

AES Mask App Manual

Introduction

The mask app is for producing binary masks used when imaging with adaptive excitation. The app is built around quirks in how our microscope produces images, so some features may not have general applicability. The mask can also encode transmission data for analogue power control with the beam modulator to account for field illumination nonuniformity, ROI intensity variation, and scattering throughout a volume.

Installation

The program is written using MATLAB app designer. It can be run either using the source code within the app designer interface, installed as a MATLAB app to run within MATLAB, or installed as a standalone app alongside the MATLAB 2023b Runtime. All code was tested with Windows 10 and Windows 11 and MATLAB versions 2022a and 2023b. Additionally, the code requires the *Signal Processing* and *Image Processing* Toolboxes.

Due to the use of the LibTiff library integrated into MATLAB for accessing Tiff stacks, versions of MATLAB prior to 9.14 (2023a) are unable to read Tiff stacks with more than 2^{16} frames. The code will truncate all data to 64000 frames if using prior releases.

Input

Input consists of a reference tiff video used to draw masks over.

Video

The video must be formatted as a Tiff stack. If multiple channels are used, they must be interlaced throughout the video.

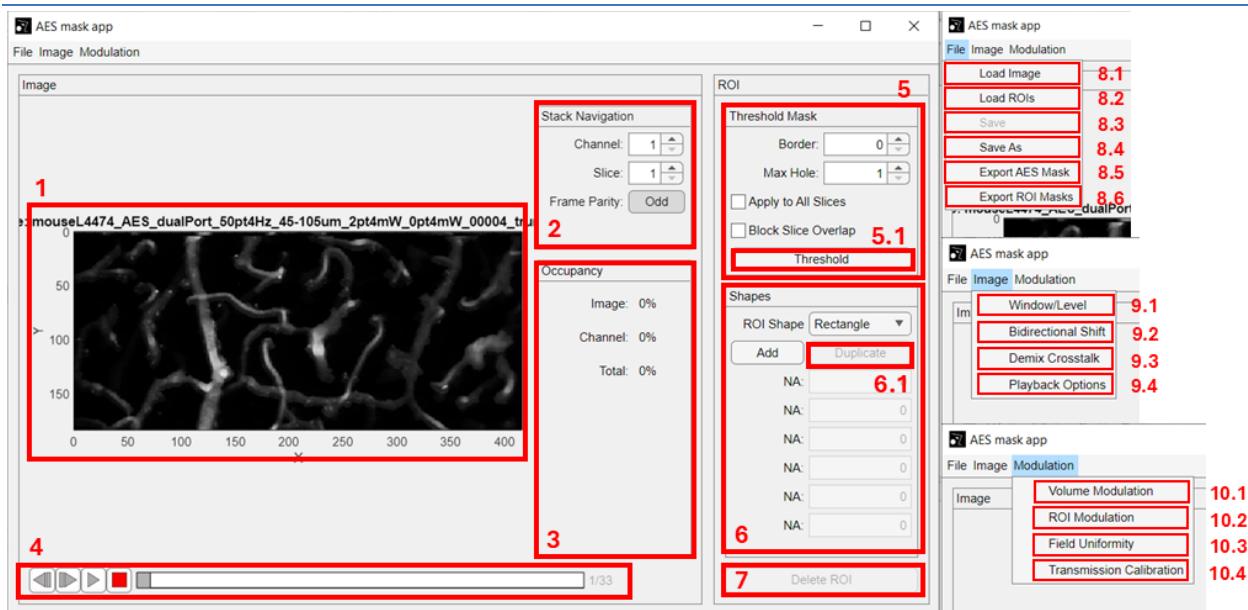
Bidirectional Slow Axis Scanning

This software was designed to work with output from a custom version of Scan Image which allows for bidirectional slow axis scanning. As a quirk of the process, each image in the stack consists of 2 frames, the first in the top half while the second is reflected vertically in the bottom half. Small electrical delays in the system caused the two adjacent frames to be offset in opposite directions. Enable the bidirectional option tells the software to apply the reverse delay and split each frame into two frames to reconstruct the video.

Volume

Volume data is assumed to be scanned bidirectionally on the depth axis. For example, for a 3 slice volume, the first 3 frames would scan from top to bottom and the next 3 frames would scan from bottom to top. Unlike single plane imaging, bidirectional y-axis scanning here means that the y-axis scans in opposite direction when imaging down the stack as opposed to when imaging up. Our system scans the z-axis continuously, meaning that the planes are slightly tilted. Scanning the y-axis bidirectional in this way ensures that the volume is sampled the same way while scanning down as while scanning up.

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1. Viewing Window

The viewing window lets you look at the masks in relation to your reference stack. Shape masks can be manipulated directly by clicking and dragging on them. Segments of the threshold mask can be selected by clicking on them. Holding “shift” allows selection of multiple threshold ROIs.

Color Key

Grayscale: reference image

Red: Base threshold mask

Yellow: Border added to threshold mask

Blue: Shape mask

Purple: Selected ROI

2. Stack Navigation Panel

The Stack Navigation Panel allows you to navigate around slices in a volume stack and channels for a multi-channel stack. The frame parity allows you to toggle between the even and odd frame for bidirectional y-scanning.

3. Occupancy Panel

The Occupancy Panel shows the current percentage of the image area filled by the AES mask for the currently viewed region, the full channel (average across slices for a volume image), and the entire stack (average across all slices and channels).

4. Video Controls

The app can display video playback of the reference stack in addition to the time average. The video file is read as needed, as opposed to be stored in memory. This trades potential speed of access in exchange for memory efficiency. The play, previous frame, and next frame behave as in any other player. The scrubber can be clicked to advance the video to a desired point. The square button toggles between the video playback and the time projection of the stack. If the square is red, the time average is showing.

5. Threshold Mask Panel

These controls generate masks by applying a minimum threshold to the reference stack.

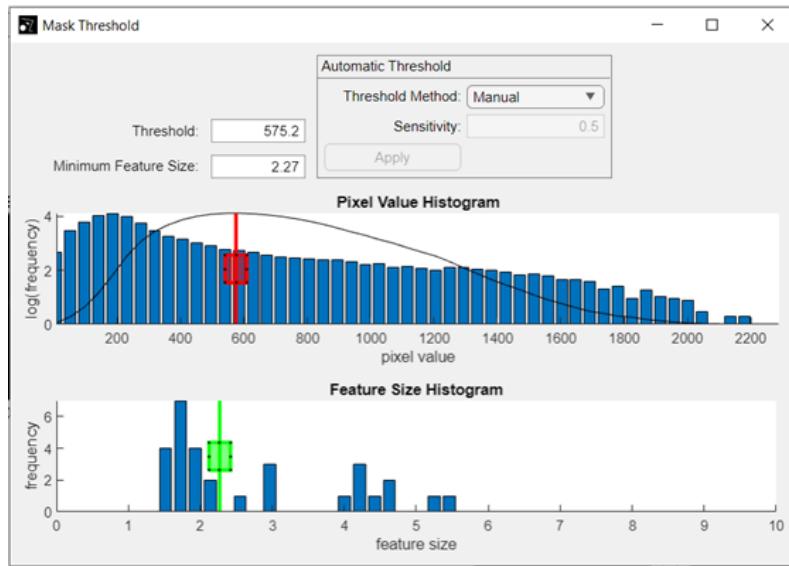
Background/Contrast do not affect the mask generation, but linear demixing for multichannel data does. The Border spinner adds an additional border around the generated threshold mask.

The Max Hole spinner eliminates any holes in masks (unselected pixels fully surrounded by selected pixels) below a certain size. This setting is always globally applied to all slices and channels.

Apply to All Slices applies any changes to the threshold mask to all slices of the current channel. It does not apply retroactively.

Block Slice Overlap eliminates overlap between threshold masks for slices of the same channel. When multiple slices have an active pixel at the same x/y location, the slice with the highest reference image brightness at that location is selected.

5.1 Threshold Mask Button



The Threshold Mask Button opens a window for tuning the threshold mask. Three methods for Threshold Selection are available. “Manual” uses a global threshold for the maximum pixel value to produce the mask by either editing the field or dragging the red bar on the pixel value histogram. “Otsu’s Method” uses the titular method to automatically correct the optimal threshold assuming a binary distribution. “Automatic” uses a built in Matlab function with a nonglobal threshold to generate a mask. The sensitivity between 0 and 1 affects how much of the image the function will select.

After setting the threshold, the mask is segmented in distinct blobs and features below a given size are ignored. The minimum blob size can be changed by editing the field or dragging the green bar in the feature size histogram.

6. Shape Mask Panel

The Shape Panel has options for generating and modifying user defined shape masks. The shape dropdown sets the next shape type to be produced. Options include rectangle, circle, ellipse, ring (ellipse without center filled), poly line, and polygon.

After clicking the add button, drag the mouse in the viewing window to generate the selected ROI type.

The duplicate button copies the ROI to other slices/channels.

The other fields in the panel list properties of the currently selected shape ROI which can be modified.

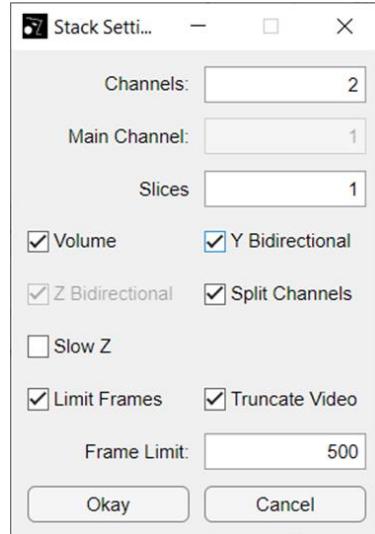
7. Delete ROI Button

The delete button deletes the current selected ROI(s), either shape masks or blobs from the threshold mask.

8. File Menu

The File menu includes the main file I/O options.

8.1 Load Image



Loads the reference image. This must be done before any other options are available. A separate window opens to describe the structure of the stack.

- Channels: Number of color channels
- Main Channel: Only available if using multiple channels and not splitting channels for mask generation. This determines the channel used to generate threshold masks.
- Slices: Only available when stack is a volume. Sets the number of planes in the volume.
- Volume: Whether the stack is a volume or a single plane.
- Y Bidirectional: If y-axis is scanned bidirectionally. Reading is specific to quirks of our microscope. When volumes are imaged bidirectionally, even frames are flipped vertically. For single plane imaging, the inverted even frame is joined below the odd frame in the same image, meaning each image in the stack holds to frames. Additionally, any delay in the galvo leads the odd frames to be shifted down while the even frames are shifted up. This shift is considered when producing masks.
- Z Bidirectional: if z-axis is scanned bidirectionally. This means that the slices for even frames have reversed order.
- Split Channels: Only available when using multiple channels. If not selected, only the main channel is considered when producing masks.
- Slow Z: Only available for volume imaging. If z is scanned after t (i.e. for 100 frames and 3 slices, you capture 100 frames at slice 1, followed by 100 frames and slice 2, etc.).
- Limit Frames: Limit the number of frames read to produce the time projection. Useful for very large files when the SNR improvement from integrating for the full duration is minimal.
- Truncate Video: stop counting frames beyond frame limit. Useful for opening very large files when you do not want to see full video playback.

8.2 Load ROIs

Loads file containing ROI's and other settings (power modulation, BC). Overrides current session.

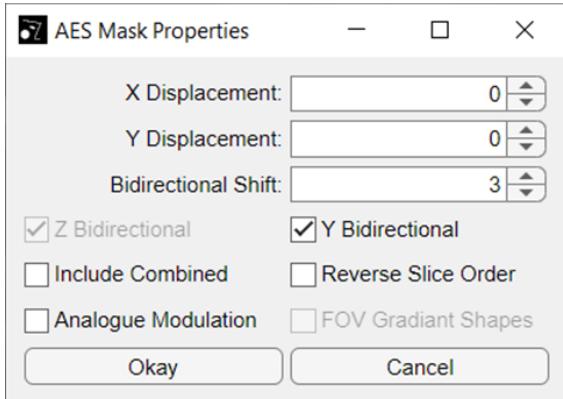
8.3 Save As

Save file containing ROI objects and other settings of current session.

8.4 Save

Over write current loaded file. If no mask file is loaded and keyboard shortcut is used, behavior defaults to "save as".

8.5 Export AES Mask



Opens window with final settings before producing AES mask to feed to the beam modulator.

- X/Y Displacement: Globally shift mask. (i.e. if the beam modulator is delayed by 1 line from the acquisition trigger, set Y to -1 to compensate).
- Bidirectional Shift: Same as editing field in app. Generated mask will shift even/odd frames to compensate for electronic desynchronization of galvanometer.
- Include Combined: Saves an additional binary mask with the sum of all channels. Sometimes useful for measuring the electrical background in the presence of crosstalk between channels during data analysis.
- Reverse Slice Order: flips the slice order in the volume.
- Analogue: If power modulation curves are produced, this encodes the voltage curve into the mask, where 1 is the minimum and 2^16 is the maximum.
- FOV Gradient Shapes: If FOV equalization modulation is selected, applies to shape masks.

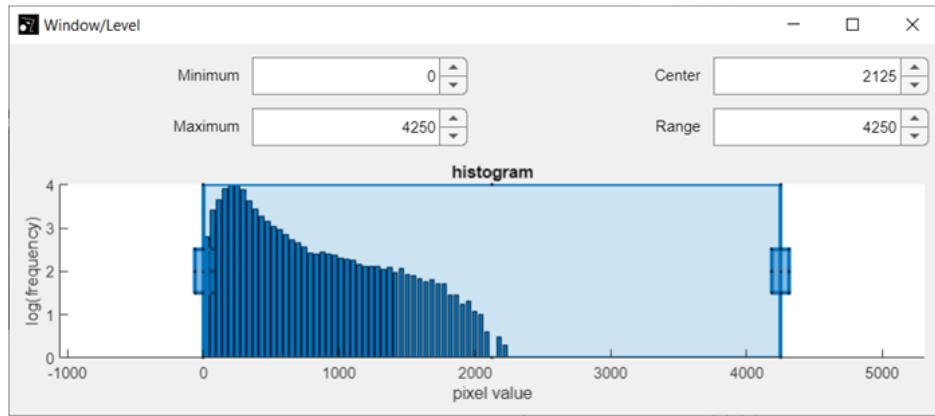
8.6 Export ROI Masks

Save separate binary tiffs for each ROI. Useful for data processing.

9. Image Menu

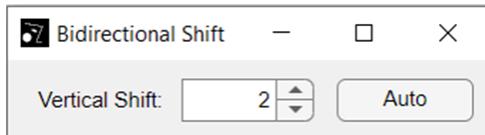
Options for changing the way the image is displayed.

9.1 Window Level



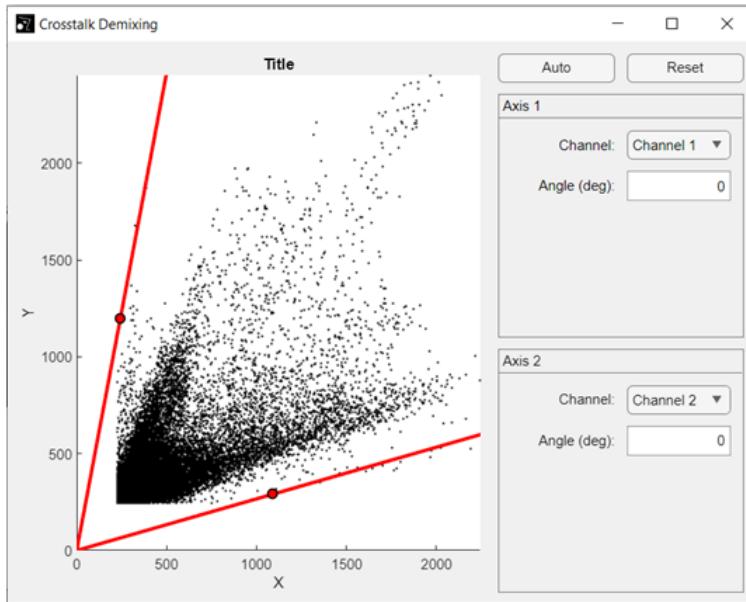
Changes the window level for the currently selected channel.

9.2 Bidirectional Shift



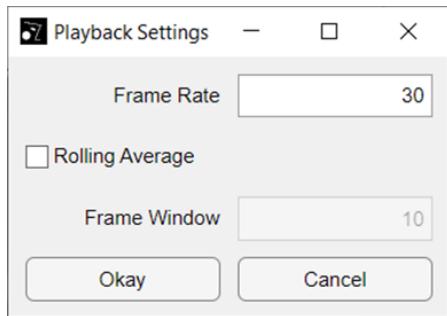
Sets the Y-shift for imaging bidirectional along the y-axis. The auto button sets the shift based on the cross-correlation between even and odd frames. This value is set automatically when loading the image.

9.3 Demix Crosstalk



Linear combination can be used to demix channel crosstalk (requires high SNR). The cross correlation between two channels is shown. The angle of the line can be dragged to set the off-diagonal elements of the crosstalk matrix. The auto button sets the angle to the minimum value while containing all pixels (only applies to the currently selected channels). The reset button undoes any demixing.

9.4 Playback Options

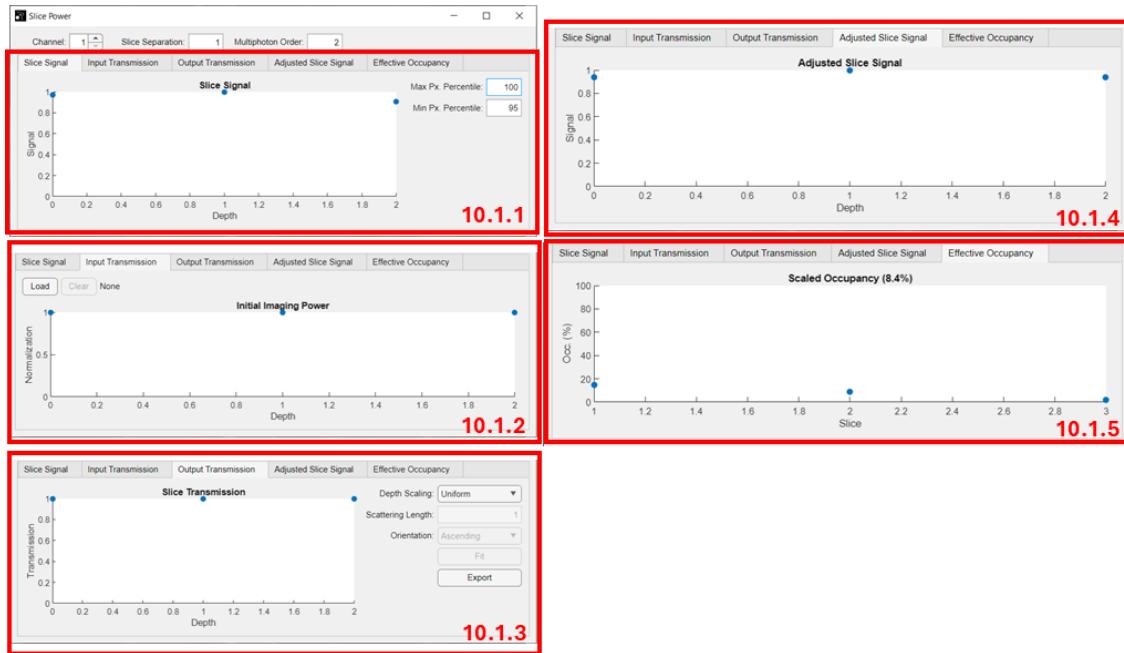


Set frame rate and rolling average for video playback.

10. Modulation Menu

Options for analogue power modulation

10.1 Volume Modulation



Set the power transmission on a slice by slice basis. Channel spinner sets the current channel being analyzed. Slice separation is only relevant if transmission is calculated using a known scattering length. Multiphoton order is used to calculate the needed transmission to equalize power across the volume.

10.1.1 Slice Signal Tab

Shows the signal of each slice based on a percentile range of pixel values from each image in the reference stack.

10.1.2 Initial Imaging Power Tab

If the reference stack was imaged with non-uniform power, that can be included in the calculation. The initial imaging power must be loaded as an Nx1 vector (.csv, .xlsx, .dat) where each element is the laser power at the corresponding slice.

10.1.3 Slice Transmission Tab

The intended transmission of the beam modulator at each slice. Can either take the inverse of the reference power (with the multiphoton order and initial imaging power consider in the calculation), uniform transmission, or apply an exponential. For a exponential modulation with depth, if the scattering length is not known, it can be based upon the slice signal.

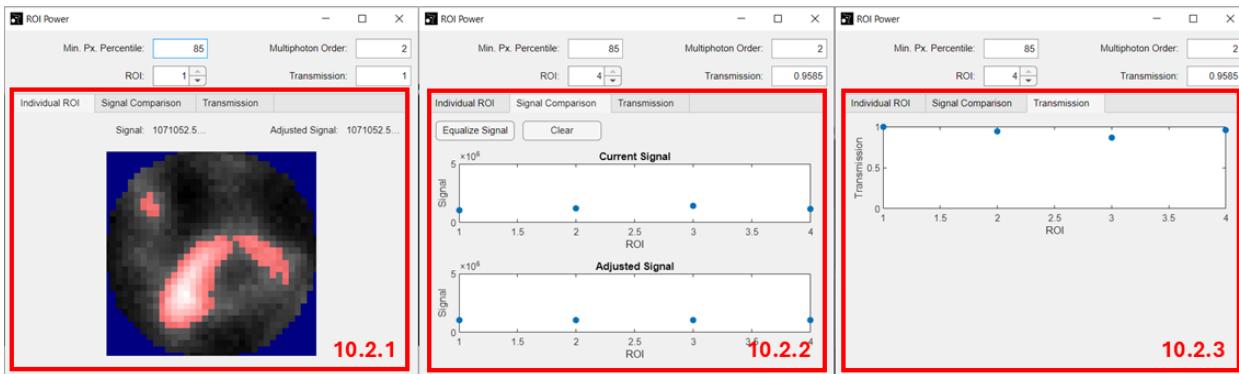
10.1.4 Adjusted Slice Signal Tab

The predicted output signal for the future imaging session based on the analogue mask transmission. This curve is applied to the Viewing Window to predict what the image will look like after power modulation.

10.1.5 Scaled Occupancy Tab

The occupancy for each slice weighted by transmission. This gives a sense of which slices are contributing the most to power exposure on the sample.

10.2 ROI Modulation



Shape mask transmission can be modulated to equalize signal between masks. Each shape within a frame/channel is indexed starting at 1. You can swap between them using the ROI number field or by clicking on the mask in the viewing window of the main app. Multiphoton order determines how transmission fit curves and expected output power are calculated. The minimum pixel percentile determine the pixels within the shape mask that are used to calculate the ROI signal. The transmission for each ROI can be automatically fit to equalize output signal or manually edited.

10.2.1 Individual ROI Tab

Shows the current pixels used to calculate ROI signal (red) based on the minimum percentile within the shape mask (grayscale). Pixels outside of the shape mask are solid blue and not included in calculations. The Signal is cumulative across active pixels (proportional to total photon count). Adjusted Signal is the expected signal value after applying the ROI transmission factor.

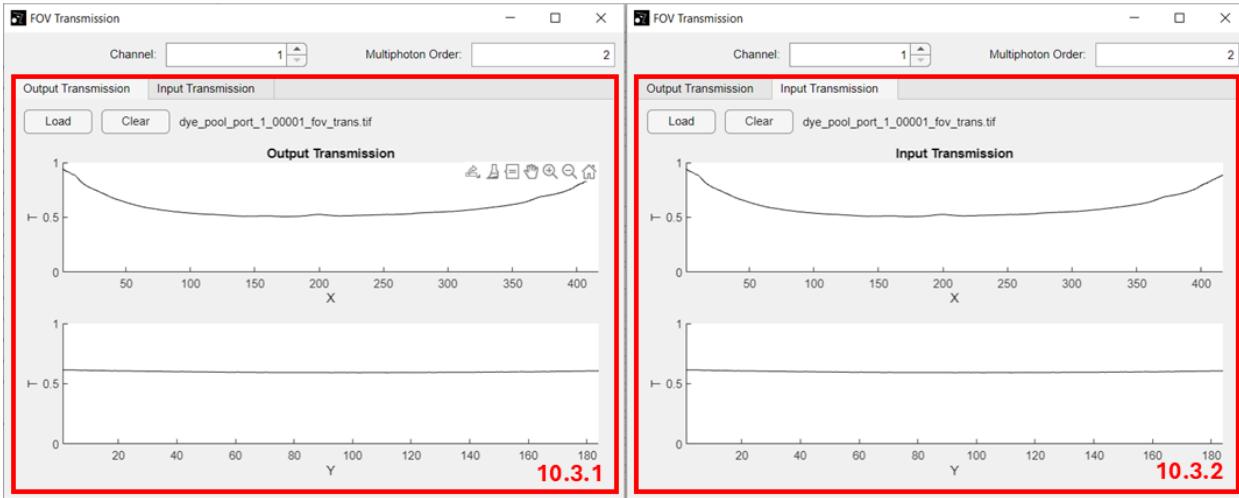
10.2.2 Signal Comparison Tab

Plots the current calculated ROI signal as a function of index before (Current) and after (Adjusted) modulation. The “Equalize Signal” button applies a transmission to balance ROI signals. If a minimum analogue transmission is set, weaker ROI’s may not be fully equalized. “Clear” sets transmission to 1 for all ROI’s.

10.2.3 Transmission Tab

Plots the transmission as a function of ROI index.

10.3 Field Uniformity



Use analogue modulation to compensate for nonuniform field illumination (i.e. for a polygon scanner). Applied separately for each channel. The FOV illumination profile is applied to the viewing window.

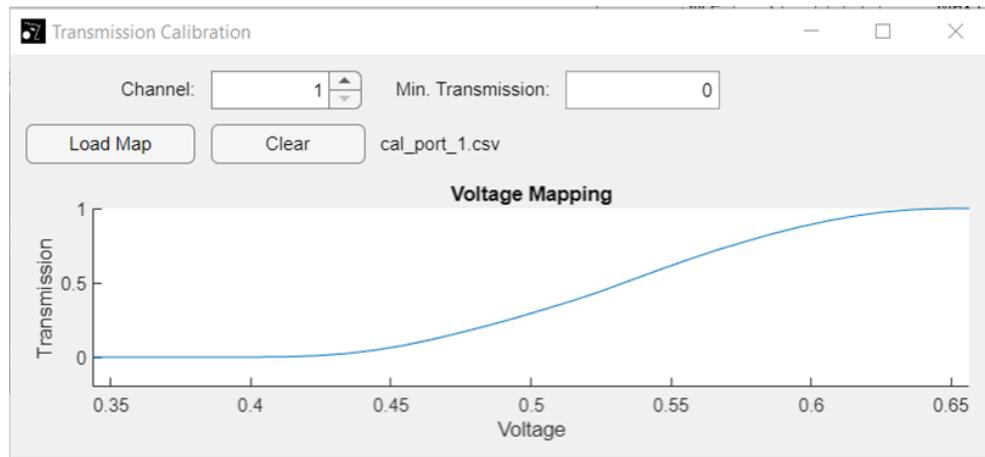
10.3.1 Output Transmission Tab

Load an output transmission profile. Expected input is a Tiff image where each pixel corresponds to the output transmission at that location in the imaging plane. Pixel values will be normalized so that the maximum value is 1. The transmission profile image dimensions will automatically scale to match the current loaded stack. The average transmission profile across the x and y axis are plotted

10.3.2 Input Transmission Tab

If the stack under evaluation was taken with FOV equalization, you can take it into account by loading the transmission profile here.

10.4 Transmission Calibration



If the modulator does not respond linearly to input signal, a calibration file can be loaded mapping the transmission to the input voltage. The calibration file is an Nx2 curve (.csv, .xlsx, .dat), where the first column is the input voltage and the second column is the output transmission. Minimum transmission prevents all output analogue transmission calculations from dropping the transmission within an illumination mask below a given value.