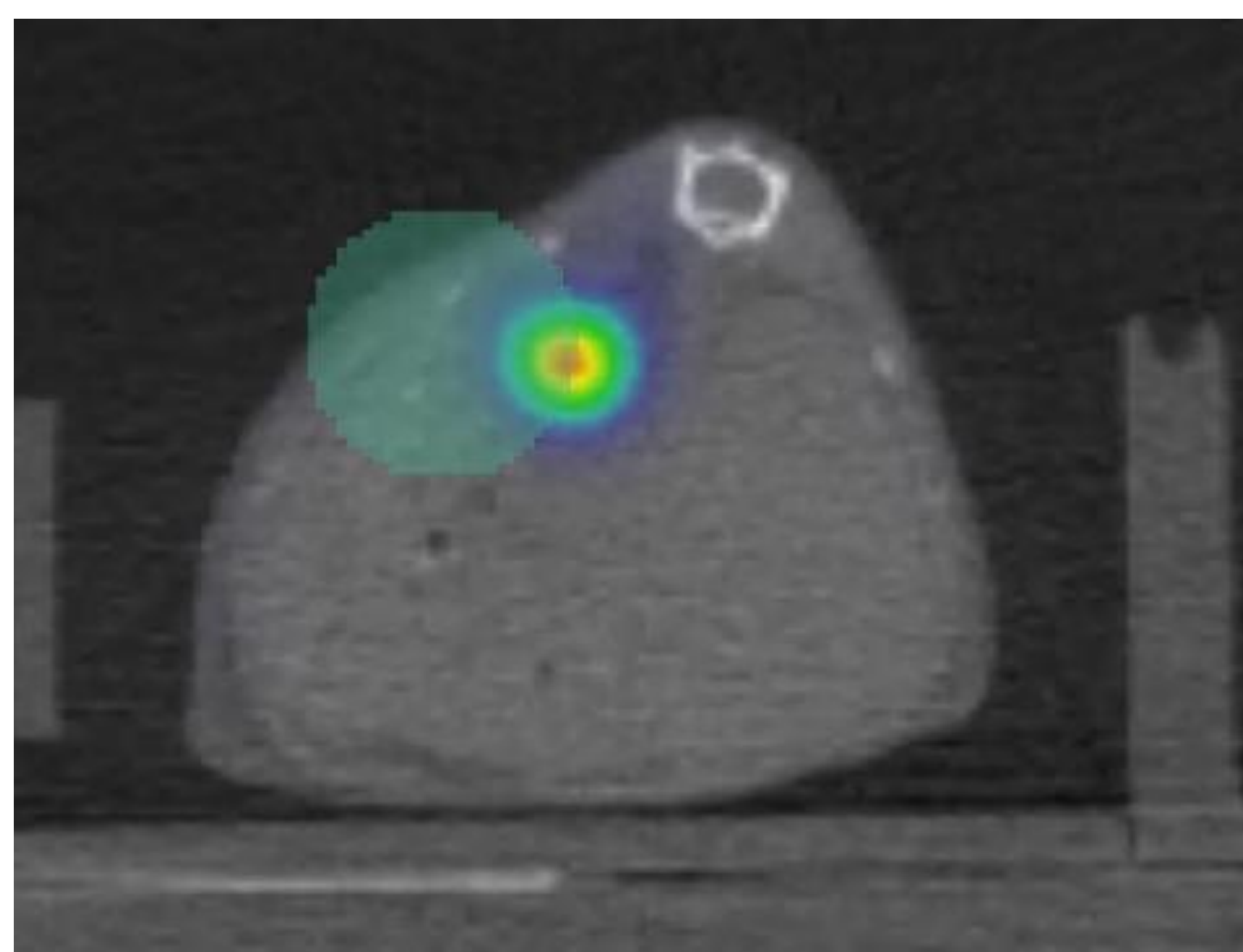
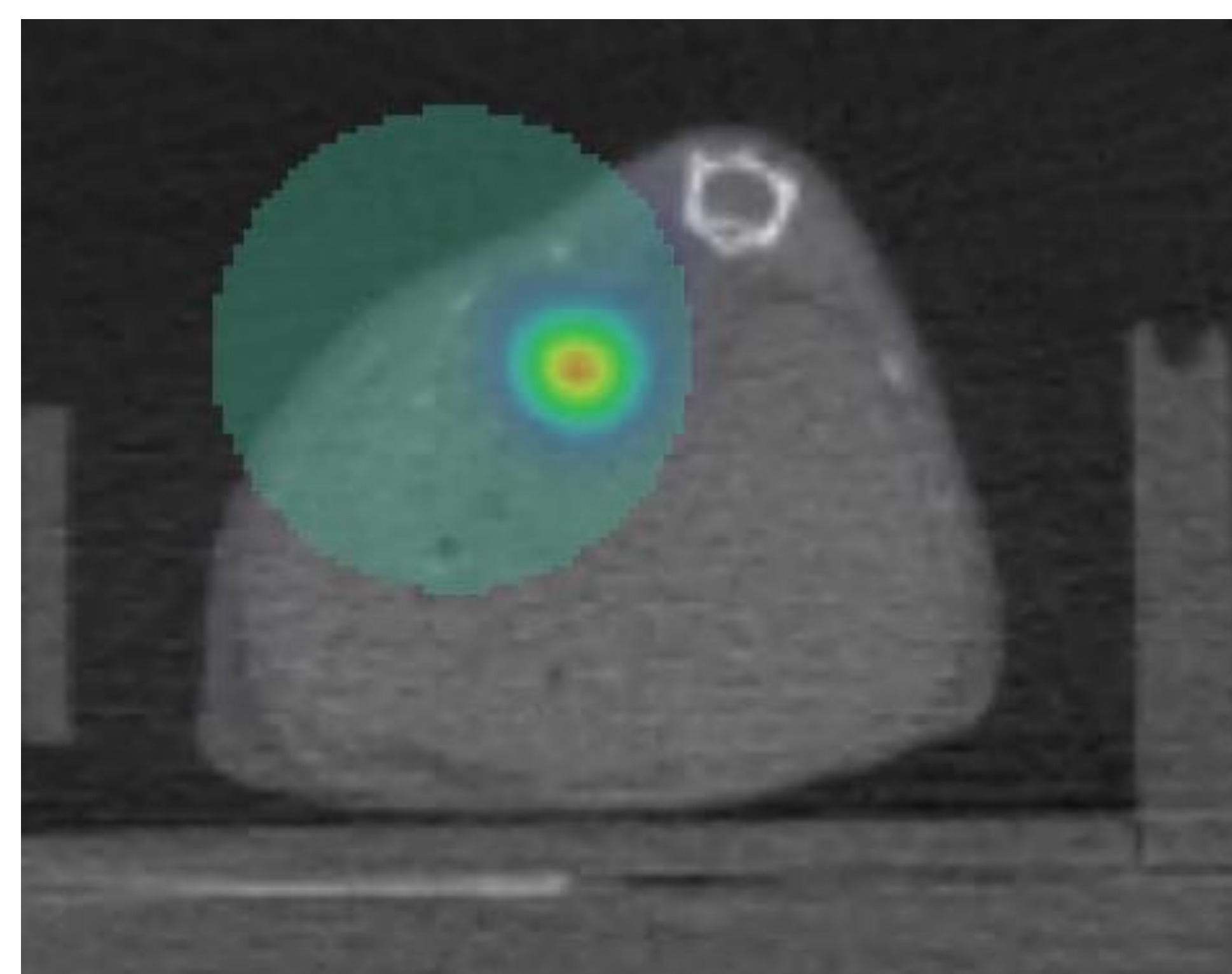


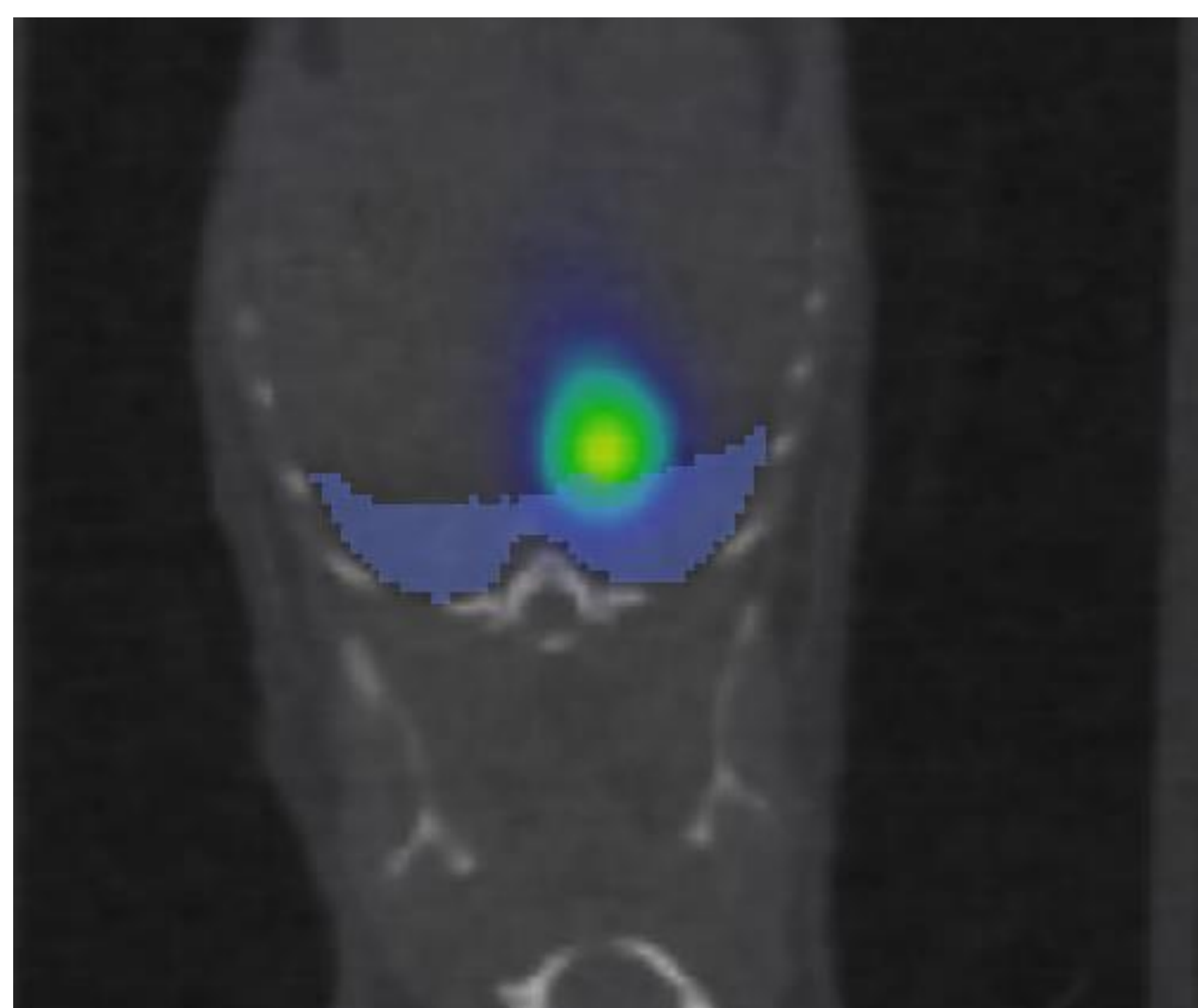
**Reconstructed spleen (sphere,  $r=10\text{mm}$ )**



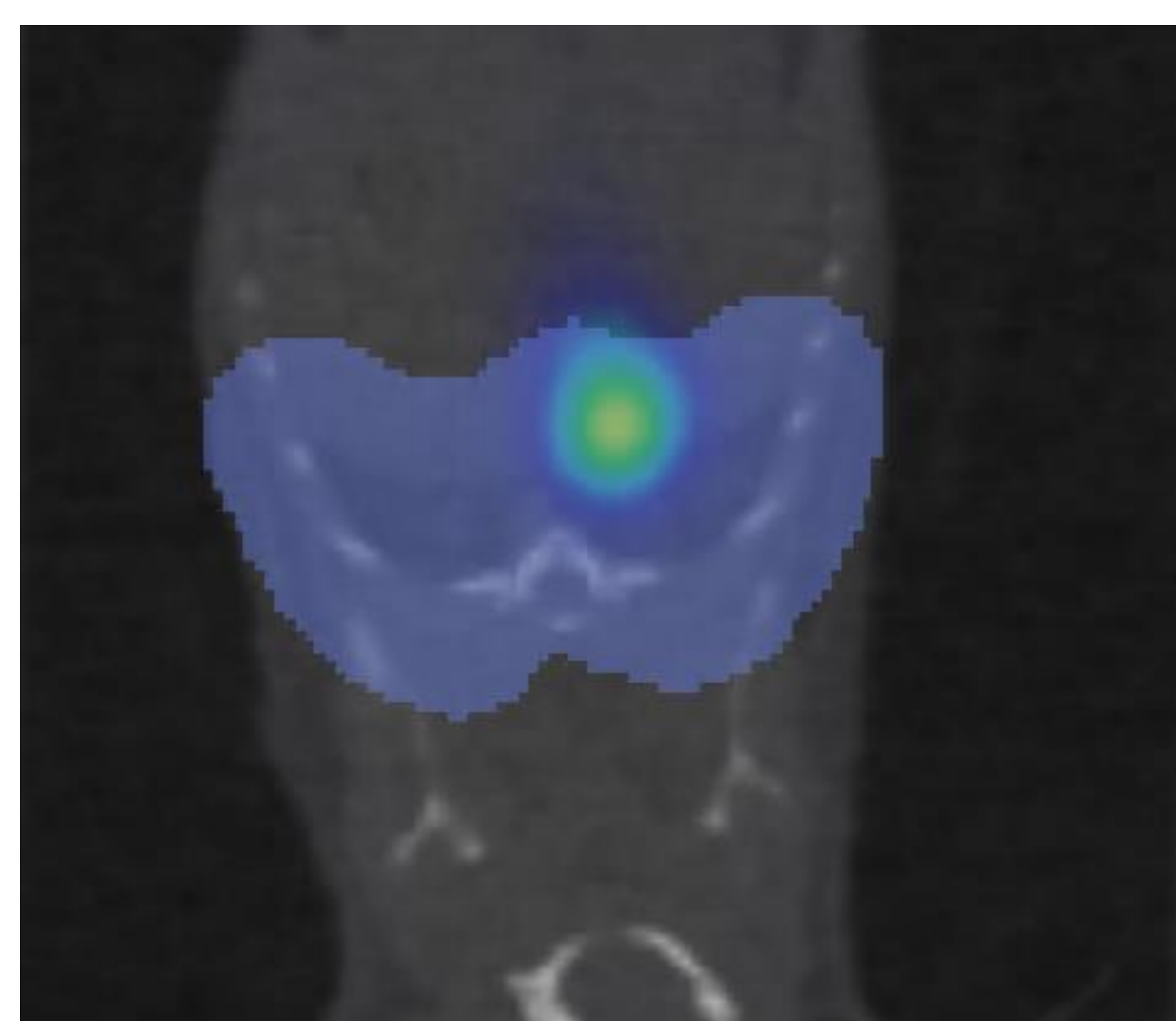
**Sphere dilatation 10x**



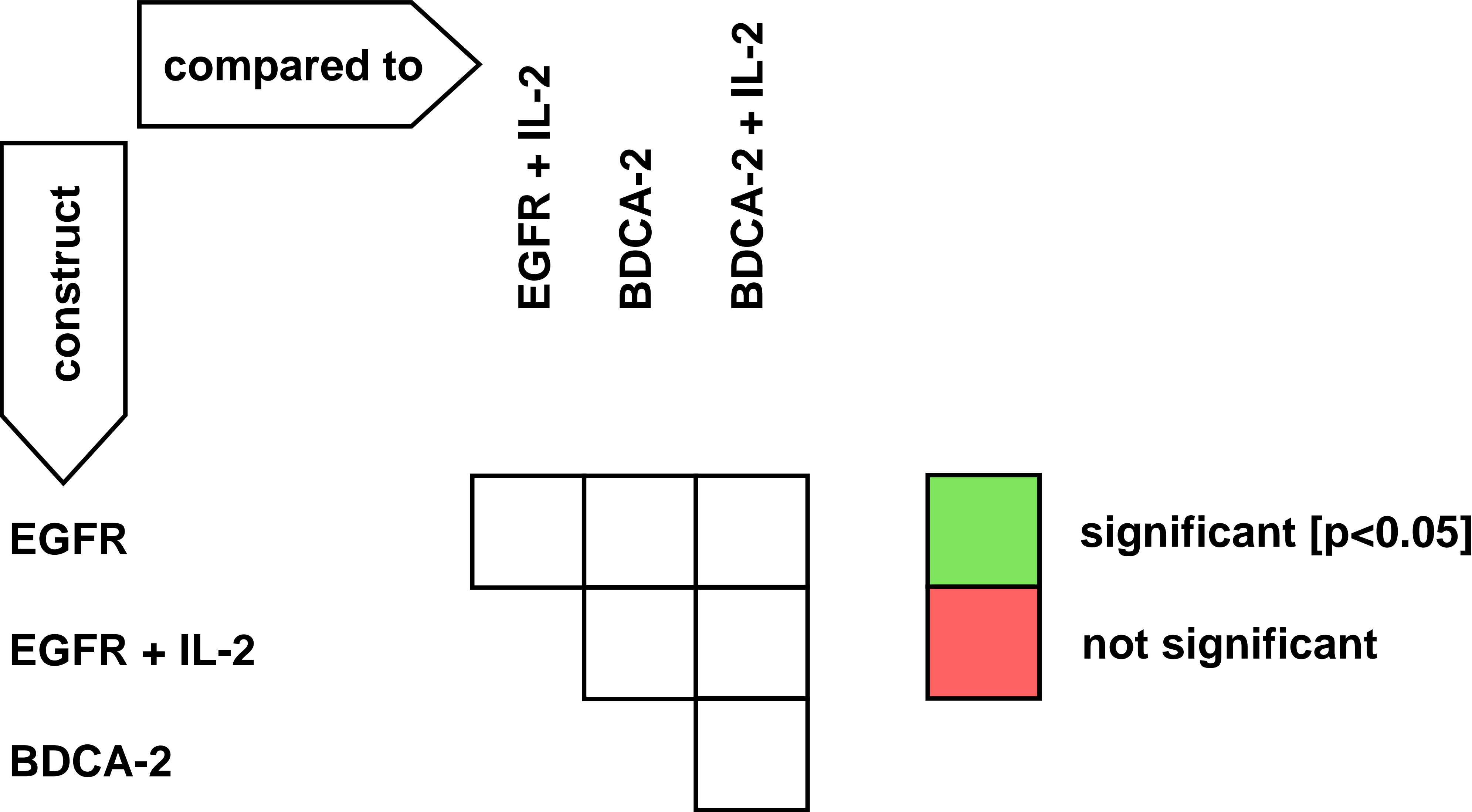
**Reconstructed lungs**



**Lungs dilatation 10x**

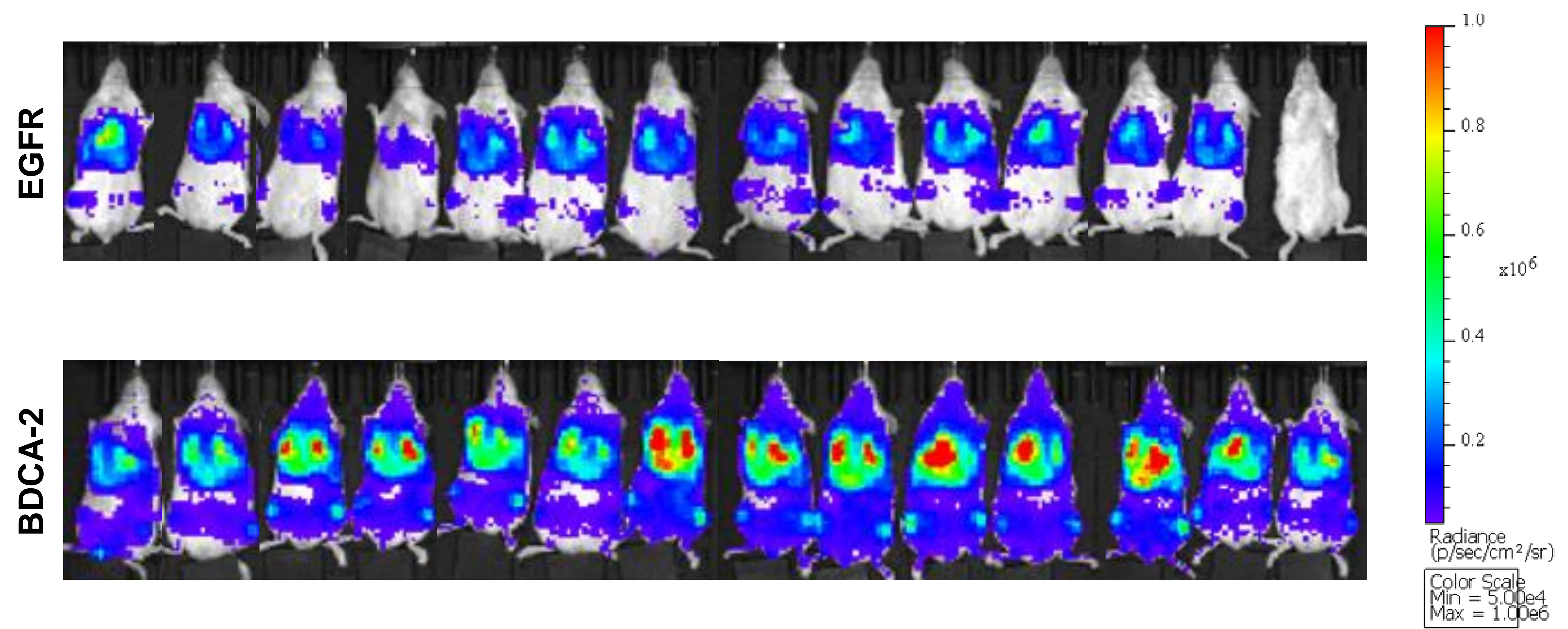


**Figure S1: Compensation of BLT signal shift in spleen and lungs.** View of representative spleen (upper) and lungs (lower) signal on Day 0 and Day 3 in BDCA-2 treated mice. Spleen was reconstructed using a sphere ( $r = 10\text{mm}$ ) (green) based on CT scan. Lungs were reconstructed using contrast-based thresholding. Shifted signal was included into the respective spleen and lungs ROI by dilatation of the ROI by factor of 10 in x-, y- and z dimension.



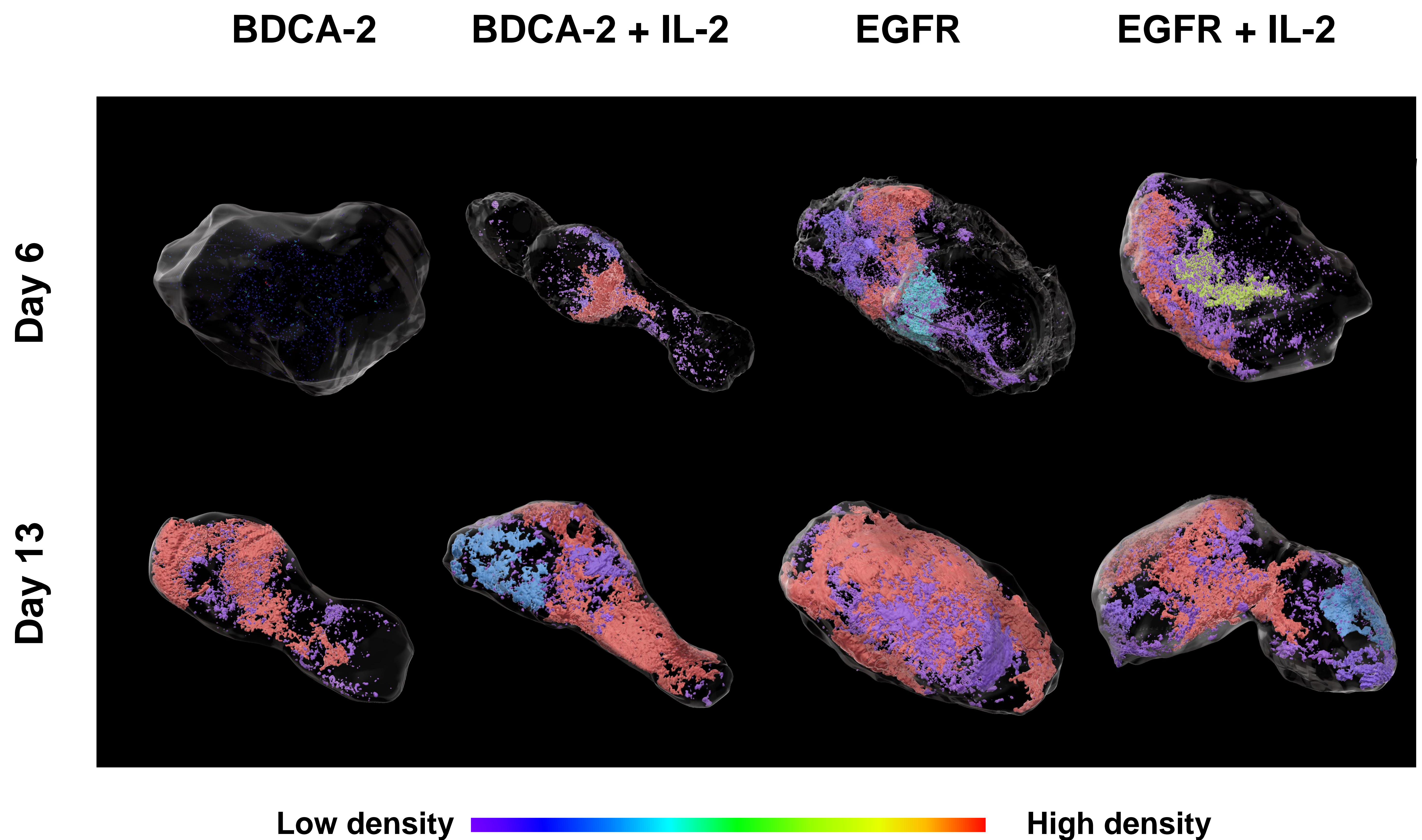
**Figure S2: Organization of the pairwise significance matrix for group comparisons.** BLT and BLI of EGFR, EGFR + IL-2, BDCA-2 and BDCA-2 + IL-2 CAR T cell treated tumors were quantified and compared. PSM  $p < 0.05$  (green),  $p > 0.05$  (red) [one-way ANOVA, multiple comparisons].





**Figure S3: Homogenous distribution of CAR T cells, 2h after injection on Day 0.** CAR T cells were injected intravenously via the tail vein. 2h post-injection, mice were intraperitoneally injected with 100  $\mu$ l D-Luciferin and measured at the IVIS Lumina *in vivo* imaging system.



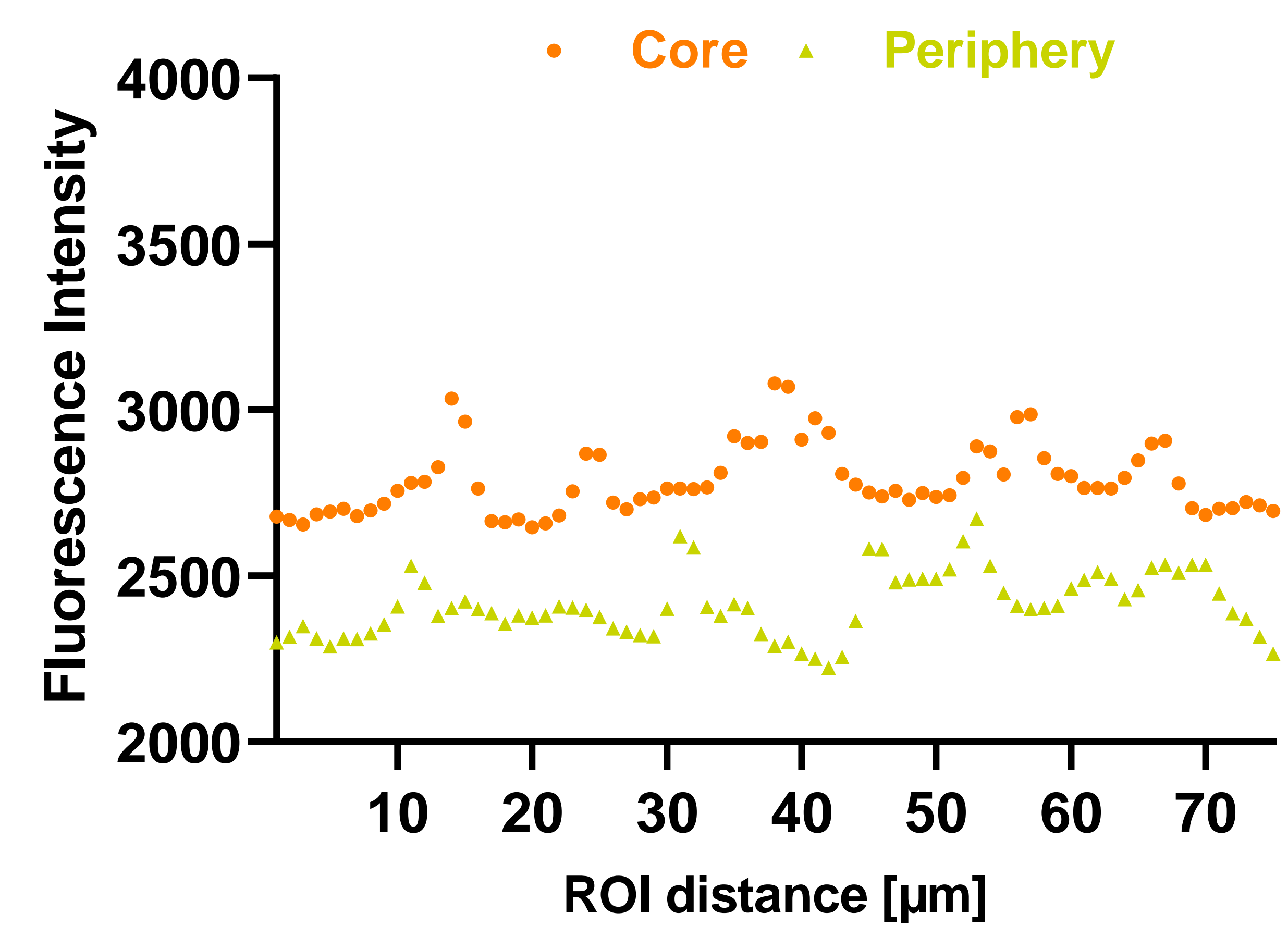
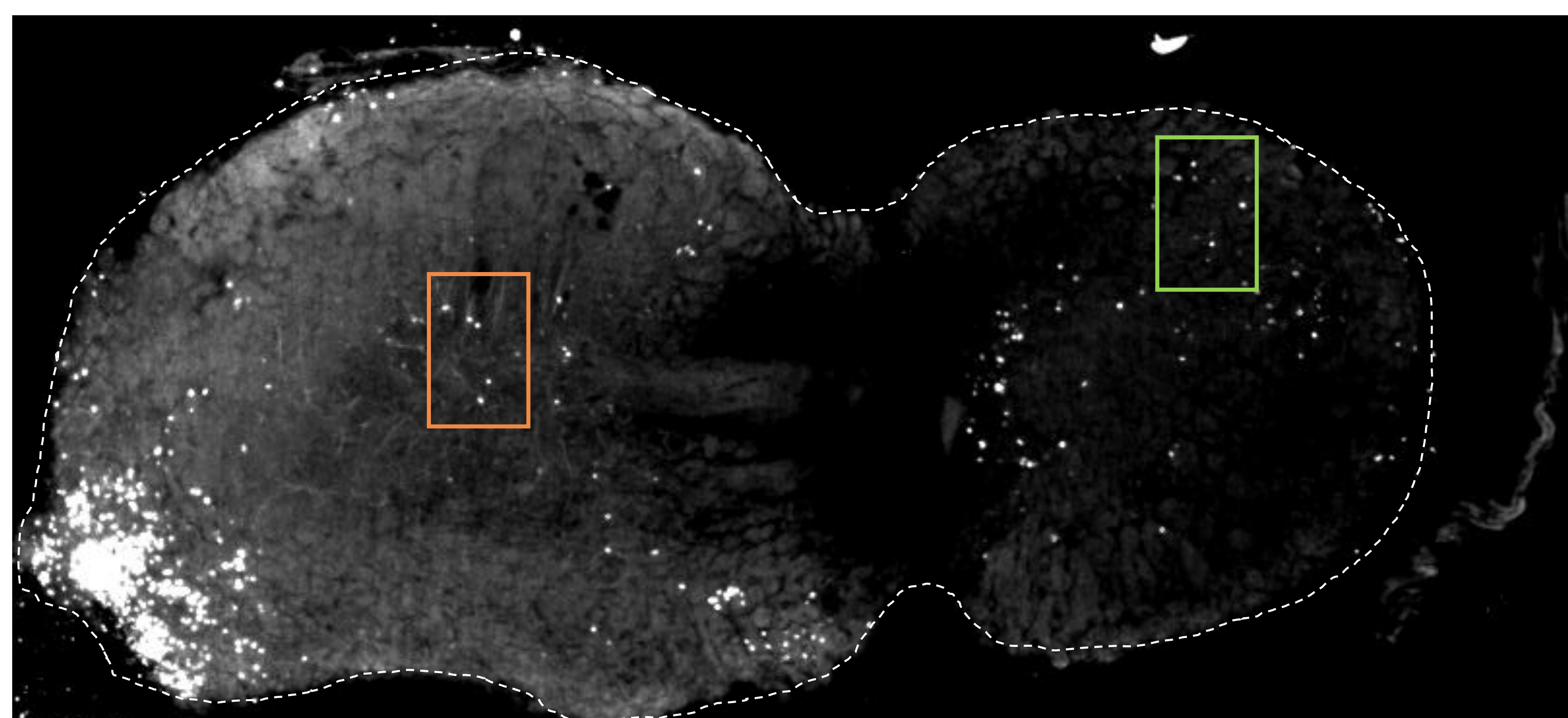
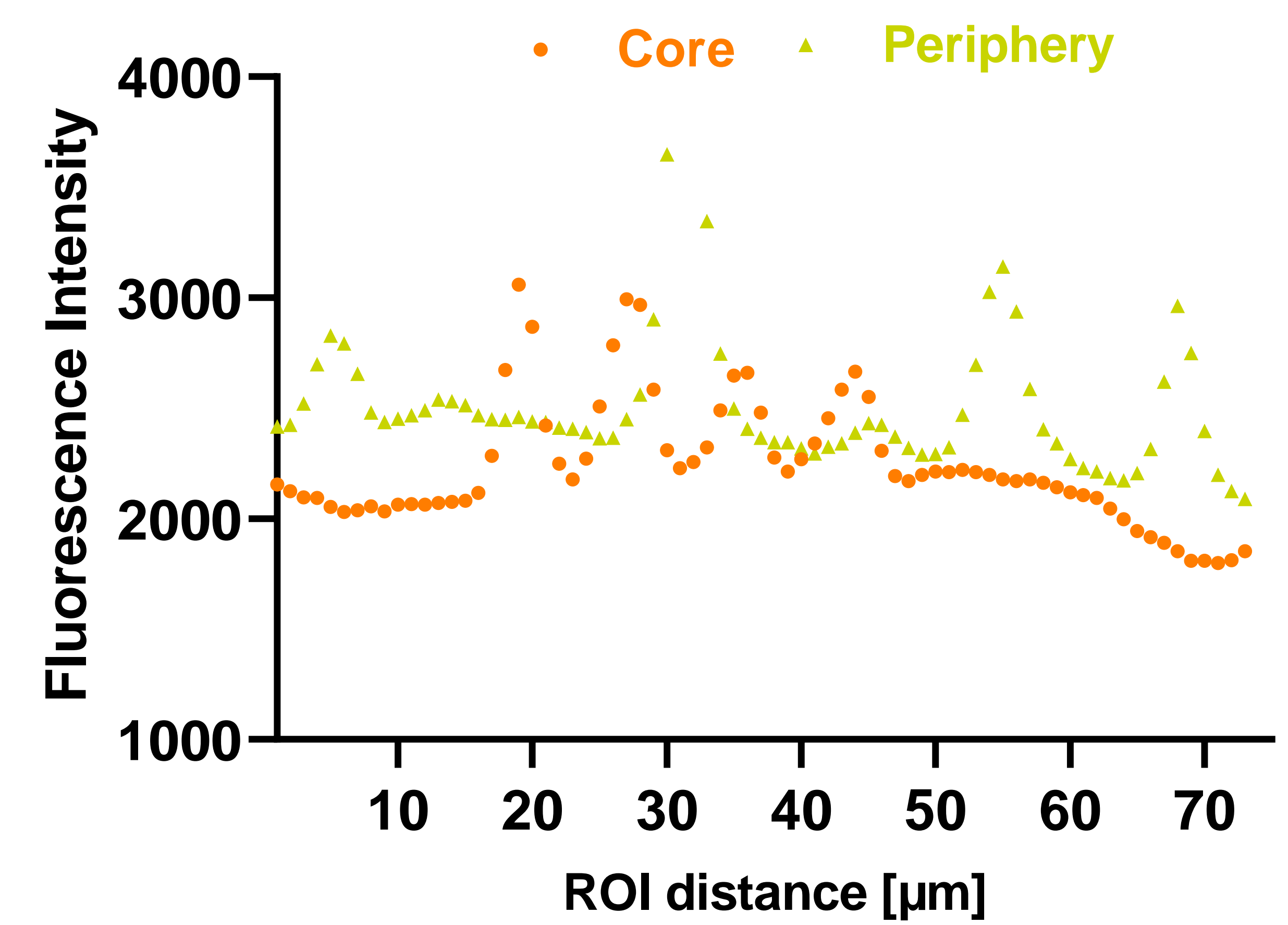
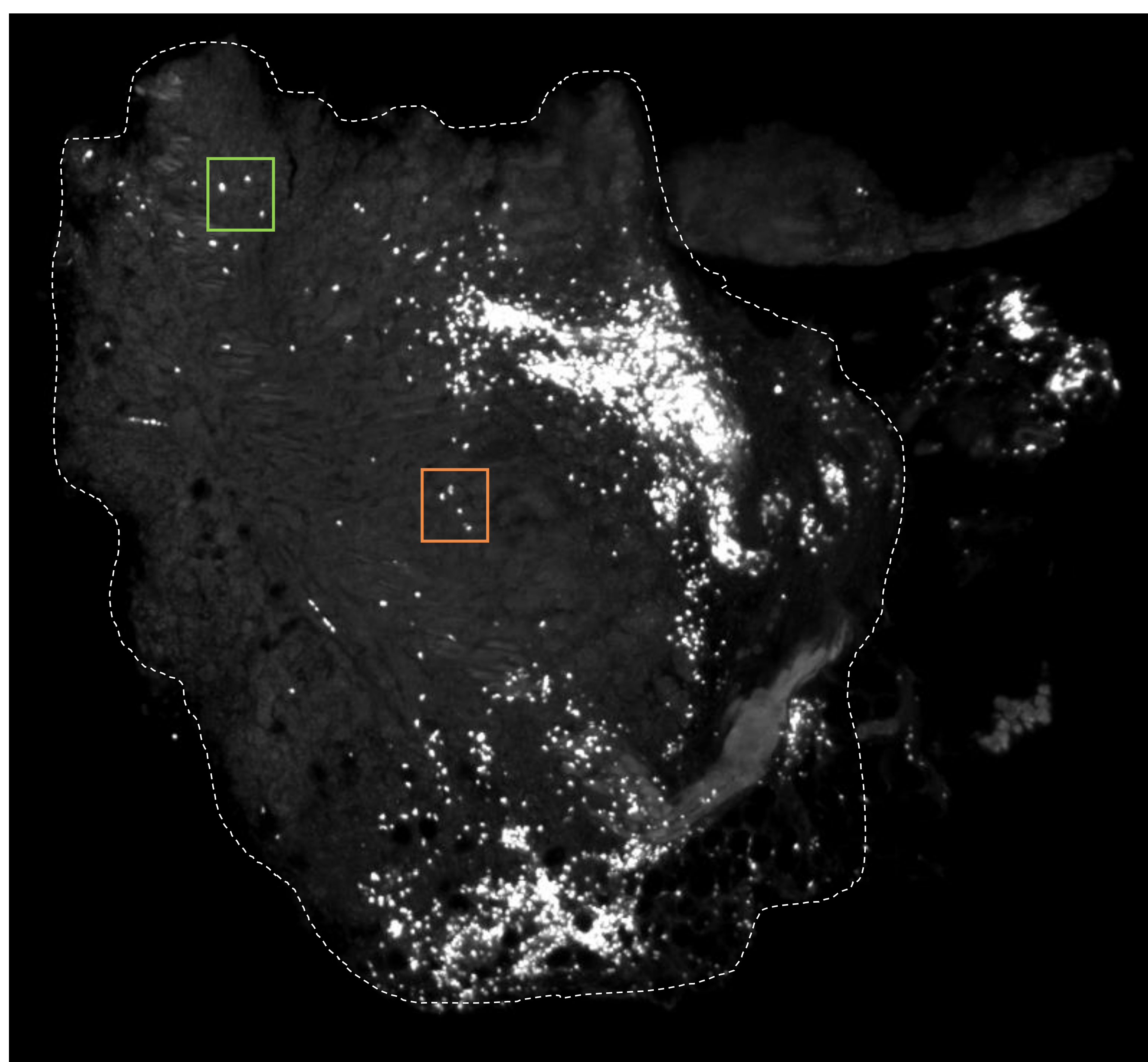
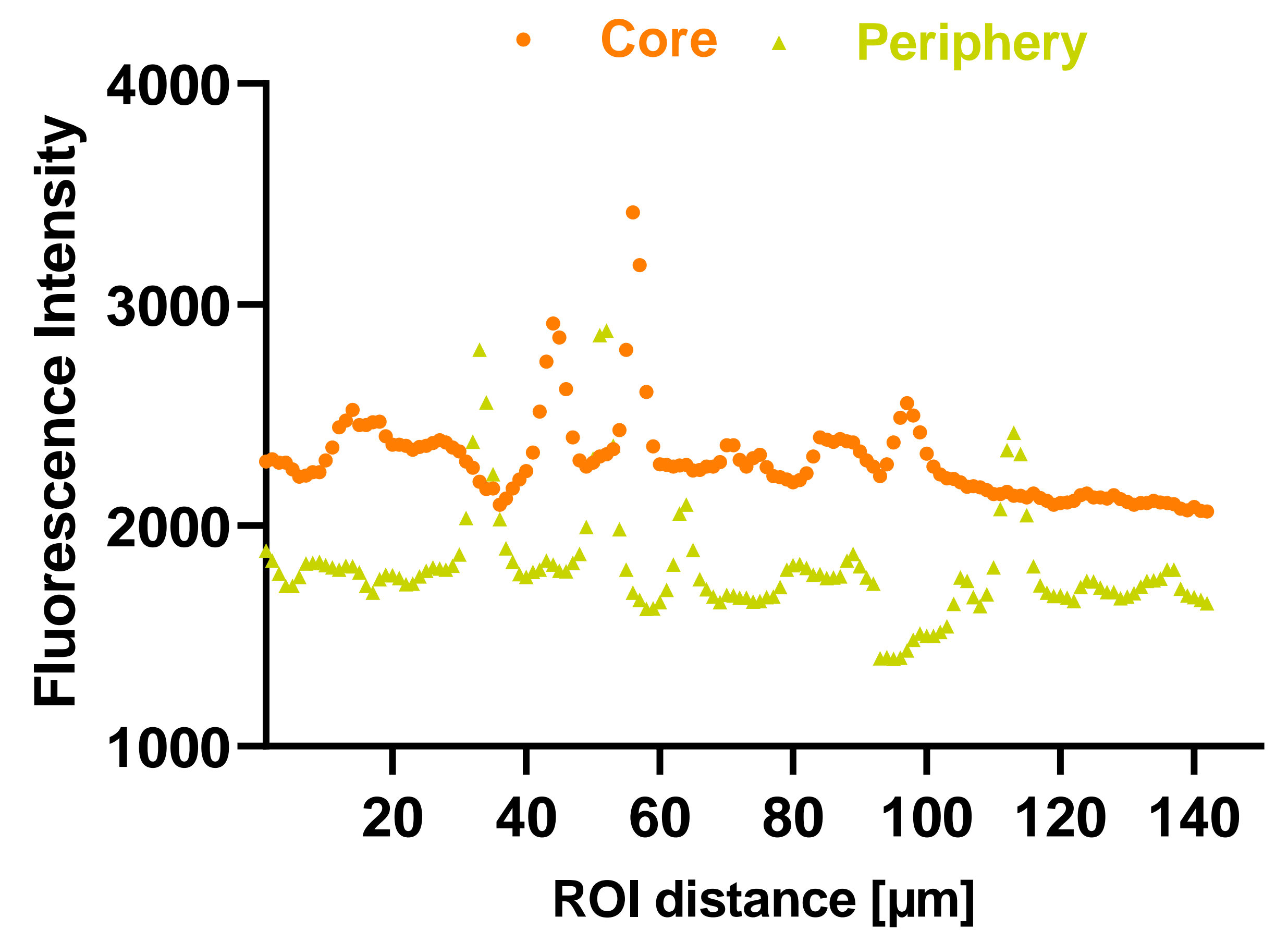
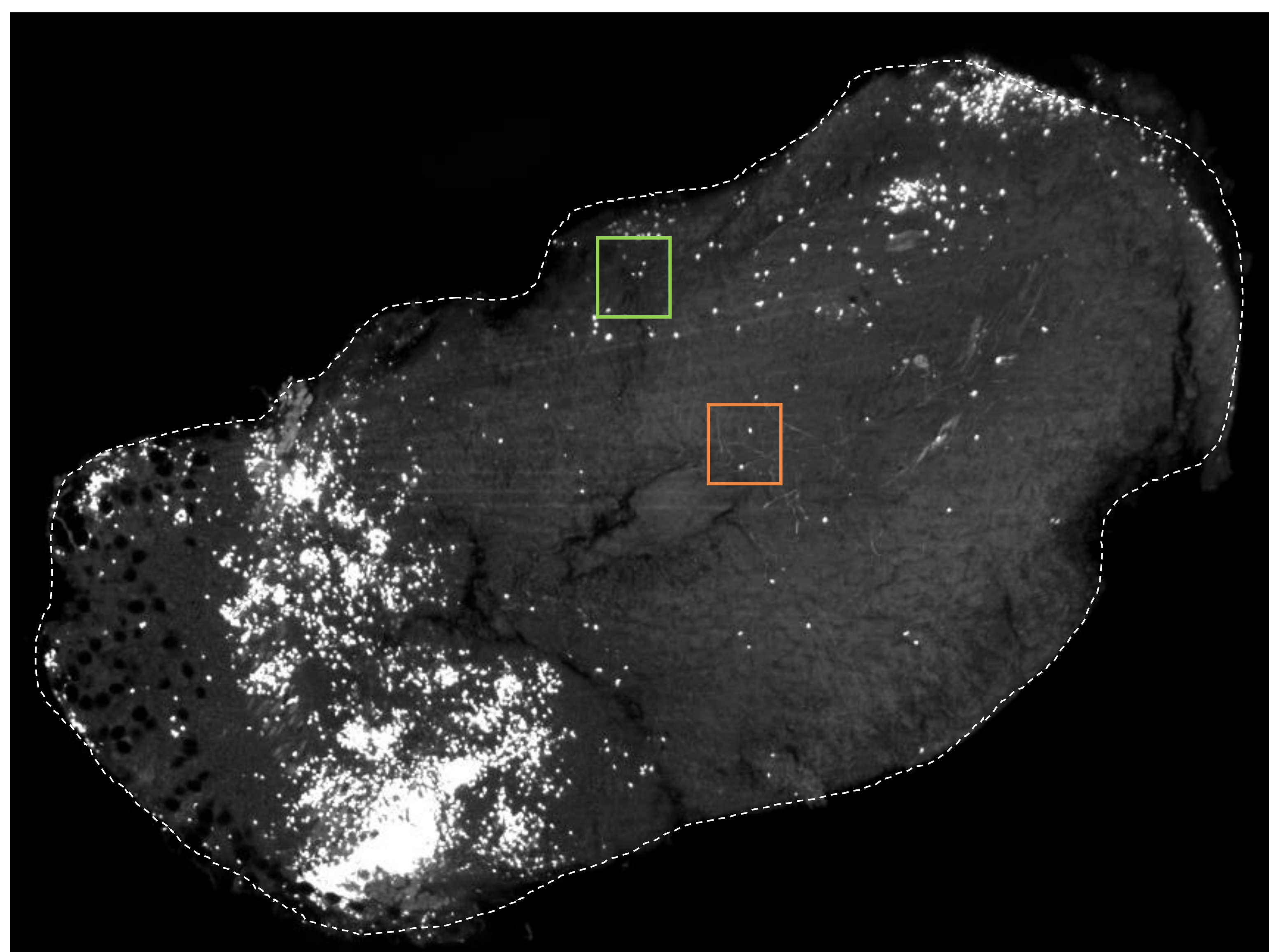


**Figure S4: Density Clustering of intratumoral T cells on Day 6.** Imaris reconstruction was applied and CD3-positive areas were color-coded based on staining intensity, in order to identify areas in the tumors with the highest density of T cells.



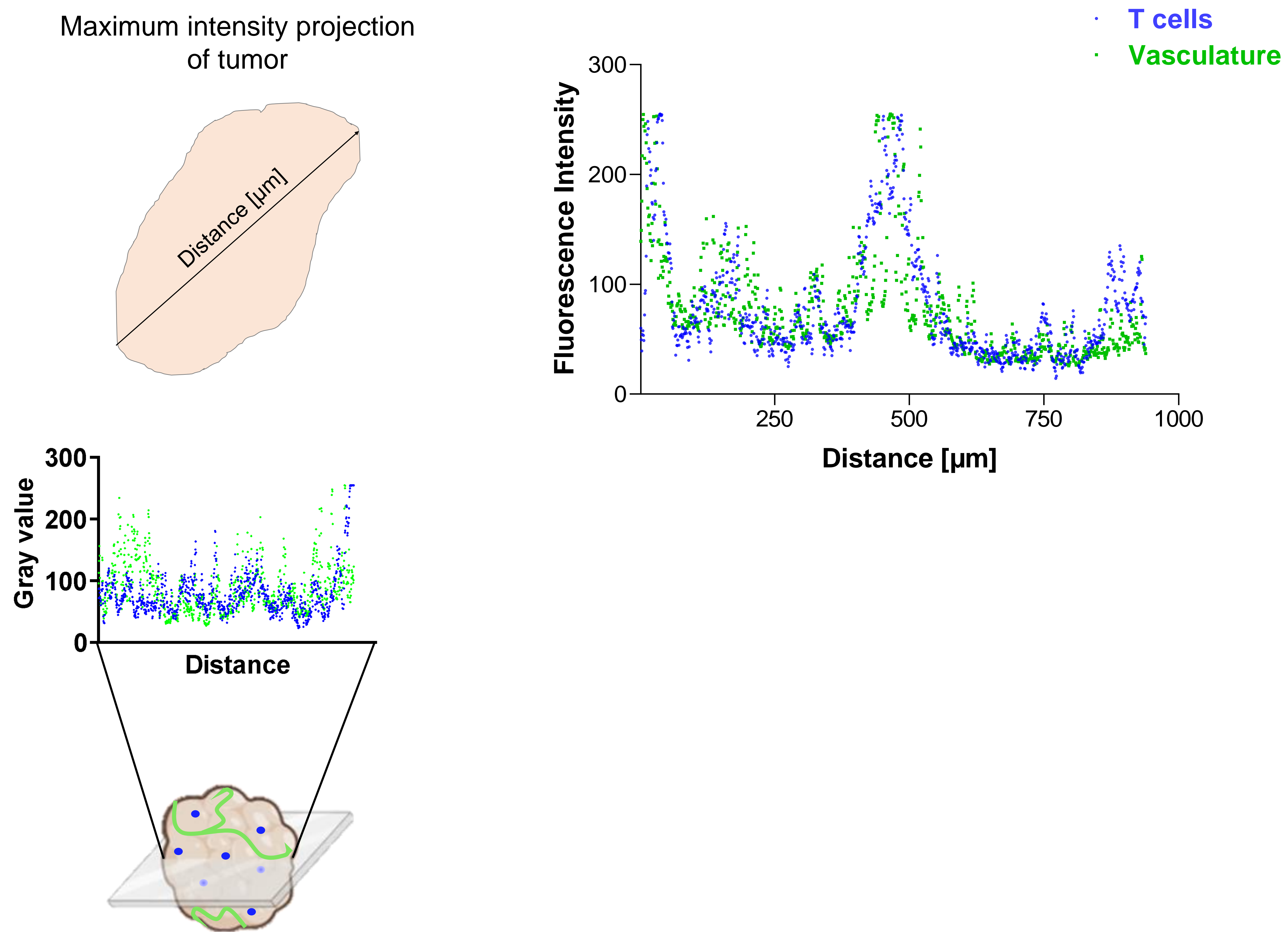




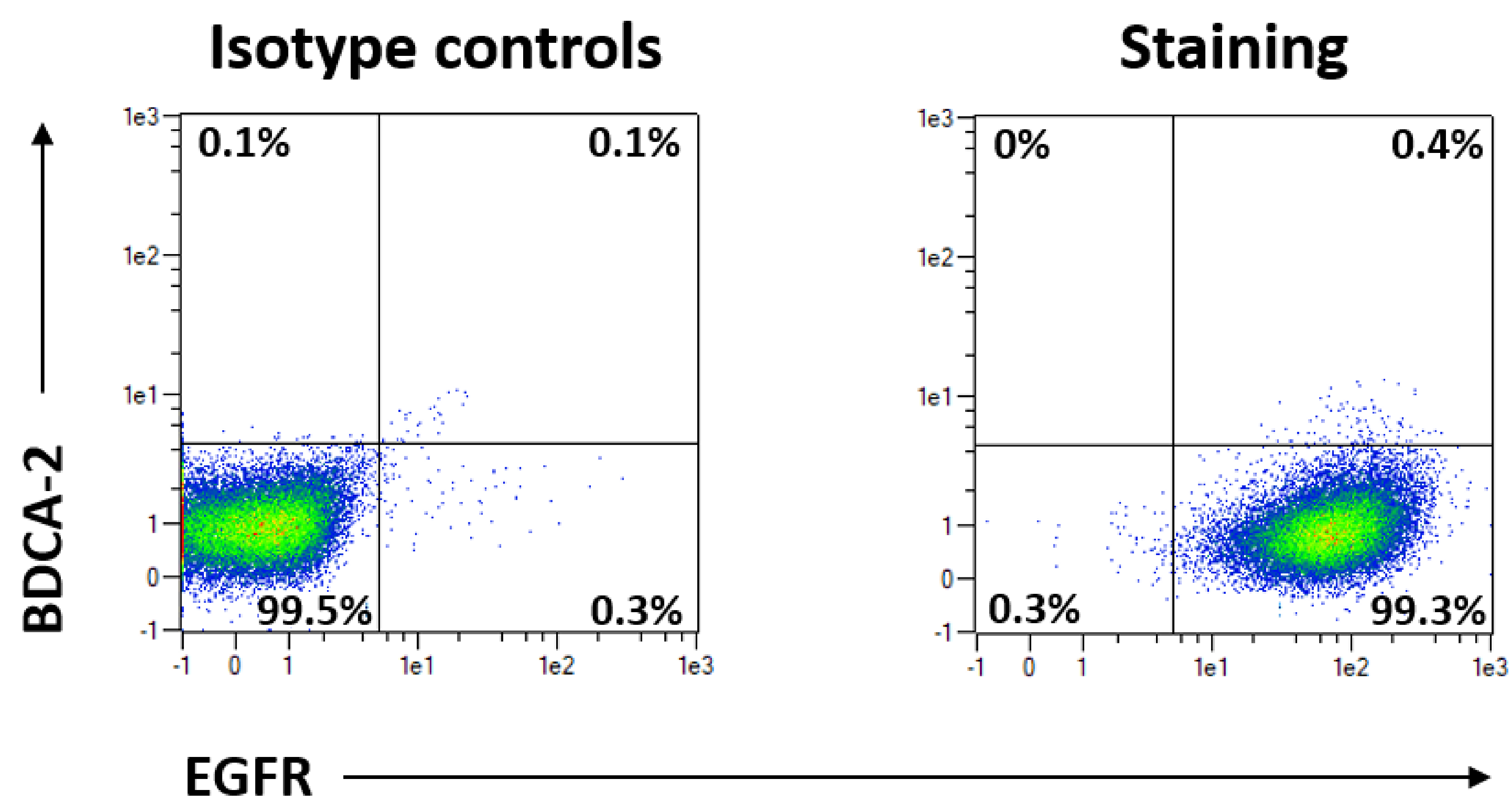


**Figure S6: Fluorescence intensity profile analysis.** In order to evaluate antibody penetration, CD3 staining intensity was analyzed in each sample using the central 20-30  $\mu\text{m}$  of the tumor, respectively. For this, the central 5 z-sections were displayed in a maximum intensity projection, rectangular ROIs were selected in the tumor core and periphery covering only individual cells and gray values were analyzed using Fiji ImageJ.





**Figure S7: Depiction of maximum intensity projection gray value profiles analysis.** The middle third of the tumor was selected for each tumor respectively and either maximum intensity of the vasculature (green) or of the CD3-positive stained areas (blue) were detected. An overlay of both maximum intensity profiles was generated to analyze co-localization of vessels and T cells.



**Figure S8: Surface expression of BDCA-2 and EGFR on the pancreatic cancer cell line AsPC-1.** Antigen expression was determined by flow cytometry using the BDCA-2-specific antibody AC114 and EGFR-directed antibody REA939.