Table 2. Parameters used in SynScope

category	module	input	user input		input format	output	guide & description
image processing	shading correction	image (.czi)	target channel		channel #	image (.tiff)	Specifies the channel for flat-field correction (BaSiC).
	chromatic shift correction	image (.tiff)	target channel		channel #	image (.tiff)	Specifies the channel to be spatially shifted to the reference. **Note:** The reference channel is automatically assigned as channel 1.
			transform parameter		Use pre-calculated parameter? yes/no (default: yes)		Determines whether to use a pre-calculated tranform metric: • **Yes:** Apply a pre-calculated transform. • **No:** Calculate new transform metrics directly from the input image.
	z-signal correction	image (.tiff)	target channel		channel #	image (.tiff)	Specifies the channel for z-signal correction.
				clean frame(s)	z frame(s)		High-SNR frames used as references for denoising. Typically the top and/or bottom frames (e.g., 1, 2, 3, 49, 50 in a 50-frame stack). **Note:** recommend using 5 or more frames.
				noisy frame(s)	z frame(s)		Low-SNR frames with degraded signals. These are usually the middle frames of the stack (e.g., 23, 24, 25, 26, 27 in a 50-frame stack). **Note:** the number of selected noisy frames should equal the number of clean frames.
				reference frame	z frame		A single frame used for histogram matching. It is recommended to use the frame with the second-highest intensity (e.g., frame 47 in a 50-slice stack).
				target range	z frame range		The range of z-frames where the correction will be applied (e.g., 1–50 in a 50-frame stack).

category	module	input	user input	input format	output	guide & description
synapse analysis	synpase detection	image (.tiff)	target channel	green channel (default: 2)	detected synapse (.nimp)	Specifies the channel containing mGRASP signal for synapse detection.
				red channel (default: 4)		Specifies the channel containing dendrite signal for synapse detection.
			threshold	auto (-1) or manual (default: -1)		Use -1 for automatic global thresholding or enter a fixed value.
			voxel size (X,Y,Z)	voxel dimension (default: 0.23, 0.23, 0.5)		Physical dimensions (μm) derived from image metadata.
		reconstucted neuron(.swc)	-	-		Reconstructed neuron structure, typically obtained from semi-automated tracing software.
	synapse assignment	detected synapse (.nimp)	-	-	assigned synpase (.nimp)	Required for synapse-centered binary decoding.
		image (.tiff)	mGRASP channel	green channel (default: 2)		Specifies the mGRASP (green only) channel to exclude from analysis.
			post-cell channel	red channel (default: 4)		Specifies channel containing the post-cell for dendrite subtraction.
			overlap threshold	# between 0-1 (default: 0.5)		Minimum colocalizing overlap required to validate a synapse.