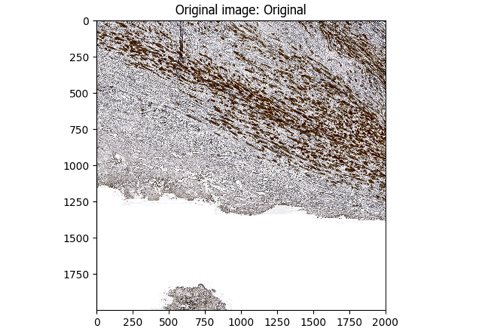
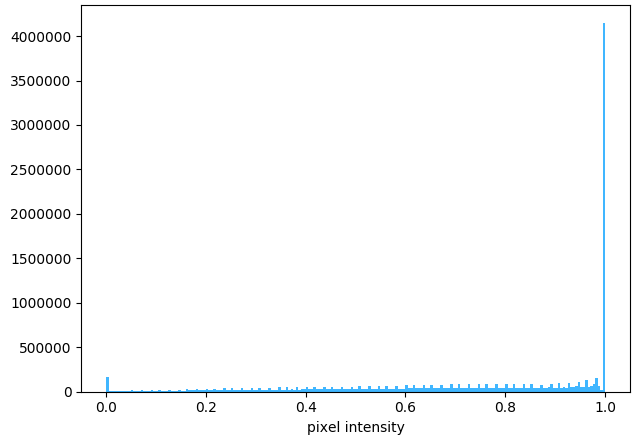
**A.**

**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).

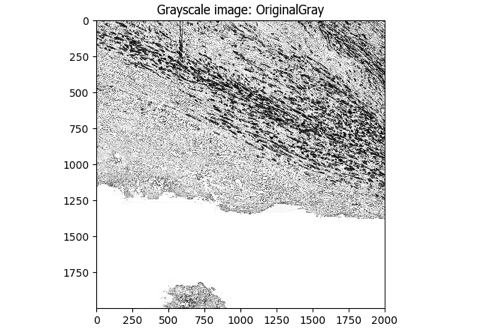
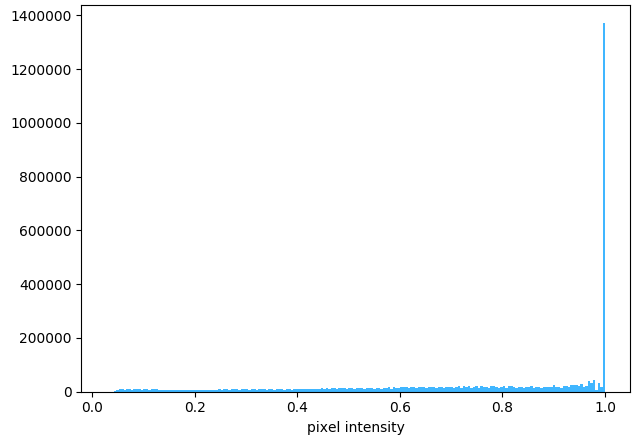
***[G].*** The DAB area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 6 pixels) are demarcated in magenta; the total DAB area size is calculated in pixels (right image) and tabulated (table).

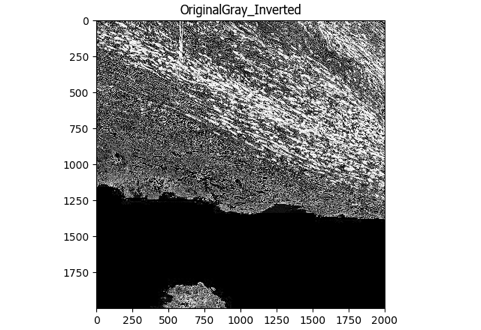
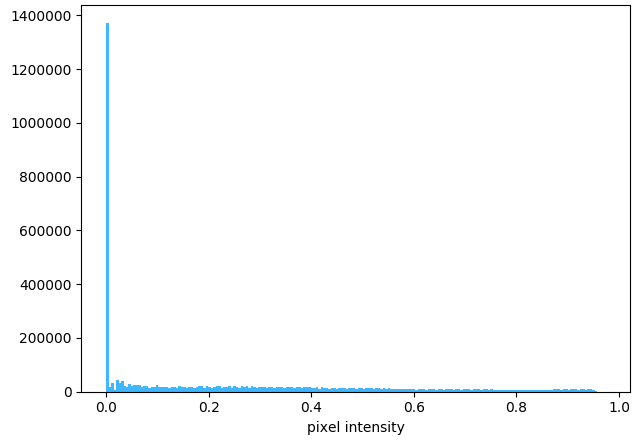
***[H].***The DAB image (left) is masked by overlaying the identified DAB-positive area (right).

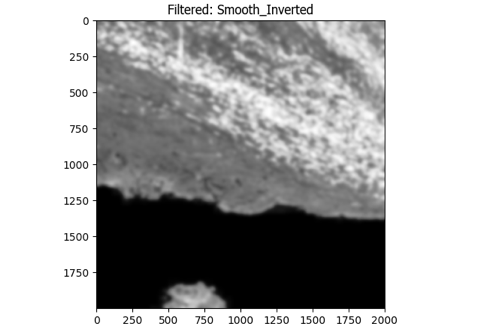
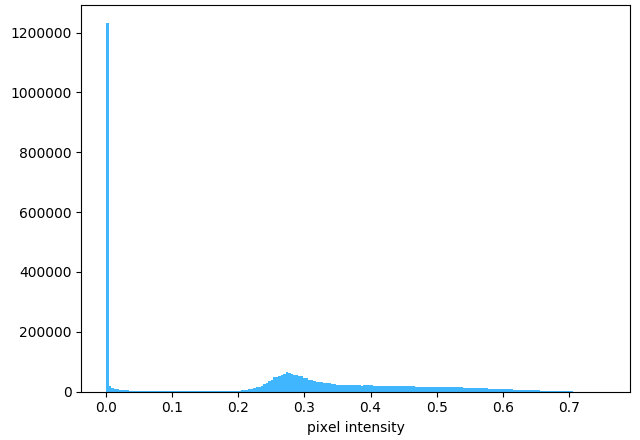
***[I].*** The DAB nuclei objects are identified, white areas in the left image; the DAB nuclei objects are demarcated by a green line in the middle image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the right image shows all the identified DAB nuclei positive objects in random colors; the total number of identified objects is calculated and tabulated (table).

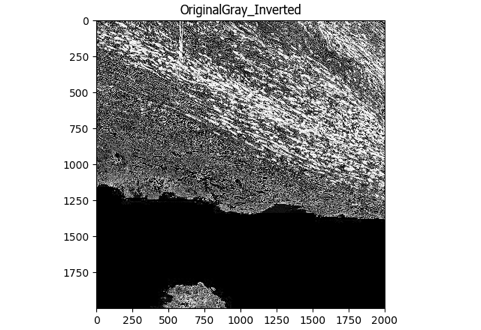
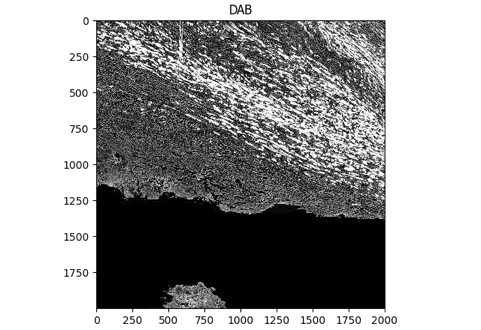
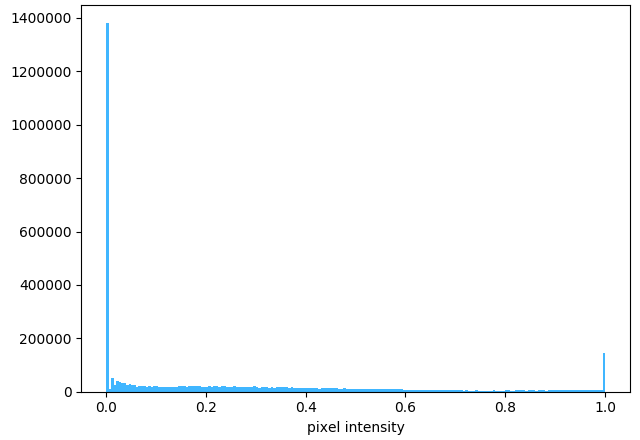
***[J].*** Finally, the data for each tile are saved in a comma-separated table, including meta-data such as tile positions, image location, object counts (there could be multiple patches of stained areas or tissue). The original image (top-left) is used to outline the DAB-positive objects. The tissue 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]

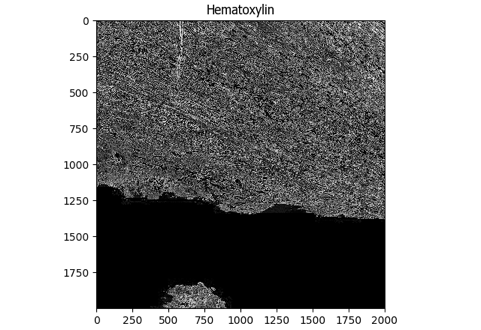
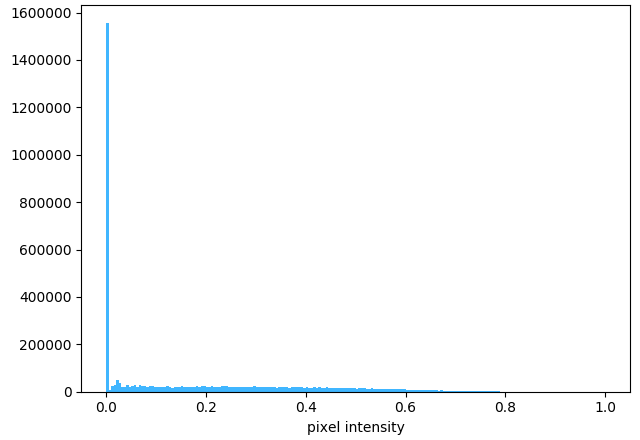
**B.**

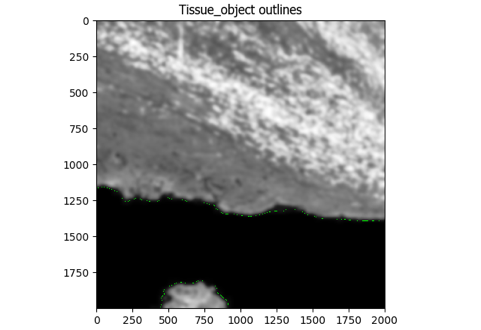
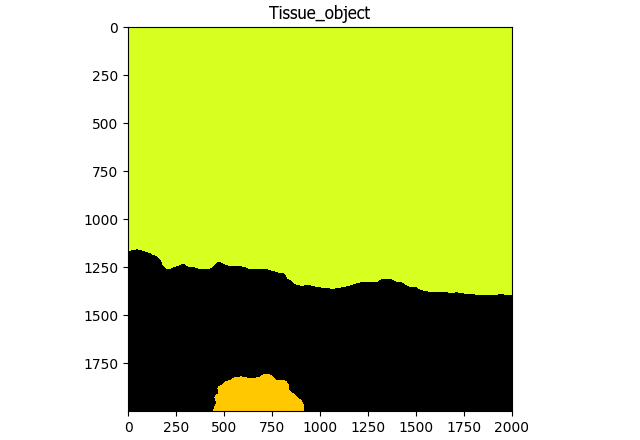
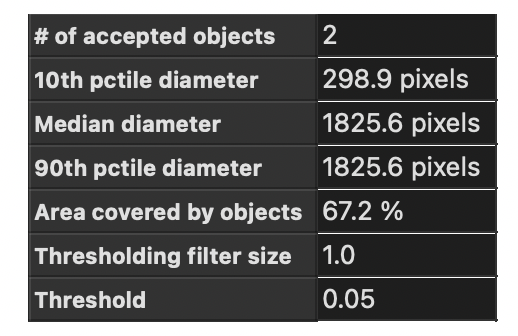
**C.**

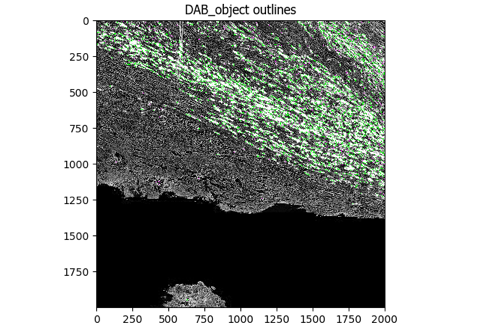
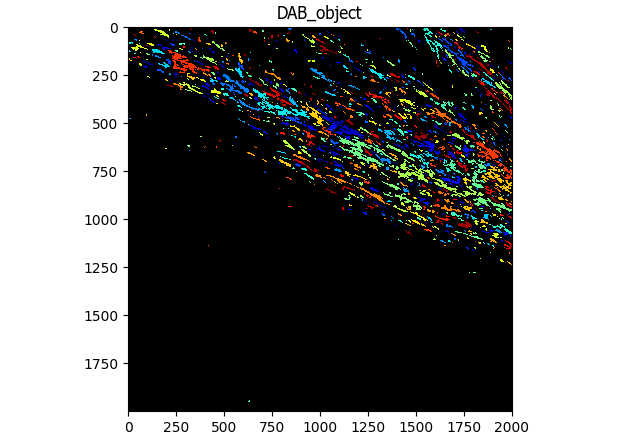
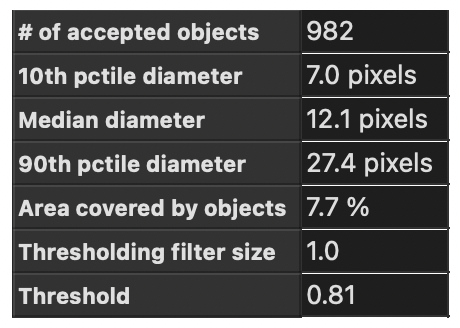
**D.**

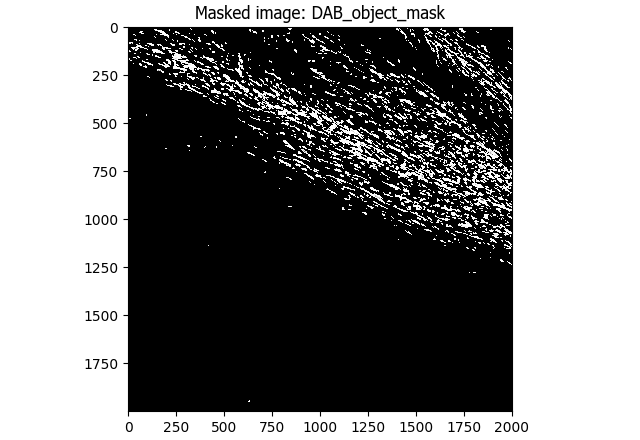
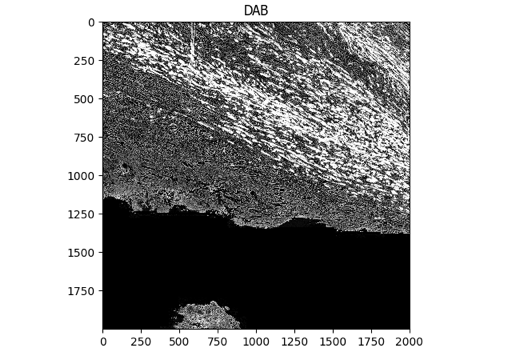
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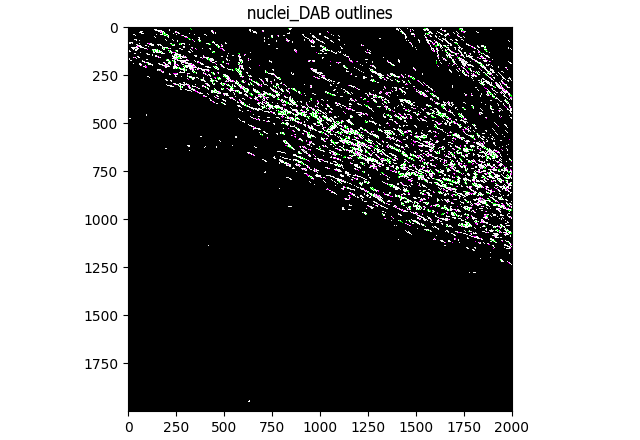
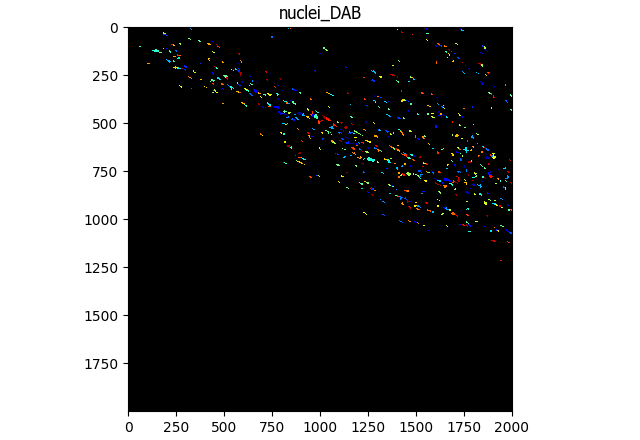
**E.**

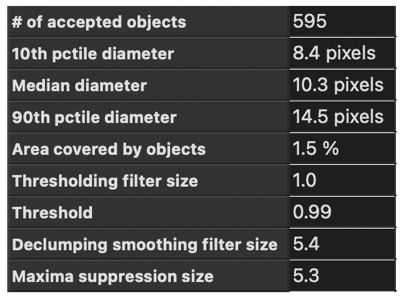
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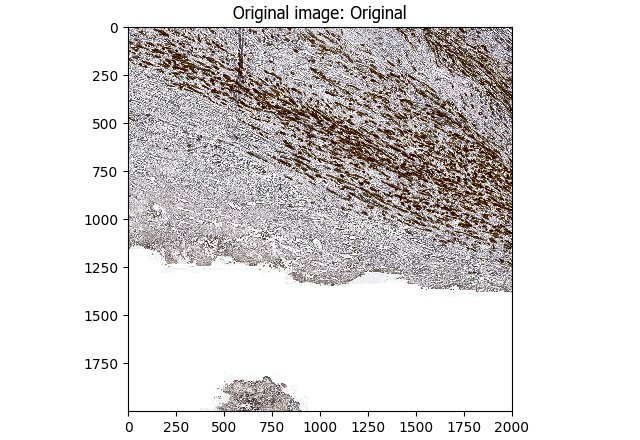
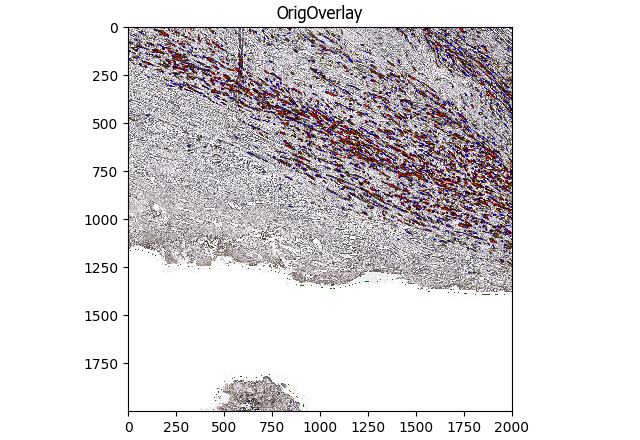
**F.**

**G.**

**H.**

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**I.**

**J.**

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