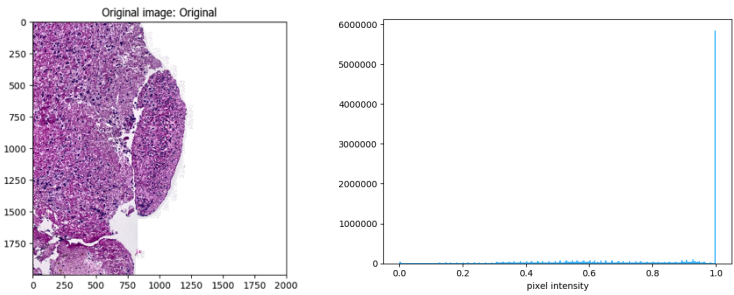
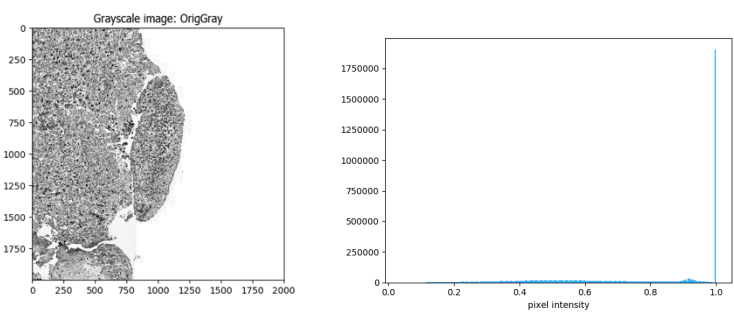


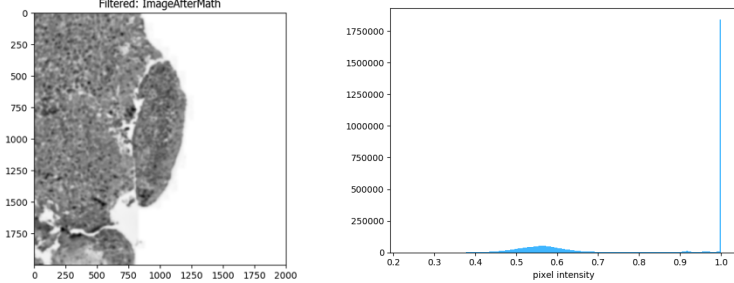
A.



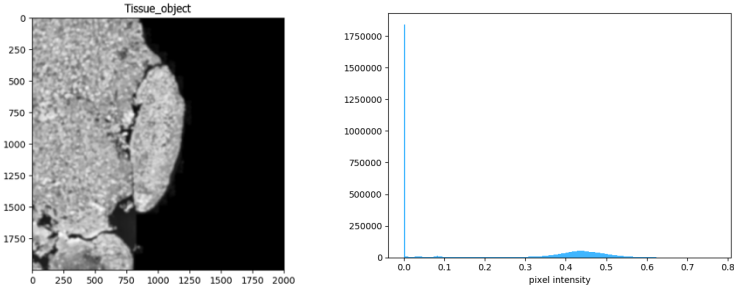
B.



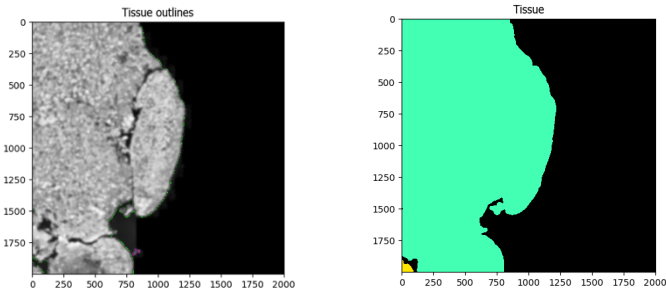
C.



D.



E.



The HE 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 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 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 colors, *i.e.* stains, are unmixed using the original image (left): HE (middle-top), and Eosin (HE, middle-bottom). The graphs (right) show the tonal distributions of HE and Eosin.

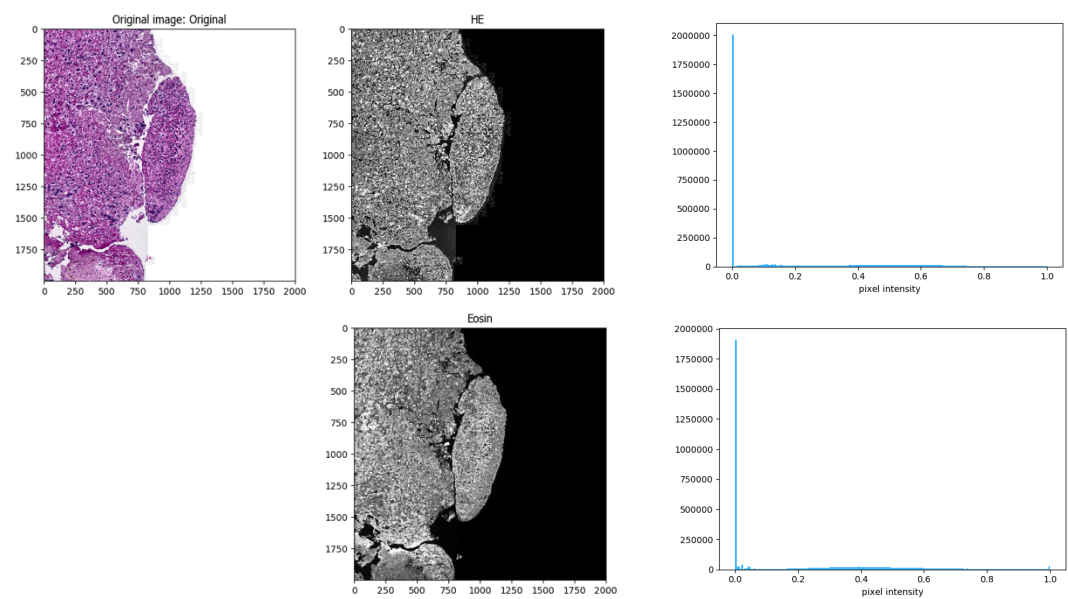
[G]. The HE area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 7 pixels) are demarcated in magenta; the total HE area size is calculated in pixels (right image) and tabulated (table).

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

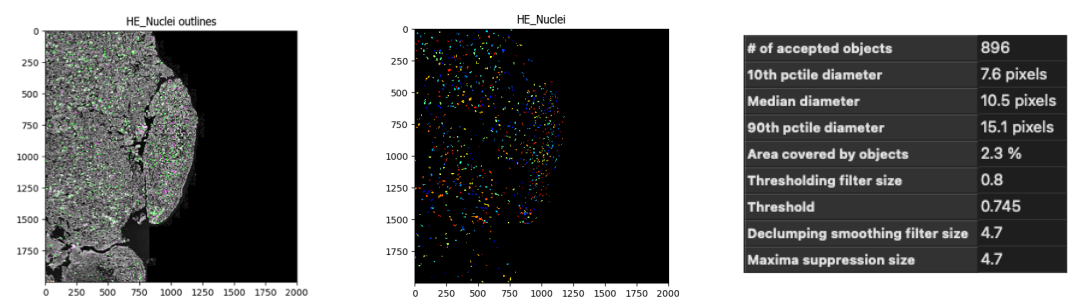
Sample used: AE2229.UMC.HE.ndpi [Tile= X22000, Y18000]

# of accepted objects	2
10th pctlle diameter	84.4 pixels
Median diameter	1591.2 pixels
90th pctlle diameter	1591.2 pixels
Area covered by objects	49.9 %
Thresholding filter size	1.0
Threshold	0.13
Declumping smoothing filter size	33.6
Maxima suppression size	7.0

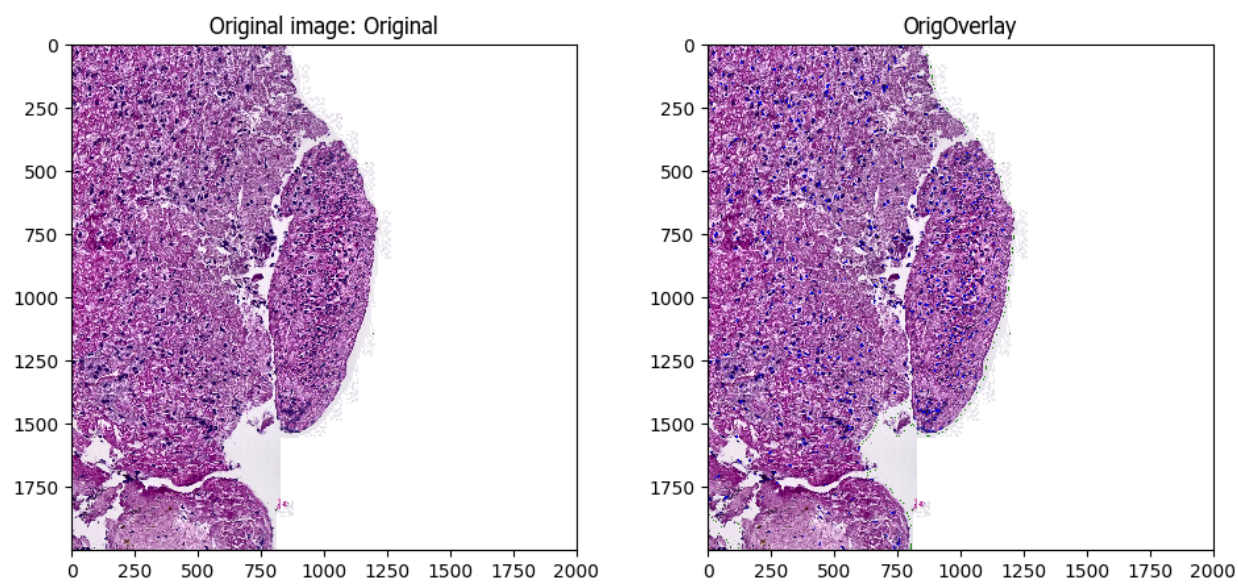
F.



G.



H.



Objects or Image	Area Occupied	Perimeter	Total Area
HE_Nuclei	90183	35600.0	4000000
Tissue	1994211	7302.0	4000000