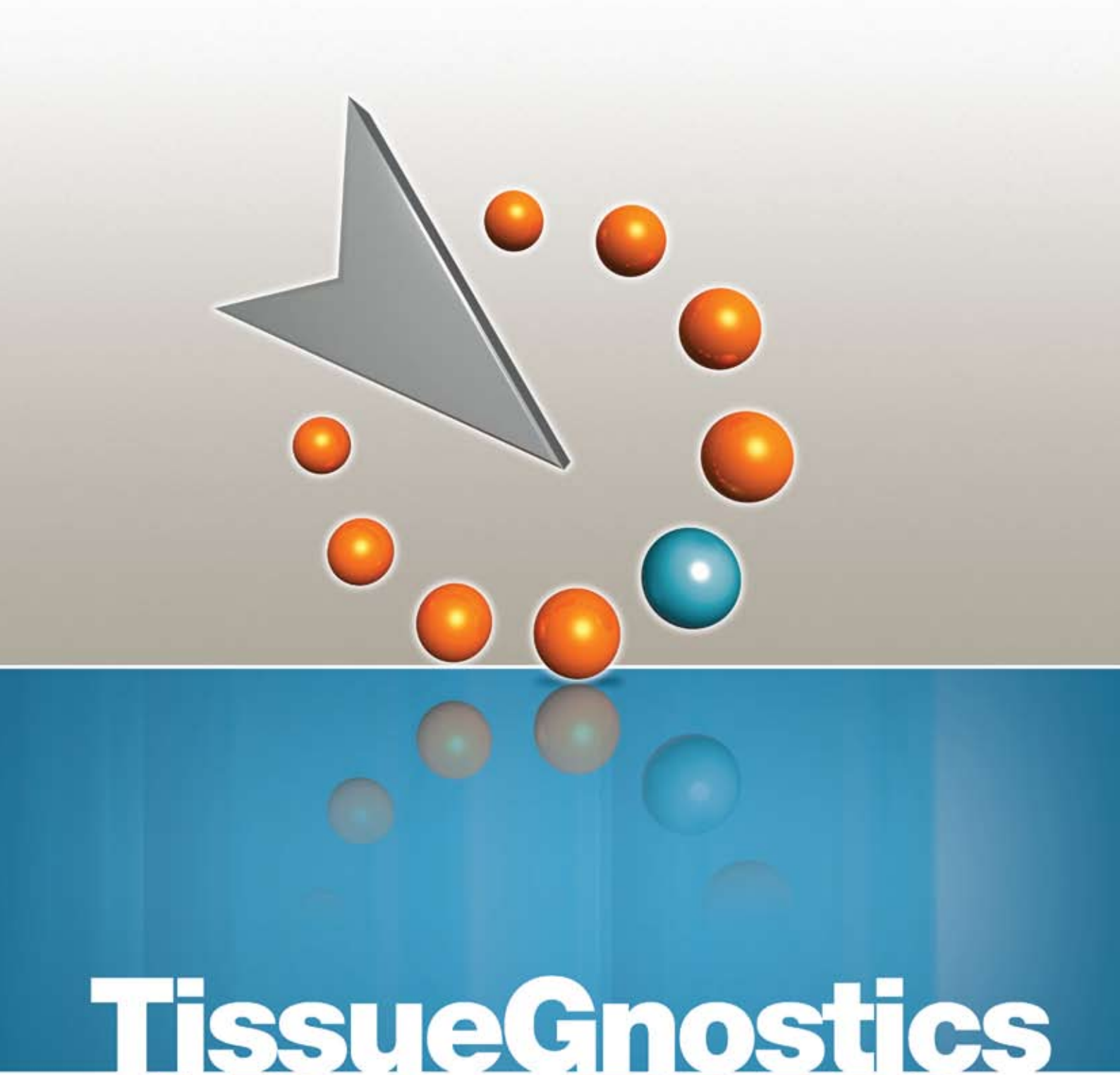


TissueGnostics

HistoFAXS

Cell Analysis System

HistoFAXS
Leading-Edge Technology

HistoFAXS

The cutting-edge tool for cell-based staining intensity analysis of immunohistochemical routine samples and research experiments

HistoFAXS® is a microscopic system that automatically acquires immunohistochemically stained sections and performs quantitative analysis of staining intensities.

HistoFAXS® is a combination of high-end hardware modules (Zeiss, Leica or Nikon-based) and two software modules:

- HistoFAXS® image acquisition and data management module.
- HistoQuest® analysis module for immunohistochemical stainings (stand alone use possible).

HistoFAXS® provides a smooth, automated workflow from image acquisition to publication quality, output of graphs and images, as well as customizable data export for further processing.

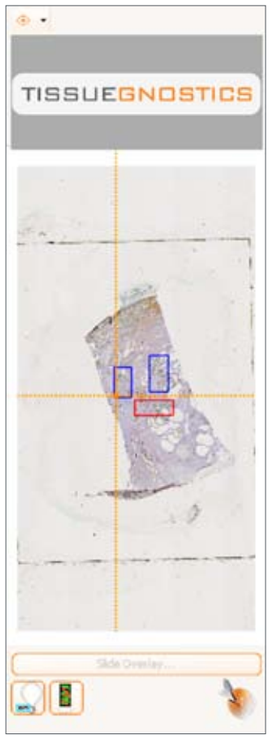
TissueGnostics HistoFAXS

HistoFAXS unique features:

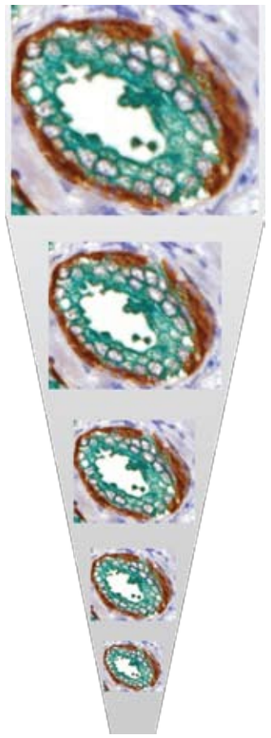
- Automatic acquisition of an unlimited number of regions of interest on up to 200 slides
- Large overview images created from individual fields of view (FOV) may be exported at user-defined resolution
- Defines regions to be analyzed (or excluded from analysis) on the acquired regions of interest
- Semi-automated color separation to extract the relevant marker information out of immunohistochemical images
- Reliable automatic nuclear segmentation with minimum user interaction
- Forward Gating from the individual cell in the image to the dot in the scattergram as well as Backward Gating from the individual dot or dot group to the corresponding cells in the sample
- DotPlot operations – Re-evaluation and further analysis of the image processing results via gating and additional DotPlots
- Histograms and overlays
- Statistics including percentage of positive cells, cells/mm^2, and mean intensity

HistoFAXS Workflow

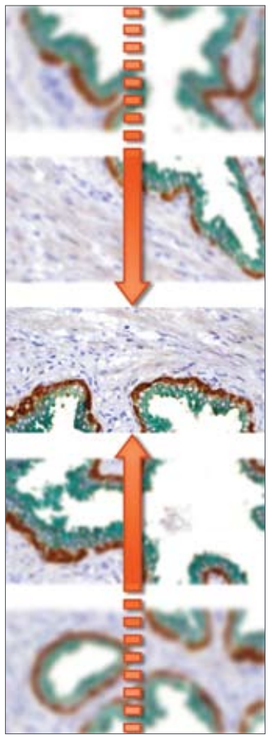
Rapid preview



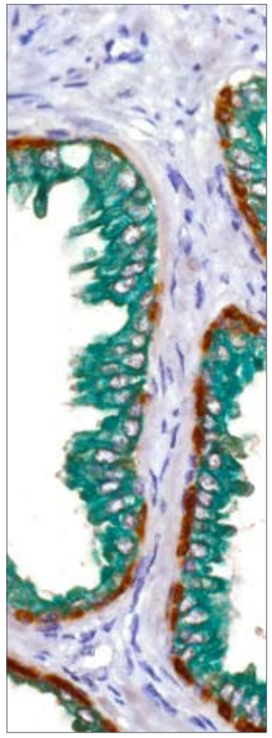
Automated acquisition at freely selectable magnifications



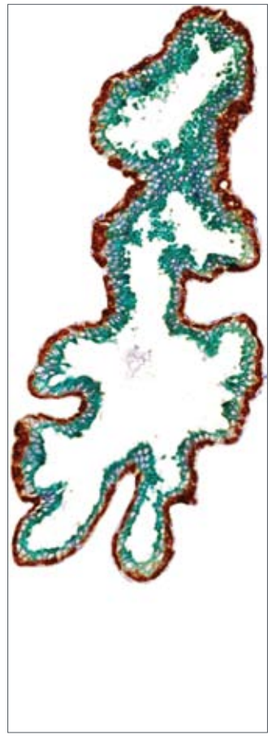
Tunable autofocus for up to 100x oil objectives



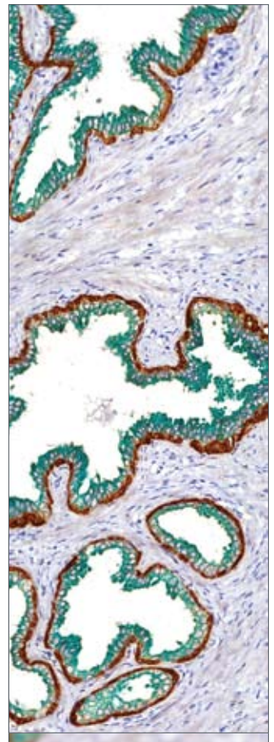
Automatic stitching function and exportable sample overview



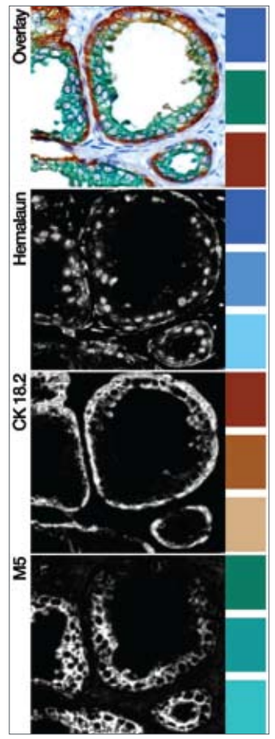
Subregion creation and export for analysis



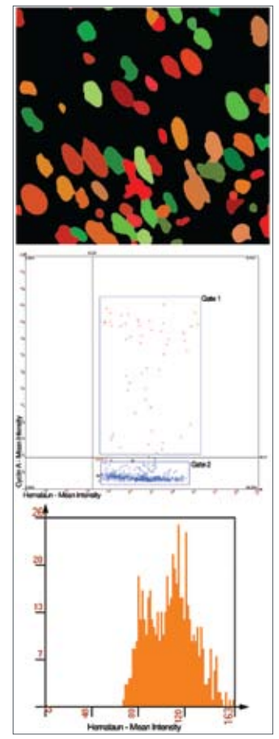
Reacquisition of single out-of-focus images

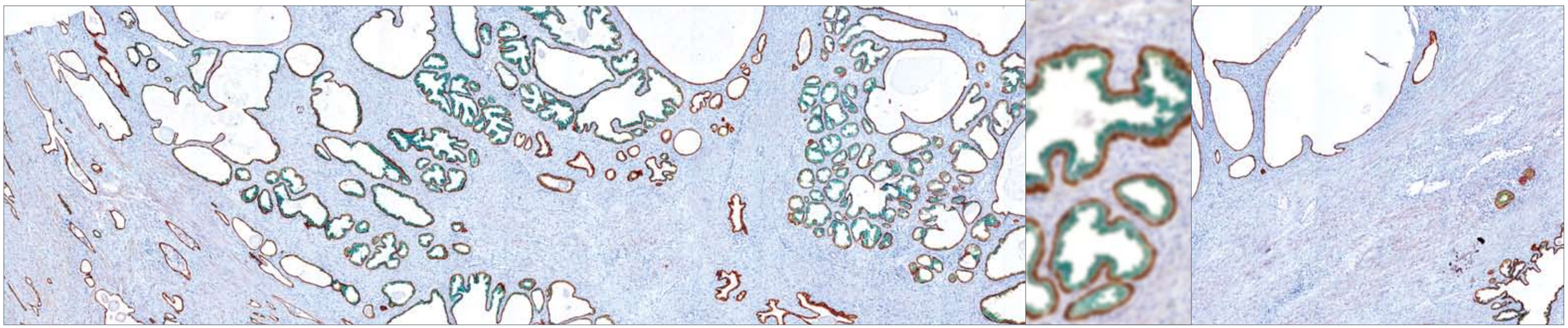


HistoQuest color separation



HistoQuest analysis of staining intensity

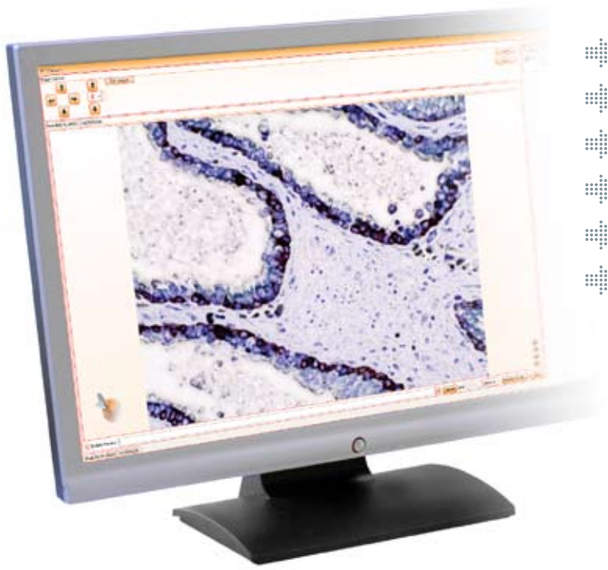




HistoFAXS
Management and Acquisition Software

The HistoFAXS management and acquisition software combines an intuitive and user friendly interface and a comfortable workflow with extensive control functions:

- Objective selection
- Light source control
- Graphical navigation function for 8 slides
- Preview window
- Automatic tissue detection
- ROI management
- Subregion creation and export
- Overview export



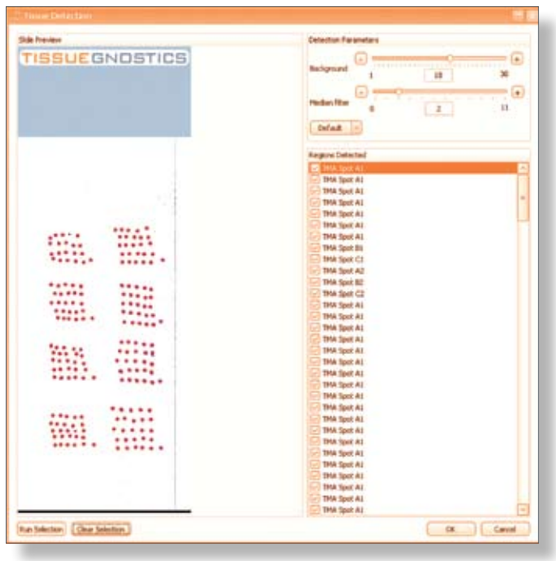
- Live image window
- Stage controls and position info
- Autofocus
- Auto-white balance
- Image export
- TMA layout

HistoFAXS
TMA workflow and features

TMA acquisition

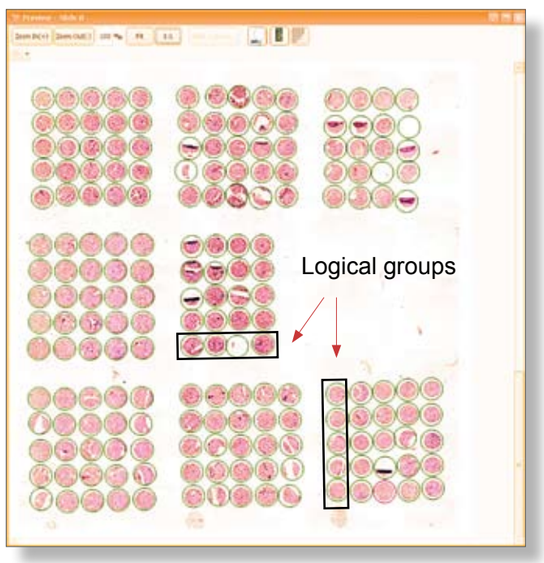
Tissue Micro Array (TMA) module is fully integrated in the HistoFAXS® system.
Acquisition is handled in the HistoFAXS® software while the analysis is done in HistoQuest®.

Core detection



TMA spots are identified on a slide preview obtained by a low magnification scan. Acquisition can be made automatically, with manual adjustment or semi-manually by projecting TMA block patterns and doing comprehensive block operations.

Each spot is given its individual ID based upon identification. Missing spots are still recognized based on block matrix.



Logical groups can be drawn and named on the preview image, thus providing one possible basis for later analysis.
After acquisition, the project is ready for analysis.

TMA analysis

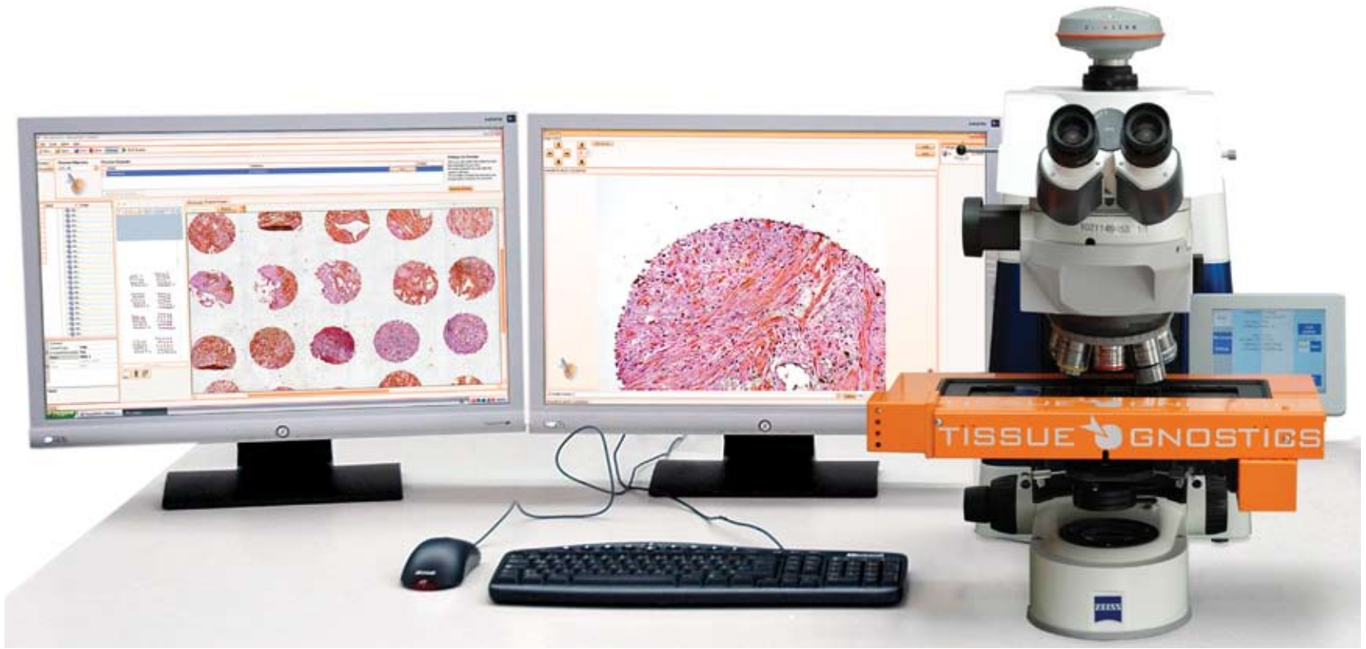
TMA projects are opened in HistoQuest®.
Logical groups are shown in HistoQuests Input Region list and are analyzed as one sample.

TMA spots not in logical groups are displayed separately in this list under their spot ID. They can be analyzed individually.

After analysis, results can be exported to Excel sheets to be linked with metadata for further examination.

A browser-type TMA explorer and a report generator completes the TMA functionalities.

Acquired and imported spot image



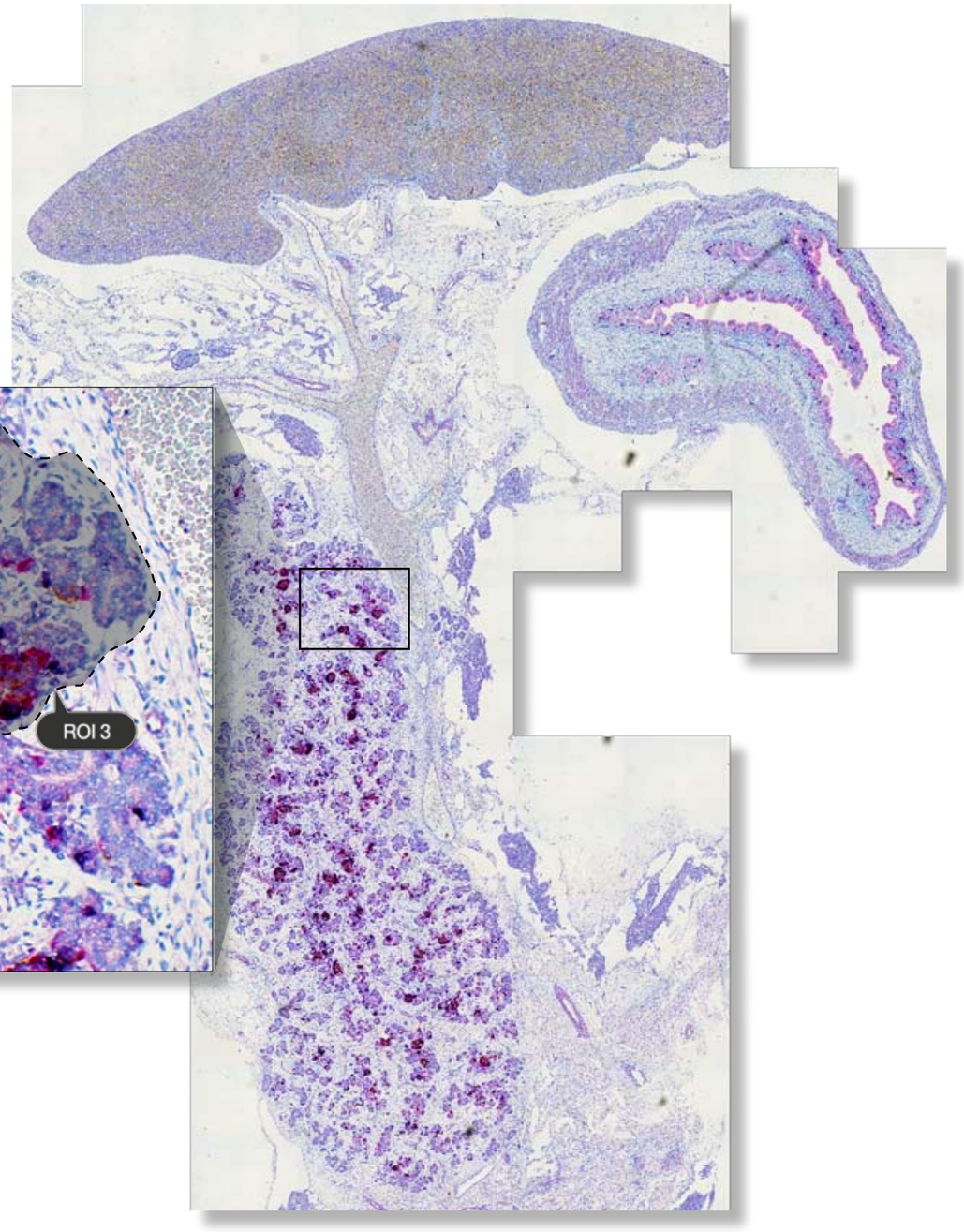
HistoQuest
Loading projects and importing images

HistoQuest® can load complete HistoFAXS® projects and display the overview of the regions of interest.

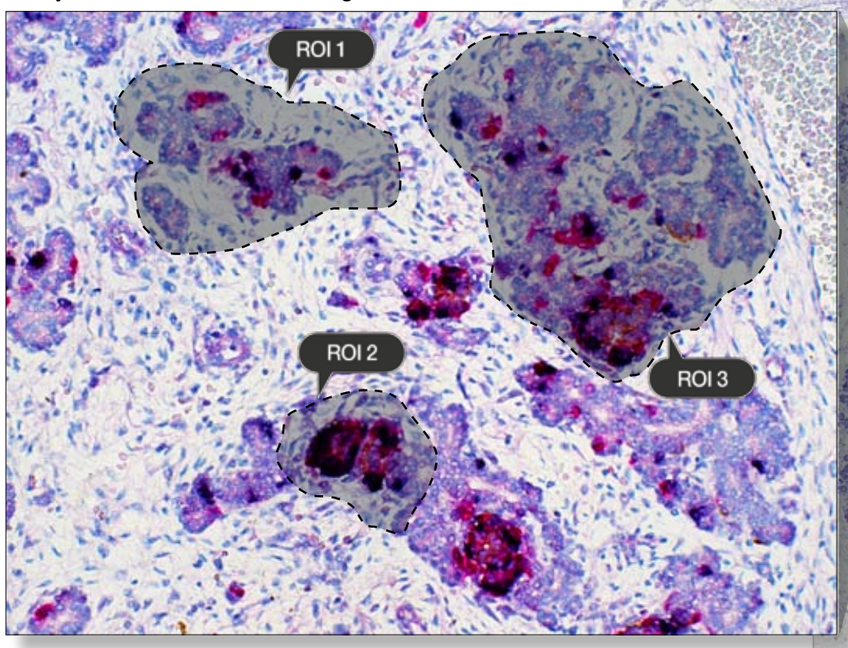
Graphical tools can be used to draw analysis selections on these areas of interest for subsequent HistoQuest® analysis (e.g. for tumor areas, etc.).

Analysis selections can be linked to form a compound analysis selection and can also be inverted.

Region of interest overview of HistoFAXS project



Analysis selections drawn on region of interest overview

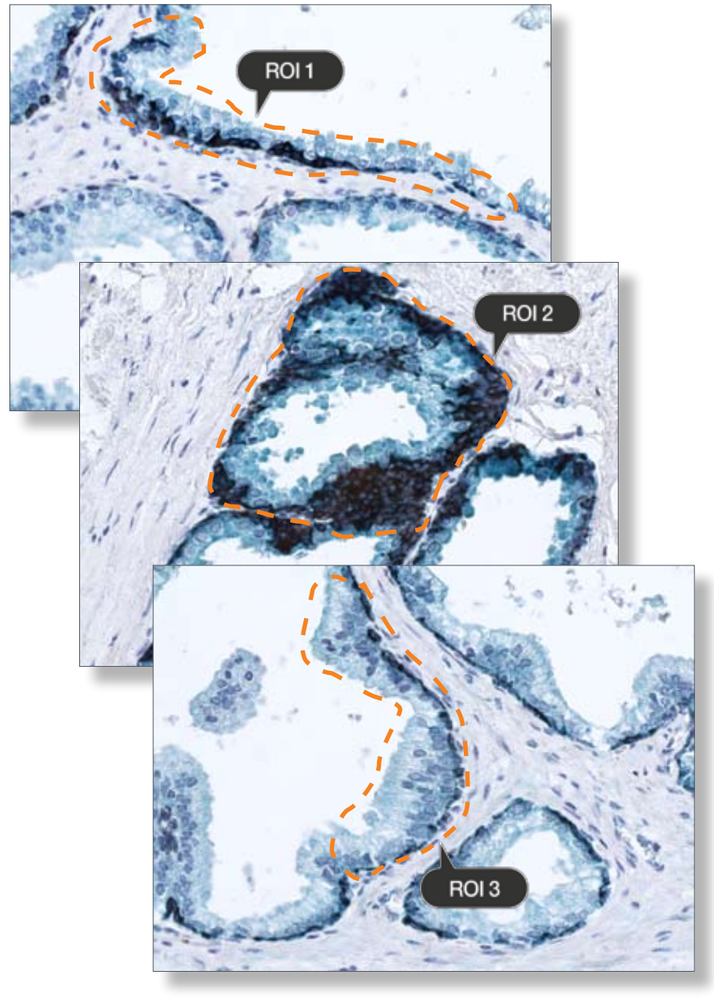


All images courtesy of O.Univ.Prof. Dr.med.Helga Fritsch and Elisabeth Richter, Medical University Innsbruck, Division for clinical functional Anatomy, 2007

Alternatively, series of images from other sources can be imported. As the spatial relation is not available, such image series cannot be reassembled into an overview. They can, however, be analyzed by HistoQuest®.

Analysis selections can be drawn on those single images which contain parts of the structure to be analyzed and linked.

Analysis selections drawn on images imported from external source



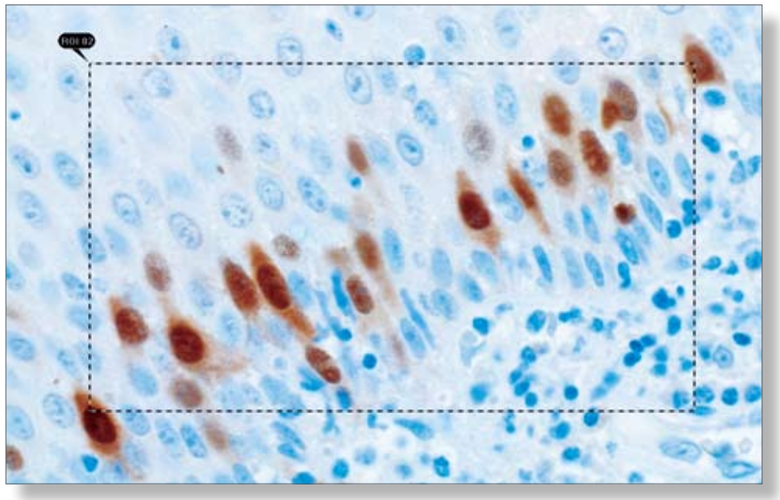
HistoQuest
Color separation

Unlike immunofluorescence, the colors of markers in immunohistochemistry images are contained in one image – in order to ease analysis HistoQuest® performs a color separation.

Color shades are picked manually and optimized by manipulation of specific values. Optimization results are displayed in near real time.

The automatic color separation produces a gray value channel image for each marker, allowing treatment with the application of TissueGnostics algorithms for single cell identification.

Original image of Cyclin A staining in skin

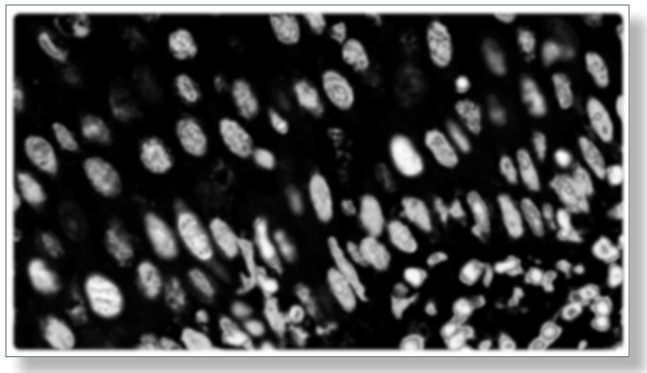


Images courtesy of Prof. Dr. Ingela Turesson, University Hospital Department of Oncology, Uppsala, Sweden

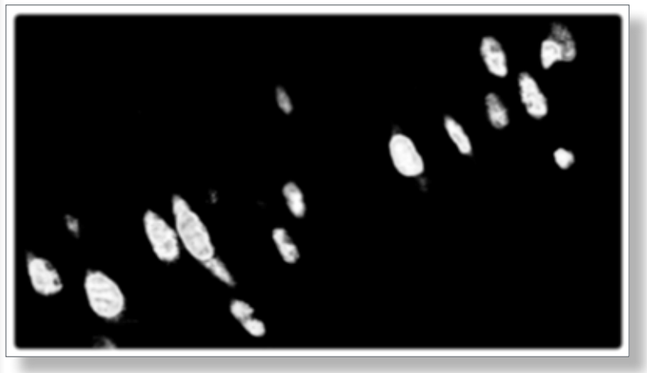
Prof. Dr. Anna Danielsson, Sahlgrenska Sjukhuset - University Hospital, Department of Oncology, Gothenburg, Sweden

Grey values after color separation

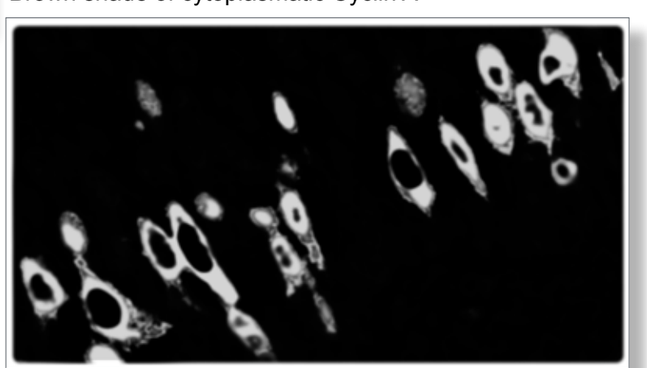
Blue shade of hemalaun



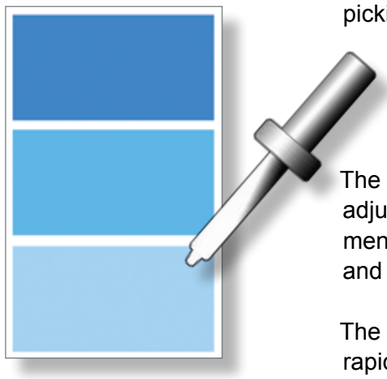
Brown shade of nuclear Cyclin A



Brown shade of cytoplasmatic Cyclin A

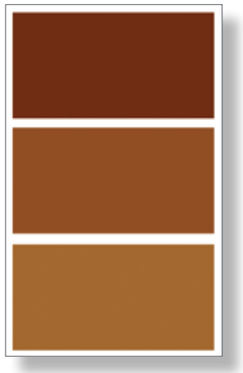


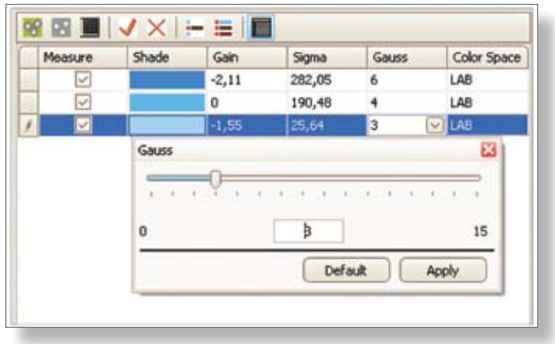
In HistoQuest®, reference shades are selected semi-automatically. RGB color values are manually chosen with a color selection tool.
An automatic pre-segmentation simplifying color picking is available.



The color shades acquired in this way can be adjusted and optimized for automatic nuclear segmentation by manipulating the gain, the color space and blur.

The values in color adjustment are calculated very rapidly with interim results.

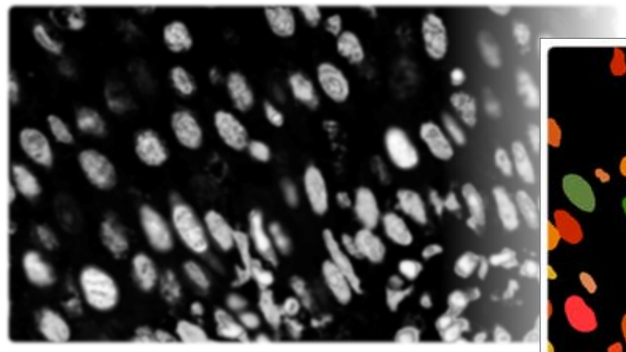




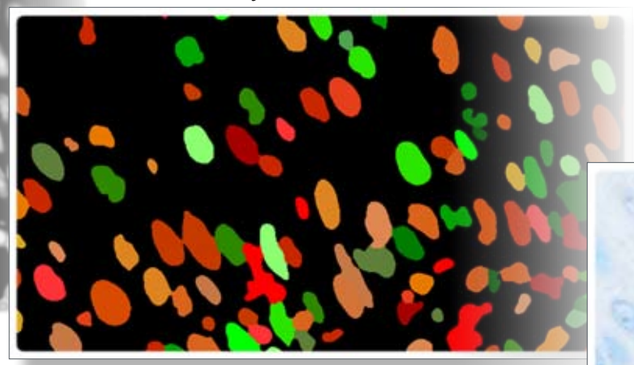
HistoQuest
Nuclear segmentation

Detection of individual cell nuclei and of cytoplasm

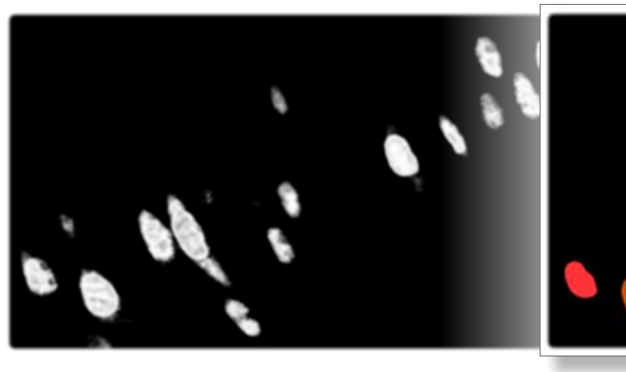
Blue shade used for nuclear detection

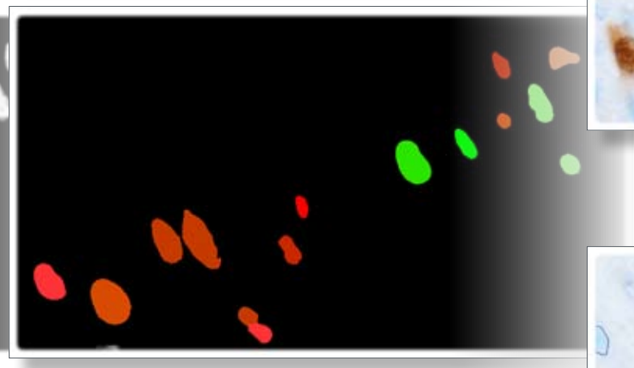


Identified objects

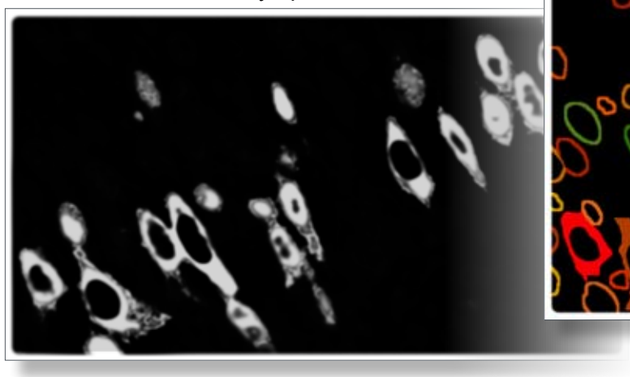


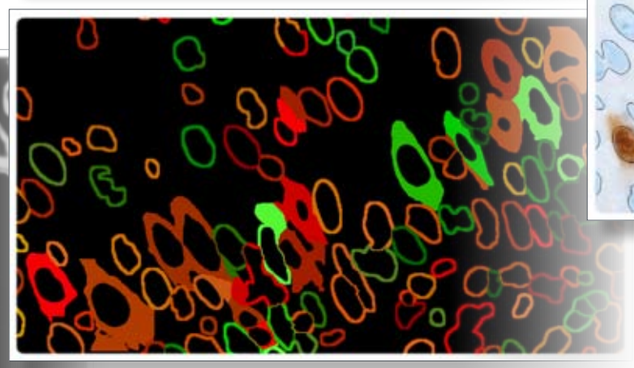
Brown shade used for nuclear mask





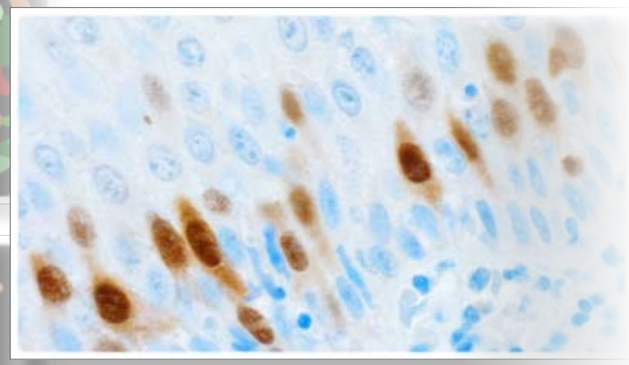
Brown shade used for cytoplasmatic mask



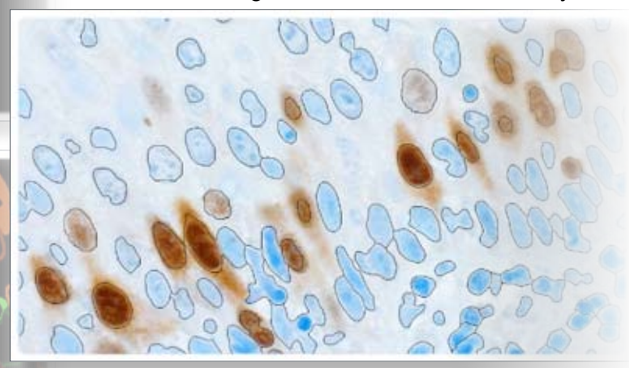


Automatic nuclear segmentation is achieved via TissueGnostics® patented set of algorithms.

Original image



Single cell identification in overlay mode



Once rapid test calculations done on representative images have established a good segmentation, nuclear segmentation as well as the measurement masks can be automatically computed for virtually any number of corresponding images.

Nuclear segmentation in HistoQuest® is completely automatic after the input of a few starting values:

- Average nuclear size
- Discrimination by area

  (Exclusion of smaller nuclear sections)
- Discrimination by gray value

  (Exclusion of weakly stained nuclei)
- Background threshold

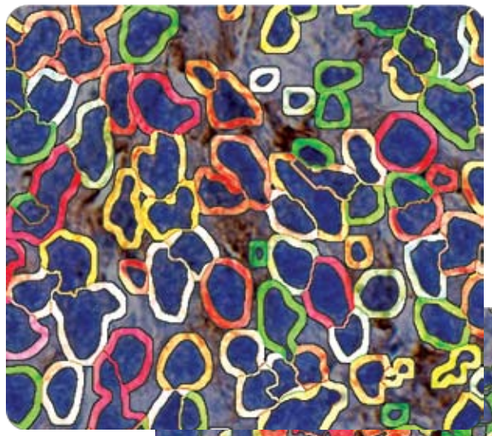
  (Default setting is automatic)

HistoQuest
HistoQuest segmentation quality

HistoQuest® provides two algorithm sets for the identification of the cell cytoplasm.

The first one creates a statistical ring mask which measures marker intensity between an interior and an exterior radius, measured from the border of identified nuclei.

Ring mask

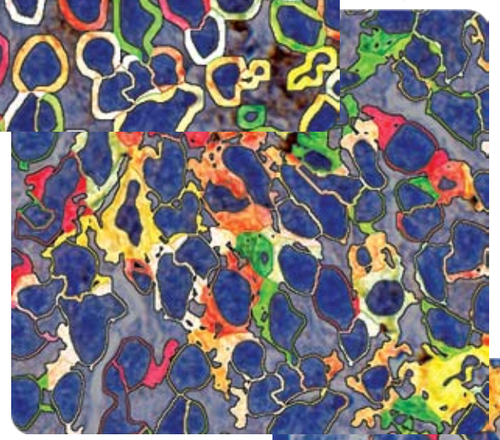


The second algorithm created on this mask identifies cell cytoplasm by effectuating simultaneous and incremental growth phases on marker intensity from the cell border.

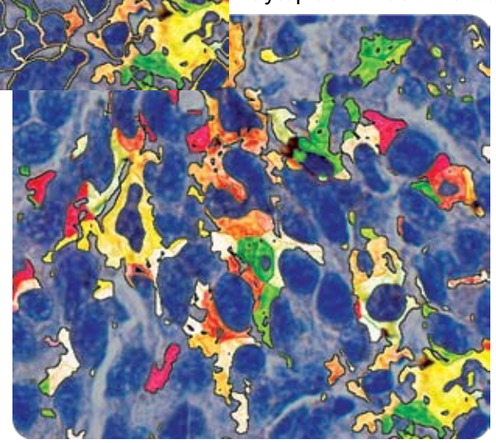
The growth is either stopped by running out of marker intensity, by reaching a predefined limit value or by two or several growth zones touching each other (overlapping growth zones are not possible).

Intensity is measured within thusly identified cytoplasmic area. The ring algorithm can be used in a support role with the Identified Cell Mask algorithm. The nuclear areas can be either included or excluded in the measurements.

Ring mask combined with cytoplasm mask



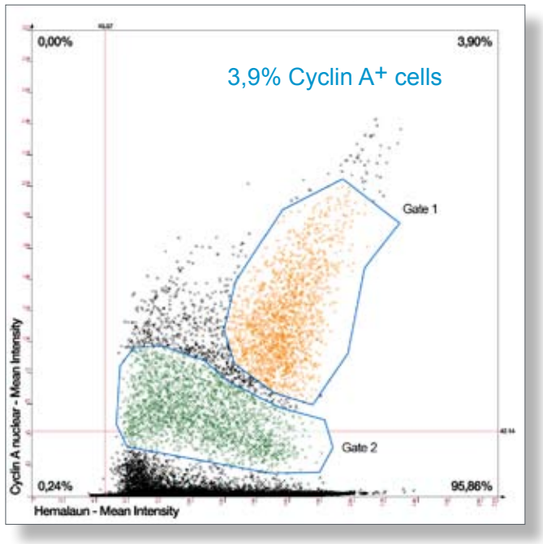
Cytoplasm mask without ring mask



HistoQuest
Working with acquired data

Data produced by segmentation and measurement are immediately plotted in default DotPlots automatically created according to the experiment setup.

Default DotPlot: Hemalaun vs. Cyclin A



These DotPlots give a visualization roughly equivalent to forward and side scatter analysis in FACS, much like the cells double positive and single positives for the markers used.

DotPlot operations are mostly analogous to those used in FACS (setting of cutoffs, gating), however, there are special features.

Chief among those are the possibilities to use Forward and Backward Gating.

New DotPlots can be generated for any combination of the available measurement parameters (currently nine).

The most important feature is that DotPlots can be generated based on any number of gates.
This allows the user to define cell subpopulations as the basis for new measurement combinations, offering near-unlimited possibilities for re-evaluating and refining data.

Statistics of scattergrams and gates

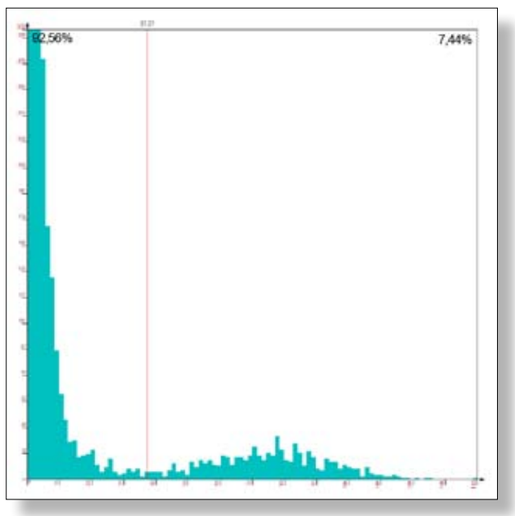
All events in scattergram					
Quadrant	Hemalaun Mean	Cyclin A Mean	Count	Percent	No./mm
UL	0,000	0,000	0	0,00%	0,000
UR	124,815	92,266	4296	3,90%	4
LL	42,249	0,972	271	0,24%	1624
LR	114,104	1,347	105694	95,86%	1694
Overall	115,104	4,889	110261	100%	63

After setting the CutOff automatically the quadrant statistics for the scattergram and each of the gates is generated.

	Quadrant	Ki67 - M...	Count	Percent	No./mm2
>	Left	2,346	7395	92,56%	184875,000
	Right	75,467	594	7,44%	14850,000
	Overall	7,783	7989	100,00%	199725,000

Histogram quadrant statistics.

Histogram



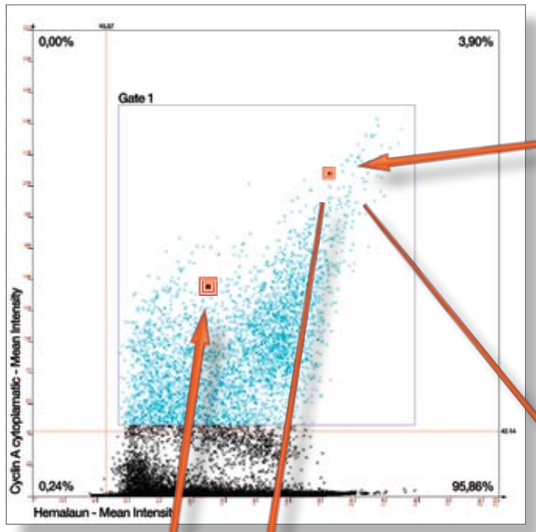
Data can also be displayed in form of histograms.

HistoQuest
Backward and Forward Gating of single cells & gates

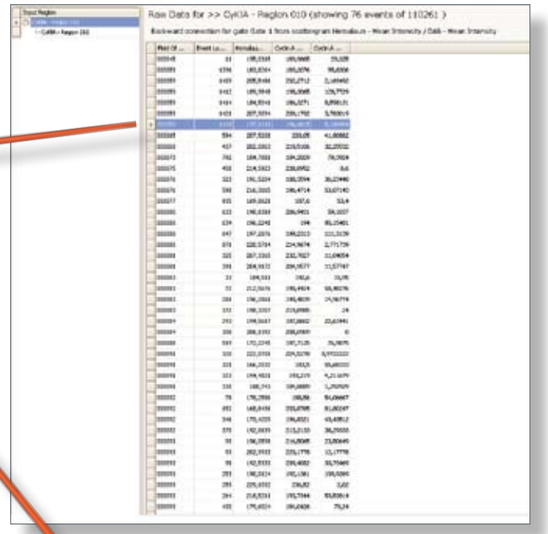
HistoFAXS offers an innovative function for visual and measurement control.

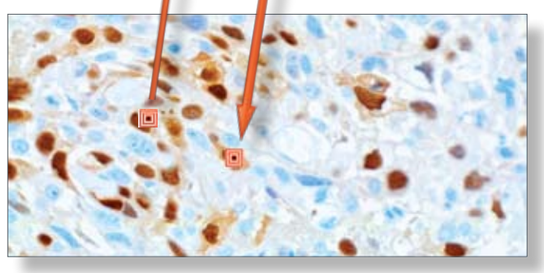
Backward Gating allows connecting backwards from the DotPlot to the images for any dot or group of dots as defined by a gate or for entire cutoff quadrants or combinations thereof.

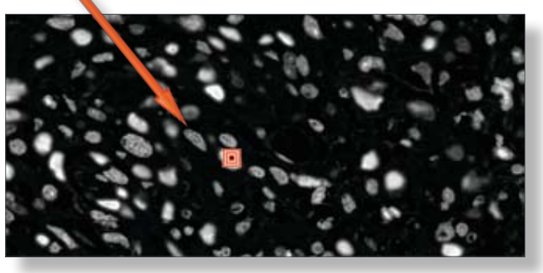
Gate with backward and forward connection



Raw data for backward connection from Gate 1







Forward Gating shows the position of any cell in the images in all DotPlots by simply double clicking on the cell in the image.

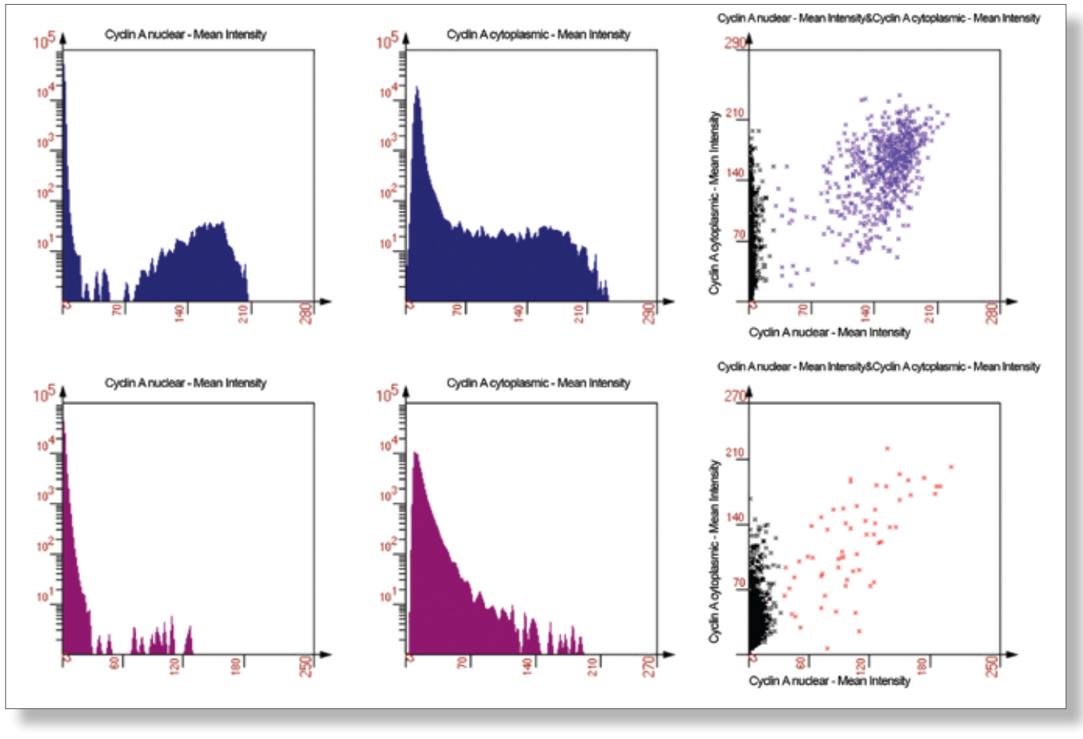
Backward Gating from dot to image

HistoQuest
Compare sets

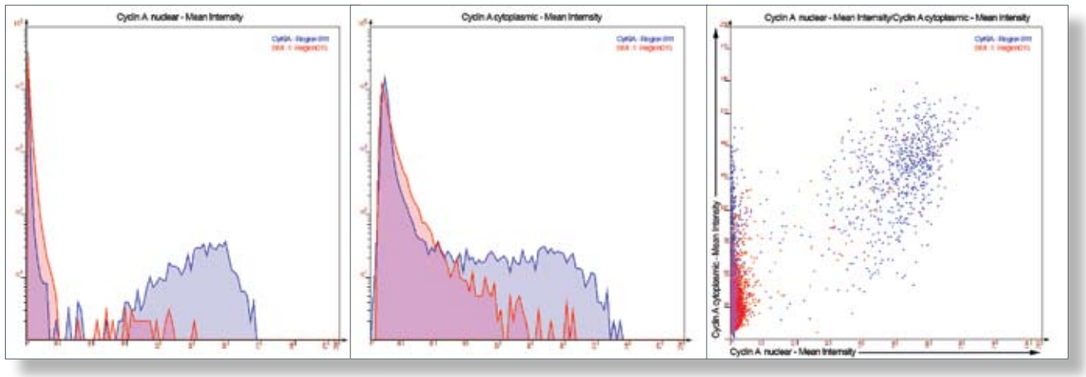
Side by side and overlay compare sets

Measurement data from different ROI's and/or Analysis Selections can be directly compared within the same diagram (DotPlot or Histogram).

Side by side vertical comparison set Cyclin A nuclear VS Cyclin A cytoplasmic



Overlay comparison set CyclinA nuclear VS Cyclin A cytoplasmic

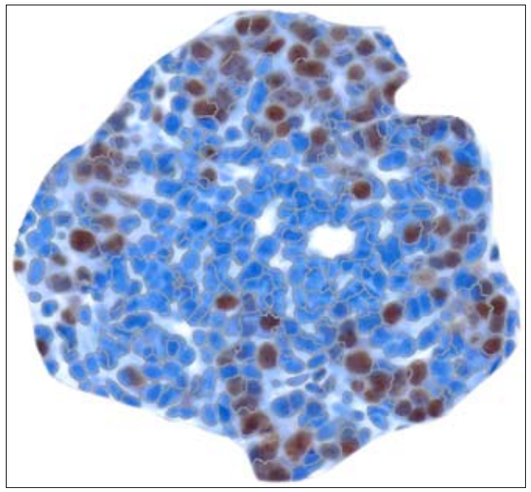


HistoQuest
Comparison of HistoQuest Analysis and manual counts

The Reinheckel Group at the Institute of Molecular Medicine and Cell Research of the Albert-Ludwigs University Freiburg, Germany, conducted validation experiments with HistoQuest®.

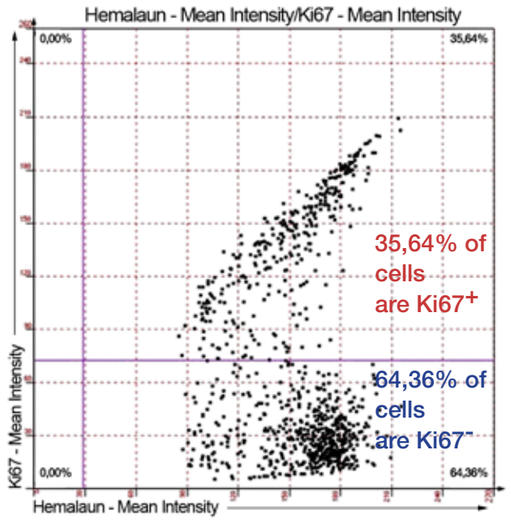
The aim was to quantify Ki67 expression in pulmonal metastasis of 14 month-old MMTV-PyMT mice with HistoQuest and do manual counts in comparison.

Sample of pulmonal metastasis quantified with HistoQuest

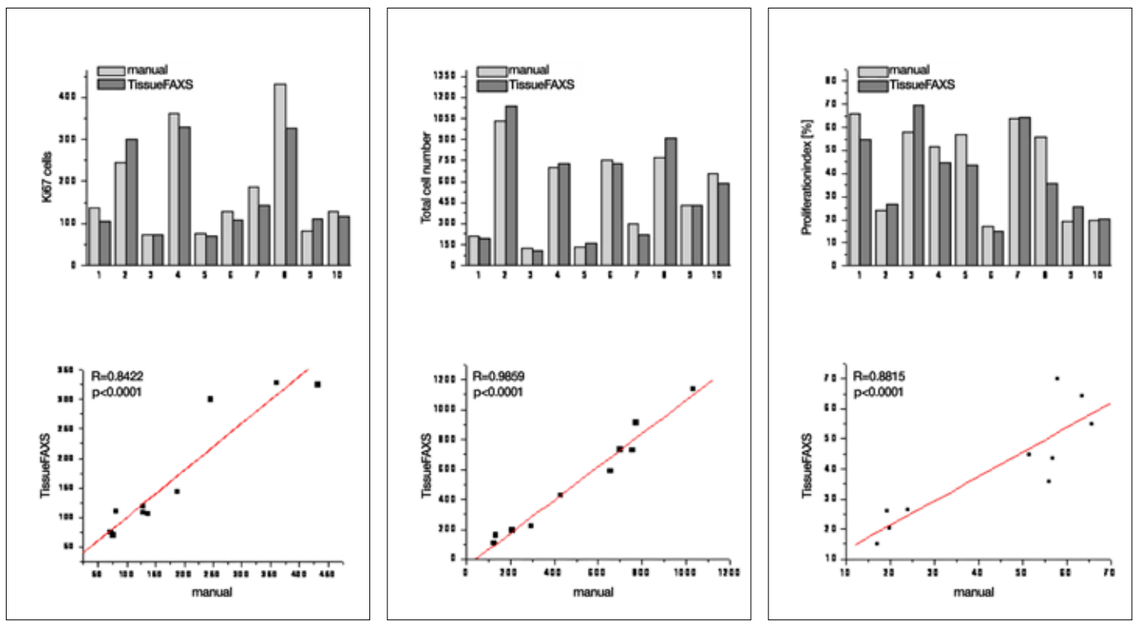


All images and graphs on this page courtesy of Dr. Thomas Reinheckel, Albert-Ludwigs University Freiburg, 2007

Quantification Ki67 positive vs. Ki67 negative cells



Comparison of HistoQuest Analysis and manual counts (ns10)

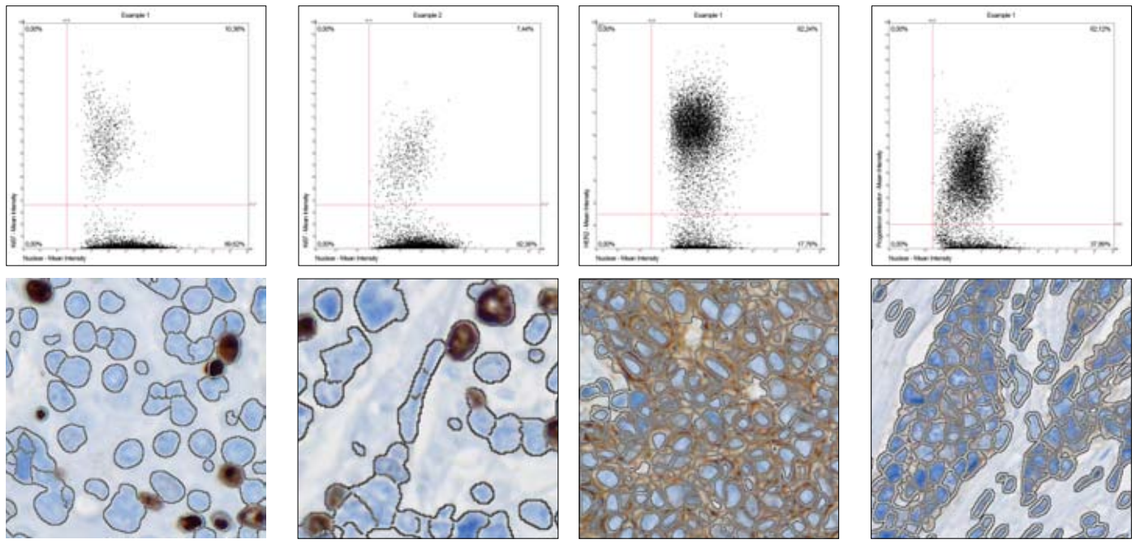


HistoQuest
Mamma carcinoma

Two typical examples of mamma carcinoma

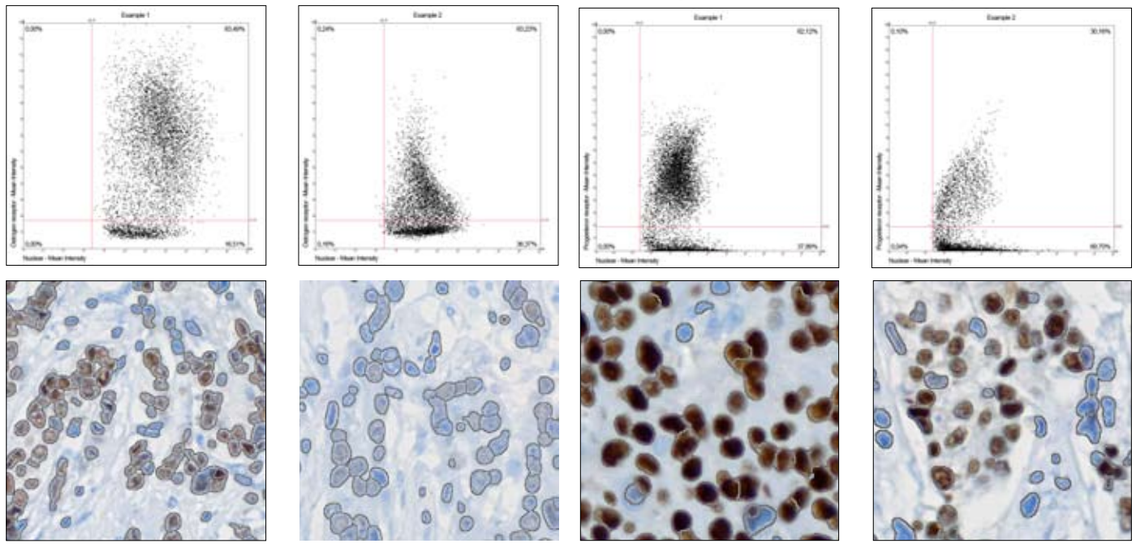
Anti-Ki67 reactivity

Anti-HER2 reactivity



Anti-estrogen receptor reactivity

Anti-progesteron receptor reactivity



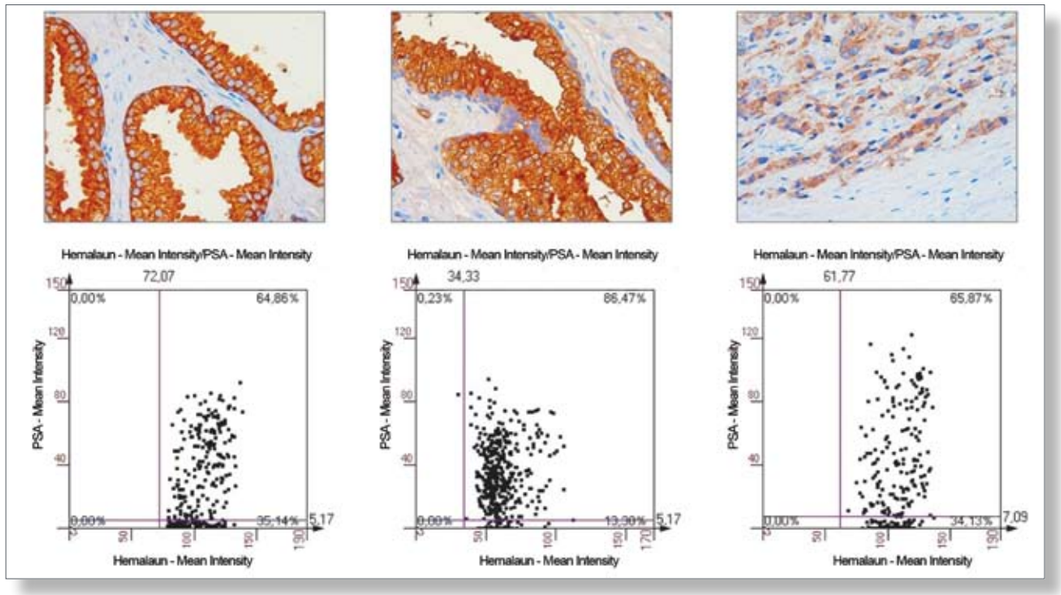
All images courtesy of Univ. Doz. Dr. Johann Feichtinger, Pathology-Bacteriological Institute, Rudolfstiftung Hospital, Vienna

HistoQuest
PSA expressions and HDAC1 overexpression

Ao. Univ. Prof. Dr. Lukas Kenner's group at the Vienna Ludwig Boltzmann Institute for Cancer Research (LBI-CR) use HistoQuest® to quantify PSA (Prostate Specific Antigen) expression and HDAC 1 (Histone Deacetylase) overexpression in prostate cancer (CPC) before and after Androgen Ablation therapy.

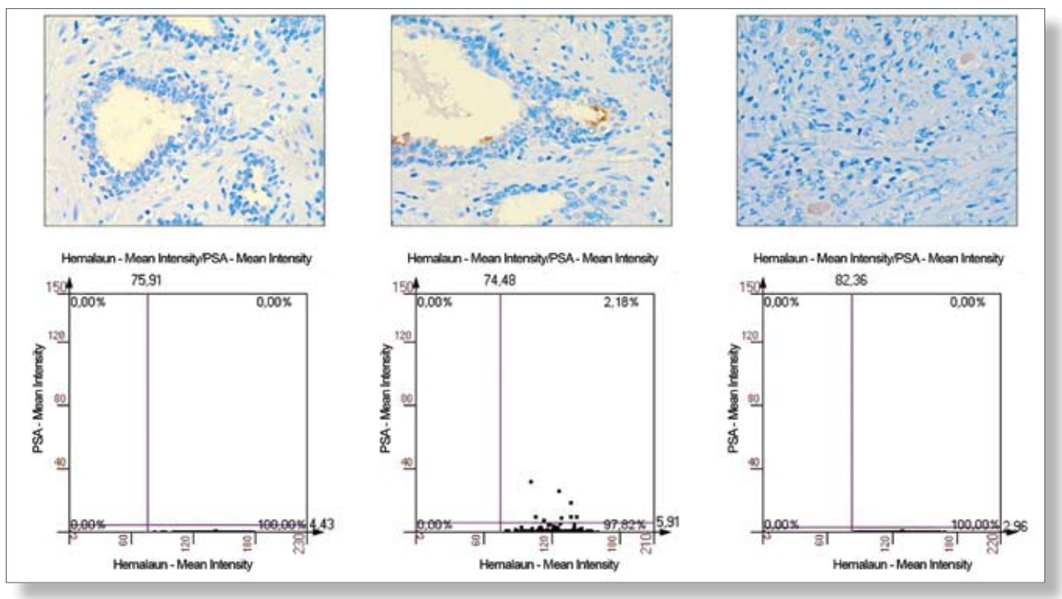
PSA expression in PC (1) - without therapy

Normal PIN CA



PSA expression in PC (2) - after Androgen Ablation

Normal PIN CA



All images and graphics courtesy Ao. Univ. Prof. Dr. Lukas Kenner, Clinical Institute of Pathology, Medical University Vienna

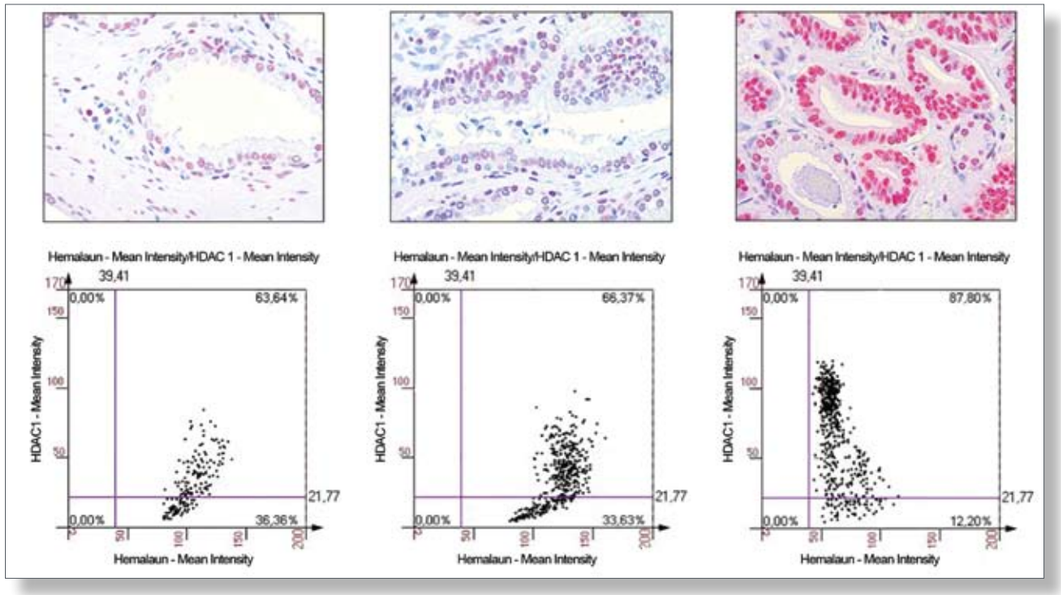
TissueGnostics HistoFAXS

"We are using HistoQuest® to evaluate our tissue arrays (prostate and lung tumors) with IHC stainings. It has enabled us to rapidly get reliable quantitative data on the expression patterns of various proteins for the first time. I can well envisage HistoQuest being used in routine diagnostics soon."

Ao. Univ. Prof. Dr. Lukas Kenner, Clinical Institute of Pathology, Medical University Vienna

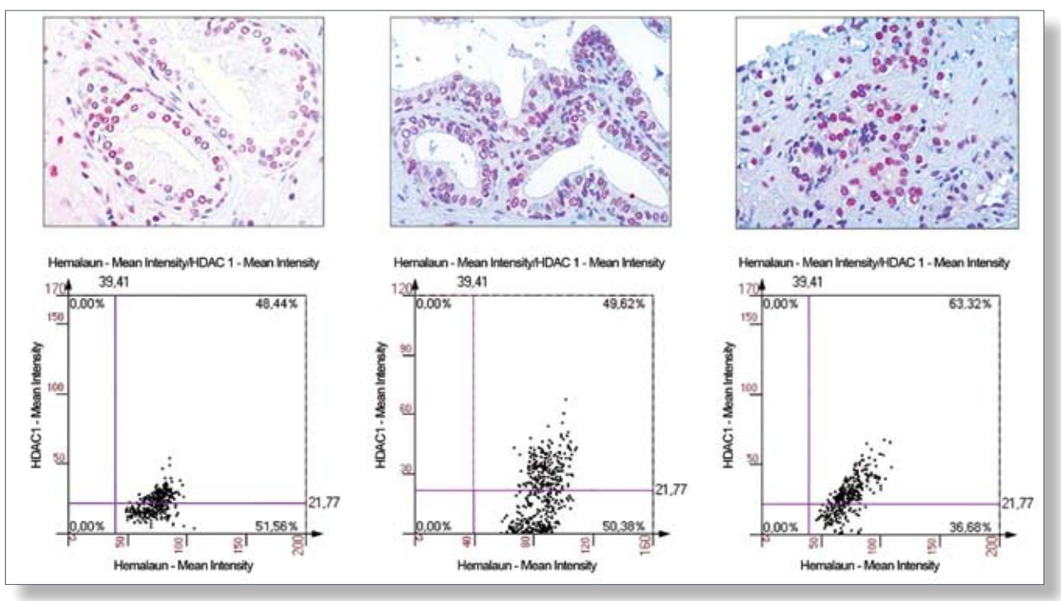
HDAC 1 overexpression in PC (1) - without therapy

Normal PIN CA



HDAC 1 overexpression in PC (1) - after Androgen ablation

Normal PIN CA



All images and graphics courtesy Ao. Univ. Prof. Dr. Lukas Kenner, Clinical Institute of Pathology, Medical University Vienna

HistoQuest
Features

HistoQuest features:

- HistoQuest® can load complete HistoFAXS® projects or import any series of .tif or .jpg images for analysis.
- Complete section overviews for ideal orientation
- Tunable color separation algorithms for optimal differentiation of colors
- Color selecting tool for adding shades
- No limitation as to the number and types of cromogenes used
- Powerful image processing algorithms for the identification of single cells in tissue based on nuclear staining
- Automatic generation of ”cell body” (cytoplasmatic) masks for intensity measurements
- Complete set of manual modification and correction tools (splitting, merging, deleting and creating nuclei)
- Powerful DotPlot operations
- Forward Gating of cells to the DotPlots and Backward Gating of dots, gates or cutoff quadrants to the images
- Unlimited custom DotPlot and histogram generation, also based on gates in existing DotPlots, allow for near-unlimited new analyses and refinement of existing ones.
- Extensive printed report and data export options

TissueGnostics HistoFAXS

Technical Specifications

Supported high-end microscopes

Zeiss AxioImager.Z1
Zeiss AxioImager.M1
Zeiss AxioObserver.Z1
Leica DM 6000
Nikon Eclipse 90i

Fully motorized base
Offers complete automation.
Up to 7 objective lenses
From 1x to 100x immersion oil.

High-performance workstation

2 x 24” TFT screens, Intel Core 2 Quad,
4 Gigabyte RAM, 1 Terabyte HD

Required space

160cm x 80cm (5.11ft x 2.55ft)

Illumination

LED Diascopic illumination (option)
Halogen 12V-100W (standard)

High-precision motorized stage

Stage for upright microscope
For up to 8 slides.
200 slides loader as option.
Stage for invert microscope
For microtiter plates and up to 4slides.
Resolution
Step size as low as 1.5 nm (.0015 µm) for smooth movement.
Repeatability
Relocation difference < 1 µm, so you can find on the slide precisely what you see on screen.
Mark and find
Finds absolute stage positions at < 4 µm accuracy (as is necessary for reliable automation).
Fast autofocus optimized for tissue samples

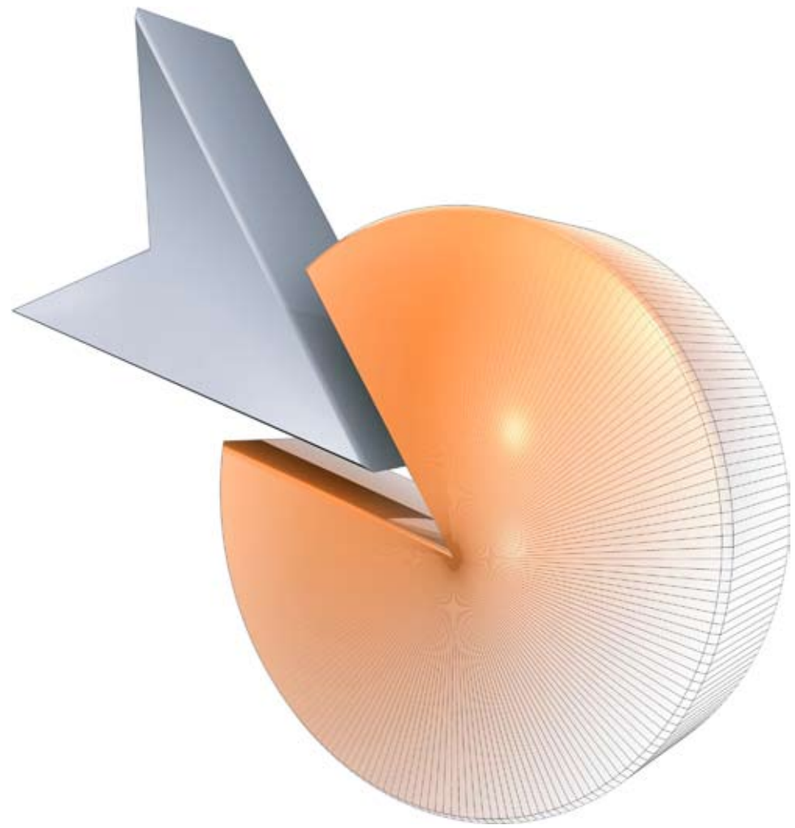
Acquisition times

Focus field	10x lens		20x lens	
	800x 600	1600x 1200	800x 600	1600x 1200
3x3	43s	75s	117s	227s
5x5	25s	37s	81s	152s
7x7	26s	37s	67s	123s

High-performance color camera for brightfield

Resolution
Up to 1.9 megapixels (1600x1200px) guarantees fine objective detail.
Frame rate
Up to 100 fps @ VGA for acquisition.
Sensor format
Up to 1” offers extremely wide fields of view.
Excellent color reproduction
Use of multiple chromophores.

Technical and application support via remote control



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