Infrared spectroscopy-based Chemical imaging for biomedical research and cancer pathology

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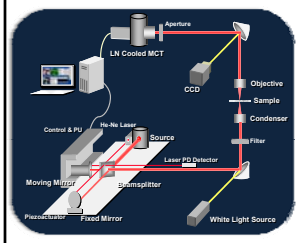
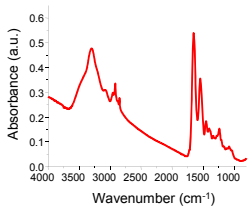
What is chemical imaging?

- Principle: Employs the intrinsic chemical contrast of tissue to provide an image: label-free method
- Chemical content is determined by spectroscopy
  - Vibrational spectroscopic imaging (*molecular spectroscopy → chemical imaging*)
    - FTIR absorption imaging (Mid-IR – 2 to 14 μm)
    - Raman scattering imaging – Raman, RRS, SERS, CARS (UV/visible/near-IR: 300 nm – 1.1 μm)
  - Other techniques of selective chemical sensitivity
    - NIR/Vis spectroscopy – overtones, refractive index
    - Intrinsic fluorescence, FLIM – important specific classes
    - MR spectroscopic imaging – metabolites
    - Mass spectroscopic imaging – proteins

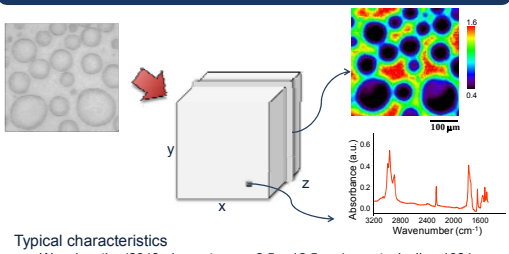
IR imaging technology





- Instrumentation based on classical techniques (40-100 years), hardware (<10 years)
- Data correlations for over 100 years
- What can we measure in tissue?
  - "Mesoscale" chemistry

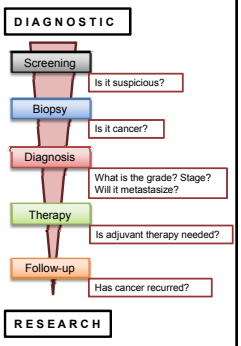
FT-IR spectroscopy → Imaging



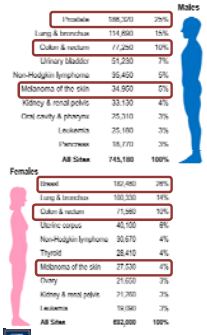
- Typical characteristics
  - Wavelengths (2048 elements over 2.5 – 12.5 µm), x, y typically ~1024
- Computation is essential to recover data
  - Manual examination is prohibitive
- Trade-offs: spatial coverage vs. resolution, spectral resolution vs. signal-to-noise ratio, time vs. data quality/size vs. information

Motivation

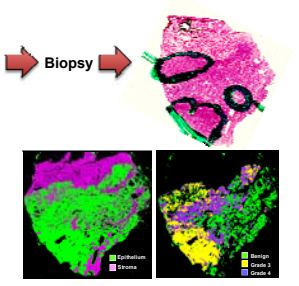
- Cancer pathology
  - Prostate cancer as a paradigm – 1 in 6 men
  - Biopsies: >1 million annually with disease ~20%
  - Diagnoses: >200, 000 annually with lethal ~20%
  - Grading is subjective, variable, leads to conflicting therapy routes
  - Prognosis tools are not perfect – 97% undergo therapy
  - "Holy Grail" of oncologic pathology
  - Primary evaluative standard for research
- Manual recognition in stained tissue



Cancer and Diagnostic Process

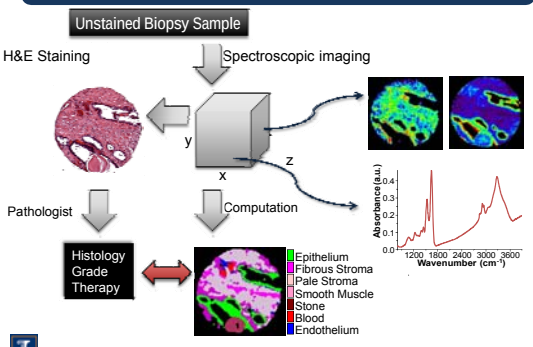


American Cancer Society, est. 2008



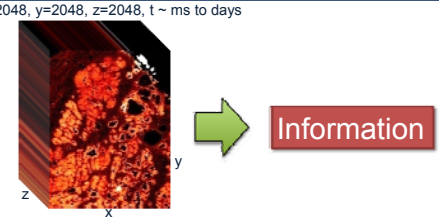
- No stains, no manual decisions

Chemical Imaging for Tissue



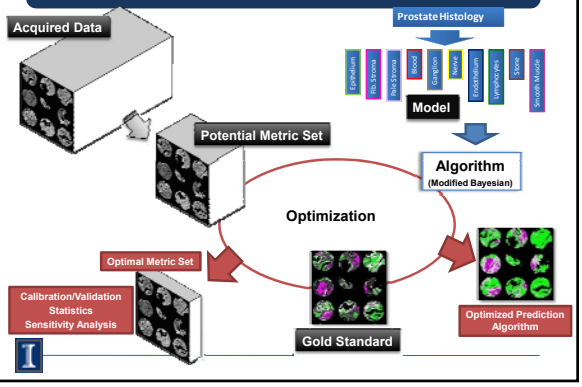
From Data to Knowledge

x=2048, y=2048, z=2048, t ~ ms to days

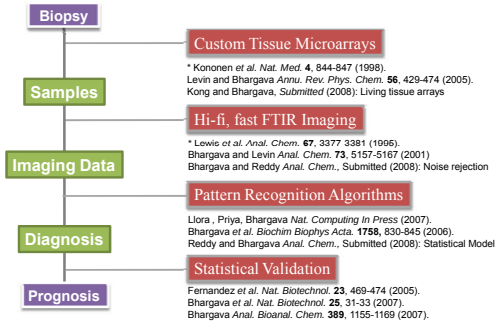


- Model based design of experiments
- Hypothesis driven analysis – supervised data analysis
- Biologically inspired statistical pattern recognition of spectra
- Approach – Model, Train algorithm, Classify, Validate

Integrative classification process



Integrative Process and Key Technologies

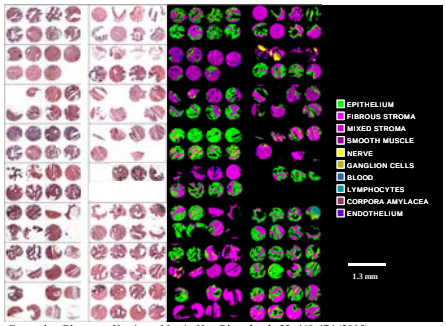


Major Translational Themes

- Translation to clinical practice
  - Comprehensive histopathology - Model-based visualizations
  - Screening Pathology
  - Prognosis
- Translation to research
  - Replicate some functions of molecular imaging
  - Multimodal imaging
  - Automated molecular analysis

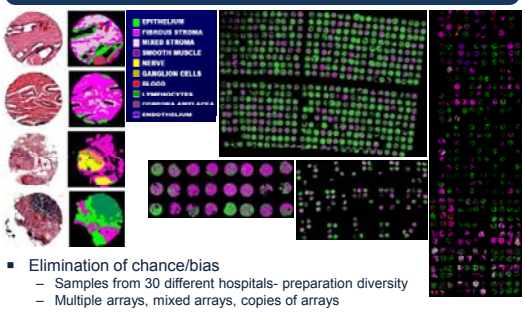
Application 1: Prostate Histology

Validation of Prostate Histology: 970 samples, 10 million spectra



Fernandez, Bhargava, Hewitt and Levin ***Nat. Biotechnol.***, 23, 469-474 (2005)

Classification examples

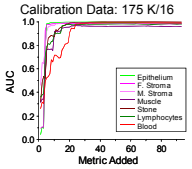


- Elimination of chance/bias
  - Samples from 30 different hospitals- preparation diversity
  - Multiple arrays, mixed arrays, copies of arrays
  - Instrument changes
  - Experimental: thickness effects, artifacts, population diversity

Metrics, Training and Validation

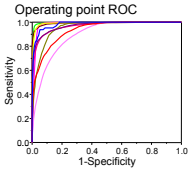
Prostate Histology

Calibration Data: 175 K/16

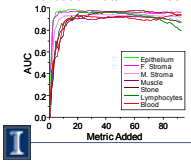


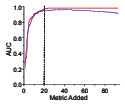
Class	AUC
Epithelium	0.994
F. Stroma	0.983
M. Stroma	0.961
Muscle	0.894
Stone	0.991
Ganglion	0.954
Nerve	0.971
Lymphocytes	0.990
Blood	0.932
Endothelium	0.983

Operating point ROC

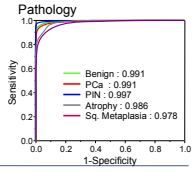


Validation Data: 2 mil/400

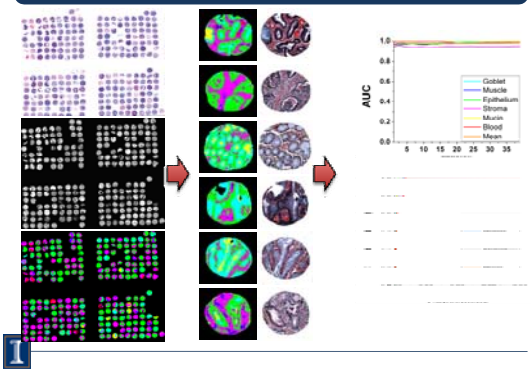




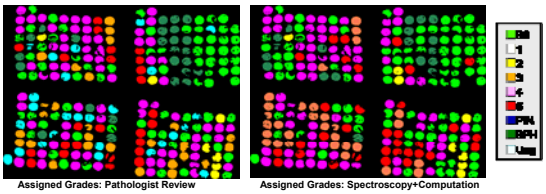
Pathology



Colon Histology



Automated Grading Results



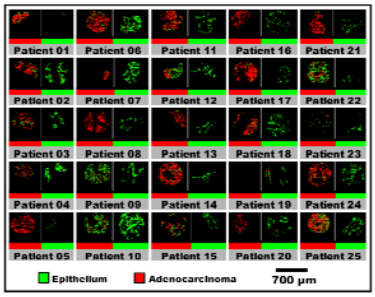
Assigned Grades: Pathologist Review | Assigned Grades: Spectroscopy+Computation

- First attempts were unsuccessful
- Questions:
  - Can cancer be detected?
  - Is morphological grading possible with chemical data?

Sensitivity	81.8%
Specificity	72.6%

2 Class Models

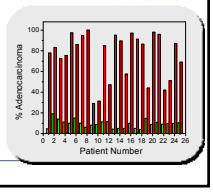
50 patient NCI validation: Cancer and Normal Adjacent Prostate (NAP)



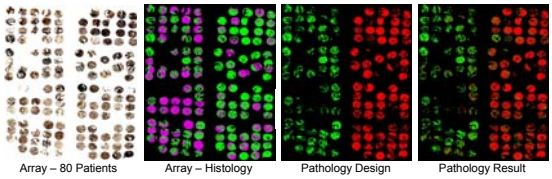
Overall Pixel Accuracy

Tuned for Epithelium Sensitivity

Ground Truth Class / Result of Classification	BENIGN EPITHELIUM	ADENOCARCINOMA
BENIGN EPITHELIUM	89.59	25.50
ADENOCARCINOMA	10.41	74.50



Prostate Cancer Diagnosis



Array – 80 Patients | Array – Histology | Pathology Design | Pathology Result

- Overall pixel accuracy ~ 88.5% ; Heterogeneity in samples?
- 1 cancer sample classified as benign (71)
- 1 benign sample classified as cancerous (69)
- Sensitivity and specificity exceeding human capabilities
- Large validation studies underway

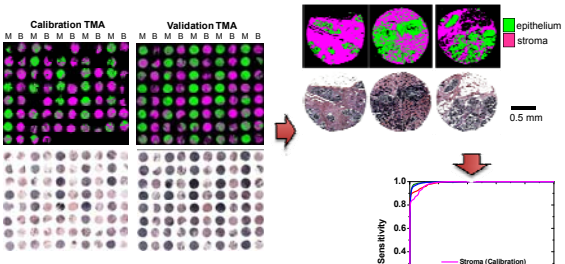
Challenges

- Opportunity to leverage both structure and chemistry without human supervision, dyes or probes
- Objective, quantitative and automated nature may be advantageous
- Clinical translation facilitated by
  - Better, cheaper and faster instrumentation
  - Multi-institution validation
  - Novel concepts
  - Integration with clinical workflow

Breast Pathology: Case Study

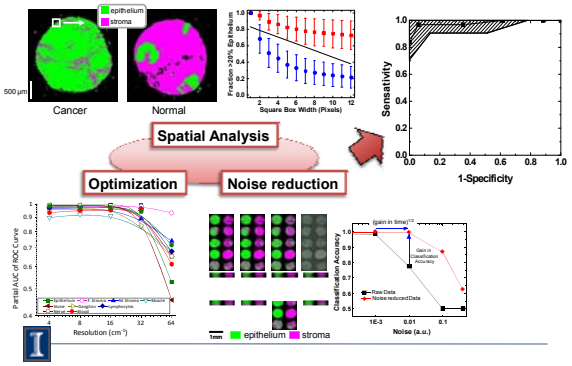
- Application: rapid screening of biopsies
  - Detect cancers rapidly
  - Quantify lesion size
- Desirable translational features
  - Accurate
  - Fast and simple to understand
  - Minimal supervision
  - Quality control steps
- Developments
  - "Bare bones" model: 2 class (epithelium/stroma; cancer/no cancer)
  - Move to larger (poorer) detectors
  - Use computation for noise rejection
  - Use spatial and spectral features

2 Class Breast Histology Model



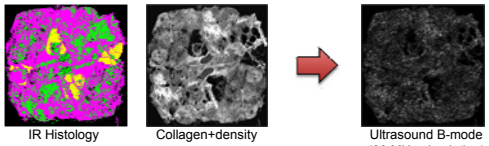
- 85% of internal human cancers are epithelial in origin
- Epithelium-stroma segmentation is the first step

Translation: Lesion Detection



Translation for pathology research

- New characterization methods
  - Tissue engineering and cellular imaging
- New technology for molecular analysis
  - High-throughput *-omics*
- Computational models for pathology
  - Design and evaluation of imaging instruments



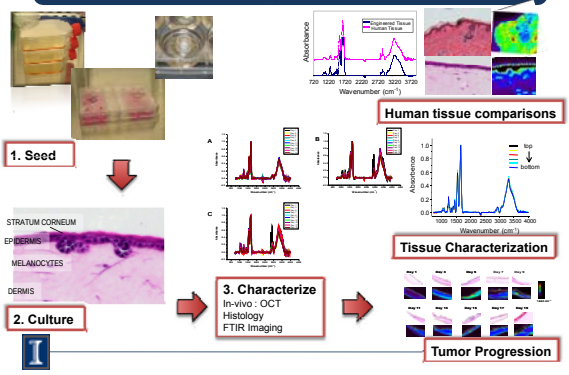
IR Histology | Collagen+density | Ultrasound B-mode (20 MHz simulation)

Cancer Models - Current state of the art

- Disease biology and progression
  - 2D cultures of single cell type are the standard
  - Recent value of 3D models emerging
  - 3D interplay of cells is critical in understanding disease progression
  - Animal models – expensive, poor control
  - Solution – Engineered tissue models
- Implications for pathology
  - Standardization
  - Fundamental statistical analyses
  - Quantification for tissue engineering

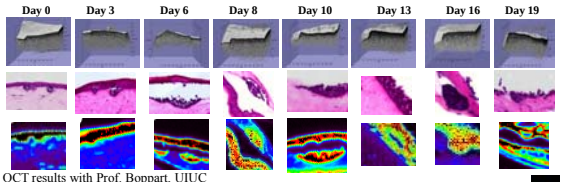
Engineered Tissue Models for Heterotypic Interactions

Model System 1: Skin



Multimodal Imaging

Tumor Growth in Engineered Skin



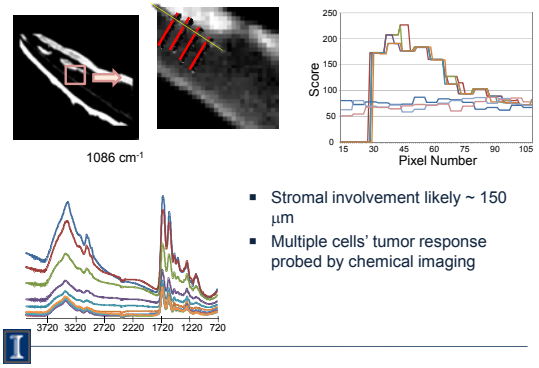
OCT results with Prof. Boppart, UIUC

4 sets of 20 engineered skin tissues were cultured. One tissue was taken out each day for analysis.

- OCT: a tendency of increasing dark areas at the epidermis/dermis boundary;
- HE and FTIR: visible increase of tumor size;
- FTIR also allows the development of automated pathology protocols;

Multimodal imaging analysis to help the early melanoma diagnosis ?

Heterotypic interactions?

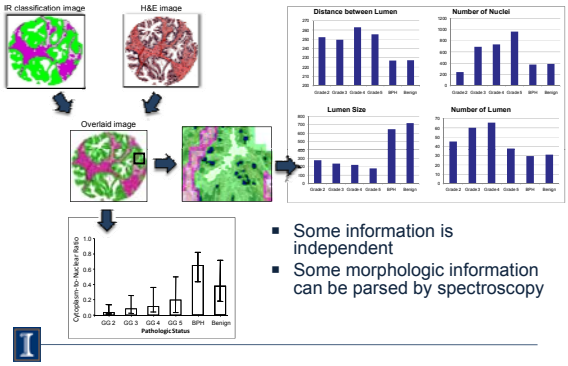


- Stromal involvement likely ~ 150 μm
- Multiple cells' tumor response probed by chemical imaging

Summary: What's in store?

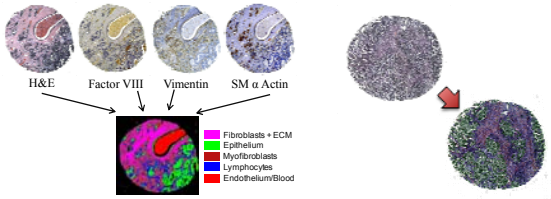
- Descriptive pathology
  - Attempt to match pathologist/molecular techniques
    - Comprehensive histology
    - Diagnostic pathology
  - Clinical samples and problems drive solutions
    - Biopsy screening
  - New technology from classical pathology knowledge
    - Tissue analysis
    - Automated LCM
- Predictive pathology
  - Newer ideas
  - Utilize full power of spectroscopic and imaging techniques
  - Key enabling technology

Tech. 1: Parsing Rules from Multimodal Imaging



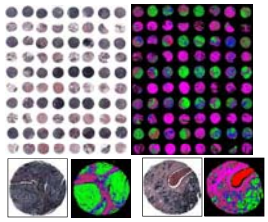
- Some information is independent
- Some morphologic information can be parsed by spectroscopy

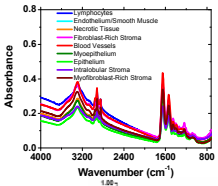
Tech 2. Molecular Imaging Vs. Chemical Imaging

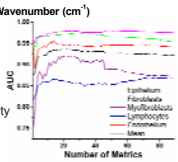


- Principle: molecular imaging measures one target – chemical imaging measures the state of the tissue
- Identification potential is preserved
- Molecular localization/origin is lost; Chemical sensitivity is gained, accuracy may be better
- Some molecular information can be parsed by spectroscopy

Molecular = Chemical Imaging

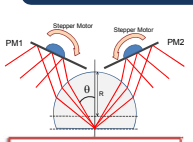




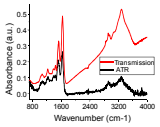


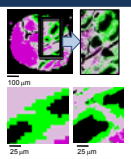
- **Reactive stroma can be characterized, classes of properties:**
  - (1) Fibroblast enrichment,
  - (2) ECM modification
  - (3) Microvessel density
  - (4) Protein structure of blood vessels
  - (5) smooth muscle proliferation
  - (6) "myo-" transitions
  - (7) collagen cross-link density (mechanical properties?)

Tech. 3: Ultra-high resolution imaging (w/glass slides)

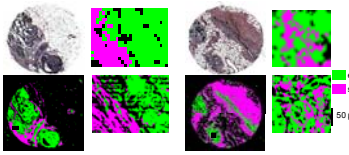


Solid Immersion Lens, ATR





Class Image



- Imaging is possible
- Spectral corrections
  - Reduced scattering
  - Absorbance recovery
- Simple models validated
- Data quality challenges

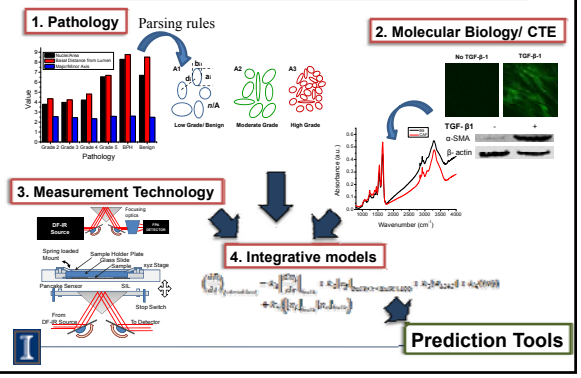
Tech 4. Analytical methods

Systems Approach to Cancer Pathology

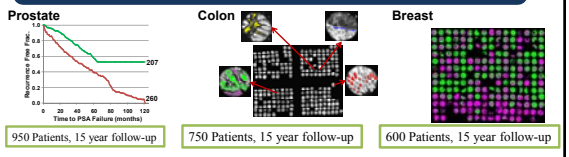
- Principle: disease is a perturbation of a network of key variables
  - Molecular systems biology: genes, proteins
  - Network of chemical and physical changes
  - Context of tissue structure
- Key components
  - Observe: often includes technology
  - Deduce
  - Model
  - Predict
- Human scale or molecular scale

Kitano, H. *Science* **2002**, 295, 1662 - 1664

Key Technologies for Systems Pathology



On-going efforts: Systems Pathology



950 Patients, 15 year follow-up | 750 Patients, 15 year follow-up | 600 Patients, 15 year follow-up

- Predictive models to inform clinicians, imaging
- Molecular and cellular information with multi-scale detail
- Challenges:
  - Information fusion, selection of sub-spaces
  - Optimized instrumentation, algorithms and software for translation

Thank you

- Students and Fellows
  - Breast: Michael Walsh, Frances Pounder
  - Prostate: Rohith Reddy, Jin-Tae Kwak
  - Colon: Jason Ip
  - Molecular and Tissue: Synthia Lane, Rong Kong, Jing Xu, Sarah Holton
  - Instrumentation/Algorithms/Probes: Charles Feng, Anil Kodali, Brynmor Davis, Spencer Brady
- External collaborators
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