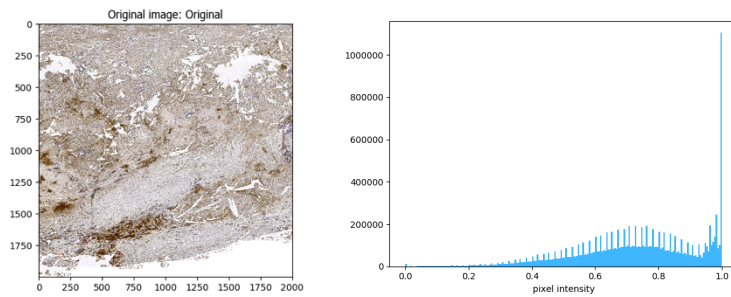
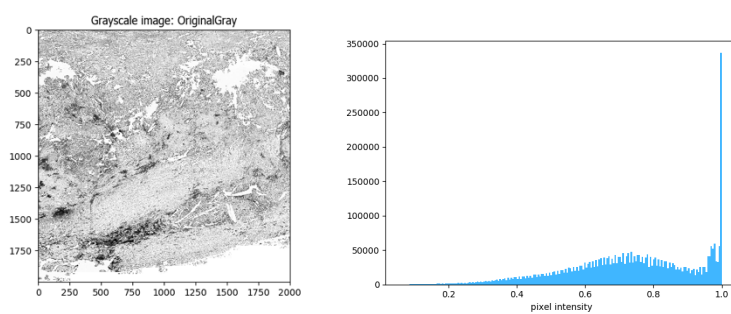


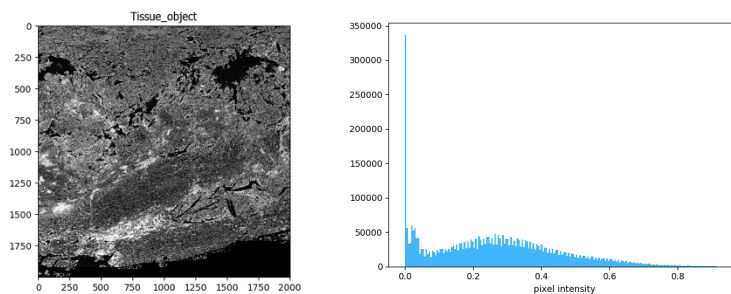
A.



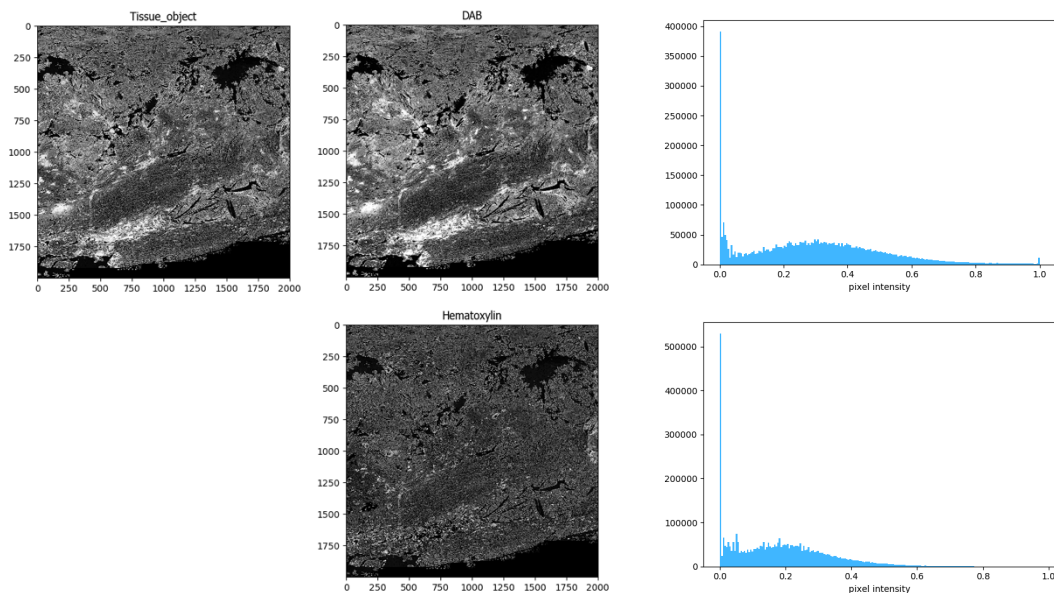
B.



C.



D.



The CD68 CellProfiler pipeline workflow. (Part 1)

[A]. The original image (left) is masked using PathProfiler Tissue Segmentation Unet and used as input by CellProfiler 4.2.6; the graph (right) shows the tonal distribution in the digital whole-slide image on a RGB scale.

[B]. The input image is converted to a gray scaled image (left); the graph (right) shows the tonal distribution in the gray scaled image.

[C]. The gray scaled image is inverted, *i.e.* non-tissue will become black (left); the graph (right) shows the tonal distribution after inverting.

[D]. The colors, *i.e.* stains, are unmixed using the original image (left): DAB (middle-top), and Hematoxylin (HE, middle-bottom). The graphs (right) show the tonal distributions of DAB and Hematoxylin.

[E]. The tissue area is identified, as demarcated by the green line in the left image; the total tissue area size is calculated in pixels (right image) and tabulated (table).

[F]. The Hematoxylin area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the total Hematoxylin area size is calculated in pixels (right image) and tabulated (table).

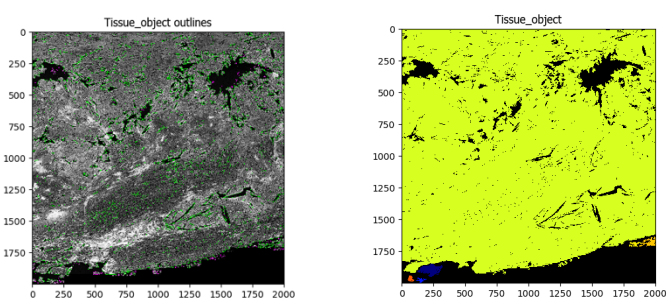
[G]. The DAB area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the total DAB area size is calculated in pixels (right image) and tabulated (table).

[H]. The DAB image (left) is masked by overlaying the identified DAB-positive area (right).

[I]. The Hematoxylin image (left) is masked by overlaying the identified Hematoxylin-positive area (right).

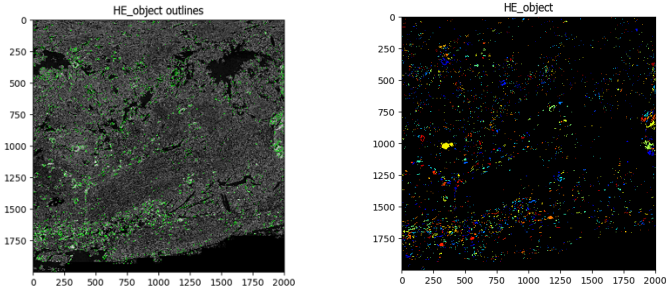
[J]. The DAB nuclei objects are identified, white areas in the left image; the DAB nuclei objects are demarcated by a green line in the middle image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the right image shows all the identified DAB nuclei positive objects in random colors; the total number of identified objects is calculated and tabulated (table).

E.



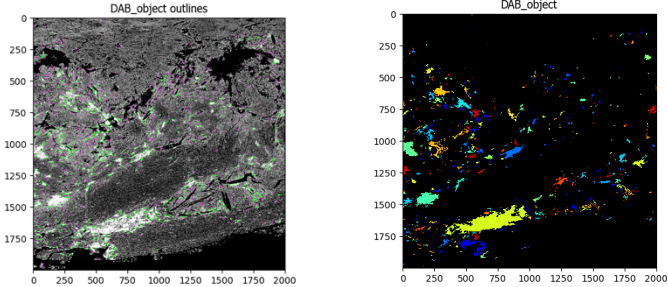
# of accepted objects	6
10th pctile diameter	42.9 pixels
Median diameter	75.1 pixels
90th pctile diameter	2074.9 pixels
Area covered by objects	85.1 %
Thresholding filter size	1.0
Threshold	0.056

F.



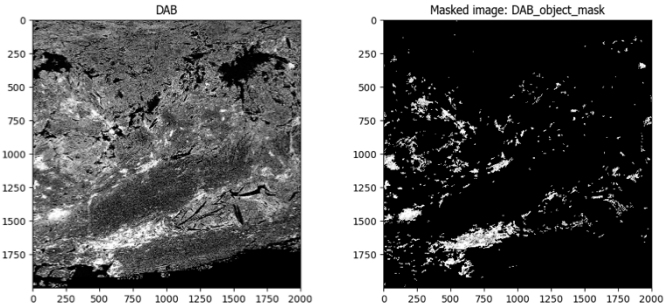
# of accepted objects	8288
10th pctile diameter	1.1 pixels
Median diameter	2.5 pixels
90th pctile diameter	7.0 pixels
Area covered by objects	3.7 %
Thresholding filter size	1.0
Threshold	0.4

G.

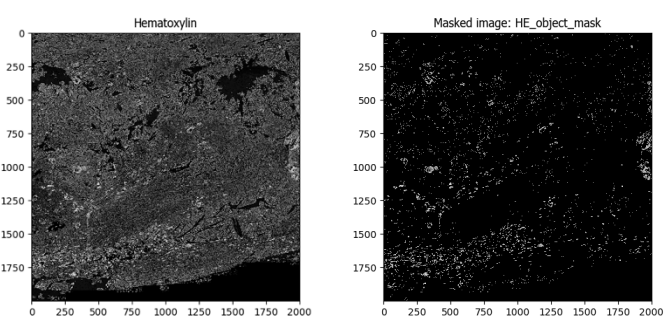


# of accepted objects	531
10th pctile diameter	8.6 pixels
Median diameter	11.8 pixels
90th pctile diameter	30.8 pixels
Area covered by objects	5.8 %
Thresholding filter size	1.0
Threshold	0.6

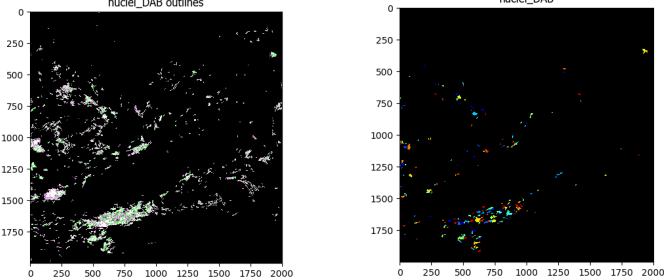
H.



I.



J.



# of accepted objects	218
10th pctile diameter	8.4 pixels
Median diameter	12.4 pixels
90th pctile diameter	22.3 pixels
Area covered by objects	1.0 %
Thresholding filter size	1.0
Threshold	0.85
Declumping smoothing filter size	5.4
Maxima suppression size	5.3

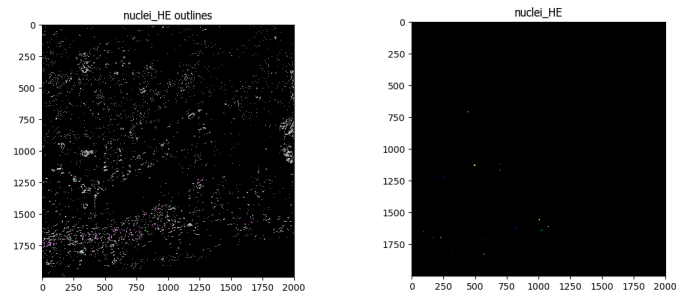
The CD68 CellProfiler pipeline workflow. (Part 2)

[K]. The Hematoxylin nuclei objects are identified, white areas in the left image; the Hematoxylin nuclei objects are demarcated by a green line in the middle image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the right image shows all the identified Hematoxylin nuclei positive objects in random colors; the total number of identified objects is calculated and tabulated (table).

[L]. Finally, the data for each tile are saved in a comma-separated table, including meta-data such as tile positions, image location, object counts (there could be multiple patches of stained areas or tissue). The original image (top-left) is used to outline the DAB- & Hematoxylin-positive objects. The tissue area (dark-green), DAB area (yellow), Hematoxylin area (light-green), DAB nuclei objects (red), and Hematoxylin nuclei objects (blue) are all demarcated in the top-right image. The table (bottom-right) shows the areas occupied by each object class.

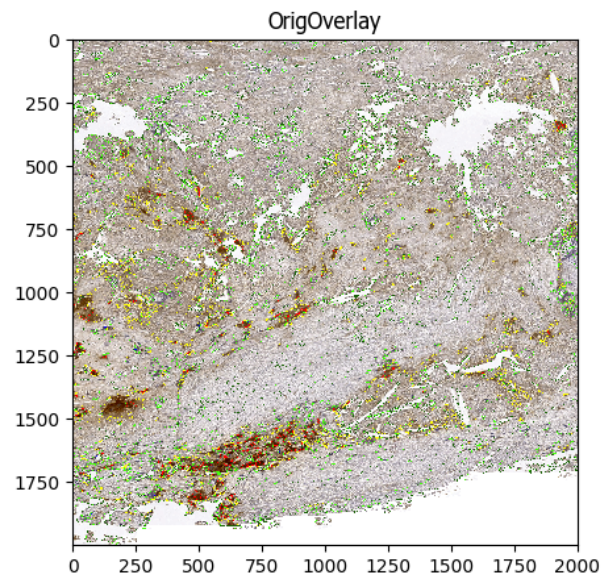
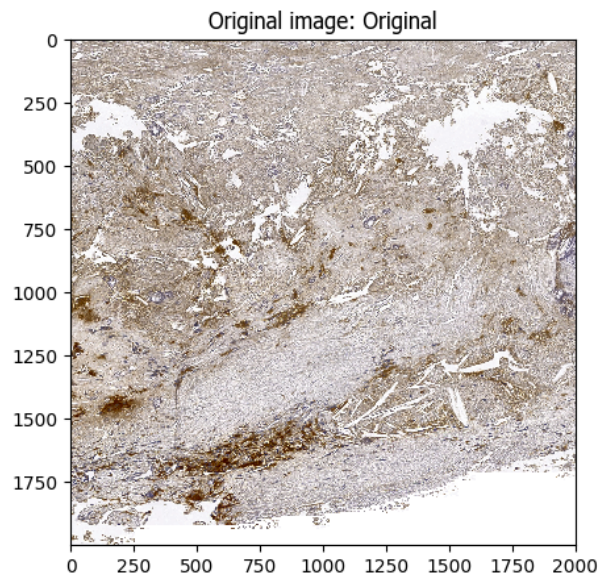
Sample used: AE9.T02-7170.CD68.20141128.TIF [Tile= X22000, Y24000]

K.



# of accepted objects	18
10th pctlile diameter	8.1 pixels
Median diameter	8.6 pixels
90th pctlile diameter	12.5 pixels
Area covered by objects	0.0 %
Thresholding filter size	1.0
Threshold	0.7
Declumping smoothing filter size	5.4
Maxima suppression size	5.3

L.



Objects or Image	Area Occupied	Perimeter	Total Area
DAB_object	232755	56523.0	4000000
HE_object	146705	85509.0	4000000
Tissue_object	3402280	105594.0	4000000
nuclei_DAB	40176	13095.0	232755
nuclei_HE	1243	610.0	146705