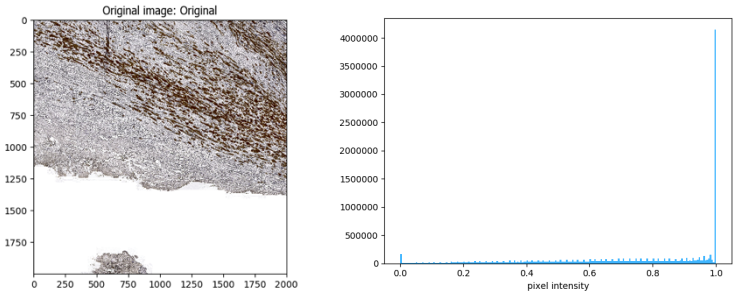
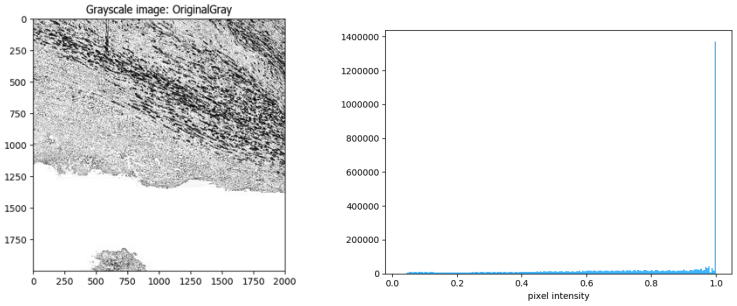
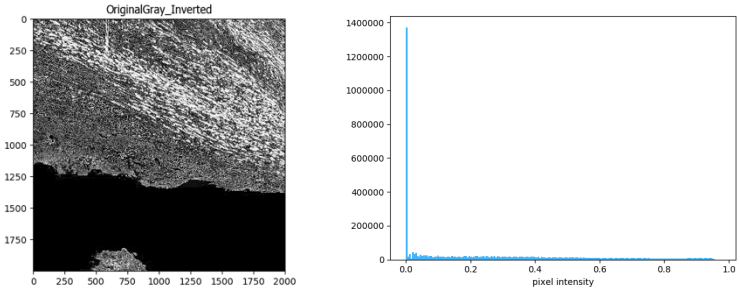
A.



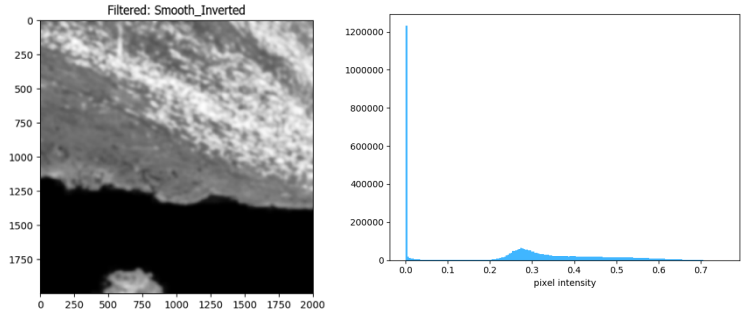
B.



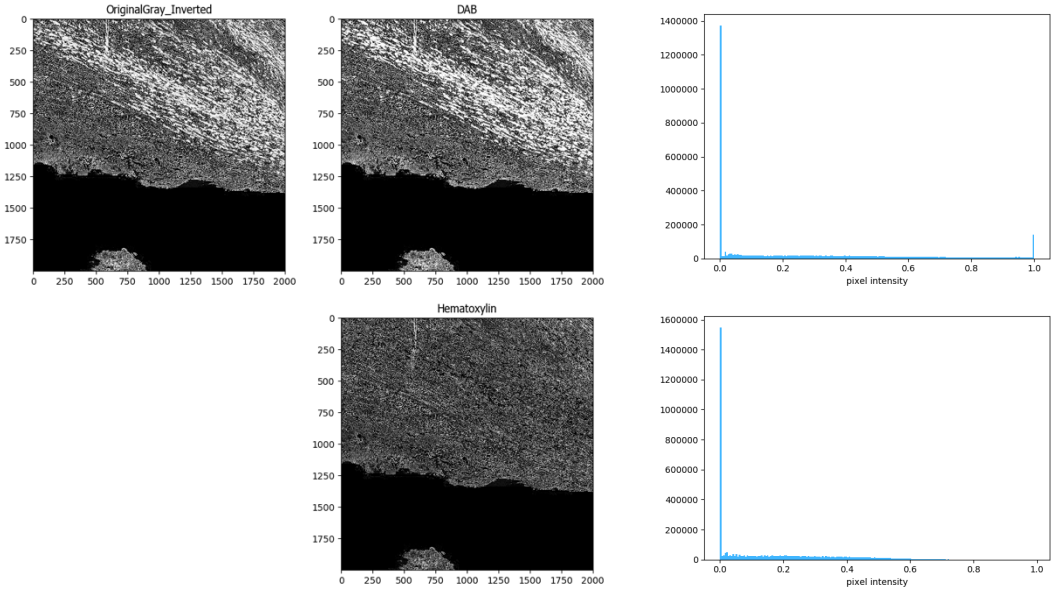
C.



D.



E.



The SMA `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).

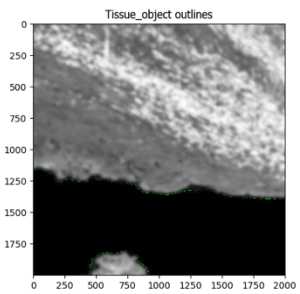
[H]. The Hematoxylin area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 7 pixels) are demarcated in magenta; the total Hematoxylin area size is calculated in pixels (right image) and tabulated (table).

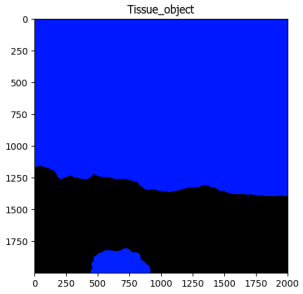
[I]. The HE-positive identified objects are filtered using the identified DAB objects. If a HE-positive object does not lay within the DAB area, it is discarded.

[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: AE9.T02-7170.SMA.20141128.TIF [Tile= X8000, Y26000]

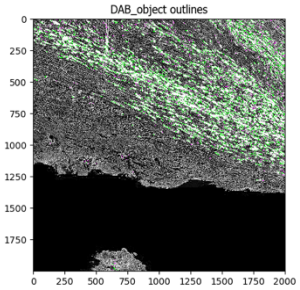
F.

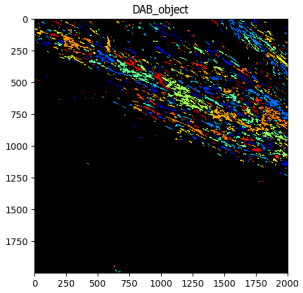




# of accepted objects	2
10th pctile diameter	298.9 pixels
Median diameter	1825.6 pixels
90th pctile diameter	1825.6 pixels
Area covered by objects	67.2 %
Thresholding filter size	1.0
Threshold	0.05

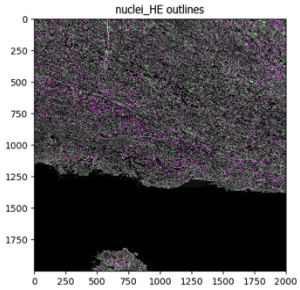
G.

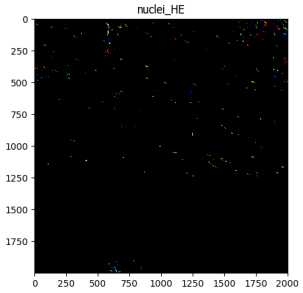




# of accepted objects	809
10th pctile diameter	9.0 pixels
Median diameter	14.1 pixels
90th pctile diameter	31.4 pixels
Area covered by objects	8.4 %
Thresholding filter size	1.0
Threshold	0.8

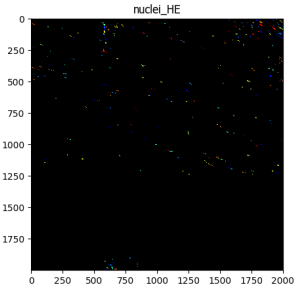
H.

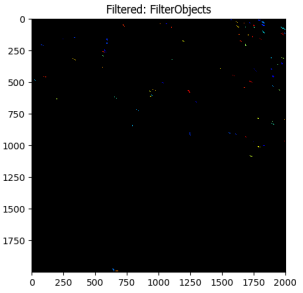




# of accepted objects	241
10th pctile diameter	7.2 pixels
Median diameter	8.5 pixels
90th pctile diameter	12.8 pixels
Area covered by objects	0.4 %
Thresholding filter size	0.0
Threshold	0.59

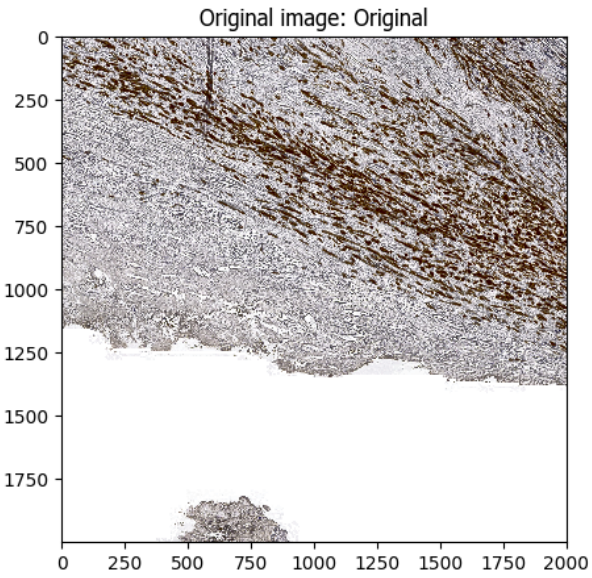
I.

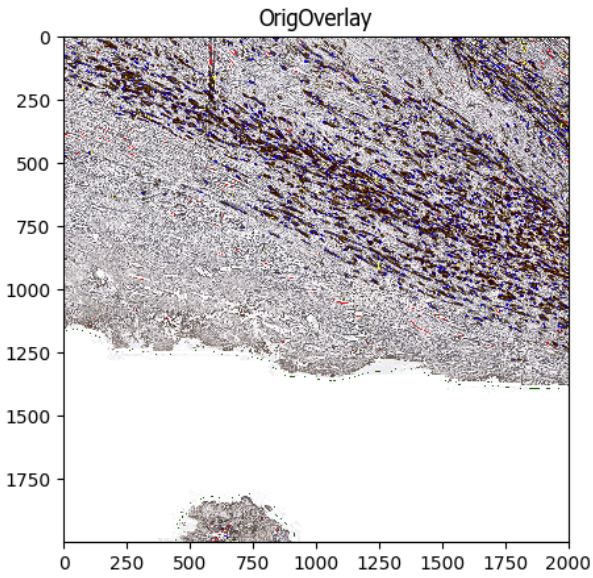




Number of objects pre-filtering	241
Number of objects post-filtering	81
Number of objects removed	160

J.





Objects or Image	Area Occupied	Perimeter	Total Area
DAB_object	334671	98404.0	4000000
FilterObjects	6452	3953.0	4000000
Tissue_object	2687722	8017.0	4000000
nuclei_HE	17587	11583.0	4000000