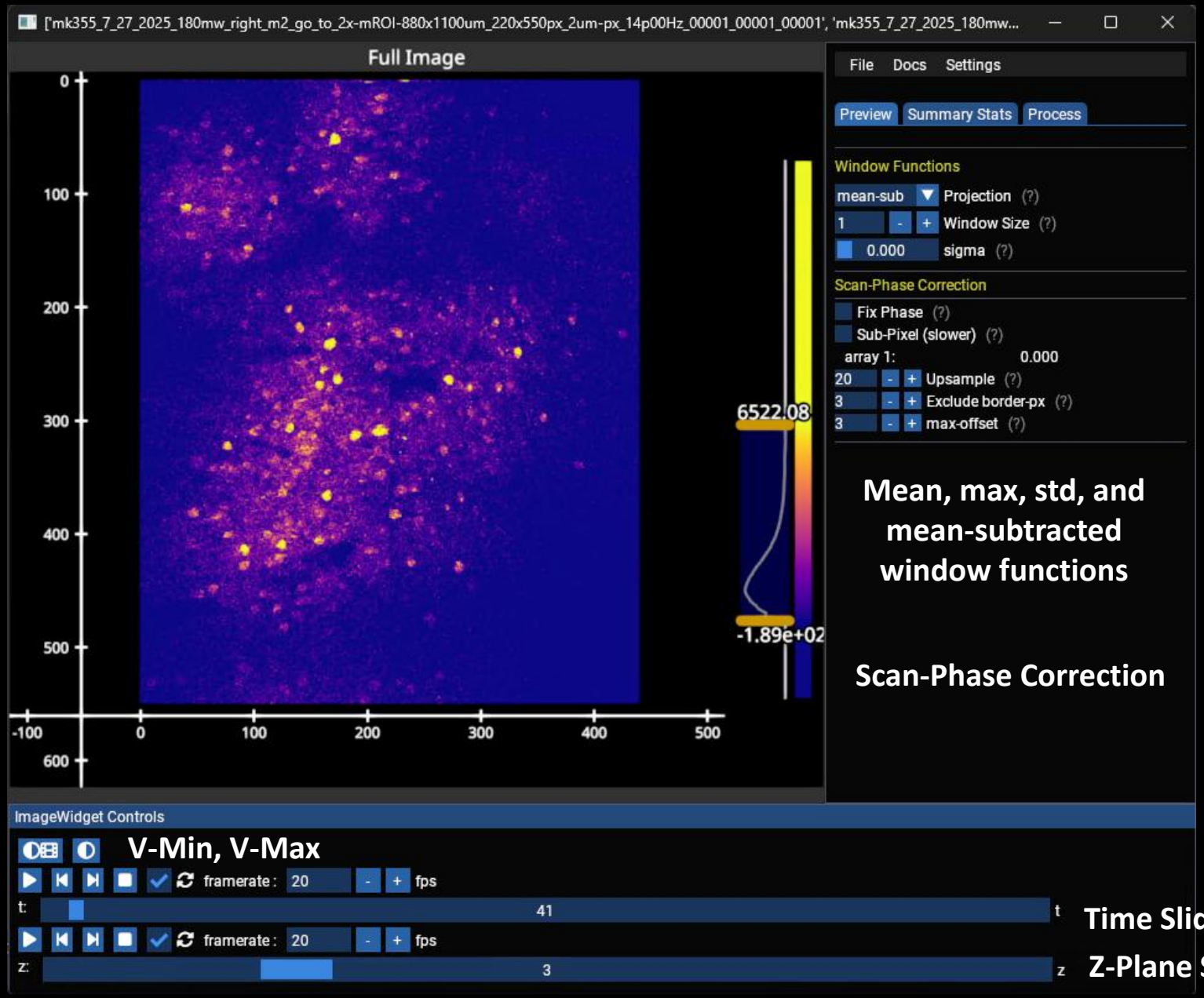
MBO GUI User Guide Overview



Mean, max, std, and mean-subtracted window functions

Scan-Phase Correction

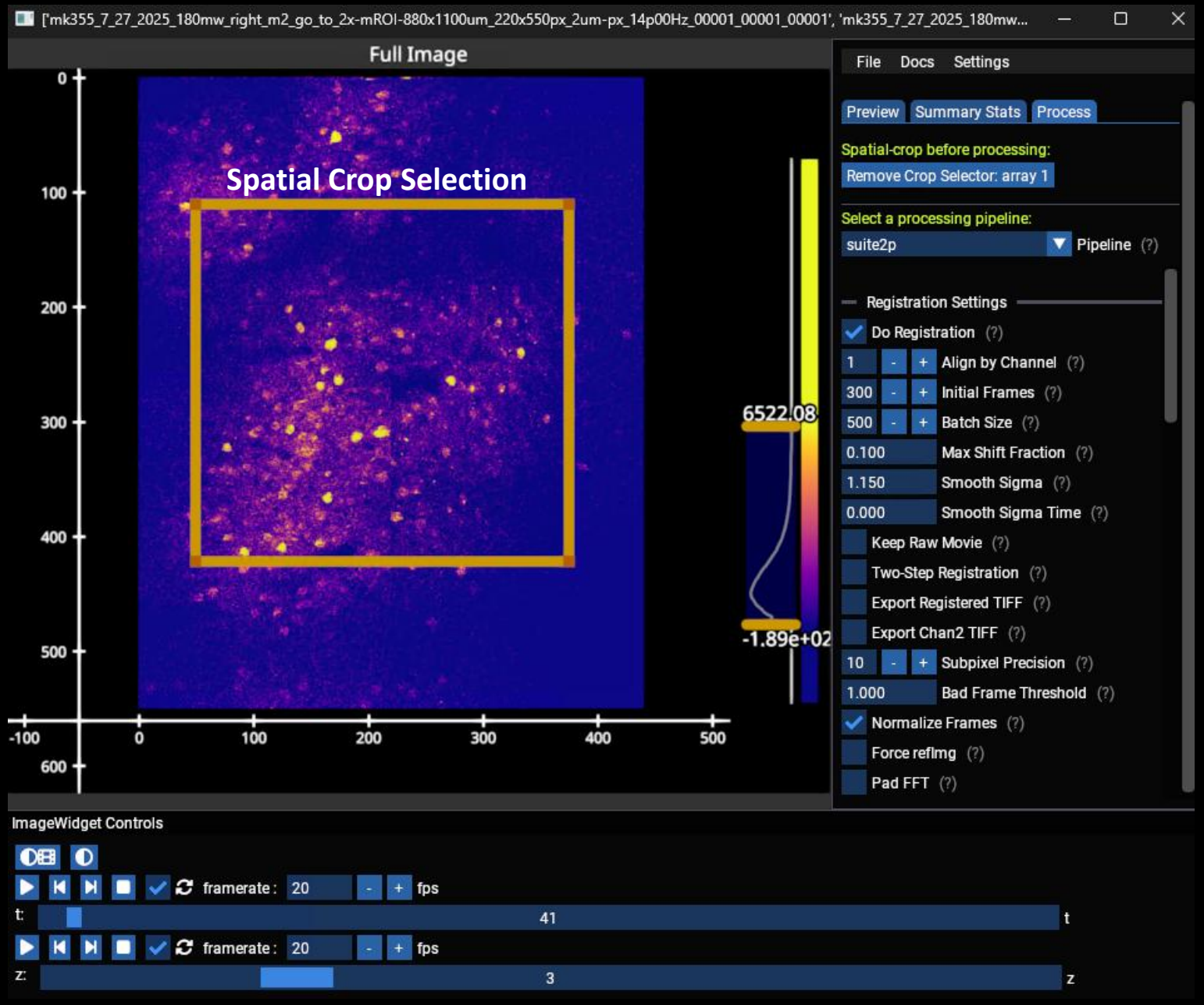
V-Min, V-Max

Time Slider

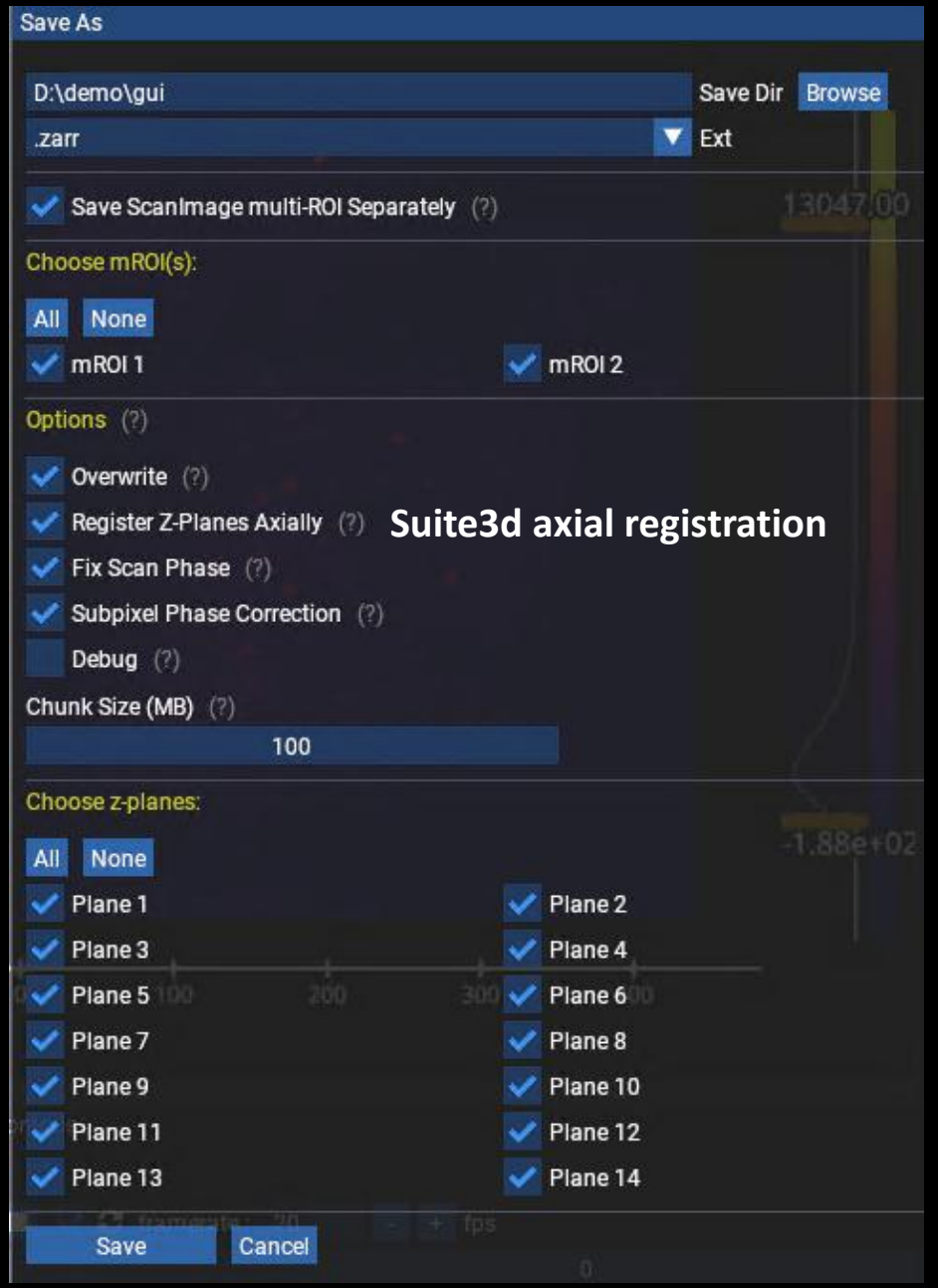
Z-Plane Slider

Multi-ROI, multi-Zplane statistics

Quickly Process with Suite2p



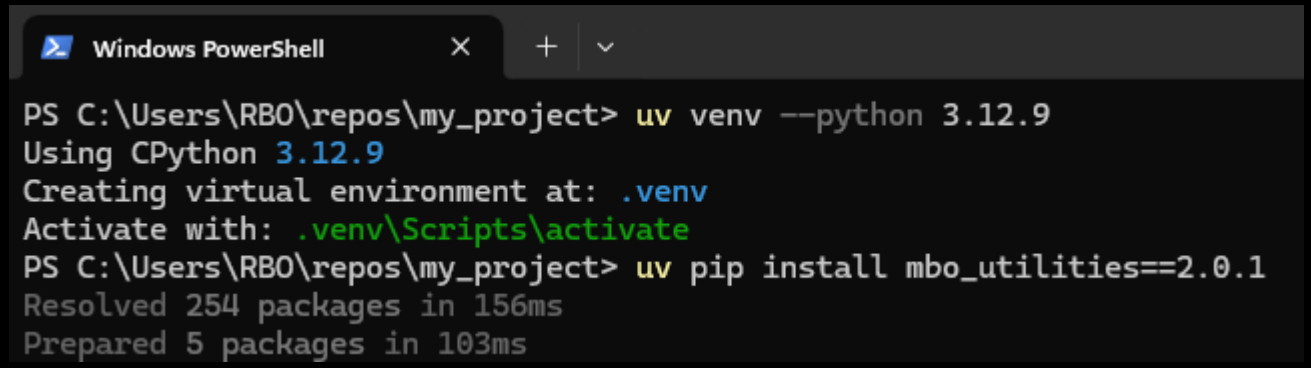
Save Full Dataset to .tiff, .zarr, .bin, or .h5

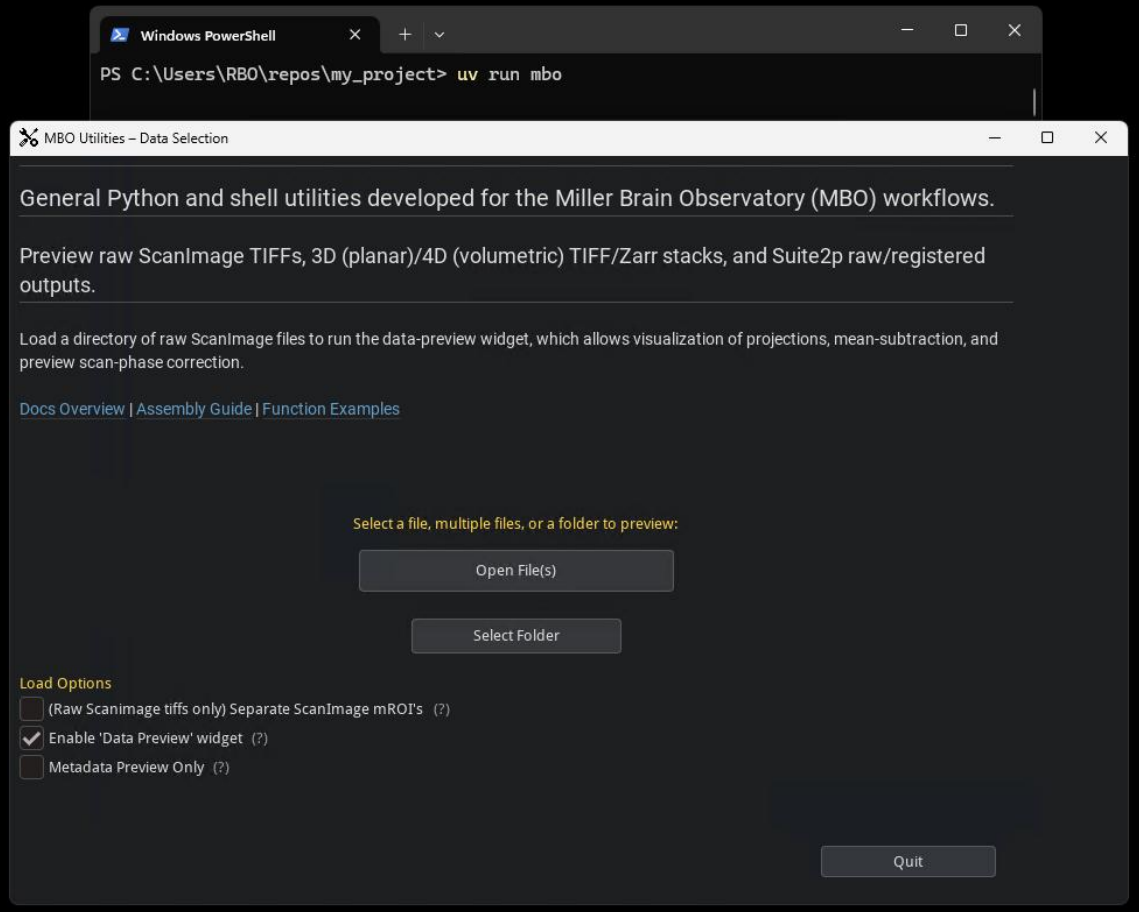


Suite3d axial registration

Installation

1. Create environment UV
2. Install mbo_utilities >=2.0.1
   (Packaged with cellpose, suite2p, suite3d, graphical utilities)
3. Start main GUI: uv run mbo





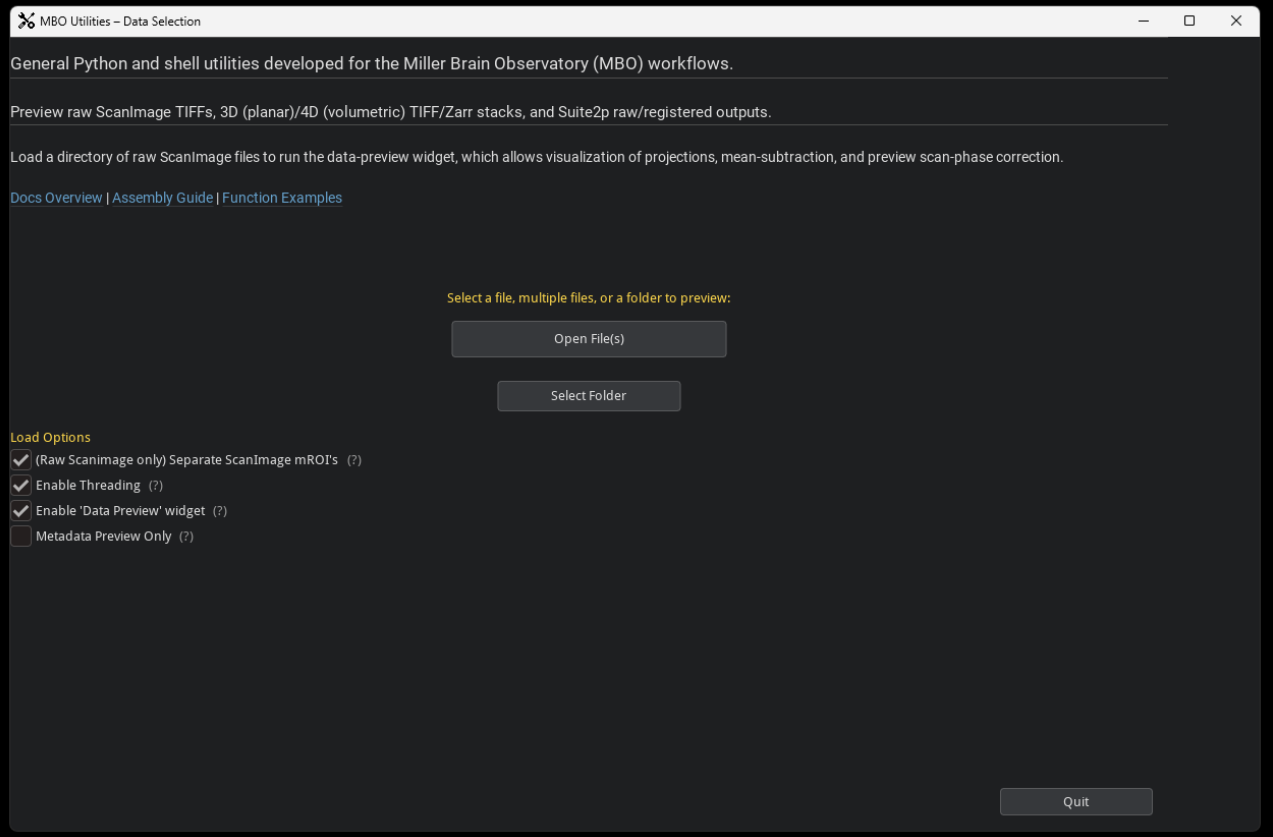
Temporary fix to io.ImFontAtlas error:
uv pip install git+https://github.com/pygfx/pygfx.git@main

Data Selection

- General data loader, opens supported filetypes (.tiff, .zarr, .bin, .h5)
- “Data Preview” widget is available for Raw ScanImage Tiffs
- Non ScanImage tiffs will open as a 3D or 4D array

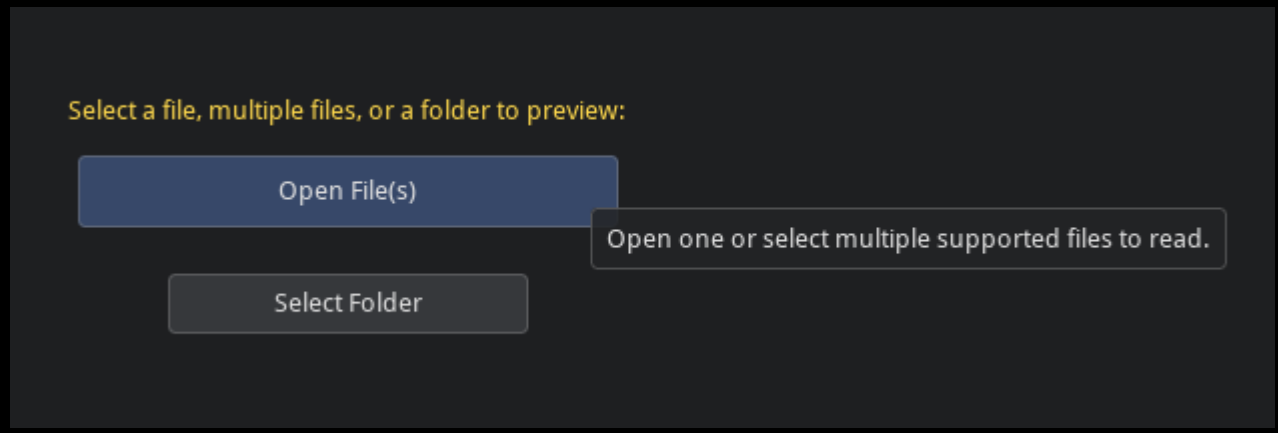
Here, we focus on the ***Data Preview widget***

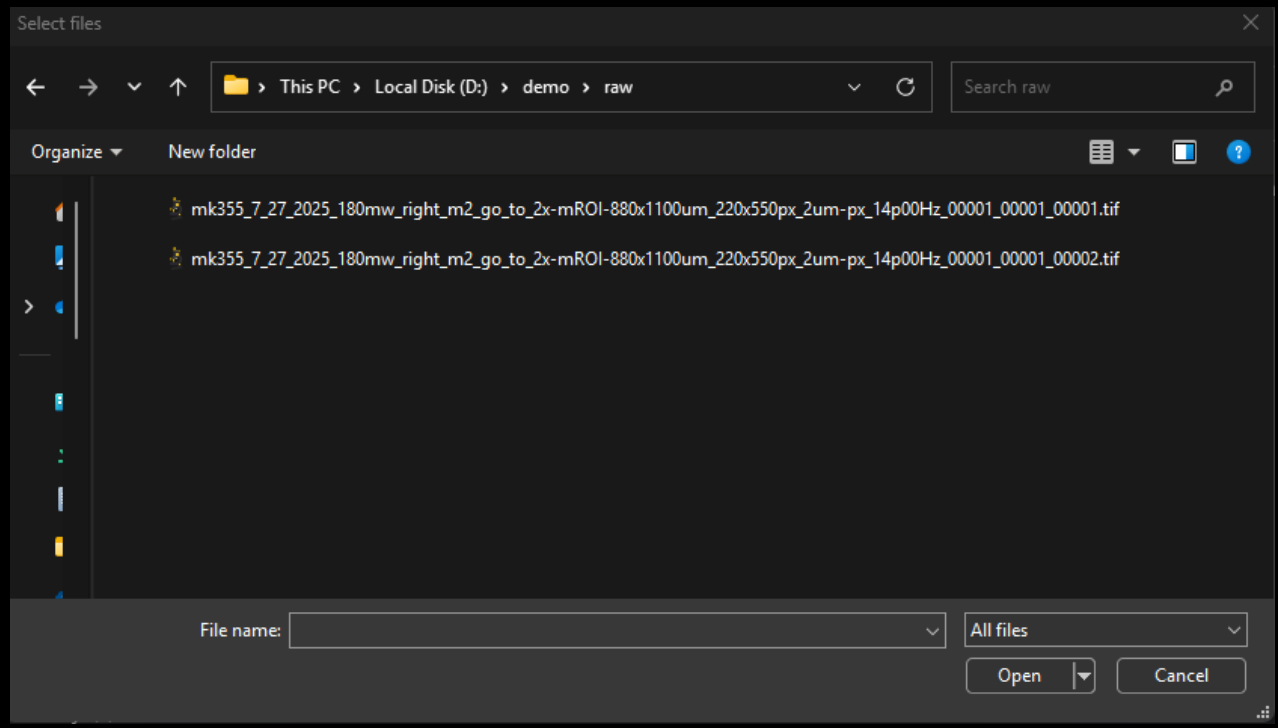
Hover (?) for tooltips, many buttons also contain tooltips on hover



Open File / Select Folder

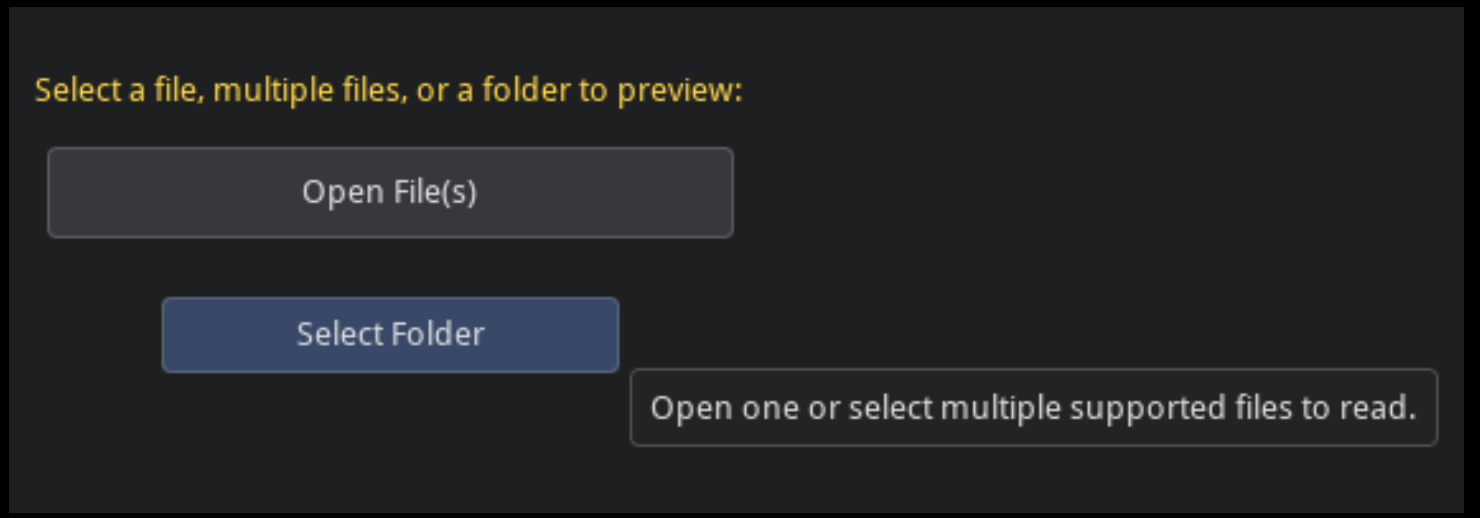
Open Files: Dialog box that shows selectable and multi-selectable

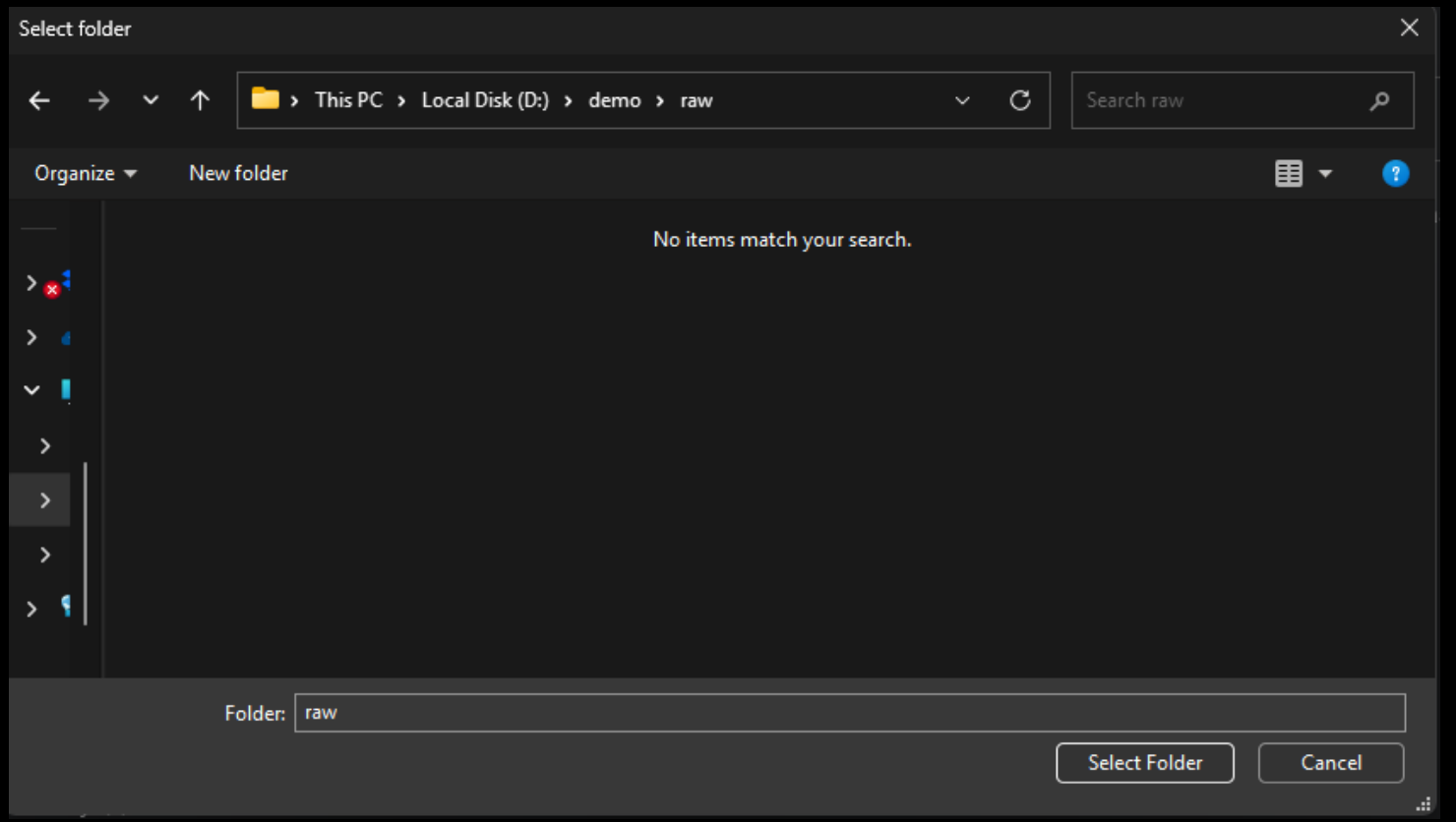




Open File / Select Folder

Select Folder: Dialog box that allows folder selection only
- Will not show you files in that folder
- Helpful for large datasets that may contain multiple files





There are files here, as seen previously.

In this case, all files in this directory will be loaded.

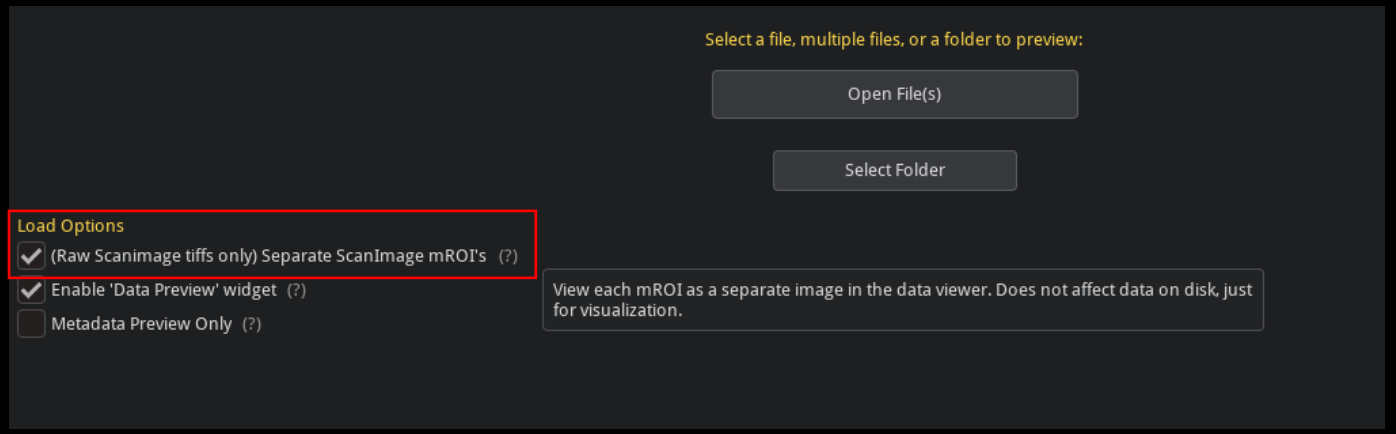
Opens tiff / zarr / bin / h5 produced by mbo.imwrite()

Load Options

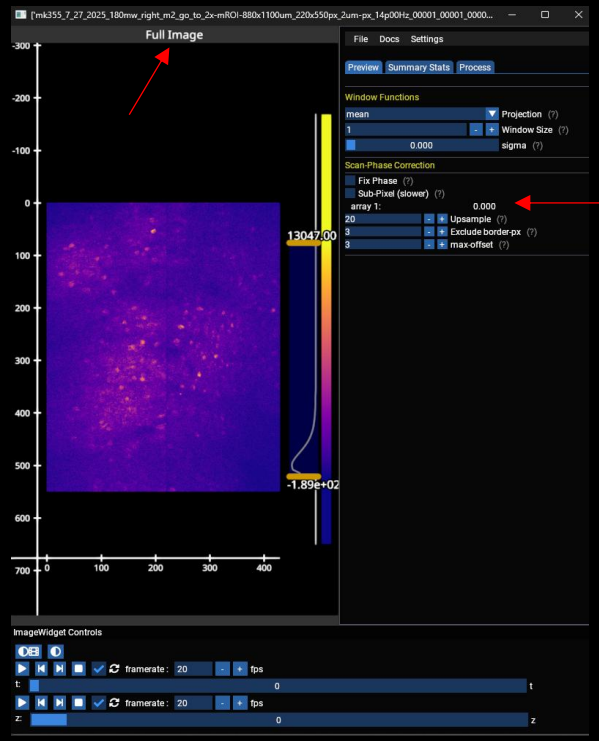
- Only affect data visualization (e.g. no mROI splitting happens on disk)

(Raw ScanImage tiffs only) Separate ScanImage mROI's

- Open ScanImage multi-ROI's as separate arrays
  - Visualizations (e.g. scan-phase correction) is done PER-ROI
  - Helpful to compare scan-phase offsets between mROI's
- If unchecked, both mROI's share an optimal phase-offset value that is calculated when frames are vertically concatenated (as they are on disk, pre-stitching)
- If checked, mROI's have distinct phase-offset values

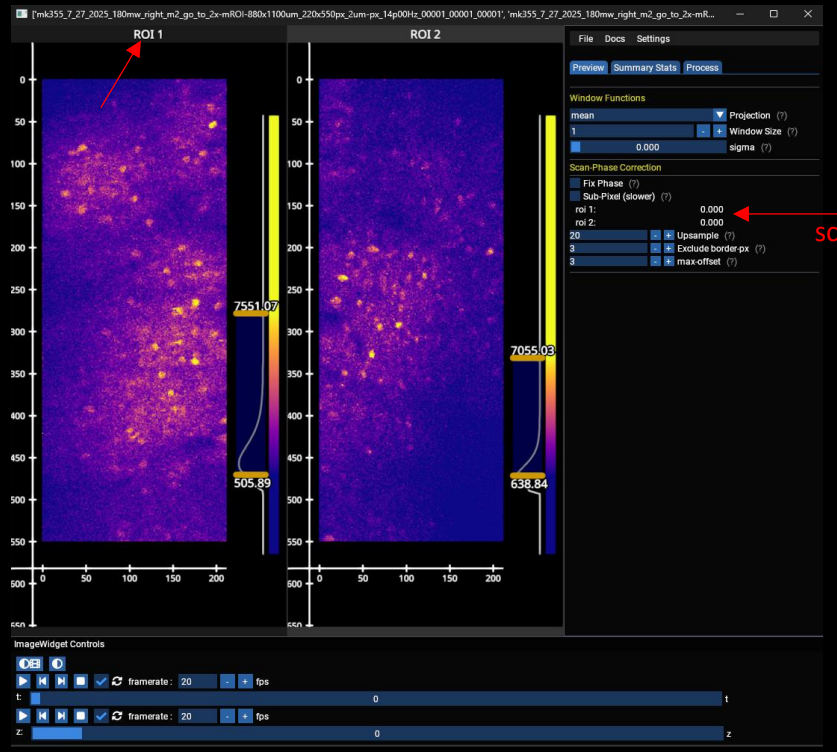


Unchecked
(stitch/concatenate mROIS)



One value for optimal scan-phase offset

Checked
(Separate mROIS)



One value for optimal scan-phase offset, for each mROI

Load Options

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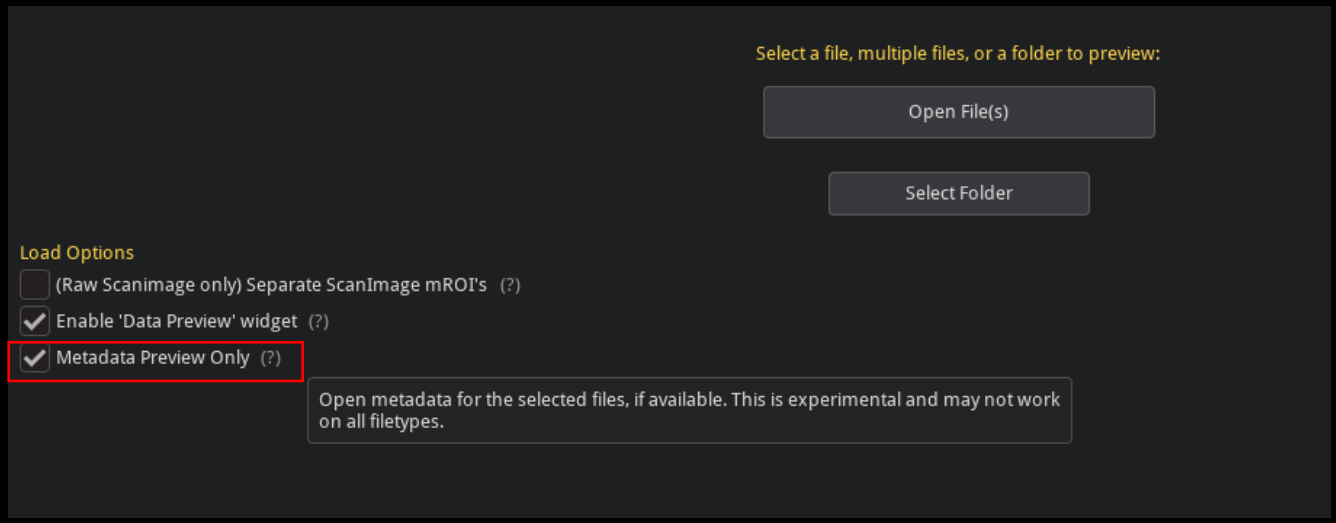
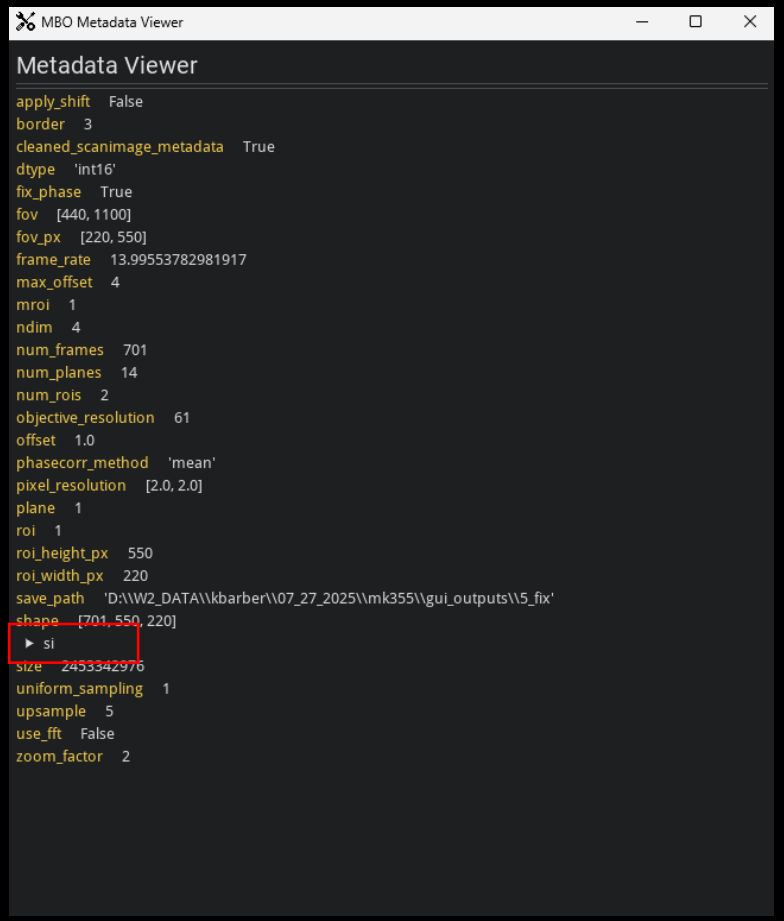
Metadata Preview (experimental)
- Quickly preview all available consolidated and raw scanimage metadata
- Supports raw ScanImage tiffs, and any extracted outputs from mbo.imwrite
- Also support suite2p ops.npy files





Collapsable subgroups

Preview Data Widget (Raw ScanImage Tiff Only)

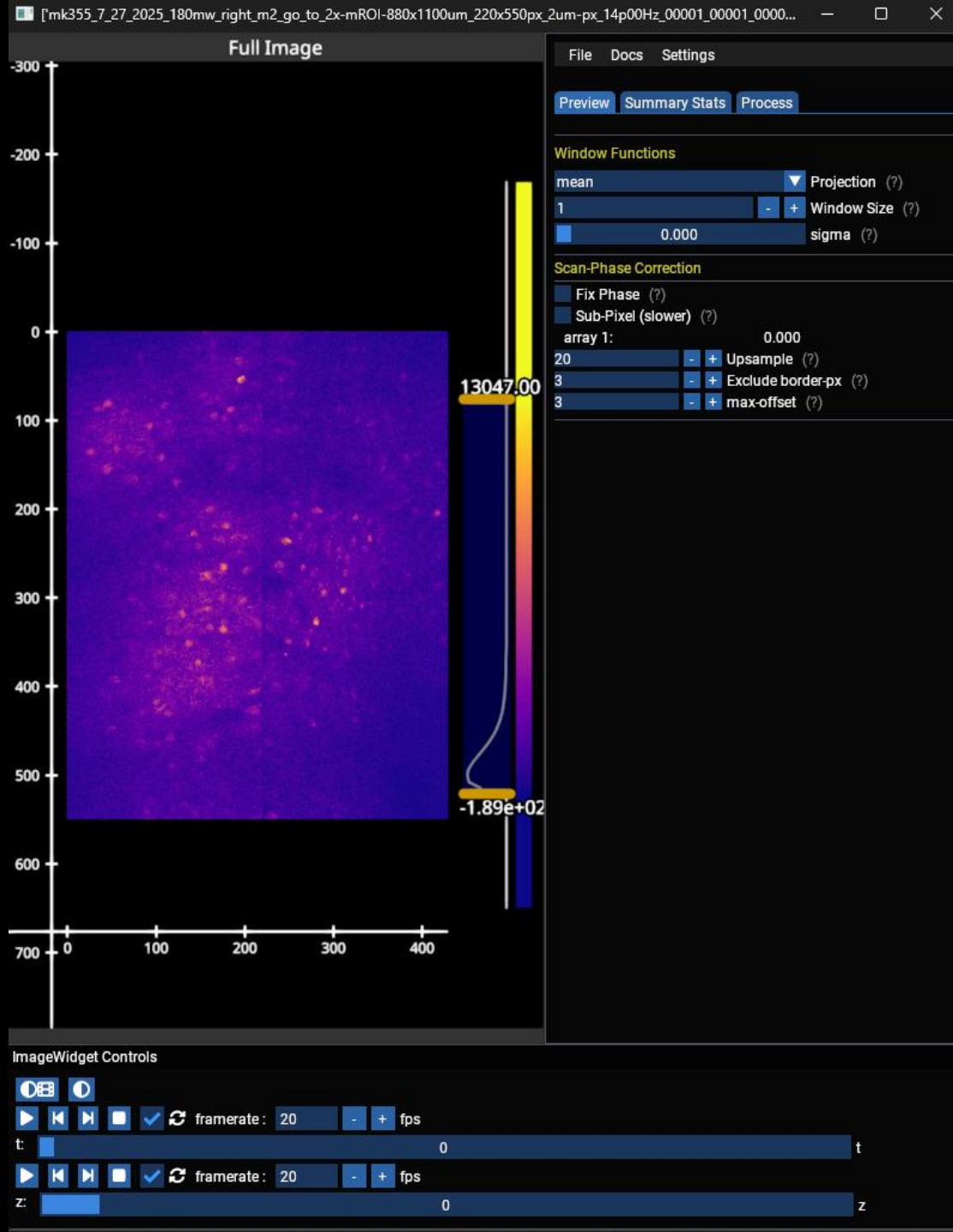
- Lazy-load a raw-scanimage tiff
- Larger tiffs (e.g. 60,000x14x400x400) could take ~30s to load on the first load
- After that, it will be cached and load in sub 5s

Window Functions

- View mean, max, std, mean-subtracted image over the given window size
- Windows > 20 frames will slow rendering
- Windows are applied over the *time dimension only*
- Windows do not take effect until Window Size > 3 frames

sigma

- Apply a spatial gaussian filter on each frame
- Helps visualize very common pre-processing step supplied as a parameter to processing pipelines
    - suite2p “smooth_sigma” parameter for registration



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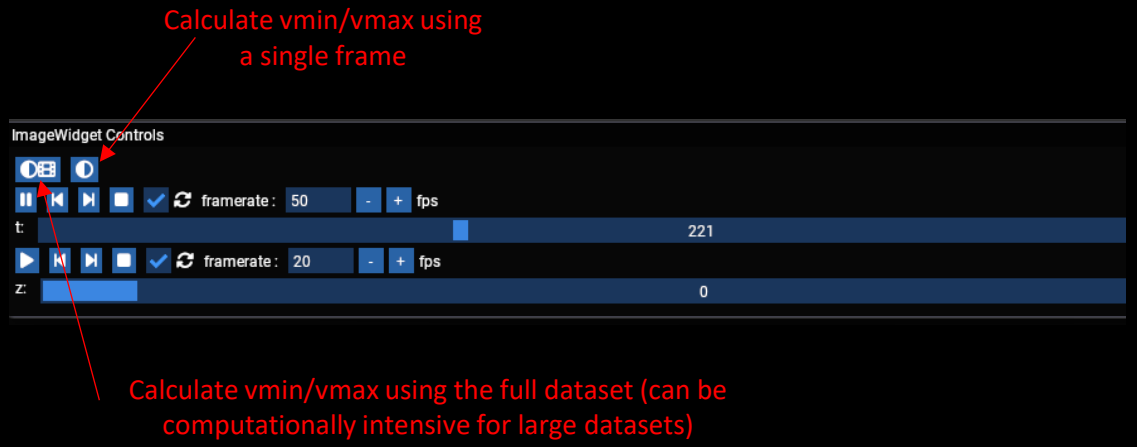
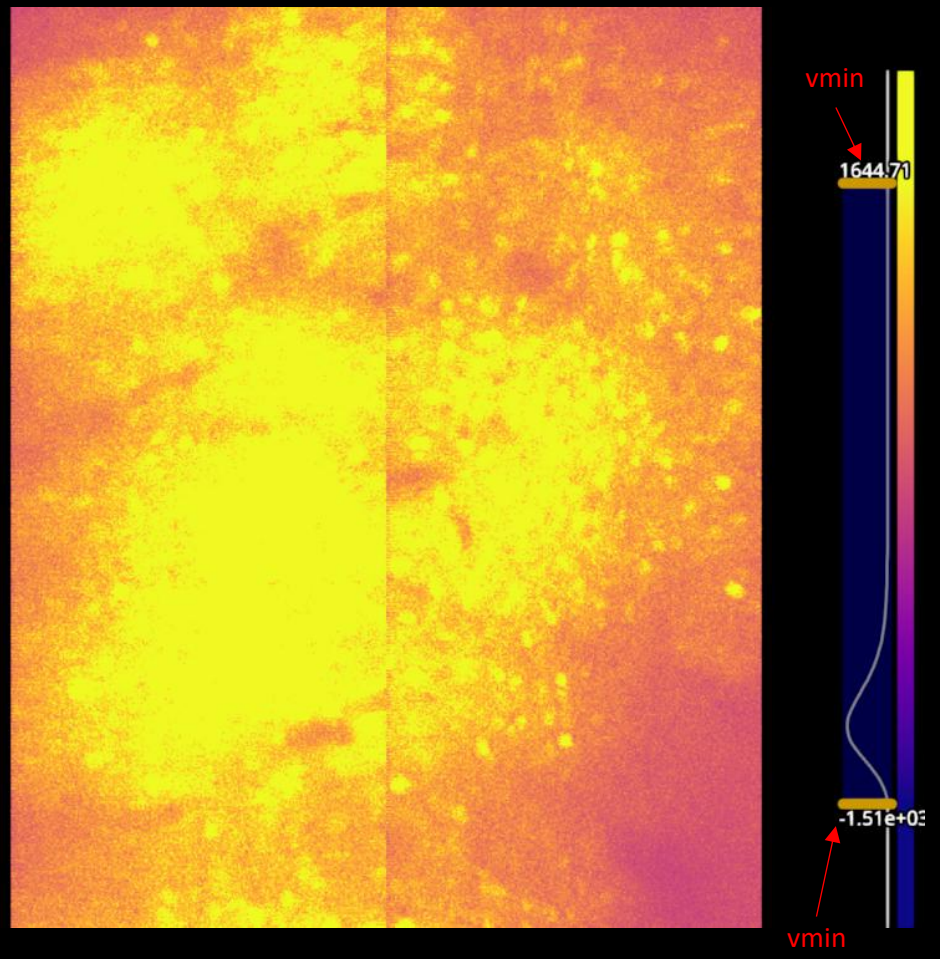
- Dynamic range can vary drastically between projections

- Use the 'auto-adjust contrast' button to automatically adjust vmin/vmax





You can also adjust the vmin/vmax manually with the histogram widget

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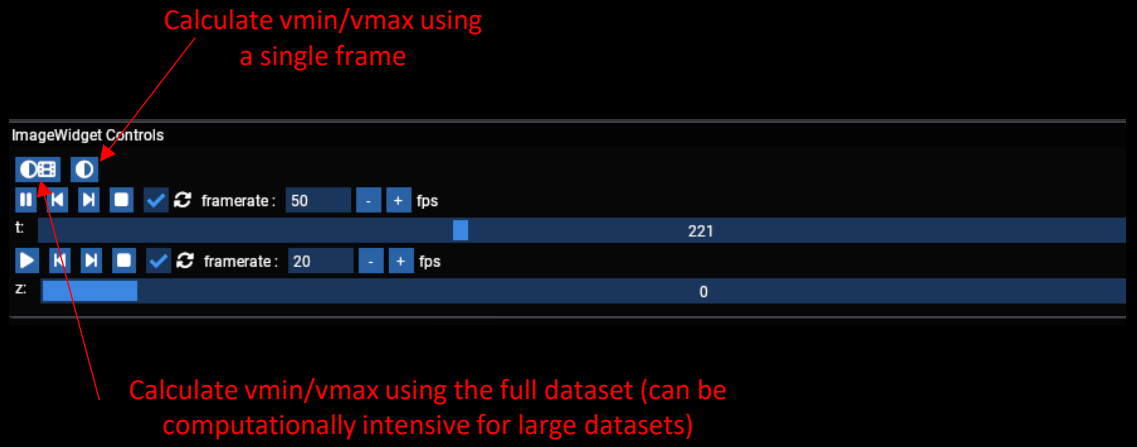

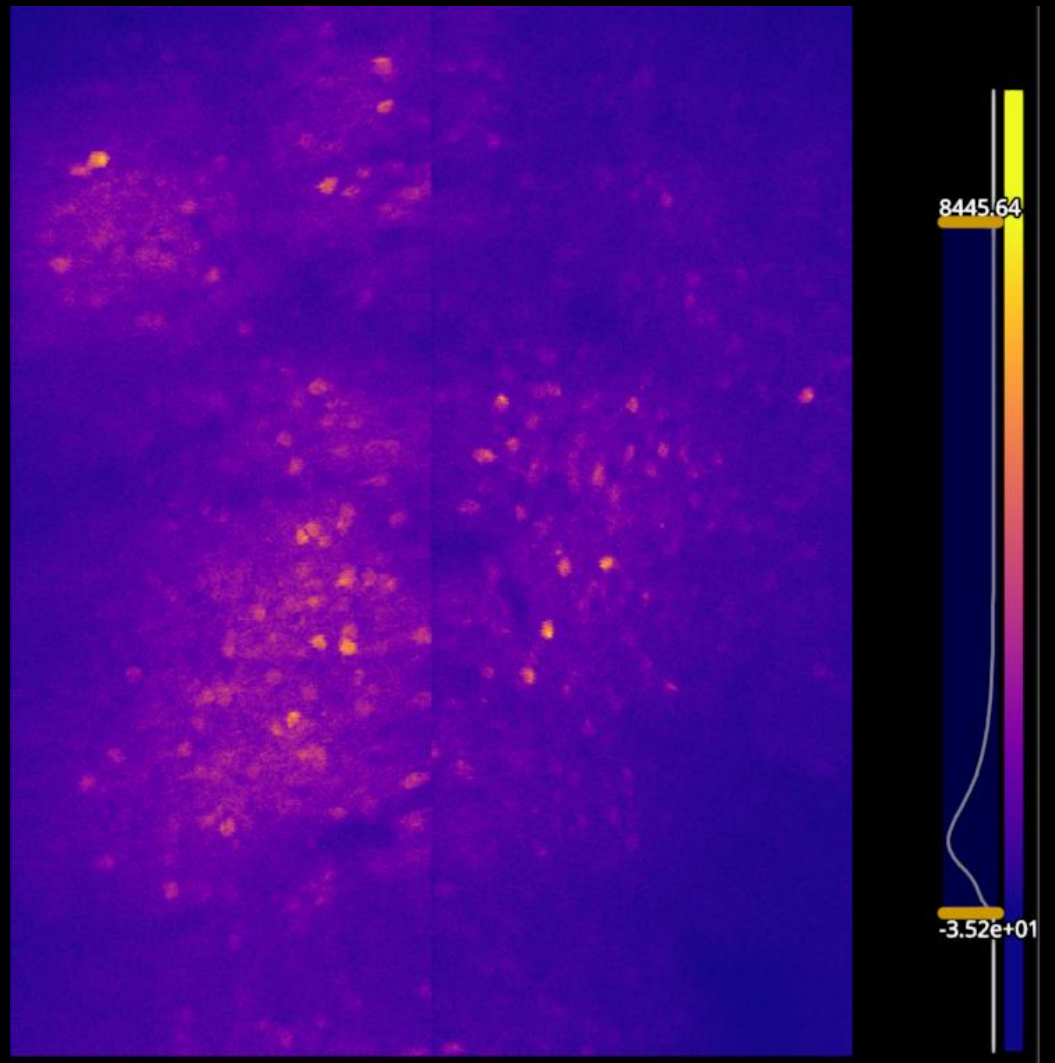

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Preview Data Widget

(Raw ScanImage Tiff Only)

Scan Phase Correction

- Compare with / without phase correction
- Use a projection with window-size of 3-20
    - Large datasets can be very slow with window size> 6
- Scan-offset results are typically reliable with a window size > 3

Fix-Phase: Enable / Disable scan-phase correction

Sub-Pixel: Enable / Disable FFT-based sub-pixel phase correction

Upsample (sub-pixel only): lines are registered within 1/upsample of a pixel

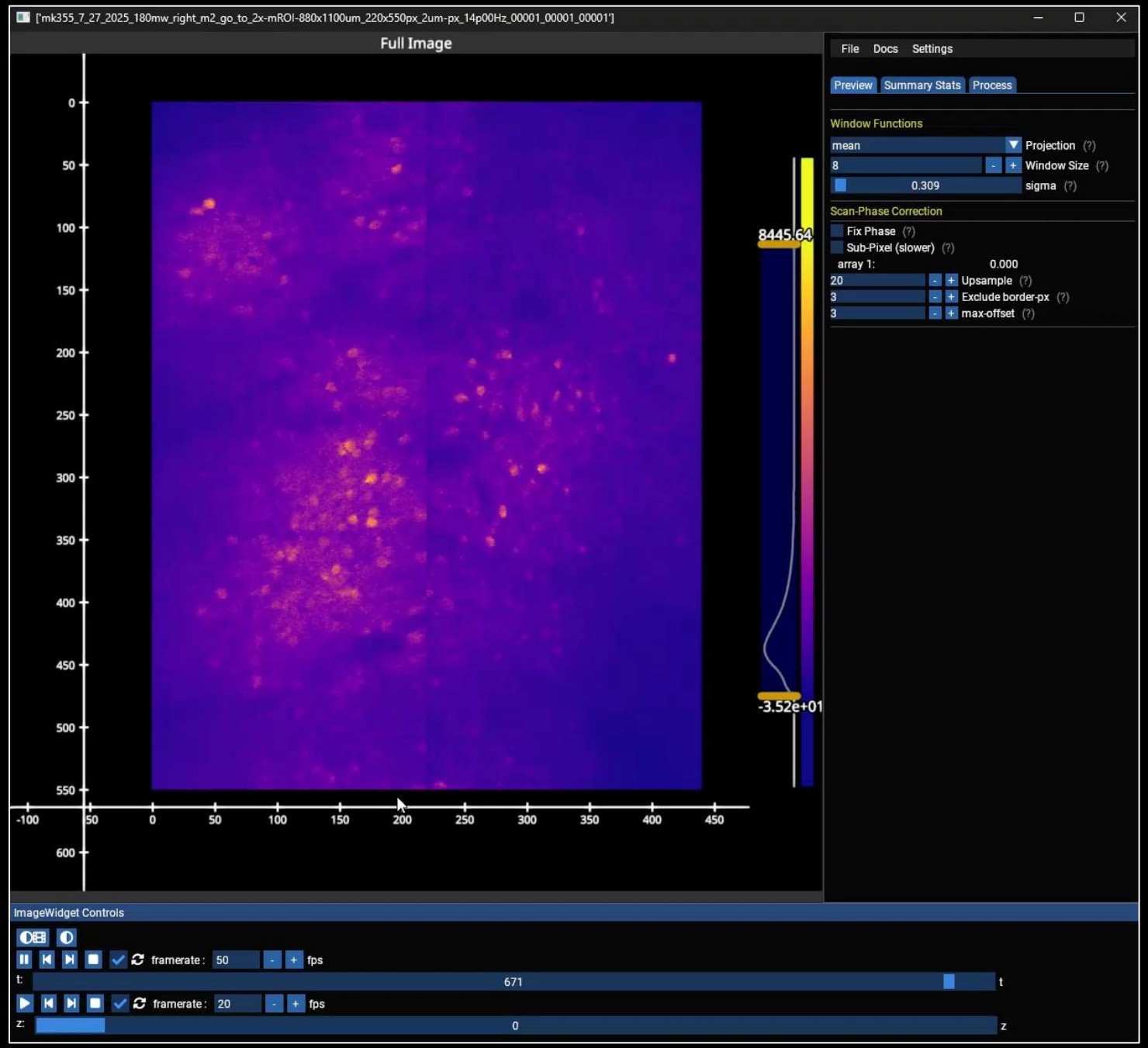
- Larger upsample = more precise shifts = more computationally intensive

Exclude border px: Pixels on the border tend to be more poorly aligned due to the non-linear angular velocity of the resonant galvo scanner. This excludes a number of pixels around the full image or individual mROI*s.*

Max-offset: Limit the allowed offset to this many pixels. Helpful in noisy recordings when some frames with minimal signal yield offsets 5+. Offsets very rarely exceed 2 or 3 pixels.

Recommended workflow:

View a mean-subtracted 3-15 window-size projection.
Check all mROI's without Fix Phase checked, and with fix-phase checked.
First adjust border/max offset, see if you can get visually reliable correction.
Turn on and off sub-pixel rapidly (may need to lower window size) and see if it improves scan-phase correction. If so, decrease upsample until you find a stable result across many frames (typically a value of 2 or 3).



Preview Data Widget

(Raw ScanImage Tiff Only)

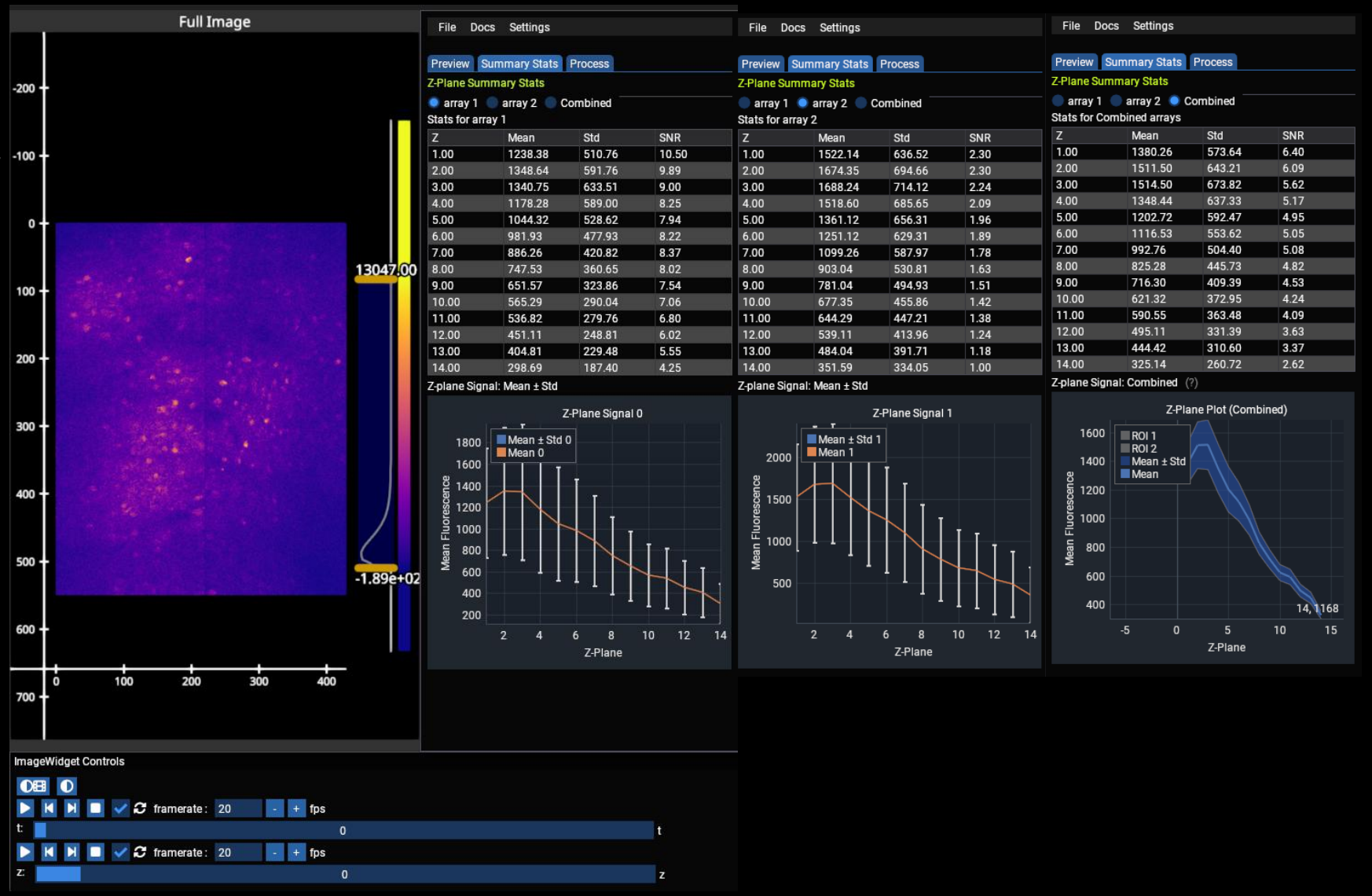
Summary Stats

- Compute mean/std/snr for each mROI regardless of splitting or stitching on every 10th frame
- SNR: mean / std_img
- Combined: Take mean of all mROI's

Useful for evaluating:

- z-plane quality as a function of depth
- mROI quality over the FOV

*note: these means are used for mean-subtracted image preview



Preview Data Widget

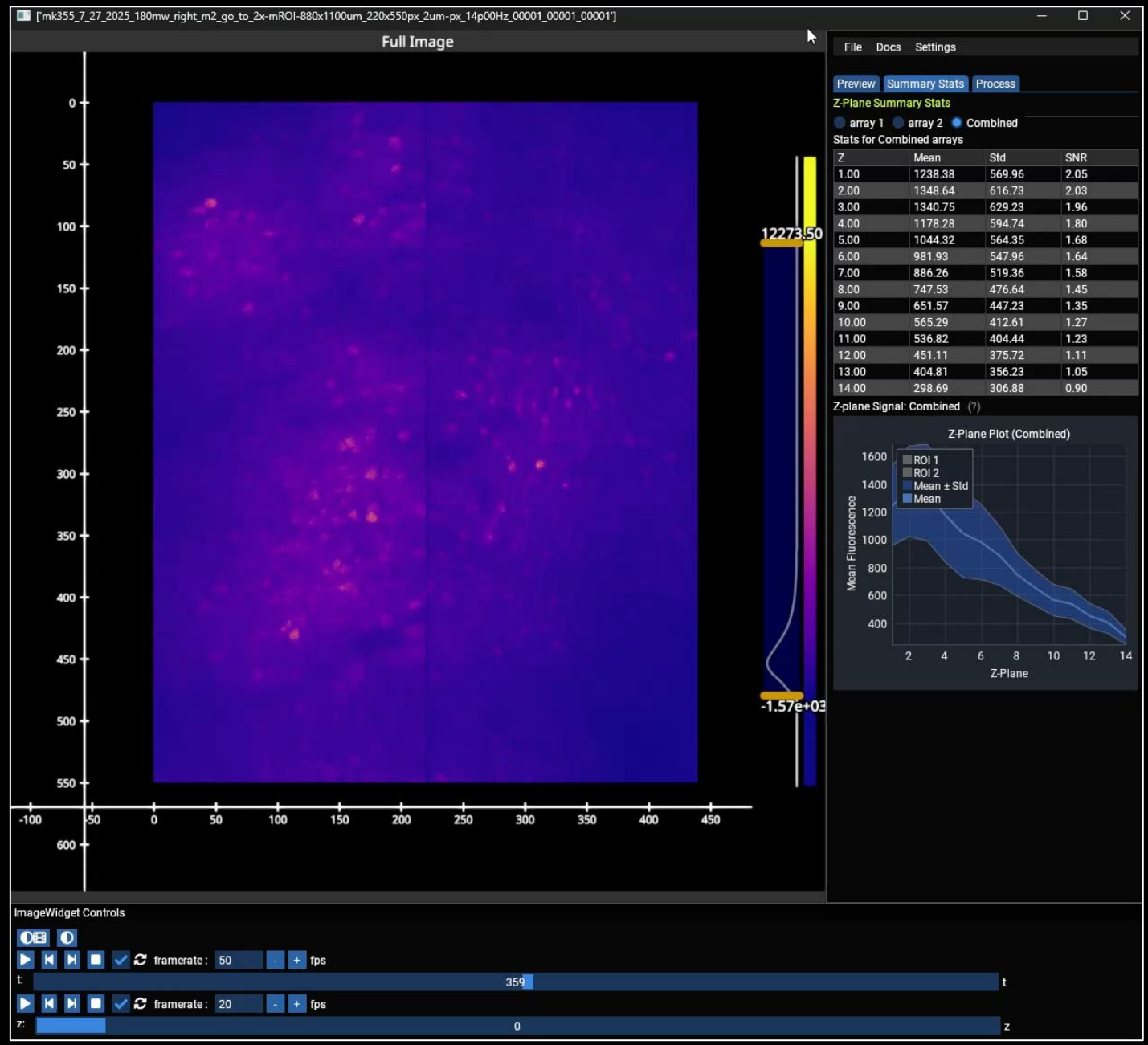
(Raw ScanImage Tiff Only)

Saving Data to Disk

- File -> Save As
- Opens a transparent save dialog

*Note: This demo is fast because it's a single ~1GB tiff

I/O speed depends on file-size, how users chose to split frames across tiffs (in scanimage during acquisition), output filetype, FFT and upsampling factor.



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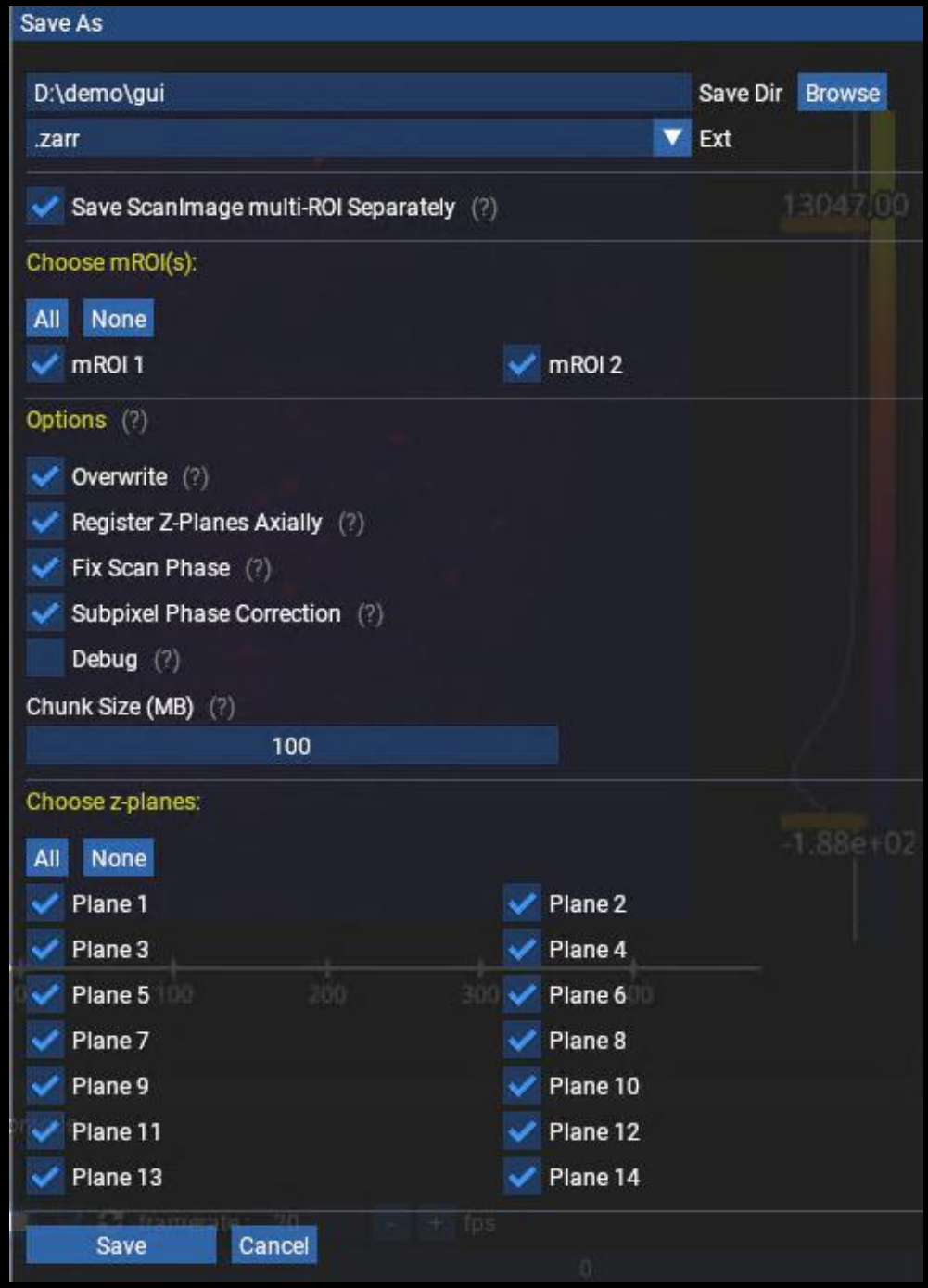
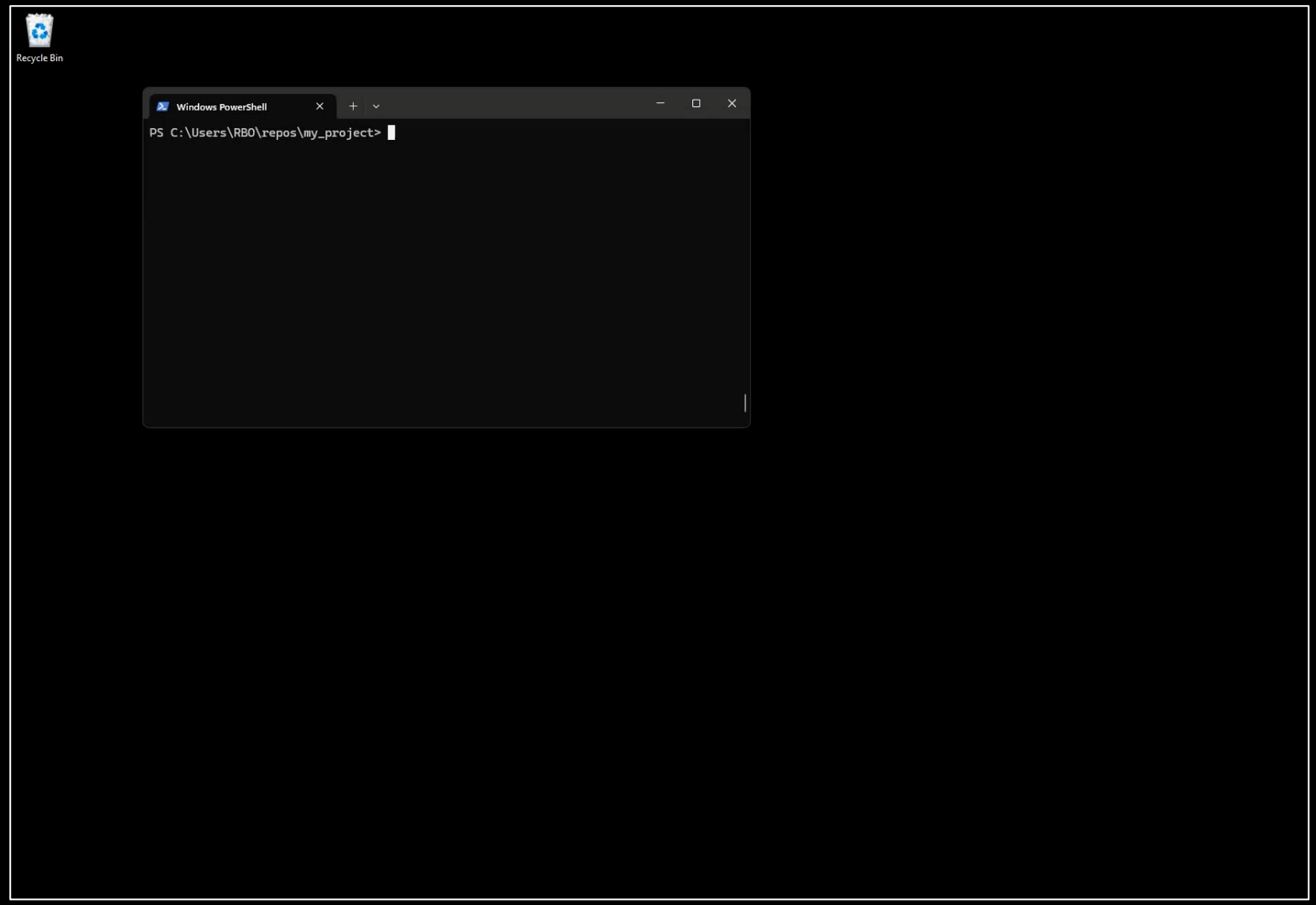


Preview Data Widget

(Raw ScanImage Tiff Only)

Reading Extracted Data

- Ensure you TURN OFF data preview or this will error
- This will not yet allow split-mROI merging



Suite2p Processing

- Run Suite2p on ***currently selected z-plane***
- All parameters, with descriptions, provided
- Use crop selector to run on a spatially cropped subset of the data