rightfield (BF) Jorescent (FL)	Analysis Module Name	Description
<u> </u>		*Modules included with any HALO package*
BF	*Area Quantification	Separate up to five chromogenic stains in a single image
	Brightfield*	Calculate percentage area of each stain, intensity of each stain, and colocalization of multiple stains
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
BF	*Color Deconvolution*	Separate chromogenic stains into channels in a pseudo-fluorescent image.
		General use add-on modules
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	Serial Section: Used in conjunction with the Tissue Classifier and a brightfield module
	Staining Registration	(typically). Register serial sections and use the tissue classifier to identify regions of interest 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).
	Modules compatible with	HALO AI nuclear segmentation (HALO 3.2+) and AI phenotyping (HALO 3.3+)
FL	HighPlex FL	Cell based analysis for unlimited channels in the nucleus, cytoplasm, or membrane
		compartments
	A4 b: 1 1110	Ideally suited for high-plex FL applications and multi-spec imaging systems
BF	Multiplex IHC	Quantify expression of up to 5 IHC markers in the nucleus, cytoplasm, or membrane Ideally multiplex IHC applications
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 sign 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
BF	ISH	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+)
		Specific use modules
BF	Axon Quantification	Quantify axons in cross sections
		Calculate axon area, myelin area, myelin thickness, inner and outer axon diameter, and G-rat
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
	Branch Structure	
FL	Branch Structure	Quantify fluorescently-labeled branched objects such as capillaries

Brightfield (BF) Fluorescent (FL)	Analysis Module Name	Description
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
		Quantify expression of up to four IHC markers in the cytoplasm and/or nucleus
BF	Cytonuclear IHC Quantification	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
FL	FISH Amplification &	Quantify up to two fluorescently labeled DNA probes to measure amplification or deletion
	Deletion Analysis FL	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
BF	Islet	Count and measure pancreatic islets, count and classify cell types within the islets
		For example, alpha cells, beta cells, or proliferating cells
FL	Islet FL	Count and measure pancreatic islets in fluorescence, count and classify cell types within the islets
		For example, alpha cells, beta cells, or proliferating cells
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
FL	Membrane	Quantify intensity of fluorescently-labeled membrane markers on a per cell basis and bin cells
	Quantification FL	according to intensity (1+, 2+ and 3+)
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	Quantify microglial activation based on length and thickness of microglial processes
DF		Calculate total number of active and inactive microglia, average process area, length and thickness, branch points and end points per cell
FL	Microglial Activation FL	Quantify microglial activation based on length and thickness of microglial processes using a
		designated fluorescent marker
		Calculate total number of active and inactive microglia, average processes area, length and thickness, branch points and end points per cell
BF	Muscle Fiber Analysis	Quantify area, mean/median/maximum diameter, and perimeter of muscle fibers stained wit Laminin or other stain to highlight fiber membrane
FL	Muscle Fiber FL	Segment and quantify the morphology of muscle fibers stained with Laminin or other dye highlighting fiber membranes
		Quantify positive fiber or membrane staining for an unlimited number of channels
BF	Object Colocalization Analysis	Count objects and measure the area, density, and stain intensity for each object
		Can measure 1-5 objects (stained with different chromogens) and can quantify areas of colocalization
FL	Object Colocalization Analysis FL	Count objects and measure the area, density and stain intensity for each object
		Algorithm can measure an unlimited number of objects (stained with different fluorophores) and can quantify areas of colocalization
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