

### Screenshot Summary for Article 68124 – Protocol Section 3 (1080p Resolutions)

- **68124\_Section3\_Screenshot\_1\_1080p.mp4**
  - 3.1.2 (Create Project is being clicked and a destination folder is being selected) **00:00-00:14**
- **68124\_Section3\_Screenshot\_2\_1080p.mp4**
  - 3.2.1 (Add images and Choose files are being clicked then QTIFF file is being navigated to) **00:00-00:15**
  - 3.2.2 (Image type is being set to Fluorescence, default settings are kept and Import is being clicked) **00:16-00:29**
- **68124\_Section3\_Screenshot\_3\_1080p.mp4**
  - 3.3.1 (The image is being double clicked and File and Save are pressed for project changes) **00:00-00:14**
  - 3.3.2 (The marker visibility is being toggled and the half-moon icon on the toolbar is being pressed to change viewing settings) **00:15-00:29**
- **68124\_Section3\_Screenshot\_4\_1080p.mp4**
  - 3.4.1 (The brush/wand tool is being used to draw an annotation around the entire tissue section) **00:00-00:15**
  - 3.4.2 (The annotation is being defined as Full\_Tissue) **00:16-00:24**
  - 3.4.3 (Negative annotations are being created) **00:25-01:04**
- **68124\_Section3\_Screenshot\_5\_1080p.mp4**
  - 3.5.1 (Objects>Annotations...>Duplicate selected annotations is being pressed under Annotations tab) **00:00-00:14**
  - 3.5.2 (SOX10 is being turned on. The duplicated annotation is being shrunk to define tumor region) **00:15-01:08**
- **68124\_Section3\_Screenshot\_6\_1080p.mp4**
  - 3.6.1 (The annotation is being selected. Then Objects>Annotations...> Expand Annotations is being clicked) **00:00-00:07**
  - 3.6.2 (The expansion radius is being set to 1  $\mu$ m and Run is pressed) **00:08-00:18**
- **68124\_Section3\_Screenshot\_7\_1080p.mp4**
  - 3.7.1 (The new annotation is being renamed as Full\_Tissue\_Expansion) **00:00-00:14**
  - 3.7.2 (The **Tumor Annotation** is being selected, right-clicked, and **Insert in hierarchy** is selected) **00:15-00:22**
- **68124\_Section3\_Screenshot\_8\_1080p.mp4**
  - 3.8.1 (The tumor annotation is being re-selected. Then Objects>Annotations...>Make inverse is being pressed) **00:00-00:08**
  - 3.8.2 (New annotation is being defined as Stroma) **00:09-00:22**
- **68124\_Section3\_Screenshot\_9\_1080p.mp4**
  - 3.9.1 (Image tab is being pressed and the pixel width and image height is being seen) **00:00-00:12**
- **68124\_Section3\_Screenshot\_10\_1080p.mp4**
  - 3.10.1 (The StarDist extension is being downloaded from Github) **00:00-00:13**
  - 3.10.2 (The extension file is being imported into QuPath) **00:14-00:24**
  - 3.10.3 (The StarDist groovy script files and model files are being downloaded from GitHub) **00:25-00:47**
- **68124\_Section3\_Screenshot\_11\_1080p.mp4**

- 3.11.1 (Tumor and Stroma annotations are being selected for the tissue selections) **00:00-00:05**
- 3.11.2 (Automate and Script Editor are being clicked and script interface is being seen) **00:06-00:10**
- 3.11.3 (The appropriate StarDist cell segmentation script is being opened) **00:11-00:20**
- 3.11.4 (stardist\_cell\_seg\_model.pb is being selected when prompted) **00:21-00:44**
- **68124\_Section3\_Screenshot\_12\_1080p.mp4**
  - 3.12.1 (The image is being saved. Then Measure> Export Measurements is clicked. Corresponding images are pressed and Export type is being set as Cells and the Separator as .csv) **00:00-00:21**
  - 3.12.2 (The output file location is being chosen and Populate is being clicked) **00:22-00:32**
- **68124\_Section3\_Screenshot\_13\_1080p.mp4**
  - 3.13.1 (The mean for markers is being selected and exported. **TXT: Export Nucleus: Mean for nuclear markers and Cytoplasm:Mean for cytoplasmic markers**) **00:00-00:26**
- **68124\_Section3\_Screenshot\_14\_1080p.mp4**
  - 3.14.1 (The exported CSV file is being opened and the column headings are being truncated to retain only marker name) **00:00-00:18**
- **68124\_Section3\_Screenshot\_15\_1080p.mp4**
  - 3.15.1 (MarkerNormalization.R script is being run and the cells are being filtered based on nuclear size.) **00:00-00:27**
- **68124\_Section3\_Screenshot\_16\_1080p.mp4**
  - 3.16.1-3.16.3 (Min-max normalization is being done) **00:00-00:18**
  - 3.16.4 (New CSV file is being saved) **00:19-00:34**