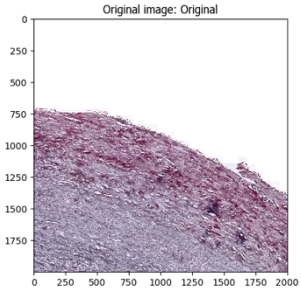
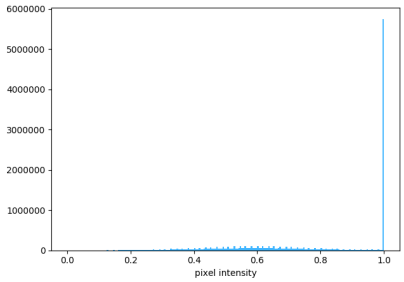
A.





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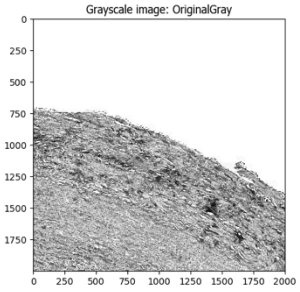

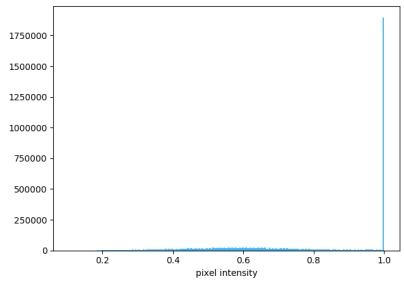

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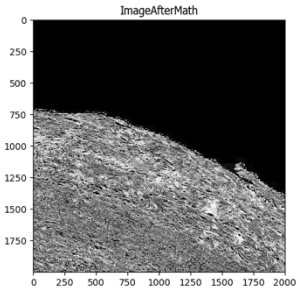
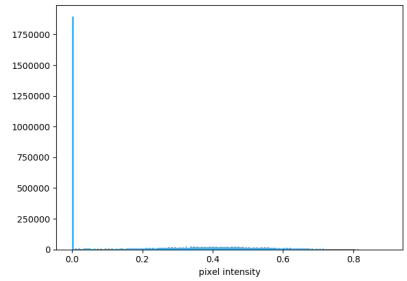
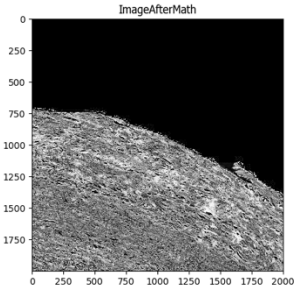
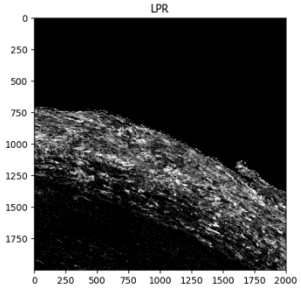
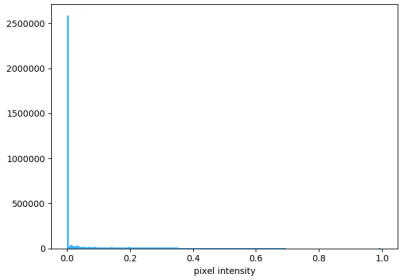


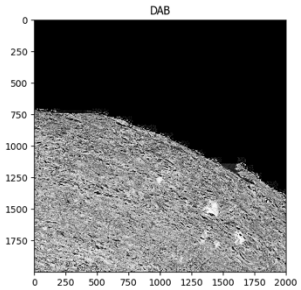


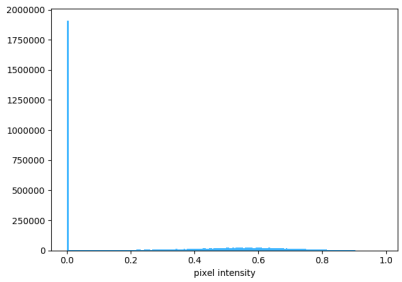
D.











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]. 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): LRP (middle-top), and DAB (middle-bottom). The graphs (right) show the tonal distributions of LRP and DAB.

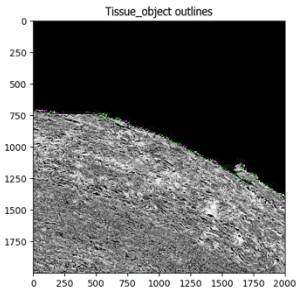
[E]. The tissue area is identified, as demarcated by the green line in the left image; the total tissue area size is calculated in pixels (right image) and tabulated (table).

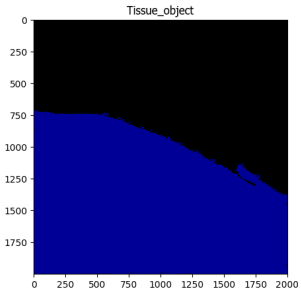
[F]. The LRP area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the total LRP area size is calculated in pixels (right image) and tabulated (table).

[G]. 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-positive objects. The tissue area (dark-green) and LRP area (red) are all demarcated in the top-right image. The table (bottom-right) shows the areas occupied by each object class.

Sample used: AE4.T02-5712.CD34.TIF [Tile= X0, Y14000]

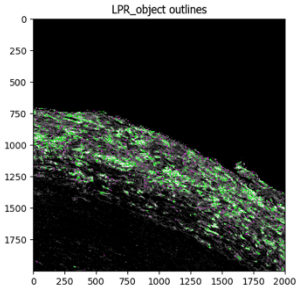
E.

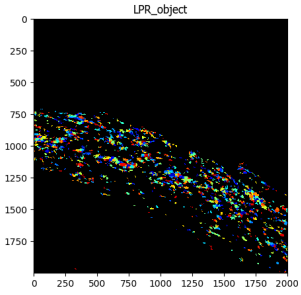




# of accepted objects	1
10th pctile diameter	1629.0 pixels
Median diameter	1629.0 pixels
90th pctile diameter	1629.0 pixels
Area covered by objects	52.1 %
Thresholding filter size	1.0
Threshold	0.1
Declumping smoothing filter size	26.9
Maxima suppression size	7.0

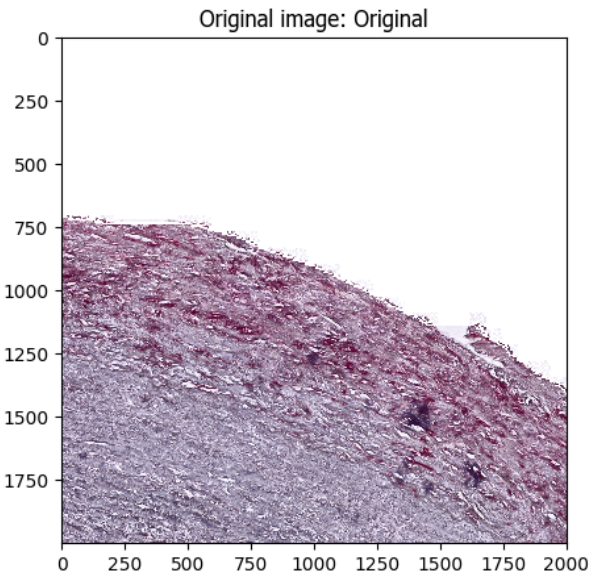
F.

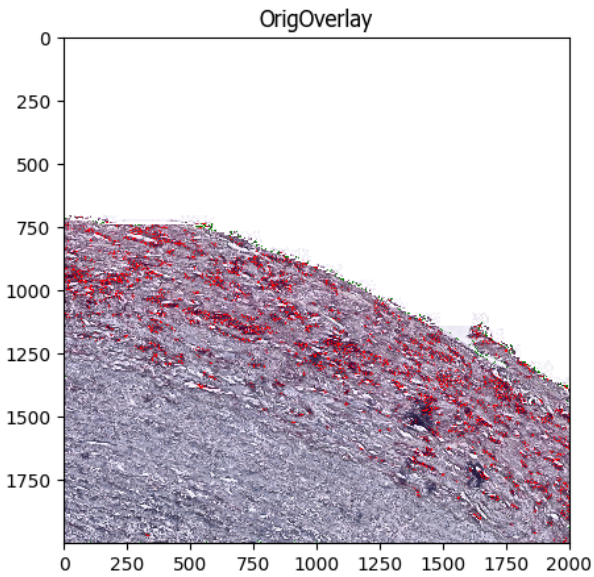




# of accepted objects	1438
10th pctile diameter	9.0 pixels
Median diameter	12.7 pixels
90th pctile diameter	20.2 pixels
Area covered by objects	6.1 %
Thresholding filter size	1.0
Threshold	0.4
Declumping smoothing filter size	5.4
Maxima suppression size	5.3

G.





Objects or Image	Area Occupied	Perimeter	Total Area
LPR_object	242236	81590.0	4000000
Tissue_object	2084265	9056.0	4000000