

# Problem with masking

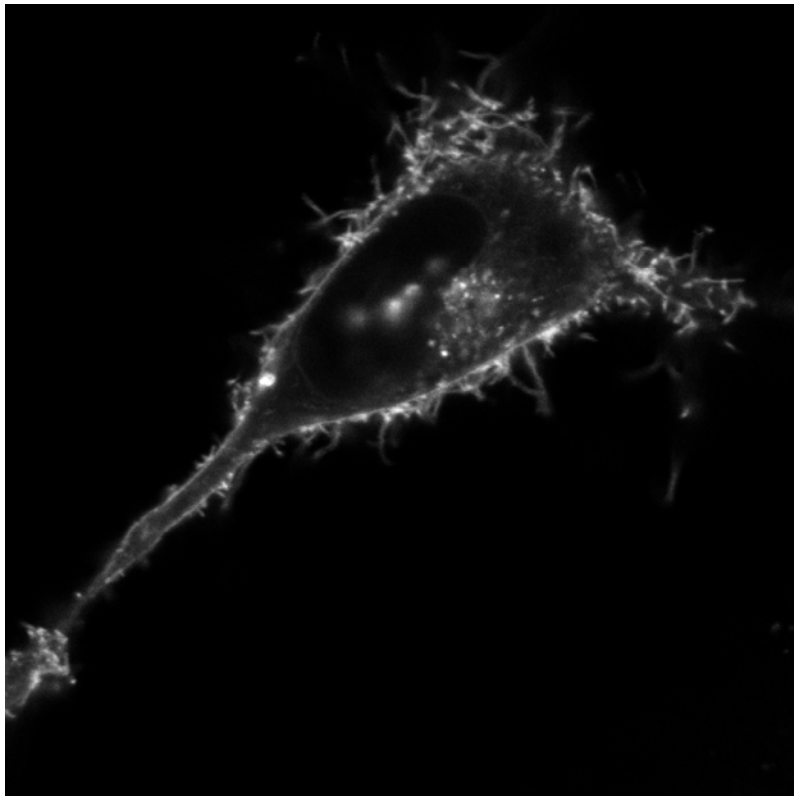
8/19/2016

# Masking Process

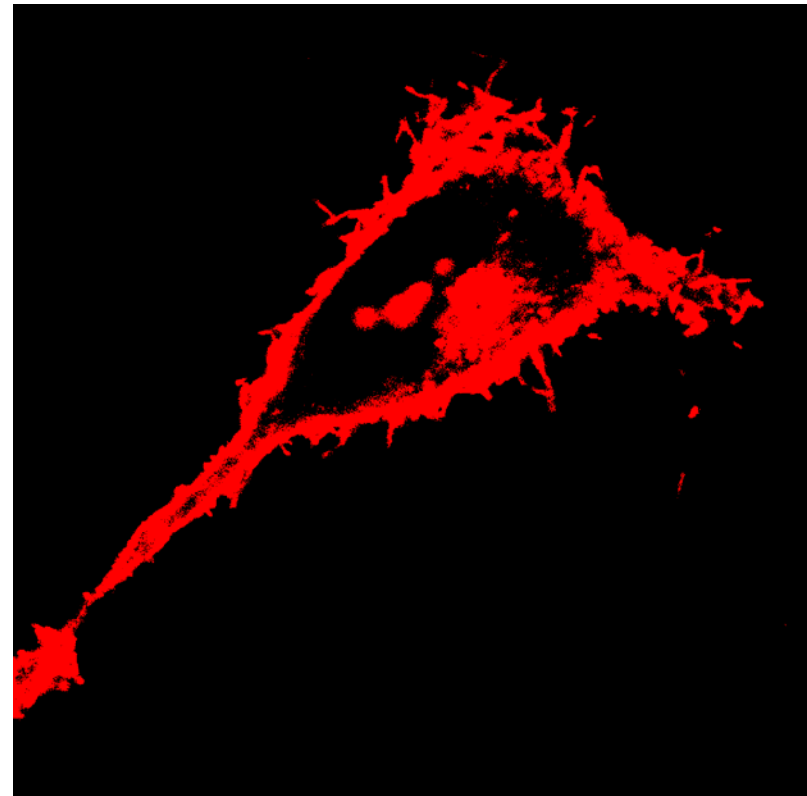
1. Find cell based on intensity and create “FullCellMask”
2. Fill cell mask with dilation and erosion followed by fill holes to create “ClosedMask”
3. Use distance transform to create “ThickMembrane” mask
4. “Membrane mask” is made by multiplying the ThickMembrane mask with the FullCellMask
5. The Membrane mask is applied to the original image to get intensity values above threshold from within the Membrane mask to create the “FinalMembrane” mask

1. Find cell based on intensity and create “full cell mask”

Original Image

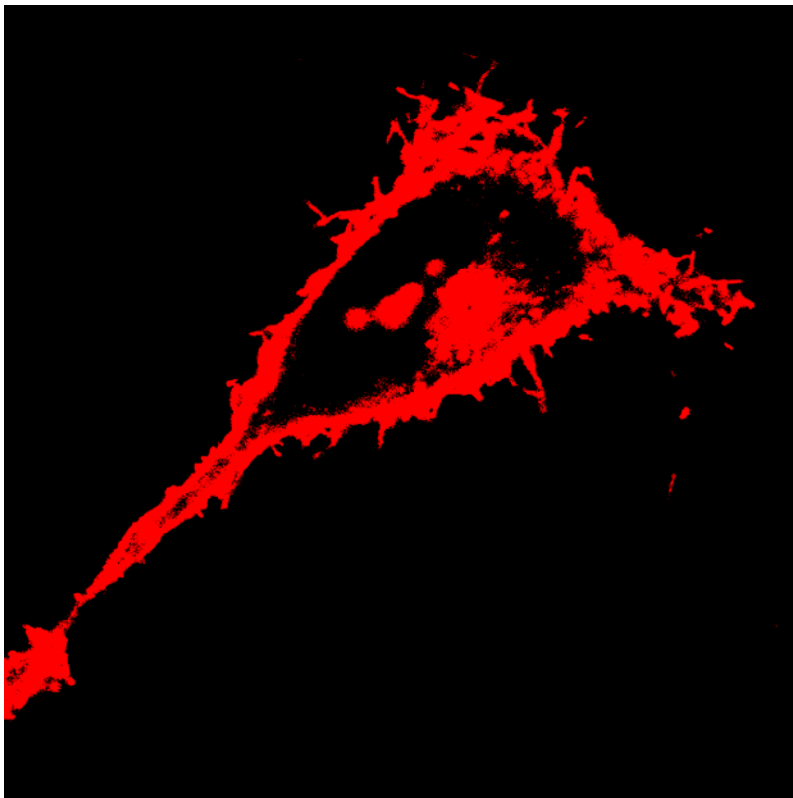


Full Cell Mask

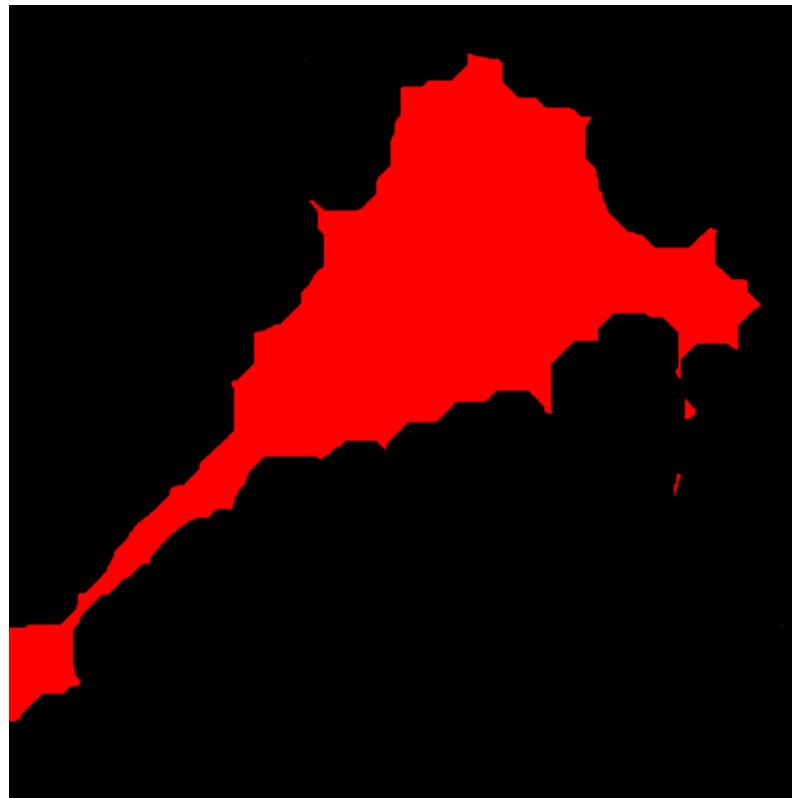


## 2. Fill cell mask with dilation and erosion followed by fill holes to create “closed mask”

Full Cell Mask



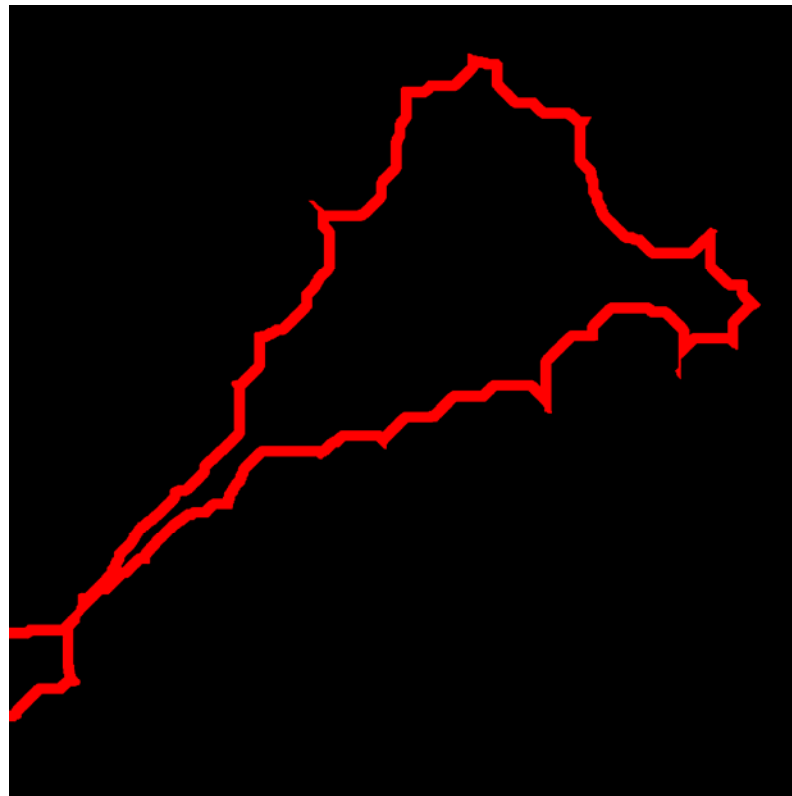
Closed Mask



You can see that the outline of the cell in the closed mask is now very crude compared to the full cell mask after the dilation and erosion, which is used to help fill in the small holes. Maybe there is a better way to fill in the Full Cell mask?

3. Use distance transform to create “ThickMembrane” mask

Thick Membrane Mask



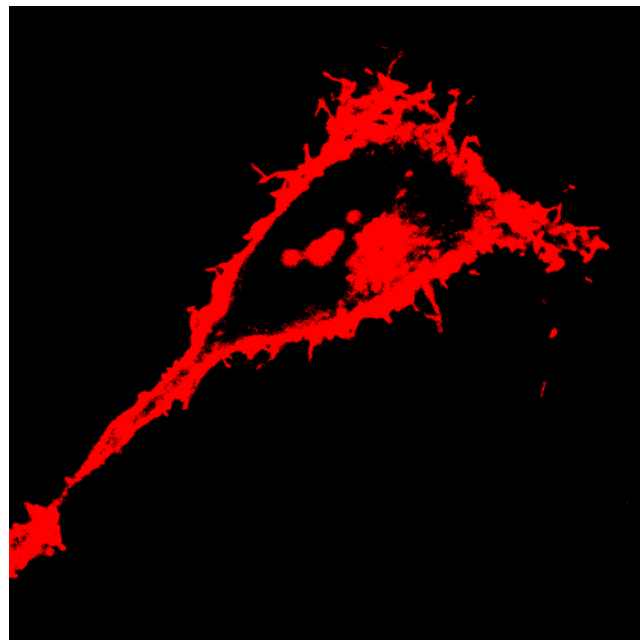
This mask is defined as 15 pixels thick

4. “Membrane mask” is made by multiplying the FullCellMask with the ThickMembrane mask

Full Cell Mask

Thick Membrane Mask

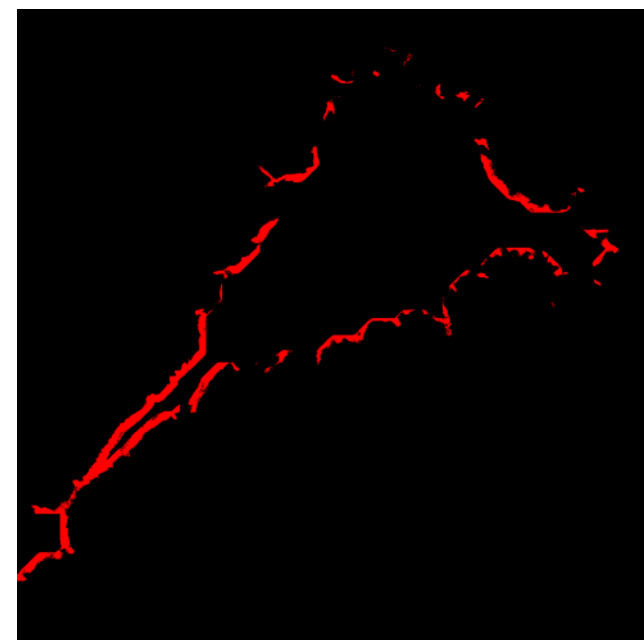
Membrane Mask



X

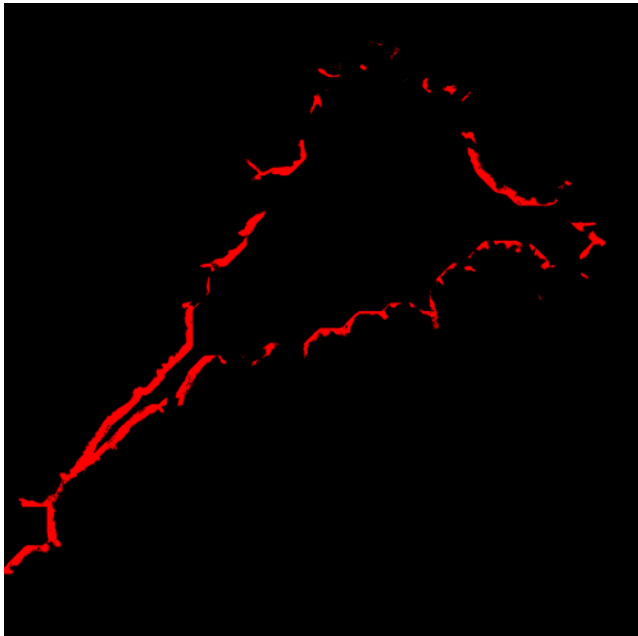


=

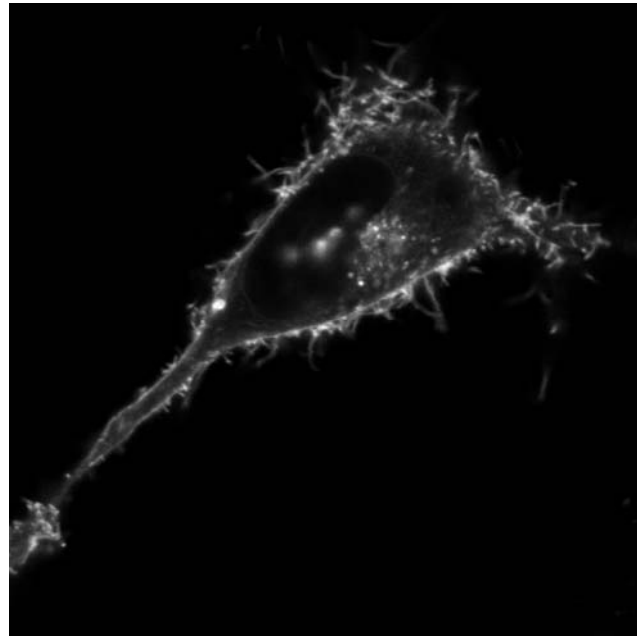


5. The Membrane Mask is applied to the original image to get intensity values above threshold from within the Membrane Mask to create the Final Membrane image

Membrane Mask



Original Image



Final Membrane Image



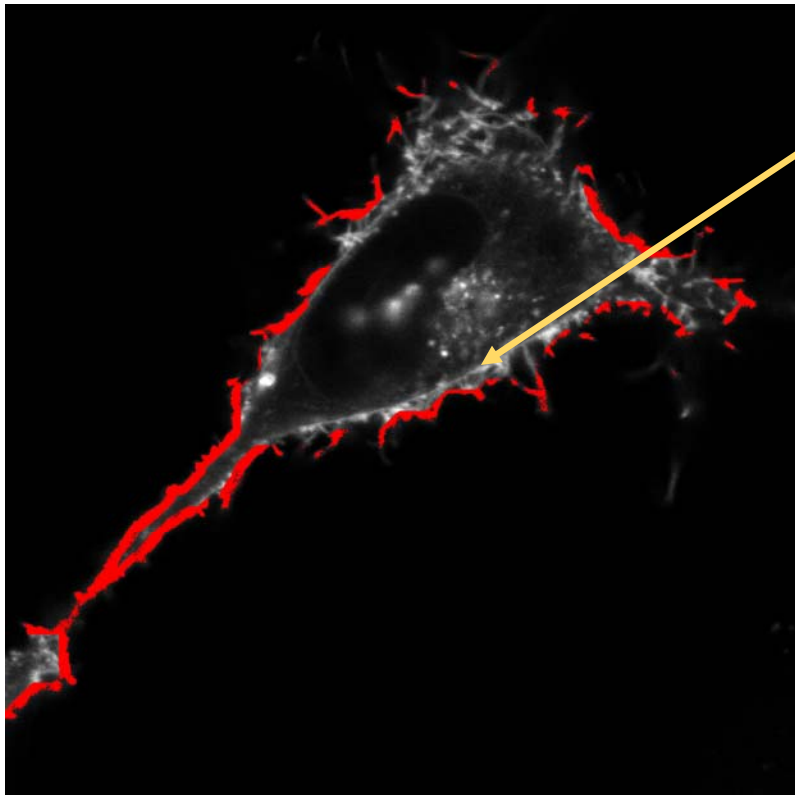
The Final Membrane image is what we have been using to get mean intensity of the cell membrane

Final Membrane Image





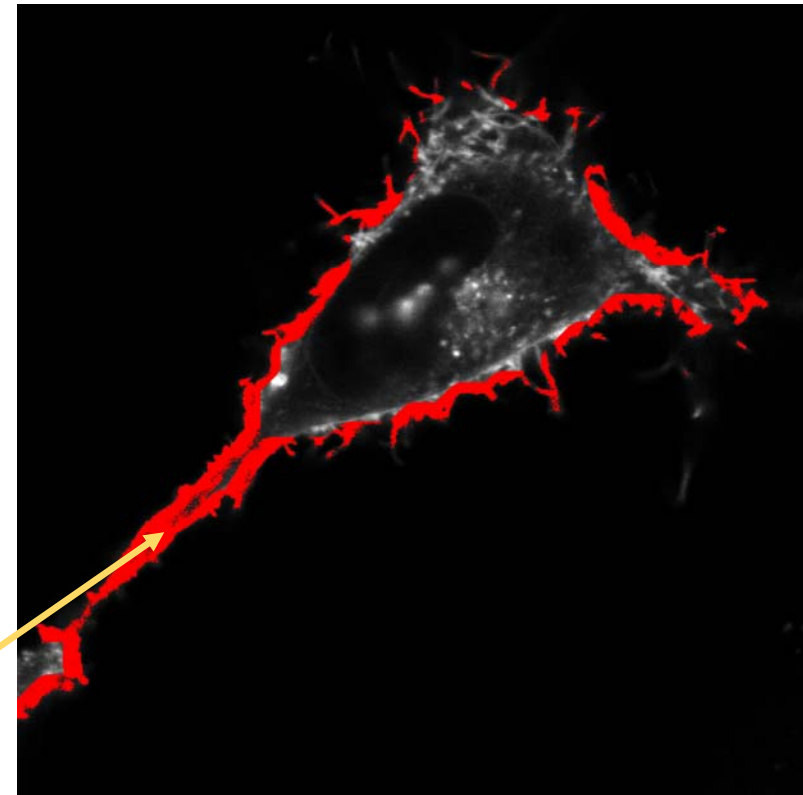
# Assessment of the Membrane Mask using an overlay with the original image



15 pixel mask thickness

You can see that a lot of the cell membrane is not getting included for analysis

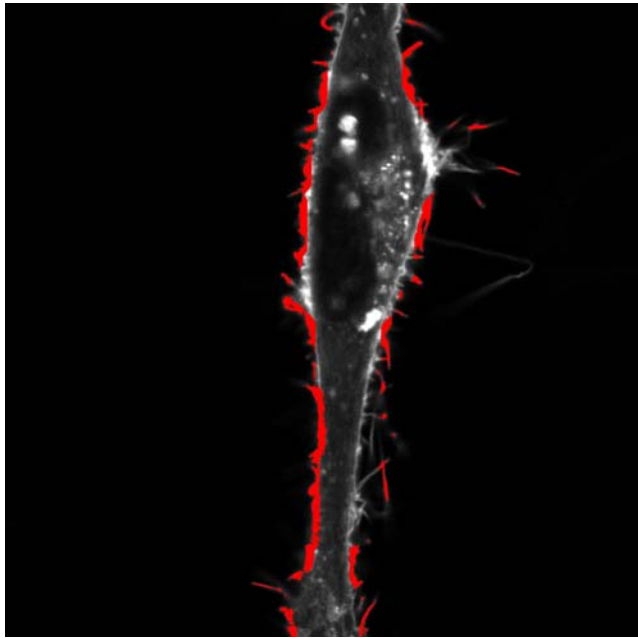
We have tried varying the "ThickMembrane" mask thickness but in this image, for example, increasing the thickness results in including intensity from the cytoplasm



25 pixel mask thickness

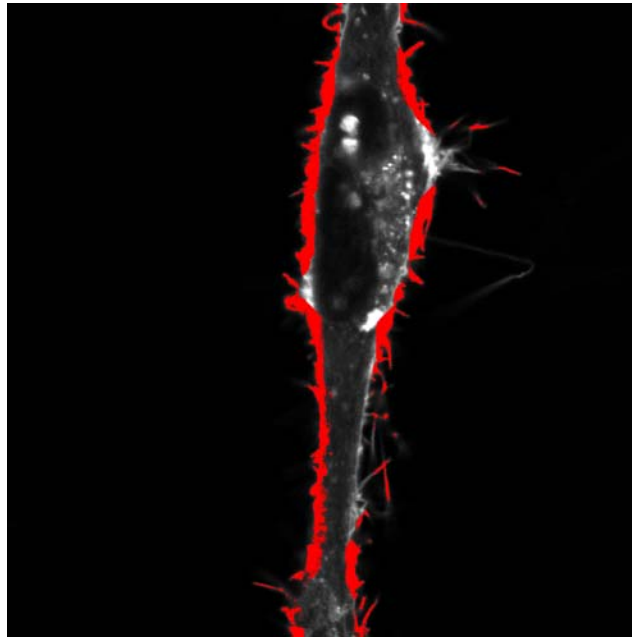
# Using different ThickMembrane mask thicknesses for different images

15 pixel mask thickness



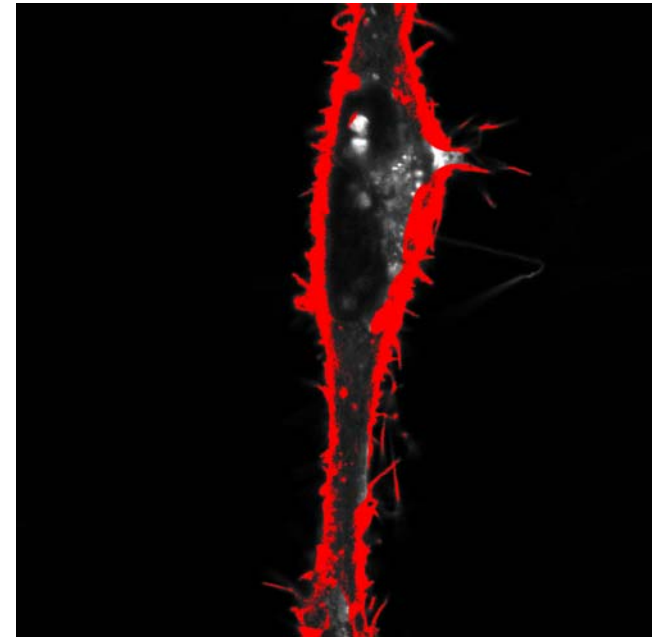
This is the mask I would use for this image

25 pixel mask thickness



Including too much

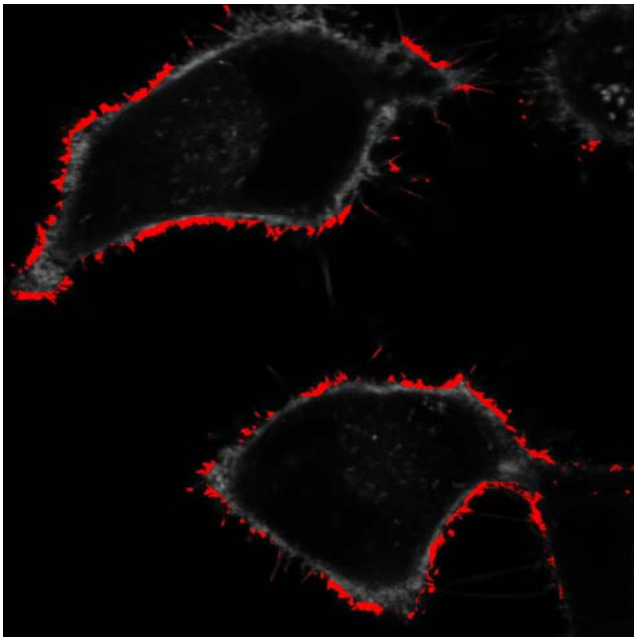
50 pixel mask thickness



Including way too much

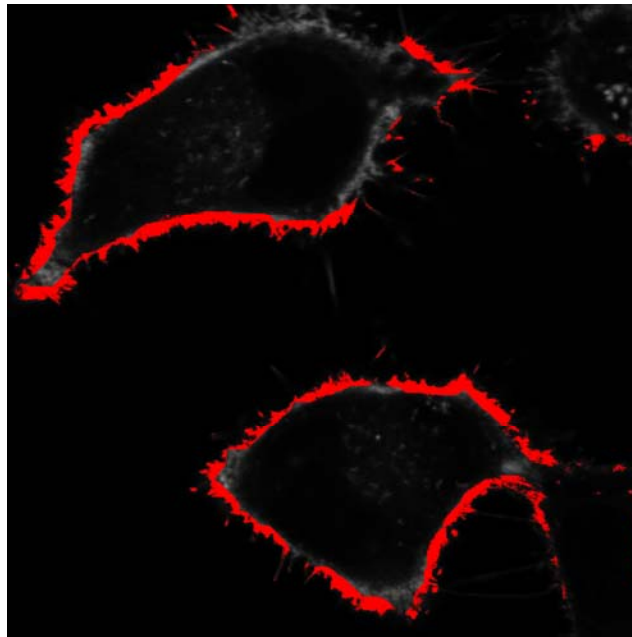
# Using different ThickMembrane mask thicknesses for different images

15 pixel mask thickness



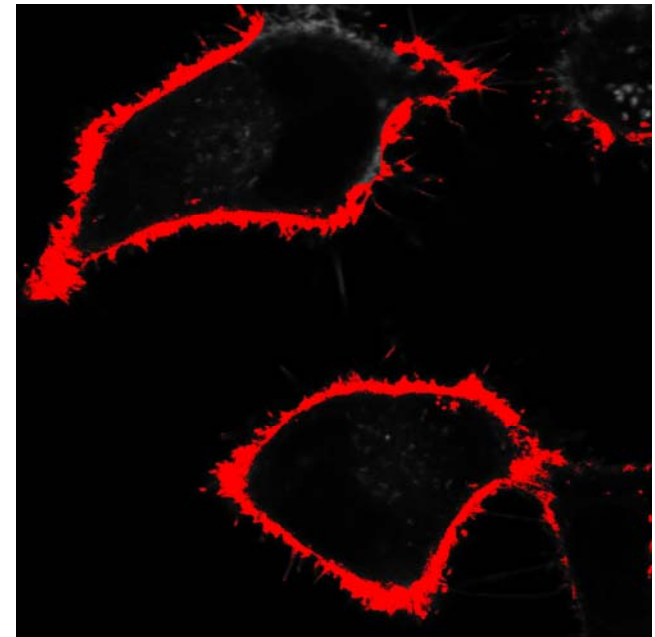
Not including enough

25 pixel mask thickness



This is the mask I would use for this image

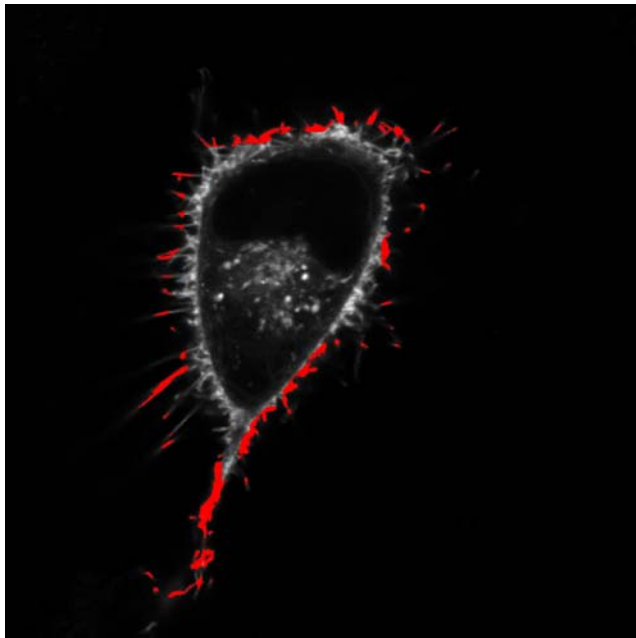
50 pixel mask thickness



Including too much

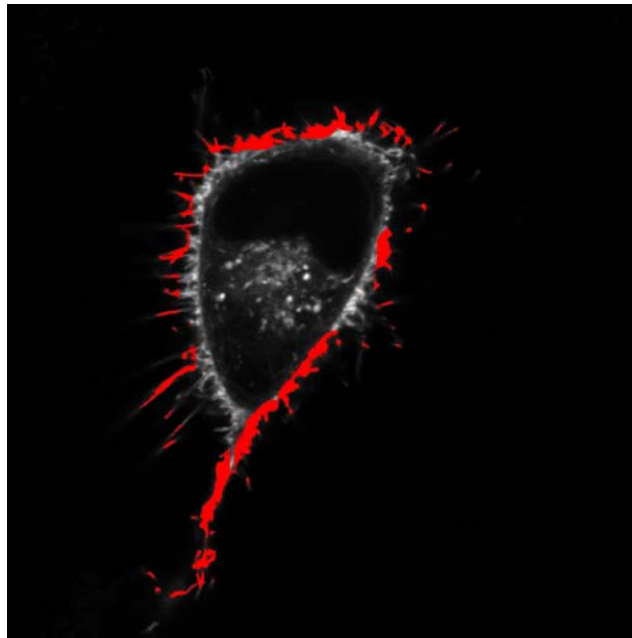
# Using different ThickMembrane mask thicknesses for different images

15 pixel mask thickness



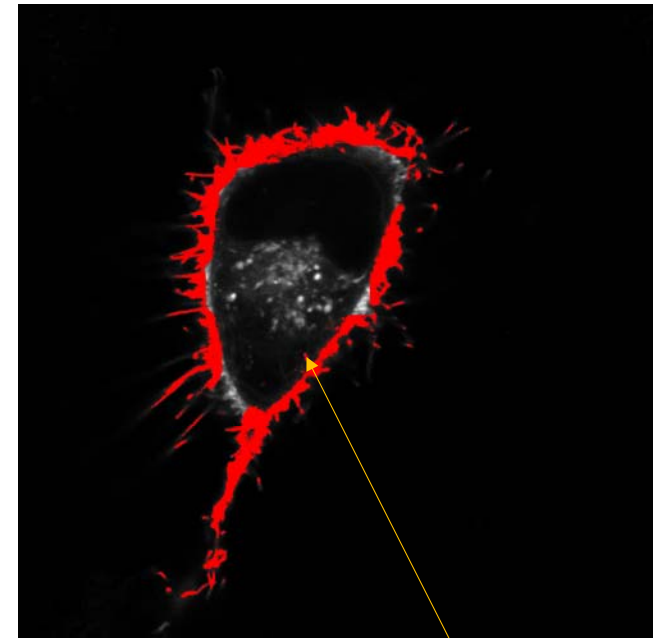
Not including enough

25 pixel mask thickness



Not including enough

50 pixel mask thickness



This is the mask I would use for this image even though it is including some cytoplasm

# Problems

- Filopodia and lamellipodia from cells makes thickness of the membrane and intensity quite variable – hard to use a single mask thickness for all images
- Work around – run every image through 3 masks (15, 25, and 50 pixels), inspect each image and choose the best option. Even within a single cell the membrane thickness can vary due to extensions so a mask that could account for that would be ideal.
- The current process is time consuming and most cells only have 25% to 50% of membrane masked, otherwise the cytoplasm becomes included in the mask.
- \*\*\* We are trying to mask the membrane to be used for FRET efficiency calculations. Only donor fluorescence at the membrane will actually FRET so it is better to miss some membrane than to include some cytosolic intensity but we want to include as much of the membrane as possible.