Does digital imaging provide?

- NOT a pathologist replacement
- Assistive technology
  - Enhance pathologist functionality
    - Reliability
    - Reproducibility
    - Capability
Slide Cytometry...The Need

Current method for assessing tumor cell for proteins by IHC

- Immunohistochemistical assay for protein or phosphoprotein (cell signalling)
- Pathologist-dependent, subjective (0-3+)
  - “Quantified” by stain area, intensity and estimated percent
  - Subjective and not reproducible
- Proteins assessed one at a time
- Dependent on pathologist time
Slide cytometry (Process) for assessing activity of signaling pathways

Immunohistologic staining of pathway components
Pathways analyzed on a cellular basis (“cytometry”) rather than pixel
Results quantitated computationally (“automated”)
Multiple pathways analyzed together (“multiparameter”)

Components
1. Multiparameter immunostaining
2. Multispectral image capture
3. Image processing to resolve individual stains based on spectra
4. Tumor segmentation
5. Computational identification of nuclei and cells in image
6. Computational assignment of immunostains to each nucleus or cell
7. Data display and Analysis
1. Multiparameter immunostaining

Current State
3 Colors, no more than 2 colors in same spatial compartment

Future
High order multiplexing with Fluorescence
2. Multispectral Hardware

• Dispersive Elements
  – Prism – PARISS
    • http://www.lightforminc.com/
  – Dispersive

• Tunable Liquid Filters
  – CRI -
    • www.cri-inc.com
Light has no color. “Color” is an observer interpretation. These two yellows appear identical to the human eye, yet they have very different spectral components.
3. Image processing to resolve individual stains based on spectra

Teach computer the spectral profile of chromogen/fluorophore A, B, C

“extract” spectra of individual stains
4. Tumor Segmentation

Manual Segmentation

Automatic Machine Segmentation
CAD – Computer Assisted Dx

- Can computer be taught to identify Prostate Cancer in whole digitized slides?
- Utility
  - Disease level Segmentation for IHC/IF,
  - CAD for Rescreening or Primary Screening
Introduction

- Most of the image is benign
- Can we reduce image size by excluding benign areas
- Combine this with scale information to greatly increase the efficiency of the detection procedure
Introduction

• Each “pass” of the image rejects pixels, and then only the positive pixels are analyzed at higher scales
• This allows us to efficiently analyze the image at higher scales by only looking at “interesting” pixels
• Increasing accuracy does NOT increase execution time
Methods

• To describe each pixel, we extract ~ 600 image features from the image
• There are three categories of features:
  – Statistical Features
  – Co-occurrence (texture) Features
  – Gabor Filter Features
• Each of these is extracted from the three channels of the image, at three different window sizes
Methods

• The individual feature likelihoods are combined by AdaBoost [3] to get a likelihood ensemble

• A small number of features are used at scale 0 to obtain the ensemble, which is then thresholded

• The process begins over again at the next scale; only pixels labeled as positive at the previous scale are analyzed
Higher scale analyses
Higher scale
CAD Conclusions

• Using the Hierarchical cascade allows for fast, accurate analysis of large biomedical images
• Such a methodology could be employed in a number of scale-sensitive image analysis systems
• Both the cascade and the analysis itself can be applied to various pathologies and imaging modalities
• The separation of classes as the scales increase indicates that more discriminatory information is available at higher scales
• Higher scales studies demonstrate the capacity to distinguish stroma vs benign glands from Gleason grade 3 and grade 4 carcinoma
5. Computational identification of nuclei in image using Farsight software
6. Computational assignment of immunostains to each nucleus

- Breast tumor stained for p-ERK (DAB) & hematoxylin
- Segmentation of nuclei (based on hematoxylin)
- p-ERK+ tumor cells (yellow)
- p-ERK- stromal cells (grey)
7. Data display & Analysis:
Frequency histogram of intensity of p-ERK staining of stromal and tumor cell nuclei in a breast tumor
Frequency histogram of intensity of p-ERK staining in carcinoma pre and post therapy
Two-parameter analysis: Germinal center stained p-ERK (SG blue), Ki-67 (VIP) and hematoxylin
Nuclear segmentation
- p-ERK+/Ki-67- (yellow)
- p-ERK-/Ki-67+ (blue)
- p-ERK+/Ki-67+ (magenta)

2-antigen scatter plot (from 1 image)
Triple stain for breast (ER/PR/Her2)
Lymphovascular Invasion Oral SCC

Cytokeratin – DAB (brown)
CD34 – Vector SG (blue)
Podoplanin – Vector VIP (purple)
Autofluorescence

Original

Spectrally separated
Future directions

• Develop automated analysis package
  – Pathway analysis (5-6 colors) on FFPE tissue
    • Endothelial cells
    • Tumor cells
    • Lymphoid cell
  – More routine analysis
    • ER, PR, Her2, EGFR, cKIT…
    • C4d in transplant rejection
  – Interact with Clinical Trial Workspace
Multidisciplinary Research Group

- **MSI work**
- Penn - Bill Lee, Wiem Lassoud
- RPI - Badri Roysam, Gang Lin
- Drexel – Youngmoo Kim
- CRI - Richard Levenson, Cliff Hoyt

- **CAD - Rutgers**
- Anant Madhabushri
  - Scott Doyle