TISSUEGNOSTICS

TG

SOFTWARE
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THE TISSUEGNOSTICS QUEST LINE

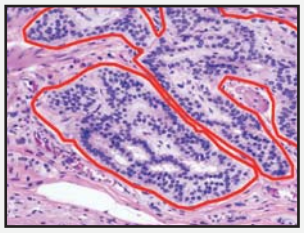
BIOMEDICAL TISSUE CYTOMETRY IMAGE ANALYSIS SOFTWARE

„This is the software I've always wanted..."

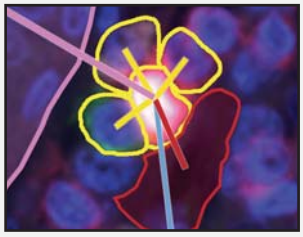
TissueGnostics Image Analysis is built on a simple principle:

- Take two powerful technologies, each of which has a drawback...
- Put them together and make them work, eliminating both drawbacks...
- Keep everything simple so non-specialists can use it...

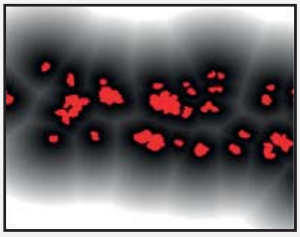
The result was TissueQuest - a Tissue Cytometry Image Analysis software package which prompted the user exclamation in the title in 2007.



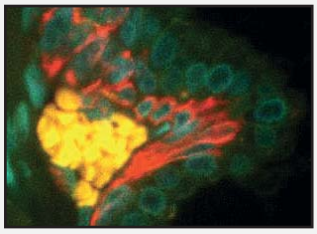
Morphology based tissue structure detection



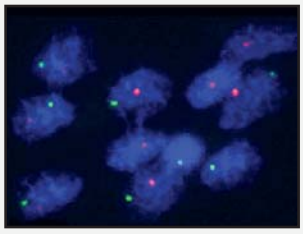
Spatial cellular micro-environment analysis



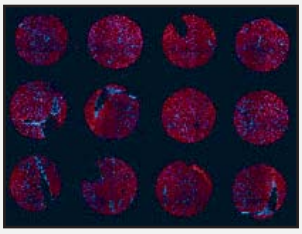
Cell distance to structure measurement



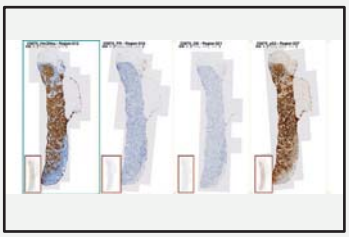
Multispectral analysis in immunofluorescence



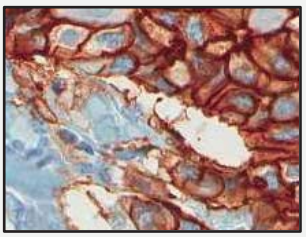
Routine pathology FISH & dot analysis solution



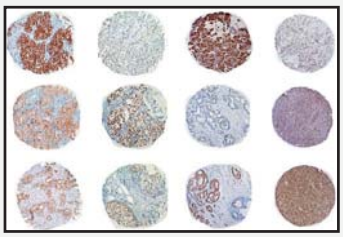
Batch immunofluorescence TMA analysis



Automated Panel analysis in Immunohistochemistry



Routine pathology membrane algorithms



Batch IHC TMA analysis

TissueQuest in its first version combined Flow Cytometry, producing large amounts of data but being essentially „blind“, with the very visual potentially data rich microscope image, which had no fast data output except for tedious manual counting or low-objectivity estimates.

By providing easy to use encapsulated image processing algorithms for extracting hard data from the images and combining them with simplified flow cytometry scattergrams and a clever feature for visualizing results the best of both worlds was made available. Natural scientists with no image analysis background could be trained to use the software in half a day to a day.

The power of TG´s analysis solutions has been growing continuously since then, but the training time much less so - we are still keeping it simple.

User friendly APPs for the analysis of specific tasks

STRATAQUEST

CONTEXT ANALYSIS SOFTWARE

Fully automated easy walk-away workflow

TISSUEQUEST

FL CELL ANALYSIS SOFTWARE

Fully automated easy walk-away workflow

HISTOQUEST

IHC/HC CELL ANALYSIS SOFTWARE

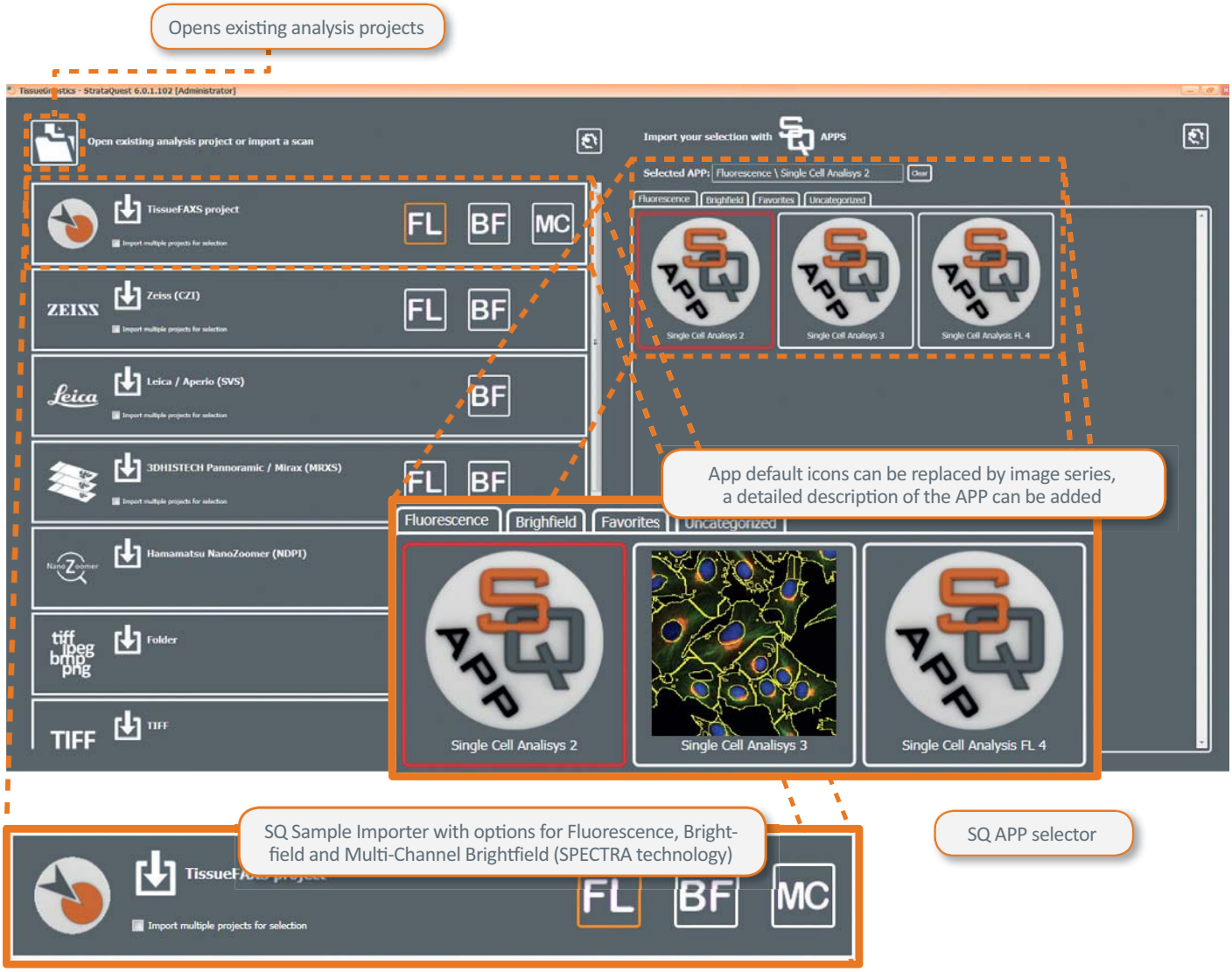
STRATAQUEST CONTEXT ANALYSIS SOFTWARE

Jack of all trades & Master of all. **Context-based Tissue Cytometry Analysis**

StrataQuest (SQ) is TissueGnostics most evolved image processing solution. It is based on an ever expanding library of ENGINES (task-specific algorithms). Most structures on a digital slide, from tumors to ISH probe signals, can be detected using the SQ ENGINES. Such detected structures and TG cell-based Tissue Cytometry analysis technology are

APPS – for biomedical researchers and clinical routine

APPS are modular prefabricated solutions for either general analysis requirements, e.g. „Multichannel IF“, or more specialized analysis, e.g. „IF Cellular Microenvironment“.
APPS come with an easy to use and commented interface, the use of which does not require any image processing know-how and provides information on the use for each step.
An unlimited number of APPS can be used in a StrataQuest installation. The APPS shown in the following are merely examples, more are availablle or can be built for customer requirements.

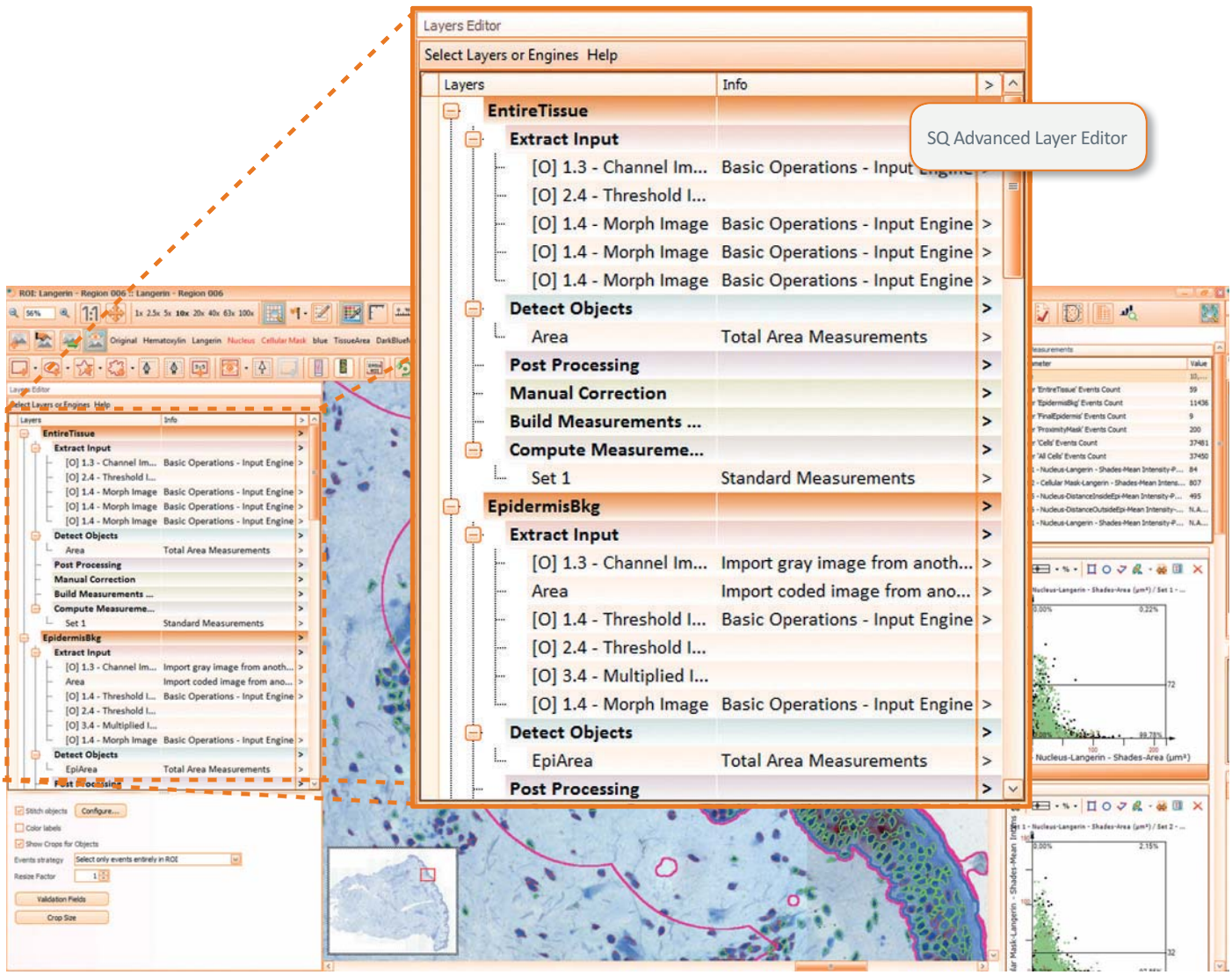


an ideal combination for detailed context-based quantitative analysis. It provides data which hitherto was difficult and time consuming to get or which could not be provided at all.
Currently, there are two ways that the capabilities of StrataQuest can be harnessed, catering to the requirements of two differents types of users:

PLUS – for core facilities & experienced image processors

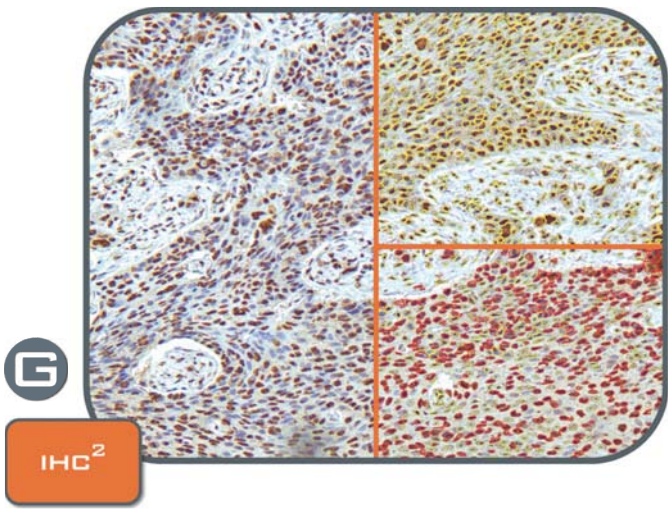
Plus provides direct access to StrataQuests Plus Layer Editor for users with image processing experience.
In the Editor, a technically unlimited number of interconnected layers (analysis pipeline steps) can be added, each containing a specific choice of ENGINES.

Build solutions to the most exacting problems – and use them to build new APPS with easy to use interfaces for the customers of a core facility.

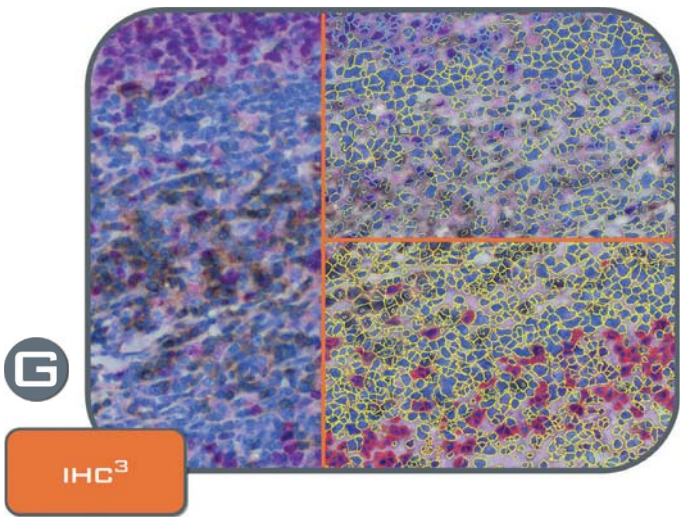


STRATAQUEST CONTEXT ANALYSIS SOFTWARE

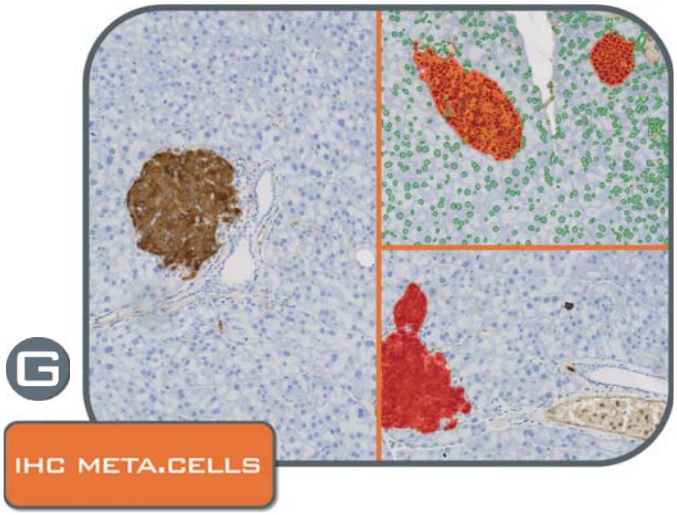
SQ APPS – General Purpose (G) and Specialized (S) APPs Immunohistochemistry/Histochemistry.
All APPs provide data export to Excel, CSV or PDF formats.



The IHC² APP unmixes two markers (e.g. chromogen and counterstain) in an IHC or HC Digital Slide and segments single cells into nucleus, and/or perinuclear area and/or cytoplasm.
Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.



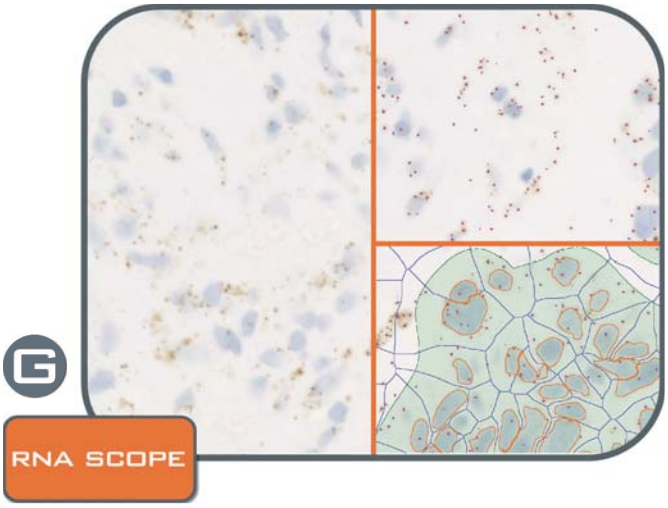
The IHC³ APP unmixes three markers (e.g. two chromogens and counterstain) in an IHC or HC Digital Slide and segments single cells into nucleus, and/or perinuclear area and/or cytoplasm.
Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.



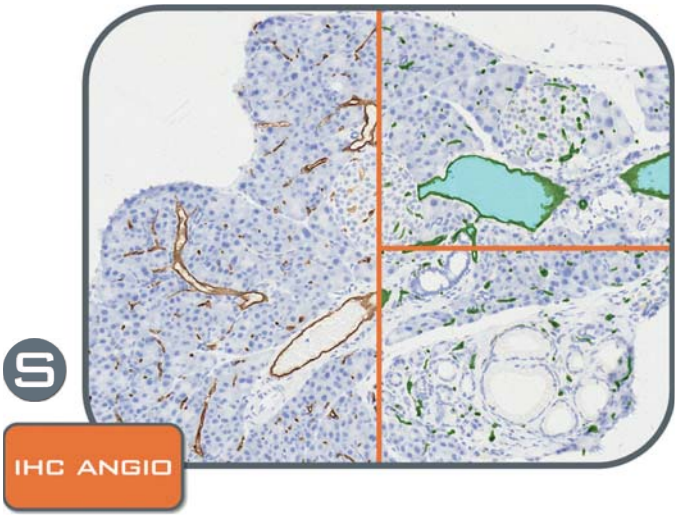
The IHC Meta.Cells APP combines the detection of IHC/HC stained metastructures (e.g. Langerhans islets) with single cell detection (segmentation of cells into nucleus, and/or perinuclear area and/or cytoplasm). Detected cells can be classified and visualized as being either within or outside of detected metastructures. Each detected area and cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.

The IHC Multi-Shades APP provides semi-automatic color separation for up to six markers or colors in an IHC or HC Digital Slide. In the above sample it has been used to detect and segment different levels of ossification based on Azan stain. Each detected area is measured for up to 20 intensity, statistic and morphometric parameters

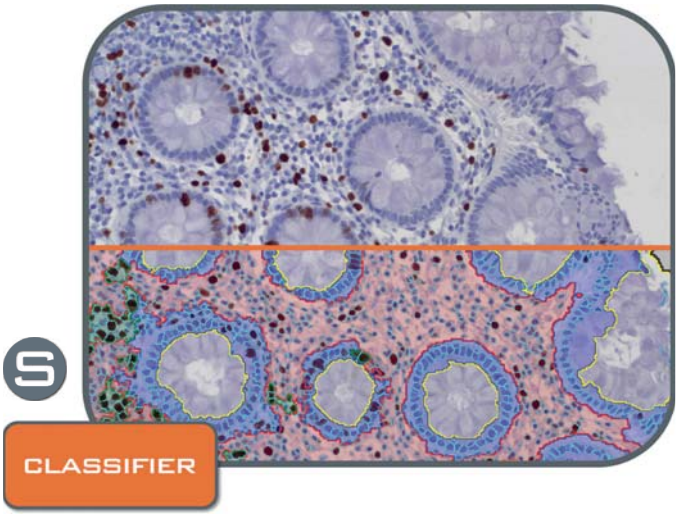
The IHC Membrane APP unmixes up to three markers in an IHC or HC Digital Slide and segments cells into nucleus and/or perinuclear area and/or cytoplasm, as well as into membrane (e.g. HER2/neu). Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters. Three more parameters are measured for membrane intensity and angle of staining.



The RNA Scope APP provides dot detection per cell within the nuclear compartment (nucleus and/or cytoplasm) for up to three markers in CISH and SISH experiments. Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters. Dot parameters are provided per cell and include count, mean intensity, total dot area, and sum of intensity as well as area and intensity lists for all single dots.



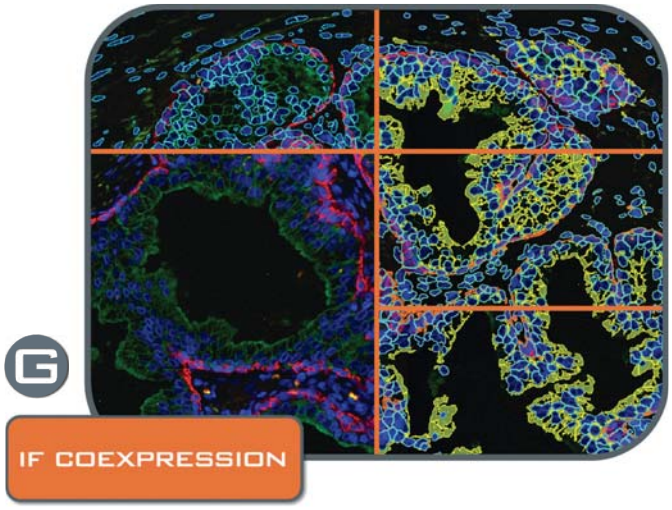
The IHC Angio APP detects blood vessels based on appropriate stains (e.g. CD31) and measures overall vessel area as well as lumen area. The vessel detection also can be set to close open stained vessel walls and to connect separated vessel sections within a definable distance. The APP outputs number and vessel density as well as areas of vessels, endothelium and lumina.



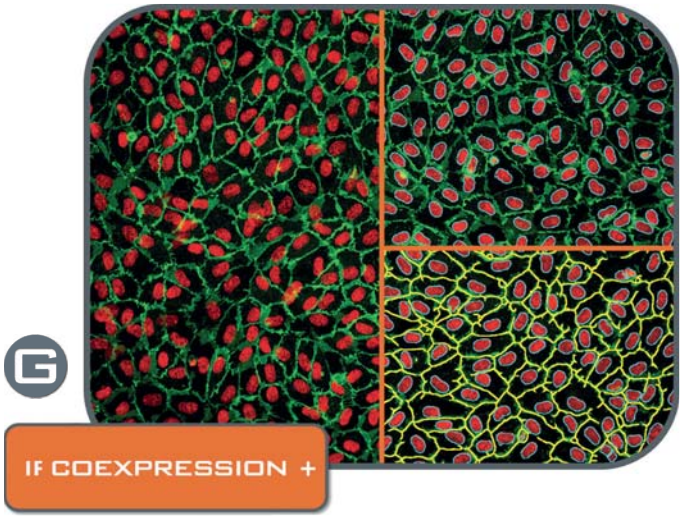
The Classifier APP is based on trainable Deep Learning technology and provides tissue metastructure detection. In the above example it separates the colon tissue into crypts, stroma and infiltration areas. Combined with the IHC2 cellular detection APP the Ki-67 + nuclei in each compartment can be quantitatively analyzed for up to 20 parameters.

STRATAQUEST CONTEXT ANALYSIS SOFTWARE

SQ APPS – General Purpose (G), and Specialized (S), APPs Immunofluorescence.
All APPs provide data export to Excel, CSV or PDF formats.

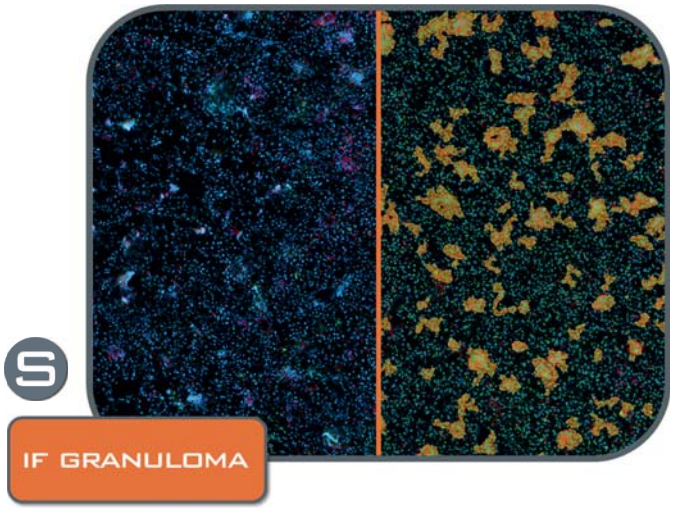


The IF CoExpression APP provides single cell based co-expression analysis for multiple immuno-fluorescent markers (the number is technically unlimited). It segments cells into nucleus and/or perinuclear area and/or cytoplasm.
Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.

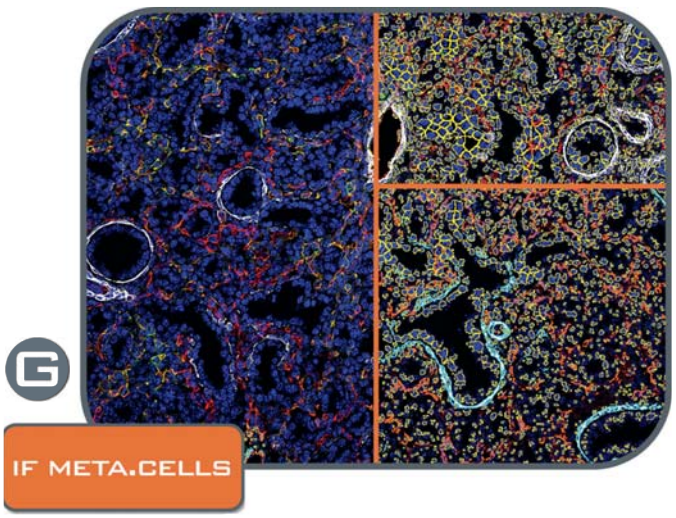


The IF CoExpression + APP provides single cell based coexpression analysis for multiple immu-nofluorescent markers (the number is technically unlimited). It segments cells into nucleus and/or perinuclear area and/or cytoplasm, as well as into membrane. Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.

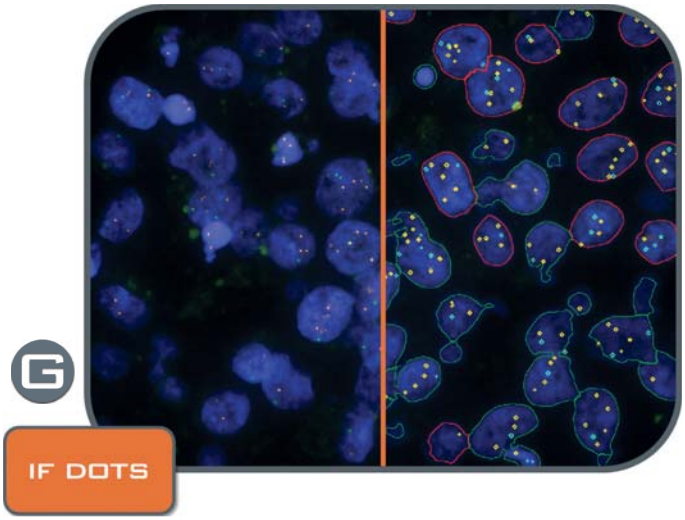
The IF Immune Status in Situ APP provides phenotyp-ic characterization of immune cells in reference to de-tected metastructures (e.g. tumors, glands, etc.) and measures the distance of detected cellular objects to the metastructure boundary (within and/or outside). Distance ranges can be defined. Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters, as is the distance of each cell to the boundary's area.



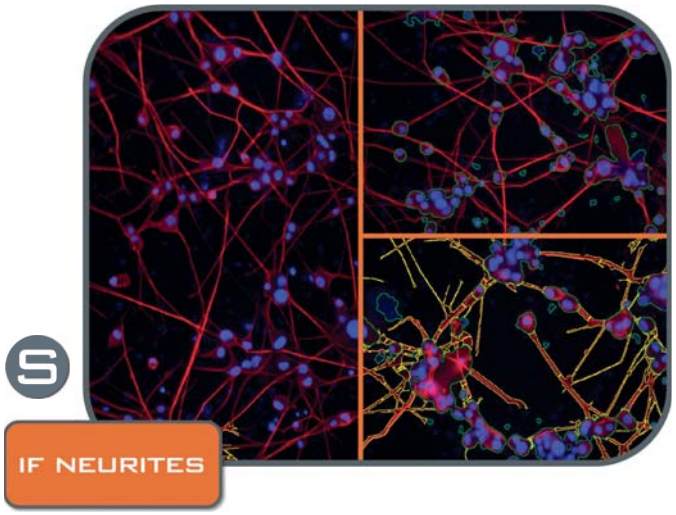
The IF Granuloma APP detects granulomas based on nuclear structure analysis and an adequate IFL staining (e.g. CD11c, CD68).
The number and area of Granulomas as well as their density is measured. Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.



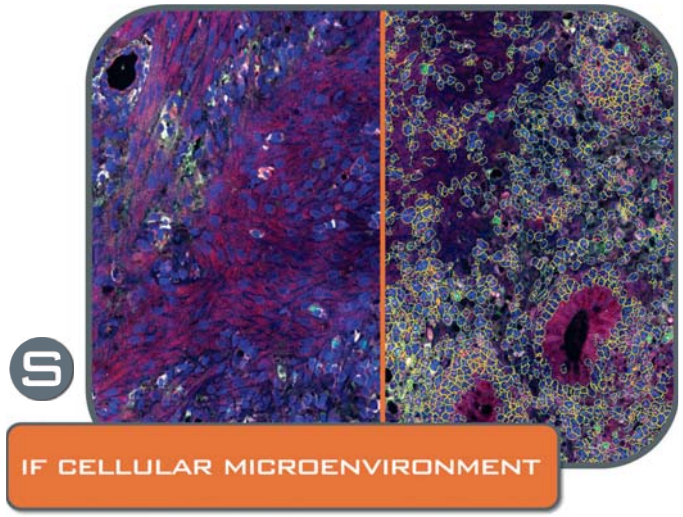
The IF Meta.Cells APP combines the detection of IFL stained metastructures (e.g. Langerhans islets) with single cell detection (segments cells into nucleus and/or perinuclear area and/or cytoplasm). Detected cells can be classified and visualized as being either within or outside of detected metastructures. Each detected area and segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.



The IF Dots APP provides dot detection per cell within the cell compartments for up to four markers in a sample (e.g. FISH, RNA, oil droplets, etc.). Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters. Dot parameters are provided per cell and include count, mean intensity, total dot area, and sum of intensity as well as area and intensity lists for all single dots.



The IF Neurite APP identifies neuronal cells and cell clusters and their neurites. It quantifies the number of neurites branching out from a specific neuron, identifies branch points and exports total neurite area, total neurite length, average neurite thickness, number of branch points, and number of end points.



The IF Cellular Microenvironment APP allows to determine the cellular phenotype of specific IF stained cell populations and establishes their spatial relationship between each other, their neighbor cells/cell populations as well as, the one with metastructures (e.g. blood vessels, tumors) in their vicinity. It is especially suited for proximity and infiltration analyses.

STRATAQUEST CONTEXT ANALYSIS SOFTWARE

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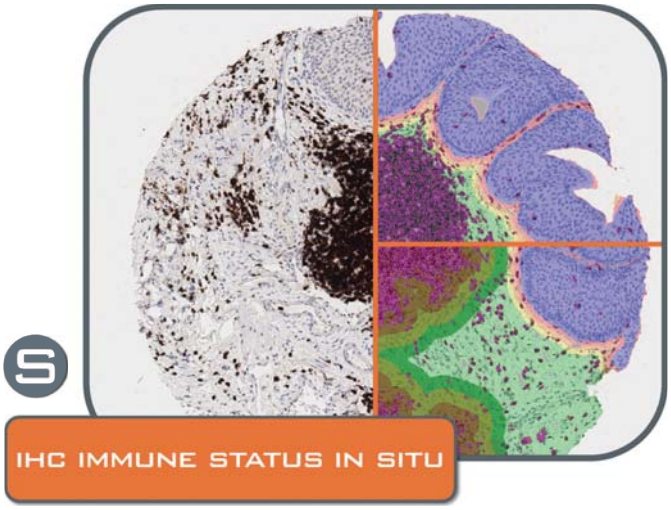
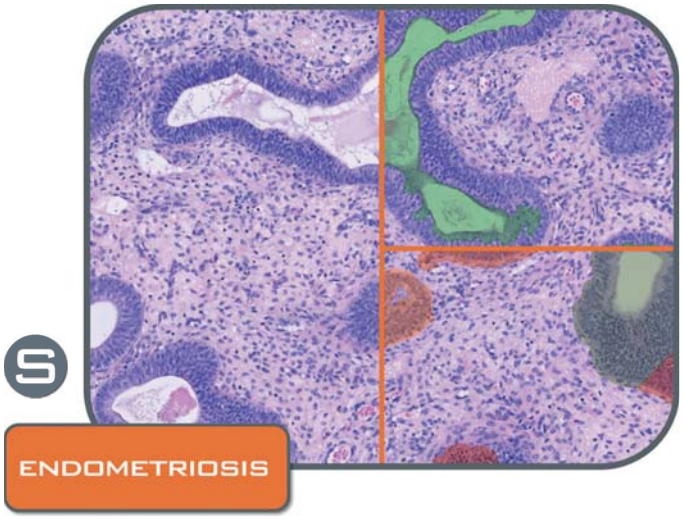
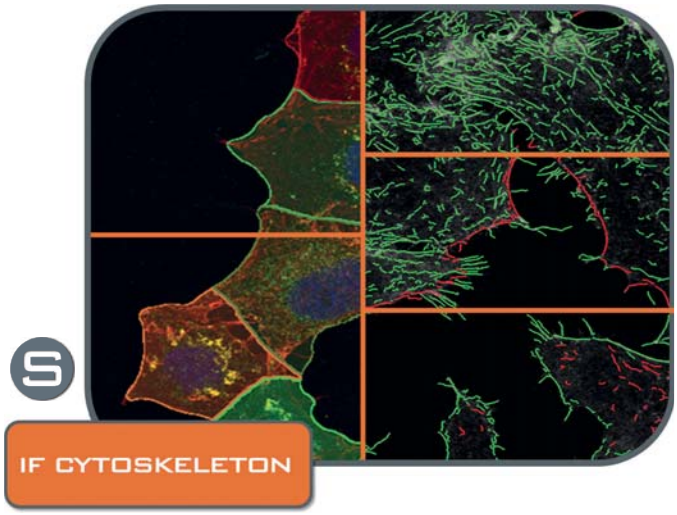


The IHC Immune Status in Situ APP provides phenotypic characterization of immune cells in context with detected metastructures (e.g. tumors, glands, etc.). It measures the distance of cellular objects to the metastructure boundary (within and/or outside). Distance ranges can be defined. Each segmented cell compartment is measured for up to 20 parameters, as is the distance of each cell to the boundary.

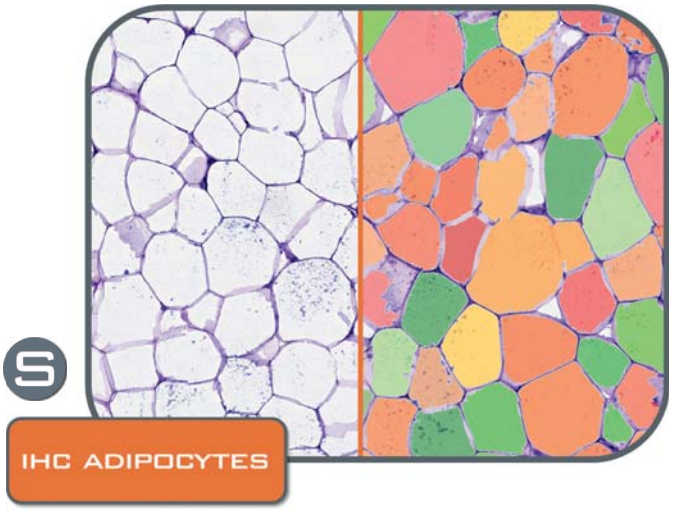


The Endometriosis APP detects specific Endometriosis structures (e.g. Glands, Gland Lumina, Stroma) on HE staining.

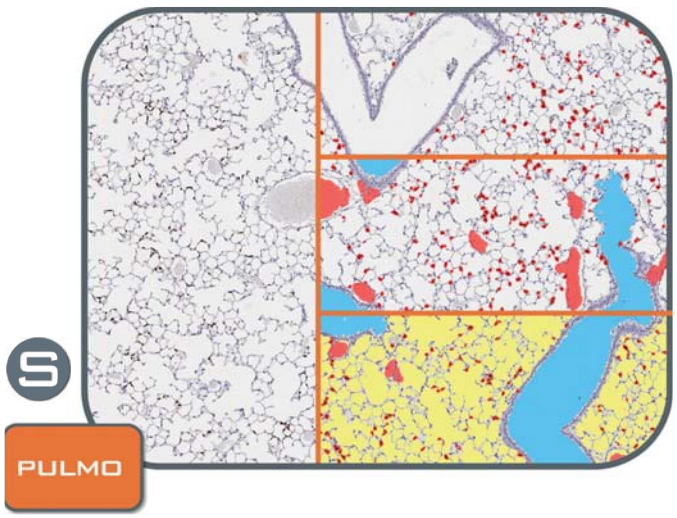
Each segmented cell compartment is measured for up to 20 intensity, statistic and morphometric parameters.



The IF Cytoskeleton APP detects cytoskeletal structures based on a specific stain. Used with other stains the cell cytoplasm can be detected and the number of cytoskeletal filaments inside of the cell, outside or on the cell membrane can be exported, as well as filament length and total filament area.

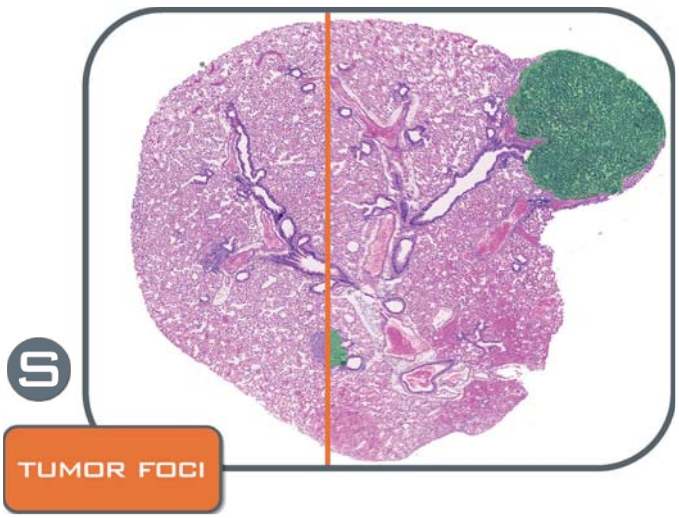


The IHC Adipocyte APP quantifies adipocytes as to their lumen in adequately stained HE samples. Small rips in adipocyte membranes are mended automatically and cell membrane artefacts in adipocyte lumina are automatically eliminated as are lumina on sample borders.
The APP outputs precise area measurements for all detected adipocyte lumina.



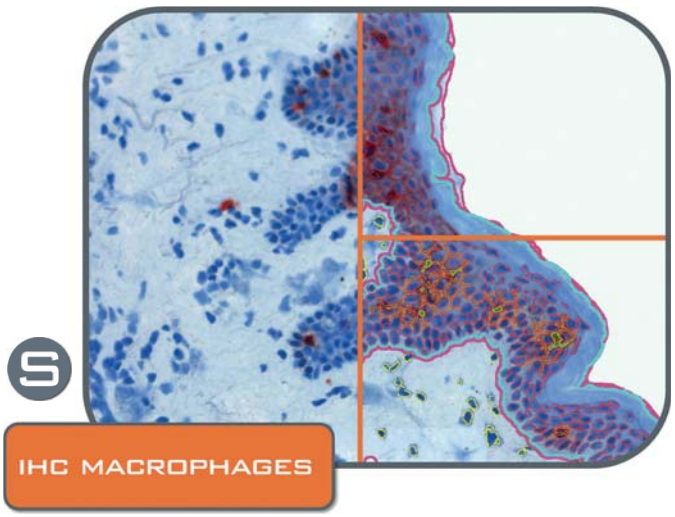
The Pulmo APP segments the metastructure components of lung, i.e. tissue, bronchioles, blood vessels and alveoles.

Each segmented metastructure is measured for up to 20 morphometric parameters.

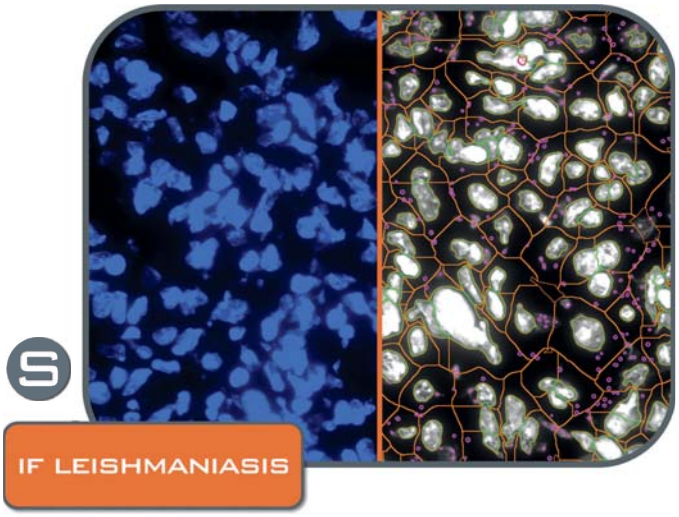


The Tumor Foci APP detects Tumor Foci based on nuclear structure analysis, mainly on HE staining.

The number and area of Tumor Foci as well as their density is measured.



The IHC Macrophages APP detects Macrophages based on adequately stained IHC samples (in the sample above, Langerin). The APP can be combined with area detection and distance range algorithms, in the sample above to determine the distance of Langerhans Cells from the border of the Epidermis within and without. Each segmented cell compartment is measured for up to 20 parameters, as is the distance of each cell to the boundary.



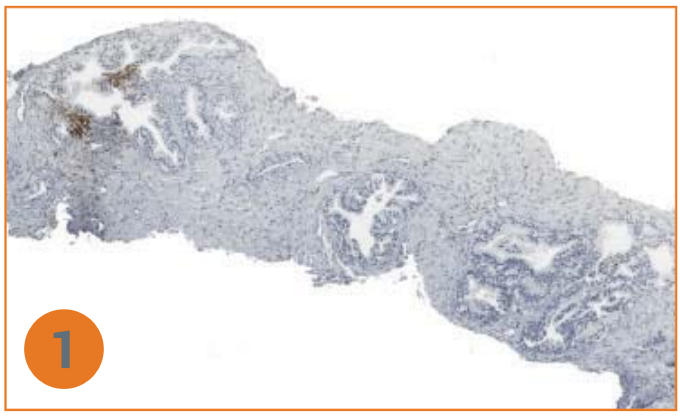
The IF Leishmaniasis APP detects intracellular Leishmania parasites and segments them in the detected host cells. The number of parasites per cell is determined and living and dead parasites can be distinguished (live/dead assays).The APP outputs the following data: 20 intensity, statistic and morphometric parameters for each segmented cell compartment per marker. Number, mean intensity, sum of intensity, and size of parasites.

STRATAQUEST CONTEXT ANALYSIS SOFTWARE

SQ PLUS: Spatial Distribution of CD3+ cells from epithelial area borders in prostate needle biopsies

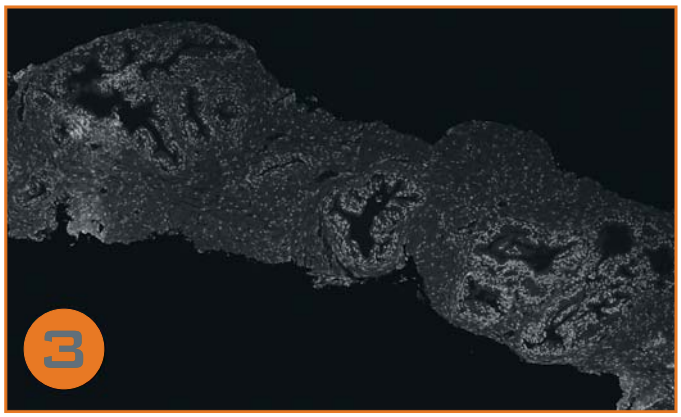
StrataQuest is ideally suited for this type of analysis which sets specific cell types and their marker expressions in context with their spatial distribution and specific metastructures.
The example shows the typical steps in this type of analysis which lends itself very well for APP building.

Apart from being integrated into TG hardware systems, StrataQuest is agnostic to a high degree and able to import many different digital slide formats as well as the main image formats (see lower right hand corner for a listing).

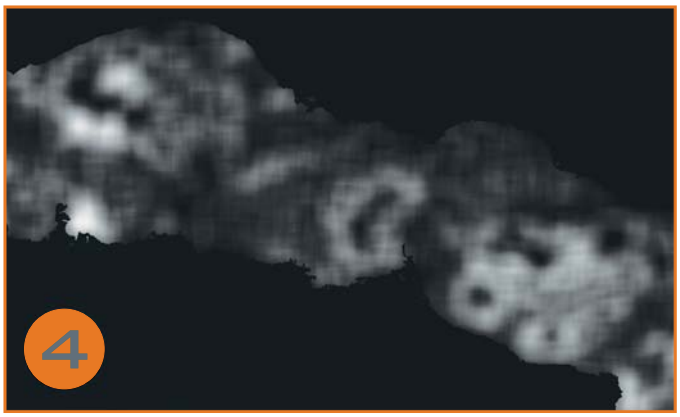


Original image
Example of an anti-CD3 stained prostate section from a clinical study of the Medical University of Vienna. The aim was an analysis of the distribution of T-cells.

Tissue detection
In the first step the tissue area was automatically identified using algorithms for tissue border and hole detection.



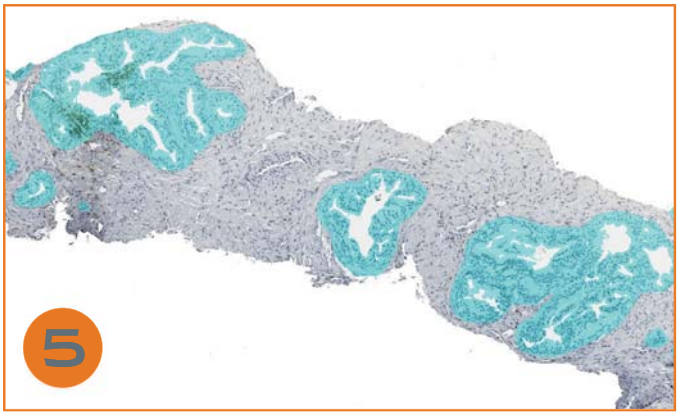
Virtual Channel of all cell nuclei
In the next step the image colors were separated into a blue and a brown channel and the nuclei were detected.



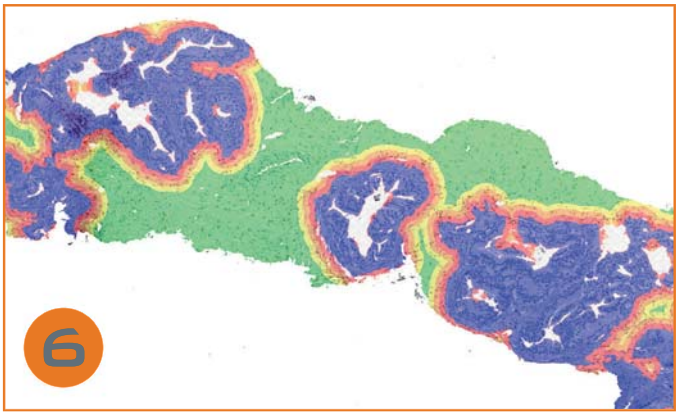
Heat map of epithelial nuclei
The morphologic features of the blue cell nuclei were used to select a subset of large and round cells and a heat map was generated from this subset.

Resulting data

- Tissue area
- Epithelial area
- Interstitial area
- % CD3+ within epithelial area
- % CD3+ within 0 to 25 µm from epi area
- % CD3+ within 25-50 µm from epi area
- % CD3+ within 50-70 µm from epi area
- CD3 intensity of T-cells
- CD3 intensity of T-cells within epithelial areas
- CD3 intensity of T-cells related to their distance from epithelia

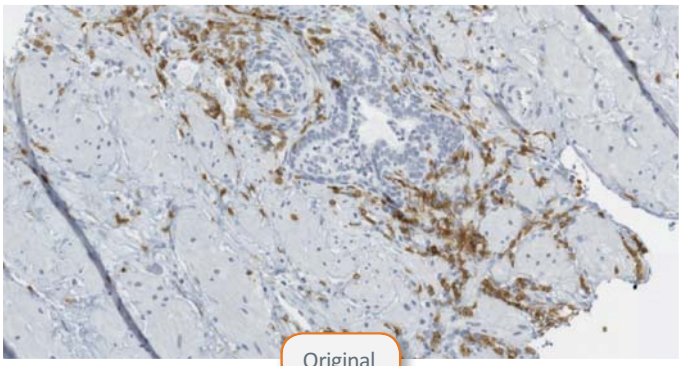


Mask for epithelial areas
The heat map was cleaned of small objects and based on it a mask for epithelial and tumor cells was generated.

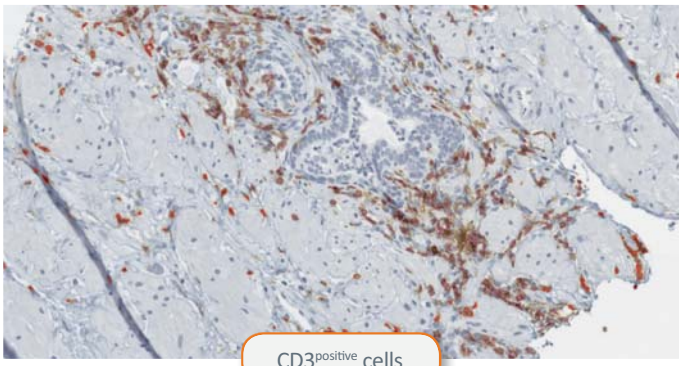


Distance measurements from epithelia
The detected epithelial strutures were used as seeds and all distances for CD3 positive cells were measured. Measurement areas were defined for T-cells within the epithelium for distance ranges of 0 to 25 µm, 25 to 50µm and 50 to75 µm.

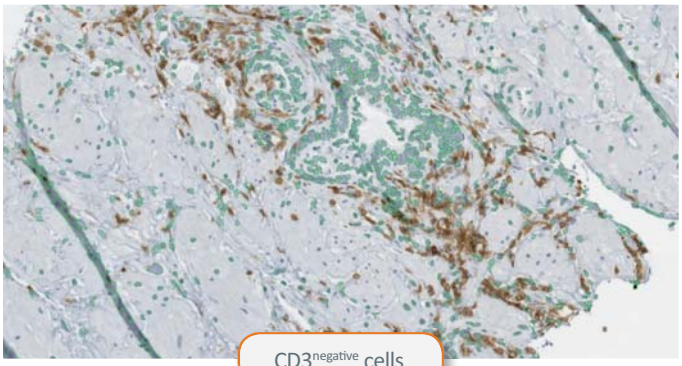
Detail views



Original



CD3^{positive} cells



CD3^{negative} cells

StrataQuest image data import capabilities:

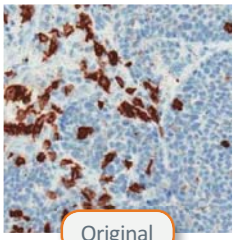
Zeiss .czi, Hamamatsu .ndp, Leica Aperio .svs, 3D Histech .mrxs , Keyence, Leica .SCN, Yokogawa CQ1, PerkinElmer .qptiff, .tiff, .jpeg, .bmp, .png (more to be added)

STRATAQUEST CONTEXT ANALYSIS SOFTWARE

SQ PLUS: Segmentation of thin stroma areas vs. tumor areas

StrataQuest is ideally suited for this type of analysis which sets specific cell types and their marker expressions into context with their spatial distribution. One of the main strenghts of StrataQuest is the capability to segment morphological structures based on a morphological stain alone. In the case shown below, this was used to detect thin areas of stroma between tumor areas in a tumor sample stained immunohistochemically for CD8+ T-cellls with a Hematoxylin counterstain. The counterstain was used for stroma detection.

Major steps of the automated analysis process

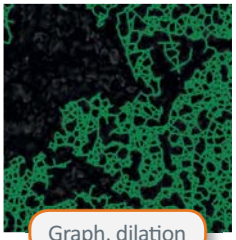

Original

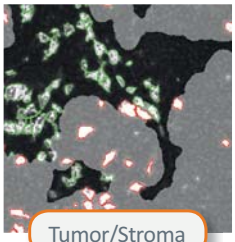

Stroma holes

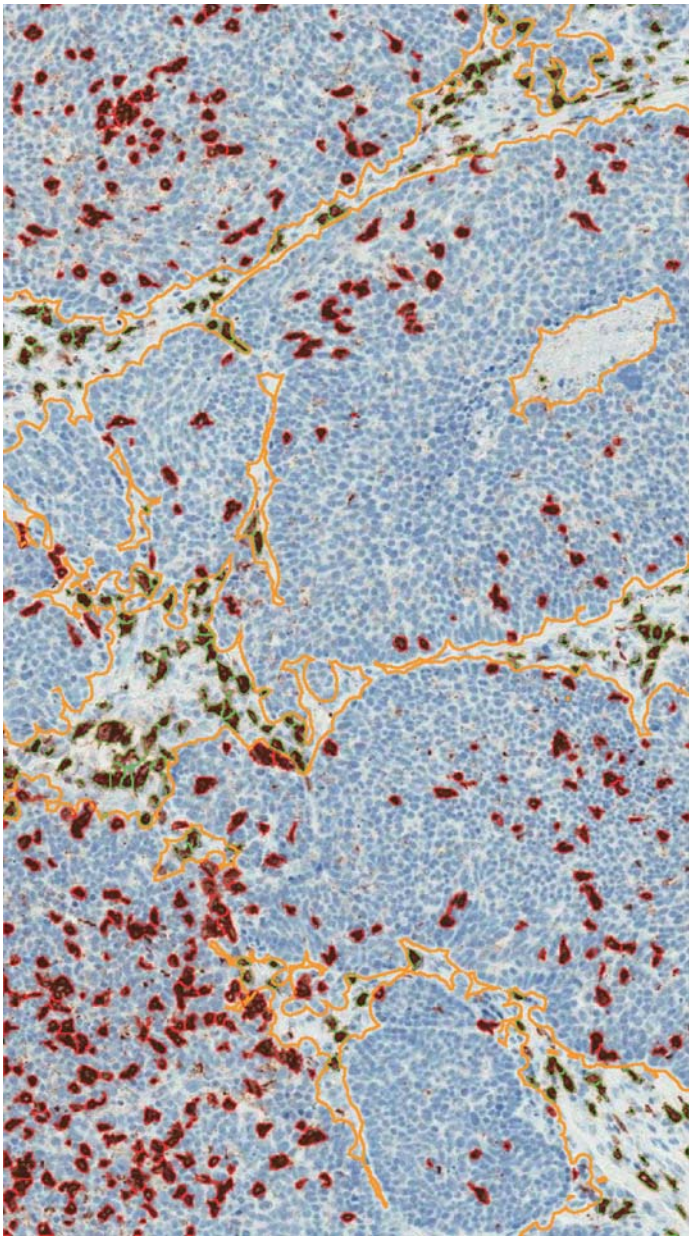

Skeletons


Clean skeletons


Stroma


Graph. dilation

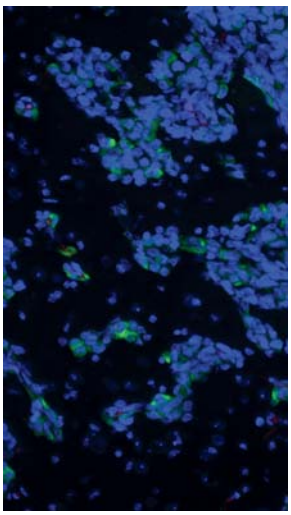

Tumor/Stroma



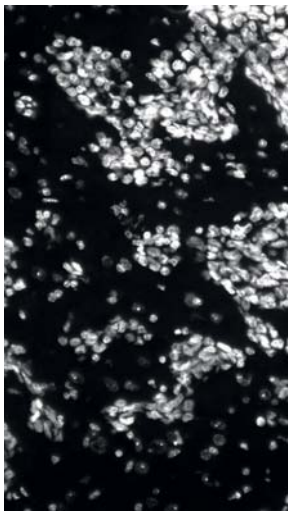
Courtesy Dr. Kathleen Williamson, Centre for Cancer Research and Cell Biology, Queen's University, Belfast, UK

Final result: Stroma areas were detected using the hemalaune counter stain only and it was shown that 17,3% of the sample are stroma (orange outline). 22% of CD8+ T-cells are located in stroma areas (outlined in green).

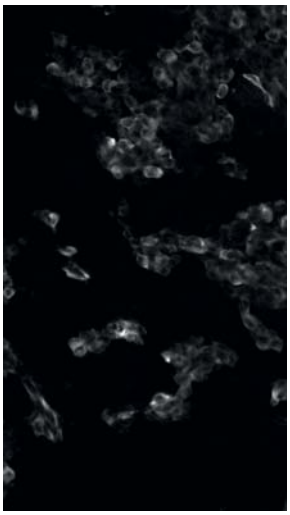
The same morphological differentiation capabilities can be used on immunofluorescent samples. Here, tumor areas are detected using anti-cytokeratin+ cells. Blood vessels are detected using anti-CD31+ structures. The distance of tumor cells from blood vessels within the tumor areas can then be measured. Some basic steps of the analysis are shown below.



3 channel overlay

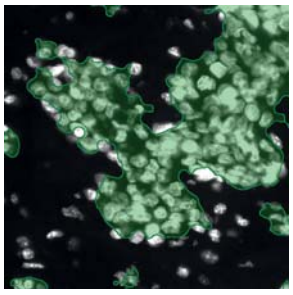


DAPI

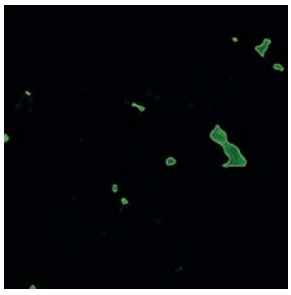


Cytokeratin (tumor)

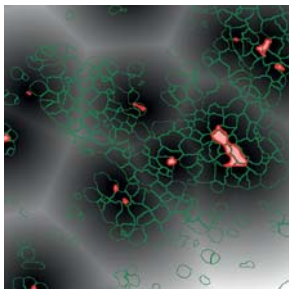
CD31 (blood vessels)



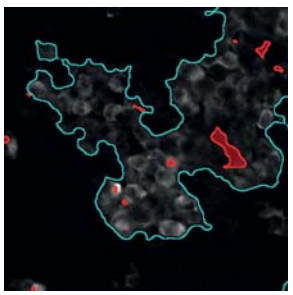
Tumor areas defined using the cytokeratin mask



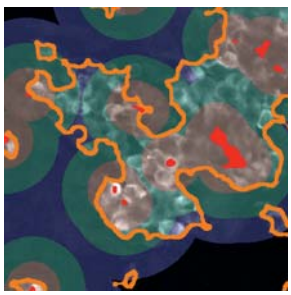
Blood vessels detected using the CD31 stain



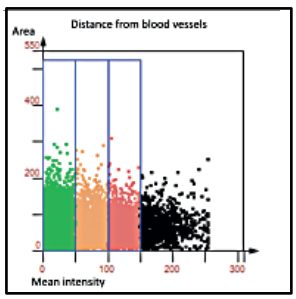
Vessels used as seeds for Distance Transformation.



Tumor groups & blood vessel overlay



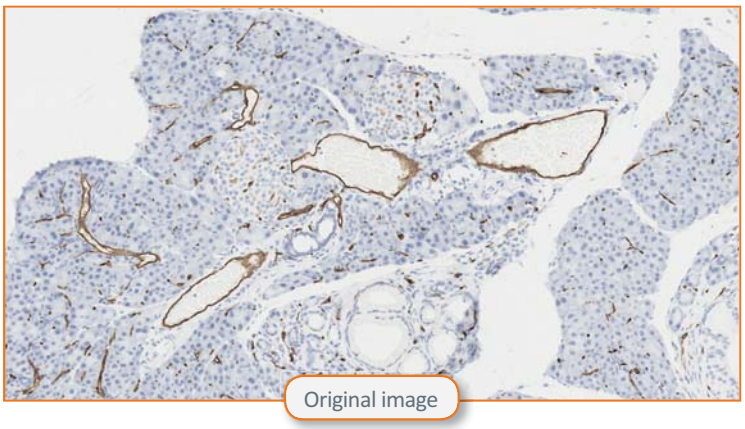
Tumor groups, blood vessels and ranges



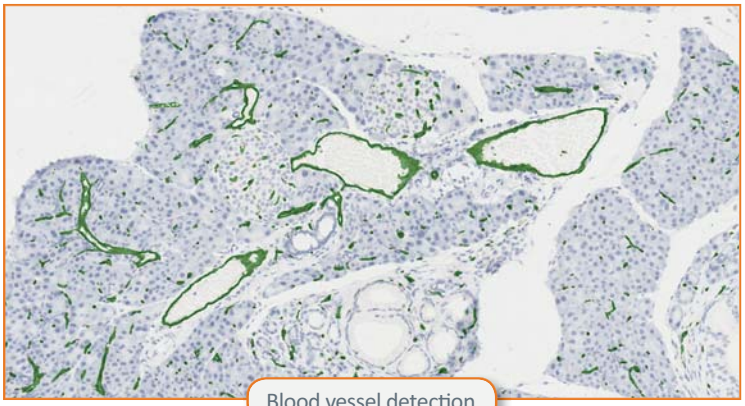
Gates for cell distances from vessels ranges

Final result - Number of tumor cells within each distance range of a blood vessel:
0-50 µm: 6.988 (52%); 50-100 µm: 3.483 (26%); 100-150 µm: 1.845 (14%)

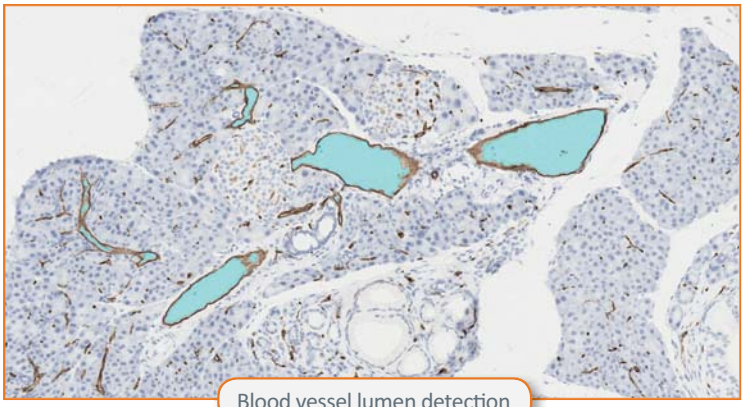
STRATAQUEST CONTEXT ANALYSIS SOFTWARE



Original image



Blood vessel detection

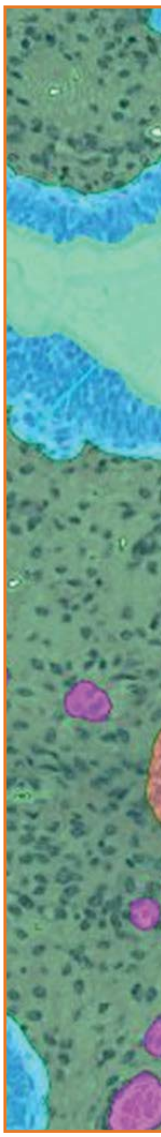


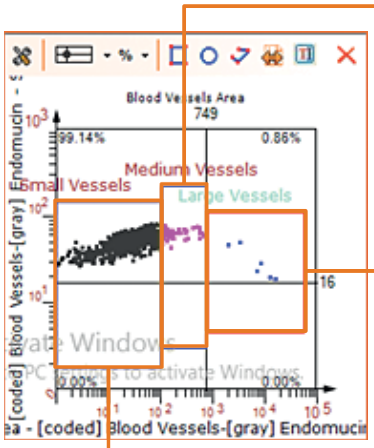
Blood vessel lumen detection

SQ ADVANCED:
Metastructure detection examples

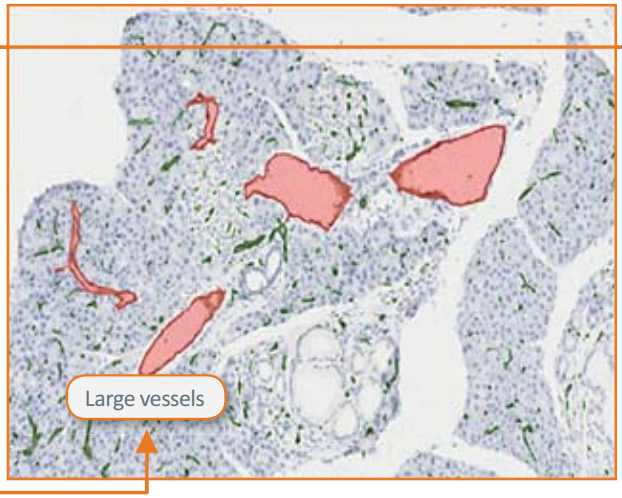
In the sample to the left blood vessels are detected on Endomucin staining. Not only are blood vessels of very different sizes detected, but also their lumina.

The image on the right shows an analysis done on tissue with endometriosis lesions. The results, detected glands (with and without lumina), blood vessels, lumina and stroma, have been obtained with different image analysis techniques and are shown in different colors.

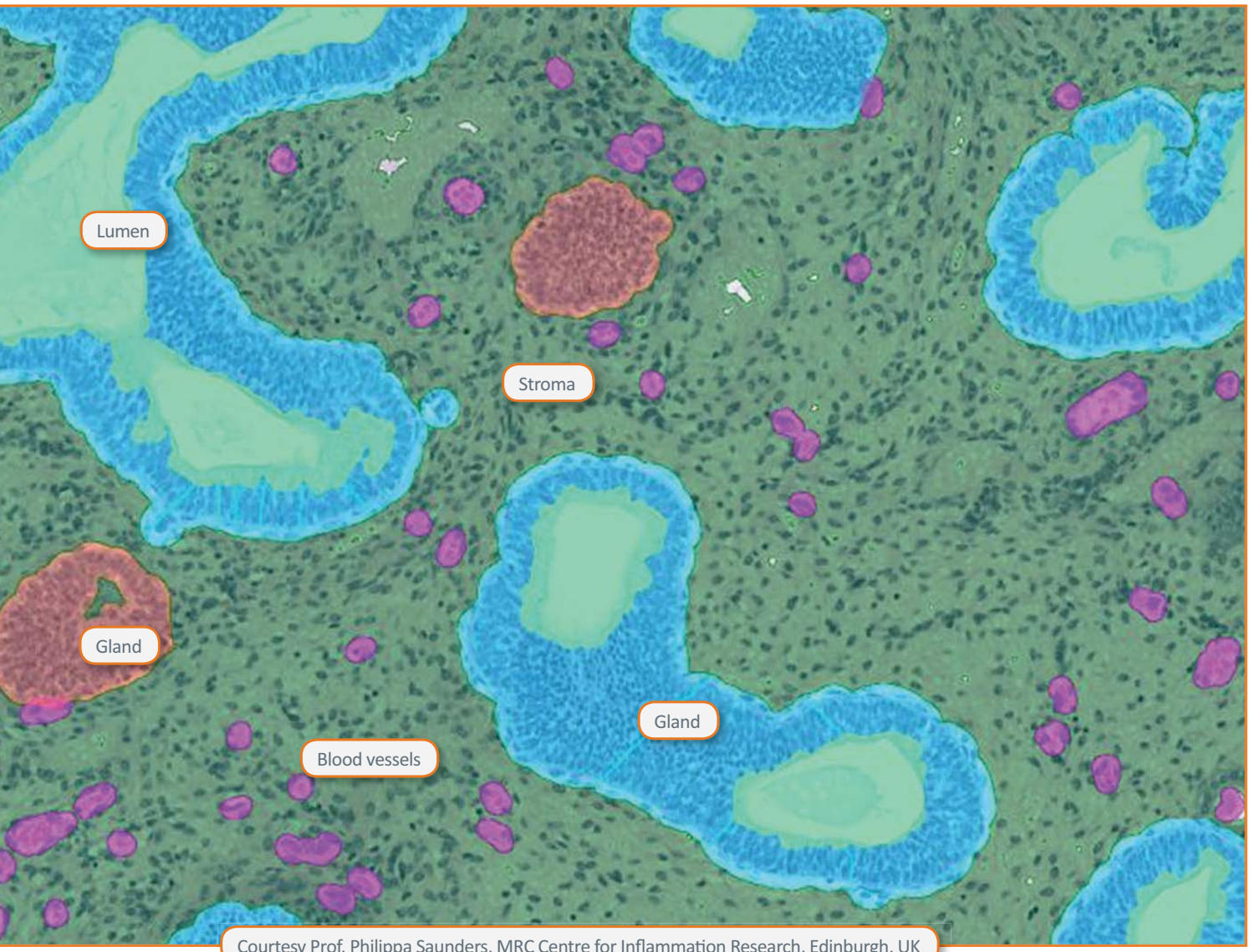




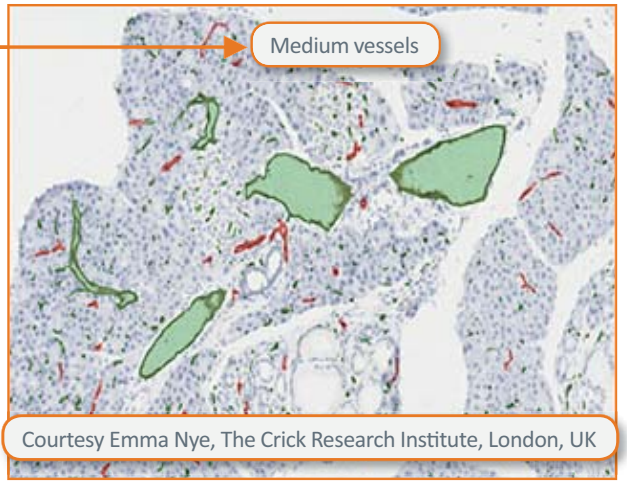
Blood vessels are easily scored by their size by using scattergrams plotting theirs area against another measurement.



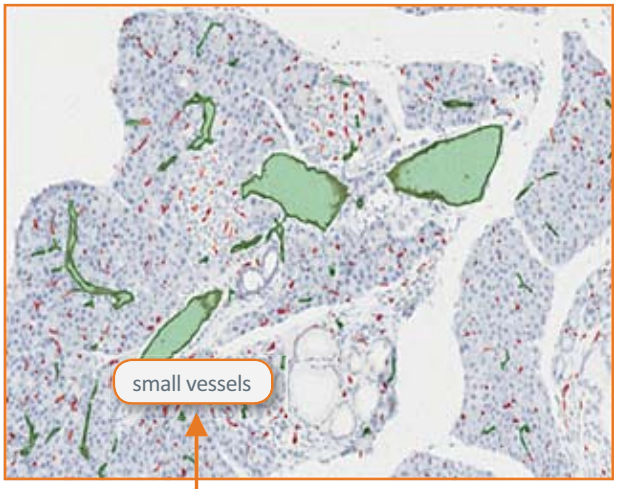
Large vessels



Courtesy Prof. Philippa Saunders, MRC Centre for Inflammation Research, Edinburgh, UK



Courtesy Emma Nye, The Crick Research Institute, London, UK

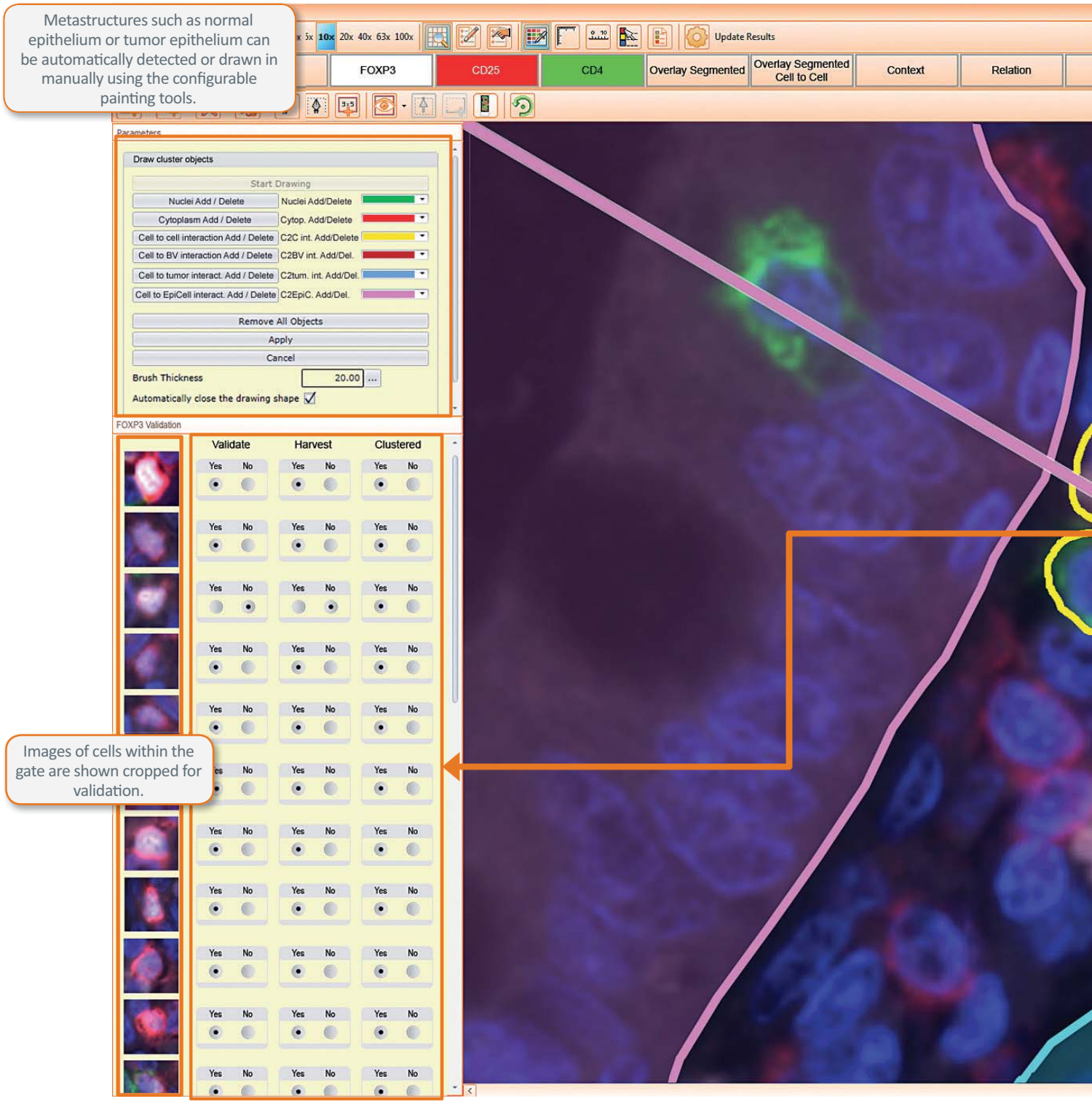


STRATAQUEST CONTEXT ANALYSIS SOFTWARE

StrataQuest Cellular Microenvironment – Extended workflow

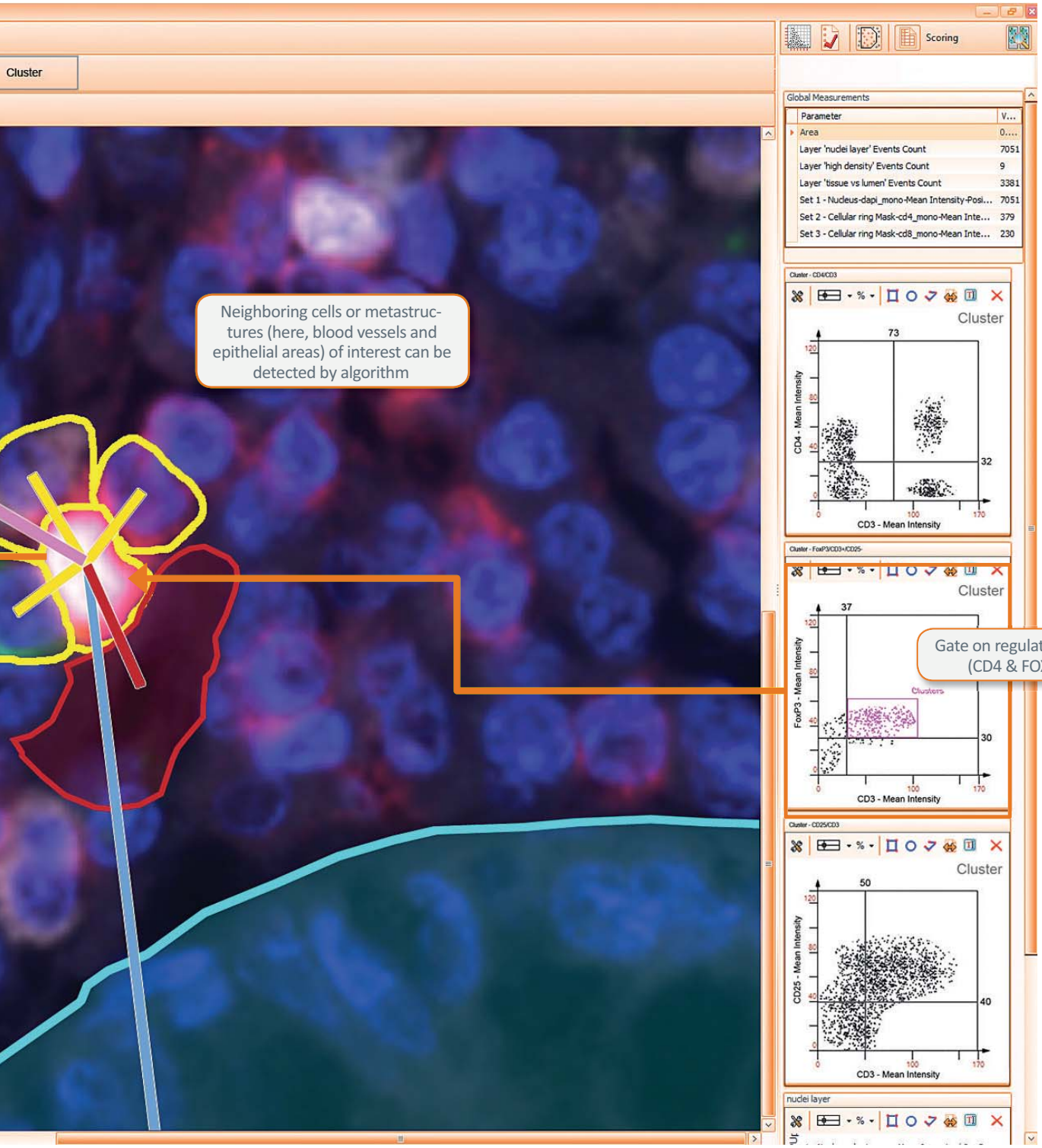
In the CELLULAR MICROENVIRONMENT APP StrataQuest provides two working modes.

The first mode is detection and spatial analysis of small cell subunits within a larger organ („clusters“in the StrataQuest nomenclature). Clusters are defined by a specific central cell and the other cluster cells by distance and optionally by specific staining.



The second mode automatically lists specific cell types for visual validation and subsequent treatment, e.g., harvesting in microdissection systems or the upcoming TissueFAXS SORT system.

The image below describes the main parts of both workflows on the basis of a colon sample stained with DAPI, and for FOXP3, CD25 and CD4.



STRATAQUEST CONTEXT ANALYSIS SOFTWARE

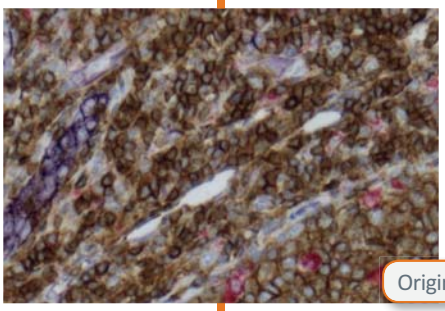
SPECTRA

MULTIPLEX? – TG SPECTRA!

StrataQuest software provides the Spectral Unmixing Engine for the TG SPECTRA technology. All TissueFAXS systems support TG SPECTRA technology by providing Brightfield and Fluorescence Multi-Channel scanning modes which scan additional Lambda Stack images for spectral unmixing.

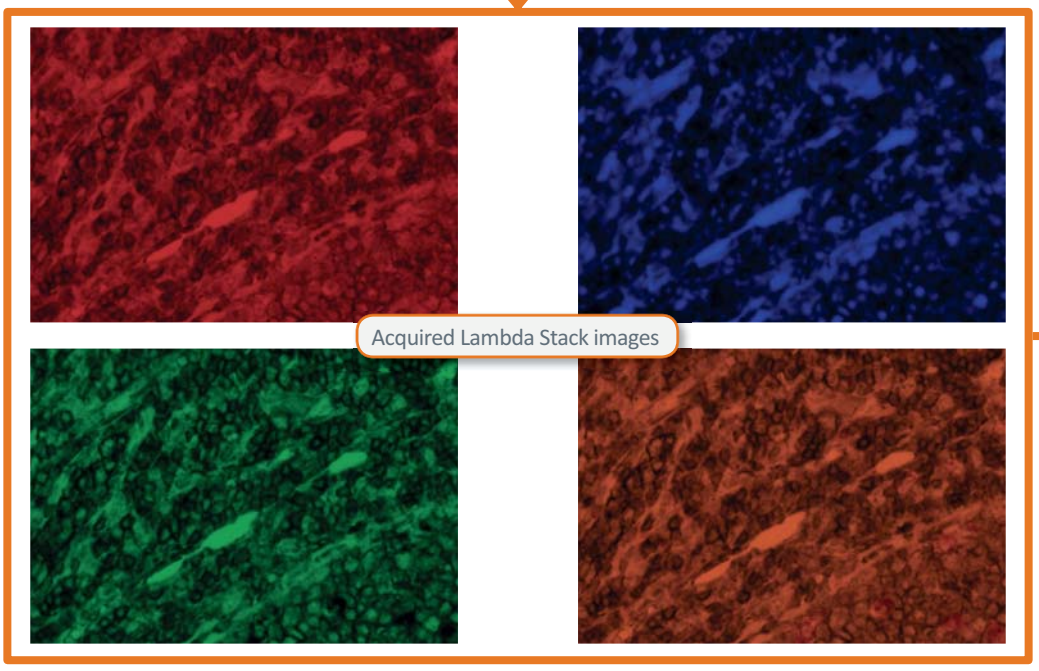
Tunable liquid crystal filter and Zyla camera

The original image of a quadruple staining is scanned in brightfield with more colour images made with a tunable liquid crystal filter.

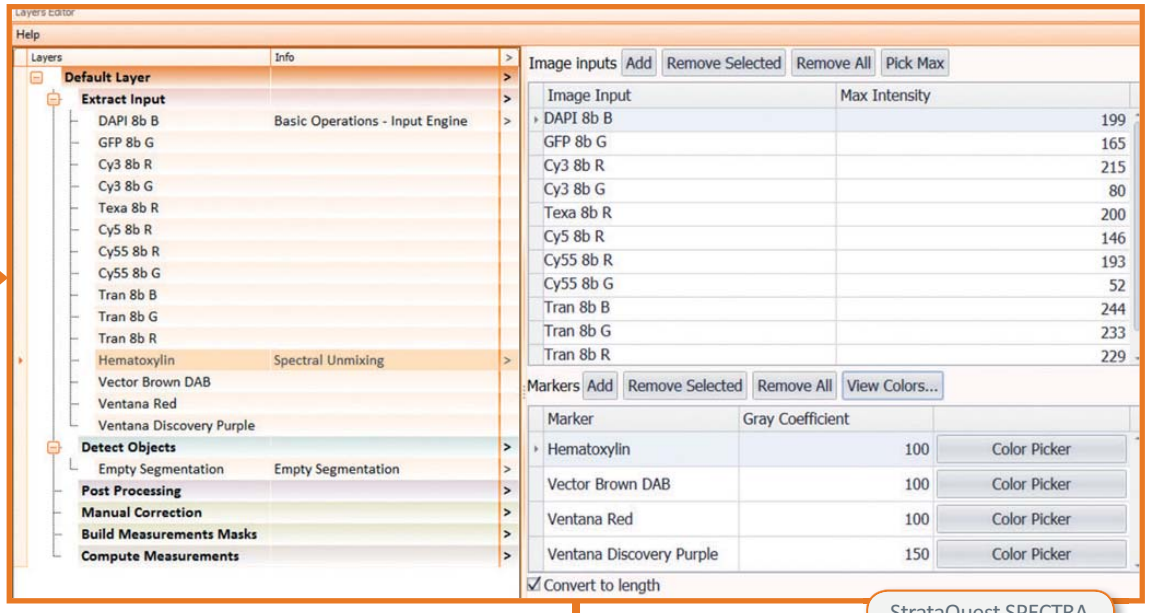


Original brightfield image

This Lambda Stack is then used for spectral unmixing in StrataQuests SPECTRA engine.



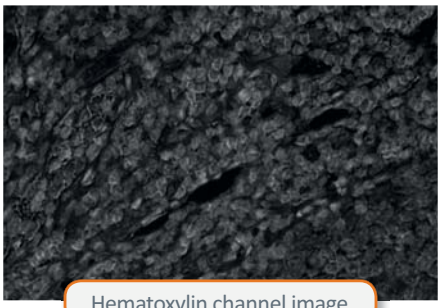
Acquired Lambda Stack images



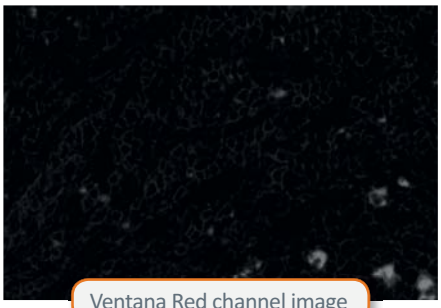
StrataQuest SPECTRA engine interface

Marker	DAPI 8b B	GFP 8b G	Cy3 8b R	Cy3 8b G	Texa 8b R	Cy5 8b R	Cy55 8b R	Cy55 8b G			Tran 8b R
Hematoxylin	158	89	93	36	106	122	172	46	173	122	119
Vector Brown DAB	55	63	117	46	116	108	146	38	75	96	134
Ventana Red	104	16	155	39	169	136	173	46	115	59	171
Ventana Discovery P	43	9	31	10	32	39	67	18	45	25	37

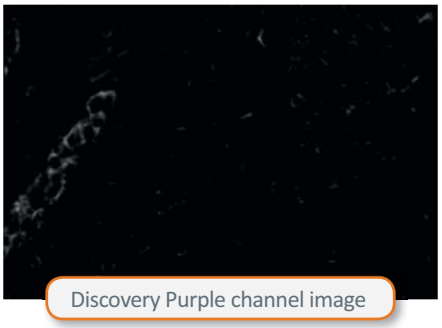
Resulting unmixed channels



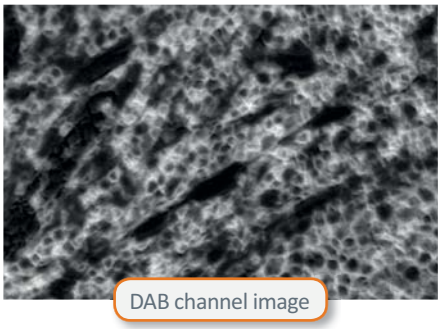
Hematoxylin channel image



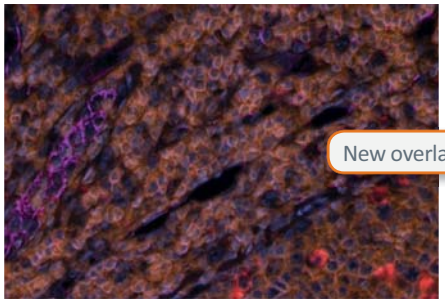
Ventana Red channel image



Discovery Purple channel image

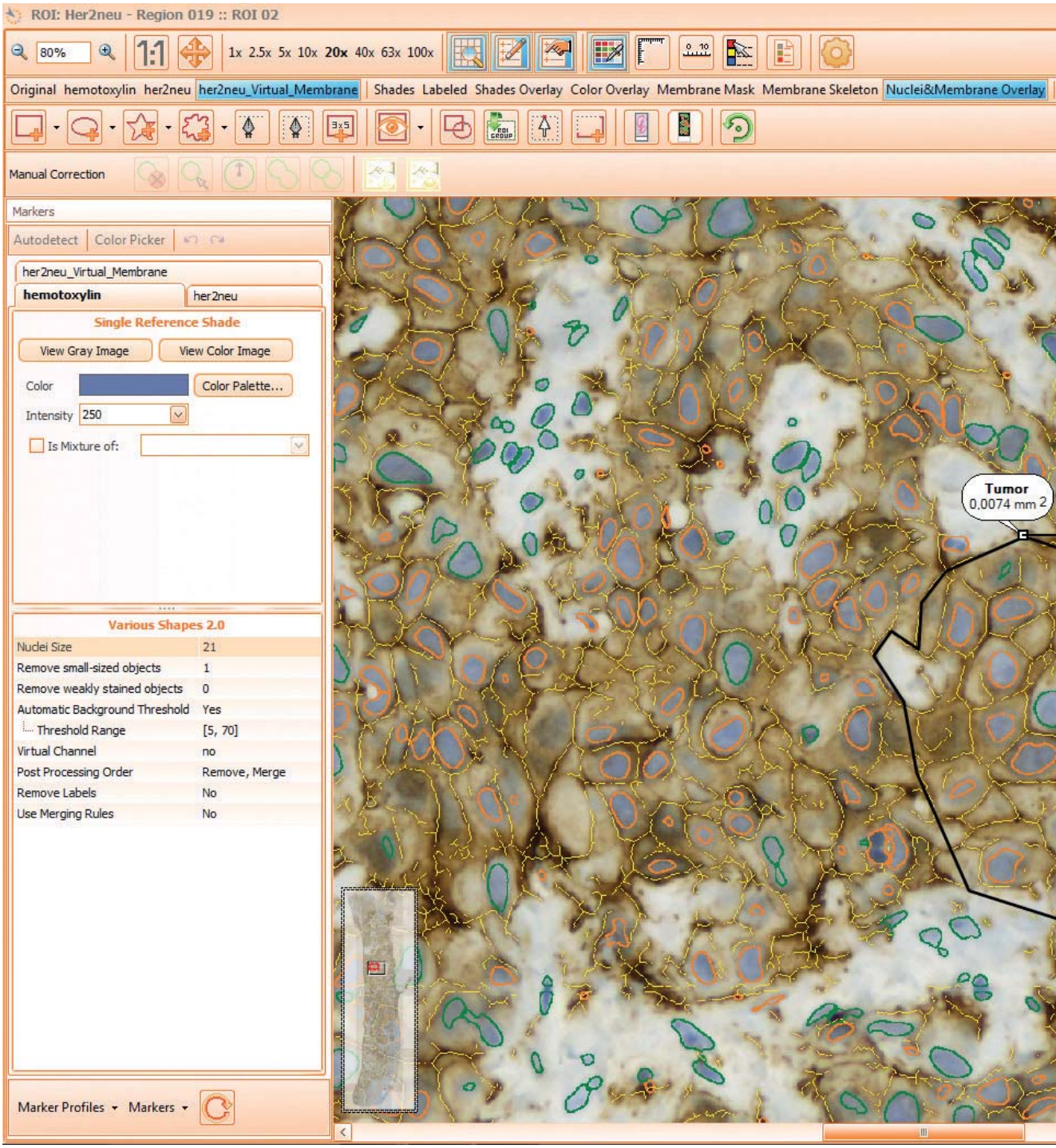


DAB channel image



New overlay composite image

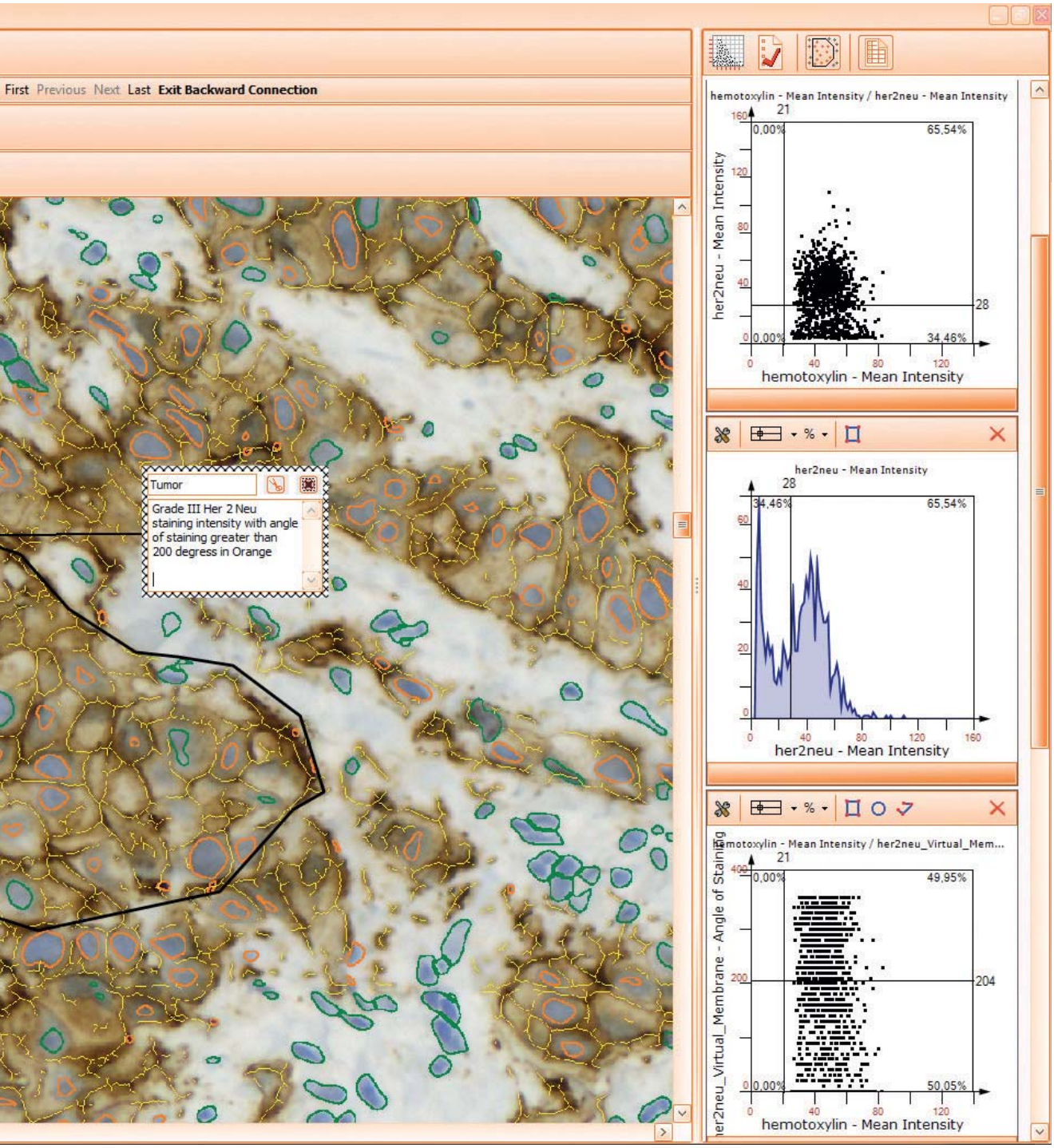
HISTOQUEST CELL ANALYSIS SOFTWARE



Breast cancer panel HER2/neu analysis, courtesy Prof. Feichtinger, Institute of Pathology, Rudolfstiftung, Vienna, Austria

HistoQuest – TGs fast-track brightfield Tissue Cytometry software

Getting introduced to HistoQuest does not take long – understanding the fundamental paradigms and running the first projects with own digital slides is easily managed in a day. Productive work can start on day two. While being a part of TGs integrated TissueFAXS systems, HistoQuest is also scanner agnostic and able to import digital slides from other scanners (see bottom of page). Given the fact that in HistoQuest (as well as in TissueQuest) tissue metastructures are drawn manually, it can also be a more cost effective solution for users that do not often require this capability.



HistoQuest image data import
Zeiss .czi, Hamamatsu .ndp, Aperio .svs, 3D Histech .mrxs , Keyence, .tiff, .jpeg, .bmp, .png (more importers can be added on demand)

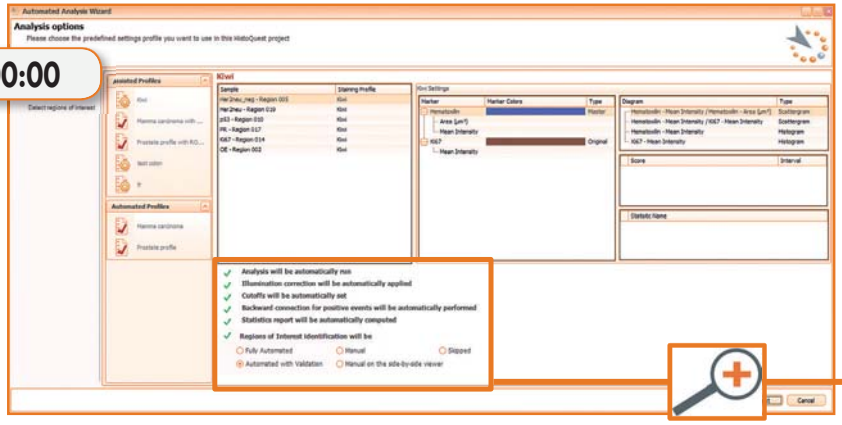
HISTOQUEST CELL ANALYSIS SOFTWARE

Just walk away.

HistoQuest provides comprehensive automation profiles for continuous analysis of samples of the same type with minimum user interaction. Profiles are established by simply saving a typical analysis project with all required options as a profile.

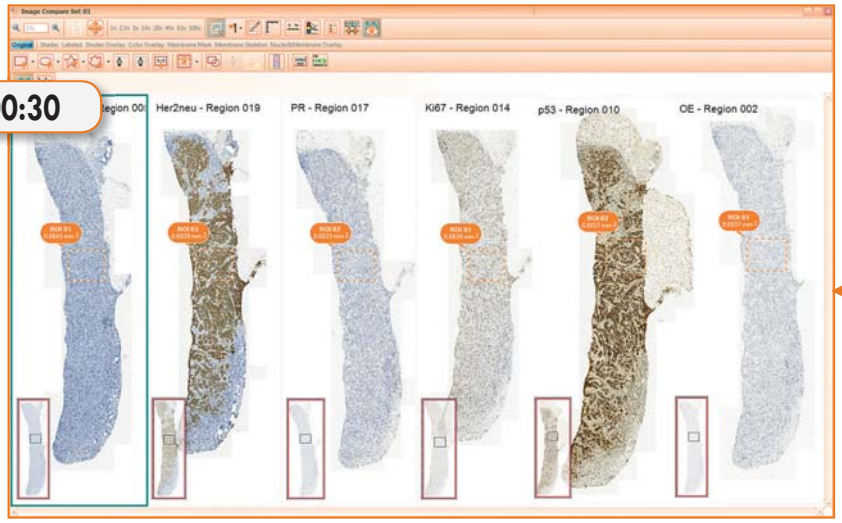
00:00:00

Start.
Analysis running on i5, 2,5 GHz, 8 GB RAM, Win 7 64 bit



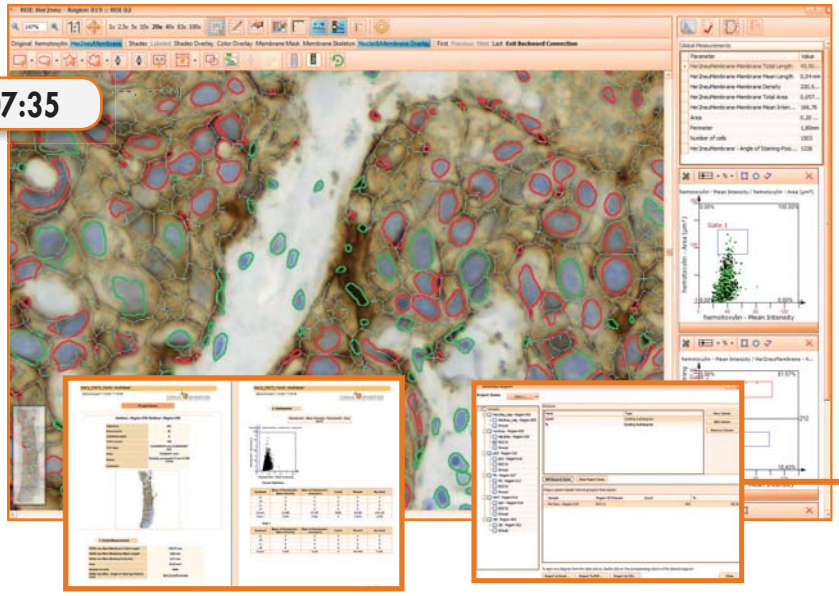
00:00:30

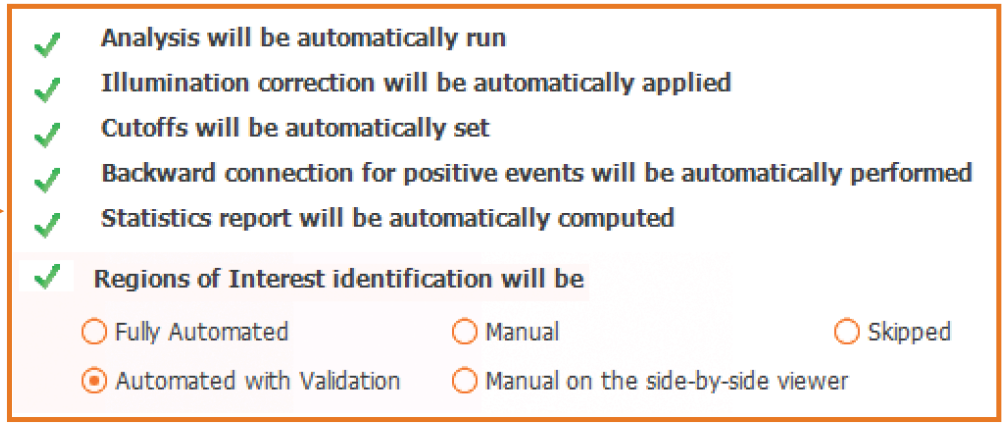
Template loaded, analysis of 6 biopsies with a rectangular region, 6 different markers, starts



00:07:35

Analysis finished, quick report and configured data export ready. Results for over 250.000 cells, 20 measurements/cell



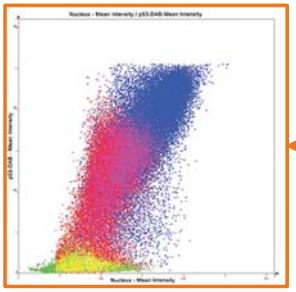


Automation profile Breast Cancer Panel

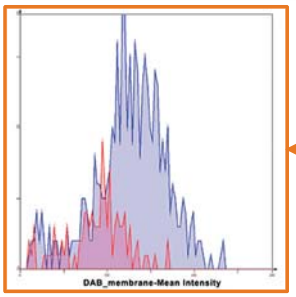
This example for fast, minimum interaction profile based Pathology Breast Panel analysis is also applicable to scientific requirements.

The biopsy consecutive section IHC stained samples are aligned with the Image Compare Set tool, a ROI drawn on one section (HE) is propagated to all of them and then analysis using the specific profile for each staining is started.

Data is automatically extracted and presented and can be exported with one click. The next sample batch is then loaded and analysed.



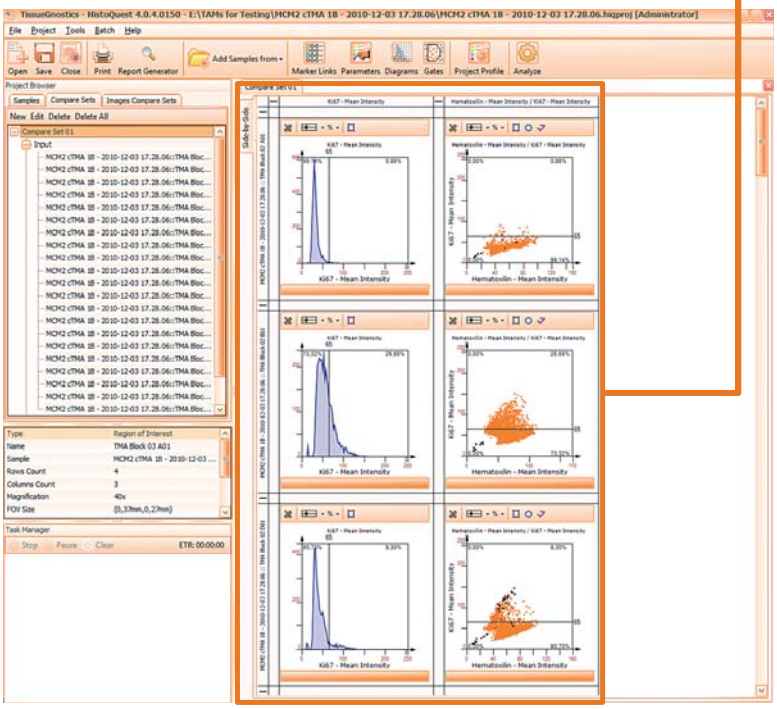
Multi-sample overlaid graphics options

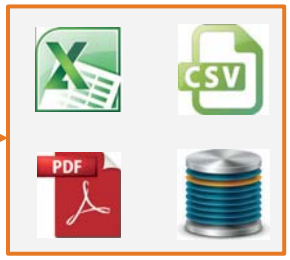


Compare Sets and Image Compare Sets

The Compare Sets tool is used to comparatively display results from multiple samples in side by side and overlaid diagrams.

Image Compare Sets is a tool for aligning consecutive sections for comparative IHC analysis or other similar applications.



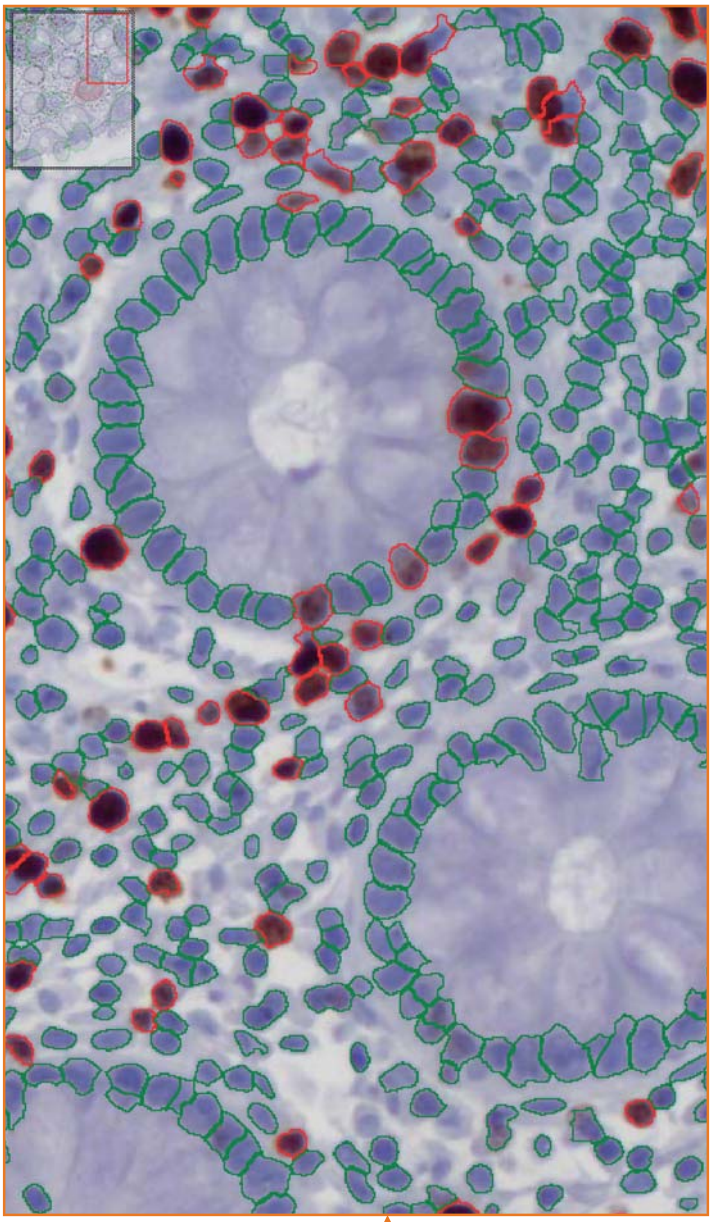


Data export

Analysis data export capabilities to Excel, CSV and PDF formats.
Data also transferrable to LIS (ASTM protocol).

HISTOQUEST CELL ANALYSIS SOFTWARE

It's a snap. HistoQuest algorithms, part I: Nuclear and Membrane



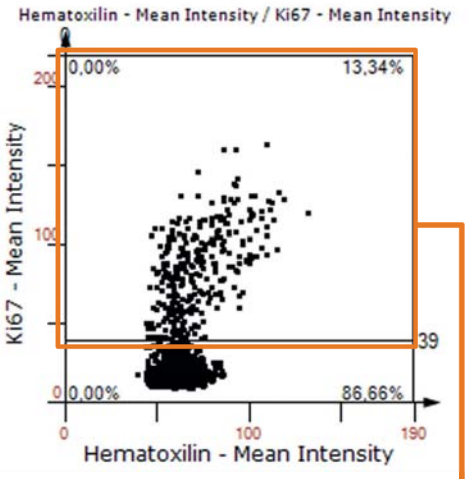
Ki-67 positive nuclei in the Upper Quadrant are displayed with red contours via HistoQuests Backward Connection feature.

HistoQuest and TissueQuest are so easy to use because all algorithms are encapsulated and only presented to the user with the necessary controls. For the nuclear detection algorithm the only essential control is a value for the nuclear size.

In this colon sample, all nuclei (contoured in green and red) are identified using this algorithm on the counterstain.

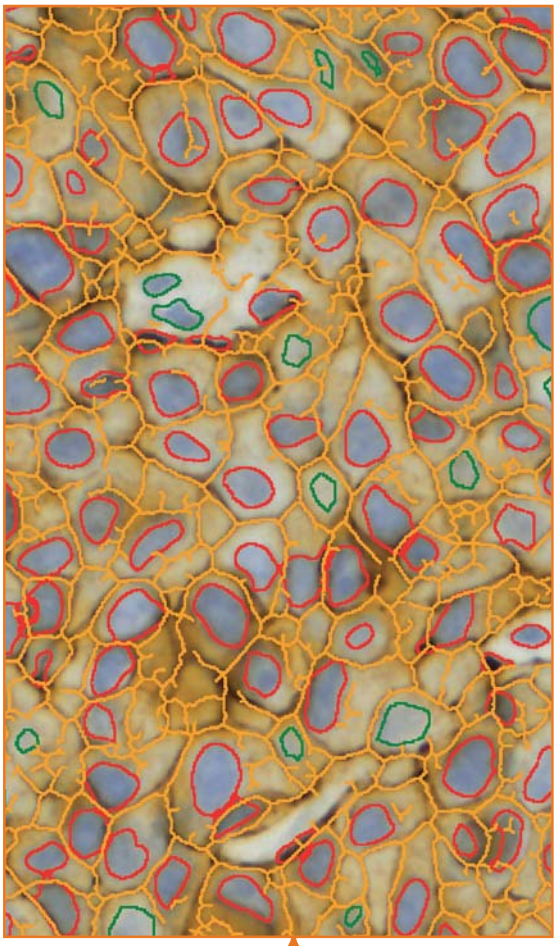
Ki-67 staining intensity is measured based on the counterstain nuclear segmentation masks.

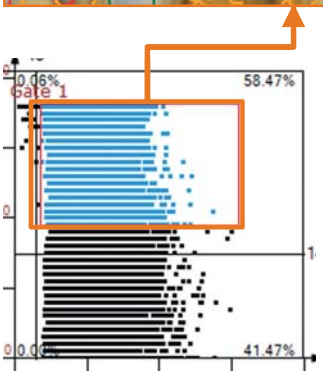
In the Tissue Cytometry workflow, scattergrams are used to plot mean intensities of Ki-67 and counterstain and a cutoff for Ki-67 is set using an automatic algorithm function.



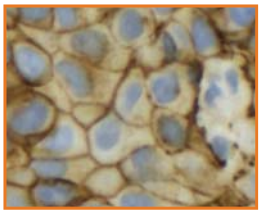
Sample	Region Of Interest	% Ki67 positive cells	% Ki-67 negative cells	Count Ki-67 positive cells	Count Ki-67 negative
Colon	Colon	13,34	86,66	308	2000

Nuclear detection and the membrane algorithm are used jointly for HER2/neu staining analysis and comparable samples.

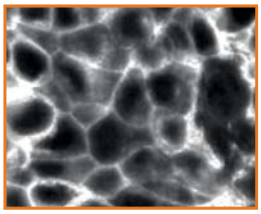




Backward gating for all cells showing more than 200° angle of staining (nuclei in red)

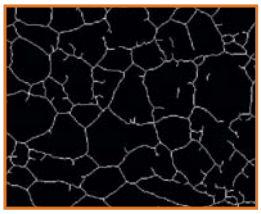


IHC staining using anti - HER2/neu and DAB

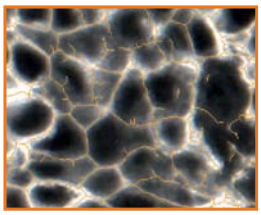


The counterstain and HER2/neu grayscale images are separated using HistoQuest automatic color deconvolution.

The HistoQuest membrane algorithm automatically detects all stained membranes.



The algorithm builds a skeleton on the membrane mask. This is used to calculate the angle of staining around nuclei.

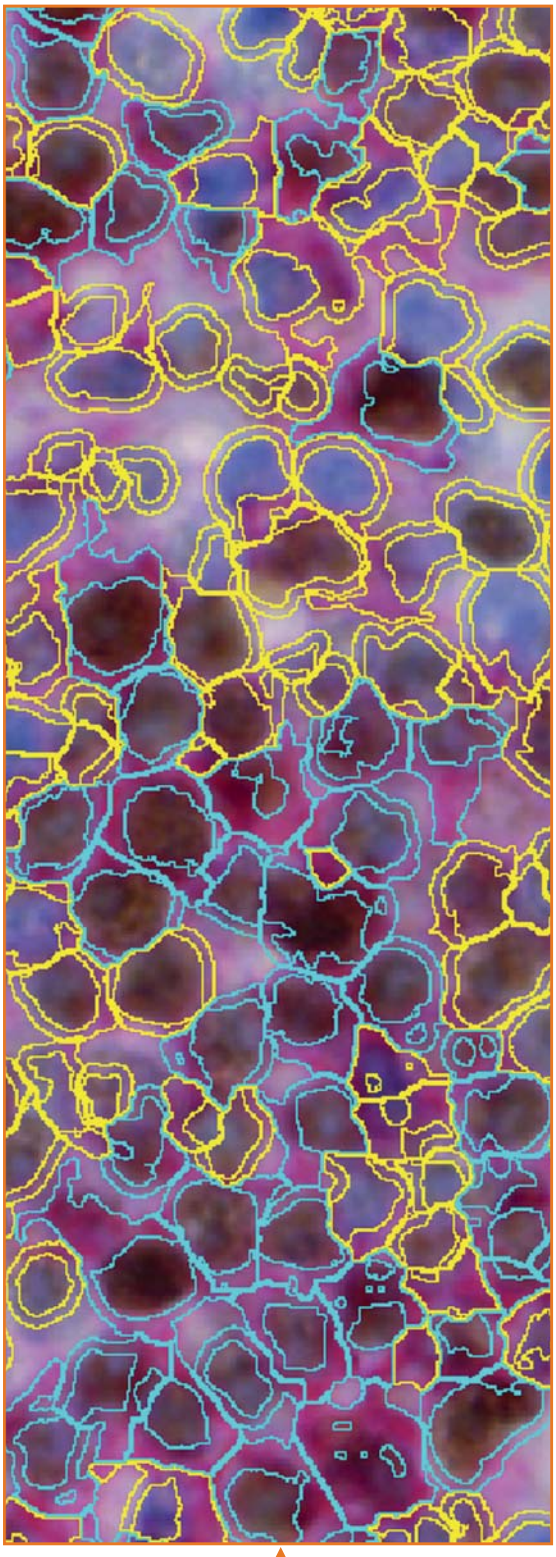


Overlay of HER2/neu shade and membrane skeleton

Sample	Region Of Interest	Membrane Density	Area (mm²)	Events Count	Membr. Pos. Cells Count	Angle of staining high
Her2/neu	ROI 06	157,73	8,15	62530	36599	58,47

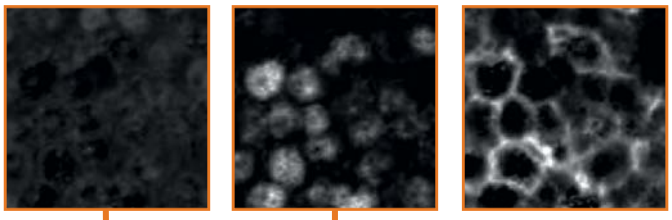
HISTOQUEST CELL ANALYSIS SOFTWARE

It's a snap. HistoQuest algorithms, part II: Nuclear and Cytoplasm; Total Area



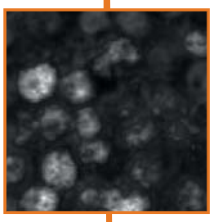
HistoQuest can easily separate three marker colours and make them available for image analysis . In this example analysis of a triple staining with two nuclear and one cytoplasmic marker is shown.

Grayscale



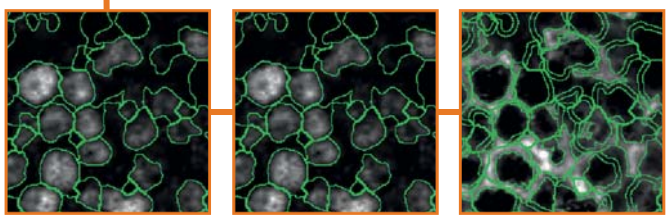
Hematoxylin | c-Myc | CD20

Virtual channel



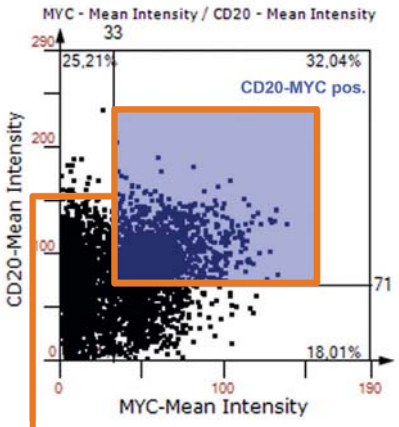
Hematoxylin being too weak for nuclear segmentation, a virtual nuclear channel with c-Myc is added. Segmentation of all channels is shown below.

Measure mask

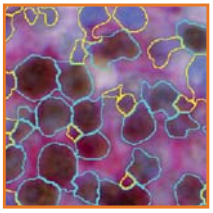


Hematoxylin | c-Myc | CD20

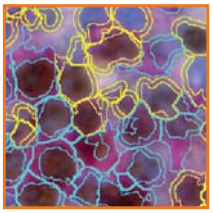
Scatterplot Myc/CD20



Setting of cutoffs for double positives



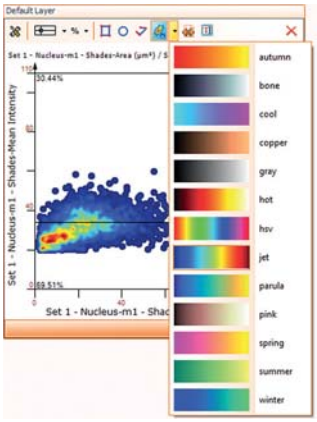
$CD20^{POS.}$/$c\text{-}Myc^{POS.}$



$CD20^{POS.}$/$c\text{-}Myc^{POS.}$

$CD20^{POS.}$/$c\text{-}Myc^{POS.}$ cells contoured in blue

Courtesy Protessor Scott Rodig, Department of Pathology, Brigham and Women's Hospital, Boston, MA. USA

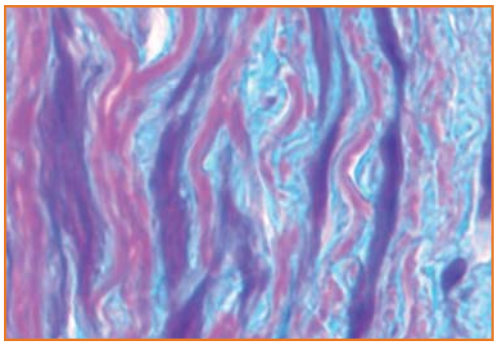


The Heatmap Scattergram feature will show areas of objects density which may not be visible in highly populated scattergrams and so deliver more information when working with them.

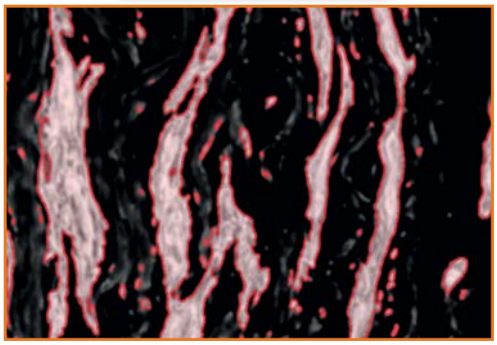
The Heatmap Scattergram option is available in all TG analysis software.

The Total Area Measurements algorithm is used to separate a technically unlimited amount of stained areas, segment them and measure size and intensity of stained objects.

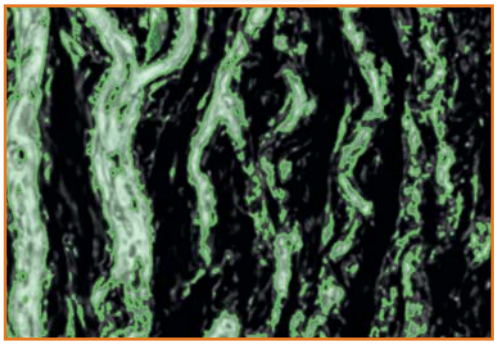
The algorithm is shown here separating parts of tissue stained with Trichrome Masson, but is equally useful to quantify, e.g. Fibrosis.



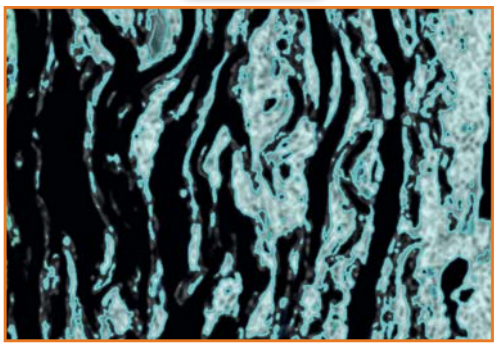
Original Trichrome staining



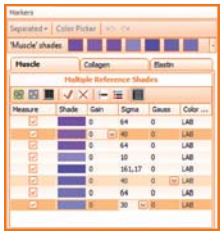
Muscle



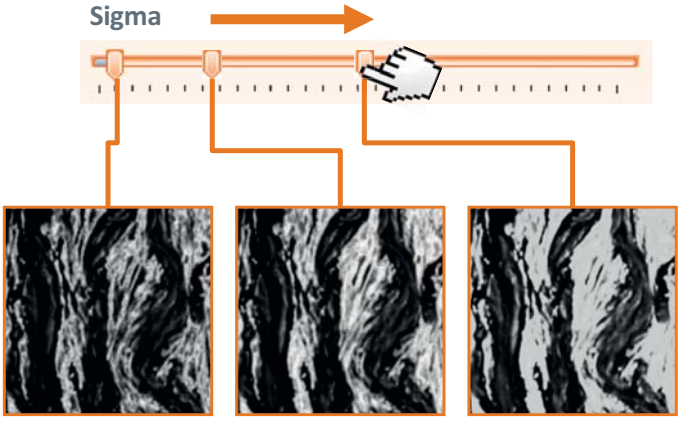
Elastin



Collagen

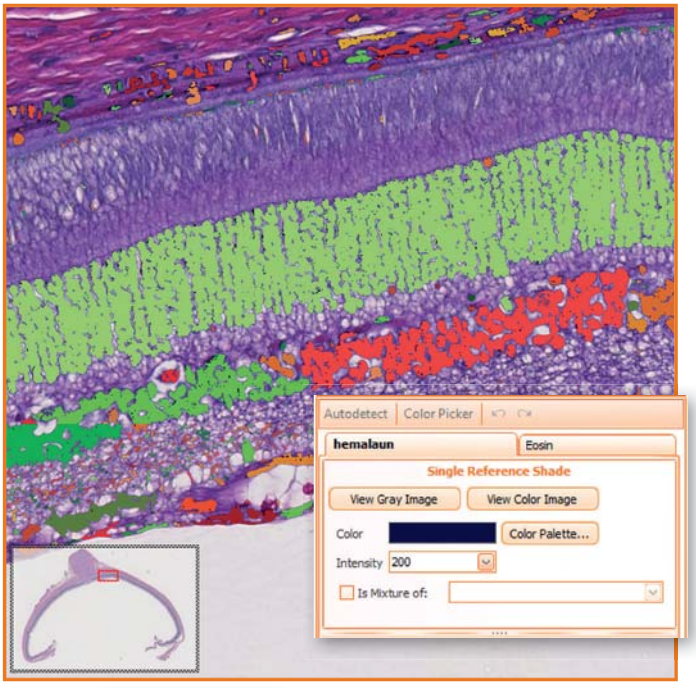


The multiple reference shades method allows for interactive grayscale channel tuning and so provides very precise results.



Muscle shade tuning

The fully automated single reference shade method can also be used for area analysis.



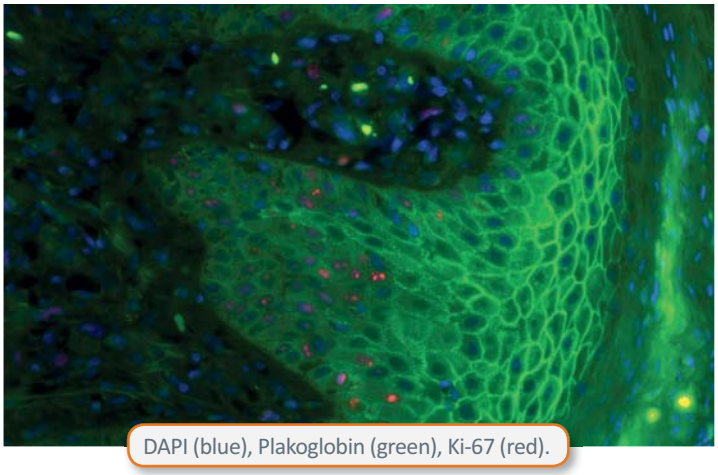
Eye retina section

TISSUEQUEST CELL ANALYSIS SOFTWARE

It's even more of a snap. TissueQuest: Versatile IF Tissue Cytometry

TissueQuest is TissueGnostics analysis software for cells and stained areas in immunofluorescence. It offers the same simple, easy workflow and algorithms as HistoQuest and is also standalone capable as well as part of TG integrated systems. As there is no need to color separate in immunofluorescence, the number of markers analysed is technically unlimited. One of the strengths of the software is the precise analysis of protein coexpression.

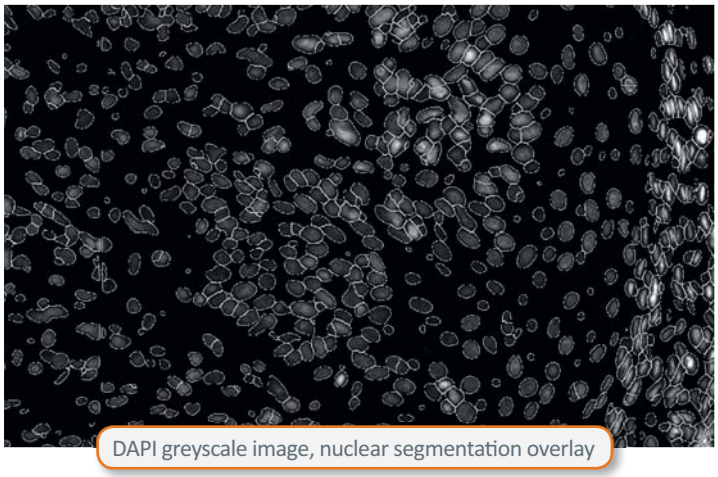
Given the fact that in TissueQuest (as well as in HistoQuest) tissue metastructures are drawn manually, it can also be a more cost effective solution for users not requiring this often.



DAPI (blue), Plakoglobin (green), Ki-67 (red).

The aim of the project shown is to quantitatively measure Ki-67 expression and to calculate the percentage of Ki-67 positive cells in the epidermis.

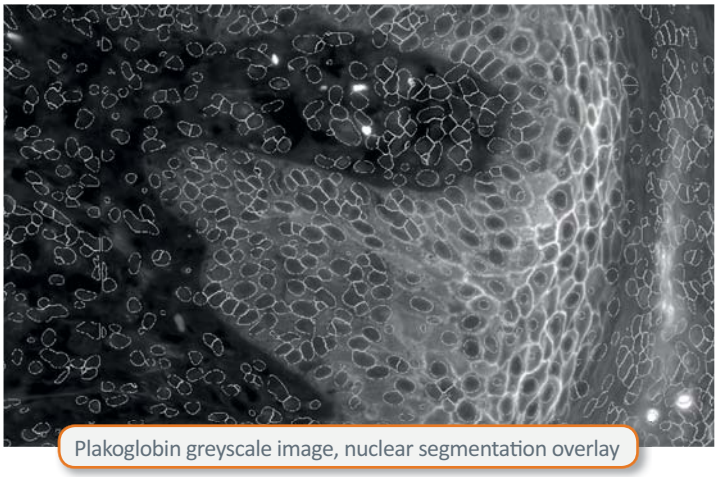
This could be achieved by using the manual region drawing tools available in all TissueGnostics analysis software to draw in the epidermis.
However, the situation allows for a more elegant approach.



DAPI greyscale image, nuclear segmentation overlay

The colocalization analysis of Ki-67 and Plakoglobin in this case can be used instead of manually drawn regions.

The first step is setting up the nuclear segmentation algorithm on the DAPI channel.



Plakoglobin greyscale image, nuclear segmentation overlay

Masks for nuclear, cytoplasmic or membrane measurements can be set up in the other, non-nuclear channels.

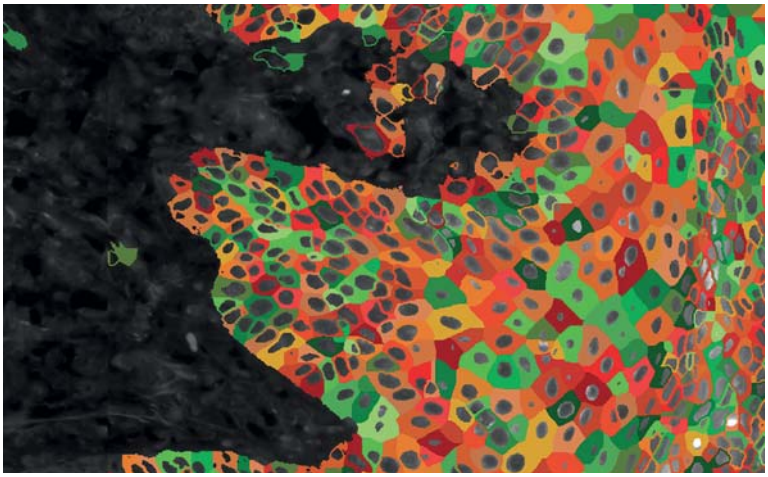
The mask for the Plakoglobin channel is set up for cytoplasmic measurement.

This way, the keratinocyte cell layers of the epidermis are reconstructed.

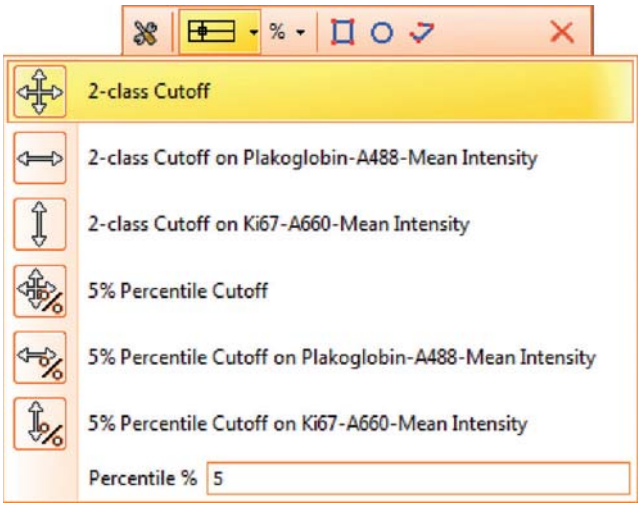
High positive cells for Plakoglobin are almost exclusively found there.

For measurement data on Ki-67 positive cells in the epidermis an automatically generated scattergram is used.

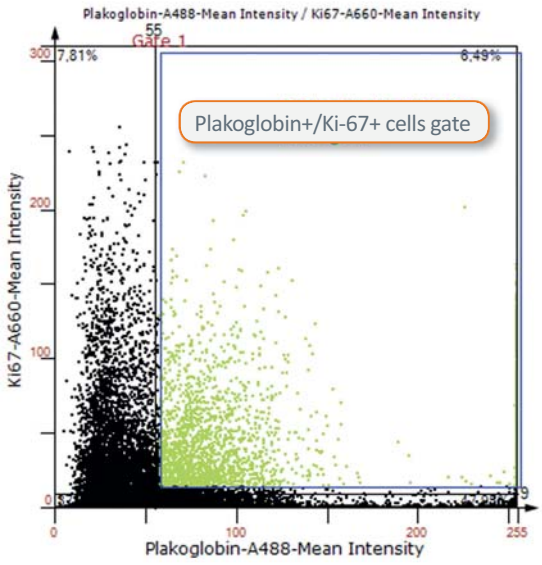
In it Plakoglobin mean intensity is plotted against Ki-67 mean intensity, with cutoffs for positive events for both markers set by an automatic algorithm, available in all TissueGnostics analysis software.



Plakoglobin channel image, backward gating for + cells, labeled overlay

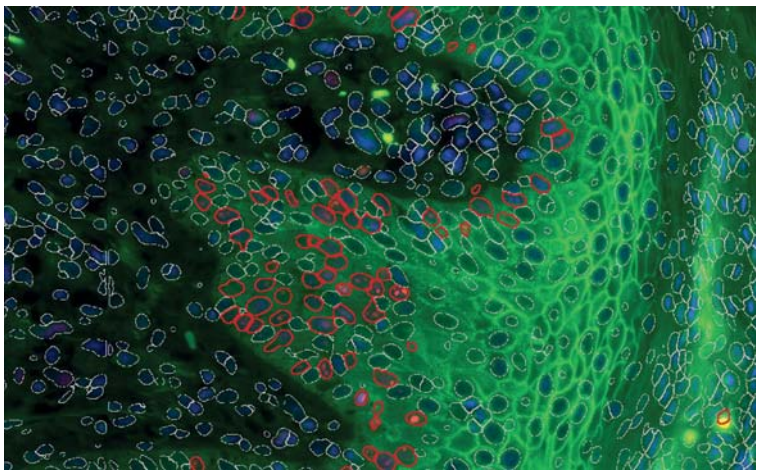


Auto-cutoff option selection menu



The 2 class-cutoff displays events positive for Ki-67 and Plakoglobin in the Upper Right scattergram quadrant.

Backward Gating on this quadrant shows the double positive events in it with red contours in the image.



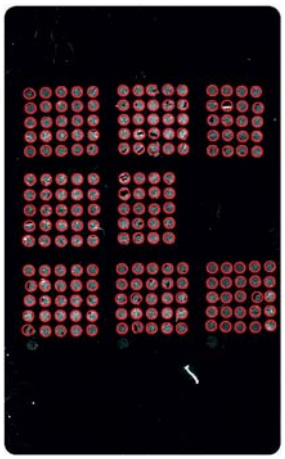
Backward gating for Plakoglobin+/Ki-67+ colocalisation

TissueQuest image data import
Zeiss .czi, Hamamatsu .ndp, Aperio .svs, 3D Histech .mrxs, Keyence, .tiff, .jpeg, .bmp, .png
(more importers can be added on demand)

TISSUEQUEST CELL ANALYSIS SOFTWARE

It's even more of a snap. TissueQuest: Versatile IF Tissue Cytometry

TissueQuests versatility extends to the analysis of Tissue Microarrays (TMA), cell cultures, small dots (e.g. FISH) and total area measurement. TMA analysis is available in all TG analysis software.

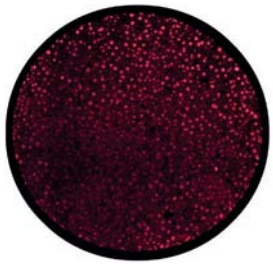


Fluorescence TMA

TissueQuest can either analyze TMA spots based on core detection of TissueGnostics TissueFAXS scanning software or the onboard core detection module can be used.

Overlay image

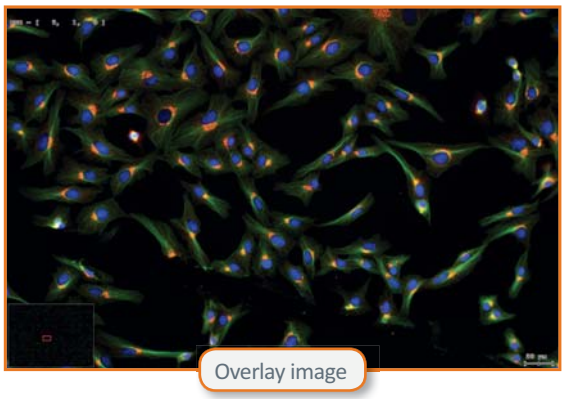
DAPI



Cy3

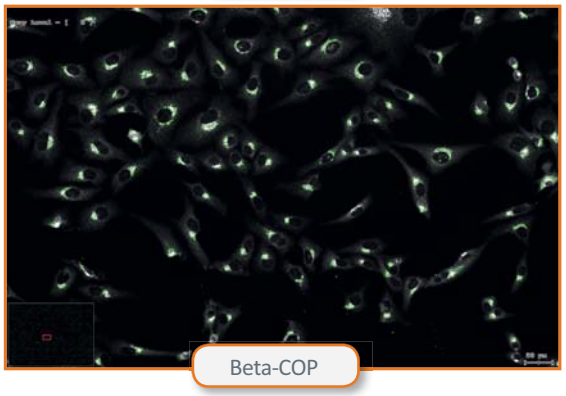
HeLa cells in culture

TissueQuest calculation is very fast. The HeLa cell culture example stained for DAPI, Beta-COP and Tubulin below was analyzed in 57,7 seconds (Intel Core i5, 2,5 GHz), with results (18 measured parameters per object & channel) for 10.947 cells.

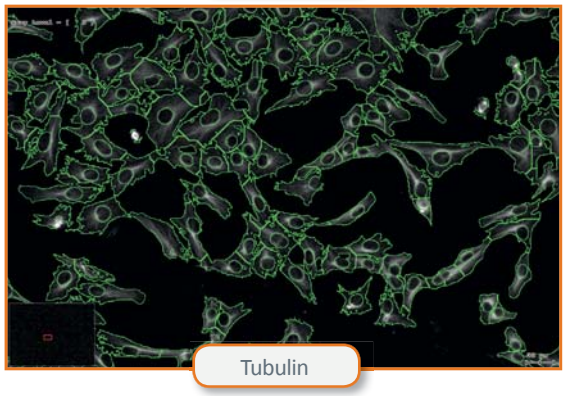


Overlay image

DAPI



Beta-COP



Tubulin

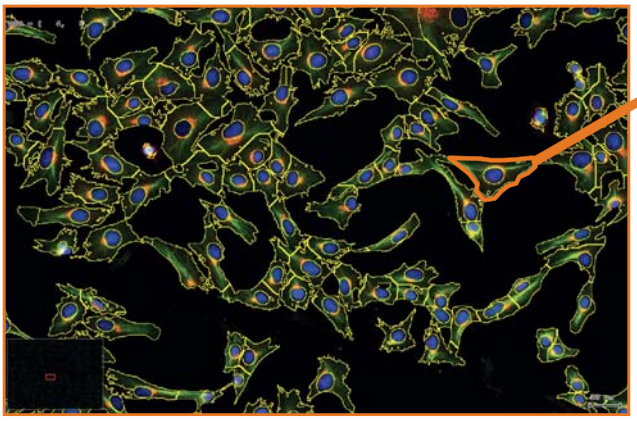
Measurement parameters

TissueGnostics analysis software measures up to 18 parameters for every single detected cellular event and for each of its marker channels.

However, if not all parameters are needed, the parameters to be calculated can be selected.

Calculating fewer parameters will use less RAM and speed up calculation.

Measured parameters can be discretely displayed on right click in an Event Data window for any object in the sample.



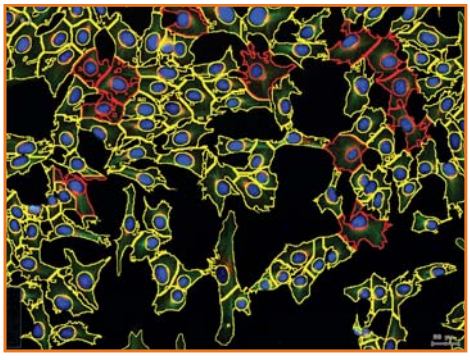
Tubulin segementation on Overlay image

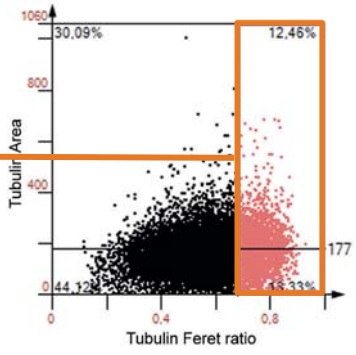
Event Data

Property	Value
Fov Id	000055
Event label	58
49 DAPI-Area (µm²)	51,85883
49 DAPI-Mean Intensity	104,2403
49 DAPI-Minimum of Intensity	0
49 DAPI-Maximum of Intensity	211
49 DAPI-Range of Intensity	211
49 DAPI-Sum Intensity	64212
49 DAPI- 25% Percentile	56
49 DAPI- 25% Percentile - Lower Mean	14,92857
49 DAPI- 25% Percentile - Upper Mean	134,0108
49 DAPI-Variance of Intensity	3639,595
49 DAPI-STD of Intensity	60,32906
49 DAPI-Equivalent Diameter (µm)	8,126185
49 DAPI-Perimeter (µm)	26,64859
49 DAPI-Compactness	0,9177511
49 DAPI-Eccentricity	0,5835705
49 DAPI-Minimum Width (µm)	7,227005
49 DAPI-Maximum Length (µm)	8,877266
49 DAPI-Feret Ratio	0,8141026
20 Rhodamine-Area (µm²)	16,07961
20 Rhodamine-Mean Intensity	167,534
20 Rhodamine-Minimum of Intensity	89
20 Rhodamine-Maximum of Intensity	255
20 Rhodamine-Range of Intensity	166
20 Rhodamine-Sum Intensity	31999
20 Rhodamine- 25% Percentile	118
20 Rhodamine- 25% Percentile - Lowe...	102,1702
20 Rhodamine- 25% Percentile - Uppe...	188,8681
20 Rhodamine-Variance of Intensity	2754,416
20 Rhodamine-STD of Intensity	52,48253
20 Rhodamine-Equivalent Diameter (µm)	4,524942
20 Rhodamine-Perimeter (µm)	29,5897
20 Rhodamine-Compactness	0,2308047
20 Rhodamine-Eccentricity	0,8750476
20 Rhodamine-Minimum Width (µm)	7,187836
20 Rhodamine-Maximum Length (µm)	11,04906
20 Rhodamine-Feret Ratio	0,6505383
10 Alexa Fluor 488-Area (µm²)	290,1906
10 Alexa Fluor 488-Mean Intensity	46,9736
10 Alexa Fluor 488-Minimum of Intensity	11
10 Alexa Fluor 488-Maximum of Inten...	182
10 Alexa Fluor 488-Range of Intensity	171
10 Alexa Fluor 488-Sum Intensity	161918
10 Alexa Fluor 488- 25% Percentile	25
10 Alexa Fluor 488- 25% Percentile - ...	19,18699
10 Alexa Fluor 488- 25% Percentile - ...	56,22506

The measured parameters can be grouped as follows:
Intensity parameters
Statistical parameters
Morphometric parameters

Morphometric parameters like Compactness allow objects to be gated based on their morphometry (On non-elongated cells in the image to the right).





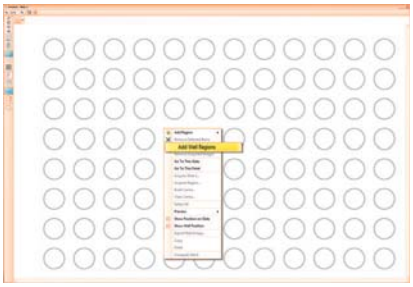
Perimeter

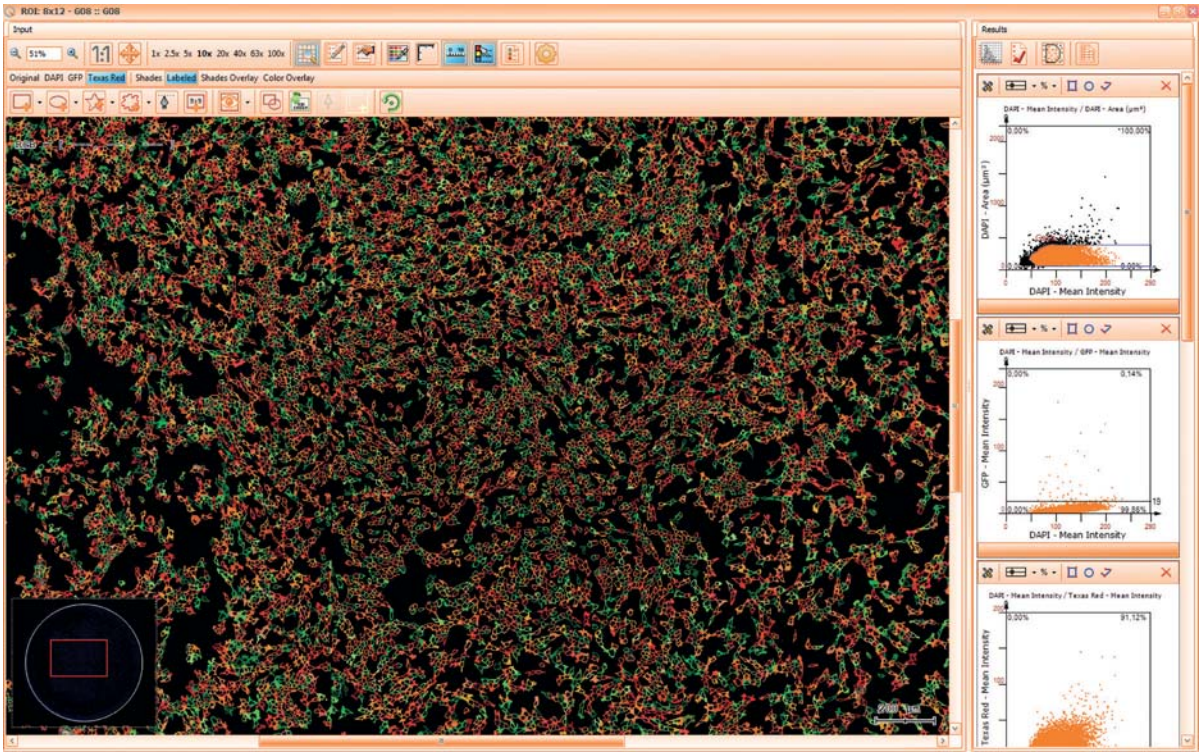
Feret Axes

TG ANALYSIS SW FEATURES

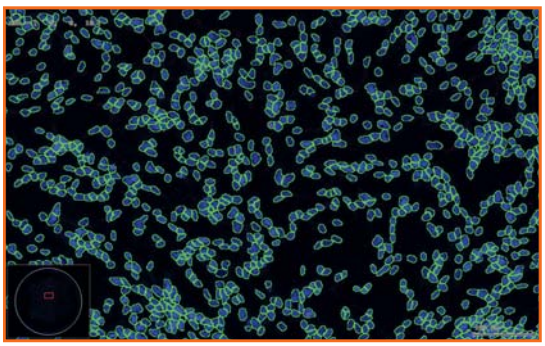
Microtiter plate analysis

Scanned projects with well content from TissueFAXS i systems can be analysed in TissueQuest, HistoQuest and Strataquest analysis software.

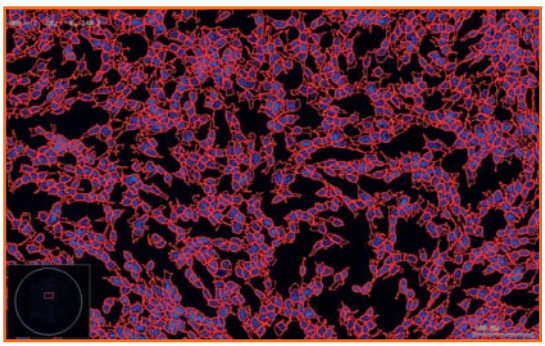




Whole well analysis, Texas Red channel, labeled view

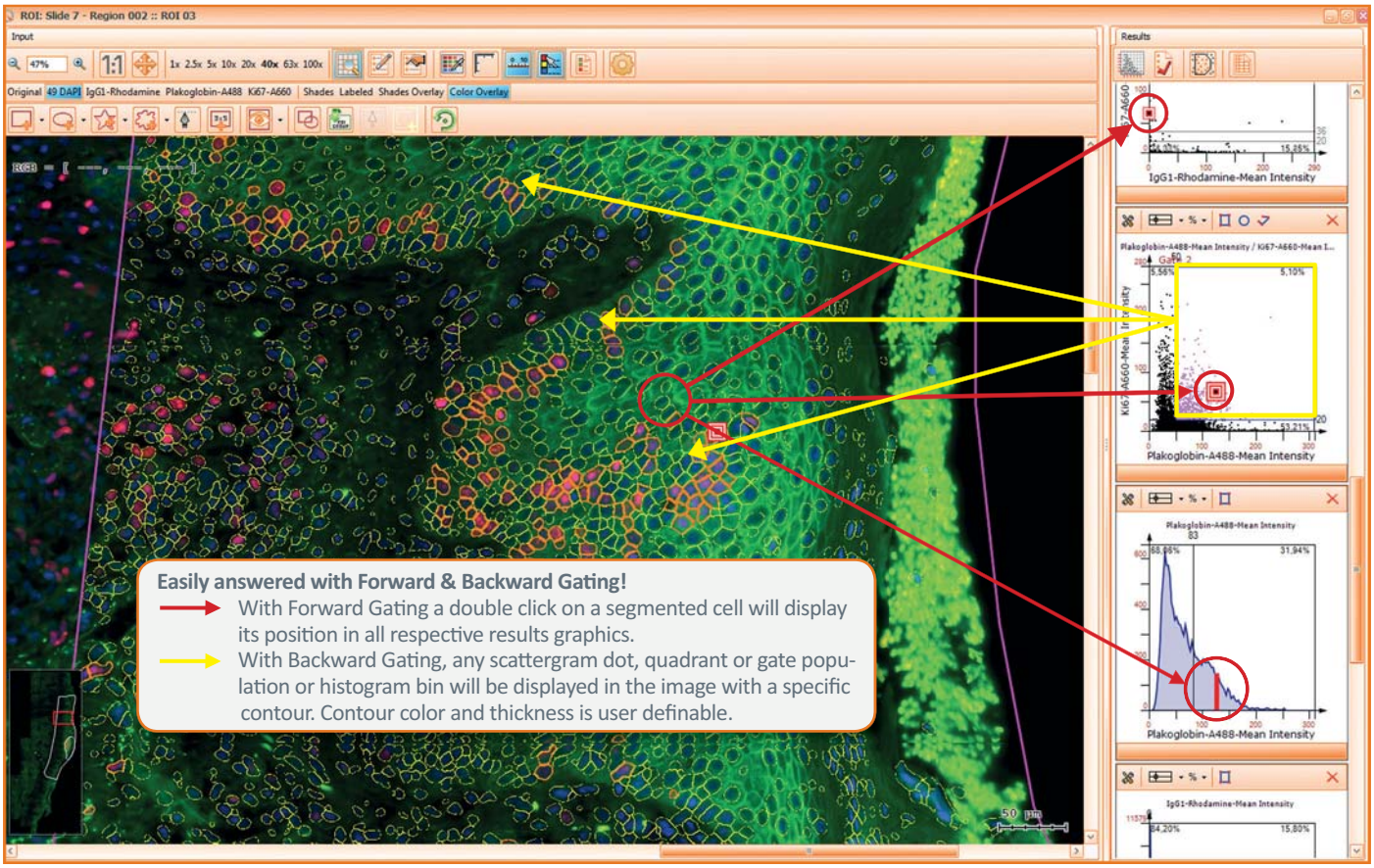


DAPI channel



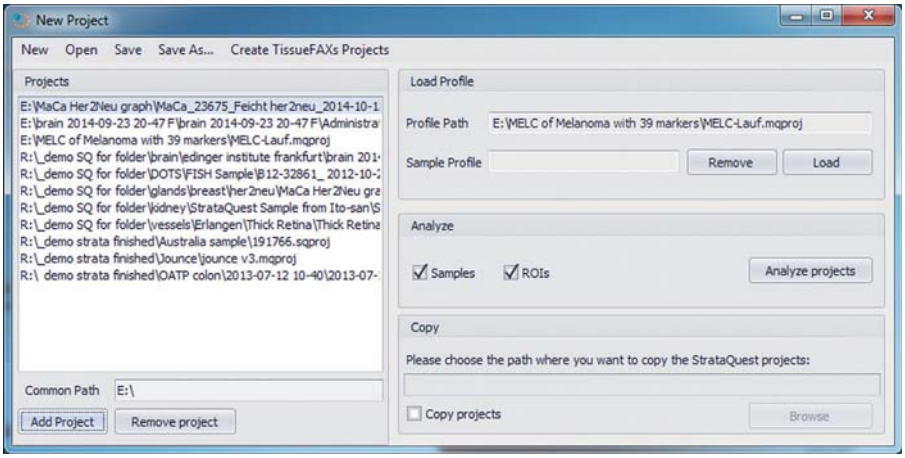
Texas Red channel

So what cells are we talking about exactly here?



Batch mode available

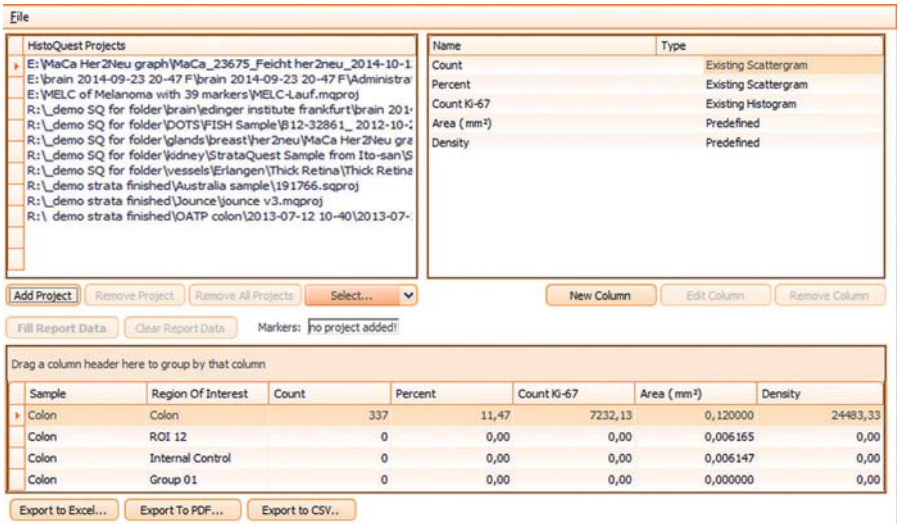
The Worker tool is integral to all TG analysis software. It lets the user group all samples of a given project into one set to be automatically analyzed.



Get your data out

The Statistics tool provides batch cross-project data export for TissueGnostics analysis software.

Values for export can be defined and exported into Excel, CSV or pdf format.



TG ANALYSIS SW FEATURES

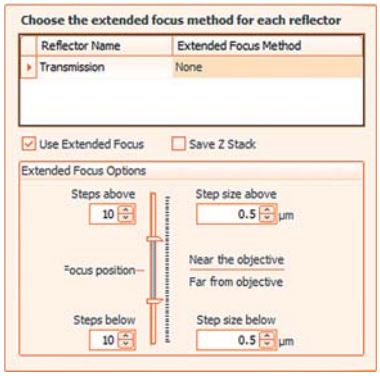
On the dot – TissueGnostics dot structure workflow

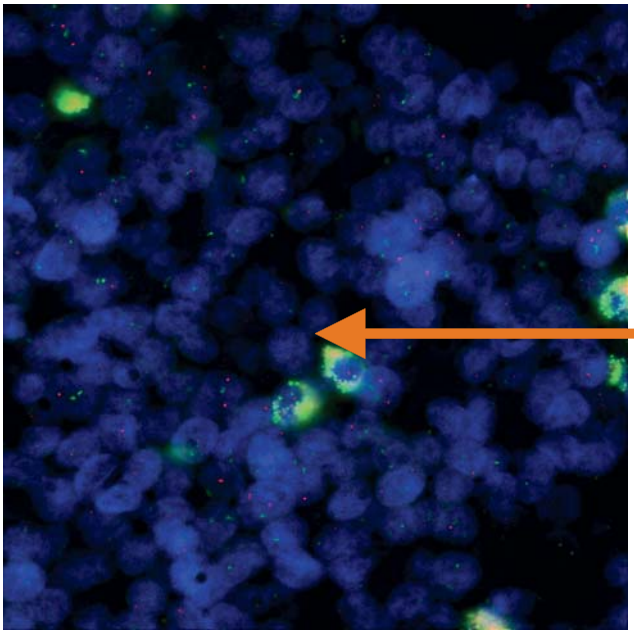
TissueGnostics analysis software provides a dedicated workflow for FISH, CISH and dot structure analysis.

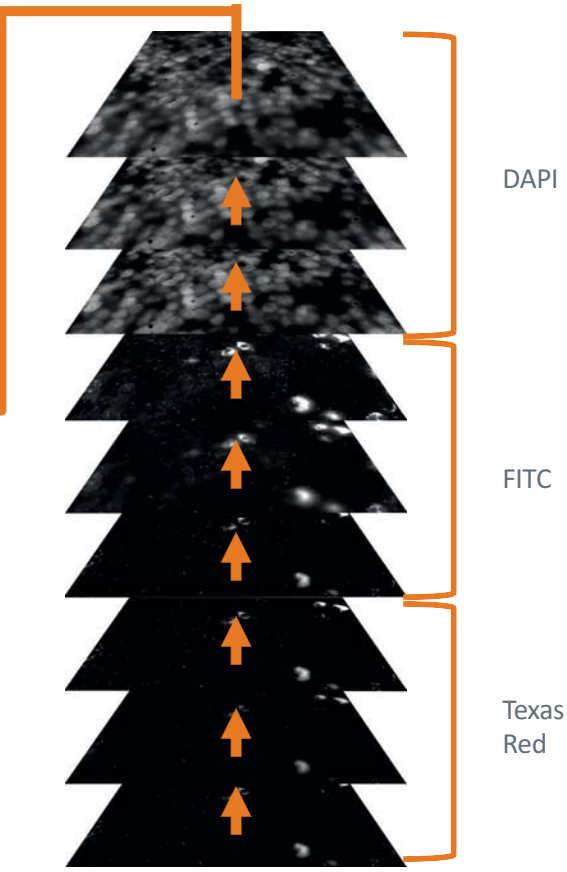
Dot analysis is an algorithm in TissueQuest and HistoQuest and an ENGINE in StrataQuest.

Dot size objects can be analysed for area, intensity and marker positivity.

The precision scanning necessary for routine FISH analysis is attained by using the TissueFAXS Z-stacking and Extended Focus Image function.

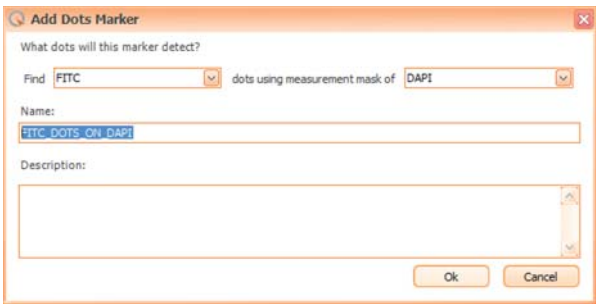






The function allows users to save either the Extended Focus Images alone, or together with all stack images, or the stack images only.

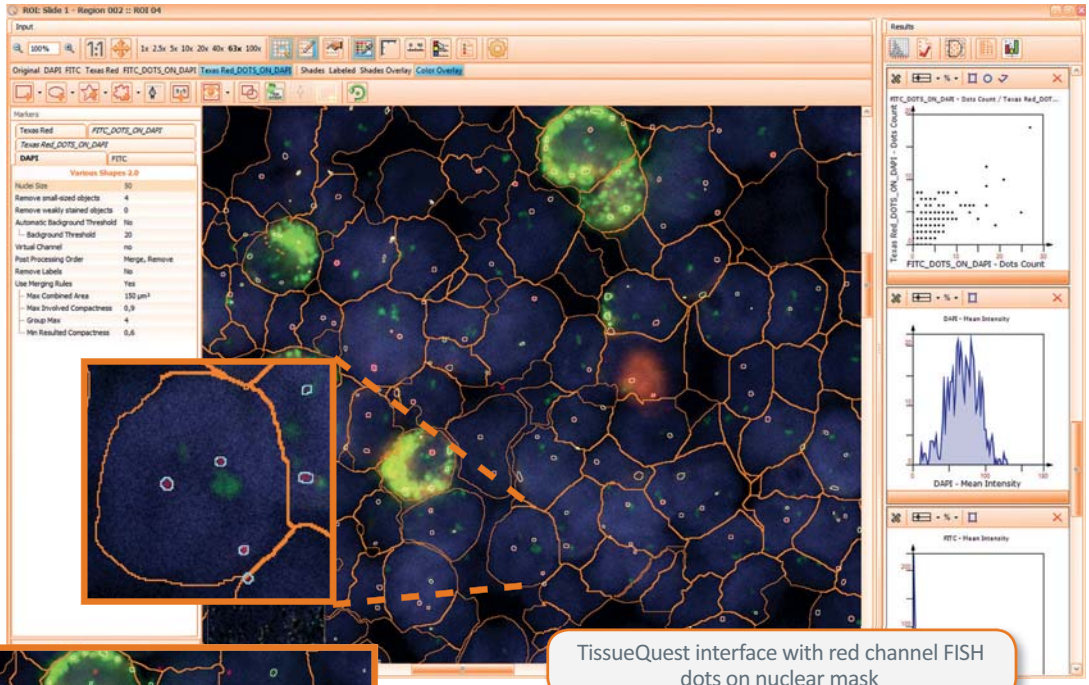
The scanned FISH or dot project can then be passed on to either the TissueQuest, HistoQuest (for ISH dots) or StrataQuest software for analysis. In the following, the TissueQuest workflow is shown.



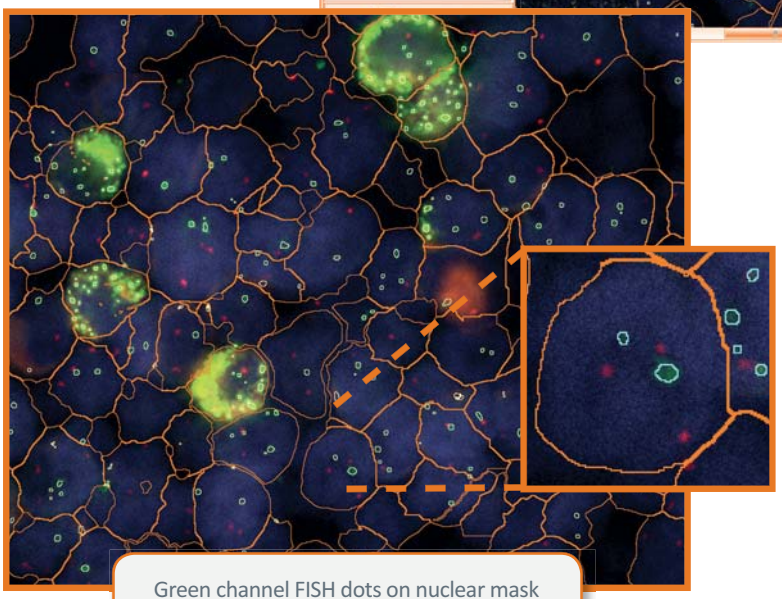
Setup of the Dot analysis algorithm is simple yet flexible. It is based on the number of markers present and permits to select on which marker channel masks dots should be analysed.

The suite of dot-associated diagrams and exports is then set up automatically in TissueQuest and HIstoQuest.

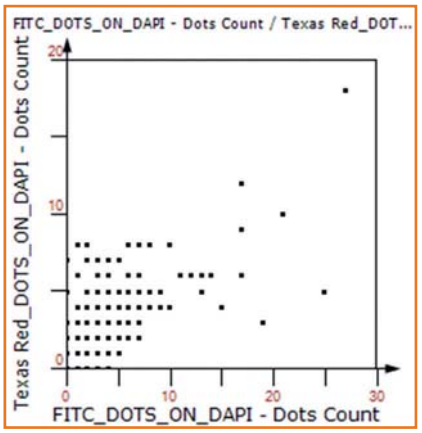
The normal channel masks (e.g. Nuclear, Cytoplasmatic) are set up with the softwares standard workflows.
Dot channel settings are chosen from options provided in the interface.



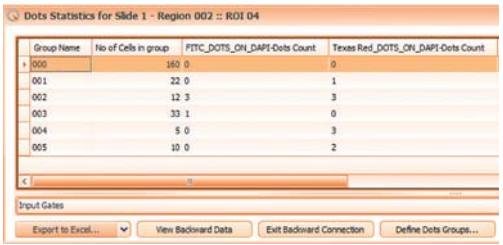
TissueQuest interface with red channel FISH dots on nuclear mask



Green channel FISH dots on nuclear mask



Red & Green dot combinations scattergram



Apart from scattergram dot data display there is a routine pathology-oriented list display of dot statistics.

This list can also be exported into Excel, CSV or PDF formats.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Group Name	No of Cells in group	FITC_DOTS_ON_DAPI-Dots Count	Texas Red_DOTS_ON_DAPI-Dots Count	FITC_DOTS_ON_DAPI/Texas Red_DOTS_ON_DAPI dots Ratio	FITC_DOTS_ON_DAPI/Texas Red_DOTS_ON_DAPI dots Colocalization	Average DAPI-Mean Intensity	Average DAPI-Area (µm²)	Average FITC-Mean Intensity	Average FITC-Area (µm²)	Average Texas Red-Mean Intensity	Average Texas Red-Area (µm²)	Average FITC_DOTS_ON_DAPI dots Mean Intensity	Average Texas Red_DOTS_ON_DAPI dots Mean Intensity
2	000	160	0	0	N. def.	N. def.	57,05	6,83	3,19	6,83	1,83	6,83	0,00	0,00
3	001	22	0	1	0,00	N. def.	63,87	16,88	2,48	16,88	2,61	16,88	0,00	70,07
4	002	12	3	3	1,00	0,00	70,00	29,87	5,95	29,87	4,81	29,87	67,74	67,66

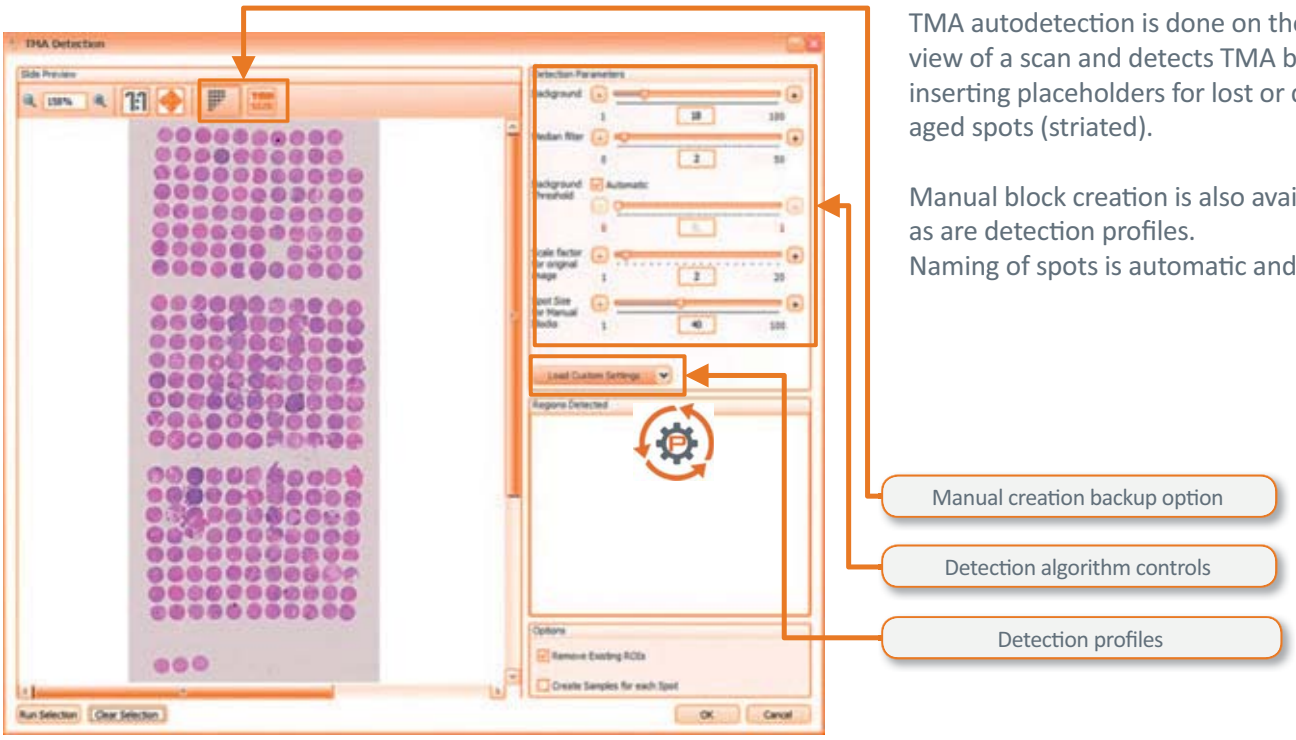
TG ANALYSIS SW FEATURES

Getting to the core. Of TMA.

TissueGnostics provides an integrated Tissue Microarray (TMA) workflow.
This makes analysis of these highly manipulation intensive samples very accessible. The workflow applies to brightfield and fluorescence TMA both.

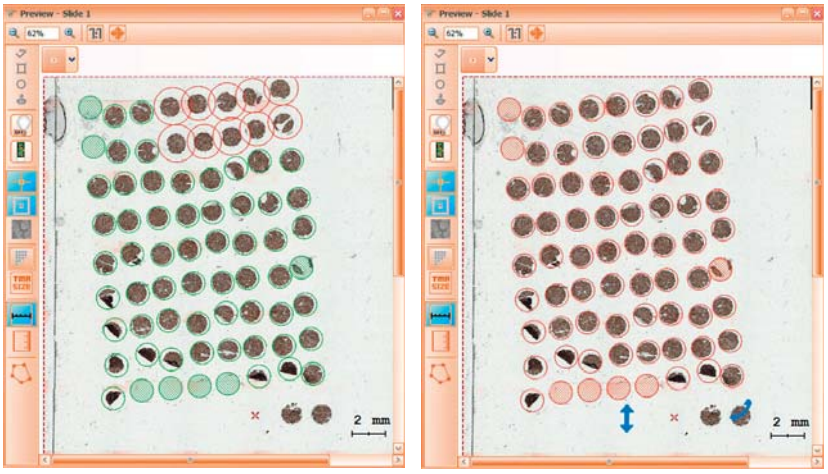
The integrated workflow is built on automatic TMA core detection algorithms available both in TissueFAXS scanning software as well as all TG analysis software. If the scan is done with a TissueFAXS system, TMA block and core detection is done there and the results are imported into analysis software.

TMA blocks and cores scanned with other scanners can be detected in TG analysis software.



TMA autodetection is done on the preview of a scan and detects TMA blocks, inserting placeholders for lost or damaged spots (striated).

Manual block creation is also available, as are detection profiles.
Naming of spots is automatic and logical.

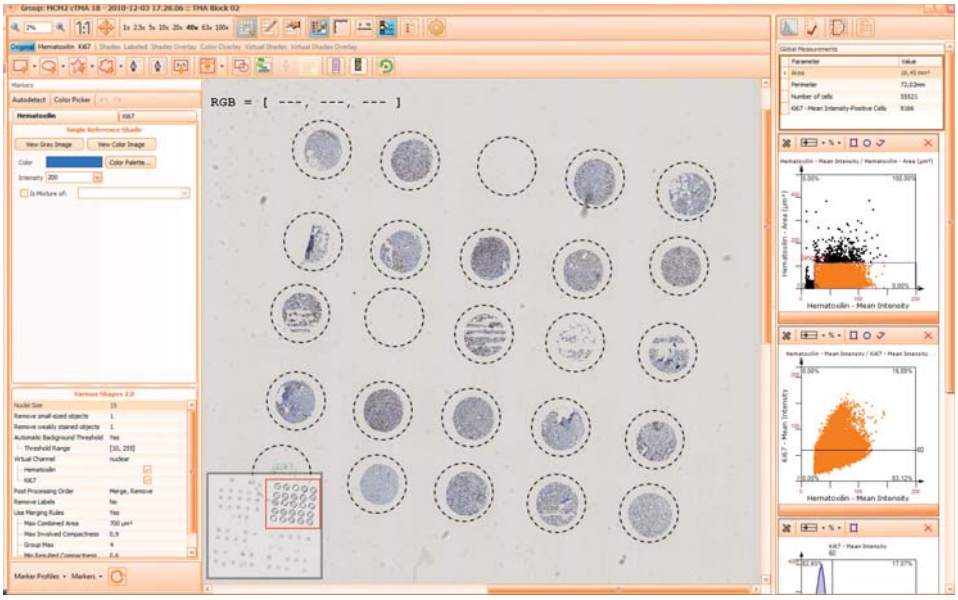


Whole blocks can be selected and rotated as well as scaled in x/y for fast rough adjustment.

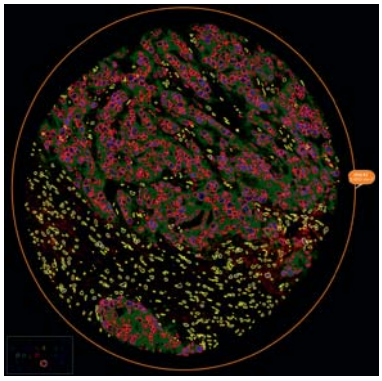
Core regions can be resized and repositioned in groups or individually.

Throughout this process, the ID of any core can be verified at any time.

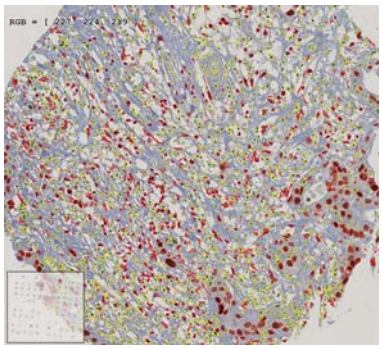
Once positioning is done, automated analysis of all cores can then be started in HistoQuest, TissueQuest and StrataQuest with identical settings and the possibility of identical cutoffs.



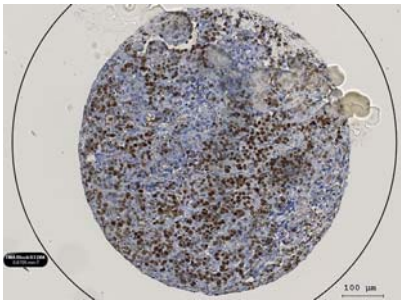
HistoQuest TMA interface

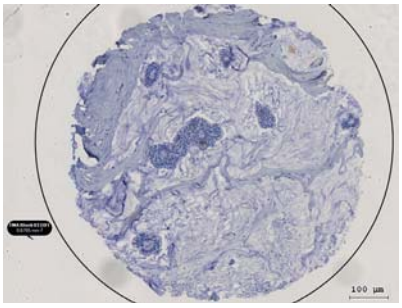


TissueQuest TMA core



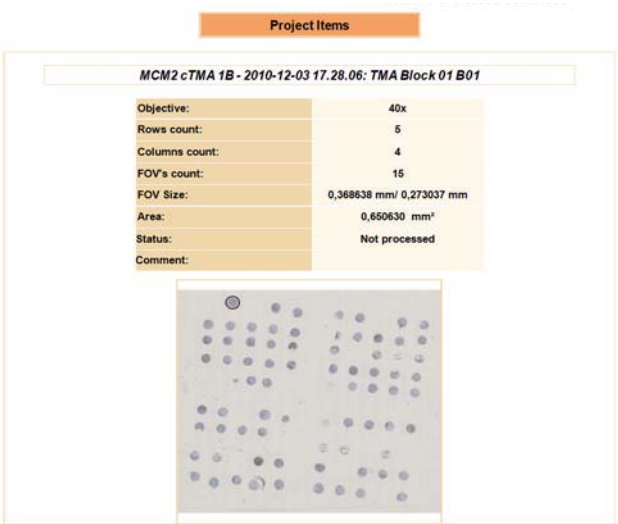
HistoQuest TMA core





However, TMA spots can, by their very nature, be very disparate,
in which case analysis with one general setting would be nearly impossible.

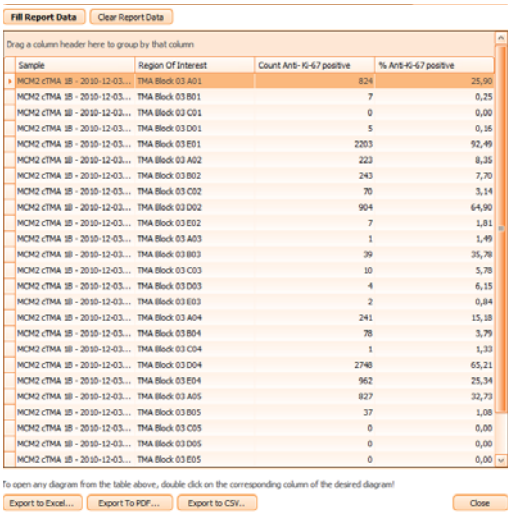
TissueGnostics TMA workflow permits the analysis of the majority with a general template
and the use of adapted templates on less conform spots.



Project Items

MCM2 cTMA 1B - 2010-12-03 17.28.06: TMA Block 01 B01

Objective:	40x
Rows count:	5
Columns count:	4
FOV's count:	15
FOV Size:	0,368638 mm/ 0,273037 mm
Area:	0,650630 mm²
Status:	Not processed
Comment:	



Fill Report Data | Clear Report Data

Drag a column header here to group by that column

Sample	Region Of Interest	Count Anti- Ki-67 positive	% Anti-Ki-67 positive
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 A01	824	25,90
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 B01	7	0,25
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 C01	0	0,00
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 D01	5	0,16
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 E01	2203	92,49
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 A02	223	8,35
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 B02	243	7,70
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 C02	70	3,14
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 D02	904	64,90
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 E02	7	1,81
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 A03	1	1,49
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 B03	39	35,78
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 C03	10	5,78
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 D03	4	6,15
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 E03	2	0,84
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 A04	241	15,18
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 B04	78	3,79
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 C04	1	1,33
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 D04	2748	65,21
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 E04	962	25,34
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 A05	827	32,73
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 B05	37	1,08
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 C05	0	0,00
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 D05	0	0,00
MCM2 cTMA 1B - 2010-12-03...	TMA Block 03 E05	0	0,00

To open any diagram from the table above, double click on the corresponding column of the desired diagram!

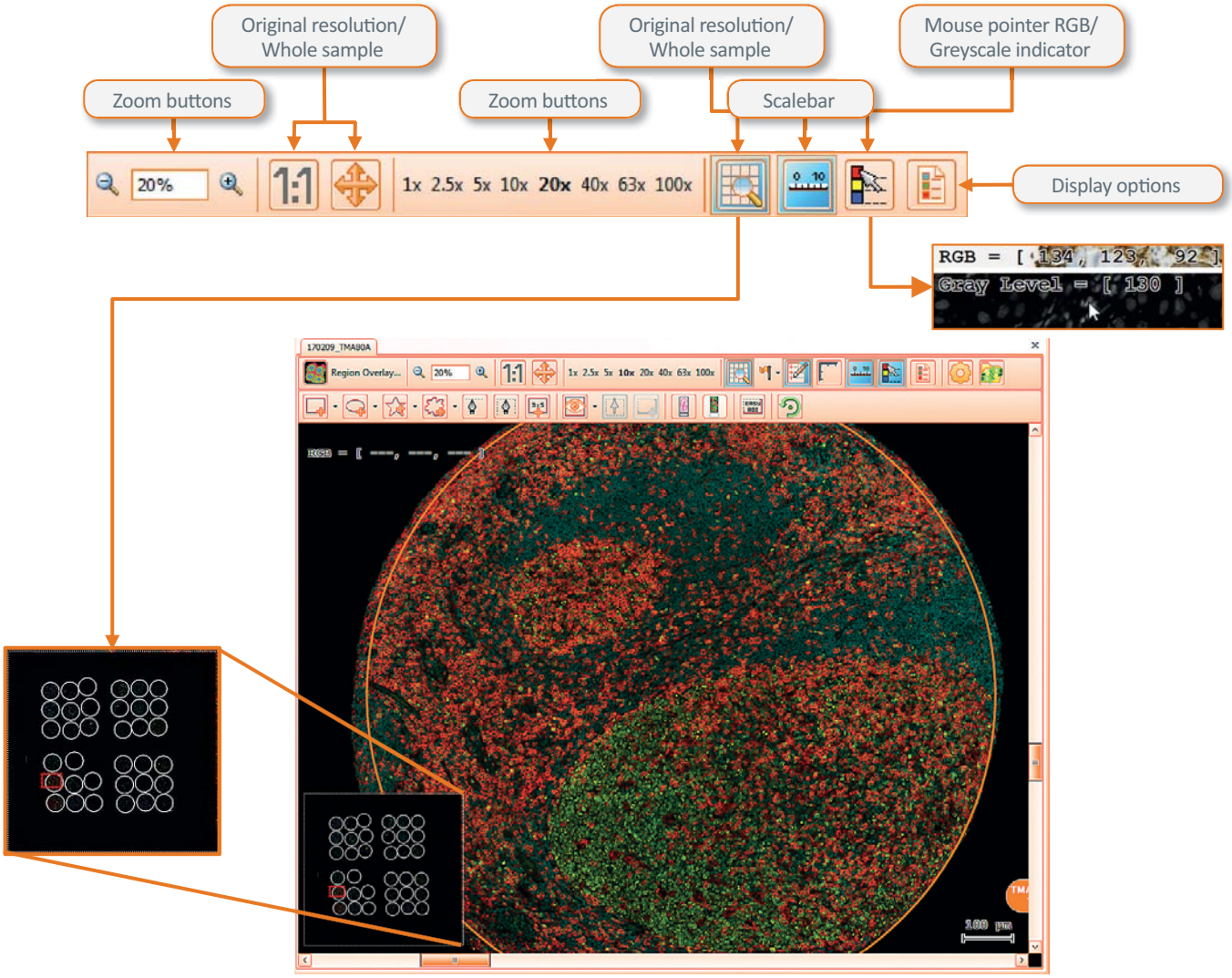
Export to Excel... | Export To PDF... | Export to CSV... | Close

TissueGnostics analysis software offers powerful report and export options for TMA analysis, always
putting easy orientation within the block structure first.

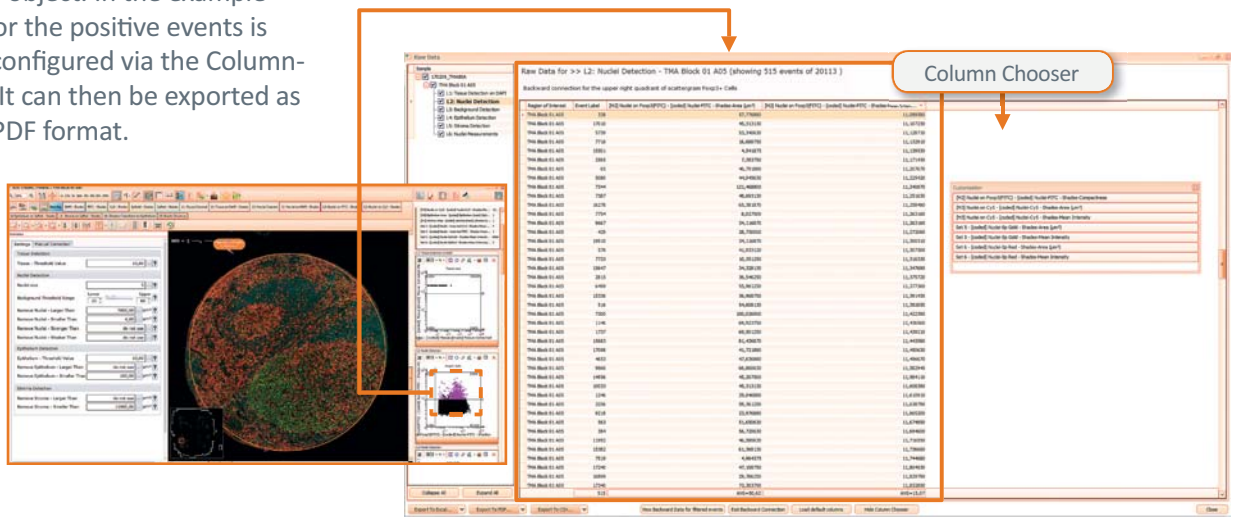
TG ANALYSIS SW FEATURES

Last, but not least... Moving around your samples & more data export

The sample viewer is central to all TissueGnostics software. Main functions are drag and drop movement of the sample as well as mouse wheel zooming (1-999%). The other main functions are shown below.



The Raw Data List is another data export mode, outputting measurements for each detected object. In the example shown, data for the positive events is selected and configured via the Column-Chooser tool. It can then be exported as Excel, CSV or PDF format.



TG IMAGING SYSTEMS

At a glimpse... The TissueFAXS imaging systems

TG imaging systems are workhorses for the fully automated scanning of slides, TMA and cell culture vessels in medium to high throughput. They are open microscope based and available as integrated systems with TG analysis software or as Scan Only. They have standard configurations with cost-effective components and can be upgraded to higher capabilities.

TISSUEFAXS

- Based on upright microscopes
- Fluorescence/brightfield scanning & analysis system for 8 slides
- Generic, TMA & FISH/CISH scanning & analysis
- Automatic Tissue Detection
- Extended Focus, Stitching & Illumination Correction
- Histo (BF), Fluo (FL) & PLUS (Both)
- Scan only available
- Upgradable to Spinning Disc Confocal

TISSUEFAXS i

- Based on inverted microscopes
- Fluorescence/brightfield scanning & analysis system for 8 slides or 1 Micro well plate or 1 Petri Dish
- Time Lapse scanning, Live Imaging capable
- Generic, TMA & FISH/CISH scanning & analysis
- Automatic Tissue Detection
- Extended Focus, Stitching & Illumination Correction
- Histo (BF), Fluo (FL) & PLUS (Both)
- Scan only available
- Upgradable to Spinning Disc Confocal

TISSUEFAXS SL

- Slide Autoloader
- Up to 120 slides
- Histo (BF), Fluo (FL) & PLUS (Both) configurations with respective capabilities
- Four scanning modes, Scan only available
- Upgradable to Spinning Disc Confocal

TISSUEFAXS CONFOCAL

- Fast Spinning Disc Confocality
- Also available in Fluo and PLUS upright and inverted configurations

TG DATASTORE

Store. Archive. Share.

TG-DataStore is a set of scalable solutions for image and metadata storage, management and archiving as well as for online image viewing.

Image management requirements ranging from single workgroups to complete research facilities or hospitals can be met.

TissueFAXS systems

TissueFAXS SL

HemoFAXS systems

Other imaging systems

PACS

HIS/LIS

Others

Supported formats

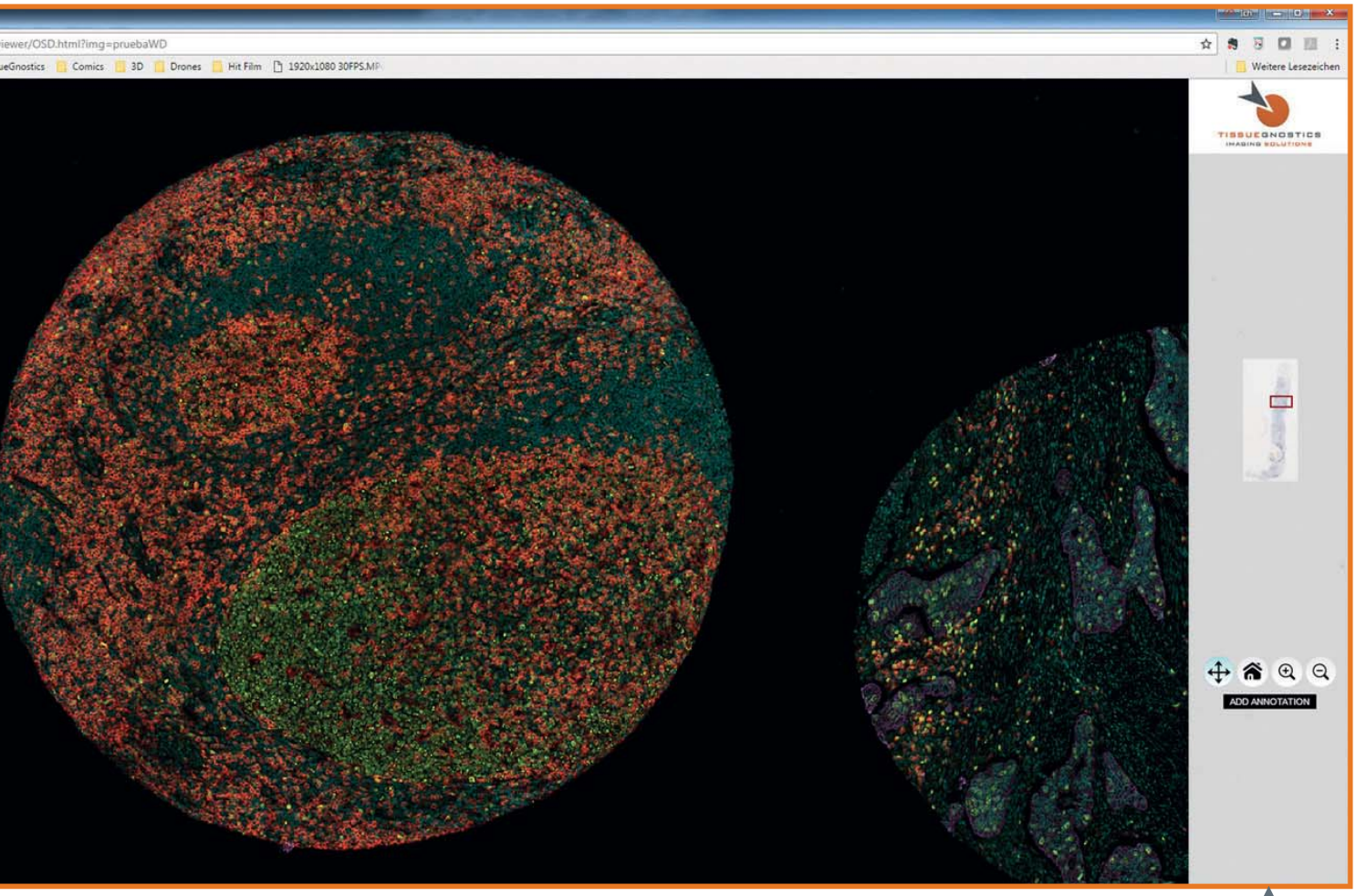
XML
SAP
HL7 MDM
DICOM
SMTP
HTTP
CIFS
NFS

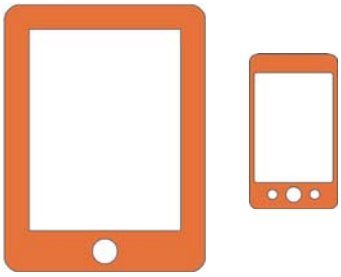
TG-DataStore has the capability to receive un-structured data from the productive storage of devices in many different formats.

Storage allows for direct data retrieval.

SAS NSAS HCP

Data archiving can have several tiers.





The TG WebViewer provides very high transfer speeds as well as annotation and measurement functions. It is one of the few web viewers which also can display fluorescence images.

Other than in webbrowsers, images can also be viewed at high transfer speeds on smartphones and tablets.

The communication server retrieves and combines images, patient and research metadata for transfer to the TG WebViewer.

SUPPORT MAINTENANCE UPGRADES

TG Support

TissueGnostics support is rendered worlwide based on a 09 to 17:30 hours workday in the UTC+2 timezone. Response time to support calls typically is between 30 minutes and 24 hours. Support can be based on a yearly contract (12 months) or a per case /time scheme.

Support response usually consists of mail or phone contact, a short consultation to start diagnostics and, typically, an online session on the installation to be supported using Teamviewer software and audio. Depending on the issue, support activity can be as short as 15 minutes.

Hardware support is usually rendered from Vienna once diagnostics have been done. Response time can be within seven days, depending on the issue.

TG Maintenance

Yearly maintenance is performed in coordination with the client. It covers complete service and recalibration of the microscope installation and of the computer(s). After the appropriate yearly maintenance, CE-conformity of the systems is assured for the period.

TG Upgrades

Upgrades for TG software are available as a yearly service. They provide one software capability enhancement and all updates of the respective year.

TG CUSTOMER EXPERIENCE

TissueGnostics has customers in 28 countries – here is what some of them say:

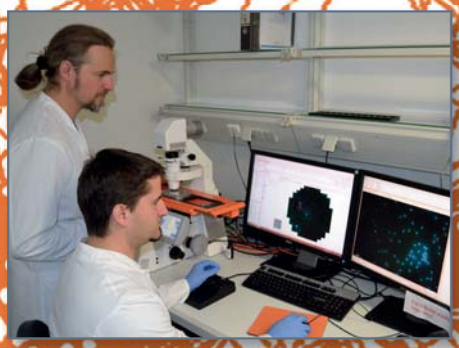
The TG System provides the highest quality images of tissues in immuno-oncology projects that are stained in a multiplex format. With the TG StrataQuest software we can extract unique data from the images and test for correlation with response to immuntherapeutics. Therefore, the TG System is ideally suited for translational research questions to develop novel drug response biomarkers and for analysis of tissues in immunotherapy clinical trials.

Beatrice Knudsen, M.D., Ph.D.
Professor of Biomedical Sciences and Pathology , Scientific Director of Translational Research Core, Cedars-Sinai Medical Center, Los Angeles, USA

TissueFAXS 200 Plus was installed in our center in August 2015, but I got to know and experienced TissueGnostics systems more than three years ago.
Since entering the Chinese market, TissueGnostics always has focused on the customers experience. Its software comes with many user-friendly features and is improved very quickly.
TissueFAXS 200 Plus provides high quality images and the patented analysis software for cell identification has high reliability, which greatly improved our research quality in histomorphology. I am really looking forward to the TissueFAXS SL PLUS Confocal upgrade with 3D reconstruction software. I believe that this will bring us more surprises.

Yu Yang, M.D
Director of Histomorphology Platform,
Research Center of Shanghai Public Health Clinical Center Affiliated to Fudan University

We are working with the TissueFAXS i Plus system to quantify distinct cell populations in situ.
Based on our experience we can strongly recommend that technique. The advantages are: i) automated image acquisition, ii) software based data analysis, and iii) 3 color phenotyping of cell subsets in situ.



Prof. Dr. Uwe Ritter
Institut für Immunologie, Universität Regensburg

Taken together, we find that the TissueGnostics system provides superior cellular biomarker quantitation within the context of the existing tissue histology for both traditionally stained tissue sections (single, color IHC analyzed by brightfield) and tissue sections stained by multiparametric immunofluorescence.

Scott J. Rodig, M.D. Ph.D.
Associate Professor of Pathology, Harvard Medical School
Tissue Microarray and Imaging Core Facility, Harvard, USA