

QuPath Protocol for Image Analysis

1. Project Setup:

- **Create a Project Folder:**

Create an empty folder on your computer that will serve as your QuPath project directory.

- **Launch and Configure the Project:**

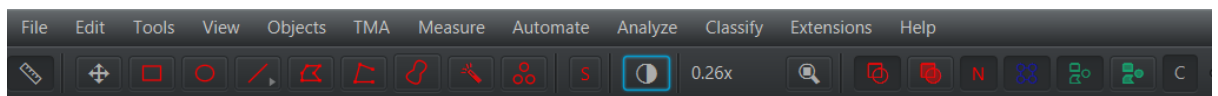
Open QuPath, navigate to Project → Create Project, and select the folder you just created.

Add your images to the project by importing them.

2. Project Configuration and Pre-processing:

- **Image Preparation:**

Open the image to be analyzed and use the **Brightness and Contrast** tool to verify the channels.



Adjust the colors if necessary to match the fluorophores used in staining.

- **Channel Selection:**

Choose one channel to initiate the analysis.

3. Creating Annotations:

- **Define Regions of Interest (ROI):**

Select an annotation shape (e.g., rectangle, circle) to delineate the area of detection. Navigate to **Analyze → Cell Detection → Cell Detection**.

- **Parameter Optimization:**

Adjust key parameters for the selected channel:

- **Threshold:** Modify to optimize signal detection.
- **Sigma:** Adjust for better cell boundary recognition.
- **Cell Expansion:** Fine-tune to ensure proper segmentation.

Iteratively modify these parameters until the desired structures are correctly detected.

Repeat this process for each channel as needed.

- **Annotation Review:**

Annotations will appear in the Annotations panel. Selecting an annotation displays the total number of detected objects.

4. Channel Name Assignment and Object Classification:

- **Populate Channel Names:**

In the Classification panel (located next to Annotations), click the three dots (⋮) at the bottom and select **Populate from Image Channels**.

This action automatically assigns channel names, easing data management.

- **Assign Classifications:**

For each annotation, select the corresponding classification and click Set Selected.

To assign a classification to all objects, go to the Hierarchy panel, select multiple objects (using Shift+Click), and assign the classification via the Annotation menu by clicking **Set Selected**.

This ensures that each object retains its assigned classification upon data export.

5. Exporting Results:

- **Data Export:**

Once the detection and classification are complete, go to **Automate** → **Directory** → **Script**. Paste the following code into the script window to start exporting data.

- https://github.com/Tatieli8/Microglia_RNAseq_Imaging/blob/R-code/export_qupath_data.groovy