Intrinsic and Extrinsic controls for FFPE tissue

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Disclosure

- I am a consultant, stockholder and scientific founder of HistoRx
- I am an author on the Yale held patent on the AQUA technology.

Use of analyte measurement to determine patient management

Clinician suspects possible diabetes

Obtain tissue sample (blood)

Measure Blood glucose levels in mg/dl (objective)

Treat with appropriate therapy

Clinician suspects possible breast cancer

Obtain tissue sample (core biopsy)

Make Histologic Dx then measure estrogen receptor levels (subjective judgment)

Treat with appropriate therapy

AQUA® method of analyte (estrogen receptor) measurement on a tissue slide

Step 1: Mask (define region of interest, exclude stroma, blank space, etc)

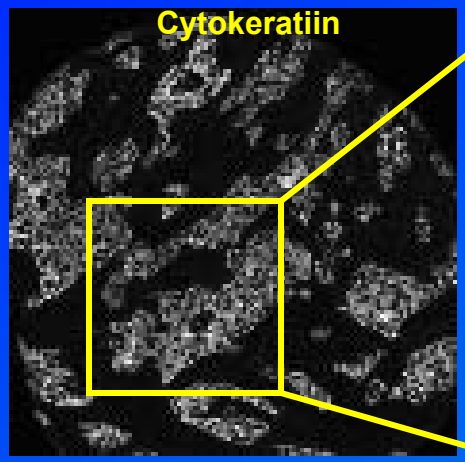
Step 2: Define the numerator and denominator

$$\text{Concentration} = \frac{\text{Numerator}}{\text{Denominator}} \Rightarrow \frac{\Sigma \text{ target intensity in compartment pixels}}{\Sigma \text{ compartment pixel area}} = \text{AQUA score}$$

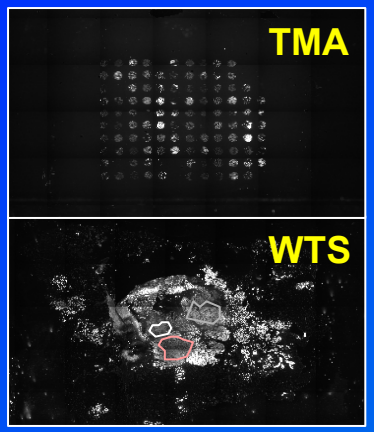
Step 3: Calculate the AQUA score

Step 4: Convert to absolute concentration or normalize to set of uniform standards

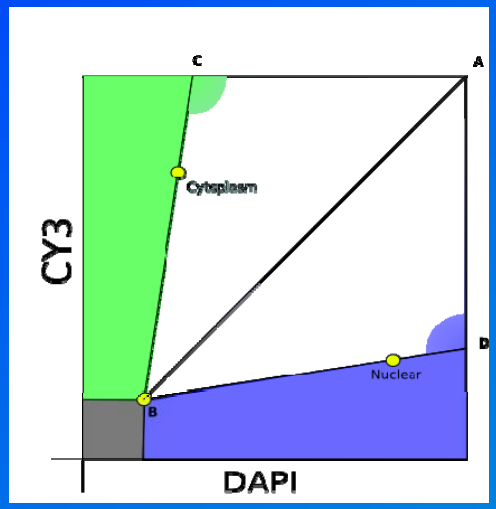
Generating the AQUA® score



Tumor Mask

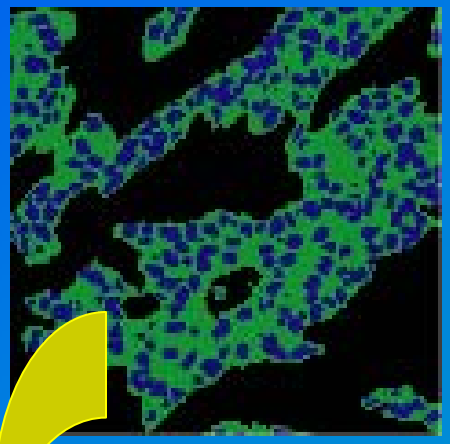


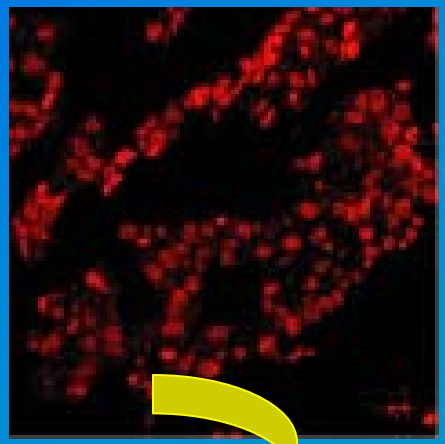
TMA-Tissue Microarray
WTS-Whole Tissue Section



Combine DAPI image and cytokeratin image then cluster to assign each pixel to a subcellular compartment

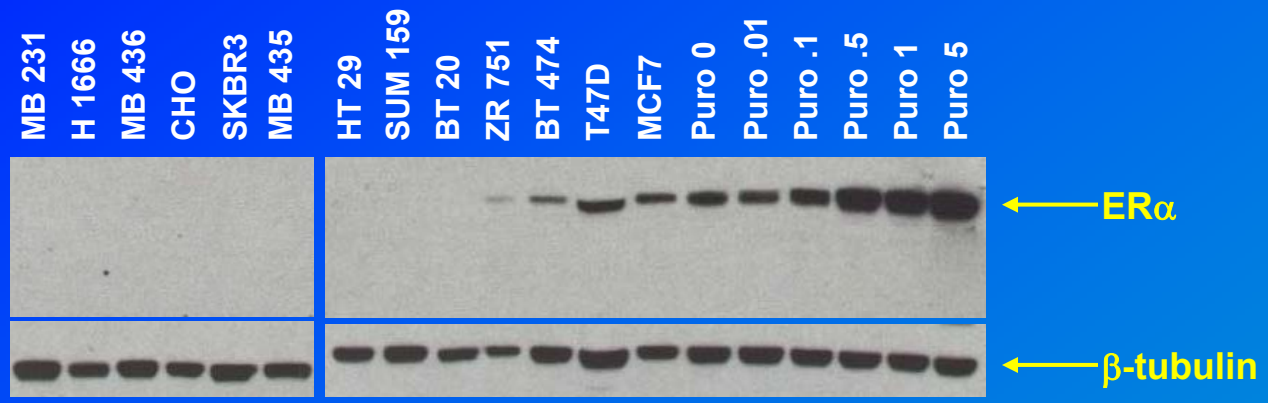
Estrogen Receptor



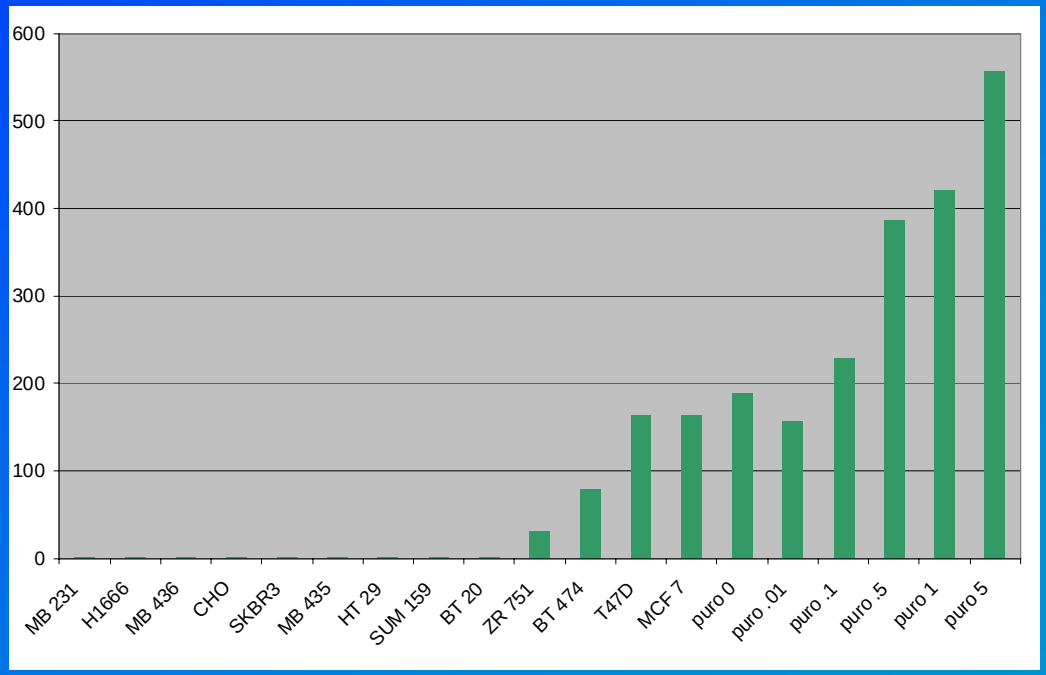


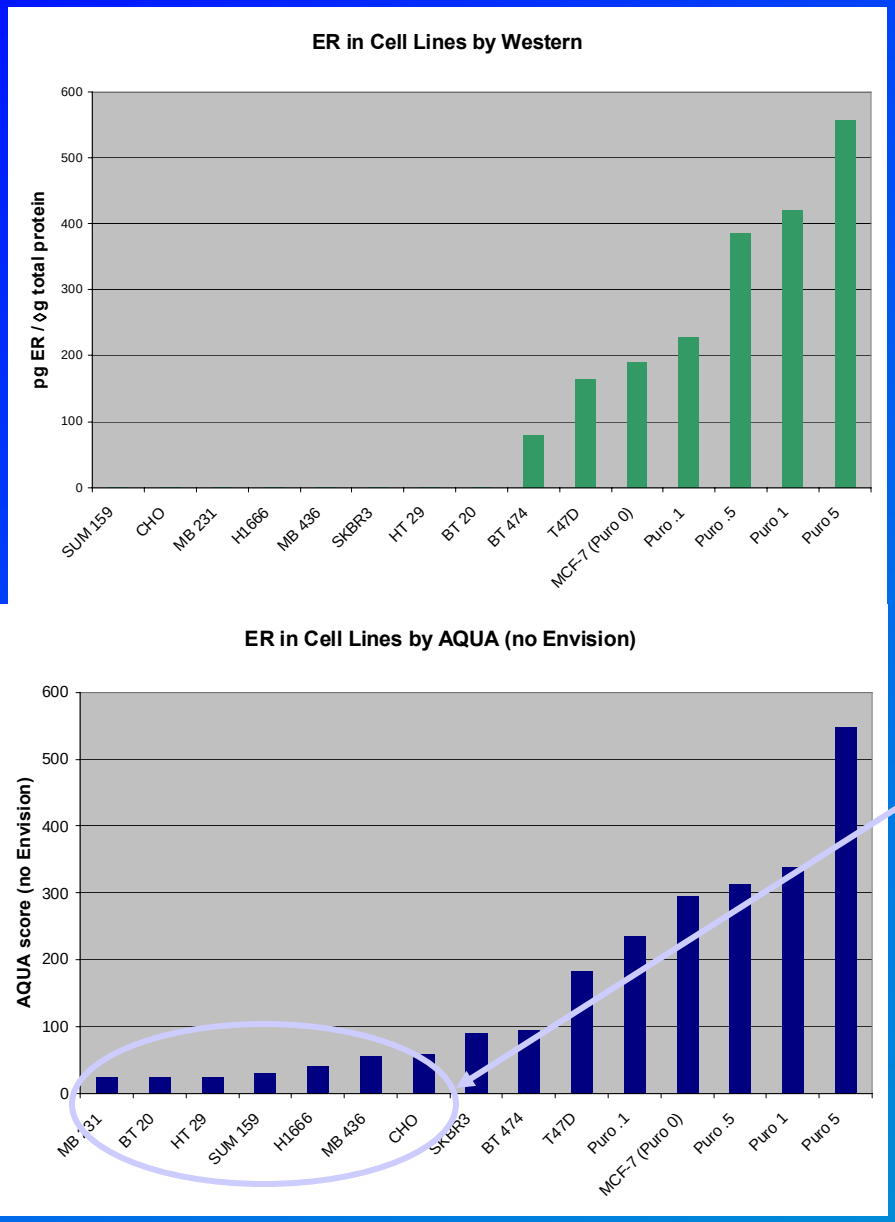
$$\frac{\Sigma \text{ target intensity in compartment pixels}}{\Sigma \text{ compartment pixel area}} = \text{AQUA score}$$

Quantification of western

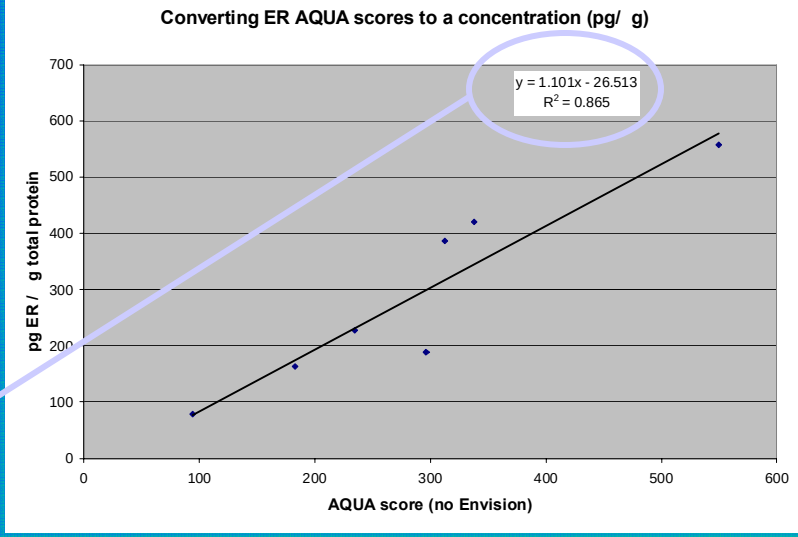


pg ER / µg total protein

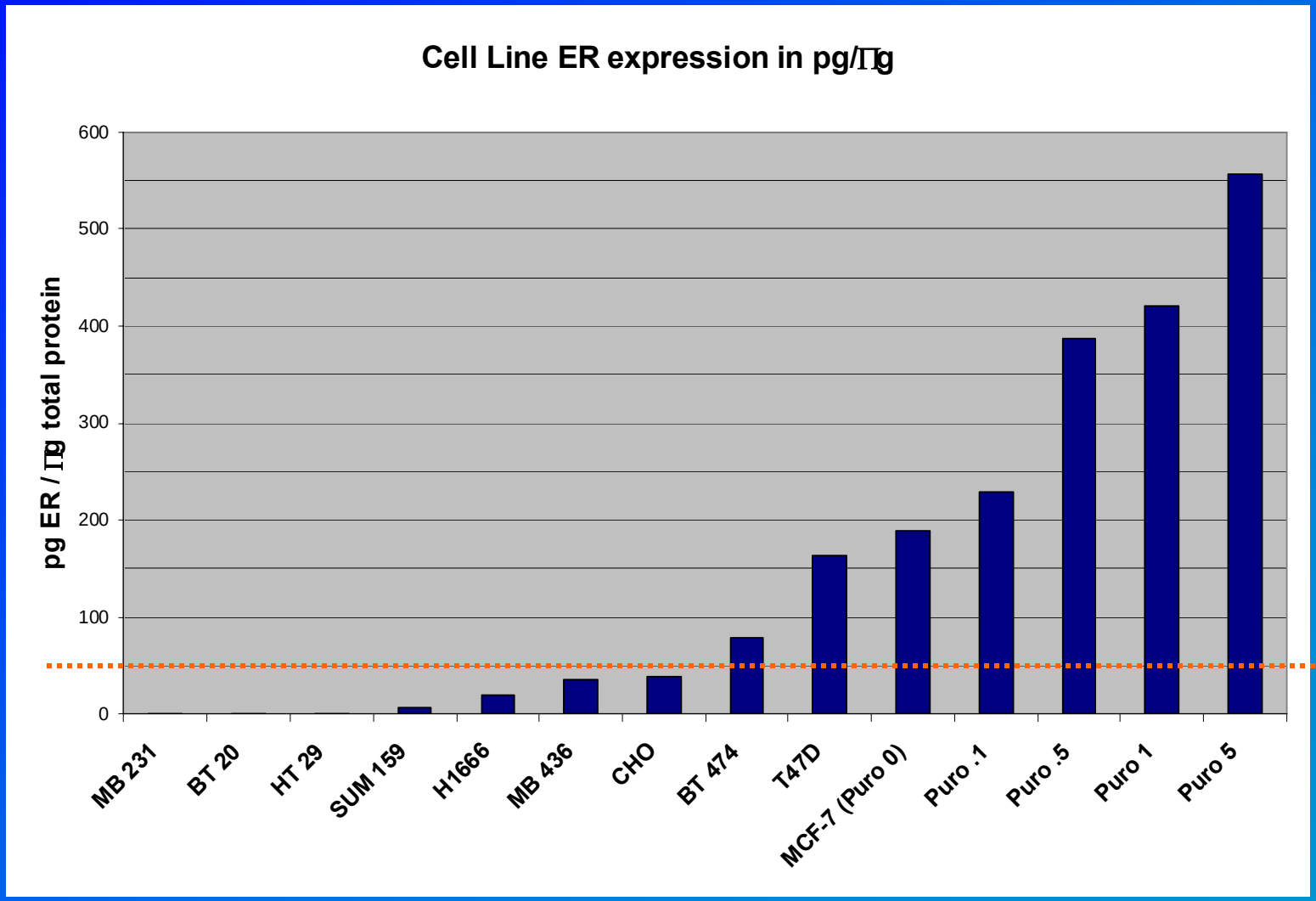




Regression without ER “negatives”

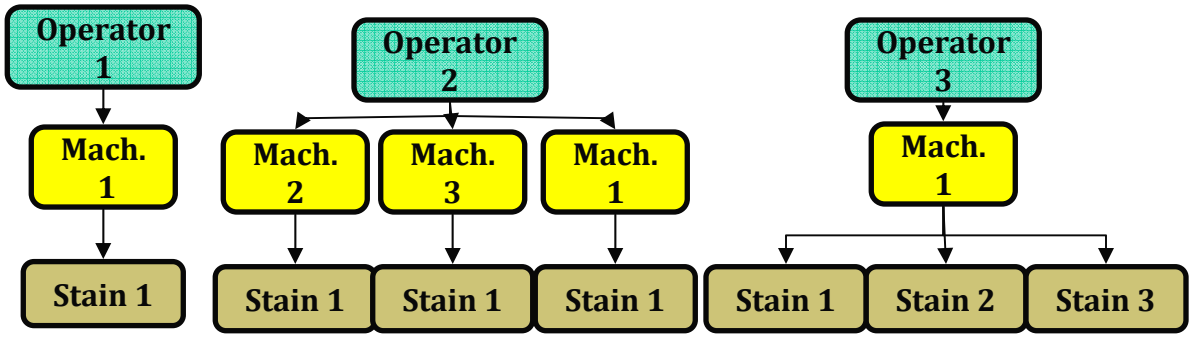


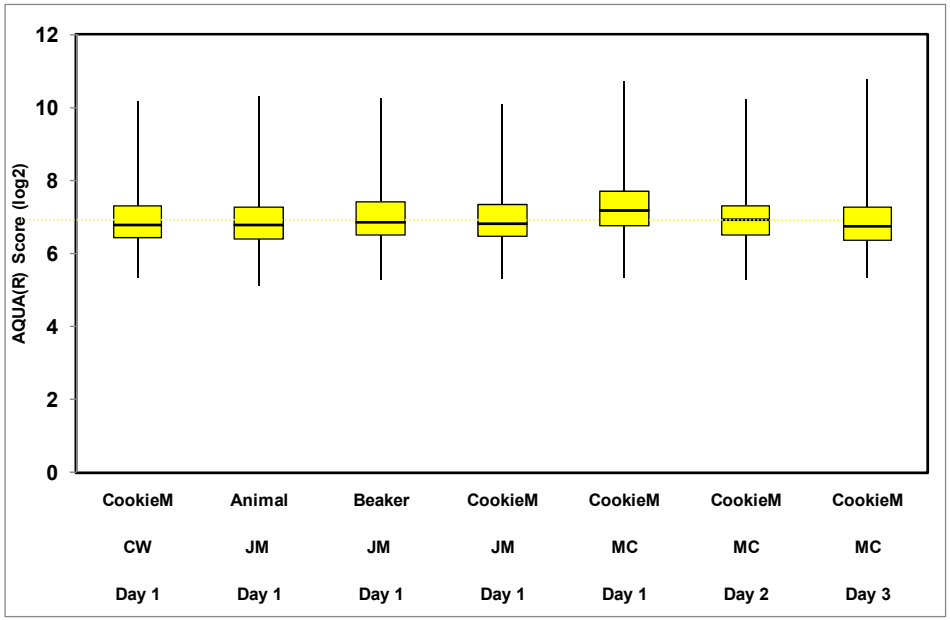
Use this equation to assign pg/μg value based on the AQUA scores for ER “negative” cell lines



Use these cell lines (present on TMAs) to convert patient ER AQUA scores from YTMA 49 to an ER concentration (pg/ug)

AQUA® analysis: Normalization/Standardization Example: HER2



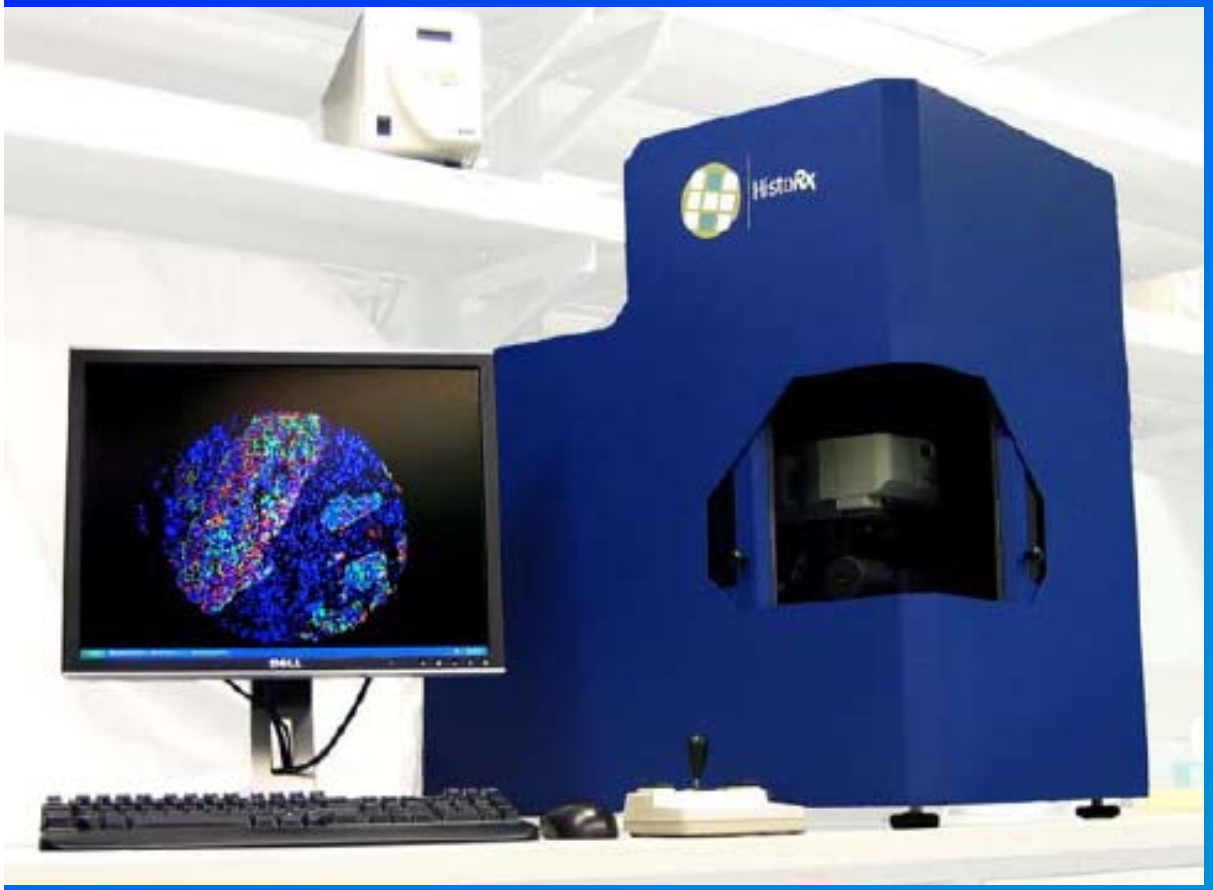


CV=4.3% Cumulative

Mark Gustavson and Jason Christiansen

The HistoRx AQUA® platform

Complete solution - Hardware, Software, Reagents



- Automated Fluorescence Microscopy
  - Expanded dynamic range of measurement
  - Multi-parametric
- Commercially available with about 16 current placements worldwide
- AQUA analysis software compatible with .tiff images
- In use by more than a dozen Pharma companies for drug development
- FDA clearance pending on AQUA software using ER as the example analyte
- US Patent 7,219,016

Summary:

- **Absolute values of Estrogen Receptor can be obtained by conversion from Western Blot to Cell Line Standards to Breast Cancer Tissue**

- **The minimal expression level detectable by this system is about 50pg/ug total protein**

- **Range of ER expression in patients is between 50pg/ug and about 1500 pg/ug**

- **Reproducibility for AQUA has shown coefficient of variation (CV) of around 5%**

- ***Someday: CLIA guidelines for Estrogen Receptor:***
Threshold for detection = 100pg/ug total protein
Acceptable variation = +/- 10% (20pg/ug)

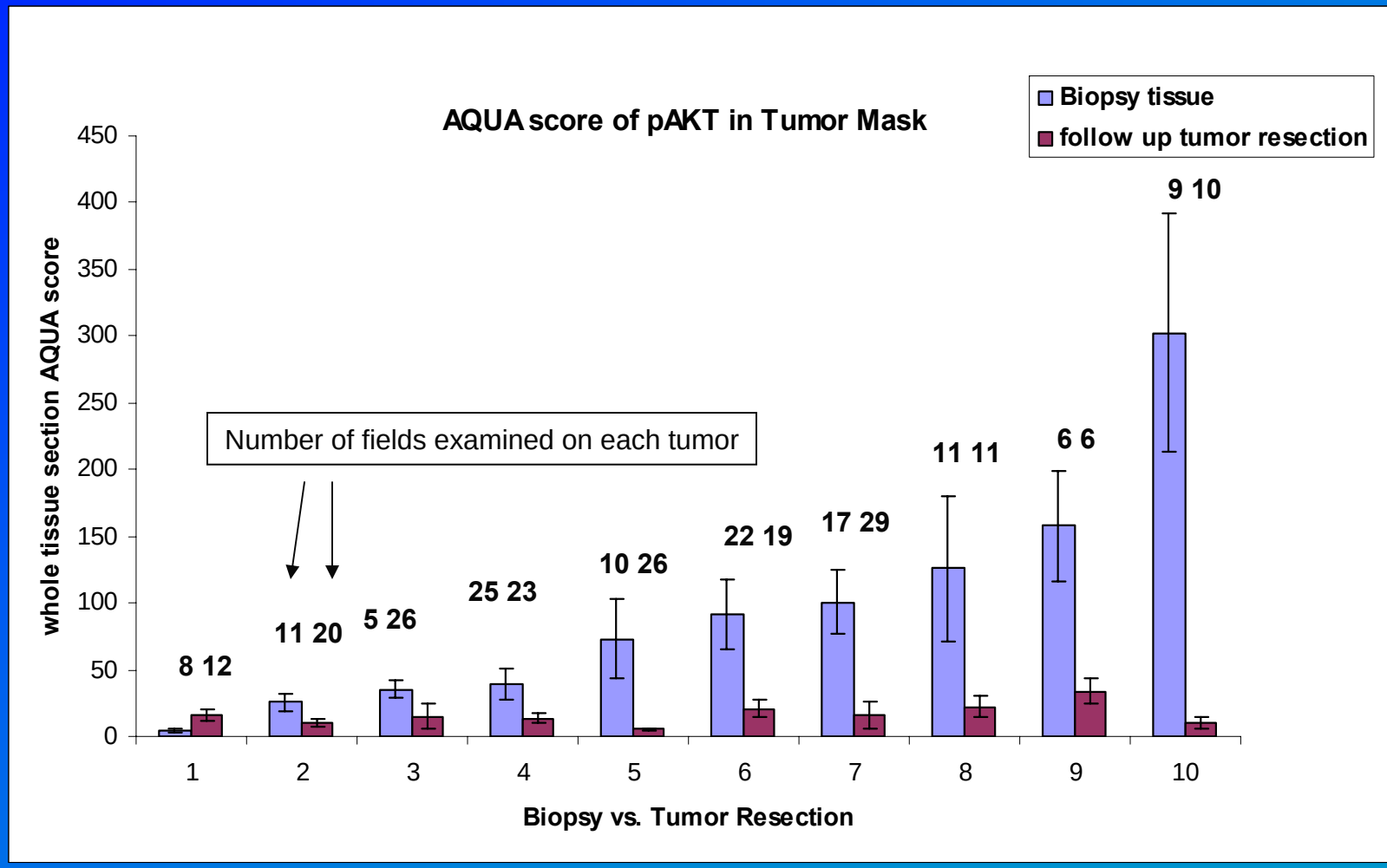
Standardization

- Extrinsic controls
  - Controls for the process of analyte detection external to the FFPE sample (ie the antigen retrieval, staining, visualization and quantification)
- Intrinsic controls
  - Controls for specimen integrity based on properties of the tissue that have been subjected to all of the pre-analytical processes in parallel with the analyte (ie controls for under-fixation, cold ischemic time, etc.)

Key Pre-analytic Variables for measuring analytes on slide in formalin-fixed paraffin embedded tissue

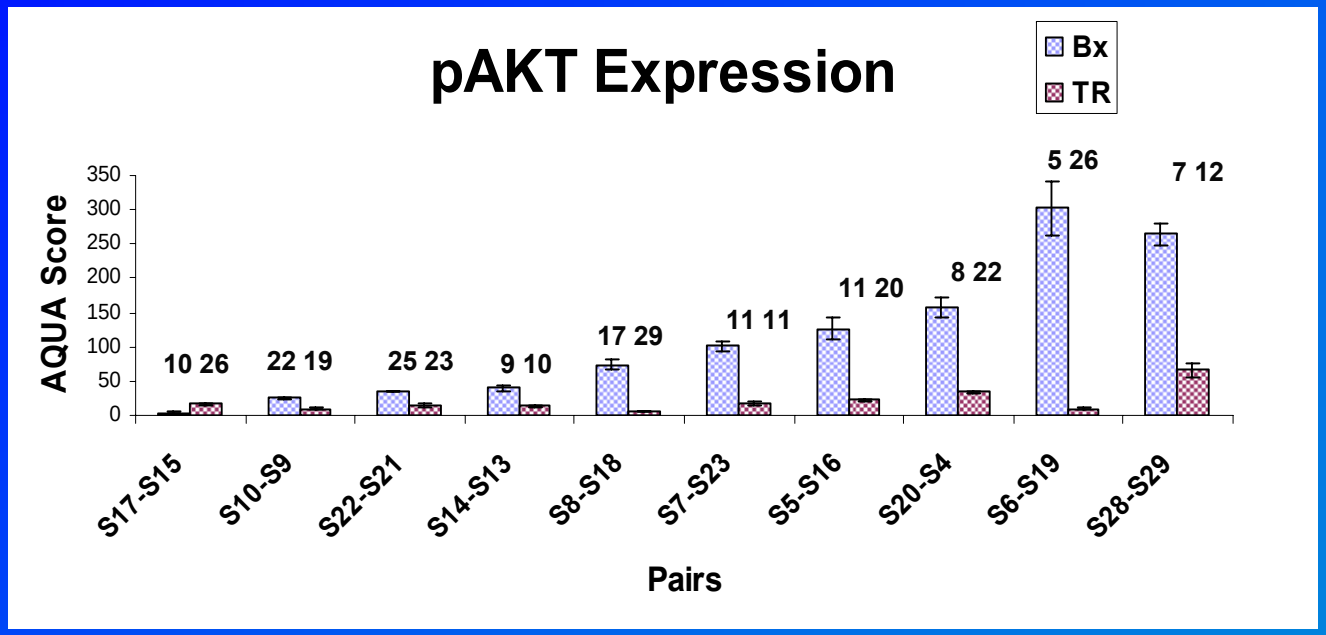
1. Time to Fixation (cold ischemic time)
2. Method of Tissue Acquisition
3. Fixative and Method of Fixation

Whole section analysis of core biopsy vs. resection to assess affect of ischemic time on stability

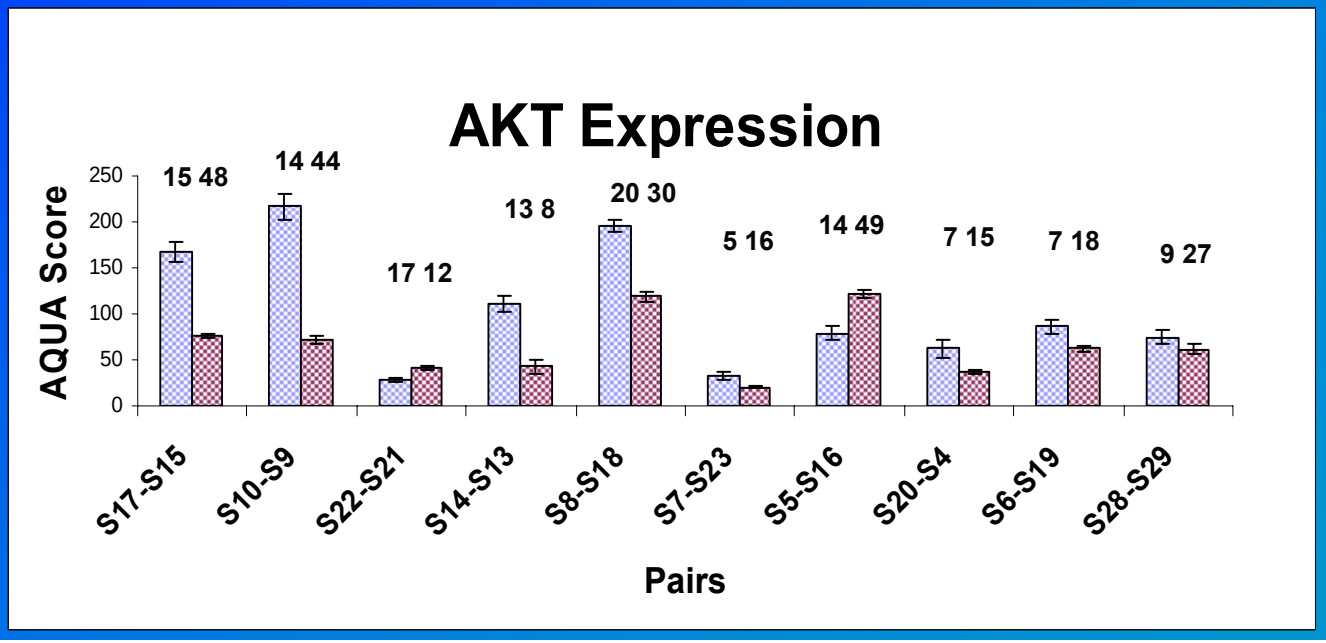


Yalai Bai and Eirini Pectacides

Lower Expression of pAKT in the resections than in CNBs

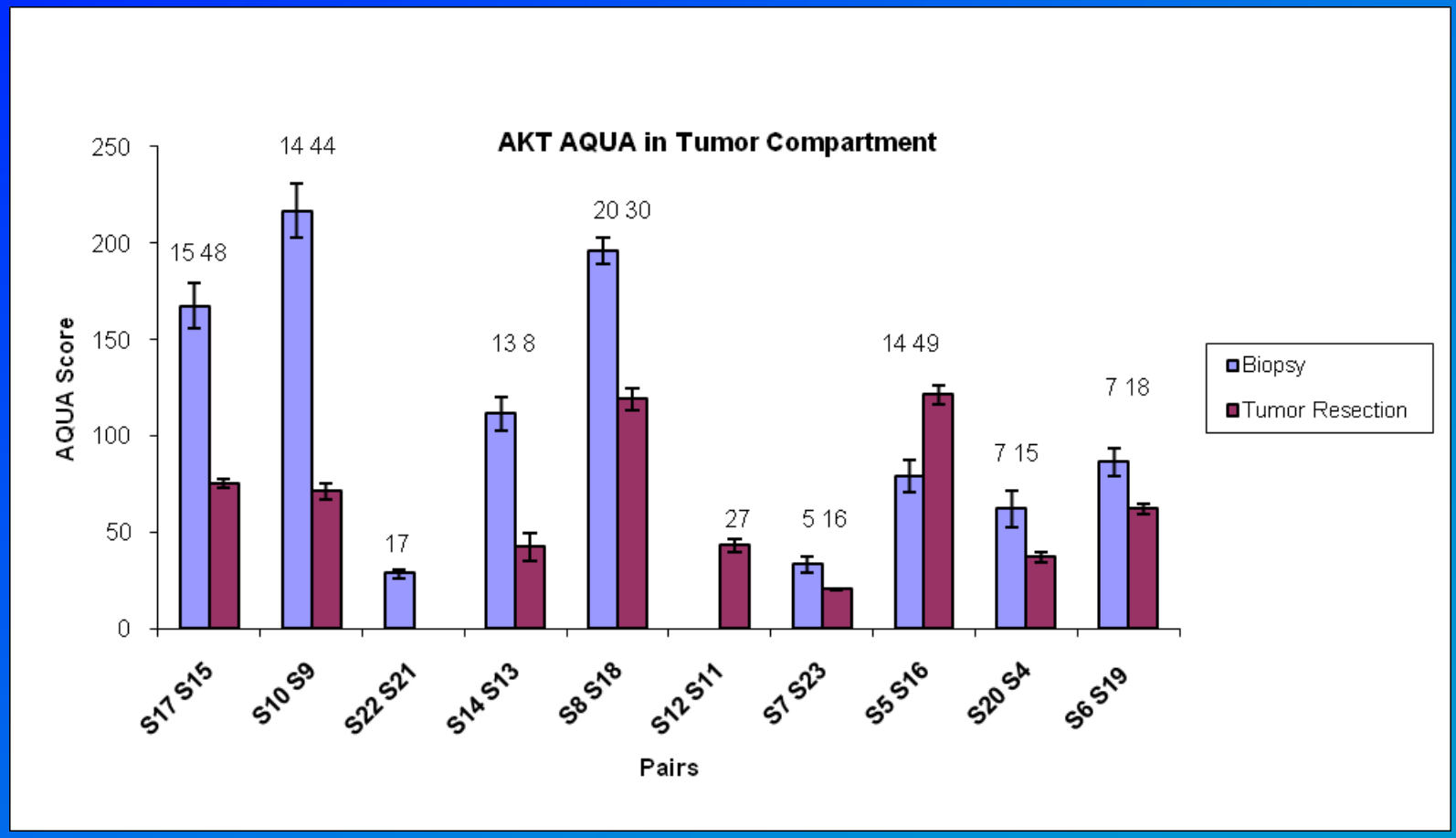


Wilcoxon Signed Ranks test
p=0.004

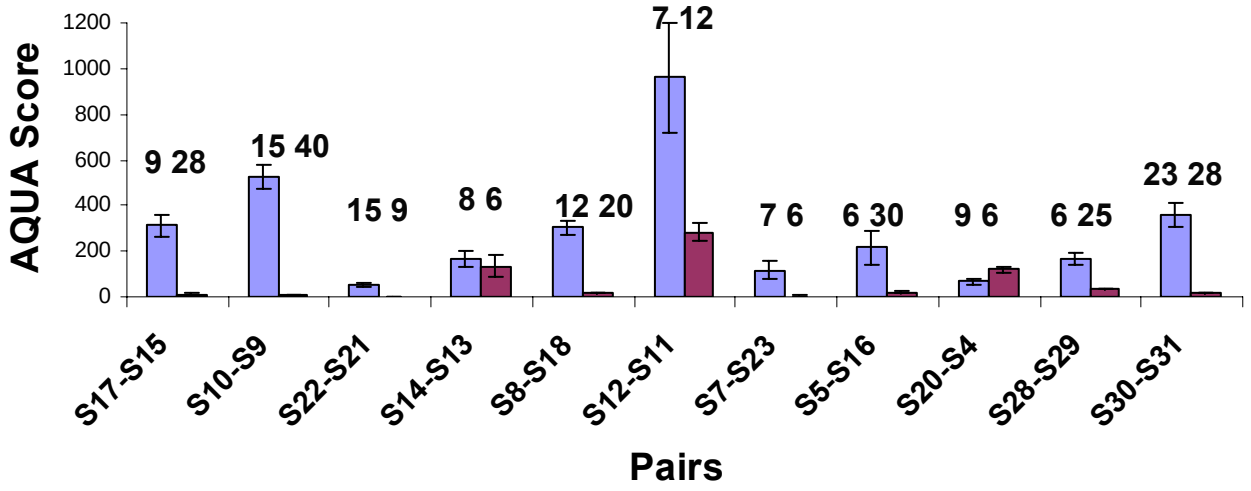


Wilcoxon Signed Ranks test
p=0.065

Whole section analysis of core biopsy vs. resection to assess affect of ischemic time on stability for Total AKT



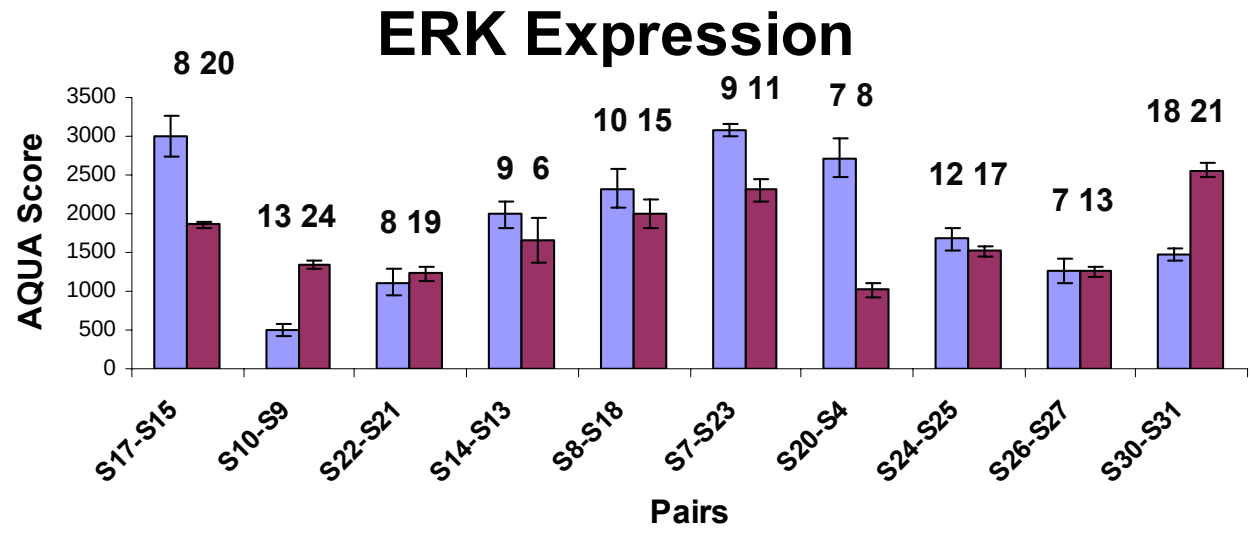
Lower Expression of pERK in the resections than in CNBs



CNBs

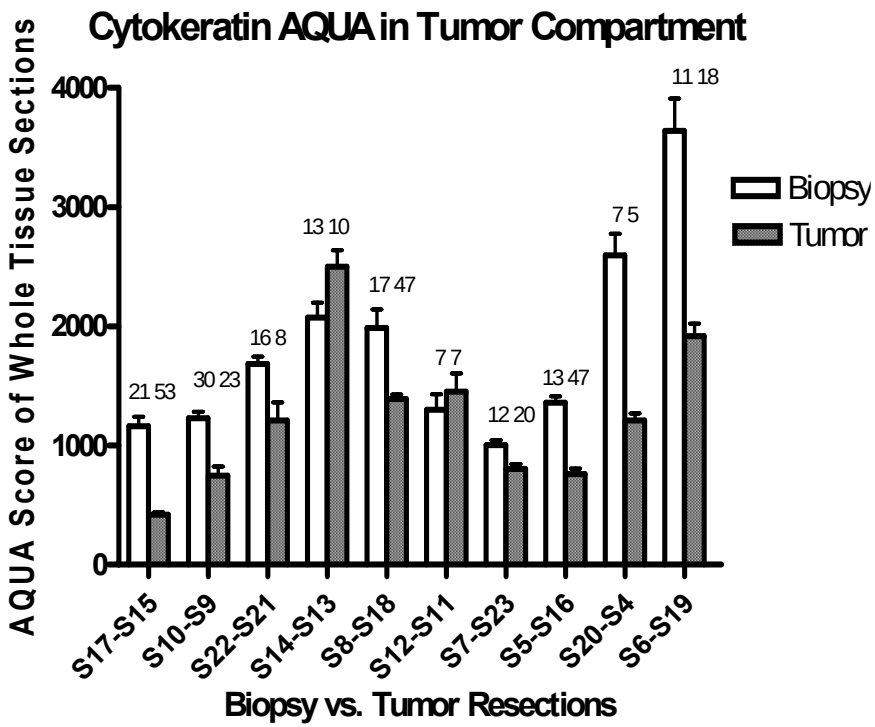
Tumor Resection

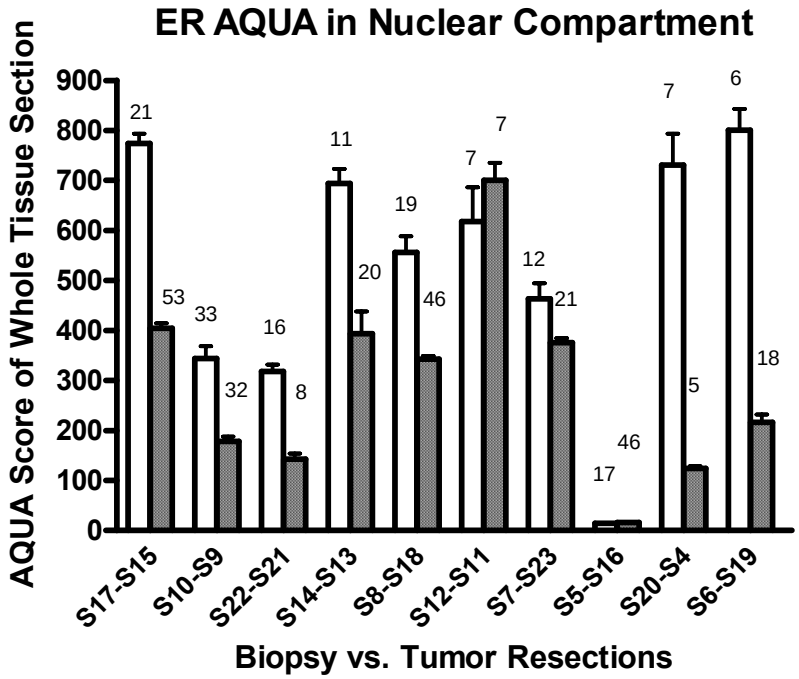
Wilcoxon Signed Ranks test
p=0.005



Wilcoxon Signed Ranks test
p=0.38

Significant Difference in ER Levels between Core Bx and Resection

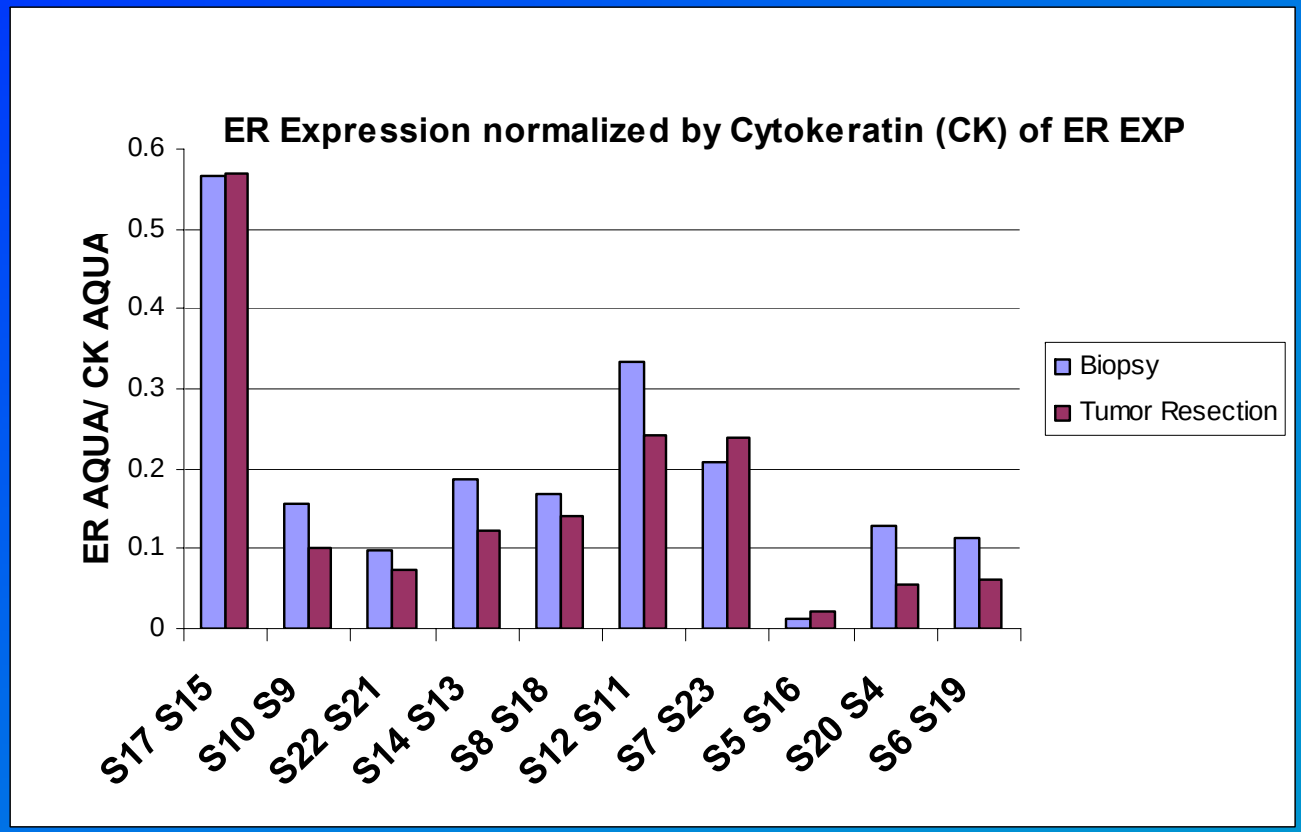


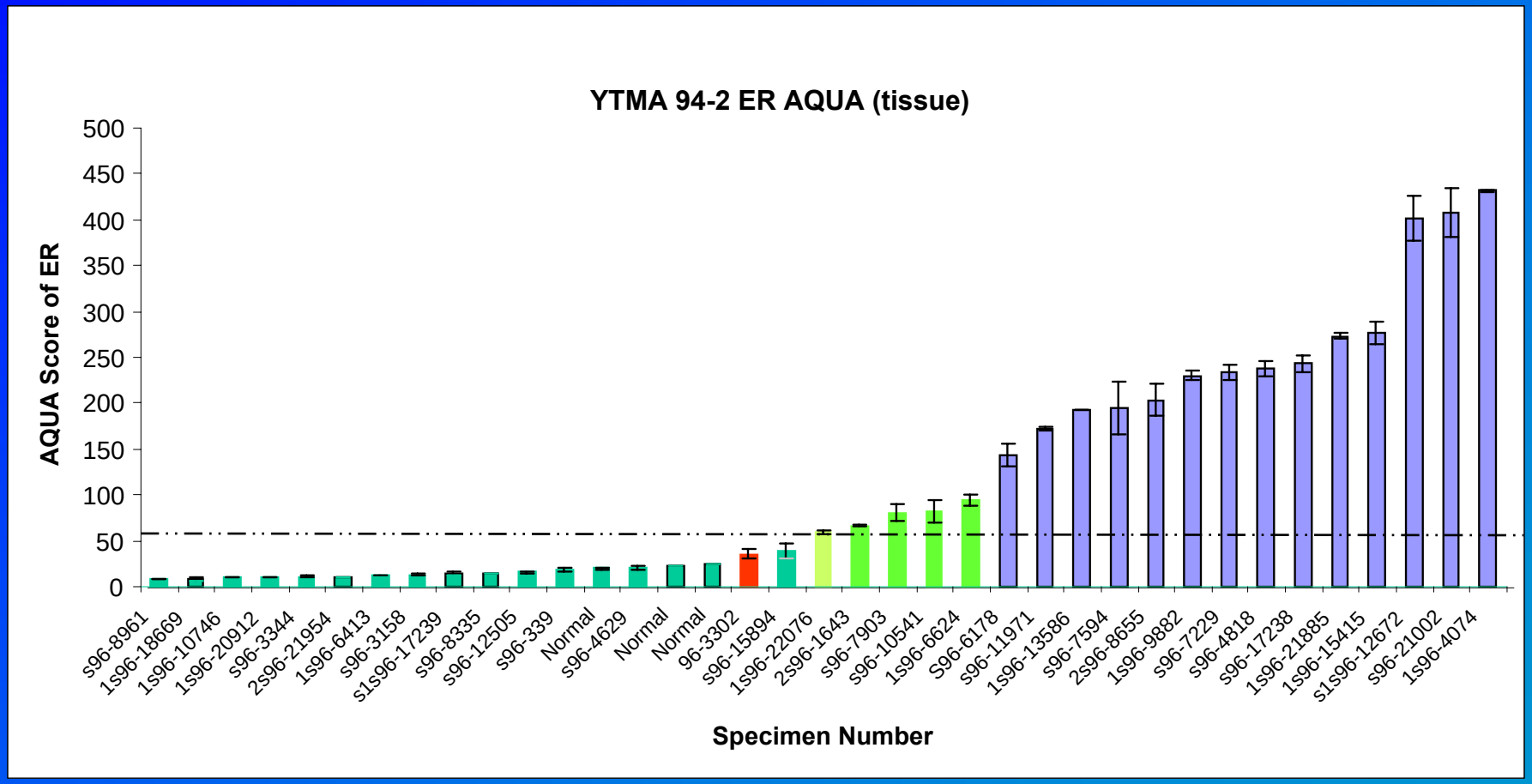


Wilcoxon signed rank test
P value 0.0098

Yalai Bai

Normalization by CK expression results in loss of statistically significant decrease in ER





	CoPath Database (Yale Pathology)	ER AQUA
	+	+
	-	+
	-	+ weak
	+	-
	-	-

Thanks to:

Rimm Group:
Robert Camp
Elsa Anagnostou
Bonnie Gould Rothberg
Veronique Neumeister
Seema Agarwal
Eirini Pectasides
Huan Cheng
Maria Baquero
Alley Welsh
Jason Hanna
Jennifer Bordeaux
Matt McRae
Bill Bradley
Camil Liceaga
Summar Siddiqui
Liz Killiam

Former Rimm Group:
Marisa Dolled-Filhart
Maciej Zerkowski
Kyle DeVito
Melissa Cregger
Tony McCabe
Satish Avvaru
Greg Tedeschi
Mark Gustavson
Gretchen Graff
Carola Zalles
Aaron Berger
Sharon Moulis
Malini Harigopal
Chris Moeder
Jena Giltnane

Tissue Microarray Facility
Lori Charette
Joe Salame
Sudha Kumar
Peter Gershkovich

Yale Collaborators
Annette Molinaro
Gina Chung
Harriet Kluger
Ruth Halaban
Lyndsay Harris
John McClaskey

Outside Yale
Lisa Ryden (Lund)
Karin Jirstrom (Malmo)
David Huntsman (BCCA)
Karen Gelmon (BCCA)
Elaine Alarid (UW)

Rimm Lab Summer ‘08

www.tissuearray.org

Intrinsic Controls for FFPE tissue

- ***Goal 1: To generate two "discovery" tissue sets to assess "pre-analytical" variability.***

Goal 2: Assessment of markers of cold ischemia ("housekeeping markers") on discovery cohorts

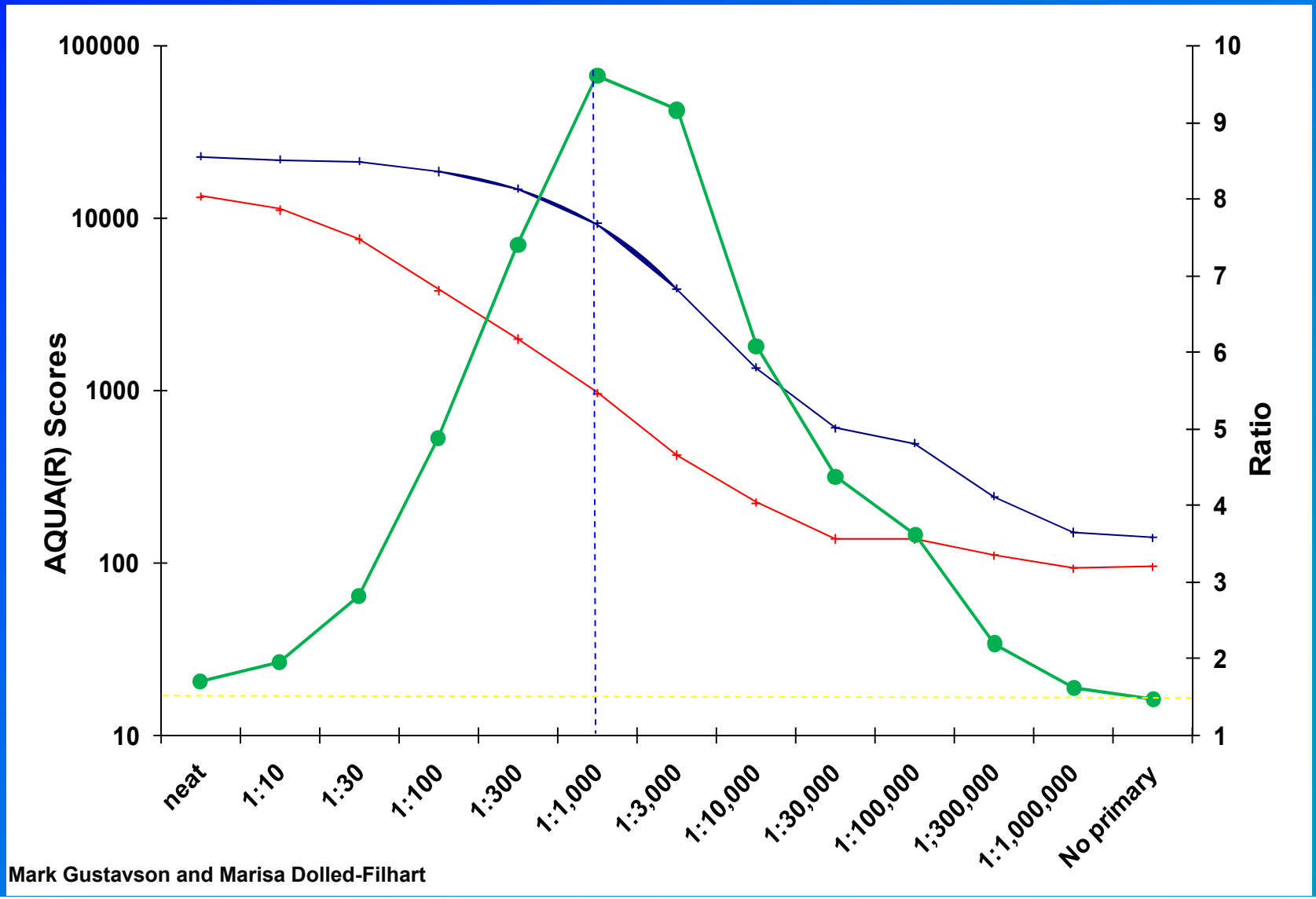
Goal 3: Assessment of markers of hypoxia on discovery cohorts

Goal 4: Generation of a Multiplexed "Tissue Immunologic Competence" (TIC) Model for normalization of tissue handling that measures tissue integrity for immunological assessment

Goal 5: Validation testing of the TIC Model in two core vs. resection specimen studies

Goal-specific Task:	Milestone:
1A. To collect tissue blocks from patients with matched pairs of core biopsies and resection specimens	1A. Establishment of a fully annotated set of 300 core needle biopsy (CNB) and resection tissue pairs from breast cancer patients
1B. To collect a cohort of patient tissues with recorded time to fixation	1B. Construction of a 2x redundancy tissue microarray (TMA) representing 100 specimens with a wide range of recorded times to fixation
2. To select, titer, validate and assess expression of a series of 15-25 proteins that could have value in monitoring cold ischemia “house-keeping genes”	2. To select and report 3-5 proteins whose change is proportional and reflective of degeneration as a function of cold ischemic time
3. To select, titer, validate and assess expression of a series of 15-25 proteins that could have value in monitoring hypoxia	3. To select and report 3-5 proteins whose change is proportional and reflective of degeneration as a function of hypoxia
4. To use Part-DSA or other methods to define a set of markers from the best results from milestones 2 and 3 to generate five “Tissue Immunologic Competence” (TIC) models	4. To report the 5 best TIC models in preparation for competitive testing to choose the best one
5A. To test the 5 TIC models to assess EGFR expression on whole sections from the Thoracic Surgery Core Series Study.	5. To report, patent and prepare to commercialize the best TIC model based on the results of Tasks 5A-B.
5B. To test the 5 TIC models on an independently collected series of breast cancer cases with matched core biopsies to test ER expression.	

Objective Optimization of Antibody Concentration



Mark Gustavson and Marisa Dolled-Filhart