Screenshot Summary for Article 68124 – Protocol Section 3

- **68124_Section3_Screenshot_1.mp4**
    - 3.1.2 (Create Project is being clicked and a destination folder is being selected) **00:00-00:11**
- **68124_Section3_Screenshot_2.mp4**
    - 3.2.1 (Add images and Choose files are being clicked then QPTIFF file is being navigated to) **00:00-00:16**
    - 3.2.2 (Image type is being set to Fluorescence, default settings are kept and Import is being clicked) **00:17-00:33**
- **68124_Section3_Screenshot_3.mp4**
    - 3.3.1 (The image is being double clicked and File and Save are pressed for project changes) **00:00-00:19**
    - 3.3.2 (The marker visibility is being toggled and the half-moon icon on the toolbar is being pressed to change viewing settings) **00:20-00:49**
- **68124_Section3_Screenshot_4.mp4**
    - 3.4.1 (The brush/wand tool is being used to draw an annotation around the entire tissue section) **00:00-00:31**
    - 3.4.2 (The annotation is being defined as Full_Tissue) **00:32-00:41**
    - 3.4.3 (Negative annotations are being created) **00:42-01:34**
- **68124_Section3_Screenshot_5.mp4**
    - 3.5.1 (Objects>Annotations…>Duplicate selected annotations is being pressed under Annotations tab) **00:00-00:11**
    - 3.5.2 (SOX10 is being turned on. The duplicated annotation is being shrunk to define tumor region) **00:12-01:10**
- **68124_Section3_Screenshot_6.mp4**
    - 3.6.1 (The annotation is being selected. Then Objects>Annotations…> Expand Annotations is being clicked) **00:00-00:09**
    - 3.6.2 (The expansion radius is being set to 1 µm and Run is pressed) **00:10-00:19**
- **68124_Section3_Screenshot_7.mp4**
    - 3.7.1 (The new annotation is being renamed as Full_Tissue_Expansion) **00:00-00:18**
    - 3.7.2 (The **Tumor Annotation** is being selected, right-clicked, and **Insert in hierarchy** is selected) **00:19-00:26**
- **68124_Section3_Screenshot_8.mp4**
    - 3.8.1 (The tumor annotation is being re-selected. Then Objects>Annotations…>Make inverse is being pressed) **00:00-00:09**
    - 3.8.2 (New annotation is being defined as Stroma) **00:10-00:21**
- **68124_Section3_Screenshot_9.mp4**
    - 3.9.1 (Image tab is being pressed and the pixel width and image height is being seen) **00:00-00:13**
- **68124_Section3_Screenshot_10.mp4**
    - 3.10.1 (The StarDist extension is being downloaded from Github) **00:00-00:10**
    - 3.10.2 (The extension file is being imported into QuPath) **00:11-00:18**
    - 3.10.3 (The StarDist groovy script files and model files are being downloaded from GitHub) **00:19-00:38**
- **68124_Section3_Screenshot_11.mp4**

  - 3.11.1 (Tumor and Stroma annotations are being selected for the tissue selections) **00:00-00:04**
  - 3.11.2 (Automate and Script Editor are being clicked and script interface is being seen) **00:05-00:09**
  - 3.11.3 (The appropriate StarDist cell segmentation script is being opened) **00:10-00:25**
  - 3.11.4 (stardist_cell_seg_model.pb is being selected when prompted) **00:26-00:53**
- **68124_Section3_Screenshot_12.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 Seperator as .csv) **00:00-00:22**
  - 3.12.2 (The output file location is being chosen and Populate is being clicked) **00:23-00:40**
- **68124_Section3_Screenshot_13.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)** **00:00-00:31**
- **68124_Section3_Screenshot_14.mp4**
  - 3.14.1 (The exported CSV file is being opened and the column headings are being truncated to retain only marker name) **00:00-00:26**
- **68124_Section3_Screenshot_15.mp4**
  - 3.15.1 (MarkerNormalization.R script is being run and the cells are being filtered based on nuclear size.) **00:00-00:31**
- **68124_Section3_Screenshot_16.mp4**
  - 3.16.1-3.16.3 (Min-max normalization is being done) **00:00-00:30**
  - 3.16.4 (New CSV file is being saved) **00:31-00:58**