



## Imaging Software Optimized for Image Acquisition and Analysis

HCImage is designed to solve a wide range of imaging applications. It includes an extensive range of image processing tools that can be used during live image capture or post acquisition analysis. For greater control, HCImage allows image capture, processing and analysis with the ability to select from over 150 measurements. Data are saved in a hierarchical file structure, allowing images and measured data to be easily displayed in a variety of graphical formats or exported to Excel.

HCImage is optimized to take full advantage of the established DCAM camera drivers, maximizing the performance and usability of Hamamatsu cameras.

HCImage provides an easy-to-use, flexible solution to many applications in Life and Material Science; including particle sizing, intensity analysis over time, object tracking and multi-dimensional time-lapse.

### Applications

- Fluorescence Microscopy
- Ratio Imaging
- Multi-dimensional Time-lapse Imaging
- Object Counting and Quantification
- High Speed Image Streaming
- Vesicle Fusion Event Monitoring
- Protein-protein Interactions
- Motion Tracking
- Particles Size Analysis

# HCIImage Live

HCIImage is designed to utilize high resolution and sensitivity from Hamamatsu cameras, and is highly optimized for DCAM driver. HCIImage provides the necessary tools for image acquisition, processing and analysis, all in a flexible, easy-to-use application. Careful thought went into the design of the user interface to maximize functionality, optimize workflow and increase efficiency.

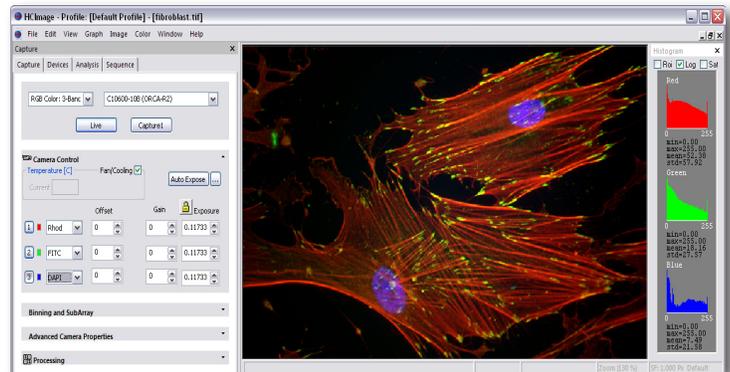
## Layout

### Dynamic Panel

A side panel provides comprehensive user control of the imaging system from Acquisition through Analysis. Panes and expanding panels provide easy access to controls.

### Custom Layout

Customize the screen layout by editing the panes and toolbar



## Time-lapse

### Visual Feedback

Use the live intensity histogram to increase dynamic range and check for saturation.

### Real-time Hardware or Software Image Processing

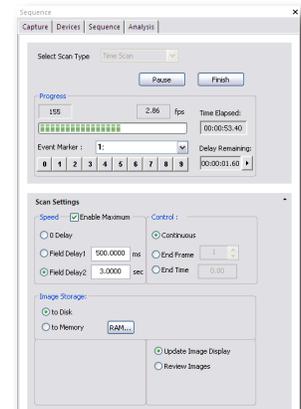
Perform background subtraction, shading correction or frame averaging while capturing images using HCIImage on a selected camera.

### Stream or Delayed Time-lapse Acquisition

Stream images at maximum speed or dynamically switch between multiple time-lapse intervals.

### TTL I/O

Use TTL to control image capture or synchronize with other events.



## Measurement

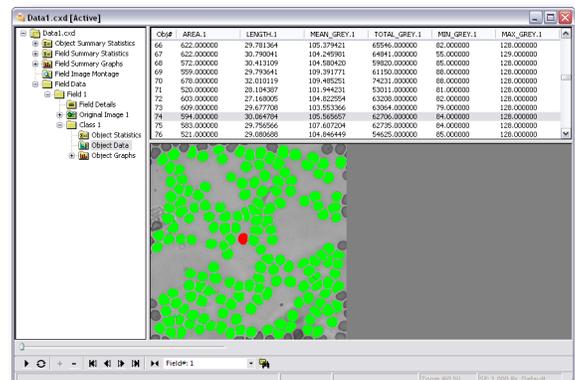
### Data Collection and Object Management

Select multiple measurements for intensity, line length, endpoint length and area on individual images with data saved in a data document or spreadsheet.

### Plot Intensity of an Object Over Time from Images Saved in an Image Sequence

### View the Data

Image sequences saved in a data document can be reviewed and measured. Measured data can be presented as a list, statistics, spreadsheet or graphically.

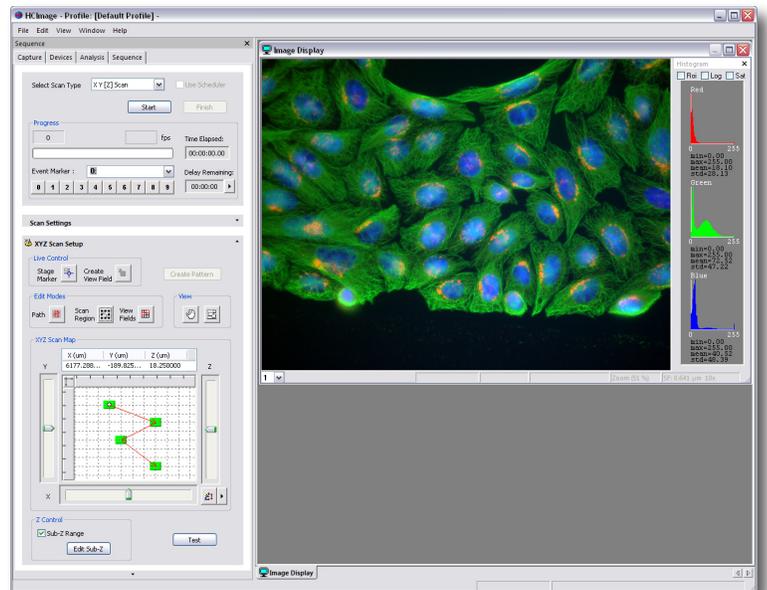


# HCIImage Acquisition

HCIImage Acquisition includes all functionality of HCIImage Live, plus the ability to perform multi-dimensional acquisition and control a wide range of motorized devices. Designed to provide a fast and flexible workflow to facilitate image acquisition, HCIImage is an elegant solution to cover many experimental designs. Multi-dimensional collection of large image sequences are organized into data tree file structure for easy access to images and measurements. Support for an extensive list of Hamamatsu cameras, microscopes, and associated peripheral hardware provides a cost effective solution to all your image acquisition requirements.

## Flexible Multi-dimensional Acquisition

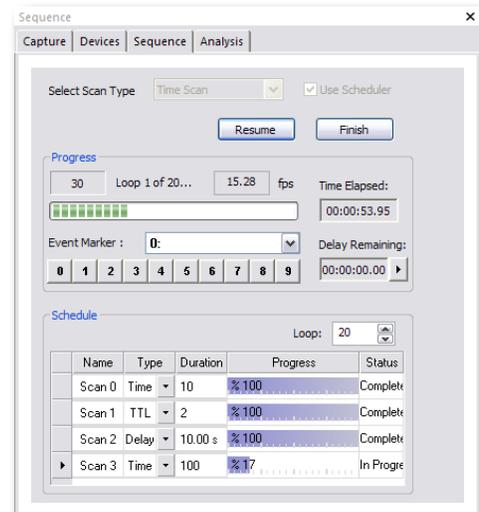
- **Multi-site Time-lapse**  
Automated capture of XYZ and wavelength scans over time
- **Multi-channel Acquisition**  
Capture up to 5 different filter settings over time
- **Z Scan Acquisition**  
Capture Z scans of a single or multiple locations
- **High Speed Image Streaming**  
Acquire large data sets at maximum camera speed
- **Modify Settings during Time-lapse**  
Modify exposure, focus and XYZ location
- **TTL Input and Output Control**  
Internal and external TTL control for acquisition and data synchronization
- **Create Large Image Montage**  
Scan large image area at high resolution for montaging



## Scheduler

Run a time-lapse with variable events, for example, Time1, Time2, TTL, Z, Delay with loops

- **Time-lapse Scheduler**
- **Variable Time Delay**
- **TTL Output Control**
- **Z Scans**
- **Multiple Loops Time-lapse**



## ■ Extensive Selection of Imaging Tools

- **Real-time Image Processing**  
Real-time noise averaging, shading and background correction
- **Exposure Protection**  
Advanced exposure protection to minimize bleaching
- **Advanced Auto-exposure**  
Customize Auto-exposure parameters to optimize image dynamic range
- **Dynamic Auto Contrast**  
Minimize sample bleaching by using short exposure
- **Sub-Array Image**  
Select sub-array on the camera CCD to increase acquisition speed
- **Auto Focus**  
Correct for focus drift by performing auto-focus at user-specified interval
- **XYZ Stage Offset**  
Automatically shift XYZ for each filter setting
- **Review Large Data Set with Ease**  
Images are organized in a data tree format for easy access to image information and analysis

## ■ Comprehensive Camera Support

- **DCAM Support**  
Support for Hamamatsu DCAM based cameras, including Imagem, ORCA-R2, ORCA-AG, C9300-221
- **Nanozoomer File Format Support**  
Support for **ndpi**, **vms**, and **vmu** NanoZoomer file formats
- **Dual Camera Support for DCAM**  
Use two identical cameras for high speed simultaneous acquisition

## ■ Support for Motorized Devices

- **Automated Microscopes**  
Support for a wide range of automated microscopes including: Olympus IX81; Nikon 90i and TE2000; Leica DMI6000; and Zeiss microscopes supported under MTB 2004
- **Wavelength Switchers**  
Sutter DG4 and DG5, TILL Polychrome V, Spectral LMM5 merge module, CAIRN Optoscan Monochromator
- **Filter Wheel Controllers**  
Hamamatsu Filter Wheels, Ludl Mac 5000 & 6000, Prior ProScan and OptiScan, Prior Lumen Pro, Sutter Lambda 10-2, 10-3, 10-B, SC and Smart Shutter
- **Shutter Controllers**  
Support for Hamamatsu shutter, Sutter, Ludl, Prior, ASI SC-2, ASI-1000, and Uniblitz controllers
- **XY Stage Controllers**  
Ludl Mac 5000 & 6000, Prior ProScan and OptiScan, Märzhäuser: Tango, LSTEP, ECO-STEP  
ASI MS-2000, Olympus, Leica, Zeiss, Nikon Biostation XYZ Stage
- **Z Motors**  
ASI-Z, Prior Z-motor, Märzhäuser, Ludl, Olympus, Nikon, Zeiss, Leica, Physik Instrument E662 and E665
- **Camera Emission Splitters**  
Image Splitter Dual View
- **I/O Devices**  
Parallel and Serial I/O support, AEQUORIA digital I/O support, National Instrument DAQ board, Visitech AOTF

# HImage Analysis

HImage Analysis includes all functionality of HImage Live and Acquisition, plus an extensive selection of image analysis tools. It features image processing and analysis tools to enable quantitative analysis on a wide range of complex images. Imaging tools are selected using customized icons to derive workfiles (macros), which are saved and can be used multiple times. Images are saved with measured data allowing dynamic interaction between images, objects, graphs, and tables to provide instant user feedback. Custom measurements can be created with a built-in equation editor expanding over 150 measurements, including count, size, shape, position, intensity and color of objects. Tracking is available for multiple objects across time separated image sequences.

## Image Processing and Analysis Tools

### Image Processing

Includes frame average, background correction, smooth, median, Laplacian, Sobel, Kirsch, erode, dilate, separate, erode, dilate, skeleton, prune, node, AND/OR/NOT/NOR plus others.

### Identify Objects

Objects are identified by intensity, color threshold, or may be manually drawn.

### Qualify

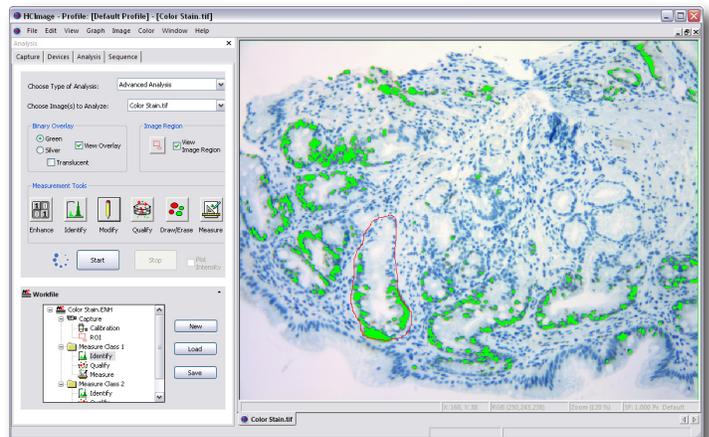
When objects do not meet the desired criteria for measurement, they can be removed with a special size, shape, intensity or position qualifier, thus ensuring distribution statistics that meet acceptable standards.

### Workfile

Workfiles are available to process single images or multiple images throughout a sequence.

### Extensive List of Measurements

Over 150 measurements including, area, diameter, length, width, perimeter, shape, position, intensity. Customize your own measurement with built-in equation editor.



## Motion Tracking

### Object Position - X, Y coordinates

### Velocity - Average speed along the track

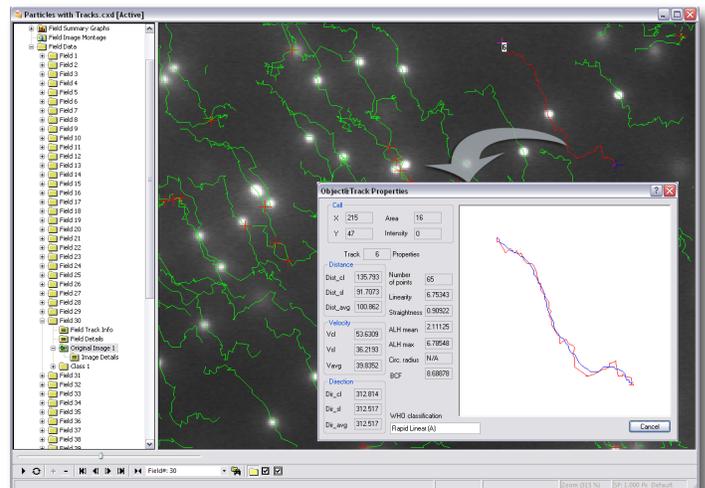
### Distance - Length of a line in calibrated units

### Direction - Angle measured in degrees

### Number of Points - How many consecutive images contain the same object in a track

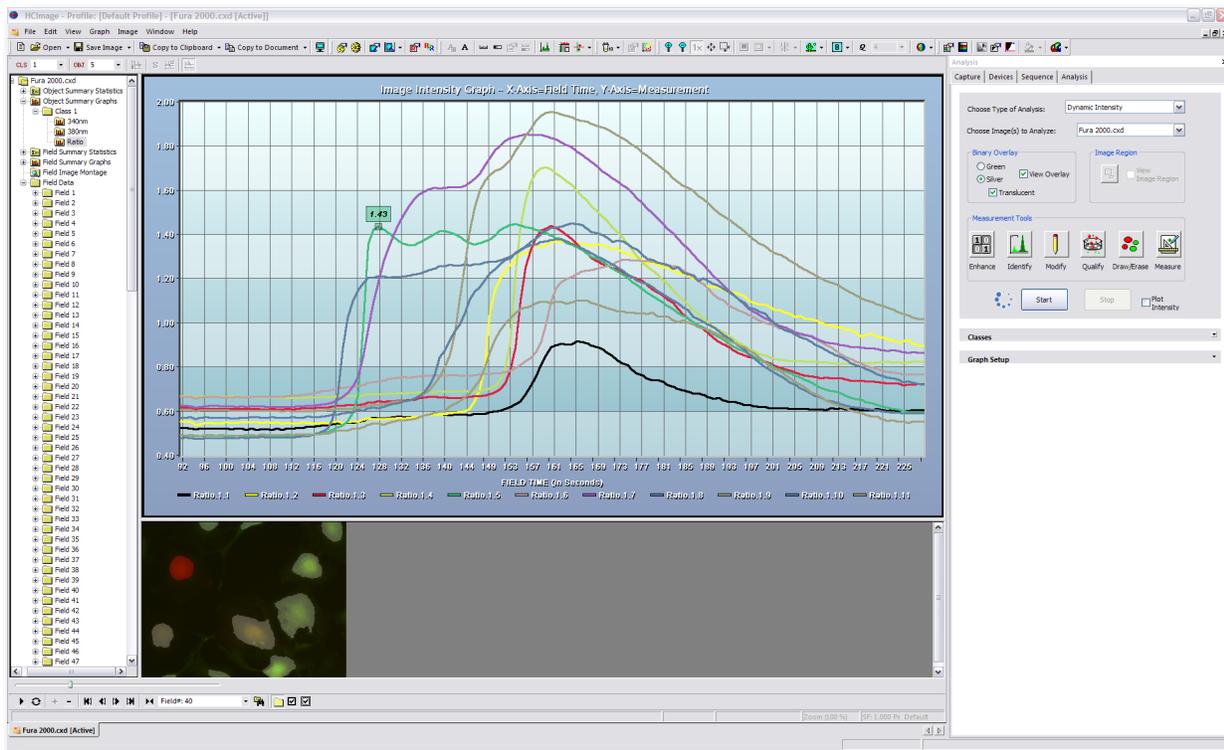
### Linearity - Numerical factor indicating how close the track is to a straight line

### BCF - Beat Cross Frequency, reports how often a track crosses its mean path



# Dynamic Intensity Analysis

HCIImage Analysis conveniently plots intensity of multiple objects in real-time or post capture on time separated image sequences. Objects can be identified by intensity, color threshold, or may be manually drawn. Critical details of an experiment are easily investigated by viewing a time course as an animation tightly linked to measured data. Custom measurements are available to deal with complex situations such as background correction on a field-by-field basis. To ensure synchronization of the experiment, event marking is possible using interactive key presses or TTL signals for automatic tagging.



HCIImage analysis is ideally suited for live cell applications including colocalization, FRET, FRAP, FURA2, BCECF, Indo, SNARF as well as other dynamic measurement techniques. These applications all benefit from the ability to identify objects in the images for plotting intensity change over time.

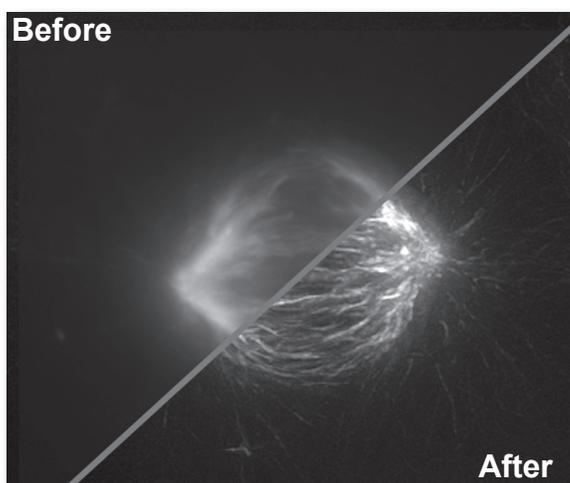
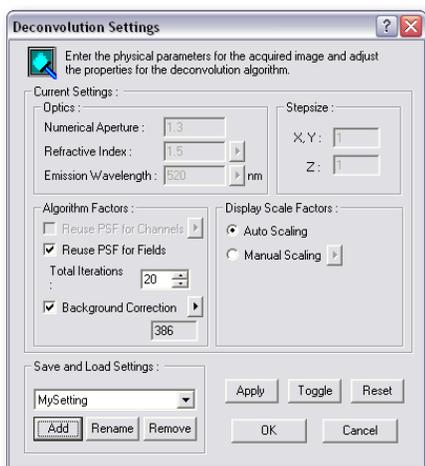
FIELD#	EVENT MARKER	380nm_1.3	Corr_380nm_1.3	380nm_1.3	Ratio_1.3
1		49.363276	24.727622	39.016602	0.633772
2		48.950583	23.904123	39.050338	0.609704
3		48.782476	24.379599	38.333893	0.636979
4		48.82479	24.06659	38.671288	0.623008
5		48.518519	23.956215	38.644987	0.617803
6		48.074644	24.691162	38.318775	0.644232
7		48.590466	24.120895	38.582494	0.626172
8		48.294900	23.592959	38.673655	0.609924
9		48.393203	24.626984	38.627043	0.637940
10		48.952707	23.824958	38.565925	0.617852
11		48.397444	24.686888	38.420848	0.623182
12		48.626328	23.870939	38.579041	0.618754
13		48.22071	23.490656	38.592066	0.608212
14		48.321191	23.796165	38.573045	0.609542
15		48.649873	23.928955	37.838636	0.633106
16		48.103703	23.752649	37.876977	0.625847
17		48.364622	23.825353	37.811914	0.619788
18		48.415955	23.827994	38.247283	0.623266
19		48.518519	23.395482	38.268671	0.611348
20		48.656689	23.781763	38.280182	0.619959
21		48.027037	23.314524	37.464641	0.622307
22		48.148146	23.561761	37.823785	0.620395
23		47.794939	22.888887	37.201824	0.609865
24		47.566952	22.899988	36.933101	0.614354
25		47.894957	22.950142	36.498787	0.620384
26		47.219373	22.648683	36.130079	0.626885
27		47.888804	22.850558	34.950444	0.652732
28		48.917379	23.181777	34.664452	0.668748
29		47.888804	24.081137	32.369486	0.742265
30		52.182336	36.666229	27.494779	0.968800
31		55.988684	30.745148	21.682763	1.417940
32		57.966995	31.982183	20.930227	1.959205
33		57.914930	32.393588	20.944955	1.546613
34		68.097476	32.769842	20.975953	1.921603
35		59.703704	31.491462	20.178690	1.565947
36		61.828793	31.221189	19.848052	1.575933
37		62.020513	30.134649	19.887382	1.512505
38		63.777835	29.462964	20.027476	1.472126
39		62.937280	28.962571	19.849458	1.412320
40		62.294296	27.973783	22.261656	1.242277
41		60.717919	27.928957	23.120955	1.189345
42		58.814815	27.492648	21.920444	1.183376

- Event Markers**  
 Acquire image sequences with dynamic I/O event marking during an experiment
- Define Objects**  
 Objects are identified by intensity, color threshold, or may be hand drawn
- Flexible Data Plot**  
 Plot one, multiple or average measurements over time
- Ratio Analysis**  
 Plot ratio intensity for Ca<sup>++</sup>, pH ratio and more
- Dynamic Data Interaction**  
 Interact between data plots and corresponding images

## Option for HImage Acquisition and Analysis

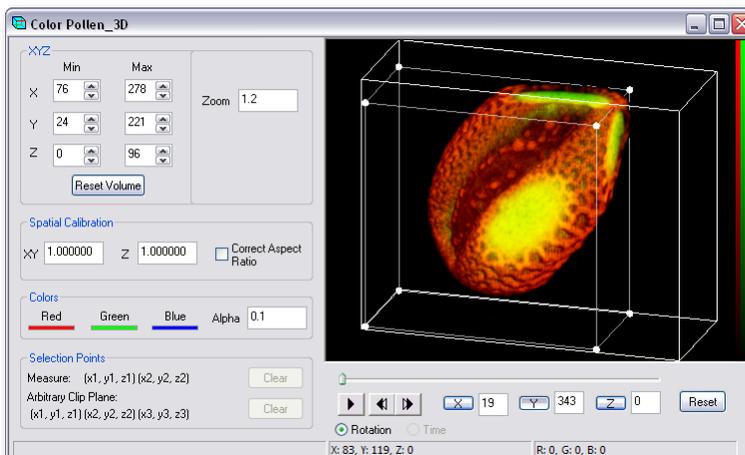
Optional 2-D Blind Deconvolution combines restorative deconvolution algorithms identifying out-of-focus haze and restoring it to its original point. A Point Spread Function (PSF) is automatically derived from an image and used in the restoration process.

- Apply to Image Sequence Automatically
- Automatically Computes PSF
- Work with Widefield, Brightfield and Confocal Images
- Ideal for Time-lapse Imaging
- Monochrome or Multi-color Fluorescence Images
- Apply to Full Image or Image Region for Fast Parameter Tuning
- Enhance Details for 3-D Reconstruction



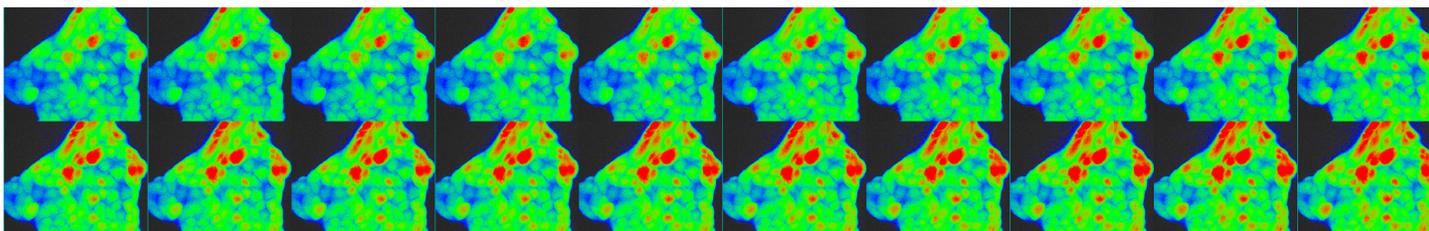
Optional Visualizer allows the user to easily view and interact with multi-dimensional data sets. A typical image data set would be a series of optical sections through a specimen. HImage Visualizer can reconstruct and render a multi-dimensional data set for interactive 3-D viewing. HImage Visualizer renders images using optimized software and hardware acceleration.

- Support for Multi-site Timepoints  
Render Z scan over time of multiple locations
- Measure Distance Between two Points  
Interactively compute the distance of two points
- Visible Volume Measurement  
Compute the volume of a visible 3-D object
- Visualize Colocalization  
Dynamically view colocalization
- Export Data to AVI or Image Sequence  
Share your rendered data set by exporting as AVI movies or as a series of tiff images



## Matrix of Levels and Functions Available

HCIImage	Live	Acquisition	Analysis	Review
Vista and XP Compatible	✓	✓	✓	✓
Multi-threaded and SSE2 Support	✓	✓	✓	✓
Full DCAM Support	✓	✓	✓	✗
TTL I/O	✓	✓	✓	✗
XYZ Stage, Filter Wheel, Shutter, and Microscope Device Drivers	✗	✓	✓	✗
Monochrome, Multi-channel or RGB Color	✓	✓	✓	✗
Time-lapse Streaming to Memory and Hard Disk	✓	✓	✓	✗
Time-lapse Scheduler, TTL, Delay, Scans over Multiple Loops	✗	✓	✓	✗
XYZλT Multi-dimensional Scans	✗	✓	✓	✗
Live Processing: Noise Reduction, Shade & Background Correction	✓	✓	✓	✗
Export to Tiff, AVI...	✓	✓	✓	✓
Image Correction and Image Processing	✓	✓	✓	✓
Intensity Threshold to Identify Objects	✓	✓	✓	✓
Area, Length, Intensity Measurement	✓	✓	✓	✓
Full Range of Morphological Measurements	✗	✓	✓	✗
Macro Building using Workfiles for Image Processing and Analysis	✗	✗	✓	✗
Data Statistics, Lists and Histograms	✓	✓	✓	✓
Analysis During Data Collection	✗	✗	✓	✗
Analysis Post Data Collection	✓	✓	✓	✗
Intensity & Ratio Plotting Live and Post Acquisition	✗	✗	✓	✗
Intensity Plotting for a Single Object Post Data Collection	✗	✓	✓	✓
Colocalization and FRET	✗	✗	✓	✗
2-D Blind Deconvolution and 3-D Visualization on image sequences	✗	Optional	Optional	✗



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