Diagnostic Development and Image analysis

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"*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?
British Journal of Cancer 77:2193-2198 (1998)

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

Observer variation in immunohistochemical analysis of protein expression, time for a change?
Histopathology 48:787-794 (2006)

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

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

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

An international multicenter study to evaluate reproducibility of automated scoring methods for assessment of Ki67 in breast cancer. The 2016 San Antonio Breast Cancer Symposium (San Antonio, TX, December 6-10, 2016) *Cancer Research* Feb 2017;77(4_Supplement):P1-03-01.

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

	Ariol	Aperio	Definiens
Correlation	0.95	0.84	**0.98**
R2	n/a	0.7102	**0.9547**
ICCC	0.93	0.7613	**0.9632**

PgR validation data

	Ariol	Aperio	Definiens
Correlation	0.97	0.87	**0.98**
R2	n/a	0.7637	**0.9538**
ICCC	0.96	0.7673	**0.9708**

- **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.

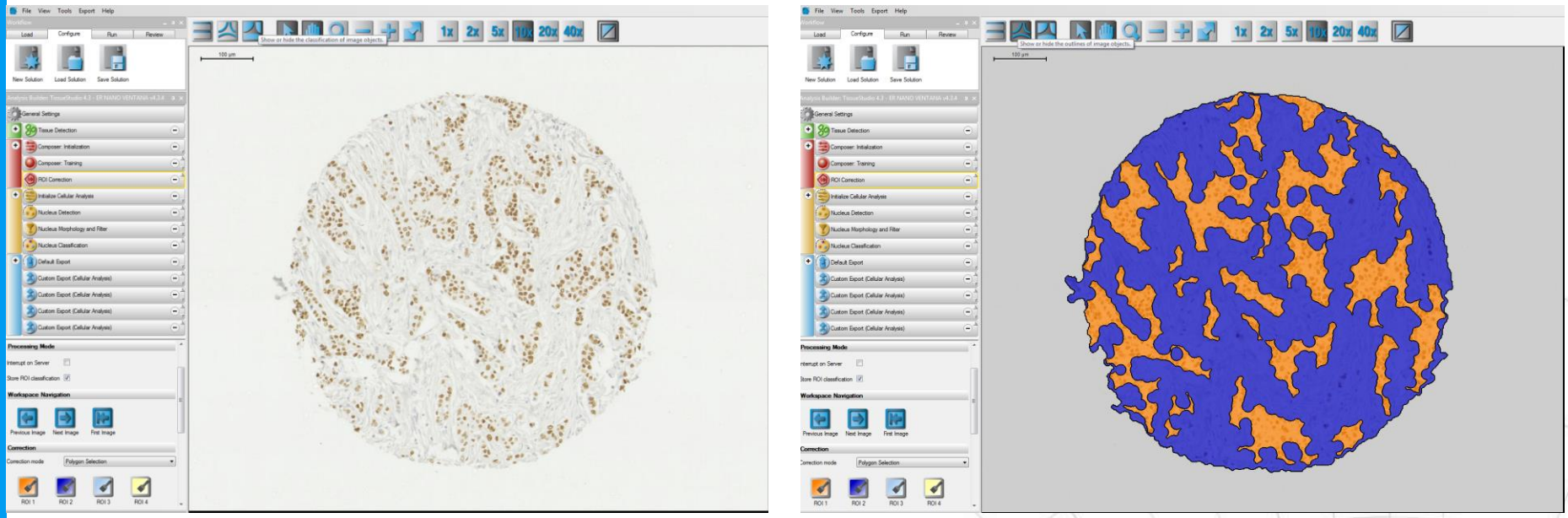
→ Detects nuclei within ROI.

→ Excludes nuclei on morphology and geometry thresholds

→ Classifies nuclei within ROI into 1/2/3+

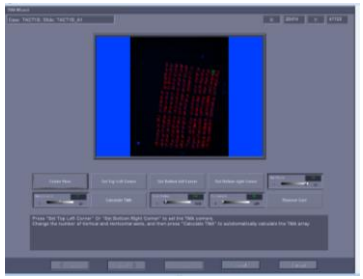
→ Select data to be exported

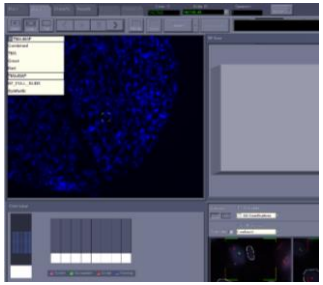
Automated tumour marking

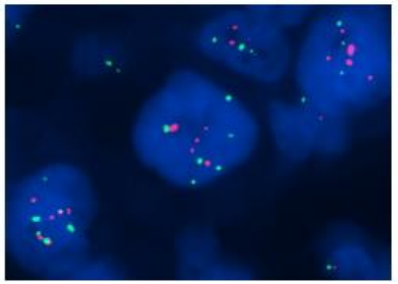


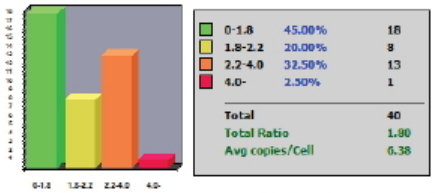
Left: Original image. Right: Image with after ROI analysis. Orange = tumour, Blue = stroma

BioView FISH Imaging and Analysis System









Low Magnification Scan

- 6-10 min depending on tissue area /TMA size

Map TMA or Mark Regions of Interest

- 10-15 min depending on the complexity of the TMA or number of selected regions of interest

High Magnification Image Acquisition

- Variable
- Dependent on tissue area, TMA size, number of regions of interest
- 1 hour-overnight

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

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-1hour

Confirm Cell Selection for Analysis

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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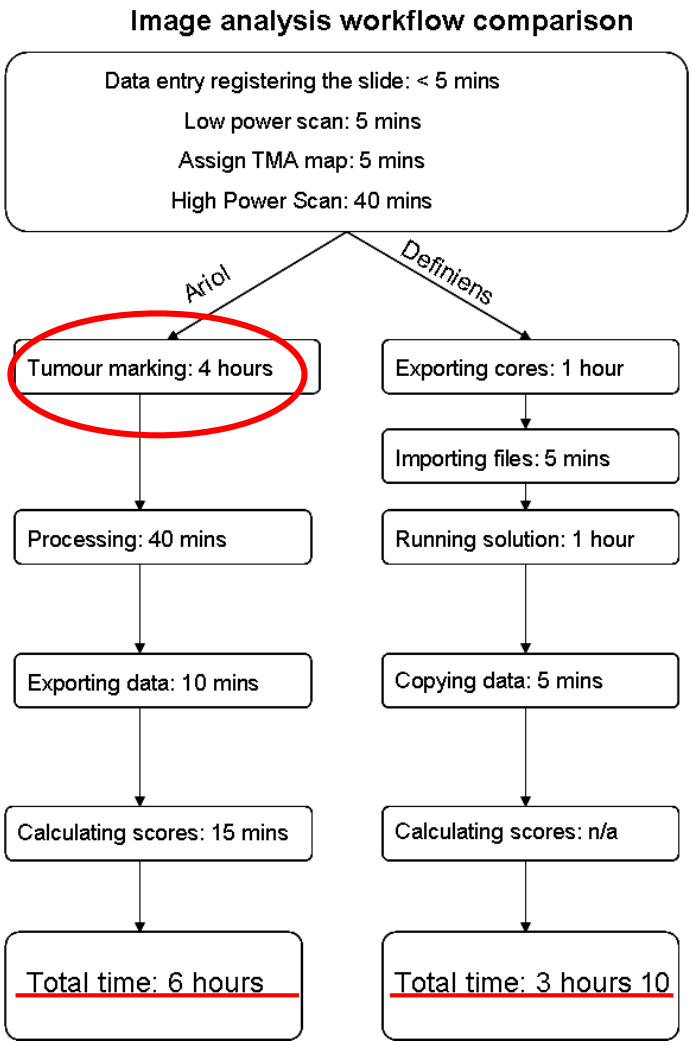

The big appeal of Definiens

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