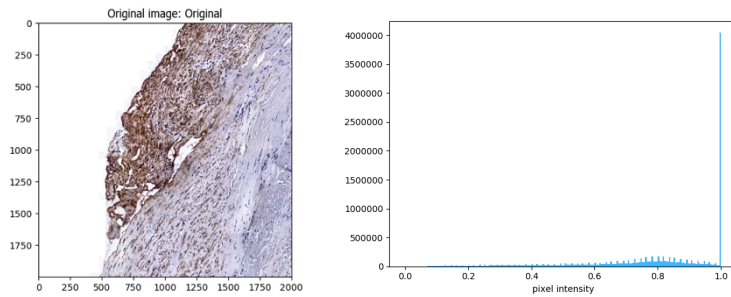
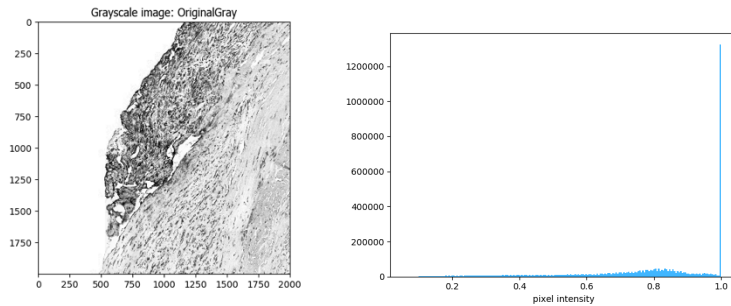


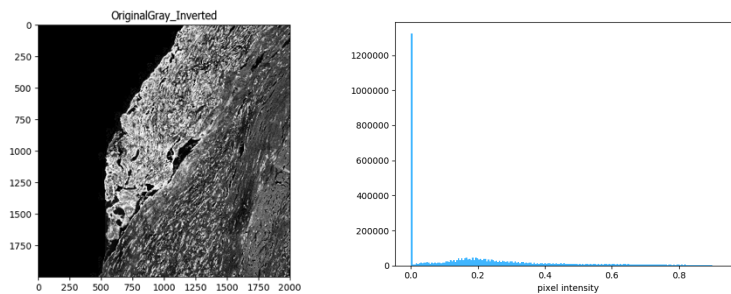
A.



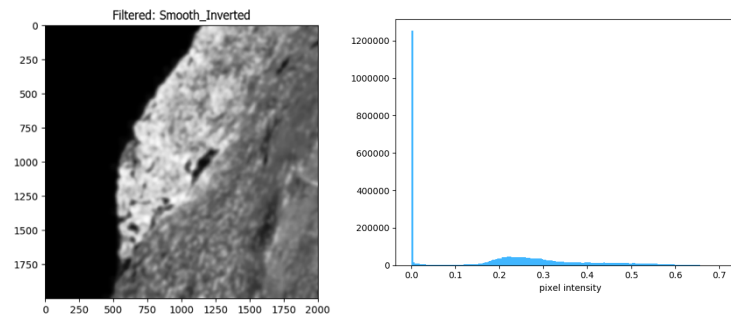
B.



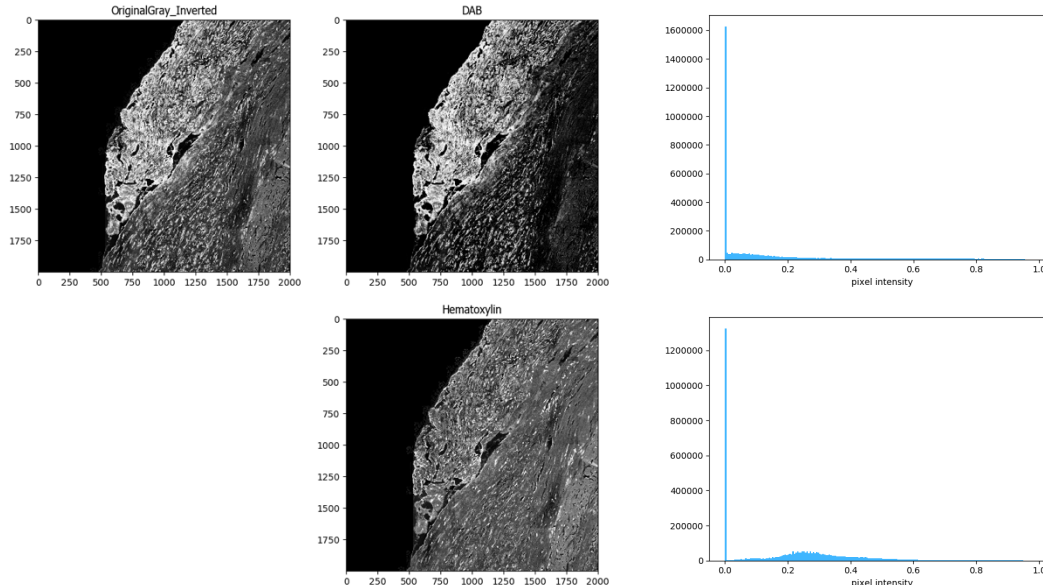
C.



D.



E.



The CD34 DAB CellProfiler pipeline workflow.

[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]. A Gaussian filter is applied to smoothen the image and reduce image artefacts (artifact size 20 pixels) and noise (left); the graph (right) shows the tonal distribution after smoothening.

[E]. 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.

[F]. 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).

[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).

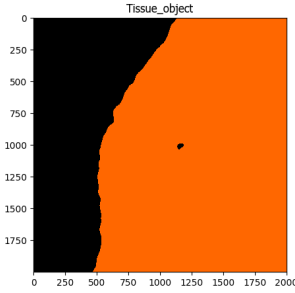
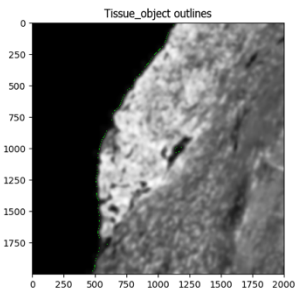
[H]. The Hematoxylin area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 6 pixels) are demarcated in magenta; the total Hematoxylin area size is calculated in pixels (right image) and tabulated (table).

[I]. The HE-positive identified objects are filtered using the identified DAB objects. If a HE-positive object does not lay within the DAB area, it is discarded.

[J]. 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 (blue), Hematoxylin objects (red), and Filtered objects (yellow) are all demarcated in the top-right image. The table (bottom-right) shows the areas occupied by each object class.

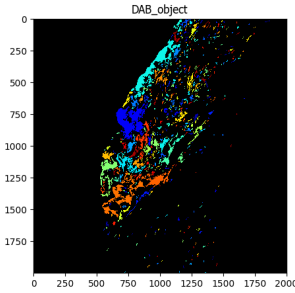
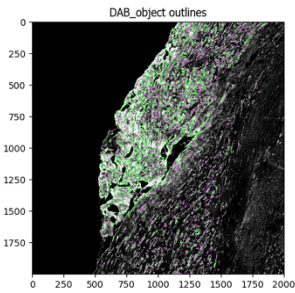
Sample used: AE2608.CD34.1010.TIF [Tile= X10000, Y4000]

F.



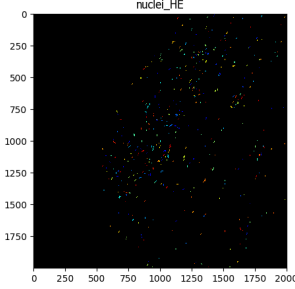
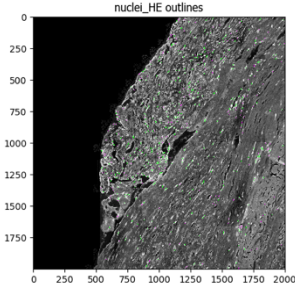
# of accepted objects	1
10th pctlile diameter	1843.7 pixels
Median diameter	1843.7 pixels
90th pctlile diameter	1843.7 pixels
Area covered by objects	66.7 %
Thresholding filter size	1.0
Threshold	0.05

G.



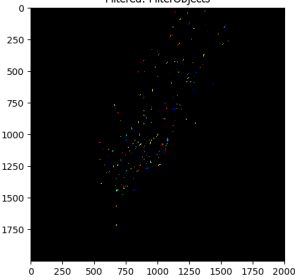
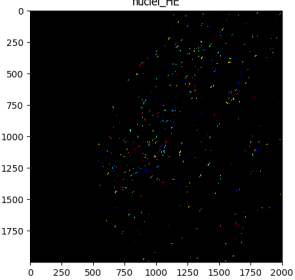
# of accepted objects	500
10th pctlile diameter	8.6 pixels
Median diameter	11.6 pixels
90th pctlile diameter	27.6 pixels
Area covered by objects	6.2 %
Thresholding filter size	1.0
Threshold	0.67

H.



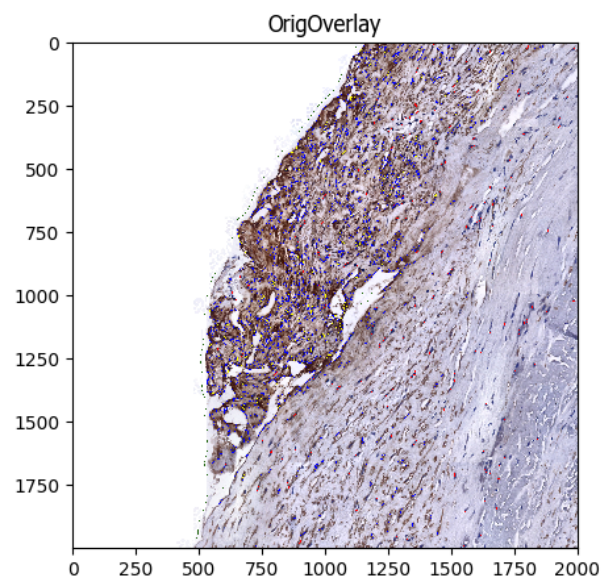
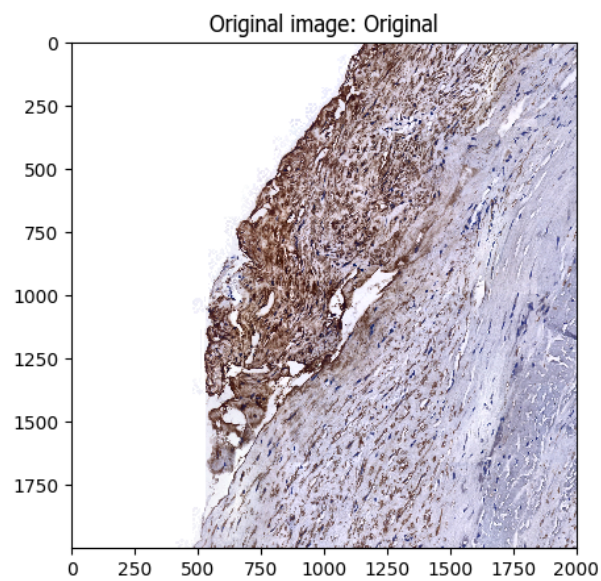
# of accepted objects	500
10th pctlile diameter	8.6 pixels
Median diameter	11.6 pixels
90th pctlile diameter	27.6 pixels
Area covered by objects	6.2 %
Thresholding filter size	1.0
Threshold	0.67

I.



Number of objects pre-filtering	426
Number of objects post-filtering	181
Number of objects removed	245

J.



Objects or Image	Area Occupied	Perimeter	Total Area
DAB_object	249420	64050.0	4000000
FilterObjects	10965	6278.0	4000000
Tissue_object	2669630	6940.0	4000000
nuclei_HE	25857	14471.0	4000000