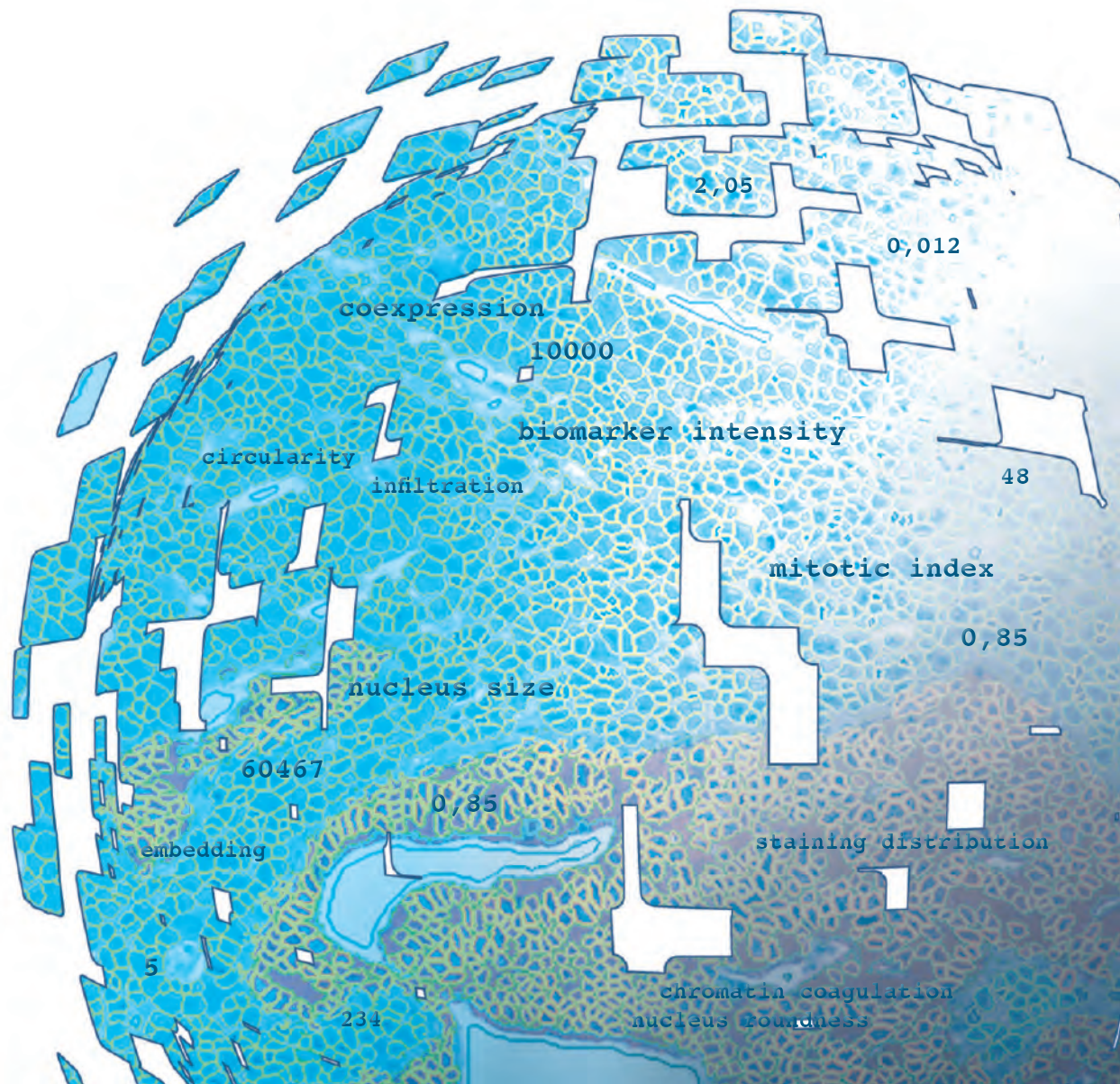


Digital Pathology Image Analysis with Definiens



Transforming Pathology into a Quantitative Science

Definiens brings the power of automated image analysis to pathology, transforming it into an accurate and quantitative science.

Whether you are performing translational research for tumor morphological and biomarker profiling, determining drug toxicity in pre-clinical safety or drug efficacy in early clinical trials, or managing a laboratory that offers digital pathology services, Definiens has the right digital pathology image analysis solution for you.

Definiens' revolutionary technology handles real world challenges such as the high degree of variability between tissue samples and staining intensities. It automatically identifies regions of interest and quantifies objects within them with great accuracy and consistency.

Definiens supports all digital image and slide formats, as well as a wide variety of staining protocols including H&E, IHC, IF, SISH and FISH. The technology is highly scalable – capable of growing from a single deployment in a research laboratory to a high production environment analyzing thousands of slides per day.

To date, more than 1,400 automated image analysis applications have been deployed using Definiens.

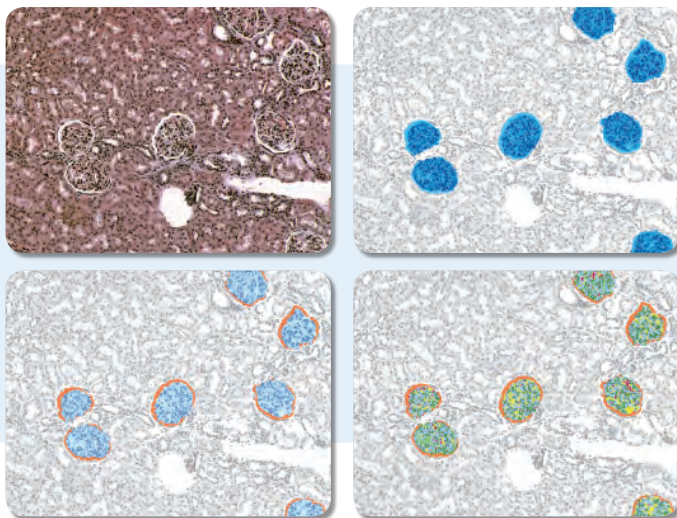


Figure 1 Detailed segmentation and quantification of glomeruli in H&E stained rat kidney tissue

“The availability of image analysis tool sets is essential as we enter the age of personalized medicine. We now have the ability to provide a number of image analysis tools across a wide range of technologies to help facilitate biomarker assessment in the clinical marketplace. The cooperation with Definiens provides us with access to a very interesting technology and a team of scientists that have extensive experience in software development and biomarker analysis.”

Ken Bloom, CMO, Clariant, Inc.

From Images to Clinical Decisions

Interpreting tissue slides manually is labor intensive, costly and carries a significant risk of human error and inconsistency. These factors can impede decision making, increase failure rates and lengthen time to-market.

Digital pathology, in which tissue slides are captured in digital form, is transforming the field, enabling images to be shared widely and creating the possibility of automated interpretation.

Automating tissue analysis brings many benefits compared to manual workflows including increased throughput, greater accuracy and enhanced reproducibility. Definiens' patented technology handles the high degree of heterogeneity in slides and stains to deliver fast, detailed and consistent results.

In the laboratory, Definiens provides detailed quantification of morphology and biomarker expression, enabling the correlation of tissue specimens with treatment, molecular information or patient outcome. This enables a better understanding of the underlying biology and directly supports translational research and the development of new diagnostic biomarkers.

In the clinic, Definiens supports pathologists in the day-to-day task of interpreting biopsies and tissue slides, helping to reduce inter- and intra-reader variability. The technology is currently being utilized in diagnostic labs processing thousands of slides per day.

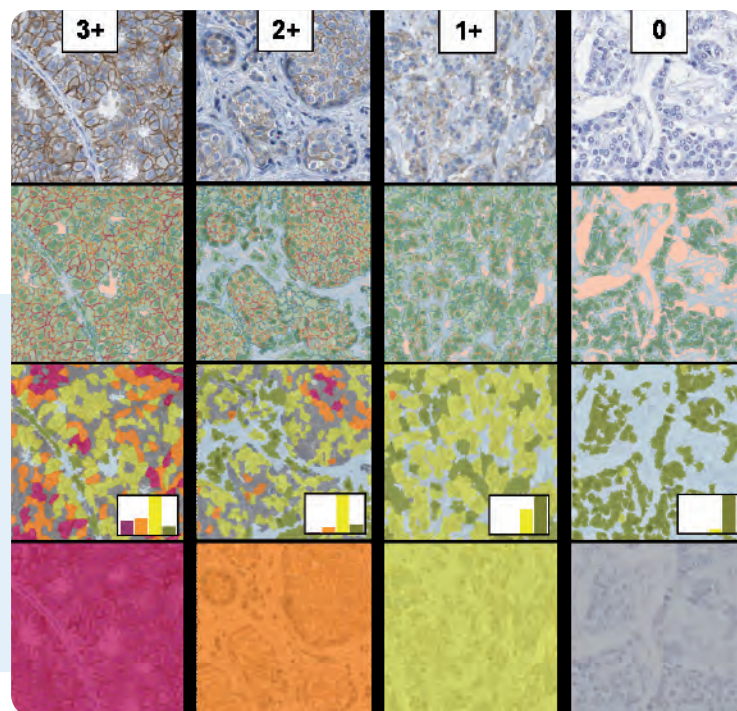


Figure 2 Analyzing the Dako Her2neu score on a true cell-by-cell basis

Use Case: Survival Prediction of Esophageal Cancer Patients

In this example, a study was conducted in collaboration with the Helmholtz Institute (Munich, Germany) comparing manual H-score evaluation (Dako Herceptest) of esophagogastric TMA cores. The aim of the study was to stratify patients based on the expression profile of IHC Dako Her2 stain in tumor tissue. A total of 391 tissue cores from 150 patients were analyzed.

Figure 3 shows an example TMA core, while figure 4 illustrates Definiens' classification algorithms which delineate 1) tumor from non-tumor regions of interest 2) nucleus, cytoplasm and membrane cellular attributes and 3) Her2 membrane biomarker expression, providing a comprehensive, multiparametric data set of morphology and Her2neu expression.

Definiens used advanced image analysis and an agnostic data mining approach to reveal a set of multiparametric features that stratified the patient cohort retrospectively by correlating these feature sets with clinical outcome (disease free survival). The Kaplan-Meier curve which was generated using manual semi-quantitative evaluation (figure 5) was not statistically significant (p -value 0.103). The Kaplan-Meier curve generated using the Definiens image analysis and data mining approach (figure 6) was not only statistically significant (p -value $< 4.07e-08$), but produced superior patient stratification as compared to the manual approach.

The study strongly indicates that there is promise in improving the diagnostic accuracy of tissue-based diagnostics that involve immunohistochemistry.

Figure 3

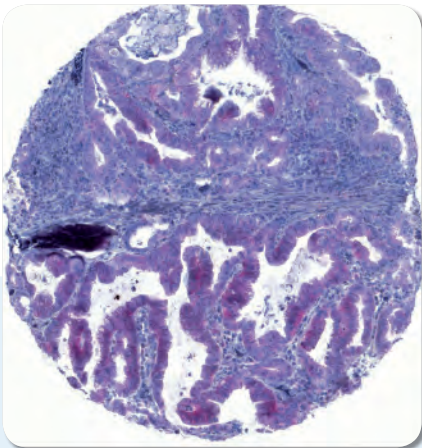


Figure 4

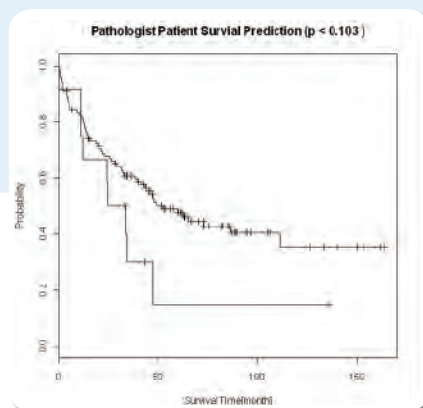
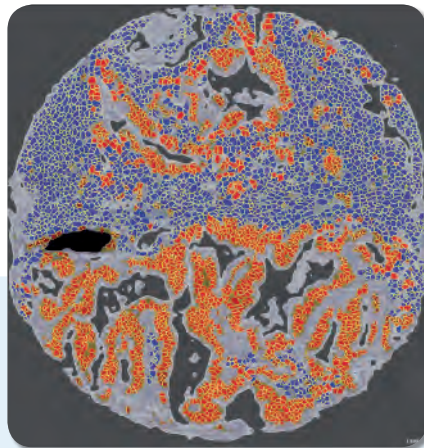


Figure 5

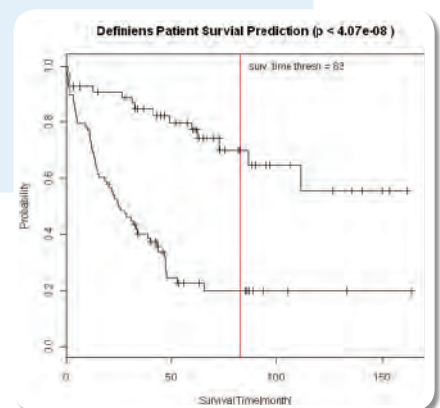


Figure 6

Use Case: Morphological Analysis of H&E Stained Tissue

H&E (hematoxylin and eosin) stained tissue poses significant challenges for automated interpretation due to the rich heterogeneity of the tissue and the lack of available contrast features. However, using a contextual, semantic approach, Definiens provides accurate and detailed segmentation and quantification of H&E tissue.

Figure 7 shows the rich detail and accuracy delivered by Definiens when used for the classification of nuclei within this esophageal tissue specimen.

Currently, there are no official guidelines establishing the pathologic grading of non-small cell lung cancer (NSCLC) tissue for patient care. Performing this task semi-qualitatively is difficult and results are often highly subjective.

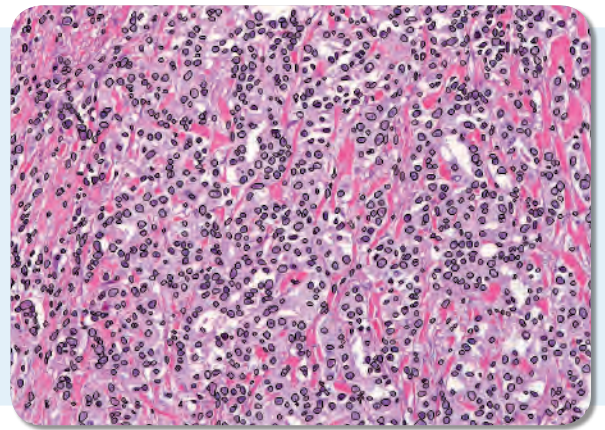


Figure 7

Figures 8 and 9 show the analysis of an adenocarcinoma region of NSCLC. Figure 8 shows the original tissue, while figure 9 shows the results “post-analysis”. Purple reveals papillary regions, while brown areas denote acinar regions. Yellow shows whitespace, and blue areas show surrounding tissue. The Definiens image analysis solution has automatically identified the location of the different regions and established the transition zones from one morphology to the next.

Images courtesy of M.D. Anderson.

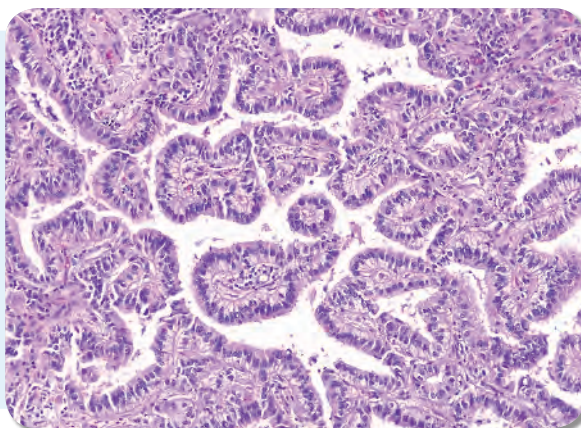


Figure 8

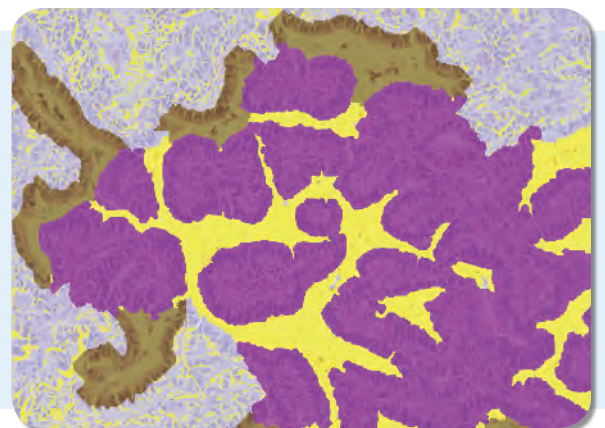


Figure 9

Use Case: SOX2 Biomarker Expression within Non-Small Cell Lung Cancer Tissue

In this study, SOX2 over-expression in NSCLC tissue was analyzed with a Definiens solution and compared with FISH analysis of SOX2 over amplification.

The aim of the study was to verify SOX2 amplification and protein over expression and to compare these results with patient and tumor characteristics. A total of 940 NSCLC tissue cores from two independent population-based cohorts containing predominantly adenocarcinomas of the lung and squamous cell lung carcinomas were evaluated.

Figure 10 shows example original TMA cores stained for nuclear SOX2 expression, while figure 11 shows the images post-processing using Definiens Tissue Studio™.

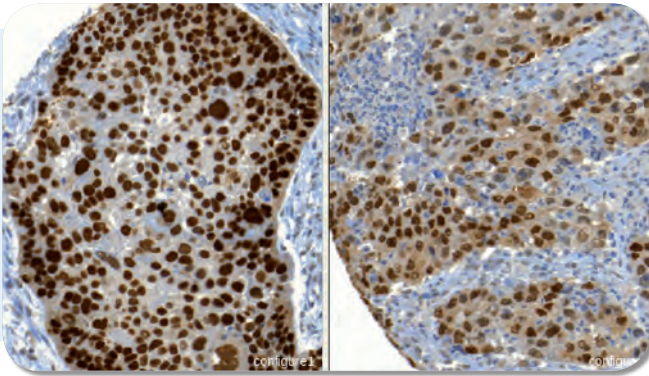


Figure 10

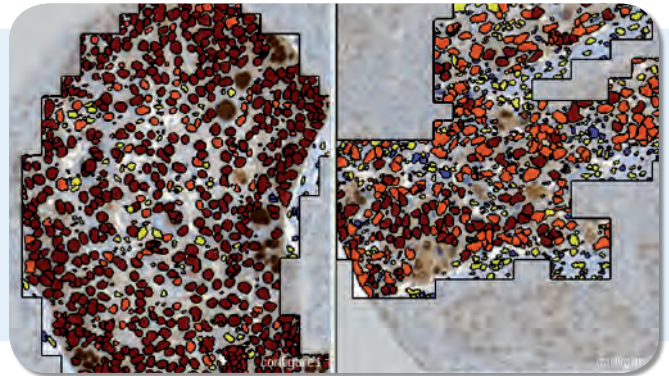


Figure 11

The study showed that nuclear expression of SOX2 assessed with Definiens Tissue Studio™ correlated with FISH analysis of SOX2 amplification. A Kaplan-Meier survival analysis (figure 12) was performed and demonstrated that over-expression of SOX2 as evaluated by both IHC and FISH correlated with longer overall survival status of the NSCLC patients in two independent cohorts.

Images courtesy of The University of Tuebingen.

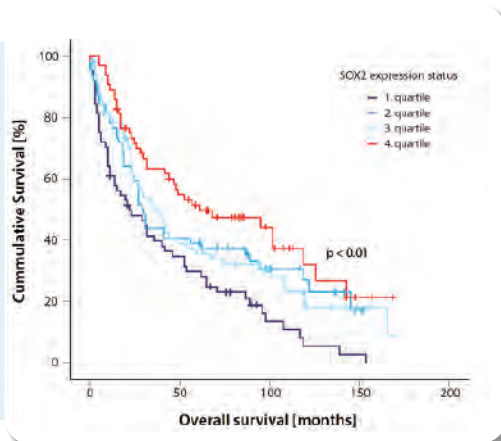


Figure 12

Use Case: Analysis of Multiplexed Immunofluorescence Assays

Multiplexed biomarker analysis is an important approach in translational research in order to better understand how biomarkers interact individually, and in concert with one another. New immunofluorescence (IF) staining technologies allow multiplexing of more than 20 proteins. Figure 13 shows the false color composite image of eight overlaid IF biomarkers.

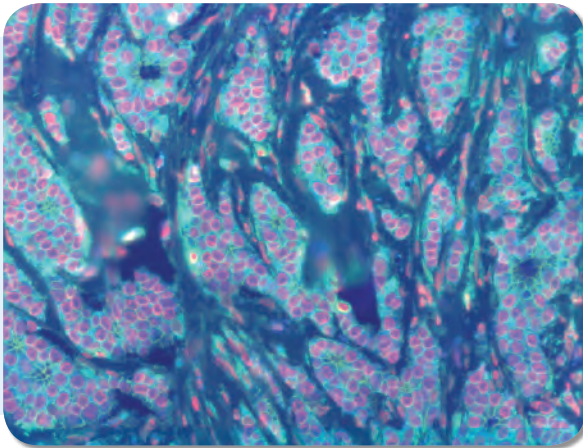


Figure 13

In this example, a Definiens solution detected cells and sub-cellular components such as membrane, nuclei and cytoplasm in a multiplexed assay. The solution also distinguishes between tumor and stromal cells based on tissue morphology (figure 14).

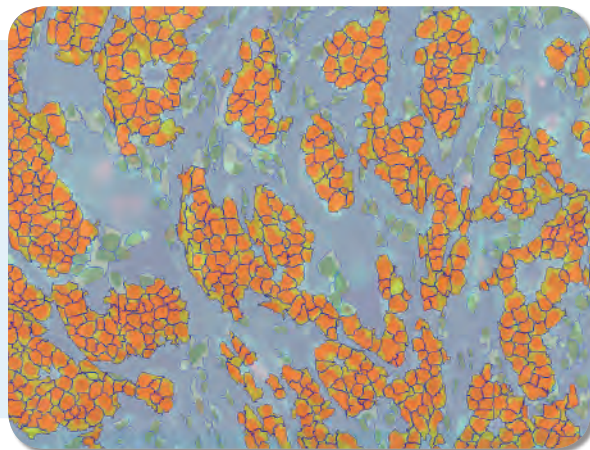


Figure 14

The quantitative analysis produced a highly detailed readout of the expression and co-expression profile of the stack of markers per tumor section, per tumor cell, and even per sub-cellular compartment within each tumor cell. The solution created a fully automated workflow and provided a rich data set to support subsequent correlation studies.

Use Case: Accelerating Pre-Clinical Safety Studies

Pre-clinical pathologists are under enormous pressure to assess the safety profile of drug candidates in order to accelerate FDA approval.

In this example, regulatory authorities requested further toxicology information in order to grant approval of a drug application submitted by Novartis.

A Definiens image analysis solution automatically detected individual crypt units across 16,000 PCNA and BrdU stained images of small intestine tissue in mice. The endpoint was the mitotic index (ratio of positive to negative cells) for each individual crypt. The high degree of morphological variation made automated nucleus and crypt detection particularly challenging.

Figure 15 (PCNA) and figure 16 (BrdU) show analysis samples from the initial benchmark study where automated results were compared against pathologist readouts. The automated solution reduced the length of the study from an estimated 18 weeks using a manual approach to just 4 weeks.

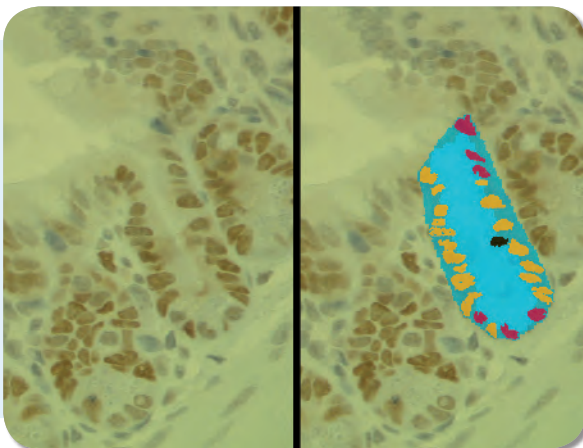


Figure 15

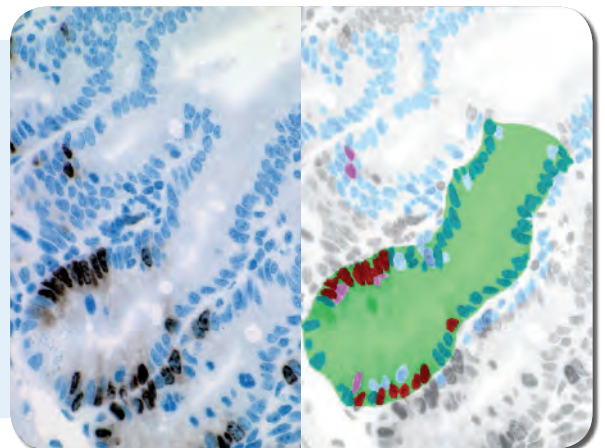


Figure 16

“Definiens solved our problem to automatically detect small intestine crypts and count proliferating and non proliferating cells which was not possible with other software solutions. (...) Beside substantial time savings on the way to FDA approval, with a fully automated process more sections/images can be analyzed thus producing more robust results.”

Dr. Elke Persohn, Head of the Cellular Imaging Group at SP&A, Pathology, at Novartis Pharma AG in Basel

Use Case: Xenograft Analysis

Xenograft tumor models play an important role in pre-clinical cancer research. By implanting human tumor cells in the tissue of animal models, the effects of compounds on tumor growth can be tested by analyzing tumor size, angiogenesis, proliferation of tumor cells or apoptosis.

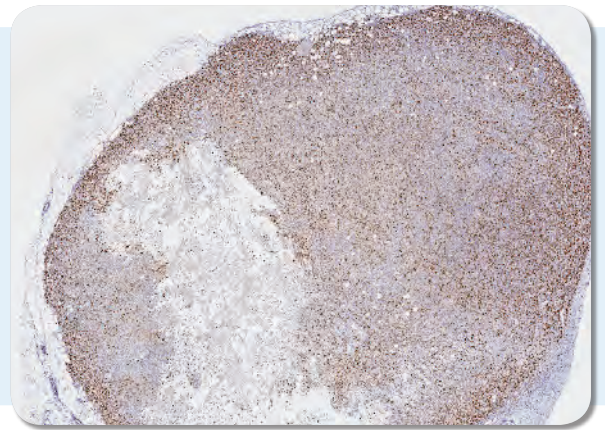


Figure 17

Definiens Tissue Studio™ supports the automated and detailed analysis of xenografts. Figure 17 shows the original xenograft tissue stained with Ki67. After separating the xenograft from the host tissue, the viable tissue sections have been distinguished from the apoptotic tissue sections (figure 18).

Within the viable sections, the highly detailed analysis of biomarker expression in the nuclei distinguishes not only positive and negative nuclei but also weak, moderate and strong biomarker expression levels (figure 19).



Figure 18

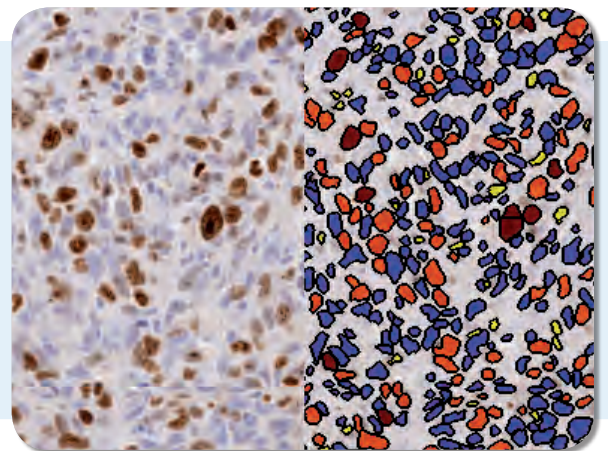


Figure 19

Application Areas Summary

Definiens solutions for digital pathology image analysis are used across a full spectrum of research and clinical applications:

Basic Research

- Discover new drug targets and disease-related biomarkers
- Elucidate signal transduction pathways
- Discover underlying biological mechanisms

Pre-Clinical Research

- Validate biomarkers for toxicity and efficacy in animal models
- Quantify biomarker expression and morphology in animal tissue as a function of disease state or drug response (e.g. dosage, time lapse)
- Understand correlations between disease markers expressed in human tissue compared to animal tissue

Translational Research

- Identify and validate tissue-based biomarkers that are predictive or prognostic
- Measure the effect of target expression, pre- and post-treatment
- Quantify disease markers, pre- and post-treatment

Clinical Trials

- Analyze human tissue pre- and post-treatment to determine pharmacodynamic and pharmacokinetic effects of treatment
- Quantify tissue-based surrogate biomarkers for toxicity

Clinical Diagnostics

- Increase accuracy and consistency of current tissue-based diagnostics
- Develop next generation tissue-based diagnostics leveraging the full potential of detailed biomarker and morphological quantification

Definiens Image Analysis Software and Support for Digital Pathology

Definiens offers a comprehensive suite of products, backed by outstanding technical and domain expertise.

Definiens Tissue Studio™ is an easy to use, out-of-the-box solution that provides a simple “learn by example” interface for the creation of custom automated image analysis solutions. Definiens Tissue Studio™ can be used for the majority of image analysis tasks related to digital pathology including translational research and biomarker investigation. More information can be found at: www.tissuestudio.com.

Definiens Developer XD is a powerful solution for the development of biomedical image analysis applications using an intuitive graphic user interface. It is used to create highly customized image analysis and data reporting solutions or best-in-class biomarker and morphological analysis for diagnostic purposes. More information can be found at: <http://developer.definiens.com>.

Both **Definiens Tissue Studio™** and **Definiens Developer XD** are highly scalable and capable of growing from a specific image analysis application for a single research project to solutions in high throughput environments.

Definiens Academy provides comprehensive training for developers and scientists, enabling them to create and use automated image analysis solutions.

Definiens Consulting comprises our in-house team of technology and domain experts who support customers in creating and deploying custom digital pathology image analysis solutions.



Summary

Definiens is transforming pathology into a quantitative science. The unique patented technology automatically identifies, quantifies and measures structures in tissue images with a high degree of detail and accuracy.

By automating tissue analysis, Definiens helps to ensure higher consistency, better understanding of the underlying biology and validation of new biomarkers in the context of personalized medicine.

Definiens' digital pathology image analysis applications are used in all areas of research and clinical diagnostics because they deliver deeper insights, faster results and better decisions.

DEFINIENS[®]

Understanding Images

DEEPER INSIGHTS
FASTER RESULTS
BETTER DECISIONS

Definiens develops software solutions for biomedical image and data analysis. The revolutionary technology provides unprecedented accuracy in identifying and quantifying biological attributes within images and turning them into knowledge. The outcome is deeper insights, faster results and better decision support in both the clinic and the laboratory.

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OBL-0002-02-030311

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