (turned off) here.
- 8. Display Settings tab
 - a. You can just use default display settings.
 - b. Display images during acquisition is usually on.
 - c. Optional: to customize display (only affects display during acquisition, does not affect raw data), select the *Manually set image display properties* option and click the *Display Images* button. Resize and position windows as desired, then click *OK*.

9. Post Acquisition tab

- a. Only turn on Auto Run Analysis if you already have optimized analysis settings for the assay.
- b. If you are not sure of analysis settings to use, make sure this option is turned off.
- 10. Summary tab
 - a. Review summary of settings and print if desired
- 11. Click on **Save Settings** and select **Save to file**. Click on **Save** and it will prompt you for a location and filename.
- 12. Click Acquire Plate to begin image acquisition.