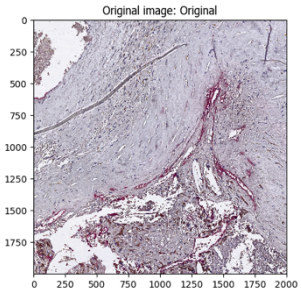
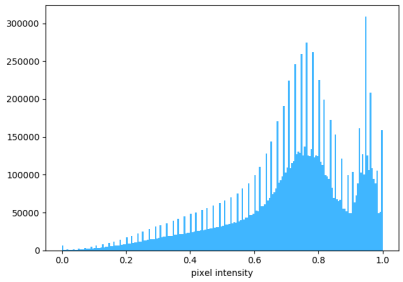
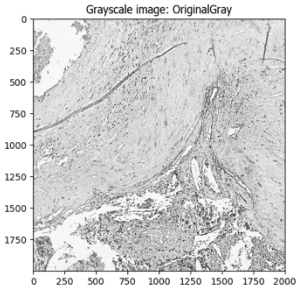
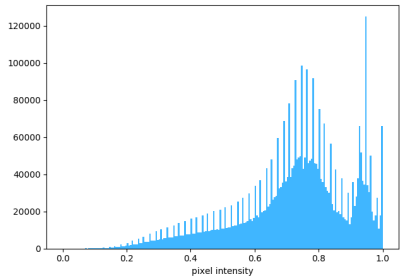
A.



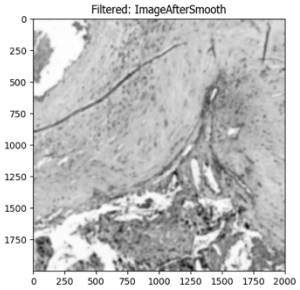


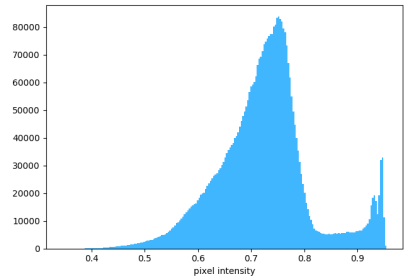
B.



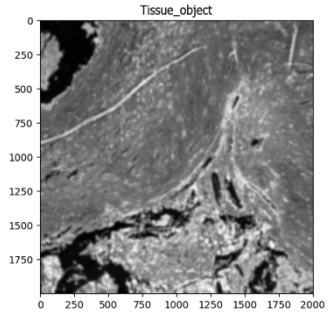


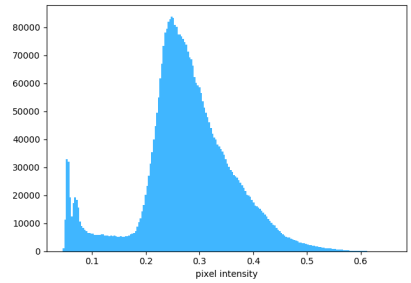
C.



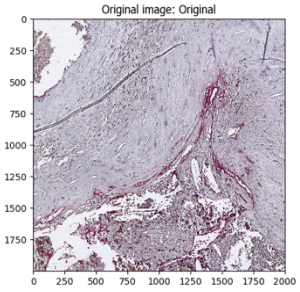


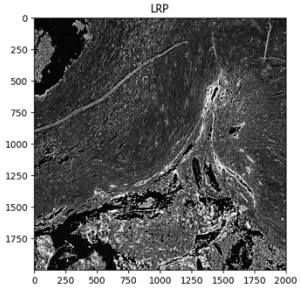
D.

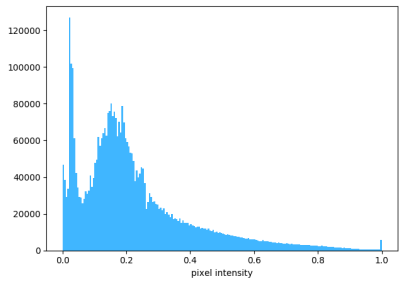


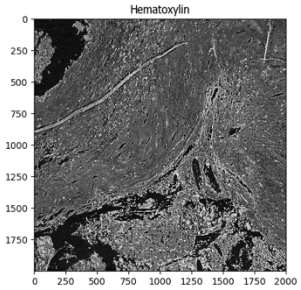


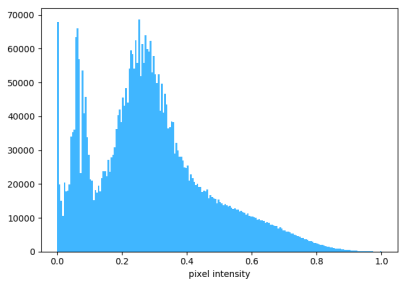
E.











The CD34 LRP `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]. 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.

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

[E]. The colors, *i.e.* stains, are unmixed using the original image (left): LRP (middle-top), and Hematoxylin (HE, middle-bottom). The graphs (right) show the tonal distributions of LRP 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 LRP area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 10 pixels) are demarcated in magenta; the total LRP area size is calculated in pixels (right image) and tabulated (table).

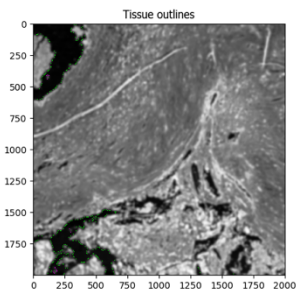
[H]. The Hematoxylin area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 6 pixels) are demarcated in magenta; the total Hematoxylin area size is calculated in pixels (right image) and tabulated (table).

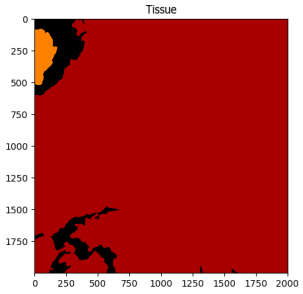
[I]. The HE-positive identified objects are filtered using the identified LRP objects. If a HE-positive object does not lay within the LRP area, it is discarded.

[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 LRP- & Hematoxylin-positive objects. The tissue area (red), LRP area (green), Hematoxylin objects (blue), 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: AE5.T02-6890.CD34.TIF [Tile= X4000, Y4000]

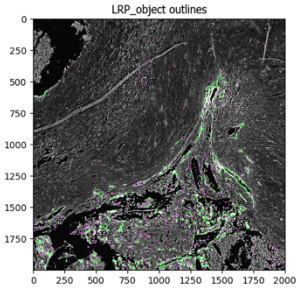
F.

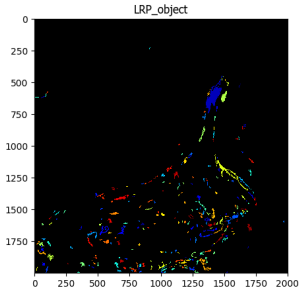




# of accepted objects	2
10th pctile diameter	260.4 pixels
Median diameter	2181.6 pixels
90th pctile diameter	2181.6 pixels
Area covered by objects	94.8 %
Thresholding filter size	1.0
Threshold	0.1

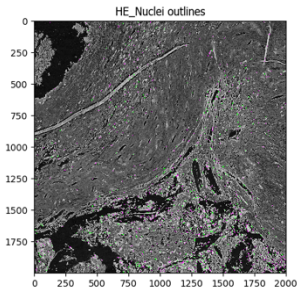
G.

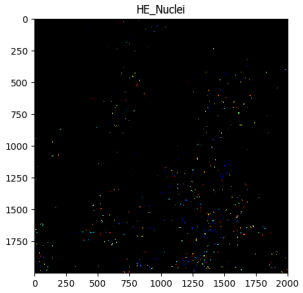




# of accepted objects	263
10th pctile diameter	10.5 pixels
Median diameter	14.7 pixels
90th pctile diameter	28.8 pixels
Area covered by objects	2.3 %
Thresholding filter size	1.0
Threshold	0.68

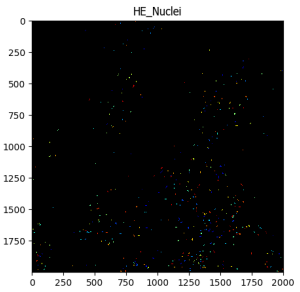
H.

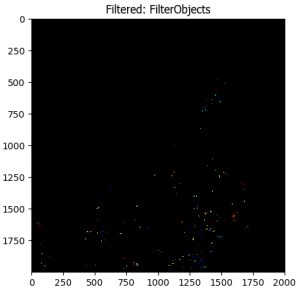




# of accepted objects	452
10th pctile diameter	6.3 pixels
Median diameter	7.7 pixels
90th pctile diameter	10.2 pixels
Area covered by objects	0.6 %
Thresholding filter size	0.0
Threshold	0.77

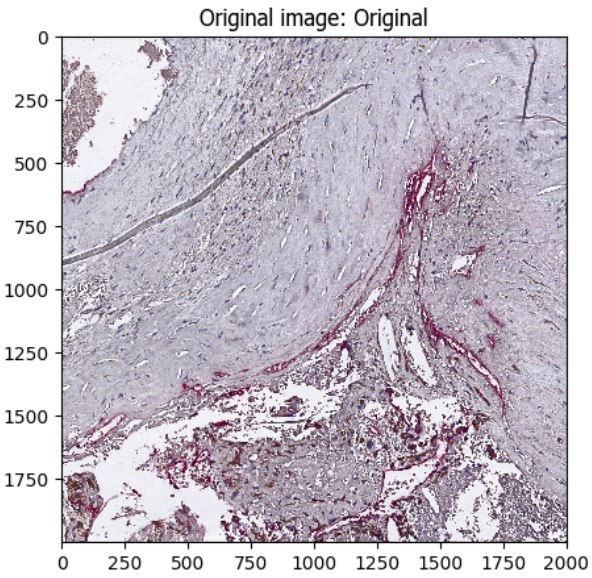
I.

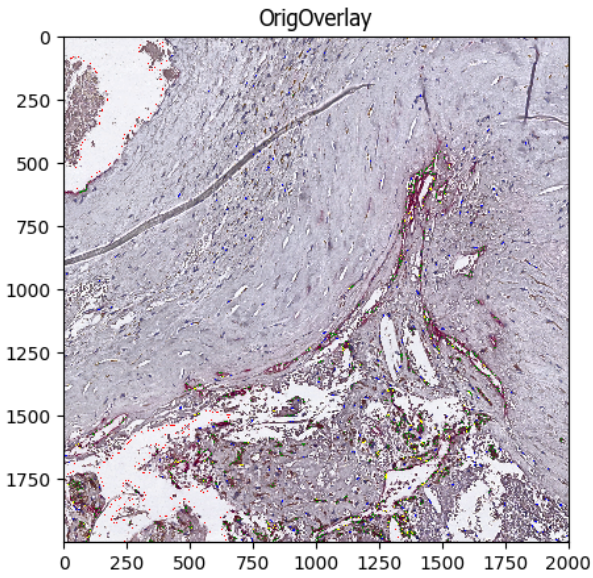




Number of objects pre-filtering	452
Number of objects post-filtering	163
Number of objects removed	289

J.





Objects or Image	Area Occupied	Perimeter	Total Area
FilterObjects	8817	5349.0	4000000
HE_Nuclei	23342	13886.0	4000000
LRP_object	93033	29437.0	4000000
Tissue	3791125	13249.0	4000000