



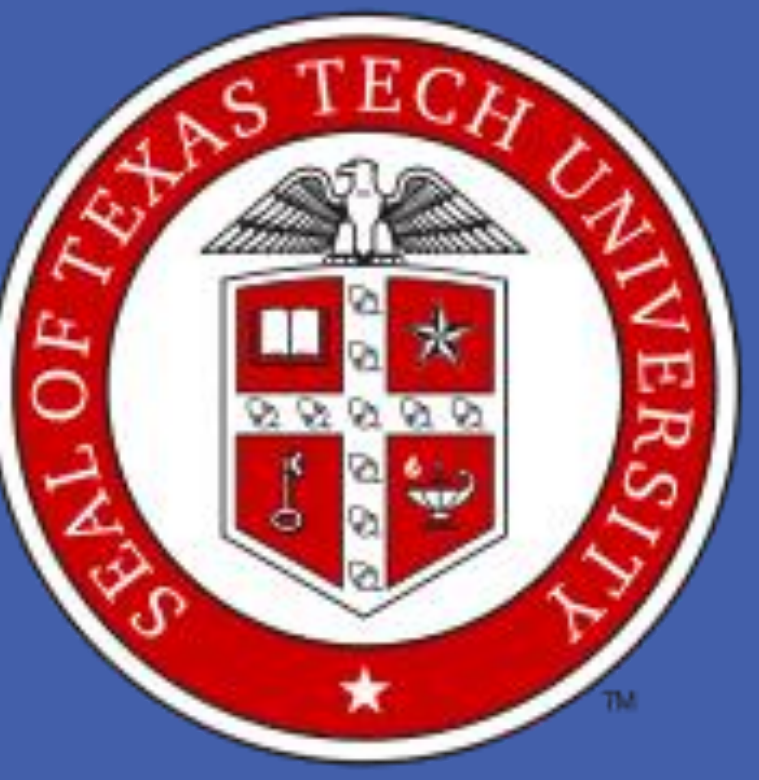
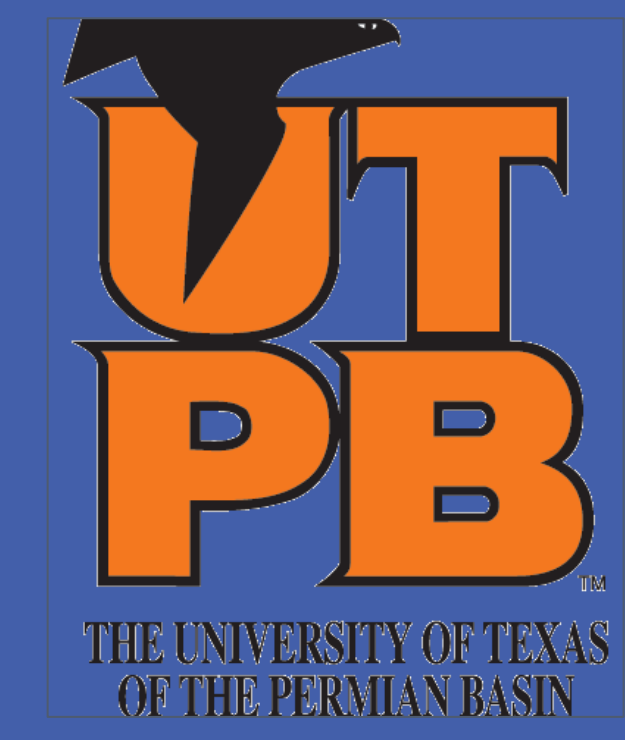
# INTER-OBSERVER COMPARISON OF THE COMPUTER-ASSISTED IMAGING ALGORITHMS FOR HISTOMORPHOMETRIC QUANTIFICATION

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## INTRODUCTION

The computer-assisted image analysis is a routine tool applied broadly in biomedical research. The image analysis is usually performed with such available tools as NISElements™, Metaview, MetaMorph®, ImageJ, Scion, Adobe Photoshop, and Image Pro Plus. However, there is no consensus regarding the usage of each program. The software, which can be freely downloaded from NIH - Image J - requires the selection of fields of view, which could be a subjective process. The more sophisticated programs have shown to have overcome these weaknesses. An example is the Aperio ScanScope® software. This on-line program allows quantification of entire slides and areas of interests under the same conditions, thus, minimizing the possibility of errors (Figure 1). The algorithms, developed to recognize and quantify the molecules of interest, still could harbor the subjective choice (Figure 2). The histoscore method was compared between observers. Despite differences in the algorithms, defining positive and negative signals, the analysis of the histoscores were similar between investigators.

## OBJECTIVES

To compare the algorithms developed by the different investigators to quantify the collagen expression in baboon heart tissues.

## MATERIALS AND METHODS

Cardiac tissues were available from the tissue bank, collected from obese and non-obese baboons (papio spp) (Farley et. Al 2009). Collagen and elastin fibers were demonstrated, using the Verhoeff-Van Gieson Elastic Stain Kit (Sigma Aldrich, LLC, USA). Slides were scanned using an Aperio ScanScope® instrument at 40X. Image analysis was performed with available positive and negative pixel count collagen algorithm (ImageScope™ v11.1.2.752 by Aperio®), additionally the method of histoscore calculation was applied, which consists in adding all the positive pixels, and determining their ratio out of the total number of pixels in the section (Brocato et al., 2013).

$$\text{Histoscore} = \left[ \left( \frac{\text{Positive pixels}}{\text{Total number of Pixels}} \right) \times 100 \right] \times 2$$

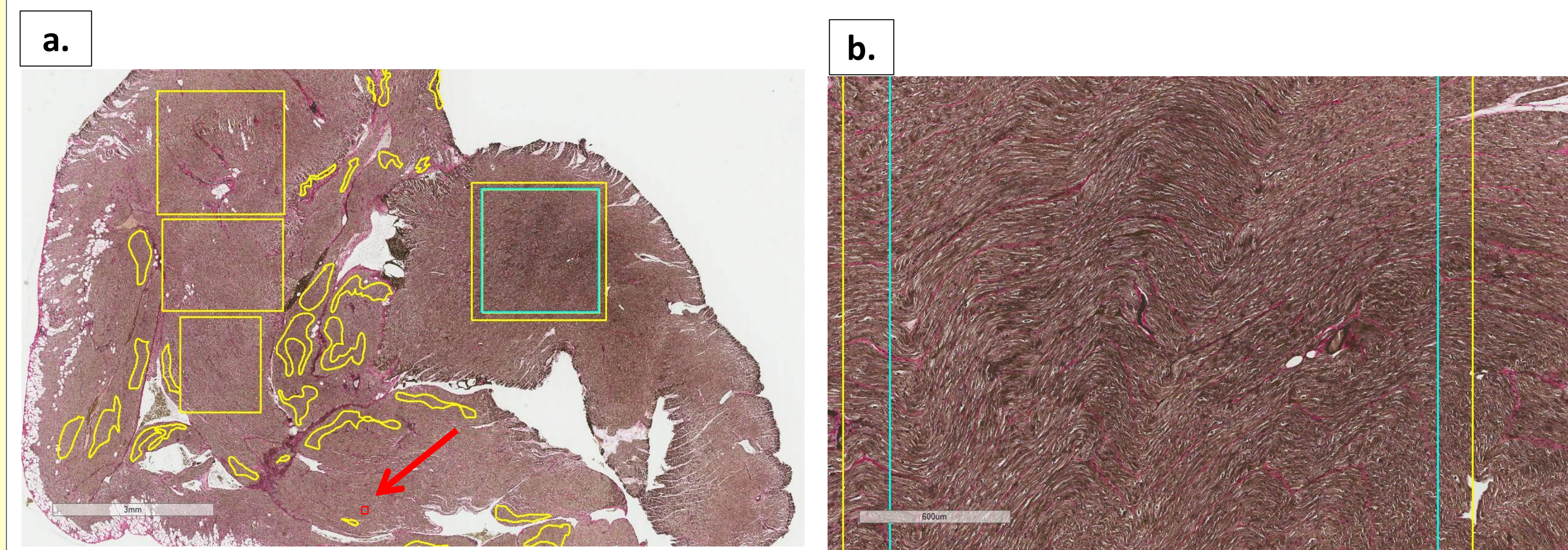


Figure 1 - Cardiac tissue used for collagen protein expression quantification. a.) View at 0.9X (bar length 3mm). Yellow outlines indicate areas that were previously quantified. Turquoise area indicates area that were selected for quantification. Red arrow is pointing at the representative image used by image J program for quantification. b.) Zoomed in section, 3.5X (bar length 600nm), showing the areas that were selected for quantification and comparison. The area was examined using collagen algorithm via Aperio ImageScope.

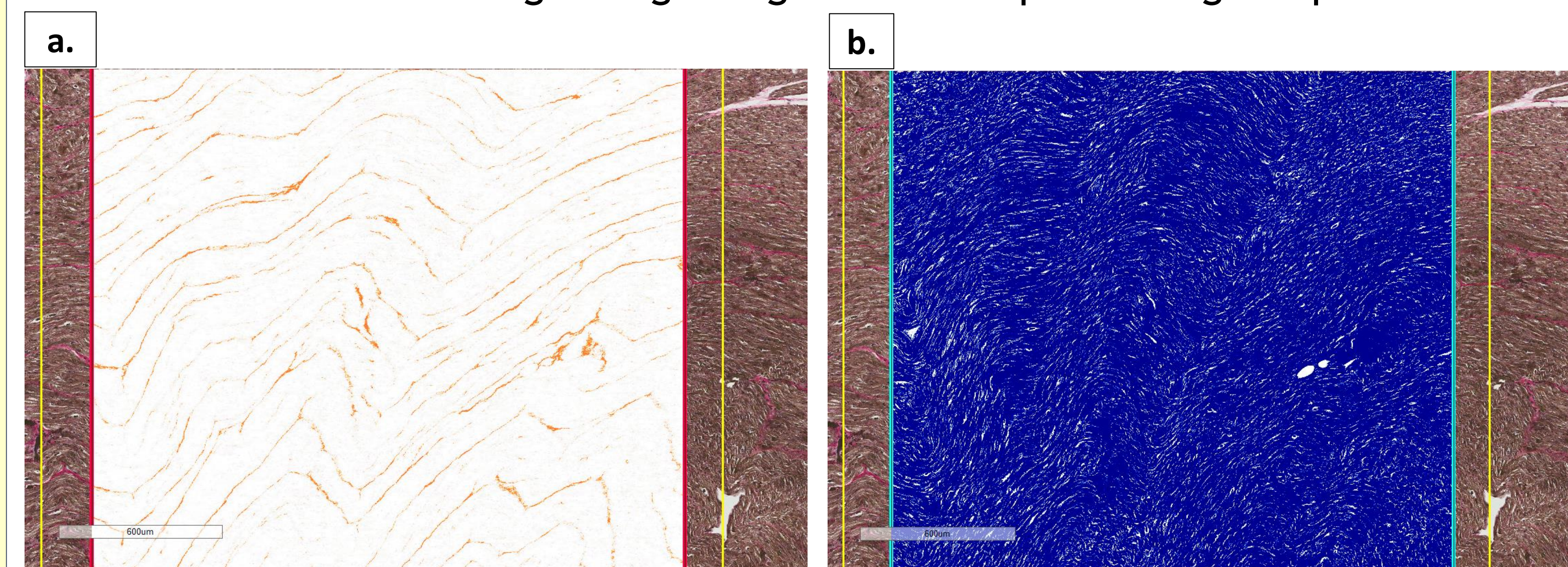


Figure 2 - a.) Tissue sections analyzed with positive pixel count of collagen algorithm. Yellow marks seen thanks to algorithm represent collagen fibers in tissue with a white background. b.) Section of tissue analyzed with negative pixel count of collagen algorithm. Blue color represent non-collagen cells in tissue.

## RESULTS

	Previous Histoscore	Most Recent Histoscore
Left Ventricle	2.1 ± 0.003	5.03 ± 0.68
Left Ventricle*	3.4 ± 0.01	5.24 ± 1.76

Table 1 - Comparison of Histoscores obtained by different investigators after analyzing same tissues with different collagen algorithms.

It was found that the histoscores for the left ventricle of non-obese and obese specimens were 5.03 ± 0.68 and 5.24 ± 1.76 respectively. From previous research, the histoscores for the left ventricle of non-obese and obese specimens were 2.1 ± 0.003 and 3.4 ± 0.01 respectively (Apostolakis-Kyrus, Katherine et al., 2014).

The Student's t calculated was not bigger than the t-tabulated for both, obese and non-obese specimens.

## CONCLUSION

The novel tool of whole slide analysis using the Aperio ScanScope® software represents a reliable method for image quantification that provides consistent results from investigator to investigator.

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