CHHE

Comparative Pathology Core Information Session

Keith E. Linder

May 3rd, 2016

NC STATE UNIVERSITY Center for Human Health and the Environment

Comparative Pathology Core (CPC)

Core Personnel / Pathologists

Dr. Keith E. Linder
DVM, PhD, ACVP,
CPC Director

Dr. Heather Shive
DVM, PhD, ACVP,
CPC Associate Director

Dr. Debra Tokarz
DVM, PhD, ACVP,
CPC Comparative Pathologist

Network of Consulting Pathologists

Dave Malarkey DVM, PhD, ACVP - *NIEHS*
John Cullen DVM, PhD, ACVP - *NCSU*
Luke Borst DVM, PhD, ACVP - *NCSU*
Jennifer Luff DVM, PhD, ACVP - *NCSU*
Mac Law DVM, PhD, ACVP - *NCSU*

NC STATE UNIVERSITY Center for Human Health and the Environment

Comparative Pathology Core (CPC)

"The CPC provides a single portal for CHHE member scientists to access unparalleled pathology expertise of a diverse range of animal models…"

Specific Aims of the Comparative Pathology Core:

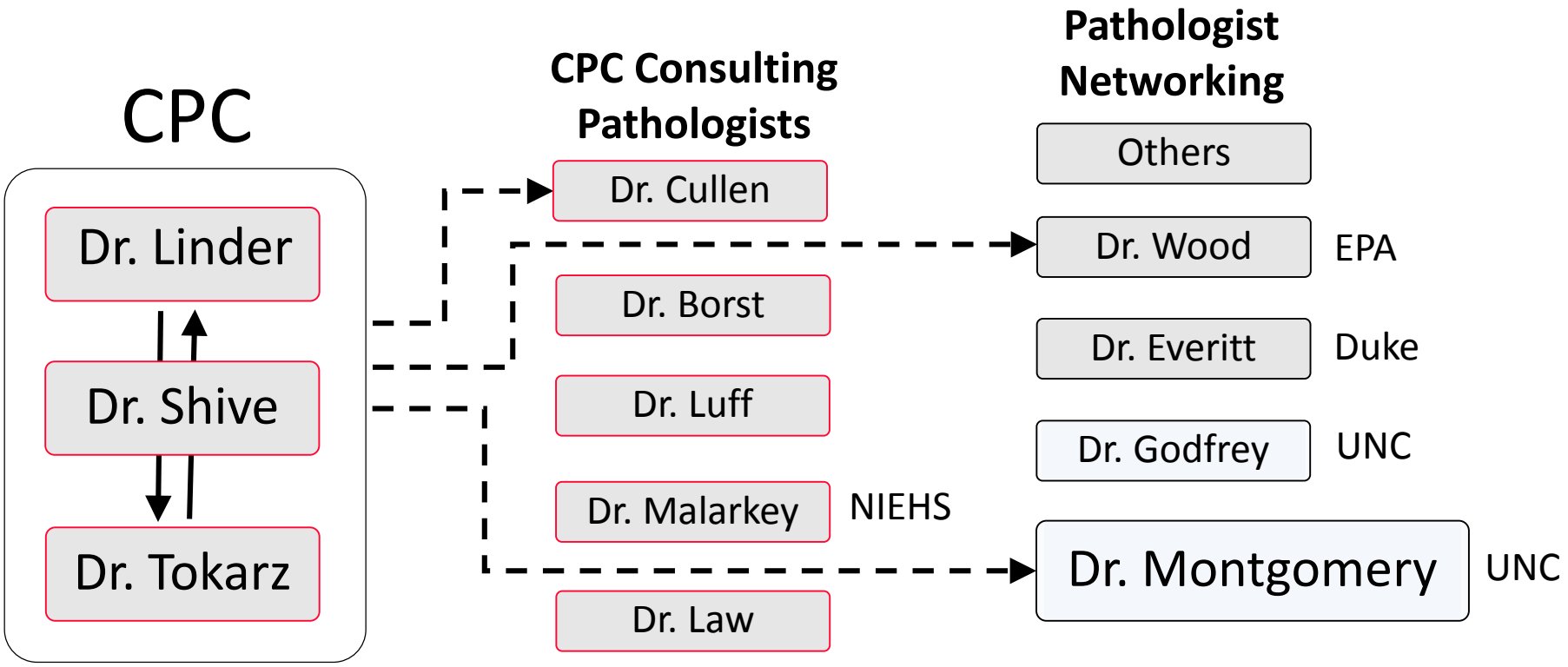
AIM 1. Provide CHHE members with expert pathology assessment of diverse animal models.

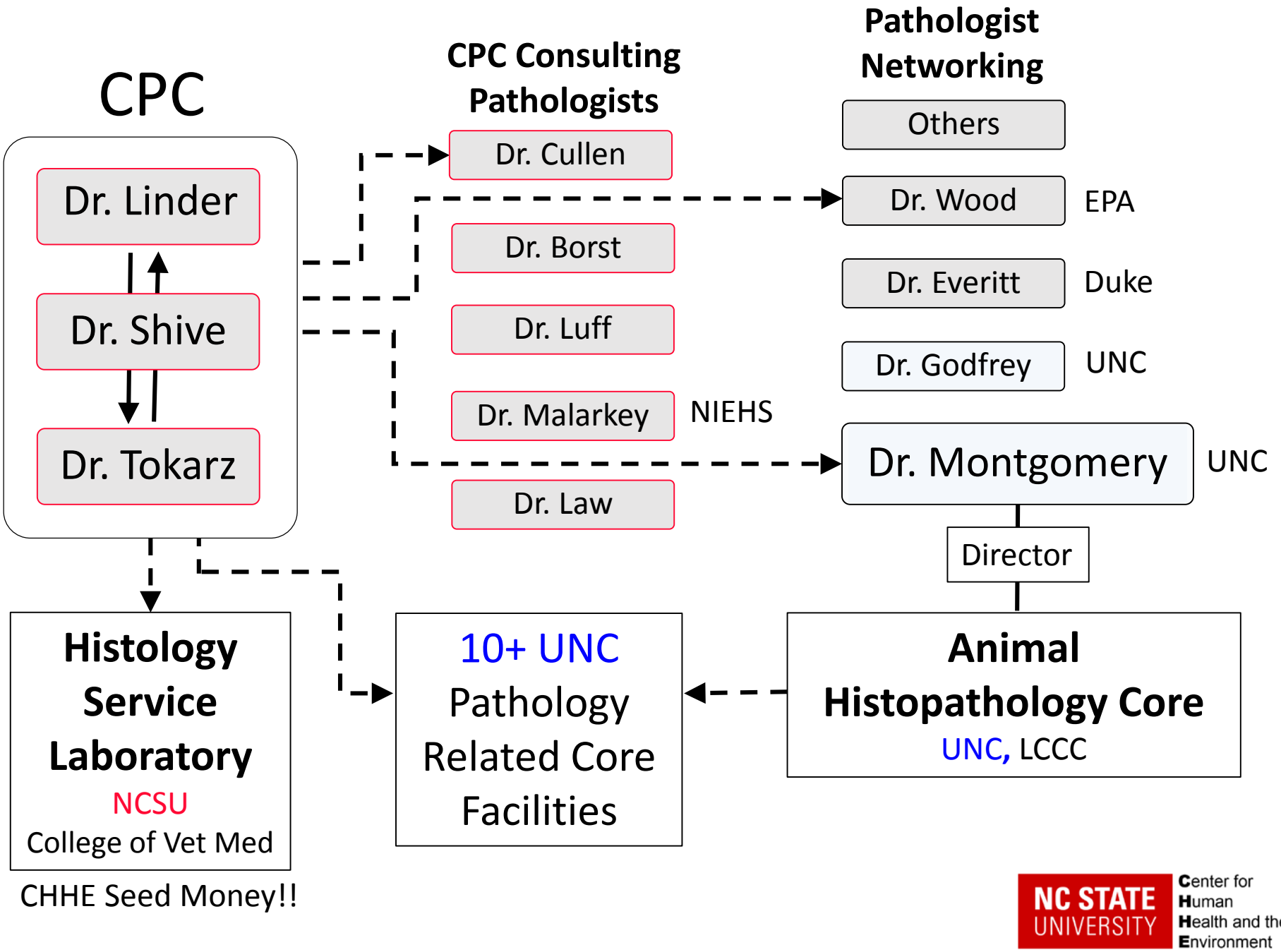
AIM 2. Foster pathology research collaborations between environmental health scientists leading to joint-authored grant applications and publications.

AIM 3. Integrate pathology (CPC) and Systems Technologies Core (STC) capabilities utilizing laser-capture micro-dissection to isolate specific cells for advanced biomolecule evaluation.

AIM 4. Provide access to animal diseased-tissue archives to study companion animals as sentinels in the human environment and possible new large animal models.

AIM 5. Provide training in pathology techniques and tissue assessment methods.


CPC
Dr. Linder
Dr. Shive
Dr. Tokarz
CPC Consulting Pathologists
Dr. Cullen
Dr. Borst
Dr. Luff
Dr. Malarkey
NIEHS
Dr. Law
Pathologist Networking
Others
Dr. Wood
EPA
Dr. Everitt
Duke
Dr. Godfrey
UNC
Dr. Montgomery
UNC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NIEHS
Dr. Law
Pathologist Networking
Others
Dr. Wood
EPA
Dr. Everitt
Duke
Dr. Godfrey
UNC
Dr. Montgomery
UNC
Director
Histology Service Laboratory
NCSU
College of Vet Med
CHHE Seed Money!!
10+ UNC Pathology Related Core Facilities
Animal Histopathology Core
UNC, LCCC
NC STATE UNIVERSITY
Center for Human Health and the Environment

Comparative Pathology Core (CPC)

Services:

- Study design
- Multiples vertebrate species
  - Rodents, fish, frogs, pigs, cats, dogs, etc.
- Necropsy methods
  - Tissue sampling, fixation and processing
- Pathology consultation
  - Gross, histologic, ultrastructural pathology
  - IHC protocol development
- Pathology assessment tools
  - Lesion scoring, morphometrics analysis, etc.

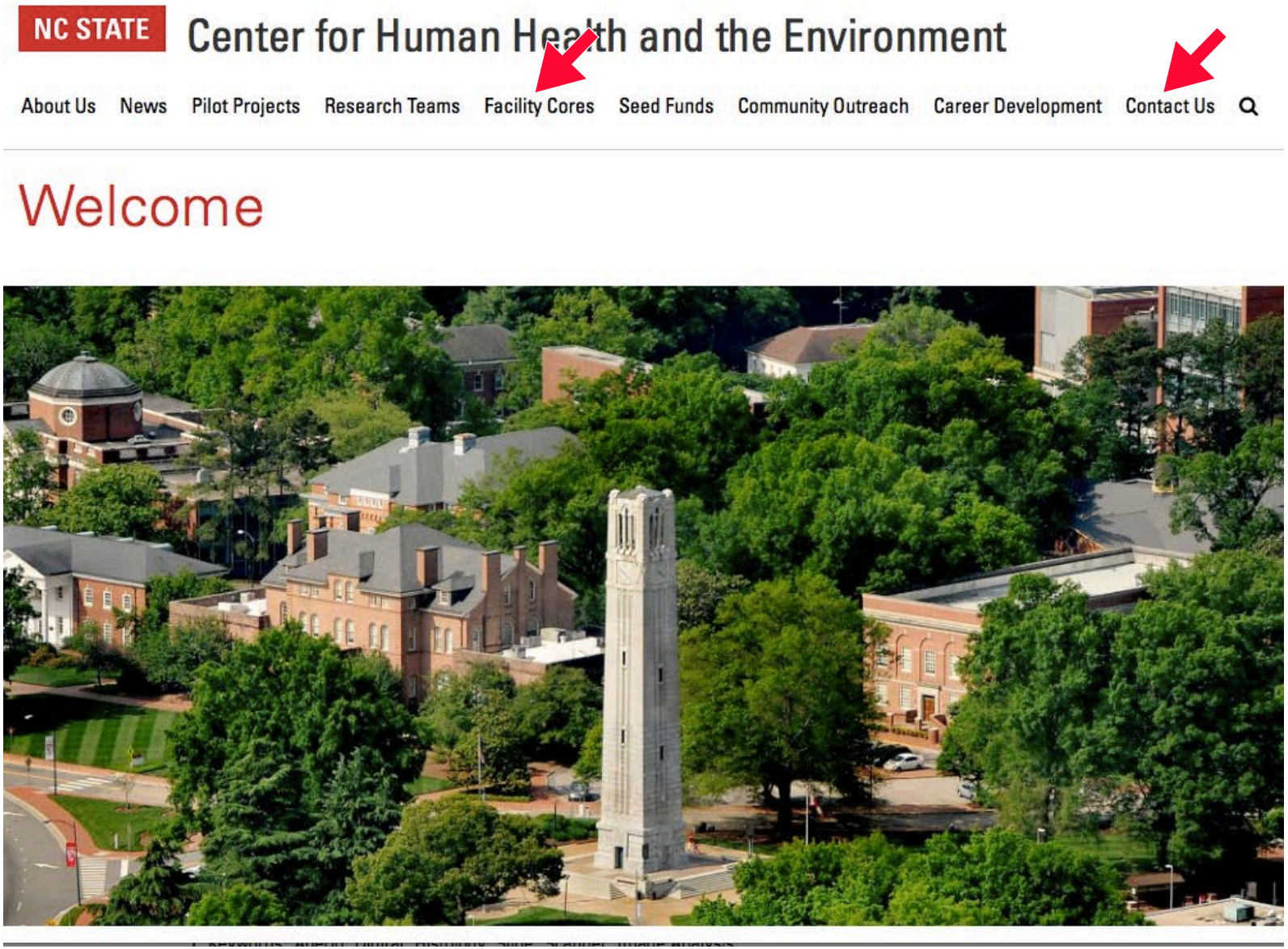
NC STATE UNIVERSITY Center for Human Health and the Environment

Comparative Pathology Core (CPC)

Services:

- Laser capture microdissection (LMD)
  - Leica LMD-7000
- Animal tissue archives
- Grant support and collaboration
  - CPC letters of support, grant methods, effort
- Publication support and collaboration
  - Text, histology images, etc.
- Training
  - Necropsy, pathology assessment methods, etc.
  - Histology / IHC techniques (limited, via histology lab)

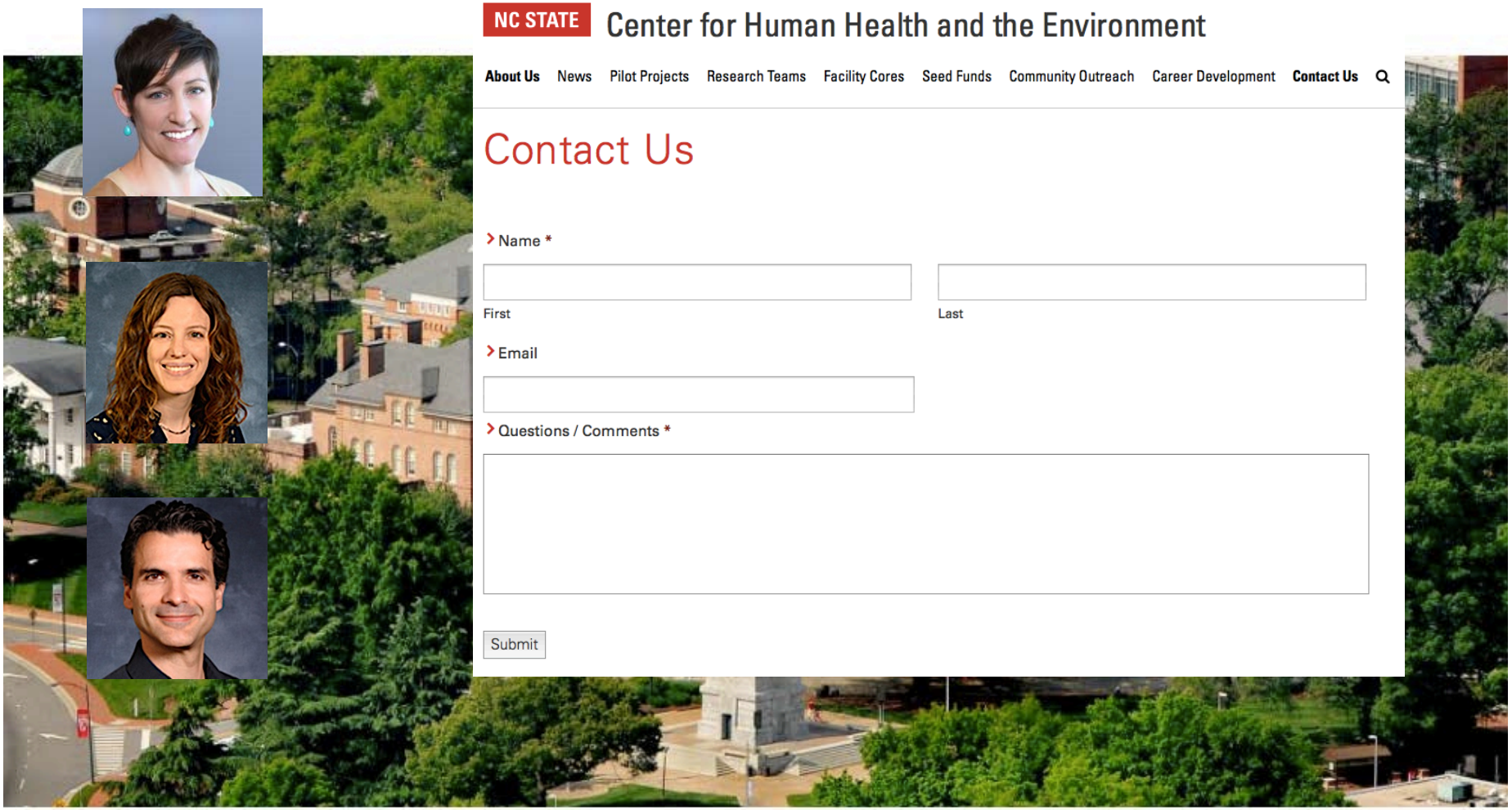
Contacting the Comparative Pathology Core



NC STATE Center for Human Health and the Environment

About Us | News | Pilot Projects | Research Teams | Facility Cores | Seed Funds | Community Outreach | Career Development | Contact Us

Welcome



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Contact Us

Name *

First

Last

Email

Questions / Comments *

Submit

SEARCHABLE CORE LISTING
Core Facilities

Core Facilities

Please remember to recognize the contributions of core facility personnel to your research project when appropriate. ABRF Recommended Guidelines for Authorship on Manuscripts can be found on the Core Facilities Policies and Guidelines Resources page of this website.

Below is an alphabetical listing of our core facilities.

Each core facility's name links to their individual website. Clicking on the 'Profile/Services'in each listing brings up a brief profile of the core, as well as a link to their equipment list if applicable.

This is a searchable database.
You can search the listing either by broad **categories**, specific **keywords** such as 'sequencing' or 'RNA', by **director name**, or by type of **equipment**, such as 'PCR'.
Please click on the sentence below 'Search for a Core Facility by Category or Keyword' to bring up the search function.
Contact the core facility for more information and to discuss your project.

Search for a Core Facility by Category or Keyword

Animal Clinical Chemistry and Gene Expression Laboratories
Contact: Hyung-Suk Kim, Director
Facility Overview: Profile/Services Click on Profile/Services for overview

Animal Histopathology Core Facility (Mice)
Contact: Dawud Hilliard, Director
Facility Overview: Profile/Services Click on Profile/Services for overview

UNC Cores of Pathology Interest

- Animal Histopathology Core*
- Translational Pathology Laboratory*
- Center for Gastrointestinal Biology and Disease (CGIBD): Histology Core
- Histology Research Core
- Tissue Procurement Core
- Microscopy Services Laboratory*
- Biomedical Research Imaging Center (BRIC): Small Animal Imaging
- Injury Biomarker Core*
- Animal Clinical Chemistry and Gene Expression Laboratories*
- Flow Cytometry Core

Animal Histopathology Core
UNC, Lineberger Comprehensive Cancer Center

- Faculty Director
  - Stephanie Montgomery (2014)
- Facility Director
  - Dawud Hilliard (2014)
- 3 Full time histotechnologists
  - Lily Wai – Histotechnologist (2009)
  - Traci Raley – Histotechnologist (2013)
  - Amanda Brown – Histotechnologist (2015)

Animal Histopathology Core

UNC, Lineberger Comprehensive Cancer Center

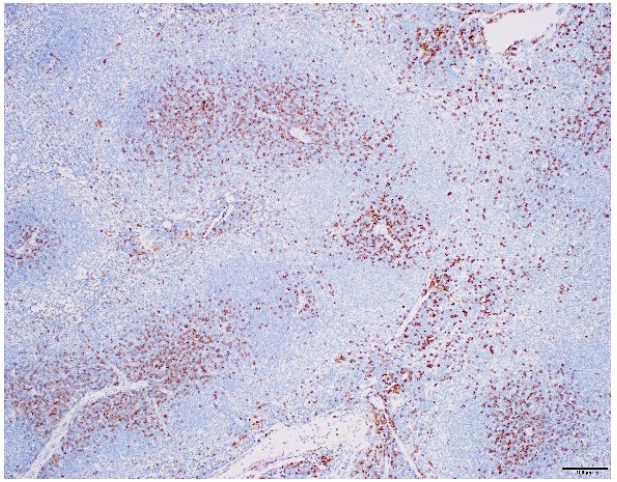
Comprehensive histopathology services

- Tissue processing and embedding
- Histology sectioning (paraffin or frozen)
- Routine and Special Stains (35+)
- Immunohistochemistry & Immunofluorescent stains
  - Multicolor, multiplex IHC or IF, automated
  - 45+ protocols available (FFPE, Mouse)
  - Investigator supplied antibody
- TUNEL staining
- Pathology consultation

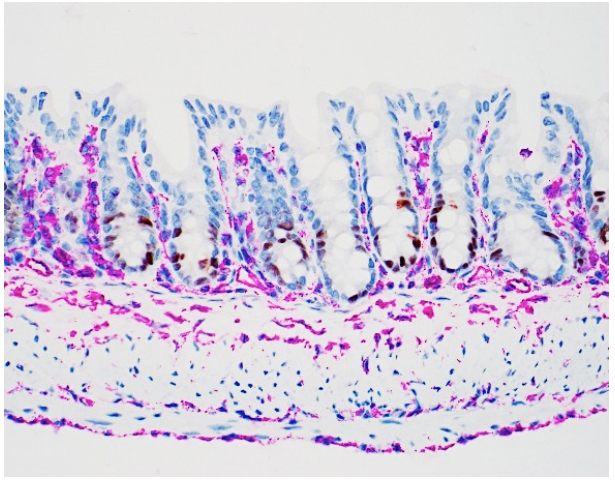
Animal Histopathology Core

UNC, Lineberger Comprehensive Cancer Center

- Alpha Fap
- Arginase 1
- AT8
- B220 (CD45R)
- Beta-Catenin
- BrdU
- Calbindin
- Cleaved Caspase-3
- CD11b
- CD26
- CD206 (mannose)
- CD3
- CD31
- **CD4**
- CD45
- CD47
- CD49b(Alpha 2)
- **CD8**
- CD95 (Fas)
- Epcam
- Fas(C18C12)
- F4/80
- FoxP3
- HT7
- iNOS
- GFP
- Glut 2
- Ly6G (Gr1)
- Ki-67
- MCI
- Myeloperoxidase
- Phospho Akt
- Phospho-Histone H2A.X
- PTEN
- RFP (tdTomato, ds Red)
- S100
- SARS N antigen
- Stat 6
- Survivin
- Tau 12
- Vimentin



CD8 IHC, mouse spleen



Ki-67 & vimentin, mouse colon

Animal Histopathology Core

UNC, Lineberger Comprehensive Cancer Center

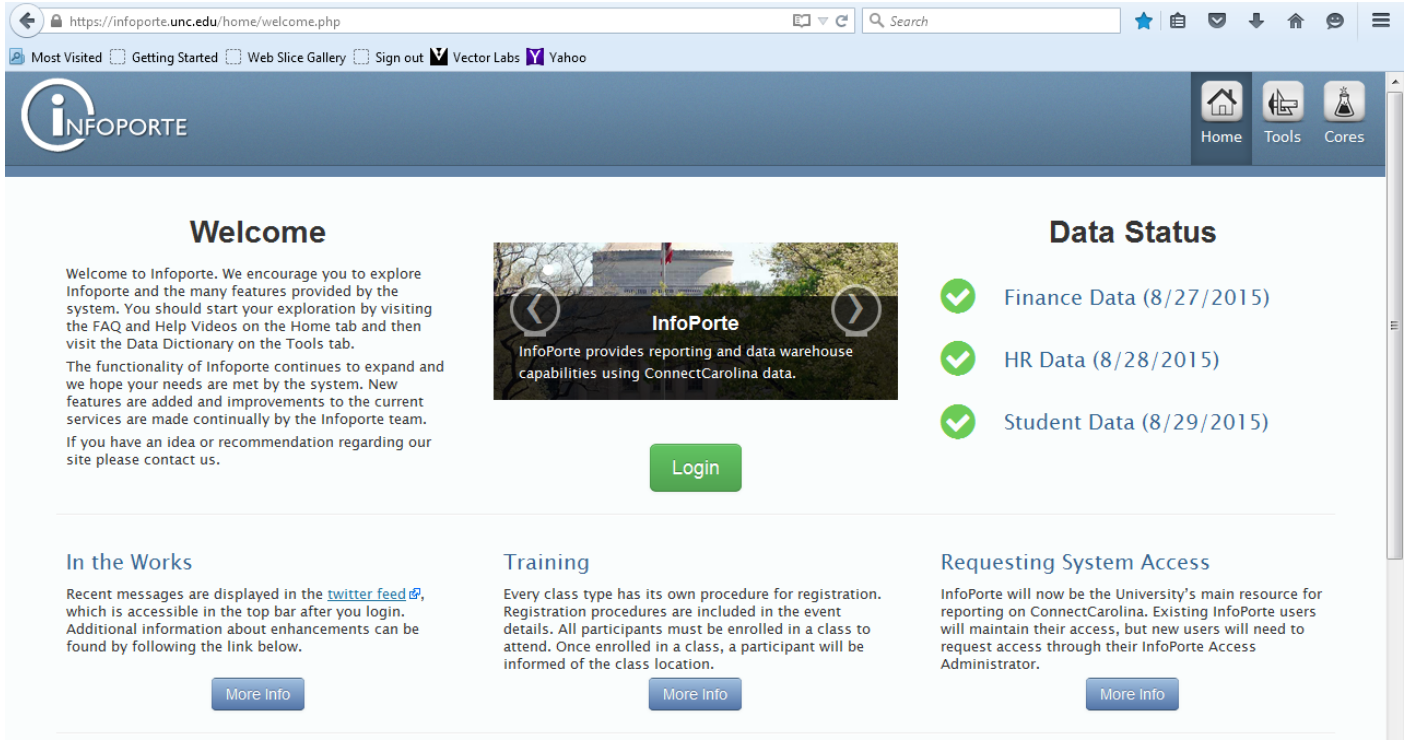
Histology Pricing	AHP UNC Investigator	Histo Lab NCSU
Process & Embed	$3.16	
Section 1 slide	$2.92	$3.90
H&E stain	$3.30	$4.60
1 H&E slide from fixed tissue	**$9.38**	**$8.30**
Special Stain - Level 1	$3.60	$14.40
Special Stain - Level 2	$4.92	
Special Stain - Level 3	$8.55	$15.40
IHC single color	**$20.54**	**$32.30**(27.10)
IF single color	$19.98	
Dual color IHC	$34	
Tech time (Special req)	$43.74/hr	
Frozen section	Tech rate+dispos	$8.00/Tissue

Animal Histopathology Core

UNC, Lineberger Comprehensive Cancer Center

Submitting an order

- First-time user: contact us to create an account
- Submit an online request
- Drop off or ship specimens



Animal Histopathology Core

UNC, Lineberger Comprehensive Cancer Center

LCCC Animal Histopathology Core

(919) 966-3653

lcccanimalistopathology@med.unc.edu

333 S. Columbia, 426 MacNider Hall

University of North Carolina

Chapel Hill, NC 27599

Stephanie A. Montgomery, PhD, DVM, Dipl. ACVP,

stephanie_montgomery@med.unc.edu

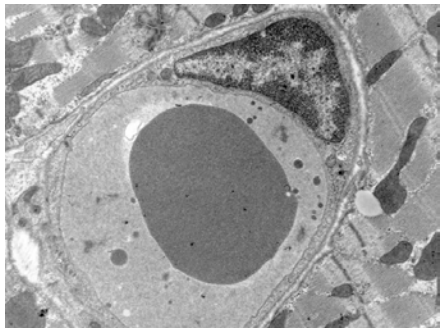
Dawud Hilliard, MPM, HTL (ASCP) QIHC,

dawud_hilliard@med.unc.edu

Microscopy Services Laboratory

UNC, Lineberger Comprehensive Cancer Center

TEM



Dr. Ariel, PhD, Director

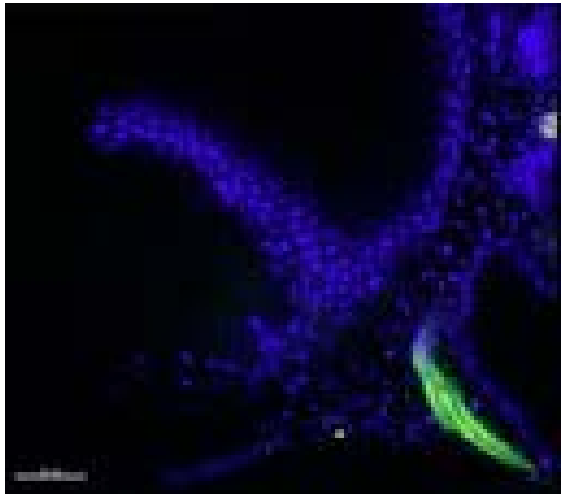
(919) 966-2413, pablo_ariel@med.unc.edu

- Electron microscopy
  - TEM, Immuno-TEM, negative staining EM, SEM
- Light Microscopy
  - Zeiss LSM 710 Spectral Confocal Laser Scanning Microscope
  - Zeiss LSM 700 Confocal Laser Scanning Microscope: 4 excitation lines, 2 fluorescent and 1 transmitted channel
  - Olympus IX70 Inverted wide field, fluorescence, Live stage housed in an incubator for long term live cell imaging.
  - Leica M420 Macroscope
- Morphometry

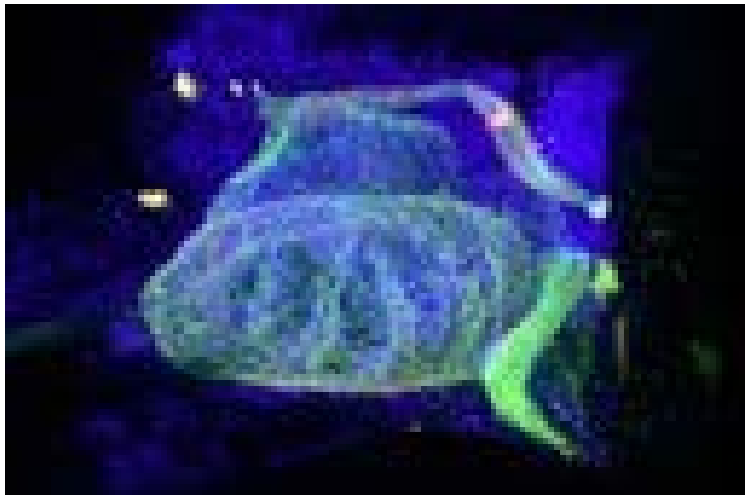
Microscopy Services Laboratory

UNC, Lineberger Comprehensive Cancer Center

3D Recon Frog Heart

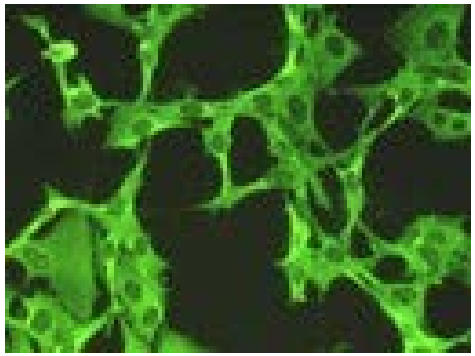


Z-Stack Frog Heart

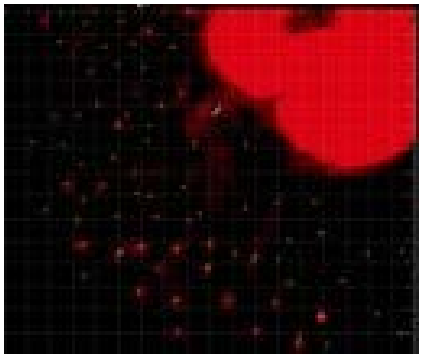


BF

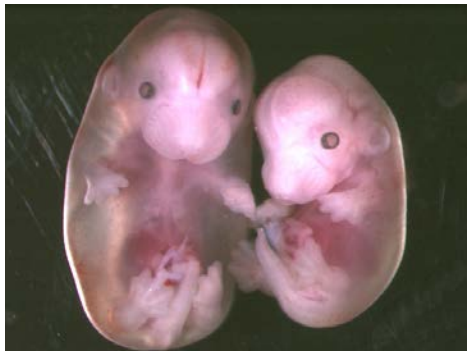
IF



Live Cell

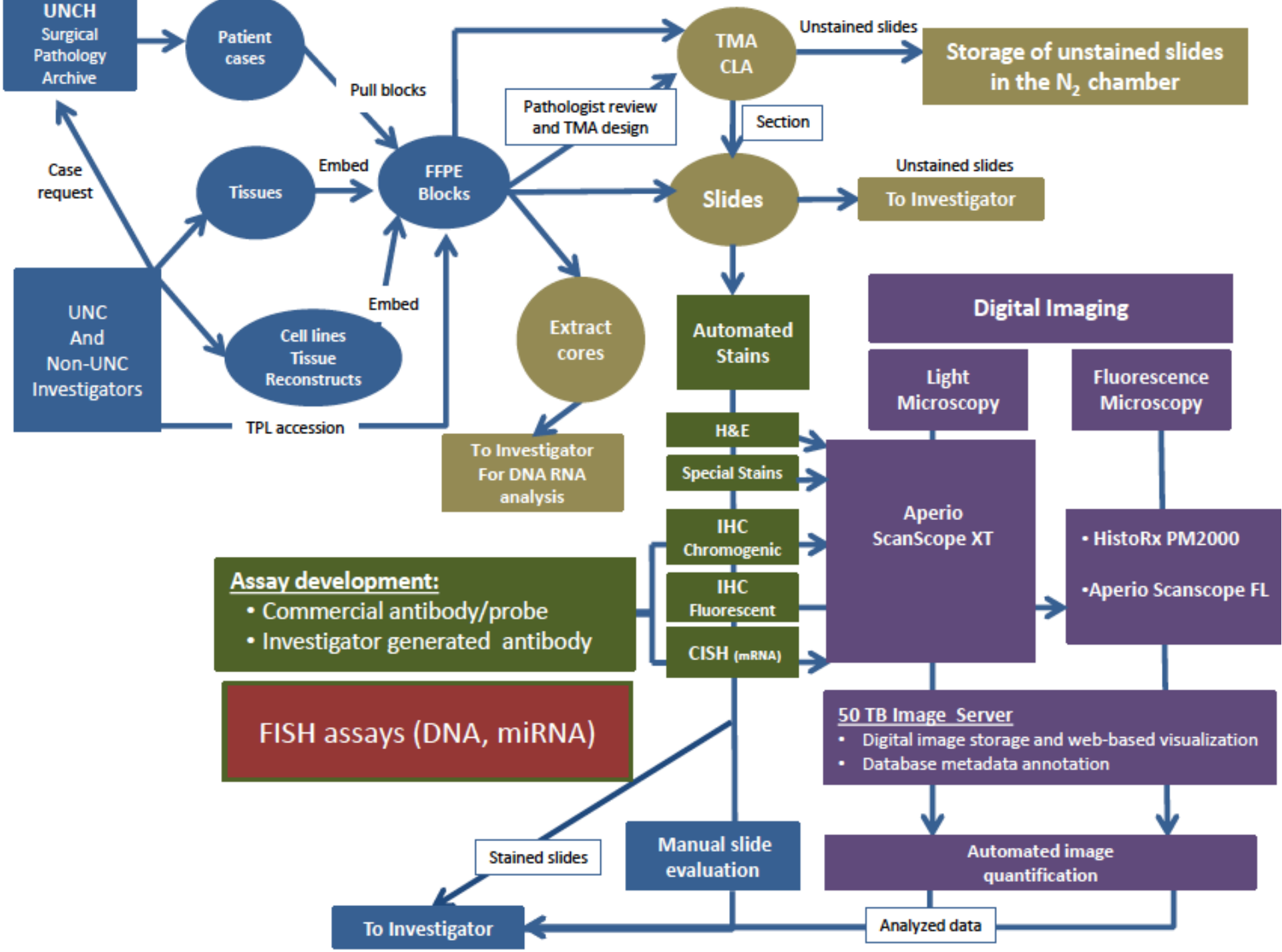


Macro



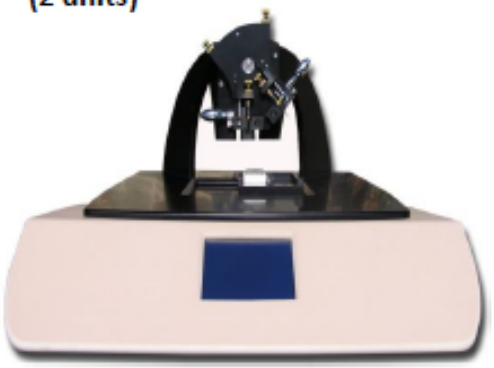
Histology and Digital Pathology services at UNC Translational Pathology Laboratory

Nana N. Feinberg
Translational Pathology Laboratory
UNC-CH
Facility Director


UNCH
Surgical
Pathology
Archive
Patient cases
Pull blocks
TMA
CLA
Unstained slides
Storage of unstained slides in the N_2 chamber
Pathologist review and TMA design
Section
Case request
Tissues
Embed
FFPE Blocks
Slides
Unstained slides
To Investigator
UNC
And
Non-UNC
Investigators
Embed
Cell lines
Tissue
Reconstructs
Extract cores
Automated Stains
Digital Imaging
Light Microscopy
Fluorescence Microscopy
TPL accession
To Investigator For DNA RNA analysis
H&E
Special Stains
IHC Chromogenic
IHC Fluorescent
CISH (mRNA)
Aperio ScanScope XT
• HistoRx PM2000
•Aperio Scanscope FL
Assay development:
• Commercial antibody/probe
• Investigator generated antibody
FISH assays (DNA, miRNA)
50 TB Image Server
• Digital image storage and web-based visualization
• Database metadata annotation
Stained slides
Manual slide evaluation
Automated image quantification
To Investigator
Analyzed data

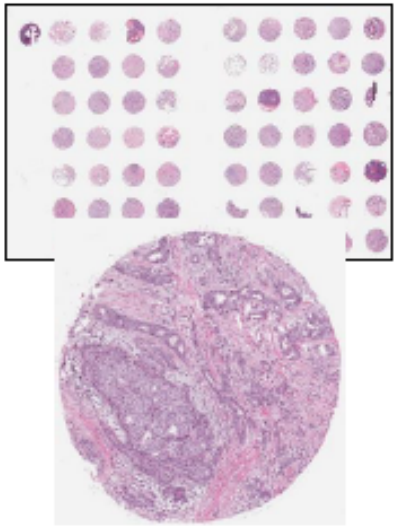
TMA Arrayer

Pathology Devices
Semi-automated Tissue Arrayer
(2 units)

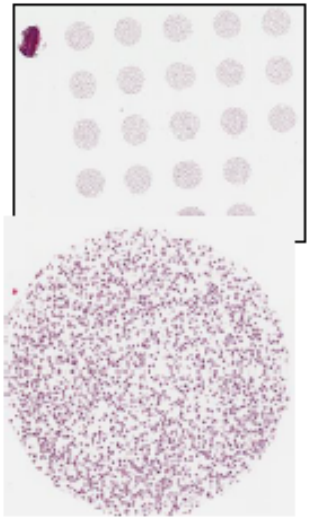


Nitrogen Cabinets for storage of unstained slides

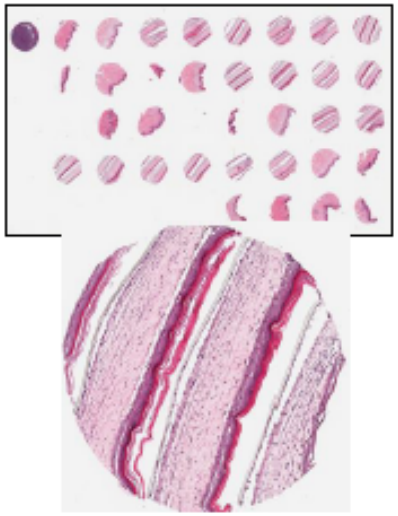
Tissue (human/mouse)

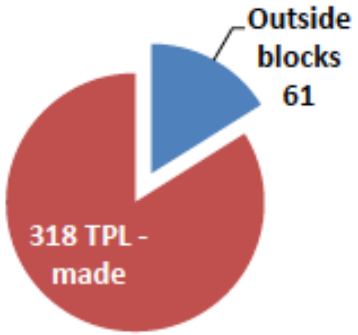


Cell lines



Tissue Reconstructs





Major slide staining equipment (Leica)

Bond max

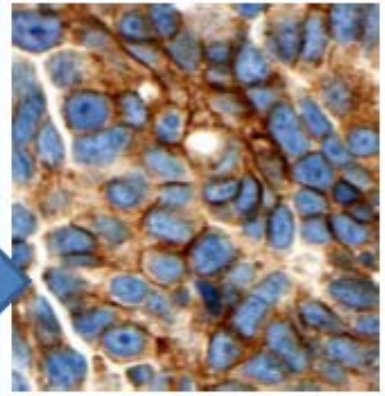
Bond III (2 units)

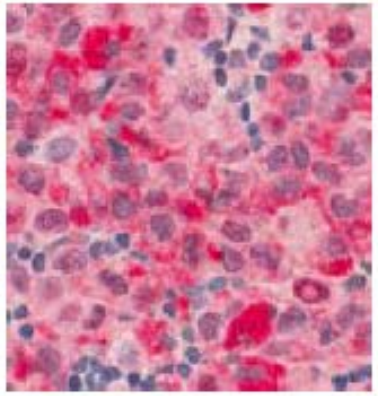
Bond Rx

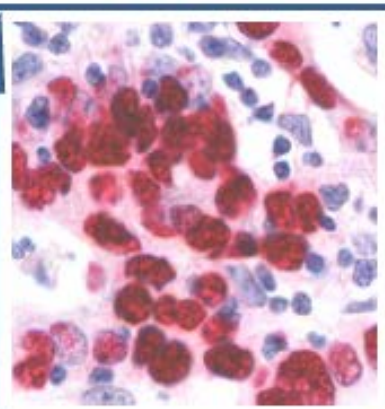
IHC

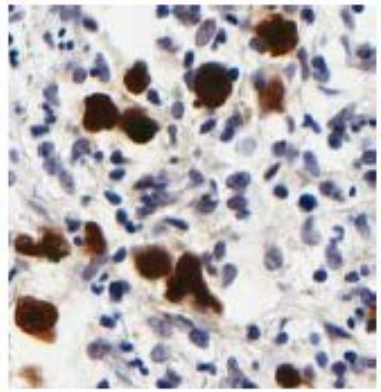
ISH

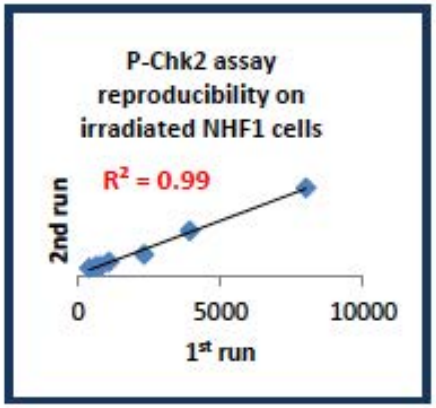
IF

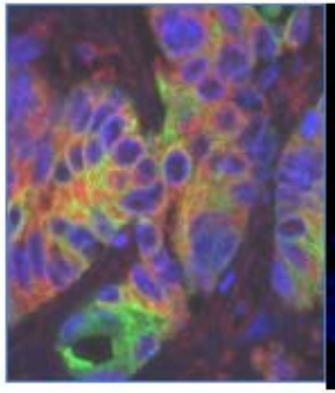


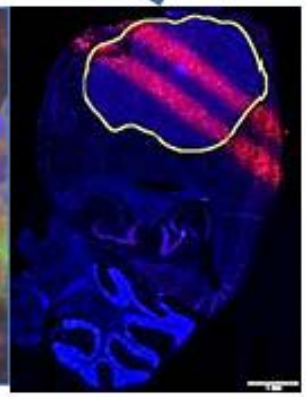


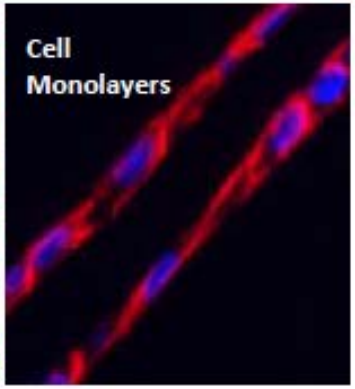


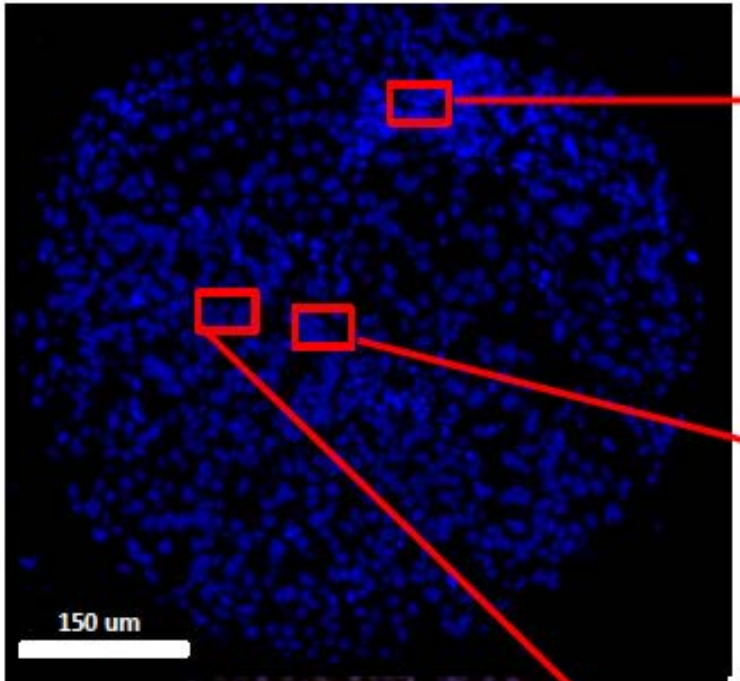


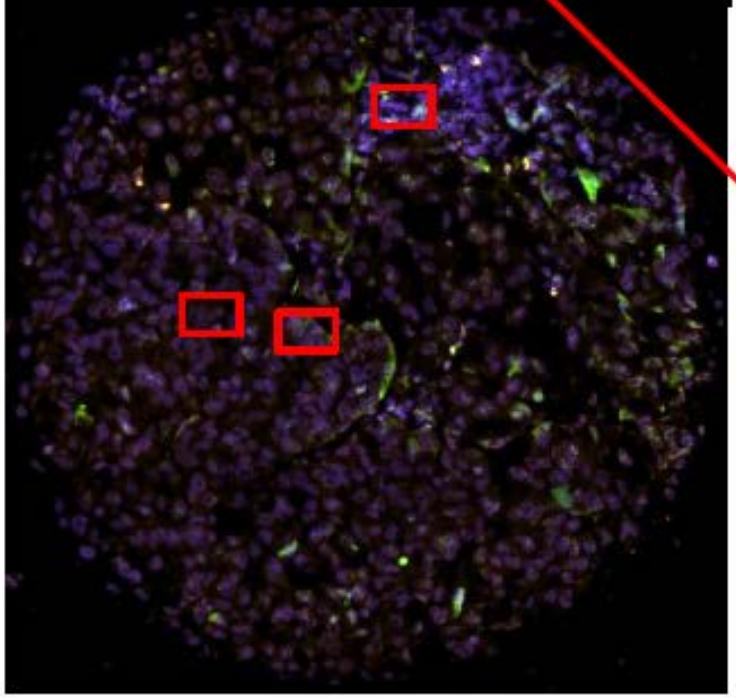


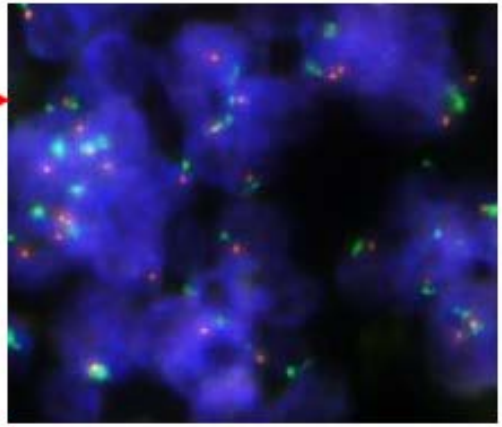


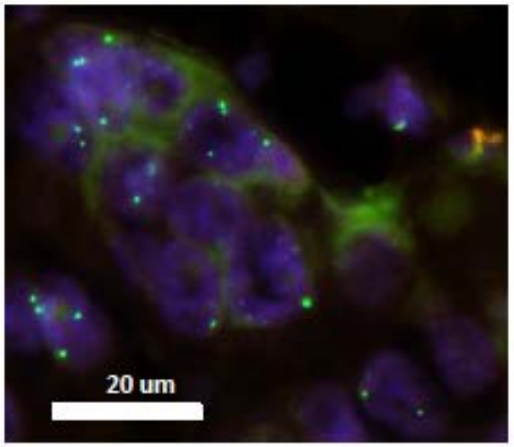


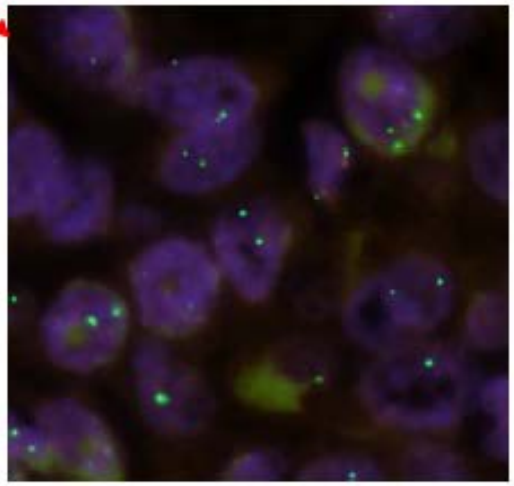


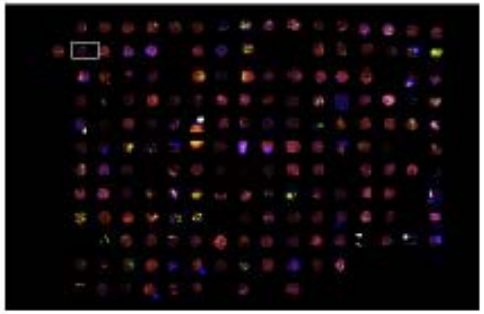












CDKN2A Red

CEP9 Green

CDKN2A FISH

TMA 100A1

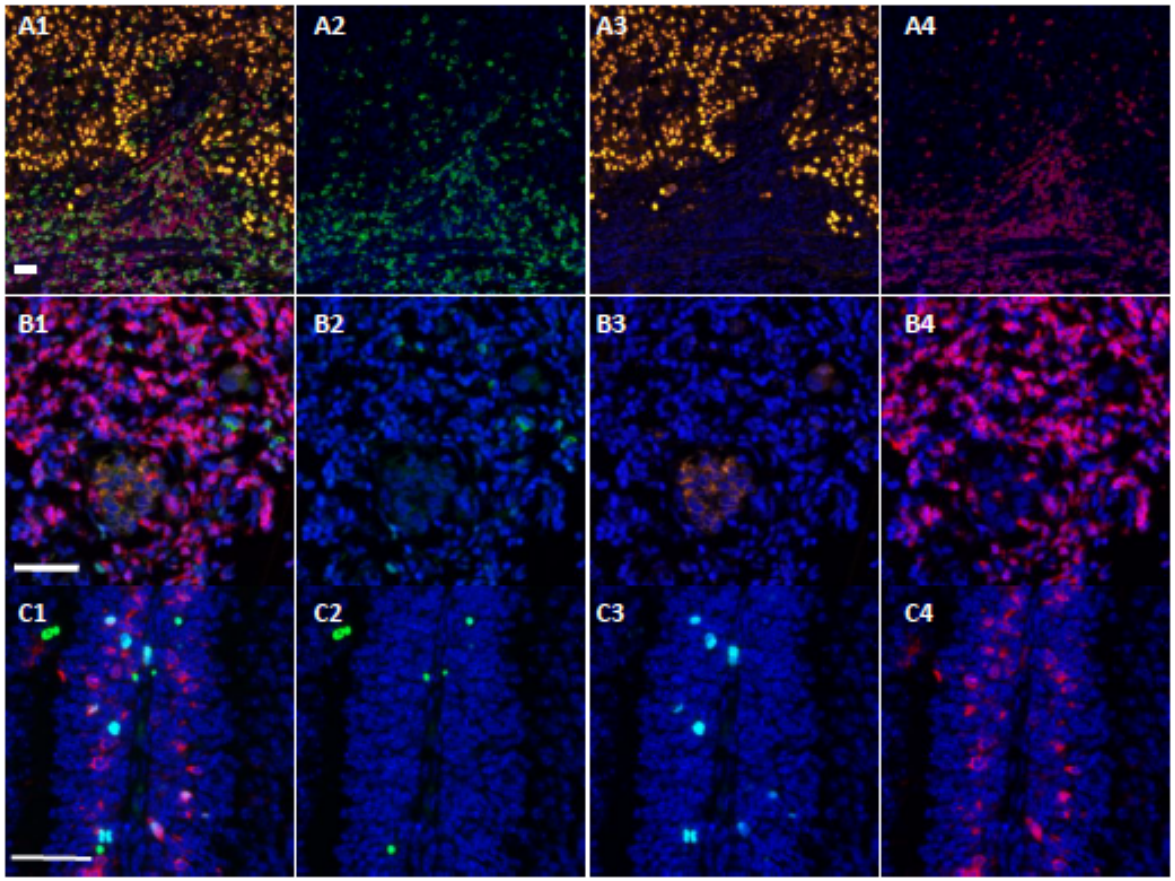
section 21

ARIOL Scan

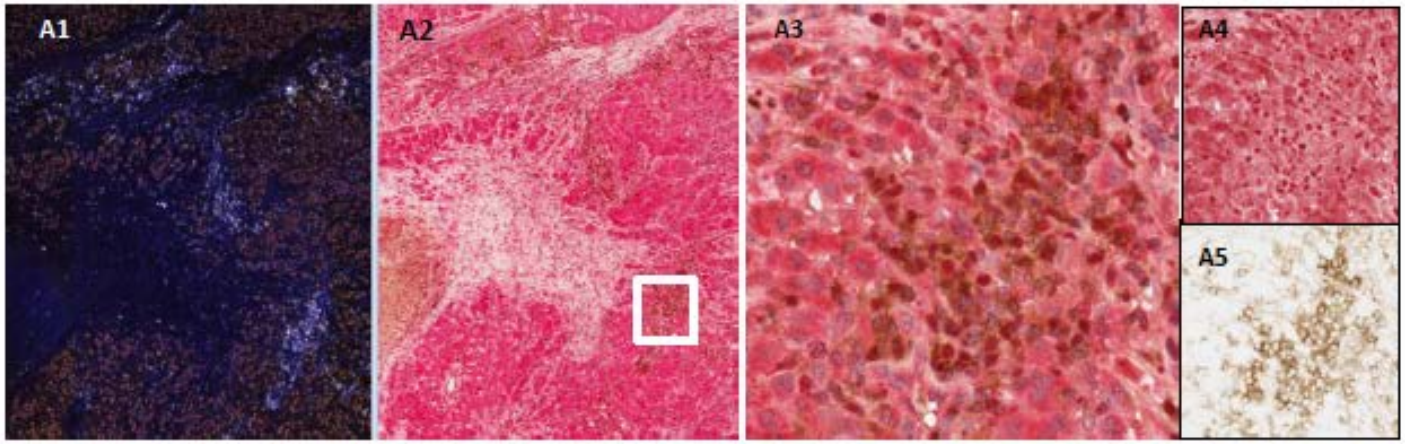
3-plex IF images done by TPL

Panel A. (A1) Human Melanoma FFPE tissue stained with Sox10 in orange (Cy3), CD3 in red (Cy5), CD8 in green (Alexa488), and Hoechst 33258 nuclear
counterstain in blue. (A2-A4) the same image shown in dual channels: (A2) CD8 and nuclear, (A3) Sox10 and nuclear, (A4) CD3 and nuclear.
Panel B. (B1) Breast carcinoma FFPE tissue stained with Palladin in green (FITC), Pan-Cytokerstin (CK) in orange (Cy3), CD3 in red (Cy5), and
Hoechst 33258 nuclear counterstain in blue. (B2-B4) the same image shown in dual channels: (B2) Palladin and nuclear, (B3) CK and nuclear, (B4) CD3 and nuclear.
Panel C. (C1) FFPE Mouse brain stained with γH2AX in green (Alexa488), phosphoH3 (pH3) in turquoise (Cy3), Cyclin B1 in red (Cy5), and
Hoechst 33258 nuclear counterstain in blue. (C2-C4) the same image shown in dual channels: (C2) γH2AX and nuclear, (C3) pH3 and nuclear, (C4) Cyclin B1 and nuclear.

The scale bars on all panels equal to 50 µm.

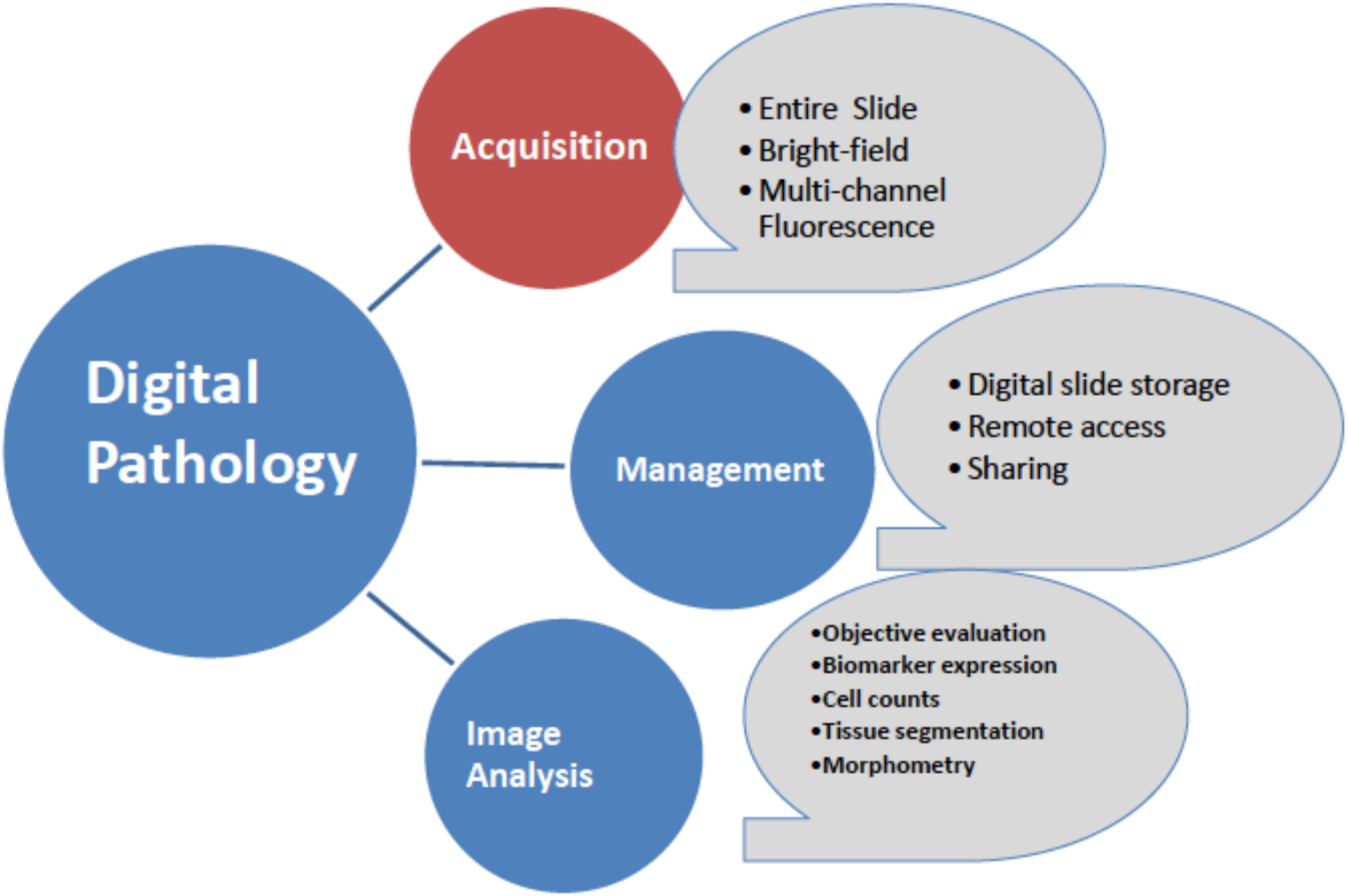


5-plex (IF+IHC)



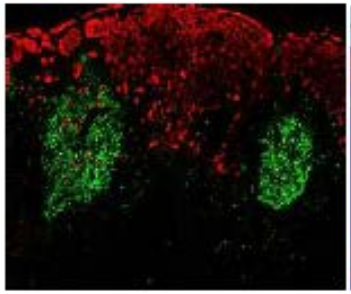
A1. Melanoma FFPE tissue (section #10) stained with Sox10 (cy3), CD3 (cy5) and CD8 (Alexa488). Sox10 is shown in orange, CD3 and CD8 in white. **A2-A5.** The same slide (section #10) shown in A1 was stained with CD45 IHC (brown) and PD-L1 (red), scanned and analyzed using Aperio color deconvolution algorithm. (A2) shows CD45-PD-L1/brown-red dual IHC image. (A3) is enlarged from the white box on A2. (A4, A5) show A3 image of the deconvolved red (PD-L1) and brown (CD45) channels respectively by the Aperio color deconvolution algorithm.

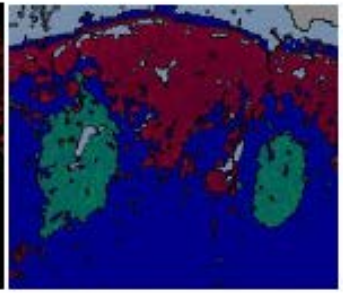
Digital Pathology:

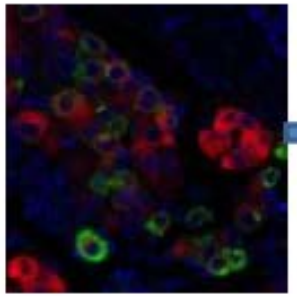


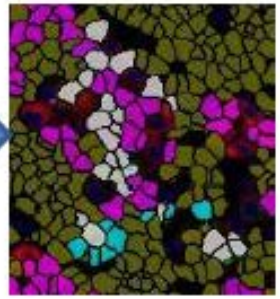
Definiens Tissue Studio

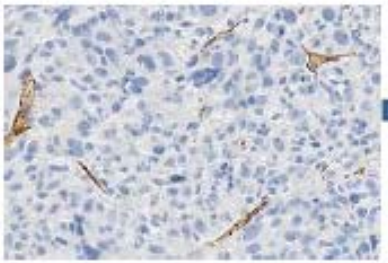
Tissue segmentation

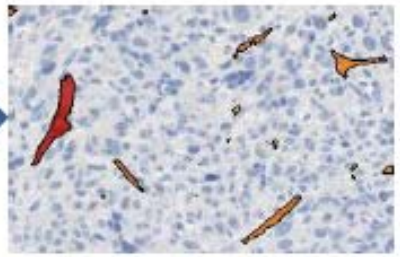




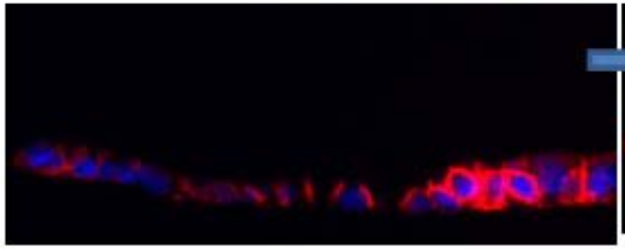


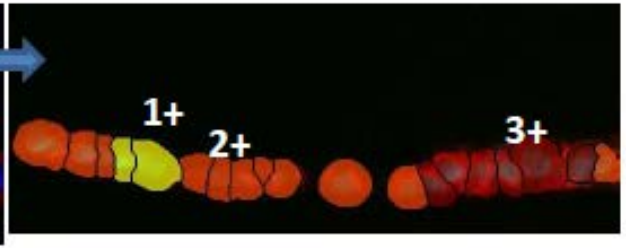


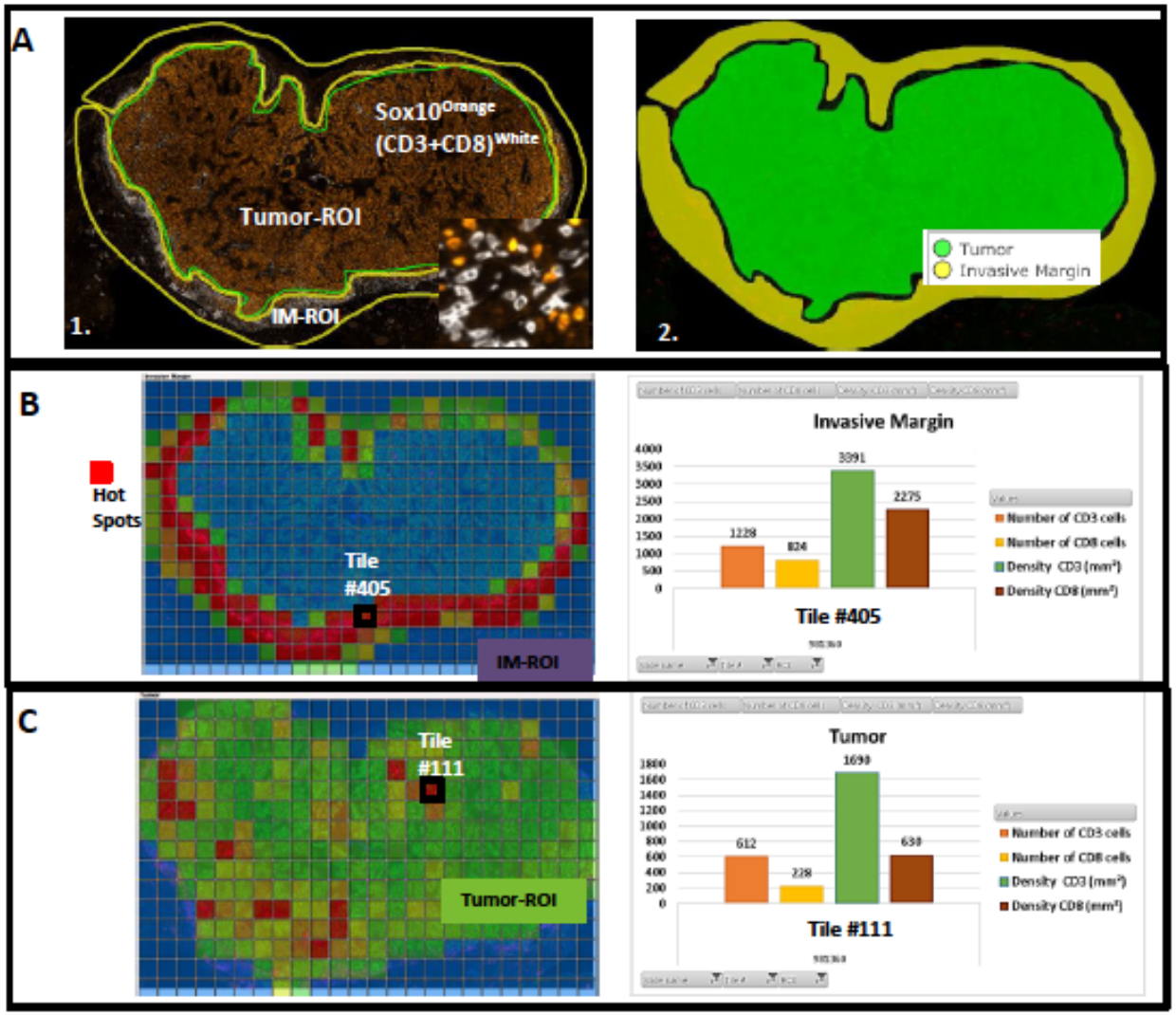




Algorithms:
Nuclear, Co-localization,
Microvessel, Membrane,

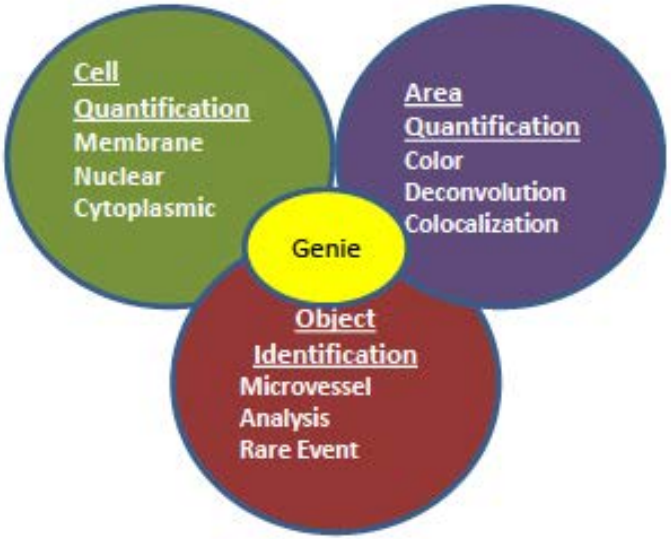


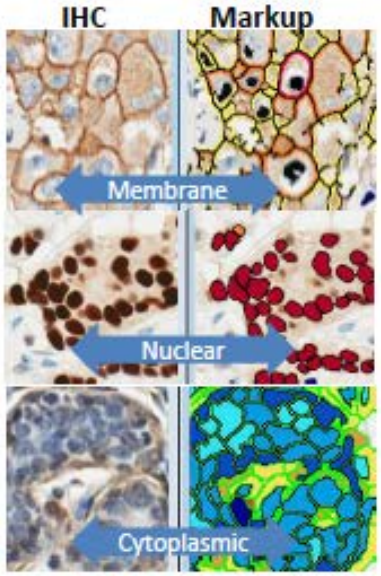


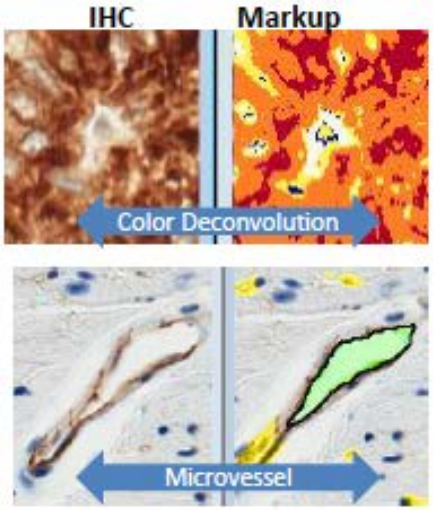


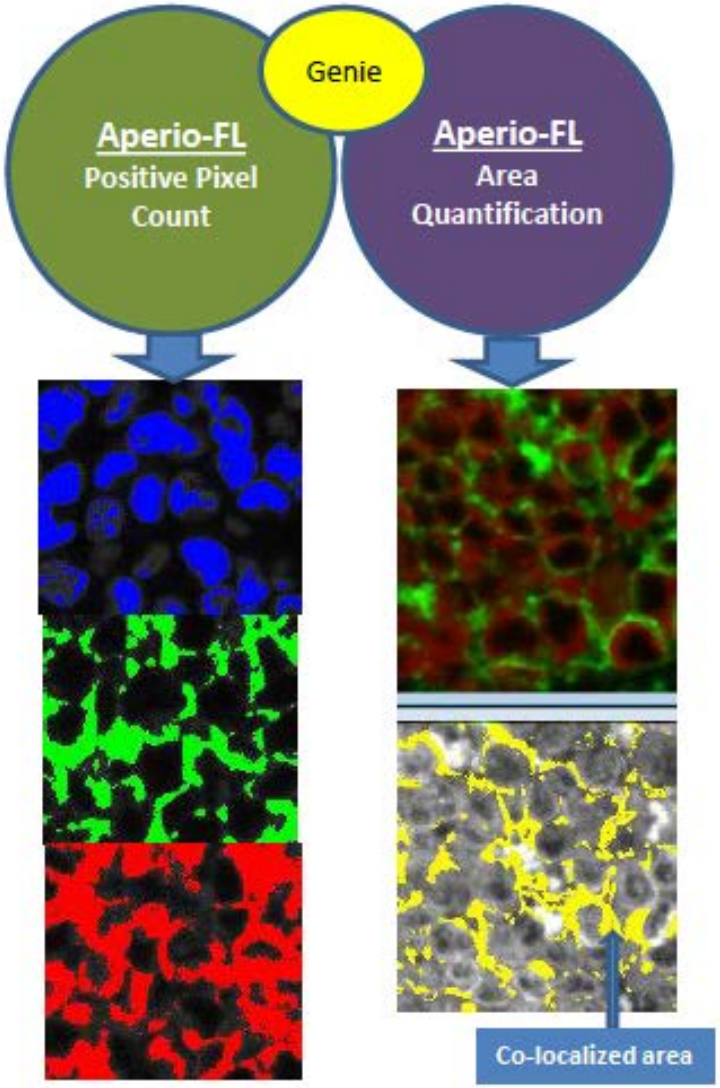
A. (1) Manually defined Tumor and IM ROIs in the Aperio Imagscope. Sox10 is shown in orange and CD3+CD8 cells in white. Sox10 positivity
B. was used to circle tumor ROI and an adjacent 0.5-1mm wide invasive margin (IM). (2) Tumor and IM defined by Definiens Tissue Studio
C. using imported ROIs from Aperio. B-C. Demonstrates Tissue Studio analysis: left panels show CD3 heat map per ROI. The tiles with high density of
D. CD3 are shown in red. The charts on the right display number and density/mm² of CD3 and CD8 cells in the black-outlined tile on the heat map.

Aperio image analysis algorithms

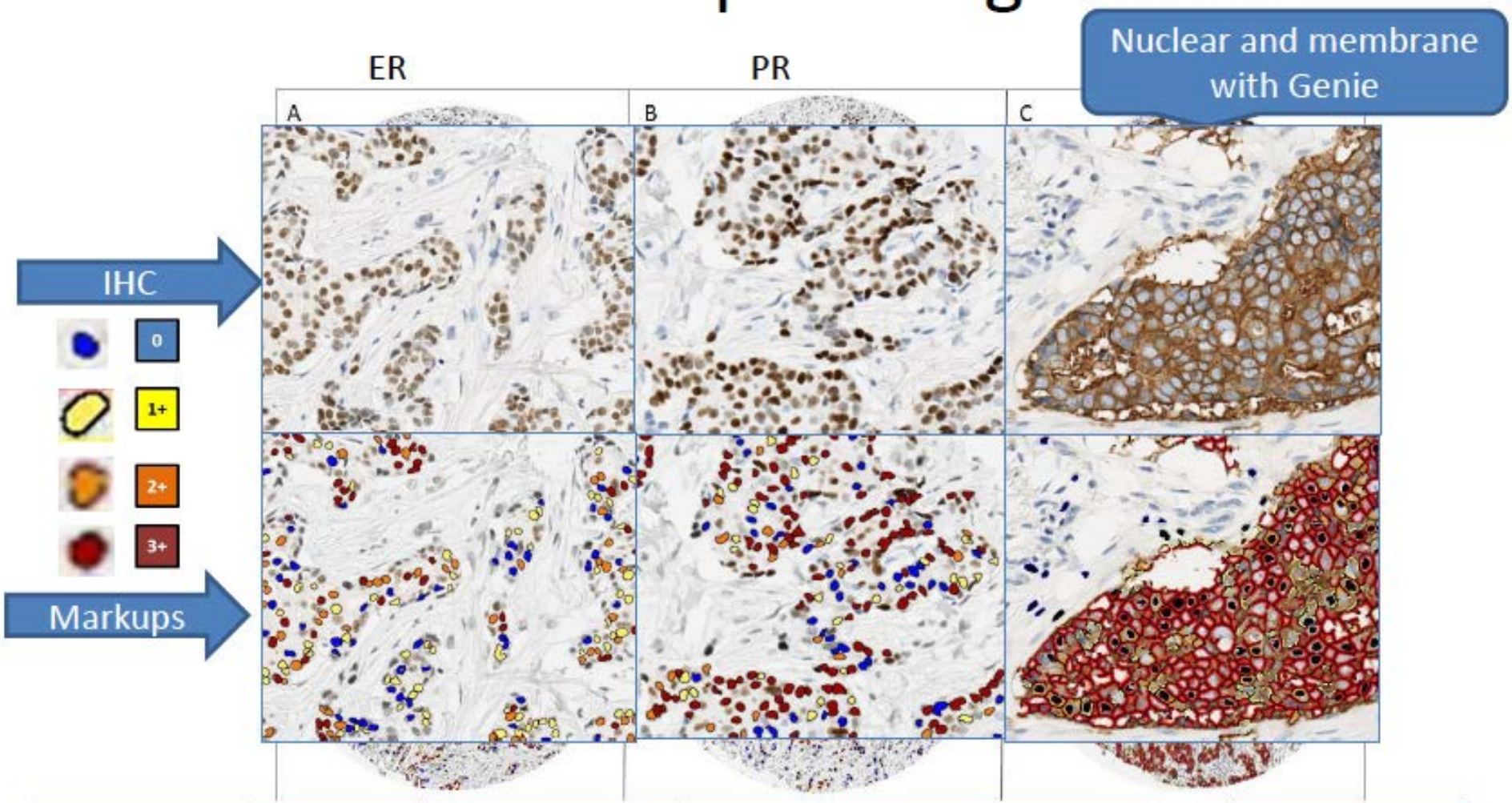








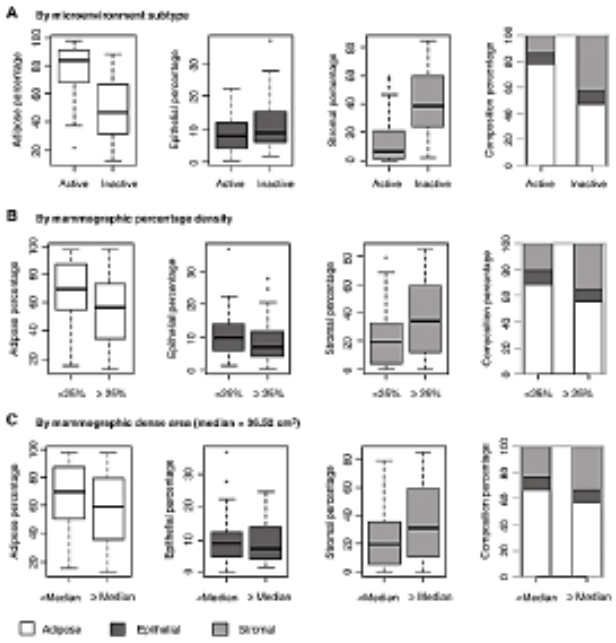
Validation of Aperio Algorithms

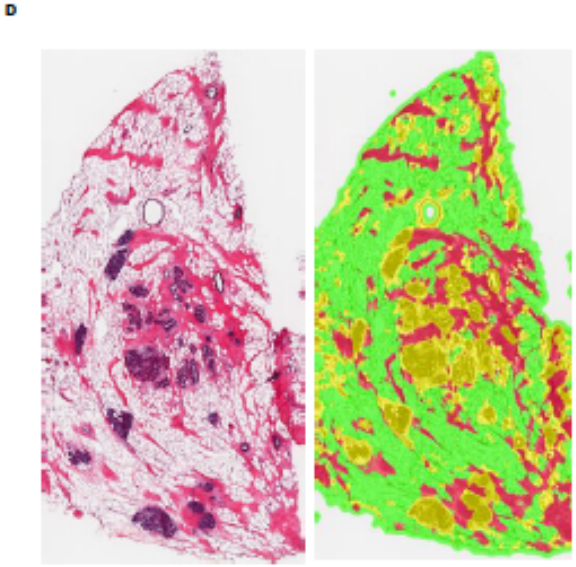


Marker	N	Observed agreement	Kappa (95% CI)	Sensitivity	Specificity
ER	242	89%	0.80 (0.73-0.86)	86%	93%
PR	280	91%	0.83 (0.78-0.89)	96%	85%
HER2	309	93%	0.79 (0.71-0.87)	98%	94%

Breast tissue composition distribution by the Active/Inactive subtype and mammographic density.

Genie Algorithm





Adipose Tissue	
Epithelium	
Stroma	
Glass	

Sun X, Gierach GL, Sandhu R, Williams T, **Midkiff BR**, Lissowska J, Wesolowska E, Boyd NF, Johnson NB, Figueroa JD, Sherman ME, Troester MA. Relationship of mammographic density and gene expression: analysis of normal breast tissue surrounding breast cancer. Clin Cancer Res. 2013;19(18):4972-82.

Animal Clinical Chemistry and Gene Expression Laboratories

UNC School of Medicine, Dept Pathology and Lab Med

Dr. Kim PhD, Director

(919) 966-3539 hskim@med.unc.edu

- Hematological tests - 25ul EDTA-whole blood per sample
  - **WBC**: Lymphocytes, Monocytes, Granulocytes
  - **RBC:** HGB, HCT, MCV, MCH, MCHC, RDW
  - **PLT:** MPV
- Clinical Chemistries - 10ul plasma, serum, or urine/test +10ul
  - **Ions**: Ammonia, Blood Urea Nitrogen, Ca, Cl, CO2, Fe, Total Iron-Binding Capacity, K, Lithium, Mg, Na, and Phosphorus
  - **Protein/Organics:** Albumin, Bilirubin, Creatinine, Cholesterol, HDL-Cholesterol, Glucose, Total Protein, C-Reactive Protein, Urine Protein, Cerebral Spinal Fluid Protein, Triglycerides, Uric Acid, and Urine Creatinine
  - **Enzymes:** Alanine Aminotransferase, Acid Phosphatase, Alkaline Phosphatase, Amylase, Aspartate Aminotransferase, Cholinesterase, Creatinine Kinase MB, GGT, Lactate, LDH, and Lipase

The University of North Carolina Organ Injury Biomarker Core

We are excited to announce the launch of The Hamner-UNC Organ Injury Biomarker Core*, a full service core designed to assist investigators in study design, measurement, and interpretation of recently discovered biomarkers of organ injury. The traditional biomarkers utilized to detect the presence of hepatic, renal and/or cardiac injury are suboptimal, lacking sensitivity and specificity. The new biomarkers not only provide enhanced sensitivity and specificity for injury detection but can additionally inform on mechanism of injury. We currently are focusing on liver, kidney, and cardiac injury biomarkers in clinical and non clinical species; however, we are willing to expand our menu of assays to include other organs based on the needs of the investigators we serve. Please contact us.

UNC ESHELMAN SCHOOL OF PHARMACY

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Advantages of working with our core:

- In-house expertise in biomarker measurement/interpretation
- Access to *in silico* modeling that incorporates release and clearance kinetics to improve study design and data interpretation (liver only at present)
- Collaborations with external experts in the fields of liver, kidney, and cardiac injury

Multiple technologies available including:

- Standard clinical chemistry analyzer
- Luminex
- Meso Scale Discovery (MSD)
- Firefly Bioworks

Services offered:

- **Study design assistance** (recommendations for biofluid sampling times, biofluid collection/processing, and pertinent biomarkers to assess)
- **Biomarker measurements** (dependent on species; including but not limited to)
  - o Hepatic: microRNA profiling, miR-122, HMGB1 (total), K18 (full length and cleaved), GLDH, total bile acids, ALT, AST, ALP, total bilirubin, direct bilirubin, LDH, and albumin
  - o Renal: KIM-1, clusterin, albumin, total protein, cystatin c, RPA-1, β2-microglobulin, osteopontin, NGAL, GSTα, GSTμ, sCr, and BUN
  - o Cardiac: microRNA profiling, cTnT, cTnI, hFABP, BNP, NT-proBNP, CK-MB, LDH, AST
- **Data Analyses/Interpretation**

MicroRNA Profiling - Now Available

MicroRNAs are small, noncoding RNA species that post-transcriptionally regulate gene expression. These species make ideal biomarkers because they are highly stable in biofluids, such as blood and urine, and can be released into circulation following organ injury. Utilizing minimal sample volume, we can now profile targeted panels (68-plex) of microRNAs in a high throughput format.

MicroRNA panels are available for the following areas of interest:
Immunology, Cardiology, Liver Toxicology, Neurology, and Oncology

Rapid funding opportunity available now

For a limited time, we are accepting one-page proposals to initiate collaborations in which biomarker measurements, analyses, and interpretations will be conducted by our core for little to no cost to investigators (up to a maximum of $2000 in consumables supplied). We are seeking projects in which liver, renal, or cardiac injury are suspected in clinical or nonclinical studies but have not yet been explored with the offered biomarkers. Please contact us for more details or to submit a proposal. Funding decision will be made within two weeks of submission.

*Supported by the UNC TraCS Institute and CTSA grant 1UL1TR001111.

For more information, to submit a study proposal, or to request a consultation,
contact: Rachel Church, Ph.D., Director
rchurch@unc.edu · 919-226-3145

Organ Injury Biomarker Core
Hamner-UNC / Eshelman School of Pharmacy

Dr. Rachel Church, PhD, Director

(919) 226-3145, rchurch@unc.edu

- Study design, data analysis/interpretation, different species
- Biomarker measurements

  Hepatic: MicroRNA, miR-122, HMGB1, K18, GLDH, Bile acids, ALT, AST, ALP, Bilirubin, LDH, albumin

  Renal: KIM-1, clusterin, albumin, total protein, cystatin c, RPA-1, beta2-microglubulin, osteopontin, NGAL, GSTalpha, sCr, BUN

  Cardiac: MicroRNA, cTnT, cTnl, hFABP, BNP, NT-proBNP, CK-MP, LDH, AST

  MicroRNA profiling - 68 plex technology, Firefly, biofluids

- Technology - *small sample size, multiplex*

  Firefly, Meso Scale Discovery (MSD), Luminex, chemistry analyzer

CHHE

Comparative Pathology Core Information Session

QUESTIONS?

Keith E. Linder

May 3rd, 2016

NC STATE UNIVERSITY
Center for Human Health and the Environment