Glossary

Below, you can find definitions for the specific terms that are used in the context of the image acquisition in the Tau mode .

Lifetime Decay Curve

The lifetime decay curve represents the fluorescence intensities (**Intensity [Counts]**) and/or the number of the measured photons in the image (mean accumulated over all pixels) over time after the excitation pulse (**Time [ns]**) in the form of a curve.

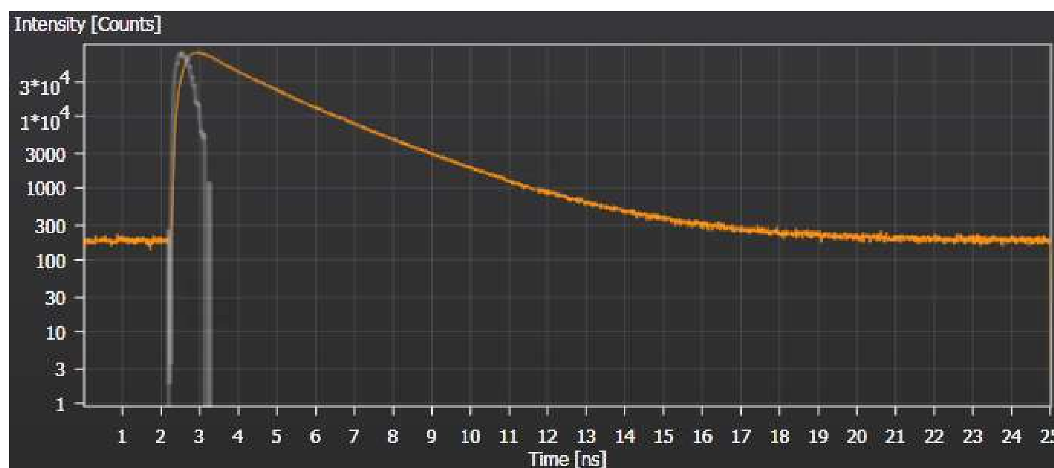

Fig. The orange curve represents a lifetime decay curve

This experimentally determined curve is composed of one or more exponential components, which are called lifetime components. A lifetime component has a value τ, the lifetime of this component, which is specified in nanoseconds.

Lifetime Diversity Curve

Using a mathematical model, it is possible to determine from the lifetime decay curve by means of a fit how frequently a given lifetime component appears in the image. As a graphic representation, the result is a diagram of the fluorescence lifetime components τ (measured in [ns]). This curve indicates how frequently each lifetime component appears in the entire image, which is why it is also called the lifetime diversity curve. Each peak indicates a lifetime component.

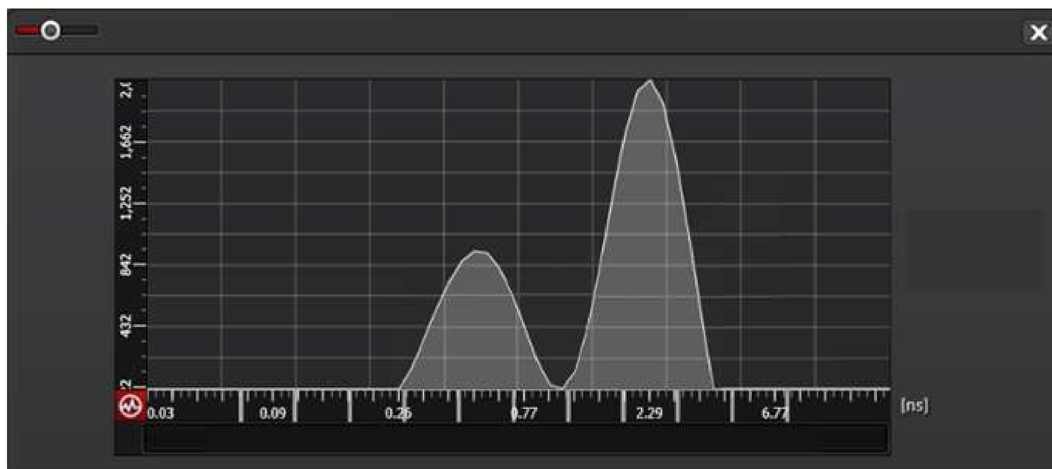

Fig. The diagram shows a lifetime diversity curve. The peaks show the lifetime components that appear frequently in the image

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Lifetime Component

A lifetime component (L) is defined by an exponential function that describes the fading behavior of the fluorescence intensity in a specimen for the respective lifetime τ. For each value τ, there is a lifetime component that occurs at a certain frequency (a).

$L = a \times e^{t/\tau}$

The sum of all lifetime components yields the lifetime decay curve.

The lifetime decay curve results from the sum of all lifetime components.

$\Sigma = (a_1 \times e^{t/\tau_1}) + (a_2 \times e^{t/\tau_2}) + (a_3 \times e^{t/\tau_3}) + \ldots$

Lifetime components that are found in a specimen at a certain frequency and are identifiable in the lifetime diversity curve by a peak can be visualized in an image acquisition in a Tau mode. To do so, see Creating a TauScan.

Species

A lifetime species, simply referred to as "species" in this context, is a target structure to be examined within the specimen that can visualized in an image acquision in a Tau mode and separated from other species.

A species is characterized by an individual fluorescence lifetime, which can be composed of multiple lifetime components. For example, dyes with multiple lifetime components or specific cell structures (cell membrane, cell nucleus, etc.) can represent a species. Identical cell structures that display different lifetimes under changed ambient conditions (e.g. pH value) also represent different species.

You are here: TauSense

TauSense: Overview

With the Leica STELLARIS system, you have the option of visualizing the fluorescence lifetimes in your specimen at the same time that the fluorescence intensities are visualized.

To do so, various gating and Tau modes for separating signals are at your disposal in the **TauModes** area. **TauGating** is for setting time gates for detection. This allows you to remove unwanted signals, such as reflections, from the images. The other Tau modes are used to analyze and separate different species in your specimen based on their fluorescence lifetimes.

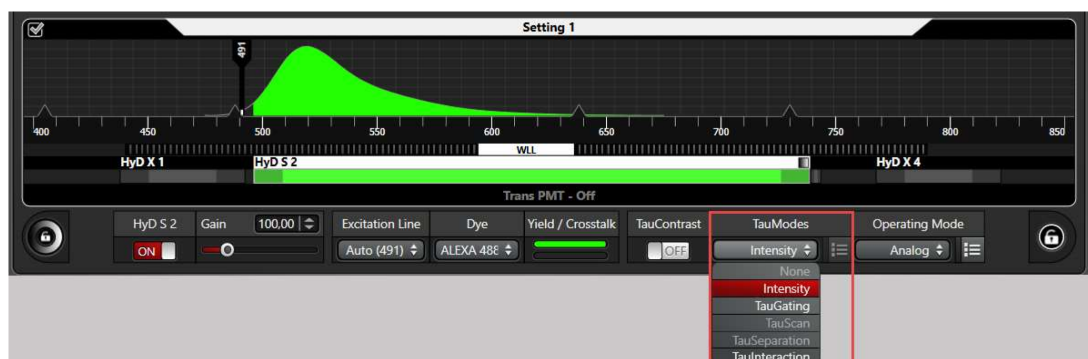
If the **TauContrast** function is switched on, a colored lifetime image is also displayed next to each fluorescence intensity image, see below.

Requirements:

- A pulsed laser is activated.
- A pulsed laser line has been selected as a reference line (**Excitation Line**). When the **Auto** function is activated, the laser line with the longest wavelength as measured from the left edge of the detector is selected automatically as a reference, see also Reference Line. For each sequence, an individual reference line can be configured.
- At least one detector of the Power HyD S or HyD X type is used.
- Under **Operating Mode**, a **Counting** mode is set as the detection mode. This happens automatically in the background whenever a Tau mode is selected or the **TauContrast** function is switched on. Refer also to Detector Operating Modes.

Definitions for the specific terms that are used in the context of the image acquisition in a Tau mode you can find here.

TauModes



In the detector configuration area, the following modes for defining a time gate (gating modes or Tau modes) are at your disposal:

Mode	Description
None	Only if the TauContrast function is switched on. The viewer only displays the lifetime image and not an intensity image of the specimen.
Intensity	Without gating. The viewer displays the full intensity image of the specimen.
TauGating	You can set the number of time gates per channel and the number of channels as you please. Every channel is displayed in a separate partition of the viewer.
TauScan	Using the Tau scan, you can obtain a spatially resolved lifetime diversity curve. With the aid of the diagram, the lifetime components in the different species of the specimen can be determined. In this way, you can also determine whether a dye contains more than one lifetime component, for example.
TauSeparation	With this method, you can carry out a separation (e.g. dye separation) of species present in the specimen based on different fluorescence lifetimes that you have determined, e.g. in a preceding Tau scan. For each lifetime component, an intensity image is generated in a separate channel and displayed in a viewer partition. The settings are also used for stage experiments in the LAS X Navigator and in the Lightning wizard (Mark&Find and tile scan).
TauInteraction	Using this method, you can define the Minimal Fraction of Donor (MFD[1]). **TauInteraction** should be selected on the detector that corresponds to the donor emission. This is a technique for carrying out FRET experiments with lifetime information in which the given average lifetime of the donor without the presence of the acceptor is compared to the measured lifetimes in the specimen. When selecting this method, the detection range is automatically set to 30 nm to avoid detection of the acceptor fluorescence signal. However, this setting can be adjusted manually.

Using the various Tau modes, intensity images are created in the default setting and displayed in the viewer.

The settings are also used for stage experiments in the LAS X Navigator and in the Lightning wizard (Mark&Find and tile scan).

TauContrast

In addition to the Tau modes, the **TauContrast** function can be switched on (a). This function determines the average photon arrival time per pixel in the detector and displays it as a color lifetime image in a separate channel (see Fig. below: right viewer partition). The box in the upper right corner of the detector bar correspondingly displays a rainbow LUT (b), refer also to Beam Path Settings: Setting the Detectors.

The colored lifetime image contains both information on the acquired intensity of the species in the specimen and on their lifetime. The image is displayed with the aid of a combined LUT that uses the brightness to illustrate the intensity and the hue to illustrate the lifetime.

The LUT range can be adapted in the Viewer.

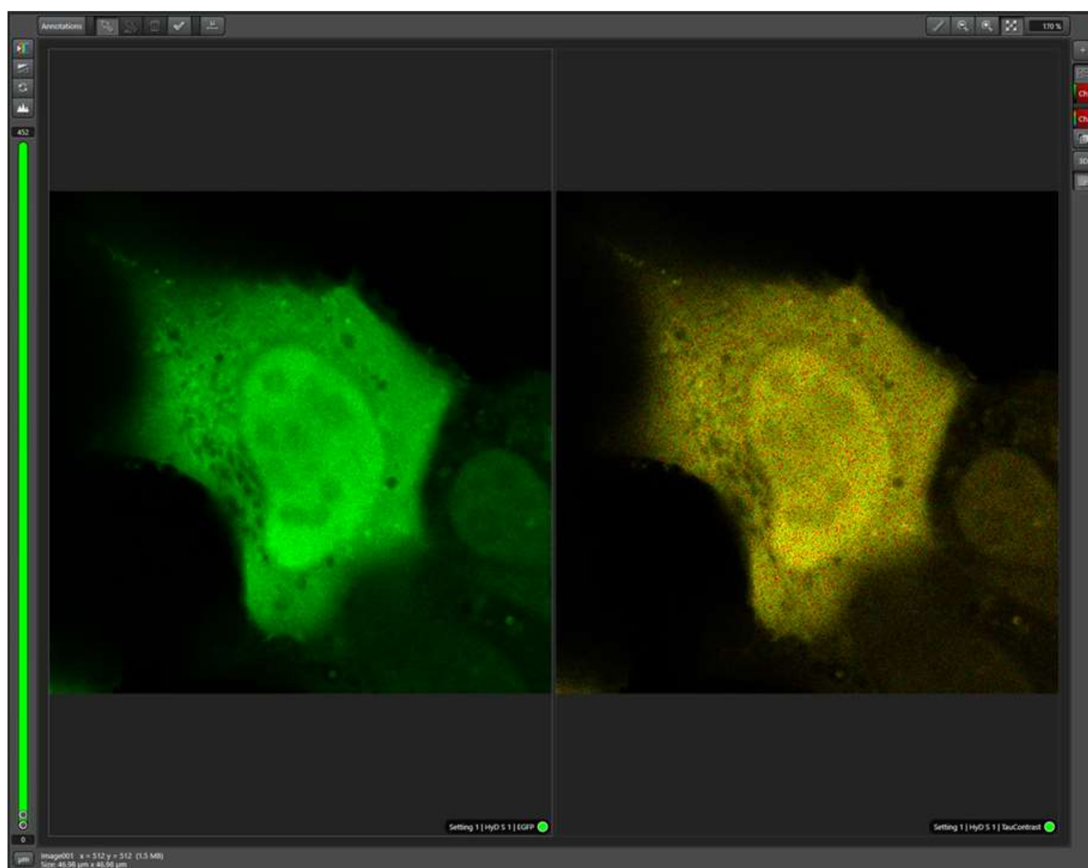


Fig. left: intensity image, right: lifetime image

Refer also to:
TauSeparation: Separating Species Based on Their Fluorescence Lifetimes .
Overview poster

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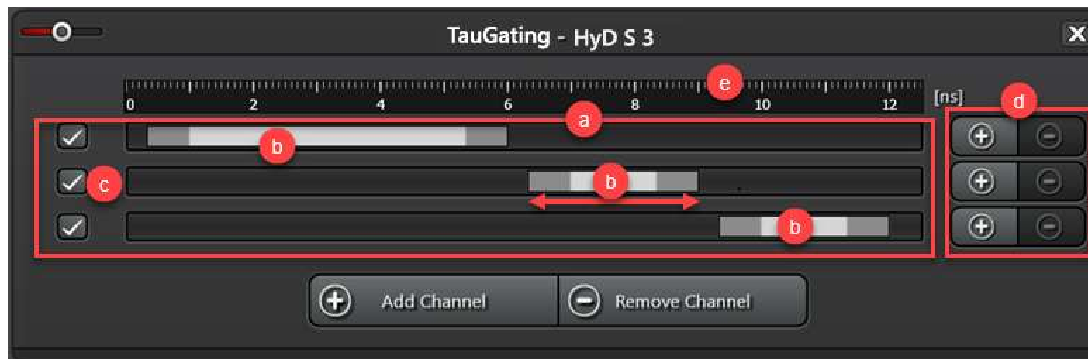
You are here: TauSense > Dialog Descriptions > TauGating

TauGating

Using the **TauGating** mode, you can create multiple channels for one detector to eliminate, for example, unwanted signals such as reflections or background noise from the acquisition. You can set the number of time gates per channel and the number of channels as you please.

Restrictions for STELLARIS 5: Max. 2 channels with 3 time gates each.



Three channels (a) with one time gate (b) each are preset. Each line represents one channel, each detection range one time gate. You can add both more channels and time gates per channel.

a	Each channel is represented in one line. Every channel is displayed in a separate partition of the viewer.
b	Each time gate is displayed as a detection range. You can use the mouse to adjust the size of the time gates and to move them (↔). In this way, you can define the beginning and end of the image acquisition (based on the laser pulse). If a channel has multiple time gates, the viewer collects them in one partition.
c	By checking the check boxes on the left margin, you can activate or deactivate the individual channels for the image acquisition.
d	Using the **Plus** and **Minus** buttons on the right margin, you can add more time gates to the respective channel or delete the currently selected time gate. A maximum of three time gates can be configured per channel.
e	Time scale: Photon arrival time after the laser pulse in nanoseconds. Here you can read the defined time gates.
Add Channel	In doing so, you add another channel below,
Remove Channel	By doing this, you delete the currently selected channel.

How to carry out an experiment: see Carrying Out an Experiment with TauGating
.

Refer also to:
TauSense: Overview

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You are here: Instructions > Carrying Out Experiments with Tau Mode > Carrying Out an Experiment with TauGating

Carrying Out an Experiment with TauGating

Using the **TauGating** mode, you can create multiple channels for one detector to eliminate, for example, unwanted signals such as reflections or background noise from the acquisition. You can set the number of time gates per channel and the number of channels as you please.

Limitations for STELLARIS 5: Max. 2 channels with 3 time gates each.

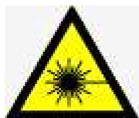
Requirements:

- A pulsed laser is activated.
- A pulsed laser line has been selected as a reference line (**Excitation Line**). When the **Auto** function is activated, the laser line with the longest wavelength as measured from the left edge of the detector is selected automatically as a reference, see also Reference Line. For each sequence, an individual reference line can be configured.
- At least one detector of the Power HyD S or HyD X type is used.

Follow these steps:

1. Switch on live mode by clicking the **Live** or **Fast Live** button.

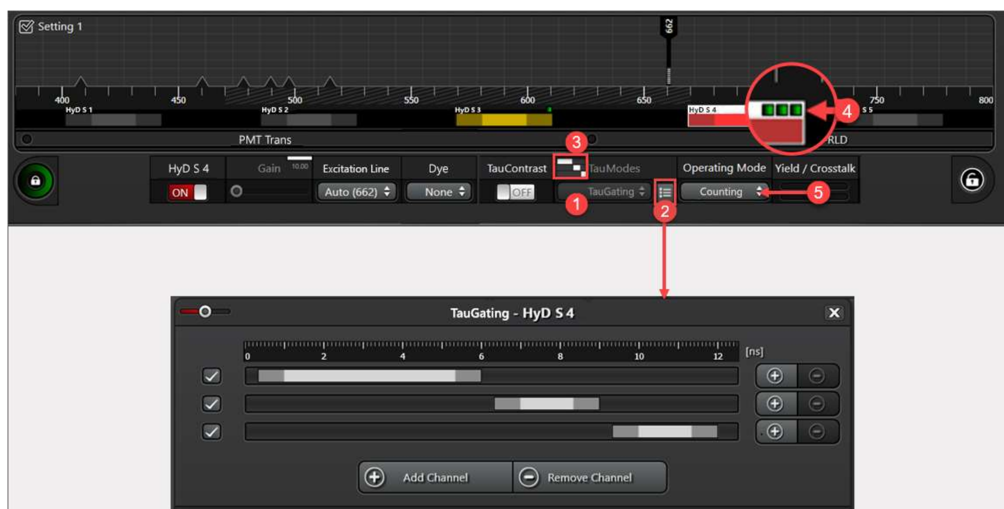
WARNING **Risk of permanent eye and skin damage from laser radiation**



From this time on, laser radiation may be present in the specimen area of the laser scanning microscope.
Make sure to follow the safety notes for operation of the system.

2. Define the instrument parameters in the **Acquire** operating step under the **Acquisition** tab; refer to Initial Image Acquisition.
3. In the area of the Beam Path Settings, configure the desired experiment settings for lasers and detectors based on the dyes used; refer to Initial Image Acquisition.
4. Switch live mode off again.
5. Under **TauModes**, select the **TauGating** entry (1) for the selected detector and click the **Options** icon (2) to open the **TauGating** dialog.

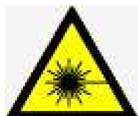
   The current setting is displayed in a thumbnail (3). One color field is displayed for each channel for the detector (4). The color corresponds to the LUT configured in each case. **Counting** is automatically set as the **Operating Mode** (5).



In the dialog, three channels are preset, each with one time gate. Each line represents one channel, each detection range one time gate. The photons detected in the time gates of a channel are combined in the intensity signal of this channel. Every channel is displayed in a separate partition of the viewer.

6. Switch live mode on again.

WARNING



Risk of permanent eye and skin damage from laser radiation

From this time on, laser radiation may be present in the specimen area of the laser scanning microscope.
Make sure to follow the safety notes for operation of the system.

7. In the **TauGating** dialog, set the time gate so that, for example, reflected light is suppressed or preferred. Alternatively, by selecting time gates correctly you can, for example, also separate long-lived dyes from short-lived dyes (refer also to **TauSeparation**).

You can add both more channels and time gates per channel. The thumbnail display adapts to the current settings.

8. Start your experiment by clicking **Start** or **Capture**.

Image acquisition starts. For each channel, an image or image series is created and stored in the project directory.
The metadata is stored in the image properties.

Refer also to:
TauSense: Overview

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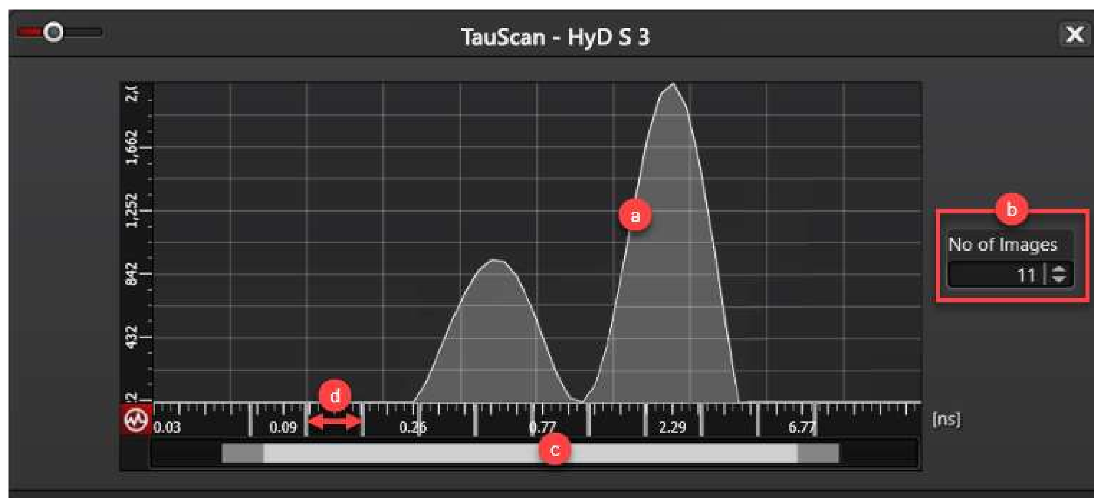
You are here: TauSense > Dialog Descriptions > TauScan

TauScan

Using a Tau scan, you can obtain a diagram of the fluorescence lifetime components (τ). This curve indicates how frequently lifetime components appear in the entire image, which is why it is also called the lifetime diversity curve.

In this dialog, you can break down the entire range of detectable lifetime components into a selectable number of sections. One channel is assigned to each section. The channel is displayed as a separate intensity image in a viewer partition. With the aid of this, you can assign the different species in your specimen to individual lifetime components. In addition, you can determine whether a species contains more than one lifetime component. You can use the **TauSeparation** method to separate the various species in your specimen if necessary.

Definitions for the specific terms that are used in the context of the image acquisition in a Tau mode you can find here.



a	Lifetime diversity curve: Graphical display of the frequency of lifetime components (τ measured in ns) in the entire image. Each peak indicates a lifetime component present in the image.
b	Set the number of channels or images here. This number corresponds to the breakdown of the entire range of detectable lifetime components in **c**.
c	Entire range of detectable lifetime components.
d	Time scale: Lifetimes τ in nanoseconds, broken down into the number of images set under **b**. The double arrow marks a section of detectable lifetime components (↔).

How to carry out a Tau scan: see Creating **TauScan**.

Note:
The **TauScan** method is not applicable in case of reflections. If the detection range is too close to the excitation line, you might see a plateau in the lifetime distribution diagram. In this case, move the detection range further to the right to increase the distance to the excitation line.

Refer also to:
TauSense: Overview
TauSeparation: Separating Species Based on Their Fluorescence Lifetimes

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You are here: Instructions > Carrying Out Experiments with Tau Mode > Creating a TauScan

Creating a TauScan

By using the **TauScan** mode (τ = fluorescence lifetime), you can identify the lifetimes of the different structures in your specimen. In addition, you can determine whether a dye contains more than one component.

A TauScan gives you a diagram of the frequencies of fluorescence lifetime components, also referred to as a lifetime diversity curve. Here, you can combine the detectable lifetime components into ranges. One channel is assigned to each range and the corresponding intensity image for each is displayed in the viewer in a separate partition. Each partition shows information about the setting, detector and corresponding lifetime. Accordingly, the different species in the specimen become visible and can be assigned based on their individual lifetimes.

The following instructions guide you through the individual procedural steps to carry out a TauScan.

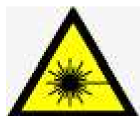
Definitions for the specific terms that are used in the context of the image acquisition in a Tau mode you can find here.

Requirements:

- A pulsed laser is activated.
- A pulsed laser line has been selected as a reference line (**Excitation Line**). When the **Auto** function is activated, the laser line with the longest wavelength as measured from the left edge of the detector is selected automatically as a reference, see also Reference Line. For each sequence, an individual reference line can be configured.
- At least one detector of the Power HyD S or HyD X type is used.

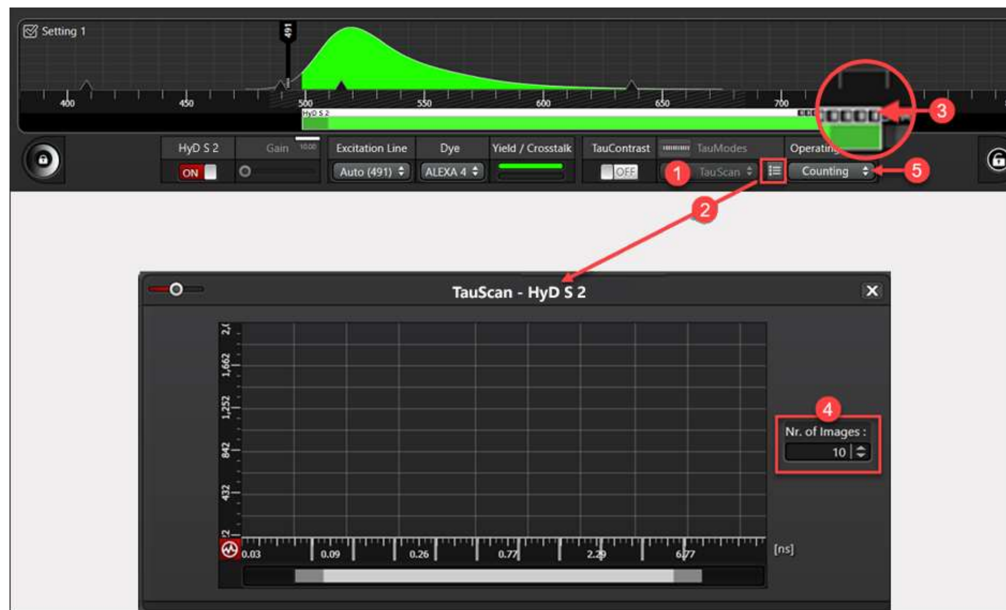
Follow these steps:

1. Switch on live mode by clicking the **Live** or **Fast Live** button.

WARNING	**Risk of permanent eye and skin damage from laser radiation**
	From this time on, laser radiation may be present in the specimen area of the laser scanning microscope. Make sure to follow the safety notes for operation of the system.

2. Select the detection channel in which you want to carry out the **TauScan** and disable all other settings.
3. Under **TauModes**, select the **TauScan** entry (1) for the selected detector and click the **Options** icon (2) to open the **TauScan** dialog.

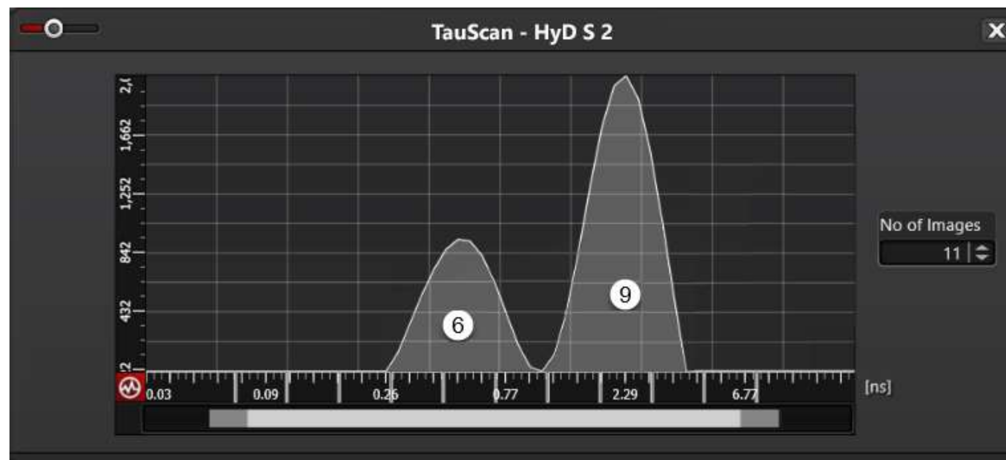
Counting is automatically set as the **Operating Mode** (5).



In the **TauScan** dialog, 10 images or 10 lifetime ranges are preset (4).

4. Use the time scale under **Nr. of Images** to set the number of lifetime ranges into which the entire detection range is to be divided, or – in other words – how many images are to be created.

Now a diagram is displayed in the **TauScan** dialog. The curve displays how frequently (counts) which lifetime components appear in the entire image. Each peak indicates a lifetime component present in the specimen (Sections 6 and 9).



Each lifetime range is represented in the viewer by a partition in a gallery display. Each partition shows an intensity image of the corresponding lifetime range. One color field is displayed for each channel (5). The color corresponds to the LUT configured in each case (6).

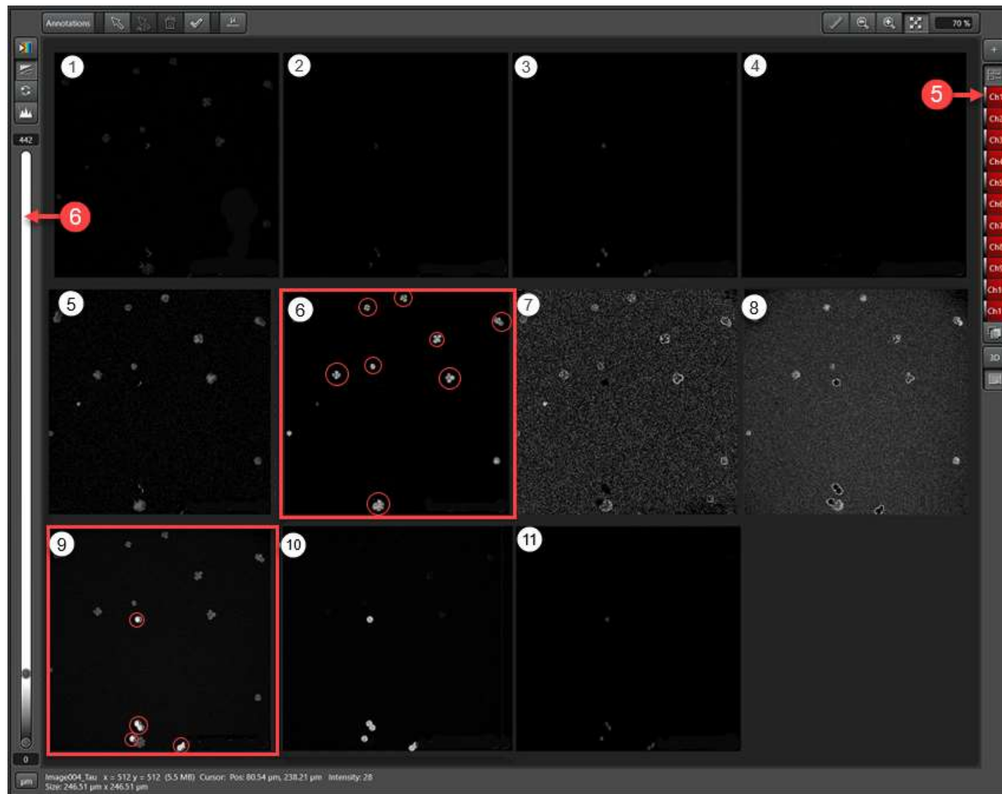


Fig. 1. Specimen with beads that is dyed with two dyes with similar emission spectra.

Based on the diagram and the visualization in the viewer, you can identify which species contains which lifetime components and how you can best assign the lifetime components to different species or channels.

You can show the lifetimes for any partition in the image channel information; refer to [Show/Hide Image Channel Information](#).

The example shows two peaks in the diagram. The first peak corresponds to image 6. It is easiest to view the cauliflower-like beads here. The second peak appears in image 9. It is easiest to view the round beads here. This supports the conclusion that the specimen has two different species with different lifetimes.

Now you can separate these based on their lifetimes using the **TauSeparation** method.

> **Note:**
> The TauScan cannot be used for reflections. If the detection range is too close to the excitation line, you may see a plateau in the lifetime distribution curve. In this case, move the detection range further to the right to increase the distance to the excitation line.

Refer also to:
TauSense: Overview

[TauSeparation: Separating Species Based on Their Fluorescence Lifetimes]

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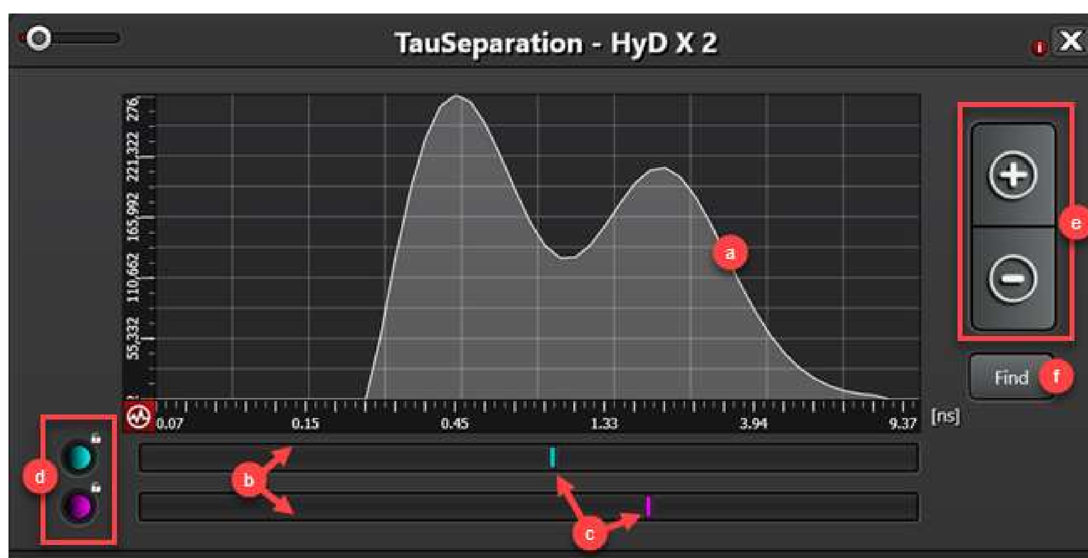
You are here: TauSense > Dialog Descriptions > TauSeparation

TauSeparation

You can use the **TauSeparation** method to separate species present in the specimen based on their fluorescence lifetimes and acquire the resulting images in separate channels. If a species has multiple lifetime components, you can also detect them separately. Configure the settings for species separation in this dialog.

Definitions for the specific terms that are used in the context of the image acquisition in a Tau mode you can find here.



a	Lifetime diversity curve: Graphical display of the frequency of lifetime components (τ measured in ns) in the entire image. Each peak indicates a lifetime component present in the image.
b	Each line represents a channel. Each species is assigned a separate channel.
c	The line represents a lifetime component for the respective channel. Move this line below a peak to assign a lifetime component to a channel and, consequently, to a species.
d	Here, you can adjust the color for the channel display. Double-clicking the color field opens a dialog for selection of a LUT. To prevent changes to the LUT assignment, you can lock it. To do so, click the open lock icon. The lock icon is closed and the LUT assignment is protected from further changes. Refer also to Beam Path Settings: Setting the Detectors.
e	Using the **+** and **-** buttons, you can add further channels or species and remove them.
f	By clicking **Find**, the analysis is carried out for all configured channels, and optimum settings for the lifetimes are determined.

Note:
The **TauSeparation** method is not applicable in case of reflections. If the detection range is too close to the excitation line, you might see a plateau in the lifetime distribution diagram. In this case, move the detection range further to the right to increase the distance to the excitation line.

Refer also to:
TauSeparation: Separating Species Based on Their Fluorescence Lifetimes

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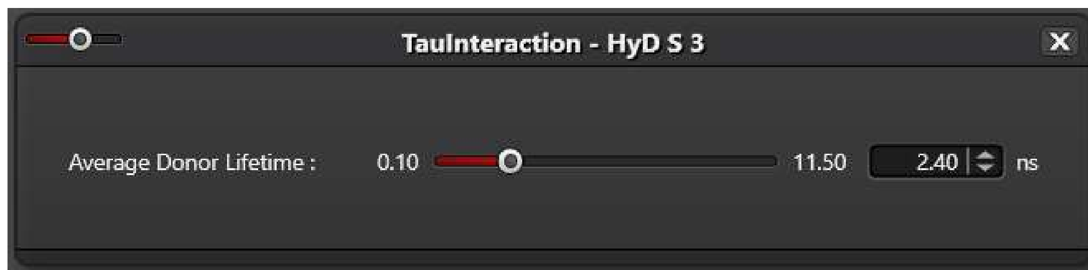
You are here: TauSense > Dialog Descriptions > TauInteraction

TauInteraction

Using the **TauInteraction** method, you can define the Minimal Fraction of Donor (MFD[1]). **TauInteraction** should be selected on the detector that corresponds to the donor emission. This is a technique for carrying out FRET experiments with lifetime information in which the given average lifetime of the donor without the presence of the acceptor is compared to the measured lifetimes in the specimen.



Average Donor Lifetime

Here, use the slider or direct input in the field to enter the average lifetime of the donor, which serves as a quantitative reference value. We recommend determining the average lifetime of the donor based on a negative control specimen (only donor). To do so, acquire images of the negative control specimen with **TauContrast** and determine the average photon arrival time.

Standard value: 2.4 ns.

The displayed value range depends on the configured pulse repetition rate of the laser:

Pulse repetition rate	Value range
40 Hz	Up to 24 ns
80 Hz	Up to 11.5 ns

When entering a value outside the permitted range, a correction to the maximum or minimum permitted average donor lifetime takes place.

When image acquisition starts, the viewer displays a fluorescence intensity image and a TauInteraction calculated image specifying the detector, the TauMode and the average lifetime of the donor. The images are stored in the project directory and the metadata are stored in the image properties.

Setting 1 | HyD X 2 | TauInteraction | Donor 2.4 ns

This method is also available in the Lightning Wizard at the detector configuration in the **Acquire** step. Refer also to: Lightning Wizard – Overview

The **Quantify** step detects the TauInteraction data and calculates the MFD values according to Padilla-Parra et. al.[2]. The MFD data are listed in the curves, the histograms and the statistics. See TauContrast/TauInteraction

Refer also to:
TauSense: Overview

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