# **INFINITY ANALYZE**

# **USER GUIDE**

Version 7







# **Document Revisions**

Date	Version Number	Document Changes	
2019-09-23	1.0	Initial Release	
2020-01-08	1.1	Updated to incorporate SW alterations and additions since the initial release, aligned with the release of INFINITY ANALYZE v7.0.2	
2021-01-15	1.2	Updated based on changes implemented for the release of INFINITY ANALYZE v7.0.3, includes workflow improvements in fluorescence acquisition, and expanded file name capabilities.	
2021-07-20	1.3	Updates for INFINITY ANALYZE v7.1.0. PC installs require Windows 10 operating system. Support for INFINITY8-2M camera model, ROI controls, and a new Fluorescence Composite edit function.	
2021-09-15	1.4	Minor revisions and corrections incorporated.	



# **Table of Contents**

Document Revisions	2
Table of Contents	3
Getting Started	5
Introduction	5
Minimum System Requirements	6
Software Installation	6
Licensing	6
Help & Resources	7
Launching INFINITY ANALYZE 7	7
Application Layout	8
Gallery	8
Navigation	10
Control Panel - Brightfield Mode	10
Capture Controls	11
Capture Options	14
Advanced Options	20
Time Lapse / Video	22
Control Panel - Fluorescence Mode	23
Fluorescence Settings	23
Capture Controls	24
Capture Options	27
Advanced Options	31
Image Processing	33
Gallery	33
Image Processing Operations	33
Menu Functions	35
File	35
Camera	38
Edit	39
View	40
Adjust	41
Enhance	43



	Combine	45
	Measure	47
	Annotate	52
	Window	54
	Help	54
Do	cked Windows	56
	Histogram	56
	Measurements	56
	Photometry	56
	Image Properties	56
Sta	tus Bar	57



# **Getting Started**

#### Introduction

The INFINITY ANALYZE version 7 application represents an evolution from the microscopy package that has been offered with INFINITY camera models for over 10 years. The development of this next generation of the INFINITY ANALYZE application is based on a 64-bit architecture, and now offers the same functionality on both the Windows and Mac platforms. Anyone familiar with the INFINITY ANALYZE release 6.X or earlier, will recognize many familiar elements in the layout and workflow. While the style and appearance of the user interface has been updated to look more current, other architectural improvements have also been made to increase memory capacity, perform post-processing operations more efficiently, and streamline commonly used functions.

Certain post-processing functions in previous releases of INFINITY ANALYZE required the purchase of an additional license on a USB key (Multi-Focus Composition and Spherical Aberration Correction). The Multi-Focus Composition has been renamed as Focus Stack Images, and is now found under the Combine menu. It is available to all users in ANALYZE 7, and no longer places an 'EVALUATION' watermark on the processed image. It is also now possible to perform post-processing operations on images acquired using INFINITY ANALYZE 7, without the need of a camera being connected. The requirement to have a USB key license for image processing without a camera has been removed.

# **Upgrades and Features**

- 64-bit architecture and improved memory management
- Windows and Mac same functionality, same structure
- Exposure enhancements include new Hybrid exposure & Peak (auto) modes
- Streamlined fluorescence image acquisition using Sequences
- Basic fluorophore definitions included as examples
- Interactive Gallery of thumbnail images
- Post processing functions include focus stacking and stitching (tiling)
- Calibrations and camera setting Presets are now managed independently, with calibrations being linked to specific Presets.
- Flat Field Correction (FFC) images can also be associated with Presets
- Settings manager utility allows for Presets, Calibrations, and Sequences to be exported and imported – as either a back-up or for sharing with other users
- Integrated video tutorials and Help dialogs
- Improved video clip capture and time lapse acquisition
- Preferences dialog provides control over specific application settings
- Metadata captured and stored as image properties
- Custom filename variables enabled in Auto file saving of images
- Option available to export fluorescence images as OME TIF
- Calibration data is retained in TIF and PNG output files



# **Minimum System Requirements**

INFINITY ANALYZE 7 requires the Windows 10 O/S, or a Mac running OSX 10.12 or higher. The application is a 64-bit program and requires a 64-bit architecture to run. There are presently no plans to support a 32-bit version of INFINITY ANALYZE 7, due to memory and storage allocation requirements. For optimal performance, the computer should have a minimum of 2 GB of additional RAM available when the operating system and any other mandatory programs are already loaded and operational. ANALYZE 7 places a significant demand on the CPU resources when displaying the live preview from high resolution, USB3.0 camera models, but a control has been provided that allows the live preview display frame rate to be limited, if high CPU usage becomes a concern. (See the File → Preferences dialog)

#### **Software Installation**

The INFINITY ANALYZE 7 software is available for download from the Teledyne Lumenera web site under the Support  $\rightarrow$  Microscopy  $\rightarrow$  Drivers & Downloads section. There is no charge to download the software. Anyone can download and install a copy of the software by completing the requested registration form, or logging into their previously created account. The software operates with or without an INFINITY camera connected. When the software runs without an INFINITY camera being detected, the application functions are restricted to image processing tasks.

INFINITY ANALYZE versions 6.X and version 7 can co-exist on the same computer. There is no requirement to uninstall an existing copy of INFINITY ANALYZE version 6. This provides the opportunity to retain the older version, while trying the latest version. Images stored in the native .SIF file format from INFINITY ANALYZE version 6 can be opened using ANALYZE 7, and are held temporarily in TIF format, until saved. The File > Convert File Format menu provides a utility to bulk convert SIF files into other formats. Select TIF or PNG as the output format if the .SIF files include annotation objects or measurements that should be preserved.

INFINITY ANALYZE 7 installs under the **Teledyne Lumenera** heading in Program Files on Windows O/S platforms, whereas the older version of INFINITY ANALYZE would be located under the **Lumenera** heading.

### Licensing

Most INFINITY camera models include a built-in license key that is identified by the application. If the INFINITY ANALYZE 7 application reports that no license was detected for INFINITY ANALYZE, contact the Technical Assistance Centre (TAC) team via email requesting to purchase a license for your camera. License keys are a permanent, one-time purchase, and will be sent electronically. The license key activation program requires a Windows computer to program the license permanently into the camera.



# **Help & Resources**

INFINITY ANALYZE 7 includes context menus that are accessed with a right-mouse-click, many tool-tips that provide guidance when the mouse hovers over a button or icon, and help dialogs to guide new users through setup. Help dialogs include an option to prevent that dialog from being displayed again. Once suppressed, help dialogs can be accessed directly from the Help menu, where there's an option to re-instate the automatic display.

Video tutorials are located on the Teledyne Lumenera web site under the Support  $\rightarrow$  Microscopy  $\rightarrow$  Training & Tutorials page, but a selection of brief video tutorials have been included, within the application, on certain Help dialogs for quick reference whether the computer has access to the internet or not.

This User Guide is intended to be a quick reference to the application features and functions. More detailed steps and instructions will be provided in the video tutorials that are featured in the INFINITY ANALYZE 7 section of the Support → Microscopy area of the web site.

# Launching INFINITY ANALYZE 7...

On a Windows computer, the INFINITY ANALYZE program installs under the **Program Files** > **Teledyne Lumenera** folder, by default. Create a shortcut on the Desktop, or add a Quick Launch icon to the Tool Tray, if desired.

When the INFINITY ANALYZE 7 launches, it connects to any available INFINITY camera model. If more than one camera is detected, a dialog is presented with a list of available cameras to choose from, as the application adjustments can only control a single camera at one time. It's possible to switch between control of multiple cameras by using the Camera > Connect... menu. Alternately, the INFINITY ANALYZE 7 program can be launched multiple times, enabling a connection to a different camera, for each instance of the application.

On a Mac computer, the INFINITY ANALYZE package should be placed in the **Applications** folder, and can be launched from there.

The application will detect multiple cameras when more than one INFINITY model is connected, and provide a camera selection dialog. To launch the application multiple times, requires that the INFINITY ANALYZE program be duplicated in the Applications folder and assigned a new name. It's then possible to launch the program and the duplicate at the same time to control two separate INFINITY cameras.



# **Application Layout**

The application functionality is presented as two distinct modes, where one mode is for typical brightfield applications and the other mode is targeted to fluorescence applications. Switching between modes is simple, with the interface showing either a light or dark appearance, by default, to indicate which mode is currently active. The File  $\rightarrow$  Preferences... menu provides the setting under Themes, to specify a preference for the application's background appearance in either mode.

The two modes include a certain amount of overlap in the camera controls, use of the gallery, calibrations, etc. The main distinction between the modes is with the configuration of the workflow for fluorescence image acquisition which uses the concept of user-defined Sequences, and the retention of additional camera settings based on the channel definitions. The Sequences control the number of channels and their associated look-up table color overlay, along with exposure and gain controls on the camera. Once a Sequence definition has been created and adjusted for the samples being used, these parameters are retained within the Sequence definition to facilitate image capture and the storage of multiple images.

The application launches initially in the Brightfield Mode for all color camera models. Subsequent launches of INFINITY ANALYZE will open it in the same configuration that was active when the application was last closed. In the initial application configuration for either Brightfield or Fluorescence Modes, a Help dialog is presented to assist new users with the steps required to setup their initial Camera Controls and/or Sequence definitions. Each Help dialog includes a check box that can be selected to prevent the dialog from being displayed. Visit the Help menu to reinstate or disable the automatic display of the Help dialog.

# **Gallery**

The image Gallery is displayed in the default layout, but can be either hidden or moved to a different location to suit individual preference for screen layout or use of available space. Captured images can be directed to the Gallery, which serves as a holding place and quick reference. Images in the Gallery will show the file name, if they are being simultaneously saved to disk. If automatic saving to file is not enabled, then a temporary name is assigned and displayed. Any unsaved images in the Gallery include an asterisk (\*) at the end of the displayed name indicating that the image has not been stored permanently.

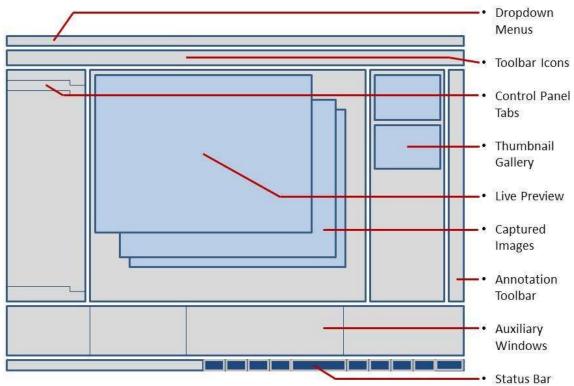
The Gallery supports operations from a right-mouse click, with the list of options varying based on whether one or multiple thumbnails have been selected. The Gallery supports click & drag or <Shift> click for selecting multiple adjacent thumbnails. A <Ctrl> click provides multiple non-adjacent thumbnail selections. Image thumbnails are added to the bottom of the current set, with the Gallery view automatically shifting downwards to show the new entry.

A variety of post-processing image operations can be accessed using the pop-up menu item "Open in Image Processing Dialog..." Refer to the section below on the Image Processing Dialog for additional details on both Gallery operations and Image Processing.



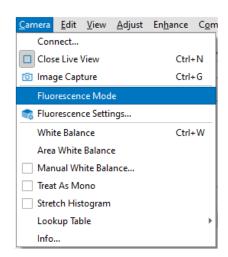
A schematic representation of the application layout (Brightfield Mode) is shown below for quick reference.

# Brightfield Mode Application Layout (Simplified)



Use the application Toolbar Icons or the Camera Menu dropdown to switch between the application s' **Brightfield Mode** & **Fluorescence Mode**.







# **Navigation**



 $\odot$ 

Q

#### Pan

When the mouse icon appears over an image as an open hand, use a left mouse click and drag operation to pan the image to position the desired field of view within the window. The icon changes to a grasping (fingers closed) icon when the mouse is clicked, to confirm that a pan operation is being performed. The image must be zoomed such that the displayed image is represented larger than the window's boundaries, for the Pan operation to function. Scroll bars appear on the right and bottom edges of the live preview or captured image window when zoomed in, and these can also be used for manipulating the preview region.

### Zoom

Several techniques are provided to zoom in or out on the active image. Use the scroll wheel on the mouse to dynamically zoom in or out on the active image until the desired zoom scale is achieved. Using either the View  $\rightarrow$  Zoom... menu item or selecting Zoom... from the right-mouse-click menu on the active image, displays an interactive zoom slider at the top of the active window. Use the tools within the zoom slider to

set a suitable zoom scale. Clicking anywhere else other than on the zoom slider will remove the control.



# **Magnifying Glass**

This function provides a pop-up window that shows a zoomed view around the mouse location in the active window. Use the View → Magnifying Glass... menu item to access this tool. Annotation and measurement overlay graphics appear in the magnified view, and a cross-hair indicates the exact center representing the precise location of the mouse pointer.

# **Control Panel - Brightfield Mode**

When INFINITY ANALYZE launches for the first time on the computer, it opens in Brightfield Mode, if the connected camera is a color model. A Help dialog is displayed as soon as the Live Preview window appears. The Help dialog provides a few simple steps that will assist a new user to understand how to quickly configure the camera for the illumination source on the microscope, by making a couple of adjustments and performing a White Balance. The Help dialog includes quick access to a short video tutorial. To suppress the Help dialog from appearing use the checkbox at the bottom of the dialog. An option is provided in the Application's File → Preferences... menu dropdown, to reinstate the automatic display of the Help dialog, if desired.





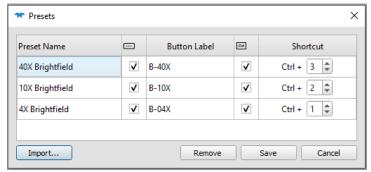
# **Capture Controls**

#### Presets

Use Presets to store camera settings for a specific microscope objective, sample type, filter, or light source. Use the Save As... button to store a new Preset by specifying a name in the pop-up dialog. One or more Presets can be created or added at any time by using the Save As... function and giving each new Preset a unique name. As each Preset is saved it is included in the dropdown list in the Presets section of the Capture Controls panel. A Preset is activated by selecting the name from the dropdown list, which restores the saved camera settings.



Click the cog icon to launch the Presets Manager dialog. A list of Presets is displayed with fields and checkboxes to the right of each Preset. Click the checkbox to the right of any Preset to include a shortcut button in the Capture Control panel. Double-click in the



Button Label field to the right of the checkbox to insert an abbreviated name for any Presets that require a shortcut button. Use shortcut buttons to provide quick (single mouse click) access to activate the most commonly used Presets. Each Preset that has an assigned Button Label will have a button displayed above the dropdown list in the Capture Controls. The second checkbox can be selected to enable a keyboard shortcut for any Preset. Configure the shortcut Buttons and hotkeys to optimize your personal workflow. Preset Names can be edited or deleted from the list using the Presets Manager dialog.

Hover the mouse over any shortcut button to see the full name of the Preset that it is associated with and the <Ctrl> key number assigned (if identified).

If any camera settings are altered while a specific Preset is active, an asterisk (\*) is displayed after the Preset name. The asterisk indicates that there are changes to be saved. Click on the Save button to update the currently active Preset with the new camera settings, or click the Reset button to alter the camera settings back to the conditions that are currently stored for the active Preset.

Choose the specific Preset labelled "(None)" to disable the use of Presets. Whenever a user-defined Preset is active the asterisk will appear after the name whenever camera settings are altered, and warning dialogs will be presented when switching to a different Preset.



#### Capture

The Capture button provides one mechanism for acquiring a still frame from the camera.



A double-left-mouse click in the Live Preview window, or a <Ctrl> g on the keyboard will also acquire a frame. See the **Capture Options** section to configure what happens when images are captured.

#### Live View / Close Live View

This button is will show / hide the camera's live preview window. The text label on the button alters to indicate the function performed if the button is clicked. A <Ctrl> n on the keyboard can be used to toggle the Live View.

#### White Balance

It is necessary to perform a White Balance operation any time that a camera is first setup on a microscope, or whenever the illumination conditions are altered. The camera relies on the White Balance operation to set the Red, Green, and Blue color channels to the appropriate levels in order to produce the best color response for the lighting conditions. Best results are obtained when the specimen is removed from the field of view, and the exposure time has been adjusted to produce a live preview of moderate brightness, prior to clicking the White Balance button. Once the camera has been properly adjusted, use the Presets to store the camera settings for the particular lighting configuration. Refer to the Help dialog for Camera Configuration instructions.

## Area WB (White Balance)

Use the Area White Balance button in a similar manner as described for the White Balance function, but when it is preferable to leave the specimen in the field of view. Once the Area White Balance function is initiated, use the mouse to select a suitable area in the live preview that represents the background illumination. The results obtained from the Global White Balance function are often more predictable.

#### Active Calibration

The Active Calibration stores the reference scale information relevant to the current magnification or objective. Use the Calibrate operation on the Measure menu to produce a Calibration with reference measurement units. An Active Calibration can be associated with a named Preset. The workflow steps of changing the microscope magnification and then selecting the appropriate Preset will restore the camera settings and the specified Calibration at the same time.

Where more than one INFINITY camera is used on the same computer, the calibration settings will be visible for each camera. Use a naming convention that identifies the calibration settings pertinent to each camera.

Captured images retain the calibration setting that was active at the time the image was acquired. Measurements made in captured images will be accurate based on the calibration setting associated with the image, and is not dependent on the currently selected calibration (that applies to the live preview). Images stored to media as either TIF or PNG format will retain the calibration settings in the file metadata. When the



image is later opened in INFINITY ANALYZE, the calibration settings are used by the software to allow for additional measurements to be made. Note: Images saved to media in a BMP or JPG format do not have the ability to store / retrieve calibration settings.

Images can no longer be saved to media using the INFINITY ANALYZE native SIF file format. These images can be imported or opened by INFINITY ANALYZE 7. Store images using the TIF or PNG format to preserve the calibration settings.

# Exposure Control Mode

The dropdown menu provides the options of Manual, Hybrid, Mean, and Peak, for the adjustment of the camera exposure time. When Manual mode is active, the Exposure and Gain adjustments can be typed into the text entry boxes, or the slider controls can be used. The exposure time is the integration time for light to reach each pixel, while the Gain is an amplification of the signal at the sensor.

The Hybrid Exposure Control Mode allows the image brightness to be changed by altering the exposure slider or changing the value in the numeric field. The actual exposure time is being simulated, by employing a relatively short exposure time while using a gain increase to boost the brightness. This allows for the camera to respond with a faster frame rate than would be normally attainable if a long exposure time were specified. This mode is of benefit under very low light conditions, so that the frame rate is sufficiently fast to allow for better framing and focusing on the sample. When the Hybrid Exposure Mode is active, be prepared for the image capture time to take a moment to respond, as the camera gain is reset to 1.0, while using the actual exposure time indicated to acquire the image. The result is an image that is far less noisy in appearance than the live preview which has simulated the longer exposure time using gain amplification.

The two other options of Mean and Peak are automatic image brightness controls that allow the target brightness to be specified using the slider or numeric field, as a percentage of saturation (maximum pixel intensity). These two options differ in that the Mean uses the average pixel intensity in the entire field of view as the value to attain, while Peak adjusts the brightness to have the upper 0.5% of the pixel intensity values at the indicated percentage. The application is using the Hybrid mode, while monitoring the pixel intensity values to achieve the specified scene brightness, so Exposure time is first increased, and then the sensor Gain (amplification) is applied to simulate longer exposure times in the live preview.

When either the Mean or Peak auto exposure modes are active, the Advanced Options controls for Brightness, Contrast, and Gamma are disabled. This is necessary to enable the auto exposure algorithm to function correctly. The Camera → Lookup Table menu selection allows for a custom Lookup control to be defined and applied to the live output.



# Exposure

The camera exposure time is the principal adjustment required to obtain an image with the desired brightness. Short exposure times are used in bright conditions and longer exposure times are required under darker conditions. Manual Exposure time adjustments are performed using either the manual slider, by typing a new exposure time in the field, or by using the up/down arrows to the right of the exposure time field. Exposure times are indicated in milliseconds (ms) [1/1000 sec]. The camera's minimum and maximum exposure times are shown on either end of the slider.

The application includes two auto-exposure adjustment modes. When either Mean or Peak are selected for the Exposure mode, the application automatically controls the camera exposure to match the specified target value as the light level changes. See the Exposure Control Mode section for details.

### Intensity (%)

Use the Intensity target slider with Mean and Peak Auto Exposure modes, or type a value into the numerical field, or use the up/down arrow buttons to the right of the numerical field, to adjust this control. Numerical values offer the range of zero % (black) to 100% (white), and represent the target scene brightness that the application uses to adjust the camera exposure. Percentage is used rather than an explicit pixel intensity number to more easily accommodate the use of 8-bit or 16-bit modes (representing 10-bit, 12-bit, or 14-bit outputs available in different camera models).

#### Gain

The Gain slider is used to adjust the amplification applied to the camera sensor's signal. Under most brightfield conditions, it's recommended to keep the camera gain in the range of 1.0 to 2.5. When low-light conditions or high magnification result in long exposure times and reduced frame rates, then using higher gain settings may be required.

# **Capture Options**

#### ROI [Region of Interest]

**Resolution -** The dropdown list provides a range of output dimensions for the live preview from the connected camera. The image sensor chip's maximum resolution is presented at the top of the list, with reduced image output sizes being presented in descending order. Frame rate increases will be observed in camera models where enabled by the image sensor. The ROI is centered on the image sensor.

Presets can be used to provide quick access to different ROI settings. Captured images will match the dimensions of the live preview based on the selected ROI.

Note: If annotation or measurement graphics are present in the live preview when a different ROI is selected, the graphics will retain their position relative to the live preview



window, rather than to the image being acquired. When the original ROI is restored, the overlay graphics will display in their original locations.

**Sampling -** Most camera models support either **Binning** or **Subsampling** options. Most CCD image sensors provide binning, where the output from adjacent pixels is summed at the sensor chip level, resulting in a lower resolution output with a brighter result. Most CMOS image sensors provide the ability to operate with subsampling. This technique skips the output from adjacent pixels, producing a lower resolution live preview, without a change in image brightness.

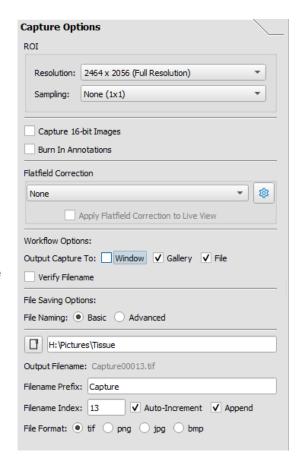
Note: Create specific calibrations to use with either binning or sub-sampling modes enabled. Since both binning and sub-sampling alter the effective pixel size, a calibration should be created for use specifically when using a sampling mode and taking measurements.

# Capture 16-bit file format

When checked, images stored in the TIF or PNG file formats will use the camera output of either 10-bits, 12-bits, or 14-bits of extended data range resulting in a 16-bit file format.

#### Burn In Annotations

When this option is checked, any measurement or annotation objects within the live preview image will be permanently burned into the output file. While the TIF and PNG file formats typically retain the overlay graphics in a dynamic (metadata) structure, enabling the Burn In Annotations setting results in the overlay graphics being permanently drawn on the image files. Burned-in graphics cannot be manipulated independently of the image background, even when next opened using INFINITY ANALYZE. All graphic objects will be visible in the images when viewed with 3<sup>rd</sup>-party software packages, when they have been burned-in. Enable this option when it is important to share images with others, and the overlay graphics must be visible. Disable this option to retain dynamic copies of overlay graphics in TIF & PNG output files. The output image file types of JPG or BMP are disabled when an image includes



overlay graphics and the Burn In Annotations check box is unselected.



# Flat Field Correction (FFC)

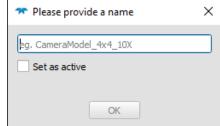
The active FFC is shown in this field. Other FFC images that have been stored will be named and can be activated from the dropdown list. FFC images are captured images that are assigned an FFC name from the option located on the right-mouse-click pop-up menu. Click the cog icon to access the Flat Field Settings Manager dialog to delete or rename existing FFC images. An active FFC image will be associated with an active Preset.

A Flatfield image can be stored for each objective magnification and/or lighting configuration that is used on the microscope. The following preparations should be made prior to setting up any Flatfield Corrections:

- Remove any sample from the field of view
- Adjust the camera exposure to a medium brightness (for example, choose the Exposure Control Mode of Mean with the Intensity % value between 50 and 75)
- Perform a White Balance operation

Follow these steps for each Flatfield Correction being performed:

- Ensure that the Flatfield Correction dropdown, in Capture Options, shows 'None'
- Capture the Live Preview image and have the result open in a Window
- Use a right-mouse-click on the image window and choose Save as Flatfield Image from the pop-up menu.
- Enter a descriptive name for this Flatfield image and click OK
- Repeat these steps for each microscope objective and optical configuration that requires a Flatfield Correction to be applied
- A list of Saved Flatfield Correction images is now available in the dropdown list.



The Settings cog icon can be used to review the list of available FlatField Corrections or to delete any unwanted Correction images.

To associate any Flatfield Correction with a Camera Preset, ensure that the desired Preset is active, then select the Flatfield Correction from the dropdown list. As soon as any change to camera settings are made, an asterisk is displayed adjacent to the Camera Preset Name. Use the Save button below the Camera Preset, and the currently active Flatfield image is immediately associated with the current Preset, and will be used each time the Preset is activated.

To remove a Flatfield Correction from a Preset, activate the Camera Preset, then select 'None' from the Flatfield Correction dropdown list. Ensure that you return to the Preset and click the Save button to update the Camera Preset with the change.



# Output Capture To

When images are acquired from the camera they can be directed to be stored in a File, displayed in a Window, or sent to the Gallery. Selecting multiple options is possible.

# Verify Filename

When this setting is enabled, the application will present a dialog coincident with each image capture, to allow for a custom file name to be entered for each image file saved to disk.

# File Saving Options

#### o Basic

Allows for single filename specification, or for the inclusion of an auto-incremented number on the end of the filename.

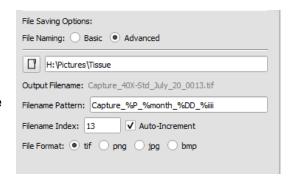
The Output Filename is indicated, based on the selections made and the Prefix provided.

File Saving Options: File Naming:   Basic Advanced			
H:\Tissue_Stains			
Output Filename: Sample_33_00005.png			
Filename Prefix: Sample_33_			
Filename Index: 5			
File Format: ○ tif ● png ○ jpg ○ bmp			

Enter the desired Filename Prefix. Use the Filename Index to specify a starting value for an appended incremental filename extension, if desired. The Append and Auto-Increment checkboxes provide the flexibility to include the auto-numbering on the end of the filename Prefix, and to choose whether the number increase is made by the application, or entered manually by the operator.

#### Advanced

Enables custom filename specifications where the inclusion of specific tokens can be included within the filename characters. See the table of available tokens below.



Use of the Advanced mode

enables the inclusion of tokens within the filenaming, if desired. Autonumbering of files is obtained by including the %iiii token anywhere in the Filename Pattern, where the number of "i" characters determines the string length for the numbering, such that an Index value of "5" will result in "00005" being used for the filename, if %iiiii is used in the Pattern field. The incremental file numbering is not restricted to the end of the Filename. A combination of different filename tokens along with user-



specified text strings provides a range of flexibility in the filenaming for captured images.

To store acquired images automatically to a file folder, specify the folder location using the folder icon or type the folder location in the text field. The Filename prefix is displayed, and can be edited in that field. Select the output file format using the Format buttons.

File storage format options include TIF, PNG, BMP, and JPG. Calibration data is stored within TIF and PNG output files only. When the Capture Option is set for 16-bit Images, the only format options are TIF or PNG. Images cannot be saved as BMP or JPG with 16-bit image data.

Measurement and Annotation data added will be retained dynamically if the output format of TIF or PNG is selected, <u>AND</u> the option to Burn in Annotations is <u>un</u>checked in the Capture Options panel. When these types of images are opened within INFINITY ANALYZE 7, the graphic objects may be repositioned, deleted, or edited. The measurements and annotations will not be visible when these types of images are opened using 3<sup>rd</sup>-party editing or viewing software. To permanently save micrometer, measurement, or annotation graphics, store 8-bit images in BMP or JPG format.

Enable the Burn in Annotations check box to commit all graphics permanently in any of the available image formats. When an image has been saved with this option enabled, the graphics will appear in the images when shared, or viewed in other image edit/view software.

When saving images to File, it is recommended that the Auto Increment Filename be activated, otherwise subsequent image capture operations will overwrite the existing saved file. When the Basic File Naming is enabled, check the Append option to enable the inclusion of the auto-increment value at the end of the user-specified file name.

# Auto-increment Filename Index

When enabled, the auto-increment function appends a sequential number to each captured image file. Enter a numerical value in the text field to specify a starting number to be appended to the root File Name entered in the field above.

#### Filename Prefix

The basic filename provision allows for the specification of an output folder location and a filename prefix to be supplied. The Auto-increment numbering is appended to the end of the supplied prefix, and incremented with each acquisition.

#### Advanced Filename Options

Advanced filename specification is possible with the inclusion of tokens. A table of available tokens is provided below. Select the desired tokens and specify them in order



of preference. When each image is captured, the tokens are resolved into text strings and the resulting filename is used to save the image to the specified folder location.

Some filename variable tokens have more than one %<char> representations, but produce the same result.

Category	Description	Token	Example & Range
Year	Numeric year 4 digits	%у	2018
		%Y	2018
		%YYYY	2018
		%year	2018
	Numeric year 2 digits	%YY	18
Month	Month of the year – full	%month	January-December
	Month of the year – abbrev.	%mon	Jan-Dec
	Month of the year - numeric	%m	00 – 12 (double digit)
		%M	00 – 12 (double digit)
		%NN	00 – 12 (double digit)
Day	Day of the week – full	%day	Sunday-Saturday
	Day of the week – abbrev.	%a	Sun-Sat
	Numeric day of the month	%d	00 – 31 (double digit)
		%D	00 – 31 (double digit)
		%DD	00 – 31 (double digit)
	Numeric day of the year	%DDD	000 – 366 (triple digit)
Time	Hour of the day – 24 hr	%hh	00 – 24 (double digit)
	Hour of the day – 12 hr	%HH	00 – 12 (double digit)
	AM / PM for 12 hr clock	%A	AM or PM
	Minutes	%mm	00 – 59 (double digit)
		%MM	00 – 59 (double digit)
	Seconds	%ss	00 – 59 (double digit)
		%SS	00 – 59 (double digit)
Counter	Incremental counter	%ii	00 - ## (digits equal to number of "i")
Sequence	Fluorescence Sequence name	%s	Eg.:Three_Channel_BGR
		%S	
Channel	Fluorescence Channel name	%с	Eg.:GFP(Green)
		%C	
Preset	Brightfield active Preset name	%р	Eg.: 40X PlanApo
		%P	

These special characters are not permitted in filenames:

\	/	:	*	?	"	<	>	1.	Λ



# **Advanced Options**

# Camera model specific settings

# Enable camera cooling

Available as an option <u>only</u> when an INFINITY3-1M or INFINITY3-1C camera model is connected. This setting is used to turn the Peltier cooling unit in the camera on or off.

# High Conversion Gain (HCG)

Available as an option <u>only</u> when an INFINITY8-2M camera model is connected. This setting is used enable or disable the camera's high conversion gain mode. In brightfield mode this setting would typically be disabled to improve the dynamic range output under bright conditions. Under low-light conditions this setting would be enabled such that shorter exposure times can be used.

# Light Source

Select the Light Source setting that produces colors in your sample that most closely match what is seen through the microscope eyepieces. This camera setting applies only to color cameras, with the purpose of reducing the bias introduced into the camera by light source types with different color temperatures.

#### Orientation

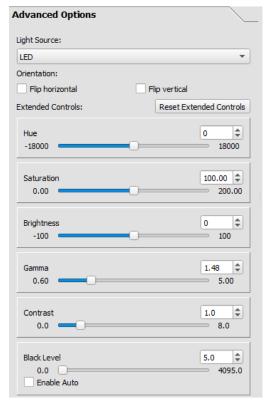
The combination of mirrors and prisms within any microscope will result in the image being produced in the camera having either the same orientation as in the eyepiece, or an image that is rotated by 180 degrees. Use both the flip horizontal & flip vertical settings to orient the camera image to match that seen in the oculars.

#### Extended Controls

Control sliders to adjust the camera settings for Saturation, Hue, Contrast, and Brightness are

provided. Under most circumstances these settings should remain at their default values, and this is especially true for Hue, Contrast, and Brightness. Changing any of these settings by any significant amount will most likely produce an undesirable image output.

**NOTE:** The adjustments for Brightness, Gamma, and Contrast are disabled when the Mean or Peak AutoExposure modes are active. Settings for these three controls must be disabled when the camera image intensity is being





controlled by the AutoExposure function, as changes to these settings impact the linearity of exposure time alterations.

The Saturation slider can be set to zero to remove all color information from a color camera image, should a monochrome result be desired [See also the menu option Camera → Treat as Mono]. Setting the saturation to a value between 1.0 and 1.5 will produce more vibrant color output, and may be especially useful when attempting to image metal objects such as copper, brass, bronze, or gold. Use the Reset Extended Controls button to return all control values in this section to the camera defaults.



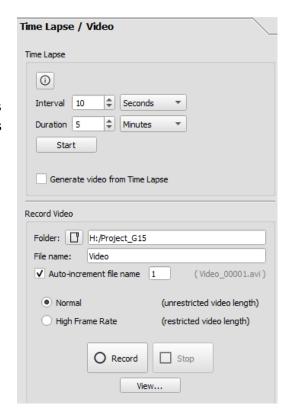
## **Time Lapse / Video**

## Time Lapse

The production of time lapse sequences of images is enabled with the controls in this section. Specify the desired interval for image capture, and the duration of the overall sequence of frames, using the numerical fields and the units dropdowns. If saving the images to file, be sure to have the option enabled for Auto-increment file name in the Capture Options panel, so that each image in the time lapse will be uniquely named. It's best to direct the images to File for time lapse operations, and to disable capture to Window.

Push the Start button to begin the acquisition. A dialog appears on-screen during the acquisition, and provides the option to pause or abort the sequence.

The application will determine the minimal interval required between frames that the application is capable of delivering with the



current settings invoked. Some image conversion settings are time consuming. It is currently not possible to acquire time lapse images with very short intervals.

If the option is checked to produce a video from the sequentially numbered output frames, then a dialog is presented on completion of the capture sequence, that allows for the AVI file name and frame rate parameter to be specified and for the video to be played once produced.

#### Record Video

Configure the Folder and File name settings appropriately. Press the Record button to initiate recording of a video, and use the Stop button to end the recording. An option can be used to add an auto incremented suffix to the video files. Choose between the two options for the technique that will be used to acquire the video clip. Use the Normal method to produce a video of longer duration. This technique will write the video to the media at a steady rate for long periods. Use the High Frame Rate method if the computer has reasonable amounts of unused RAM (memory), that the software can use as a buffer while recording short but fast video clips.

Video files are stored as **.wmv** format on a Windows system, and in **.avi** format on a Mac system.



**NOTE:** The Video Preview utility in ANALYZE reports recorded video files as having no content on the Mac. However, using QuickTime (or a 3<sup>rd</sup>-party player) will allow the .avi files to be viewed correctly.

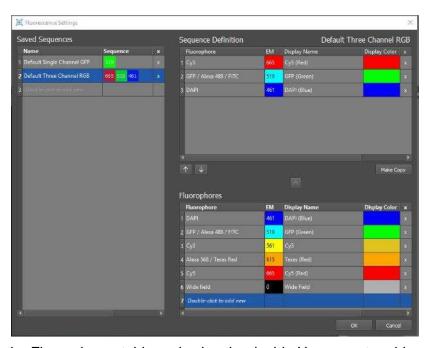
**NOTE:** Choosing to limit the Live View Refresh Rate in the File > Preferences > Display panel, or closing the Live Preview window during video recording will likely result in an increase in the video recording framerate.

# **Control Panel - Fluorescence Mode**

When INFINITY ANALYZE first switches into Fluorescence Mode, a Help dialog provides a quick overview of the Fluorescence setup required to produce a custom Sequence. The Sequences are used to define the camera settings and the order of operations for collecting individual images used to produce a fluorescence color composite. The Help dialog includes quick access to a short video tutorial. To suppress the Help dialog from appearing use the checkbox at the bottom of the dialog. An option is provided in the Application's Help menu dropdown, to reinstate the automatic display of the Help dialog, if desired.

# **Fluorescence Settings**

The Fluorescence Settings dialog holds the definitions of available fluorophores to be grouped into capture Sequences. Add New Sequence names by double-clicking in the last row under Saved Sequences. Add your own Fluorophores to the default list by double-clicking in the bottom row of the table and filling in the fields, including the selection of a color Look-Up Table (LUT) to be applied to the channel output. Use the 'X' at the end of any row to delete a specific entry from either of the three tables. Promote



Fluorophores by selecting them in the Fluorophores table and using the double Up arrow, to add them to the Active Sequence. Reorganize the order for image acquisition by selecting any row in the current Sequence Definition table and selecting the Up or Down arrow to relocate it. The 'Make Copy' button will duplicate an existing Sequence allowing you to provide it with a new name and make any necessary adjustments.



# **Capture Controls**

## Sequence

The concept of Sequences is similar to the Presets used in Brightfield Mode, however with a Sequence the camera settings are stored for a defined set of fluorescence channels. When a specific sequence is active, the workflow for the fluorescence capture is simplified as the application will restore the saved camera settings and apply the chosen color Look-Up Table (LUT) to each defined channel. If the camera settings are adjusted as part of a composite image acquisition, the Sequence name will be displayed with a trailing asterisk (\*) to show that the standard settings have been adjusted. When the mode is switched a dialog is presented to allow for the altered settings to be retained in the current Sequence, or discarded, or for a new Sequence definition to be added.

Use the Fluorescence Settings dialog to create a new sequence or to modify an existing sequence. Or use the Save As... button to create a sequence with the same fluorophores but with different camera settings. This allows for multiple Sequence definitions to be saved for use with samples that have different camera requirements.

Default Sequences are provided as a starting point, and can be either copied or renamed. If all sequences are deleted from the list, the application will re-generate the default sequences next time it launches.

Specify the desired Sequence from the drop-down list to make it the active Sequence.

#### Start Sequence

Click this button to initiate a new multi-channel acquisition using the active Sequence. Rotate the filter cube to the appropriate position for the first channel, make any necessary microscope adjustments for focus and field-of-view. You can zoom in on portions of the Live Preview image to facilitate focus adjustments. Make any camera settings adjustments for image quality. When ready, use the Capture button to acquire the image. The application then activates the next channel in the Sequence to repeat the channel captures until the Composite image is produced at the end of the Sequence.

During the setup for a new sequence, or when making adjustments to the configuration settings, disable the setting labelled Auto-Select Next Channel in Sequence, to allow repeated Capture button clicks without advancing to the next channel in the Sequence. This allows for the settings to be adjusted and confirmed on one channel, prior to changing to the next channel. Enable the checkbox and click Capture again, to advance to the next Channel. Once the Sequence is completed, this option must be enabled for the Composite result to be generated.

**Note:** When a Single Channel Sequence is active, it is not necessary to click either the Start Sequence or the Save Images button, as the workflow settings are optimized to simply allow for the Capture button to be pressed to acquire each single channel image and save it according to the auto saving options defined in the Capture Options panel.



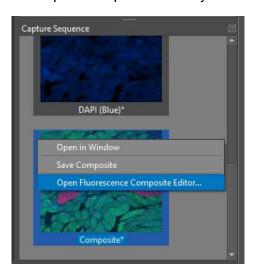
# Save Images

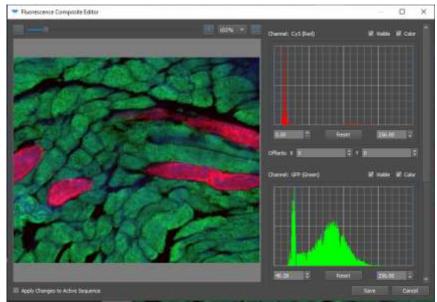
Images are transferred from the Capture Sequence into the Gallery, and saved to disk using the file name and folder specified in the Capture Options.

### Capture

Use either the Capture button or double left-mouse-click on the live preview image to acquire an image from the camera. Captured images are displayed in the Capture Sequence Gallery, and the next channel in the sequence becomes active. Return to a previous channel by selecting the thumbnail image in the Capture Sequence Gallery.

On completion of a Multi-Channel capture sequence, a thumbnail representation of the Composite result is displayed in the Capture Sequence panel. Use a right-mouse click on the Composite thumbnail, and select **Open Fluorescence Composite Editor**, to launch a post-processing window with controls to manipulate each channel using the histogram and (X,Y) position offsets, as desired. Checkbox controls permit toggling channel visibility on/off, or showing a channel without the color LUT only during the edit session, these controls do not impact the appearance of the composite result.





The Composite Editor includes a checkbox to optionally apply the channel edits to the



active Sequence.

#### Close Live View / Live View

The Live Preview Window can be removed or reinstated at any time using this button.

#### Active Calibration

Select from the list of defined calibrations to capture images with the desired scale reference for the magnification being used. Annotations included in the Live Preview (eg. Micrometer scale bar) are not transferred to the Composite or Single Channel fluorescence images within the Fluorescence Mode. Open a composite image in the Brightfield mode to add micrometer bars or measurements.

# Exposure Control Mode:

Choose between Hybrid, Manual, Mean, and Peak options for the image exposure. The Hybrid mode is active by default, and uses a combination of exposure time and sensor gain to produce a preview image that maintains a refresh speed of more than 2 frames per second – allowing sufficient updates to frame the sample and focus the image. When the image is captured, the acquisition will take the indicated exposure time, as the gain is adjusted back to unity for the image capture. Select Manual as the Exposure Control Mode if you want direct control over the camera's exposure and gain using the sliders or text fields. Selecting either Mean or Peak Exposure modes enables the application to control the image brightness using Hybrid mode, based on the Mean or the Peak pixel intensity values to achieve the target brightness for the image as indicated in the slider for Intensity (%)

# Exposure

Manual Exposure adjustments can be made using the Exposure slider or by typing numerical values in the field.

#### Gain

Use the Gain slider adjustment or numerical input box to amplify the camera signal. A gain setting increase will assist with maintaining shorter exposure times, but the quality of the image output usually includes increased noise in the data, if the gain is increased too much.

# Select Input Source

Choose between using all color channels or a single color input channel for a color camera.

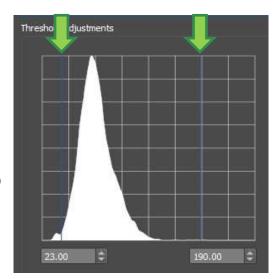
# Apply Display Color to Channel

The current channel is displayed using the Look-Up Table (LUT) color defined by the Sequence definition. Use this control to disable or enable the color LUT. When disabled, the camera output is displayed in greyscale.



# Threshold Adjustments / Histogram

The Threshold Adjustments control provides a histogram display in real time for the live preview. Use the graphic to monitor how the exposure and gain settings are influencing the camera response to the sample. The horizontal axis represents pixel intensity values from dark on the left to bright on the right. The vertical axis represents the number of pixels with a specific intensity value in the image. Two (blue) vertical bar adjustments are mouse controlled. Moving the left bar to the right clips the bottom of the image data to increase the black level cut-off. Moving the right bar to the left increases the amount of data that is shifted towards being saturated.



Individual channel images can be stored without the Threshold Adjustments being applied, by selecting the Capture Option setting labelled Include Raw Component Images.

# Show Component Channels

Use the buttons displayed in this section to control which Sequence channels are currently included in the output color composite image. By default all color channels are enabled. Disable or enable individual channels and they are immediately removed or added back into the displayed composite result.

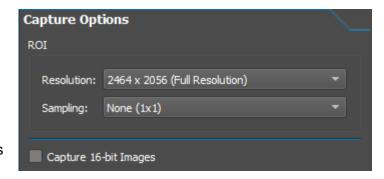
# Shift Component Image

Horizontal and Vertical adjustments can be applied to the currently active channel to provide alignment of the sample that is introduced by the wavelength shifts in the optics. Adjustments are made in single pixel increments using the numerical values entered manually or with the up/down controls adjacent to the numbers.

# **Capture Options**

ROI [Region of Interest]

Resolution - The dropdown list provides a range of output dimensions for the live preview from the connected camera. The image sensor chip's maximum resolution is presented at the top of the list, with reduced image output sizes being presented in descending





order. Frame rate increases will be observed in camera models where enabled by the image sensor. The ROI is centered on the image sensor.

A specific Sequence can be used to retain an ROI specification. All channels within a capture sequence will have the same ROI. Captured images will match the dimensions of the live preview based on the selected ROI.

Note: If annotation or measurement graphics are present in the live preview when a different ROI is selected, the graphics will retain their position relative to the live preview window, rather than to the image being acquired. When the original ROI is restored, the overlay graphics will display in their original locations.

**Sampling -** Most camera models support either **Binning** or **Subsampling** options. Most CCD image sensors provide binning, where the output from adjacent pixels is summed at the sensor chip level, resulting in a lower resolution output with a brighter result. Most CMOS image sensors provide the ability to operate with sub-sampling. This technique skips the output from adjacent pixels, producing a lower resolution live preview, without a change in image brightness.

Note: Create specific calibrations to use with either binning or sub-sampling modes enabled. Since both binning and sub-sampling alter the effective pixel size, a calibration should be created for use specifically when using a sampling mode and taking measurements.

# Capture 16-bit Images

When checked, images stored in the TIF or PNG file format will use the camera-specific output of either 10-bit, 12-bit, or 14-bits of extended data range resulting in a 16-bit file format. The BMP and JPG formats are not able to store the 16-bit data format.

#### Flatfield Correction

Choose from the list of stored flatfield correction images to apply the correction to captured images. Enable the option to Apply Flatfield Correction to Live View to verify the performance. It is not advisable to leave this option enabled due to the reduction in frame rate, however, it is useful for verifying the results that are to be expected in the captured image.

# Workflow Options:

# Output Capture to: Window

Each individual channel is captured to a Window, unless this option is disabled. Captured images are automatically sent to the Capture Sequence thumbnail panel.

# Auto-Select Next Channel in Sequence

Enable this setting to ensure that the next channel is activated as each capture operation is performed. Choose to optionally disable this setting when defining a new Sequence with specific camera settings. When this setting is disabled, the same Channel remains active with subsequent Capture operations, allowing for the impact of the settings adjustments on a single channel to be refined within the



Sequence. Disable this checkbox and click Capture again, to move to the next Channel in the Sequence.

# • Auto-Save Capture Sequence

Enable this setting for optimal workflow, allowing the application to auto-save the acquired images after the last channel image is acquired. With this option enabled, the captured images are saved using the storage file types and naming conventions specified in the File Saving Options.

With this option unchecked, captured image channels and the resulting Color Composite will be copied into the Gallery and to File Storage, when the Save Images button in the Capture Controls is clicked.

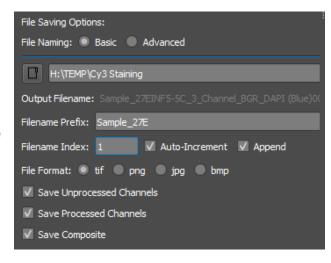


# File Saving Options

# o Basic

Allows for single filename specification, or for the inclusion of an auto-incremented number on the end of the filename, combined with the automatic use of the sequence and channel names within the filename.

The Output Filename is indicated, based on the selections made and the Prefix provided.



Enter the desired Filename Prefix. Use the Filename Index to specify a starting value for an appended incremental filename extension, if desired. The Append and Auto-Increment checkboxes provide the flexibility to include the auto-numbering on the end of the filename Prefix, and to choose whether the number increase is made by the application, or entered manually by the operator.

**Unprocessed Channels** – image channels stored without color LUT applied and without histogram stretch

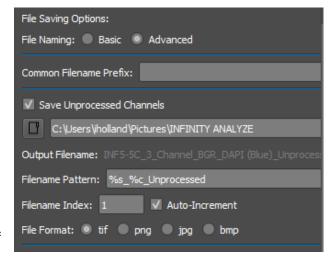
**Processed Channels** – image channels stored with any specified histogram stretch and the color LUT applied

**Composite** – image resulting from the combined Processed Channels

## Advanced

Enables custom filename specifications where the inclusion of specific tokens can be included within the filename characters. See the table of available tokens at the end of the Capture Options section for Brightfield.

With the Advanced File Naming selected, folder locations and naming conventions can be specified for each different type of output file produced.





#### File Format:

Use the fields in this section to specify the file types for saving the captured color composite and component images. Available file types are TIF, PNG, JPG, BMP. Data export to a multi-channel OME-TIF file is also available to users familiar with this format.

OME-TIF files will contain the raw input channels collected under the current sequence, but can only be viewed in  $3^{rd}$ -party software that supports the OME-TIF structure, such as ImageJ. Enable this option from the File  $\rightarrow$  Preferences dialog, under the Fluorescence Mode panel.

# **Advanced Options**

## Camera model specific settings

# Enable camera cooling

Available as an option <u>only</u> when an INFINITY3-1M or INFINITY3-1C camera model is connected, this setting is used to turn the Peltier cooling unit in the camera on or off.

# High Conversion Gain (HCG)

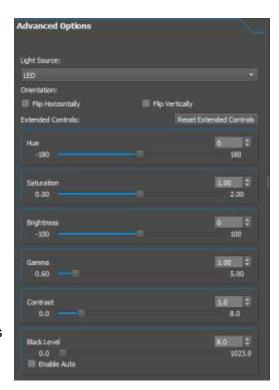
Available as an option <u>only</u> when an INFINITY8-2M camera model is connected, this setting is used enable or disable the camera's high conversion gain mode. In fluorescence mode this setting is enabled by default to improve the sensor response under low-light conditions. Disabling this setting will result in longer exposure times being required.

#### Orientation

The combination of mirrors and prisms within any microscope will result in the image being produced in the camera having either the same orientation as in the eyepiece, or an image that is rotated by 180 degrees. Use both the Flip Horizontally & Flip Vertically settings to orient the camera image to match that seen in the oculars.

#### Extended Controls

Control sliders to adjust the camera settings for Saturation, Hue, Contrast, and Brightness are provided. Under most circumstances these settings should remain at their default values, and this is especially true for Hue, Contrast, and Brightness. Changing any of these settings by any significant amount will most likely produce an undesirable image output. The





Saturation slider can be set to zero to remove all color information from a color camera image, should a monochrome result be desired. Setting the saturation to a value between 1.0 and 1.5 will produce more vibrant color output, and may be especially useful when attempting to image metal objects such as copper, brass, bronze, or gold. Use the Reset Extended Controls button to return all control values in this section to the camera defaults. Saturation and Hue adjustments have no impact on monochrome camera output.

Black Level should typically be left enabled in the Auto function. This allows the camera to automatically set the best black-level adjustment based on the current camera settings. Disable the Auto function to shift the camera output so that the minimal camera output is further along the histogram.



# **Image Processing**

# **Gallery**

The post-processing of images begins with them being accessible within the Gallery. Images can be captured directly into the Gallery or moved to the Gallery from the display window. The Gallery is a collection of working images, represented by their thumbnails. Images stored in the Gallery can be viewed as a reference, or can be selected for further processing. When the checkbox is activated adjacent to *Gallery* in the Capture Options, all acquired images are stored in the Gallery. When copies of images are held solely in the Gallery, a prompt to Save the images is presented prior to the application being closed or the images being removed.

Save the entire Gallery of images, or restore a set of images saved from a previous Gallery, using the pop-up menu options that appear when using a right-mouse click in the border area that surrounds all thumbnail images. Double-click on any image in the Gallery to open it in the main window.

Select a single image in the Gallery with a mouse click, or select multiple images with either a <Shift> click, or a click & drag with the mouse. With one or more images selected in the Gallery, use a right-mouse click on any of the selected images and choose Open in Image Processing Dialog... from the pop-up menu. The Image Processing functions can also be accessed from the Combine → Image Operations... menu

The thumbnail image size used for Gallery images is controlled from the Gallery option presented in the File  $\rightarrow$  Preferences menu.

# **Image Processing Operations**

Use the Image Processing Operations dialog to perform post-processing on one or more selected images. Begin by identifying the desired image processing function from the dropdown list of Operation Settings in the upper left corner of the dialog. Available operations are:

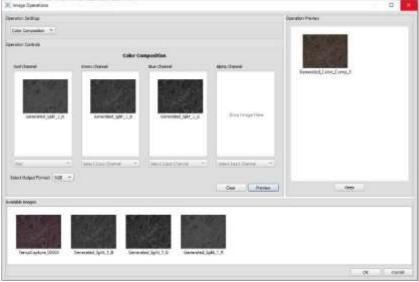
- Add
- Subtract
- Multiply
- Divide
- Merge
- Color Composition

- Split
- Min
- Max
- Average
- Stitch
- Focus Stack

Thumbnails of the currently selected images are shown in the lower section of the Image Operations dialog. When images are ineligible for processing with the currently selected operation, those images will be greyed out and their names shown in italics. Images that do not have a matching bit depth, matching size, or format may be ineligible for the selected operation. Depending on which operation is chosen, panels are shown in the Operation Controls area of the dialog. Drag the candidate images from the Available Images section, into the input panels. Select the Preview button to invoke the selected Process. The results appear in the Operation



Preview panel (right side).
Choose whether to Keep the result or choose Clear or Cancel. If the result is kept, then it is transferred into the collection of Available Images, for further processing (if desired), and copy is transferred back into the Gallery. The results of Image Processing operations are held in the Gallery, but must still be Saved to disk for permanent storage.





# **Menu Functions**

The list of menu functions appears across the top of the application with dropdown lists of operations presented below each heading. Menu items that are greyed out are either not available in the present application state, or have not yet been implemented

A few menu items are located under different headings on the Mac platform. The File  $\rightarrow$  Preferences feature in Windows is located under INFINITY ANALYZE  $\rightarrow$  Preferences on the Mac, while the Help  $\rightarrow$  About INFINITY ANALYZE in Windows is found under INFINITY ANALYZE  $\rightarrow$  About INFINITY ANALYZE on the Mac.

#### **File**

## Open...



Presents a File Open dialog with options to Browse to a specific folder, specify full or partial filenames, and file types. Select one or more images that will be opened into the working area, and into the Gallery of the application when the Open button is selected.

## Close

Close currently active window in the main panel

### Close All Images

Close all image windows in the main panel

#### Save



Save the currently active window in the main panel using the file name associated with the image.

#### Save As...

Assign a new folder location / file name to the currently active window

# Create video from images...

Display a dialog prompting the selection of a series of image files that will be converted into a video file.

#### Video Viewer...

Open a video view panel with controls to select a video for playback from an identified folder.

#### Convert file format...

Change image files from an existing format into another image file format. Images with annotation or measurement objects in the original files, can be preserved when converting into TIF or PNG formats. The graphic objects can then be burned into JPG or BMP files by first opening the TIF or PNG files in ANALYZE and selecting the Edit > Burn In menu option, then saving the results in any file format.



# • Settings Manager...

Provides functions to Import, Export, or Delete stored Calibrations, Presets, Fluorophores, and Sequences.

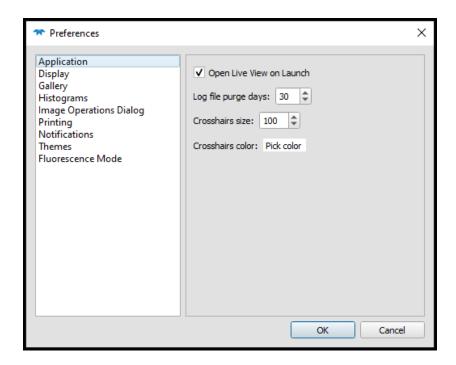
# Preferences

Opens a dialog with several options allowing the application to be customized. Options are available for:

	l a					
Application	Open Live View on Launch	Controls whether the live preview window is opened automatically on application launch				
	Log file purge days	Number of days log files are kept				
	Crosshairs size	Size of crosshair overlay in pixels				
	Crosshairs color	Choose the desired color for the crosshair from the palette				
Display	Limit Live View Refresh Rate	Enable to restrict the upper range for display frame rate to reduce CPU usage				
	Maximum Live View Refresh Rate:	Set the upper limit for the Live Preview in frames per second. Lower numbers reduce CPU usage, but make the image refresh less fluid.				
	Limit Auto Exposure Refresh Rate	Enable to reduce the application CPU usage by limiting the frequency of checks for auto-exposure adjustments being made.				
	Maximum Auto Exposure Refresh Rate:	Set the upper limit in times per second that the Auto Exposure routine uses to compute exposure time. Lower numbers reduce fluctuations and improve CPU performance, but may cause the response to lag.				
	White Balance Digital Gains	Enable this option to allow the application to fine tune the camera white balance by applying digital adjustments in addition to the analog intervals available on each sensor.				
Gallery	Item size	Choose Gallery thumbnail representation from: Small, Medium, Large, Extra Large				
Histograms	Display	Path, Bar, or Outline are the style options				
	Scaling	Uniform adjusts the vertical scale for all channels based on any channel saturating, while Channel adjusts the vertical scale independently for each color channel.				
	Clip Peaks	Enable to exclude oversaturated or undersaturated peaks from impacting the histogram scaling				
	Opacity	Set the opacity of the histogram channels as a percentage, where 100% is a solid color while lower values increase the transparency.				
Image Operations	Preview item width	Preview thumbnail width in pixels				
Dialog	Preview item height	Preview thumbnail height in pixels				
	Focus stacking median filter size	Median filter matrix size in pixels impacts results in focus stacking algorithm (default=3)				
	Focus stacking Gaussian filter size	Gaussian filter matrix size in pixels impacts results in focus stacking algorithm (default=7)				
Printing	Images per page	Number of images to be printed on a page (default=4)				



	Print all images	Check to print all images that are open
	Show file names	Check to include file names on the print
Notifications	Show calibration instruction	Check to display the reminder dialog at the start of each calibration
	Show annotation save options Save As or Burn-in	
Themes	Brightfield Mode Theme	Specify preference for dark or light application theme in Brightfield Mode (default=Bright)
	Fluorescence Mode Theme	Specify preference for dark or light application theme in Fluorescence Mode (default=Dark))
Fluorescence Mode	Allow OME TIF export	Advanced file export type enabled as an option for fluorescence sequence images



## Print Preview

Show the selected images as they will appear when printed given the current Print Layout.

#### Print

Send the identified images to the Printer in the specified Layout

## Exit

Close the INFINITY ANALYZE application. The current application settings are preserved and will be restored when the application is next launched. If there are



unsaved image files a prompt will be displayed to select how the images are to be saved or cleared prior to closing the application.

## Camera

## Connect...

Use this function to prompt the application to detect a camera that was not available when the software launched. INFINITY ANALYZE can only connect with a single camera at one time. If multiple cameras are connected to the same computer, this function will produce a dialog allowing a choice of cameras to select from.



#### Close Live View / Live View



This button toggles between providing the option to either close or launch the live preview. The name and icon will change based on whether the Live View is currently active or not. By default the image preview is started when the application launches. An option is available in the Help  $\rightarrow$  Settings to disable the automatic open of the video preview on application launch.



### Image Capture

Capture an image from the camera using the current settings. Equivalent to using the Capture button, <Ctrl> g, or double left mouse click on the live preview.

## Record Video...

Display the dialog to specify settings and record a video clip.



## Fluorescence Mode / Brightfield Mode

Switch the application controls between Brightfield Mode and Fluorescence Mode. See the descriptions above for details on the Brightfield and Fluorescence Modes.

The default presentation for Brightfield Mode has a lighter tone, while the presentation switches to a darker tone when Fluorescence Mode is active. The light or dark theme can be controlled by the operator, using the Help  $\rightarrow$  Settings dialog, under the category of Themes.



## Fluorescence Settings...

Launch the dialog that defines the Fluorescence Sequences and available Fluorophores to facilitate the workflow of capturing a multi-channel image to create a color composite.

# White Balance

If the image brightness is within a normal range, and the field of view is empty, this option will adjust the color channel balance to produce a uniform grey image.

## Area White Balance

After selecting this item, click the mouse in an unoccupied region of the image (ie. the light source background) and the computation for white balance will be performed using the pixel intensities surrounding the mouse click.



## Manual White Balance...

Use the sliders in the dialog presented, to increase or decrease the amount of channel gain adjustment applied to the sensor for each of the Red, Green, or Blue pixels to influence the color output from the camera.

## Stretch Histogram

Enable or disable this selection to automatically alter the image histogram to improve contrast. This may result in the image colors becoming distorted.

# Lookup Table

Two options are presented under this menu item. One is a check box to either apply or disable the image data passing through a Look-Up Table (LUT). The second option opens a dialog to Define... the parameters of the LUT. The adjustments are made graphically to each color channel, and can be saved or loaded from the dialog.

#### Info...

Displays a pop-up dialog showing version information and camera details. The dialog includes the USB connection status, showing whether the camera is detected as operating with USB2.0 or USB3.0. Slow frame rates result when a USB3.0 camera connects as a USB2.0 device.

## **Edit**



#### Drag

Cancel the currently active operation and return mouse function to pan operations.



## Select

Use the mouse to activate a single graphic overlay object with point/click, or drag a rectangular selection to activate all graphic objects contained within.



## Select All

Activate all graphic objects in the image.



#### Delete

Remove the selected graphic object(s) from the image



# Bring to Front

Move the selected graphic object(s) to the top of the stack.



#### Send to Back

Move the selected graphic object(s) to the bottom of the stack.



#### Bring Forward

Move the selected graphic object(s) by one position up in the stack.



## Send Backward

Move the selected graphic object(s) by one position down in the stack.



# Copy to Clipboard

Copy the contents of the current image to the clipboard. This allows for a Paste operation in a report or other open document.

## Undo

The most recent operation performed on the captured image will be rolled-back. Can be invoked using a <Ctrl> Z on the keyboard.

#### Redo

The most recent operation Undo operation on the captured image will be reinstated. Can be invoked using a <Ctrl> Y on the keyboard.

# Duplicate on Live View

Transfer a copy of all the graphic objects in the current image onto the Live Preview.

#### Burn In

Convert active graphic objects into permanent, static objects in the current image. This prevents further alterations being made to the graphic objects, but allows them to be captured in JPG or BMP formats, and ensures that they are visible in 3<sup>rd</sup>-party image viewing software, whether the resulting image is stored as TIF, PNG, BMP, or JPG.

#### **View**

#### Toolbars and Docked Windows →

The flyout menu provides access to the list of available toolbars and docked windows that can be enabled or disabled to customize the application and the use of available screen real estate.

A right-mouse-click in any Menu bar or Toolbar at the top of the application window provides quick access to these options.

Simple Mode		
Select All		
▼ Control Panel		
<b>▼</b> Gallery		
<b>✓</b> Histogram		
<b>✓</b> Measurements		
<b>✓</b> Properties		
▼ Standard Toolbar		
Measure Toolbar		
Annotate Toolbar		
Annotation Edit Toolbar		
▼ Status Bar		
☐ Full Screen		

# Zoom...

Selecting this option produces a Zoom control at the top of the currently active window, whether a captured image of the Live Preview. Use the slider bar to adjust the zoom level manually, or choose a pre-defined zoom percentage from the dropdown list. The icon on the far right of the Zoom control will fit the current image to the window dimensions, without distortion.

Click anywhere outside of the active image to close the Zoom control.

# Magnifying Glass...



Activate the Magnifying Glass feature to launch a floating viewer that shows a magnified area of the active window, around the cursor position. The viewer only tracks the cursor when over an image. Drag the Magnifying Glass viewer to the desired location on the screen, resize the viewer using a drag operation in the corners, and change the zoom percentage using the slider at the bottom of the viewer.

Close the Magnifying Glass from the View Menu, or by clicking on the 'X' in the upper right corner.

#### Full Screen

Toggle between Full Screen mode and the regular view mode by selecting the Full Screen menu item. To return to regular mode use the F11 key on the keyboard or choose the Full Screen item again.

On the Mac, this menu changes alters between Enter Full Screen and Exit Full Screen. If the menu bar is hidden on the Mac when in Full Screen mode, simply move the mouse to the top of the screen to display the Menu.

#### Crosshairs

Enable or disable the display of a cross-hair in the center of the image. To adjust the size or color of the crosshair access the File  $\rightarrow$  Preferences... menu and select the Application option

# Saturation Overlay

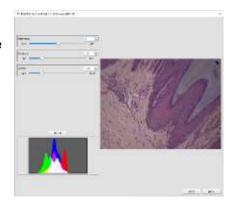
Enable or Disable the display of a saturation mask wherever pixel intensity values reach maximum intensity. Currently, the mask is set to display the overlay color as Red only. Frames that are showing a zero pixel value will be highlighted in Cyan. Options to adjust the desired overlay color will be included in a future release. Enable or Disable the Saturation Overlay on the Live Preview or Captured images as desired.

#### **Adjust**

Items available on the Adjust menu apply to a captured image that is open and active, in the working area of the application.

# Brightness / Contrast / Gamma...

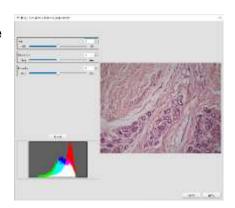
A dialog is presented showing a representation of the active image, along with a histogram, and the three slider controls to adjust Brightness, Contrast, and Gamma. Adjustments made to the controls take effect on the interim image within the dialog. The controls can be reset to defaults by clicking on the Reset button. The alterations to the image can be Cancelled or Applied to the parent image, by selecting the appropriate button.





# Hue / Saturation / Intensity...

A dialog is presented showing a representation of the active image, along with a histogram, and the three slider controls to adjust Hue, Saturation, and Intensity. Adjustments made to the controls take effect on the interim image within the dialog. The controls can be reset to defaults by clicking on the Reset button. The alterations to the image can be Cancelled or Applied to the parent image, by selecting the appropriate button.

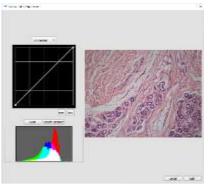


## Area White Balance

Use the eye-dropper mouse pointer to indicate an area in the active image that represents white (or a shade of grey). The area immediately around the mouse click location is used to perform a Red, Green, Blue channel adjustment on the active image, to adjust the white balance. This adjustment is independent of the live preview from the camera, where the white balance is controlled through the buttons in the Capture Controls.

# Lookup Table...

A dialog is presented showing a representation of the active image, along with a histogram and a graphic window. Above the graphic window is a dropdown selector providing the look-up control over either All Channels, or the individual channels of Red, Green, or Blue. Additional buttons on this dialog include a Reset, Histogram Stretch, Load, and Save.



To adjust the distribution of pixels across the histogram click and drag the location of the end nodes (dots) on the diagonal line in the graphic window. The left side of the graphic represents dark pixels, while the right side of the graphic represents bright pixels. New nodes can be added anywhere along the diagonal line in the graphic window, using a double-mouse click. New nodes can be dragged to different positions, with their impact shown on the histogram and in the image representation on the right side. Once a desired LUT adjustment has been made, the result can be Saved and Loaded later using the buttons below the graphic window. The Stretch Histogram button imparts a histogram adjustment based on the min/max

## Mirror Vertical / Mirror Horizontal

distribution of pixel intensities in the current image.

Selecting Mirror Vertical flips the active image top to bottom, while selecting Mirror Horizontal flips the active image left to right.

Rotate Counter Clockwise 90 degrees / Rotate Clockwise 90 degrees
 Selecting either of these options will cause the active image to be rotated by 90 degrees in the selected direction.

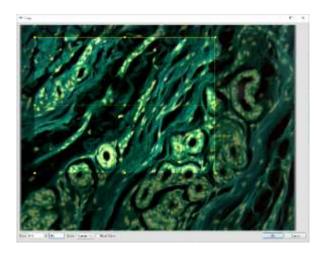


## Rotate 180 degrees

Selecting this option will result in the active image being rotated by 180 degrees.

# Crop...

The Crop dialog is presented with a representation of the active image and a dynamic crop overlay graphic. Use the mouse to drag any of the yellow 'handles' on the crop overlay to locate and size the crop window over the desired area of interest. Alternately, use the keyboard to enter numeric values in the Width and Height fields shown in the lower left corner of the dialog, to specify an exact crop dimension (eg.: 800x600 or 640x480). A custom ratio dropdown allows for the specification of a desired width/height ratio

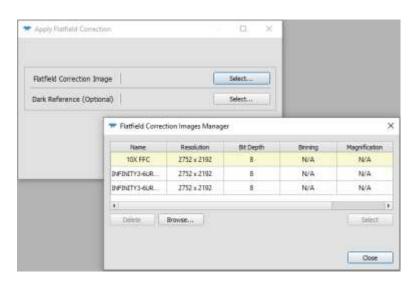


for the crop. The Fixed Ratio check box locks the ratio of width and height while dragging the crop area using the handles, when checked. Choose the OK button to close the dialog and apply the selected crop region to the active image, or choose the Cancel button to discard the crop adjustments.

#### **Enhance**

## Flatfield Correction...

Provides access to stored FFC images, which can then be applied to the currently active image as a post-processing correction. A selection dialog is presented, from which the suitable FFC image can be chosen and applied. This tool also provides access to the selection of a stored dark-frame image, to include a dark-frame offset as part of



the correction. The process to capture and store a dark-frame image requires the camera parameters to be configured in the same manner as for the image to be corrected, but a fully dark image (no light source) is captured and saved. This dark frame reference can then be imported from a saved location at the time of the FFC processing.



## **Pseudo Flatfield Correction**

Uses an algorithm to perform a flatfield correction based on mathematical patterns rather than on the actual illumination detected by the camera. Useful under circumstances where adjustments are required on images captured without an actual flatfield image being available.

#### Denoise

A filter is applied to the image resulting in a reduction of apparent sensor noise.

#### Remove Bad Pixels

Non-uniform pixels in the image are replaced using an average from the neighbouring pixels.

## Sharpen

Applies a sharpening algorithm to the image.

## Filter Settings...

Presents a dialog with options to influence the behavior of three operations. Settings are provided for Denoise, Bad Pixel Removal, and Sharpen, where the numerical field represents the number of pixels in the field of influence for each filter.

## Max Contrast

Performs a histogram stretch operation on the image.

#### Sketch

Replaces the image contents with an outline of edges resembling a drawing or sketch

## Adaptive Edge Emphasis

Enhances the contrast along the boundaries between light and dark objects in the image.

# Equalization

Performs a histogram equalization operation.

#### Monochrome

Converts a color image to a monochrome representation

## Negative

Inverts the distribution of light and dark pixels in the image. For a monochrome image the black and white areas are reversed, whereas the individual Red, Green, and Blue channels are inverted in a color image.

## Spherical Aberration Correction

Reduces haze in an image resulting from spherical aberrations in the optical system.



## **Combine**

The items in the Combine menu become active when there are one or more images open in the working area, or held in the Gallery.

# Image Operations...

Selecting this menu item results in the Image Operations dialog being displayed. The same dialog can be accessed using a right-mouse-click within the Gallery dockable window.

Use the Operation Settings menu to choose the desired image processing function. The appearance of the Image Operations dialog will adjust depending on which operation is being performed. Candidate input images



are displayed in the area at the bottom of the dialog. When images are ineligible for the currently selected operation, they are greyed out. Exclusion may be based on bit-depth or width and height, etc.

The left side of the dialog holds the selection of input images. Drag images from the Candidate area into the Input area. A Clear button will remove all input images. The Preview button performs the selected operation on the input images with a representation of the result showing on the right side panel. Choose the Keep button to preserve a copy of the results to the Candidate area.

Use the Cancel button to terminate the operations without saving the results. Use the OK button to keep copies of the results in the Gallery and in the Working area. These images must be saved to disk prior to closing the application.

#### Add

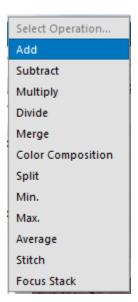
Sums together the overlapping pixels in two images. A series of image pairs can be placed in the Input area to be processed simultaneously.

#### Subtract

The values of the overlapping pixels in the image on the left are subtracted from the pixels in the image on the right. A series of image pairs can be placed in the Input area to be processed simultaneously.

#### Multiply

The values of the overlapping pixels in the two images are multiplied together. A series of image pairs can be placed in the Input area to be processed simultaneously.





## Divide

The values of the overlapping pixels in the image on the left are divided by the pixels in the image on the right. A series of image pairs can be placed in the Input area to be processed simultaneously.

## Merge

Applies only to monochrome images that are the result of a previous Split operation. Three input channels are blended together to form a 3-channel color result.

# Color Composition

Allows for input channels from color images to be combined into a new color composite.

## Split

Color images placed in the Input area will be split into their three channel (R,G,B) components.

#### Min.

The minimum pixel value is selected from the pair of overlapping pixels for all pixels in the images loaded into the Input area.

#### Max.

The maximum pixel value is selected from the pair of overlapping pixels for all pixels in the images loaded into the Input area.

### Average

The average pixel value is computed for the pair of overlapping pixels for all pixels in the images loaded into the Input area.

#### Stitch

Images loaded into the Input area are processed and stitched together. The algorithm works on the content of the images, and requires that adjacent images have at least 30% overlap.

#### Focus Stack

Images loaded into the Input area are processed as a stack collected in the Z-direction. The algorithm processes the stack to select the pixels with the best focus, to create the output image.

Use the Align Images checkbox for a stack of images collected from a stereoscope. It lengthens processing time but reduces artifacts in the output. Apply the Median Filter and/or Gaussian Filter to fine tune the output results.

#### Stitch Images...

This menu item is a shortcut that opens the Image Operations dialog with the Image Stitching operation active. See the description above for the usage.



# Focus Stack Images...

This menu item is a shortcut that opens the Image Operations dialog with the Focus Stacking operation active. See the description above for the usage.

#### Measure

A variety of measurement operations can be used on the live preview or captured images. If an objective calibration has not been prepared or selected, then the results of measurement operations are reported in pixels. Refer to the descriptions for Calibration... in this section, to know how to produce calibrations for the objectives on the microscope being used with the camera.

#### Distance

Click and drag the mouse between any two points in the live preview or captured image to display the computed distance.

## Rectangular Region

Click and drag the mouse to measure the dimensions of any rectangular area. Hold down the Shift key on the keyboard while dragging the mouse, to lock the aspect ratio of the rectangle into a square.

# Elliptical Region

Click the mouse and drag it to shape the X and Y axes of the elliptical area being drawn. Release the mouse to close the region. Hold down the Shift key on the keyboard while dragging the mouse, to lock the aspect ratio of the ellipse into a circle.

## Polygonal Region

Click the mouse at a starting point and continue to click on each vertex of the polygonal shape. To close the polygon, the last mouse click must align with the first point. A red circle symbol appears over the starting point when the mouse is within working distance to close the polygon.

### Circle from 3 Points

Click the mouse at a starting point on the perimeter of a circular feature. Next click a second point anywhere along the perimeter. When the mouse is moved a circle appears. Move the mouse to a third point along the perimeter of the circle and make a final click to draw the circle from 3 points.

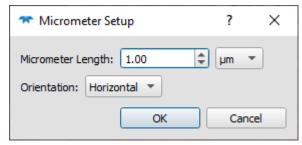
# Angle

A mouse click starts a line segment. A second mouse click at the vertex, and the last mouse click on the second segment completes the angle. The measured angle is displayed as text.



#### Micrometer

After selecting this menu item, use a left-mouse-click in the image where the Micrometer bar will be drawn, close to the desired position. The Micrometer Setup dialog will be displayed. Enter the length for the Micrometer bar, and units. The default orientation is



Horizontal, but Vertical can be selected from the dropdown, if preferred. Click OK to draw the Micrometer and length label. Use the Annotate → Select function to highlight the Micrometer bar and alter the graphic properties. The Annotation Edit toolbar must be enabled to gain access to the graphic property controls.

Label text for the Micrometer is displayed by default. To hide the measurement text open the Measure -> Measure Options... dialog and click to remove the checkbox from the Line Segment Length type. The Measure Options... dialog also has the property that controls the decimal places displayed with line segment length, which impacts the text label for Micrometer.

## Counting

This manual counting function assists the operator by annotating numbers on-screen when counting items in the live preview or captured images. A circle graphic displays a sequential number at the location of each mouse click. The Annotation Edit Toolbar is used to alter the color of the circle and label text, along with the font and font size. Use a double-left-mouse click to conclude a counting session, or select on the grab & move icon in the Annotation toolbar to exit from Counting. The <Esc> key will also terminate a count.

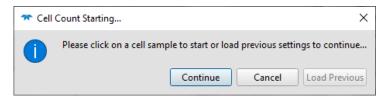
To resume counting from the last number in a counting sequence, use the Annotate > Select menu or tool bar icon to choose a manual counting number on-screen. Next, use a right-mouse-click to bring up the menu, and select Resume Count. The next left-mouse-click will be placed as a continuation of the selected set of count numbers.

**Note:** When placing or modifying an elliptical or rectangular annotation or measurement shape, holding down the Shift keys constrains the aspect ratio to a circle or square, while holding down the Ctrl key causes the shape to be resized from the center.



## Cell Count...

This function enables automated counting of objects in a captured image. This function is not enabled



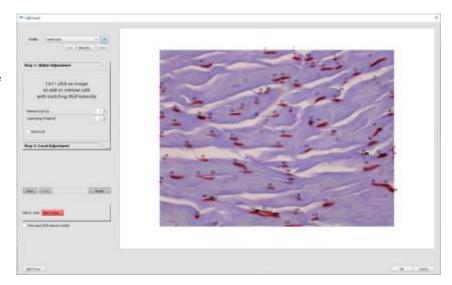
in Live Preview. Select the Measure → Cell Count... with a captured image in the working area. A pop-up dialog prompts to click on a cell or region within the image to initiate the cell count process. Options in the dialog allow for the operation to be Cancelled, for a Previous Cell Count setting to be loaded, or to Continue with the mouse selection of an object in the image. The Load Previous button is unavailable if no

previous session settings have been stored.

If a previous Cell Count setting is requested, the Settings icon at the top of the dialog can be used to access the Cell Count Profile Manager dialog. Use the fields presented to manage existing saved settings.



The Cell Count dialog is displayed with preliminary results, if the mouse click was used to launch the operation, or if an existing settings file produces results from the image that's active. The results from cell counting are based on the Red, Green,



Blue pixel intensities within the image. A high contrast image will yield more reliable results. A saved settings file will yield different results in an image with a different pixel intensity range between cells and their backgrounds.

The Cell Count dialog includes controls on several tab panels to allow for alterations and adjustments to the results being shown in the representative window. The results will change as the settings and options are adjusted. Global Adjustments will impact results across the entire image based solely on matching pixel intensities, whereas Local



Adjustments allow for individual regions or cells to be included or excluded from the results. Controls are also provided for the Marker Color selection.

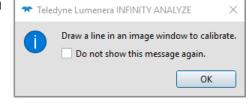
## Calibration...

Use this function to produce a calibration setting that will be used by the application to associate image pixels with known distances, for each objective. Calibrations are stored in the application using custom name assignments. An object of known dimensions or a stage micrometer are required when performing the Calibration setup.

You can capture images of objects of known dimensions, or of the stage micrometer at different magnifications, and use the Measure  $\rightarrow$  Calibrate operation to perform a calibration on each captured image; or you can perform the steps directly on the Live Preview.

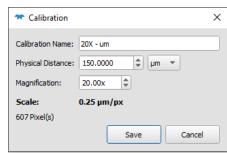
Follow these steps to produce one or more Calibrations using the Live Preview:

- 1. Position the desired objective magnification on the scope, or set the stereoscope zoom on the microscope.
- 2. Adjust the camera settings and microscope focus such that the object of known dimensions, or stage micrometer markings are clearly visible.
- Choose the Measure → Calibration... menu item. Alternate methods are also available to initiate a Calibration. Either use the right-mouse-click pop-up menu and choosing Measure → Calibration... when the mouse is over the Live Preview or a



Captured image, or choose the Calibrate... button on the Capture Controls panel. The Calibrate... button can also be used to toggle in and out of Calibration mode.

4. Follow the prompt on the instruction dialog to click and drag a distance. Note, this dialog can be hidden so that it doesn't reappear each time, by clicking the option on the dialog. To later restore the information message, visit File → Preferences, and find the option on the Notifications panel.



- 5. Use the fields in the Calibration pop-up dialog to provide a Calibration Name, enter the distance drawn in Step 4, specify the measurement units, and enter the numeric value for the current magnification or zoom.
- 6. Click on the Save button to store the Calibration or click Cancel
- 7. Switch to another objective or zoom level, adjust the camera settings and repeat the steps from 3 to 6.



The list of calibrations can be found in the drop-down list in the left-side panel of Capture Controls. Simply pick the appropriate calibration from the Calibration drop-down list whenever the matching objective or zoom-level is enabled on the microscope.

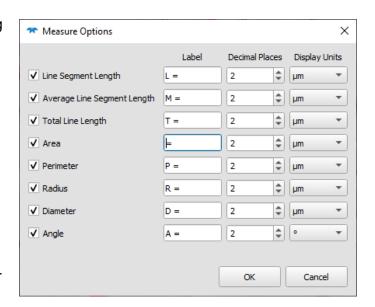
To associate a specific calibration with a Preset for camera settings, ensure that the desired calibration is showing in the Calibration field, and then click the Save button below the currently active Preset. Alternately, create a new Preset while a specific Calibration is active, and the two will be paired together.

Presets and Calibrations can be cleared using the File  $\rightarrow$  Settings Manager... dialog.

Images captured while a Calibration is active will be automatically associated with the Active Calibration, for Brightfield images. Any images in ANALYZE can be associated with a Calibration by using the Right-Mouse-Click menu in either the Gallery or the Displayed image window. Choose to calibrate using the Active Calibration, or choose another available Calibration. This operation can be performed on multiple images by selecting more than one thumbnail and using the Right-Mouse-Click menu in the Gallery. Multiple open images in the main display area can also have a Calibration applied in a single operation, by using the right-mouse click operation.

# Measure Options...

Use the Measure Options dialog to identify the Labels, displayed decimal places, and preferred measurement units to be displayed when measurements are taken on images. The display units can be different that the current calibration. If a calibration is created with the units of microns (um), it's possible to specify that the reported length and area measurements show the results in either um, inches, mm, cm, or custom units. This dialog also



provides the option to display or not display the length or area measurements alongside the measurement graphics, by using the checkbox to the left of the measurement type.



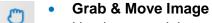
#### **Annotate**

The annotation properties for all annotation objects are set using the Annotation Edit Toolbar. By default, this toolbar is displayed just below the main toolbar icons. The toolbar can be hidden, if desired, from the View → Toolbars and Docked Windows menu, or use a right-mouse-click in the menu bar (or other toolbar) at the top of the application, and click next to the Annotation Edit Toolbar.



To select any annotation item placed on an image (or live preview), use the Annotate  $\rightarrow$  Select menu, to put the mouse cursor into the select mode. Alternately, use the Annotate Toolbar icons to invoke Select or Select All. The Annotate Toolbar is visible by default, but can be hidden using View  $\rightarrow$  Toolbars and Docked Windows menu, or with a right-mouse-click in the menu bar (or other toolbar)

There are several other annotation controls that can be applied in conjunction with the mouse clicks being used to draw shapes. Pressing the <CTRL> key, when starting rectangles, lines and ellipses will result in them being drawn relative to their center (as the initial mouse click). Combining <CTRL> and <SHIFT> allows for the creation of squares or circles from a center point when used with the ellipse or rectangle functions. For lines, the <SHIFT> key aligns to angle increments, and <CTRL> also makes the first point draw from the center. All annotations will align to an invisible grid when the <ALT> key is pressed. Combinations of CTRL,SHIFT and ALT can be used simultaneously as desired, either when initially drawing a shape or when editing the shape after initial placement. For squares and rectangles, the <SHIFT> key will lock the current aspect ratio, and not necessarily keep a square or circle shape if it was changed from its original shape. The <ALT> key is especially useful for Grid annotations as it helps users draw perfectly formed grids with integer multiples of the spacing.



Used to cancel the current operation and return the mouse control to a pan operation. The mouse icon changes from an open hand to a closed hand when the mouse is clicked for a pan operation.

Line
 Click & drag a line on the image using a left mouse click

# Rectangle Click & drag a rectangle on the image using a left mouse click. Hold the shift key to produce a square.



# Round Rectangle

Click & drag the rounded rectangle on the image using a left mouse click

# Ellipse

Click to begin the ellipse, and drag the desired shape. For a circle, hold down the Shift key while moving the mouse, and the width and height of the ellipse will be locked to produce a circle.

# Polygon

Click the mouse at the first vertex or starting point for the polygon. Use repeated mouse clicks to define other vertices, with a rubber-band graphic showing the path from the last defined point. The polygon shape will be closed when the last point is clicked in close proximity to the starting point. It may be necessary to zoom in on the image near the polygon start point to be close enough to close the shape. It's also possible to produce an open polyline using a double-click on the end-point.

#### Arrow

Click the mouse at the tip of where the arrow head is pointing, and drag the tail away from the head of the arrow.

#### Text

団

Click the mouse at the desired start location for the text box. A single click produces a dashed vertical rectangle on the image. Begin typing text using the keyboard. The selection tool can be used to highlight a text box, and a click within the box enables further typing or editing of the text. Use the Annotation Edit toolbar to control the Font, size, and color of the displayed text.

#### Select

Activate the Annotate → Select function to choose a specific annotation object or measurement object for manipulation. A selected object can be dragged to a new location, and many objects can be re-sized by clicking on the corner handles. Hold down the <Ctrl> key while clicking on annotation objects to select multiple objects. Annotation objects that are positioned below larger objects will not be selectable, until they are first moved to the top of the stack.

Use the delimiting rectangle's handles to resize or reshape an object. Hold the <Shift> key while scaling a circle or square, to retain the aspect ratio. Rotate any object using the handle in the top center of the object. Hold the <Shift> key to constrain the rotation to a limited set of rotation angles.

## Select All

Use this option to select all annotation objects in the active image with the click of the button.

×

4

4

0



×

#### Delete

While one or more annotation objects are presently selected, clicking on the Delete operation will permanently remove them from the image.



# Bring to Front

Move the selected annotation object to the top of the stack



#### Send to Back

Move the selected annotation object to the bottom of the stack

## **Window**

Items in the Windows menu dropdown list provide for predefined layout styles or adjustments in the presentation of image windows.

#### Cascade

Images are stacked one above the other with an offset so that the edges of the images below can be seen.

## Tile Horizontally

Fit windows across the active area by placing them one beside the other in a row.

## Tile Vertically

Fit windows across the active area by placing them one below the other in a column.

## Tile Grid

Display the windows as small tiles arranged across the active window in a grid formation

#### Tabbed View

Image windows are displayed with their titles along the bottom of the active area window, in tabs. Select the tab to bring an image to the foreground.

Arrow buttons are presented on the lower right corner of the tab area to provide scrolling through the image titles.

# Help

# Initial Camera Configuration...

Displays the Help Dialog for the Initial Camera Configuration. Shows how to configure a color camera to optimize the image for the lamp source in Brightfield Mode.

## • Fluorescence Settings...

Displays the Help Dialog for the Sequence configuration. Shows how to configure a set of parameters for a multi-channel image acquisition in Fluorescence Mode.

# Manual

The User Guide for INFINITY ANALYZE 7.x is launched as an Adobe PDF document



# Online Help

An internet browser application is launched displaying the INFINITY ANALYZE 7.X Training and Tutorials web page.

# About INFINITY ANALYZE...

Displays a dialog with the version information for the current release of INFINITY ANALYZE 7.X



# **Docked Windows**

Docked Windows are displayed by default at the bottom of the screen just above the Status Bar. These are dockable windows that can be dragged to other areas of the application layout using the mouse.

## **Histogram**

The histogram docked window presents a monochrome or 3 color channel result showing the relative count of pixels at their intensity readout within the image. The horizontal axis of the histogram represents the pixel intensity range from zero on the left to fully saturated on the right.

#### **Measurements**

The Measurements docked window presents a tabular record of any measurements taken on the currently active image. Data records can be selected with a mouse drag, and then using a right click - Copy / Pasted into an open Excel spreadsheet or other compatible 3<sup>rd</sup>-party package.

This capability will be implemented in a subsequent update - Individual row measurement results can be selected and the corresponding graphic will be highlighted in the active image. If a data record is deleted from the table, the associated graphic will be deleted from the image. If a measurement graphic is selected in the image the corresponding table row will be highlighted. Deleting the graphic from the image will result in the record being deleted from the table. Records may be sorted by clicking on the column header.

# **Photometry**

This capability will be implemented in a subsequent update - The Photometry table reports on pixel intensity results from operations performed in areas identified within the active image. Data from the Photometry table can be drag & dropped into an Excel Spreadsheet.

#### **Image Properties**

Click on any captured image to populate the Image Properties dockable window, as a table. The Image Properties represent the metadata stored with the image at the time of capture. Data shown in the table includes:

- Bits Per Sample [8 or 16] For 16 bit image storage, the actual data range will be either
   10, 12, or 14-bits, depending on the specific image sensor in the camera
- Brightness
- Camera Serial Number
- Created Time
- Image Height (pixels)
- Image Width (pixels)



- Camera Vendor
- Camera model
- Contrast
- Exposure
- Gain
- Saturation
- Gamma
- Light Source [Camera light source Color Correction Matrix setting]

# **Status Bar**

The Status Bar is enabled by default along the bottom of the Application. It features the camera frame rate on the left side, and a selection of display-related characteristics arranged along the right. If the display of this information is not required, the Status Bar can be turned off to make additional screen real-estate available. The View → Toolbars and Docked Windows menu function provides access to toggle the Status Bar. The layout of the fields represented in the Status Bar is indicated in the table below.

X= 242, Y= 413	Mouse cursor location in pixels from (0,0) in the upper left corner
RGB=(32,64,129)	Pixel intensity values at the mouse cursor location (Red, Green, Blue)
45%	Current image zoom expressed as a percentage
1936 x 1456 px	Image sensor output width and height in pixels
10X	Objective magnification entered for the current calibration
W=900um, H=670um	Image width and height in units specified by the calibration

Display FPS: 27.4	The live preview frame rate is displayed at the left end of the status bar whenever the camera Live Preview window is operational. This value may be lower than the frame rate being output by the camera, either due to lack of resources or if the File > Preferences > Display settings have been configured to reduce the live preview frame rate to reduce CPU load.
Received FPS: 27.4	The current frame rate is presented to the right of the Display rate. This value shows the rate at which image data is being transferred over USB between the camera and the computer.