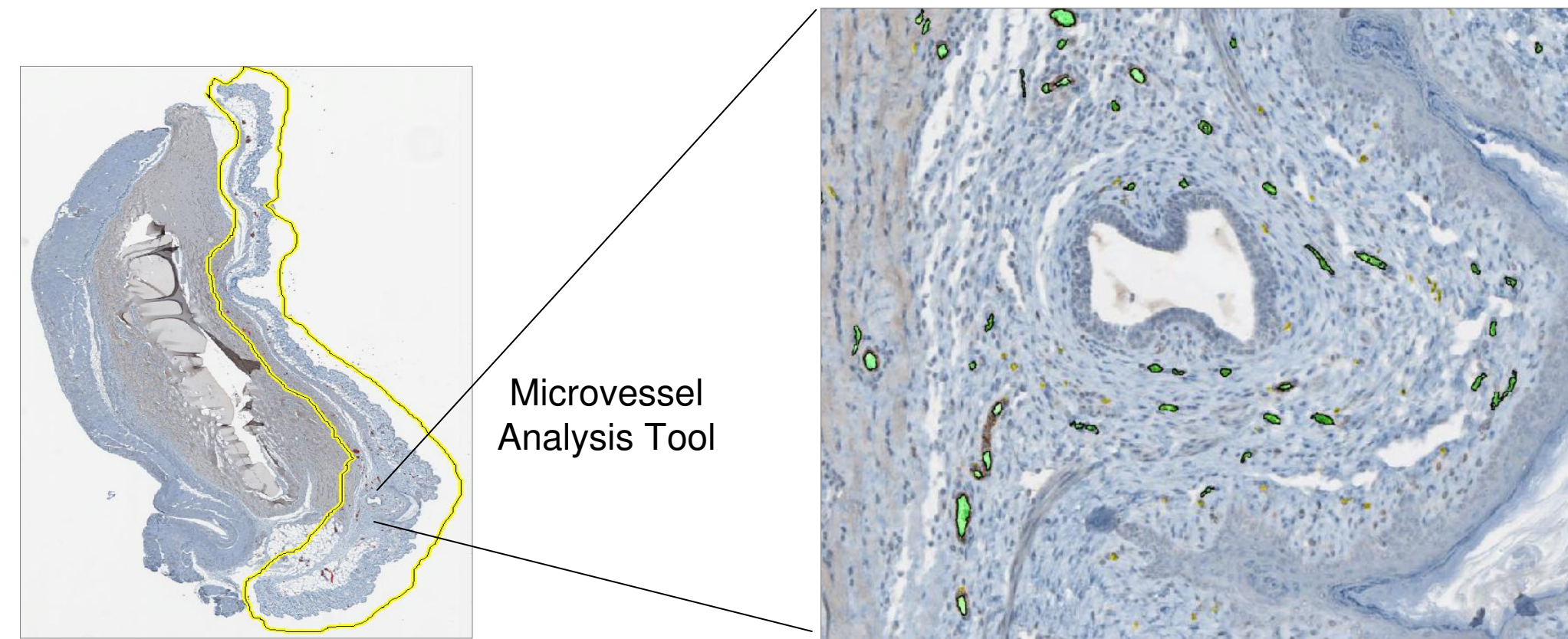


Introduction

The ability to evaluate microvessels in tissues is important across many therapeutic areas in both preclinical studies as well as clinical trials which seek potential biomarkers of vascular injury.

There is an urgent need for more accurate, reproducible microvessel measurements that do not require tedious, manual counting under a microscope. The time spent by a pathologist in training others to manually count vessels and to review the work is a bottleneck in this area.

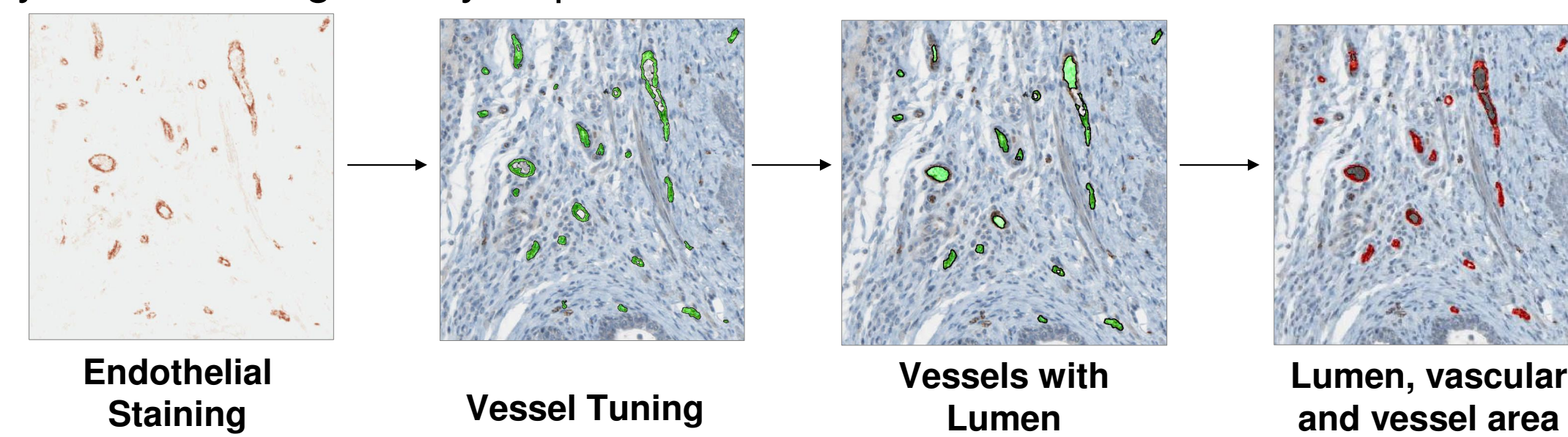
In this study, the Aperio Microvessel Analysis tool, an image analysis tool that identifies and counts vessels stained with an endothelial cell marker, was compared to manual counting for its accuracy and precision in mouse matrigel slides.



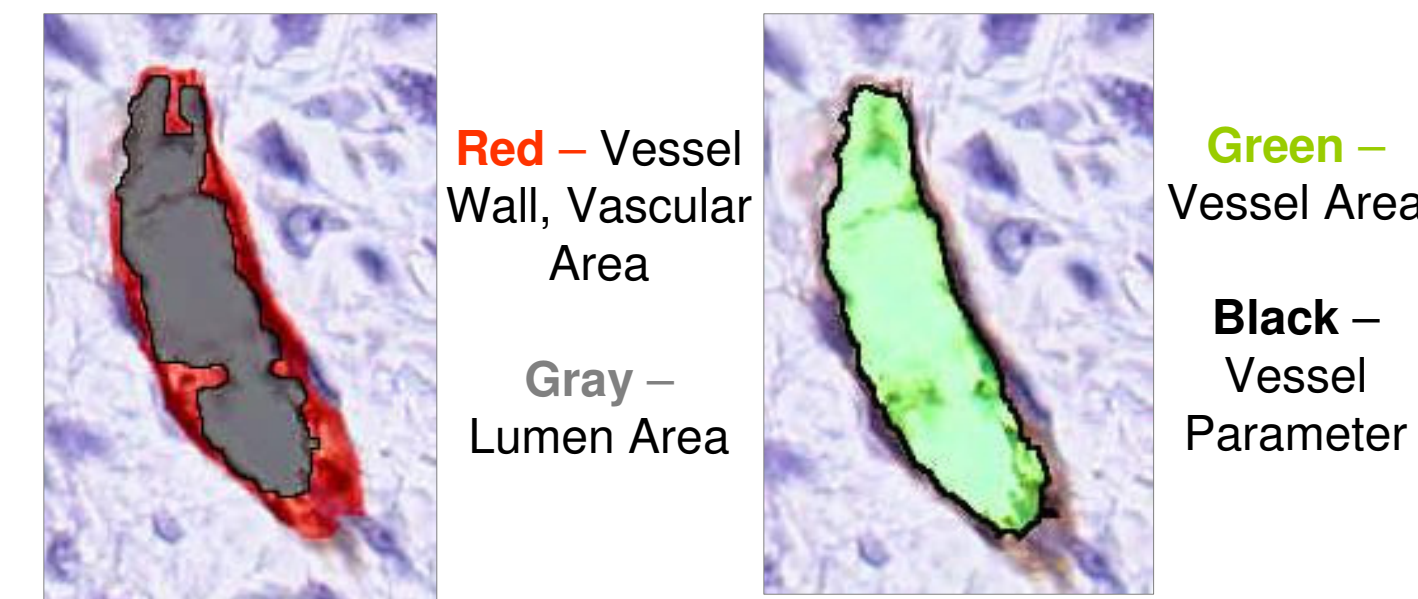
Materials and Methods

Image Analysis Process

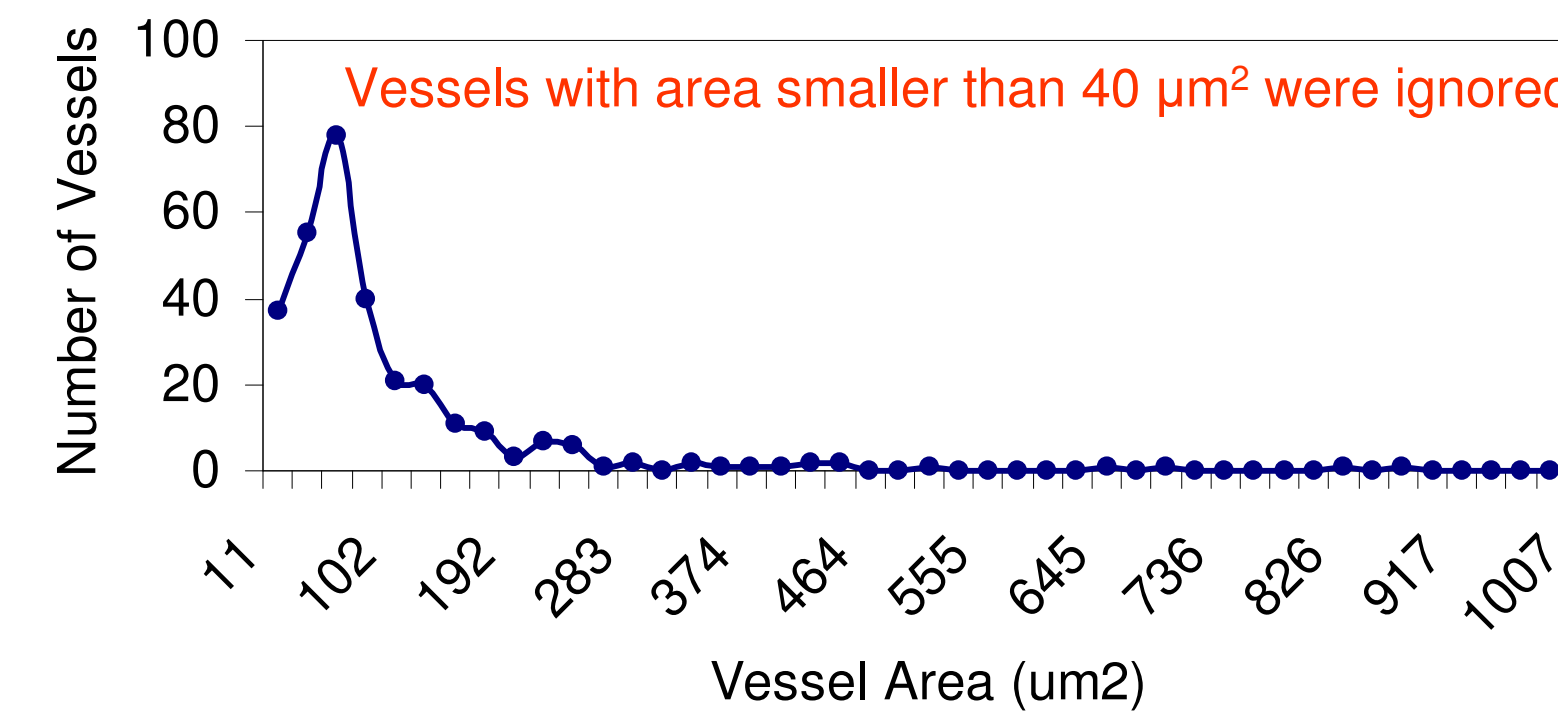
The Microvessel Analysis tool in Aperio Technologies' Spectrum Plus™ software was utilized to automate the identification of stained endothelial cells and vessels in matrigel slides. Tissue sections from eight matrigel slides from individual mice were scanned at 20x with a ScanScope, and then analyzed with Microvessel Analysis. The image analysis process is shown below:



The process for detecting endothelial cells, vessels, and lumen. First, the DAB stain from Factor VIII stained endothelial cells is isolated with color deconvolution. Second, a novel dual stain thresholding is used to isolate endothelial cells from non-specific staining. Third, stained areas are merged by the computer into vessels, with lumen detected (when present). For matrigel analysis, vessels smaller than 40 μm² were excluded (shown in yellow at right). Statistics are calculated on each vessel, including lumen area, vascular and vessel area, and vessel perimeter.



For each vessel, the above four statistics are calculated. Vessels less than 40 μm² were ignored in the study, as shown in the distribution at left from one slide.



Study design

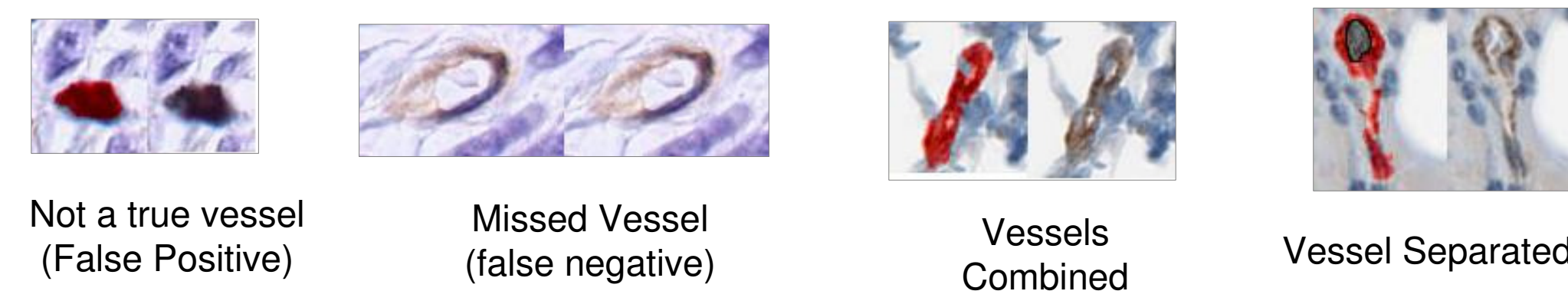
Two ACVP board certified pathologists reviewed slides with two PhD scientists who were involved in scoring of blood vessel tissue sections on slides evaluated in this study in order to ensure concordance in identification of blood vessels based on morphology.

A) Precision of manual identification of vessels

Two PhD scientists independently identified 346 vessels across annotated regions of tissue sections that included epidermis, dermis, subcutis, and the leading edge of a matrigel plug in two whole slide images. The number of vessels that both observers identified, and the number of vessels that at least one scientist identified were marked and counted.

B) Accuracy of computer based identification of vessels

One PhD scientist independently counted vessels on all digital slides, and compared these values with the computer computation. The two scientists then jointly reviewed the results generated by automated microvessel analysis to determine accuracy. Four general types of errors were encountered with vascular image analysis, as show below:



Results

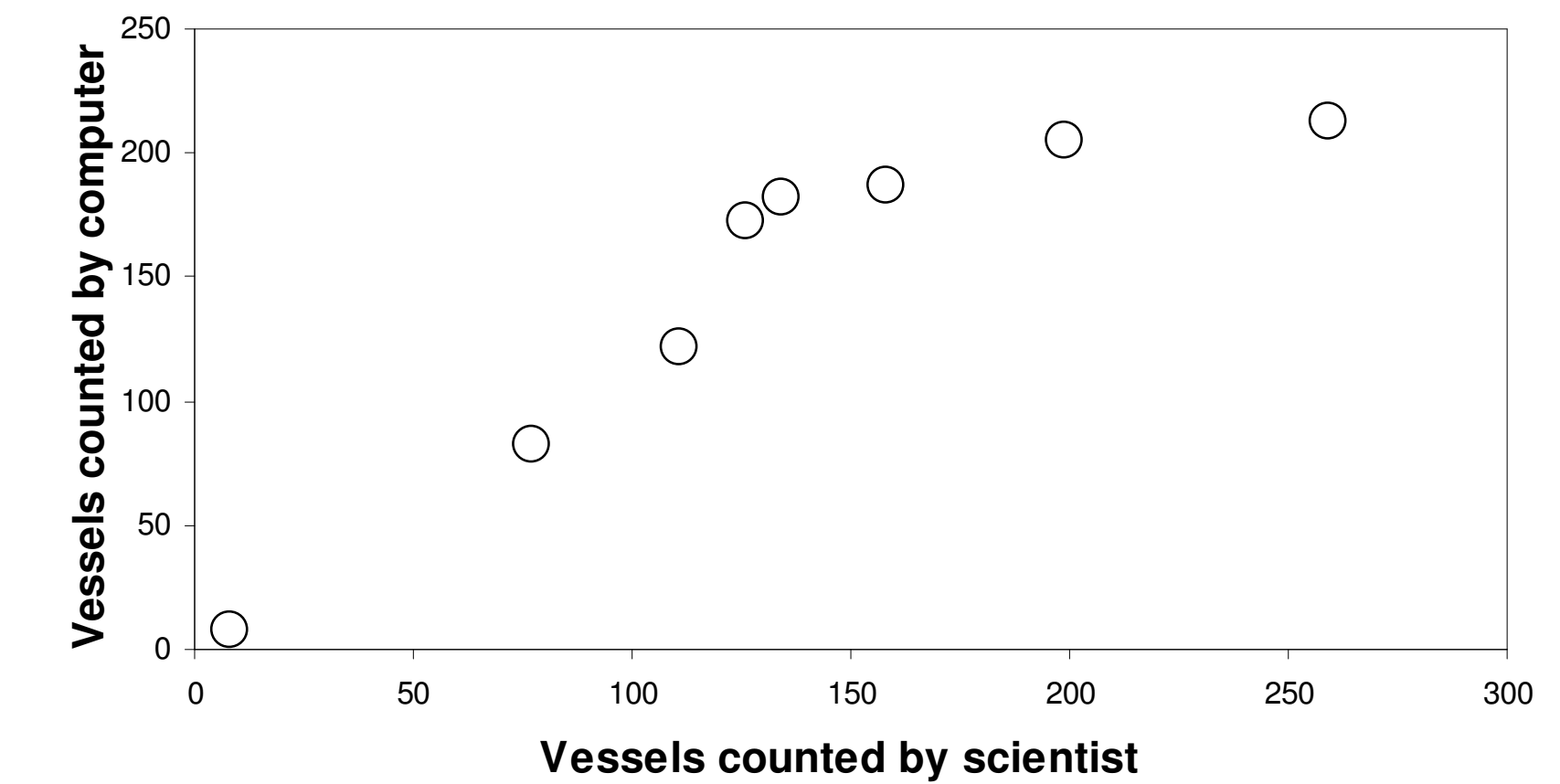
The results from the precision study of manual identification of vessels is shown below. Only 60% of the 346 vessels were identified consistently by both scientists, even with the smaller vessels (<40 μm²) not being included in the study.

Total possible vessels observed	Found by both scientists	Found by only 1 st scientist	Found by only 2 nd scientist	Number of vessels not in agreement
346	247	51	48	99
Percentage	100%	21%	19%	40%

Table 1. Precision of manual identification of vessels in matrigels.

Aperio products are FDA cleared for In-Vitro Diagnostic (IVD) use for specific clinical applications, and are intended for Research Only Use (ROU) for other applications.

Despite low precision between scientists the concordance between scientist and computer analysis was strong as shown in the figure at right. Table 2 shows the assessment of accuracy by both scientists together, and the types of errors observed



Specimen ID	Computer Analysis reviewed by both parapatologists						
	Correct	False positive	False negative	Vessels combined	Vessel separated	% vessel errors for individual statistics ^a	% vessel errors for microvessel area density ^b
5843	172	21	0	0	11	18.6%	12.2%
6068	66	5	2	0	2	13.6%	10.6%
5841	181	36	4	2	3	24.9%	22.1%
5839	144	15	3	1	9	19.4%	12.5%
4838	1	1	1	0	0	NA ^c	NA
5837	146	17	1	1	1	13.7%	12.3%
5835	144	14	1	1	2	12.5%	10.4%
5827	83	8	2	0	1	13.2%	12.0%
Totals / Median	238	26	2	0	13	13.6%	12.2%

Table 2. Assessment of Microvessel Analysis accuracy across 8 slides.

^aAny of the four error types will lead to errors in individual statistics
^bOnly false positives and false negatives will leave to errors in microvessel area density, since with area measurements of vascularity per slide it doesn't matter if the vessel is combined or separated into two vessels
^cThis slide only had one vessels across the entire section. While the statistics should not be included for analysis, due to the low number of vessels, it is instructive that only one false positive was found in this section.

Conclusions

•In this pilot study, we compared precision and accuracy between automated Microvessel Analysis and manual counting on whole slide images.

•Manual counting of blood vessels by two PhD scientists had a low precision rate with a concordance of only 60% compared to precision by the automated approach of 87.4%. Expected error rates averaged 13.6% for statistics that were based on individual vessels (e.g. vessel perimeter or individual vessel area) and 12.2% for microvessel density measurements.

•Other advantages to automated microvessel analysis on whole slides include speed, both the scanning and analysis time can be accomplished in minutes. Additional advantages include quantitative vessel measurements and statistics which are calculated by the software algorithm.

•Further work will expand the validation to other tissue types and will compare concordance between trained pathologists and between trained pathologists and automated analysis.