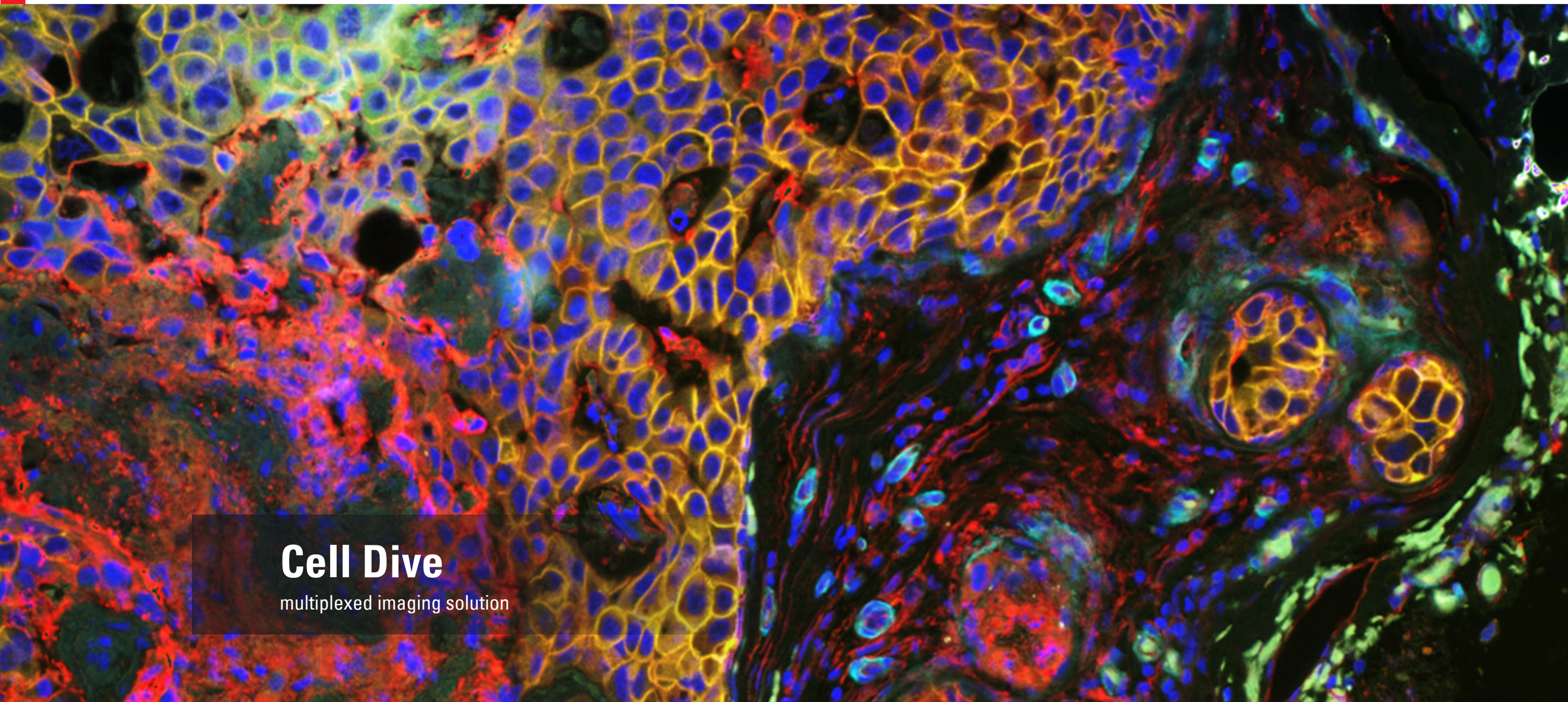
From Eye to Insight

Leica
MICROSYSTEMS



Cell Dive

multiplexed imaging solution

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Leica MICROSYSTEMS

> **Intro**

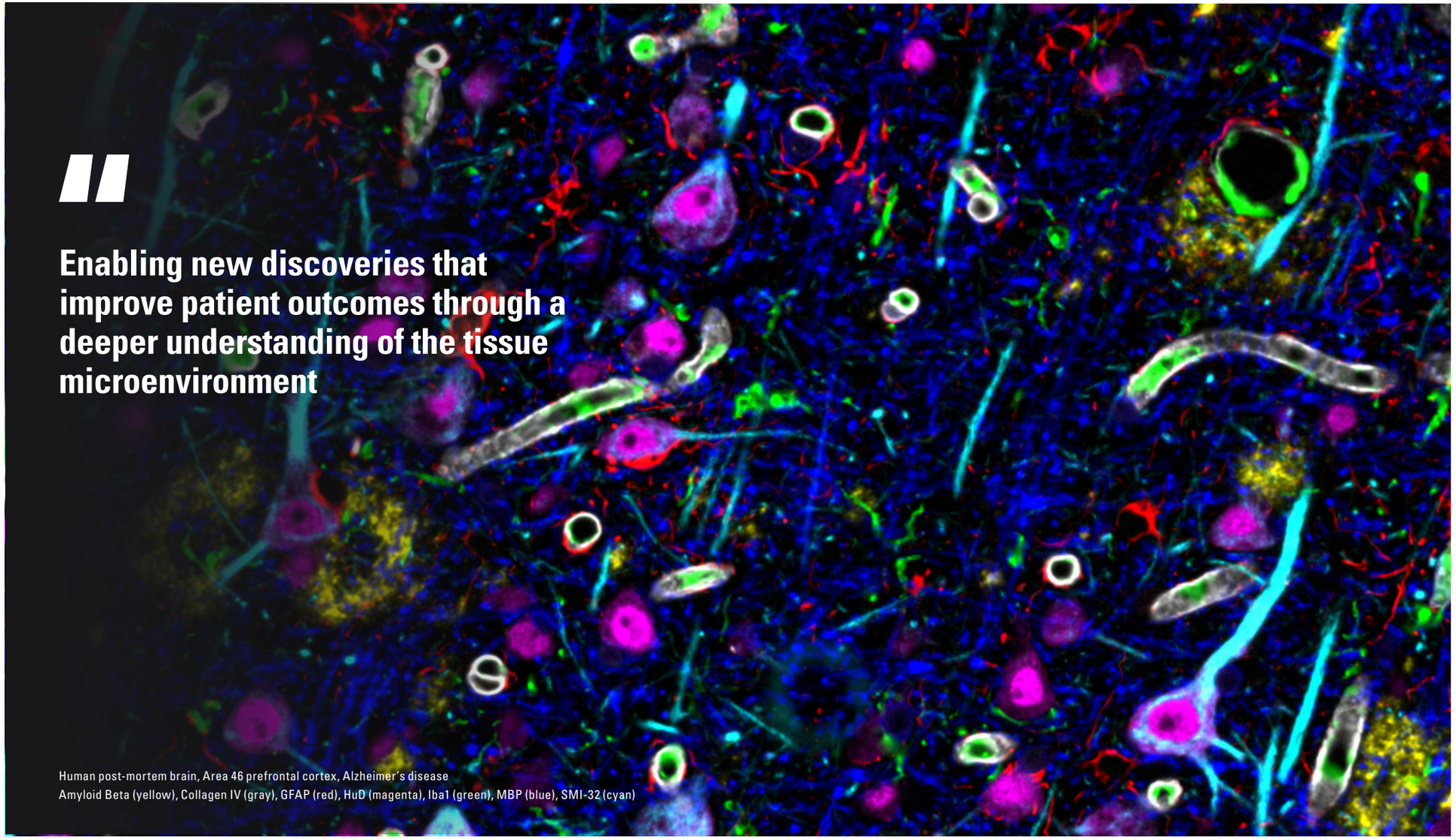
> Component Overview

> Quantifying Biomarkers

> Multiplexing Overview

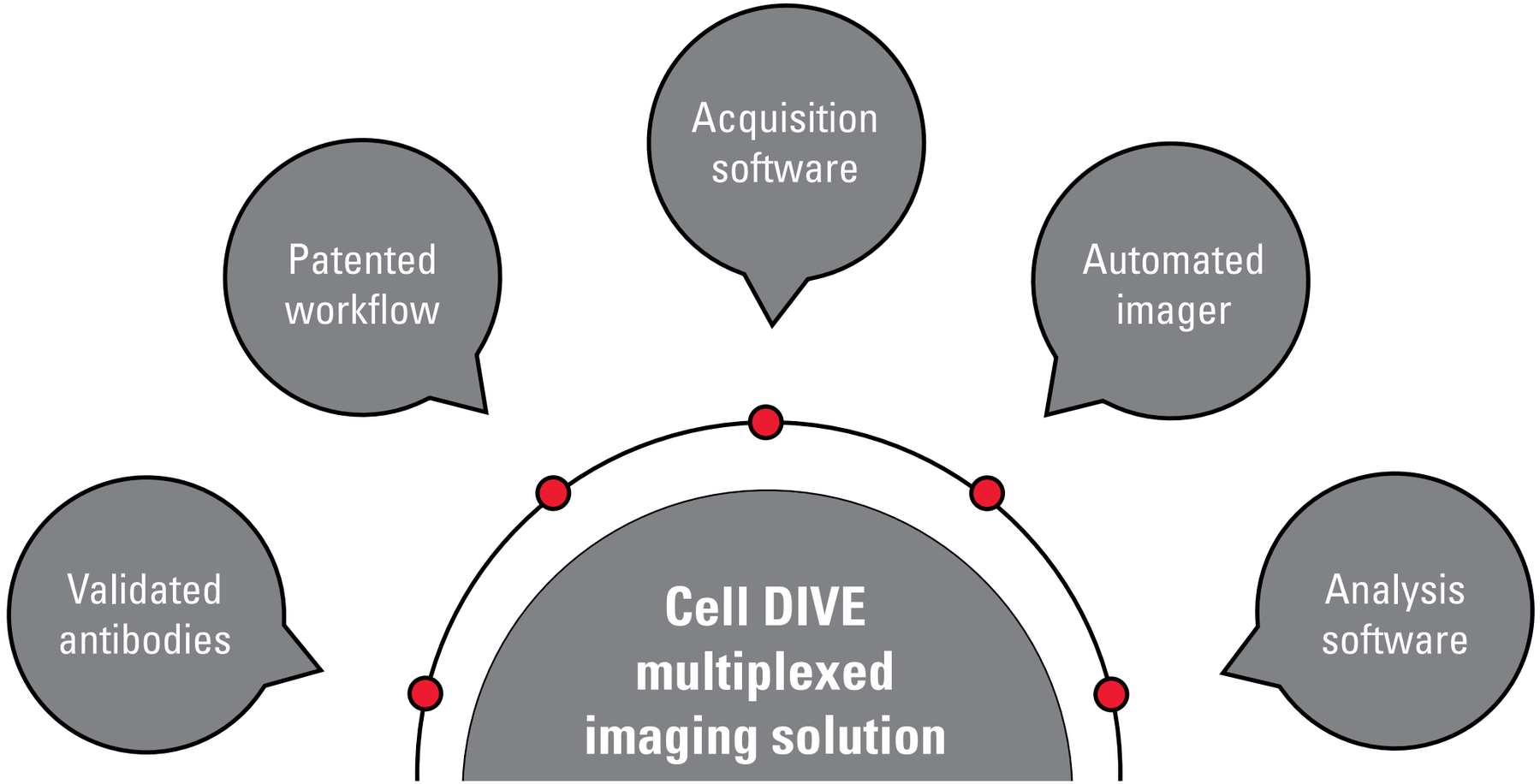
> Imaging Steps

> Studies and Examples



> Intro
> **Component Overview**
> Quantifying Biomarkers
> Multiplexing Overview
> Imaging Steps
> Studies and Examples

COMPONENTS THAT WORK TOGETHER TO GIVE YOU RELIABLE, REPRODUCIBLE RESULTS



From Eye to Insight

> Intro

> Component Overview

> **Quantifying Biomarkers**

> Multiplexing Overview

> Imaging Steps

> Studies and Examples

Clearly visualize, identify, and quantify significant biomarkers

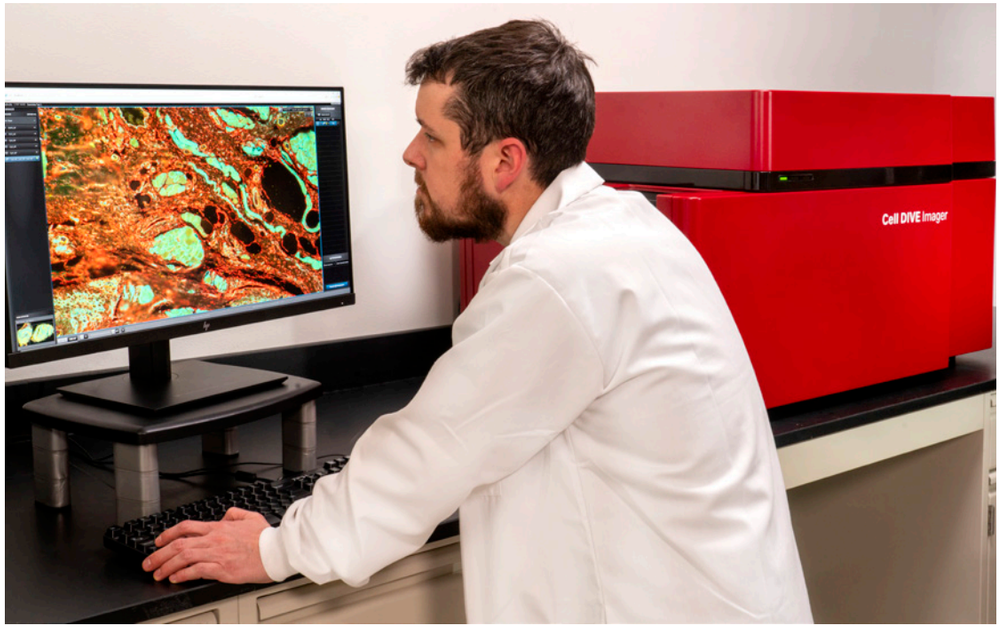
Customizable: design and configure experiments exactly how you want

> Use any formalin-fixed paraffin-embedded (FFPE) tissue sample format, including whole tissue sections or tissue micro arrays (TMA)

> Design custom biomarker panels from 350+ validated antibodies, or validate your own

> Choose any combination of 60+ biomarkers for visualization

> Decide what portion of the sample is imaged and analyzed, from whole slides to specific regions of interest



Precise: collect the data valuable to you, and trust in your results

> The Cell DIVE automated imager is engineered for precision, speed, and sensitivity

> Superior image resolution enables spatial biomarker mapping at a single-cell level

> Automated distortion correction, image alignment, and stitching yield precise spatial maps

> Advanced imager calibration enables correlation across samples and imagers*

*Correlation across imagers not supported in near-infrared (IR) channel

Proven: years of experience plus three tested and refined technologies are merged into one solution

High-resolution imager (Cytiva)

> 20+ years of experience developing automated fluorescence microscopes, from super resolution to high content analysis

> Thousands of publications spanning the full imaging portfolio

Patented workflow (GE Research Center)

> Developed by scientists, for scientists, over the course of ten years

> Validated by collaborators globally

> Technology used in over 40 peer-reviewed publications

Comprehensive analysis software (Indica Labs, Inc.)

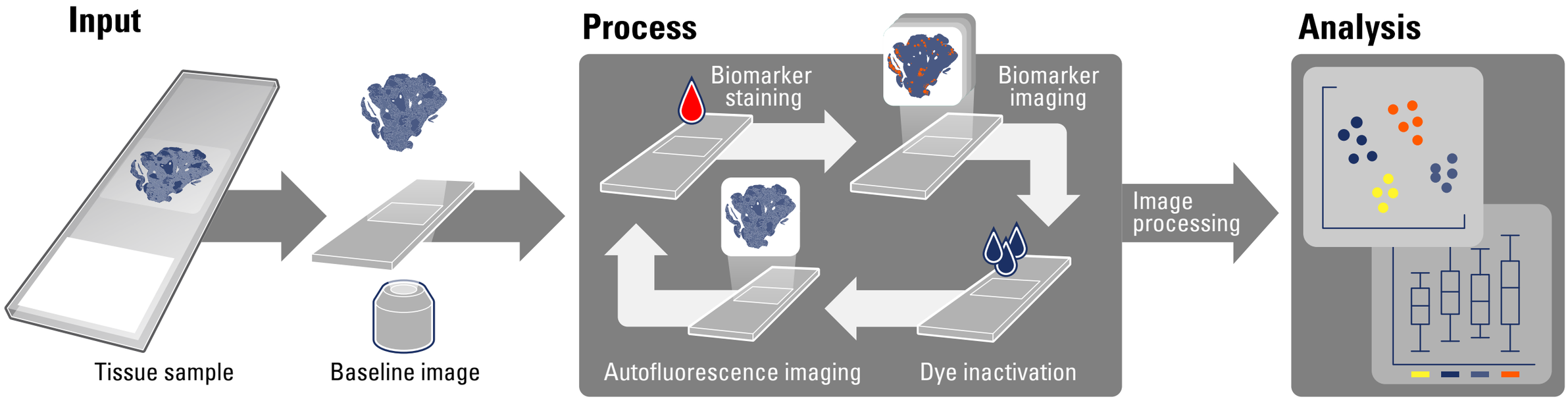
> With a focus on easy-to-use modules, extensive analytics, and scalability, HALO™ image analysis platform is the gold standard for quantitative tissue analysis

> 900+ installations worldwide, 350+ publications citations, and direct technical support in North America, Europe, China, and Japan

> Intro
> Component Overview
> Quantifying Biomarkers
> **Multiplexing Overview**
> Imaging Steps
> Studies and Examples

COMPLETELY CUSTOMIZABLE, PRECISE MULTIPLEXING

Spatially map 60+ biomarkers from just one tissue section, at the single-cell level



> Intro

> Component Overview

> Quantifying Biomarkers

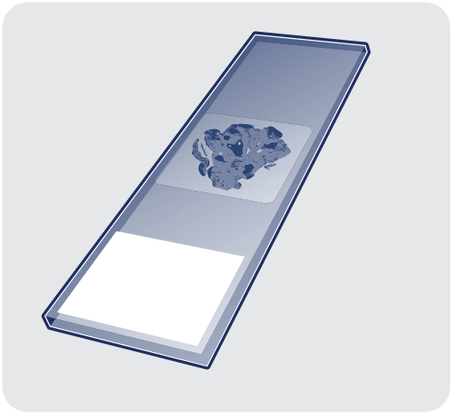
> Multiplexing Overview

> **Imaging Steps**

> Studies and Examples

CELL DIVE IMAGING STEPS

Image processing starts



1. Sample preparation

Prepare any FFPE tissue sample or tissue microarray.

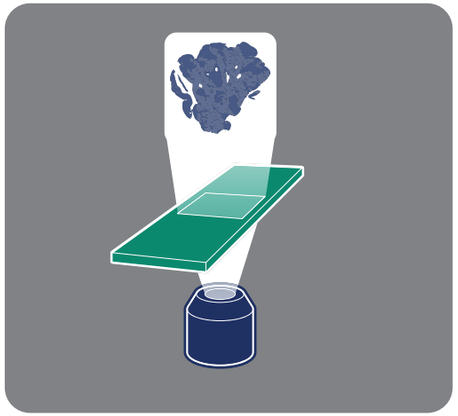
> Unique two-step antigen retrieval ensures broad epitope unmasking

2. Region selection

Select regions of interest (ROI) from a virtual hematoxylin and eosin (H&E) image of the entire sample.

> Capture only the data you want with ROIs of any shape or size

> Capture regions up to 45 mm × 20 mm

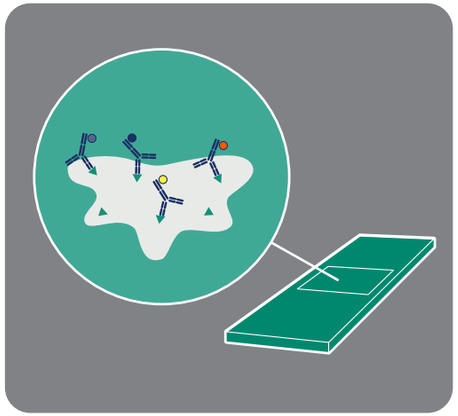


3. Autofluorescence imaging

Capture 20x images for downstream autofluorescence removal.

> Autofluorescence imaging is performed on tissue stained with DAPI only.

> During image acquisition, images are automatically processed to perform distortion correction, blank glass subtraction, and flat-field correction.



4. Biomarker staining

Stain the tissue sample with DAPI and up to four dye-conjugated antibodies at one time.

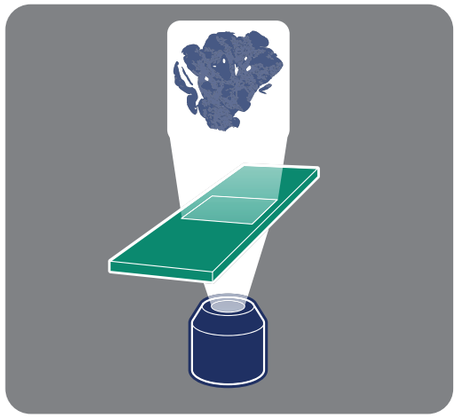
> Create a custom panel of biomarkers from our list of over 350+ validated antibodies, or validate your own

> Validated antibodies are tested for specificity, sensitivity, and antigen effects.

> Intro
> Component Overview
> Quantifying Biomarkers
> Multiplexing Overview
> **Imaging Steps**
> Studies and Examples

CELL DIVE IMAGING STEPS

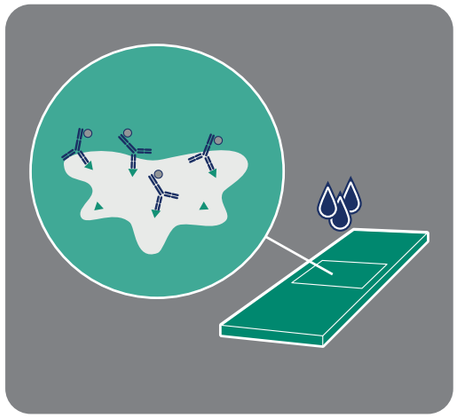
Image processing continues



5. Biomarker imaging

Capture the stained biomarker image.

> During image acquisition, images are automatically processed to perform distortion correction, blank glass subtraction, and flat-field correction.

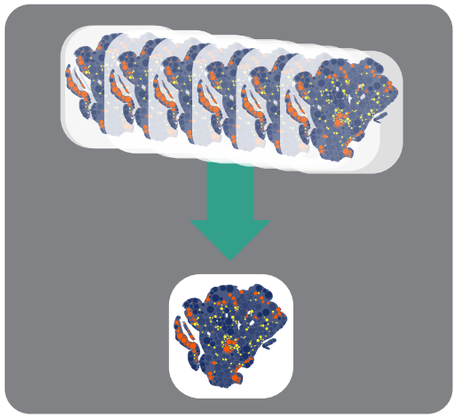


6. Dye inactivation

Use our patented dye-inactivation process to turn off dye molecules without damaging the sample, allowing for additional staining cycles with new biomarkers.

> Validated antibodies have been evaluated for antigen effects due to repeated exposure to dye-inactivation solution

Repeat steps 3 through 6 for 60+ biomarkers.



7. Image processing

Conduct true single-cell analysis due to the automated, patented, post-acquisition processing that delivers seamlessly stitched and precisely aligned data.

> Autofluorescence removal
> Stitching
> Image alignment.

8. Segmentation and analysis

Segment, extract features, and analyze data to determine abundance and localization of specified biomarkers for comprehensive spatial mapping.

> Seamless import into HALO™ image analysis platform
> Non-proprietary.TIFF file type compatible with other analysis software platforms.

Leica MICROSYSTEMS

> Intro

> Component Overview

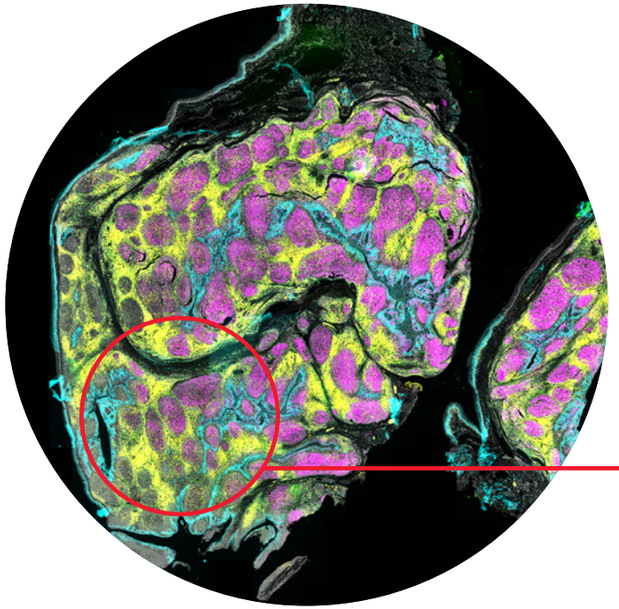
> Quantifying Biomarkers

> Multiplexing Overview

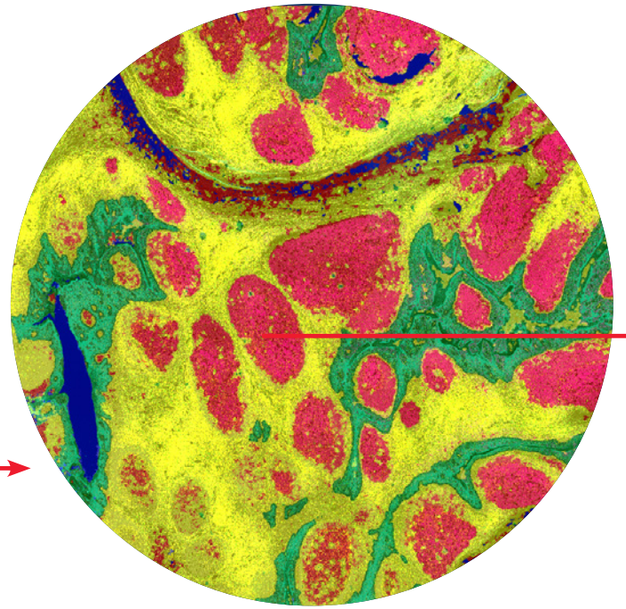
> **Imaging Steps**

> Studies and Examples

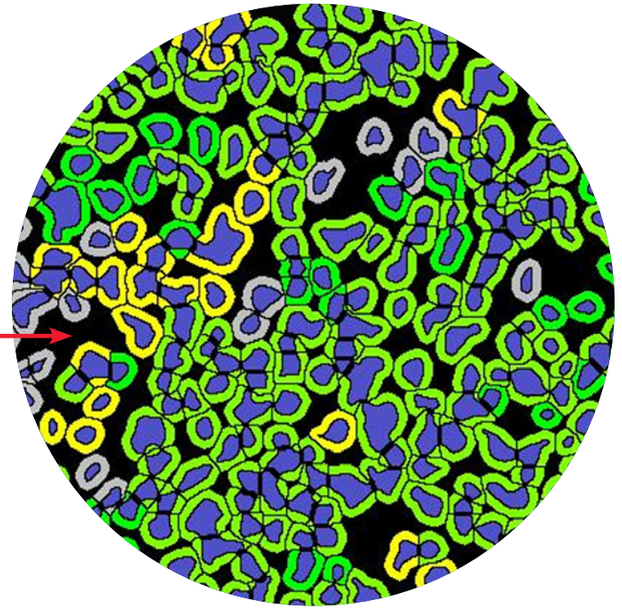
CELL DIVE IMAGING STEPS SINGLE-CELL ANALYSIS OF TONSIL TISSUE STAINED WITH 30 MARKER CELL DIVE PANEL



Immunofluorescence image of tonsil tissue stained with a 30 marker Cell DIVE™ panel. The image shows five of the 30 markers used in the study – HER2, CD8, CD20, CD3, and DAPI.



Compartmental masks originating from classification analysis of tonsil tissue.
Germinal centers are marked in red, squamous epithelium in green, and undesignated tissue in yellow.



Single-cell analysis of cellular phenotypes following nuclear segmentation. Nuclei are masked in blue. Green bands around the nuclear mask mark killer T cells (CD3+, CD8+) and yellow bands around the nuclear mask mark non-killer T cells (CD3+, CD8-).

From Eye to Insight

> Intro

> Component Overview

> Quantifying Biomarkers

> Multiplexing Overview

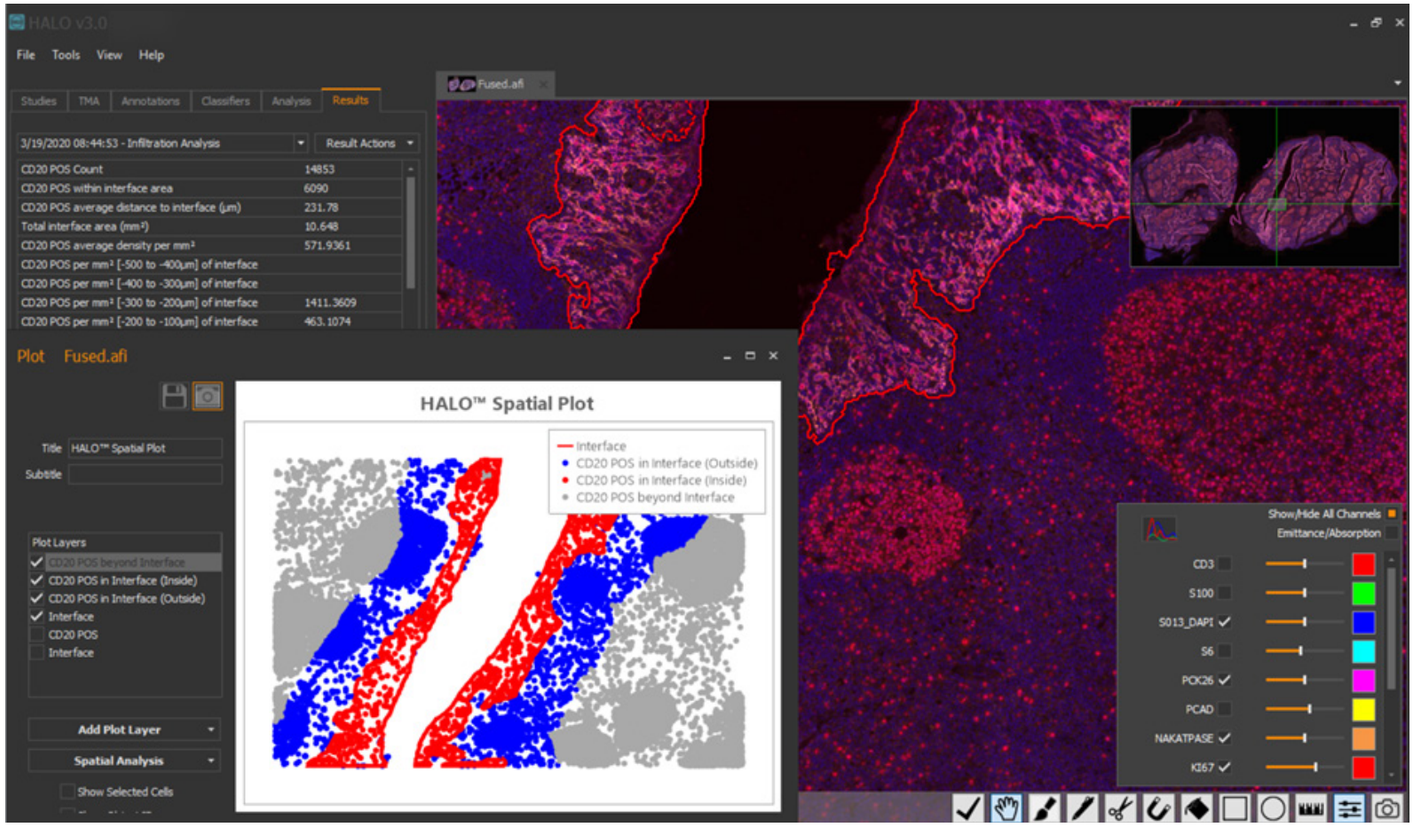
> Imaging Steps

> **Studies and Examples**

Analyzing thousands of data points from even just one single tissue section can be a struggle.

We have simplified the process by providing direct input into the industry-leading HALO™ image analysis platform.

- Unmatched scalability for high-through-put quantitative tissue analysis
- Simplified analysis workflow with add-on modules so you can spend more time analyzing data
- Easy, intuitive interface requiring no previous bioinformatics experience
- Quickly analyze large tissue regions and expression patterns of an unlimited number of
biomarkers in any cellular compartment
- Precisely define cell phenotypes and spatial relationships.



> Intro

> Component Overview

> Quantifying Biomarkers

> Multiplexing Overview

> Imaging Steps

> **Studies and Examples**

CELL DIVE IMAGING AT WORK

This study was completed by the Royal College of Surgeons in Ireland (RCSI) in collaboration with GE Research and Queen's University Belfast and the University of Stuttgart. With a focus on clinical and patient-centered research, RCSI succeeds in leading impactful discoveries which address global health challenges

The impact of heterogeneity in the microenvironment of primary colorectal cancer on apoptosis marker

RCSI Leading the world to better health

Introduction

Studies have shown that apoptosis sensitivity of tumors is a predictor of patient outcome in stage III colorectal cancer (CRC). An understanding of apoptosis resilience in tumors in relation to tumor cell heterogeneity may contribute to a better understanding of treatment options for patients.

Patient cohort

- 165 colorectal cancer patients
- 128 stage III patients
- Folinic acid (leucovorin), fluorouracil, and oxaliplatin (FOLFOX) adjuvant chemotherapy
- Retrospective
- 65-month median follow-up

Aims

1. To provide detailed insight of the spatial heterogeneity of CRC on a cellular level
2. To study apoptosis resilience in individual cells in combination with spatial markers
3. To identify potential novel cellular biomarkers to predict CRC patients' response to adjuvant chemotherapy

Summary

- Tumor cells determine the apoptopic expression. Immune cells barely contribute to the overall mean level.
- The primary tumors' cell type composition might be an exploitable prognostic
- The presence of PQ1+ regulatory T cells in primary tumor tissue was associated with increased risk for recurrence

Collaboration between

RCSI RESEARCH CENTRE FOR SYSTEMS MEDICINE · GE · QUEEN'S UNIVERS BELFAST · University Stuttgart

From Eye to Insight

> Intro

> Component Overview

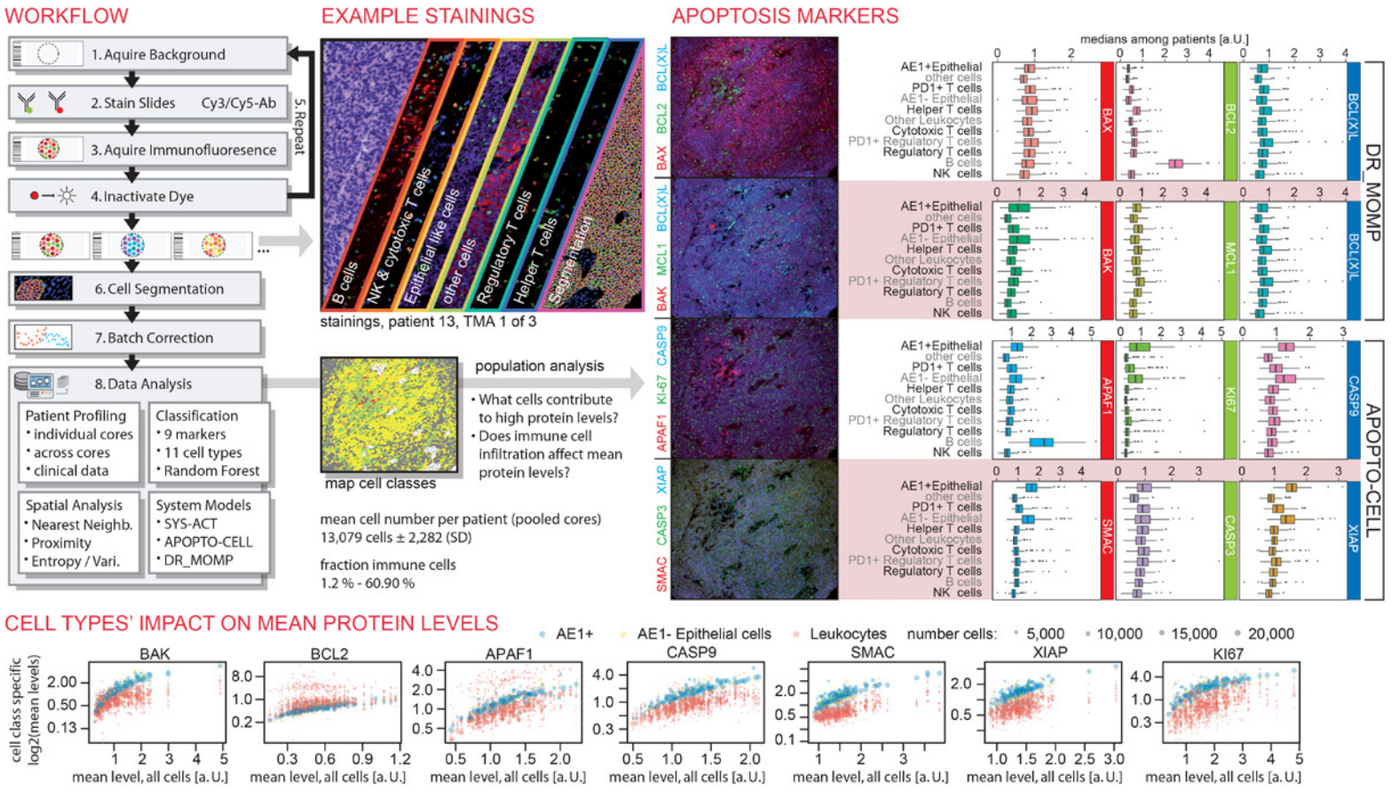
> Quantifying Biomarkers

> Multiplexing Overview

> Imaging Steps

> **Studies and Examples**

Exploratory multiplex tissue image analysis of the impact of heterogeneity in the microenvironment of primary colorectal cancer on apoptosis marker in patients



AU. Lindner[1], M. Salvucci[1], X. Stachtea[2], S. Carberry[1], PD. Dunne[2], A. Sood[3], EI. McDonough[3], S. Cho3, P. Laurent-Puig[4], S. Van Schaeybroeck[2], M.Salto-Tellez[2], JF. Graf[3], M. Rehm[5],M. Lawler[2], DB. Longley[2], F. Ginty[3], JHM. Prehn[1].

1. Royal College of Surgeons in Ireland, Dublin, Ireland; 2. Queen's University, Belfast, United Kingdom; 3. General Electric, Niskayuna, NY, USA; 4. Université Paris Descartes, France; 5. University of Stuttgart, Stuttgart, Germany.

Background corrected multiplex immunofluourescence staining levels [Cell DIVE™] of AE1, BCL2, CD3, CD4, CD8, CD45, FOXP3, and PCK26 were used to identify B cells (red), NK and cytotoxic T cells (orange), epithelial cells (yellow), regulatory T cells (dark green), helper T cells (blue), and other cells (light green square). Markers for the plasma membrane (Na+K+ATPase), nucleus (DAPI) on cytosol (RPS6) were used for cell segmentation (pink).

This study is part of a larger investigation on Systems Modeling of Tumor Heterogeneity and Therapy Response in Colorectal Cancer and led by an international consortium funded by the US National Institute of Health (GE Research and MSKCC, award R01CA208179-01A1), Science Foundation Ireland (Royal College of Surgeons (RCSI), Northern Ireland Public Health Agency (Queen's University, Belfast).

> Research reported here was supported by National Cancer Institute of the National Institutes of Health under award numbers R01 CA208179. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

> Lindner A., et al. Exploratory multiplex tissue image analysis of the impact of heterogeneity in the microenvironment of primary colorectal cancer on apoptosis markers in patients. In: Proceedings of the American Association for Cancer Research Annual Meeting 2019; 2019 Mar 29-Apr 3; Atlanta, GA. Philadelphia (PA): AACR; Cancer Res 2019;79(13 Suppl):Abstract nr LB–088

From Eye to Insight

> Intro

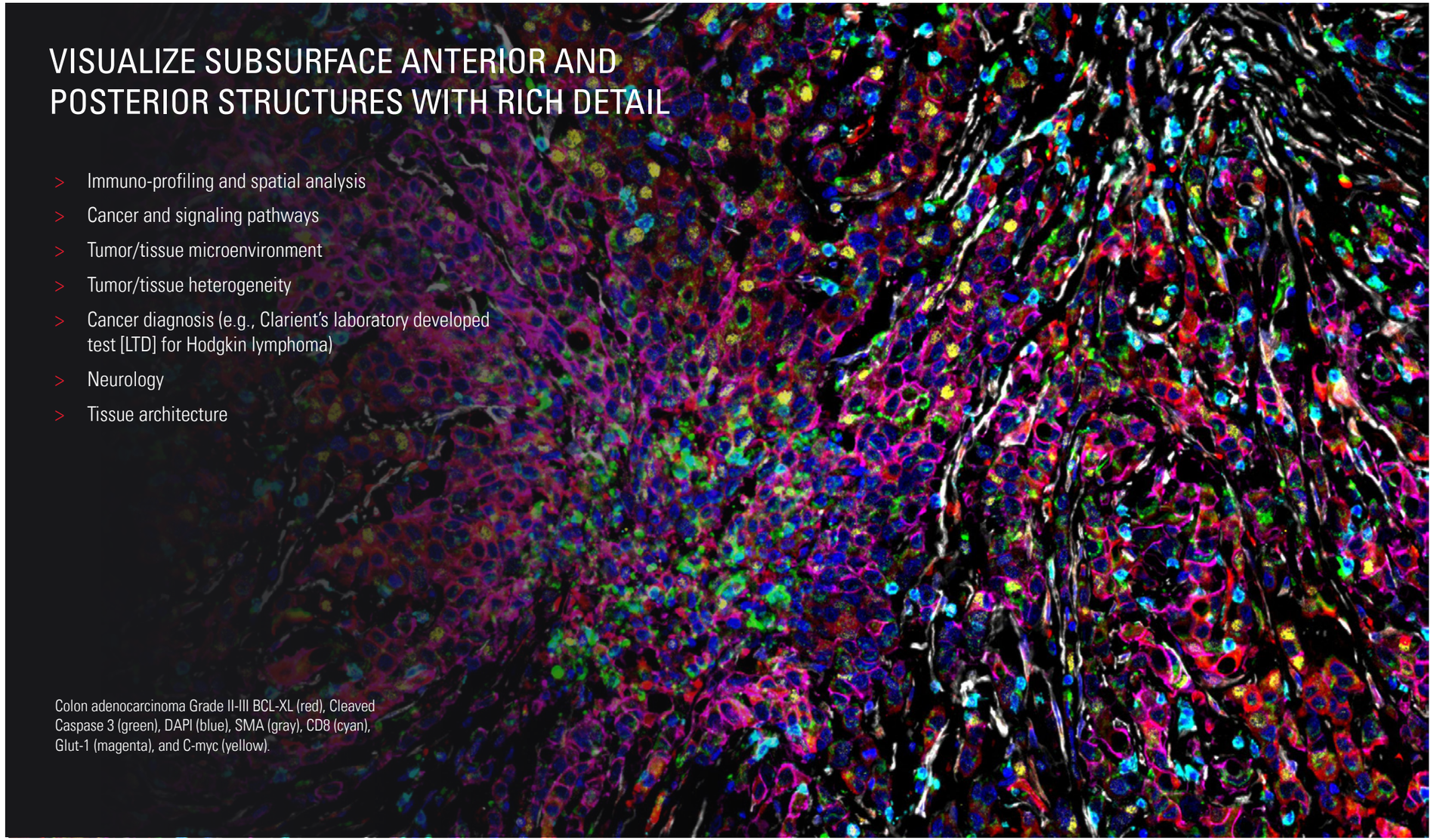
> Component Overview

> Quantifying Biomarkers

> Multiplexing Overview

> Imaging Steps

> **Studies and Examples**



From Eye to Insight

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