Automated assessment of ER and ki67 in individual tumor cells in intact tissue sections using multicolor IHC, multispectral imaging, and pattern-recognition-based image analysis software.

C Hoyt¹, H Gardner², ¹Cambridge Research and Instrumentation, Inc, Woburn, Massachusetts, USA, ²Oncology Translational Laboratories, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, USA.

Abstract

Background: A promising area of research is the analysis of multiplexed nuclear proteins on a per-cell basis, and correlating multi-protein-based cell phenotype statistics with clinical patterns. Many believe that capturing this information from individual tumor cells, rather than average values for proteins across tumor cell populations, as obtained in single-stained serial sections, may hold key information about disease state, and thus offer valuable information for diagnosis, prognosis and therapy selection.

Objective: Our objective is to demonstrate that multicolor IHC staining and multispectral imaging can be used to quantify reliably multiple nuclear proteins on a per-cell basis, without significant inter-stain interference or cross talk. Additionally, our objective is to offer an example of the additional information afforded by multiplexed per-cell IHC. ER and ki67 were chosen for this demonstration because of the recent finding by Penalva-Lorca et al. that ki67 identifies a subset of ER-positive patients who could be sensitive to hormonal treatment.

Methods and Materials. For each of two breast tissue samples, two serial sections were stained singly for ER with SC and PR with VIP IHC stains, and a third for both ER and PR, for a total of six slides. Spectral images were acquired with the CRI Nuance camera, and analyzed with CRI’s pattern-recognition-based image analysis software, inForm™, which can be trained to automatically detect tumor cells, and unmix the overlapping IHC signals using the spectral signatures of each stain. The extracted data was then used to calculate the average optical density of both markers for each of the six stains. For the ER/PR stain, a TMA was stained for ER with vector red and ki67 with DAB. Multispectral images were acquired with a CRI Vectra™, which is an automated multistain slide analysis system that scans at 4x to detect the TMA, and collects 20x multispectral images of each core. In this case, per-cell multi-protein data was exported for data analysis with Matlab, for cell-phenotype classification.

Results. Results suggest that, if staining is performed carefully, independent and reliable measurement of individual nuclear proteins on a per-cell basis, in a multiplexed protocol, are possible. Additionally, the flow cytometry-like analysis can be used to reveal per-cell multi-parameter expression profiles. In particular, percent double negativity, single positivity for each protein, and double positivity. This tissue cytometry-like detail about protein expression state may offer additional metrics upon which to base correlations with clinical patterns.

4X mosaic of whole array

20x multispectral image of a core

Example cores with quadrant analysis

Eight example cores were selected that show the range of co-expressions detected in this TMA. CRI inForm analysis software was used to segment areas of tumor, segment individual cells within those areas, and extract spectrally unmixed signal for ER and ki67. Per-cell data from inForm was then analyzed in Matlab, to perform quadrant analysis.

Testing the independence of IHC stains when multiplexed

Two archival breast cancer samples were used for this experiment. One sample was double-positive for ER and PR, and the other was ER single-positive. Tissue sections were stained with a goal of having positive staining in the range of 2-4 IOD, to facilitate accurate spectral unmixing. As shown in the table below, agreement between double and single-stained samples was within 10%. We included the PR negative sample because it demonstrates true negative vs. false positive due to unmixing.

Conclusions

- The combination of multispectral imaging and automated image analysis provides an unprecedented opportunity to analyze interactions among different oncological pathways.
- Multi-parameter molecular assessments of cells in intact tissue sections can be performed, thus enabling ‘flow-on-a-slide’ with conventional immunohistochemical stains.
- Per-cell co-expression can be detected, using spectrally unmixed signals in individual cells, without disaggregating the tissue, retaining tissue context and avoiding the averaging of signals from many cell types.