TMA: A Valuable Clinical & Research Resource

Helen L. Fedor

National Society for Histotechnology

Seattle, Washington

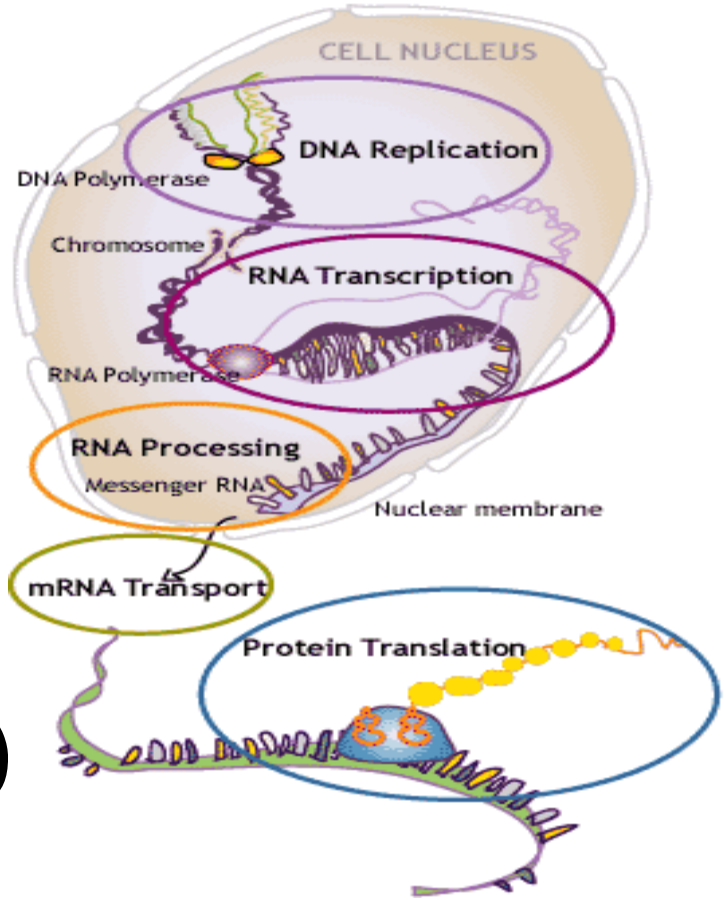
September 28, 2010

Genes to Protein

DNA (Data Storage)

RNA (Read Out)

Protein (Product)



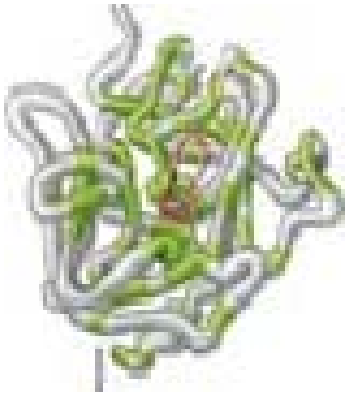
How Does DNA Specify the Sequence of a Protein?

- DNA sequences must be “decoded” to make a protein
- This decoding requires creation of an RNA template
- Creation of “messenger RNA” is called **transcription**
- Creation of protein from the mRNA is called **translation**

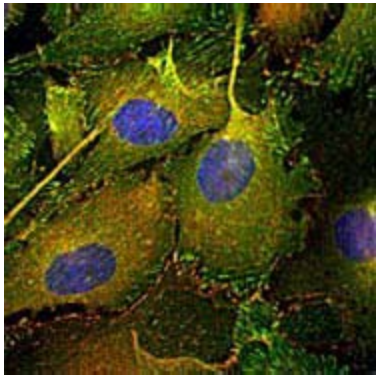
Protein to Cellular Components

Protein

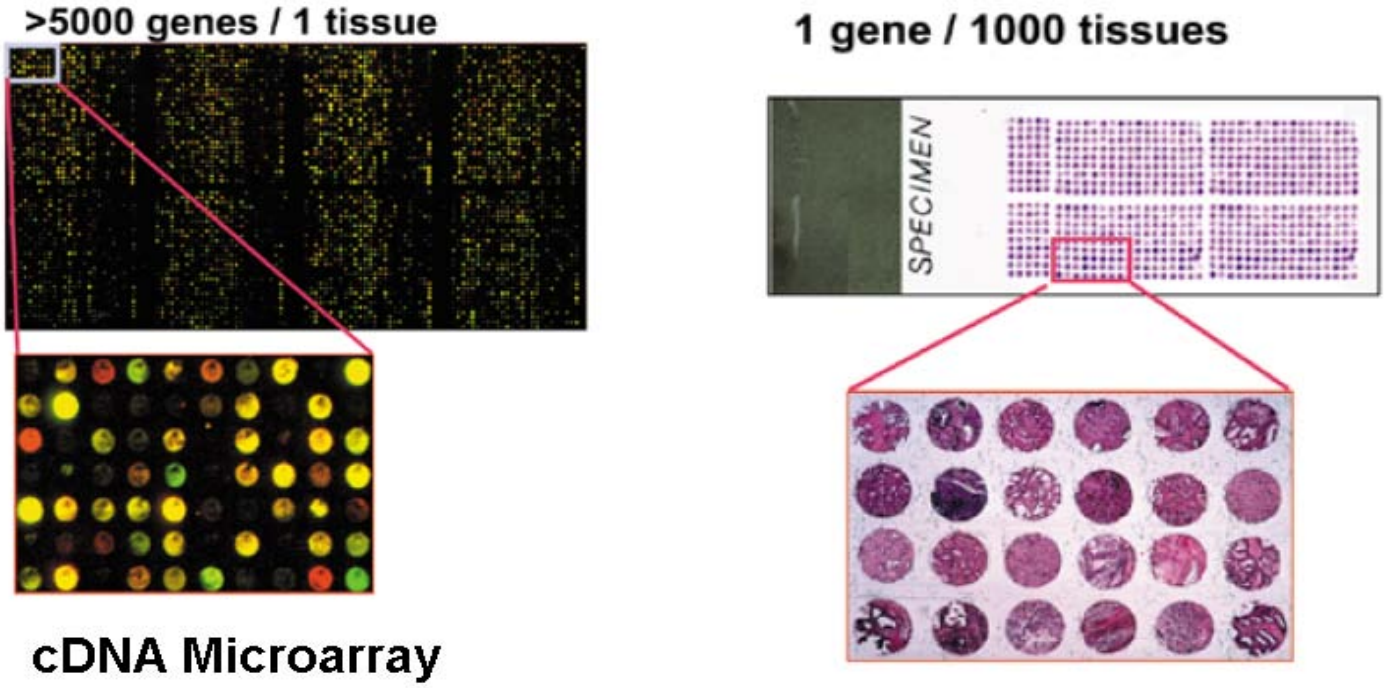
Folding to form tertiary structure



Assembly of proteins to
from cellular components



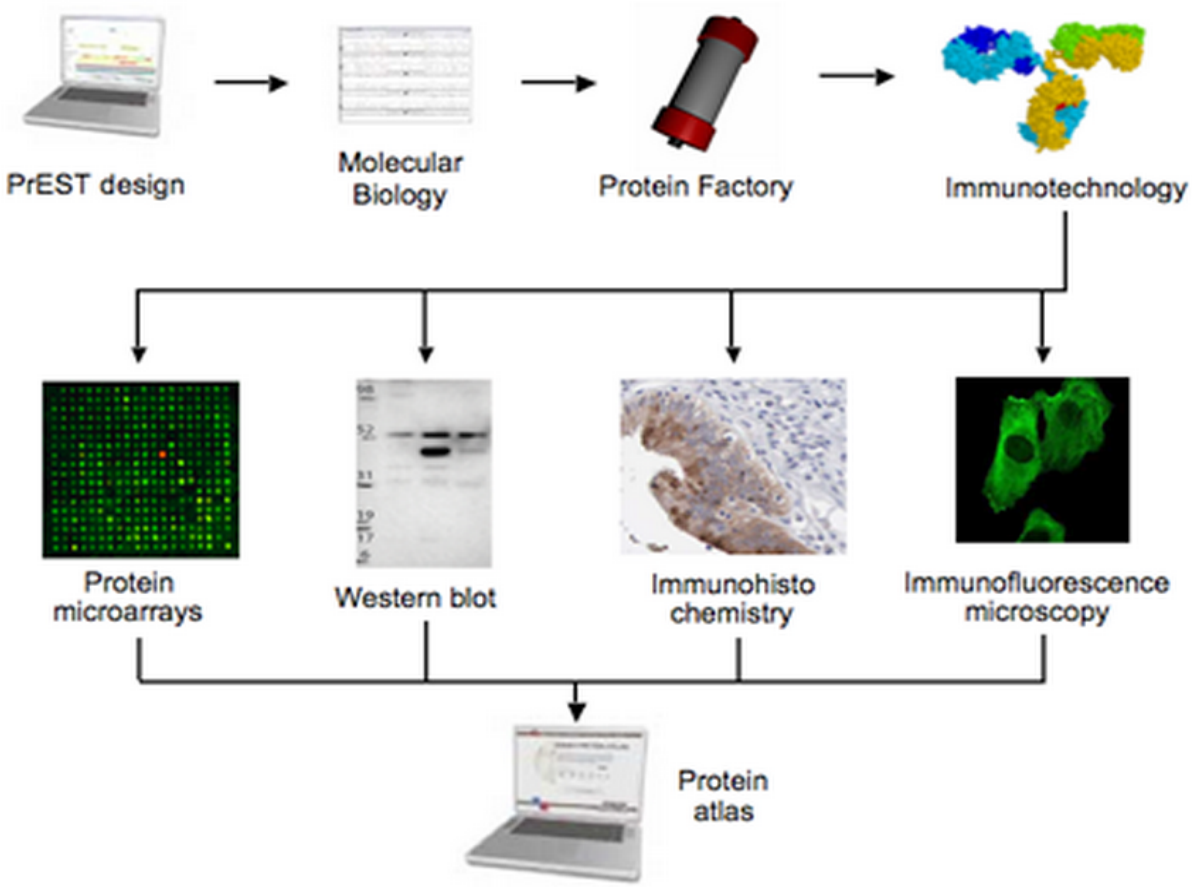
Arraying Technologies



Tissue microarray technology for high-throughput molecular profiling of cancer Kallioniemi O et.al. Human Molecular Genetics, 2001, vol. 10, No. 7

Nilsson et al (2005). Towards a human proteome atlas: High-throughput generation of mono-specific antibodies for tissue profiling. Proteomics. 5(17):4327-37

Pontén F et al (2008). The Human Protein Atlas - a tool for pathology. J Pathology. 216(4):387-93.



Discovery of Tumor Markers

- Human Protein Atlas
  - A wide scale screening effort underway to catalog all human proteins
  - Ability to produce mono-specific antibodies
  - Develop markers for disease process

hpr | the project | protein atlas | dictionary | disclaimer | submission of antibodies | help

HUMAN PROTEIN ATLAS

The human protein atlas shows expression and localization of proteins in a large variety of normal human tissues, cancer cells and cell lines with the aid of immunohistochemistry (IHC) images and immunofluorescence (IF) confocal microscopy images.

Enter search: search

Advanced search

Select a chromosome:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

OTHER

Or a protein class:

Enzymes | GPCRs excl olfactory receptors | Kinases | Peptidases | Transcription factors | Transporters | More...

Filter search to show genes with tissue profiles

2009-06-16

A new version (5.0) has been released including antibodies targeting protein products from 1/3 of the human protein-coding genes. Tutorials on how to use the Human Protein Atlas have also been added.

The Human Protein Atlas now contains subcellular localization data (IF) for 3541 genes.

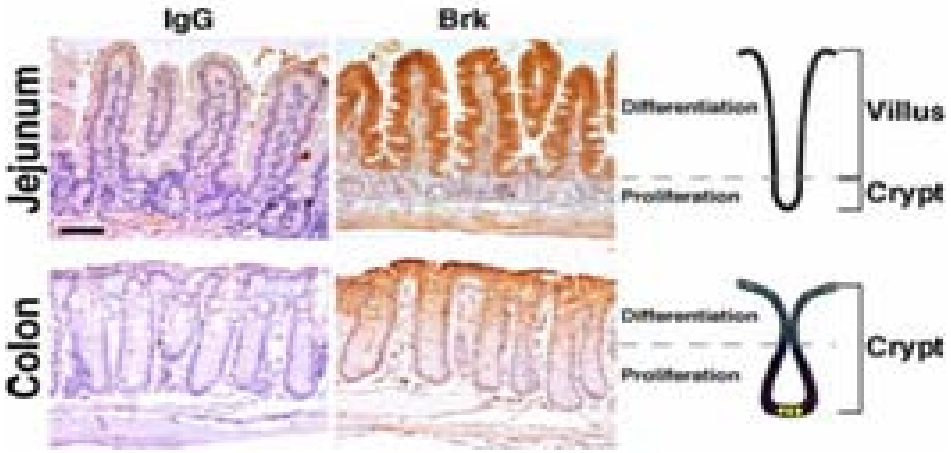
See release history for further details.

Version: **5.0** Atlas updated: **2009-06-16** (release history)
Atlas content: **8832** antibodies and **7,334,244** images.

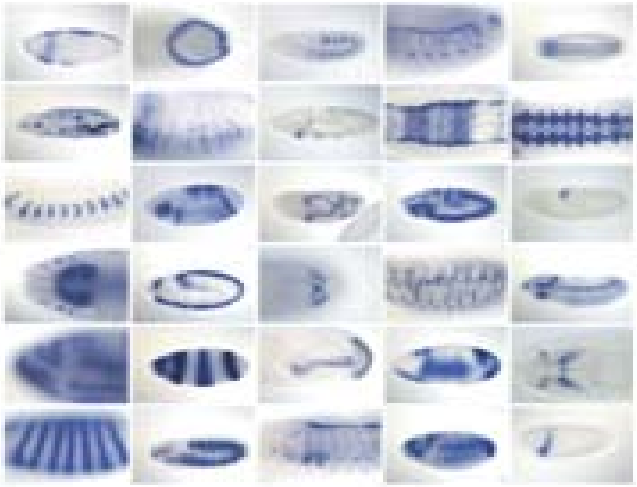
Knut och Alice Wallenbergs Stiftelse

The HPR project is funded by the Knut & Alice Wallenberg foundation. The atlas is part of the HUPO Human Antibody Initiative (HAI).

Determine Pattern of Protein/RNA Expression Across Tissues and Cell Types Provides Insight into Gene Function and Relevance



http://edoc.hu-berlin.de/dissertationen/haegebarth-andrea-2005-08-18/HTML/chapter3.html



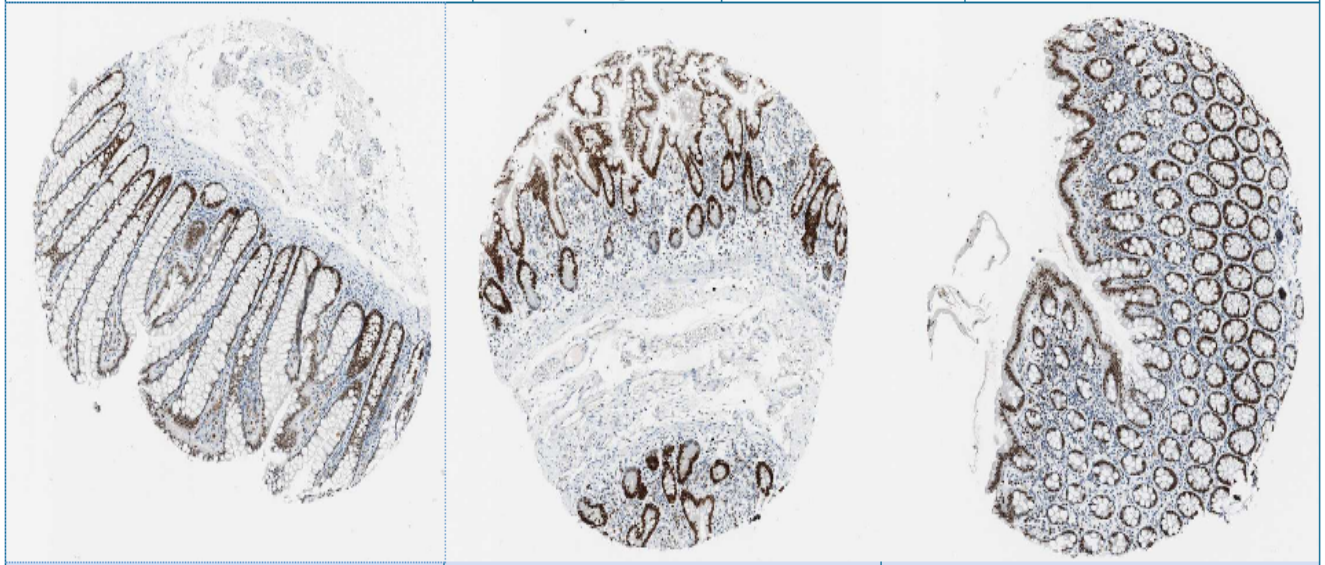
http://www.mpi-cbg.de/research/research-groups/pavel-tomancak.html

Human Protein Atlas: Example, EZH2 in Normal Colon Tissue

hpr | the project | protein atlas | dictionary | disclaimer | submission of antibodies | help

Colon [EZH2]

Cell Type	Intensity	Quantity	Localization
Glandular cells	strong	>75%	nuclear



Female, age 55 **Colon** (T-67000) **Normal tissue, NOS** (M-00100) **Patient id:** 1506	**Male, age 73** **Colon** (T-67000) **Normal tissue, NOS** (M-00100) **Patient id:** 3266	**Female, age 80** **Colon** (T-67000) **Normal tissue, NOS** (M-00100) **Patient id:** 2944

Brown color indicates presence of protein, blue color shows cell nuclei. Image Usage Policy

Navigation

Home
Search result
CAB009589
- Expression profiles - IHC
  - Normal tissues
  - **Colon**
  - Cancer tissues
  - Cells
- Gene/Protein info
- Antigen/Antibody info

Validation Summary

IH WB

Search

search

Introduction

- Many proposed new disease target genes with *diagnostic*, *prognostic*, and *therapeutic* applications
- Validation requires many samples
- Quantitative RT-PCR or protein arrays have disadvantages
  - Genes may be expressed in multiple different cell types
- In situ analyses: ideal but generally slow
- Tissue microarrays address some of these problems

Discovery of Tumor Markers

- Discovery based assays destroy the tissue, hence no morphology
- Tissue allows the researcher to evaluate candidate makers in the cellular micro – environment
- Some markers behave differently in context than it does homogenized
- TMA's allow the researcher a variety of tissue to evaluate the effects of candidate marker.

Why Do We Use Paraffin Tissue Versus Other Biological Samples?

- Abundant Resource
- Clinical information attached
- Can be stored for a long period of time
- Retains most of it's nucleic information
- Allow users to evaluate information in a tumors' micro-environment

Frozen TMAs

- Post fixation can be tightly controlled
- Some antibodies do not bind to formalin fixed epitopes
- The quality of nucleic acids (DNA/RNA) is generally much higher
- Hoos and Cordon-Cardo have developed a simple devise, independent of the Beecher Instruments devise for frozen TMA construction (*Lab Invest 81:1331-1338, 2001*)
- Fejzon have adapted the Beecher Instrument machine using dry ice to keep the donor and recipient blocks frozen (*Am J Pathol 159:1645-1650, 2001*)

Histological Evaluation

- Paraffin blocks
  - Best method for histomorphologic examination
  - Cost effective method of storing surgical specimens
  - Can last upwards of 50 years
- H&E stained slides
  - Most universal diagnostic stain

Historical Background

- Diagnostic test or research was done on a whole mount section
- Little standardization from run to run or from technician to technician
- Hard to compare samples
- High cost for reagents
- Time prohibitive

Tissue Microarrays

- 1986: H. Battifora – "Multi-Tissue Tumor Block"
- 1998: Kononen et al. – "Tissue Microarray" (TMA)

TMA allows many more specimens that are precisely arranged

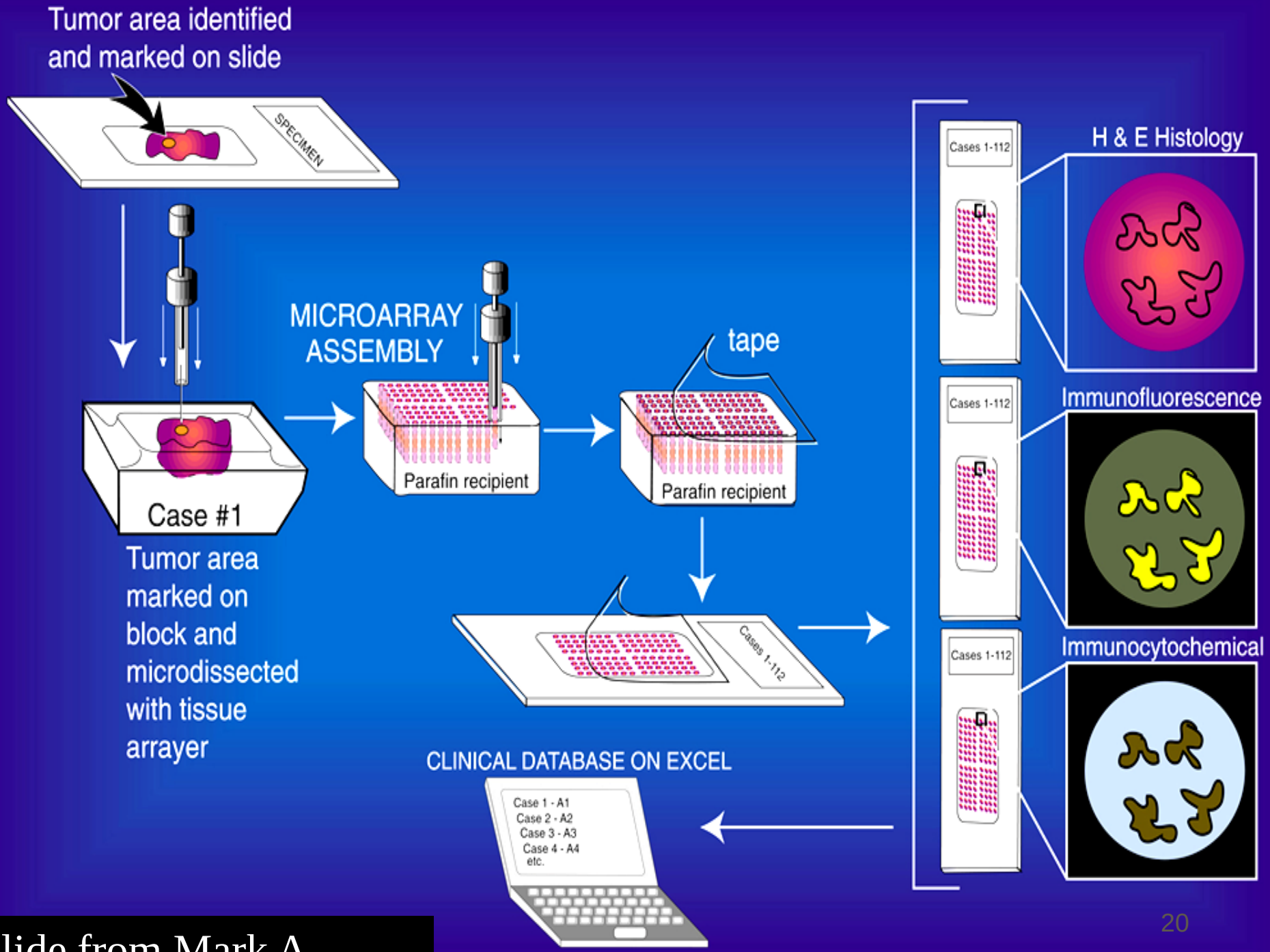
Why TMA Technology Was Created

- "...analysis of hundreds of specimens from patients in different stages of disease is needed to establish the diagnostic, prognostic and therapeutic importance of each of the emerging cancer gene candidates."

Tissue microarray technology for high-throughput molecular profiling of tumor specimens Kononen J et.al. Nature Medicine, 1998, vol. 4, No. 7

Tissue Microarray Advantages

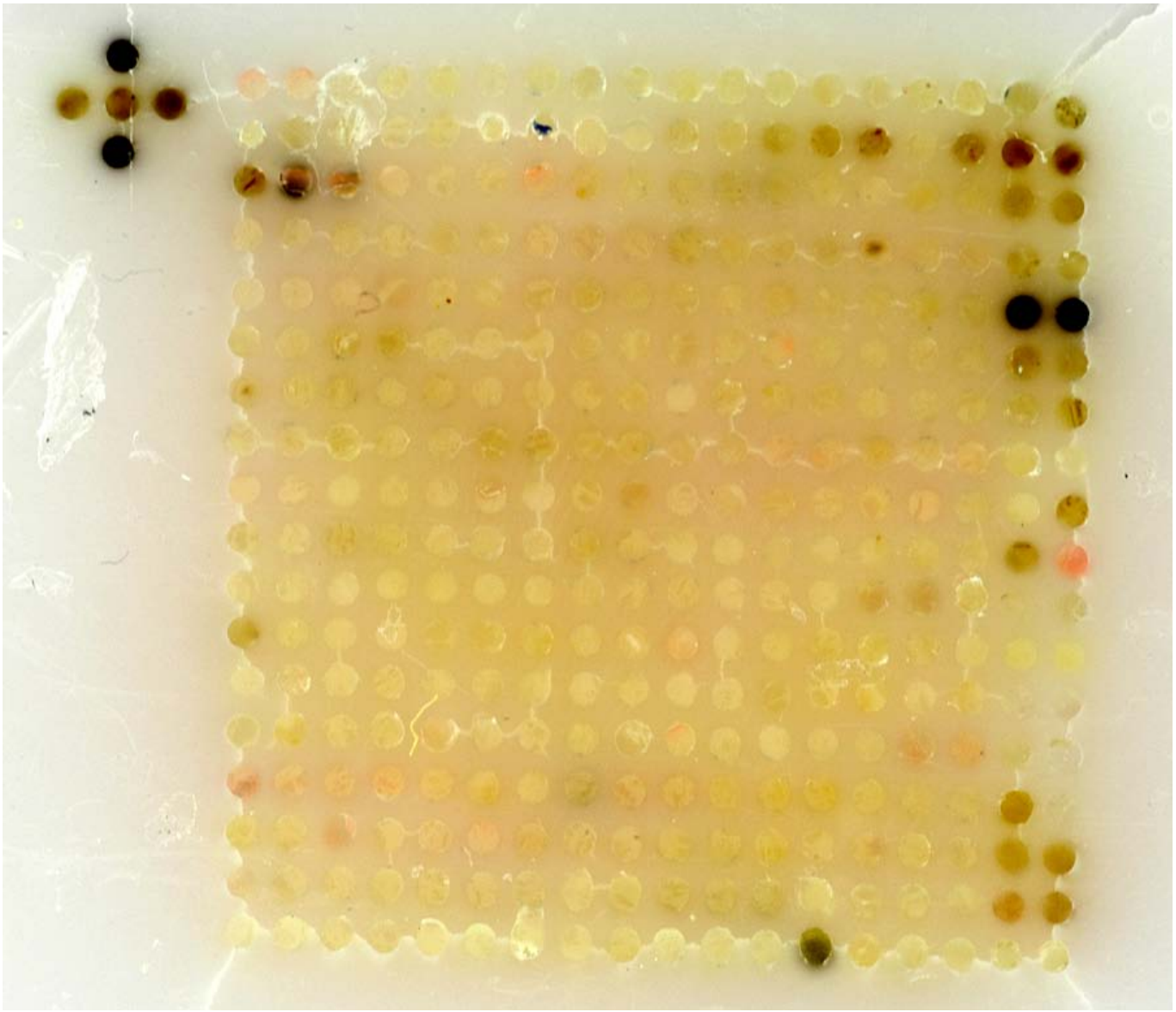
- High throughput
- Expands tissue use
- Uniform reaction conditions
- Built-in controls
- Economize use of reagents
- Facilitates data recording and linking to clinical data



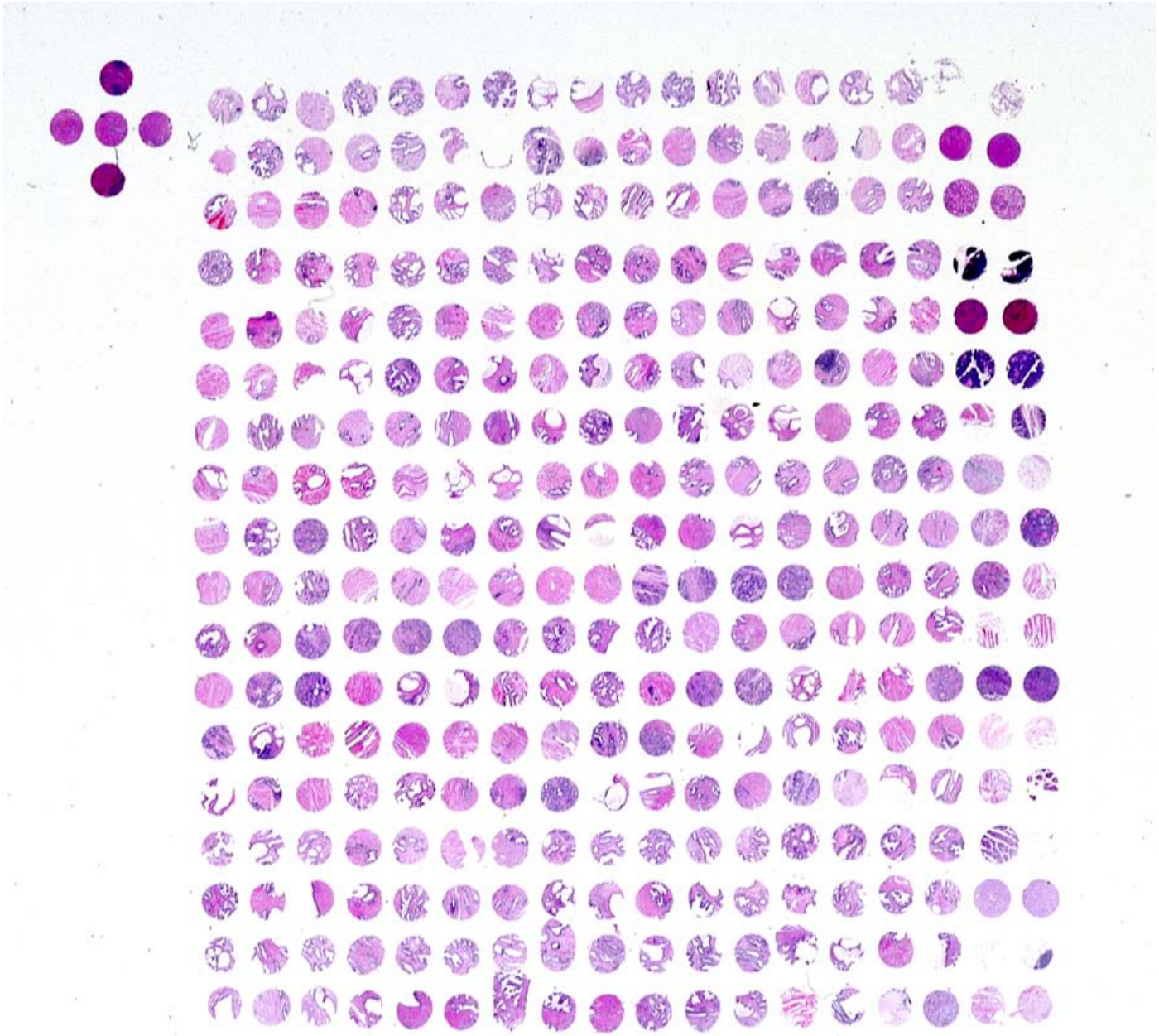
Slide from Mark A.

Donor Block Sampling

Transfer to Recipient Block

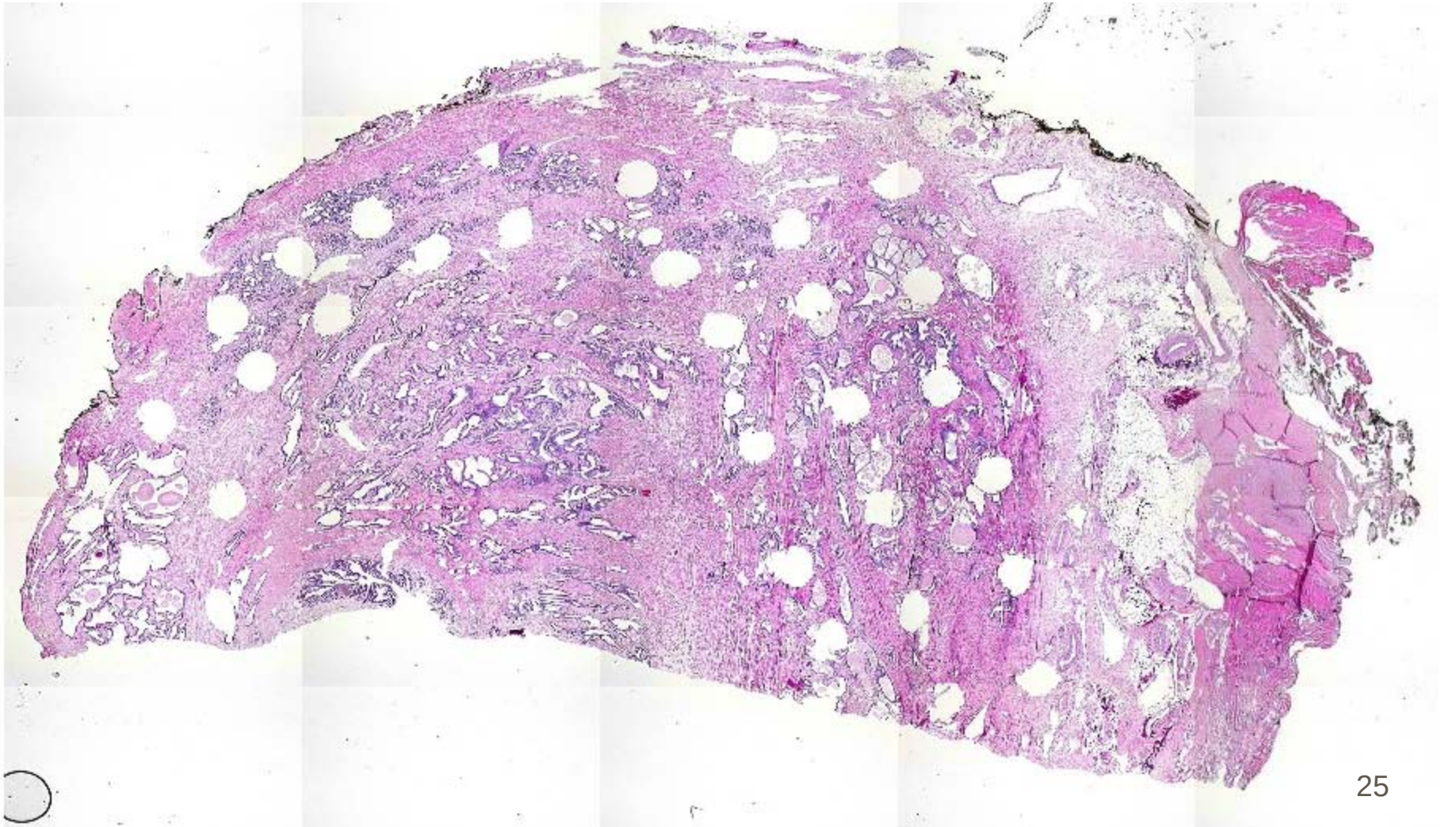


JHU Tissue Microarray – 400 cores, 0.6 mm



Tissue Microarray – 400 cores, 0.6 mm each – H&E of 4 μm section

Effects of Tissue Removal on Paraffin Blocks



Uses for TMAs

- Pattern of gene expression across normal and diseased tissues (*prevalence arrays*)
- Work up new antibodies or probes
- Compare staining methods
- Compare tissue fixation/processing methods

Uses for TMAs

- Clinico-pathological studies: correlation of gene expression with outcome (*prognostic arrays*)
- Validation of RNA profiling studies
- Comparing effects of therapeutic interventions (*Therapeutic effect arrays*)
- Many others

What Is the Purpose of the Array?

- Human control tissues
- Specific disease type
- Specific disease type with matched normal tissue
- Cell Lines
- Drug treatment arrays (progression)
- Human tissue before and after treatment in clinical trials

What Is the Purpose of the Array

- New antibody workups, many tissue types at the same time
- Animal study tissues
- Zoo organ collections
- Xenograft tumors
- Mixed animal model with human counterpart

Prostate TMAs at JHU – Contain Tissues From > 700 Total Patients

- **New Marker Prevalence Phase I Arrays**
  - from 4-8 patients/cases each
- **New Marker Prevalence Phase II Arrays:**
  - 20-40 patients/case each
- **Prognostic/Grade-Stage Phase II Arrays:**
  - used to correlate marker expression with Gleason grade and pathological stage in a small number of cases (20-40).
- **Prognostic/Grade-Stage Phase 2 Arrays**
  - are similarly used but contain samples from up to 240 patients (6 arrays).
- **Prognostic/Natural History of Prostate Cancer Recurrence after radical prostatectomy**
  - up to 317 patients (287 with biochemical recurrence and long term follow-up)
- **Untreated Metastatic Prostate Cancer**
- **Matched Primary-Metastatic Prostate Cancer Arrays**
- **High Grade PIN** –
- **Atrophy** –

Progression TMA

- Usually has clinical outcomes data linked
- Looking for a biological expression change
- Maybe be done with patient samples that have under gone therapies
- Maybe multi-institutional

Tumor Specific TMA

- Possible clinical data attached
- Designed to study a specific tumor or disease
- Looking for tumor wide specific changes
- Includes some normal tissues
- Or includes matched normal tissues

New Antibody Workups

- 4-8 cases of Tumor and adjacent normal from one cancer type
- With a redundancy of four

New Antibody Workups

- When evaluating a new antibody we use an TMA that has 20 different tissue types

Human Control Tissues

- Discarded human tissues from surgical specimens
- Ideal for working up new antibodies or probes for *in situ*

Tissue Types

Stomach	Thyroid	Skin
Colon	Uterus	Lung
Gall bladder	Skeletal muscle	Brain
Pancreas	Ovary	Kidney
Liver	Seminal Vesicle	Spleen
Thymus	Prostate	Tonsil
Lymph node	Salivary gland	Breast

Research Arrays

- May have tissue from hundreds of cases
- May contain tumor and normal from each of the cases
- The tissue may be put into more than one array block

Evaluating Prognostic Markers

The prospect of survival and recovery from a disease as anticipated from the usual course of that disease or indicated by special features of the case

Research Article

Protein Expression Profiling Identifies Subclasses of Breast Cancer and Predicts Prognosis

Jocelyne Jacquemier,[1,2] Christophe Ginestier,[1] Jacques Rougemont,[6] Valérie-Jeanne Bardou,[3] Emmanuelle Charafe-Jauffret,[1,2,7] Jeannine Geneix,[1] José Adélaïde,[1] Alane Koki,[8] Gilles Houvenaeghel,[4] Jacques Hassoun,[2,7] Dominique Maraninchi,[5,7] Patrice Viens,[5,7] Daniel Birnbaum,[1] and François Bertucci[1,5,7]

[1]Institut de Cancérologie de Marseille, Département d'Oncologie Moléculaire, [2]BioPathologie, [3]BioStatistiques, [4]Chirurgie, and [5]Oncologie Médicale et Investigation Clinique, Institut Paoli-Calmettes and UMR599 Institut National de la Santé et de la Recherche Médicale; [6]ERM206 Institut National de la Santé et de la Recherche Médicale; [7]Université de la Méditerranée, UFR de Médecine; and [8]Ipsogen S.A., Marseille, France

Abstract

Breast cancer is a heterogeneous disease whose evolution is difficult to predict by using classic histoclinical prognostic factors. Prognostic classification can benefit from molecular

and St-Gallen; refs. 3, 4), decisions on whether to treat patients with node-negative cancer with or without adjuvant chemotherapy are currently being made with scant information on risk for metastatic relapse. In addition, identifying among the patients who receive chemotherapy, those who will benefit and those who will not

x 10.88 in

0 (1 of 13)

Cancer Res 2005; 65: (3). February 1, 2005

Discovery of Tumor Markers

- Collect a series of markers and apply them to a TMA to help discern function

- Usually use already characterized antibodies, but there is a wide-scale screening effort underway

Challenges of Working with Tissue

- Autolysis of tissue
- Fixation alters epitope sites
- Need multiple samples for validation
- Time prohibitive
- In Situ studies require well characterized specimens

Validation – Breast Cancer I

- Number of TMA spots required to represent ER, PR and Her2/neu
  - Two spots: similar to the whole tissue in more than 95% of the cases

Camp RL, Charette LA, Rimm DL: Validation of tissue microarray technology in breast carcinoma. Lab Invest 80:1943-1949, 2000

Validation – Prostate Cancer

- 10 replicate tumor samples from 88 cases of prostate cancer to evaluate Ki-67 expression
- Four cores provided optimal sampling to determine PSA recurrence
- Fewer TMA samples: significantly increased Ki-67 variability and a larger number did not significantly improve accuracy

Rubin, MA, et al., Am J Surg Pathol 2002, 26:312-9

Validation - Summary

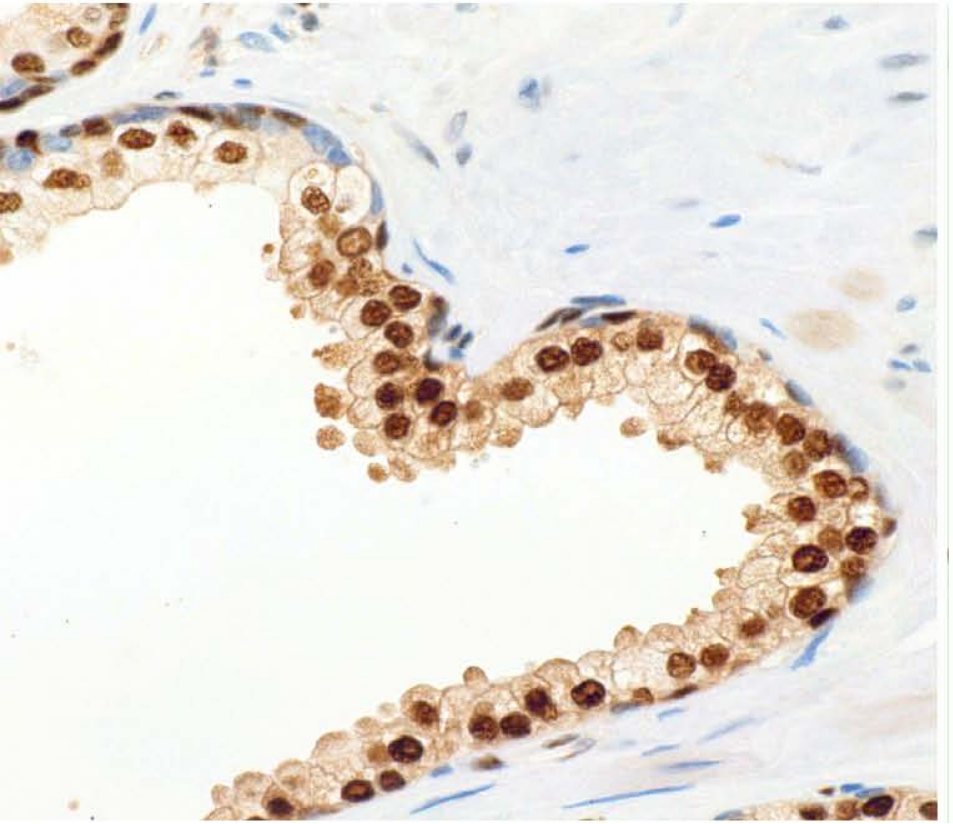
- All published studies indicate there is agreement between the use of TMAs and standard tissue sections for clinico-pathological studies
- In theory, the number of cores needed will depend on the variability in staining
- 2-4 cores are representative of 95% of the tissue
- 5-6 cores does not improve concordance rates

Most Important Aspect of Tissue Microarray Construction

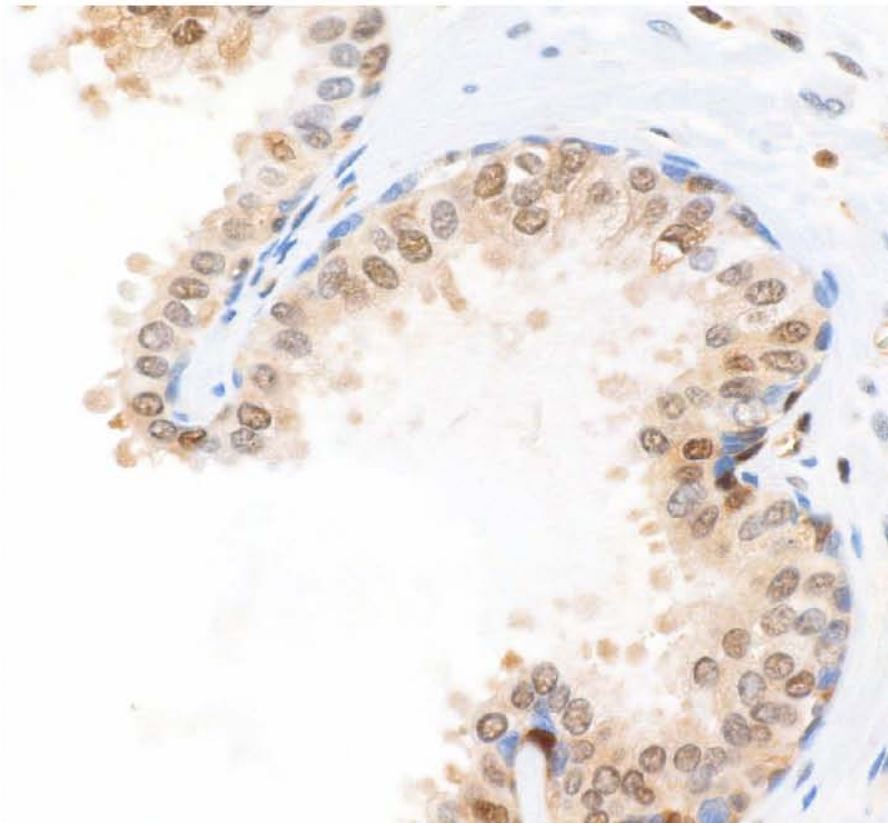
- Fixation
- Fixation
- Fixation

p27Kip1 Fixation Artifact

Peripheral Aspect



Interior Aspect



p27Kip1 Fixation Study

- Prostate specimens fixed in formalin at for 0, 1, 2, 3, 7 or 8 days
- TMA were constructed containing (564 samples 0.6 mm in diameter) of benign prostate tissue
- TMAs stained against $p27^{Kip1}$ by Immunohistochemistry
- Quality of staining graded based on estimate of percent nuclei in normal secretory cells with strong staining

Hum Pathol. 2002 Jul;33(7):756-60.

Inadequate formalin fixation decreases reliability of p27 immunohistochemical staining: probing optimal fixation time using high-density tissue microarrays.

De Marzo AM, Fedor HH, Gage WR, Rubin MA

Results

- When categorized regarding fixation time as 0, 1, or 2 or more days, there was a significant increase ($P < 0.0001$) in the percentage of cores scored as strong (>50% nuclei strong) as fix time increased
- Even at 8 days of fixation, staining was superior to same day processing and equal to 2 days

Build, Buy or Collaborate?

- Construction is not particularly difficult, especially if done in a core facility
- Obtaining tissue can be very difficult and time consuming!
  - Obtaining clinical follow-up can be even more difficult
- Always plan to construct and use TMAs in conjunction with a **histopathologist!**
- Buying TMAs is possible, but ...
- Collaborations are good, but this may involve MTAs, and significant support for collaborator

Companies Selling TMAs

- Cybri
- Isbio
- Stratagene
- Zymed
- WVU Pathology
- Gentaur
- Biosciences
- Innogenex
- Invitrogen
- Lifespan
- BD Pharmingen
- Imgenex(Super Bio Chips)
- Petagen (MTR Scientific)
- Ambion LandMark
- Chemicon
- Clinomics
- Biogenex
- Oligene
- Koma Biotech
- Bio Chain

Challenge of Interpretation

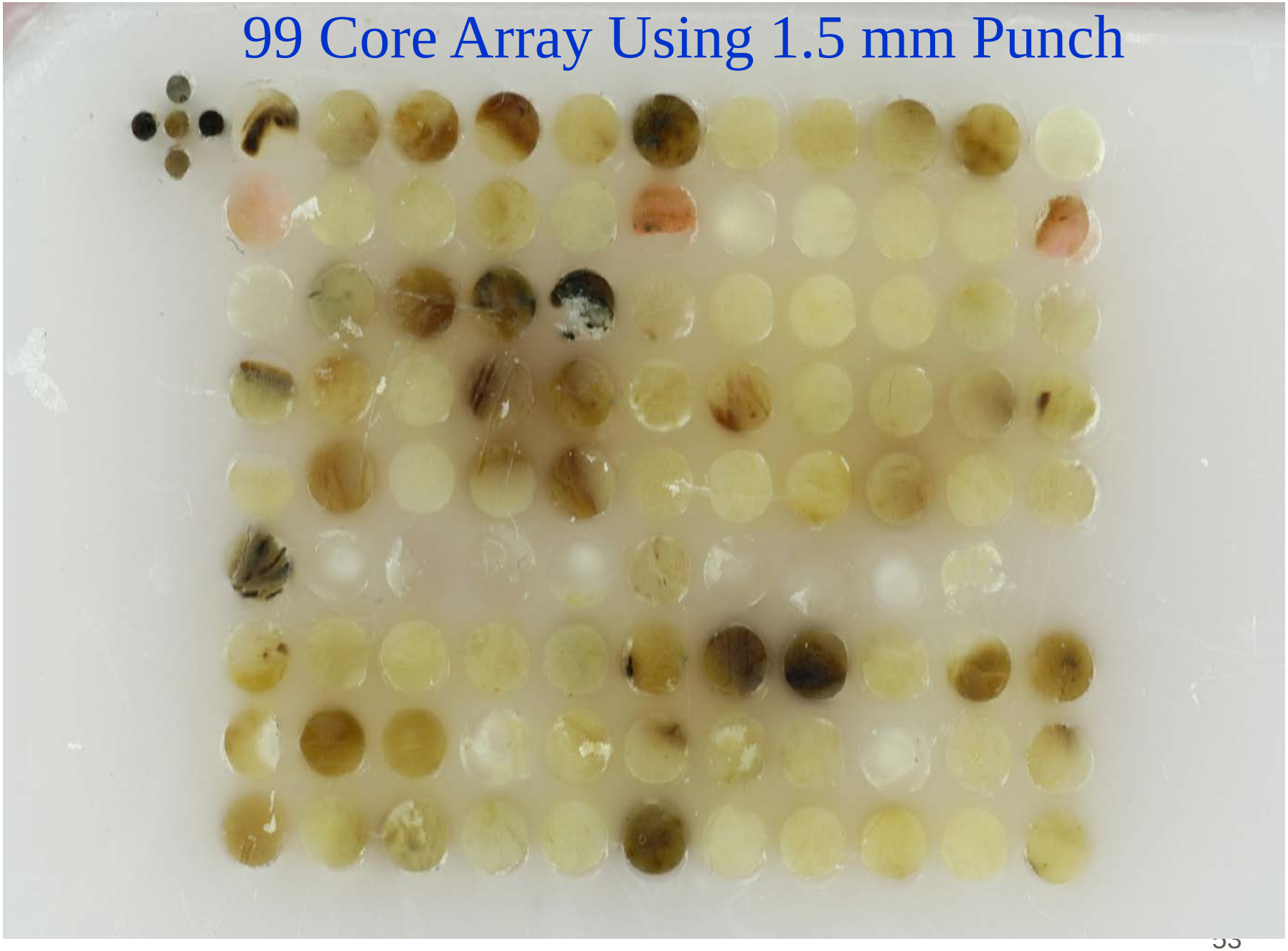
- Validation of antibodies need to be performed on your tissue(s) of interest
- Pilot TMAs should always be used if possible and these should be used after antibody/ in situ protocol is already validated
- Histopathology interpretation is required for most TMA studies on every slide!

Challenge of Interpretation

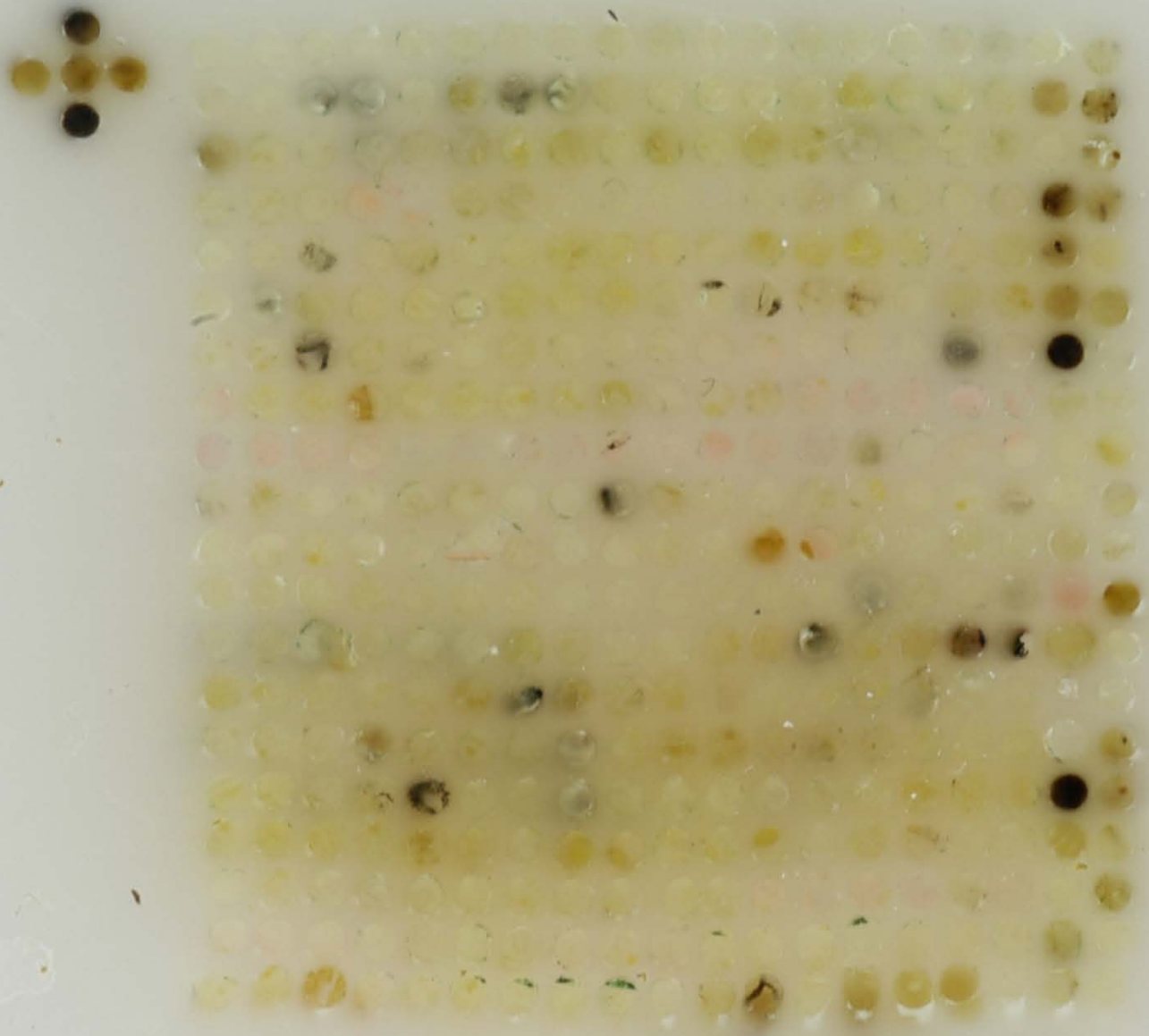
- "The TMA platform remains challenging because working with tissue remains challenging"

Hewitt, S.M. Tissue Microarrays as a tool in the discover and validation of tumor markers. In Methods in molecular Biology, Tumor Biomarker Discover, vol 520, humana press

99 Core Array Using 1.5 mm Punch



400 Core Array Using 0.6 mm Punch



Percent Increase in Tissue Used

Core Size	Area	Percent Difference
0.6 mm	0.28 mm Square	
1.0 mm	0.78 mm Square	64% More Tissue
1.5 mm	1.77 mm Square	84% More Tissue

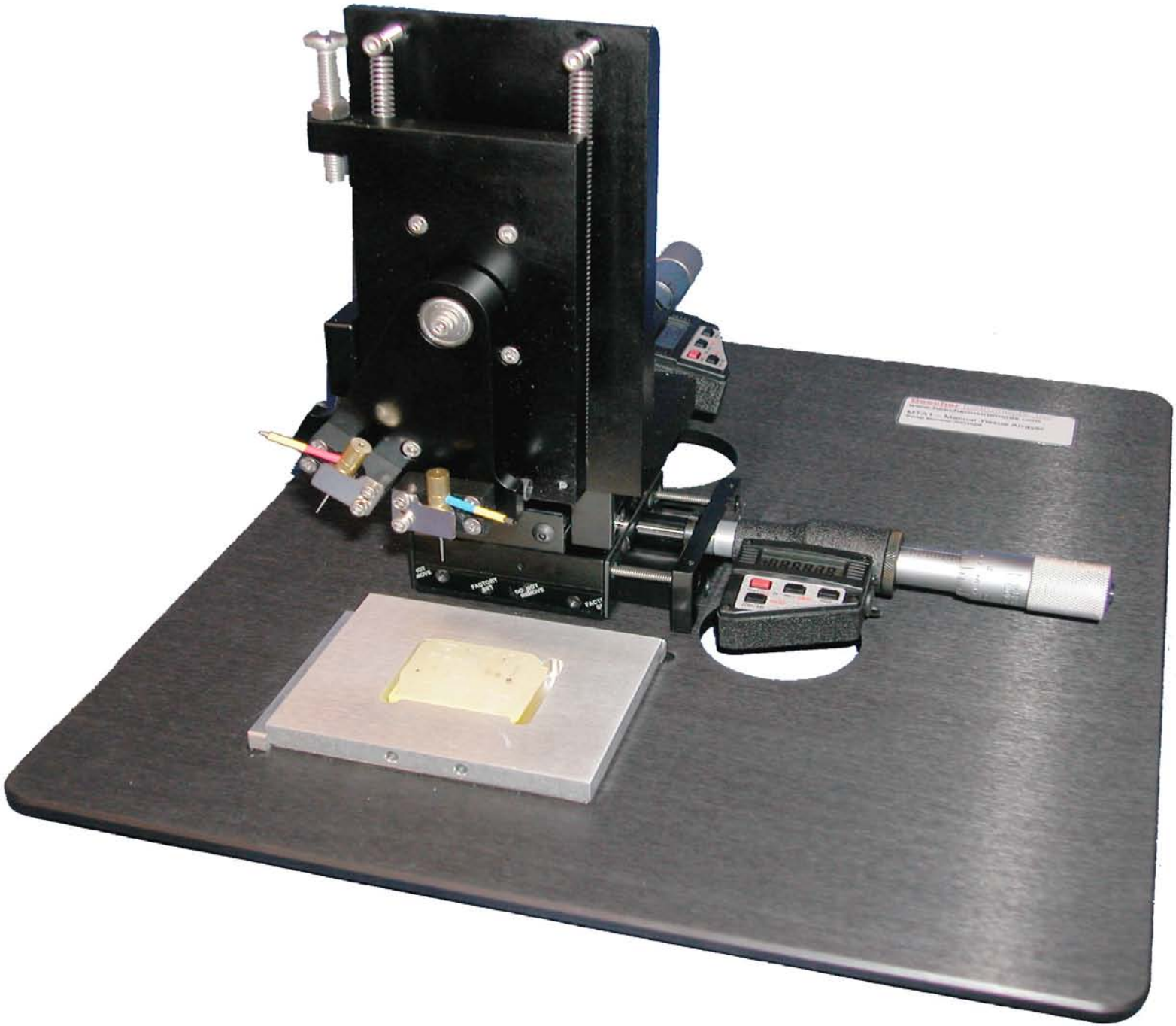
0.6mm Core

- Maximum utilization of valuable tissue
- Number of samples on a slide is maximized

0.6mm Core

- Damage to the donor and recipient block is minimized

- Many studies have shown that this size gives as much information as a standard tissue section



Constructing Tissue Microarrays Without Prefabricating Recipient Blocks: A Novel Approach

- Used double-sided adhesive tape attached to x-ray film as an adhesive platform on which the tissue cores were placed securely

- The array of tissue cores then was embedded in an embedding mold

- TMAs with up to 220 cores

- 2 to 3 hours

Chen N. J Clin Pathol. 2005 Jul;124(1):103-7.

CEMA Cutting Edge Matrix Assembly

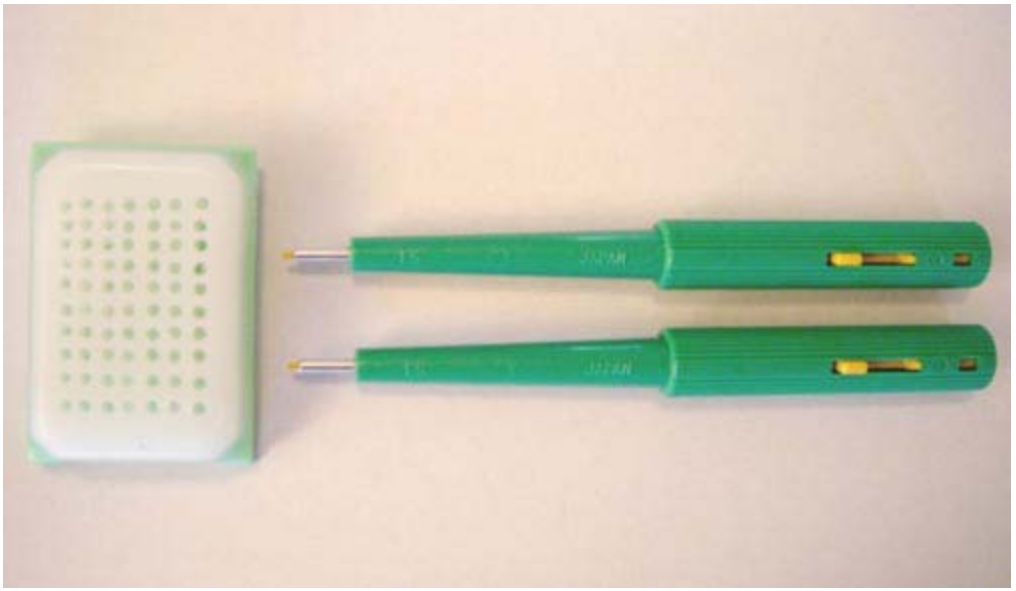
- Ultra high density arrays
- Cut tissues 250-1000um thick
- Place each sample on top of each other and bond to make a stack
- Re-embed In paraffin
- Cut primary stacks into secondary plates of 250-100um

CEMA Cutting Edge Matrix Assembly

- Secondary plates are bonded, oriented , re-embedded in paraffin
- Final array slides are cut at 5um

Nature Methods **2**, 511 - 513 (2005)
Ultrahigh density microarrays of solid samples
Matthew J LeBaron, He

EZ-TMA™ Manual Tissue Microarray Kit 1



- sales@ihcworld.com

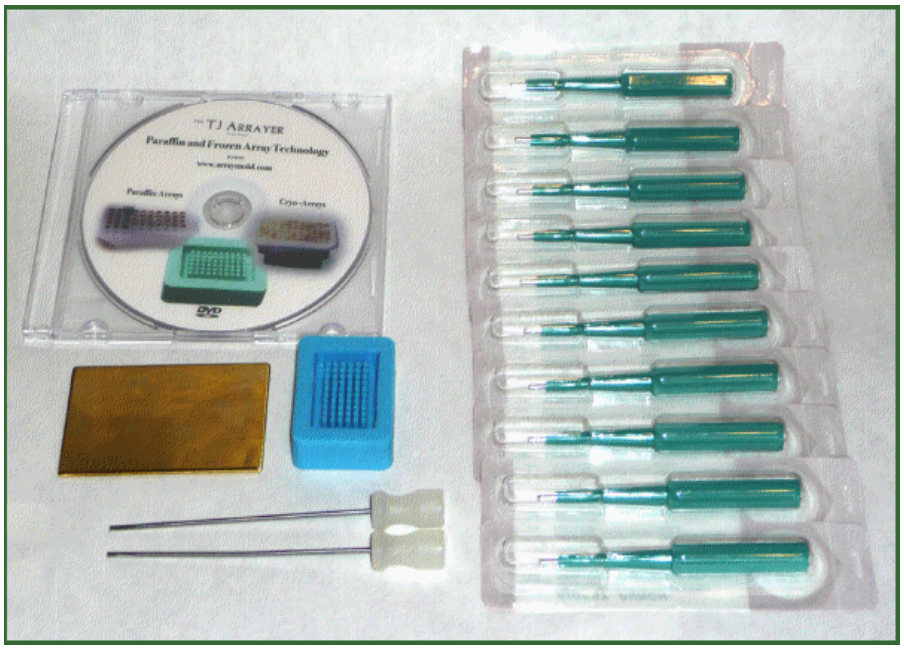
Array Builder from Lab Vision

- http://www.labvision.com/

Tissue-Tek®
Quick-Ray™ Tissue Microarray System

http://www.sakura-americas.com/products/tisstek-quickray.html

Arraymold Manual Tissue Microarrayer



For Frozen
or
Paraffin
arrays

http://www.ihcworld.com/products/Arraymold.htm

Slide Storage

Antigenicity is greatly affected by ambient air over time.

Divito et al. reported that here was a significant loss of ER staining by 6 days in ambient temperatures

Lab Invest. 84, 1071-1078(2004)

Tissue Microarray Slide Storage

- Few studies have been completed looking at the viability of tissue components on paraffin slides over time
- The slides are precious and need to be stored in a fashion that will protect the quality of all the tissue characteristics
- We cut 20 sections at one time

Tissue Microarray Slide Storage

- They are dried overnight in a vertical position
- Then they are placed front to front (we use chemate or probe-on Plus slides)
- And then placed in small Ziploc bags labeled, and stored at -20 degrees

Slide Storage

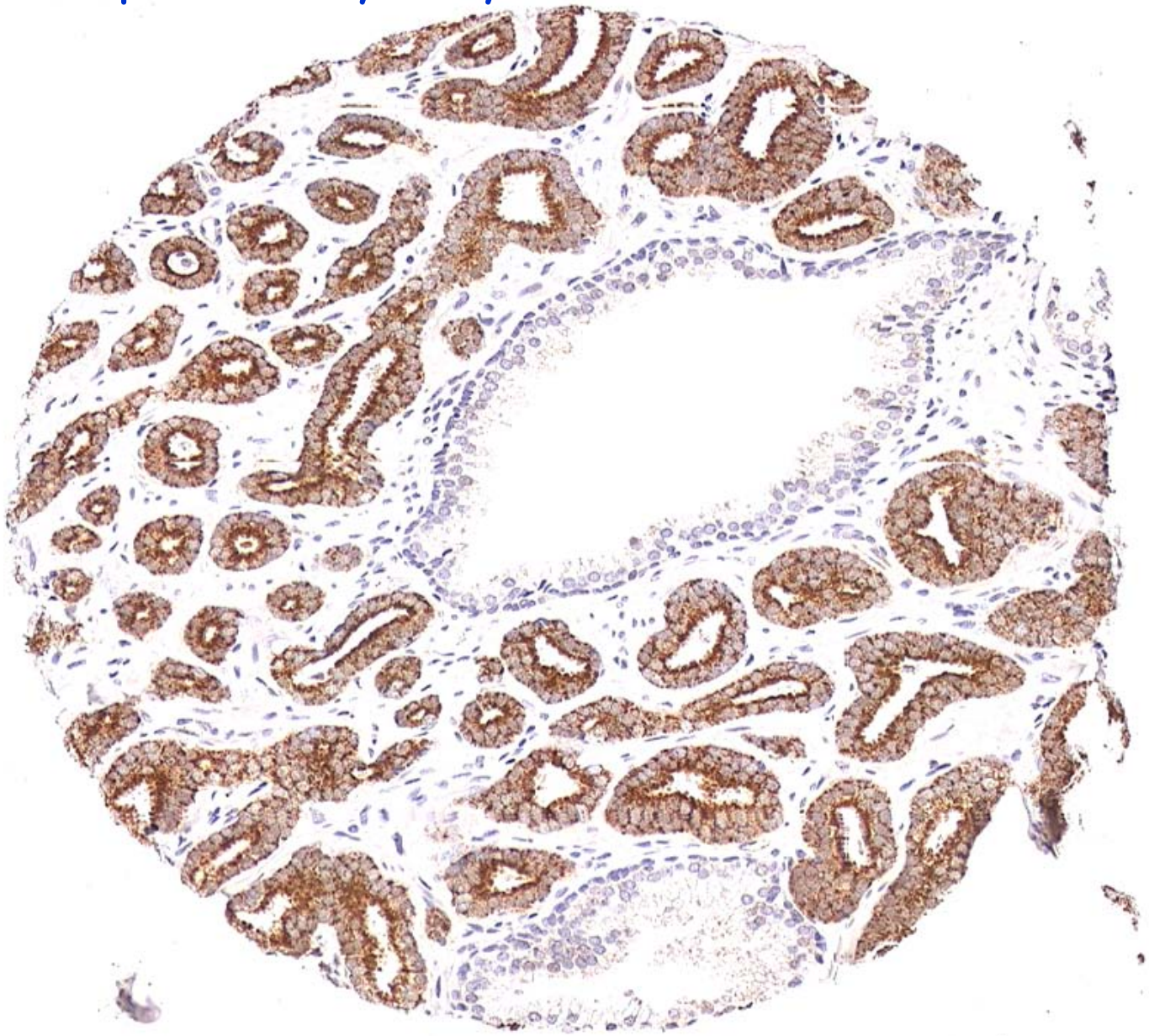
Others are using various procedures to retain the quality of their slides

- After the slides are dry they are dipped in liquid paraffin
- Or stored at -80 degrees C
- Stored under nitrogen gas
- Dipped and stored at 4 degrees

Staining Tissue Microarray Slides

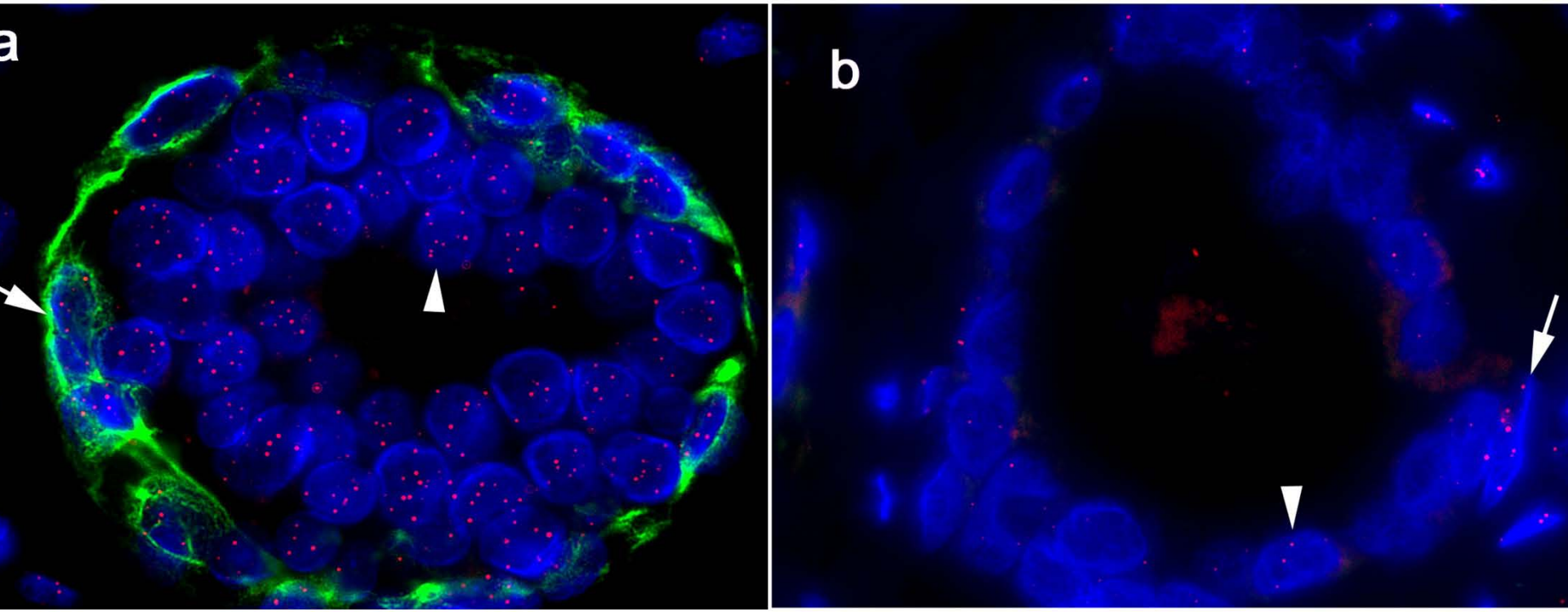
- Tissue microarray slides may be stained by any means that conventional slides may be stained
- They do not need any special treatment

Alpha Methyl Acyl CoA Racemase



Cancer Res. 2002 Apr 15;62(8):2220-6

Combined FISH and IHC in Paraffin



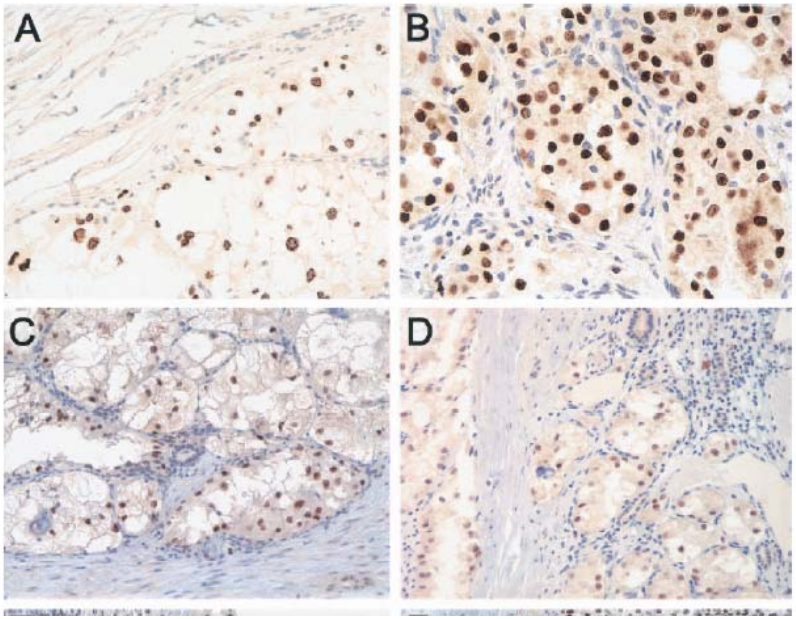
Alan K. Meeker, et al., Am J Pathol 2002 160:1259-68.

The American Journal of Surgical Pathology *27(6): 750–761, 2003*

Aberrant Nuclear Immunoreactivity for TFE3 in Neoplasms With *TFE3* Gene Fusions

A Sensitive and Specific Immunohistochemical Assay

Pedram Argani, M.D., Priti Lal, M.D., Brian Hutchinson, M.A., Man Yee Lui, B.A., Victor E. Reuter, M.D., and Marc Ladanyi, M.D.



39/40 tumors pos – Sen 97.5%

6/1476 other tumors – Spec 99.6%

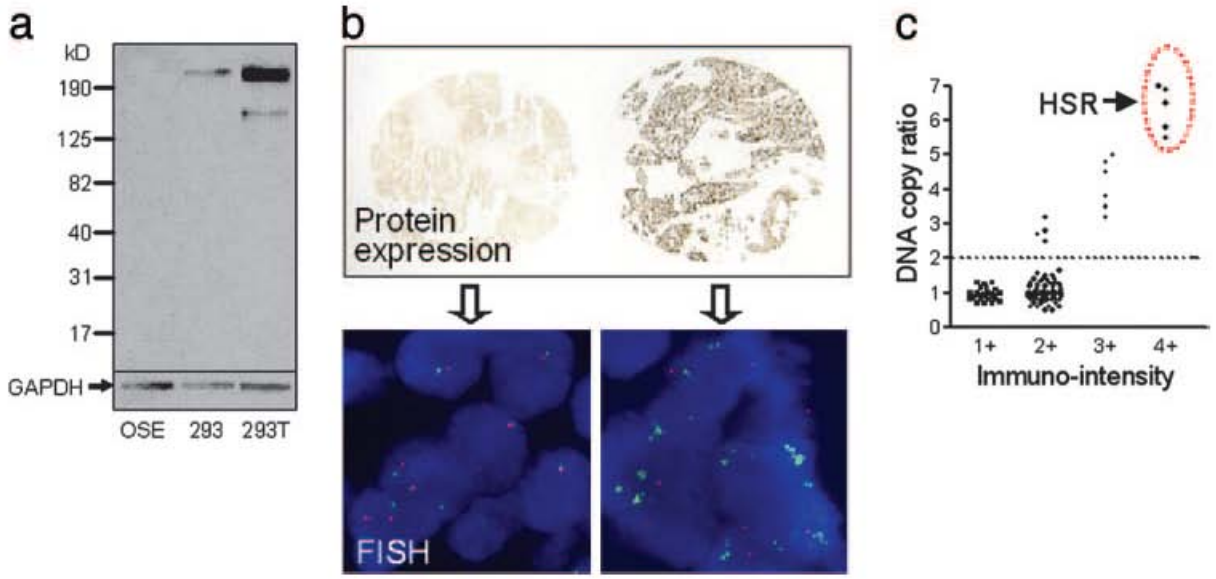
Am. J. Surg Path, 2003, 27:750-61.

Amplification of a chromatin remodeling gene, Rsf-1/HBXAP, in ovarian carcinoma

Ie-Ming Shih*†, Jim Jinn-Chyuan Sheu*, Antonio Santillan*, Kentaro Nakayama*, M. Jim Yen*, Robert E. Bristow*, Russell Vang*, Giovanni Parmigiani*‡, Robert J. Kurman*, Claes G. Trope§, Ben Davidson§, and Tian-Li Wang*†

*Departments of Pathology, Gynecology, and Oncology, ‡Department of Biostatistics, The Johns Hopkins University School of Medicine, Baltimore, MD 21231; and §Departments of Pathology and Gynecologic Oncology, Norwegian Radium Hospital, University of Oslo, Montebello, N-0310 Oslo, Norway

Edited by Bert Vogelstein, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, and approved August 15, 2005 (received for review May 20, 2005)



14004–14009 PNAS September 27, 2005 vol. 102 no. 39

Cell Culture Donor Blocks

- Resuspend cells in 10% formalin and fix overnight
- Pellet the cells and then wash in 1 X PBS
- Pellet and resuspend cells in 0.8% agarose at 42 degrees
- Prefill tapered end of a 0.6ml microfuge tube with agarose and let solidify

Cell Culture Donor Blocks

- Transfer the agars cell mixture to the microfuge tube with the solidified agarose
- Add a wooden toothpick to the tube, this facilitates removal of the plug form the microfuge tube
- This plug is removed after it solidifies and can be cut in half to yield two blocks of cells
- Process the plugs using standard paraffin processing schedules

Array Construction Tips and Techniques

- Sources of information
  - Protocols, tips techniques, and trouble-shooting
    - Review : Jensen TA, Hammand MEH: the tissue microarray-a technical guide for histologists. The journal of Histotechnology 24:283-287, 2001
    - A detailed web site with protocols: http://www.Yalepath.Org/dept/research/ycctma/ytma_protocol.Pdf
    - Sites developed by the NIH:
      - Http://resresources.Nci.Nih.Gov/tarp/

Some Web Sites

- Rimm Lab, Yale

  http://www.yalepath.org/dept/research/ycctma/ytma_protocol.pdf

- Johns Hopkins TMA Lab

  http://tmalab.jhmi.edu/

- Beecher Instruments

  http://www.beecherinstruments.com


TISSUE
MICRO
ARRAY

Need for Data Management

200 TMAs from Johns Hopkins TMA lab

Record Block

- The record block has 1200 cores
- If you could get 300 sections from this block
- That would yield 360,000 samples

However, we do not recommend going over 600 punches in one block

Database Design

American Journal of Pathology, Vol. 159, No. 3, September 2001

Technical Advance

Relational Database Structure to Manage High-Density Tissue Microarray Data and Images for Pathology Studies Focusing on Clinical Outcome

The Prostate Specialized Program of Research Excellence Model

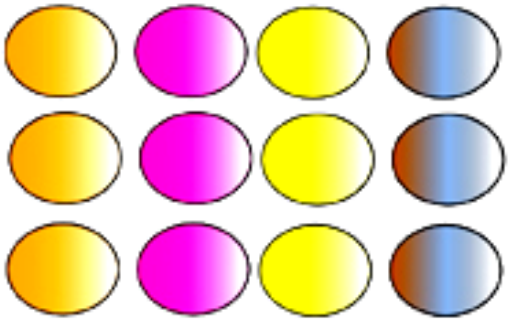
Sargum Manley,* Neil R. Mucci,†
Angelo M. De Marzo,‡ and Mark A. Rubin*§

From the Departments of Pathology, Internal Medicine, Division*

histochemical biomarkers including E-cadherin, p
tate-specific antigen, p27^{Kip1}, and Ki-67 labeling i
This system facilitates the statistical analysis of

The TMAJ Software Project

http://tmaj.pathology.jhmi.edu



What is *TMAJ?*

- *TMA-J* is a set of open source software tools and backend database structure to facilitate management and analysis of tissue microarrays and associated pathology and image data

What Does *TMAJ* Do?

The Database Tracts:

- Clinical information about pathology specimens
- Pathology tissue blocks
- Tissue Microarray cores
- TMA Blocks
- TMA Slides
- TMA core images
- TMA image scoring data: manual or semi-automated

What Does *TMAJ Do*?

The software applications provide a platform for:

- Entering pathology data
- Designing TMAs
- Viewing and scoring TMA images online
- Viewing multiple TMA images from the same spot
- Controlling access by managing users and projects

DATA

Tissue Microarrays	750
Specimens	51,900
Tissue Blocks	41,300
ArrayCores	106,800
ArraySlides	8501
ArrayImages	212,256

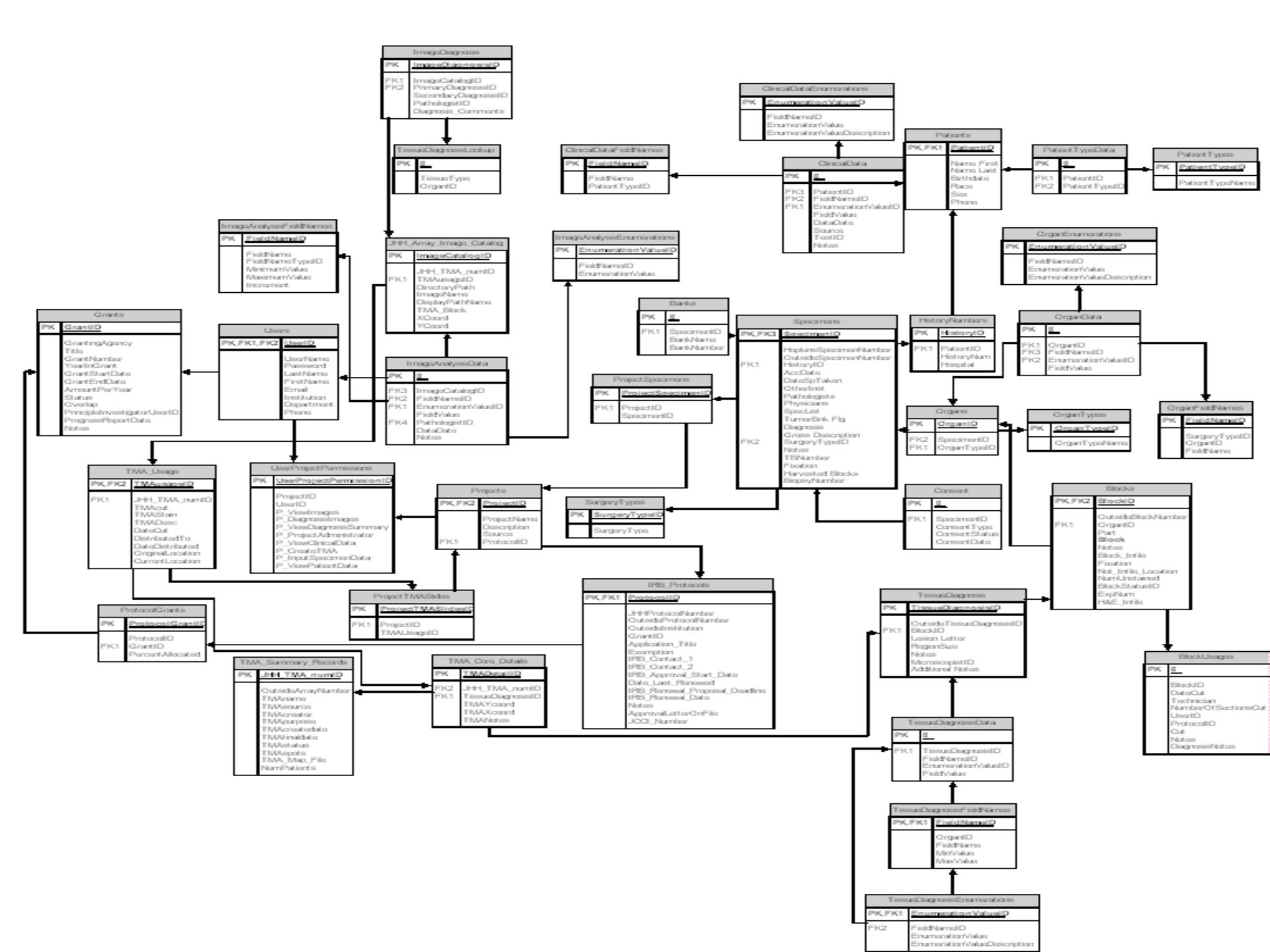
*286 users, 39 Institutions, updated August, 2010

Primary Goals of System

- Address security issues
- Remove or isolate patient identifiers
- Manage multiple organ systems
- Develop web based interface
- Scalable to accommodate large number of simultaneous users
- Storage of large sets of images with diagnoses
- Data structure compatible with emerging standards for easy data exchange
  - CaBIG compatibility (to be defined)
  - The tissue microarray data exchange specification:
    - Berman et al., **(http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=165444)**

Security: Protecting Patient Information

- Database stored on a secure server
- Identifiable patient information in encrypted tables (Approved by the IRB)
- Researchers have no access to patient identifiers
- Creates virtual separate entities: “clinical database” and “research database”


ImagejDiagnosis
PK
ImagejDiagnosisID
FK1 ImagejCatalogID
FK2 PrimaryDiagnosisID
SecondaryDiagnosisID
PathologistID
Diagnosis_Comments
TissueDiagnosisLookup
PK
ID
TissueType
OrganID
ClinicalDataEnumerations
PK
EnumerationValueID
FieldNameID
EnumerationValue
EnumerationValueDescription
ClinicalDataFieldNames
PK
FieldNameID
FieldName
PatientTypeID
ClinicalData
PK
ID
FK3 PatientID
FK2 FieldNameID
FK1 EnumerationValueID
FieldValue
DateDate
Source
TextID
Notes
Patients
PK, FK1
PatientID
Name First
Name Last
BirthDate
Race
Sex
Phone
PatientTypesData
PK
ID
FK1 PatientID
FK2 PatientTypeID
PatientTypes
PK
PatientTypeID
PatientTypeName
ImagejAnalysisFieldNames
PK
FieldNameID
FieldName
FieldNameTypeID
Minimum Value
Maximum Value
Increment
JHH_Array_Image_Catalog
PK
ImagejCatalogID
FK1
JHH_TMA_numID
TMAusageID
DirectoryPath
ImageName
DisplayPathName
TMA_Block
XCoord
YCoord
ImagejAnalysisEnumerations
PK
EnumerationValueID
FieldNameID
EnumerationValue
OrganEnumerations
PK
EnumerationValueID
FieldNameID
EnumerationValue
EnumerationValueDescription
Grants
PK
GrantID
GrantingAgency
Title
GrantNumber
YearInGrant
GrantStartDate
GrantEndDate
AmountPerYear
Status
Overlap
PrincipalInvestigatorUserID
PrognosisReportDate
Notes
Users
PK, FK1, FK2
UserID
UserName
Password
LastName
FirstName
Email
Institution
Department
Phone
ImagejAnalysisData
PK
ID
FK3 ImagejCatalogID
FK2 FieldNameID
FK1 EnumerationValueID
FieldValue
FK4 PathologistID
DateDate
Notes
Banks
PK
ID
FK1
SpecimenID
BankName
BankNumber
Specimens
PK, FK3
SpecimenID
HopkinsSpecimenNumber
OutsideSpecimenNumber
FK1
HistoryID
AccDate
DateSpToken
OtherInst
Pathologists
Physicians
SpecList
TumorSink_Flg
Diagnosis
Gross Description
SurgeryTypeID
Notes
TBNumber
Location
Harvested Blocks
SequryNumber
FK2
HistoryNumbers
PK
HistoryID
FK1
PatientID
HistoryNum
Hospital
OrganData
PK
ID
FK1
OrganID
FK3
FieldNameID
FK2
EnumerationValueID
FieldValue
ProjectSpecimens
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ProjectSpecimenID
FK1
ProjectID
SpecimenID
Organs
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OrganID
FK2
SpecimenID
FK1
OrganTypeID
OrganTypes
PK
OrganTypeID
OrganTypesName
OrganFieldNames
PK
FieldNameID
SurgeryTypeID
OrganID
FieldName
TMA_Usage
PK, FK2
TMAassignedID
FK1
JHH_TMA_numID
TMAcut
TMAStain
TMADate
DateCut
DistributedTo
DateDistributed
OriginalLocation
CurrentLocation
UserProjectPermissions
PK
UserProjectPermissionID
ProjectID
UserID
P_ViewImages
P_DiagnosisImages
P_ViewDiagnosisSummary
P_ProjectAdministrator
P_ViewClinicalData
P_CreateTMA
P_InputSpecimenData
P_ViewPatientData
Projects
PK, FK2
ProjectID
ProjectName
Description
Source
ProtocolID
FK1
SurgeryTypes
PK
SurgeryTypeID
SurgeryType
Consent
PK
ID
FK1
SpecimenID
ConsentType
ConsentStatus
ConsentDate
Blocks
PK, FK2
BlockID
FK1
OutsideBlockNumber
OrganID
Part
Block
Notes
Block_Info
Location
Not_Info_Location
NumUnstained
BlockStatusID
ExpNum
H&E_Info
ProjectTMASlides
PK
ProjectTMASlideID
FK1
ProjectID
TMAUsageID
IRB_Protocols
PK, FK1
ProtocolID
JHHProtocolNumber
OutsideProtocolNumber
OutsideInstitution
GrantID
Application_Title
Exemption
IRB_Contact_1
IRB_Contact_2
IRB_Approval_Start_Date
Date_Last_Renewed
IRB_Renewal_Proposal_Deadline
IRB_Renewal_Date
Notes
ApprovalLetterOnFile
JOS_Number
TissueDiagnosis
PK
TissueDiagnosisID
FK1
OutsideTissueDiagnosisID
BlockID
Lesion Letter
PlugletSize
Notes
MicroscopistID
Additional Notes
BlockUsages
PK
ID
BlockID
DateCut
Technician
NumberOfSectionsCut
UserID
ProtocolID
Cut
Notes
DiagnosisNotes
ProtocolGrants
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ProtocolGrantID
FK1
ProtocolID
GrantID
PercentAllocated
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TMAname
TMAsource
TMAcreator
TMApurpose
TMAcreatedate
TMAmeltdate
TMAstatus
TMAslots
TMA_Map_File
NumPatients
TMA_Core_Details
PK
TMACoreID
FK2
JHH_TMA_numID
FK1
TissueDiagnosisID
TMAYcoord
TMAXcoord
TMANotes
TissueDiagnosisData
PK
ID
FK1
TissueDiagnosisID
FieldNameID
EnumerationValueID
FieldValue
TissueDiagnosisFieldNames
PK, FK1
FieldNameID
OrganID
FieldName
MinValue
MaxValue
TissueDiagnosisEnumerations
PK, FK1
EnumerationValueID
FK2
FieldNameID
EnumerationValue
EnumerationValueDescription

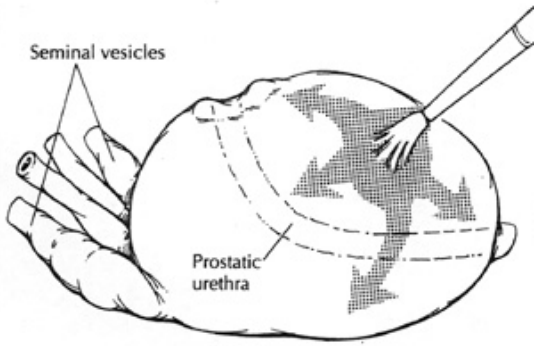
Patients, Specimens, & Blocks

Patients	
PK	**PatientID**
	PatientHash
U1	**Hospital**
U1	**HistoryNumber**
	NameFirst
	NameLast
	BirthDate
	Race
	Sex
	OutsideHospital1
	OutsideHistoryNumber1
	Notes
	IsEncrypted
	InputLog

Specimens	
PK	**SpecimenID**
FK1,I3	**PatientID**
I2,U1	**SurgPathNumber**
	OutsideSurgPathNumber
	OutsideResearchNumber
U1	**Hospital**
	SpecimenTypeID
	SpecimenList
	AccessionDate
	DateSpecimenTaken
	Pathologists
	Physicians
	Diagnosis
	GrossDescription
	Notes
	TumorBankNumber
	HarvestedBlocks
	TumorBankFlag
	SpeciesType
	InputLog
I1	StudyID
	Age
	FixationID
	MarginsPositive

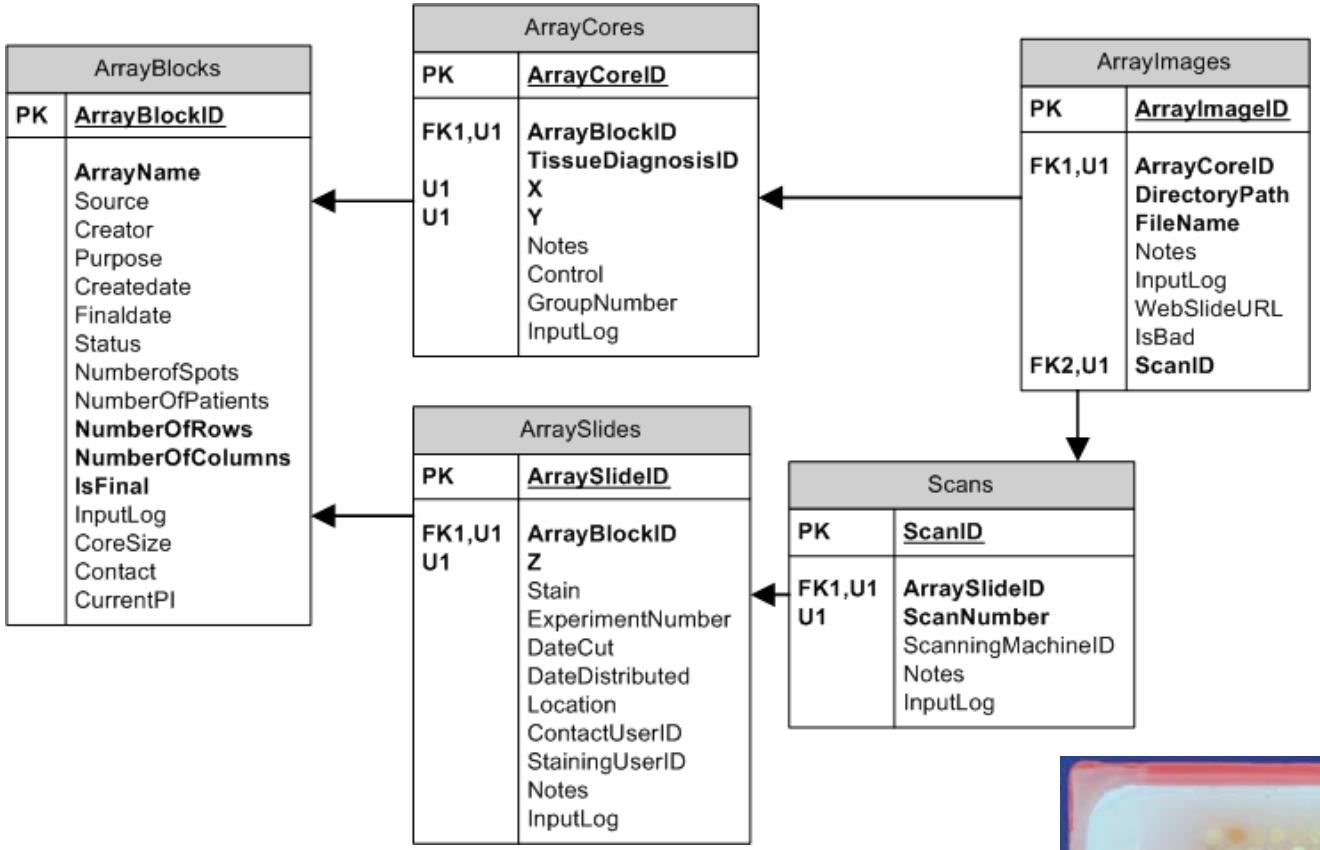
Blocks	
PK	**BlockID**
FK1,U1	**SpecimenID**
U1	**Part**
U1	**BlockDesignation**
	Notes
	Block_In
	Fixation
	Not_InFile_Location
	NumberUnstained
	BlockStatusID
	ExpNum
	HasHandE
	OutsideBlockID
	ContactUserID
	Area_mm2
	InputLog
	IsDivided

TissueDiagnosis	
PK	**TissueDiagnosisID**
FK1,U1	**BlockID**
	OutsideTissueDiagnosisID
	TissueTypeID
U1	**LesionLetter**
	RegionSize
	MicroscopistID
	Notes
	Area_mm2
	InputLog

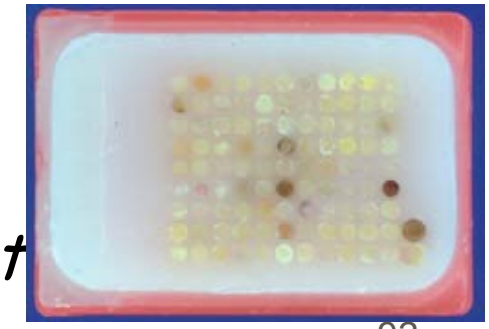


tables all form a

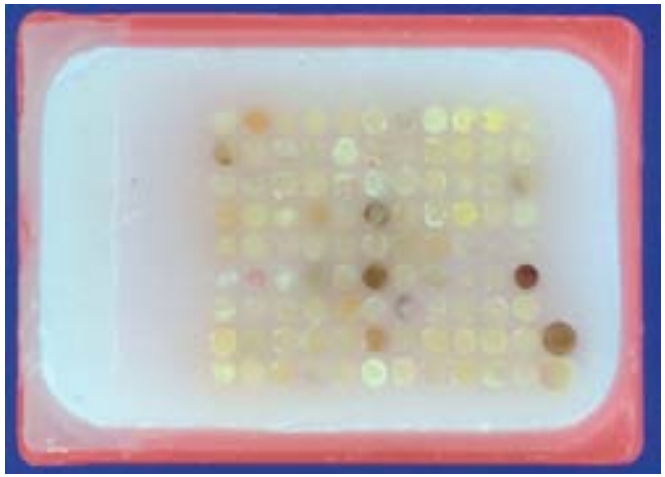
ArrayBlocks



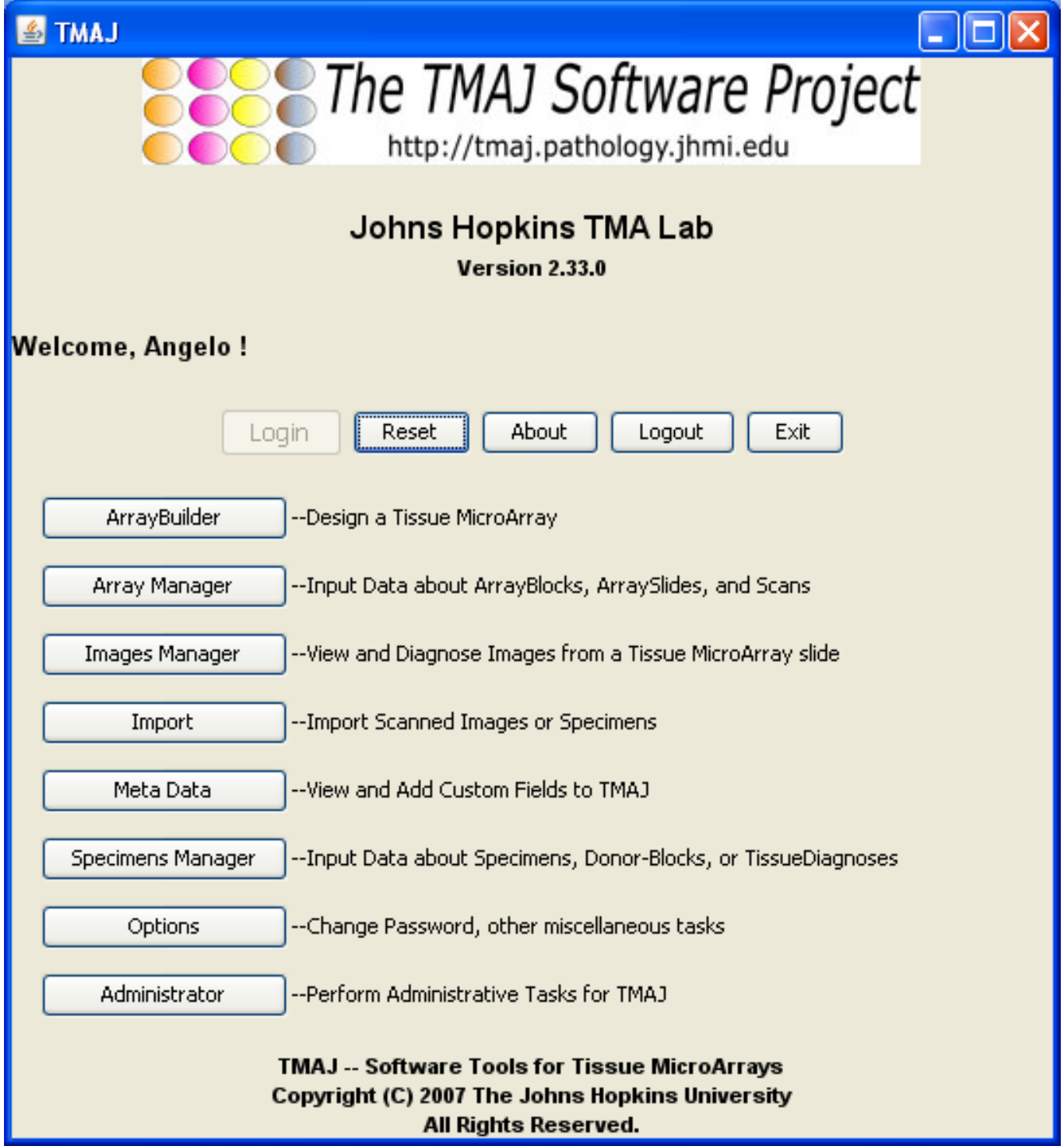
The Schema of ArrayBlock-relat



- **Array_Cores**
  - **Array_CoreID**
  - **Array_BlockID**
  - **Tissue_DiagnosisID**
  - **X**
  - **Y**

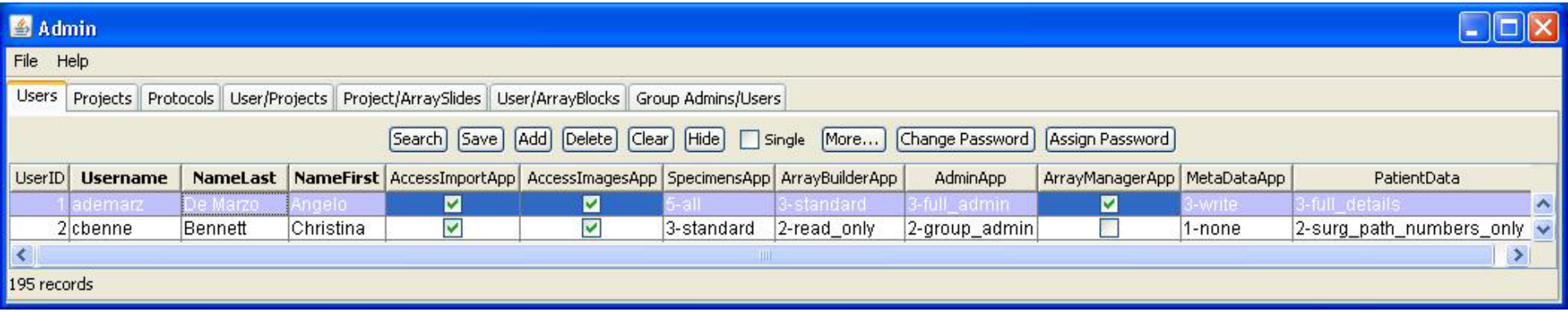


Applications & Screenshots


TMAJ
The TMAJ Software Project
http://tmaj.pathology.jhmi.edu
Johns Hopkins TMA Lab
Version 2.33.0
Welcome, Angelo !
Login
Reset
About
Logout
Exit
ArrayBuilder
--Design a Tissue MicroArray
Array Manager
--Input Data about ArrayBlocks, ArraySlides, and Scans
Images Manager
--View and Diagnose Images from a Tissue MicroArray slide
Import
--Import Scanned Images or Specimens
Meta Data
--View and Add Custom Fields to TMAJ
Specimens Manager
--Input Data about Specimens, Donor-Blocks, or TissueDiagnoses
Options
--Change Password, other miscellaneous tasks
Administrator
--Perform Administrative Tasks for TMAJ
TMAJ -- Software Tools for Tissue MicroArrays
Copyright (C) 2007 The Johns Hopkins University
All Rights Reserved.

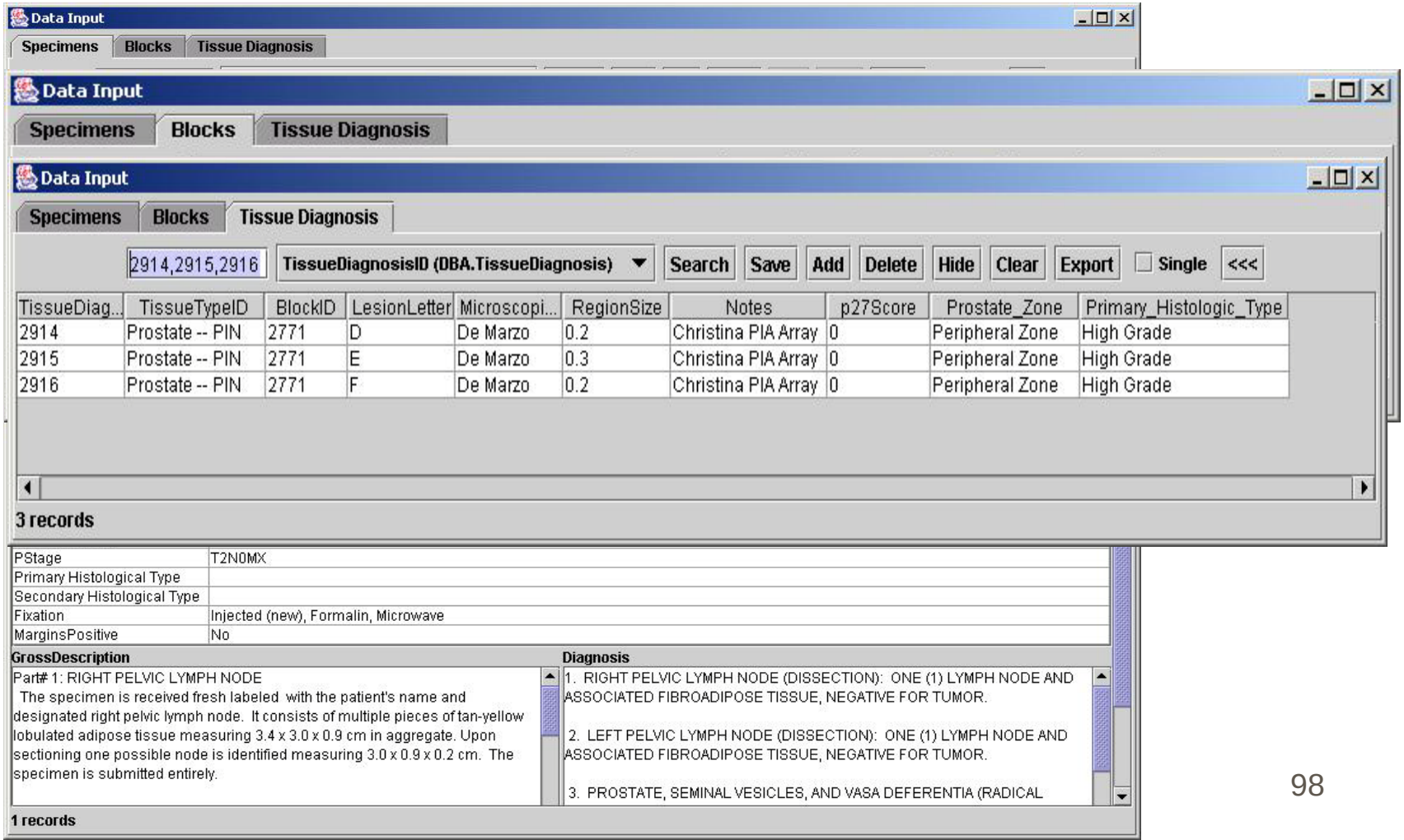
Security Options: Specimens

- Users may only access specimens to which they have permission.
- Admins may assign a user permission to a specimen by using the Users-Specimens tab in the Administrator application.



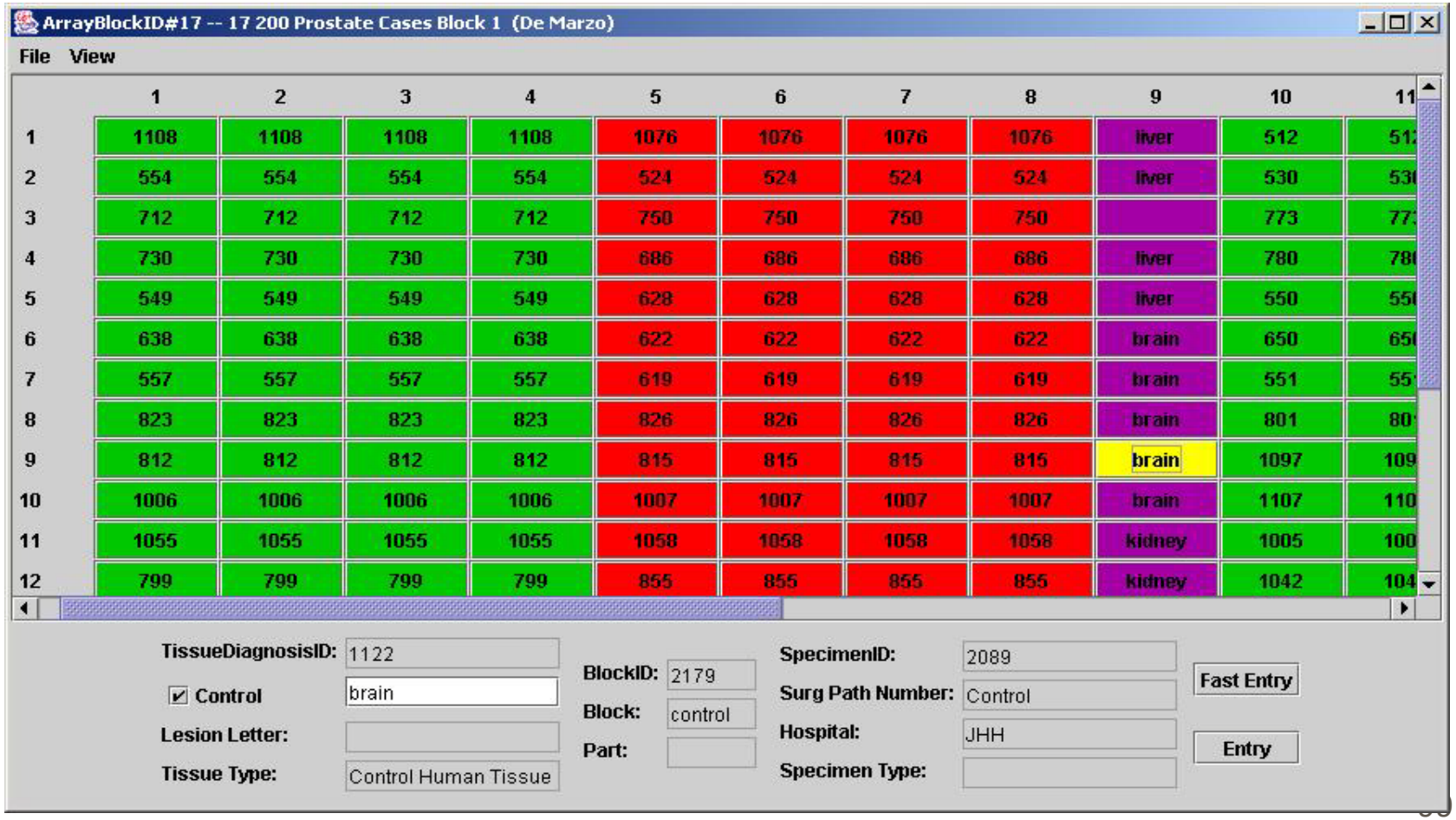
Data Input Application

- This application allows for detailed input of data on individual specimens and donor-tissue-blocks.

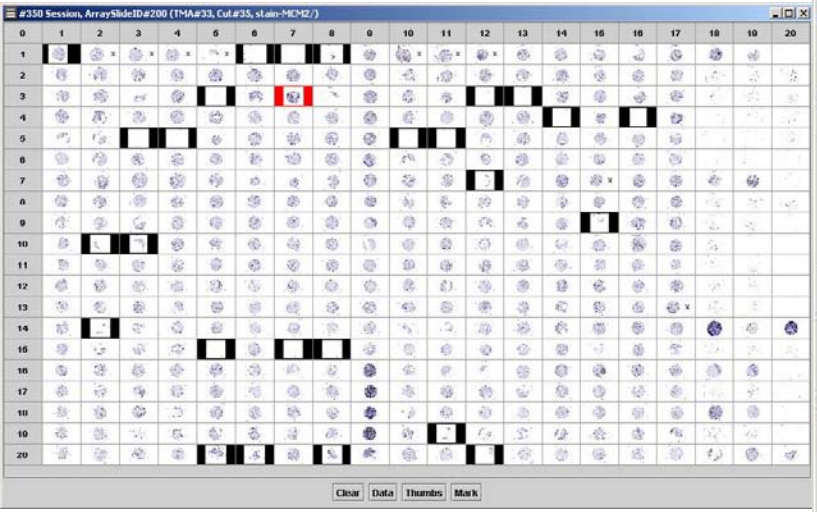


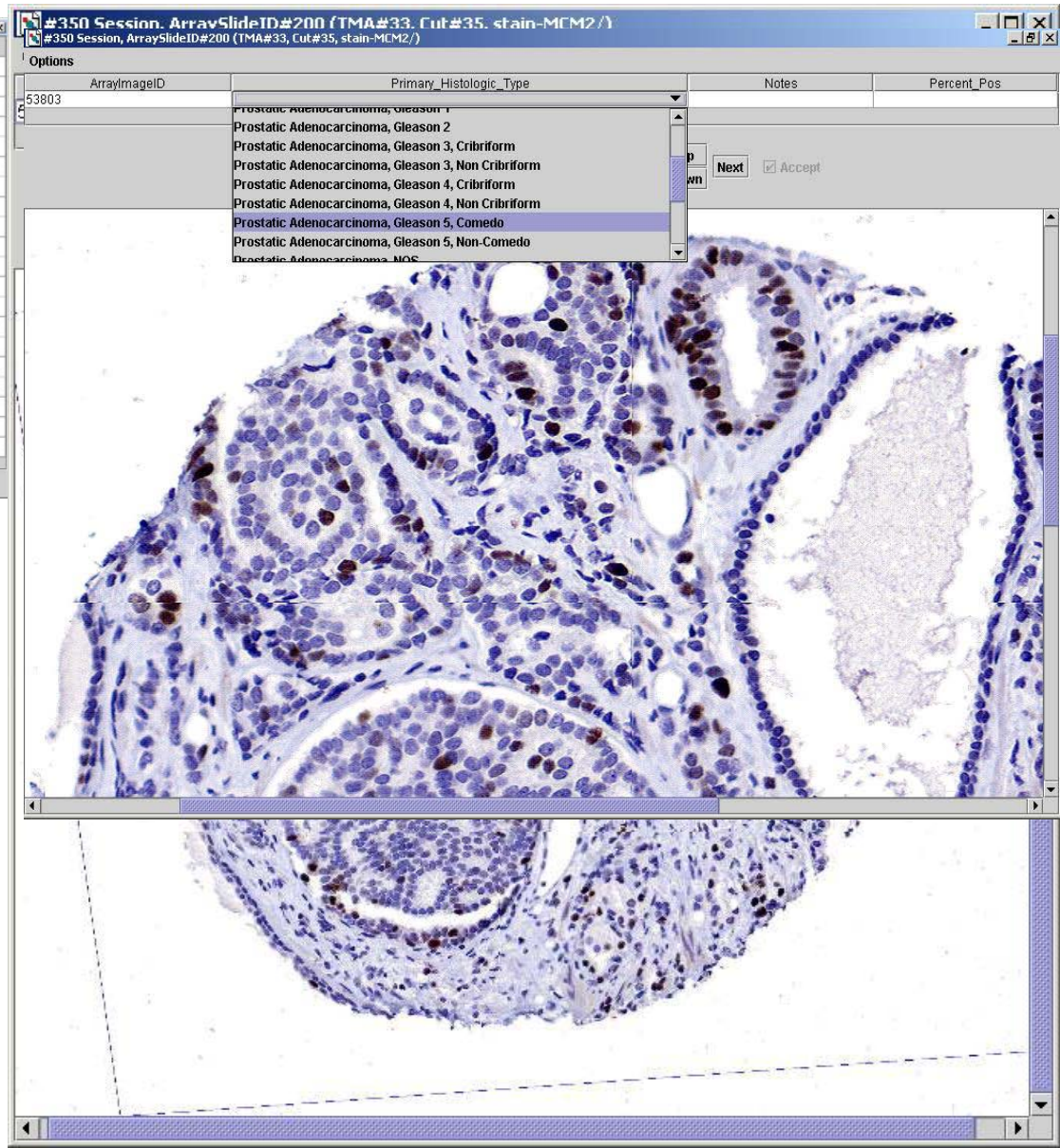
ArrayBuilder Application

- The ArrayBuilder application allows users to design Tissue MicroArrays.



Images Application





Digital Image Acquisition

- Can use conventional microscopes
- Record data in spreadsheet: diagnoses and interpretations
- Or the data can be recorded on paper for later entry into a spreadsheet or database
- Major Problem:
  - Easy to loose track of the x and y coordinates of given spots

Institutions Using TMAJ

- Johns Hopkins University
- Harvard Dana Farber Cancer Institute
- Cleveland Clinic
- University of Texas Southwestern
- Vanderbilt University

For More Information

- http://tmaj.pathology.jhmi.edu
- To see published images
  - login to tmaj as a guest and then click the Images button.
    - Username: guest
    - Password: guest

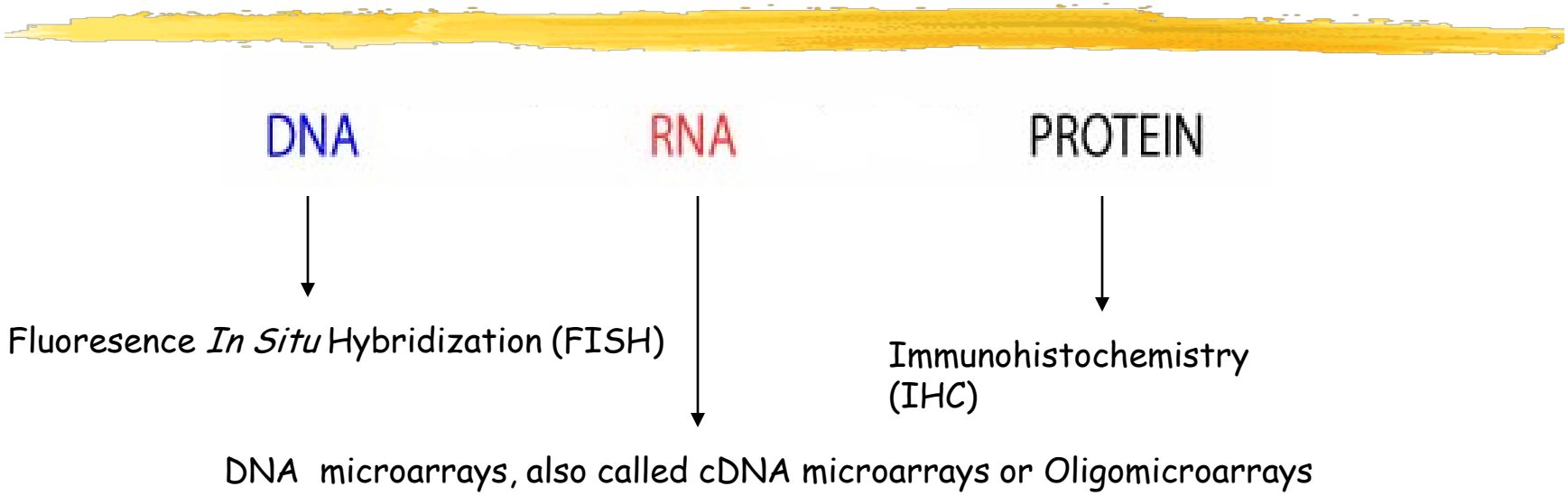
http://users.skynet.be/J.Beever/hosepipe.html

Human Genome Project

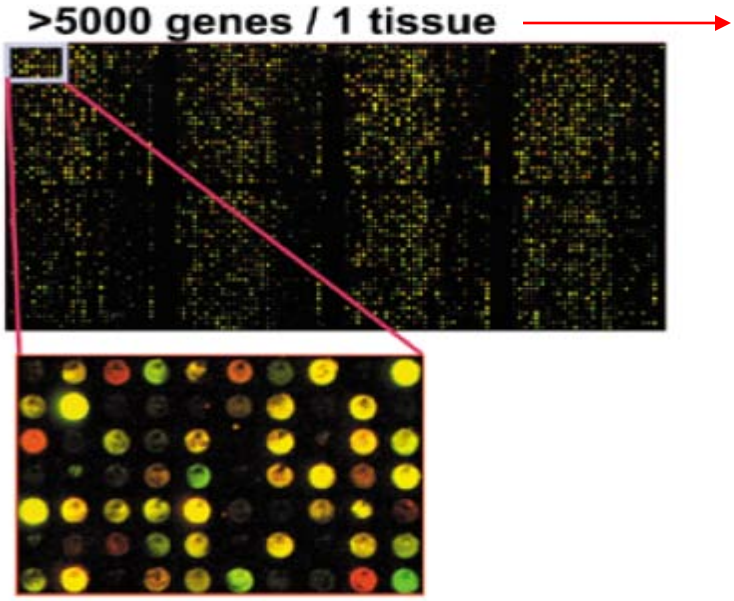
- Sequencing of the human genome has yielded an estimate of 20,000–25,000 protein-coding genes

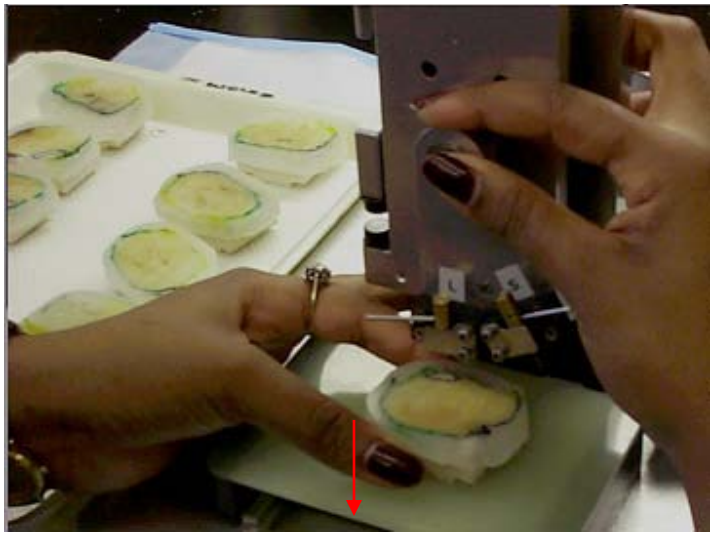
http://www.genome.gov/12011238

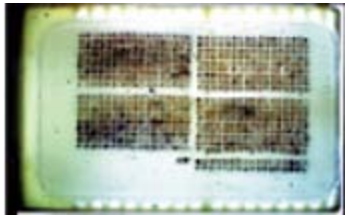
Detection Techniques



First Gene Discovery, Then Validation







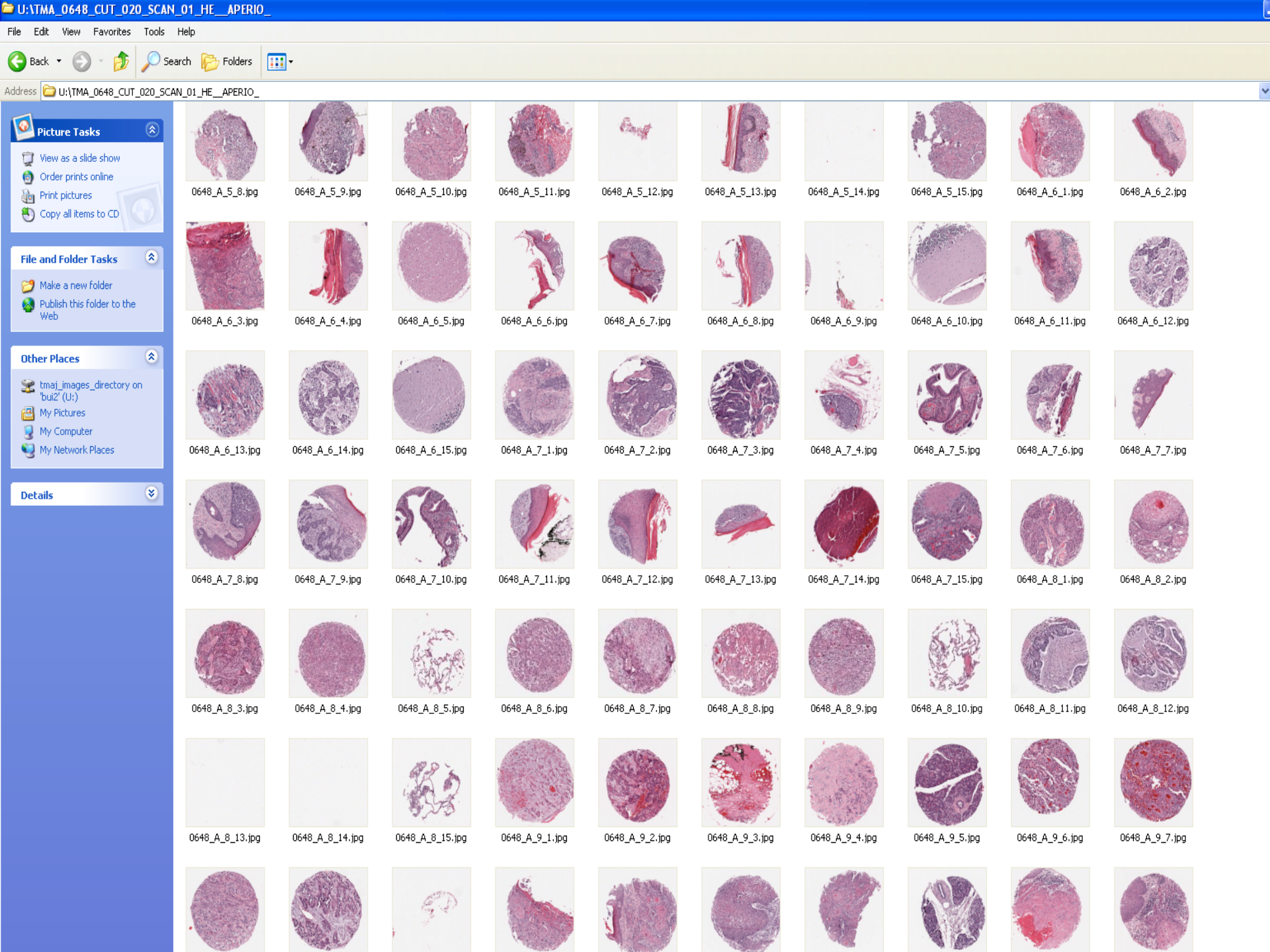
Imaging Tissue Microarray

Reason #1

- Permanent electronic record which can be stored on a server and accessed from any computer with internet access

Digital Image Acquisition

- Can use conventional microscopes
- Record data in spreadsheet: diagnoses and interpretations
- Or the data can be recorded on paper for later entry into a spreadsheet or database
- Major Problem:
  - Easy to loose track of the x and y coordinates of given spots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File Edit View Favorites Tools Help
Back Search Folders
Address U:\TMA_0648_CUT_020_SCAN_01_HE__APERIO_
Picture Tasks
View as a slide show
Order prints online
Print pictures
Copy all items to CD
File and Folder Tasks
Make a new folder
Publish this folder to the Web
Other Places
tmaj_images_directory on 'bui2' (U:)
My Pictures
My Computer
My Network Places
Details
0648_A_5_8.jpg
0648_A_5_9.jpg
0648_A_5_10.jpg
0648_A_5_11.jpg
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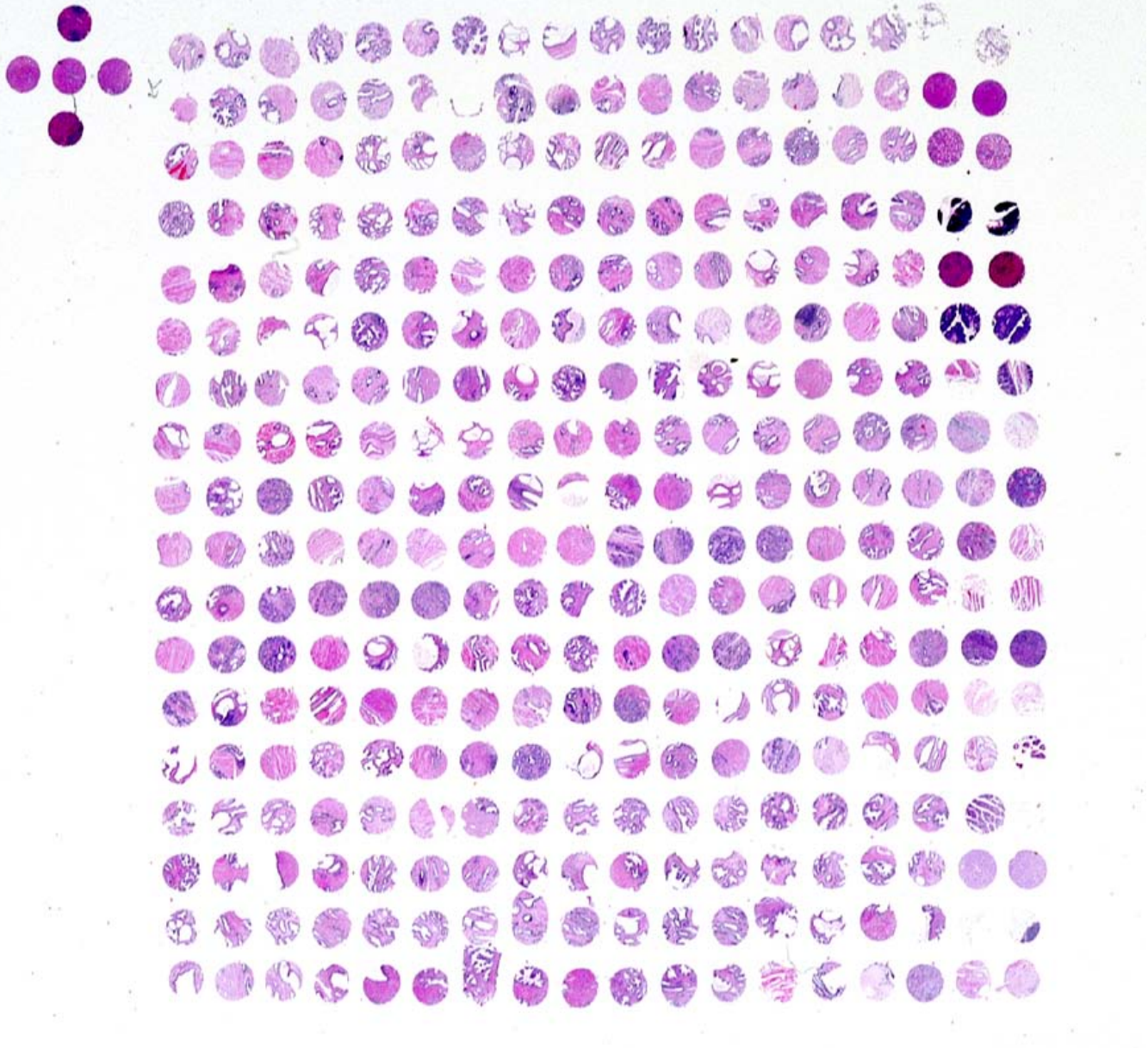
0648_A_9_4.jpg
0648_A_9_5.jpg
0648_A_9_6.jpg
0648_A_9_7.jpg

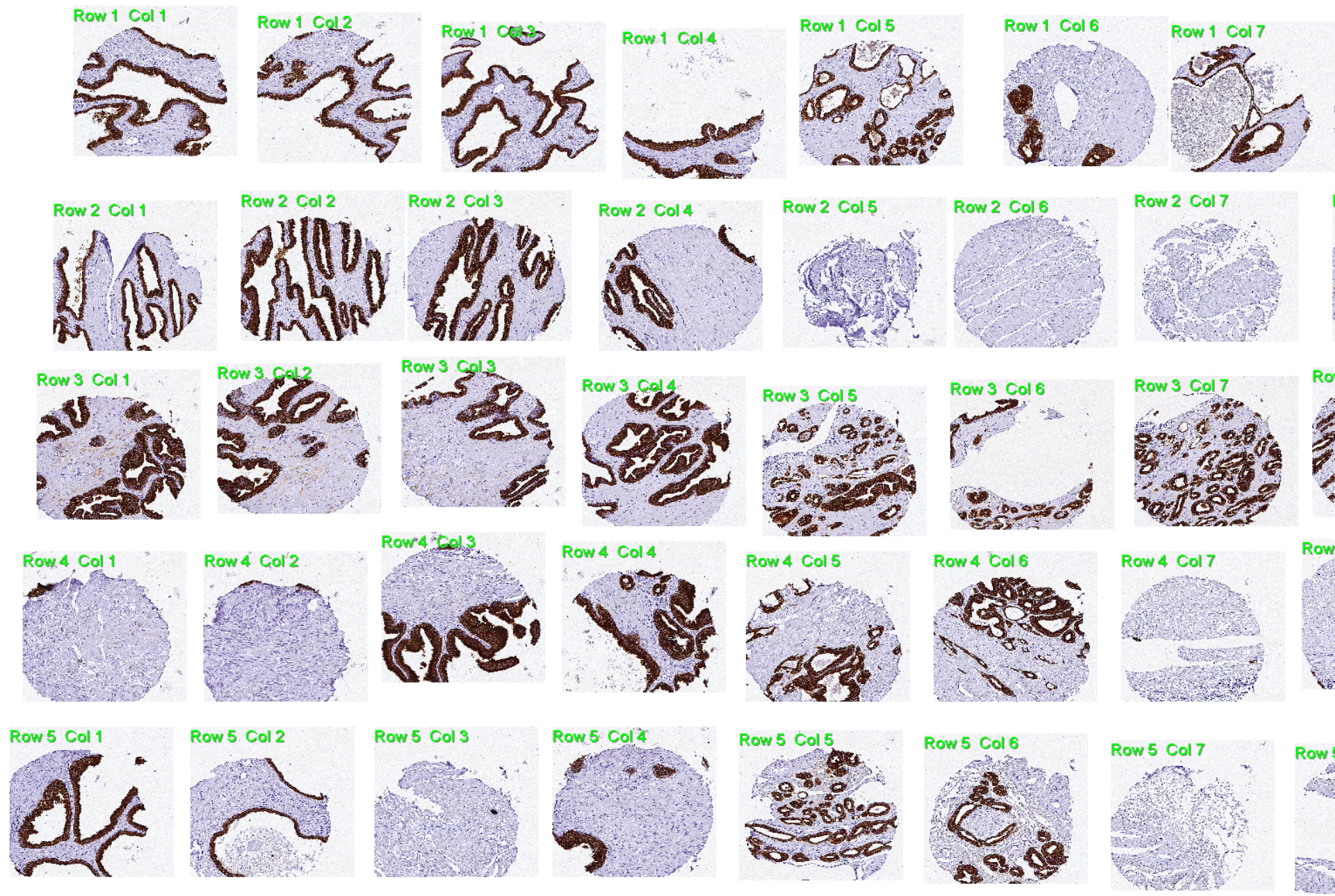
Imaging Tissue Microarray

Reason #2

- Keeps track of the x, y spot coordinates, making spot review and data entry MUCH easier



Tissue Microarray – 400 cores, 0.6 mm each – H&E of 4 µm section



Tissue Microarray – 400 cores, 0.6 mm each – CK8 of 4 μm section

Imaging Tissue Microarray

Reason #3

- To collaborate efforts between pathologists via telepathology

https://secondslide.com/

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"SecondSlide improves turnaround time for consultations by eliminating glass slide logistics."
– *Juan Rosai, MD*

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Background

Data Sheet
Application Sheet

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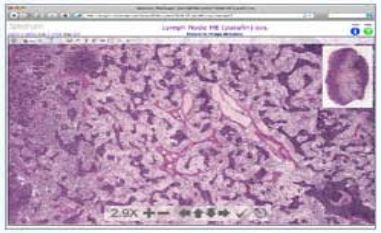
- Gain access to subspecialty expertise



+ View Sample Digital Slide

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Clinical


Research


Education

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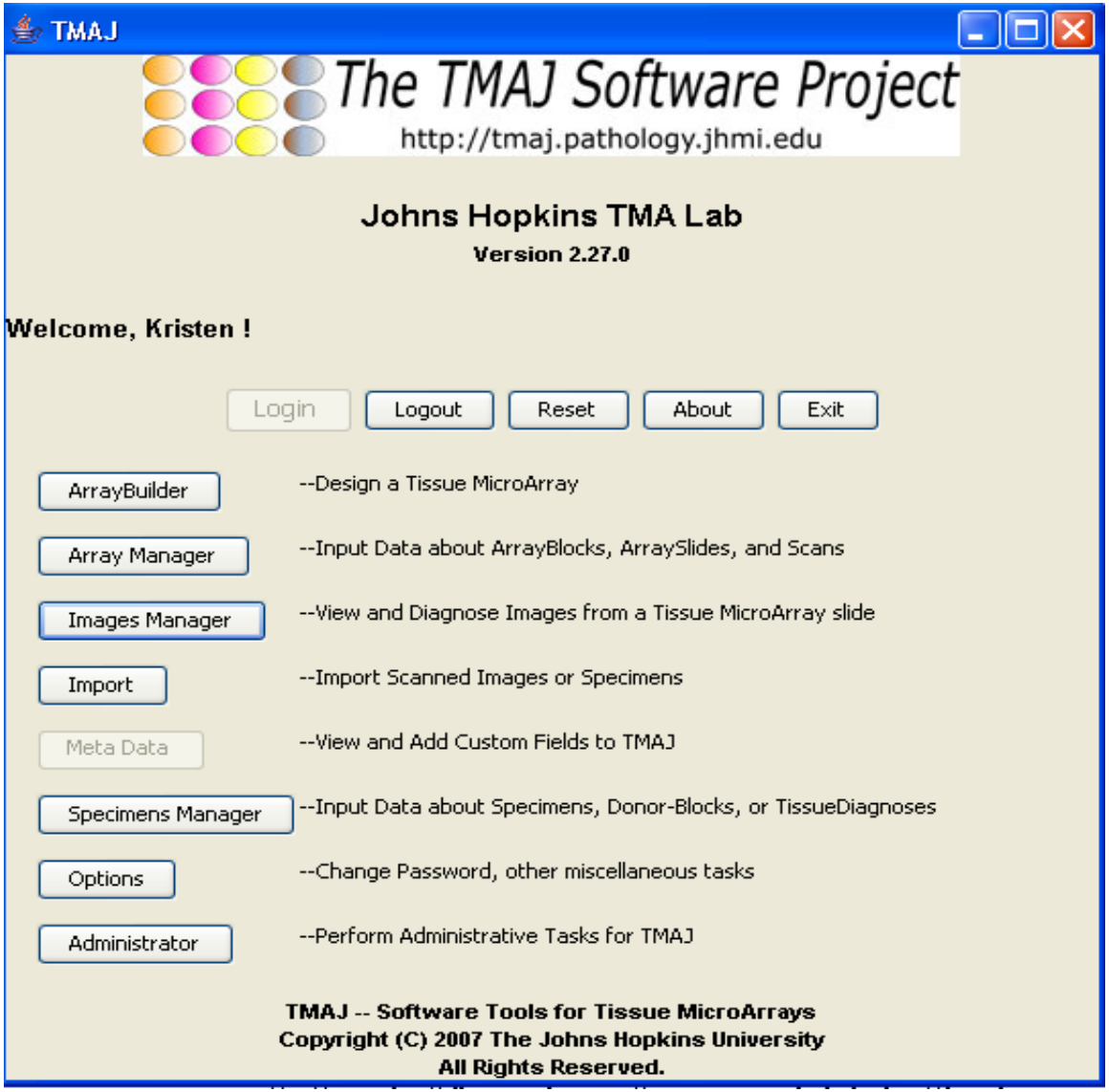
Imaging Tissue Microarray

Reason #4

- Ability to import images into a database, which is linked to image, pathology, and clinical information.
- And then can export data to automate data analysis

TMA Databases

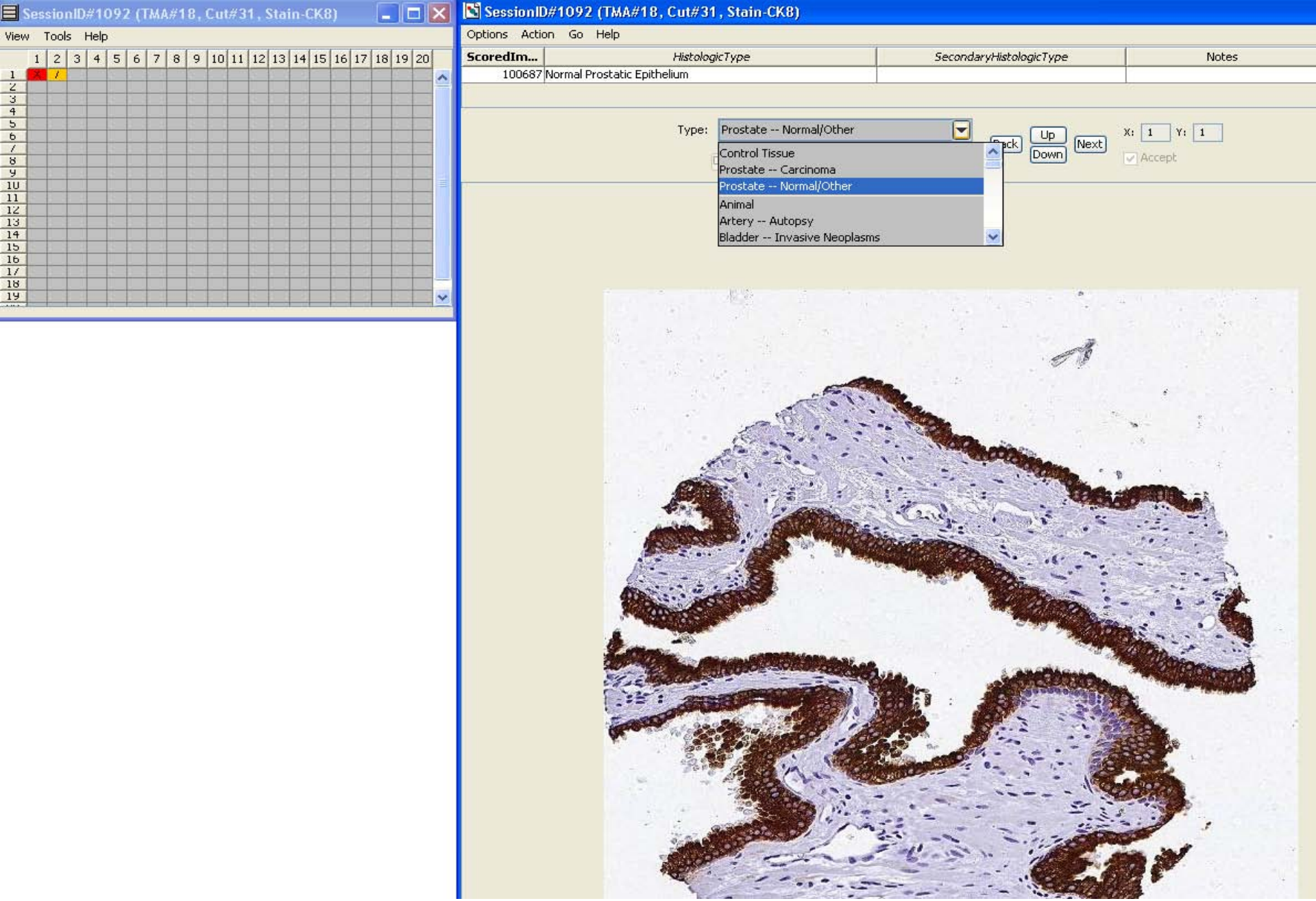
Institution	Name of Database	Webpage
Stanford University, California, USA	TMAD	http://tma.stanford.edu/cgi-bin/home.pl
Johns Hopkins University, Maryland, USA	TMAJ	http://tmaj.pathology.jhmi.edu/
Graz University of Technology, Austria	TAMEE	https://esus.genome.tugraz.at/tma/
MD Anderson Cancer Center, Texas, USA	TAD	http://bioinformatics.mdanderson.org/tad.html



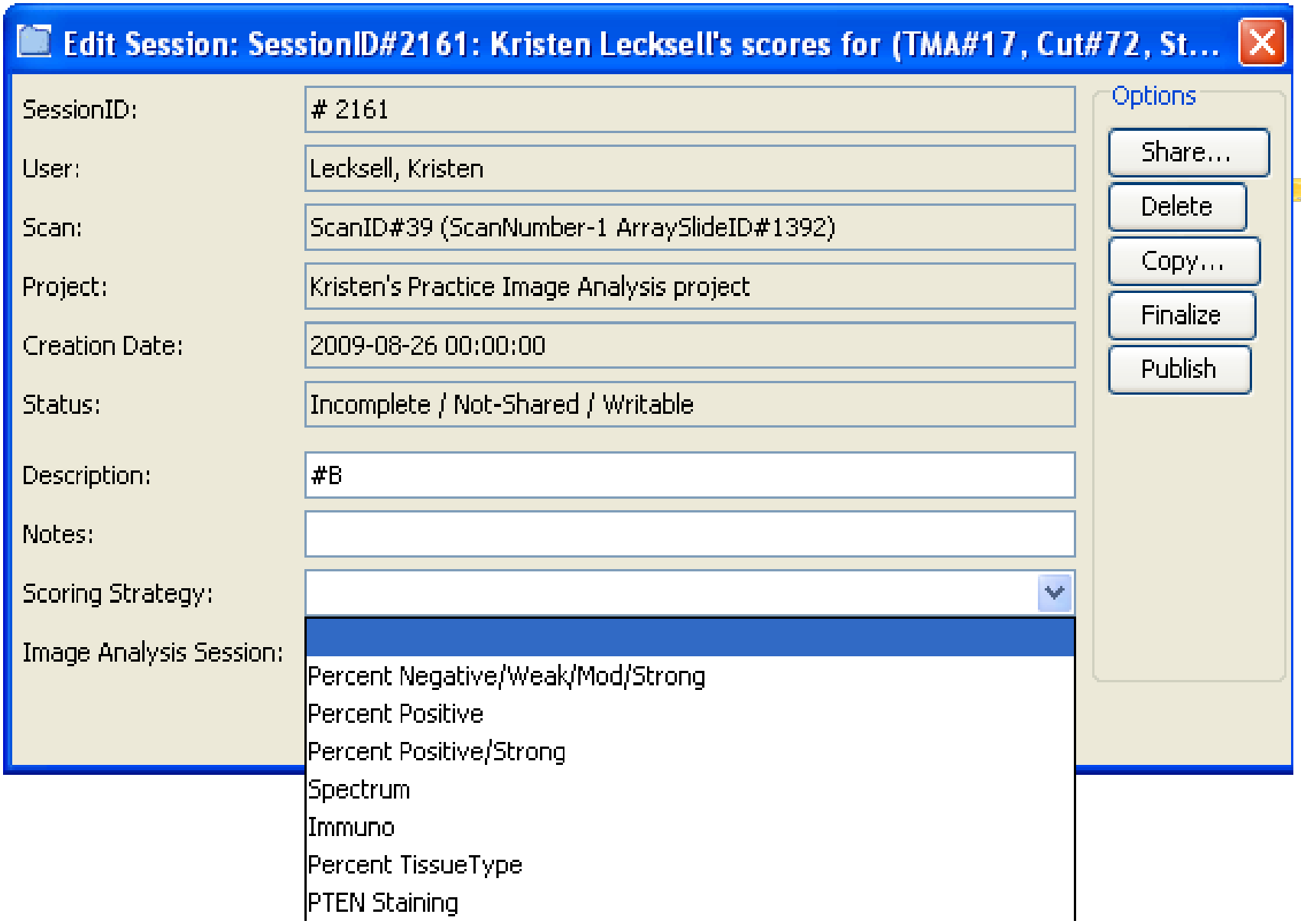
James D. Morgan

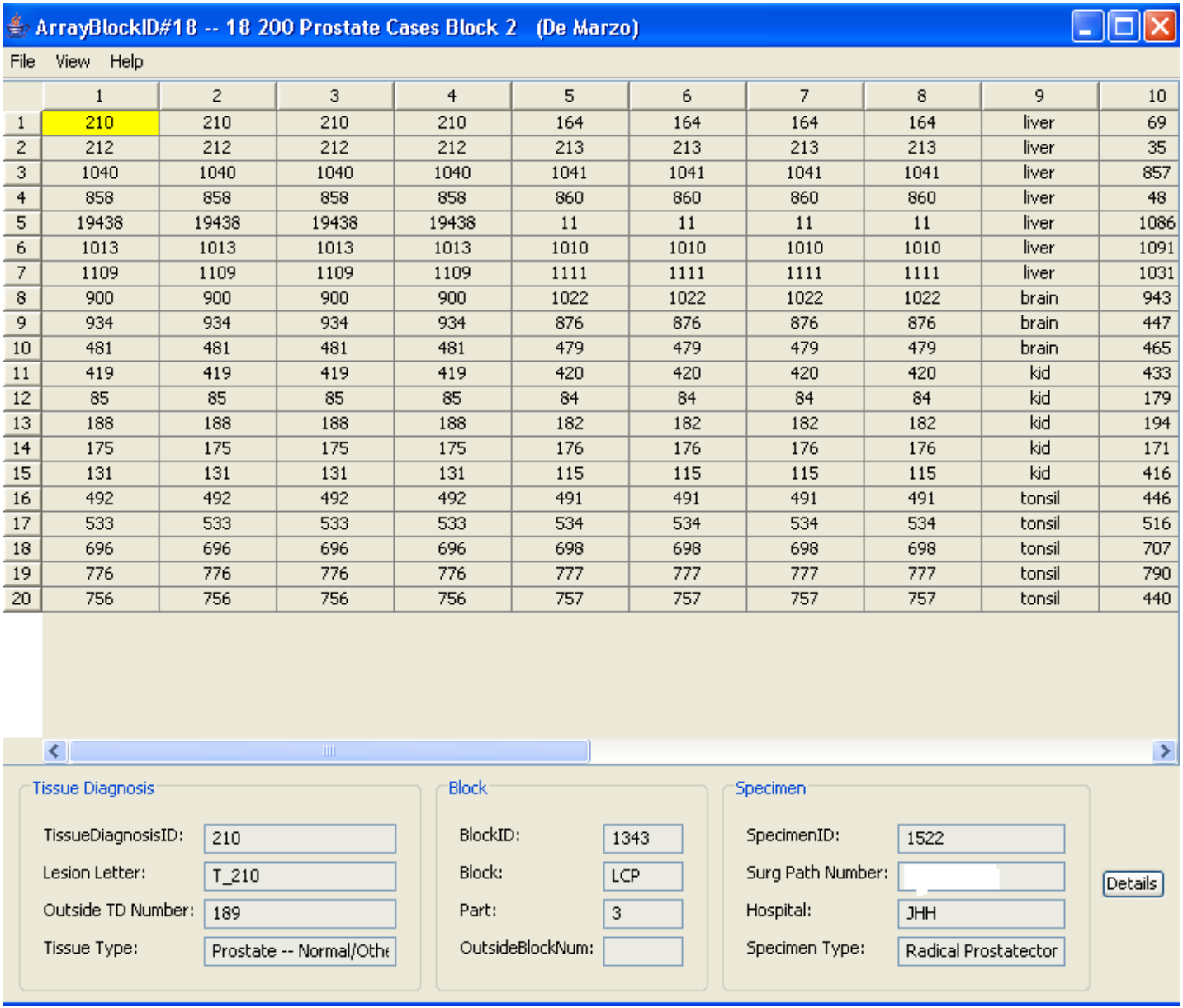
Angelo M. De Marzo

http://tmaj.pathology.jhmi.edu/



http://tmaj.pathology.jhmi.edu/


Edit Session: SessionID#2161: Kristen Lecksell's scores for (TMA#17, Cut#72, St...
SessionID:
2161
User:
Lecksell, Kristen
Scan:
ScanID#39 (ScanNumber-1 ArraySlideID#1392)
Project:
Kristen's Practice Image Analysis project
Creation Date:
2009-08-26 00:00:00
Status:
Incomplete / Not-Shared / Writable
Description:
#B
Notes:
Scoring Strategy:
Image Analysis Session:
Percent Negative/Weak/Mod/Strong
Percent Positive
Percent Positive/Strong
Spectrum
Immuno
Percent TissueType
PTEN Staining
Options
Share...
Delete
Copy...
Finalize
Publish


ArrayBlockID#18 -- 18 200 Prostate Cases Block 2 (De Marzo)
File View Help
1 2 3 4 5 6 7 8 9 10
1 210 210 210 210 164 164 164 164 liver 69
2 212 212 212 212 213 213 213 213 liver 35
3 1040 1040 1040 1040 1041 1041 1041 1041 liver 857
4 858 858 858 858 860 860 860 860 liver 48
5 19438 19438 19438 19438 11 11 11 11 liver 1086
6 1013 1013 1013 1013 1010 1010 1010 1010 liver 1091
7 1109 1109 1109 1109 1111 1111 1111 1111 liver 1031
8 900 900 900 900 1022 1022 1022 1022 brain 943
9 934 934 934 934 876 876 876 876 brain 447
10 481 481 481 481 479 479 479 479 brain 465
11 419 419 419 419 420 420 420 420 kid 433
12 85 85 85 85 84 84 84 84 kid 179
13 188 188 188 188 182 182 182 182 kid 194
14 175 175 175 175 176 176 176 176 kid 171
15 131 131 131 131 115 115 115 115 kid 416
16 492 492 492 492 491 491 491 491 tonsil 446
17 533 533 533 533 534 534 534 534 tonsil 516
18 696 696 696 696 698 698 698 698 tonsil 707
19 776 776 776 776 777 777 777 777 tonsil 790
20 756 756 756 756 757 757 757 757 tonsil 440
Tissue Diagnosis
TissueDiagnosisID: 210
Lesion Letter: T_210
Outside TD Number: 189
Tissue Type: Prostate -- Normal/Oth
Block
BlockID: 1343
Block: LCP
Part: 3
OutsideBlockNum:
Specimen
SpecimenID: 1522
Surg Path Number:
Hospital: JHH
Specimen Type: Radical Prostatector
Details

Data in TMAJ Specimens Table

- Patient Data (Encrypted, not accessable)
  - PatientID
  - Age
  - Race
- Surgical Pathology Data
  - SurgPathNumber
  - DateSpecimenTaken
  - PStage
  - HistologicalType (Gleason for radical prostatectomies)
  - MarginsPositive

SessionID#1092 (TMA#18, Cut#31, Stain-CK8)

Options Action Go Help

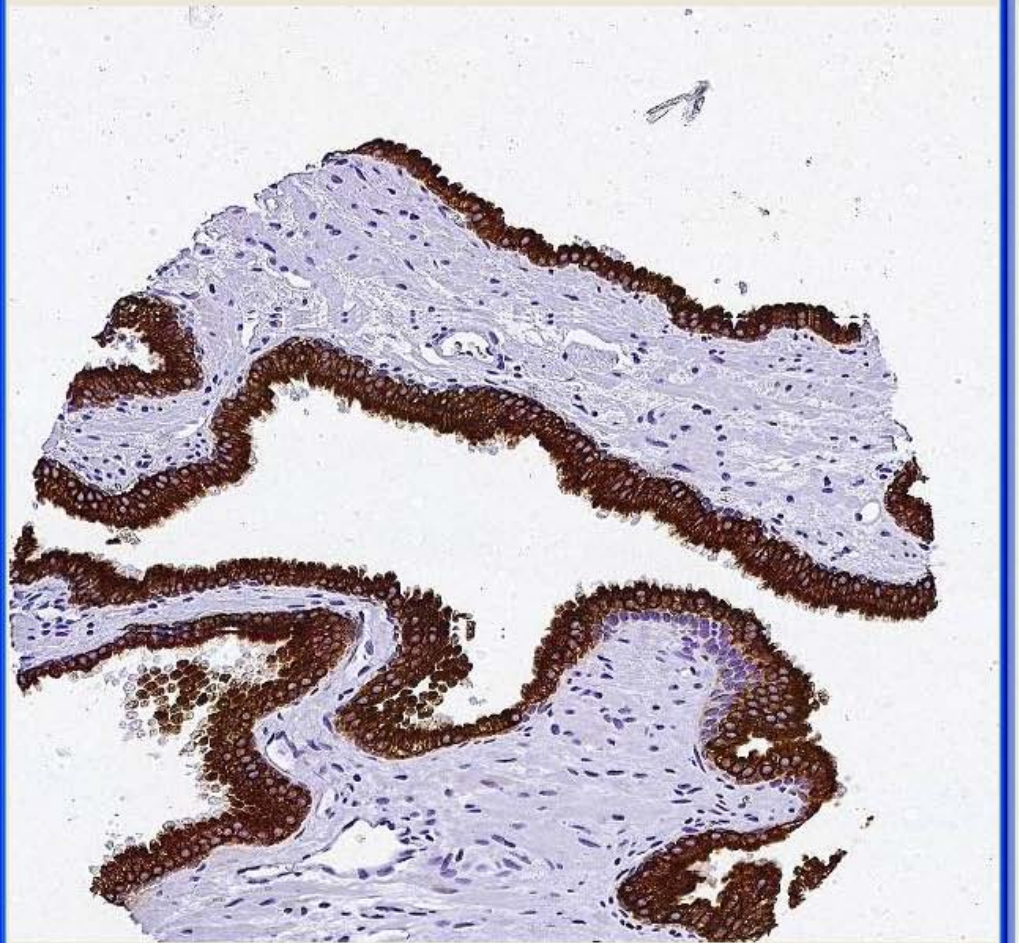
ScoredIm...	HistologicType	SecondaryHistologicType
100687	Normal Prostatic Epithelium	

Type: Prostate -- Normal/Other

Back Up Down Next

X: 1 Y: 1

Draw Save Log Link Info Large Accept



SessionID#1049 (TMA#18, Cut#30, Stain-MCM2)

Options Action Go Help

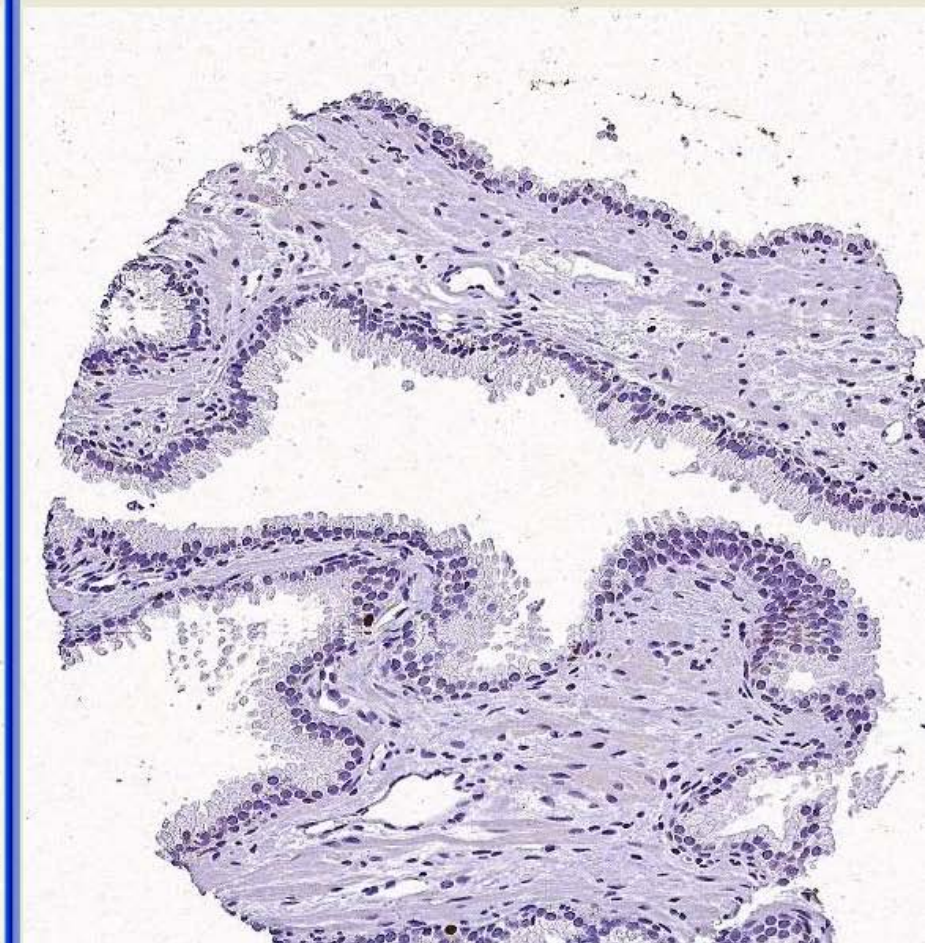
ScoredIm...	HistologicType	SecondaryHistologicType
84911	Normal Prostatic Epithelium	

Type: Prostate -- Normal/Other

Back Up Down Next

X: 1 Y: 1

Draw Save Log Link Info Large Accept



Systems for TMA Image Acquisition

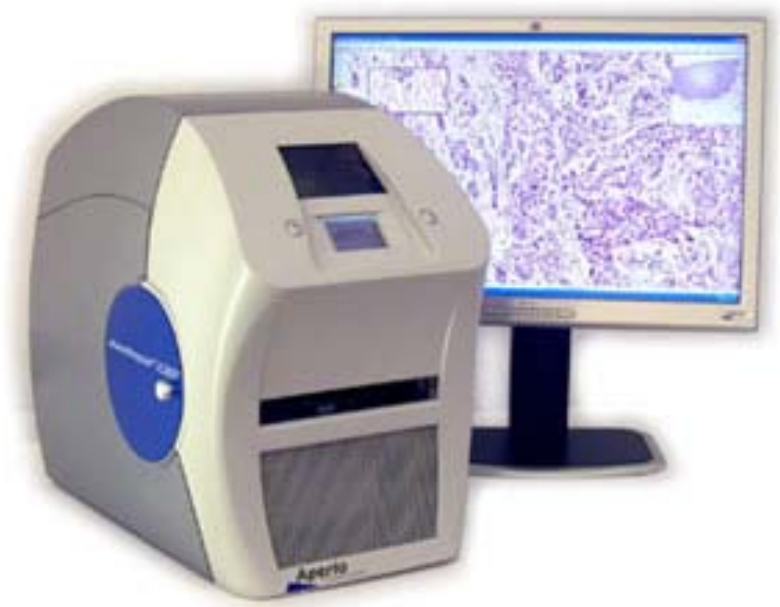
Omnyx	In development	http://www.omnyx.com/html
Olympus	Nanozoomer	http://www.olympusamerica.com
Aperio Technologies	ScanScope™	http://www.aperio.com
Genetix	Ariol	http://www.genetix.com
CompuCyte	Laser Scanning Cytometer	http://www.compucyte.com
HistoRx	Aqua	http://www.historx.com

Olympus/Bacus Scanners

BLISS

Nanozoomer

Tissue Microarray Image Acquisition



Aperio ScanScope

Dako/Zeiss Scanner

HistoRx Scanner

PM-2000™
(fluorescence only)
by HistoRx

Hardware of Most Scanning Systems

- Brightfield microscope, objectives (Plan Apochromat: 1.25x, 5x, 10x, 20x, 40x & 60x) and motorized stage
- Color video camera such as a 3 CCD (charge-coupled device)
- High resolution monitor
- Slide loader
- CPU

Software of Most Scanning Systems

- Proprietary
- Composite
- Controls the hardware
  - Stage
  - Objectives
  - Video camera
- TMA analysis
  - Morphometric measurements
  - IHC quantification
  - Histology pattern recognition

Image Capture

- Digitizing the tissue or cells on a glass microscope slide, so that the personal computer becomes the microscope
- Can view under different magnifications
- Can represent whole tissues or TMAs
- Working with pixels instead of a real image

20x Camera Capture

	Pixel # / Micron
Aperio: ScanScope CS	2
Olympus/Bacus Labs: BLISS	2.37

Megabytes & Gigabytes

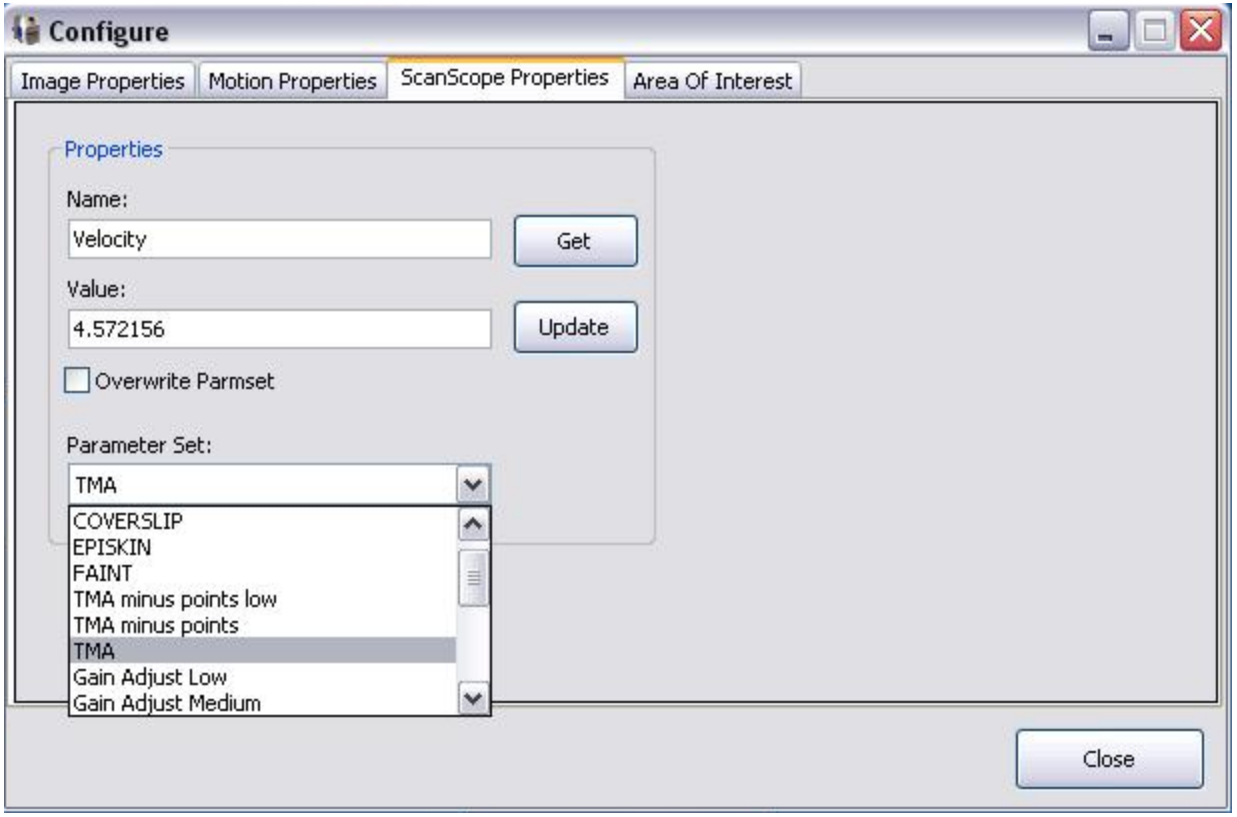
- Digitized TMA: 100 MB to 3 GB
- Storage is an issue
- BUT storage is getting cheaper
- The higher the magnification the greater the storage space needed

Huge files

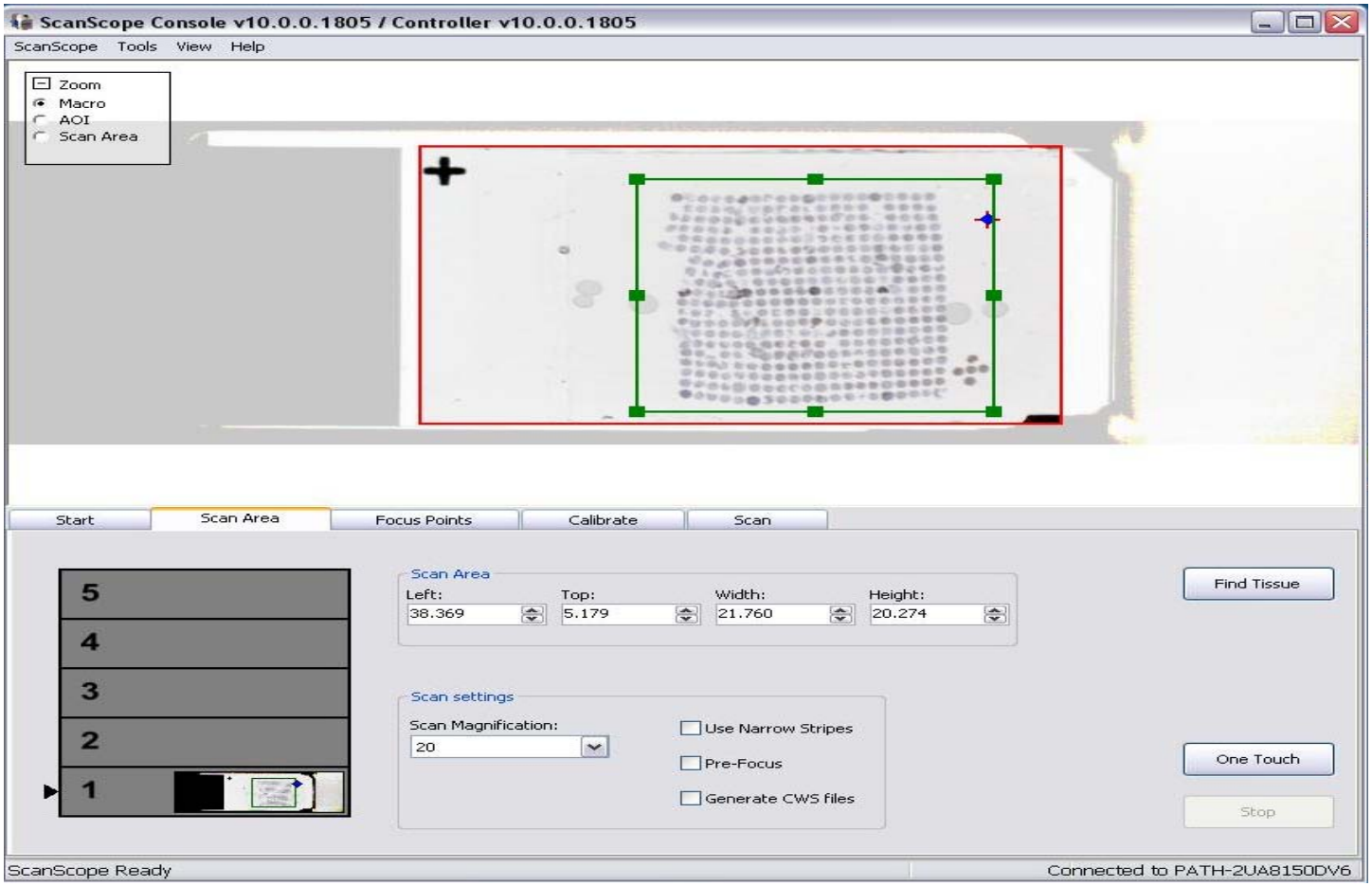
- Opening, Moving, Storing of files is a real issue.
- Costs time and money
- Backing up

- Getting better

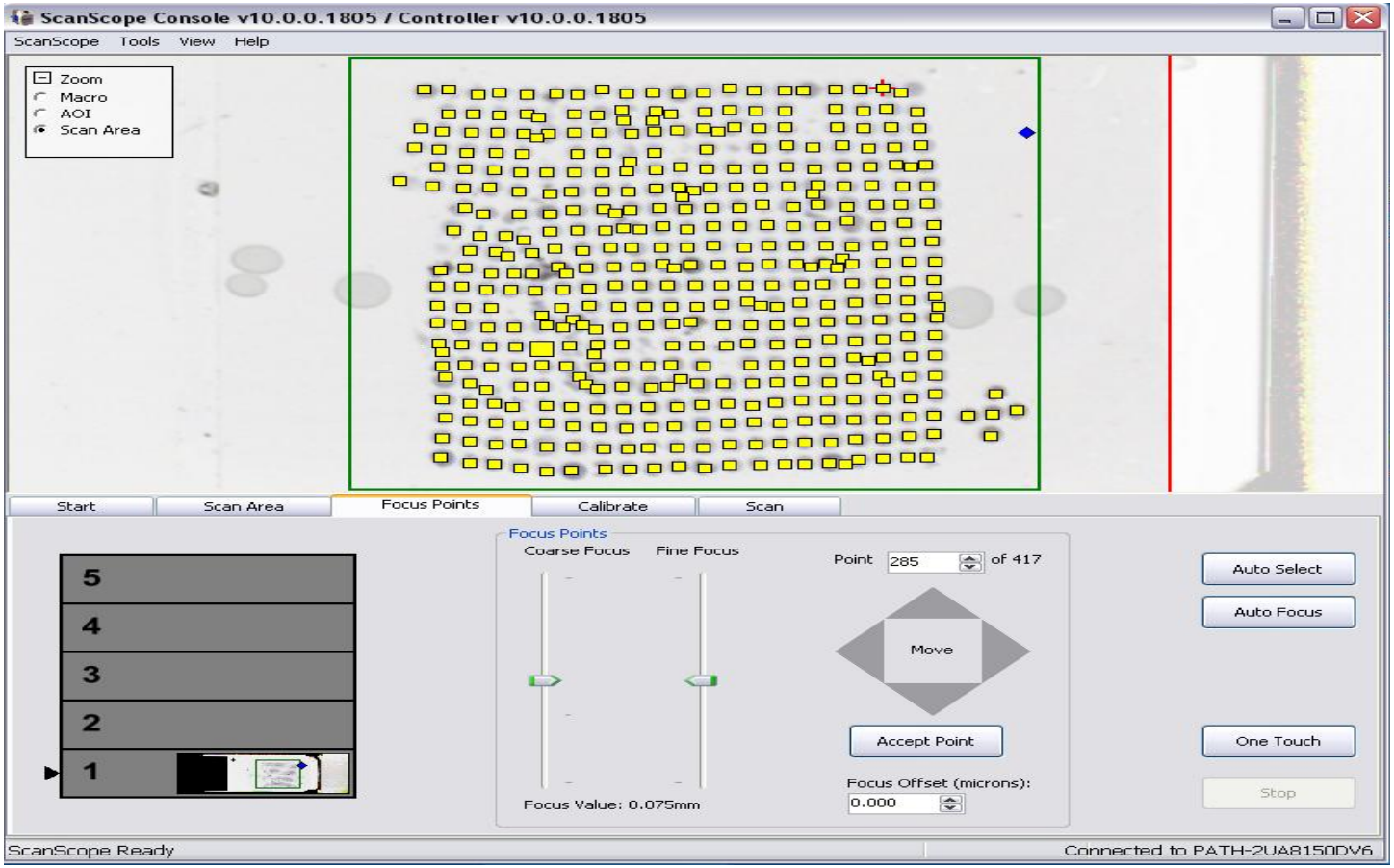
Scanning: Aperio



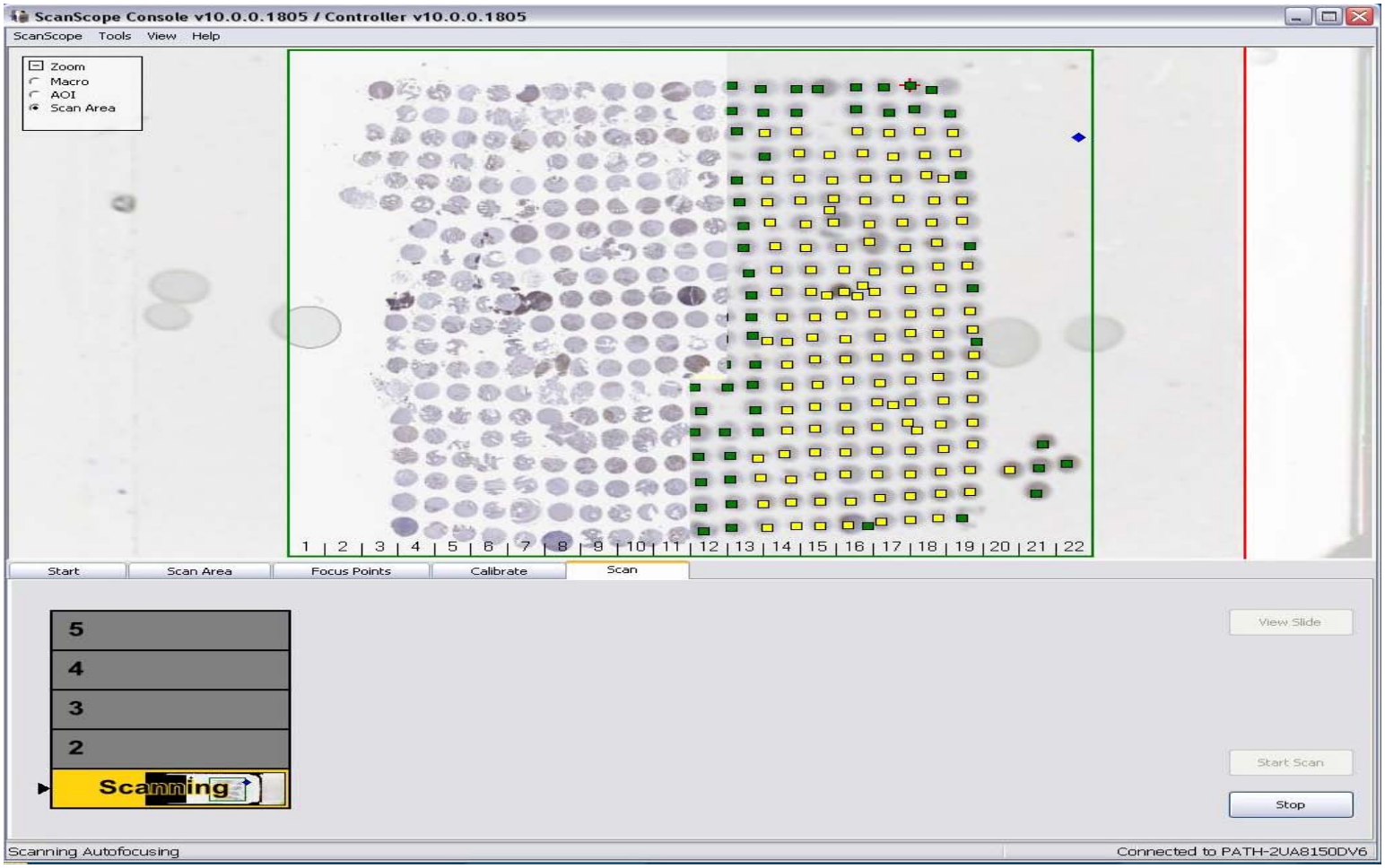
Scanning: Aperio



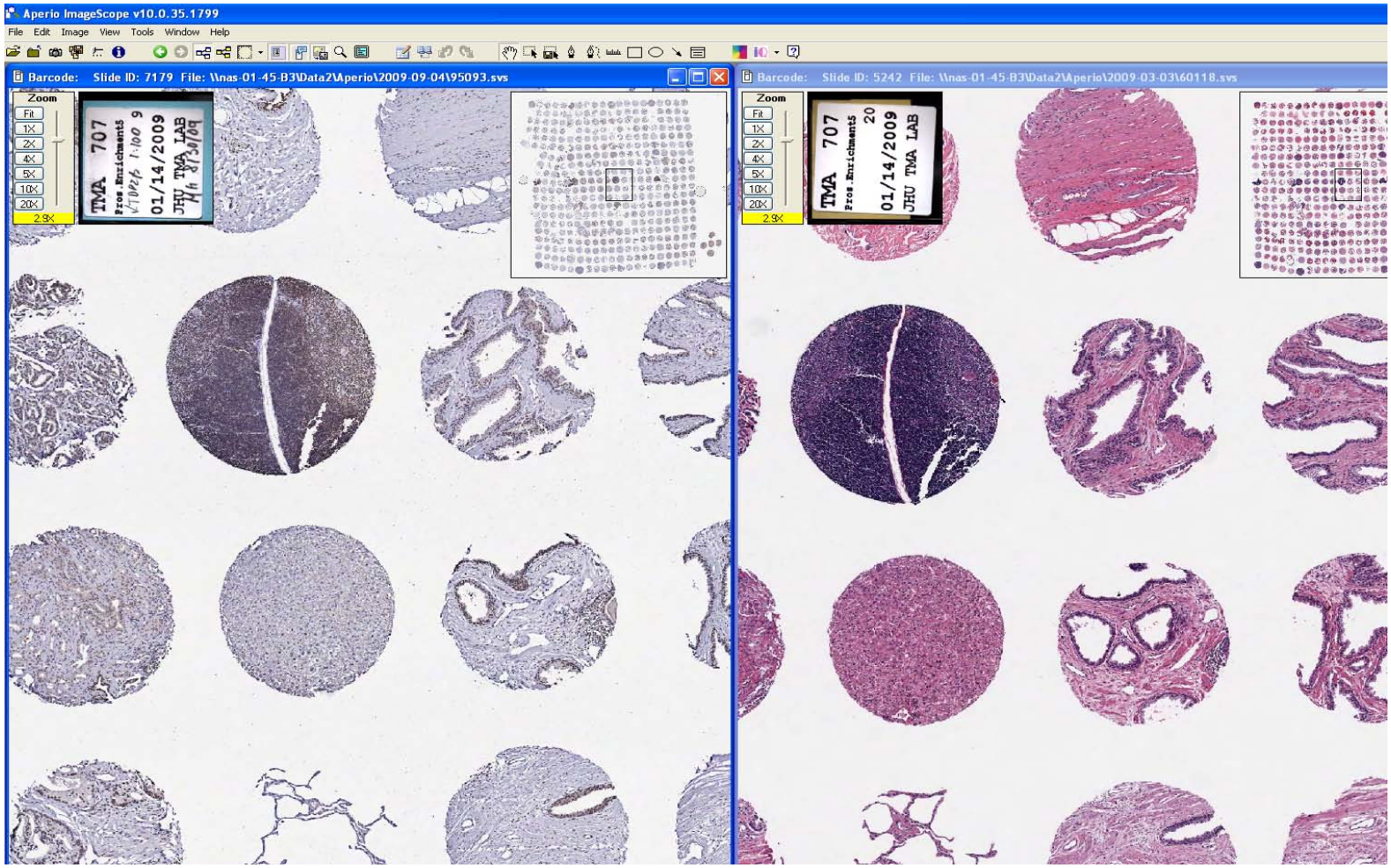
Scanning: Aperio



Scanning: Aperio



Viewing an Image: Aperio



Tissue Microarray – 400 cores, 1.5 mm each – stained with TOP2B on the left and H&E on the right - 4 μm section each

Spectrum™

User: **Lecksell, Kristen**, Role: **_SysAdmin**

Projects | Specimens | Digital Slides | TMA Blocks | Analysis | SecondSlide | Ad

Edit TMA Map

Sectors

Add Sector | Rows: 20

Delete Sector | Cols: 20

Center Spots | Spot Size: 1650

Auto Segment

Coordinate Labels

Row Labels: Numeric

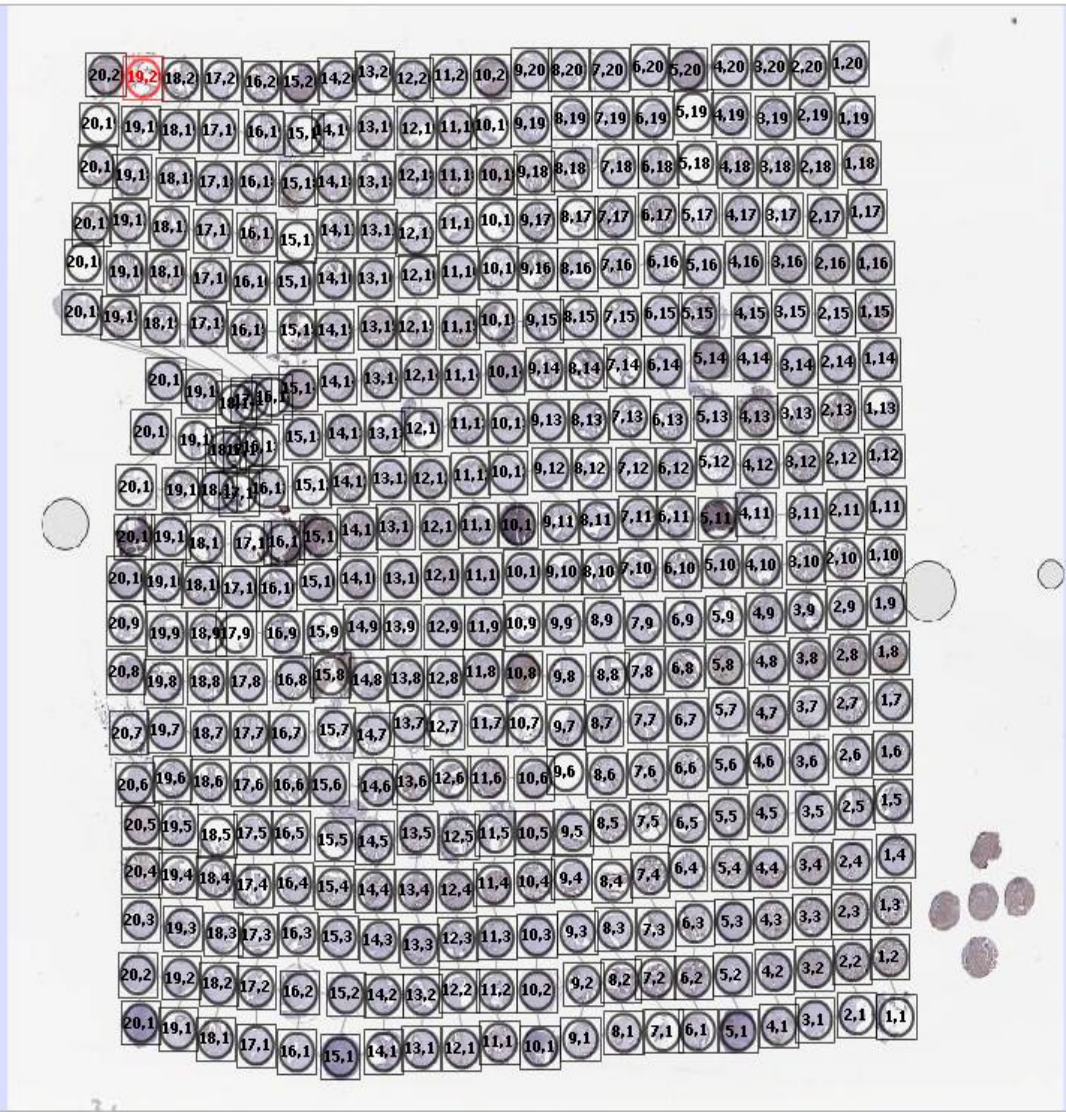
Col Labels: Numeric

Sector Labels: Alphabetic (upper case)

Reverse Rows

Reverse Columns

Submit | Cancel



TMA Composites

- Made by software that combines or stitches together the original multiple images for each spot to form one image/spot
- Contains some naming convention that identifies the X & Y coordinates of each spot

Pathologist-based Analysis

- Standard on IHC studies
- Semi-quantitative
- Qualitative
- Inter-observer variation
- Intra-observer variation
- Difficult to reproduce

Pathologist-based analysis of HER2 on HerceptTest

Score	Criteria
Weakly Positive: 2+	Weak to moderate complete membrane staining in >10% of tumor cells
Strongly Positive: 3+	Strong complete membrane staining in >10% of tumor cells

Image Analysis

"It is the extraction of meaningful information from images; mainly from digital images by means of digital image processing techniques. Image analysis tasks can be as simple as reading bar coded tags or as sophisticated as identifying a person from their face."

http://en.wikipedia.org/wiki/Image_analysis

Typical Image Analysis

- Includes determining where the edges of an object are, counting similar objects, calculating the area, perimeter length and other useful measurements of each object.
  - Counting and measuring anatomic structures
    - Nuclei
    - Microvessels
    - Lymphoid cells
  - Counting and measuring pixel color intensities

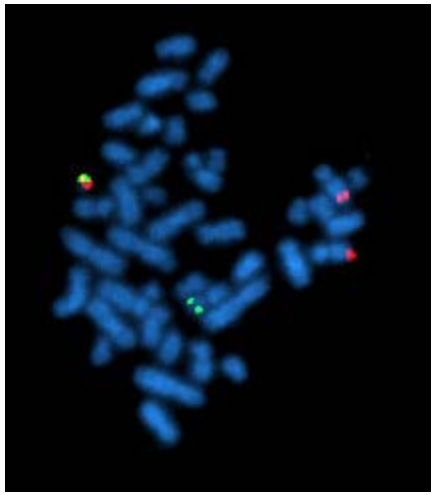
http://en.wikipedia.org/wiki/Microscope_image_processing

ImmunoHistoChemistry

- Chromagenic Dyes
  - Brown
  - Blue
  - Red
- Fluorescent Dyes
  - Red
  - Blue
  - Green

Fluorescence In Situ Hybridization

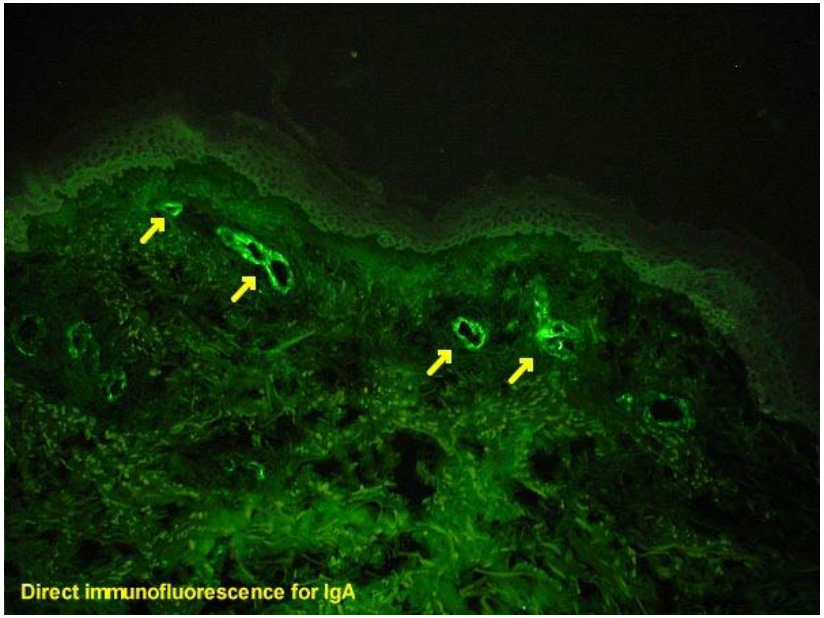
- Cytogenetic technique
- Detects and localizes the presence or absence of specific DNA sequences on chromosomes



http://en.wikipedia.org/wiki/Fluorescent_in_situ_hybridization

Immunofluorescence

- The labeling of antibodies or antigens with fluorescent dyes
- Used to visualize subcellular distribution of biomolecules



http://en.wikipedia.org/wiki/Immunofluorescence

Users of Image Analysis

- Oncology
- Pathology
- Toxicology within the pharmaceutical industry

Quantitative Image Analysis

- Image information on the pixels is quantified
  - Hue (Color) – stain color
  - Saturation (Color Purity) – amount of stain
  - Luminosity (Intensity) – specimen density

Why Use It

"The continuous staining intensity values provided by the quantitative image analysis allows for better discrimination of subtle protein expression differences, which may not be apparent in the pathologist categorical evaluation."

Alpha-Methylacyl-CoA Racemase Protein Expression Is Associated with the Degree of Differentiation In Breast Cancer Using Quantitative Image Analysis Witkiewics AK et. al. Caner Epi Biomarkers Prev, 2005, vol. 14, no.6

Publications

Research Article

Decreased NKX3.1 Protein Expression in Focal Prostatic Atrophy, Prostatic Intraepithelial Neoplasia, and Adenocarcinoma: Association with Gleason Score and Chromosome 8p Deletion

Carlise R. Bethel,[1] Dennis Faith,[4] Xiang Li,[2] Bin Guan,[2] Jessica L. Hicks,[3] Fusheng Lan,[5] Robert B. Jenkins,[5] Charles J. Bieberich,[2] and Angelo M. De Marzo[3]

[1]Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health; [2]Department of Biological Sciences, University of Maryland Baltimore County; [3]Departments of Pathology and Urology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, The Johns Hopkins University School of Medicine, Baltimore, Maryland; [4]The University of Minnesota School of Medicine, Minneapolis, Minnesota; and [5]The Mayo Clinic, Rochester, Minnesota

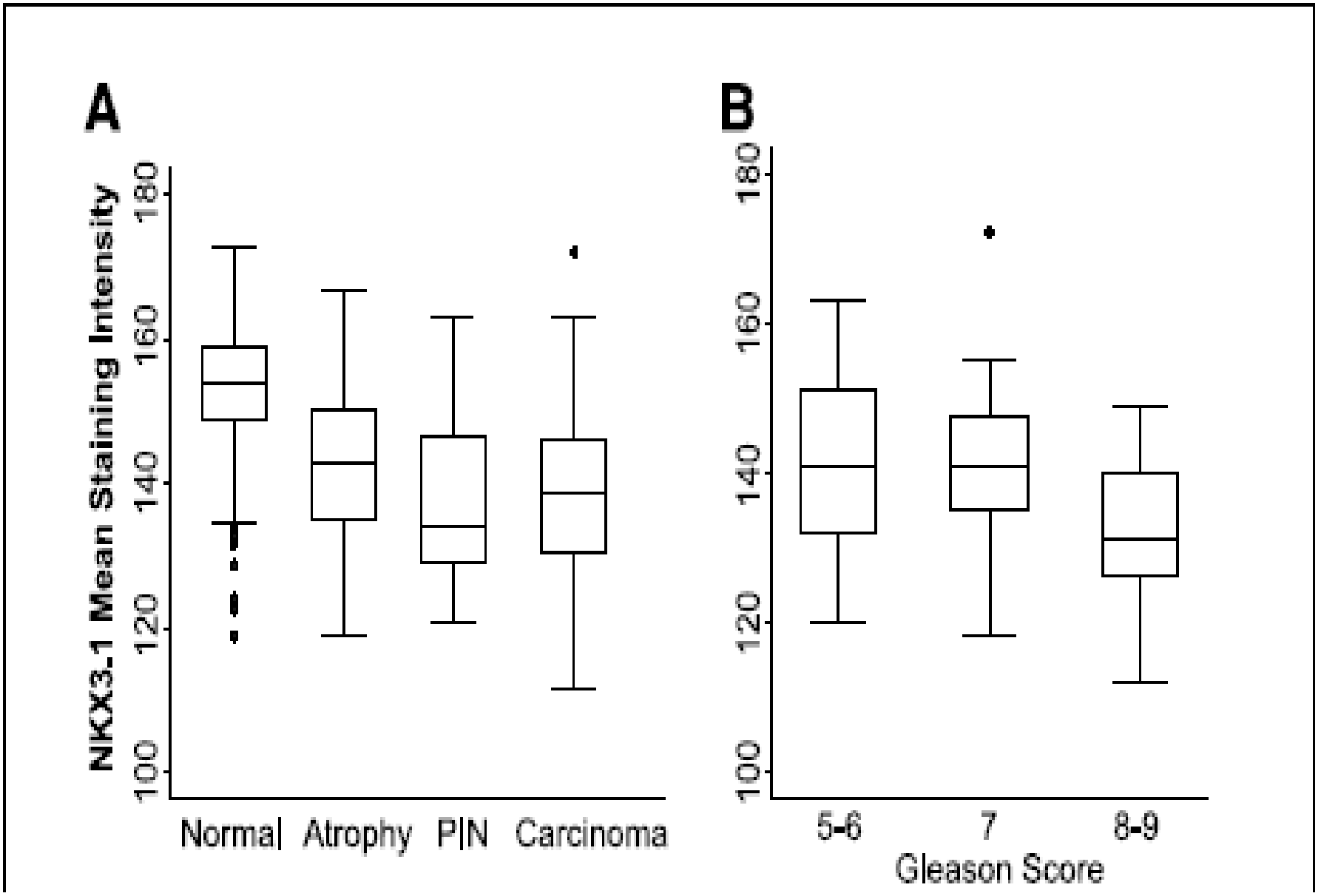
Abstract

NKX3.1 **is a homeobox gene located at chromosome 8p21.2, and one copy is frequently deleted in prostate carcinoma. Prior studies of *NKX3.1* mRNA and protein in human prostate cancer and prostatic intraepithelial neoplasia (PIN) have been conflicting, and expression in focal prostate atrophy lesions has not been investigated. Immunohistochemical staining for NKX3.1 on human tissue microarrays was decreased in most focal atrophy and PIN lesions. In carcinoma, staining was inversely correlated with Gleason grade. Fluorescence *in situ* hybridization showed that no cases of atrophy had loss or gain of 8p, 8 centromere, or 8q24 (*C-MYC*) and only 12% of high-grade PIN lesions harbored loss of 8p. By contrast, NKX3.1 staining in carcinoma was correlated with 8p loss and allelic loss was inversely related to Gleason pattern. Quantitative reverse transcription-PCR for *NKX3.1* mRNA using microdissected atrophy revealed a concordance with protein in five**

in approximately 50% to 85% of cases (2, 3). Given that mutations in the remaining allele of *NKX3.1* have not been detected (4, 5), *NKX3.1* may function as a haploinsufficient tumor suppressor gene. That loss of one allele of *NKX3.1* occurs early in prostate carcinogenesis is evidenced by the finding that LOH on chromosome 8p has been reported to occur in high-grade prostatic intraepithelial neoplasia (PIN), a lesion that is a putative precursor to many invasive prostatic carcinomas (6), at a frequency between 20% and 80% (7–9).

Targeted disruption of *Nkx3.1* in mice results in abnormal prostate ductal morphogenesis and protein secretion (10–12). Although *Nkx3.1* homozygous mutant mice do not develop invasive carcinoma, epithelial hyperplasia and PIN lesions arise with age. Compound mutant mouse studies indicate that cooperativity exists between *Nkx3.1* and the tumor suppressors *Pten* and *Cdkn1b* (encoding p27; refs. 13–17). These compound mutants develop PIN lesions that progress to invasive carcinomas and at times to metastatic disease. Because the effects are seen in *NKX3.1*

Cancer Res 2006, vol. 66, no. 22



Decreased NKX3.1 Protein Expression in Focal Prostatic Atrophy, Prostatice Intraepithelial Neoplasia, and Adenocarcinoma: Association with Gleason Score and Chromosome 8p Deletion: *Cancer Res* 2006, vol. 66, no. 22

The Prostate 61:215–227 (2004)

Trefoil Factor 3 Overexpression in Prostatic Carcinoma: Prognostic Importance Using Tissue Microarrays

Dennis A. Faith,[1] William B. Isaacs,[1,3] James D. Morgan,[2] Helen L. Fedor,[2] Jessica L. Hicks,[2] Leslie A. Mangold,[1] Patrick C. Walsh,[1] Alan W. Partin,[1] Elizabeth A. Platz,[4] Jun Luo,[1]** and Angelo M. De Marzo[1,2,3]*

[1]*Brady Urological Institute, The Johns Hopkins University, School of Medicine, Baltimore, Maryland*
[2]*Department of Pathology, The Johns Hopkins University, School of Medicine, Baltimore, Maryland*
[3]*The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University, School of Medicine, Baltimore, Maryland*
[4]*Department of Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, Maryland*

BACKGROUND. Human intestinal trefoil factor 3 (TFF3) is a member of a family of polypeptides encoded by a cluster of genes on chromosome 21. Through gene expression profiling studies TFF3 mRNA has been found to be overexpressed in prostate cancer.

METHODS. We used immunochemistry on tissue microarrays and software tools, collectively

Use of Human Vascular Tissue Microarrays for Measurement of Advanced Glycation Endproducts

Marc K. Halushka, Elizabeth Selvin, Jie Lu, Anne M. Macgregor and Toby C. Cornish

Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland (MKH,JL,AMM,TCC), and Departments of Epidemiology and Medicine, Johns Hopkins Bloomberg School of Public Health and the Johns Hopkins University School of Medicine, Baltimore, Maryland (ES)

Correspondence to: Marc K. Halushka, MD, PhD, Ross Building, RM 632L, 720 Rutland Avenue, Baltimore, MD 21205. E-mail: mhalush1@jhmi.edu

Advanced glycation endproducts (AGEs) are present in the vasculature and are associated with vascular disease. We determined levels of AGEs in eight distinct adult vascular tissues using tissue microarray (TMA) technology and associated these levels with clinical characteristics. Medium-to-large caliber blood vessels were harvested from 100 adult autopsies to create 17 TMAs. AGE levels were evaluated by IHC using a polyclonal anti-AGE antibody on over 700 unique blood vessels. Slides were digitally scanned, and quantitative analysis was performed using a color deconvolution image analysis technique. Medial AGE staining was strongly correlated between all eight blood vessels. In the media, AGE staining levels were significantly higher at older ages (p=0.009), in white subjects (p<0.001) and with longer postmortem interval (PMI; p<0.0001). These associations remained significant after simultaneous adjustment for age, race/ethnicity, PMI, and diabetes status. Diabetes was associated with elevated AGE levels but only after adjustment for confounding by clinical variables including race/ethnicity, hypertension, and kidney function. This extensive vascular study shows that AGE accumulation in the macrovasculature is a global process affecting atherosclerosis-prone and -resistant vessels. It also suggests ethnicity has a previously undescribed role in vascular tissue AGE levels. This manuscript contains online supplemental material at http://www.jhc.org. Please visit this article online to view these materials. **(J Histochem Cytochem 57:559–566, 2009)**

α-Methylacyl-CoA Racemase Protein Expression Is Associated with the Degree of Differentiation in Breast Cancer Using Quantitative Image Analysis

Agnieszka K. Witkiewicz,[1] Sooryanarayana Varambally,[2] Ronglai Shen,[4] Rohit Mehra,[2] Michael S. Sabel,[3,5] Debashis Ghosh,[4] Arul M. Chinnaiyan,[2,5] Mark A. Rubin,[1] and Celina G. Kleer[2,5]

[1]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; Departments of [2]Pathology, [3]Surgery, and [4]Biostatistics; and [5]Comprehensive Cancer Center, University of Michigan, Ann Arbor, Michigan

Abstract

α-Methylacyl-CoA racemase (AMACR) is an enzyme involved in the metabolism of fatty acids and is an important tissue biomarker in the prostate to distinguish normal glands from prostate cancer. Here, for the first time, we evaluated the expression of AMACR protein in normal breast, ductal carcinoma *in situ*, and invasive carcinomas. By immunofluorescence and immunohistochemistry, AMACR was seen in cytoplasmic granules consistent with a mitochondrial and peroxisomal localization. AMACR expression was determined by immunohistochemistry on 160 invasive carcinomas with long follow-up, using a high-density tissue microarray, and evaluated by two methods: standard pathology review and quantitative image analysis. AMACR was overexpressed in 42 of 160 (26%) invasive carcinomas, and it was associated with a decrease in tumor differentiation, a feature of aggressive breast cancer. Quantitative analysis allowed for better discrimination and more accurate evaluation of low-intensity staining. In conclusion, AMACR protein is expressed in normal breast and its expression seems to increase in invasive carcinomas. We observed stronger AMACR protein expression in high-grade carcinomas when compared with low-grade ones. Quantitative image analysis is a novel way to accurately and reproducibly evaluate immunohistochemistry in breast tissue samples using high-density tissue microarrays. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1418–23)

Introduction

Epidemiologic studies show that red meat and diary products, which are both sources of branched chain fatty acids, are associated with breast cancer risk. Case control studies from geographically disparate areas have found a significant positive association between the intake of meat, red meat, and high-fat meat and the risk of developing breast cancer (1-3). Breast cancer incidence is associated with per capita fat consumption and interestingly these rates change cancer, very little is known about AMACR expression in normal breast and in breast cancer.

At this time, pathologist-based analysis is the current standard for the evaluation of immunohistochemistry; however, because of its semiquantitative nature, it is at times difficult to reproduce by different observers. For example, the interpretation of HER-2/neu by immunohistochemistry using the Hercept test varies among pathologists, raising doubts

Automated subcellular localization and quantification of protein expression in tissue microarrays

ROBERT L. CAMP, GINA G. CHUNG & DAVID L. RIMM
Department of Pathology, Yale University School of Medicine, New Haven, Connecticut, USA
Correspondence should be addressed to D.L.R.; email: david.rimm@yale.edu

Published online 21 October 2002; doi:10.1038/nm791

The recent development of tissue microarrays—composed of hundreds of tissue sections from different tumors arrayed on a single glass slide—facilitates rapid evaluation of large-scale outcome studies. Realization of this potential depends on the ability to rapidly and precisely quantify the protein expression within each tissue spot. We have developed a set of algorithms that allow the rapid, automated, continuous and quantitative analysis of tissue microarrays, including the separation of tumor from stromal elements and the sub-cellular localization of signals. Validation studies using estrogen receptor in breast carcinoma show that automated analysis matches or exceeds the results of conventional pathologist-based scoring. Automated analysis and sub-cellular localization of beta-catenin in colon cancer identifies two novel, prognostically significant tumor subsets, not detected by traditional pathologist-based scoring. Development of automated analysis technology empowers tissue microarrays for use in discovery-type experiments (more typical of cDNA microarray

ment for compartmentalization of expression) utilizes fluorescent tags to separate tumors from stroma and to define subcellular compartments. The distribution of a target antigen is then quantitatively assessed according to its co-localization with these tags. As subcellular compartments (for example, membrane, cytoplasm, nuclei and so forth) of different tissues and tumors vary widely in size and shape, traditional methods of defining compartments based on morphometric criteria (that is, feature extraction) perform poorly on a large-scale basis. Rather than counting target-containing features, PLACE delineates target expression as the sum of its intensity divided by the total size of the assayed compartment.

As the thickness of tissue sections makes it difficult to discriminate between overlapping subcellular compartments, we have also developed a novel, rapid exponential subtraction algorithm (RESA), which subtracts an out-of-focus image, collected slightly below the bottom of the tissue, from an in-focus image, based on pixel intensity, signal-to-noise ratio, and the

Quantitative Image Analysis Data

Region	Label	Brown Area	Intensity	Blue Area	Percentage	Region Score	Range	Region Area	Average Brown Intensity	Row	Col
73 -->	A y_1 x_1	13,887	131.00	679,681	2.00	2.59	0	3,360,516.00	131	0	8
64 -->	A y_1 x_2	2,340	127.00	508,384	0.46	2.49	0	4,091,863.00	127	0	7
55 -->	A y_1 x_3	4,630	137.00	636,202	0.72	2.73	0	4,443,115.00	137	0	6
46 -->	A y_1 x_4	2,248	128.00	372,074	0.60	2.52	0	4,680,475.00	128	0	5
37 -->	A y_1 x_5	1,455	131.00	561,304	0.26	2.59	0	4,222,184.00	131	0	4
28 -->	A y_1 x_6	5,080	136.00	243,279	2.05	2.71	0	3,732,823.00	136	0	3
19 -->	A y_1 x_7	2,183	129.00	136,396	1.58	2.54	0	3,617,851.00	129	0	2
10 -->	A y_1 x_8	3,115	124.00	456,341	0.68	2.42	0	3,839,037.00	124	0	1
1 -->	A y_1 x_9	14,488	141.00	307,735	4.50	2.83	0	3,512,177.00	141	0	0
74 -->	A y_2 x_1	21,641	142.00	78,344	21.64	2.85	0	3,226,102.00	142	1	8
65 -->	A y_2 x_2	5,624	133.00	691,468	0.81	2.64	0	3,990,782.00	133	1	7
56 -->	A y_2 x_3	1,952	134.00	788,298	0.25	2.66	0	3,816,783.00	134	1	6
47 -->	A y_2 x_4	1,762	130.00	397,287	0.44	2.56	0	3,416,594.00	130	1	5
38 -->	A y_2 x_5	3,089	132.00	526,069	0.58	2.61	0	3,685,898.00	132	1	4
29 -->	A y_2 x_6	2,843	129.00	741,022	0.38	2.54	0	4,264,986.00	129	1	3
20 -->	A y_2 x_7	3,512	126.00	413,834	0.84	2.47	0	3,850,962.00	126	1	2
11 -->	A y_2 x_8	2,227	131.00	382,420	0.58	2.59	0	3,714,733.00	131	1	1
2 -->	A y_2 x_9	2,982	130.00	438,740	0.68	2.56	0	3,557,805.00	130	1	0
75 -->	A y_3 x_1	26,783	145.00	378,032	6.62	2.92	0	3,743,370.00	145	2	8
66 -->	A y_3 x_2	13,145	130.00	350,378	3.62	2.56	0	4,004,523.00	130	2	7
57 -->	A y_3 x_3	4,469	128.00	235,968	1.86	2.52	0	3,332,755.00	128	2	6
48 -->	A y_3 x_4	6,607	131.00	246,994	2.61	2.59	0	3,879,280.00	131	2	5
39 -->	A y_3 x_5	9,497	134.00	395,068	2.35	2.66	0	3,814,716.00	134	2	4
30 -->	A y_3 x_6	2,518	126.00	334,253	0.75	2.47	0	4,141,314.00	126	2	3
21 -->	A y_3 x_7	537	132.00	66,964	0.80	2.61	0	4,295,700.00	132	2	2
12 -->	A y_3 x_8	5,265	122.00	634,025	0.82	2.37	0	4,222,104.00	122	2	1

Other Uses of Scanning:

- Telepathology
  - Diagnosis
  - Consultation
- Education

Publishing TMA Images and Scoring Data Over the Internet

- Roughly modeled after Stanford Microarray Database
- Concept:
  - Once a study is published by a journal, all TMA diagnoses, image, scoring and non-protected clinical data can be “published” as supplemental data to the Internet for public online viewing or down loading
  - In addition, some TMAJ Images now linked to “Proteinpedia” database

  (http://humanproteinpedia.org) by Akhilesh Pandy, MD PhD.

Im

#599 Session, ArraySlideID#477 (TMA#358, Cut#2, Stain-PTEN)

Options

ScoredImageID	Primary_Histologic_Type	Secondary_Histologic_Type	Percent_Positive	Percent_Strong	Nc
	Normal Prostatic Epithelium		100	80	

Type: Prostate -- Normal/Other

Back | Up | Down | Next

X: 12 Y: 1

Accept

Draw | Save | Link | Info | Large

Projects

Open Sessio

Recent Sess

#5 Session,

#7 Session,

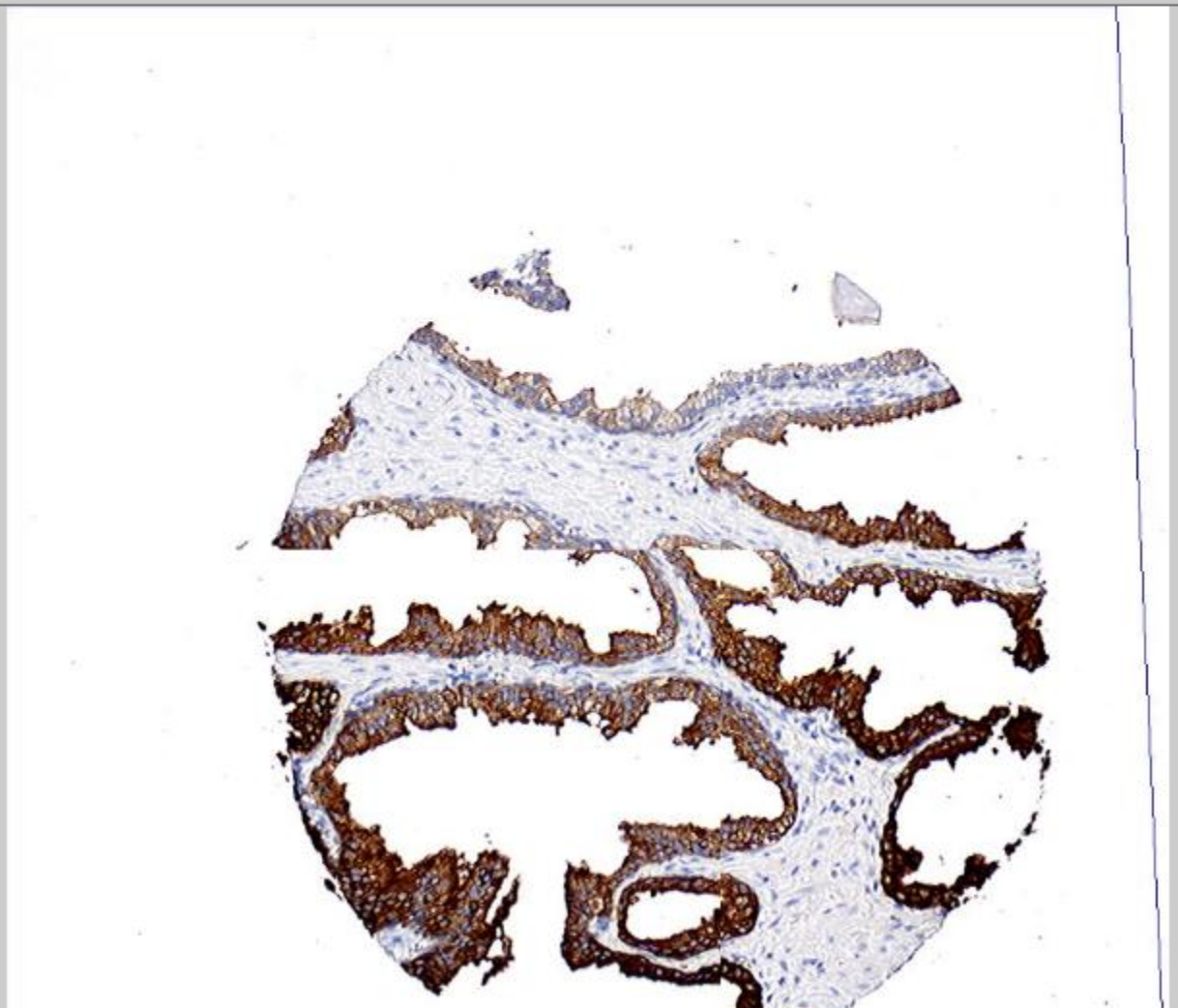
#46 Session

All Sessions

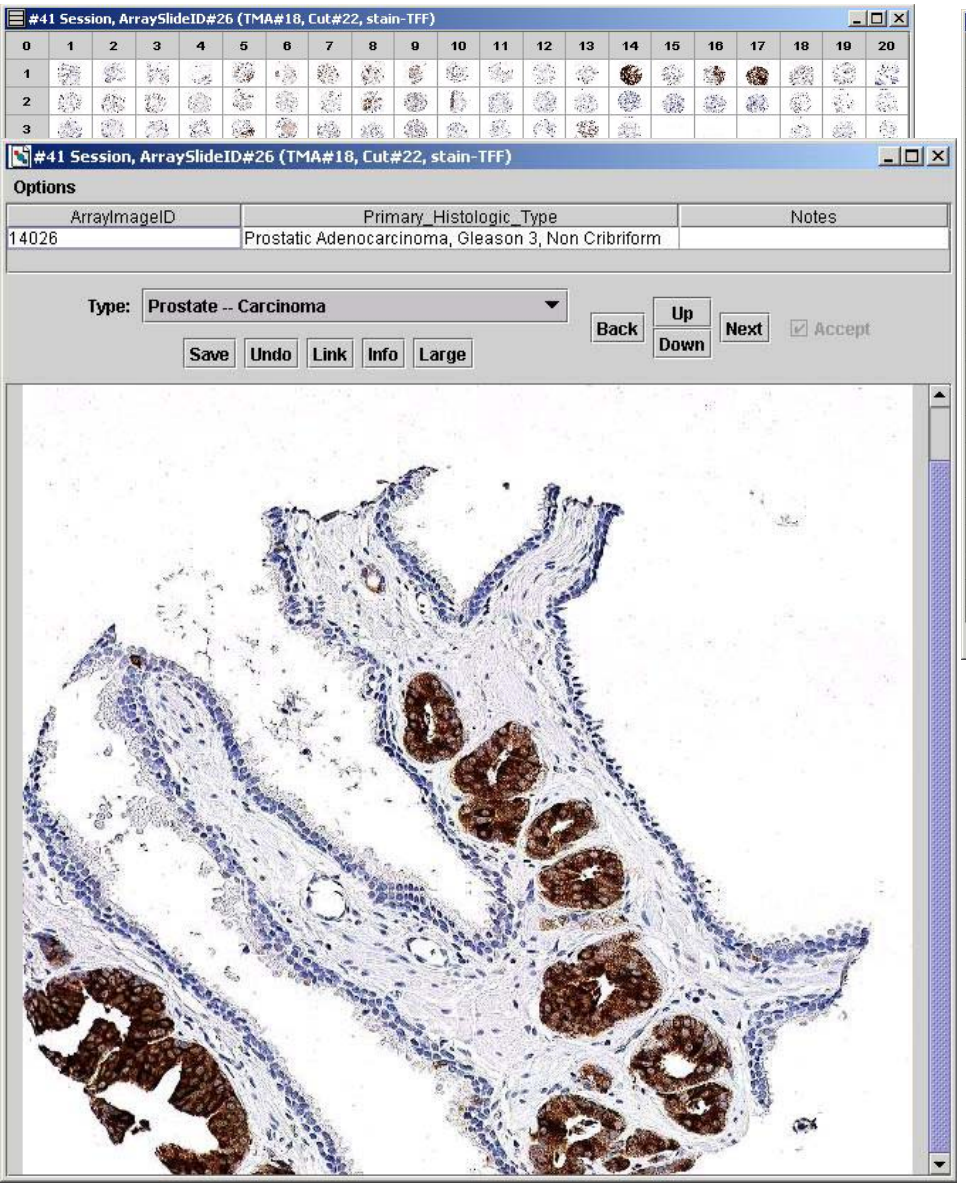
My Proje

Shared S

Publishe



Images Application: Viewing 2 Stains



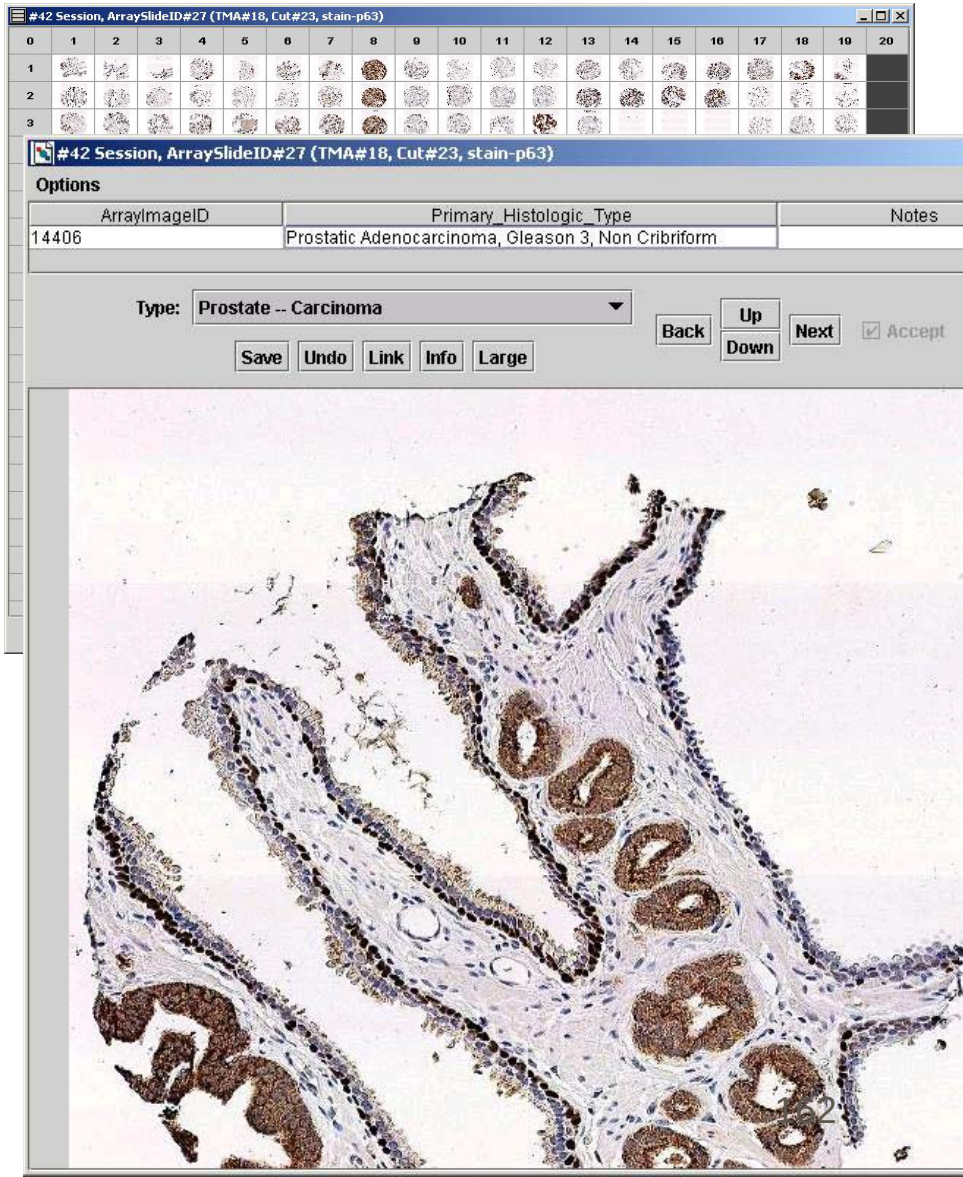


Image Application: Filtering

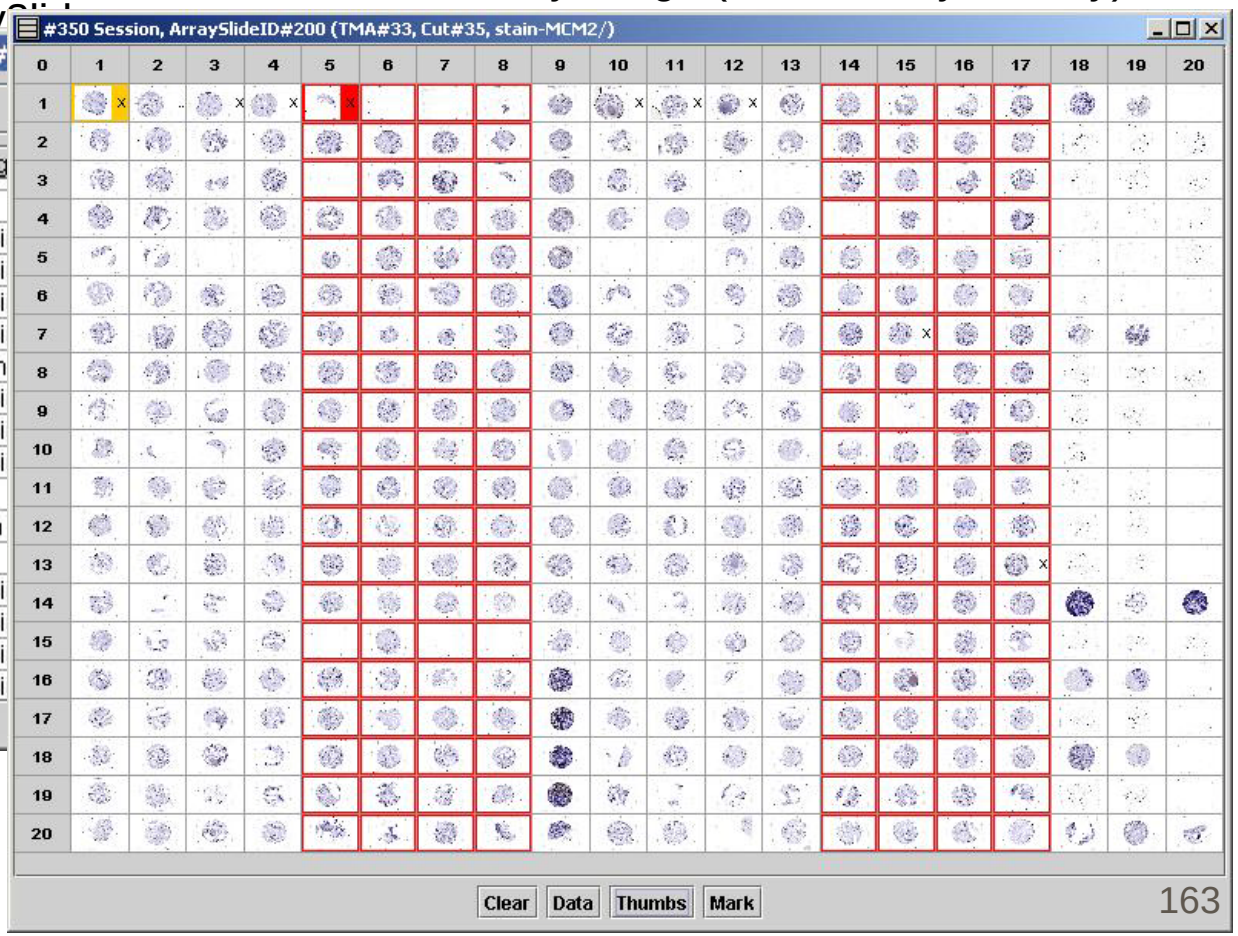
- The table shows information about every image (identified by x and y) in an Array[illegible]

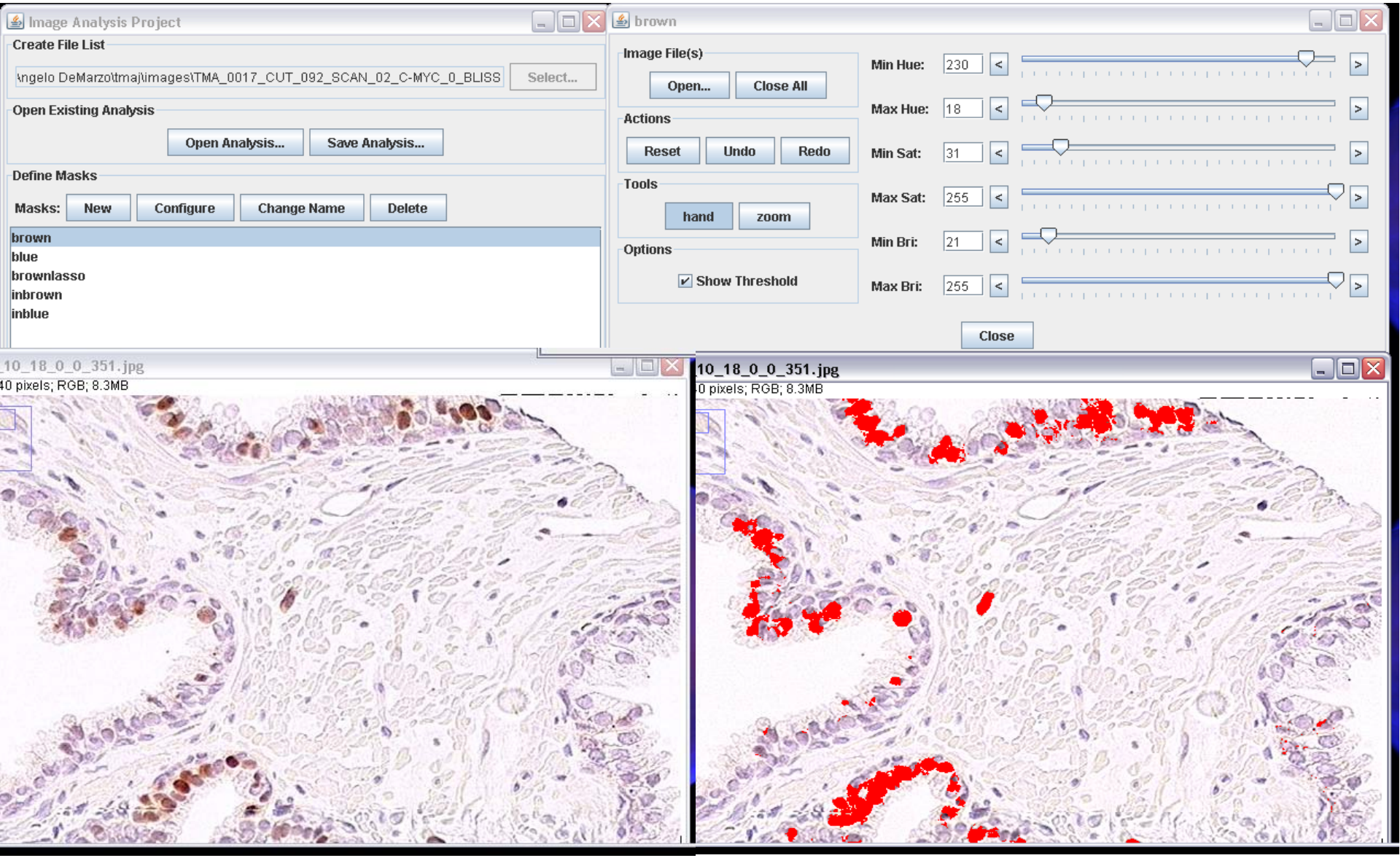
#3 Session, ArraySlideID#3 (TMA#18, Cut#

Remove

X	Y	Control	Primary_Histolog
9	17	tonsil	Not Specified
10	17		Normal Prostatic Epitheli
11	17		Normal Prostatic Epitheli
12	17		Normal Prostatic Epitheli
13	17		Normal Prostatic Epitheli
14	17		Prostatic Adenocarcinom
15	17		Normal Prostatic Epitheli
16	17		Normal Prostatic Epitheli
17	17		Normal Prostatic Epitheli
18	17	vas	Not Specified
19	17	vas	Normal Prostatic Stroma
20	17	brain	Not Specified
1	18		Normal Prostatic Epitheli
2	18		Normal Prostatic Epitheli
3	18		Normal Prostatic Epitheli
4	18		Normal Prostatic Epitheli

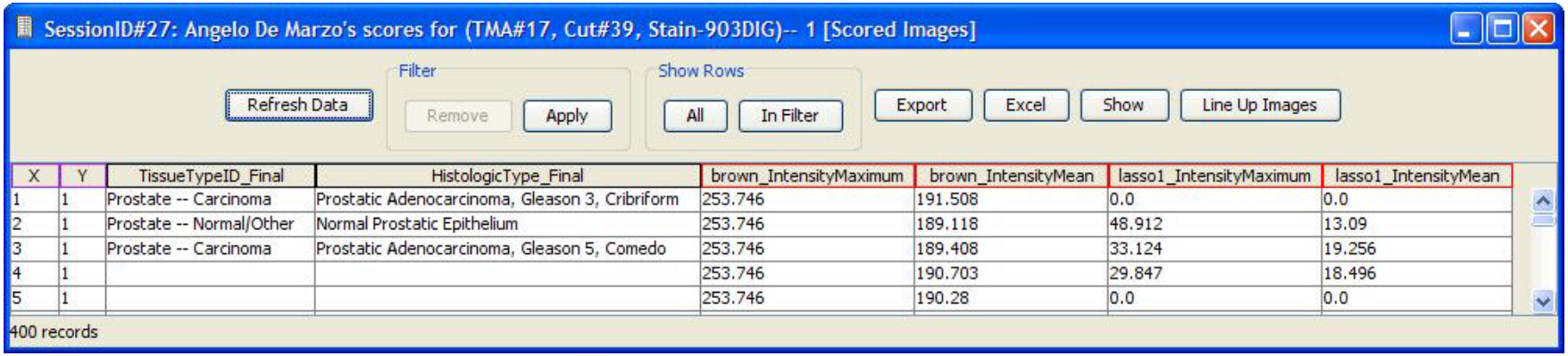
399 records





T. Cornish, MD PhD, J. Morgan – Frida

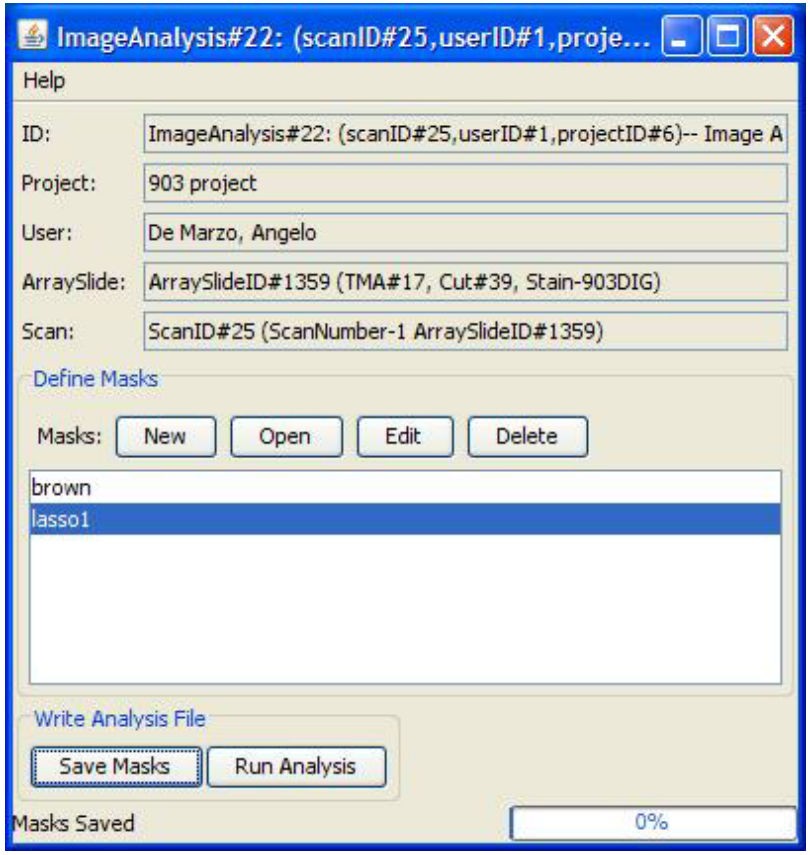
Viewing Image Analysis Results



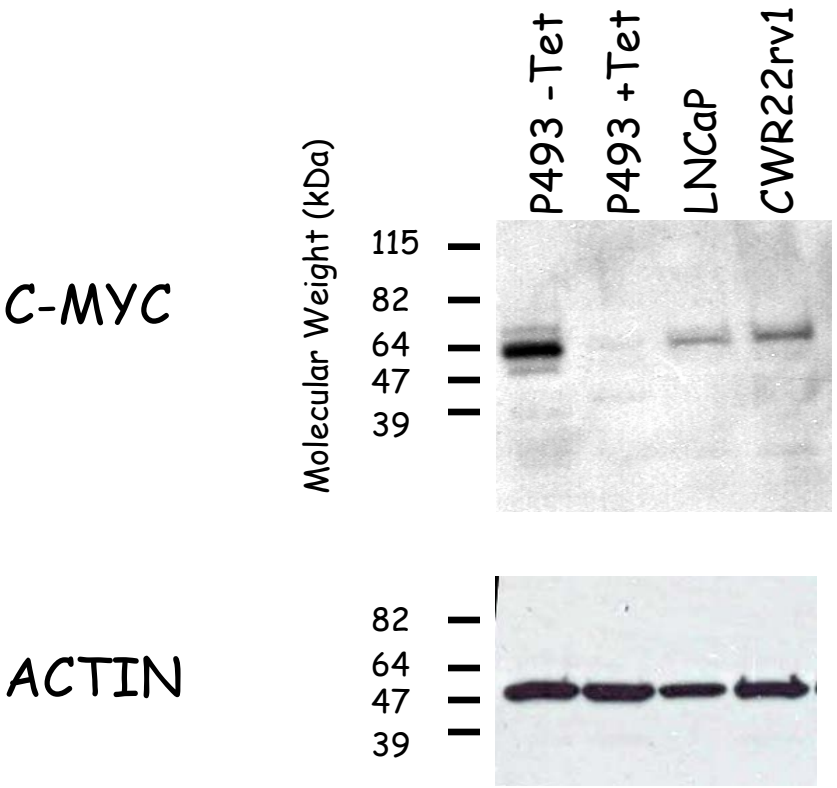
- Image Analysis Results may be viewed side-by-side with a regular scoring session

TMAJ & Frida Integration

- The image analysis software package Frida has been integrated with TMAJ

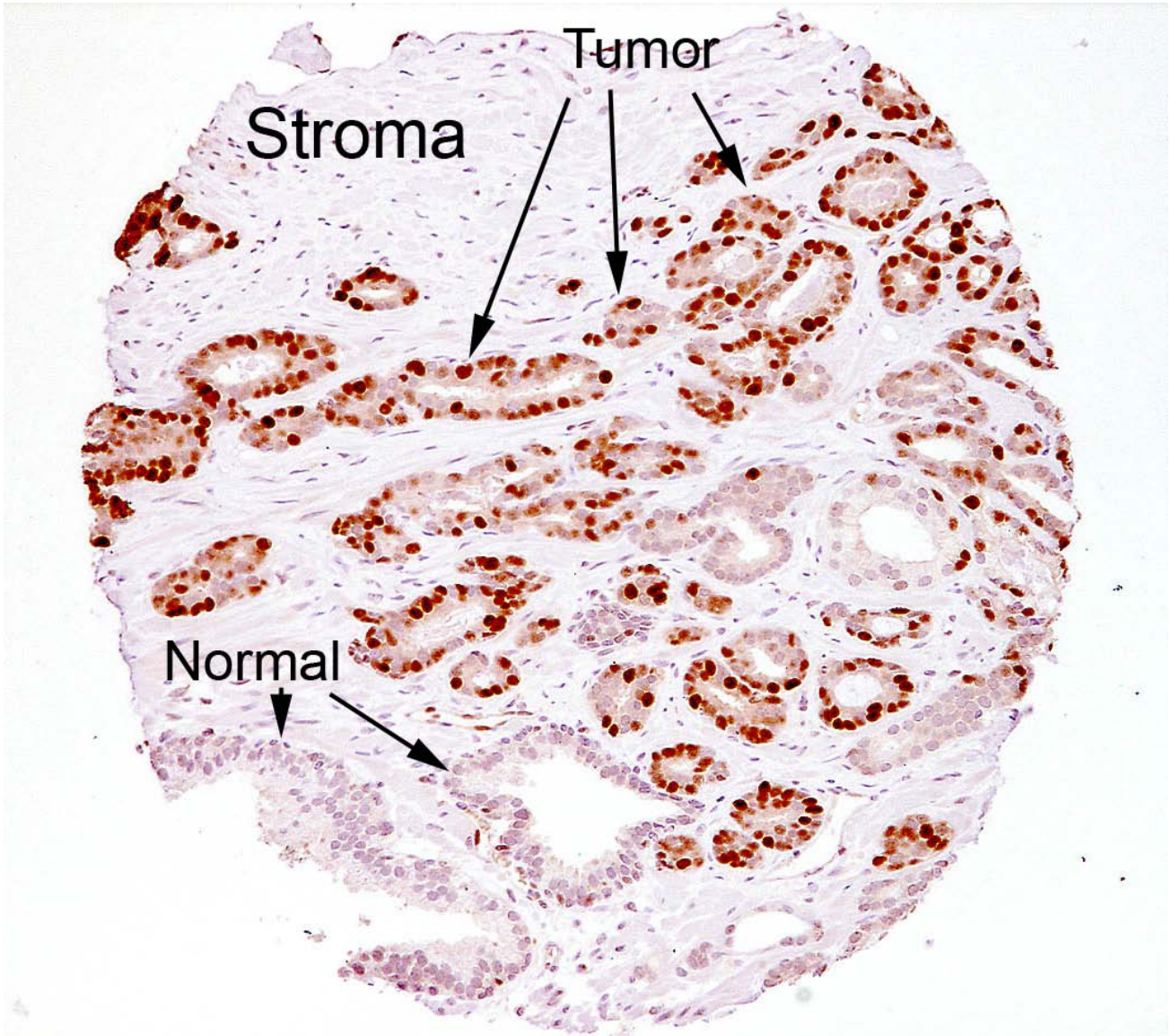


MYC Western



C. Koh, Unpublished

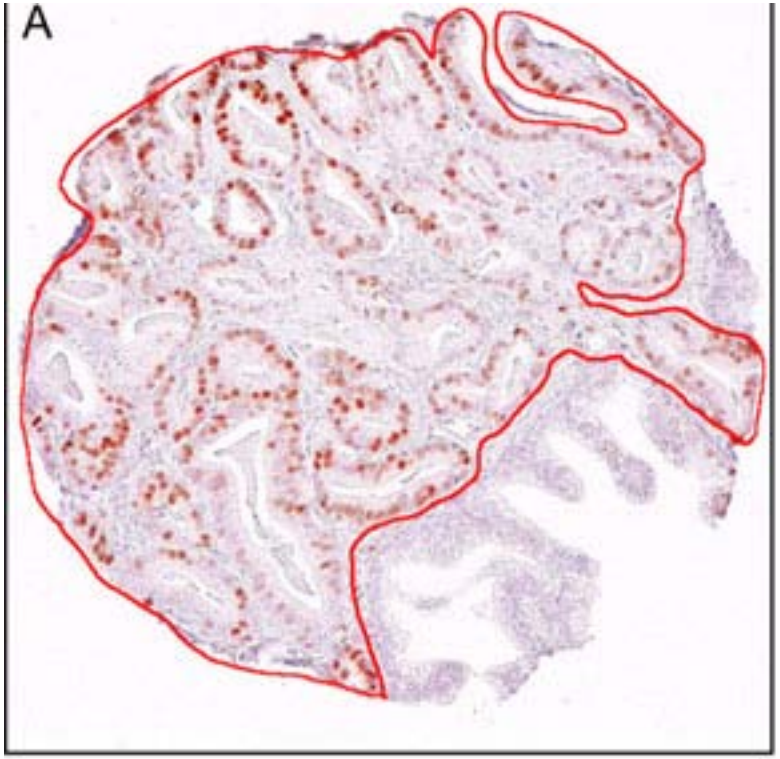
C-MYC Staining in Human Prostate Tissues



B. Gurel, T. Iwata, C. Koh, et al, AM De Marzo, Modern Path, 2008 In Press

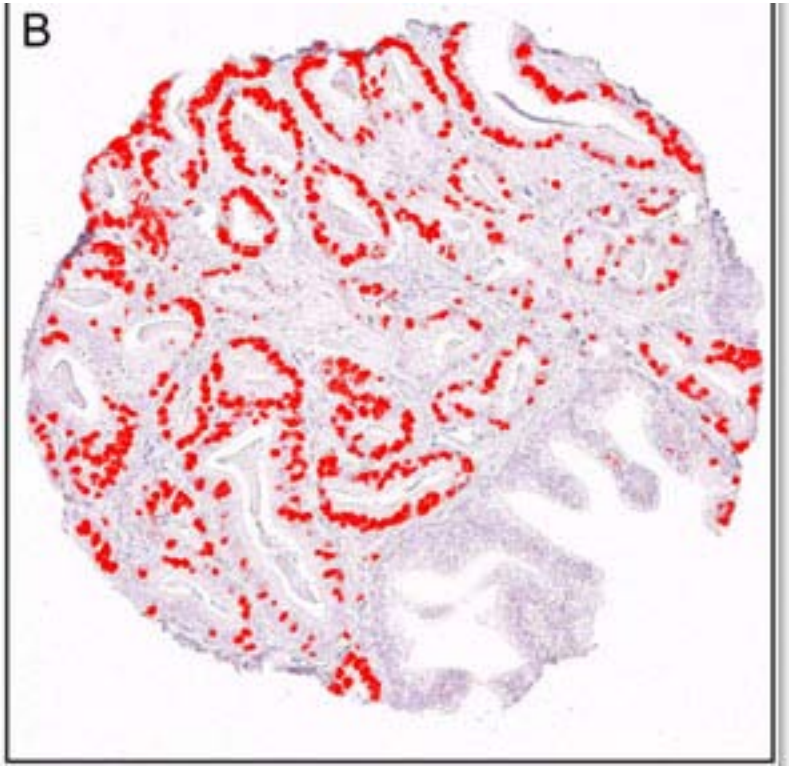
The FRIDA image analysis process

A. Creation of the “Lasso” mask, enabling the user to disregard the benign prostatic epithelium in the TMA spot.

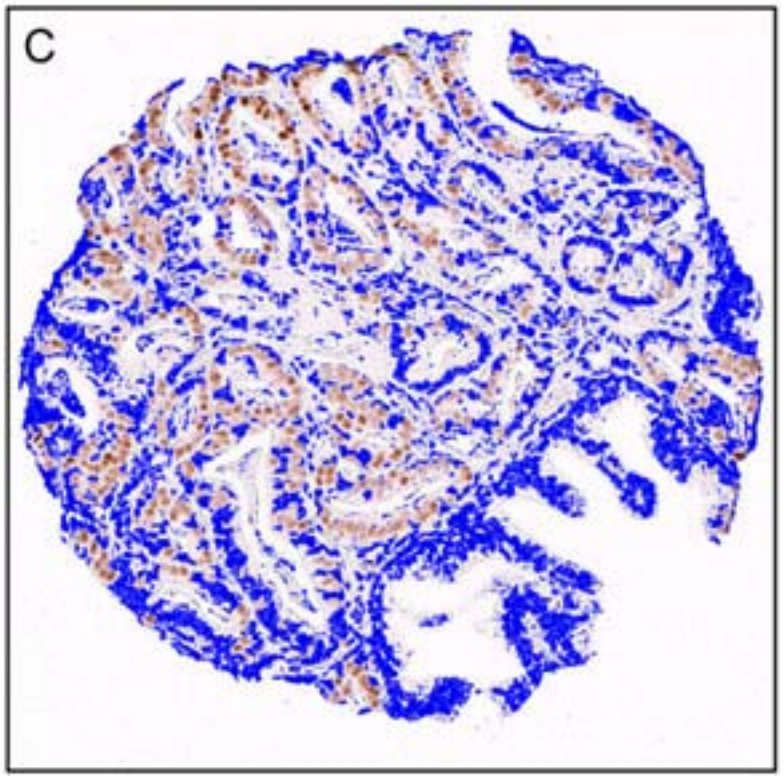


The FRIDA image analysis process

B.“Brown” and “Blue” color masks, differentiating the brown DAB stain and the blue hematoxylin stain, respectively.



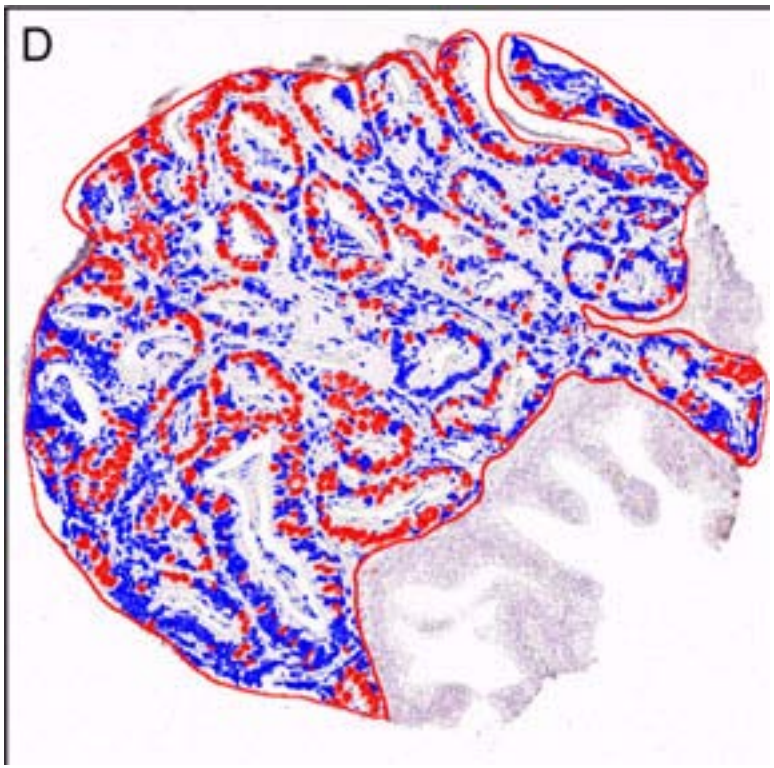
The FRIDA image analysis process



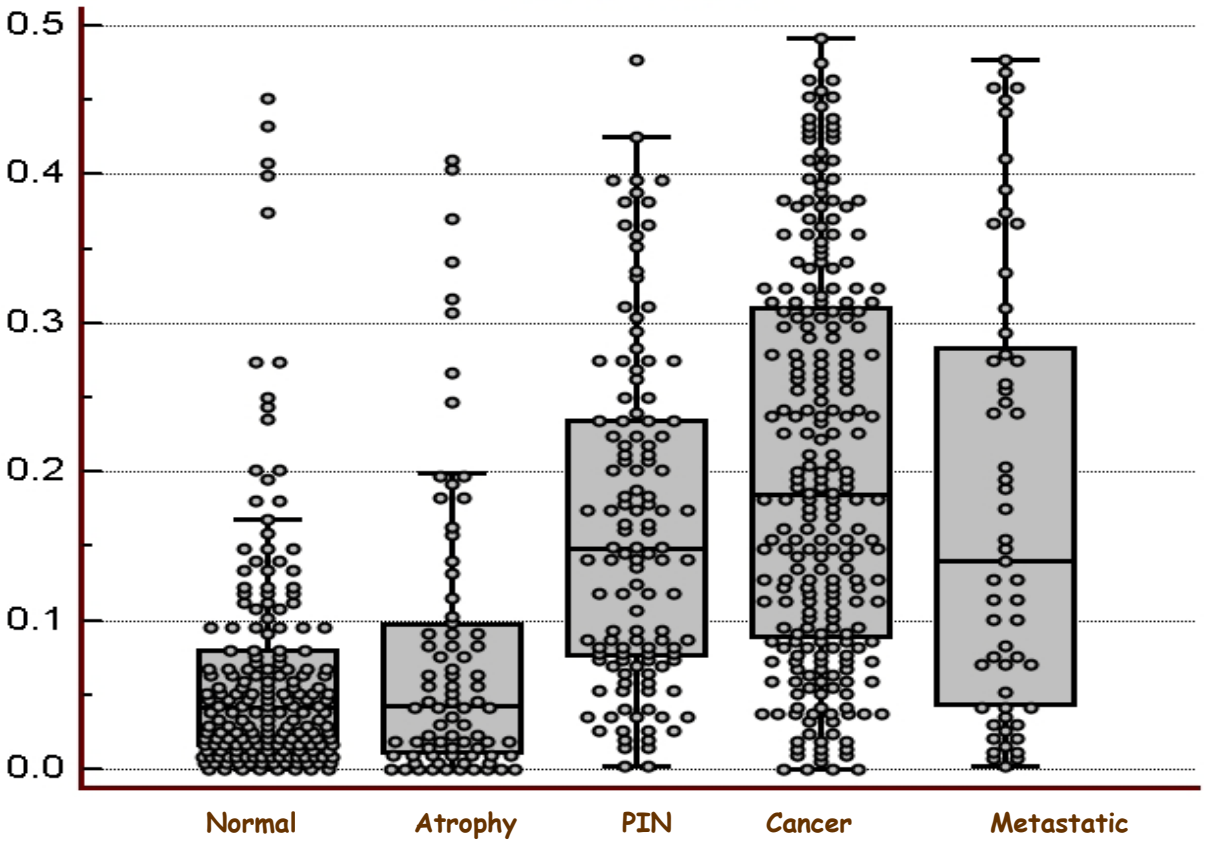
The FRIDA image analysis process

D. The “Meta-Mask” created by combining the lasso and color masks using Boolean logic.

“and”, “or”, “not”



C-MYC Staining



B. Gurel, T. Iwata, C. Koh, et al, AM De Marzo, Modern Path, 2008 In Press

ebiotechnology

Infrared spectroscopic imaging for histopathologic recognition

Daniel C Fernandez[1,3,4], Rohit Bhargava[1,4], Stephen M Hewitt[2] & Ira W Levin[1]

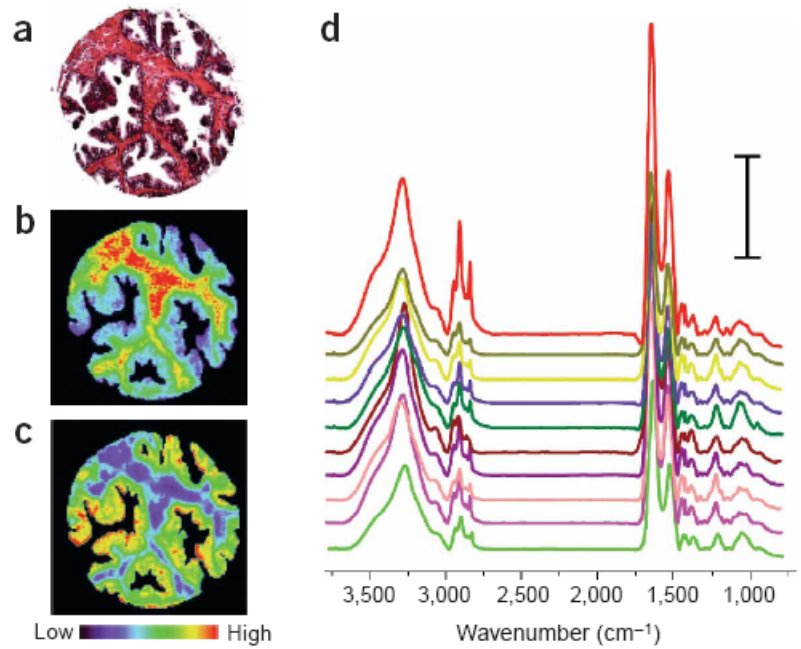


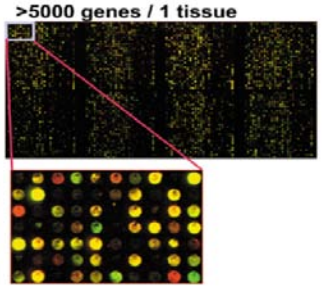
Figure 1 Chemical staining and infrared spectroscopic characterization of prostate tissue. (**a**) H&E-stained biopsy section of human prostate tissue showing epithelial cells defining the lumen (top). (**b**,**c**) The relative protein (middle, **b**) and phosphodiester (bottom, **c**) concentrations were determined, without the use of external reagents, from infrared spectral absorbance at 1,545 cm^{-1} and 1,080 cm^{-1}, respectively, from a corresponding, unstained section, demonstrating noninvasive, nondestructive tissue segmentation similar to that achieved by stains. Absorbance intensities are indicated by the color bar at the bottom. (**d**) Characteristic infrared absorbance spectra of ten histologic classes comprising prostate tissue are (from bottom to top) from normal epithelium, fibrous stroma, mixed stroma, muscle, nerve, lymphocytes, stone, ganglion, endothelium and blood. The bar indicates an absorbance of 0.2 absorbance units.

characteristic spectral pattern descriptors that establish differences between specific cell types, and term these spectral features 'metrics' (**Supplementary Table 1** online). For example, the ratio of absorbance of the phosphodiester-specific spectral peak at 1,080 cm^{-1} to the amide II vibration-specific absorbance at 1,545 cm^{-1} provides a definitive metric for discriminating epithelial cells from stromal cells. In contrast to the morphologic identification of specific cell

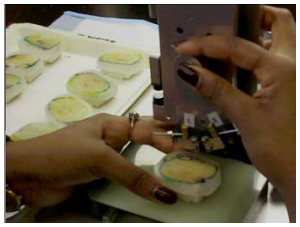
nature.com/naturebiotechnology

Nature Biotechnology, 23: 469-474, 2005

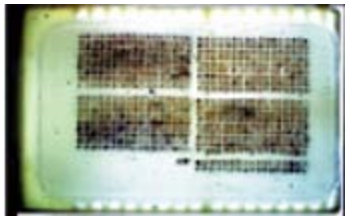
Recap



gene discovery



tissue microarray



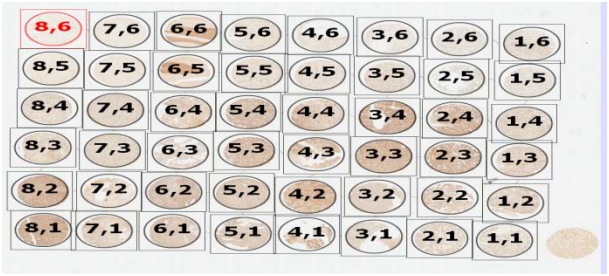


image acquisition

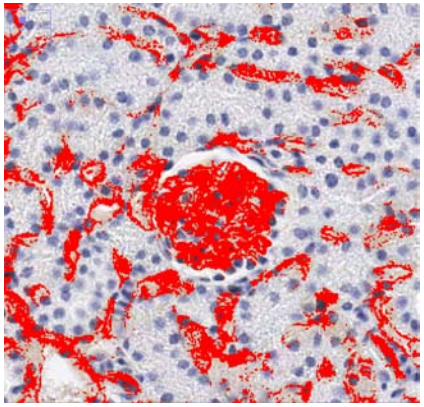


Image analysis

For More Information…

- Visit http://tmalab.jhmi.edu (about our TMA lab, with links to software)
- View the Images Application
  - Log on to http://www.tmaj.com
  - Install Java Web Start
  - Click on the "Images" link
  - Log in as Username: **guest** Password: **guest**
  - Click on "Published Sessions" (session data can be viewed but not modified)
  - Click on "Angelo De Marzo"
  - Click on any of the available sessions

Tissue Microarray Lab Roster

Kristen Lecksell Imaging Specialist

Angelo Demarzo Director

James Morgan Software Engineer

Marcella Southerland TMA Specialist

Marc Halushka Co-Director

Helen Fedor Manager