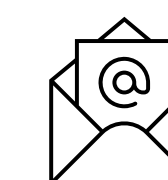


# A Flexible, User-Friendly, and Versatile Graphical User Interface for Region of Interest Image Segmentation

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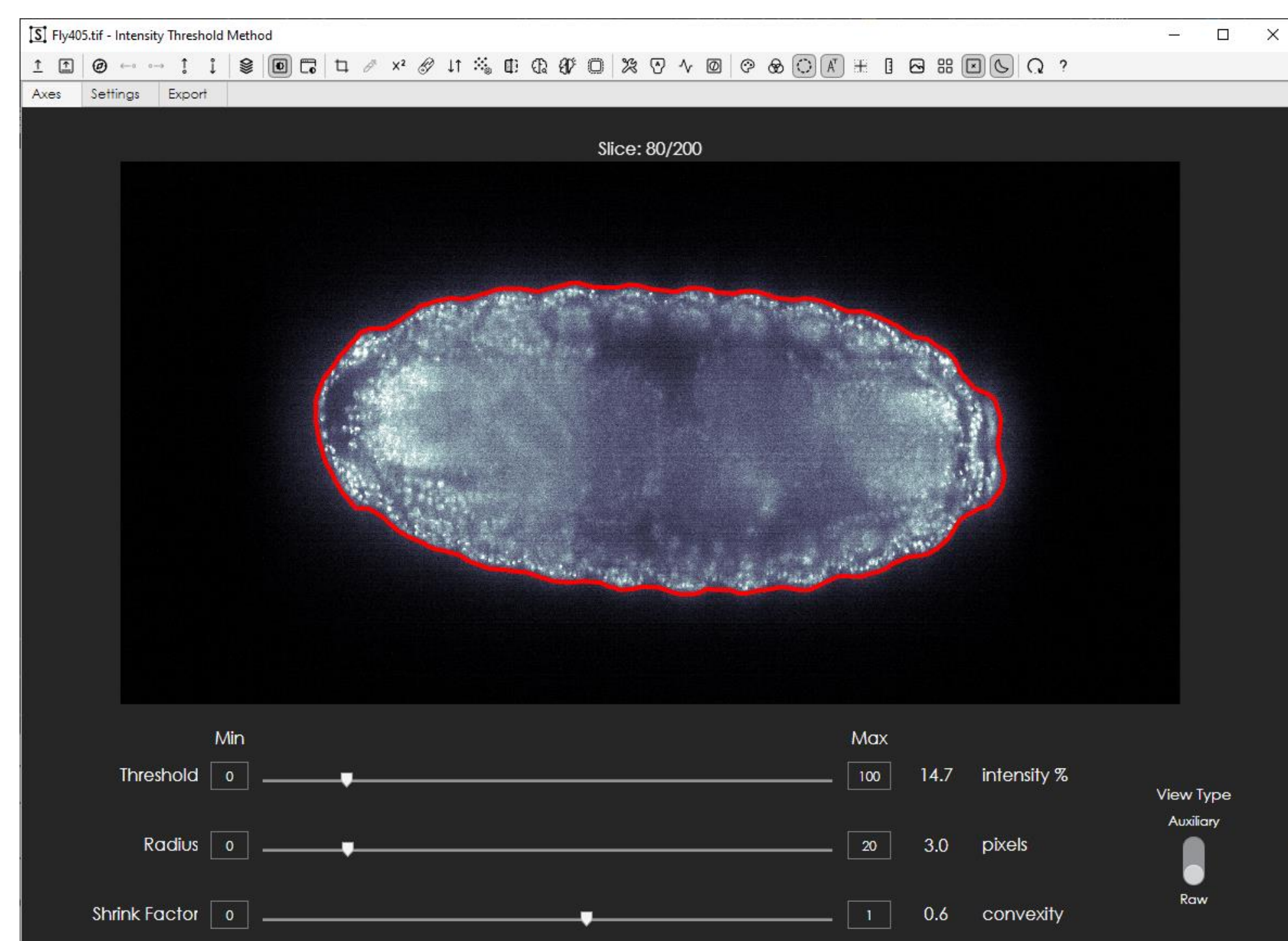
## Abstract

Image segmentation is utilized in many areas of biological research for identifying specific image regions, extracting features, and quantifying signals in microscopy images. Furthermore, automated segmentation approaches can be utilized to reduce the size of data sets by cropping out the regions of interest (ROIs) for subsequent image processing and are especially relevant when handling large datasets, as is common in many light sheet modalities. Here we describe a modular graphical user interface developed in MATLAB which enables the user to navigate and visualize multidimensional data, apply various preprocessing options, and performs segmentation with only 3 adjustable parameters. Preprocessed data is segmented using traditional parametric methods, which can be further refined by post processing options, including active contours and manual adjustments. Resulting datasets from the segmentation can be used to train a deep neural network to perform semantic segmentation on regions for various needs.

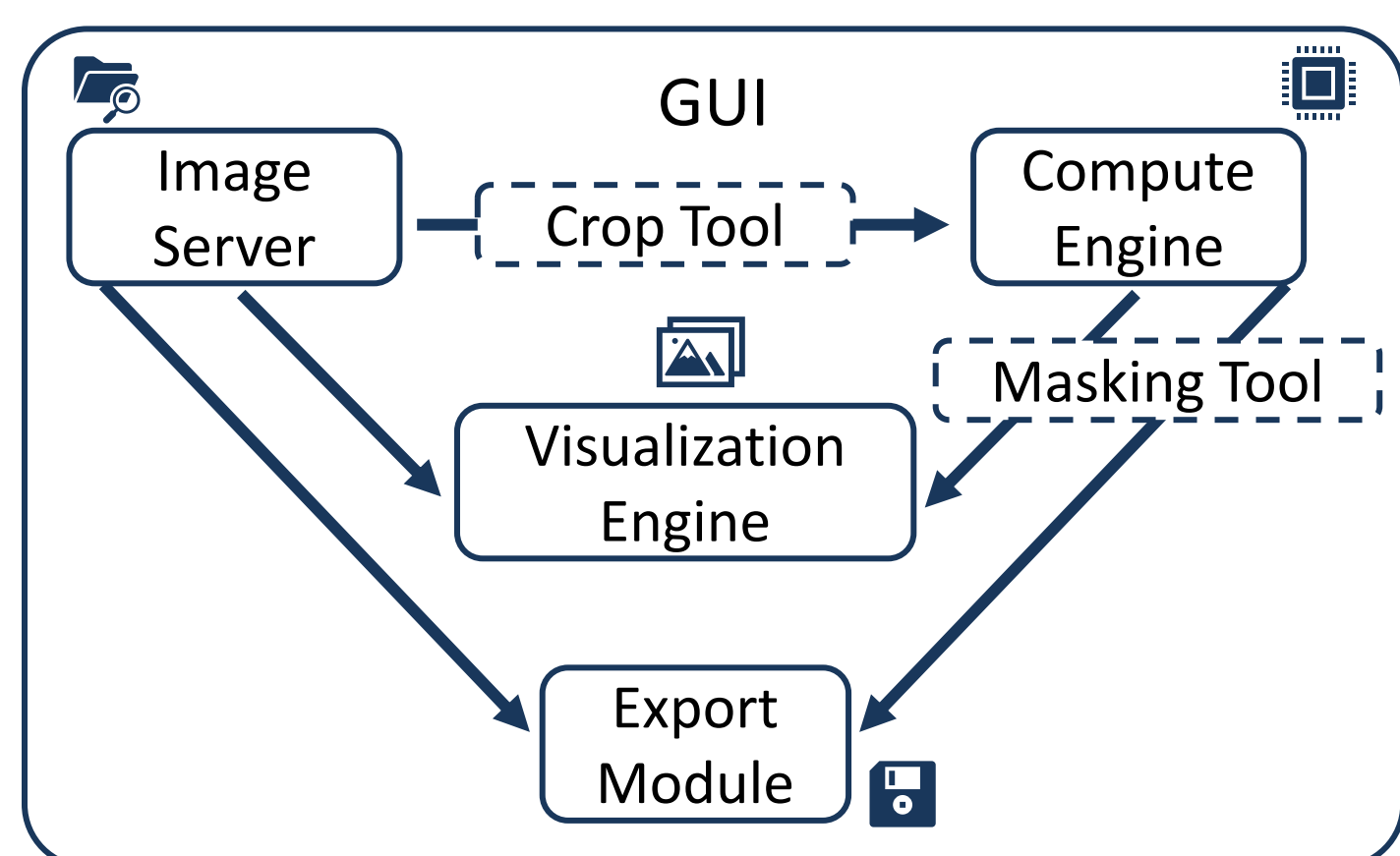
To demonstrate the use of this GUI, we segmented a subset of SiR-actin labeled butterfly ovary images from a dual-view inverted Selective Plane Illumination Microscope (diSPIM) to produce a dataset to train a deep neural network to recognize a region occluding desirable signal in a maximum intensity projection. We further demonstrate the versatility of the software by segmenting a DIC image.

## Software

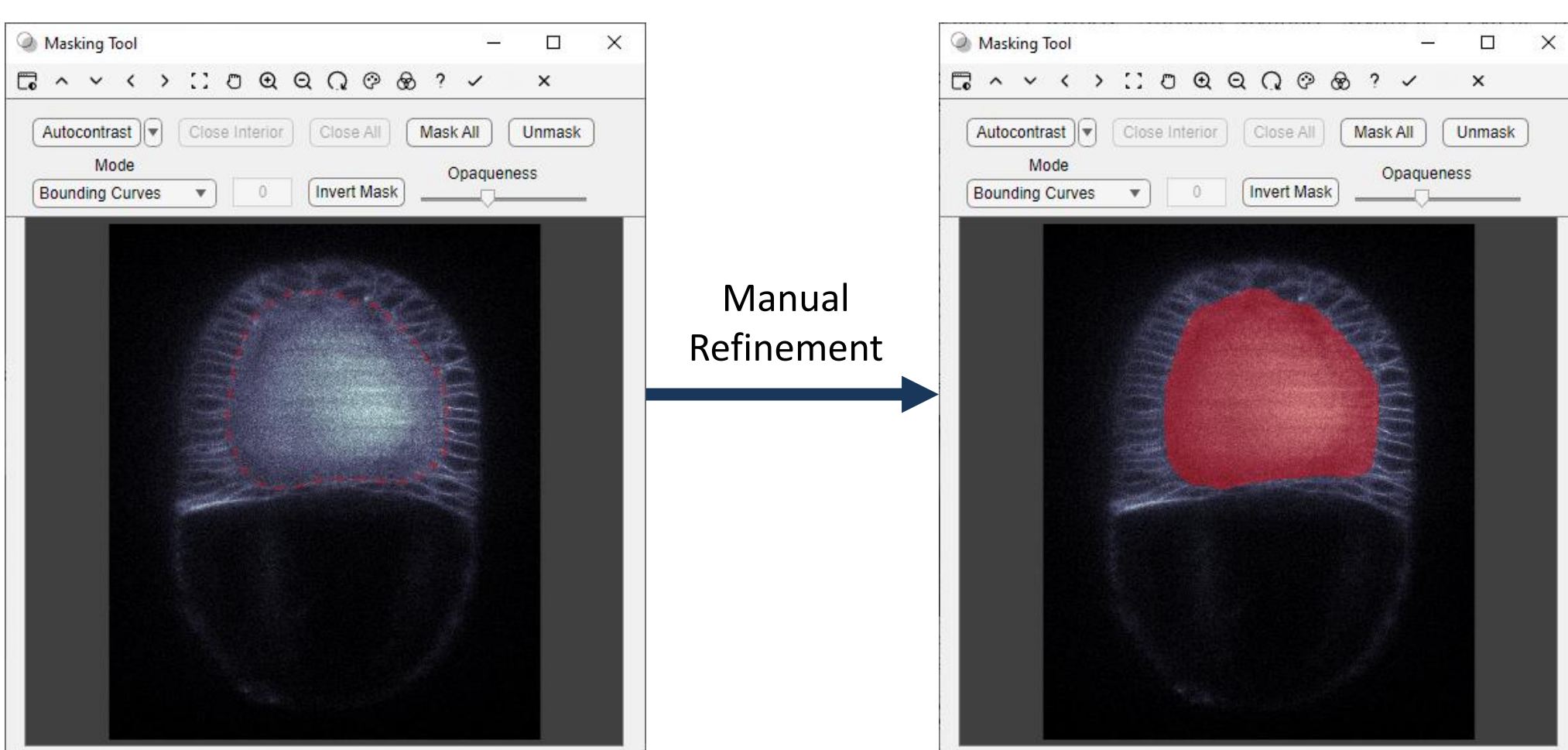
A.



B.



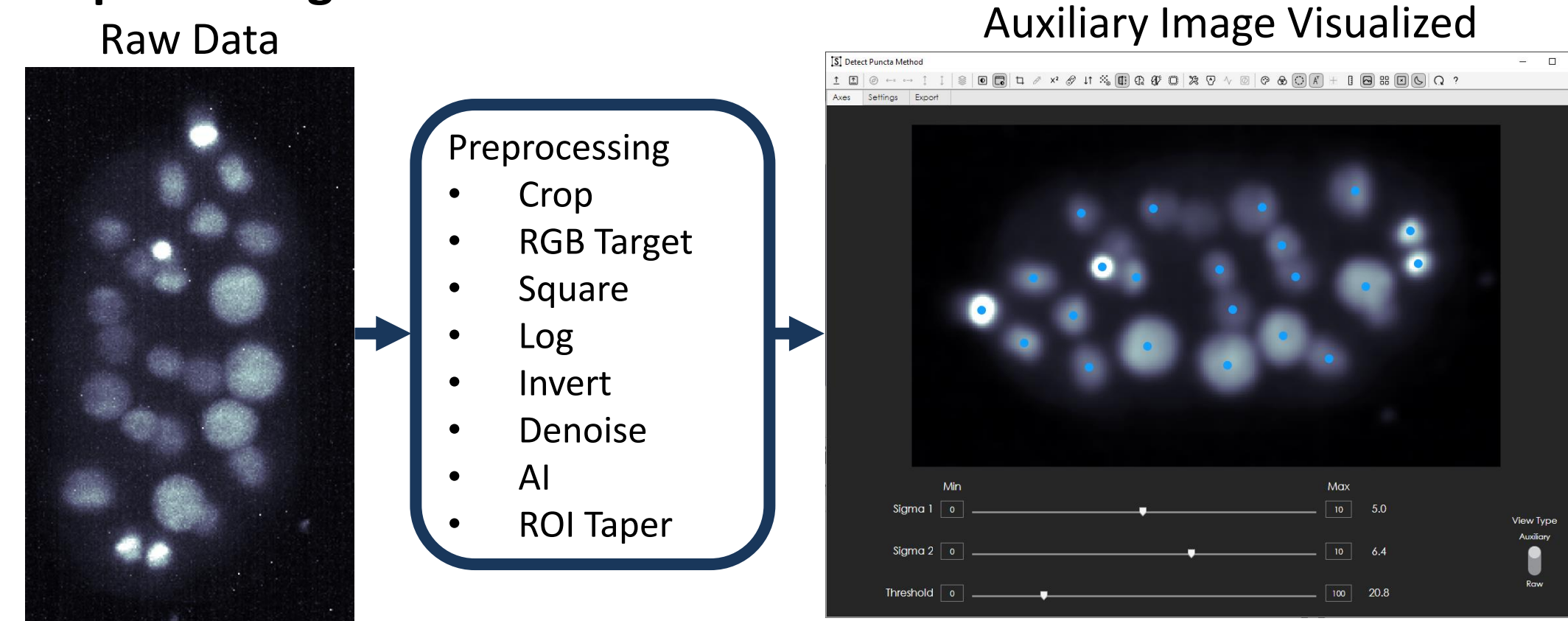
C.



**Fig 1. Overview of Key Components.** (A) Screenshot of segmentation software with *D. melanogaster* (nuclear marker) imaged on a line scanning confocal with contour overlay enabled. (B) Software architecture (C) Manual segmentation of butterfly ovary (SiR-Actin) in Masking Tool used to make a training dataset to train a model to identify an undesirable ROI across space and time. This enabled removal of the ROI across the dataset.

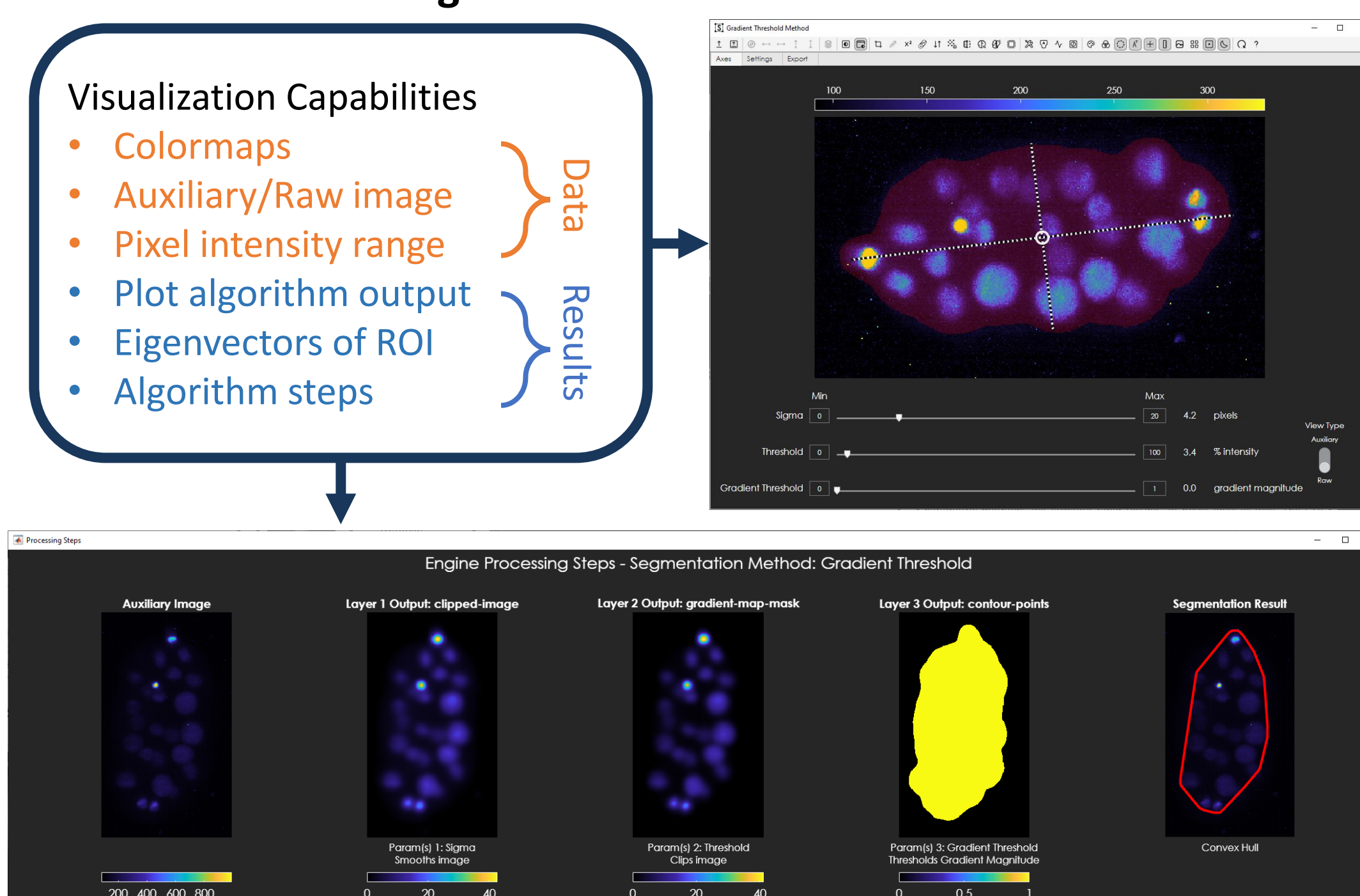
## Applications of Software Features

### Preprocessing Raw Data



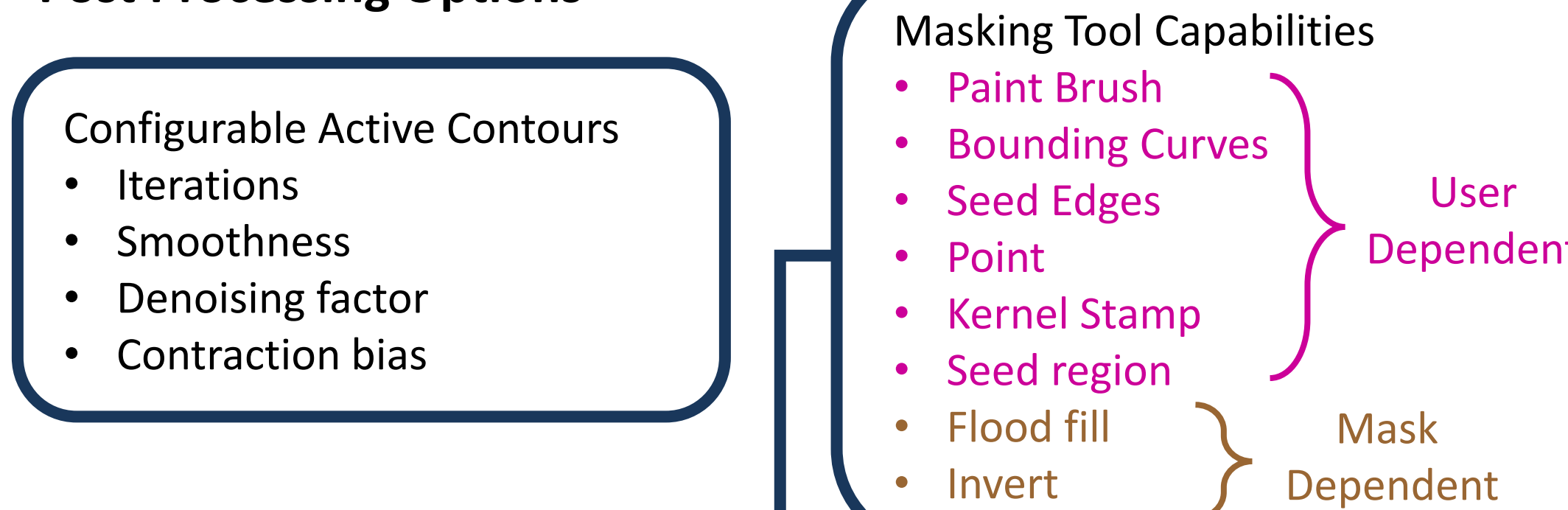
**Fig 2. Segmenting and counting Nuclei.** diSPIM image of *C. elegans* embryo was cropped in the GUI by setting a bounding box in the crop tool and then the auxiliary image is computed by denoising the cropped raw data. The parametric algorithm aims to find blobs and the output result is a point cloud representing detected centroids.

### Visualize Data and Algorithm

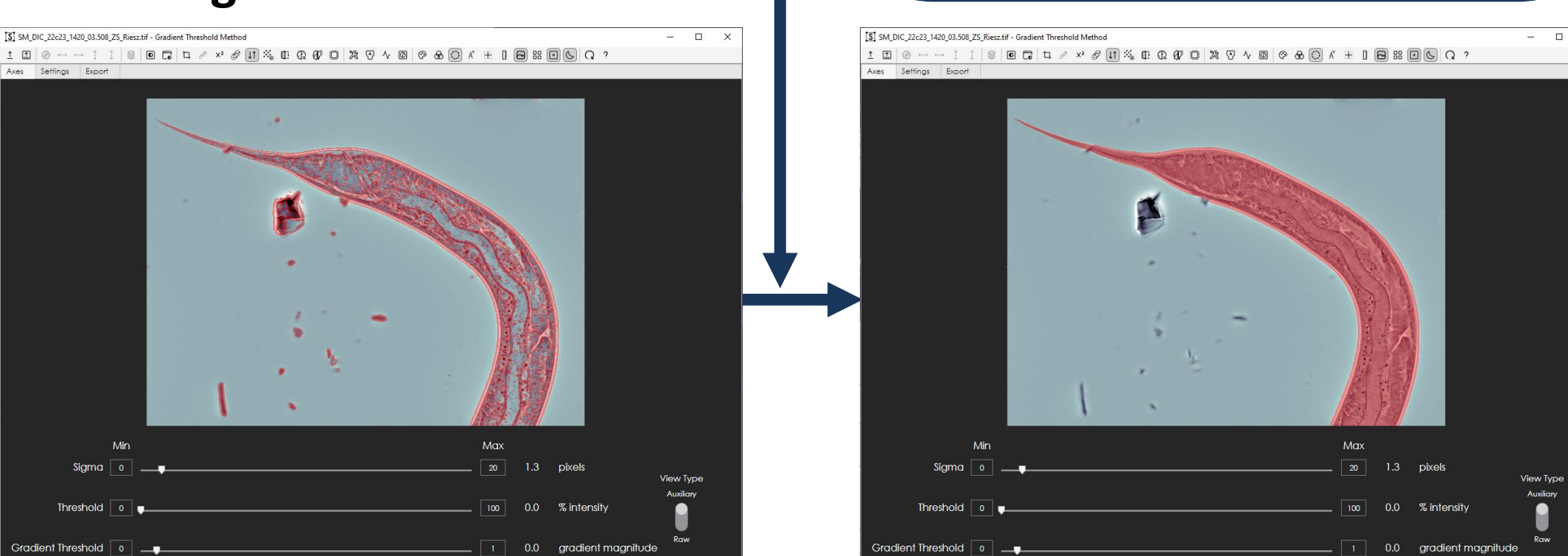


**Fig 3. Visual Feedback and Step by Step Pipeline Control.** The GUI shows the same *C. elegans* embryo as in figure 2 with a different colormap and shows the segmentation mask's eigenvectors to provide orientation information. Feedback from the engine algorithm visualization allows the user to troubleshoot their parameter selection and decide if they want to stop at an earlier step in the algorithm pipeline. The GUI then shows a mask result from the pipeline at step 3.

### Post Processing Options

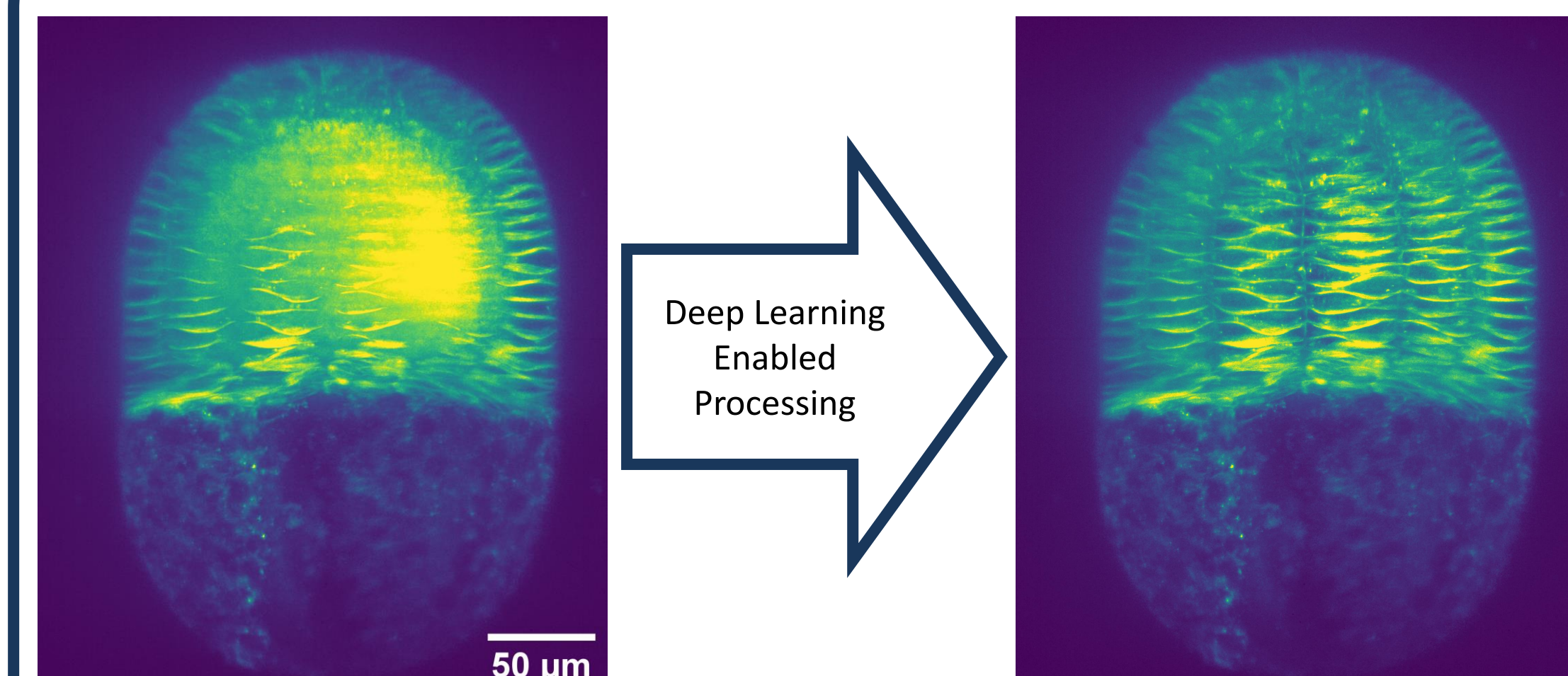


### Masking Tool Refinement



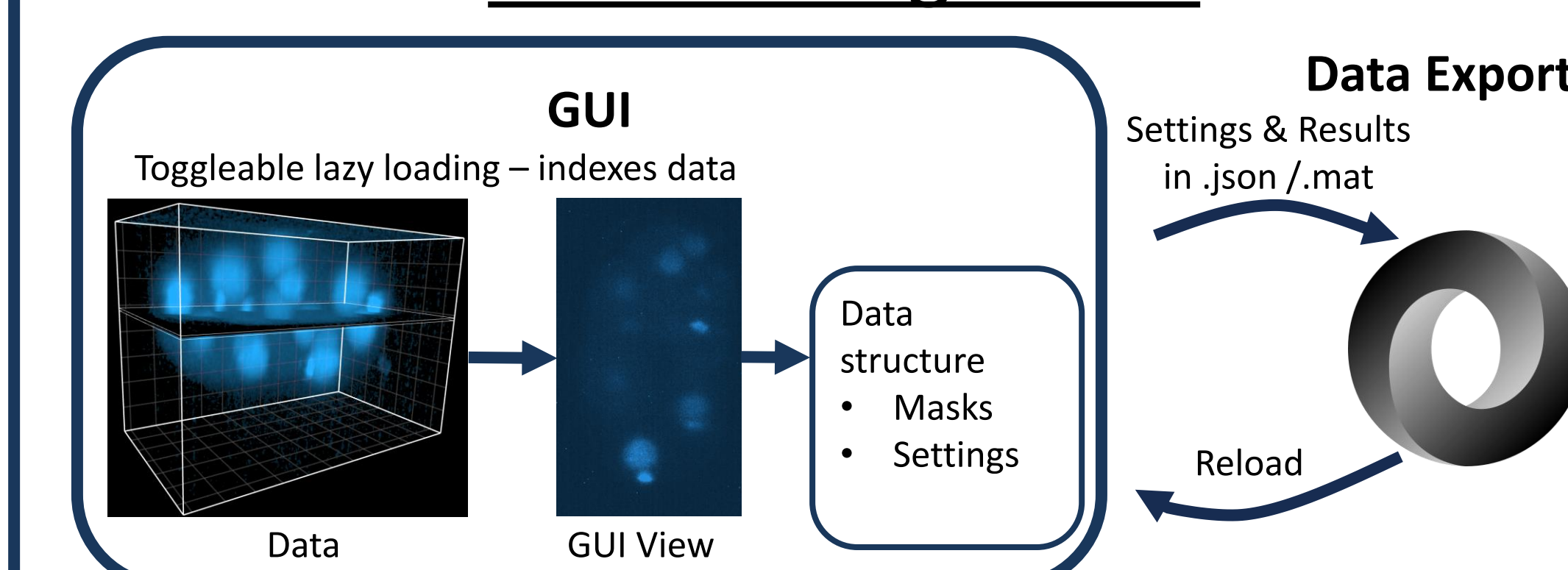
**Fig 4. Segmenting DIC data.** A DIC image of a *C. elegans* with the nematode segmented with a gradient based thresholding method. Initial results are computed with this parametric method and the results are refined in the masking tool. The user left clicked and dragged the mouse around undesired areas while in the bounding curves mode to eliminate them. The user then closed off any open boundaries in the masked ROI and clicked the "Close all" button in the masking tool to flood fill the rest of the worm in the image.

## AI Results



**Fig 5. Removing ROIs in Volumetric Timeseries.** We manually segmented 350 images, taken from a volumetric time series (70 slices and 80 timepoints total). This data was used to train a deep network, DeeplabV3+, to identify ROIs in butterfly ovary images acquired on a diSPIM system. The undesirable regions were removed by masking out segmented ROIs across slices prior to a maximum intensity projection. While only 350 images were used to train the model, 5600 images were successfully processed by the deep network.

## Data Management

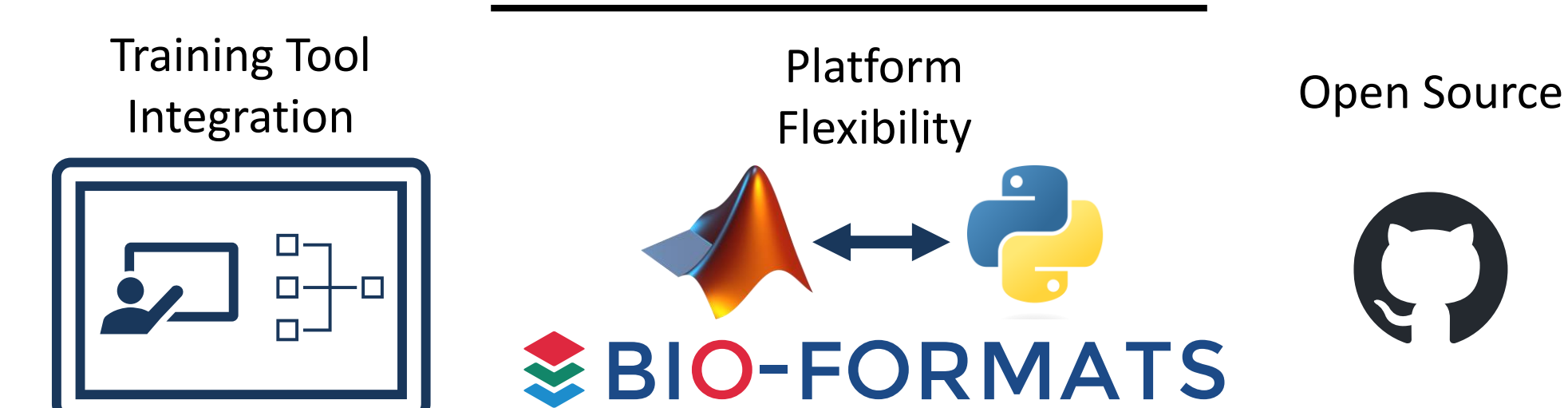


**Fig 6. Multi-format Compatibility, Saving Settings, and Batch Processing.** The software can read 2D images (.png, .tif, .jpg) and 3D+ data from .tif, M-Data (.mat), and videos (.mp4, .avi, .mpg) and can perform lazy loading for memory efficiency. As user navigates to different indices and computes on a 2D image, settings and results are saved. This information can be reloaded into the GUI and batch processing can be performed by applying settings to all indexed slices with a single click.

## Conclusions

Segmentation remains a difficult computer vision task for microscopy data given the diversity in features, varying signal to noise ratio, multiple imaging modalities, and sparse signal distribution. Rather than attempting to solve all problems, we developed a user-friendly, modular framework with graphical tools and real time visual feedback for testing image analysis pipelines. In addition to prepackaging some classical parametric methods, we facilitate rapid testing of novel algorithms. The GUI tools' visual feedback enables users to troubleshoot steps in their pipeline both at the parameter selection optimization stage as well as the algorithmic design stage. Furthermore, users can leverage state-of-the-art deep learning to transform their data for customizable applications. This tool combines the power of advanced filtering techniques simplified down to 3 parameters and the non-linear nature of deep neural networks, facilitating analysis with little to no coding experience required.

## Future Directions



## Acknowledgements

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