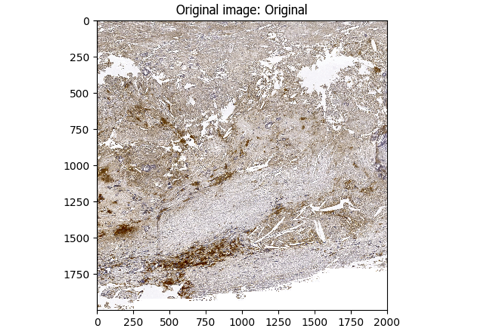
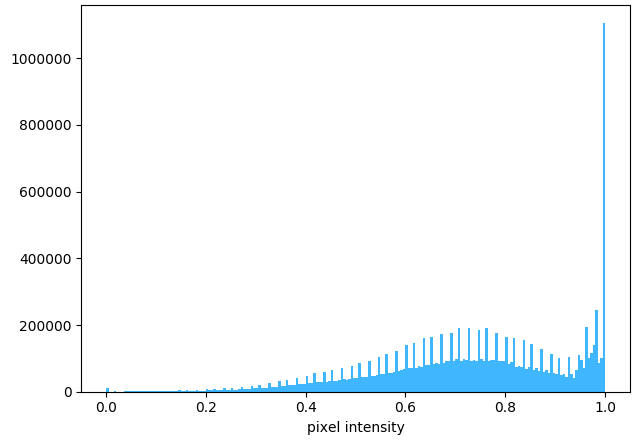
**A.**

**The CD68 CellProfiler pipeline workflow. (Part 1)**

***[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 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.

***[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).

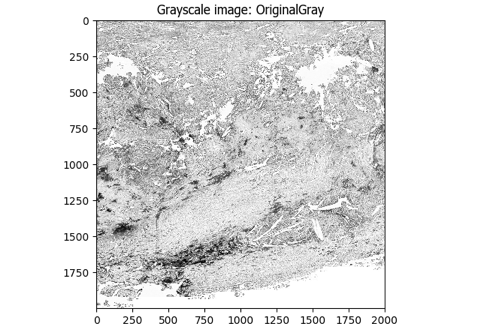
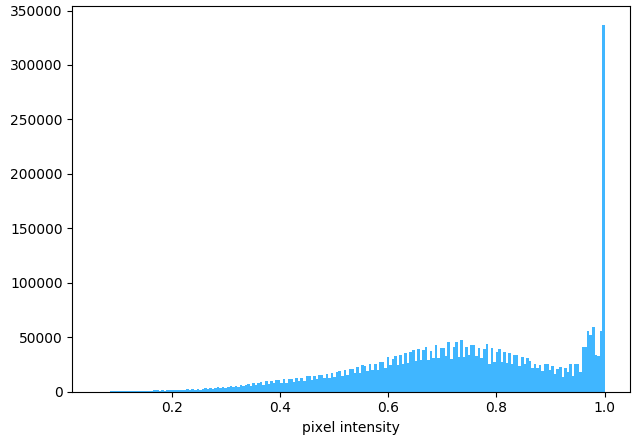
***[F].*** The Hematoxylin area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the total Hematoxylin area size is calculated in pixels (right image) and tabulated (table).

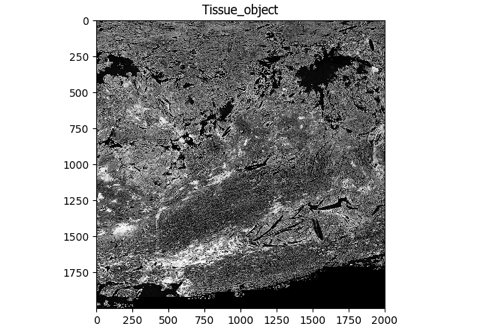
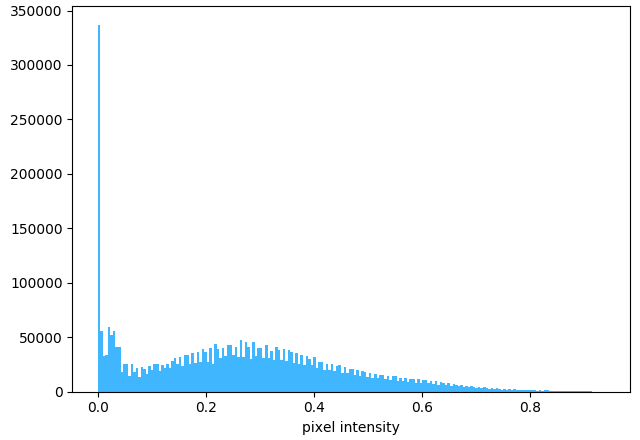
***[G].*** The DAB area is identified, as demarcated by the green line in the left image, areas that are excluded due to size (minimal size 8 pixels) are demarcated in magenta; the total DAB area size is calculated in pixels (right image) and tabulated (table).

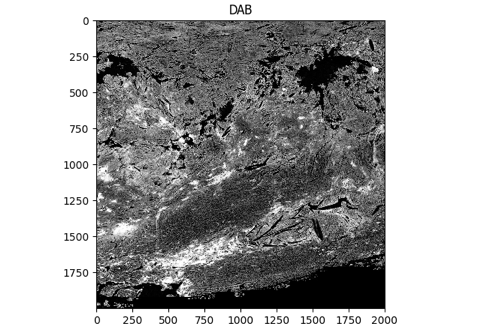
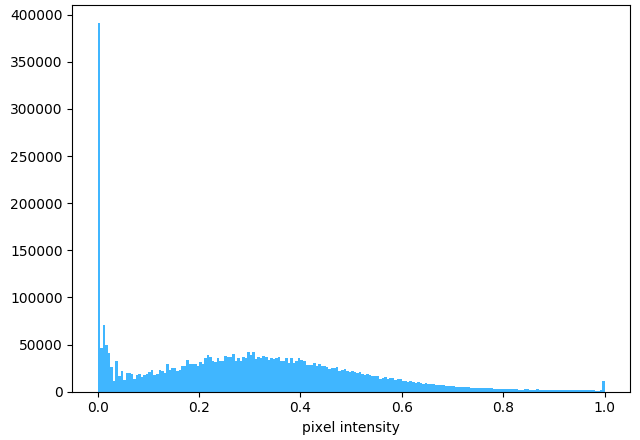
***[H].***The DAB image (left) is masked by overlaying the identified DAB-positive area (right).

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

***[J].*** 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).

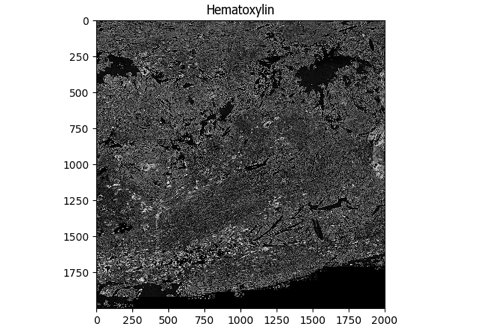
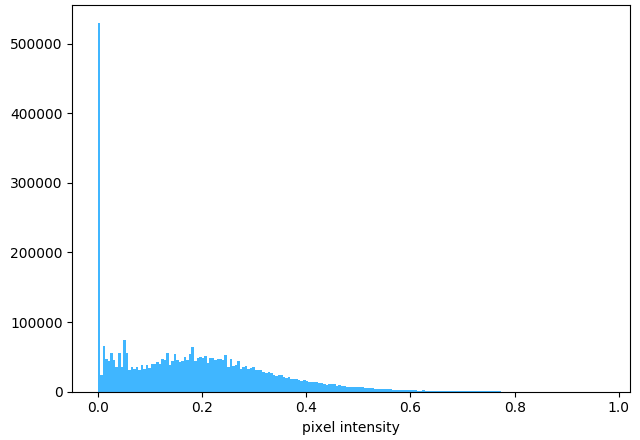
**B.**

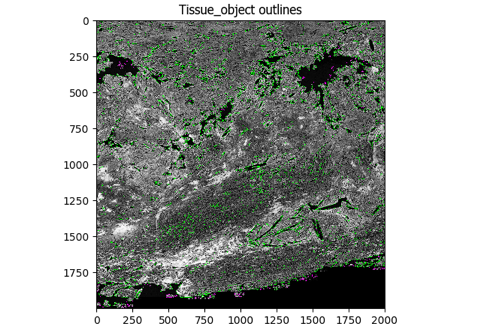
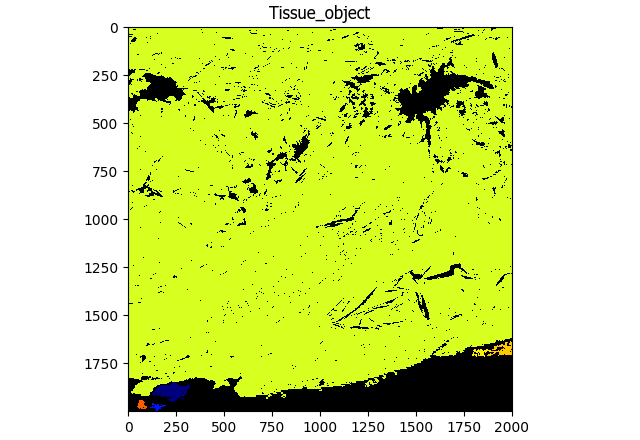
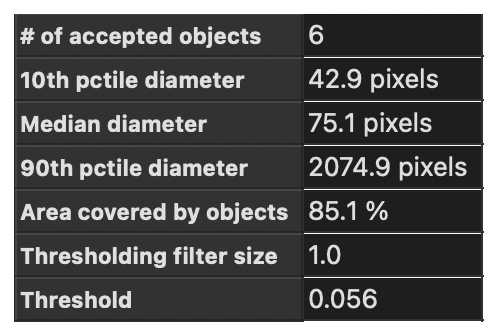
**C.**

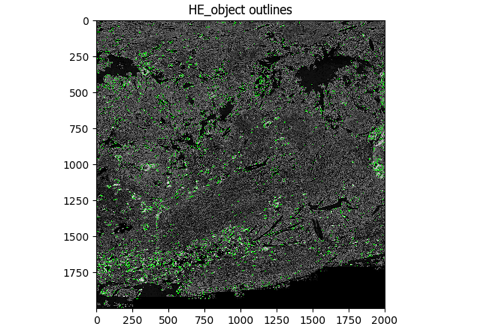
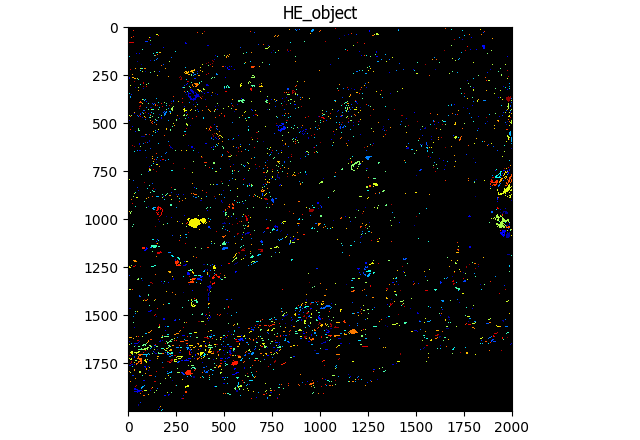
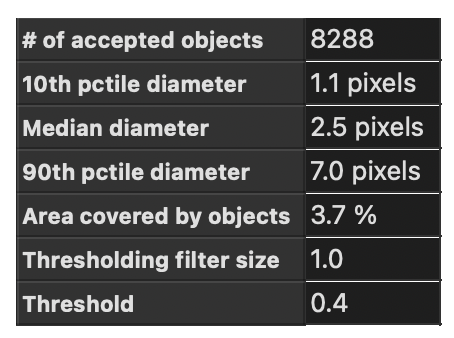
**Afbeelding met tekst, natuur, krater, schermopname

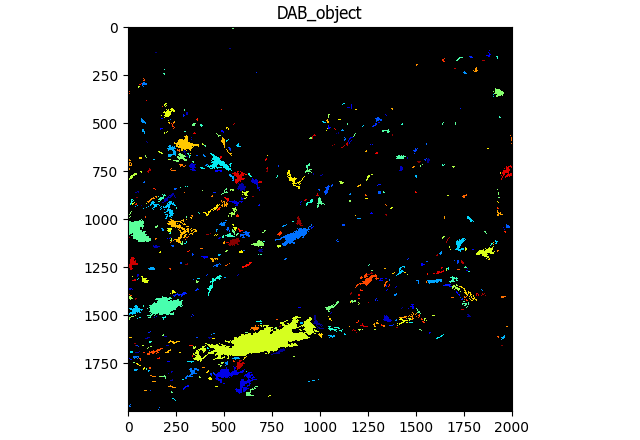
Automatisch gegenereerde beschrijving**

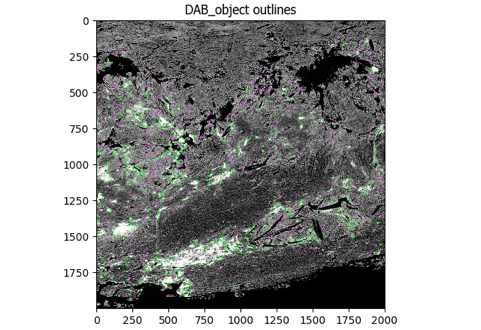
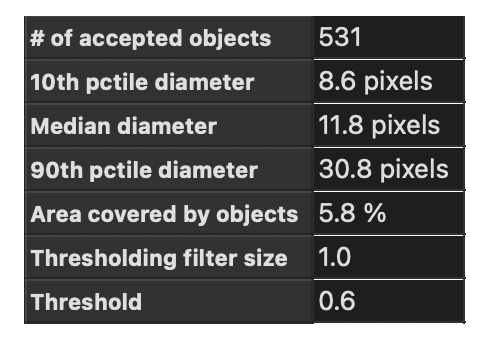
**D.**

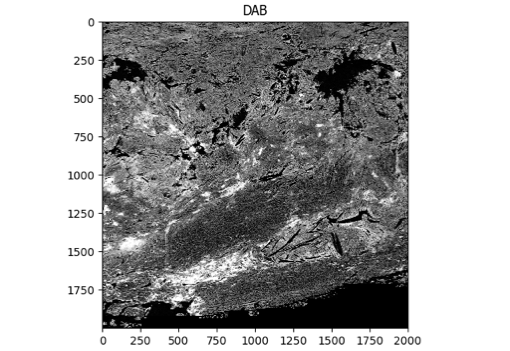
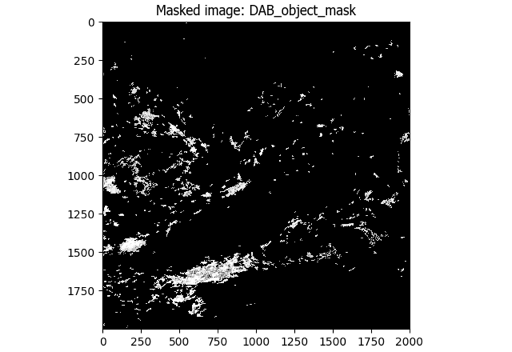
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**E.**

**F.**

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**G.**

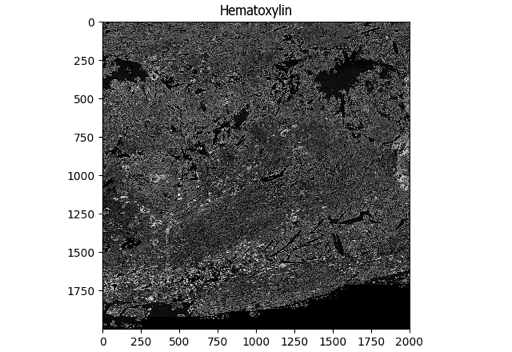
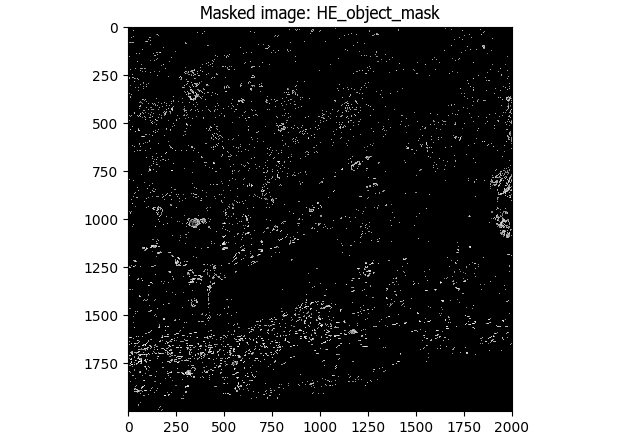
**H.**

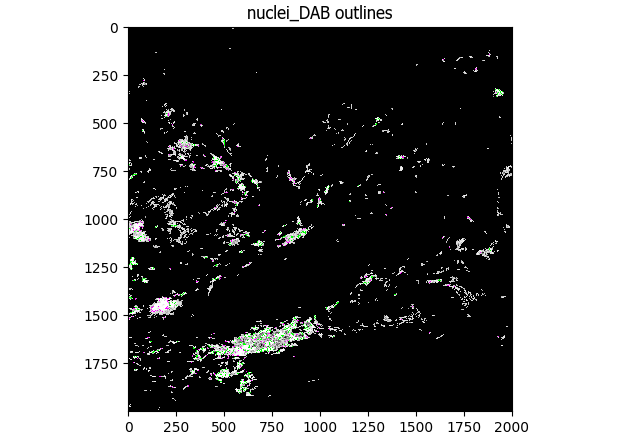
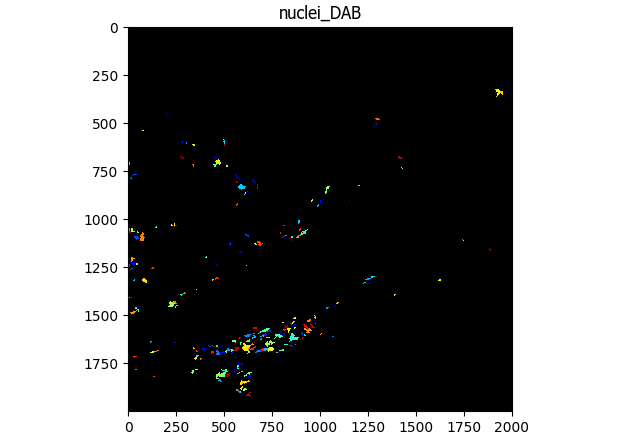
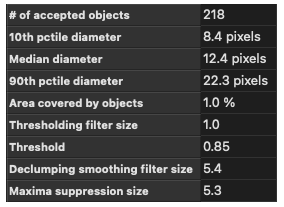
**The CD68 CellProfiler pipeline workflow. (Part 2)**

***[K].*** The Hematoxylin nuclei objects are identified, white areas in the left image; the Hematoxylin 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 Hematoxylin nuclei positive objects in random colors; the total number of identified objects is calculated and tabulated (table).

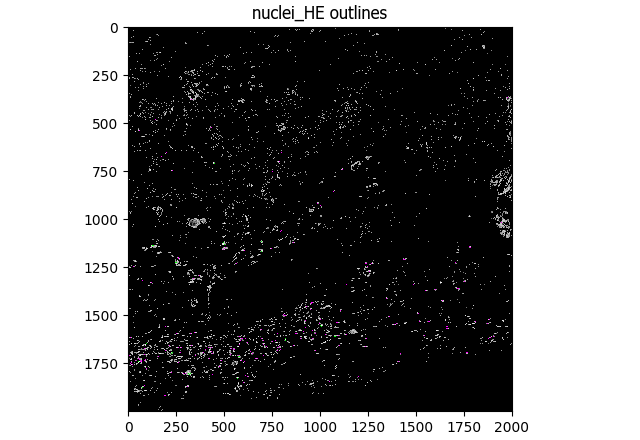
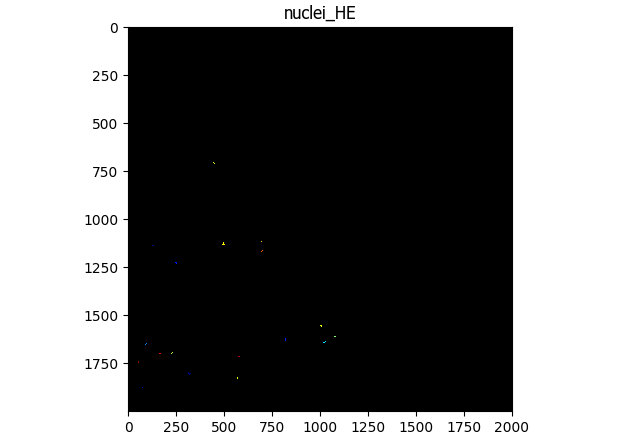
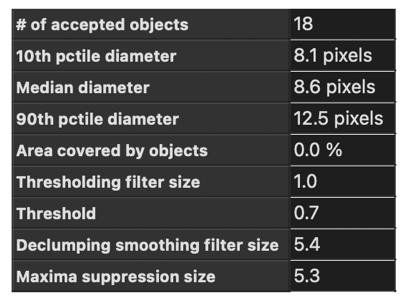
***[L].*** Finally, the data for each tile are saved in a comma-separated table, including meta-data such as tile positions, image location, object counts (there could be multiple patches of stained areas or tissue). The original image (top-left) is used to outline the DAB- & Hematoxylin-positive objects. The tissue area (dark-green), DAB area (yellow), Hematoxylin area (light-green), DAB nuclei objects (red), and Hematoxylin nuclei objects (blue) are all demarcated in the top-right image. The table (bottom-right) shows the areas occupied by each object class.

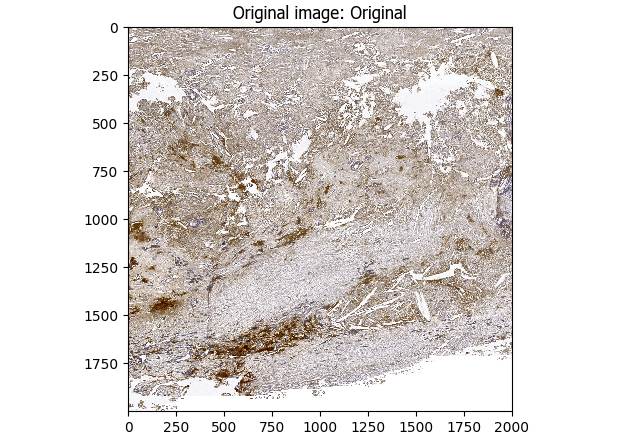
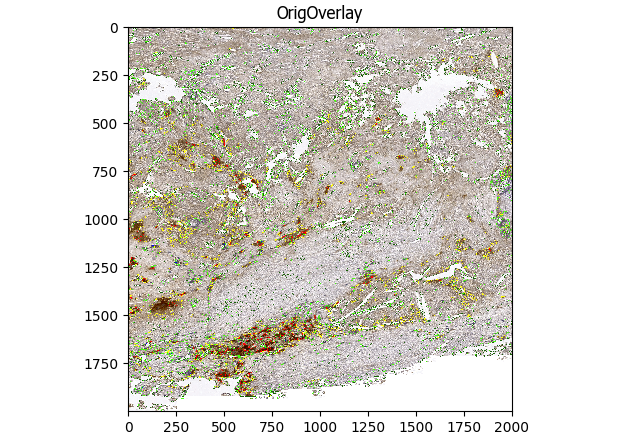
Sample used: AE9.T02-7170.CD68.20141128.TIF [Tile= X22000, Y24000]

**I.**

****

**J.**

**K.**

**L.**

**Afbeelding met tekst, schermopname, Lettertype, nummer

Automatisch gegenereerde beschrijving**