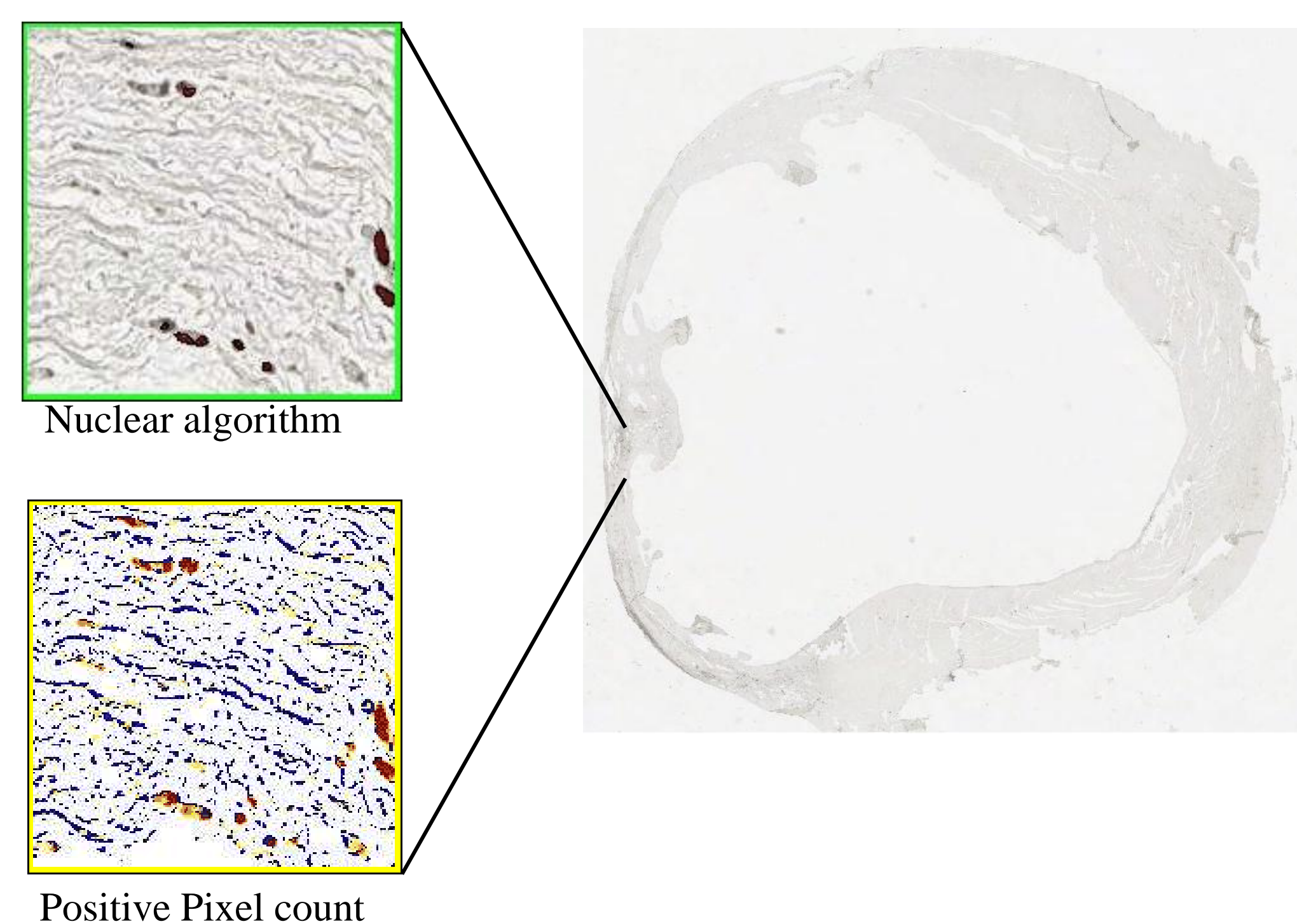


OBJECTIVE

Conduct a validation of concept to determine if Feature Analysis on Consecutive Tissue Sections (FACTS*) and digital analysis could be used for quantitative analysis of the tissue response in post implant histology.

BACKGROUND

The ability to evaluate the specific tissue response the host presents to an implanted material is a critical evaluation in determining biocompatibility. There is an urgent need for more accurate and reproducible measurements that do not require tedious manual counting through a microscope. While the histological evaluations are important and required for biocompatibility testing, these evaluations create a bottleneck in the testing of biocompatibility. In this study, FACTS analysis was used in conjunction with a nuclear counting algorithm along with an algorithm that allows the investigators to determine positivity.



Validation of FACTS® Analysis

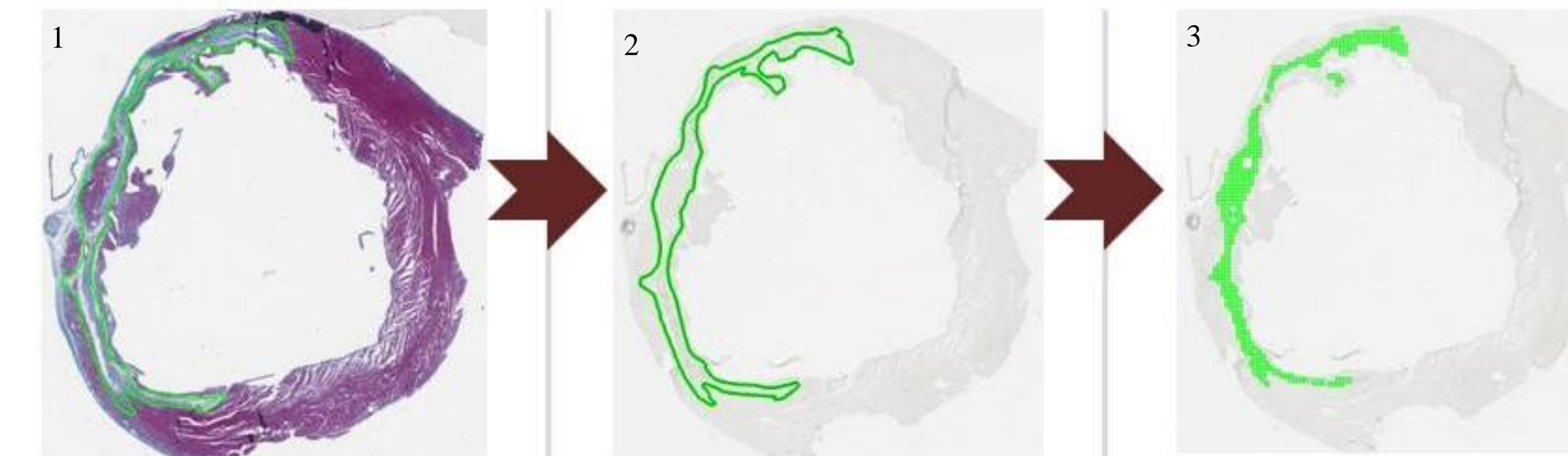
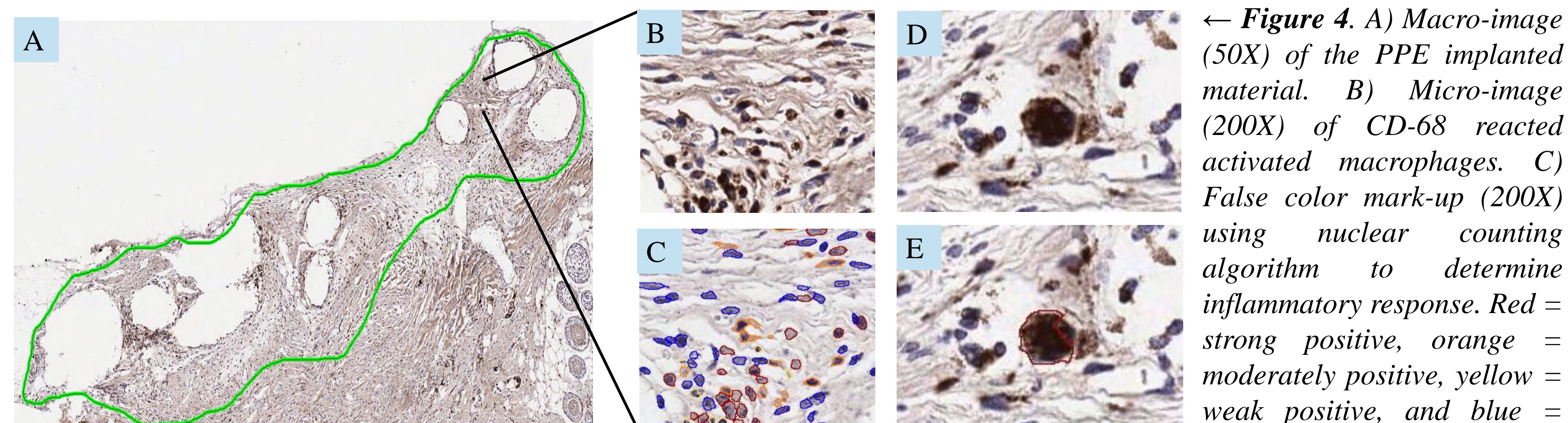


Figure 3. Using FACTS analysis on the left ventricle of a rodent heart.

- Determine the area of infarct based on the increased deposition of collagen in the myocardium using Masson's Trichrome stain.
- Using **F**eature **A**nalysis on **C**onsecutive **T**issue **S**ections (FACTS®) transpose the area of infarct onto a CD-68 stained slide. This will give us accurate area of analysis.
- Import a 100 x 100 pixel grid, each grid is 400.4µm². This fills in the area of infarct and gives individual fields of view.
- Fields of view that don't meet specified criteria are deleted. The criteria are:
 - All four corners of the box should be touching tissue.
 - Only regions to be counted are within the infarct zone.
 - All folds, artifacts, nonspecific staining will be excluded
- When all of the excluded grids were deleted, a random number generator was used to pick the ten fields of view to be counted.
- Two investigators counted the CD-68 stained activated macrophages in each of the fields of view selected by step 5.
- Digital analysis was then run across the grids and the counts were compared to the counts made by the two investigators.

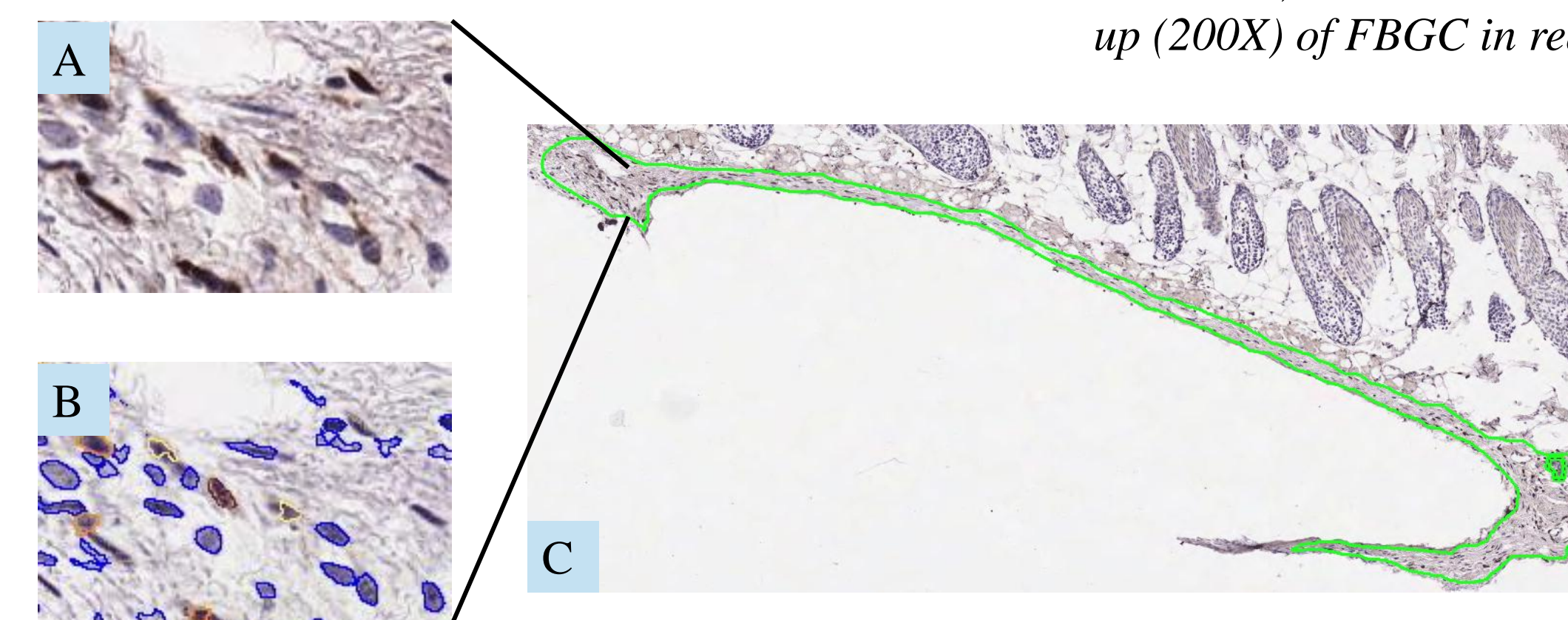
MATERIALS AND METHODS

In an effort to perform biocompatibility testing, implants and explants were performed in a Mus muscularis model. Subcutaneous implants of PPE and ePTFE were performed. The explanted materials and surrounding tissues were stained with hematoxylin and eosin, Milligan's Trichrome, and reacted with CD-68 antibody for identification of activated macrophages. These samples were used as control samples to assist with validation of a nuclear algorithm and an algorithm used to count foreign body giant cells (FBGC).



← Figure 4. A) Macro-image (50X) of the PPE implanted material. B) Micro-image (200X) of CD-68 reacted activated macrophages. C) False color mark-up (200X) using nuclear counting algorithm to determine inflammatory response. Red = strong positive, orange = moderately positive, yellow = weak positive, and blue = negative. D) micro-image (200X) of FBGC reacted with CD-68. E) False color mark-up (200X) of FBGC in red.

Figure 5→. A) Micro-image (200X) of CD-68 reacted macrophages. B) False color mark-up (200X) of the nuclear counting algorithm, Red = strong positive, orange = moderately positive, yellow = weak positive, and blue = negative. C) Macro image of the region of interest around an implant of ePTFE. The material is not present and the majority of the measurement was performed on the superficial surface of the implant.

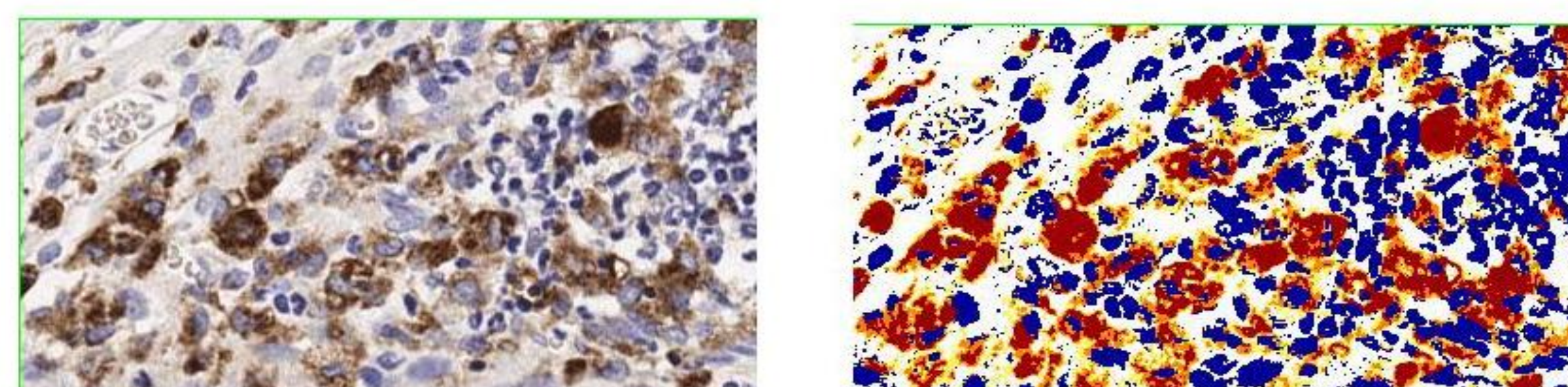


What is Positivity?

Positivity is the measure of positive staining, whether the staining is specific or non-specific. Positive pixel analysis counts pixels of a designated color. Once the specific color has been determined and thresholds set, the algorithm outputs five specific thresholds; strong positive, medium positive, weak positive, negative and background. Positivity is a calculation of the total number of positive pixels divided by the total number of pixels in the area analyzed. This analysis was used to determine immunohistochemistry (IHC) optimization for CD-68 reacted sections in the following experiments.

A side-by-side comparison using positive pixel count.

Red=strong positive
Orange=medium positive
Yellow= weak positive
Blue=negative
White=background



RESULTS

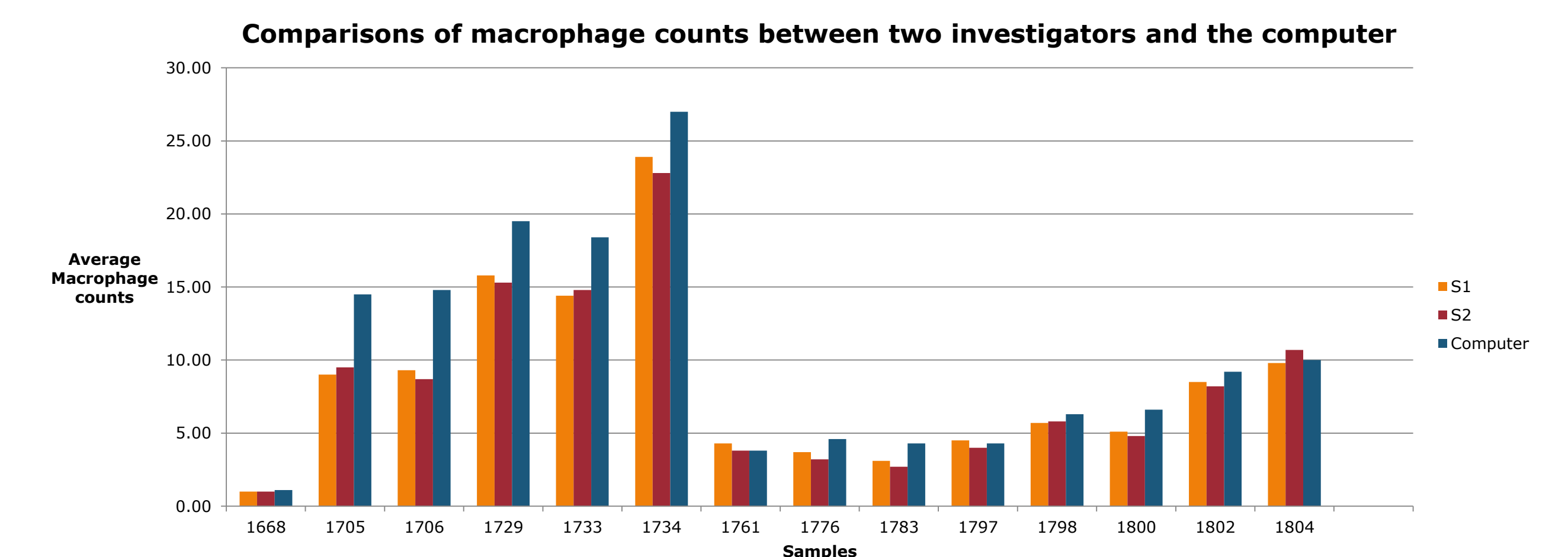
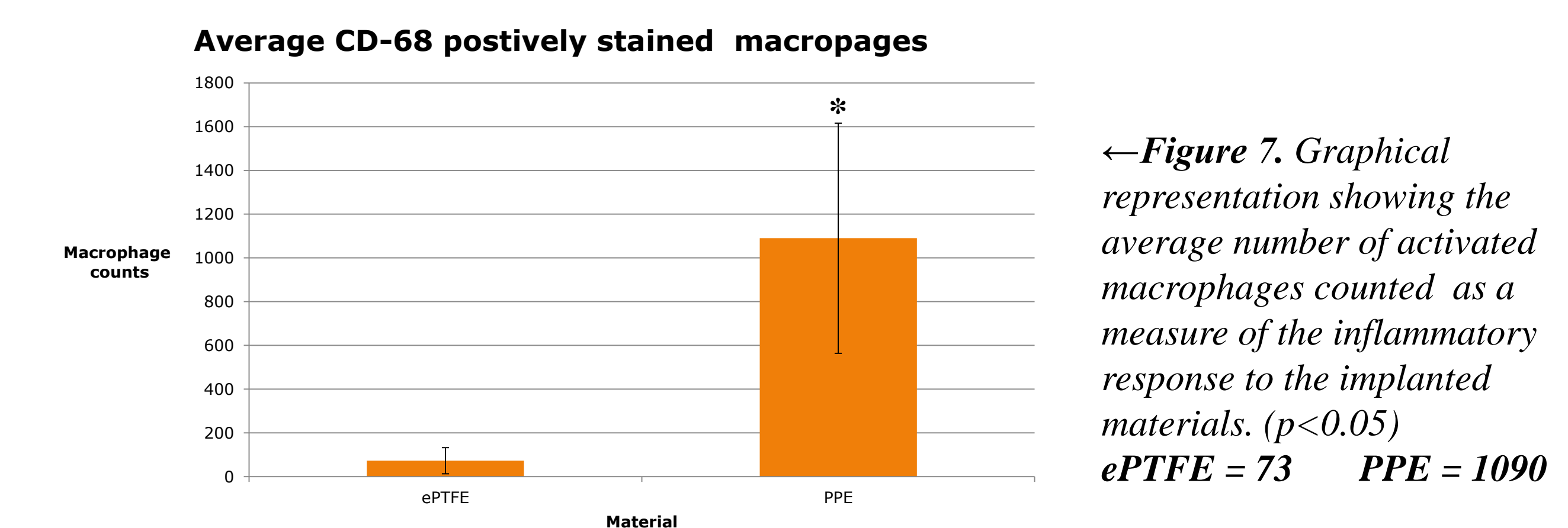
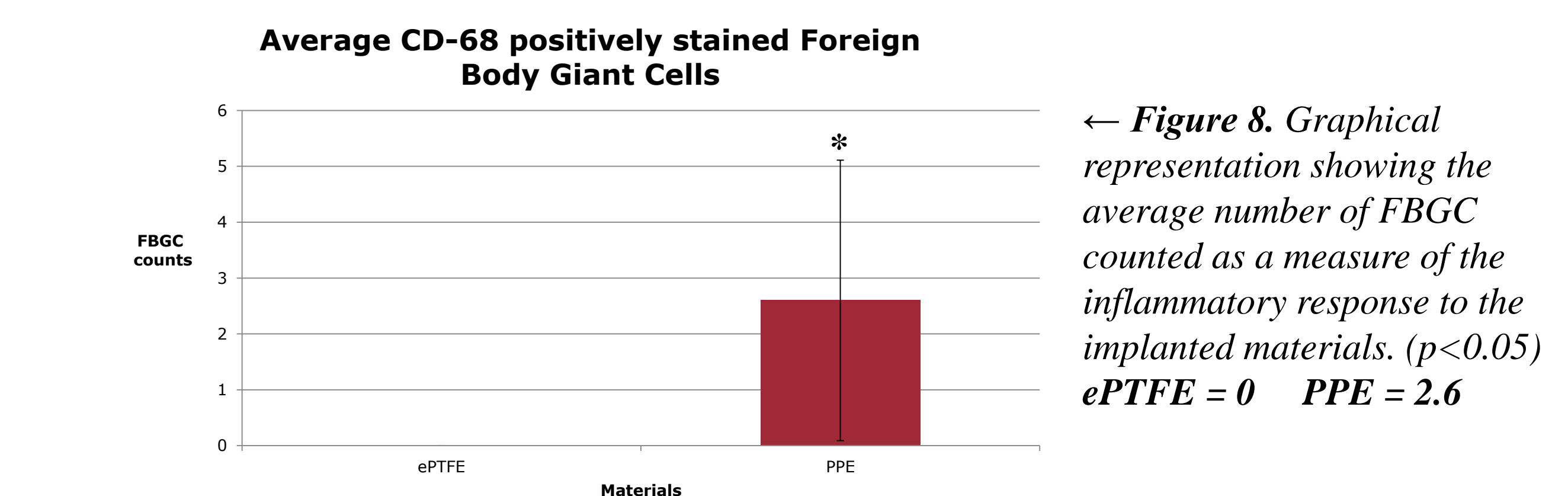


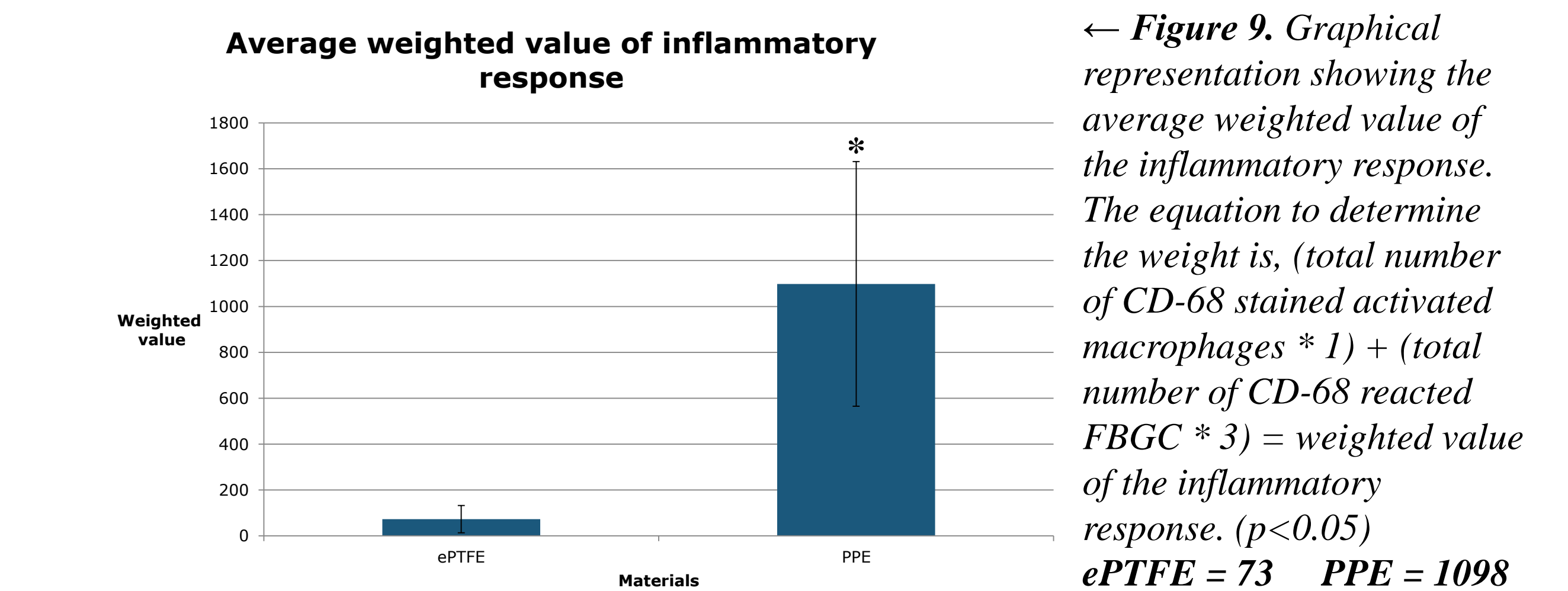
Figure 6. Graphical representation comparing the counts of CD-68 activated macrophages between two investigators and the nuclear counting algorithm across 10 fields of view within the area of infarct on the left ventricle of a rodent.



← Figure 7. Graphical representation showing the average number of activated macrophages counted as a measure of the inflammatory response to the implanted materials. ($p < 0.05$)
ePTFE = 73 PPE = 1090



← Figure 8. Graphical representation showing the average number of FBGC counted as a measure of the inflammatory response to the implanted materials. ($p < 0.05$)
ePTFE = 0 PPE = 2.6



← Figure 9. Graphical representation showing the average weighted value of the inflammatory response. The equation to determine the weight is, (total number of CD-68 stained activated macrophages * 1) + (total number of CD-68 reacted FBGC * 3) = weighted value of the inflammatory response. ($p < 0.05$)
ePTFE = 73 PPE = 1098

CONCLUSIONS

The advantages of digital pathology and morphometry over traditional manual methods include:

- Rapid throughput** -- once algorithms have been developed, the analysis can be automated on all samples and performed in a matter of minutes.
- Consistency of results** -- evaluations can be performed using identical parameters and assumptions on all samples with no inter-investigator bias.
- Whole slide analysis** -- Using whole slide scanning we are able to generate data from the entire implant specimen instead of selected fields of view through manual methods.