Nikon

CL-Quant Ver.3.10

Instructions

Thank you very much for choosing Nikon.
This manual is prepared for users of the CL-Quant Ver.3.10 analysis software.
For trouble-free operation, read this manual before using the program.

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Chapter 1

**Getting Started
with CL-Quant**

Introduction

Welcome to Nikon's CL-Quant 3.10 Analysis Software. CL-Quant provides everything you need to create high performance microscopy image recognition analyses, on par with custom written code, for your imaging experiment. Whether you are performing image based assays, or developing new ones, CL-Quant has the tools to get great results.

This guide is designed to introduce you to CL-Quant.

Learning CL-Quant

The software can be used in two primary modes: application execution mode and application teaching mode. This guide is divided into three main chapters accordingly.

- Chapter 1 Getting Started with CL-Quant

Chapter 1 provides a useful overview of the software. The quick start guide−covering recipe and procedure execution, data analysis and procedure teaching−may be the only section you need to read.

- Chapter 2 Using CL-Quant

Chapter 2 provides detailed description of the software interfaces and functions for image viewing, data analysis and procedure execution.

- Chapter 3 Teaching CL-Quant

Chapter 3 provides detailed instructions for updating and creating application recipes and procedures in CL-Quant.

1.1 Installing the Software

The software must run on a Windows PC running Microsoft .NET version 4.0 or greater.

If you have a new Windows PC, it probably already has .NET installed. Otherwise you can download it freely over the Internet from Microsoft, or from technical support. The CL-Quant installer will automatically detect if .NET is not installed, and will provide you with a link to download the free installer.

Once you have installed .NET, you can install CL-Quant. Double click on the executable provided to you, and follow the installation instructions.

NOTE:

- CL-Quant is available only in 64-bit version. Make sure you have the appropriate version for your PC.

- You must have administrator privileges to install CL-Quant.

The software has been tested and characterized on platforms similar to the following:

Recommended Platform

Processor	Intel Core 2 Duo or later
Processor speed	3.0+ GHz
RAM	8+ GB high speed memory
Operating System	Windows 7, 64 bit
Disk space	512 GB minimum

1.2 Usage Guidelines

The following usage guidelines parallel how the software is tested. The guidelines are size limitations for the different types of usage (e.g. processing, displaying, teaching, etc.) that can be done with multi-dimensional data sets in the software. The limitations assume you have a PC configuration that meets or exceeds the recommended platform specifications. Please see the discussion session at the end for a detailed explanation.

- Limitation for CL-Quant Ver. 3.10

Dimension	Usage	Max Supported
X,Y	Load and display	400 MB
	Examine	400 MB
	Processing	400 MB
	Segmentation Teaching	36 MB
	Stitching	400 MB
Z	Load and display	300 planes
	Examine	300 planes
	Processing	300 planes
	Segmentation Teaching	300 planes
C	Load and display	20 channels
	Examine	20 channels
	Processing	20 channels
	Segmentation Teaching	20 channels
T	Load and display	NA
	Examine	NA
	Processing	NA
	Segmentation Teaching	NA
M	Load and display	NA
	Examine	NA
	Processing	NA
	Segmentation Teaching	NA
Additional	Max objects per frame	256,000
	Max tracks per M	256,000
Total limits	Total load and display limit (XYZCM)	16 GB
	Total examine limit (XYCM)	4 GB
	Total processing limit (XYC)	2 GB
	Total teaching limit (XYZCM)	1 GB
	Total session limit (XYZCTM)	360 GB

Discussion

Limitations are presented in terms of multi-dimensional image dimensions as well as total limits. For example, processing is limited in x,y to 400 MB (20,000 x 20,000 8-bit pixels) while display can support up to 400 MB (20,000 x 20,000 pixels). Total limits are defined in terms of their relevant dimensions. For example, total examine limits are a function of x,y,c (channel), and m (position or FOV), while total processing limit does not include the m dimension. This is because examination functions (i.e. LUT, threshold overlay, mask overlay, etc.) can be applied to multiple FOVs simultaneously, whereas processing (i.e. procedure execution) is done one FOV at a time.

CL-Quant's stitching function is only supported through Nikon's BioStation CT CSV format. Simply load the CSV file, and the stitching is done automatically.

"Load and display" covers loading a list of FOVs, and navigating (zoom, animate in time or Z) them, but without applying examination functions (listed in the examination menu).

1.3 CL-Quant Basics

1.3.1 Window Layout

When you open the software, you will see a large blank workspace (the main panel in the figure below) and two additional panels on the right. The top panel on the right is the data explorer, a repository for data objects including "Fields of View" (FOVs) and "Procedures". The multi-tabbed Controls panel at the bottom contains data object information ("**Properties**" tab) and key controls in the "**Properties**" and "**Controls**".
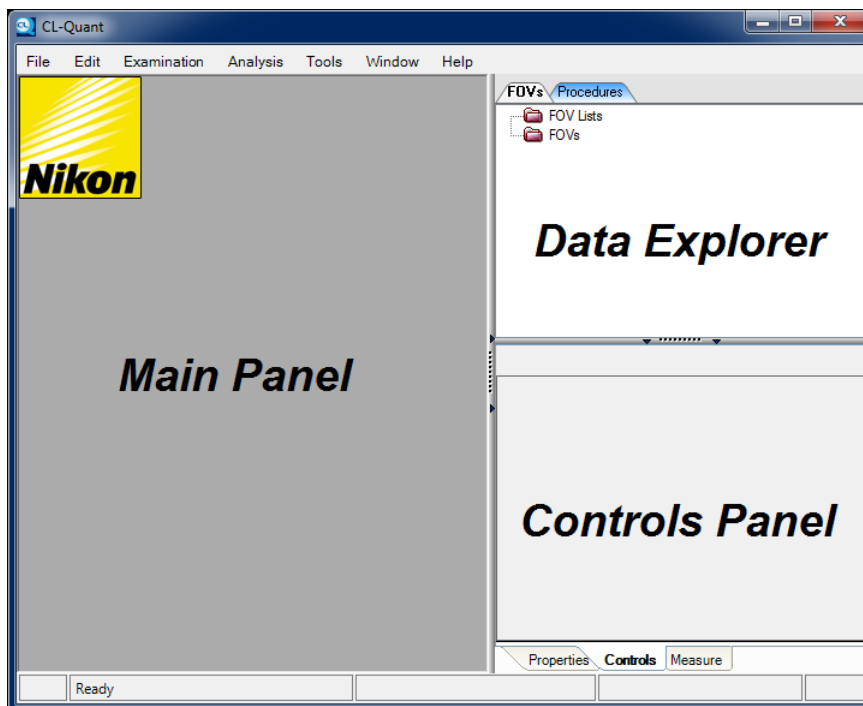
When you display procedures and FOVs they will appear in RecognitionFrames (RFrames). RFrames are the primary graphical interface in the software. RFrames are described in Section 2.1.2, "RecognitionFrame".

In this section, we describe the supported data objects and file types. Additional information about how to use these features for tasks is presented in Chapter 2, "Using CL-Quant" and Chapter 3, "Teaching CL-Quant".

1.3.2 File Types

CL-Quant generates the following file types:

- CL-Quant TIFF (*.TIFF)
 CL-Quant FOVs (see next section) are saved to TIFF format. The channel, mask and temporal arrangement of the FOV's images will be maintained. The format is an extension of the Open Microscopy Environment (OME). The format should be readable in OME compliant software.

- CL-Quant Procedure (no extension)
 All CL-Quant procedures can be saved to a data file.

- CL-Quant Procedure List (no extension)
 A procedure list contains an ordered collection of procedures for a single application. It can be saved to a data file that does not have an extension. See the Teaching CL-Quant section for instructions on assembling procedure lists.

- CL-Quant Recipe (no extension)
 Functionally equivalent to a procedure list, a recipe is a finalized collection of procedures. Unlike a procedure list, recipe update can only be done in the Recipe Console.

- BioStation Experiment Files (*.JSON)
 BioStation experiments are saved to the JavaScript Object Notation (JSON) format. The JSON file format is a data-interchange format that collects the file paths of all BioStation experiment images. When a JSON file is loaded, the BioStation CT viewer mode will be activated.

1.3.3 Data Objects

Data objects include Fields of View (FOVs) and procedures. When loaded, data objects will appear in the data explorer where they can be selected and displayed.

(1) Fields of View (FOV)
 The FOV and its objects are the primary units of analysis. An FOV can contain:

 - images,

 - segmentation masks,

 - field measurements,

 - object measurements,

 - object measurement statistics,

 - object regions of interest (ROIs).

 The FOV objects may also have multiple subset or class memberships. Initially an FOV contains sets of images, aligned in x,y and arrayed in up to three dimensions; time, channel and z.

 - FOV with time extent (a movie)
  A set of images, aligned in x,y, which show a time course experiment.

- FOV with channel extent (multi-channel)

A set of images, aligned in x,y, where each channel image provides different information about the x,y field. These could be images taken where the light has passed through different fluorescent emission filters, or through different objectives (fluorescence, phase, DIC imaging, etc.). All that matters is that the images have the same x,y dimensions; conceptually they should also represent the actual Field of View in the real world under the scope.

- FOV with z extent (z-stack)

A set of images, aligned in x,y, where each z plane shows an image frame acquired at a different depth of focus.

CL-Quant currently supports 5 dimensional imaging (x, y, z, time and channel). When a set of FOVs is loaded into an FOV list, a positional dimension is added, which enables users to navigate through multiple FOV positions ("M" dimension).

FOV List

FOVs can be organized into an FOV list. This can be done via drag and drop in the Data Explorer. Also, when sets of FOVs are loaded together they will be organized into an FOV List. All the FOVs in the list are presented together in the RFrame. The List enables batch procedure execution and group based data analysis. Therefore, you should consider loading FOVs from the same type of experiment together into a single FOV list. This will facilitate batch processing and integrated data analysis. It is less convenient to process and analyze data across RFrames.

(2) Procedures

Procedures encode the processing rules for analyzing FOVs. Procedures enable the software to be general purpose (because they can be taught for any application), and yet also provide "one-click" application execution and automation for high volume application.

We strive to make creation of procedures (what we call procedure teaching) easy and flexible, so that any scientist can create powerful and high performance analyses for their application, on par with custom written code.

The software currently provides import, enhancement, segmentation, partition, measurement, decision and tracking procedures.

- Import procedure:

Automates the importing of images on file into collections of FOVs.

- Enhancement procedure:

Automates traditional image and binary mask operations and common actions.

- Segmentation procedure:

Automates the innovative, machine-learning based confidence mapping.

- Measurement procedure:

Automates the calculation of field and objects measurements.

- Decision procedure:

Automates the application of innovative, machine-learning based object classifiers which assign FOV objects into non exclusive classes (e.g. live cell, dead cell, responder, non responder, artifact, etc.).

- Tracking procedure:
Automates the tracking of objects in movies and calculation of kinetic measurements.

CAUTION

- Your ability to create procedures is controlled by the configuration of the software you purchased.

<u>Procedure List</u>

Procedures can be grouped, saved and reloaded as a procedure list file. The procedure list provides a way to group and order a set of procedures for consecutive execution. You may have been provided with a procedure list for your application when you purchased the software.

<u>Recipes</u>

Functionally equivalent to a procedure list, a recipe is a finalized collection of procedures. Unlike a procedure list, recipe update can only be done in the Recipe Console.

1.3.4 Definitions

BMP (Bit-Map)	Image file format that separately stores the Red, Green and Blue components of each pixel in the corresponding image. Images of this type usually have a file suffix of ".bmp".
Channel	An FOV can be composed of multiple channels, which can be processed either independent of one another or in combination. A BMP image for example, once imported is composed of three separate channels for the Red, Green and Blue components of the image.
Decision Module	The interface that provides the tools necessary to teach and apply a Probabilistic Classification Tree to a series of input data or images. This mechanism allows the user to label objects and teach the software so that it can differentiate between objects considered to be from different categories or classes.
Enhancement Module	The interface that provides the ability to enhance an image using general purpose image processing functions such as Add, Subtract, Convolve, Erode, Dilate, etc.
FOV (Field of View)	A collection of images, organized by channel / mask, z, and temporal "dimensions" that are aligned in x,y. Conceptually, FOVs also contain the measurements and objects derived from the images. For example, an FOV could contain a two channel phase contrast and GFP fluorescence movie, aligned in x,y showing both the morphology and protein expression of a moving cell over time. After segmentation processing, a mask dimension is added with the same x,y,t extent as the original channels that defines the cell boundaries dynamically over time. After measurement processing, data and object regions of interest are associated with the moving cells and stored in the FOV.

Import	The process by which images or movies are loaded into the software in preparation for processing or viewing. Import can be automated with import procedures.
List	A data object that contains a collection of procedures or FOVs. The procedure list can be saved and loaded as a single file. The procedure list allows for ordering and batch execution of individual CL-Quant procedures. The FOV list allows for the same processing of groups of FOVs as well as integrated image and data review.
Mask	A mask is a binary image that is typically used as an overlay for a particular FOV channel or image. Each Channel can have any number of associated masks defined. These masks can be manipulated using the Segmentation and Enhancement modules and can serve as arguments for many image processing functions.
Measurement Module	The module responsible for performing a variety of quantitative measurements on FOV images, channels or movies. For example, this module can be called upon to measure the area associated with the nucleus of a cell or the area of its corresponding cytoplasm.
Movie	An FOV whose channels have temporal extent, meaning a series of x,y aligned image frames taken at regular time intervals. The movie, for example, may be a time series taken of live cells as they move about on a substrate.
Procedure	A processing step. There are several types of procedures such as enhancement procedure, segmentation procedure, measurement procedure, etc. Procedures can be saved and subsequently loaded into the software to process new data as necessary. They can also be modified to accommodate new operations or a new processing flow.
Recipe	An ordered collection of processing or measurement steps that when executed and applied to an FOV performs the specified operations in the appropriate sequence and stores the results for later manipulation or examination.
RecognitionFrame (RFrame)	A user interface that presents one or more FOVs. Complete application processing can be applied to the FOV(s) through this one interface. All image and data analysis can be done through the single interface as well. Both procedures and FOVs are viewed in the RFrame interface.
TIFF (Tagged Image File Format)	Image file format originally created by the company Aldus, jointly with Microsoft, for use with PostScript printing, TIFF is a popular format for high color depth images, along with JPEG and PNG.

1.3.5 Hot Keys

Hot keys are keyboard shortcuts that allow the user to quickly perform a specific function without having to click or navigate. Below is a list of available hot key functions.

- List of hot keys

Function	Context	Hot Key
Open	Global	Ctrl+O
Save as	Global	Ctrl+S
Reload	Global	Ctrl+R
Print	Global	Ctrl+P
Undo	Global	Ctrl+Z
Redo	Global	Ctrl+Y
Next timeframe	Global	Right arrow
Previous timeframe	Global	Left arrow
First timeframe	Global	Up arrow
Last timeframe	Global	Down arrow
Next Z Depth	Global	Ctrl+Up arrow
Previous Z Depth	Global	Ctrl+Down arrow
Hide bottom/right panel	Global	Ctrl+H
Toggle bottom/right panel on/off	Global	Ctrl+T
Length	Global	F7
Area	Global	Shift+F7
Angle	Global	Ctrl+F7
Annotation and measurement	Global	Ctrl+Alt+E
Histogram	Global	Ctrl+Alt+H
Object Histogram	Global	Ctrl+Alt+O
Mask edit tool	Global	Ctrl+Alt+M
LUTs	Global	Ctrl+Alt+L
LUTs on/off	Global	L
Maximize RFrame	Global	Ctrl+Alt+Shift+F
Zoom in	Global	=
Zoom out	Global	-
Fit to screen	Global	`
Best fit	Global	1
Zoom actual pixel	Global	2
First channel (red)	Global	R or Ctrl+1
Second channel (green)	Global	G or Ctrl+2
Third channel (blue)	Global	B or Ctrl+3
Delete	Global	Delete
Zoom to selected object	Global	Z
Swap Z/T	Global	Ctrl+Shift+Delete+D
Copy overlays to next timeframe	Mask editor	A
Copy selected drawn to next timeframe	Mask editor	S
Closed freehand shape tool	Mask editor	C
Polygon tool	Mask editor	P
Straight line tool	Mask editor	I
Freehand line tool	Mask editor	F
Confirm current shape	Mask editor	Enter
Remove last drawn shape	Mask editor	Esc
Go to previous zoom level	Zoom to rectangle	Esc
Clear annotations	Annotation	Ctrl+Shift+Delete

Function	Context	Hot Key
Set regions as Want	Soft Matching	W
Set regions as Don't Want	Soft Matching	D
Set regions as Background	Soft Matching	B
Cancel region drawing	Soft Matching	Esc

■ 1.4 CL-Quant Preferences

CL-Quant preferences are options that can be configured to give you important control over CL-Quant performance and usability.

To access the **Options** window, select "**File** > **Options**" from the "**File**" menu. At the top level are buttons to reset, apply or cancel your changes. The reset button will restore the original settings from when you first installed CL-Quant.

1.4.1 General Options

The top level window of the general options allows you to configure the file opening and display policies.

- **Load multiple images and procedures as lists**

  When this is checked, if multiple images or procedures are loaded at once, they will be grouped into FOV and procedure lists respectively.

- **Display when multiple files not in list**

  This determines how files are displayed when they are not loaded in a list.

  First   If this is selected, the first file only will be displayed.

  All   If selected then all the files loaded will be displayed.

  None   If this is selected, no files will be displayed. They will appear as FOV data objects in the data explorer.

  Ask   If selected, the user is prompted to choose between displaying all and displaying none.

- **Stitching Options**

  The software provides automated stitching of Nikon BioStation CSV format composite images in three modes:

  Fixed   CSV file meta-data, which indicates the expected frame overlap, is used to stitch the mosaic image without automatic alignment.

  User   In this mode you can specify the stitched image overlap. When loading, a window will appear in which you can type the desired overlap.

  Automatic   The software will automatically align the input stitching images to create the stitched composite. For multi-channel inputs, the first channel (Channel 0) alignment will be used for all channels.

- **Load and save objects and data**

  When this option is checked, FOV data and object ROIs/tracks are saved when saving FOVs. If it is unchecked, only images (channels and masks) will be saved.

- **Splash screen**

  If checked, the splash screen will be displayed when the software is launched.

- **Confirm exit**

  If checked, the software will prompt the user to confirm that he wishes to exit the software.

1.4.2 Graphics and Log

Sub-sections contain graphics and log settings.

(1) Graphics

- **Overlay mouse margin**

    How sensitive the software is to mouse-over detection. If the mouse pointer is within the pixel range, it will detect the object of interest.

- **Save displayed image line thickness**

    Sets the line thickness of overlain object ROIs and tracks in the saved image when using the "save displayed image" function.

- **Colorize component display**

    If an image channel has an assigned color other than brightfield, check this option to show individual channels in their assigned color. Uncheck this option to show individual channels as brightfield image, regardless of assigned color.

- **RGB merge**

    This specifies the method that will be used to merge a number of color assigned grayscale channels into a single R,G,B channel. Note that while the combined channel is primarily for visualization, it can also be processed by segmentation procedures. So the mode of combination will affect combined channel segmentation processing.

Simple	Each R,G,B component from the color input channels is summed and truncated at a maximum 255 value per component.
Best Fit	Each R,G,B component from the color input channels is summed, and then each component is scaled so that its maximum intensity is 255.
Saturation Best Fit	Each R,G,B component from the color input channels is summed. The R,G,B components are summed and the 95th percentile value is determined. The components are then scaled individually to 255 based on the value.

(2) Log

In the log section you can adjust the characteristics of CL-Quant's log file.

- **Verbosity**

    Determines the amount of information recorded to the log file.

- **Log File Enabled**

    Determines whether a log file will be saved.

- **Formatting**

    If checked, a time-stamp will be added to log file output.

1.4.3 Advanced Options

- **On demand loading**

    When this is checked, CL-Quant will load the images you need into memory when needed, rather than up front. This can make CL-Quant large image or large image set loading appear more responsive, however batch processing will be slower. Check this when you don't want to wait a long time for a large data set to load, and you are unconcerned about total processing time.

- **Always name according to file name**

    If checked, FOV data objects will have the same name as the saved file. If unchecked, FOV will be named using the image meta-data.

- **Memory usage limit**

    Limitation used for AVI export and combined channel display. If the value is greater than zero, preset limitations are enforced for recipe apply (64 MB) and stitching (324 MB).

- **Large data working folder**

    You can specify the location for CL-Quant's internal database. This is important if the drive your operating system is on is small.

- **ImageJ executable**

    You can specify the location for the ImageJ program executable. This is needed in order to run ImageJ functions in CL-Quant.

- **Compress saved image**

    If checked, compression methods will be applied to saved FOVs to reduce file size.

- **Movie compression**

    Options for saving image sequences to AVI format.

None	No compression will be used.
Basic	Simple compression will be used.
Prompt	You will be prompted to choose between basic and no compression.

1.4.4 Recipes and Procedures Options

- **Reload recipes and procedures from previous session**

  If checked, the recipes and procedures that were loaded when the software was exited will be re-loaded when the software is re-started.

- **Save teaching data with recipes and procedures**

  When this is checked, recipe or procedure teaching data will be saved, which can get quite large. If users only care to apply recipes or procedures rather than understand how they work, it is best to turn this off. However, if teaching data is not saved, then the recipe or procedure will not be updatable.

- **Compress saved recipes and procedures**

  When this is selected the software will automatically compress images saved with recipes and procedures.

- **Auto save FOVs data after recipe apply**

  If this option is checked, during batch processing a copy of each processed FOV is saved to the user specified folder. This provides some protection in case the batch run is disrupted.

Chapter 2

Using CL-Quant

Chapter Overview

This chapter describes using CL-Quant for image examination, applying recipes and procedures, and performing data analysis. Creating recipes and procedures is described in Chapter 3, "Teaching CL-Quant".

2.1 User Interface

When you open the software, you will see three panels:

- Main panel:

A large blank workspace

- Data explorer:

Top panel on the right. A repository for data objects including "**FOVs**" (Fields of View), and "**Procedures**" (and also recipes).

- Controls panel:

Multi-tabbed panel at the bottom. Contains data object information in the "**Properties**" tab and key controls in the "**Properties**" and "**Controls**" tabs.
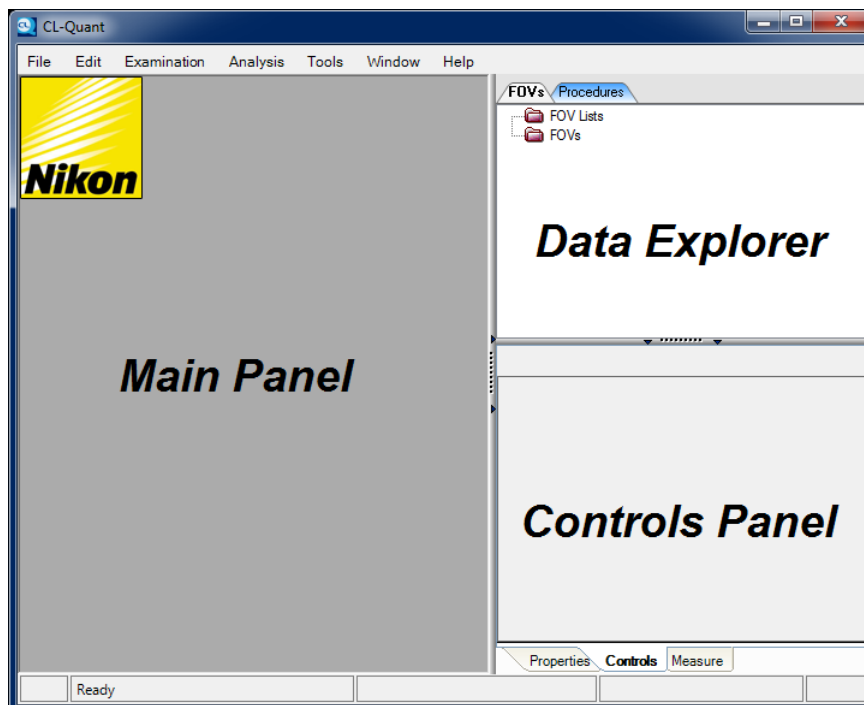
When you display FOVs, they will appear in RecognitionFrames (RFrames). RFrames are the primary graphical interface in the software. RFrames are described in Section 2.1.2, "RecognitionFrame".

2.1.1 Data Explorer

The data explorer, pictured below, is the central repository for data objects including FOVs, FOV lists, procedures, and procedure lists. Through this interface you can display, hide, save, rename, and reload the data objects.

(1) Renaming data objects

Renaming a data object in the data explorer is straightforward: right-click on the data object you wish to rename (it does not have to be highlighted) and select "**Rename**" from the options list that appears.

(2) Saving data objects

To save a select data object in the data explorer, right-click on the object you wish to save and click on the "**Save**" option from the menu. This will launch the save dialog.

(3) Displaying data objects

To display any data object in the explorer, simply right-click on the object you wish to display, and click on the "**Display**" option from the menu.

If you have multiple objects highlighted, you may choose the option "**Display All**". As the name suggests, this option will open new windows in the main panel, each one displaying an object that was highlighted when you chose the option.

(4) Hiding data objects

Right-clicking on any data object in the data explorer will display the "**Hide**" option. This will close the open window containing the data object.

To hide multiple data objects, select multiple data objects in the data explorer by holding the "**Shift**" or "**Ctrl**" key while left-clicking them. When finished selecting, right-click on one of the selected objects and then left-click on the option "**Hide All**".

(5) Reloading data objects

The reload command allows you to recover a modified data object and restore it to its previously-saved state. Right-click on the data object you wish to recover and click on the "**Reload**" option from the context menu.

(6) Removing data objects

To remove a data object from the data explorer, right-click on the object you wish to remove, and select the respective option.

> **NOTE:**
>
> If you have not saved that object to disk, removing it from the data explorer will mean losing any changes you made.

To remove multiple data objects, select them in the data explorer, right-click on one of the selected objects, and then left-click on the option "**Remove**".

(7) Selecting data objects

Selection in the data explorer is done by left-clicking on any data object or folder. You may select all objects under a certain folder at once by right-clicking on the folder and then left-clicking on the option "**Select All**".

To specifically select only a few objects, hold down the "**Ctrl**" key and click on any unselected object.

> **NOTE:**
>
> Using the "**Ctrl**" key to click on a selected object will deselect it.

2.1.2 RecognitionFrame

When you load one or more multi-dimensional images they will be displayed in the RecognitionFrame (RFrame). The RFrame is the primary graphical user interface in the software and can display a single FOV, or multiple FOVs in an FOV list. It contains all the elements of an imaging experiment in a single window. The primary elements of the interface are:
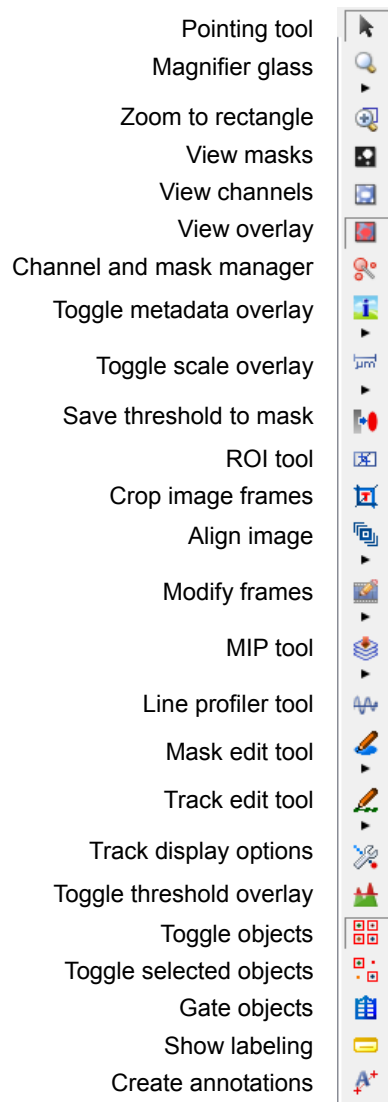
   - Image panel,

   - Content panel,

   - Toolbar.

The figure below shows the RFrame layout:
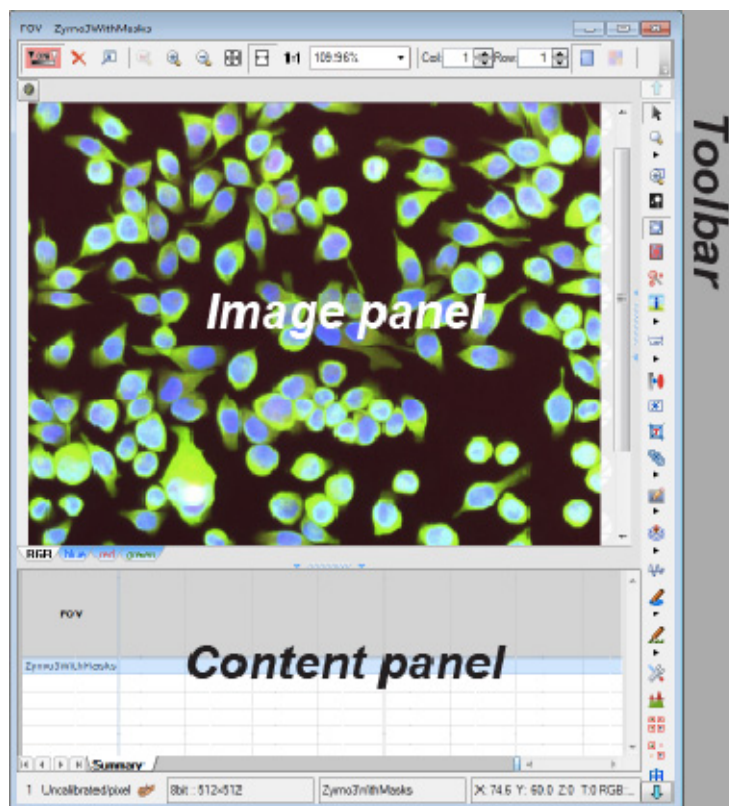
2.1.3 Toolbar

The RFrame toolbar provides key functionalities for image and mask examination, manipulation, and data analysis. It also provides ways to create enhancement, gating and decision procedures.

Pointing tool	
Magnifier glass	
Zoom to rectangle	
View masks	
View channels	
View overlay	
Channel and mask manager	
Toggle metadata overlay	
Toggle scale overlay	
Save threshold to mask	
ROI tool	
Crop image frames	
Align image	
Modify frames	
MIP tool	
Line profiler tool	
Mask edit tool	
Track edit tool	
Track display options	
Toggle threshold overlay	
Toggle objects	
Toggle selected objects	
Gate objects	
Show labeling	
Create annotations	

These tools are described in more detail in the sections below.

- **Pointing tool** - Standard mouse mode.

- **Magnifier glass** - Zooms in on the region under your mouse.

- **Zoom to rectangle** - Zooms to region specified on the image.

- **View masks** - Shows only the mask.

- **View channels** - Shows only the image.

- **View overlay** - Shows mask overlay on the image.

- **Channel and Mask Manager** - Use the Channel/Mask Manager.

- **Toggle metadata overlay** - Shows file metadata overlay on the image.

- **Toggle scale overlay** - Shows scale bar with calibration (if available) overlay on the image.

- **Save threshold to mask** - Saves intensity threshold overlay as binary mask.

- **ROI tool** - Specify a region of interest (ROI) for cropping.

- **Crop image frames** - Crops the FOV to a specified time window and sampling steps.

- **Align image** - Realigns images in a sequence based on ROI inputs.

- **Modify frames** - Manipulate T- or Z-stack image sequence.

- **MIP tool** - Creates an intensity projection (MIP) image from Z-stack.

- **Line profiler tool** - Use the line profiler tool.

- **Mask edit tool** - Use the mask editing tool.

- **Track edit tool** - Use the track editing tool.

- **Track Display Options** - Use the Track Display Options.

- **Toggle Threshold Overlay** - Toggles the intensity threshold overlay tool.

- **Toggle objects** - Toggles object bounding box or track display.

- **Toggle selected objects** - Toggles object bounding box or track display for selected objects.

- **Gate objects** - Creates gated object subset from selected objects.

- **Show labeling** - Toggles object label display.

- **Create annotations** - Generates annotation measurement on user-specified regions.

2.1.4 Zoom Controls

Zoom controls are located at the top of the RFrame.
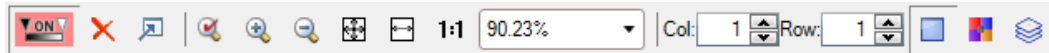
- **Turn on / off LUT**

This button will apply the look up table (LUT) to the image.

> **NOTE:**
>
> Turn LUT on at all times when viewing/processing 16-bit images to ensure best picture contrast.

- **Reset LUT**

This button will reset any LUT settings, and turn off the LUT.

- **Toggle LUTs window**

This button will open the LUT panel to the right of the image.

- **Zoom to selected object**

This button will zoom into the image to best fit selected objects.

- **Zoom in / Zoom out**

Click these buttons to zoom in or out of the image.

- **Fit to screen**

The image will be zoomed to fit completely on the screen.

- **Best fit**

The image will be zoomed to fit the smaller of its height or width to the screen.

- **Zoom 1:1**

This will show the actual pixels, meaning one display pixel for one image pixel, or 100% zoom level.

- 88.5% ▾ **Zoom level drop down**

  Here you can select the desired zoom level from the drop down box.

- Col: 1 ▲▼ Row: 1 ▲▼ **Column and row settings**

  When viewing an FOV list, use the textbox provided to adjust how images are displayed on a grid.

- ▫ **Switch to Main view mode**

  This option toggles the default image view mode.

- ▦ **Switch to Gallery view mode**

  This option toggles image view to Gallery View mode.

- ▨ **Switch to MIP view**

  This option toggles image view to mean intensity projection (MIP) view for Z-stacks.

2.1.5 Minimap

Minimap control is located under the zoom controls toolbar in the RFrame. To turn on minimap display, click on the minimap control icon.

> **NOTE:**
>
> Minimap display will not appear if the entire image is visible. Zoom in anywhere on the image to view.

Minimap display

When enabled, the minimap display is shown in the upper left corner of the image panel overlaid on the image. Note that masks and object overlays on the image are not visible in the minimap view. The minimap is an abstract representation of the entire image. The displayed region is shown as a neon-green rectangle that shows the current view in relation to the entire image.
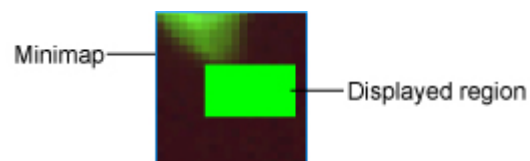
On the minimap, you can:

- click anywhere on the minimap to navigate to a different region; and

- click-and-drag the display region to pan across the minimap.

2.1.6 Metadata Overlays

In CL-Quant, metadata (such as time and length calibrations) can be overlaid on the image to provide additional context information about the files. There are two types of overlay options; metadata overlay and scale overlay.

(1) Metadata overlay

Image metadata provides descriptive information about image context. To toggle metadata display, click on the "**Toggle metadata**" icon on the toolbar (shown below). The metadata overlay is displayed on the lower left hand corner of the image. This function supports time calibration display.

> **NOTE:**
>
> Metadata information may not be available for all images.

You can burn the metadata overlay display on the image by selecting on the "Burn Metadata" option accessed by clicking on the arrowhead below the option with Metadata overlay enabled.
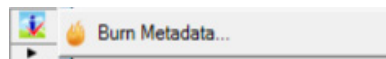
> **NOTE:**
>
> This action will modify the image and cannot be reversed. Any pixels and image information behind the overlay burnt region will be lost.

(2) Scale overlay

The scale overlay displays a scale bar on the image to provide length context within the image. If calibrations are specified, the scale bar will display the calibrated distance with the scale bar. To toggle scale overlay, click on the "**Toggle scale overlay**" icon on the toolbar (shown below). The scale overlay is displayed on the lower right hand corner of the image.

You can burn the scale overlay display on the image by selecting on the "**Burn Scale**" option accessed by clicking on the arrowhead below the option with Scale overlay enabled.
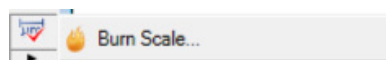
> **NOTE:**
>
> This action will modify the image and cannot be reversed. Any pixels and image information behind the overlay burnt region will be lost.

2.1.7 ROI Tool

The ROI tool allows the user to select a region of interest (ROI) by clicking-and-dragging a rectangle across the image. Additional options for ROI operations can be selected from the context menu by right-clicking on the ROI frame. To use the ROI tool, click on the ROI region selection tool from the toolbar (shown below).

When selected, click-and-drag over an area on the image to create a region of interest. A pink, rectangular border will indicate the selected region of interest. You can move the ROI by holding down the left-click while dragging inside the ROI. To resize the ROI, hold down the left-click while dragging on the ROI border.

(1) ROI options

The "**ROI**" context menu (shown below) is accessed by right-clicking inside a selected ROI.
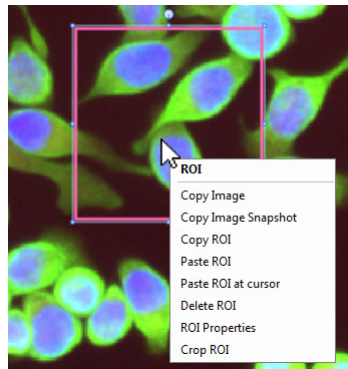
- **Copy image**
  Copies image inside ROI onto clipboard scaled relative to the actual image size

- **Copy image snapshot**
  Copies image inside ROI as-is onto clipboard relative to displayed size

- **Copy ROI**
  Copies the ROI frame only

- **Paste ROI**
  Pastes ROI at the original location as the copied ROI

- **Paste ROI at cursor**
  Pastes ROI at the tip of the cursor

- **Delete ROI**
  Deletes the ROI frame

- **ROI properties**
  Displays ROI properties in a new window

- **Crop ROI**
  Crops image to ROI frame

(2) ROI properties

The ROI properties pop-up displays information about the ROI frame and enables the user to edit the location and size of the ROI. To display the "**ROI Properties**" dialog, select "**ROI Properties**" from the "**ROI**" context menu.
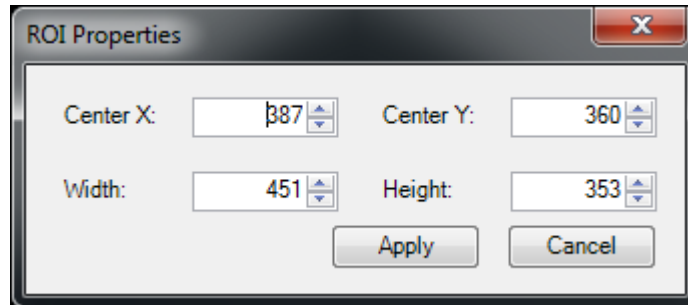
- **Center X**
  Horizontal position of the ROI center

- **Center Y**
  Vertical position of the ROI center

- **Width**
  Width of the ROI

- **Height**
  Height of the ROI

Use the textbox to adjust the ROI attributes as needed. Click "**Apply**" to update ROI position and size.

◼ 2.2 Loading and Saving Files

This section covers loading and saving files and other data objects, and exporting FOVs and data.

To learn how to configure import procedures, which can automate the import of FOVs and FOV elements (masks and channels), see Chapter 3, "Teaching CL-Quant".

2.2.1 Loading FOVs

CL-Quant supports standard image file types such as raw, TIFF, PNG, GIF, BMP, and JPEG. CL-Quant supports multi-dimensional formats. CL-Q N dimensional FOVs are saved to CL-Q TIFF format (an extension of the OME standard). In addition, CL-Quant can load BioStation CT CSV and JSON formats, ICS/IDS and NEX, and AVI.

Image frames can be imported to create a multi-dimensional FOV (see Section 3.4.1, "FOV Import Procedure").

To load an FOV, either:

- Select the file in a Windows Explorer window and drag it into the software.

or

- Click on the "**File**" menu and then click on the "**Open**" option, which will prompt a dialog window that allows you to browse your computer and local network to the FOVs you wish to load into the software.

When you load an FOV into CL-Quant, it will be displayed in an RFrame. It will also appear in the data explorer panel under the "**FOVs**" tab.

If you load multiple image files, by default they will be loaded into an FOV List. Loading an ICS/IDS NEX file will load all of the linked files into an FOV list. Also loading BioStation CT 3.0 format CSV file will load any linked FOVs (i.e. from a well plate) into an FOV list. You can also create an FOV list by right clicking on the FOV list folder.

You can modify the CL-Quant policy for displaying FOVs when loading, and also for grouping FOVs into lists in the CL-Quant general options.

2.2.2 Loading Stitched FOVs

Large, stitched mosaic FOVs can be loaded and stitched automatically using the BioStation CSV format meta-data. Multi-channel FOVs are supported. If the FOV is time-lapse, you will be prompted to select just a single time frame for stitching. The arrangement of files into the large, stitched mosaic image, and of multiple such stitched images into a multi-channel FOV is specified in the BioStation CSV format meta-data.

The loading of 20 x 20 (1,000 x 1,000 pixels) frame, two channel FOVs has been validated for the recommended platform.

There are three ways of loading BioStation CSV format stitched images, which are configurable in the CL-Quant options settings (under "**File** > **Options**"):

- Fixed stitching:
Pixel overlap between adjacent frames specified in the CSV format meta-data is used for stitching.

- User stitching:
The user can specify the pixel overlap amount.

- Auto stitching:
CL-Quant will automatically align the input image frames for stitching.

When you load or import the stitched FOV, a load window will appear that gives you the option of selecting a time range or sampling interval for loading, the amount of pixel overlap for stitching (when in "**User**" mode).

The arrangement of frames into stitched images, and stitched images into multi-channel FOVs can also be accomplished through stitched image FOV import (see Section 3.4.1, "FOV Import Procedure").

2.2.3 Loading Other File Types

Loading a recipe
You can always load recipes by simply dragging and dropping them into the software, or by clicking on the "**Open..**" option found in the "**File**" menu.

Loading a procedure
Procedures can also be opened via drag and drop or "**File** > **Open**".

If you have to load several procedures at once (and do so several times), you should create a list of those procedures. This is described below in Section 2.2.6, "Saving other file types".

2.2.4 Saving the FOV

Save an entire FOV by selecting the FOV in the data explorer, and then clicking "**File** > **Save**". This will save the FOV to TIFF format, which will preserve the channel, time, z and mask structure of the FOV.

You can also save the FOV to TIFF format by right-clicking on it in the data explorer under the "**FOVs**" tab. Click on the option "**Save**", and a save window will open.

On the "**General Options**" page you can select the "**Load and save objects and data**" option, which will allow CL-Quant to save FOV's objects, data and subsets.

You may save multiple FOVs by selecting multiple FOVs ("**Shift**" or "**Ctrl**" click) at once, or by selecting the FOV list data object.

2.2.5 Saving FOV Images

You can save individual image frames, thumbnails, masks, and also the displayed image.

(1) Saving a mask

If you only want to save a specific mask of an FOV, right click on the mask tab and select "**Save Mask**". This will save the current frame's mask only.

(2) Saving a displayed image

If you wish to save whatever is currently being displayed in the RFrame (e.g. masks and / or ROIs overlain on the image), navigate to the **File** menu and click on "**Save Displayed Image**". You may apply this action to multiple, selected FOVs. In this case whatever would be displayed on the FOV, if you displayed it (it is not necessary to display it), is saved shown as displayed on the underlying image frame. The images are saved at their actual resolution.

(3) Saving a snapshot

"**File** > **Save Snapshot...**" saves the image panel thumbnails as a "**Save Displayed Image**" format, where all overlaid masks, ROIs etc. are saved to the image. The function saves the composite image at the displayed layout and zoom level. All of the thumbnails above the displayed position are saved.
For example, if you have a list of FOVs and you wish to create a thumbnail composite of all of them, you would set the zoom level at the level you wish to save, arrange the horizontal width appropriately, and the scroll down in image panel to the very last thumbnail. When you execute the function, all of the thumbnails above your position in the image panel will be saved.

> **NOTE:**
>
> "**Save displayed image**" saves one image at its original resolution. "**Save Snapshot**" saves multiple FOVs displayed in the RFrame image panel exactly as they appear on your monitor.

(4) Saving a movie

"**File** > **Save Movie...**" saves the displayed time-lapse FOV to AVI format. This can be done in either "**Save displayed image**" or "**Save snapshot**" mode.

2.2.6 Saving Other File Types

(1) Saving a procedure

In the data explorer you'll see a tab for procedures. Right-click on any of the procedures you wish to save and click on "**Save**", which will prompt a window where you can choose where to save the procedure.

Segmentation, measurement and decision procedures can be saved with or without the teaching images. This is a configurable option under the options menu. See Section 1.4, "CL-Quant Preferences".

If you save and reload the procedure without the teaching images, you won't be able to view the teaching images used to create the procedure.

CAUTION

- Procedures can be created in all versions of CL-Quant. However, procedures can only be saved if your version has support for procedure saving.

(2) Create and save a procedure list

When you load multiple procedure files by drag and drop (or "**File** > **Open**"), a new procedure list data object will appear in the "**Procedure Lists**" folder (located in the data explorer under the "**Procedures**" tab), with the loaded procedures arrayed under it in a list. You can also create a procedure list by right clicking on the "**Procedure Lists**" folder and selecting "**New**". This will create a new procedure list data object. You can then add procedures to the list by dragging and dropping them from their folders in the data explorer.

You must specify the processing order of the procedures by arranging their order in the list. You can lock the list order by right clicking and selecting "**Lock Procedure List**". When a procedure list is locked, it will appear as a single data object (you cannot see the sub-procedures), which can reduce the apparent complexity for users, and the sub-procedures cannot be accessed.

To save, right click on the procedure list data object and choose "**Save**", or select the data object and then choose "**File** > **Save**". The procedure list will be saved to a single file on the file system.

2.2.7 Exporting FOVs

(1) Measurements Export

The measurements export option saves the FOV's data to a destination directory of your choosing. The images are not saved, and the .csv file created cannot be reloaded. To export FOV's data, select "**File** > **Export** > **Measurements**".

(2) FOV Export

This option will export multi-dimensional FOV's image frames (including masks) to individual files on the file system. Select "**File** > **Export** > **FOV**". You can select t and z ranges and sampling for the export.

(3) Print to Excel

Selecting "**File** > **Print to Excel**" will export the spreadsheet of the selected FOV(s) to Excel format. Any displayed chart will also be exported.

(4) Print Tracks to Excel

Selecting "**File** > **Print Tracks to Excel**" will export FOVs containing track data to Excel.

(5) Print Object Histogram to Excel

This function will export existing object histograms to Excel format.

(6) Save Track Locations to CSV

This saves only the x, y, t information from tracks to a CSV format.

(7) Save Track Measurements to CSV

This function saves only the trajectory measures to CSV format. These are measures which are one per track, such as total length. It does not save the motility measurements which are one per time point.

2.3 Manipulating FOVs

An FOV can contain images, segmentation masks, field measurements, object measurements, object measurement statistics, and object ROIs. FOV objects may also have multiple subset or class memberships. Initially a FOV contains sets of images, aligned in x,y and arrayed in up to three dimensions; z, time and channel.

Once an FOV is loaded, you can manipulate the FOV by manually editing its channels and masks, calibrating its spatial and intensity units, merging and appending multiple FOVs into one, and cropping the FOV in x,y and t dimensions.

2.3.1 Edit FOVs

(1) Editing channels

You may save the selected channel, add new channels, remove unwanted ones, and rename them. Channels tabs are located at the bottom of the image panel in the RFrame.

- Adding a channel

To add a channel (be sure to have an FOV loaded), right-click on the bottom strip (in the blank area or on a channel tab itself) of the FOV and select "**Add Channel**".

- Inserting a channel

To insert a channel, click on the channel tab positioned just after where you wish to insert a channel, right click on the channel tab and select "**Insert Channel**".

- Removing/renaming a channel

To remove or rename a channel, simply right-click on the channel tab you wish to remove or rename, and select the appropriate option.

(2) Editing masks

Masks can be added, removed/deleted, and renamed. In addition, you may change the color of the mask.

- Adding a mask

To add a mask (be sure to have an FOV open), you can right-click on the mask strip (along the top of the image panel) of the FOV and select the only option, which is to "**Add Mask**".

- Removing/renaming a mask

To remove or rename masks, simply right-click on the mask tab of the mask you wish to remove or rename and select the appropriate option.

2.3.2 Merge FOVs

(1) Merging FOVs by channel

It is sometimes useful to load one FOV into a destination FOV's Channel. For example, you might want to combine two fluorescence channels into a single FOV for analysis. This can be done by a simple drag and drop operation.

To merge the two FOVs, display the destination FOV in an RFrame, and then drag the FOV to be merged from the data explorer onto the channel tab of the destination FOV−a new channel will be created automatically. The image you want to merge must have the same dimensions (x, y) as the existing FOV.

(2) Merging FOVs by mask

It is sometimes useful to load one FOV into a destination FOV's mask. For example, you may have a mask you created from one procedure that you want to compare with another procedure. To bring that mask image into the destination FOV, you first need to have the destination FOV RFrame displayed. The image you want to merge must have the same dimensions (x, y) as the existing FOV.

Simply drag the FOV to be merged onto the mask strip of the destination FOV−a new mask will be created automatically.

2.3.3 Append FOV

Fixed point and time-lapse FOVs can be inserted along the time dimension into any other fixed point or time-lapse FOV. To append an FOV into the destination FOV, you first need to have the first FOV's RFrame window open. The FOV you want to merge must have the same x,y dimensions as the destination FOV.

You may either append the FOV before or after the current frame. You may merge as many FOVs into the destination FOV's time dimension as you like. Display the destination FOV in an RFrame. To insert before the current frame, drag the FOV to be merged from the Data Explorer to the left of the time slider. To insert a movie after Frame C, drag the FOV to be merged to the right of the slider.

2.3.4 Crop FOV

(1) X,Y crop

FOVs can be cropped using the ROI tool. Draw a rectangle around the area you wish to crop to a new FOV. Right-click on the ROI frame and select "**Crop ROI**" from the context menu. This will copy the FOV (all t, z, channels, and masks) within the ROI region to a new FOV. Please refer to the ROI tool section on creating ROI region tools.

Your cropped FOV will appear in the data explorer under the FOVs tab and folder.

(2) Time crop

Click on the "**Crop image frames**" icon (see below) on the toolbar to launch the time crop dialog. The time crop dialog allows you to select a range and frequency for the time crop.

Once you select your options, click "**OK**" and a new FOV will be created. It will appear in the data explorer.

2.3.5 Image Alignment

The software provides the capability of aligning FOVs so that objects (typically cells) in the time series are located at the same x and y position in the spatial "imaging system domain". In live cell assays, alignment may be necessary because cells tend to move over the course of time making it difficult to monitor the growth or behavior of a single specific cell.

Image alignment may also be necessary in fluorescence or other dead-cell assays when the mechanical system incorrectly positions a sample for microscopic analysis. In this case, alignment can be called upon to adjust the image so that samples taken a various times and positions can be aligned in the spatial domain.

It is important to note that software image alignment always results in some degree of x, y cropping of the aligned images. This is because the images are shifted in x and y the exact amount necessary to align the two images. When an image is shifted, the pixels around the border of the image that previously existed outside the bounds of the original image are now brought into the image area. Since this data is "unknown", the new pixels are set to zero so there can be no misinterpretation in the resulting image. For this reason, the aligned image will always contain less pixel data than either of the two images provided as input. The resulting aligned image will contain valid pixel information for the area where the two images intersect.

The following three alignment tools are provided depending on the application requirements. The ROI-based alignment operations require at least one ROI defined anywhere within the time series. When multiple ROIs are specified, they must be specified at the same time index (same image).

(1) Alignment types

ROI-based Global Alignment (RGA)

RGA uses up to ten user specified statically positioned ROIs to align all images in the T series to the reference image containing the initial ROIs. If an image is selected in the middle of the time series, then RGA will propagate the alignment both backward and forward in time. All masks associated with the time series will also be shifted by the same amount as the associated image. The resulting aligned images and their associated masks will be padded with zeros outside the region containing valid pixel data.

In RGA, all ROIs are statically positioned and do not move over the course of the alignment analysis. RGA is useful if you have an object, or series of objects, that do not move over the entire course of the time series. For example, a Petri dish may have a reference marker etched into the glass or an imperfection in the glass that can be used as a reference. In this case the user would select a ROI around the reference marker so RGA can align all image data to that reference.

ROI-based Incremental Alignment (RIA)

RIA uses up to ten user specified dynamically updated ROIs to align each of the images in the T series to the reference image containing the initial ROIs. If an image is selected in the middle of the time series, then RIA will propagate the alignment both backward and forward in time. All masks associated with the time series will also be shifted by the same amount as the associated image. The resulting aligned images and their associated masks will be padded with zeros outside the region containing valid pixel data.

In RIA, the positions of all ROIs are adjusted with each successive frame in the time series.

Thus, RIA is capable of tracking multiple objects that may be uniformly drifting in position during the course of an experiment. This alignment mechanism should not be used in applications where there is differential movement between the objects as the differential movement may send contradictory feedback to the alignment engine.

Image-based Global Alignment (IGA)

IGA does not require the use of any user specified ROIs. This alignment mechanism uses all the information within the image to assess and correct for any misalignment. Unlike the ROI-based alignment tools, IGA only corrects for misalignment going forward through the times series starting with the first image of the time series. IGA adjusts the x, y position of each image relative to the first image in the series and makes the same corrections to the associated masks.

(2) Guidelines for using ROI-based operations

Try the following suggestions that will help improve the performance and robustness of the ROI-based alignment tools.

Tips for Selecting Alignment ROIs

- Select small objects (ROIs) rather than larger objects both for alignment robustness and speed.

- Select objects that are stationary relative to all the other cells in the image (RGA only).

- Select objects that move in unison (RIA only).

- Select objects with good contrast over those with poor contrast.

- Select objects that have unique size and shape characteristics.

- When possible, select objects in the center of the field of view, so that they are less likely to move outside the field of view.

- Select objects in the middle of a long movie so that overall drift is minimized.

- Select objects that remain within the field of view the longest.

- Select multiple objects when no single object remains within the field of view for the entire movie.

- Select multiple objects to improve the accuracy and robustness of the alignment.

Alignment Limitations

Image movement must be less than a quarter of the x and y dimensions of the image. In our tests, the image dimensions were 1,000 x 1,000 pixels so the limit for maximum movement was 250 pixels. Movement between successive images greater than 250 will result in loss of alignment.

2.3.6 Modify Frames

FOVs containing multiple frames (such as a time sequence or Z-stack) can be loaded into CL-Quant for analysis. The "**Modify frames**" function on the toolbar allows you to manipulate the frames of the FOVs. Click on the "**Modify frames**" icon on the toolbar and select an option.

- **Reverse T**

  This option will reverse the FOV's time dimension and the frame order of the T-series.

- **Reverse Z**

  This option will reverse the FOV's axial/Z dimension and the frame order of the Z-stack.

- **Swap T/Z dimensions**

  This option will swap the FOV's time and Z dimensions. For image sequences that contain only T or only Z series, this option will convert the sequence to the other dimension.

2.3.7 MIP Functions

MIP functions are intensity projection operations that flatten a Z-stack image sequence into a single image based on specified image statistic. The MIP functions will generate a new FOV that contains the result output of the MIP operation. To generate an MIP image, first make sure you have a Z-stack image sequence loaded into CL-Quant. Click on the "**MIP tool**" icon on the toolbar (shown below) and select an option for the MIP image output.

There are five types of MIP that can be generated using the MIP function.

- **Min Projection**

  Outputs an MIP image containing the minimum intensity for each pixel over the entire Z-stack. (Figure 1).

- **Max Projection**

  Outputs an MIP image containing the maximum intensity for each pixel over the entire Z-stack. (Figure 2).

- **Mean Projection**

  Outputs an MIP image containing the mean intensity for each pixel over the entire Z-stack. (Figure 3).

- **Median Projection**

  Outputs an MIP image containing the median intensity for each pixel over the entire Z-stack. (Figure 4).

- **HSDEF Projection**

  Outputs an MIP image containing the 2D extended depth of focus (EDF) intensity projection image. The EDF projection combines the in-focus pixels of each Z-stack image into one image, ensuring that the projection output is all in focus. (Figure 5)
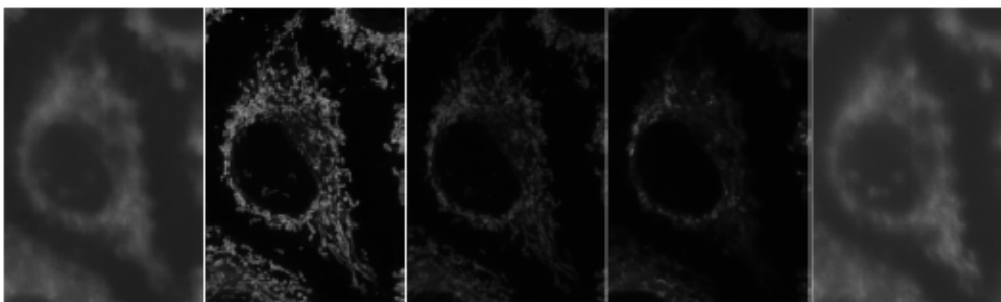
| Figure 1 | Figure 2 | Figure 3 | Figure 4 | Figure 5 |

2.4 Examining FOVs

The examination toolbar contains functions related to image, mask and object region of interest viewing. This section describes many common and useful interface features.

2.4.1 Freehand Measurement Tools

The freehand measurement tools will show calibrated image measurement based on user input. Use the tool to measure straight-line length, area, and angle of a drawn object. To launch the annotations tool, click on the "**Create annotations**" icon (shown below) on the toolbar.

When toggled, the annotation tools will be displayed in the "**Annotations**" tab in the Controls panel.

There are three tools provided for freehand measurements:

- **Length measurement**

  Measures the straight line length of a drawn line

- **Area measurement**

  Measures the area of a drawn region

- **Angle measurement**

  Measures the angle between two drawn lines

Click on any of the measurement tools and start drawing on the image. The annotations will appear in the Fields panel in the "**Annotations**" tab under the measured image name.

If the image is calibrated, users can change the measurement unit by selecting a desired unit from the display unit dropdown menu.

2.4.2 Navigate the FOV or FOV List

FOV navigation refers to changing the displayed image pixels in the image panel. You can do this by moving through the x, y, z, channel, and time dimensions of the FOV. In addition, each image frame may have one or more masks that you can display. You can also navigate through multiple FOVs in an FOV List.

(1) Horizontal and vertical scrollbars

If the image is larger than the RFrame image panel, scrollbars will appear on the bottom and right side of the image. You may use these to navigate your image(s) in x and y directions.

In addition, there is the "**Grabber**" tool which allows you to navigate in x,y by dragging your mouse when this tool is selected. Click and hold your mouse pointer on the image to get the grabber tool.

(2) Mask tabs

At the top of the RFrame are the zoom controls. Just below those is the mask strip where the mask tab appears. You may display the mask overlay by clicking on their respective tabs.

(3) Time, M, and Z controls

Just below the RFrame image panel are the movie controls. They are the same for navigating in time, m, and z.

Each green block represents an image in the sequence. The green arrow plays the sequence. Next to that are controls for stepping through the sequence and advancing to the end of the sequence. You can adjust the playing speed using the +/- buttons or set playing speed to real time (R) or maximum (M) using the controls on the right.

(4) Channel tab

You'll notice that the channel strip (which behaves just like the mask strip) is directly below the movie controls. Click on a channel tab to display the image from the next channel. If there are multiple channels, the combined channel will be automatically shown. You can include or exclude channels from the combined channel by right clicking on their channel tab.

(5) FOV list display grid

When you view all images in an FOV list in an RFrame, you can adjust how the display grid is organized by changing the number of columns and rows of FOV to show. The column and row display selection are located above the image in the zoom control toolbar.

2.4.3 Look Up Table

To the right of the image panel is the look up table. It provides tools for enhancing the image display.
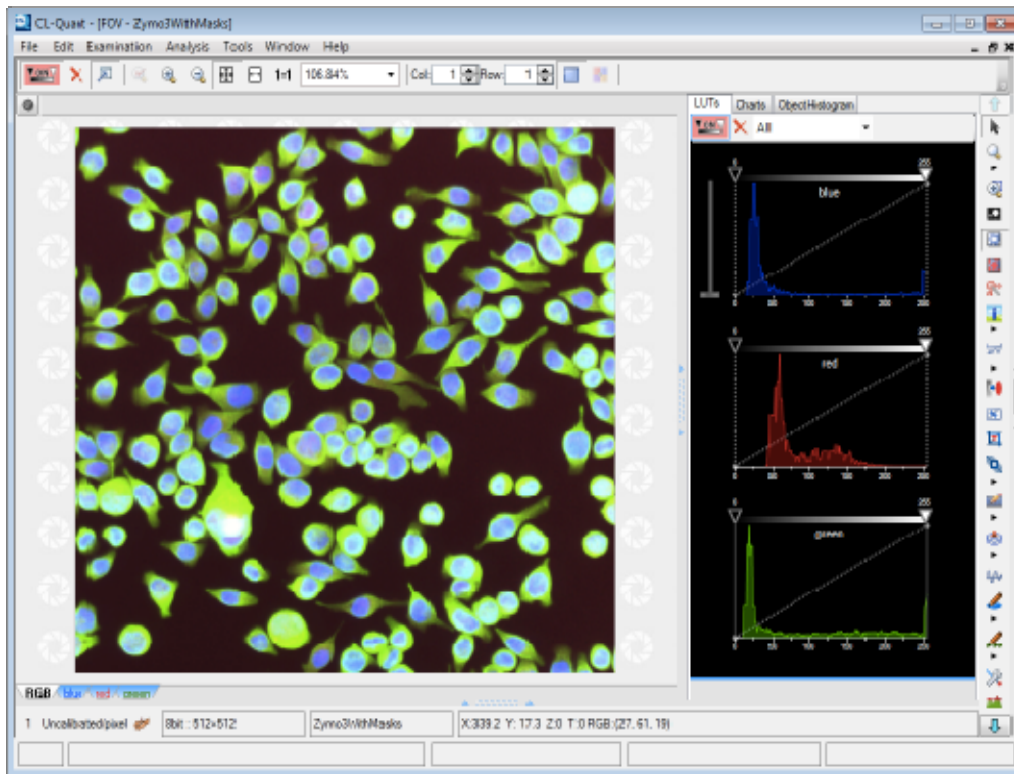
Access the LUT panel by clicking on the "**Toggle LUTs window**" button on the zoom control toolbar ⤢ or pressing "**Ctrl**"+"**Alt**"+"**L**" on the keyboard.

2.4.4 Magnify Tool

If there are features in an image that are too small to see, you may zoom into that area for a closer look.

You may use the zooming features built into the RFrame, or if you just need to take a quick look at a particular feature in the FOV, you can use the magnify tool. The icon is found in the examination tools area of the tool bar.

While activated, the image will be magnified while you hold down the left mouse key.

You can also zoom to a specific region of the image using the "**Zoom to Rectangle**" tool on the toolbar. Left-click and drag over the region you wish to magnify and release the mouse button to zoom. The "**Zoom to Rectangle**" tool icon is shown below.

2.4.5 Display Intensity Threshold

The "**Threshold**" icon on the toolbar will activate the threshold tool in the controls panel. The tool enables you to visualize the selection of a range of values in the image. Values that fall within the range will be overlain in red (the color red is reserved for the threshold overlay).

To use the tool simply:

1. Click the "**Show**" or "**Hide**" button to visualize the selected pixels in the range.

2. Move the slider to adjust the range.

3. Modify the text in the boxes to adjust the text.

The threshold tool is described in detail in Section 3.2.1.3, "Thresholding".

Save display threshold

The current displayed threshold can be saved to mask using the "Save threshold to mask" function. Click on the icon shown below (located below the "**Threshold**" icon in the toolbar) to save the threshold overlay to mask.

2.4.6 Line Profiler Tool

The line profiler tool will show you a trace of the intensity values along a line you draw on the image.

To access the tool click on the "**Toggle line profiler tool**" icon.

When draw a line across the image, a trace of the intensity values are shown overlain on the image as shown in the figure below.
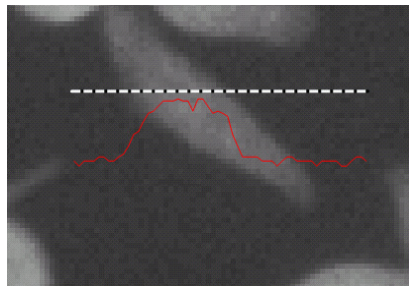
2.4.7 Channel and Mask Manager

The channel and mask manager displays a list of channels and masks that are available on the image. User can modify channel and mask display from the manager. To launch the channel and mask manager, click on the "**Channel and mask manager**" icon on the toolbar (shown below).

The channel and mask manager can be used to display multiple masks as well as changing the attributes of individual channels and masks. An example of the channel and mask manager dialog is shown below.
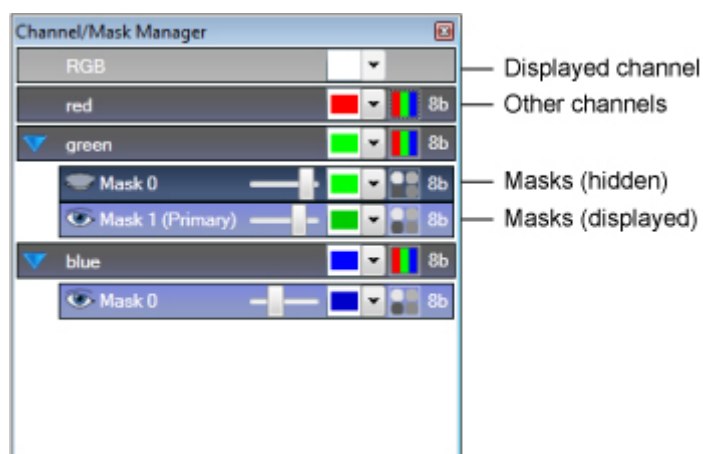
Channels are shown in gray with masks from the respective channels displayed in blue and organized under the specific channels. A lighter color indicates the channel/mask is displayed on the image.

Channel options

To rename a channel, right-click on the channel panel and select "**Rename channel**" from the context menu. User can also specify the channel color (the color which appears in the RGB, if enabled) and include/exclude the channel from the RGB-combined channel using the options shown above.

Mask options

Multiple masks can be displayed by toggling the eye icon in the mask options. Use the slider to adjust the mask transparency and the mask color pulldown menu to specify mask color. User can opt to color individual mask component differently by enabling the Color by label option. Note that the Color by label option when enabled will override the displayed mask color. If a detection mask is used for object measurements, the primary mask for measurement will be indicated with the suffix "(Primary)".

2.4.8 Calibration Properties

Calibration properties allow you to set and adjust the pixel and temporal calibration of the image. The pixel calibration information is displayed on the lower left hand corner of the RFrame below the channel tabs.

Edit calibration icon
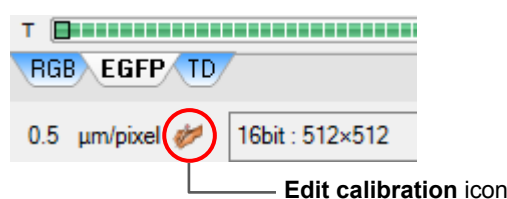
Change calibration settings

To change calibration settings, click on the "**Edit calibration**" icon next to the pixel calibration display (see above). This will launch the "**Calibration Properties**" dialog.
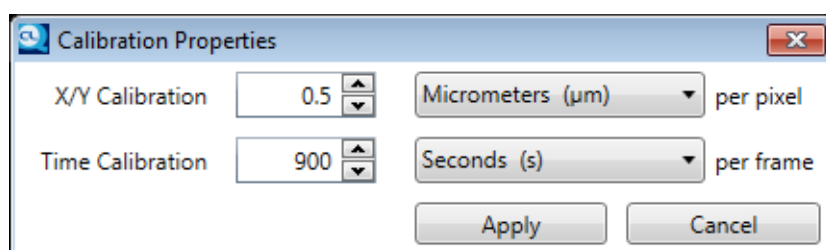
Use the textbox provided to adjust the time and X/Y calibration settings value. Click on the measurement drop down menu to the right to select the calibration units, or disable calibration by selecting the "**Uncalibrated**" option from the drop down menu.

2.4.9 Gallery View

Gallery view displays thumbnails of selected objects on a grid allowing for the visualization of multiple objects simultaneously. Whole image is displayed alongside the thumbnail gallery allowing users to quickly reference and identify selected thumbnail objects.

To launch Gallery view, you must have objects or trajectories selected. Click on the "**Gallery View**" icon on the magnification toolbar above the image.

User interface

The Gallery View user interface is split into three sections:

   - Whole image preview

   Displays the entire image with selected objects highlighted for quick reference

   - Thumbnail gallery

   Displays the selected objects as small thumbnails. Options are provided for sorting and displaying specific image statistic.

   - Filmstrip

   Allows user to "focus" on a single image thumbnail and displays the selected object at other time points.
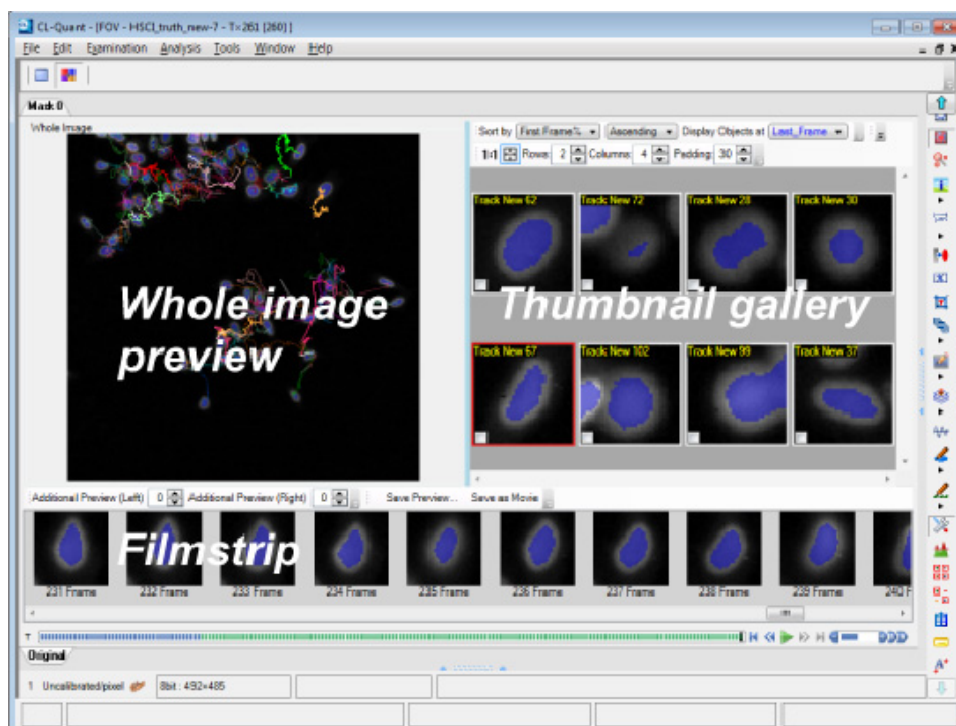
2.4.10 BioStation CT Data Viewer

BioStation CT (BioCT) is an automated incubator/imaging platform developed by Nikon for live cell imaging and analysis. BioCT has the ability to capture multiple whole-well images over time for extended experiments. For BioCT with the version 3.8 firmware upgrade, image file paths and experiment information are stored to a JSON header file which can be read in the BioStation CT data viewer. The BioStation CT data viewer parses the JSON header file and allows fine-tuned selection of experiments, plates, wells, and FOVs directly from the viewer.

To launch BioCT data viewer, go "**File > Open**", and load a BioCT JSON file.

> **NOTE:**
>
> The data explorer, along with files loaded into it, will not be accessible in the BioStation CT data viewer mode. You must exit the BioStation CT data viewer mode to view non-JSON image data.

User interface

The BioStation CT data viewer has a specialized user interface that allows the user to quickly and easily select experiments and FOVs to view.

There are two main panels:

- Image panel

  Shows the stitched and loaded BioCT images.

- FOV selection panel

  Allows the user to load and select experiments, plates, samples, and FOVs to be displayed in the image panel.
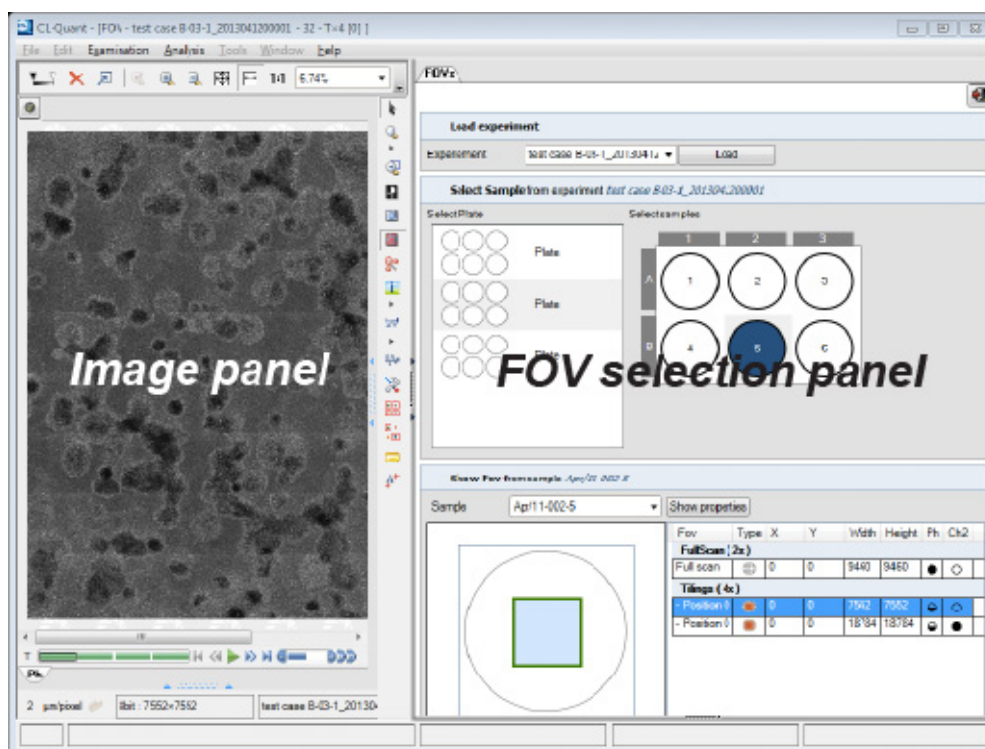
2.5 Apply Procedure

When you have loaded a procedure in the data explorer, activate the Controls panel at the bottom of the workspace and select the "**Measure**" tab.

To apply a procedure:

1. Select a procedure in the data explorer.

2. Select the FOVs that you will apply the procedure in the image panel.

3. Click the "**Apply**" button (red box) in the "**Measure**" tab.

Note that for FOVs with time and z extent you can select a range and sampling for apply.
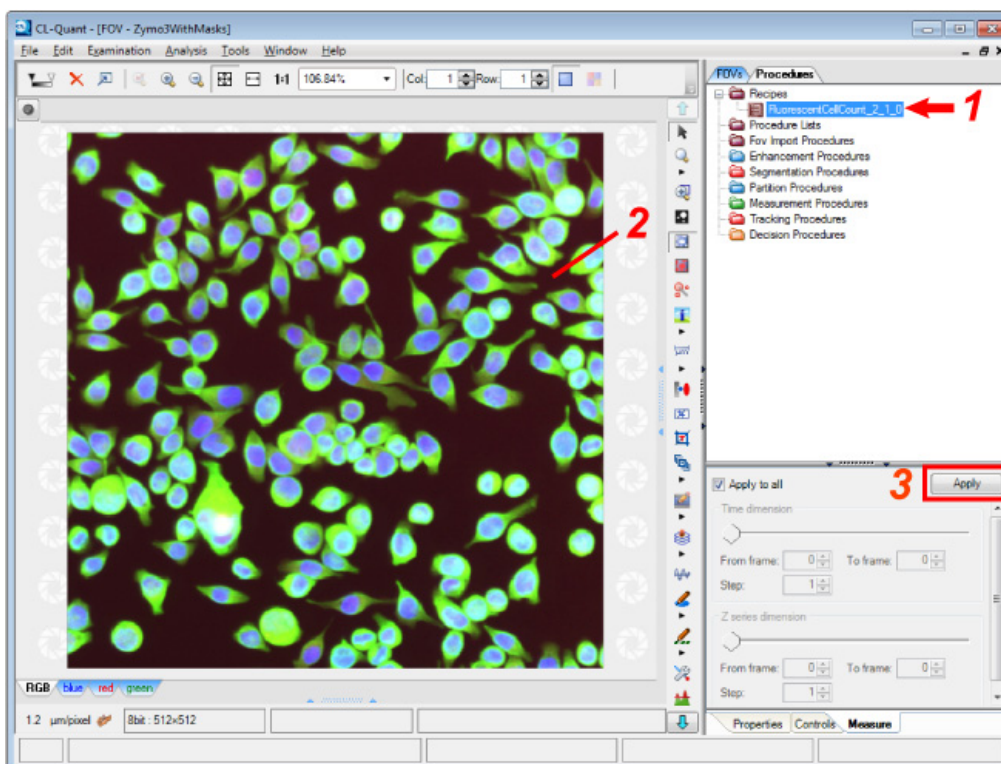
2.6 Data Analysis

CL-Quant allows the user to view measurement data and provides tools to review or gate object data. This section provides information on performing fixed-point analysis and kinetic analysis on the image.

2.6.1 Fixed Point Analysis

Fixed point analysis features data presentation, and integrated data - image - chart based experiment analysis. It provides object and data linking to facilitate image and data understanding, charting, and the manual creation, or gating, of sub-populations of objects.

(1) Objects and subsets

Objects

When you apply object segmentation and measurement procedures to an FOV it will create objects. Objects are a primary unit of analysis that would likely correspond to cellular or subcellular phenomena depending on your application.

An object is associated with its set of measurements, and can be represented as an ROI overlain on the FOV, a row in the object tab of the spreadsheet, or as a data point in the charts.

Each FOV in the FOV List will have its own object tab in the spreadsheet which will contain the object measurements as shown in the screen capture below.
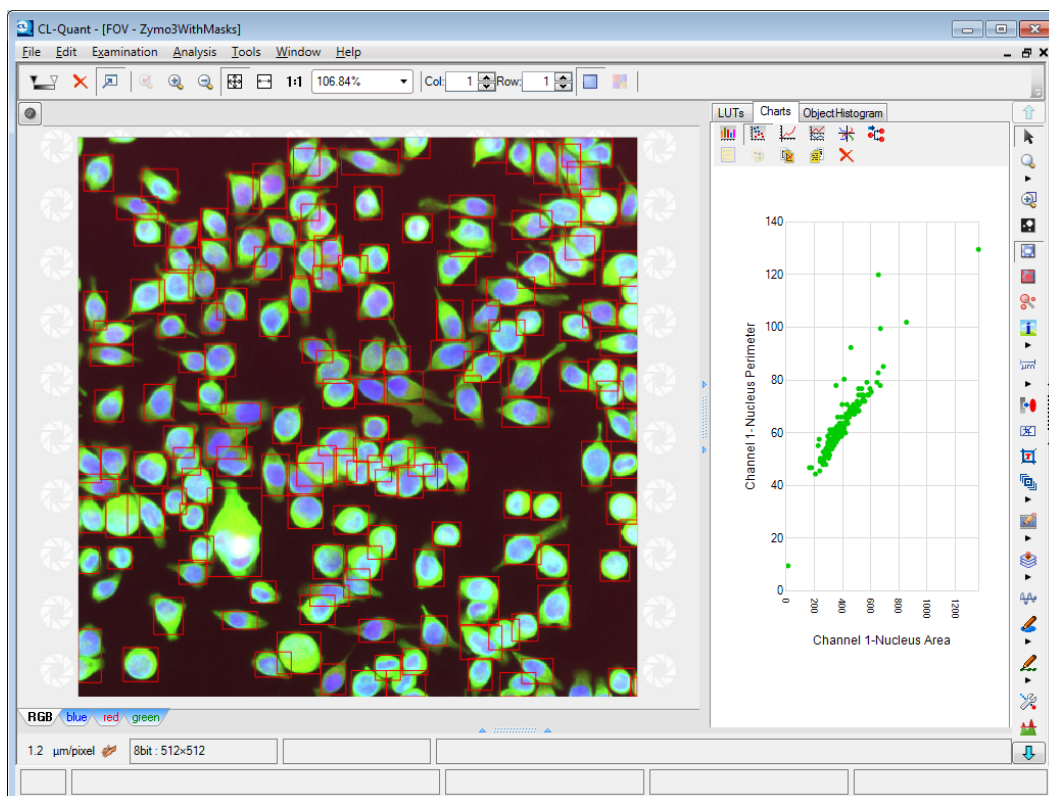
Subsets

Object subsets are collections of objects from one or more FOVs. A subset can be created manually by gating or through the application of a decision procedure. Subset objects are not removed from their original FOV, they exist in the subset as well as the FOV. When a subset is created, a new object tab is created in the spreadsheet containing the member object data.

> **CAUTION**
>
> - Automatic subset creation can only be done with decision procedures. Decision procedures can only be created if your version supports the decision module.

Object linking

Any object you select, regardless of where you selected them (e.g. image panel, chart, spreadsheet), will automatically be highlighted in the spreadsheet, charts, and image views.

(2) Adjusting object bounding boxes

After applying an object measurement procedure, the segmented objects are represented by bounding boxes. The bounding boxes enable object linking to support integrated image and data analysis. The image masks are used for measurement.

Show/hide object bounding boxes

To show or hide object bounding boxes, select the "**Toggle objects**" icon in the tool bar, shown below:

Under the "**Toggle Objects**" icon, there is an icon for "**Toggle selected objects**". In this mode, only the bounding boxes for objects selected in charts or spreadsheets will be displayed.

Color objects

Objects can be colored and identified based on their FOV or subset membership. If there are object subsets in the FOV, you can click on the "Controls" tab located at the bottom of the rightmost panel to display the object color options.

- **Color by Subset/Color By FOV**

  To color by subset, click on the arrow underneath the icon and choose "**Color by Subset**" (shown below).

The first highlighted "**F**" icon adjusts color options for the color by FOV mode.

The "**S**" icon will allow you to adjust the color by subset mode options.

The checkbox in the middle turns coloring on or off.

You can adjust the opacity of the colors by clicking on the drop-down menu (you may have to scroll to the right to see everything).

(3) Gating

You can manually gate a subset by selecting objects in charts, on images, or in the spreadsheet.

CAUTION

- Gating of object tracks is not currently supported.

Gate from chart

With one or more FOVs with object data and ROIs displayed in the RFrame, select objects from the dot plot or bar of the histogram in the information panel. You can select objects in dot plots by clicking them with your mouse or dragging your mouse to cover a region in the chart. Once selected, the object data points will turn red.
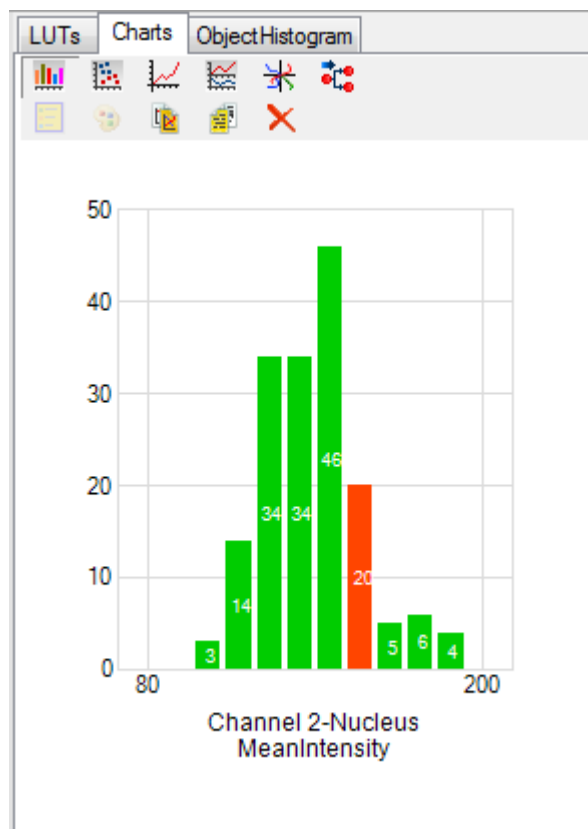
You can select objects in a histogram by clicking one or more of the histogram bars, which turn red when selected (as shown here). To gate these objects select "**Analysis** > **Gate**" from the menu bar.

Gate from spreadsheet

With one or more FOVs with object data and ROIs displayed in the RFrame, select the objects you wish to gate in the spreadsheet by clicking the rows in the object set tab. Select "**Analysis** > **Gate**" from the menu bar to create the new subset.

Gate from image

With one or more FOVs with object data and ROIs displayed in the RFrame, select the ROIs in the image panel, then select "**Analysis** > **Gate**" from the menu bar to create the new subset.

2.6.2 Kinetic Analysis

Kinetic analysis features include displaying object tracks, reviewing track data, charting track data and loading and saving FOVs with track data.

(1) Displaying Object Tracks

After applying a tracking procedure, you can review the object tracks. Tracks are similar to object ROIs, but since the object now has temporal extent, tracks show how the object moves over time.

Viewing object tracks

To view object tracks, click on the "**Toggle objects**" button on the toolbar.

This will display the object tracks. Tracks are a series of connected vertices showing the location of the tracked object at various time points.
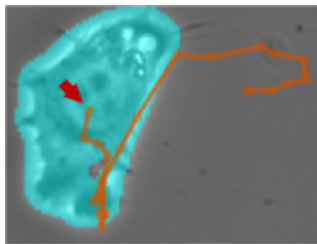
The figure above shows a track overlaid in orange on a moving cell (the segmentation mask is also shown in cyan). The current location of the object is shown as a green, circular ROI (red arrow, above). The x,y location of the object at previous time points is shown as orange boxes connected by a colored line.

<u>Adjusting tracks</u>

You can adjust the track length with "**Track Visibility**" parameter from the "**Track Display Options**" dialog. To launch the dialog, click on the "**Track display options**" button on the toolbar (shown below).

The "**Track Display Options**" dialog has many options to adjust the track appearance and presentation. The dialog is shown below.
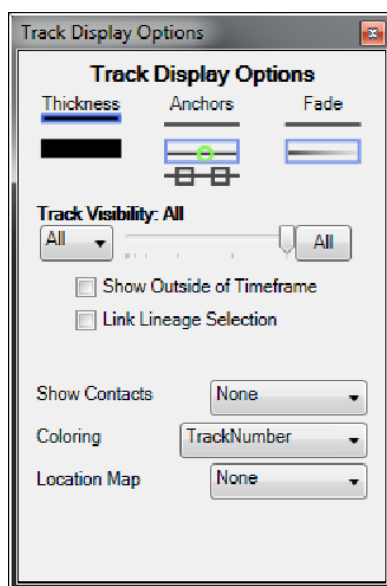
- **Track Display Options**

  You can modify how tracks are displayed using options below:

  Thickness   You can adjust the thickness of the track by selecting the desired thickness here.

  Anchors   You can choose to hide all or some anchors or show all anchors. Anchor sizes can be adjusted by changing the track thickness.
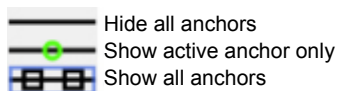
  Fade   You can change how long tracks are displayed to maintain same track appearance for all track points or to have the track fade with older track points.

- **Track Visibility**

  When this parameter is set to 1, only the current track location, denoted by a green circle showing the active anchor is visible. When this parameter is set to all, the whole track is displayed. The pull-down menu and the slider provide precise step definition that can be set to show a set number of previous track points with current point.

● **Show Outside of Timeframe**

Enable this option to show all tracks, including those that do not appear in the current time frame.

● **Link Lineage Selection**

If lineage is enabled in tracking, enable this option to display the lineage links between the parent and daughters tracks.

● **Show Contacts**

Use the pull-down menu to specify how the location of which two tracks intersect is shown.

● **Coloring**

Use the pull-down menu to specify how track colors are shown. You can specify the track colors by state, direction, lineage, or track number.

● **Location Map**

Use the pull-down menu to enable the display of specific location of a track at a certain time point. There are two location map options - first frame and last frame.

(2) Reviewing Track Data

After applying a tracking procedure, track data will appear in the FOV spreadsheet. There are two types of data; dynamic data and track data, which appear on separate tabs.

Data types

- Dynamic data

Name	Acceleration	Average Intensity	Compactness	Ellipse Fitting Angle	Major-to-minor Ratio	Match Score	Max P... Radi...
Track 9	0.000	1973.676	1.353	7.015	5.434	1.000	9
Track 10	0.000	1848.867	3.153	96.527	1.796	1.000	1
Track 11	0.000	1906.337	2.583	17.771	1.207	1.000	9
Track 12	0.000	1936.929	1.893	35.704	2.234	1.000	10
Track 13	0.000	1909.262	1.771	107.615	1.610	1.000	7
Track 14	0.000	1947.139	0.492	3.517	1.045	1.000	2

Motility_demo_output / Motility_demo_output - Tracks /

As shown in the figure above, dynamic data appears in the first tab. Dynamic data characterizes a single time point. It could be instantaneous information, such as the average intensity of the object at that time point. It could also be accumulated information, such as the curvilinear velocity of the object from its initial time point until the current time point, or within a moving window t frames before the current time point.

- Track data

Track data appears in the second tab with the "-Tracks" suffix. Track data, such as total length, first frame, etc. characterize the entire track.

Spreadsheet display options

To view spreadsheet display options, right-click anywhere in the spreadsheet panel to bring up the display options context menu. Here, you can change how the objects are organized in the spreadsheet and modify refresh options for time-lapse sequences.
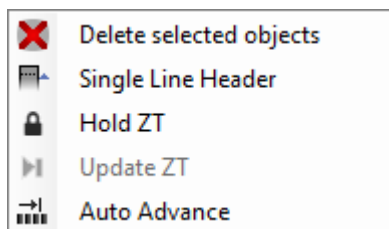
- **Delete selected objects**

  Select this option to remove selected objects from the spreadsheet. This action is irreversible.

- **Single Line Header**

  Select this option to reduce the header width to one line; names longer than the allotted cell space will be truncated in this display mode.

- **Hold ZT**

  By default, every time you advance one frame, the spreadsheet will update with the dynamic data for the current time frame, which can be inefficient. You can suspend spreadsheet update by selecting the "**Hold ZT**" option to disable automatic update.

- **Update ZT**

  Select this option to display the dynamic data for the current frame.

- **Auto Advance**

  Select this option to automatically advance image sequence to the first frame of when the selected object appeared.

(3) Integrated Kinetic Characterization

Tracking analyses generate tracks and data. The integrated data and track linking feature is a powerful way to find objects of interest, outliers, or potential errors. Track data can be plotted in dot plot and histogram, and both track and dynamic data can be plotted in the trace plot.

Track measurement in the trace plot

The figure below shows a tracking measurement charted in the trace plot. To modify the data shown in the trace plot, click on the vertical measurement name (red rectangle) and choose a different measure.
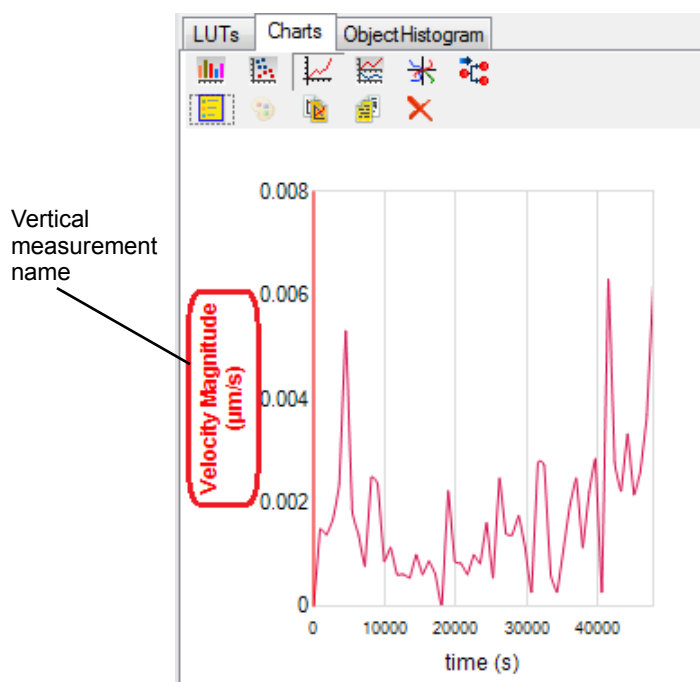
- Using a secondary axis

You can plot additional measurements on the same axis by selecting additional measurements. To plot new measurements on a secondary axis, right-click on the charts panel and select "**Secondary axis**" from the context menu. In addition, you can change how the secondary measurements are displayed in the "**Secondary data style**" submenu.
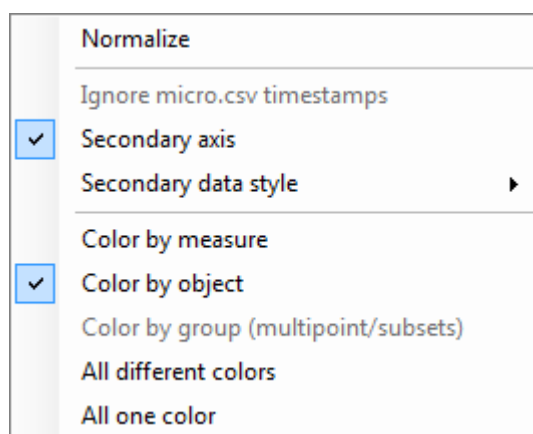
Click on the axis title of the secondary axis to select a measurement to plot. Measurement being plotted on the primary y-axis will be displayed in parentheses in the measurement selection menu. An example of a two-axes trace plot with acceleration and velocity magnitude charted is shown below.
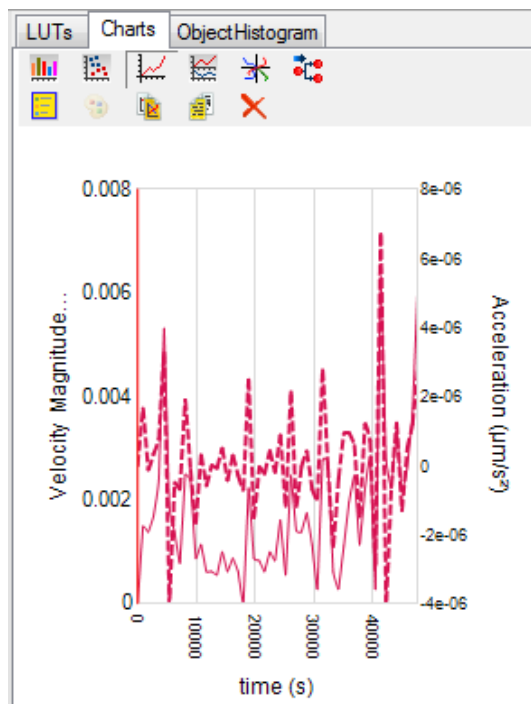
Track measurement in the dot plot and histogram

Track measurements can also be shown in the dot plot and histogram. When you select the objects in the charts (circled below), they are also automatically selected in the image overlay and spreadsheet.
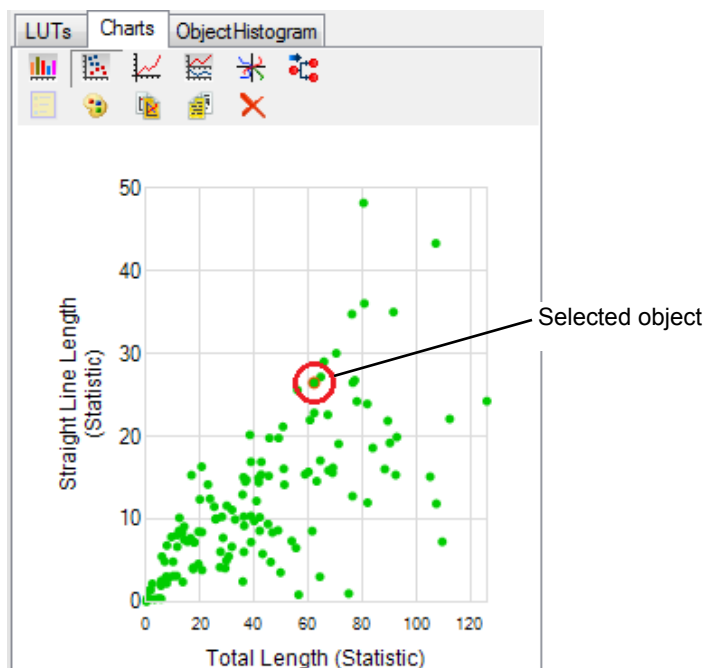
The axes value can be adjusted by clicking on the min and max values of the respective axes (circled below). A textbox will replace the axis labels. Enter a new value into the textbox and press Enter to confirm. The axis will be renumbered based on the inputs.
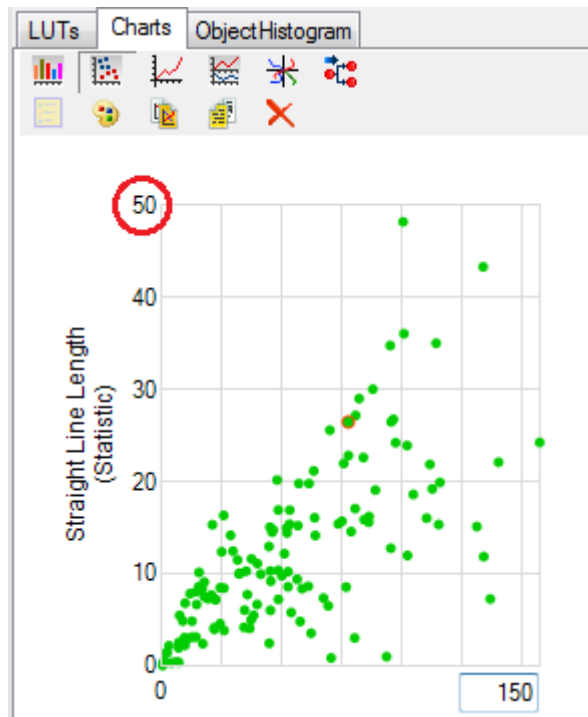
2.6.3 Charting

Multiple chart types are supported. To change the type of chart you are using, click on the "**Charts**" tab in the Controls panel and select the available option as shown below.
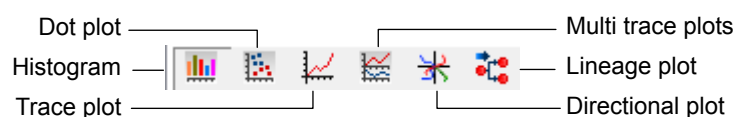
> 🖊 **NOTE:**
>
> Charts will only become enabled after applying a recipe. Not all chart types are available for all recipes.

Chart options

Additional chart options are shown on the charts toolbar. These options allow you to modify the chart appearance and export chart data.

- **Show/hide legends**

  Toggles legends display on charts.

- **Assign dot colors** (Dot plot only)

  Modifies the color of data points in a dot plot. This tool is available for dot plots only.

- **Copy chart to clipboard**

  When selecting this option, a snapshot of the chart as displayed will be copied to the clipboard.

- **Copy chart data to clipboard**

  Data values associated with the displayed chart will be copied to the clipboard. For histograms, the displayed value for each histogram bar will be copied.

- **Reset axes**

  Resets chart axes to default.

- Chart legends

When plotting multiple objects, the plots are shown in different colors. The chart legends provide information about the object plots and the corresponding objects. To toggle legends display, click on the "**Show/hide legends**" icon in the charts toolbar. The legends are overlaid on the chart in the upper right corner of the chart.
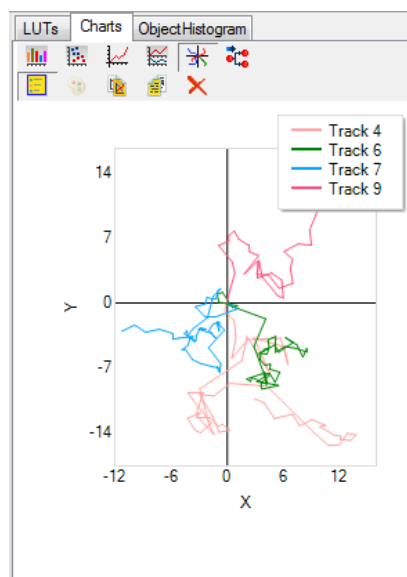
- Chart axes

User can change the chart axes range by clicking on the minimum value and the maximum value on each axes. The cursor will change to an I-beam when hovering over the axes values indicating that they can be adjusted. User can reset the chart axes back to default using the "**Reset axes**" option.

(1) Histogram

Histogram present data for one or more object sets (images and subsets). When you select the object set in the summary tab of the spreadsheet, its data will be plotted in the chart.

Histogram bin adjustment

The number of bins plotted is adjustable by the user from a minimum of two bins. Click on the chart and use your scroll wheel to adjust.
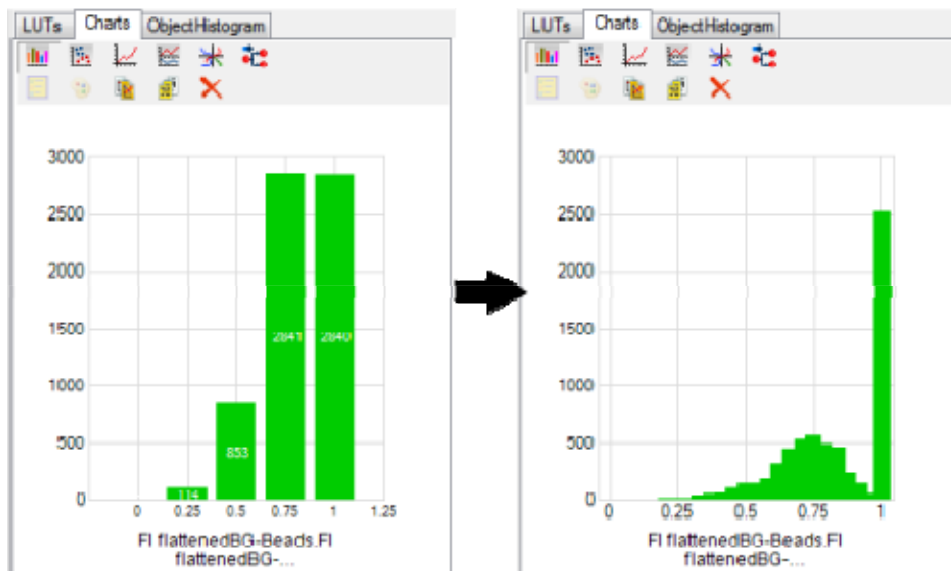
Multiple axis scales

"**Linear**", "**Log e**", "**Log 2**" and "**Log 10**" scales are supported for X and Y axes. Right click on the chart to choose as shown below:
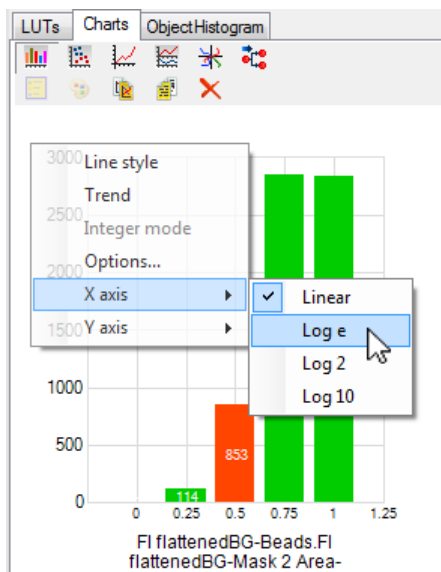
Line plot mode

The histogram can be plotted as a bin, or as a line. Right click on the chart and select "**Line style**" as shown below.
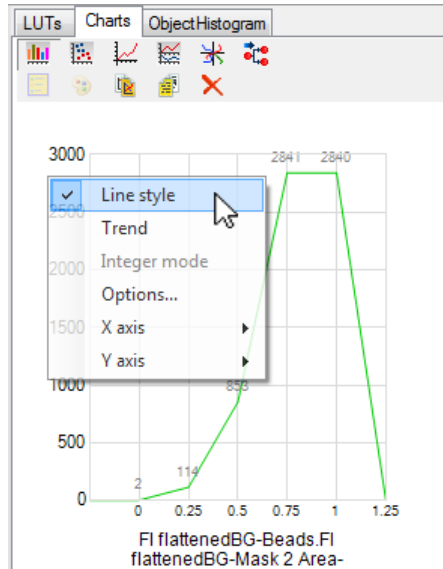
(2) Dot plot

Dot plot present data for one or more object sets (images and subsets). When you select the object set in the summary tab of the spreadsheet, its data will be plotted in the chart.
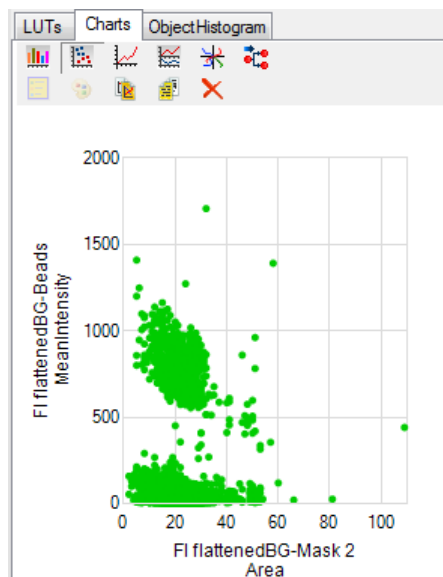
Assigning dot colors

You can assign different colors for the objects in the chart. In the above image, there are two separate clusters of dots differentiated by their y-axis value (mean intensity). Click on specific dots or hold down the left click while dragging to select multiple dots.

To change the color of the selected dots, click on the palette icon 🎨 on the charts toolbar

and select a new color from the list provided or add a new color.
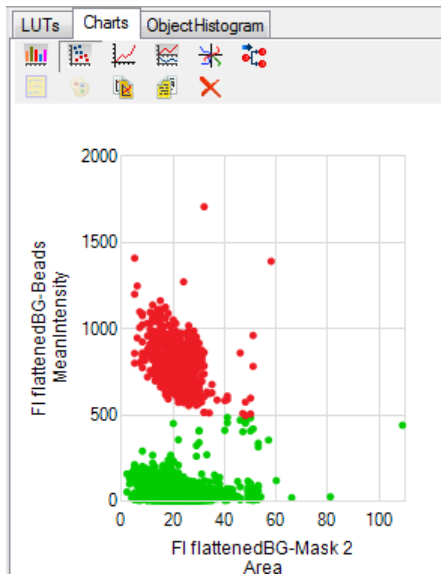
(3) Trace plot

Trace plot displays object or field measurements data plotted against time. This charting option is applicable to multi-frame image sequences only. A tooltip is provided when you hover over the trace plot to indicate measurement values at the given time point.
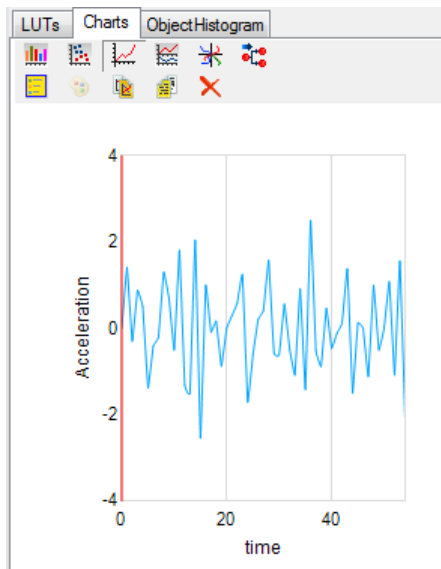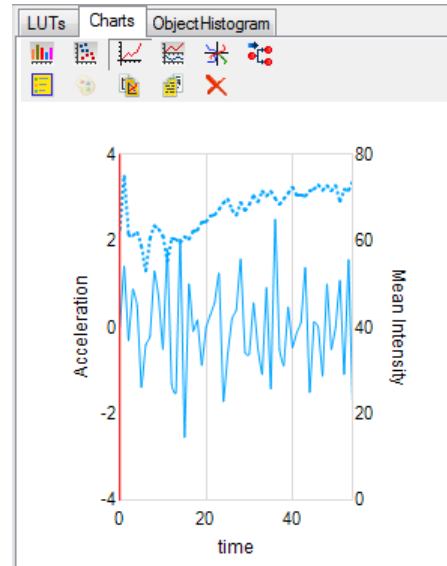

Plotting multiple objects

To plot a track, click on its trajectory on the image. Hold down the "**Shift**" key while clicking on object trajectories to select multiple objects on display. The trace plot will update automatically with the trace plots of the selected object.

Plotting multiple measurements

Click on the Y-axis label and select from the list of available measurement to plot additional measurements. The axis label will turn red when you hover over it. A check mark denotes the measurement that is being plotted.
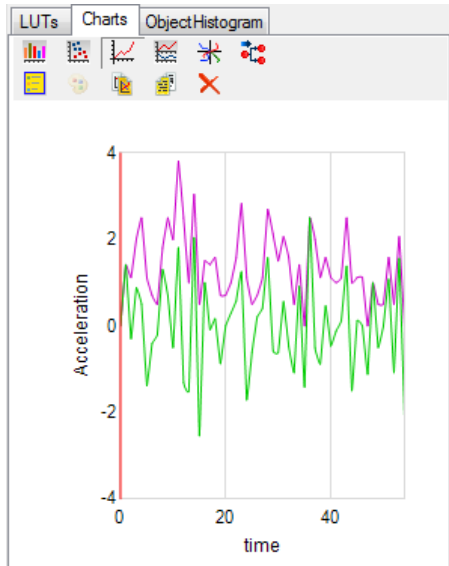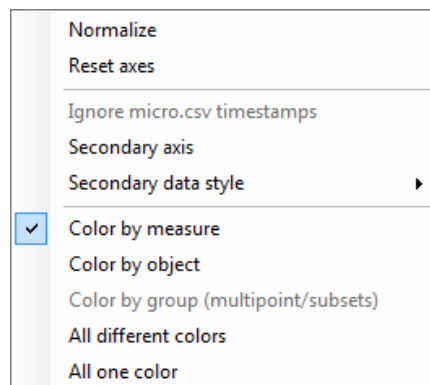

Two measurements colored by measure   Two measurements on two Y-axes

Trace plot options

You can adjust the display of the trace plot, add a second Y-axis, or normalize the plotted measurements from the trace plot context menu. Available options are shown below.

- **Normalize**

  Normalizes the measurement axis to a fixed scale between 0 (min) and 1 (max).

- **Reset axes**

  Resets chart axis.

- **Secondary axis**

  Adds a secondary Y-axis to the trace plot on the right. You can click on the axis label in the secondary axis to plot secondary measurement.

- **Secondary data style**

  This option allows you to change the trace plot display for the secondary axis measurement.

- **Color by measure**

  Colors object trace plot by measurement type.

- **Color by object**

  Colors object trace plot by object.

- **Color by group**

  Colors object trace plot by selected multipoint or subset membership.

- **All different colors**

  Colors all object trace plot with a different color.

- **All one color**

  Colors all object trace plot with the same color.

(4) Multiple trace plot

If you have a multi-point image file with multiple image sequence, you can plot object trace plots from each of the multi-point images. Select an object in each image sequence.
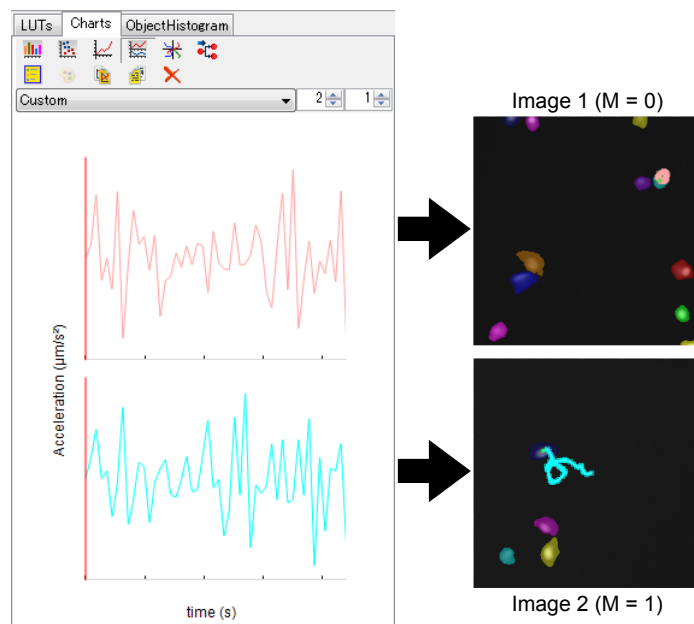
Object trace plot for selected trajectories in image 1 (top) and image 2 (bottom)

As shown above, objects can be individually-selected from each of the multi-point image and plotted in the Multiple trace plot window. The same specified measurement trace plots are plotted for both the multi-point images.

Trace plot options

The same trace plot options are provided in the multi-trace plot as the options in the trace plot.

Adjusting chart layout

You can adjust the way the charts are organized using the dropdown menu and the textboxes below the chart options. Note that the charts are limited to the display of the same measurements (i.e. it is not possible to plot acceleration at the top for one multi-point image and plot velocity at the bottom for the other multi-point image).

(5) Directional plot

Directional plot displays the X and Y displacement of selected object tracks for the duration of the tracks centered at a common origin. This chart can be used to investigate migration behavior of tracked objects.
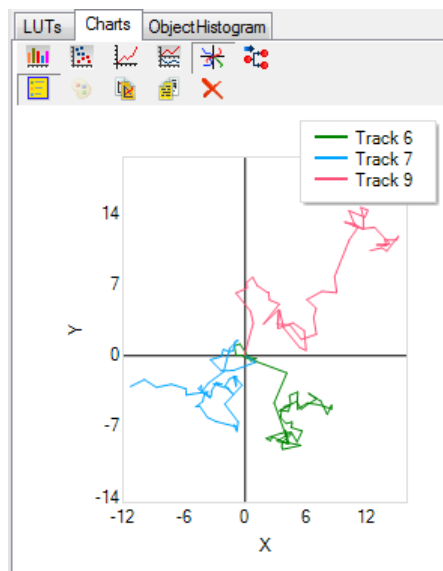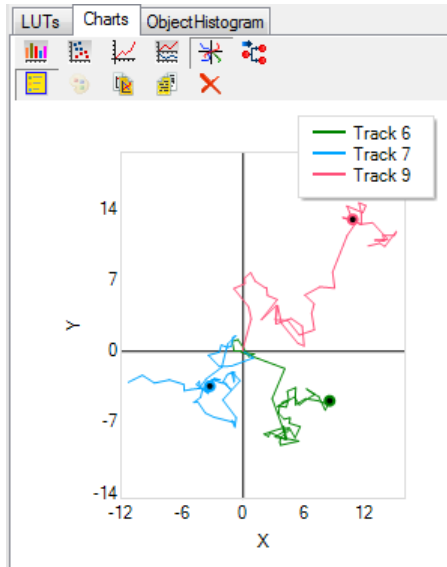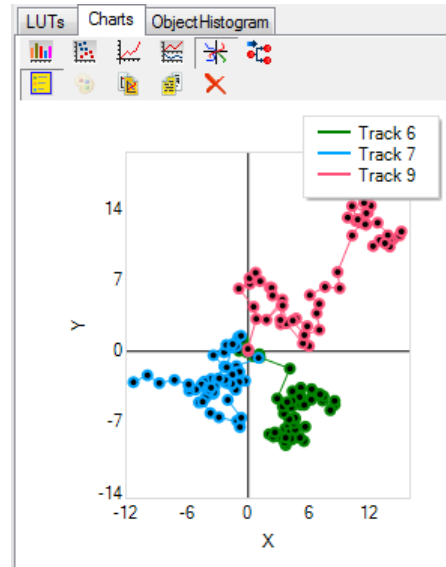

Directional plot with all anchors off

Click on an object trajectory on the image to select and plot the track. You can plot multiple trajectories by holding down the "**Shift**" key while selecting trajectories from the image. To plot all object tracks on the directional plot, right-click on the chart and select the "**All**" option in the context menu to plot all objects.

Track display options

The directional plot display is linked to the "**Track Display Options**" window which allows the user to adjust the appearance of the trajectory on the image. All track point anchors are displayed as small points on the directional plot when the "**Show all anchors**" option is selected; whereas only the current anchor is displayed as a point on the directional plot when the "**Show active anchor only**" option is selected. To disable all track point display in the directional plot, select the "**Hide all anchors**" option from the "**Track Display Options**" window.
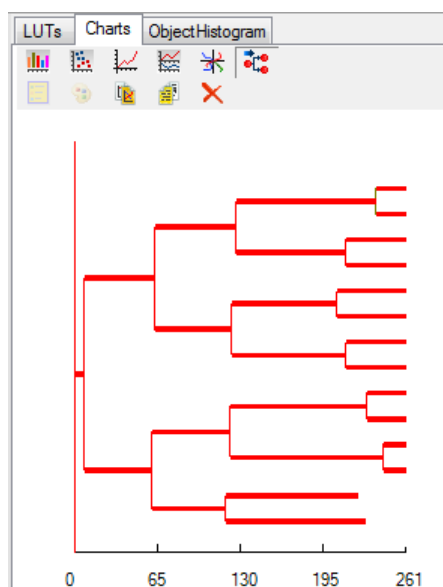

Directional plot with current anchor only

Directional plot with all anchors

(6) Lineage plot

Lineage plot traces the temporal evolution from a single ancestor track to descendant tracks by division in an image sequence.

Track measurement in lineage

You can plot track measurement on the lineage plot. To select a track measurement to plot on the lineage plot, click on the X-axis label on the chart and click on a measurement to show. Track measurements are plotted as a function of color. The color bar below the measurement label provides calibration information that correlates the displayed color to measurement value.
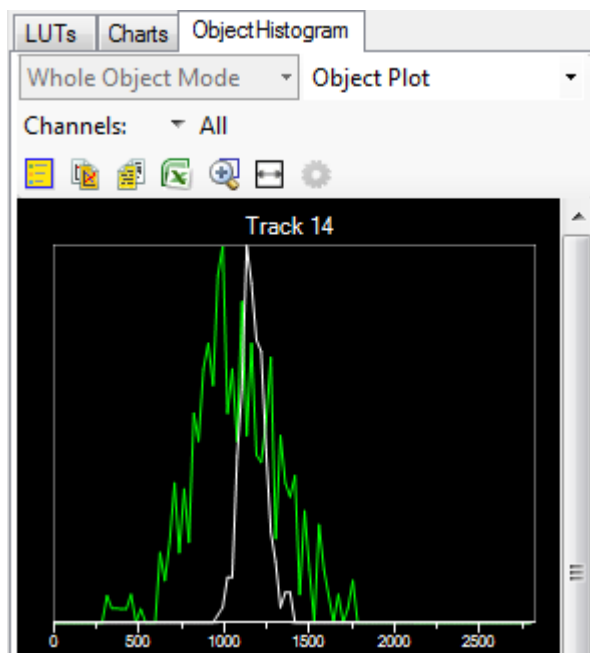

2.6.4 Object Histogram

The object histogram function displays the intensity histogram of selected objects. Intensity values for all pixels in the selected object masks are plotted on the object histogram. To show the object histogram panel, click on the "**Object Histogram**" tab by the "**LUT**" and "**Charts**" tab.
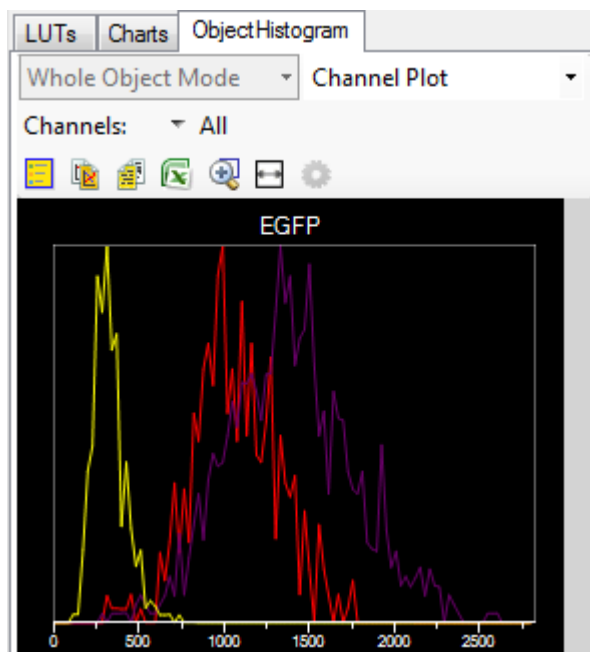
To plot object histograms, click on the object bounding boxes or trajectories on the image. The intensity histograms for individual object are plotted on the histogram plot.

Viewing object histogram by object

By default, object histograms are plotted for each individual channel. You can change the object histogram display to show each object in a separate histogram with the intensity of each channel plotted. To view object histogram by objects, click on the "**Channel Plot**" drop down menu and select "**Object Plot**".

Selecting channels

You can specify the channels in objects histogram by clicking on the dropdown menu next to the "**Channels**" label. You can select individual channels, all, or no channels from the menu and the object histogram plot will be adjusted accordingly.

Object histogram tools

Object histogram provides several chart options tool for manipulating the histogram chart as well as provide options for histogram exports. The six object histogram tools are as follows:

- **Show/hide legends**

  Toggles legends display for object histograms.

- **Copy histograms to clipboard**
  Exports object histogram as displayed to clipboard.

- **Copy histogram data to clipboard**
  Exports object histogram data to clipboard.

- **Export histogram data to Excel**
  Exports object histogram data to Excel spreadsheet format.

- **Zoom to histogram region**
  Toggles zoom tool to zoom into highlighted histogram region.

- **Best fit zoom for all histogram**
  Resets zoom level to best-fit range for all histograms.
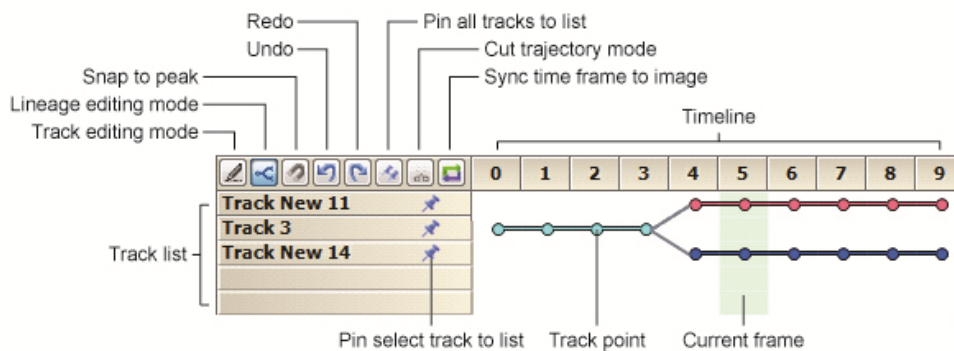
■ 2.7 Track and Lineage Editing

CL-Quant provides a way for the user to create new tracks or modify existing tracks and/or lineage in an image sequence to optimize the result. To launch the track and lineage editing mode, click on "**Toggle trajectory editor**" icon on the toolbar (below).

2.7.1 Track Editing Grid

Clicking on the "**Toggle trajectory editor**" icon will launch the Track Editing Grid below the image. Controls for the Track Editing Grid are shown below:

- **Track editing mode**

  Enables the user to use the grid to connect and disconnect multiple tracks and to trim track points.

- **Lineage editing mode**

  Enables the user to use the grid to view track lineages and to connect and disconnect lineages.

- **Snap to peak**

  Enable this option to use peak detection algorithm and detect likely track point location when interpolating track points.

- **Undo**

  Undo the previous track editing action.

- **Redo**

  Redo the last undone track editing action.

- **Pin all tracks to list**

  Toggle this icon to pin/unpin all tracks in the track list.

- **Cut trajectory mode**

  This mode toggles the track cutting tool to split tracks.

- **Sync time frame to image**
  Synchronizes selected time frame to current frame and displays the image.

Track list

Selected tracks will appear in the track list. To remove a track from the track list, click on the pushpin icon (📌) next to the track name to "unpin" the track from the grid. You can also click on the "**Pin all tracks to list**" icon (📌) to unpin all tracks. An empty outline of the pushpin icon (📌) indicates the track is unpinned. Click anywhere on the image to remove the unpinned track from the track list. Note that the track will not be removed from the image, only removed from view in the grid.

Timeline

Track points of listed tracks are displayed in the timeline. Tracks appear like a "string of pearls" in the timeline view, showing the start and end frame of the trajectory. The green highlight illustrates the current time frame; double-click on a frame to change the current frame position.

2.7.2 Editing Tracks on the Grid

The user can edit tracks using the Track Editing Grid or directly manipulating track points on the image. This section provides information on managing track edits on the Grid.

(1) Select tracks

To begin editing tracks, select the track you wish to edit by clicking on the track in the image. The selected track will appear in the track list below the image. You can select additional tracks by clicking on other tracks, your previous selection(s) are preserved in the track list if the track is "pinned". A pushpin icon (📌) next to the track name indicates the track is pinned to the track list.
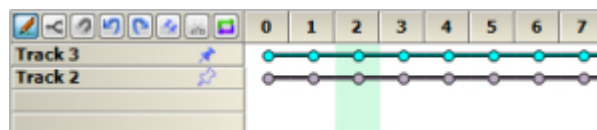
After selecting the tracks you wish to edit, you can begin manipulating the tracks using the Track Editing Grid. You can connect two tracks at different point in time or break a track into multiple components. Following sections will provide instructions on how to connect and disconnect tracks in the Track Editing Grid.

(2) Connect tracks

You can connect any points on two tracks to create a new, connected track by dragging a track point on one track on the timeline to another track. In the example below, Track 2 and Track New 11 are connected end-to-front to create a continuous track.
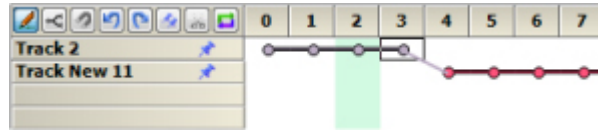
Two tracks can be joined together even if they do not overlap or appear in adjacent time frames. The software will automatically insert the missing time points and interpolate the position.

There are two ways that the track point position is interpolated - by mean distance traveled or by peak detection.

- Mean distance traveled:

The intermediate points are estimated by dividing the straight line distance between two connecting track points by the number of frames between them.

- Peak detection:

The software will attempt to interpolate the intermediate points based on their most likely position (i.e. at locations where image intensity is the highest). You can turn on peak detection by toggling the magnet icon ( ) in the track editing toolbar.

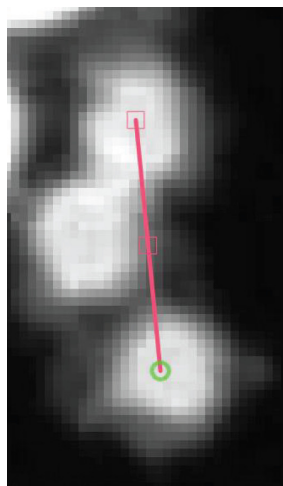A comparison between the interpolation approaches are shown below.
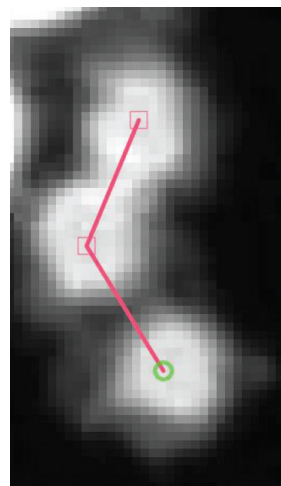

| Figure 1 | Figure 2 |

The mean distance traveled method (Figure 1) may place interpolated track points incorrectly if they are not near the linear path whereas the peak detection method (Figure 2) will attempt to place the interpolated point based on the pixel intensity of the image.

(3) Disconnect tracks

You can break a track into multiple trajectories by dragging a track point down to an open slot in the track list at the time point selected. The current track will terminate at the time point selected and a new track beginning at the following time point will be created. You can break a trajectory as many times as the track length allows.

In the example below, Track 2 is split into two separate tracks, one spanning frames 0 through 3 and the other starting from frame 4, by dragging the track point at frame 3 down to an open row in the Track Editing Grid.
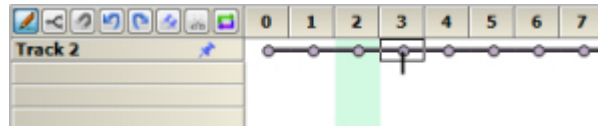
Separate a track by dragging a track point to an open row in the Track List
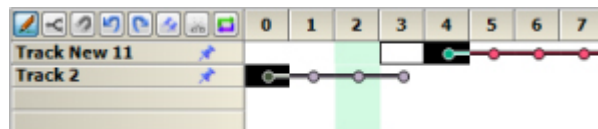
Old track will terminate at the track point selected and a new track begins in the frame after it.

(4) Trimming tracks

You can trim the trajectories by highlighting the track points you wish to remove. Press the "**Delete**" key on your keyboard to delete the selected track points. You can also remove an entire trajectory by double-clicking on the track and press the "**Delete**" key.
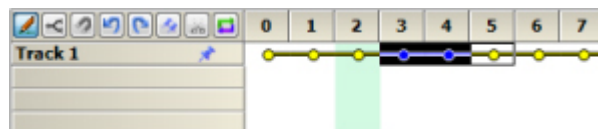
Highlight the point you wish to remove (hold "**Shift**" key to select multiple points)
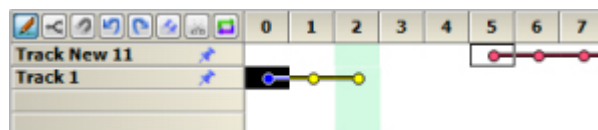
Press "**Delete**" to remove selected track points. The track is trimmed.

2.7.3 Editing Tracks on an Image

In addition to the Track Editing Grid, the user can also edit tracks directly on the image. Track editing on the image provides user the additional option to create new tracks, extend existing tracks, and move track point positions.

(1) Create new tracks

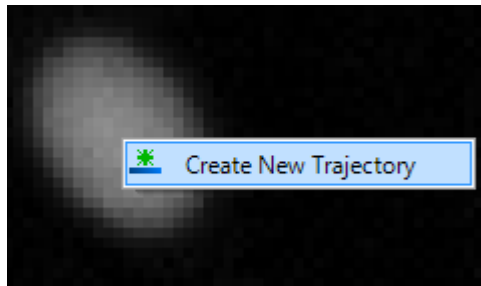User can draw track trajectories by creating new tracks. This option allows the user to generate manual tracks that can be compared against software-generated tracks. To start drawing your tracks, right click on the image where you'd like to place the track point and select "**Create New Trajectory**".

When you start the process of creating a new trajectory, the time lapse sequence will advance by one frame automatically. A red arrow shows the direction of the cursor with respect to the previous track point location. Left-click on a location on the image where you'd like to set the track point and the sequence will advance by another frame. The drawn track will be shown as a red line overlaid on the image.
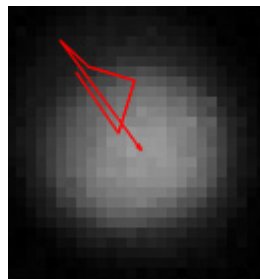
Please note that you will not be able to modify previous track points or to skip frames when defining track location while you are in track creation. You may navigate the time sequence using the time control below the image. Further track edits are possible after the completion of track creation as explained later in this section.

When you are done with drawing the track, right-click anywhere on the image and select "**End Trajectory**" from the context menu. A new track will be created.
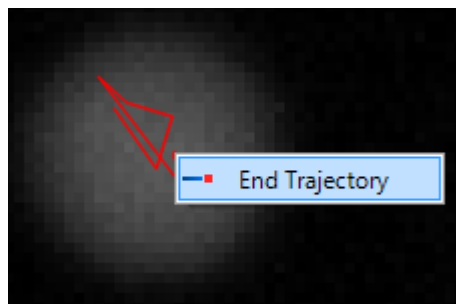
(2) Extending existing tracks

Track extension is very similar to creating a new track with the difference being that a new track is created as a continuation of an existing track. To begin track extension, right-click on any track point on a track and select "**Extend Track**" from the context menu. The RFrame will automatically navigate to the frame after the end of the selected track.
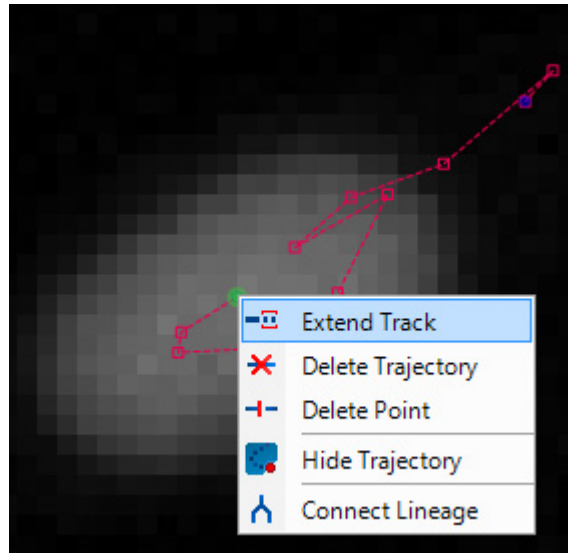
Like in track creation, a red arrow originating from the last track point will indicate the direction your cursor is pointing. Left-click on the image to set the location of the track point, the sequence will then advance to the next frame.

When you are done with track extension, right-click anywhere on the image and select "**End Trajectory**" from the context menu to complete the operation. For track creation and extension, you may also connect two tracks by right-clicking on a track point and select "**End Trajectory**" from the context menu. You will be prompted to either end the track at the selected track point (connect tracks) or create a new track point and maintaining the existing tracks.
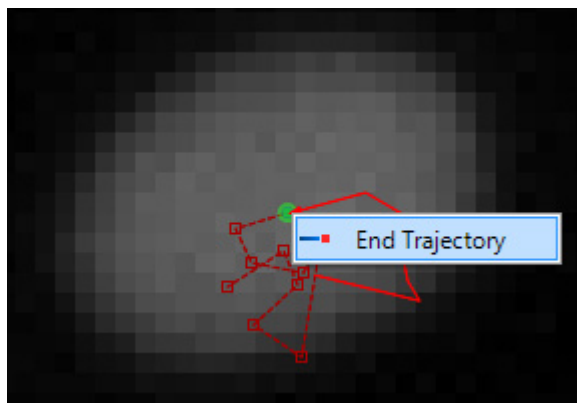
(3) Move track points

Track points are placed at the center of the tracked mask component. However, a slight shift in the detected mask may affect the correct placement of a track point. To move a track point, left-click and drag the point you wish to move. Release the left-click at the desired location.
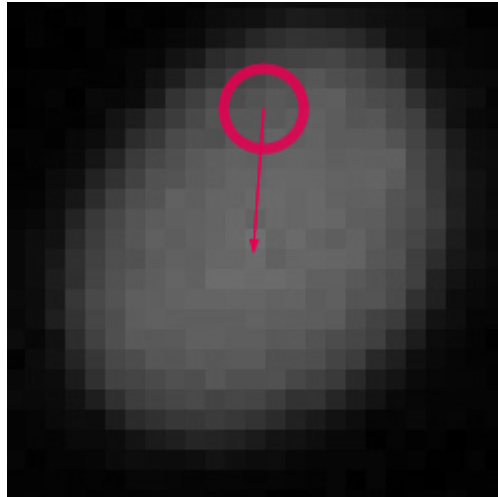
(4) Additional track editing options

There are additional track editing options available by right-clicking on a track point to pull up the context menu. An explanation of the functions not covered above is shown below.
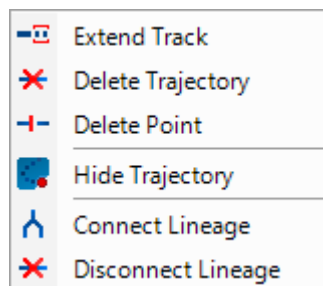
- **Delete Trajectory** - Removes the entire trajectory.
- **Delete Point** - Remove the selected track point. Multiple points can be selected by selecting two points on the same track while holding down the "**Shift**" key.
- **Hide Trajectory** - Remove the selected trajectory from view. This may be useful when editing overlapping tracks.
- **Connect Lineage** - Create a lineage from one parent track and two children tracks.
- **Disconnect Lineage** - Disconnect children tracks lineage from the selected track.

Connecting and disconnecting lineages will be further explained in the next section, Editing lineage.

2.7.4 Editing Lineage

Certain tracking procedure allows the user to track cell division over time in the form of a lineage. The lineage editing tool enables the user to define and edit cell lineage plot to track division events.

Enable lineage editing

To enable lineage editing, click on the Lineage icon ( ) to get started. If you have several tracks of a lineage pinned to the Track Editing Grid, enabling lineage will rearrange the tracks to display the connected lineage. Tracks without lineage will be displayed normally.
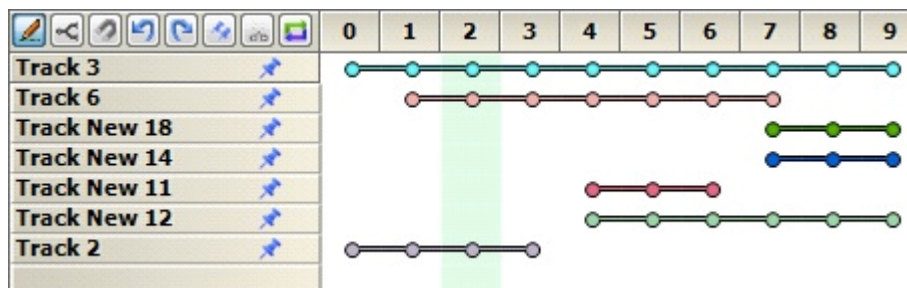
Enable lineage editing off
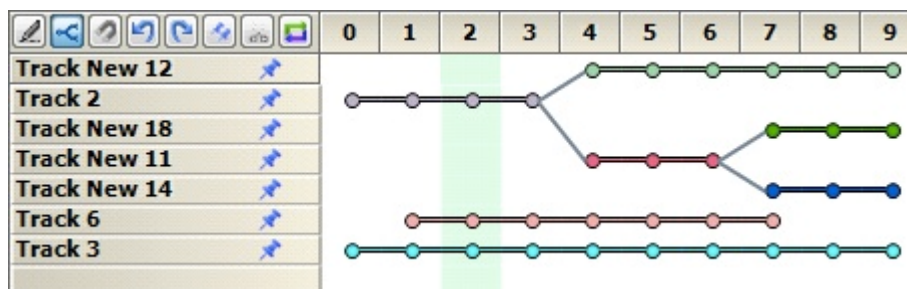
Enable lineage editing on

(1) Connecting lineages

In order to connect lineages, you must have a minimum of three tracks - a parent track and two children tracks. The parent track must terminate before the children tracks, which must both start at the same frame.

To connect a lineage, drag a point on the parent track to any point on a child track to connect. Unlike Track Editing, drag-and-connect will not merge the two tracks, the pending connections are shown as a gray dotted line connecting the parent to the child. Drag a point on the parent track to any point on the second child track to complete the lineage connection.
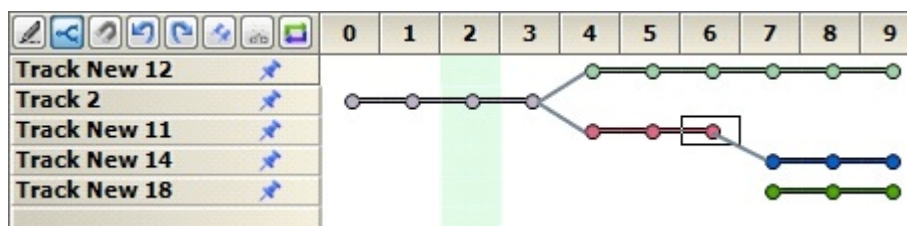
Click-and-drag parent track to child track to connect
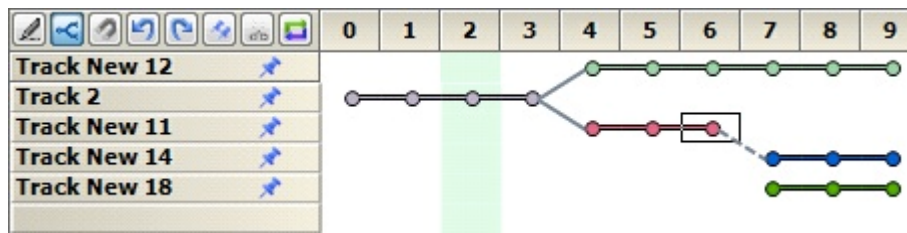
Pending connections shown as gray dotted line
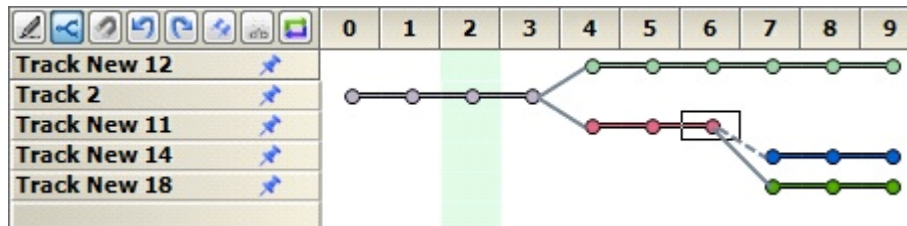
Click-and-drag parent track to second child track to complete lineage connection
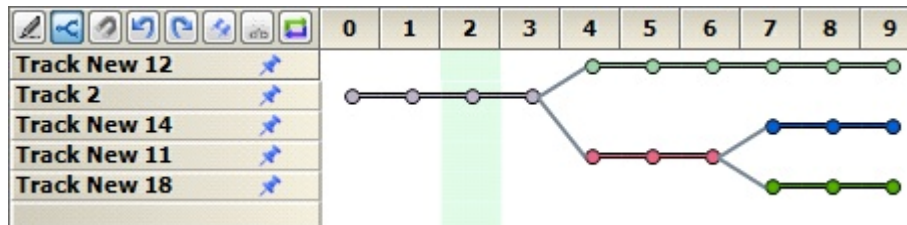
Completed lineage connection

You can also connect lineages on the image using "**Connect Lineage**" option from the context menu. You must have three tracks selected.

(2) Disconnecting lineages

To disconnect a lineage, click-and-drag the termination point on the parent track to any open space in the Track Editing Grid.
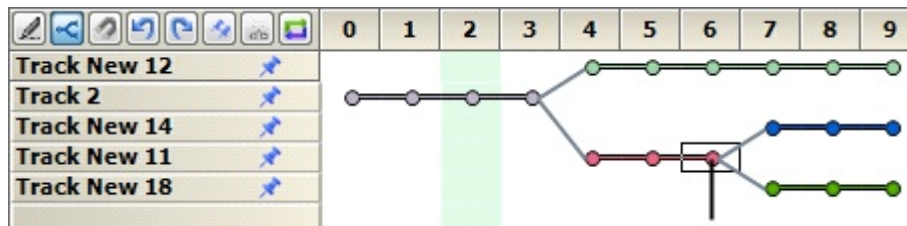
You can also right-click on a track with lineage on the image and select "**Disconnect Lineage**" from the context menu to disconnect lineage from the selected track.

2.7.5 Update Track Measurements

Track measurements will need to be updated after editing to ensure accurate calculation of track characteristics. You may choose an existing tracking procedure (from a recipe) or load a new tracking procedure to update the measurements. This process will modify the track measurements while preserving the tracks.
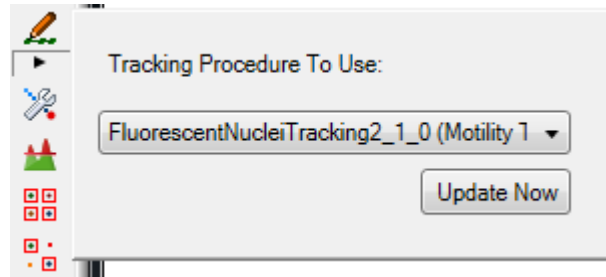
To update track measurements without exiting the track editing mode, click on the arrowhead icon below the track editing button in the toolbar to see additional track editor options. Select the tracking procedure you wish to use for the update in the drop down menu. Click the "**Update Now**" button to update the track measurements and stay in track editing mode.

Track measurements will be updated automatically when exiting the track editing mode.

2.8 Mask Editing

CL-Quant allows the user to create new mask objects or edit existing mask objects to optimize segmentation. Click on the Mask Editor icon (below) to begin.

(1) Draw mask regions

Once in the mask editing mode, you can use the mouse cursor to draw mask regions on the image directly as shown below. Newly created mask regions will be overlaid on the image and masks.
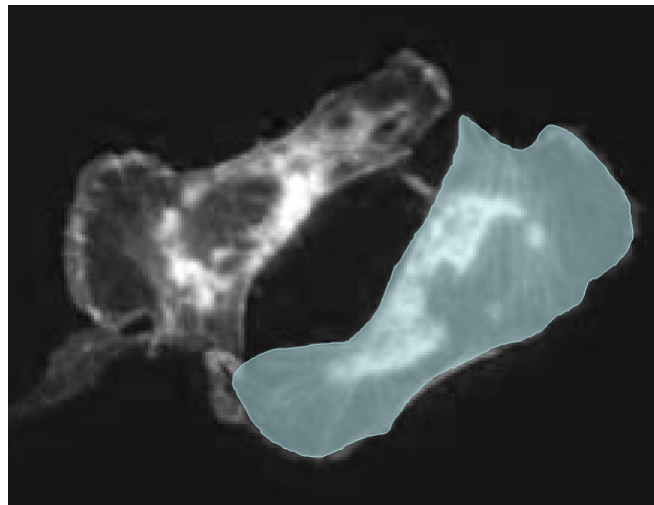
New mask region created using the Mask Editor

Click on the mask region to bring up the object bounding boxes. You can move the mask region by holding down the left-mouse button while dragging the mask region; or resize the region by clicking and dragging the corners and edges of the bounding box.
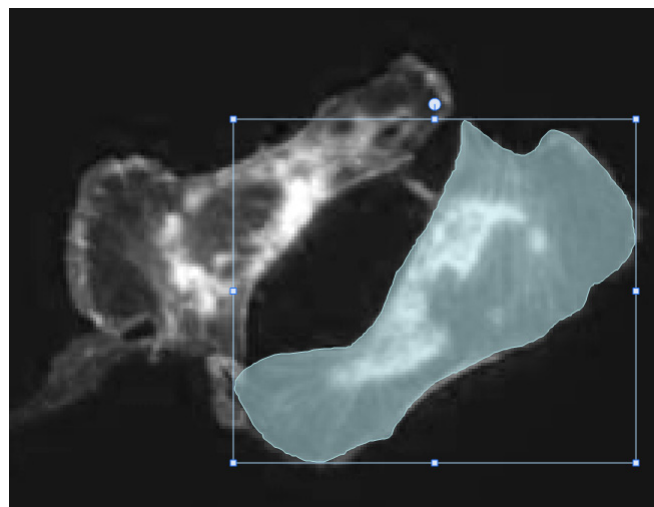
Mask region with object bounding box

Drawing tools

User may specify a different mask drawing tool in the "**Mask Edit Tool**" tab. Open the "**LUTs**"/"**Charts**" panel and click on the "**Mask Edit Tool**" tab to view the available drawing modes.
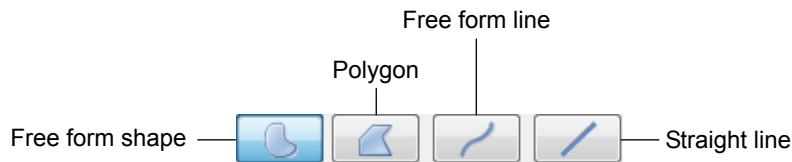
- **Free form shape**
  User can create any shape mask regions using the free-form shape. To create a region, hold down the left mouse button on the image and move the mouse around regions desired.

- **Polygon**
  User can specify straight-edged polygons using the polygon tool. To create a region, left-click anywhere on the image, click once to specify a point on the polygon

- **Free form line**
  User can create a free form line mask region using the free form line tool. To create a region, hold down the left mouse button on the image and draw the line region desired.

- **Straight line**
  User can specify a straight-line mask using the line tool. To create a region, left-click anywhere on the image, click once to specify the end point of the line.

For free form and polygon tools, press "**Enter**" or double-click on the image to complete a region.

Copy mask region

User may copy selected custom regions or all custom regions to the next time frame using the "**Copy Selected**" or "**Copy All**" function respectively. The mask regions will be added to the next time frame and the user will have the ability to add or subtract said region from mask.

(2) Add and remove regions

After drawing the mask region you wish to edit, you can add or subtract the manual region from the working mask using the context menu.

Add regions to mask

If your original mask appears under-segmented, you can add mask region to an existing mask by drawing in the filling region. Right-click on the edited region and select "**Add To Mask**" in the context menu to combine the existing mask with the new region.
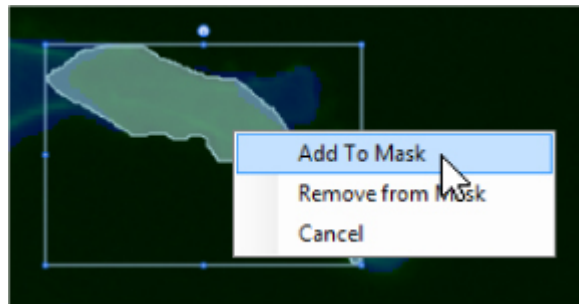
Remove regions from mask

If your mask appears over-segmented or if false detections are included, you can remove mask region from an existing mask by drawing in the region you wish to subtract from the original mask. Right-click on the edited region and select "**Remove from Mask**" in the context menu to remove region from the existing mask.
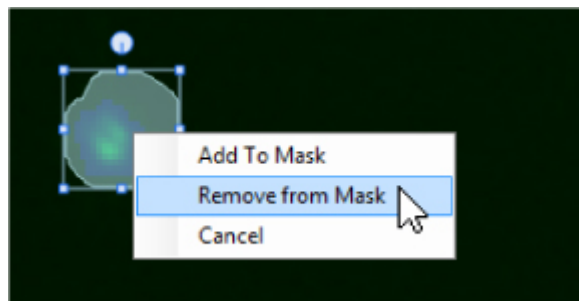
Cancel drawn region

You can cancel/remove the drawn region from the image without editing the image mask using the "**Cancel**" option. Right-click on a drawn region and select "**Cancel**" in the context menu to remove the drawn region from the mask editor. No changes will be made to the existing image mask and the drawn region will be removed from the mask edit history panel.

(3) View mask edit history

In the mask editor mode, user actions are tracked in mask edit history. To view mask edit history, open the "**LUTs**"/"**Charts**" panel and select the "**Mask Edit Tool**". Mask edit history are displayed as a list with actions and coordinates of the mask edit, as shown below.

Action	Detail
Delete	{X=-456.1524, Y=-274.6284}
Add	{X=-455.5504, Y=-276.2748}
Delete	{X=-438.9122, Y=-270.4602}
Delete	{X=-443.3664, Y=-290.6797}
Drawn	{X=451.6, Y=259.2}
Delete	{X=-447.837, Y=-300.1256}

Mask edit history

Click on an action to display the hand-drawn region on the image. Mask regions that are added to the image are shown in green on the image and those that are removed from the image are shown in red on the image. Regions that are drawn, but with no actions specified are shown in light blue and are listed as "**Drawn**" in the "**Action**" column.

(4) Update mask measurements

Field and object measurements on the mask will need to be updated after mask editing to ensure accurate calculation of the mask metrics. Click on the arrowhead underneath the "**Mask edit tool**" icon and select the measurement procedure you wish to use (see below). Click "**Update Now**" to update the measurements immediately. Otherwise, the measurements will be updated when you exit from the Mask Editor.
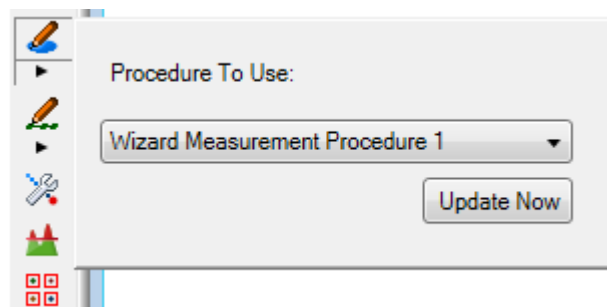
2.9 Action History

Action history displays a list of all operations done on an FOV. To view action history, go to "**Tools** > **View Action History**".
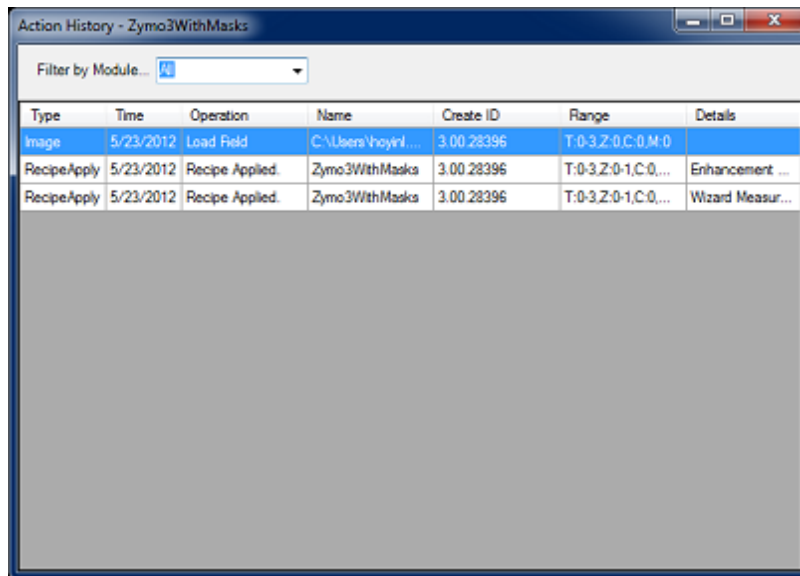
Action History window

The "**Action History**" window shows the type of operations done on the FOV and contains information about the completed operations. Information such as timestamp and description of the operations is displayed. A list of operations visible in the action history window is shown below.

Image	Mask editor	Track Editor	Mask	Channel	Recipe
Load	Add to Mask	Track Move	Add	Add	Apply
Crop	Remove from Mask	Track Delete	Delete	Delete	
		Connect Track	Insert	Insert	
		Disconnect Track	Rename	Rename	
		Connect Lineage			

You can view only filter action history view by action module by selecting on the action group pulldown menu.

Chapter 3

Teaching CL-Quant

Chapter Overview

This chapter covers functionality used to teach CL-Quant for new applications. If you are interested in creating novel experimental analyses, this is the place to start.

Two main types of teaching are supported: you can update an existing recipe (provided by Nikon), or create your own, new procedure list from scratch.

Recipe Update

Recipe update is done using the Recipe Console. The Recipe Console provides key parameters for updates along with instructions and guidelines.

Procedure List Creation

Procedure lists are functionally equivalent to recipes, but can be assembled by you from component procedures. For most applications, only segmentation and measurement procedures are needed to achieve the desired result. Partition, tracking and decision procedures provide additional image recognition functionality. Import and enhancement procedures provide additional functionality.

- Core Procedures (teaching wizards provided)

Segmentation procedure:	This type of procedure defines objects of interest in your images using segmentation masks. The masks are used to quantify the objects.
Partition procedure:	Many times the initial segmentation mask will cover multiple objects. This optional procedure can be used to separate them into individual components.
Measurement procedure:	This procedure quantifies the objects in your images.

- Additional Recognition Procedures

Motility tracking procedure:	This type of procedure is used to track objects in images over time. You can also specify tracking measurements (it is not necessary to use the measurement procedure if you use a tracking procedure).
Decision procedure:	This procedure can be used to classify objects based on their measurements.

- Other Procedures

FOV import procedure:	This procedure automates the import of image frames into multi-dimensional FOVs, and also multiple FOVs into FOV lists.
Enhancement procedure (teaching wizard provided):	This procedure automates traditional image processing functions, and common tasks (such as adding channels, deleting channels, bit depth conversions, etc.).

Innovative teaching wizards are provided to make it easy to create core procedures. These are described in the section below. You can combine multiple procedures in procedure lists and recipes to streamline image processing and analysis. The recipes can be edited using the Recipe Console described in the next section. The creation and configuration of the other types of procedures are described in the following sections.

> **NOTE:**
>
> Procedure creation requires that you have purchased the required functionality. Tracking is included in the "**Tracking**" option, and Decision is included in the "**Decision**" option.

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3.1 Recipe Console

The Recipe Console allows the user to view procedures and to modify key parameters within a recipe. The user can also disable or duplicate specific steps of a recipe to fit their image processing needs.

3.1.1 Launch the Recipe Console

First load a recipe, and then right-click on the recipe in the Data Explorer. Select the "**Update with recipe console**" from the context menu to launch the Recipe Console.
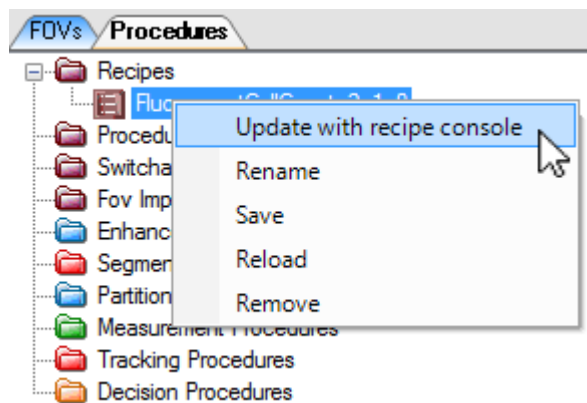
The Recipe Console will prompt you to choose an image; if no image is loaded into the data explorer, click on the "**Load**" button to load an image into the Recipe Console. The Recipe Console provides step-by-step preview such that you can also quickly modify key parameters without evaluating its performance on whole image sequences.

3.1.2 Recipe Console Interface

The Recipe Console interface is divided into two panels: control panel and main panel as shown in the figure below. An additional Descriptions window can be displayed when clicking on the "**?**" icon in the main panel. Result of adjustments to the parameters in the Recipe Console is previewed directly on the image.

Control panel ———
Recipe Console panel ———
(Main panel)
Recipe step header ———
(with recipe step box shown)

Recipe step header ———
(with recipe step box hidden)
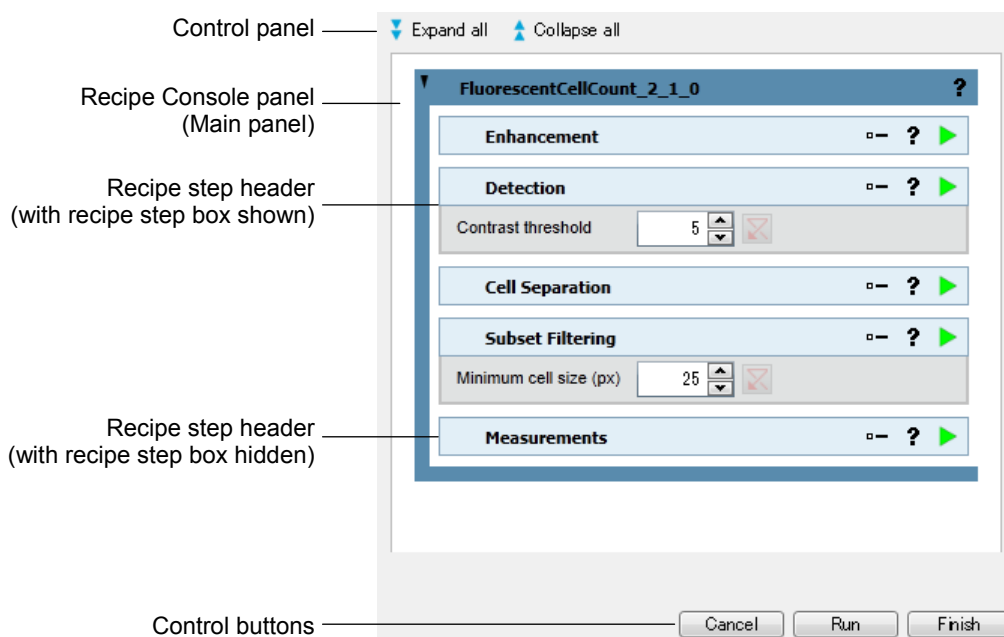
Control buttons ———

Control panel

The parameter display control panel is at the top of the Recipe Console UI that allows the user to expand or collapse all visible parameters in a recipe. There are two buttons, as shown below:

- **Expand all**
  Shows all visible parameters in the recipe steps.

- **Collapse all**
  Hides all visible parameters in the recipe steps.

Main panel

The main panel (Recipe console panel) allows the user to make changes to the recipe step-by-step with additional descriptions to assist the user in optimizing the parameter settings. Each step individually can be expanded or collapsed; or user may opt to expand all or collapse all recipe steps by clicking on the "**Expand all**" or "**Collapse all**" button. One example of a recipe step box is displayed below.

Collapsed recipe step box (header only)
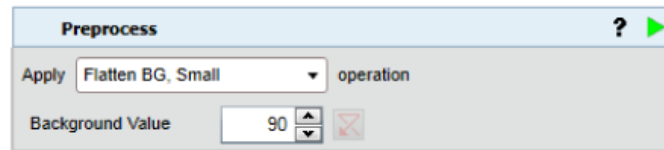
Expanded recipe step box

The recipe step headers have different number of buttons to enable easy access to description, apply recipe step, and recipe options depending on the configuration of the recipe step. Descriptions of the buttons are shown below.
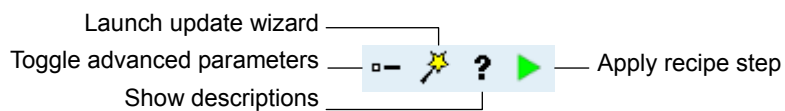
- **Toggle advanced parameters**

  If available, shows advanced recipe parameters.

- **Launch update wizard**

  If available, launches procedure update wizards interface.

- **Show descriptions**

  Shows description of the current step in a new window.

- **Apply recipe step**

  Applies selected step to the entire image.

3.1.3 Review or Update Key Parameters

Some recipe steps will provide one key parameter for your update in the console using controls similar to the one shown in the figure below. The parameter panel is hidden by default. To view available adjustable parameters, double-click on the recipe step header to expand the recipe step. You can adjust the parameter using the slide bar or textbox provided. Use the pulldown menu to select an alternate operation for the recipe step (if available). Click on the "**Show descriptions**" button to display the description popup (not shown). The popup provides important information about the recipe step and description to updating key parameters.
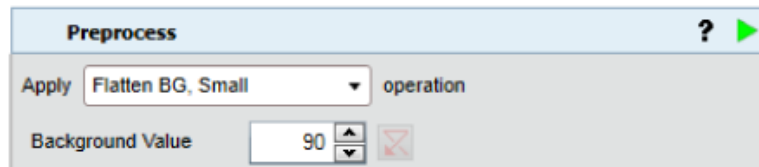
In some recipe steps, an additional option to modify the teaching may be available by clicking on the "**Open**" (magic wand) icon. Please refer to the Procedure update wizards section for more information on modifying the procedure teachings.

When modifying a parameter, recipe changes are previewed on the image instantly. Click the "**Apply recipe step**" button to save changes to the parameters and to apply the recipe step to the entire image sequence.

To revert to recipe default values, click on the "**Revert**" button next to the parameter textbox. Please note that certain functions (such as segmentation and partition updates) cannot be reverted.

View image output

Recipe Console has a built-in function that allows the user to view image output of the whole recipe without exiting the Recipe Console. Click the "**Run**" button at the bottom of the Recipe Console page to run the analysis recipe on the loaded image. All image processing steps in the analysis recipe will be applied to the image.

Alternately, you can click the "**Apply recipe step**" button on the last step of the recipe to apply the whole recipe to the image.

Finalize recipe update

If you are satisfied with the recipe results, you can exit the Recipe Console and save the changes by clicking on the "**Finish**" button at the bottom of the Recipe Console page. All changes to the analysis recipe will be saved.

3.2 Procedure Creation Wizards

Launching procedure creation wizard

To launch the procedure creation wizard, click on the "**Wizard**" function under the "**Tools**" menu and select the procedure type you wish to create (segmentation, measurement, partition, enhancement, tracking, decision, recipe).
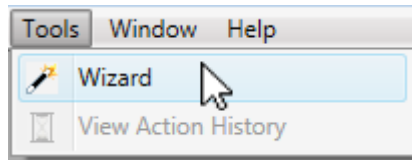
You can also right click on the segmentation, partition or measurement folders in the data explorer and select "**New**". This will launch the respective wizard.
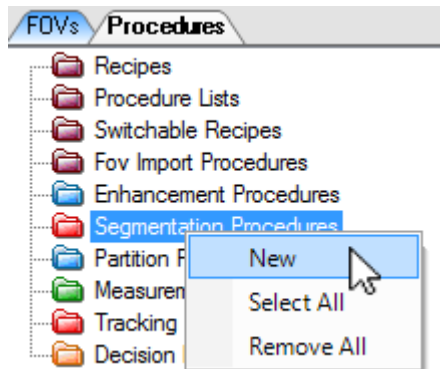
3.2.1 Segmentation Teaching Wizard

A segmentation procedure is used to detect structures of interest in the microscopy image so that they can subsequently be quantified as field or object measurements. The detection is represented as a detection mask that covers pixels belonging to the structures of interest. The segmentation procedure creates the detection mask.

The segmentation teaching wizard will guide you through the steps of creating a segmentation procedure. It provides instructions to guide you.

The wizard is designed to create a single detection mask .To create more than one mask, just run the wizard again on the image with its first mask. This will then create a second procedure that will create a second mask on the image.

3.2.1.1 Select FOVs

Select FOVs
The first step in the wizard is to select the FOVs you want to use for teaching. Multiple FOVs can be used. If the FOVs have temporal or Z extent, the first frame in T and the middle frame in Z will be used.

> **NOTE:**
>
> You can exit the wizard and use the FOV crop tool to extract specific frames from the multi-dimensional FOV, and then re-launch the wizard and use them for teaching.

> **CAUTION**
>
> - The FOVs must have the channel and mask configuration to be included in the same wizard session.

Select channel and mask
If the FOVs have multiple channels, you will be prompted to select one channel for teaching. If the FOV already has one or more masks, you will be prompted to select the mask position or create a new mask.

3.2.1.2 Soft Matching

In this step, you will be asked if you want to perform Soft Matching, or go to thresholding.

Soft matching is teachable image pattern recognition technology in the software. It generates transformed images called a confidence image with enhanced and suppressed patterns based on user teaching. A segmentation procedure encodes the user teaching and can be used to automatically transform input images in a high volume mode. The transformed confidence map can then be thresholded in the next step to generate the detection mask.

Therefore the goal of segmentation teaching using soft matching technology is to teach a transformation where the structures of interest are cleanly separated (made either dark or bright) from other structures.
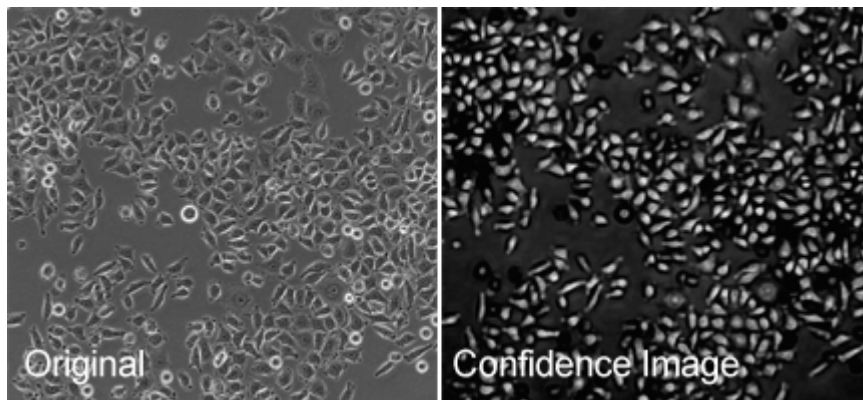
In this example above, a segmentation procedure was taught to enhance the internal cell patterns (such as nuclei), and suppress cell borders and bright structures. This confidence image can be simply thresholded and the mask counted for an accurate measure of cell count in the image. See the guidelines for successful soft matching in the next section.

Steps for Soft Matching

1. Select a single image as your teaching image

Teaching is done one image at a time, though you can later review the result of applying the function to all teaching images. Select one image to get started.

2. Draw teaching regions

This step requires teaching regions of interest (ROI) specified by type. The types include "**Enhance**", "**Suppress**", and "**Background**" patterns.

To create a teaching specification, proceed as follows:

(1) Using the region drawing tools, draw the region(s) for one of the patterns of interest.

Drawing Tools
Drawing tools include freehand line tool and freehand region tool.

- Freehand region tool: a general purpose tool
- Freehand line tool: specialized to enhance edges

To draw an ROI, simply depress the tool icon and then draw on the image frame. When you draw the ROI it will be active, and the segments of the ROI will appear to move around the region.

Freehand line tool ——— Freehand region tool
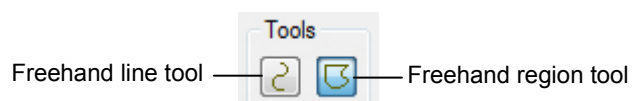
(2) Assign the active (moving) regions the pattern type (Enhance, Suppress, or Background).

After drawing an ROI, the user should assign a pattern type to the active ROIs. The pixels inside the ROIs become a teaching set for the confidence mapping operation.

Pattern Types

- Enhance:

Types of image patterns that you want to isolate from the rest of the image, mask and quantify.

- Suppress/Background:

Types of patterns that you do not want to include in the mask.

The "**Background**" pattern is processed more quickly. It can be considered as a first pass, "**Suppress**" pattern. The image is then considered more carefully to distinguish between "**Enhance**" and "**Suppress**" patterns.
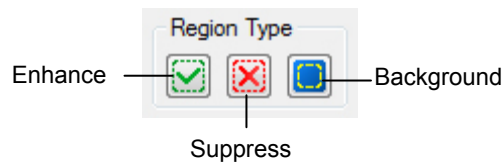
To assign pattern types to ROIs, click on the appropriate icon as shown above; the green check mark is for "**Enhance**" type, the red "**X**" is for "**Suppress**" type and the yellow square on blue background is for "**Background**" type. When you click on the icon, all the active ROIs will be assigned the type of the icon you click.

Activating, deleting and re-assigning ROIs

Initially when you draw the ROI, it is moving and in an active state. ROIs can only be assigned and deleted when they are active. After assignment, the ROI will become inactive. In order to re-assign or delete the ROI, click on it to activate it.

CAUTION

- If an ROI becomes deactivated before it is assigned, and has no assignment, it will be removed during the soft matching process.

Add/Remove region types

You may add an additional set of "**Enhancement**" and "**Suppression**" ROI types by clicking on the "**Add**" button (circled red below). The additional types allow multiple simultaneous soft matching with the results combined. It can be used when one set of regions is inadequate for detection. Up to four sets are supported. However, each additional set will increase processing time.
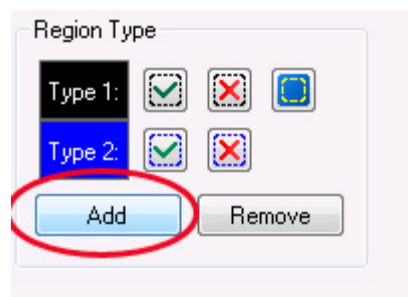
(3) Repeat for the other patterns.

(4) Adjust as needed.

3. Set threshold and review confidence image

After assigning ROIs and clicking "**Next**", soft matching will create a matching model based on your teaching. The model is then immediately applied to the teaching image and the result is displayed. The results include a confidence image and a threshold overlay calculated using the confidence image and shown overlain on the original image as shown below.
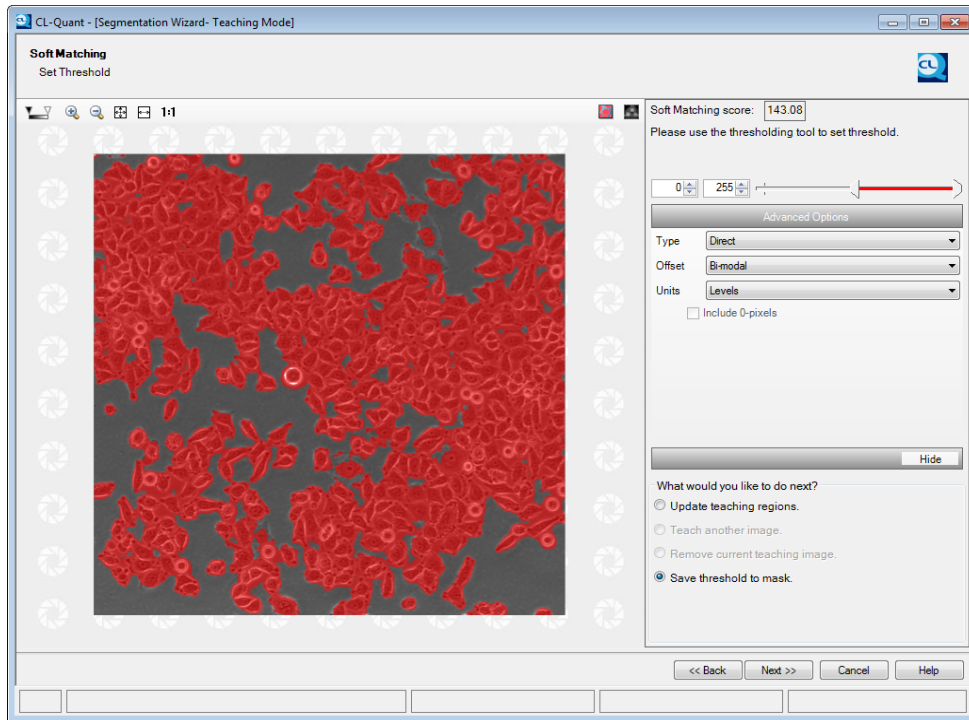
(1) Review the confidence image

To review the confidence image, toggle off the threshold overlay by clicking the "**Hide**" button (red rectangle), followed by the view confidence image icon (green circle). The confidence image will appear in the image panel.

Select to display the confidence image

Click to hide the threshold overlay
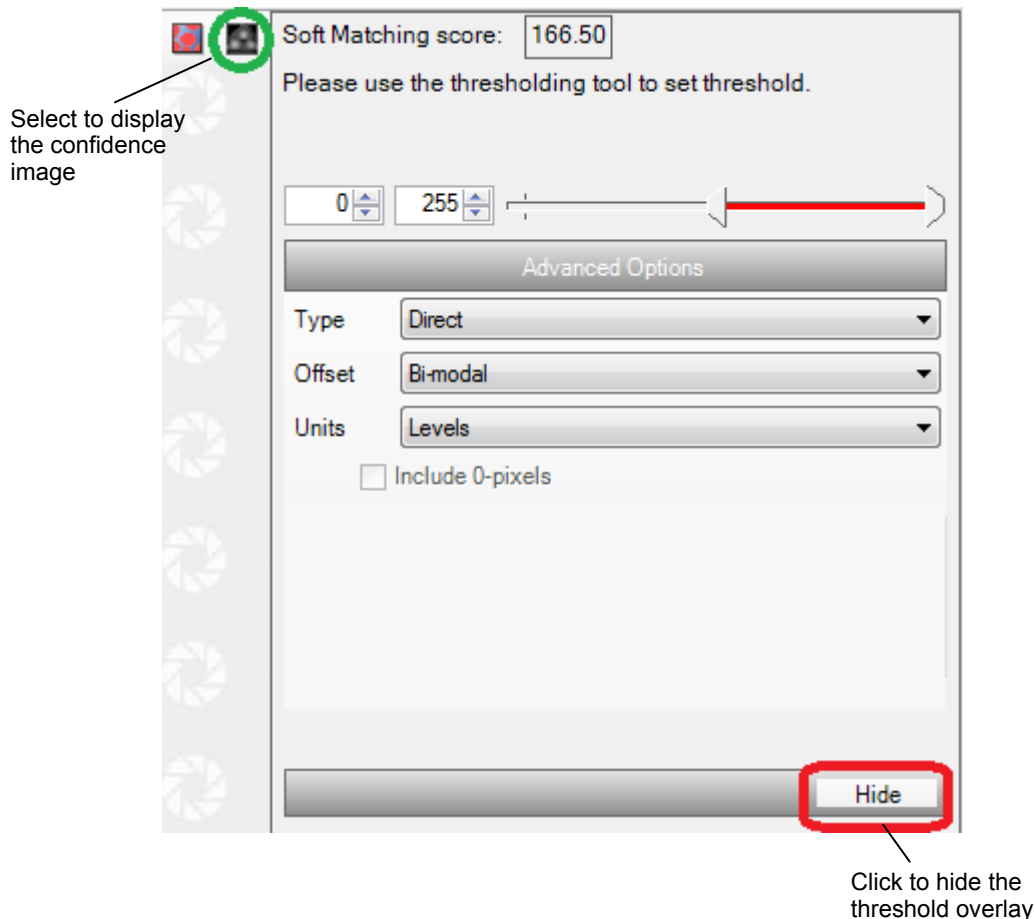
(2) Adjust the threshold overlay

The threshold is a range of confidence values in the confidence image. Values that fall within the range will be overlain in red (red color is reserved for the threshold overlay). If you advance to the next step, these pixels will be saved to your segmentation mask. You can adjust the threshold range by typing in values or dragging the sliders. The threshold tool is described in Section 3.2.1.3, "Thresholding".

- **Preview on All**
  If you select this option the current procedure will be applied to all teaching images for your review.

(3) Next steps

The wizard provides several options for your next step. These are:

- **Update teaching regions**
  If you are not satisfied with your result, you can choose to update the teaching regions using the current active image. This will take you back to the draw soft matching regions step described above. You can then add or delete teaching regions.

- **Teach another image**

  If you have multiple images in your teaching set, you can now choose to teach on a second image. The teaching from multiple images can be included in the soft matching model.

- **Remove current teaching image**

  If multiple teaching images are used in Soft Matching, you can remove the current teaching image using this option. Select this option to remove the last-taught image from the segmentation procedure. All other existing teaching images will be preserved.

- **Remove all teaching data**

  Select this option to remove all teaching image and data from the segmentation procedure, you will be taken back to the Soft Matching teaching page. This operation cannot be reversed.

- **Save threshold to mask**

  If the result looks good, select this option. The pixels included in the threshold overlay will be saved to create your segmentation mask.

Soft Matching score

Above the threshold tool settings is the soft matching score. The soft matching score is the difference of the mean confidence value of enhancement ROIs minus the mean confidence value of the suppression ROIs. A higher score indicates better separation between the enhancement and suppression classes.

Soft Matching score: 166.50

Improve soft matching detection

The soft matching score can serve as a guide to improve soft teaching. Creating teaching regions with clearer distinction between enhancement ROIs (dotted green region) and suppression ROIs (red dotted region) will yield a higher score. The figure below shows an example of good teaching (top) and bad teaching (bottom) with their respective confidence maps.

Good teaching
(High soft matching score)

Bad teaching
(Low soft matching score)
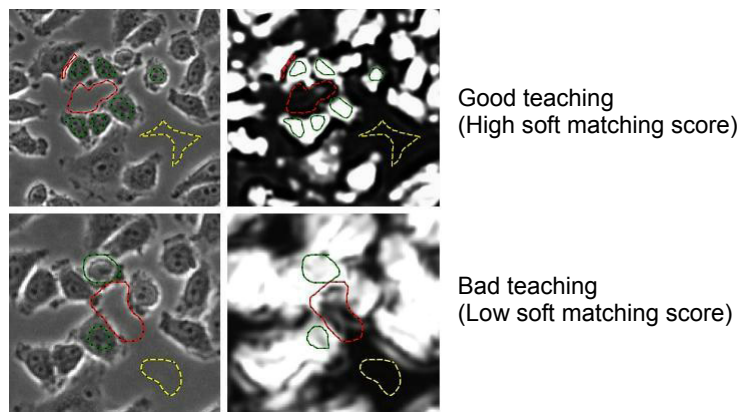
3.2.1.3 Thresholding

Use the threshold tool (shown below) to create a segmentation mask. The tool highlights a range of pixels in the image (original, processed or confidence) whose values fall between the parameters you select. If the pixels are within the values, they will be shown having a red overlay in the image panel. Thus, if you select all the possible values, then the entire image panel will be covered in red.
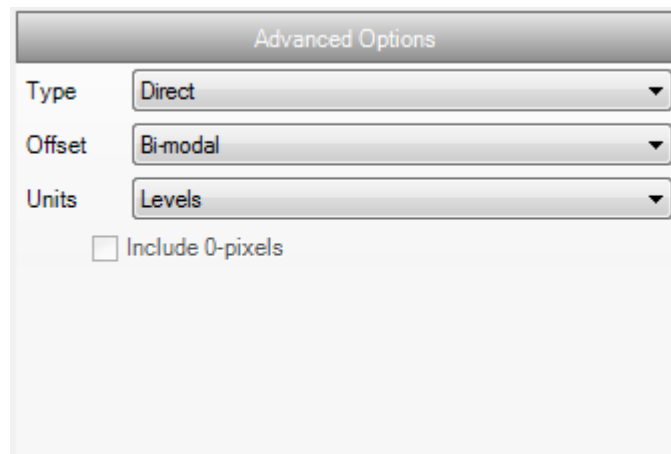
The tool is used to select pixels for inclusion in a segmentation mask. This is the detection mask which will be quantified later.

To use the tool simply:

1. Click the "Show" or "Hide" button to visualize the selected pixels in the range

2. Move the slider to adjust the range

3. Modify the text in the boxes to adjust the text

Once you select the appropriate range, click "**Save threshold to mask**" and the selected pixels (in red) will be saved to your segmentation mask.

The threshold tool has many advanced options which are described below. To access the advanced options, click on the small button circled in red in the figure above. This powerful tool has 2 types of thresholds, 4 offset choices and 2 types of units. The default mode is a direct type, bi-modal offset, and levels units. The default should work fine in most cases.

<u>**Threshold options**</u>

● **Threshold type**

"**Direct**" and "**Adaptive**" types of thresholding are supported. The adaptive type combines thresholding with image processing steps into a simple one step operation.

Direct	With the direct type threshold, the values you select represent the real pixel values in the image (i.e. no offset). Thus if you select 0 and 100, pixels in the image that have values from 0 to 100 will be covered in the red overlay. This is the standard type of threshold in most image analysis software.

> **NOTE:** If you have performed Soft Matching, the overlay is being calculated using the pixels in the hidden confidence image, and displayed on the original, visible image.

Adaptive	Often objects of interest cannot be isolated within a single direct threshold. This could be due to intensity gradients from uneven illumination, or from global intensity undulations in the image due to accumulation of fluorescence in biological structures. For example, it is desirable to be able to threshold puncta in a bright region of the image as well as a darker region of the image using a single threshold.

The adaptive threshold accomplishes this by normalizing the background before applying the threshold. The size parameter determines the degree of normalization. A low number is more normalized (to detect smaller structures), and a higher number is less normalized (to detect larger structures). The size parameter should be set to be roughly the same size as the structures of interest in the image (in total pixels).

Comparison with direct type

Adaptive type differs from direct type threshold in that the values you select for the threshold are interpreted with respect to the normalized image, not the displayed image in the image panel. The hidden normalized image will have a much smaller range of values than the original.

● **Dynamic Offset**

Dynamic offsets enable the threshold values to adjust dynamically as a function of the image content. The tool supports three types of Bi-modal offsets.
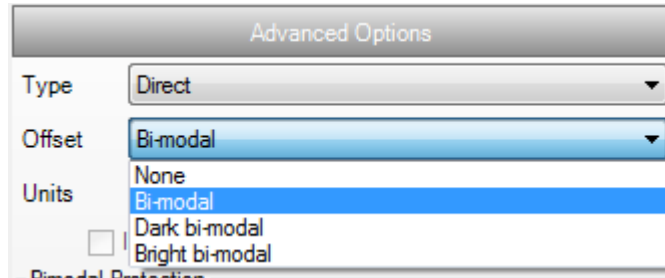
Bi-modal	The tool default offset. Usually the pixel values in an image have a bi-modal frequency distribution corresponding to foreground (structures of interest) and background pixels. The software automatically determines the bi-modal point in the image's pixel intensity distribution and sets that point to zero, thus making the threshold settings dynamic when applied to a set of images (each image's threshold range will vary slightly). The threshold range is then set with respect to this bi-modal zero point from -255 to 255.
Dark/Bright bi-modal	Sometimes an application will be more likely to have occurrences of uni-modal or tri-modal distributions. In the uni-modal case the bi-modal point can move over the peak causing large changes from image to image. The dark or bright bi-modal offsets should be used in these cases. The algorithm assumes a tri-modal distribution and then sets the offset to favor either dark or bright detection. Select "**Dark bi-modal**" offset if you want to segment dark pixels, and "**Bright bi-modal**" offset if you want bright pixels.

● **Threshold Units**

The tool supports levels and histogram percentile scales.
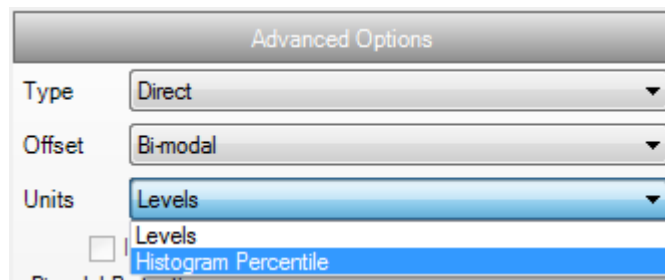
Levels	This is the standard type of threshold and default unit. Here the unit is image gray level. So for an 8 bit image, the threshold values range from 0 to 255 (range type) or -255 to 255 (bi-modal type).
Histogram Percentile	The units in this case are percentiles. The percentile is calculated with respect to the pixel intensity distribution of the image's pixels. Thus the threshold values range from 0 to 100 (range type) or -100 to 100 (bi-modal type). So for example, if you increase the threshold value to accommodate one more unit, you will bring pixels into the overlay that corresponds to increased 1% selection from the pixel intensity distribution.

3.2.1.4 Advanced options

The final step of the wizard prompts you to choose among advanced options:

- **Enable update for this procedure**
  If checked, the procedure can be updated in the future using the segmentation teaching wizard interface. If unchecked, the procedure will not be updateable, but can be reviewed.

- **Save Soft Matching confidence image**
  If you used Soft Matching, any soft matching confidence image will be saved to create a new channel if this option is checked.

3.2.2 Segmentation Review and Update Wizard

Existing segmentation procedures can be reviewed and updated using the update and review wizard. If update was allowed for the procedure when it was created, you will be able to update the procedure parameters using the wizard. If update was not allowed, then you can review the teaching and parameters that were used. As you step through the wizard it will apply the procedure to the teaching images in real time and show you the steps including pre-processing functions, thresholding, mask refinement, and saving options.

To update or review an existing procedure, load the procedure and right click on it in the data explorer and select "**Review with Wizard**" or "**Update with Wizard**" as shown below.
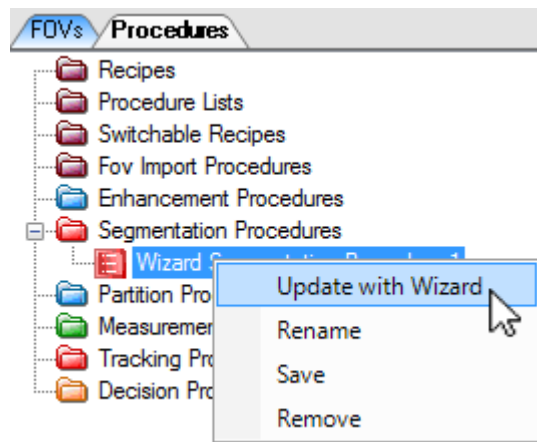
3.2.2.1 Review FOVs, channels and masks

At first the wizard will orient you to the FOVs used for teaching. It will show you the FOVs used for teaching, the specific channel used, and the mask position that was taught.

3.2.2.2 Review or update soft matching regions

If Soft Matching was used, you will be able to review the teaching images as shown below.
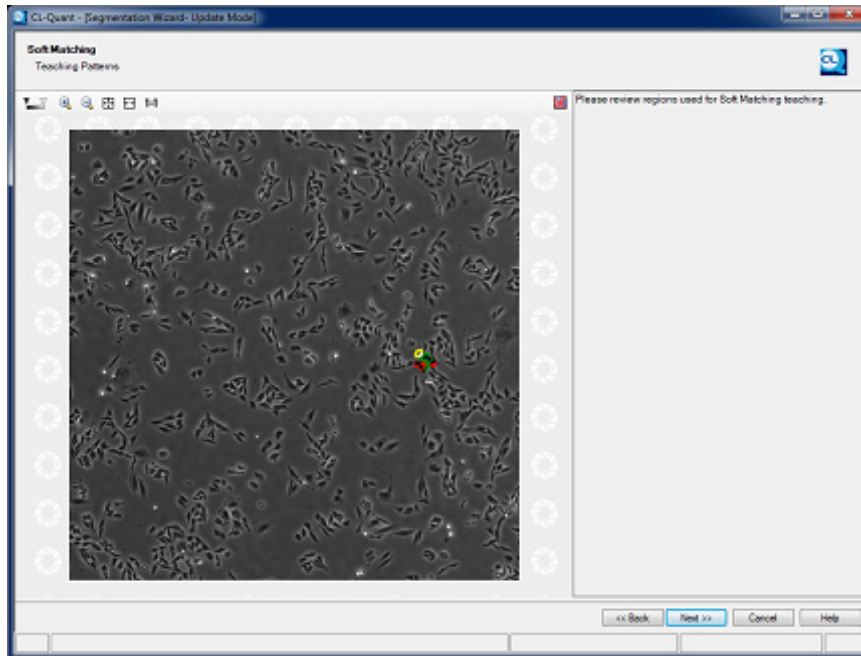
On the next page, you will be able to review the soft matching results, as well as additional options for updates and reviews. To review teaching data, select "**Review teaching data**" and click "**Next**".
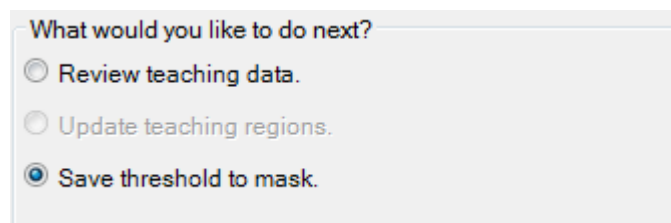
3.2.2.3 Review and update threshold parameters

At this step, you can review the confidence image, review the detection mask, review the threshold overlay and modify the threshold overlay (which will change the detection mask).

<u>**Steps**</u>

1. Review default threshold overlay

At this step, a detection mask with the threshold settings from the procedure will be displayed. To toggle the detection mask click on the "**View overlay**" icon.

2. Review confidence image

You can also review the confidence image by clicking the "**Show Confidence Image**" icon shown below.

3. Adjust threshold settings

You can adjust the threshold parameters (to make the detection mask more or less sensitive) by adjusting the threshold slider (green circle) and associated text box. The updated threshold setting is overlaid automatically on the image. The "**Reset Value**" button (red rectangle) will restore the procedure's original setting.
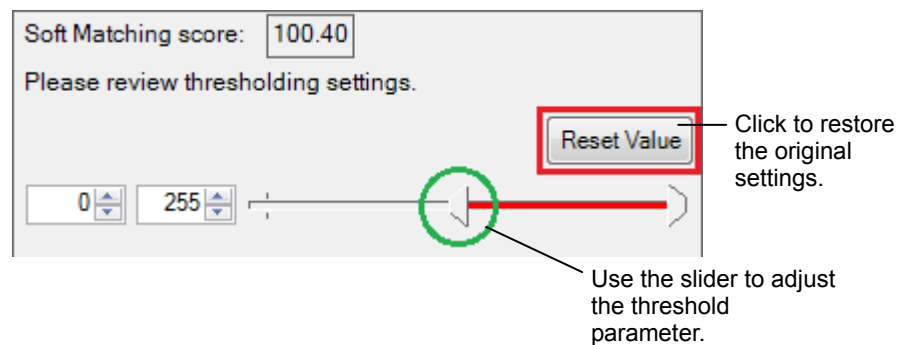
3.2.2.4 Advanced options

Here you can change the selection for intermediate image saving (confidence image). Intermediate images will be saved as a channel in the FOV after the procedure is applied.

You can also elect to make the procedure non-updateable.

3.2.3 Partition Teaching Wizard

A partition procedure is used to divide a segmentation mask into discrete components corresponding to individual structures of interest. The partition wizard will guide you through the steps of creating a partition procedure. It provides instructions to guide you. When completed, you will have created a partition procedure.

The wizard includes the following steps:

1. Select FOV:
   More than one FOV can be selected for teaching.

2. Teach cell pattern:
   Teach the software what your cells look like.

3. View teaching result:
   Based on the partition result, give the software more teaching examples to improve performance.

3.2.3.1 Select FOVs

Select FOVs
The first step in the wizard is to select the FOVs you want to use for teaching. Multiple FOVs can be used. If the FOVs have temporal or Z extent, the first frame in T and the middle frame in Z will be used. The FOV must have a mask before it can be used for partition teaching.

> **NOTE:**
>
> You can exit the wizard and use the FOV crop tool to extract specific frames from the multi-dimensional FOV, and then re-launch the wizard and use them for teaching.

Select mask
If the FOVs have multiple masks you will be prompted to select one mask for teaching. The wizard can only be used to teach partitioning for one mask at a time (multiple procedures can be used to partition multiple masks).

3.2.3.2 Teach cell pattern

Steps for teaching

1. Soft fitting

To teach soft fitting, use the region selection tools in the Soft Fitting section (shown below). You can select existing regions and define new regions using the tools.

Tools and buttons of the Soft Fitting area

The Soft Fitting section provides three types of region selection tools and buttons:

- **Pointer**

  The pointer tool allows the user to select existing regions on the image.

- **Draw regions to cut**

  Use this tool to specify the cut regions (i.e. cell boundaries)

- **Draw regions to keep**

  Use this tool to specify the keep regions (i.e. cell bodies)

- **Add Image -** Use this button to add a teaching image.

- **Delete Image -** Use this button to delete a teaching image.

- **Smoothing kernel size** - Specify the smoothing kernel size for smoothing the Soft fitting confidence image.

- **Teach -** Click this button to view soft fitting confidence map.
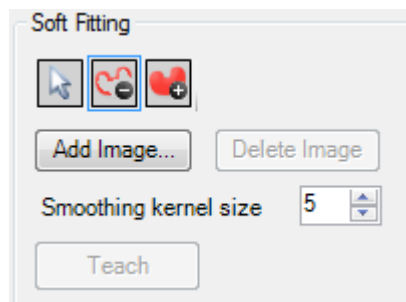
Soft fitting section of Partition Wizard

Select the region tool you wish to use and draw directly on the image. Soft fitting uses two region types - Cut and Keep - as guides for separating confluent cell regions. The Cut regions are shown with a red dotted border while the Keep regions are shown with a green dotted border.
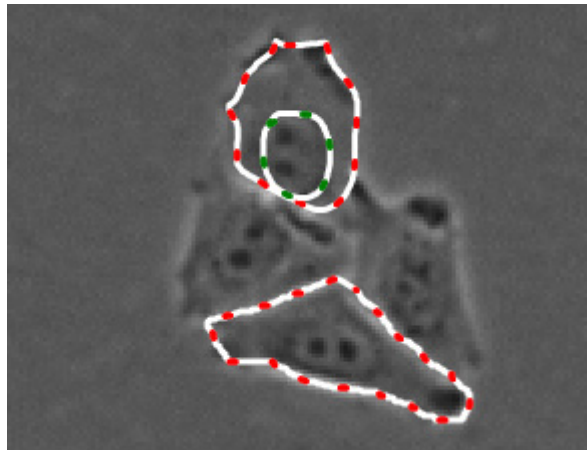
Example of Soft Fitting teaching with Cut (red) and Keep (green) regions specified

For best results, define a Keep region inside each Cut region to ensure optimal pattern recognition of the partition regions. When you are finished, click the "**Teach**" button to view the confidence image.

2. Review confidence image

When you click the "**Teach**" button, a confidence map showing the boundaries and cell bodies is generated in the confidence channel. Keep regions should appear bright in the confidence channel and should be clearly separated by dark regions that represent the Cut regions and background (below).
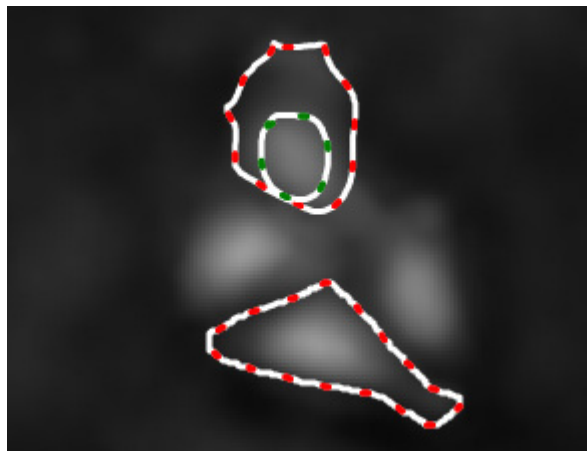
Soft fitting confidence image with regions overlay

As shown in the confidence image above, there are good intensity separation between the cell boundaries and cell bodies. At this point, you can define addition cell regions and click "**Teach**" again to generate a new confidence image.

Next, seed masks are generated that will serve as the guide for partitioning the confluent cell region masks.

3. View seed masks

Seed masks are used as a guide for cell partition. The masks are generated from thresholding the confidence image. Three seed masks will be generated for partition - large, small, and medium. To view the seed masks, click the "**Create Seeds**" button in the Partition section.

The seed masks are generated in the confidence channel image and can be viewed by toggling their respective masks tab above the image.
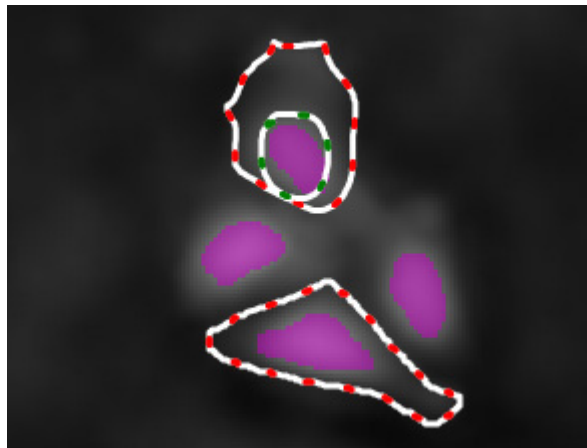
Adaptive seed mask overlay on the confidence channel

You can modify the sensitivity of the seed masks by adjusting the threshold slider in the Partition section. A higher threshold will result in more refined seeds but may also remove certain seeds. For best results, use the lowest possible threshold value that will provide you with good small seeds for all cells.

3.2.3.3 View partition result

After you have optimized the seed masks, use the cell size slider to specify the approximate sizes of each cut region. The Partition wizard will attempt to cut cell regions into discrete cell components that are within the specified cell size range.

The Create Annotation tool A^+ on the toolbar can assist you with specifying the size range. For best results, measure the region size of the smallest and largest cell in the image.

To view the partition result, click "**Apply**". The partition output will be generated in the "Output" mask in the original image channel. If you are not satisfied with the output, you can re-teach soft fitting by editing the teaching regions by drawing. If you are satisfied with the result, click "Save" to save the teaching and "Finish" to quit out of the Partition update wizard.

Select input and output

User can specify the input channel and mask, as well as the output channels and mask in the "**Input & Output**" tab of the Partition Wizard.

Note that existing data in the specified output channel or mask will be overwritten by the Partition Wizard output.

3.2.4 Partition Procedure Update Wizard

Existing partition procedures can be reviewed using the procedure review wizard. Unlike all other procedures, partition procedure update is not supported at this time. You can use the wizard to review the teaching and parameters that were used. As you step through the wizard it will apply the procedure to the teaching images in real time and show you the steps involved.

To review an existing procedure, load the procedure and right click on it in the data explorer and select "**Review with Wizard**".

Steps for reviewing procedures

1. Review FOVs, channels and masks
At first, the wizard will orient you to the FOVs used for teaching. It will show you the FOVs used for teaching, the specific channel used, and the mask position that was taught.

2. Review soft fitting teaching
Next, the review wizard will show you the regions drawn for soft fitting teaching. At this step, you can add or remove additional teaching images or redraw soft fitting teaching regions.

3. Review soft fitting confidence
Click "**Teach**" to run the soft fitting algorithm on the image to generate a confidence map of cell body and cell boundary.

4. Review threshold and seeds
Click "**Create Seeds**" to see the seed masks that will be used as cell partition guides. You can modify the threshold to change seed detection sensitivity and specify desired size range.

5. Review partition mask
Click "**Apply**" to apply image partition to the detection mask.

3.2.5 Measurement Configuration Wizard

A measurement procedure is used to quantify structures of interest in your images. This wizard will guide you through the steps of creating a measurement procedure. It provides instructions and tutorials to guide you. When completed, you will have created a measurement procedure for your application. The wizard includes the following steps:

1. Teaching image selection

2. Measurement type selection

3. Measurement selection

4. Additional options

3.2.5.1 Teaching image selection

Choose an image representative of your application. Its channels and masks must be in the same positions as the FOVs on which you will apply the procedure.

3.2.5.2 Measurement type selection

Choose between object or field types of measurement.

- Object measurements:
Calculated for discrete mask components individually. An example of an object measurement would be the mean intensity and area for each individual cell in an image.

- Field measurements:
Calculated for the entire mask. An example of a field measurement would be the wound size in a scratch wound assay.

Specify the primary mask for object

If you choose object type measurements and have more than one mask, you will be prompted to select the primary mask. The primary mask has a one to one relationship with the biological object in the image. For example, a nuclear stain would usually be used for a primary mask. One mask component in the primary mask can be associated with multiple components in the non primary masks.

When you apply an object measurement procedure, ROIs will appear overlain on the image identifying individual objects. See Section 2.6.1, "(1) Objects and Subsets".

3.2.5.3 Measurement selection

(1) Measurement selection for single mask

Measurement selection is easy for most applications. The figure below shows the measurement selection interface for an application with two channels (Ph and Fl) and one mask. You simply select the morphology measures you want, and then select the intensity measures for each channel. The intensity measures are configured for each channel independently. In this case no intensity measures are selected for the Ph channel (red arrow), while all intensity measures are selected for the Fl channel (green arrow).
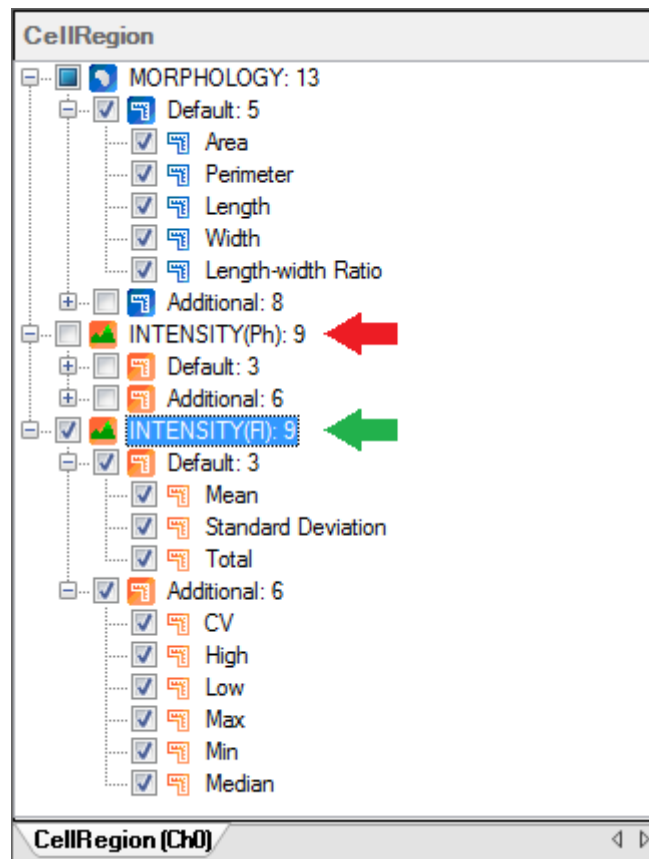
(2) Measurement selection for multi-mask

Measurement selection in the multi-mask and multi-channel scenario is similar to the one-channel, one-mask case. However, in addition, morphological measures can be specified for each mask. Intensity measures can be specified for each mask on each channel.

In the wizard UI, each mask has its own tab. Click on the tabs at the bottom (blue and red circles below) to switch between them. For each mask, specify its morphological and intensity measures. Intensity measures are specified for each channel individually. When you switch between masks, the displayed name at the top of the panel will change to display the current mask name (arrows).
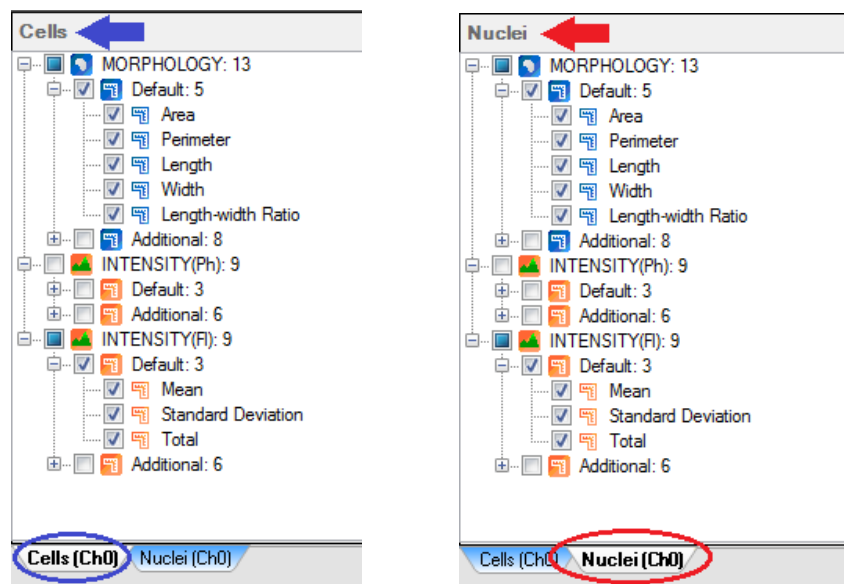
(3) Measurement definitions

The following morphology and intensity measurements are provided:

Morphology measurement

Area	The number of pixels in the mask.
Area to image size ratio	The ratio of the mask area to the area of the whole image field of view (FOV).
Centroid X	The x coordinate of the mask centroid. The coordinate origin is this top left corner of the image frame. Centroid is essentially the average x location of the mask (sum of x coordinates divided by the total number of pixels in the mask). Centroid is only calculated for binary images (not using the image intensity).
Centroid Y	The y coordinate of the mask centroid. The coordinate origin is this top left corner of the image frame. Centroid is essentially the average y location of the mask (sum of y coordinates divided by the total number of pixels in the mask). Centroid is only calculated for binary images (not using the image intensity).
Compactness	A measure of the masks elongatedness and compactness. It is defined as perimeter^2 / area.
Component Count	The number of discrete units in the mask.
Length	The length of the mask is defined as the number of pixels in the major axis of the best fitting ellipse to the mask.
Length-width ratio	The ratio of length to width.
Orientation	Orientation measured with respect to top left corner of image. Angle in radians.

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Perimeter	The boundary of the mask. It is the pixel count of the boundary pixels just inside the mask. Internal holes in the mask are counted.
ROI Width	The width of the bounding box encompassing the object.
ROI Height	The height of the bounding box encompassing the object.
Width	The width of the mask is defined as the number of pixels in the minor axis of the best fitting ellipse to the mask.

Intensity measurement

Coefficient of variation in intensity	The coefficient of variation is defined as the ratio of the standard deviation of intensity to the mean of intensity.
High intensity	The 95% intensity value measured in the mask, measured on the grayscale image. 95% is the value below which 95% of the pixel intensity values fall. The pixels belong to the mask and are measured on the grayscale image.
Low intensity	The 5% intensity value measured in the mask, measured on the grayscale image. 5% is the value below which 5% of the pixel intensity values fall. The pixels belong to the mask and are measured on the grayscale image.
Max intensity	The maximum of the intensity values measured in the mask. The maximum intensity value is the highest value measured from the pixels in the mask on the grayscale image.
Mean intensity	The mean of the intensity values measured in the mask, measured on the grayscale image. Mean is defined as the sum of the intensity values in the mask divided by the number of pixels in the mask.
Median intensity	The median is the number separating the higher half of the sample from the lower half. Here the sample is the pixel intensity values in the mask measured on the grayscale image.
Min intensity	The minimum of the intensity values measured in the mask. The minimum intensity value is the lowest value measured from the pixels in the mask on the grayscale image.
Standard deviation of intensity	The standard deviation is a measure of the spread of the intensity values measured in the mask on the grayscale image. The formula for the standard deviation of a random variable is used.
Total intensity	The sum of the intensity values measured in the mask using the grayscale image.

3.2.5.4 Additional options

You can create advanced measurements (derived from the base measures) and you can rename measurements. On the final step you can set whether the procedure can be modified or not.

Measurement renaming

The default naming convention is *channelName.maskName measurementName*. You can define your own measurement names (for both regular and derived measures) by right clicking on the measurement and typing a new name. When you apply the procedure, the name will appear just as you've written it.
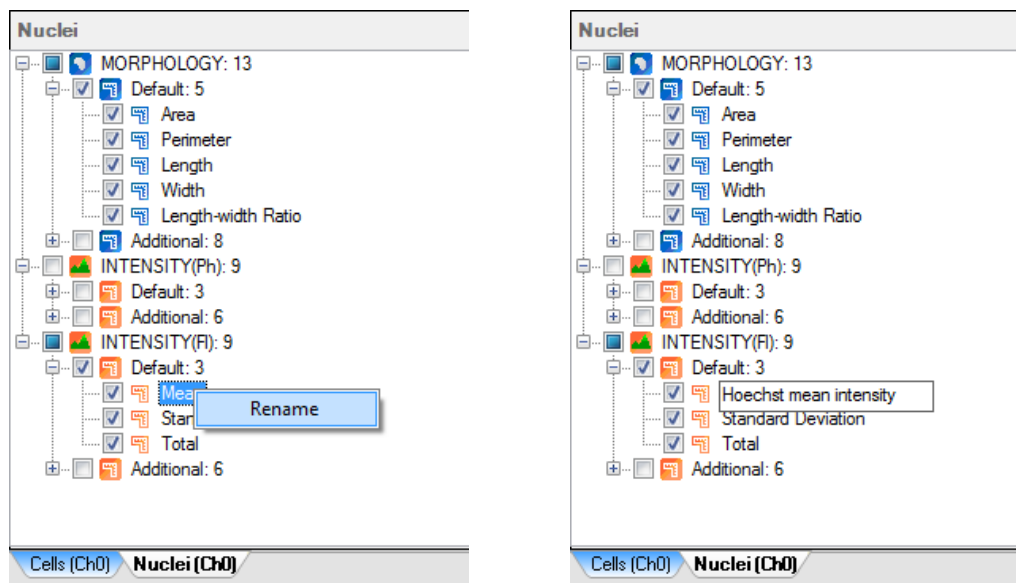
Advanced measurements

You can derive measurements using existing measures you created in the selection step. Common types of derived measures include ratios and differences. When you click on the "**Add**" button, a new tab will appear in the wizard containing the derived measurement.

Please select operation for creating an
advanced measurement:

Ratio ▼

Then select measurements to be used for the
advanced measurement from the dropdown.

Measurement 1:

Value ▼   Morphology.IndividualCells.Perimeter ▼

Measurement 2:

Value ▼   Morphology.IndividualCells.Area ▼

☐ Use an Equation
first[T] for the current T of Measure 1, second[T + 1] etc

[   ]

[Add   ]   [Add Template]

Note: You can rename any measurement by
right-click on the measurement name.

When you're done, click "Next" to continue.

- **Use an equation**

  Enable this option to define a customized advanced measurement equation using "**first**" and
  "**second**" for Measurements 1 and 2 respectively. You can create temporal measurement for
  the measurement equations by modifying the time value in the square bracket following the
  measurement notations. You can click on the "**Add Template**" button to create an equation
  template for multiple measurements.

3.2.6 Measurement Procedure Update Wizard

Existing measurement procedures can be reviewed and updated using the update and review wizard. If update was allowed for the procedure when it was created, you will be able to update the procedure measurements using the wizard. If update was not allowed, then you can review the teaching and parameters that were used.

To update or review an existing procedure, load the procedure and right click on it in the data explorer and select "**Review with Wizard**" or "**Update with Wizard**".

Steps for reviewing and updating procedures

1. Review teaching image

In the first step, the wizard will show you the model image used for configuration. The procedure logic is tied to the FOV's channel and mask structure.

2. Confirm object or field measurement

Next, you will be shown whether the procedure is a field or object measurement procedure. If the latter, you will be shown which mask is used as the Primary Mask (see previous section). Even if the procedure is updatable, you are not allowed to change these settings.

3. Review measurements

Here you can review the procedure's measurements. If the procedure is updatable you may modify the measurements.

4. Define advanced measurements

Here you can review the procedure's advanced measurements. If the procedure is updatable you may modify the measurements.

5. Enable update

Lastly you can confirm the update status of the procedure. If update is enabled, you can change the procedure to be non updatable.

3.2.7 Enhancement Procedure Wizard

An enhancement procedure is a powerful tool that contains a large set of traditional and innovative image analysis functions. Advanced users and customer support can use the wizard interface to create custom procedures for specialized applications. The enhancement procedures can be incorporated into template or custom recipes for even more robust applications.

This section covers how to create an enhancement procedure. Teaching an enhancement procedure is similar to creating a macro using other tools. Your processing order, parameters, inputs and outputs are recorded to the RFrame history. You can then save this history to an enhancement procedure and select additional advanced editing options.

3.2.7.1 Basic operations

(1) Select FOVs

The first step in the wizard is to select the FOVs you want to use for teaching. Multiple FOVs can be used. Click "**Next**" to continue.

> **NOTE:**
>
> You can exit the wizard and use the FOV crop tool to extract specific frames from the multi-dimensional FOV, and then re-launch the wizard and use them for teaching.

(2) Enhancement functions

The enhancement wizard interface (shown below) is similar to the enhancement module interface. There are three tabs on the right to provide specific function sets for the user to choose from.
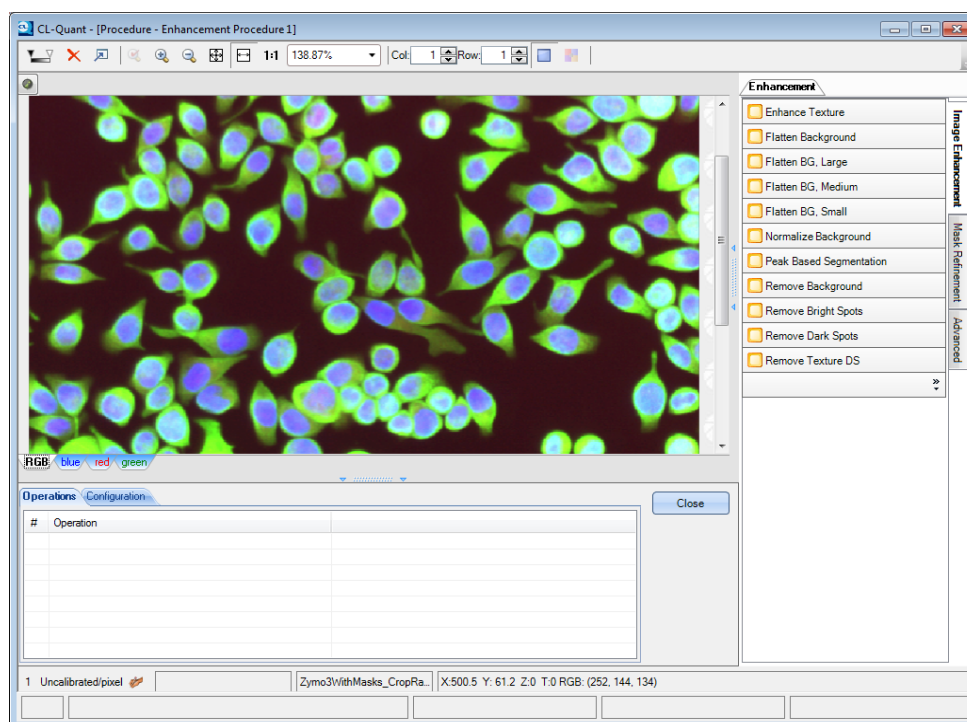
- Image Enhancement:
  A selection of powerful image enhancement functions (such as background normalization
  and flatten background) which are commonly used in standard and template recipes.

- Mask Refinement:
  A selection of powerful mask manipulation functions (i.e. partition and gating of single
  components) which are commonly used in standard and template recipes.

- Advanced:
  The complete set of image processing functions.

(3) Execute enhancement functions

Select the enhancement function you'd like to apply from the list. The function parameter
panel appears, providing a thumbnail for the input and output channel or mask.
Drag-and-drop the channel or mask onto the input thumbnail and drag-and-drop the output
thumbnail to an existing or new channel or mask to define the input and outputs.
In the example below, the "red" channel (red arrow) is designated as input and output the
operation to "Channel 3" (blue arrow).
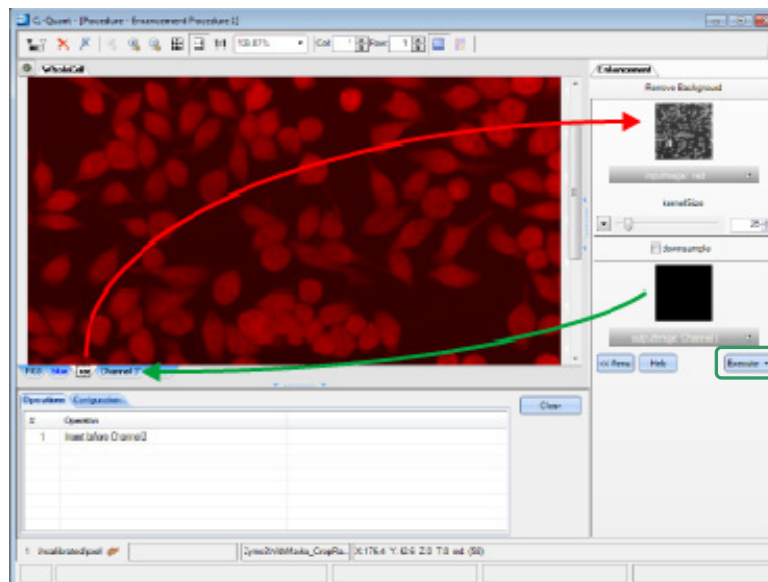
Some functions provide several key adjustable parameters. Use the slider bar or the textbox
to change the parameter values. Press "**Execute**" (green box, above) to apply the function to
the image. Note that the executed function is saved automatically in the operations history.

Click "**Menu**" to return to the function selection screen to add new enhancement functions.

(4) Configure temporal parameters

You can apply the functions between time frames by setting the temporal parameters. You can set an offset from either the current time point, or the beginning or end of the movie. Note that the enhancement procedure will be subject to any apply procedure parameters such as range or frequency, in which case the first and last frame of the movie would be replaced by the first and last frame of the apply procedure range.

In the example above, the "green" image at the current time (in Input1) is added to the "green" image of the next frame (in Input2) and the resultant image is output to "Channel 3".

(5) Edit enhancement functions
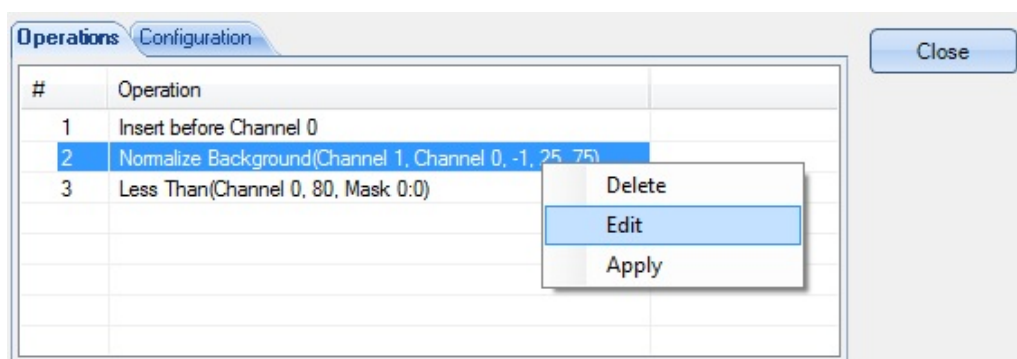
- Delete an operation:

To delete an operation, right-click on the item in the operations history and select "**Delete**" from the context menu. Note that changes to the image cannot be undone by deleting the operation.

- Edit an operation:

To edit an enhancement function, double-click on the operation or right-click on the operation and select "**Edit**" from the context menu. You will be taken to the edit mode where you can modify the input, output, and key parameters. Press "**Update**" to save changes to the function. Press "**Menu**" to return to the function selection screen.

> 🖊 **NOTE:**
>
> You can edit another function while in the edit mode by clicking on a different operation.

- Apply an operation:

To apply a specific operation, select the operations from the list. Right-click on the selected operations and choose "**Apply**" from the context menu. This function will apply the selected operations.

(6) Configure additional options

You can set additional enhancement procedure options by selecting the "**Configuration**" tab above the operations history panel.

● **Create fixed frame output**

Makes the procedure act as a single frame procedure. When applied to a time-lapse FOV, a single frame procedure will act only on one frame. This will create a "fixed point" channel or mask in the time-lapse FOV. This is a special static type of mask or channel that can be associated with the rest of the time-lapse FOV. This is very useful for image normalization, or for using a single detection mask across the time-lapse FOV.

(7) Create the procedure

When you have finished creating the enhancement procedure, press "**Close**" to return to the wizard screen. The enhancement procedure you have created will be executed and shown in the preview image on the left side.

Click "**Finish**" to complete the Enhancement Wizard and return to the main screen.

3.2.8 Enhancement Function Definitions

This section describes various enhancement functions for enhancement procedures. Each item defines a group of operations as it appears in the Enhancement Module.

3.2.8.1 Image Enhancement

Image enhancement functions provide powerful tools to enhance the input image to optimize detection and analysis. There are eight image enhancement functions and the function definitions are shown below.

- **Enhance Texture**

   Adds texture to the image to better improve foreground-to-background separation.

   EnhanceTexture (image input, bool useDiscKernel, int32 discKernelSize, int32 xKernelSize, int32 yKernelSize, int32 enhanceFactor, single downsamplePct, image output)

- **Flatten BG**

   Normalizes the background intensity of an image to a desired value to correct tilted image or uneven illumination.
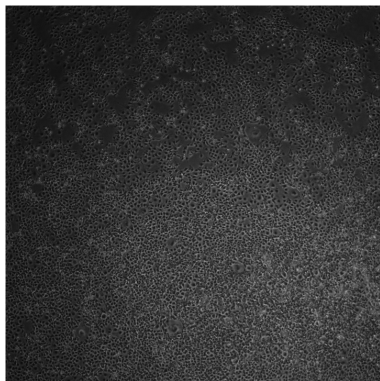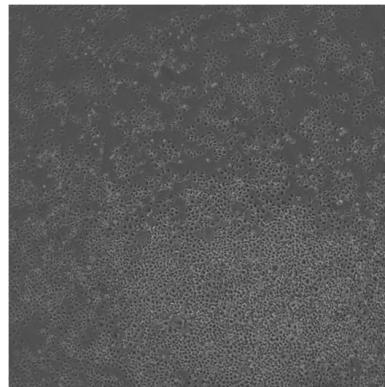
Figure 1

Figure 2

There are three Flatten BG functions to correspond to kernel sizes:

FlattenBG, Large (image input, int32 bgValue, image output)	Flattens image background to the desired bgValue using a kernel size of 255.
FlattenBG, Medium (image input, int32 bgValue, image output)	Flattens image background to the desired bgValue using a kernel size of 65.
FlattenBG, Small (image input, int32 bgValue, image output)	Flattens image background to the desired bgValue using a kernel size of 7.

- **Normalize Background**

  Normalizes the image intensity of an input image to a desired range. This function is particularly useful for image sets with very different brightness.

  NormalizeBackground (image input, int32 desiredRange, int32 ReferencePercentile, int32 ImageHeight, image output)

- **Peak Based Segmentation**

  Takes a distance-transform image and performs iterative guided partition to separate touching mask components.

  PeakBasedSegmentation (image input, image output, int32 StartingLevel, int32 EndingLevel, int32 largestSeed, int32 smallestSeed, int32 step)

- **Remove Background**

  Performs an open residue operation on the original image to remove the background of the image.

  RemoveBackground (image input, int32 kernelSize, image output)

- **Remove Bright Spots**

  Removes spots that are relatively brighter than the kernel surrounding it.

  RemoveBrightSpots (image input, int32 filterSize, image output)

- **Remove Dark Spots**

  Removes spots that are relatively darker than the kernel surrounding it.

  RemoveDarkSpots (image input, int32 filterSize, image output)

- **Remove Texture DS**

  Performs iterative opening followed by closing to remove texture in the image. This function also downsamples the image.

  RemoveTextureDS (image input, int32 filterSize, image output, single percentDS, image output)

3.2.8.2 Mask Refinement

Mask refinement enhancement functions allow the user to refine input detection masks and prepare the image masks for further post-processing and analysis operations. There are six mask refinement functions and their function definitions are listed below.

- **Dilate**

  Takes the maximum value of all pixels in the kernel neighborhood of a pixel in the input image and propagates the pixel maximum value to all the pixels in the kernel neighborhood. The height and width of the kernel is the same.

  Dilate (image input, int32 kernelSize, image output)

- **Erode**

  Takes the minimum value of all pixels in the kernel neighborhood of a pixel in the input image and propagates the pixel minimum value to all the pixels in the kernel neighborhood. The height and width of the kernel is the same.

  Erode (image input, int32 kernelSize, image output)

- **Fill Holes**

  Fills in small, enclosed gaps in the input mask and stores result to output.

  FillHoles (image input, int32 sizeValue, image output)

- **Guided Partition**

  Segments a contiguous binary input mask into discrete objects. Partition follows an input reference seed mask. Partitioned mask is stored to output.

  GuidedPartition (image input, image seed, bool fourConnected, image output)

- **Peak-Based Partition**

  Performs a distance transform operation on the input mask followed by iterative guided partition to separate touching object masks.

  PeakBasedPartition (image input, image output, int32 StartingLevel, int32 EndingLevel, int32 largestSeed, int32 smallestSeed, int32 step)

- **Remove Objects**

  Removes input mask objects that fall below a specified cutoff size.

  RemoveObjects (image input, int32 sizeValue, image output)

3.2.8.3 Advanced

Power users wishing to perform additional image enhancement operations may select enhancement functions from the "**Advanced**" section. All enhancement functions in the "**Image Enhancement**" and "**Mask Refinement**" sections can be found here along with additional arithmetic, logic, morphological, and more post-processing functions.

This section will describe the advanced function in more detail.

(1) 3D operations

3D operations are used to process Z-stack images and generate intensity projection output in 2D.
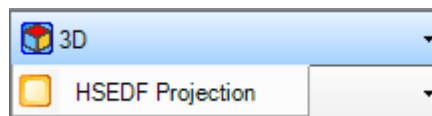
- **HSEDF Projection**

  Takes an input Z-stack and generates a 2D extended depth of focus (EDF) intensity projection image. The EDF projection combines the in-focus pixels of each Z-stack image into one image, ensuring that the projection output is all in focus.

HSEDF Projection(input image, output image)	This uses an extended depth of focus (EDF) algorithm to combine in-focus pixels of all Z-stacks into one projection image.

(2) Arithmetic operations

Arithmetic operations apply basic image arithmetic to manipulate the pixel intensity value of the input image. A list of arithmetic operations is shown below.
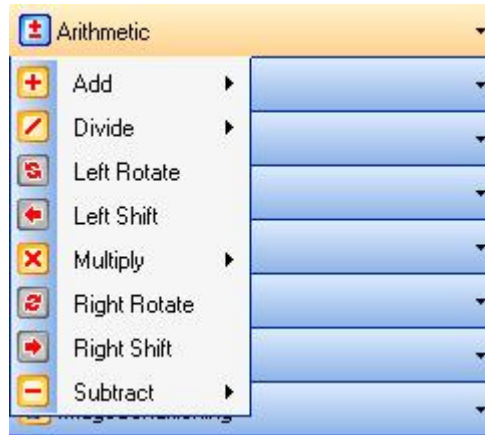
Descriptions of operations and their sub-operations are given below, along with their input parameters.

- **Add**

Combines the intensity of two inputs to generate an output image. Two sub-operations are available:

Add (constant) **(image input, int32 c, image output, arithmeticScheme)**	This function takes an image and an integer as inputs and adds the integer to the pixel intensity value of the input image.
Add (image) **(image input1, image input2, image output, arithmeticScheme)**	This function takes two images for inputs and combines their pixel intensity value and store results to output image.

- **Divide**

Divides the intensity of the first input by the second input to generate an output image. Three sub-operations are available:

Divide (constant) **(image input, int32 c, image output)**	This function divides the pixel intensity value of the input image by the integer constant c and stores result to output image.
Divide (decimal constant) **(image input, single c, image output)**	This function divides the pixel intensity value of the input image by a single precision float constant c and stores result to output image.
Divide (image) **(image input1, image input2, image output)**	This function divides the pixel intensity value of input image 1 by input image 2 and stores result to output image.

- **Rotate**

  Shifts the bits of each pixel in the input image by the number of bits specified by shiftCount. Two functions are available:

LeftRotate (image input, int32 shiftCount, image output)	This function performs a left shift on the bits wrapped around from Most Significant Bit (MSB) to Least Significant Bit (LSB) and stores result to output image.
RightRotate (image input, int32 shiftCount, image output)	This function performs a right shift on the bits wrapped around from LSB to MSB and stores result to output image.

- **Shift**

  Moves the bits of each pixel in the input by the number of bits specified by shiftCount. Two functions are available:

LeftShift (image input, int 32 shiftCount, image output)	This function performs a left shift on the bits with the MSB discarded and results are stored to output image.
RightShift (image input, int32 shiftCount, image output)	This function performs a right shift on the bits with the LSB discarded and results are stored to output image.

- **Multiply**

  Multiplies the intensity of the first input by the second input to generate an output image. Three sub-operations are available:

Multiply (constant) (image input, int32 c, image output, arithmeticScheme)	This function multiples the input image by an integer constant c and stores result to output image.
Multiply (decimal constant) (image input, single c, image output, arithmeticScheme)	This function multiples the input image by a single precision float constant c and stores result to output image.
Multiply (image) (image input1, image input2, image output, arithmeticScheme)	This function multiplies input image 1 by input image 2 and stores result to output image.

- **Subtract**

  Subtracts intensity of the second input from the first input to generate an output image. Two sub-operations are available:

Subtract (constant) (image input, int32 c, image output, arithmeticScheme)	This function subtracts an integer constant c from the input image and stores result to output image.
Subtract (image) (image input1, image input2, image output, arithmeticScheme)	This function subtracts input image 2 from input image 1 and stores result to output image.

(3) Bitwise operations

Bitwise operations perform pixel-wise boolean function to the image and generates binary output image corresponding to the true/false state of each pixel. The list of bitwise operations appears below.

Descriptions of operations and their sub-operations are given below, along with their input parameters.

• **Pixel And**

Performs boolean "and" operation between the two inputs and stores result to output image. Two sub-operations are available:

PixelAnd (image) **(image input1, image input2, image output)**	This function performs boolean "and operation" between each similarly-positioned pixels in inputs 1 and 2.
PixelAnd (constant) **(image input1, int32 c, image output)**	This function performs boolean "and" operation between each pixel in image input and integer constant c.

• **Pixel Not**

Performs a boolean "not" operation between each pixel in the input image. This function inverts the image input and stores result to output image. Function input parameters are described below.

PixelNot (image input, image output)

• **Pixel Or**

Performs a boolean "or" operation between the two inputs and stores result to output image. Two sub-operations are available:

PixelOr (image) **(image input1, image input2, image output)**	This function performs a boolean "or" operation between each similarly-positioned pixel in inputs 1 and 2.
PixelOr (constant) **(image input, int32 c, image output)**	This function performs a boolean "or" operation between each pixel in image input and integer constant c.

● **Pixel Xor**

Performs a boolean "exclusive or" ("xor") operation between two inputs and stores result to output image. Two sub-operations are available:

PixelXor (image) **(image input1, image input2,** **image output)**	This function performs a boolean "xor" operation between each similarly-positioned pixel in inputs 1 and 2.
PixelXor (constant) **(image input, int32 c, image** **output)**	This function performs a boolean "xor" operation between each pixel in image input and integer constant c.

(4) Comparison operations

Comparison operations perform pixel-wise comparison between two inputs to generate output result corresponding to the comparison metric. A list of comparison operations is shown below.

Descriptions of operations and their sub-operations are given below, along with their input parameters.

● **Between**

Checks each pixel in input and returns true (255) if pixel intensity is between the upper and lower value and returns false (0) if outside the lower and upper value limits. Input parameters of the between function is shown below.

Between (image input, int32 lower, int32 upper, image output)

● **Equals**

Checks each pixel in the first input and returns true of the pixel intensity is equal to the value of the second input. If equal, the function returns true (255) for that particular pixel and false (0) if not equal. Two sub-operations are available:

Equals (image) **(image input1, image input2, image output)**	This function checks the pixel intensity of each equivalent pixel in input images 1 and 2 and returns true if the equivalent pixel intensities are equal.
Equals (constant) **(image input, int32 c, image output)**	This function checks the pixel intensity of each pixel in the image input against an integer constant c and returns true if the pixel intensity equals the value of c.

- **Greater Than**

  Checks each pixel in the first input and returns true if the pixel intensity is greater than the value of the second input. If greater than, the function returns true for that particular pixel and false if value is less than or equal to the first input. Two sub-operations are available:

GreaterThan (image) **(image input1, image input2, image output)**	This function compares the pixel intensity of each pixel in input image 1 against the intensity of each equivalent pixel in image 2 and returns true if the equivalent pixel intensity of input 2 is less than or equal to the pixel intensity of input 1.
GreaterThan (constant) **(image input, int32 c, image output)**	This function compares the pixel intensity of each pixel in the input image against an integer constant c and returns true if c is less than or equal to the pixel intensity.

- **Greater Than or Equals**

  Checks each pixel in the first input and returns true if the pixel intensity is greater than or equal to the value of the second input. If greater than or equal to, the function returns true for that particular pixel and false if value is less than the first input. Two sub-operations are available:

GreaterThanOrEquals (image) **(image input1, image input2, image output)**	This function compares the pixel intensity of each pixel in input image 1 against the intensity of each equivalent pixel in image 2 and returns true if the equivalent pixel intensity of input 2 is less than the pixel intensity of input 1.
GreaterThanOrEquals (constant) **(image input, int32 c, image output)**	This function compares the pixel intensity of each pixel in the input image against an integer constant c and returns true if c is less than the pixel intensity.

- **Less Than**

  Checks each pixel in the first input and returns true if the pixel intensity is less than the value of the second input. If less than, the function returns true for that particular pixel and false if value is more than or equal to the first input. Two sub-operations are available:

LessThan (image) **(image input1, image input2, image output)**	This function c compares the pixel intensity of each pixel in input image 1 against the intensity of each equivalent pixel in image 2 and returns true if the equivalent pixel intensity of input 2 is greater than or equal to the pixel intensity of input 1.
LessThan (constant) **(image input, int32 c, image output)**	This function compares the pixel intensity of each pixel in the input image against an integer constant c and returns true if c is greater than or equal to the pixel intensity.

● **Less Than or Equals**

Checks each pixel in the first input and returns true if the pixel intensity is less than or equal to the value of the second input. If less than or equal to, the function returns true for that particular pixel and false if value is greater than the first input. Two sub-operations are available:

LessThanOrEquals (image) **(image input1, image input2,** **image output)**	This function c compares the pixel intensity of each pixel in input image 1 against the intensity of each equivalent pixel in image 2 and returns true if the equivalent pixel intensity of input 2 is greater than the pixel intensity of input 1.
LessThanOrEquals (constant) **(image input, int32 c, image** **output)**	This function compares the pixel intensity of each pixel in the input image against an integer constant c and returns true if c is greater than the pixel intensity.

● **Lower Threshold**

Checks each pixel in the input image and sets pixel intensity to an integer constant c if the intensity is below the constant c. Pixel intensity higher than c will not be affected by this function.

LowerThreshold (image input, int32 c, image output)

● **Maximum**

Compares the pixel intensity of the two inputs and stores the higher intensity value of the two to output. Two sub-operations are available:

Maximum (image) **(image input1, image input2,** **image output)**	This function compares the pixel intensity of the equivalent pixels of input images 1 and 2 and stores the higher pixel intensity of the two to output.
Maximum (constant) **(image input, int32c, image** **output)**	This function compares the pixel intensity of the input image and integer constant c and stores the higher value of the two to output.

● **Minimum**

Compares the pixel intensity of the two inputs and stores the lower intensity value of the two to output. Two sub-operations are available:

Minimum (image) **(image input1, image input2,** **image output)**	This function compares the pixel intensity of the equivalent pixels of input images 1 and 2 and stores the lower pixel intensity of the two to output.
Minimum (constant) **(image input, int32c, image** **output)**	This function compares the pixel intensity of the input image and integer constant c and stores the lower value of the two to output.

- **Not Equals**

  Checks each pixel in the first input and returns true of the pixel intensity is not equal to the value of the second input. If not equal, the function returns true (255) for that particular pixel and false (0) if equal. Two sub-operations are available:

NotEquals (image) **(image input1, image input2,** **image output)**	This function checks the pixel intensity of each equivalent pixel in input images 1 and 2 and returns true if the equivalent pixel intensities are not equal.
NotEquals (constant) **(image input, int32 c, image** **output)**	This function checks the pixel intensity of each pixel in the image input against an integer constant c and returns true if the pixel intensity do not equal the value of c.

- **Upper Threshold**

  Checks each pixel in the input image and sets pixel intensity to an integer constant c if the intensity is greater than the constant c. Pixel intensity lower than c will not be affected by this function.

  UpperThreshold (image input, int32 c, image output)

(5) Distance Transform operations

<u>Overview</u>

Distance transform functions output a topographical map of an input mask, and encode the pixel distance to the mask boundary.

Given an image I, the distance transform assigns each foreground pixel (inside the mask) the shortest distance between the pixel and a background pixel (outside the mask).

The distance transform can be computed sequentially by a two-pass procedure. The first (forward) pass scans in a left-right top-bottom raster scan order. The second (backward) pass scans in a reverse right-left bottom-top order. In the forward pass for a foreground distance transform, the output U(x,y) at pixel position (x,y) is determined by the value of input image I and the values at previously computed positions of U by

$$U(x, y) = \begin{cases} \min_{(i,j) \in N^B(x,y)} \{U(i, j) + l(i, j)\}; \forall I(x, y) \in foreground \\ \\ 0; \forall I(x, y) \in background \end{cases}$$

In the unbounded case, we handle the boundary condition by setting U(x,-1) = 0 and U(-1,y) = 0. Otherwise, we handle the boundary condition by setting U(x,-1) = 1 and U(-1,y) = 1.

$N^B(x,y)$ is the backward neighbors of the pixel (x,y). The backward neighbor contains a selected set of adjacent neighbors that are already scanned in the forward pass. $l(i,j)$ is the neighbor position dependent distance length. When $l(i,j)$ = 1 for all (i, j) and $N^B(x,y)$ is the upper half of the 4-connected neighbor, that is, $N^B(x,y)$ = {(x-1,y), (x,y-1)}, the resulting distance will be a 4-connected city block distance (L_1). When $l(i,j)$ = 1 for all (i, j) and $N^B(x,y)$ is the upper half of the 8-connected neighbor, that is, $N^B(x,y)$ = {(x-1,y), (x-1,y-1), (x,y-1), (x+1,y-1)}, the resulting distance will be an 8-connected chessboard distance (L_∞). For the 8-connected neighbor, when $l(x-1,y)$=1, $l(x-1,y-1)$= $\sqrt{2}$, $l(x,y-1)$=1, and $l(x+1,y-1)$= $\sqrt{2}$, the resulting distance will be the Euclidean distance (L_2).

The backward pass produces the distance transform image D by

$$D(x, y) = \min\{ \min_{(i,j) \in N^F(x,y)} (D(i, j) + l(i, j)), U(x, y)\}$$

$N^F(x,y)$ is the forward neighbors of the pixel (x,y). The forward neighbor contains a selected set of adjacent neighbors that are already scanned in the backward pass. For 4-connected city block distance, $N^F(x,y)$ is the lower half of the 4-connected neighbor. That is, $N^F(x,y)$ = {(x+1,y), (x,y+1)}. For 8-connected chessboard distance and Euclidean distance, $N^F(x,y)$ is the lower half of the 8-connected neighbor. That is, $N^F(x,y)$ = {(x+1,y), (x+1,y+1), (x,y+1), (x-1,y+1)}.

The images in Figure 1, illustrates the three distance operators applied to a Euclidean disc. Figure 1(a) shows the Euclidean disc; Figure 1(b) shows the Euclidean distance transformed image of (a); Figure 1(c) shows the 4-connected (cityblock) distance transformed image of (a); Figure 1(d) shows the 8-connected (chessboard) distance transformed image of (a).

(a)　　　　　　　(b)　　　　　　　(c)　　　　　　　(d)
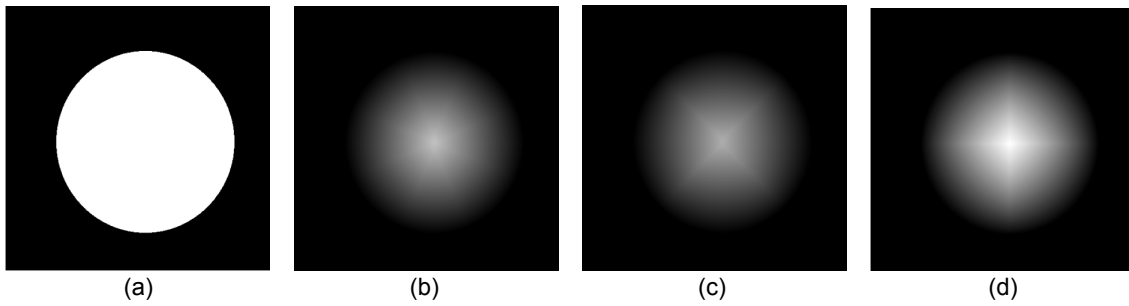
Figure 1

The images in Figure 2, illustrates bounded and unbounded effect. Figure 2(a) shows a Euclidean disc hole; Figure 2(b) shows the Euclidean distance transformed image of (a) when the distance is bounded by the image boundary; Figure 2(c) shows the Euclidean distance transformed image of (a) when the distance is unbounded by the image boundary.
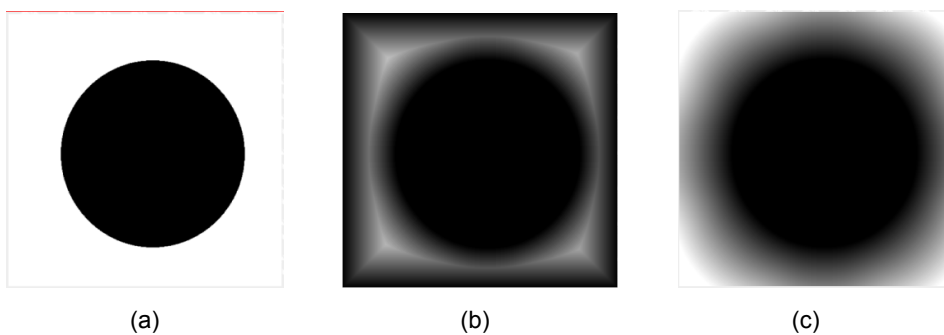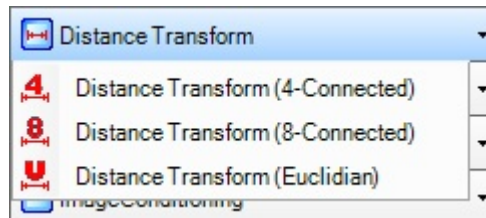

(a)　　　　　　　(b)　　　　　　　(c)

Figure 2

Usage

In CL-Quant, the three distance transform operations - 4-connected, 8-connected, and Euclidean - that defines how the pixel distances are measured. The list of distance transform operations are shown below.

DistanceTransform (4-connected) (image input, image output, boolean unbounded, outputDepth)	This function outputs a topographical map of an input mask. Pixel distance from the mask boundary is measured from four adjacent pixels in the cardinal directions.
DistanceTransform (8-connected) (image input, image output, boolean unbounded, outputDepth)	This function outputs a topographical map of an input mask. Pixel distance from the mask boundary is measured from eight adjacent pixels in the cardinal and ordinal directions.
DistanceTransform (Euclidean) (image input, image output, boolean unbounded, outputDepth)	This function outputs a topographical map of an input mask. Pixel distance from the mask boundary is calculated as the Euclidean distance from the boundary, or the square root of the sum of the squares of the x- and y-distances.

NOTE:

All distance transform functions now have the option of 8-bit and 16-bit output depth in the outputDepth dropdown menu. It is highly recommended that 8-bit depth used for object radius less than 255. For very large object with radius exceeding 255-px, use of 8-bit output will truncate the object intensity near the center.

Application

We will describe two typical applications of distance transform (A) Medial Axis; (B) Seeds for partition.

(A) Medial Axis

Medial axis is initially introduced for shape description. The following hypothetical scenario can help visualize a medial axis. Let the shape be a region of grass surrounded by bare soil. Simultaneously at all boundary points of the grass region light a fire that burns uniformly from the border of the grass region to its interior. Eventually the fire burning from one part of the border will meet the fire burning from another part of the border. Since all the grass will have been burned at the meeting place, the fire will quench and be extinguished. The locus of points on the arc of fire extinction is the medial axis of the region. Another way to think about the medial axis is as the loci of centers of bi-tangent circles that fit entirely within the foreground region being considered. Figure 3 illustrates this for a rectangular shape.
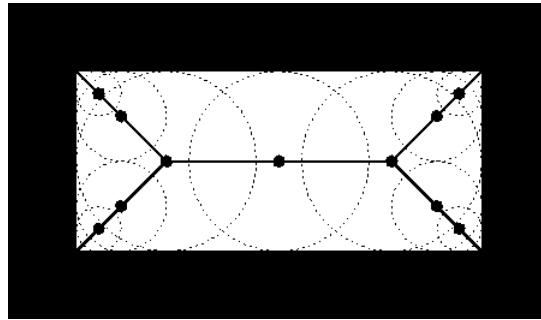
Figure 3

Each point on the medial axis has an associated value that is proportional to the time it took for the fire to reach the given point from the time the grass fire was set. The medial axis with its medial axis distance function is called the medial axis transform. It is an information preserving representation of shape. To see this, just consider running the grass fire backward. With time running in reverse, set a grass fire on each point of the medial axis exactly at the time the original grass fire is extinguished at that point. The boundary of the fire at time t=0 would be the boundary of the original given shape.

The medial axis can be produced by first calculating the distance transform of the image. The medial axis then lies along the singularities (i.e. creases or curvature discontinuities) in the distance transform. The medial axis is often described as being the `locus of local maxima' on the distance transform. If the distance transform is displayed as a 3-D surface plot with the third dimension representing the grayscale value, the medial axis can be imagined as the ridges on the 3-D surface. Figure 4 shows a rectangular region (a) and its Euclidean distance transformed image (b). Figure 4 (c) shows the overlay (in red) of the peak detection on the distance image. The peak is detected by morphological opening residue as follows:

$I_{peak} = I_{distance} - Open (I_{distance}, 7 \times 7)$

$M_{overlay} = Greater Than (I_{peak}, 0)$

(a)   (b)   (c)
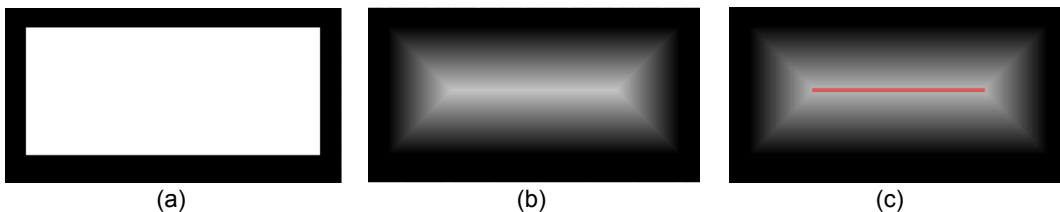
Figure 4

(B) Seeds for partition

To separate overlap objects, partition method is often used (see ZOI section for details). The partition operation divide a mask based on the seeds. It is important to create good seed regions to prepare a mask for partition. For this purpose, distance transform can be applied to the mask and the peak (maximum regions) in the distance image could be used as seed regions. Figure 5 (a) shows a mask containing two overlapped circular regions. Figure 5 (b) shows its Euclidean distance transformed image. Figure 5 (C) shows the overlaid seed regions in red. They are created by

$I_{seed} = Greater Than (I_{distance}, 81)$

(a)   (b)   (c)

Figure 5

(6) Filtering operations

Filtering operations apply image filters to reduce image noise on the input image. The list of filtering operations is shown below.

Descriptions of operations and their sub-operations are given below, along with their input parameters.

● Average Filter

Averages the pixel intensity of each pixel in the input image with its neighboring pixels within the filter window defined by its kernel size. Two sub-operations are available:

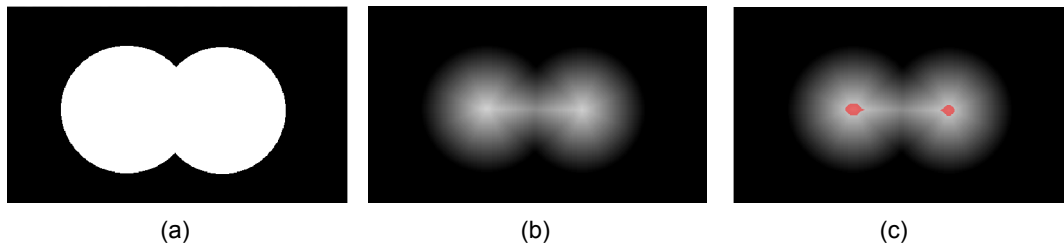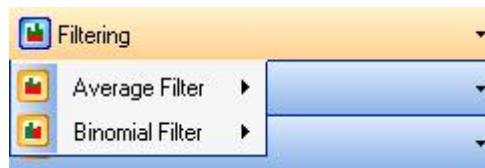|	
AverageFilter1 (image input, int32 kernelSize, image output)	This function averages the pixel intensity of each pixel in the input image with its neighboring pixels within the filter window. Both dimensions of the filter window are the same size and defined by one integer constant.
AverageFilter2 (image input, int32 kernelWidth, int32 kernelHeight, image output)	This function averages the pixel intensity of each pixel in the input image with its neighboring pixels within the filter window. The dimensions of the filter window are determined by two integer constants.

● Binomial Filter

Performs a binomial weighted average of the pixel intensity of each pixel in the input image with its neighboring pixels within the filter window defined by its kernel size. Two sub-operations are available:

|	
BinomialFilter1 (image input, int32 kernelSize, image output)	This function performs a binomial weighted average of the pixel intensity of each pixel in the input image with its neighboring pixels within the filter window. Both dimensions of the filter window are the same size and defined by one integer constant.
BinomialFilter2 (image input, int32 kernelWidth, int32 kernelHeight, image output)	This function performs a binomial weighted average of the pixel intensity of each pixel in the input image with its neighboring pixels within the filter window. The dimensions of the filter window are determined by two integer constants.

(7) Image Conditioning operations

Image conditioning operations are enhancement operations that re-condition the image appearance to correct or improve specific image attributes. The list of image conditioning operations is shown below.
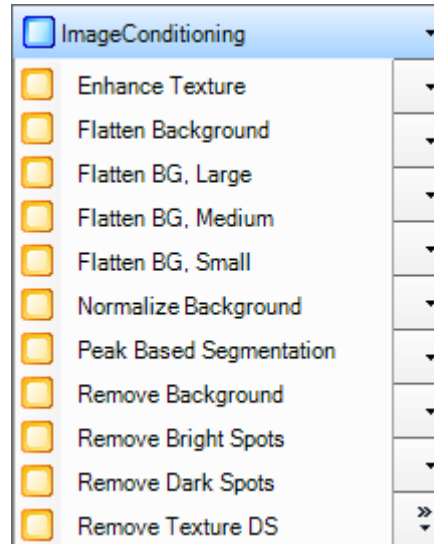
Descriptions of operations and their sub-operations are given below, along with their input parameters.

- **Enhance Texture**

    Adds texture to the image to better improve foreground-to-background separation.

    EnhanceTexture (image input, bool useDiscKernel, int32 discKernelSize, int32 xKernelSize, int32 yKernelSize, int32 enhanceFactor, single downsamplePct, image output)

- **Flatten BG**

    Normalizes the background intensity of an image to a desired value to correct tilted image or uneven illumination.
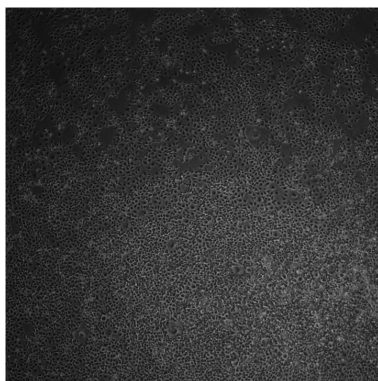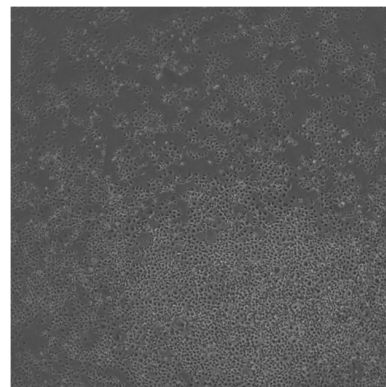

| Figure 1 | Figure 2 |

Three Flatten BG functions are available to correspond to the kernel size used:

FlattenBG, Large (image input, int32 bgValue, image output) — This function flattens image background to the desired bgValue using a kernel size of 255.

FlattenBG, Medium (image input, int32 bgValue, image output) — This function flattens image background to the desired bgValue using a kernel size of 65.

FlattenBG, Small (image input, int32 bgValue, image output) — This function flattens image background to the desired bgValue using a kernel size of 7.

- **Normalize Background**

  Normalizes the image intensity of an input image to a desired range. This function is particularly useful for image sets with very different brightness.

  NormalizeBackground (image input, int32 desiredRange, int32 ReferencePercentile, int32 ImageHeight, image output)

- **Peak Based Segmentation**

  Takes a distance-transform image and performs iterative guided partition to separate touching mask components.

  PeakBasedSegmentation (image input, image output, int32 StartingLevel, int32 EndingLevel, int32 largestSeed, int 32 smallestSeed, int32 step)

- **Remove Background**

  Performs an open residue operation on the original image to remove the background of the image.

  RemoveBackground (image input, int32 kernelSize, image output)

- **Remove Bright Spots**

  Removes spots that are relatively brighter than the kernel surrounding it.

  RemoveBrightSpots (image input, int32 filterSize, image output)

- **Remove Dark Spots**

  Removes spots that are relatively darker than the kernel surrounding it.

  RemoveDarkSpots (image input, int32 filterSize, image output)

- **Remove Texture DS**

  Performs iterative opening followed by closing to remove texture in the image. This function also downsamples the image.

  RemoveTextureDS (image input, int32 filterSize, image output, single percentDS, image output)

(8) Image operations

Image operations are applied to the entire field of view (FOV) of the image and modify its image property. A list of image operations is shown below.
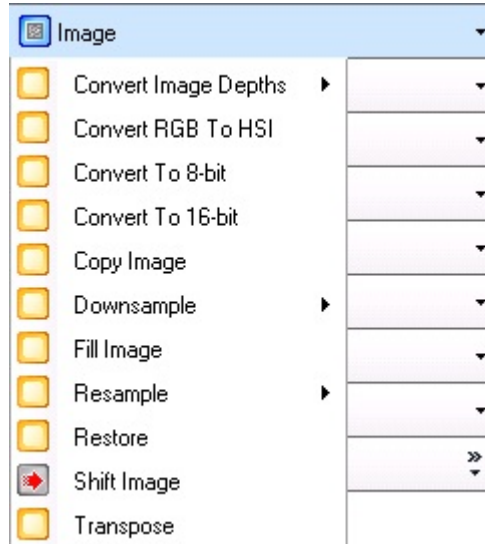
Descriptions of operations and their sub-operations are given below, along with their input parameters.

- **Convert Image Depths**

    Converts the pixel depth of an image and mapping pixel intensity of the input image to new values in the output. Two sub-operations are available:

ConvertImageDepths1 (image input, mappingType, image output)	This function converts pixel depth of an image using one of five mapping options.
ConvertImageDepths2 (image input, int32 sourceMin, int32 destMin, int32 sourceMax, int32 destMax, image output)	This function converts pixel depths of an image and maps the source minimum and maximum pixels values to specified destination min and max values.

- **Convert RGB To HSI**

    Converts an input image in the RGB (red-green-blue) color domain to the HSI (hue-saturation-intensity) domain and stores to output.

    ConvertRGBtoHSI (image input red, image input green, image input blue, image output hue, image output saturation, image output intensity)

- **Convert To 8-bit**

    Converts an input image with 16-bit pixel depth to an 8-bit image. Source upper and lower intensity percentile in the 16-bit domain is mapped to their corresponding 8-bit values.

    ConvertTo8-bit (image input, single lowerPercentile, single upperPercentile, single lowerScale, single upperScale)

- **Convert To 16-bit**

  Converts an input image with 8-bit pixel to a 16-bit image, allowing pixel intensity values up to 65535.

  ConvertTo16-bit (image input, image output)

- **Copy Image**

  Creates a duplicate copy of the input image and stores to output.

  CopyImage (image input, image output)

- **Downsample**

  Takes an input image and downsamples it to a new, reduced size in the output image. Four downsample sub-operations are available:

DownsampleBySize (image input, int32 newWidth, int32 newHeight, bool saveOriginal, bool applyToAll)	This function downsamples the input image to new width and height, measured in pixels as shown in Figure 1.
DownsampleByPercent (using average filter)(image input, single percentFactor, image output)	This function downsamples the input image to new width and height, measured in percentage with respect to its original resolution.
DownsampleByPercent (image input, single percentWidth, single percentHeight, image output)	This function downsamples the input image to new width and height, measured in percentage with respect to its original width and height as shown in Figure 2.
DownsampleByPercent (in-place) (image input, single percentWidth, single percentHeight, bool saveOriginal, bool applyToAll)	This function downsamples the input image to new width and height, measured in percentage with respect to its original width and height, and stores output to the input image.

- **Fill Image**

  Creates a uniform output image based on a specified statistic of pixel intensity of the input image.

  FillImage (image input, statistic, bool ignoreZero, image output)

- **Resample**

  Takes an input image and resamples it to a new size in the output image. This function can be used both for up- and downsampling the input image depending on input parameters. Two sub-operations are available:

Resample1 (image input, image output)	This function resamples an input image or mask and save result to an output mask.
Resample2 (image input, int32 newWidth, int32 newHeight, output image)	This function resamples the input image to a different dimension and stores result to output.

• **Restore**

Takes an image that has been downsampled and restores the original FOV. FOV masks, object ROIs and measurements will be upsampled. The active FOV will be selected as its input as shown in Figure 3.

Restore (image input, bool applyToAll)

📍 **NOTE:**

The restore function recipe must be created at the same time as the downsample recipe, otherwise there will be no image available for restoration. These can be saved to one or two recipes.

• **Shift Image**

Translates the image from original to new position specified by x- and y-shifts and stores result to output. Image is not wrapped around in translation and blank pixels from the movement will be set to specified uniform intensity value.

ShiftImage (image input, image output, int32 xShift, int32 yShift, int32 padValue)

• **Transpose**

Transposes the input image and stores result to output. When the function is applied, rows of the input image will become columns of the output and the columns from input will become the rows of the output.

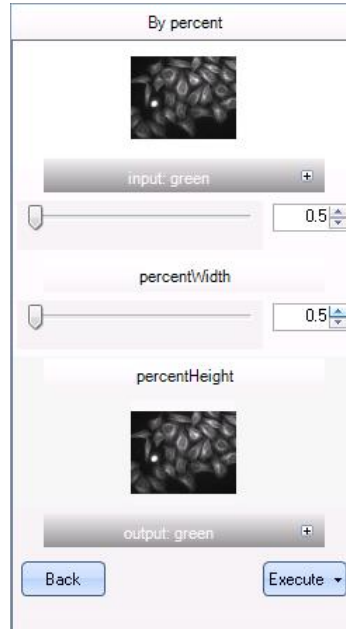**Transpose (image input, image output)**

Figure 1

Figure 2

Figure 3

(9) Labeling Operations

Labeling operations takes a binary mask as input and generates a grayscale mask output with pixel intensity value corresponding to its label value. The list of labeling operations is shown below.

- **Create Field Centroid Mask**

  Generates a binary output image of one pixel at the center of mass of the entire image.

  CreateFieldCentroidMask (image input, image output, int32 pixelVal)

- **Create Object Centroid Mask**

  Generates a binary output mask of one pixel at the center of mass of each binary mask component.

  CreateObjectCentroidMask (image input, image output, int32 pixelVal)

- **Group Objects**

  Assigns labeled values to each object on the input reference mask based on the zone of influence.

  GroupObjects (image referenceMask, image objectMask, bool useZoi, image output)

- **Labeling (4-Connected)**

  Assigns unique labeled values for each binary object in the input image and stores the assigned labels to output. Pixels are considered to be connected and of the same object if it is adjacent to a non-zero pixel in the four cardinal directions. Three sub-operations are available:

Labeling (image input, image labeling, bool autoSetOutputDepth)	This function assigns unique label to each object.
Labeling : Morphological filter (image input, image labeling, int32 maxObjects, int32 minSize, int32 maxSize, single minCompactness, single maxCompactness, bool autoSetOutputDepth)	This function assigns unique label to each object that falls within particular size and compactness criteria.

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| **Labeling : Advanced filter (image mask, image background, image labeling, int32 maxObjects, int32 minSize, int32 maxSize, single minMean, single maxMean, single minStdev, single maxStdev, int32 minPixelSum, int32 maxPixelSum, bool autoSetOutputDepth)** | This function assigns unique label to each object that falls within particular size and image intensity criteria. |

- **Labeling (8-Connected)**

  Assigns unique labeled values for each binary object in the input image and stores the assigned labels to output. Pixels are considered to be connected and of the same object if it is adjacent to a non-zero pixel in the four cardinal directions and the four ordinal directions. Three sub-operations are available:

Labeling (image input, image labeling, bool autoSetOutputDepth)	This function assigns unique label to each object.
Labeling : Morphological filter (image input, image labeling, int32 maxObjects, int32 minSize, int32 maxSize, single minCompactness, single maxCompactness, bool autoSetOutputDepth)	This function assigns unique label to each object that falls within particular size and compactness criteria.
Labeling : Advanced filter (image mask, image background, image labeling, int32 maxObjects, int32 minSize, int32 maxSize, single minMean, single maxMean, single minStdev, single maxStdev, int32 minPixelSum, int32 maxPixelSum, bool autoSetOutputDepth)	This function assigns unique label to each object that falls within particular size and image intensity criteria.

- **Propagate ZOI Labels**

- **Remove NonOverlapped Objects**

  Discards objects from input 1 that do not overlap with the reference input 2 and stores result to output.

  RemoveNonOverlappedObjects (image input1, image input2, image output)

- **Remove Overlapped Objects**

  Discards objects from input 1 that overlap with the reference input 2 and stores result to output.

  RemoveOverlappedObjects (image input1, image input2, image output)

(10) Logical Operations

Logical operations are pixel-wise operations that generate a binary output image corresponding to the logic function applied. The list of logical operations is shown below.

- **Logical And**

Performs pixel-wise logical "and" operation between the first and second input and returns a binary output of non-zero pixels on both inputs. Two logical and sub-operations are available:

LogicalAnd (image) (image input1, image input2, image output)	This function performs a pixel-wise logical and operation that returns 255 if both input1 and input2 are nonzero.
LogicalAnd (constant) (image input, int32 c, image output)	This function performs a pixel-wise logical and operation that returns 255 if both input and integer constant c are nonzero.

- **Logical Not**

Performs pixel-wise logical "not" operation on the input image and stores binary result to output. This function will return a value of 255 for all zero pixels and 0 for all non-zero pixels.

LogicalNot (image input, image output)

- **Logical Or**

Performs pixel-wise logical "or" operation between the first and second input and returns a binary output of non-zero pixels of either inputs. Two logical or sub-operations are available:

LogicalOr (image) (image input1, image input2, image output)	This function performs a pixel-wise logical or operation that returns 255 if either input1 or input2 is nonzero.
LogicalOr (constant) (image input, int32 c, image output)	This function performs a pixel-wise logical or operation that returns 255 if either the input image or the integer constant c is nonzero.

(11) Morphological Operations

<u>Overview</u>

Morphological operations process images based on shapes. It applies a structuring element to the input image to modify the neighborhood surrounding the pixel of interest. By choosing the size and shape of the structuring element, one can construct a morphological operation that is sensitive to specific shapes in the input image.

The basic morphological operations are dilation and erosion. Binary erosion removes pixels on object boundaries while binary dilation adds pixels to the boundaries of object masks. The number of pixels added or removed from the object masks depends on the size and shape of the structuring element.

- Binary Erosion

Let E be a Euclidean space or an integer grid, and B a binary image in E. The erosion of B by the structuring element S is defined by:

$$B \ominus S = \{z \in E \mid S_z \subseteq B\}$$

where Sz is the translation of S by the vector z, i.e., $Sz = \{ s + z \mid s \in S\}, \ \forall z \in E$

The erosion of B by S can also be described by the expression:

$$B \ominus S = \bigcap_{s \in S} B_{-s}$$

- Binary Dilation

The dilation of B by the structuring element S can be similarly defined by:

$$B \oplus S = \bigcup_{s \in S} B_s$$

Figure 1(a) shows a rectangular shape. The erosion of the rectangular shape by a disk structuring element (illustrated in Apricot), resulting in the Medium purple smaller rectangular region in (b). The dilation of the rectangular shape in (a) by the same disk, resulting in the addition of cyan region with rounded corners in (c).
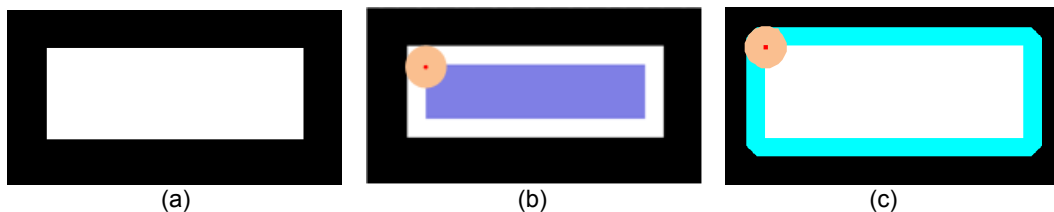

(a)   (b)   (c)

Figure 1

- Binary Opening
The opening of B by S is obtained by the erosion of B by S, followed by dilation of the resulting image by S:

$$B \circ S = (B \ominus S) \oplus S$$

The opening is also given by,

$$B \circ S = \bigcup_{S_i \subseteq B} S_i$$

which means that it is the locus of translations of the structuring element S inside the image B. In the case of the square of side 20, and a disc of radius 4 as the structuring element, the opening is a square of side 20 with rounded corners, where the corner radius is 4.

- Binary Closing
The closing of B by S is obtained by the dilation of B by S, followed by erosion of the resulting image by S:

$$B \bullet S = (B \oplus S) \ominus S$$

The closing can also be obtained by

$$B \bullet S = (B^c \circ S^s)^c$$

where P^c denotes the complement of P relative to E. That is

$$P^c = \{p \in E \mid p \notin P\}$$ and S^s is the symmetric of S.

The above means that the closing is the complement of the locus of translations of the symmetric of the structuring element outside the image B.

Opening together with closing are the basic workhorse of morphological noise removal. Opening removes small objects, while closing removes small holes.

Figure 2(a) shows the opening of the rectangular shape in Figure 1(a) by a disk structuring element (illustrated in Apricot), resulting in the yellow rectangular region with rounded corners. Figure 2(b) shows a region of two overlapped circles. The closing of the shape (b) by the same disk, resulting in the addition of pink regions that fill the shape corners in (c).
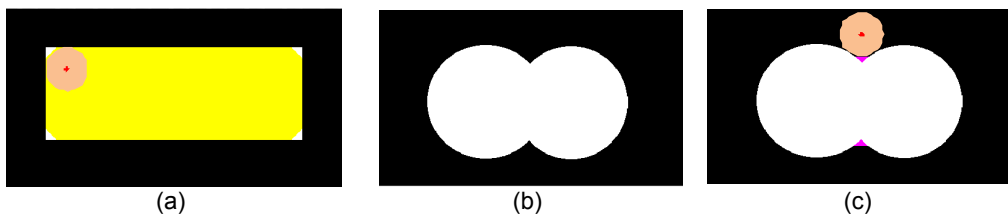

| (a) | (b) | (c) |

Figure 2

- Grayscale morphological operations

In grayscale morphology, images are functions mapping a Euclidean space or grid E into a functional space of grayscale intensity. Grayscale structuring elements are also functions of the same format, called "structuring functions".

Denoting a one-dimensional image by *I(x)* and the structuring function by S(*x*), the grayscale dilation of I by S is given by

$$(I \oplus S)(x) = \max_{z \in S}\{I(x-z)+S(x)\}$$

Similarly, the erosion of *I* by S is given by

$$(I \ominus S)(x) = \min_{z \in S}\{I(x+z)-S(x)\}$$

Figure 3 illustrates a one dimensional profile in (a) that is processed by a structuring element in (b). The dilation of the profile by the structuring element resulting in the red profile in Figure 3(c). The erosion results in the blue profile in Figure 3(d). The closing of the profile by the same structuring element resulting in the yellow profile in Figure 3(e) where the pit is filled. The opening results in the purple profile in Figure 3(f) where the peaks are removed.

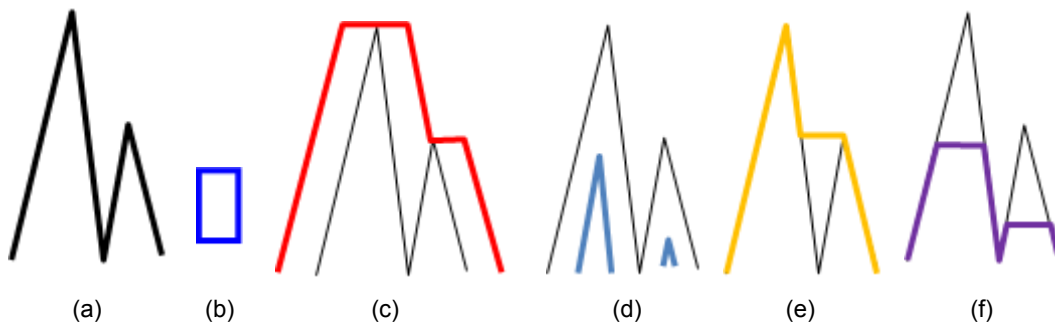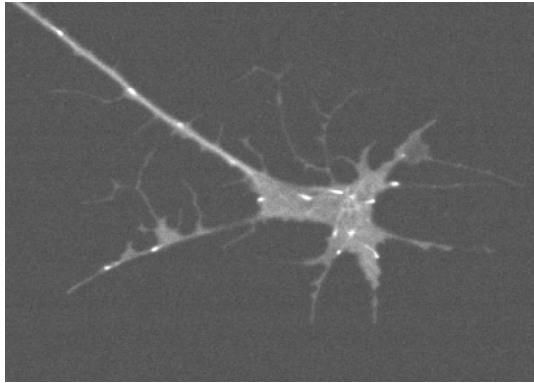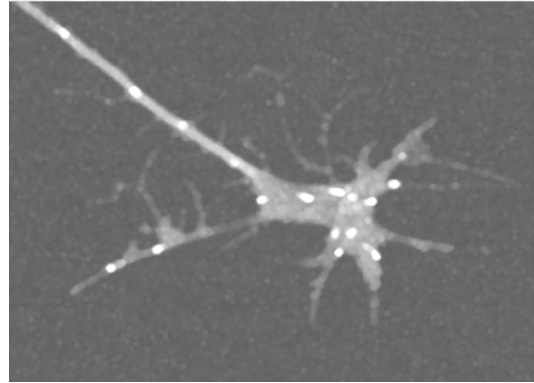(a)   (b)   (c)   (d)   (e)   (f)

Figure 3

The operations can be directly extended to 2-D images. Figure 4(a) shows a neuron growth cone image with bright microtubule plus tips. The dilation of the image by a rod structuring element of radius 7 resulting in the image in Figure 4(b) where bright spots are expanded. The erosion of the image by the same structuring element resulting in the image in Figure 4(c) where bright spots are much reduced. the opening of the image by the same structuring element resulting in the image in Figure 4(d) where the bright spots are dimmed. the closing of the image by the same structuring element resulting in the image in Figure 4(e) where the cellular background is brighter since darker textures are filled in.

(a)

(b)
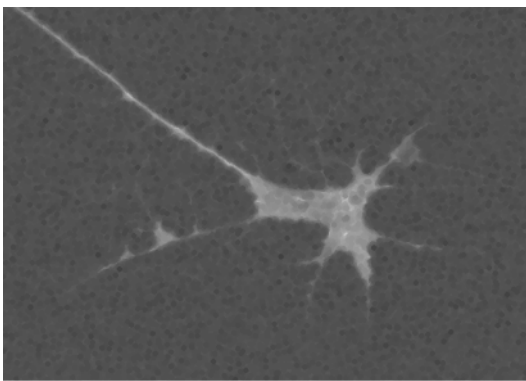
(c)
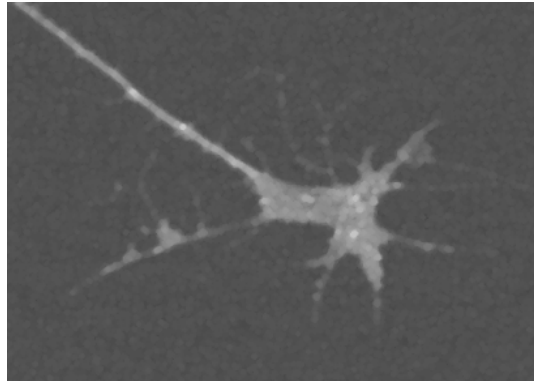
(d)
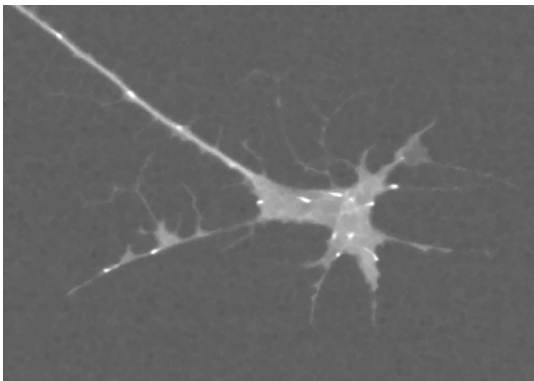
(e)

Figure 4

Usage

The list of morphological operations appears below. The variations are mostly related to different configurations of structuring elements.
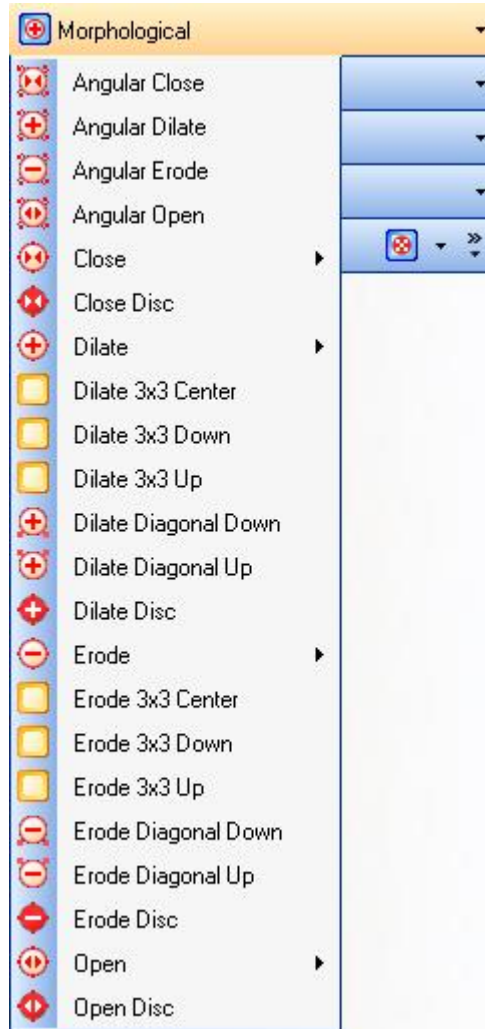
Descriptions of operations and their sub-operations are given below, along with their input parameters.

- **Angular**

  Performs specified operation on the input image with structuring kernel specified by the angle in radians and stores result to output image. Four functions are available:

AngularClose (image input, int32 kernelWidth, int32 kernelHeight, single angleInRadians, image output)	This function closes the input image with structuring kernel at the angle specified.
AngularDilate (image input, int32 kernelWidth, int32 kernelHeight, single angleInRadians, image output)	This function dilates the input image with structuring kernel at the angle specified.

AngularErode **(image input, int32 kernelWidth, int32 kernelHeight, single angleInRadians, image output)**	This function erodes the input image with structuring kernel at the angle specified.

AngularOpen **(image input, int32 kernelWidth, int32 kernelHeight, single angleInRadians, image output)**	This function opens the input image with structuring kernel at the angle specified.

- **Close**

  Performs a dilation operation followed by an erode operation using the same structuring element. Two sub-operations are available:

Close1 **(image input, int32 kernelSize, image output)**	This function performs a closing operation using a structuring element of the same width and height.

Close2 **(image input, int32 kernelWidth, int32kernelHeight, image output)**	This function performs a closing operation using a structuring element of different height and width.

- **Close Disc**

  Performs a closing operation using a disc-shaped structuring element instead of a square kernel.

  CloseDisc (image input, int32 kernelSize, image output)

- **Dilate**

  Takes the maximum value of all pixels in the kernel neighborhood of a pixel in the input image and propagates the pixel maximum value to all the pixels in the kernel neighborhood. Two sub-operations are available:

Dilate1 **(image input, int32 kernelSize, image output)**	This function performs a dilation operation using a structuring element of the same width and height.

Dilate2 **(image input, int32 kernelWidth, int32kernelHeight, image output)**	This function performs a dilation operation using a structuring element of different height and width.

- **Dilate 3x3**

  Performs a dilation operation with a 3x3 structuring element of specific arrangement and stores result to output image. Three dilate 3x3 operations are available:

Dilate3x3Center **(image input, image output)**	This function performs a dilation operation with a 3 x 3 centered structuring element shown in Figure 5.

Dilate3x3Down **(image input, image output)**	This function performs a dilation operation with a 3 x 3 down structuring element shown in Figure 6.

Dilate3x3Up **(image input, image output)**	This function performs a dilation operation with a 3 x 3 up structuring element shown in Figure 7.

| Figure 5 | Figure 6 | Figure 7 |
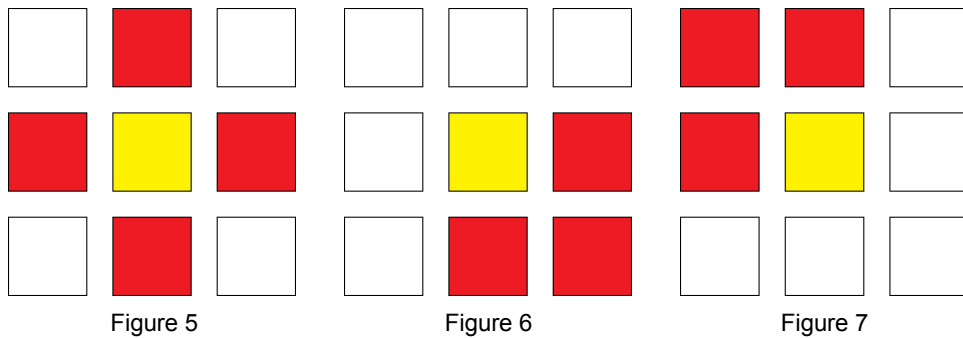
- **Dilate Diagonal**

  Performs dilation operation with an n x n diagonal structuring element and stores result to output image. Two dilate diagonal operations are available:

DilateDiagonalDown (image input, int32 kernelSize, image output)	This function performs a dilation operation with an n x n diagonal structuring element as shown in Figure 8.
DilateDiagonalUp (image input, int32 kernelSize, image output)	This function performs a dilation operation with an n x n diagonal structuring element as shown in Figure 9.

| Figure 8 | Figure 9 |

- **Dilate Disc**

  Performs a dilation operation with a disc-shaped structuring element and stores result to output image.

  DilateDisc (image input, int32 kernelSize, image output)

- **Erode**

  Takes the minimum value of all pixels in the kernel neighborhood of a pixel in the input image and propagates the pixel maximum value to all the pixels in the kernel neighborhood. Two sub-operations are available:

Erode1 (image input, int32 kernelSize, image output)	This function performs an erosion operation using a structuring element of the same width and height.
Erode2 (image input, int32 kernelWidth, int32kernelHeight, image output)	This function performs an erosion operation using a structuring element of different height and width.

- **Erode 3x3**

  Performs erosion operation with a 3x3 structuring element of specific arrangement and stores result to output image. Three erode 3x3 operations are available:

 | | |
  |---|---|
  | **Erode3x3Center (image input, image output)** | This function performs an erosion operation with a 3 x 3 centered structuring element shown in Figure 5. |
  | **Erode3x3Down (image input, image output)** | This function performs an erosion operation with a 3 x 3 down structuring element shown in Figure 6. |
  | **Erode3x3Up (image input, image output)** | This function performs an erosion operation with a 3 x 3 up structuring element shown in Figure 7. |

- **Erode Diagonal**

  Performs erosion operation with an n x n diagonal structuring element and stores result to output image. Two erode diagonal operations are available:

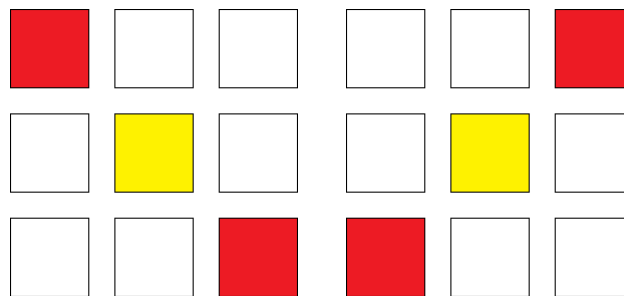 | | |
  |---|---|
  | **ErodeDiagonalDown (image input, int32 kernelSize, image output)** | This function performs an erosion operation with an n x n diagonal structuring element as shown in Figure 8. |
  | **ErodeDiagonalUp (image input, int32 kernelSize, image output)** | This function performs an erosion operation with an n x n diagonal structuring element as shown in Figure 9. |

- **Erode Disc**

  Performs an erosion operation with a disc-shaped structuring element and stores result to output image.

  ErodeDisc (image input, int32 kernelSize, image output)

- **Open**

  Performs erosion operation followed by a dilation operation using the same structuring element. Two sub-operations are available:

Open1 (image input, int32 kernelSize, image output)	This function performs an opening operation using a structuring element of the same width and height.
Open2 (image input, int32 kernelWidth, int32kernelHeight, image output)	This function performs an opening operation using a structuring element of different height and width.

- **Open Disc**

  Performs an opening operation using a disc-shaped structuring element instead of a square kernel.

  OpenDisc (image input, int32 kernelSize, image output)

Application

Morphological operations are the basis for our structure-guided image processing approach. In the following subsections, we will describe structure-guided image feature enhancement and structure-guided image feature extraction.

- Structure-guided image feature enhancement

Image features could be noisy and could contain irrelevant information. The structure-guided image feature enhancement efficiently enhances image features of interest and removes noisy and irrelevant information. The structure-guided image feature enhancement is accomplished by grayscale morphological opening and closing operations applied alternatively to image foreground and background. Morphological opening and closing operations possess an increasing property that maintains inclusion relationships on the images they apply. If an inclusion relationship exists for structuring elements A and B, that is:

$$A \subset B,$$

then combinations of opening and closing have the following property:

$$(((I \bullet A) \circ A) \bullet B) \circ B \subset (I \bullet B) \circ B$$

$$(((I \circ A) \bullet A) \circ B) \bullet B \supset (I \circ B) \bullet B$$

Therefore, processing sequences that progressively apply combinations of openings and closings are less severe and introduce less distortion when a small structuring element is used before a larger one. Application domain structure information is encoded into the size and shape of structuring elements to achieve structure-guided enhancement using increasing idempotent processing sequence. Figure 10 illustrates a structure-guided feature enhancement processing sequence on a noisy edge intensity profile (see Figure 10(a)). Structuring elements A and B (see Figure 10(b)), where $A \subset B,$ are used for the processing.

Figure 10 (c) illustrates the effect of opening and closing by A. Note that only partial resulting profile is shown in the illustration. Figure 10 (d) illustrates the effect of further opening and closing by B. The resulting feature enhanced image is shown in Figure 10 (e). The feature enhancement process removes noise and preserves the structure of the features of interest. There is no blur, ringing, overshoot or pre-shoot normally caused by phase distortion of linear filtering.

By choosing the proper structuring elements, structure-guided feature enhancement can be efficiently accomplished. Features of different structures can be enhanced using directional elongated structuring elements. The direction of the structuring element is chosen to align with the primary direction of the features to be enhanced. The largest size of the structuring element in the processing sequence should be smaller than the smallest size of features to be enhanced.

The structure-guided feature enhancement process could start with grayscale opening followed by grayscale closing or start with grayscale closing and followed by opening. Opening first will enhance dark features and closing first will enhance bright features. Each opening and closing iteration could use the same size structuring element for detailed feature refinement or could use increased size structuring element for aggressive feature refinement. Elongated structuring element of orthogonal directions could be alternatively or sequentially applied in the enhancement processing sequence.
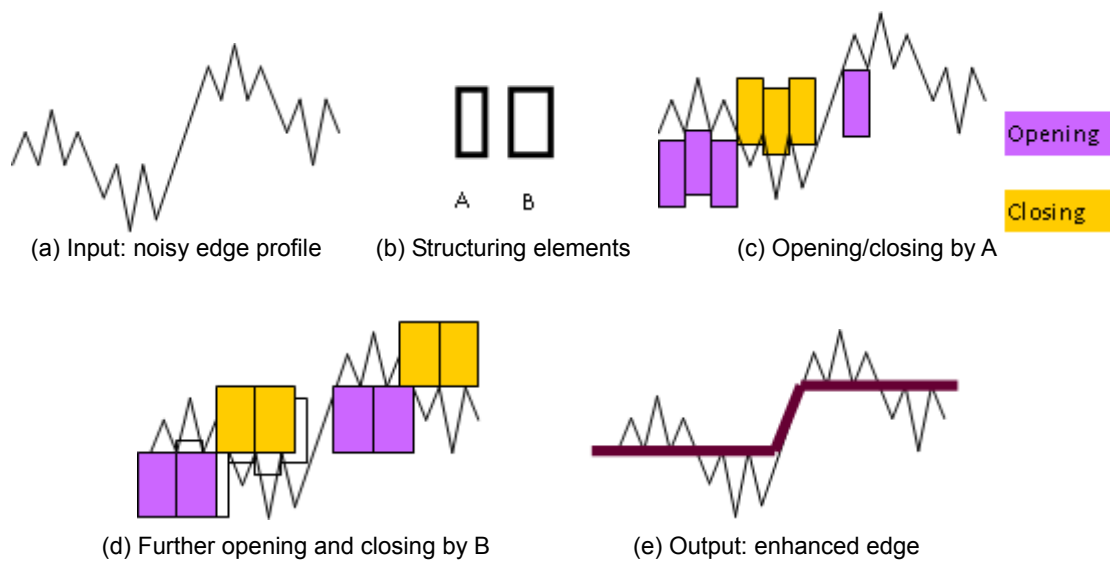
(a) Input: noisy edge profile   (b) Structuring elements   (c) Opening/closing by A

(d) Further opening and closing by B   (e) Output: enhanced edge

Figure 10

- Structure-guided image feature extraction

Image features are characterized by their grayscale intensity distributions and their spatial structures. Major image features include linear features such as bright edge, dark edge, general edge, bright line, dark line and general line. Major image features also include image regions such as bright region, dark region and general region, etc. Linear features can be arranged vertically, horizontally, and/or in different spatial directions. Regions can exhibit different shapes such as circular or rectangular, etc. They can be arranged in a given structure including relative locations, orientation or symmetry. Other image features include small regions or points such as corners of regions or intersection points of different features (linear features and/or regions).

The structure-guided image feature extraction approach efficiently extracts image features of interest and removes noisy and irrelevant information. This is accomplished by a sequence of grayscale morphological processing that encodes structure information into directional elongated structuring elements that can be efficiently implemented.

1. Image Feature Extraction

The following sub-sections describe some feature extraction processing sequence in the preferred embodiment of the invention.

(A) Bright edge extraction

Bright edges can be extracted by a grayscale erosion residue processing sequence defined as:

$$I - I \ominus A$$

Where I is an input image and A is a structuring element.

Figure 11 illustrates the grayscale erosion residue operation applies to a one dimensional ramp edge I shown in Figure 11(a). Figure 11(b) shows the structuring element A and Figure 11(c) shows the eroded profile of I by A. The erosion residue result is shown in Figure 11(d). As shown in Figure 11, grayscale morphological bright edge detection does not introduce undesired phase shift or blurry effect normally caused by linear filters.

(a) Input ramp edge image: I

(b) Structuring element: A

(c) Eroded image: I ⊖ A
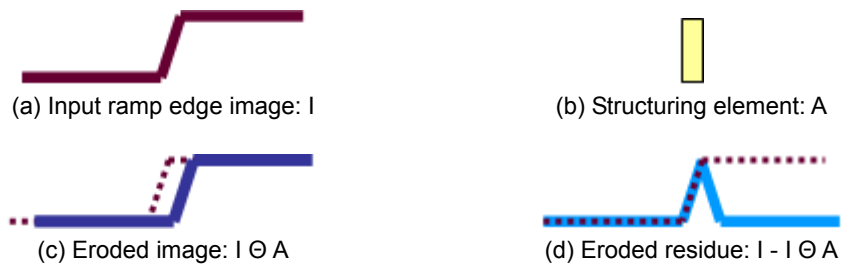
(d) Eroded residue: I - I ⊖ A

Figure 11

(B) Dark edge extraction

Dark edges can be extracted by a grayscale dilation residue processing sequence defined as:

$$I \oplus A - I$$

Figure 12 illustrates the grayscale dilation residue operation applied to the one dimensional ramp edge I shown in Figure 11(a). Figure 12(a) shows the dilated profile of I by A. The dilation residue result is shown in Figure 12(b). As shown in Figure 12, grayscale morphological dark edge detection does not introduce undesired phase shift or blurry effect.

(a) Dilated image: I ⊕ A

(b) Dilation residue: I ⊕ A - I

Figure 12

(C) General edge extraction

General edges (both dark and bright edges) can be extracted by the difference of grayscale dilation and erosion defined as:

$$I \oplus A - I \ominus A$$

Figure 13 illustrates the difference of grayscale dilation and erosion operation applied to the one dimensional ramp edge I shown in Figure 11(a).

Figure 13 (a) shows the dilated and eroded profile of I by A. The difference of grayscale dilation and erosion result is shown in Figure 13(b). As shown in Figure 13, grayscale morphological edge detection does not introduce undesired phase shift or blurry effect.

(a) I ⊕ A (green) and I ⊖ A (blue)

(b) I ⊕ A - I ⊖ A

Figure 13

(D) Bright line/region extraction

Bright lines/regions can be extracted by a grayscale opening residue processing sequence defined as:

I - I O A

Figure 14 illustrates grayscale opening residue operation applied to a one dimensional image profile shown in Figure 14(a). Figure 14(b) shows the opening result of image I by a sufficiently large structuring element. The opening residue result is shown in Figure 14(d). As can be seen in Figure 14(d), grayscale morphological line/region detection does not introduce undesired phase shift or blurry effect.

(a) Input image gray level profile

(b) Closing (orange) and opening (purple) results

(c) Closing residue

(d) Opening residue
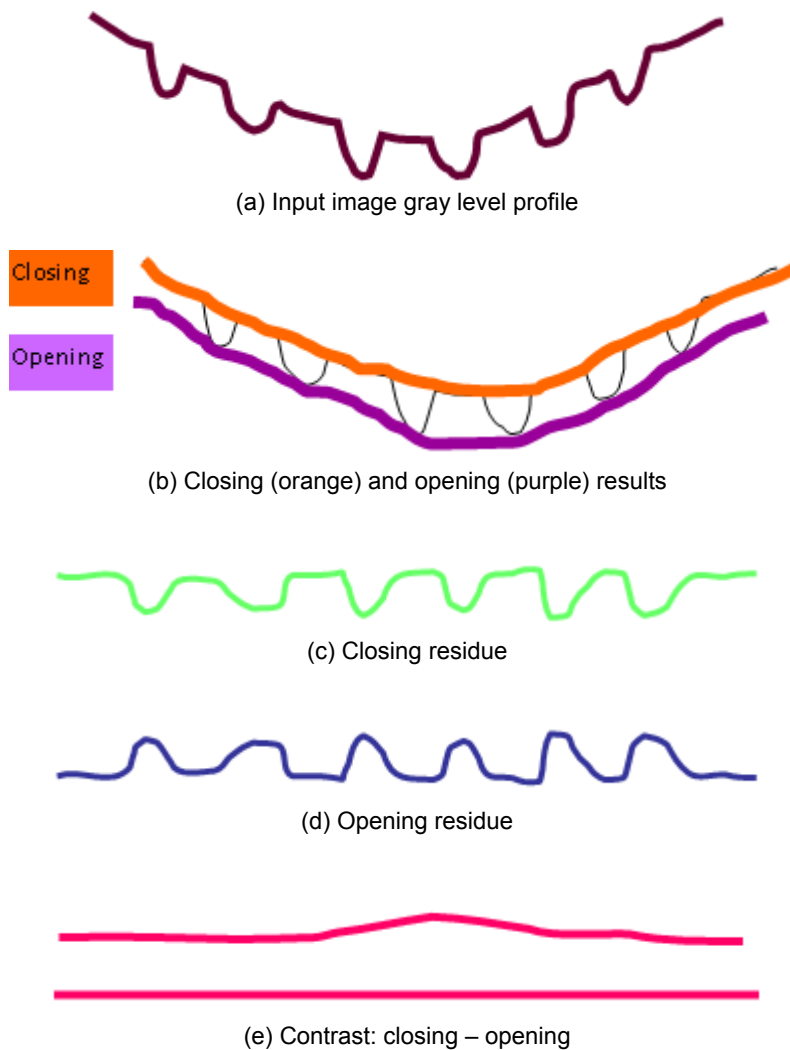
(e) Contrast: closing – opening

Figure 14

(E) Dark line/region extraction

Dark lines/regions can be extracted by a grayscale closing residue processing sequence defined as:

$$I \bullet A - I$$

Figure 14 illustrates grayscale closing residue applied to the one dimensional image profile shown in Figure 14 (a). Figure 14 (b) shows the closing result of I. The closing residue result is shown in Figure 14(c). As can be seen in Figure 14(c), grayscale morphological line/region detection does not introduce undesired phase shift or blurry effect.

(F) Region contrast extraction:

Region contrast can be extracted by the difference of grayscale closing and opening. The processing sequence is defined as:

$$I \bullet A - I \bigcirc A$$

Figure 14 illustrates the difference of grayscale closing and opening applied to the one dimensional image profile shown in Figure 14(a). Figure 14 (b) shows the closing and opening results of I. The difference of grayscale closing and opening is shown in Figure 14(e). As can be seen in Figure 14(e), the morphological region contrast extraction does not introduce any undesired phase shift or blurry effect.

(G) Region boundary extraction

Since two sides of a line are also edges, edge extraction operations will extract lines as well as edges. When using the same structure element, the edge extraction results, sub-sections A-C, include the corresponding line/region extraction results, sub-sections D-F. A region boundary consists of only edges that are in the boundaries of large regions and excludes edges from narrow lines.

Bright region boundary can be defined as the difference between grayscale morphological opening and erosion:

$$I \bigcirc A - I \ominus A$$

Similarly, dark region boundary can be defined as the difference between grayscale morphological dilation and closing:

$$I \oplus A - I \bullet A$$

And general region boundary can be defined as the difference between the summation of grayscale morphological opening and dilation and the summation of grayscale morphological erosion and closing:

$$(I \bigcirc A + I \oplus A) - (I \ominus A + I \bullet A)$$

2. Structure-guided Feature Extraction Processing Sequence

By choosing the proper structuring element for the feature extraction processing sequence, structure-guided feature extraction can be efficiently accomplished. Features of different structures can be extracted using directional elongated structuring elements. Directional elongated structuring elements are 1 pixel wide or have limited width in one of its dimension. The direction of the elongated structuring element is chosen to be approximately orthogonal to the primary direction of the features to be extracted.

Figure 15 shows an example of the structure-guided edge extraction that extracts horizontal edges and another example that extracts vertical edges from an input step edge image using directional elongated structuring elements. Figure 15 (a) is a two-dimensional step edge image. To extract vertical edges, a horizontal elongated structuring element is used (Figure 15(b)). The general edge extraction processing sequence, difference of grayscale dilation and erosion, is applied and the vertical edges are extracted (see Figure 15 (c)). Similarly, a vertical elongated structuring element is used (Figure 15(d)) for horizontal edge extraction and the horizontal edges are extracted (see Figure 15 (e)). In these examples, the width of the directional elongated structuring element is one pixel and the length of the structuring element determines the width of the extracted edges. Note that the medial axes of the extracted edge lines correspond to the position of the input step edge and no phase shift or blurry effect are introduced in the process.

(a) Input step edge image   (b) Horizontal structuring element H   (c) $I \oplus H - I \ominus H$

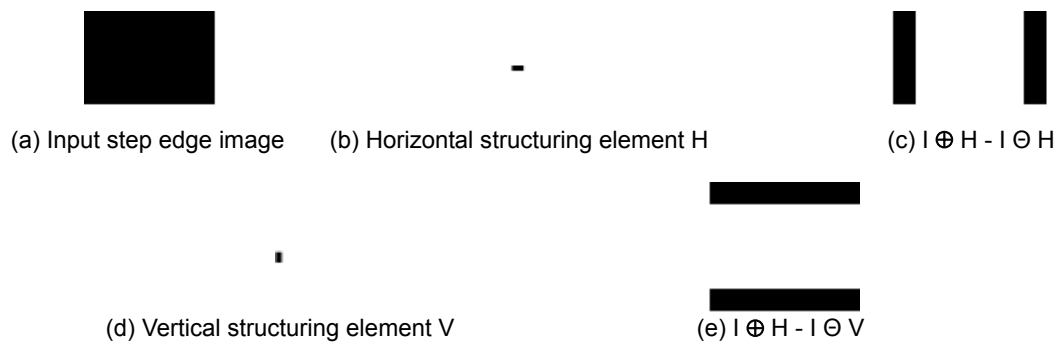(d) Vertical structuring element V   (e) $I \oplus H - I \ominus V$

Figure 15

The structure-guided processing can be applied to different feature extraction processing sequences as described above. Figure 16 shows an example of the structure-guided line extraction that extracts horizontal lines and another example that extracts vertical lines using directional elongated structuring elements. Figure 16 (a) is an input frame image. To extract vertical lines, a horizontal elongated structuring element is used (Figure 16 (b)). The dark line extraction sequence, closing residue, is applied and the vertical lines are extracted (see Figure 16 (c)). Similarly, a vertical elongated structuring element is used (Figure 16 (d)) for horizontal line extraction and the horizontal lines are extracted (see Figure 16 (e)). In these examples, the width of the directional elongated structuring element is one pixel and the length of the structuring element is longer than the width of the extracted lines. The structure-guided feature extraction allows the separate extraction of features of interest defined by their structures and ignore/remove irrelevant features. For example, if only the horizontal edges are of interest, it can be easily extracted as shown in Figure 15 (e) and no vertical edge components are included in the result.

(a) Input frame image   (b) Horizontal structuring element H   (c) Closing residue: I ● H – I

(d) Vertical structuring element V   (e) Closing residue: I ● V – I
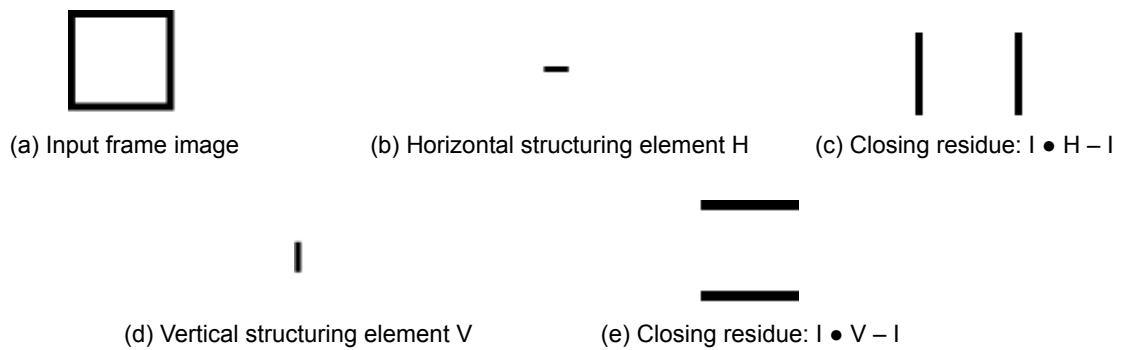
Figure 16

The extraction of features from any directions can be accomplished with the structure-guided feature extraction approach and the features extracted from multiple directions can be combined by a union (maximum) of multiple directional features. Furthermore, two-dimensional structuring elements of different size and shape can be used to extract desired regions.

(12) Noise operations

Noise operations add a well-defined noise signal to the input image.
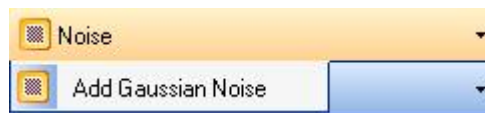
● **Add Gaussian Noise**

Adds noise to the field of view with distribution of pixel intensities following a normal distribution centered on the mean specified. Result image is stored to output.

AddGaussianNoise (image input, int32 mean, int32 stdDev, image output)

(13) Normalization operations

Normalization operations re-map image intensity to make use of the full dynamic range.
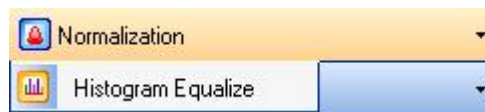
● **Histogram Equalize**

Redistributes input image intensities to utilize the full dynamic range available. Gain for each gray level is separately determined by its frequency of occurrence in the input. Result is stored to the output image.

HistogramEqualize (image input, image output)

(14) Post-Processing operations

Post-processing operations are used to enhance initial detection masks. These operations allow for additional segmentation mask editing and refinement for specific image analysis.
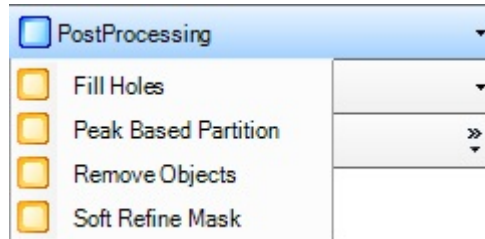
- **Fill Holes**

  Fills in small, enclosed gaps in the input mask and stores result to output.

  FillHoles (image input, int32 sizeValue, image output)

- **Peak Based Partition**

  Performs a distance transform operation on the input mask followed by iterative guided partition to separate touching object masks.

  PeakBasedPartition (image input, image output, int32 StartingLevel, int32 EndingLevel, int32 largestSeed, int32 smallestSeed, int32 step)

- **Remove Objects**

  Removes input mask objects that fall below a specified cutoff size.

  RemoveObjects (image input, int32 sizeValue, image output)

- **Soft Refine Mask**

  Performs Fill Hole and Remove Object operations simultaneously on the input mask and stores result to output.

  SoftRefineMask(image input, int32 sizeValue, image output)

(15) ZOI operations

<u>**Overview**</u>

- Zones of Influence

ZOI operations take a binary input image and calculate the zones of influence (ZOI) of individual objects.

Figure 1 (a) shows an image consisting of 4 circular component regions. In Figure 1 (b), the foreground pixels in the component regions are labeled by their component labels. Its background (non-component regions) can be partitioned into zones of influence (ZOI) as shown in Figure 1 (c). In the ZOI image, each pixel is associated with a component label. The background pixels are labeled by the labels of the component that is closest to the pixel. Figure 1(d) shows the ZOI image overlaid with the component regions.
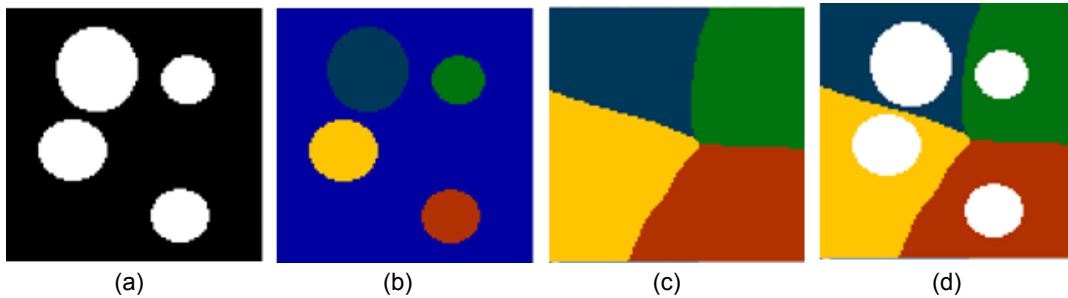

(a)   (b)   (c)   (d)

Figure 1

- Image region partition

The boundary of ZOI can be used as cutting lines to partition mask regions of an image. Figure 2 (a) shows an image mask containing two overlapped circular regions. Figure 2 (b) shows the overlay (in red) of two seed components created by thresholding the Euclidean distance transformed mask image. Figure 2(C) shows the image region partition using the seed component ZOI boundary as the cutting line.
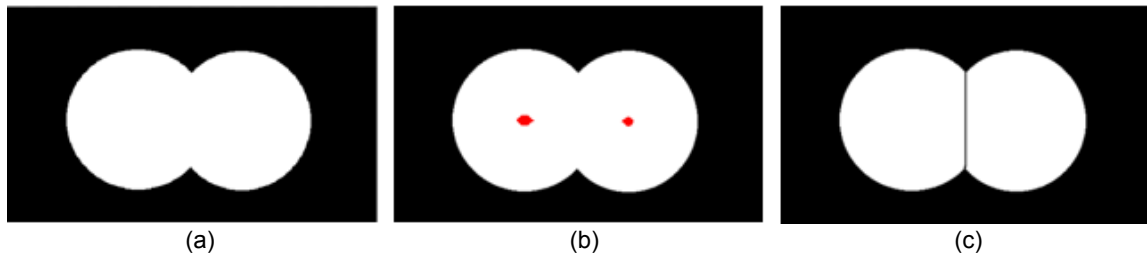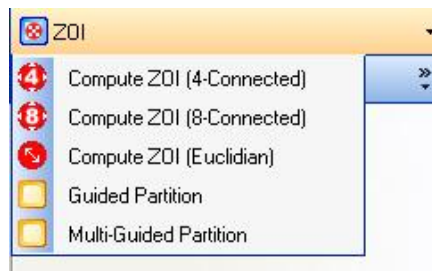

(a)   (b)   (c)

Figure 2

<u>**Usage**</u>

In CL-Quant, there are three ComputeZOI operations - 4-connected, 8-connected, and Euclidean - that defines how the ZOI distances are measured. There are two partition operations - Guided Partition and Multi-Guided Partition. The operations selection menu is shown below.

● **Compute ZOI**

Takes a binary input image and generates a grayscale Zones of Influence (ZOI) map where each ZOI is determined based on their distance between neighboring objects. Three sub-operations are available:

ComputeZOI (4-connected) (image input, image output, bool bounded)	This function generates a grayscale ZOI map based on distance between neighboring objects. Distance between masks is measured from adjacent pixels in the four cardinal directions.
ComputeZOI (8-connected) (image input, image output, bool bounded)	This function generates a grayscale ZOI map based on distance between neighboring objects. Distance between masks is measured from adjacent pixels in the four cardinal directions plus the four ordinal directions.
ComputeZOI (Euclidean) (image input, image output, bool bounded)	This function generates a grayscale ZOI map based on distance between neighboring objects. Distance between masks is calculated as the square root of the sum of the squares of the x-distance and y-distance.

● **Guided Partition**

Segments a contiguous binary input mask into discrete objects. Partition follows an input reference seed mask. Partitioned mask is stored to output.

GuidedPartition (image input, image seed, bool fourConnected, image output)

● **Multi-Guided Partition**

Multi-guided partition function takes three reference seed masks as inputs. It also inputs a size range containing a lower bound and an upper bound. It stores result to output. The operation includes three iterations:

(1) 1st iteration: performs guided partition on regions from the input image that is larger than the size lower bound using the largeSeed and retain resulting regions that are within the size range in the output image. The remaining regions greater than the upper bound will be continued partitioned in the 2nd iteration.

(2) 2nd iteration: performs guided partition on remaining large regions using the smallSeed and retain resulting regions that are within the size range in the output image. The remaining regions greater than the upper bound will be continued partitioned in the 3rd iteration.

(3) 3rd iteration: performs guided partition on remaining large regions using the adaptiveSeed and retain all resulting to be included in the output image.

Multi-GuidedPartition (image input, image largeSeed, image smallSeed, image adaptiveSeed, sizeRange, image output)

3.3 Additional Procedures

Other types of key image recognition procedures include motility tracking and decision procedures. These are described in the sections below.

3.3.1 Tracking Procedure Wizard

To create a new tracking procedure, go to "**Tools** > **Wizard**". In the wizard launch dialog, select "**Tracking**" and press "**Launch**". This will launch the tracking procedure wizard. The wizard will guide you through setting the appropriate parameters to optimize tracking performance and will enable you to select track measurements to include. The following sections will assist you in creating your own tracking procedure.

> **NOTE:**
>
> Tracking procedure creation requires the "**Live Tracking**" license.

3.3.1.1 Track measurements

Track
A track is the path traveled by a tracked object. The track links individual objects at each time frame. Tracking measurements characterize the tracks in several different ways:

- **Trajectory measurements:**
  Characterize the track in its entirety. These are one per track for all time. For example, straight line length is the distance the track object travels "as the crow flies".

- **Motility measurements:**
  Characterize the track at each time point. For example, velocity angle measurement $\theta 4$ in the figure below is the angle between the direction of the track object at frame 4 and frame 3.

- **Running window measurements:**
  Summarize motility measurements within a user defined running window. For example, the sum of the velocity angle could be measured over a three frame running window.
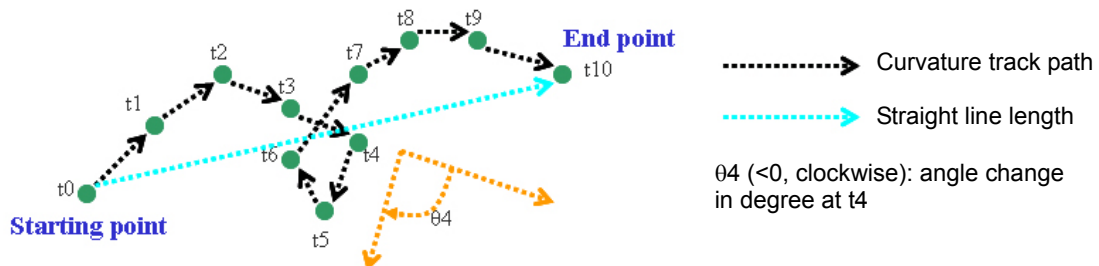
- **Polar measurements:**
  With polar measurements, the object mask is transformed from the image (x,y) coordinates to polar coordinates in (r, θ) where r is the distance from the center of the object and θ is the angle from the image x axis ($\theta = 0$ is parallel to the x axis). Measurements are then made on the mask in the polar domain.
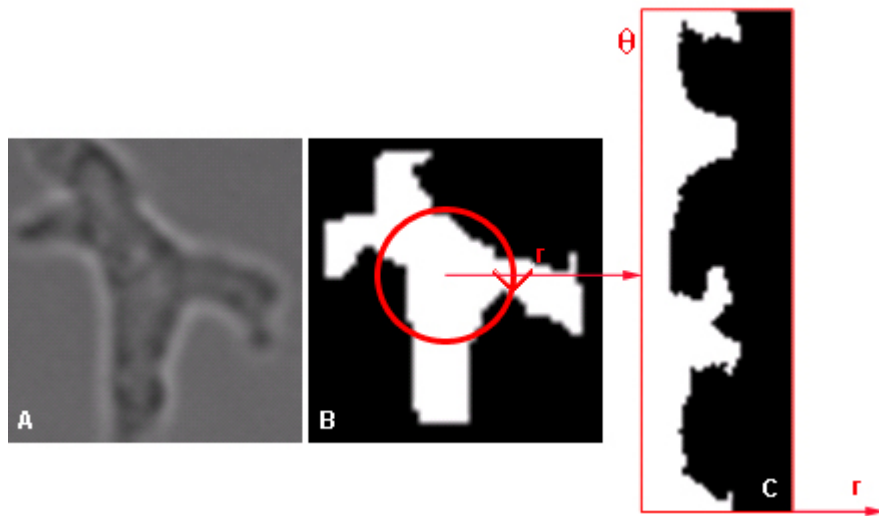
3.3.1.2 Configure a motility procedure

Motility procedures are general-purpose tracking procedures that have been extensively validated across multiple cell lines. Use motility for tracking large objects or objects in phase contrasts.

- **Channel and Mask Selections**

The motility tracking procedure requires a segmentation mask input on each image frame. Use the "**Channel index**" and "**Mask index**" textboxes to specify the location index of the channel and mask used for tracking.

- **Tracking Parameters**

Sample window size	Defines the width (number of frames) of a moving window in which measurements, such as velocity, are calculated.
Min object size	Objects in the segmentation mask below this size (pixels) are not tracked.
Max search range	Specifies the search radius (in number of pixels) for connecting track points from previous track point position.
Split Threshold	Threshold for split detection. A lower threshold value will bias toward more splits and vice versa.
Merge threshold	Threshold for merge detection. A lower threshold value will bias toward more merges and vice versa.
Min frames for overlapping	Minimum threshold number of image frames for which two touching objects are considered in an overlapped state.
Min trajectory length	Minimum threshold number of frames that a tracked object must appear in to be considered a trajectory.

- **Tracking Options**

Object Split	Given one object, object A in the previous frame, the object A is split if two objects, object B_1 and object B_2, matches object A in the current frame. **- Allow split** Two new tracks will be created in the current frame for a trajectory that satisfies the condition above. Select this option for cells undergoing division. - **Ignore split** A new track will be created for one of the new objects in the current frame for a trajectory that satisfies the condition above; the other new object will inherit the previous trajectory.
Object Merge	Given two objects, object A_1 and object A_2 in the previous frame, the trajectories of objects A_1 and A_2 are merged if one object, object B, matches both A_1 and A_2 in the current frame. **- Merge with partition** Object masks that satisfy the conditions above will be partitioned by their appearance and previous trajectories will continue in the current frame. **- Allow merge** Object masks that satisfy the conditions above will be merged to a single mask and a new trajectory will be created in the current frame.

- Ignore merge
Object masks that satisfy the conditions above will be merged to a single mask and inherit the trajectory of one of the merged components in the current frame.

Enable lineage interface	When this option is checked, a track object will be ended when a mask component separates (i.e. cell division), and two new tracks (i.e. daughter cells) will be created. This option allows for tracking lineage (previous generation) of splitting cells. When this option is enabled, lineage measures (parent / child track number, generation, descendants) are automatically created. These measures are defined in the next section.
Enable robust measurement	When this option is checked, track measurements of large cell clusters will be separated from track measurements of isolated cells.
Object-to-object overlap	Given an object A in the previous frame and object B in the current frame, one of two methods is used to determine whether A "overlaps" B, and therefore, is a possible match for B: 1) The pixels of A intersect the pixels of B, or 2) The zone of influence (ZOI) of A intersects the pixels of B or the ZOI of B intersects the pixels of A. This option specifies which method to use. If this option is TRUE, method 1) is used, otherwise, the less restrictive method 2) is used. The default value is TRUE. In situations where the objects move slowly, i.e., there's pixel overlap from frame to frame, then method 1) may be better since it is more restrictive about possible matches. In situations where objects move quickly and the distance moved between frames is larger than the size of the object (no pixel overlaps), then method 2) must be used.
Remove short track when merging	When two objects are considered in a merge state, checking this option will terminate the shorter track at the merging time point.

When you are done with parameter configurations, click "**Next**" to define track measurements.

3.3.1.3 Track Measurement Definitions

There are four main categories of motility measurements.

- **- Motility Measurements:**
  Motility measurements are state metrics for each track point at time *t*.
- **- Trajectory Measurements:**
  Trajectory measurements are metrics for the entire track up to *t*.
- **- Running Window Stats:**
  Running window stats are track measurements within the sample window size.
- **- Field Measurements**
  Field measurements provide metrics for counting object overlaps for all tracks combined.

Motility measurement definitions

Object ID	For a track at time *t*, the Object ID is the unique identification number of the object on the track at time *t*.
Predecessor	For a track at time *t*, the Object ID of the object on the track at time *t*-1.
Successor	For a track at time *t*, the Object ID of the object on the track at time *t*+1.
X	X coordinate of the track object at time *t*.
Y	Y coordinate of the track object at time *t*.
Object size	Pixel count of the track object mask at time *t*.
Average intensity	Mean of the pixel values measured in the track object mask at time *t*.
Median intensity	The median of the pixel values measured in the track object mask at time *t*. The median is the number separating the higher half of the pixel values from the lower half.
Top 5% intensity	The top (or high) 5% intensity value measured in the track object mask at time *t*.
Top 10% intensity	The top (or high) 10% intensity value measured in the track object mask at time *t*.
Top 15% intensity	The top (or high) 15% intensity value measured in the track object mask at time *t*.
Top 20% intensity	The top (or high) 20% intensity value measured in the track object mask at time *t*.
State	Sometimes internal states are calculated by the tracking algorithm and will be reported if this measurement is selected.

Overlap State	This reports whether a mask object is touching/overlapping another mask object (10) or not (0).
Velocity magnitude	The displacement of the track object from *t*-1 to t divided by the time increment.
Velocity angle	The angle between the track object from *t*-1 to *t*.
Acceleration	The change in the velocity magnitude from *t*-1 to *t*.
Match score	This is the matching confidence over time. 1 is perfect confidence. As a cell touches other cells or enters into a cluster of cells its match score will fall.
Compactness	The square of the perimeter of the track object mask divided by the object size.
Major to minor ratio	The ratio of the major axis of the track object mask to the minor axis of the track object mask.
Ellipse fitting angle	Angle of fitting ellipse (of the object mask) to x axis of FOV.
Max polar radius	Maximum radius of the points along the mask from the origin (object center) when transformed in polar coordinates.
Min polar radius	Minimum radius of the points along the mask from the origin (object center) when transformed in polar coordinates.
Norm mean radius	Mean polar radius divided by maximum polar radius.
Process count	Number of peaks in the track object mask in polar coordinates.
Norm process mean radius	Mean of all process max polar radii, divided by the track object's max polar radius.
Relative process angle mean	Mean of the relative process angle divided by the track object ellipse fitting angle.
Relative process angle std	The standard deviation of the relative process angles.
Polar local maximum radii	Local maximum radii of points along the mask centered at the origin when transformed into polar coordinates. A maximum of ten (10) local maxima may be displayed.
Polar local minimum radii	Local minimum radii of points along the mask centered at the origin when transformed into polar coordinates. A maximum of ten (10) local minima may be displayed.

Trajectory measurement definitions

Total Time	Total time span of a track (from starting to end point)
First Frame	Time of the track starting point

Last Frame	Time of the track end point
Sum Vx	Sum of incremental velocity in x direction over the whole track
Sum Vy	Sum of incremental velocity in y direction over the whole track
SqSum Vx	Sum of square of incremental velocity in x direction over the whole track
SqSum Vy	Sum of square of incremental velocity in y direction over the whole track
Straight Line Length	Length of the straight line connecting track starting and end points
Straight Line Velocity	Straight line length divided by Total Time
Total Length	Total incremental distances of curvature track path
Curvilinear Velocity	Total Length divided by Total Time
Curvature Rate	Sum of angle change within the length of the track, it includes the sign to reflect the direction of change
Track Linearity Percentage	Ratio of Straight Line Velocity to Curvilinear Velocity
Bounding Box Area	The area of a bounding box generated from the minimum and maximum values of x- and y-coordinates over track lifetime.
Overlapped Frame Count	Total number of frames of which clustering of two masks is observed.
Overlapped Frame Percentage	Ratio of Overlapped Frame Count to length of image sequence.
Non-overlapped Frame Count	Total number of frames of which no clustering occurs.
Non-overlapped Frame Percentage	Ratio of Non-overlapped Frame Count to length of image sequence.
Mean Overlapped Velocity	Mean velocity of clustered mask objects in the overlapped state.
Mean Non-overlapped Velocity	Mean velocity of standalone mask objects.
StdDev of Overlapped Velocity	Standard deviation of the velocity of clustered mask objects in the overlapped state.
StdDev of Non-overlapped velocity	Standard deviation of the velocity of standalone mask objects.

Running window stats

Statistics such as minimum, maximum, average, sum, difference with previous frame, and difference from start can be calculated for all selected measurements within the sample window. For example, if you select "**Sum**" from the running window statistics and set the running window to five frames, the sum of the measurement for the current and prior four frames will be reported. So for the "**velocity magnitude**" measurement in this case, sum (velocity magnitude) would report the total displacement of the track object of the object in the current and preceding four frames.

Field measurement definitions

Overlapped Count	Total number of tracked objects in overlapped state.
Overlapped Pct	Percent of tracked objects in overlapped state in all tracked objects.
Non-overlapped Count	Total number of tracked objects in non-overlapped state.
Non-overlapped Pct	Percent of tracked objects in non-overlapped state in all tracked objects.
Mean Overlapped Velocity	Mean velocity of all tracked objects in overlapped state.
Mean Non-overlapped Velocity	Mean velocity of all tracked objects in non-overlapped state.

3.3.2 Decision Procedures

The decision module contains CL-Quant's next generation, teachable, phenotype classification tools. Decision teaching refers to creating a decision tree based classifier. This can be done directly in the images. Decision teaching can only begin after you have calculated measurements by applying a measurement procedure. Decision teaching can be done on more than one image simultaneously. It consists of the following steps:

1. Create and load a decision template

2. Create a teaching set by labeling

3. Create a decision procedure

> 🖊 **NOTE:**
>
> Decision procedure creation requires the Decision module license configuration. Decision procedures cannot be applied to classify trajectories.

3.3.2.1 Select user image

To start decision teaching, you will need to load a user image. Select the image you want to use for decision teaching by clicking on the image in the image selection dialog. Only images with objects can be used in the decision wizard. Click on the "**Load more images**" button to load additional images.
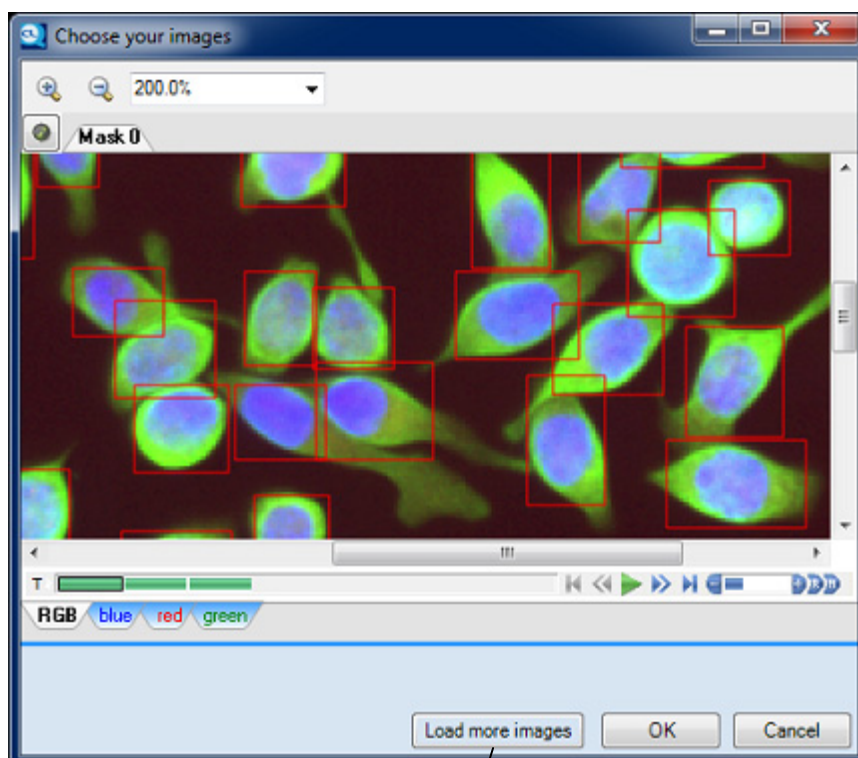
Load more images button

3.3.2.2 Create category names

On the "**Select Category Labels**" page, you can create a new decision template for teaching. The template labels will be saved to the decision procedure.

Add labels to a decision template

Here you define the names and colors for your classification categories. To create a category, just click on the "**Add Label**" icon (red circle).

Click to create a category.
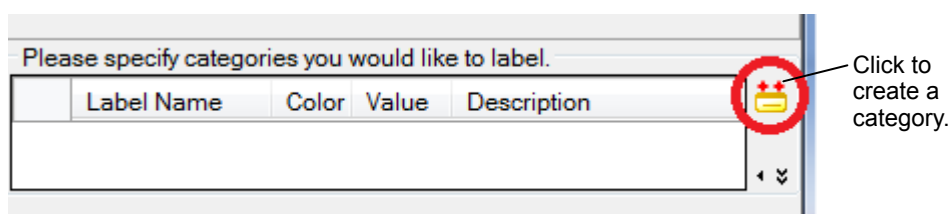
The category name can be modified, and the category can be removed (by clicking the red X icon under the "**Add Label**" icon). At least two categories are required for creating a decision procedure. When you are finished creating your classification categories, click "**Next**."

3.3.2.3 Create a teaching set by labeling

On this page you can teach the decision tree or review previously-taught trees. In teaching mode, any object ROI you click on will be assigned the selected class in the control window. In the example below the selected class (arrow) is the Responders class.
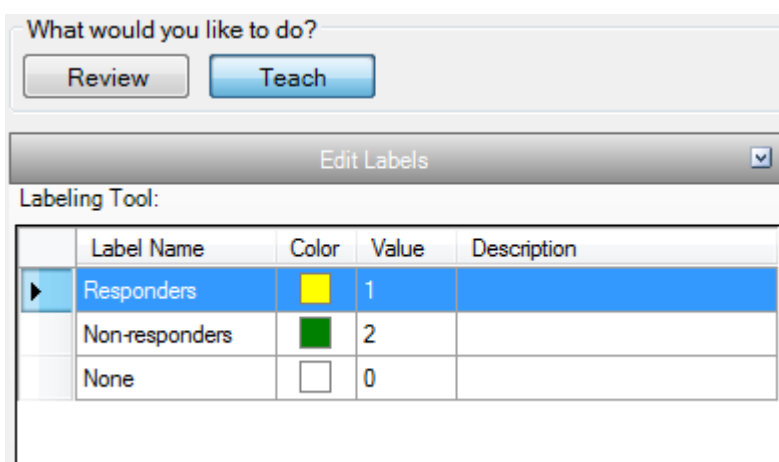
Edit Labels

Create a teaching set by highlighting a class in the control window, and clicking on object ROIs (this process is called labeling). When you click on a ROI, that object is assigned the highlighted class. Click on the ROI a second time to un-assign the class, or you can assign the "**None**" class. You can label objects in as many FOVs as you want.

In this example, we have labeled several representative object ROIs for each class (yellow is "**Responders**" and green is "**Non-responders**"). These labeled objects comprise the teaching set. The software will use this information to create decision rules.
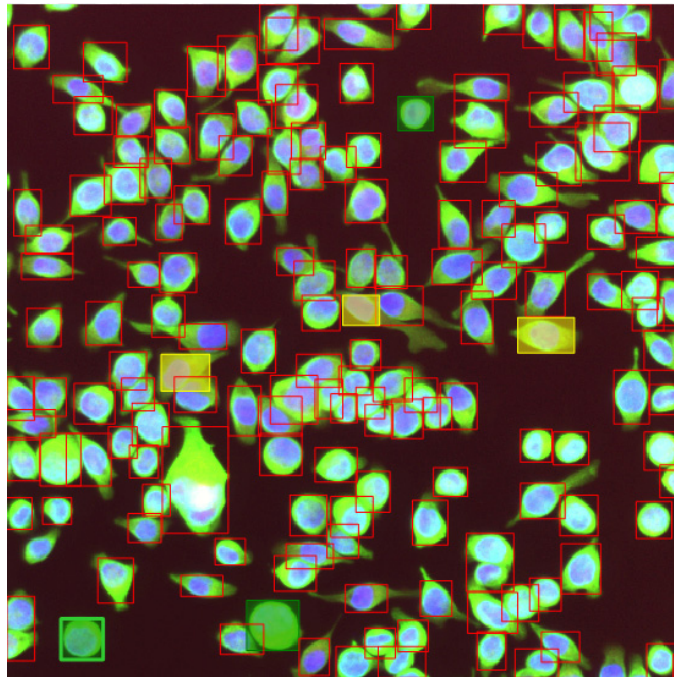
Edit Training Image

You can review original image or add/remove new teaching image(s) using the options found in the "**Edit Training Image**" section beneath the decision template.
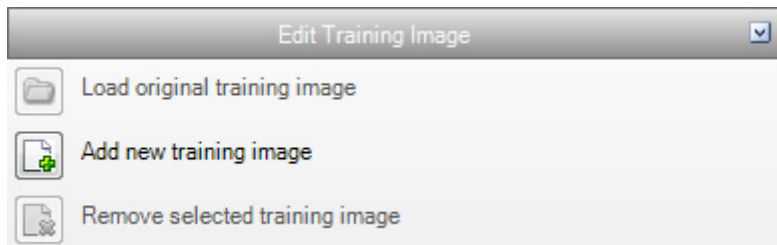

If you are satisfied with your decision teaching, click "**Next**" to continue and to finish the decision wizard.

### 3.3.2.4 Understanding the decision tree

The decision tree is a set of classification rules, developed using the measurements calculated by the measurement procedure. The decision procedure represents these rules as a decision tree. The tree is visible in the configuration tab of the procedure, and is easy to understand and explore. Specialized charts and graphs facilitate knowledge discovery.

(1) Transparent tree structure

The figure below shows an example decision tree. The tree is created automatically based on user labeling of the teaching set of objects. It creates the tree that can best distinguish the labeled classes. The tree is transparent and amenable to user review and update.
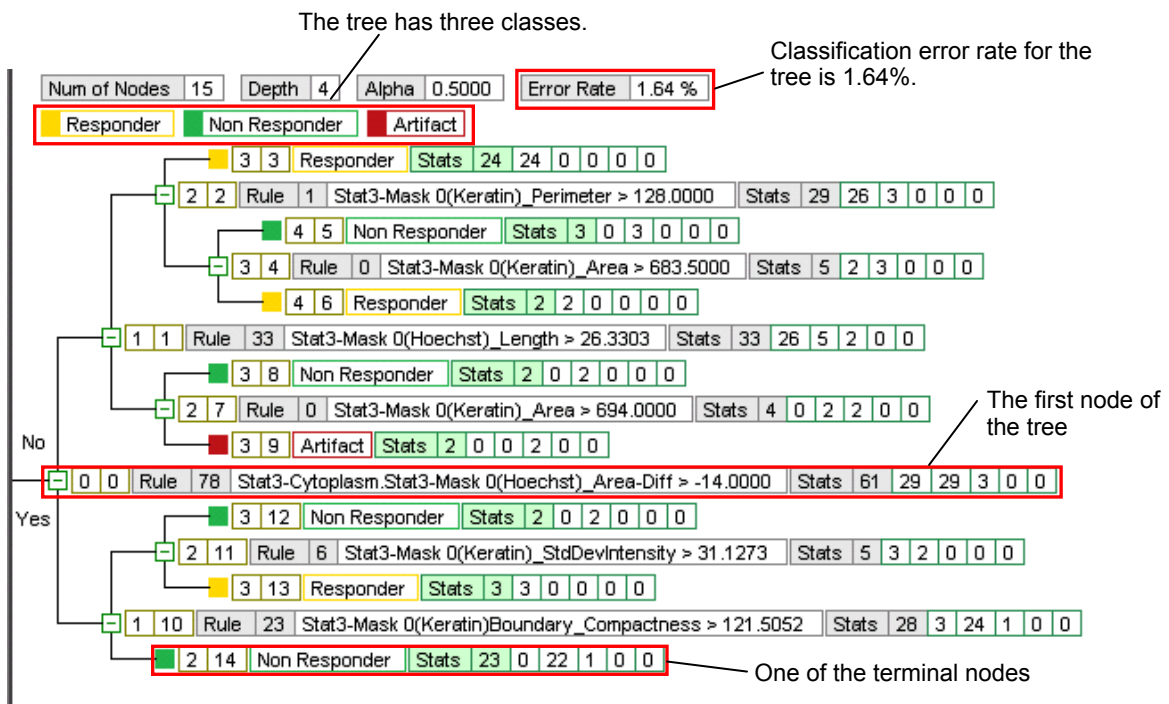
In this example, the tree has three classes, "Responder", "Non Responder", and "Artifact". The tree is able to classify the user labeled teaching set with 1.64% error. Each node in the tree is a rule. The rule is applied to the incoming objects. If the answer to the rule is yes, then the object moves down to the next node below, if the answer is no, then the object moves up to the next node above. The object moves through the tree in this fashion until it reaches a terminal node where its classification class (as opposed to its taught or labeled class) is assigned. Note that each class can have more than one terminal node.
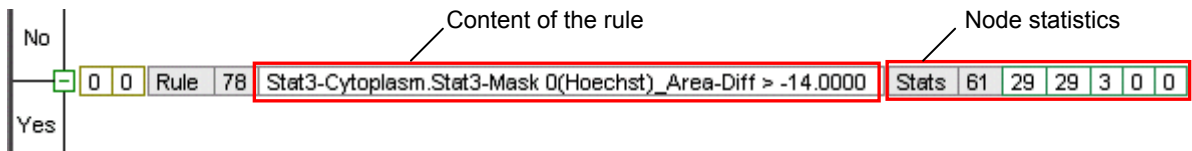
The figure above shows the first rule in the tree, Rule 78. If an object's Cytoplasm - Nucleus Area difference is greater than -14.0, it will move down to the next rule, rule 23. Otherwise it will move up to the next rule, Rule 33. The node statistics tell you the total number of samples in the node, and the number belonging to each of the three classes. The order corresponds to the class listing at the top of the tree panel. Here, the order is "Responder", "Non Responder", then "Artifact" class. The additional 0 spots are for unused classes. Since this is

top node, all the statistics for the teaching set are shown. 61 total objects were labeled, 29 Responder class, 29 Non Responder class and 3 Artifact class.

Clicking on any node in the tree will highlight the objects overlays on the images.

> ✏ **NOTE:**
>
> The tree only shows the classification performance on the teaching set objects where the truth is known. The error rate tells you how well the tree performs given that you know "the truth". To ascertain the tree performance on all the FOV objects you must apply the procedure to the training FOVs to classify all the objects (shown below).

The figure below shows one of the tree's terminal nodes. Objects that arrive in this terminal node are classified as "Non Responder". In this case, there are 23 teaching set objects in the node. Of these 0 were taught as "Responder", 22 were taught as "Non Responder" and 1 was taught as an "Artifact" (this is the only error in the tree).
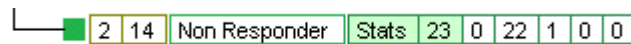
(2) Tree charts and graphs

The decision recipe also provides a specialized table and chart to assist with tree understanding. Click on the charts button at the bottom of the information panel to bring up the charting window. There you will see the contingency table icon and the parallel coordinate plot icon.

 Contingency table

Contingency Table icon —

The contingency table presents the tree's overall classification accuracy on the teaching set. Read from left to right along the rows, it tells you the classification error for the labeled teaching sets. Read from top to bottom down the columns, it tells you the classification error for the classes. The tree only has one misclassified object, but its error can be thought of in these two different ways. Reading along the row we see that of three labeled "Artifacts", one was mis-classified. Reading down the column we see that of 30 classified "Non Responders", one was actually an "Artifact".

Category	Respo...	Non Re...	Artifact	#Total	#Error	Error %
Responder	29	0	0	29	0	0.00 %
Non Resp...	0	29	0	29	0	0.00 %
Artifact	0	1	2	3	1	33.33 %
#Total	29	30	2	61		
#Error	0	1	0		1	
Error %	0.00 %	3.33 %	0.00 %			1.64 %

To understand the error case better, we can go to the parallel coordinates plot.

Parallel plot
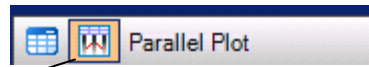
Parallel Plot icon

You can populate the plot objects by clicking on a node in the tree. The example plot below shows the objects in the terminal node shown above, which contains the tree's only error. As described above this node contains 23 objects classified as "Non Responders", of these 1 object was labeled as "Artifact".

The vertical bar represents the measurement used at the decision nodes leading to the terminal node. Since this node is in a shallow position, there are only two measurements used to reach it. The parallel coordinate plot enables you to see the distribution of the node objects with respect to the measurements. Each object is represented by a line. The "Non Responder" labeled objects are shown in green, and the "Artifact" object is shown in red. The shading on the bars represents a histogram of the entire teaching set population distribution for the measurement; darker shading indicates a higher frequency of occurrence.
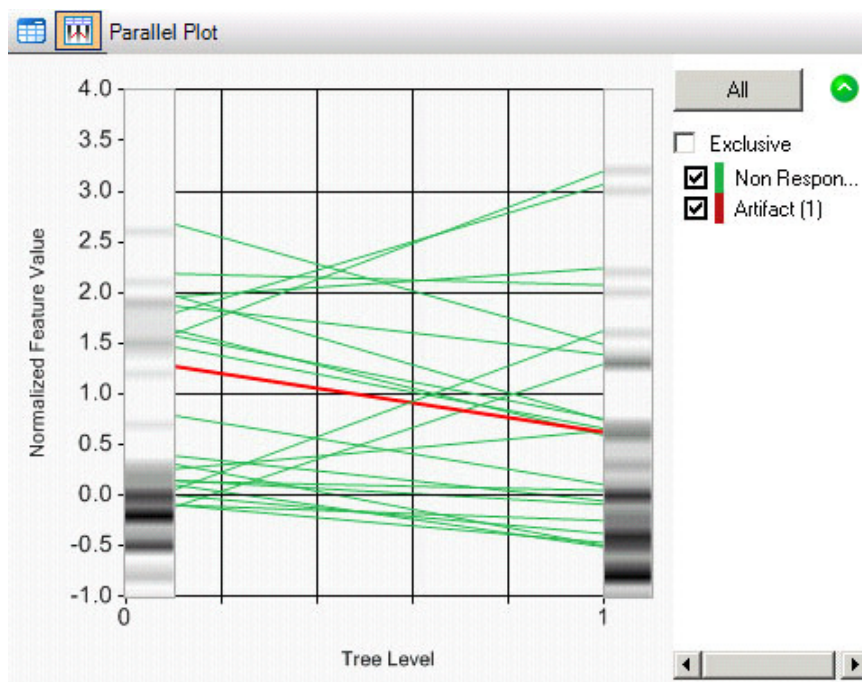
(3) Manual Tree Modifications

Manual tree modifications include pruning and changing the node label. When a terminal node is pruned, the tree performs as if the node has been removed. However, pruned nodes will be recovered after save and reload. To permanently remove pruned nodes you must select "**Remove Pruned Nodes**" from the context menu.

Right clicking on the tree will bring up the context menu for manual tree modifications as shown below. The first two entries will toggle the contingency table and parallel coordinate plot. The remaining items are described below.
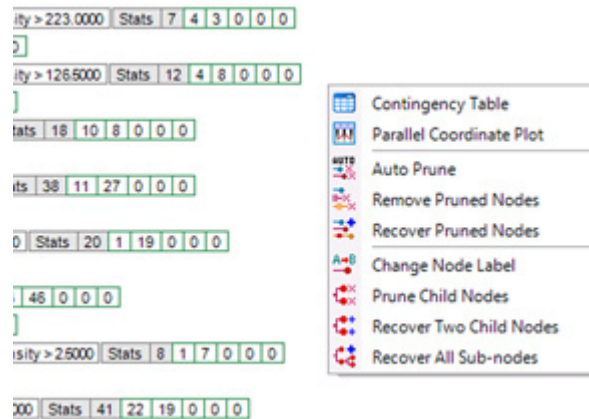
- **Auto Prune**

  This operation will automatically prune the tree.

- **Remove Pruned Nodes**

  Will permanently remove pruned nodes.

- **Recover Pruned Nodes**

  Re-activates pruned nodes to normal status.

- **Change Node Label**

  Allows the user to manually re-assign a node to a different class.

- **Prune Child Nodes**

  Prunes all the nodes below this one.

- **Recover Two Child Nodes**

  Recovers this node's child nodes, but their children remain pruned.

- **Recover All Sub-nodes**

  All nodes below the current node are recovered.

3.4 Other Procedure

CL-Quant provides additional utility procedures such as basic enhancement procedures and FOV import procedures.

3.4.1 FOV Import Procedure

An FOV import procedure can be executed to either: import an FOV list from FOVs on the file system, or create a time-lapse FOV from image frames on the file system.

To create the procedure, right click on the "**FOV import procedures**" folder in the data explore and select "**New**". This will create a new import procedure data object. Double click on the data object to launch the procedure in a new window.

The FOV import procedure has the same organization as the FOV RFrame except that the FOV list panel is treated differently. In the normal FOV RFrame, the FOV list panel shows the FOVs in an RFrame list. In this case, the panel shows the folder or images on file that will be imported to the channel or mask. To specify individual images or folders for importing to the FOV, simply drag and drop them from the Windows Explorer. Most supported image types can be used.

For example, say you want to create a three-channel movie. On the file system you have the individual files arranged in folders. To create an import procedure, you simply drag and drop the first folder from file into the FOV list panel. The folder on file will be associated with Channel 0, and when you execute the procedure, images from that folder will be loaded into Channel 0. To create the second channel, right click on the channel strip and select "**Add Channel**". Repeat the same steps as for Channel 0 until all the channels are created, and their import directories or images defined.

You can add masks to the channels in the same way, but first be sure to click on the mask strip and select "**Add Mask**". You'll notice when you click on the new mask tab that the image list panel is now blank. After adding files from file system, the image list panel will show the images associated with Mask 0 (if you click on the "**Channel 0**" tab, it will show the images associated with Channel 0). To create a second mask, just right click on the mask strip and select "**Add Mask**". Masks can be added, removed/deleted, and renamed. In addition, you may change the color of the mask. When the procedure is executed the imported masks will be assigned the selected color.

At any time, you may click on "**Clear List**" or "**Cancel**" to erase your channel or mask import specification.

- **Relative path**

  You may also check the "**Relative path**" box. In this mode, the directory structure will be interpreted relative to the location of the import procedure. This allows you to create movable procedures which do not rely on the absolute file system path. If it is kept unchecked, the procedure will always import files from the same absolute file path.

- **Load all channels of multi-channel images**

  When you check the load all channels option, all the channels of the input images will be loaded. If unchecked, only the first channel of the input image will be loaded.

- **Load as FOVs**

  When this option is checked, the files will be loaded into FOVs or an FOV list. If unchecked, they will be loaded into a single time-lapse FOV.

● **Load as T Movie**

When this option is selected (red box) the files will be loaded into a single time-lapse FOV in T series. Note that the dimension of the images imported must be the same to use this option.

● **Load as Z Movie**

When this option is selected (red box) the files will be loaded into a single time-lapse FOV in Z series. Note that the dimension of the images imported must be the same to use this option.
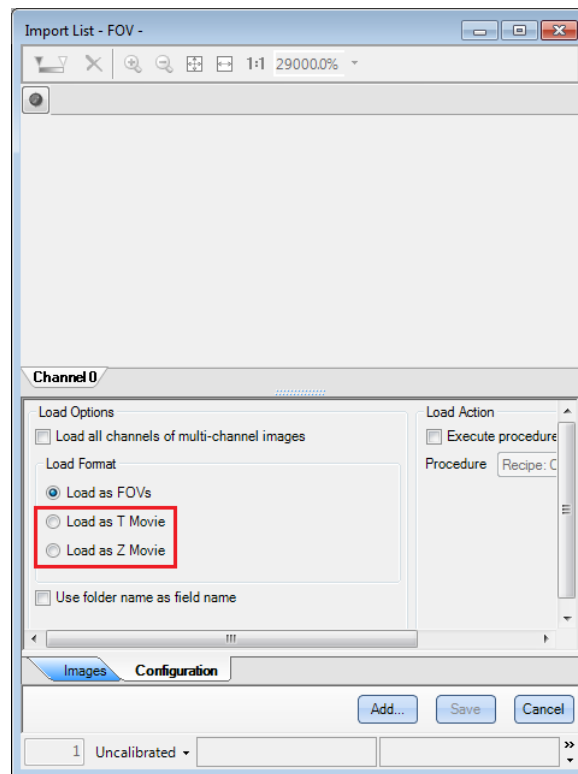
● **Use folder name as field name**

In this mode, the folder name will be used for the FOV name. When multiple FOVs are imported as an FOV list in this mode, the name of all individual FOVs will be the same as the folder name.

● **Execute procedure after loading**

When this option is checked, you may select an action procedure to execute on each image after it is imported. An action procedure is a previously loaded procedure or procedure list that is already in your procedure data explore panel.

To finalize the procedure, click "**Save**".

En Symbol for separate collection applicable in European countries

This symbol indicates that this product is to be collected separately.
The following apply only to users in European countries.
• This product is designated for separate collection at an appropriate collection point. Do not dispose of it as household waste.
• For more information, contact the retailer or the local authorities in charge of waste management.

De Symbol für getrennte Wertstoff-/Schadstoffsammlung in europäischen Ländern

Dieses Symbol zeigt an, dass dieses Produkt separat entsorgt werden muss.
Folgendes gilt für Verbraucher in europäischen Ländern:
• Dieses Produkt darf nur separat an einer geeigneten Sammelstelle entsorgt werden. Eine Entsorgung im Hausmüll ist unzulässig.
• Wenden Sie sich für nähere Informationen bitte an Ihren Händler oder die örtlich für Abfallentsorgung zuständigen Behörden.

Fr Symbole pour la collecte sélective applicable aux pays européens

Ce symbole indique que ce produit doit être collecté séparément.
Les mesures suivantes concernent uniquement les utilisateurs européens.
• Ce produit doit être jeté séparément dans un point de collecte approprié. Ne jetez pas ce produit dans une poubelle réservée aux ordures ménagères.
• Pour plus d'information, contactez le détaillant ou les autorités locales responsables de la gestion des ordures.

Es Símbolo para recogida separada aplicable en países Europeos

Este símbolo indica que este producto se recogerá por separado.
Lo siguiente sólo se aplicará en países Europeos.
• Este producto ha sido designado para su recogida en un punto de almacenamiento apropiado. No lo tire como un desecho doméstico.
• Para más información, contacte con el vendedor o autoridades locales al cargo de la gestión de residuos.

Dk Symbol for special bortskaffelse af denne type produkter i de europæiske lande

Dette symbol angiver, at dette produkt skal bortskaffes specielt.
Det efterfølgende er kun til forbrugere i de europæiske lande.
• Dette produkt skal bortskaffes på fx en genbrugsplads el .lign. Det må ikke smides væk som normalt husholdningsaffald.
• For yderligere information kontakt din forhandler eller de lokale myndigheder, som fx teknisk forvaltning.

Nl Symbool voor gescheiden inzameling zoals dat wordt gebruikt in Europese landen

Dit symbool betekent dat dit product apart moet worden ingezameld.
Het volgende is alleen van toepassing op gebruikers in Europa
• Dit product dient gescheiden ingezameld te worden op een daartoe bestemd inzamelpunt. Niet weggooien bij het normale huisvuil.
• Neem voor meer informatie contact op met het verkooppunt, of met de lokale instantie die verantwoordelijk is voor het verwerken van afval.

Pt Símbolo para recolha de resíduos em separado utilizado nos países Europeus

Este símbolo indica que este produto é para ser recolhido separadamente.
Esta norma aplica-se só para os utilizadores nos países Europeus.
• Este produto está designado para recolha de resíduos em separado num recipiente apropriado. Não deitar no caixote do lixo doméstico.
• Para mais informações, contactar o revendedor ou as autoridades locais responsáveis pela gestão dos resíduos.

It Simbolo per la raccolta differenziata applicabile nei paesi europei

Questo simbolo indica che il prodotto va smaltito separatamente.
La normativa che segue si applica soltanto agli utenti dei paesi europei.
• Il prodotto è designato per lo smaltimento separato negli appositi punti di raccolta. Non gettare insieme ai rifiuti domestici.
• Per maggiori informazioni, consultare il rivenditore o gli enti locali incaricati della gestione dei rifiuti.

This symbol is provided for use in the People's Republic of China, for environmental protection in the fields of electronic information products.

このマークは、中国のお客様に向けたもので、電子情報製品分野における環境保護を目的としています。

No Symbol for kildesortering i europeiske land

Dette symbolet indikerer at produktet skal kildesorteres.
De nedenstående punktene gjelder for alle europeiske brukere.
• Dette produktet skal kildesorteres og innleveres til dedikerte innsamlingspunkter. Må ikke kastes med normalt husholdningsavfall.
• For mer informasjon, ta kontakt med din forhandler eller lokale myndigheter.

Se Symbol för separat upphämtning i euopeiska länder

Den här symbolen anger att hämtas måste hämtas separat.
Följande gäller bara användare i europeiska länder.
• Den här produkten är avsedd för separat upphämtning vid ett lämpligt uppsamlingsställe. Produkten får inte kastas i hushållsavfall.
• För mer information, kontakta återförsäljaren eller de lokala myndigheter som ansvarar för avfallshantering.

Fi Erillisen keräyksen merkki Euroopan maissa

Tämä merkki osoittaa, että tuote kerätään erikseen.
Seuraavat maininnat koskevat vain eurooppalaisia käyttäjiä.
• Tämä tuote kerätään erikseen asianmukaisista keräyspisteistä. Älä hävitä tuotetta talousjätteiden mukana.
• Lisätietoja saat jälleenmyyjältä tai paikallisilta jätehuoltoviranomaisilta.

Ru Символ сортировки мусора, использующийся в европейских странах

Данный символ означает, что этот продукт должен утилизироваться отдельно от других.
Приведенная ниже информация касается только пользователей из стран Европы.
• Данный продукт должен утилизироваться отдельно от других в соответствующих приемных пунктах. Не выбрасывайте данный продукт вместе с бытовым мусором.
• Дополнительную информацию Вы можете получить у продавца или у местных властей, отвечающих за утилизацию мусора.

Gr Σύμβολο για την ξεχωριστή αποκομιδή απορριμμάτων στις Ευρωπαϊκές χώρες

Αυτό το σύμβολο υποδηλώνει ότι η αποκομιδή αυτού του προϊόντος πρέπει να γίνει ξεχωριστά.
Τα κάτωθι απευθύνονται μόνο σε Ευρωπαίους χρήστες.
• Αυτό το προϊόν είναι σχεδιασμένο έτσι ώστε να γίνεται η αποκομιδή του σε ειδικά σημεία. Μην το πετάτε μαζί με τα υπόλοιπα απορρίμματα.
• Για περισσότερες πληροφορίες, επικοινωνήστε με τον διανομέα του προϊόντος ή με τις υπεύθυνες τοπικές αρχές για θέματα διαχείρισης απορριμμάτων.

Pl Symbol oznaczający segregowanie odpadów, stosowany w krajach Europy

Ten symbol oznacza, że produkt musi być wyrzucany oddzielnie.
Poniższe uwagi mają zastosowanie tylko dla użytkowników z Europy.
• Ten produkt jest przeznaczony do oddzielnej utylizacji i powinien być dostarczony do odpowiedniego punktu zbierającego odpady. Nie należy go wyrzucać z odpadami gospodarstwa domowego.
• Aby uzyskać więcej informacji, należy skontaktować się z przedstawicielem przedsiębiorstwa lub lokalnymi władzami odpowiedzialnymi za zarządzanie odpadami.

Hu Európai országokban érvénes "Elkülönített hulladékgyűjtés" jelzése

Ez a jelzés azt jelenti, hogy ezt a terméket elkülönítve kell gyűjteni.
Az alábbiak csak az európai országokban élő felhasználókra érvényes.
• Ezt a terméket a megfelelő hulladékgyűjtőhelyen, elkülönítve kell gyűjteni. Ne dobja ki háztartási hulladékként.
• További információkért forduljon a forgalmazóhoz, vagy a helyi hatóság hulladékgyűjtésért felelős részlegéhez.

Cz Symbol pro oddělený sběr odpadu platný v evropských zemích

Tento symbol znamená, že tento produkt se má odkládat odděleně.
Následující pokyny platí pro uživatele z evropských zemí.
• Tento produkt se má odkládat na místě sběru k tomuto účelu určeném. Neodhazujte spolu s domácím odpadem.
• Více informací o způsobu zacházení s nebezpečným odpadem vám podá příslušná místní instituce.

Jp ヨーロッパにおける廃棄物個別回収のシンボルマーク

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• 詳細については販売代理店または地域の廃棄物処理機関にご連絡ください。

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