Measuring response of lung cancer therapy with quantitative tissue cytometry of a panel of signaling markers

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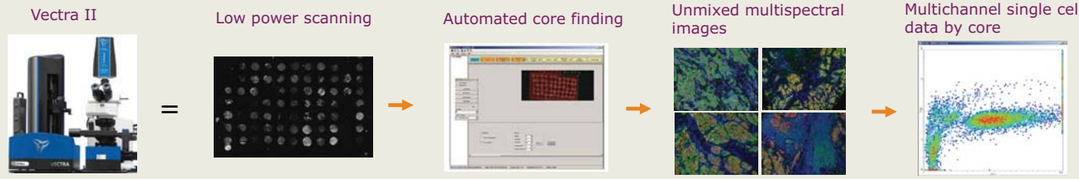
Abstract

Background: Monitoring of the signaling activity is a critical part of determining whether certain therapeutic agents are effective. However, given the complexity of the up- and down-regulation of AKT and ERK pathways, it can be difficult to determine the state of each cell in a solid tumor section using single-marker staining methods. Tissue cytometry (or *in situ* cytometry) is a means of obtaining quantitative, per-cell, multi-marker antigen expression data from a tissue section. While determining the per-cell, multi-marker phenotype of samples containing whole cells has become a common, if not ubiquitous, part of flow cytometry and high-content screening, the same multi-marker analysis on samples such as solid tumors, bone or other solid tissues, which are not easily broken down into individual cells, are not easily performed. Immunohistochemical (IHC) and immunofluorescence (IF) methods have been developed for labeling such samples; however, in order to obtain quantitative multi-marker results from IHC and IF samples there are several challenges that must be overcome to give analogous "tissue cytometry" results.

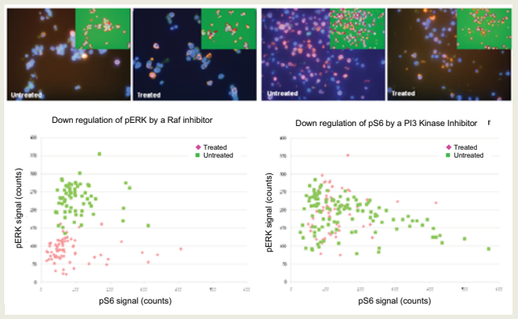
Methods: To address this, a tissue cytometry platform was developed that integrates: a) multiplexed immunofluorescence staining protocols; b) a multispectral imaging system (Vectra™ or Nuance™) that to isolate IHC or IF marker signals from one another and, for IF, from tissue autofluorescence; and c) a new pattern-recognition-based image analysis package (inForm™) for automatically performing morphologic segmentation and extracting data from cells-of-interest; and d) easy-to-use, optimized staining kits for panels of markers. For this study, a staining panel was developed targeting phosphoepitopes of AKT, ERK, and S6, using antibodies of three different isotypes, with secondaries conjugated to Alexa fluorophores (A488, A555, and A647). DAPI was used as a counterstain. Proof of principle of the suitability of the marker panel and imaging and analysis methods was done in cell lines, monitoring the down regulation of pS6 by a PI3 Kinase Inhibitor and the down regulation of pERK by a Raf inhibitor. Clinical samples pre- and post-case for treatment of a lung cancer with a PIK3CA inhibitor were analyzed and both nuclear and cytoplasmic signal levels for all three markers obtained.

Results: Results from cell lines matched expected down regulation. In the clinical samples, down regulation of pERK in lung was observed two varying degrees across cases.

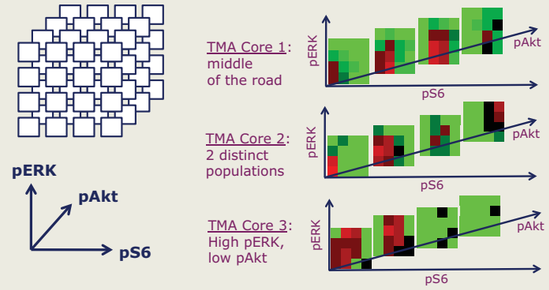
Multispectral imaging and automated scanning technology for tissue microarrays



Assay validation on cancer cell lines

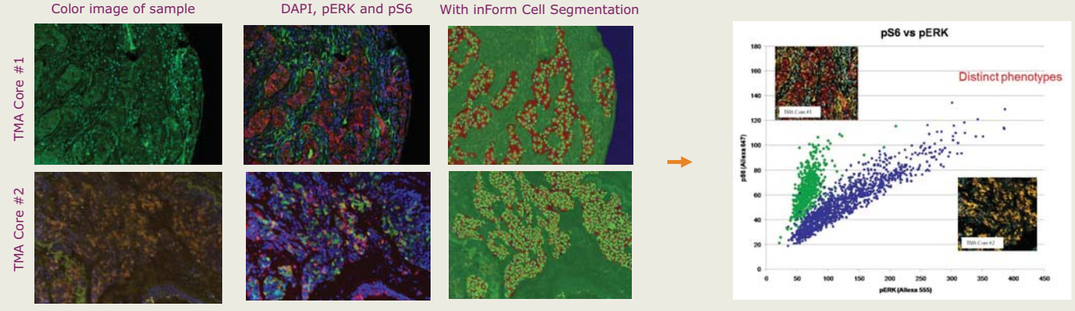


Cluster analysis of cores in tissue micro-array



Cells from each sample divided into quartiles of pS6, pAkt and pErk expression, and thence placed into 64 bins. Number in each bin represented as a heat map

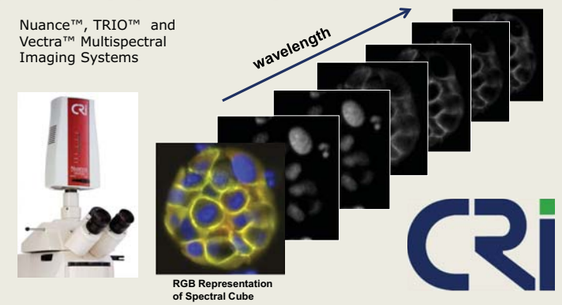
Assessment of intratumoral diversity:
Analysis of pAKT / pERK / pS6 labeled FFPE biopsy sections



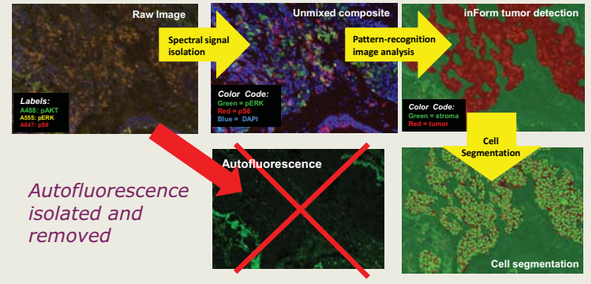
Multispectral Data Acquisition

Spectral Imaging and Unmixing

Nuance™, TRIO™ and Vectra™ Multispectral Imaging Systems



Multispectral imaging and automated analysis workflow



Multispectral images of FFPE lung caner specimens were unmixed into their constituent components, removing the autofluorescence and greatly increasing both legibility and quantitative accuracy. The images were automatically segmented into tumor, stroma and blank space. Within the tumor regions, cells were found using the DAPI counterstain and fluorescence intensities extracted from nuclear and cytoplasmic regions of each cell

Conclusions

- Tissue segmentation accuracy was estimated at greater than 90%, based on visual review by pathologists.
- Performance of the platform for automated multiplexed tissue cytometry analyses supports its application to routine clinical studies and works for both IHC and IF staining methodologies.
- In this particular example, the monitoring of signaling in these clinical samples was effective and easy to perform.

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