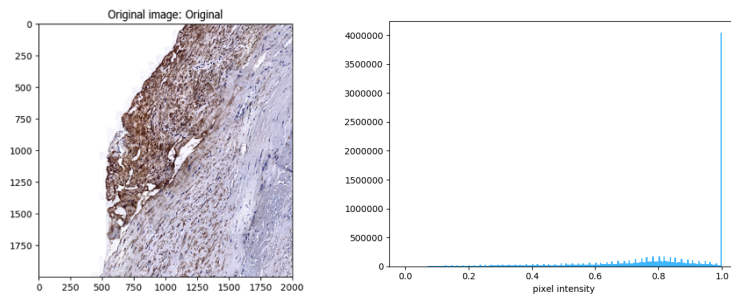
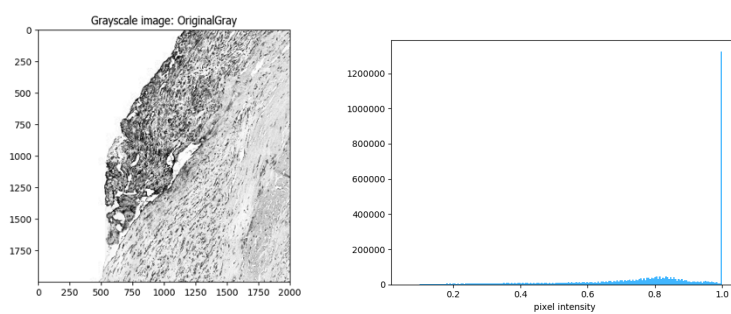


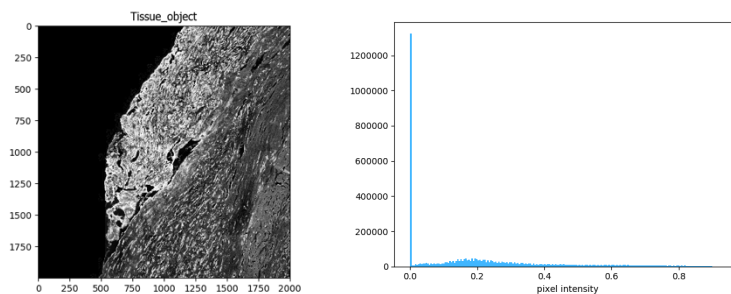
A.



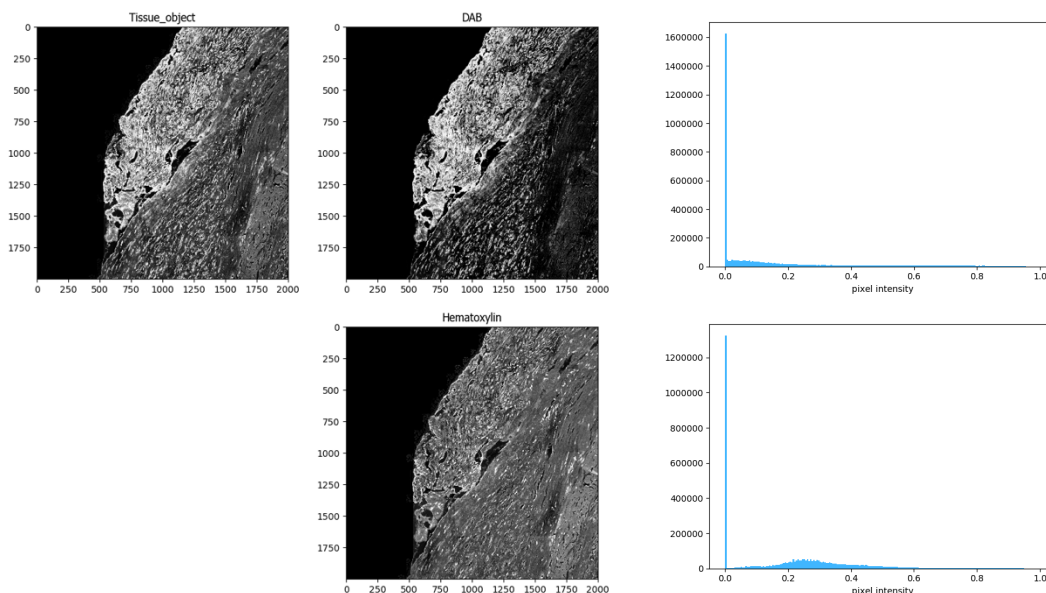
B.



C.



D.



The CD34 DAB 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).

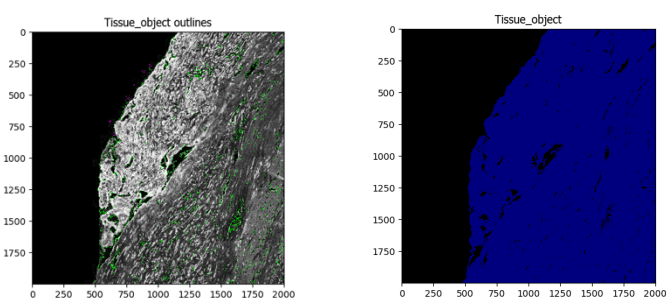
[G]. The DAB area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the total DAB area size is calculated in pixels (right image) and tabulated (table).

[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).

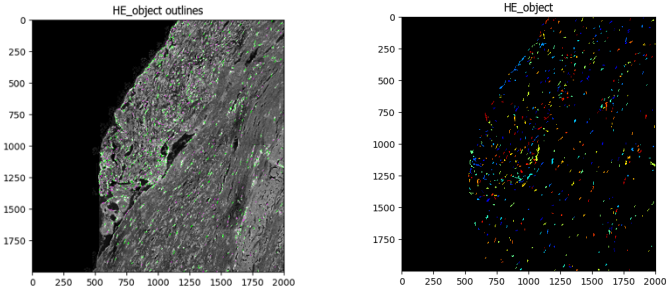
[J]. The DAB nuclei objects are identified, white areas in the left image; the DAB nuclei objects are demarcated by a green line in the middle image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the right image shows all the identified DAB nuclei positive objects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

E.



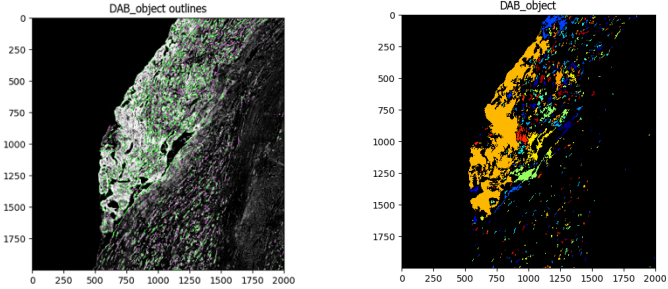
# of accepted objects	1
10th pctlile diameter	1800.2 pixels
Median diameter	1800.2 pixels
90th pctlile diameter	1800.2 pixels
Area covered by objects	63.6 %
Thresholding filter size	1.0
Threshold	0.0626

F.



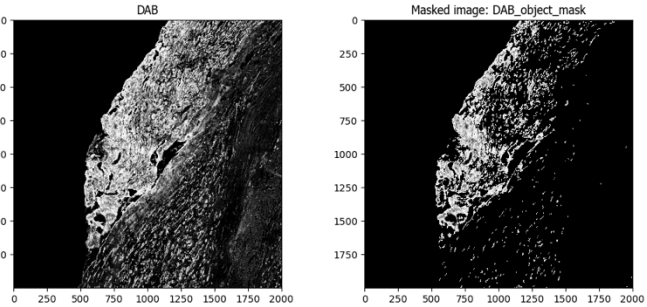
# of accepted objects	596
10th pctlile diameter	8.7 pixels
Median diameter	11.5 pixels
90th pctlile diameter	16.7 pixels
Area covered by objects	2.0 %
Thresholding filter size	1.0
Threshold	0.65

G.

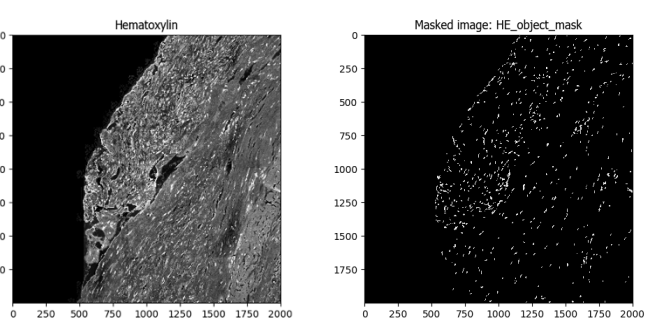


# of accepted objects	580
10th pctlile diameter	8.5 pixels
Median diameter	12.0 pixels
90th pctlile diameter	23.7 pixels
Area covered by objects	9.7 %
Thresholding filter size	1.0
Threshold	0.6

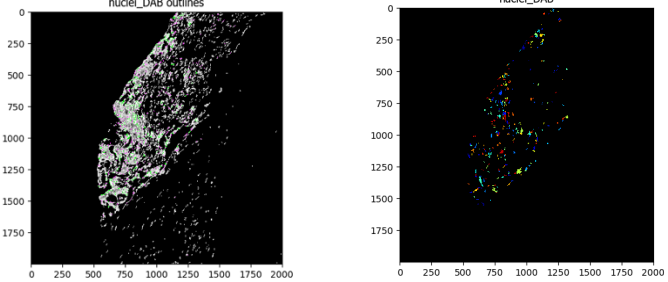
H.



I.



J.



# of accepted objects	259
10th pctlile diameter	8.6 pixels
Median diameter	11.2 pixels
90th pctlile diameter	17.6 pixels
Area covered by objects	0.9 %
Thresholding filter size	1.0
Threshold	0.85
Declumping smoothing filter size	5.4
Maxima suppression size	5.3

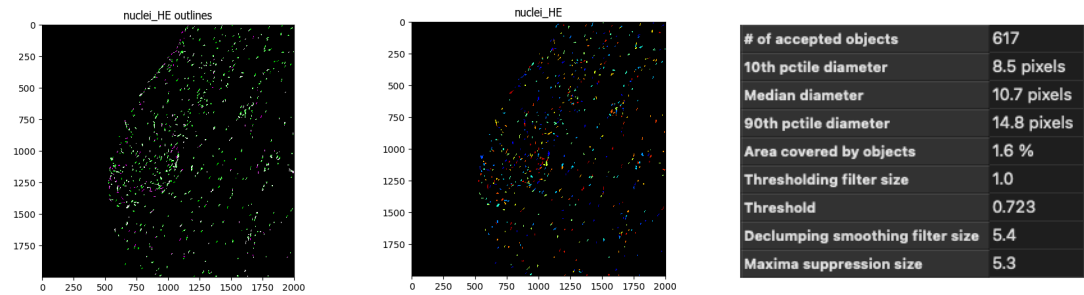
The CD68 CellProfiler pipeline workflow. (Part 2)

[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: AE2608.CD34.1010.TIF [Tile= X10000, Y4000]

K.



L.

