TIDE™ Imaging System

User Guide

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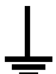
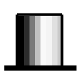
Chapter 1 Warning Symbol Definitions

Below is a list of warning symbols you may encounter in this manual or on your device.

Symbol	Description
	Direct Current
	Alternating Current
	Both Direct and Alternating Current
	Earth Ground Terminal
	Protective Conductor Terminal
	Frame or Chassis Terminal
	Equipotentiality
	On (Supply)
	Off (Supply)
	In Position of a Bi-Stable Push Control
	Out Position of a Bi-Stable Push Control
	Caution: Risk of Electric Shock
	Caution: Hot Surface
	Caution: Risk of Danger
	Warning: Laser Radiation
	Caution: Spinning Blades May Cause Harm
	

Chapter 2 Safety

SHOCK WARNING

Before applying power to the system, make sure that the protective conductor of the three-conductor main power cord is correctly connected to the protective earth contact of the socket outlet. Improper grounding can cause electrical shock resulting in severe injury or even death. Make sure that the line voltage rating agrees with your local supply and that the appropriate fuses are installed. Fuses should only be changed by qualified service personnel. Contact Thorlabs for assistance. Do not operate without cover installed. Refer servicing to qualified personnel.

Make sure the included power cords are the correct type for the service you are connecting to and connect to a properly grounded power outlet (100-240 VAC; 50-60Hz)

CAUTION

Do not obstruct the air-ventilation slots in the computer housing. Do not obstruct air-ventilation into the bottom of the base unit or out of the exhaust fan on the rear of the unit.

Mobile telephones, cellular phones, or other radio transmitters should not to be used within the range of three meters of this unit, since the electromagnetic field intensity may exceed the maximum allowed disturbance values according to IEC 61000-6-1:2005.

WARNING

Class 1M Invisible LED emission (800nm to 1000nm) is present from the microscope nosepiece when system is initialized through objectives and open nosepiece ports.

Do not look into the optical output when the device is operating. The invisible LED radiation can cause damage to your eyesight.

All empty nosepiece ports must remain capped if unused.

Autofocus Engine, AF-OCT-SP2, must be powered off when servicing objectives, nosepiece, or AF1 Autofocus head (Refer to 4.1).

The following LED class information only pertains to the collimated beam inside the Autofocus head, AF1, which is not user serviceable. This class information is also stated on the laser safety label on top of the autofocus head housing.

Class 3R Invisible LED Radiation When Open
Avoid Exposure to Beam
No User Serviceable Parts *Inside*

Class 1M LED Emission may be present:

a) From the objective (intended use).

b) From the back of the Autofocus Engine, AF-OCT-SP2, when the fiber cap is removed and the fiber is disconnected.

c) From the output of the fiber when the fiber is not connected to a scanner.

INVISIBLE LASER RADIATION

DO NOT STARE INTO BEAM OR VIEW DIRECTLY WITH OPTICAL INSTRUMENTS

CLASS 1M LED PRODUCT

CLASSIFIED ACCORDING TO DIN EN 60825-1:2014

FINGER TRAP WARNING

Do not place hands or objects in the area of the slide scanner during operation

Chapter 3 Description

3.1. Experiment Architecture

3.1.1 Overview

The TIDE™ system generates high magnification large format images and allows for inspection and scanning of a desired area. This chapter describes the general layout and file architecture.

Figure 1 Typical Slide

Figure 1 illustrates a typical slide. The entire slide image itself is called a “Prescan”, and this slide also includes one ROI (Region of Interest) in the center of the slide that is outlined by a dotted line. The image data for the Prescan is located in the “Prescan” folder which is just under the top-level slide folder.

All image data, whether for a Prescan or an ROI scan, is organized the same way, with folders under the top-level folder for 1x, 1xRaw, 2x, 4x, 8x and 16x images depending on how far a user has zoomed in on an image. The images in the 1xRaw folder are not “flat fielded” or “color balanced”, but other than that are the same as those images in the 1x folder. The 1xRaw is not used for display purposes. The contents of the 1x folder are used for the full resolution image display. Each folder contains images that are identical in size (pixel width and height) and named by row and column, with the row and column values starting at a 0 (zero) value. For example, an image tile name might appear as “Tile_0000_0002.jpg”, with the column number appearing first followed by the row number. So for “Tile_0000_0002.jpg”, the image tile would correspond to column 0 (zero) and row 2.

Images can be saved as JPEG or TIFF. The full resolution (1x folder) color images are saved as 8bit or 16 bit RGB images. Monochrome images are saved as either 8bit or 16bit TIFF or JPEG. All monochrome images are false colored based on stated emission wavelength in the application. All monochrome images saved to disk are NOT false colored.

3.1.2. Scan Hierarchy

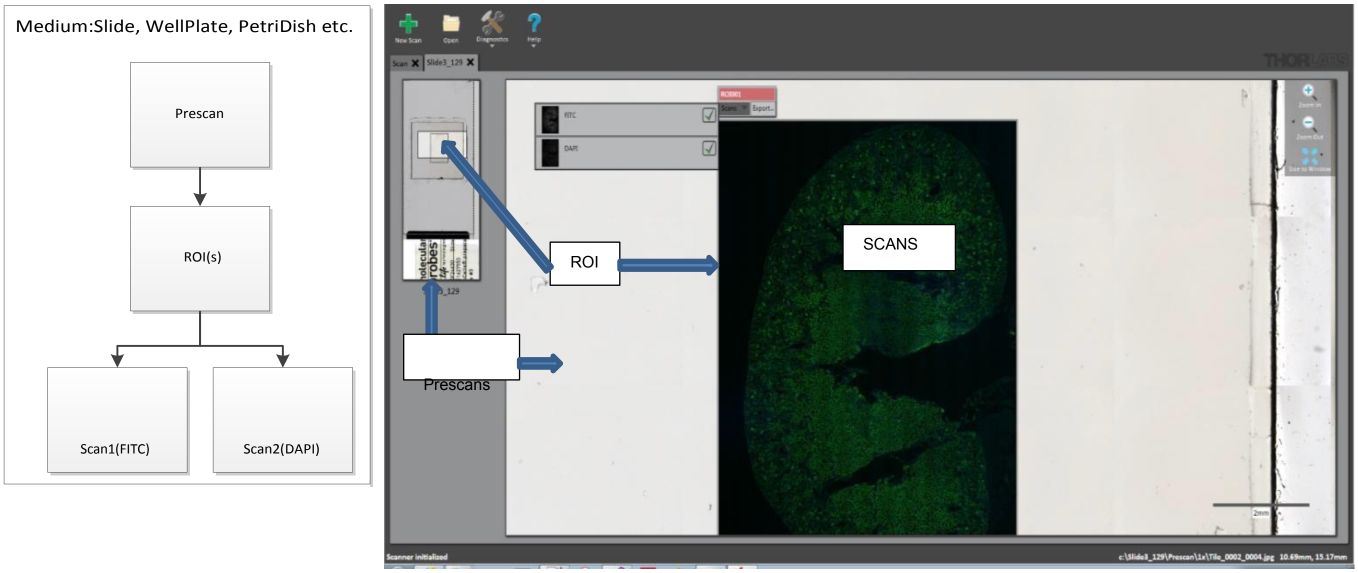


Figure 2 Representative Hierarchy of Two Channel FL Scan with Single ROI

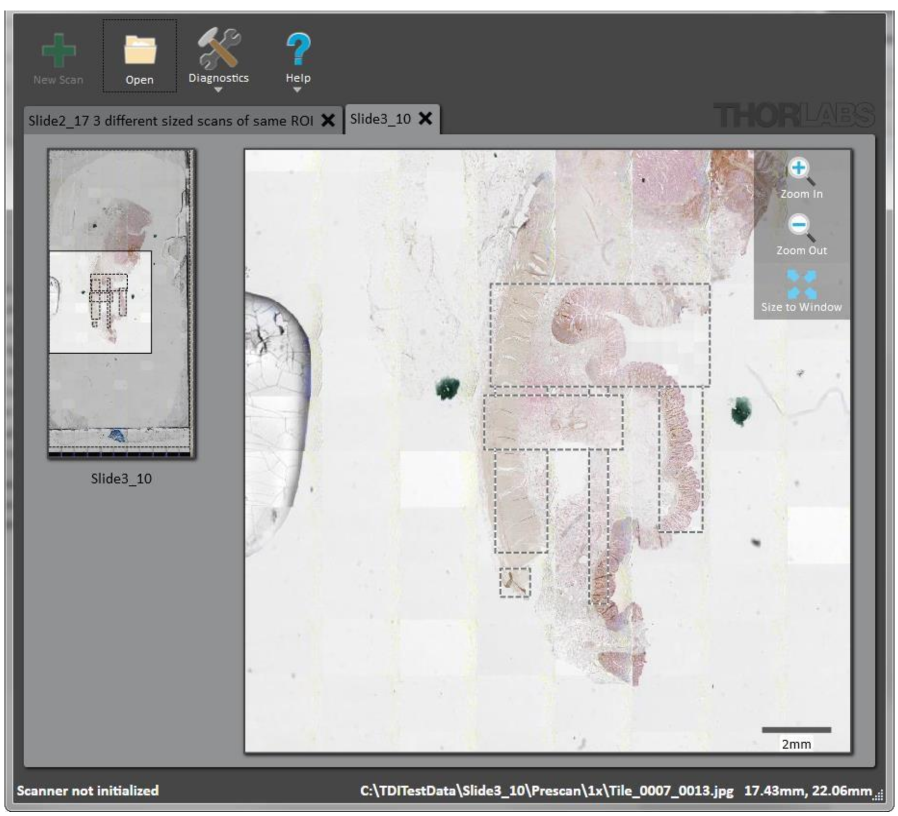


Figure 3 Multiple ROIs defined by user. Notice in the above figure that an ROI can overlap one or more defined ROIs.

An ROI "requested area" can have ONE or MORE "scans" associated with it. Each scan is a separately performed microscope examination of the ROI, and each scan can be independently configured, for things such as the

Objective (magnification lenses) used, exposure time, light intensity, wavelength etc. The ROI in Figure 4 has two scans associated with it, one for the FITC channel and one for DAPI.

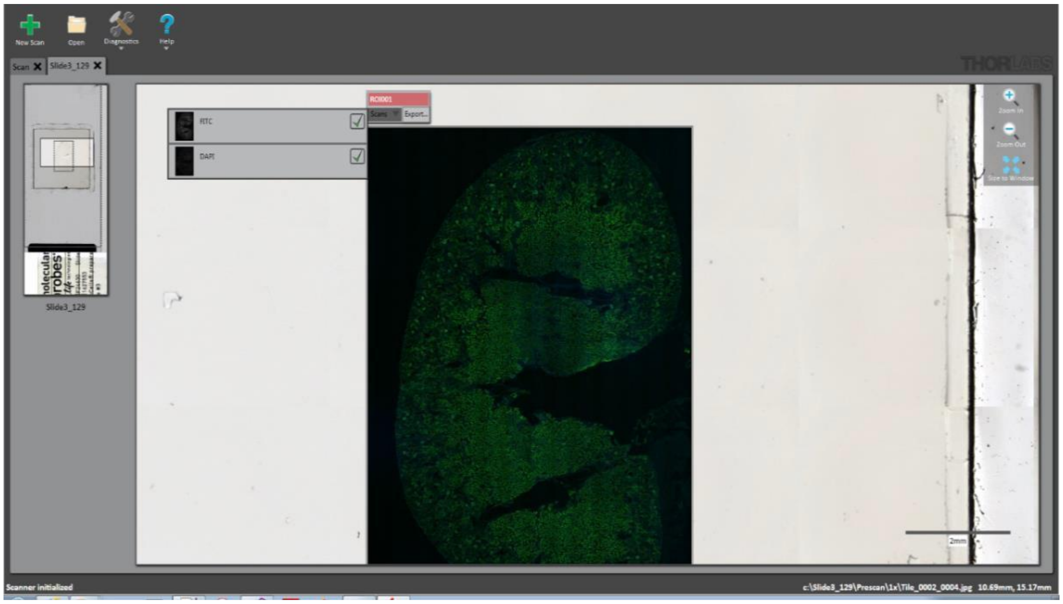


Figure 4 ROI with Two Scans

3.1.3. Experiments

Experiment data references the collection of scan information. This information is saved as an experminet.xml file in the root folder, as shown below, and is structured in the same way that the information is communicated in the scan hierarchy section above. This file contains all the pertinent information to recreate an experiment or properly display the data. All experiments can be viewed by browsing to the “experiment.tdix” file.

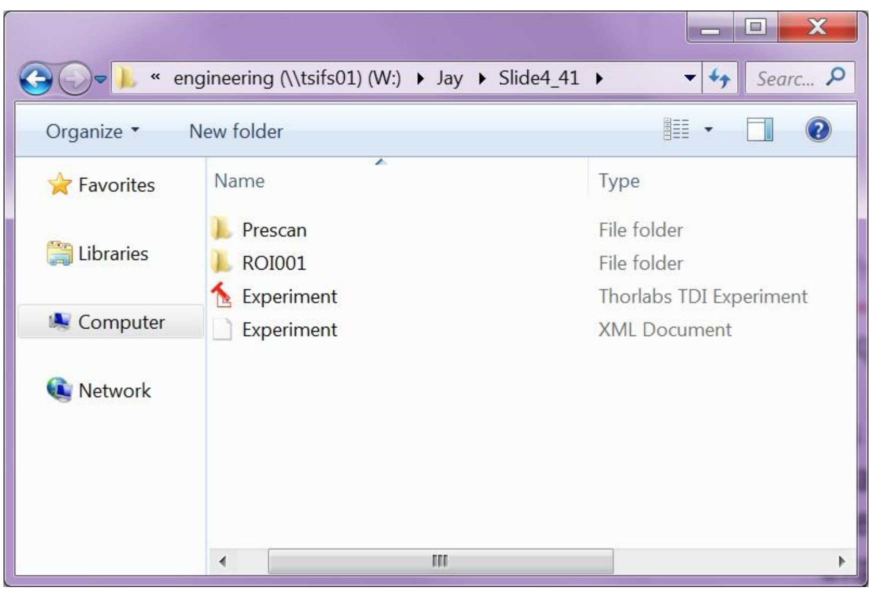
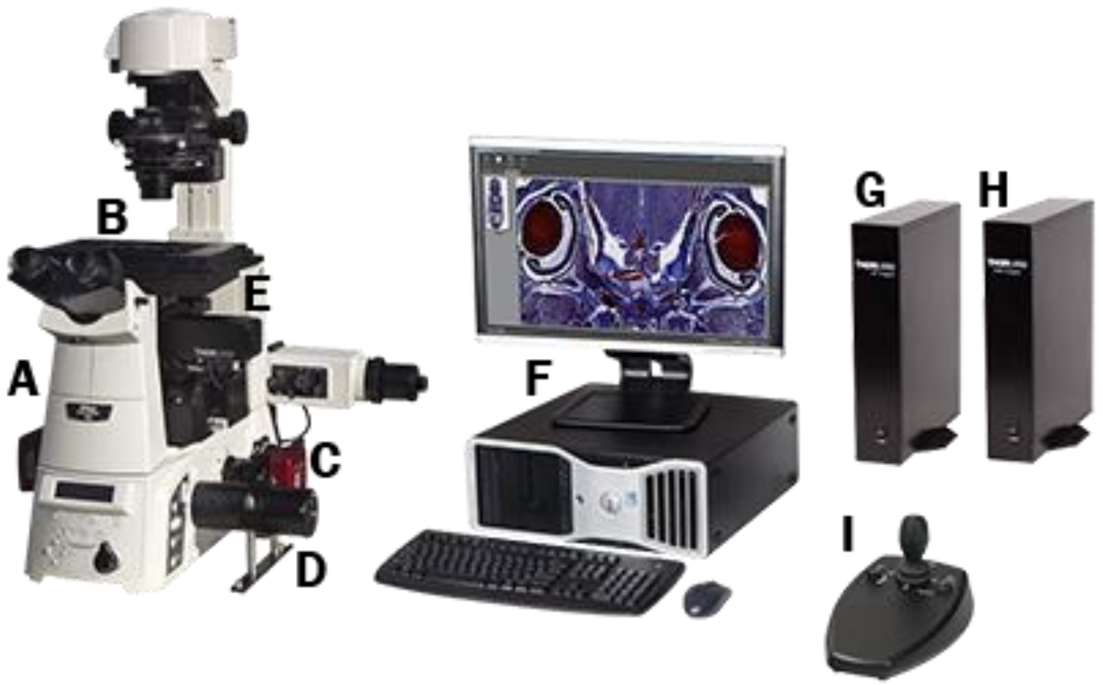


Figure 5 Experiment.xml File in Root Folder

Chapter 4 Operation

4.1. System Components



A – Microscope Base
B – Slide Holder/Stage (Refer to Chapter 1 Safety)
C – Digital Camera – OSCAR Enabled
D – Focus Motor
E – Auto-focus Head (Refer to Chapter 1 Safety)
F – Host Computer and Monitor (Not Sold with System)
G – AF-OCT-SP2 "Auto-Focus Engine" (Refer to Chapter 1 Safety)
H – OE1 "OSCAR Engine"
I – Joy Stick

4.2. Experiment Setup

Select the experiment format (i.e four slides, well-plate, petridish). If the system is only configured for one experiment format, this step is not necessary as it will default to the correct medium.

Insert the medium to be scanned. For slides, install the desired number of slides to be scanned. Orientation of the slide is critical to ensure desired operation of the system. All slides should be installed on the stage in the orientation shown on the scan tab (see Section 4.2.1). For example, for inverted microscopes with a four slide holder the coverslip should be face down and the label should be on the side closet to the user.

Full instructions on operating the software during a scan and export can be found in Section 4.2.

4.3. Graphical User Interface

TIDE LS software is used to control all elements of the TIDE Imaging System. There are three main screens for viewing information: Experiment Setup Screen, Live View Mode, and Results.

4.3.1. Experiment Setup Screen (Scan Tab)

The Experiment Setup Screen, also referred to as the “Scan” tab, is the initial screen that appears when selecting a new scan. This screen is essentially the home screen of the experiment. This allows the user select the medium they would like to scan, name the experiment, and access the live view for determining the parameters for the prescan. All experiments start with a prescan on this screen. The user should select each slide they wish to scan. Depending on the system configuration up to 4 slides can be selected to be scanned at one time. The functions of this screen are as follows:

Open-This allows the user to open a saved scan result. To open the results of a past scan the user should browse to the folder and select the experiment.tdix file.

New Scan-The new scan button serves two purposes. When the system is first started, the new scan button will initialize the scanner. This can take up to one minute depending on the system configuration. The new scan button is also used to initiate a new scan tab. Once scans are completed, a new scan tab must be opened to initiate a new scan.

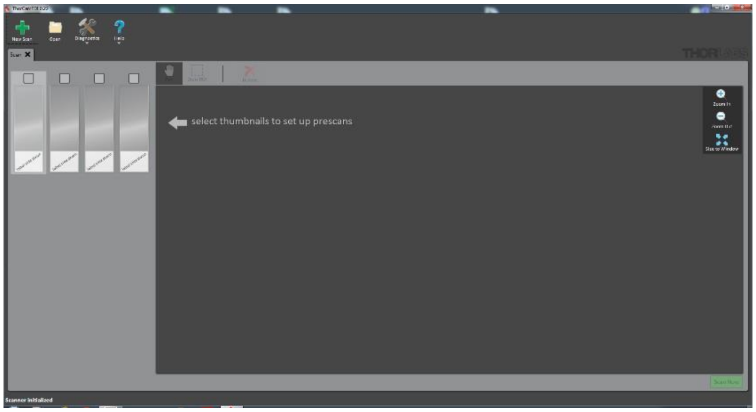


Figure 6 Representative Scan Tab without Slide Selected

Including a Slide-Slides can be included and excluded by either clicking on the slide or the check box above the slide. Once a slide has been scanned it cannot be excluded. In order to rescan a slide, the scan tab must be closed and a new scan tab opened.

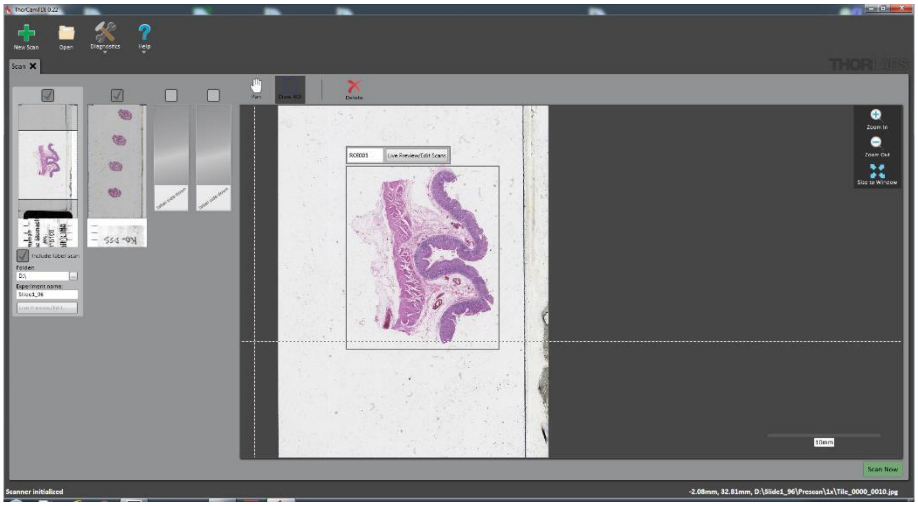


Figure 7 Scan Tab with Two Completed Prescans and ROI Drawn

Include Label Scan-Label scanning is enabled by default. By unchecking the include label scan when the scan is completed it will ignore the label.

Folder-Allows the user to define the path where the experiment will be saved. This cannot be changed once a scan has commenced.

Experiment Name-Allows the user to name the experiment. This cannot be changed once a scan has commenced.

Live Preview/Edit-This will launch the live view with the settings that will be used for the scan. While it is possible to enter the live view once a scan is complete, the settings cannot be edited once the scan is complete.

Pan-The pan function allows the user to pan around the resulting prescan.

Delete-Allows the user to delete an ROI and all its associated scan settings. Once an ROI is deleted it cannot be recovered.

Scan Now-This executes on any of the scans that have been configured. This button will enable when at least one scan is configured to execute. While it is possible to scan from one to four slides at a time, it is most efficient to set up all available slides before scanning.

Draw ROI-Allows the user to select a region of interest for further scans. Multiple ROI's can be drawn on a single prescan. *TIP:Given the nature of the way the system scans it may be more efficient to scan a single larger area than a collection of smaller ROI's (i.e. small dense tissue micro arrays).*

ROI Name-The ROI name is displayed at the top of the ROI. The user can rename the ROI by clicking on the name. The ROI can only be renamed before the ROI scan has completed.

ROI Live Preview/Edit-Allows the user to setup the parameters for the ROI scans and preview. Once an ROI scan(s) are complete, the setup parameters cannot be changed. However, the user may still add additional scans to the ROI.

4.3.2. Live View/Edit Window

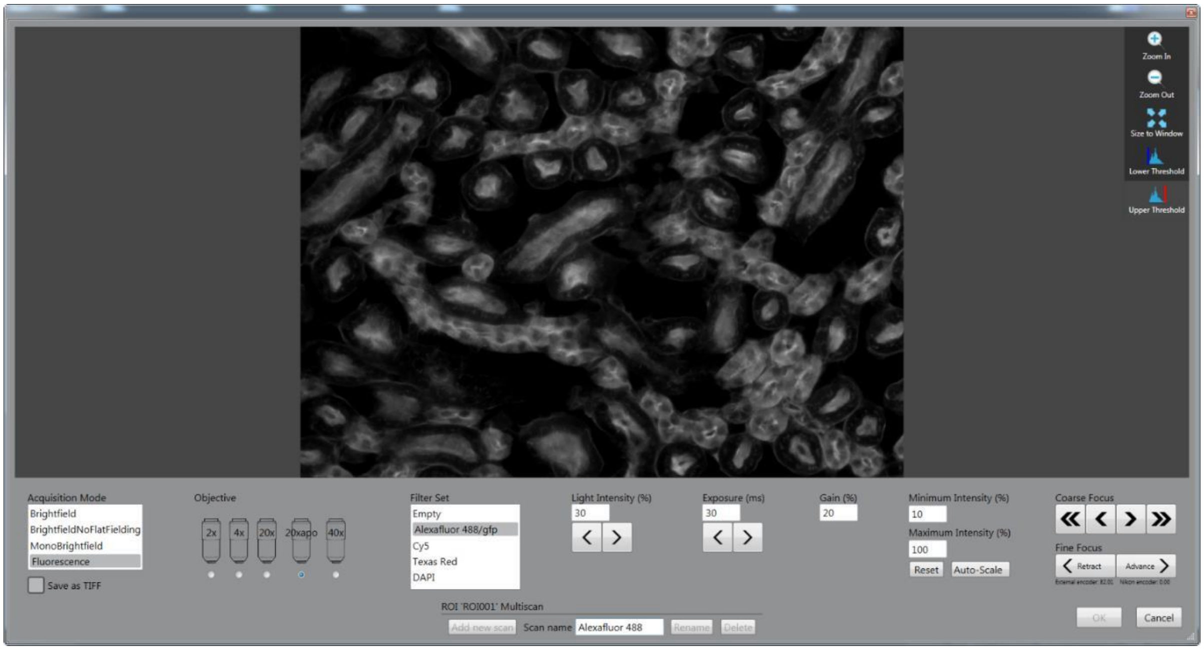


Figure 8 Live View/Edit Window

The live view window is the area where a user selects all the parameters used to image and provides a live window that is representative of what the final image will look like. The user should expect the end scan to have the same quality that is displayed in the live window. The main functions of this view are as follows:

Acquisition mode-Defines the camera to be used to image as well as the illumination path.

Save as TIFF-The system default is to save scans in TIFF. If the box is unchecked, the system will save scans as JPEG. TIFF is recommended where data preservation is needed.

Objective-Defines the objective that is to be used for a given scan.

Filter Set-This will display any preconfigured filter set options in the system. This view will only show for acquisition modes configured to work with filter sets.

Light Intensity-This applies to a given light source assigned to an acquisition mode and is displayed in percent intensity. The range of available intensity may be limited by the light source configuration.

Exposure-The desired exposure to use for acquisition. Note: At longer exposures the available exposure options may be limited. In the event an exposure is entered that is invalid the system will provide the two nearest exposure values.

Gain-This is adjustable from 0-100% in 1% increments. This is the relative gain applied to the analog signal from the image sensor. While increasing gain will increase signal, this will also slightly increase noise.

Min/Max Intensity-This sets a minimum and maximum intensity threshold and persists to the raw image. Setting a minimum threshold will block any signal below the value set. Reducing the maximum value will effectively scale the signal so the camera will digitally saturate at a lower value. Unlike the Gain setting above which scales the camera ADC, this setting does not impact the camera but rather the image itself.

Focus Control-The focus controls will appear on systems equipped with a compatible focus motor. There are three levels of resolution: two levels of coarse adjustment and one level of fine adjustment. These controls can be activated with a single click or, for moving larger distances, the button can be held down.

Zoom In/Out-Zooming in and out in the live view can be done by using the mousewheel or the zoom in and out buttons.

Scale to Fit-Scales the live image to fit the available window.

Lower Threshold-Enabling lower thresholding will paint any pixel value below 2%, in the live window, blue. This does not persist to the final image. This is only intended to be a tool for setting up a scan.

Upper Threshold-Enabling upper thresholding will paint any pixel value above 98% in the live window red, indicating the camera is approaching saturation. This does not persist to the final images. This is only intended to be a tool for setting up a scan.

Add New Scan-This button will add a new scan to the selected region of interest. For example, in applications such as multichannel fluorescence, a user may add up to 5 different scans, one for each color channel to be imaged. Add a scan will commit any changes made to the previous live window before adding the new scan.

Scan Name-This displays the name of the scan currently being edited. If multiple scans have been added, this field will display as a drop down box and can be used to select a specific scan to edit.

Rename- This will turn the scan name into a user editable field. A name change can be committed by hitting the enter key.

Delete-This will delete the current scan settings.

OK-Hitting this will commit all the changes made in the current live window session. In the event that a scan has been completed, this button will show as return to results.

Cancel-This will close the live view and dispose of any changes made to the current live view session.

4.3.3. Results Tab

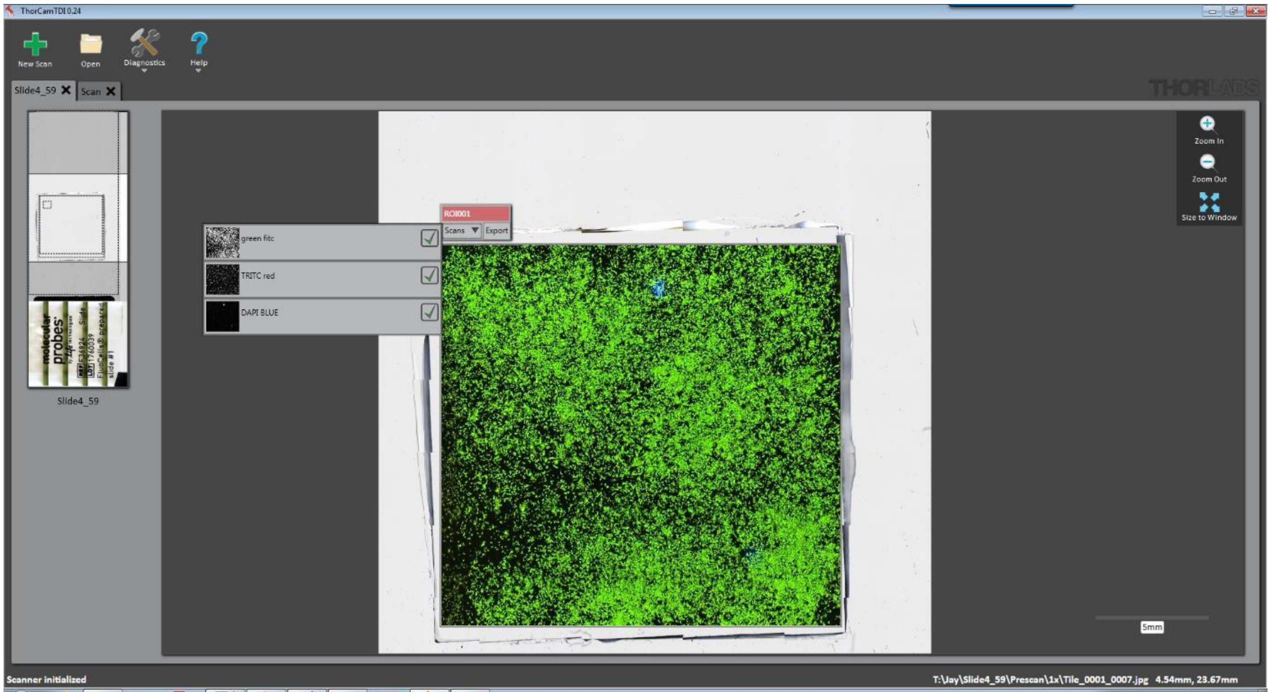


Figure 9 Results Tab

Results are displayed as tabs using the experiment name, once all the scans for a given experiment are complete. A thumbnail of the slide is shown on the left and the zoomable window on the right. This screen allows users to toggle between scan layers, zoom in to look at high magnification scans and access the image export features.

Toggle ROI's-When there are several ROI's that overlap, these can be selected by right clicking on the ROI. The box at the top of the ROI will display the ROI being viewed.

Selecting Scans-Scans can be turned on and off using the check boxes in the scan drop down.

Export-The export feature is accessed from the ROI toolbar. To export an ROI and its associated scans, selected the ROI and then click the export button. More information on export can be found in Section 4.3.

4.4. Export

Slide scanner systems allow for large area high resolution image creation. This results in very large file sizes that can be a challenge to manage and work with. The included image exporter allows users to easily export images in a number of ways, whether it be breaking full resolution data sets into smaller subsets for analysis or conversely combining full resolution images into larger images or down-sampling images for publications.

4.4.1. Features

The following features are available:

- Export to jpeg or TIFF
- Creating Large Single Images
- Combine data sets into a single large image with an option for down-sampling (ideal for publications and notes) or preserving the full resolution
- Create custom tiled images - preserves full resolution while allowing users to choose the size of images.

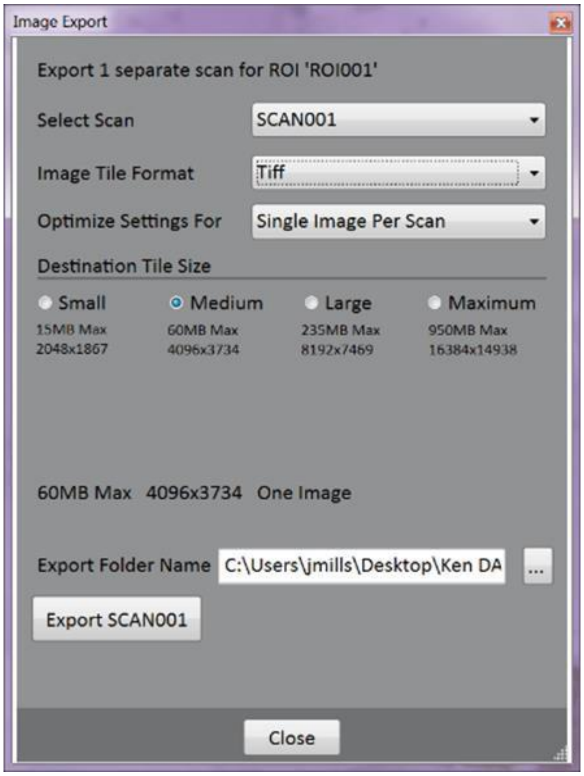


Figure 10 Image Export Options

Select Scan-This dropdown allows the user to select which scan to export for the selected ROI.

Image Tile Format- Users can select JPEG or TIFF. *Note: If a scan was conducted in a JPEG format, selecting TIFF as an export format will maintain the same data integrity as the JPEG. Converting from TIFF to JPEG will down-sample.*

Optimize Settings for:

> **Single Image**-This will create one large single image. The limit to the single image size is based on available RAM on the PC.
>
> **Custom Tiled**-This will create a collection of tiles of the size specified in the destination tile section.

Destination Size-Allows the user to select the maximum file size for a given file. This will display in pixels as well as file size. Depending on the scan size and machine requirements, some options may not be available.

Export Folder Name-Allows the user to define an export path.

Chapter 5 Trouble Shooting

NOTE: There are no user serviceable parts in the system. Please see Chapter 2 for warnings related to servicing the system.

Dropped Frames- Dropped frames are generally associated with high CPU or network usage. Limit any amount of internet usage or processes that consume CPU usage (i.e panning of large images, image processing, downloading or uploading large files) while scanning.

Autofocus Error- Ensure the sample is installed in the correct orientation as indicated in the scan tab.

Triggers are not being generated- Ensure the trigger cable on the camera and TIDE engine are connected.

Live view does not show image/black image- Ensure shutters on microscope light path(s) are not closed.

Chapter 6 Care and Maintenance

Handle the system with care during transportation and unpacking. Banging or dropping the system can damage the unit or lower system performance. If the system is mishandled during shipment, the optical components may become misaligned, which could lead to a decrease in image quality. If this occurs, the system will need to be realigned by qualified personnel. Please contact Thorlabs technical support for more information.

- Do not store or operate in a damp, closed environment.
- Do not store or operate on surfaces that are susceptible to vibrations.
- Do not block or obstruct vent holes or fans.
- Do not expose to direct sunlight.
- Do not use solvents on or near the equipment.
- Keep away from dust, dirt, and air-borne pollutants (including cigarette smoke). The system is not designed for outdoor use. Protect the equipment from rain, snow, and humidity.
- Do not expose to mechanical and thermal extremes. Protect the equipment from rapid variation in temperature.
- Handle all connectors, both electrical and optical, with care. Do not use unnecessary force, as this may damage the connectors.
- Handle the optical fiber with care. Mechanical stress can decrease performance and potentially destroy the fiber. Continual bending of the optical fiber can cause damage.

6.1. Service/Configuration

All TIDE imaging systems are aligned and configured by a technician at the time of install. Please contact the Thorlabs service department for alignment and configuration needs.

Only trained and approved Thorlabs personnel are allowed to service the system. Please contact Thorlabs technical support for more information. Opening or servicing of the system by anyone other than Thorlabs approved personnel will void the warranty.

6.2. Accessories and Customization

The scanner system can easily be adapted for custom accessories. To achieve the listed specifications however this system should only be used with the accessories that Thorlabs provides. Any modification or maintenance by unqualified personnel will render the warranty null and void, leaving Thorlabs free of liability. Please contact Thorlabs technical support for questions on customization.

Chapter 7 Regulatory

As required by the WEEE (Waste Electrical and Electronic Equipment Directive) of the European Community and the corresponding national laws, Thorlabs offers all end users in the EC the possibility to return “end of life” units without incurring disposal charges.

- This offer is valid for Thorlabs electrical and electronic equipment:
- Sold after August 13, 2005
- Marked correspondingly with the crossed out “wheelie bin” logo (see right)
- Sold to a company or institute within the EC
- Currently owned by a company or institute within the EC
- Still complete, not disassembled and not contaminated

Wheelie Bin Logo

As the WEEE directive applies to self-contained operational electrical and electronic products, this end of life take back service does not refer to other Thorlabs products, such as:

- Pure OEM products, that means assemblies to be built into a unit by the user (e. g. OEM laser driver cards)
- Components
- Mechanics and optics
- Left over parts of units disassembled by the user (PCB’s, housings etc.).

If you wish to return a Thorlabs unit for waste recovery, please contact Thorlabs or your nearest dealer for further information.

Waste Treatment is Your Own Responsibility

If you do not return an “end of life” unit to Thorlabs, you must hand it to a company specialized in waste recovery. Do not dispose of the unit in a litter bin or at a public waste disposal site.

Ecological Background

It is well known that WEEE pollutes the environment by releasing toxic products during decomposition. The aim of the European RoHS directive is to reduce the content of toxic substances in electronic products in the future.

The intent of the WEEE directive is to enforce the recycling of WEEE. A controlled recycling of end of life products will thereby avoid negative impacts on the environment.

Chapter 8 Thorlabs Worldwide Contacts

For technical support or sales inquiries, please visit us at www.thorlabs.com/contact for our most up-to-date contact information.

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