Imaging and Image Analysis of Tissue Microarrays

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NSH: 35th Annual Symposium Convention Birmingham, AL

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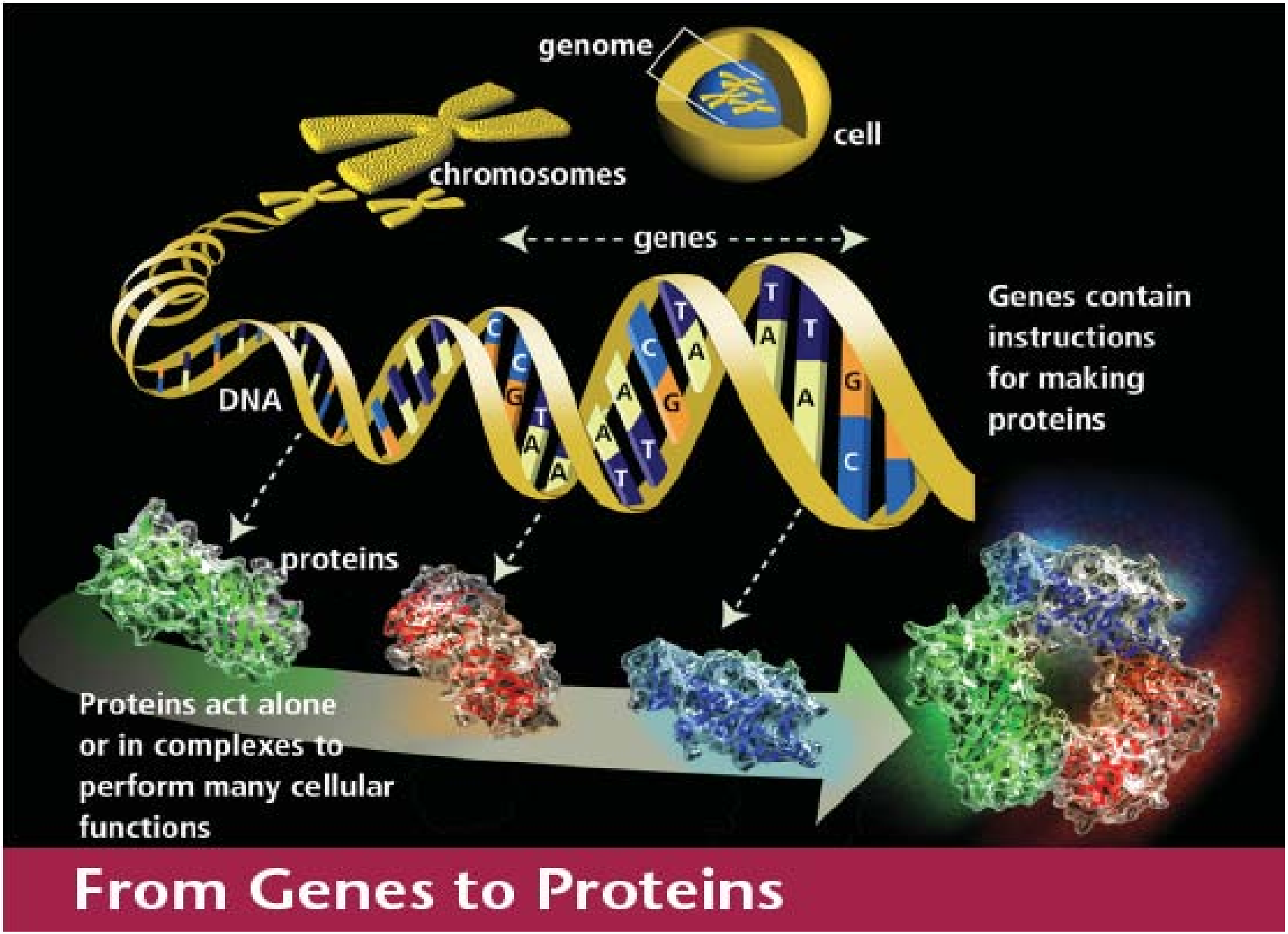
Topics

- Background Information
- Imaging Tissue Microarrays Reasons
- TMA Imaging Systems
- Image Capture
- Quantitative Image Analysis
- Other Uses of Scanning

Human Genome Project

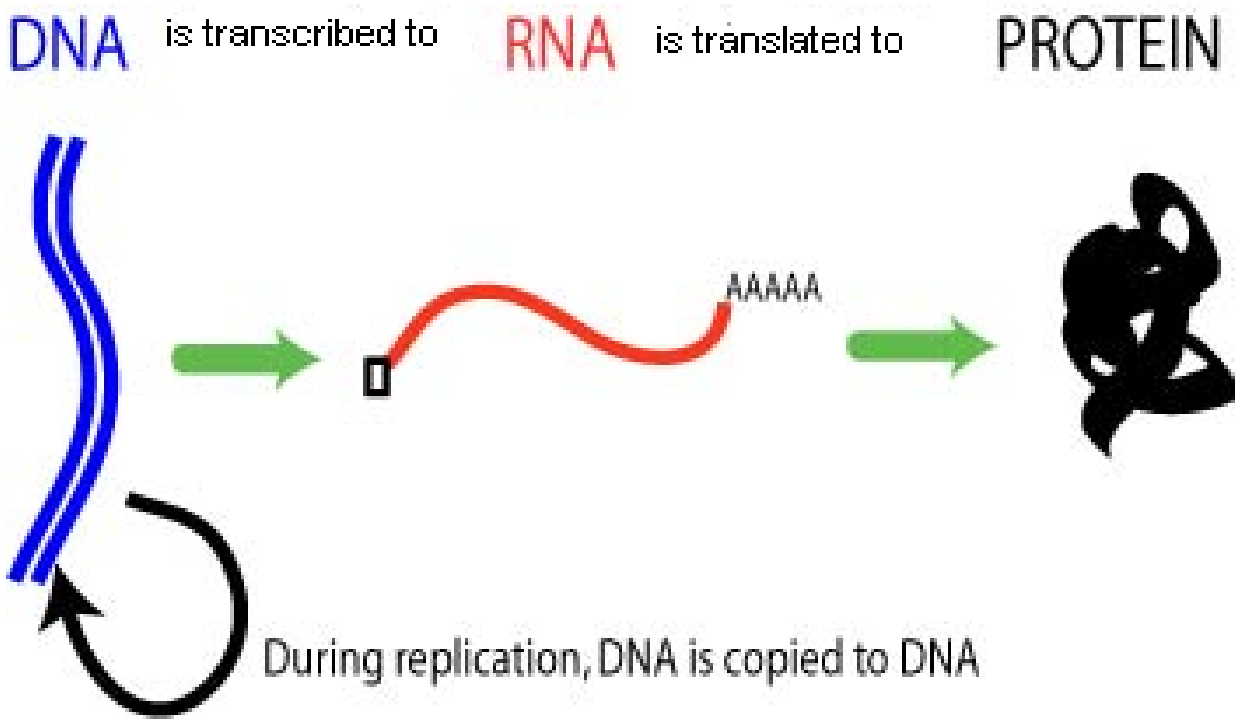
- Sequencing of the human genome has yielded an estimate of 20,000–25,000 protein-coding genes

http://www.genome.gov/12011238



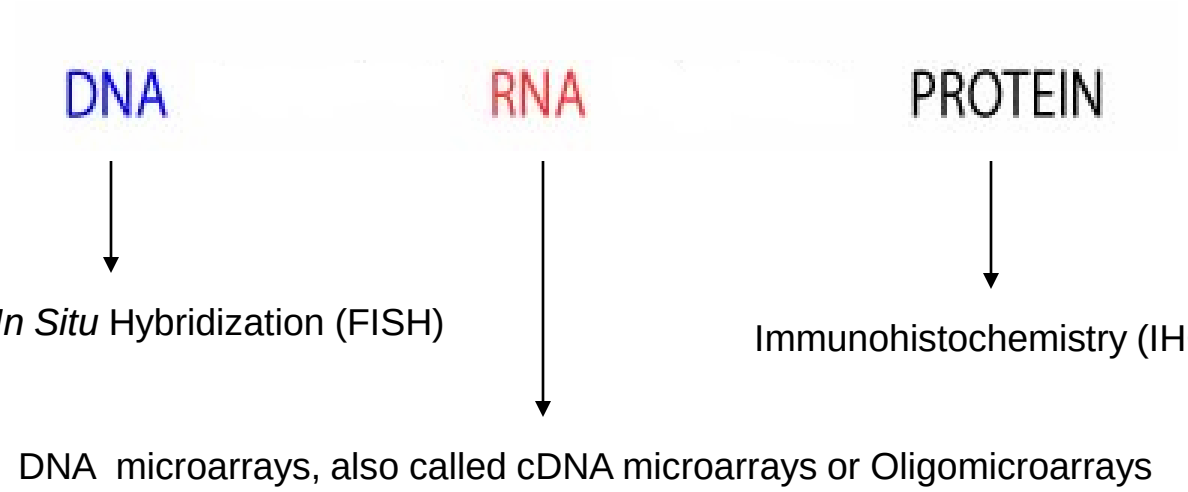
http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer2001/PrimerColor.pdf

Central Dogma of Genetics

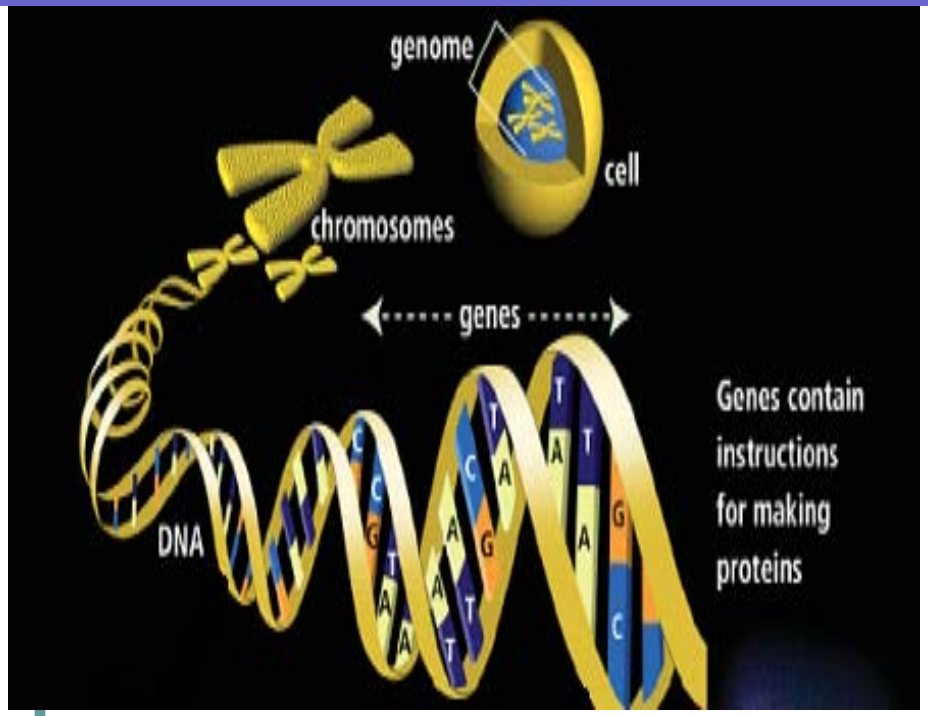


http://cnx.org/content/m11415/latest/

Detection Techniques



Complementary Base Pairing

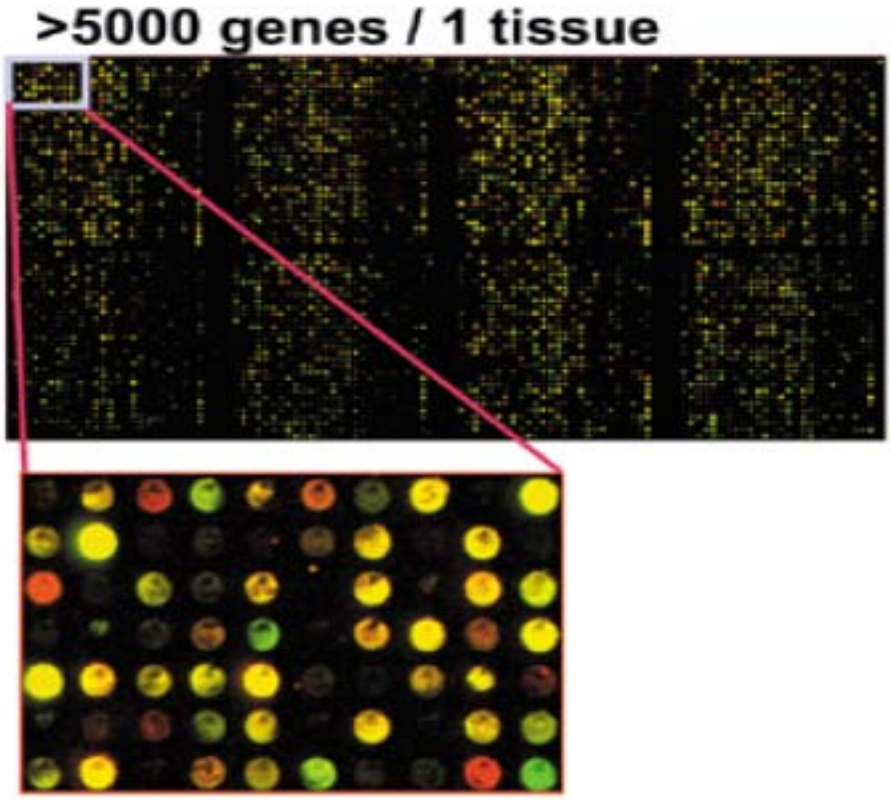


A = Adenine ⟷ T = Thymine

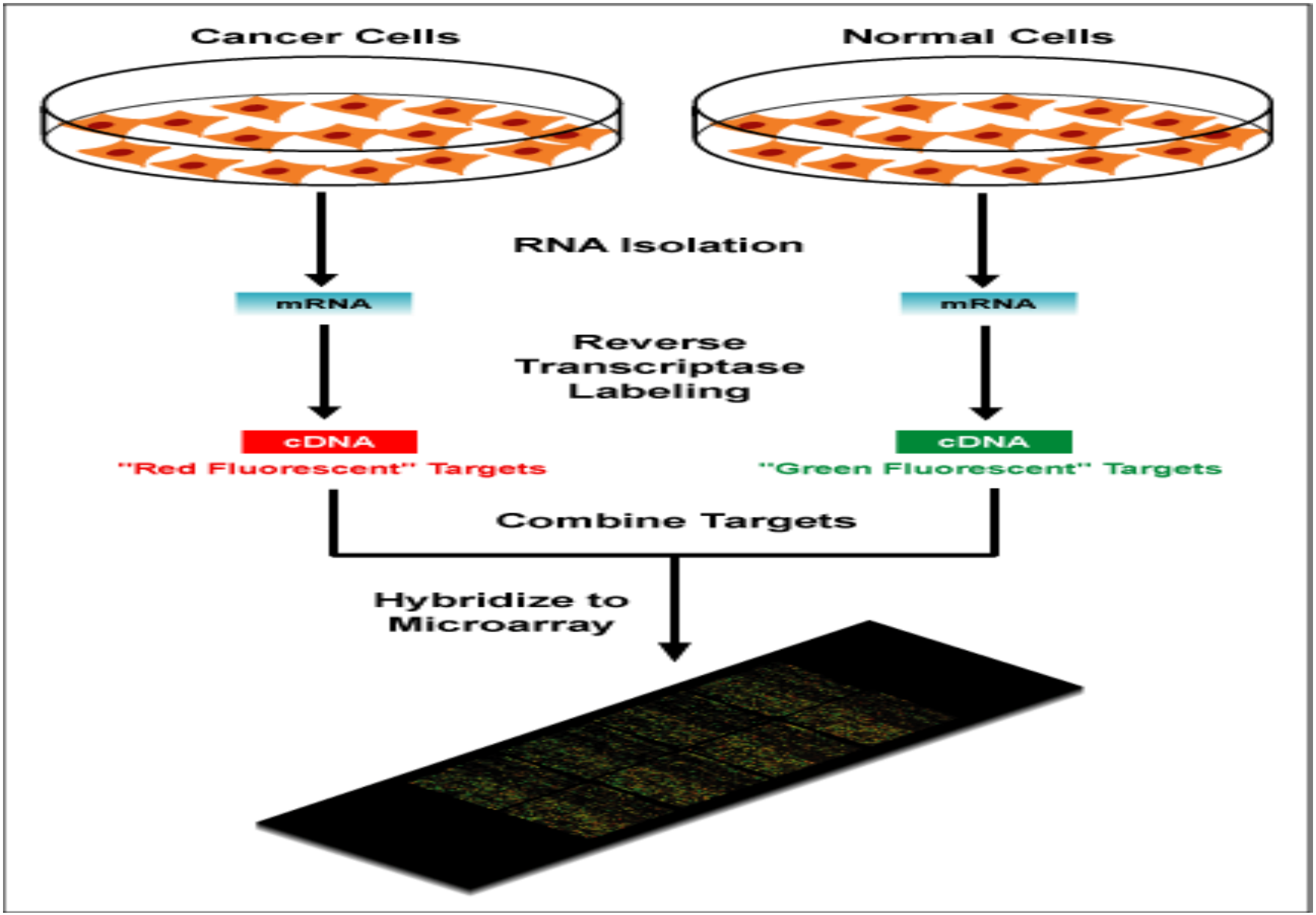
G = Guanine ⟷ C = Cytosine

http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer2001/PrimerColor.pdf

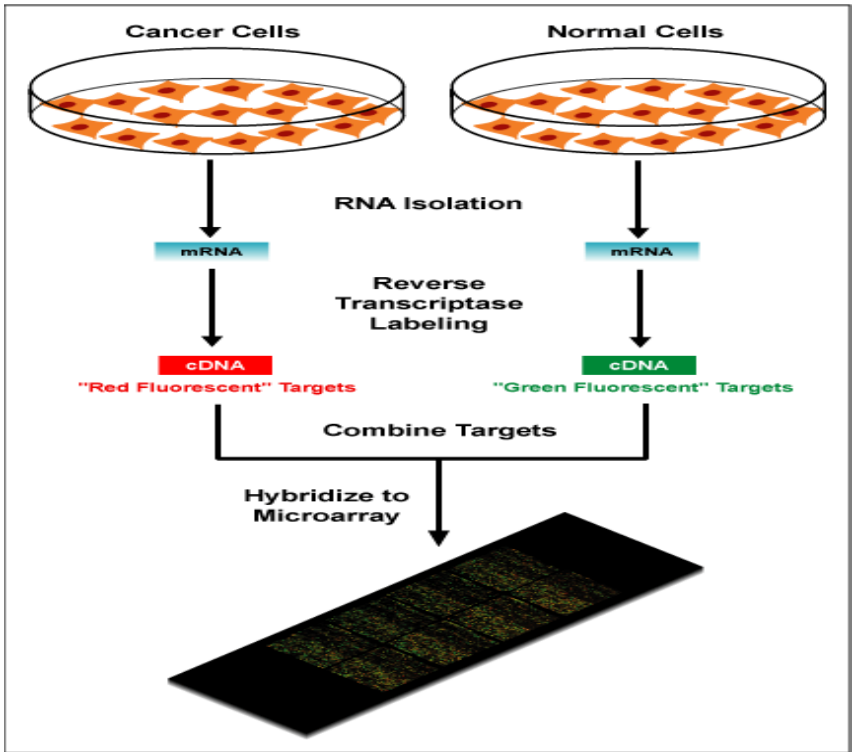
DNA Microarray

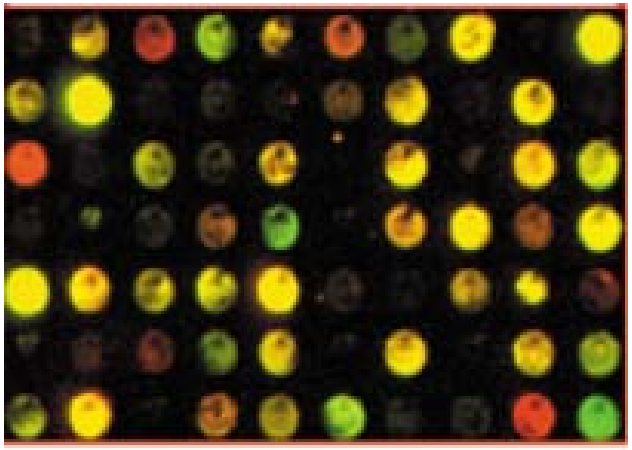


Tissue microarray technology for high-throughput molecular profiling of cancer Kallioniemi O et.al. Human Molecular Genetics, 2001, vol. 10, No. 7



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





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

Gene expression

Red Spot = Cancer

Green Spot = Normal

In-Between Spot = Both

High Throughput Techniques

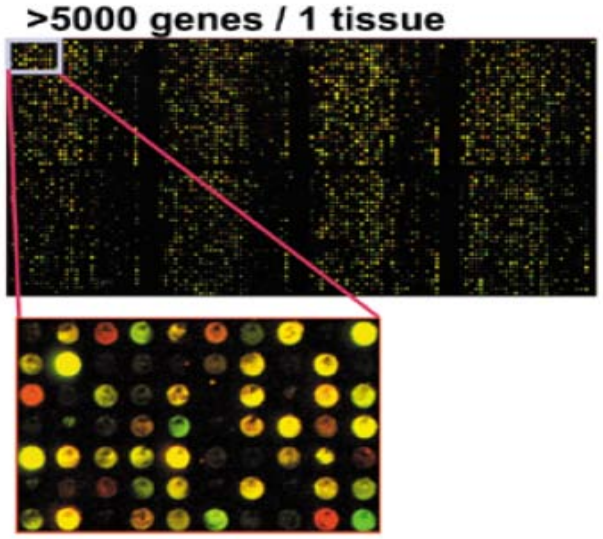
- High throughput techniques such as DNA microarrays, serial analysis of gene expression (SAGE) and proteomic surveys have produced many new potential diagnostic, prognostic and therapeutic targets that may lead to clinically useful applications

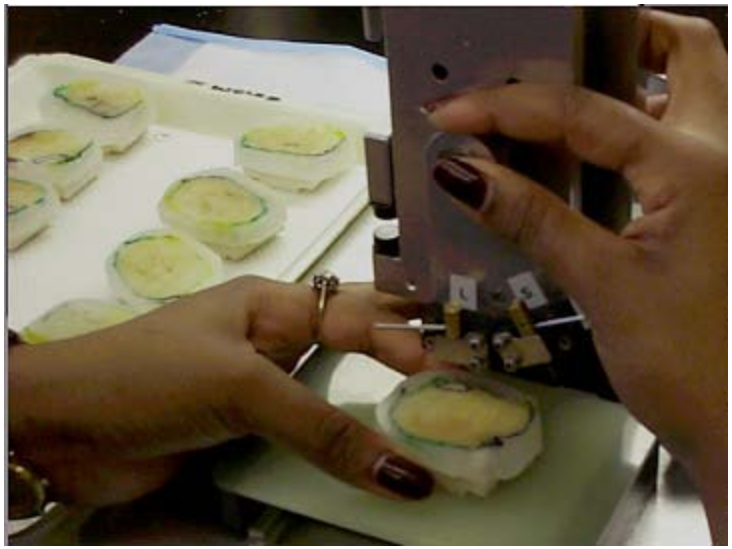
Tissue microarray technology for high-throughput molecular profiling of tumor specimens
Kononen J et.al. Nature Medicine, 1998, vol. 4, no. 7, pp. 844-847

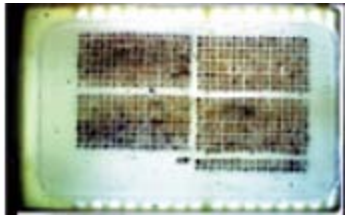
Disadvantages of Techniques

- Histology is not preserved (sample ground up)
- Genes may be expressed in multiple different cell types
- Validation requires many samples

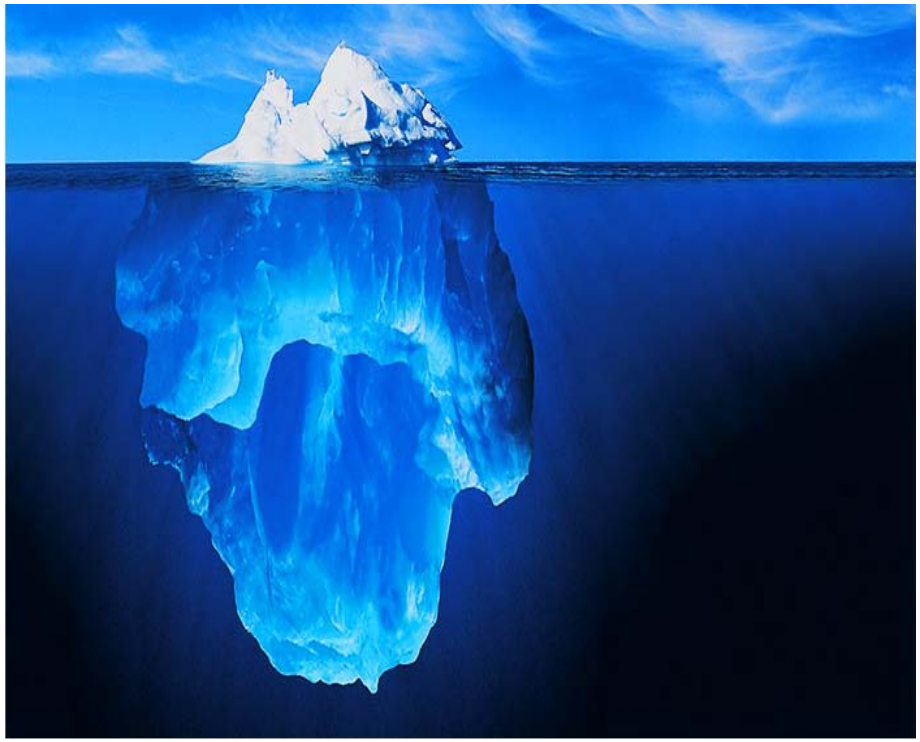
First Gene Discovery, Then Validation







HER2, ER & PR



http://www.ralphclevenger.com/

Other Targets

Angiogenesis Inhibitors	Target	Phase of Testing	Cancer Type
Avastin (bevacizumab)	VEGF	Filed for approval	Kidney
Cilengitide	Integrins	III	Brain
Pazopanib	VEGFR and PDGF	III	Kidney, Inflammatory Breast

http://www.curetoday.com/index.cfm/fuseaction/article.show/id/2/article_id/1133

Other Targets

HER Family Inhibitors	Target	Phase of Testing	Cancer Type
Erbitux (cetuximab)	EGFR (HER 1)	III	Lung, Pancreatic
Trastuzumab-DM1	HER2	II	Breast
Vectibix (panitumumab)	EGFR (HER1)	III	Head and Neck

http://www.curetoday.com/index.cfm/fuseaction/article.show/id/2/article_id/1133

Histotechnologists Needed

- To make TMAs
- To do IHC on TMAs
- To image slides
- To run image analysis

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

Imaging Tissue Microarray Reason #1

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

Birmingham, AL Become a Fan

Wall Info Photos

Birmingham, AL's Albums

Profile Pictures
2 photos

Fan Photos 14 photos
View Comments

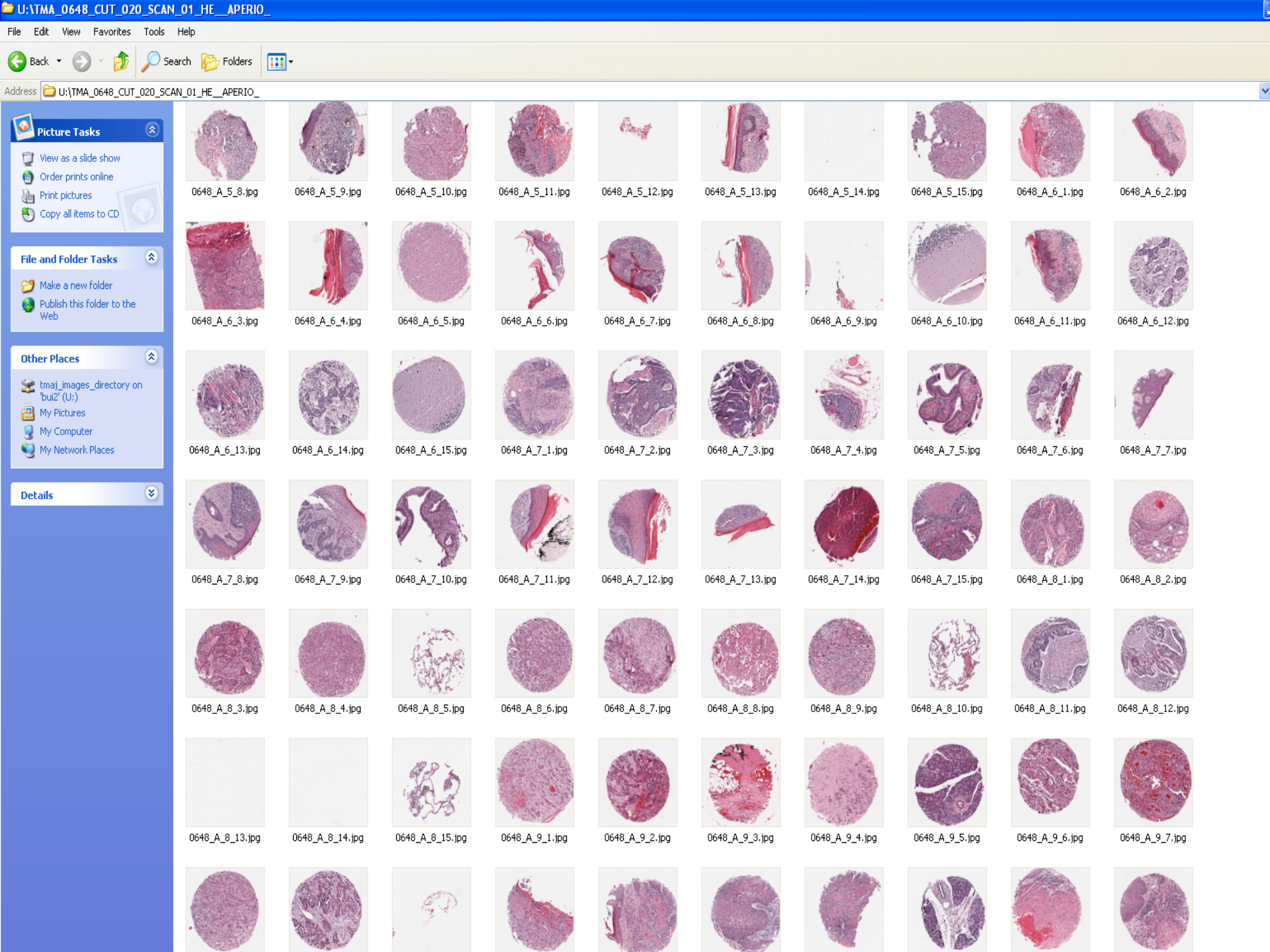








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File Edit View Favorites Tools Help
Back Search Folders
Address U:\TMA_0648_CUT_020_SCAN_01_HE__APERIO_
Picture Tasks
View as a slide show
Order prints online
Print pictures
Copy all items to CD
File and Folder Tasks
Make a new folder
Publish this folder to the Web
Other Places
tmaj_images_directory on 'bui2' (U:)
My Pictures
My Computer
My Network Places
Details
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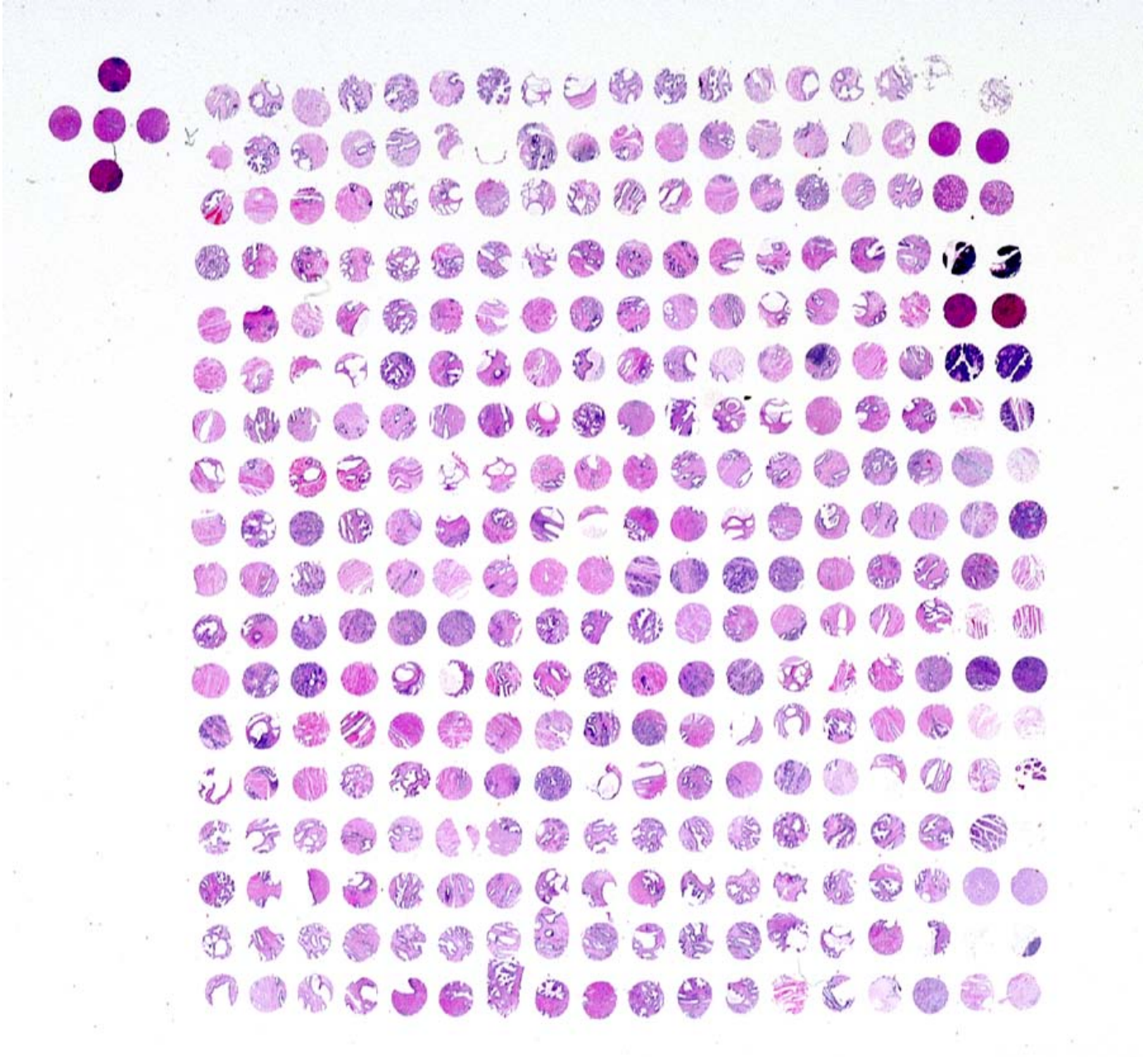
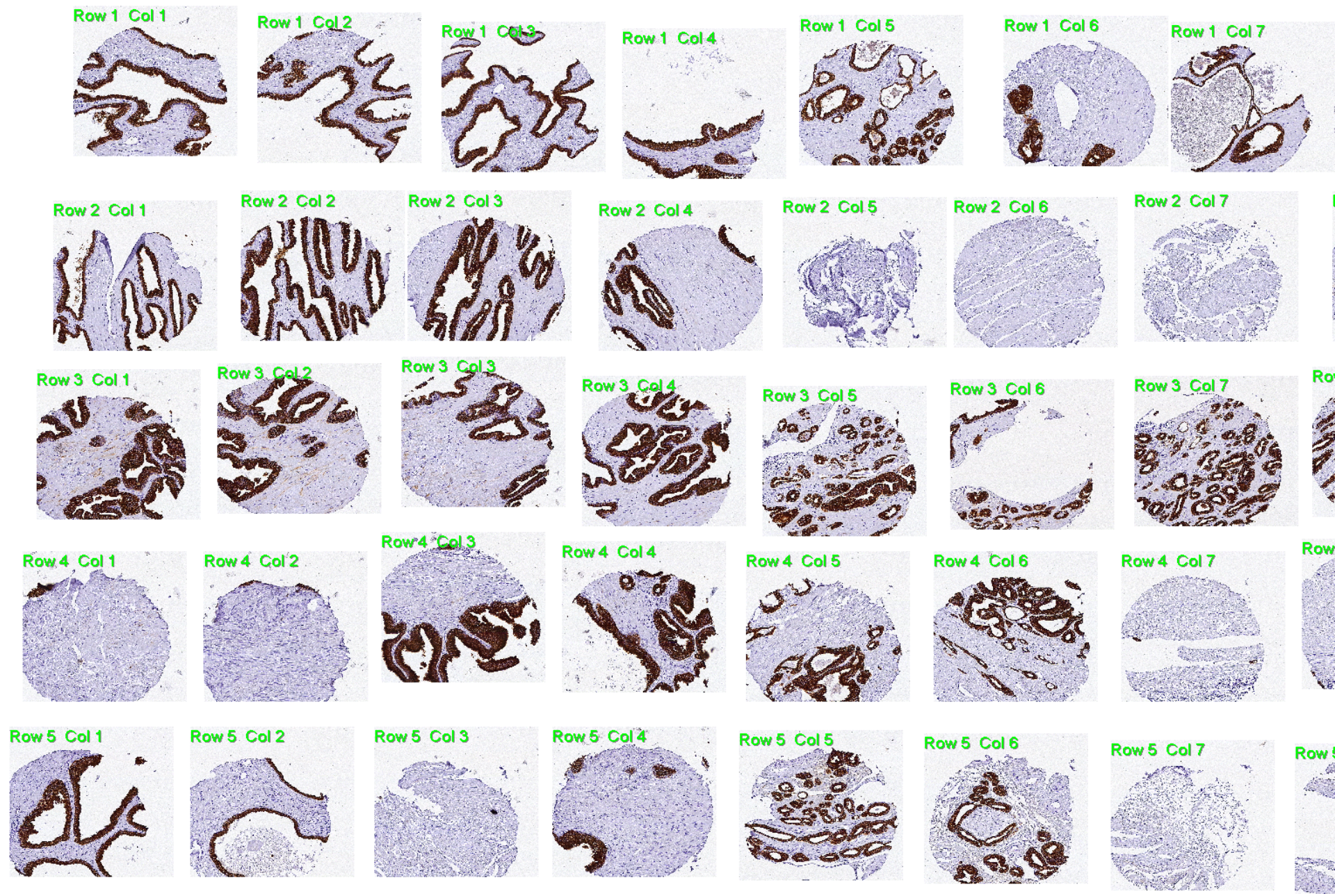
0648_A_9_5.jpg
0648_A_9_6.jpg
0648_A_9_7.jpg

Imaging Tissue Microarray Reason #2

- Keep track of the x, y spot coordinates, making spot review and data entry M**UCH** 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/

HOME | HOW IT WORKS | FAQ | CONTACT US

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

FREE SERVICE

Background

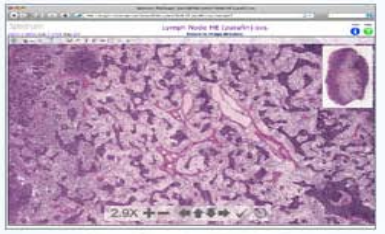
Data Sheet
Application Sheet

SecondSlide Webinars

Free Digital Slide Sharing Service for Pathology

SecondSlide makes slide sharing easy:

- Share slides with anyone you choose, regardless of geographical location
- Improve turnaround time
- Eliminate glass slide logistics
- Provide pathology services to remote hospitals
- Gain access to subspecialty expertise



+ View Sample Digital Slide

SecondSlide Applications


Clinical


Research


Education

Please contact us with questions or + Sign Up for A Free Trial

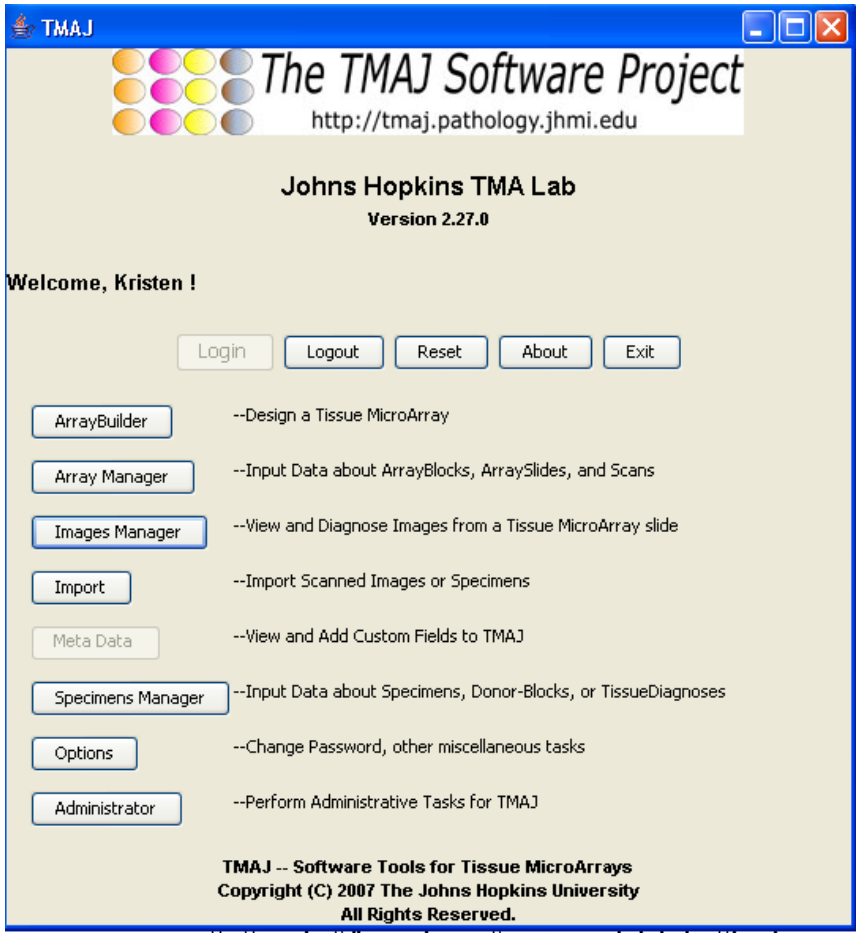
Imaging Tissue Microarray Reason #4

- Can import images into a database linked to image, pathology, and clinical information.

Publicly Available TMA Databases

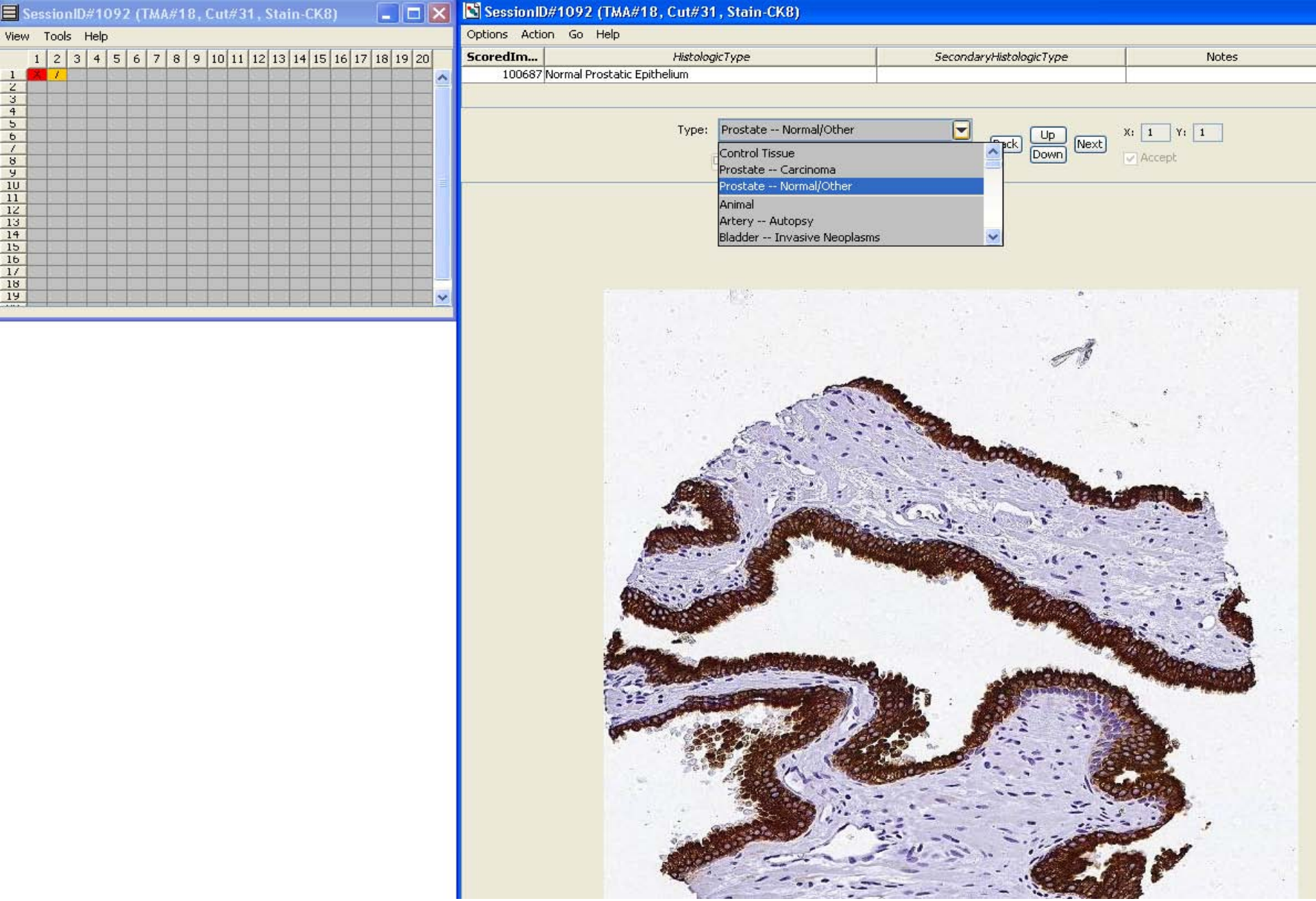
Institution	Name of Database	Webpage
Stanford University, California, USA	TMAD	http://tma.stanford.edu/cgi-bin/home.pl
Johns Hopkins University, Maryland, USA	TMAJ	http://tmaj.pathology.jhmi.edu/
Graz University of Technology, Austria	TAMEE	https://esus.genome.tugraz.at/tma/
MD Anderson Cancer Center,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

Johns Hopkins Online TMA Database

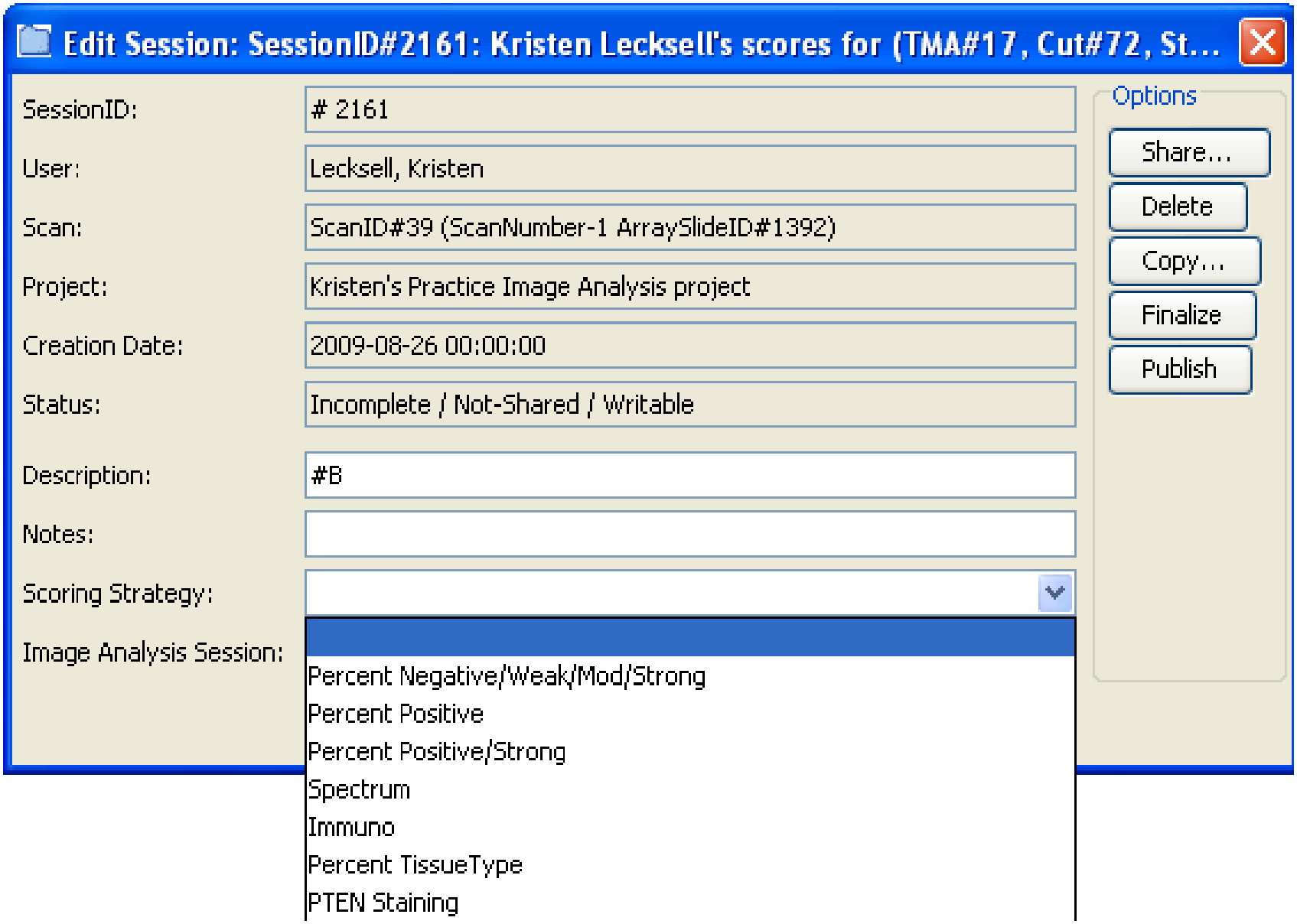


Dennis Faith
Brian Razzaque
James D. Morgan
Helen Fedor
Angelo M. De Marzo

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/Othe

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
  - PatientID
  - Age
  - Race
- Surgical Pathology Data
  - SurgPathNumber
  - DateSpecimenTaken
  - PStage
  - HistologicalType (Gleason for radical prostatectomies)
  - MarginsPositive
- Clinical Data
  - PreOpPSA (for radical prostatectomies)

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

Options Action Go Help

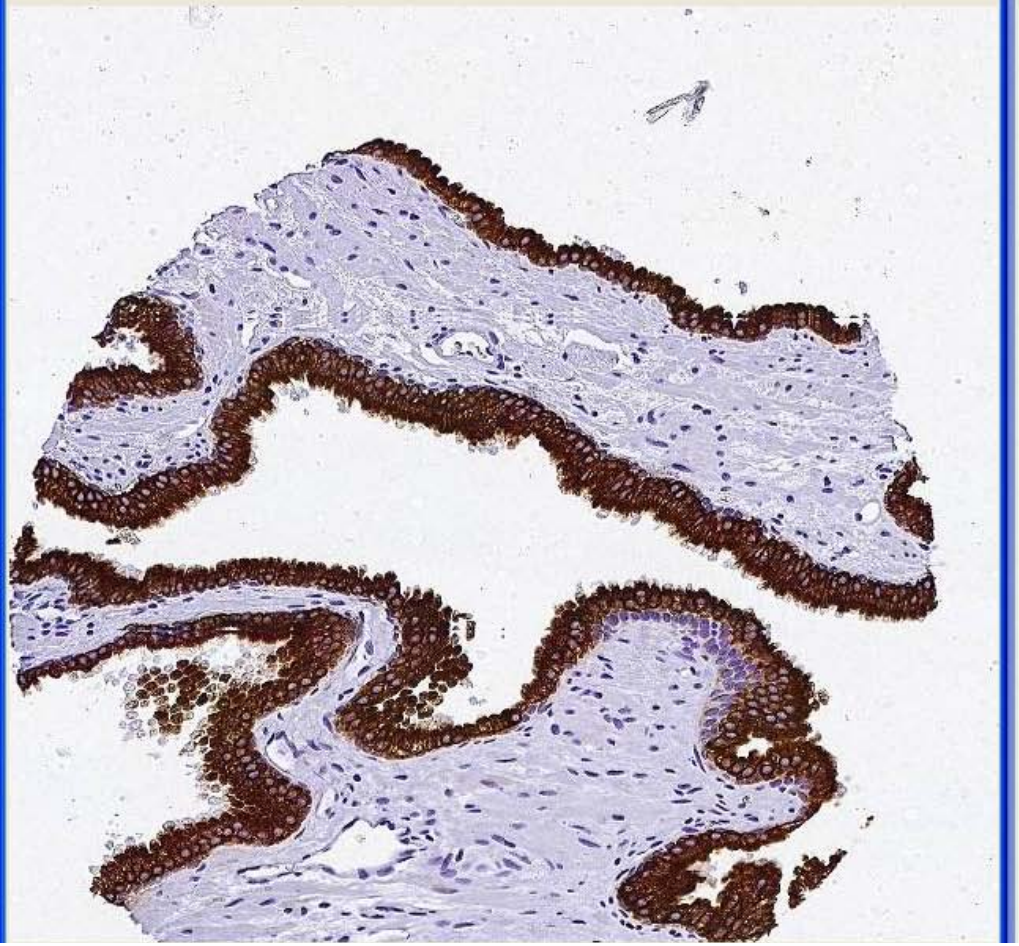
ScoredIm...	HistologicType	SecondaryHistologicType
100687	Normal Prostatic Epithelium	

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

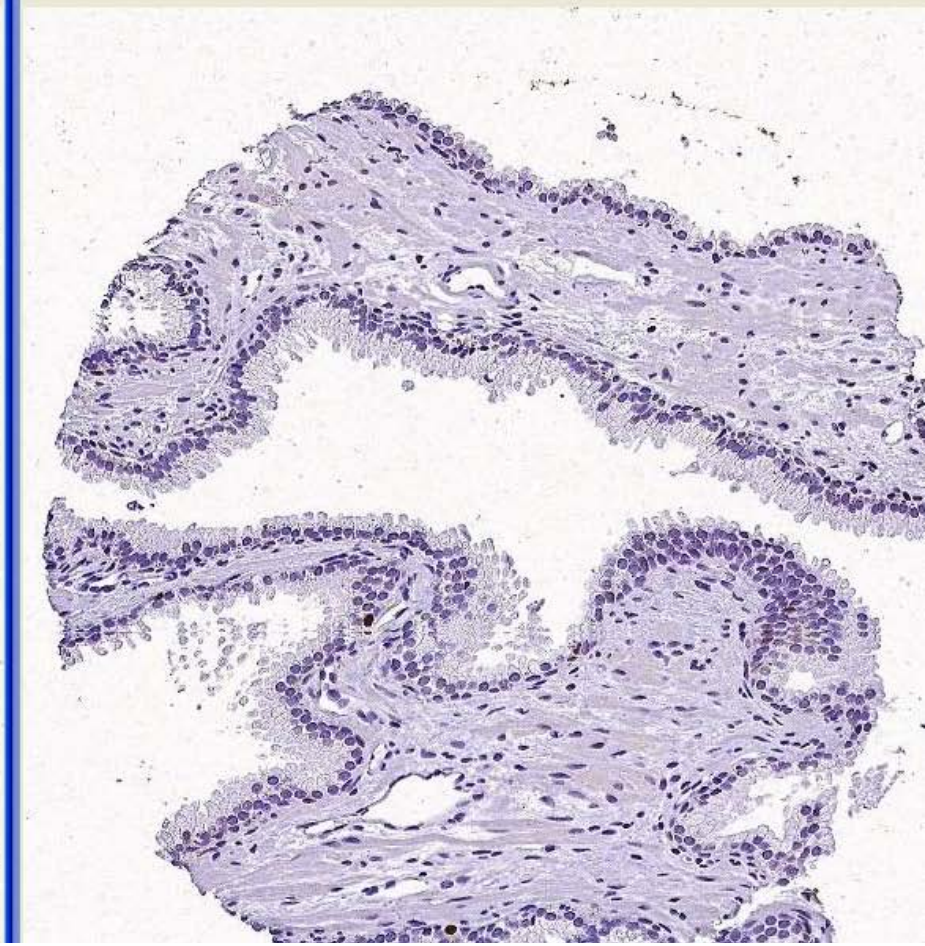
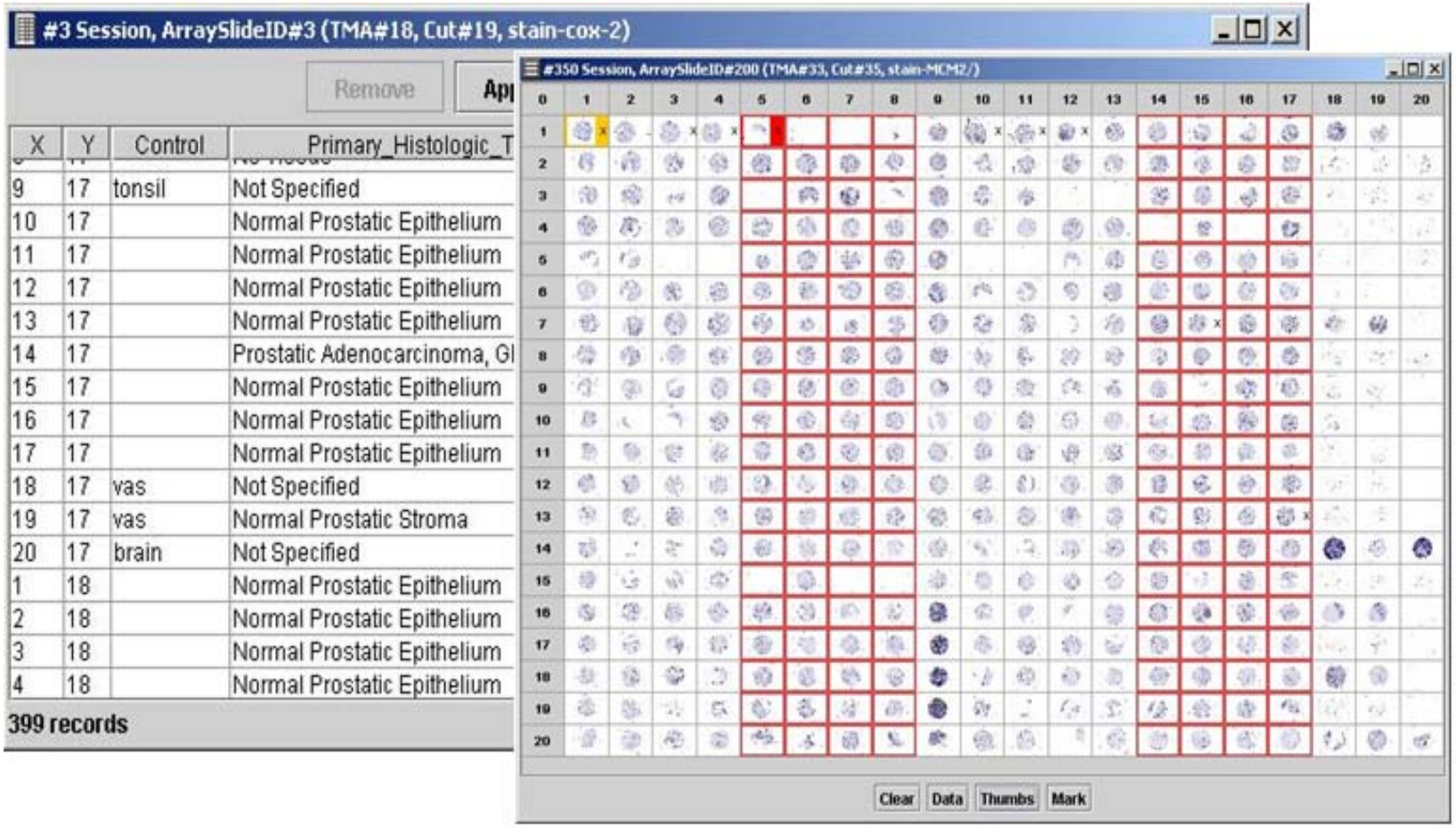


Image Application: Filtering



Scanners

Aperio Technologies	ScanScope™ XT, GL, CS, OS & FL	http://www.aperio.com
Olympus and its wholly owned subsidiary Bacus Laboratories Inc. (BLI)	BLISS™ and Nanozoomer™ RS & HT	http://www.olympusamerica.com/
Dako – think it's now Zeiss, but Dako web link still works	ACIS III™	http://www.dako.com/go/acis.htm
HistoRx	PM-2000™ (fluorescence only)	http://www.historx.com/

Aperio Scanners

Olympus/Bacus Scanners

BLISS

Nanozoomer RS

Nanozoomer HT

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 & 100x) 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 – it's movement
  - Objectives
  - Video camera
- Image analysis
  - Morphometric measurements
  - IHC quantification
  - Histology pattern recognition

Three Ways to Capture a Virtual Slide

- “Taking static images of a glass slide using a conventional light microscope and then collating them together;”
- “Remotely operating a microscope that is capable of dynamically changing its field of view and capturing an image at a user-defined resolution;”
- “High-resolution scanning of the whole glass slide.”

Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev Mol Diagn. 2008 Nov;8(6):707-25. Review.

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 paper image

20x Camera Capture

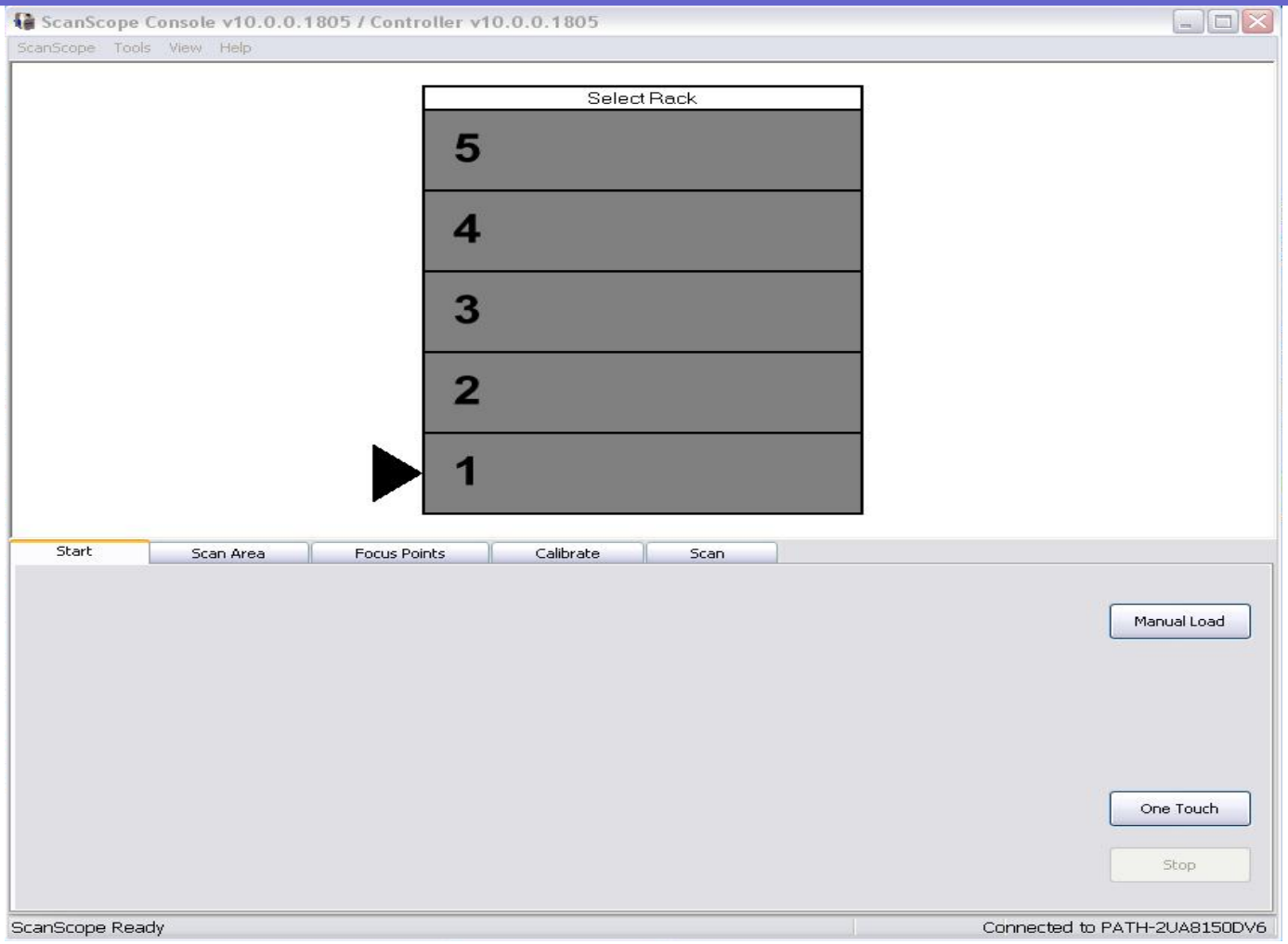
	Pixel # / Micron
Aperio: ScanScope CS	2
Dako/Zeiss: ACIS II	2
Olympus/Bacus Labs: BLISS	2.37

Megabytes, Gigabytes & Terabytes

- Digitized TMA: 100 MB to 3 GB
- Storage is an issue
- BUT storage is getting cheaper

Scanning: Aperio

Scanning: Aperio



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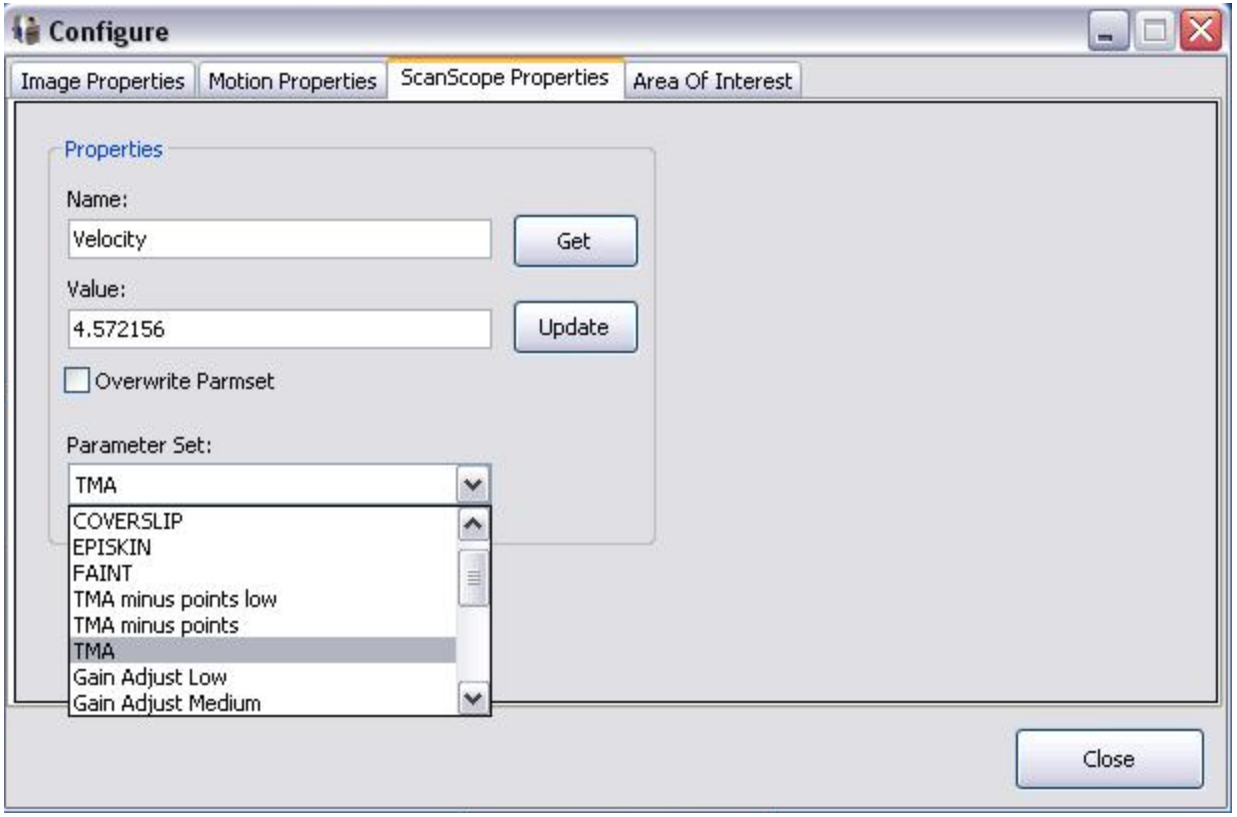
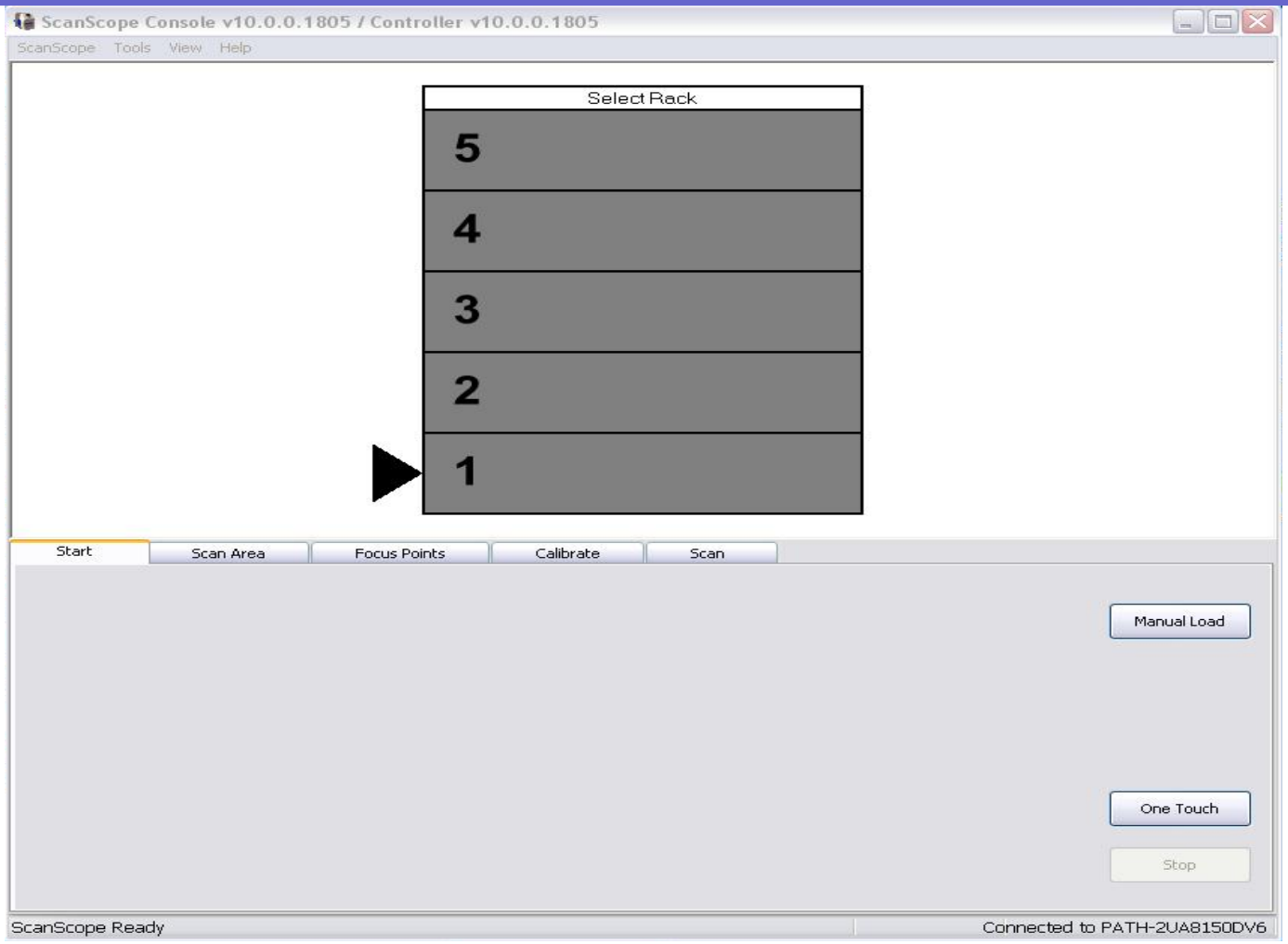


Scanning: Aperio



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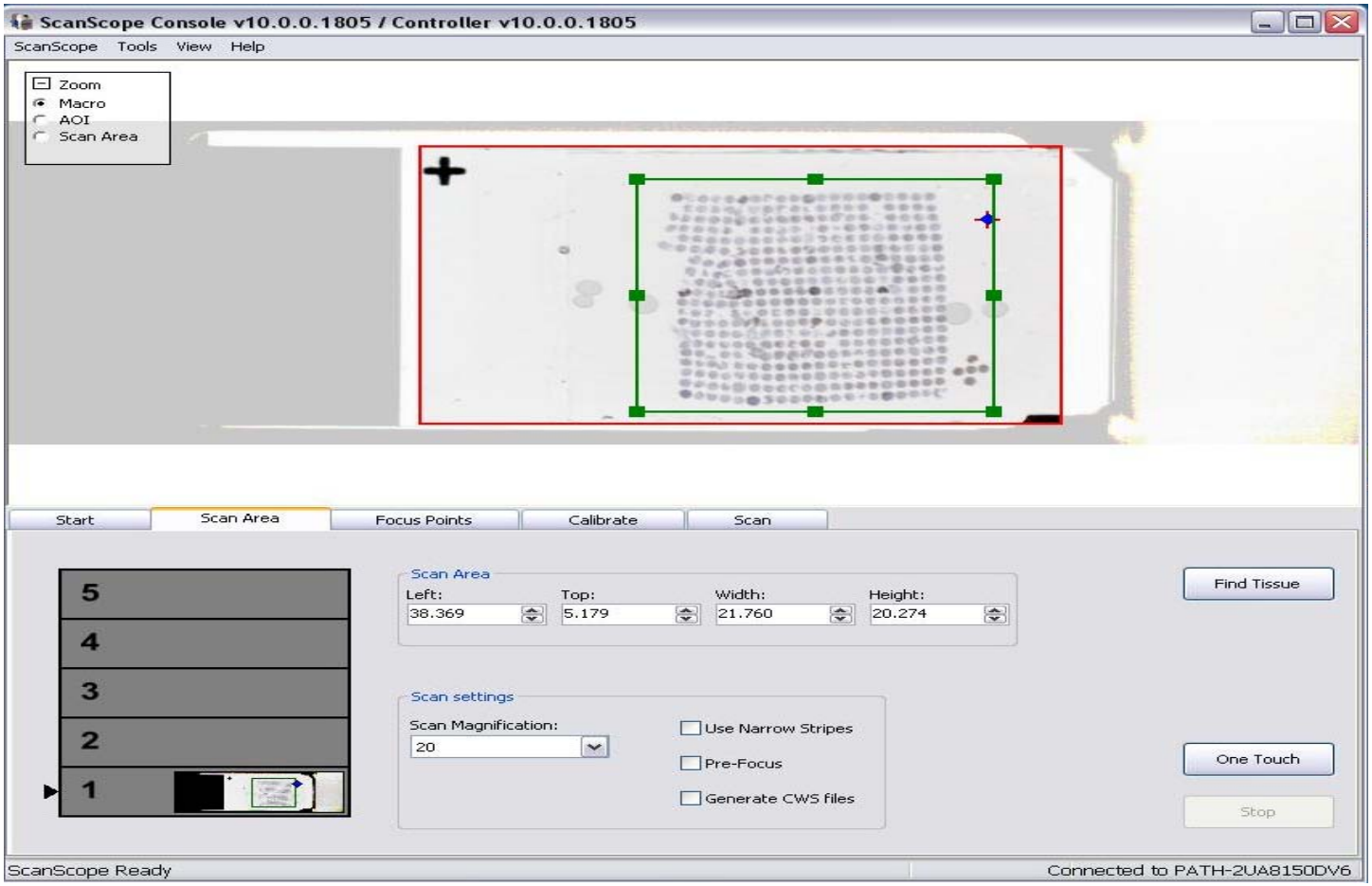

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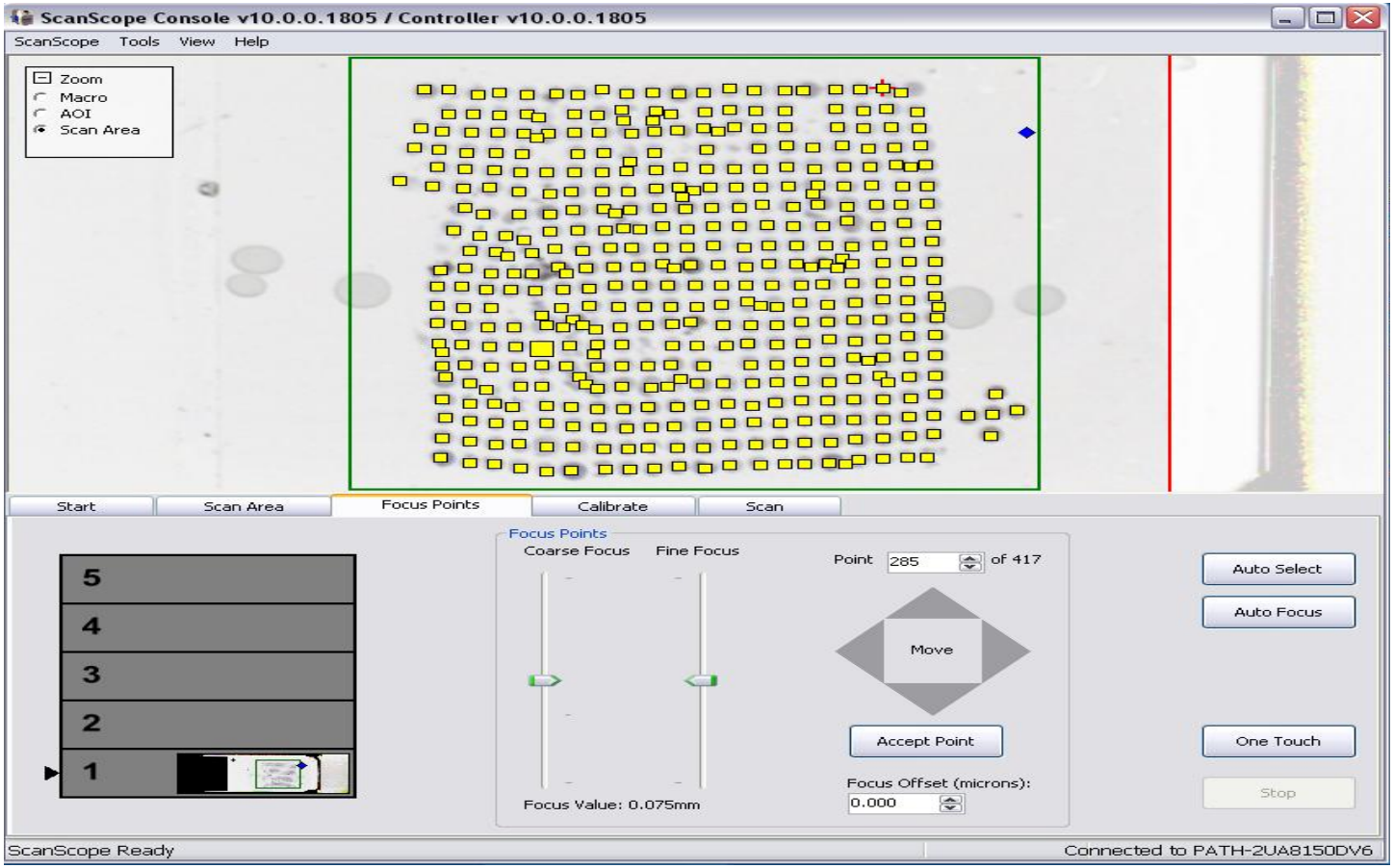

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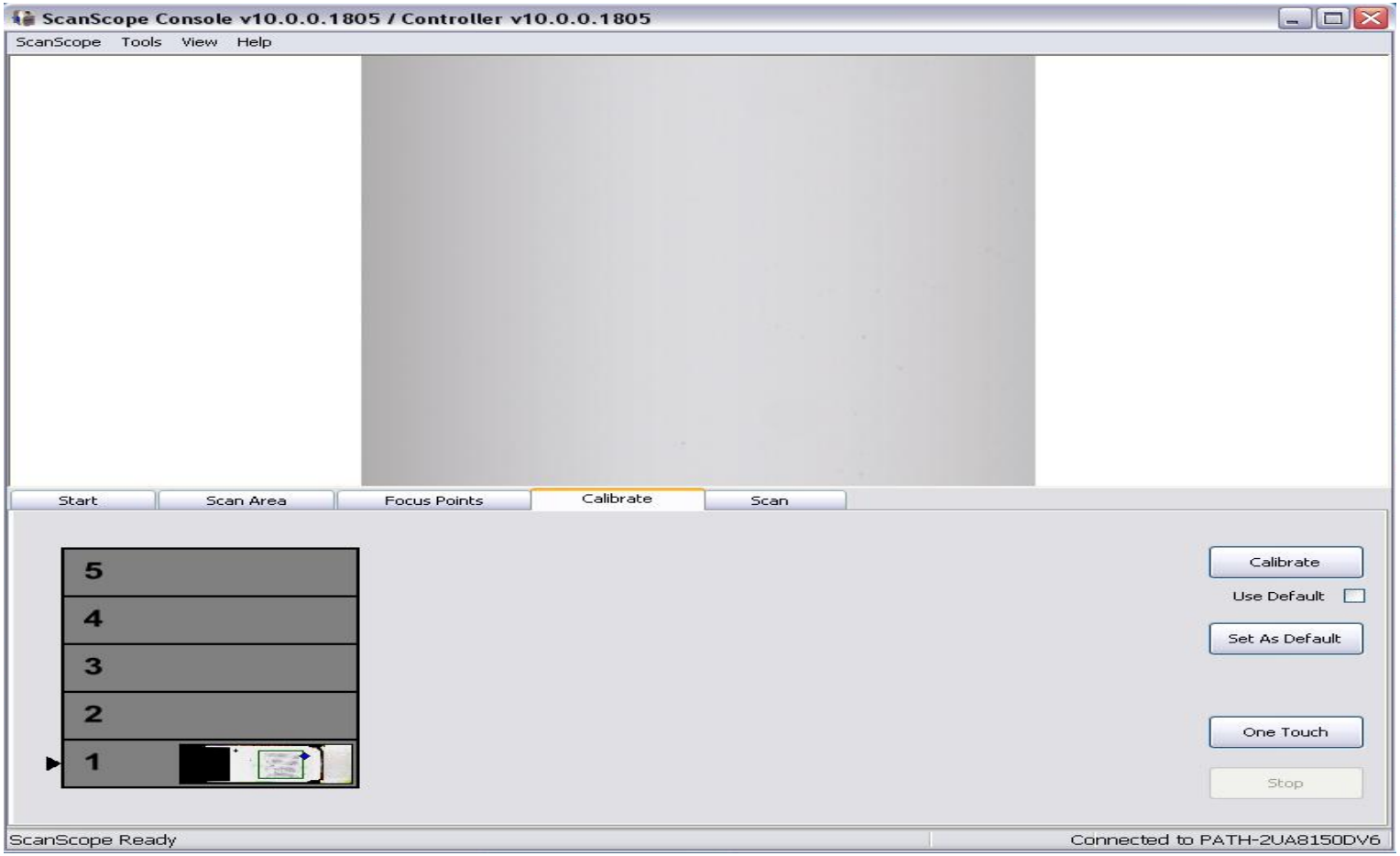
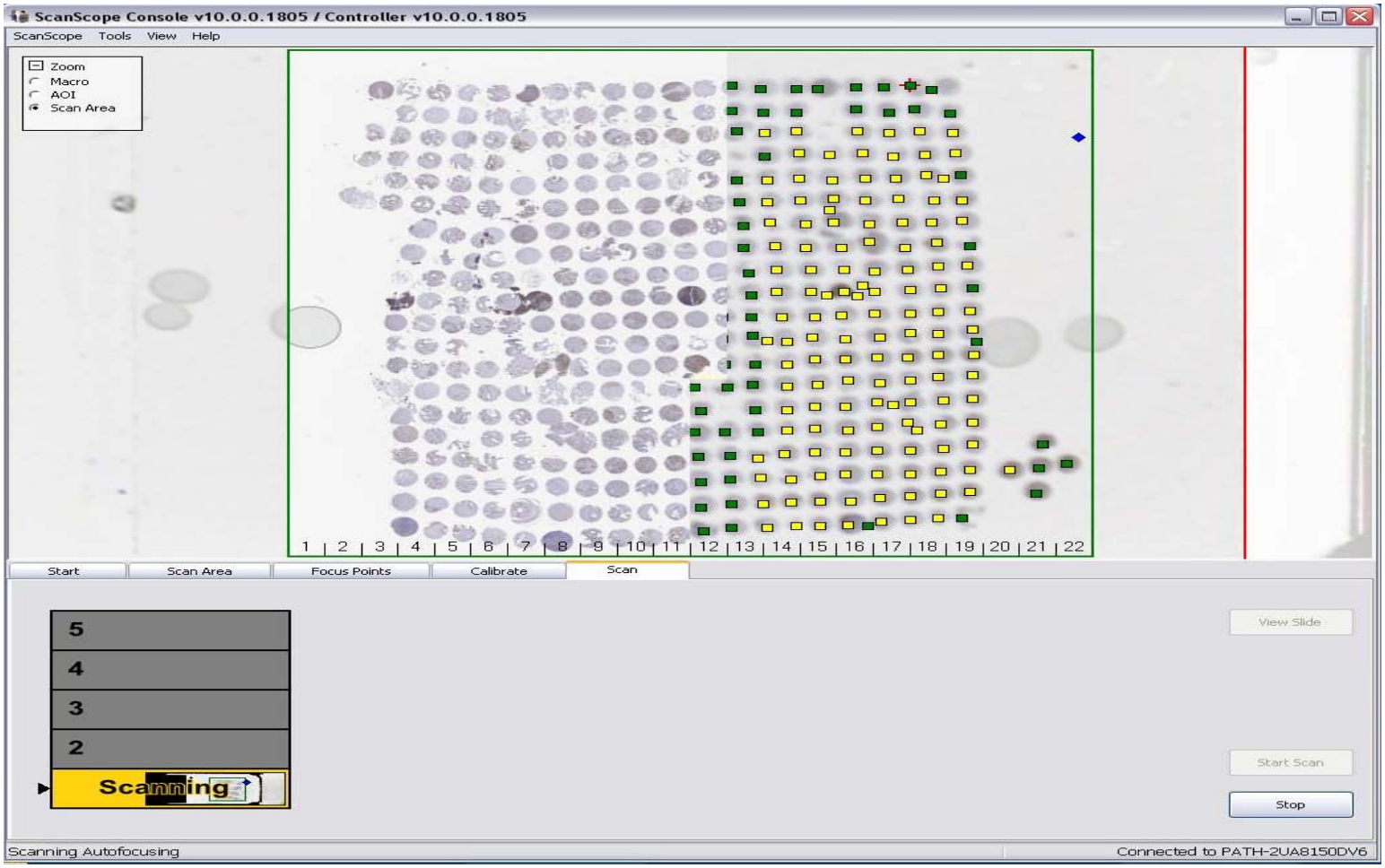
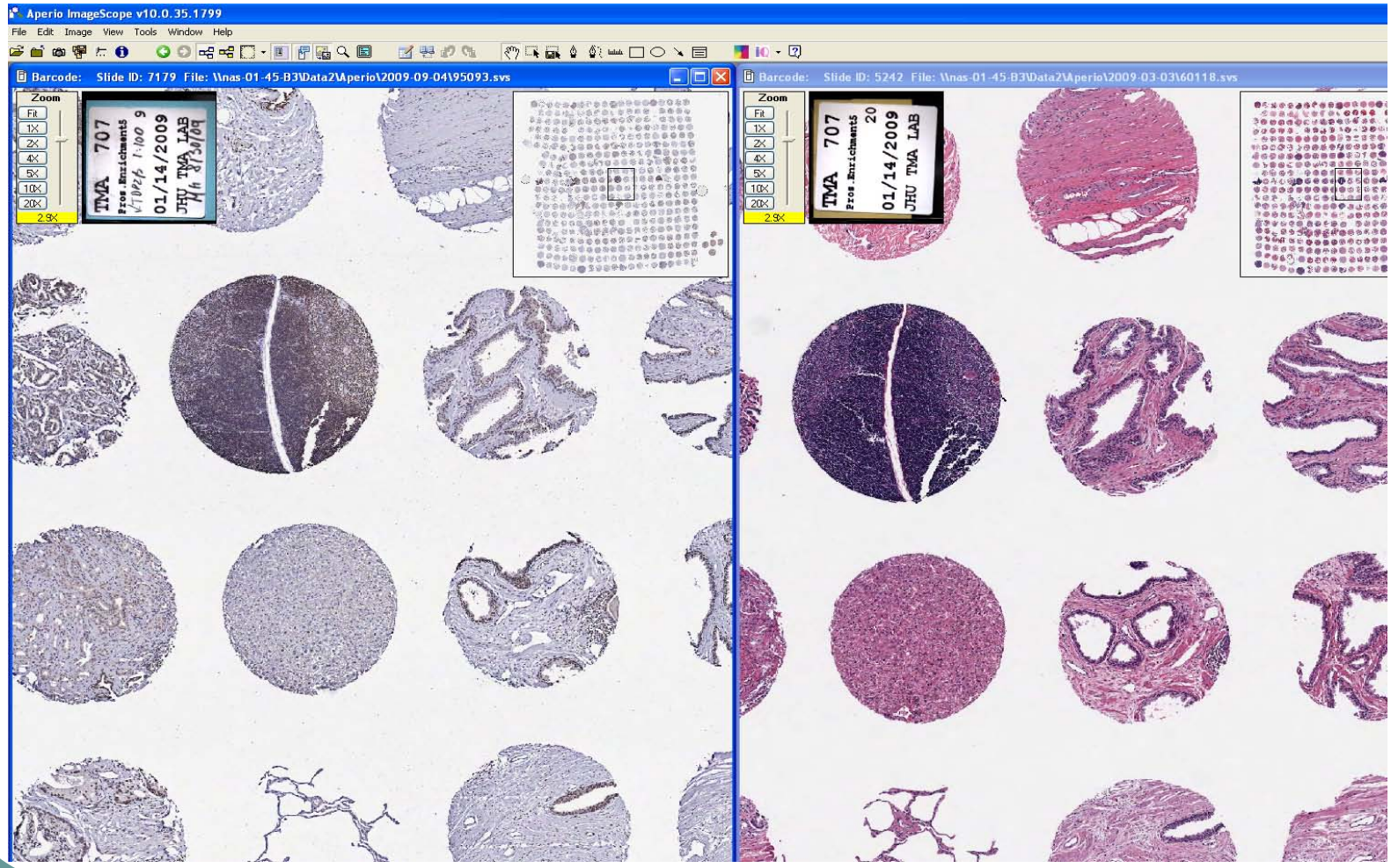


Scanning: Aperio



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

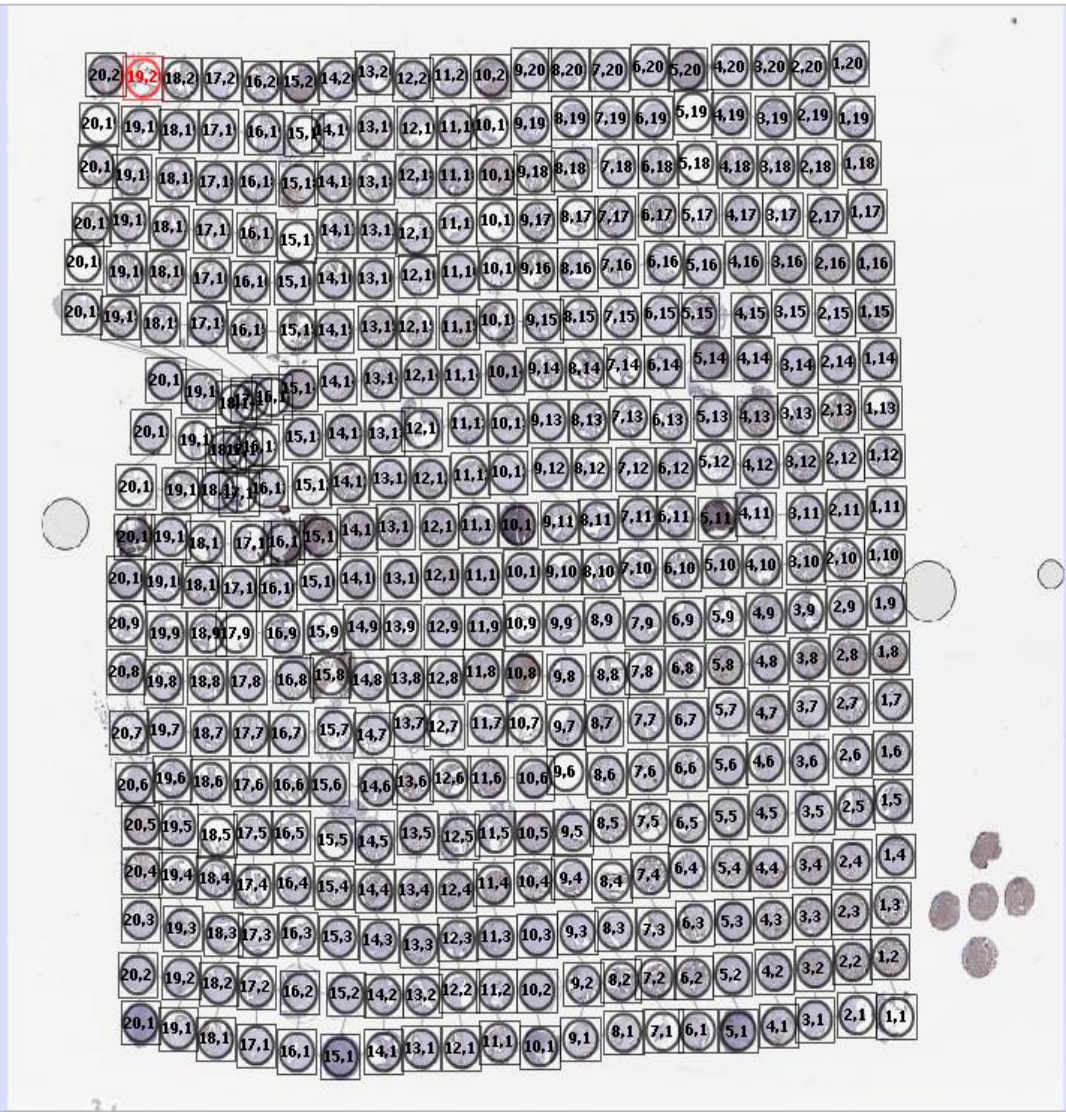
Col Labels: Numeric

Sector Labels: Alphabetic (upper case)

Reverse Rows

Reverse Columns

Submit | Cancel



TMA Composites

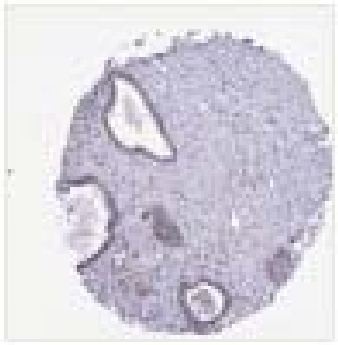

0707_A_1_5_.jpg


0707_A_1_6_.jpg


0707_A_1_7_.jpg


0707_A_1_8_.jpg


0707_A_1_12_.jpg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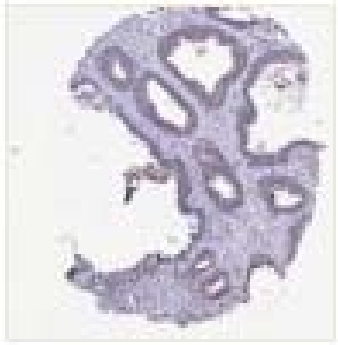

0707_A_1_14_.jpg


0707_A_1_15_.jpg

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

Quantitative 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

Quantitative Image Analysis

"With automated image analysis, complex computer algorithms are used to enhance the slide image, making interpretation straight-forward and facilitating a more objective interpretation."

Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev Mol Diagn. 2008 Nov;8(6):707-25. Review.

Algorithm

"An algorithm is a computer-based specific set of instructions for carrying out a task or solving a problem."

Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev Mol Diagn. 2008 Nov;8(6):707-25. Review.

Typical Quantitative Image Analysis

- Separating stains (color deconvolution)
- Quantifying a particular stain (measuring pixel color intensities)
- Identifying a region of pathological interest (cancer vs. normal)

Mulrane L, Rexhepaj E, Penney S, Callanan JJ, Gallagher WM. Automated image analysis in histopathology: a valuable tool in medical diagnostics. Expert Rev Mol Diagn. 2008 Nov;8(6):707-25. Review.

Color Deconvolution

Analytical and Quantitative Cytology and Histology®

Color Deconvolution for the Analysis of Tissue Microarrays

Toby C. Cornish, M.D., Ph.D., and **Marc K. Halushka,** M.D., Ph.D.

OBJECTIVE: To analyze tissue microarrays (TMAs) using color deconvolution, a method for separating component dyes in digital images, and compare the results to observer scoring.
STUDY DESIGN: TMAs were constructed from tissues from 100 adult autopsies and immunohistochemically stained for connective tissue growth factor. A region of interest (ROI) was created for each core image using 3 binary masks—tissue area, inclusion area, and exclusion area. The diaminobenzidine (DAB) and hematoxylin sig-

istry, staining, tissue microarray.

Immunohistochemistry (IHC) is a powerful method for evaluating protein expression in tissues. The advent of high temperature antigen retrieval methods in the 1990s improved the repeatability and quality of IHC, and it is now routinely used for both clinical and research applications.[1] A significant advantage of IHC over techniques such as Western blotting and enzyme-linked immunosorbent assay

Cornish TC, Halushka MK. Color Deconvolution for the Analysis of Tissue Microarrays. Anal and Quant Cytology and Histology. 2009 31(0) ahead of print.

Color Deconvolution for TMA Images Website

- http://colordecontmas.sourceforge.net/

Typical Quantitative Image Analysis

- Determine where the edges of an object begin and end
- Count similar objects
- Calculate the area, perimeter & length of objects such as:
  - Nuclei
  - Microvessels
  - Lymphoid cells

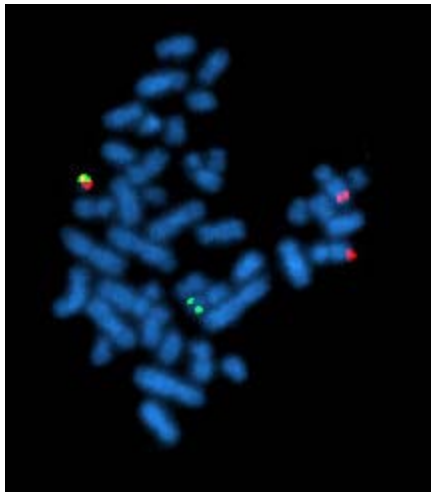
http://en.wikipedia.org/wiki/Microscope_image_processing

ImmunoHistoChemistry

- Method of protein detection and localization
- Can use frozen or formalin-fixed paraffin-embedded tissue

Fluorescence In Situ Hybridization

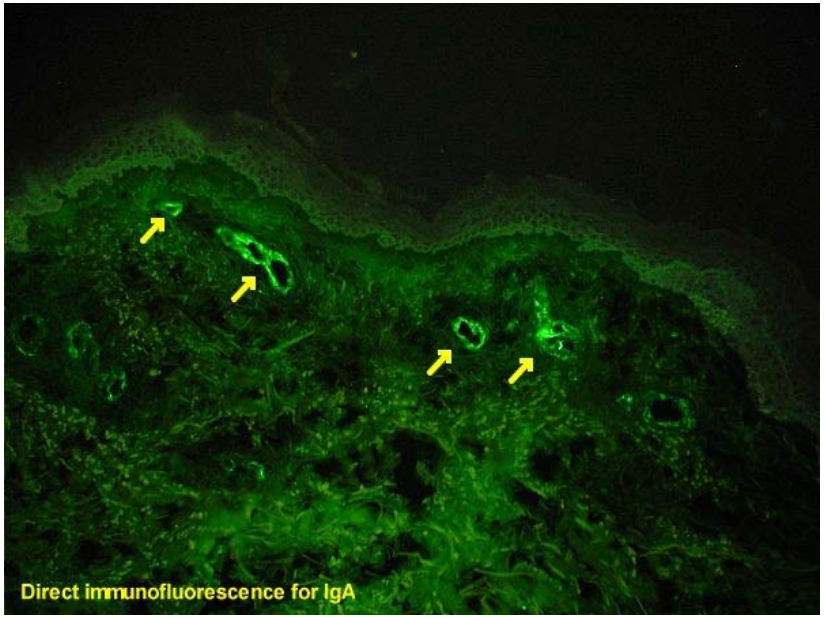
- Cytogenetic technique
- Detects and localizes the presence or absence of specific DNA sequences on chromosomes



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 Quantitative Image Analysis

- Oncology
- Pathology
- Toxicology within the pharmaceutical industry

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

Commercially Available Software Packages for Quantitative Image Analysis

Software Package	Company
ScanScope Systems®	Aperio Technologies, Inc., CA, USA
BLISS™ workstation with TMAscore	Bacus Laboratories, Inc., IL, USA
Automated Quantitative Analysis (AQUA®)	HistoRX, CT, USA
HistoQuant	3DHistech, Budapest, Hungary

Quantitative Image Analysis Article

Review

EXPERT REVIEWS

Automated image analysis in histopathology: a valuable tool in medical diagnostics

Expert Rev. Mol. Diagn. 8(6), 707–725 (2008)

Laoighse Mulrane,
Elton Rexhepaj,
Steve Penney,
John J Callanan and
William M Gallagher†
†Author for correspondence
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Fax: +353 1283 7211

Virtual pathology, the process of assessing digital images of histological slides, is gaining momentum in today's laboratory environment. Indeed, digital image acquisition systems are becoming commonplace, and associated image analysis solutions are viewed by most as the next critical step in automated histological analysis. Here, we document the advances in the technology, with reference to past and current techniques in histological assessment. In addition, the demand for these technologies is analyzed with major players profiled. As there are several image analysis software programs focusing on the quantification of immunohistochemical staining, particular attention is paid to this application in this review. Oncology has been a primary target area for these approaches, with example studies in this therapeutic area being covered here. Toxicology-based image analysis solutions are also profiled as these are steadily increasing in popularity, especially within the pharmaceutical industry. Reinforced by the phenomenal growth of the virtual pathology field, it is envisioned that the market for automated image analysis tools will greatly expand over the next 10 years.

FrIDA: An open source framework for image dataset analysis

- FrIDA is image analysis software.
- Developed by the Johns Hopkins University Tissue Microarray Core Facility.
- Open source and written in 100% Java.
- Makes use of functionality from the NIH's ImageJ application.

http://bui2.win.ad.jhu.edu/frida/

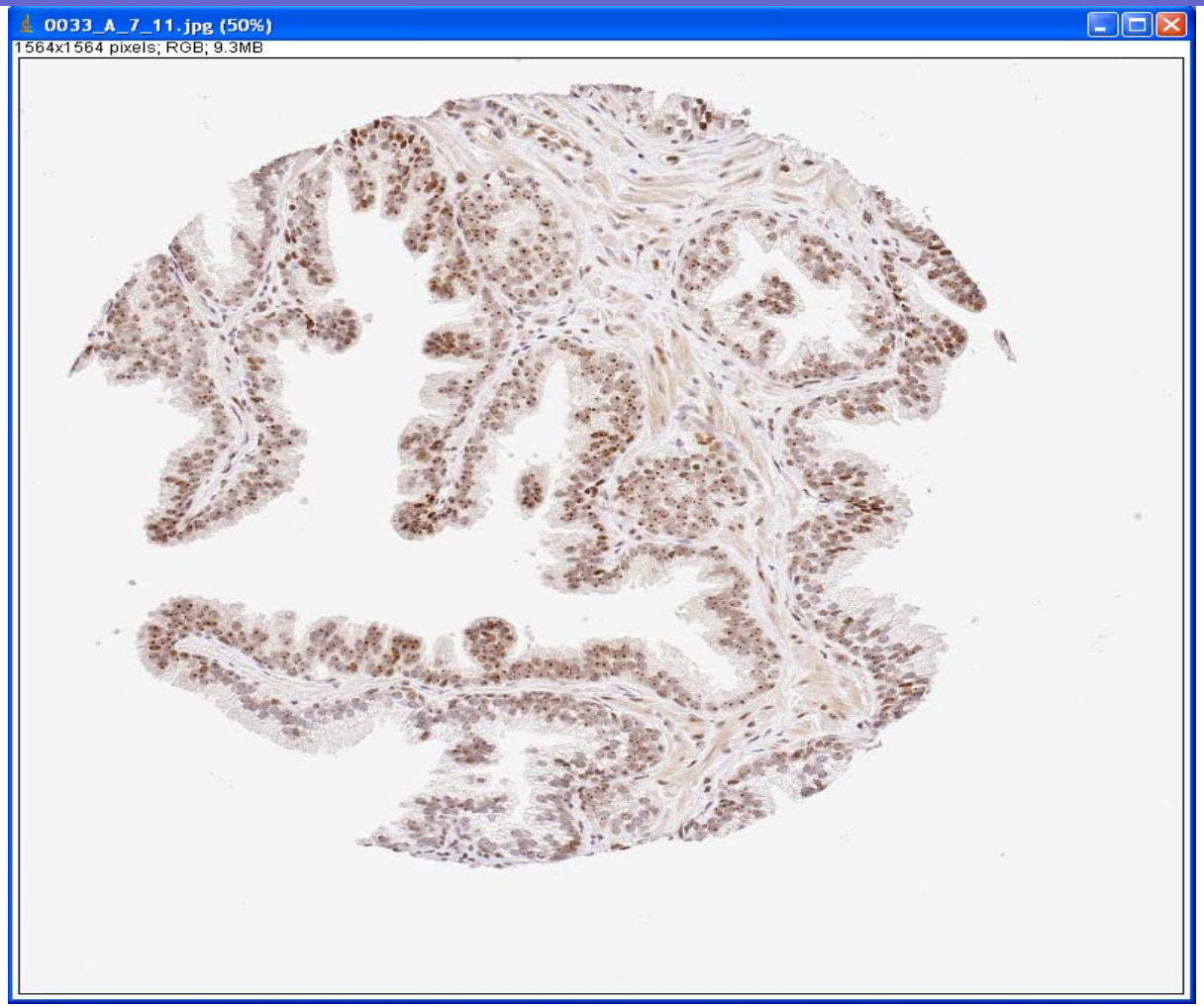
Image information on the pixels is quantified

- Hue (Color) – stain color
- Saturation (Color Purity) – amount of stain
- Luminosity (Intensity) – specimen density
- Good description is found at http://en.wikipedia.org/wiki/HSL_and_HSV

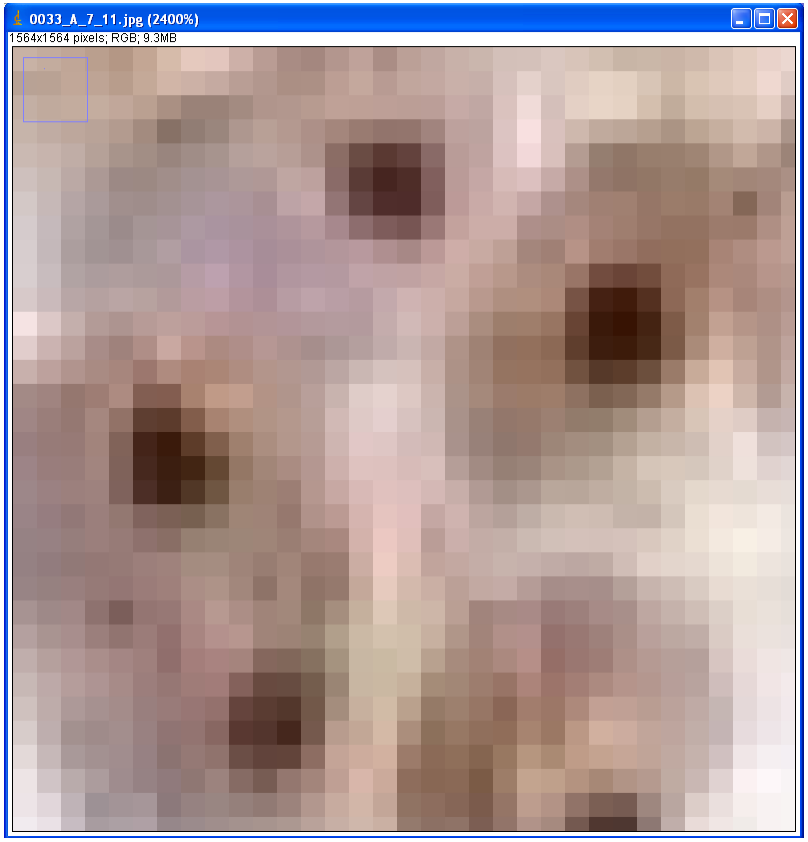
Setting Up a Quantitative Image Analysis

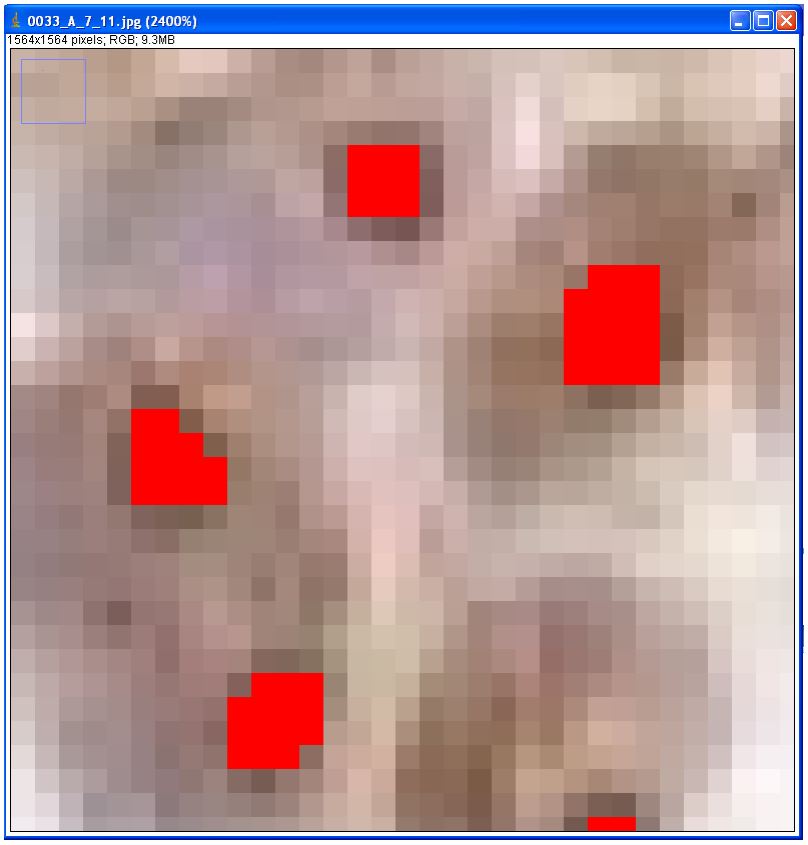
- Create a color mask
- Create a lasso mask
- Create a meta-mask

Creating a Color Mask

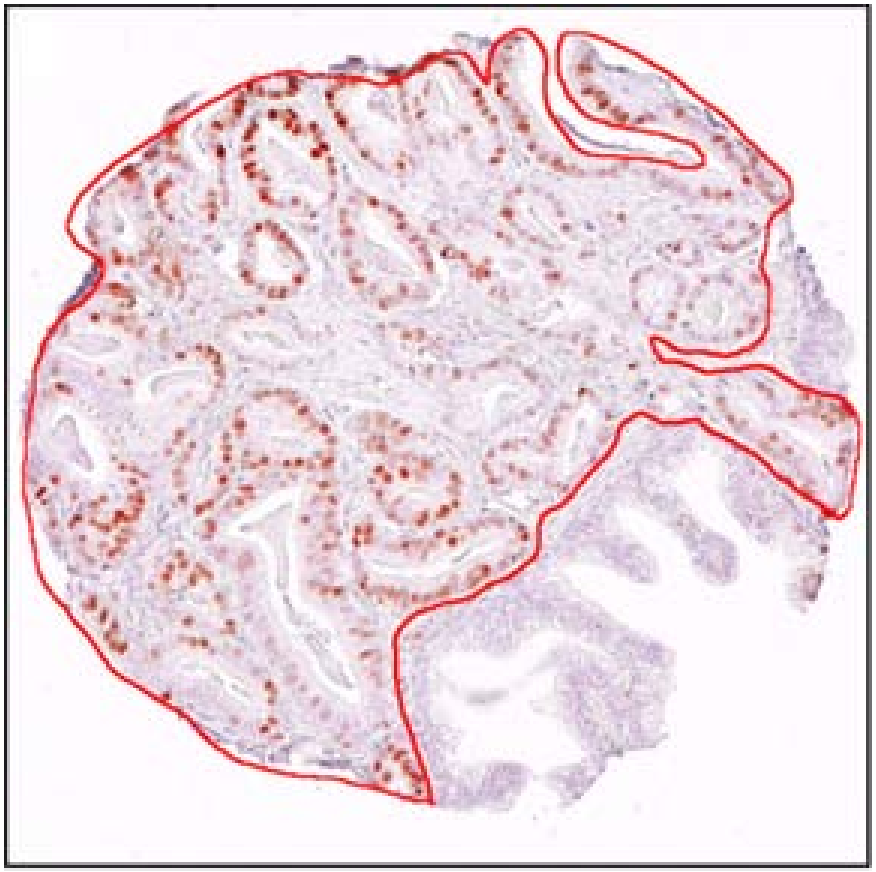


Creating a Color Mask

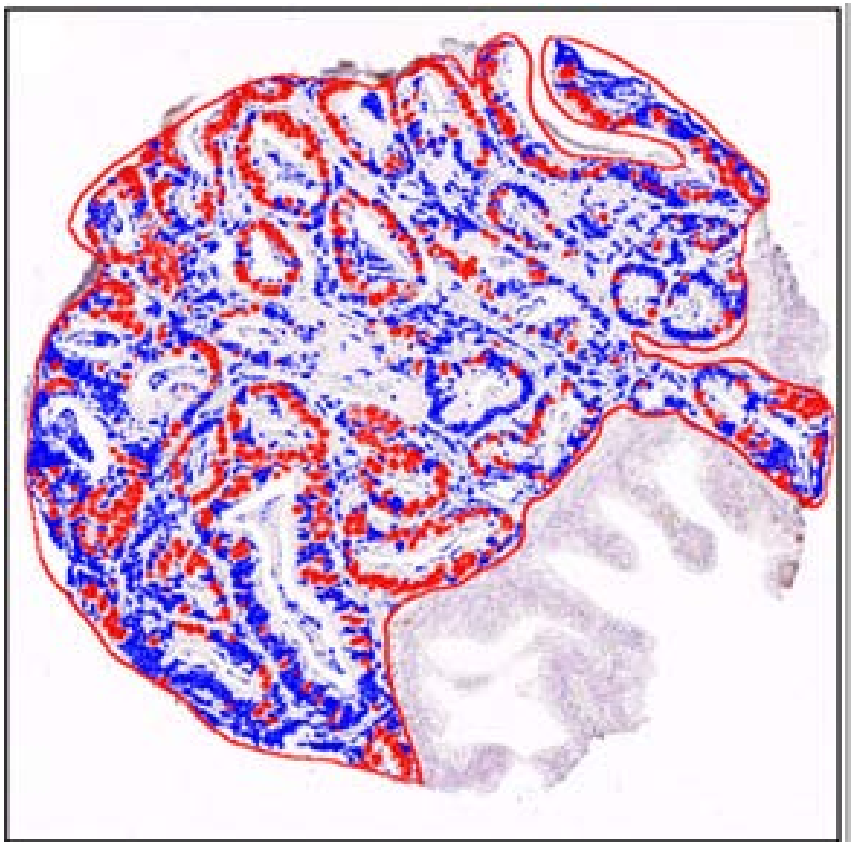




Creating a Lasso Mask



Creating a Meta-Mask Using Boolean Logic



Quantitative Image Analysis Data: Intensity Values

- 0 = no stain
- >0<255 = some stain
- 255 = maximum stain

Quantitative Image Analysis Data: FrIDA

- Blue Intensity Mean
- Brown Intensity Mean
- Blue Total Area
- Brown Total Area
- Brown and Tumor Intensity Mean
- Lasso + Blue or brown Intensity Mean

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 are now linked to “Proteinpedia” database

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

Published TMAJ Images

- 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

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

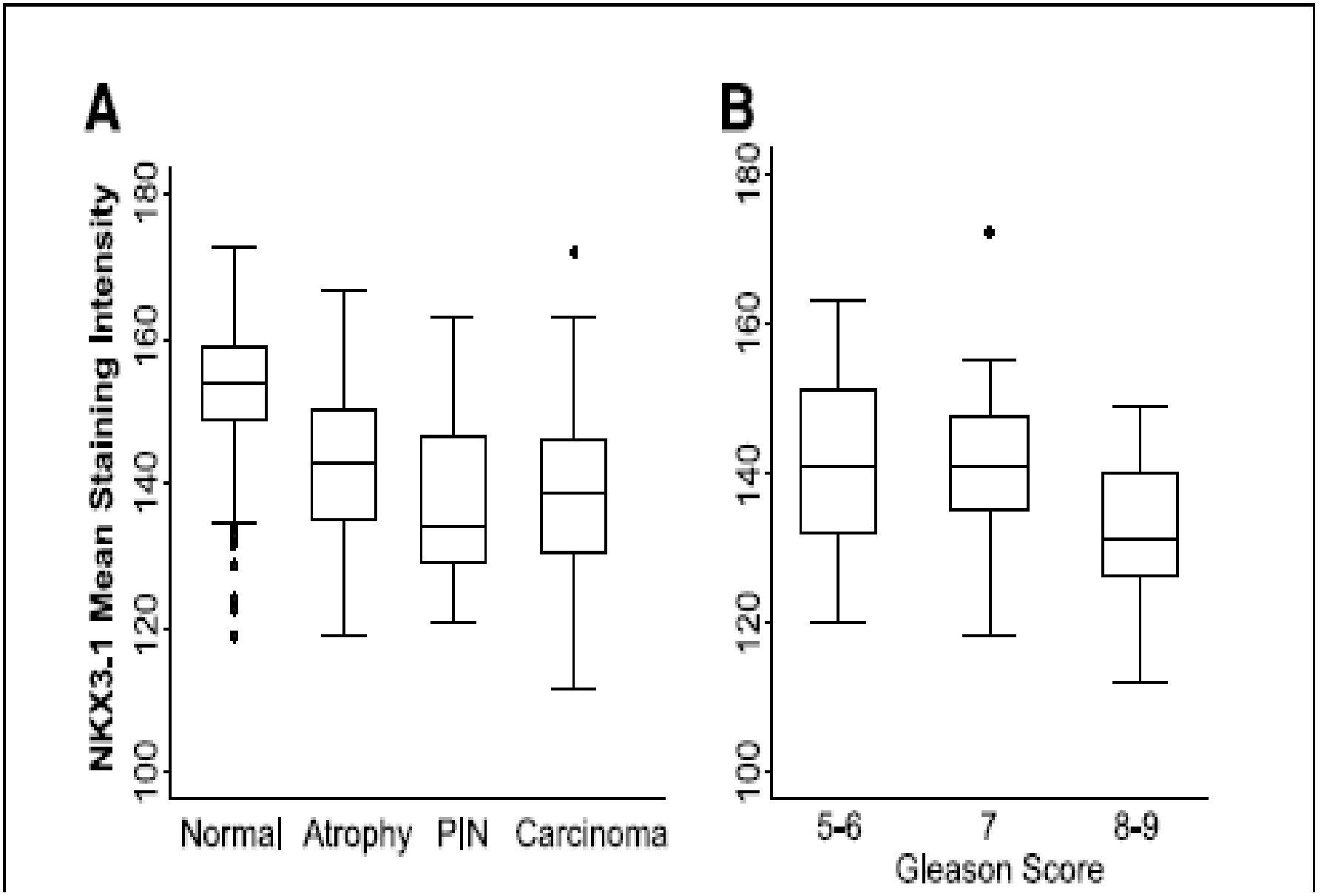
Abstract

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

Modern Pathology (2008) 21, 1156–1167

www.modernpathology.org

Nuclear MYC protein overexpression is an early alteration in human prostate carcinogenesis

Bora Gurel[1], Tsuyoshi Iwata[1], Cheryl M Koh[1], Robert B Jenkins[2], Fusheng Lan[2], Chi Van Dang[3], Jessica L Hicks[1], James Morgan[1], Toby C Cornish[1], Siobhan Sutcliffe[4], William B Isaacs[5,6,7], Jun Luo[5,7] and Angelo M De Marzo[1,5,6,7,8]

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The MYC onco-protein is a transcription factor that regulates cell proliferation, metabolism, protein synthesis, mitochondrial function and stem cell renewal. A region on chromosome 8q24 encompassing the *MYC* locus is amplified in prostate cancer, but this occurs mostly in advanced disease suggesting that *MYC* alterations occur late in prostate cancer. In contrast, *MYC* mRNA is elevated in most prostate cancers, even those of relatively low stage and grade (eg Gleason score 6) suggesting that MYC plays a role in initiation. However, since MYC protein levels are tightly regulated, elevated *MYC* mRNA does not necessarily imply elevated MYC protein. Thus, it is critical to determine whether MYC protein is elevated in human prostate cancer, and if so, at what stage of the disease this elevation occurs. Prior studies of MYC protein localization have been hampered by lack of suitable antibodies and controls. We utilized a new anti-MYC antibody coupled with genetically defined control experiments to localize MYC protein within human tissue microarrays consisting of normal, atrophy, PIN, primary adenocarcinoma, and metastatic adenocarcinoma. Nuclear overexpression of MYC protein occurred frequently in luminal cells of PIN, as well as in most primary carcinomas and metastatic disease. MYC protein did not correlate with gain of 8q24, suggesting alternative mechanisms for MYC overexpression. These results provide evidence that upregulation of nuclear MYC protein expression is a highly prevalent and early change in prostate cancer and suggest that increased nuclear MYC may be a critical oncogenic event driving human prostate cancer initiation and progression.
Modern Pathology (2008) **21**, 1156–1167; doi:10.1038/modpathol.2008.111; published online 20 June 2008

Keywords: MYC oncoprotein; prostatic carcinoma; prostatic intraepithelial neoplasia

Automated subcellular localization and quantification of protein expression in tissue microarrays

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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

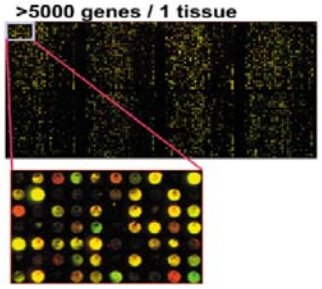
ment for compartmentalization of expression) utilizes fluorescent tags to separate tumors from stroma and to define subcellular compartments. The distribution of a target antigen is then quantitatively assessed according to its co-localization with these tags. As subcellular compartments (for example, 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

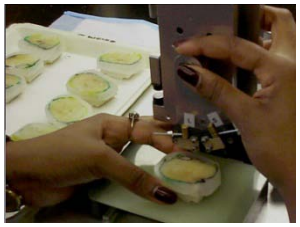
Other Uses of Scanning:

- Telepathology
  - Diagnosis
  - Consultation
- Education

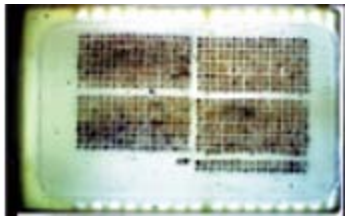
Recap



gene discovery



tissue microarray



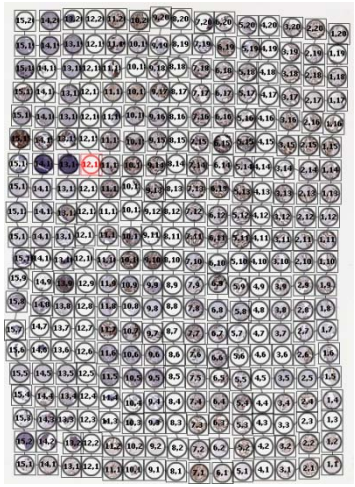


image acquisition and segmentation

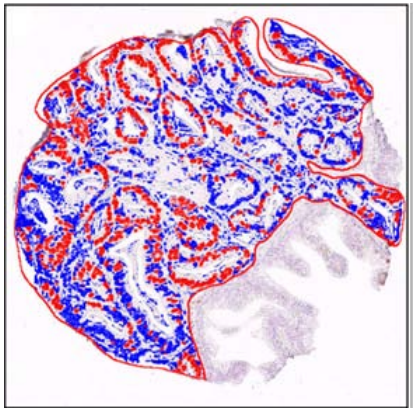


Image analysis

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Helen Fedor, B.S.

Toby Cornish, M.D, Ph.D.

Marcella Southerland, B.S.

James Morgan, B.S.

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