Multiplexed immunochemical quantitative molecular phenotyping via multispectral imaging and automated segmentation

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Quantitative Molecular Phenotyping using Multiplespectral Imaging

Semi-Automated Assessment of Histochemical Stains

- Image Processing Steps
  - Step 1: Find Cancer Areas at Low Power (2X)
  - Step 2: Extract Several 2X Fields
  - Step 3: Classify Cancer and Segmentation

Validation Results: Concordance between Manual and Semi-Automated Assessments

- Summary Table

<table>
<thead>
<tr>
<th>Marker</th>
<th>Concordance</th>
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<tbody>
<tr>
<td>PR</td>
<td>0.84</td>
</tr>
<tr>
<td>CK</td>
<td>0.68</td>
</tr>
<tr>
<td>AEB1</td>
<td>0.75</td>
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</table>

- Performance criteria for manual assessments (spearman correlation with r values of 0.50 to 0.84)
- Concordance between manual and automated assessment was generally good, as it was rated 0.84. While 12 samples per hour on average could be analyzed manually, results of the automated analysis system could be reviewed, edited, and accepted, at a rate of 200 to 500 samples per hour. Overall, it took about 20 days to manually assess the 2000 tissue section images, while the same task was greatly enhanced. While 12 samples per hour on average could be analyzed manually, the automated system analysis could be reviewed, edited, and accepted, at a rate of 200 to 500 samples per hour. Overall, it took about 20 days to manually assess the 2000 tissue section images, while the same task was greatly enhanced.

Conclusions

- Semi-automated machine-learning-based segmentation, chromogen signal unmixing, and facilitated review can augment manual assessment rates 20-fold. Throughput rises from 12 cases per hour (manual) to 250 per hour (semi-automated).
- Machine-learning techniques are capable of producing automated tissue classification and segmentation algorithms that agree with manual assessment and are reliable across variability of human tissue samples and tissue sample preparation (r values of 0.9 to 0.94).
- Spectral imaging can be used to perform multiplexed assessments of weakly expressed signaling pathway proteins with signals as low as 10% of tissue autofluorescence levels.