



| MetaXpress <sup>®</sup> Software – Acquisition Journal: |  |  |  |  |  |  |
|---|--|--|--|--|--|--|
|   | StreamAcquisition_AfterEachImage_revF  |  |  |  |  |  |
| File Name(s)  | StreamAcquisition_AfterEachImage_revF.JNL  |  |  |  |  |  |
| Description   | The Stream Acquisition function lets you configure the ImageXpress system for<br>rapid acquisition of images as a continuous data stream and save the images into a<br>stack. This journal allows you to automate the stream acquisition over a whole<br>plate. Individual stacks are saved to a folder on the local computer. |  |  |  |  |  |
| Compatibility   | MetaXpress versions 5 and 6  |  |  |  |  |  |
|   | ImageXpress Micro or Nano system   |  |  |  |  |  |
| Prerequisites   | None   |  |  |  |  |  |
| Notes   | Images are not acquired to the MDCStore database.  |  |  |  |  |  |
|   | A local folder should be used for saving.  |  |  |  |  |  |
|   | Most streaming experiments only use one wavelength. If multiple wavelengths are selected in the Plate Acquisition settings, the journal will stream each wavelength one at a time unless an IF-THEN statement is added selecting a specific wavelength.  |  |  |  |  |  |
| Author  | Paula Gedraitis  |  |  |  |  |  |
| Date  | November 1, 2018   |  |  |  |  |  |

## **Instructions: Using the Journal**

- 1. Save the provided StreamAcquisition\_AfterEachImage\_revF.JNL file to a convenient location on the ImageXpress computer.
- In the MetaXpress software, go to Journal > Edit Journal (in the MX6 simplified menu, go to Control > Journal > Edit Journal). Select the StreamAcquisition\_AfterEachImage\_revF.JNL file.
- 3. Click on the **Descriptions** tab at the top of the Journal Editor window. Edit these three settings as necessary for your experiment:
  - a. NumberOfFrames
  - b. ParentDirectory this must be a valid directory that the MetaXpress software can write to. A local directory is highly recommended; using a network location or a USB drive may cause performance issues. If the location is unavailable (e.g. network location and computer is not on network), this may cause the software to freeze. Within the parent directory, subdirectories with the plate name and plate ID will be created for each experiment.
  - c. FileExtension this must be ".stk" or ".tif". If the streams are expected to be 4 GB or larger, then the ".tif" option should be selected. When using the ".tif" option, make sure that the legacy format preference is deselected (Edit > Preferences > Save tab).

| Windows Processing Open Save Meas   | ure Objects   Jo        | oumals |
|---|-------------------------|--------|
| Query for Close Options on Close All<br>Close All Options:  |                         |        |
| <ul> <li>Close without saving open images</li> <li>Close and query each image for saving</li> <li>Query all images at once, then close all</li> </ul> |                         |        |
| Query for Exit Options on Exit<br>Exit Options:   |                         |        |
| <ul> <li>Exit without saving open images</li> <li>Exit and query each image for saving</li> <li>Query all images at once, then exit</li> </ul>        |                         |        |
| Save Thumbnails for Multi-Dimensional Appli<br>Notify user if some information will be lost dur   | cations<br>ing the save |        |
|   |                         |        |
| Save Images Using Legacy Format (.stk., old   | format .tif)            |        |

| Builtin Functions Descusses 1   | l _loumat  |       |
|---|--|-------|
| /iew:   | coons   Countain<br>  C:\Users\paula.gedraitis\Documents\Journals\Acquisition\StreamAcquisition AfterEachImage_revF.JNL  |       |
| Menu  | Functions Descriptions   |       |
| Image: Second | StreamAcquisition_AfterEachImage_revF - MX6         Paula Gedinatis - July 2014. Revised again January 2016 to clean up file saving         2018-11-01, PG: Revised to add user-configurable file extension, use StreamtoRam as default.         Set binning and custom field of view in Plate Acquisition Setup         Insert this journal ''After each image'' and enable option to ''Prevent asynchronous hardware moves''         Do not use timelapse option in Plate Acquisition Setup (in MX6, set number of timepoints to 1)         Streaming settings         NumberOfFrames = 100       2. Select the setting to modify         Saving settings: ParentDirectory must exist and have sufficient space. FileExtension should be either '',tif'' or '',stk''.         Using local storage is highly recommended.         ParentDirectory = IF(NIGHT/Frame)         Set up stream saving         1: Select Image[Current At Start]]         2: Create Directory ''2ParentDirectory. 1) = ''V', ParentDirectory. ParentDirectory + ''V']         3: Create Directory ''2ParentDirectory + Streen Status.PlateName + ''V''         Assign To Variable         Variable:         NumberOfFrames         100         4: Save the modified journal | 2<br> |

- 4. Save the modified journal. If desired, you can go to File > Save As within the Journal Editor to save it with a different name. If the journal will be used with different settings for different assays, it is recommended to create multiple copies with different names.
- 5. Go to Screening > Plate Acquisition Setup and create or load acquisition settings/protocol.
- 6. Set the binning (Objective and Camera tab) and custom field of view (Sites to Visit tab) parameters appropriately for your experiment. Note: Faster frame rates can be achieved with higher binning settings and smaller fields of view, but only if these are acceptable for the assay.
- 7. Set the plate acquisition for single time point acquisition.
  - a. In **MX5**: On the **Timelapse** tab, make sure the number of time points is set to 1.

## StreamAcquisition\_AfterEachImage\_revF

| periment- Paula stream test to HD | Number of timepoints:    | 1          | -        | -        |     |   |  |   |
|-----------------------------------|--------------------------|------------|----------|----------|-----|---|--|---|
| Names and Description             | Number of unepoints.     | P          |          |          |     |   |  |   |
| Objective and Camera- 10X Plan I  | Perform time series for: | One        | well the | en the n | ext | - |  |   |
| Plate- Greiner 96-well plastic    | Approximate minimum      | n time ini | terval:1 | .4 sec   |     |   |  |   |
| Wells to Visit- 2 of 96           | Interval:                | 1          | <u> </u> | 880      |     |   |  |   |
| Sites to Visit- multi-site        | interver.                | E.         |          | lace     |     |   |  |   |
| Timelapse- 1 time point(s)        | Duration:                | 0          | <u></u>  | sec      | -   |   |  |   |
| Fluidics                          |                          |            |          |          |     |   |  |   |
| Acquisition Loop                  |                          |            |          |          |     |   |  |   |
| Autofocus                         |                          |            |          |          |     |   |  |   |
| W1 Cy3                            |                          |            |          |          |     |   |  |   |
| W2 FITC                           |                          |            |          |          |     |   |  |   |
| Journals- 1 selected              |                          |            |          |          |     |   |  |   |
| Display Settings                  |                          |            |          |          |     |   |  |   |
| Post Acquisition                  |                          |            |          |          |     |   |  |   |
| Summary                           |                          |            |          |          |     |   |  |   |
|                                   |                          |            |          |          |     |   |  |   |
|                                   |                          |            |          |          |     |   |  |   |
|                                   |                          |            |          |          |     |   |  |   |
|                                   |                          |            |          |          |     |   |  | Ç |

b. In **MX6**: On the **Acquisition** tab, make sure the option to "Acquire Time Series" is disabled, and the option to "Run Journals During Acquisition" is enabled.

| Objective and Camera- 10X Plan | Autofocus options   |   |
|--------------------------------|---|---|
| Plate- Costar 96-well Plastic  | Enable laser-based focusing   |   |
| Sites to Visit- single site    | Enable image-based focusing (for acquisition or laser recovery)   |   |
| Acquisition                    | Acquisition options   |   |
| Autofocus                      | Acquire Time Series   |   |
| Wavelengths                    | Acquire Z Series  |   |
| W1 DAPI                        |   |   |
| Journals- 1 selected           |   |   |
| Display                        | <ul> <li>Use Ruidics</li> <li>Run Journals During Acquisition</li> <li>Analyze Images After Acquisition</li> <li>Perform shading correction</li> <li>Directory</li> <li>C:\Shading Images\</li> </ul> |   |
|                                |   | ß |

8. On the **Journals** tab, enable the journal option "After Each Image" and select the streaming journal. Make sure that the option to "Prevent asynchronous hardware moves" is also enabled.

| Objective and Camera- 10X Plar   | Acquisition Step                           |                                  | Journal   |
|----------------------------------|--|----------------------------------|---|
| Plate- BD Falcon 96-well Plastic | Before each image                          |                                  | [None]  |
| Sites to Visit- single site      | After each image                           | 1                                | StreamAcquisition AfterEachImage revF 🛛 🔫 🛶 🛶 🛶 |
| Acquisition                      | Before focusing                            |                                  | [None]  |
| Autofocus                        | Start of z                                 | 12                               | [None]  |
| Wavelengths                      |  | - 71                             | [None]  |
| W1 FITC                          | End of z                                   |                                  | INONET  |
| Journals- 1 selected             | Start of site                              | 13                               | [None]  |
| Dis <mark>play</mark>            | End of site                                | 12                               | [None]  |
|                                  | Start of well                              |                                  | [None]  |
|                                  | End of well                                | Ľ                                | [None]  |
|                                  | Start of time point                        |                                  | [None]  |
|                                  | End of time point                          |                                  | [None]  |
|                                  | Start of plate                             |                                  | SPA Start Plate Nov2015                         |
|                                  | End of plate                               |                                  | SPA Stop Plate Nov2015                          |
|                                  | Prevent asynchronou<br>(recommended if any | is hardware mo<br>journals are d | oves<br>ependent on hardware positioning).      |

9. Save your acquisition settings/protocol and run the plate acquisition when ready.

## Note on file handling for large streams

Streams expected to create files > 4 GB must be saved to Meta TIFF (.tif) format. Saving these streams to .stk or legacy .tif format will result in data corruption.

When you save a large stream as the multi-page .tif, the MetaXpress software splits the image up into 2GB portions. If you open any one of the partial files, the entire stack opens automatically. Looping an analysis journal over the directory of images will handle these correctly, analyzing each stream only once.

| _Stream_B02_s1_FITC.tif         | 10/30/2018 2:03 PM | TIF File | 2,075,063 |
|---------------------------------|--------------------|----------|-----------|
| _Stream_B02_s1_FITC-file002.tif | 10/30/2018 2:04 PM | TIF File | 2,075,063 |
| _Stream_B02_s1_FITC-file003.tif | 10/30/2018 2:04 PM | TIF File | 2,013,428 |

Alternatively, you can create smaller streams. There are 3 ways to do this:

1. Reduce the number of frames.

2. Reduce the field of view size (use the "Custom Field of View" option on the Sites to visit tab of Plate Acquisition Setup).

3. Increase the binning.

## Note on stream to hard disk option for advanced users

Streaming Acquisition provides the option to stream to RAM or stream to hard disk. The stream to RAM option performs better and is the recommended (default) option.

If you go to the actual Stream Acquisition dialog (Acquire > Stream Acquisition, or in the MX6 simplified menu, Control > Acquire > Stream Acquisition), you can see how much memory is required for a particular setting. If you are limited in the number of frames you can collect by the computer RAM, you can change to stream to hard disk. This setting is farther down in the journal. The default value for StreamToRAM is "Y". Set to "N" to stream to hard disk.

While streaming to RAM saves the stream in a stack or multi-plane TIF file, streaming to hard disk saves a separate file per frame. This may be more challenging for file handling and image analysis. The frames can be opened together as a stack using the Build Stack function in MetaXpress (**File menu > Open Special > Build Stack > Numbered Names**).

| Build Stack: Nu                                     | umbere                               | d Name                               | es                             | ×              |
|---|--------------------------------------|--------------------------------------|--------------------------------|----------------|
| First Image:  | Stream I                             | F Hard D                             | lisk 100_St                    | ream_C04_s1_TI |
| Last Image:   | Stream I                             | F Hard D                             | lisk 100_St                    | ream_C04_s1_TI |
| Options for list o<br>Show any va<br>Only allow the | of possib<br>ariation f<br>ie digits | ole Last I<br>from base<br>at the er | mages<br>e name<br>nd of the n | ame to vary    |
| Z Dist. Between                                     | Planes:                              | 1                                    | -                              |                |
| Increment Name By:                                  |                                      | 1                                    | *                              |                |
| Pad number w  | with lead                            | ling O's                             |                                |                |
| ОК  |                                      |                                      |                                | Cancel         |