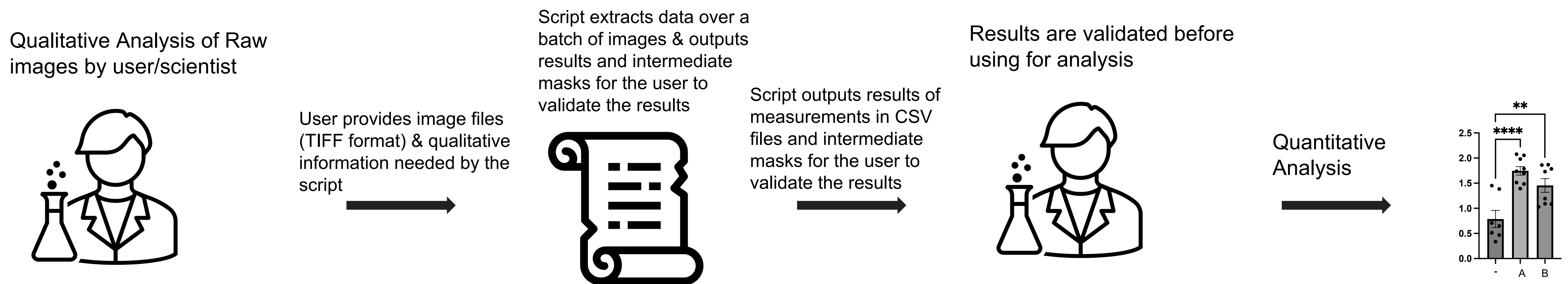


# Automation of data extraction from primary/hPSEC Endothelial cells IF images - method to detect, count & measure protein marker fluorophore expression.

The purpose of this automation tool is to automate the non-qualitative process of data extraction from biology images. We are using this tool to extract data from endothelial cell IF images, however the tool is generic and can be easily adapted to use with any cell type/marker.

## Automation Workflow:



## Script:

**GITHUB:** <https://github.com/kelkarm/ec-data-extraction/blob/main/src/ec-data-extractor.ijm>

Save the script file 'DataExtractor.ijm' in the Fiji/ImageJ application, Plugins->Scripts folder

- The script can be edited by navigating to: Plugins->Macro->Edit and opening the file.
- Test mode can be enabled by setting 'TEST\_MODE' to 1 in the code.
- To test different threshold methods:

```
method = "Otsu"; // Otsu is one of the thresholding algorithms
setAutoThreshold(method);
call("ij.plugin.frame.ThresholdAdjuster.setMethod",method); setThreshold(minThreshold,maxThreshold);
```

- Script can be edited to take segmentation method as input parameter

## Execute the Script:

Fiji/ImageJ Application->Plugins->Macro->Run-> 'DataExtractor.ijm'.  
For debugging, use Debug->Debug Macro from script editor menu.

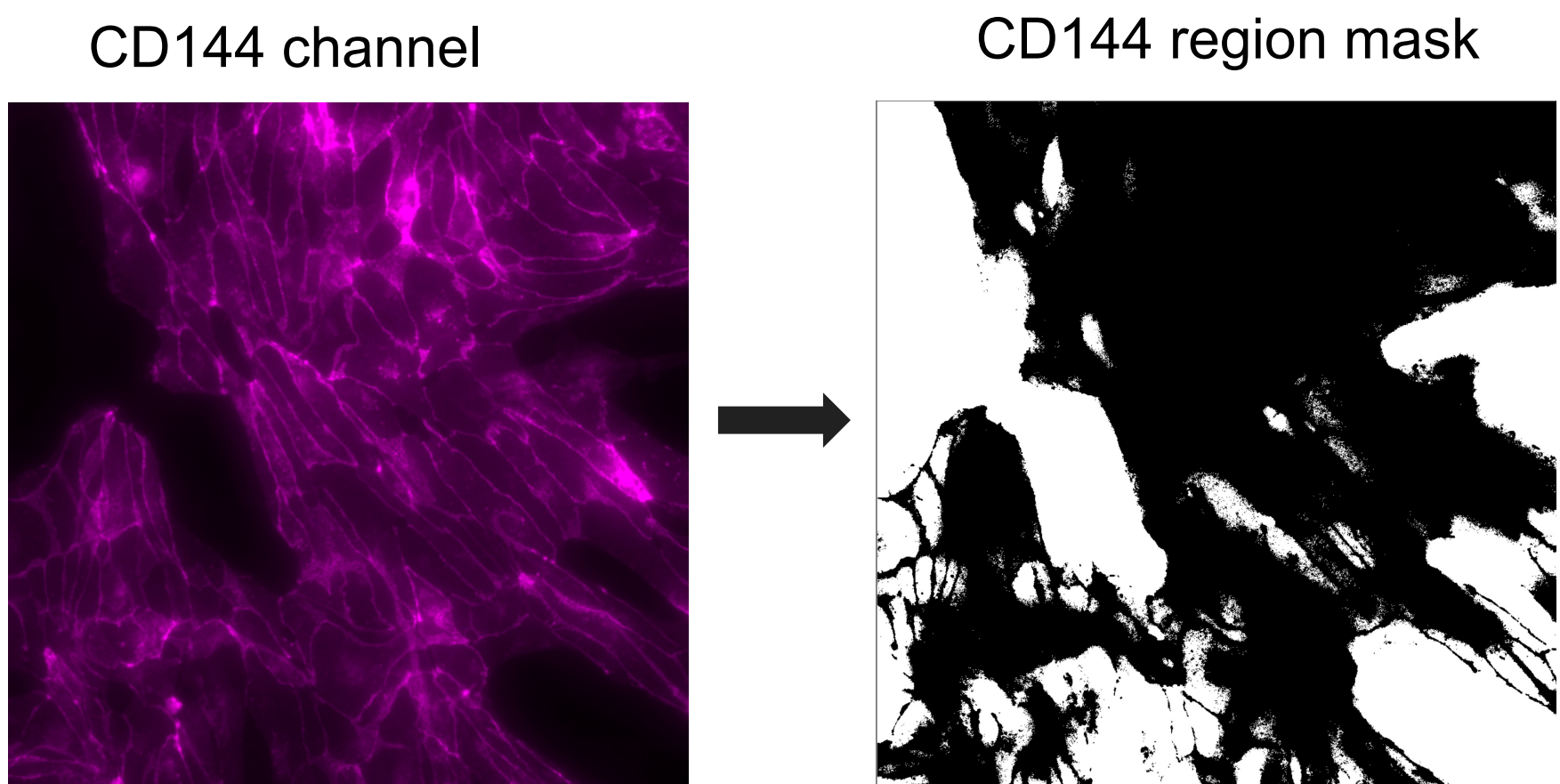


(A) To identify endothelial cells and count them from IF assay images of hPSEC cultures

(I) Create masks representing region of interest

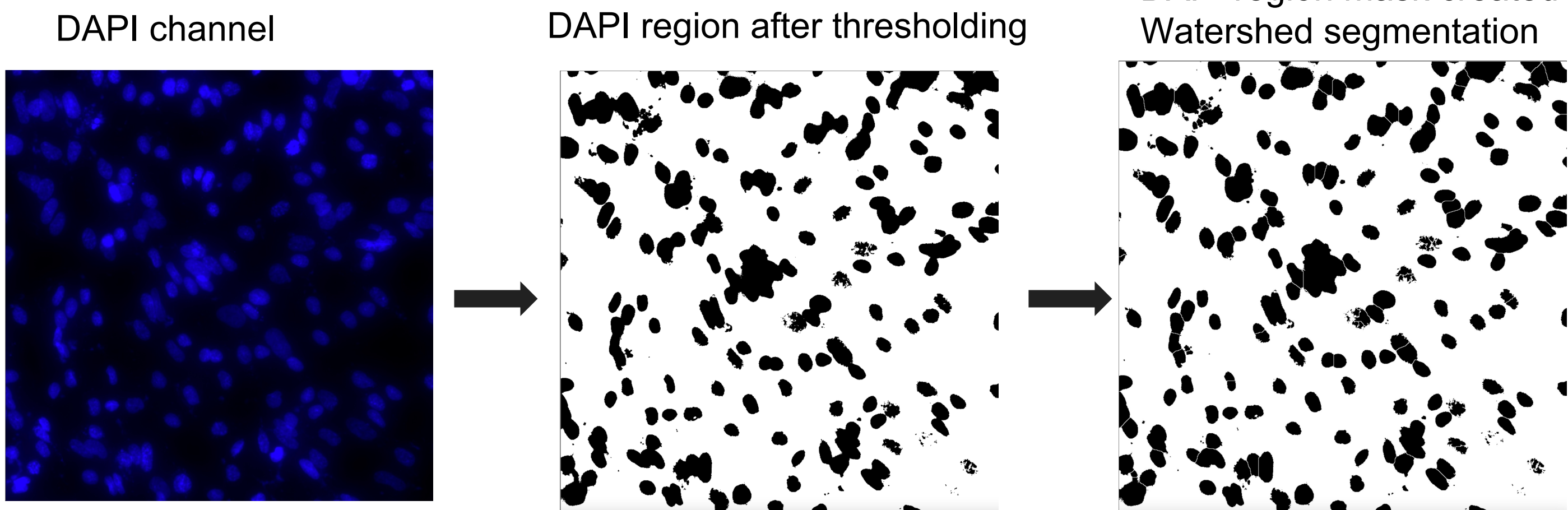
NOTE: Compare different thresholding algorithms to choose the best one the accurately represents the region

**Set Threshold** (White background, Default algorithm in Fiji), **Create Mask** (Dark region represents region of interest)



NOTE: Areas with nuclei clusters are connected. We need to separate them using a segmentation algorithm.

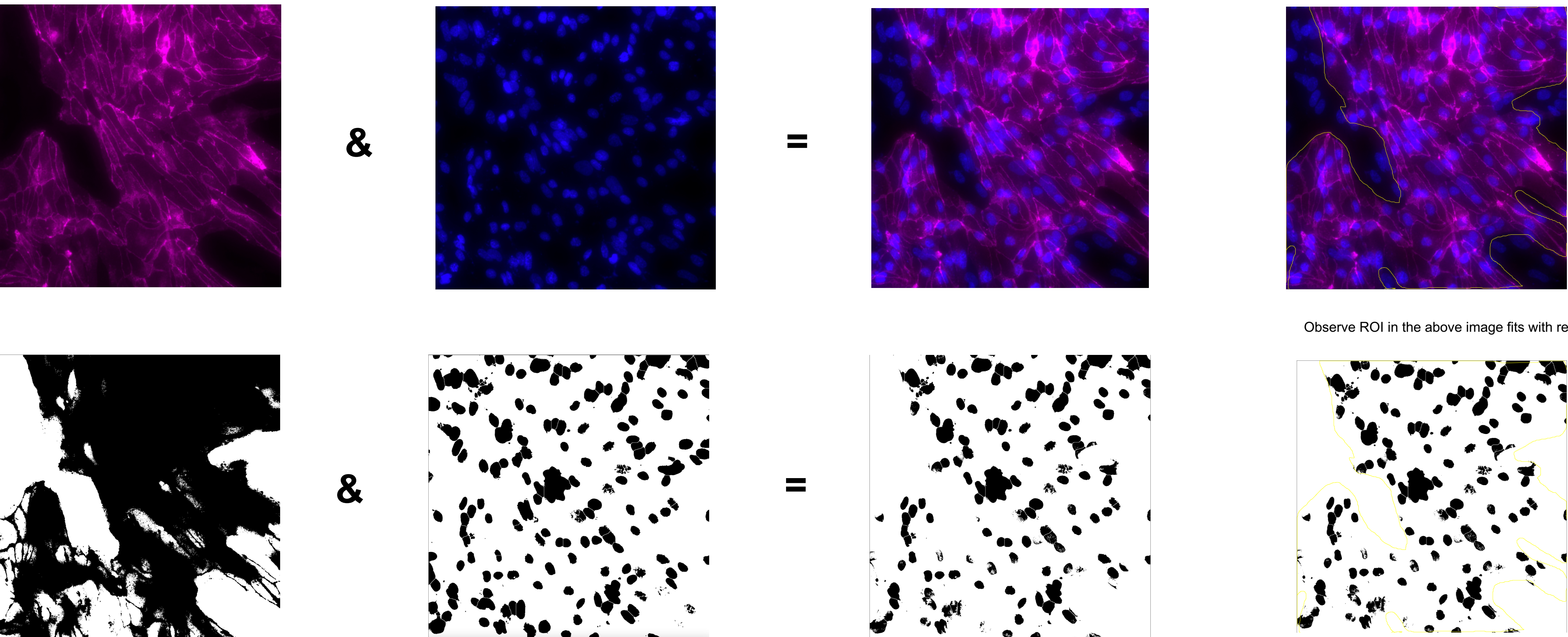
**Make Binary, Watershed, Create Mask**



(II) Identify endothelial cell

*ImageCalculator('AND create', DAPI\_mask, CD144\_mask)*

Overlap of nuclei region with endothelial region gives us endothelial nuclei



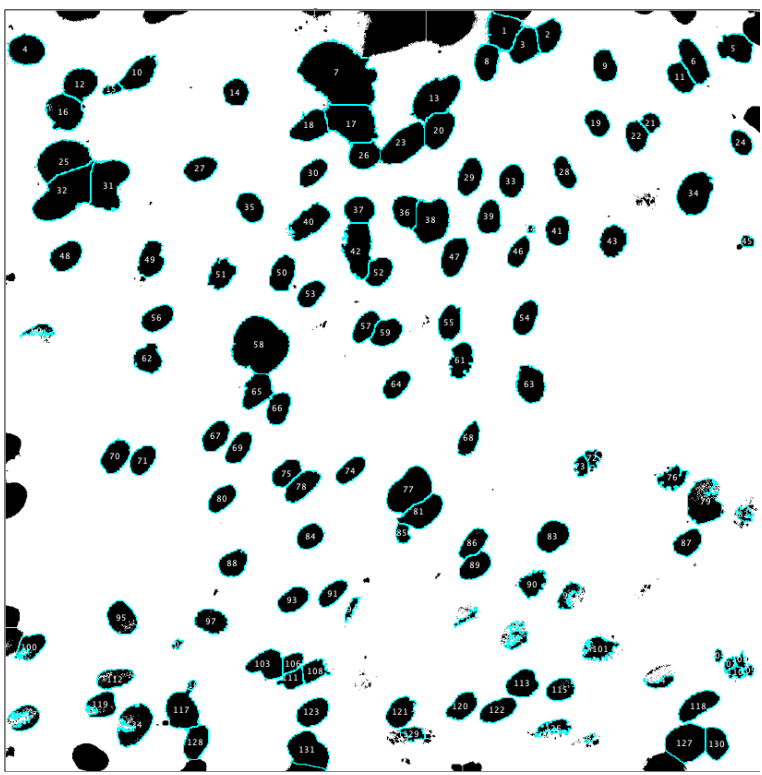


(III) Count endothelial cells

Method (1)

Using 'Analyze Particles'. In this example, minimum Nuclei size is '10' pts, so analyze particles 10-Infinity  
\*Result of dapi\_mask is the overlap region from step (II)

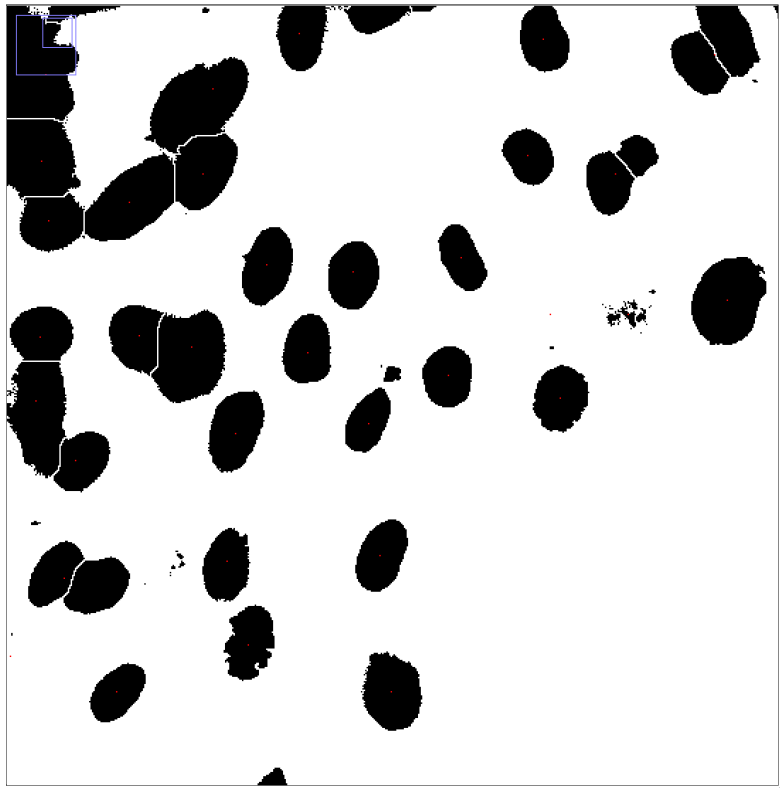
Summary						
Slice	Count	Total Area	Average Size	%Area	Mean	IntDen
Result of dapi_mask	132	9050.314	144.321	15.056	255	36801.742



Method (2)

Using ITCN plugin.

Results	
Image: Result of dapi_mask	
Number of Cells: 132 in 26529.2438 square microns	
Density: 0.0018 cells per square micron	
.....	



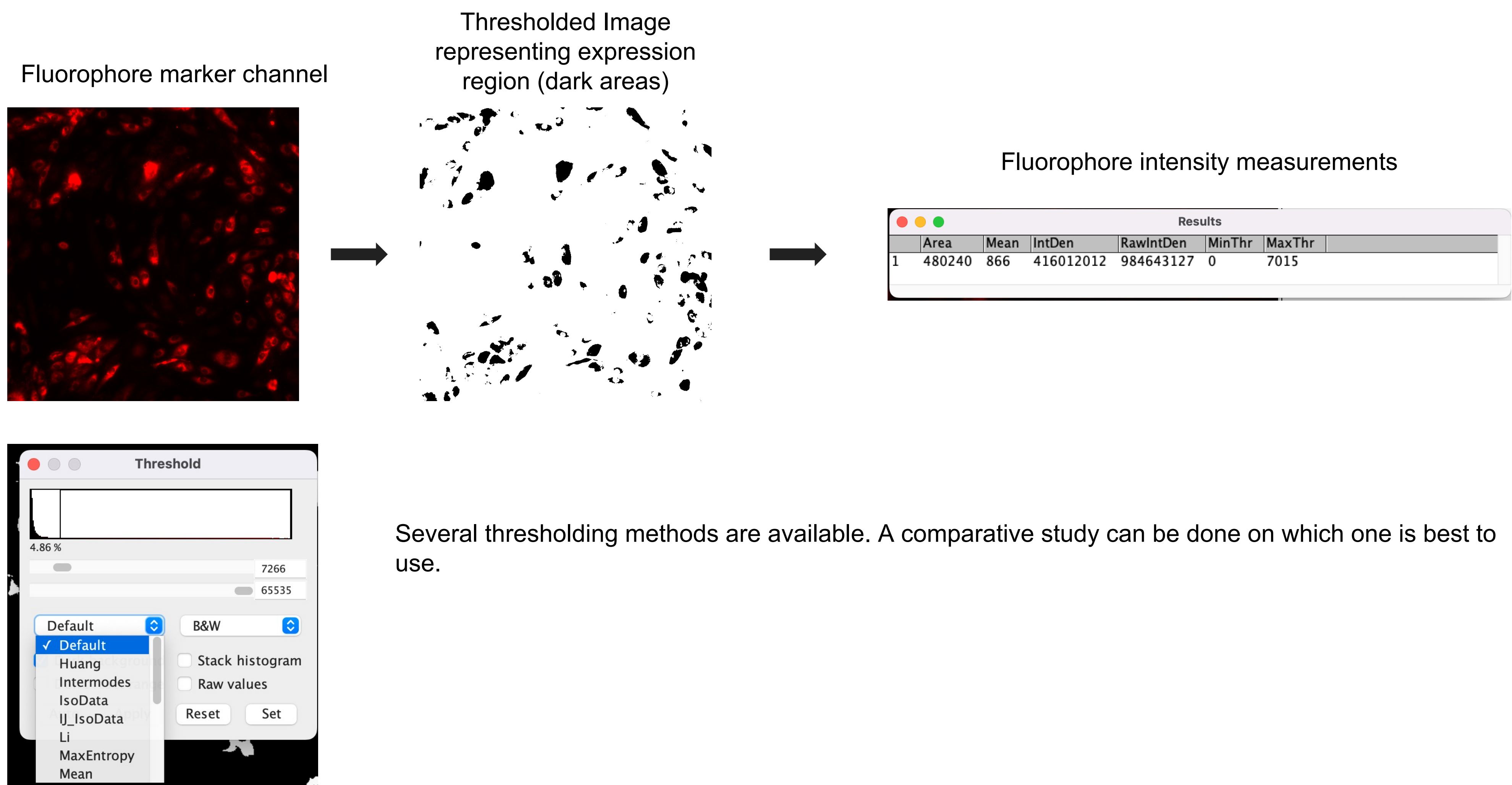
Conclusion:

Of the two methods to count endothelial cells, Analyze Particles algorithm gives a better control over particle counting by allowing min-max particle size range setting and is not dependent on nuclei being evenly distributed. ITCN is no longer supported and has very high processing time to use in automation. ITCN is also highly sensitive to 'width' parameter set by user. If distance between nuclei has high variance in the image, the ITCN accuracy is low.

(B) Measure IF marker fluorophore levels in cells from IF assay images of hPSEC cultures

(I) Identifying ROI & Measure fluorophore intensity

Use thresholding (Default in Fiji tool) to identify regions of marker expression.



Threshold

4.86 %

7266

65535

Default

Default

Huang

Intermodes

IsoData

IJ\_IsoData

Li

MaxEntropy

Mean

B&W

Stack histogram

Raw values

Reset

Set

Several thresholding methods are available. A comparative study can be done on which one is best to use.

**NOTE:** hPSEC culture samples contain a mix of differentiated ECs and undifferentiated hPSCs. This method works for protein studies where the marker protein are specific to ECs. For study of non-specific markers expressed in ECs, EC regions first need to be identified using CD144/other endothelial marker. Then this EC mask can be overlayed on the marker image to measure flurophore expression in EC positive region.