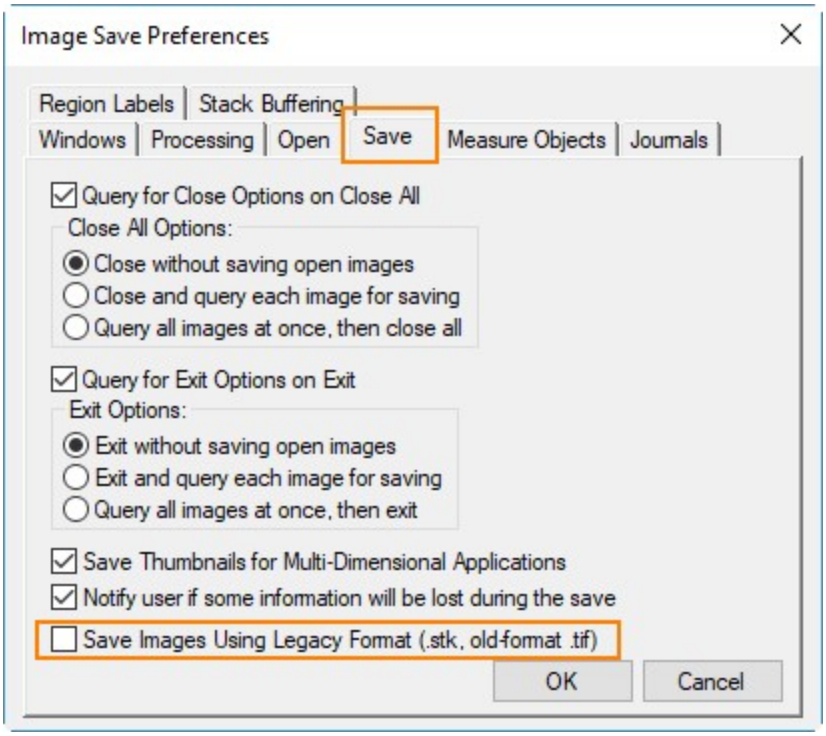
MetaXpress® Software – Acquisition Journal: StreamAcquisition_AfterEachImage_revF	
File Name(s)	StreamAcquisition_AfterEachImage_revF.JNL
Description	The Stream Acquisition function lets you configure the ImageXpress system for rapid acquisition of images as a continuous data stream and save the images into a stack. This journal allows you to automate the stream acquisition over a whole 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

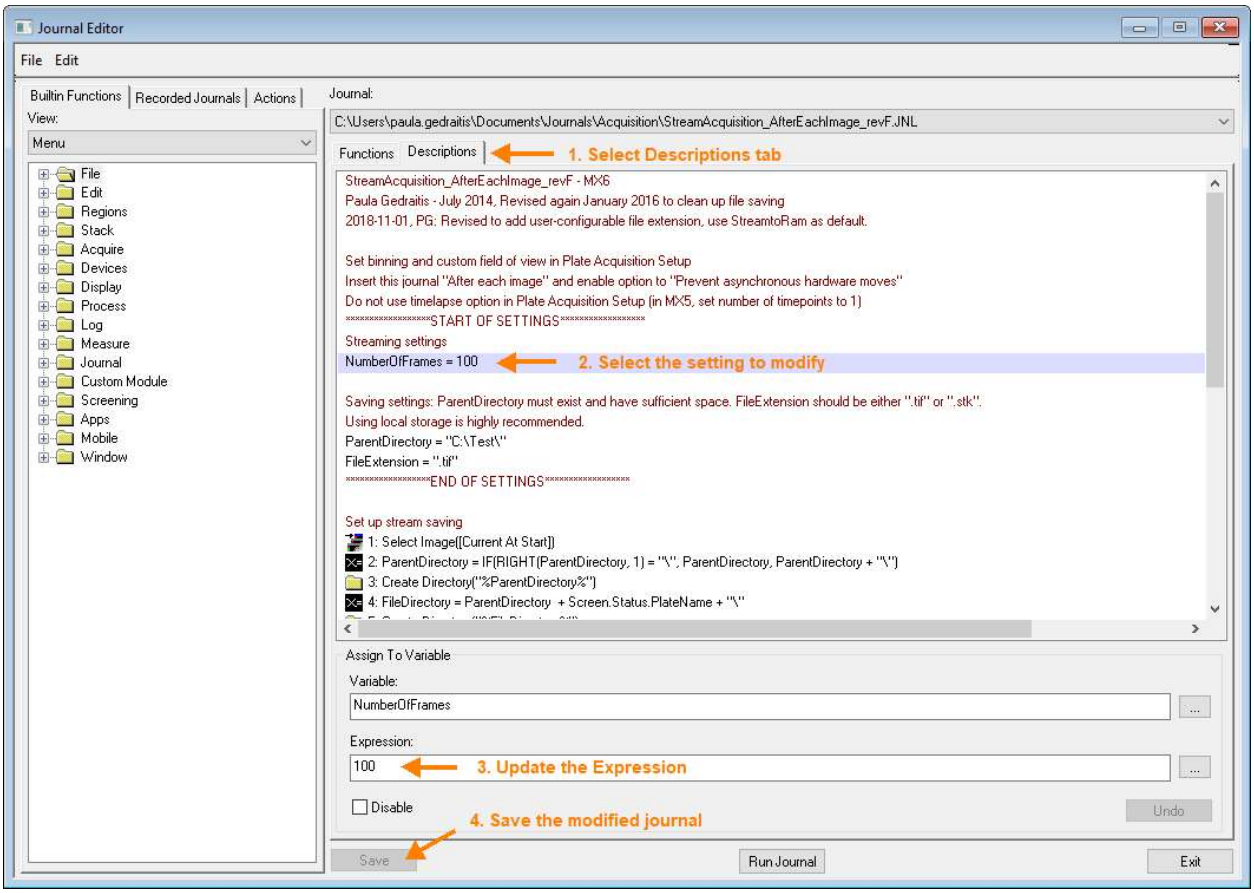
Instructions: Using the Journal

1. Save the provided StreamAcquisition_AfterEachImage_revF.JNL file to a convenient location on the ImageXpress computer.

2. 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**).





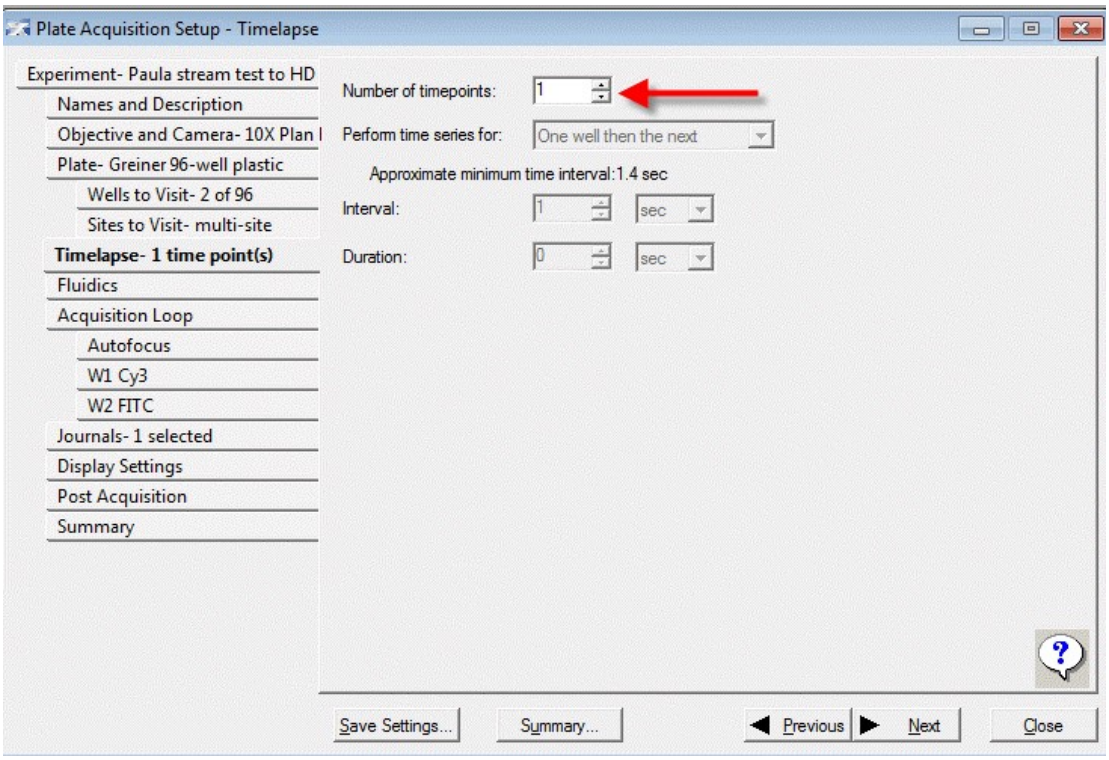
4. Save the modified journal. If desired, you can go to **File > Save As** within the Journal Editor to save it with a different name. If the journal will be used with different settings for different assays, it is recommended to create multiple copies with different names.

5. Go to **Screening > Plate Acquisition Setup** and create or load acquisition settings/protocol.

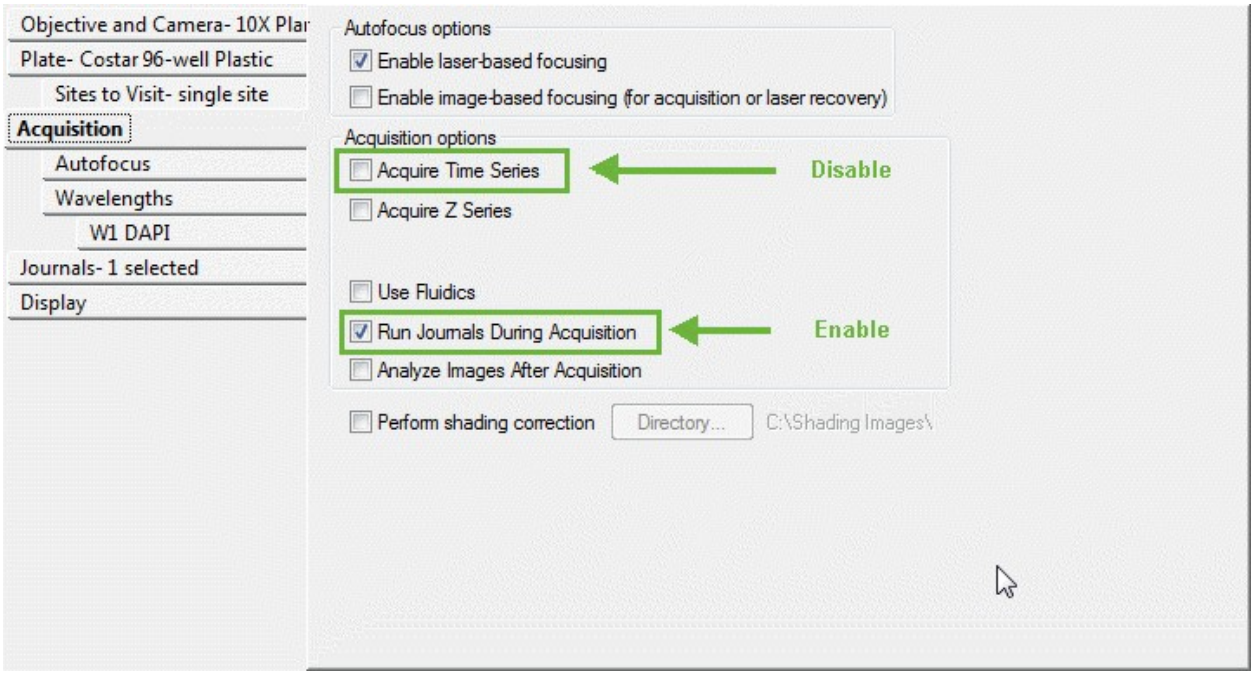
6. Set the binning (**Objective and Camera** tab) and custom field of view (**Sites to Visit** tab) parameters appropriately for your experiment. **Note:** Faster frame rates can be achieved with higher binning settings and smaller fields of view, but only if these are acceptable for the assay.

7. Set the plate acquisition for single time point acquisition.

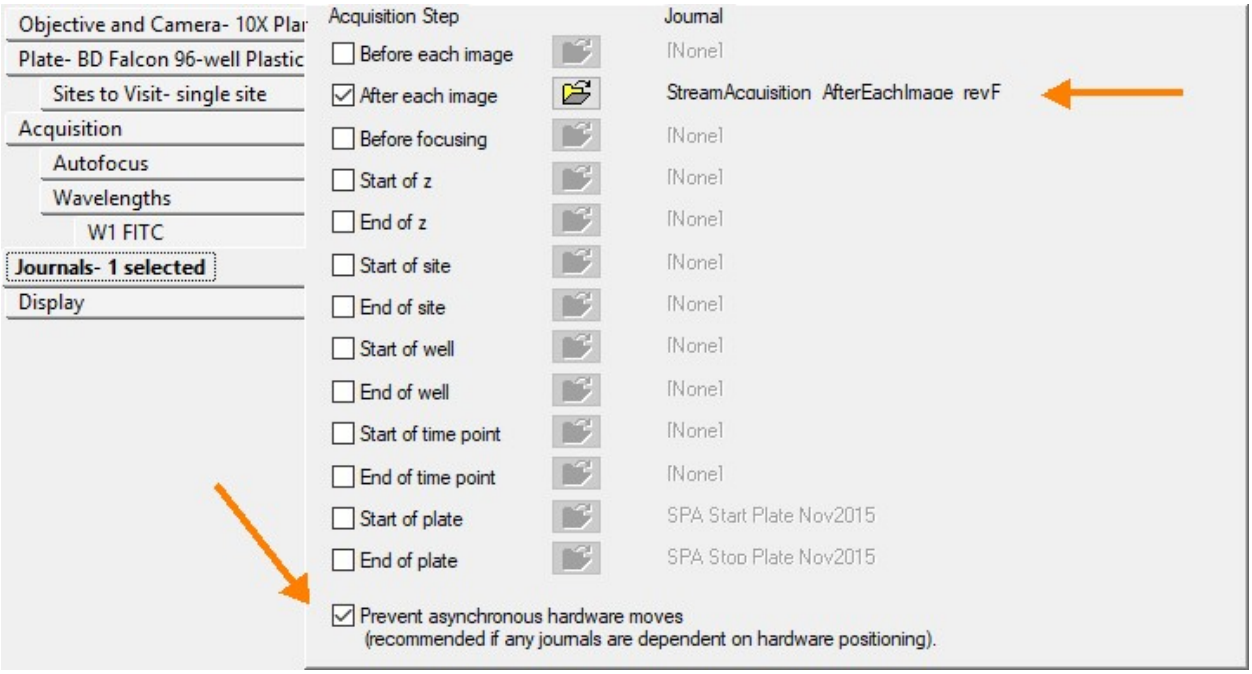
    a. In **MX5**: On the **Timelapse** tab, make sure the number of time points is set to 1.



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.



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.



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

<u>Note on file handling for large streams</u>

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	2,075,06
_Stream_B02_s1_FITC-file002.tif	10/30/2018 2:04 PM	TIF File	2,075,06
_Stream_B02_s1_FITC-file003.tif	10/30/2018 2:04 PM	TIF File	2,013,42

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**).