HALO Image Analysis Software/Workstation

Data Analysis workroom: DRC I Room 1036

Request permission to access

AMCF core staff (402-559-6659, advancedmicroscopy@UNMC.edu)

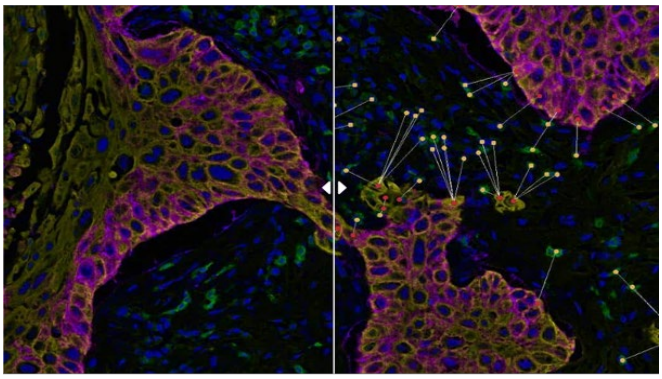
1) card access to room
2) reservation permissions in RSS.

IMPORTANT: All workstation access/use must be scheduled in RSS. Failure to reserve time will result in loss of access.

Directly open several image types

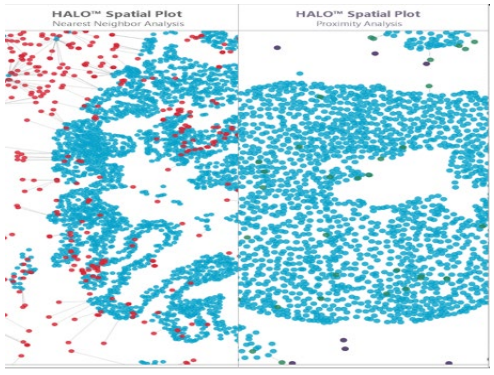
1.Non-proprietary (JPG,TIF,OME.TIFF)
2. Nikon (ND2)
3. 3D Histech (MRXS)
4. Akoya (QPTIFF, component TIFF)
5. Olympus (VSI)
6. Hamamatsu (NDPI, NDPIS)
7. Aperio (SVS, AFI)
8. Zeiss (CZI)
9. Leica (SCN, LIF)
10. Ventana (BIF)
11. Philips (iSyntax)
12. KFBIO (KFB)
13. DICOM (DCM*) *whole-slide image

Available HALO Modules



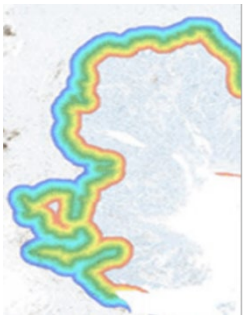
Spatial Analysis | Indica Labs

Particularly useful to those involved in the immune-oncology field, the Spatial Analysis module offers a suite of analysis tools to identify proximity and relative spatial distribution of objects, cells, and/or features across single tissues or serial sections (nearest neighbor, proximity, infiltration, density heat map). This module can be used in conjunction with any cell-based analysis module for brightfield or fluorescence.

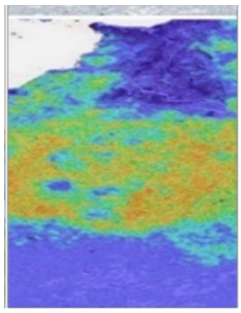


NEAREST NEIGHBOR ANALYSES Determine the average distance and number of unique neighbors between any two cell or object populations. For example, cytokeratin positive cells (blue) and CD4 positive cells (red) connected with grey lines show CD4 positive cells next to the nearest pan cytokeratin positive cell.

PROXIMITY ANALYSES Calculate the number of objects or cells within a certain distance of another object or cell. For example, CD8 positive cells within 30 um of a pan cytokeratin positive cell (blue) are labeled green while CD8 positive cells greater than 30 microns from pan cytokeratin positive cells are labeled purple. A corresponding proximity histogram is automatically generated.



INFILTRATION ANALYSES Determine the number of objects/cells within a set range. When using this tool, a tumor boundary is defined/annotated in green. The infiltration analysis tool defines the invasive margin inside of tumor (yellow and red) and the invasive margin outside of the tumor (blue and purple) automatically. For example, CD8 positive cells are quantified within the margin with an automatically generated histogram showing CD8+ cell density inside the tumor boundary (-1 to -500um), at the tumor boundary (0), and outside the tumor boundary (+1 to +500).

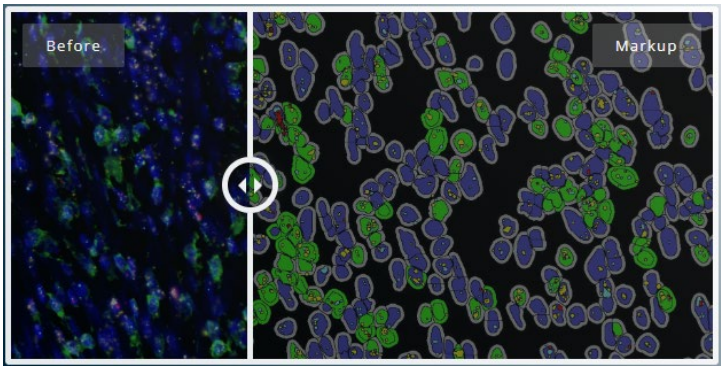


DENSITY HEATMAPS The density heatmap spatial analysis algorithm measures the density of a selected cell population from your object data within a certain radius. The scaling and colors of the markup are customizable. Reported in the summary data, the entire tissue area is analyzed, and all cells of the desired population are counted. The average cell population and the average cell population density is calculated within the user-defined radius, as well as the minimum and maximum densities.

For example, a density heatmap analysis with a radius of 25 um can be used to measure the density of immune cell positivity across a whole slide. The markup image, with adjustable transparency, depicts areas of higher density in red and orange, moderate density in yellow, and areas of lower density in green and blue.

Spatial Analysis Training webinars (free access with UNMC email)

- Masterclass Webinar: Performing Highly Multiplexed IF and Spatial Analysis Workflows with HALO®
- A role for HALO® in characterizing cell heterogeneity across organ systems: from the liver to the brain
- Advanced Multiplex and Spatial Analysis Methods using HALO Data
- Optimizing quantitative analysis of highly multiplexed CODEX images in HALO
- Whole-slide Quantitative RNAscope Image Analysis: Applications and Methods
- Tumor-specific tumor-resident cytotoxic T cells predict recurrence in stage III melanoma patients treated with adjuvant immunotherapy



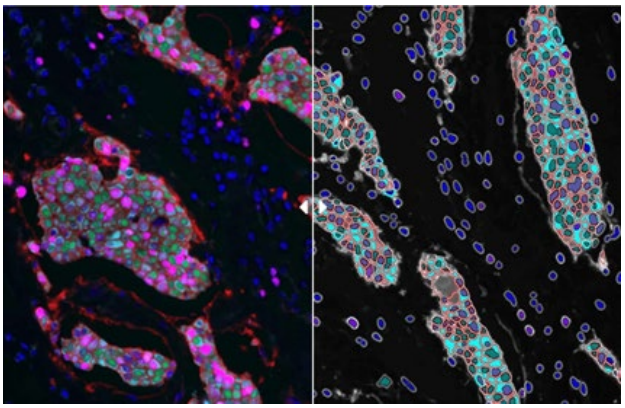
FISH-IF Quantification | Indica Labs

This module measures any number of fluorescently-labeled DNA/RNA ISH probes (or punctate marker such as PLA) and immunofluorescent (IF) protein biomarkers on a cell-by-cell basis. Users can rapidly contextualize corresponding protein and gene expression profiles for every cell. HALO® FISH-IF analysis is designed to work with single or dual IHC-ISH assays including RNAscope™ and supports the H-score protocol for RNAscope as recommended by the manufacturer, ACD, a Bio-techne brand.

- Download the latest Quantitative RNAscope Image Analysis Guide
- Review the latest high-impact publications using HALO RNAscope Image analysis.

FISH-IF Quantification Training webinars (free access with UNMC email)

- Getting Started with RNAscope™ Image Analysis in HALO
- Whole-slide Quantitative RNAscope Image Analysis: Applications and Methods
- Masterclass Webinar: Optimizing RNAscope Image Analysis
- HALO Image Analysis Masterclass Series

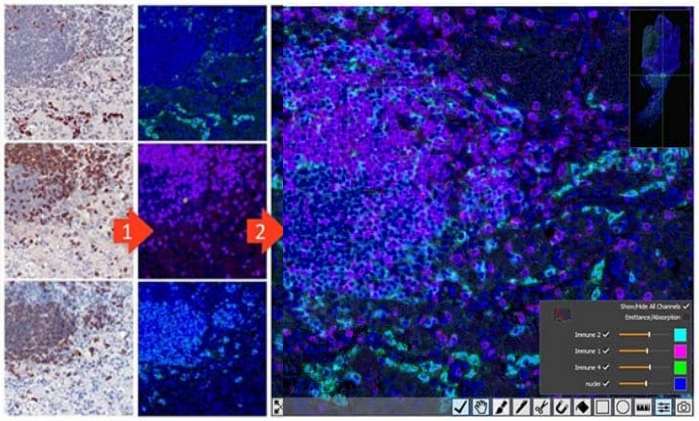


Highplex FL | Indica Labs

Simultaneous analysis of an unlimited number of fluorescent markers in any cellular compartment – nucleus, cytoplasm, and/or membrane. With the option to define specific cell phenotypes according to marker positivity, this module is ideally suited for applications in immuno-oncology where multiple markers are required to characterize distinct immune and tumor cell populations within the tissue. When used in conjunction with our Spatial Analysis module, the spatial relationships between any number of cell populations may be interrogated.

Highplex FL Training webinars (free access with UNMC email)

- Masterclass Webinar: Performing Highly Multiplexed IF and Spatial Analysis Workflows with HALO®
- Advanced Multiplex and Spatial Analysis Methods using HALO Data
- Multiplex Immunohistochemical Phenotyping of T cells in Primary Prostate Cancer
- Optimizing quantitative analysis of highly multiplexed CODEX images in HALO
- Tumor-specific tumor-resident cytotoxic T cells predict recurrence in stage III melanoma patients treated with adjuvant immunotherapy
- Automated detection and quantitation of gastric immune cells in a mouse model of Helicobacter pylori-driven preneoplastic progression
- High Dimensional Spatial Biology: from Discovery to High Throughput Studies
- HALO Image Analysis Masterclass Series

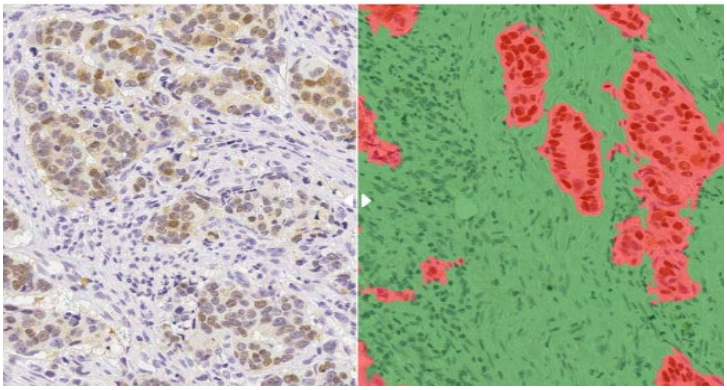


Serial Registration Analysis | Indica Labs

Serial section analysis allows users to create a tissue classifier based off a reference slide (e.g., H&E or Pan-CK) and then apply the resulting classification mask to serial section(s) stained for additional IHC markers (e.g., HER2, ER, PR, Ki-67, CD8, CD4). The serial section add-on takes the Classifier created from the H&E image and allows the user to apply the resulting classification mask to IHC-stained serial sections (e.g., ER, HER2) as regions of interest. Serial registration analysis is compatible with *both brightfield and fluorescent images* and any HALO module(s) can be used for the final analysis step. Where serial section analysis involves different tissue sections that have been stained with different markers, serial stain fusion facilitates analysis of single slides that have been stained, stripped and restained (see Romain Remark et al. *Science Immunology, 2016)*. Serial Stain Registration uses an Image Consistency Phase, a Similarity-Only Phase, and a High-Resolution Registration to precisely align single cells. The Single Channel Registration option allows the user to align the images using one consistent fluorescent channel, typically DAPI, to improve the registration. Next, registered serial stain images are fused into a single composite fluorescent image that can be analyzed using Highplex FL or (any other FL module).

Serial Registration Training webinars (free access with UNMC email)

- CD38 in the Prostate Tumor Microenvironment
- Spatial Organization of Immune Cells in the Pancreatic Ductal Adenocarcinoma (PDAC) Tumor Microenvironment
- The Use of Image Fusing in the Deployment of a 7-plex Immunofluorescent Assay
- HALO® IMAGE ANALYSIS MASTERCLASS WEBINAR SERIES

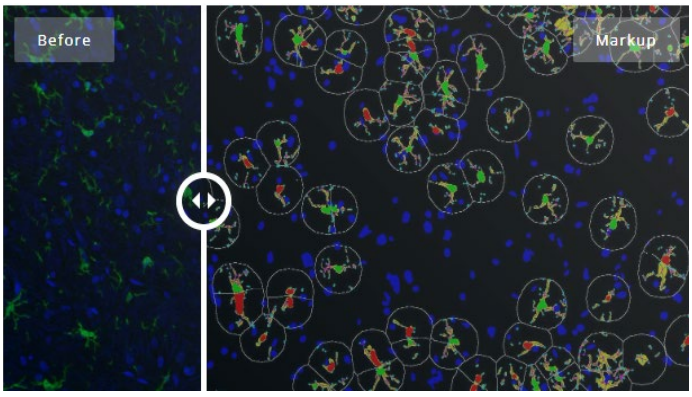


Tissue Classifier | Indica Labs

The Tissue Classifier Add-on utilizes a state-of-the-art machine learning algorithm to identify tissue types based on color, texture, and contextual features. Utilizing a "learn-by-example" approach, the user highlights a few distinct tissue types and within seconds the software learns to categorize tissue. The tissue classifier add-on can be used in conjunction with any of our cell-based analysis modules for BF or FL analysis, ultimately minimizing manual outlining of regions of interest.

Tissue Classifier Training webinars (free access with UNMC email)

- Spatial Biology: Revolutionizing Tissue Imaging and Analysis Using Spatial profiling,
- A role for HALO® in characterizing cell heterogeneity across organ systems: from the liver to the brain,
- Maximizing use of HALO® and HALO AI for a comprehensive image analysis for HUMAN Brain FFPE Tissue samples in Alzheimer's Disease,
- Optimizing quantitative analysis of highly multiplexed CODEX images in HALO



Microglial Activation FL Module | Indica Labs

The Microglial Activation FL module measures fluorescently-stained microglia cell activation in whole slide images. This module detects microglia, soma, and processes, and ultimately reports the number of activated microglial cells, the number of inactivated microglial cells, and the number of negative (non-microglia) cells detected. In addition to cell classification and counting, this tool precisely measures cell processes. It reports the total process area and length, as well as the average process area, length, and thickness, and quantifies process branching by counting the number of branch points and end points per cell. An interactive markup image output option enhances visualization of results by selecting which of the following are displayed: activated microglia, inactivated microglia, microglial processes and skeleton, branch points, end points, as well as the microglia radius.

Microglial Activation FL Training webinars (free access with UNMC email)

- Neurobiology Image Analysis with HALO and HALO AI