

STAC Proposal: Cardiovascular Specialty Group

Removal of Bovine Products from CV Tissues

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STAC CV Subcommittee

(Nov, 2007)

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STAC CV Subcommittee

(Nov, 2007)

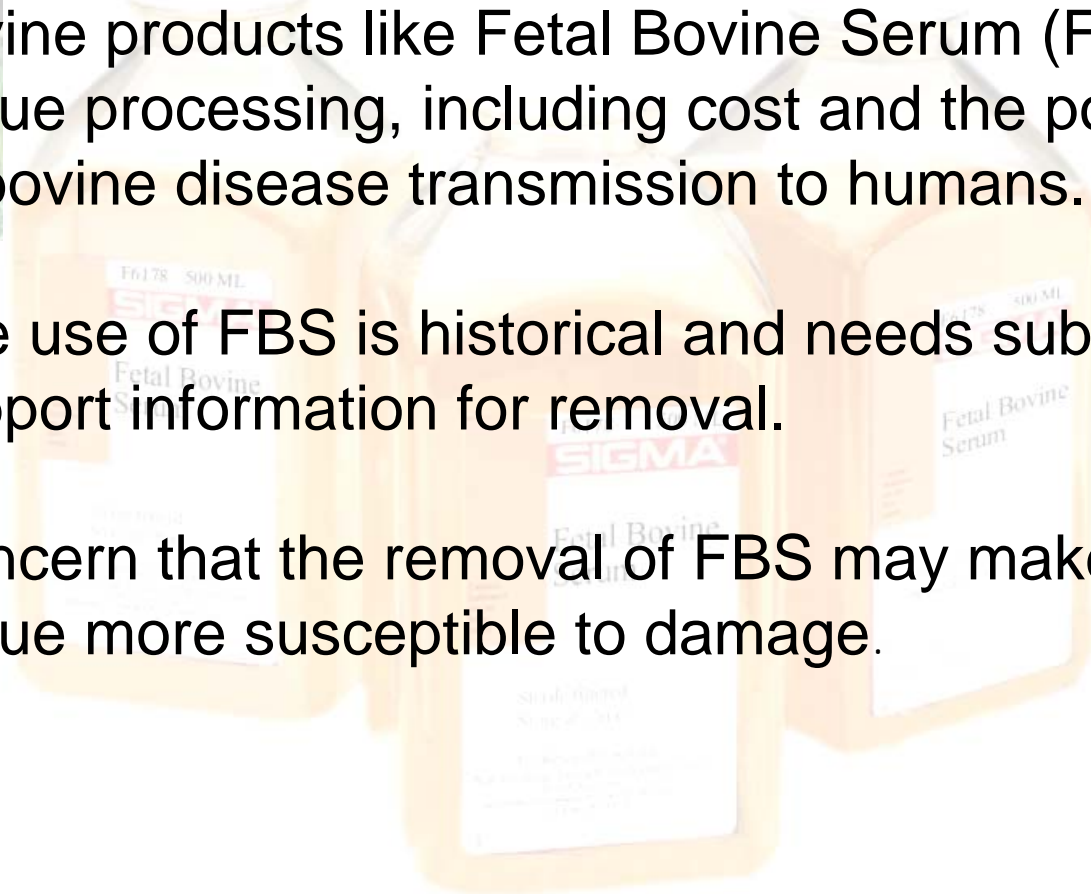
Topics discussed included:

- ▶ Development of analytical methods for heart valve assessment.
- ▶ Issues related to decellularization strategies?
- ▶ Further studies of heart valve ischemia?
- ▶ Evaluate loss of endothelial cells using current methods and optimize their removal.
- ▶ Testing of new antibiotics?
- ▶ Evaluation of deviations from a straight line cooling curve?
- ▶ Does ice in the tissue effect quality?
- ▶ Removal of bovine products?
- ▶ Temperature deviations
- ▶ Cracking? Vapor versus liquid storage? Mechanical storage freezers?
- ▶ Processing steps in the surgery?

Removal Of Bovine Products From The Processing Flow Of Human Tissues



1. There are several downsides to the inclusion of bovine products like Fetal Bovine Serum (FBS) in tissue processing, including cost and the potential of bovine disease transmission to humans.
2. The use of FBS is historical and needs substantial support information for removal.
3. Concern that the removal of FBS may make the tissue more susceptible to damage.





Removal Of Serum Makes No Difference To Cell Viability

In Vitro Cell.Dev.Biol.—Animal (2007) 43:269–275
DOI 10.1007/s11626-007-9039-z

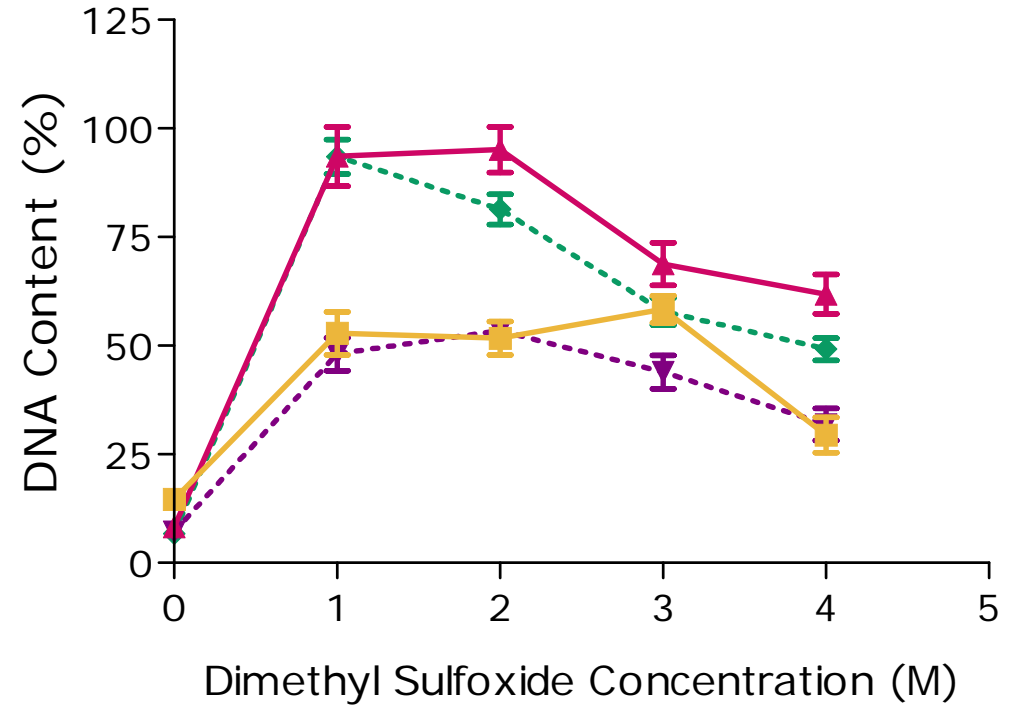
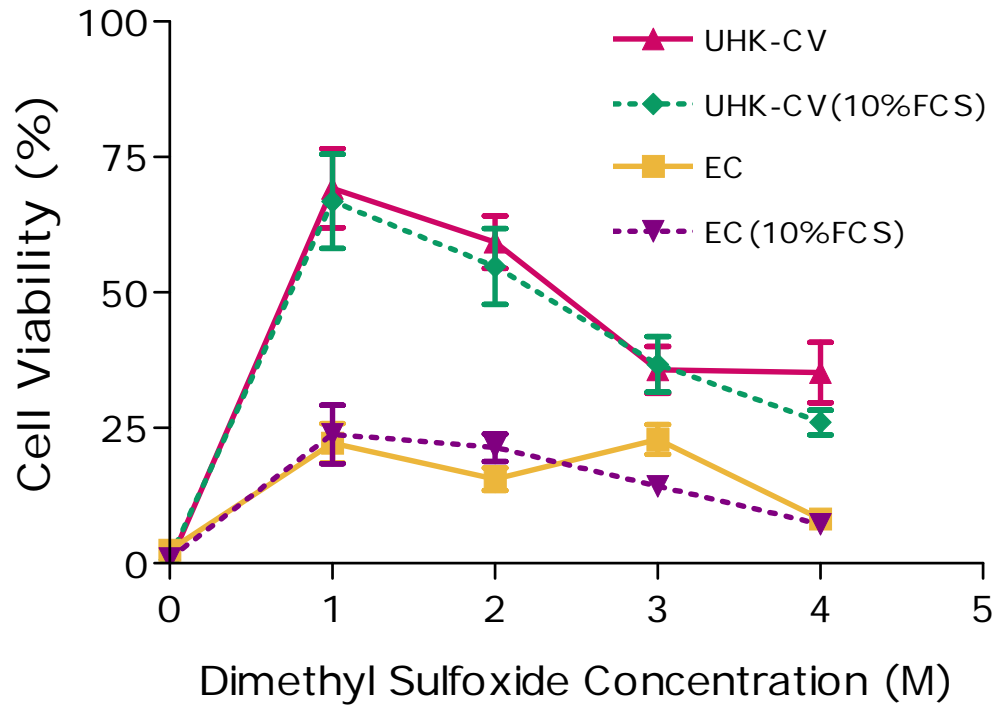
REPORTS

Serum-free solutions for cryopreservation of cells

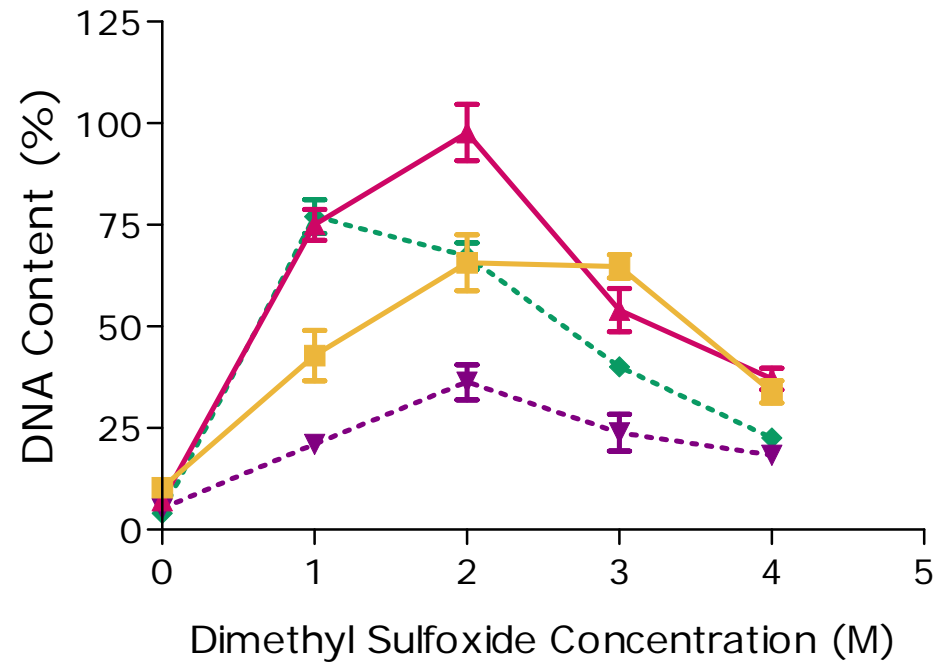
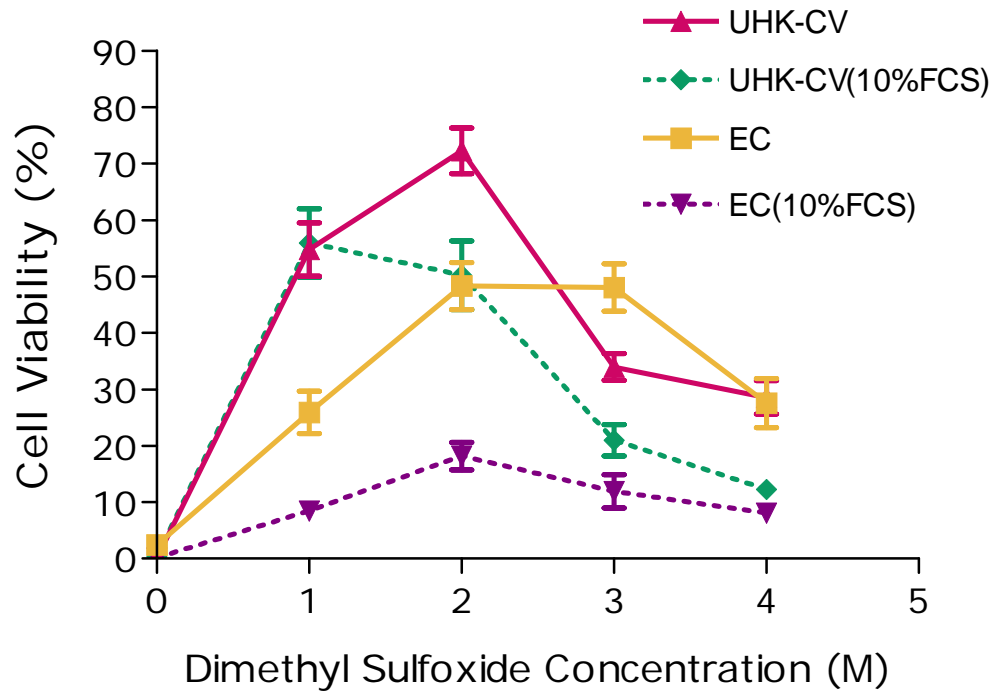
Lia H. Campbell • Kelvin G. M. Brockbank

Received: 1 February 2007 / Accepted: 14 May 2007 / Published online: 19 September 2007 / Editor: J. Denry Sato
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A10 Cell Viability After Cryopreservation



BCE Cell Viability After Cryopreservation



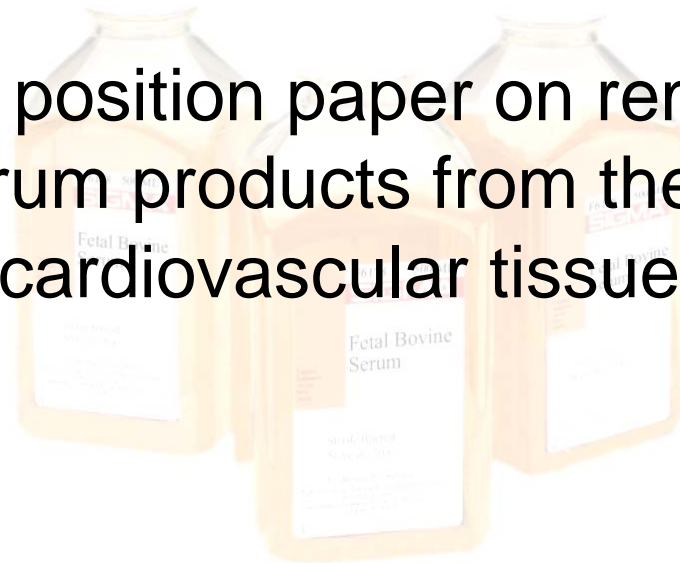


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Conclusions:

- ▶ Prepare a cardiovascular testing guidance document
- ▶ Prepare a position paper on removal of bovine serum products from the processing of human cardiovascular tissues





Study Milestones and Deliverables

Milestone	Deliverable to AATB	Estimated Delivery Date
Contract Initiation	Signed Quote and Research Agreement	August 2008
Study Design and Preparation	<ol style="list-style-type: none"> 1. Written description of proposed methods including process flowchart from donation to completion of testing. 2. Statistical method justification 	September 2008
Procurement & Processing of test articles	<ol style="list-style-type: none"> 1. Tabulated summary describing each experimental group 2. Photos illustrating representative test articles for each treatment group 3. Description of any unforeseen or inadvertent events observed at receipt or during treatment 	October 2008
Test Evaluation and Preliminary Reports	<p>Study report to AATB that includes</p> <ul style="list-style-type: none"> • Representative photos that illustrate analytical method • Draft tabulated or photographic summary describing value (histology, materials, and mechanics) for each experimental unit • Preliminary statistical analysis comparing treatment groups • Preliminary conclusions • Preliminary comparison of study values to any relevant literature 	November 2008 January 2009
Scientific Dissemination	<ol style="list-style-type: none"> 1. Presentation at AATB Annual meeting 2. Submission for publication in appropriate journal 	February 2009



Experimental Design

Treatment Groups[@]	Preservation Solution Composition
Fresh Control	Stored overnight in DMEM cell culture medium at 4°C
Frozen with serum	1.4M DMSO in DMEM cell culture medium plus 10% FBS
Frozen Serum-free	2.0M DMSO in base Solution [*]
Frozen Serum-free plus colloid	2.0M DMSO in Solution plus 6% Dextran 40

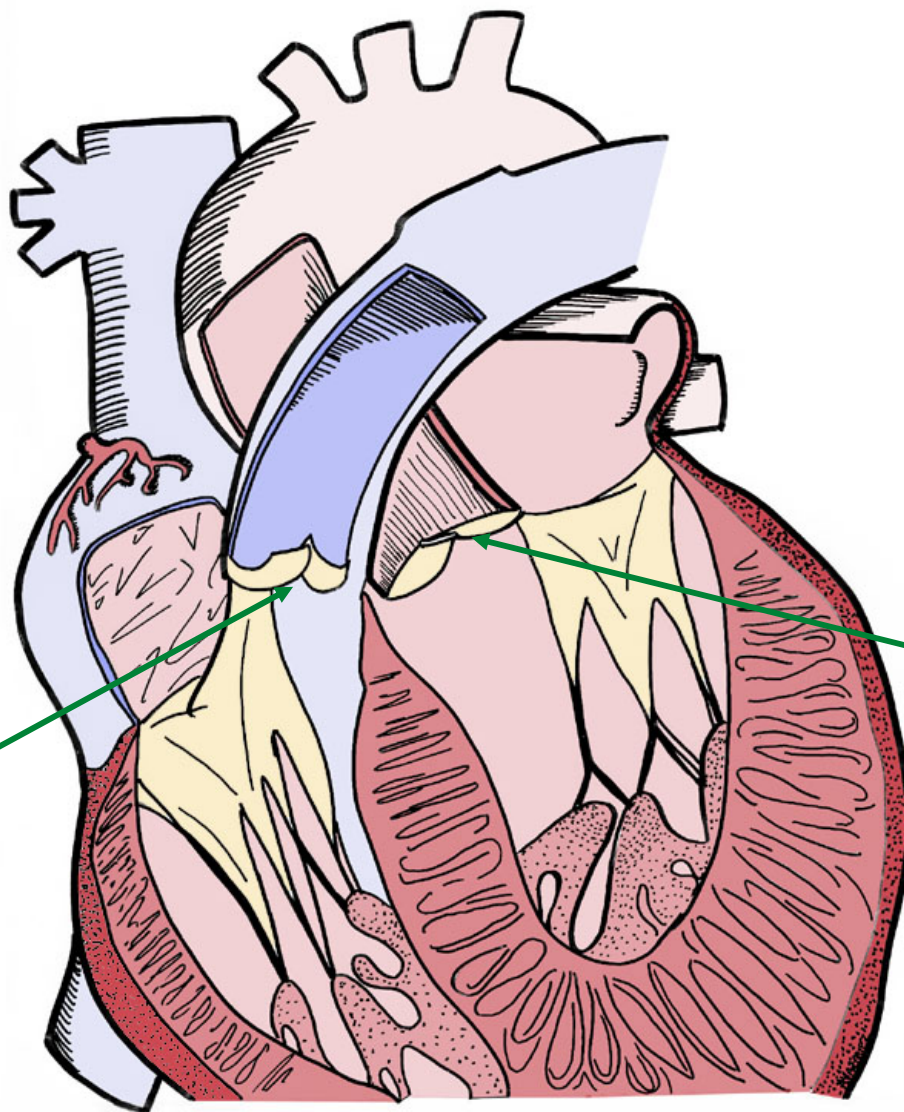
[@]Group size and statistical methods pending biostatistician review.

^{*}Campbell & Brockbank. Serum-Free Solutions for Cryopreservation of Cells, 2007.

Decision - Which Valve?

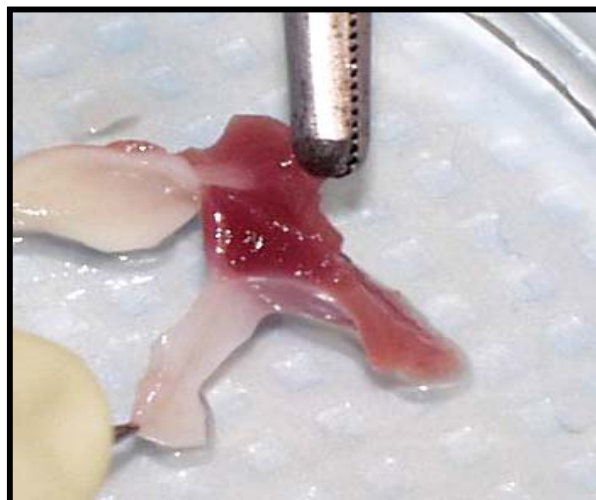
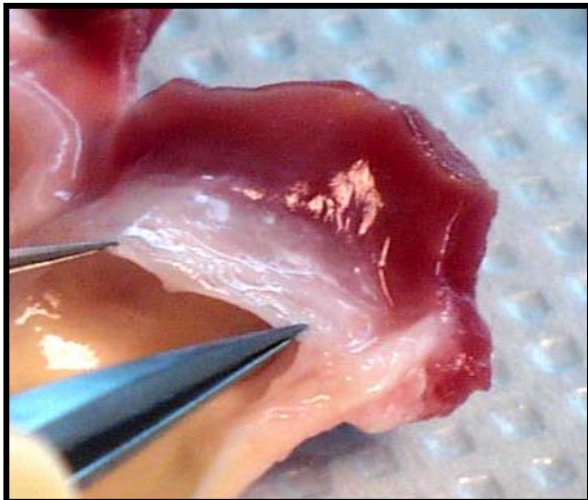


Pulmonary Valve



Aortic Valve

Preparation of Valve Sections





Focus on Leaflet Biomechanics Testing

▶ Physiologic Load Testing

1. Leaflets loaded to physiologically relevant loads (e.g., 400 kPa for aortic leaflets)
2. Measure low load modulus and extensibility

▶ Stress Relaxation Testing

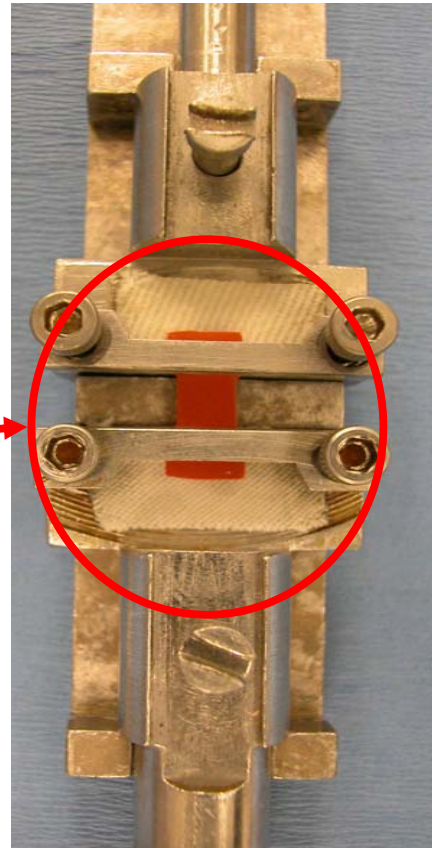
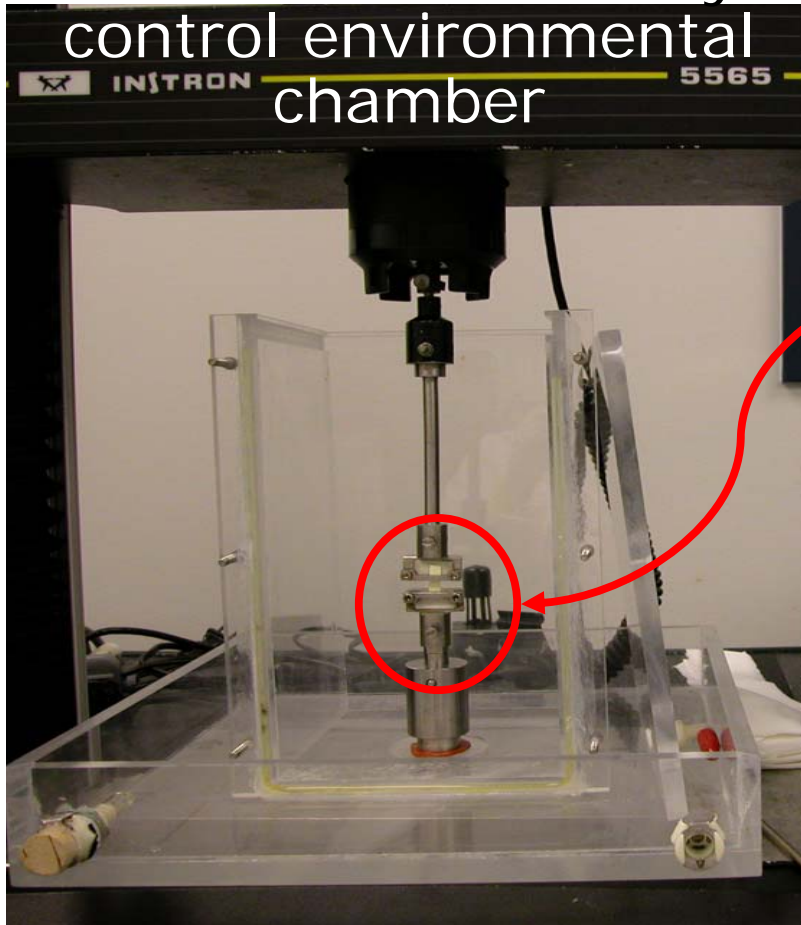
1. Leaflets held under constant strain (to produce physiologic load above) and stress decay (viscoelastic creep) is measured
2. Measure instant and 1000 sec stress relaxation, and relaxation rate

▶ Ultimate Tensile Testing

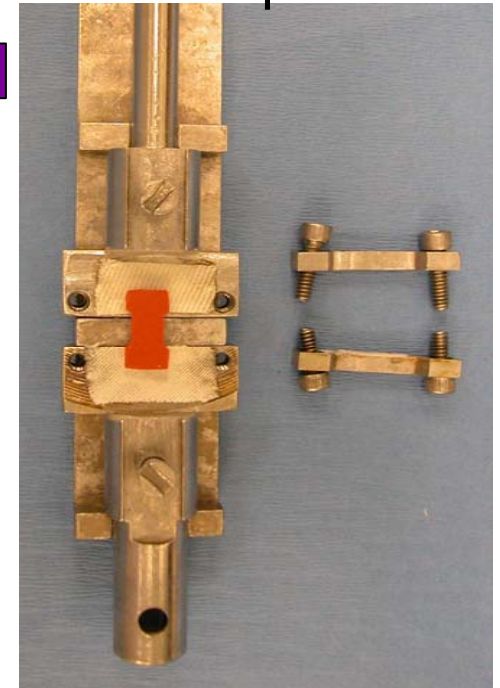
1. Leaflets are loaded until tensile failure occurs
2. Measure high load modulus, ultimate tensile strength, and ultimate elongation

Leaflet Biomechanics Test Set-up

Standard Instron load frame with thermally control environmental chamber



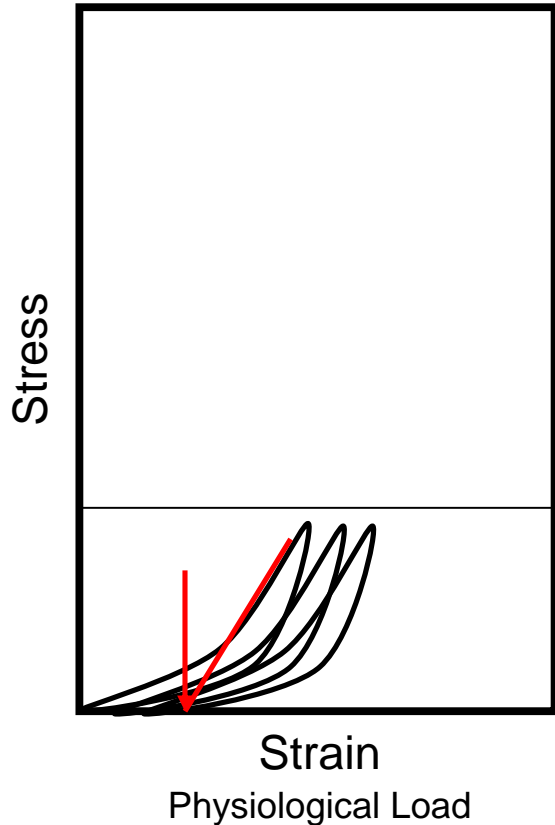
Die cut leaflet micro-tensile samples



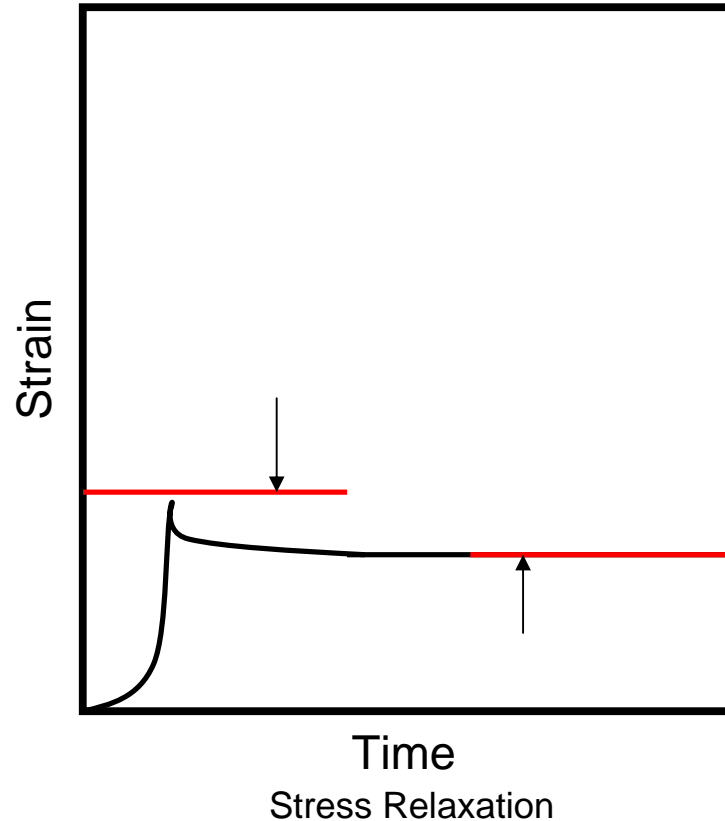
Mounting fixtures to assure reproducible sample orientation

Leaflet Biomechanics Analyses

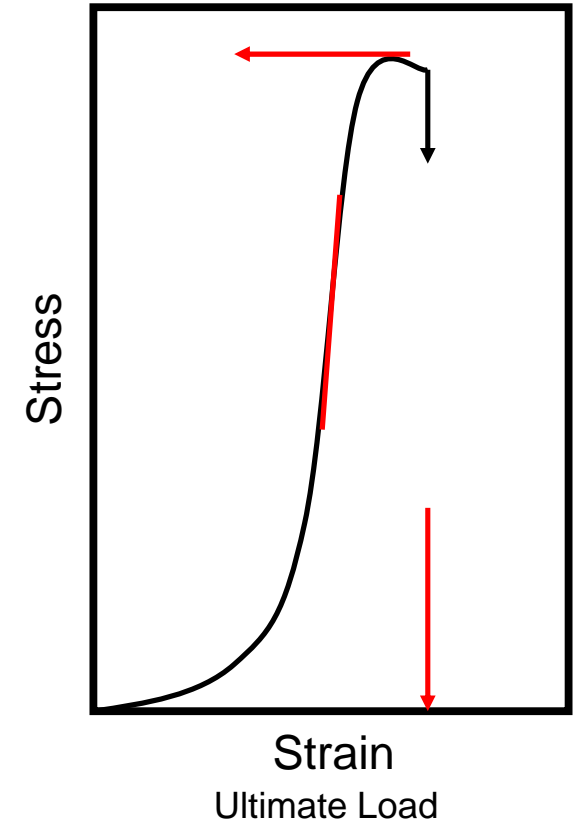
Low load Modulus
Extensibility



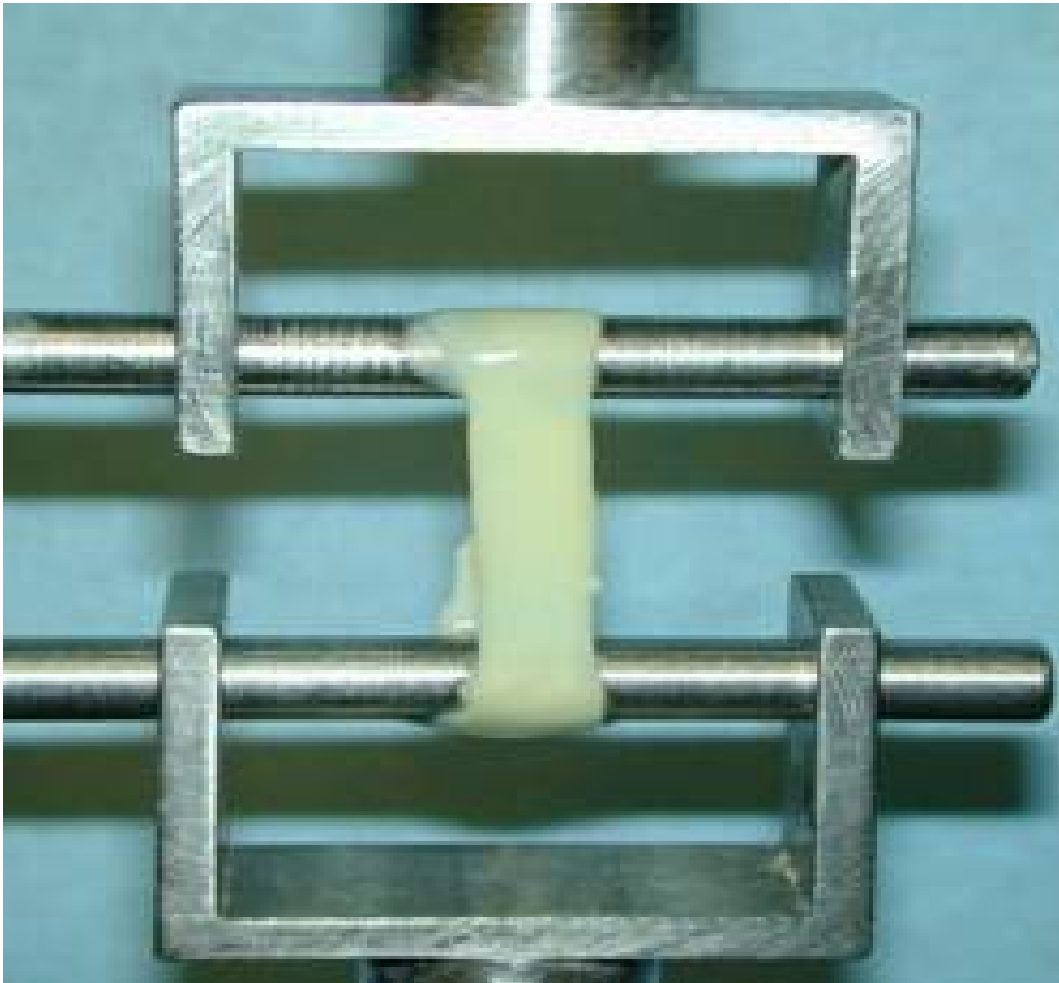
Instant and 1000 sec
relaxation
Relaxation rate



High load Modulus
Ultimate tensile strength
Ultimate elongation



Conduit Biomechanics



Conduit Hoop Strength Jig

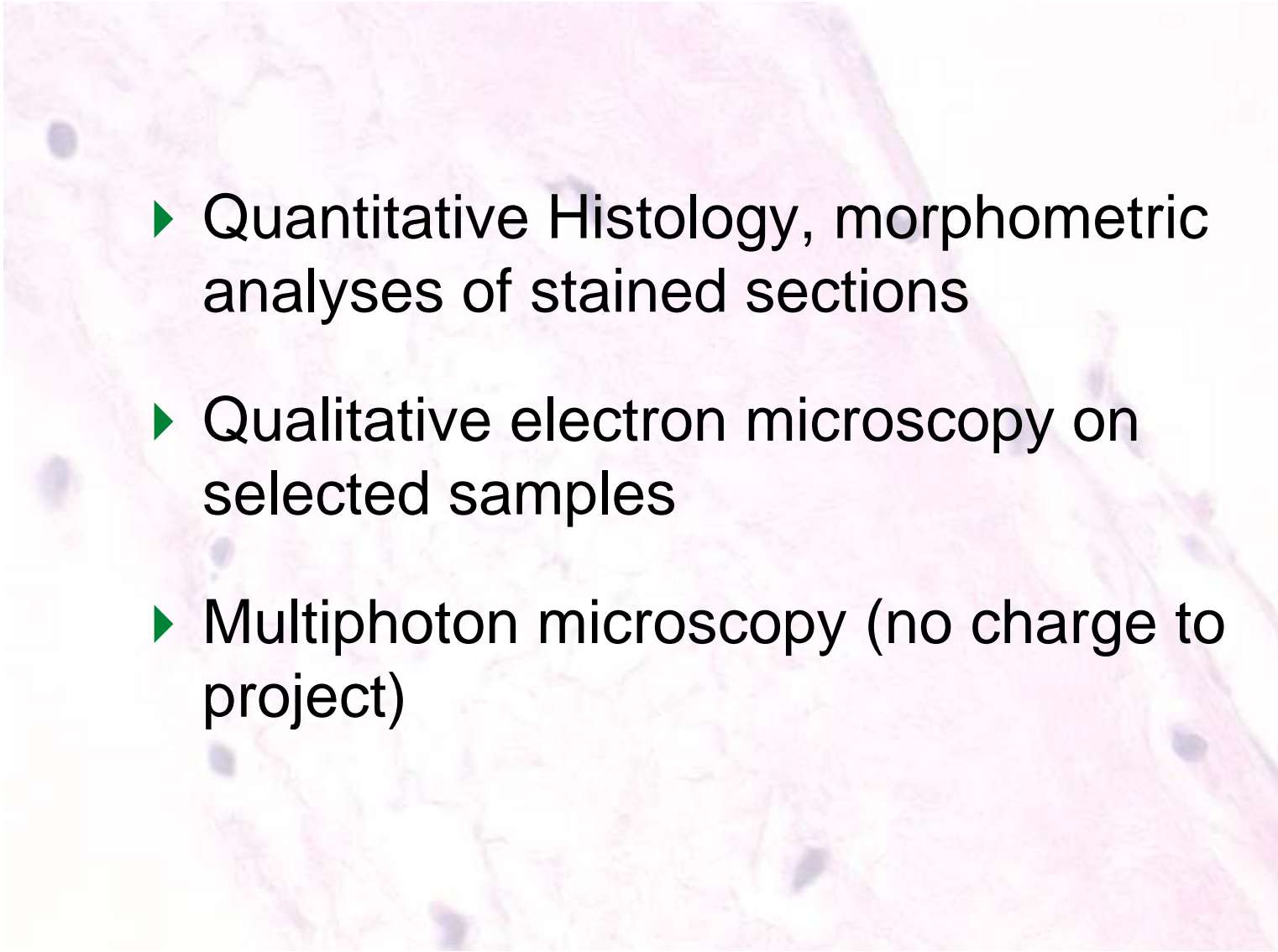
Conduit segments taken above the S-T junction

Mounted using custom stirrup jigs

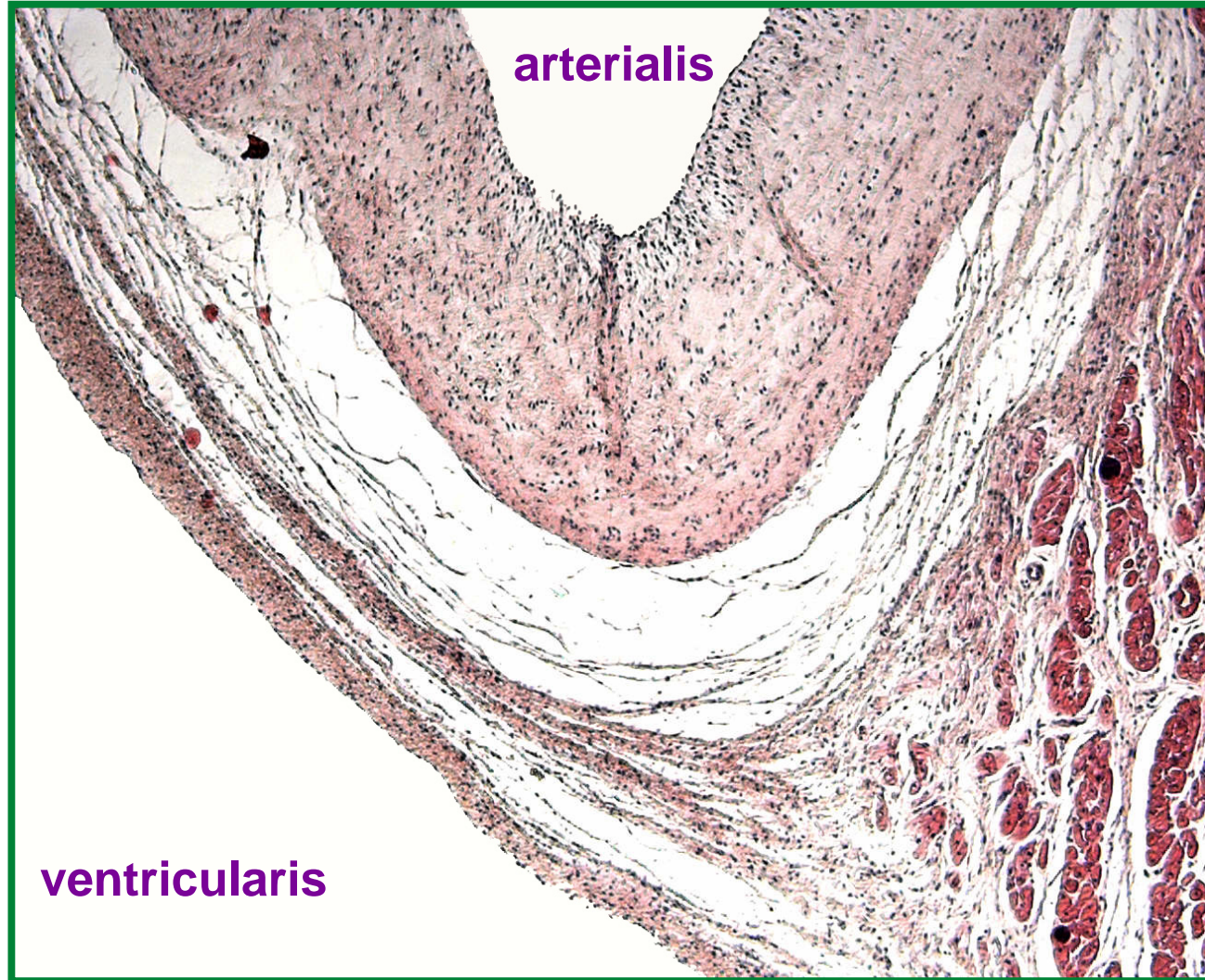
Ultimate hoop strength measured by loading to failure



Focus on Morphology

- 
- ▶ Quantitative Histology, morphometric analyses of stained sections
 - ▶ Qualitative electron microscopy on selected samples
 - ▶ Multiphoton microscopy (no charge to project)

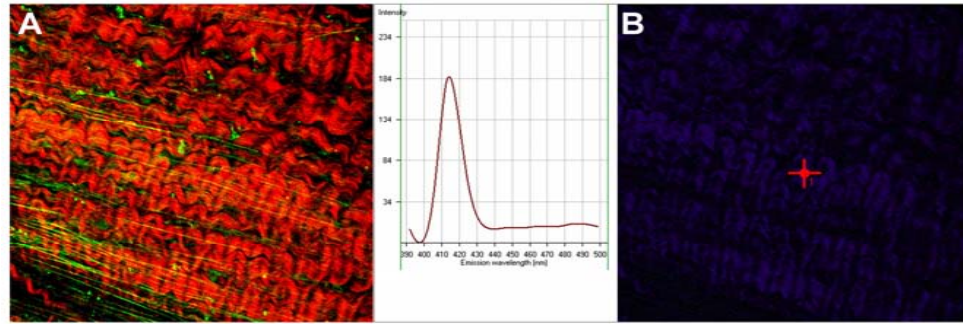
H & E Section



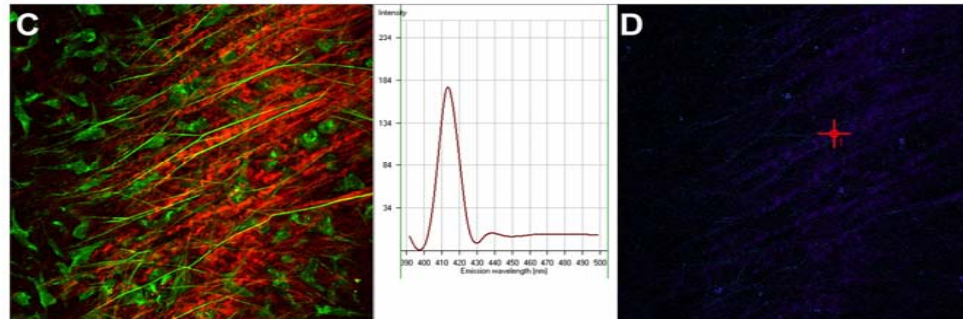
Multiphoton-induced (Laser) Autofluorescent Imaging of Porcine Aortic Leaflets

Arterialis

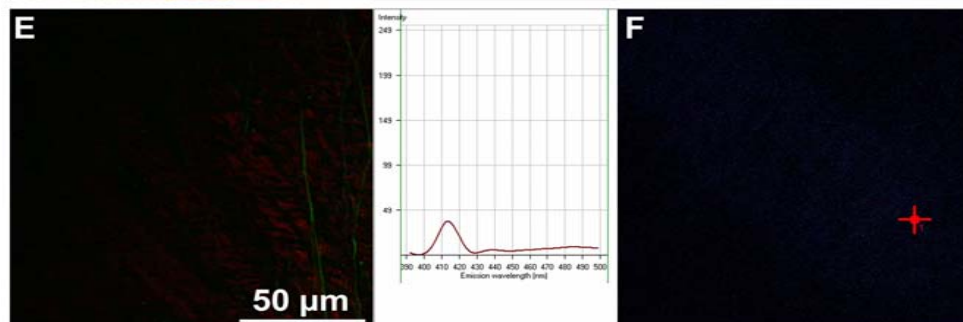
Fresh



Vitrified
VS83

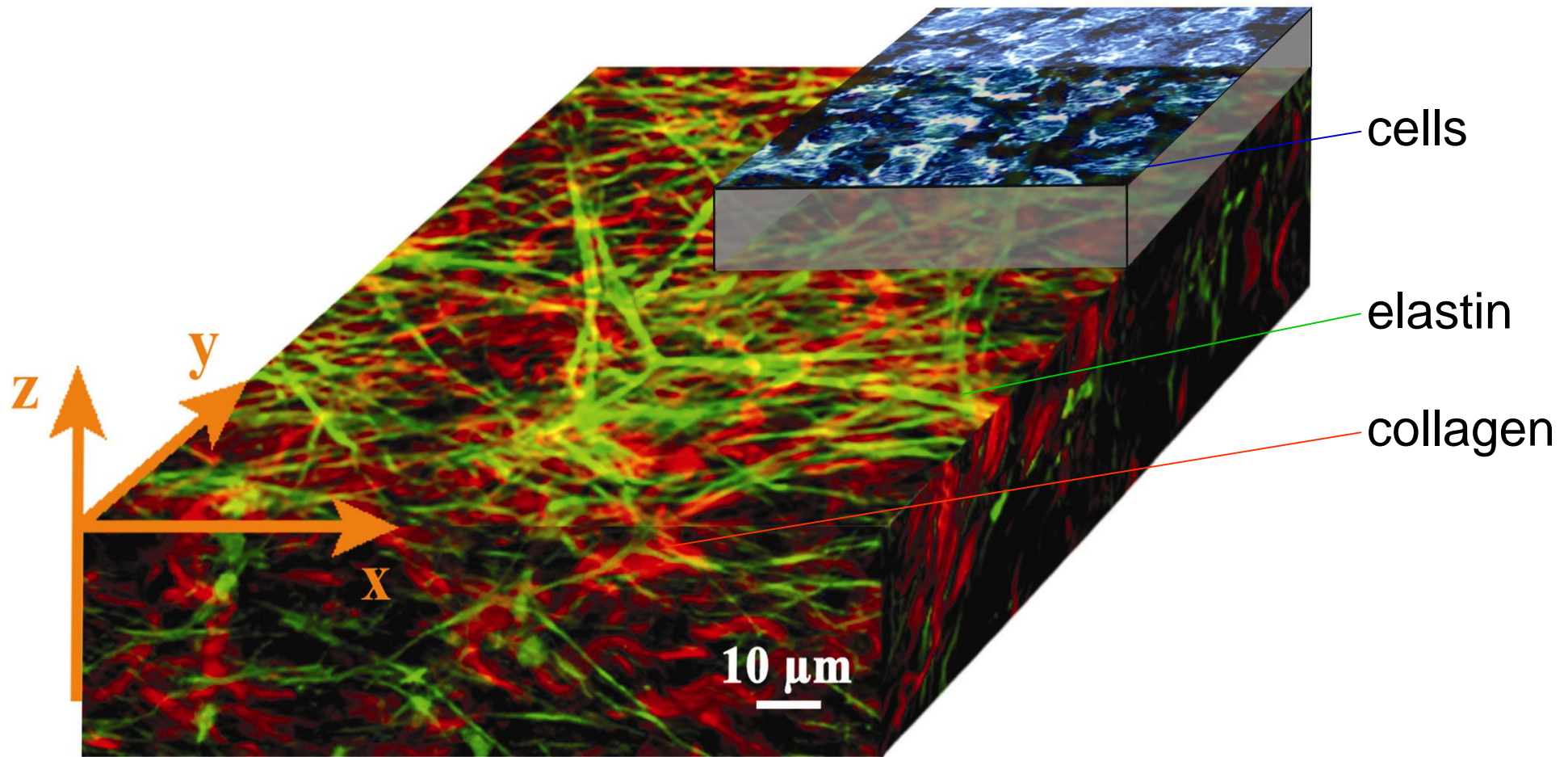


Frozen



- Collagen & cells (760nm)
- Elastin (840nm)
- SHG signal used to quantify collagen (392-499nm)

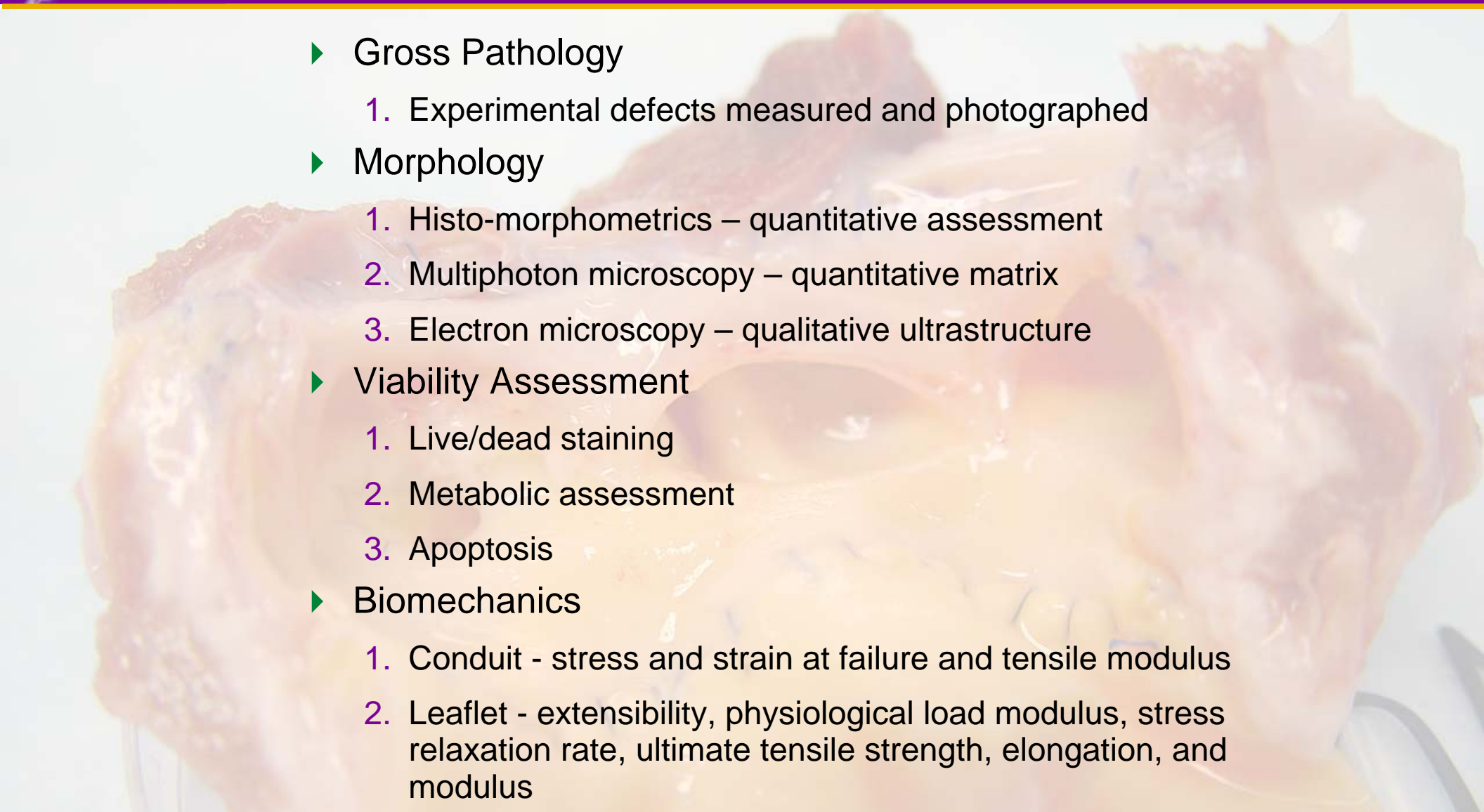
Multiphoton Microscopy



3D-reconstruction of a leaflet, each component can be quantified.



Overview of Tissue Assays

- ▶ Gross Pathology
 1. Experimental defects measured and photographed
 - ▶ Morphology
 1. Histo-morphometrics – quantitative assessment
 2. Multiphoton microscopy – quantitative matrix
 3. Electron microscopy – qualitative ultrastructure
 - ▶ Viability Assessment
 1. Live/dead staining
 2. Metabolic assessment
 3. Apoptosis
 - ▶ Biomechanics
 1. Conduit - stress and strain at failure and tensile modulus
 2. Leaflet - extensibility, physiological load modulus, stress relaxation rate, ultimate tensile strength, elongation, and modulus
- 



Summary Milestones and Deliverables

Milestone	Estimated Delivery Date
Contract initiation	August 2008
Study design and preparation	September 2008
Procurement & Processing of test articles	October 2008
Test Reports	November 2008 January 2009
Scientific Dissemination*	February 2009

*Prepared for presentation at next AATB meeting and manuscript ready for submission to journal