SUPPLEMENTARY NOTES ON DEVELOPMENT OF FLUORESCEIN PIXEL INTENSITY EXPORT PROTOCOL FROM PENTERO VIDEO SCREENSHOTS USING PHOTOSHOP

ROI SIZE

In the Photoshop images provided in ExampleFiles, note that (as described in the protocol), there are layers for “FIELD”, “A”, “B”, and “C”.

- The actual size of the biopsy was determined empirically (for example, the distance between the BrainLab-registered forceps) by a member of the team who was present at the time of surgery.
- ROI-B is a circle approximately the same diameter as the biopsy, including minimal area covering spurious objects (e.g., cotton, forceps, gloved surgeon fingers).
- ROI-A is a circle 0.5X the diameter of ROI-B.
- ROI-C is a circle 2X the diameter of ROI-B.
- ROI-D is a closely-traced shape mimicking the size and shape of the biopsy, either as witnessed or described.

J.A.N., T.H.U., and J.S.S. independently exported ROIs A, B, C, D, and FIELD from several biopsy images and found the “mean gray intensity” to be most reproducible among the A fields. Thus, all fluorescence intensity values in the manuscript reflect “A” ROIs.