Whole Slide Image (WSI) Analysis with QuPath

Tutorial

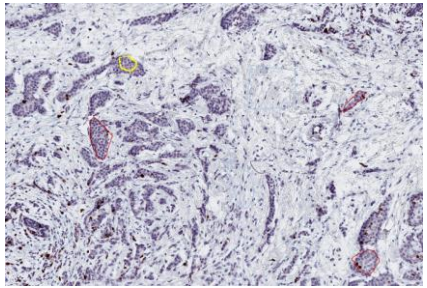
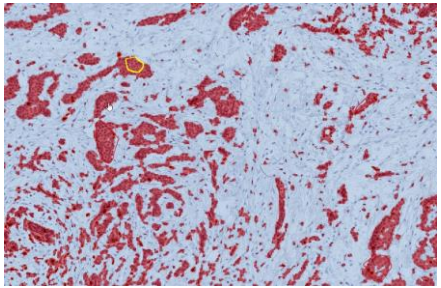
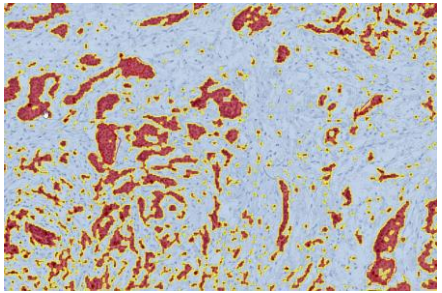
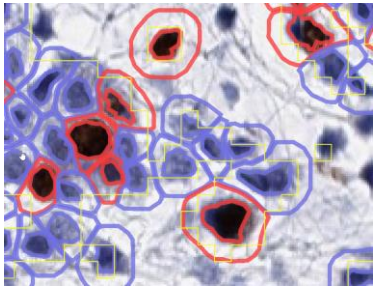
Tutorial created by
Markus Plass and Michaela Kargl
Medical University Graz, Diagnostics- and Research Institute for Pathology

This tutorial was created within the EMPAIA-AT project funded by Bundesministerium Klimaschutz, Umwelt, Energie, Mobilität, Innovation und Technologie FFG

Whole Slide Image (WSI) Analysis with QuPath

Objectives:

1. learn how to get started with the open-source software QuPath
2. train QuPath to distinguish tumor / non-tumor
3. use QuPath for Ki67 Analysis
4. apply the trained algorithm to other regions of interest

(A) Set-Up Project in QuPath

(1) Download & Install QuPath
(2) Create Project & Add Whole Slide Images
(3) View & Explore WSI

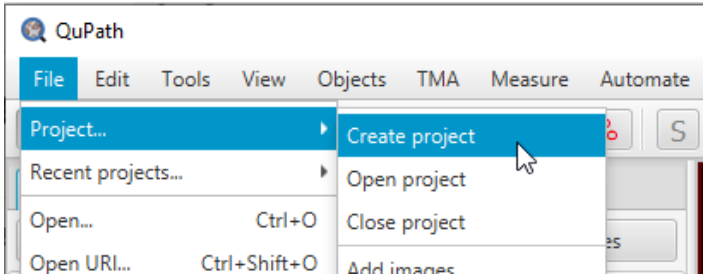
(1) Download, Install and Open QuPath

1. Download & install the QuPath Software suitable for your operating system from https://qupath.github.io/

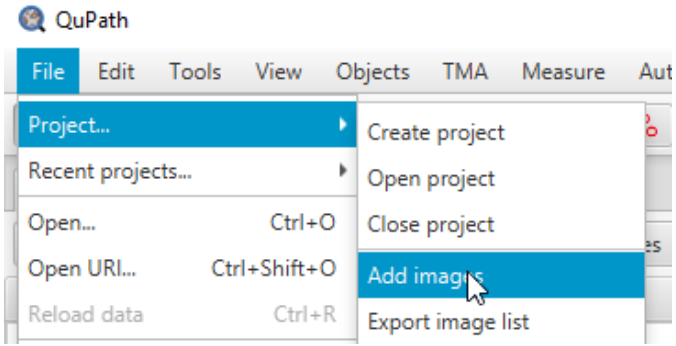
2. After successful installation, you can find the QuPath Icon on the desktop:

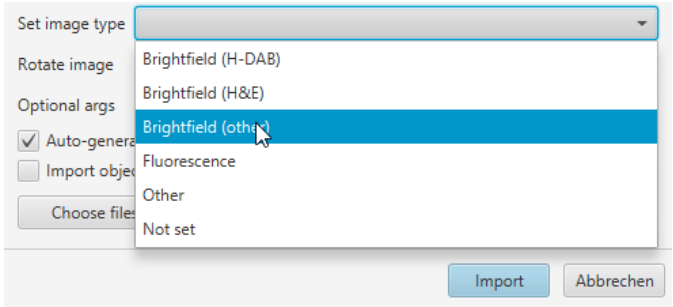
(2) Create Project in QuPath and Add Images

1. Create Project:
   “File” > “Project” > “Create project”



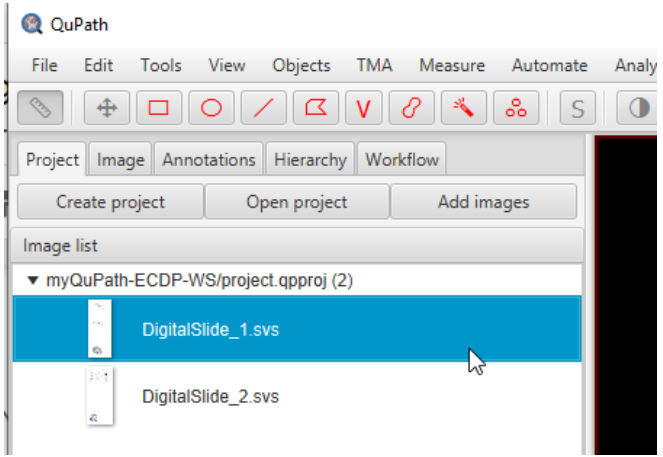
2. Add WSI to Project:
   a. “File” > “Project” > “Add images”
   b. drag&drop Ki67 image to pop-up window, and set image type “Brightfield (other)”





(3) View & Explore WSI in QuPath

1. Open WSI
   (double-click on image name in the image list)



2. Explore the image:
   scroll to zoom in and out…
   press left mouse key to drag the image…

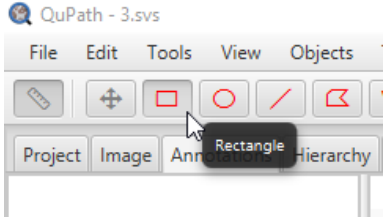
(B) Train Classifier for Tumor/Non-Tumor

(1) Make ROI with Region*
(2) Annotate Tumor Areas within that region
(3) Annotate Non-Tumor Areas within that region
(4) SELECT that annotation
(5) Train Classifier

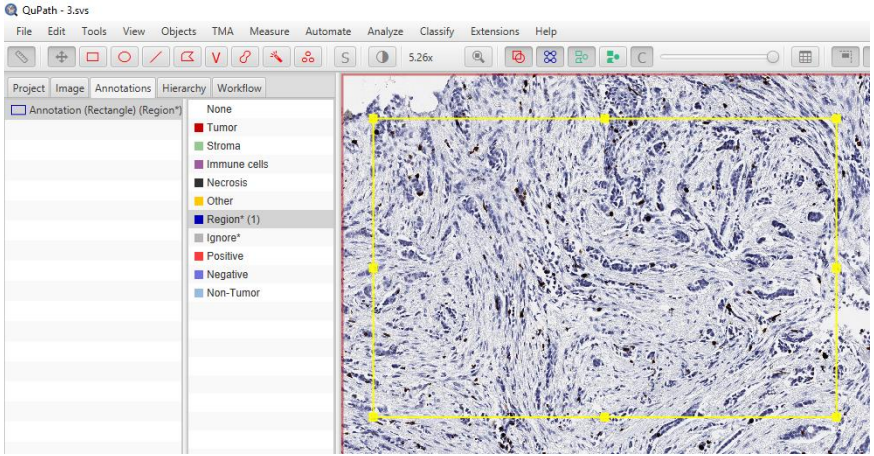
(1) Annotate Region of Interest

1. Step 1: Select Annotation-Class “Region*”:
   > 1. click on ”Annotations”
   > 2. click on “Region*”
   > 3. click on “Auto set”

2. Step 2: Select Annotation Tool: (e.g. Rectangle)



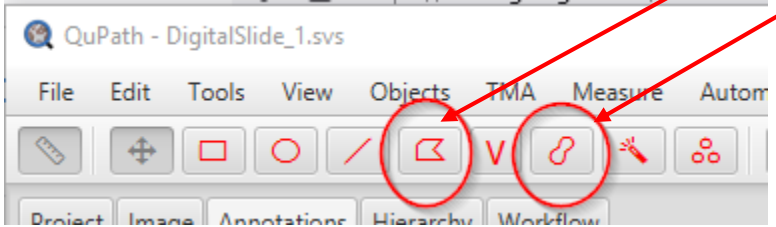
3. Step 3: Draw Annotation
   to mark Region of Interest



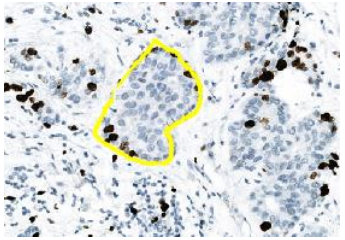
(2) Annotate Tumor Areas

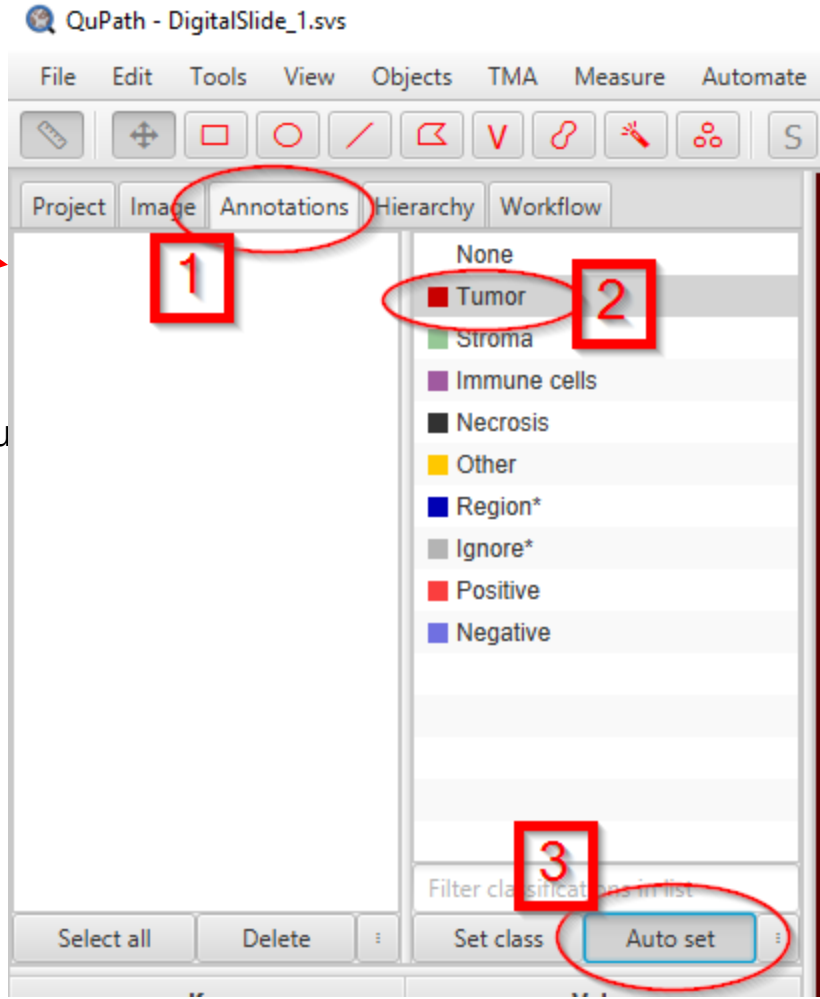
1. Step 1: Select Annotation-Class “Tumor”:
   > 1. click on ”Annotations”
   > 2. click on “Tumor”
   > 3. click on “Auto set”

2. Step 2: Select Annotation Tool: (e.g. Polygon or Bru



3. Step 3: Draw Annotations
   to Mark Tumor Areas



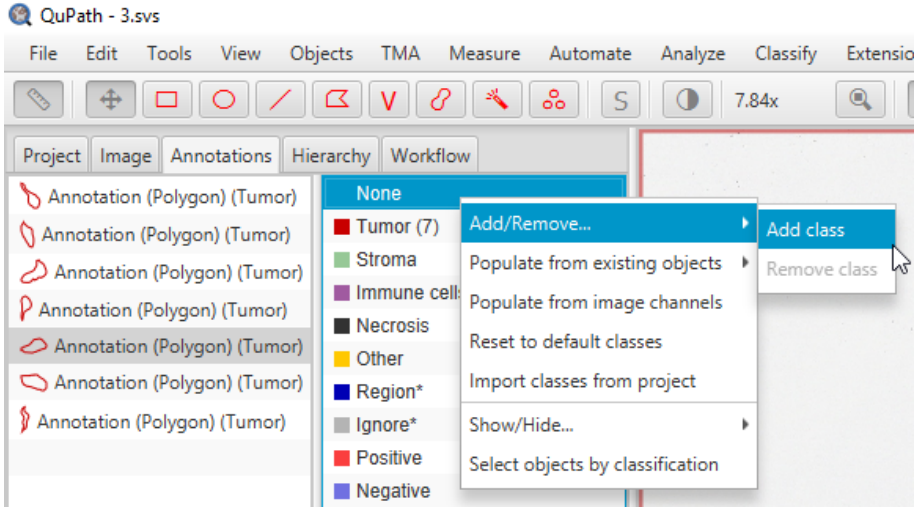


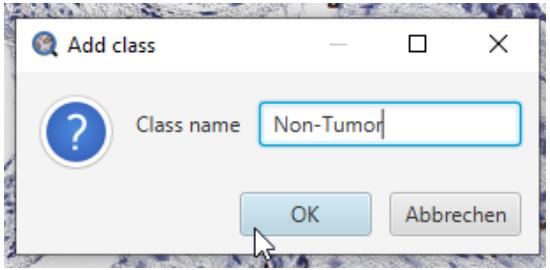
(3a) Create Annotation Class “Non-Tumor”

1. Select

   “Annotations” > “Add/Remove…”Add class”

2. Name the new class



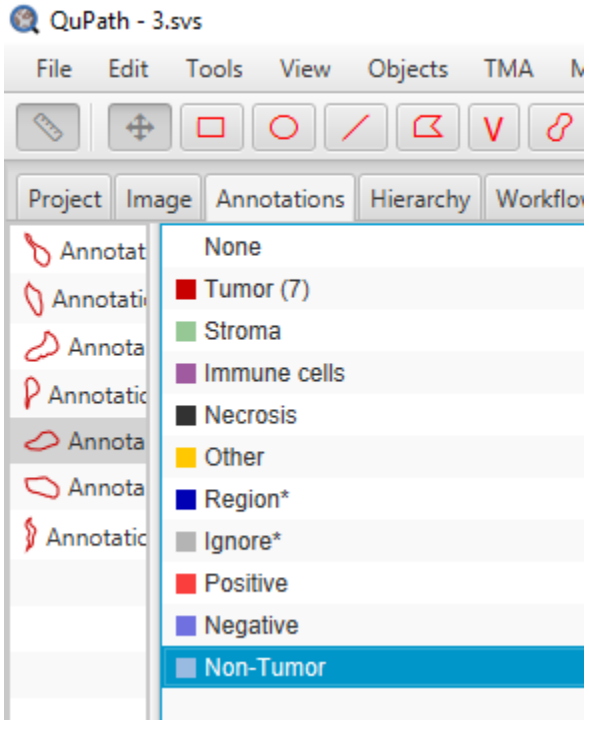


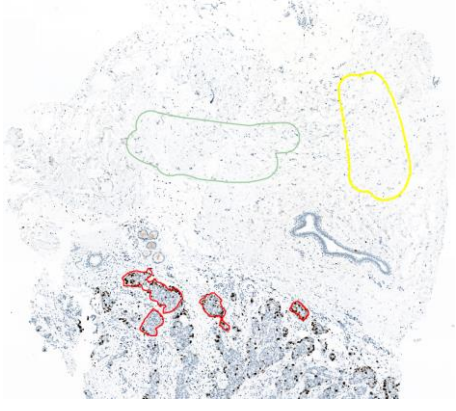
(3b) Annotate Non-Tumor Areas

1. Step 1: Select Annotation-Class “Non-Tumor”
   > 1. click on ”Annotations”
   > 2. click on “Non-Tumor”
   > 3. click on “Auto set”

2. Step 2: Select Annotation Tool:
   (e.g. Polygon or Brush)

3. Step 3: Draw Annotations
   to Mark non-tumor Areas

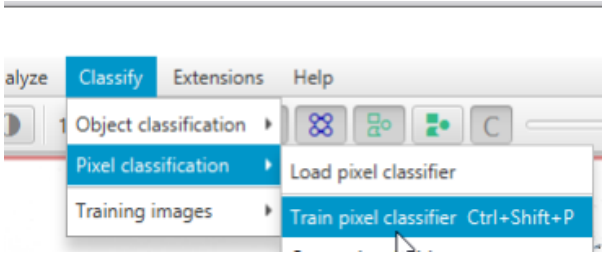




(3d) Train Classifier

1. Select the Region of Interest

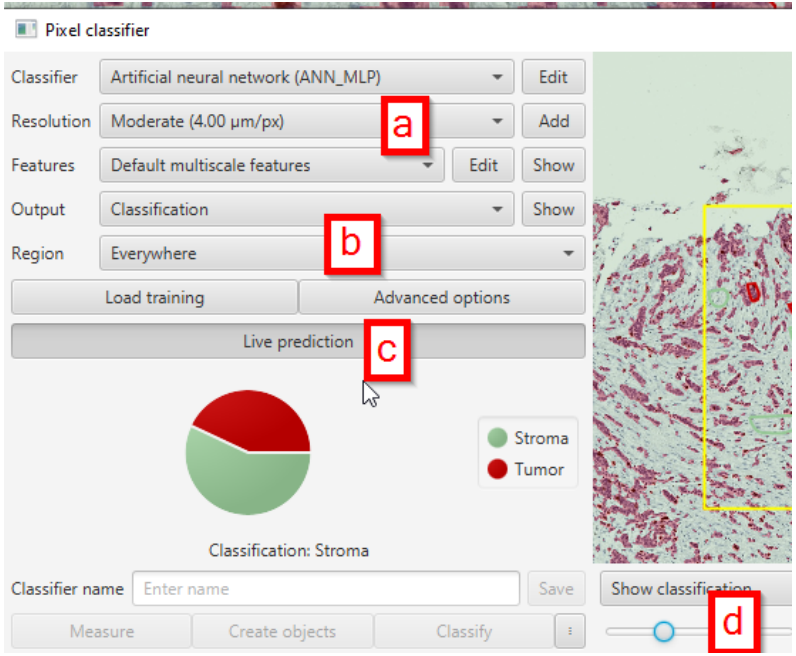
2. Step 2: Select “Train Pixel Classifier”



1. Step 3: Configure Pixel Classifier
   a. set Resolution to “Moderate”
   b. set Region to “Everywhere”
   c. click on “Live prediction” (and wait for result)
   d. use the slider to adjust the transparency of the result visualisation so that you can check the plausibility of the result

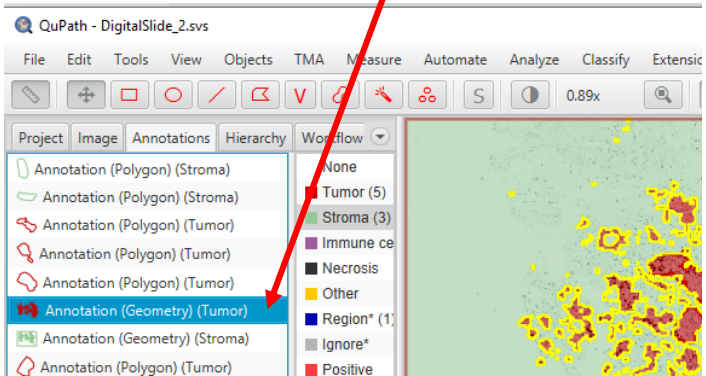
   → if the result is not good yet, try another resolution (e.g. “Extremely low” ...)

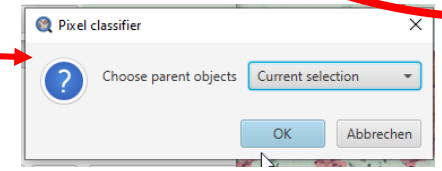
2. Step 4: Name and Save your Classifier

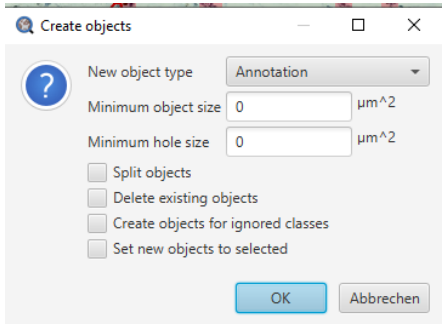


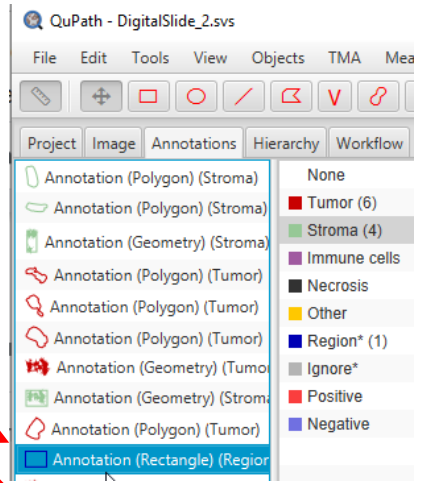
(4) Automatic Tumor Annotations

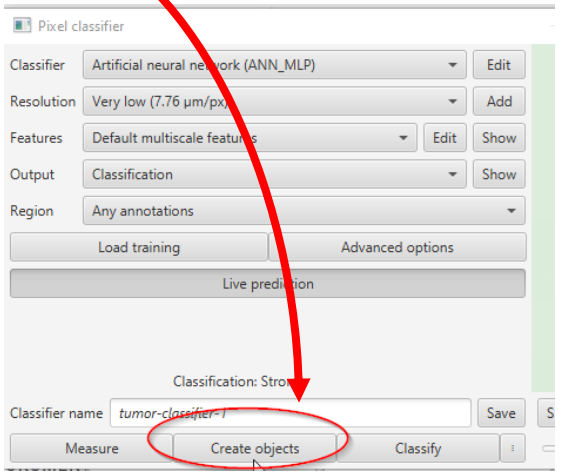
1. Step 1: Select Region of Interest from Annotations' list
2. Step 2: In Pixel classifier window select "Create objects"
3. In pop-up windows choose parent object "Current selection" and object type "Annotation"
4. → find automatic annotation of detected tumor areas in the Annotations' list









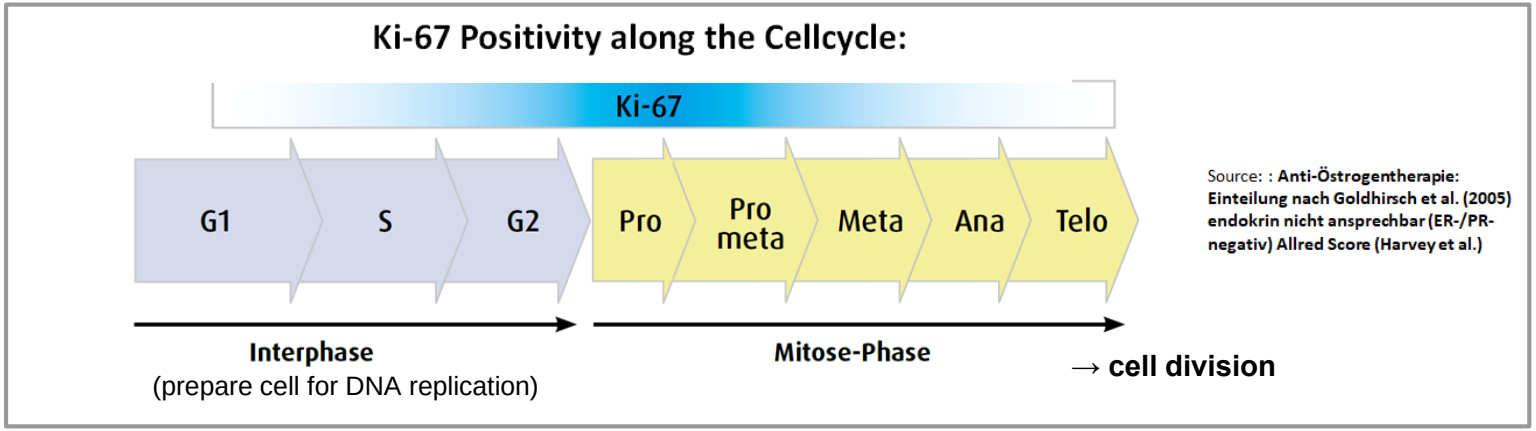


(C) Ki67 Analysis

(1)Select/Define Area for Analysis
(2)Detect Positive Cells

ad (C) Ki67 Analysis: Background Information

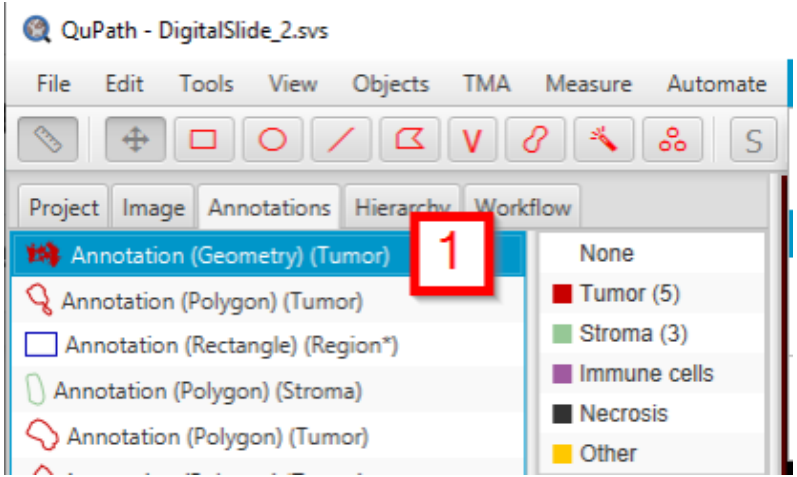
Ki-67 is a commonly used indicator of cellular proliferation in cancer



$$\text{Ki67\_index [\%]} = \frac{\text{[number of positively stained tumor cells]}}{\text{[total number of tumor cells]}}$$

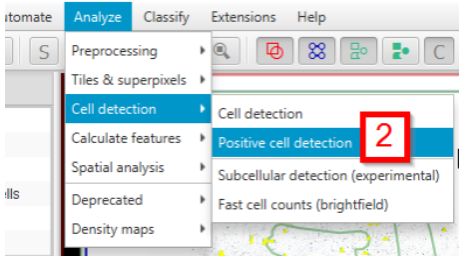
(1) Select/Define Area for Analysis

1. in the Annotations' list select the annotation of a tumor area (e.g. the annotation of the automatically detected tumor areas)



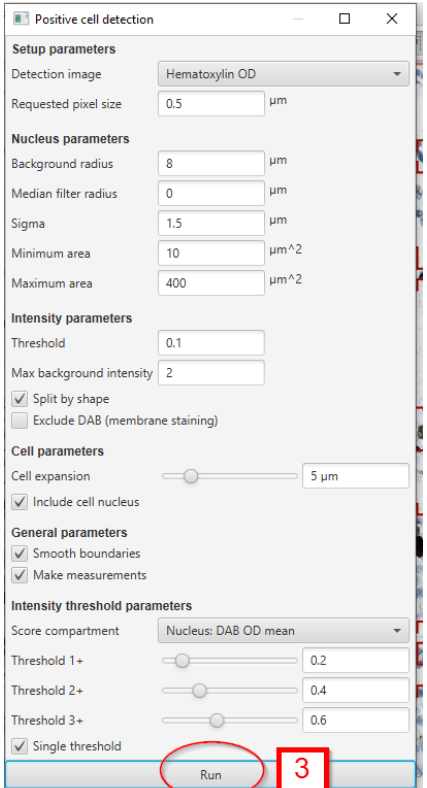
(2) Ki67 Analysis: Detection of Positive Cells

2. from the QuPath menu select “Analyze”>”Cell detection”>”Positive cell detection”



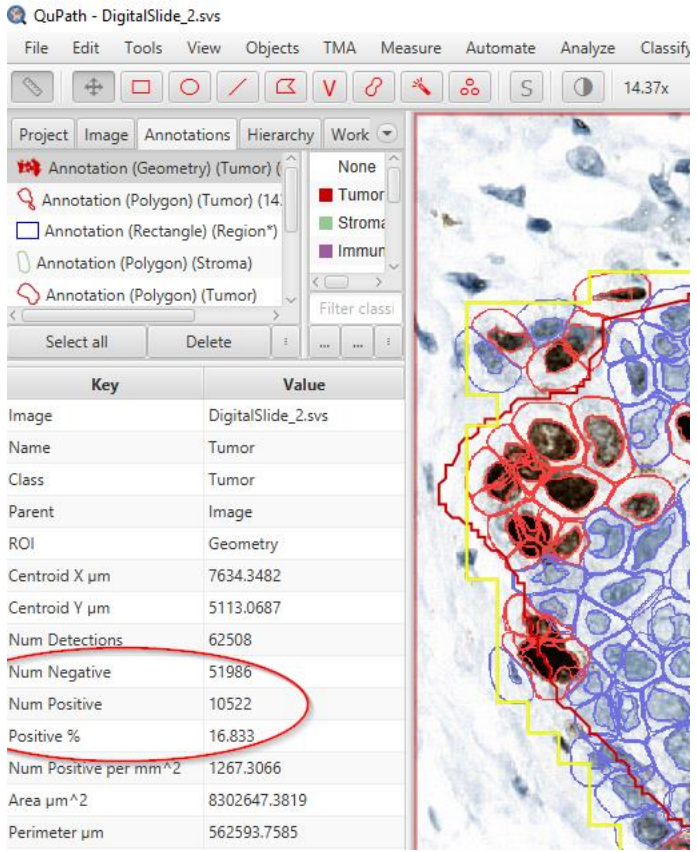
3. in the “Positive cell detection” pop-up window click on “Run”

4. Wait for the result…



(3) Ki67 Analysis Result

4. Find the result



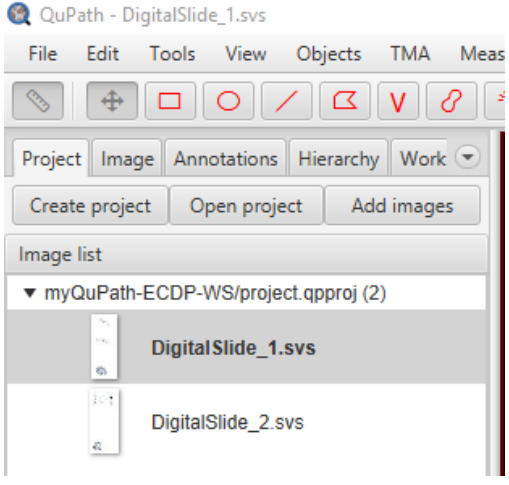
(D) Apply Trained Algorithm to Other Region

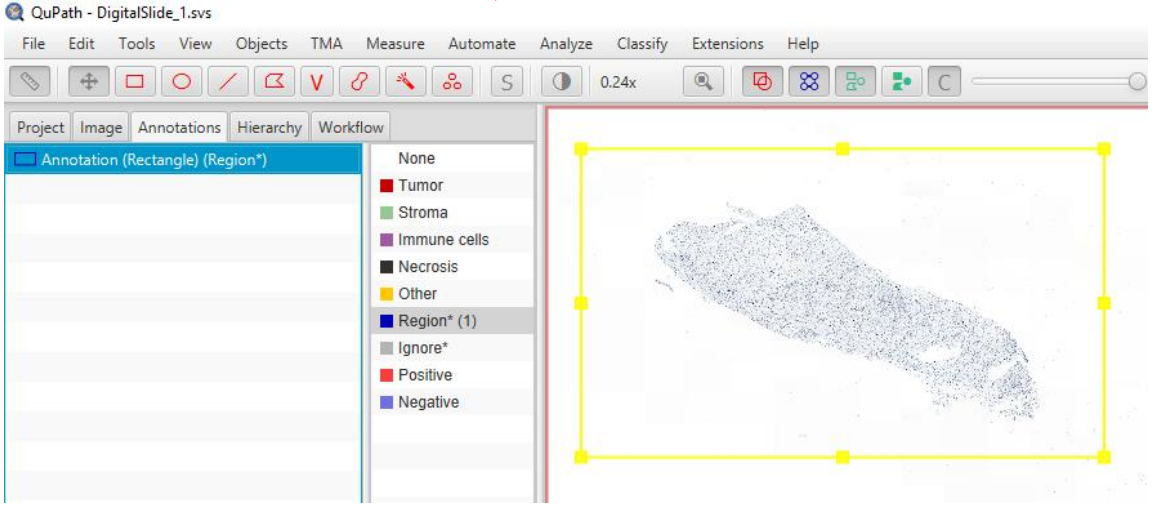
(1) Define another Region of Interest
(2) Load Pixel Classifier
(3) Cell Detection

(1) Define Region of Interest

1. In the Project's Image list double-click on the file name to open the respective WSI

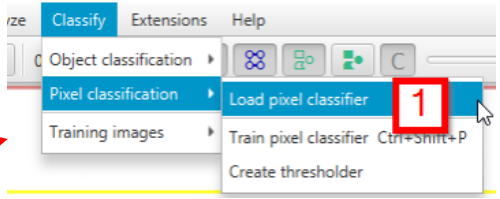
2. Draw Region of Interest (e.g. rectangle annotation)

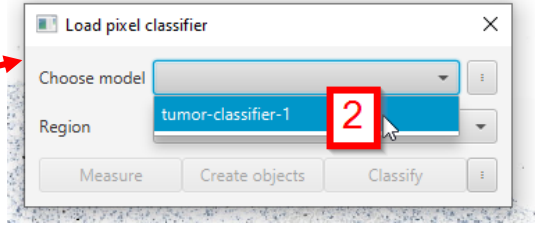




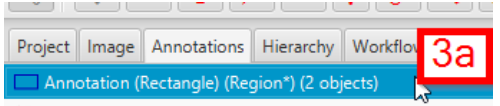
(2) Load Pixel Classifier

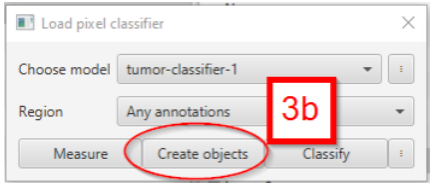
1. From the menu bar select
   “Classify”>”Pixel classification”>”Load pixel classifier”
2. In the “Load pixel classifier” pop-up window choose the (previously trained) tumor classifier
   → *result: classifier is applied and tumor areas are detected*

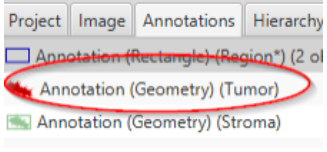


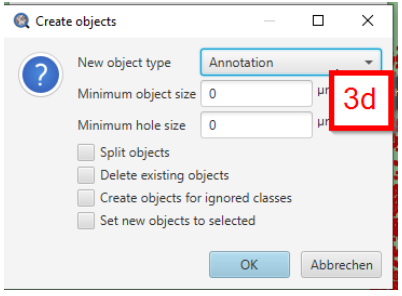


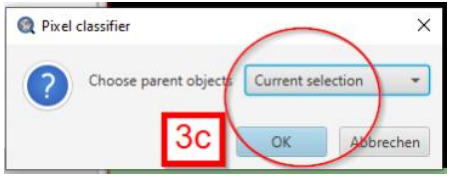
3. Create the annotation object for the detected tumor areas
   Step 3a: in the Annotations’ list select the Region of Interest
   Step 3b: in the “Load pixel classifier” window click on “Create objects”
   Step 3c: in the pop-up window choose “Current selection” as parent object
   Step 3d: select “Annotation” as new object type
   → *result: find annotation for the detected tumor areas in the Annotations’ list*





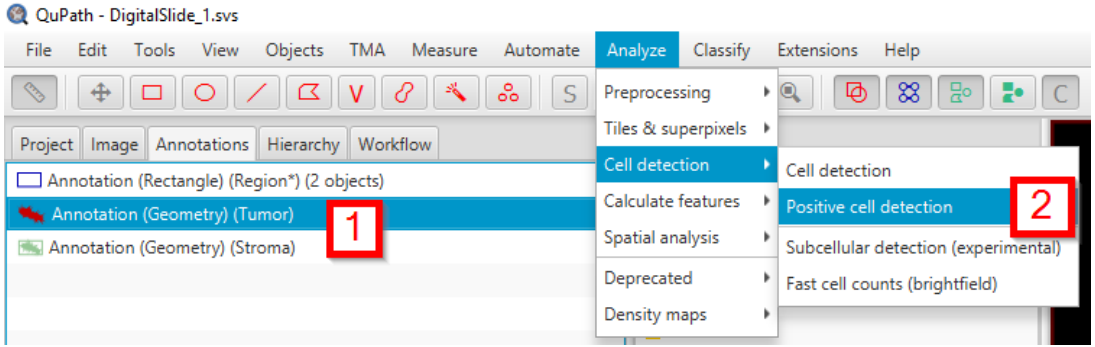




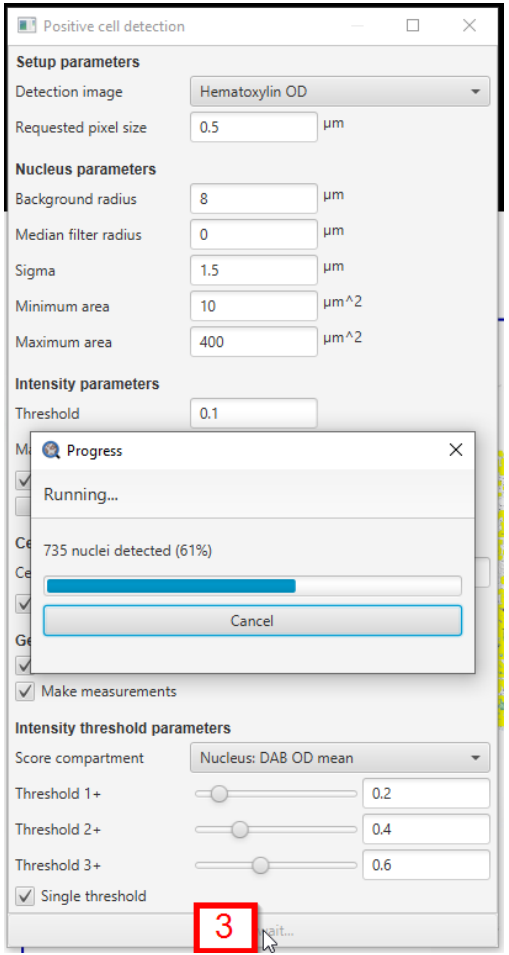


(3) Cell Detection

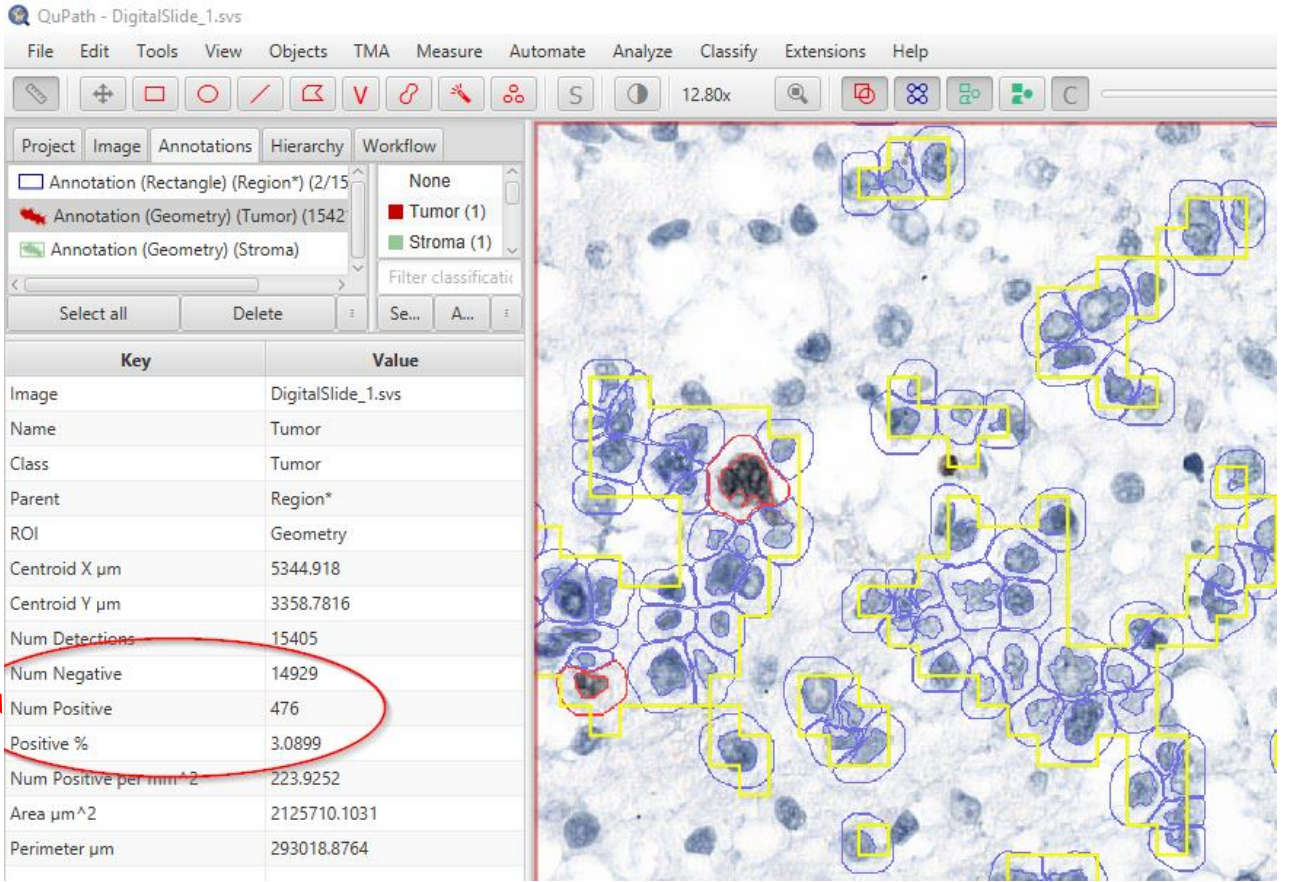
1. In the Annotations' list select the previously generated annotation of the detected tumor areas
2. In the menu bar go to
   "Analyse" > "Cell detection" > "Positive cell detection"



3. In the "Positive cell detection" window click on "Run"
   …and wait for result…



(4) Cell Detection Result = Ki67 Analysis Result



Further Reading:

https://qupath.readthedocs.io/en/stable/docs/tutorials/cell_detection.html#

Tutorial created by
Markus Plass and Michaela Kargl
Medical University Graz, Diagnostics- and Research Institute for Pathology

This tutorial was created within the EMPAIA-AT project funded by Bundesministerium Klimaschutz, Umwelt, Energie, Mobilität, Innovation und Technologie FFG