

Challenges in Whole Slide Image Based 3D Imaging



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Contents

- PICT Center
- WSI based Histology 3D Imaging
- Applications:
 - Lung Adenocarcinoma
 - Coronaries of transplanted mice hearts
 - Glioblastoma
- MicroCT

MGH Pathology Imaging and Communication Technology Center

Automated Histology Lab

- Gross Imaging
- WSI
- Associated information

Other Imaging System

Hardware System

Imaging Lab.

Research Application Software

Mini WSI Scanner

WSI MultiSpectral version

Multi Spectral Imaging System
(microscope based)

High Volume/High Speed multipurpose
WSI Scanner (Fluorescence, BF, 3D)

High Volume/Ultra high speed WSI scanner

**High Volume/High Speed WSI
Scanner (Fluorescence, BF)**

RFID Research system

Next Generation Optical Microscope System

LCM, xMD

Gross Imaging Researches: Management
3-D

WSI Research: 1. Basic (Image Management,
Quality, GUI, Compression,
Standard, Human Interface, etc)
2. 3-D reconstruction,
visualization & Analysis

3. Image Analysis

4. Decision Support System

5. Multispectral Imaging Basic

6. MSI Application

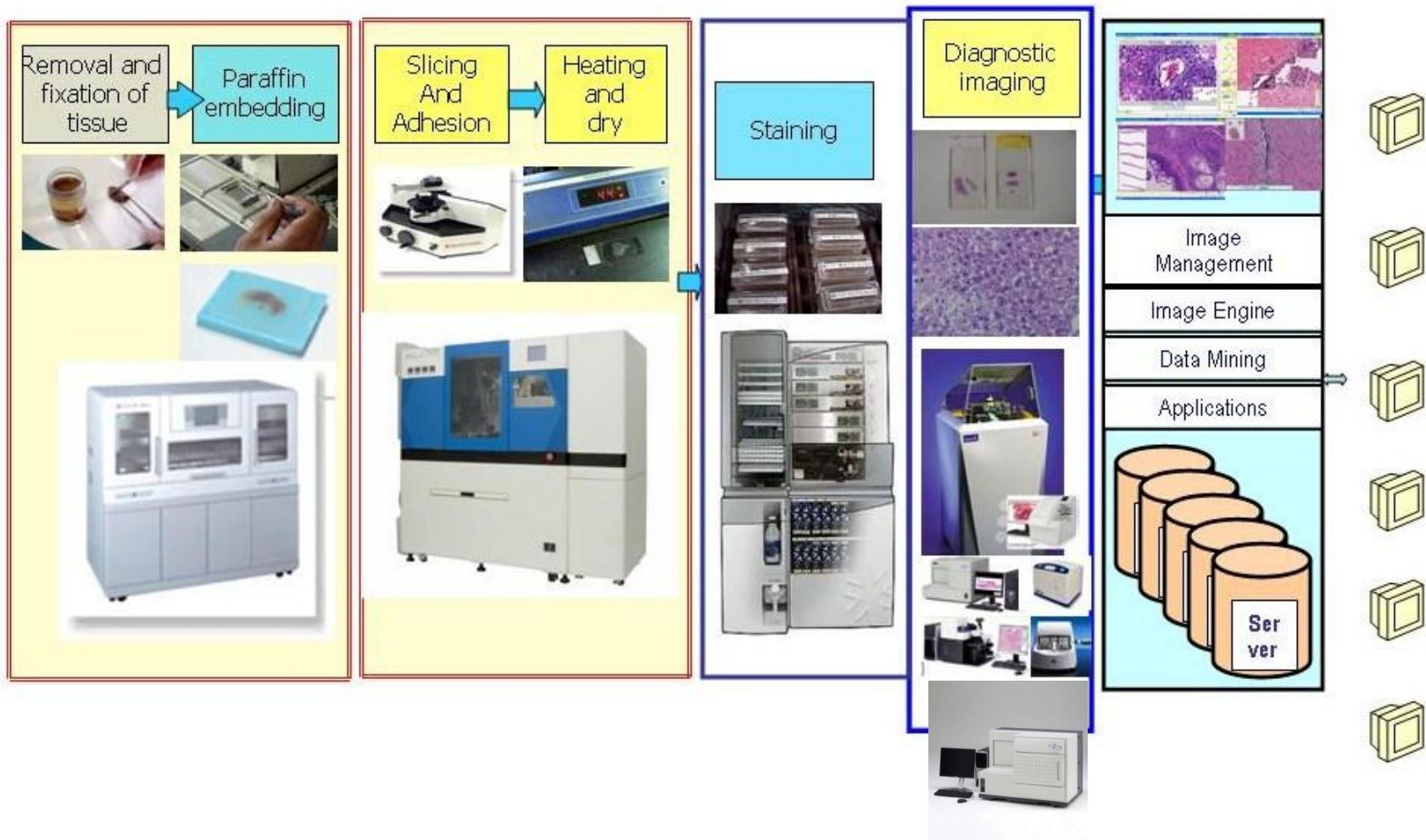
Image/Data Management System

Education

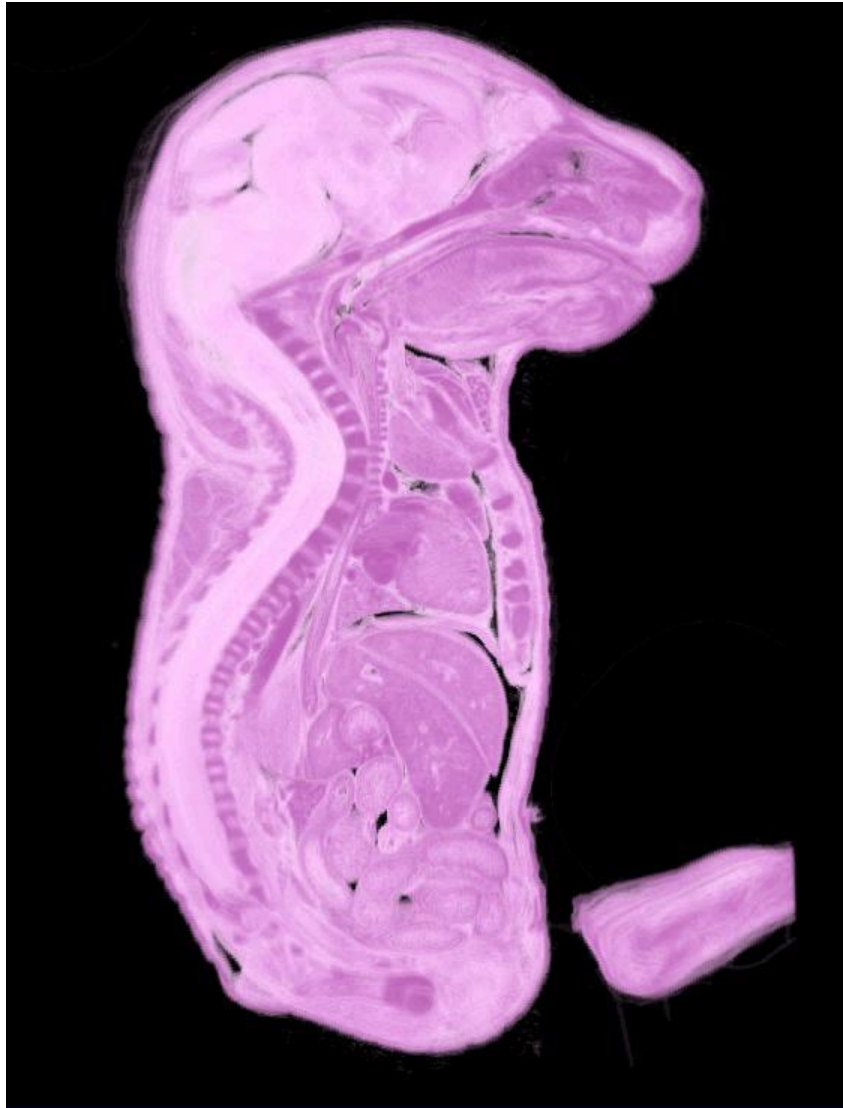
1. Conference
2. Distance Learning
3. CME (online/onsite)
4. Lecture
5. Virtual Simulation

Security Management System

Development of Automation Histology Lab at MGH since 2007



3D Imaging in Pathology

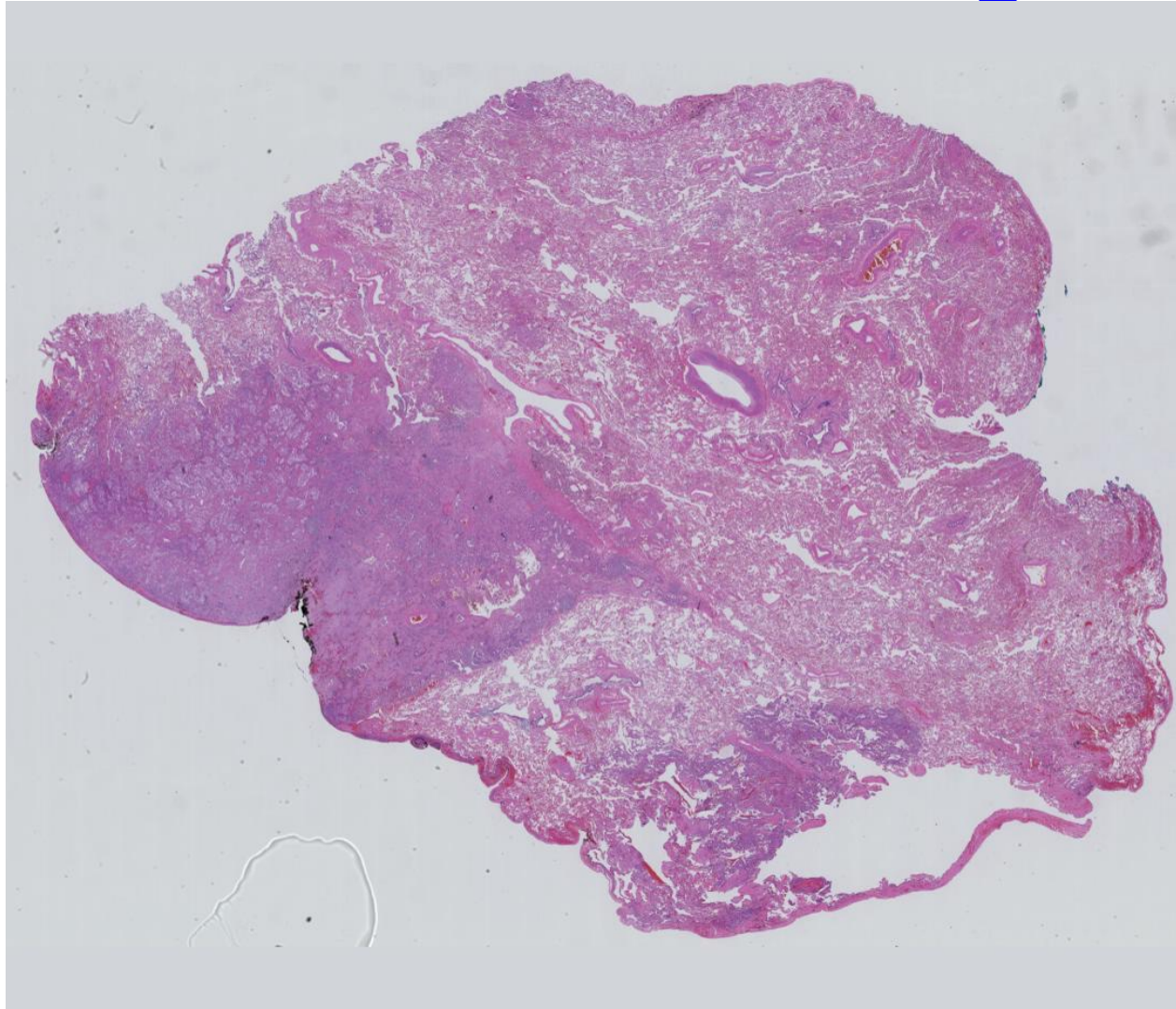


Many pathologists have been interested in 3D for many years.

Background

- WSI technologies and rendering software have now improved to the point that 3D reconstruction of large structure at microscopic scale from hundreds of serial sections is possible. The challenges in this approach include section registration, quality of tissue, effects of tissue processing and sectioning, and the huge amount of data that can be generated.

Whole Slide Image



Whole Slide Image

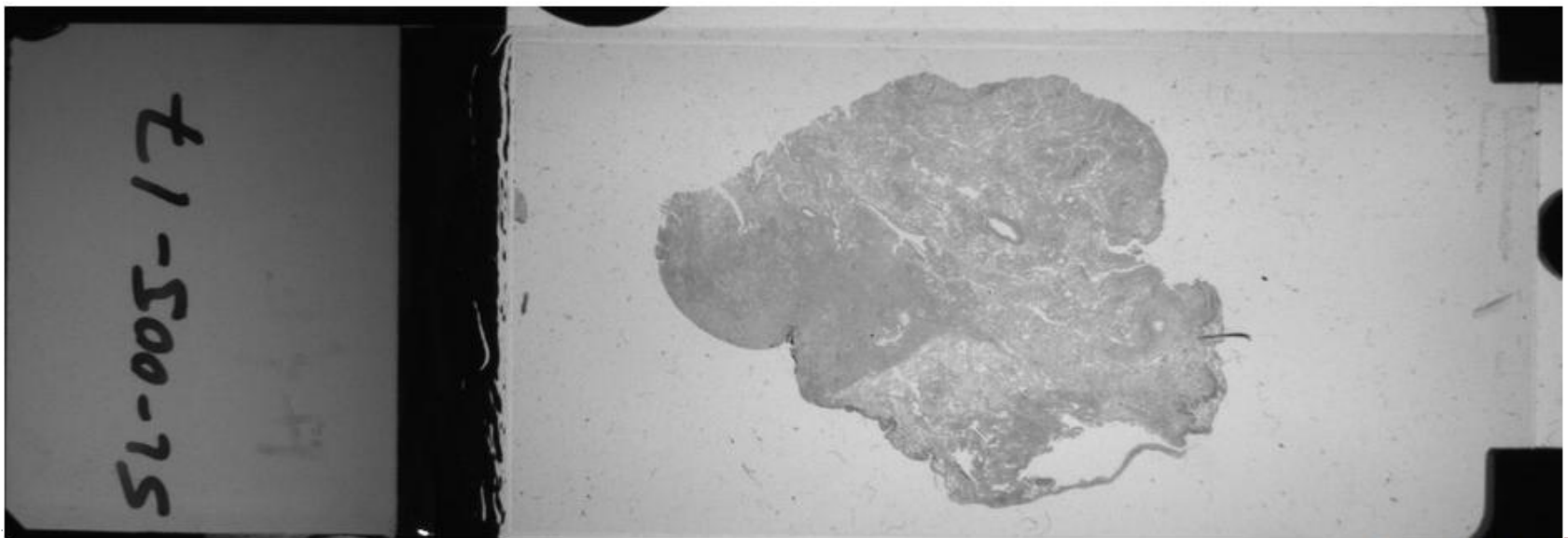
Status X

Current Lens 40x
Source Lens 40x

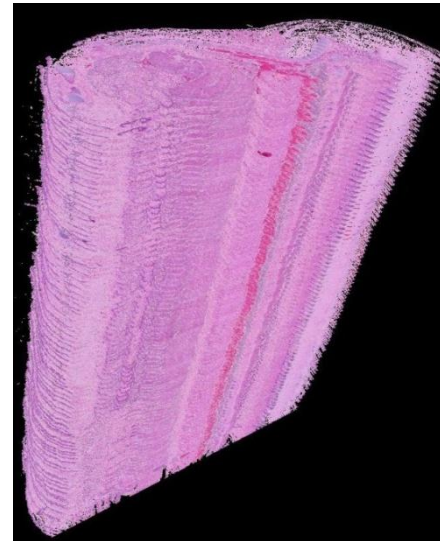
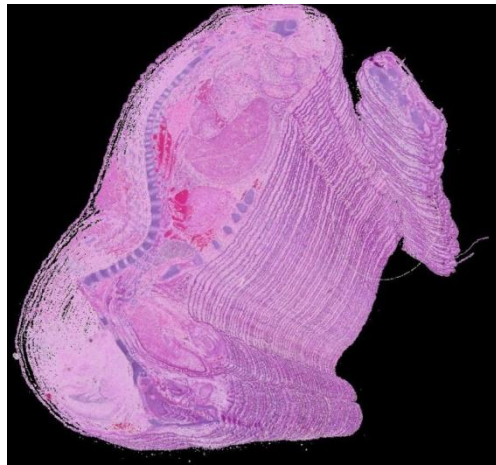
Focusing available No

Source filename SL-005-17 - 2012-07-26 17.47.09 on 172.20.142.167
Reference SL-005-17
Filesize 2 GB (**uncompressed** 33.6 GB)

Current screen width 216 μm
Current screen height 211 μm



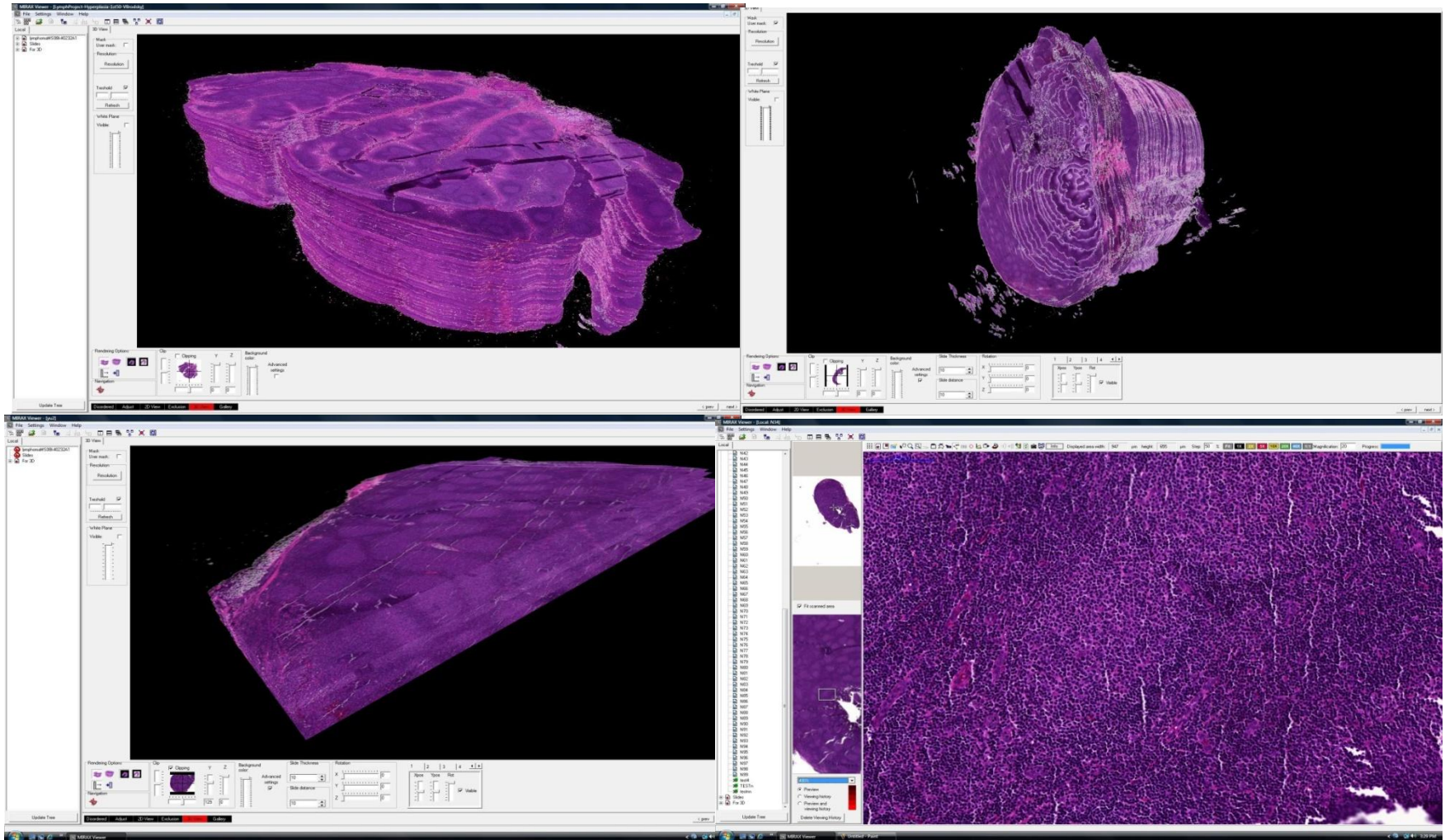
History of WSI based Histology 3D Imaging at MGH since 2007



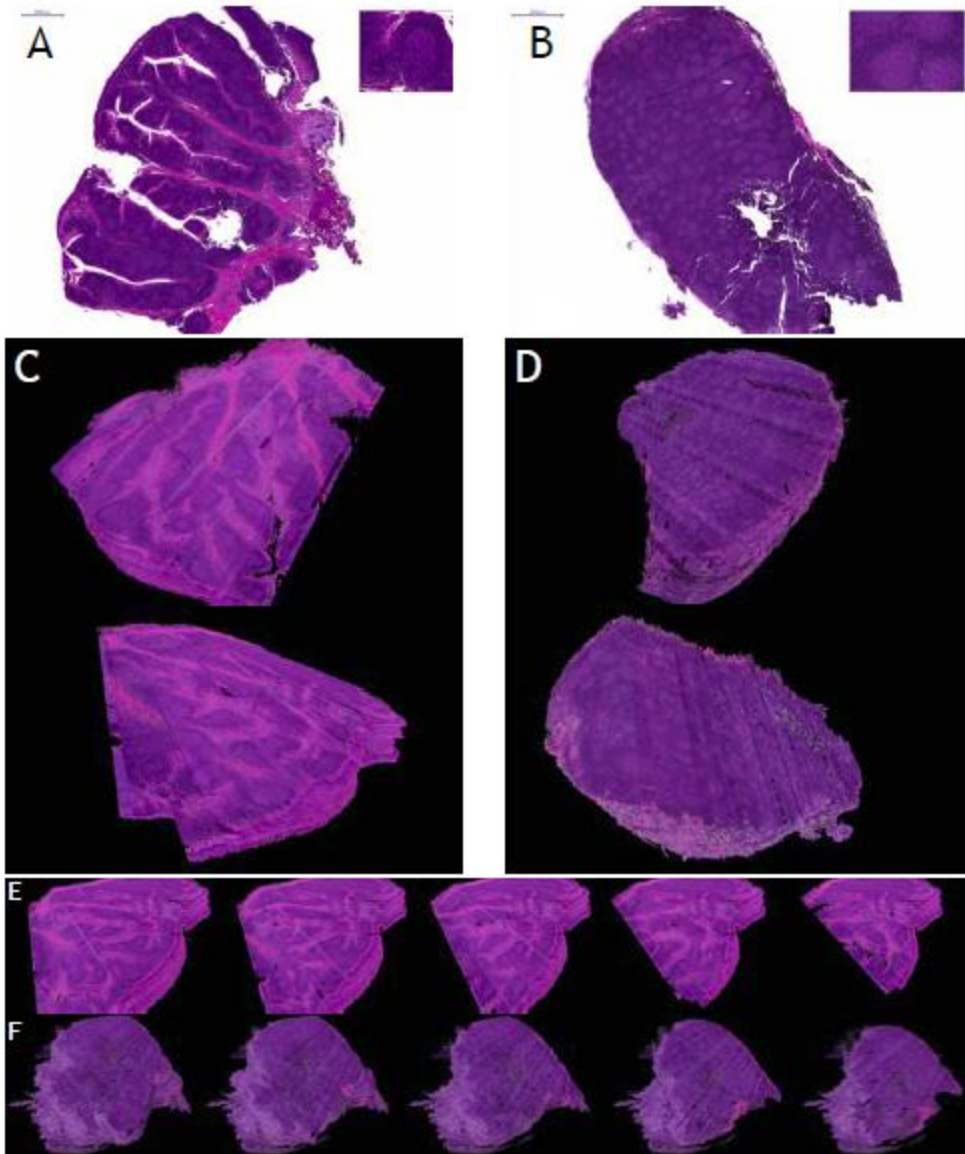
Lymphoma (2007-2008)

Partially supported by 3D Histech

Early stage (2007-2008) of WSI based 3D Imaging



Follicular Lymphoma (work with Dr. Sohani) Benign-Malignant
by MiraxScan and Mirax 3D Software



Certain low-power morphologic features that help distinguish benign from malignant follicular lymphoid proliferations may be enhanced by 3D analysis. This analysis may be cumbersome for routine diagnostic use in straightforward cases of RFH and low-grade FL, but may be useful in helping to distinguish **RFH from grade 3FL** which share many higher-power morphologic (increased number of centroblasts, mitoses and tingible-body macrophages) and IHC (high Ki67, Bcl20negative) features within follicles. In the future computational power will increase to allow higher resolution 3D analysis

Conventional 2D examination of a case of reactive follicular hyperplasia involving tonsil (A) and grade 1-2 of 3 follicular lymphoma involving a lymph node (B). 3D reconstruction of the same two cases (C: reactive follicular hyperplasia; D: follicular lymphoma) after alignment of serial sections and stacking of images emphasizes relative amounts of cortex and paracortex and features and patterns of the follicular structures in each case. Dynamic varying of the plane of section of the 3D models further exposes 3D relationships between structures (E: reactive follicular hyperplasia; F: follicular lymphoma).

Issues

Morphologic features were often enhanced upon 3D reconstruction, although the relatively low resolution of the 3D model precluded extensive analysis of cellular interactions. **The reconstruction process was made more difficult by tissue processing effects such as wrinkle, stretch, bubble, variable thickness across the tissue section.**

- Total file sizes to create one 3D model were 50-100 GB/model.

Technical issues

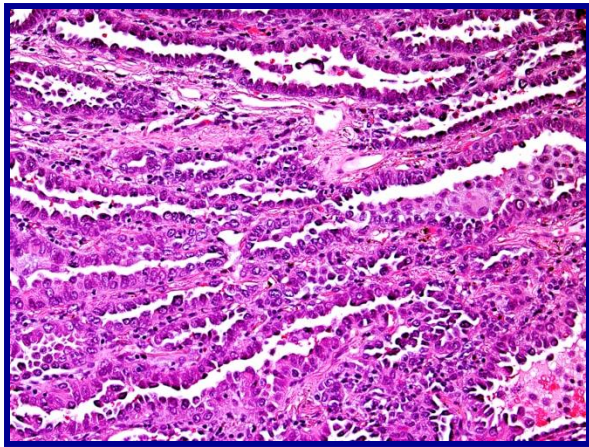
- **Registration by block and by slide**
- **Slide Quality and image quality**
- **Stability of Staining**
- Tissue features by organ and tissue processing
- Exact size of spaces between slides
- Computer Memory and performance
- 3D image Resolution is limited by: (i) PC specifications; (ii) size of original 2D image; (iii) and number of slides
- Speed to manipulate 3D model was effected by the size of 3D model
- Cost for the staining
- Luck of information

Lung Adenocarcinoma (2010-)

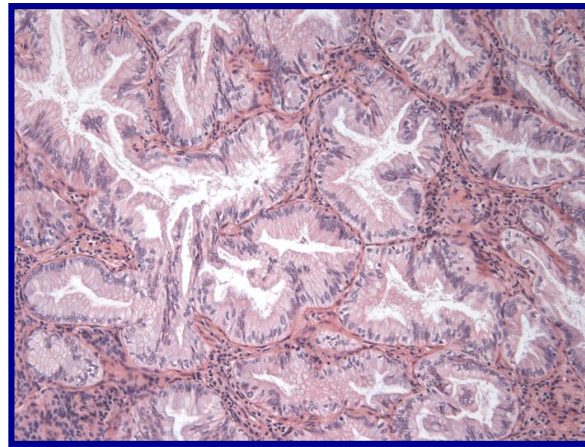
3D Histech system + Automated Sectioning System

3D for Histologic Patterns of Lung Adenocarcinoma

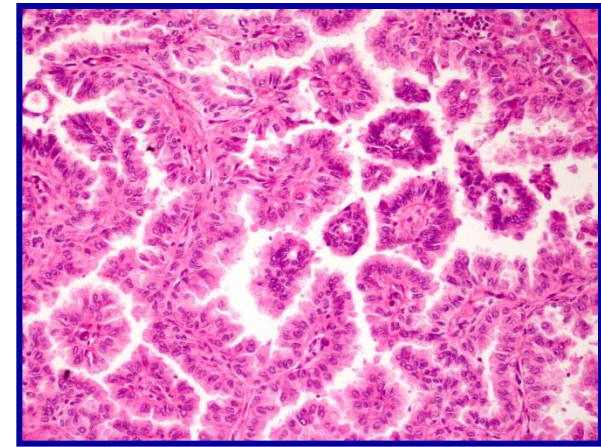
ISALC/ATS/ERS international multidisciplinary classification of lung adenocarcinoma (work with Dr. Mino-Kenudson's group)



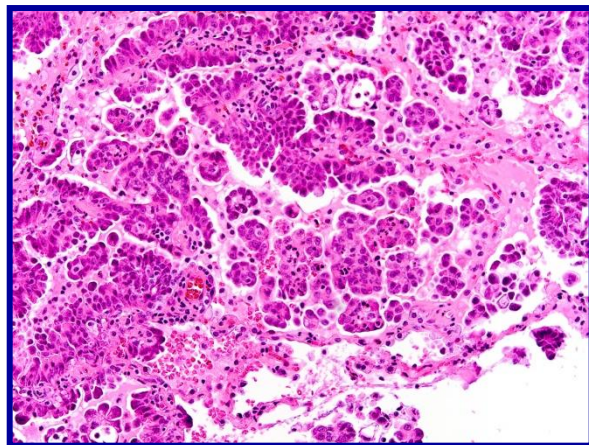
Lepidic (bronchioloalveolar))



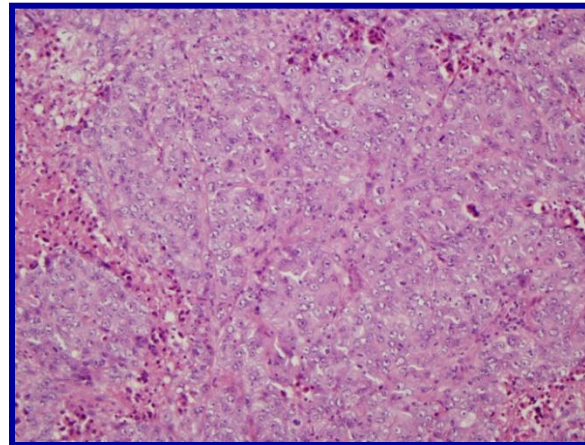
Acinar



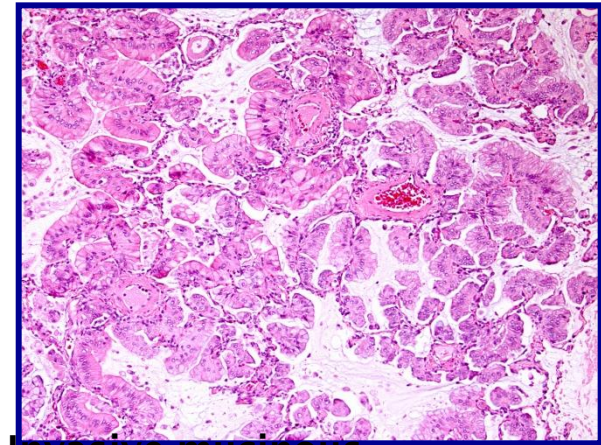
Papillary



Micropapillary



Solid

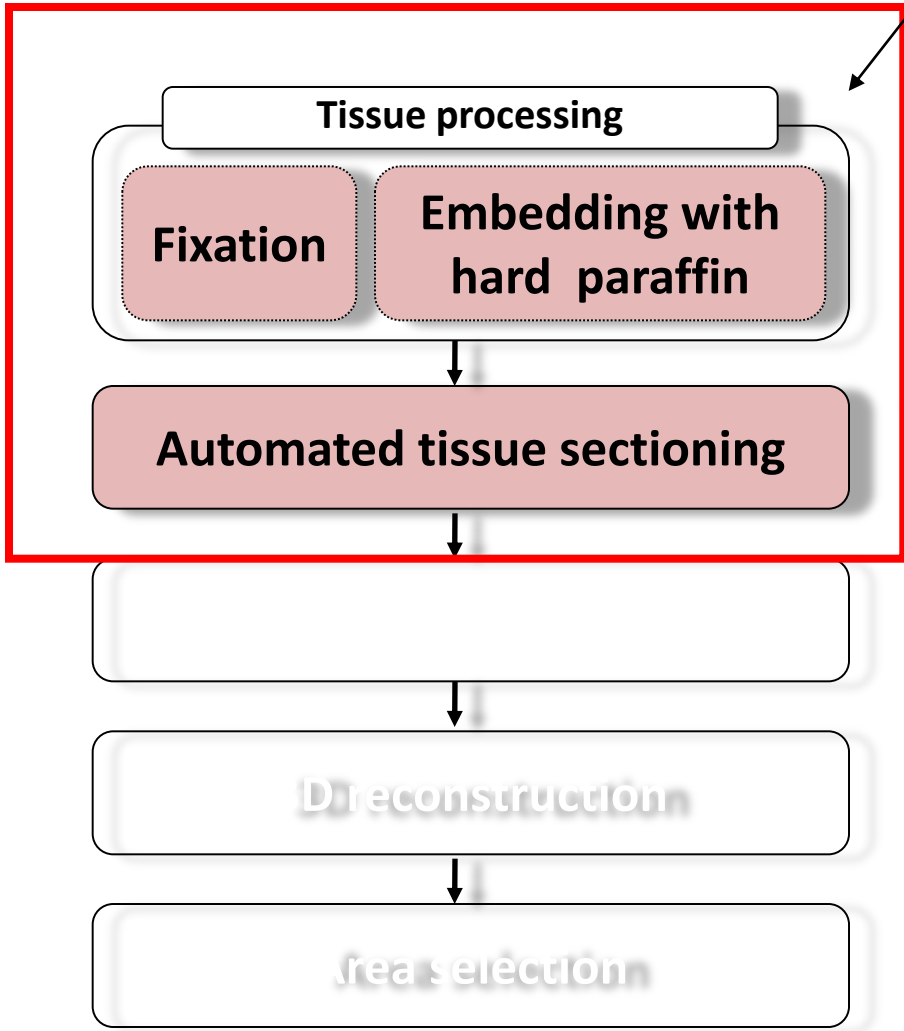


Invasive mucinous adenocarcinoma (mucinous BAC)



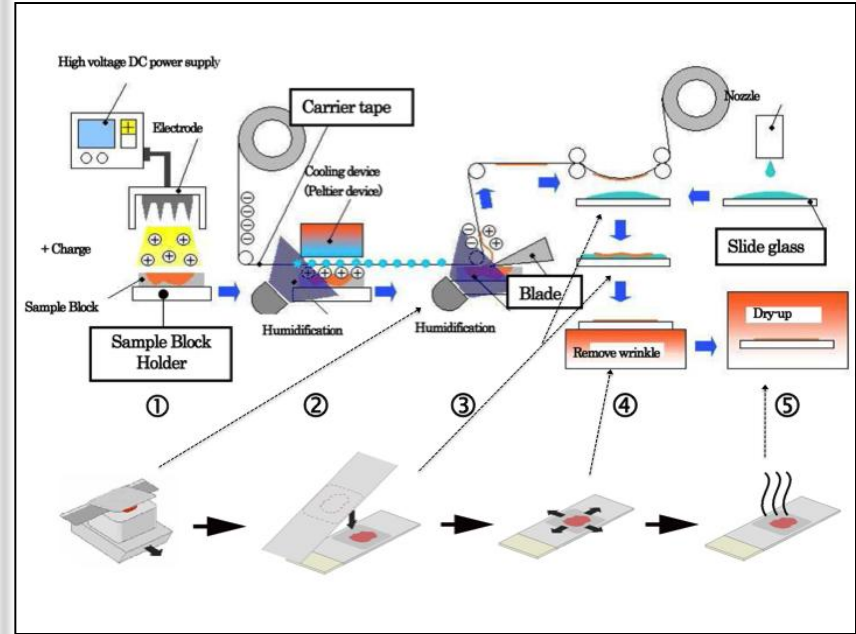
Automated Sectioning System

Work flow-1: Sectioning

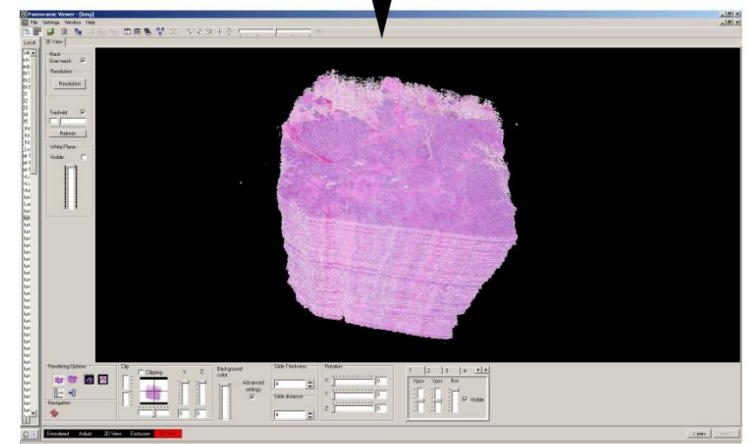
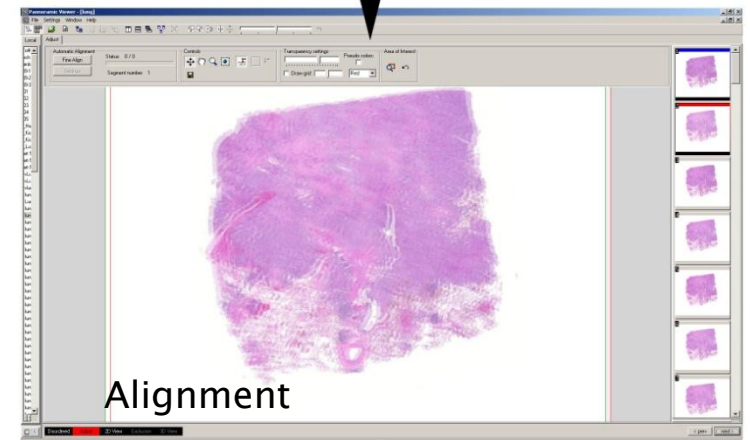
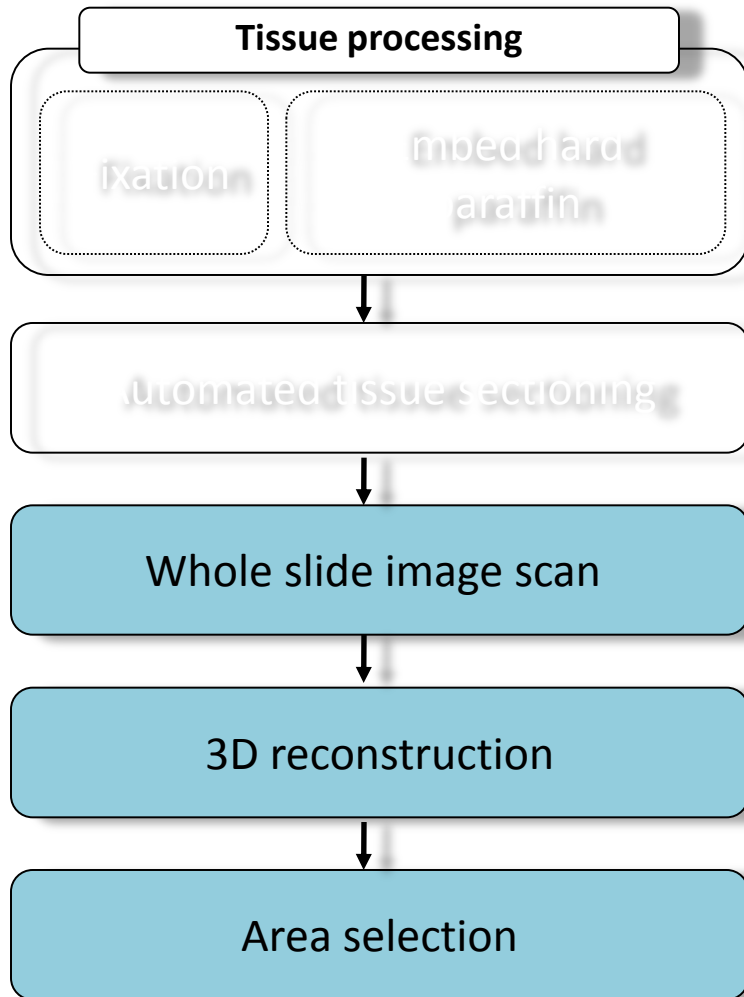


Since 2009, we do the tissue processing by ourselves to control the quality of a tissue block

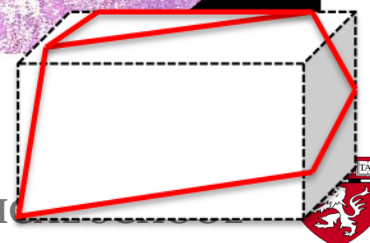
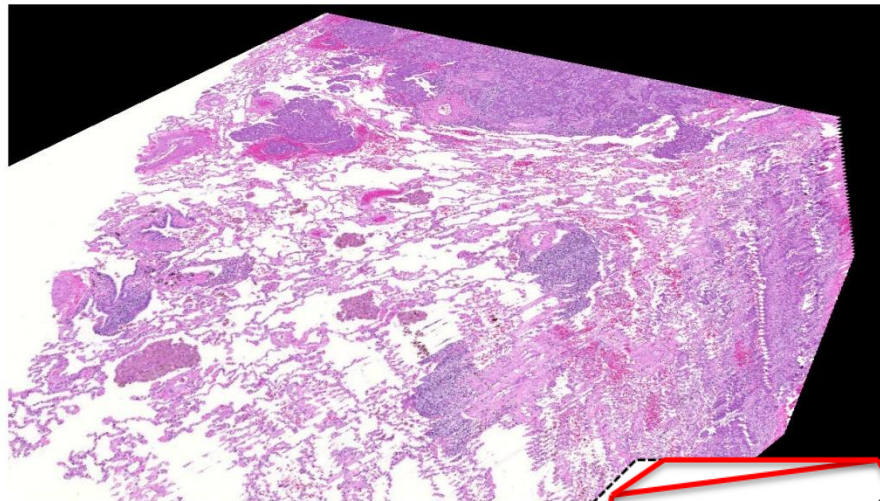
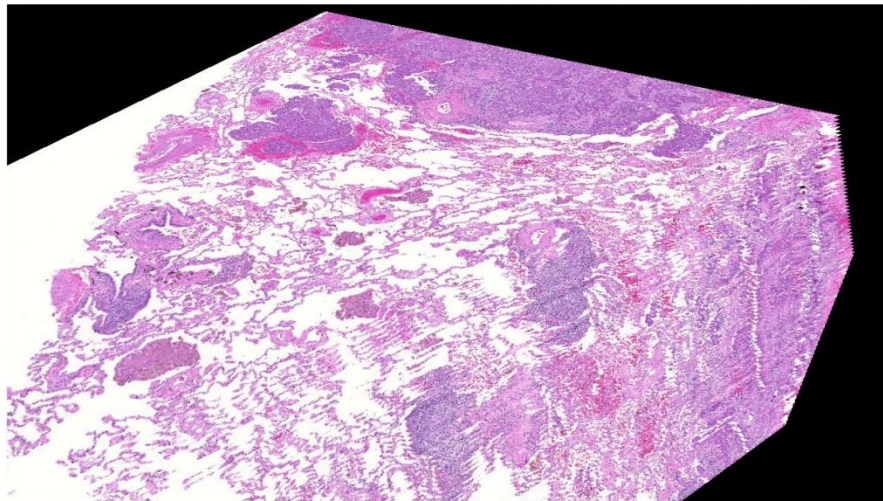
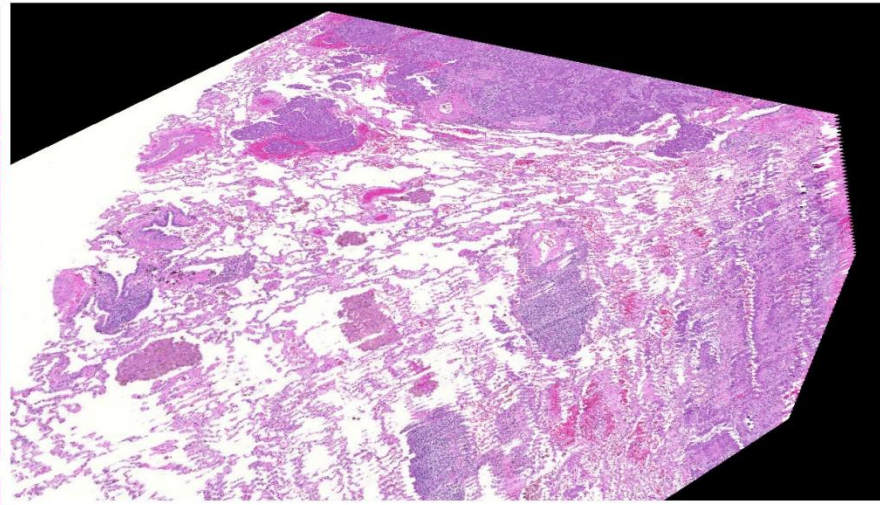
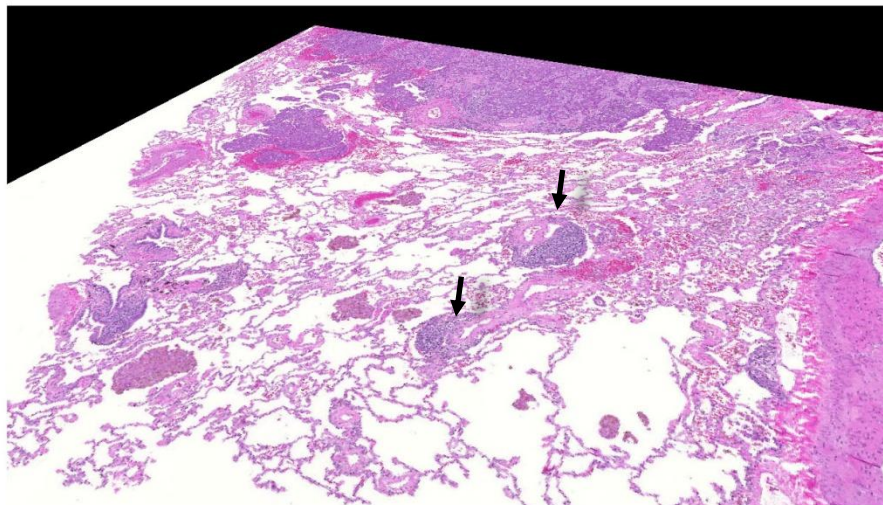
KURABO- Automated Tissue Sectioning Machine



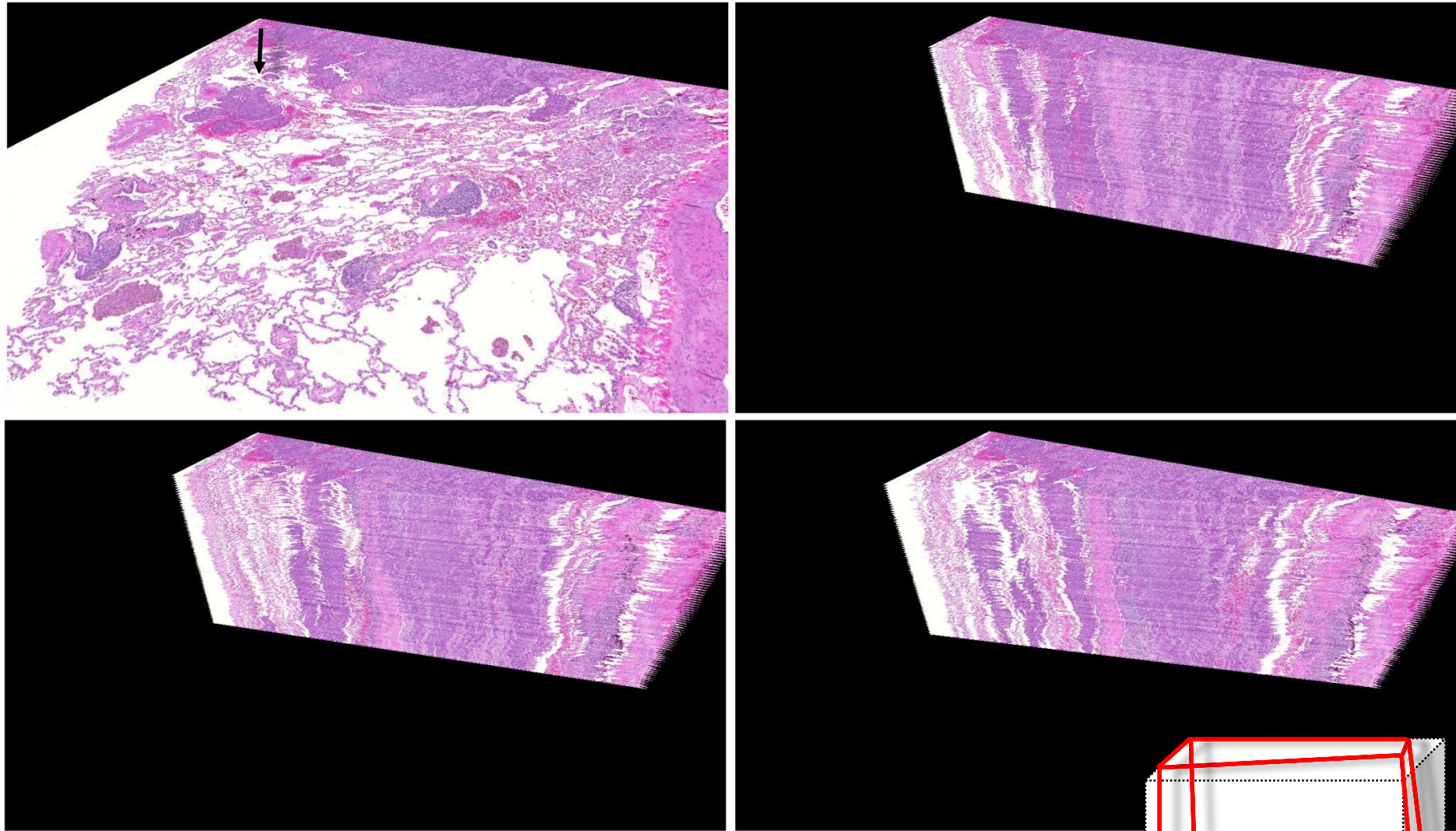
Work flow-2: Imaging & Reconstruction



3D Reconstruction of Lung Adenocarcinoma: “Islands of Tumor Cells”



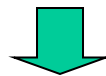
3D Reconstruction of Lung Adenocarcinoma: “Islands of Tumor Cells”



Next step

To improve 3D images with a higher magnification in order to further analyze the transition from one pattern to another

To assess the clinical implication of additional information brought by 3D reconstruction (such as inclusion of “the islands of tumour cells” in a solid pattern)

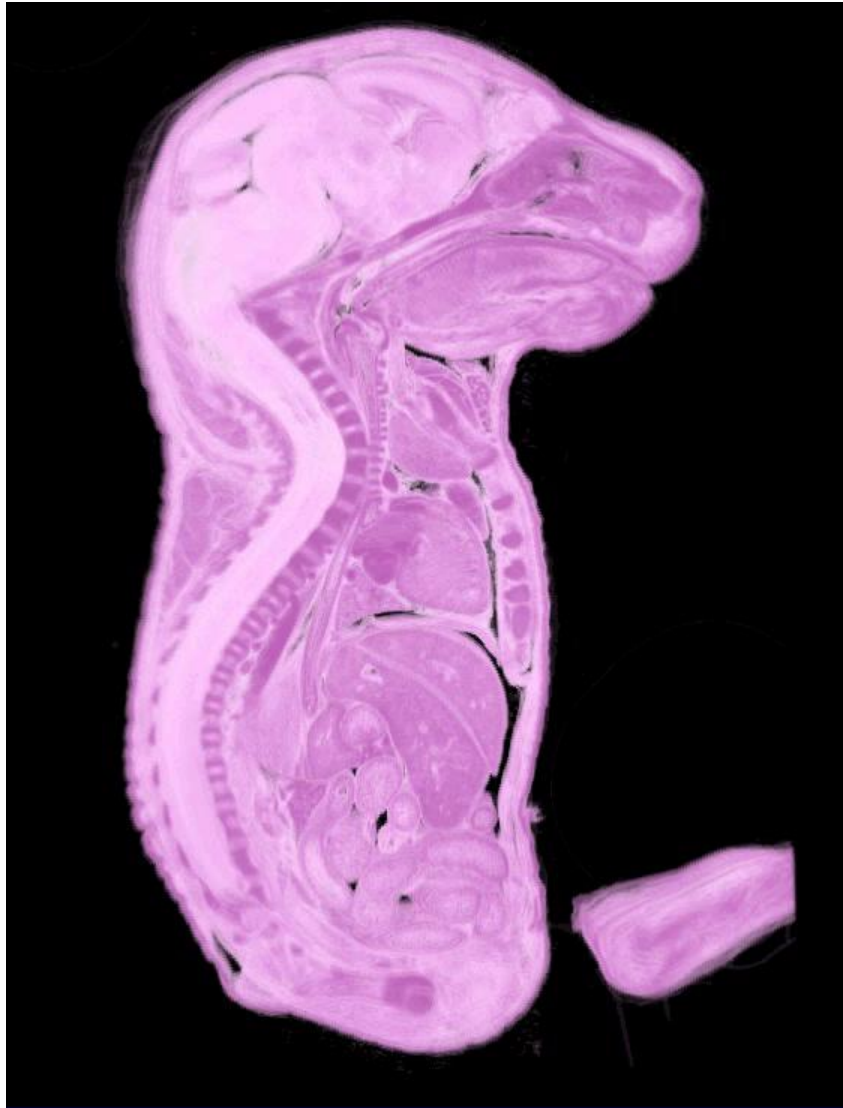


Am J Surg Pathol. 2013 Feb;37(2):287-94. doi: 10.1097/PAS.0b013e31826885fb.

Tumor islands in resected early-stage lung adenocarcinomas are associated with unique clinicopathologic and molecular characteristics and worse prognosis.

Onozato ML., Kovach AE, Yeap BY, Morales-Oyarvide V, Klepeis VE, Tammireddy S, Heist RS, Mark EJ, Dias-Santagata D, Iafrate AJ, Yagi Y, Mino-Kenudson M.

New approach (2011-)

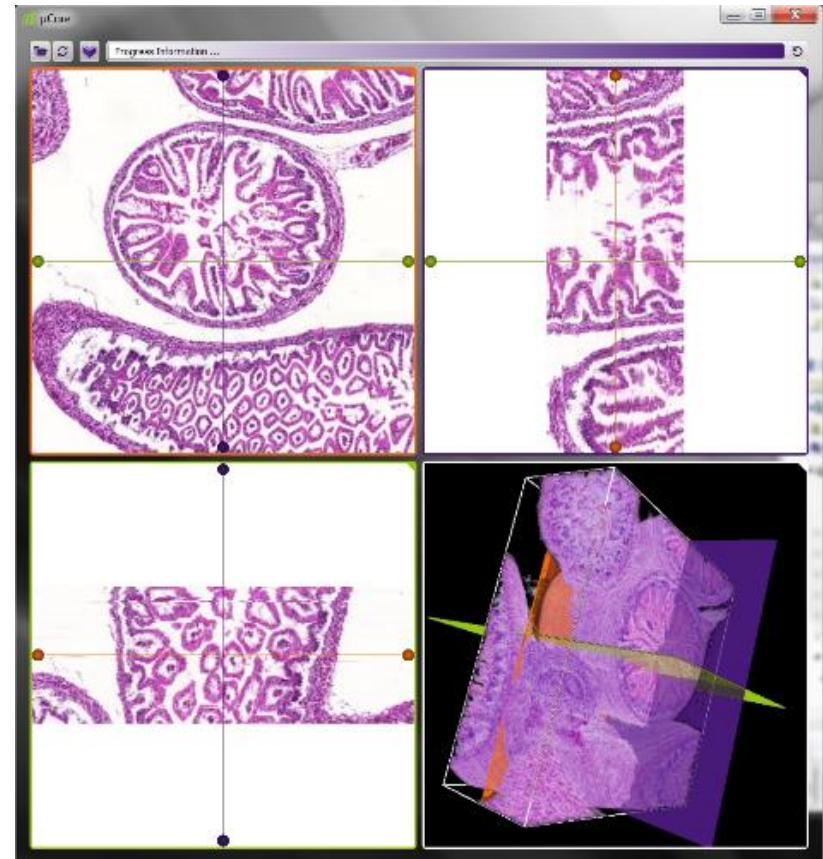


Working with Technical
University of Munich
(microDimensions)

Focused on Speed and
Quality

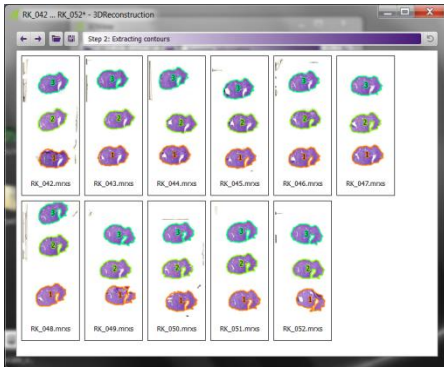
3DView: 3D Whole Slide Imaging

- 3D reconstruction of stacks
- Up to original scanned resolution <math>< 1 \mu\text{m}</math> (40x)
- Easy handling of virtual slides
- Bright-field, fluorescence, confocal
- Volume analysis
- Visual volume editing
- Supporting multiple formats including ndpi

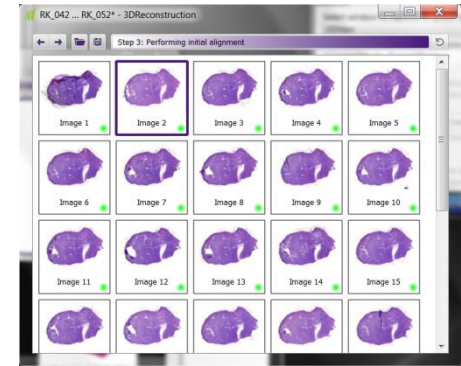
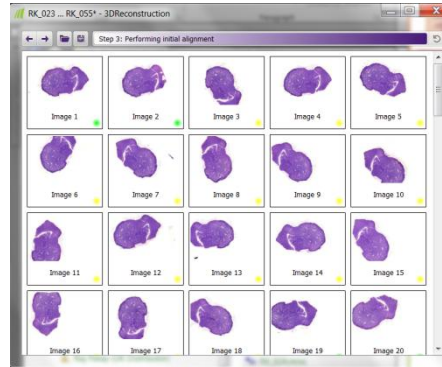


3D reconstruction

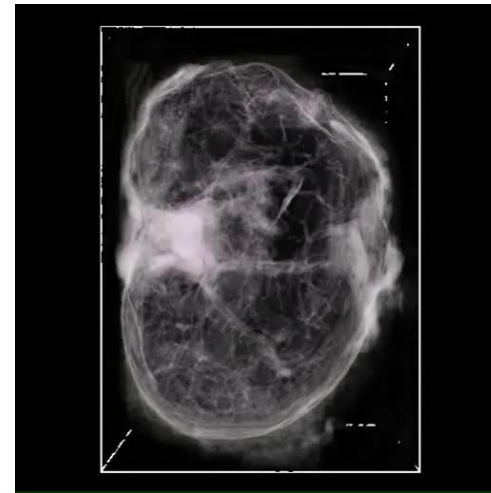
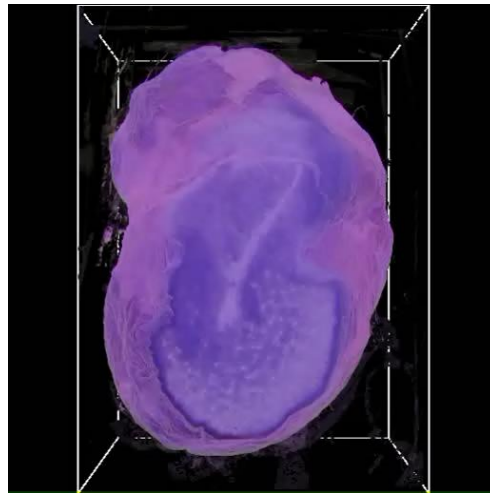
- From virtual slides



contour extraction



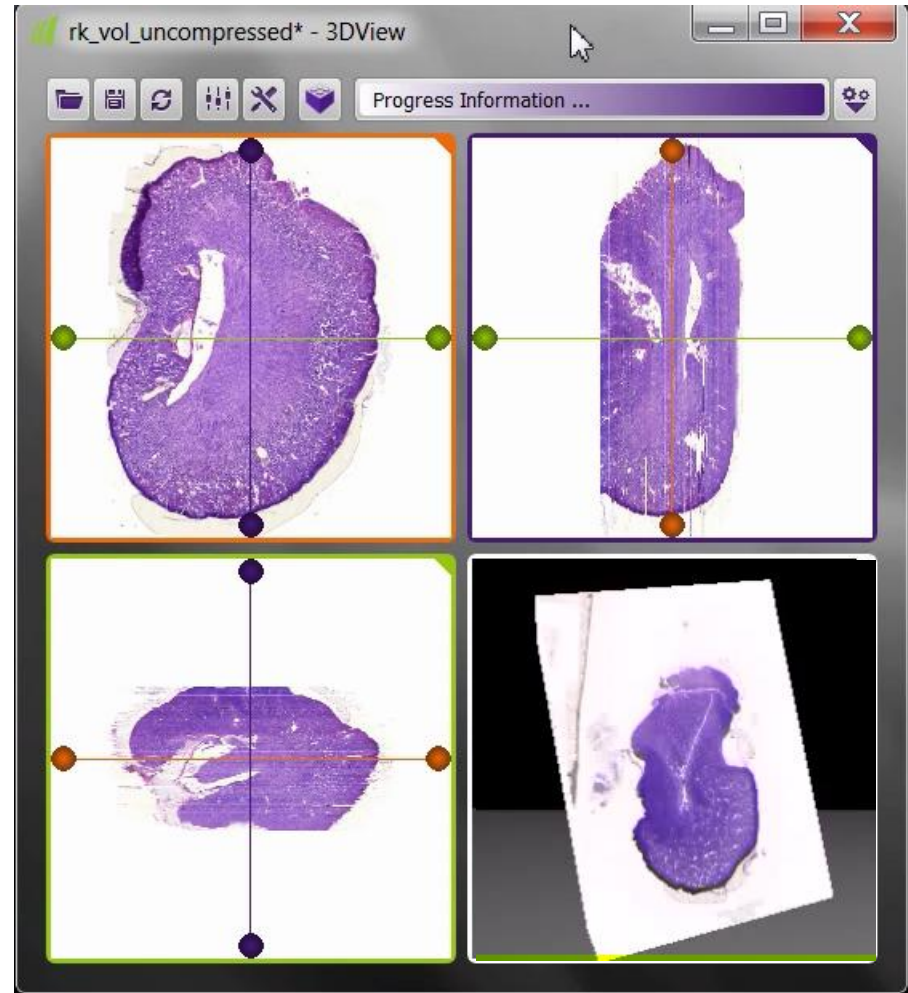
alignment



Yukako Yagi, PhDAPIII 2008

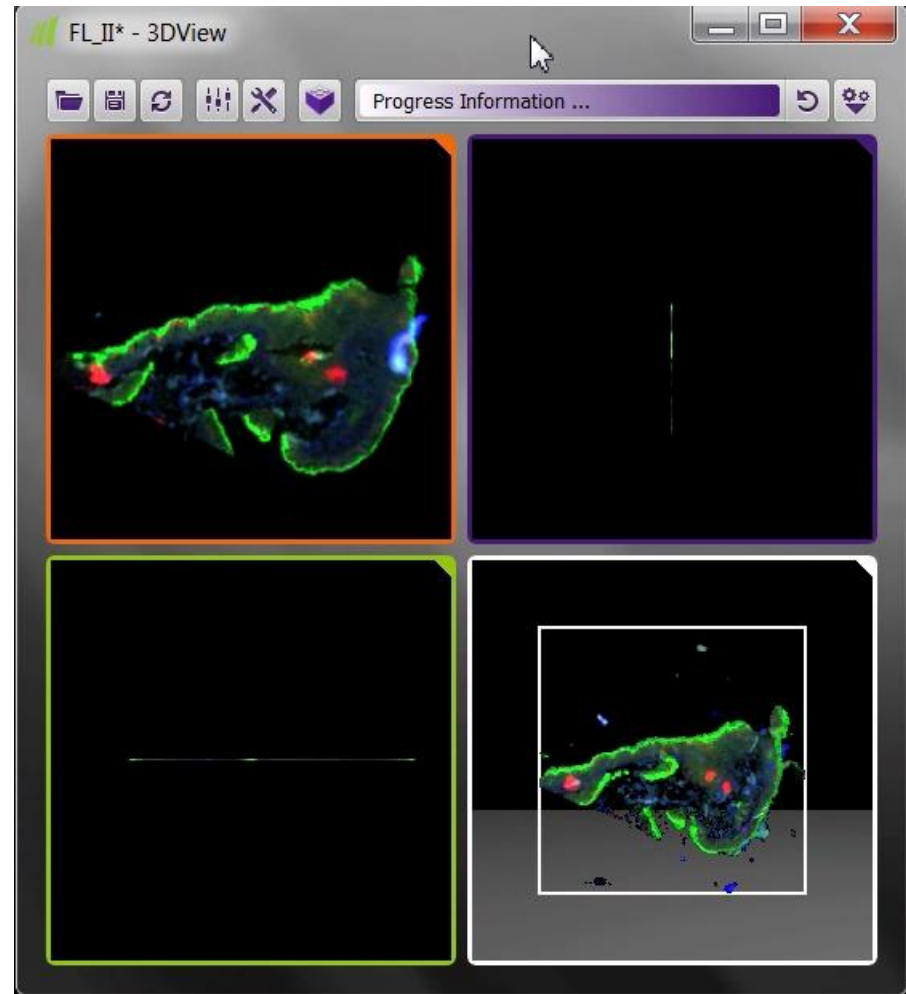
3D visualization

- Virtual sectioning planes create any view in the volume
- rotate and zoom the data freely
- Transparency adjustments help us to observe inside the volume



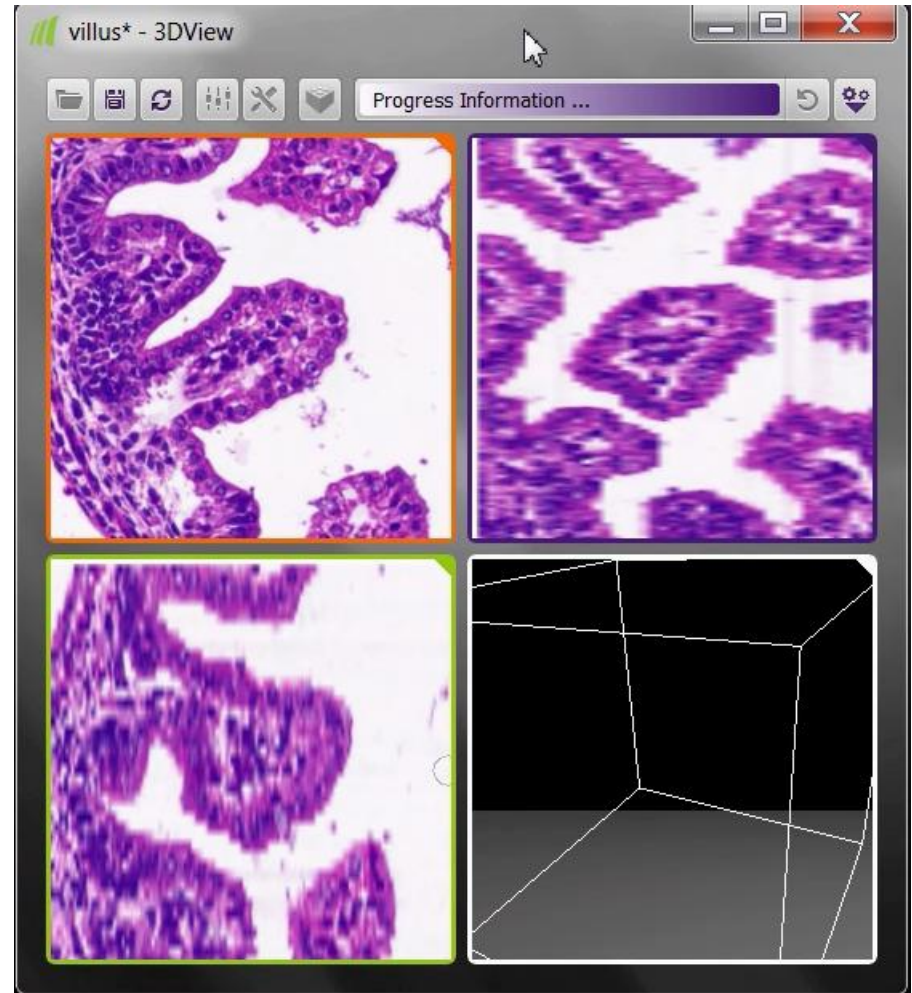
3D magnification levels

- Resolution can be adapted to any magnification level (1x-40x)
- Zoom update functionality guarantees optimal alignment on each resolution level
- Select a region of interest and visualize it on maximal resolution

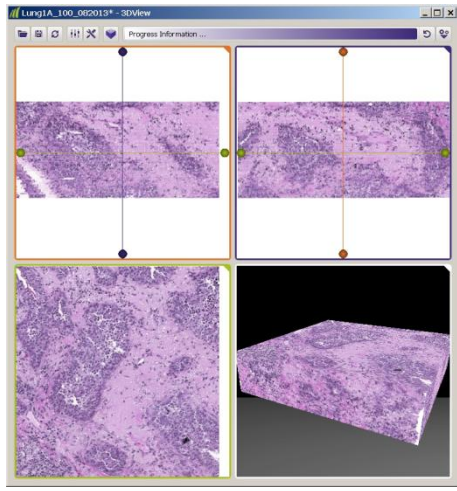


3D segmentation

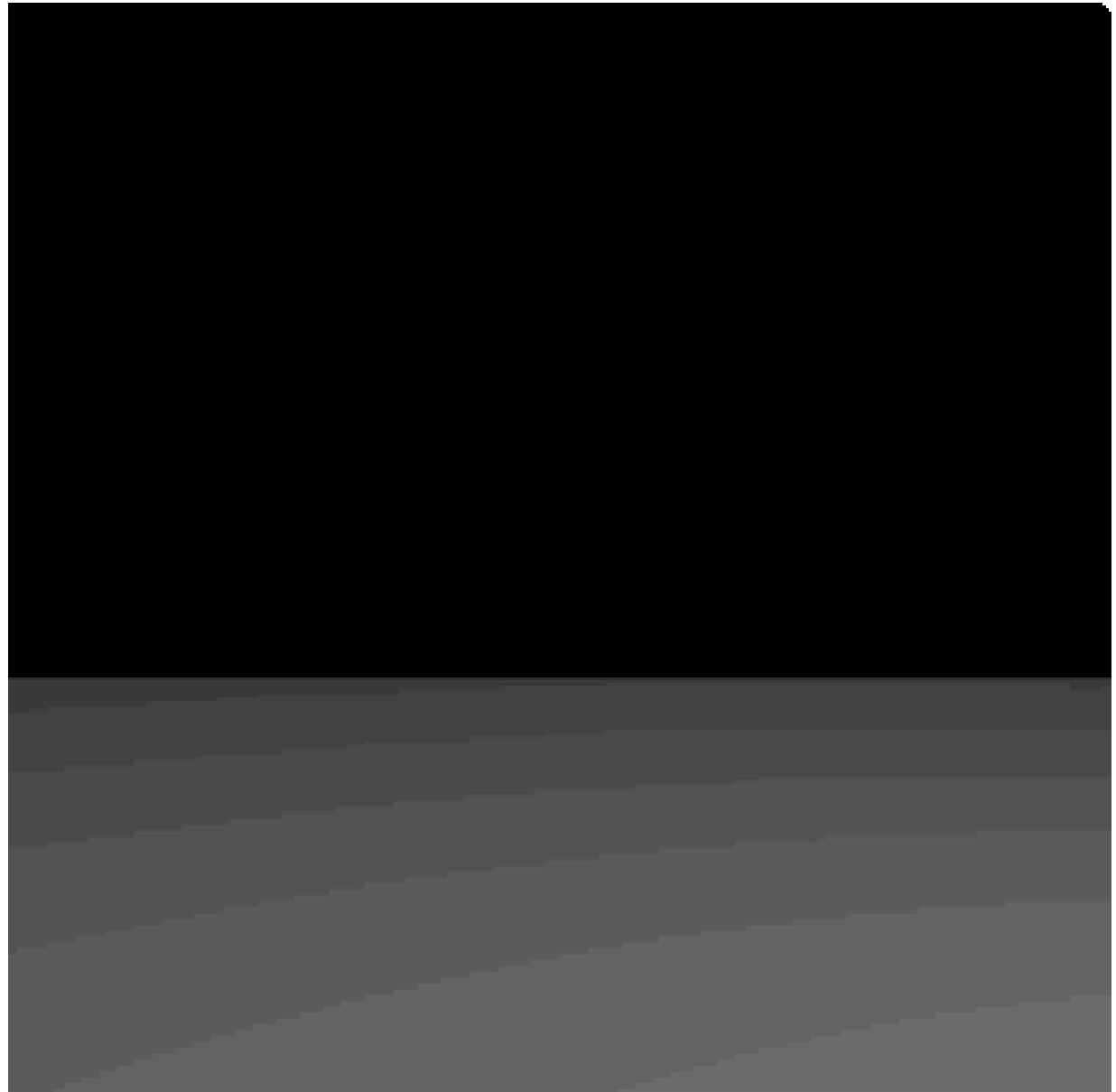
- Segmentation functionality lets us extract anatomy
- Extract with only a few brush strokes (green = object, red = background)
- Measure the anatomy as volume

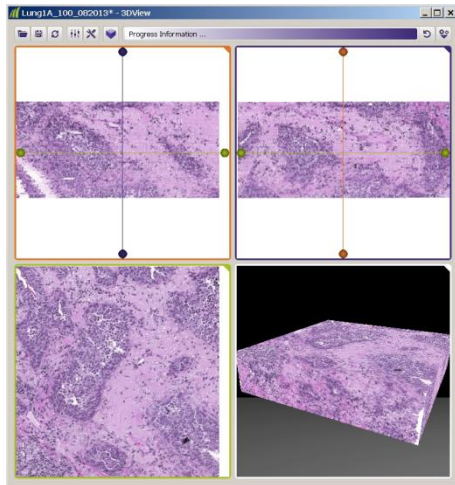


Lung Adenocarcinoma with New software



Collaboration
with
microDimensions
and 3DHistech.





Collaboration
with
microDimensions
and 3DHistech.

3D reconstruction of vascular structures using whole slide digital imaging

Imaging of coronaries of transplanted mice hearts

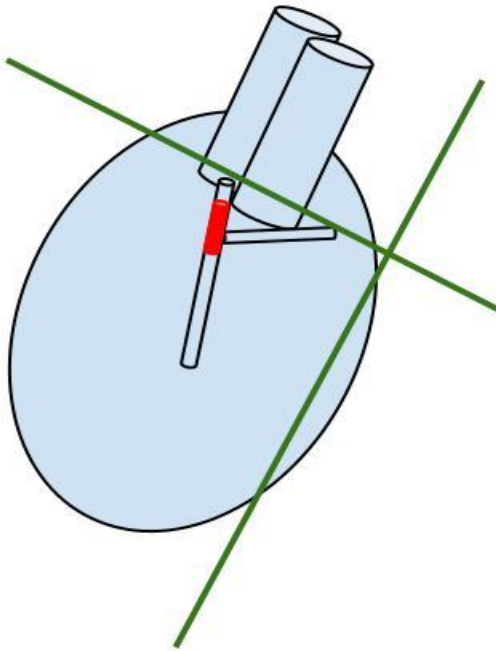
- After transplantation, a sign of chronic rejection is the thickening of the coronary lumen (proximal to the origin) because of intimal proliferation and infiltration of different lymphocytes

Aims

- To provide a solution for the imaging of the involved coronary segment
- 3D reconstruction of digital slides to
 - Find the involved coronary area
 - Perform exact measurements on the thickening

Why 3D?

- **Current Problem with microscope observation (2D) 1**



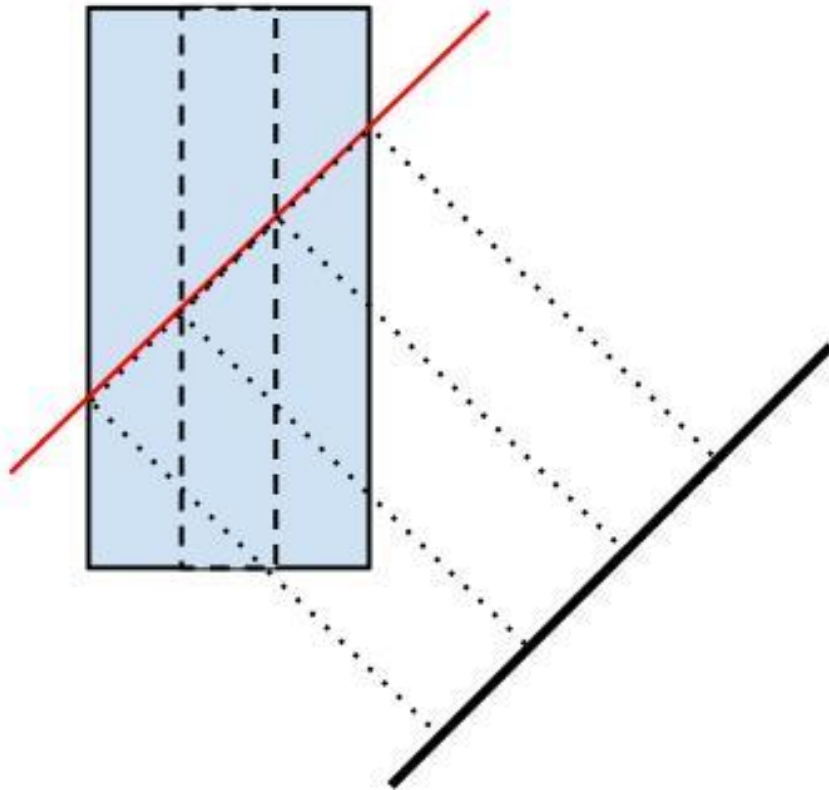
- The involved coronary portion is very small (approx. 1 mm short and the diameter is around 0.1 mm)
- The orientation of the embedding could result in losing a proper cross section
- With manual sectioning it is easy to miss the small

coronary

Yukako Yagi, PhD, AFIP 2008

Why 3D?

The method, simply measuring the "thickness" of the subendothelial layer of any accidentally found vessel leads to more variation of the parameter than variation caused by the experimental setting.

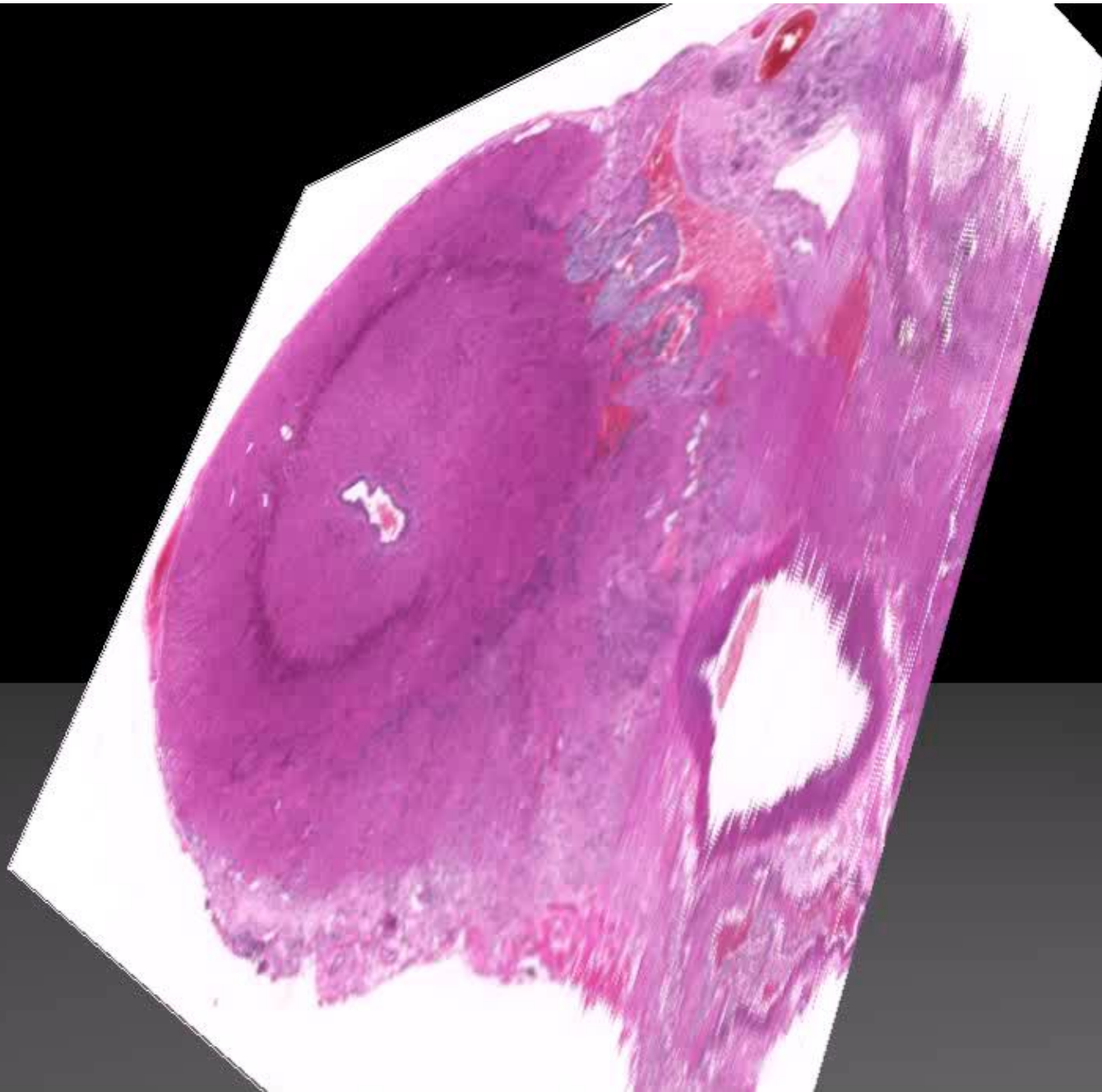


Current Problem with microscope observation (2D) 2

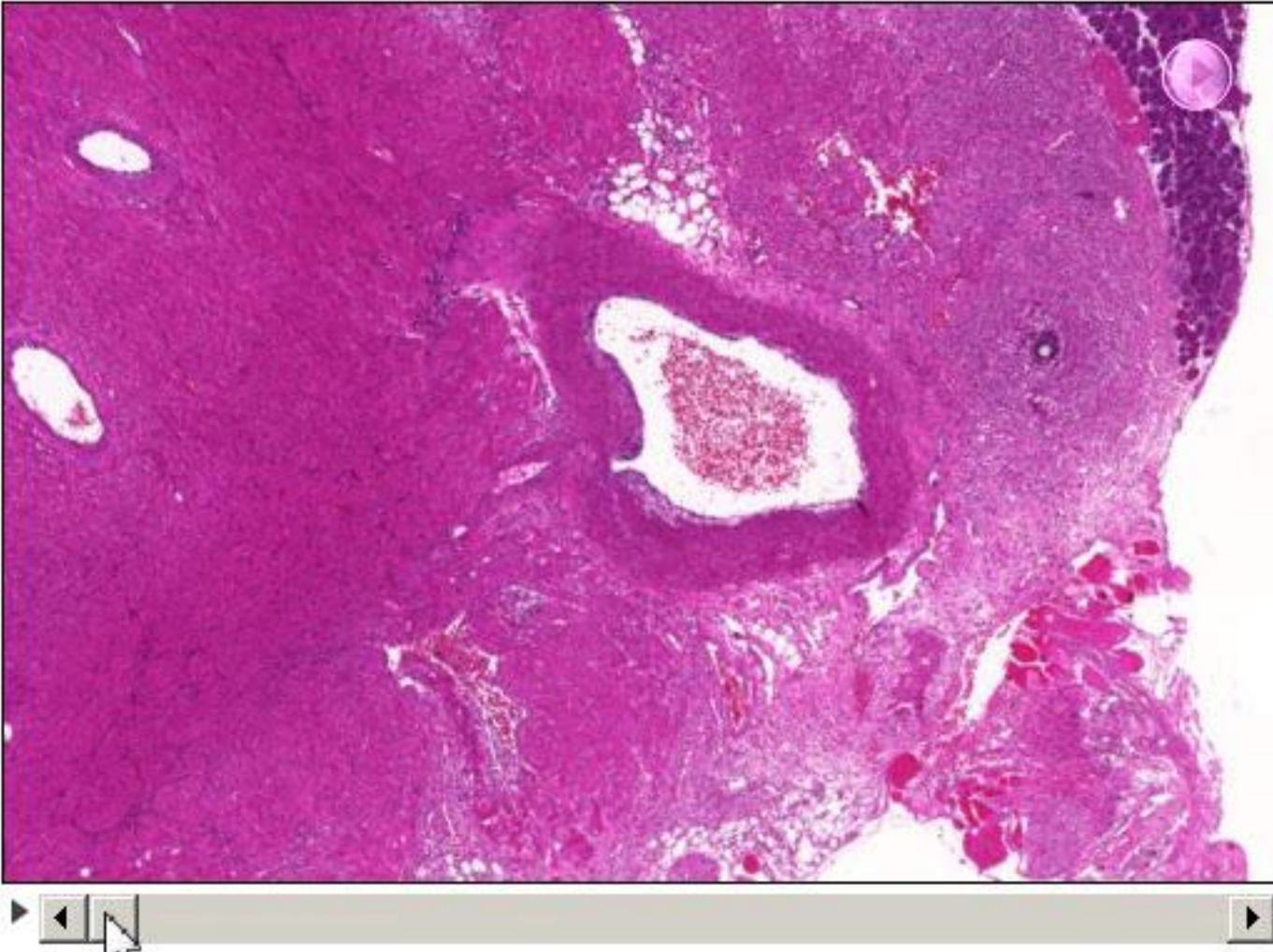
- Even finding the coronary, exact measurement of the thickening is impossible because of the angle of sectioning

Sample 1 (normal, overview from about 287 slides)

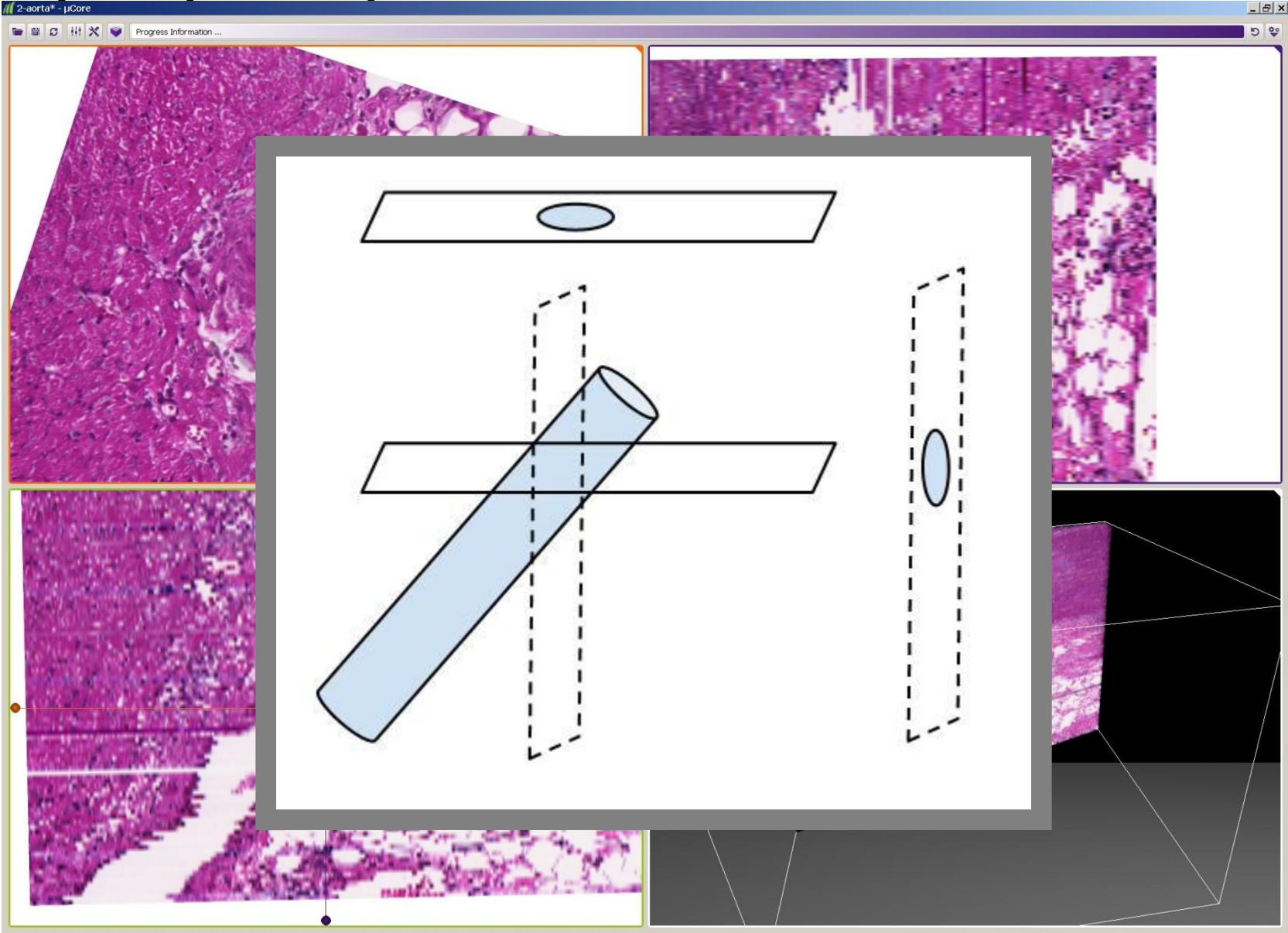




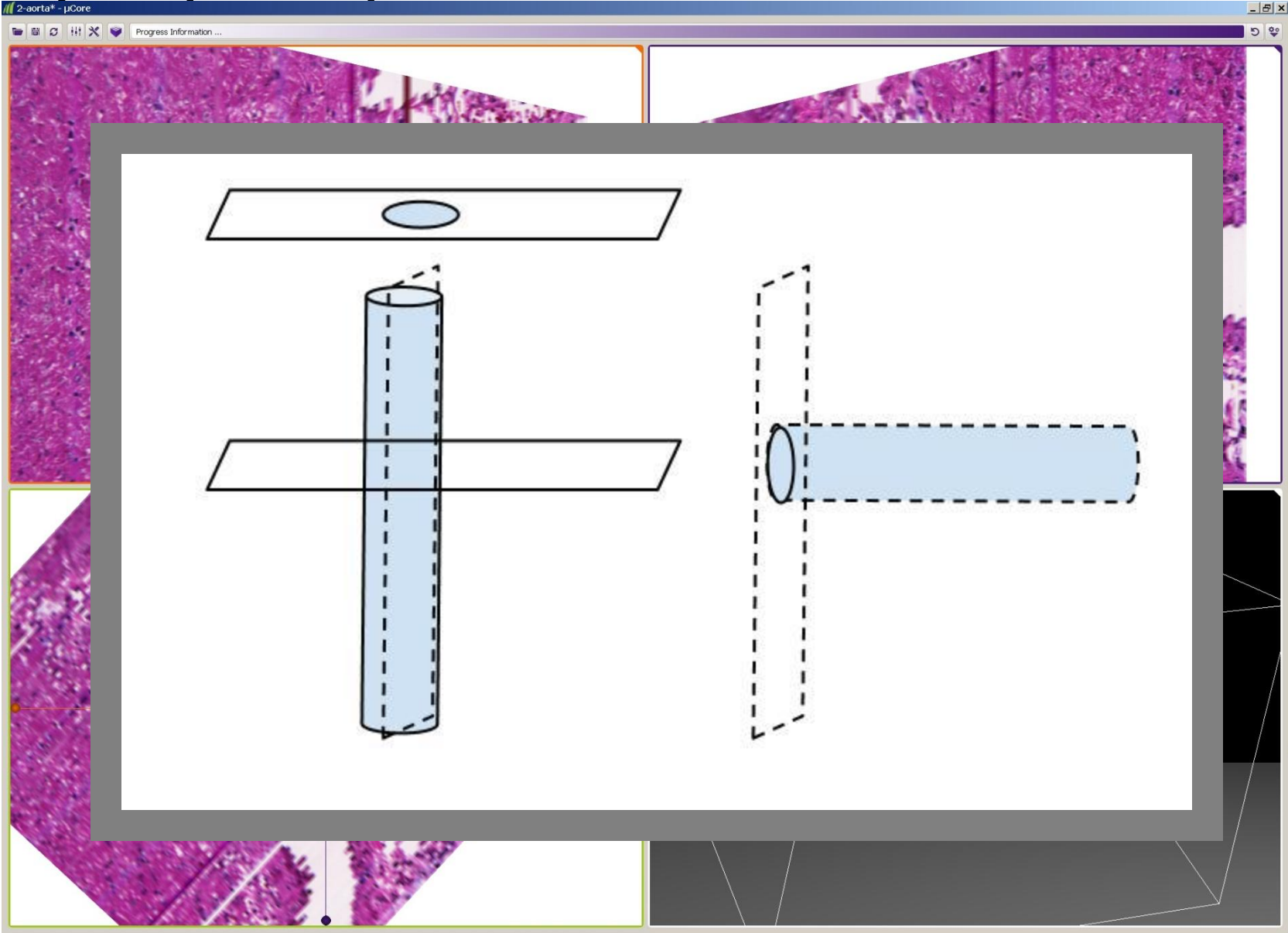
Sample 1 (normal, aorta)



Sample 1 (normal)



Sample 1 (normal)

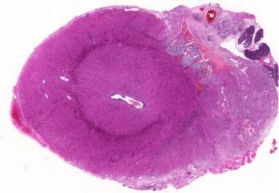


Color Normalization Program

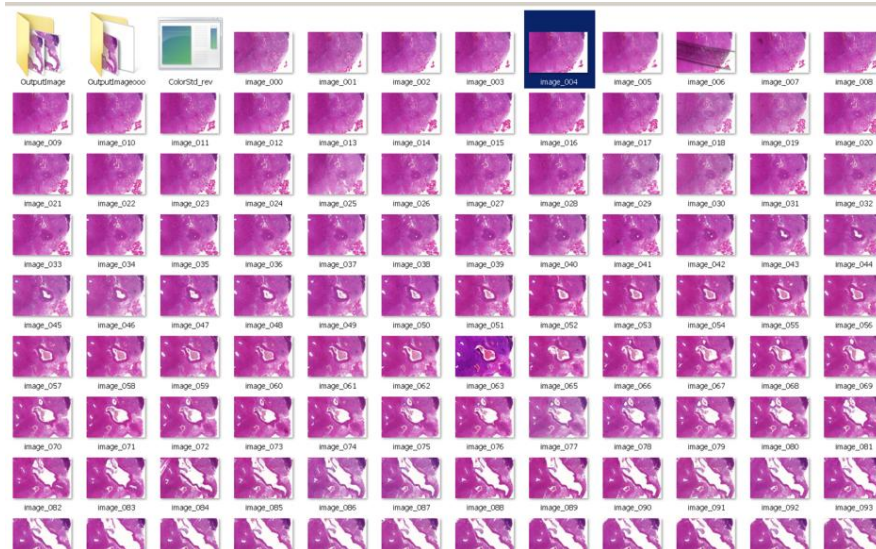
Start Program



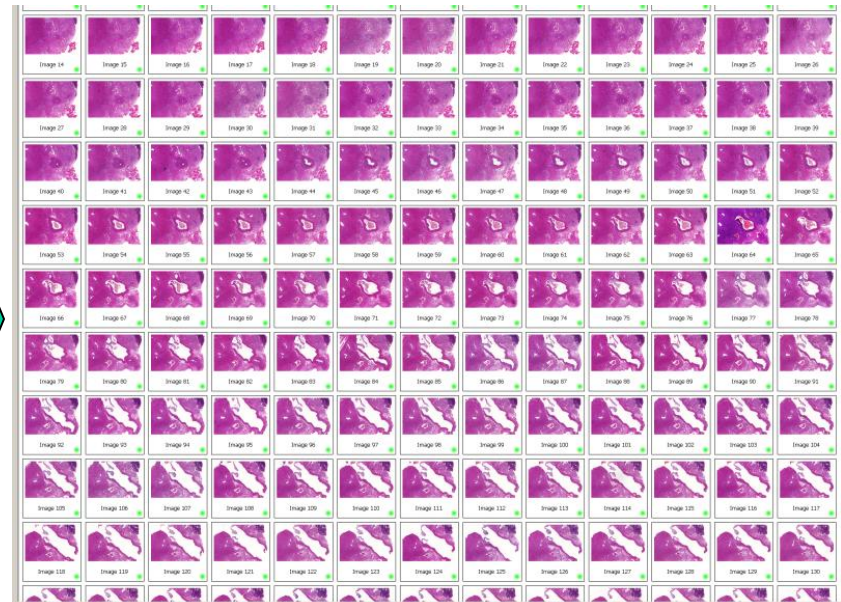
Enter Reseference
(Target Color) Image



Automatically normalize all
images in the folder

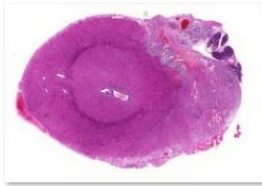


Results go to new folder

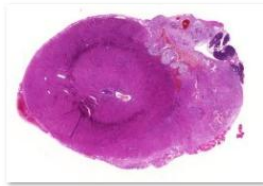




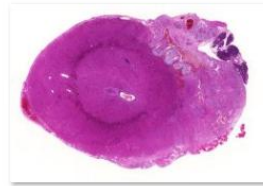
OutputImage



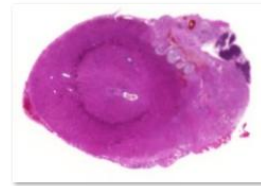
image_001.tif



image_03.tif



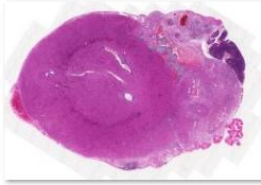
image_04.tif



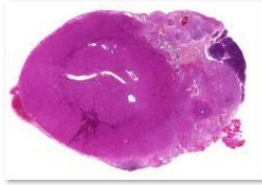
image_005.tif



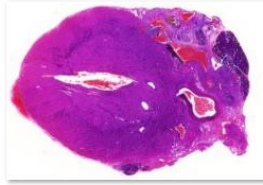
image_006.tif



image_019.tif



image_028.tif



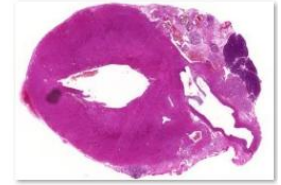
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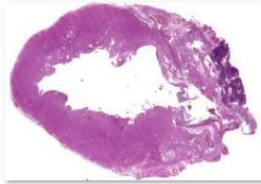
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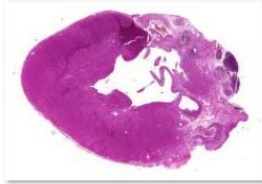
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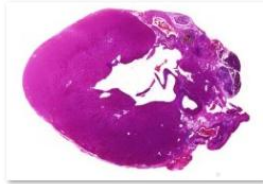
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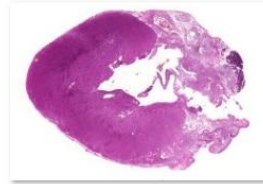
image_000_std



image_001_std



image_03_std



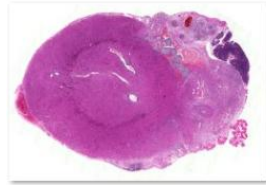
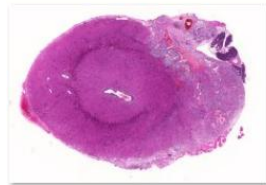
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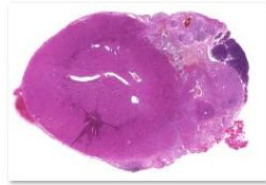
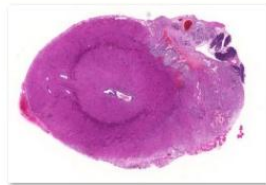
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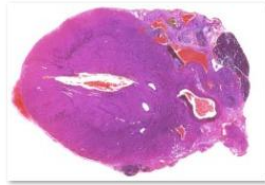
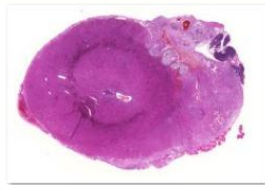
image_006_std



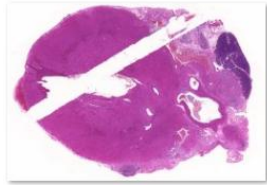
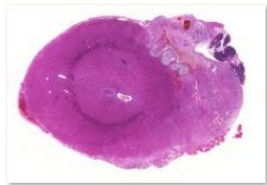
image_019_std



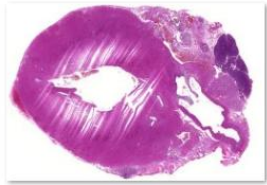
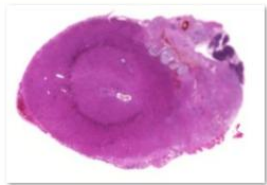
image_028_std



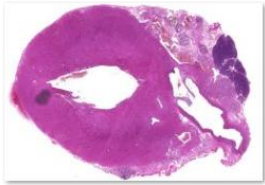
image_063_std



image_071_std



image_084_std

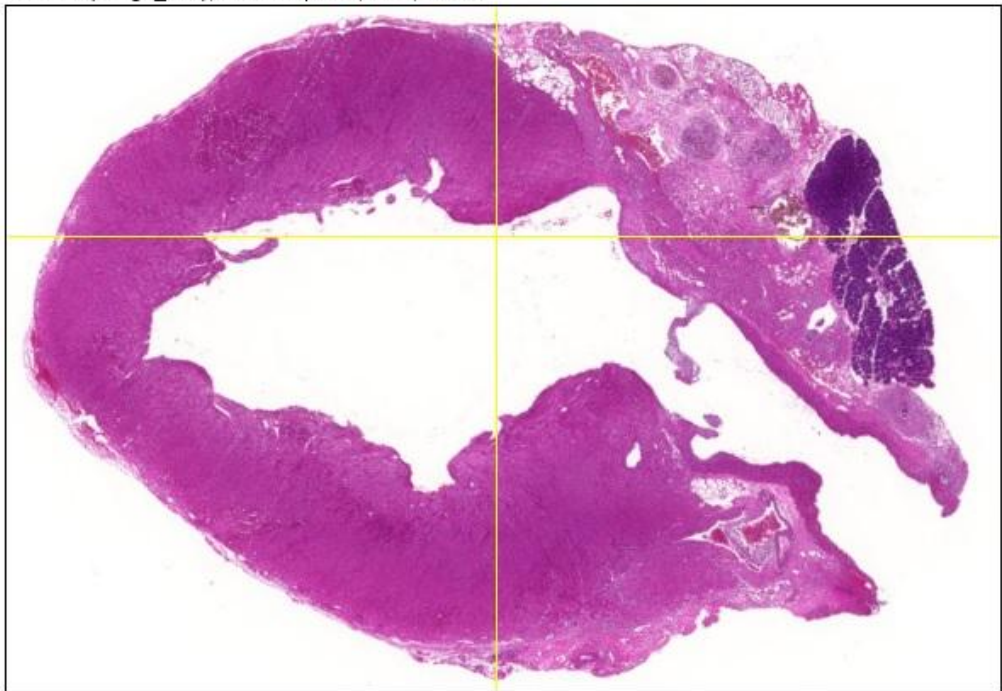


image_088_std

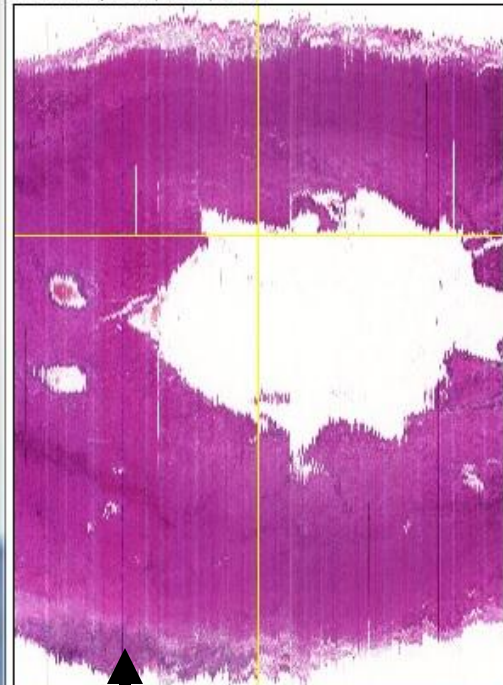


Before color normalization

144/288 (image_143); 585x403 pixels; RGB; 259MB

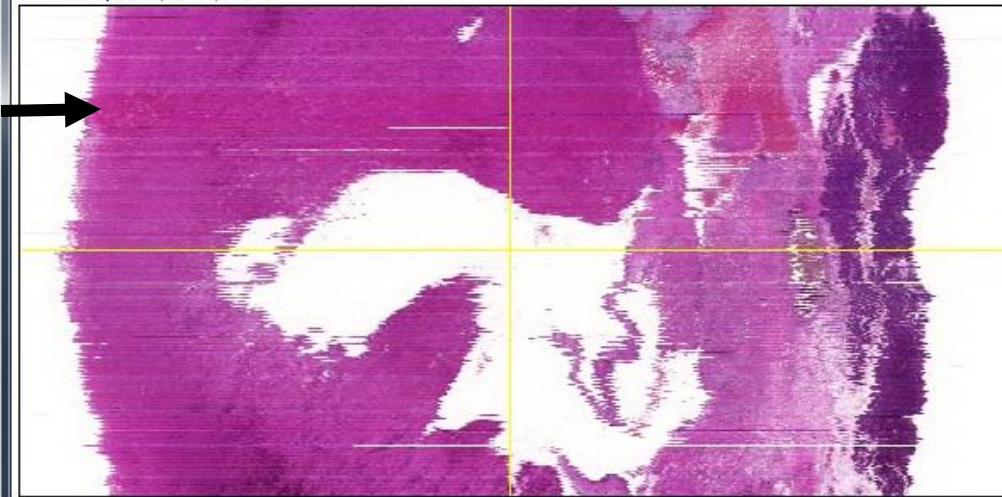


288x403 pixels; RGB; 453K



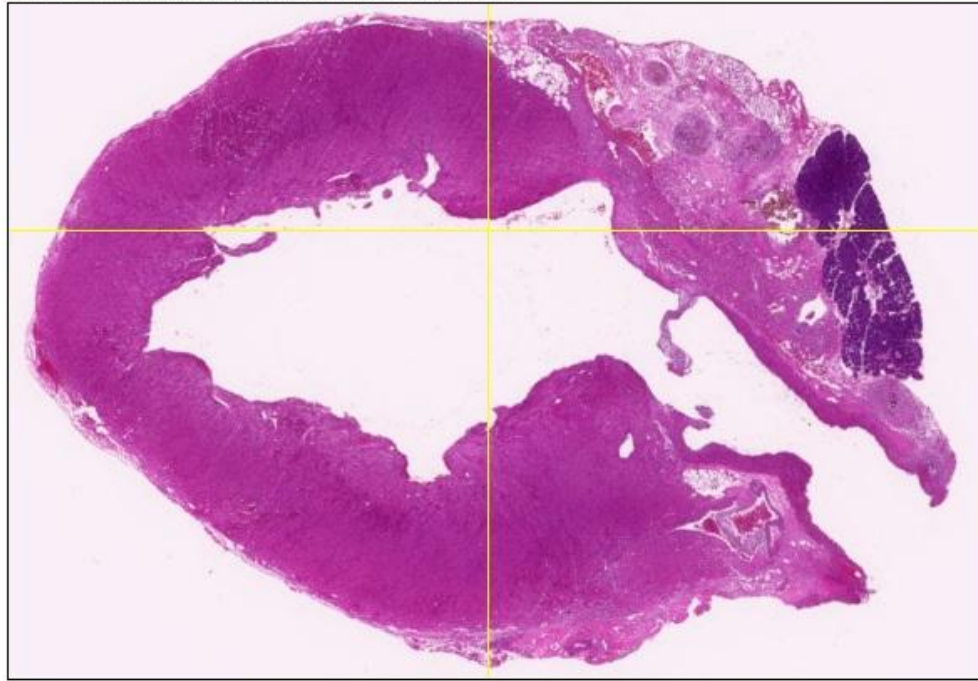
XZ 135

585x288 pixels; RGB; 658K

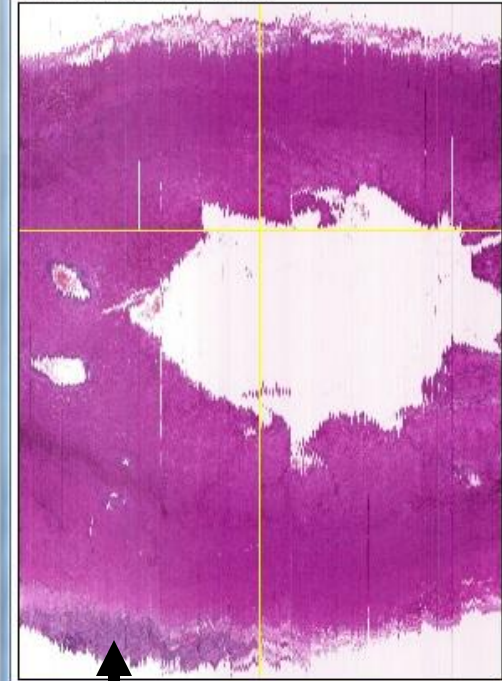


After color normalization

144/288 (image_143_std); 585x403 pixels; RGB; 259MB

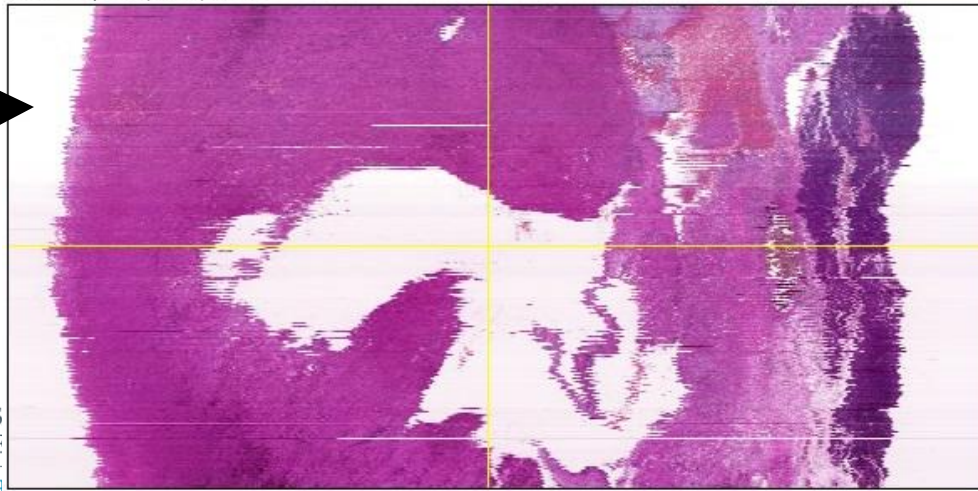


288x403 pixels; RGB; 453K



XZ 135

585x288 pixels; RGB; 658K



Multi-modality imaging

Brain: Glioblastoma

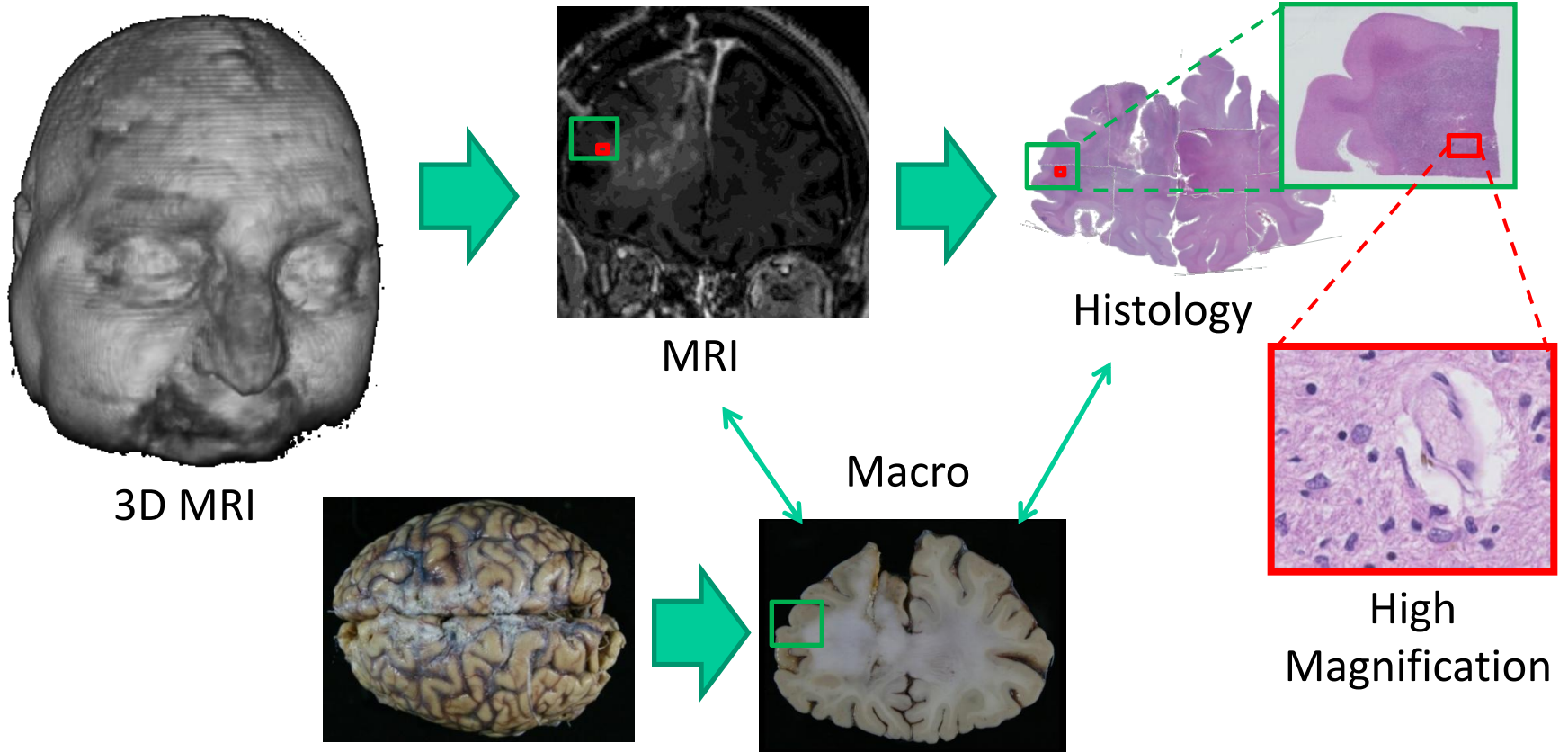


Working with Noriaki Hashimoto, Toru Tanaka,
Hiedeaki Haneishi, Jennie TAYLOR (Clinician),
Matija SNUDERL (Pathologist), Martinos
Center

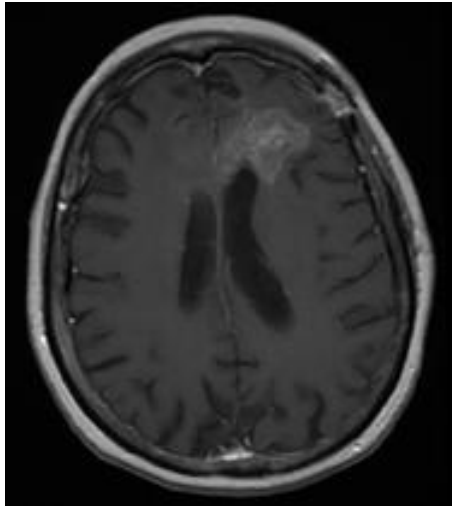
Registration Experiments

- 2D Histology – 2D Macro
- 3D MRI – 2D Macro

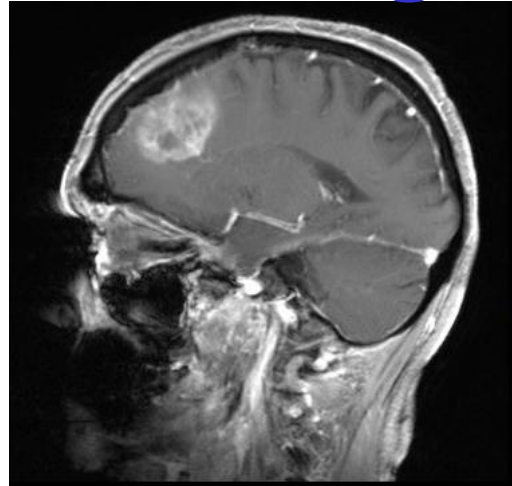
Backgrounds



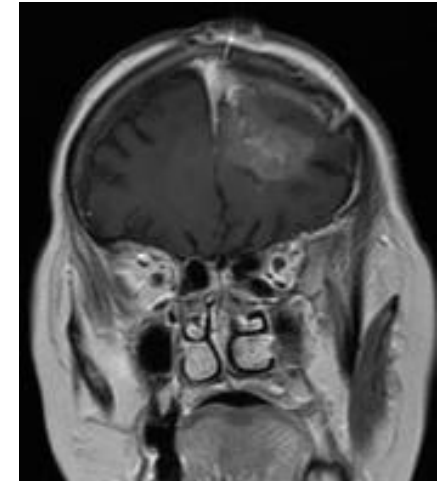
MRI Images



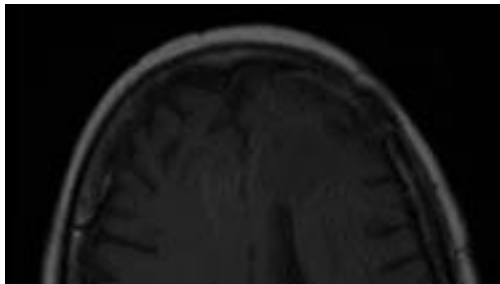
Axial



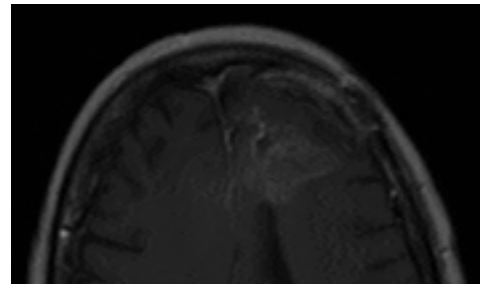
Sagittal



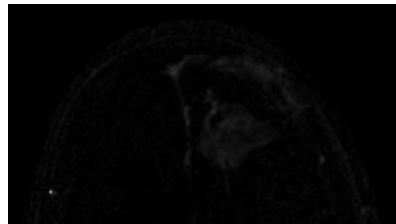
Coronal



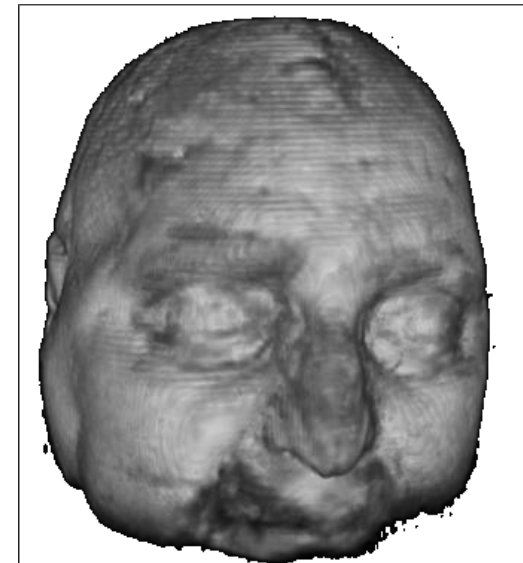
Before Injection



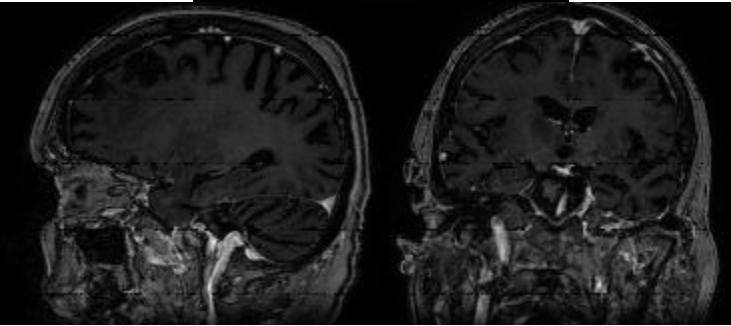
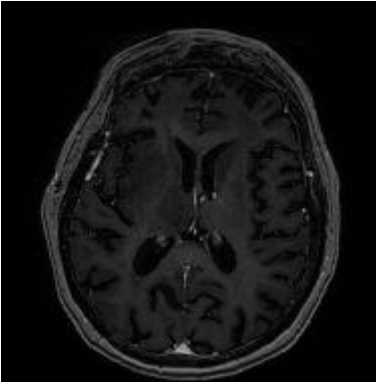
After Injection



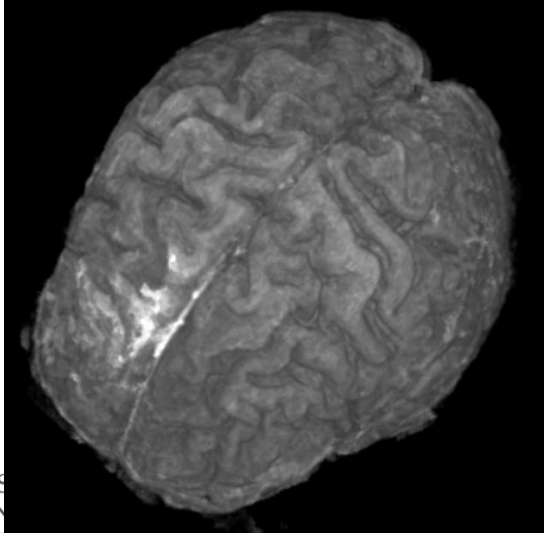
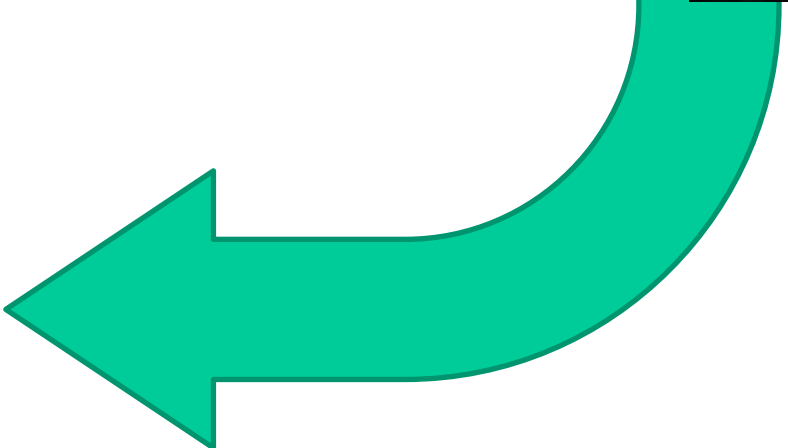
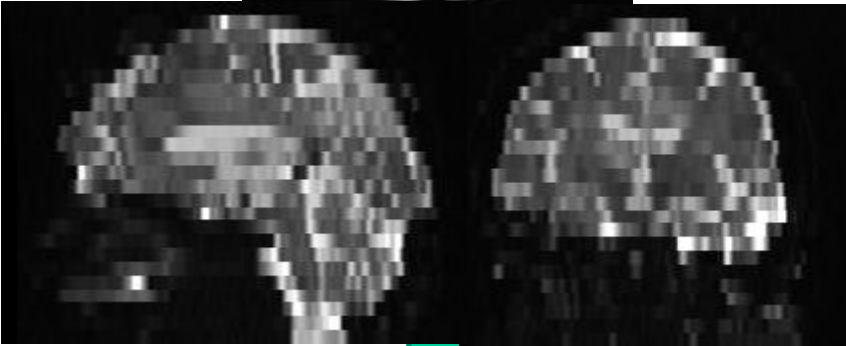
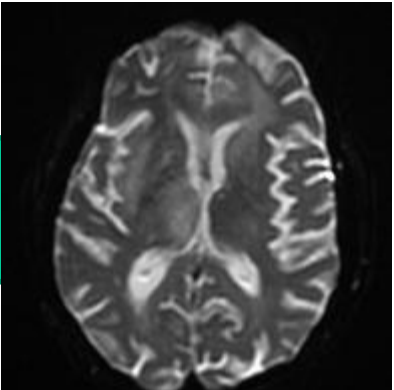
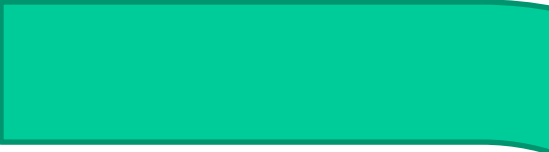
Emphasized Region of Tumor



3D MRI Image

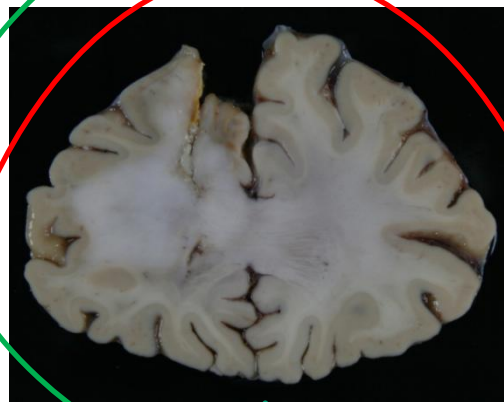


Highest Resolution Image

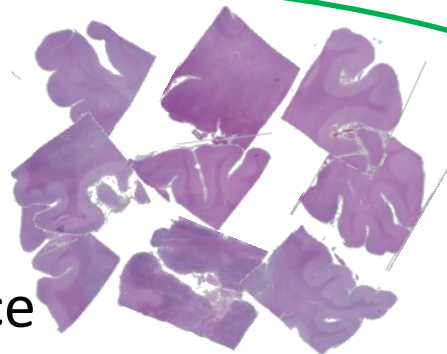


Extracted Brain

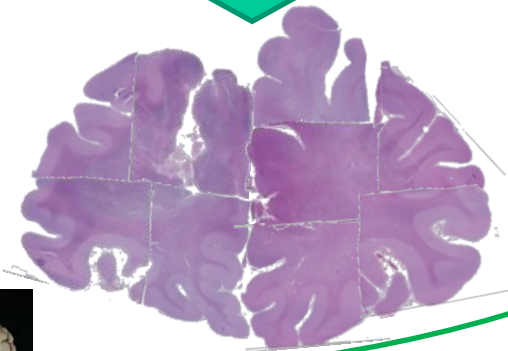
Work Flow



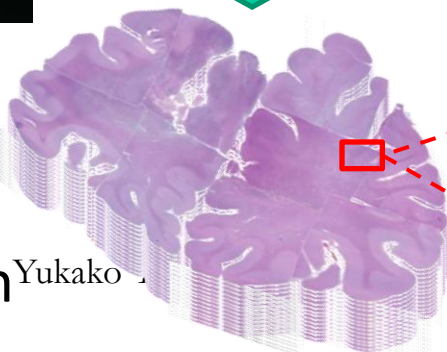
Reference



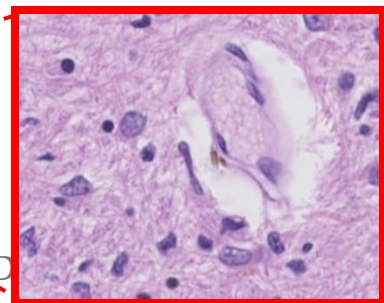
2D Reconstruction



3D Reconstruction



3D Reconstruction



MED

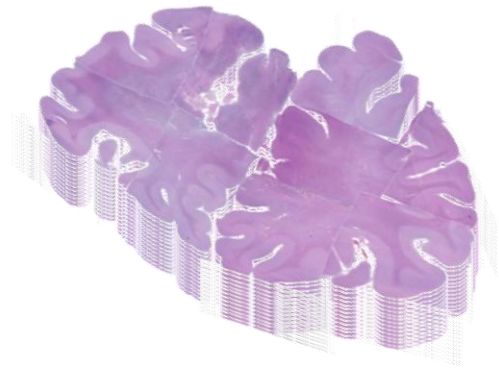


Correlation

Yukako

Reference





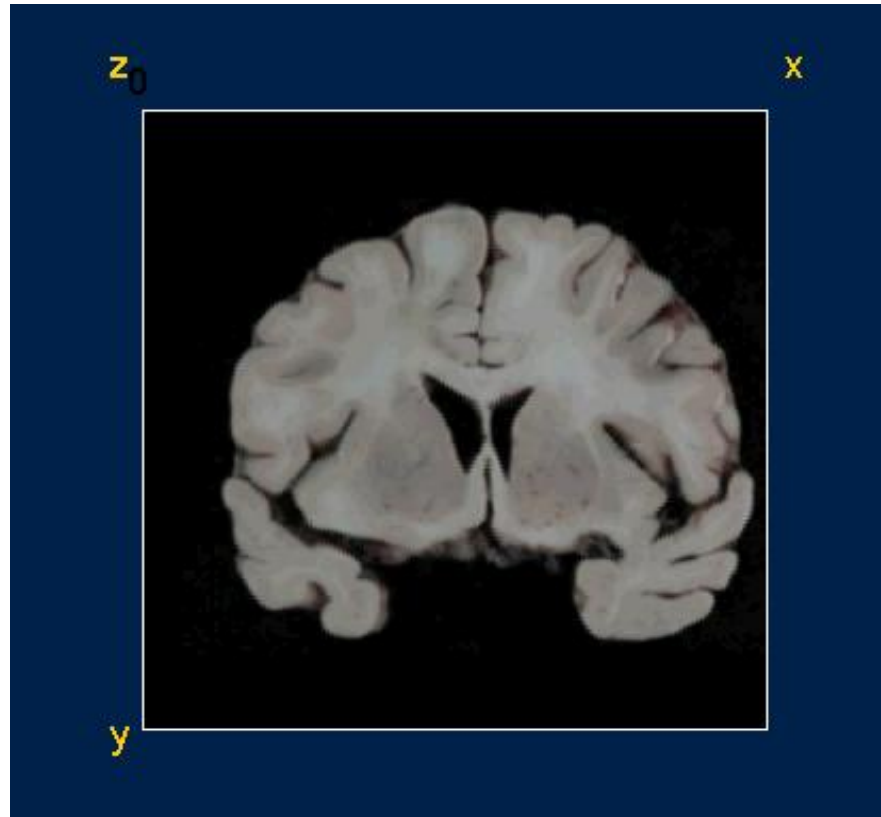
Histology

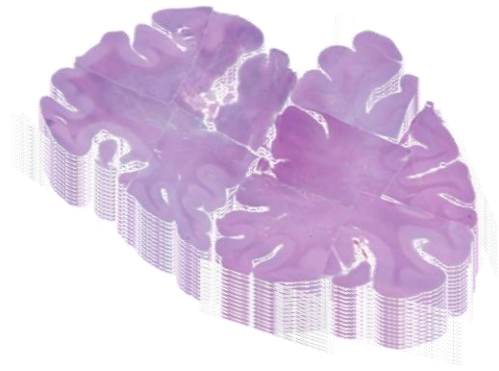


Macro



MRI





Histology



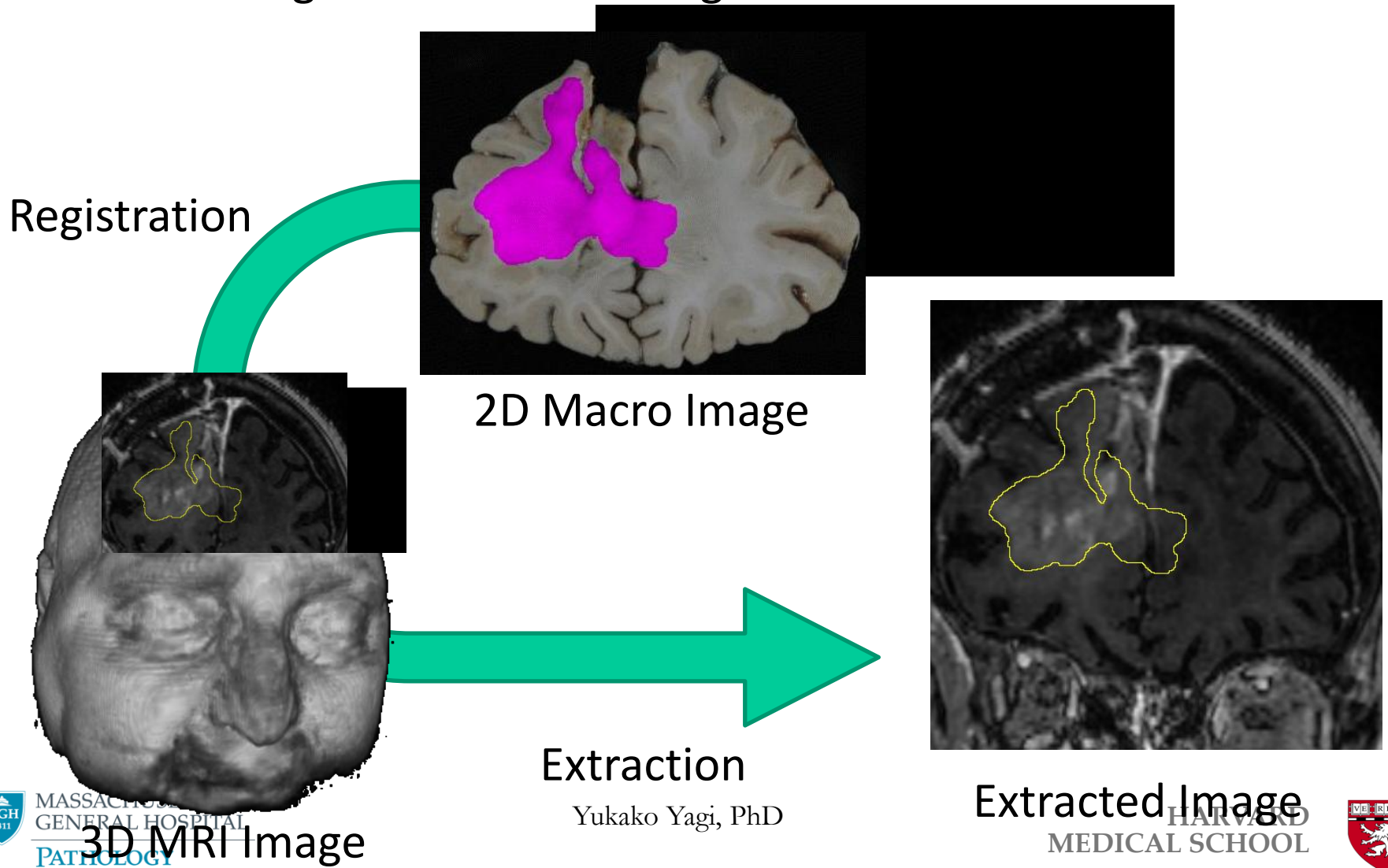
Macro



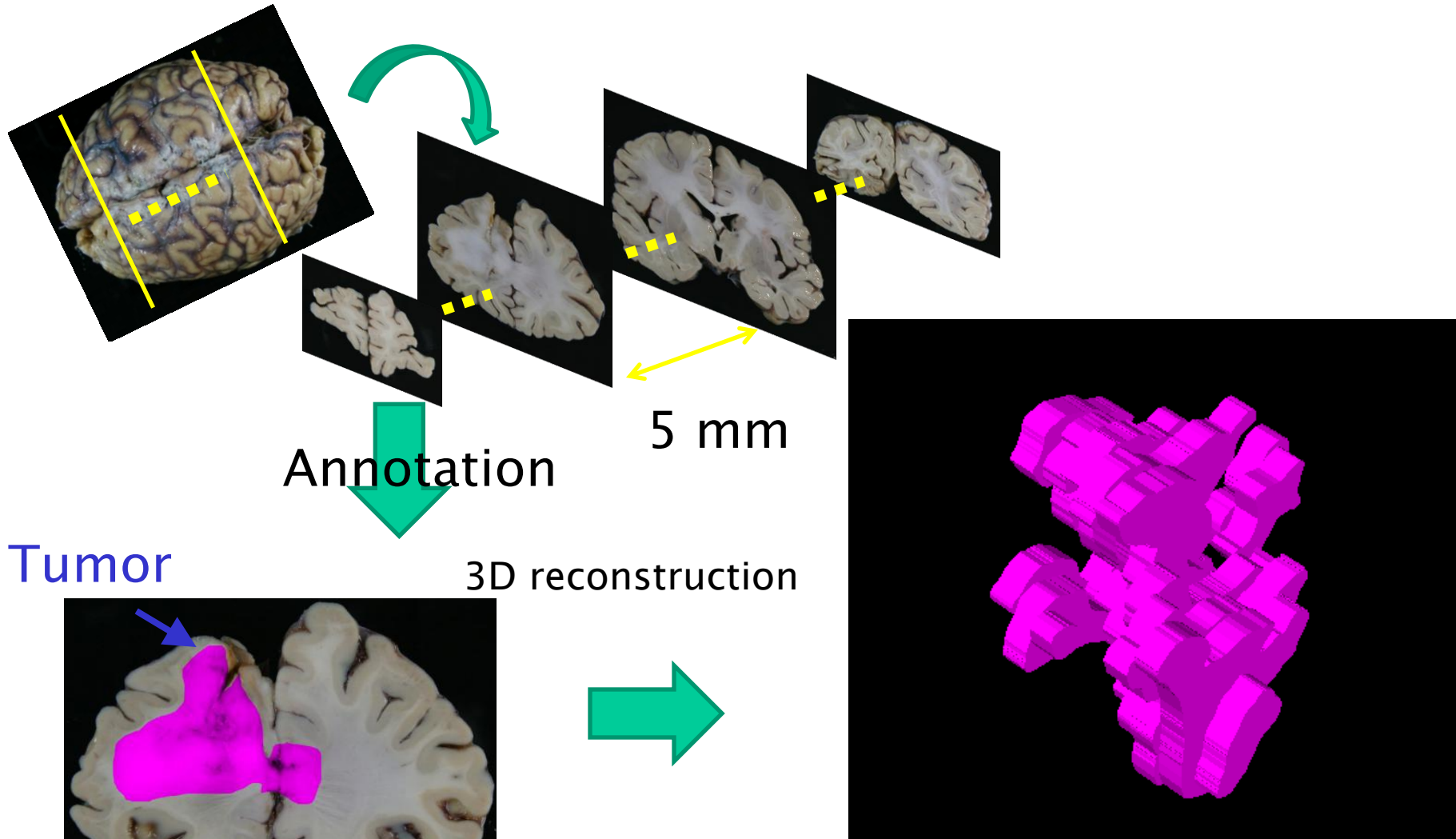
MRI

2D Macro - 3D MRI

This method can extract a section image which is most similar to the macro image from 3D MRI image.



Gross Macro Images



Required Technologies before Whole Brain 3D

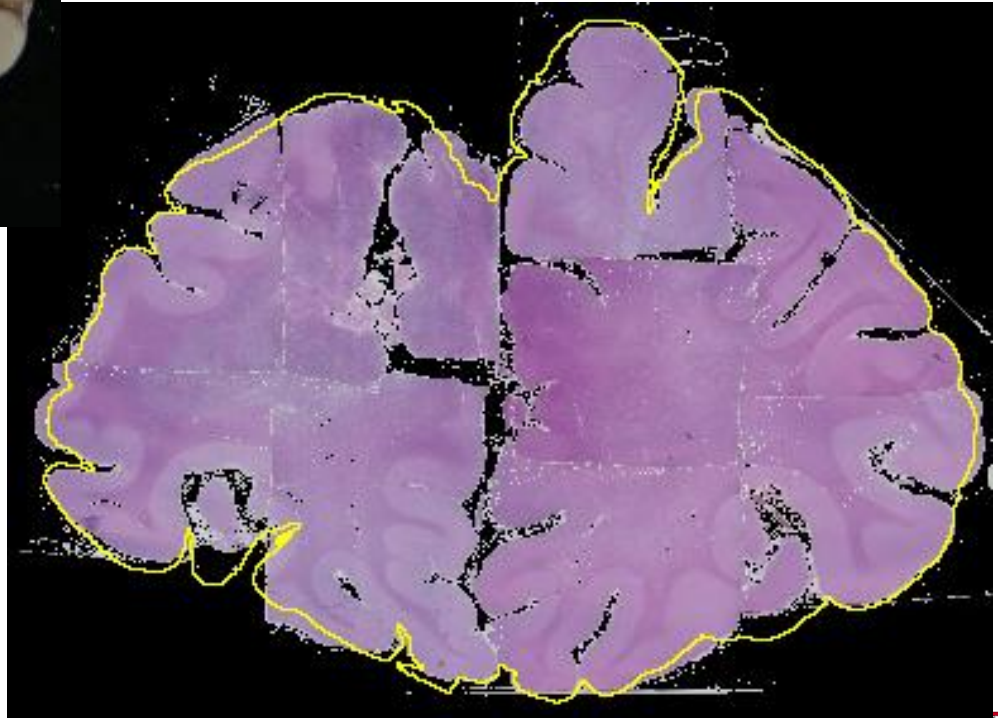


1. Find location of each block



The blocks were handed to us after a clinical diagnosis was rendered..

2. Multiple WSI viewer



Template matching

- Automatic estimation of location and angle
 - Correlation coefficient of each image is calculated at all pixels at different rotation angles
 - Put on the pixel which has the highest correlation
 - $$R \downarrow NCC = \frac{\sum_{j=0}^{N-1} \sum_{i=0}^{M-1} I(i,j)T(i,j)}{\sqrt{\sum_{j=0}^{N-1} \sum_{i=0}^{M-1} I(i,j)^2} \times \sqrt{\sum_{j=0}^{N-1} \sum_{i=0}^{M-1} T(i,j)^2}}$$

Template T

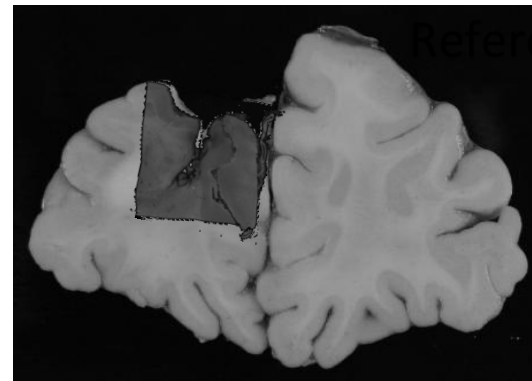
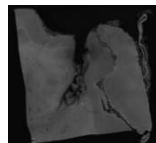
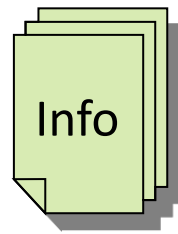
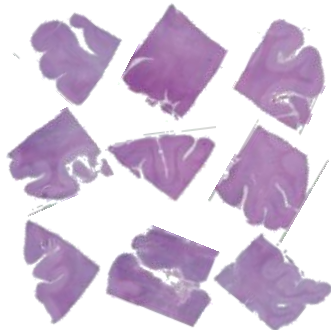


Image I

Fast registration using low-resolution image

- Export information of location and rotation using low-resolution images (less than 1x)
 - Information is used for merging high-resolution images

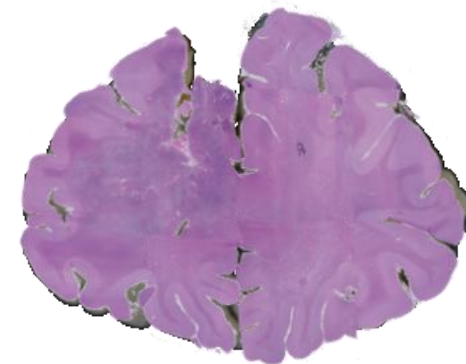
Low-resolution images from multiple tissues



Location and rotation angle for each block



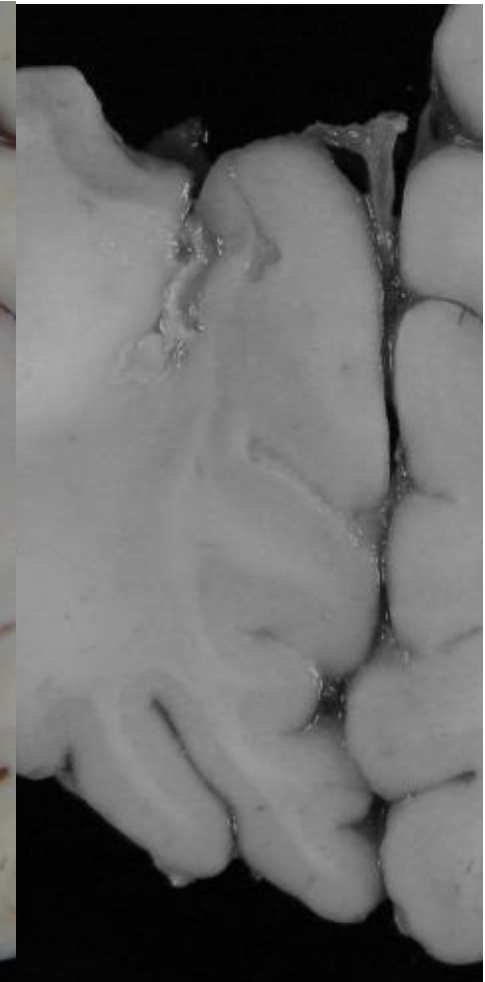
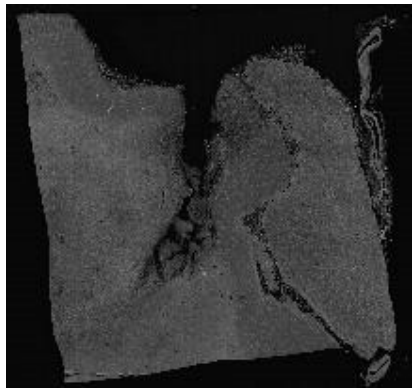
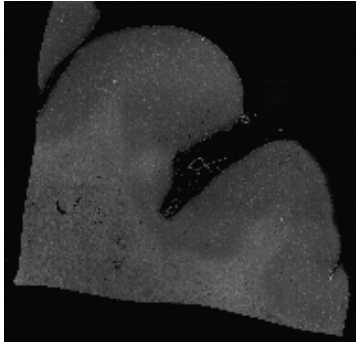
Registration



Low-resolution image of entire tissue

Yukako Yagi, PhDAPIII 2008.

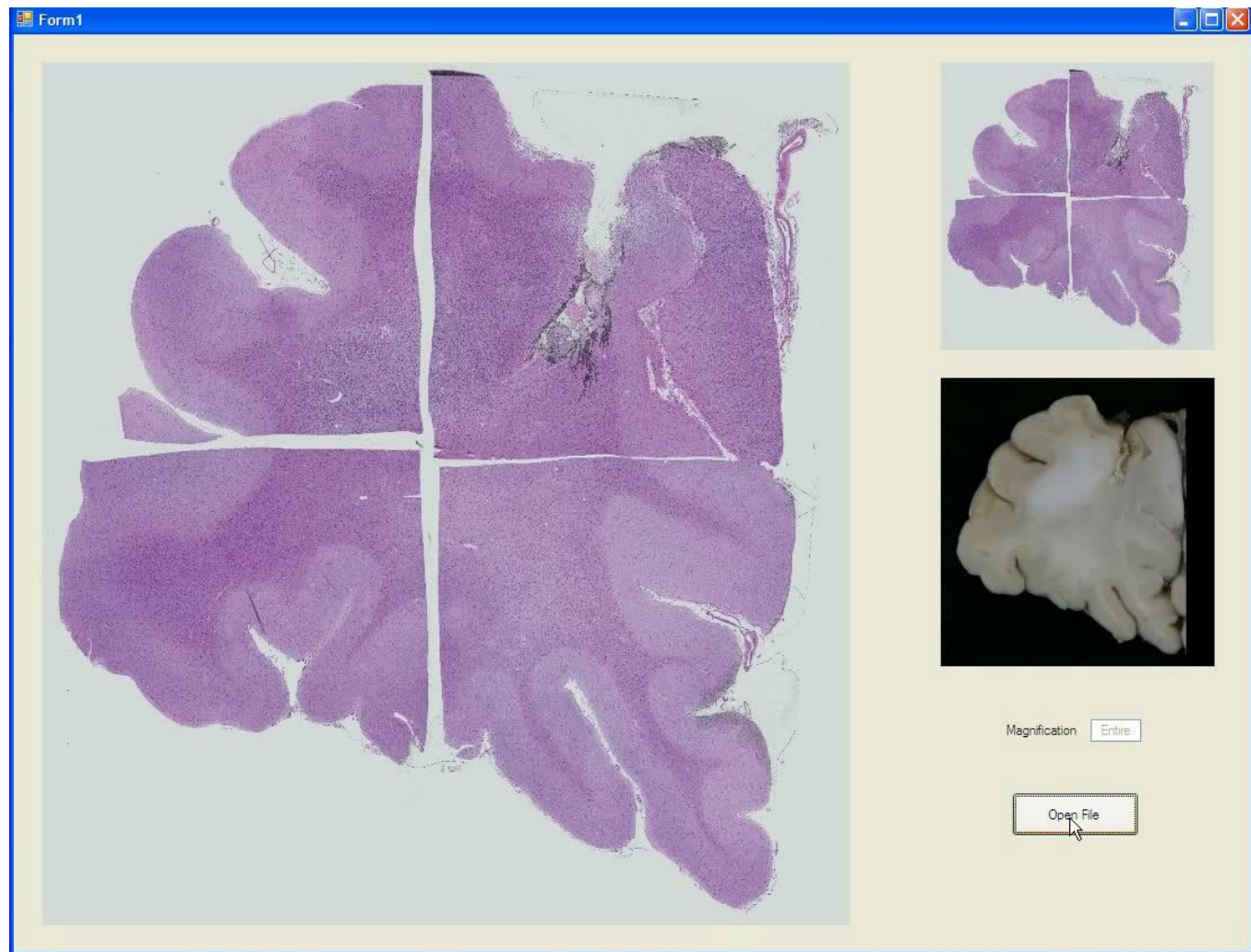
Results



Results



Multiple WSI viewer



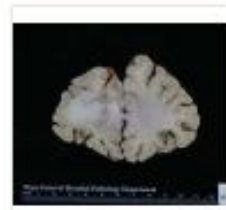
Results of all slices



A11-397 Slice 2.JPG



A11-397 Slice 3.JPG



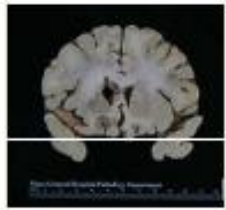
A11-397 Slice 4.JPG



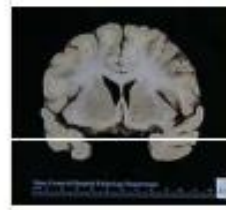
A11-397 Slice 5.JPG



A11-397 Slice 6.JPG



A11-397 Slice 7.JPG



A11-397 Slice 8.JPG



A11-397 Slice 9.JPG



A11-397 Slice 10.JPG



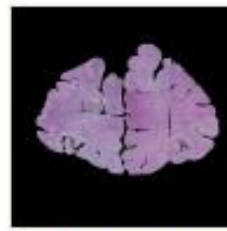
A11-397 Slice 11.JPG



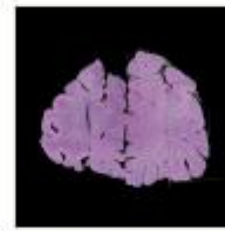
S02RGB.tif



S03RGB.tif



S04RGB.tif



S05RGB.tif



S06RGB.tif



S07RGB.tif



S08RGB.tif



S09RGB.tif

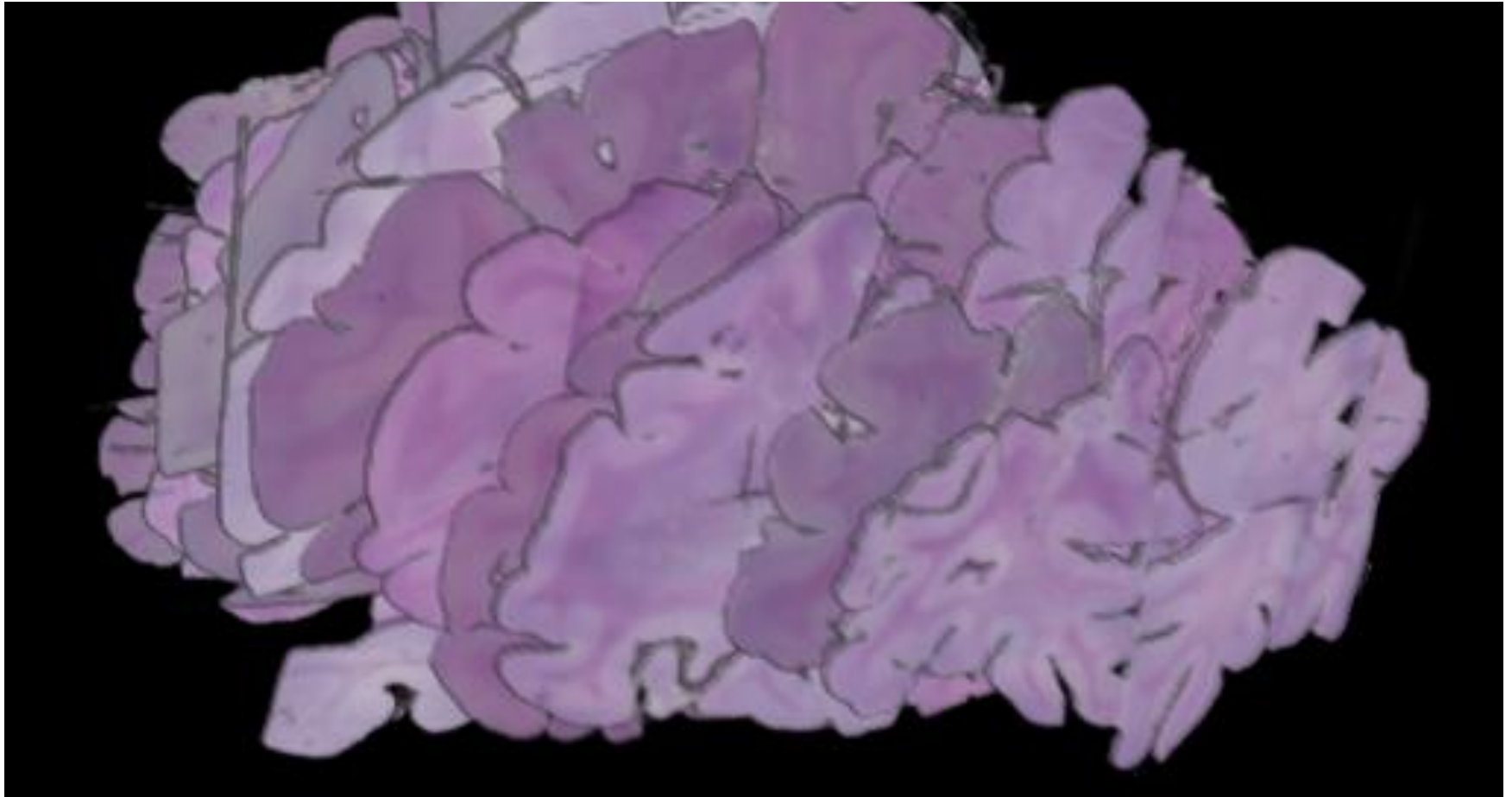


S10RGB.tif

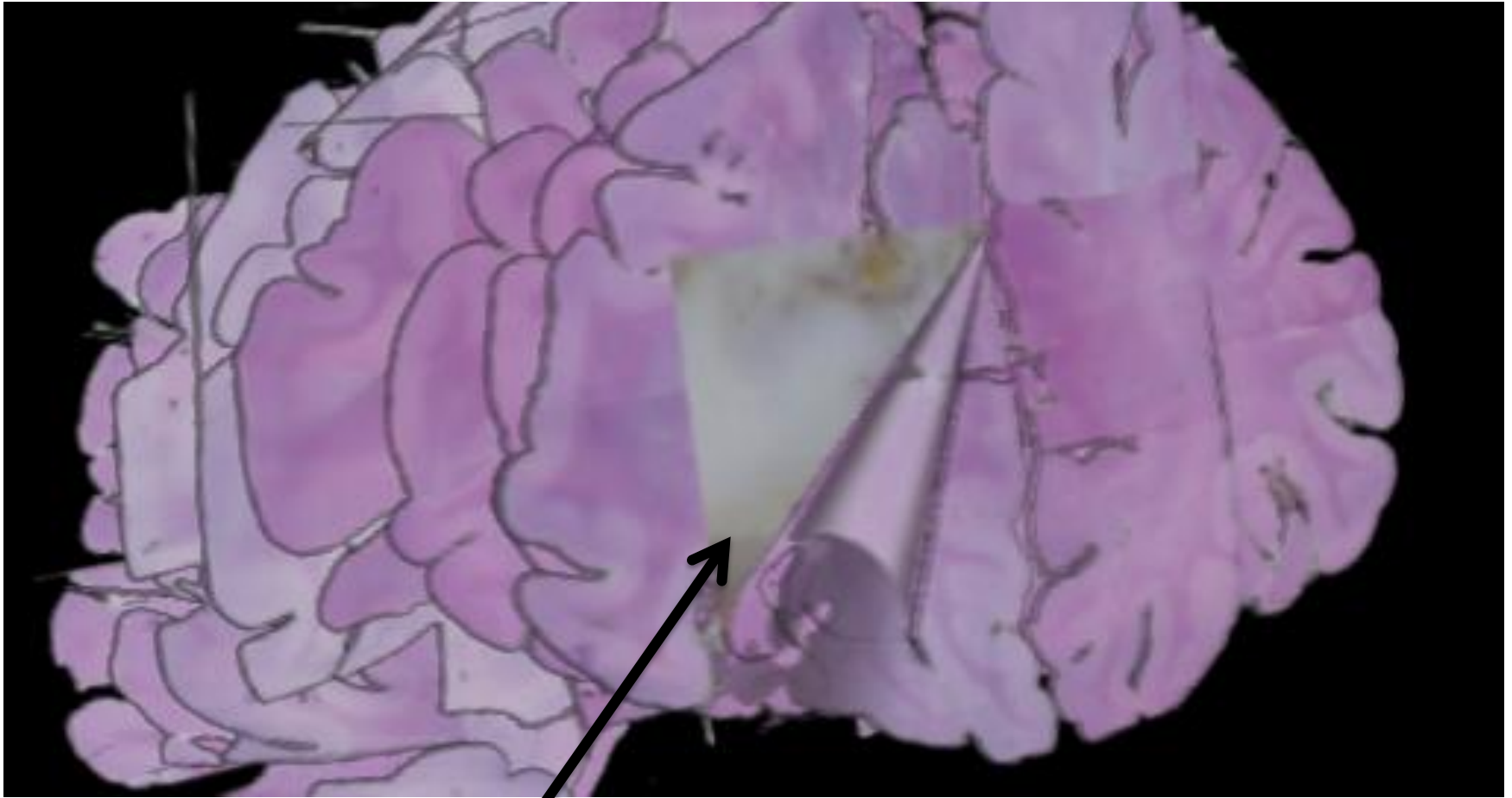


S11RGB.tif

3D Images from 9 slices



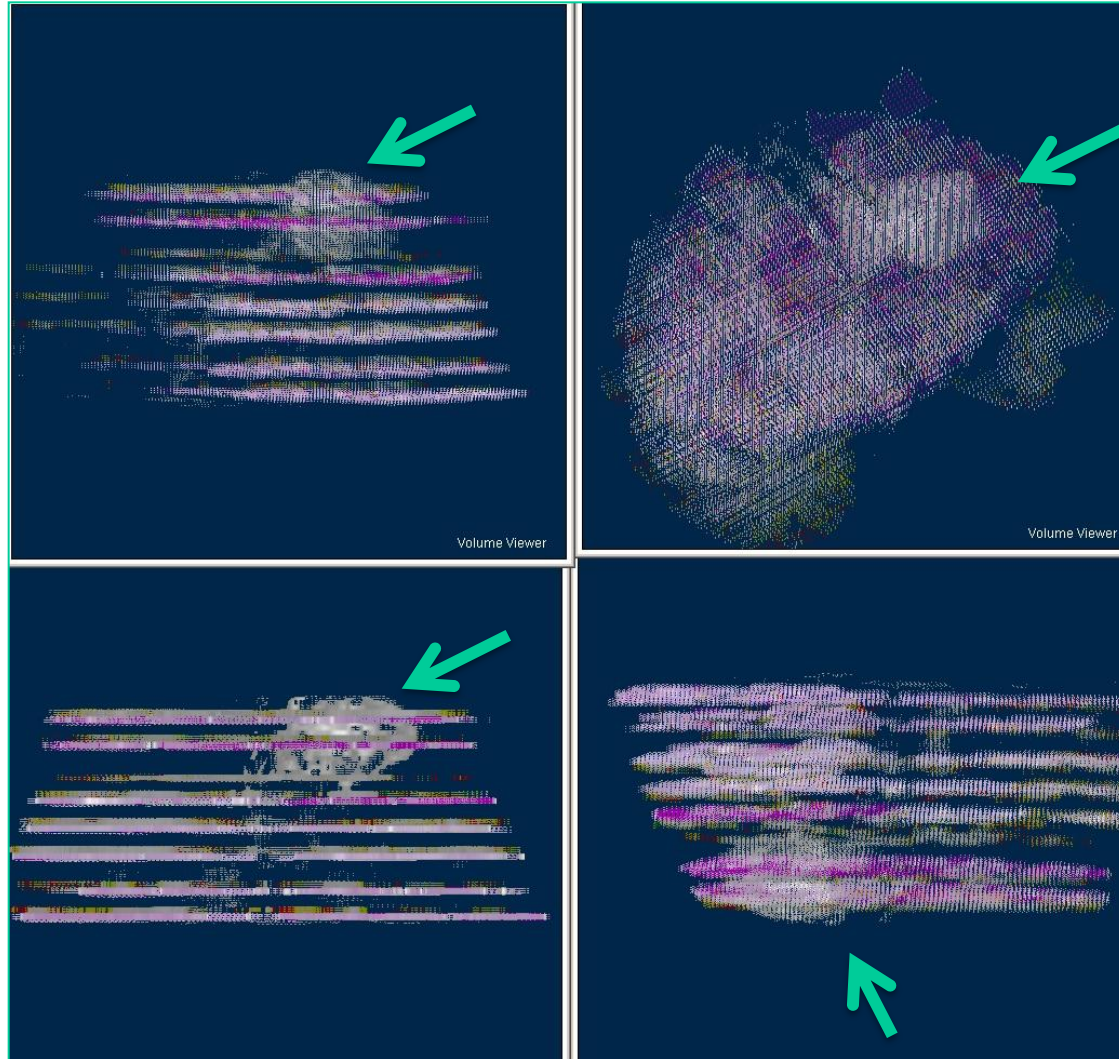
3D Images from 9 slices



Gross macro images are behind histology images

Yukako Yagi, PhDAPIII 2008

Histology, Macro and MRI 3D of Whole Brain



Tumor?

Histology, Macro and MRI 3D of Whole Brain

- There are still many things to overcome to successfully create Whole Brain Histology 3D image
- We would like to have a whole brain to make a perfect multi-modality 3D imaging model

Summary

- WSI based histology 3D imaging is becoming very popular and it is showing the important role in Pathology research.
- Data analysis with other modalities, such as radiology, molecular data, and more is important
- Producing Accurate and reliable image data is the key for the future of digital pathology
- Scanners with functionality which fit to a specific purpose will be required.

Summary

Image Application:
Combination of morphological analysis and spectral analysis

Tissue Processing



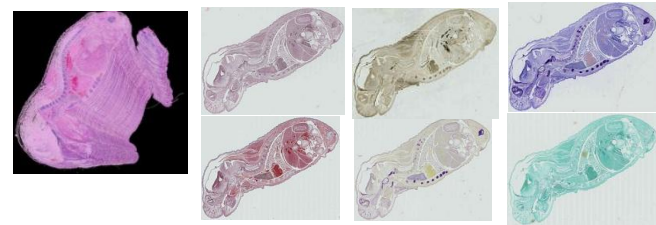
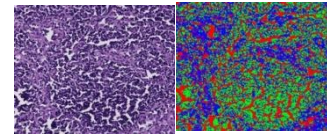
Accurate Results require Good Images, Good images require good slide, good slides require good block.....

Paraffin block

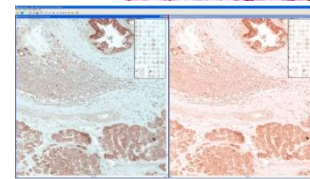
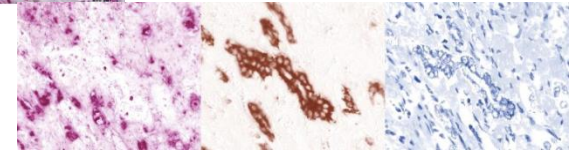
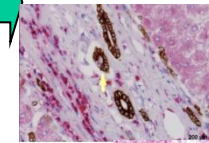
Sectioning



Scanning



Digital Stains

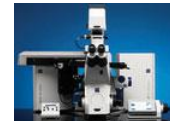


.....

Staining
Cover glass



Stained slides



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- Authors acknowledge to all the collaborators, Toru Tanaka,, Drs. László Fónyad, Kazunobu Shinoda, Evan A. Farkash, Divya P. Sebastian, Robert B. Colvin, Mari Mino-Kenudson, Veronica Klepies, Pinky Bautista, Jennie Taylor, Matija Snuderl, Noriaki Hashimoto.





Thank You!

