

# Snap-embalming tissue - cell signaling and tissue morphology frozen in time at room temperature

**NCI Grant 1R21CA125698-01A1**

## **BRN Symposium 2011**

March 28, 2011

Claudius Mueller, PhD

# Preservation Matters!



Rosalia Lombardo († 1920)

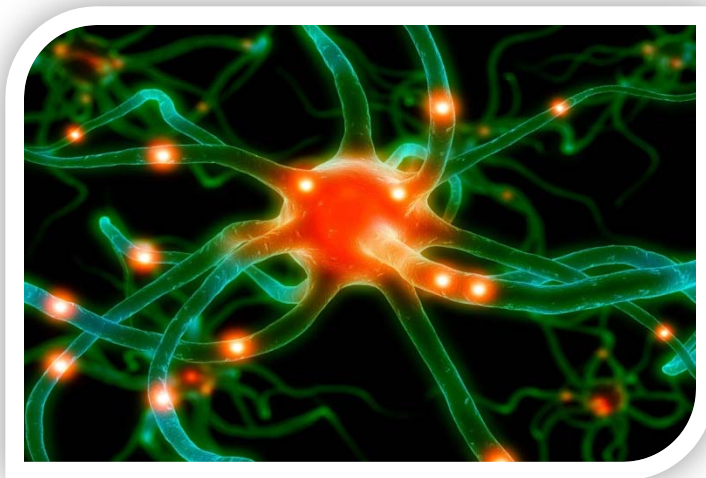


Ötzi († 3000 BC)

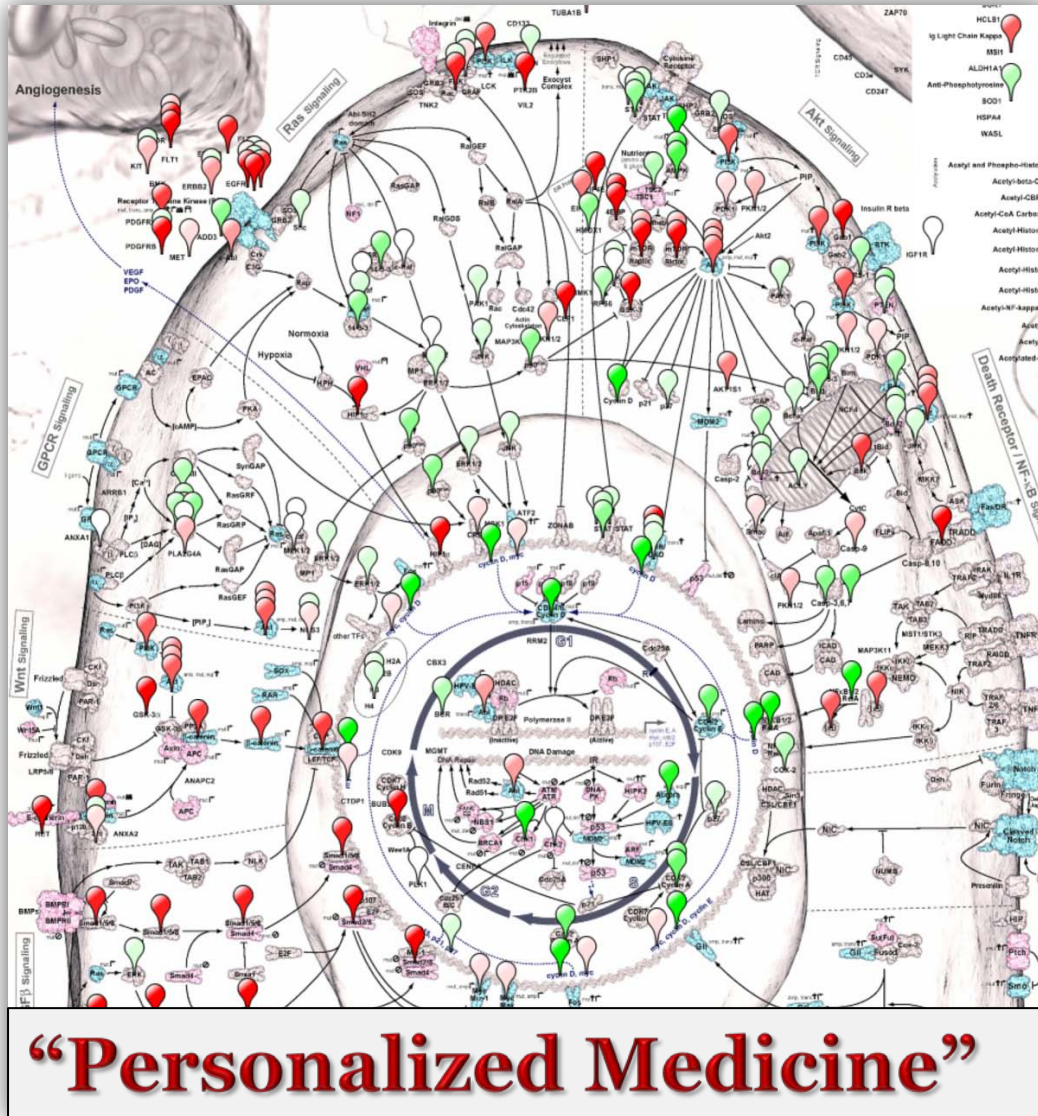
## Our Novel Fixation Chemistry makes a Difference in Clinical Trials

<b>Breast Cancer (USO, GSK)</b>	Phase II trial of Trastuzumab and/or Lapatinib plus chemotherapy for Her2+ breast cancer	Opened Sept 2008, Accrual complete (n=100)
<b>Multiple Myeloma (Virginia Oncology Services)</b>	Targeted inhibitor screening	Opened May 2007, Accrual complete
<b>Breast Cancer (NSABP)</b>	Phase II Randomized Clinical Trial Evaluating Neoadjuvant Therapy Regimens	Opened Nov 2010, Targeted enrollment n=100

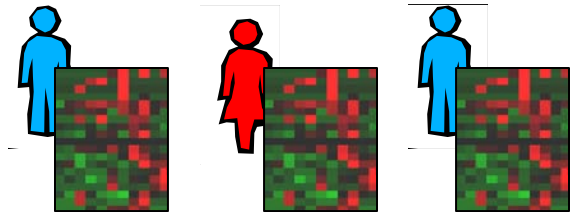
# Background



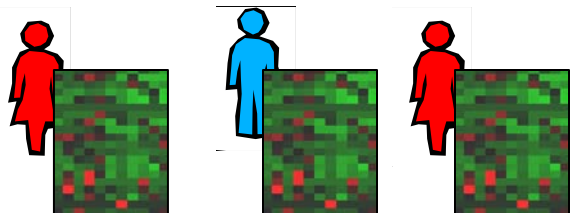
# Quantification of Phosphoproteins is Key



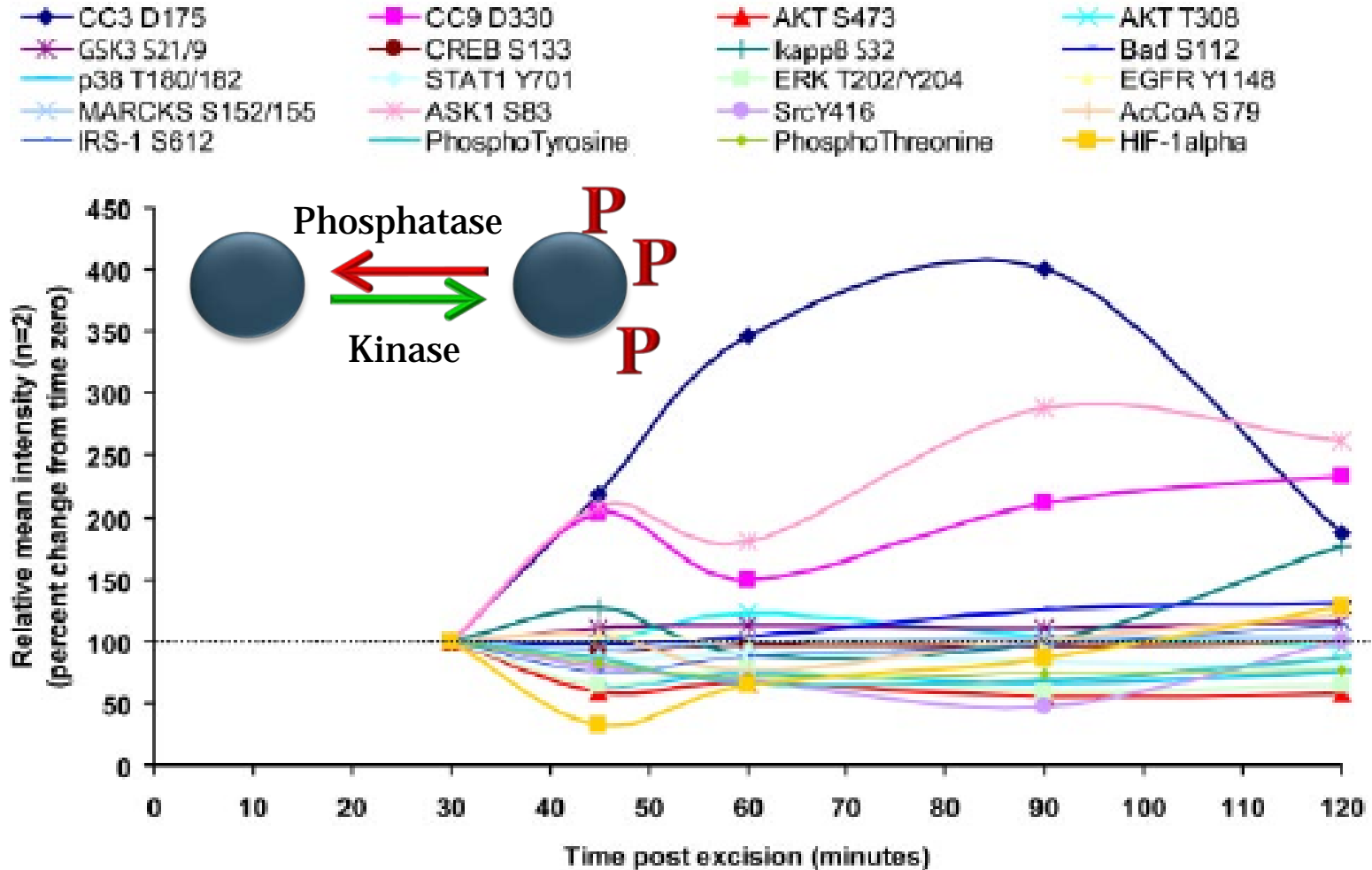
Kinase Inhibitor A



Kinase Inhibitor B



# Protein Phosphorylation Fluctuates Rapidly



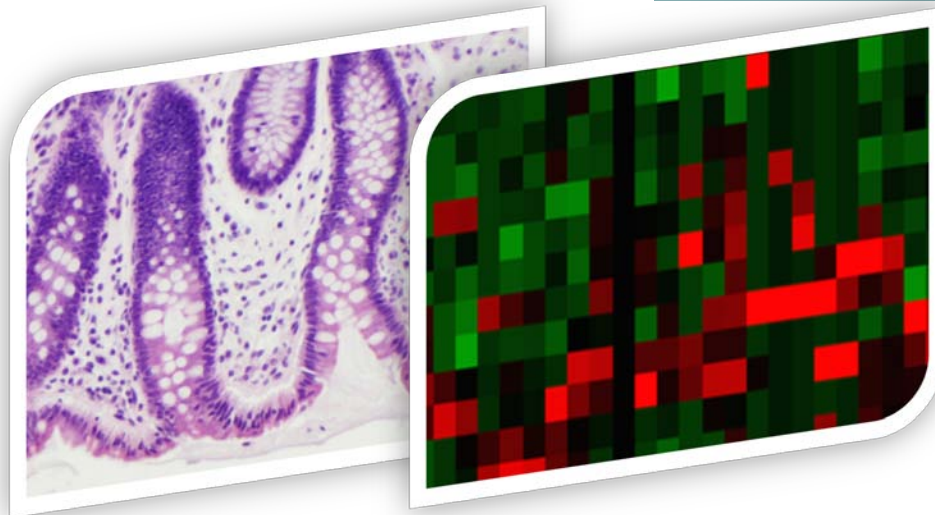
Human Uterus at RT

Espina et al., *Mol Cell Proteomics* (2008)

Espina et al., *Proteomics Clin Appl* (2009)



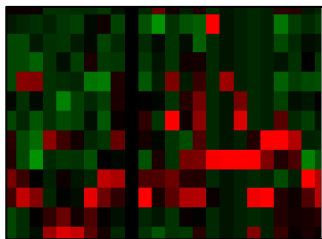
# Biomarker and Histology Preservative (BHP)





## Paraffin Block

**Phospho-  
proteins**



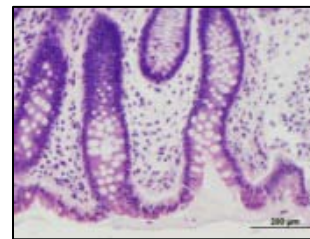
↓  
Equivalent to  
**Snap-Frozen**

**Extractable  
Protein Yield**



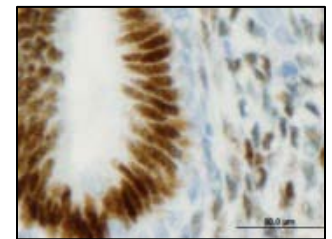
↓  
Equivalent to  
**Snap-Frozen**

**Diagnostic  
Morphology**



↓  
Equivalent to  
**Formalin**

**Immuno-  
histochemistry**



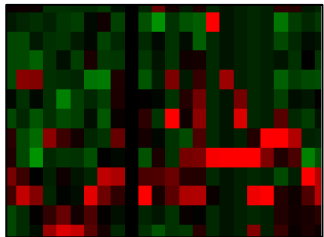
↓  
Equivalent to  
**Formalin**





**Paraffin  
Block**

**Phospho-  
proteins**



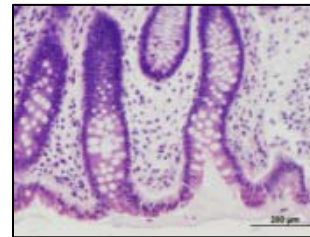
Equivalent to  
**Snap-Frozen**

**Extractable  
Protein Yield**



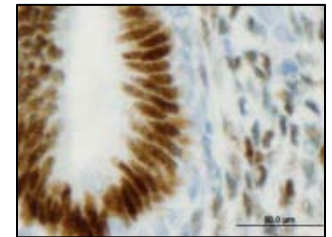
Equivalent to  
**Snap-Frozen**

**Diagnostic  
Morphology**



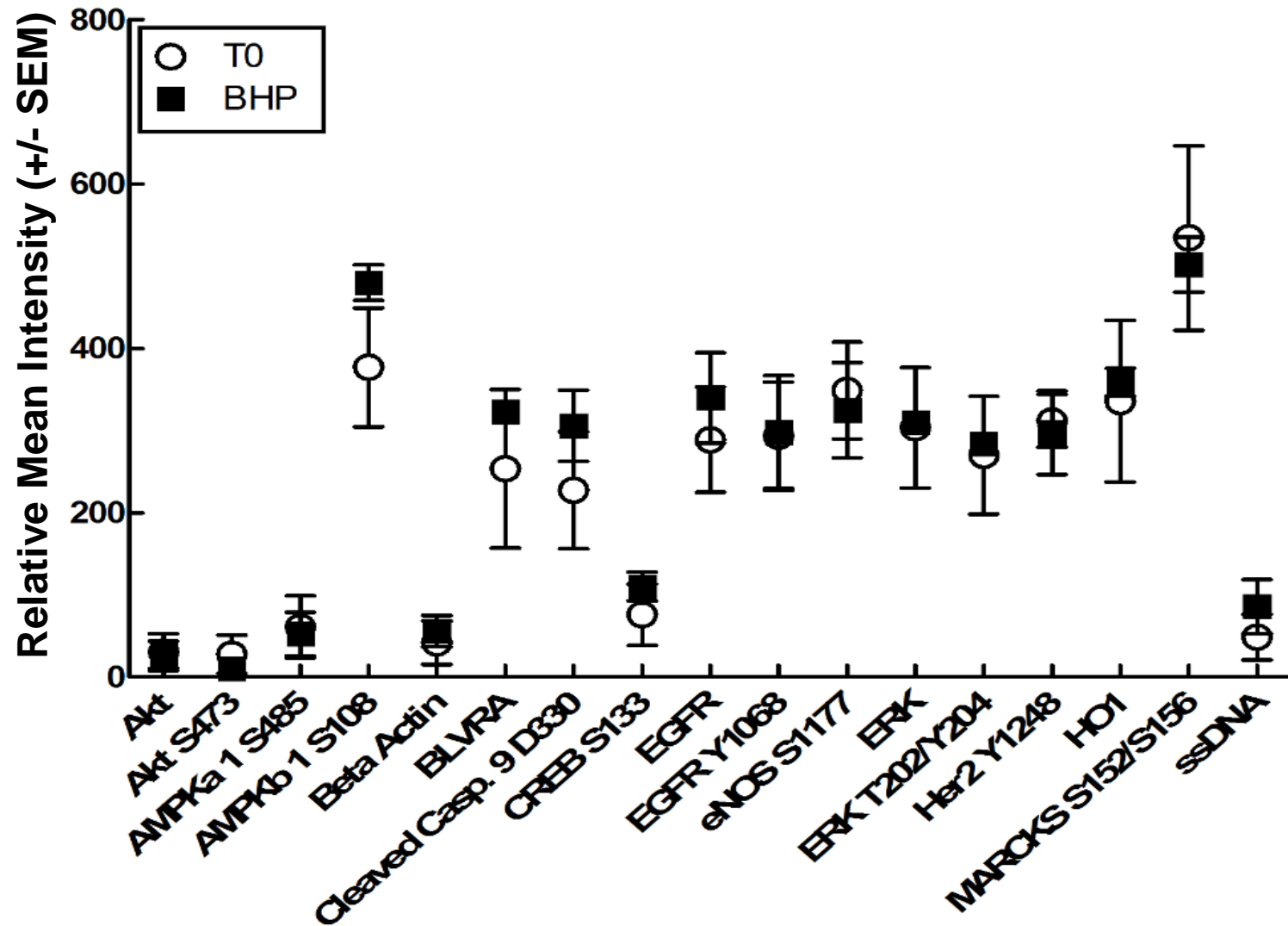
Equivalent to  
**Formalin**

**Immuno-  
histochemistry**



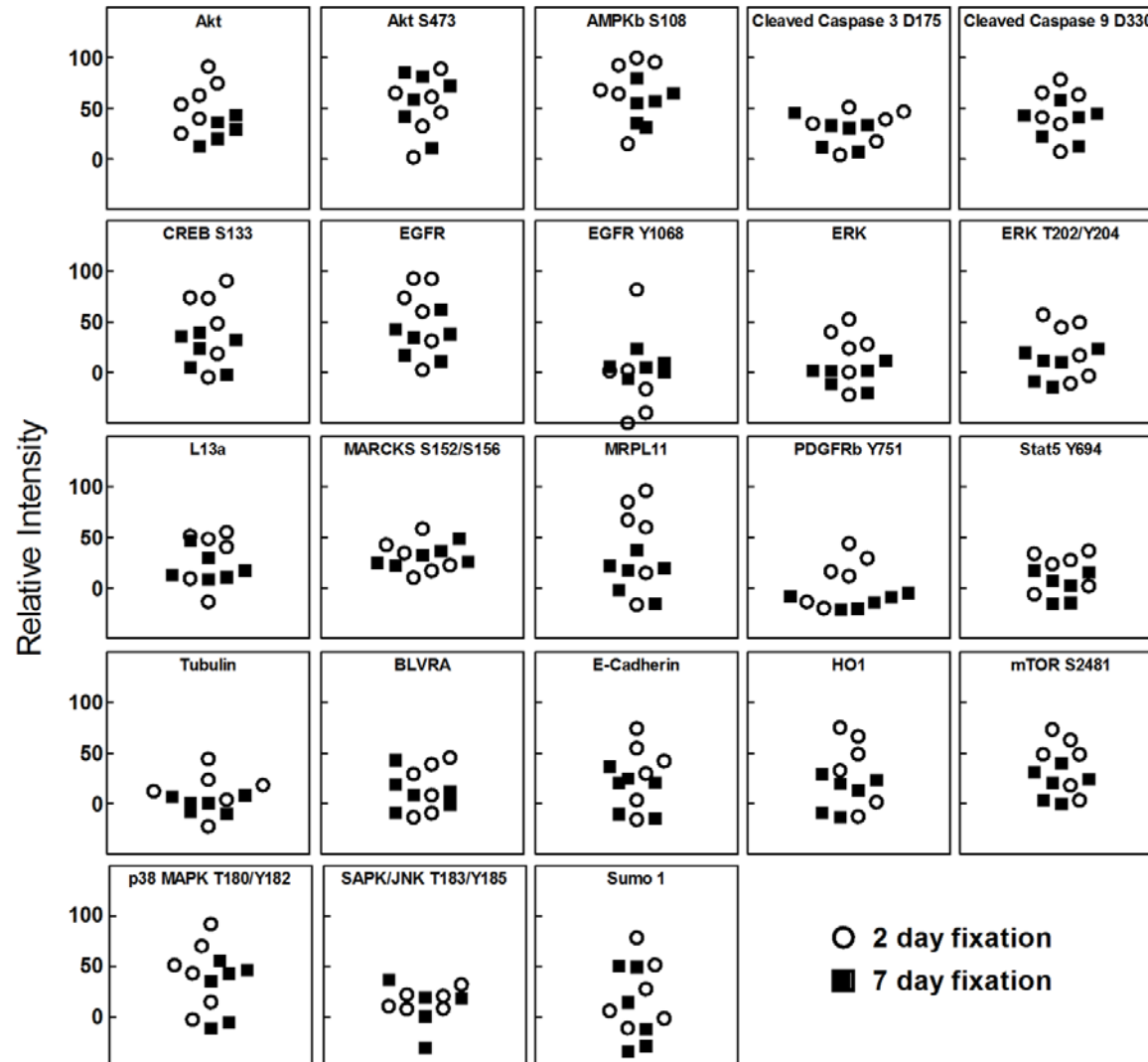
Equivalent to  
**Formalin**

# Phosphoprotein Levels Comparable to Snap-Frozen Mouse (n=3) Liver - measured by RPMA



# Protein Phosphorylations Stable Over Fixation Time

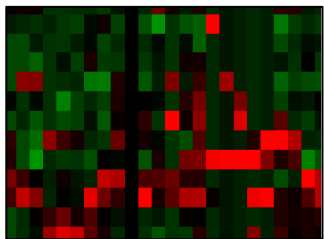
## Human Colon Mucosa (n=6) - $p > 0.05$





## Paraffin Block

Phospho-  
proteins



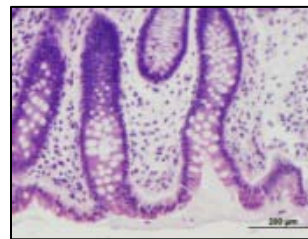
Equivalent to  
**Snap-Frozen**

Extractable  
Protein Yield



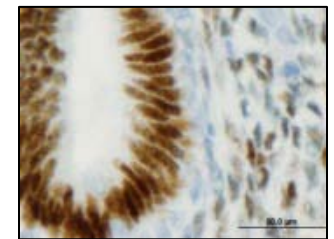
Equivalent to  
**Snap-Frozen**

Diagnostic  
Morphology



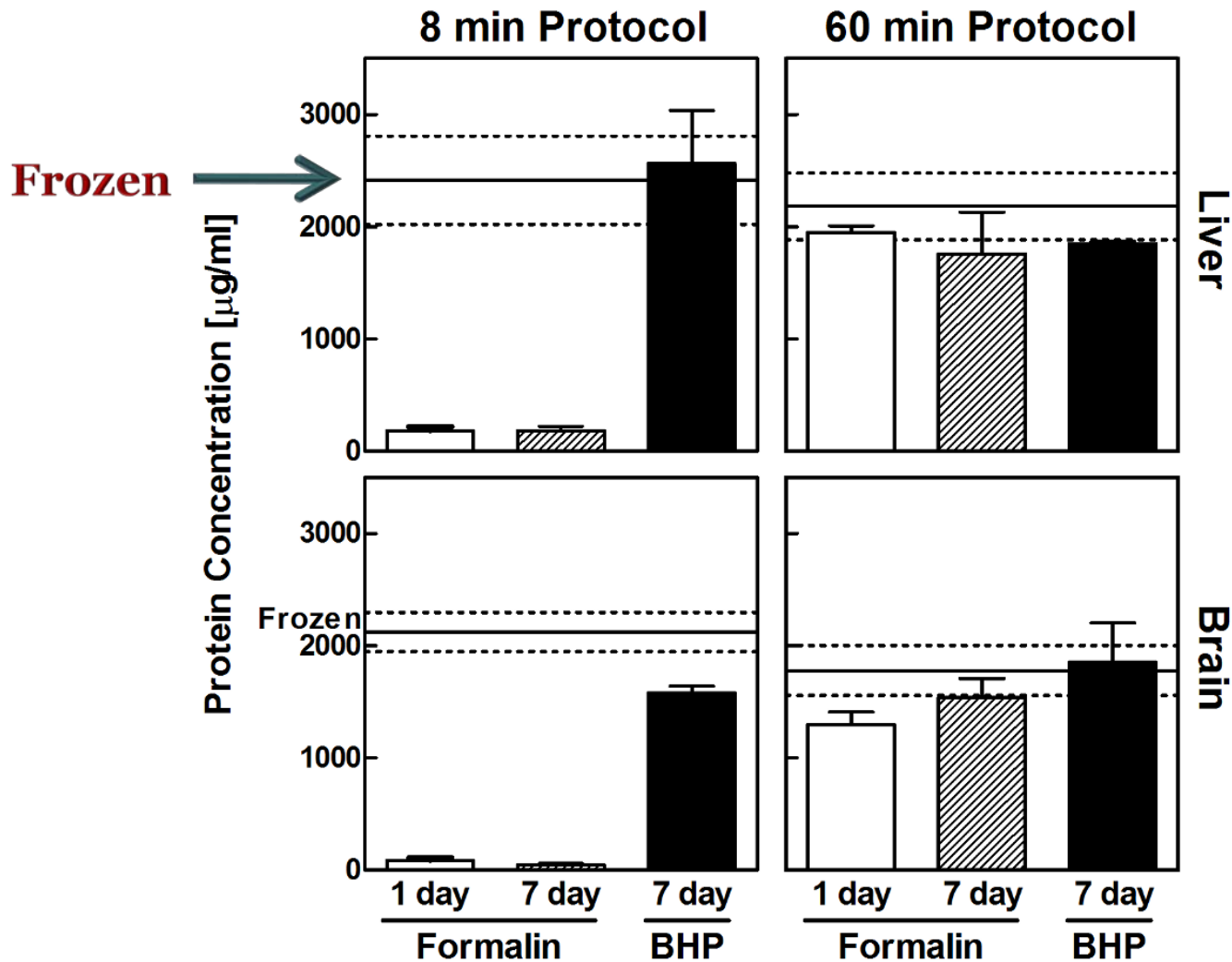
Equivalent to  
**Formalin**

Immuno-  
histochemistry



Equivalent to  
**Formalin**

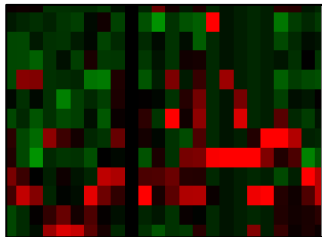
# Protein Yield from Paraffin Block is Comparable to Matched Snap-Frozen Tissue





**Paraffin Block**

**Phospho-proteins**



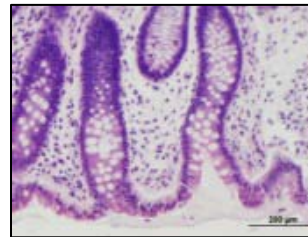
↓  
Equivalent to  
**Snap-Frozen**

**Extractable Protein Yield**



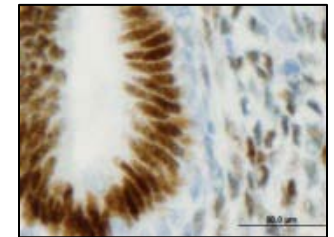
↓  
Equivalent to  
**Snap-Frozen**

**Diagnostic Morphology**



↓  
Equivalent to  
**Formalin**

**Immuno-histochemistry**



↓  
Equivalent to  
**Formalin**



# Morphology tested in > 25 different Tissues

## Human Tissues

Breast	Colon
Uterus	Bone Marrow
Lymph Node	Prostate

## Feline Tissues

Testes

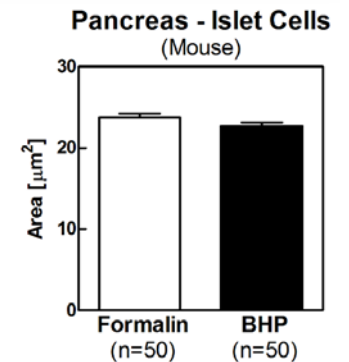
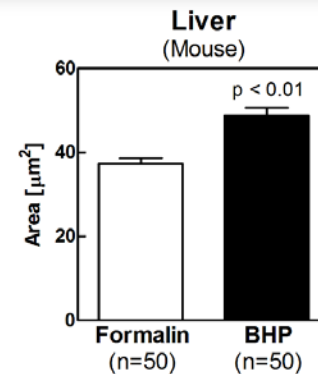
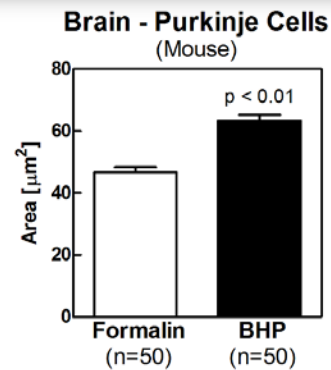
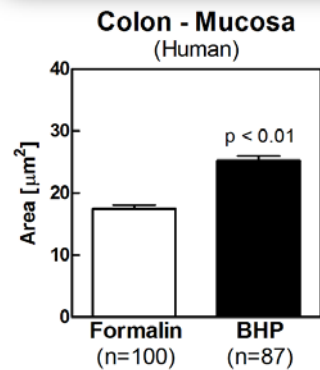
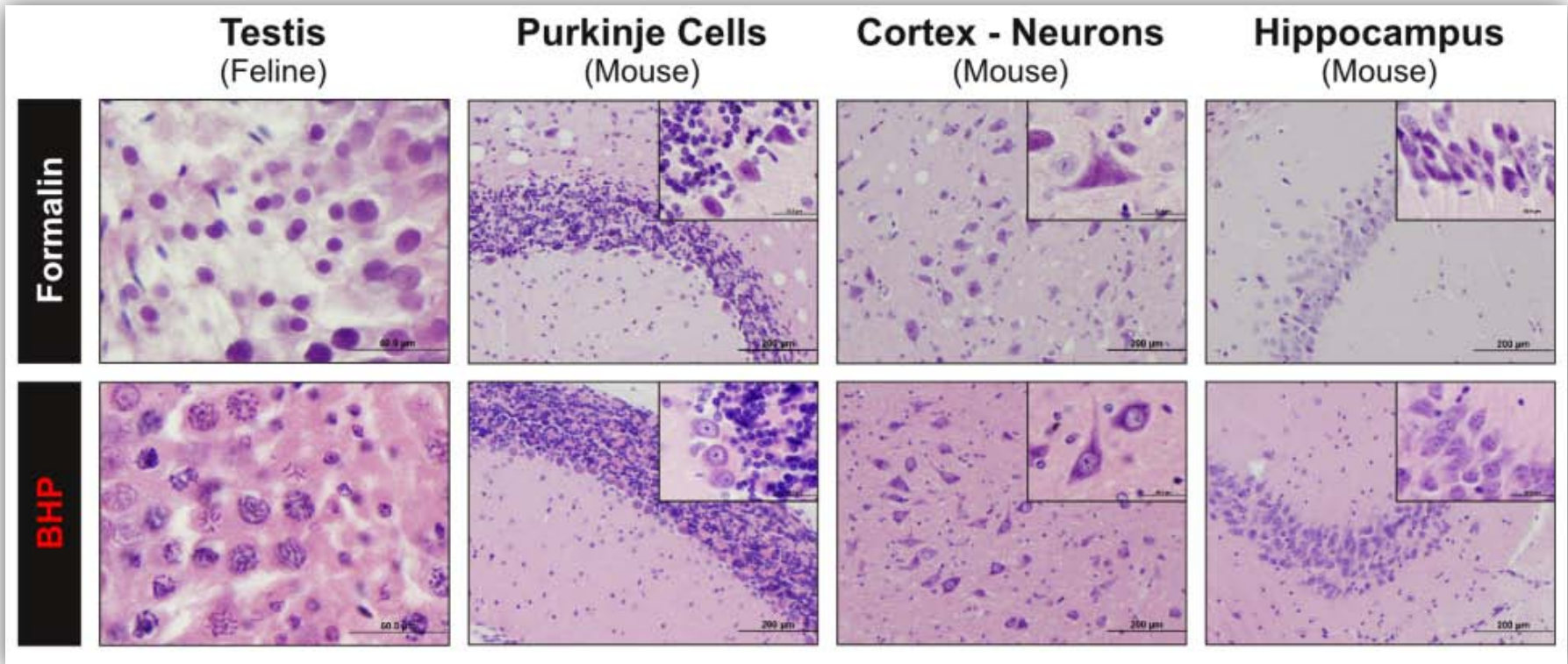
## Mouse Tissues

Brain	Tongue	Spleen
Eye	Stomach	Ovary
Ear (Cartilage)	Small Intestine	Fallopian Tube
Salivary Glands	Colon	Femur
Heart	Kidney	Rib
Mammary	Liver	Sciatic Nerve
Lung	Pancreas	Tail

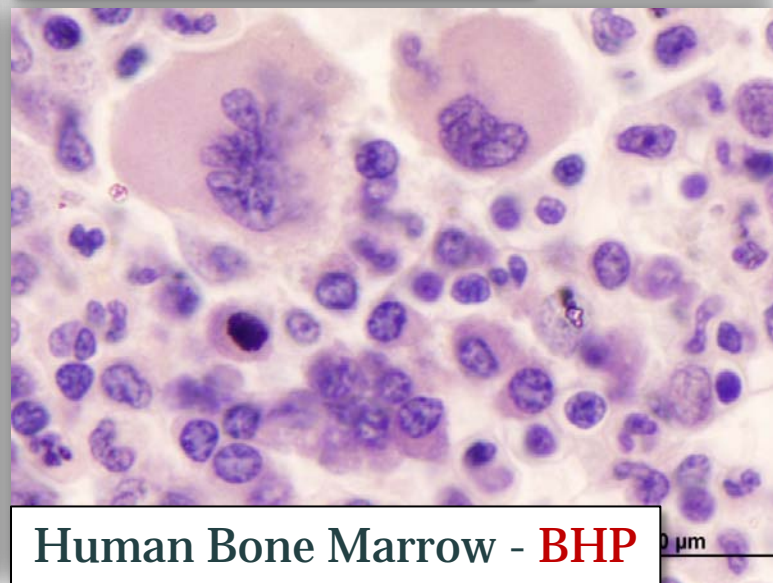
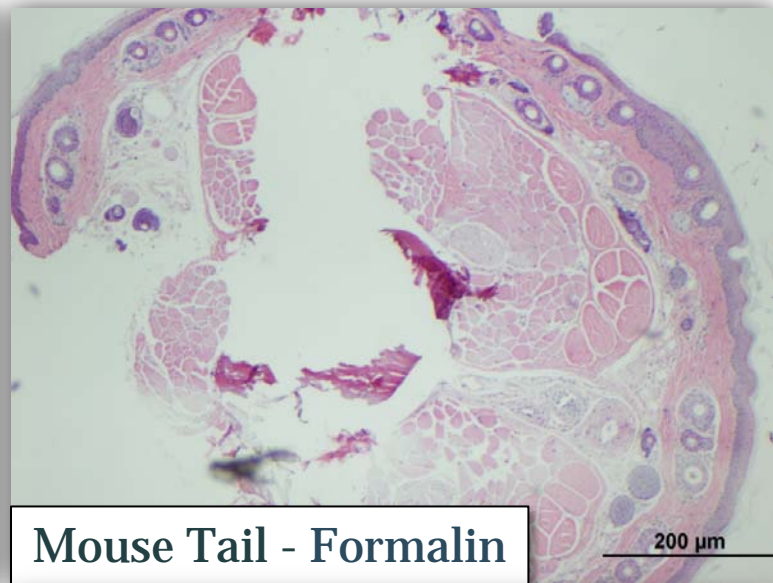
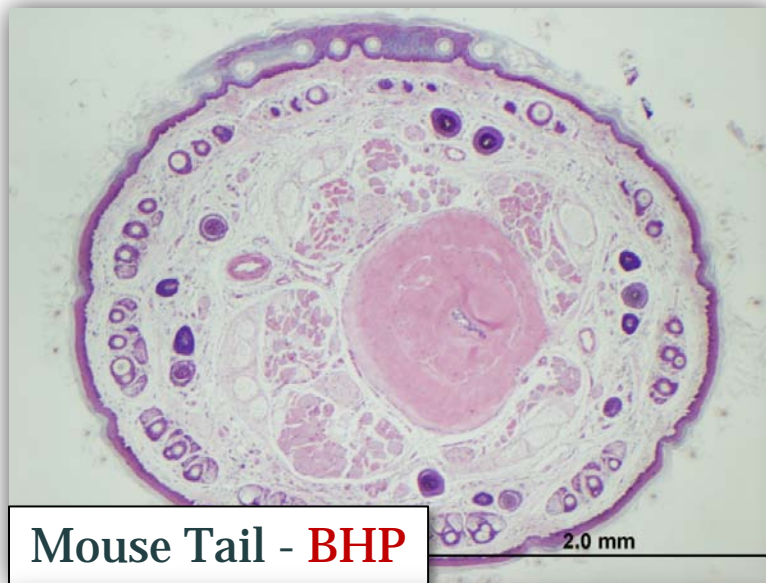
# Reviewed by Independent Pathologists

<b>Criteria</b>	<b>Worse than Formalin</b>	<b>Equal to Formalin</b>	<b>Better than Formalin</b>
<b>Overall colour fidelity</b>		✓	
<b>Cell size</b>		✓	
<b>Preservation of nuclear membrane</b>		✓	
<b>Preservation of nucleoli and nuclear chromatin</b>			✓
<b>Preservation of overall cell structure</b>		✓	
<b>Nuclear: Cytoplasmic ratio maintained</b>		✓	

# Preservation of Nuclear Volume and Chromatin

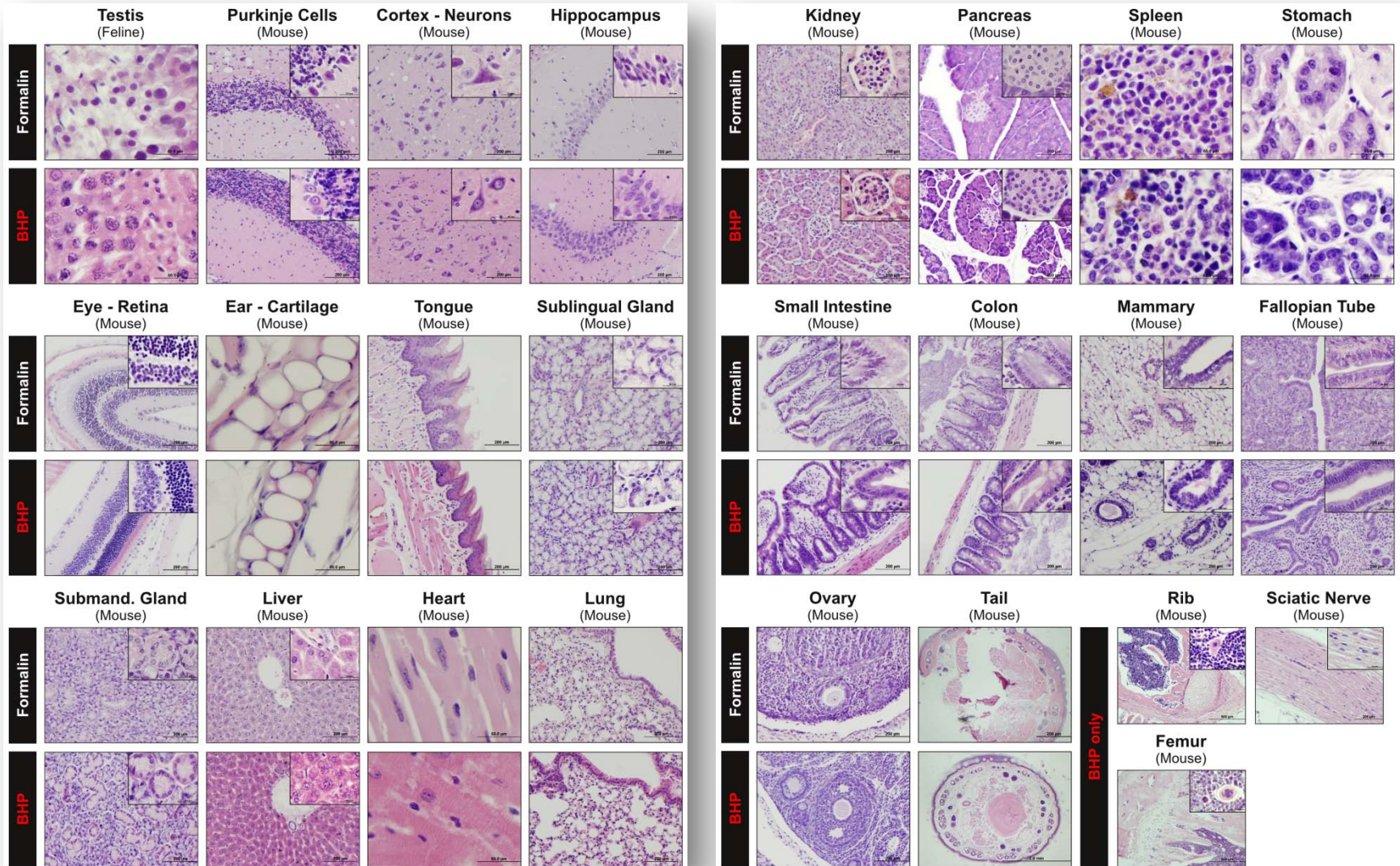


# No De-Calcification Needed





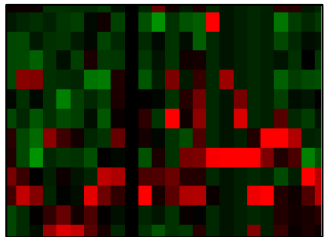
# Morphology Examples: Come see Poster #6!





**Paraffin  
Block**

**Phospho-  
proteins**



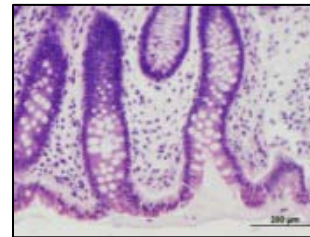
↓  
Equivalent to  
**Snap-Frozen**

**Extractable  
Protein Yield**



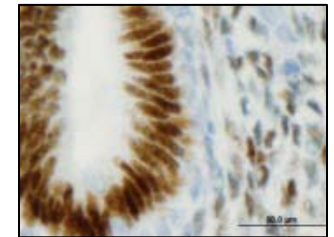
↓  
Equivalent to  
**Snap-Frozen**

**Diagnostic  
Morphology**



↓  
Equivalent to  
**Formalin**

**Immuno-  
histochemistry**

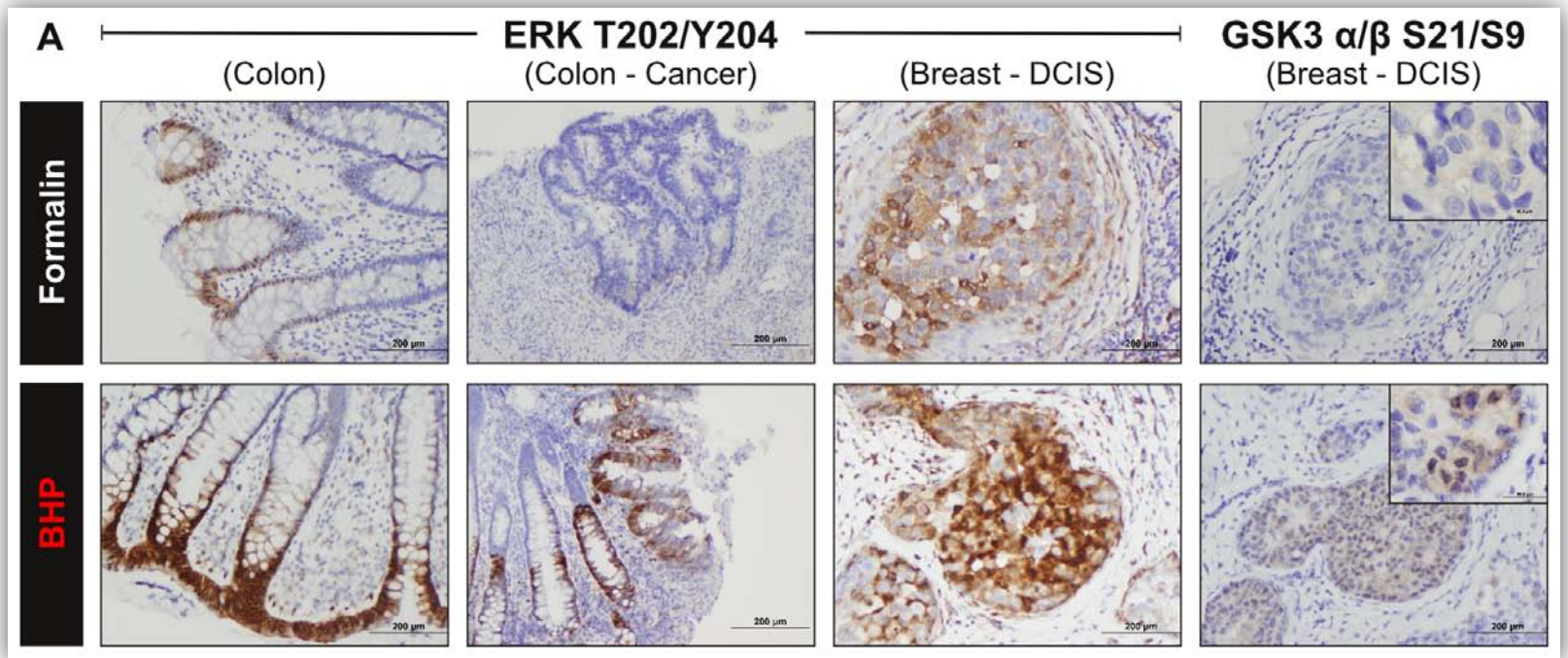
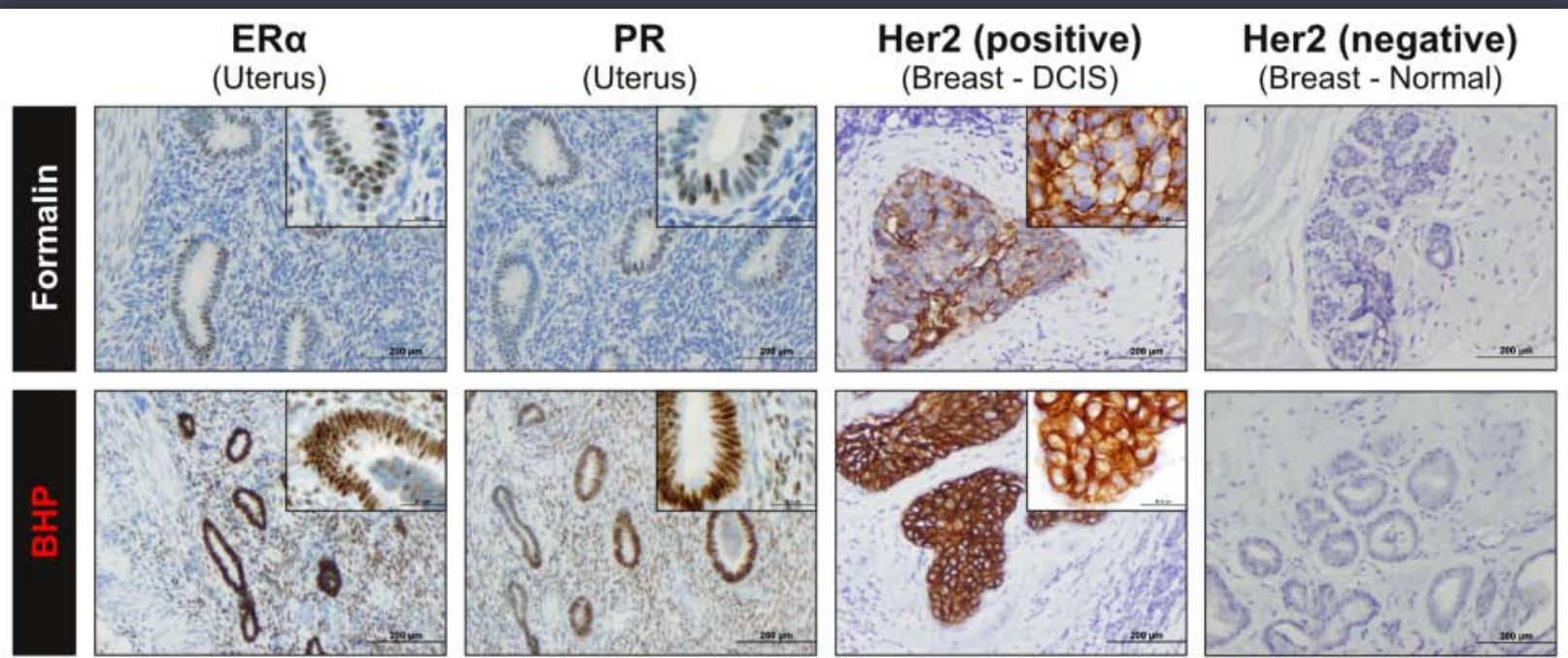


↓  
Equivalent to  
**Formalin**



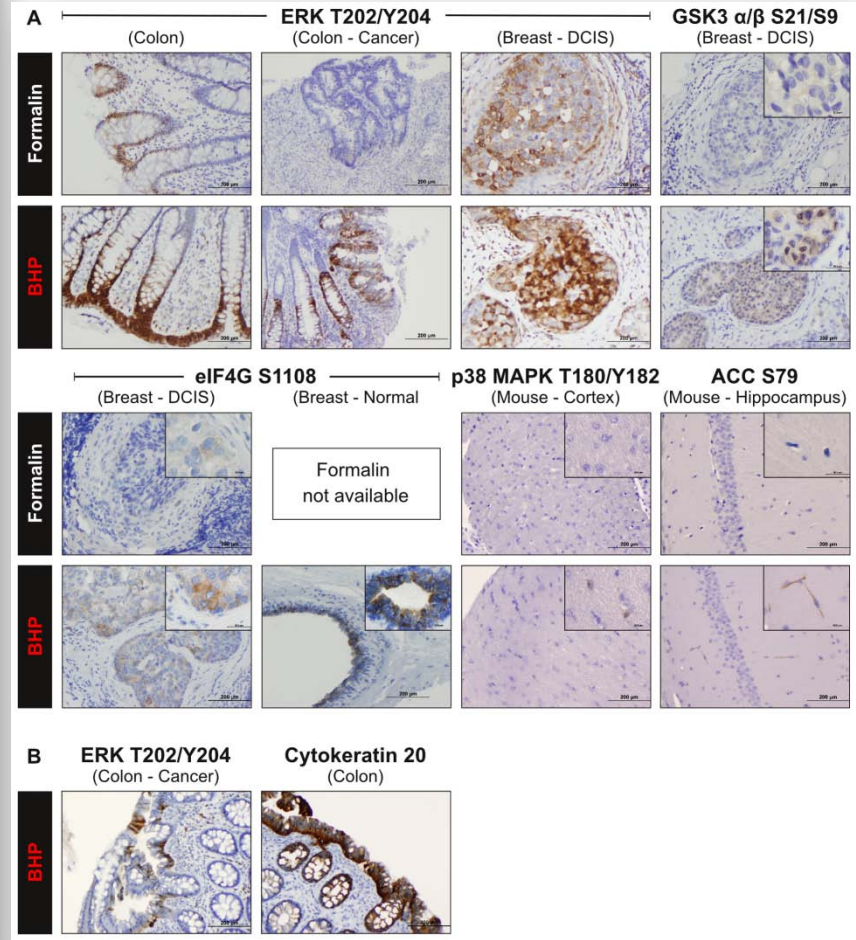
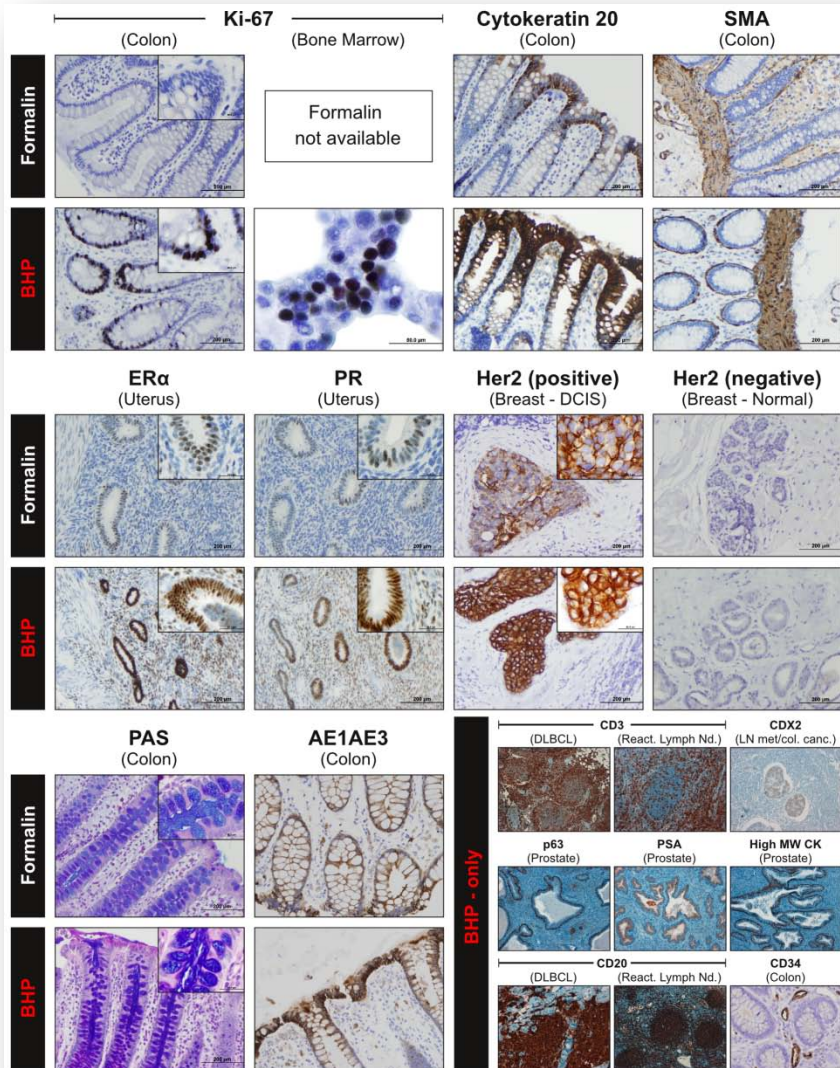
## Immunohistochemistry Endpoints Verified in Paraffin Sections

1) Ki-67 (clone: MIB-1)	10) Cytokeratin 7	19) Phospho-ERK (Thr202/Tyr204)
2) Her2	11) Cytokeratin 20 (clone: Ks20.8)	20) Phospho-GSK3 $\alpha/\beta$ (Ser21/Ser9)
3) Estrogen Receptor $\alpha$ (clone: 1D5)	12) CD3	21) Phospho-eIF4G (Ser1108)
4) Progesterone Receptor (clone: PgR 636)	13) CD20	22) Phospho-Akt (Ser473)
5) PAS	14) CD31	23) Phospho-p38 MAPK (Thr180/Tyr182)
6) DPAS	15) CD34	24) Phospho-Acetyl-CoA Carboxylase (Ser79)
7) AE1/AE3	16) CD38	25) Phospho-Bcl-2 (Ser70)
8) Smooth Muscle Actin (clone: 1A4)	17) CD45	
9) EGFR (clone: DAK-HI-WT)	18) CDX2	

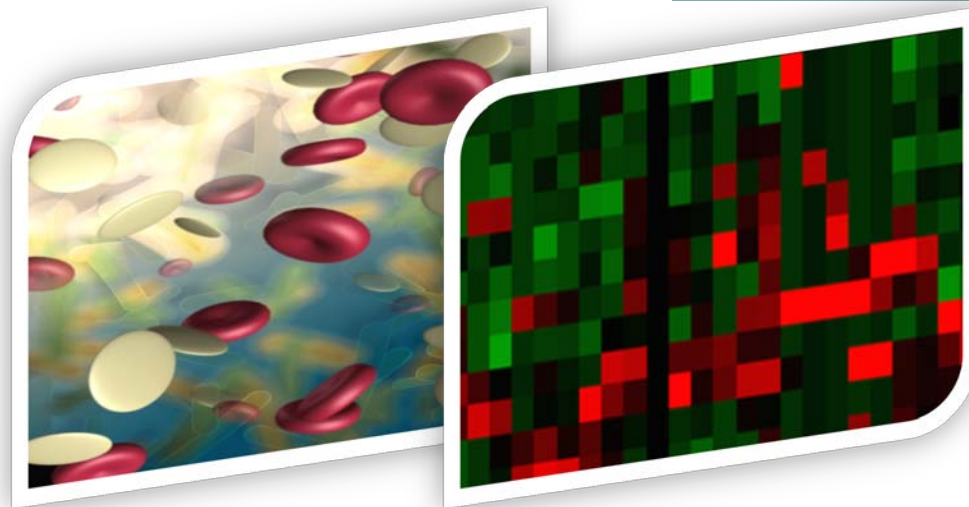




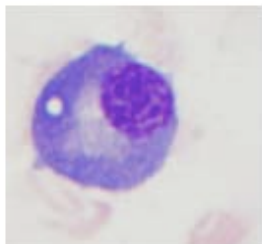
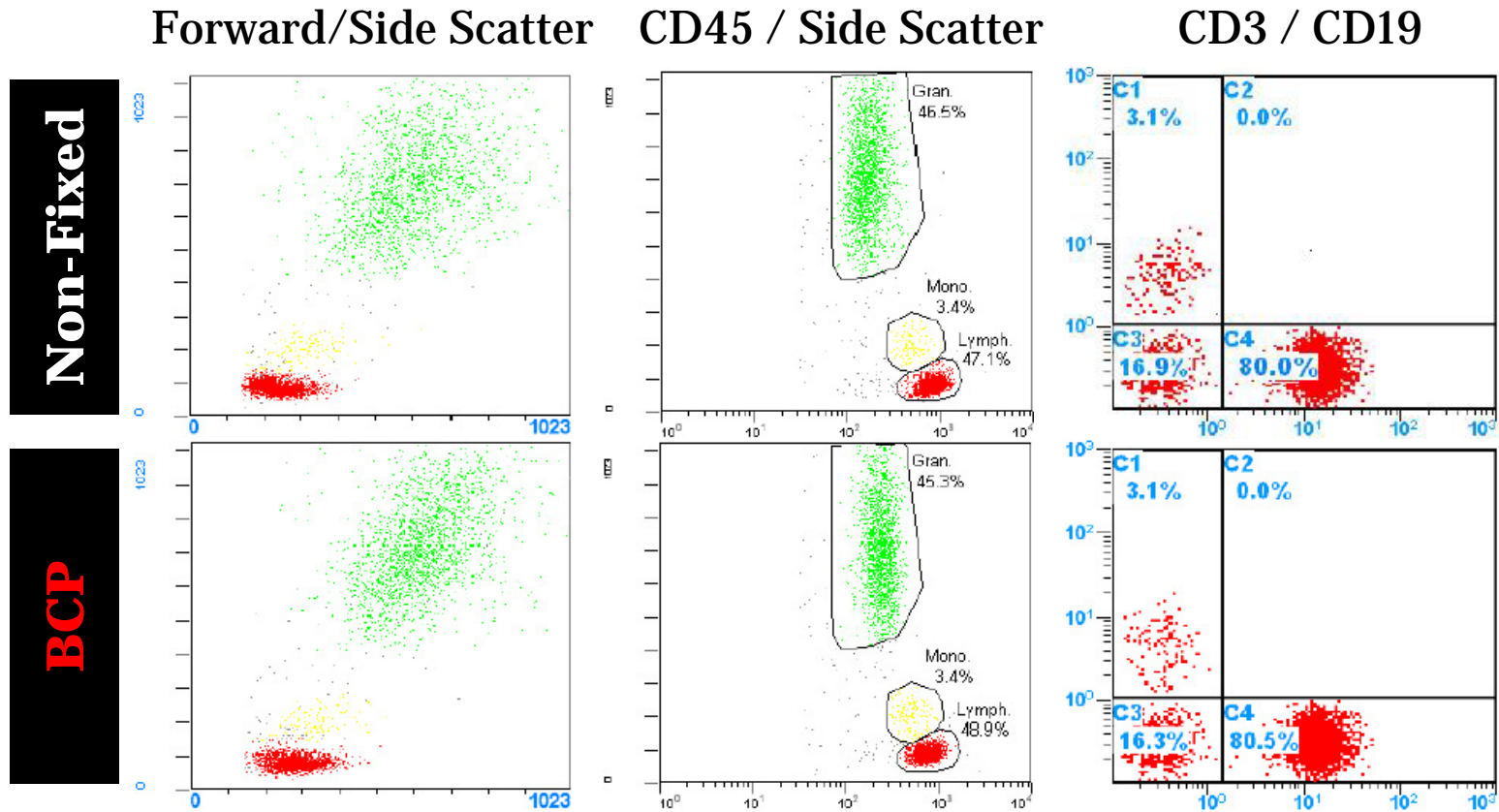
# IHC Examples: Come see Poster #6!



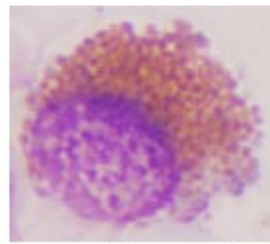
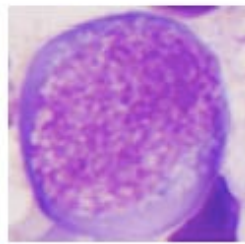
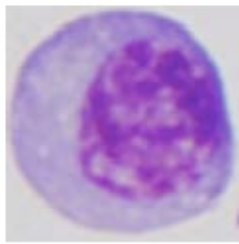
# Biomarker and Cytology Preservative (BCP)



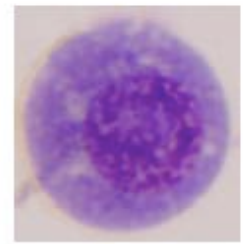
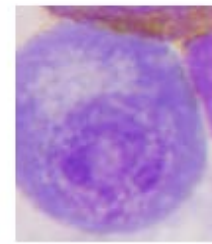
# Cell Morphology and Antigenicity is Retained



**Plasma cell**



**Eosinophil**



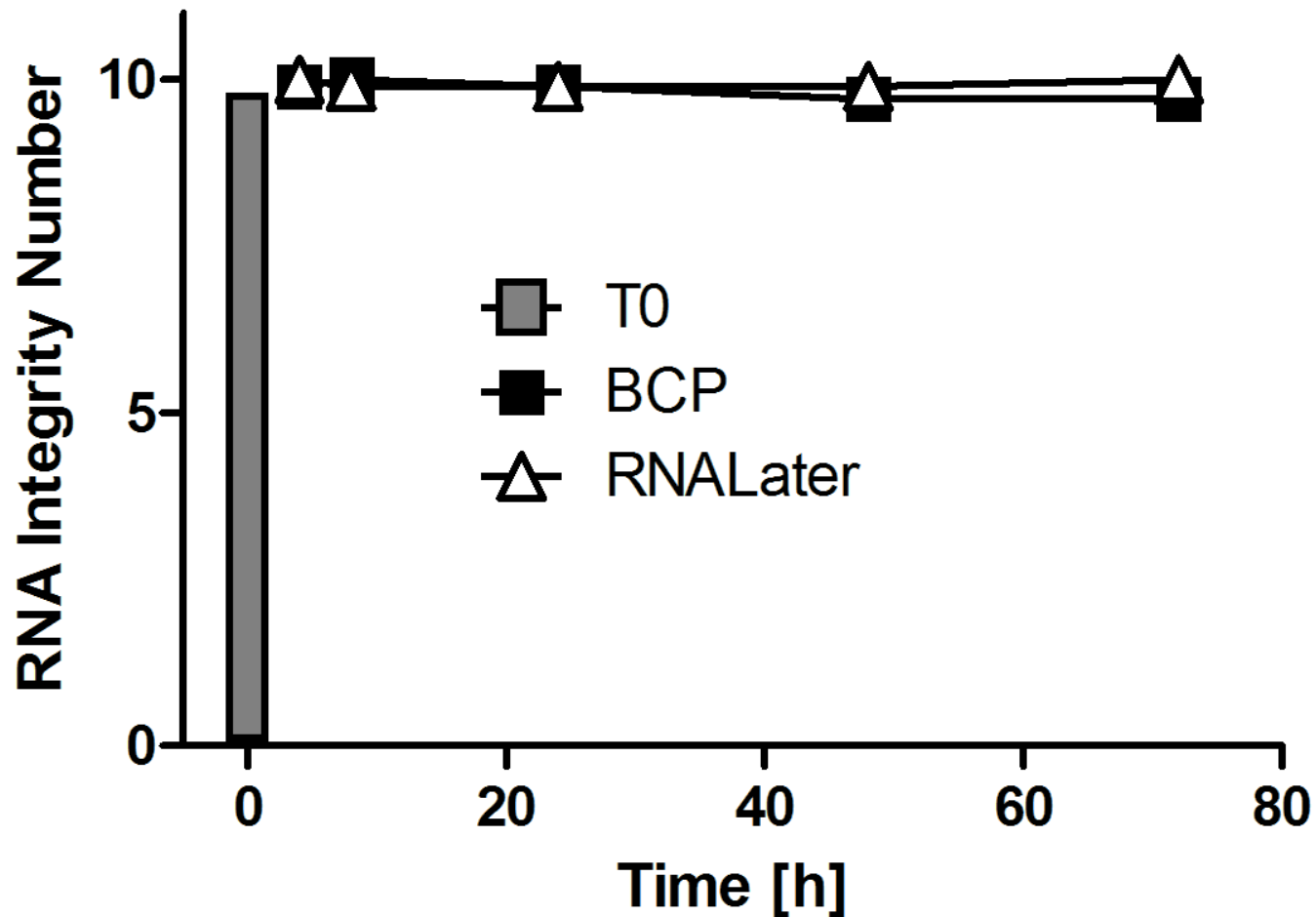
# DNA/RNA



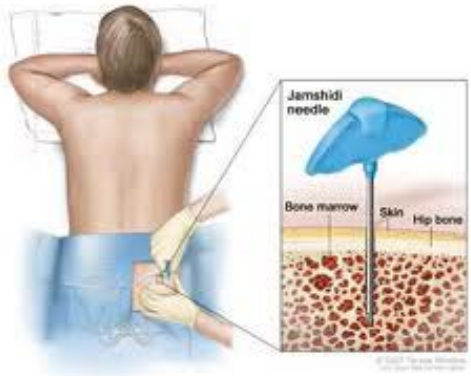


# RNA is Preserved during 72 hour Timecourse

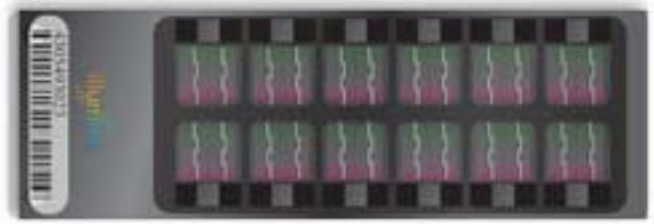
## RNA Integrity – T47D Cell Line



# Preservation of DNA for high fidelity SNP CNV analysis



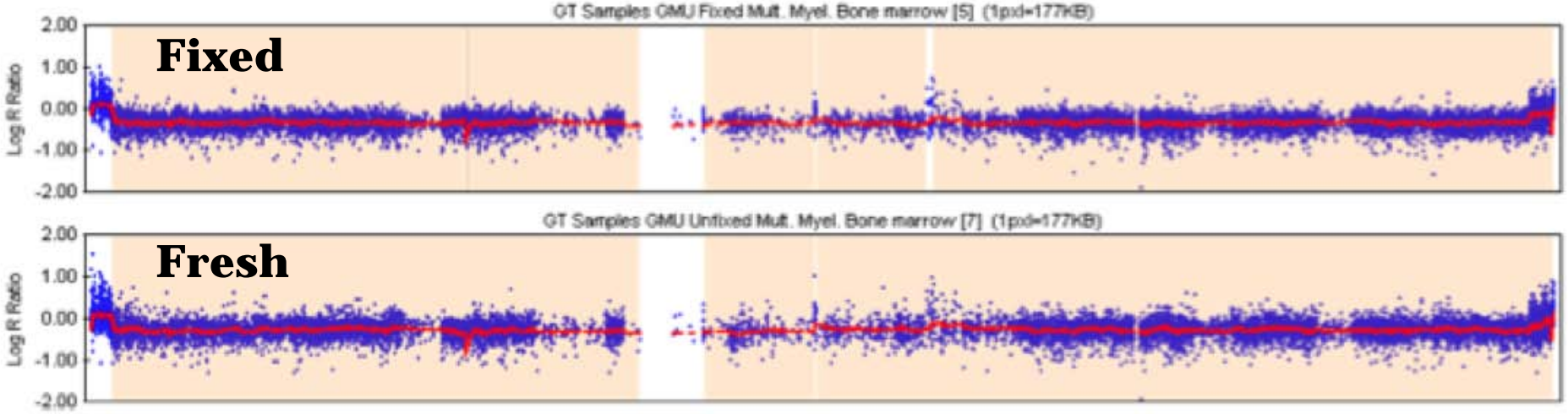
- 1) Mix aspirate with BCP
- 2) Room-temp. transport
- 3) Direct DNA extraction



Illumina CytoSNP Bead Array

Bone Marrow BX/ Aspirate

## Chromosome X

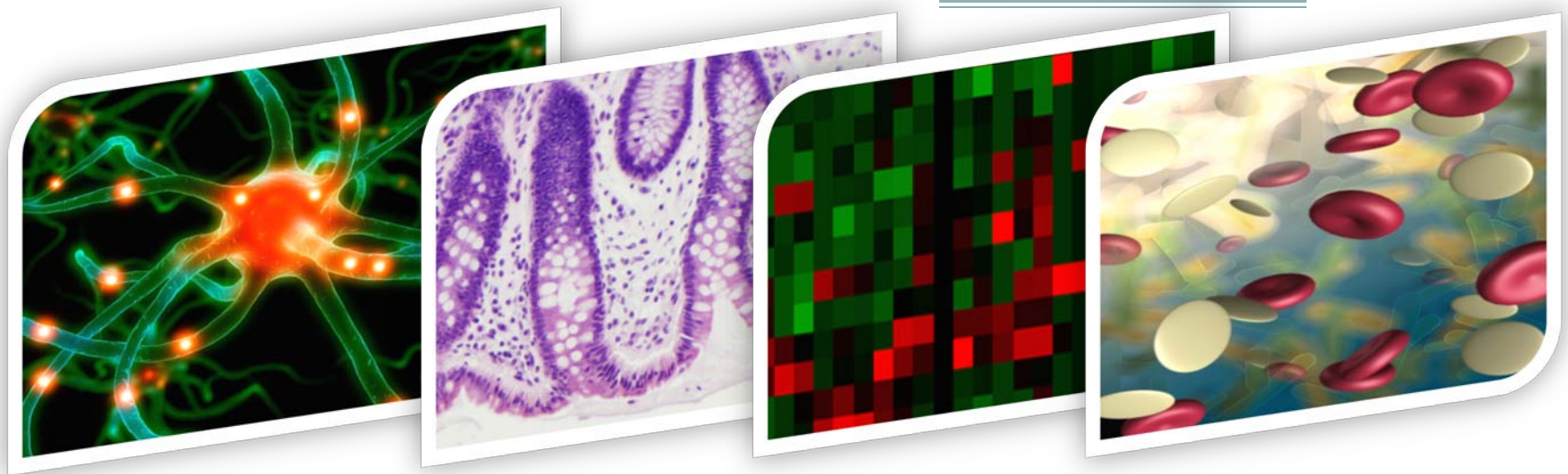


# BHP/BCP Licensed by Theranostics Health

- **Developed under the IMAT program**, licensed through George Mason University to Theranostics Health
- Pre-commercialization studies (shelf-life, etc.) are currently under way
- Theranostics Health is willing to provide **access to the fixative** prior to its commercial release through one-off supplier agreements.



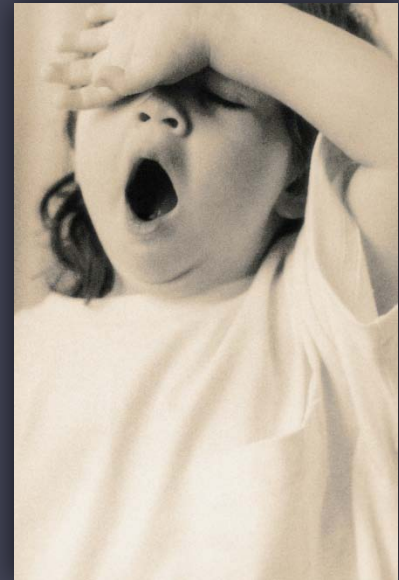
# Summary



# BHP Preserves Phosphoproteins + Morphology

- **1-Step, Room-Temperature** use
- **Paraffin** embedding compatible
- **Phosphoprotein** levels are comparable to matched **snap-frozen** samples
- **Histomorphology** is **comparable** to FFPE
- **No de-calcification** necessary
- **Antigenicity** is **stronger** than FFPE

The End



cmuelle1@gmu.edu



# Acknowledgements

## **George Mason University (Manassas, VA, USA)**

- **Ginny Espina**
- **Lance Liotta**
- Alessandra Romano

## **St. James's Hospital (Dublin, Ireland)**

- Eoin Gaffney
- Ciara Ryan

## **Mediterranean Institute of Oncology (Catania, Italy)**

- Lorenzo Memeo
- Cristina Colarossi



## **NCI Grant 1R21CA125698-01A1**

**“Surrogate and Sentinal Technologies to Monitor Stability of Cancer Phosphoproteins”**

# **Poster #6**