Diagnostic Development and Image analysis

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Program Director Diagnostic Development
CCTG Breast Group Steering Committee
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ASCO-CAP HER2 Panel

“As is your Pathology, so is your Medicine.” Sir William Osler. 1849-1919.
Automated Image analysis – why am I a fan?

Is chromosome 9 loss a marker of disease recurrence in transitional cell carcinoma of the urinary bladder?

Observer variation in immunohistochemical analysis of protein expression, time for a change?

Estrogen Receptor and Progesterone Receptor As Predictive Biomarkers of Response to Endocrine Therapy: A Prospectively Powered Pathology Study in the Tamoxifen and Exemestane Adjuvant Multinational (TEAM) Trial
J Clin Oncol Apr 2011;29(12):1531-8

An international multicenter study to evaluate reproducibility of automated scoring methods for assessment of Ki67 in breast cancer.

Manual FISH – 200 nuclei from up to 6 areas scored 2-4 times (max 14400 nuclei/chromosome/case – 85 cases)

Manual IHC – 8661 cases dual scored by 6 pairs of observers (7 different proteins) ICC 0.90

4781 Cases – 6 TMA cores/case, 2 markers – 57372 cores individually “tumour marked” prior to semi automated quantitative image analysis

Can we finally use Ki67 as a diagnostic biomarker

Automated image analysis: Faster, more accurate, reproducible, portable, and provides continuous rather than discontinuous data (more powerful stats) – what’s not to like?
## Systems cross comparison – and Quality control/assurance

- Hardware AND Software updates require re-validation of algorithms/solutions

### ER validation data

<table>
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<th>Ariol</th>
<th>Aperio</th>
<th>Definiens</th>
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<tbody>
<tr>
<td>Correlation</td>
<td>0.95</td>
<td>0.84</td>
<td><strong>0.98</strong></td>
</tr>
<tr>
<td>R2</td>
<td>n/a</td>
<td>0.7102</td>
<td><strong>0.9547</strong></td>
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<tr>
<td>ICCC</td>
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### PgR validation data

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<td>ICCC</td>
<td>0.96</td>
<td>0.7673</td>
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- A 600 core set of images in triplicate is re-analysed – can be used for cross validation and cross comparison of systems/approaches.
- Data compared to previous ‘gold-standard’ generated data for validation set
- Full documentation of validation process and updated SOP generated
Diagnostic development: Definiens workflow – user friendly.

- Set the magnification & resolution of image.
- Detects the tissue within the image.
- Segments the tissue for training.
- Train ROI.
- Allows for ROI correction before cellular analysis.
- Selects ROI from composer stage for analysis.
- Detects nuclei within ROI.
- Excludes nuclei on morphology and geometry thresholds.
- Classifies nuclei within ROI into 1/2/3+
- Select data to be exported.
Automated tumour marking

Left: Original image. Right: Image with after ROI analysis. Orange = tumour, Blue = stroma
BioView FISH Imaging and Analysis System

1. Low Magnification Scan
   - 6-10 min depending on tissue area /TMA size

2. Map TMA or Mark Regions of Interest
   - 10-15 min depending on the complexity of the TMA or number of selected regions of interest

3. High Magnification Image Acquisition
   - Variable
   - Dependent on tissue area, TMA size, number of regions of interest
   - 1 hour-overnight

4. Review Fields of View and Signal Detection Sensitivity
   - Variable
   - HER2/CEP17 signal detection settings should be assessed
   - Dependent on overall signal strength, presence of background, number of fields of view
   - Average 30 min

5. Manual Refinement in Segmentation of Selected Cells
   - Variable
   - Dependent on extent of cell clustering, number of fields of view, quality of signal and hybridization, number of cells required for analysis
   - Average 45 min-1 hour

6. Confirm Cell Selection for Analysis
   - Variable

7. Select Required Number of Cells for Analysis
   - As cells are selected for analysis, scoring is performed by the system in real time
Diagnostic Development

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The big appeal of Definiens

Image analysis workflow comparison

Data entry registering the slide: < 5 mins
  Low power scan: 5 mins
  Assign TMA map: 5 mins
  High Power Scan: 40 mins

Axiol

Definiens

Tumour marking: 4 hours

Processing: 40 mins

Exporting data: 10 mins

Calculating scores: 15 mins

Total time: 6 hours

Exporting cores: 1 hour

Importing files: 5 mins

Running solution: 1 hour

Copying data: 5 mins

Calculating scores: n/a

Total time: 3 hours 10

200 core TMA
Funding for the Ontario Institute for Cancer Research is provided by the Government of Ontario.
Current ER/PgR protocol

- Upload images as single core TIFF files
- Load solution
- Run solution
- Solution pauses after running ROI script allowing for mid-analysis QA/ correction of ROI selection.
- Continue run of solution
- Post analysis QA of fully analysed images
- 10% comparison of analysed images with manual scoring methods