Gradient Segmentation

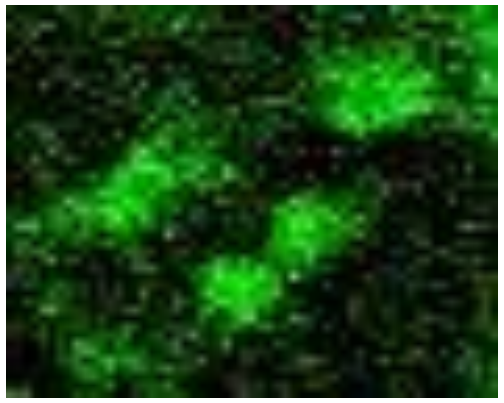
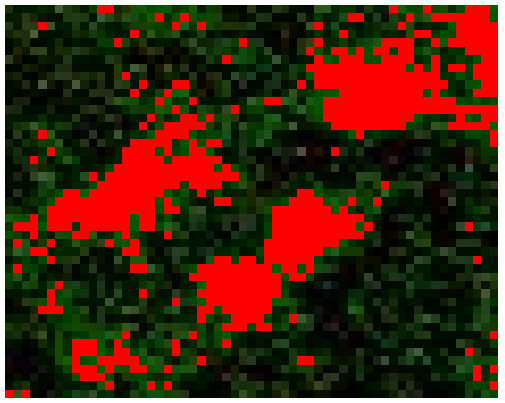
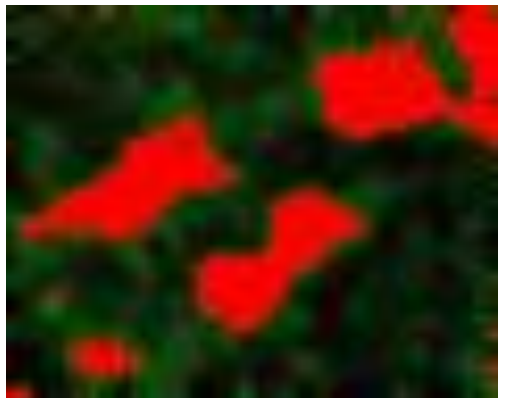
Segmentation

- To quantify cell shape and motion, cells must be identified in 3D and then tracked over time
  - Velocity
  - Distance
  - Dendrite Extension
  - Cell-Cell interacions

- Many programs exist to do this, with varying levels of success

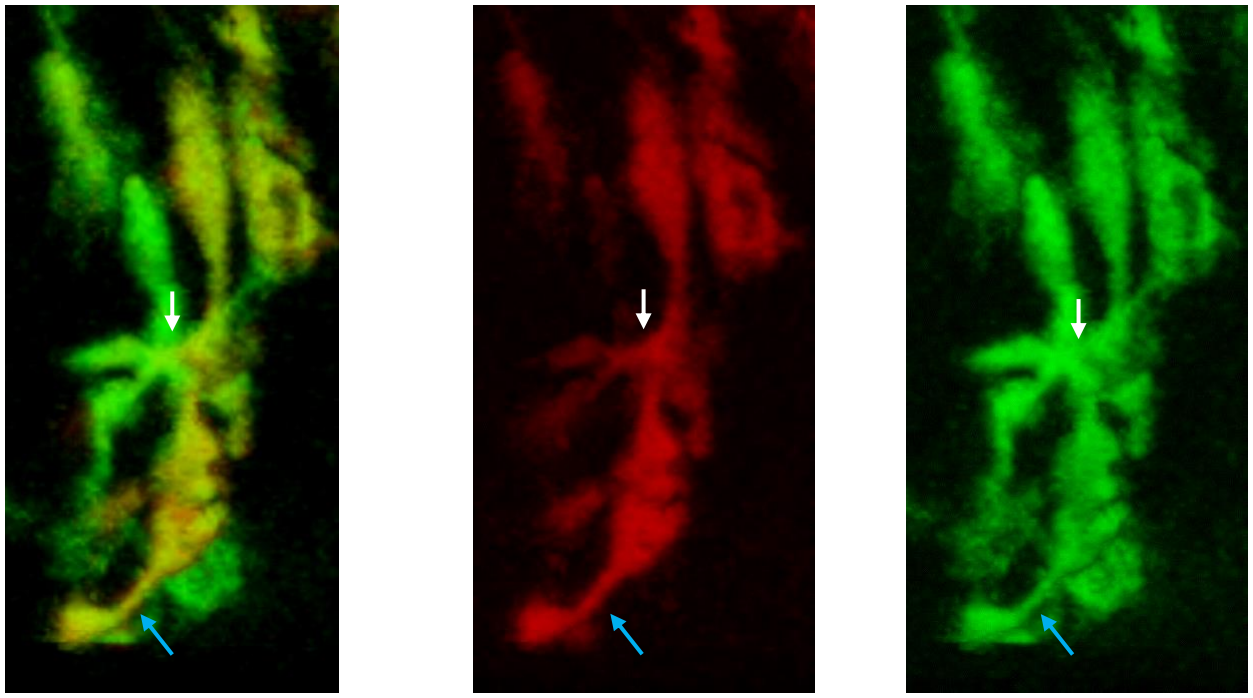
Imaris Algorithm

- On 1 channel, set a threshold separating foreground and background
- Smooth off corners, filter small objects
- Watershed transform to separate touching objects
  - Relies on indentation points

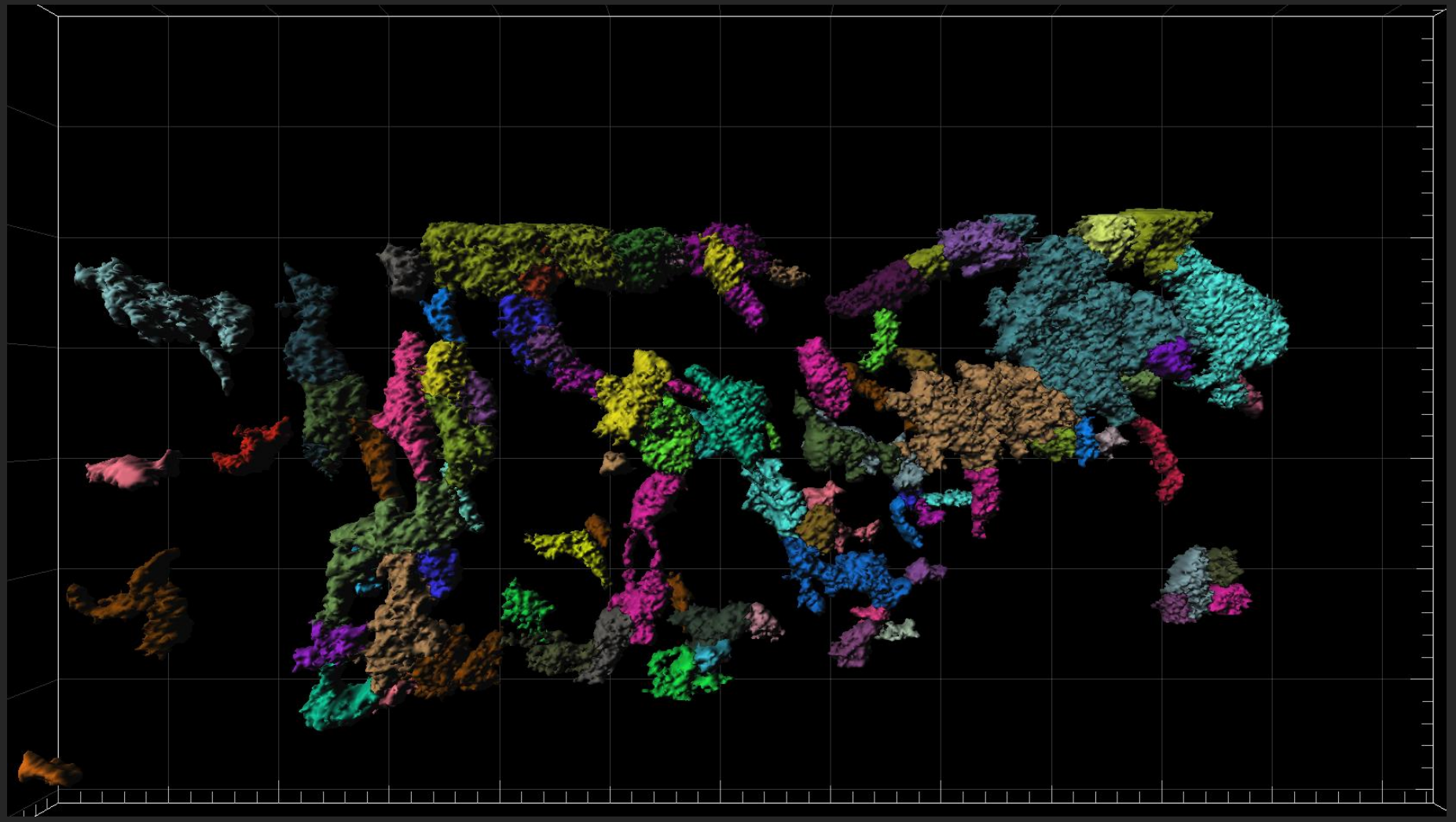

Problems with Imaris Segmentation

- Assumes spherical cells that are slightly touching
- Assumes sharp change in intensity on cell boundaries

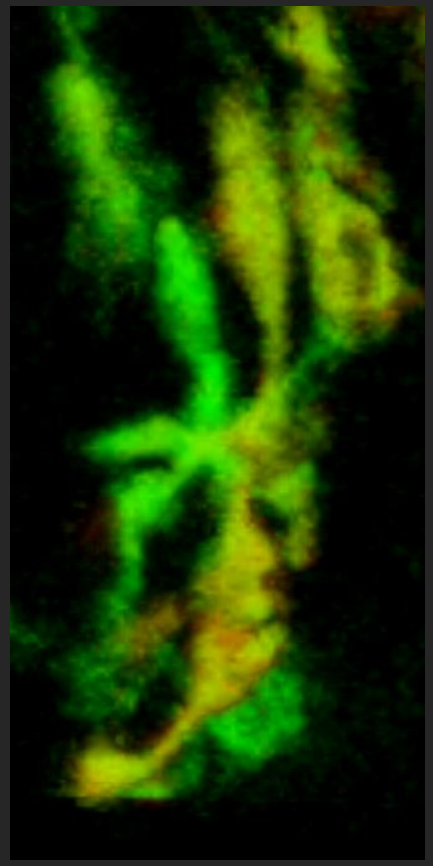


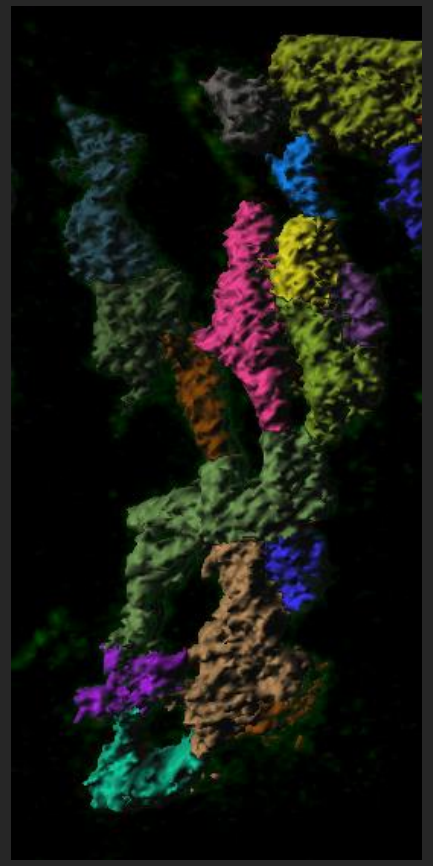
- Aortic Macrophages:
  - Low resolution
    - 0-1 pixels between cells
  - Many true double positives, with small difference in ratio of fluorophores
    - Thresholding on GFP or YFP will always pick up both cells
    - Using GFP/YFP or YFP/GFP ratio is very noisy and condenses all double positives into a small range
  - Dendritic-shaped with indentation points that do not correlate to cell boundaries

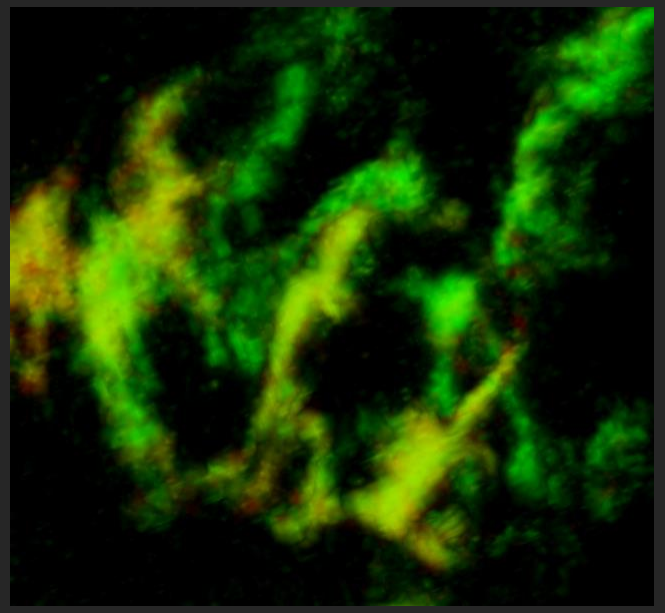
Imaris Segmentation- Giant Errors

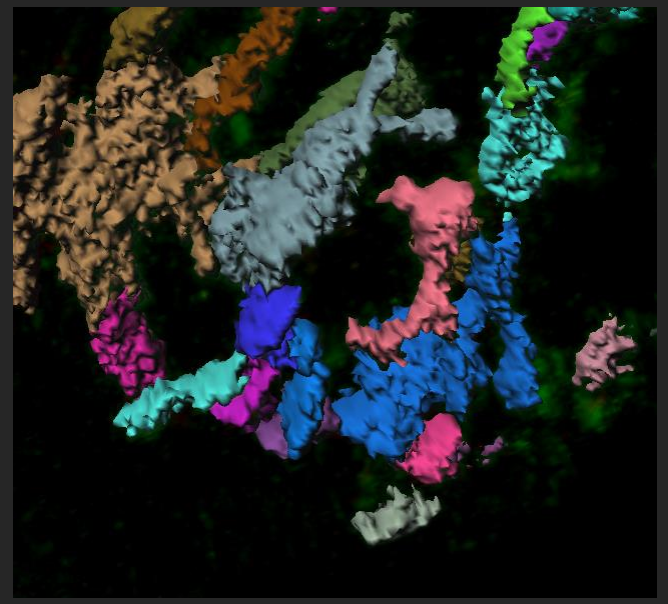


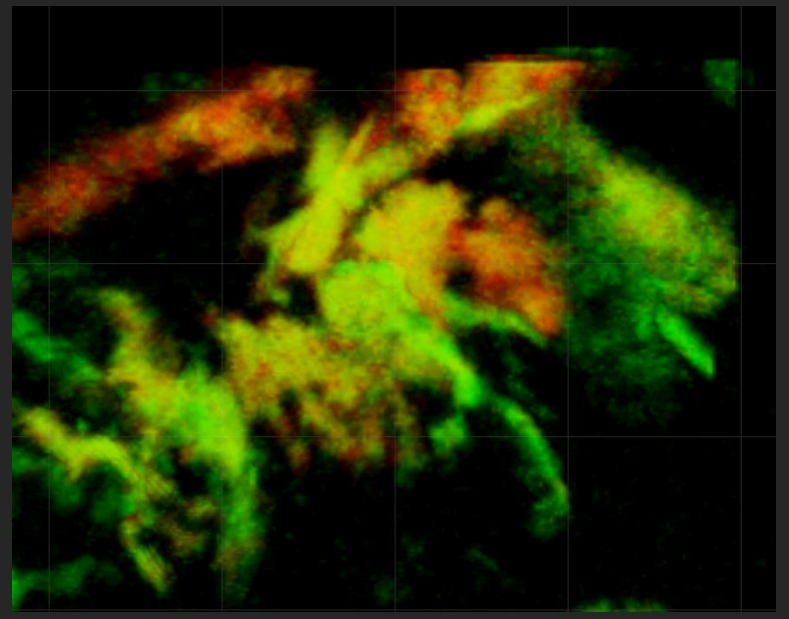
Errors

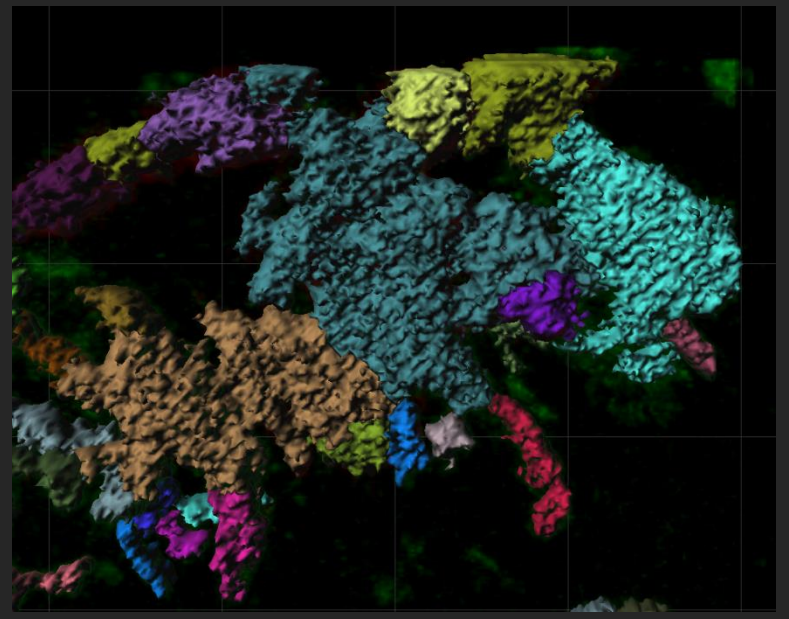












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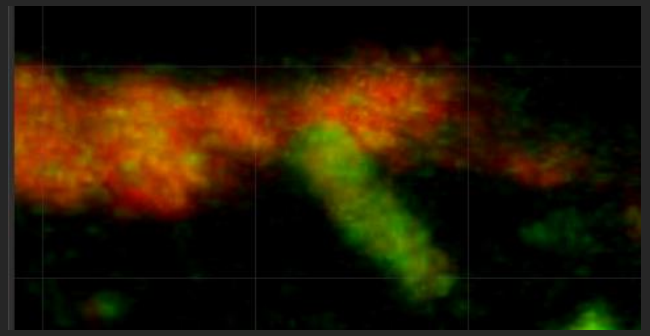

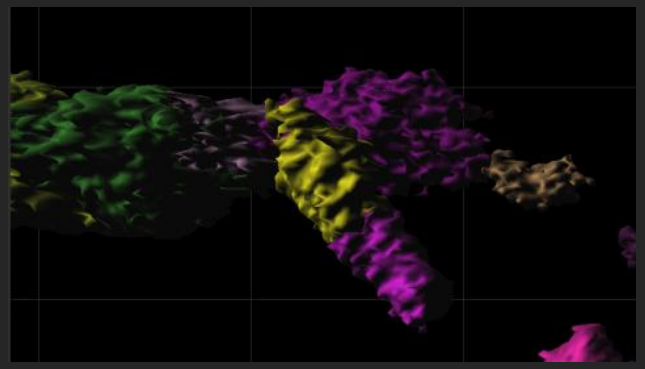

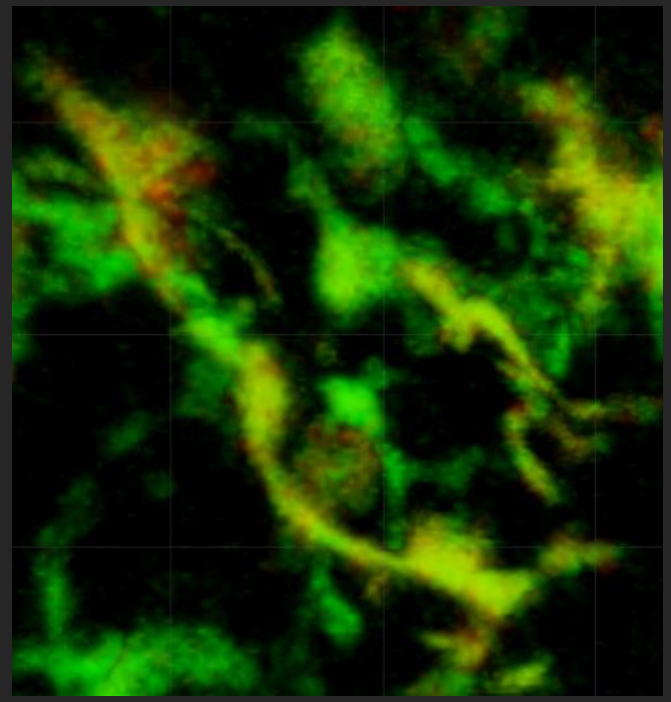

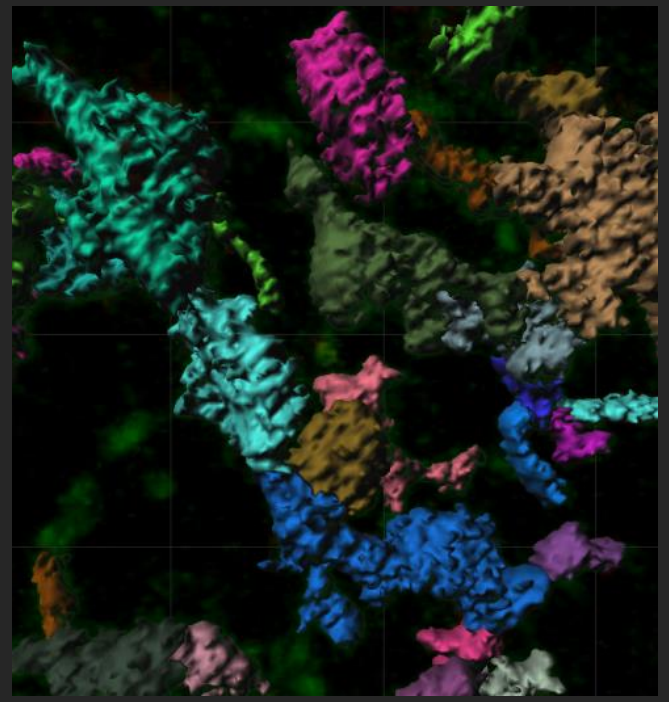

Macrophage Segmentation

- Often the boundary between cells isn't defined by a sharp change in GFP or YFP intensity, but a change in the apparent color

- Need a segmentation algorithm that:
  - Uses both GFP and YFP information simultaneously (NOT the ratio)
  - Blind to shape

Dynamic Color Gradient Segmentation

Unsupervised color image segmentation using a dynamic color gradient thresholding algorithm

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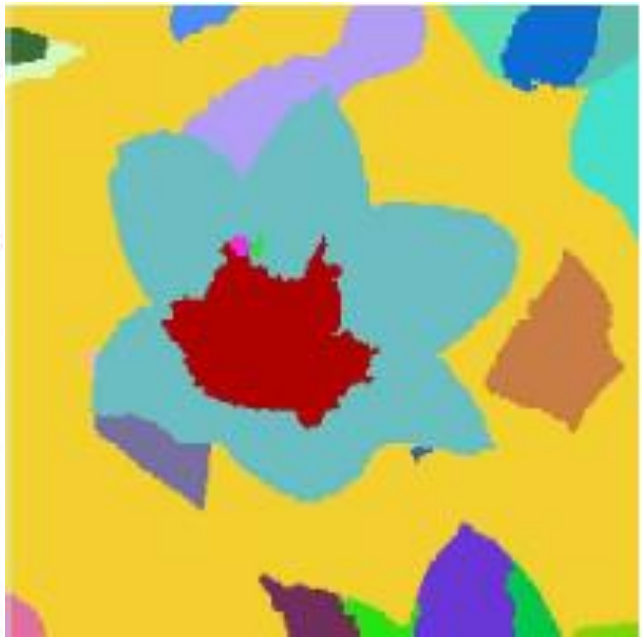
Human Vision and Electronic Imaging XIII, edited by Bernice E. Rogowitz, Thrasyvoulos N. Pappas,
Proc. of SPIE-IS&T Electronic Imaging, SPIE Vol. 6806, 68061H, © 2008 SPIE-IS&T · 0277-786X/08/$18

Original

Segmented

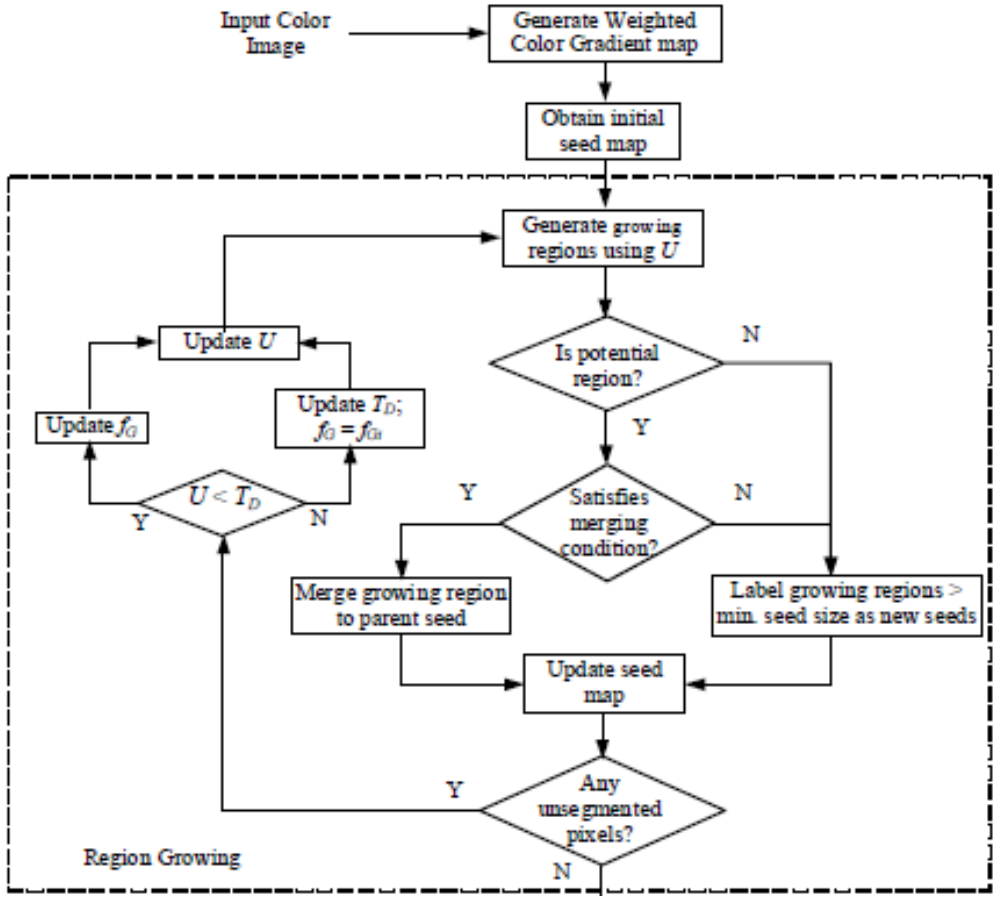


Dynamic Color Gradient Segmentation

- Uses multiple color gradients simultaneously
- Includes “texture” (measure of variance within a cell)
  - Dim cells have more “texture” than bright cells

- My adaptations:
  - 3D instead of 2D
  - Does not process background pixels to greatly increase efficiency
  - Multiple rounds of processing to better incorporate known facts about cells

Algorithm Overview



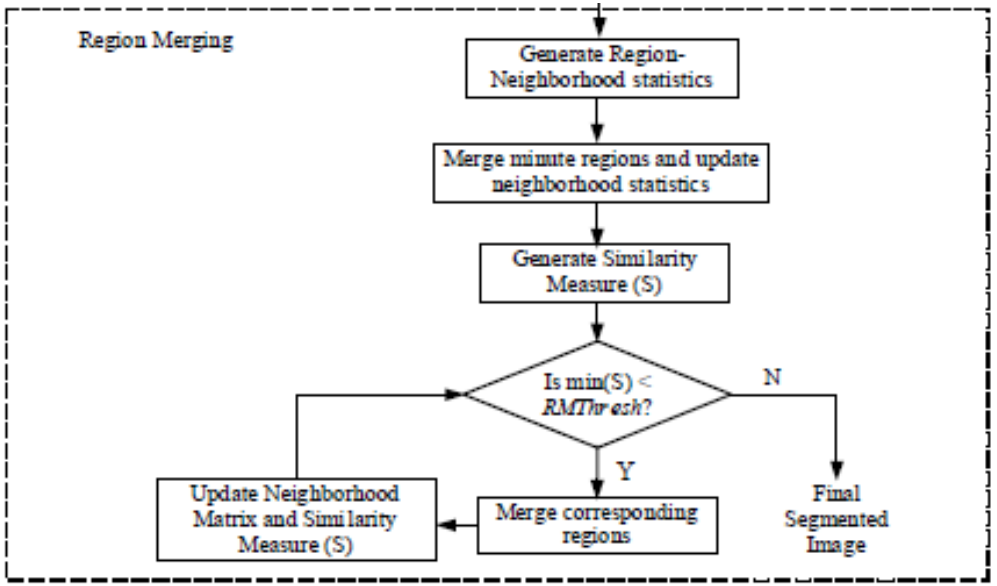
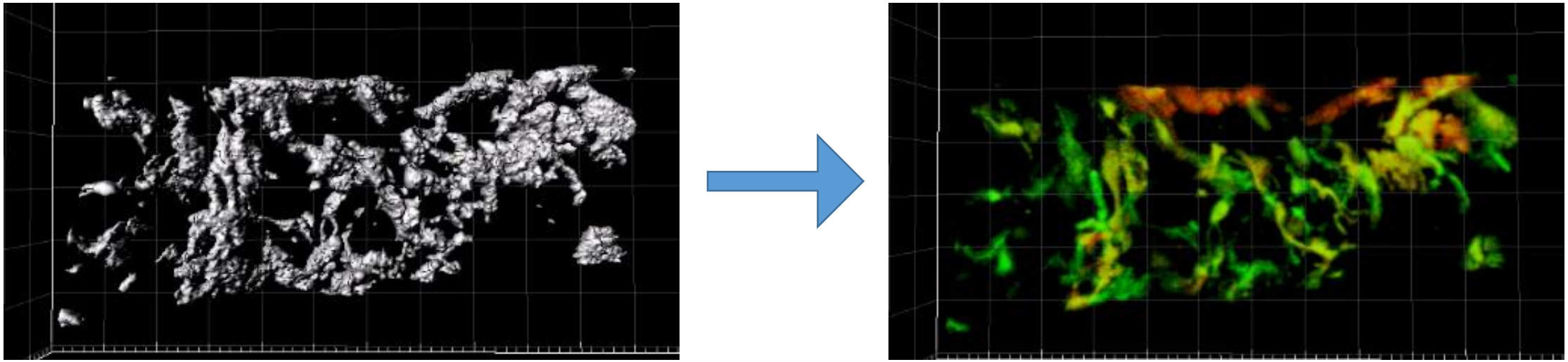


Fig. 2. Flowchart of the proposed algorithm.

1- Remove Background

- In Imaris, make surfaces over GFP+YFP channel to cover cell volume
- Mask those pixels and force all background pixels to be 0
- Import the cleaned GFP and YFP data into Matlab

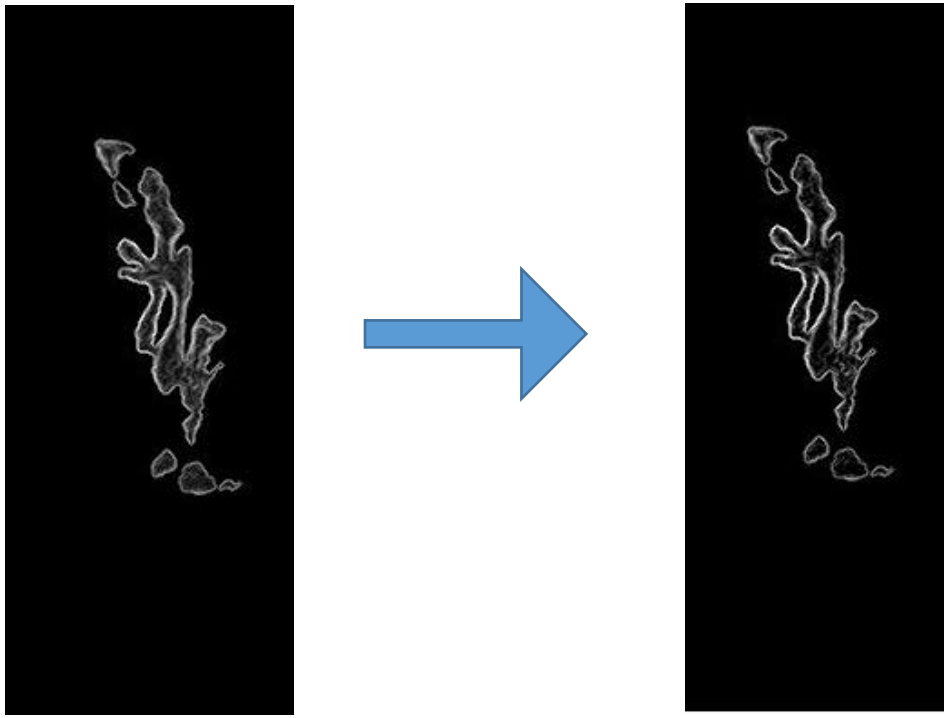


2- Calculate 2D dual color gradient maps

- For each pixel calculate the total gradient in both colors in 2D
- Set all background pixels to 0 without calculating

3- Enhance the Gradient Map

- Using a combination of high, low, and middle thresholds, increase the contrast on the gradient map
  - Some are set automatically, some are set manually and will need to be tweaked

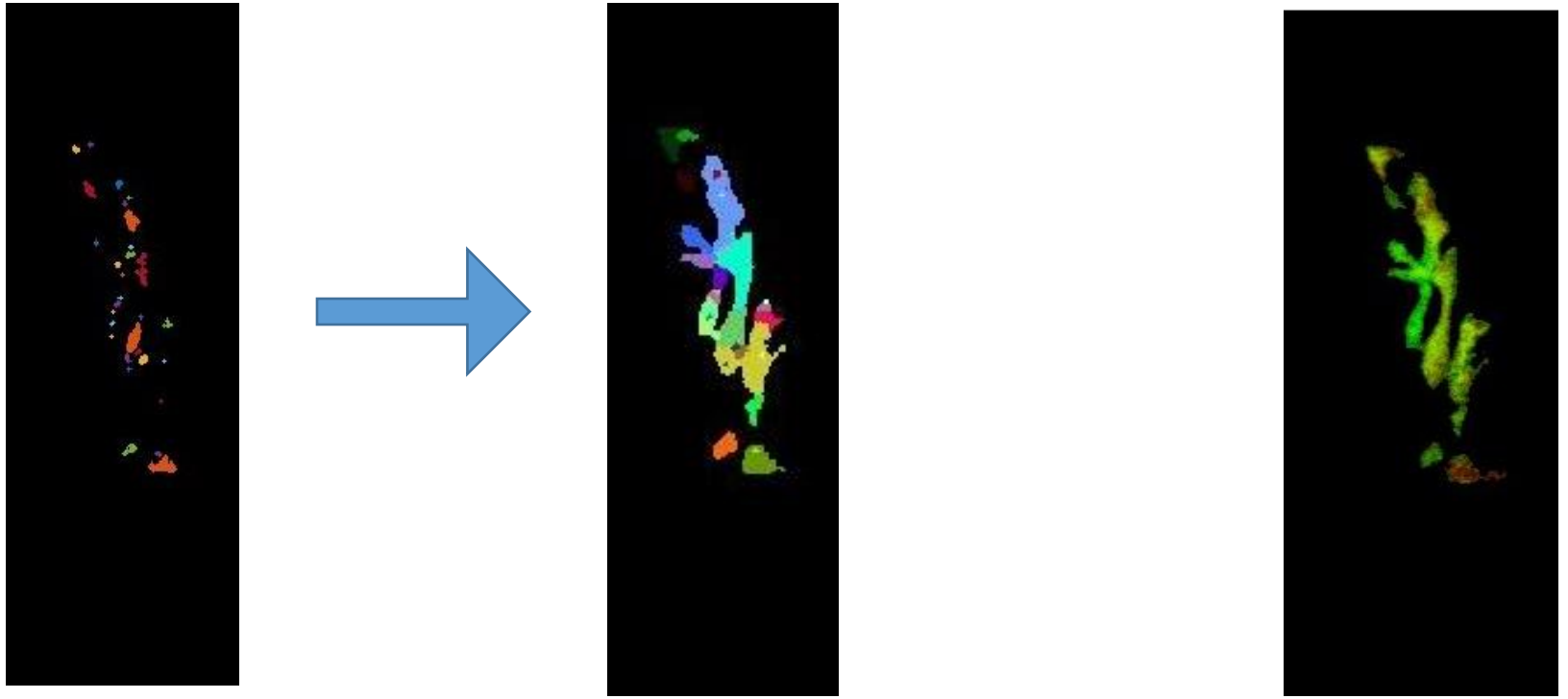


4- Seed generation

- Find regions below a threshold on the 2D gradient map (even color in raw data)
- These are the starting seeds of region growing
  - Size and threshold can be adjusted

5- Region Growing

- Increase the max threshold on the gradient map
- For each newly selected area, decide if it should be merged with an existing region or start it's own region
  - Based on size and comparison of median intensity of GFP and YFP



6- Region Merging Round 1

- Region growing results in a highly oversegmented image (~3000 regions / timepoint in this example)
  - If neighboring cells are merged here, stop and change some variables
- **In 3D**, make a graph of all adjacent neighbor regions
- For each touching pair, compute a similarity measure:

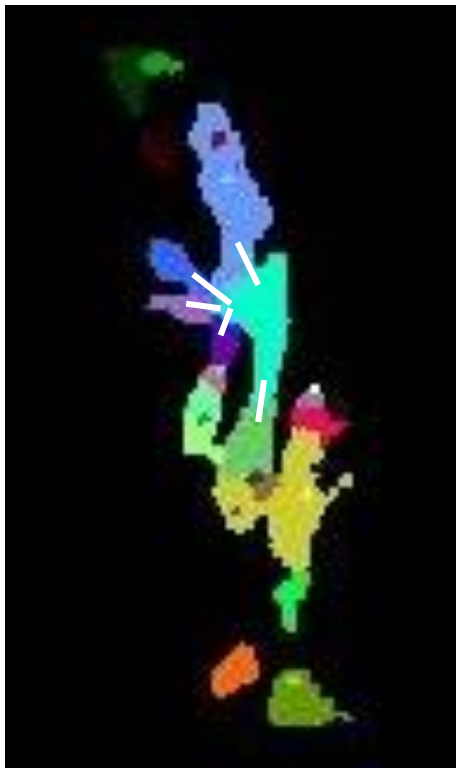
$$S_{A,B} = (\mu_A - \mu_B)^T * inv(\mathrm{cov}_A + \mathrm{cov}_B) * (\mu_A - \mu_B)$$

Difference in mean intensities in both channels

Texture, or covariance between GFP and YFP

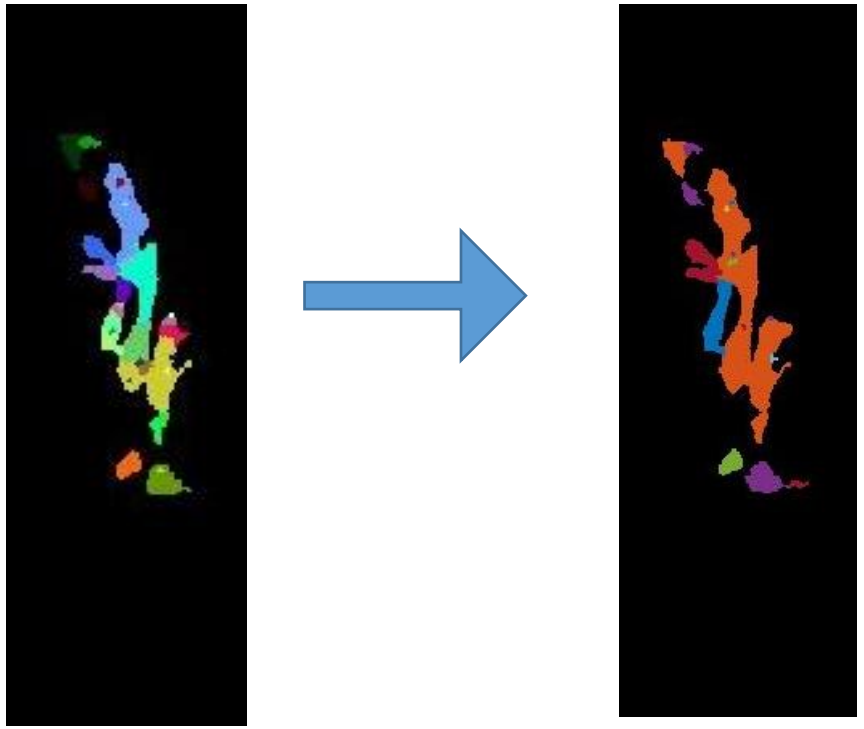
Lower means more similar

- Result is a list of similarities measurements between touching regions



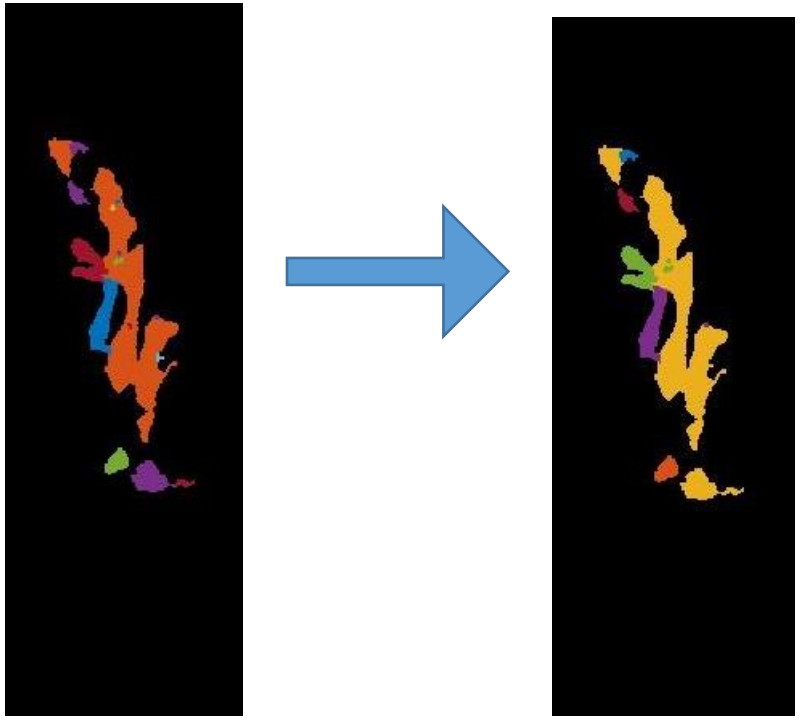
6- Region Merging

- Merge the most similar regions (balance efficiency vs accuracy)
- Iteratively repeat calculating connections graph and similarity measurements until smallest similarity measure is above a threshold
  - Most sensitive variable in the program
- Regions are now in 3D



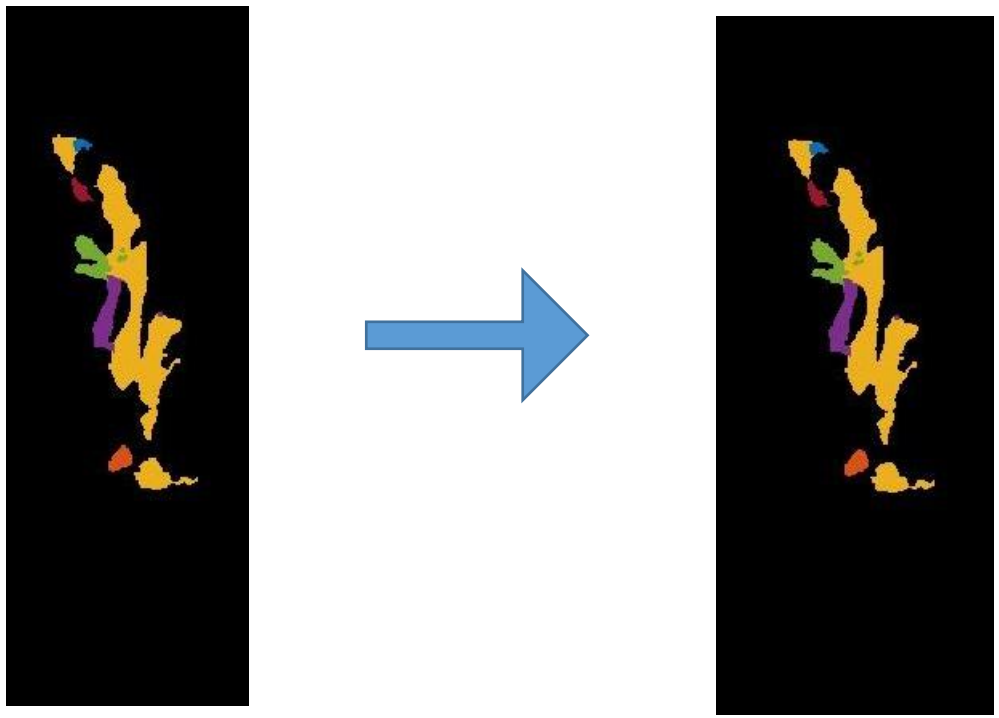
7- Smart Region Clean Up

- Forcibly merge regions to most similar touching neighbor that fail certain criteria:
  - Minimum size
  - Landlocked- Completely enclosed by one or more cells
    - Does not apply to all potential imaging, but does work for atherosclerotic macrophages



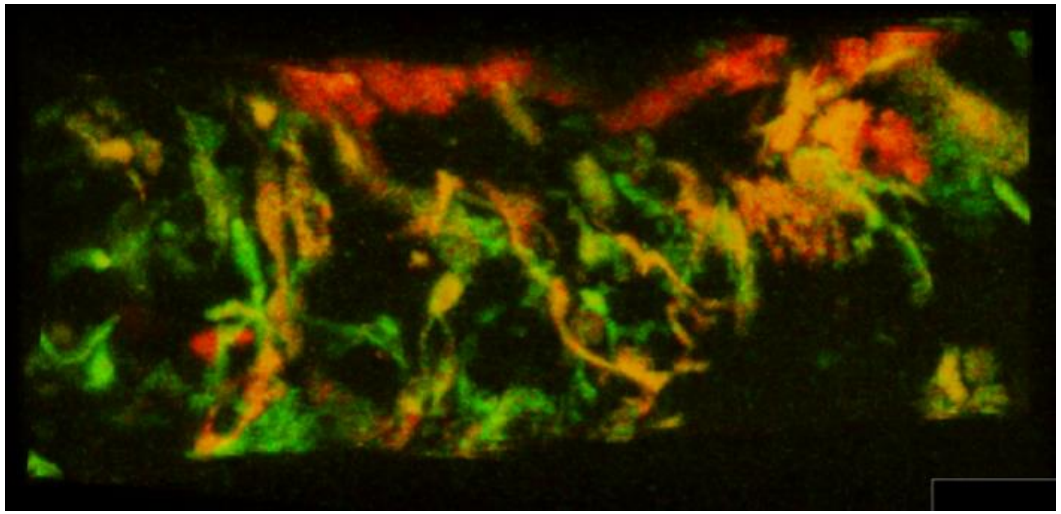
8- Region Merging Round 2

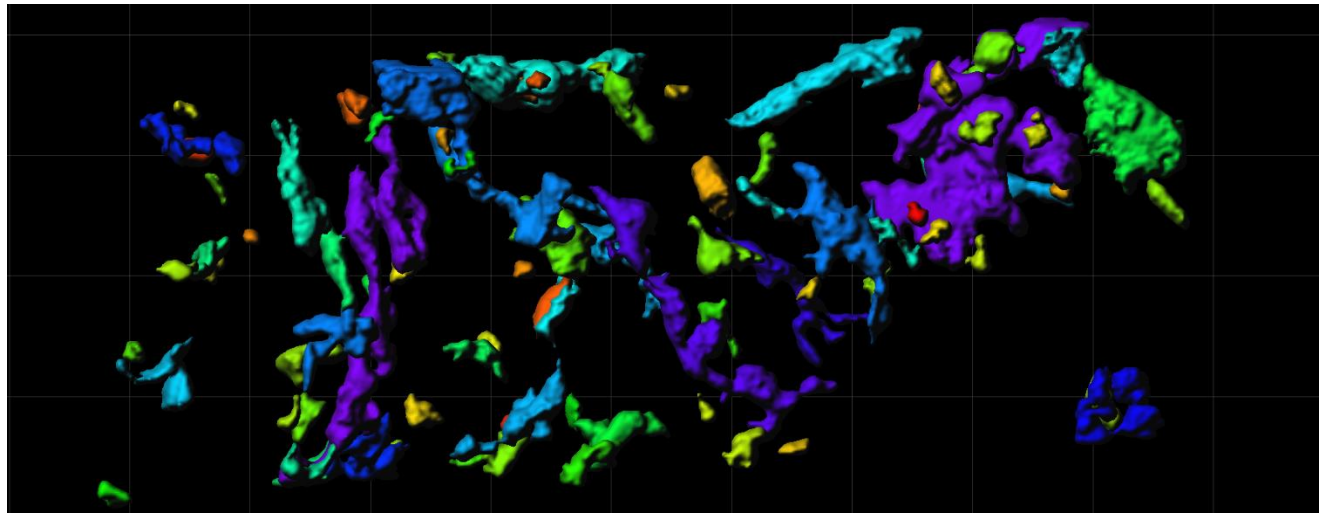
- Repeat merging procedure on “cleaned up” regions to the same threshold



9- Form Surfaces

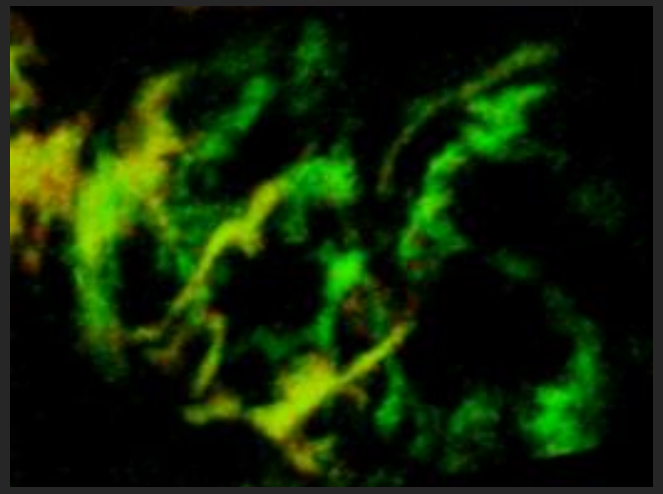
- For each identified region, import the intensity of the voxels back into Imaris, and have Imaris create a surface around it
  - Smoother than having Matlab do it



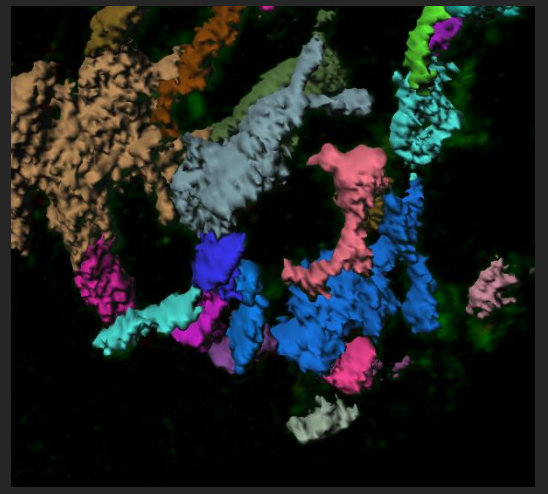


Improvements with this algorithm

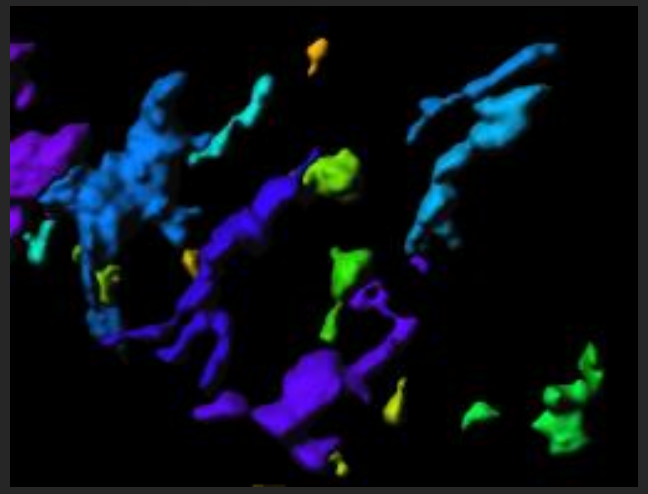
Raw



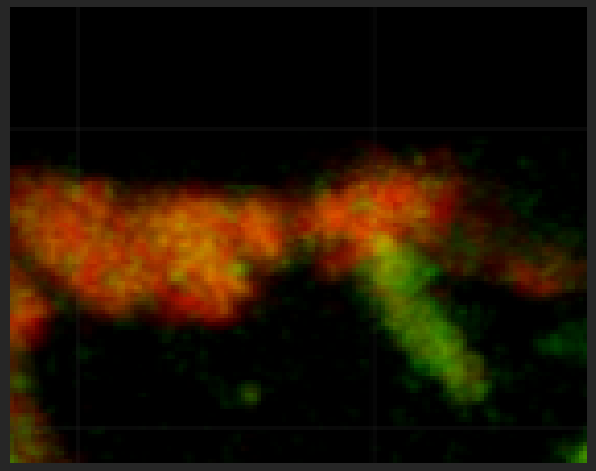
Imaris Default



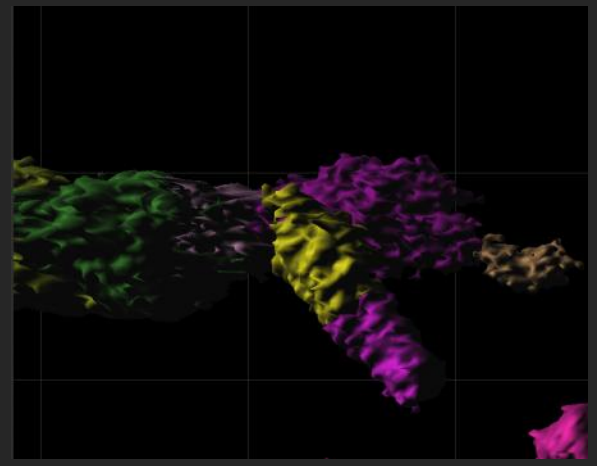
Color Gradient



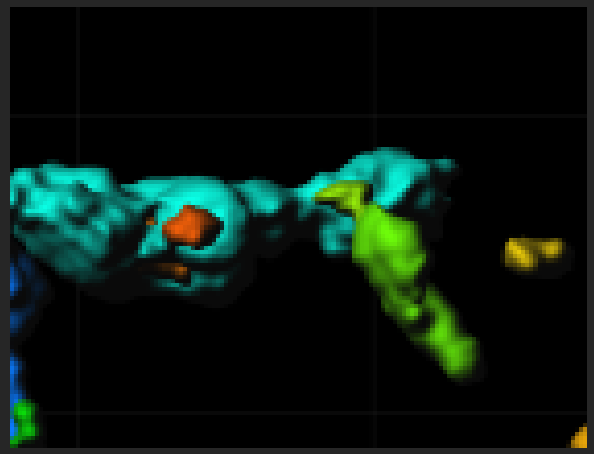
Raw



Imaris Default

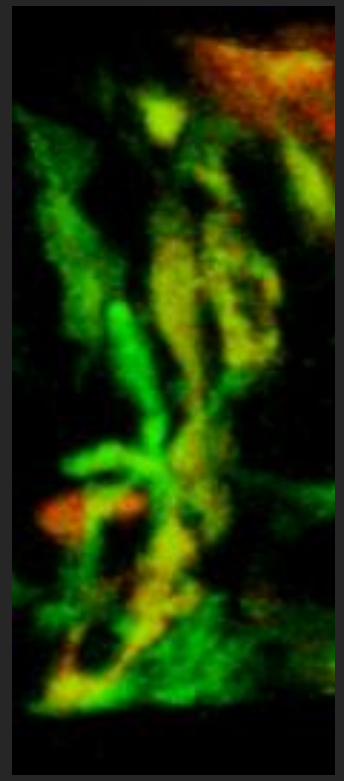


Color Gradient



Improvements

Raw



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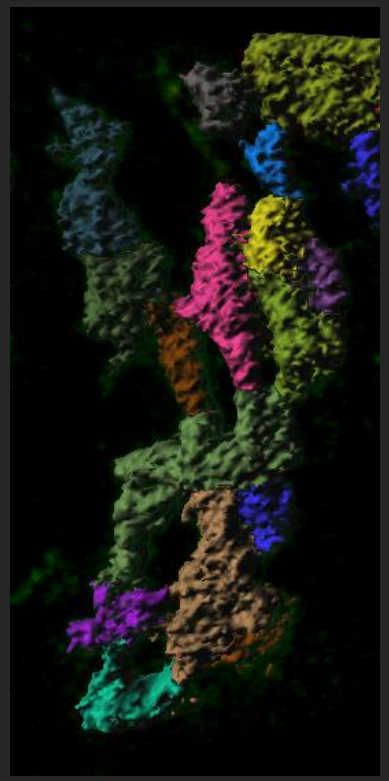
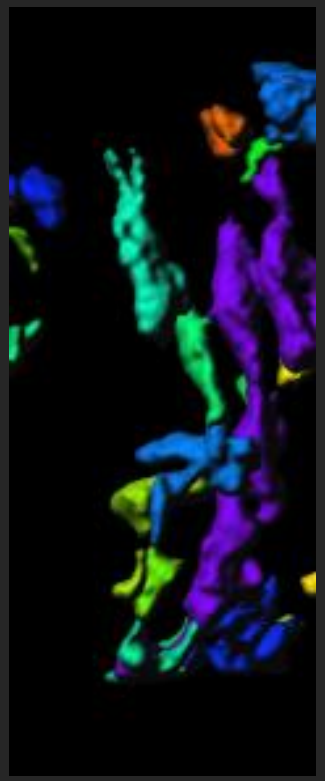


Color Gradient



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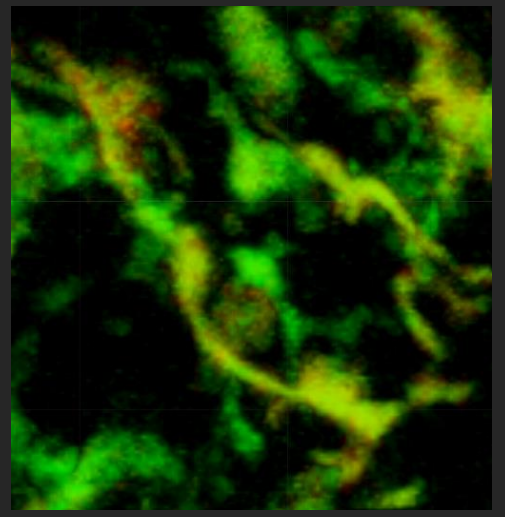

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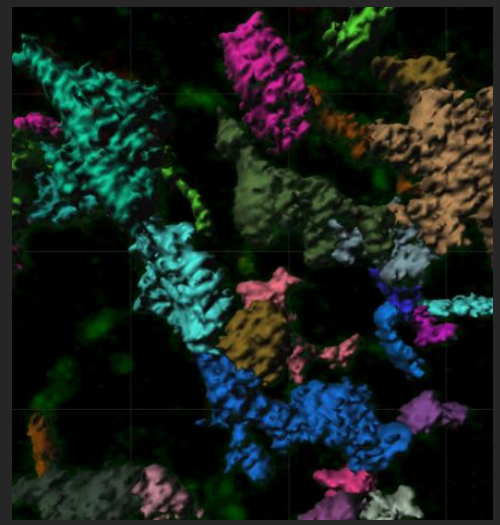
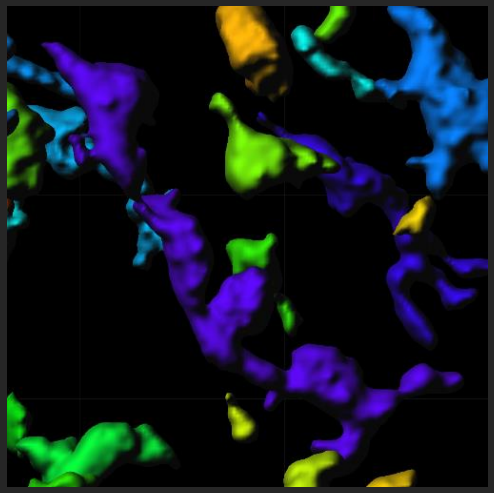
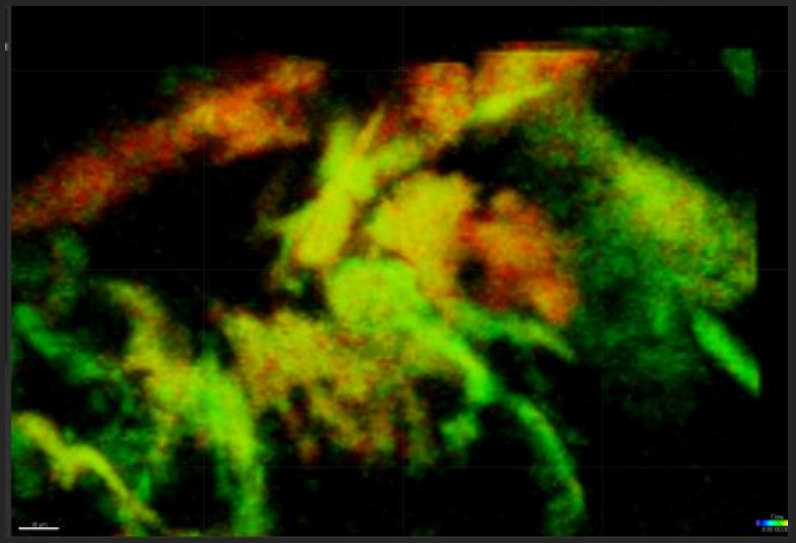


Color Gradient



Errors in this algorithm

Raw



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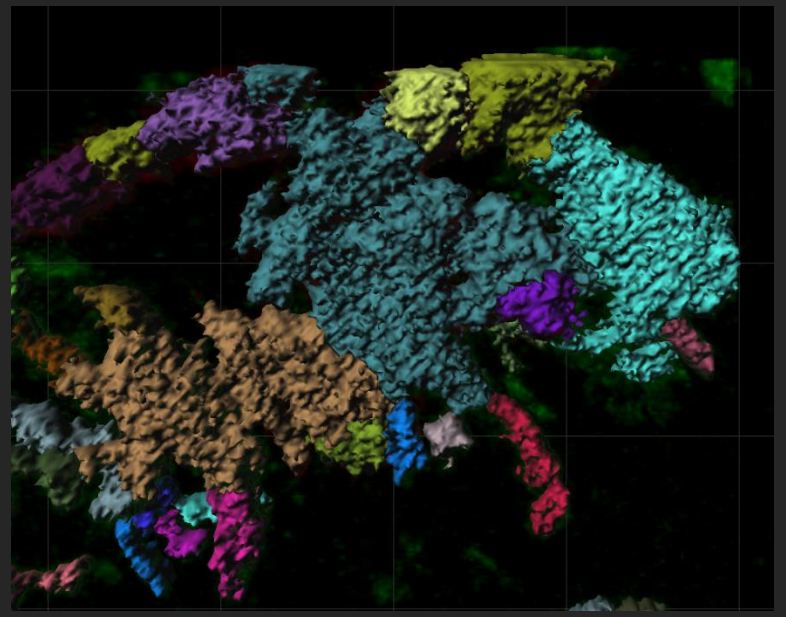

Color Gradient