TissueGnostics
TissueFAXS
Cell Analysis System

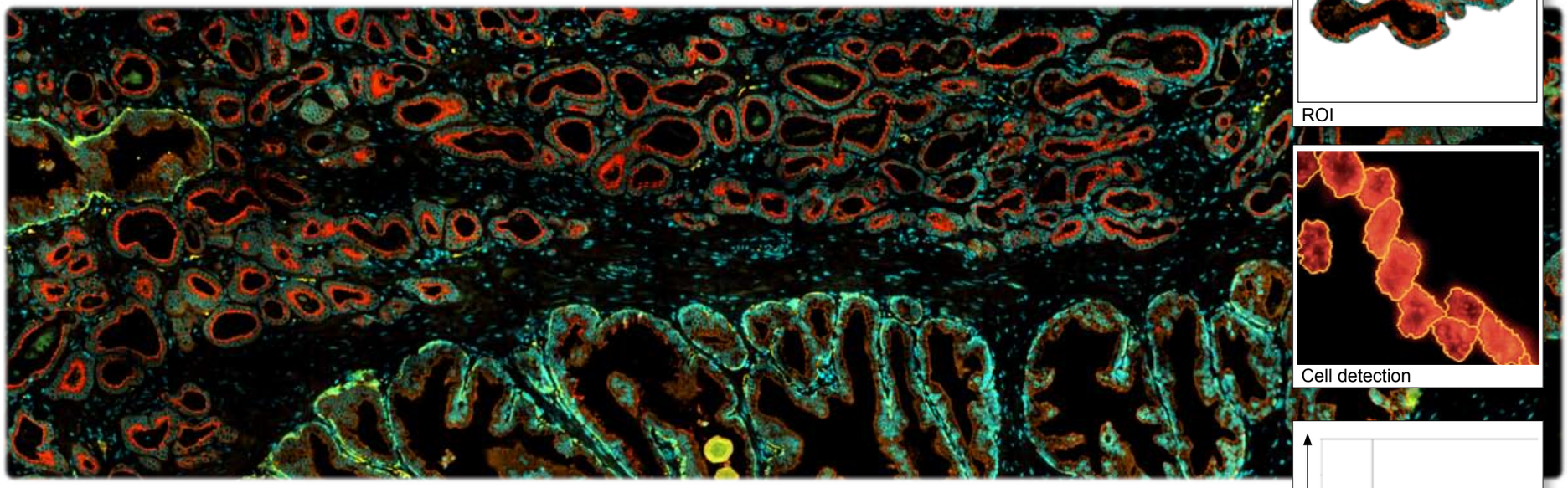
TissueGnostics TissueFAXS

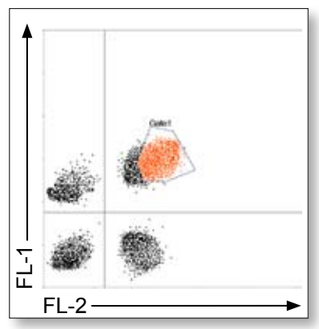
TissueFAXS

The equivalent to flow cytometry in tissue

Microscope-based cell analysis system for cells in tissue sections and smears.

TissueFAXS high-throughput acquisition and automated analysis capabilities permit major time savings.





TissueFAXS® is a unique analytical instrument that combines the advantages of state of the art:
- multi-channel microscopy
- automated high resolution imaging with the scientific accuracy of flow cytometry.

Instead of bleaching away in the fridge, data of entire fluorescence specimens is available on mouse click to be discussed with your colleagues and students.

The quantitative and objective analysis tools provided by the TissueQuest® software module complete the TissueFAXS performance. TissueFAXS is successfully used in cancer research, developmental biology, pathology, immunology, dermatology, urology, drug development and clinical testing.

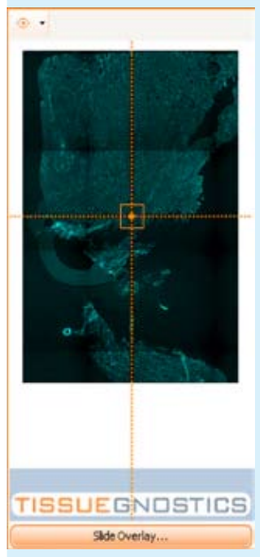
- Automated multi-channel image acquisition
- Specimen overview and cellular detail in one interface
- Individual regions of interest (ROI) definable by graphical tools
- Automated single cell detection by patented algorithms
- Objective quantification by control based background subtraction
- Dotplot operations and gating on subpopulations
- Backward and Forward Gating from dotplot to image and image to dotplot
- Statistical data in tables and export in Excel as well as ASCII formats

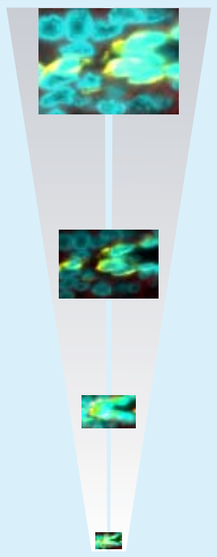
All events in scattergram					
	x-Mean	y-Mean	Events	No./mm^2	%
UL	48.24	56.23	463	2625.16	17.00
UR	71.73	57.48	103	584.00	4.00
LL	47.08	21.10	1710	9695.50	61.00
LR	71.39	24.39	542	3073.08	19.00
SUM	52.85	28.83	2818	15977.73	100.00

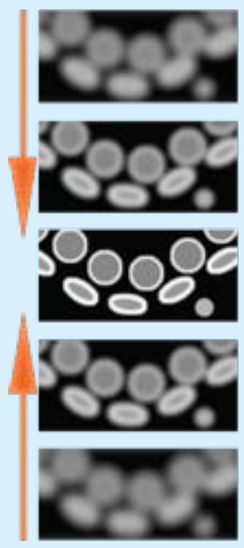
TissueGnostics TissueFAXS

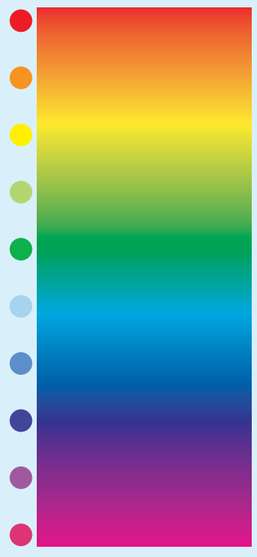
TissueFAXS
Workflow

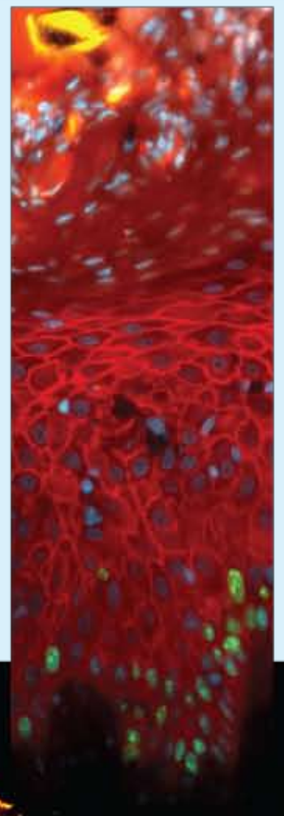
Rapid preview	Automated ROI or whole slide acquisition at any magnification	Tunable auto-focus for up to 100x oil objectives	Up to 10 different fluorescence filter cubes	Automatic stitching function generates an exportable sample overview	ROI creation and export	Re-acquisition of single out of focus images	TissueQuest analysis

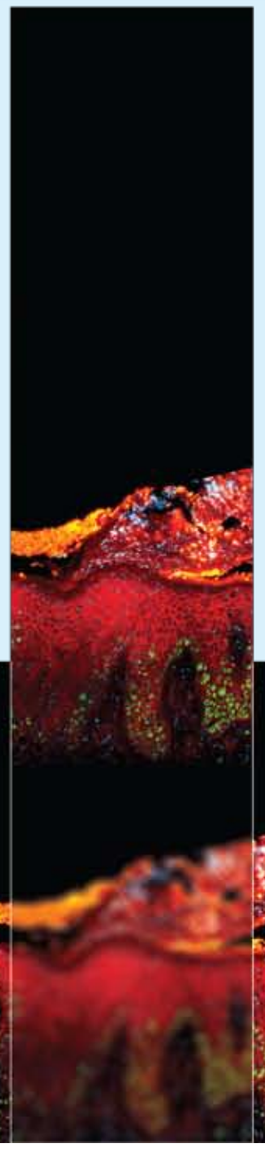


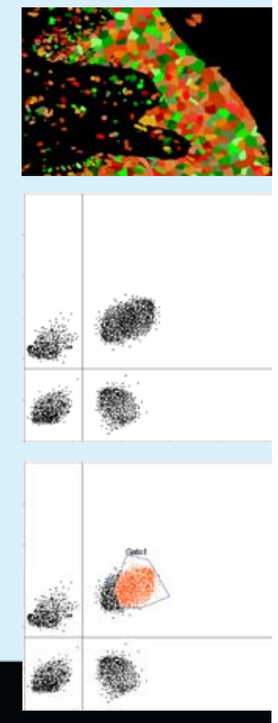


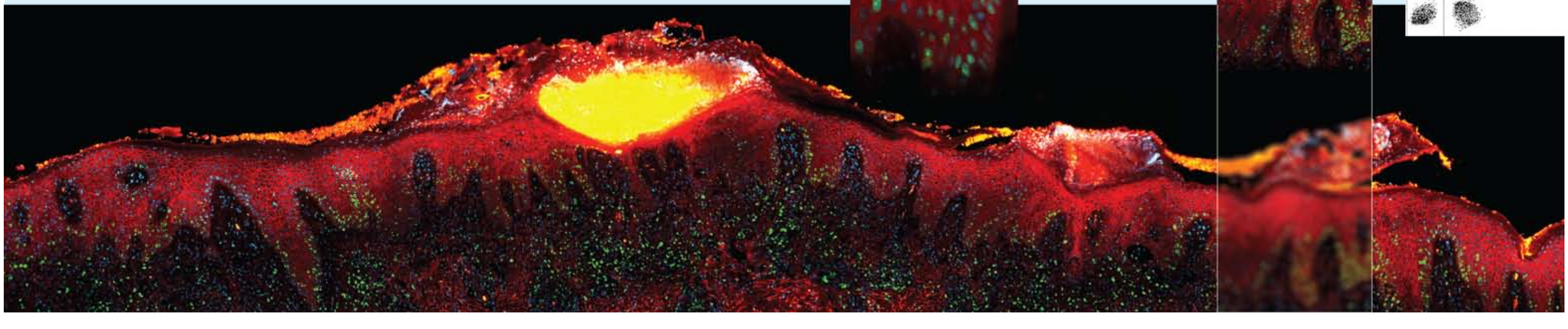








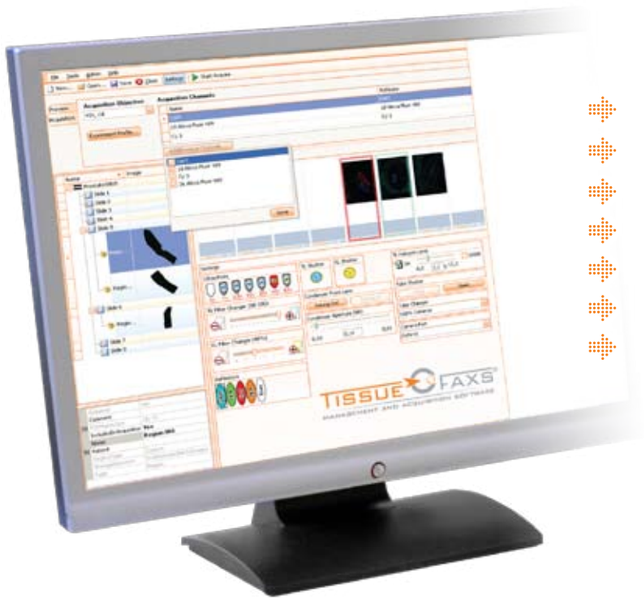




TissueFAXS

Management and Acquisition Software

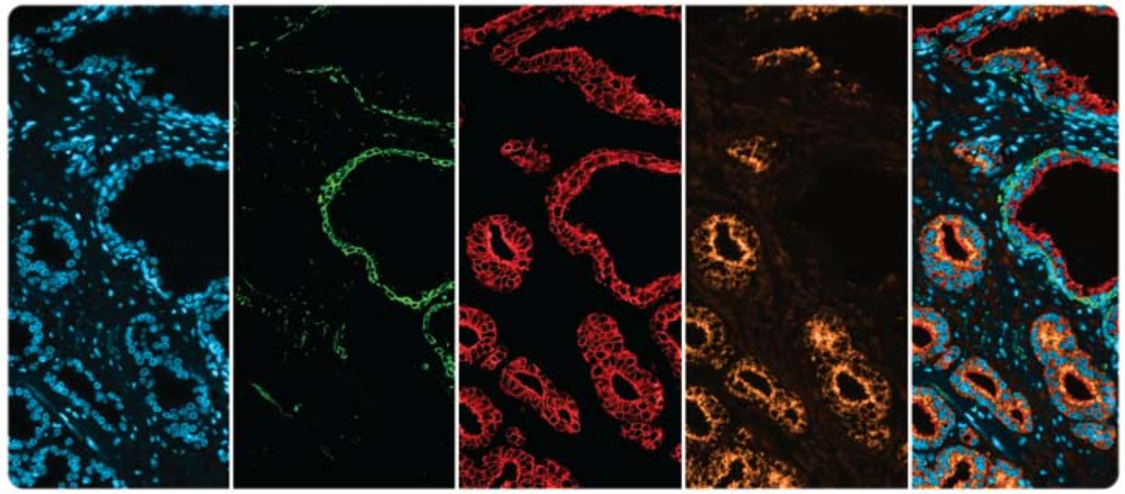
The TissueFAXS acquisition and management software combines an intuitive and user friendly interface and a comfortable workflow with extensive control functions:



- Objective selection
- Light source control
- Filter selection
- Graphical navigation function for 8 slides
- Preview window
- ROI management
- Well plate layout

- Live image window
- Stage controls and position info
- Autofocus
- White balance
- Image export
- Colour overlay controls
- TMA layout

Four colour staining overlay



Nuclei | Basal EC | Epithelial cells | Tumor marker | Overlay

TissueQuest

Cell Analysis Software Module

TissueQuest is the first software solution worldwide to identify and analyse individual cells in their tissue environment

Cell identification is not confined to isolated cells. The TissueQuest software also identifies cells that are agglomerated in dense clusters or have been reduced to ring structures (as it is typical of cancer cells).

Regardless of the type and density of cells involved, TissueQuest recognizes their nuclei due to patented image processing algorithms.

By combining specific stainings and algorithms, one can measure staining intensities that are specific for various cell types.

Cells are clearly distinguished even

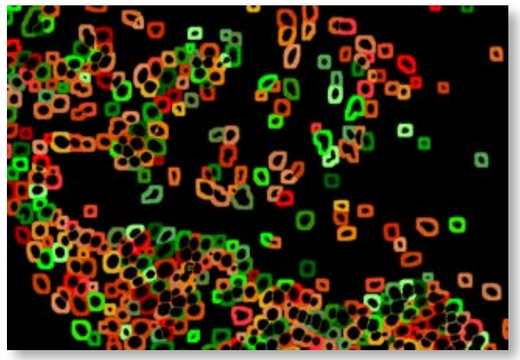
- in solid tissue structures
- if embedded in clusters
- if nuclei are uniformly stained
- if staining involves ring structures

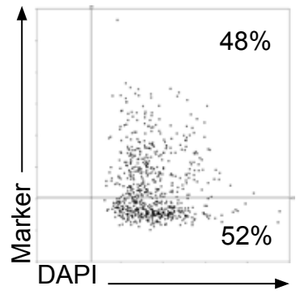
TissueQuest

Different mask options and quantitative analysis

Analysis of staining intensities can be restricted to the area of the nucleus or can be extended into the cytoplasm. In case of cytoplasmic antigens TissueQuest provides an image processing algorithm that is growing in stained areas around the nucleus.

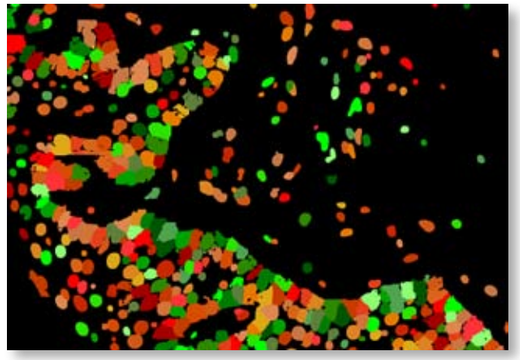
Ring mask

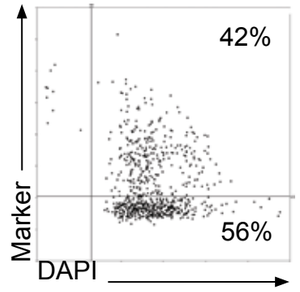




48%$^{+}$ cells
Mean intensity: 47

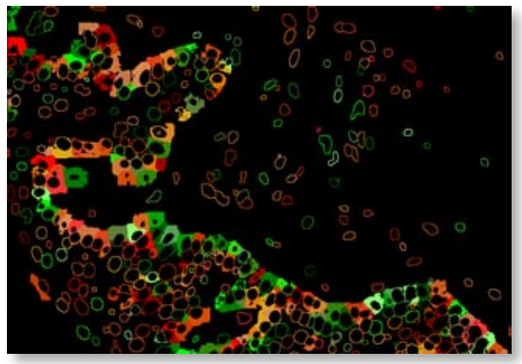
Growing mask

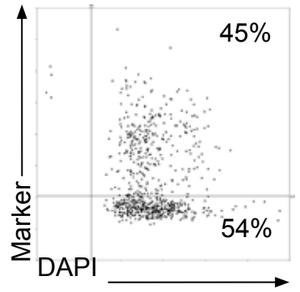




42%$^{+}$ cells
Mean intensity: 47

Growing mask/ring mask without nucleus





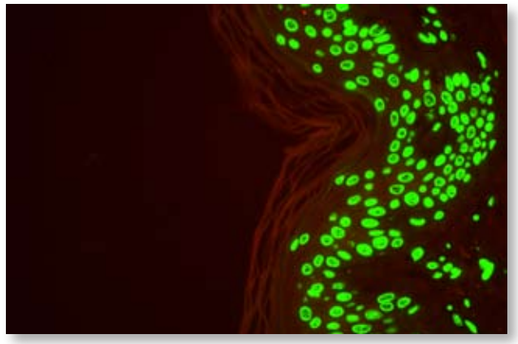
45%$^{+}$ cells
Mean intensity: 51

TissueQuest

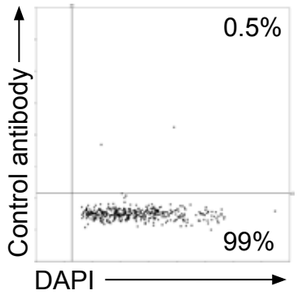
Quantitative analysis of one marker

Effect of therapy on the density of antigen expression in human skin:

Normal control

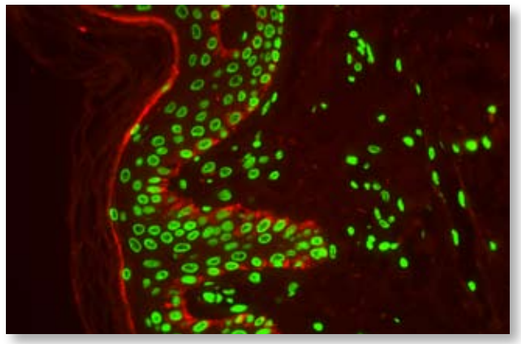


Negative Control Antibody

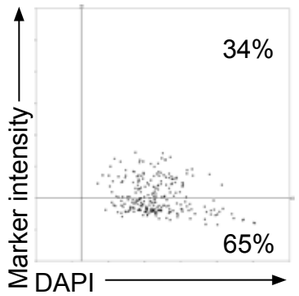


Mean intensity: 29

Normal skin before therapy

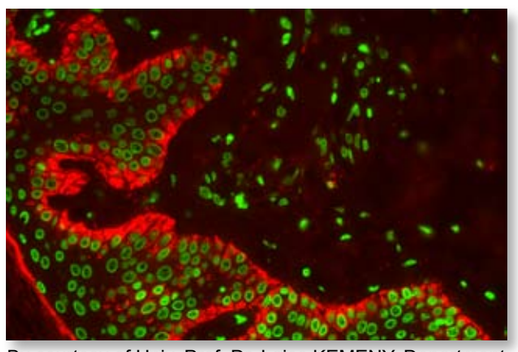


Marker expression before therapy

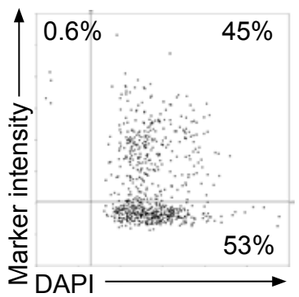


Before therapy the percentage of cells that react with the marker was 34% and the mean intensity was 39.

Normal skin after therapy



Marker expression after therapy



After therapy the percentage of cells that react with the marker was 45 and the mean intensity was 51.

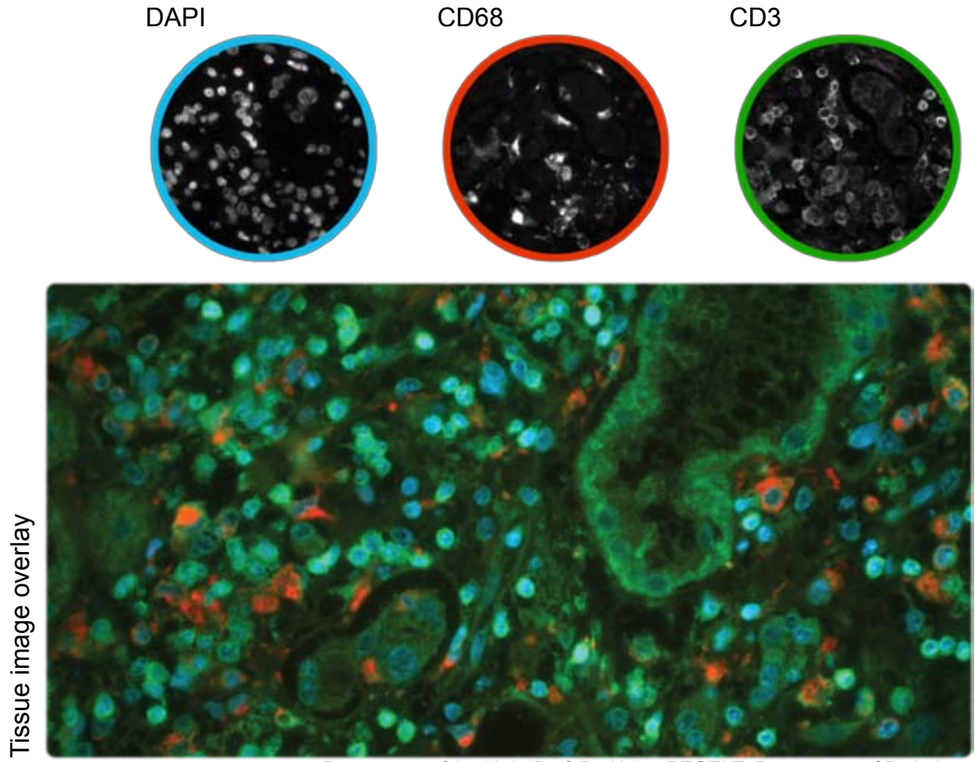
By courtesy of Univ. Prof. Dr. Lajos KEMENY, Department of Dermatology and Allergology, University of Szeged, Hungary

It was demonstrated that therapy goes hand in hand with an up-regulation of antigen expression indicated by an increase in the percentage of positive cells from 39% to 45% and in the mean intensity from 39 to 51, respectively.

TissueQuest

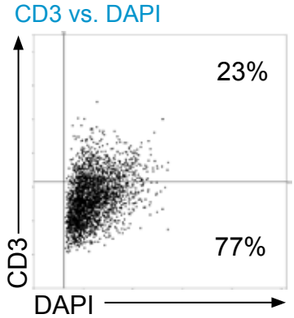
Leucocytic infiltrate in rejected kidney: Triple staining

Tissue: Paraffin section of rejected kidney allograft
Staining protocol: DAPI, CD68, CD3

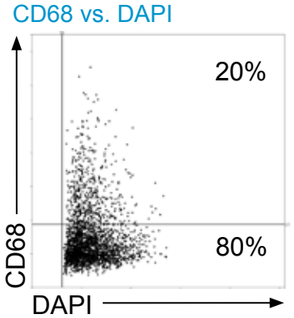


By courtesy of Ao. Univ. Prof. Dr. Heinz REGELE, Department of Pathology, Medical University of Vienna, Austria

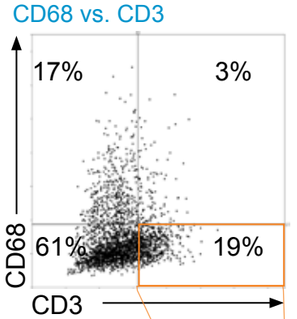
CD3 vs. DAPI



CD68 vs. DAPI



CD68 vs. CD3



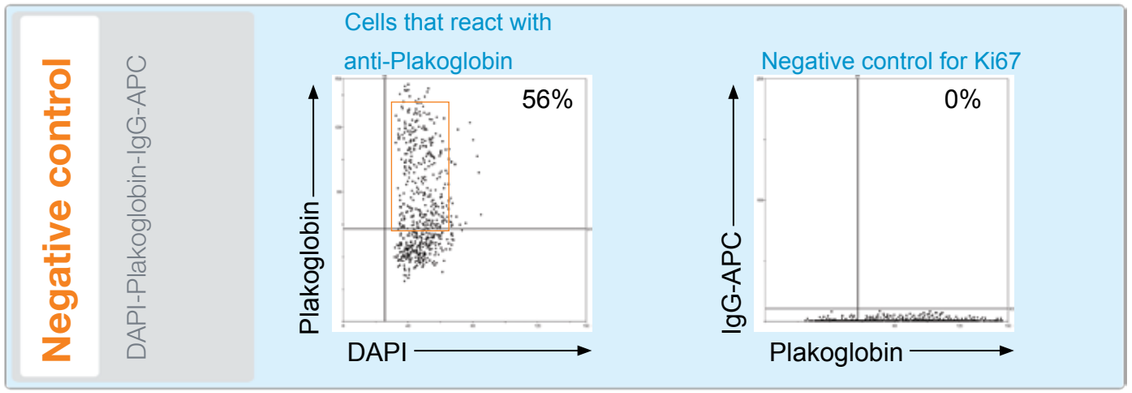
Cut-offs and/or gates will immediately update the data in the tables.
The export function also outputs the quadrant statistics for all dotplots into Excel and ASCII format.

All events in scattergram					
	x-Mean	y-Mean	Events	No./mm^2	%
UL	48.24	56.23	463	2625.16	17.00
UR	71.73	57.48	103	584.00	4.00
LL	47.08	21.10	1710	9695.50	61.00
LR	71.39	24.39	542	3073.08	19.00
SUM	52.85	28.83	2818	15977.73	100.00

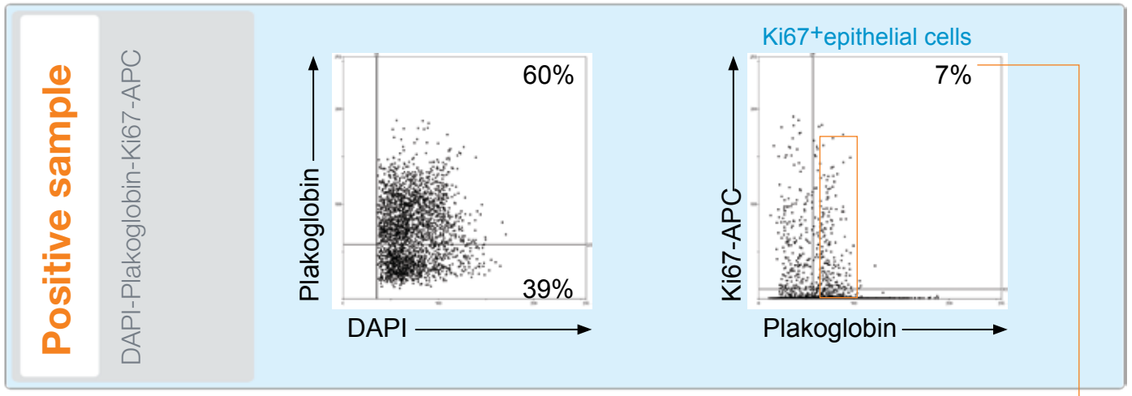
TissueQuest

Skin Project: Triple staining

The staining combination includes anti-Plakoglobin to identify all keratinocytes and Ki67 to analyse the proliferative activity of all cells. That means that Plakoglobin$^+$/Ki67$^+$ cells are proliferative keratinocytes and Plakoglobin$^-$/Ki67$^+$ cells are proliferative non-epithelial cells.



The use of isotype matched non-reactive control antibodies allows the setting of precise cut-offs.



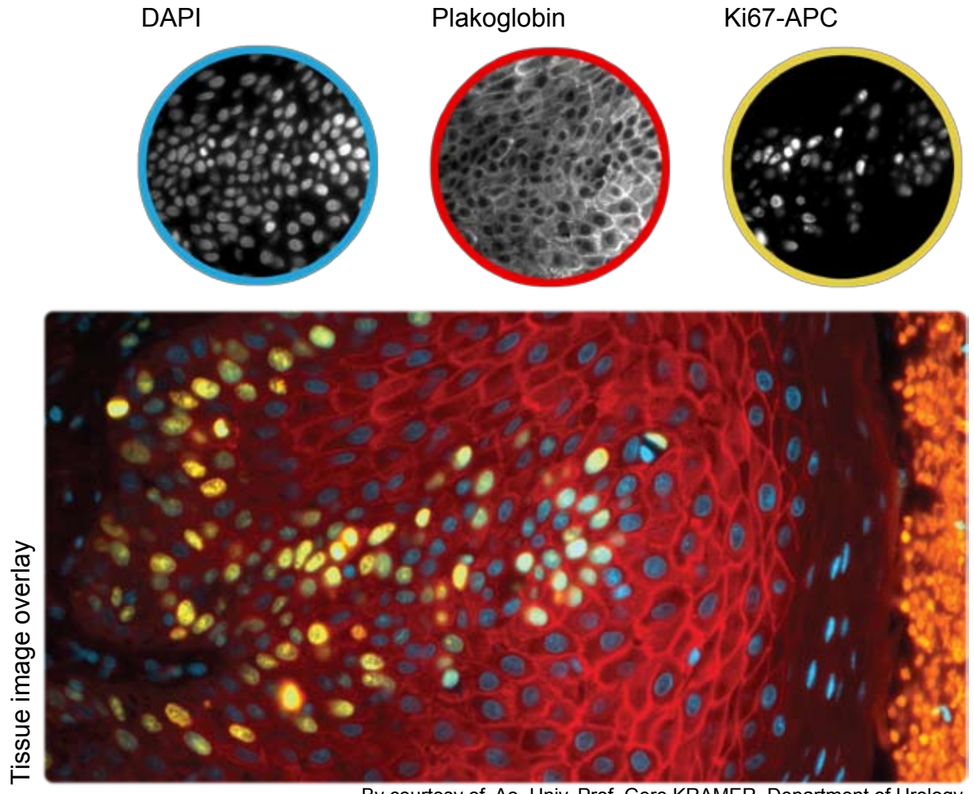
Statistical results are displayed when cut-off or gates are set.
The parameters include mean intensity, relative/absolute events, and percentage of reactive cells as well as sums.

7.15% Plakoglobin$^+$/Ki67$^+$cells

All events in scattergram					
	x-Mean	y-Mean	Events	No./mm^2	%
UL	36.81	62.84	192	1451	7.38
UR	73.78	60.17	186	1406	7.15
LL	36.35	0.43	827	6251	31.80
LR	94.65	0.38	1396	10553	53.67
SUM	70.35	9.28	2601	19663	100.00

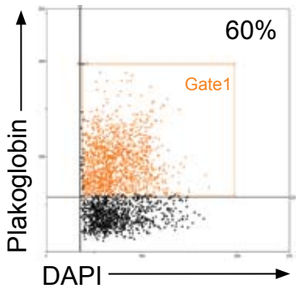
The quadrant statistics (UL-Upper Left, UR-Upper Right, LL-Lower Left, LR-Lower Right) are presented in tabular format.

Tissue staining protocol: DAPI, Plakoglobin-Alexa488, Ki67-APC or IgG$_1$-APC

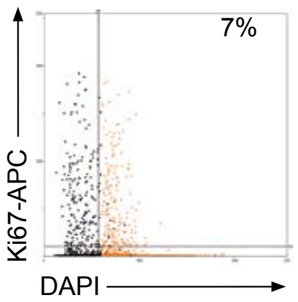


By courtesy of Ao. Univ. Prof. Gero KRAMER, Department of Urology, Medical University of Vienna, Austria

Gating on
Plakoglobin$^+$cells

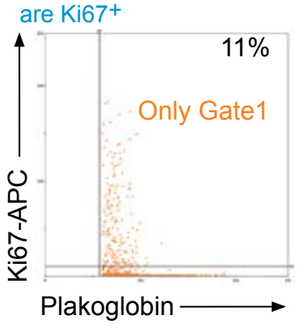


By the use of gates the analysis is restricted to subpopulations with defined phenotype.



Cell subpopulations can be visualised in distinctive colours by gating.

11% of keratinocytes
are Ki67$^+$

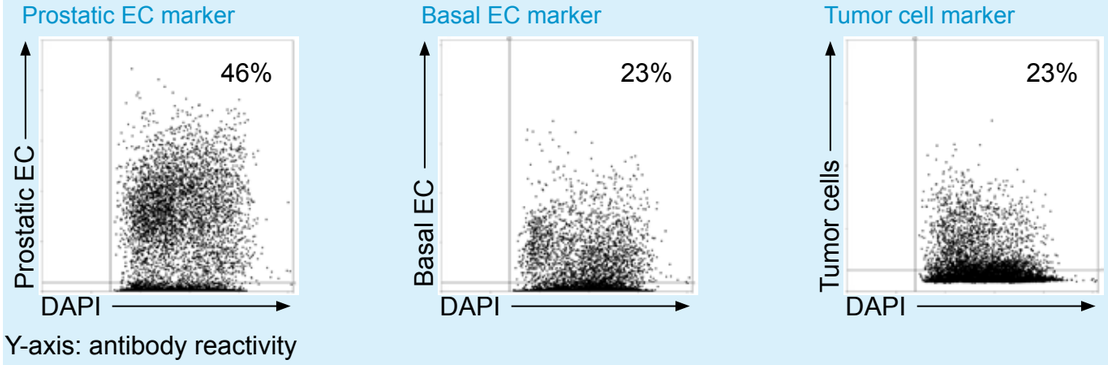


Gates can be used as input gates for new scattergrams

TissueQuest

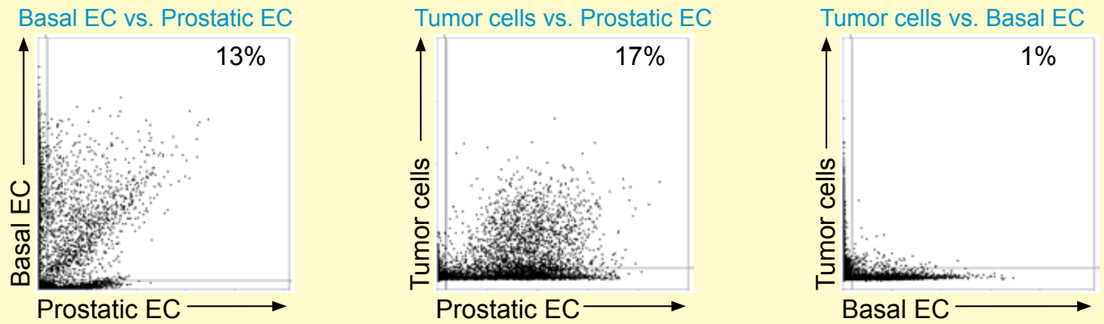
Prostate Project: Four colour staining

Single antibody reactivities



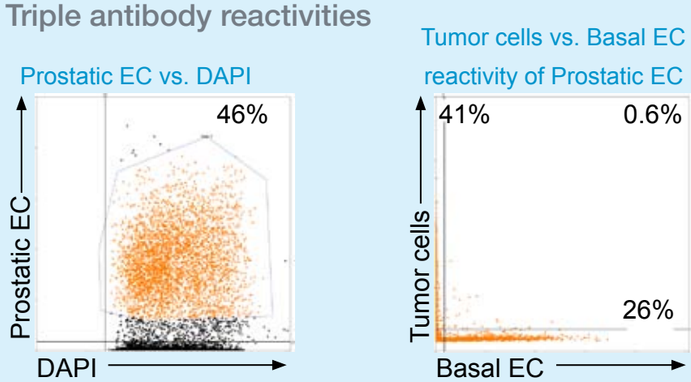
Y-axis: antibody reactivity

Double antibody reactivities



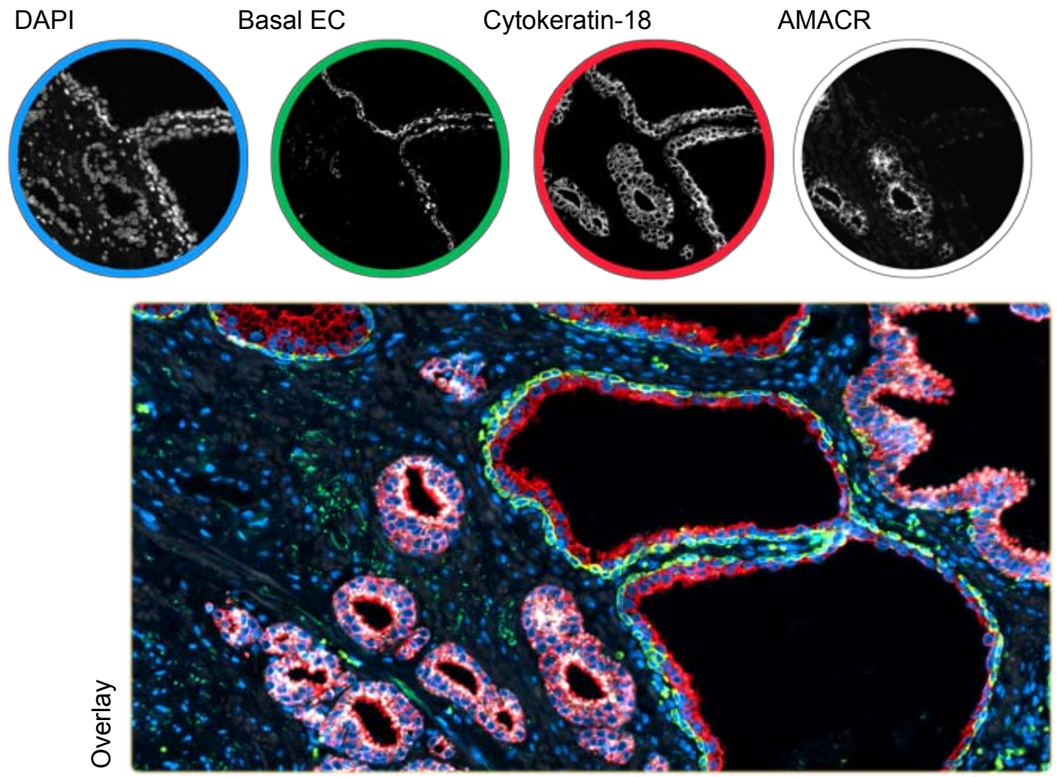
Analysis of coexpression pattern reveals that 17% of all cells express AMACR and that 99% of the AMACR$^+$ tumor cells co-express the marker of prostatic EC. In contrast, only 1% of AMACR$^+$ tumor cells were shown to co-express the marker for basal EC.

Triple antibody reactivities

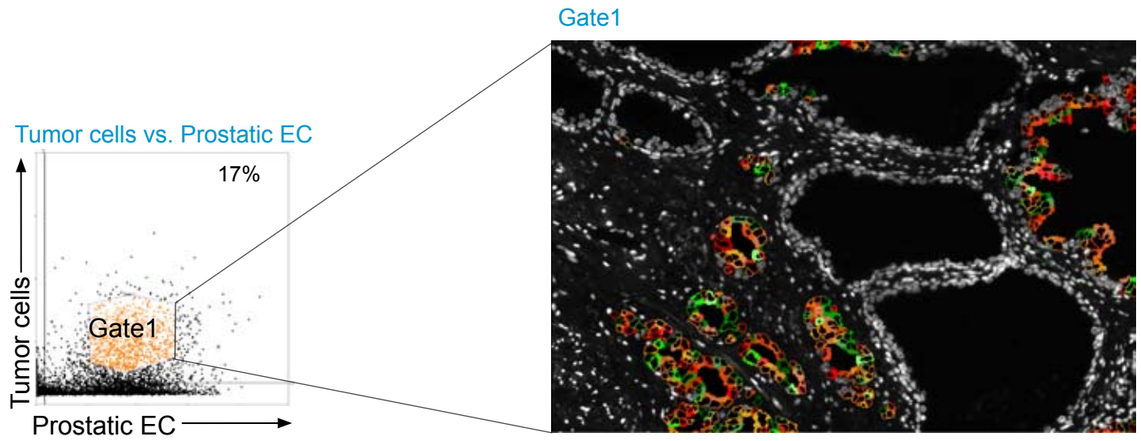


To analyse reactivity of a specific subset of cells with two different antibodies a gate is set on the subpopulation. This population (in orange) is then used as input for additional dotplots.

Tissue: Paraffin section of carcinoma of the prostate
Staining protocol: DAPI, basal epithelial cells (basal EC), cytokeratin-18 (prostatic EC), AMACR (tumor cells)



By courtesy of A. Univ. Prof. Dr. Helmut KLOCKER, Department of Urology, Medical University of Innsbruck, Austria



Statistic for Gate1					
	x-Mean	y-Mean	Events	No./mm^2	%
UL	0.32	21.81	568	3680.56	6.00
UR	86.60	40.60	1707	11061.13	17.00
LL	0.66	9.10	4525	29321.40	48.00
LR	74.56	10.25	2695	17463.24	29.00
SUM	37.32	15.85	9495	61526.33	100.00

TissueQuest

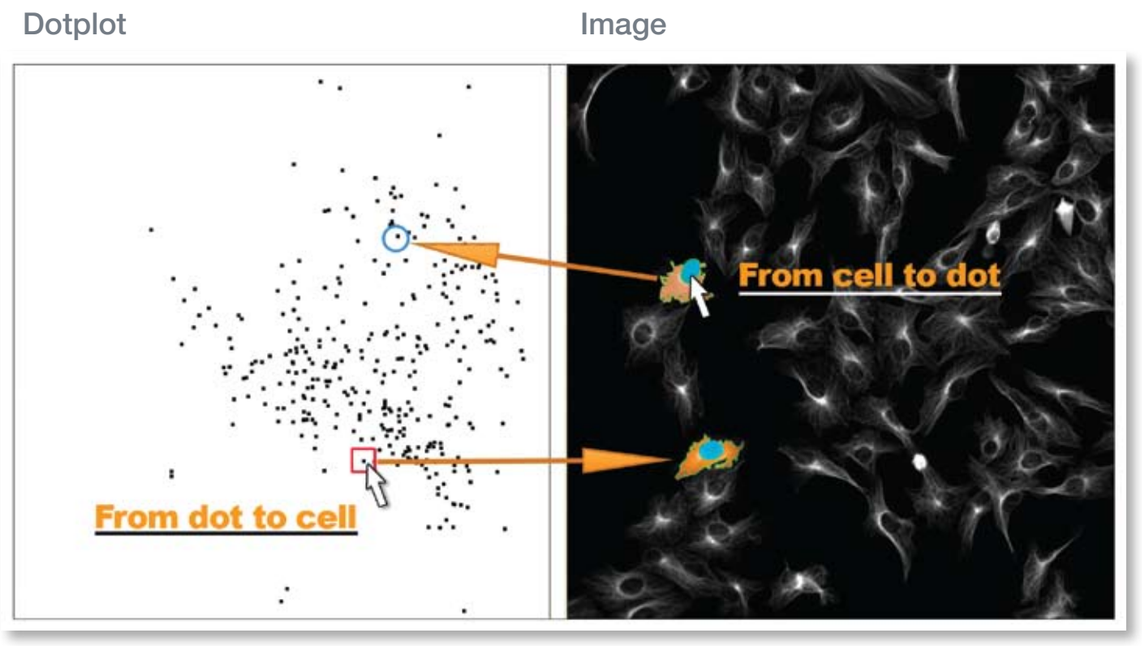
Backward and Forward Gating of single cells & gates

TissueQuest offers an innovative function for visual and measurement control.

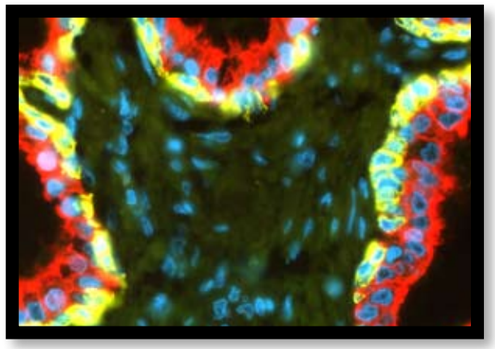
Backward Gating enables the user to connect backwards from a dot in the dotplot to the specific corresponding cell in the image.

By Forward Gating the user is able to locate the dot in the plot corresponding to a specific cell in the image.

The function “View event data” reads out all measurement data for an individual cell.

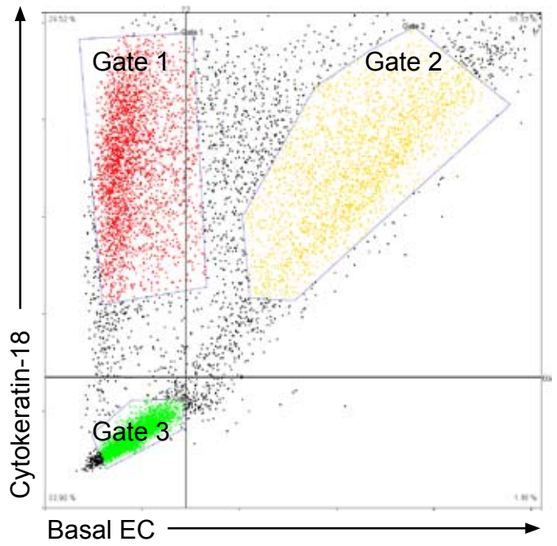


Overlay

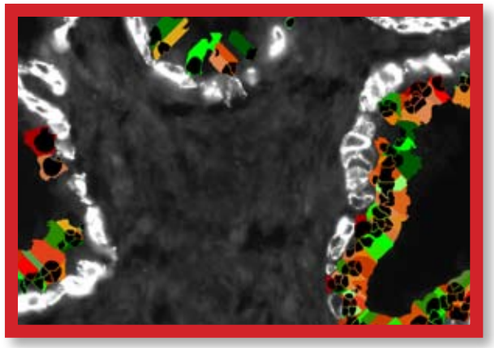


Backward Gating is an easy to use tool that helps in setting cut-offs and in the re-confirmation of image processing data.

It also helps in tracking phenotypically and/or morphologically distinct subsets of cells from the dotplot to the tissue.

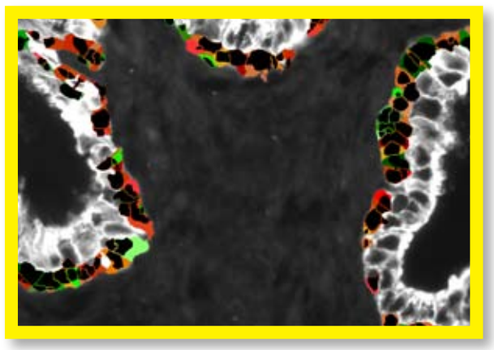


Gate 1



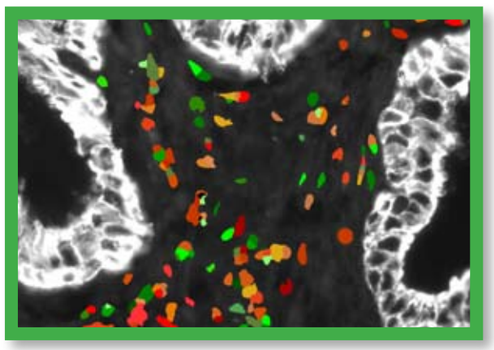
Cytokeratin-18^{+} and basal marker negative secretory epithelial cells

Gate 2



Cytokeratin-18^{+} and basal marker positive basal epithelial cells

Gate 3



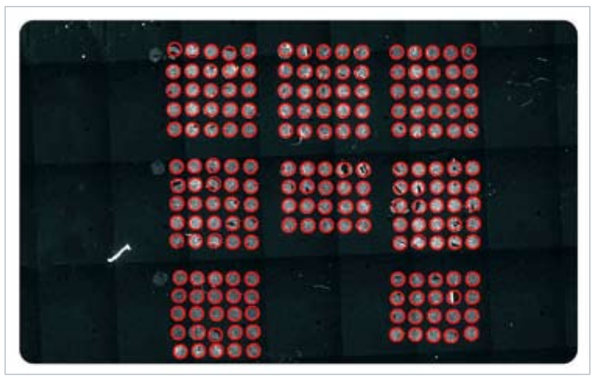
Cytokeratin-18 negative and basal marker negative non-epithelial cells

TissueFAXS
TMA Module

The TissueFAXS TMA module :

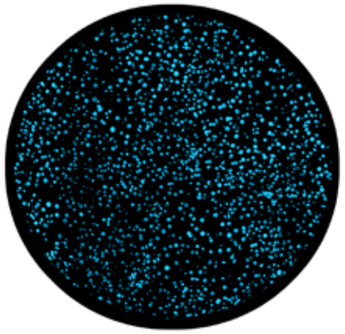
- is located in the new project wizard
- either recognizes cores in full auto mode or provides software-assisted manual core definition
- is tolerant of missing cores
- creates a meta annotation including all properties and a thumbnail of the core
- provides an option for generation of core ID
- allows for the re-naming and change of core location and grouping
- imports and exports from and into Excel sheet templates
- permits automatic acquisition at chosen magnification
- allows for logical grouping
- exports images into TissueQuest and HistoQuest
- provides a TMA-Explorer
- offers a report generator

TMA layout

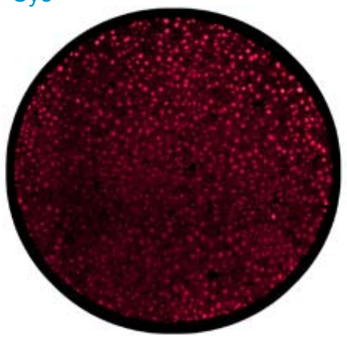


By courtesy of Univ. Prof. Dr. Kurt ZATLOUKAL,
Department of Pathology, Medical University of Graz, Austria

DAPI



Cy3



Overlay

TissueFAXS
Features and Benefits

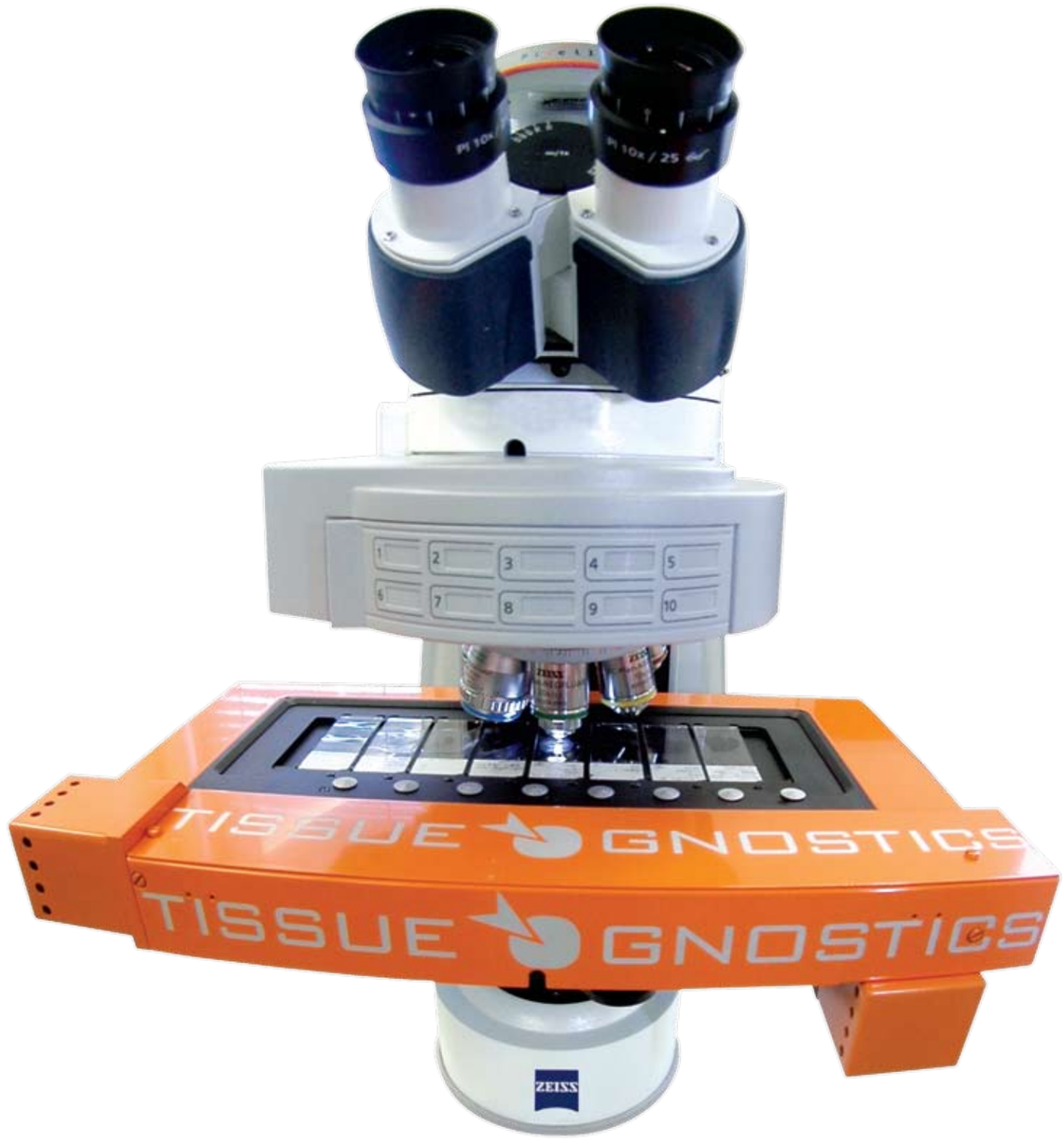
Features

- Preview scan (1x to 10x) „Fluorescence” with zoom function
- Preview scan (1x to 10x) „Brightfield“ with zoom function
- Automatic multi-channel acquisition of fluorescence images (up to 10 channels)
- Freely selectable magnification for acquisition
- Accurate autofocus for 1x to 100x oil objectives
- One push white balance function
- Detail Windows with zoom in and zoom out function
- Generation of overview images using TissueStitching function
- Export function for detail images and overview with selectable resolution and file size calculation

Benefits

- Easy to use Project Wizard
- Objective quantification
- Observer independent results
- Easy Handling
- Accuracy
- Freely selectable regions of interest (ROI)
- Selection of individual fields of views by setting flags for re-acquisition or export
- Relocate function
- Customised colours and controlable intensity in overlay images
- Virtual slide creation
- Effective bleaching prevention by minimisation of open shutter times
- Saving of time and resources due to high degree of automatisation
- High throughput acquisition
- Forward and Backward Gating

Fast, Precise and Automated Image Acquisition!
Easy, observer independent automated tissue analysis!



TissueFAXS i

Inverted Cell Analysis System

TissueFAXS i® provides the TissueFAXS capabilities on an inverted microscope basis.

This way, quantitative analysis can easily be performed on chamber slides, well plates, microtiter plates, culture flasks as well as standard slides and smears.

The system can be optionally upgraded with third party devices for other inverted microscope applications.

Very detailed information about marker distribution, marker localisation and molecular transport on a sub cellular basis is provided by TissueFAXS i.
Due to this function experiments like intra-nuclear translocation studies can be easily analysed.

The analytical capabilities of TissueFAXS i offer the possibility to efficiently implement quality control measures in the pharmaceutical field as well as in cell therapy, where the viability, the phenotype and the activity status of cells can be monitored.

TissueGnostics TissueFAXS

Technical Specifications

Supported high-end microscopes

Zeiss AxioImager.Z1
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
Up to 10 fluorescence filter cubes
DAPI, Alexa 488, Cy3, APC are standard

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

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

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-precision motorized stage

Stage for upright microscope
For up to 8 slides.
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)
Autofocus for brightfield and fluorescence applications

High-performance monochrome camera for fluorescence

Superior quantum efficiency
Frame rate
12 fps @ 1392 × 1024 for rapid specimen acquisition.
Sensor format
2/3” offering wide fields of view.
Resolution
1.4 megapixels guarantees fine object details.
Dynamic range
69.5 dB covering different expression levels of biological markers.

High-performance colour camera for brightfield (optional)

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 colour reproduction
Use of multiple chromophores.

Technical and application support via remote control

TissueGnostics
Publication list

Is Benign Prostatic Hyperplasia (BPH) an Immune Inflammatory Disease?
Kramer G, Mitteregger D, Marberger M. - Eur Urol. 2007 May 51 (5): 1202-16. Epub 2006 Dec 11.

Glycogen Synthase Kinase 3beta (GSK3beta) Regulates Differentiation and Proliferation in Neural Stem Cells from the Rat Subventricular Zone
Maurer MH, Bromme JO, Feldmann RE Jr, Jarve A, Sabouri F, Burgers HF, Schelshorn DW, Kruger C, Schneider A, Kuschinsky W. J. Proteome - Res. 2007 Mar; 6(3):1198-208.

3D Parallel Coordinate Systems – A New Data Visualization Method in the Context of Microscopy-Based Multicolour Tissue Cytometry (MMTC)
M Streit, Rupert C Ecker, K Österreicher, Georg Steiner, H Bischof, C Bangert, T Kopp, R Rogojanu -Cytometry Part A, 2006 Jul; 69(7):601-11.

An improved method for discrimination of cell populations in tissue sections using Microscopy-Based Multicolour Tissue Cytometry
Rupert C Ecker, Radu Rogojanu, Marc Streit, Katja Oesterreicher and Georg Steiner - 2006, Cytometry Part A, 69(3):119-23.

Leukocyte segmentation and classification in blood-smear images
Herbert Ramoser, Vincent Laurain, Horst Bischof, Rupert C Ecker - Proc. Of the Int. Conf. Of the IEEE Engineering In Medicine and Biology Society (EMBS); September 01-04, 2005.

Fast Automatic Segmentation of Nuclei in Microscopy Images of Tissue Sections
Vincent Laurain, Herbert Ramoser, Christoph Nowak, Rupert C Ecker - Proc. Of the Int. Conf. Of the IEEE Engineering In Medicine and Biology Society (EMBS); September 01-04, 2005.

Pimecrolismus leads to an apoptosis-induced depletion of T cells but not Langerhans cells in patients with atopic dermatitis
W Hoetzenecker, Rupert C Ecker, T Kopp, A Stuetz, G Stingl, A Elbe-Bürger - 2005, Journal of Allergy and Clinical Immunology 115(6): 1276-83.

Autocrine IL-10 partially prevents differentiation of neonatal dendritic epidermal leukocytes into Langerhans cells
S.Chang-Rodriguez, Rupert C Ecker, G Stingl, A Elbe-Bürger - 2004, Journal of Leukocyte Biology: 76(3): 657-66.

Application of Spectral Imaging Microscopy in Cytomics and Fluorescence Resonance Energy Transfer (FRET) Analysis
Rupert C Ecker, R de Martin, GE. Steiner, J A. Schmid - 2004, Cytometry 59A(2): 182-90.

Microscopy-Based Multicolour Tissue Cytometry at the Single Cell Level
Rupert C Ecker and Georg. Steiner - 2004, Cyometry 59A(2):172-81.

Corticosteroids but not Pimecrolimus affect viability, maturation and immune function of murine epidermal Langerhans cells
W Hoetzenecker, JG. Meingassner, Rupert C Ecker, G Stingl, A Stuetz, A Elbe-Buerger - 2004, Journal of Investigative Dermatology 122(3): 673-84.

Inhibition of Restenosis by Tissue Factor Pathway Inhibitor: in vivo and in vitro evidence for suppressed Monocyte Chemoattraction and Gelatinase Activity
CW. Kopp, T Hölzenbein, S Steiner, R Marculescu, H Bergmeister, D Seidinger, I Mosberger, C Kaun, M Cejna, R Horvat, J Wojta, G Maurer, Rupert C Ecker, R de Martin, E Minar - 2004, Blood 103(5): 1653-61.

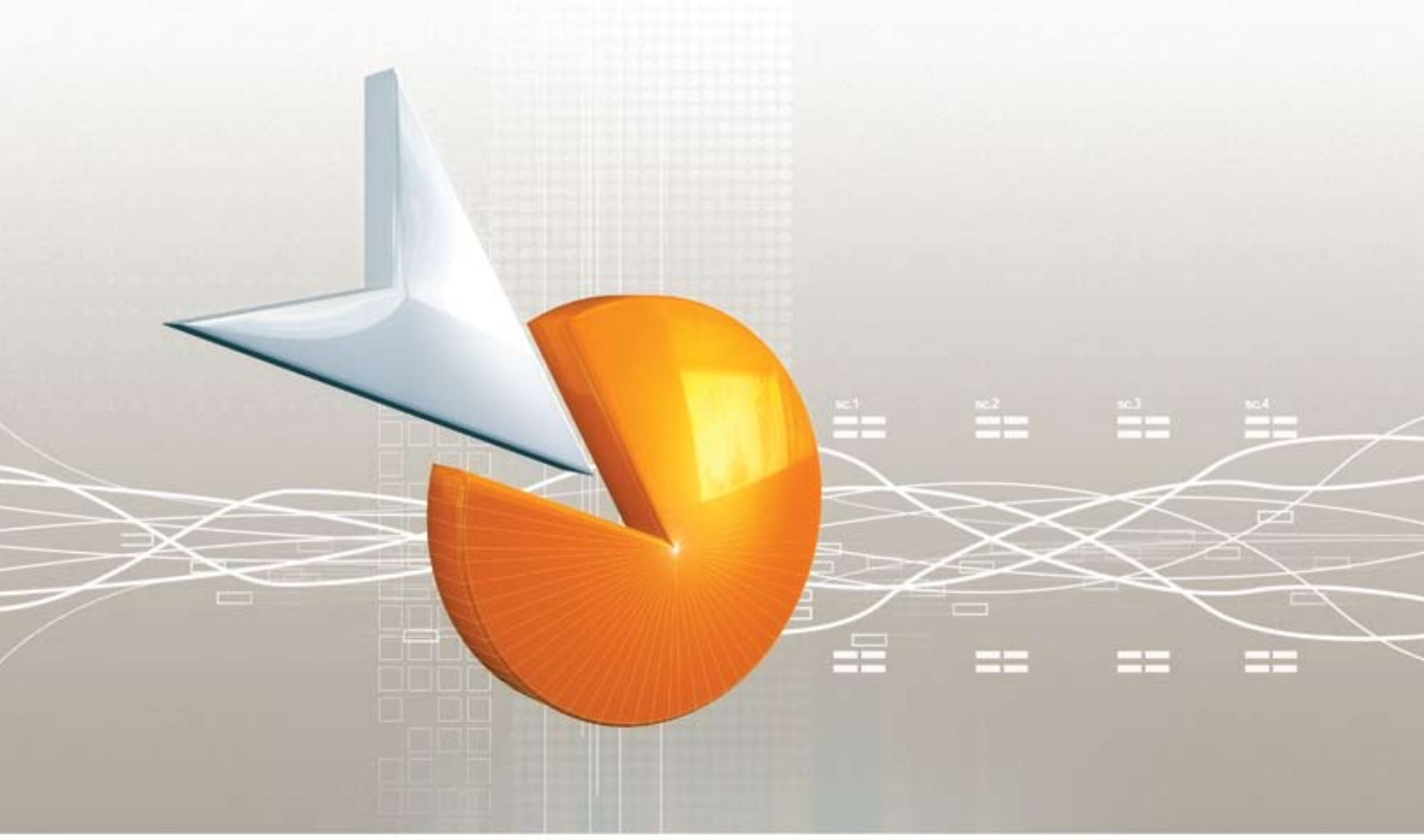
Overexpression of anti-CD75 reactive proteins on distal and collecting renal tubular epithelial cells in calcium-oxalate stone forming kidneys in Egypt
G Kramer, Georg Steiner, C Neumayer, M Prinz-Kashani, M Hohenfellner, M Gomha, M Ghoneim, M Newman, M Marberger - 2004, British Journal of Urology International 93(6): 822-826.

Cell-suface matrix proteins and sialic acids in cell-crystal adhesion; the effect of cristal binding on the viability of human CAKI-1 renal epithelial cells
G Kramer, Georg Steiner, M Prinz-Kashani, B Bursa, M Marberger - 2003, British Journal of Urology International 91: 554-559

Histopathologische Quantifizierung immunologischer Prozesse
Rupert C Ecker, R de Martin, and Georg Steiner - 2003, Biomedizinische Technik 48 (Suppl. 1): 546-547.

Automated data acquisition by confocal laser scanning microscopy and image analysis of triple stained immunofluorescent leukocytes in tissue
Georg Steiner, Rupert C Ecker, G Kramer, F Stockenhuber, M J Marberger - 2000, J Immunol Methods 237(1-2): 39-5.

Published by:
TissueGnostics GmbH
1020 Vienna, Austria
Tel. +43-1-2161190
Fax +43-1-2161190 90
www.tissuegnostics.com



TissueGnostics GmbH
Taborstrasse 10/2/8
A-1020 Wien, Austria
Tel.: +43 1 2161190
Fax: +43 1 2161190 90
www.tissuegnostics.com
E-mail: office@tissuegnostics.com

TissueGnostics USA Ltd.
420 N Larchmont Boulevard
CA 90004, Los Angeles, USA
Tel.: +1 323 4660499
Fax: +1 323 4660499
www.tissuegnostics.com
E-mail: office@tissuegnostics-usa.com

TissueGnostics Romania SRL
Nicolae Iorga Boulevard nr. 51C
700213 Iasi, Romania
Tel.: +40 332 405866
Fax: +40 332 405867
www.tissuegnostics.com
E-mail: office@tissuegnostics.ro

TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS