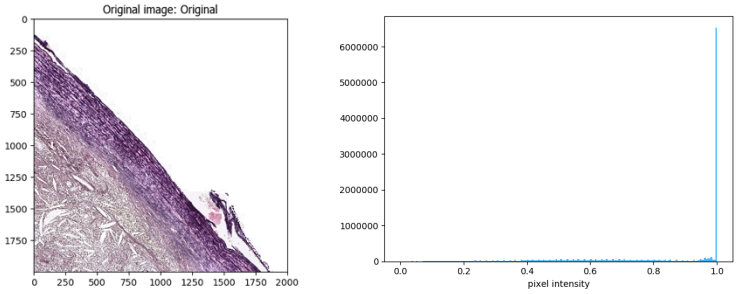
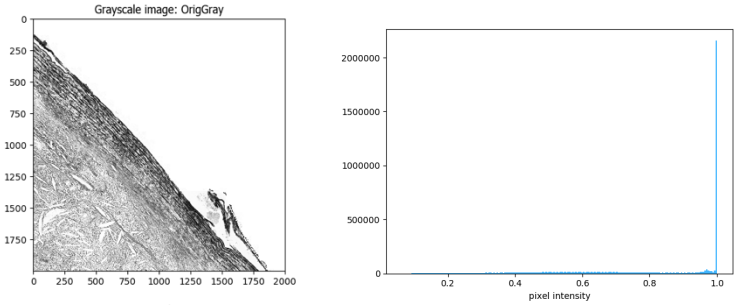
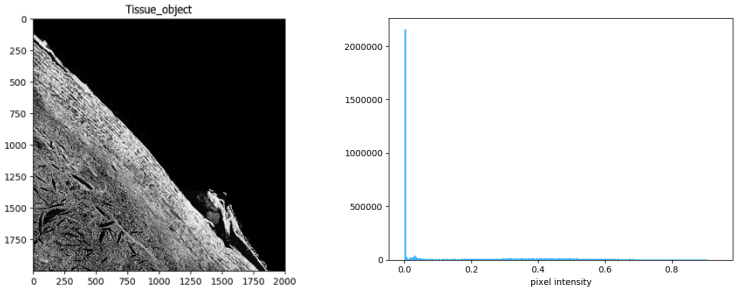
A.



B.



C.



The EVG `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 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).

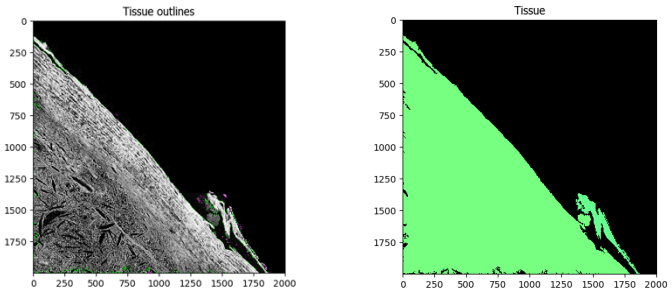
[E]. The colors, *i.e.* stains, are unmixed using the original image (left): EVG (middle), and EVG_IDENTIFYING (HE, right). The graph (right) shows the tonal distribution of the EVG_IDENTIFYING image.

[F]. The EVG-positive objects, *i.e.* elastic tissue, are identified, white areas in the left image; the EVG-positive objects are demarcated by a green line in the middle image, areas that are excluded due to size (minimal size 1 pixels) are demarcated in magenta; the right image shows all the identified EVG-positive objects in random colors; the total number of identified objects is calculated 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 EVG-positive objects. The tissue area (green), and EVG-positive objects (red) are all demarcated in the top-right image. The table (bottom-right) shows the areas occupied by each object class.

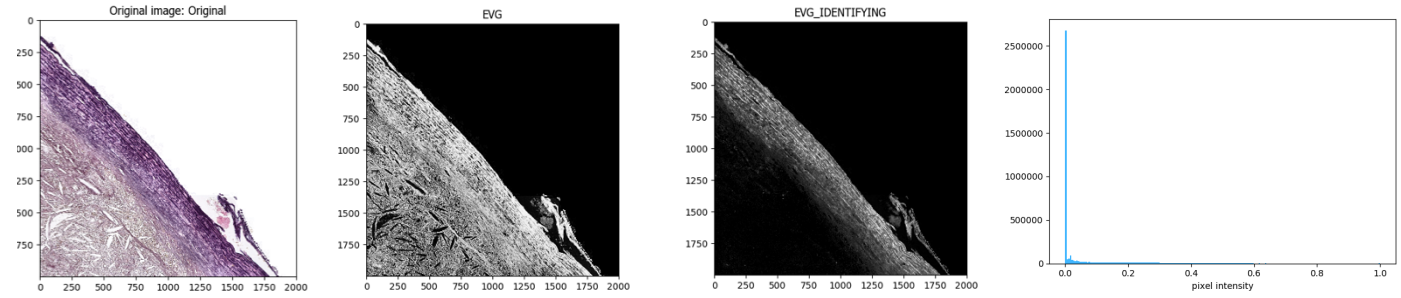
Sample used: AE9.T02-7170.EVG.TIF [Tile= X8000, Y24000]

D.

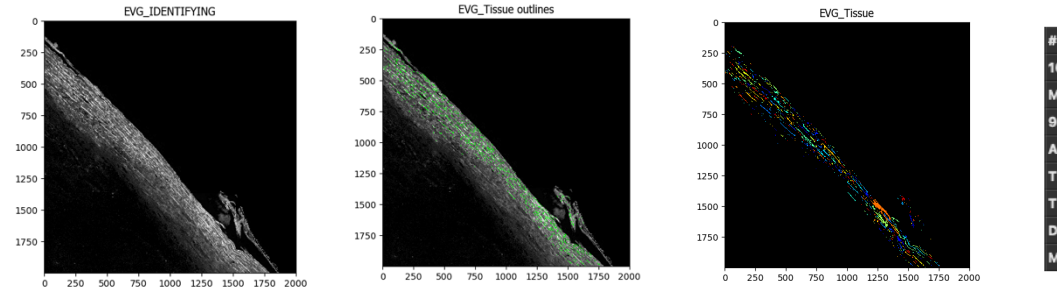


# of accepted objects	2
10th pctile diameter	236.9 pixels
Median diameter	1464.3 pixels
90th pctile diameter	1464.3 pixels
Area covered by objects	43.2 %
Thresholding filter size	1.0
Threshold	0.165
Declumping smoothing filter size	26.9
Maxima suppression size	7.0

E.

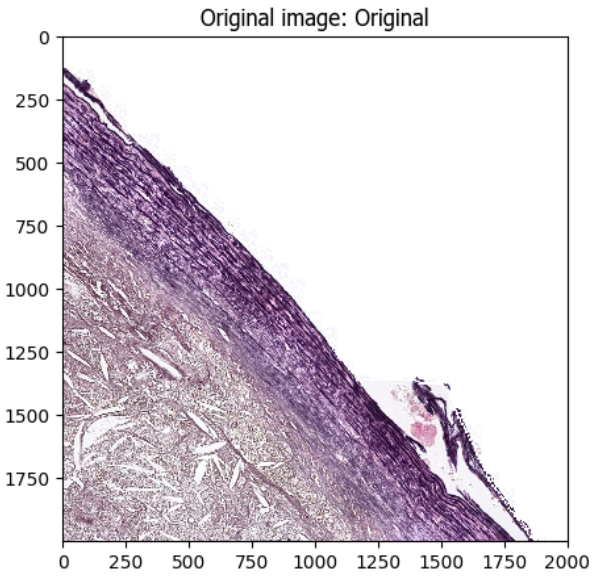


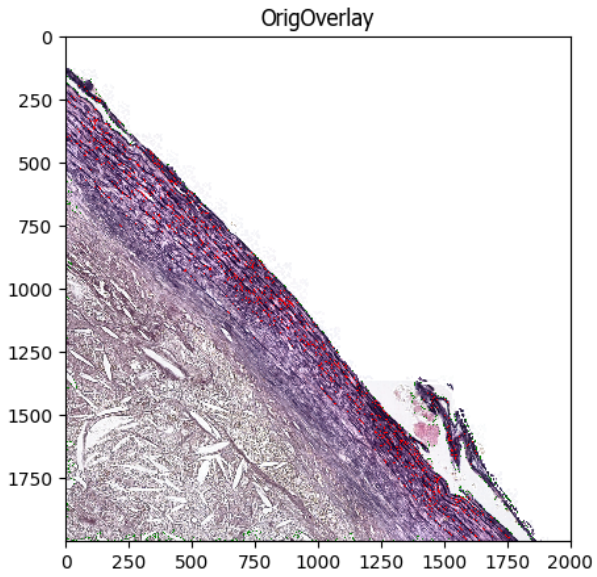
F.



# of accepted objects	1008
10th pctile diameter	1.6 pixels
Median diameter	4.1 pixels
90th pctile diameter	12.9 pixels
Area covered by objects	1.7 %
Thresholding filter size	1.0
Threshold	0.53
Declumping smoothing filter size	0.7
Maxima suppression size	7.0

G.





Objects or Image	Area Occupied	Perimeter	Total Area
EVG_Tissue	68181	33470.0	4000000
Tissue	1728224	16514.0	4000000