Table S1

Step I

i. STEERABLE FILTER : Thick Ridge (Stem) Detection

Function	Segmentation Parameter	Default	Units	Recommendations
gcaMultiScaleSteerableDetector.m	*BBScale* (σ of Gaussian Filter)	[5,6,7,8,9,10]	pixels	Set based on the radius of the stem ridges to detect. Thicker stems require larger σ values. Finer intervals of the σ values allows for smoother integration of multiple scale ridges at the expense of computational cost.

ii. THICK RIDGE CLEANING

Function	Segmentation Parameter	Default	Units	Recommendations
GCAgetNeuriteOrient.m	*ThreshNMSResponse*	25th	percentile	As just a rough threshold required typically no reason to modify
GCAgetNeuriteOrient.m	*MinCCRidgeBeforeConnect*	3 (~ 0.6)	pixels (μm)	Adjust to equivalent distances based on pixel size of image.
GCAgetNeuriteOrient.m	*MinCCRidgeAfterConnect*	5 (~ 1)	pixels (μm)	No adjustment necessary if entered in μm or for simple veil/stem morphologies.

iii. THICK RIDGE LINKING

Function	Segmentation Parameter	Default	Units	Recommendations
gcaConnectLinearRidges.m	*MaxRadiusLargeScaleLink*	10 (~ 2)	pixels (μm)	Adjust to equivalent distances based on pixel size of image.
gcaConnectLinearRidges.m	*MaxRadiusNoGeoTerm*	3 (~ 0.6)	pixels (μm)	No adjustment necessary if entered in μm or for simple veil/stem morphologies with high signal to background stem regions.
gcaConnectLinearRidges.m	*GeoThresh* (θRL)	90 (0)	degrees (cos(θRL))	Not modified in this study

iv. DEFINING NEURITE ENTRANCE RIDGE CANDIDATES

Function	Segmentation Parameter	Default	Units	Recommendations
GCAgetNeuriteOrient.m	*MinCCEntranceRidgeFirstTry*	5	pixels	Designed to be self-adaptive.
GCAgetNeuriteOrient.m	*MaxDistBorderFistTry*	10	pixels	

Step II

i. CHECK NEURITE ORIENTATION

Function	Segmentation Parameter	Default	Units	Recommendations
GCAneuriteOrientConsistencyCheck.m	*SizeOfConsistencyRestraint*	5	pixels	If localization of the centerline of the entering neurite stem is ambiguous, due to asymmetric/changing filopodia structures, or if the stem itself is relatively mobile, parameter may need to be increased. If the neurite entrance point and a distractor object are in close proximity, value may need to be decreased. However, this parameter-value only needed to be adjusted for a handful of cases in this study.
GCAneuriteOrientConsistencyCheck.m	*CheckOrient*	false	logical	Of use in cases when the temporal majority does not reflect the correct orientation of the neurite. Allows user to perform a quick quality control check, and if need be, choose the correct cluster to which all the neurite frames will be aligned. Note, none of the data included formally in the manuscript required manual refinement. However the visualization did help decide the SizeOfConsistencyRestraint in a handful of cases. The manual step was primarily added in case the user is studying a growth cone interaction with another persistent ridge like object.

Table SI (cont)
Step III

i. INITIAL THRESHOLDING

Function	Segmentation Parameter	Default	Units	Recommendations
GCAReconstructVeilStem.m	*NonGCImage*	*false*	logical	Flag to turn off the growth cone veil/stem reconstruction for non-growth cone images. If set to false will NOT use the large ridge information from Steps I-II. Set to *true* for images **Fig. S3E-G** and **Fig. S4E-G.**
GCAReconstructVeilStem.m	*threshType*	*'Local'*	**Options:** 'Local' 'Global' 'External'	'Local': Implements local Otsu thresholding 'Global': Implements the global thresholding specified by segmentation parameter 'threshMethod'. 'External': Loads a pre-run binary mask (For example, can utilize any segmentation method implemented in Fiji and use this flag to import it as the initial thresholding mask for the veil/stem). Dependent upon the intensity histogram of the image. While local thresholding helps better filter unresolved clusters of filopodia, it can perform poorly for veil/stem estimation if there are significant intracellular inhomogeneities within the veil/stem.
GCARunGlobalSegmentation Process.m	*threshMethod*	*'Otsu'*	**Options:** 'Otsu' 'MinMax' 'Rosin' 'Gradient'	Only applicable if 'threshType' is set to 'Global' Performance dependent upon the intensity histogram of the image.
GCAReconstructVeilStem.m	*maskDirectory*	*[ ]*	character array (path name)	Only applicable if 'threshType' is set to *'External'* Specifies the path folder where a sequence of externally run initial threshold mask files are located.
gcaThresholdOtsu_local.m	*LocalThresholdPatchSize*	75	pixels	Only applicable if 'threshType' is set to 'Local' Dependent upon the size of the growth cone. Typically the larger the growth cone, the larger the optimal patch size. Very large patch sizes essentially converge to global thresholding.

ii. MORPHOLOGICAL OPENING

Function	Segmentation Parameter	Default	Units	Recommendations
gcaMorphologicalOpeningWith GeometryConstraints.m	*DiskSizeLarge*	6 (~1.3)	pixels (μm)	Set by the size of the low-fidelity filopodia information to remove (higher fidelity ridge detection will be performed in the next step), and effectively defines what size protrusion is considered part of the 'veil'. If filopodia have strong signal and are crossing-values slightly larger than a filopodia may be required to remove these under-thresholded pieces.
gcaMorphologicalOpeningWith GeometryConstraints.m	*DiskSizeSmall*	3 (~0.65)	pixels (μm)	Only applicable if *considerGeometry* is set to *true.* The morphological structuring element is shrunk to this size for thin regions of the veil/stem (defined by *DiskSizeLarge*) which span two larger amorphous regions. The *considerGeometry* option is a practical solution for removing under thresholded filopodia while maintaining the larger veil/stem path.
gcaMorphologicalOpeningWith GeometryConstraints.m	*considerGeometry*	*true*	logical	Flag to reduce disk size of the morphological operation based on geometry considerations. A value of true is always recommended for growth cone images to help facilitate veil/stem reconstruction, while a value of false is typically recommended for non-growth cone images - though we found setting this option to true can sometimes be advantageous even for non-growth cone images, specifically when segmentation of two smaller amorphous features spanned by a thin region is necessary.

iii. BRIDGE GAPS IN THICK RIDGE PATHS TO EXTEND SEARCH

Function	Segmentation Parameter	Default	Units	Recommendations
GCAReconstructVeilStem.m	*MaxRadiusBridgeRidges*	5 (~1)	pixels (μm)	Adjust to equivalent true distances based on pixel size of image. No adjustment typically necessary if entered in μm or for simple veil/stem morphologies.

Table SI (cont)

Step VI

i. STEERABLE FILTER: Thin Ridge (Filopodia) Detection

Function	Segmentation Parameter	Default	Units	Recommendations
gcaMultiscaleSteerableDetector.m	*FiloScale*	1.5	pixels	Based on the thickness of the filopodia objects in the image

ii. THIN RIDGE CLEANING

Function	Segmentation Parameter	Default	Units	Recommendations
GCAReconstructFilopodia.m	*filterBackEst*	*true*	Logical flag to approximately locate high intensity objects in the image based on the image intensity histogram of the full image.	Recommended: Saves computational time and reduces false positive branching structures. However, many false negatives indicates the threshold is not sufficiently permissive and that *dilateObjectMask* may need to be = true. (See Below)
GCAReconstructFilopodia.m	*dilateObjectMask*	*'test'*	**Options:** *logical flag (true/false)* *'test'*	It is recommended when switching to a new imaging modality to check once the full intensity histogram of the example image to ensure 2*σ of the first fitted Gaussian produces a sufficiently permissive rough localization of high intensity objects in the image. Depending on the shape/separation of the signal to background, this estimation may not define a sufficient local region around the high intensity veil/stem objects to search for lower intensity filopodia detections. If this parameter is set to 'test', GCA calculates the percent of the image is defined as background from the 2*σ estimate. If the background is greater than *percentCutOff* of the total image the dilation will be automatically be turned on, if not the 2*σ background estimate is considered accurate.
GCAReconstructFilopodia.m	*percentCutOff*	50	% of image initially estimated as background	Only applicable if *dilateObjectMask* is set to 'test'. Set according to your expected object to background ratio.
GCAReconstructFilopodia.m	*LRDilRad*	10	pixels	Flexible: just needs to be set such that the object masks are relatively permissive and do not exclude underlying filopodia/branch signal. If the initial object masks are too small it results in false negative filopodia. If too large it may increase the computational time unnecessarily and there is a risk of additional false branch structures.
GCAReconstructFilopodia.m	*multSTDNMSResponse*	3	σ (std) of first Gaussian fit of NMS response histogram	Not modified in this study
GCAReconstructFilopodia.m	*minCCRidge*	3 (~0.6)	pixels (μm) (connected component)	Adjust to equivalent true distances based on pixel size of image. No adjustment typically necessary if entered in μm.
gcaAttachFilopodia StructuresMain.m	*filterBasedOnVeilStemAttachedDistr*	*true*	Logical flag to further filter the disconnected filopodia segment pool for subsequent filopodia/branch network linking by estimating the distribution of the mean thin ridge responses attached to the veil/stem in the first iteration of the filopodia/branch reconstruction.	Recommended to further reduce false positives filopodia segments as long as the initial iteration seed filopodia population is sufficient to provide reliable thresholds. Software will automatically flag and turn off option if have obviously low numbers of attached filopodia, such as in **Fig. 4G**.

Table SI (cont)

iii. FILOPODIA SEGMENT BUILDING

Function	Segmentation Parameter	Default	Units	Recommendations
gcaConnectLinearRidges	*maxRadiusLink*	5 (~1)	pixels (μm)	Adjust to equivalent true distances based on pixel size of image. No adjustment typically necessary if entered in μ
gcaConnectLinearRidges	*geoThresh* (θFL)	25.8 (0.9)	degrees (cos(θFL))	Not modified in this study

iv. TRADITIONAL FILOPODIA/BRANCH LINKING

Function	Segmentation Parameter	Default	Units	Recommendations
gcaConnectFiloBranch.m	*maxRadiusConnectFiloBranch*	15 (~3)	pixels (μm)	Adjust to equivalent true distances based on pixel size of image. No adjustment typically necessary if entered in μm. However, increasing may be beneficial if a crossing filopodia is mislabeled as a branch, particularly if the intersecting filopodia are thick and the orientations are such that the two structures have a number of overlapping pixels.
gcaConnectFiloBranch.m	*geoThreshFiloBranch* (θFL)	60 (0.5)	degrees (cos(θFL))	Not modified in this study

v. EMBEDDED ACTIN BUNDLE LINKING (OPTIONAL- For Actin Labels Only)

Function	Segmentation Parameter	Default	Units	Recommendations
gcaConnectEmbedded RidgeCandidates.m	*maxRadiusLinkEmbedded*	10 (~2)	pixels (μm)	Adjust to equivalent true distances based on pixel size of image. No adjustment typically necessary if entered in μm.
gcaConnectEmbedded RidgeCandidates.m	*geoThreshEmbedded*	60 (0.5)	degrees (cos(θFL))	Not modified in this study
gcaReconstructEmbedded.m	*curvBreakCandEmbed*	0.05	1/Pixels	Not modified in this study