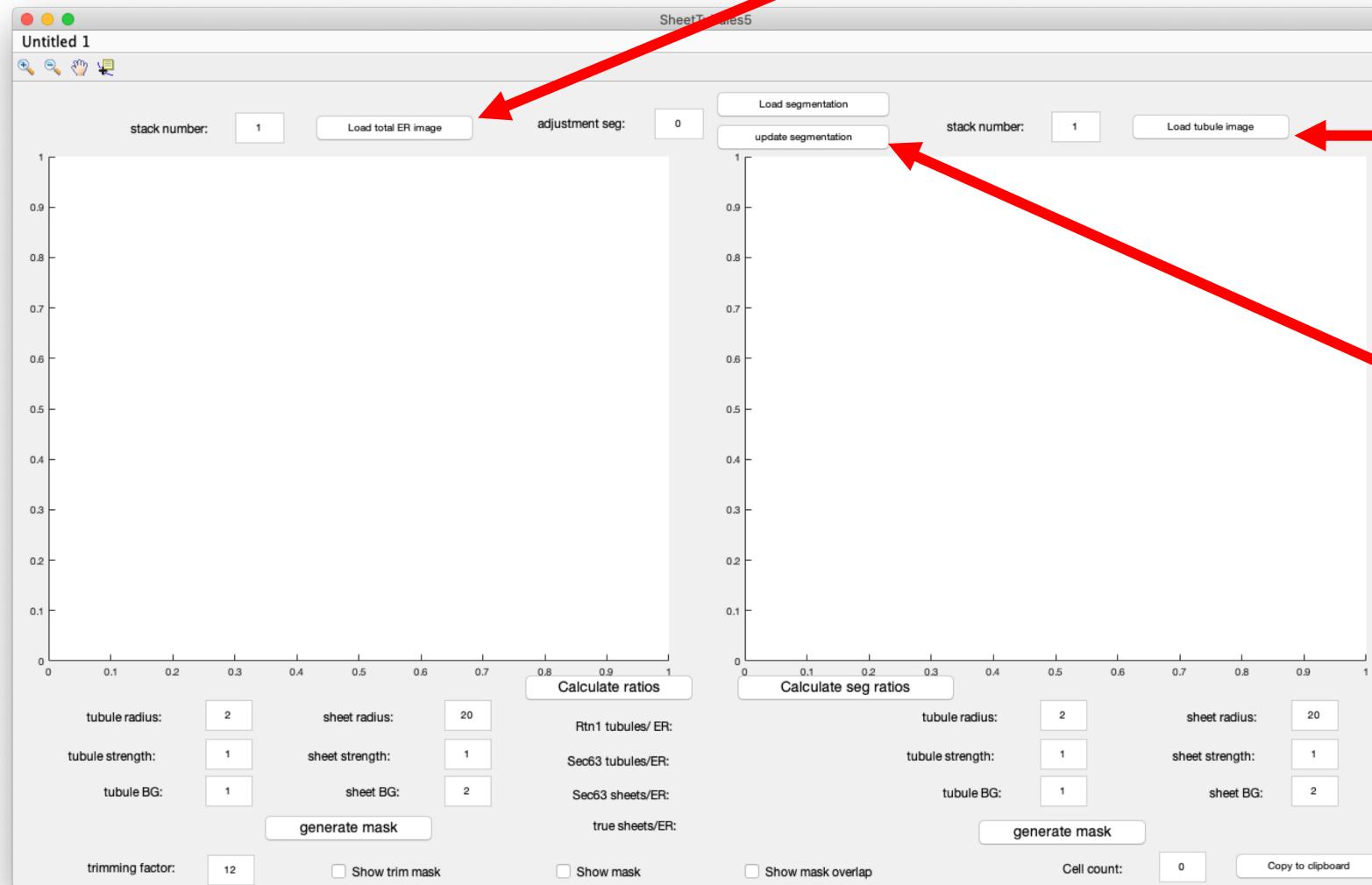


Loading images (cortical sections)

1.) Load total ER 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.

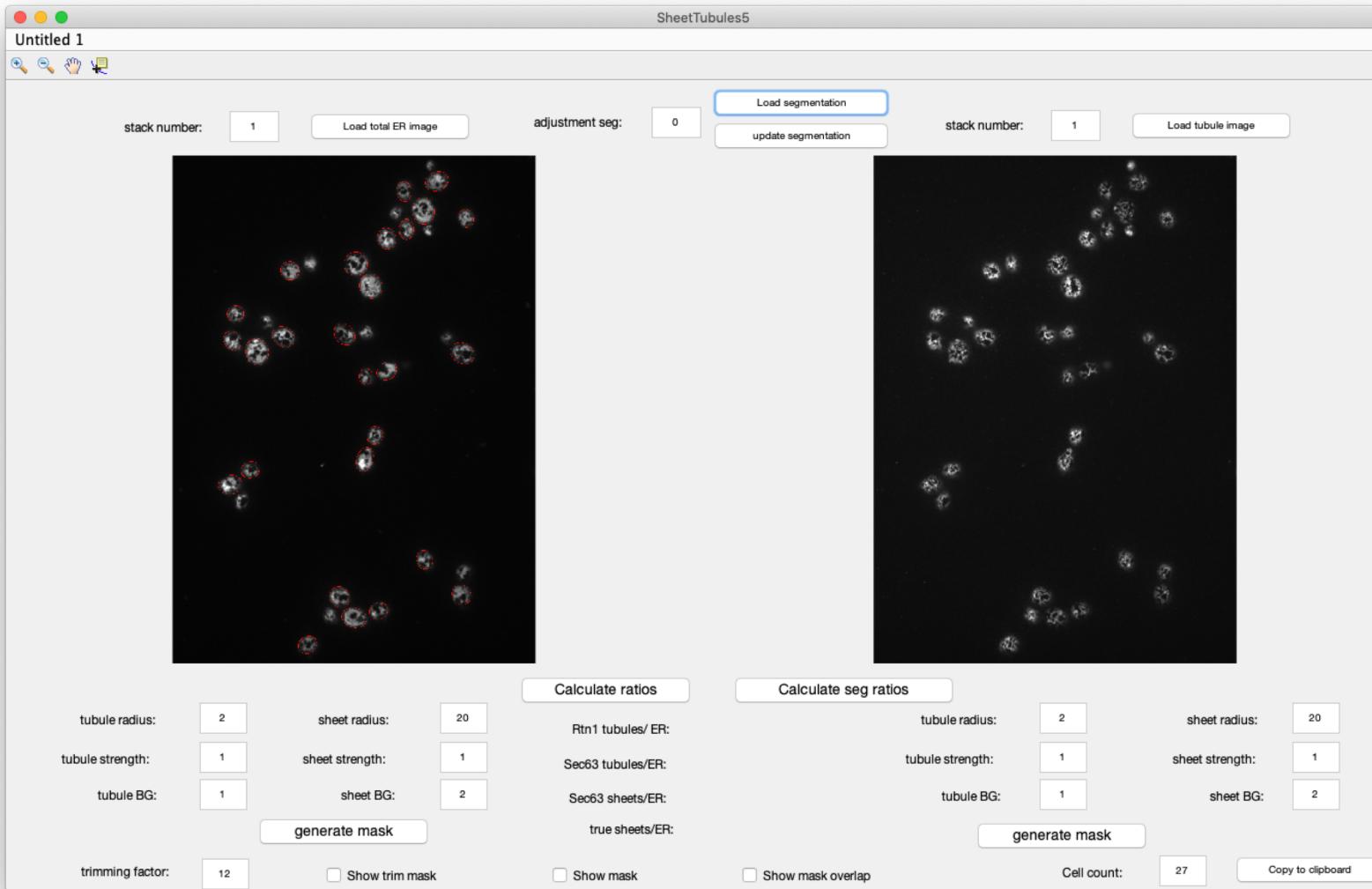
3.) Load segmentation mask
(optional, this calculates ER per cell,
rather than across the entire image).

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

Updating image segmentation

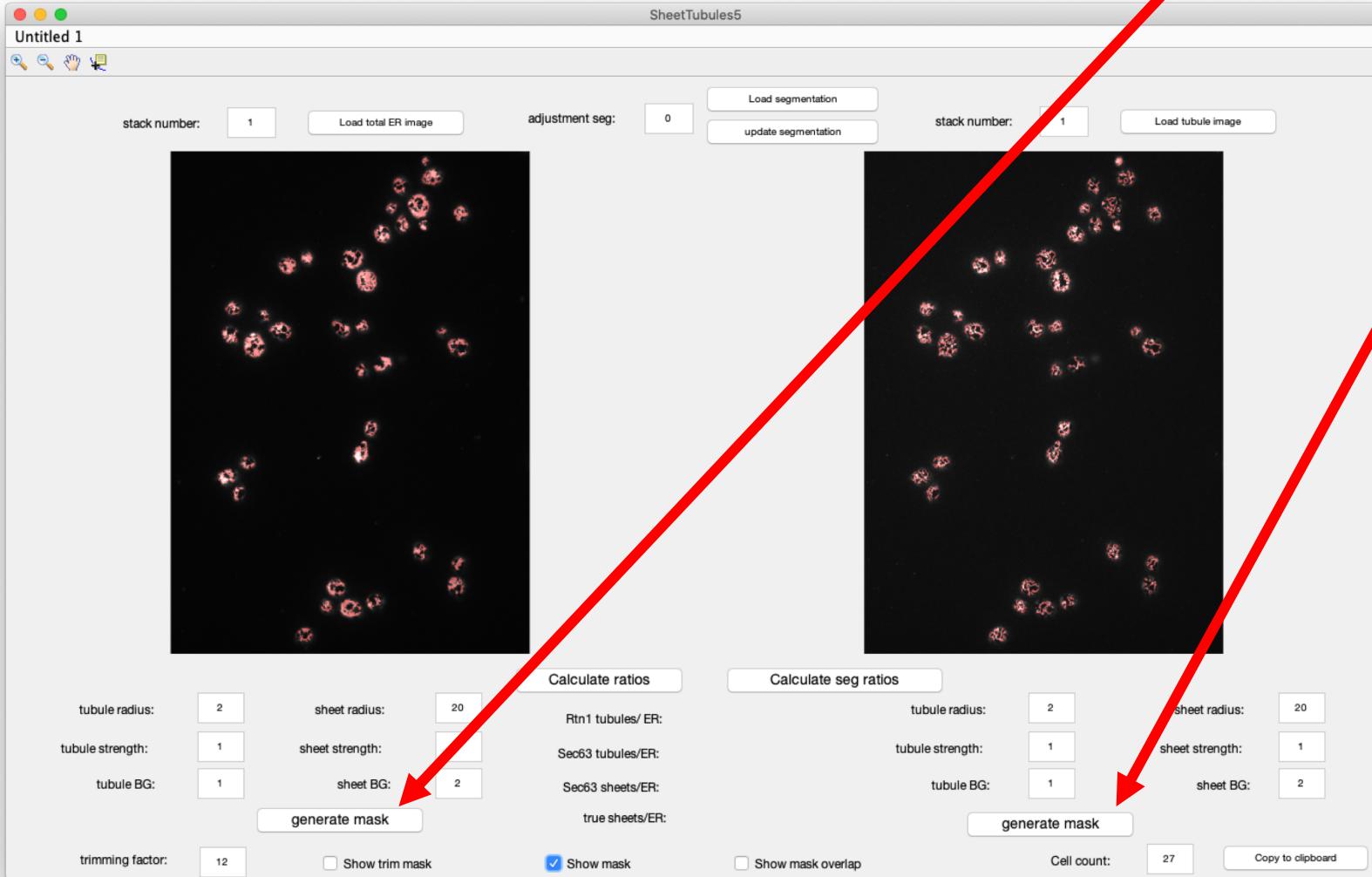
The image should load and look like this. Total ER (Sec63) on the left and tubules (Rtn1) on the right.

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.



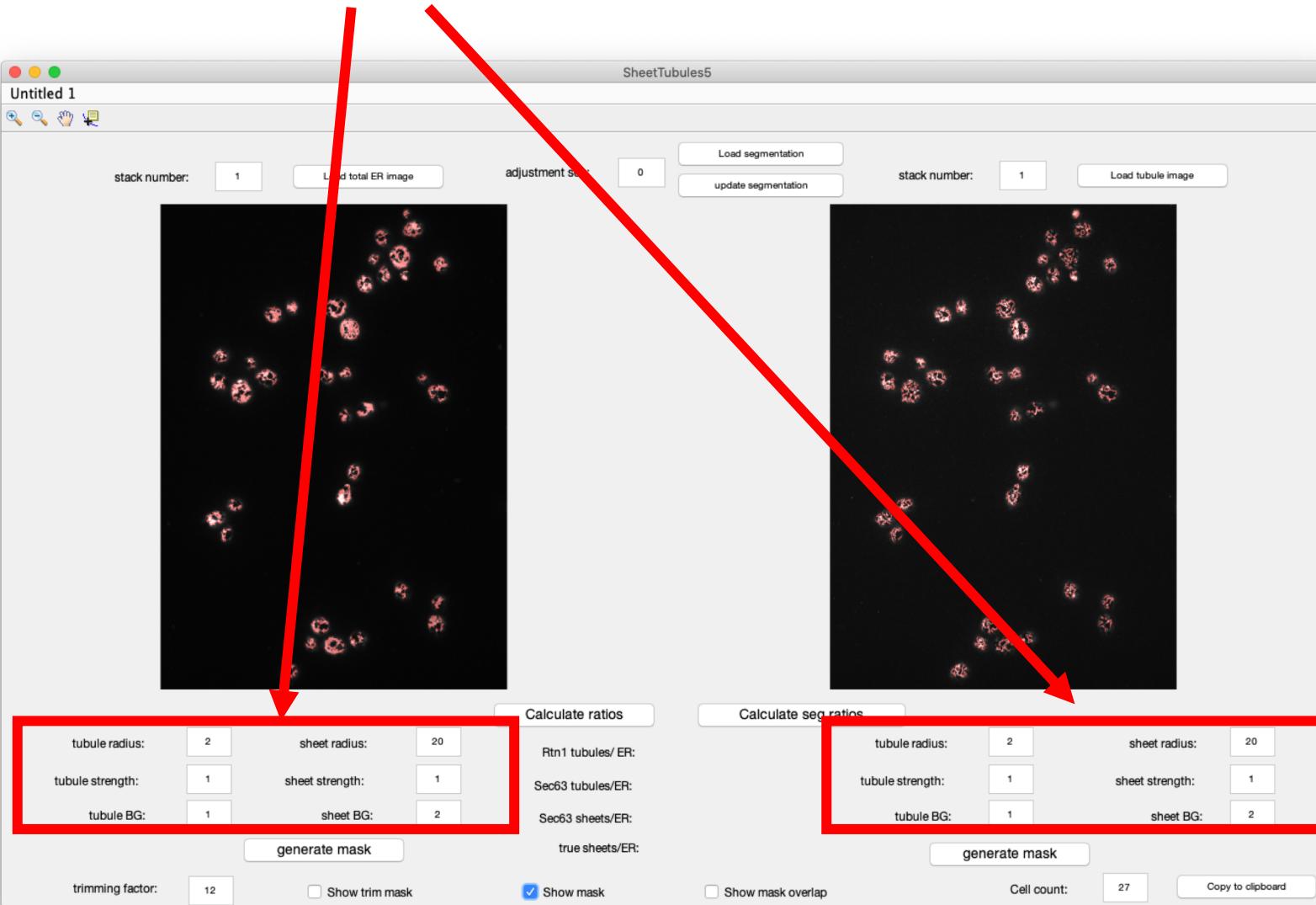
Generating ER masks:

To generate ER masks press the 'generate mask' button for the total ER 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: Controls how strong the background removal is.

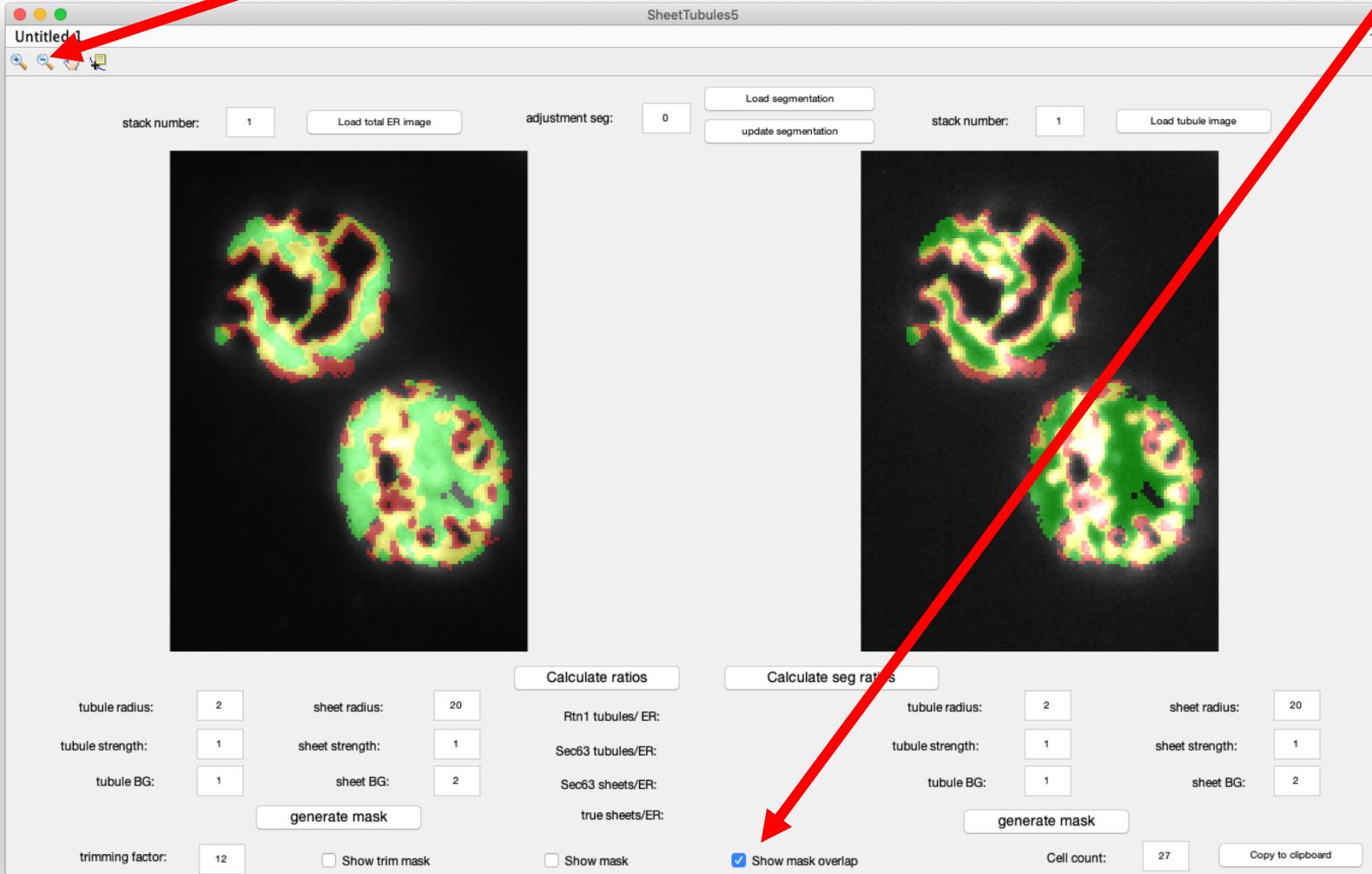
The algorithm runs in two parts. First optimized for finding tubule-like structures and second for sheet-like structures. This runs in both the total ER (Sec63) and tubule (Rtn1) images.

Depending on the fluorescent proteins used and how long it takes to focus on the cortical sections, fluorescent signal can vary across images due to bleaching and these settings 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.

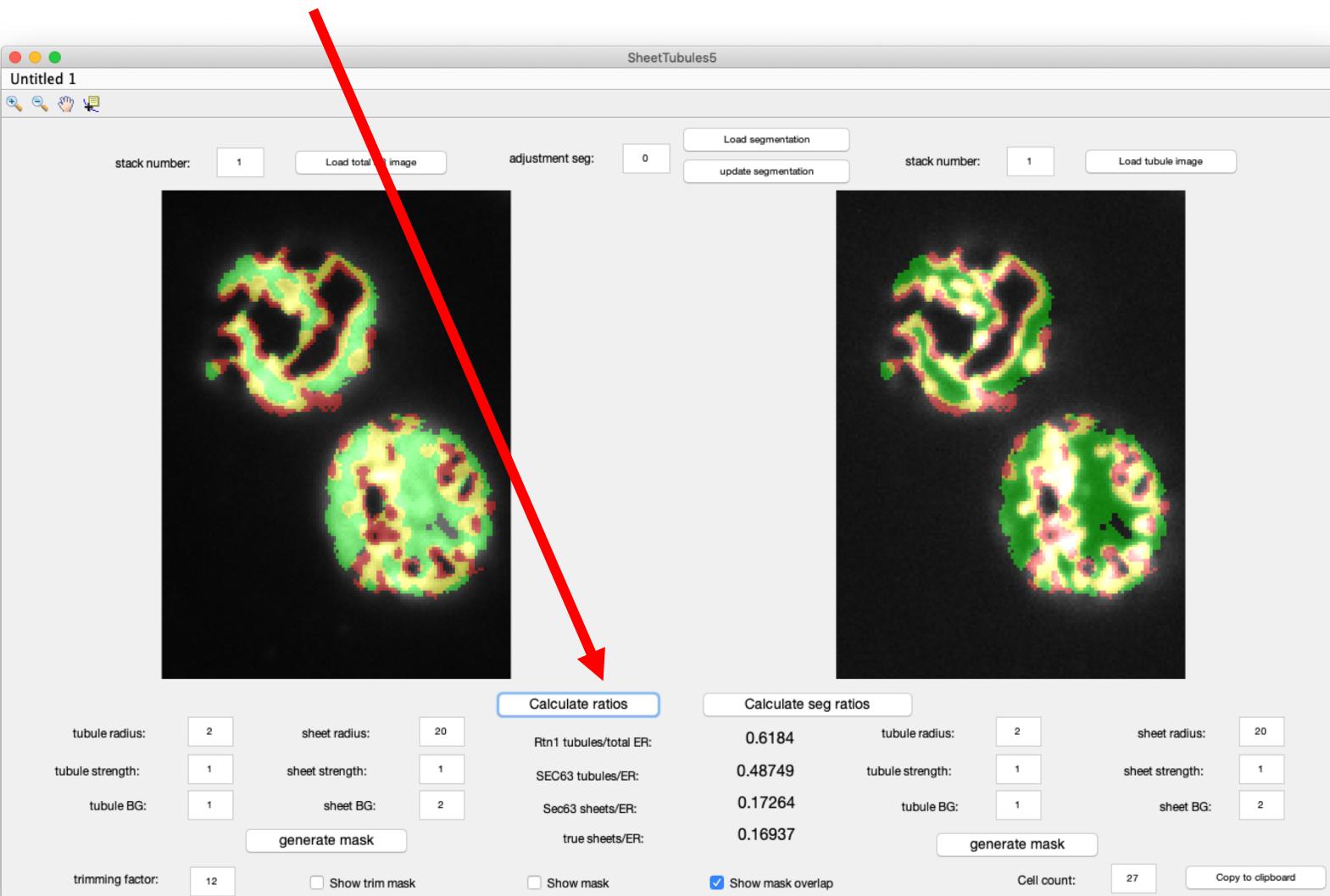
You can use the zoom tools to get a better look at individual cells.



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

Calculating tubule and sheet ratios:

Press the ‘calculate ratios’ button to get metrics on sheet-to-tubule ratios. These outputs are based on the entire image and not single cell measurements.

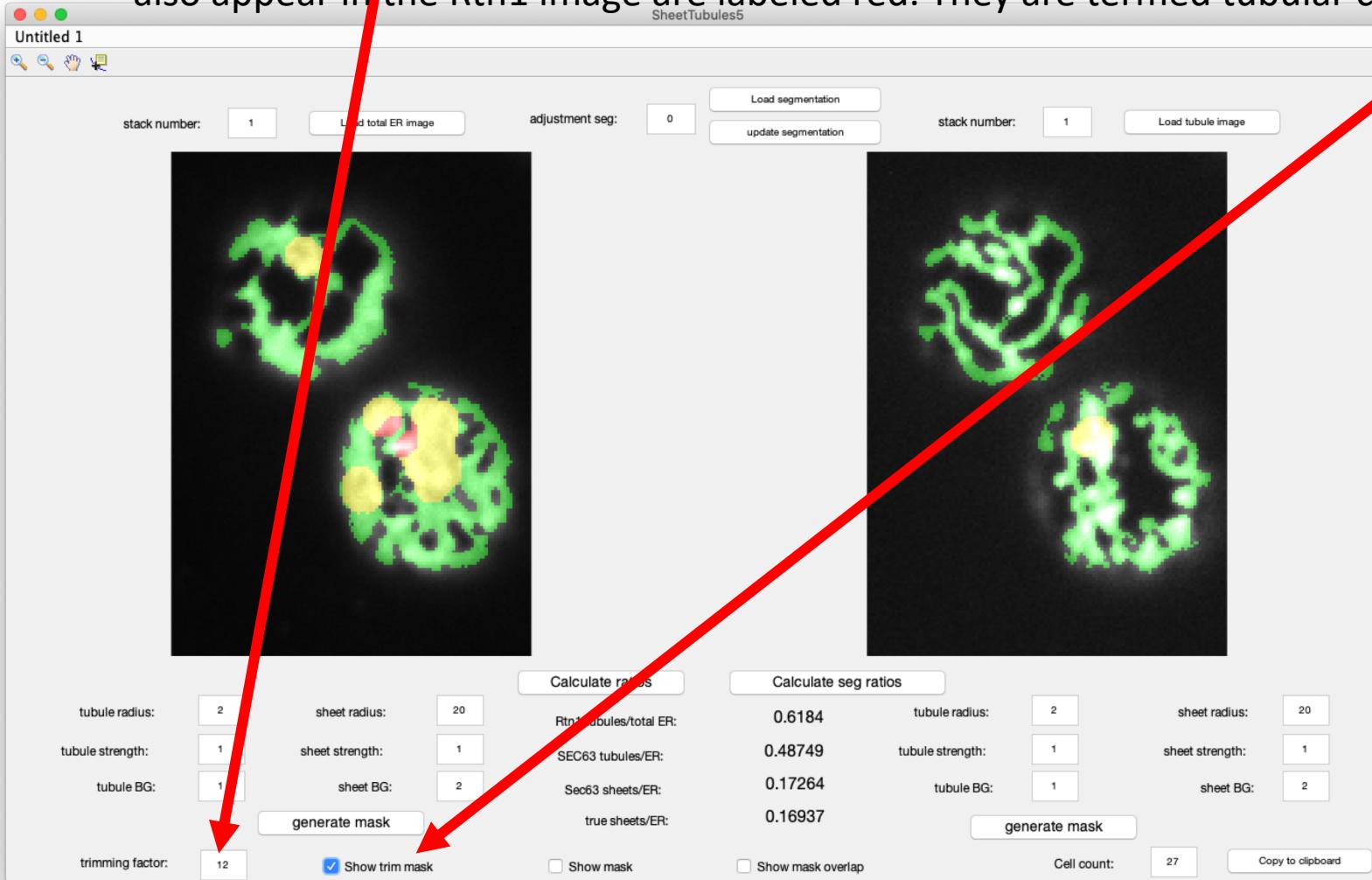


Rtn1 tubules/ER: is the tubules identified in the Rtn1 image divided by the total ER (Sec63) mask.

Sec63 tubules/ER: is the tubules identified in the Sec63 image divided by the total ER (Sec63) mask.

Calculating tubule and sheet ratios:

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 and can be fine tuned using the “trimming factor” and re-calculating the ratios. The Sec63 sheets that also appear in the Rtn1 image are labeled red. They are termed tubular clusters.

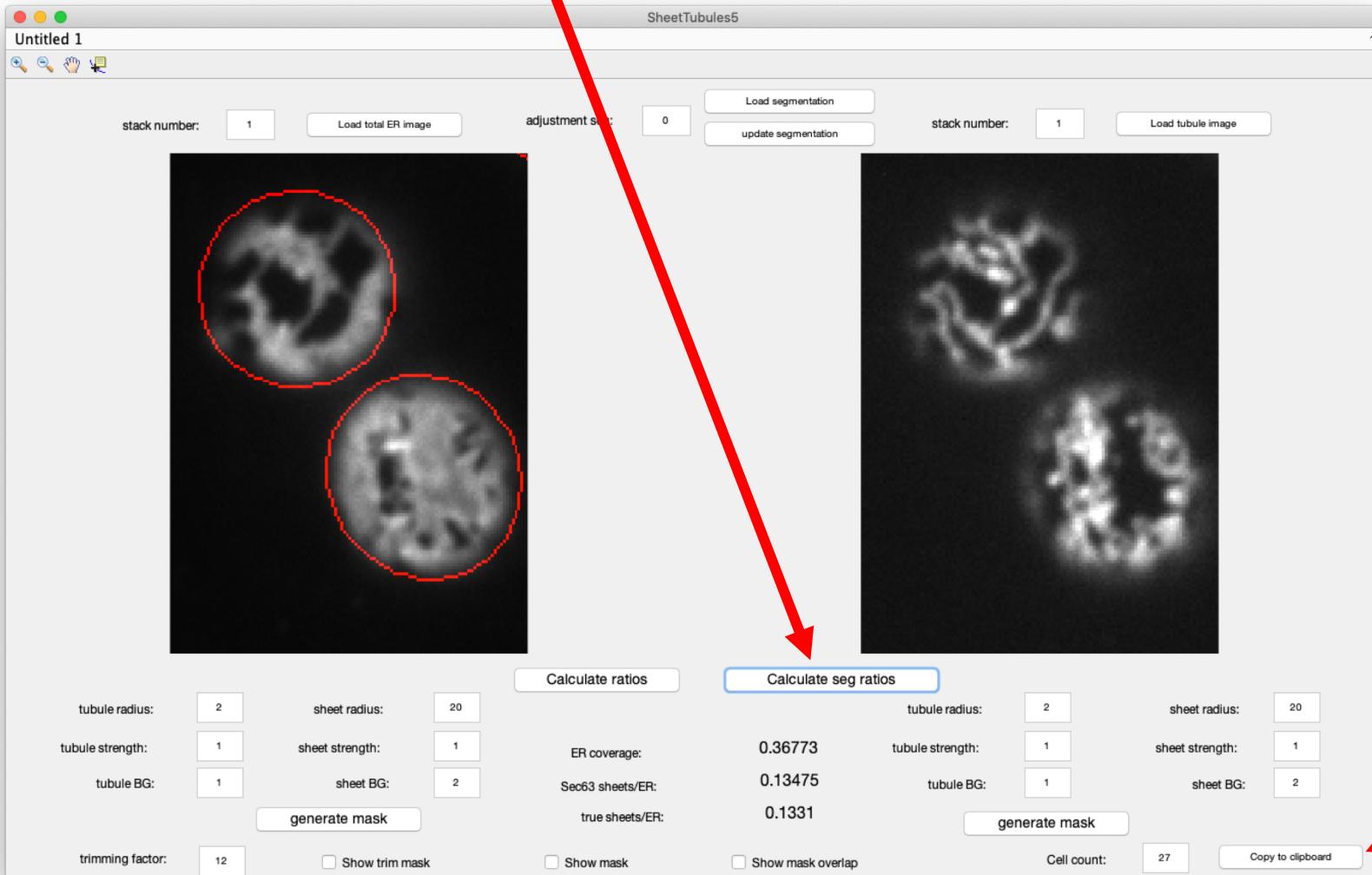


Sec63 sheets/ER: is the total sheets identified in the Sec63 image, divided by the total ER (Sec63) mask.

true sheets/ER: is the Sec63 sheets that are not sheet-like in the Rtn1 image (Sec63 sheets minus tubular clusters), divided by the total ER (Sec63) mask.

Calculating segmented tubule and sheet 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.

Sec63 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.

Pressing the “copy to clipboard” button copies all given parameters and also the calculated ratios (or segmented ratios, if that button is pressed) to the clipboard.