

Integrating Biotechnology into Translational Cancer Research

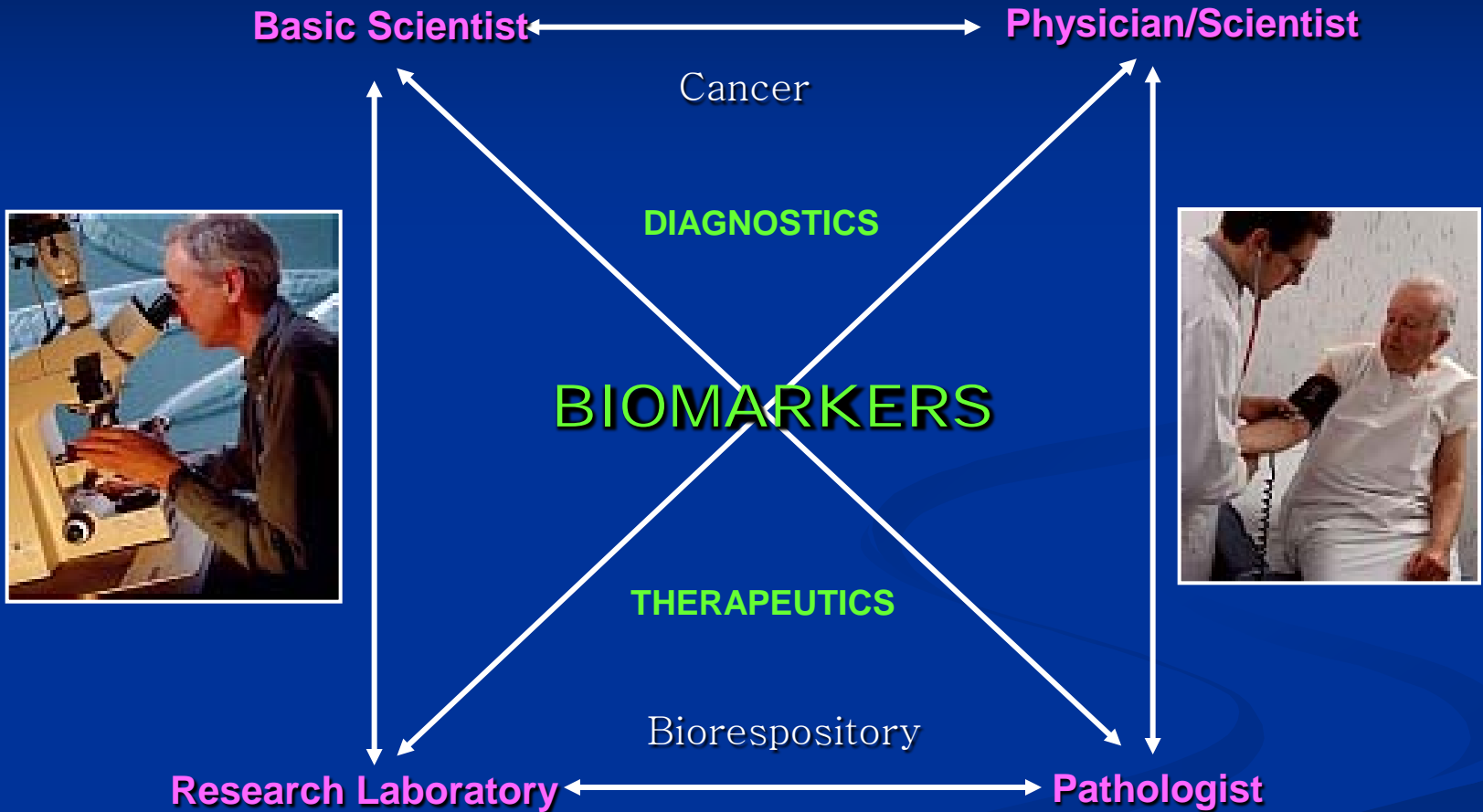
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Director

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Eastern Virginia Medical School
Norfolk, VA***

*Eastern Virginia Medical School
Discovery Laboratory*



TRANSLATIONAL RESEARCH PROGRAM



BIOMARKER DISCOVERY



“Fishing Expedition”
Unbiased Discovery



“Maybe Peyton Manning is good at baseball”
“Maybe my favorite gene is a biomarker”
Targeted Discovery

Rational Design



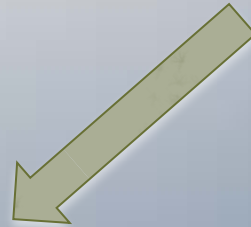
“Data Analysis”



“Orthogonal Confirmation”
“Death by Validation”



“Systems Biology”



“Integromics”

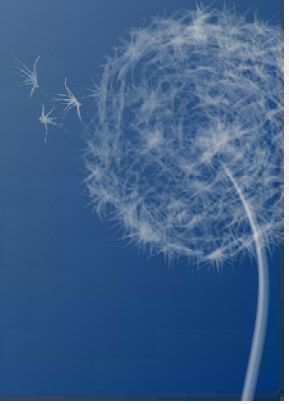


CBIDRC Biotechnology Expertise

Genomics

Proteomics

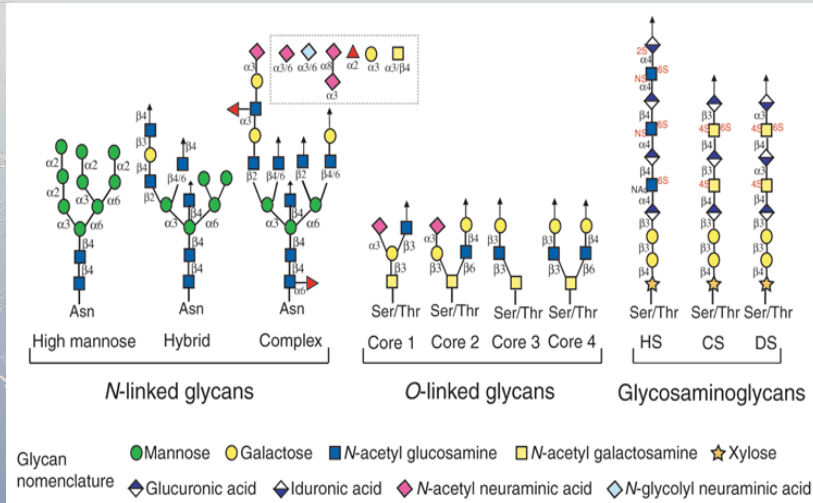
Metabolomics



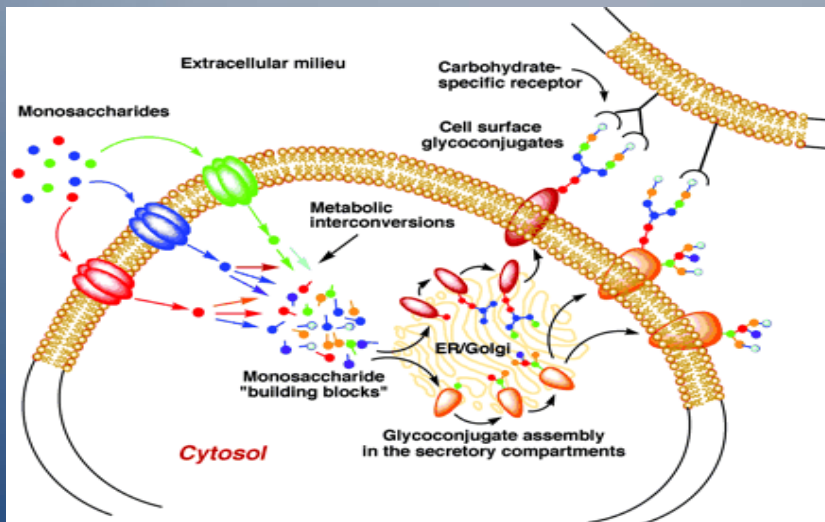
Example Technologies

- ✿ Glycoproteomics
- ✿ Imaging Mass Spectrometry
- ✿ Quality Human Tissues for PRoBE compliant study

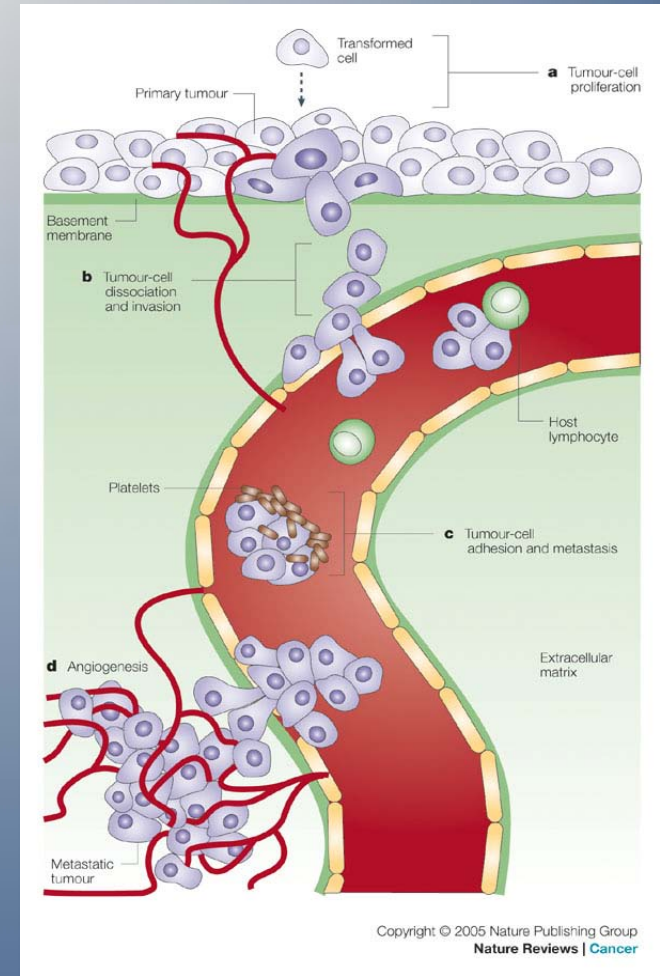
Glycoproteomics



Rasman, R. Nature methods 2, 817 (2005)



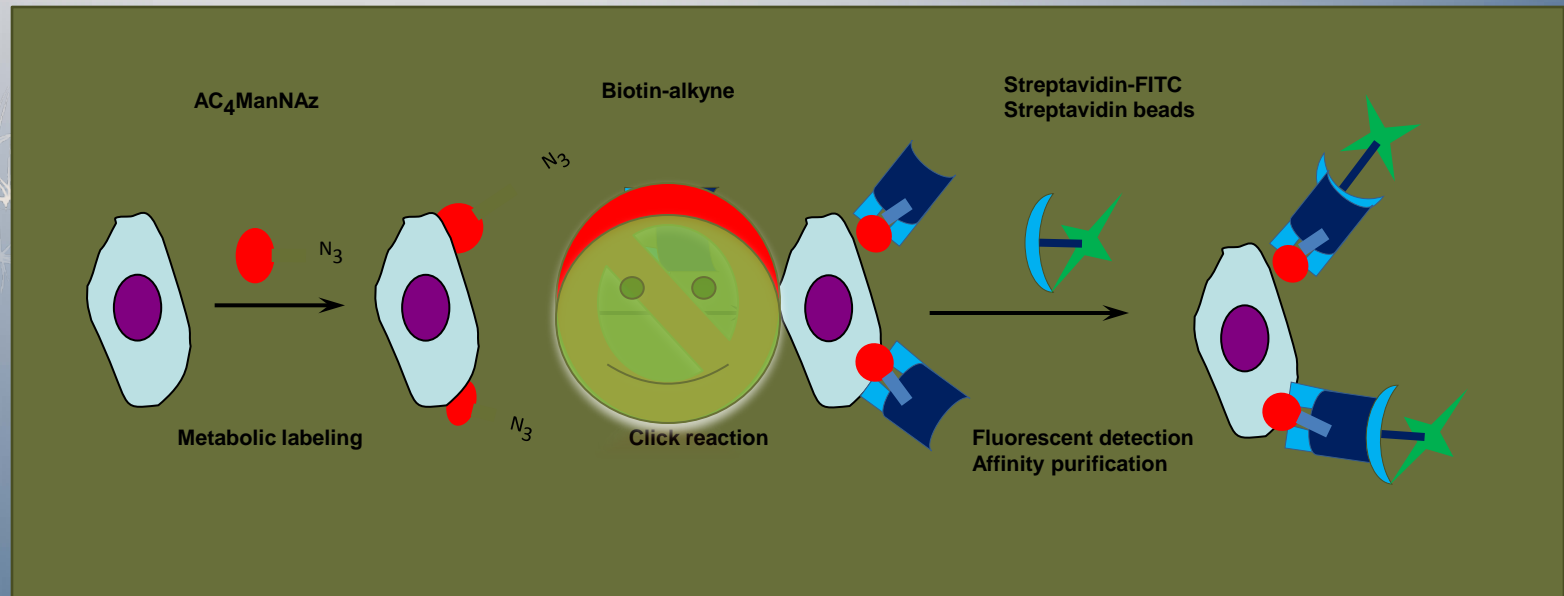
Bertozzi, CR. Science 291, 2357 (2001)



Fuster MM, Nature review cancer 5, 526 (2005)

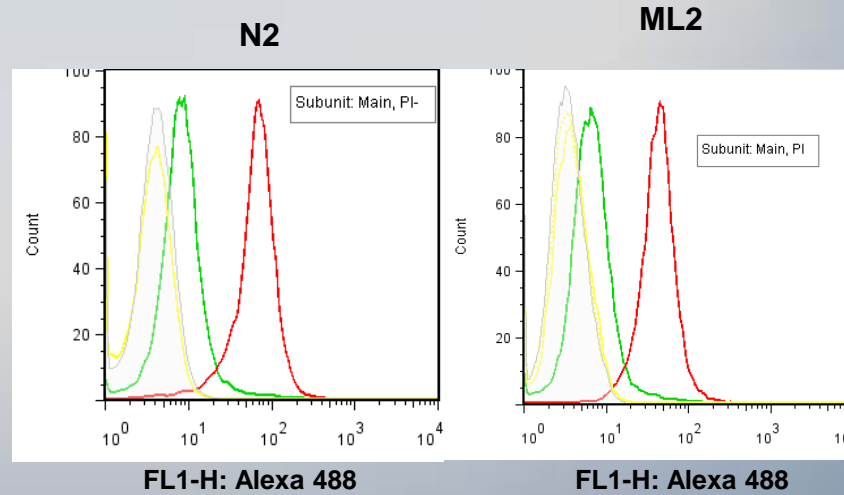
Can we identify a cell surface glycoprotein involved in metastasis?

N2 versus ML2

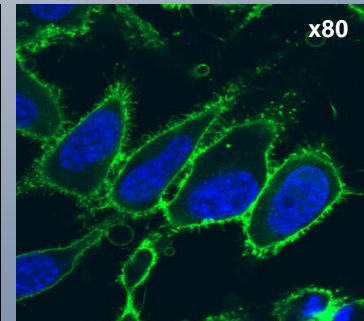
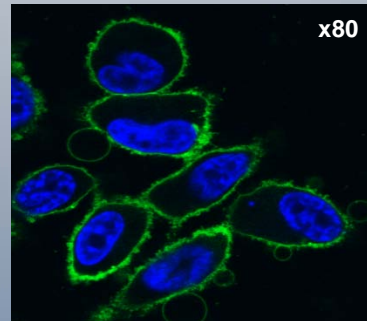


1. Metabolic labeling and chemoselective conjugation “CLICK”
2. Affinity detection and enrichment of labeled cell-surface sialylated proteins.
3. Streptavidin-captured protein subjected to quantitative LC-MS/MS.

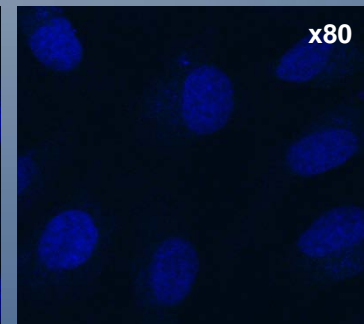
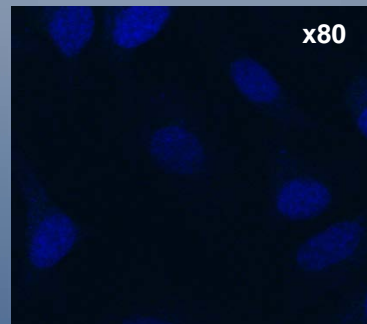
Imaging cell-surface sialylation with ManNaz and dcfb-65



ManNAz



ManNAc

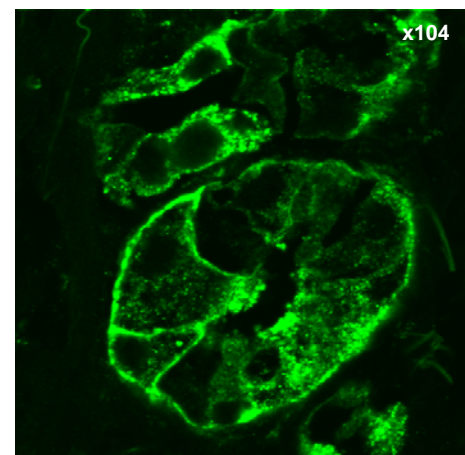
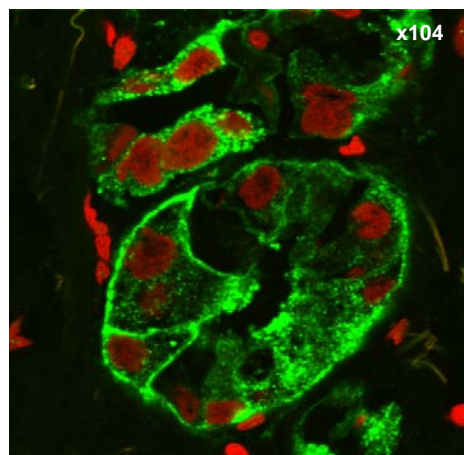
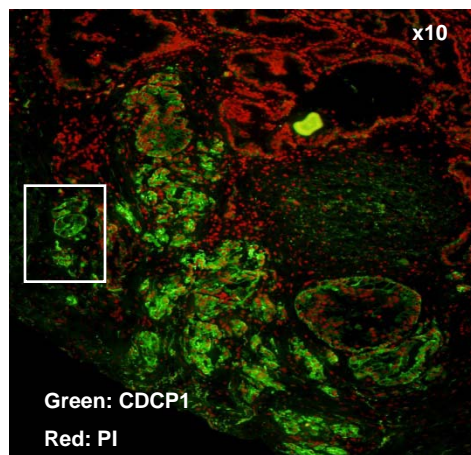


A. Flow cytometry analysis of fluorescent labeling. grey histogram, untreated cells; yellow, SAv-A488 only; green, control ManNAc treated cells; red, ManNAz treated cells.

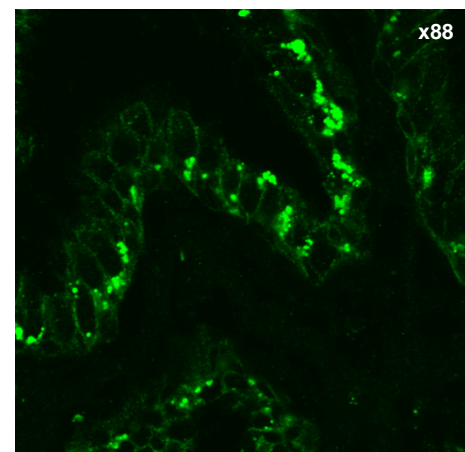
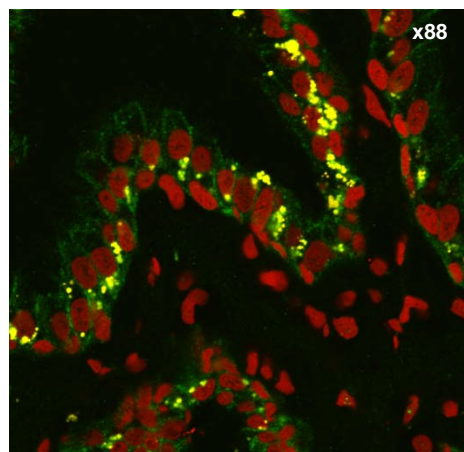
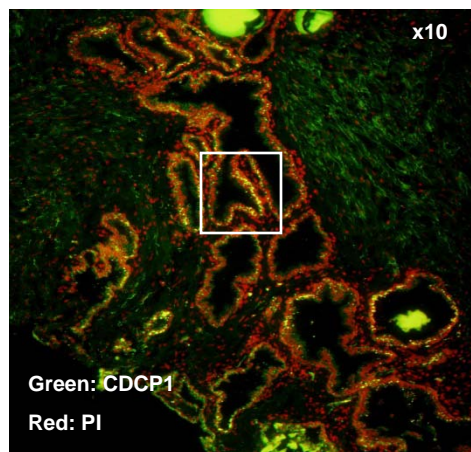
B. Microscopic analysis of fluorescent labeling on live cells. TO-RPO-3 was used to stain nuclei.

CDCP1 Subcellular Localization in PCa tissue

Tumor gland



Normal gland



CDCP1 mAb 41-2

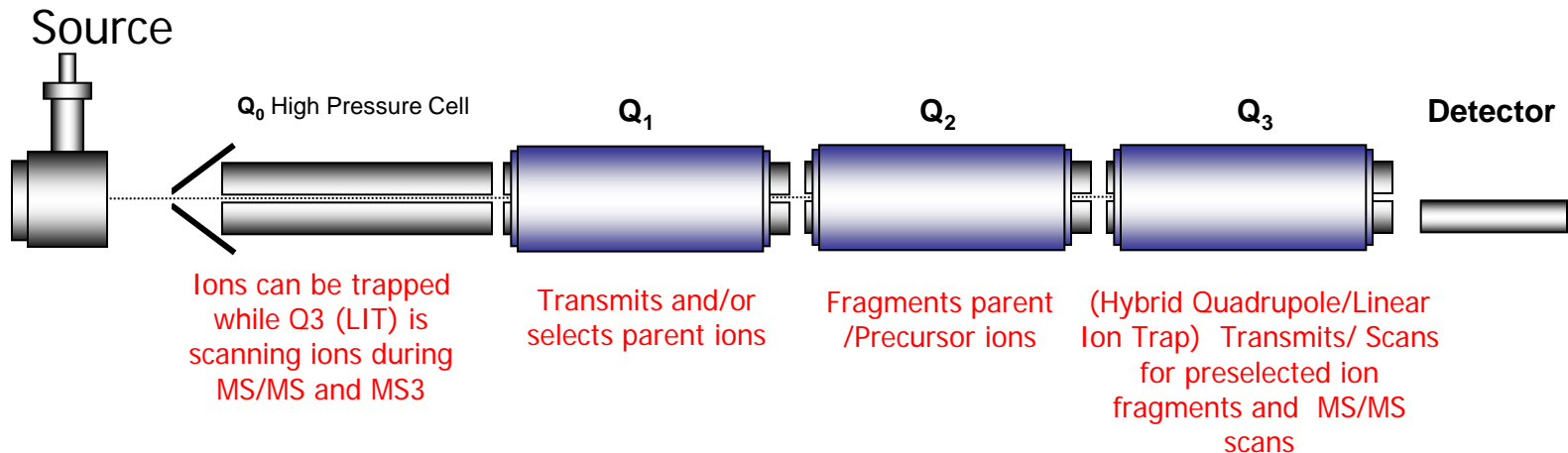
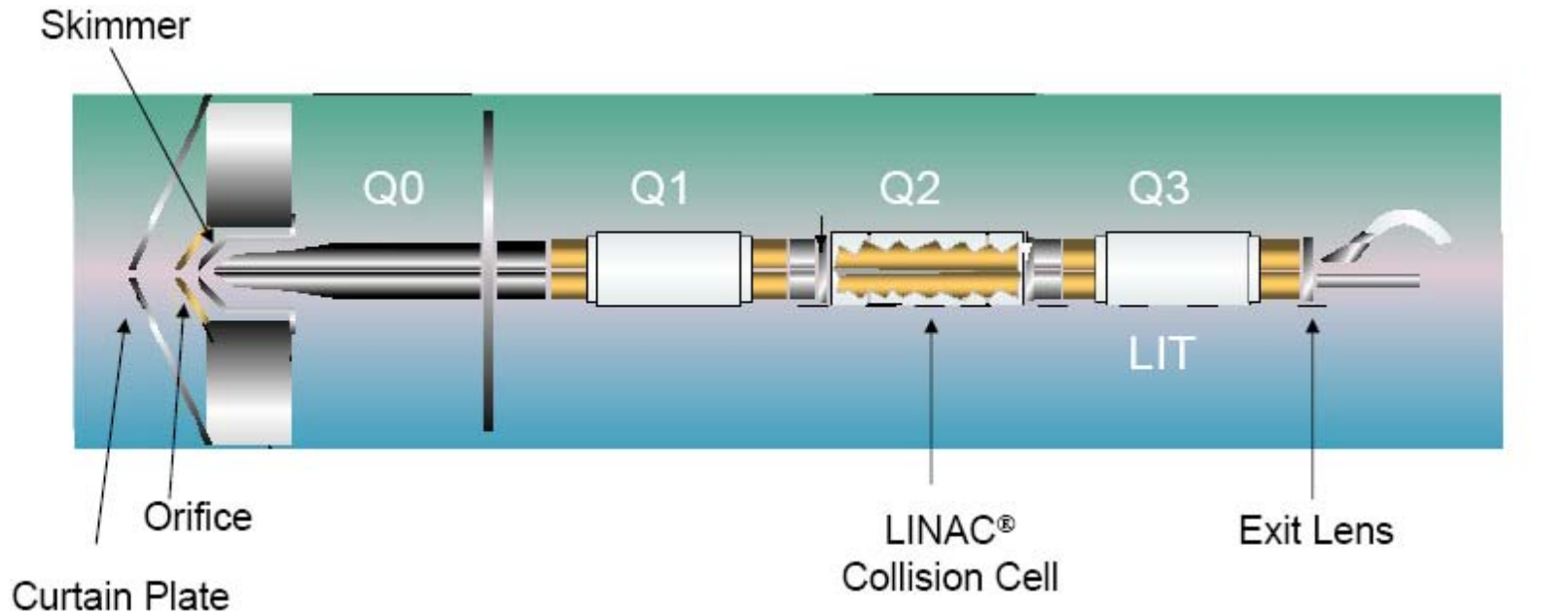
Characterization and Quantitation of Target Glycopeptides

How to overcome the “either peptides or sugars” technology barrier?

Peptide bonds are stronger and more uniform than glycanyl/glycyl bonds.

Application of MRM and QqQ technology

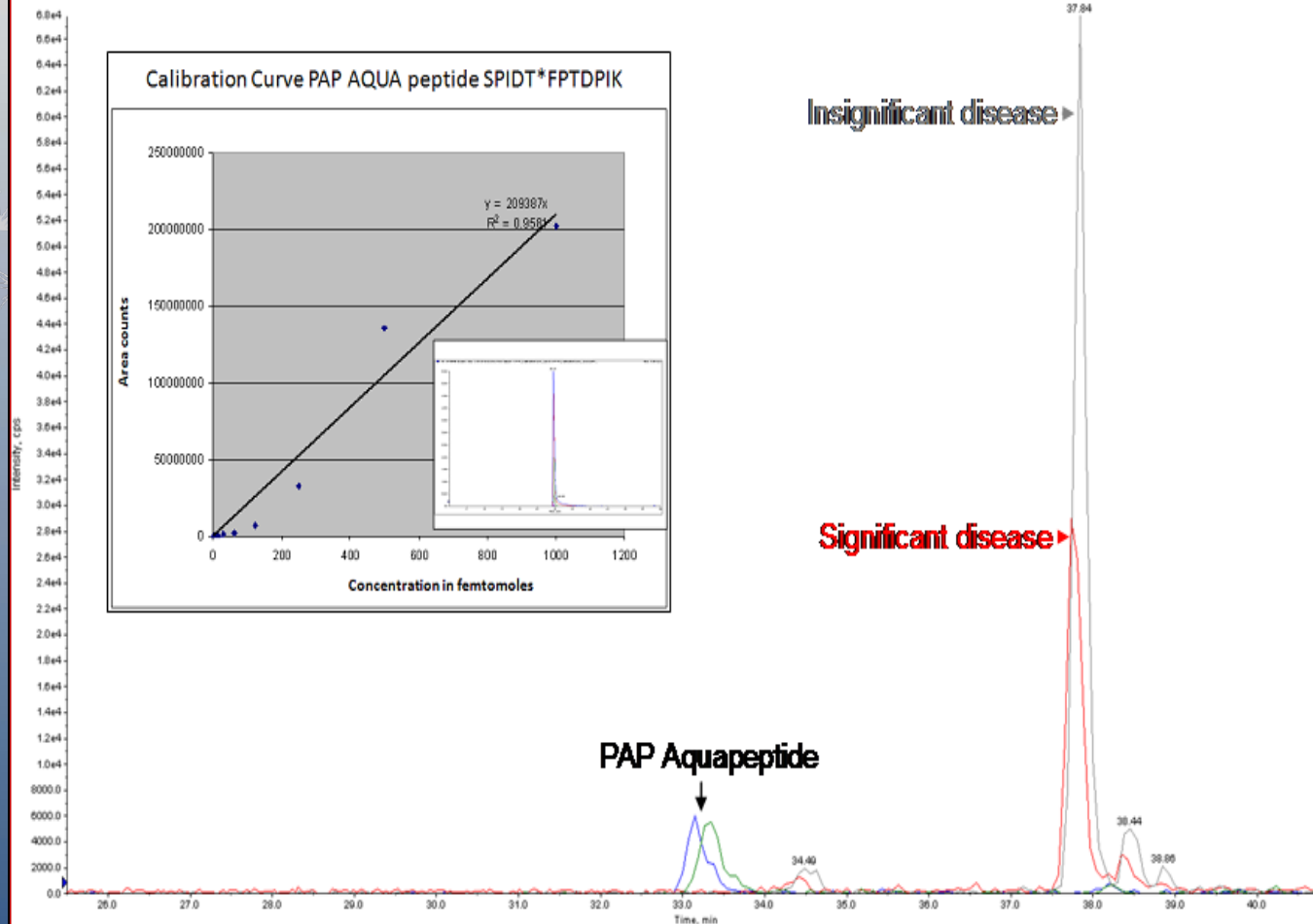
4000 Q-TRAP™ System



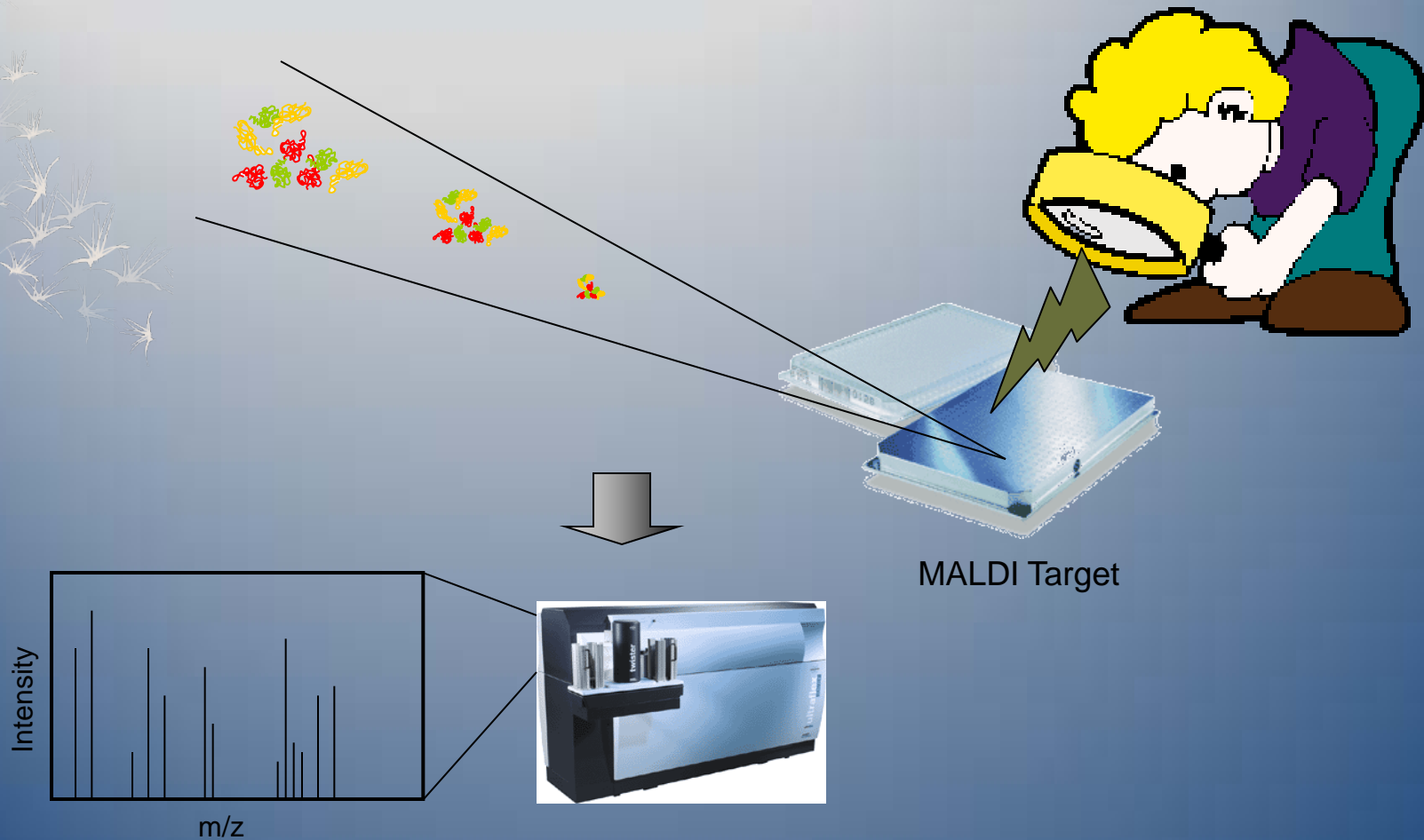
Discovery and Diagnostics

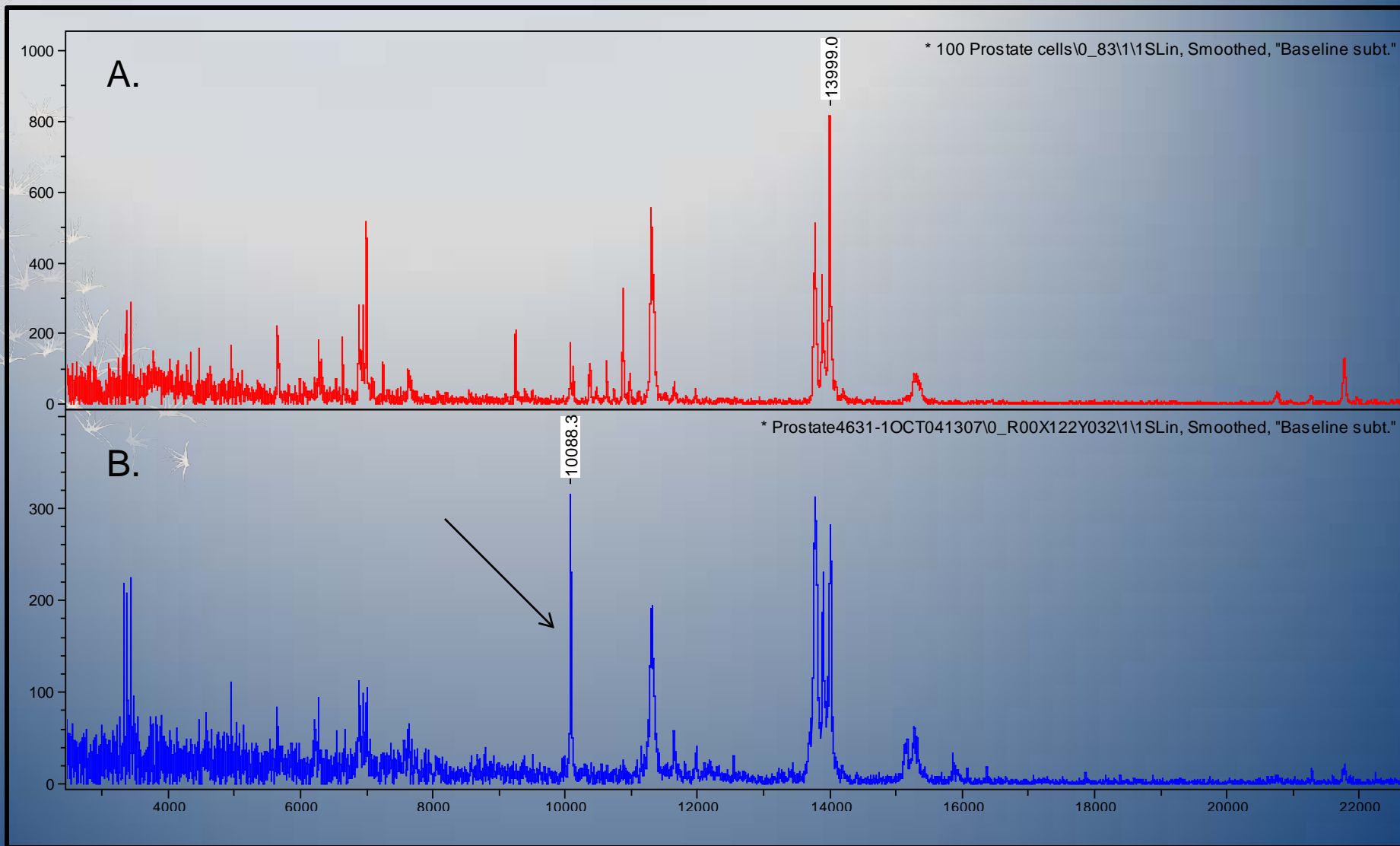
MRM quantitation for PAP Aquapeptide and Man₆N₂-Asn301 Glycopeptide

XIC of MAN₆N₂ conjugated GEYFVENIYYRNETQHEPPYPLMLPGCCSPSCPLER



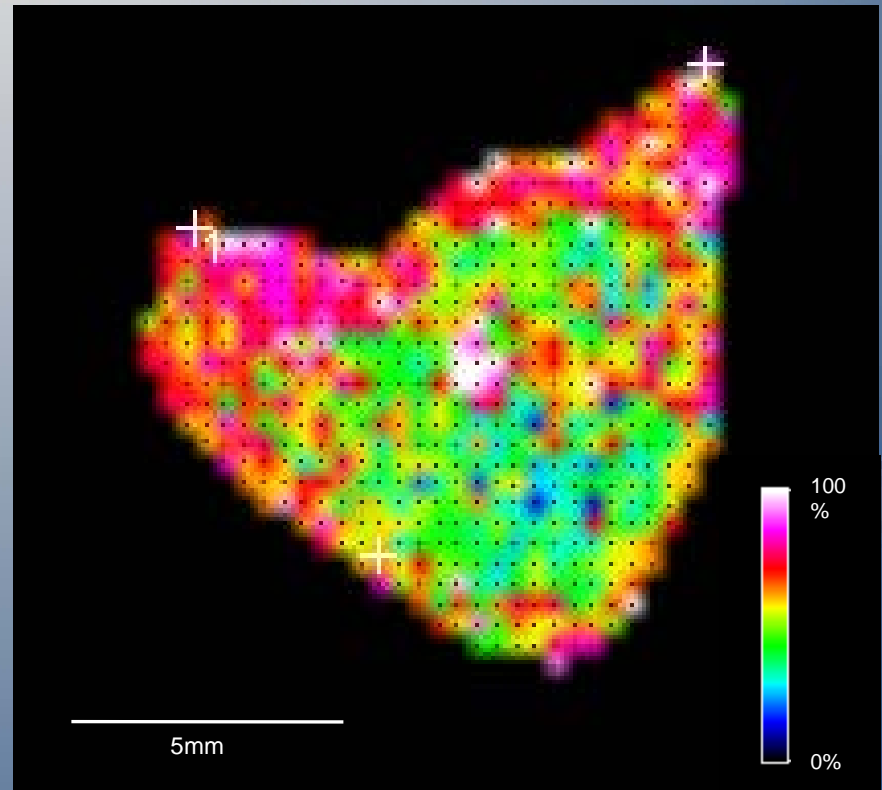
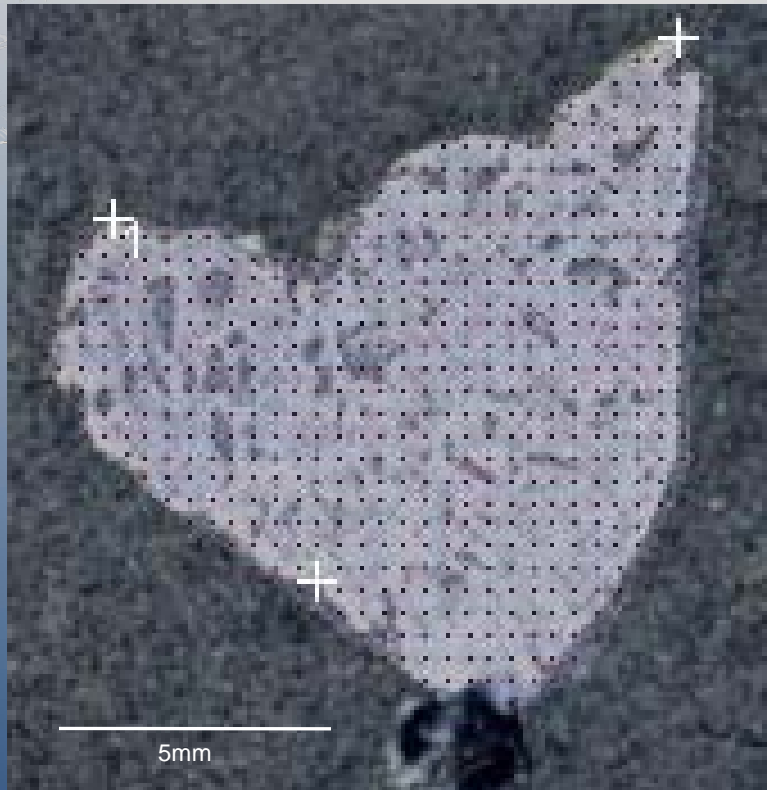
Application of Imaging Mass Spectrometry to the Clinical Management of Prostate Cancer

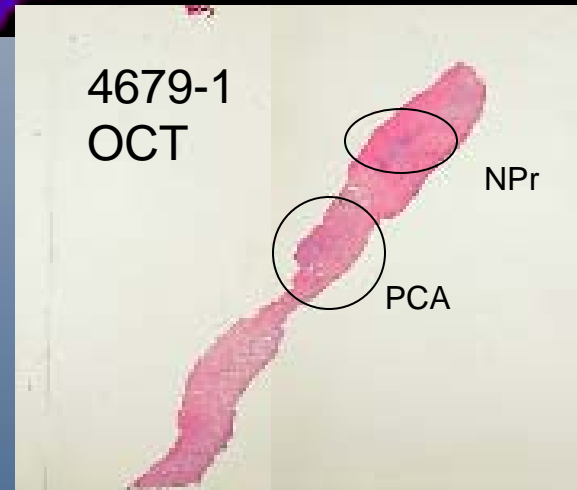
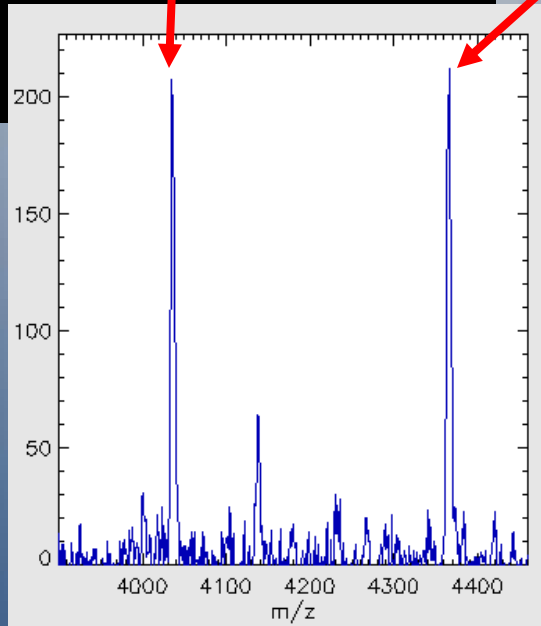
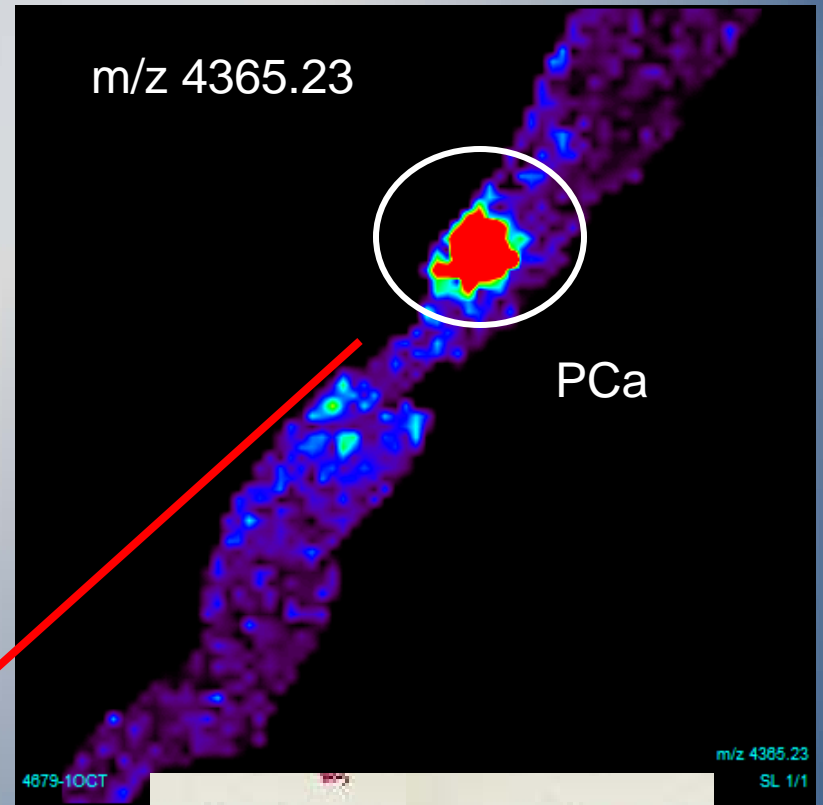
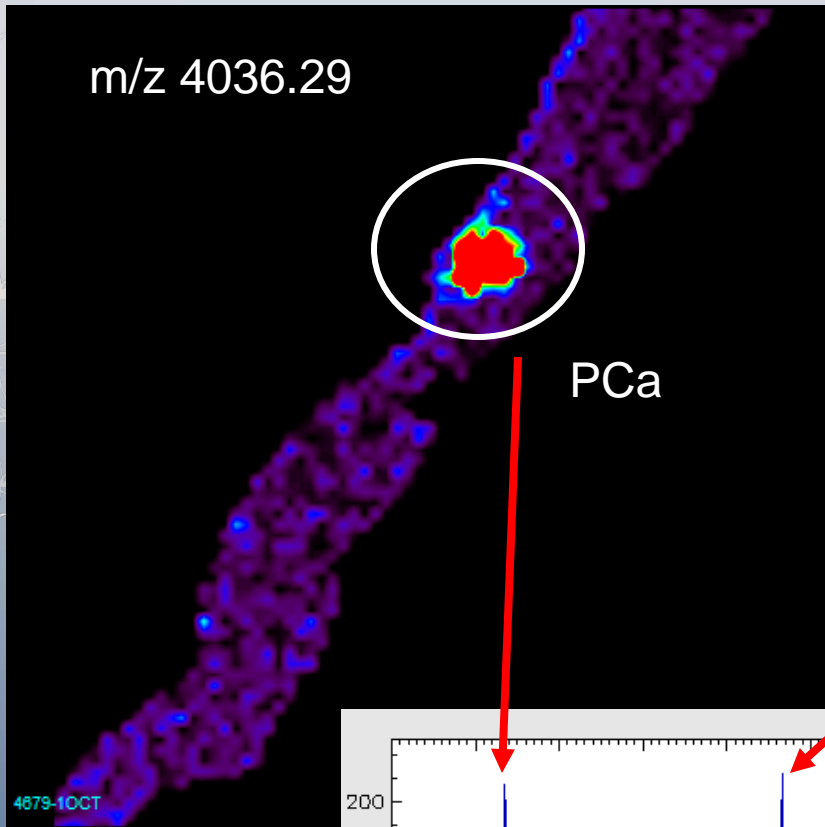




Comparison of MALDI spectra acquired from LCM cells versus intact tissue. A) Representative spectra generated from 100 prostate adenocarcinoma cells microdissected and mounted onto the MALDI target. B) Representative spectra from the same tissue in an area of prostate adenocarcinoma collected via direct analysis of intact tissue.

Registering Expression Levels of Selected m/z to Tissue Grid Position





Evaluating The Utility of MEKK2 IHC For PCa Stage/Grade

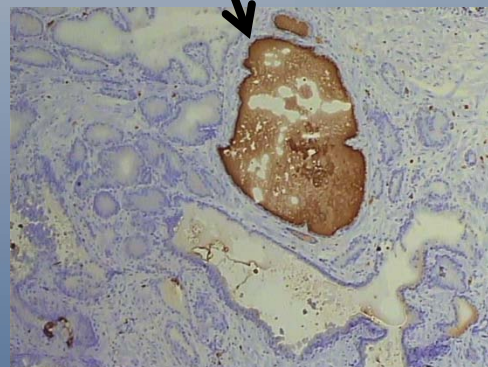
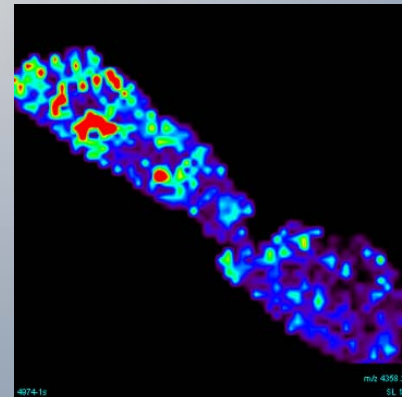
H & E



MEKK2 stain



MALDI-MSI (m/z 4355)

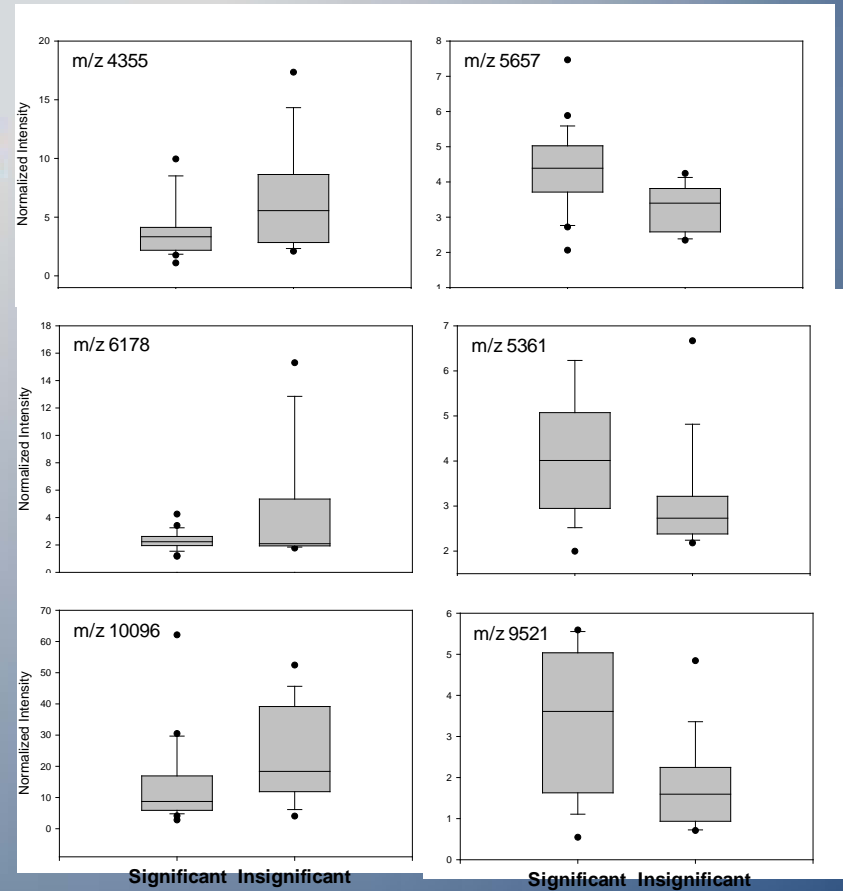
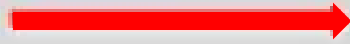


Area of intense staining

47 Prostates

11 Insignificant Disease

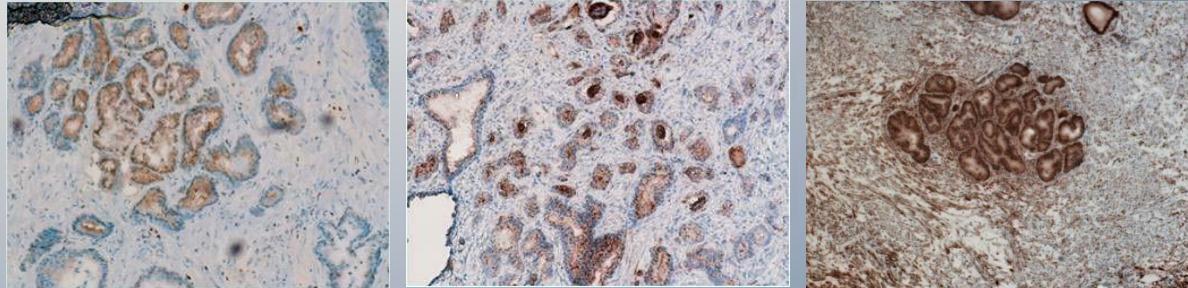
36 Significant Disease



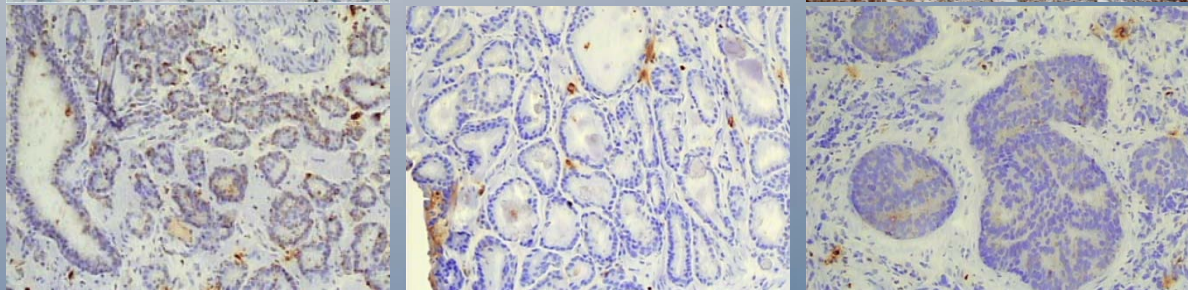
4355 sequence identified as MEKK2

MEKK2 is Over-expressed in Insignificant Disease

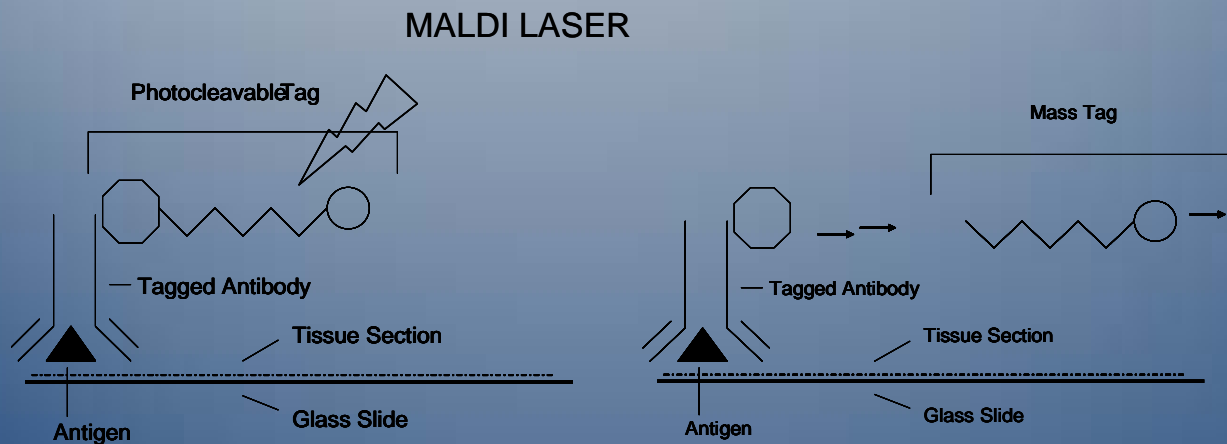
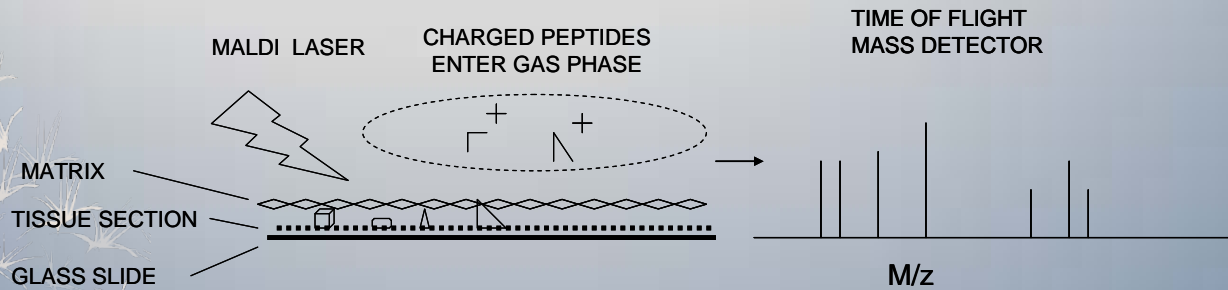
Insignificant



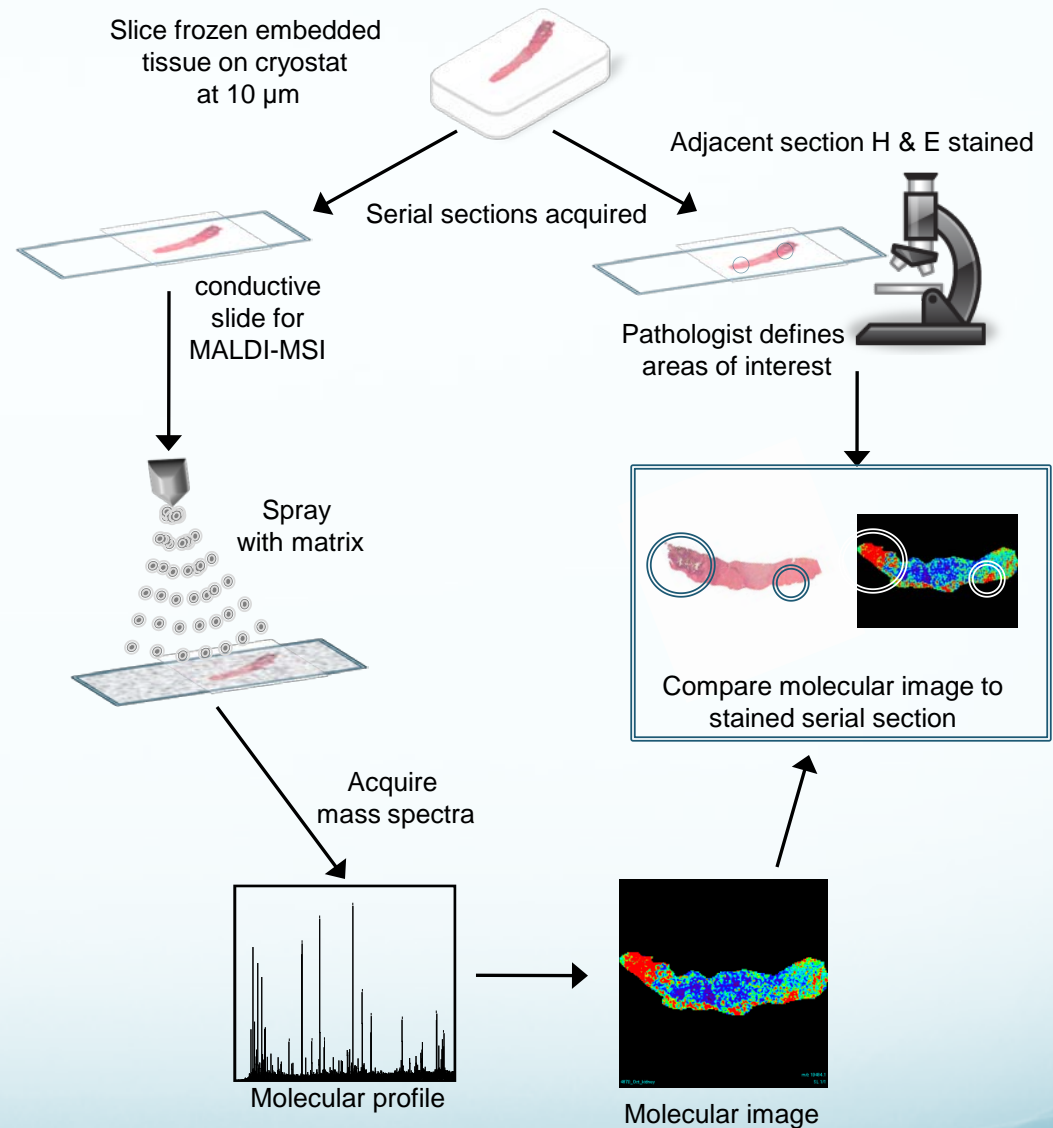
Significant



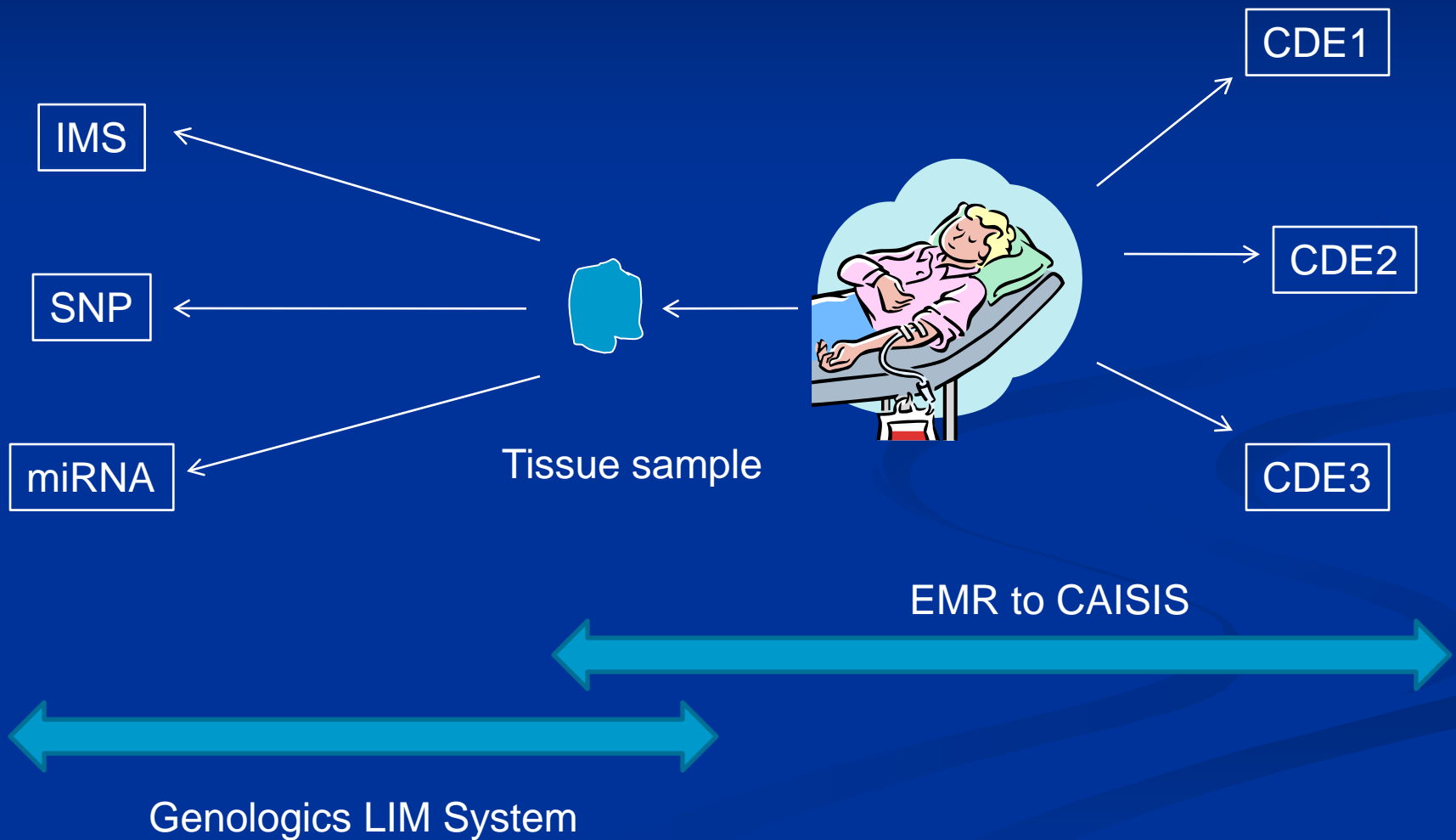
Direct IMS Diagnostics or Standard IHC Diagnostics??



Pathology-Directed Discovery Process



Integration is Critical to Success in Translational Research



CBIDRC RESOURCES

Clinical Sites

Dermatology
Internal Medicine
Ob/Gyn
Otolaryngology
Pathology
Pediatrics
Oncology
Urology
Surgery

Clinical Biorepository

Prostate, Breast,
Bladder, Renal,
H&N, Melanoma
36,000 serum
4,000 urine
1,700 plasma
7,000 frozen tissue
600 EPS

Study Compliance

35 Grant submissions*
26 approved IRB protocols
6 patents

Informatics

Infrastructure

CAISIS
Genomics
EMR Portals

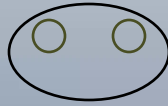
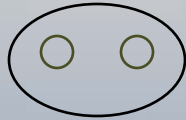
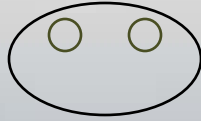
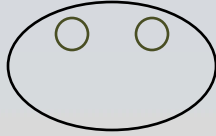
Molecular

Pathology

IHC/SlidePath
Tissue processing
LCM
Tissue Staging



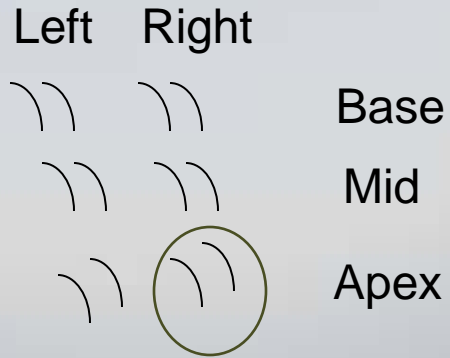
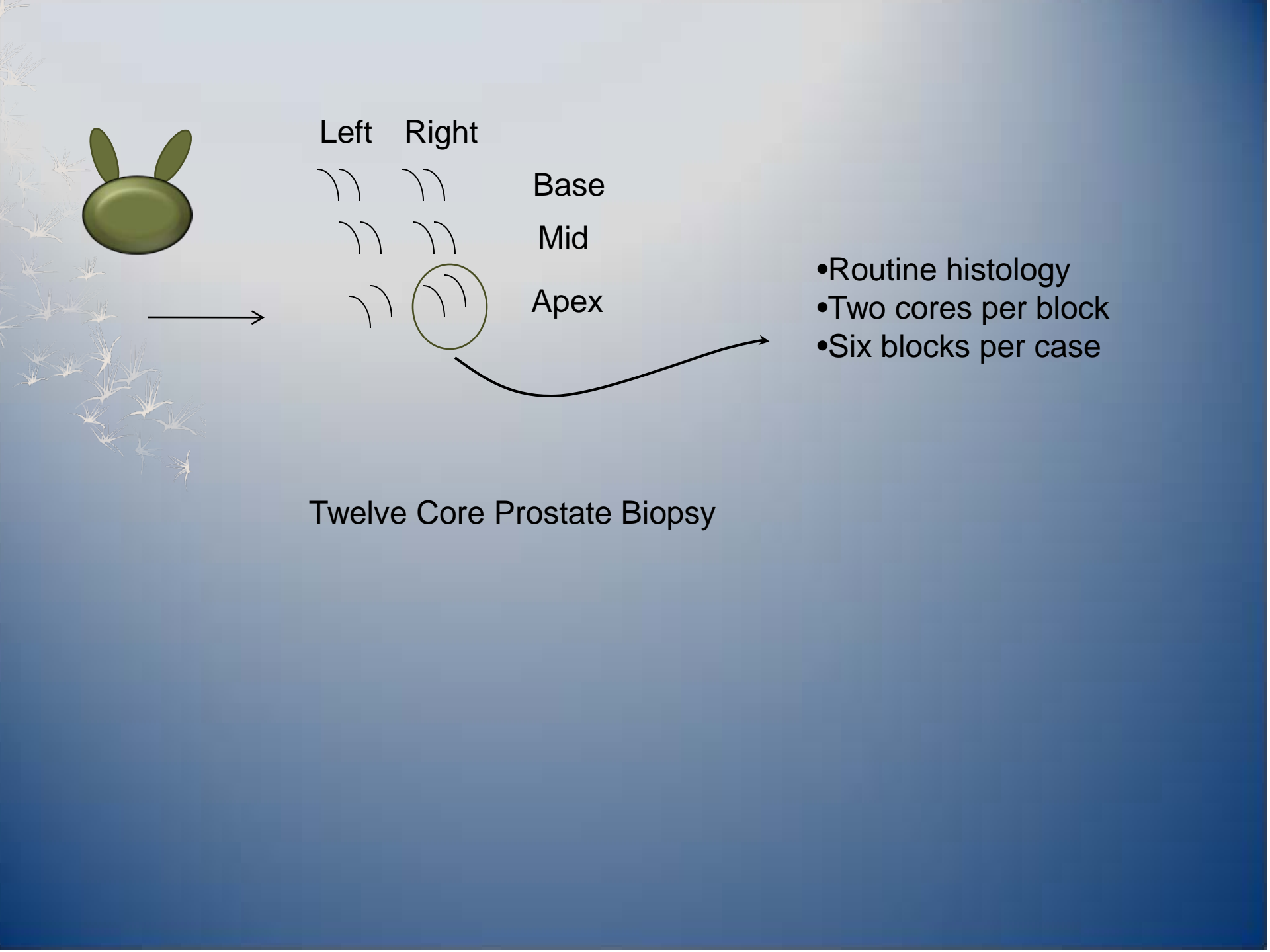
Fresh Prostate



Frozen Cores



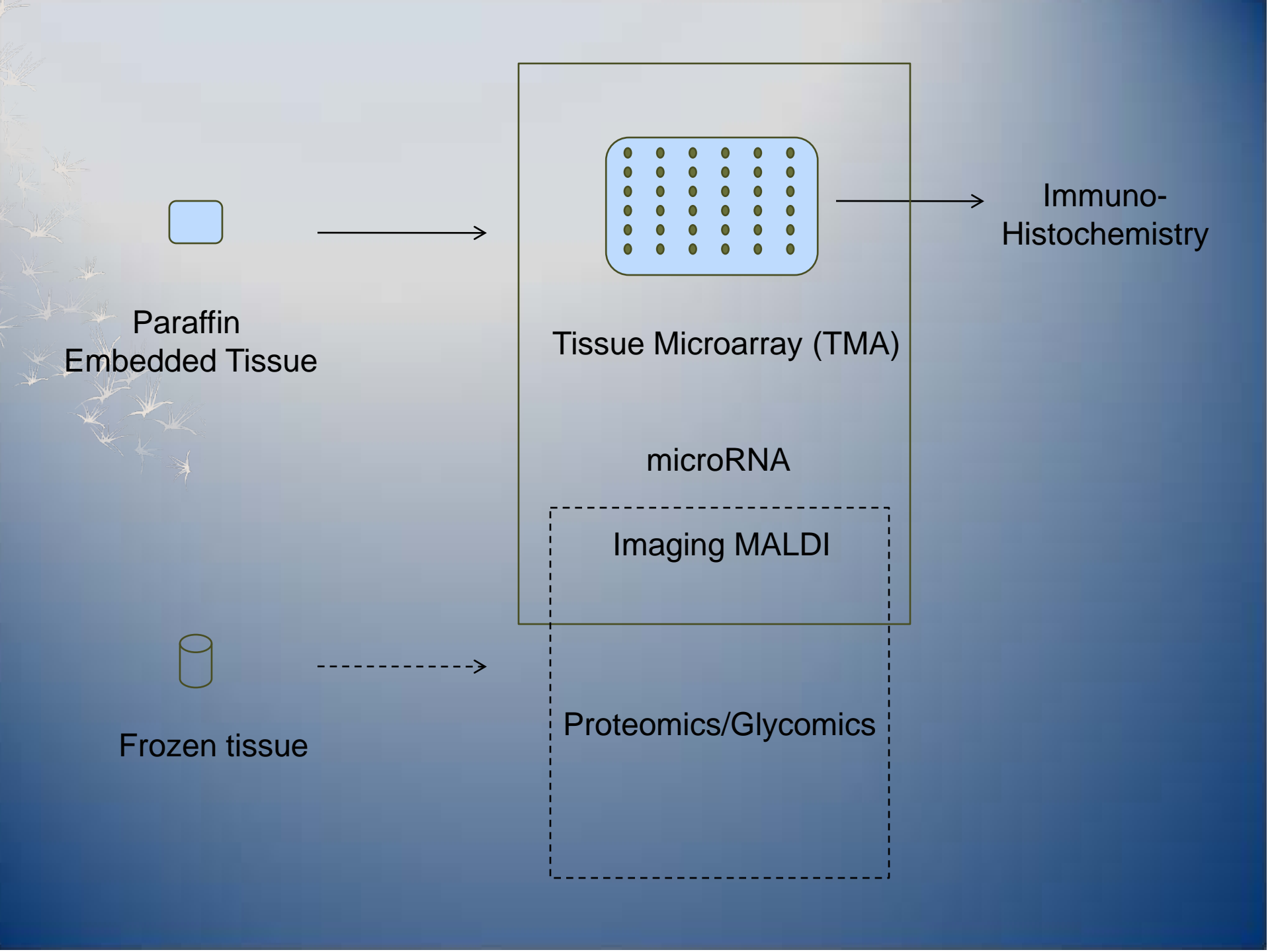
Routine Histology



- Routine histology
- Two cores per block
- Six blocks per case



Twelve Core Prostate Biopsy



Cancer Biology and Infectious Disease Research Center

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Michael Ward

Chris Wilkins

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Yvonne Oden

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Clinical Research Activity

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