COLLEGE of AMERICAN PATHOLOGISTS

New histology, same rules?

Approach to validation of slide-free multiphoton histology for clinical use

Richard Torres, MD, MS
DCPC Webinar Series

April 6, 2022

Richard Torres, MD, MS, FACP

- Adjunct Associate Professor of Laboratory Medicine, Yale School of Medicine
- Co-Founder, Co-CEO, Applikate Technologies, Inc.
- Practicing hematopathologist
- Research areas: flow cytometry, medical laboratory instrumentation, multiphoton microscopy

Disclaimer

- The CAP does not permit reproduction of any substantial portion of the material in this Webinar without its written authorization. The CAP hereby authorizes attendees of the CAP Webinar to use the PDF presentation solely for educational purposes within their own institutions. The CAP prohibits use of the material in the Webinar – and any unauthorized use of the CAP's name or logo – in connection with promotional efforts by marketers of laboratory equipment, reagents, materials, or services.
- Opinions expressed by the speaker are the speaker's own and do not necessarily reflect an endorsement by the CAP of any organizations, equipment, reagents, materials, or services used by participating laboratories.

Disclaimer

- **The Clearing Histology with MultiPhoton platform that will be discussed has not been evaluated by the FDA and is not yet approved for primary diagnosis in the US. The performance characteristics described are for the purpose of engendering dialogue and education, and not meant as an endorsement of clinical use.**

Disclosure

- **Current management role and ownership interest in Applikate Technologies, Inc., a company commercializing the Clearing Histology with MultiPhoton (CHiMP) platform**

Sections

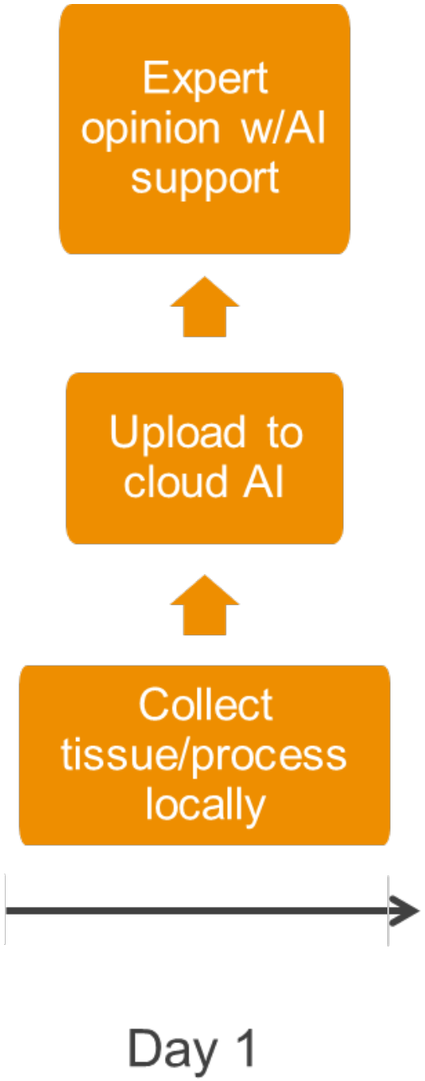
- **Motivation for multiphoton microscopy**
- Physical slides, WSI, and multiphoton comparative risks
- Validation considerations from pathologist point of view
- Validation examples
- Conclusions

Quoted use cases for ex vivo microscopy (EVM)

- Preliminary intraoperative evaluation (e.g. margins)
- Adequacy of core biopsies
- Adequacy of transplant tissue
- Identification of tissue for ancillary studies
- Identification of tissue for biorepositories

The true potential of EVM

- A replacement for physical slides / whole slide imaging
  - Faster turnaround time
  - Direct path to digital – consultation and digital analysis
  - Reduced labor (cost) requirements
  - More levels, more data, 3D patterns
  - Preserved tissue for ancillary studies

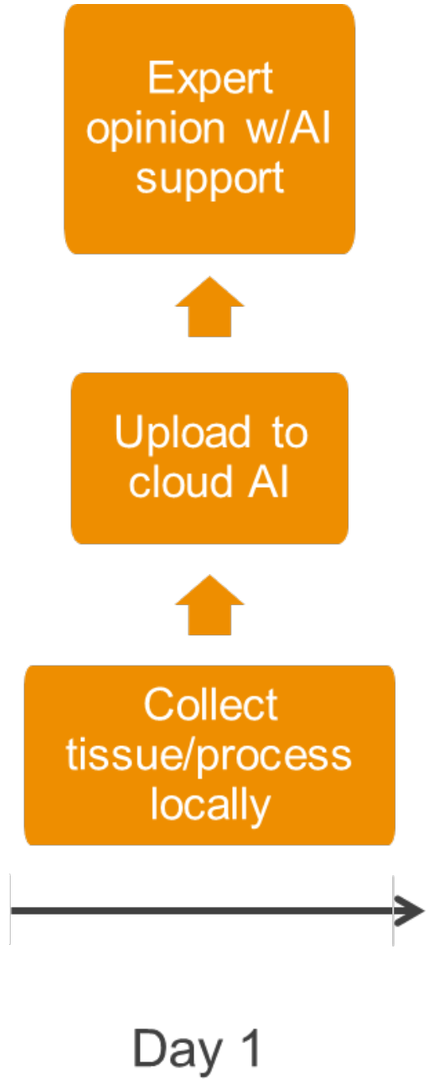


The true potential of EVM

- A replacement for physical slides / whole slide imaging
  - Faster turnaround time
  - Direct path to digital – consultation and digital analysis
  - Reduced labor (cost) requirements
  - More levels, more data, 3D patterns
  - Preserved tissue for ancillary studies

What is holding us back?

Inferior image quality would mean physical slides still required



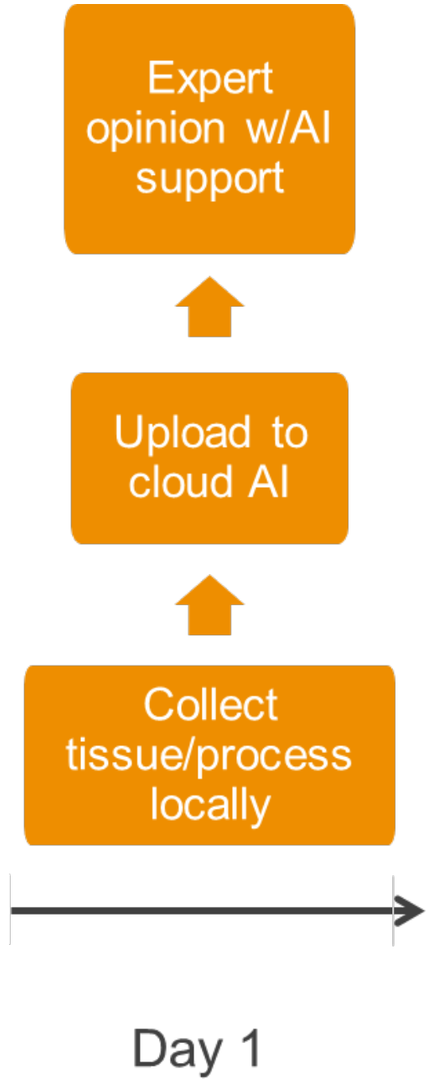
The true potential of EVM

- A replacement for physical slides / whole slide imaging
  - Faster turnaround time
  - Direct path to digital – consultation and digital analysis
  - Reduced labor (cost) requirements
  - More levels, more data, 3D patterns
  - Preserved tissue for ancillary studies

What is holding us back?

Inferior image quality would mean physical slides still required

+ cost + effort + labor



The whole slide imaging (WSI) parallel

- **Ease of remote review and consultation could improve diagnostic accuracy/precision**
- **Digital analysis tools could improve diagnostic accuracy/precision**

What is holding us back?

Inferior image quality would mean physical slides still required

The whole slide imaging (WSI) parallel

- **Ease of remote review and consultation could improve diagnostic accuracy/precision**
- **Digital analysis tools could improve diagnostic accuracy/precision**

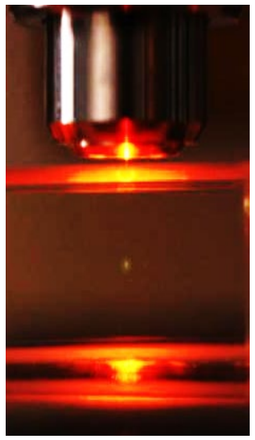
What is holding us back?

Inferior image quality would mean physical slides still required

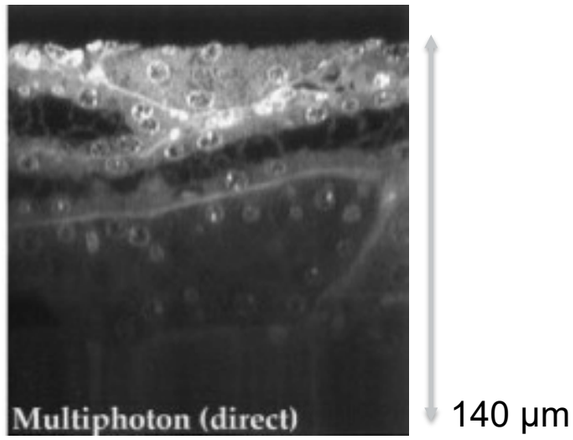
+ cost + effort + labor

Multiphoton has high image quality potential

- Focusing a pulsed infrared laser generates sufficient energy to excite fluorescence in only a tiny volume



Steve Ruzin, UCBerkley



Centonze et al, Biophys J, 1998

- Features:
  - High resolution potential in thick tissue
  - Some depth
  - Low tissue damage risk
- Perceived limitations
  - Inferior images to physical slides/WSI
  - Expensive
  - Complicated to set up and use
  - Additional personnel

CHiMP is a multiphoton approach for histology

CHiMP = Clearing Histology with MultiPhoton Microscopy

Collect tissue

Place in holder

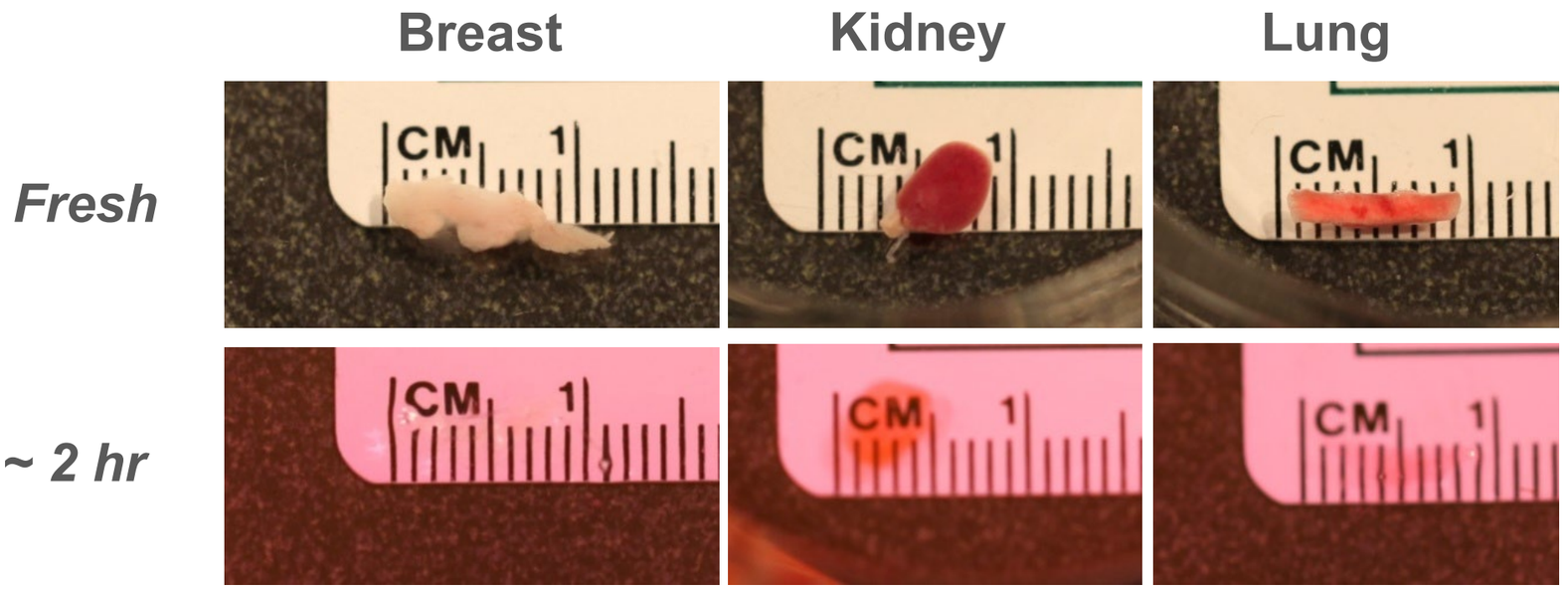
Process to clearing

Place in imager

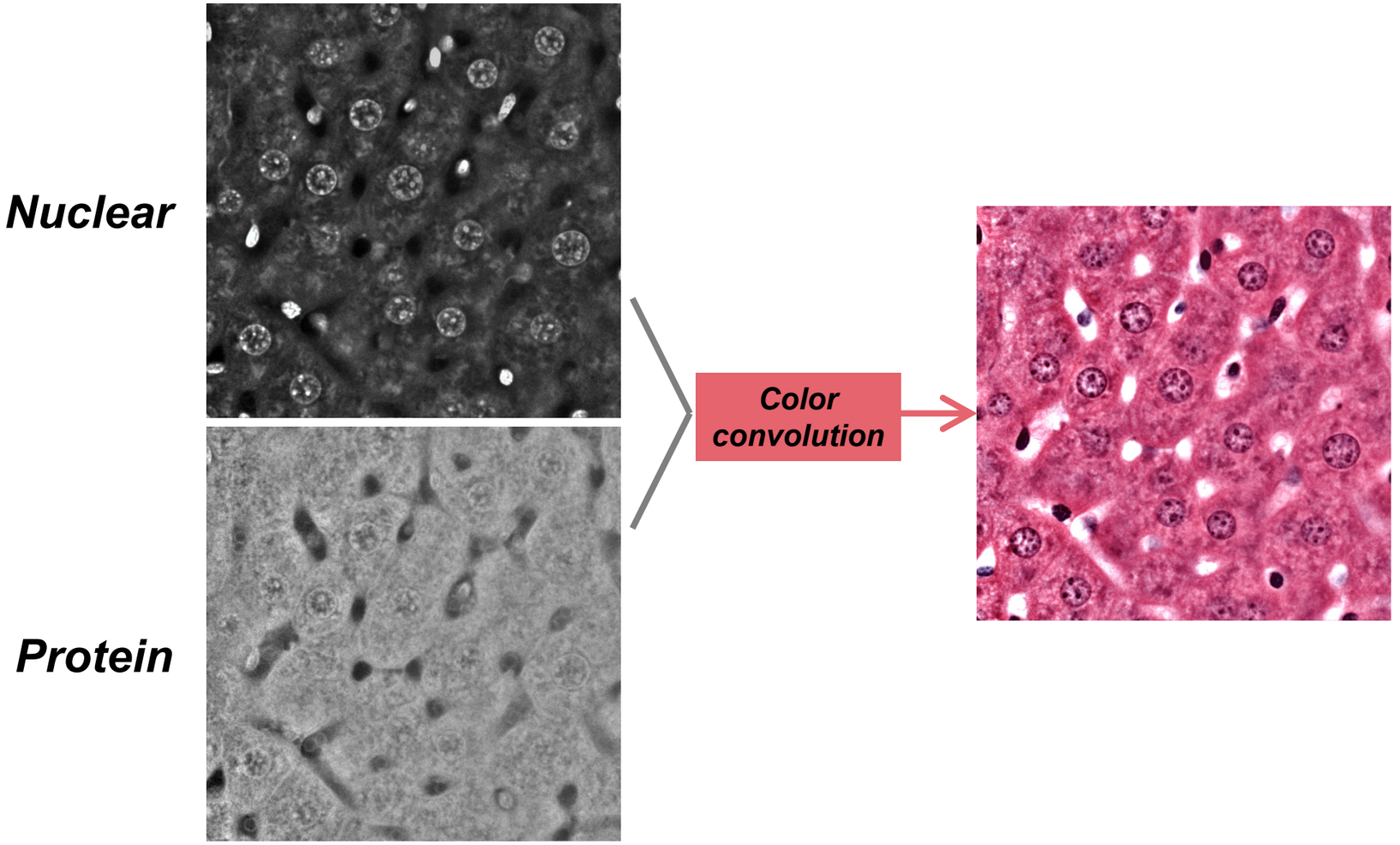
View remotely

Clearing improves quality and depth imaging

- Refractive index differences distort and dim
- Refractive index: water = 1.33, proteins and membranes ≈ 1.55, xylene ≈ 1.49
- Benzyl Alcohol/Benzyl Benzoate BABB ≈ 1.55

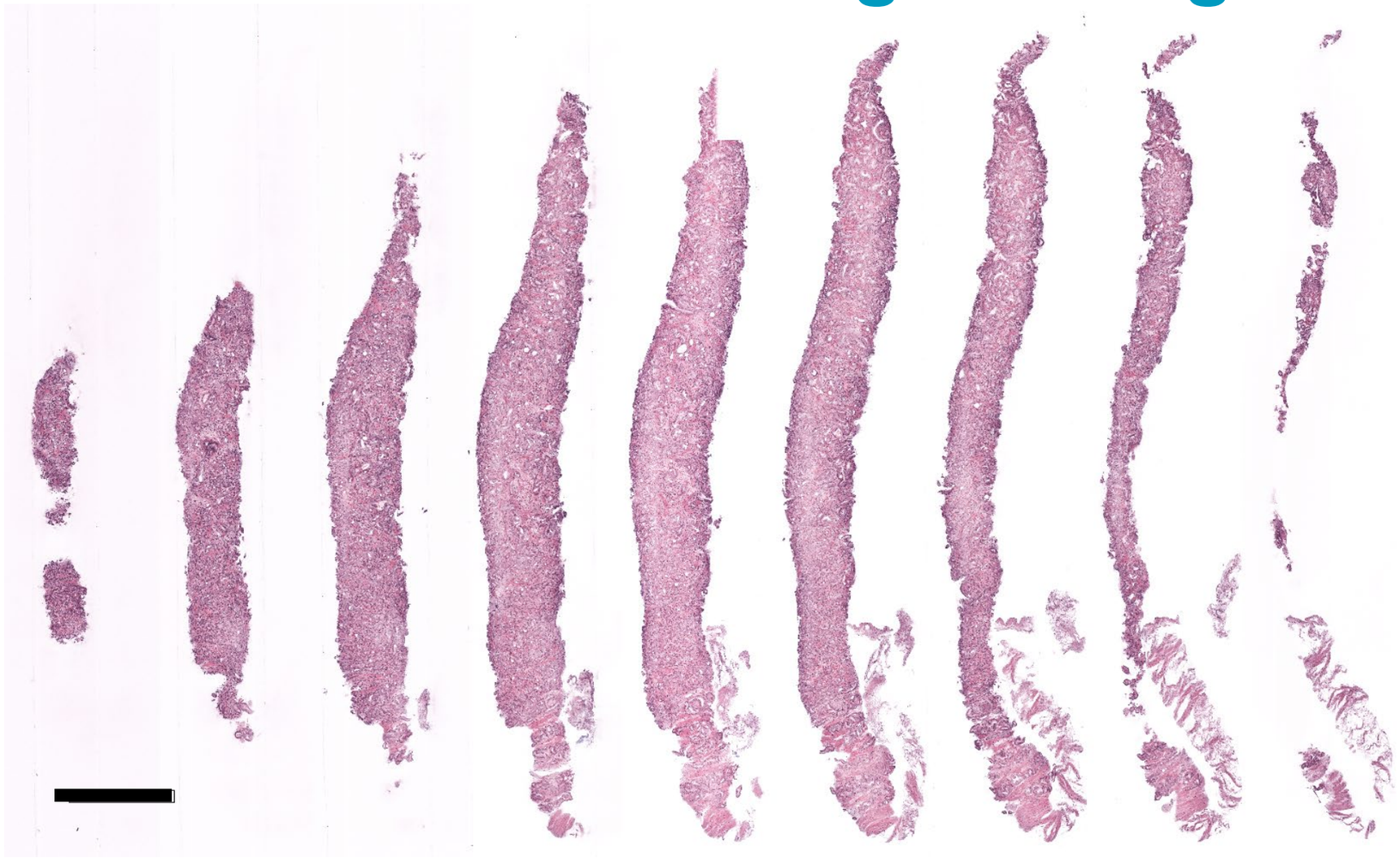


Fluorescent dyes recreate H&E



Torres et al, *Arch Path Lab Med*: June 2013

Intact full cores can be imaged using CHiMP



Human prostate biopsy imaged through full thickness with CHiMP. Slice spacing 100µm, scale bar 1mm.

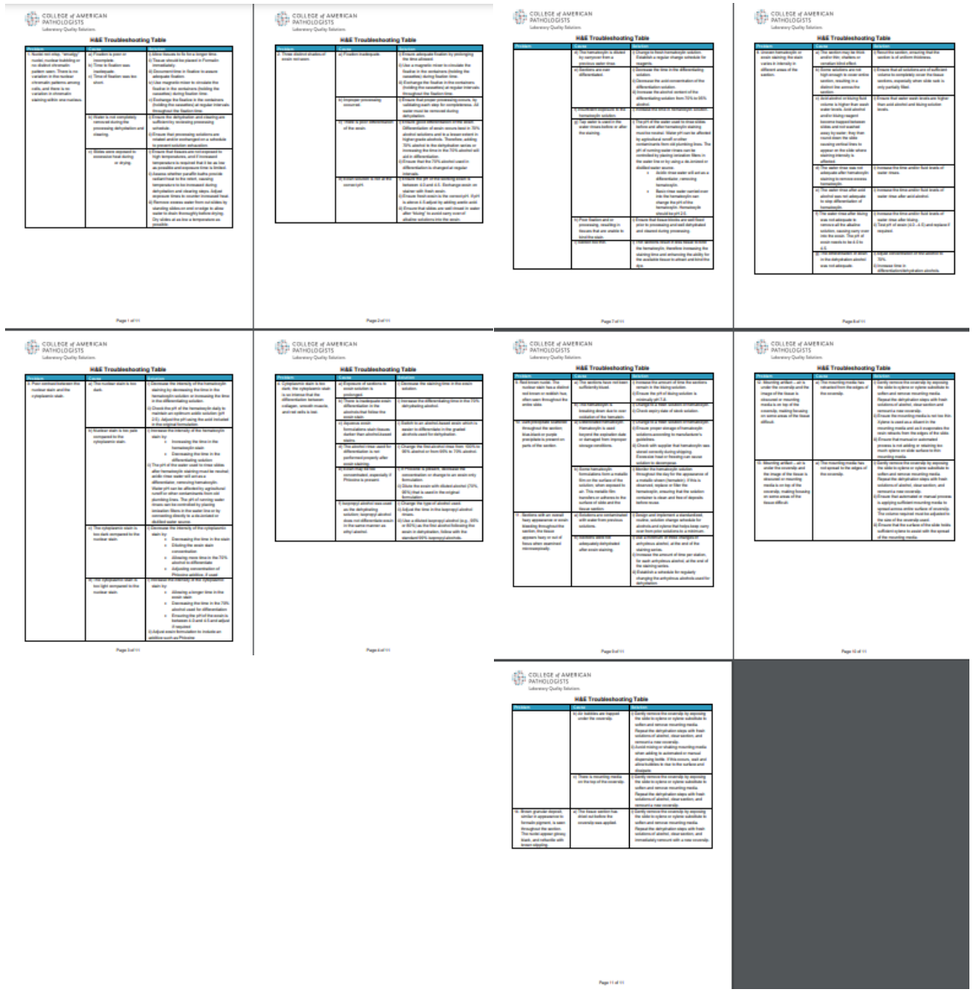
Sections

- Motivation for multiphoton microscopy
- **Physical slides, WSI, and multiphoton comparative risks**
- Validation considerations from pathologist point of view
- Validation examples
- Conclusions

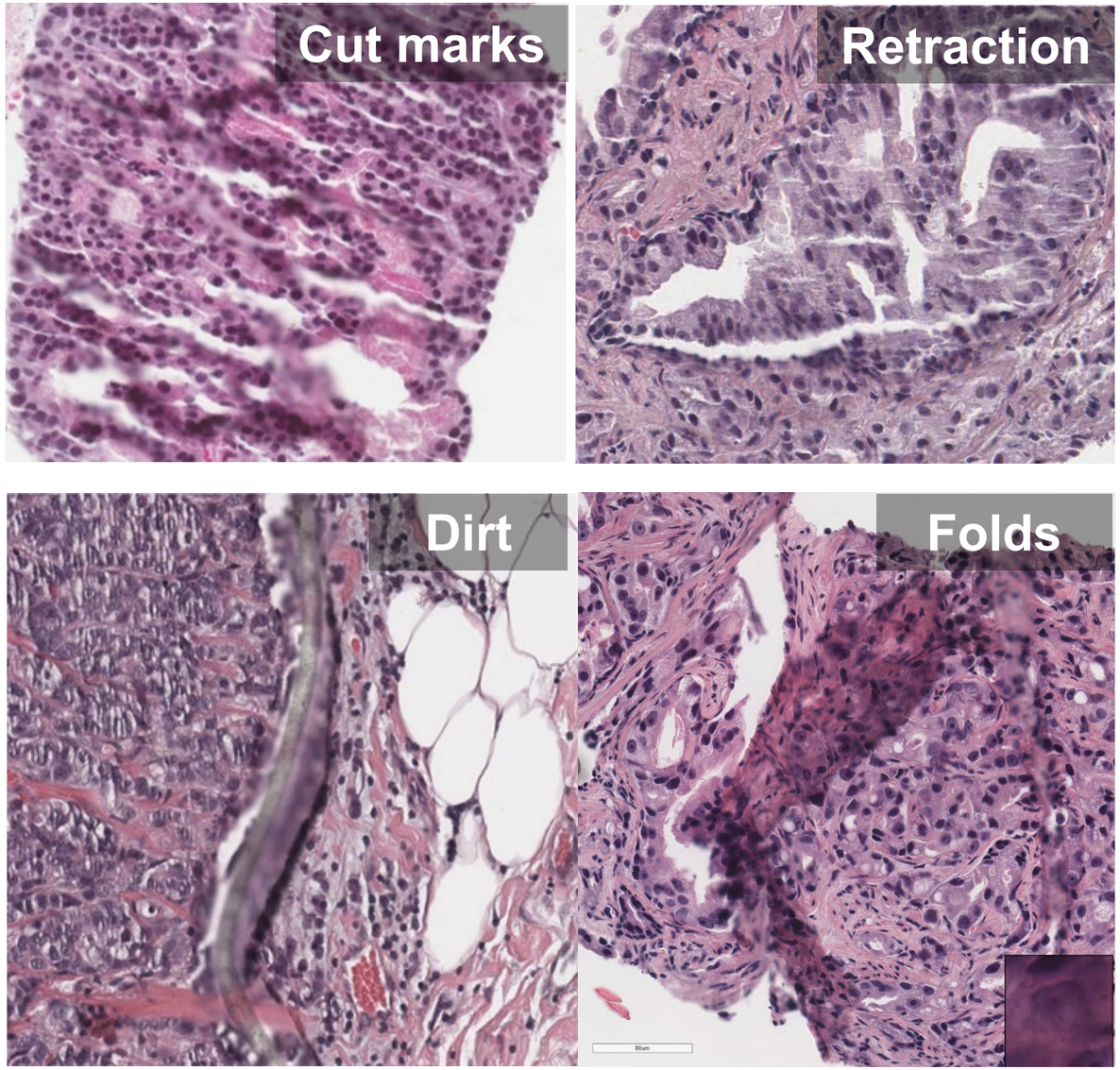
Histology has many intrinsic quality risks

- **List from the CAP 'H&E Troubleshooting Guide'**

1. Nuclei not crisp, “smudgy” nuclei, nuclear bubbling.
2. Three distinct shades of eosin not seen
3. Poor contrast between nuclear and cytoplasmic stain
4. Cytoplasmic stain is too dark
5. Cytoplasmic stain is too light
6. Nuclear stain too dark
7. Nuclear stain too light
8. Uneven hematoxylin or eosin staining
9. Red brown nuclei
10. Dark precipitate scattered throughout
11. Hazy appearance or eosin bleeding throughout
12. Air under the coverslip or mounting media on top
13. Brown granular deposit



Cutting and mounting increases artifact risk

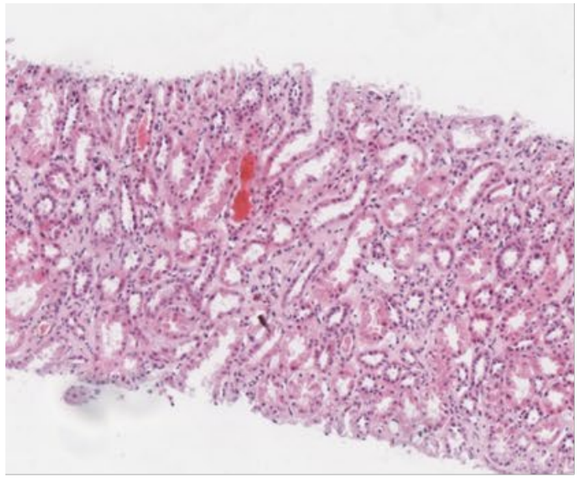


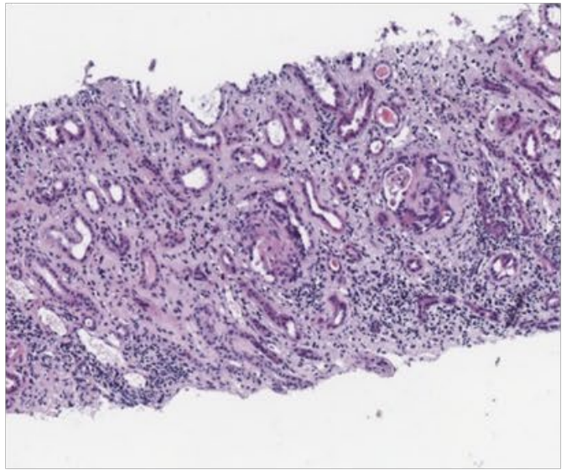
Study: 367/388 slides showed 406 artifacts

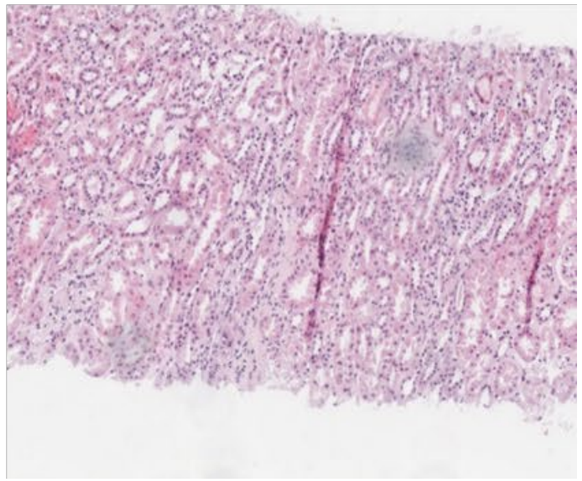
Patterns	*f*
Prefixative artifacts	
Heat*	1
Crush[†,§]	28
Split[¶,†]	36
Contaminant**	3
Hemorrhagic***	1
Total	69
Fixative artifacts	***f***
Formalin pigment[††,§§]	60
Tissue processing artifacts	***f***
Microtomy[¶¶,‖]	75
Fold[†††,‡]	134
Total	209
Staining and mounting artifacts	***f***
Residual wax[‡‡,§§§]	19
Stain deposit[¶¶¶,****]	29
Contaminant[‡‡‡]	3
Air bubble entrapment[††††,§§§§]	17
Total	68
Grand total	406

Igho OE, Aimakhume A. Artifacts in histology: A 1-year retrospective study. *Ann Bioanthropol* 2017;5:34-9.

WSI compounds intrinsic histology artifacts

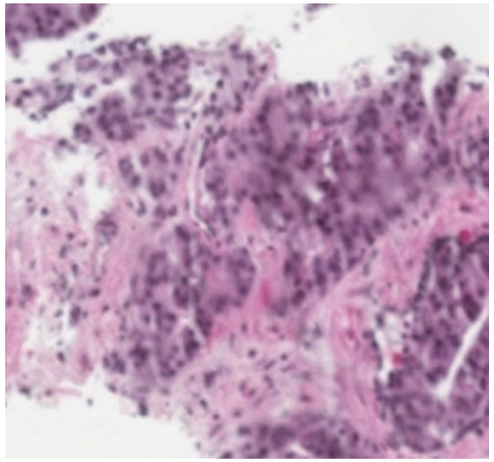






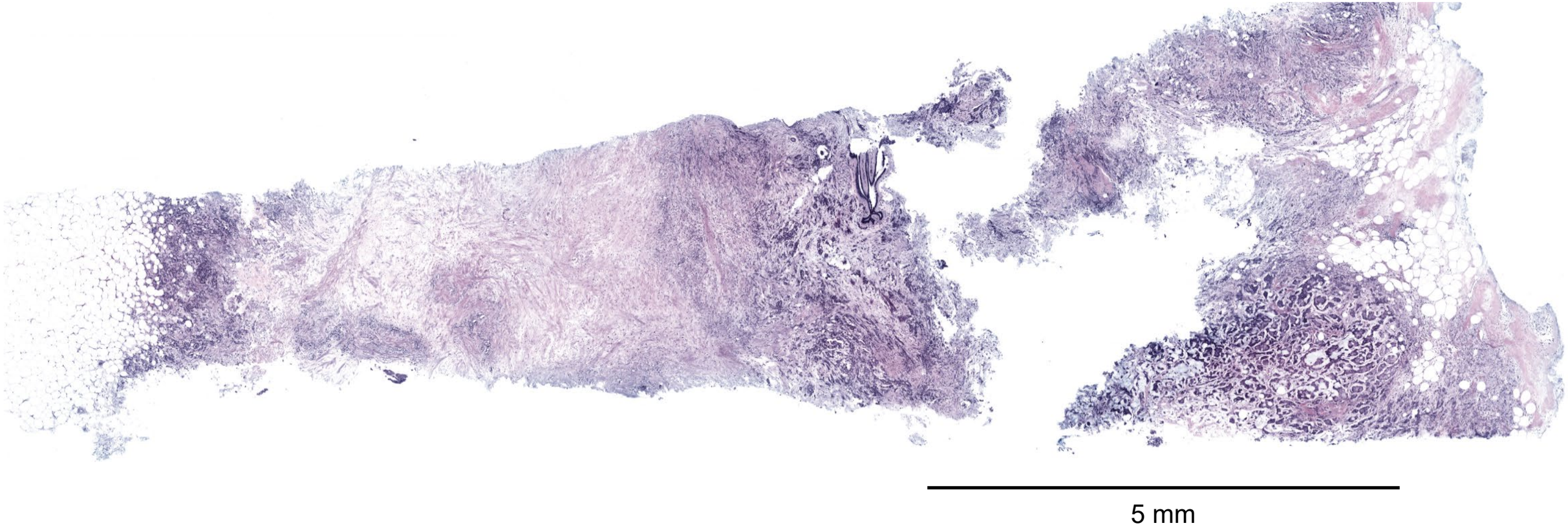
Hue/intensity variability
dyes + imager + monitor

Loss of Focus



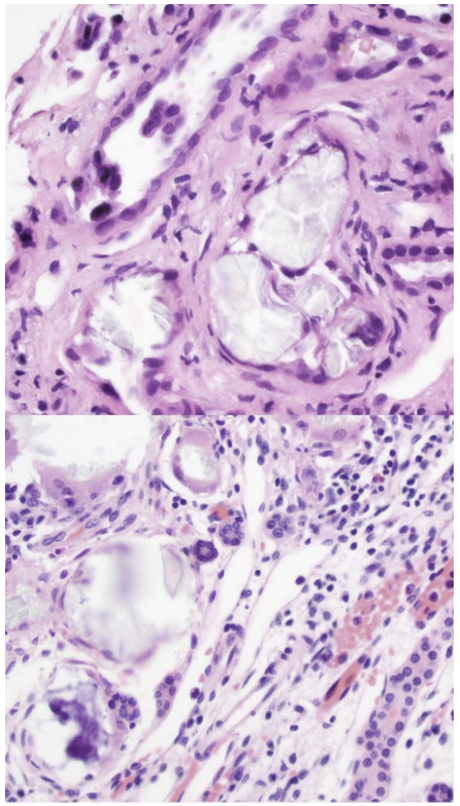
Multiphoton avoids many standard artifacts

e.g. breast tissue that could be very difficult to cut

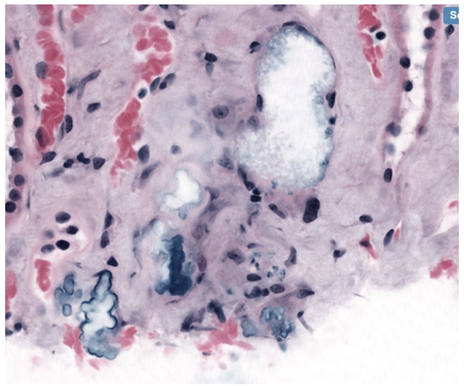


Multiphoton could introduce new artifacts

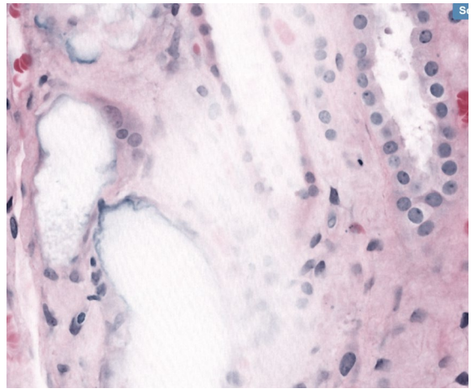
e.g. oxalate crystals



Physical slide



50 um depth

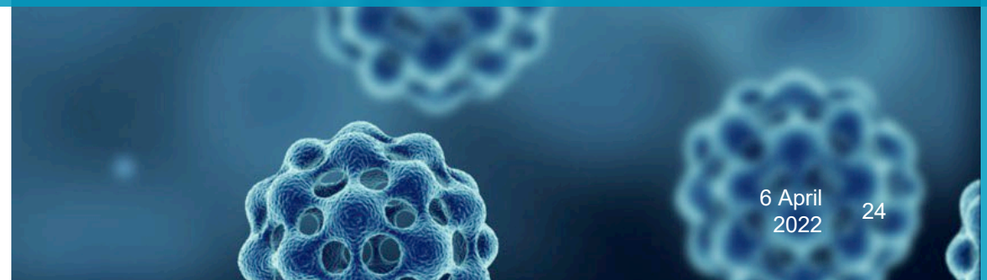


200 um depth

Multiphoton

Sections

- Motivation for multiphoton microscopy
- Physical slides, WSI, and multiphoton comparative risks
- **Validation considerations from pathologist point of view**
- Validation examples
- Conclusions



CAP EVM validation principles

Ex Vivo Microscopy checklist items

ANP.23560 EVM - System Validation **Phase I**

The laboratory performs validation studies before the Ex Vivo Microscopy (EVM) technology is used for the intended purpose(s).

NOTE: The specific components of the validation study are left to the discretion of the laboratory. However, studies should be performed using an adequate number of cases, data should be evaluated, and a summary statement provided prior to implementation. Records of how discordant data or unacceptable variations from the expected were resolved are required.

As general guiding principles, the validation process should:

- *Closely emulate the real-world environment and involve tissue types and clinical settings relevant to the intended use(s)*
- *Be carried out by or under the supervision of a pathologist adequately trained to use the EVM system*
- *Encompass the entire EVM system, with reevaluation if a significant change is made to a previously validated system.*

CAP EVM validation principles

ANP.23570 EVM - Function Checks **Phase II**

Regular function checks are performed and records maintained on the Ex Vivo Microscopy (EVM) system/instrument.

NOTE: Function checks include confirmation that an instrument or item of equipment operates according to manufacturer's specifications before routine use, at prescribed intervals, or after minor adjustment. Depending on the type of system, function checks may include calibration.

ANP.23580 EVM - Method Performance Specifications Availability **Phase II**

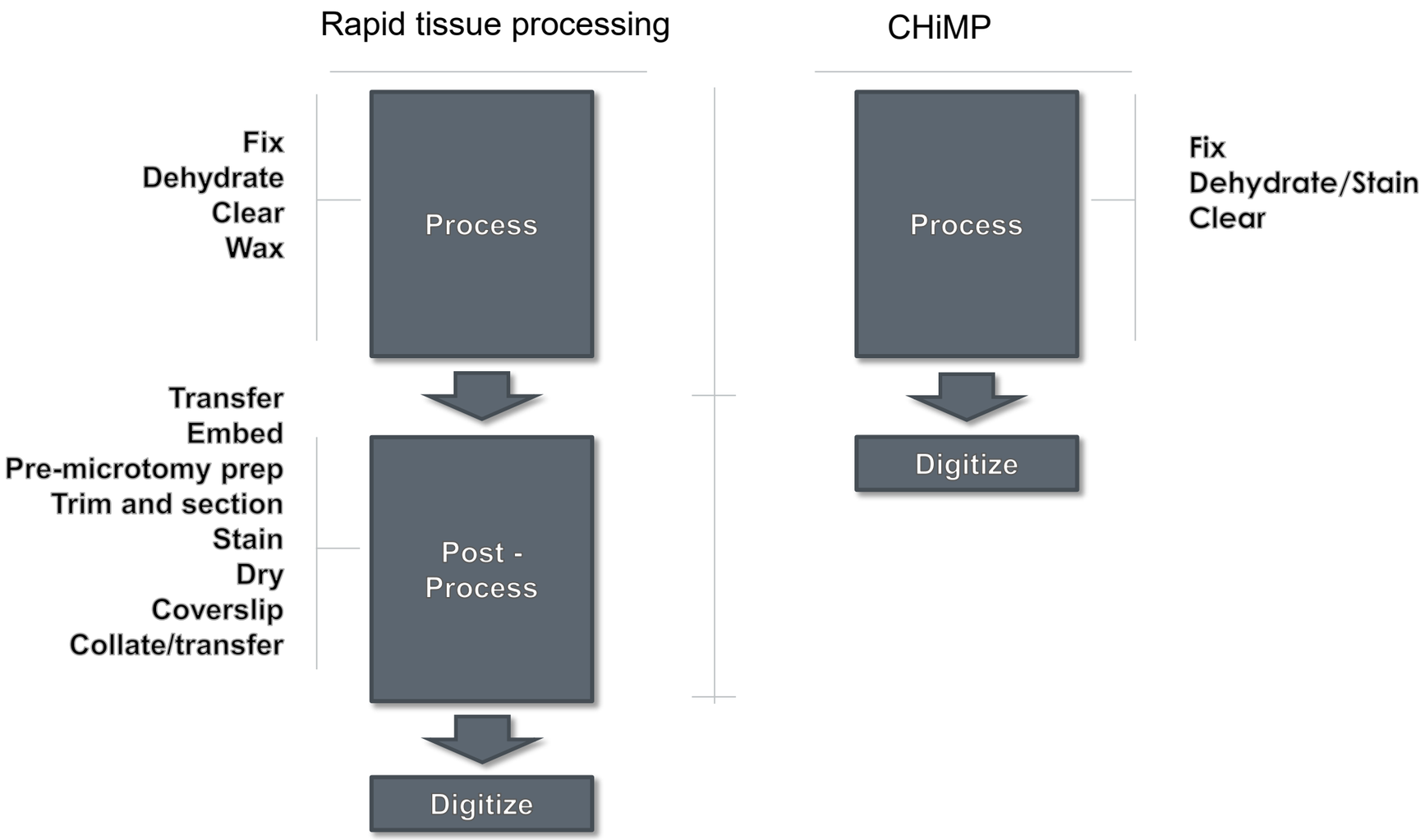
The current Ex Vivo Microscopy (EVM) methods and all significant changes to analytical methodology, including performance specifications and supporting validation data, are maintained by the laboratory.

ANP.12500 Record Retention **Phase II**

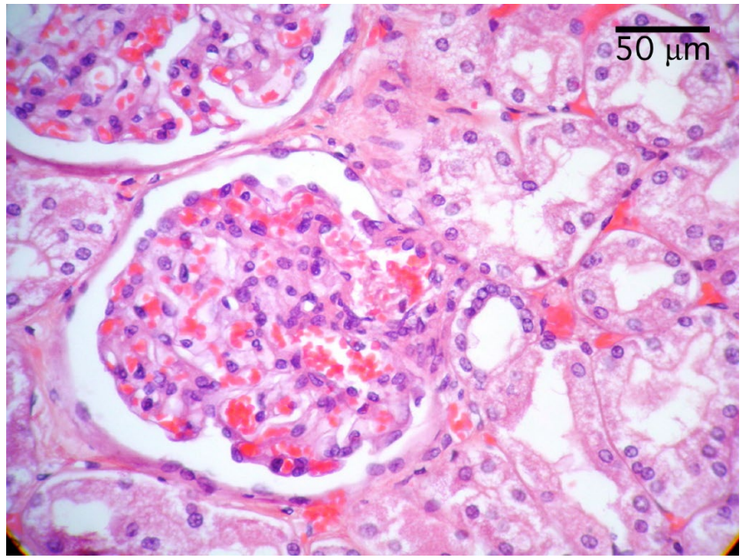
Surgical pathology records and materials are retained for an appropriate period.

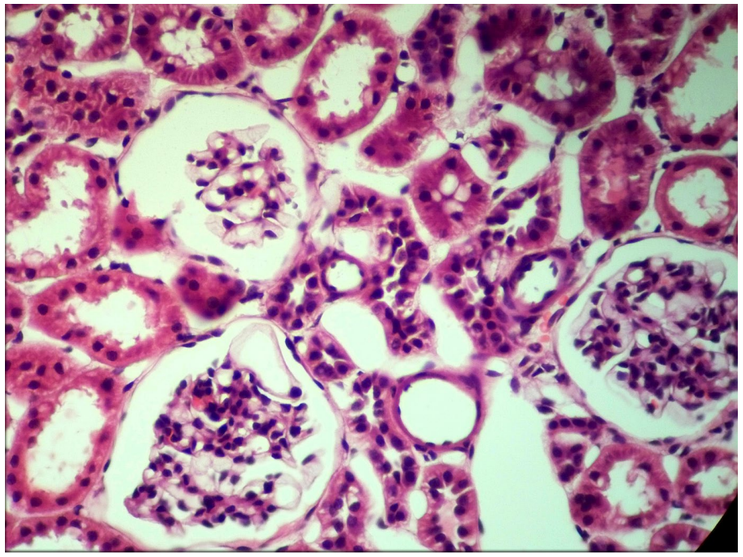
Digital images used for primary diagnosis	10 years if original glass slides are not available
Datasets from In-Vivo Microscopy (IVM) or Ex Vivo Microscopy (EVM) systems used to aid in interpretation or diagnosis	10 years - data must be retrievable for this period (Subject to Note 6 below)

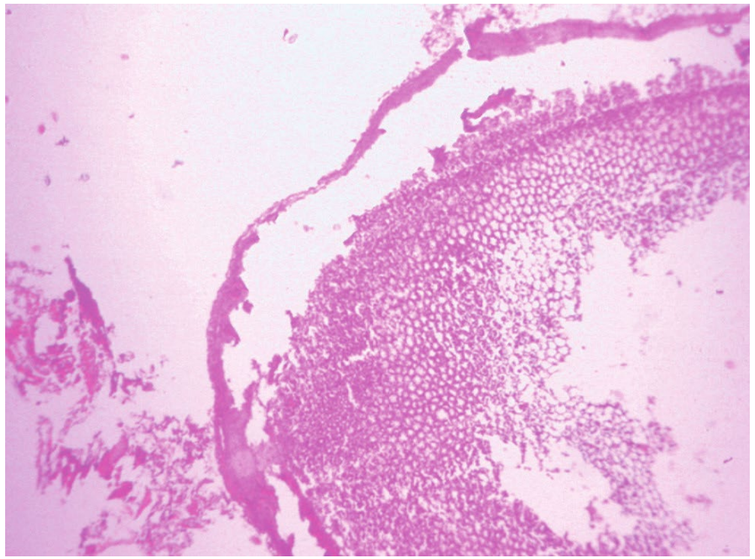
CHiMP preparation is a variation of standard



Even light microscopes can accentuate variability







How do we know that it is good enough?

CAP validation principles

AP checklist items

ANP.11734 Slide Quality **Phase II**

Slides are of sufficient quality for diagnosis.

NOTE: Histopathology slides must be of adequate technical quality to be diagnostically useful. Criteria to evaluate include adequate tissue fixation, processing, thickness of sections, absence of interfering tissue folds and tears, and good staining technique and cover slipping. For hematoxylin and eosin and other routine stains, the patient slide serves as the internal control to ensure adequate staining technique. The sections must be cut from sufficient depth in the block to include the entire tissue plane.

CAP validation principles

AP checklist items

ANP.23120 Tissue Processing Programs **Phase II**

Tissue processing programs are validated.

NOTE: To validate new processing programs, laboratories should run tissue samples of the same size, thickness and fixation in duplicate. Reagents on the processor(s) should be comparable, e.g. all fresh reagents. Process, embed, cut, and stain slides at the same time and evaluate the quality of the blocks, e.g. firmness, ease of cutting. The slides should be evaluated by the pathologist without knowledge of which processing program was used and graded on quality of section and staining. The new processing program must be of adequate quality before being put into use.

This method may also be used to verify a routine processing program before putting a new processor into clinical service.

WSI CAP / API / ASCP validation principles

Validating Whole Slide Imaging Systems for Diagnostic Purposes in Pathology: 2021 Guideline Update

Summary of recommendations
At least 60 samples per use case (e.g. H&E of fixed tissue, frozen, hematology)
Reflect spectrum and complexity of routine work
Another 20 cases for additional applications
Establish concordance between digital and glass for same observer
If <95% concordance, investigate
At least 2 week washout between reviews

1. Pantanowitz L, Sinard JH, Henricks WH, Fatheree LA, Carter AB, Contis L, Beckwith BA, Evans AJ, Lal A, Parwani AV. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Archives of Pathology and Laboratory Medicine*. 2013 Dec;137(12):1710-22.

2. Evans AJ, Brown RW, Bui MM, Chlipala EA, Lacchetti C, Milner Jr DA, Pantanowitz L, Parwani AV, Reid K, Riben MW, Reuter VE. Validating whole slide imaging systems for diagnostic purposes in pathology: guideline update from the College of American Pathologists in collaboration with the American Society for Clinical Pathology and the Association for Pathology Informatics. *Archives of Pathology & Laboratory Medicine*. 2022 Apr;146(4):440-50.

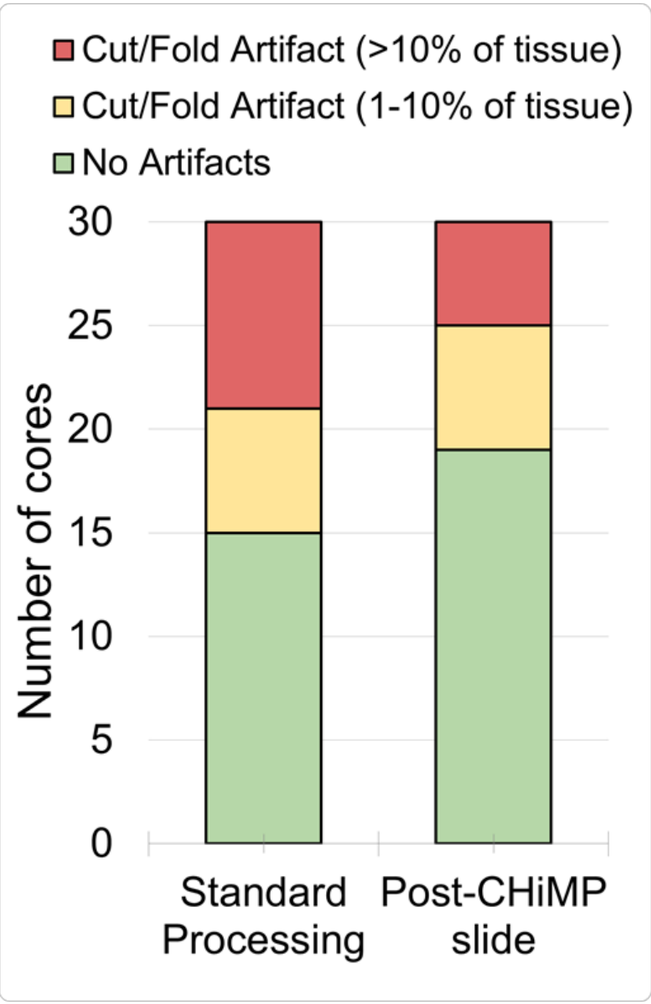
Drug analogue conceptual framework for EVM primary diagnosis

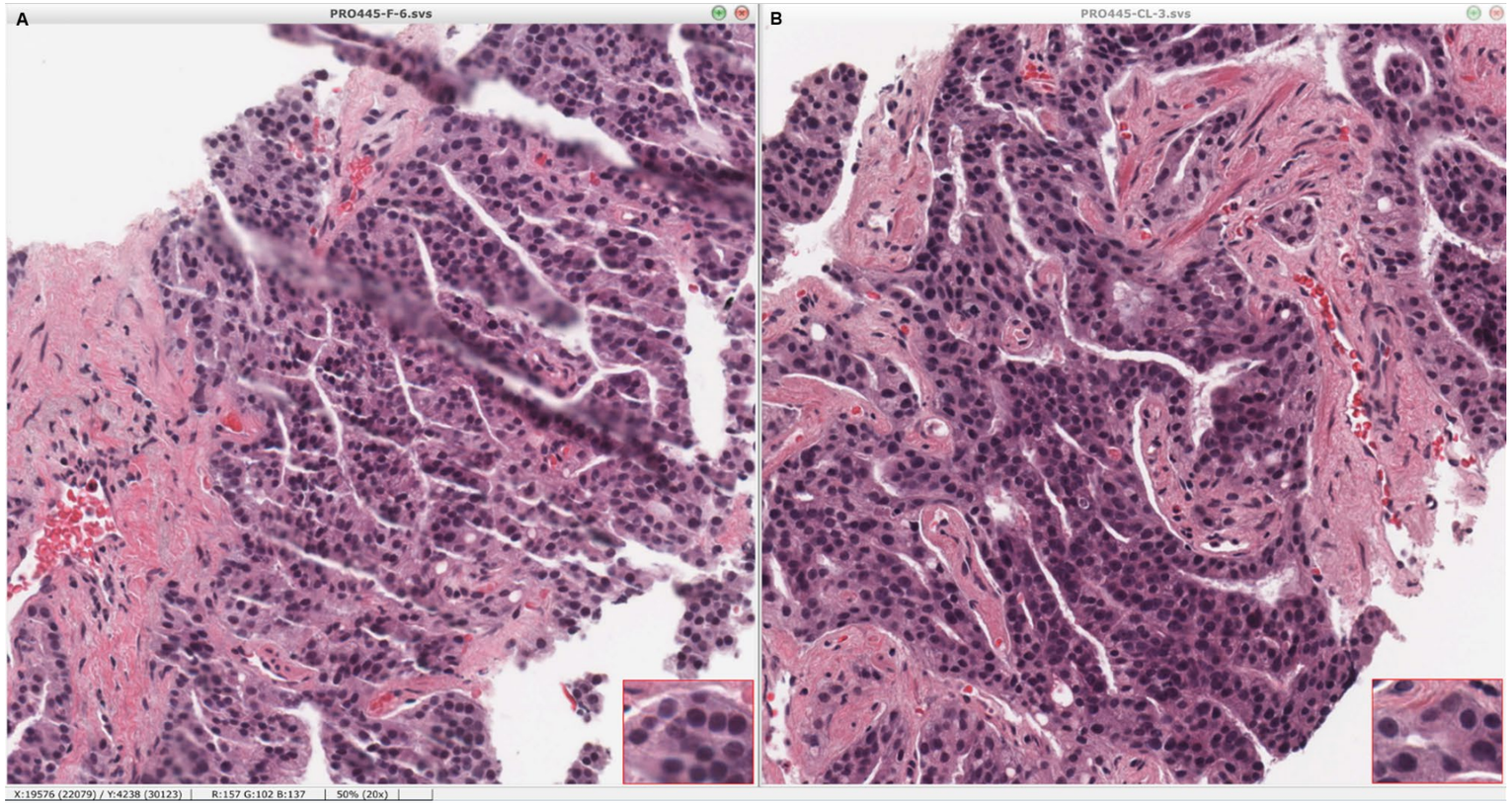
Phase	Specimen source
'Phase 1': 'Safety' – Are gold-standard diagnostic processes adversely affected?	**Paired animal specimens** **Paired/unpaired excess human samples**
'Phase 2': 'Efficacy' – Are diagnostic features identifiable at least as well as in physical slides?	**Paired/unpaired excess human samples** **Fresh excess human samples**
'Phase 3': 'Implementation trial' – How well does it work in routine clinical practice compared to standard?	**Clinical specimens used in diagnosis**

Sections

- Motivation for multiphoton microscopy
- Physical slides, WSI, and multiphoton comparative risks
- Validation considerations from pathologist point of view
- **Validation examples**
- Conclusions

Phase 1 examples

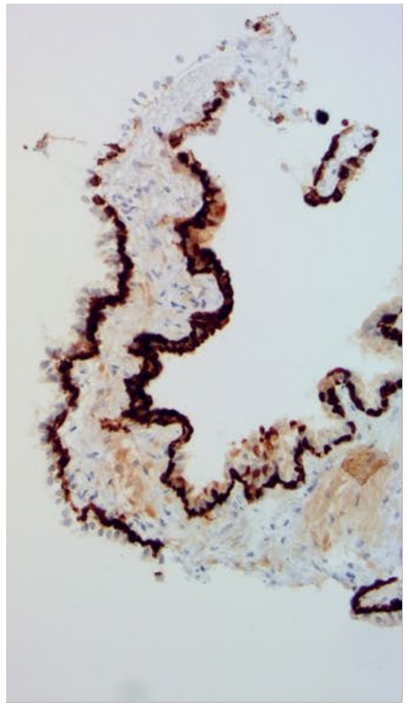




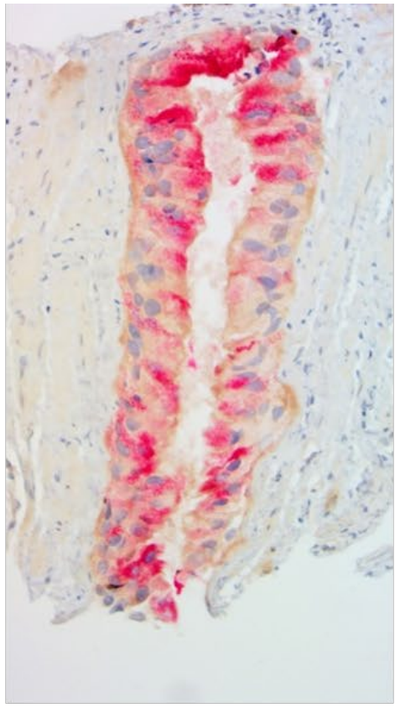
Standard processing **Post-CHiMP**

Phase 1 examples

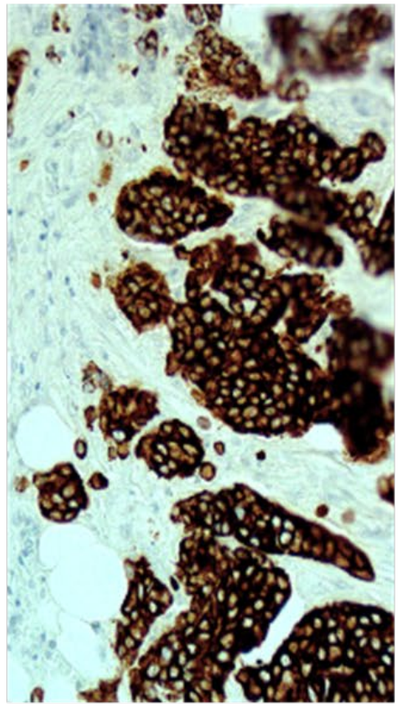
Antibody testing post-CHiMP processing and imaging, internally controlled
Excess human samples



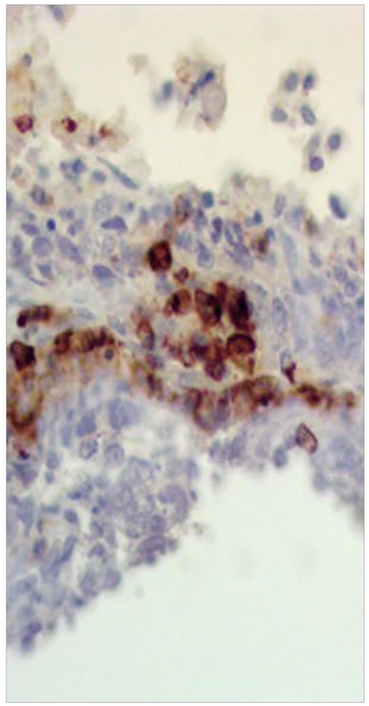
PIN-4 Normal



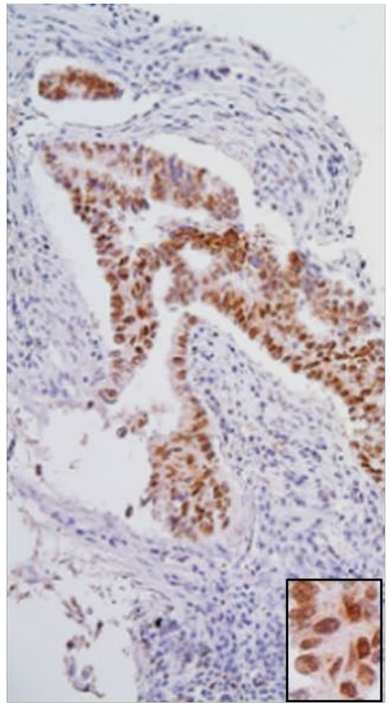
PIN-4 Tumor



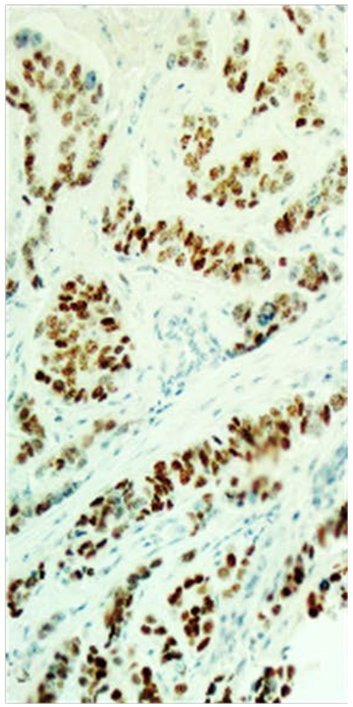
AE1/AE3



CD45



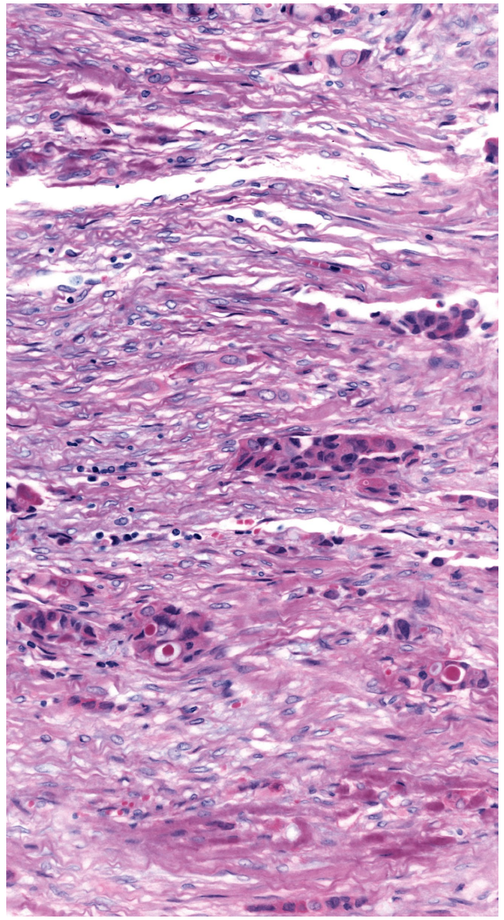
TTF-1



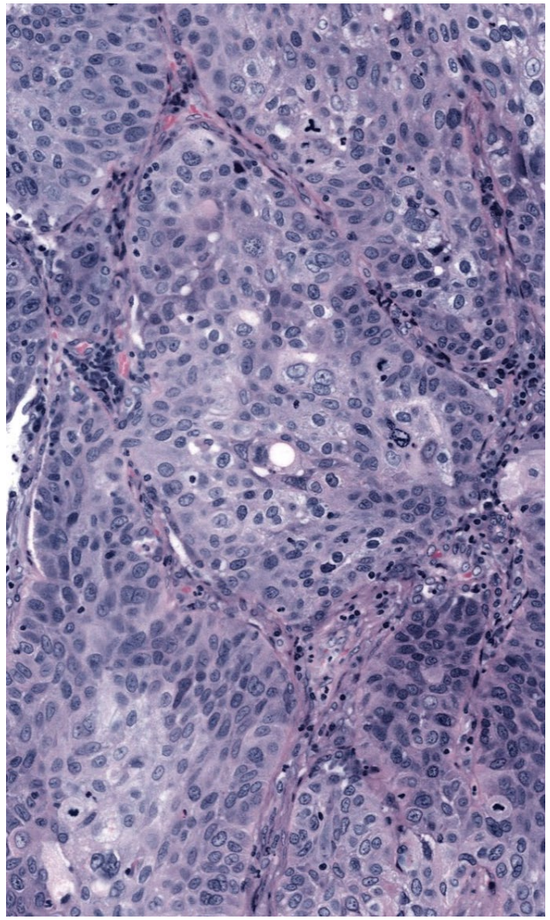
ER

Phase 2 examples

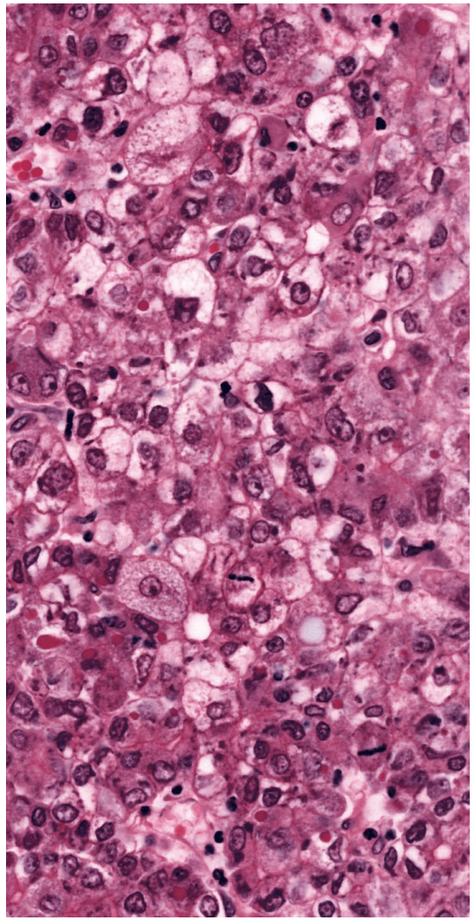
Excess human tissue sample multiphoton images



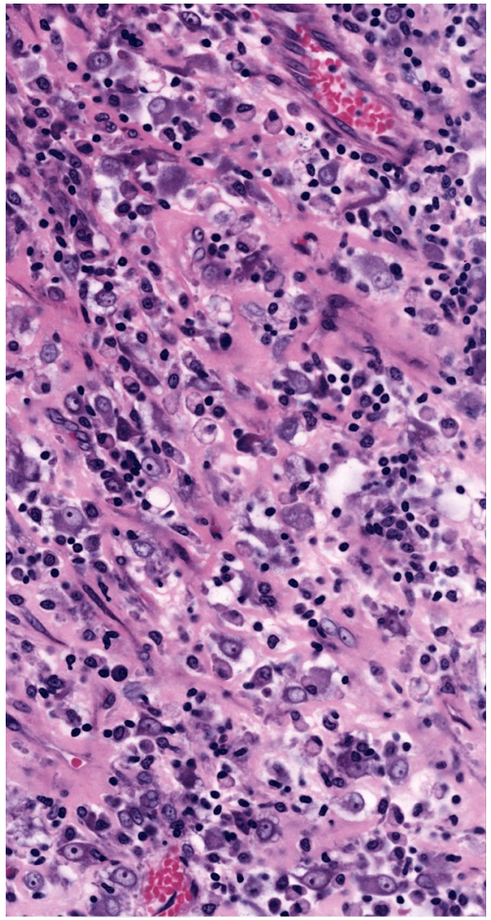
Breast Ca



Lung Ca



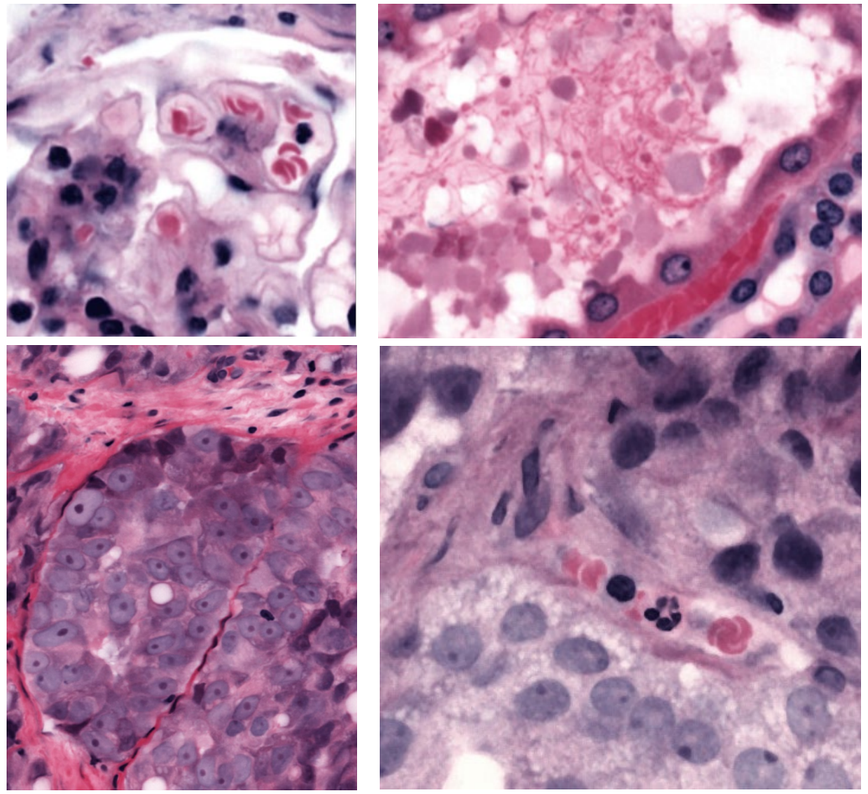
Liver Ca



Renal Ca

Phase 2 examples

Excess human samples



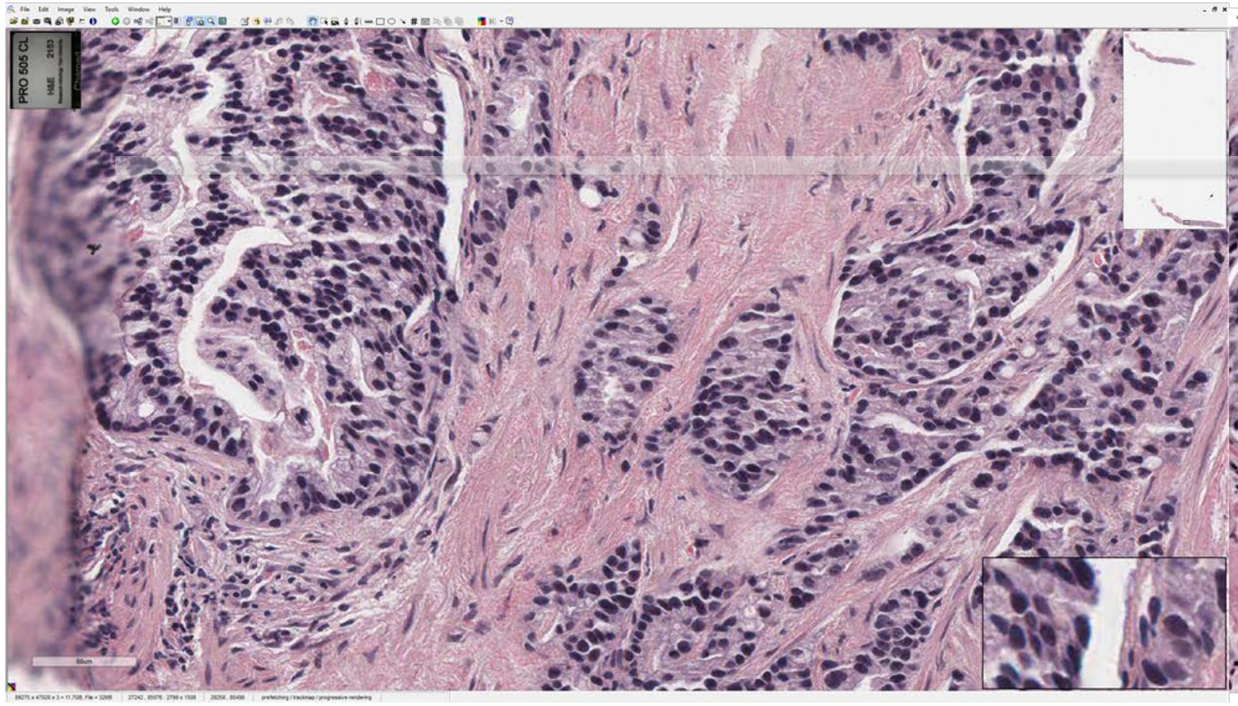
Fine features

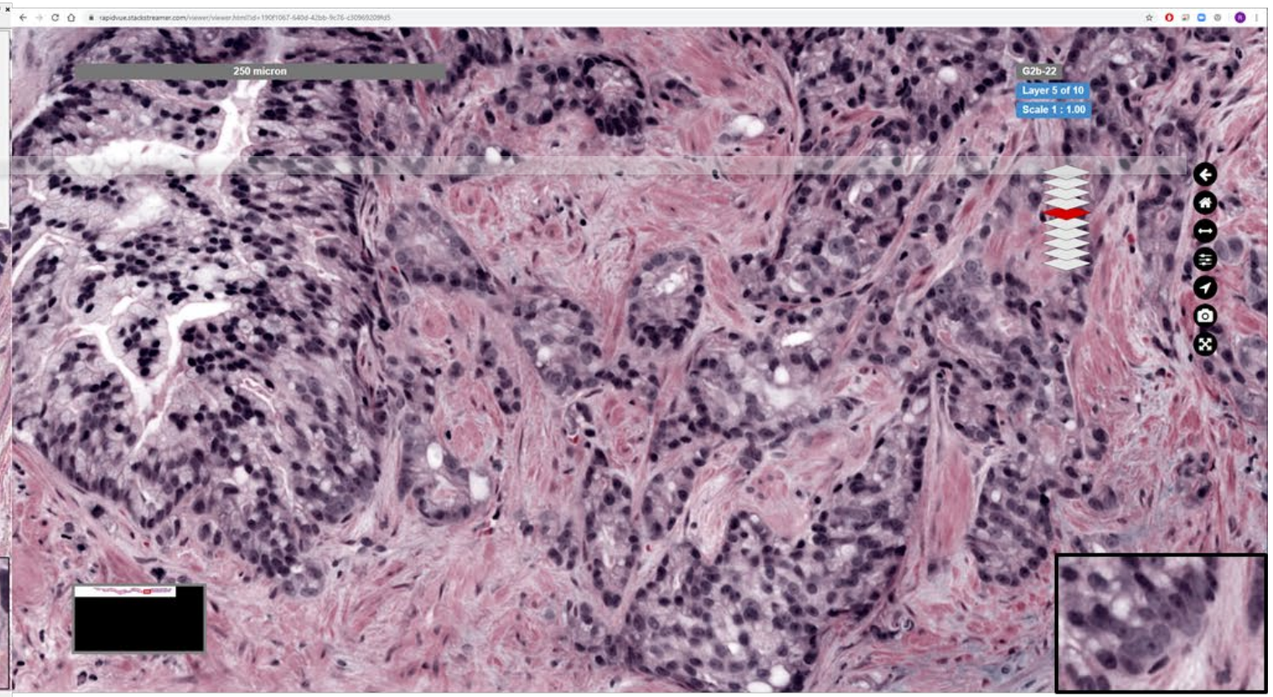
Phase 2/3 examples

Paired fresh clinical samples for research (not used in clinical care)

Physical slide

CHiMP multiphoton





Phase 2/3 example

Paired fresh clinical samples for research (not used in clinical care)

A

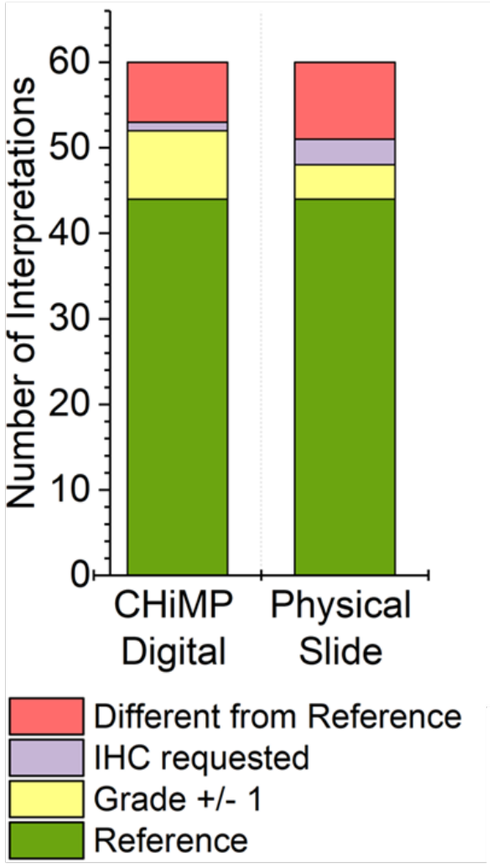
Physical Slide Diagnosis

Reference Diagnosis	NFC	ASAP	HGPIN	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	IHC
NFC	21								
ASAP		0							
HGPIN	2		1						*1*
Grade 1				14	1				*3*
Grade 2	3			2	3				
Grade 3				1		2			
Grade 4							2	1	
Grade 5	1					2		1	*2*

Digital Diagnosis

Reference Diagnosis	NFC	ASAP	HGPIN	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	IHC
NFC	21								
ASAP		0							
HGPIN	1		1	1					
Grade 1	1	2	1	11					*1*
Grade 2				4	4	1			
Grade 3						2	1		*1*
Grade 4							3		
Grade 5	1					2		2	*1*

B



Torres et al, *Arch Path Lab Med*: 1 May 2021

Phase 3 example

Paired fresh clinical samples used in clinical care

Specimen source:

- Kidney needle core biopsies (n > 60)
- Medical or Transplant

Specimen prep:

- Standard formalin biopsy bottle to path lab
- Prepped/imaged/returned in < 4 hrs

Post-imaging:

- Standard processing + stains

Diagnostic comparison:

- Standard core and post-CHiMP core used for clinical diagnosis
- Diagnosis on CHiMP images post-washout

Sections

- Motivation for multiphoton microscopy
- Physical slides, WSI, and multiphoton comparative risks
- Validation considerations from pathologist point of view
- Validation examples
- **Conclusions**

Conclusions

- Current histology standard has known limitations, including quality
- There are existing frameworks for assessing new ex vivo microscopy technology validations
- Whole slide imaging is a relevant case study for EVM validation
- Safety, efficacy, and implementation is a potentially applicable stepwise validation concept
- Overall diagnostic quality is the essence of new EVM technology validation from pathologist perspective
- Pathologist assessment can determine overall diagnostic quality

Acknowledgements

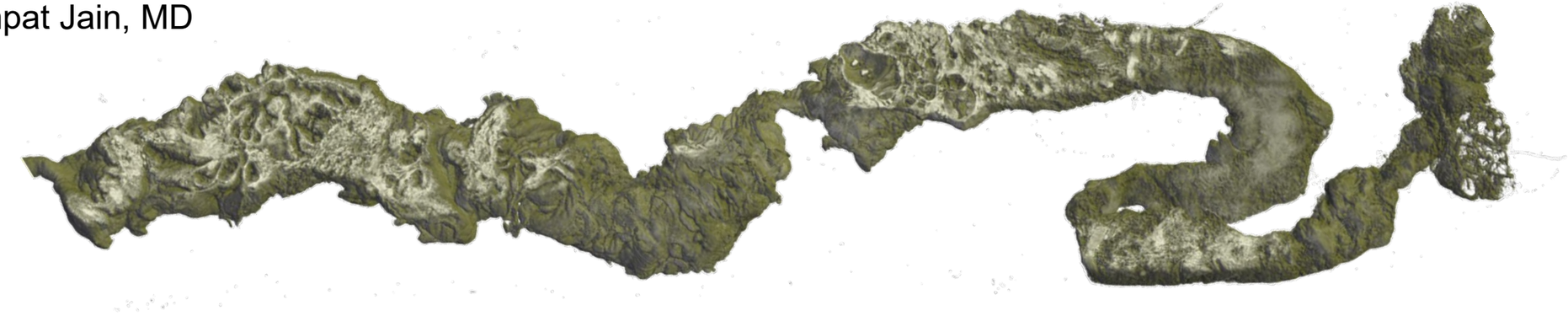
Applikate Technologies
- *Michael J. Levene, PhD*
- *Eben Olson, PhD*

Yale Pathology
- John H. Sinard, MD, PhD
- Peter Humphrey, MD, PhD
- Sudhir Perincheri, MD, PhD
- Robert Homer, MD, PhD
- Gilbert Moeckel, MD, PhD
- Dhanpat Jain, MD

Yale Laboratory Medicine
- *Brian R. Smith, MD*

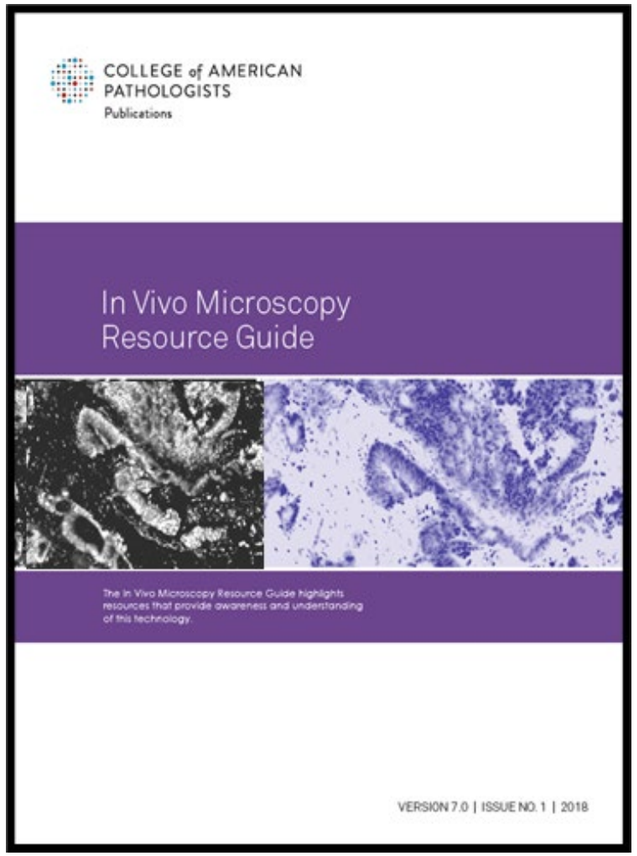
Yale School of Medicine
- Lloyd Cantley, MD, PhD
- Joerg Bewersdorf, PhD
- Gary Desir, MD
- Robert Safirstein, MD
- Preston Sprenkle, MD, PhD
- Darryl Martin, PhD

Support
- NIH/NCI R44 CA189522-02
- NIH/NIBIB R43 EB21171-01
- 3UL1TR001863 - 02W1NIH (NCATS)



Resources of Digital and Computational Pathology Committee

- List resources
  - SPECs
  - Resources Guides
  - Topic Center Pages & AI pages
  - AI@CAP.ORG email address



THANK YOU!

Thank you for attending our webinar "New histology, same rules? Approach to validation of slide-free multiphoton histology for clinical use" by Richard Torres, MD, MS. For comments about this webinar or suggestions for upcoming webinars, contact AI@cap.org

NOTE: There is no CME/CE credit available for today's complimentary webinar. The recording of the presentation will be sent out in about 1 week.

COLLEGE of AMERICAN PATHOLOGISTS