

# **Optical Coherence Tomography**

# ThorImage OCT Operating Manual



Version 4.4



Version: 4.4 Date: 9/28/2016

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Thorlabs GmbH



This manual contains "NOTES" which highlight important behavior of this software package.

Please read these advices carefully!

# 1 Introduction

# **1.1 Important Notices**

Please read the operating manual carefully before using your Thorlabs OCT System. All statements regarding safety and technical specifications will only apply when the unit is operated correctly. Refer to the OCT System Operating Manual for information regarding to the hardware components of this system. This software is intended for laboratory use only and is not certified for medical applications, including but not limited to life support situations.

# **1.2 Software Overview**

The Thorlmage OCT software is an advanced imaging and data management application that works with Thorlabs Optical Coherence Tomography imaging systems. It runs on the Microsoft Windows 7 operating system and provides a comprehensive user interface for acquiring, viewing, and managing OCT data.

ThorImage OCT features 1D, 2D and 3D OCT data acquisition and processing. In addition, advanced processing techniques such as Optical Coherence Angiography (Speckle variance contrast imaging) and Doppler OCT complete the main feature set of the application. The software offers a high degree of flexibility by enabling easy point-and-click scan pattern creation and being able to edit scanning parameters directly. Visual enhancements of the OCT image view can be achieved by adjusting contrast and brightness, choosing among different colormaps and views or switching between sectional view, surface view, or volume rendering in 3D mode.

Acquired OCT data can be stored, opened, and viewed in 1D, 2D and 3D formats. OCT data can also be exported in standard image formats (BMP, JPEG, PNG, PDF, and TIFF). In addition, users can export and convert OCT data into VTK data, VFF data, FITS data, and binary raw processed data (RAW format), which is supported by open-source image processing software such as ImageJ, ParaView, or MicroView.

Since version 4.0 of ThorImage OCT, OCT data files have been using an extendable proprietary file format. The state in which OCT data is stored inside the file can be determined in the <u>Settings dialog</u>; options include processed data, spectral raw data, or both (the processed data format covers most requirements whereas storing raw spectral data aims for the advanced scientific user intent on using their own processing algorithms on the OCT data).

Starting with version 4.2, legacy data files acquired with Thorlabs SDOCT software having the *RAW* or *SDR* extension (spectral data) can be loaded and exported with Thorlmage OCT.

# 1.3 First Steps

Upon receipt of your Thorlabs OCT system, you will find that the Thorlmage OCT software application is pre-configured for a quick start. No options need to be adjusted when running the Thorlabs OCT system for the first time.

After setting up the Thorlabs OCT imaging system according to the instructions in the Hardware User's Guide, turn on the OCT device and the computer. Open the Thorlmage OCT application by either double-clicking the Thorlmage OCT icon on the desktop or navigating to All Programs -> Thorlmage OCT and clicking the Thorlmage OCT shortcut. The user interface of the application will appear after the OCT device has been initialized and the following default view is displayed:



Desktop Icon of ThorImage OCT



After starting the ThorImage OCT software, the main window will open.

This manual will walk you through the functionality of the software including 2D imaging, scan control, 3D imaging, recording, playback, and exporting of OCT data.

# 2 Overview

This chapter gives an overview of the ThorImage OCT user interface and its main controls. The user interface is divided into several regions providing different options, actions, and properties.



# **Mode Selector**

Use the mode selector to switch between the OCT system's acquisition modes:

- 2D Mode: Acquisition of 2D data (B-scans)
- <u>3D Mode</u>: Acquisition of volume scans
- Doppler Mode: Acquisition of Doppler OCT data
- Speckle Variance Mode: Acquisition of Speckle Variance data
- 1D Mode: Acquisition of 1D data (A-scans)
- Data Viewing Mode: Viewing of captured datasets of any of the former acquisition modes

#### Action Toolbar

This toolbar provides controls to perform actions in the current acquisition mode. Data acquisition can be started and stopped from here using the Scan/Stop button. Single snapshots or entire scans can be captured by clicking the Record button. The bar with the two sun symbols displays the current reference intensity if applicable to your OCT system.

When viewing datasets, the action toolbar will display options for opening the dataset in ImageJ (if installed) or exporting the data to various export formats.

#### **Display Control Toolbar**

When viewing live or offline OCT data, the display can be adjusted using the controls available here. These include setting color modes, selecting aspect ratios, performing distance measurements on the current data, or showing a single A-scan in a B-scan.

#### **Captured Datasets**

The captured dataset list displays all datasets acquired during a ThorImage OCT session. To get a good overview of the captured datasets, for each dataset the list shows the acquisition mode in which it was acquired along with a preview picture of both the sample monitor and the OCT data, as well as the study name, the experiment number and the storage format. Left-clicking a list entry will activate and display the selected dataset.

# **Dataset Parameters**

When acquiring data, this panel contains the parameters used in the currently running acquisition. In data viewing mode, the parameters used to acquire the currently active dataset are displayed. These include their identifier (study name and experiment number), the refractive index of the data, user-defined comments for the current dataset, acquisition parameters (like B-scan size, volume size, scan orientation, etc.), and processing and averaging parameters that influence imaging quality.

#### **Sample Monitor**

The sample monitor displays the live video image of the probe camera. A blue boundary depicts the maximal scan range. The current scan line or scan area is drawn in red, and can be set with the mouse pointer by using the <u>Draw & Scan</u> feature. When viewing a captured dataset, this will usually contain a still image of the probe's camera at the time of acquisition.

## Note

The contents of the action toolbar, the display control toolbar and the dataset parameters control depend on the currently active acquisition mode and are explained in the corresponding chapters of this manual.

# 2.1 Viewing Options

All controls, except for the OCT display in the center and the mode switching buttons on the extreme left, are arranged in "*tool windows*". Tool windows can be undocked and rearranged within and outside of the application window by clicking on the tool window title bar and dragging to a different location. A tool window can remain undocked in a floating position or docked to different target areas. To dock a tool window to a different location in the application screen, drag the window until it hovers over a highlighted target area. Then release the window for it to dock at that new location. Additionally, tool windows can be automatically hidden or arranged in tabbed interfaces. For the former click on the down area in the upper right corner of a tool window and select *Auto Hide*. For the latter drag the tool window on top of another tool window. The two tool windows will then share a common tab-interface.

The window configuration will be restored when closing and restarting the application.



Drop markers and highlighted target area during a tool window dragging operation

Two different default window layout configurations can be loaded by choosing the respective entries from the *View* menu:



Viewing options in the View menu

• **Default View**: The default configuration for wide screen displays. The Captured Datasets, Dataset Parameters, and Sample Monitor controls are located on the right.

• **Small Screen View**: This configuration is for small screens with a 4:3 aspect ratio. The Captured Datasets, Dataset Parameters, and Sample Monitor controls are located at the bottom.



Left: The Default View configuration; Right: The Small Screen View configuration

# 2.1.1 Menu Bar

The menu bar is invisible by default. The visibility of the menu bar can be toggled either by using the Alt key or the F10 key or by changing the corresponding setting in the <u>settings dialog</u>.

ThorImage	OCT 4.1	-	Frank Station	Automation allow			
<u>F</u> ile <u>V</u> iew	<u>P</u> lugins <u>H</u> elp						
$\square$	) Scan	) Record	<b>J</b> ia Snapshot	S Advanced Snapshot	•	*	*
ThorImage	OCT 4.1	-	Contraction in	Automatical States			
	) Scan	) Record	<b>J</b> Snapshot	Advanced Snapshot	•	*	*

Hiding and showing the menu bar can be toggled by the Alt or F10 keys.

# 2.1.2 Fullscreen Mode

The fullscreen mode can be toggled by either pressing *F11* or by using the *Fullscreen OCT* entry in the view menu. Activating fullscreen mode also removes the window decorations (the application's window title bar and icons) and the task bar.



Application in regular appearance (title bar and window decorations on)...

... and in fullscreen mode with title bar and window decorations off.

# 2.2 Settings Dialog



The settings button at the right end of the toolbar

The Settings Dialog is accessible through the settings button in the right part of the Action Toolbar. It provides global options that are used throughout the entire application.

T Settings		×
General Hardware Feedb	Jack	
Dataset and Export		
Dataset Folder	C:\OCTData\	Change
Export Folder	C:\OCTExport\	Change
ImageJ Command	C:\Program Files\Fiji.app\ImageJ-win64.exe	Change
One Click Export Data Format	PDF	
One Click Export Slice Direction	XZ Slice	
Storage Format	Store Processed Data	
X Open default image viewer	after one click export	
Mode Switch	between acquisition modes sition	
Appearance Show Menu Bar		
	O	Cancel

The Settings dialog provides ThorImage OCT's global configuration options.

# 2.2.1 General: Dataset and Export Options

Dataset and Export					
Dataset Folder	C:\OCTData\	Change			
Export Folder	C:\OCTExport\	Change			
Imagel Command	C:\Program Files\Fiji.app\ImageJ-win64.	Change			
One Click Export Data Format	PDF				
One Click Export Slice Direction	XZ Slice				
Storage Format	Store Processed Data				
X Open default image viewer	after one click export				

Export options in the Settings dialog

## Dataset Folder

Recorded datasets will be automatically stored in this folder.

## **Export Folder**

When exporting data, this folder will be used as default location.

#### ImageJ Command

This option sets the default command and path to be used to start the ImageJ application. A valid executable needs to be selected in order for the ImageJ command to show up when viewing datasets.

#### **One Click Export Data Format**

Describes the default image export format that is used, for example, if the viewed dataset is to be opened by an external image viewer. Available options include PNG, BMP, JPG, PDF, and TIFF.

#### **One Click Export Slice Direction**

When exporting volume datasets as images they need be sliced into single images. The direction in which the slicing is performed by default is described by this option.

#### Storage Format

In the storage options, one can specify which data should be stored in the dataset files. Storing the processed data has the advantage that dataset loading and switching in the dataset list is fast. Storing spectral raw data enables to reprocess data offline after it has been acquired. Storing both combines these advantages but comes for the cost of increased file size.

#### Open default image viewer after one click export

If this option is checked, the default windows image viewer is opened after performing the one click export.

# 2.2.2 General: Mode Switch Options

Mode Switch

 Image: Setting setting setting setween acquisition modes

 Image: Setwate dataset after acquisition

#### Mode switch options in the settings dialog

#### **Transfer Geometry**

When activating the option Transfer geometry settings between acquisition modes, the scan pattern is

# ThorImage OCT

maintained if possible when switching acquisition modes. When switching for instance from 2D to Doppler mode, the complete scan pattern is maintained. When switching from 2D to 3D mode, the B-scan length (FOV x), the Center position, and the Angle is maintained.



The scan pattern will be transferred when switching from 2D (left) to 3D mode (right) if the *Transfer geometry settings* between acquisition modes option is enabled.

#### Activate Dataset

When activating the option *Activate dataset after acquisition*, the dataset is activated and shown in the display after acquisition. Deactivating this option is especially useful in speckle variance mode for disabling processing of the data, which can take longer than the acquisition itself. To achieve this, the *Storage Format* has to be set to *Spectral Raw Data*.

# 2.2.3 General: View Options



The view options portion houses a checkbox to toggle the visibility of the menu bar on and off. When this box is not checked, the menu bar can be brought up by pressing the *F10* or the *Alt* key. See also <u>Viewing</u> <u>Options</u>.

# 2.2.4 Hardware: Probe Configuration

Probe	
Probe Configurations	ProbeLSM03.ini
Add/Edit Probe Config	Probe.ini
	ProbeLSM02.ini
Device Configuration	ProbeLSM03.ini
	ProbeLSM04.ini

#### Probe configuration choice

If you have different probe objectives at your disposal, you will also have different configurations for each of the objectives. These configurations contain information about the optical properties of the objective lens as well as correction factors for the draw & scan feature. Make sure to have the matching probe

configuration selected in this dropdown when changing probe or probe objectives.

If the probe objective is not listed, a new probe configuration can be generated by clicking the *Add/Edit Probe Config* button Please refer to the <u>probe calibration</u> section of this manual for learning about the probe calibration process.

# 2.2.5 Hardware: Handheld Probe Configuration (optional)

Handheld Probe Button Functions		Probe Keys
Button 1 Start/Stop measurement / Preview (3D)		
Button 2 Snapshot	-	\\_ • /
Button 3 Autoset signal thresholds		3 2
Handheld Probe Audio feedback		
<ul> <li>Start/Stop measurement / Preview (3D)</li> <li>Start/Stop recording / Scan (3D)</li> <li>Ringlight intensity up/down</li> </ul>	<ul> <li>Snapshot</li> <li>Advanced snapshot</li> <li>Autoset signal thresholds</li> </ul>	

Handheld probe configuration

If you have a Thorlabs OCTH handheld probe connected to your OCT system, the settings dialog will provide some options specific to the handheld probe. These comprise the configuration of the three buttons of the handheld probe, as well as toggling audio feedback on key system events.

# Handheld Probe Button Functions

You can assign one of a number of functions to each one of the three buttons in the handheld probe. Choices are:

- Start/Stop measurement / Preview (3D): Toggle image acquisition on/off; in 3D mode this will start and stop the preview function.
- Start/Stop recording / Record (3D): Toggle recording on/off; in 2D/Doppler modes, when this is pressed during a running acquisition, the acquisition will not stop after recording has been stopped.
- Increase ringlight intensity: same as moving the ringlight slider upwards (see Sample Monitor/Ringlight)
- Decrease ringlight intensity: see above
- Snapshot: see <u>Snapshot</u>
- Advanced snapshot: see <u>Advanced Snapshot</u>
- Autoset signal thresholds: see Coloring Control

#### Handheld Probe Audio Feedback

Use the checkboxes to toggle audible feedback for the handheld probe button functions listed above.

# Note

You may need extra hardware (speakers/headphones) to hear the audio feedback. Modern computers usually come with onboard sound cards; there should be one or more 1/8in jacks on the computer's backpanel for connecting headphones or speakers. Please refer to the appropriate hardware manual to

learn how to connect audio hardware.

# 2.2.6 Hardware: Device Configuration



Resetting the device configuration in the Settings dialog

Thorlabs' Spectral Domain OCT systems have the capability to restore their default device configuration. During regular use there should be no need to adapt/restore the device configuration.

# Note

The restore device configuration functionality is not available for Swept Source OCT devices and for Spectral Domain OCT systems that were delivered with a software version 3.2 or lower.

# 2.2.7 Feedback

Visual Feedback on key events	
<ul> <li>Start/Stop measurement / Preview (3D)</li> <li>Recording (2D/Doppler)</li> <li>Snapshot</li> </ul>	<ul> <li>Advanced snapshot</li> <li>Autoset signal thresholds</li> </ul>
Audio Feedback on key events	
Play Snapshot Sound	

Audiovisual feedback options

# Visual Feedback on key events

Toggling these checkboxes will provide visual feedback on the listed events. Visual feedback will be either the toolbar or the display background flashing.

# Audio Feedback on key events

These checkboxes, when checked, will provide an audio signal on the listed events. Currently only the snapshot event provides optional audio feedback.

Please see also the <u>note</u> in the Handheld Probe Configuration section.

# 2.3 Hardware: Device Configuration 2

Device Configuration		
Restore Device Configuration		

Resetting the device configuration in the Settings dialog

Thorlabs' Spectral Domain OCT systems have the capability to restore their default device configuration. During regular use there should be no need to adapt/restore the device configuration.

# Note

The restore device configuration functionality is not available for Swept Source OCT devices and for Spectral Domain OCT systems that were delivered with a software version 3.2 or lower.

# 2.4 Data Viewing Mode

ThorImage OCT will start up in data viewing mode when no OCT device is connected and switched on. A warning will pop up saying that the OCT device could not be initialized which can safely be disregarded if acquiring data is not required. All data acquisition modes will be disabled in this mode.



Warning indicating that the device could not be found

In this mode, data files can be opened using the *Open Dataset* or, if available, the *Recent Datasets* button in the captured datasets tool window. The datasets will be added to the dataset list and can be handled in the same way as if the device was connected.



Software when started in Data Viewing Mode; Open Dataset and Recent Datasets buttons in the upper right corner are marked in red.

# **3 OCT Imaging**

selector.

2D Mode

• 3D Mode

<u>1D Mode</u>

Doppler Mode

mode's manual section):

Speckle Variance Mode

Data Viewing Mode



Mode selector

# 3.1 Reference Intensity

The reference intensity shows the amount of light from the reference arm that is reaching the detector.



OCT imaging can be performed in different modes which can be selected using the mode

If the software finds a Thorlabs OCT device connected and switched on, all five operational modes are available through the mode selector. If no OCT device is present,

From top to bottom (you can also click in the image on the left to open the corresponding

only the data viewing mode for viewing and exporting OCT data will be available.

Different states of reference intensity. For optimal image quality, the reference intensity needs to be in the green range.

# Note

For optimal imaging the reference intensity needs to be set accordingly. Please refer to the hardware manual of your Thorlabs OCT device for instructions on how to adjust reference intensity.

#### Note

The reference intensity control is not available for Swept Source OCT engines. Setting the reference intensity is not required in these systems.

# 3.2 Reference Length (VEGA Imaging Module)

If you have a Thorlabs VEGA imaging module as shown below, the toolbar will have reference stage controls in all data acquisition modes. The VEGA imaging module contains a motor-controlled integrated reference stage. The reference length can be set through a set of controls in Thorlmage OCT.



**Thorlabs VEGA Imaging Module** 

When ThorImage OCT starts, the imaging module will calibrate the motorized reference stage whenever it requires calibration. During this process the reference stage controls will be blocked. You can however use the other aspects of ThorImage OCT while calibration is taking place.

Zeroing reference stage		
Reference Stage Control		
Reference stage during calibration process		

Usually the process of calibrating the reference stage should not take more than ~20 seconds, provided that the software has been closed properly before switching off the imaging module after its last run.

# Note

The reference length displayed in the input field is relative to the zero delay line positioned at the very top of the OCT image. Thus, when the relative reference length is set to 0.000mm, a good starting point for measuring can be obtained by positioning the probe height so that the primary peak of your sample is at the top of the OCT image.

# Note

The reference length / zero delay line calibration depends on the probe objective used. Thus, if you switch probe configurations using the <u>Settings Dialog</u>, the reference stage will keep its position relative to the zero delay line with the new objective.



The reference stage controls allow you to move the stage in different ways:

• By clicking and holding the jog buttons. Clicking one of the smaller arrows will move the reference stage slowly, comparable to a turn of the OCTG probe's reference length adjuster. Using the larger arrows will result in faster movement.

Clicking and holding an arrow button will move the stage in the indicated direction until the mouse button is released or the maximum/minimum length is reached.

• By entering a number into the input field and clicking the Move button to the right. This will immediately move the reference stage to the given length; the controls are locked during the move.

# Note

ThorImage OCT will remember the last used reference length after each restart and subsequently move the stage during initialization. When the stage needs recalibration, the controls will be locked for the duration of the zeroing process (this may take approx. 30-40 seconds and will not happen very often).

# 3.3 Background Calibration

For optimal image quality, a background measurement must be performed. To achieve correct results, remove the sample, ensure that no signal is left in the OCT image, and click the *Calibrate Background* button in the action toolbar.



Background measurements can be performed by pressing the calibrate background button.

# Note

*The calibrate background button is only visible for Swept Source OCT systems*. When working with Spectral Domain OCT systems (Callisto, Ganymede, Hyperion, Telesto, and Telesto II), manual background calibration is not necessary as the background measurement is automatically performed during device initialization.

# 3.4 Sample Monitor

The sample monitor tool window displays a live video image of the sample if one of the acquisition modes is selected. The sample monitor proides visual aid in positioning the sample. The focus can be adjusted by focusing the sample in the video image. Additionally, the scan position can be determined in the sample monitor.

When an offline dataset is selected, a still video image from the acquisition of the offline dataset will be displayed.



Sample monitor showing a live image of the video camera (scan region display switched on)

# Scan Region / Draw & Scan

The current scan region can be displayed as an overlay on the video image. In the 2D mode the overlay takes the form of an arrow representing start and end of the B-scan line. The tip of the arrow indicates the last acquired A-scan of the B-scan corresponding to the rightmost vertical line in the OCT display.

In 3D mode a rectangle indicates the area to be scanned whereas in 1D mode the beam position is indicated by a red cross. The visibility of the scan region can be switched on and off by using the scan region button below the video image. The scan region can be directly modified by using the draw & scan feature. The scan pattern can be drawn directly into the video image by clicking the start and the end points of the scan.

In all modes, the maximum possible scan region is displayed as a blue rectangle. No part of the scan arrow and the scan rectangle can thus lay outside of the area, respectively.

# **Ring Light**

The standard probe included with the Thorlabs SDOCT systems provides a ring light to illuminate the sample for the video image. The intensity of the ring light can be adjusted by clicking the ring light button and moving the slider.



Ring light slider in the sample monitor control (scan region display switched off)

# Note

The ring light and the ring light control might not be available for your OCT system.

# 3.5 2D Mode

In 2D Mode, the probe beam is scanned in one lateral direction while reflectivity profiles (A-scans) are collected in the depth direction. This collection of A-scans builds a 2D cross-sectional image called B-scan. As an alternative to using the scan parameters window the scan line can be defined by using the Draw & Scan feature.

# 3.5.1 Toolbar

In the 2D mode, the action toolbar looks as follows:



#### Scan

Using the scan button starts and stops 2D OCT data acquisition.

#### Record

The *Record* button can be used to acquire a stack of many B-scans, acquired as quickly as possible after each other. The acquired stack of B-scans is shown as a single entry in the dataset list. It can be viewed by clicking on the respective entry in the dataset list.

## Note

Using the *Record* button can not guarantee a consecutive stack of B-scans. To make sure that no scans in between get lost please use *Advanced Snapshot* button or <u>3D Mode</u> with FOV y equals zero.

#### Snapshot

When the OCT system is acquiring 2D data, a single image can be stored using the *Snapshot* button. The acquired B-scan appears in the dataset list and can be selected in order to view it.

#### **Advanced Snapshot**

For acquiring high quality 2D data the *Advanced Snapshot* button can be used. By pressing the down arrow right next to the button options for this specific mode can be selected. It provides advanced averaging options and performs more averaging compared to the live acquisition yielding high quality images. When performing B-scan averaging it registers the images and adjusts their relative position to reduce the effects of bulk sample motion. Except for the averaging options all parameters are identical to the live data acquisition parameters. The averaging options set in the averaging section of the dataset parameters are ignored.

The acquired B-scan appears in the dataset list and is automatically selected when taking the high quality shot.

# Note

When using a handheld probe (Thorlabs OCTH), the toolbar will provide for manual calibration of the apodization spectrum due to the fact that this particular scanner type does not acquire dedicated apodization spectra for system-inherent performance reasons. By default, "Use scan for apodization" is checked so manual calibration is not required unless intended.

X	Use scan for apodization spectrum
	Calibrate Spectrum
Ма	nual calibration might be desired

when using an OCTH scanner.

# Note

If you are operating a VEGA imaging module, the toolbar will also provide controls for moving the integrated reference stage. For more information please go to OCT Imaging / <u>Reference Length (VEGA Imaging Module)</u>

# 3.5.2 Scan Pattern

In the 2D imaging mode the scan pattern can be set by either specifying its parameters or by using the Draw & Scan feature. The Scan Pattern parameters can be found in the <u>Dataset Parameters</u> window.

Scan Pattern								
	3	¢		z				
Size		1024	÷		1024 🌲	pixel		
FOV		7.66	÷		2.82 🌻	mm		
Pixel Size		7.48			2.76 🌻	μm		
Fixed Pixel Size								
	3	¢		У				
Center		1.07	÷		0.48 🌻	mm		
Angle					78.17 韋	۰		
# Frames					0			

Parameters that can be set to specify the scan pattern

#### Draw & Scan

The Draw & Scan feature provides an easy way to specify the scan pattern by clicking the end points of the B-scan in the video image. On top of the video image a blue rectangle indicates the maximum scan

range, i.e. no portion of the scan may be outside of this rectangle.

## Size

Size x specifies the number of A-scans in a B-scan while Size z is the number of depth pixels in each A-scan. The measurement depth shown under FOV z depends directly on the number of pixels in the A-scan. If the number of pixels is reduced the imaging depth reduces as well. By increasing the number of A-scans in a B-scan (Size x) the imaging quality can be increased, however, imaging will become slower.

**Please note:** Increasing the Size x parameter will only make sense as long as the X spacing remains below or equal to the lateral resolution of the system, which in turn depends on the type of OCT system and probe objective. Oversampling / averaging can be controlled by the <u>averaging parameter(s)</u> in "<u>General Dataset Parameters</u>".

# Field of View (FOV)

The parameters in field-of-view (FOV) determine the scan length of the B-scan (FOV x) and the maximal imaging depth (FOV z). The former parameter can be automatically set by using the *Draw & Scan* feature.

## **Pixel Size / Fixed Pixel Size**

The pixel size parameters indicate the pixel dimensions in the respective axes. Checking *Fixed Spacing* enables the *Spacing* parameter and disables the *Size* parameter for the x-axis; the pixel size is adapted automatically, keeping the x-axis pixel size when changing the field of view.

## Center

The physical coordinate of the center of the B-scan can be specified by the *x* and *y* coordinates, where the reference point is the center of the maximum scan range rectangle.

#### Angle

The angle of the B-scan can be specified by this parameter. The scan direction is rotated by increasing/ decreasing the value.

#### #Frames

The number of captured B-scans.

# 3.5.3 Recording

In the recording options one can specify the number of B-scans that are recorded when pressing the *Record* button in the action toolbar. By default, the number of recorded frames is not limited. When selecting the *stop recording* checkbox one can specify the number of frames that are recorded.



Recording can be automatically stopped after a predefined number of frames.

# 3.5.4 Display

The display toolbar provides control over the settings affecting the way in which B-scan OCT data are viewed. It contains various options to influence the aspect ratio of the display, perform length measurements of structures in the B-scan, or to display a single A-scan of the B-scan. Additionally, the display toolbar provides controls to adjust the coloring parameters, such as the contrast and the brightness of the OCT image.



Display toolbar settings for the 2D display

# **Aspect Ratio**

This combo box sets the aspect ratio of the B-scan. The following options are available:

- **Physical**: The physical aspect ratio is used. The image size depends on the scan pattern FOV parameters.
- **Pixel**: The aspect ratio depending on the number of A-scans in the B-scan and the number of depthpixels. In this mode the image pixels are quadratic.
- **Stretch**: The maximum available display space is used, ignoring the size relations of the actual scan pattern.

# Zoom

By using the mouse wheel anywhere on the screen the region can be zoomed in and out.

# Fit to Screen

When the fit to screen button is pressed, the size of the displayed B-scan will be adjusted such that it fits on the screen while respecting the current aspect ratio. The image will be entirely zoomed out.

# Marker Tool

Using the marker tool, the size of structures that appear in the B-scan can be measured. By clicking on the *Add Marker* button the marker tool is armed and a click somewhere on the B-scan defines the start point of the measurement. A second click on the B-scan defines its end point.

The length of the marker is shown directly in the B-scan. Additional information can be obtained by clicking on the *Show Markers* button. A new window appears showing all added markers with their respective coordinates, their line length, and their angle to the x-axis. From this window you may delete markers, change their color, and export the marker list to a csv file by clicking the *Export* button.

# ThorImage OCT



Length and angle measurements can be done by adding markers to the image in 2D mode.

# Note

The refractive index of the sample also influences the measurement results. It can be set in the <u>General</u> <u>Dataset</u> options.

#### **A-Scan View**

When the A-scan view is activated, a single A-scan of the B-scan at the position of the vertical red line that is shown in the B-scan will be displayed. Moving the line with the mouse selects the A-scan to be displayed.

In the A-scan view, the x-axis is the signal strength in dB and the y-axis is the depth in mm. The range of the x-axis is determined by the dynamic range that is set in the <u>Coloring Control</u>.



A-Scan view in the 2D display

## Pepper Filter

The pepper filter searches the displayed image for bright and dark spots which mostly occur due to speckle and noise in the acquired data. It removes them based on the surrounding data values and can thus improve the visual appearance of the B-scans. The pepper filter will not have any influence on exported images or data but merely improves the visual appearance within the ThorImage OCT software.

# Flip Image

Flip Image inverts the *z*-axis of the displayed B-scan. In general, images can be acquired upside-down when using OCT, as it cannot be distinguished between positive and negative time delays. If this is the case, the flipped image button orients the image back to its physical orientation.

The upside-down imaging can also be used deliberately, as imaging quality in general lowers in large imaging depth. By putting the upper structures in the lower imaging regions, sometimes better images can be achieved.

# Note

This option is only available in two-dimensional B-scan display.

# **Coloring Control**

The coloring control specifies the dynamic range and the colormap that is used for creating false color images. By adjusting the position of the left and right thumb the dynamic range can be increased or decreased. If the distance between the thumbs increases the *contrast* of the images will decrease. By moving the entire slider, the *brightness* of the images can be controlled.

Right-clicking the coloring control brings up a popup menu where the desired colormap can be selected.

Clicking the blue arrow symbol will set the dynamic range to a reasonable default value depending on the average intensities of the currently acquired image.



Open the colormap popup by right-clicking the coloring control.

# Frame Selection

If a 2D dataset contains more than one B-scan, the frame slider can be used to scroll through the single B-scans in the dataset, displaying each in the 2D display.

# 3.6 3D Mode

In 3D mode 3-dimensional OCT scans can be acquired. In order to achieve this, the scan pattern contains a stack of B-scans. This mode is best suited for samples with little movement since the measurement usually takes some time depending on the number of A-scans per B-scan and B-scans per volume taken. As an alternative to using the scan parameters window the scan region can be defined by using the <u>Draw & Scan</u> feature.

# 3.6.1 Toolbar

In 3D mode, the action toolbar looks as follows:



Action toolbar options in the 3D acquisition mode

#### Preview

The 3D mode provides a preview button that can be used to rapidly acquire 3D volumes at a very fast rate. In this mode, multiple volumes are acquired and visualized online in the 3D display until the acquisition is stopped by the user. The data that is acquired using the preview mode is not stored on disk. Use the scan button to create high quality OCT images of the examined samples.

# Note

The size of the preview dataset is defined in the *Preview* group of the *Dataset Parameters* control. This size will generally be significantly lower than the actual volume acquisition size applied when using the scan button.

#### Scan

The scan button can be used to acquire a single volume with the scan pattern and resolution specified in the dataset parameters. It is used for single shot acquisition.

During the scan a progress bar with a cancel button is shown. The current acquisition can be stopped by pressing the cancel button.



Scan progress and cancel button

## **Time Series**

Using the time series button one can invoke a series of 3D measurements. In the drop-down list of the button one can adjust the number of volumes to be acquired and the scan pause between successive volumes. Note that for each measured volume a new 3D dataset is created.

# Note

The minimum available scan pause time is limited by the time calculated for a single scan/storage process.

#### **Export Rendering**

Using the *Export Rendering* button one can export the currently displayed volume rendering as a single image, much like a screenshot of the display area.

# Note

When using a handheld probe (Thorlabs OCTH), the toolbar will provide for manual calibration of the apodization spectrum due to the fact that this particular scanner type does not acquire dedicated apodization spectra for system-inherent performance reasons. By default, "Use scan for apodization" is checked so manual calibration is not required unless intended.



# Note

If you are operating a VEGA imaging module, the toolbar will also provide controls for moving the integrated reference stage. For more information please go to OCT Imaging / <u>Reference Length (VEGA Imaging Module)</u>

# 3.6.2 Scan Pattern

In the volume imaging mode the scan pattern can be set by either specifying its parameters in the dataset parameters control or by using the Draw & Scan feature.

Scan Pattern								
	x	У	z					
Size	400 🗘	200 🌻	1024 🌻 pi	ixel				
FOV	8.00 🗘	3.92 🗘	2.82 🗘 m	nm				
Pixel Size	20.00	19.60 🌲	2.76 🜲 µ	m				
Center	0.84 🗘	0.48 🗘	] m	nm				
Angle			0.00 😂 °					
Fixed Pixel Size								

Parameters that can be set to specify the scan pattern in 3D mode

#### Draw & Scan

The Draw & Scan feature provides an easy way to specify the scan pattern by clicking two opposing corners of the volume in the video image. In the video image a blue rectangle indicates the maximum scan range. No portion of the scan region may be outside of this rectangle.

#### Size

Size x specifies the number of A-scans in a B-scan whereas Size y specifies the numbers of B-scans in the volume, and Size z gives the number of depth pixels in an A-scan. The product of the three sizes specifies the number of voxels to be acquired in the complete volume. The measurement depth shown under FOV z depends directly on the number of pixels in the A-scan. If the number of pixels is reduced the imaging depth reduces as well. By increasing the number of A-scans in a B-scan (Size x) the imaging quality can be increased, however, imaging will become slower.

**Please note:** Increasing the Size x or Size y parameter will only make sense as long as the X and Y pixel sizes remains below or equal to the lateral resolution of the system, which in turn depends on the type of OCT system and probe objective. Oversampling / averaging can be controlled by the <u>averaging</u> <u>parameter(s)</u> in "<u>General Dataset Parameters</u>".

## **Pixel Size / Fixed Pixel Size**

The pixel size parameters indicate the pixel dimensions in the respective axes. Checking *Fixed Pixel Size* enables the *Pixel Size* parameter and disables the *Size* parameter for the *x* and *y*-axis; the pixel size is adapted automatically, keeping the x and y-axis pixel sizes when changing the field of view.

#### Field of View (FOV)

The parameters in field-of-view (FOV) determine the scan length (FOV x), the scan width (FOV y), and the imaging depth (FOV z) of the imaging volume. The former two parameters can also be set by using the Draw & Scan feature.

## Center

The physical coordinates of the lateral center of the volume can be specified by the x and y coordinates, where the reference point is the center of the maximum scan range rectangle.

#### Angle

The angle of the volume can be specified using this parameter. The scan direction is rotated by increasing/decreasing the value.

# 3.6.3 Volume Display

ThorImage OCT provides capabilities to render volumetric OCT data with different view options.

In general the 3D view can be manipulated using the mouse:

- Rotation: Moving the mouse while holding the left mouse button, the volume can be rotated.
- **Translation**: Moving the mouse while holding the right mouse button, the volume can be shifted laterally.
- Zooming: Using the mouse wheel, the volume can be zoomed in and out.

The toolbar of the 3D display looks as follows:



Display toolbar settings for the 3D display

The coloring control behaves as described in the <u>2D Display</u> section. By clicking on the first combo box one can switch between the Volume View, the Sectional View, and the Surface View. Both are described in the following sections.

# **Volume View**

The volume view displays a 3D rendering of the acquired dataset.

# **Rendering Method**

view

**Clip Plane** 





The clip plane is activated by checking the check box in the clip plane controls.

Using the clip plane makes parts of the volume transparent. By default, the transparent part of the volume is always perpendicular to the view direction and can be moved along the viewing axis by using the clip plane slider. Clicking the pin icon locks the clip plane to the volume. The transparency button to the right toggles the clip plane's opacity on or off.



Left: Clip plane using the transparent mode; Right: Clip plane using the opaque mode

# **Sectional View**

The sectional view displays single slices in each major axis of the 3D volume. The individual slices can be switched on and off using the corresponding check boxes in the display toolbar. The position of the displayed slices can be adjusted with the sliders in the display toolbar.



Sectional view parameters in the display toolbar of the 3D volume display



Sectional view mode of the 3D display

## **Surface View**

The surface view performs a simple segmentation of the outer surface of the object viewed in the OCT section. The surface is then rendered with an additional color between red and white indicating the actual depth.

## Depth coloring control

The depth coloring control can be used to specify the region of depths that are used for the color-coding of the depth in the rendered volume.

#### **Median Filter**

A median filter can be applied to reduce the effect of noise, e.g. speckle, on the segmented OCT data surface.



Surface view mode of the 3D display

# Frame Box & Scale Bar

By default the volume is surrounded by a frame box and scale axes are shown for each axis (x, y, and z). These may be toggled on/off.



Left: Frame box and scale bar are shown; Right: Frame box and scale bar are hidden (also, the dynamic range coloring is different)

# **Normal Views**

In the upper right corner of the 3D display there are three buttons which can be clicked to activate a normal view. Clicking the icon twice reverses the orientation.



Left: No normal view is active; Right: The XY normal view is active.

# 3.6.4 Slice Display

The 3D slice display can be activated in the action toolbar of the data viewing mode. It provides twodimensional views of slices of an entire 3D-volume along the three orthogonal axes, x, y, and z. The corresponding display toolbar for the 3D slice display toolbar looks as follows:



Display toolbar settings for the 3D slice display

An example of an en-face image in the slice display is shown next:



Enface view of a volume shown in the 3D slice display

The options Aspect Ratio, Fit to Screen, A-scan View and the Intensity Coloring Control are identical to that in the <u>B-scan Display</u>.

#### **Slice Direction**

Specifies the axis along which the three-dimensional volume is sliced. B-scans along the fast (X) and the slow axes (Y) can be viewed, as well as en-face images of the volume (Z).

#### Frame Selection

The frame selection specifies the position of the visible frame within the volume. By moving the slider different slices along the specified axis (slice direction) can be viewed.

# 3.7 Doppler Mode

Doppler OCT is used to measure flow velocities of scattering structures, i.e. in addition to structural B-scan data, as available in 2D mode, this method uses additional post-processing to extract phase differences between successive A-scans. The flow velocities will be colored using a red/blue color scheme, where blue and red indicate the maximum positive and negative flow velocity in scanning direction, respectively. The maximum absolute flow velocity  $v_{max}$  can be computed by

$$v_{\rm max} = f \cdot \lambda / 4$$
,

where *f* is the A-scan rate of the system and  $\lambda$  is the central wavelength. The effective A-scan rate *f* can be adjusted directly by changing the <u>device speed</u>. The effective A-scan rate is further reduced by dividing the A-scan rate of the device by the <u>spectrum averaging</u> parameter.

Usage of Doppler OCT is similar to <u>2D Mode</u>.

# Note

Due to the nature of the OCT scan, only the phase shift component in direction of the beam can be

detected. For blood flow observations this does not pose a problem, since a vector component parallel to the beam direction is present in general, however, quantitative results depend on the direction of blood flow with respect to the beam direction which is unknown to the OCT system.

# Note

The velocities that can be detected using Doppler imaging depend on the acquisition speed (A-scan rate) and on the angle between flow velocity vector and detection beam. If the detection window and the actual velocity do not match it is oftentimes advantageous to adjust the acquisition speed of the OCT system. The faster the acquisition rate, the higher are the velocities that can be detected. However, acquisition speed should approximately correspond to the highest expected axial velocity, in order to optimally detect lower velocities. Acquisition speed is modified by setting the <u>device speed</u> and/or the <u>spectrum averaging</u> parameter. The device parameters influence the physical acquisition speed of the device, while the effective acquisition speed of the OCT device is further reduced by the spectrum averaging parameter.

# 3.7.1 Toolbar

The action toolbar in the Doppler acquisition mode is similar to that in the <u>2D acquisition mode</u>. The Advanced snapshot feature is not available in Doppler mode.

# Note

When using a handheld probe (Thorlabs OCTH), the toolbar will provide for manual calibration of the apodization spectrum due to the fact that this particular scanner type does not acquire dedicated apodization spectra for system-inherent performance reasons. By default, "Use scan for apodization" is checked so manual calibration is not required unless intended.



# Note

If you are operating a VEGA imaging module, the toolbar will also provide controls for moving the integrated reference stage. For more information please go to OCT Imaging / <u>Reference Length (VEGA Imaging Module)</u>

# 3.7.2 Doppler Averaging

The first averaging option in Doppler mode is spectrum averaging. It can be used to increase the signalto-noise ratio while decreasing the imaging speed. Hence, spectrum averaging can simulate an increased acquisition time and in turn changes the effective A-scan rate *f*.

There are two additional averaging parameters in Doppler Mode. They specify how many A-scans are used to compute phase differences and intensity mean values (lateral), and how many data-points in each A-scan are averaged to reduce the influence of phase and intensity noise (axial).

By selecting oversampling the data acquisition will compute for each acquired pixel a suitable phase value by using the neighboring pixel values. If no suitable neighboring values exist (at the edges of the sampled data) no value will be computed. Without oversampling only every *n*th pixel is evaluated, where *n* is determined by the lateral or axial averaging, respectively.
🔿 Averaging / Imaging Speed
Spectrum Averaging 1
Lateral Averaging 2
Axial Averaging 1
Oversampling

Doppler averaging parameters

# 3.7.3 Scan Pattern

The scan pattern parameters used in Doppler mode are identical to the scan patterns in 2D mode. See <u>scan pattern in 2D mode</u>.

# 3.7.4 Recording

The recording options for Doppler mode are the same as in 2D mode, with the exception of the phase data being recorded in addition to the intensity data. See <u>2D mode recording</u>.

# 3.7.5 Display

The toolbar of the Doppler display looks as follows:



Display toolbar settings for Doppler display

The following controls are the same as shown in 2D mode and are described <u>there</u>: Aspect ratio, Fit to Screen, Frame Selection, Intensity Coloring Control. Doppler mode specific display parameters are described below.

### Overlay

Intensity image and Phase/Velocity image are overlaid in a single view. Phase/Velocity images are alpha blended onto the intensity images, but only if a certain intensity threshold is exceeded.

### Blur

Intensity and Phase/Velocity Images are blurred to reduce effects of noise.



Doppler display with Overlay switched off. The intensity image is shown on the left, the phase/velocity image is shown on the right.



Doppler image with overlay switched on. Phase/velocity information is blended onto the intensity image.

### Intensity Threshold

The intensity threshold specifies the minimum intensity for each data point to display data in the Phase/ Velocity image. The intensity threshold option is available for overlay and separated intensity/phase images.





### Phase/Velocity Coloring Control

Specifies which phases/velocity values are colored and determines contrast of the Doppler information. The phase region specified by the control will not be colored, the minimum and maximum phases will always be blue and red, respectively.

## 3.8 Speckle Variance Mode

Speckle Variance OCT can be used for angiographic imaging. It visualizes blood vessels without requiring significant blood flow and without requiring a specific acquisition speed window.

### Note

For optimal image quality, the sample has to be stabilized. Furthermore, a gel should be used for matching the refracting index at the sample interface.

### 3.8.1 Toolbar

In the Speckle variance mode, the action toolbar looks as follows:



Action toolbar of the Speckle Variance acquisition mode

#### Scan

The scan button starts to acquire a single volume with the scan pattern and resolution provided. This is used for single shot acquisition with high sampling rate.

### Note

When using a handheld probe (Thorlabs OCTH), the toolbar will provide for manual calibration of the apodization spectrum due to the fact that this particular scanner type does not acquire dedicated apodization spectra for system-inherent performance reasons. By default, "Use scan for apodization" is checked so manual calibration is not required unless intended.

×	Use scan for apodization spectrum
	Calibrate Spectrum
Ма	anual calibration might be desired when using an OCTH scanner.

### Note

If you are operating a VEGA imaging module, the toolbar will also provide controls for moving the integrated reference stage. For more information please go to OCT Imaging / <u>Reference Length (VEGA Imaging Module)</u>

### 3.8.2 Speckle Averaging

Speckle averaging parameters indicate how many successive A-scans (*Averaging Fast Axis*) and/or B-scans (*Averaging Slow Axis*) are to be used when computing variance and mean values. Larger values make for better signals but will require more acquisition time and are also more sensitive to bulk motion artifacts.

Averaging / Imaging Speed	
Averaging Fast Axis -	2
Averaging Slow Axis -	2
Speed / Sensitivity Low Speed (1 kHz)	

Averaging parameters in speckle variance imaging

### 3.8.3 Scan Pattern

The scan pattern parameters used in speckle mode are identical to the scan patterns in volume mode. See <u>scan pattern in 3D Mode</u>.

# 3.8.4 Display

The toolbar of the speckle variance display looks as follows:



Display toolbar settings for the Speckle Variance display

The parameters Slice Direction, Aspect Ratio, Fit to Screen, Intensity Coloring Control, Autoset Intensity Coloring, and Slice Selection are identical to that in the <u>3D Slice Display</u>.

### Overlay

Mean intensity image and variance image are overlaid in a single view. Variance images are alpha blended onto the intensity images, but only if a certain intensity threshold is exceeded.



Default speckle view; Left: Mean Intensity projection image; Right: Speckle variance projection image

### 3 OCT Imaging



Overlay image of mean intensity and speckle variance

### Projection

Projection performs a maximum intensity projection (MIP) along the selected axis of the OCT data. Especially in *en face* display, angiographic images show improved imaging quality when this option is enabled.



Left: Mean intensity and speckle variance overlay with no projection; Right: Mean intensity and speckle variance overlay image with projection

### Blur

Intensity and Variance Images are blurred to reduce effects of noise when using this button.

### Variance Coloring Control

Specifies how variance values are colored and determines contrast and brightness of the angiographic information. The colormap can be changed by right clicking on the middle thumb of the coloring control, which causes a colormap popup to appear.

#### Intensity Threshold

The intensity threshold is only visible if the Overlay option is activated and specifies the minimum intensity for each data point to overlay data with the speckle variance image.

## 3.9 1D Mode

In 1D Mode, the probe beam is positioned statically in space while reflectivity profiles (A-scans) are collected in the depth direction at the beam position. As an alternative to using the scan parameters window the beam position can be defined by using the <u>Draw & Scan</u> feature.

### 3.9.1 Toolbar

In the 1D mode, the action toolbar looks as follows:

	17 I. C. M.	11721		
			AScan Display	
Dentinu		Collinget Consistences	- scan bispidy	
Preview	Snapshot	Calibrate Spectrum		
-				

Action toolbar options in 1D acquisition mode

### Preview

Using the Preview button starts and stops 1D OCT data acquisition.

### Snapshot

When the OCT system is acquiring 2D data, a single image can be stored using the *Snapshot* button. The acquired M-scan appears in the dataset list and can be selected in order to view it.

#### Note

Raw Data storage is currently not supported in 1D snapshots. When the Spectral Raw Data storage format is selected, a 1D snapshot will yield processed data regardless.

#### Calibrate Spectrum

Spectral domain systems require a calibration spectrum that can be captured by pressing the Calibrate Spectrum button. To achieve correct results, remove the sample, ensure that no signal is left in the OCT image, and click the Calibrate Spectrum button in the action toolbar.

#### Note

The *Calibrate Spectrum* button is not available for Swept Source OCT engines as it is not required in these systems.

### Note

If you are operating a VEGA imaging module, the toolbar will also provide controls for moving the integrated reference stage. For more information please go to OCT Imaging / <u>Reference Length (VEGA Imaging Module)</u>

## 3.9.2 Scan Pattern

In the 1D imaging mode the scan pattern can be set by either specifying its parameters or by using the Draw & Scan feature. The scan pattern parameters can be found in the <u>dataset parameters</u> window.



Parameters that can be set to specify the scan pattern

#### Draw & Scan

The Draw & Scan feature provides an easy way to specify the beam location by clicking into the video image; a red cross indicates the A-scan position. The blue rectangle in the video image indicates the maximum scan range, so positioning the scan point outside of these bounds will not be possible.

### Center

The physical coordinate of the A-scan position can be specified by the x and y coordinates relative to the center of the scan area.

### # Scans

The number of captured A-scans that are kept in a ring buffer (A ring buffer works in a way that it will always contain the last <#Scans> A-scans

### **Repetition Time**

This parameter specifies the time between two successive A-scan measurements. Increasing the averaging parameter will simultaneously increase repetition time.

### 3.9.3 Averaging

In 1D mode there are two averaging options: spectrum averaging and A-scan averaging. While spectrum averaging is faster, A-scan averaging mitigates phase washout that can occur when moving the sample. Both averaging parameters can be combined; their product is however limited to 1000.

Averaging / Imaging Speed	
Spectrum Averaging	L
A-Scan Averaging — 109	9
Speed / Sensitivity Video rate (28 kHz)	

1D mode averaging parameters

### Note

Changing the averaging parameters will automatically influence repetition time.

### 3.9.4 Timing

Data acquisition in 1D mode does not guarantee that every successive A-scan will be acquired. When the repetition time between successive A-scans is too high, scans will be lost and a warning will be displayed.

In order to avoid timing issues, you have to adjust the repetition time until the warning disappears. This can be achieved by using the <u>repetition time slider</u> or by increasing at least one of the <u>averaging parameters</u>.

### Note

To acquire consecutive A-scans please use the <u>2D Mode</u> with FOV x equals zero and set the entry "SizeOfApodization" to zero in the currently used Probe.ini located in the <u>config folder</u>. Make sure to save the ini-file after the manipulation and restart the software. Please remember to insert the default value 25 again to the ini-file if you want to have high quality OCT images and are not interested in consecutive spectra only.

## 3.9.5 Display

There are two different display modes in the 1D imaging mode: the A-scan display which shows the last acquired spectrum and A-scan, and the M-scan display which shows a sequence of A-scans along the temporal axis.

### A-Scan Display

The A-scan display shows the spectral raw data and the processed A-scan data as a line profile. The graphs can be zoomed in and out using the mouse wheel. The data can be fit to the available screen space using the Fit to Screen button. For offline data the A-scan to be displayed can be selected using the slider titled **"A-scan #**. This control is not available in online mode which only displays the currently acquired A-scan.



A-Scan display showing the spectral raw data (top) and the processed A-Scan (bottom); Fit to Screen button on top left.

### **M-Scan Display**

The M-scan display provides an alternative to the line profile view. Here depth information is encoded in the vertical axis while time information (A-scan number) is encoded in the horizontal axis. This is usually also referred to as an M-scan. The dynamic range slider provided here works analogous to the one in 2D mode.



M-Scan display

# 4 General Dataset Parameters

Dataset Parameters
Study Default  Vumber 0
Refractive Index 1.00
Acquisition Time: 00.061 sec
O Comment
📀 Scan Pattern
Averaging / Imaging Speed
A-Scan Averaging 1
B-Scan Averaging 1
Speed / Sensitivity Default (30 kHz A-scan rate)
• Processing
Image Field Correction
Undersampling Filter
Autocorrelation Compensation
Dispersion Compensation
None Calibrated Quadratic
►
Dispersion Factor 0 A.U.
Apodization Window Hann
Oevice Info
OCT Model Ganymed
Serial Number M00261114
Probe
Center Wavelength 1300 nm

General dataset parameters common to all imaging modes

This section describes the options in the Dataset Parameters control which are common to all acquisition modes.

In data viewing mode, most of these parameters are disabled and represent their state in the currently displayed dataset. Some parameters, such as Comment or Refractive Index, can be modified later on; these changes can be saved when closing the respective dataset.

The paragraphs below each deal with one of the sections seen in the screenshot on the left:

- General
- <u>Comment</u>
- <u>Averaging/Imaging Speed</u>
- Processing
- Device Info
- <u>External Triggering</u> (for select OCT systems)

# 4.1 General

The general section of the dataset parameters contains information used to identify the dataset and options specific to the current measurement. A *Study* is intended to comprise one or more datasets in the same scope and is freely editable. Each additional measurement will cause the (Experiment) *Number* field to be automatically incremented, thus each measurement is uniquely identified by study name and number.



Fields in the general section are used to uniquely identify each dataset. Additional options that are specific to the sample can be set here.

### Study

User defined name to identify the current set of measurements. For each study several measurements can be performed whereas each scan is identified by a unique Experiment Number. The Experiment Number is determined automatically by ThorImage OCT and cannot be assigned manually.

### Note

The study name determines the storage location for the dataset (see **Dataset Location**).

#### **Experiment Number**

The running number uniquely specifies the measurement in the current study. It is automatically assigned.

#### **Refractive Index**

For each measurement a refractive index of the sample can be specified. The physical axial depth of the image will be scaled by the inverse of the refractive index. The refractive index can also be modified for already stored measurements. It influences the measurements made with the <u>marker tool</u>.

#### Acquisition Time

This label shows the acquisition time per single scan. For 2D and Doppler modes the acquisition time is calculated for a single B-scan whereas for 3D and speckle variance modes the time for a single volume/ variance scan will be shown.

#### Load/Save Dataset Parameters

Using the Save Dataset Parameters button, the currently set dataset parameters can be stored to a file. Note, that this parameter file contains the dataset parameters for all imaging modes. The dataset parameters can be restored by using the *Load Dataset Parameters* button and selecting a stored parameter file.

### 4.2 Comment

The comment field is an optional text field that can be used to add a descriptive text to each dataset. The comment field can be modified and will be saved in the currently displayed dataset's OCT file.

<ul> <li>Comment</li> </ul>			

The comment field can be used to store additional information and user specified metadata that is specific for a dataset.

# 4.3 Averaging/Imaging Speed Parameters

ThorImage OCT comes with averaging options that provide trade-offs between imaging speed, imaging quality, and sensitivity. Different processing options show different vulnerabilities to motion induced imaging artifacts. The averaging options available depend on the active imaging mode.

Averaging / Imaging Speed
A-Scan Averaging 1
B-Scan Averaging 1
Speed / Sensitivity Default (30 kHz A-scan rate)

Averaging parameters to be used in OCT processing to be set in acquisition modes. Available parameters might vary depending on the operating mode.

### A-scan Averaging

A-scan averaging improves the imaging quality by flattening the noise floor and increasing the sensitivity at the cost of slower acquisition. Sample motion does not result in phase washout or blurring of the images; the signal remains intact, however motion can induce distortions within the acquired data.



Left: B-scan of a bug with no averaging applied; Same sample with A-scan averaging and slight motion; no artifacts occur

### **B-scan Averaging**

B-scan averaging improves the imaging quality by flattening the noise floor at the cost of slower acquisition. The computation is done by a moving average so that the update rate on screen remains intact. B-scan averaging can in some situations produce better images, but creates artifacts if sample motion is present. If motion artifacts are a limiting factor when using B-scan averaging, consider using the *Advanced Snapshot* button.



Left: B-scan of a bug with no averaging applied; Middle: Same sample with B-scan averaging; Right: Same sample with B-scan averaging and slight motion resulting in imaging artifacts

### Note

B-scan averaging is not available when using the spectral raw data format. However, you can achieve similar images by using the *Advanced Snapshot*.

#### Speed/Sensitivity

The Ganymede, Hyperion, Telesto, and Telesto II OCT engines feature speed preset switching. The Speed/Sensitivity combobox provides different presets affecting the spectrometer inside the OCT engine. These presets usually change the spectrometer's integration time and consequently the sensitivity. As a rule of thumb, the lower the frequency, the higher the sensitivity. Changing a preset also changes acquisition time as indicated in the <u>General</u> section.

### Note

If the speed/sensitivity settings are changed, the reference intensity needs to be adjusted to allow for optimal imaging (see <u>reference intensity</u>).

## 4.4 **Processing Parameters**

ThorImage OCT provides various processing options, which can improve imaging quality, although at the cost of processing speed. These parameters can be either set before or during data acquisition (in 2D and Doppler mode). Furthermore, when using spectral raw data format, it is possible to reprocess the data in offline data. The *Reprocess Data* button is disabled when the currently displayed OCT data has been processed with the currently shown processing parameters.

### ThorImage OCT

Processing	• Processing
Image Field Correction	Reprocess Data Restore Parameters
Undersampling Filter	Image Field Correction
Autocorrelation Compensation	Undersampling Filter
Dispersion Compensation	Autocorrelation Compensation
● No ○ Calibrated ○ Quadratic	Dispersion Compensation
Dispersion -10 👻	No Calibrated Quadratic
Dispersion Factor — -11 A.U.	Dispersion 70 👻
Apodization Window Hann	Dispersion Factor —11 A.U.
	Apodization Window Hann

Left: Processing parameters that can be set in different imaging modes. Right: When storing spectral raw data there is the option to reprocess the data with different parameters in offline mode.

### Image Field Correction

When acquiring large image fields an offset to the optical path length of the sample arm depending on the lateral scan position might occur. As a result, a flat surface can appear to be curved. Image field correction provides a numerical correction for this and corrects the geometry of the surface by using calibrated factors.



Left: B-scan with image field distortion visible; Right: B-scan with image field correction enabled

### Note

Please note that processing speed may suffer when the image field correction is activated.

#### Note

Please note that the image field correction is not available in the 1D mode.

#### **Undersampling Filter**

Scatterers and reflecting surfaces that are positioned close to the largest possible measurement depth might show aliasing artifacts. These artifacts can partially be compensated by activating the undersampling filter.



Left: B-scan with visible aliasing artifacts; Right: B-scan with undersampling filter enabled

### Note

Please note that processing speed may suffer when the undersampling filter is activated.

#### **Autocorrelation Compensation**

At low acquisition speed, the standard Fourier reconstruction technique can suffer from autocorrelation artifacts that are due to interference of the sample light with itself. These artifacts can partially be reduced by activating the autocorrelation compensation.



Left: B-scan with autocorrelation artifacts; B-scan with autocorrelation compensation enabled

### Note

Please note that processing speed may suffer when the autocorrelation compensation is activated.

#### **Dispersion Compensation**

Group velocity dispersion mismatch between sample arm and reference arm in general causes axial blurring of the acquired signals as the optical path length differences vary with the wavelength. Using the dispersion correction settings of ThorImage OCT, the effects of dispersion mismatch can be reduced. Commonly dispersion mismatch is either introduced by the imaging probe or by the sample, as for example when imaging through several millimeters of water.



Left: B-scan with visible blurring due to dispersion in the sample arm; Right: B-scan with dispersion compensation enabled

- **Calibrated Dispersion Compensation**: For static dispersion, as created by the imaging probe, a calibrated dispersion vector can be used. This does not work for correcting dispersion that is introduced by the sample. Note that the controls for selecting calibrated dispersion compensation presets are only shown when calibration data is available.
- Quadratic Dispersion Compensation: Using the quadratic dispersion compensation and moving the dispersion slider the dispersion mismatch to be corrected can be specified during imaging. This way, the user is able to correct the exact amount of dispersion present in the images.

#### Note

Please note that processing speed may suffer when the dispersion compensation is activated.

#### **Apodization Window**

In the processing of the OCT data, spectral shaping is applied. By selecting a suitable apodization window a compromise between axial FWHM resolution and side-lobes is achieved. In general, the Hann window yields good and artifact free imaging quality. For high-resolution (small FWHM) imaging, the tapered cosine window can be used although at the cost of imaging artifacts, especially from high signal-to-noise ratio of reflecting surfaces.

### 4.5 Device Info

Information about the device and probe in use can be found in the *Device Info* section. This is especially useful when working with multiple devices or with different objectives in order to know the device and probe that were used to acquire a specific dataset.



Device Info section showing engine type, serial number, center wavelength and spectral width.

# 5 Dataset Handling

Dataset management is realized using the *Captured Datasets* control. It shows a list of previously acquired and/or loaded datasets along with their identifiers (study name and experiment number), the acquisition mode in which the dataset was acquired and preview pictures of the probe camera and OCT images.

Left-clicking on a dataset highlights and displays it in the probe monitor and OCT displays. Several datasets of different acquisition modes can be opened simultaneously while only the OCT data of the currently active and displayed dataset is kept in main memory. Datasets with unsaved changes (e.g. changed processing parameters) are indicated by an asterisk (\*) after the study name.

Search Bar				(	Open File	
Captured Datas	sets					
Search				2	19 🧐	Recent Files
Study	#	Mode	Format	Camera	Data	
Default	78	$\square$	Proc		V	
Default	83	đ	Raw		1	
Default	82	đ	Raw			
Default	81	X	Raw+ Proc		E. E. J	
Default*	80	$(\mathbf{\hat{e}})$	Raw+ Proc			
Default*	79	$\square$	Raw+ Proc	See.	1	

Captured Datasets control giving an overview about all opened datasets

# 5.1 Dataset Location

Each captured dataset is stored in the dataset folder. Its location can be changed in the <u>settings dialog</u>. The dataset folder contains for each study a folder that contains the actual OCT data files. This allows to group several OCT datasets using the same study name. Each dataset in a study has a unique experiment number, which is increased each time a new dataset is created. The full path of the dataset is composed thus:

### DatasetFolder \ Study \ Study\_ExperimentNumber\_Mode.oct

The datasets are stored in the proprietary Thorlabs OCT data format (see <u>section OCT data format</u> for an introduction into the file format).

# 5.2 Closing / Deleting Datasets

By right clicking on one or several selected datasets in the dataset list the selected file(s) can be closed or deleted. Closing removes them from the list, while deleting also deletes the dataset file from disk.



Context menu that appears when right clicking on a dataset

# 5.3 Opening Datasets

There are different possibilities to open a dataset from disk:

- Click open file in the file menu
- Press the open file button in the "Captured Datasets" control
- Use the recent files button in the "Captured Datasets" control
- Drag and drop a file from the Windows file explorer into ThorImage OCT
- Use the "Recent Items" feature of the Windows jump list



**Recent files list in the Captured Dataset control** 

### Note

The files neither need to be located in the dataset folder, nor need they have the default file naming scheme in order to be opened in ThorImage OCT; however changing the study name is only possible when the file is located in the dataset folder and follows the specified naming scheme.

# 5.4 Changing Datasets

The following parameters can be changed in offline datasets:

- Study
- Refractive Index
- Comment

In 2D datasets the markers can also be changed. When using the spectral raw data format, processing parameters can be changed in offline datasets as well. An unsaved dataset is indicated in the dataset list with an asterisk (\*) after the study name. When closing a dataset which has pending changes confirmation is required to either save or lose the changes.

### Note

Changing the study name will move the dataset file into a different study folder. For opened files that are not located in the dataset folder, the study name cannot be changed.

# 5.5 Data Export

Captured datasets can be exported to various file formats using the export buttons in the toolbar. Exported files cannot be reimported into ThorImage OCT; for loading previously saved data please refer to <u>Opening Datasets</u>.



The default export folder can be changed in the settings dialog.

# 5.5.1 Standard Export

The export button in the toolbar opens a dialog for specifying the export filename, the file format and, for image formats and 3D data, the slice direction.



Options when exporting to image formats using the standard export

### 5.5.2 Quick Export

The One Click export button in the toolbar directly exports the data into the export folder without asking the user for the file format or the filename. Instead these parameters are globally defined in the <u>settings</u> <u>dialog</u>. When the export is finished, the standard Windows application for viewing the exported data will be launched (e.g. a PDF reader for PDF files).

### Note

The One Click export only supports image file formats. In contrast to the standard export, the One Click export stores not only the OCT image but also the sample monitor image.

## 5.5.3 Camera Export

The currently displayed image of the video camera (<u>sample monitor</u> window) can be exported into various image formats using the camera export button.

## 5.5.4 Export Rendering

Using the Export Rendering button when viewing rendered 3D data, a shot of the rendered view can be exported to a JPG file.

### Note

This button is only visible in the volume view of an offline 3D data set.

### 5.5.5 Export Video

For 3D datasets a video export feature is provided, creating an animated video of the 3D rendering for the current dataset. Supported formats are multipage TIFF, GIF, and uncompressed AVI. Two animation options are available.



Options available for video export

### Rotate volume around z-axis

The video will show a rotation of the volume of 360° around the z-axis.

#### Fly through volume

The clip plane will be activated and will move through the volume and back once.

It is possible to use both export options, in which case the two parts of the video will play in a row, rotation first and then flythrough.

### Note

This button is only visible in the volume view of an offline 3D data set.

### 5.5.6 Image Formats

ThorImage OCT supports PNG, JPG, BMP, PDF, and TIFF as image export formats. The coloring in the exported images will be the same as shown in the current dataset display. Slices of temporal 2D or 3D data are stored in a single file for the TIFF and the PDF file format. For the other image formats, each slice will be stored into a separate file and the slice number will be encoded in the filename.

By default a scale bar is shown in the lower right corner of the exported image indicating the physical size of the image. Furthermore, for 2D datasets, the markers, if any, are shown in the exported images. Both scale bar and markers can be toggled in either the export dialog for the standard export or the settings dialog for the <u>One Click export</u>.

During image export there is the option to maintain the physical aspect ratio. Note that when this is option is checked no downsampling will be performed, i.e. the images might be larger than the actual amount of data.

# 5.5.7 Data Formats

Supported data export formats are RAW/SRM, FITS, VTK, VFF, and TIFF (32bit float). For all these formats the uncolored OCT data is exported in a lossless fashion as 32bit floating point data. Note, however, that the scan metadata, such as for instance the study name, is only stored in the native OCT file format.

- **RAW/SRM**: When selecting the RAW export format two files will be generated: A raw file that contains a little-endian binary stream of the 32bit processed floating point data. The SRM file is a human-readable text-file that contains information about the dimensions of this binary stream.
- FITS: The Flexible Image Transport System (FITS) file format
- VTK: The Visualization Toolkit (VTK) file format
- VFF: The Sun TAAC graphic file format. Also known as Sun IFF
- **TIFF (32bit float)**: When selecting the TIFF (32bit float) format the data is stored in a TIFF format that supports 32bit uncolored floating point data. Note that this file cannot be opened correctly by many image viewers (e.g. GIMP).
- TXT: Text file format.
- CSV: Comma separated values file format.

### Note

Scale bar and markers will only be exported for image and not data formats.

# 5.6 3rd Party Applications

ThorImage OCT offers built-in functionality to open the acquired datasets automatically using third-party software. The corresponding options are provided in the action toolbar when viewing a captured dataset.

### 5.6.1 Explorer

This button opens the Microsoft Windows file explorer at the location where the currently selected dataset is stored.

### 5.6.2 ImageJ

This toolbar button opens the currently viewed dataset with ImageJ. For this option to be available, the ImageJ path needs to be set in the <u>settings dialog</u>. The ImageJ distribution Fiji comes preinstalled on the OCT system's computer but can also be manually installed from the OCT software DVD.

(Thorlabs does not provide support for third party software. For more information about ImageJ and Fiji, please visit <u>http://imagej.nih.gov/ij/index.html</u> and <u>http://fiji.sc/Fiji</u>.)

# 6 Probe Calibration

When changing the objective of the OCT probe, the probe configuration has to be changed as well. This can be done in the settings dialog. If no configuration file for an objective is available, a new probe configuration can be generated using the probe calibration tool. It can be opened by clicking *Add/Edit Probe Config* in the settings dialog. The probe calibration tool will then guide you through the various steps that are necessary to generate the configuration file.

For the procedure we recommend to mount the probe on its stand so that the objective points downwards. The probe calibration requires a calibration sample to be positioned under the probe. Under certain circumstances, depending e.g. on the OCT wavelength and lighting conditions, you may also need the IR card included in your Thorlabs OCT system package.





Left: Probe calibration sample; Right: IR card

# 6.1 Edit Probe Type

In the first step of the probe configuration process the probe type must be specified. This includes the used objective (LSM02, LSM03, LSM04) and the probe serial number that is printed on the probe reference arm (the latter is optional).

Probe Calibration		×
Enter Probe Type This Wizard will walk you though the probe of	calibration. In the first step please o	enter/edit the probe type.
Position Calibration Sample	Probe Objective Probe Serial Number (Optional)	LSM04
Calibrate Camera Scaling		
Calibrate Image Field		
	Cano	el < Back Next > Finish

Enter probe type

# 6.2 Position Calibration Sample

In the second step, the calibration sample must be placed under the probe such that the three holes are positioned in the center of the video image. It is also essential that the sample is in the focus of the probe to ensure a sharp image. Please adjust the height of the probe using the height adjustment knobs of the stand such that a sharp image is visible.

If the sample is detected correctly, a red cross will be displayed in the center of the markings which needs to match with the blue square. For some objectives it is also required that the calibration sample is positioned in the same orientation as the camera image.



Positioning of the calibration target

When the calibration sample is positioned correctly, the cross and the square will turn green.



Correctly positioned calibration target

## 6.3 Camera Scaling and Galvo Factors

After positioning the calibration sample, the camera scaling and galvo factors will be automatically determined. If this step fails, you can either retry by clicking back and next again, or retry the calibration procedure using an IR card.



Recalibration of galvo factors using an IR card

# 6.4 Image Field Calibration

In the last step the image field will be calibrated. If the IR card has been used in the previous steps, please remove it and place the calibration sample below the probe once more.

Image field calibration is done in two steps. First, a continuous B-scan preview is used to adjust reference intensity and reference path length in such a way that the surface of the calibration target is clearly detected. In the second step, the full image field is measured.

If everything has been adjusted correctly, the B-scan of the image field preview looks as shown in the figure below and automatic image field calibration will take place.



Surface successfully detected

If the sample surface fails to be detected, please follow the instructions given in the following paragraphs to improve imaging quality.

#### **No Surface Detected**



No surface detected during image field calibration

When the image looks as in the above figure, no surface has been detected yet. There are different reasons for a failing surface detection:

- The reference intensity might not be set correctly. It can be adjusted using the knob in front of the probe.
- The reference path length might be misaligned. It can be adjusted using the knob on top of the probe.
- The calibration target might not be in the focus of the probe.

Please adjust these parameters individually until the detected surface shows up green.

#### **No Holes Scanned**

The preview image field calibration should scan through one or more holes of the calibration sample. If the scan looks as follows, the previous calibration could not be determined correctly. Please go back and recalibrate the galvo factors.



No holes scanned during image field calibration

#### **Image Flipped**

If the reference path length is wrong, the image might be flipped upside down. In this case, the reference path length needs to be adjusted using the knob on top of the probe until the holes are below the remaining surface.



The image is flipped upside down; the holes in the calibration sample are shown on top.

#### Surface found: Image Field Calibration

If the surface precalibration has been successful, the image field calibration will be performed.

Probe Calibration	
Calibrate Image Field Image field calibration successful!	
<ul> <li>Enter Probe Type</li> <li>Position Calibration Sample</li> <li>Calibrate Camera Scaling</li> <li>Calibrate Galvo Factors</li> <li>Calibrate Image Field</li> </ul>	0 5 10 15 0 2 4 6 8 10 12 14 16 x (mm)
	Cancel < Back Next > Finish

Successful image field calibration

Clicking the finish button closes the probe calibration dialog and the resulting probe configuration file will be written. The new probe configuration will afterwards be available in the probe combo box in the settings dialog.

# 7 Software Development Kits

For maximum flexibility, ThorImage OCT offers several possibilities to implement customized solutions using the software development kits (SDKs). Experienced software developers will be able to use these in a multitude of programming environments to tailor the use of Thorlabs OCT systems to their requirements.

### ANSI C SDK

The C SDK is the core development kit. It allows to directly control the OCT system, implement customized scan patterns and execute processing routines. The C SDK documentation can be found in

C:\Program Files\Thorlabs\SpectralRadar\Manual\C Reference.pdf

Several small demonstration programs are in our <u>C++-Demo</u> and are a good starting point to get to know our SDK.

### LabVIEW SDK

On top of the C SDK we have developed a LabVIEW SDK that exposes most of the functionality from the C SDK in LabVIEW, including a collection of demo VIs to get you started in developing customized software solutions. The documentation can be found in

C:\Program Files\Thorlabs\SpectralRadar\Manual\LabVIEW Reference.pdf

### Matlab SDK

The Matlab SDK contains a collection of scripts for Mathworks Matlab which enable users to open OCT files and read processed as well as raw data files in Matlab. It does not depend on the C SDK and is limited to reading OCT files into Matlab.

C:\Program Files\Thorlabs\SpectralRadar\Matlab\Readme.txt

### Note

A customized solution using the ANSI C SDK can have all functionalities like the GUI ThorImageOCT since this software is based on the ANSI C SDK.

# 8 OCT File Format

With version 4.0 of this software package, a new flexible file format has been introduced. Its file extension is .oct and it is a zip container (compressed folder) which can be accessed by changing the file extension to .zip and opening the file with a zip viewer such as Windows explorer.

An OCT file contains an XML file named Header.xml which contains metadata describing the captured dataset, e.g. study name, scan pattern and the processing parameters are stored in the Header.xml file.

For all imaging modes, the actual data is stored in a folder named data, which contains the following files:

- VideoImage.data: The image of the sample monitor as 32bit BGRA data stream.
- **PreviewImage.data**: The preview picture of the data as a 32bit BGRA data stream (as displayed in the Captured Dataset list).

When using the *Store Processed Data* option in the settings dialog, the reconstructed OCT data is stored as a stream of uncolored 32bit (single-precision) floating point data in little endian byte order. File sizes can be read from the Header.xml file in the root folder of the ZIP container. Depending on the imaging mode the following files are stored in the OCT file:

- 2D and 3D mode: One file Intensity.data, containing the intensity values in decibel (dB).
- **Doppler mode**: Two files *Intensity.data* and *Phase.data*, containing the intensity in decibel (dB) and phase values, respectively.
- **Speckle Variance mode**: Two files *Intensity.data* and *Variance.data*, containing the intensity values in decibel (dB) and the variance values, respectively.

When using the *Store Spectral Raw Data* option in the settings dialog, the spectral raw data is stored in the data folder into files named *Spectral\*.data*, where \* indicates the running number of the B-scan. Besides the actual spectral raw data, several calibration files required for reprocessing the dataset are also stored in the OCT file:

- OffsetErrors.data: The spectrum used to remove the offset errors from the camera.
- Chirp.data: The vector used to linearize the data from ë- to k-space.
- **ApodizationSpectrum.data**: The mean of the apodization spectra used for apodization (offset errors already removed).
- Probe.ini: The used configuration file from the probe.

#### Note

An example on how to import *Spectral\*.data* files from an OCT file is given in the Matlab script "OCTFileGetRawData.m" found in the SpectralRadar\Matlab folder.

#### Note

The Header.xml file inside an OCT file contains all necessary metadata to import the spectral raw data with third-party software, e.g. SpectrometerElements and BytesPerPixel information and the exact dimensions of the data in pixels and mm. The following instruction can be used to import the data with ImageJ:

- 1. Rename the .oct file to .zip or extract the files directly with any file archiving tool able to handle ZIP compressed files.
- Open the header.xml file and extract the dimensions of the scan (e.g.: <DataFile Type="Raw" SizeZ="2048" SizeX="553" SizeY="1" RangeZ="5.12" RangeX="4.06399" RangeY="1" BytesPerPixel="2" ApoRegionStart0="0" ApoRegionEnd0="25" ScanRegionStart0="25" ScanRegionEnd0="553">data\Spectral0.data</DataFile>)
- 3. Open ImageJ and import the raw data in the Spectral0.data from the "data" folder: ImageJ->File->Import->raw with the following settings:

- a) Image-Type: (BytesPerPixel\*8)-bit unsigned (from the above example: **16-bit unsigned**)
- b) Width: **2048**; height e.g. **553**; Offset to first image: 0 Number of images: e.g. 1 gap between images: 0
- c) Little-endian byte order checked

# 9 Processing algorithm

To obtain the depth information (A-scan) from a spectrum acquired by the camera, several processing steps are necessary. The main steps are as follows:

- **Remove offset errors**: Removes offset errors from the camera by subtracting the Offset.data from all spectra (including all apodization spectra).
- **Background subtraction**: Removes the DC spectrum by subtracting the mean of all apodization spectra (in general 25 apodization spectra are acquired during each measurement).
- Apodization: Creates the apodization vector by dividing an apodization window function, e.g., a Hann window, by the the mean of all apodization spectra. The used apodization window function can be seen in the <u>Processing Parameters</u>. One applies the apodization vector by multiplying all spectra with the apodization vector.
- **Re-Sampling**: Interpolation of all data to obtain spectra that are linearly sampled in k-space. The original positions, i.e., positions in ë-space, are given by the Chirp.data.
- **Image formation**: Fast Fourier-Transforming (FFT) the spectra and taking the absolute value of the complex data gives the A-scan. The data are generally stored in dB, which are computed by taking the logarithm to the base 10 and multiplying the data by 20.
- **Display**: Color mapping is applied to the dB-values.

### Note

The above described steps do not specify the algorithm in detail since the processing chain depends on the chosen settings in the software.

#### Note

Please see the chapter <u>OCT File Format</u> how to handle the data, e.g. spectral data from the camera or saved offset errors.

# **10 External Triggering**

With version 4.4 of this software package, the possibility to trigger A-Scans externally has been implemented. If the checkbox to activate the external trigger mode under "Hardware" in the "Settings" dialog for external A-scan triggering is still not enabled when the device is switched on this feature is not supported for your hardware. By activating the external trigger mode a new tab shown in the following figure will appear in <u>General Dataset Parameters</u>.

🐼 External Trigger		
Maximal trigger frequency	94507 +	Ηz
Number of required trigger pulses:		
Total scan	0	
For each B-scan	0	
Preparation of each B-scan	0	
Imaging of each B-scan	0	
Trigger Timeout	5 🔶 s	ec

All parameters except trigger timeout are read only and depend on the imaging sensitivity and scan pattern.

For a correct operation of the External Trigger Mode it is crucial to supply the system with a correct trigger signal. The trigger signal level on the base unit and the computer must be *low* before the measurement is started on the computer. Sending a trigger signal (rising edge) while the following message is visible can lead to a misalignment of the preparation and scan regions.

	Do not send trigger signals! Preparing acquisition	
L		J
	The preparation of the acquisition is	

running. During this phase, sending a trigger signal can cause a timing problem.

### Trigger signal prerequisites

Each A-Scan in a measurement will be triggered by a **rising edge** on the trigger signal line. The trigger signal train must fulfill the following requirements:

- The trigger signal is a typical TTL signal (low TTL level below 0.8 V, high TTL level above 2.0 V).
- The first trigger signal starts after the preparation of the measurement has been completed (see above figure)
- The first trigger signal is a complete and clear trigger signal. Do not disconnect and connect the trigger cable to simulate this.
- The trigger signal frequency must not exceed the maximum trigger frequency.
- The width of the low pulse should be at least 1 µs.

### Maximum trigger frequency

The maximal trigger frequency shows the maximum valid frequency for the trigger signal. If the frequency of the trigger signal exceeds this value, the behavior of the camera can not be predicted and may cause errors. This parameter depends on the chosen <u>Averaging/Imaging Speed</u>.

### Number of required trigger pulses

### ThorImage OCT

Each B-Scan consists of a preparation region followed by the imaging region. The preparation region is used to acquire the apodization scans and positions the scanner at the start of the scan pattern. These parameters depend on the set imaging speed / sensitivity, the averaging parameter, the flyback time and the sizes of the scan pattern and apodization (Note: Flyback time and apodization scan count are part of the probe configuration and will usually be set at the factory).

### **Trigger Timeout**

After starting the measurement the software is waiting for a trigger signal to occur. The measurement will stop if no signal is received after the trigger timeout set in the External Trigger parameters. It is necessary to stop the current measurement before starting a new one.

### Note

All changes of parameters will stop/start the measurement and therefore it is necessary that no trigger signal be sent during initialization of the acquisition.

#### Note

If you are using our SDK and the external trigger mode please make sure that your trigger signal starts after startMeasurement() and ends after stopMeasurement(). The ExternalTriggerDemo() function in the  $C^{++}$  Demo shows an example for the timing to start and stop the trigger signal.

# 11 Troubleshooting

### OCT Engine

### Question:

After starting the software, the toolbar displays a message "Could not find device. Please switch on the device." even though the OCT system is switched on; all operation modes except data viewing mode are disabled.

### Answer:

Close the OCT software, switch the OCT engine off and check all connections between the computer and the engine and also the probe cable and fiber. Make sure all cables are seated correctly and the POWER and SYS OK indicators on the OCT engine light up when you switch it on. When all connections are good, switch the device on and start the software.

### **General Data Acquisition**

### **Question:**

I try to acquire 2D images but the OCT display remains dark. What is happening?

#### Answer:

- Try clicking the *Autoset Dynamic Range* button. This should adjust the dynamic range boundaries into an area where a signal is visible in the OCT display. If that does not help, check if the reference intensity bar in the toolbar shows a green bar. If that is not the case, adjust your reference intensity according to the SDOCT hardware manual.
- Maybe the distance between probe lens and sample or the reference length adjustment is wrong. Refer to the SDOCT hardware manual on how to adjust probe height and reference length.
- Check if the optical fiber is correctly seated in the jacks on the probe and on the OCT device. Refer to the SDOCT hardware manual if in doubt.

#### Question:

My OCT image looks blurry.

#### Answer:

- The focus plane of the probe is misaligned. Refer to your OCT hardware manual on how to adjust focus and probe distance.
- If there is dispersion between the sample and Check whether the <u>dispersion compensation</u> is set correctly.

#### Question:

The software is not able to acquire very large acquisition sizes (taking into account pixel sizes in x and y as well as averaging parameters).

#### Answer:

Adding more RAM to the computer that operates the OCT system might improve the situation. Memory availability depends on a multitude of parameters which ThorImage OCT cannot influence, due to general operating system principles. If you experience problems acquiring the same large acquisition size several times, upgrading the RAM in your computer might resolve the problems.

# 12 End User License Agreement (EULA)

The End User License Agreement is part of this software distribution. <u>Click this link to view the document</u> (<u>PDF reader required</u>).
# **13 Thorlabs Worldwide Contacts**

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



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