



MetaXpress [®] Software – Acquisition Journal:				
Pipette Wait Read_revB				
File Name(s)	Pipette Wait Read_revB.jzp			
Description	This journal will pipette to wells with a defined wait time prior to imaging.			
	RevB adds interactive set up of the SecondsToWait and SecondsPerWell parameters.			
Compatibility	IXM with Fluidics optionOnly tested in MX6			
Prerequisites	Fluidics option			
Notes	 The following setting is hard-coded in the journal. This can be modified by editing the journal, or can be moved to interactive prompt: Plate Directory is C:\MX6\ 			
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Instructions: Journal Installation

- 1) Save the provided .jzp file to a convenient location on the ImageXpress computer.
- Go to Control > Journal > Import Journal Suite (simplified menu) or Journal > Import Journal Suite (standard menu).



3) Select the provided .jzp file and a convenient folder on the ImageXpress computer. Click **Import**. Note: No confirmation is displayed. If an older version of files already exist in the folder, they will be overwritten with the newer version.

elect Journal Suite
^
~
ect Import Location
Close

4) **Close** the Import Journal Suite window. The journal is now installed and ready to be set up in a protocol.

Instructions: Configuring a Protocol with the Journals

- 1) Go to Screening > Plate Acquisition Setup.
- 2) Load or create a suitable protocol with the plate type, magnification, and wavelengths already configured.
- 3) Go to the **Acquisition** tab. Make sure that "Use Fluidics" and "Run Journals during Acquisition" are enabled. It is recommended to disable the "Acquire Time Series" option.

Objective and Camera- 10X Plar	Autofocus options
Plate- Eppendorf 24 Well film b:	Enable laser-based focusing
Sites to Visit- multi-site	Enable image-based focusing (for acquisition or laser recovery)
Acquisition	Acauisition options
Autofocus	Acquire Time Series
Wavelengths	Acquire Z Series
W1 DAPI	
Fluidics	
Journals- 3 selected	Use Fluidics
Display	Run Journals During Acquisition
	Analyze Images After Acquisition
	Directory for Stored Correction Images C:\

Objective and Camera- 10X Plar				Configure Stations
Plate- Eppendorf 24 Well film b:	Scheduled Events:			Conligure Stations
Sites to Visit- multi-site	Time Event			
Acquisition				
Autofocus				
Wavelengths				
W1 DAPI				
Fluidics				
Journals- 3 selected				
Display				
	Reset Tips	Add new Event	Delete Event	Edit Event
	Reset Tips	Add new Event	Delete Event	Edit Event

4) Go to the **Fluidics** tab. No events should be scheduled here.

5) Click **Configure Stations** and make sure that the compound plate types, tip types, and other fluidics options are set appropriately here.

Notes:

- The XY offset should be set to 0 here. If you need to use an XY offset, this will be set in the journal.
- You can specify a Z offset here as needed.
- Track Volume and Track Liquid Surface are optional and generally recommended.
- Click **Reset Tips** to reset Tips and Liquid Levels as appropriate.
- Click **System Properties** to adjust other parameters such as Dispense Rate. These settings are saved with your protocol.



6) **Close** the Configure Fluidic Stations dialog.

- 7) Go to the **Journals** tab. Enable the following journals and select the matching journal files from the journal import. Also enable the option to "Prevent asynchronous hardware moves".
 - a) After each image
 - b) Start of well
 - c) Start of plate

Configure Run			Sna	p Start Live	Focus	Test	Preview
Objective and Camera- 10X Plar	Acquisition Step		Journal				and a start
Plate- Eppendorf 24 Well film b	Before each image	13	[None]				
Sites to Visit- multi-site	After each image	1	Pipette Wait Read After	EachImage			
Acquisition	Before focusing	13	[None]				
Autofocus	Start of z	13	[None]				
W1 DAPI	End of z	12	[None]				
Journals- 3 selected	Start of site	12	[None]				
Display	End of site	12	[None]				
	Start of well	B	Pipette Wait Read Start	of Well			
	End of well	12	[None]				
	Start of time point	2	[None]				
	End of time point	12	[None]				
	Start of plate	B	Pipette Wait Read Start	of Plate			
	End of plate	13	[None]				
	Prevent asynchronous (recommended if any	s hardware journals are	moves dependent on hardware po:	sitioning).			
Save Protocol*			(?	Close	<< S	ummary

8) **Save** the modified protocol.

Instructions: Running the Protocol

- Before running the acquisition, it is recommended to go to Window > History Window to open the history window for viewing. Position it somewhere on the screen where it will not get covered up by images or the Plate Acquisition Status dialog. If necessary, clear the old history.
- If necessary, go to Plate Acquisition Setup > Fluidics > Configure Stations > Reset Tips and reset tips and liquid levels, as appropriate.
- 3) Click **Acquire Plate** to run the Acquisition.
- 4) When prompted, enter the number of seconds to wait. This is the incubation time that you want between the start of pipetting and the start of imaging for each well. The default time is 600 seconds (10 minutes).

Seconds to Wait	×
Specify the incubation time between the com	pound addition and the start of imaging, in seconds:
Number:	600
ОК	Cancel

5) When prompted, enter the number of seconds per well. This is the expected time it takes to image each well. The default time is 180 seconds (3 minutes).

Seconds Per Well	×
Specify the expected time to acquire a single well	, in seconds:
Number: 180	
OK Cancel	

6) The journal will calculate and display the number of wells it will be able to process per group, based on the Seconds to Wait and Seconds Per Well values. If this looks reasonable, click **Continue** to proceed. Otherwise, click **Cancel** and re-evaluate the experiment setup and the times that you entered.

Calculated 3 wells will be pro	ocessed in each group. 🔺

7) When prompted, enter the name of the plate. The journal will look for the plate file in the plates folder **C:\MX6\Plates**. If necessary, the default directory can be changed by editing the journals. If desired, a default plate name can be hard coded into the journal to save on entry time.

Plate Na	ıme	×		
Confirm o	or enter plate n	ame:		
String:	: Eppendorf 24 Well film bottom			
	ок	Cancel		

Hint: The easiest way to find the name is to copy it from the plate file:

Windows (C:) \rightarrow N	∕IX6 > pla	ates		
 Name 3-Slide 384-we 	Holder -s	lides in est.plt	colum	nnsplt
or 🥥 1536w 🦳 Bead P 🥥 Bead P	·789866.plt late IXM-4 late IXM-C	t I.plt 2.plt		
Costar	6-well pla	stic squ	are.pl	t
Costar	6-well pla	stic.plt		
Costar	384-Well P	Plastic.p	lt	
8C Eppen	dorf 24 We	ll film b	otton	n.plt
Undo				
Cut				1655986.p
Сору			1	ilt
Paste			V	5
Delete				
Select All				
Right to left Reading	g order			
Show Unicode cont	rol charact	ers		
Insert Unicode cont	rol charact	er	>	
Open IME				
Reconversion				

8) Next, select the wells that will be imaged during the experiment. The selection must match the wells selected for imaging in the protocol. If there is a mismatch, there may be unexpected results in the experiment workflow. Note: the wells are listed in the order that they will be acquired by the program.

ict the weils you will be imaging (i	NUST MATCH PROTOCO
A01	~
☑ B01	
C01	
✓ D01	
✓ D02	
C02	
☑ B02	
A02	
A03	
B03	×

9) Select Compound Plate 1 or 2:

Select Compound Plat	e X
Select the compound pla	te you are pipetting from:
0 0	1 2
ОК	Cancel

10) Enter the volume of compound to pipette to each well.

Compound Volume X
Enter the volume (ul) to dispense to each well:
Number: 25
OK Cancel

11) If desired, enter an X and Y offset for pipetting into the sample plate. Using the default values of 0 will cause it to pipette to the center of each well. Offsets can be positive or negative, but take care not to enter too large of a value, which could result in the pipette tip crashing into the sample plate.

X Offset	×
Enter the X offset (um) from well center	r (should be less than 6600):
ОК	Cancel
Y Offset	×
Enter the Y offset (um) from well cente Number:	r (should be less than 6600):
ОК	Cancel

12) When ready, click Continue to begin the experiment.

Click Continue to start the experiment.	^
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- 13) The progress of the experiment will be displayed in the History Window. The timings displayed are from the start of each group of wells (for example, with the default timings, 3 wells per group is calculated based on the wait time of 10 minutes and read time of 3 minutes per well). Note: the screenshots show shorter wait times which were used for testing.
- 14) When it is waiting to pipette or image, a "Waiting" dialog box is displayed so that the user has an idea of where it is in the wait time. If you accidentally click **Continue**, this will not upset the timing, but you will no longer see the countdown.

Plate Acquisition Status-	
Press <esc> to cancel Start Cancel Waiting X Waiting to pipette to well A04</esc>	History Window
Timeout: 27 Continue	Start pipetting to well D04 at 0.008 sec Get Next Tip Draw from Compound Plate 1, Row 4, Column 4, Volume 3 ul Dispense 3 ul to Sample Plate, X: 72900, Y: 69700 Eject Tip Wating to pipette to well B04 Start pipetting to well B04 at 30.976 sec Get Next Tip Draw from Compound Plate 1, Row 2, Column 4, Volume 3 ul Dispense 3 ul to Sample Plate, X: 72900, Y: 33700 Eject Tip Waiting to pipette to well A04
	O Start Recording History Image: Start Recording History Clear Save As

15) **Optional**: When the experiment is done, go to the History Window and click **Save As** to save the experiment log to a .txt file.

History Window		×
Get Next Tip		~
Draw from Compound Plate 1, Row 1, Co	olumn 6, Volume 25 ul	
Dispense 25 ul to Sample Plate, X: 1089	00, Y: 15700	
Eject Tip		
Waiting to image well D06		
Start imaging well D06 at 100.171 sec		
Group 6, Well 2 of 3		
Waiting to image well B06		
Start imaging well B06 at 130.855 sec		
Group 6, Well 3 of 3		
Waiting to image well A06		
Start imaging well A06 at 160.524 sec		
		v

pwr example.txt - Notepad X File Edit Format View Help Group 1, Well 1 of 3 Start pipetting to well A01 at 0.006 sec Get Next Tip Draw from Compound Plate 1, Row 1, Column 1, Volume 25 ul Dispense 25 ul to Sample Plate, X: 18900, Y: 15700 Eject Tip Waiting to pipette to well B01... Start pipetting to well B01 at 30.057 sec Get Next Tip Draw from Compound Plate 1, Row 2, Column 1, Volume 25 ul Dispense 25 ul to Sample Plate, X: 18900, Y: 33700 Eject Tip Waiting to pipette to well D01... Start pipetting to well D01 at 60.106 sec Get Next Tip Draw from Compound Plate 1, Row 4, Column 1, Volume 25 ul Dispense 25 ul to Sample Plate, X: 18900, Y: 69700 Eject Tip Waiting to image well A01... Start imaging well A01 at 100.162 sec Group 1, Well 2 of 3 Waiting to image well B01... Start imaging well B01 at 130.902 sec Group 1, Well 3 of 3 Waiting to image well D01... Start imaging well D01 at 160.577 sec Group 2, Well 1 of 3 Start pipetting to well D02 at 0.007 sec Get Next Tip Draw from Compound Plate 1, Row 4, Column 2, Volume 25 ul Dispense 25 ul to Sample Plate, X: 36900, Y: 69700 Eject Tip Waiting to pipette to well B02... Start pipetting to well B02 at 30.057 sec Get Next Tip Draw from Compound Plate 1, Row 2, Column 2, Volume 25 ul Dispense 25 ul to Sample Plate, X: 36900, Y: 33700 Eject Tip Waiting to pipette to well A02... Start pipetting to well A02 at 60.105 sec