Towards Precision Medicine: Translational Diagnostics, Feasibility Research and OME

5-June-2014

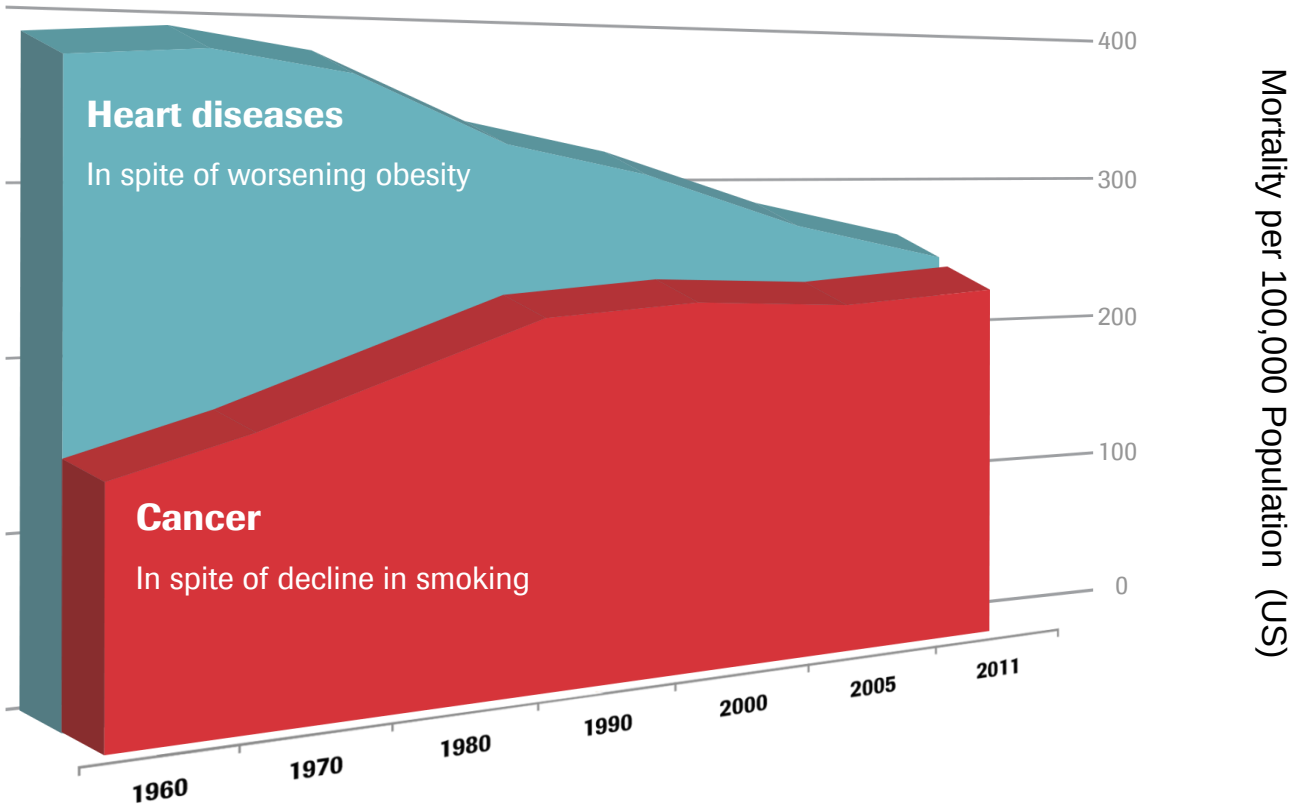
Karl Garsha

Principal Scientist

Digital Pathology & Workflow

Ventana Medical Systems Inc., Tucson, AZ

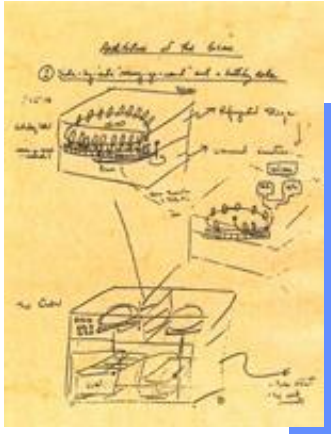
Cancer death rates remain stubbornly high



To Improve the Lives of all Patients afflicted with Cancer

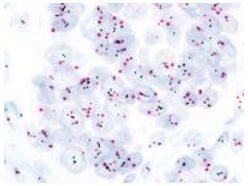
> *Change the practice of medicine by inventing tools that didn't exist before*, rather than building a better microscope.
>
> —*Tom Grogan, MD*
> *Ventana Founder*

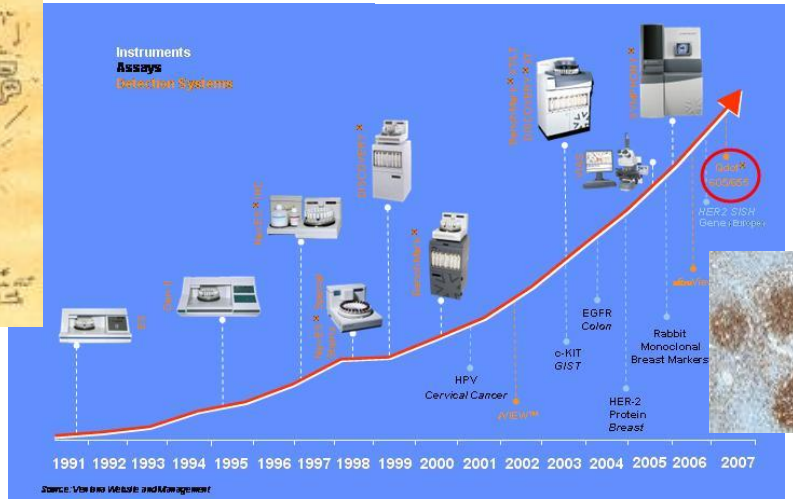
1991

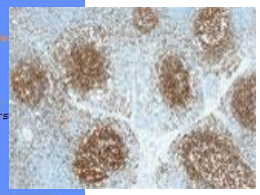


2007

2012



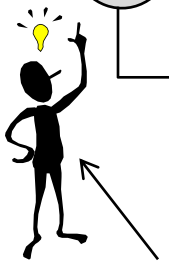




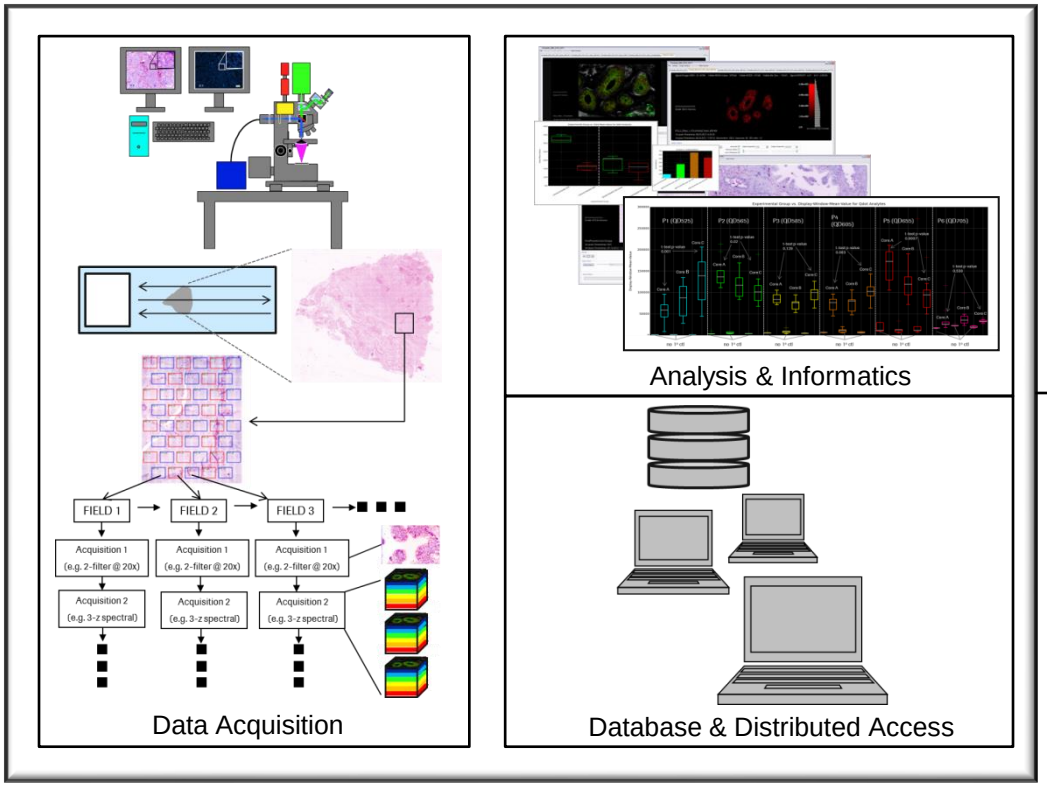
Who are we?

Digital Pathology: Translational Technology & Applications

Broadly-Defined Applied-Research Project



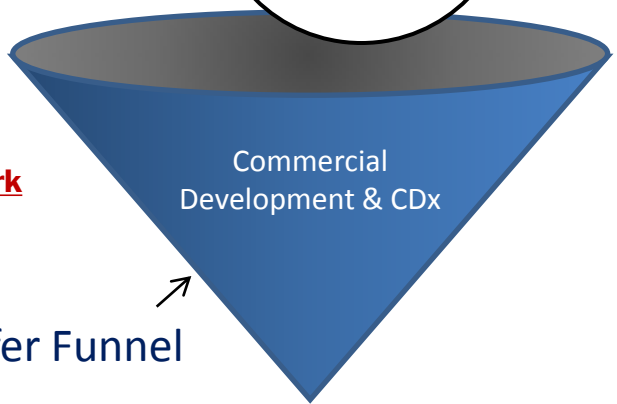
"Ideas Person"



(Business Case)

Clearly-Defined High-Value Application

- **Engage high risk**-unknown outcome, plenty of inventing, little precedent
- **Succeed fast or change direction**: Moving parts and evolving priorities
- **'Scope creep' comes with the territory** (adapt and overcome)
- **Enable** exploratory **data collection** for high-value – not an end in itself
- **Can't be "shrink-wrapped"-heavy reliance on microscopy and preliminary work with chemistry/detection/biology scientists to rapidly evolve solutions**
- **Rapid progression to GO:NO-GO commercial decision point for opportunities**



Technology Transfer Funnel

Cancer is a complex disease

Multiple genetic and epigenetic changes can cooperate to drive cancer and confound cancer therapy

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

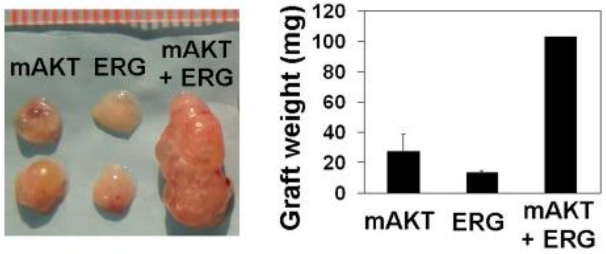
Gerlinger et al. N Eng J Med 2012 366, 10: p883

Identification of a Cell of Origin for Human Prostate Cancer

Goldstein et al. Science 2010 329, 5991: p568

ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells

Zong et al. PNAS 2009 106, 30: p12465



ETS family transcription factors collaborate with PTEN pathways to induce carcinoma from prostate cells.

The clonal and mutational evolutionary spectrum of primary triple-negative breast cancers

Shah et al. Nature, 2012 486, 395-399

Clonal Evolution in Cancer

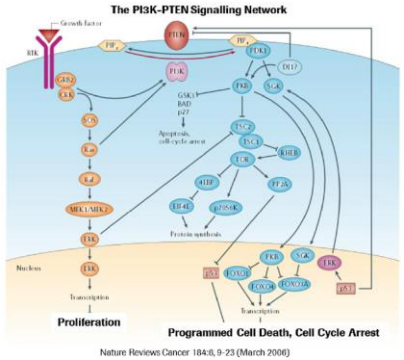
Greaves & Maley, Nature, 2012 481, 306-313

PIK3CA Mutations Frequently Coexist with RAS and BRAF Mutations in Patients with Advanced Cancers

Janku et al. PLoS ONE 2011 6, 7: 222769

Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis

Cully et al. Nature Reviews: Cancer 2006 184, 6: p184

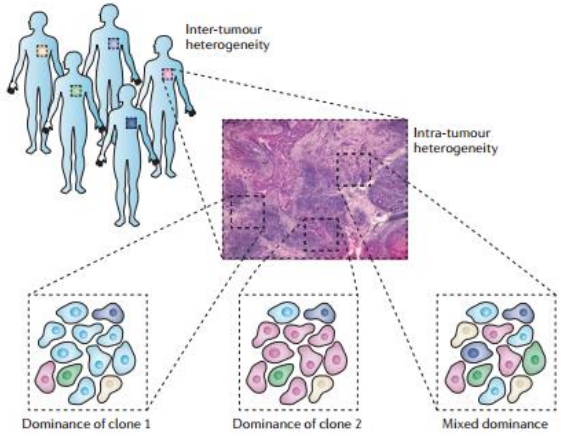


Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing

Ding et al. Nature, 2012 481, 506-510

Intra-tumour heterogeneity: a looking glass for cancer?

Ding et al. Nature Reviews Cancer, 2012 12, 323-334



Our Strategy: Synergy Enables Emergent Capabilities

Pre-analytic Preservation

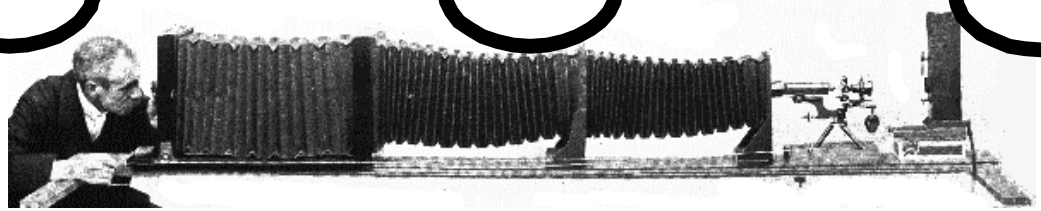
Molecule-Targeted Probes

Multiplex Reporting Technology

Tissue Anatomy Visualization and Anatomic Pathology Selection

Analytical Imaging and Computational Analysis

Databasing, Informatics, Distributed Access



 Arthur Smith, 1904

Analytical Imaging Technology: The 4 Pillars

Analytical Multiplexing **ENABLES** Ventana special research projects – technology platform for **advancing personalized medicine and companion diagnostics**

1. Preserve Anatomic Context

1. Provides expert-guided selection of anatomic pathology
2. Cell morphology indicates cell type and anomolous expression patterns
3. Spatial heterogeneity of genotype and phenotype in tumor

2. Multiplexed Biomarkers

1. Reporters indicate multivariate changes in same cells; increased information density from limited tissue
2. Deconvolution of multiplexed signals is important for:
    1. Interpretation-extends limits of human perception
    2. Segmentation
    3. Quantitation

3. Photometric Measurement Standardization and Quantitation

1. Permits inter-slide and intra-slide comparison of marker levels over extended time periods
2. Greater objectivity in measurement of expression on a continuous scale
3. Higher dynamic range of measurement
4. Facilitates concordance of measurement results between instruments

4. Enable statistically-powered analysis and informatics tools

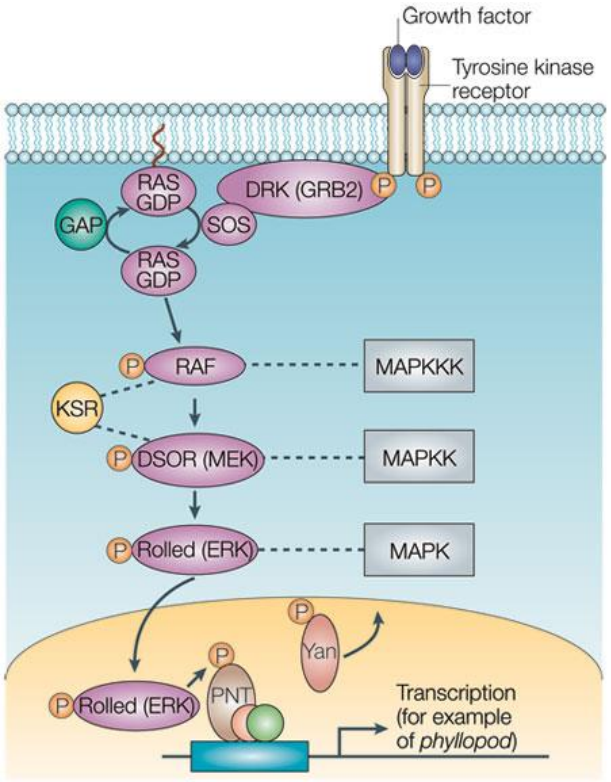
1. Permits scientifically defensible hypothesis testing for assays without medical precedent
2. Enables objective interpretation of subtle, but significant, differences in genotype or phenotype

What are we doing?

We are developing technology to enable **multiplexed** identification of **molecular changes** at key nodes in complex molecular **signaling pathways**

- Cells receive chemical signals at the membrane through receptors (HER2, EGFR, HER3)
- Signals are relayed through protein phosphorylation cascades
- Signal transduction regulates the genetic machinery to alter cellular 'behavior'
- Each pathway may influence several biological outcomes
- Chemical 'action-items' are decided through interaction between signalling pathways at 'nodes'
- Crosstalk between tumorigenic signalling pathways can contribute to dysregulation and malignancy

The Canonical RTK Signalling Pathway

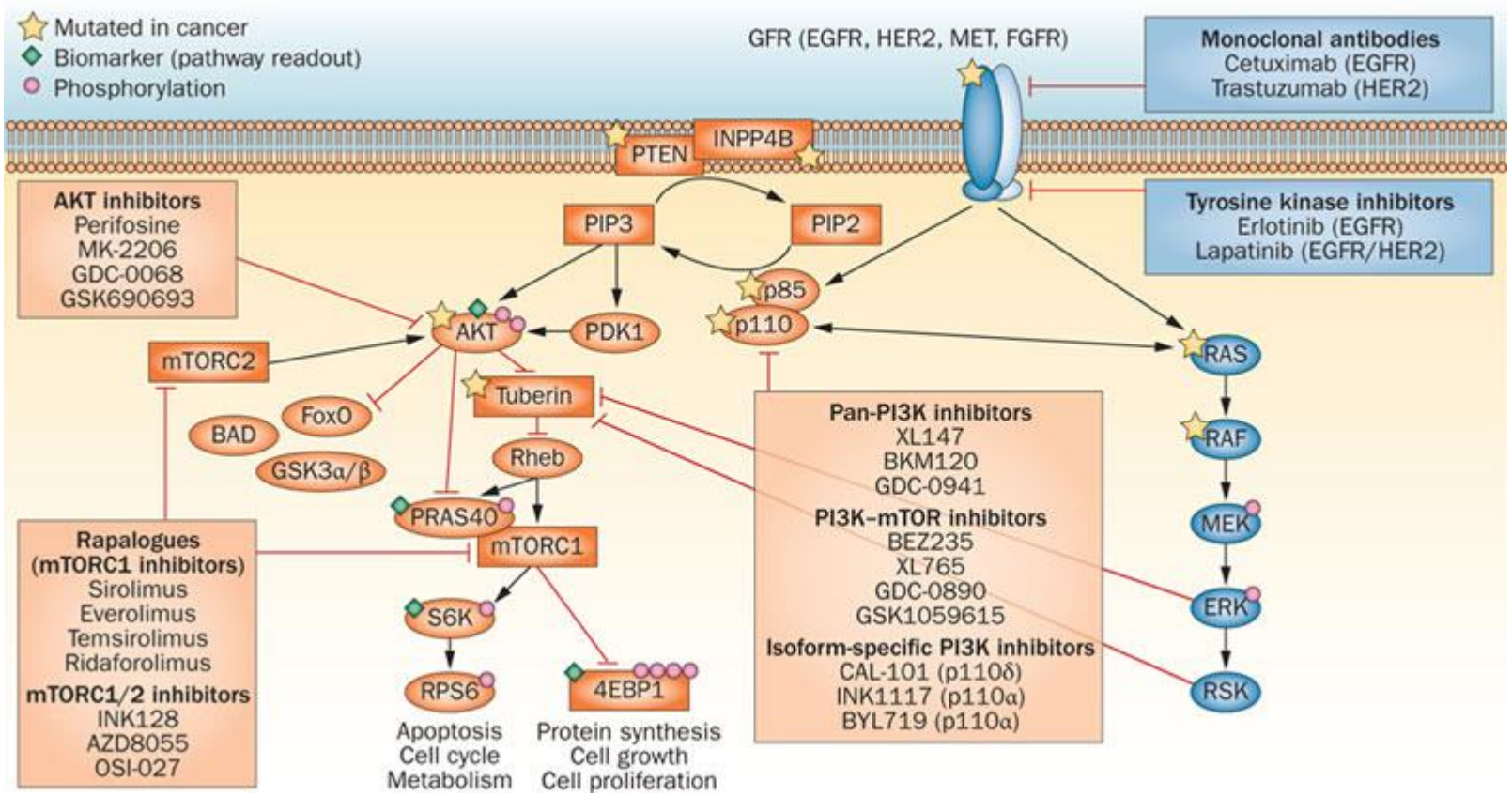


Nature Reviews | Genetics

Nature Reviews Genetics 6, 9-23 (January 2005)

Why are we doing this?

Existing cancer medicines directed to PI3K signaling pathway



Rodon J et al, Nat Rev Clin Oncol 10.1038 143-153 (March 2013)

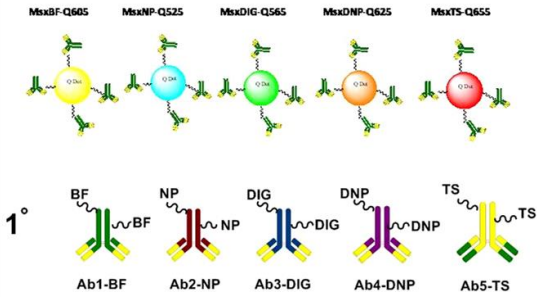
'Doing now what patients need next':

Analytical Assay for Multiplex Molecular Expression in Tissue

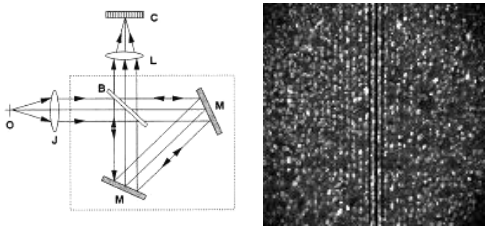
Multiplex QD Reporting Chemistry

(Kosmeder et al.)

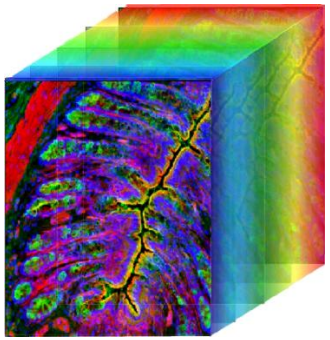
Multiplex stain with Quantum Dots

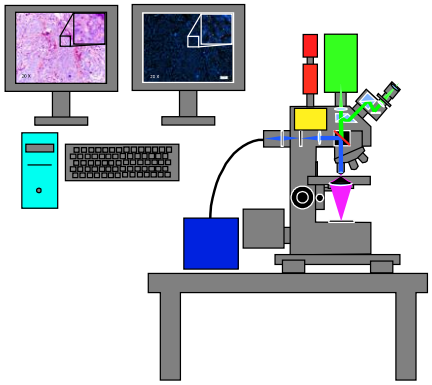


Analytical (Spectral) Imaging

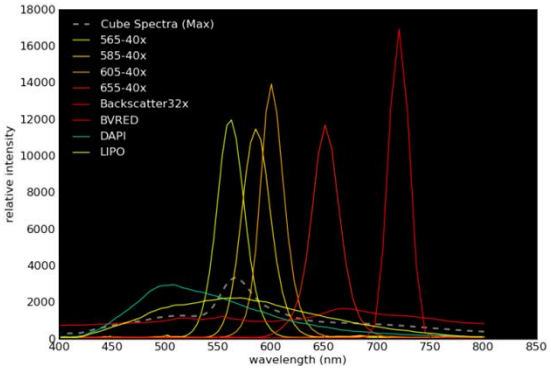


J. Microscopy, Vol 182, Pt. 2, May 1996, pp. 133-140





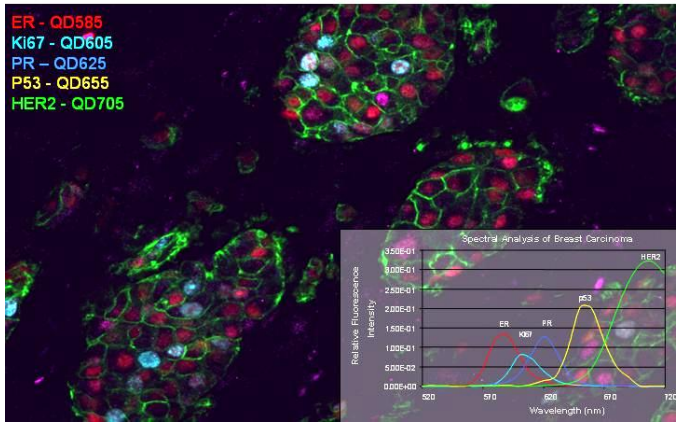
Computational Analysis and Quantitation



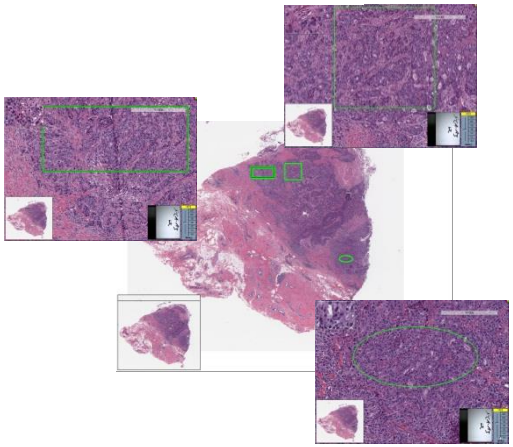
fluorescence reference spectra analyte

$$\begin{bmatrix} F_{\lambda_1} \\ F_{\lambda_2} \\ \vdots \end{bmatrix} = \begin{bmatrix} S_{\lambda_1}^{a_1} & S_{\lambda_1}^{a_2} & \cdots \\ S_{\lambda_2}^{a_1} & S_{\lambda_2}^{a_2} & \cdots \\ \vdots & \vdots & \ddots \end{bmatrix} * \begin{bmatrix} a_1 \\ a_2 \\ \vdots \end{bmatrix}$$

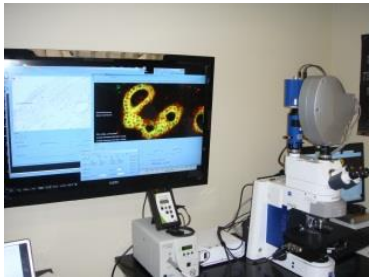
Infiltrating Ductal Breast Carcinoma - IHC Multiplexing with Haptens (Ashworth-Sharpe et al., 2008)



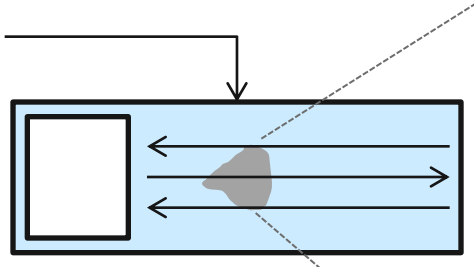
Doing now what patients need next:
Interrogating Anatomic Pathology for Multiplex Molecular Expressior



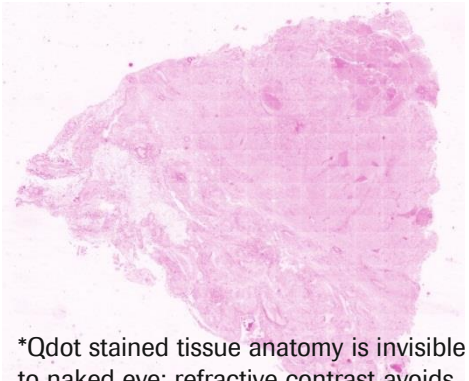
1. Pathologist selects regions on primary stained section (digitized)



Ventana Research System (Vu Lab OHSU)

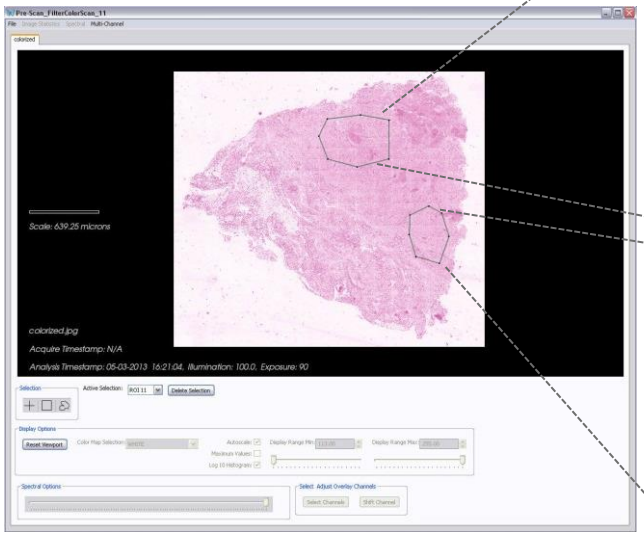


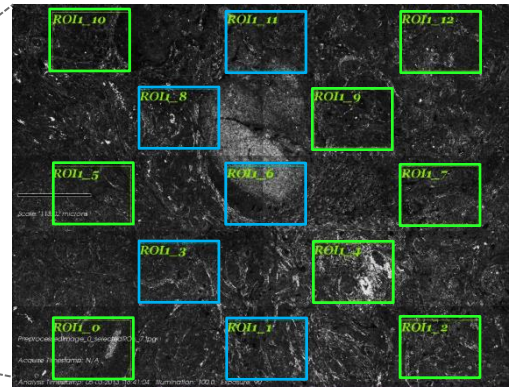
2. Transparent tissue section digitized using 'Bright Vision' near-IR contrast on Ventana Analytical Imaging System (VAIS)

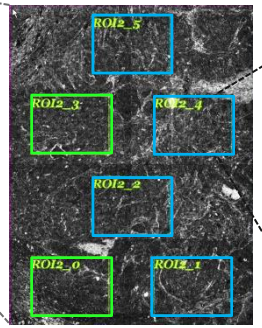


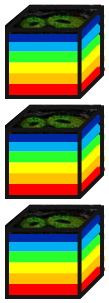
*Qdot stained tissue anatomy is invisible to naked eye; refractive contrast avoids photodamage to section and permits fast overview scan

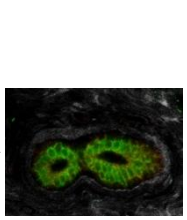
3. Digital image of tissue structure is viewed and marked for regions of interest in VAIS software

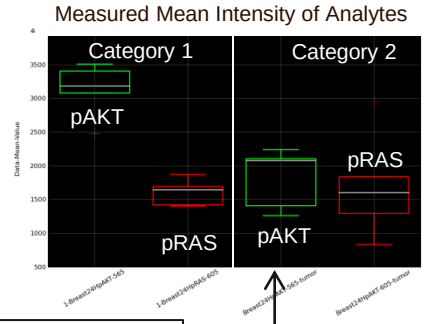








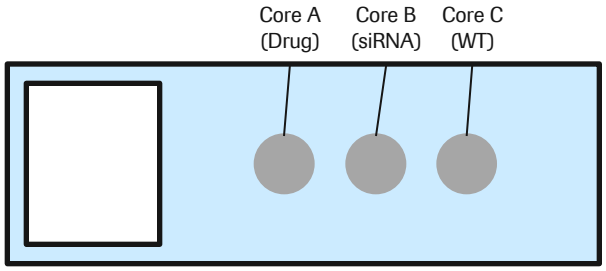


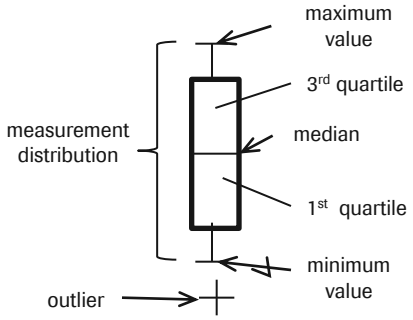


4. Large regions are spectral-spatial imaged at regular grid intervals to acquire accurate multiplex molecular information efficiently

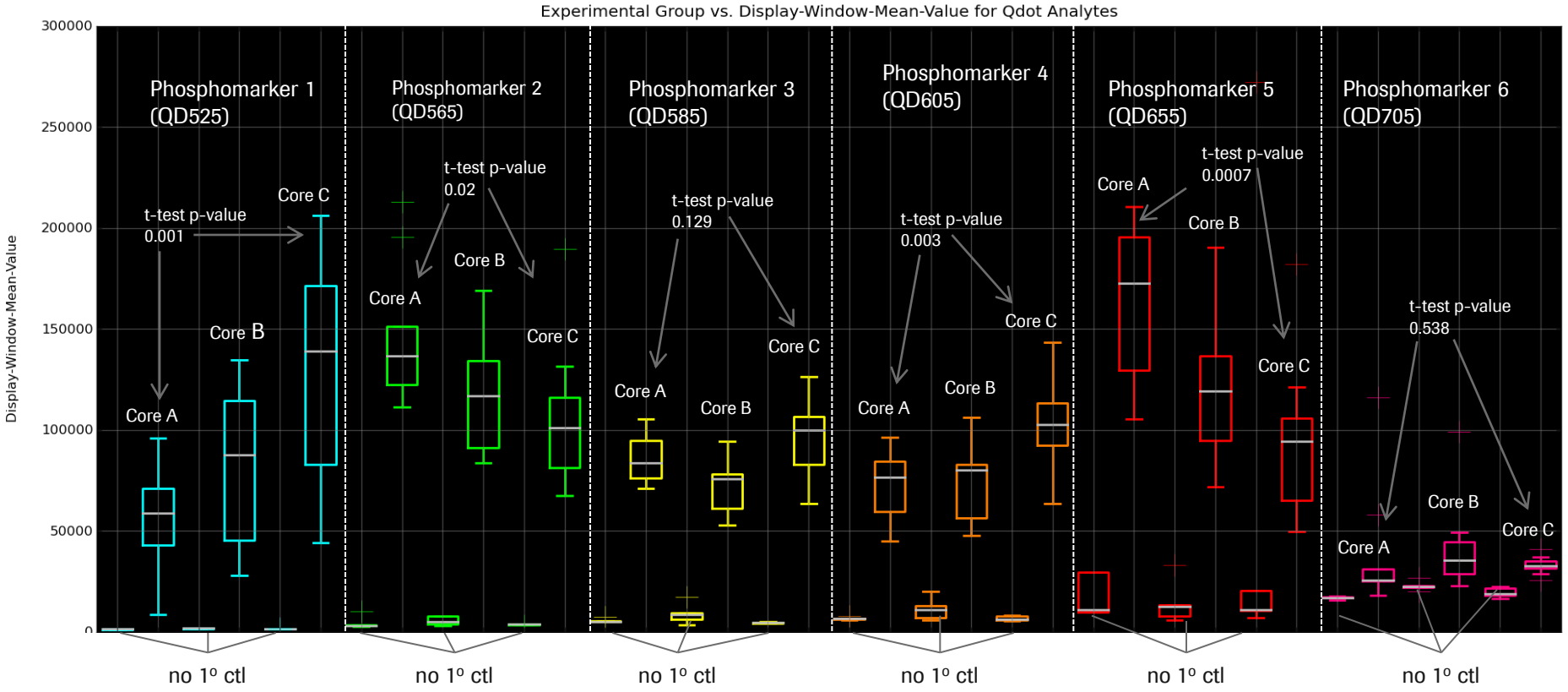
PI3K Quantitative 6-plex: Photometric Quantitation

SKBR3 cell lines 3-in-1 slides: GSK690693 (AKT inhibitor), AKT1-3 si wild-type (WT)





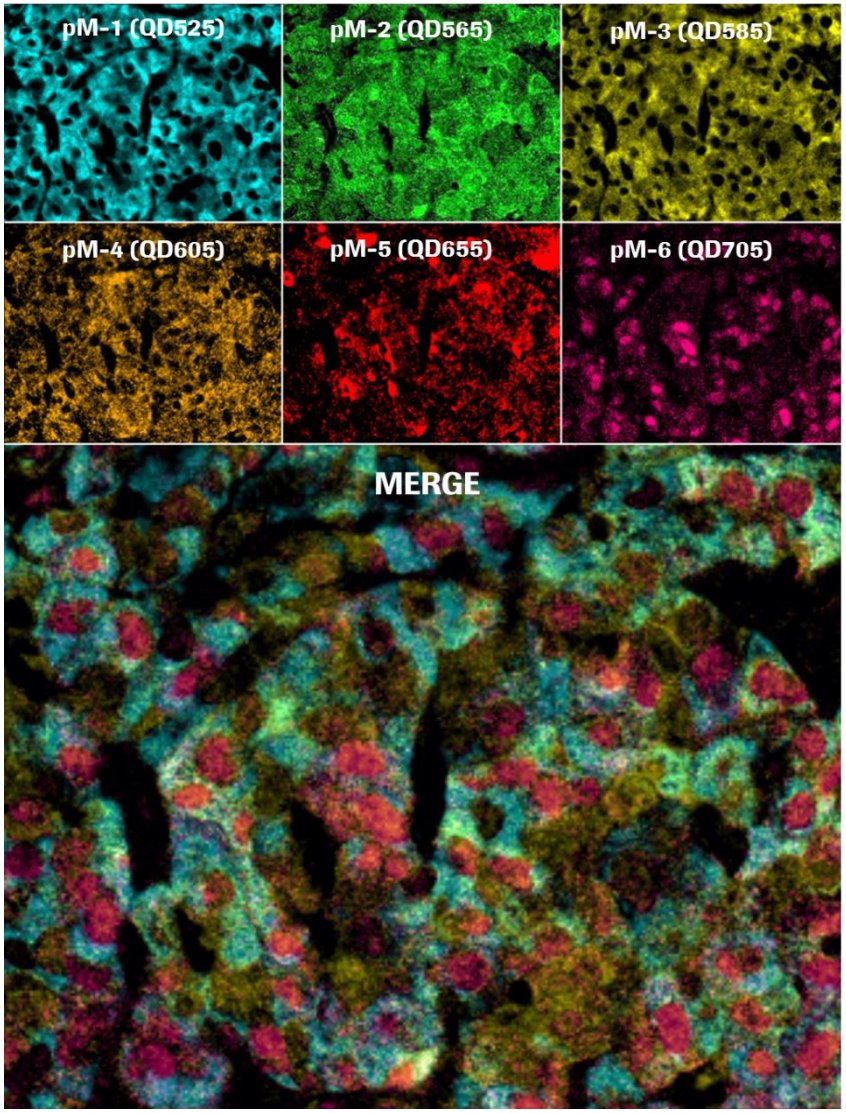
- Each distribution represents 10 fields per core, 2 replicate slides (5 fields each core, for each slide)
- No primary controls (no 1º ctl) indicate low non-specific background levels
- 6-Plex measurements are consistent with images of single marker DAB staining experiments on 3-in-1 slides



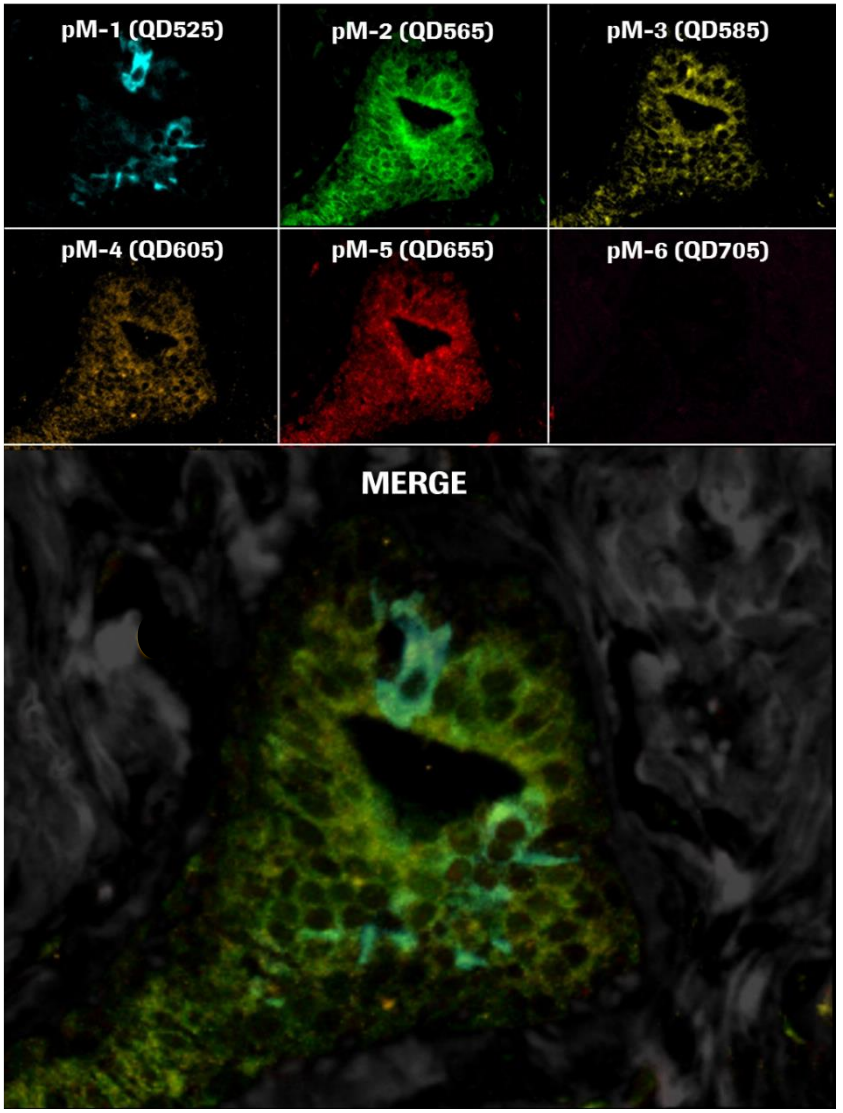
Expression Level Heterogeneity

Can we describe this objectively and identify the competing phenotypes?

TUMOR

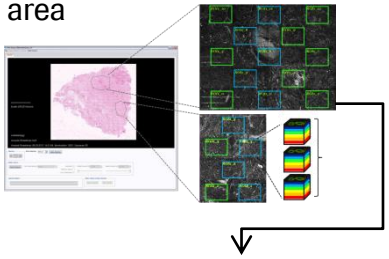


TUMOR ADJACENT

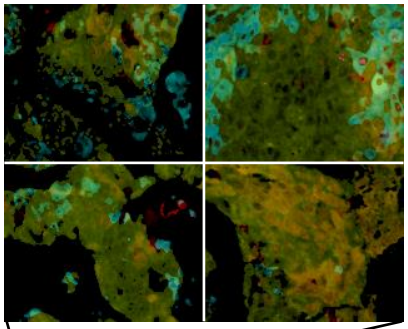


From Spectral Unmixing to Biological Unmixing

Unmixed Layer Stacks sampled over tumor area



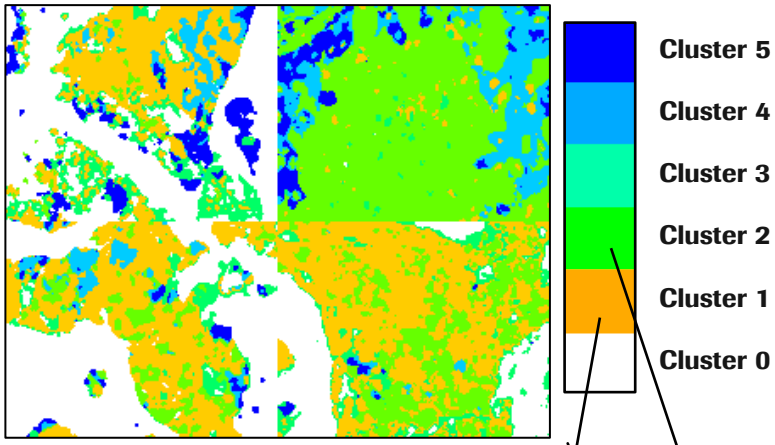
Multiple ROI dataset



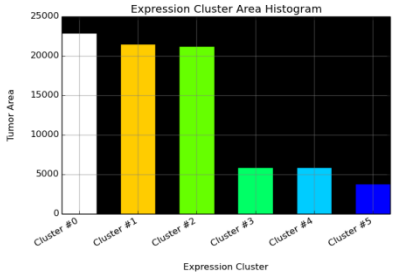
Each unit area has 6 scalar values that define a vector (6 unmixed analyte values)

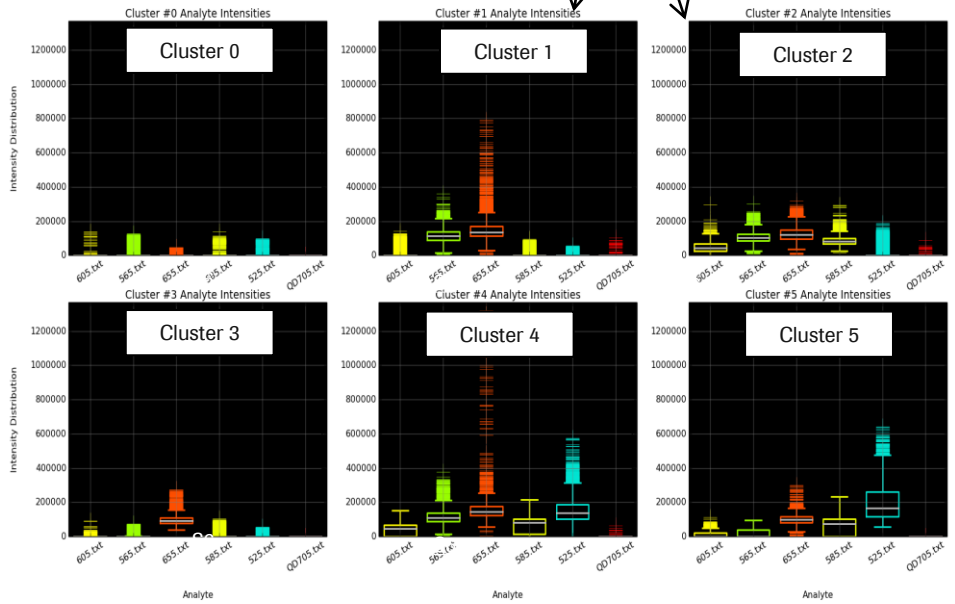
Cluster Algorithm

EXPRESSION CLUSTER MAP:
heterogeneity of marker expression pattern mapped as colors



Tumor Histogram by 'expression cluster' (color coded to expression map)



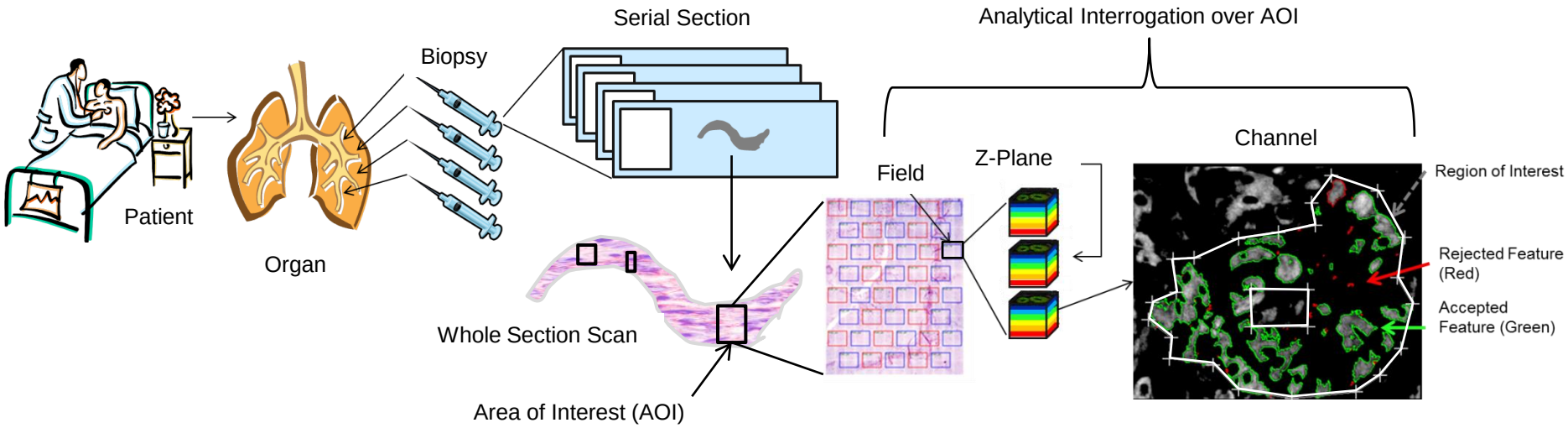


Plot values for each marker grouped by 'expression cluster'

OME in Precision Pathology: Challenges and Opportunities

Analytical Digital Pathology applications have different technical and workflow requirements from conventional research microscopy

1. Data Model for analytical tissue pathology organized relative to anatomic context



2. Lack of file formats and/or rare proprietary file formats (PFF)

1. Rare and unusual file formats hamper 'out-of-the-box' adoption of turnkey OME tools
    1. FT spectral imaging 'raw' spectral cube format
    2. Mass-spec ratio imaging data
    3. Ventana Digital Pathology 'bif' format
2. Implementation of OME internal data model will provide improved framework for managing such data
3. Internal proprietary standards and commercial development toolkits ('Feeding the Monster')

OME in Precision Pathology: Challenges and Opportunities

Our strategy makes extensive use of Python-wrapped, natively-compiled libraries to facilitate high plasticity; our analysis software provides a rudimentary (proof-of-concept) client interface to OMERO

1. We leverage well-established scientific computing library interfaces (native compiled C and C++, Java)
    1. Translating high-level data representations can be tricky
    2. **Type-handling** and **debugging** requires some knowledge of underlying library source and language
2. **Managing** Python-version and other **dependencies** with large wrapped libraries is non-trivial
    1. Resources, knowledge & time for re-compiling libraries from source on Windows are very limited
    2. Our current development environment is Python 2.7, 64-bit (Windows 7) & 32-bit (Windows XP)
    3. ICE framework / OMERO.blitz are KEY, however:
        1. we historically have run into challenges with the combination of Windows, ICE and Python 2.7
        2. Sync OMERO.blitz & ICE with scientific Python distributions (e.g. Enthought)?

OME in Precision Pathology: Suggestions to Enable Precision Medicine

Further development of machine learning tools, server-side data mining and handling of data from heterogenous sources will help advance precision diagnostics research

1. It will be important to **consolidate** access/storage/analysis of **sequence data, mass spec data*, and other analytical technologies**
    1. Link analytical data to image data based on tissue pathology data model
    2. Provide consolidated access (client capabiities) to access and mine information in other databases
2. **Machine learning and pattern recognition** tools are important technologies to next-generation precision digital pathology
    1. Identifying/Segmenting morphological patterns to interrogate
    2. Categorizing molecular phenotype
    3. Multivariate index assays (MIA)
3. Ability to reference and access **multimodal images at hierarchical anatomic levels**
    1. Non-invasive (MRI, CT)
    2. Digitized tissue sections (primary stain and single stain IHC)
    3. Analytical imaging (multiplex molecular tags)

* See: Schramm, et al., 2012 **"imz-ML – A common data format for the flexible exchange and processing of mass spectrometry imaging data"**, J. Proteomics 75, 16: 5106-5110. Also: **www.imzml.org**

Acknowledgements

- **Ventana Medical Systems (Technology & Applied Research)**
  - David Chafin
  - Michael Otter
  - Ben Stevens
  - Daniel Bauer
  - Don Johnson
  - Julia Ashworth-Sharpe
  - Esteban Roberts
- **Ventana Medical Systems (Digital Pathology and Workflow)**
  - Christophe Chef'dhotel
  - Franklin Ventura
- **Ventana Medical Systems (Medical Innovation)**
  - Tom Grogan
  - Eric Walk
  - Kandavel Shanmugham
  - Michael Barnes
- **Oregon Health and Science University (OHSU)**
  - Damien Ramunno-Johnson
  - Thomas Jacob
  - Chris Corless
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