

## List of Available HALO Modules

Brightfield (BF) Fluorescent (FL)	Module Name	Description/Use-Cases
BF and FL	Tissue Classifier	Identify multiple tissues classes using a learn-by-example approach Can be used in conjunction with all other modules (fluorescent and brightfield) to select specific tissue classes for further analysis
BF and FL	Tissue Microarray (TMA)	A workflow module for Tissue Microarrays (TMA) organization, analysis, and manual scoring Can be used in conjunction with all other analysis modules
BF and FL	Spatial Analysis	Perform nearest neighbor, proximity, infiltration, & density heatmap analysis Use in conjunction with other modules (fluorescent and brightfield) to analyze spatial relationships between identified objects
BF and FL	Serial Section and Staining Registration	Serial Section: Used in conjunction with the Tissue Classifier and a brightfield module (typically). Register serial sections and use the tissue classifier to identify regions of interest on one slide then quantify another marker within the corresponding regions on a serial slide. Serial Stain: Used with fluorescent or pseudo-fluorescent slides that are first registered together and next fused to create a single slide (.tif or .serial).
BF	*Area Quantification Brightfield*	Separate up to five stains in a single image Calculate percentage area of each stain, intensity of each stain, and colocalization of multiple stains
BF	Axon Quantification	Quantify axons in cross sections Calculate axon area, myelin area, myelin thickness, inner and outer axon diameter, and G-ratio
BF	Branch Structure Analysis	Quantify branched objects such as capillaries in brightfield Measure number of branch points and end points, length, thickness and area of branches
BF	Cytonuclear IHC Quantification	Quantify expression of up to four IHC markers in the cytoplasm and/or nucleus Calculate intensity of stain in the cytoplasm and nucleus for each cell, bins cells according to stain intensity (1+, 2+, and 3+) and quantify cell phenotypes on a per cell basis
BF	Deconvolution	Generate deconvolution settings for serially stained brightfield images to create pseudo-fluorescent images
BF	Immune Cell	Count immune cells and optionally classify them based on proximity to another marker For instance use a Pan Cytokeratin to find tumor regions, then measure proximity of TILs to the tumor margins
BF	ISH Quantification	Quantify up to three probes across tissues, bin cells according to signal count (1+, 2+, 3+, and 4+), determine number of probe signals per cell basis Can be used to analyze CISH, SISH, and RNA ISH
BF	ISH-IHC	Quantify up to four total in situ hybridization (ISH) probes along with a protein (IHC) markers to identify RNA expression in specific cell populations Determine number of probes per cell and bin according to signal count (1+, 2+, 3+, & 4+)
BF	Islet Analysis	Count and measure pancreatic islets, and count and classify cell types within the islets For example, alpha cells, beta cells, or proliferating cells
BF	Membrane IHC Quantification	Quantify intensity of membrane stains on a per cell basis and bin cells according to stain optical density (1+, 2+ and 3+)
BF	Microglial Activation Quantification	Quantify microglial activation based on length and thickness of microglial processes Calculate total number of active and inactive microglial, average number of processes and area, length, and thickness or processes per cell
BF	Multiplex IHC	Quantify expression of up to 5 IHC markers ion the nucleus, cytoplasm, or membrane Ideally multiplex IHC applications
BF	Muscle Fiber Analysis	Quantify area, mean/median/maximum diameter, and perimeter of muscle fibers stained with Laminin or other stain to highlight fiber membrane
BF	Object Colocalization Analysis	Count objects and measure the area and stain intensity for each object Can measure one or two objects (stained with different chromogens) and can quantify colocalization of the two objects where applicable
BF	Vacuole Analysis	Quantify vacuole area, diameter, and perimeter and number of vacuoles per cell Can be used to quantify lipid droplets in liver unstained or stained (Oil Red O), lipid droplets in adipose tissues, and measure alveolar area in lung

\* Included with any HALO package \*

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FL	*Area Quantification FL*	Analyze an unlimited number of fluorophores in a single image Calculate percentage area and intensity of each fluorophore across analysis area and colocalization of multiple fluorophores
FL	Branch Structure Analysis FL	Quantify fluorescently-labeled branched objects such as capillaries Measure number of branch points and end points, length, thickness and area of branches
FL	Cytonuclear Quantification FL	Quantify expression of an unlimited number of IF markers in the cytoplasm and/or nucleus Algorithm outputs intensity of stain in the cytoplasm and nucleus for each cell, bins cells according to stain intensity (1+, 2+, and 3+) and measures co-expression of stains on a per cell basis
FL	FISH Amplification & Deletion Analysis FL	Quantify up to two fluorescently labeled DNA probes to measure amplification or deletion Count number of signals and ratio of two probe signals (test:control) and bin cells automatically according to signal ratios (low, average, high, and very high signal ratios)
FL	FISH Breakapart & Fusion Analysis FL	Measure gene rearrangements detected using break-apart fusion probes Signals which come into close proximity or are overlapping are considered fused and fusion distance can be set within the algorithm
FL	FISH FL	Measure intensity and co-expression for an unlimited number of fluorescent in situ hybridization probes on a per cell basis Bin cells according to signal count (1+, 2+, 3+, and 4+), determine number of FISH probe signals per cell
FL	FISH-IF FL	Measure intensity and co-expression of an unlimited number of fluorescent in situ hybridization probes or protein expression on a per cell basis Bin cells according to signal count (1+, 2+, 3+, and 4+), determine number of probe signals per cell
FL	High-Plex FL	Cell based analysis for unlimited channels in the nucleus, cytoplasm, or membrane compartments Ideally suited for high-plex FL applications and multi-spec imaging systems
FL	Islet FL	Count and measure pancreatic islets in fluorescence, and count and classify cell types within the islets For example, alpha cells, beta cells, or proliferating cells
FL	Object Coexpression Analysis FL	Measure co-expression of a fluorescently-labeled object with up to three other fluorescently-labeled marker Can be used to quantify negatively stained objects such as muscle fibers or adipose cells
FL	Object Colocalization Analysis FL	Count objects and measure the area and stain intensity for each object Algorithm can measure one or two objects (stained with different fluorophores) and can quantify colocalization of the two objects where applicable
FL	Membrane Quantification FL	Quantify intensity of fluorescently-labeled membrane markers on a per cell basis and bin cells according to intensity (1+, 2+ and 3+)

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