Quantitative Image Analysis and 3-D Digital Reconstruction of Aortic Valve Leaflet

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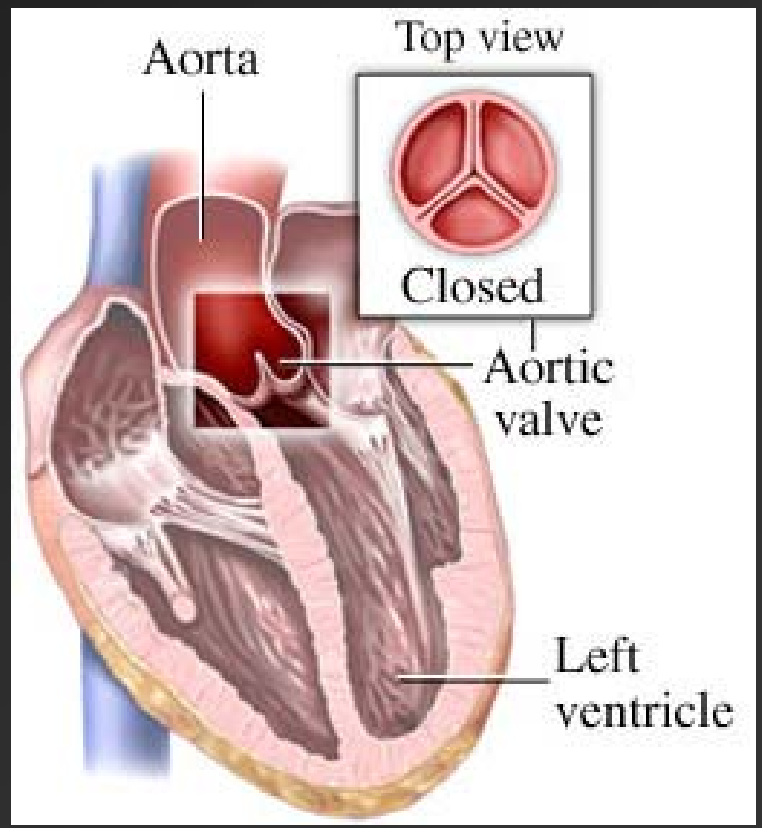
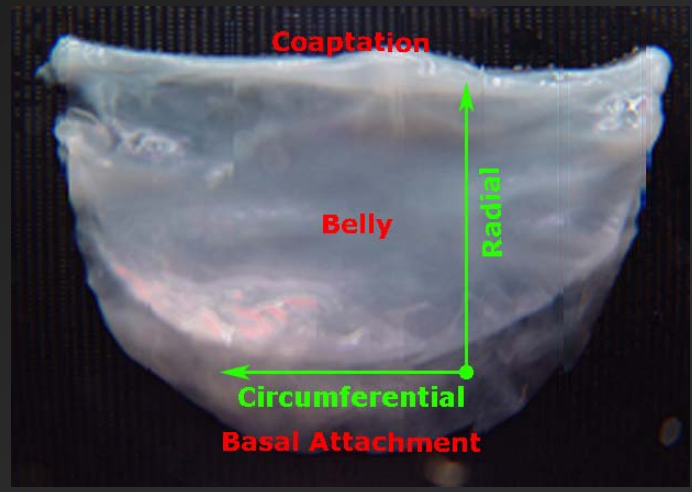
Introduction

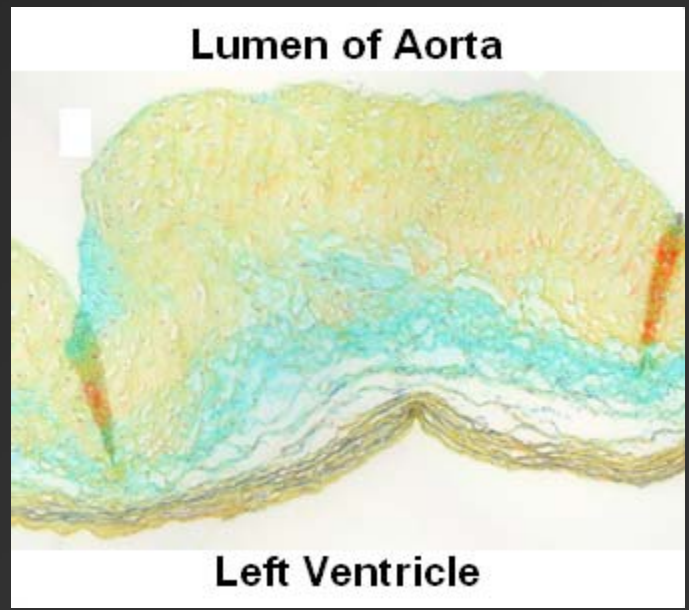
- Aortic valve (AV): most commonly replaced valve
- Tissue engineering (TE) valve offers customized availability, growth potential, durability, and bio-compatibility.
- Must first understand structure of the valve to replicate its function by TE



Background

- Each valve is composed of three leaflets (right-coronary, left coronary, non-coronary)
- Each leaflet is composed of 3 cell layers
  - Fibrosa: Collagen and little elastin
  - Spongiosa: Glucosaminoglycans (GAGs)
  - Ventricularis : Collagen and elastin

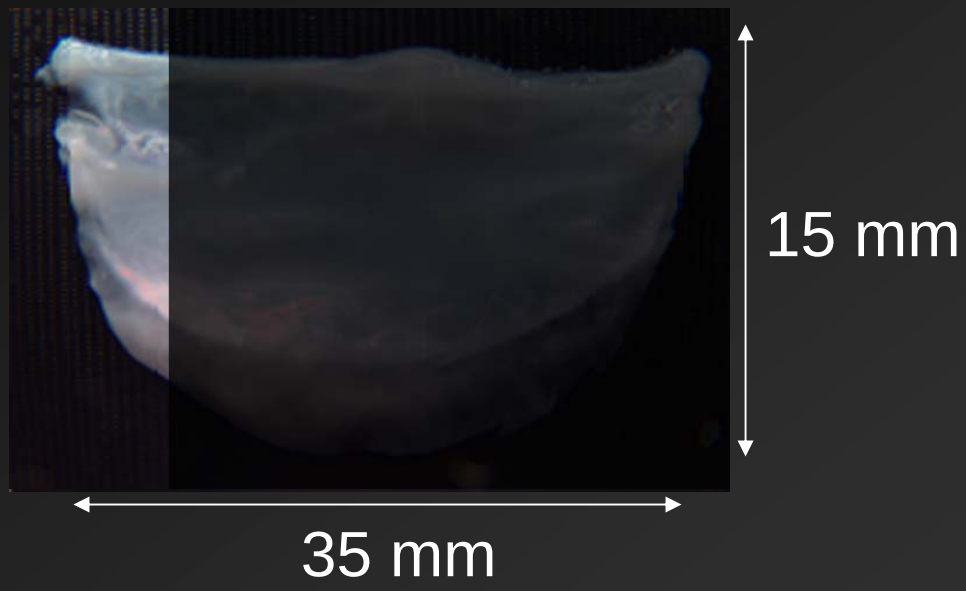




Objectives

- Original objective: Construct 3D representation of a porcine right coronary AV leaflet containing…
  - Cell count and distribution
  - Layer thickness variations
  - Track collagen and elastin fibers
- Technological limitations bifurcated the objective
  - Quantification
  - 3D reconstruction

Status



- Total histological sections: ~ 300 slides
- Imaged, quantified, and 3D reconstructed 50 slides

Histology and Image Acquisition

- 5 μm thick circumferential slices fixed in formalin stained with Movat's pentachrome
  - Collagen = Yellow
  - GAGs = Blue
  - Nuclei and Elastin = Dark Purple
- Acquisition
  - Quantification: 17 slices, spaced 90 μm apart, were digitally captured using bright field microscopy at 20x and montaged
  - 3D Reconstruction: 50 slices, spaced 10-15 μm apart were scanned individually using slide scanner

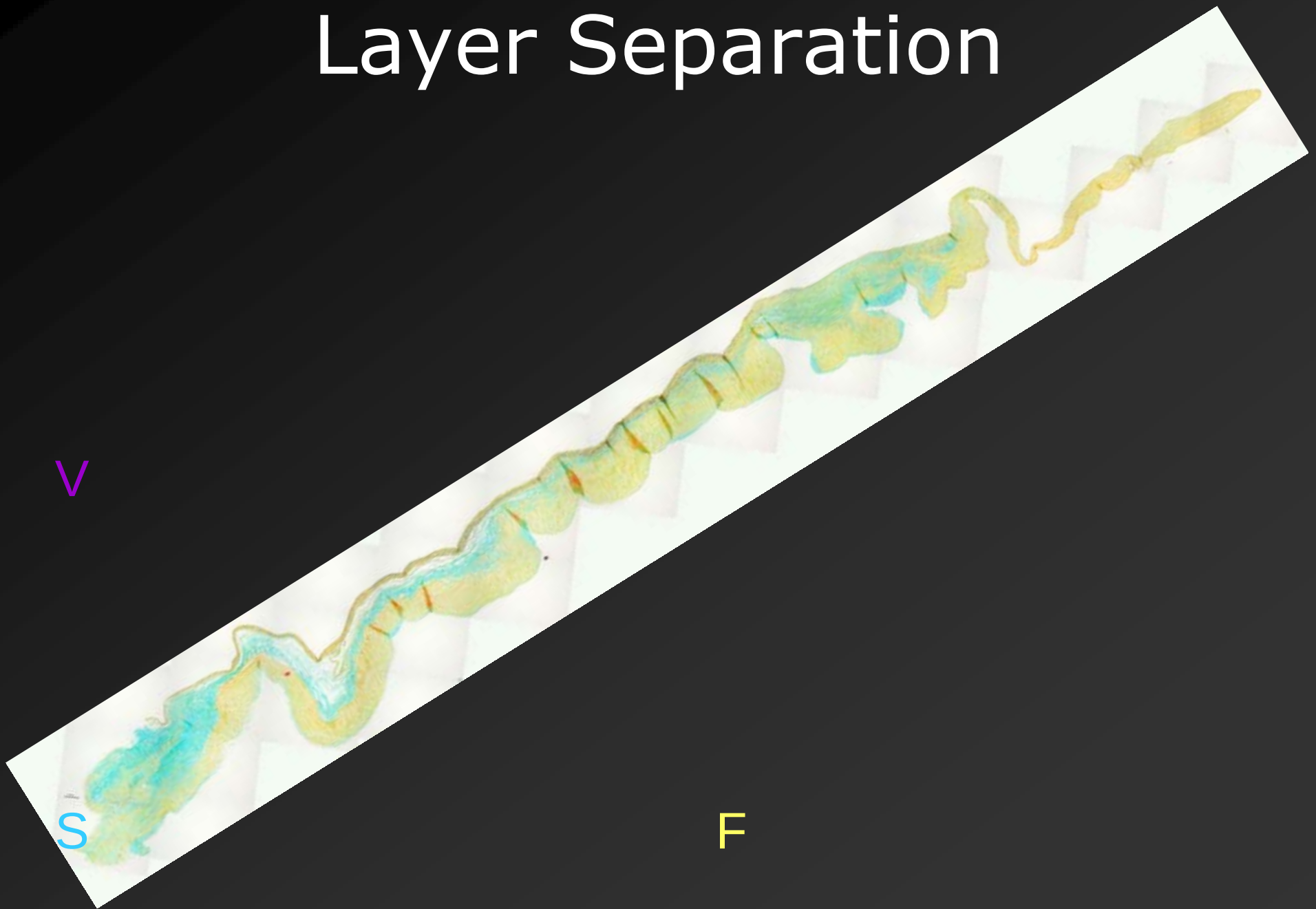
Principles of Image Analysis

- Contrast
  - 256 shades of gray in a 8-bit monochrome image
  - Defines edges/borders of objects

- Threshold
  - Enhances contrast by dividing image into two category
  - Objects within the image (subsets of image data) defined by contiguous pixels similar in intensity
  - Automatic threshold selection uses mode pixel intensity

Color Separation

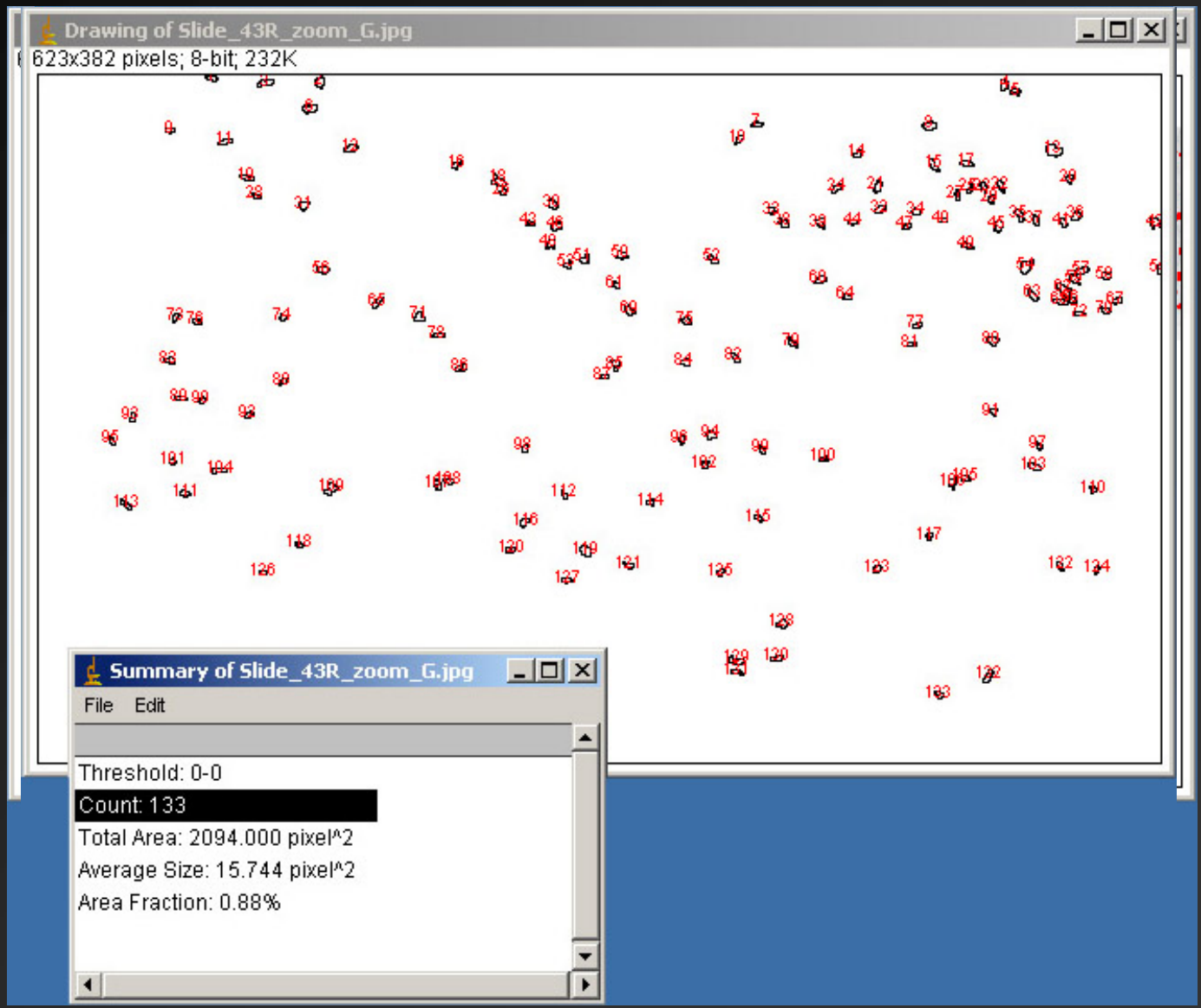
Layer Separation



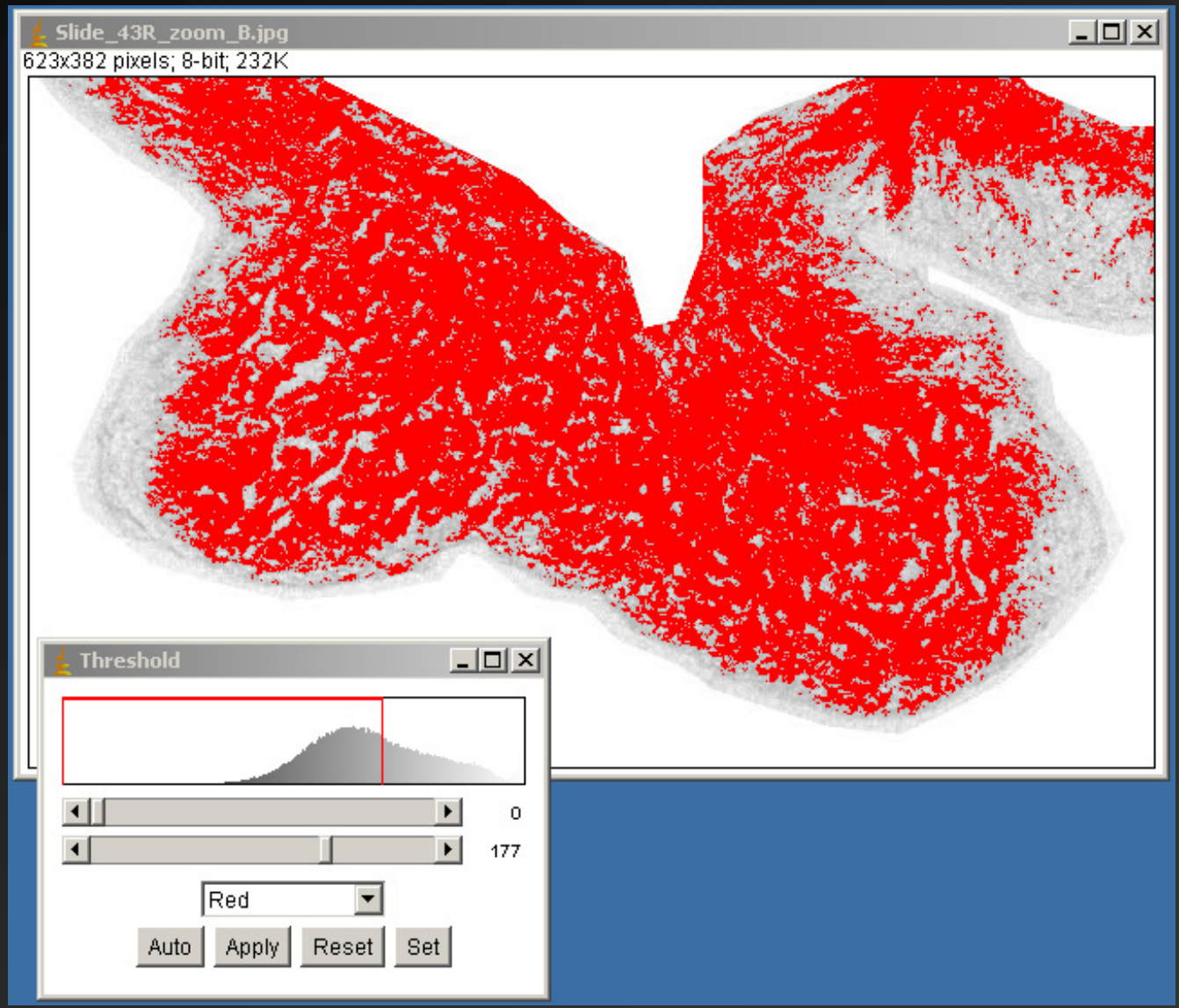
Cytometry

- Cell nuclei counting completed with particle analysis function in NIH's Image J
- RGB image converted to monochrome on the green channel
- Threshold is user defined
- Definition of a cell
  - size: 9-100 pixel^2
  - circularity: 0.6-1.0

Particle Analysis of Nuclei



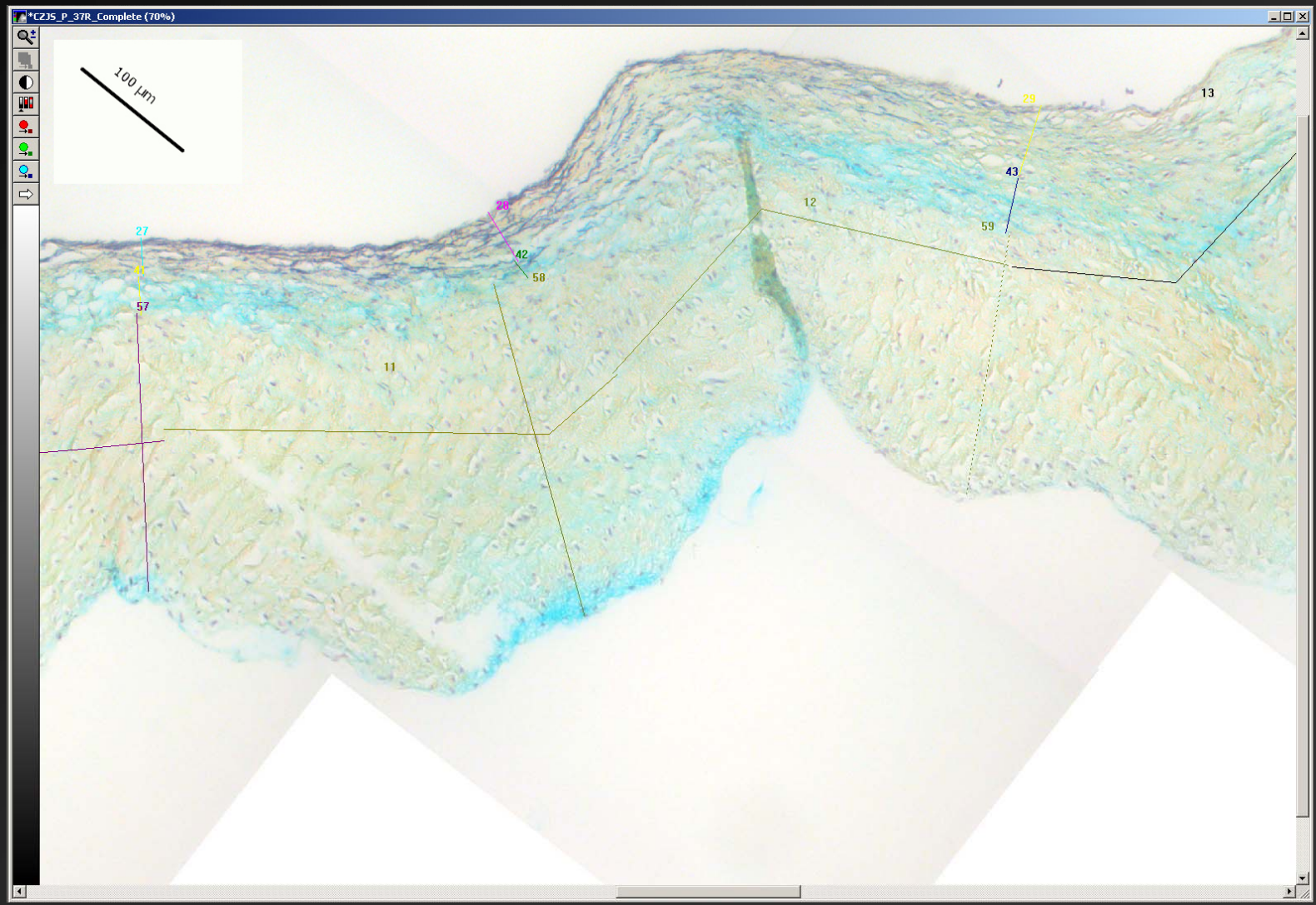
Collagen and Elastin Content



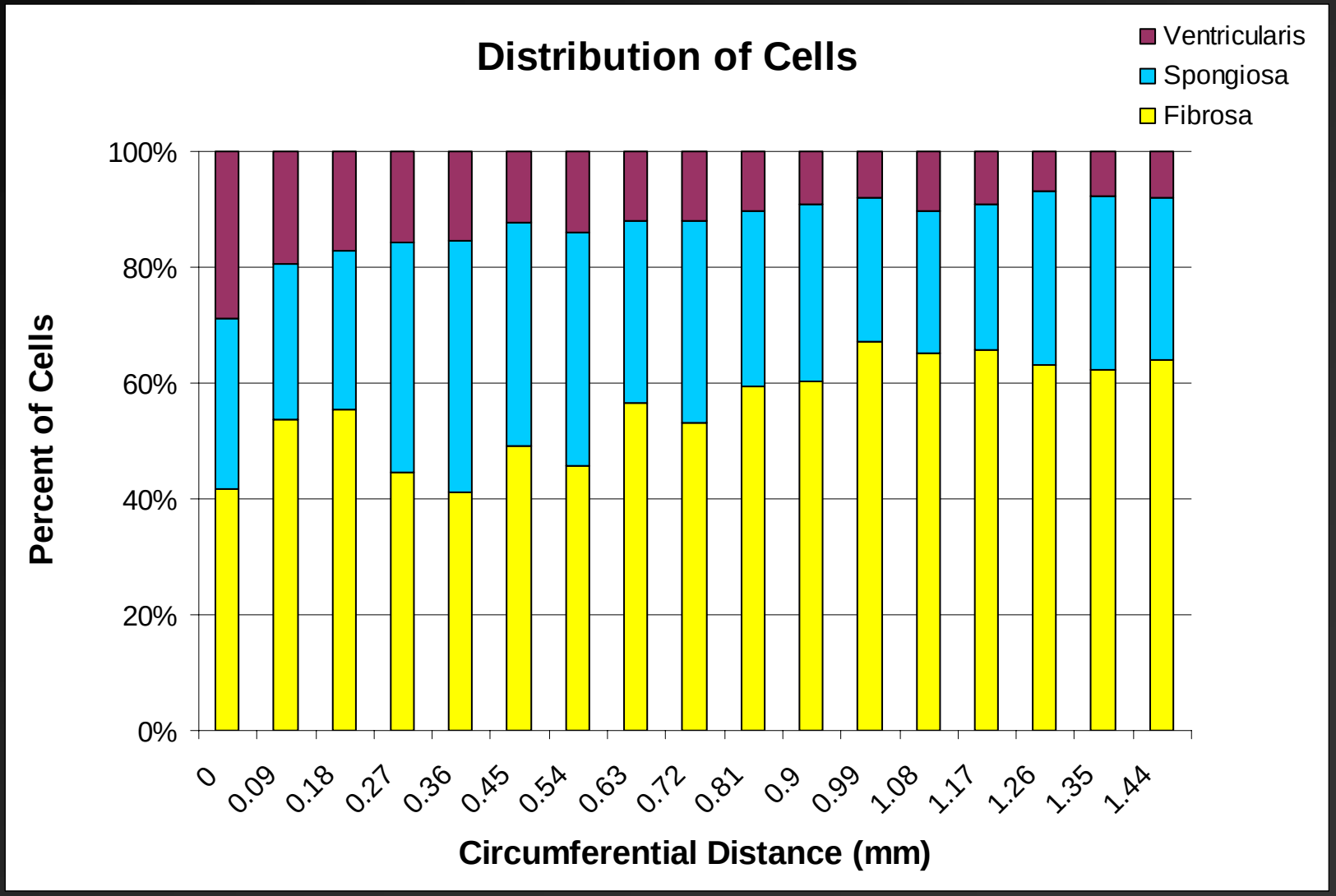
Layer Thickness Measurement

- Scale bar added during image acquisition used to establish calibration between pixels and μm in Metamorph™
- Take representative samples, spaced apart by 500 μm, along the radial length of each slice
- Results indicate
  - Average layer thickness
  - Topographic representation
  - Local variations

Measuring Thickness



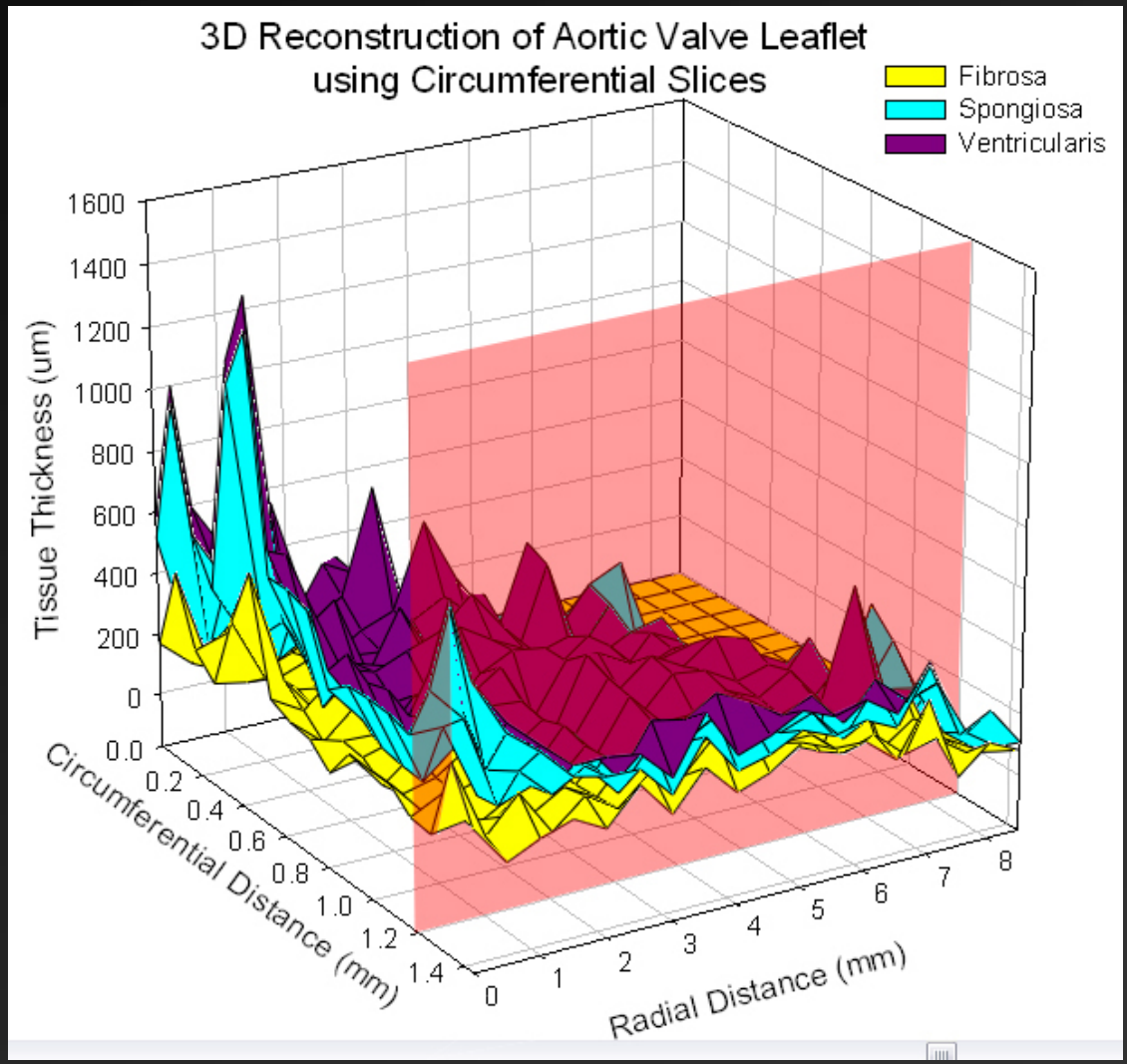
Quantification Results

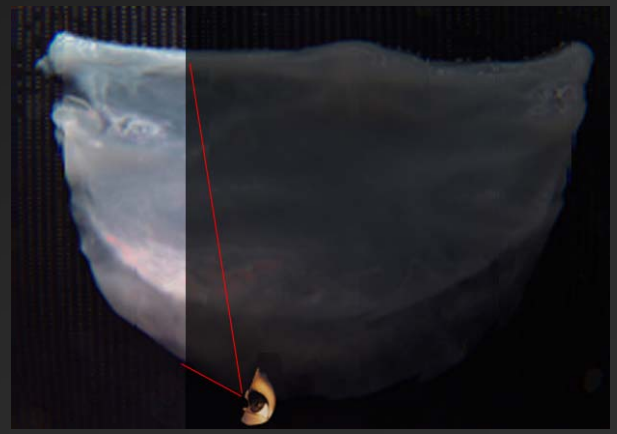


Quantification Results (cont'd)

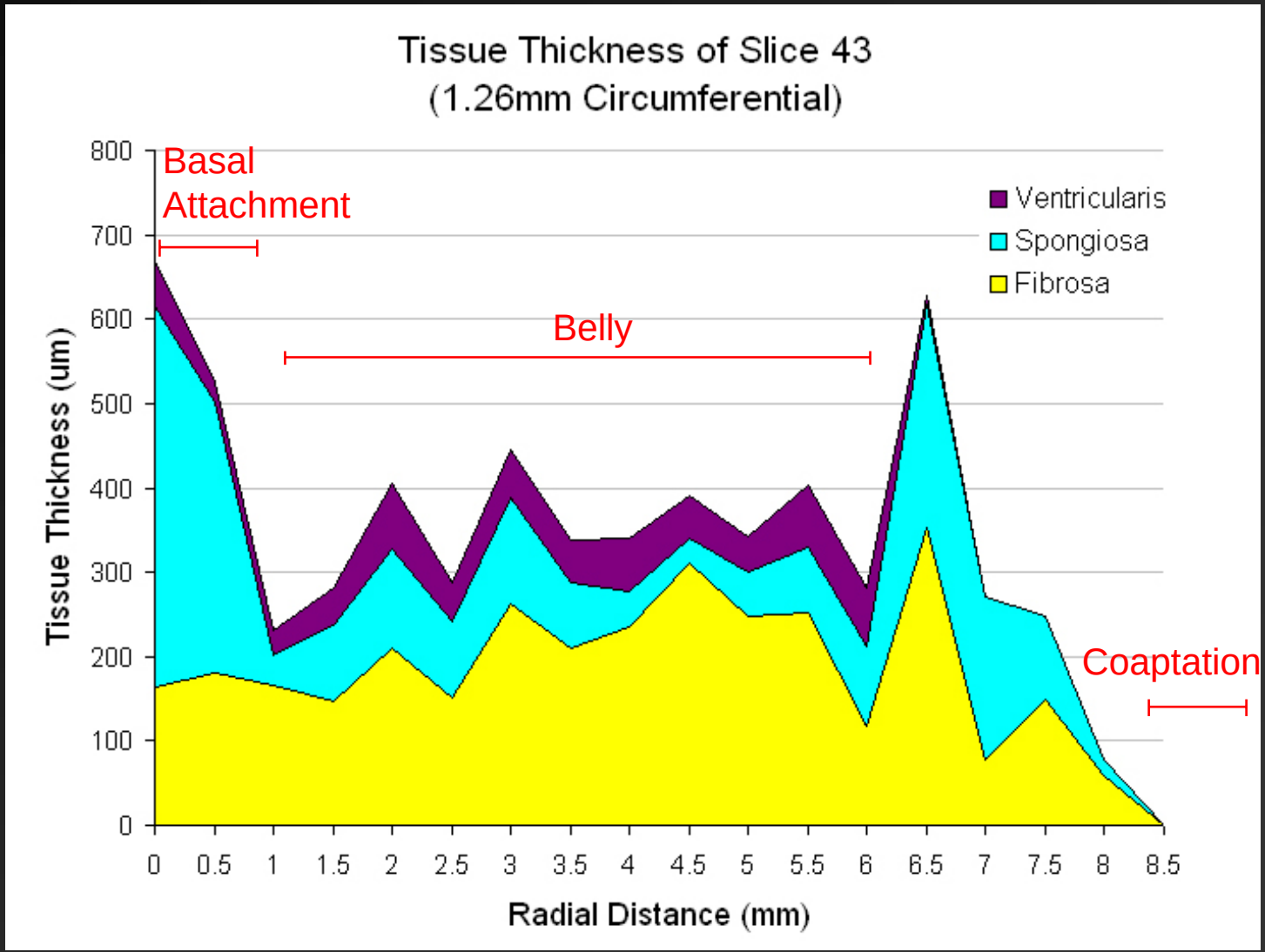
- Collagen and Elastin Content
  - Fibrosa: 48.2±6% area occupied by collagen
  - Ventricularis: 54.3±6% collagen
    39.3±5% elastin
  - Need for comparision with literature values (entire tissue composition: 13% elastin and 50% collagen by dry weight)
- Average thickness
  - Fibrosa: 150-230 μm (~100-350 μm)
  - Spongiosa: 110-200 μm (~70-250 μm)
  - Ventricularis: 40-60 μm (~50-150 μm)

Topography of Layer Thickness





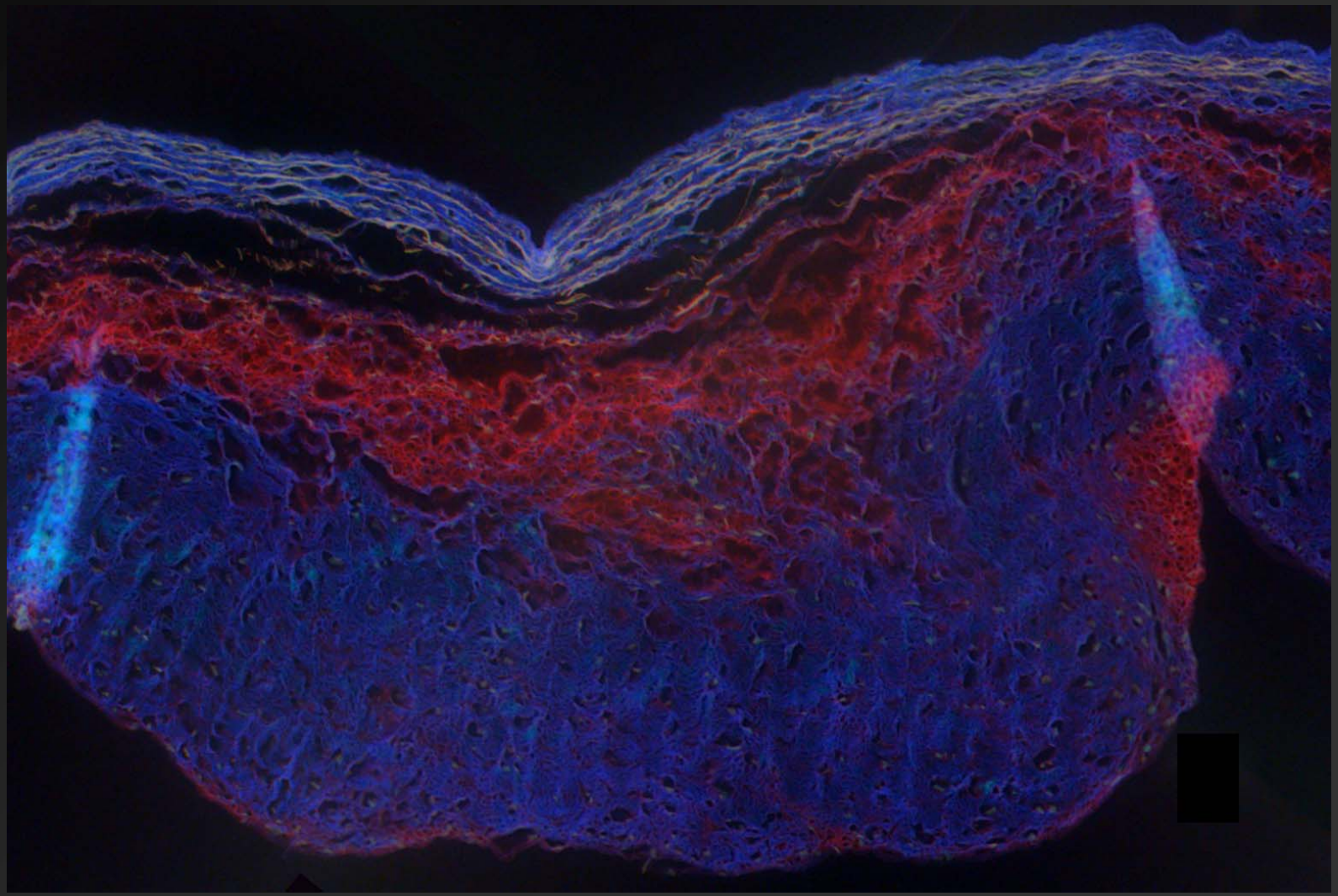
Regional Variation



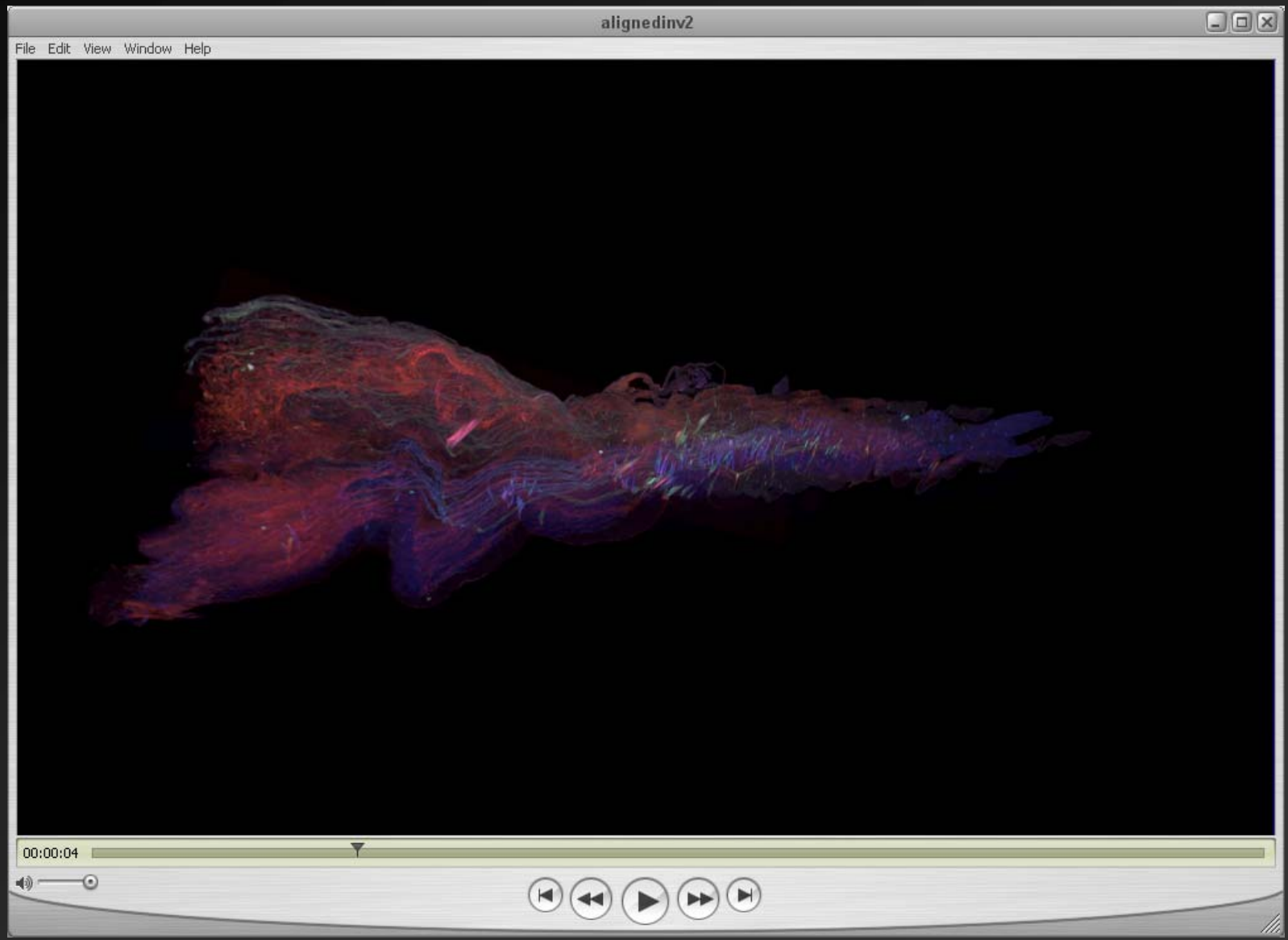
3D Reconstruction

- Images of 50 slices, spaced 10-15 μm apart, were digitally aligned by morphology and stacked to construct a 3D representation
- Software designed for fluorescent microscopy so image colors had to be inverted
  - Collagen = Blue
  - GAGs = Red
  - Nuclei and Elastin = Yellow
- 3D representation allows user to visualize the leaflet better than topography

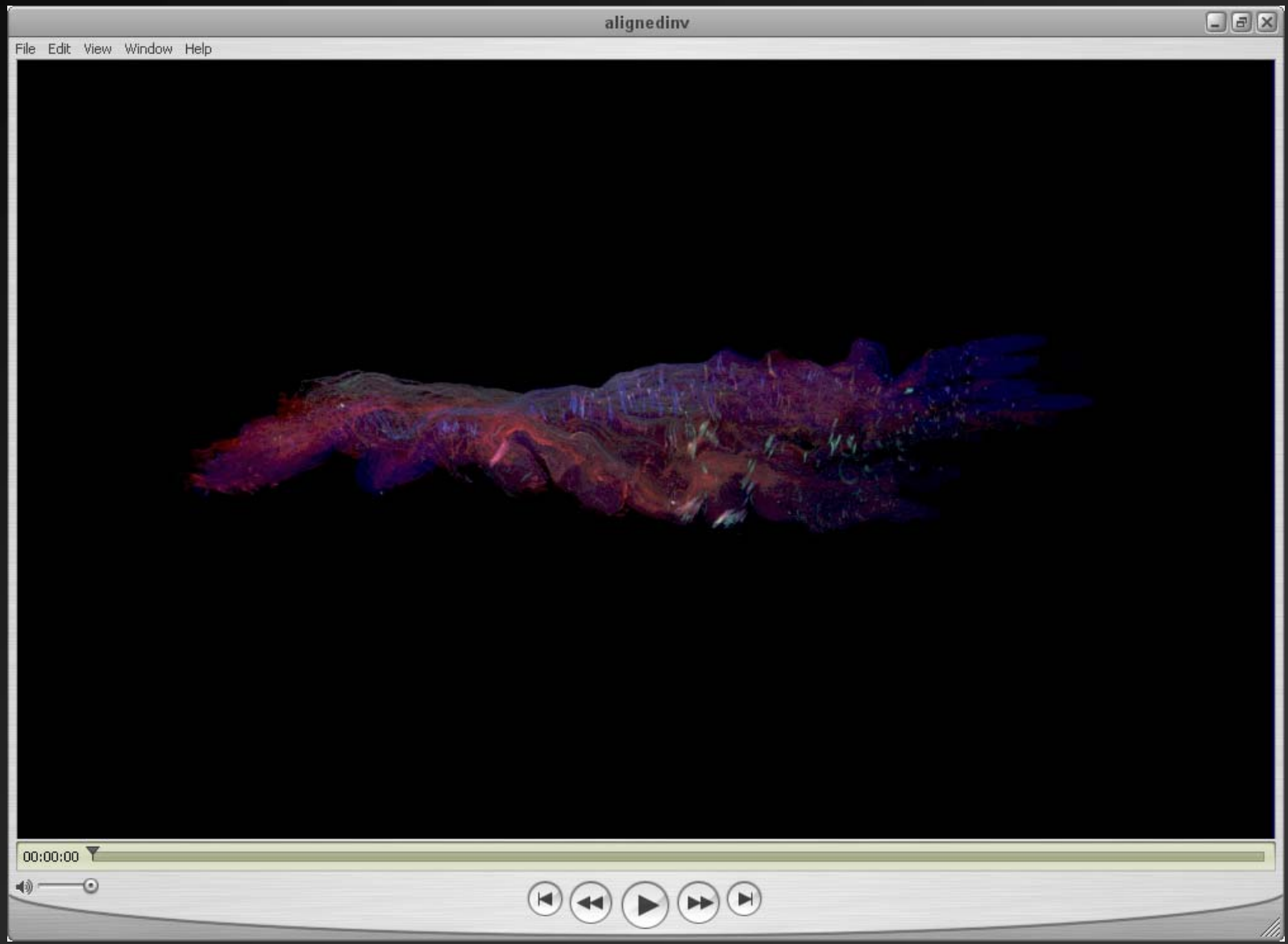
Inversion



Ooo....



Ahh...



Conclusion and Future Work

- Preliminary results calls for completion of the entire leaflet
- Statistical validation of quantification upon completion
- Explore other imaging techniques (fluorescent microscopy, X-ray, ultrasound, acoustic microscopy, SEM)
- Construct a 3D representation containing quantitative information
- Use 3D reconstruction to simulate and visualize dynamic response to applied load

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- Department of Computational Biology, University of Pittsburgh
- Developers of NIH's Image J

References

- Scott, M. and Vesely, I. Aortic valve cusp microstructure: the role of elastin. *Ann. Thorac. Surg.* 1995. 60:S391-4.
- Lacefield et al. Three-dimensional visualization and thickness estimation of aortic valve cusps using high-frequency ultrasound. *Physiol. Meas.* 2004. 25:27-36
- Boughne et al. A precise radiographic technique for the measurement of dimensionalchanges in heart valve biomaterials following fixation. *Journal of Biomechanics*. 2002. 35:983–987
- Lu et al. Evaluation of progression in nonrheumatic aortic valvular stenosis by scanning acoustic microscopy. *Ultrasound in Medicine & Biology*. 2000. 26:4:563-569
- P. Chieco, A. Jonker and C.J.F. van Noorden. *Image Cytometry*. 1st edition. Springer. 2001.

¿Questions?

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