

## **MetaXpress® 6 Software Guide**

Running the Laser Autofocus Wizard for Multi-well Plates



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The purpose of this chapter is to guide the user through the **Laser Autofocus Wizard** when creating and configuring new multi-well plate templates. This process includes entering the plate dimensions in **Plate Acquisition Setup** and running the **Laser Autofocus Wizard**.

However, this chapter <u>will not</u> cover creating new Multi-well plates or advanced Laser Autofocus settings and troubleshooting. For this information refer to corresponding chapters.





## When Do I Use Laser Autofocus Wizard?

- When setting up a new plate type
- If using a new objective on a previously-configured plate
- When using environmental control for the first time with the plate (allow system and plate to equilibrate for at least 2 hours)
- If you there are experiencing focus issues with laser auto focus when acquiring





## Before You Begin, You Will Need

ImageXpress<sup>®</sup> platforms are capable of scanning most clear-bottom plate formats. You will need:

- Plate dimensions from manufacturer (usually available from their website)
- Empty plate (recommended, but not required)

#### AND

• Plate with only media (or sample) in all wells which are at least 50% full

\*NOTE\* If the plate will be used with environmental control, Molecular Devices recommends to allow the system to equilibrate for at least two hours. Plate(s) being tested should also be equilibrated at the same temperature for at least 30 minutes



Example Plate Drawing Containing Plate Dimensions From <u>Greiner Website</u>





## What is Laser Autofocus (LAF)?

An algorithm that adjusts the focus until the signal from a laser spot reflected off the sample plate bottom is detected

The laser autofocus mechanism uses a 690nm laser + dedicated camera







The software finds the plate by moving the objective up and down until the brightest reflection is detected from the laser, which occurs when the objective is focused at the plate bottom



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#### **Principles of Laser Autofocus**



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#### **Running Laser Autofocus Wizard**



- In the Plate Acquisition Setup dialog, select the Configure tab, then the Plate tab
- 2. In the **Plate Name** drop-down menu, select the plate to be configured
- 3. Make sure that a 10X Plan Fluor objective is in the system \*NOTE\* If you don't have a 10X Plan Fluor objective, then contact technical support for recommendations
- 4. Click on the Laser Autofocus Wizard button



#### **Running Laser Autofocus Wizard**



- 5. The **Plate Acquire LAF Setup Wizard** screen will appear
- 6. The LAF Wizard has two phases:
  - Phase 1 Dry plate. Measure plate bottom thickness across the plate and calculate mean thickness and several variation parameters.
  - Phase 2 Wet plate. Determine laser exposure times needed to find the plate bottom and well bottom surfaces
- 7. Click on the **Next >>** button to begin





Measure Bottom Variation
Well selection         ○ All wells       Selection pattem:         ○ Groups of wells       Checkerboard with gaps, including A1         Group size (NxN):       2         Selecting more wells takes longer to finish mesaurement but provides more robust autofocus settings, especially for plates with a large amount of variation.
Sites per well Performing measurements on multiple sites/well is slower
Single site     Single site     faster site-to-site LAF.
1 2 3 4 5 6 7 8 9 10 11 12
Time estimate: 0:06:00
<< Back Next>> Cancel

- 8. The next step is to configure parameters using a **dry plate** (recommended but not required, a plate with sample can be used)
- 9. Under Well Section
  - Select **All wells** for greatest accuracy. \*NOTE\* This can be quite time consuming
  - To complete a quicker LAF test, select
     Checkerboard with gaps including A1.
     You can adjust Group size and Gap
     between groups to change the test
     pattern (reflected in the plate map below)
- 10. Under Sites per well
  - Select **Multiple sites**: **5** sites is best, **3** should be the minimum
- 11. Click on the **Next>>** button



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sure Bottom Variation
- following manufication activity will be used to measure slate bottom unitation :
10x Plan Fluor
ess <b>Next&gt;&gt;</b> to measure new bottom variation settings.
cquire LAF Setup Wizard
sure Bottom Variation
e following magnification setting will be used to measure plate bottom variation:
10X Plan Fluor
DTE- bottom variation settings already exist for this plate. These settings were not culated by the setup wizard. It is recommended that new settings be calculated.
DTE-bottom variation settings already exist for this plate. These settings were not culated by the setup wizard. It is recommended that new settings be calculated.



- 12. The **Measure Bottom Variation** dialog will appear
  - This screen indicates which objective will be used to measure plate bottom thickness (10x Plan Fluor)
  - If you are running the Laser Autofocus (LAF)
     Wizard for the first time on this plate type or if you want to recalculate bottom thickness, click on the Next>> button
  - If LAF wizard has previously been run on this pate type and you want to configure a new objective, click the Use Existing Settings button. This will skip the dry plate setup and take you directly to the wet plate steps



	riation		
	Load a DRY plate int	to the instrument.	
	Open door/clamp	Close door	
C Existing value Calculated va (based on ob	(fine step slues (fine step jective N.A.]	= 0.0, coarse step = 0.0) = 1.4, coarse step = 11.1	1
Allow Extreme V	ariation (Example: Thin	bottom plastic plate)	

13. Load a dry plate into the system

- i. Click on the **Open door/clamp** button to open the stage door and load a dry plate (no media or cells)
- ii. Click on the Close door button
- iii. Select Calculated Values
- iv. In general, do <u>not</u> check the **Allow Extreme Variation** box as it may give inaccurate measurements
- v. Click on the Start>> button





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14. The Measure Bottom Variation Status dialog appears

- This screen will tell you the status of the laser autofocus search
- It will determine the mean optical thickness and variation of the plate bottom
- The tested wells are color coded.
   Green = all searches were successful; yellow = some failures occurred; red = all searches failed
- The status line will indicate the number of failed plate bottom or well bottom searches



	e			
Typical thickness (u	m): 112.5	Variation	n (+/-): 8.0	
Plate bottom variation	Minimum	Maximum	Averane	Std Dev
Adjacent wells (um):	5.2	17.0	9.5	3.7
ntra-well (um):	0.0	13.6	5.5	3.8
<sup>o</sup> late maximum variatio	on (um): 374.1			
	I AF failures - 67 ol	ate bottom 67 w	ell bottom	
		•		
••		•		
The number of fa measurements	ailed LAF searches	should not affect	t plate bottom o	dimension
The number of fa measurements.	ailed LAF searches	should not affect	t plate bottom o	dimension
The number of fa measurements.	ailed LAF searches ments (replace existing	should not affect	t plate bottom o	dimension

15. After LAF has completed its scan, the **Measure Bottom Variation Summary** dialog will appear

 This screen shows the Typical thickness of the plate
 \*NOTE\* Write this value down as you will need it to

adjust objectives with correction collars in the next steps and when you use this plate

 If there are objectives with correction collars (usually objectives > 10X) in the system, you may need to exit the LAF Wizard to change them before starting next steps. Click **Cancel** to exit the wizard; any settings will be saved

The value for the correction collar is **typical thickness** \* plate bottom refractive index. Refractive index is **1.52** for glass bottom plates and **1.59** for plastic bottom plates



The number of f measurements.	ailed LAF searches should not affect plate	bottom dimension
late bottom measure	ements	
late bottom measure Save settings	ments (replace existing measurements)	
late bottom measure ) Save settings ) Discard settings	ments (replace existing measurements) (keep existing measurements)	





- 15. The **Measure Bottom Variation Summary** dialog will also tell you if the number of failures exceeds the ability to find focus consistently
  - If you receive this message, click Cancel and refer to Advanced Laser Autofocus Wizard and Troubleshooting Guide
  - If the message indicates the search results can be accepted, select Save settings, then click on the Next >> button



\*Note\* Correction collars must be set before running this step (**collar = typical thickness \* refractive index**) . You will need to exit out of the LAF Setup Wizard to use the Main Taskbar to adjust the correction collar and return to resume LAF setup. Refer to the slide at the end of this document titled **Run Laser Autofocus Wizard for New Objectives** for instructions.

	Exposure Param	eters			
Gelect t nagnific	he medium that will b ation setting to use f	e used when runr or calculating exp	ing this plate typ osure times.	be. Then select t	he
	Medium: Aqueous medi Magnification s	a 🚽 Refr setting for calculat	active index: 1	.33	
	20x Super Plan	n Fluor ELWD		·]	
Selec	t LAF step sizes (um) wisting values Calculated values based on objective N	(fine step = ) (fine step = )	1.0. coarse step 1.6. coarse step	= 0.0) = 4.9)	
Press N	ext>>to measure n	ew exposure setti	ngs.		

- The next step is to configure parameters using a wet plate. The Calculate Exposure Parameters dialog will appear
  - This phase determines laser exposure times. These steps will need to be repeated for every objective in the system including the 10X.
    - i. Select the type of **Medium** (usually Aqueous) from the drop-down menu. If you are unsure of what to use, select **Unknown**
    - ii. Select the objective for testing from the drop-down menu
    - iii. Select Calculated Values under the Select LAF setup sizes (um) section
    - iv. Click on the **Next >>** button



Plate Acquire LAF Setup Wizard	<b>—</b> ×
Calculate Exposure Parameters	
Well selection     Selection pattem:	
Selecting more wells takes longer to finish mesaurement but provides more rob autofocus settings, especially for plates with a large amount of variation.	ust
Sites per well       Performing measurements on multiple sites/well is but provides intra-well variation data, which may r faster site-to-site LAF.	slower result in
1 2 3 4 5 6 7 8 9 10 1	1 12
Time estimate: 0:06:00	
<< Back Next>> C	ancel
16	

17. The **Calculate Exposure Parameters** dialog appears

#### 18. Under Well Section

- Select **All wells** for greatest accuracy. \*NOTE\* This can be quite time consuming
- To complete a quicker LAF test, select
   Checkerboard with gaps including A1.
   You can adjust Group size and Gap
   between groups to change the test
   pattern (reflected in the plate map)

#### 19. Under Sites per well

• Select **Multiple sites**: **5** sites is best, **3** should be the minimum

20. Click on the Next>> button



nstrument. elected wells c	ontain the medi	um that will be used	
door/clamp	Close door		
Plate and we refractive ind nearly idention based medic	ell bottom is rec lex of the plate cal (e.g. glass p m)	ommended unless ti and medium are late with glycerol-	ne
1			
	Anstrument. elected wells co door/clamp Plate and we refractive inc nearly identic based mediu	Instrument.         elected wells contain the medi         door/clamp       Close door         Plate and well bottom is recrefractive index of the plate nearly identical (e.g. glass plased medium)         based medium)	Instrument.         elected wells contain the medium that will be used         door/clamp       Close door         Plate and well bottom is recommended unless the refractive index of the plate and medium are nearly identical (e.g. glass plate with glycerol-based medium)         based medium)       Close door



- i. Click **Open door/clamp** and replace the dry plate with a plate containing media in all wells
- ii. Click on the **Close door** button
- iii. Under LAF search type
  - Select **Plate and well bottom** for 10X and higher objectives.
  - Select Plate bottom only for low magnification objectives (1X, 2X, 4X) or plates containing glycerol or mounting media
- iv. Click on the Next>> button



xposure Measurer	nent Summary			
Exposure variation	Minimum	Maximum	Averace	Gtd Day
Plate bottom exposure	e (us): 1.0	1.0	Average 1.0	0.0
Well bottom exposure	(us): 18.0	47.0	29.7	6.9
LAF search step size	s (um)			
Fine step = 1.4	Co	arse step = 11.1		
Plate bottom exposur	es (us)	Well botto	m exposures	(us)
1st pass-	1.0	1s	t pass-	24.0
2nd pass-	2.0	2r	nd pass-	40.0
3rd pass-	5.0	3n	d pass-	46.0
	LAF failures- 8	plate bottom, 8 we	Il bottom	
			10 State 1	
			:: :	
=_			::: :	
==			::: : ::	
=-			: "	
-	:		•	
The number of f	ailed LAF search	es should be insign	ificant for cal	culating
The number of f successful LAF	ailed LAF search	es should be insign with this objective	nificant for cal	lculating
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The number of f successful LAF	ailed LAF search exposures to use	es should be insign with this objective	anificant for cal	Iculating
The number of f successful LAF	ailed LAF search exposures to use	es should be insign with this objective	anificant for cal	Iculating
The number of f successful LAF	ailed LAF search exposures to use Save sett	es should be insign with this objective ings and exit wiza	anificant for cal	Iculating
The number of f successful LAF	ailed LAF search exposures to use Save settings and m card settings and m	es should be insign with this objective ings and exit wiza reasure with anoth measure with anoth	nificant for cal	

#### 22. The Exposure Measurement Summary

dialog will tell you if the number of failures exceeds the ability to find focus consistently

- If there are too many failures, click on Discard Settings and measure with another objective or Cancel to exit the wizard
  - Refer to the Advanced Laser Autofocus
     Wizard and Troubleshooting Guide for help
- If the wizard was successful, select one of the Save settings options
- Repeat the exposure measurement steps for all objectives in the system
- When settings for all objectives have been establish, click on the Save settings and exit wizard button





#### **Testing Laser Autofocus Settings**



- Open the **Plate Acquisition Setup** dialog and load a plate with samples
- Load a saved protocol or create a new acquisition protocol for the plate. Refer to corresponding chapters for details on this processes.
- Right-click in the Plate Map to move the stage to a well and site of interest
- Click the **Focus** button to test the laser autofocus routine on the active wavelength.
  - The autofocus routine should run and snap an image fairly quickly. If it takes longer than 2-3 seconds, refer to Advanced Laser Autofocus Wizard and Troubleshooting Guide



#### Run Laser Autofocus Wizard for New Objectives

To re-run Laser Autofocus Wizard for a particular objective or to configure a new objective:





- Start the Laser Autofocus Wizard (Click on the **Plate** tab on the **Plate Acquisition Setup** dialog)
- . Click the **Next>>** button twice (x2) to get to the **Measure Bottom Variation** dialog
- iii. Click on the Use existing settings button. This will take you to the Calculate Exposure
   Parameters dialog
- iv. Select the medium and your new objective from the drop-down menus
- v. Select Calculated values, then press Next>>
- vi. Continue the configuration as described earlier



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## Before Contacting Tech Support for LAF Help

Obtain a copy of the following

- Protocol settings (.hts) file you are currently working with
- Plate file (.plt)
- MetaXpressAutofocusReport.txt
- MetaXpressAutofocusStatus.txt

\*Note\* The plate file can be located in C:\MX6\plates

The Autofocus report and status can be located in Windows Temp folder by:

- Navigating to C:\Users\xxxxxx\AppData\Local\Temp
- Type %temp% in Windows search





#### Support Resources

- F1 / HELP within MetaXpress® Software
- Customer Care portal and Knowledge Base:
  - <u>https://support.moleculardevices.com/</u>
- Technical Support can also be reached by telephone:
  - https://www.moleculardevices.com/contact





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