

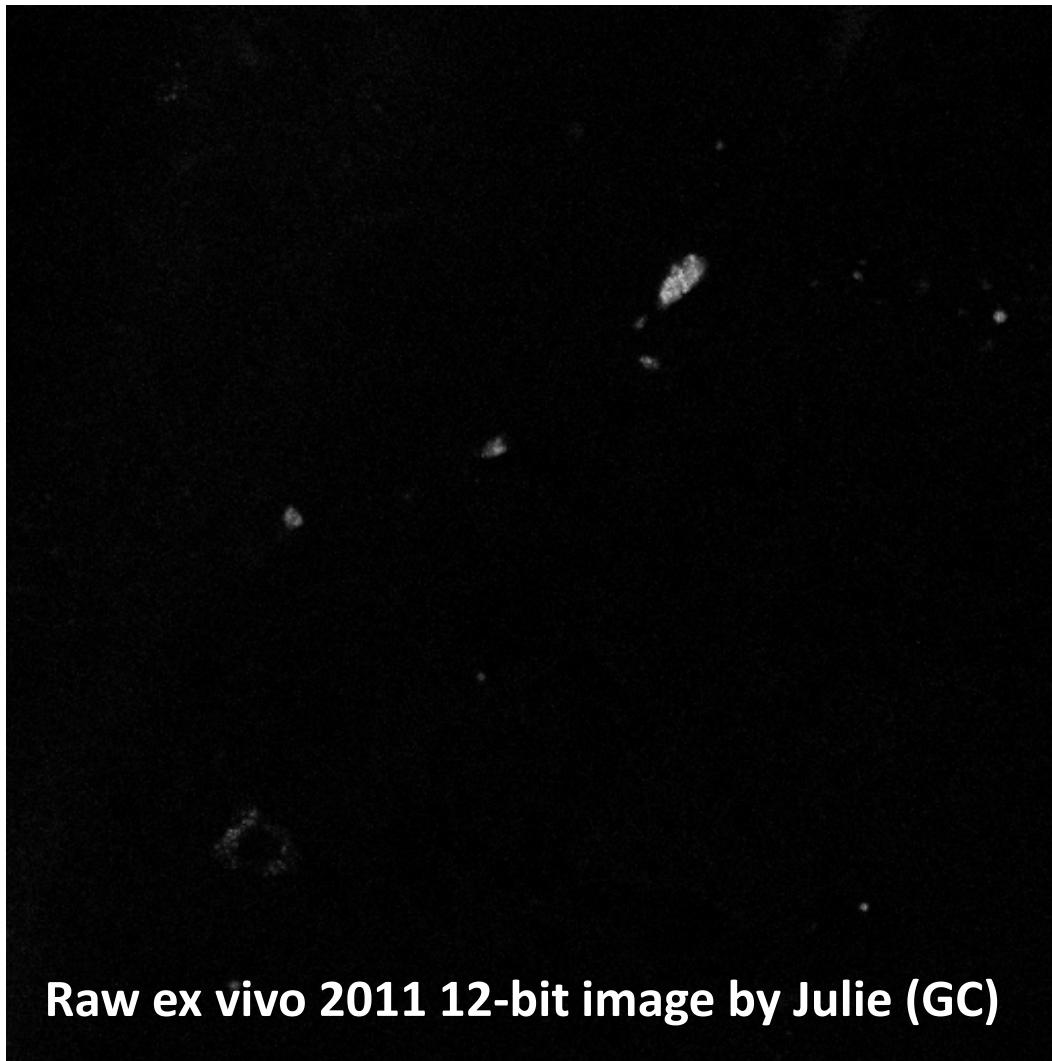
Analysis of AMDX2011 aggregates in retinal images

Alex Matov
October 2022

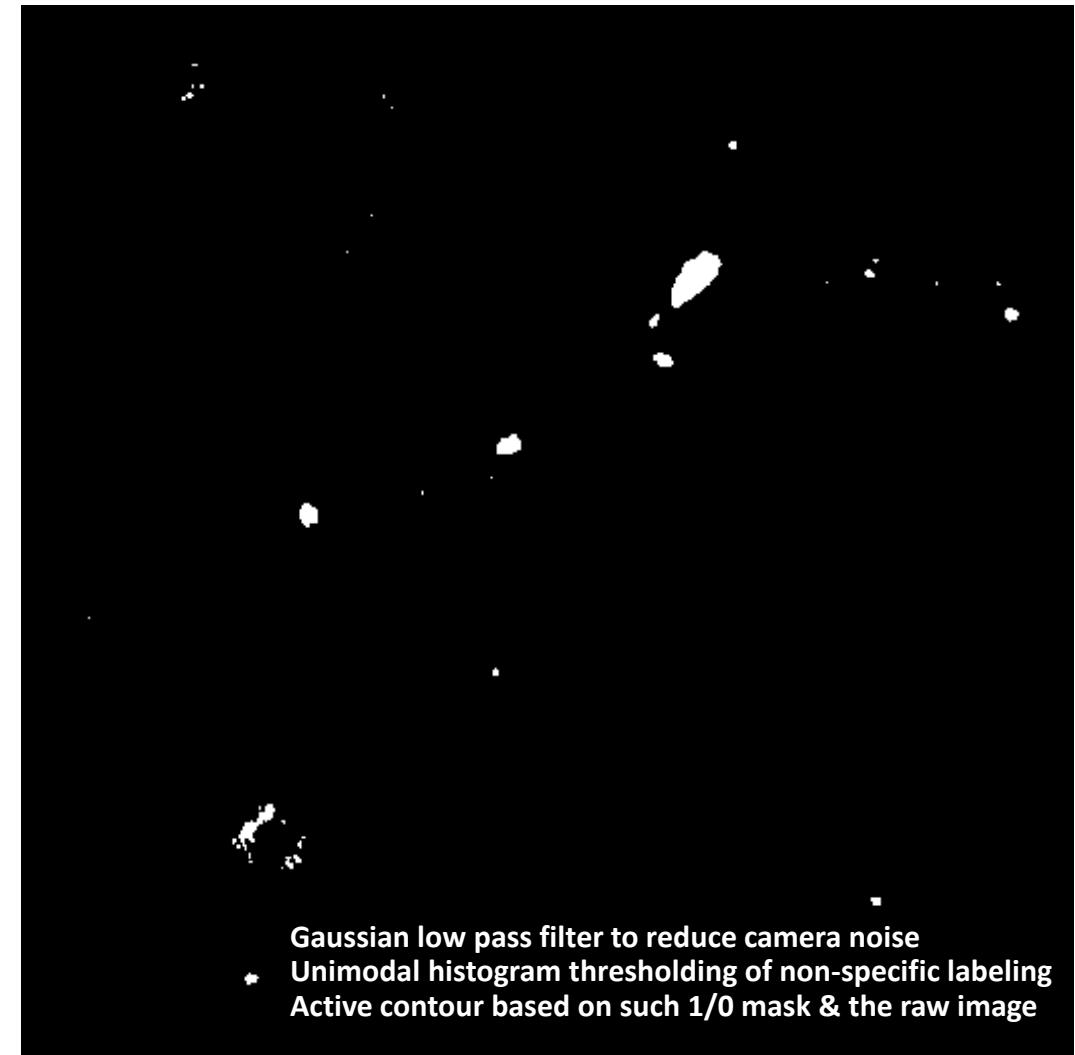
Image classification & disease detection

- Computer vision metrics (brightness, morphology, localization within the retina, etc.)
- Deep learning with neural networks (classification into different disease types/stages)
- Database image storage (rapid delivery of a searchable pool of informative analytics)

Preliminary analysis



Raw ex vivo 2011 12-bit image by Julie (GC)



- Gaussian low pass filter to reduce camera noise
- Unimodal histogram thresholding of non-specific labeling
- Active contour based on such 1/0 mask & the raw image

Preliminary analysis

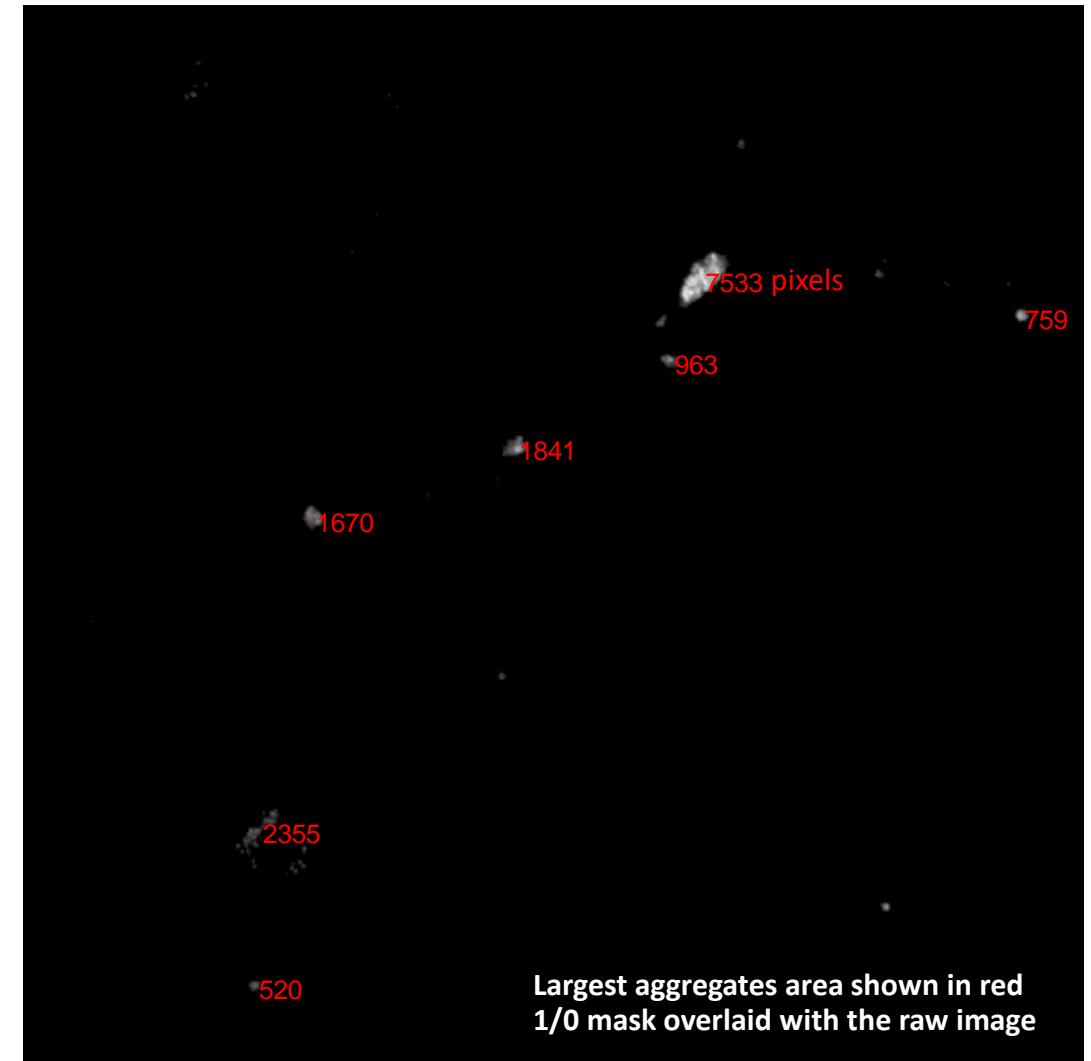
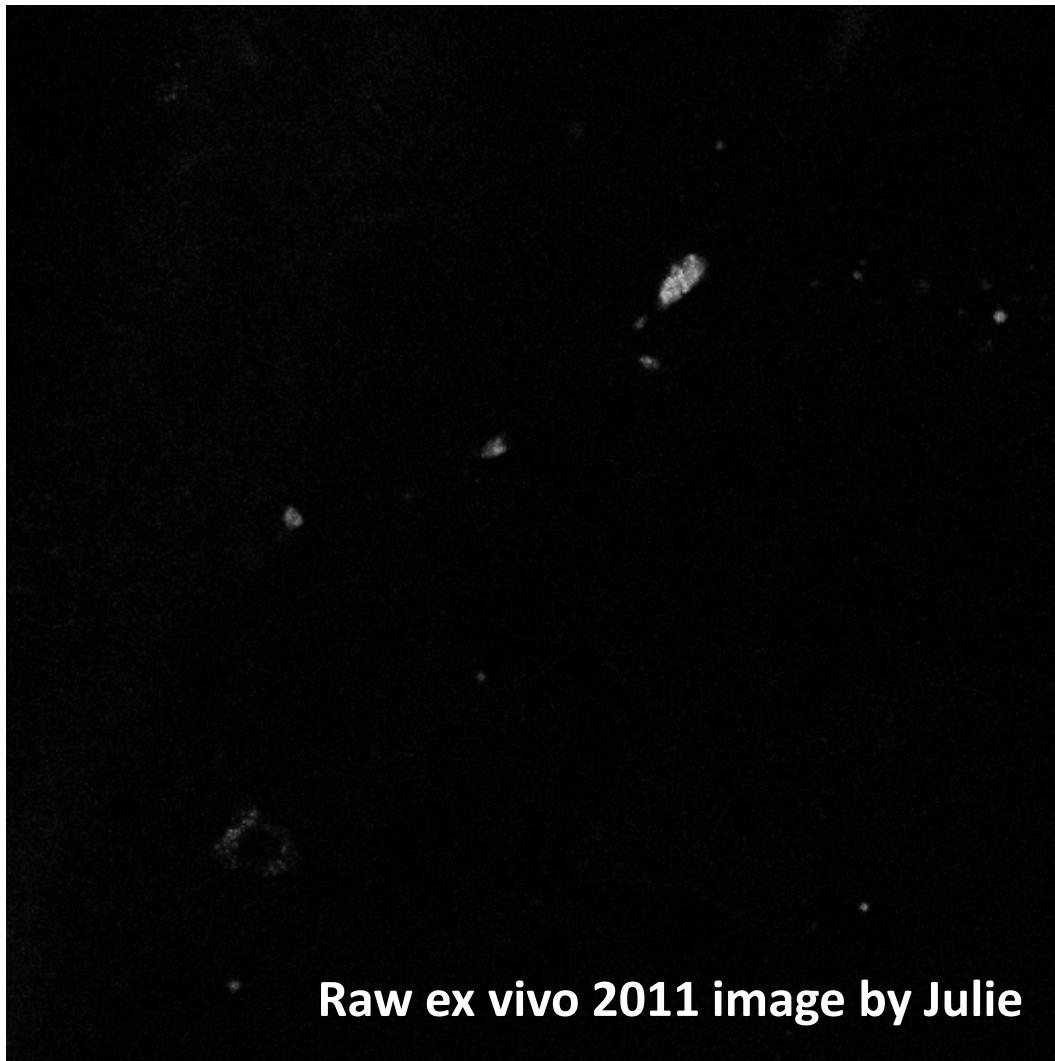
45x1 struct array w fields:

```
Area  
Centroid  
BoundingBox  
SubarrayIdx  
MajorAxisLength  
MinorAxisLength  
Eccentricity  
Orientation  
ConvexHull  
ConvexImage  
ConvexArea  
Image  
FilledImage  
FilledArea  
EulerNumber  
Extrema  
EquivDiameter  
Solidity  
Extent  
PixelIdxList  
PixelList  
Perimeter  
PerimeterOld
```

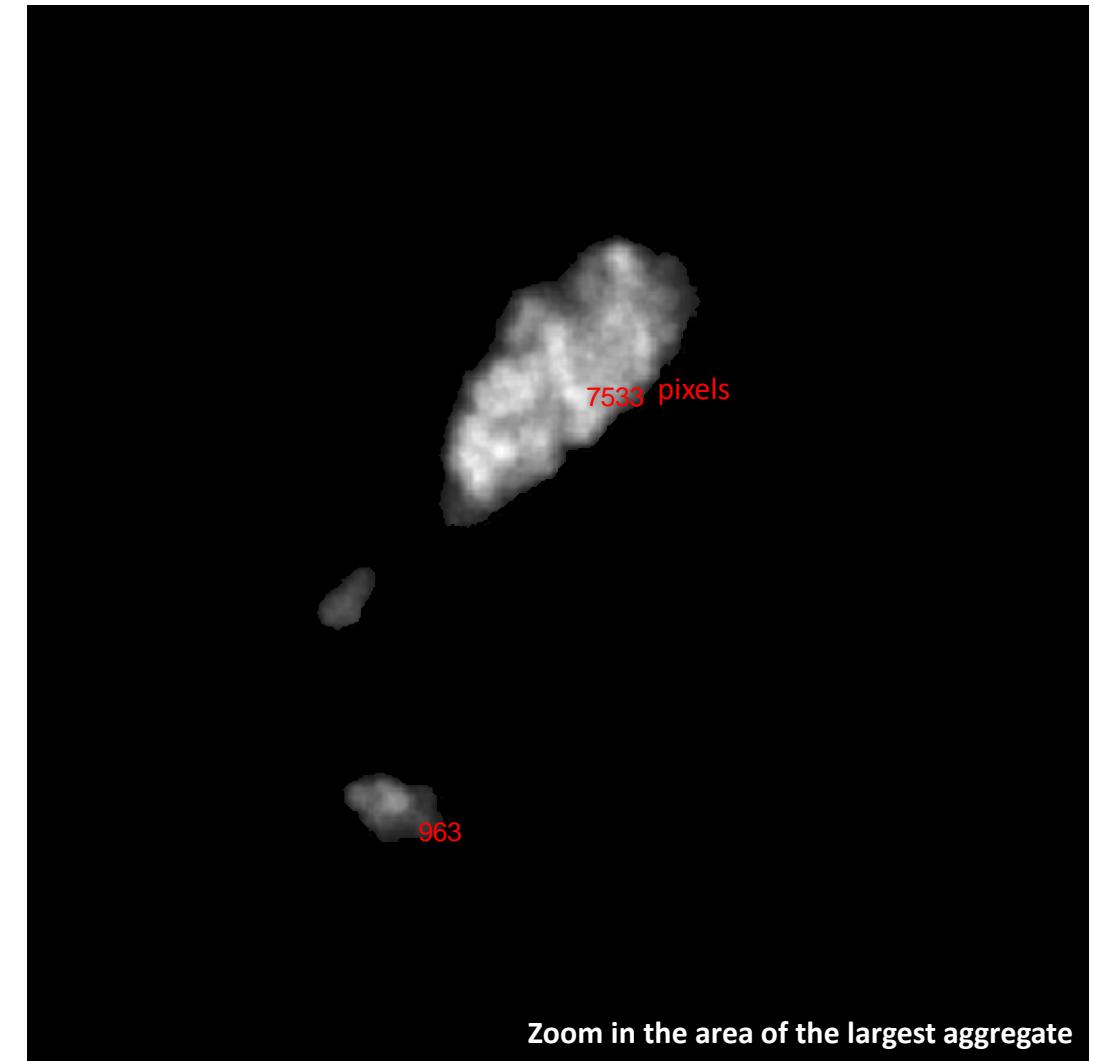
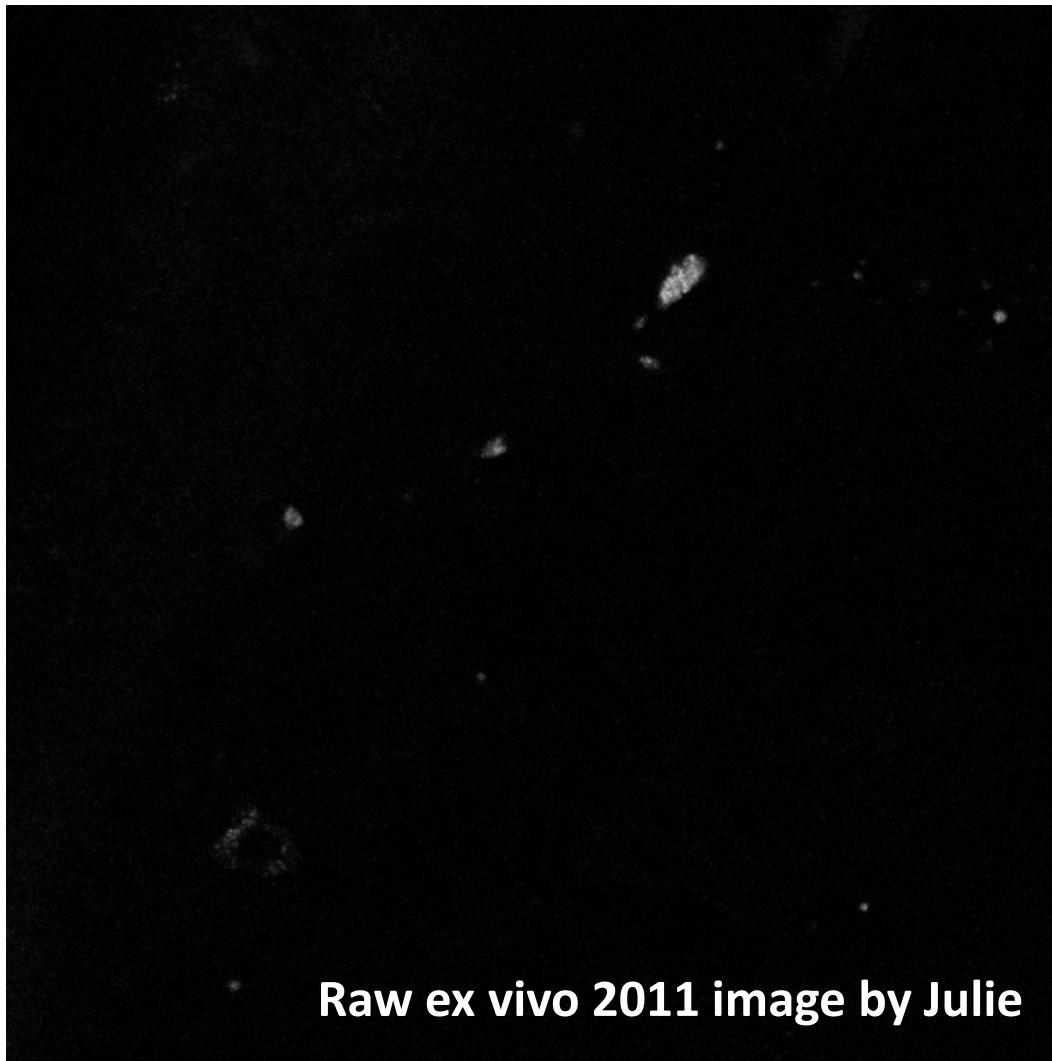
Raw ex vivo 2011 image by Julie

Area: 7533 (example)
Centroid: [1.4850e+03 601.4648]
BoundingBox: [1.4325e+03 543.5000 112 124]
SubarrayIdx: {[1x124 double] [1x112 double]}
MajorAxisLength: 146.6684
MinorAxisLength: 66.7536
Eccentricity: 0.8904
Orientation: 48.0603
ConvexHull: [49x2 double]
ConvexImage: [124x112 logical]
ConvexArea: 7978
Image: [124x112 logical]
FilledImage: [124x112 logical]
FilledArea: 7533
EulerNumber: 1
Extrema: [8x2 double]
EquivDiameter: 97.9353
Solidity: 0.9442
Extent: 0.5424
PixelIdxList: [7533x1 double]
PixelList: [7533x2 double]
Perimeter: 377.2390
PerimeterOld: 398.8600

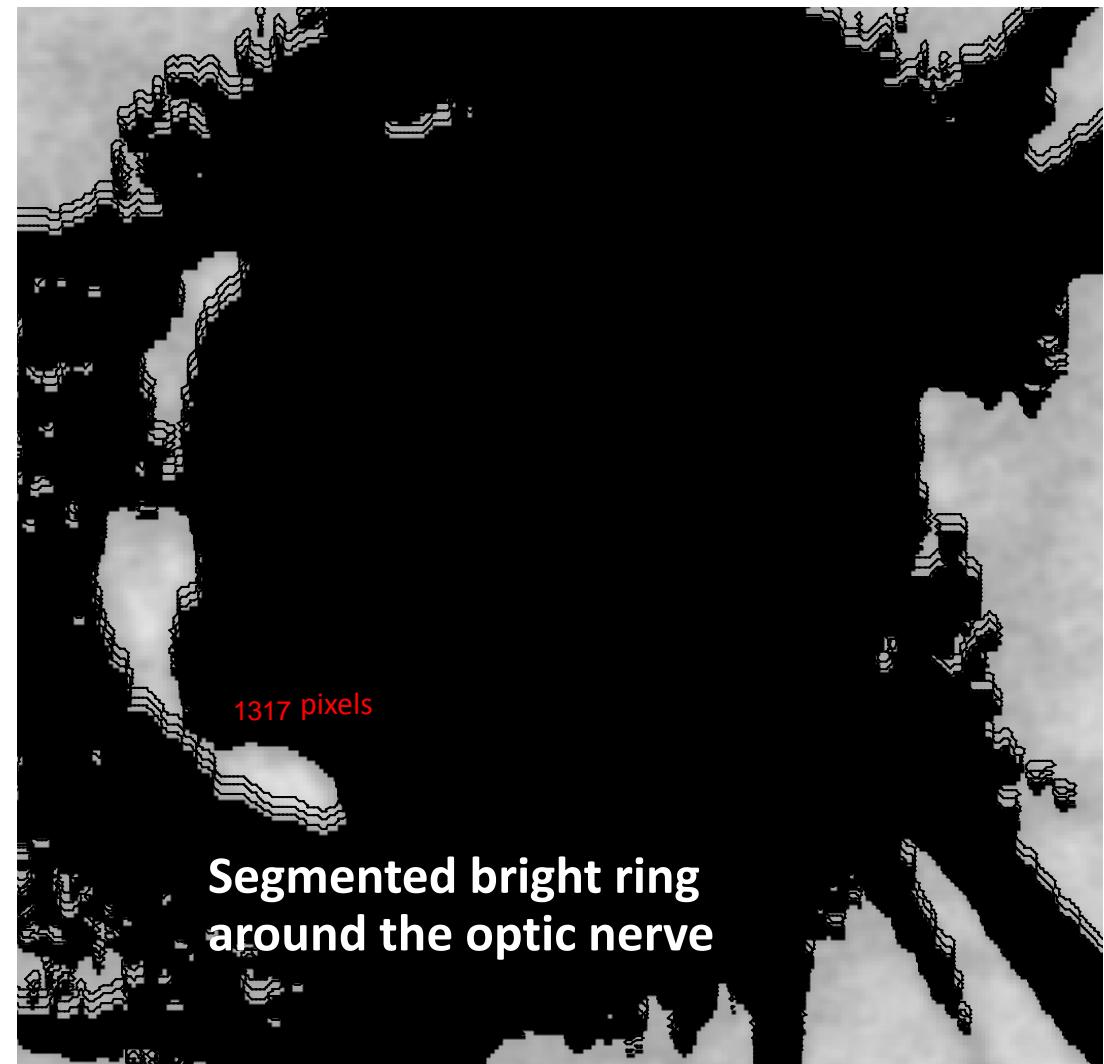
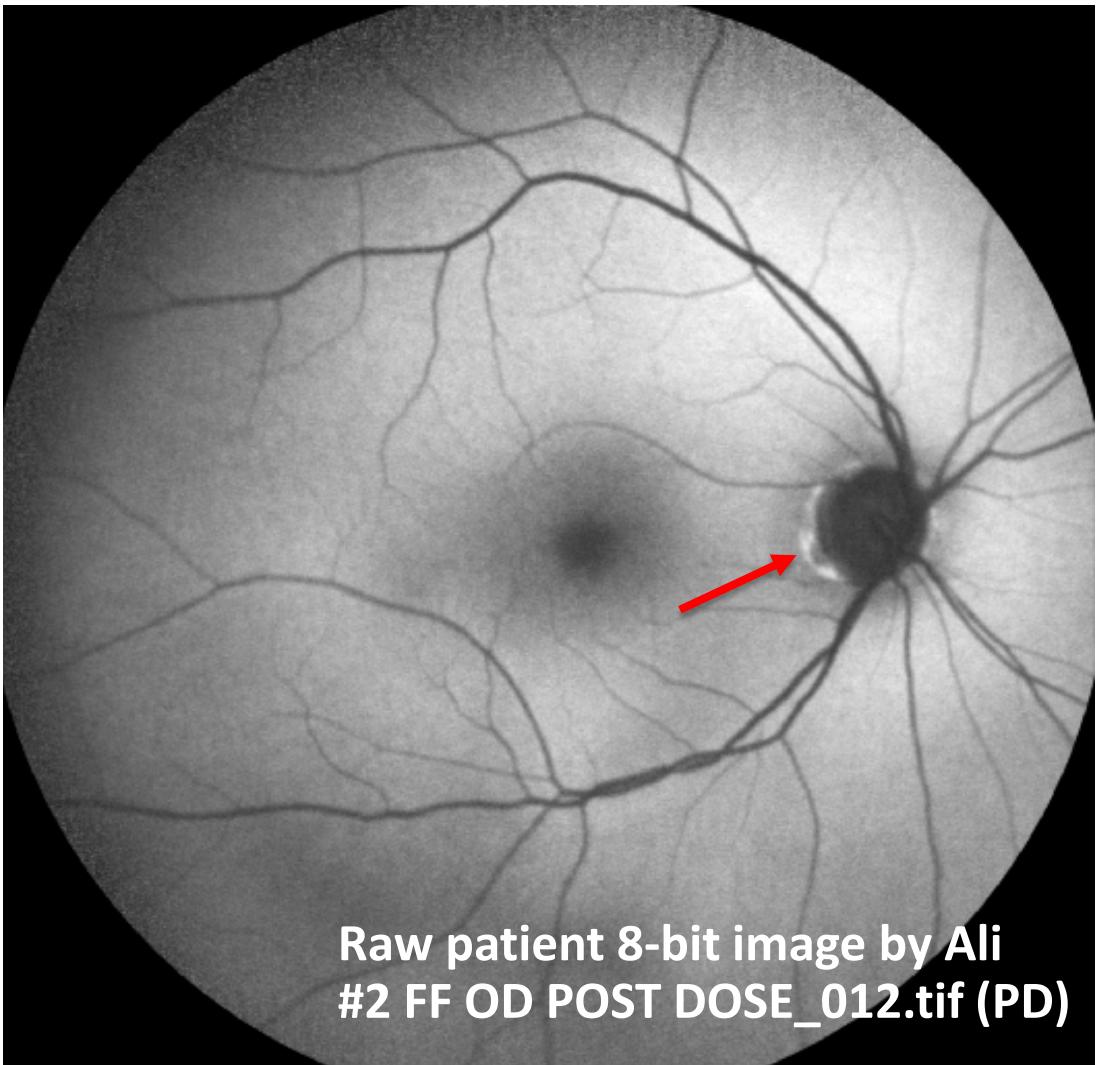
Preliminary analysis



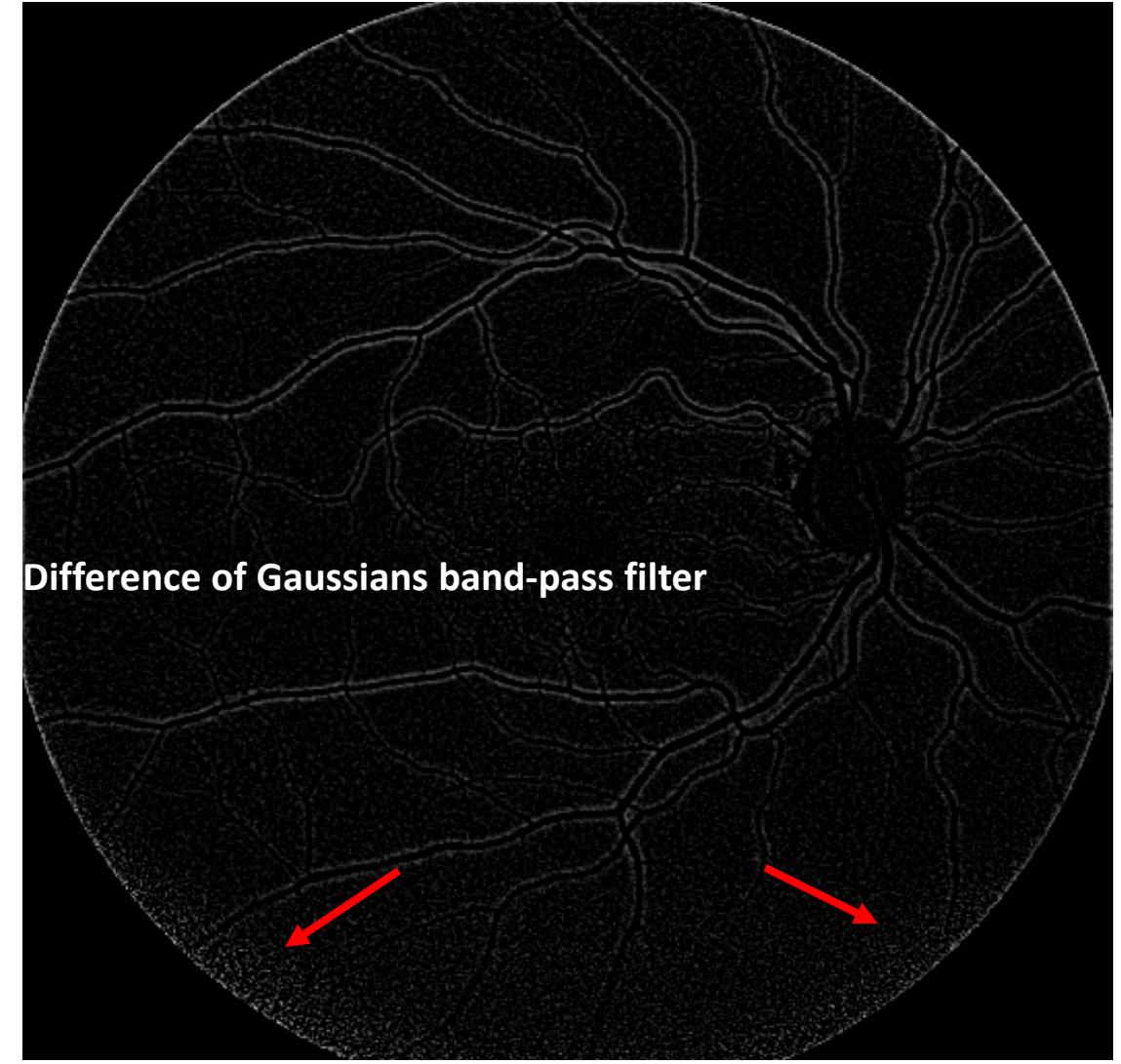
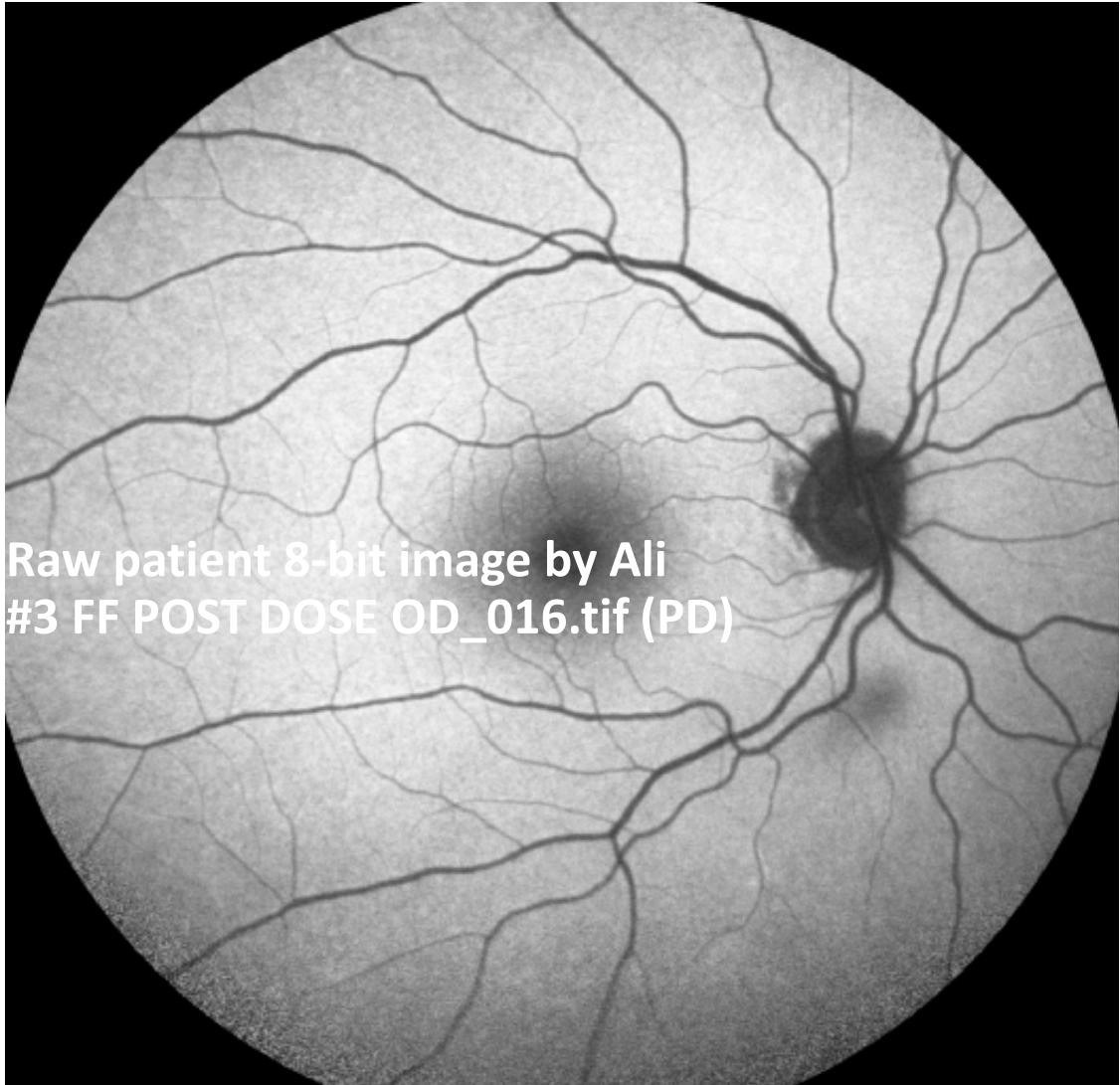
Preliminary analysis



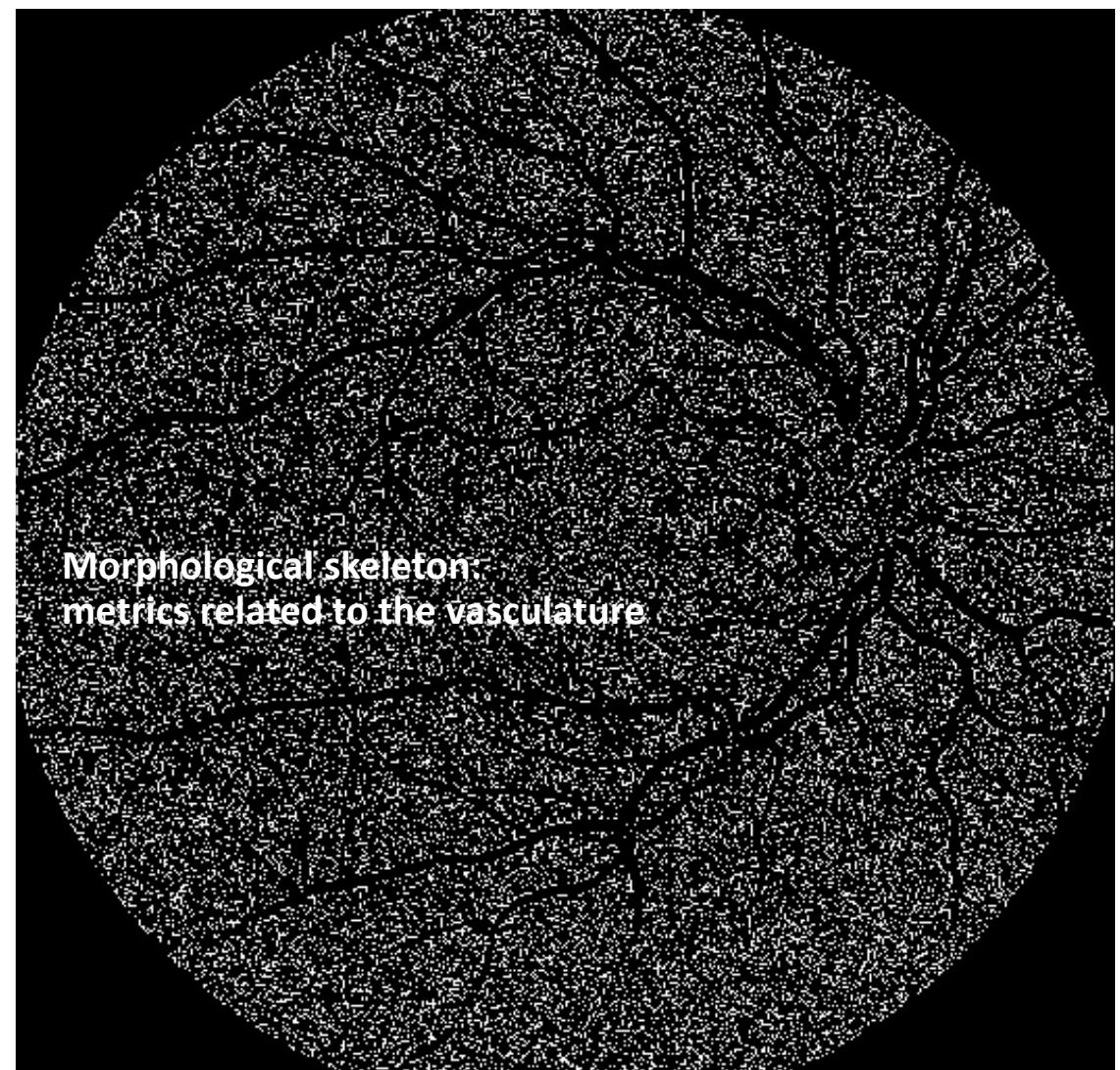
Preliminary analysis



Preliminary analysis

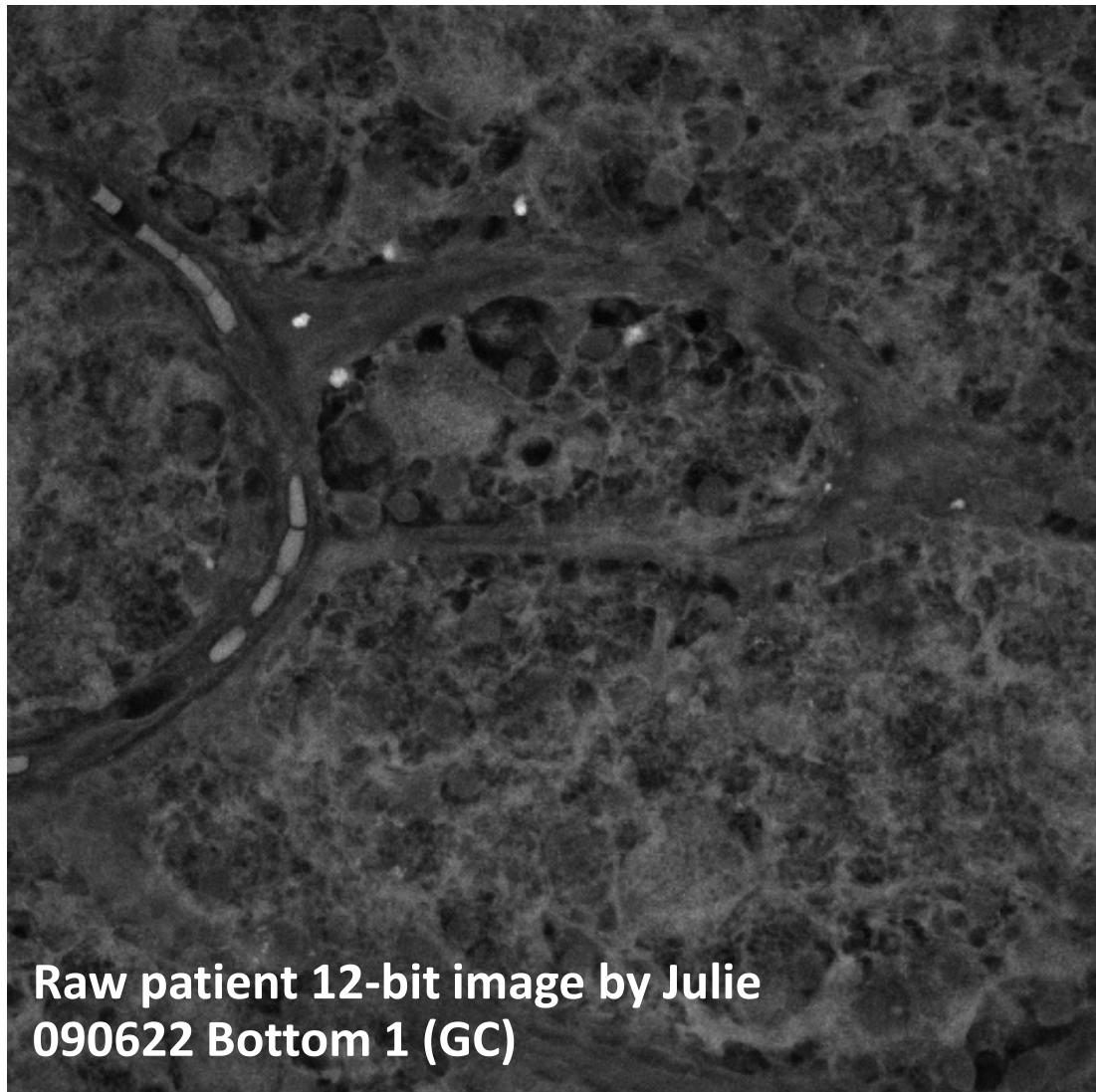


Preliminary analysis

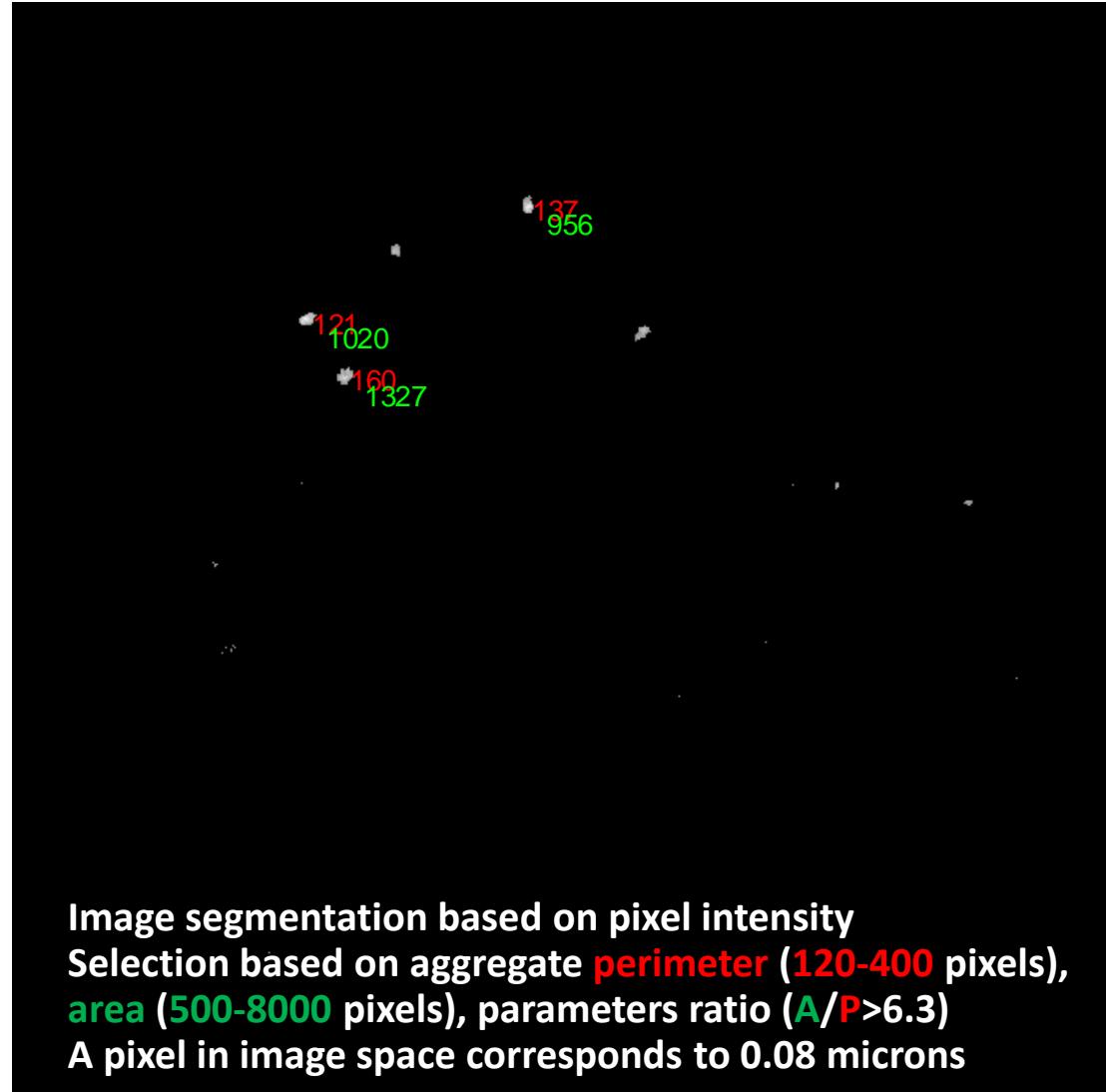


Preliminary analysis

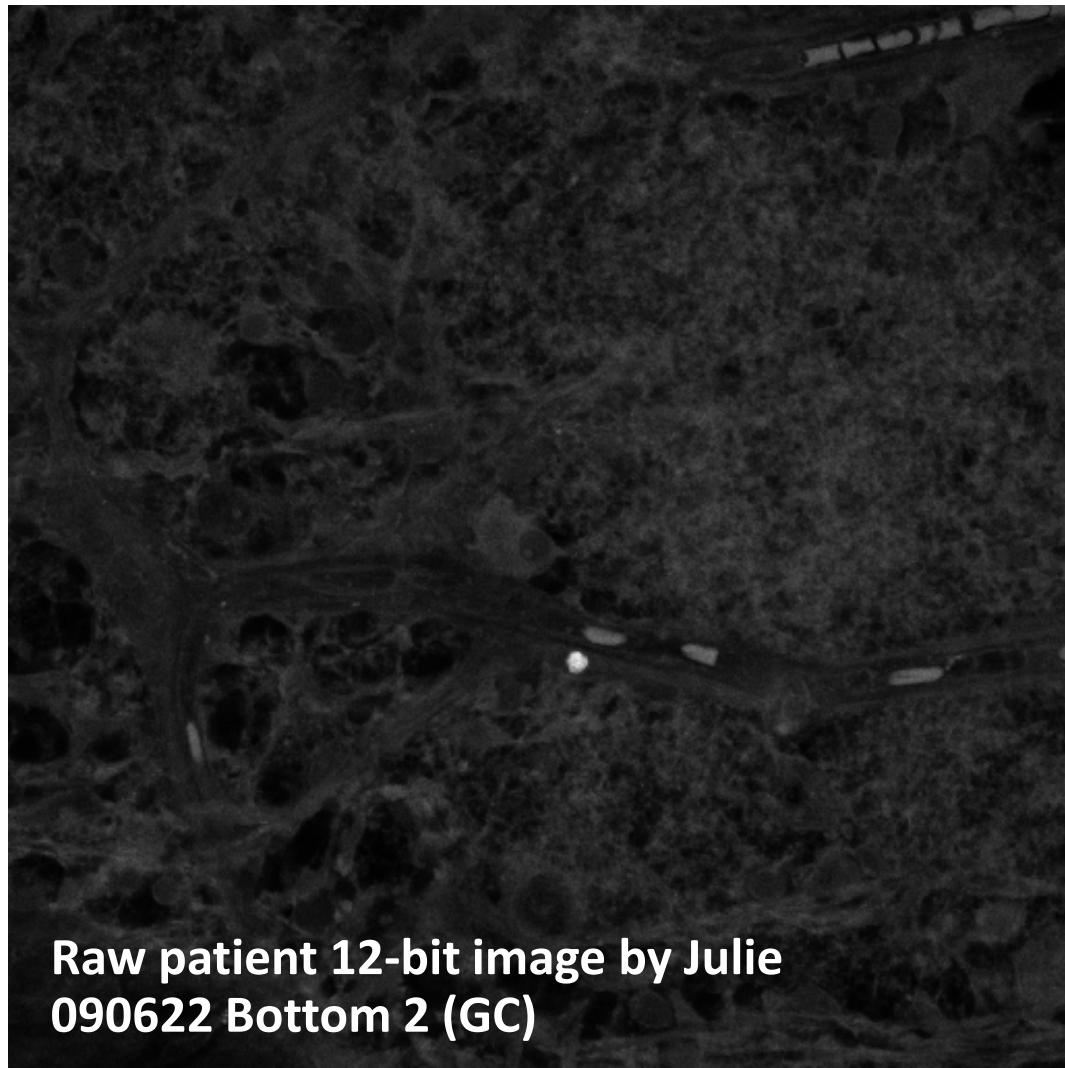
3 aggregates detected, Aggregate perimeter in pixels (red), Aggregate area in pixels (green)



Raw patient 12-bit image by Julie
090622 Bottom 1 (GC)



Preliminary analysis



Raw patient 12-bit image by Julie
090622 Bottom 2 (GC)

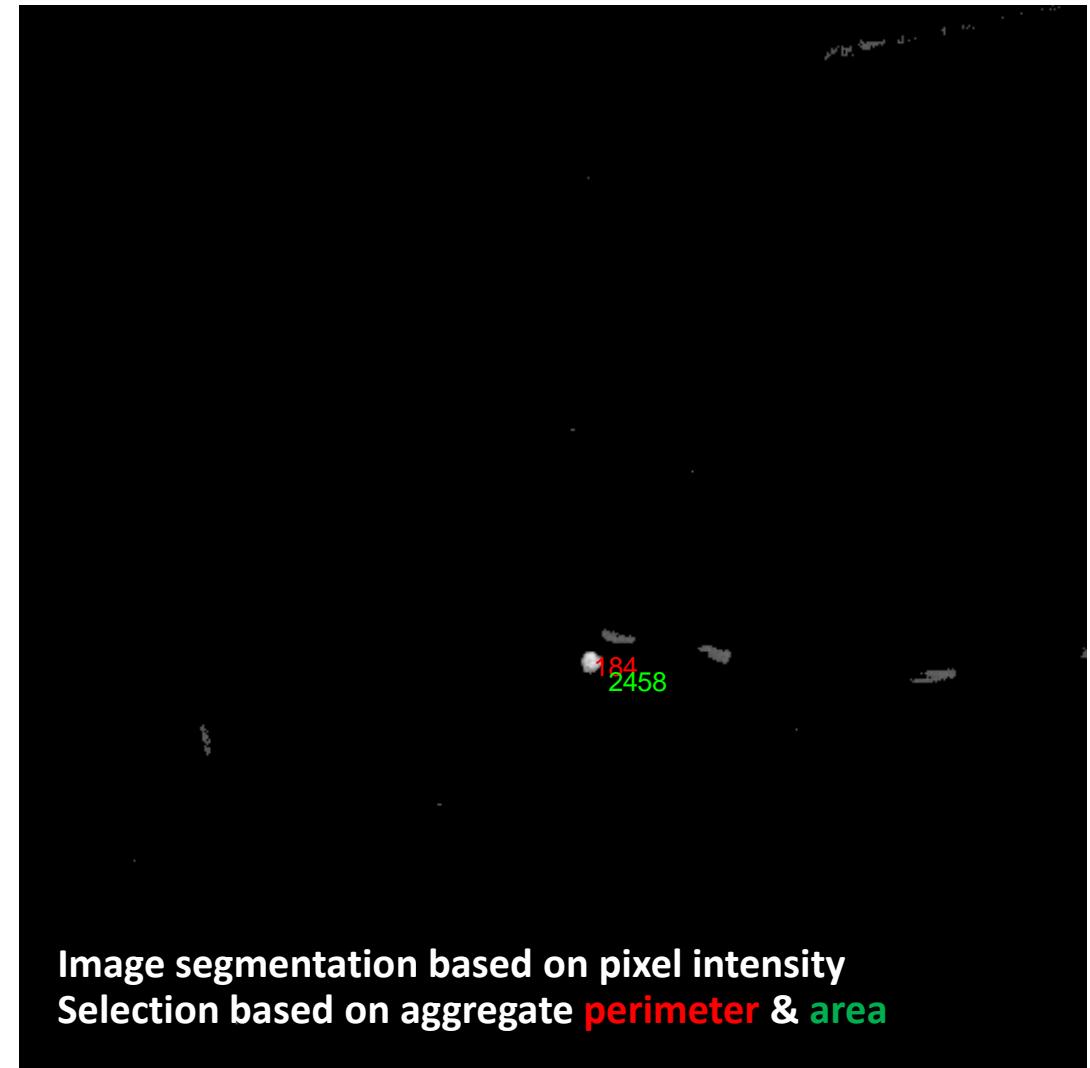
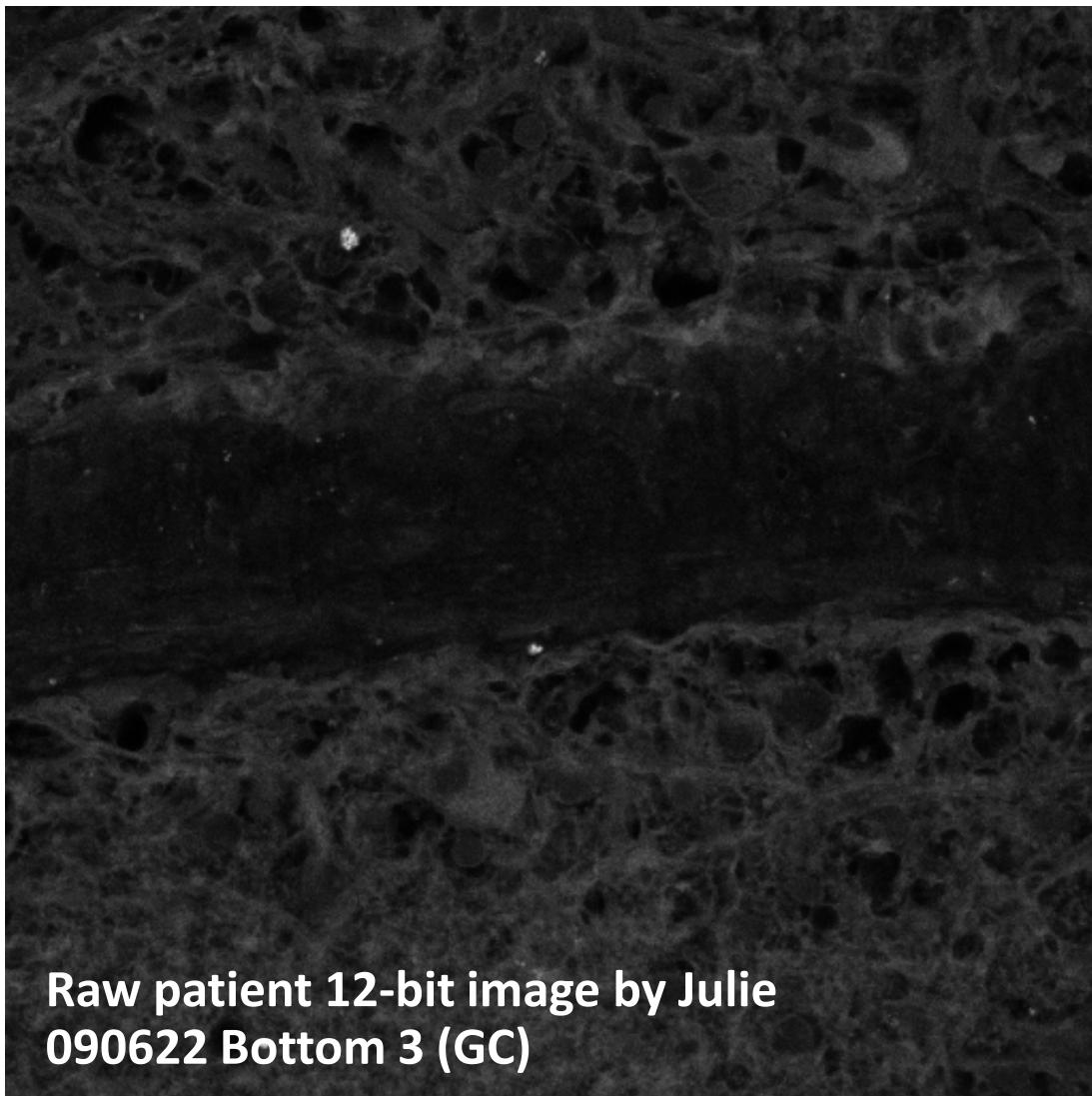


Image segmentation based on pixel intensity
Selection based on aggregate **perimeter** & **area**

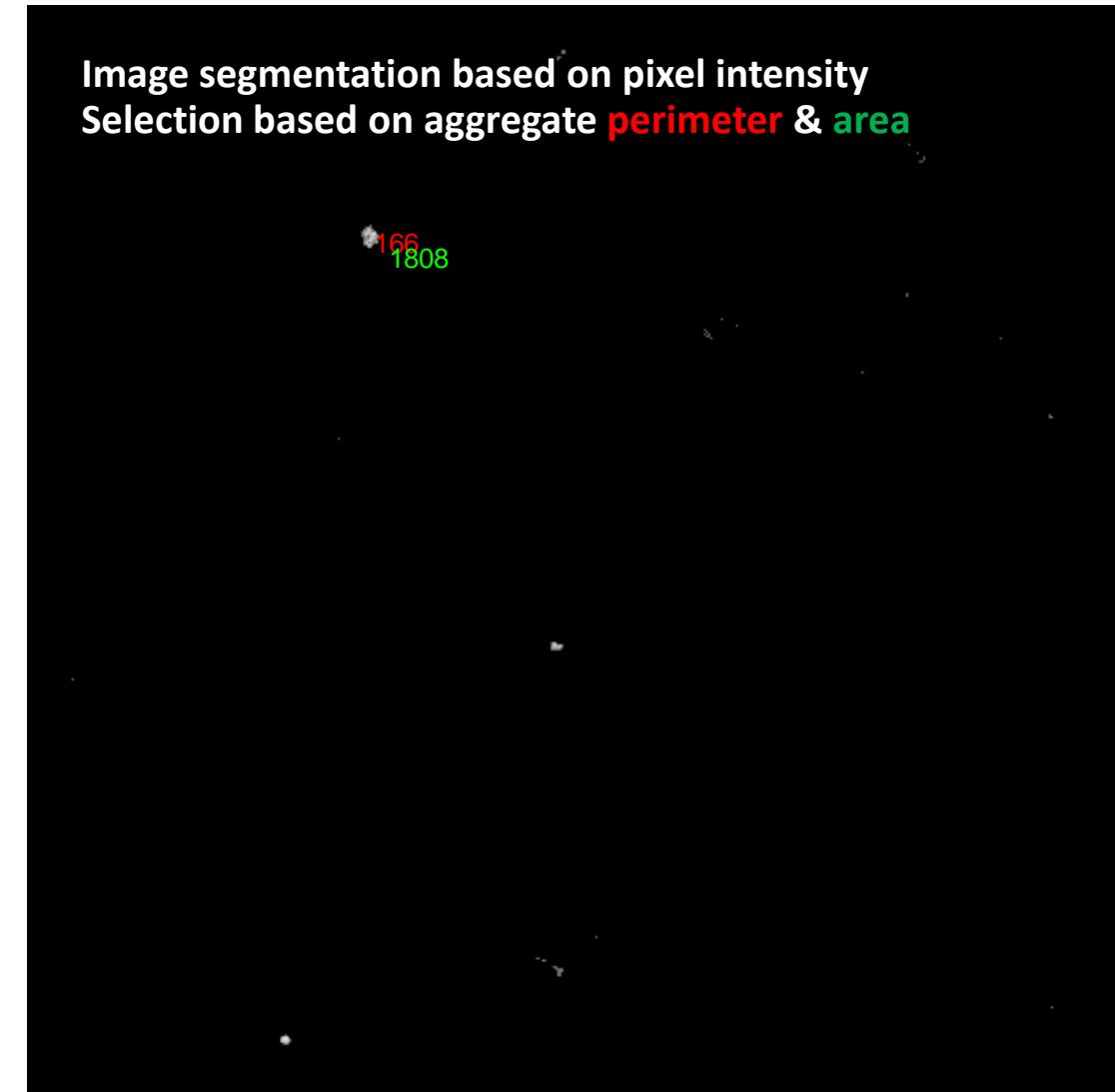
Preliminary analysis

Aggregate perimeter in pixels (red), Aggregate area in pixels (green)

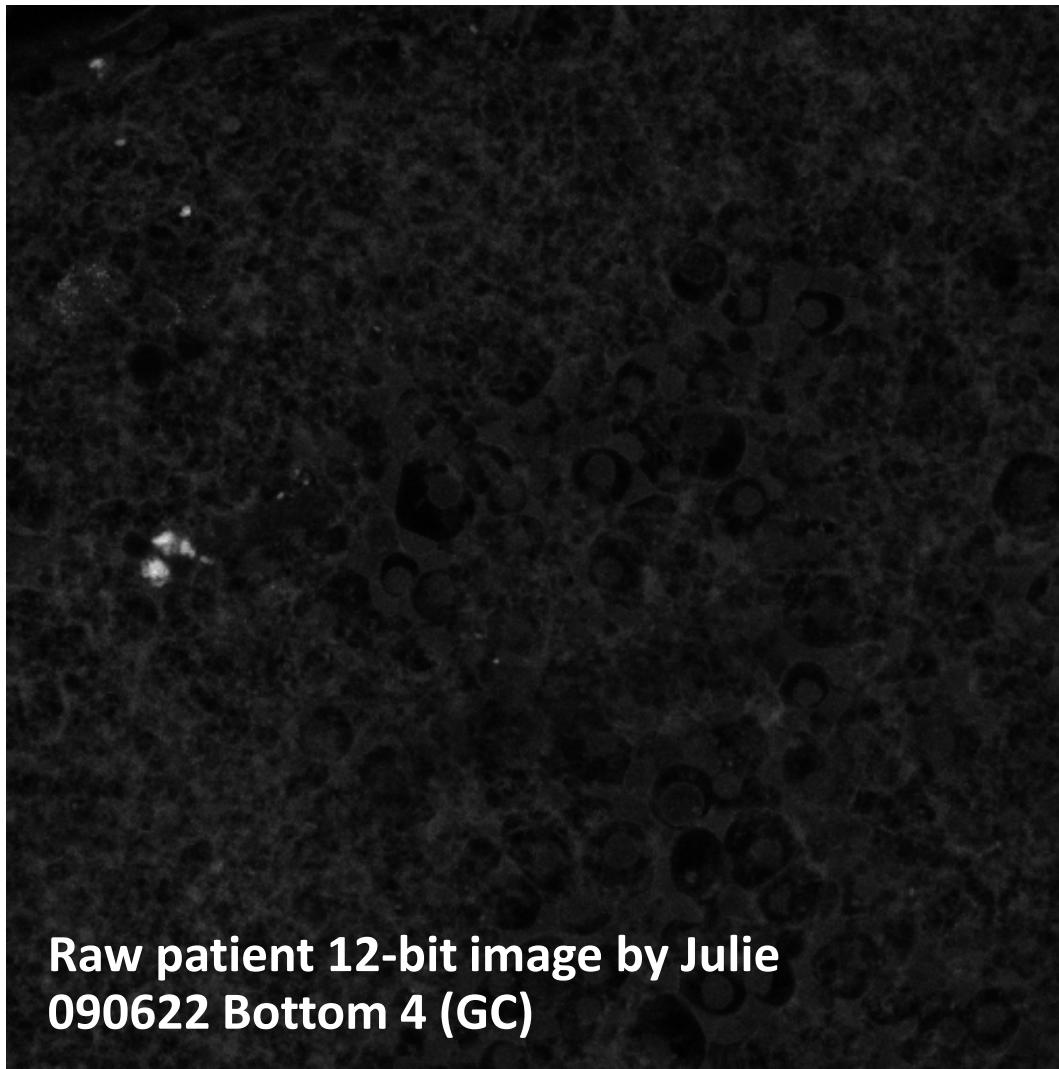


Raw patient 12-bit image by Julie
090622 Bottom 3 (GC)

Image segmentation based on pixel intensity
Selection based on aggregate **perimeter** & **area**

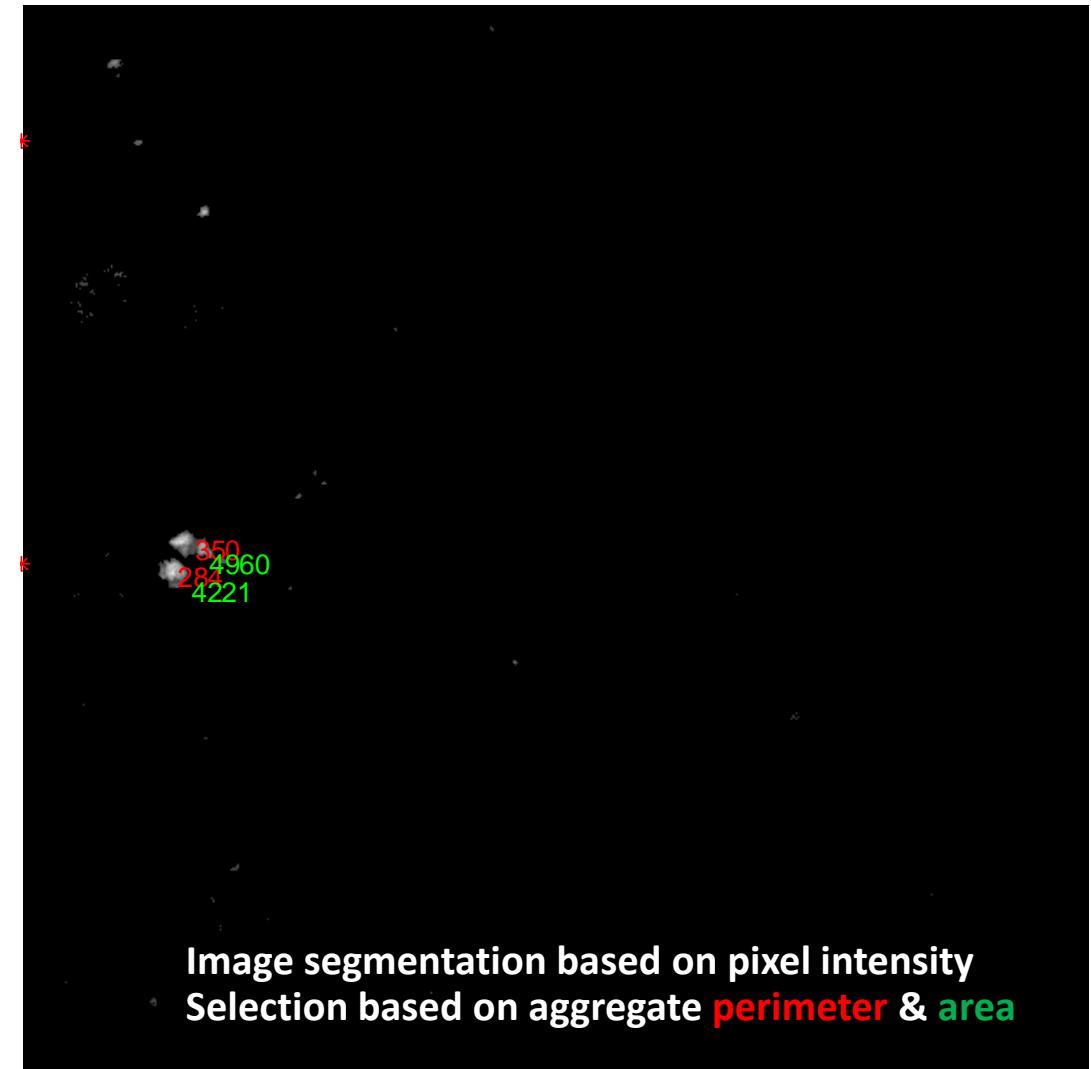


Preliminary analysis



**Raw patient 12-bit image by Julie
090622 Bottom 4 (GC)**

Aggregate perimeter in pixels (red), Aggregate area in pixels (green)



Preliminary analysis

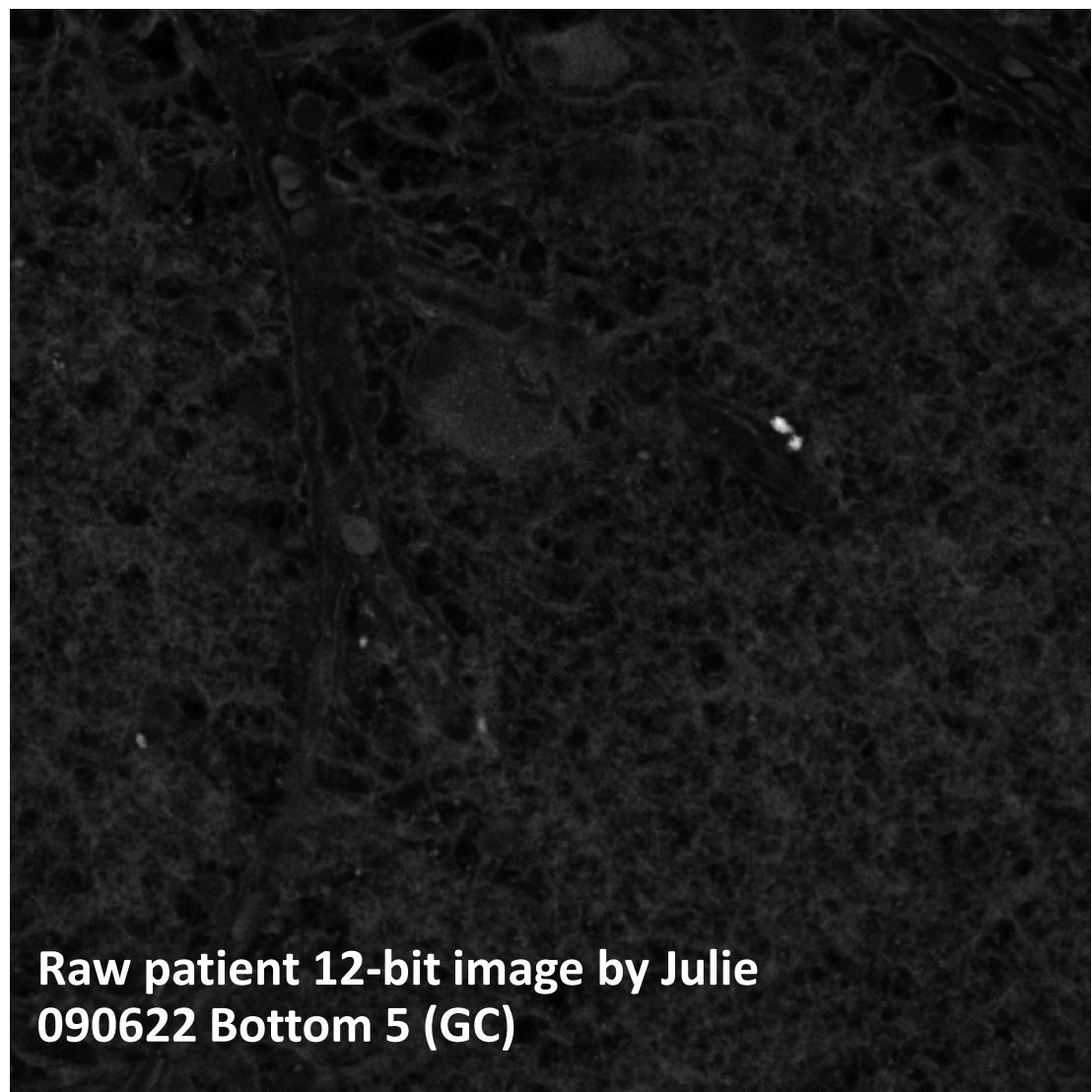


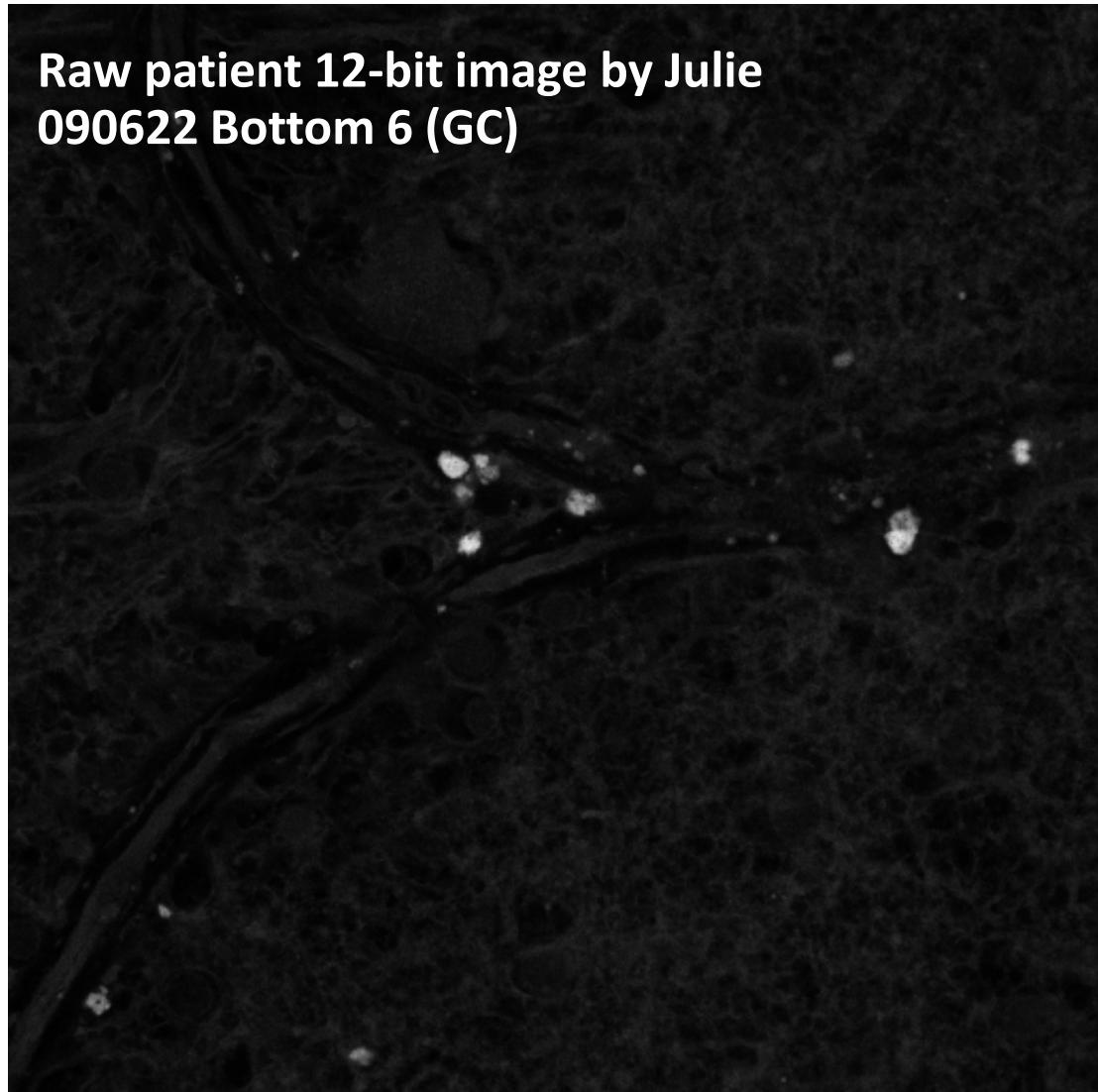
Image segmentation based on pixel intensity
Selection based on aggregate **perimeter** & **area**

166
1231
1064

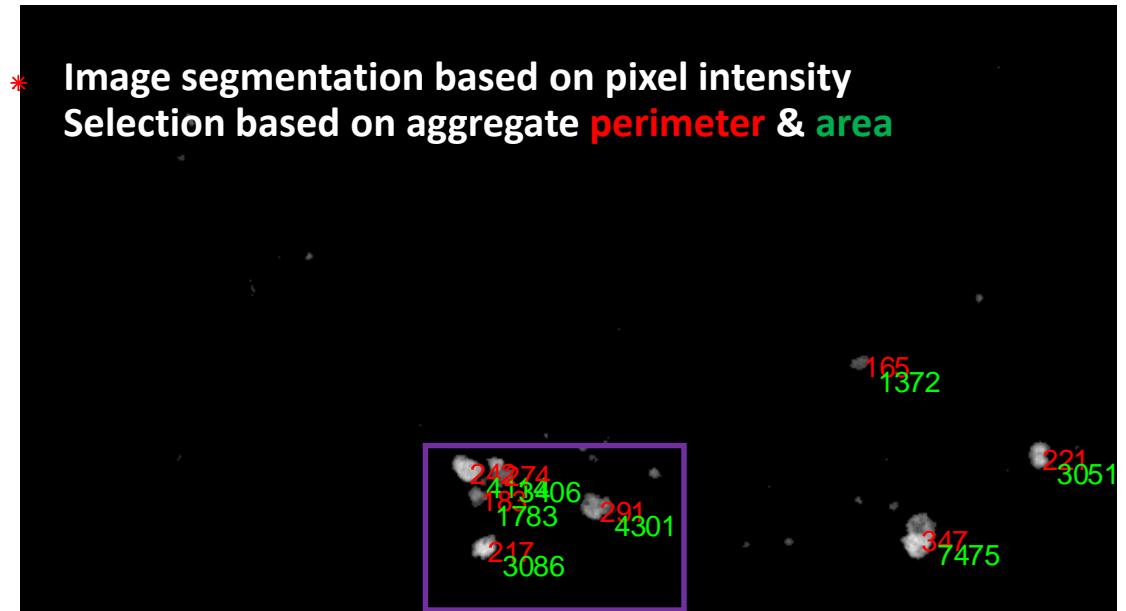
Preliminary analysis

10 aggregates detected, Aggregate perimeter in pixels (red), Aggregate area in pixels (green)

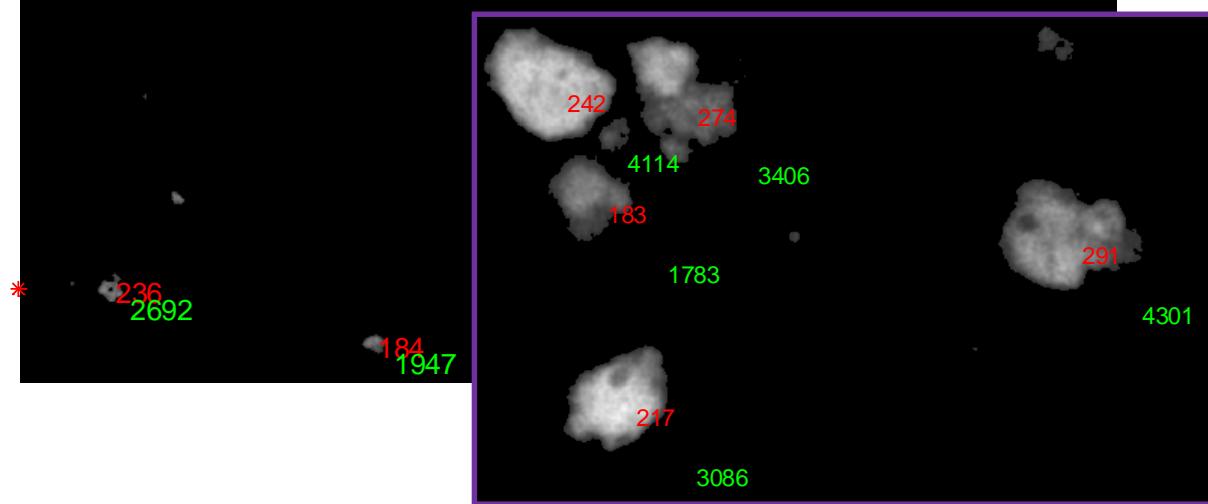
Raw patient 12-bit image by Julie
090622 Bottom 6 (GC)



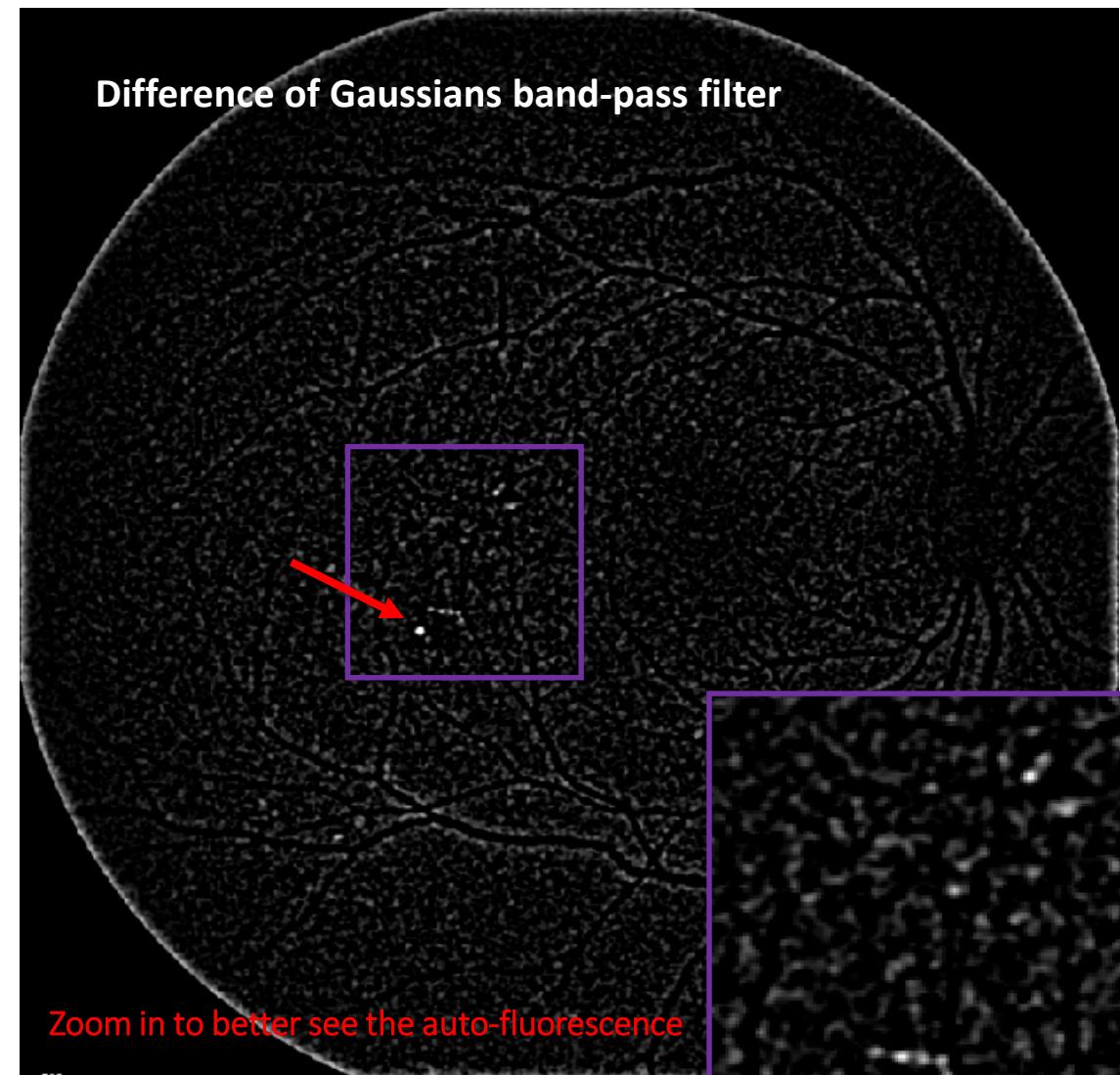
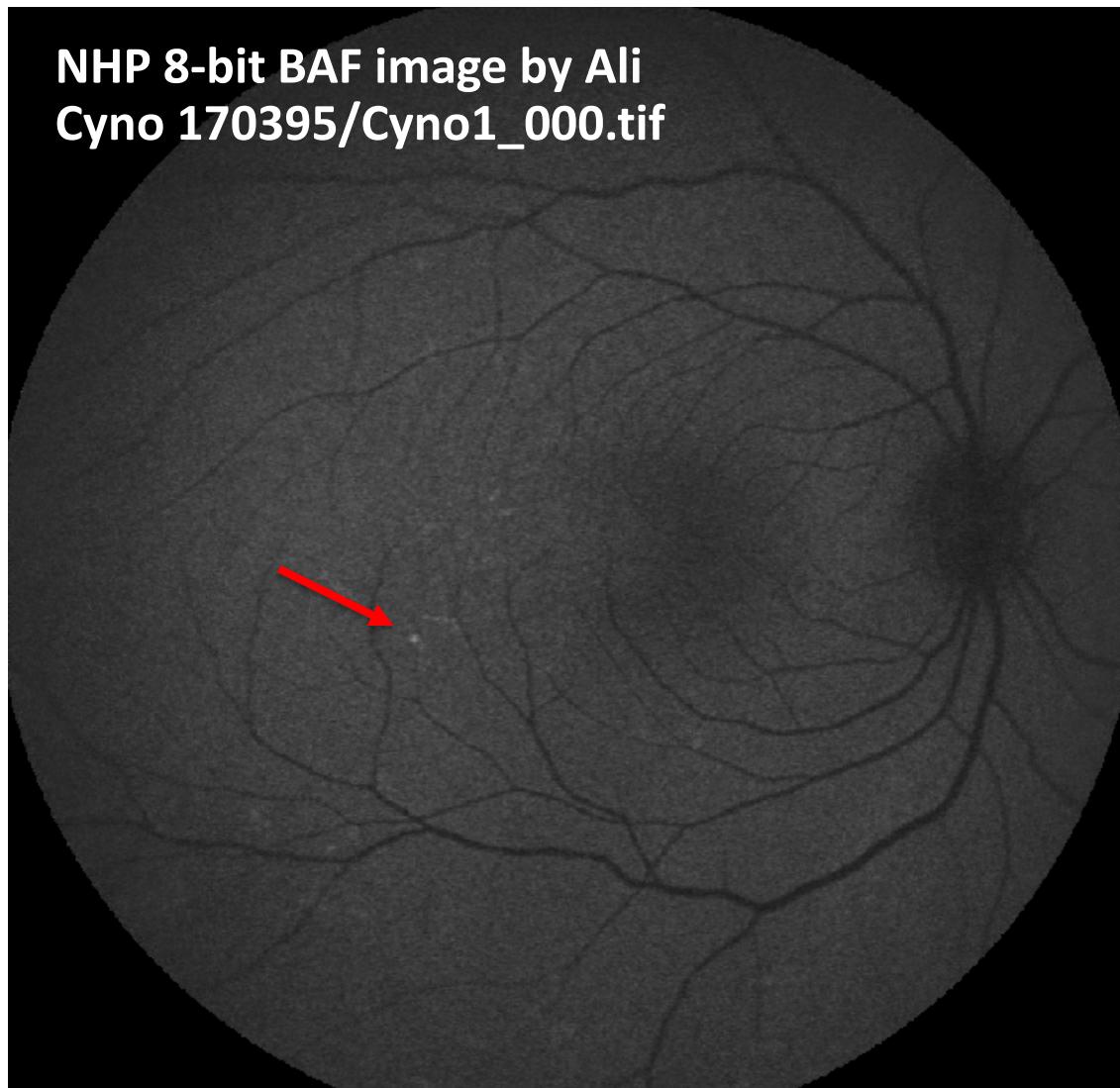
- * **Image segmentation based on pixel intensity
Selection based on aggregate **perimeter** & **area****



Zoom in to better see the five aggregates

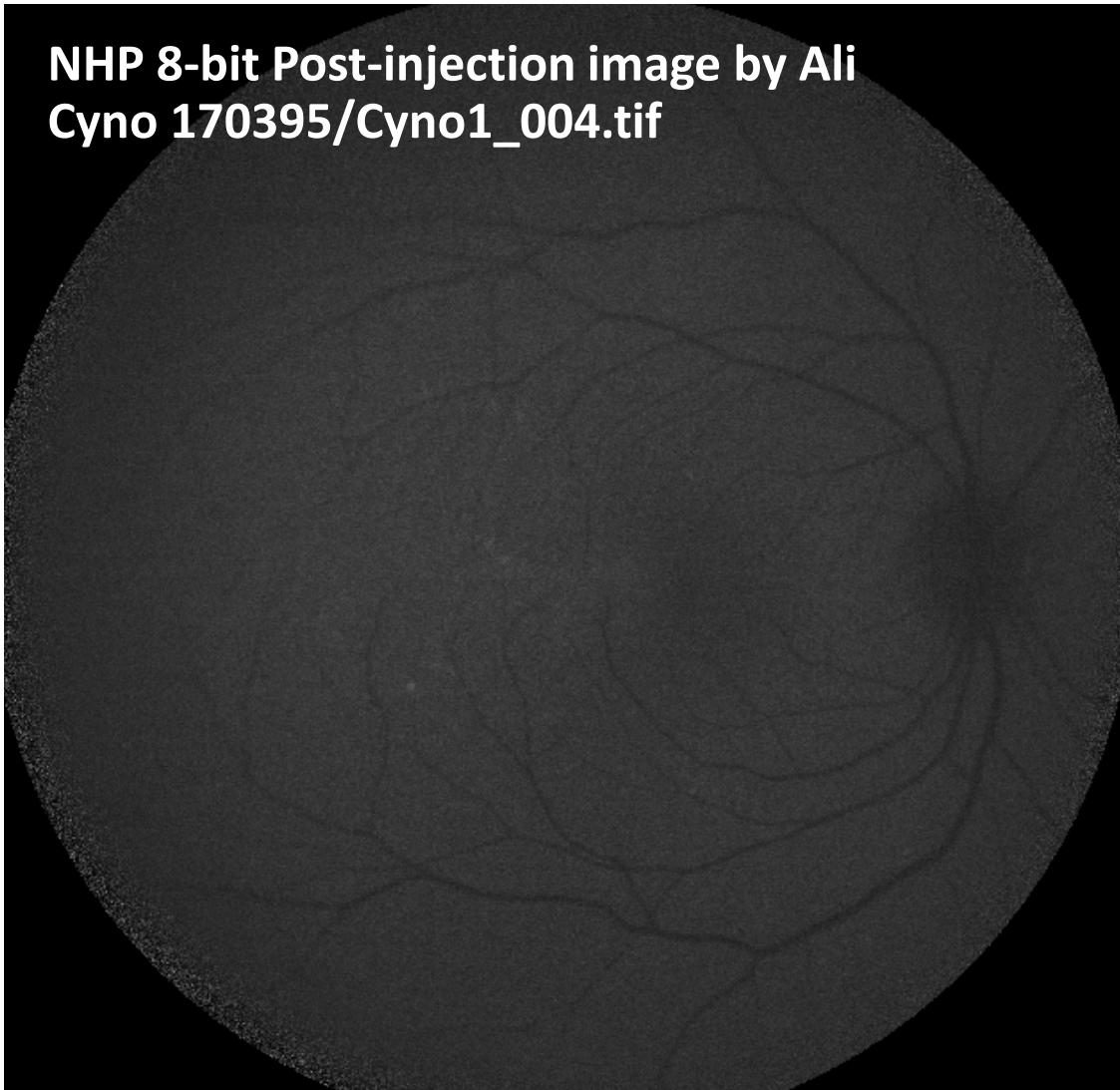


Preliminary analysis

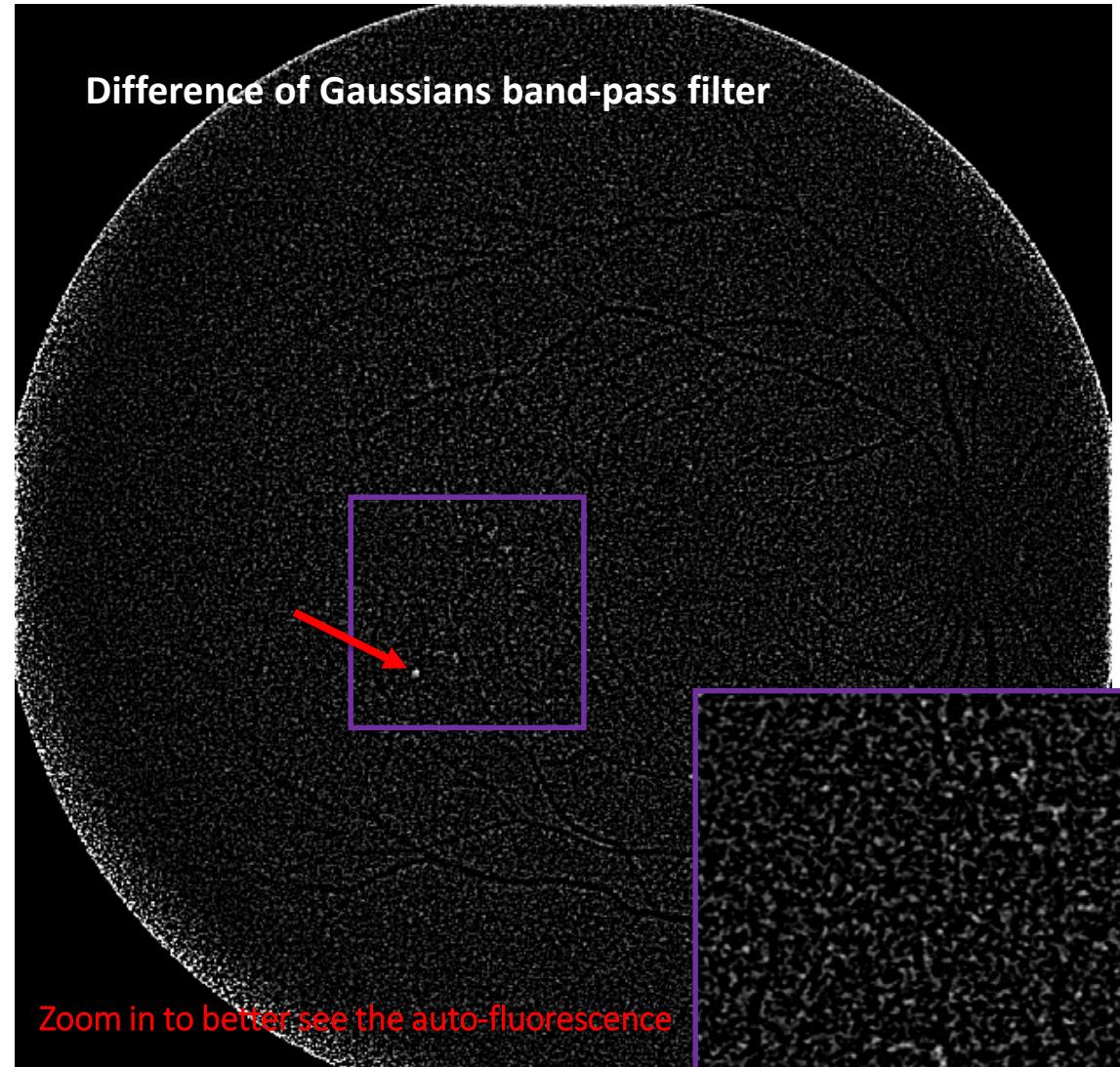


Preliminary analysis

NHP 8-bit Post-injection image by Ali
Cyno 170395/Cyno1_004.tif



Difference of Gaussians band-pass filter



Zoom in to better see the auto-fluorescence

Examples of image metrics

SIZE AND DISTRIBUTION OF AGGREGATES

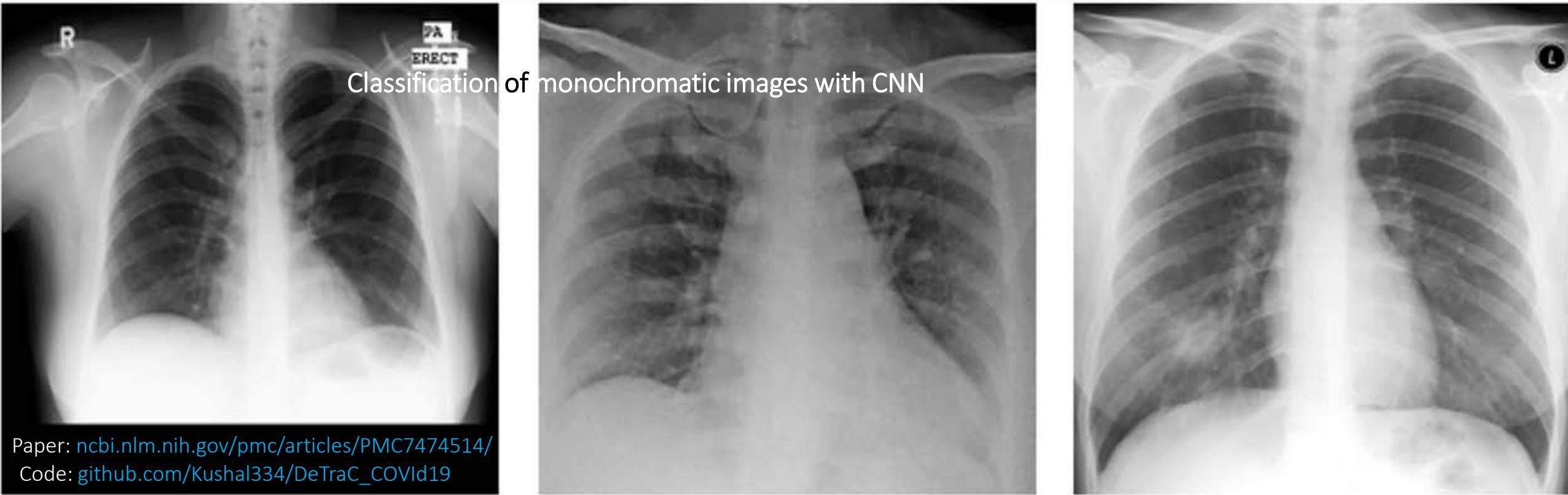
- 1) object: number – number of fluorescent aggregate in image
- 2) object: EulerNumber - number of objects in the image minus the total number of holes in those objects – distinguishes reticular or mesh-like patterns vs more uniformly distributed aggregates
- 3) object_size:average - The average number of above-threshold pixels per object – captures information about the size of the aggregates
- 4) object_size:variance - The variance of the number of above-threshold pixels per object – quantifies the homogeneity of fluorescent objects in cells
- 5) object_size:ratio - The ratio of the size of the largest aggregate to the smallest within the retina – assessing the distribution of fluorescent aggregate sizes
- 6) object_distance: average – average aggregate distance to the optical nerve – provides information about how individual aggregate are distributed throughout the retina
- 7) object_distance: variance – variance of object distance to the optical nerve – captures information about the distribution of objects around a central point
- 8) Object_distance: ratio – ratio of largest to smallest distance to the optical nerve

AGGREGATES EDGE FEATURES

- 9) edges:area_fraction - fraction of the non-zero pixels in an aggregate that are along an edge – distinguishes protein that localizes along the aggregate edges
- 10) edges:homogeneity - Measure of edge intensity homogeneity - captures homogeneity of edge gradients, or ‘are the edges primarily steep or more gradually sloping?’
- 11) edges:direction_maxmin_ratio - Measure of edge direction homogeneity 1 – captures homogeneity of edge direction, or are the edges primarily in one direction or are they more evenly distributed? images with patterns containing edges oriented predominantly along a particular direction result in edge gradient histograms
- 12) edges:direction_maxnextmax_ratio - Measure of edge direction homogeneity 2 – ratio of the largest to the next largest value in the histogram from above feature
- 13) edges:direction_difference - Measure of edge direction difference - this feature distinguish aggregate patterns in which there are parallel edges

SHAPE OF THE AGGREGATES

- 14) obj_skel_len - The average length of the morphological skeleton of the aggregates (or blood vessels in the eye)
- 15) obj_skel_hull_area_ratio - The ratio of aggregate skeleton length to the area of the convex hull of the skeleton, averaged over all aggregates
- 16) obj_skel_branch_per_len - The ratio of the number of branch points in skeleton to length of the vascular skeleton
- 17) convex_hull: fraction of overlap - fraction of convex hull occupied by protein fluorescence (above-threshold pixels)
- 18) convex_hull: shape_factor



Paper: [ncbi.nlm.nih.gov/pmc/articles/PMC7474514/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7474514/)
Code: github.com/Kushal334/DeTraC_COVID19

(a)

Examples of a) normal, b) COVID-19, and c) SARS chest X-ray images

- 80 normal chest images ($4,020 \times 4,892$ pixels)
- 105 COVID-19 images ($4,248 \times 3,480$ pixels) 8-bit depth (max. intensity value 256)
- 11 SARS images ($4,248 \times 3,480$ pixels)

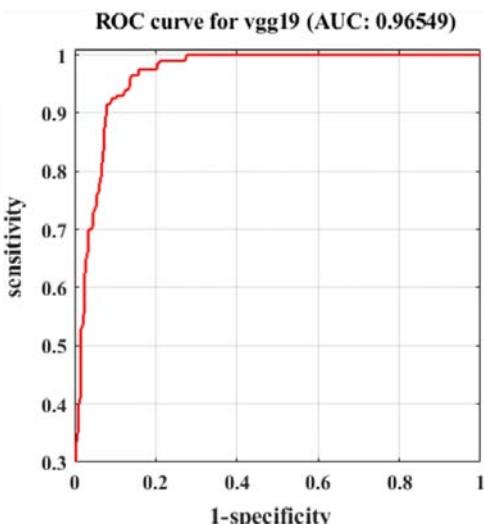
Transfer learning scenarios:

- “shallow tuning” adapts only the last classification layer & freezes the parameters of the remaining layers without training
- “deep tuning” retrains all parameters of the pre-trained network from end-to-end
- “**fine-tuning**” gradually trains more layers by tuning the learning parameters until a significant performance boost is achieved

(b)

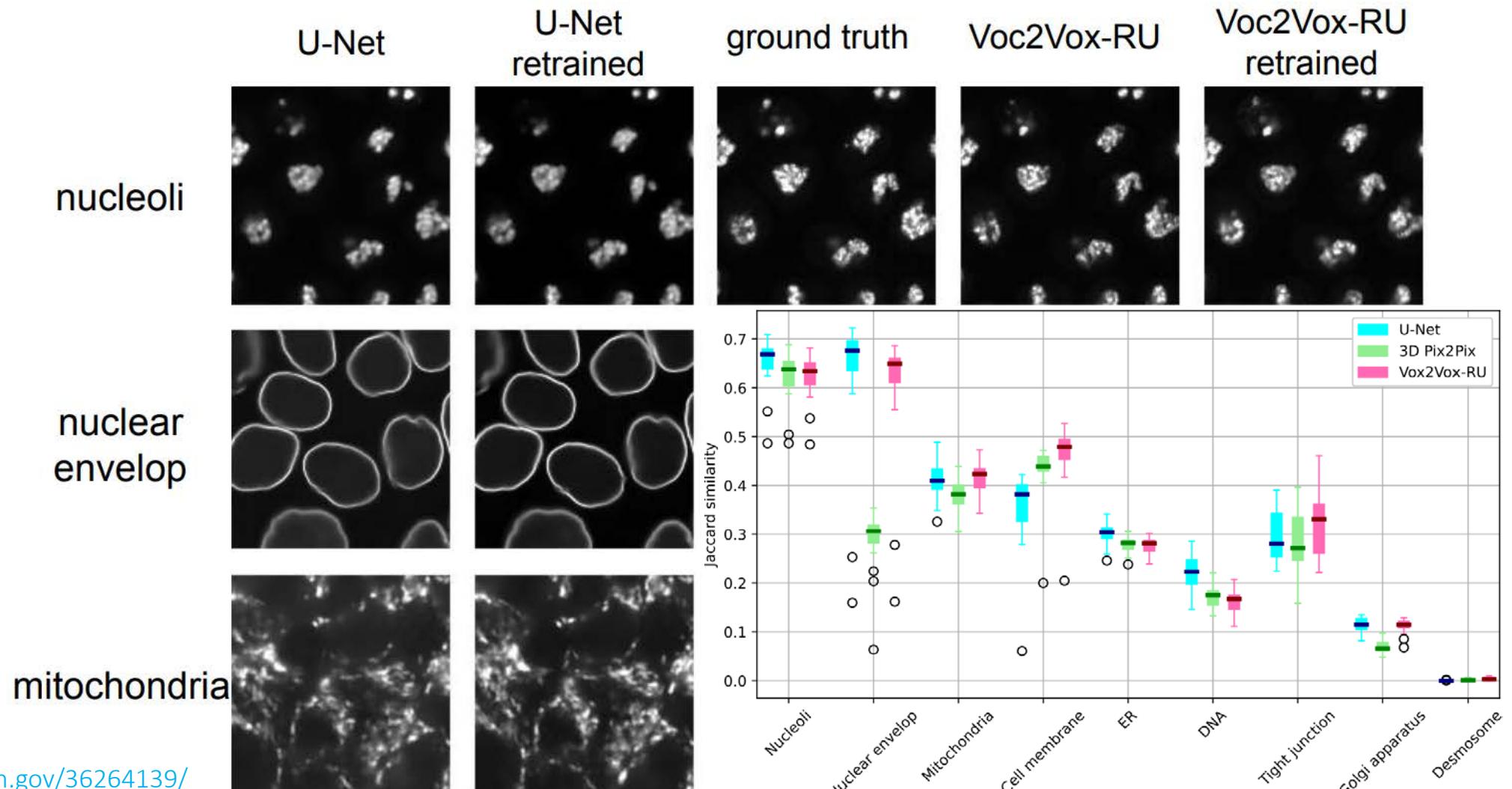
ImageNet (15 million images)
pre-trained CNN networks:

- AlexNet
- VGG19 keras.io/api/applications/vgg
- ResNet
- GoogleNet
- SqueezeNet



Improving and evaluating deep learning models of cellular organization

similarity with
AMDX-2011P
aggregates



Paper: pubmed.ncbi.nlm.nih.gov/36264139/

Code: murphylab.cbd.cmu.edu/Software/2022_insilico/

Storage of image data & delivery of analytics

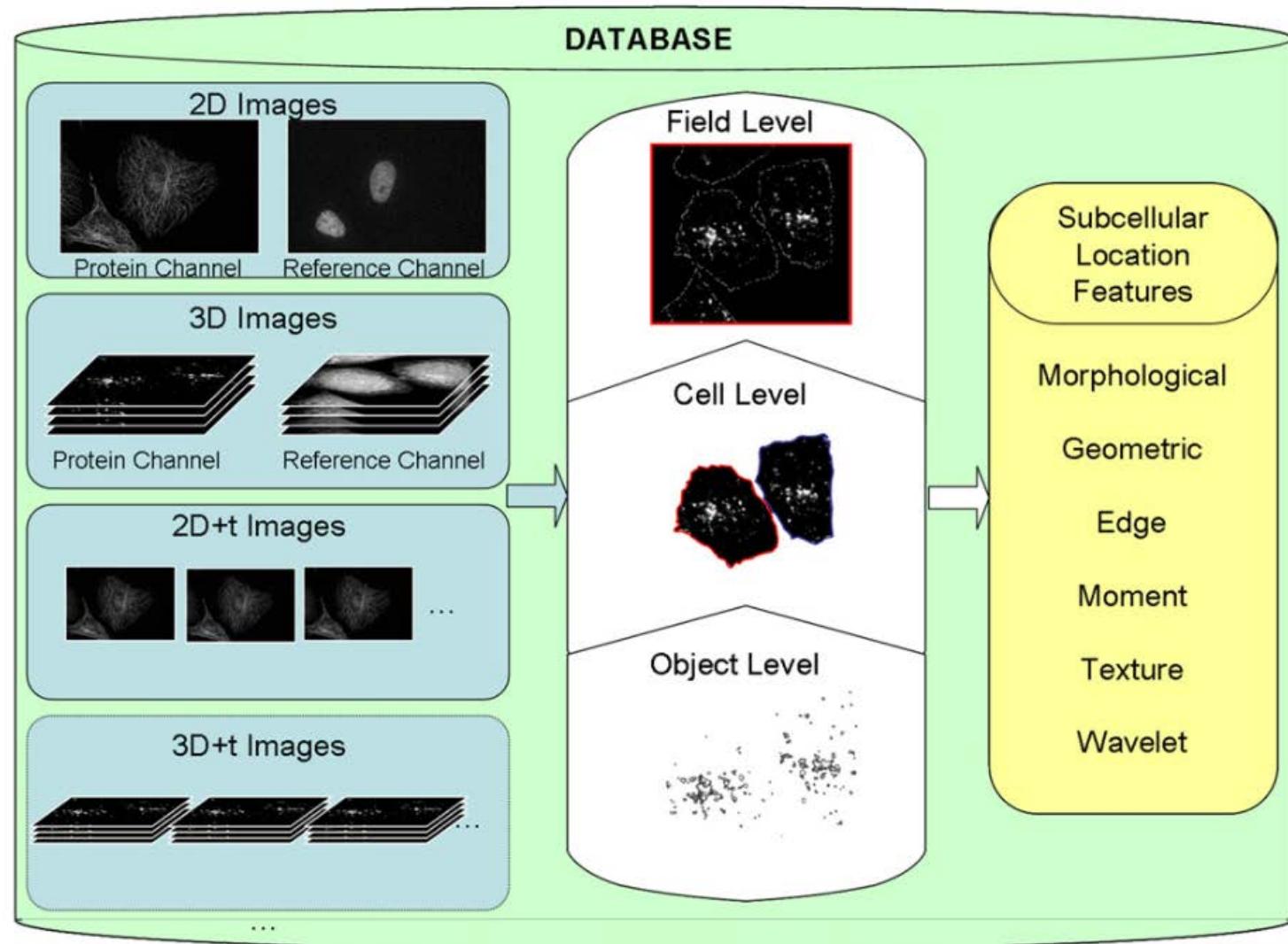


Figure 1. The Image Database Depicted Contains Images with Related Biological Protocol, Acquisition Parameters, and Subcellular Location Features