

# SPCDynamics

Visualisation and Analysis of Dynamic Effects in TCSPC / FLIM Data

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*USER GUIDE*

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## Thank you...

... for choosing a quality product from Becker&Hickl. This guide will lead you through the installation process of the SPCDynamics application and some simple operating commands to get even more out of your hardware.

## Use SPCDynamics if you...

... if you have data recorded with the B&H measurement suite SPCM.exe or similar and want to scan through this data along its timeline.

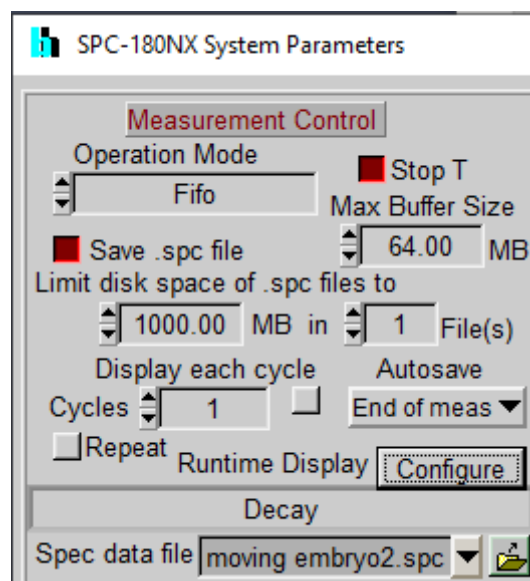
In the [SPCDynamics Particle](#) section you can check the behaviour of molecules with different lifetimes in your probe. In a pre-recorded data set you can look for entry and exit times of molecules passing the excitation area. Molecules of different lifetimes can be separated.

In the [SPCDynamics Image](#) section you can display single frames of an image recording and bin them together or delete them.

## Pre-Requisites

SPCDynamics is programmed to load and analyse single photon data files as created by the B&H measurement suite SPCM.exe or similar. The file type should be \*.spc and comes in pairs with a \*.set setup file of the same name.

It should have been recorded in **Fifo** or **Fifo Image** operating mode and with the **Save .spc file** enabled.



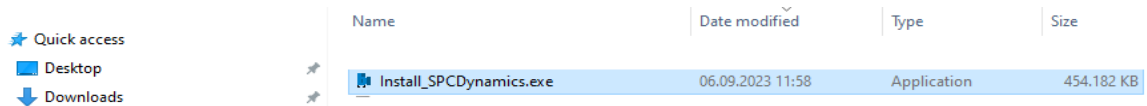
*SPCM.exe System Parameters*

## Software Installation

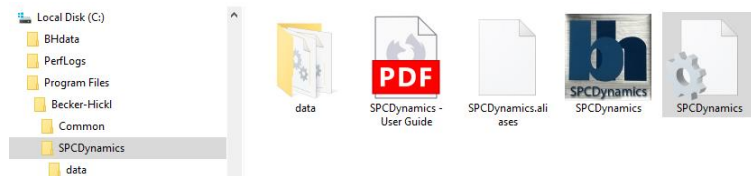
The SPCDynamics application installer can be downloaded from [www.becker-hickl.com](http://www.becker-hickl.com).

### Run Installer

- Execute the Install\_SPCDynamics.exe to run the installer.



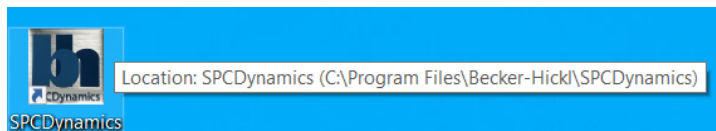
- The default installation path is C:\Program Files\Becker-Hickl.



The required **LabView Runtime Engine 2017 SP1** is also installed. The runtime can be used to operate software written with LabVIEW without needing a LabVIEW license. It can also be downloaded from [www.ni.com](http://www.ni.com).

The default destination is C:\Program Files\National Instruments\

- The SPCDynamics software shortcut is now available on your desktop. Another link can be found in the start menu.



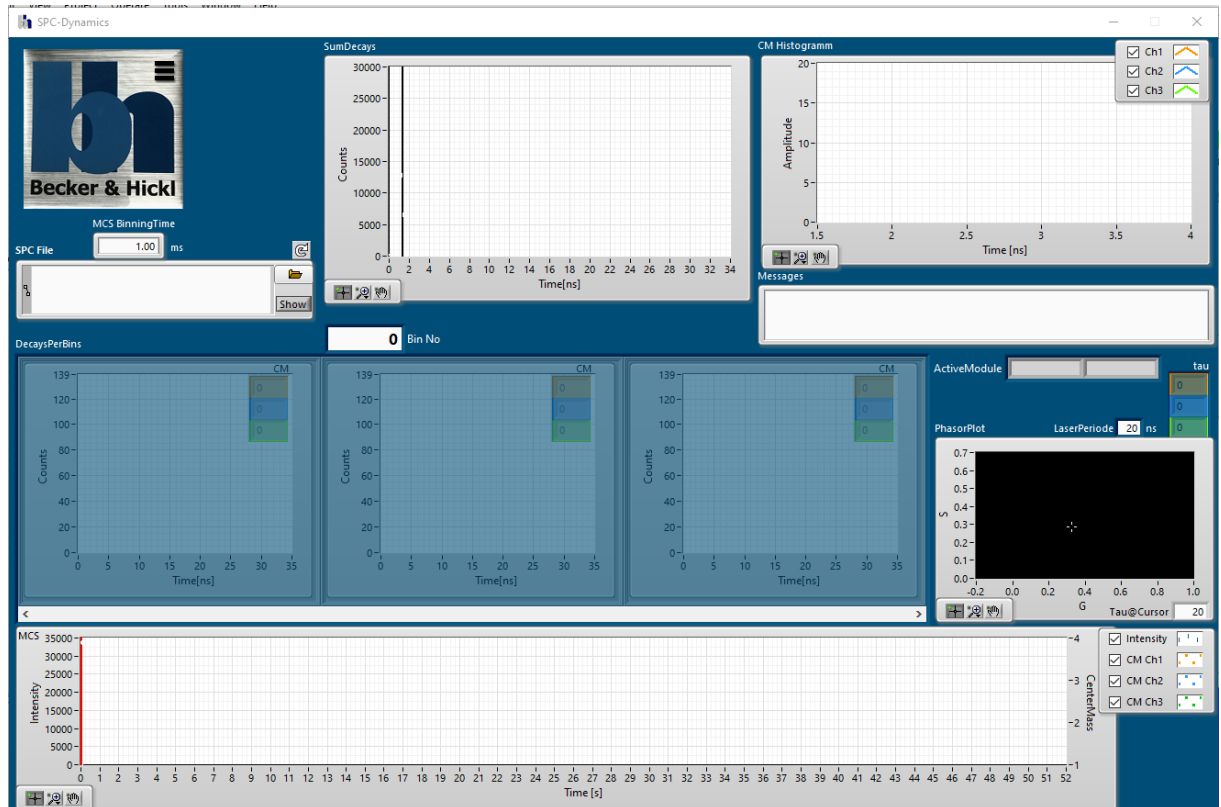
- Running SPCDynamics requires a valid license key from Becker&Hickl.

## Using the Particle Dynamics Application

Particle Dynamics is part of the SPCDynamics suite. It displays the timeline of a measurement where single photons are binned to groups of photons within a selectable milliseconds bin. Each such bin can be displayed and a rough lifetime analysis is performed. Bins which fall into a certain lifetime range can be exported or deleted from the measurement data.

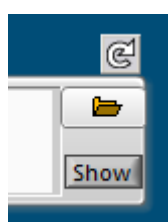
### Starting Particle Dynamics

Double-click the desktop icon or use the link provided in the start menu.




The Particle Dynamics window starts in the default configuration and is ready to read a \*.spc file.

Should you have closed SPCDynamics at your last usage in the Image Dynamics mode, SPCDynamics will open in that mode again. Use the [Menu](#) to switch to Particle Dynamics.



Press the folder button to bring up a default file select box where you can select the \*.spc file you wish to display. Note, that a \*.set file with the same filename has to reside in the same folder.

Since the application memorizes the last loaded file you can simply press the reload button  to load that file from disk again.

Pressing the Show button will bring up the file's directory in the standard browser.

### MCS Binning Time



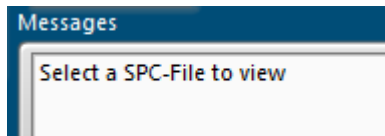
The Binning Time describes how many milli-seconds of the measurement will be sampled into one time bin. Each of those bins will then be displayed as data point in the MCS graph and the decay curve for each such bin can be displayed.

A reasonable value for the binning time is crucial for a successful data analysis. While small binning times will give you a more detailed view of fast changing events, there might be a too low photon rate in each bin for an accurate decay curve calculation. Also, the amount of data

points to be held in memory increases and can slow down significantly the applications response time.

Consider also the overall time of your measurement in the \*.spc file. Longer measurements lead to more bins that have to be calculated.

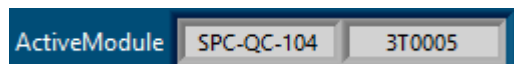
### Message Box



The Message Box display information about analysis progress or hints as to what steps could be taken next.

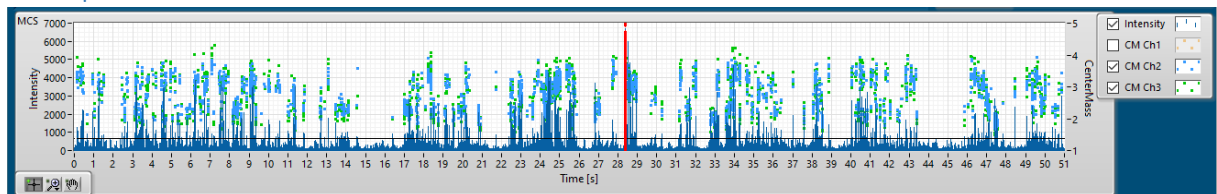
You might also see messages about processing errors there. Unknown or corrupt data files might be such errors – accompanied by a short red-flashing of the application.

### Active Module




When a \*.spc file is successfully loaded these fields show the type and serial number of the B&H card used to record the data.


### MCS Graph



The MCS graph displays the \*.spc data on a macro time scale. In this example over the full 51 seconds measurement time.

The dark blue needles rising from the zero line indicate the photon count in each time bin. The number of photons can be read on the left Y-axis as Intensity. In this view you can determine in which time bin fluorescent molecules have past the detectors field of view. The low signals between higher needles can be considered noise or even dark count rates.

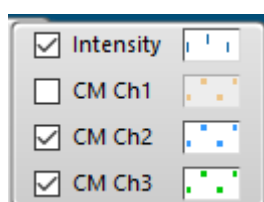
In order to eliminate bins with a low count rate from the analysis you can move the horizontal thin black cursor to a level above the noise. Select the  button to turn the mouse pointer into a tool you can use to shift the line up or down. Only bins with a needle height above this line will be used in further calculations. When starting Particle Dynamics this threshold will be set to 10% of the highest photon count of all bins.

The button  brings up a menu from which you can select several tools to zoom in or out of the graph.



Note, that the MCS graph is just over 1,000 pixel wide whereas in this example we have over 10,000 time bins, meaning that only a tenth of the lower needles are displayed when not zooming in.

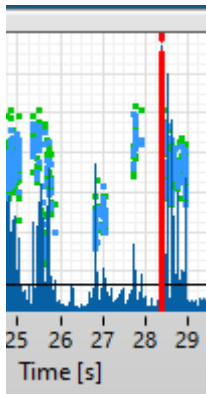
The hand  tool allows shifting of the graph.




For each time bin with a photon count of at least 100, the centre-of-mass CM or so called 1<sup>st</sup> moment is calculated. Displayed as dots above the bin's needles are only values for bins with a higher photon count rate than the black cursors line. The centre-of-mass' Y-axis is on the right. It is auto-scaled to distribute the dots to the upper part of the graph.

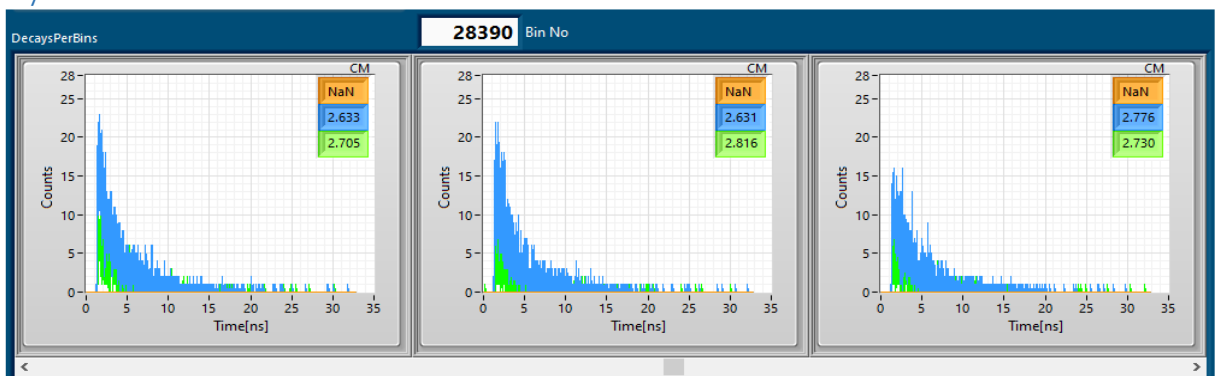
In all graphs you can click on the axis' max or min values and modify them to your liking. The CM-dots are coloured orange, light-blue and green for the three measurement channels of an SPC-QC or the first three routing channels of an SPC module.

If it helps you to unclutter the graph you can choose to disable certain plots by removing the check mark in the plot legend.



The vertical thick red cursor marks the position of the currently displayed decay curve in the DecaysPerBin graph above the MCS graph. When zoomed out the cursor homes in on the highest needle in the pixel's neighbourhood and displays the corresponding decay curve in the middle of the three graphs. You can use the  button to turn the mouse pointer into a tool to move the red cursor from left to right. When loading a new \*.spc file the red cursor automatically jumps to the highest needle in the graph.

## Decays Per Bins



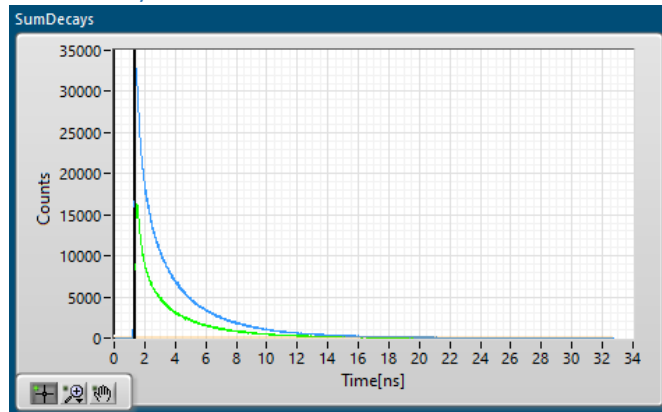
The DecaysPerBins graph shows the decay curves in three consecutive time bins. The number of the bin in the middle is displayed above the graph as Bin No. You can click in the Bin No field and manipulate the bin number to display. Use the up and down arrow keys to in- or de-crease the digit. Alternatively, you can use the right and left arrow keys anytime to move one bin up or down. The slider below the graph let you scroll quickly through the bins.

When changing the Bin No the red cursor in the MCS graph is moving accordingly showing you the current position in the measurement.


When using data from an SPC-QC the card's three channels are displayed as orange, light blue and green plots. When using an SPC card the plots reflect the first three routing channels. With more than 100 photons in a decay curve a centre-of-mass algorithm is used to determine the lifetime. The results are displayed in the corresponding colours in the top right corner of the graphs. Note, that this is not an exponential fit and can't be used to determine any multi-exponential lifetime.



## Sum of Decays






The SumDecays graph adds up the decay curves of all time bins. The plot's colours orange, light blue and green correspond to the SPC-QC card's three channels or the SPC card's first three routing channels.

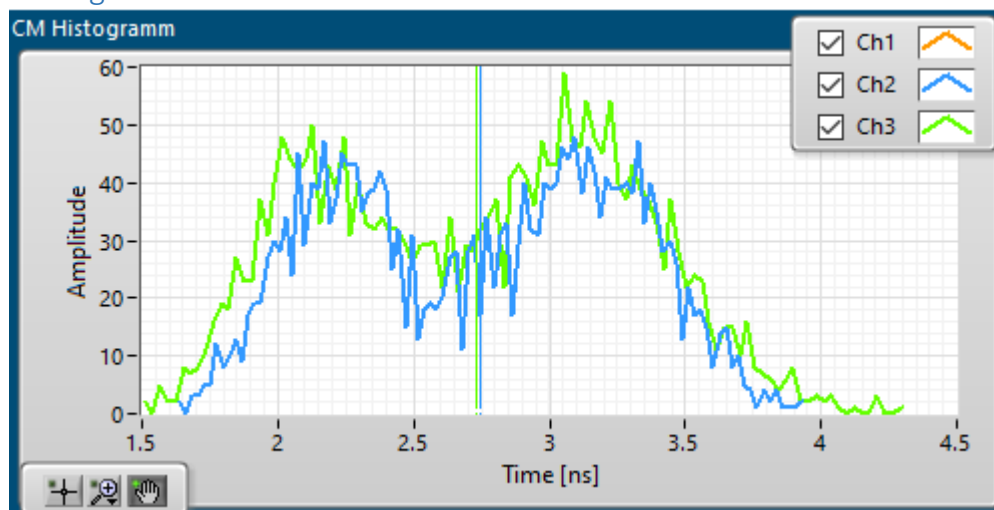
This graph is used to determine the start position (typically the laser pulse) of the decay curves. Particle Dynamics finds the maximum in each plot and subtracts that time from the centre-of-mass calculation of all time bins. You can manually change this start position for each channel by moving the black vertical cursors right or left, using the mouse pointer in the  tool style. Your changes will cause a recalculation for all CM and tau values.

A mathematically more accurate start point would require intimate knowledge of the system's instrument response function (IRF) which Particle Dynamics can't produce.

Note, that the sum of decay curve should not be used for exponential fit to determine the lifetime of your probe. Typically, you might have molecules of different lifetimes passing the observation area in different bins. The sum graph does add them all up.

You can use the    buttons to zoom in and out of the plots or move them around.

## CM Histogram



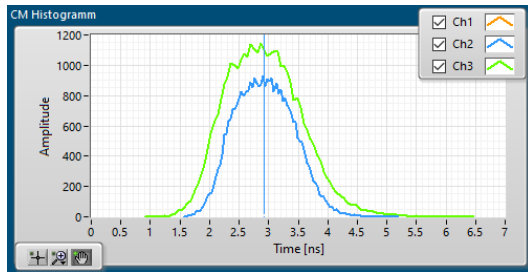
The centre-of-mass histogram displays a statistic over the calculated lifetimes in all time bins.

Use this information to determine different lifetimes in your probe at different times.

In this example we have two distinct lifetimes, one with 2.2 ns and another with 3.3 ns.

The plot's colours orange, light blue and green correspond to the SPC-QC card's three channels or the SPC card's first three routing channels.

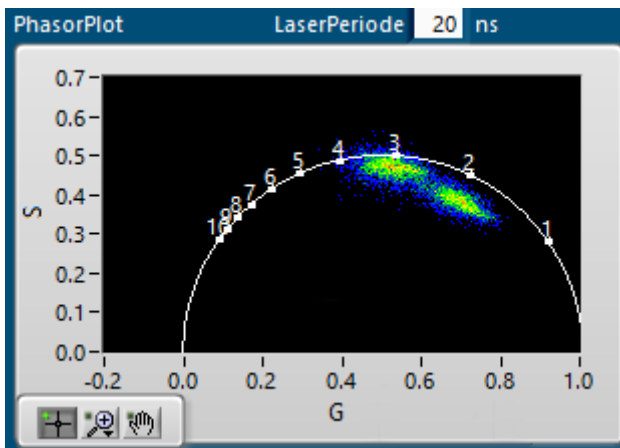
The coloured vertical cursors are located at the average lifetime over all time bins.



The character of this graph can change significantly with the threshold position of the black horizontal cursor in the MCS graph. Here an example of the same data with a threshold of zero; meaning all time bins are included as long as their count rate is at least 100 photons. The noise in low populated bins has obscured completely the two distinct lieftimes of the brighter bins.

For further usage of the CM Histogram see the section [Manipulating Data](#).

## Phasor Plot



The PhasorPlot graph is an attempt to display more clearly the lifetimes calculated for each time bin. For this reason the decay curves in each time bin are multiplied with a sine and cosine curve of the length of a laser period, all measurement points summed up and normalized. This creates an S and G phase coordinate for each bin. All phases are inserted into the graph. Coordinates that appear with the highest incidence are coloured red, going over yellow, green and blue to the lowest incidence and black for none at all.

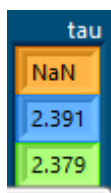
The so-called universal circle is the location on which the phases of perfect exponential curves would lay, with their lifetimes measured in nanoseconds. Do the coordinates fall inside the curve, then this is an indicator of a multiexponential decay. Where the lifetime of at least one component of a double-exponential decay is known, the other can be geometrically calculated. Coordinates outside the circle are mathematically unsound, but appear because of noise in the signal.

Particle Dynamics extrapolates the measured decay curves over their actual time length to the length of a laser period to increase validity of the results. For this and for a calibration of the ns-timescale the laser period needs to be entered manually into the data field. This value is not included in the measurement's \*.spc and \*.set files.

For further details about the Phasor Plot see the Becker & Hickl TCSPC Handbook.

In the above example we see two multi-exponential decay curves with an average of 3.1 ns and 2.2 ns. The plotted incidence is the sum of all three possible channels.

Tau



The tau field shows the lifetime results for the three possible channels. The calculation is done using the formula

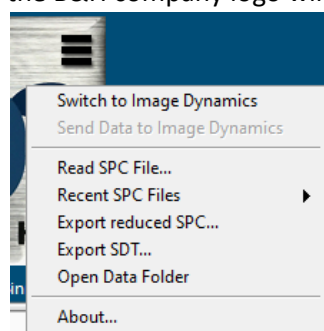
$$\tau = \frac{1}{\omega} \left( \frac{s}{g} \right)$$

Note, that the displayed values are an average of all time bins included in the calculation. The above seen separation in two distinct lifetimes is not observable in this view. A value of NaN appears when the calculation fails, or like in this example the channel wasn't used.

## The Particle Dynamics Menu



Clicking anywhere on the B&H company logo will bring up the application's menu.



### Switch to Image Dynamics


Change the SPCDynamics mode to the Image Dynamics window.

### Send Data to Image Dynamics

You might want to view your current \*.spc-file in Image Dynamics. Use this menu tag as shortcut.

Note, that this menu tag is greyed out when your current \*.spc-file doesn't include markers.

### Read SPC File...

Brings up the Windows standard browser and invites you to select a \*.spc file to load and analyse. The effect is the same like clicking the  button.

Note, that an equally named \*.set file needs to be present in the same folder.

Should there be no equally named \*.set file a file select box will open to ask you for a corresponding \*.set file to use in conjunction with your chosen \*.spc file.

### Recent SPC Files

Hovering on this menu line will bring up a submenu offering to load one previously loaded file again. Up to 10 files are memorized here.

### Export Reduced SPC...

This option allows you to export a new \*.spc file where all photon infos are deleted of time bins where there is no CM value displayed in the MCS graph.

Time bins without CM value occur when

- there are less than 100 photons in that bin.
- the black cursor in the MCS graph is set to photon threshold that is not reached in that bin. See [MCS graph](#) for details.
- you have selected a range of CM values which does not include that bin's value. See [Manipulating Data](#) for details.

You can use this new \*.spc just like the original, but cleared of unwanted photon data.

### Export SDT...

This option allows you to create an \*.sdt file containing the summarized decay curve over all selected time bins. You can tell whether a time bin is part of the selection by a displayed CM value dot in the CM graph.

Time bins without CM value occur when

- there are less than 100 photons in that bin.
- the black cursor in the MCS graph is set to photon threshold that is not reached in that bin. See [MCS graph](#) for details.
- you have selected a range of CM values which does not include that bin's value. See [Manipulating Data](#) for details.

You can use \*.sdt files in programs like Spcm.exe or SPCImage.exe and several LabVIEW example applications to display and analyse the decay curve stored in an \*.sdt file.

Note, that in this format the individual photon's absolute time information is lost.

### Open Data Folder

Opens the folder of the last accessed file in the default file browser.

### About

Opens the B&H [about and copyright box](#).

## Manipulating Data

Particle Dynamics allows you to modify your measurement data.

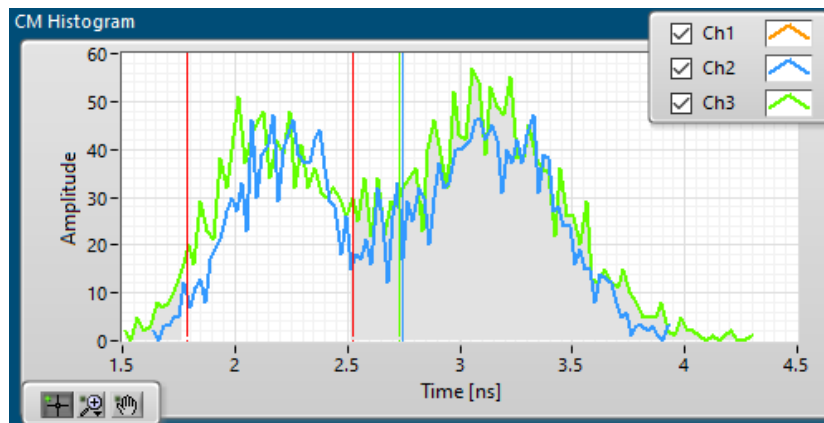
A typical scenario would be a measurement where different types of particles have been recorded, but only one type is of further interest.

Time bins with a distinct lifetime value can be selected and exported. Either as \*.spc file containing all timing information of the measured photons in those time bins, or as \*.sdt file that contains the accumulated decay curves of all photons in the selected time bins.

In both file types all photon's info of not selected time bins is erased.

The created \*.spc file can be reloaded into Particle Dynamics and further analysed. For example, for transient times.

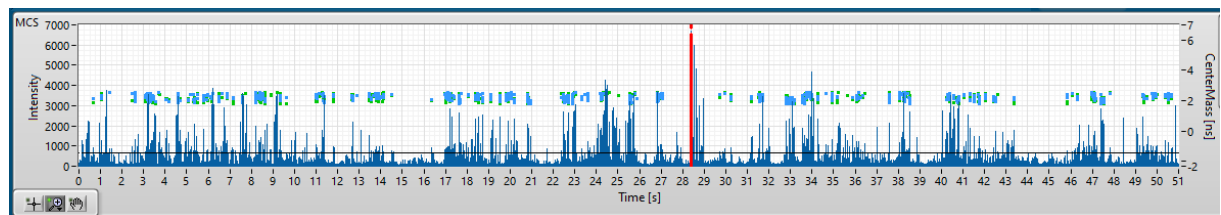
The \*.sdt file can be loaded into SPCImage for a detailed multiexponential lifetime analysis.



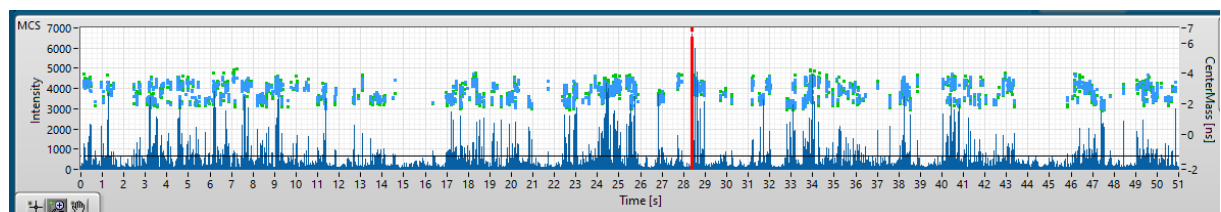
*In this example we selected only lifetimes between 1.8 ns and 2.5 ns.*

To select only time bins with lifetimes in a certain range click into the CM Histogram graph and drag the red cursor to a different CM value. The plots outside the chosen range will be greyed out and the corresponding CM values in the MCS graph will be removed.

You can redo the selection anytime by clicking and dragging in the CM Histogram graph. A single click in the graph will clear the selection and all CM values in the MCS graph will reappear.



*Only time bins with CM values in the range of 1.8 - 2.5 ns and intensity higher 800 photons are selected.*



*The CM selection is removed and all time bins with an intensity higher than 800 photons are selected.*

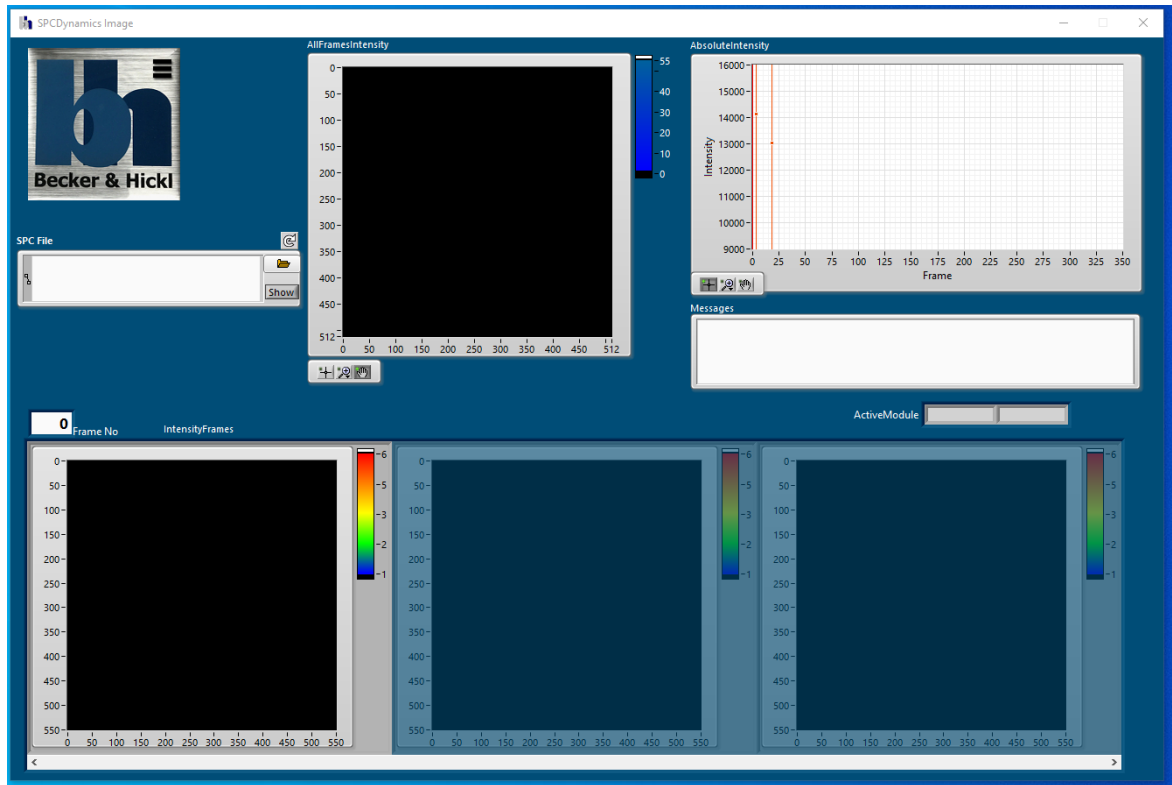
Use the [Menu>Export Reduced SPC](#) to export the selected time bins to files.

## Using the Image Dynamics Application

Image Dynamics is part of the SPCDynamics suite. It displays single frames recorded in an \*.spc/\*.set-file pair. Single frames can be deleted or exported to different formats or binned together to increase the photon rates.

### Starting Image Dynamics

Double-click the desktop icon or use the link provided in the start menu.




The Image Dynamics window starts in the default configuration and is ready to read a \*.spc file. Should you have closed SPCDynamics at your last usage in the Particle Dynamics mode, SPCDynamics will open in that mode again. Use the [Menu](#) to Switch to Image Dynamics.



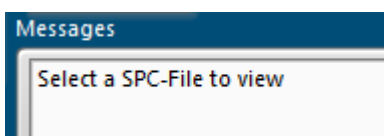
Press the folder button to bring up a default file select box where you can select the \*.spc file you wish to display. Note, that a \*.set file with the same filename has to reside in the same folder.

Only \*.spc-files that have been recorded with valid pixel-, line- and frame-marker can be displayed in Image Dynamic.

Since the application memorizes the last loaded file you can simply press the reload button  to load that file from disk again.

Pressing the Show button will bring up the file's directory in the standard browser.

### Message Box

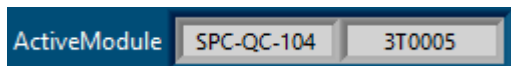


The Message Box display information about analysis progress or hints as to what steps could be taken next.

You might also see messages about processing errors there.

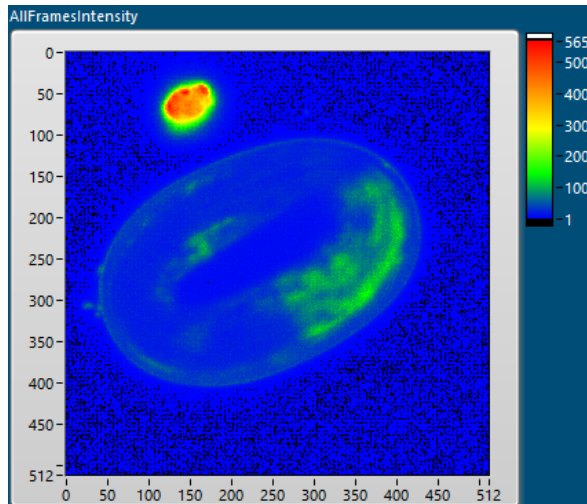
Unknown or corrupt data files might be such errors – accompanied by a short red-flashing of the application.

## Active Module




When a \*.spc file is successfully loaded these fields show the type and serial number of the B&H module used to record the data.

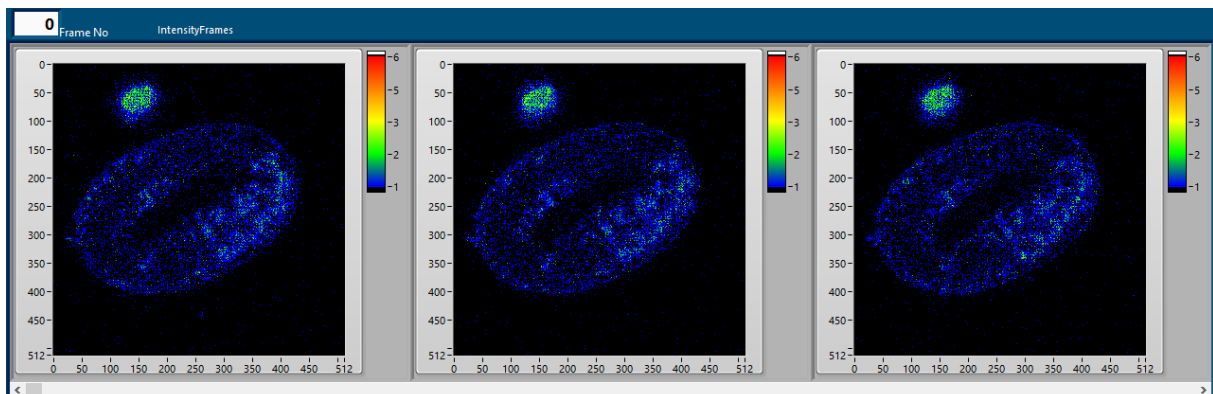
## AllFramesIntensity



When an \*.spc-file is successfully parsed for pixel, line and frame markers the intensity sum of all frames is displayed in the AllFramesIntensity. The intensity, that is the sum of photons per pixel is colour coded. See the colour bar to the right for values. Note, that this display shows the colour coded amount of photons in each pixel – don't confuse with lifetimes. This display is similar to the presentation in Spcm.exe.


You can use the  tools to zoom into this display if desired.

## IntensityFrames

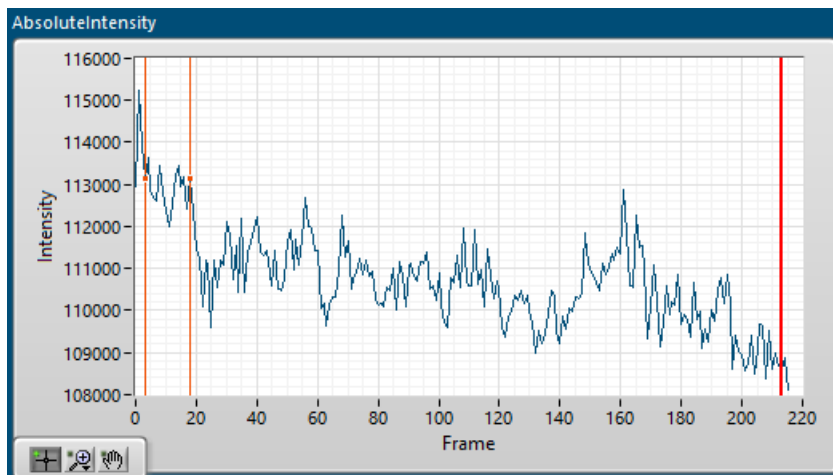


The linear photon file \*.spc is parsed of pixel-, line- and frame-markers and the photon rate in each pixel is displayed in their according frames. Typically, the photon rates per pixel are rather low, which is why measurements are performed over a series of frames. This on the other hand can cause motion blur or whole frames might even be flawed which will disturb the accumulated image as displayed in **AllFramesIntensity**. In **IntensityFrames** you scroll through all frames and lookout for such flaws.



Note, that this display shows the colour coded amount of photons in each pixel – don't confuse with lifetimes. A lifetime analysis on this level is typically not possible due to the low amount of photons.

 The **Frame No** field shows the frame number of the left image in the display. Use this field to scroll through all single frames or use the scroll bar below the display. You can also use the thick red cursor in the **AbsoluteIntensity** graph to scroll through the frames.

## AbsoluteIntensity



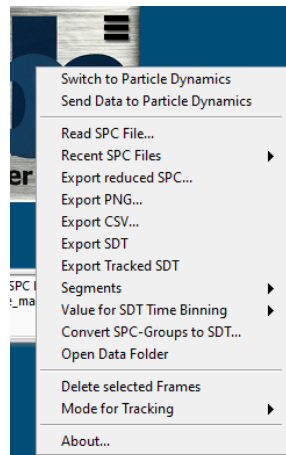
The absolute intensity graph shows the sum of photons in each frame. Like this you can detect an over-all deterioration of your probe like in the example above or possible sudden image changes at certain frames.

Use the thick red cursor with the  tool to scroll through the **IntensityFrames** display to the corresponding frame. The  tool allows you to zoom into the graph.

## The Image Dynamics Menu



Clicking anywhere on the B&H company logo will bring up the application's menu.




### Switch to Particle Dynamics

Change the SPCDynamics mode to the Particle Dynamics window.

### Send Data to Particle Dynamics

You might want to view your current \*.spc-file in Particle Dynamics. Use this menu point as shortcut.

### Read SPC File...

Brings up the Windows standard browser and invites you to select a \*.spc file to load and analyse. The effect is the same like clicking the  button.

Note, that an equally named \*.set file needs to be present in the same folder.

Should there be no equally named \*.set file a file select box will open to ask you for a corresponding \*.set file to use in conjunction with your chosen \*.spc file.



## Recent SPC Files

Hovering on this menu line will bring up a submenu offering to load one previously loaded file again. Up to 10 files are memorized here.

## Export Reduced SPC...

This option allows you to export a new \*.spc file where all photon infos are deleted of frames that you have deleted before.

You can use this new \*.spc just like the original, but cleared of unwanted frames.

## Export PNG...

You can export all intensity frames as \*.png images. After clicking the menu tag Export PNG... a file select box will open to ask you for a folder location to save all frame images to.

## Export CSV...

You can export all intensity frames as comma separated text tables. After clicking the menu tag Export CSV... a file select box will open to ask you for a file name and location to save the table to. All frame's intensities will be written to one file.

## Export SDT...

This option allows you to create an \*.sdt file containing the summarized frames over all frames still on display.

You can use \*.sdt files in programs like Spcm.exe or SPCImage.exe and several LabVIEW example applications to display and analyse the decay matrixes stored in an \*.sdt file.

Note, that in this format the individual photon's absolute time information and frame index is lost.

Note the setting at [Menu>Value for SDT Binning](#).

## Export Tracked SDT...

If you have used the SPCDynamics [Feature Stabilization](#) tool, this option allows you to create an \*.sdt file containing the summarized frames over all frames with your stabilization result.

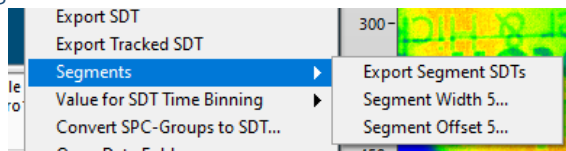
You can use \*.sdt files in programs like Spcm.exe or SPCImage.exe and several LabVIEW example applications to display and analyse the decay matrixes stored in an \*.sdt file.

Note, that in this format the individual photon's absolute time information and frame index is lost.

Note the setting at [Menu>Value for SDT Binning](#).

Note, that this menu tag is greyed out when your haven't started a tracking, yet.

## Segments



To observe changes in your probe over a longer time period it is useful to bin several successive frames and compare them with frames of different measurement times.

With the Export Segment SDTs option you can create \*.sdt files containing binned frames of selectable Width and Offset. Single frames typically don't contain enough photons to support robust decay analysis. Therefore, binning is a necessity.

The menu Segment Width X... shows the currently selected number of frames to include in one \*.sdt file. By clicking on this menu this value can be changed.

The menu Segment Offset X... shows the currently selected starting point for the next \*.sdt file. By clicking on this menu this value can be changed.

With Segment Offset smaller than Segment Width a moving average of frames will be

created. Offset and Width identical will put each frame in only one \*.sdt file. While Offset larger than Width some frame's information will not be taken into account. Note the setting at [Menu>Value for SDT Binning](#).

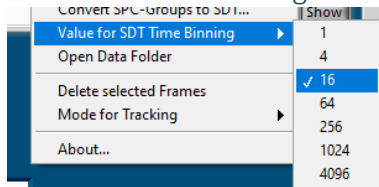
### Convert SPC-Groups to SDT...

This option is similar to [Menu<Segments](#) but aimed at longer measurements where Spcm.exe might be forced to create groups of \*.spc files with equal base names but consecutive numbering. This batch converting is asking you for the \*.spc files in a group and a corresponding \*.set file to use for all \*.spc files in that group. The number of frames to include in one \*.sdt file must be entered in a message box. The offset option is not available in this mode.

The created \*.sdt file will be placed in the same directory where the original \*.spc files reside. \*.sdt are not overridden, but numbered consecutively.

Note the setting at [Menu>Value for SDT Binning](#).

### Value for SDT Time Binning



For all \*.sdt export options the SDT Time Binning factor is used.

The photon raw data stored in an \*.spc file includes timing information in 12bit, that is 4096 bins, regardless of the ADC-resolution entered in Spcm.exe.

When creating \*.sdt files in SPCDynamics, the default value as set in Spcm.exe can be used or a different resolution chosen here. While smaller values will lessen the accuracy of an exponential fit, large values will increase the file size beyond a PC's capacity or in case of low photon rates not create a significant decay curve at all.

### Open Data Folder

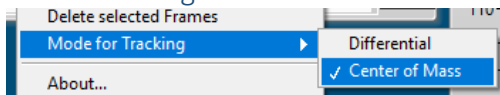
Opens the folder of the last accessed file in the default file browser.

### Delete Selected Frames

After selecting a range of frames, as described in [Deleting Data](#), use this option to eliminate the photon data collected in those frames.

Note, that deleting only happens on the display. To actually save the shortened data to file use the [Menu>Export reduced SPC](#).

### Mode for Tracking



See the section about [Feature Stabilization](#) for a description of this option.

### About

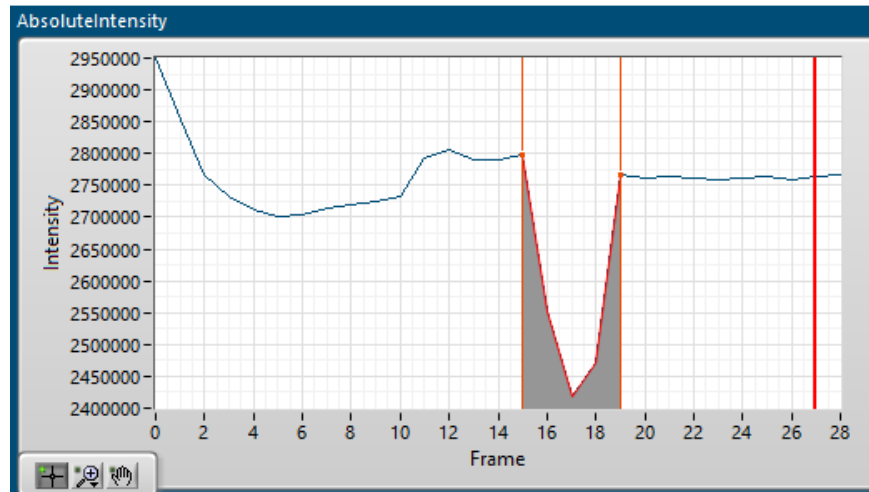
Opens the B&H [about and copyright box](#).


## Manipulating Data

Image Dynamics allows you to modify your measurement data.

### Deleting Data

You might want to delete some frames. A typical scenario would be some disturbance in one or several frames. Accidental shake of the probe, passing incidence or change in environment would be examples for that.



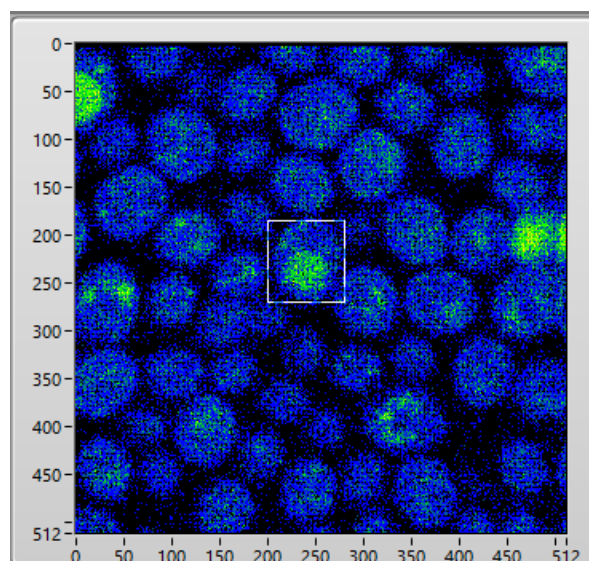
Use the thin red cursors with the  tool to select a range for frames. The **IntensityFrames** display will scroll to the lowest frame of your selection.

Use the [Menu<Delete selected Frames](#) to delete your selection from the data. Note, that deleting only happens on the display. To actually save the shortened data to file use the [Menu>Export reduced SPC](#).

### Feature Stabilization

Image Dynamics incorporates a simple image tracking tool.

A typical scenario would be a slow-moving object in your probe. The accumulation of all frames would show a motion blur.



Click in the left display of the **IntensityFrames** and drag a frame over the feature you like to track through the frames.

When successful the **AllFramesIntensity** will show a sharpening and intensity increase of the feature, whereas the surrounding area might become blurrier since the frames have been moved from their original position to compensate for the feature's traversing. Check the success by scrolling to the last frame and observe the position of the detection-frame. It should still be sitting over your feature.

Note, that stabilization only happens on the display. To actually save the moved data to file use the [Menu>Export Tracked SDT](#).

The feature stabilization is still in development process and strongly depends on clear detectable features. Give it a try with different detection-frame sizes and verify a successful outcome.

Currently there are two tracking algorithms implemented. In the [Menu>Mode for Tracking](#) you can choose between Differential (where two successive frames are subtracted for minimal difference) and Centre of Mass (where the centre of mass in each frame is calculated and matched to the previous one).

## The About Box

Using the menu by clicking on the B&H logo in the top-left corner you can open the about box window.



The Becker&Hickl company logo and the copyright information is displayed, alongside with contact details, the application's version and build date and the open VI Server port for remote controlling the application from other LabVIEW apps.

## Closing the SPCDynamics Application



You can terminate the SPCDynamics application, as you are used to from any other Windows application, by clicking on the red X in the top right corner. The current setup will be stored to disk and reloaded when you start SPCDynamics again.

## The \*.spc File Format

Deeper understanding of the \*.spc format is not required for users of the SPCDynamics application. The information is included here for developers interested in creating their own modifications.

\*.spc-files contain binary raw data as produced by Becker&Hickl SPC and SPC-QC cards. Coded is each single photon received during a measurement and its precise timing information.

Information about the measurement condition is saved to a separate file with the same name and a \*.set extension. Applications like Spcm.exe, several example applications written in LabVIEW and others can write \*.spc / \*.set file pairs to disks.

\*.spc files are coded 32-bit wide, where the first 32-bit include special information about the Macro Time clock and the number of routing channels used during the measurement.

byte 0, 1, 2 = macro time clock in 0.1 ns units (for 50 ns value 500 is set)

byte 2: bit 7 = 1 ExpressFLIM-mode in SPC-QC cards (not valid for SPCDynamics)

byte 3: bit 7 = 1 (Invalid photon),

bits 3-6 = number of routing bits,

bit 2 = 1 file with raw data (for diagnostic mode only)

bit 1 = 1 file with markers (optional for SPCDynamics)

bit 0 reserved

All following data represent so called events encoding information about single photon occurrences or electronic markers. See following table for the SPC card's encoding:

PHOTON EVENT									
	Bit 7	Bit 6	Bit 5	Bit 4	Bit 3	Bit 2	Bit 1	Bit 0	
Byte 0	MT[7]	MT[6]	MT[5]	MT[4]	MT[3]	MT[2]	MT[1]	MT[0]	MT[0-11] - Macro time, 12 bits
Byte 1	R[3]	R[2]	R[1]	R[0]	MT[11]	MT[10]	MT[9]	MT[8]	R[0-3] - Routing bits
Byte 2	ADC[7]	ADC[6]	ADC[5]	ADC[4]	ADC[3]	ADC[2]	ADC[1]	ADC[0]	ADC[0-12] - ADC value
Byte 3	INVALID	MTOV	GAP	0	ADC[11]	ADC[10]	ADC[9]	ADC[8]	GAP - if =1 Possible FIFO Overflow occurred MTOV - Macrotime counter overflow event INVALID - invalid photon or not a photon
Macro Time Overflow EVENT									
	Bit 7	Bit 6	Bit 5	Bit 4	Bit 3	Bit 2	Bit 1	Bit 0	
Byte 0	CNT[7]	CNT[6]	CNT[5]	CNT[4]	CNT[3]	CNT[2]	CNT[1]	CNT[0]	CNT[0] - CNT[27] - number of MT overflows
Byte 1	CNT[15]	CNT[14]	CNT[13]	CNT[12]	CNT[11]	CNT[10]	CNT[9]	CNT[8]	
Byte 2	CNT[23]	CNT[22]	CNT[21]	CNT[20]	CNT[19]	CNT[18]	CNT[17]	CNT[16]	
Byte 3	1	1	0	0	CNT[27]	CNT[26]	CNT[25]	CNT[24]	
MARKER EVENT									
	Bit 7	Bit 6	Bit 5	Bit 4	Bit 3	Bit 2	Bit 1	Bit 0	
Byte 0	MT[7]	MT[6]	MT[5]	MT[4]	MT[3]	MT[2]	MT[1]	MT[0]	
Byte 1	M[3]	M[2]	M[1]	M[0]	MT[11]	MT[10]	MT[9]	MT[8]	M[0-3] Markers 0 to 3
Byte 2	x	x	x	x	x	x	x	x	
Byte 3	1	MTOV	GAP	1	x	x	x	x	
MARKER EVENT SPC-160									
	Bit 7	Bit 6	Bit 5	Bit 4	Bit 3	Bit 2	Bit 1	Bit 0	
Byte 0	MT[7]	MT[6]	MT[5]	MT[4]	MT[3]	MT[2]	MT[1]	MT[0]	INT[0-12] - Intensity counter
Byte 1	M[3]	M[2]	M[1]	M[0]	MT[11]	MT[10]	MT[9]	MT[8]	
Byte 2	INT[7]	INT[6]	INT[5]	INT[4]	INT[3]	INT[2]	INT[1]	INT[0]	
Byte 3	1	MTOV	GAP	1	INT[11]	INT[10]	INT[9]	INT[8]	

Note, that in SPC cards the 12-bits ADC value are time reversed. Not so in SPC-QC cards.

In SPC-QC cards the marker events are encoded slightly different. See following comparison:

SPC	SPC-QC
MT-Overflow ×40000000	MT-Overflow ×80000000
Frame Marker ×90004000	Frame Marker ×40004000
Line Marker ×90002000	Line Marker ×40002000
Pixel Marker ×90001000	Pixel Marker ×40001000
Invalid Marker ×80000000	GAP ×C0000000

Further information about \*.spc files can be found in the Becker&Hickl SPC\_data\_file\_structure.h

## The \*.set File Format

Deeper understanding of the \*.set format is not required for users of the SPCDynamics application. The information is included here for developers interested in creating their own modifications.

The setup files \*.set consist of:

- 1 - a file header containing structural data which are used to find the other parts of the file
- 2 - the 'file information' which was created when the file was saved
- 3 - the system setup data for hardware and software in an ASCII human readable section and a binary section

SPCDynamics draws only on a small portion of information included in a \*.set file. Therefore it might be possible to “fake” a \*.set file for a certain measurement should the original file be not available. Here is a list of values that must be set correctly for a successful analysis through SPCDynamics:

- The **BHModuleType** as encoded in the binary file header
- The module's **Ser.No.** as mentioned in the ASCII file information section
- The **tac\_range** as mentioned in the ASCII system setup section in the line after #SP [SP\_TAC\_R,F,
- The **tac\_gain** as mentioned in the ASCII system setup section in the line after #SP [SP\_TAC\_G,I,
- The **adc\_resolution** as mentioned in the ASCII system setup section in the line after #SP [SP\_ADC\_RE,I,

You can use Spcm.exe in emulation mode to create a \*.spc file which will contain the correct values for your measurement.

Alternatively you could use the BH-LabVIEW example Vi FileIO\SPC\SPCWriteSETforSPC.vi

When using a text editor to patch the values make sure to use an editor that is capable to save binary files like Notepad++. The classic Notepad does not preserve the binary sections.

Further information about \*.set files can be found in the Becker&Hickl SPC\_data\_file\_structure.h

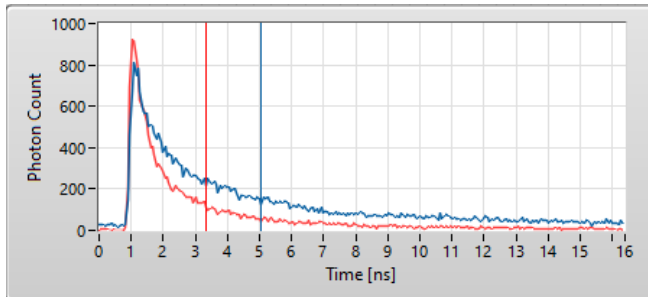
## 1<sup>st</sup> Moment Algorithm

In order to reach the throughput necessary to calculate lifetimes for several thousand time bins in reasonable time some mathematical and computational simplification have been done.

The full analysis of a decay curve would need an exponential curve fit, folded with an instrument response function (IRF). Sometimes even multi-exponential curves are observed. Modern PCs are still not offering this sort of computational power for large data sets.

We at Becker&Hickl understand, that sometimes a quick and possibly not so accurate result is desired, especially when dealing with rapid changes or moving objects.

The examples in the next image you see the red decay curve emitted from a molecule with a shorter lifetime while the blue curve has a longer lifetime (slower decay).



We assume, that the declining part of the curve can be characterized by an exponential decay curve.

$$N(t) = N_0 e^{-t/\tau}$$

Now, it is a characteristic of the exponential decay curve, that its lifetime value tau can be approximated in first order by the centre of mass below the curve. Also called the 1<sup>st</sup> moment.

$$\mathbf{R} = \frac{1}{M} \sum_{i=1}^n m_i \mathbf{r}_i,$$

where  $M = \sum_{i=1}^n m_i$  is the total mass of all of the particles.

Of course, we still need to subtract the start position of the decay curve. SPCDynamics uses the maximum of the curve which can be only an approximation of a real IRF.

Another uncertainty lays in the shape of the curve itself: The 1<sup>st</sup> moment algorithm can't realize or compensate for multi-exponential decays.

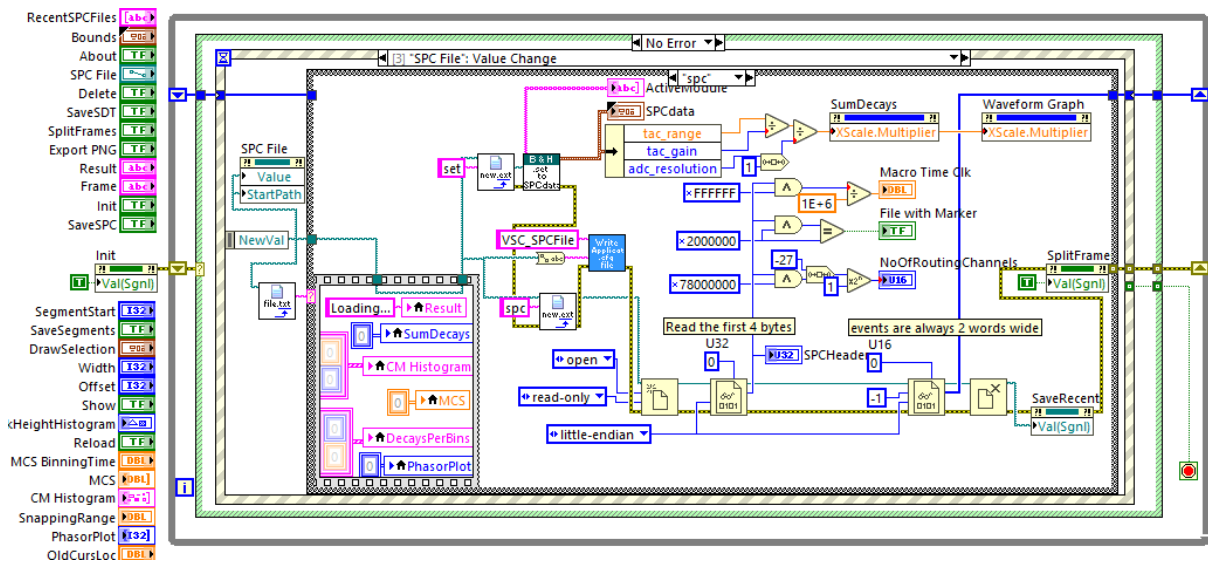
## Remote Controlling SPCDynamics

SPCDynamics offers an interface using the VI-Server so that other LabVIEW applications or code can connect to it. Commands can be sent by writing well defined strings to a control, while the result can be read from another string control.

Please contact [info@becker-hickl.com](mailto:info@becker-hickl.com) to request a list of all available remote commands alongside with handshake requirements.


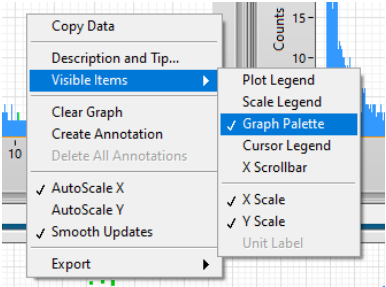
## Source Code Distribution

For developers the complete LabVIEW 2017 source code (Virtual Instruments) of the SPCDynamics application is available for your modification requirements. Please contact [info@becker-hickl.com](mailto:info@becker-hickl.com) for pricing and licenses.





## Troubleshooting

The app's backdrop flashes red	Check the message window for error messages
Error: File Not Found	The mentioned *.set file was not found. There should always be a *.set file with an equal name to the selected *.spc file in the same folder
The message box displays "Creating Time Bins" but nothing is happening	There might be too many time bins to calculate. Try increasing the MCS BinningTime or shorten your overall measurement time
All CM fields display NaN and there are no CM-dots in the MCS graph	There might be not enough photons in all of the time bins. Minimum for a solid evaluation is 100 photons in a bin. Some bins with less than 100 are not an issue
I don't see a regular decay curve in the SumDecays graph	You might have to adjust the measurement parameters in your measurement software
The ns time markers on the PhasorPlot's universal circle are wrong	The LaserPeriode value needs to be manually inserted according to your measurement setup
I don't see any signals in the PhasorPlot	<ul style="list-style-type: none"> <li>• Check whether the SumDecays graph shows regular decay curves. The PhasorPlot algorithm will fail for non-exponential data.</li> <li>• Confirm, that the LaserPeriode is showing the same value as your measurement setup</li> </ul>
I can't move the cursors in the MSC or SumDecay graph	You need to click on the  button in order to obtain the cursor move tool
I'd like to zoom in on a certain feature in the DecaysPerBin graph but there are no zoom tools	<ul style="list-style-type: none"> <li>• You can right click on any graph and select Visible Items&gt;Graph Palette to display the zoom tools</li> <li>• You can click on any of the graph's min or max x- or y-scale's digit and enter a value manually</li> </ul> 
I'd like to hide a plot in the DecaysPerBins graph but there is no visibility checkbox	You can right click on any graph and select Visible Items>Plot Legend to display the Plot visibility checkboxes
I tried everything but my problem persists	Feel free to contact <a href="mailto:info@becker-hickl.com">info@becker-hickl.com</a> with a detailed description of your issue together with a screenshot of SPCDynamics. Consider to email us in addition a copy of your *.spc / *.set pair.