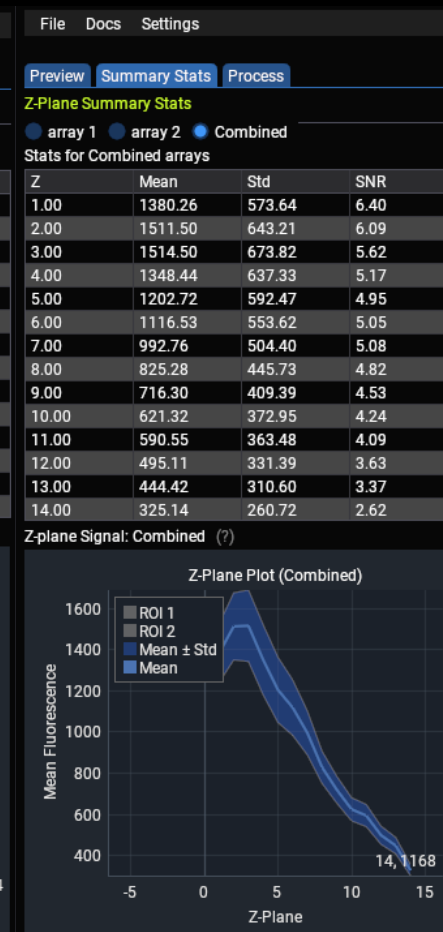
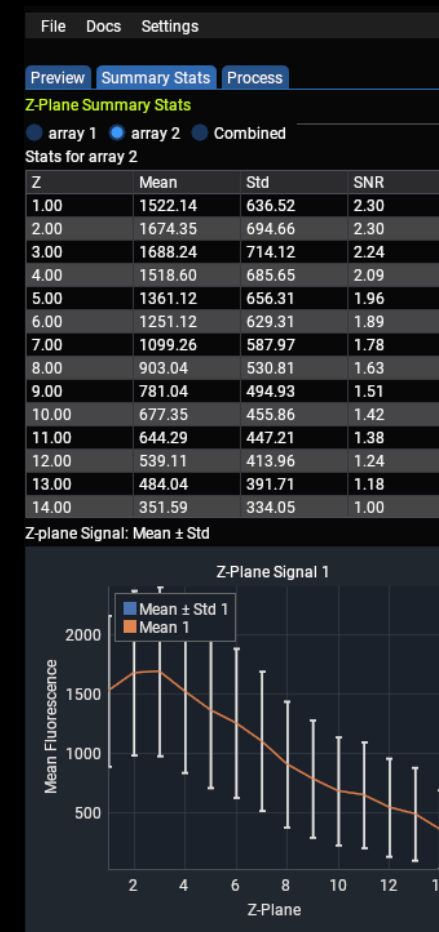
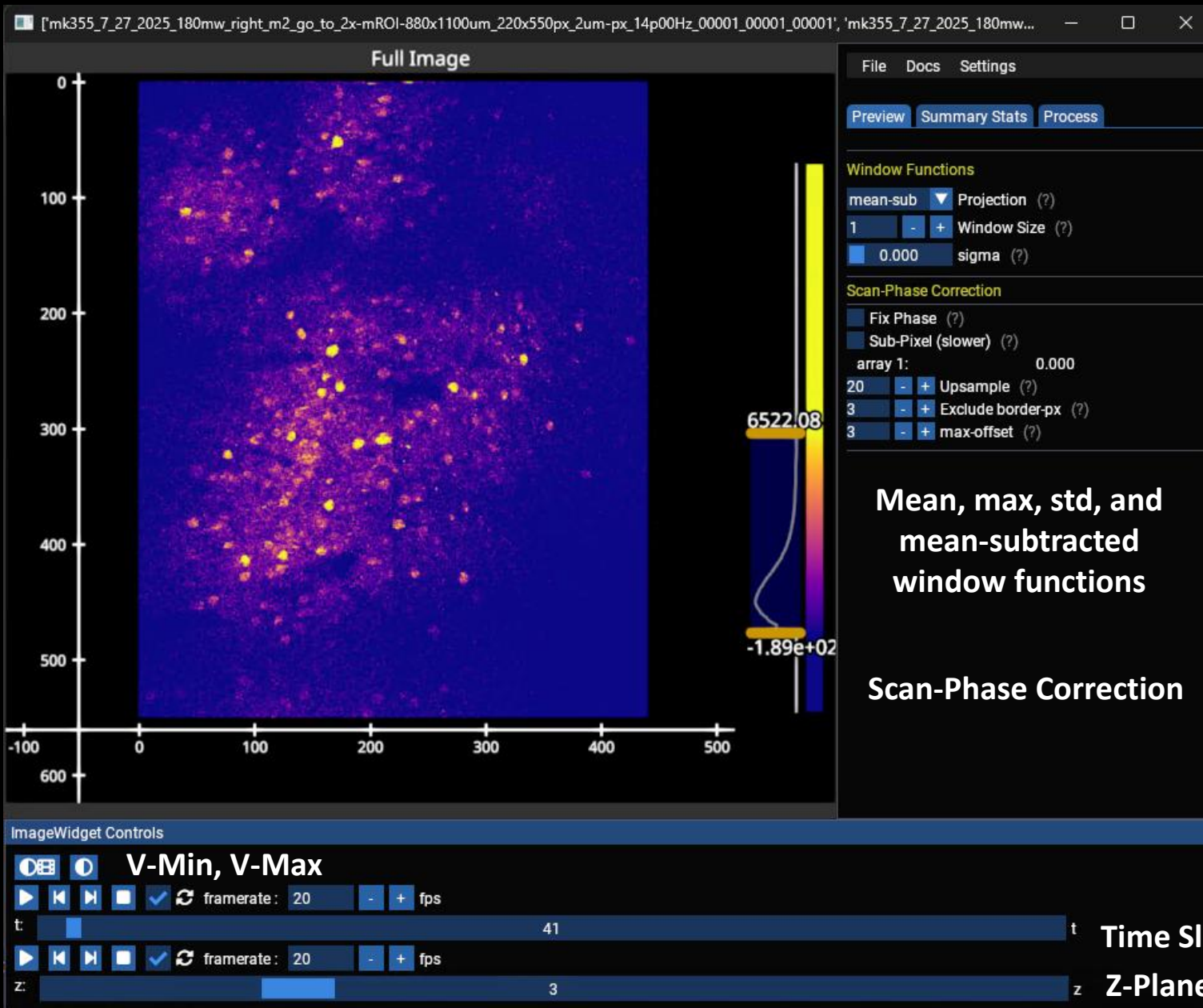
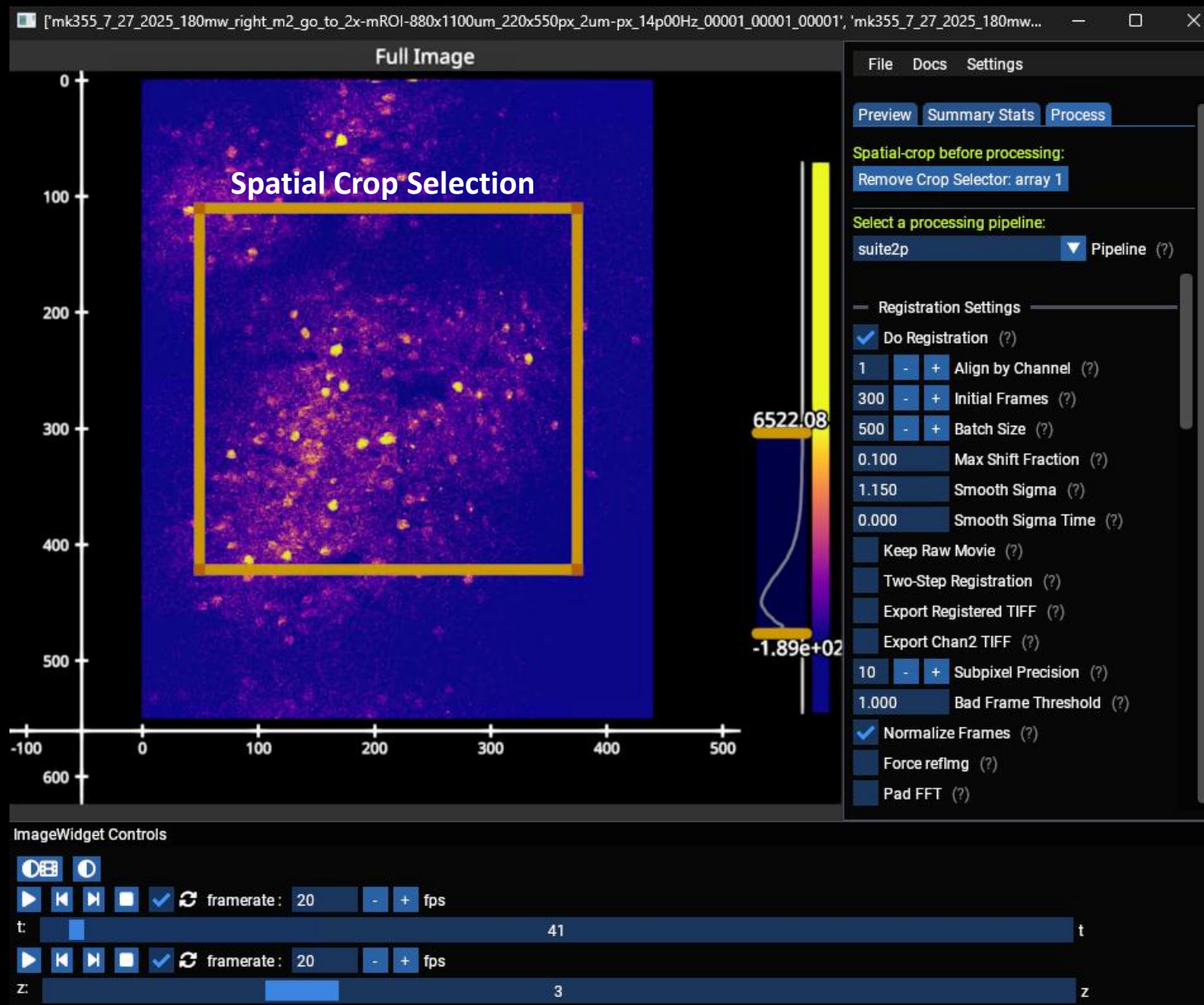


MBO GUI User Guide Overview



Multi-ROI, multi-Zplane statistics

Quickly Process with Suite2p



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

Save As

D:\demo\gui Save Dir Browse

.zarr Ext

☒ Save ScanImage multi-ROI Separately (?)

Choose mROI(s):

All None

☒ mROI 1 ☒ mROI 2

Options (?)

☒ Overwrite (?)

☒ Register Z-Planes Axially (?) Suite3d axial registration

☒ Fix Scan Phase (?)

☒ Subpixel Phase Correction (?)

☐ Debug (?)

Chunk Size (MB) (?)

100

Choose z-planes:

All None

☒ Plane 1 ☒ Plane 2

☒ Plane 3 ☒ Plane 4

☒ Plane 5 ☒ Plane 6

☒ Plane 7 ☒ Plane 8

☒ Plane 9 ☒ Plane 10

☒ Plane 11 ☒ Plane 12

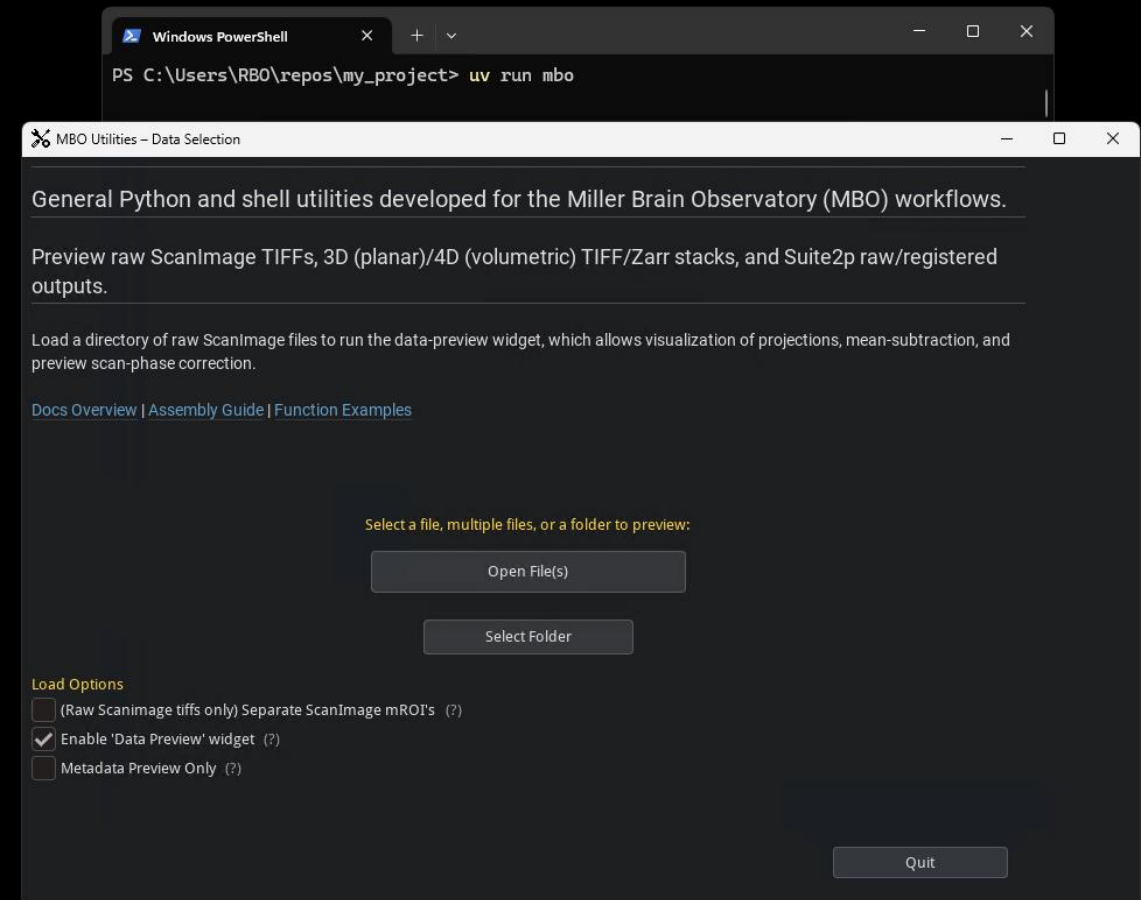
☒ Plane 13 ☒ Plane 14

Save Cancel

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

```
Windows PowerShell
PS C:\Users\RBO\repos\my_project> uv venv --python 3.12.9
Using CPython 3.12.9
Creating virtual environment at: .venv
Activate with: .venv\Scripts\activate
PS C:\Users\RBO\repos\my_project> uv pip install mbo_utilities==2.0.1
Resolved 254 packages in 156ms
Prepared 5 packages in 103ms
```



Temporary fix to io.ImFontAtlas error:

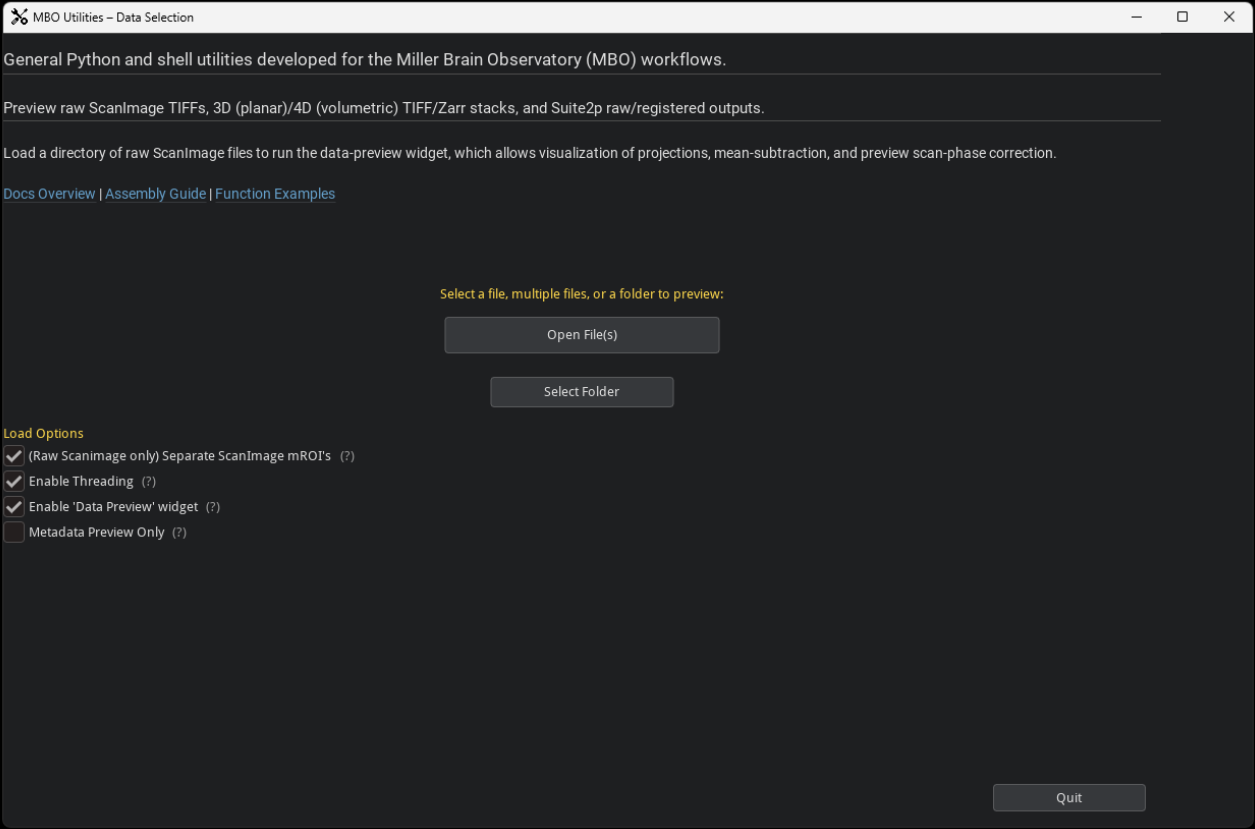
`uv pip install git+https://github.com/pygfx/pygfx.git@main`

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

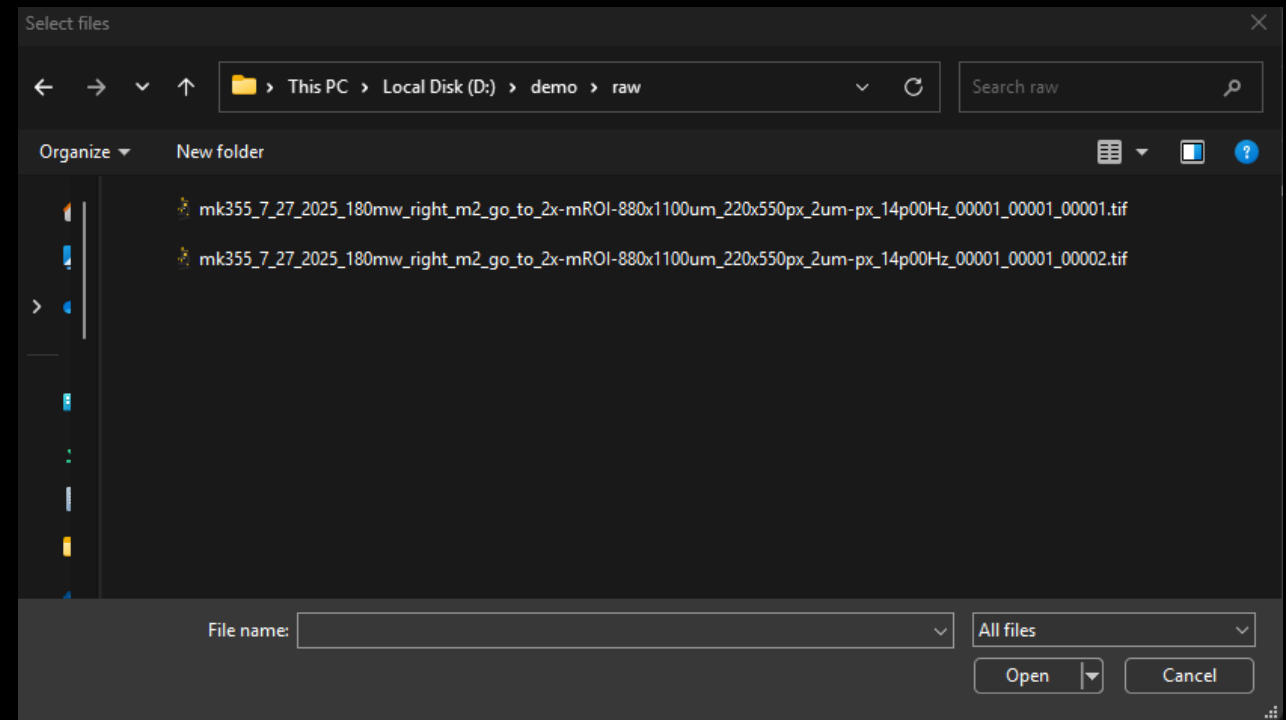
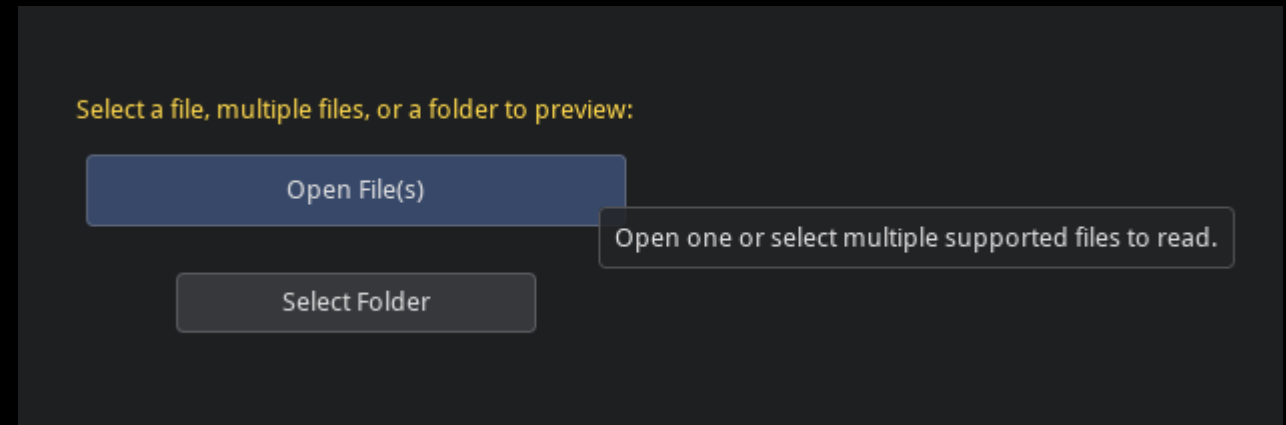
Here, we focus on the ***Data Preview widget***

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.

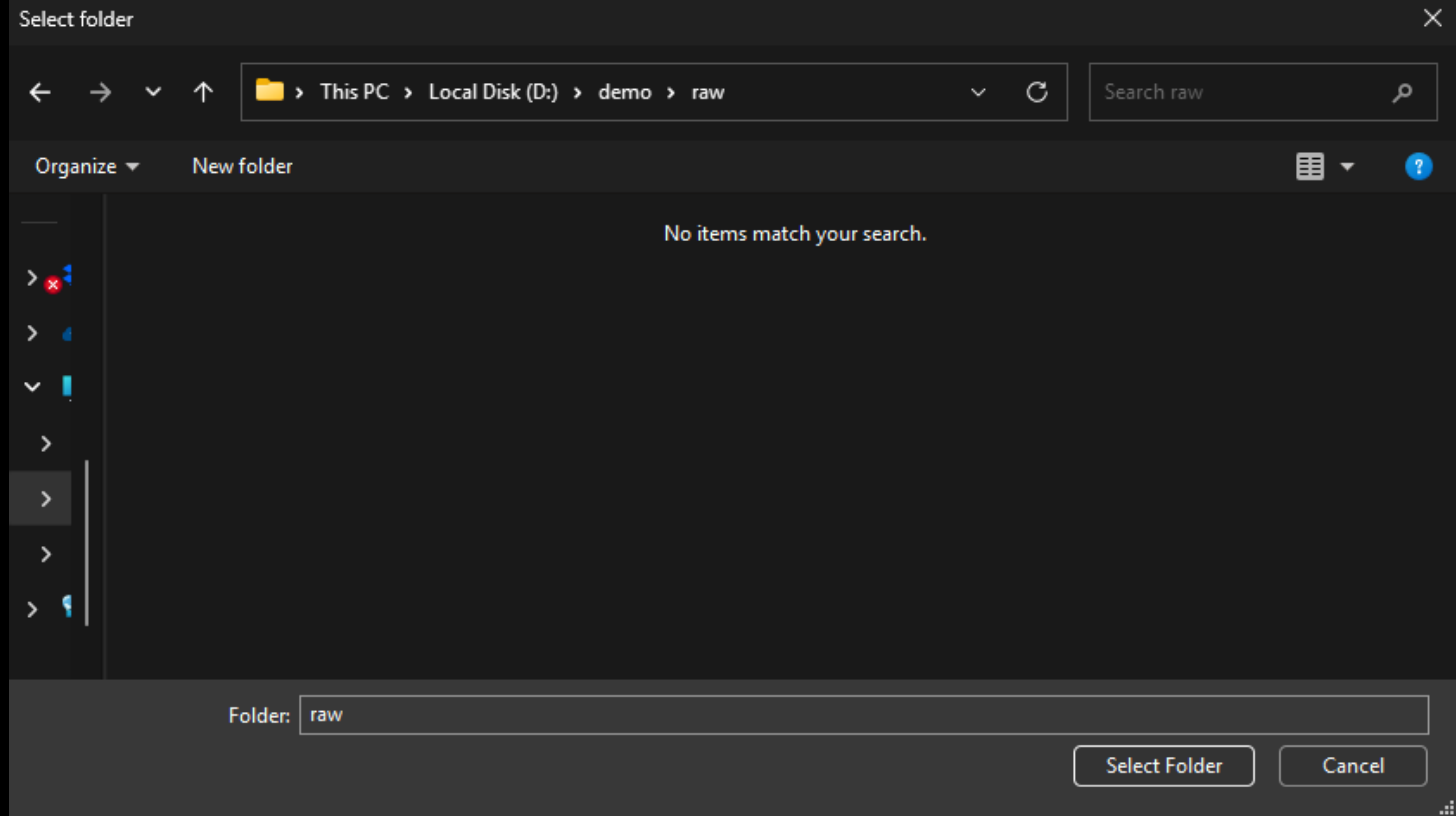
Opens tiff / zarr / bin / h5 produced by
`mbo.imwrite()`

Select a file, multiple files, or a folder to preview:

Open File(s)

Select Folder

Open one or select multiple supported files to read.



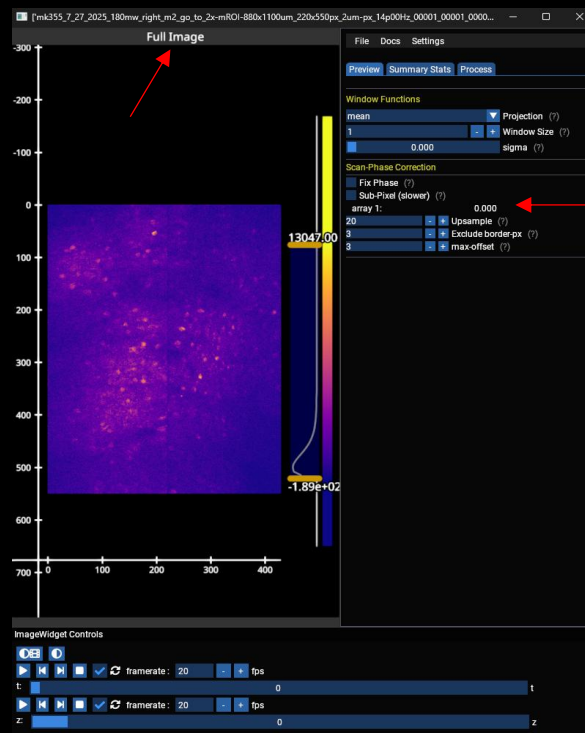
Load Options

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

(Raw ScanImage tiffs only) Separate ScanImage mROI's

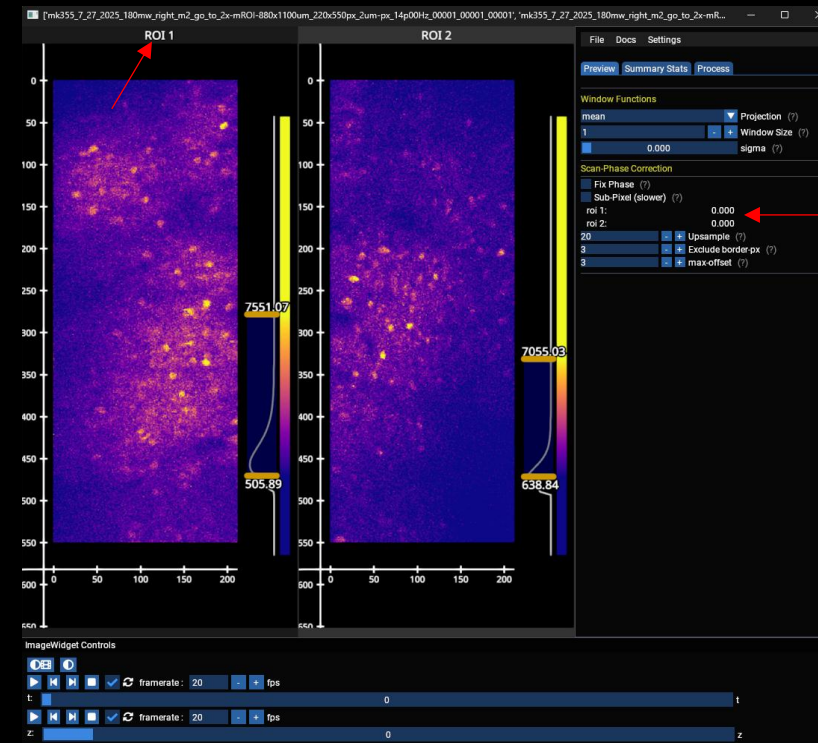
- Open ScanImage multi-ROI's as separate arrays
 - Visualizations (e.g. scan-phase correction) is done PER-ROI
 - Helpful to compare scan-phase offsets between mROI's
- If unchecked, both mROI's share an optimal phase-offset value that is calculated when frames are vertically concatenated (as they are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

Unchecked (stitch/concatenate mROIs)

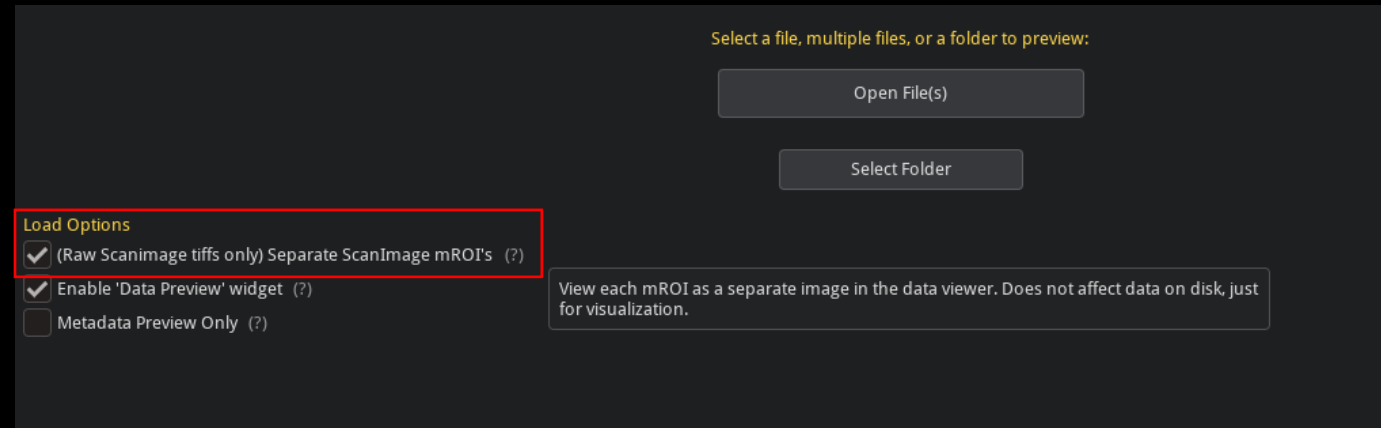


One value for optimal scan-phase offset

Checked (Separate mROIs)



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

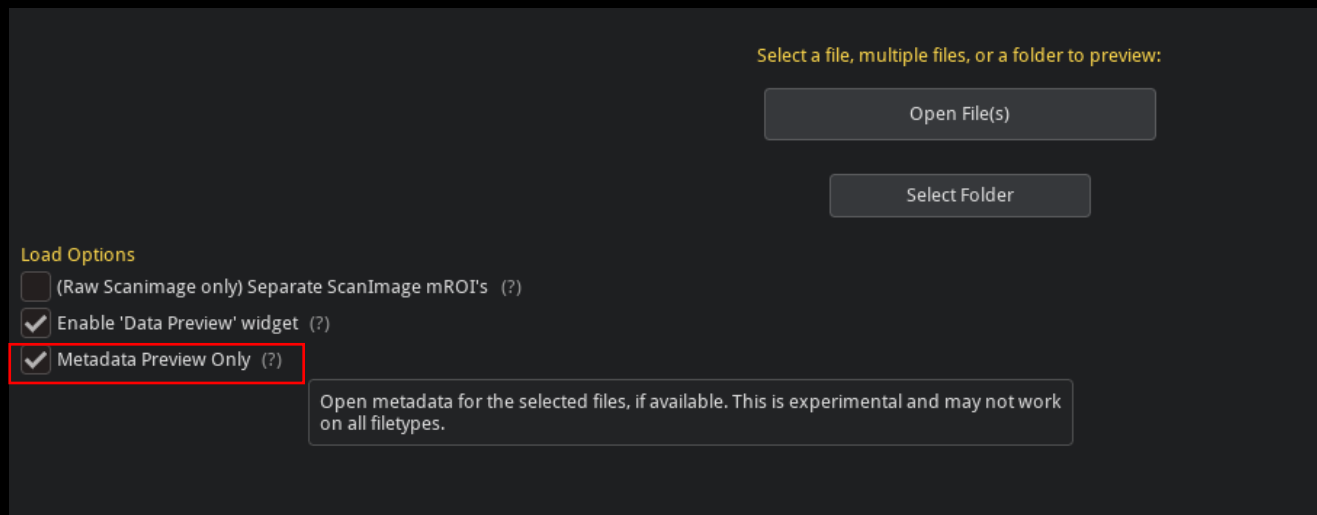
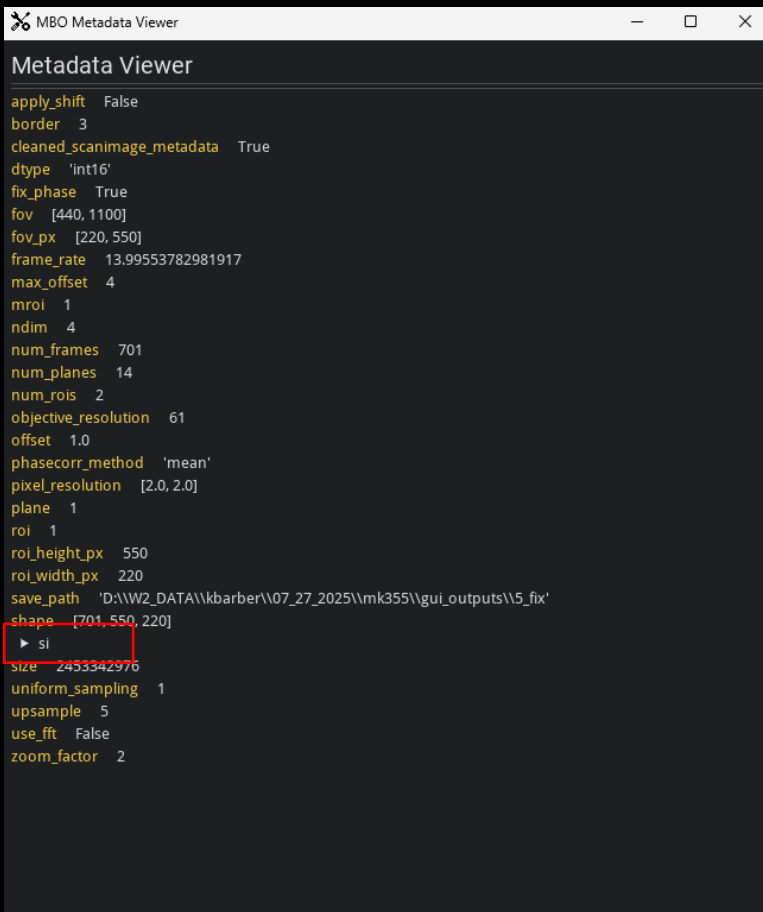


Load Options

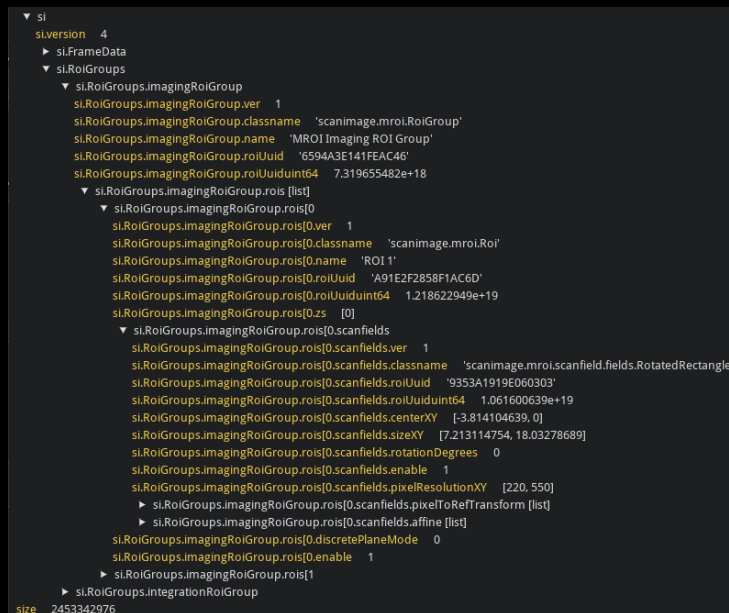
- Only affect data visualization (e.g. no mROI splitting happens on disk)

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



Collapsible 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

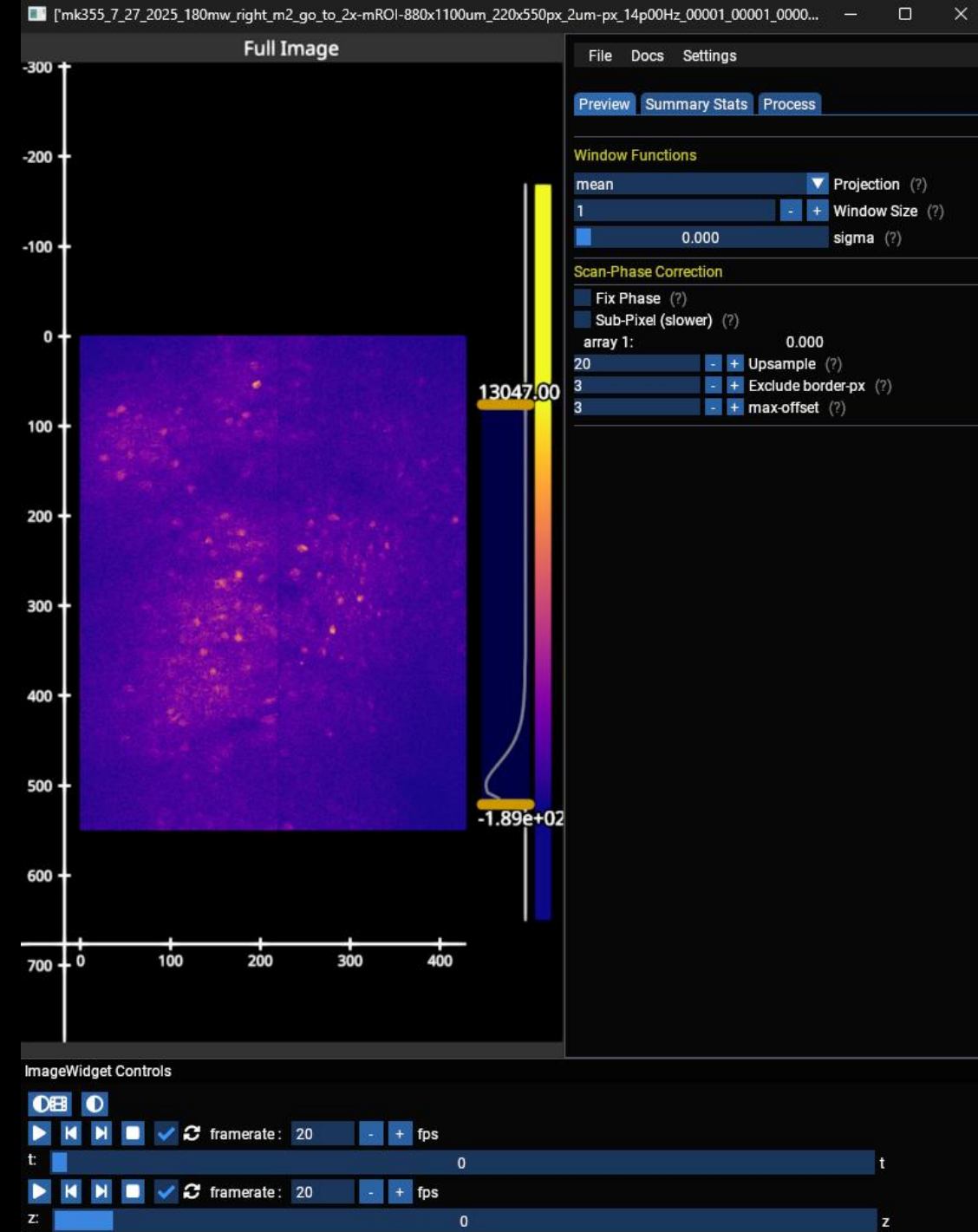
Window Functions

- 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



Preview Data Widget (Raw ScanImage Tiff Only)

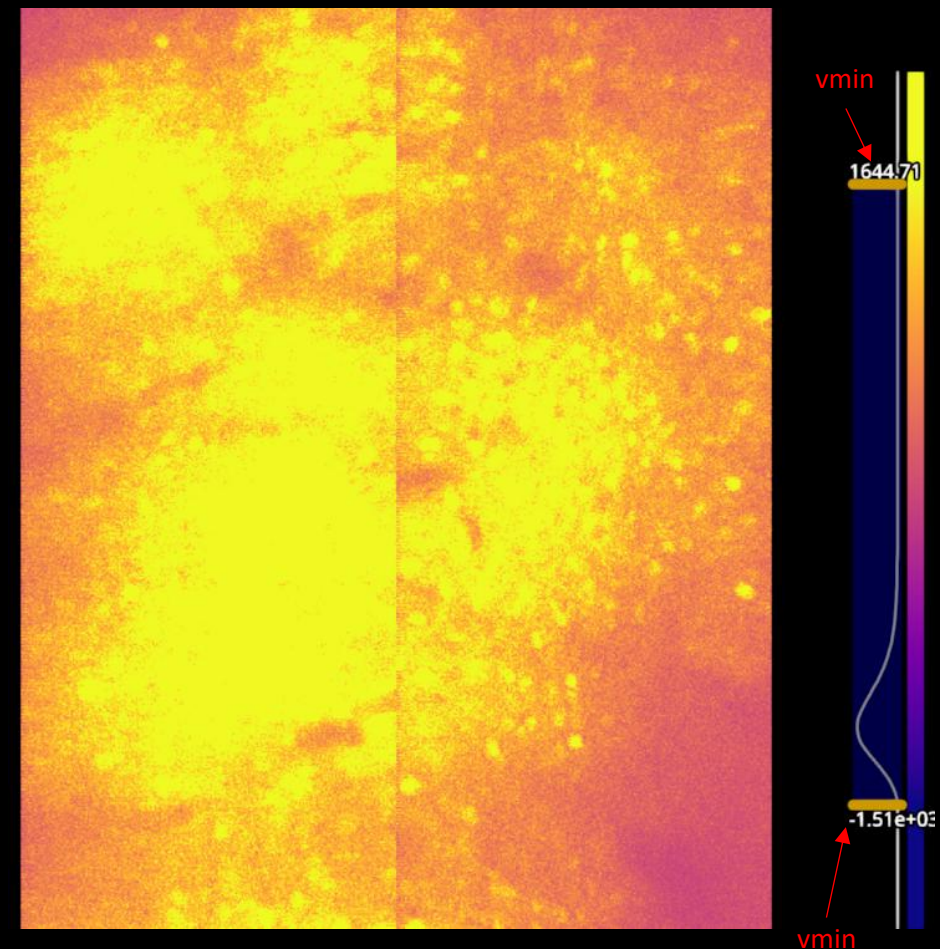
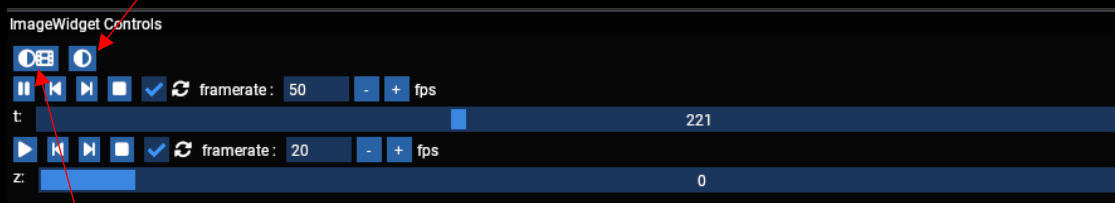
- Lazy-load a raw-scanimage tiff
- Larger tiffs (e.g. 60,000x14x400x400) could take ~30s to load on the first load
- After that, it will be cached and load in sub 5s

Window Functions

- View mean, max, std, mean-subtracted image over the given window size
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- Windows do not take effect until Window Size > 3 frames

- 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

Preview Data Widget (Raw ScanImage Tiff Only)

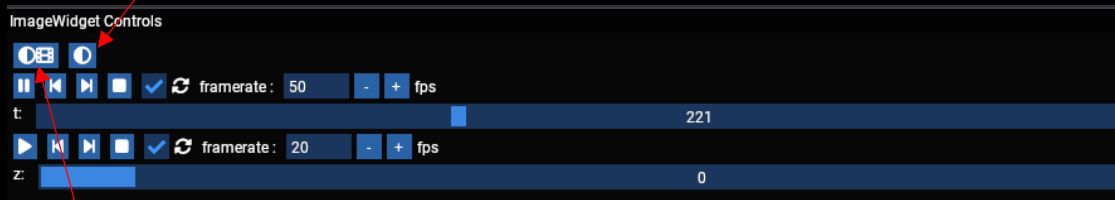
- Lazy-load a raw-scanimage tiff
- Larger tiffs (e.g. 60,000x14x400x400) could take ~30s to load on the first load
- After that, it will be cached and load in sub 5s

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

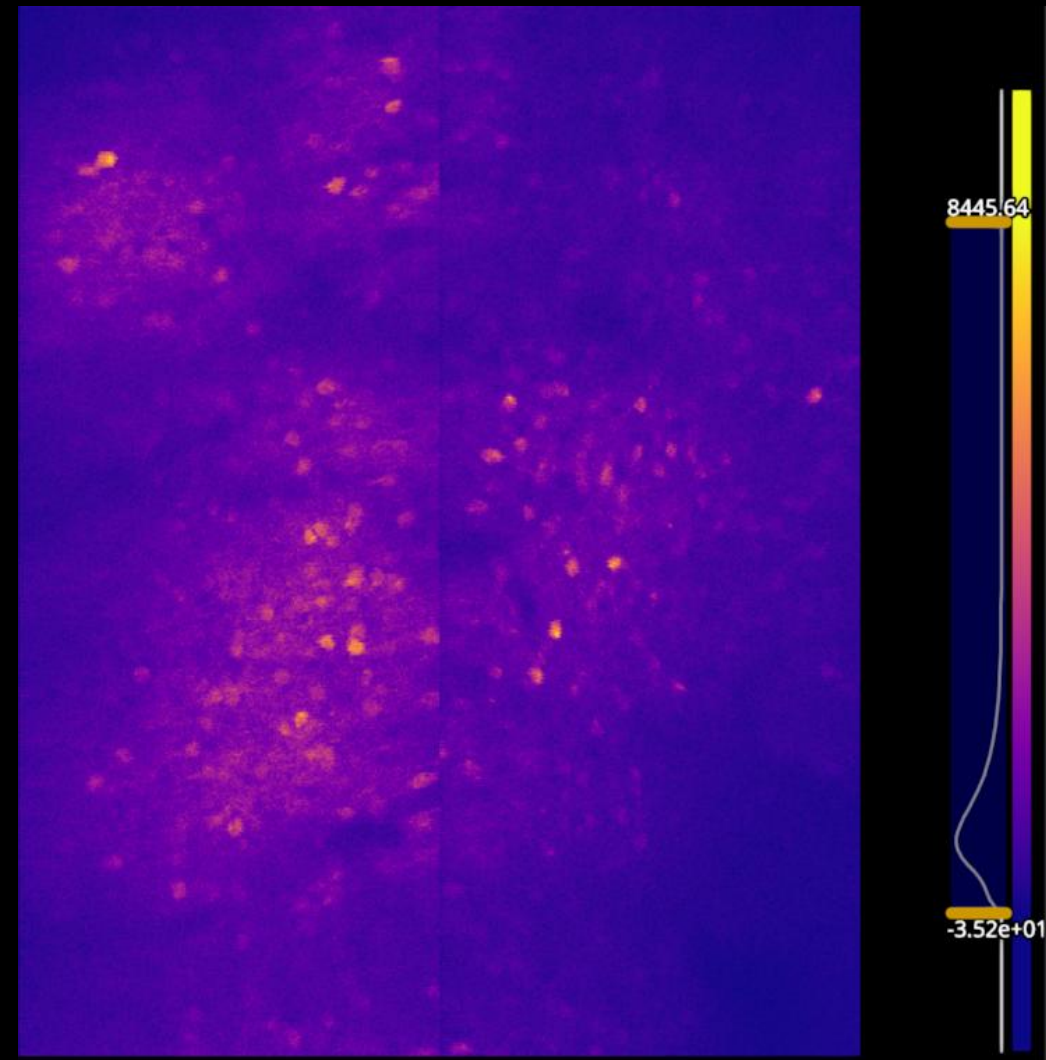
- Dynamic range can vary drastically between projections

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



Calculate vmin/vmax using
a single frame

Calculate vmin/vmax using the full dataset (can be
computationally intensive for large datasets)



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

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 mROIs.

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:

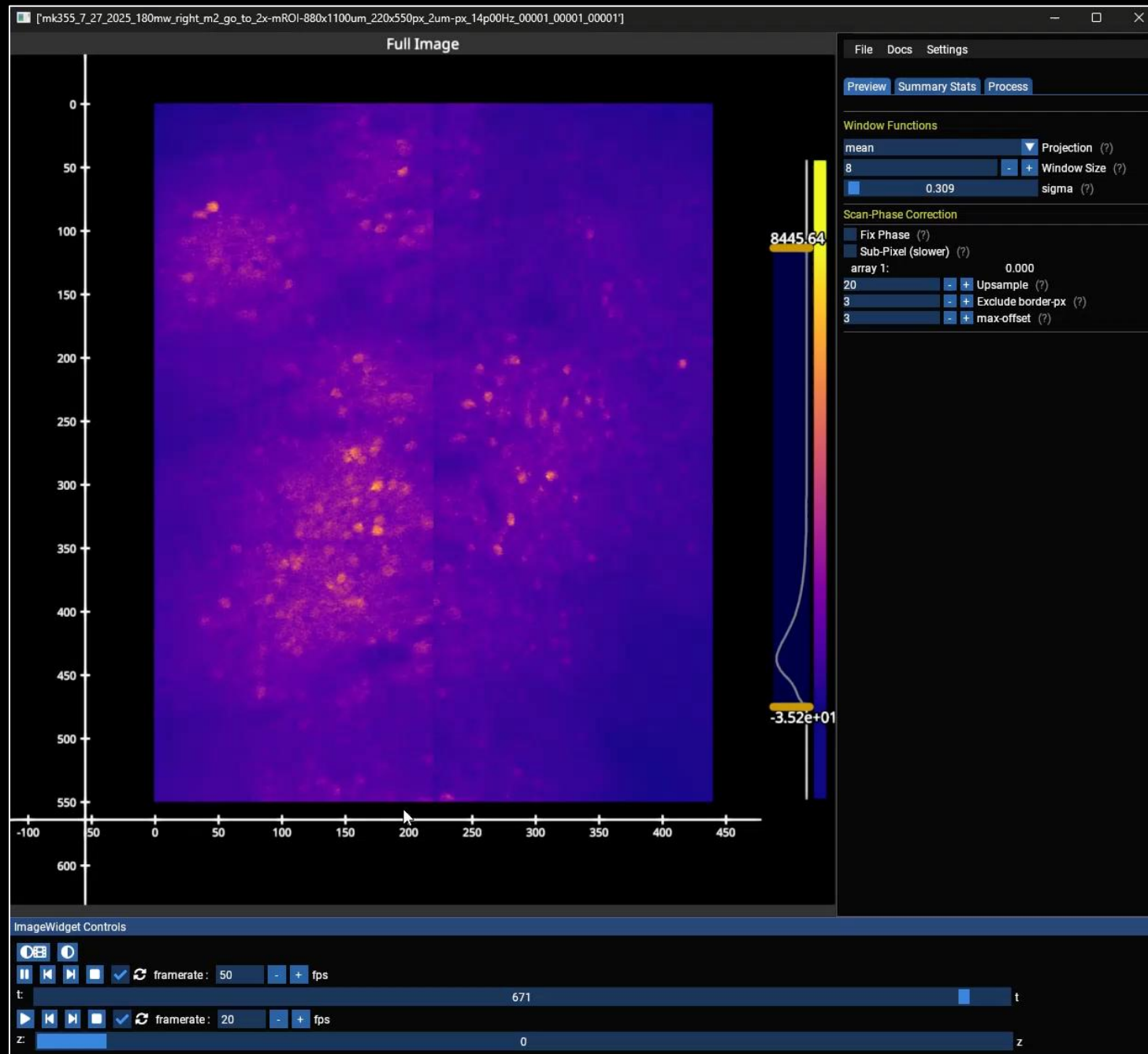
View a mean-subtracted 3-15 window-size projection.

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)

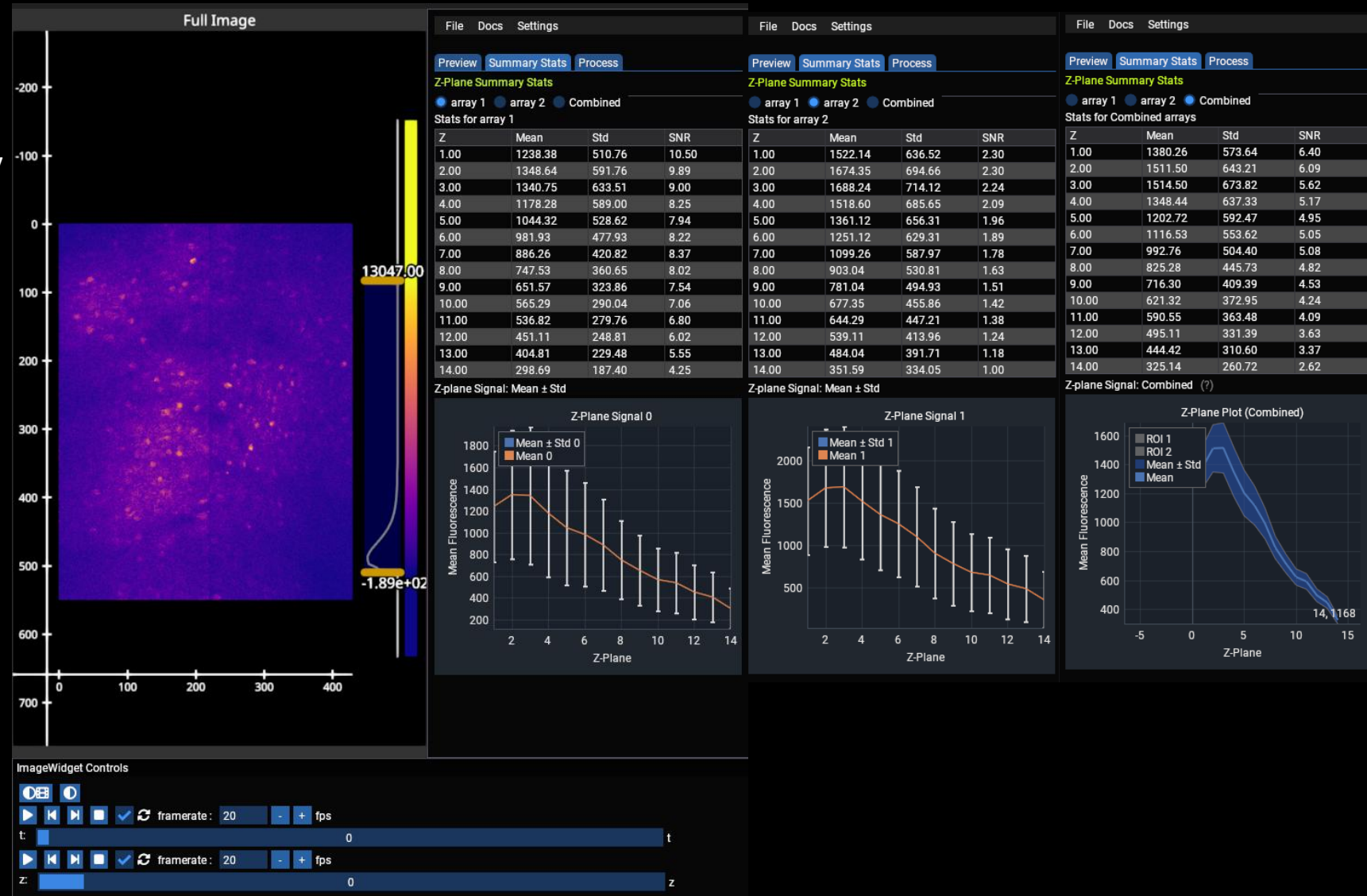
Summary Stats

- 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

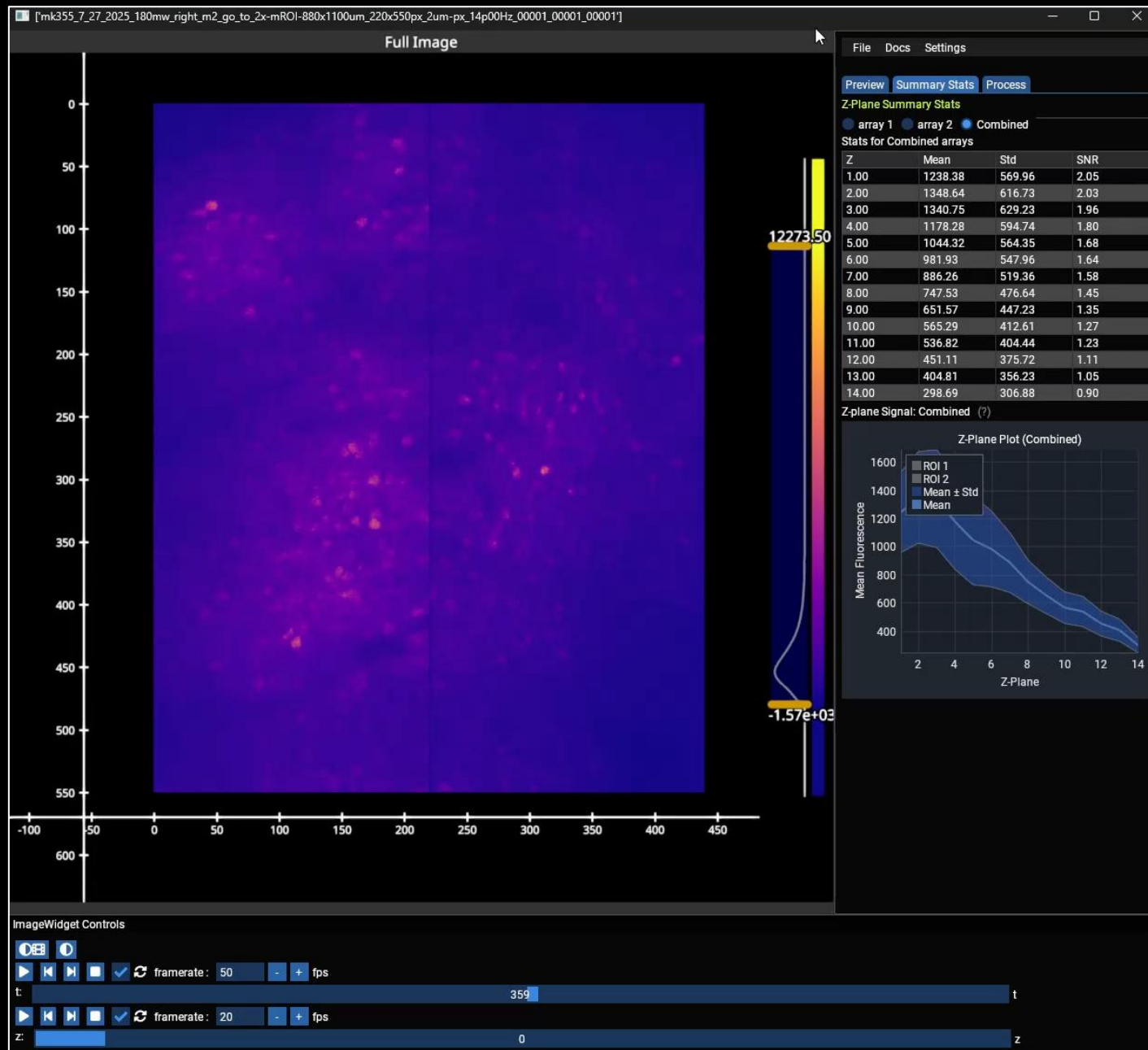
(Raw ScanImage Tiff Only)

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.



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.

The image shows a 'Save As' dialog box with a dark blue header and a light blue body. The background is a blurred screenshot of a data visualization with a color scale from 0 to 1.3047e+02 and a value of -1.88e+02. The dialog contains the following elements:

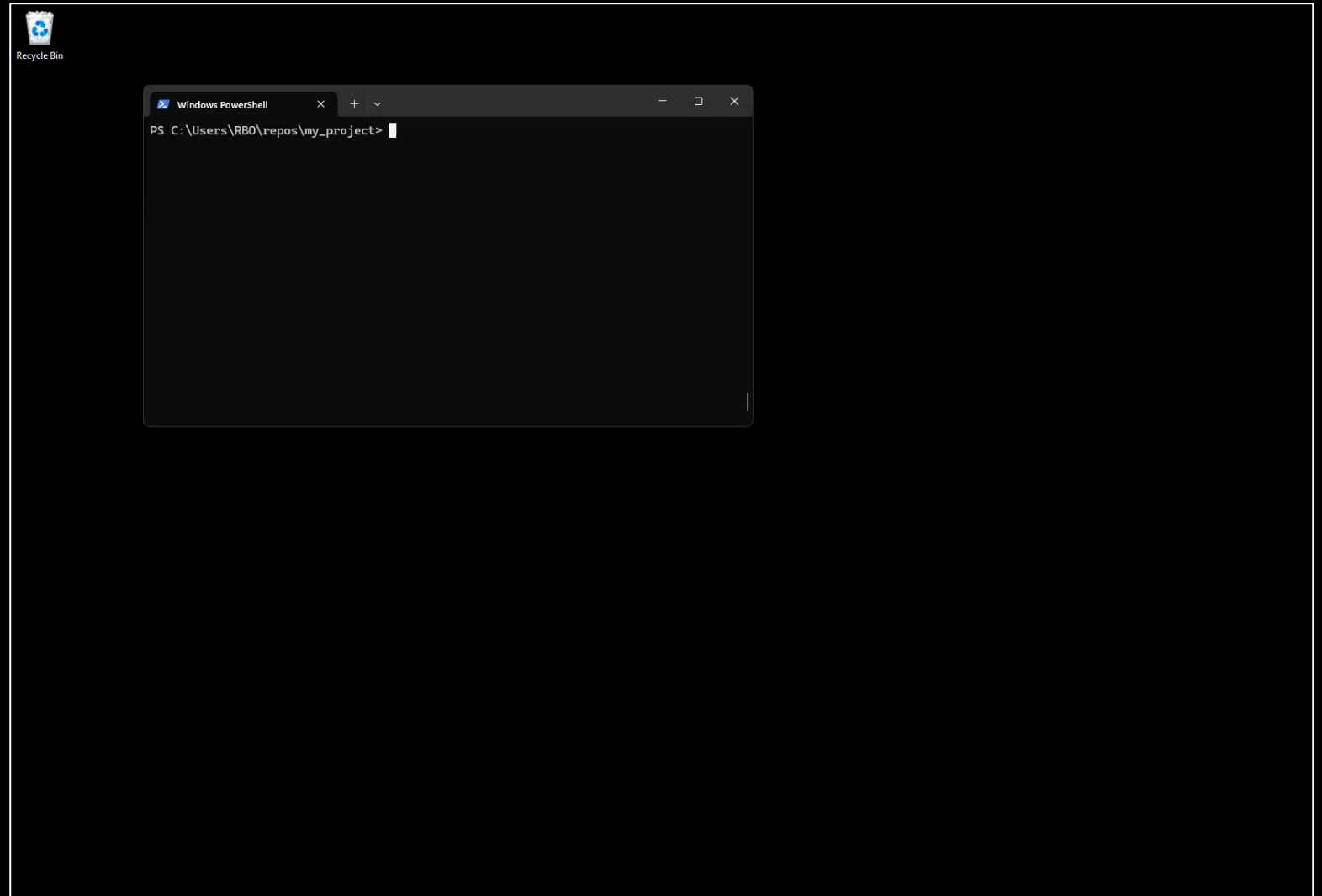
- Save Dir:** A text field containing 'D:\demo\gui' and a 'Browse' button.
- Ext:** A dropdown menu showing '.zarr'.
- Save ScanImage multi-ROI Separately (?):** A checked checkbox.
- Choose mROI(s):** A section with 'All' and 'None' buttons, and two checked checkboxes for 'mROI 1' and 'mROI 2'.
- Options (?):** A section with five checked checkboxes: 'Overwrite (?)', 'Register Z-Planes Axially (?)', 'Fix Scan Phase (?)', 'Subpixel Phase Correction (?)', and 'Debug (?)'.
- Chunk Size (MB) (?):** A slider set to '100'.
- Choose z-planes:** A section with 'All' and 'None' buttons, and two columns of checked checkboxes for 'Plane 1' through 'Plane 14'.
- Buttons:** 'Save' and 'Cancel' buttons at the bottom.

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

