Drug Development Facilitated by Digital Pathology: A Global Challenge

Sam V. Machotka DVM, PhD, DACVP, DABT
Merck & Co., Inc.
Pathology Visions 2008
San Diego, CA
Oct-26-28-2008

MERCK

Outline

- Merck & Co, Inc
  - Global Company
- Pharmaceutical Discovery & Development
  - Process
  - Study Types/Design
    - Exploratory (non-GLP)
    - GLP-CFR 21 Parts 58 & 11
  - Histopathology Evaluation-unique requirements
- Use of Digital Histological Tissue Images
  - Why?
  - How?
  - Challenges
  - Lessons Learned
  - Efficiency Needs
  - Conclusions

Merck Research Laboratories Global Safety Assessment

Mirabel (France)

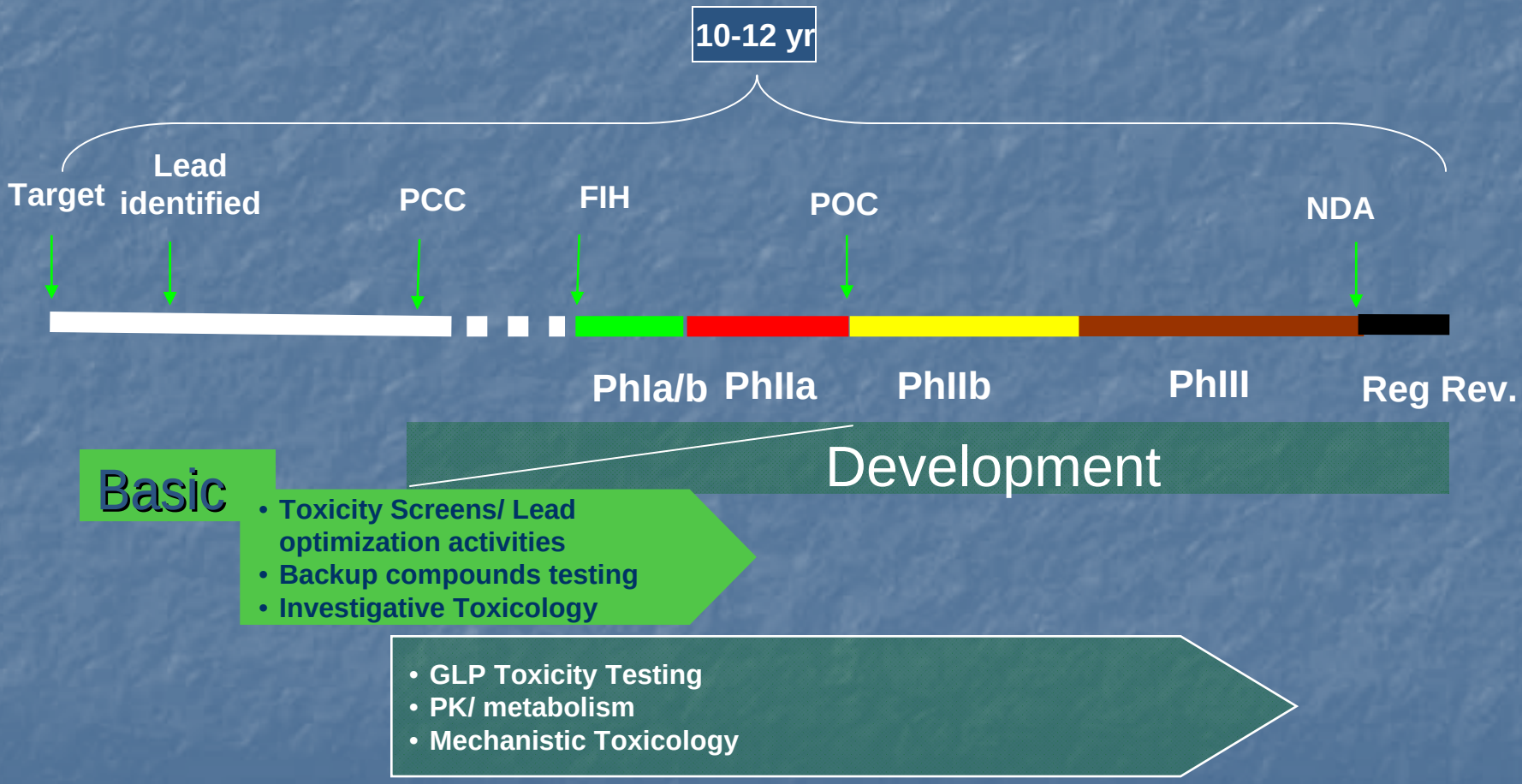
Tsukuba (Japan)

West Point (US)

Pharmaceutical Discovery and Development

- Discovery
  - Internal
    - Basic Research
  - External (In-license)
- Development
  - Pre-clinical
    - Drug metabolism
    - Drug formulation & manufacturing for testing
    - Safety assessment
  - Clinical
    - Clinical trials
- Statistics, Regulatory Affairs, Manufacturing, Marketing

Safety Assessment in Drug Discovery and Development



Study Types

- Exploratory (non-GLP)
  - Investigative, dose-ranging, and certain preliminary studies
- GLP (Good Laboratory Practice)
  - According to FDA 21 CFR Part 58 (GLP) & Part 11 (Electronic Signature, Audit Trail)
  - Required for “pivotal” non-clinical safety studies to support safe conduct of clinical studies and registration

Study Design

- Pre-clinical Safety Study for New Drug Submissions
  - Species: Rats, Mice, Dogs, Monkeys
- Typical Study Duration
  - Acute/sub-chronic: ≤ 3 months
  - Chronic: 6-12 months
  - Carcinogenicity:
    - 2 years (rats, mice)
    - 6 months (mouse transgenic)

Histopathology Evaluation Unique Requirements

- Multiple dose groups: 1-10
- Numerous animals (both sexes): 2-500
- Numerous tissues/slides: 4-25000/10-5000
- Interim, end of dosing and recovery evaluations
- Target tissues: 0-20
- Evaluation & report cycle time: 1 day-3 months

Example Histopathology Table

Sex	Female				Male			
Group	**Control**	**5 mkd**	**10 mkd**	**20 mkd**	**Control**	**5 mkd**	**10 mkd**	**20 mkd**
Animal	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5
Brain								
Dx 1	1 - 1 - -	1 - - 1 -	- - - 1 -	1 - 2 1 3	- - - 1 -	1 - - 1 -	1 - - 1 -	1 2 2 1 -
Dx 2	- 1 - - -	- - 2 - -	- - - - -	- - - - -	- - - - -	- - 1 - -	- - - - -	- - - - -
Dx 3	- - - - 1	- - - - -	- - 1 - -	2 - - 1 -	1 - - - -	- - - - -	- - - 2 -	- - - - -
Heart								
Dx 1	1 - - 1 -	- - - - -	- 2 - - -	1 - - - -	- - - - -	1 - - 1 -	- - - - -	1 - - 1 -
Dx 2	- - - - -	- - - - -	1 - 1 - 2	2 - 3 2 2	- - 1 - -	- 1 - - -	- - 1 1 1	3 3 1 2 2
Dx 3	- - 2 - -	- 3 - - -	- - - - 2	- - 1 - -	3 - - - -	- - - 1 -	- 2 - - -	- - - - 1
Kidney								
Dx 1	- - - 1 -	1 - - - -	- - - 2 -	- - - - -	1 - - 1 -	- - - - -	1 - - - -	- - - 1 -
Dx 2	- - - - -	- - - - -	- - - - -	- - 2 - -	- - - - -	- - - - -	- - 2 - -	- - - - -
Dx 3	- - - - -	- - - 1 -	- - - - -	- 1 - - -	- - - - -	- - 2 - -	- - - - -	- - - - -
Liver								
Dx 1	- - - - -	1 - 2 1 -	2 3 - 1 1	3 3 2 4 4	- - - - -	2 3 - 2 1	3 3 3 4 4	4 3 3 4 4
Dx 2	- - - 2 1	- - - - -	- 1 - 1 -	1 2 1 - -	- 1 - - 1	- - - - -	1 - 1 1 -	2 1 1 2 -
Dx 3	- P - - -	- - - - P	- - - - -	P - - P -	- - P - -	- - - - p	- - - - -	- - - - -

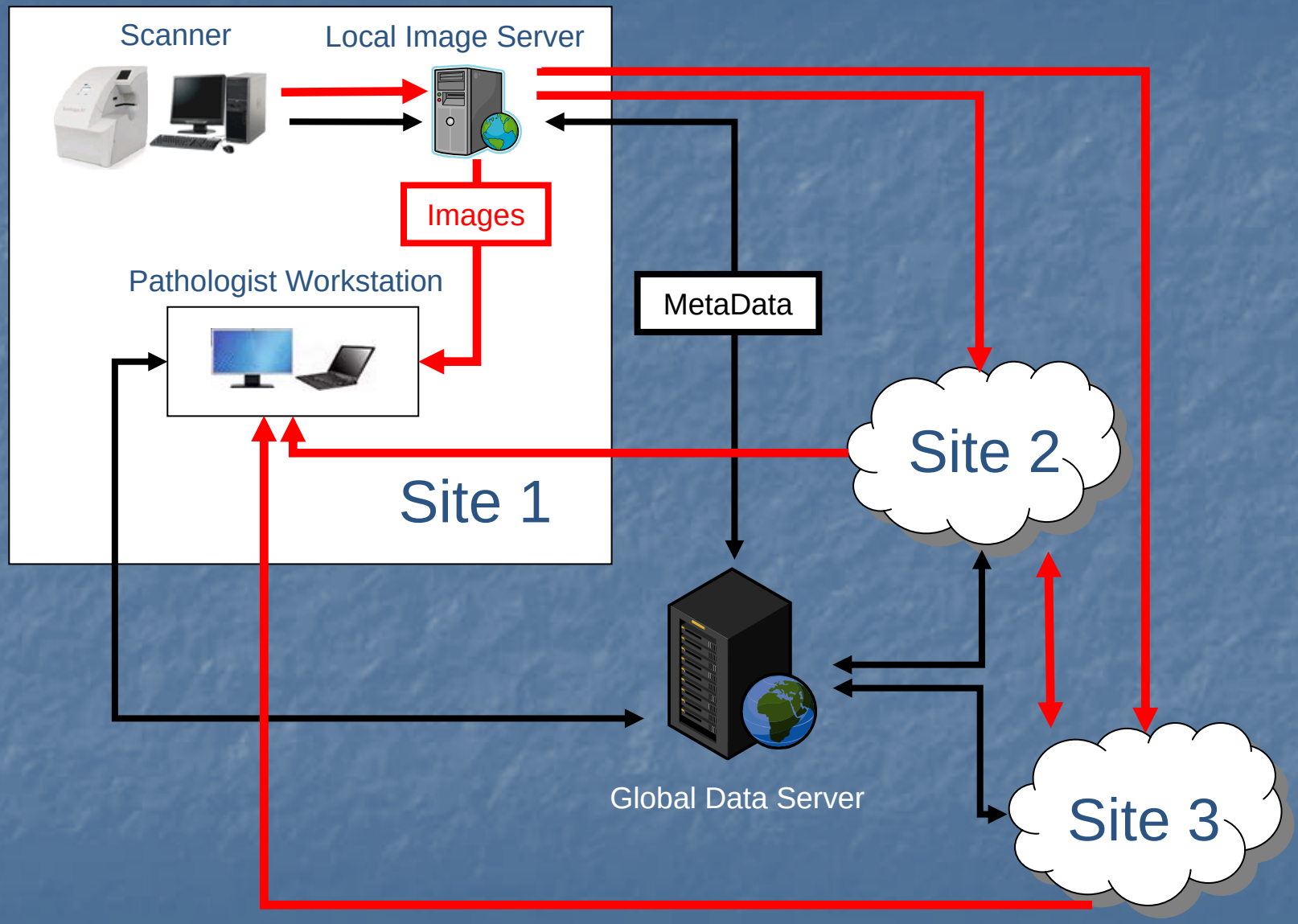
Use of Digital Histological Tissue Images-Why?

- Worldwide shortage of qualified, experienced veterinary pathologists
- Efficient use of pathologist resource
  - Global (remote) access for training, consultation, study evaluation, peer review-without microscope
  - Reduce report cycle time
  - Interactions with CRO partner
- Glass slides not distributed (less chance of breakage/loss)
- Digital image not so susceptible to quality deterioration
- Organ changes and size can be compared (tiling) & measured easily
- Digital images are ideal medium for computer analysis
- Digital image database for historical controls, unusual lesions & test article-related changes

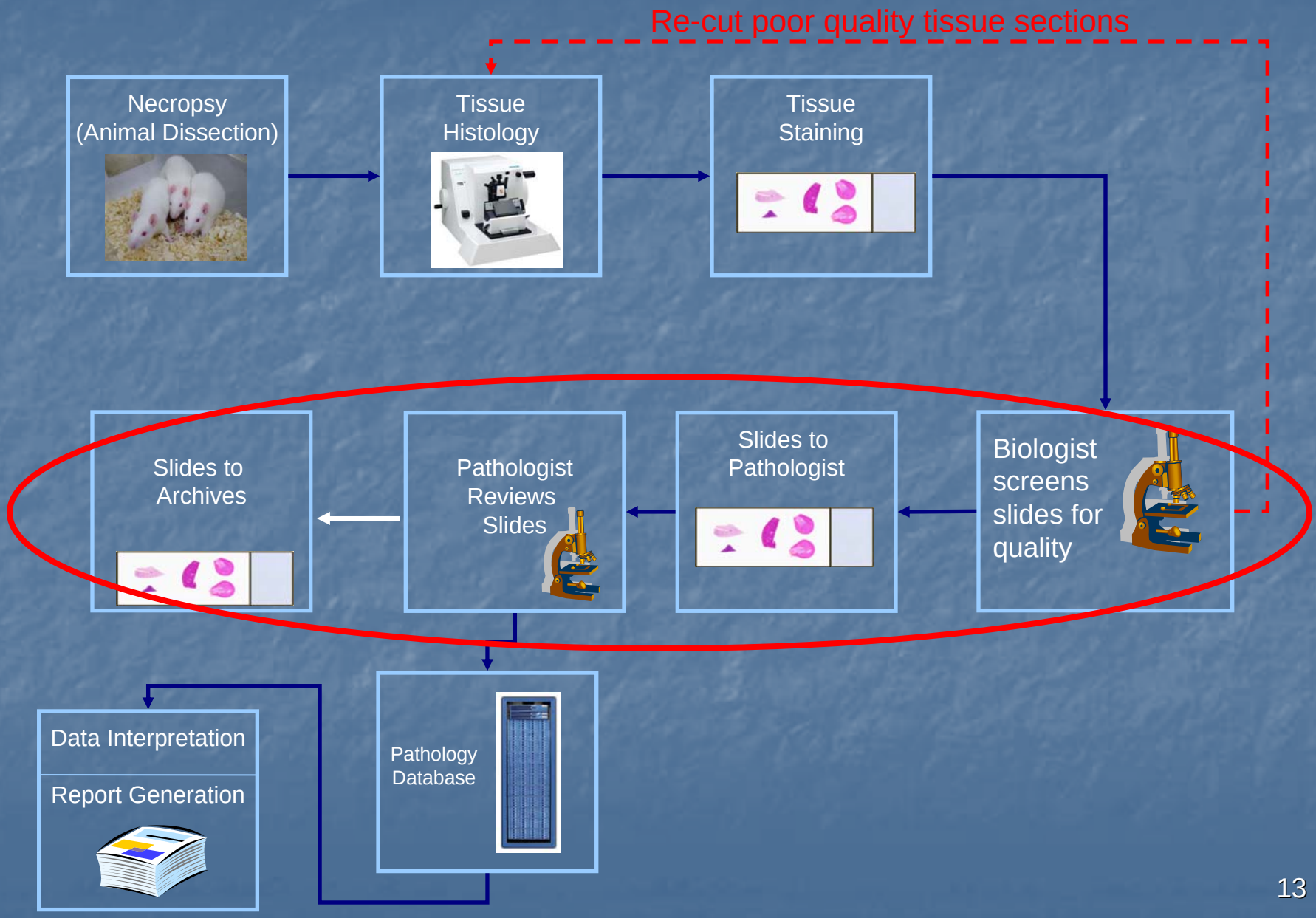
Use of Digital Histological Tissue Images-How?

- Infrastructure/System Architecture
- Discovery/Basic Research
- Pre-Clinical Development
  - Work Flow
  - Consultation/educational
  - Global Interesting Slide Seminar
  - Selective Organ Comparison (Tiling)
  - Study Evaluation
    - User Acceptance Test
    - Computer analysis
      - Investigative
      - Computer Assisted Pathology

System Architecture



Current Pathology Work Flow



Work Flow Using Digital Images

Re-cut poor quality tissue sections

Necropsy (Animal Dissection)

Tissue Histology

Tissue Staining

Digital Scanner

Slides to Archives

Pathologist accesses images

Pathologist trains system

Global Image Database System

Pathologist Reviews Non-Normal Tissues

Non-Normal Tissues

"Heatmap"

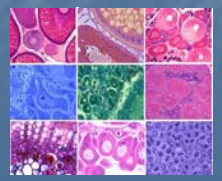
Image Quality Check

Data Interpretation

Report Generation

Pathology Database

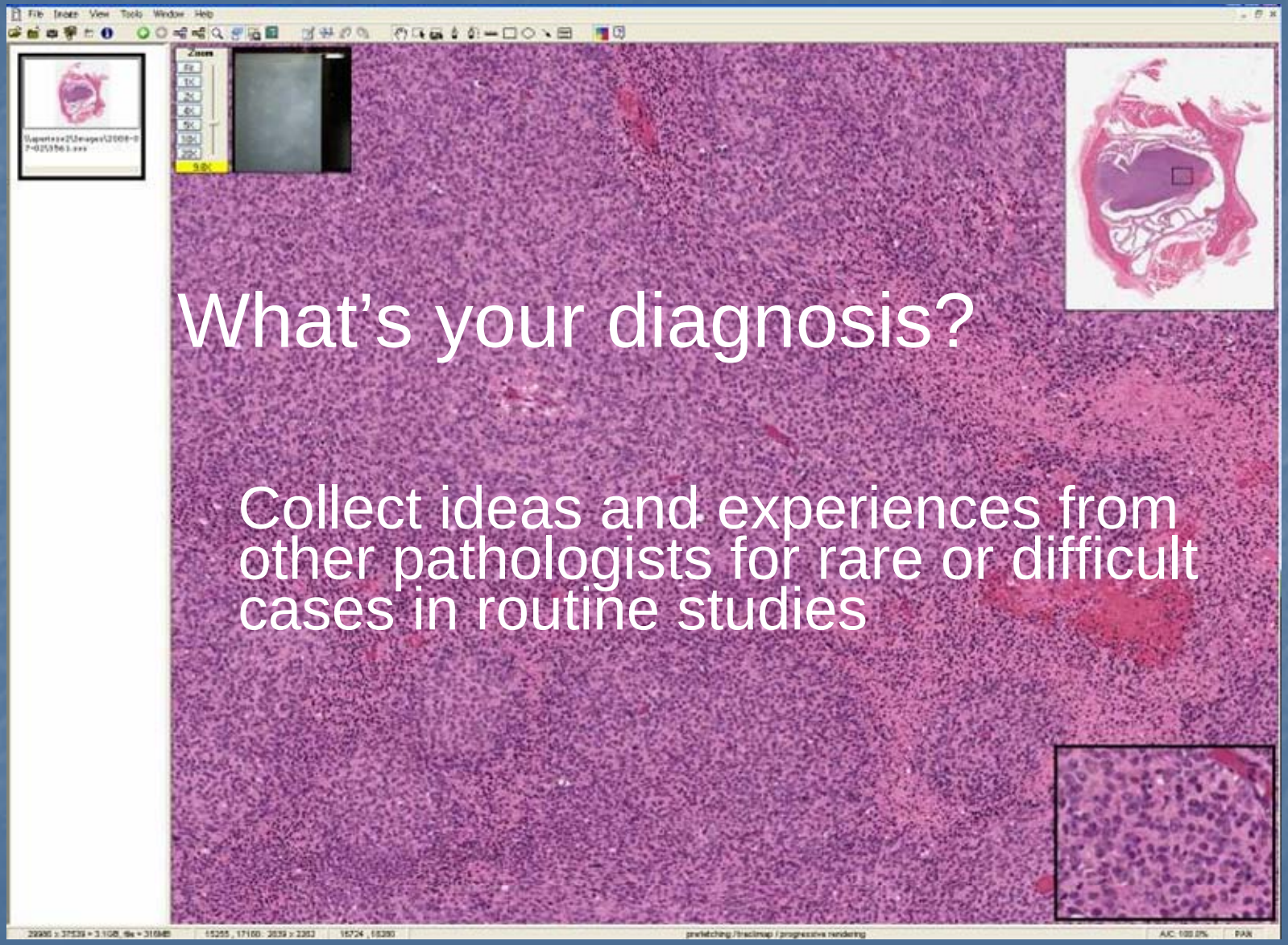
Normal



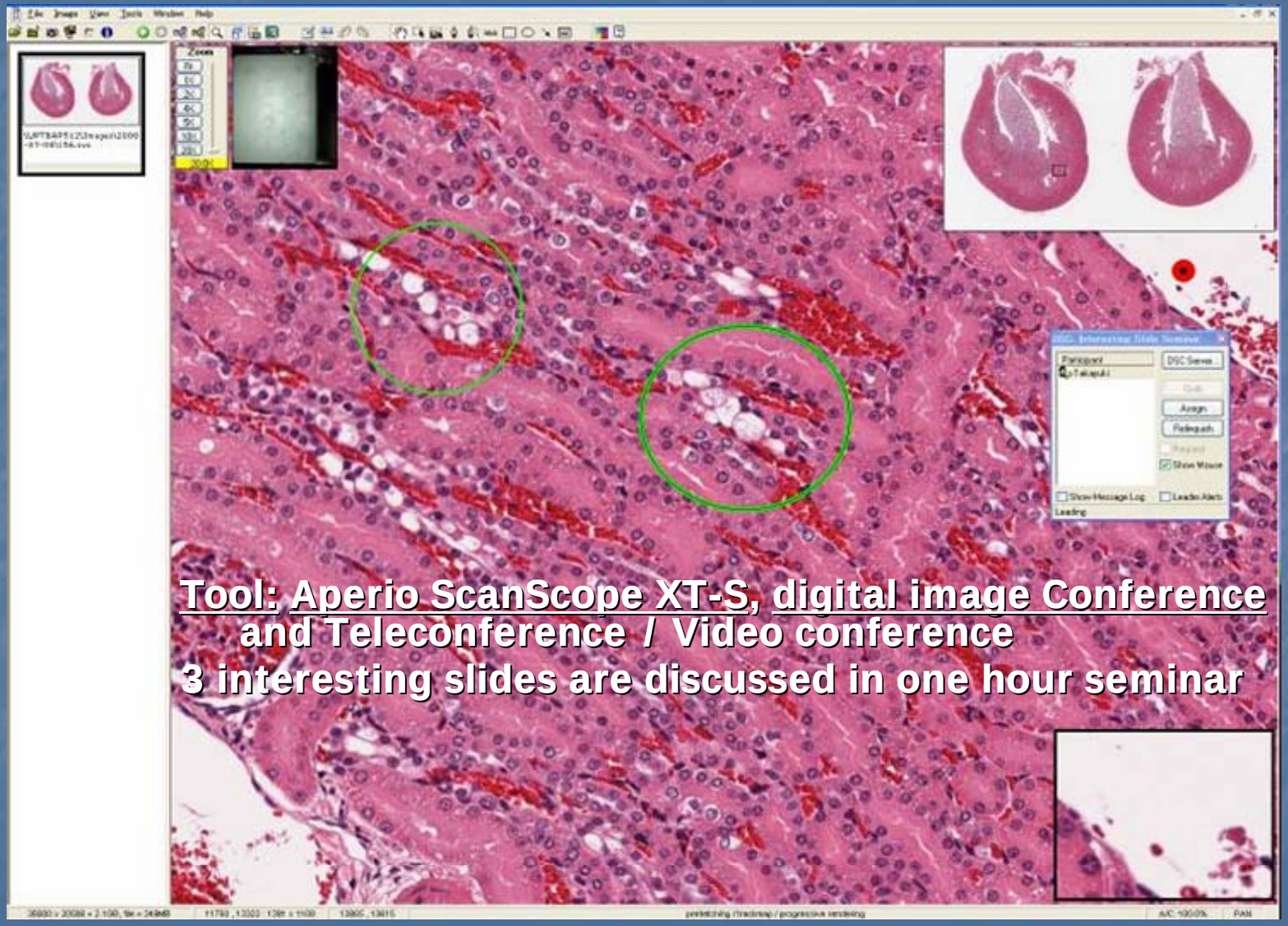
Consultative Use

- Standardize Terminology and Severity Grade of Findings Among Studies
  - Important for
    - Comprehensive pathology data package for submission of compounds
    - Building a robust historical data
- Education/Training
  - Expand pathologist's experience
  - Utilize digital images as atlas of toxicologic pathology

Consultation



Global Interesting Slide Seminar



Selective Organ Comparison (Tiling)

- Control vs. Treated Tissue Morphology
  - Liver
- Control vs. Treated Organ/Tissue Size
  - Spleen
  - Ovary
  - Uterus
- Macro View

Image Tiling: Liver

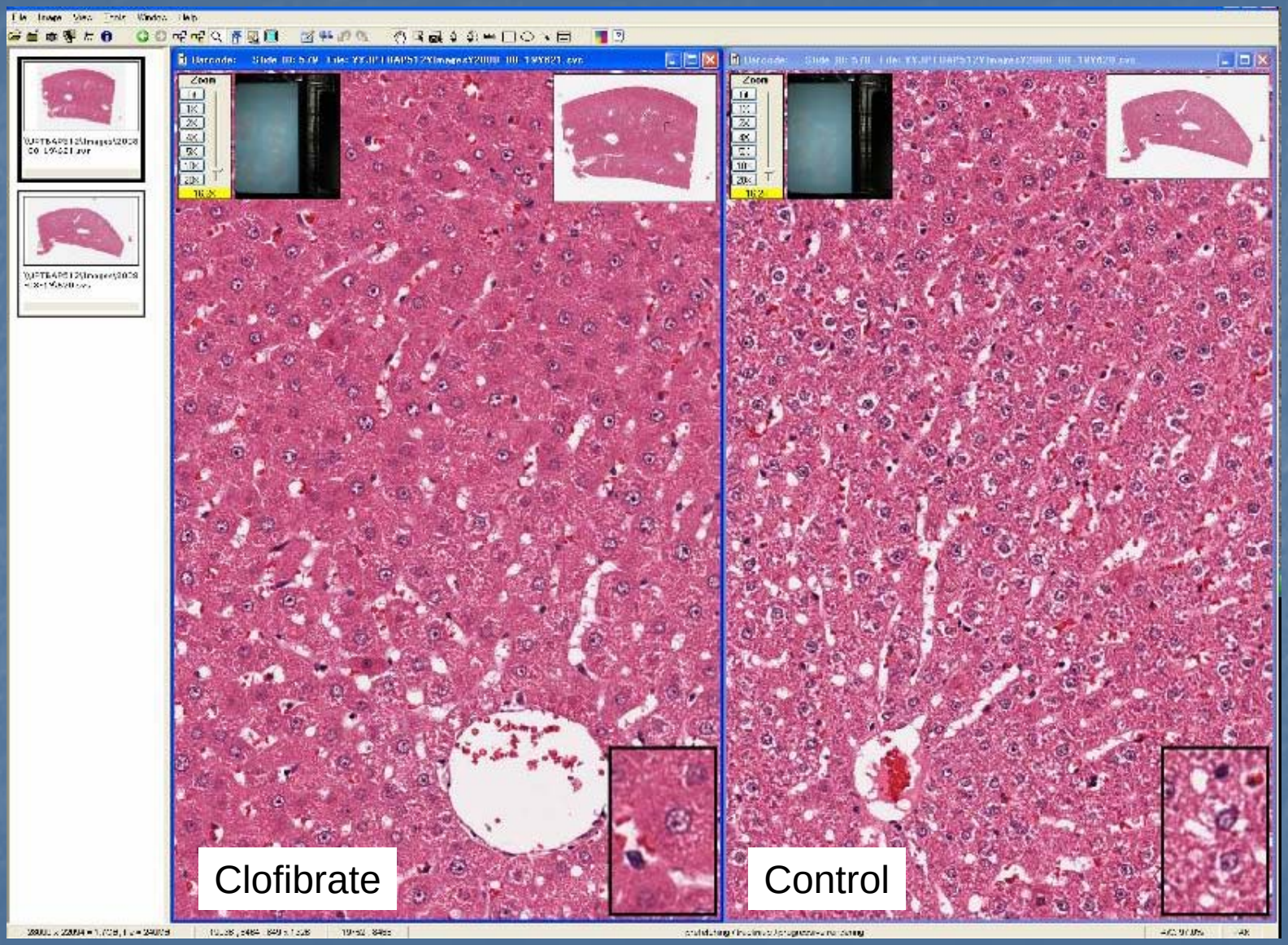


Image Tiling: Spleen

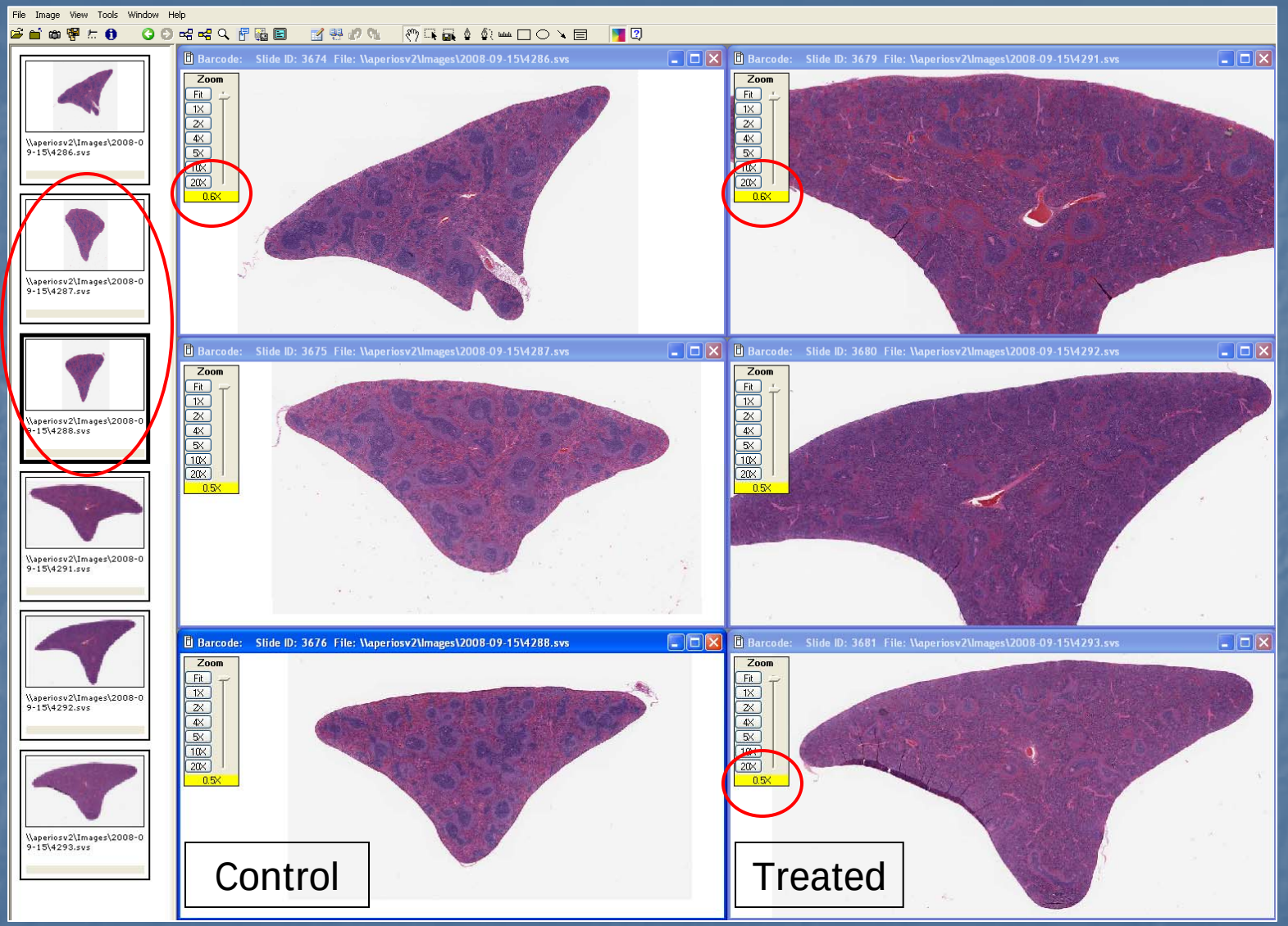


Image Tiling: Spleen

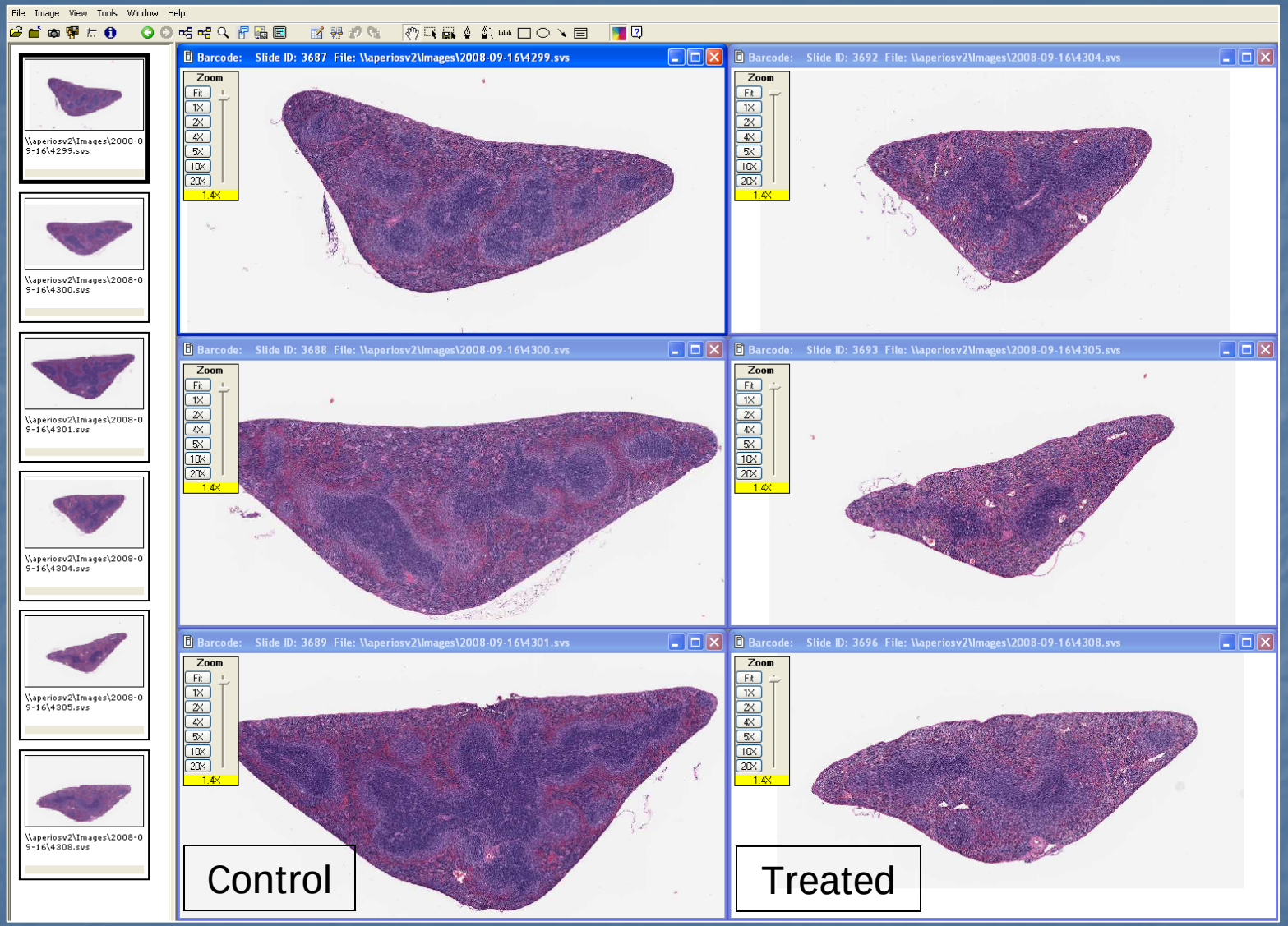
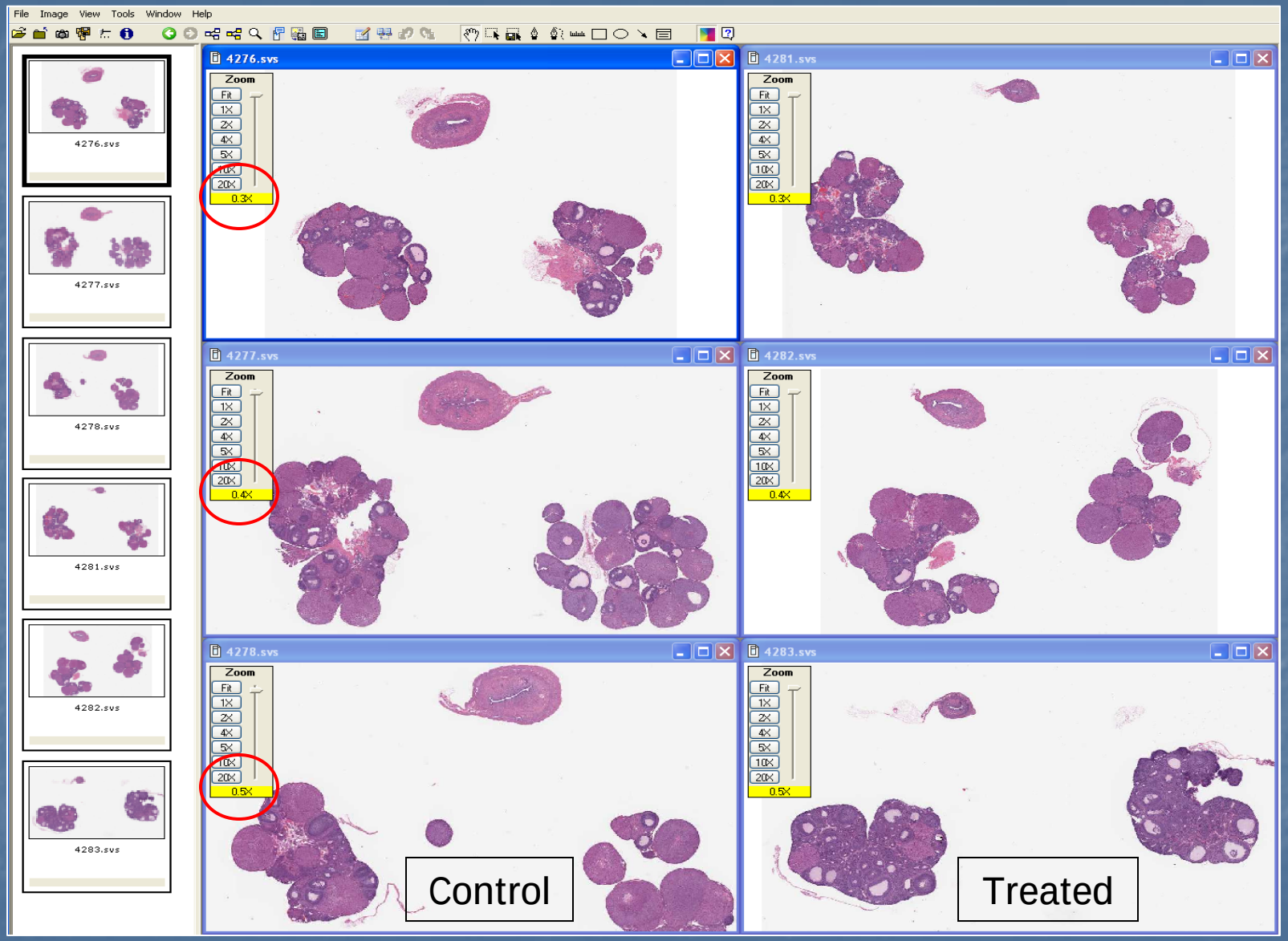
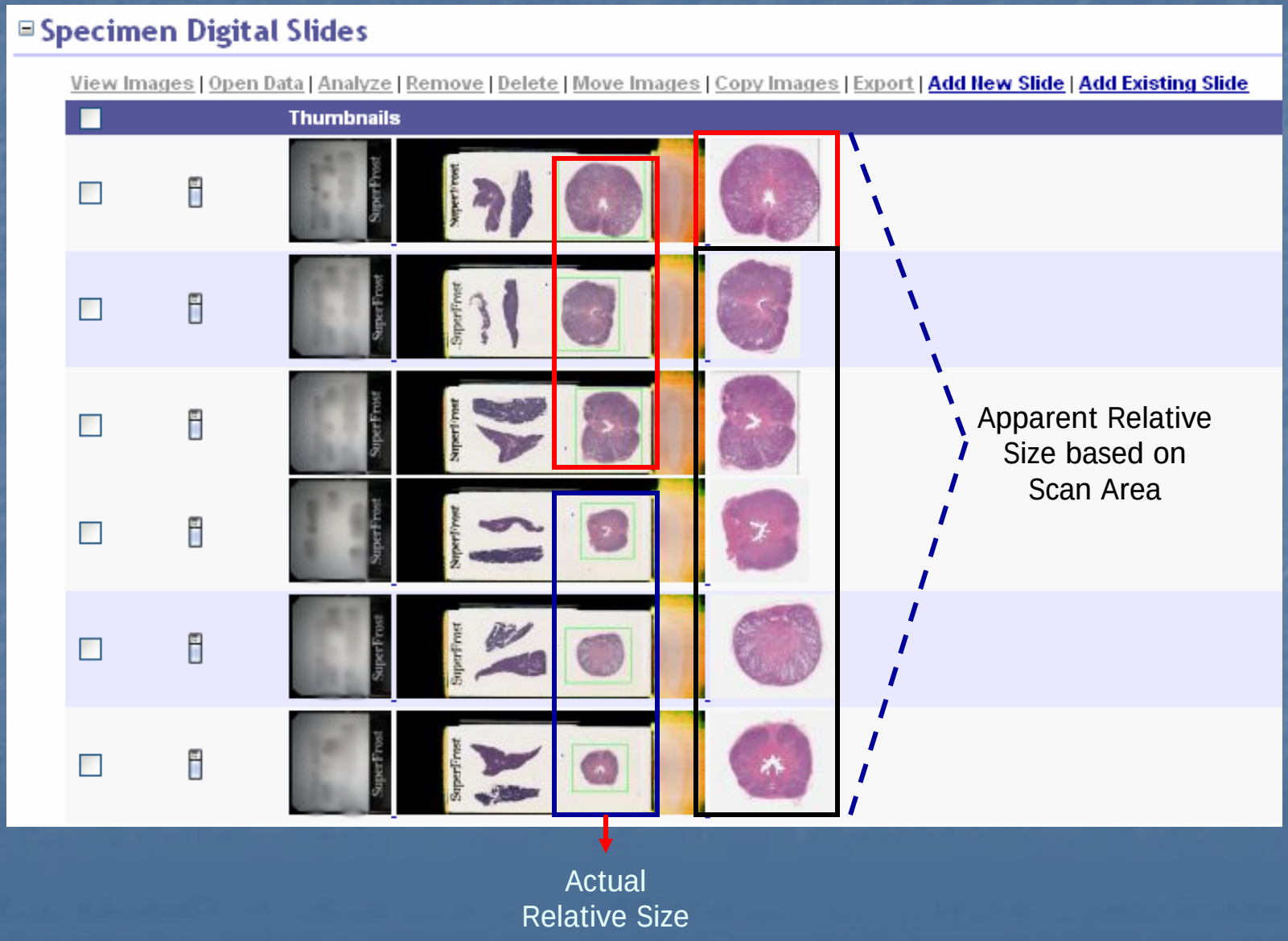


Image Tiling: Uterus/Ovary



Macro View: Dog Prostate



Digital Images for Study Evaluation User Acceptance Test

- Study pathologist conducts evaluation using glass slides/microscope
- Study pathologist confirms findings using digital images
- Group of reviewing pathologists evaluate digital images w/o knowledge of findings (“blinded read”)
- Study and reviewing pathologists complete questionnaire
  - Compare results between glass slide/microscope and digital image evaluation
  - Ergonomic feedback-image management, quality, etc.

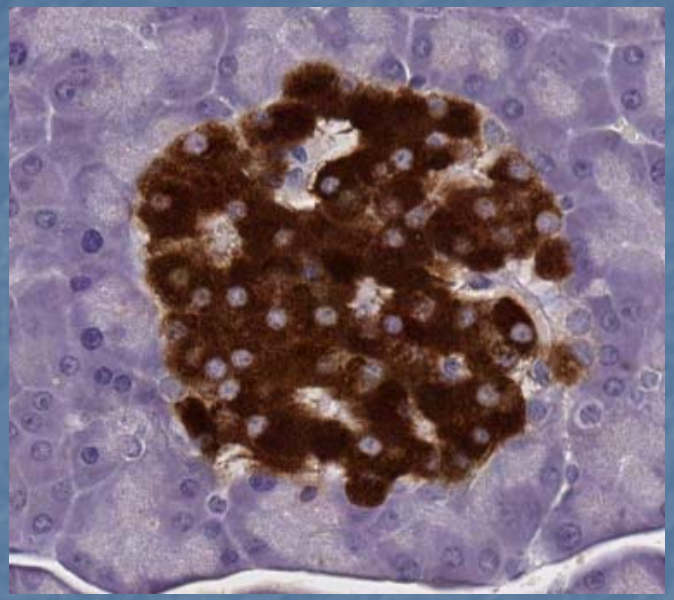
Computer Analysis of Digital Images

- Investigative Studies
  - Pancreatic Islet Area
  - Aortic Root Analysis
- Computer Assisted Pathology
  - Histology Quality
  - Histopathology

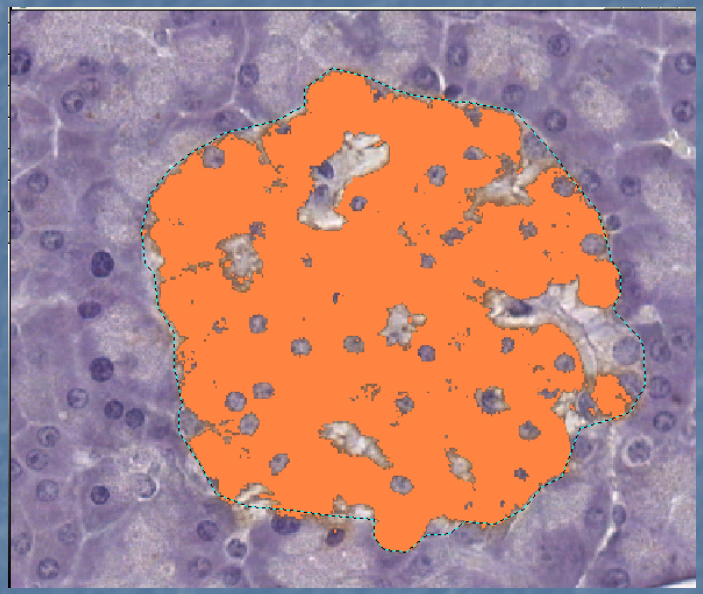
Percent Area of Islets in Rat Pancreas Containing Insulin

- IHC staining of rat pancreas with monoclonal antibody to insulin
- Area of insulin containing cells in pancreatic islets was determined manually on glass slides using a grid system.
- Digital images of the stained pancreas were prepared and analyzed with analytical software.
- Islets were segmented manually and auto-thresholding was used when appropriate (semi-automated).
- The analytical software results were within 10% of the manual counts for % insulin area.

Quantitation of Insulin Producing Area



Digital Image

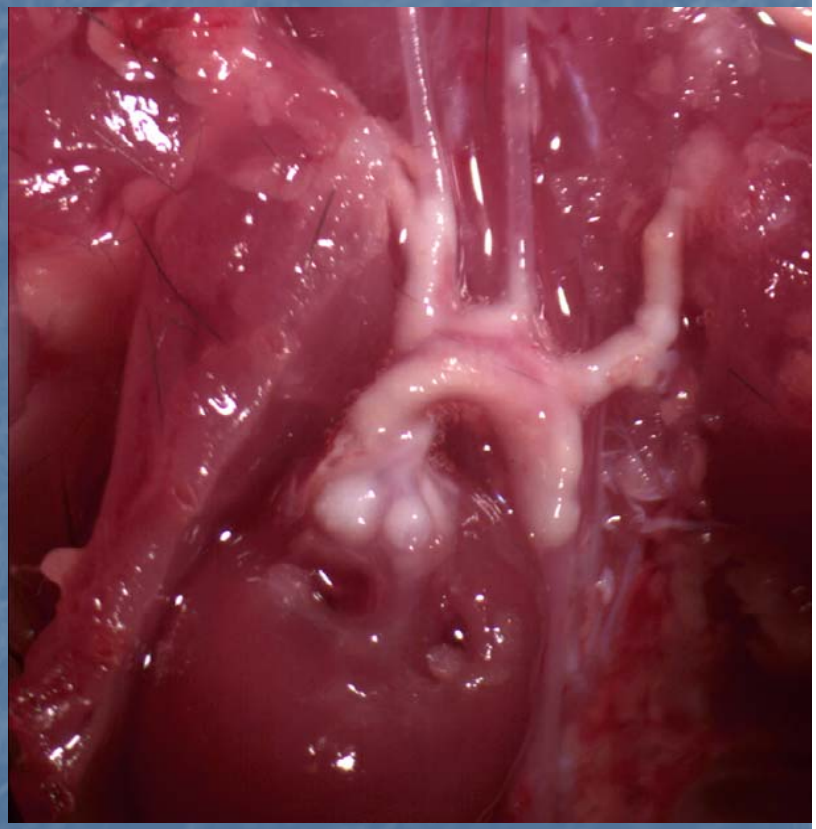


Analytical Thresholded Image

Total # of islets: 32
Mean islet area (microns^2): Manual (6692) vs Software (7974)
Insulin positive area (microns^2): Manual (4937) vs Software (5499)
% Insulin area: Manual (73%) vs Software (69%) 94% of manual method
Amount of time to perform analysis was reduced by 33%

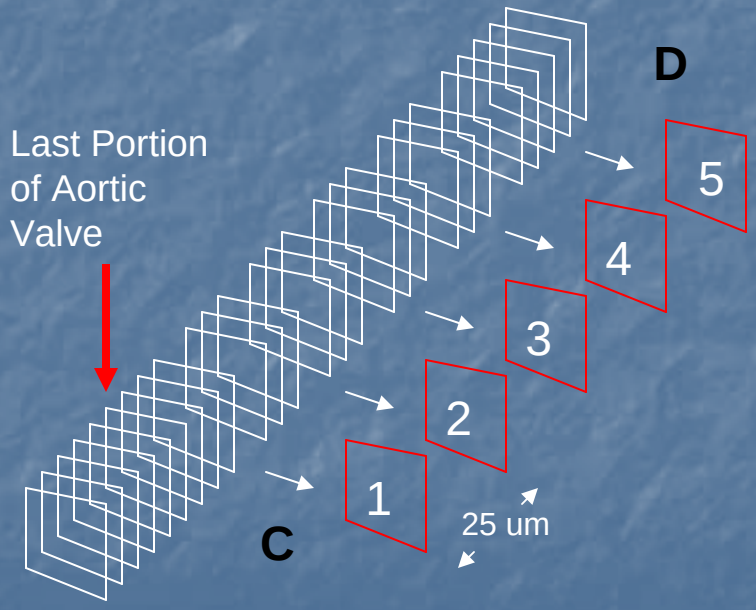
Aortic Root Analysis: Atherosclerosis in Mice

Heart and Aorta Removed



Gross Appearance

Aortic Root Analysis : Microtomy Method



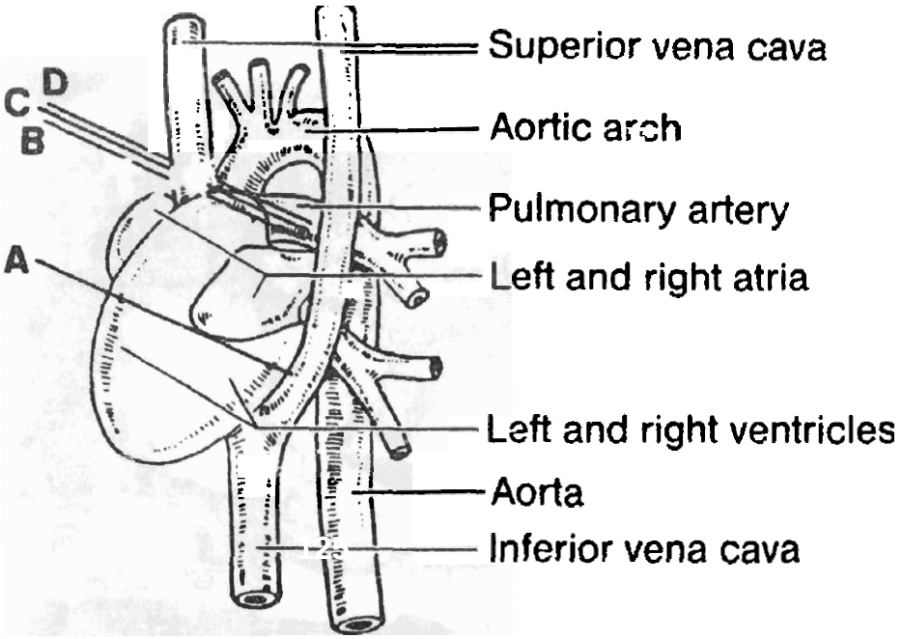
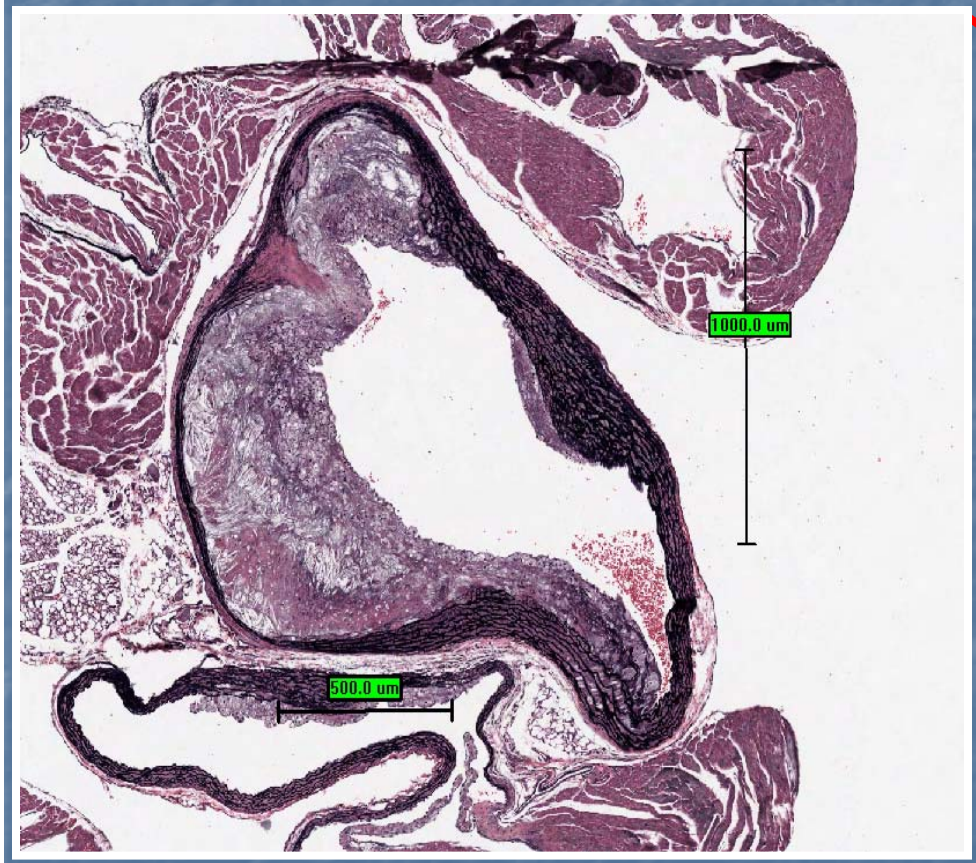


Fig. 1. Anatomy of a mouse heart and aorta. The area evaluated is an approximately 125 μm section between C and D. The figure is redrawn from *Biology of the Laboratory Mouse*, E. Green (ed.), 1966, with permission.

Aortic Root Analysis : Microtomy Method



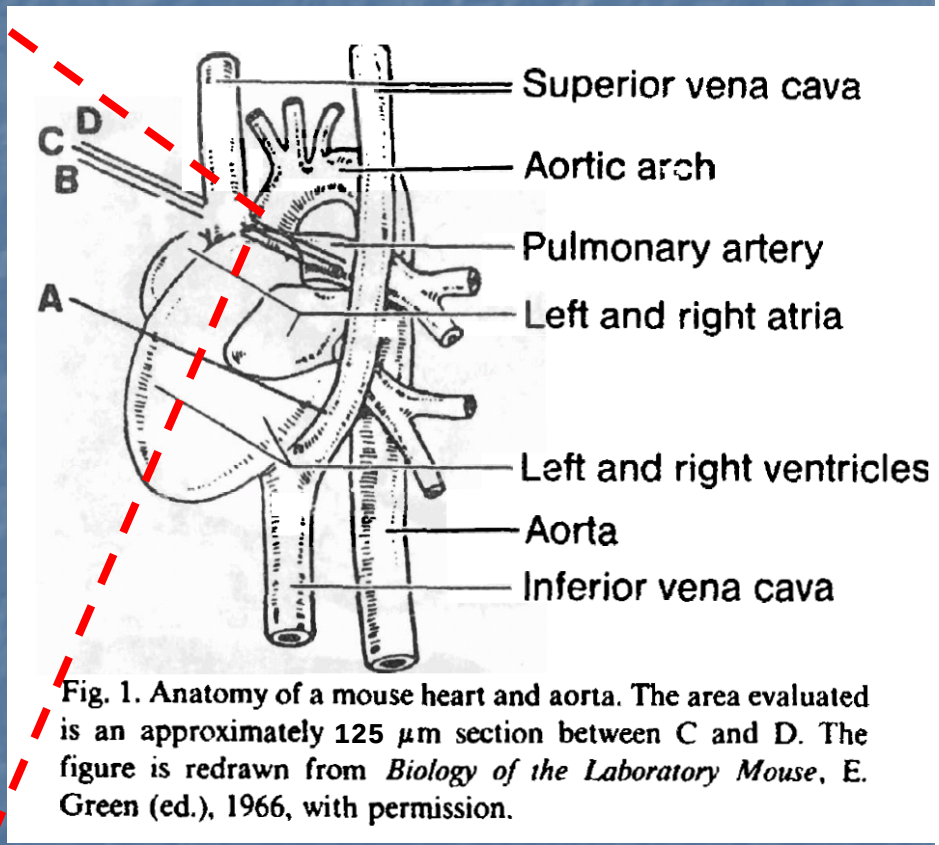
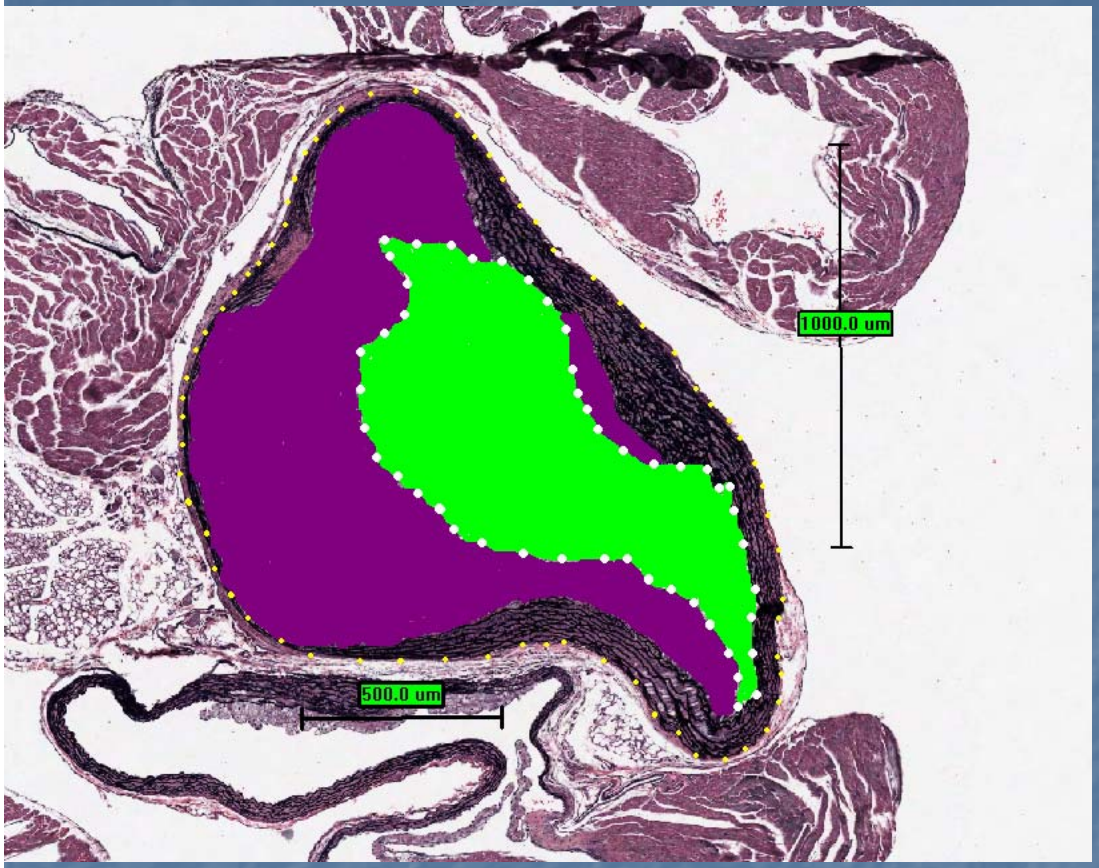
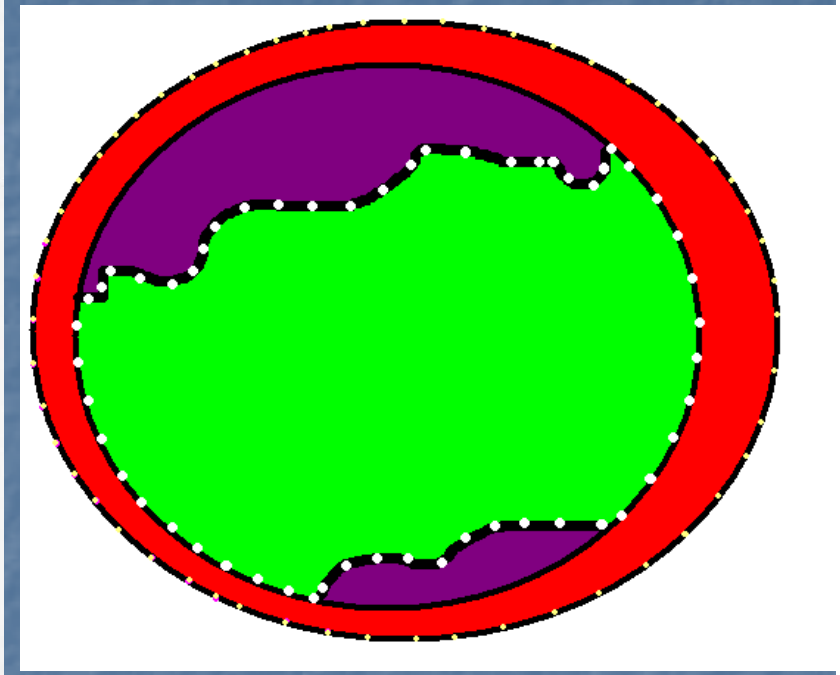


Fig. 1. Anatomy of a mouse heart and aorta. The area evaluated is an approximately 125 μm section between C and D. The figure is redrawn from *Biology of the Laboratory Mouse*, E. Green (ed.), 1966, with permission.

Atheromatous Change: Elastin staining, slide selection, scanning. Images saved at 3X as TIFF files with addition of 500 and 1000 micron standardization bars.

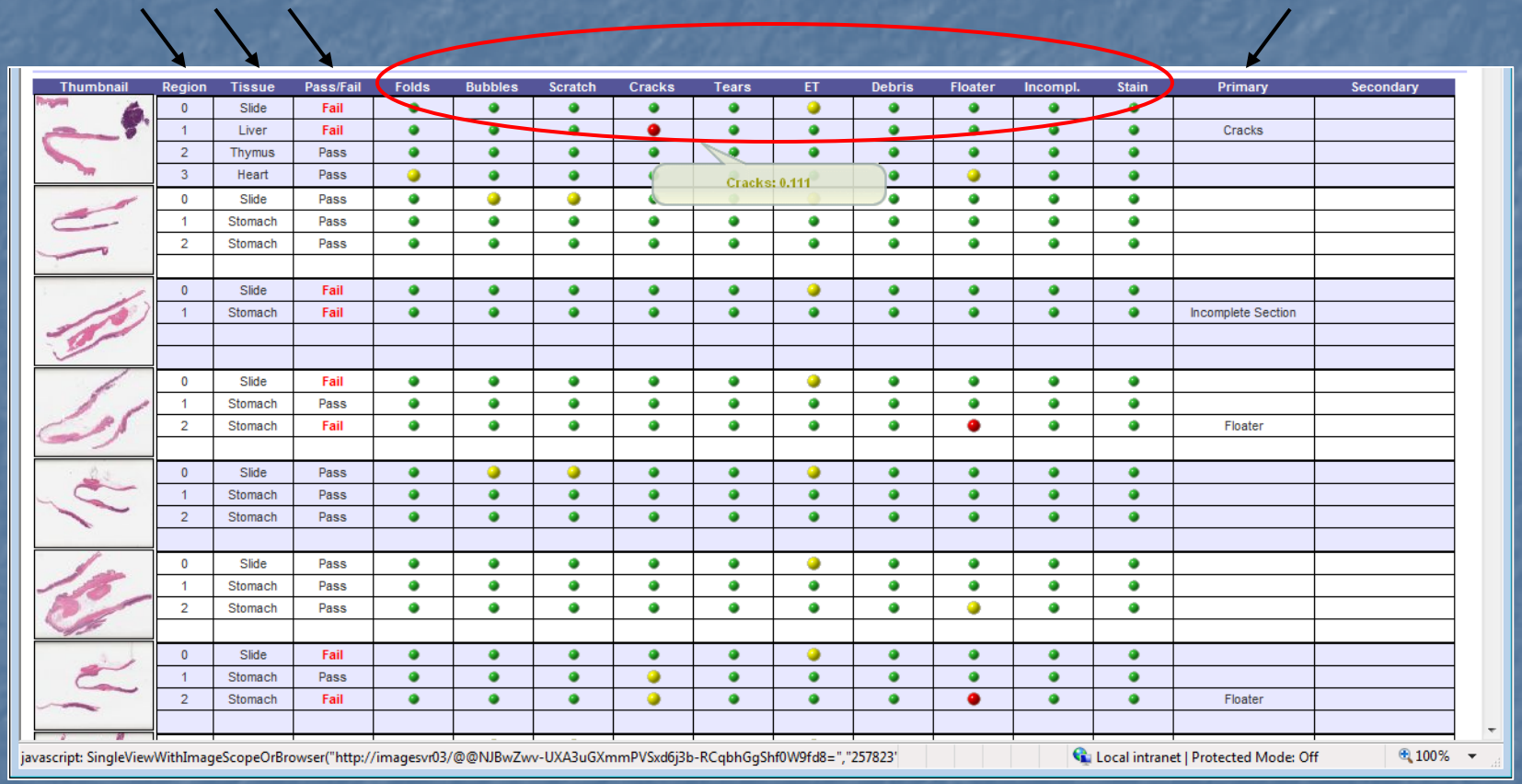
Image Analysis



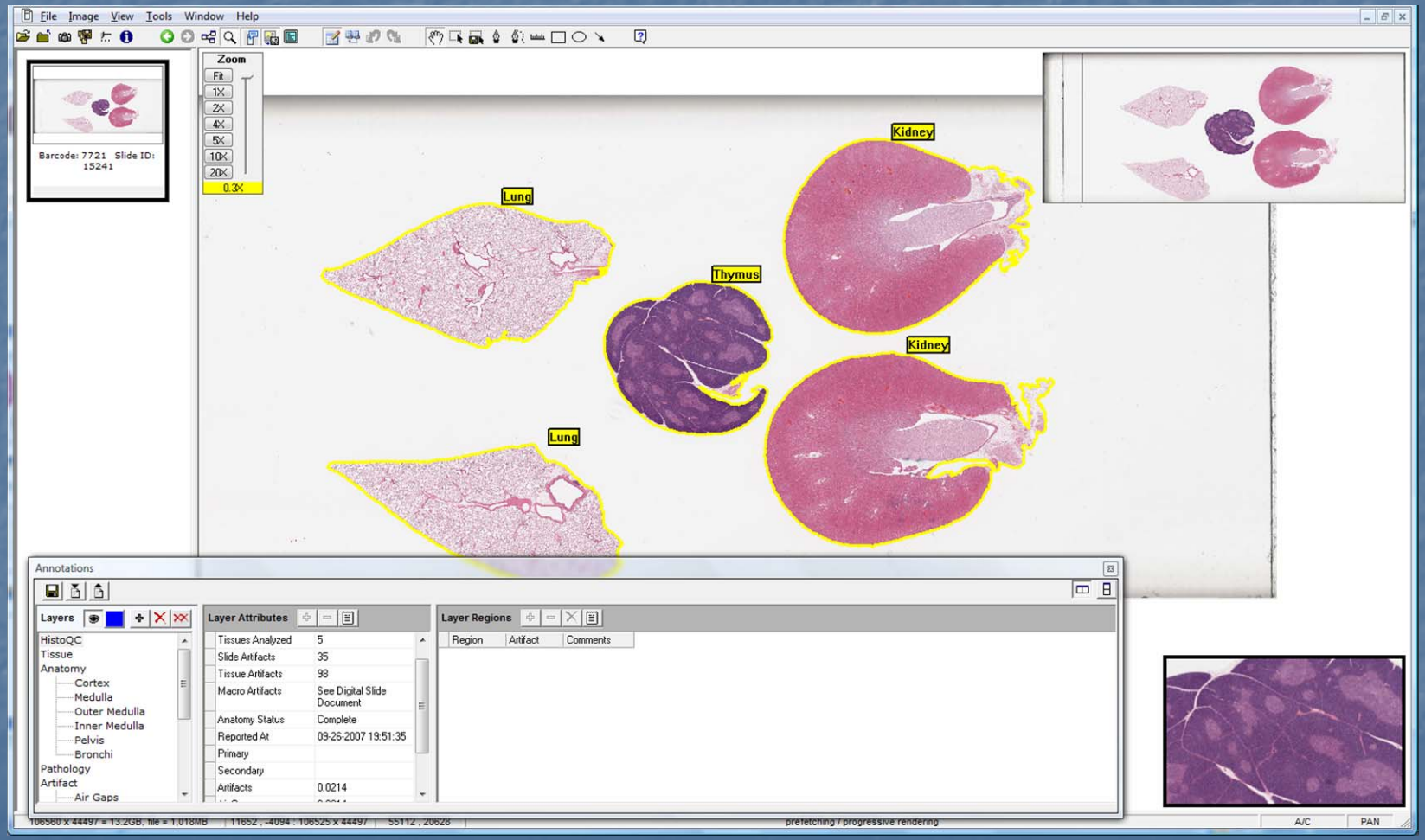


- **Purple**: Plaque area
- **Red**: Aortic wall area (Black elastin staining in the section)
- **Green**: Aortic lumen
- Plaque area/Plaque area + Vessel Wall Area normalizes for vessel size variation.

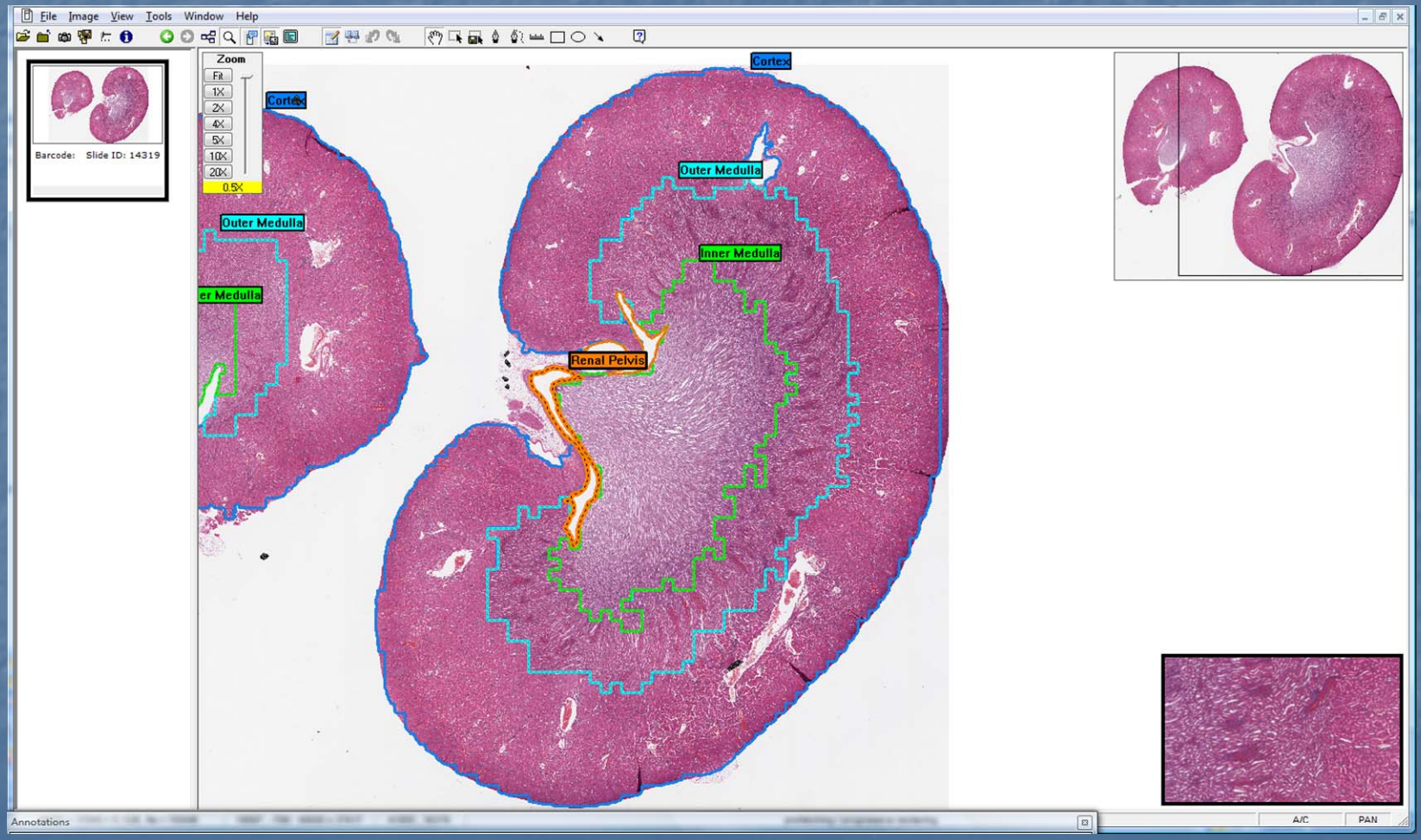
Computer Assisted Pathology Histology Heatmap



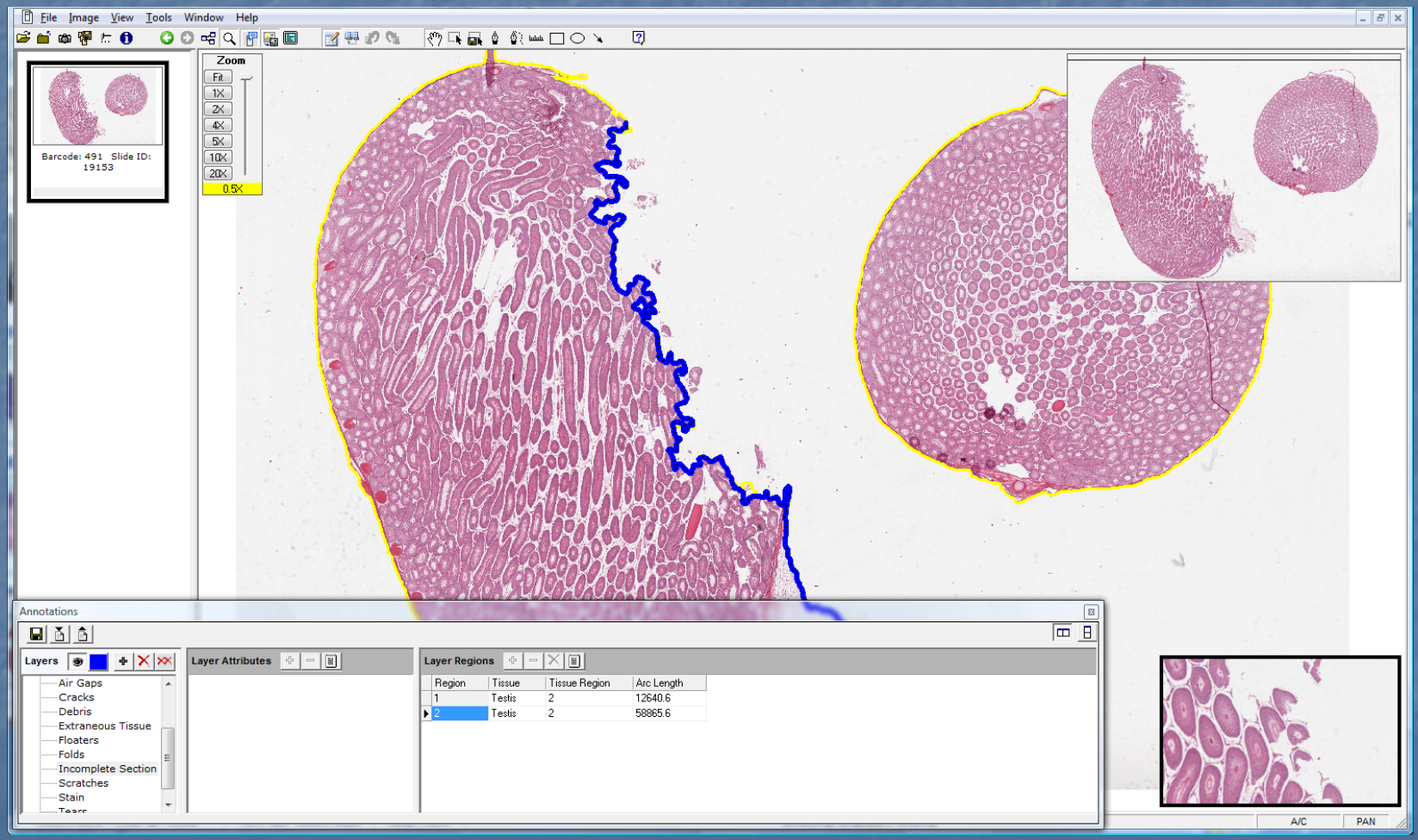
Tissue Recognition



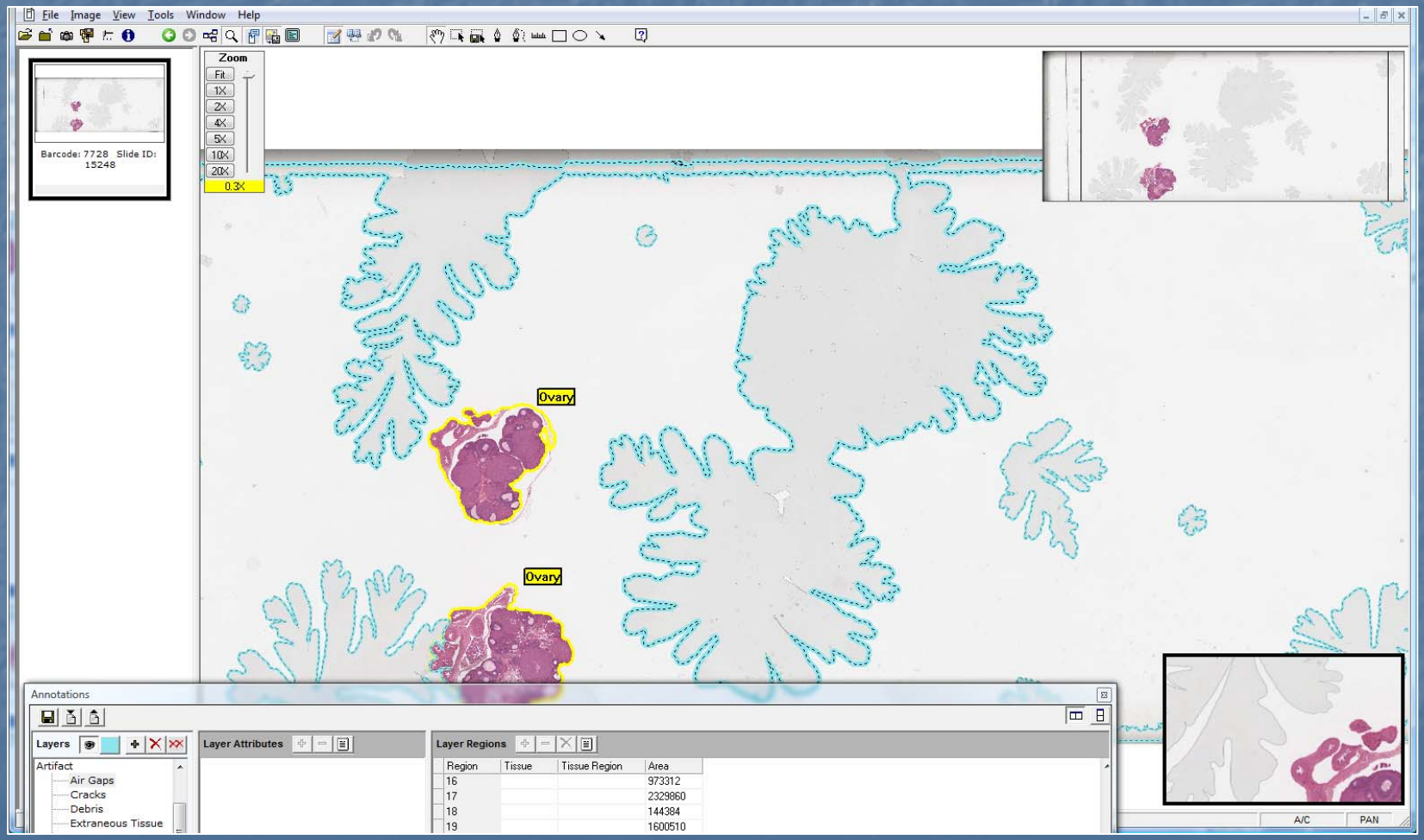
Anatomy Verification



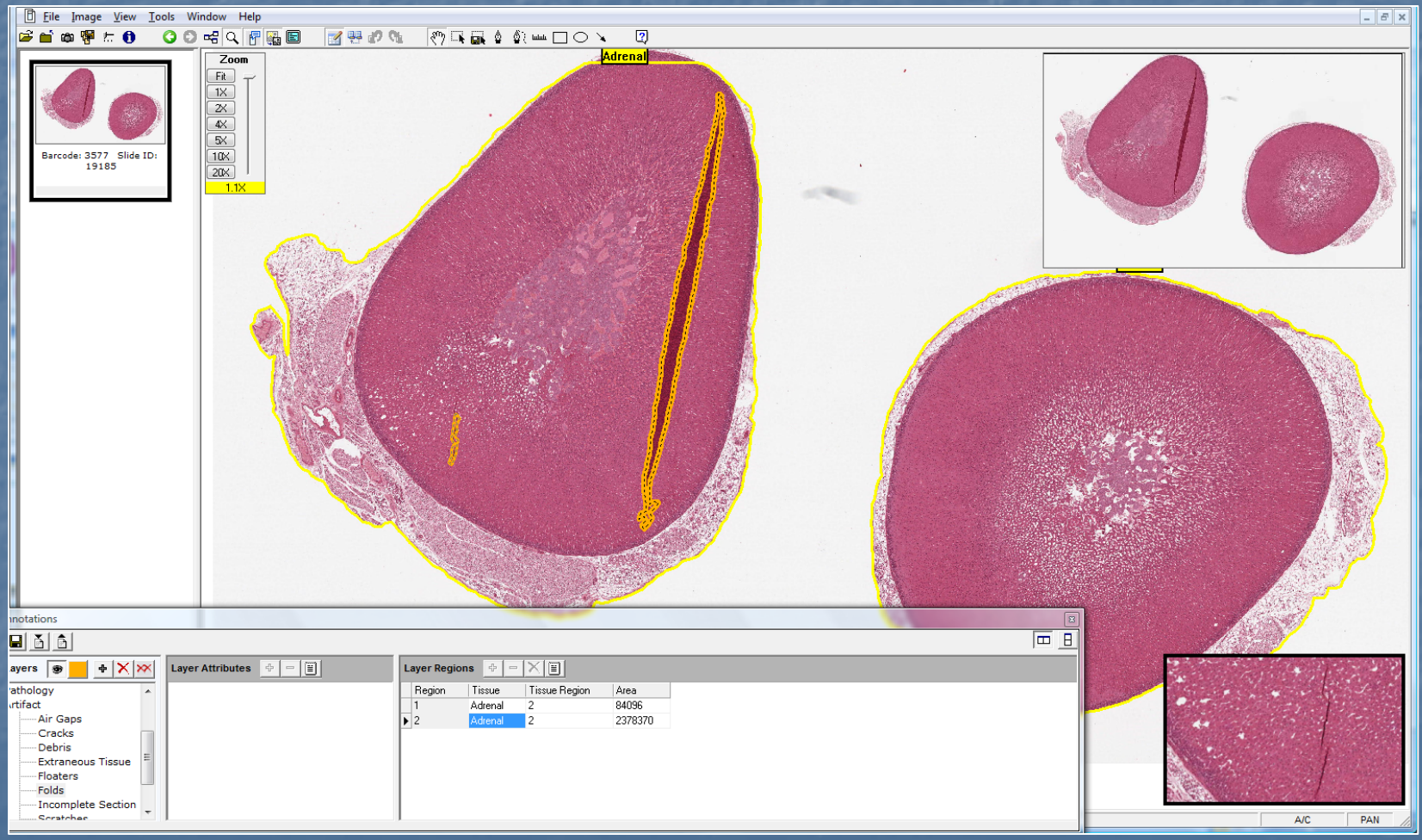
Incomplete Section-Tears



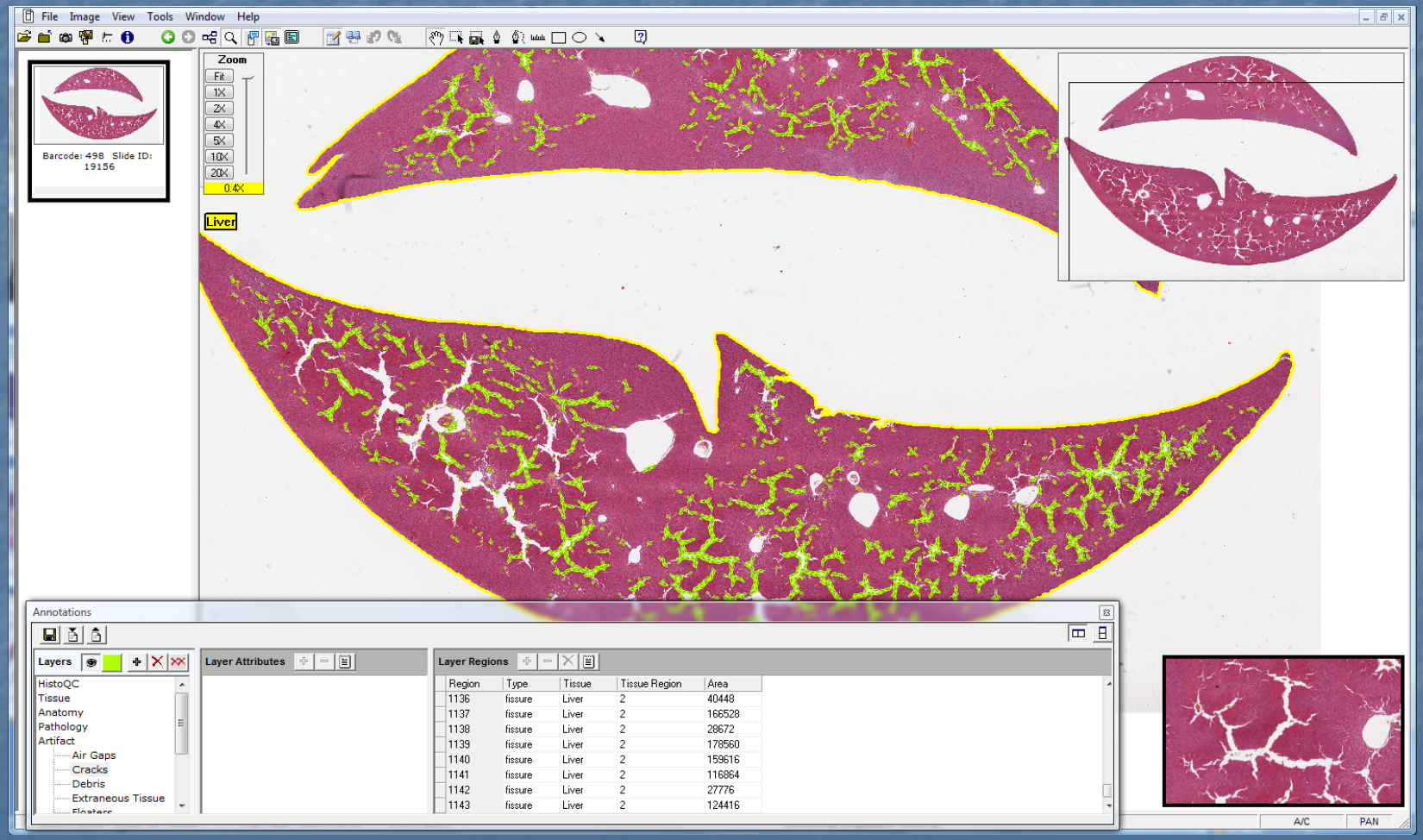
Air Gaps & Bubbles



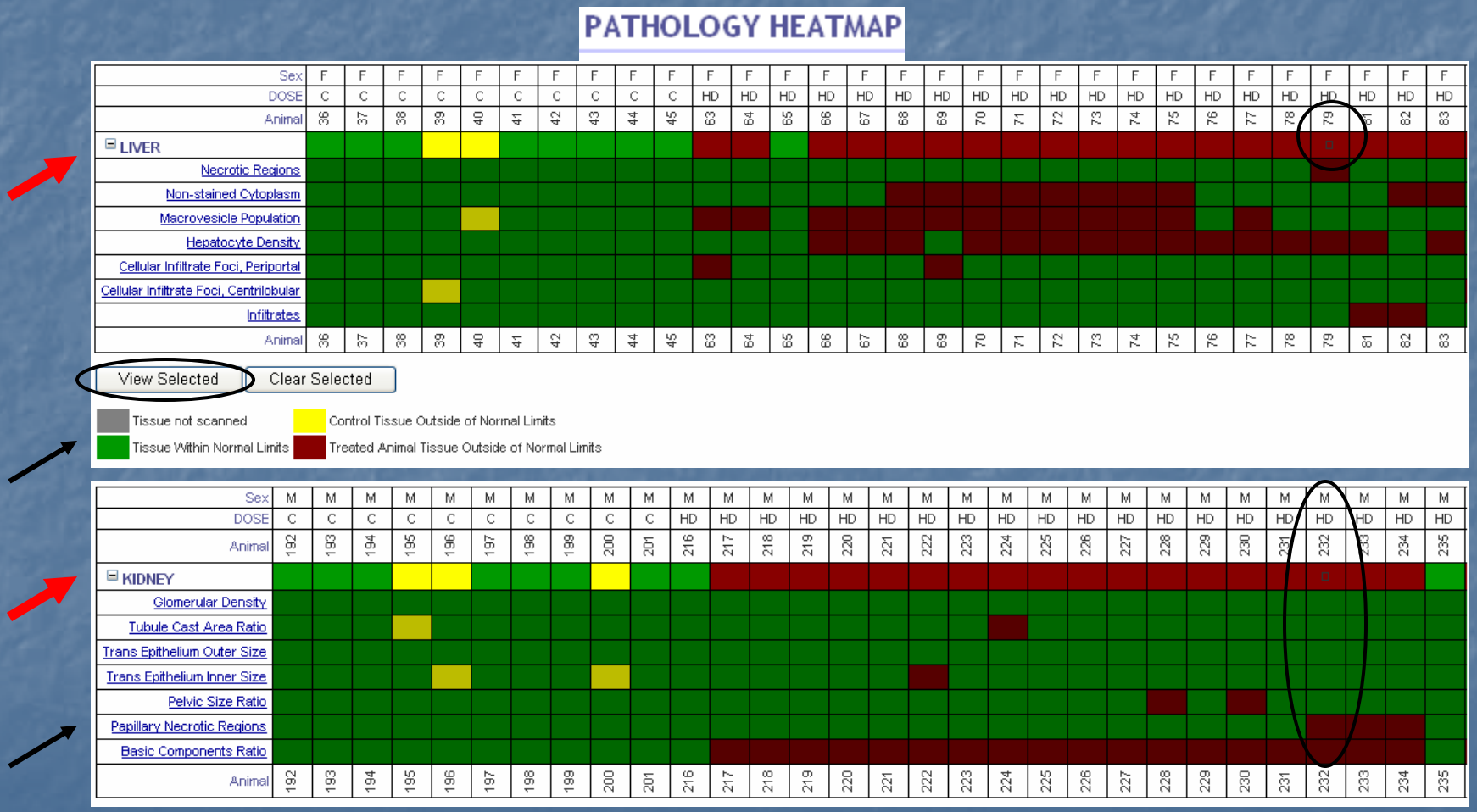
Tissue Folds



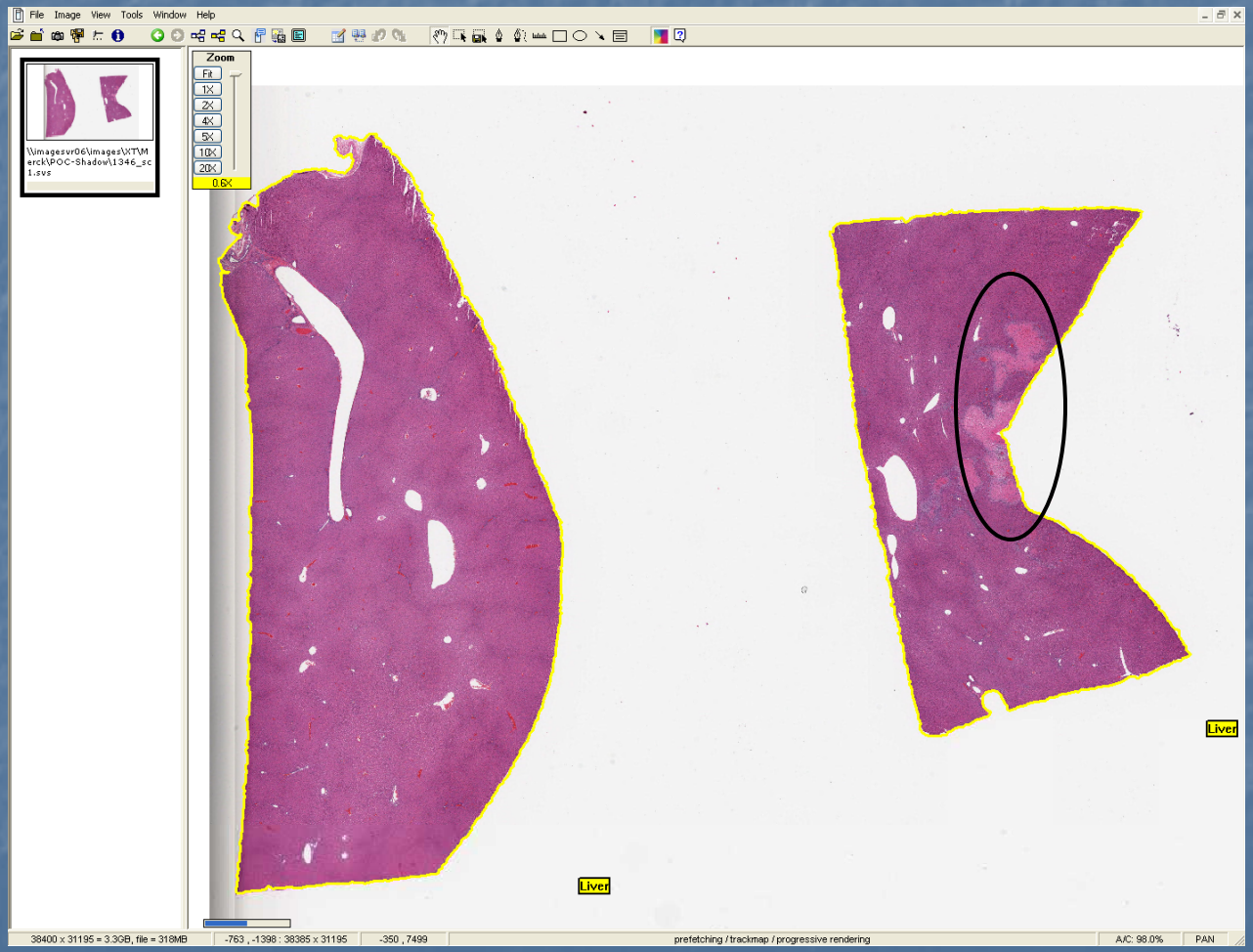
Tissue Cracks



Computer Assisted Pathology

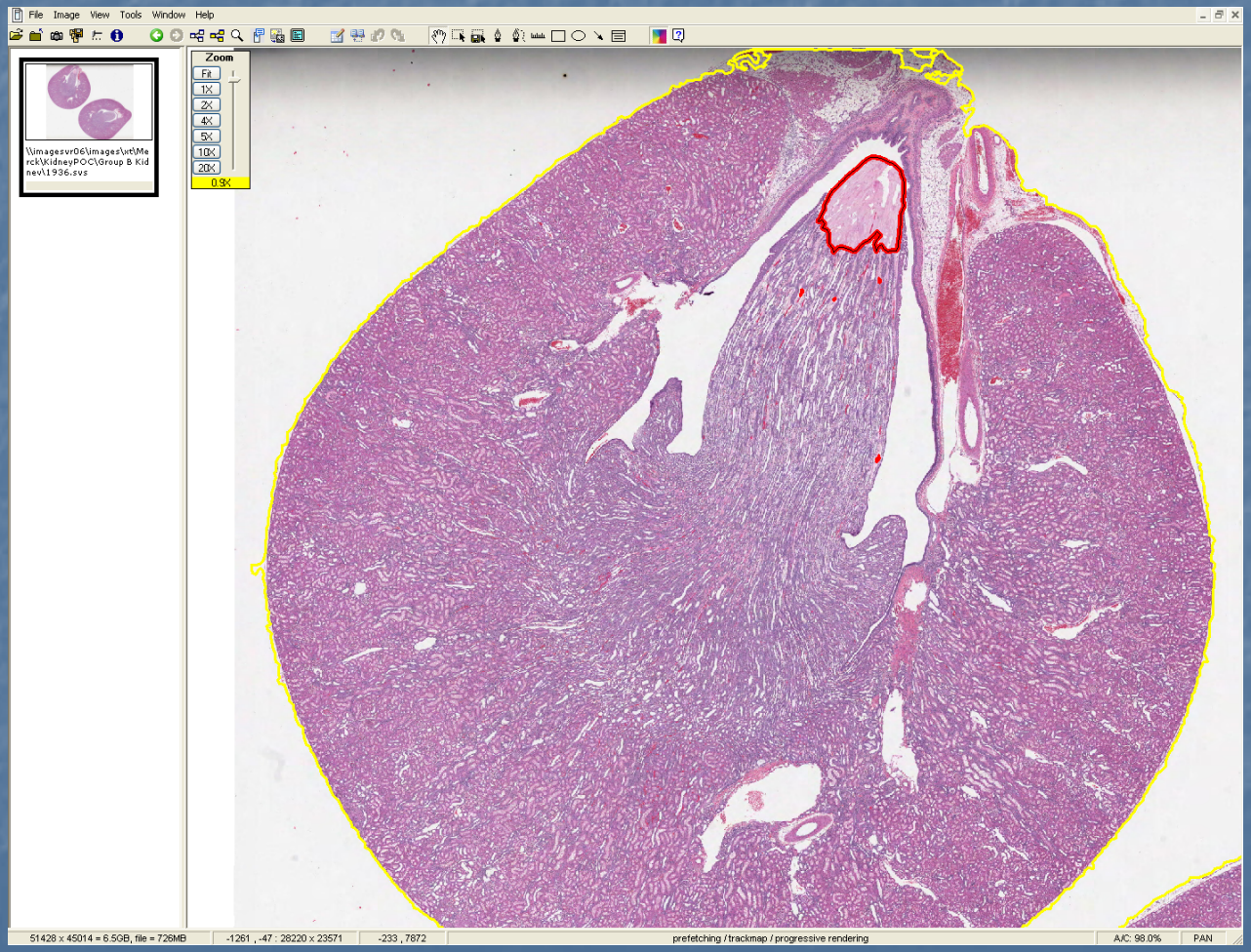


Liver Image


File Image View Tools Window Help
Zoom
Fit
1X
2X
4X
5X
10X
20X
0.6X
\\imagesvr06\images\XT\Merck\POC-Shadow\1346_sc1.svs
Liver
Liver
38400 x 31195 = 3.3GB, file = 318MB
-763 , -1398 : 38385 x 31195
-350 , 7499
prefetching / trackmap / progressive rendering
A/C: 98.0%
PAN

Kidney Feature Chart

Kidney Image



Lessons Learned
IT Related

- Scanner & Server location
- High Speed (1Gbps) Network Connection
- Image Backup & Storage
- Monitor Storage Capacity/utilization
- Security Issues
  - Firewall could prevent remote access
  - Security patches/upgrades may affect system
  - Group assignment for access control
- Workstations
  - Sufficient RAM, hard drive space, network access
  - PC/laptop must be able to support large, high resolution monitor
  - Ergonomic considerations

Lessons Learned
Scanning Related

- Digital Scanner
  - Format & compression quality
  - Manual preview/adjust scan area
  - Determine run time based on production specimens
  - Tissue sections 2-3 mm from slide edge
  - Clean/dry slides
- Digital Slide Data Base
  - Structure of data base fields are work flow dependent
  - Permission architecture is work flow dependent

Lessons Learned
Image Viewing/Evaluation Related

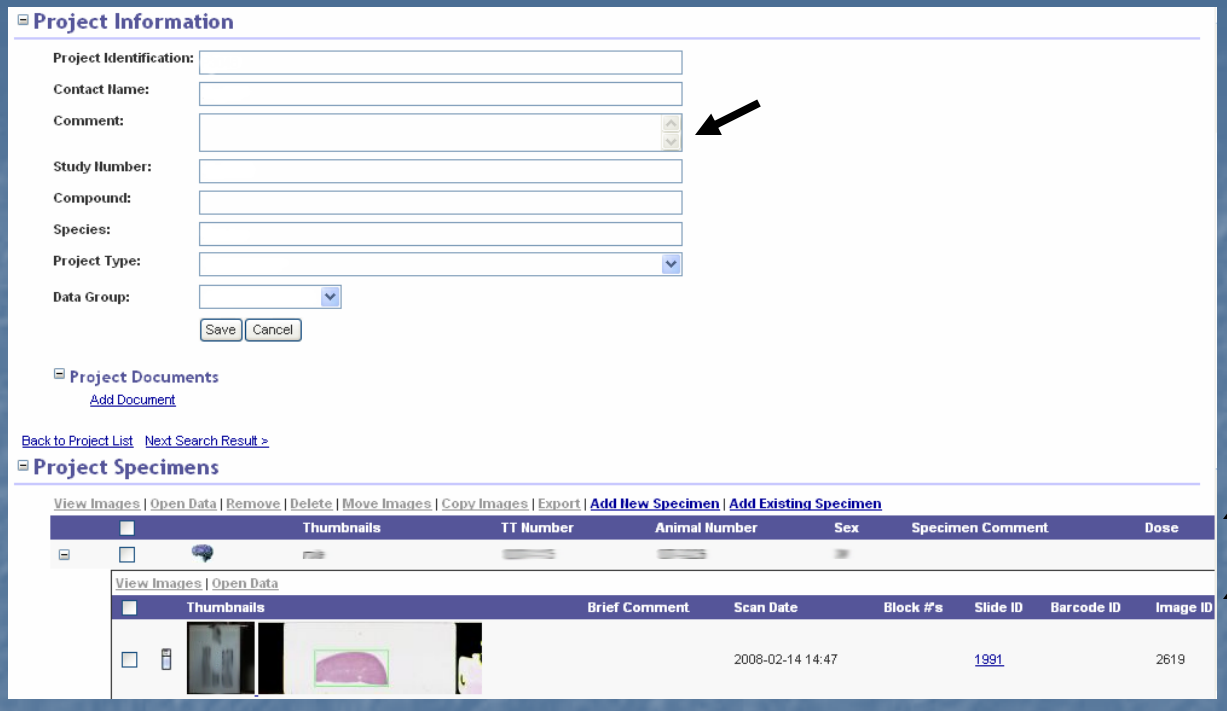
- PC/laptop-addressed under IT related
- Monitor type & configuration
  - High resolution (1920 x 1200, 60 Hz)
  - Sufficient dimensions (24” diagonal)
- Digital Slide Database
  - Efficient image accessibility
  - Efficient loading of images into viewing software
- Image Viewing Software
  - Tiling
  - Adjust to new paradigm (image vs. slide)

Efficiency Needs

- Scan several areas from a single digital slide, reducing scan time and file size. Uniquely associate multiple tissues per slide, and multiple diagnoses per tissue in database, i.e., 1 tissue/scan area.
- Scan the entire 1”x3” slide with uniform quality to capture tissues close to margin’s edge.
- Place all data fields (Project, Specimen, and Digital Slide) on a single page for data entry/review, image selection, and Boolean searches; eliminating the need for multiple check boxes and redundant header links.
- Display user-defined data fields with filmstrip thumbnails. Select filmstrip thumbnails to order, place, size, and tile images in main window. Repeatedly add/subtract images in filmstrip from database.

Multiple Scan Regions

Data Fields


Project Information
Project Identification:
Contact Name:
Comment:
Study Number:
Compound:
Species:
Project Type:
Data Group:
Save
Cancel
Project Documents
Add Document
Back to Project List
Next Search Result >
Project Specimens
View Images | Open Data | Remove | Delete | Move Images | Copy Images | Export | Add New Specimen | Add Existing Specimen
Thumbnails
TT Number
Animal Number
Sex
Specimen Comment
Dose
View Images | Open Data
Thumbnails
Brief Comment
Scan Date
Block #'s
Slide ID
Barcode ID
Image ID
2008-02-14 14:47
1991
2619

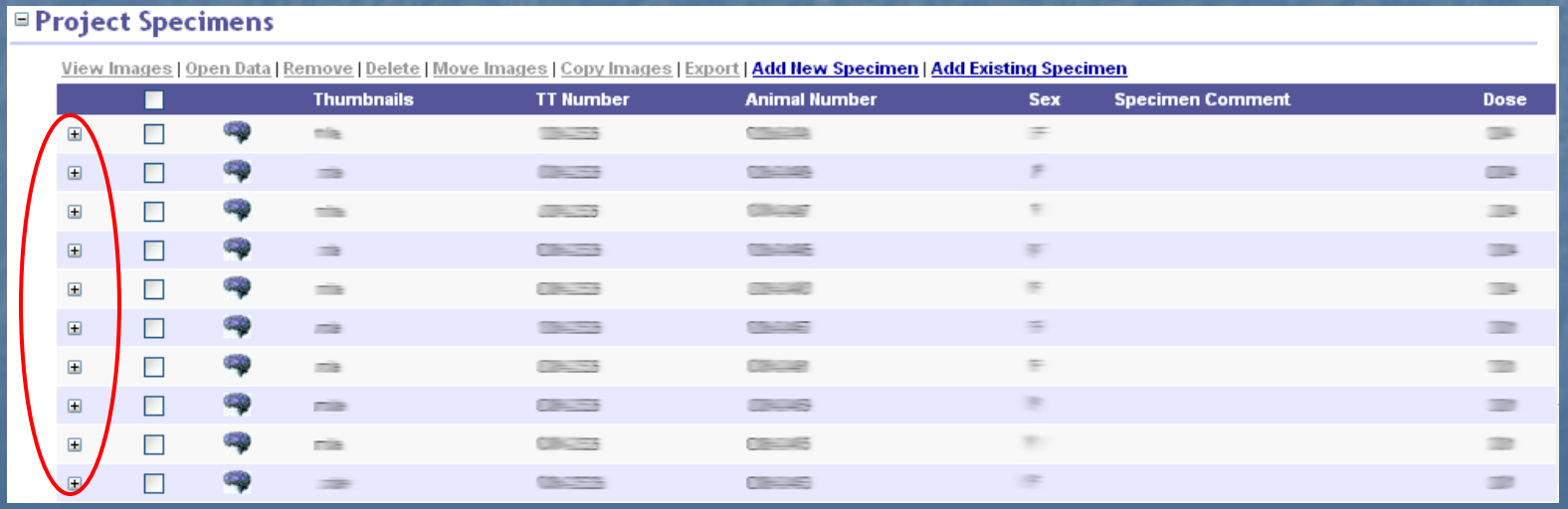

Project Specimens
View Images | Open Data | Remove | Delete | Move Images | Copy Images | Export | Add New Specimen | Add Existing Specimen
Thumbnails
TT Number
Animal Number
Sex
Specimen Comment
Dose

Image Tiling

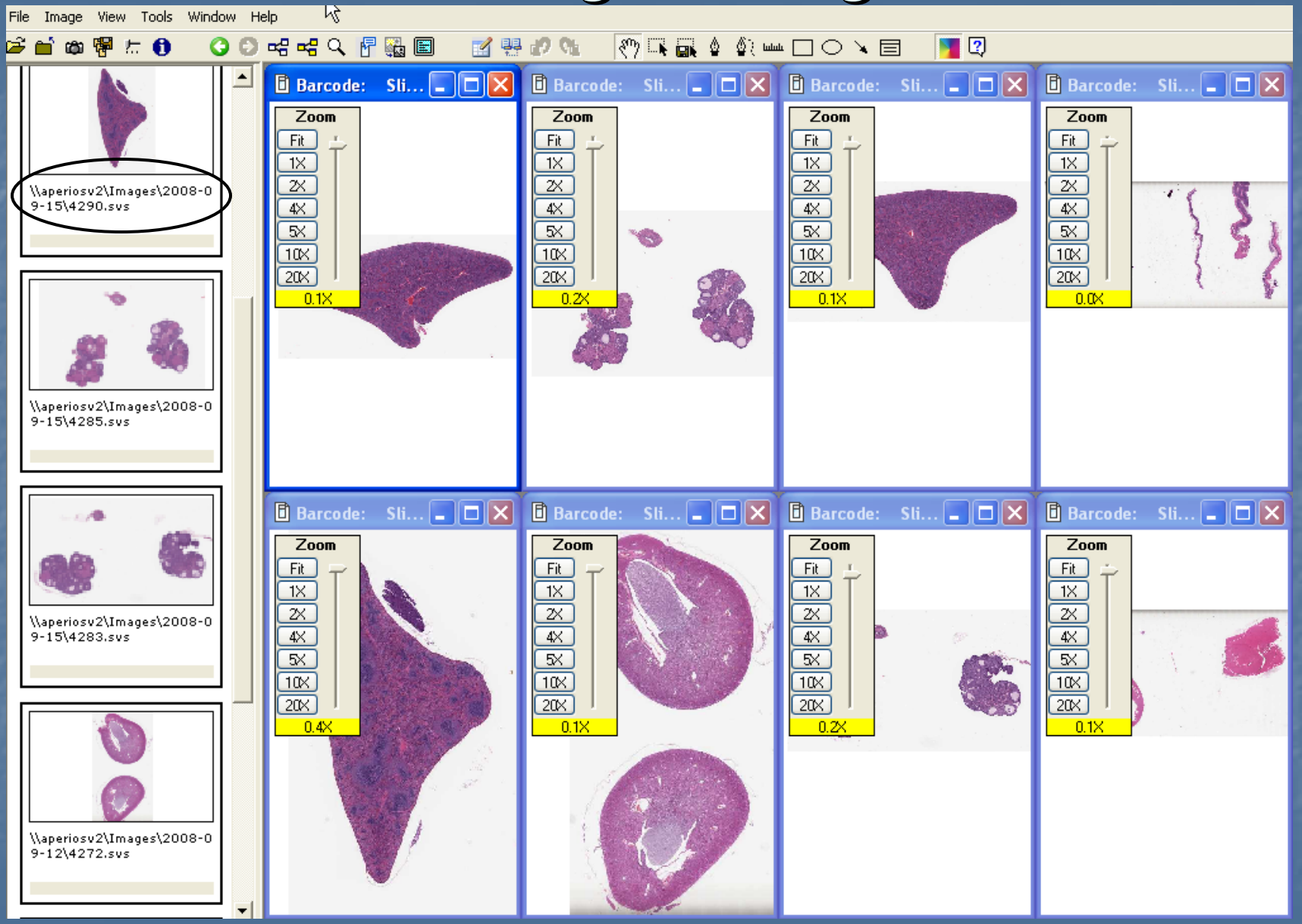
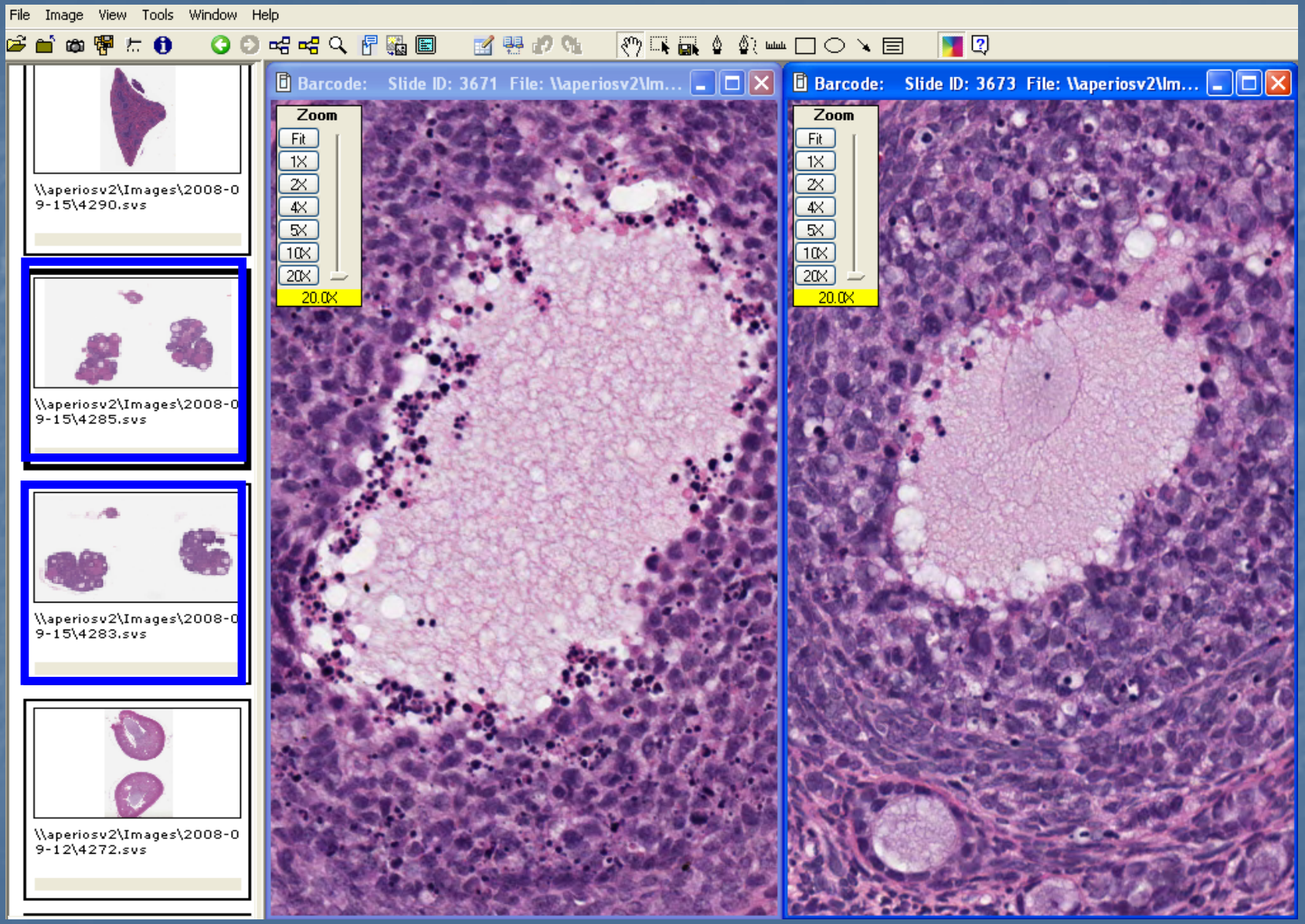


Image Tiling



Efficiency Needs (cont.'d)

- Create and save frequently used searches, e.g., a control/high-dose, males link. Place links in e-mails for specified projects, specimens, or digital slides.
- Save and associate projects, specimens, or digital slides to digital slide conferences using a drag/drop tool.
- Login system using Company username and password. Specified user(s) are automatically notified of new digital slides upon login.
- Develop/retrofit new features to existing systems, e.g., larger capacity loader tray, polarizing and fluorescence modules, etc...

Use of Digital Histological Tissue Images-Challenges

- Acceptance (Industry, Regulatory Agencies)
- Validation/GLP, Electronic Signature, Audit Trail
- Infrastructure/Network Architecture
- ROI (Return on Investment)
- Scanning time
- Screen refresh-remote viewing
- Limited image magnification

Conclusions

- Use of digital images in the pharmaceutical industry offers many benefits
  - Digital images with analysis are used extensively in the discovery/basic research arena
  - Digital images alone or with analysis in the GLP regulated safety arena can also be utilized with significant benefit.
    - However, there are challenges around user and regulatory acceptance and ROI (cost effectiveness, efficiency, report cycle time).

Acknowledgments

- Chris DeMaula
- Tom Forest
- Tim Johnson
- Sylvain Molon-Noblot
- Takayuki Tsuchyia
- Srinivasa Prahalada
- John Mardi
- Jason Flor
- Deb Harner
- Adam Smith
- Terry Greber

MERCK