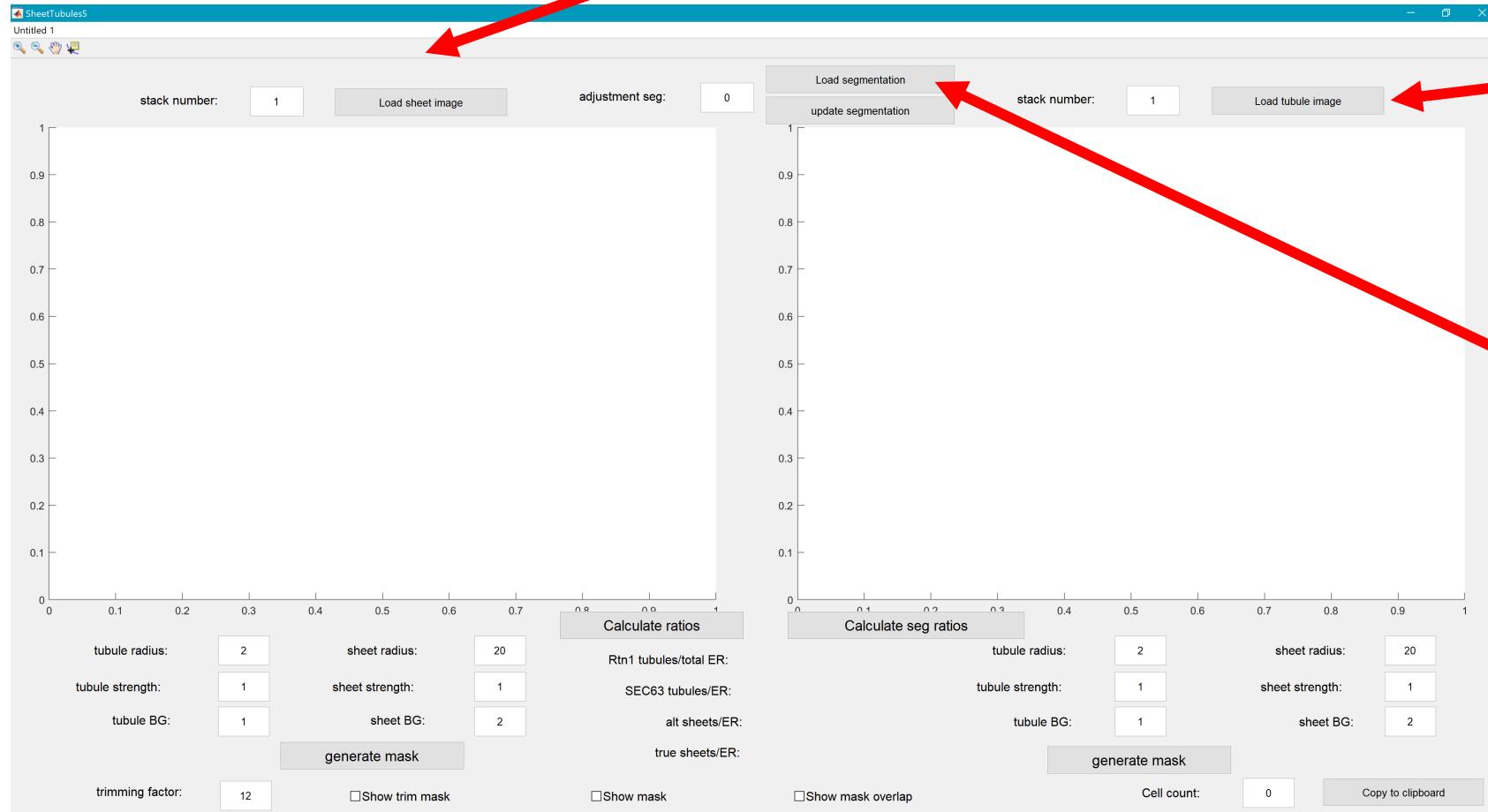


# Loading images (cortical sections)

1.) load sheet image, in this case Sec63-mNeon.  
if you have a stack of images you can select a different image by  
changing the 'stack number' before loading the image



2.) load tubule image,  
in this case Rtn1-mCherry

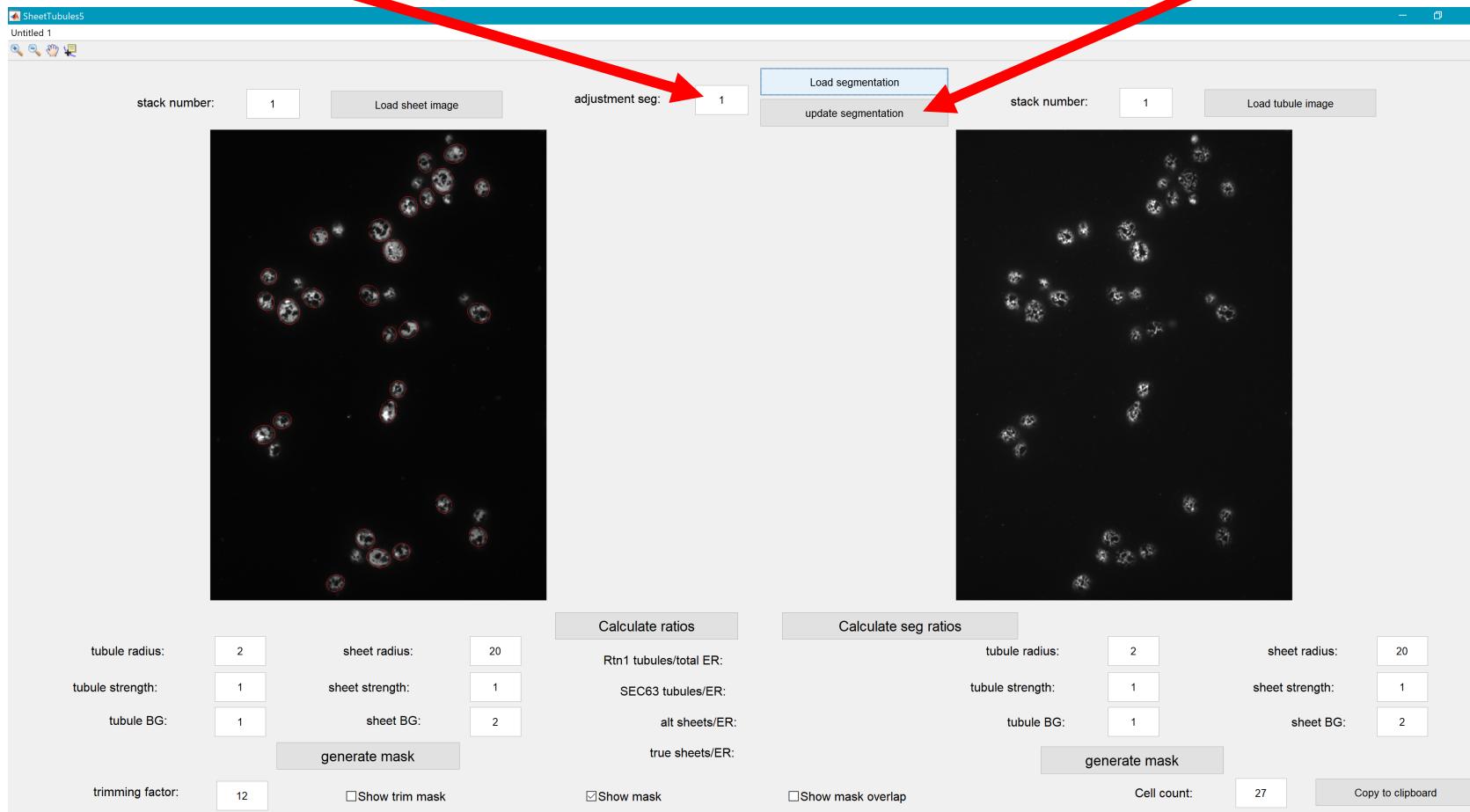
3.) load segmentation mask  
(optional, this calculates ER per cell,  
rather than across the entire image)

The segmentation file is just a binary  
image to labelling cells. In this case  
made from a bright field image in  
imageJ

# Updating image segmentation

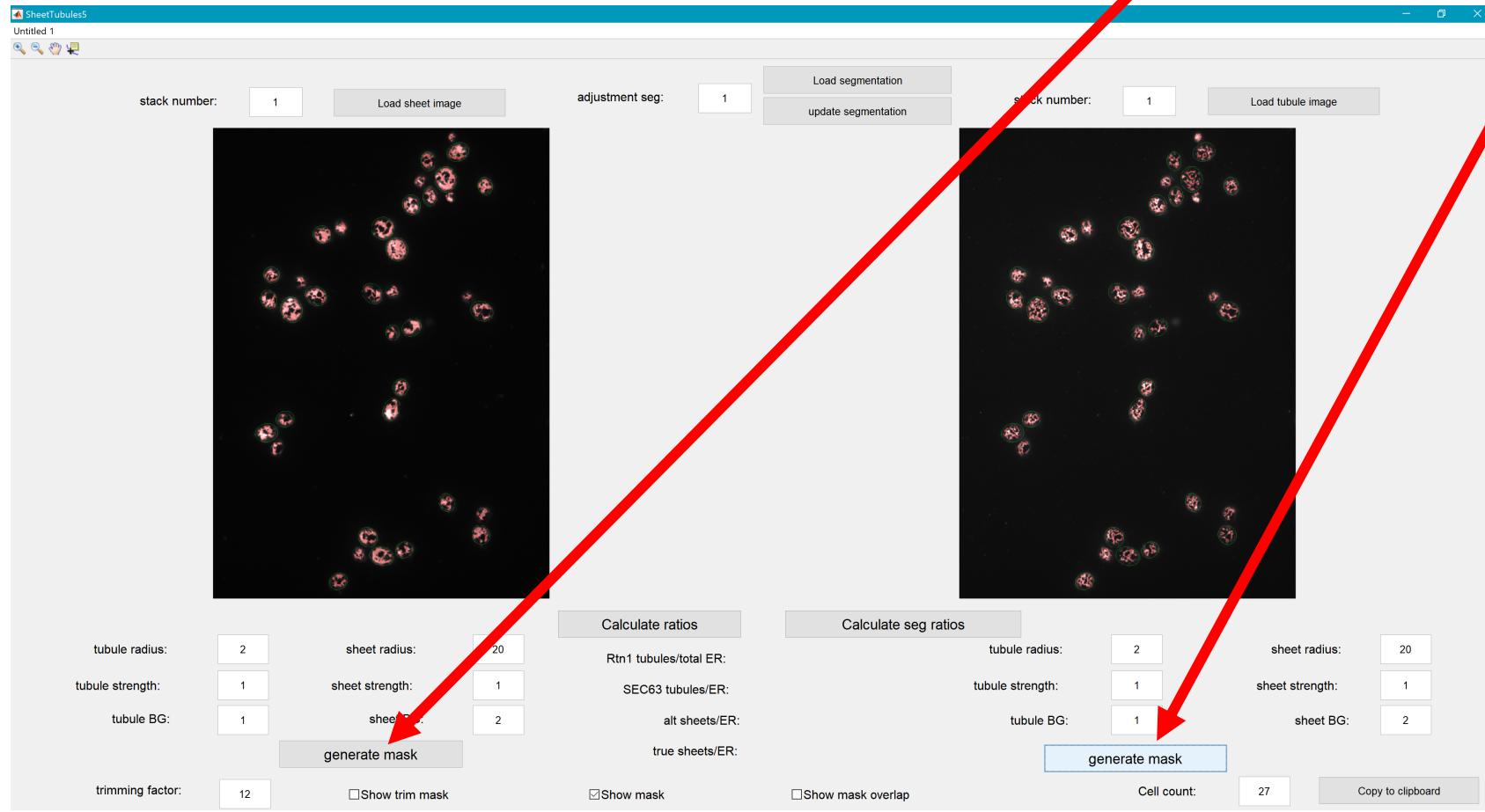
The image should load and look like this. Sheets (Sec63) are on the left and tubules (Rtn1) are on the right.

The cell segmentation mask can be seen from the red outline. If this outline is too big or small, it can be adjusted with the 'adjustment seg' value (positive or negative) followed by the 'update segmentation' button



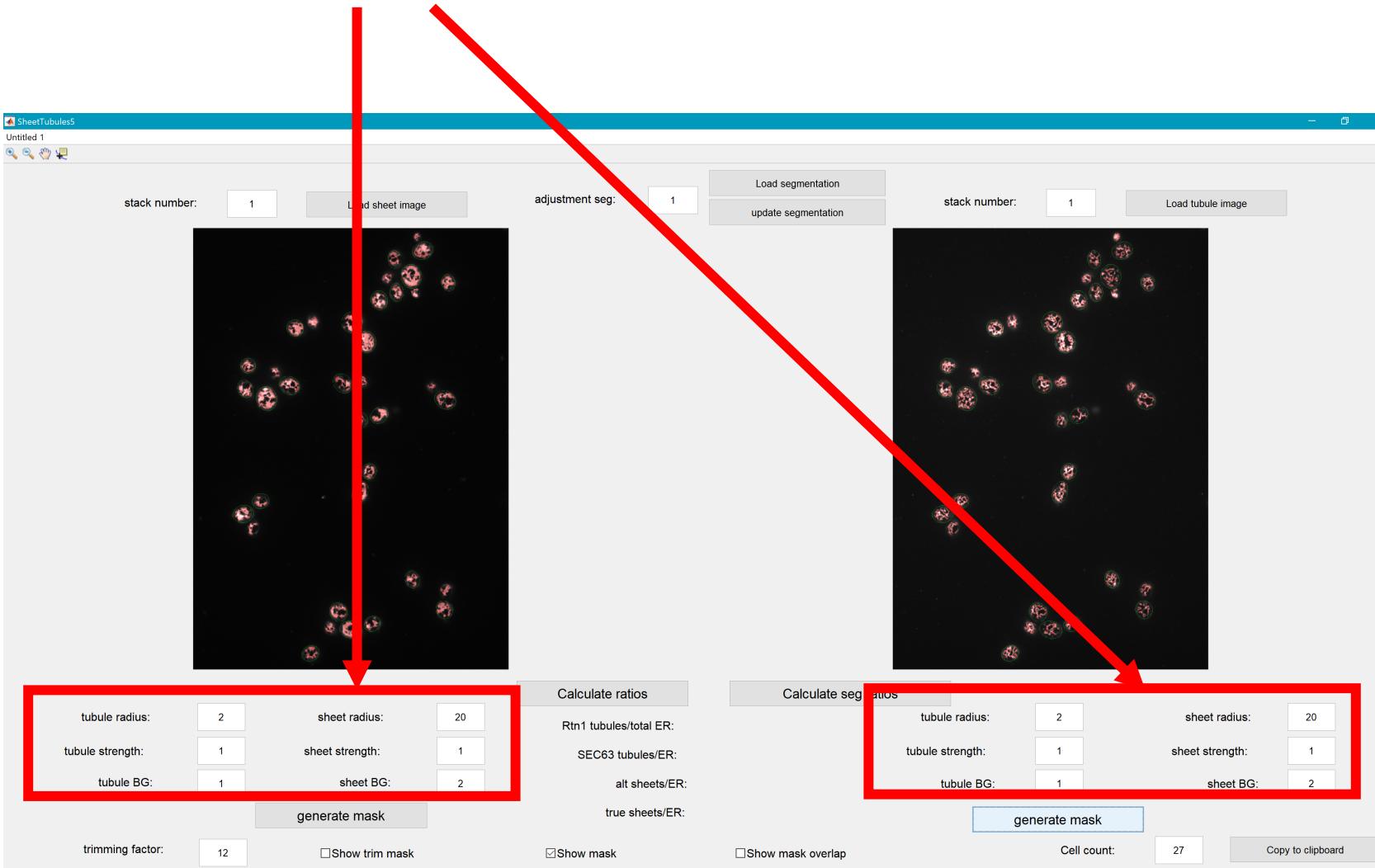
# Generating ER masks:

To generate ER masks press the 'generate mask' button for the Sheet image and the tubule image



# Adjusting ER masks:

The masks then appear in red. These are controlled by adjusting the following parameters:



**radius:** controls how large the filter radius is when searching for tubules.

**strength:** controls how aggressive the filter is

**BG:** is how strong the background removal is

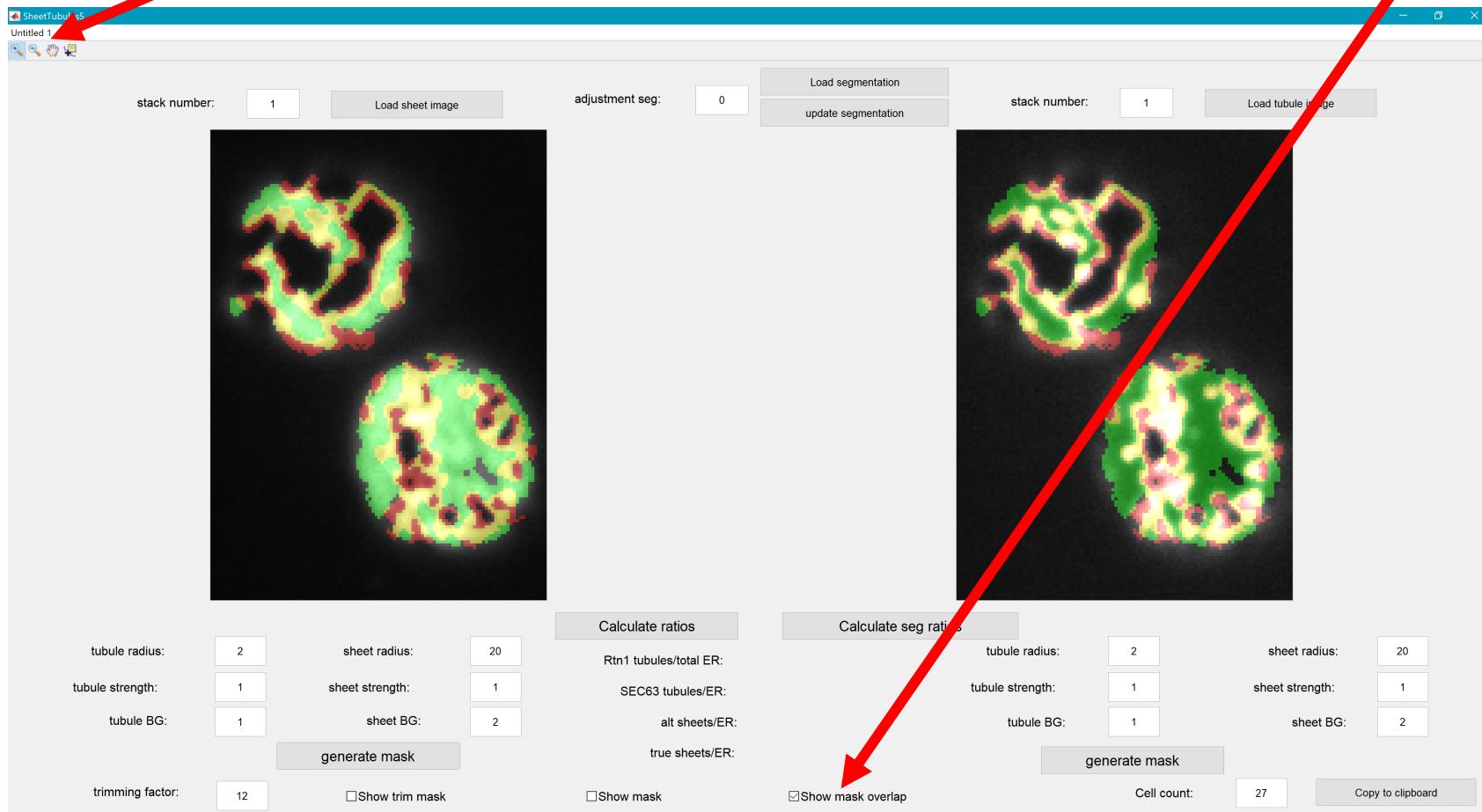
The algorithm runs in two parts. First optimized for finding tubule-like structures and second for sheet-like structures. This runs in both the sheet (Sec63) and tubule (Rtn1) images.

For us we found our fluorophores lost a lot of signal when searching for cortical sections so we often have different signal intensity across images and these setting may need to be adjusted per image to get good segmentation if there is a lot of variation

# Comparing ER segmentations

To compare the ER segmentation from the Sec63 and the Rtn1 image tick the ‘show mask overlap’ button

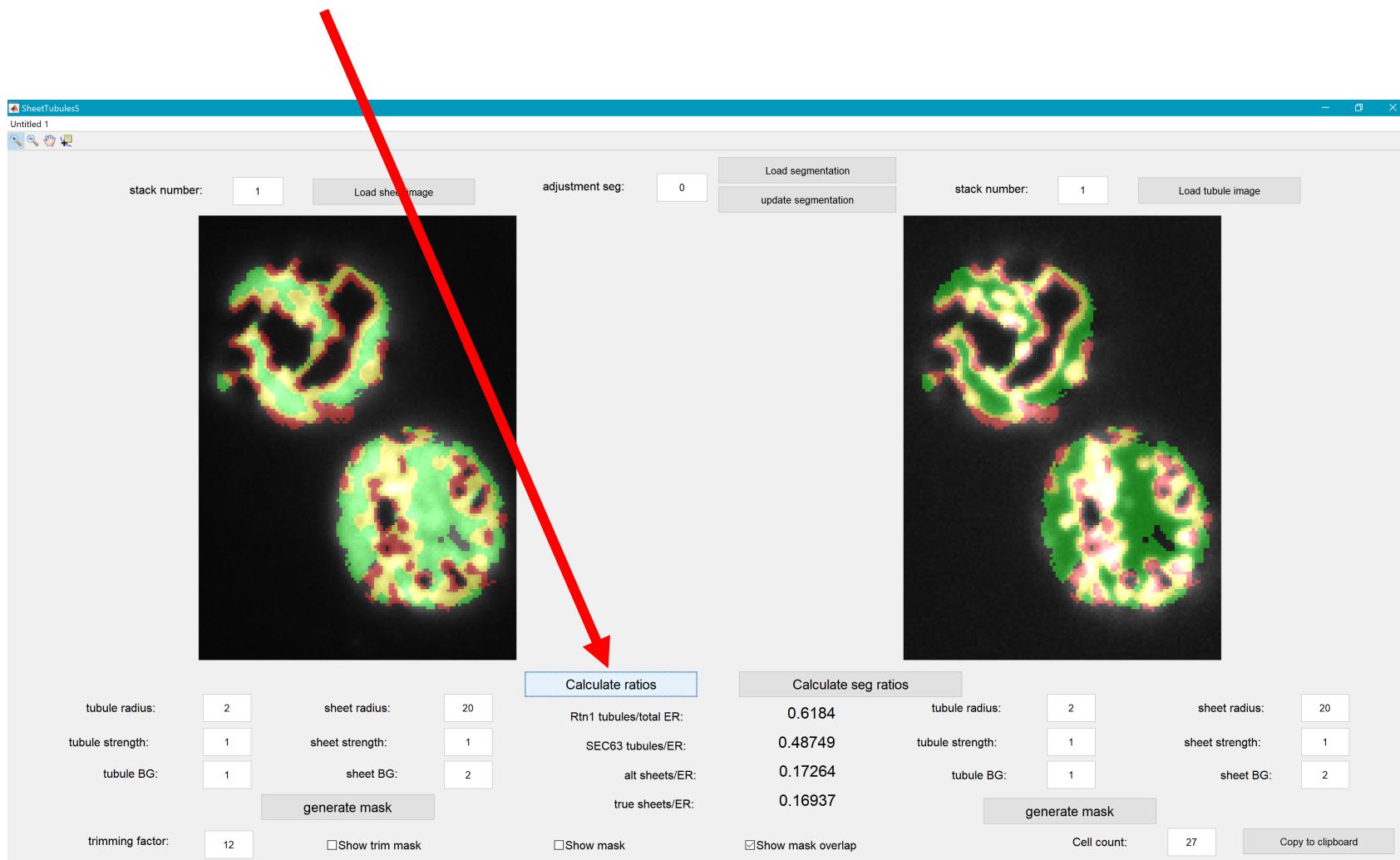
You can use the zoom tools to get a better look at individual cells



This will show the Sec63 segmentation in green, the Rtn1 segmentation in red and the overlap as yellow

# Calculating sheet:tubule ratios

Press the 'calculate ratios' button to get metrics on sheet:tubule ratios. These stats are based on the entire image and not single cell measurements

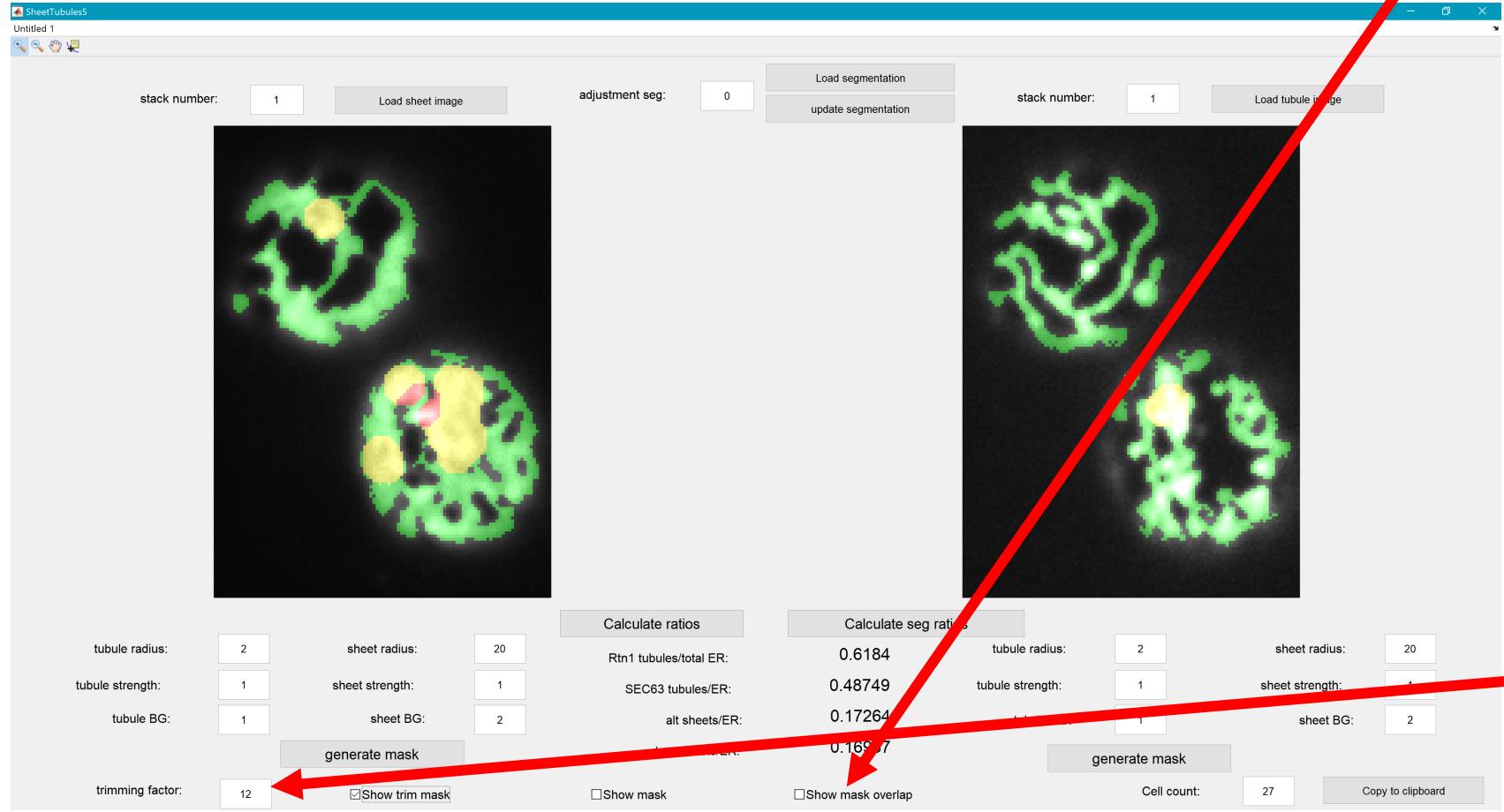


**Rtn1 tubules/total:** is the tubules identified in the Rtn1 image divided by the total sec63 ER mask

**Sec63 tubules/ER:** is the tubules identified in the Sec63 image divided by the total sec63 ER mask

# Adjusting ER sheet masks

You can visualize what has been considered sheet-like ER by pressing the 'show trim mask button'. What is considered sheet-like in each channel appears yellow. The Sec63 sheets that also appear in Rtn1 are labeled red, this are possibly Dense tubular structures



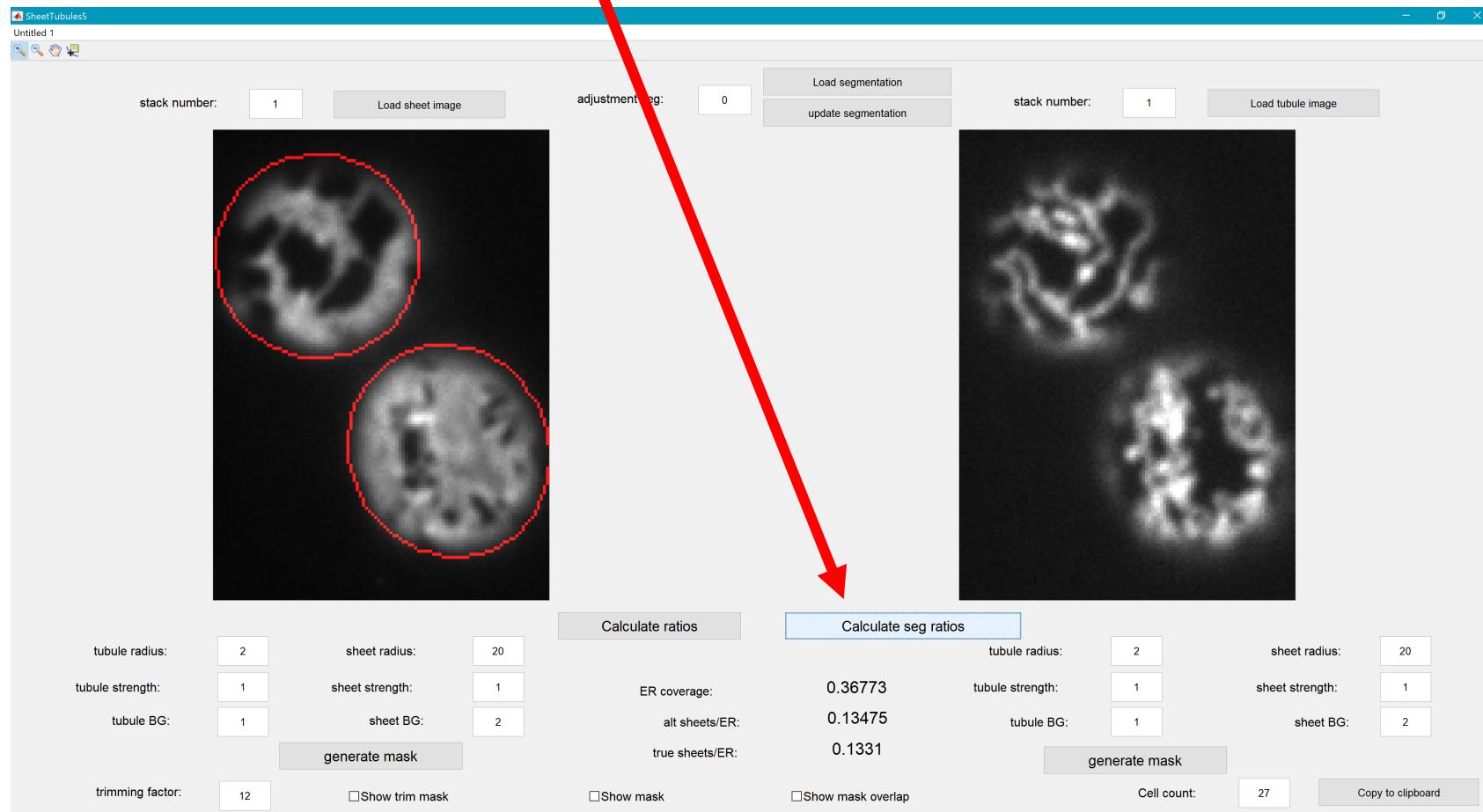
**Alt sheets/ER:** is the total sheets identified in the Sec63 image divided by the total sec63 ER mask

**true sheets/ER:** is the is the Se63 sheets that are not sheet-like in the Rtn1 image (i.e. not just densely clustered tubules) divided by the total sec63 ER mask

What is considered a sheet can be fine tuned using the 'trimming factor' value and re-calculating the ratios

# Calculating segmented sheet:tubule ratios:

Pressing the 'calculate seg ratios' button, calculates similar ratios but based on the single cell segmentation.



Here we also gain information about the ER coverage of the cell surface.

**ER coverage:** is the average proportion of the cell surface covered by ER.

**Alt sheets/ER** and **true sheets/ER** are similar to the previous measurements but are now giving the median per cell measurement instead of the total image measurement.