Image Aquisition	Functions	Contents/Description
Image Formats:	• Image import	bmp, tif, jpg, gif, tga, png, j2k, jp2, mac, msp, ras, pct, eps, wmf, psd, img, cmp, zvi, lsm, czi
	• Image export	bmp, jpg, tif, tga, png, psd, cmp, avi, lsm, mov, j2k, jp2, pcx, tga, wmf, pcf
Camera Control*:	• Exposure time adjustment	Manual adjustment, exposure time measurement, automatic mode
	• Gray value scaling	Conversion to 8 bit dynamic range
	• Digital gain	Adjustment of digital signal amplification
	• White balance	Interactive or automatic adjustment of optimum neutral balance of the color channels
	• 3200 K	Default value for white balance, optimized for halogen light source at 3200° K
	• Resolution	Selection of Microscanning resolution modes (AxioCam HR)
	• Binning	Increased camera sensitivity by combining the signals of adjacent pixels
	• Histogram	Intensity distribution histogram for all three color channels
	• Color adjustment	Manual adjustment of the color reproduction of the white balance
	• Frame	Interactive selection of an image sensor sub frame
	• Black reference	Image background optimization for low light level applications
	• White reference	Correction of optical and illumination inhomogeneities in bright background
	• Image orientation	Rotation and mirroring of image orientation for optimum image display
	• Shutter control	Trigger of external shutter, synchronous to image acquisition
	• Live image frame rate	Selection: slow/medium/fast
	• Focus/exposure frame	Activation of measurement frame for focus and exposure information
Image Processing		
Annotate:	• Annotation	Addition of text, marking of elements (arrows, scale bars, etc.)
Adjust:	• Brightness/contrast	Adjustment of brightness, contrast and gamma
	• Color Balance	Manual adjustment and readjustment of color rendition
Lightness:	• Hue/Lightness/Saturation	Adjustment of hue and saturation
	• Shading correction	Correction of uneven illumination
Geometric Tranformation:	• Pixelshift	xyz-shift with subpixel-accuracy
	• OrthoView	Display of orthogonal 3D image cub
Smooth:	• Gauss, Sigma	Image smoothing using gauss or sigma filter
Sharpen:	• Enhance Contour	Image sharpening using contour enhancement
Resample:	• Resample	Image zoom-out and zoom-in
Image Analysis		
Interactive measurement tools and parameters:		
	• Scaling	Scaling in geometric units
	• Length	Distance between 2 points
	• Outline	Measurement of diameter, area, perimeter, length and width of the circumscribing rectangle, radius, center of gravity, mean density, standard deviation of mean density of gray value
	• Angle 3, Angle 4	Definition through 3 or 4 points
	• Circle	Measurement of diameter, area, perimeter, length and width of the circumscribing rectangle, radius, center of gravity, mean density, standard deviation of mean density of gray value
	• Events	Counting of events
	• Interactive Measurement Wizard	Directed interactive measurement
Documentation		
• Image gallery		Clear presentation of loaded images as thumbnails
• Search functionality		Full text search in all information fields
• Information window		Display of all information on the image
• Printing of images/data		Print of images
• Reports		Creation of user definable reports
Configuration		
• Toolbars/Dialogs/Workflows		Creating individual toolbars, dialogs and workflows
• Short-cuts		Allocation of keyboard entries with AxioVision functions

** Depending on the camera used*

Module	Functions	Contents/Description
Mark&Find	**Recording and retrieving of specimen**	
	• Database	Management of projects and samples in a database
	• Mark interactively	Color assignment of sample positions in the database
	• Visualize	Visualization of the selected points. Relocation by clicking on the colored marker
	• Focus position	Relocation with optional use of stored focus position
	• Calibrate	Calibration using a "Home slide"
Autofocus	**Automatic Focusing**	
	• Calibrate	Calibration by specifying the optimum focus position, usage of current microscope settings with motorized microscopes
	• Focus	Automatic calculation of the focus plane. Suited for transmitted light, reflected light as well as bright field, dark field, fluorescence
Extended Focus	**Calculation of sharp images from several focus positions**	
	• Execute	Capture of an extended depth of focus image by combining images of different focus postions
MosaiX	**Automatic scanning of large surfaces**	
	• Execute	Scanning of entire sample (motorized stage required)
Panorama	**Formation of overview images**	
	• Execute	Seamless composition of images
Multichannel Fluorescence	**Image acquisition in several fluorescence channels**	
	• 8 channels	Simultaneous acquisition of up to 8 independent channels per image
	• Channel configuration	Adjustment of exposure time and microscope settings independent for each channel
	• Optimal display	Channel display as pseudo colored merge image or monochrome display of every single channel
	• Color coding	Free assignment of pseudo colors to channels with easy choice from list
	• Extended parameters	Listing of all channels in a spreadsheet format with extended parameter settings
	• Dye selection	Choice of most commonly used fluorescence dyes from list
	• Focus position	Assignment of different focus positions to individual channels with correct aberrations
	• Channel pool	Storing of channel configurations in a channel pool for easy recombination into other experiments
	• Image information	Display of channel specific information as annotation
	• Experiment	Saving of channel configurations as experiment for exact reproduction of experimental set up
	• ReUse	Extraction of channel settings from previously acquired images for the exact reproduction of an experimental set up
Z-Stack	**Acquisition of image series from different focus positions**	
	• Focus control	Automatic adaptation of the minimal possible step size according to microscope type
	• Z-stack configuration	Definition of start and stop position (or center position) as well as desired interval between individual Z-planes
	• Nyquist criterion	Automatic calculation of the optimal Z-interval for 3D Deconvolution or ApoTome
	• Navigation	Precise stepwise navigation through defined Z-stack or to the start, stop or center position
	• Experiment	Saving of Z-stack definitions as experiment for exact reproduction of the experimental set up
	• ReUse	Extraction of Z-stack definitions from previously acquired images for the exact reproduction of an experimental set up
Time Lapse	**Acquisition of image series over time**	
	• Time configuration	Definition of interval as well as number of cycles or total time
	• Exposure time	Automatic measurement of the correct exposure time for the first timepoint
	• Display	Easy control of the experiment through continuous display of most recently acquired image
	• Image information	Time of acquisition as annotation in image
	• Autosave	High data security during long time lapse acquisitions due to autosave-function

Module	Functions	Contents/Description
	• Experiment	Saving of time lapse configurations as experiment for exact reproduction of experimental set up
	• ReUse	Extraction of time lapse settings from previously acquired images for the exact reproduction of an experimental set up
	• Image size	Acquisition of images as large as required by experimental conditions (limited only by hard disk capacity)
3D Deconvolution	**Restoration of Z-stack images**	
	• Automatic PSF Calculation	Automatic extraction of all necessary microscope parameters from the ZVI image for calculation of an optimized "Point Spread Function" (PSF)
	• Nearest Neighbor	Method for rapid contrast improvement and blur removal from all Z-stack images
	• Regularized Inverse Filter	Filter for rapid 3D restoration of Z-stacks
	• Constrained Iterative	Iterative filter for the quantitative 3D restoration of Z-stacks
	• Preview function	Deconvolution within a user definable small region of interest for fast preview
	• Optimal noise treatment	Automatic calculation of the optimal strength of restoration by determination of image noise levels through "General Cross Validation"
	• Auto-Stop	Iterations stop automatically upon reaching optimal image improvement
	• Display	Three normalization methods for individual adaptation of result images (Clip, AutoLinear, MatchInput)
ApoTome	**Optical sectioning of fluorescent samples with "structured illumination"**	
	• Image acquisition	Automated acquisition of three temporary images followed by online processing to an optical section
	• Scanner control	Automatic and precise shift of grid pattern in the object plane
	• Grid calibration	Calibration of grid focus for correct acquisition of varying fluorescence wavelengths
	• Correction algorithms	Automatic correction of fluctuations in illumination as well as signal degradation due to bleaching
Inside4D	**Visualization in 3D**	
	• Volume display	Volume display of Z-stack fluorescence images with up to 8 channels with selective switching between different channels or view in merged pseudo color mode
	• Shadow projection	Creation of animations with strong sense for spatial conditions
	• Transparency rendering	Presentation of transparent tissues or cultures
	• Surface rendering	Enhancement of individual structures
	• Maximum projection	Ideal for prints and publication
	• Spatial interaction	Free positioning of the 3D volume in space with free choice of angles for x,y and z, lateral position and zoom factor
	• 3D inside view	Orientation within a volume
	• Annotations	Optional display of volume edges, color coding and scaling of axes
	• Animations	Generation of animations as rendered image series with export options in popular video formats (avi, QuickTime)
	• Maximum rendering speed	Acceleration of rendering methods through modern graphic boards (support of OpenGL-standard)
Imaging Plus	**Processing, Gray Morphology, Fourier Transformation, Color Transformation**	
	• Adjust	
	– Contrast	Contrast enhancement using interactive/automatic histogram adaptation
	– Negative	Calculation of inverted image (negative)
	– Gray transformation	Adjustment of gray values using transformation tables
	• Geometric Transformations	
	– Rotate, Shift	Rotation around an axis
	– Mirror	Mirror along horizontal or vertical axis
	– Alignment	Alignment of two images using reference points
	• Smooth	
	– Lowpass	Lowpass filter (floating average value)
	– Median	Median filter (non-linear method)

Module	Functions	Contents/Description
	– Rank	General rank operator
	• Sharpen	
	– Delineate	Enhancement of edges
	• Edges	
	– Sobel	Edge detection using Sobel filter
	– Laplace	Laplace filter
	– Highpass	Highpass filter
	• Morphology	
	– Gray Erode, Gray Dilate	Erosion or dilation of objects
	– Gray Open, Gray Close	Erosion followed by dilation or dilation followed by erosion
	– Tophat White	Removal of bright regions
	– Tophat Black	Accentuation of dark regions
	– Gray Gradient	Morphological gradient to detect contours
	– Watersheds	Watersheds – algorithm for separation/reconstruction
	• Arithmetics	
	– Add, Substract	Addition or subtraction of two images
	– Add Constant	Addition of a contant value
	– Multiply, Divide	Multiplication or division of two images
	– Multiply Constant	Multiplication with a constant value
	– Average	Average of two images
	– Maximum, Minimum	Maximum or minimum of two images
	– Square, SquareRoot	Square or squareroot of an image
	– Logarithm, Exponential	Logarithm or exponent of an image
	– Combine	Linear combination of two images
	• Fourier Transformation	
	– Transform	Fourier transformation on an image
	– Spectrum	Calculation of power or phase-spectrum
	– Filter	Filtering in the frequency domain using a defined filter
	– Inverse	Inverse Fourier transformation
	• Utilities	
	– Copy Region	Copying of image regions
	– Color Model	Transformation of RGB color space into HLS color space and vice versa
	– Split Channels	Split RGB image into single color channels
	– Combine Channels	Combine single color channels to a color image
	– Convert Pixel Format	Conversion of pixel formats (e.g. "8 bit integer" to "float")
	– User Filter	Filtering an image with used defined filter matrix
Interactive Measurement	**Expanded interactive measurement techniques**	
	– Distance, Line, Calipers	Measurement of length
	– Multiple Calipers	Measurement of the length of multiple lines, perpendicular to a base line
	– Multiple Distance	Measurement of distance between multiple parallel lines and a base line
	– Curve, Curve (Spline)	Measurement of length of the drawn curve
	– Aligned rectangle or free orientation, outline, outline (spline), circle	Measurement of geometric and densitometric object features to the center
	– Circle	Drawing of a circle from the contour to the center, clicking on contour points
	– Marker	x- and y- coordinates of a point
	– Interactive Measurement Program Wizard	Guided generation of a program for interactive measurement

Module	Functions	Contents/Description
AutoMeasure	**Creation of easy measurement programs with a measurement wizard**	
Creation of measurement programs	• Automatic Measurement Program Wizard	Guided generation of a program for automatic measurement
	– Image enhancement	Contrast, brightness, Gamma, noise reduction (Sigma), shading correction, improvement of edges
	– Segmentation	Global or local definition by clicking or circumscribing objects, specification of thresholds using the image histogram
	– Binary image clean-up	Deletion of artefacts, filling of holes
	– Automatic object separation	Erosion and dilatation, watersheds
	– Interactive editing of the measurement mask	Drawing of separation lines, deletion of objects, addition of objects
	• Selection of measurement parameters	Region specific, field specific, geometric and annotation parameters
	– Definition of measurement conditions ("objectfilter")	Logical concatination (AND/OR) of region specific parameters, definition by simple clicking on reference objects
	– Defintion of a measurement frame	Rectangle, circle, freehand
	– Measurement	Measurement of geometric and densitometric features for single objects or the entire image
	– Documentation	Marking of measured objects and display of freely selectable measurement parameters in the graphics plane
	– Data storage	Saving of measurement data in an Excel-compatible file format (csv)
Execution of measurement programs	– Image acquisition	Image acquisition via camera, all images of a folder, all loaded images
	– Autom. assignment of scaling	With ZVI image format
	– Control of program execution	Activation/deactivation as well as changing of a function during the program execution
	• Program information	
	– Display	List of executed functions with parameter settings
AutoMeasure Plus	**Segmentation, binary image processing, automatic measurement**	
	• Segmentation	
	– Thresholds	Adjustement of thresholds using histogram
	– RegionGrowing	Detection of associated regions (gray values within user defined tolerance level)
	– Automatic	Automatic determination of thresholds using a histogram
	– Dynamic	Threshold detection by using size information
	– Valleys	Detection of dark lines (valleys) in images with bright background
	– Canny	Edge detection considering "steepness" of edges
	– Marr	Detection of edges and associated regions
	• Binary processing	
	– Bin Erode, Bin Dilate	Erosion or dilation of binary objects
	– Bin Open, Bin Close	Erosion followed by dilation or dilation followed by erosion
	– Bin Fill, Bin Scrap	Filling of holes, removal of artifacts
	– AND, OR, XOR, NOT	Bitwise logic operation on the image
	– Bin Endidean Distance	Generation of a "distance map", indicating the distance of each pixel to the object border
	• "Skeletonizing" of binary images	
	– Thinning	Thinning of binary objects to 1 pixel wide lines (skeleton)
	– Exoskeleton	Skeletonize the image background
	• Measure	Automatic measurement of geometric and densitometric object features

Module	Functions	Contents/Description		
Cumulus	**Image cataloging and archiving**		Single User	Workgroup
	Organizing catalogs, categories and key words		X	X
	Storage of assets on a server			X
	Network access for workgroup members			X
	Central management of access rights			X
	Local administration of catalogs		X	
	Data and category fields		X	X
	Voice annotation for assets		X	X
	Free definable queries to all data fields		X	X
	Short response time to complex queries		X	X
	Asset export to HTML		X	X
	On the fly e-mail assets		X	X
	TCP/IP Client/Server architecture			X
	Access to the same pool of assets – even from different operating systems			X
	User rights control for asset access			X
	ODBC compatibility		X	X
Commander	**Recording and automatic execution of steps**			
	Protocol, save	Record procedures and save protocol		
	Start	Automatic run of recorded protocol		
	Edit	Edit protocol		
VBA	**Programming environment**			
	Visual Basic Editor	VBA environment with full access to AxioVision functionalities		

Application kits	Functions	Contents/Description
AxioVision FRET	**Measurement of molecule interaction**	
	• User modes	User mode for routine measurements
	• Evaluation agent	Adjustment of system parameters for the acquisition: exposure time, method (acceptor bleaching or correction method), time lapse parameter
		Acquisition of multichannel images according to definition
		Background measurement in all channels
		Signal measurement in all channels
		Definition of ROIs by threshold, circles or freehand
		Choice of evaluation method Youvan, Gordon, Xia, Acceptor Ratio, Siegel
		Presentation of raw data as table
		Storage of raw data
	• Display	Display of all raw data as histograms:
		– channel by channel (FRET-Donor, FRET-Acceptor, Donor-Acceptor)
		– all FRET-methods against time
		Generation of false color images for FRET signals for each method (Youvan, Gordon, Xia, Akzeptor Ratio, Siegel)
KS ELISPOT	**Exact immune response measurement**	
	• User modes	Administrator mode – to set up and tune the system
		User mode – for routine measurements
	– Direct evaluation of wells	Definition of the well area on the motor stage for evaluation
		Selection of a configuration file for evaluation
		Start of image acquisition including measurements
		Storage of raw data
	– Evaluation of stored images	Definition of the image folder
		Definition of the well area in the plate field
		Selection of a configuration file for evaluation
		Start of evaluation
		Storage of raw data
	• Display	Result presentation in internal rtf format
	• Spot Teaching	Training the system using the unique "Teach mode"
	• Report	Creating a report as a Word document

Measurement parameters

Entry-level program	IM: Interactive Measurement	IM incl. measurement assistent	AutoMeasure		
Region specific parameters					
				• Geometric	
		X	X	AcpX, AcpY	x- and y-coordinates of the top object point
X	X	X	X	Area	Area of the region
		X	X	AreaConvex, AreaFilled	Area of the convex shell of the region and of the filled region
X	X	X	X	CenterX, CenterY	x- and y-coordinates of the geometric center of gravity of the region
		X	X	EllipseMajor, EllipseMinor	Length of the main axis and the secondary axis of the ellipse with the same geometric moment of inertia as the region
		X	X	Ellipse Angle	Angle of the main axis of the ellipse with the same moment of inertia
X	X	X	X	Perimeter	Perimeter of the region
		X	X	Perimeter Convex	Perimeter of the convex shell of the region
		X	X	Perimeter Filled	Perimeter of the filled region
		X	X	Perimeter Crofton, Perimeter Crofton Filled	Perimeter of the region and perimeter of the filled region according to Crofton
		X	X	Perimeter X, Perimeter Y	x- and y-projection of the perimeter
		X	X	Perimeter XF, Perimeter YF	x- and y-projection of the perimeter of the filled region
		X	X	Perimeter XY, Perimeter XYF	Diagonal projection of the perimeter and the perimeter of the filled region
		X	X	BoundTop, BoundBottom, BoundLeft, BoundRight	x- and y-coordinates of the bounding box
X	X	X	X	BoundWidth, BoundHeight	Width and height of the bounding box
		X	X	AreaFrame	Area of the measurement frame
		X	X	FeretMinimum, FeretMaximum	Minimum and maximum feret of the region
		X	X	FeretMinimumAngle, FeretMaximumAngle	Angle of the minimum and the maximum feret of the region
		X	X	FeretRatio	Ratio of the ferets ($\frac{FeretMin}{FeretMax}$)
X	X	X	X	Diameter, radius	Diameter, radius of the circle with equivalent area
		X	X	FormCircle	Circular shape factor of the region
		X	X	FibLength	Length of a fiber-like thin region
		X	X	ID	Explicit characteristic of the region, of the squares
X	X	X		Distance measurement	Distance between 2 points
X	X	X		Angle measurement	Angle in °
				• Densitometric	
X	X	X	X	Mean	Densitometric mean value of the region (gray and color values)
X	X	X	X	Standard deviation	Standard deviation of the densitometric values of the region (gray and color values)
		X	X	Minimum, Maximum	Minimum and maximum densitometric value (gray and color values)
		X	X	Sum, Sum Square	Sum of the densitometric values of the region, sum of the squares (gray and color values)
Field specific parameters					
				• Geometric	
			X	FldArea	Area of all regions
			X	FldAreaPer	Percentage area of all regions in the measurement frame
			X	FldCount	Number of the measured regions
			X	FldPerim	Sum of all region perimeters
				• Densitometric	
			X	FldDensMean	Densitometric value of all regions (gray and color values)
			X	FldDensStd	Densitometric value standard deviation in all regions (gray and color values)
			X	FldDensMin, FieldDensMax	Minimum and maximum densitometric value in all regions (gray and color values)
Further parameters					
X	X			Counting events	
	X	X		Marker	