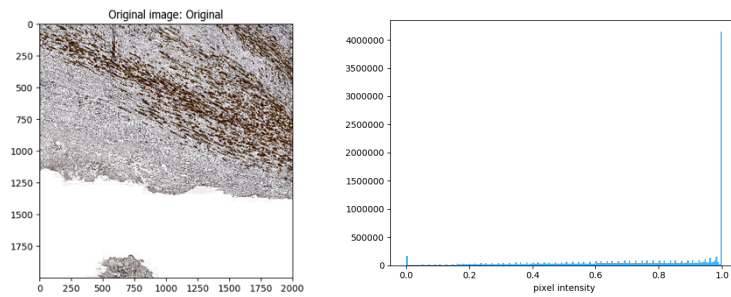
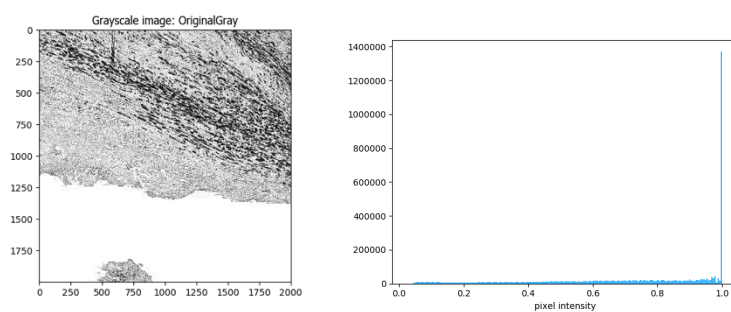


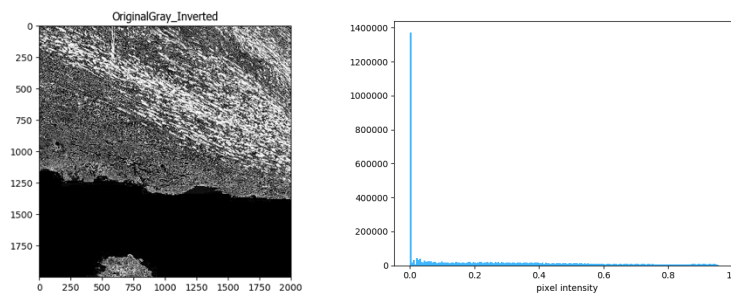
A.



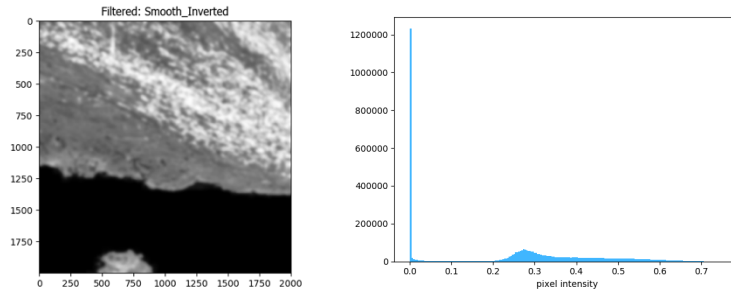
B.



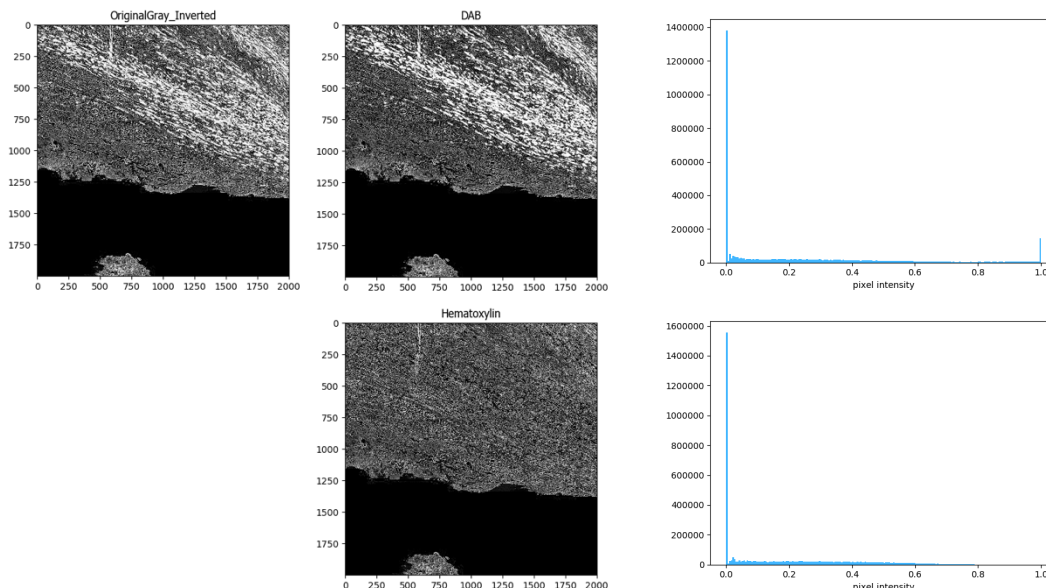
C.



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 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 6 pixels) are demarcated in magenta; the total DAB area size is calculated in pixels (right image) and tabulated (table).

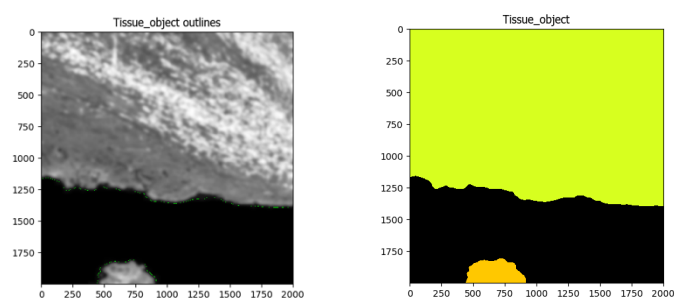
[H]. The DAB image (left) is masked by overlaying the identified DAB-positive area (right).

[I]. The 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).

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

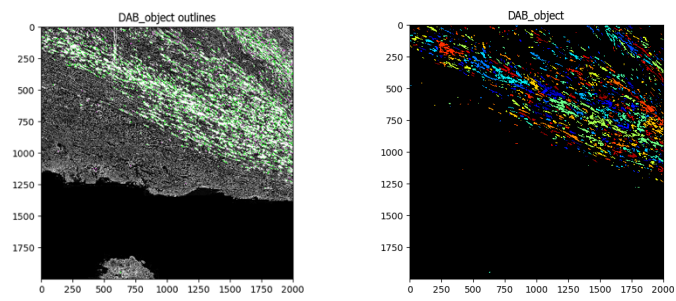
Sample used: AE9.T02-7170.SMA.20141128.TIF [Tile= X8000, Y26000]

F.



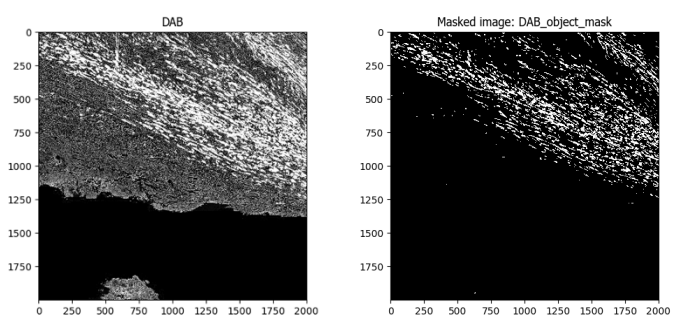
# of accepted objects	2
10th pctlile diameter	298.9 pixels
Median diameter	1825.6 pixels
90th pctlile diameter	1825.6 pixels
Area covered by objects	67.2 %
Thresholding filter size	1.0
Threshold	0.05

G.

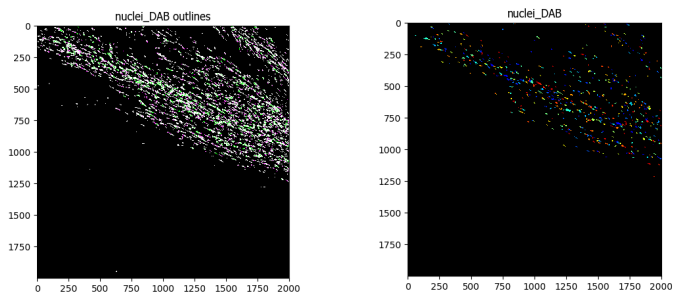


# of accepted objects	982
10th pctlile diameter	7.0 pixels
Median diameter	12.1 pixels
90th pctlile diameter	27.4 pixels
Area covered by objects	7.7 %
Thresholding filter size	1.0
Threshold	0.81

H.

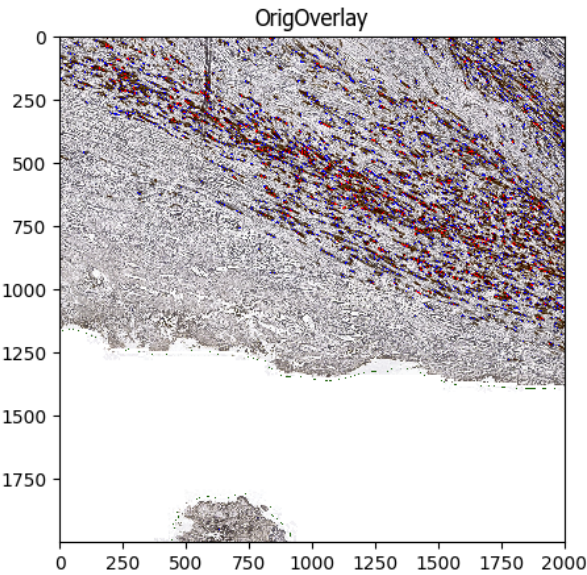
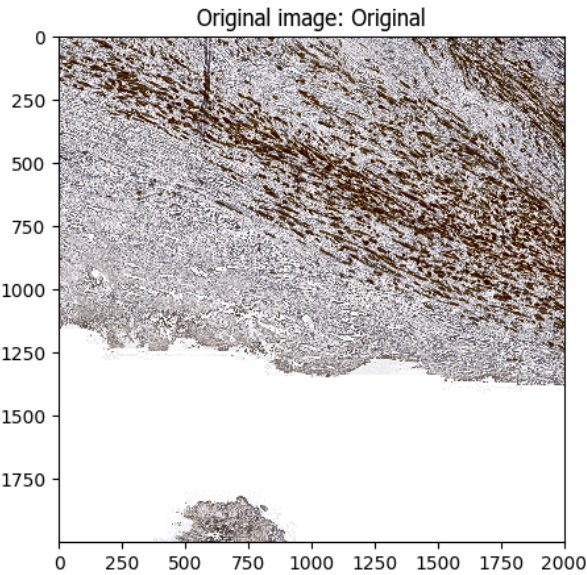


I.



# of accepted objects	595
10th pctlile diameter	8.4 pixels
Median diameter	10.3 pixels
90th pctlile diameter	14.5 pixels
Area covered by objects	1.5 %
Thresholding filter size	1.0
Threshold	0.99
Declumping smoothing filter size	5.4
Maxima suppression size	5.3

J.



Objects or Image	Area Occupied	Perimeter	Total Area
DAB_object	306296	94685.0	4000000
Tissue_object	2687722	8017.0	4000000
nuclei_DAB	59628	25908.0	306296