

# QuPath Introduction

DCPL

2022

# QuPath

- What is QuPath

*QuPath* is an open source , powerful, flexible, extensible software platform for whole slide image analysis. Some typical applications include: cell counting, positive cell determination, cell classification, multiplex fluorescence analysis, etc.

- Minimum requirements to run QuPath

- \* Qupath can work on 64 bit Windows, MAC and Linux environment
- \* Recommended hardware setup: intel core i7 or above, 16GB RAM
- \* If you are dealing with large data set (for example: Z-stack, multiplexed image), >16GB RAM is strongly recommended .

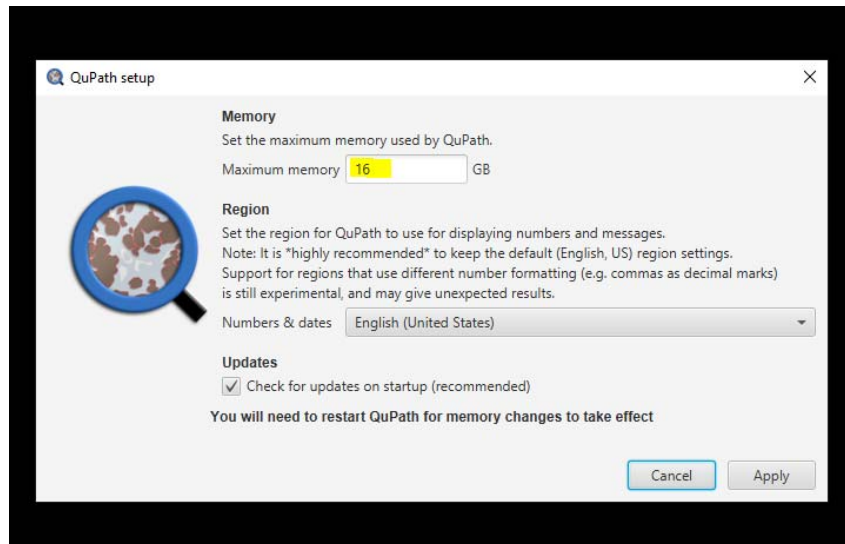
# Qupath versions and download

- Current stable version  
Qupath Version 0.2.3
- Download link: <https://github.com/qupath/qupath/releases/tag/v0.2.3>
- FAQ site: <https://forum.image.sc/tags/qupath>
- What to download
  - \* For windows (two options, Option 1 recommended):
    - Option 1 (without administrator permission) : QuPath-0.2.3-windows.zip**  
Unzip it and run QuPath-v0.2.3.exe
    - Option 2 (with administrator permission): QuPath-0.2.3-Windows.msi**  
Standard windows installer
  - \* For MAC: **QuPath-0.2.3-Mac.pkg**
  - \* For Linux: **QuPath-0.2.3-Linux.tar.xz**

# Image Format supported by QuPath

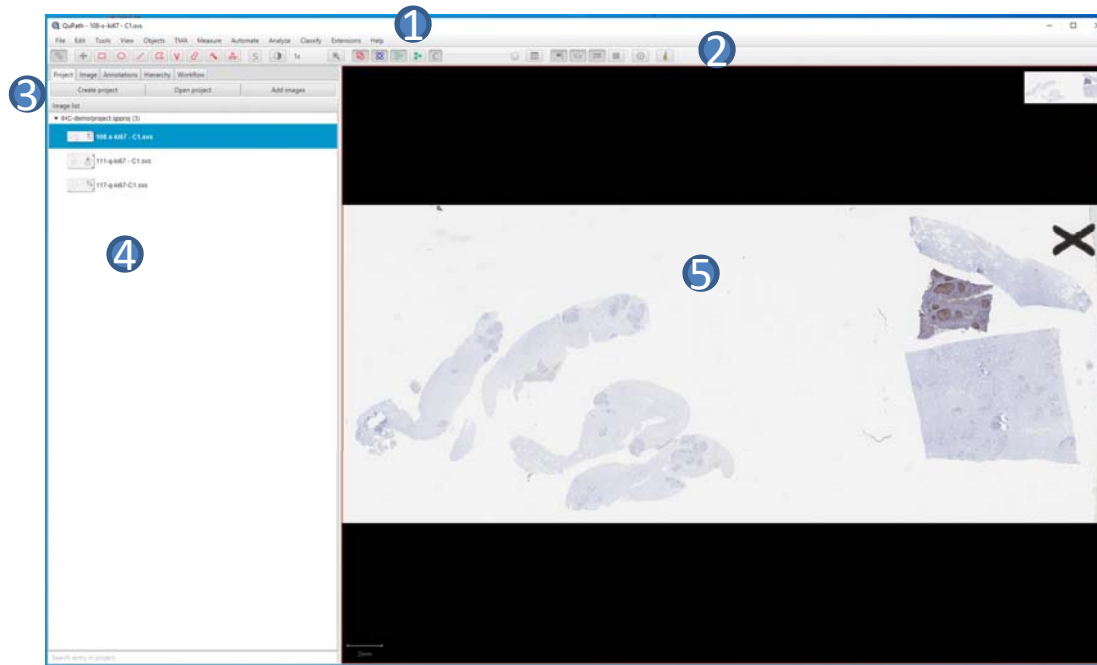
- QuPath supports a wide range of image formats, including many whole slide formats.
- To support different image formats, two essential open source libraries are used in QuPath. For details please check following links respectively:
  - \* **Bio-formats:** <https://docs.openmicroscopy.org/bio-formats>
  - \* **OpenSlide:** <https://openslide.org>
- As a quick summary, following WSI image formats are supported:  
Aperio SVS/SCN, Ventana BIF, Zeiss CZI, Hamamatsu NDPI, 3D-Histech MRXS  
(may need to convert to OME-TIFF), JPG/JP2/Some TIFF formats

# QuPath-Setup



When running QuPath for the first time, you will see a setup window, shown on left. QuPath will request 50% of the total memory available in your system, this is the default setting. Generally speaking, the software will perform better if your system has more memories.

# QuPath- Software Layout



Software Layout:

1 Command menu

2 Functional keys

3 Operational tabs

4 Information summary window

5 Image viewer

# QuPath-Project Tab

## Project view & folder structure

We can view and analyze single image using QuPath. However in digital pathology, we always need to manage and process multiple images in batch mode. It is highly recommended to use **Project** function. Especially, if you want to analyze our Versa SCN fluorescence image, you have to use **Project** function to open/load the image properly.

**Create Project: File-> Project...-> Create Project**

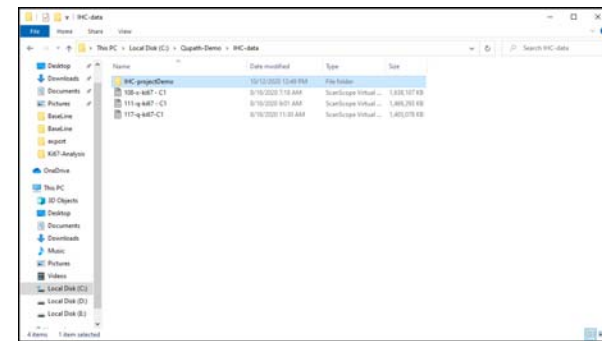
**Project** provides a way to:

- Group together related images
- Easily switch between images (by clicking on thumbnails in the project image list)
- Organize your data files, along with scripts, classifiers and other useful things
- View a larger thumbnail and some file data by hovering the mouse over an image, without needing to open it fully

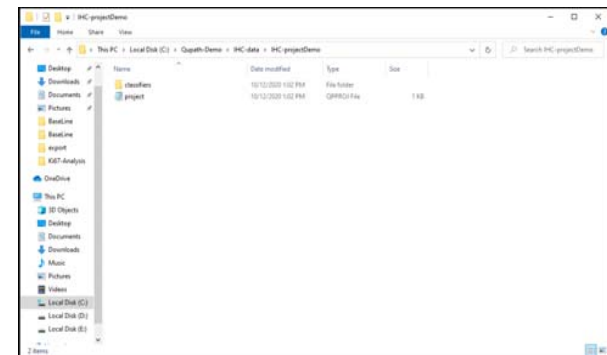
### Caution:

**When you want to transfer /share your project with other user, please make sure both of you are using the same QuPath version. Project created by version 0.2.x cannot be opened by version 0.1.x. Also, when you transfer the project to another computer, you also need to maintain the folder structure.**

For example: if you have image files at C:\Qupath-Demo\IHC-data, you can create a blank subfolder first: IHC-projectDemo

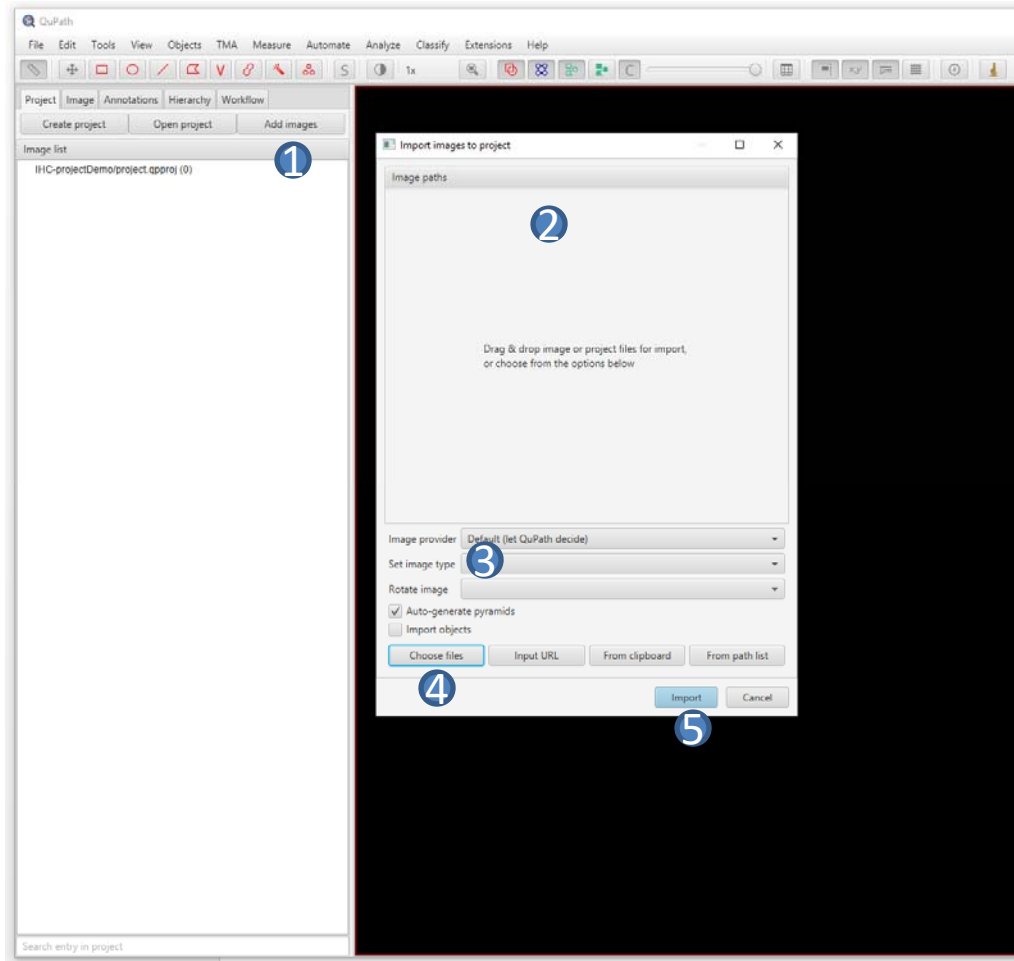


When you use QuPath to create the project, assign the above IHC-projectDemo folder to be the project folder, QuPath will then generate a project file and some subfolders for future computational purposes, as shown below:



# QuPath-Project Tab

## Add images to project



Add images to project:

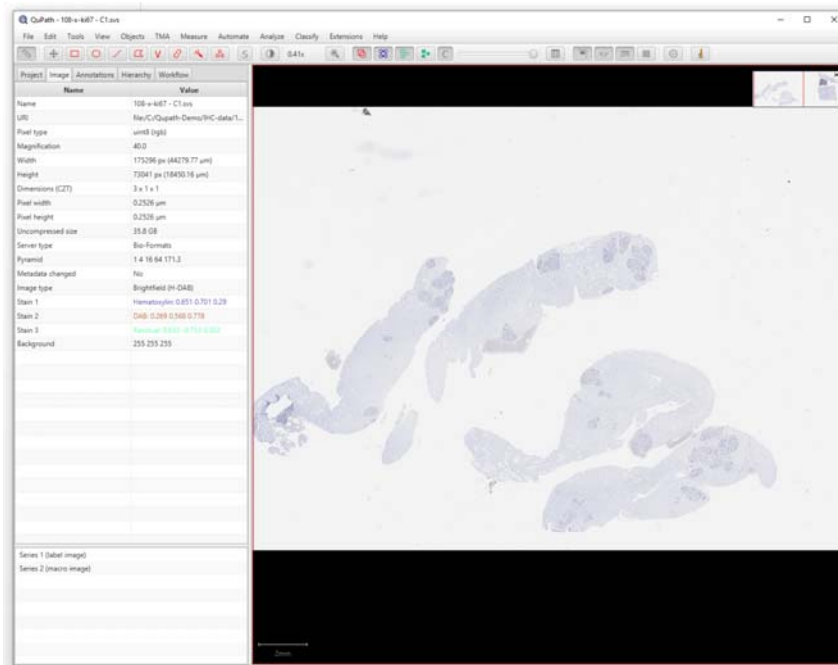
- 1: click **Add images** from top left;
- 2: Import images to project: you will see this dialogue window;
- 3: Set image type: H&E / H-DAB /H-other/FL, etc.;
- 4: click **Choose files** to select images you want to add to the project;
- 5: then click **Import**

After importing images, you will see a image list on the left, if you select one image and click, that image will be shown in the viewer on right:



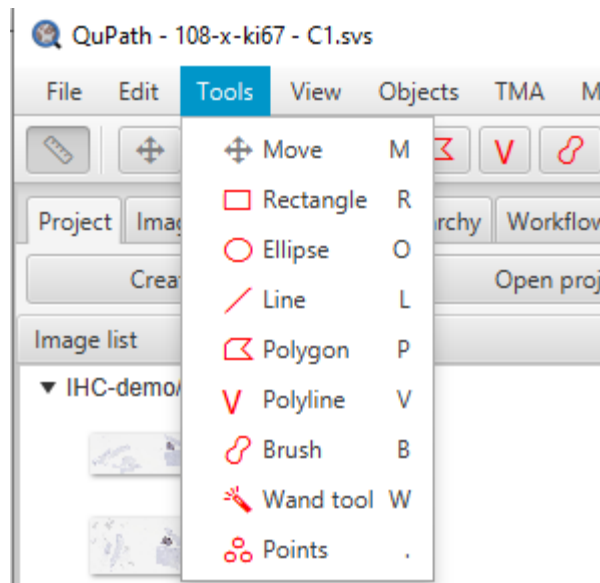
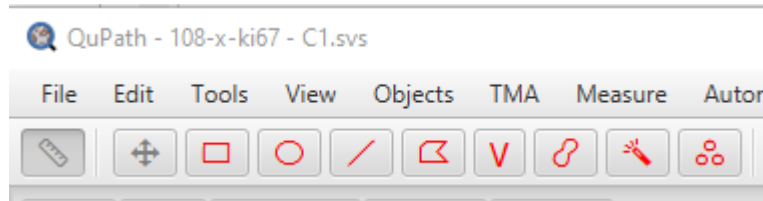


# QuPath-Image Tab



When an image is selected, you can check and view the image properties from the **Image Tab**. Some fundamental image information will be listed here, for example: magnification, image size, pixel size, etc.

# QuPath-Annotation

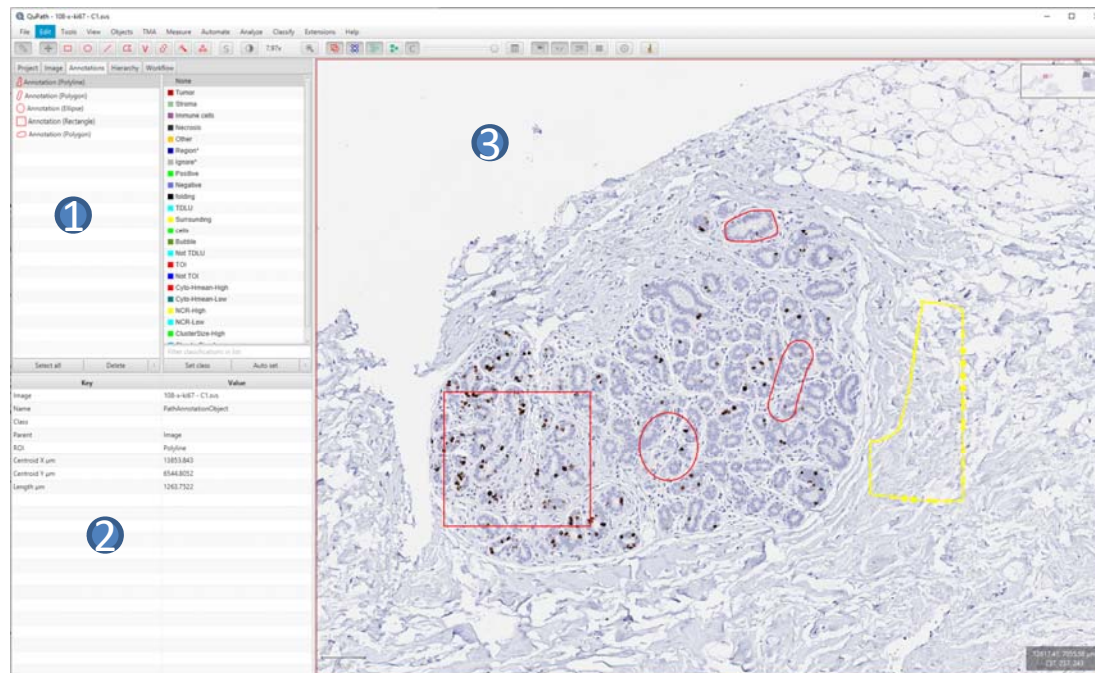


In digital pathology image processing, the first step is typically annotation- defining your region of interest. You can access annotation tools either from command menu – Tools or from functional keys from top left. You can define your ROI by regular shapes or polygon or brush, etc.



: This is the Move key, when click, you can pan the image within the image viewer window.

# Qupath-Annotation Tab



Annotation example (shown on left):

1: all annotation regions are organized/ listed in **Annotation Tab**;

2: Information summary window: annotation summary information will be listed here;

3: Image viewer: couple of ROIs are highlighted in image viewer.

Some online annotation tutorials:

<https://www.youtube.com/watch?v=FAoUHu40dk0>

<https://www.youtube.com/watch?v=lGj3uyxUdgs>

# QuPath-Annotation

## Shortcut keys

- Shortcut keys for the tools and commands you use most frequently can help a lot to get things done quickly, and with less frustration.
- *ALT Key*
  - \* When the *Move* tool is selected, use the *Alt* key to click on multiple objects to select them.
  - \* When the *Brush* or *Wand* tools are selected, use the *Alt* key to switch to 'eraser' mode when drawing
- *Shift Key*
  - \* When the *Brush* or *Wand* tools are selected, use the *Shift* key to continue adding to an existing selected annotation (rather than creating a new one).
- **Note for Linux users**  
Replace *Alt* with *Alt + Super*

### Drawing and navigation

Tool	Shortcut
Move	M
Move (temporary)	Spacebar
Rectangle	R
Ellipse	O
Line	L
Polygon	P
Brush	B
Wand	W
Points	.

# QuPath – Other Shortcut keys

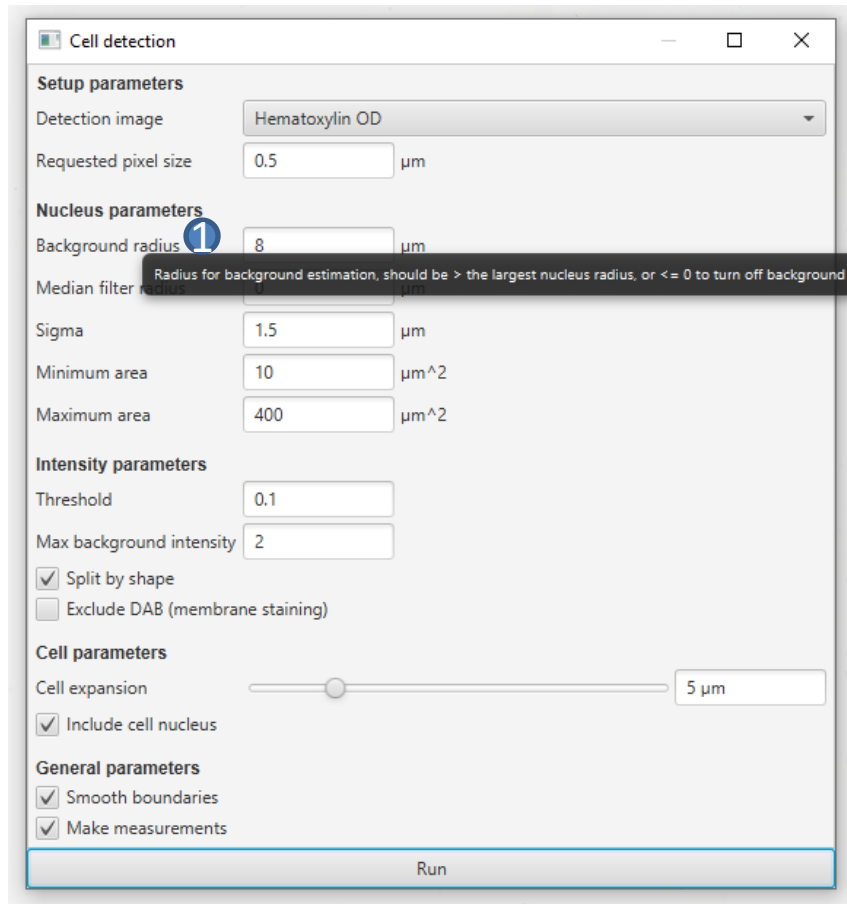
## Display Shortcuts

Action	Shortcut
Show/hide <b>detections</b>	H
Show/hide annotations	A
Show/hide TMA grid	G
Show/hide counting grid	Shift + G
Fill/unfill detections	F
Fill/unfill annotations	Shift + F
Show/hide color channel	<i>Numeric keys</i>

## Command Shortcuts

Command	Shortcut
File → Save	Ctrl + S
File → Revert	Ctrl + R
View → Show command list	Ctrl + L
View → Brightness/Contrast	Shift + C
View → Show log	Shift + Ctrl + L
Objects → Duplicate annotation	Shift + D
Objects → Restore last annotation	Shift + E
Objects → Create full image annotation	Shift + Ctrl + A
Objects → Select → Reset selection	Shift + Ctrl + R
Classify → Create detection classifier	Shift + Ctrl + D

# QuPath – Cell Object Detection



Now images are organized in project and ROIs are defined by annotations. We can start to investigate the data set and understand different processing functions in QuPath.

The fundamental concept in QuPath is object detection-more specifically, nucleus object detection. Watershed segmentation technique is applied for this purpose.

WaterShed Cell Detection :

**Analyze->Cell detection->Cell detection**

Parameters used for cell detection :

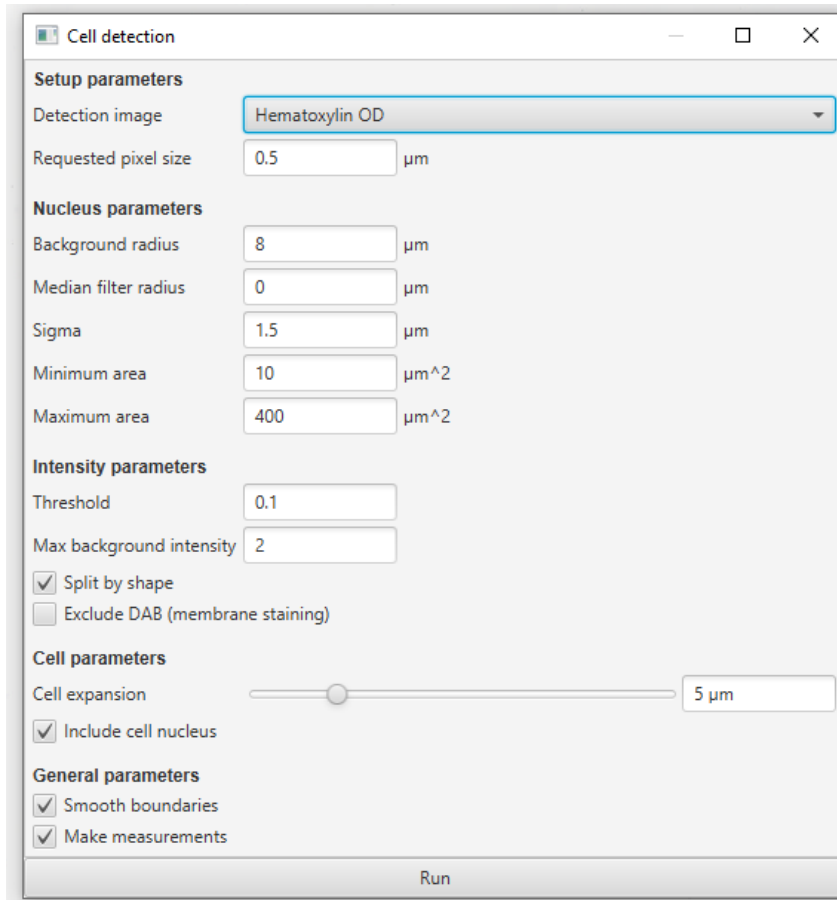
- \* Setup parameters
- \* Nucleus parameters
- \* Intensity parameters
- \* Cell parameters

Recommendations:

- When using QuPath processing functions, you can always start with default parameter settings, then compare result image with original image to see if any parameter needs to be modified;
- Parameter definition can be viewed by moving mouse cursor on top of the parameter name, for example highlighted by ① :

Background radius: radius for background estimation

# QuPath – Cell Object Detection



## WaterShed cell detection details:

### \* Setup parameters

Detection image: Hematoxylin optical density or Overall optical density

Requested pixel size: pixel size for computation

### \* Nucleus parameters

Background radius : if background subtraction is considered, 0 means no background subtraction

Median filter radius: reduce image texture / smooth intensity vibrations

Sigma: control /reduce the noise effect, smaller number you will get more nucleus (more fragments)

Minimum/Maximum area: define the range of nucleus size

### \* Intensity parameters

Threshold : the lower intensity threshold for nucleus object

Max background intensity: the upper intensity threshold for nucleus object

### \* Cell parameters

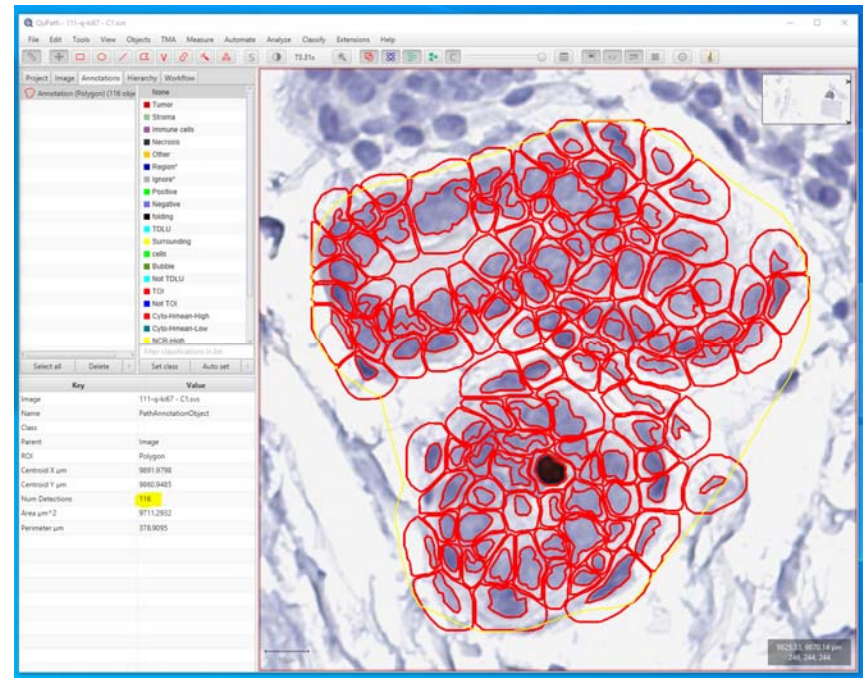
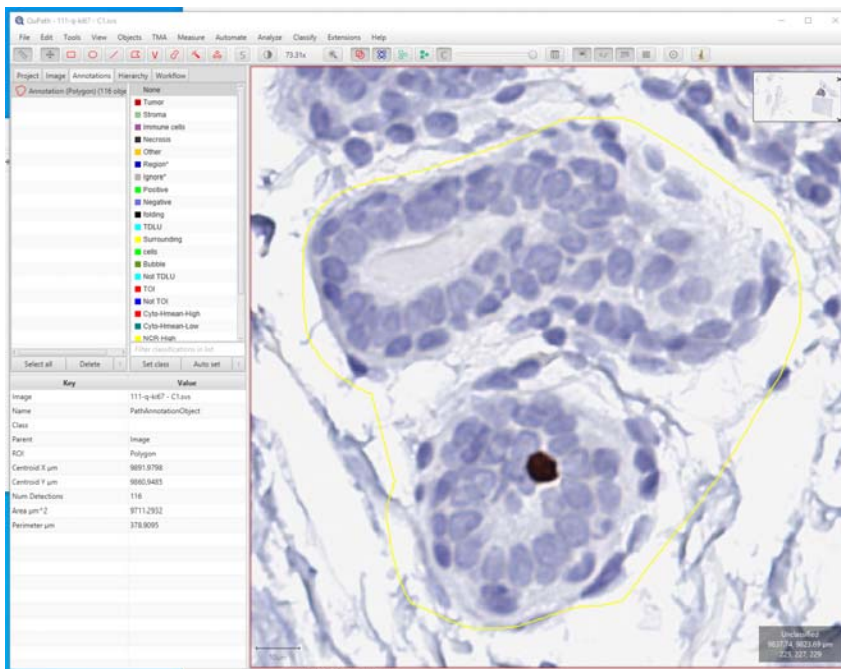
Cell expansion : based on detected nucleus objects, define cell size



# QuPath – Cell detection example

Yellow ROI containing some cell objects

116 cells are identified, highlighted in red





# QuPath- Cell positivity

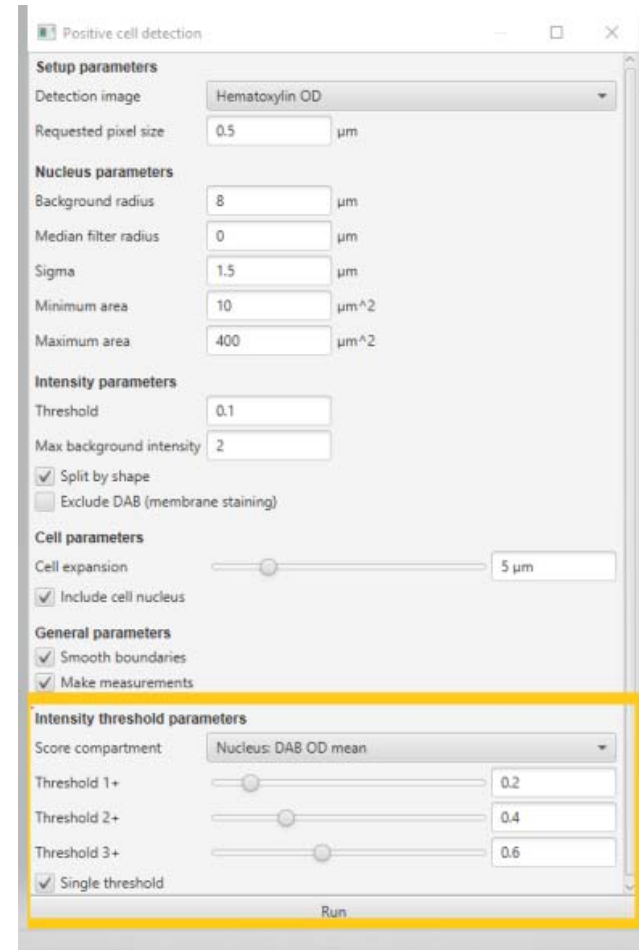
In IHC (H-DAB) image analysis, after cell objects are detected based on Hematoxylin channel, we can further determine positive cells stained by DAB (brown channel) .

Positive cell detection:

**Analyze->Cell detection-> Positive cell detection**

Parameters in the yellow region (Shown in left image) can be adjusted to determine positive cells (cells with brown staining).

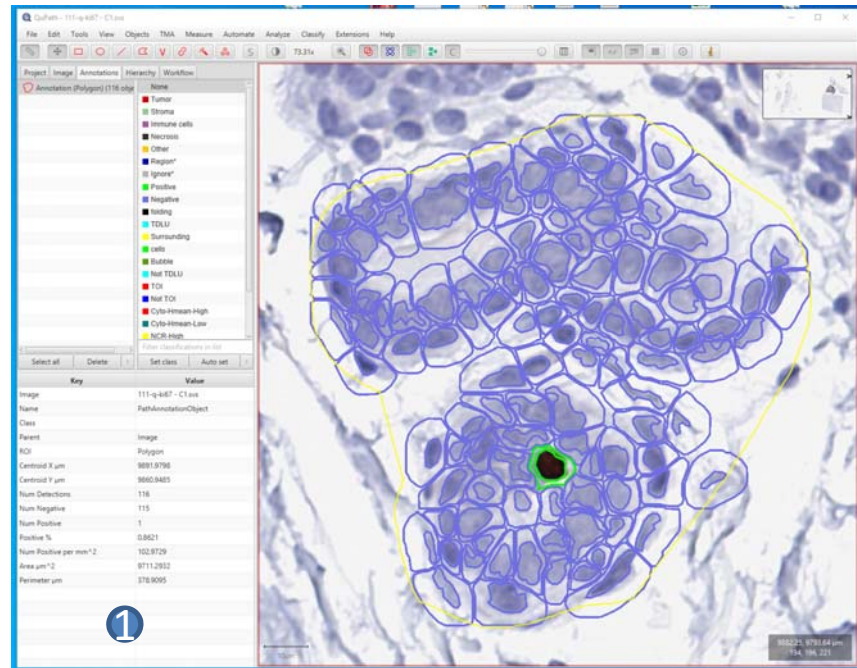
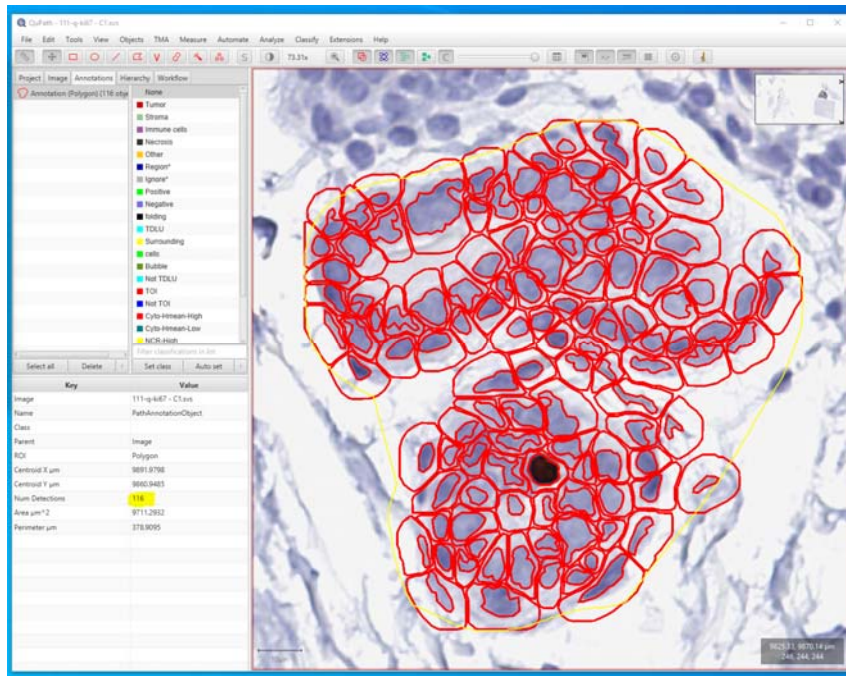
H-score can be computed after this positivity determination.



# QuPath – Cell positivity example

116 cells are detected from previous step

Applying single thresholding: Nucleus DAB mean intensity  $>0.2$ , one positive cell is identified and highlighted in green

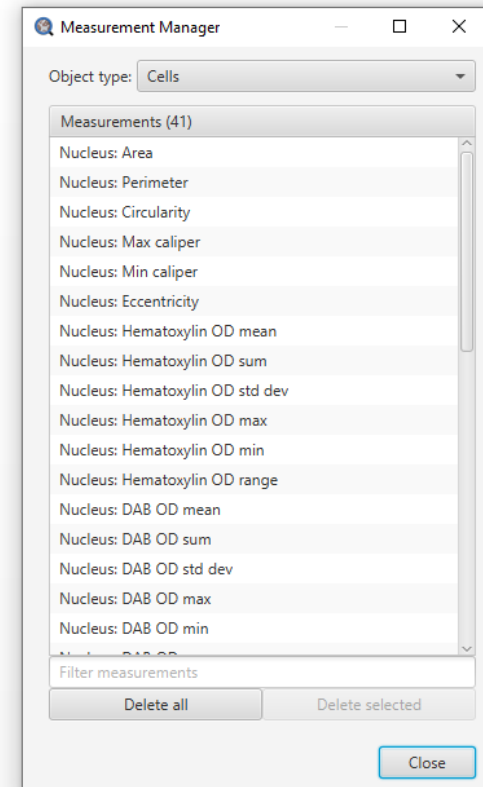
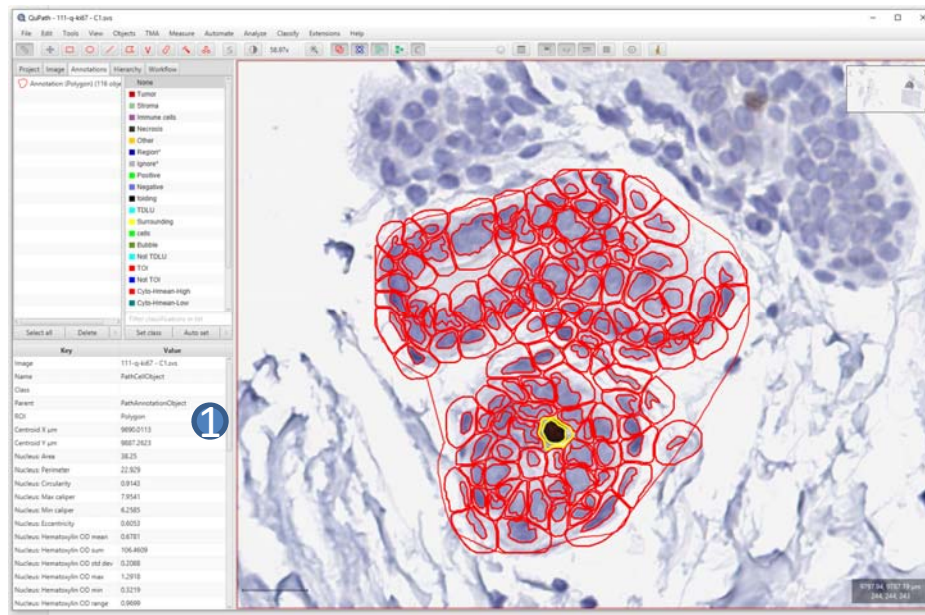


Summary information is listed in region ①

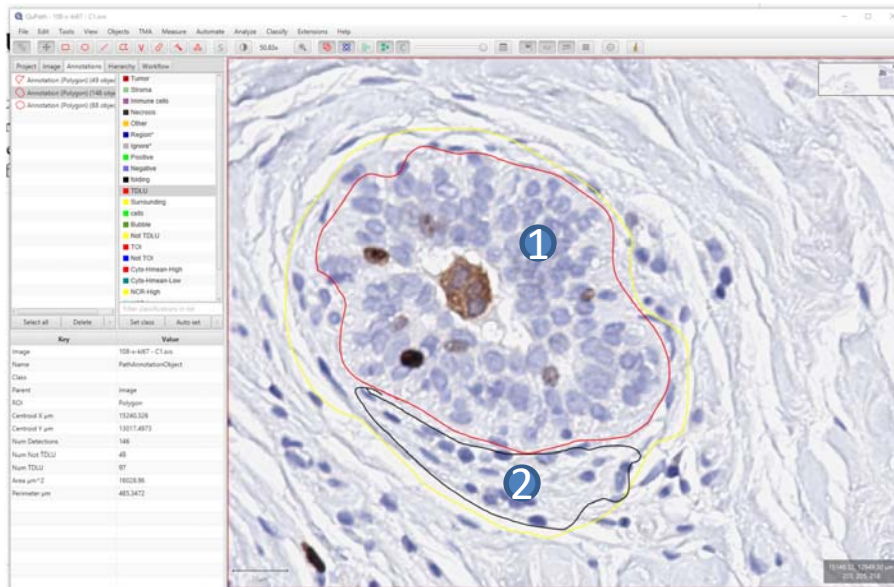
# QuPath- Cell Classification-Object Features

At WaterShed cell detection step, cell object features are computed. If you click one identified cell object (yellow one) in the viewer, feature measurements are shown in region **1**

You can also find the cell object feature list by clicking:  
**Measure -> Show measurement manager**  
The total feature number is 41 at this point.



# QuPath- Cell Classification-Feature selection



In digital pathology image processing, another popular application task is classification-to separate different cell types based on cell object features.

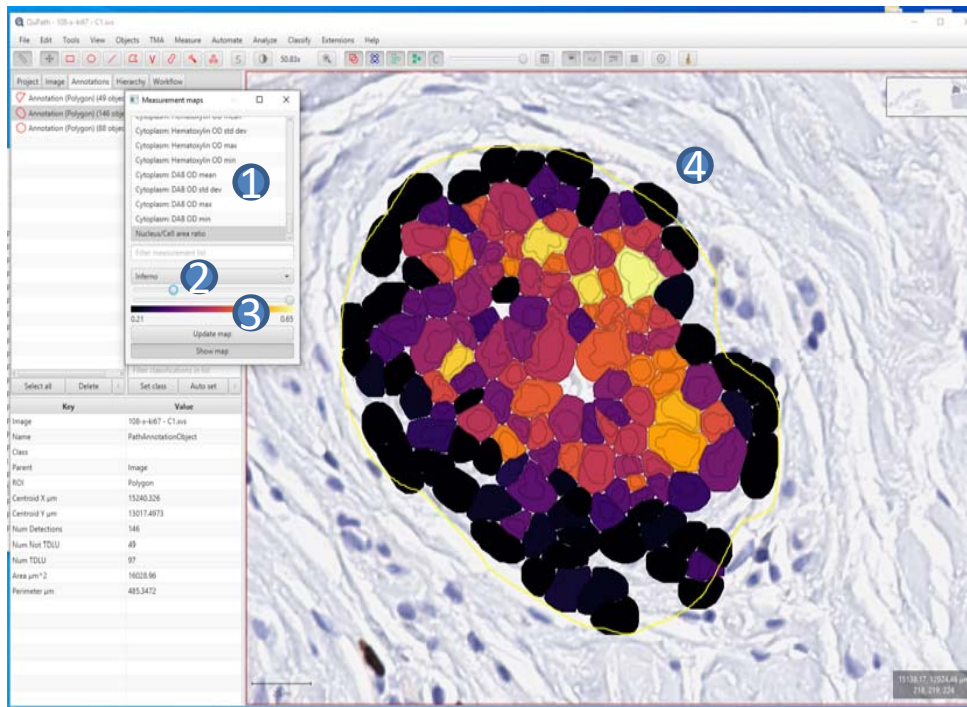
For example, if we want to separate TDLU cells (red region ① ) and Not TDLU cells (black region ②), we need to investigate their object feature measurements and select the proper feature /feature combination for optimal separation.



# QuPath- Cell Classification-Feature investigation

You can investigate object features by clicking:

## Measure – Measurement maps



- 1 Dialogue window for Measurement maps  
41 cell object features are listed here  
Current selected feature: Nucleus/Cell area ratio
- 2 Color template to highlight the difference  
Current selected: inferno
- 3 Feature threshold  
By moving the control bar, you can change the threshold; current threshold is 0.21;
- 4 Highlighted result in Image viewer  
Dark objects are rejected : Not TDLU cells

# QuPath- Cell Classification- Single measurement classifier

From feature investigation step, we find a possible object feature- **Nucleus/Cell area ratio** for separation, now we can create a simple classifier by clicking:

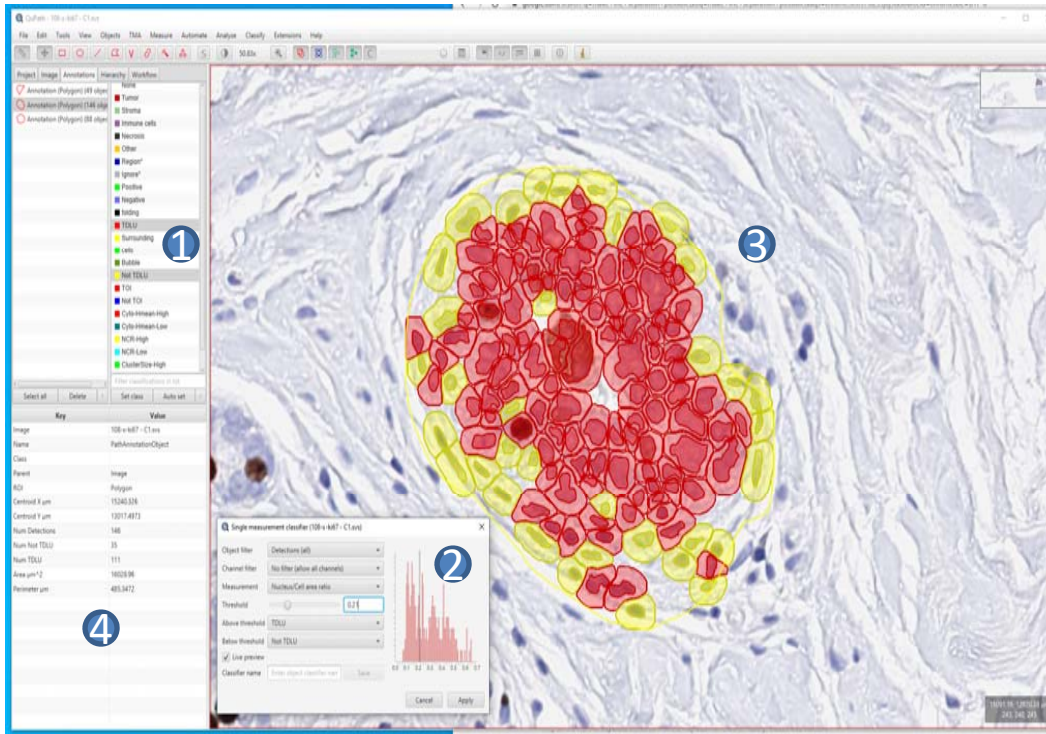
**Classify -> Object classification -> Create single measurement classifier**

① At this window, you can add new class definition by right clicking your mouse button, in this example, two classes are added –TDLU (red) and Not TDLU (yellow);

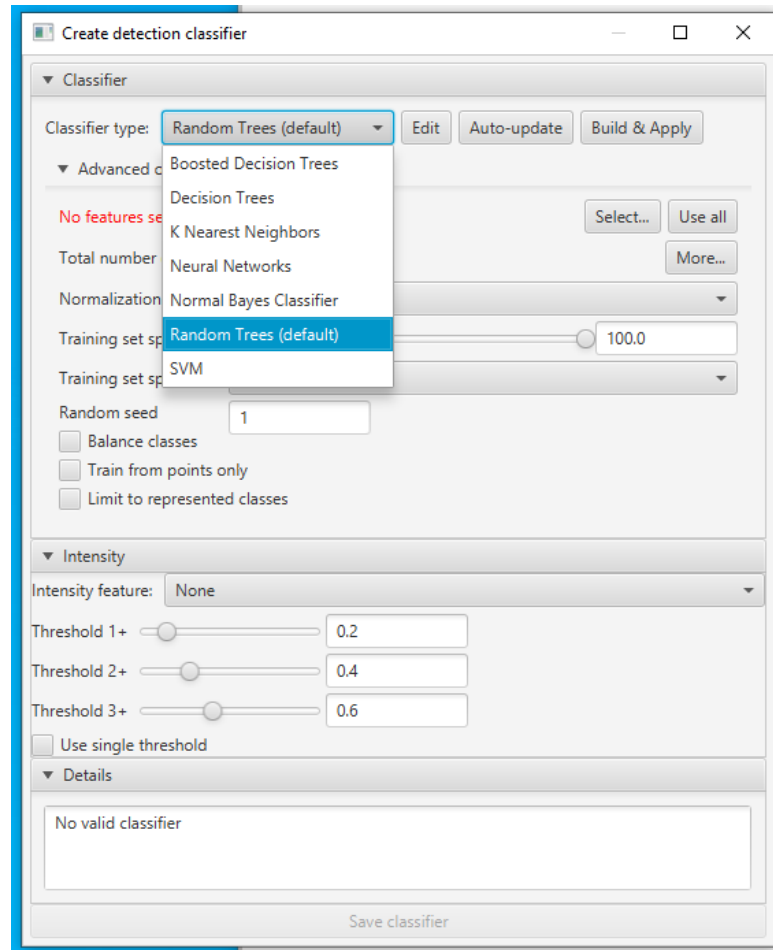
② In this classifier window, you can choose **Nucleus/Cell area ratio** as selected feature for separation, then define your separation threshold as 0.21;

③ In image viewer window, the result will be shown: red object representing TDLU cells, yellow color representing Not TDLU cells;

④ TDLU cell counts and Not TDLU cell counts will be summarized in this summary window.



# QuPath- Cell Classification- Cell classification-Classification models



In practical, separation result based on single feature is not good enough. Then we need to apply classification models to include more features into consideration.

Find **Create detection classifier** window by clicking:  
**Classify -> object classification -> older classifier-> Create detection classifier**

From this window, you can find some popular classification models: Boosted Decision Trees/Decision Trees/K Nearest Neighbors/Neural Network/Normal Bayes Classifier/ Random Trees/SVM

Model performance is basically data-dependent. Due to robustness and less training input, **Random Tree** is a suggested option for initial data investigation.

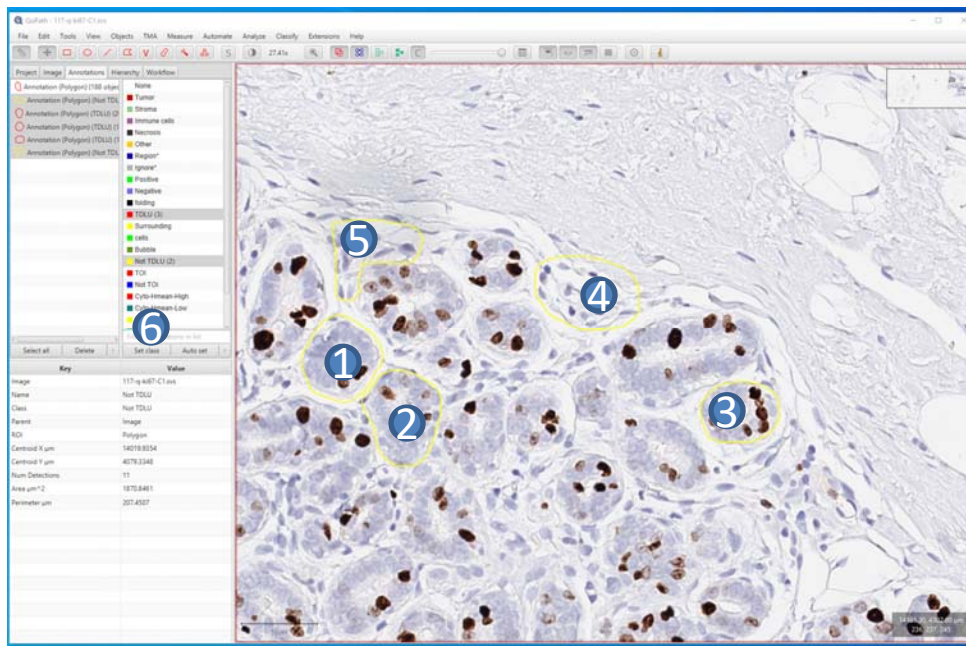
Here is a link to describe the concept of random trees:

<https://towardsdatascience.com/understanding-random-forest-58381e0602d2>

Classification procedure contains three steps:

- \* Training: annotate and select cell objects for different cell types
- \* Validating: prepare a region with mixed cell types
- \* Building classifier: select classifier model and features, build the classifier using training set and apply the classifier on validating region

# QuPath- Cell Classification- Cell classification- Build Random Trees Classifier

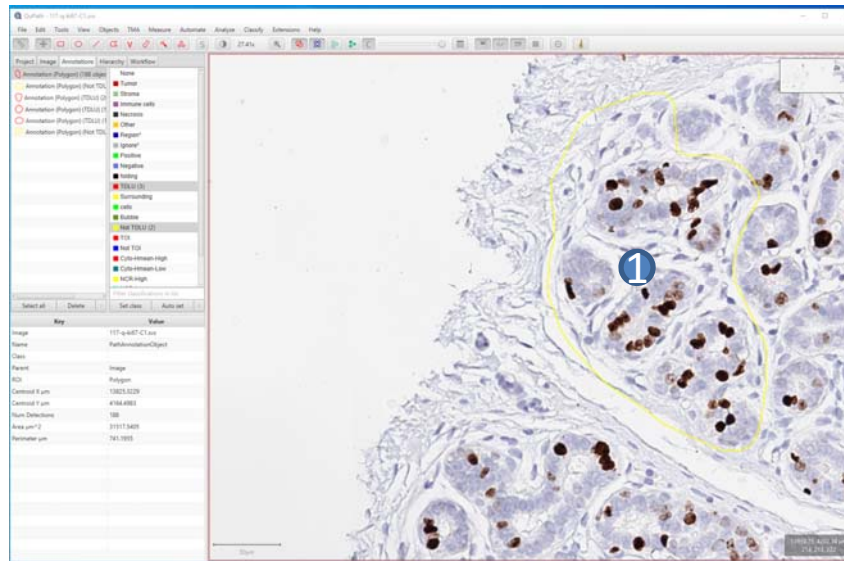


Training data input:

- In image viewer, use annotation tools to define TDLU cells ( ① ② ③ ) and Not TDLU cells ( ④ ⑤ ), and assign them to proper class respectively by clicking **Set class ⑥** ;
- Then use Watershed cell object detection to obtain the feature measurements, totally 41 cell object features will be computed.



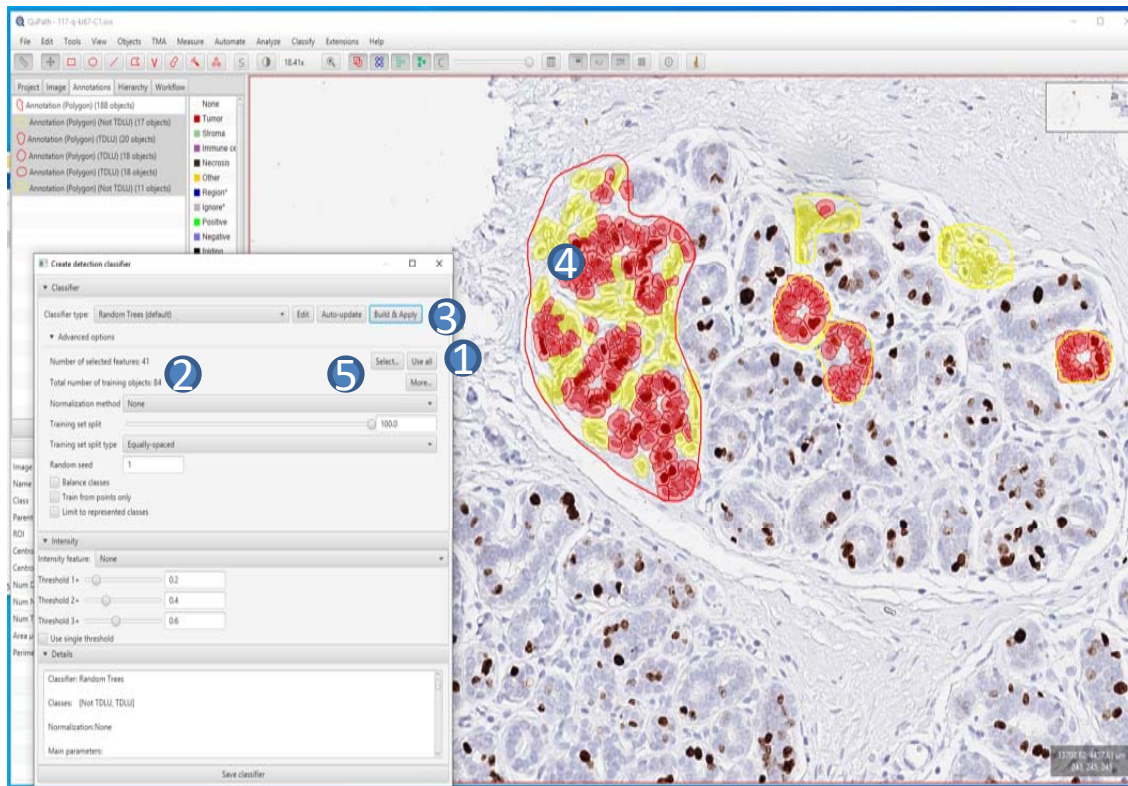
# QuPath- Cell Classification- Cell classification- Build Random Trees Classifier



Prepare validation set :

- In image viewer, use annotation tools to define another region with mixed TDLU & Not TDLU cells ①
- Then use Watershed cell object detection to detect cell objects in this region.

# QuPath- Cell Classification- Cell classification- Build Random Trees Classifier

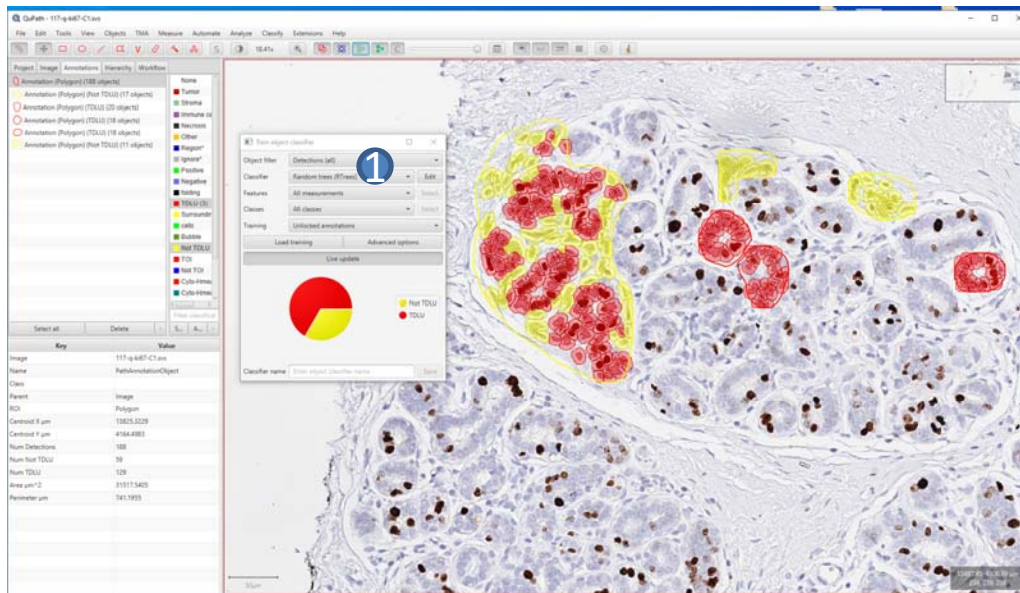


Build classifier and apply it to validation region:

Now, open the Create detection classifier window, load the training data by clicking **Use all** ①, for this example, totally 84 objects and 41 features ② are used to compute the classifier. Then, click **Build & Apply** ③, result will be shown in validating region ④. Sometimes, based on result, you can add/remove object features for purpose by clicking **Select** ⑤.

Now in validation region, TDLU cells are highlighted in red, Not TDLU cells are in yellow.

# QuPath- Cell Classification-Update



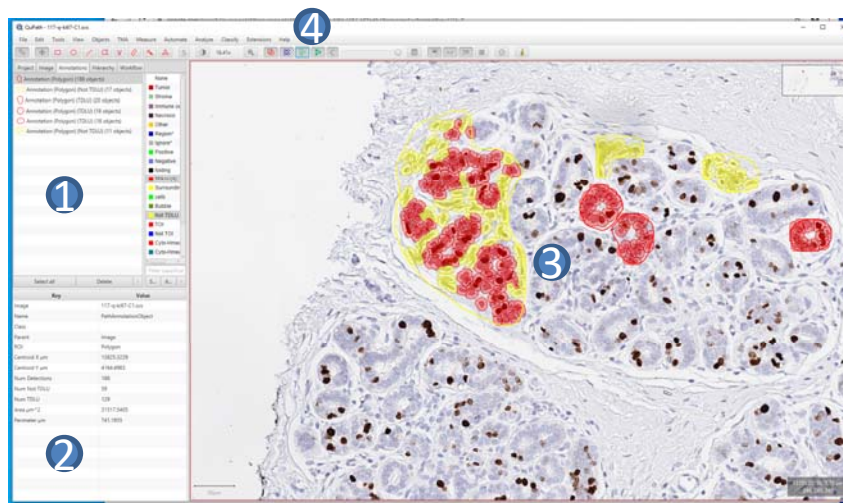
From current version, QuPath starts to change the dialogue window layout/appearance. For example, **Train object classifier** will replace the **Create detection classifier** in future, **Train object classifier** can be found by clicking:

**Classify->Object classification-> Train object classifier** or Shortcut : **Ctrl+Shift+D**

**Train object classifier** ① still uses the same classification concepts, it gives you an extra classification pie chart. You can define a name to the classifier and save the classifier for future use.

# QuPath-Result

Result :



- ① Annotation summary list
- ② Detection result summary
- ③ Detection highlighted in viewer
- ④ On/Off detection markup

