

# ExpressFLIM

Real Time Fluorescence Imaging Software using the Becker&Hickl SPC-QC-104 Card

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*USER GUIDE*

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## Thank you...

... for choosing the quality Becker&Hickl SPC-QC-104 card. This guide will lead you through the installation process of the ExpressFLIM application and some simple operating commands to get even more out of your hardware.

## Use ExpressFLIM if you...

... want to see lifetime images in real time. The colour coding allows you to see any changes in lifetimes of your probe with up to 5 frames per second. May it be because of external stimulation or because the probe has moved.

Up to three single-photon detectors can be simultaneously recorded in three channels.

Don't use ExpressFLIM if you need high precision timing values or you are interested in the multi-exponential decay characteristic of your probe. ExpressFLIM uses a [1<sup>st</sup> moment approach](#).

## Hardware Requirements



You should have a Becker&Hickl SPC-QC-104 inserted in a PCIe slot of Windows 64-Bit computer running at least Windows 10 or later.

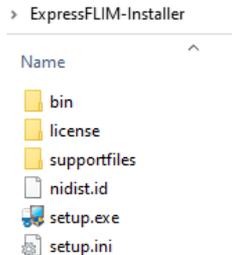
For seamless operation it is recommended to use a Becker&Hickl DCC-100 or DCC-100PCIe or DCU-400 module to supply power to the used single-photon detectors and a Becker&Hickl GVD-120 as scan controller. The system can be operated with a wide range of third-party detectors and scanners but compatibility needs to be verified by the user.

## Software Installation

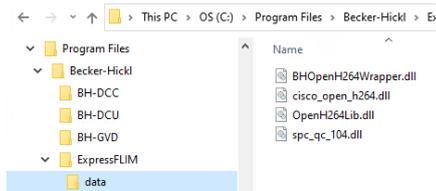
The ExpressFLIM application installer can be downloaded from [www.becker-hickl.com](http://www.becker-hickl.com).

### Run Installer

- Execute the Setup.exe contained in the installation package to run the installer.



- The default installation path is C:\Program Files\Becker-Hickl.
- ExpressFLIM and three support apps will be copied during the installation process to their corresponding folders.
- Four support dll are copied to the ExpressFLIM \data subfolder.



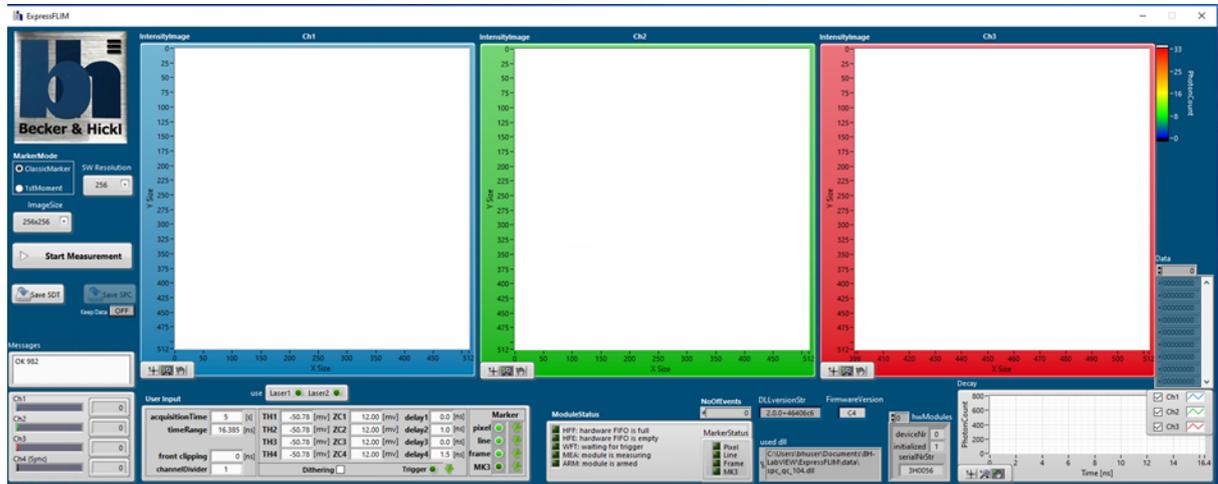
- The required **LabView Runtime Engine 2017 SP1** is also installed. The runtime can be used to operate software written with LabView without needing a LabView license. It can also be downloaded from [www.ni.com](http://www.ni.com). The default destination is C:\Program Files\National Instruments\
- The ExpressFLIM software shortcut is now available on your desktop. Another link can be found in the start menu.



## Using the ExpressFLIM Application

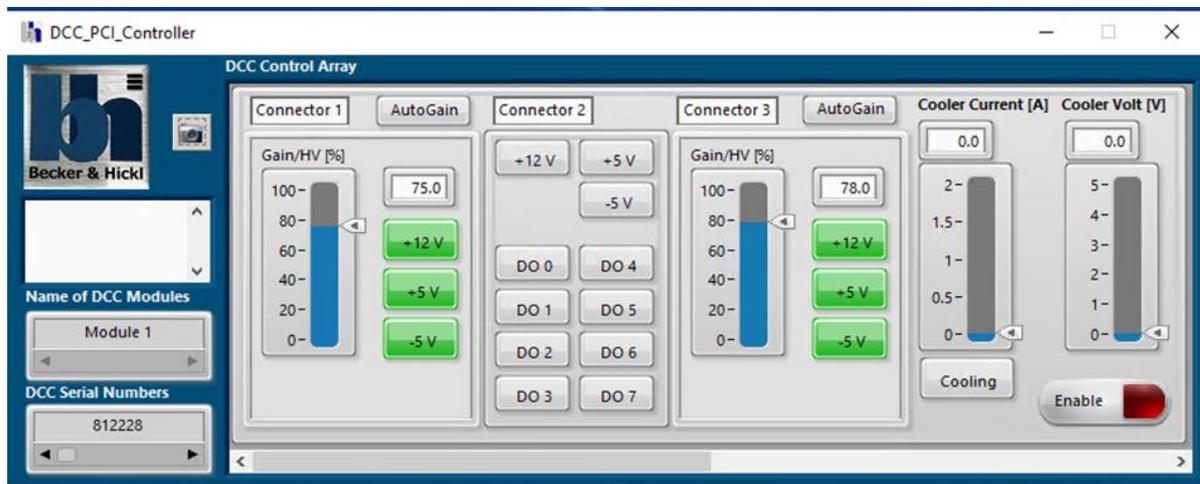
### Starting ExpressFLIM

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

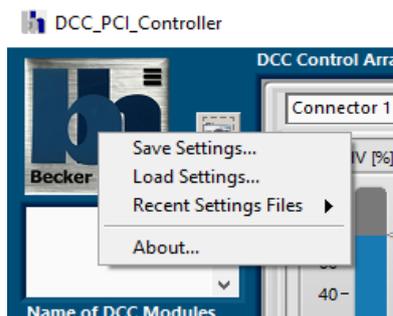


The ExpressFLIM window starts in the default configuration and will automatically start the GVD-control and DCC-control applications.

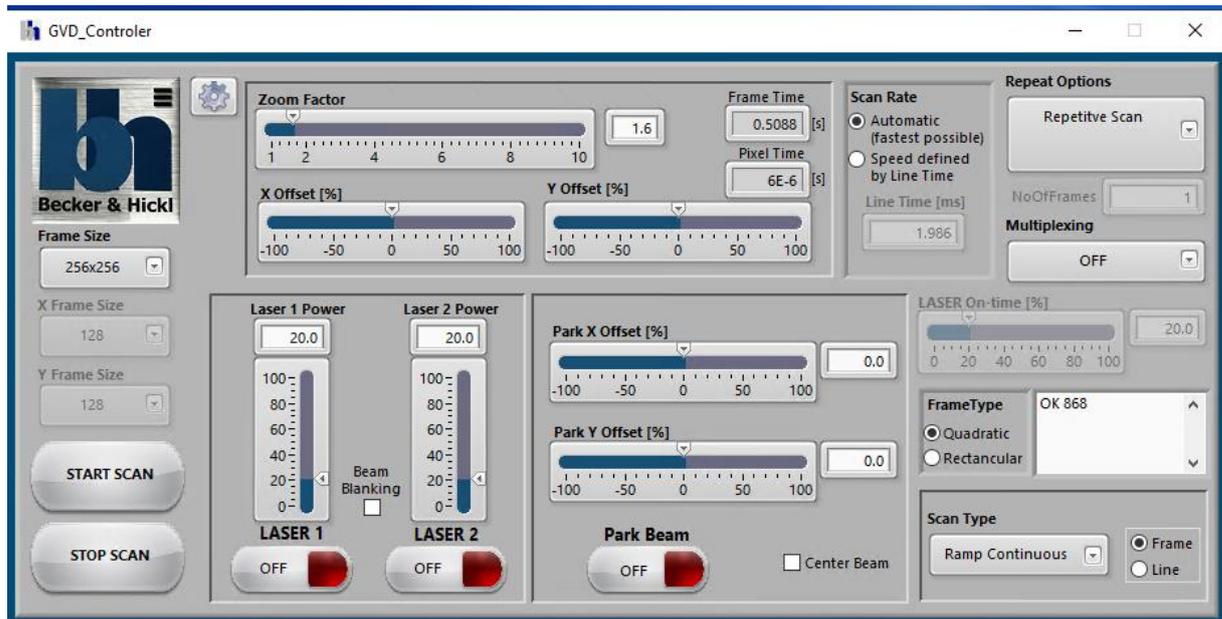
### The DCC-Control App



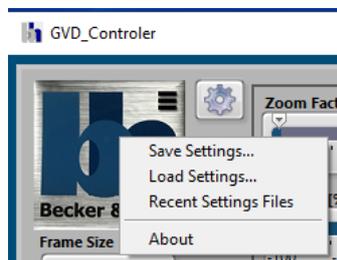
You can save and load settings from files using the menu available by clicking on the B&H logo in the top left corner. With every new start of the DCC-controller app the last loaded settings are brought back into the panel.



## The GVD-Control App

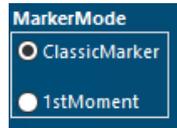


You can save and load settings from .cfg files using the menu available by clicking on the B&H logo in the top left corner. With every new start of the GVD-controller app the last loaded settings are brought back into the panel.



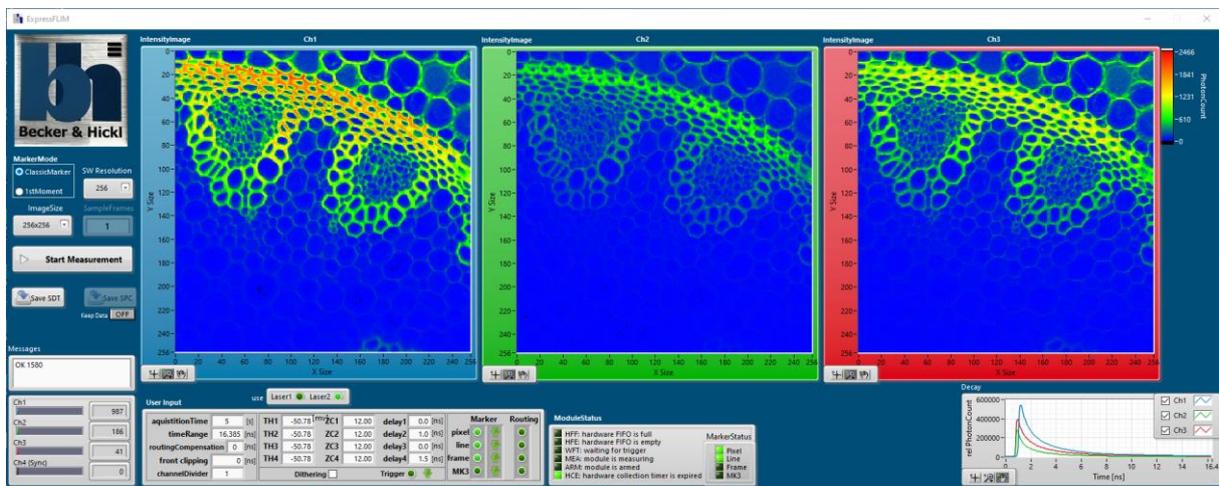
## ExpressFLIM Measurement Modes

ExpressFLIM can run in two different measurement modes with a SPC-QC-104 module.



### Classic Marker Mode

The **Classic Marker** mode as is available in the Becker&Hickl classic Spcm.exe measuring suite. The photon event stream read from the card can be saved to files either as raw data in a **\*.spc** file or as pre-processed decay curves per pixel in a **\*.sdt** file. Both file types are compatible and readable by the Spcm.exe application, or can be exported to SPCImage.exe



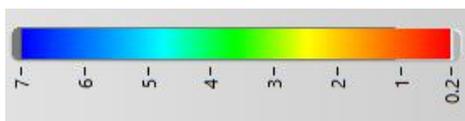
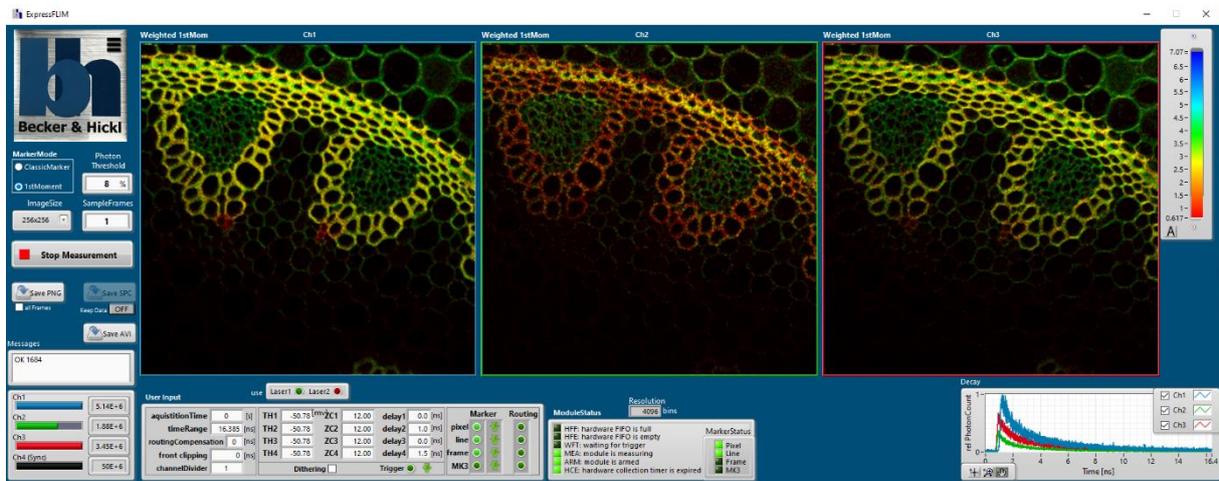
Note: The **Intensity Image** displayed in this mode don't show the lifetimes at the pixel spots, but are a false colour representation of the photon intensity at that spot.

Starting with black or dark blue for low intensity and going over yellow to red for highest intensity.

Use this mode to quickly get an overview of your probe, even with low photon rates per pixel – or if a **\*.sdt** file is required for post-processing in Spcm.exe or the Becker&Hickl advanced FLIM analysis software SPCImage.exe

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

The **1<sup>st</sup> Moment** mode provides the express FLIM images in real time. Here the false colour scale represents average lifetimes in [ns] at the respective pixel.



Starting in red with the shortest lifetimes and going over green to blue for the longest lifetime in the image. Technically the [centre of mass](#) of the decay curves in each pixel is calculated, the rising position on the time scale subtracted and mapped to the colour code bar.

The brightness of the colours is weighted with the local intensity at each pixel.

In this mode you have the options to save the raw data to a [\\*.spc](#) file or save the single images as [\\*.png](#) files or create an [\\*.avi](#) movie.

## The ExpressFLIM Menu

The menu is available by clicking on the B&H logo in the top left corner.



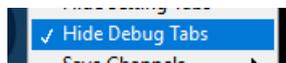
- By selecting **Hide Settings Tabs** some adjustment fields will be hidden in order to provide a “cleaner” look and let you focus on your measurement. Use this mode once your setup is done and you are happy with the measurement results. You can always bring the adjustment fields back by removing the checkmark.



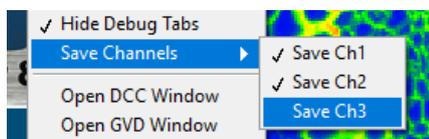
- By selecting **Hide Debug Tabs** some fields that provide information about the internal workings of ExpressFLIM will be hidden in order to provide a “cleaner” look and let you focus on your measurement.

Use this mode once the application has successfully started, photons are counted and you don’t need to provide feedback to the Becker&Hickl support.

You can always bring the debug fields back by removing the checkmark.



- With **Save Channels** you can select which of the three channels will be included in the saving process when creating [\\*.png](#) or [\\*.avi](#) files. The default is to save all three channels, but you can remove the checkmark next to a channel to omit it in order to save hard-drive space.



When saving you will be visually warned about any channel not currently recording.

- By clicking on **Open DCC Window**, the DCC-controller app is brought into view or restarted. Use this when you have accidentally closed the DCC-controller app.
- By clicking on **Open DCU Window**, the DCU-controller app is brought into view or restarted. Use this when you have accidentally closed the DCC-controller app.
- By clicking on **Open GVD Window**, the GVD-controller app is brought into view or restarted. Use this when you have accidentally closed the GVD-controller app.
- You have the option to save a configuration file using menu command **Save Settings**. All measurement parameters, the measurement mode ([MarkerMode](#)) as well as the window screen position will be saved to a [\\*.cfg](#) of your choice.  
Note, that independent of your configuration file the current state upon closing the app will be stored to C:\Users\- From your choice of configuration file, you can load all measurement parameters, the measurement mode ([MarkerMode](#)) as well as the window screen position using the menu command **Load Setting**.  
Note, upon starting the application the last state of the app is restored using the file C:\Users\- The last 10 used configuration files will be displayed under **Recent Settings Files** for you to choose from.
- A previously saved [\\*.spc](#) file can be loaded back into the application and reinterpreted using **Read SPC-File**.
- For easy access to the last saved data file use the menu command **Open Data Folder**, which will open the file location in your default windows browser.
- Clicking the menu command **About** will open a new window displaying the about box with copyright and system information.

## 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 ExpressFLIM Application



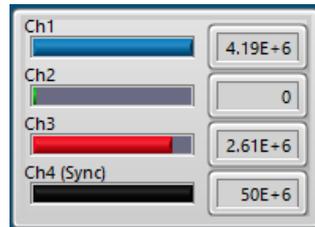
You can terminate the ExpressFLIM application, as you are used to from any other Windows application, by clicking on the red X in the top right corner.

The current setup will be stored to disk and reloaded when you start ExpressFLIM again. All references to the SPC-QC-104 will be closed and used memory freed.

Note, that you won't be asked to save your last measurement. Any data still in memory will be lost.

## ExpressFLIM Outputs

### Photon Count Rates



This window displays the count rate of all photons that have made it each second through the discriminating process ([TH](#) and [ZC](#)).

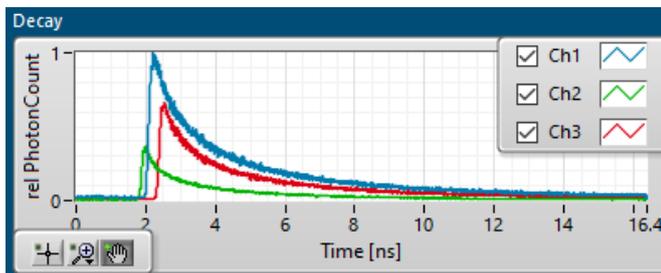
For channels 1 to 3 a count rate higher 1E+3/second is desirable. (In this example channel 2 is obviously not detecting anything.)

Rates much higher than 1E+7 are likely to create problems for the detectors – going into overload shutdown or even damage.

On channel 4 all sync pulses are detected. In this example we are working with a 50MHz pulsed laser. The count rates are not affected by the [Measurement Mode](#).

The most likely adjustment for improper count rates is the laser power or filter and pinhole position. But you should also take timing issues like [aquisitionTime](#) and [delay](#) into account. Even bad or wrong cables can be a root cause.

### Decay



This window displays the average decay curves of the whole image. Therefore, an exponential fit to these curves will not yield a lifetime value for any specific pixel.

Its purpose is solely to provide you with a feedback about the validity of the measurement's timing parameters.

The Time axis runs in nanoseconds the length of the observation time interval, the [timeRange](#).

In [Classic Marker Mode](#) the amplitude of the curves is showing the accumulated photon count during the measurement.

In [1<sup>st</sup> Moment Mode](#) the amplitude is normed to the photon count of the highest channel. Therefore showing the relative photon counts of each channel.

You can use the controls in the lower left corner of the window to zoom in or out of the graphic. By removing the checkmarks  next to the channel's name you can hide the curve in the display.

For a valid measurement it is recommended to position the rising edge of the decay curves near the first 10% of the observation interval. Use the [delay](#) parameter for that.

## Resolution



Only in [1<sup>st</sup> Moment Mode](#) this window describes in how many equidistant time bins the hardware has divided the observation time interval.

The corresponding value in [ClassicMarker Mode](#) is the SW Resolution which is adjustable.

## Messages



This window displays messages from the ExpressFLIM application to you. Sadly, most of the messages will contain errors that will inhibit a successful measurement. Error messages will be accompanied by a red flashing of the application backdrop, to alert you even more about the issue. In this example the underlying .dll was not found at its default place and user action is required before continuing.

A message like “OK” followed by number on the other hand is of no concern and simply declares a successful communication to the DDC-control or GVD-control applications.

## Debug Information

The default style window of ExpressFLIM will display several windows with debug information. Their contents is of low importance during normal operation. They will aid nevertheless in trouble shooting either by you or when contacting the Becker&Hickl support.

You can hide all debug information by checking the option [Menu>Hide Debug Tabs](#)

### used dll

The most likely cause for a non-operational ExpressFLIM application is a missing spc\_qc\_104.dll. This dll is [installed](#) alongside with ExpressFLIM.exe and should reside in a \data subfolder next to it. In a successful installation this path will be displayed here.

### DLLversionStr

displays the version of spc\_qc\_104.dll.

### FirmwareVersion

displays the FW version of your SPC-QC-104 card.

### hwModules

The spc\_qc\_104.dll is capable of handling up to four SPC-QC-104 modules in one PC. All modules will be displayed here with their initialized state and serial number.

Nevertheless, ExpressFLIM can currently handle only one SPC-QC-104 module.

### Data

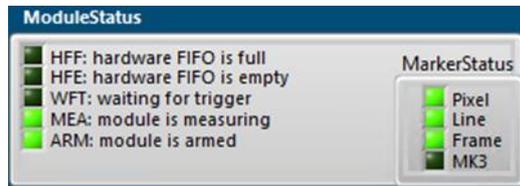
displays the raw event data as received from the SPC-QC-104 module. Each line represents one event in hexadecimal notation. The interpretation of such events requires knowledge beyond the scope of this manual. The event coding is also different in ClassicMarker and 1stMoment modes. These raw data events are stored in [\\*.spc](#) files. Please contact Becker&Hickl if you require detailed information about the \*.spc formats.

Further knowledge about the Data format is not required, since ExpressFLIM will interpret the data and bring it accordingly to display.

### NoOfEvents

displays how many raw [Data](#) events have been transferred from the SPC-QC-104 module to ExpressFLIM. Use this information to confirm a successful communication to the hardware.

### Module Status



This window shows flags describing the current state of the module. During a measurement this might be particularly interesting.

- HFF indicates an overflowing transfer FIFO in the SPC-QC-104. Typically, this appears when data cannot be processed at the incoming pulse rate. Reducing the intensity might mitigate this problem
- HFE indicates the transfer FIFO is empty and no events are being processed. Permanently lit would indicate a problem with the hardware, since even with zero photon rate, at least the marker event would need to be transferred.
- WFT would light up when you have setup a measurement to expect a [Trigger](#), but there has no trigger level change occurred yet.
- MEA indicates a running measurement
- ARM indicates the module is armed for measurement. In this state it is ready to accept an external trigger to actually start measuring.

### Marker Status

These flags indicate which type of marker events have been received since the last transfer.

## Measurement Parameters

### Start Measurement



You can start a measurement any time by pressing this button. The current [measurement parameters](#) will be taken and a photon acquisition will be attempted. Even if the measurement parameters might not be perfectly adjusted to your setup, there is no risk of damage to the SPC-QC-104. Feel free to start a measurement and adjust the parameters during the run to optimize them. Still, be aware of the laser power output. There you run the risk of damaging your detectors or even burning your probe. START WITH LOW LASER POWER.

During a running measurement the button will look like this:



You can press this button any time to interrupt the measurement.

When using an [acquisitionTime](#) larger than zero the measurement will automatically stop after the selected number of seconds. You can start the next measurement immediately by pressing the **Start Measurement** button again.

### Effect on DCC-Control

When starting a measurement with ExpressFLIM the DCC-control outputs are automatically enabled

and the control button changes from  to .

You should make sure, that the Gain/HV values are set to the recommended levels for your photodetectors.

Automatic disabling of outputs when stopping a measurement is currently not implemented since the delay in enabling will falsify the beginning of a new measurement.

You can minimize the DCC window if desired. It doesn't have any influence on its operation.

### Effect on GVD-Control

Similar to the DCC-Control outputs, with **Start Measurement** the GVD-controller will automatically turn the selected lasers on with their appointed power and start the scanner.



Therefore, a careful adjustment of scanning parameters and laser powers is recommended.

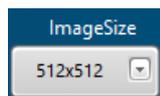
When ExpressFLIM has stopped a measurement, the **Stop Scan** and **Laser OFF** commands are automatically send to the GVD to prevent damage to the probe or detectors.

You can minimize the GVD window if desired. It doesn't have any influence on its operation.

### Using 3<sup>rd</sup> Party Controllers

ExpressFLIM can't communicate with 3<sup>rd</sup> party controllers, but will still operate as desired when scanning, sync und photon signals are presented.

## Image Size

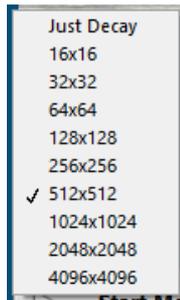


You can select the image size from a variety of choices. Your selection will be automatically relayed to the GVD-controller preparing the scanner for the next measurement.

Note, that only square choices are currently support by ExpressFLIM.

The default size is 512x512 pixels which represents the window sizes of the three channel displays in 1:1 scaling.

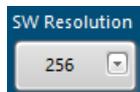
Smaller image sizes will give you the option of higher frame rates or sampling, with the downside of higher granulation. The images will be scaled up to fit the channel displays.



Larger sizes on the other hand will reduce the frame rate and might be limited by your PC's capabilities. The images will be scaled down to fit the channel displays, giving you no higher resolution than the 512x512. [\\*.png](#) and [\\*.avi](#) files will be stored with the higher resolution nevertheless.

**Just Decay** will stop the scanner and take measurements form one spot only. Resulting in no image.

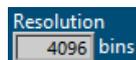
## SW Resolution



Only in [ClassicMarker Mode](#) can you select in which timing resolution you want to save your image data. It describes in how many time bins you want to divide the observation time interval. Visualizing the **Intensity Images** is independent from this value. Only the amount of details stored in [\\*.sdt](#) files is adjusted.

The maximum resolution is 4096, but this will most likely exceed the PC's memory capacity and memory allocation might slow down processing speed.

The corresponding value in [1<sup>st</sup> Moment Mode](#) is the Resolution in bins, but this is given by the hardware and not adjustable.



## Laser Usage



In ExpressFLIM you can select which of the two controllable lasers you want to use for the next measurement. The selected laser will be marked in bright green. During measurement the indicator will turn red and relay the information to the GVD-controller turning the laser on.

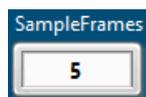
## Photon Threshold



Low photon rates in a pixel will distort the lifetime calculation since the decay curve can't be properly determined. To reduce that type of "noise" you can adjust the photon threshold to accept only pixels with higher photon rates for lifetime calculation and tint them in the according colour.

Pixel that fall below the threshold will be displayed in grayscale representing only their relative photon count.

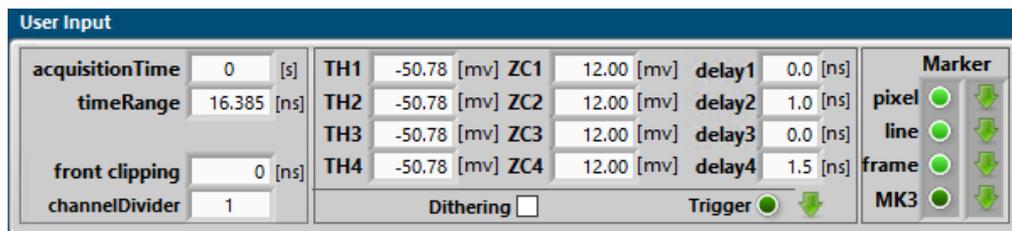
## Sample Frames



In low photon rate condition, you might want to sample more than one frame before calculating the lifetime. This will reduce the measurement jitter significantly, but also cause an image delay and for moving probes a movement blur.

The algorithm provides a moving average calculation using for every new frame a sampling with the selected number of previous frames.

## User Input



User Input				Marker							
acquisitionTime	0 [s]	TH1	-50.78 [mv]	ZC1	12.00 [mv]	delay1	0.0 [ns]	pixel	<input checked="" type="checkbox"/>	↓	
timeRange	16.385 [ns]	TH2	-50.78 [mv]	ZC2	12.00 [mv]	delay2	1.0 [ns]	line	<input checked="" type="checkbox"/>	↓	
front clipping	0 [ns]	TH3	-50.78 [mv]	ZC3	12.00 [mv]	delay3	0.0 [ns]	frame	<input checked="" type="checkbox"/>	↓	
channelDivider	1	TH4	-50.78 [mv]	ZC4	12.00 [mv]	delay4	1.5 [ns]	MK3	<input checked="" type="checkbox"/>	↓	
				Dithering <input type="checkbox"/>				Trigger <input checked="" type="checkbox"/> ↓			

Several parameters control how the SPC-QC-104 records photons. Not all of them need adjusting for a successful ExpressFLIM measurement. Your input will be checked by the SPC-QC-104 firmware and possibly rounded to the next possible values.

### Acquisition Time

A started measurement might run unlimited until **Stop Measurement** is pressed, or until a selected number of seconds has past.

You might use a limited time especially in [ClassicMarker Mode](#) to avoid accumulating data in the channel's **Intensity Images** and photon count in the [Decay](#) window.

In [1stMoment Mode](#) you might like to use infinite measurement by entering "0" in the acquisitionTime field to observe lifetime changes in real time.

### Time Range

Time Range is the length of the observation time interval after a sync pulse. Photon pulses are only registered if the time difference to the last Sync lies within Fehler! Verweisquelle konnte nicht gefunden werden..

You will find the selected value as maximum value of the [Decay](#) window.

Note, that not all values are acceptable by the hardware, so ExpressFLIM is rounding your input to the nearest possible value.

Note also, that very long recording time intervals may require lower laser repetition to avoid double exposure.

### Front Clipping

Front clipping sets the minimum time delay between Sync and photon signal, for a pulse to be processed in the SPC-QC-104. Photon pulses with shorter time difference to the Sync will be discarded.

This can be helpful to remove unwanted artefacts at the beginning of the decay curve. Check in the [Decay](#) window for that.

### Channel Divider

With channel divider ratios larger than one, recording will go over multiple periods of the Sync signal.

## Dithering

Dithering is a mechanism to reduce the Differential Non-Linearity (DNL) of the TDC module inside the SPC-QC-104. It should always be enabled unless pulse rates per channel exceed 90 MHz. Above that rate, dithering can lead to undesired artefacts.

## Trigger

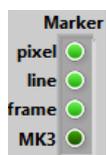
Press the Trigger field to turn it bright green informing the SPC-QC-104 to wait with a measurement start until the trigger input on its micro Sub-D connector is switched. In dark green condition the measurement will commence immediate with pressing the [Start Measurement](#) button.

## Trigger Polarity



You can select whether to start a measurement on the rising or falling edge of the trigger signal on the micro Sub-D connector.

## Marker



The photon event raw data stream received from the SP-QC-104 card includes markers as signalled to the micro Sub-D connector from the scanning system.

ExpressFLIM expects marker0 to be included at the beginning of each **pixel**. Marker1 will signal a new **line** and marker2 the beginning of a new **frame**. Therefore, a disabling of these markers is not possible in ExpressFLIM.

Marker3 (**MK3**) is typically not used with ExpressFLIM.

## Marker Polarity

Check with your scanner whether a rising or falling edge will signal the beginning of a [Marker](#).

### TH1-4, ZC1-4, delay1-4

Technically the SPC-QC-104 comprises of four identical time tagging channels.

The input signals from the detectors and the laser are received by four identical constant-fraction discriminators, CFD1 through CFD4. The CFDs provide defined trigger pulses, the temporal position of which is independent of the amplitude of the input pulses.

For principle of the CFDs and parameter setup please see the bh TCSPC Handbook, section 'Constant-Fraction Discriminators' and 'System Optimisation'.

For ExpressFLIM applications channel 4 is expected to always be connected to a synchronizing signal (typically the laser trigger). Channel 1 to 3 are the channels used for imaging. Ensure, that your hardware is cabled that way.

Still, all channels share the same type of parameters for [Threshold](#), [ZeroCross](#) and [Delay](#).

#### Threshold, Zero Cross Level

Your detectors should have been delivered with practical values for **TH** and **ZC** in mV. You can insert them into the corresponding fields.

For third-party detectors these parameters may have to be determined through extra tests.

If in doubt you can start off with the default values and adjust them according to your signal quality and the [Count Rates](#) window.

As a last resort contact the support at Becker&Hickl.

#### Delay

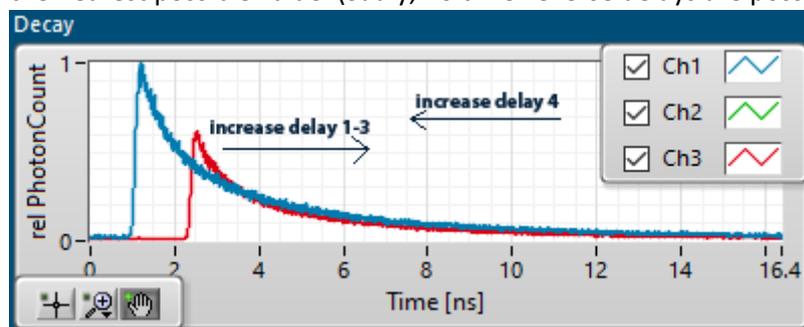
The cable length between detectors and sync source on one side and the SPC-QC-104 card on the other will influence the timing in the observation interval.

It is recommended to position the rising edge of the decay curves near the first 10% of the observation interval. Check the [Decay](#) window for that. This way any pre-exaltation effects can be observed (and eliminated) while a fair amount of visible decay will produce reliable results.

You can use the **delay** fields to add an electronic delay. The delay can be adjusted in steps of 0.5 ns with values up to 129 ns for each channel. For channels 1 through 3 an increase of the delay will result in a right shift (to larger times) of the corresponding decay curve.

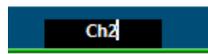
Since channel 4 is reserved as sync signal an increase of the delay value will result in a left shift (to shorter times) of all channels.

Note, that not all values are acceptable by the hardware, so ExpressFLIM is rounding your input to the nearest possible value. (Sadly, no time-reverse delays are possible!)



It is not necessary to have all channels rising at exactly the same time. ExpressFLIM will automatically detect the rising edge of each channel and take that position into account when calculating the [1<sup>st</sup> moment lifetimes](#). The delay is mostly used to make sure all relevant data is in the selected **Fehler! V erweisquelle konnte nicht gefunden werden**. and undesired artefacts are excluded.

## Channel Name



Centered above the image windows, **IntensityImage** or **Weighted 1stMom**, you can click on the channel names and edit them. The default values are Ch1, Ch2 and Ch3. It might be useful for you to name them according to your measurement requirements. The legends will be updated in the [Photon Count Rates](#) window and the [Decay](#) window. The names will also be used when saving [\\*.png](#) and [\\*.avi](#) files.

## Lifetime Autoscaling



In **1stMoment Mode** the colour code bar on the right offers an autoscaling button.

While the colour bar itself never changes – it always runs from red (shortest lifetime) to blue (longest lifetime) – the scaling can be adjusted.

With autoscaling selected, for each frame all pixels in all channels are searched for the shortest and longest lifetimes. These time values will be inserted at the bottom and top of the scale.

You will see quite a fluctuation of values and colour tinting of the images during your measurement. This is because of uncertainties in the [1<sup>st</sup> moment algorithm](#) and because of noise when using a small number of [sampled frames](#) and [photon threshold](#).

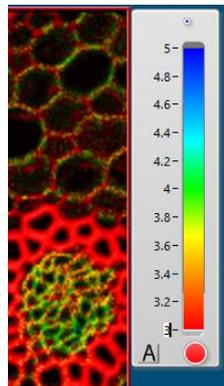
You also won't be able to see lifetime changes that will occur over the whole image, because the short and long values are constantly adjusted.

Nevertheless, this mode comes in handy to determine initial values for short and long lifetime values to be expected in your probe/image. When setting up a measurement autoscaling should be your starting point.

With all measurement parameters set to satisfaction it is recommended to deselect autoscaling. Now your images keep their colour tinting and the scale values are static.

In this mode you will be able to see changes in lifetime of sections in your probe or even the whole image.

But be aware, that certain pixels might be exceeding the shortest or longest lifetime and therefore tinted in the shortest red or longest blue colour.



To warn you of this clipping condition, below and above the colour bar are a red and blue dot that will increase in size in relation to the number of pixels not covered by the scale.

With autoscaling disabled you can click on the lowest and highest scale number and edit its value manually. Your entry will remain static until you change it again or select lifetime autoscaling. The image will be colour tinted according to your entry.

Use this option if you want to zoom in on a certain lifetime range.

## Saving Data

Depending on the [Measurement Mode](#) different file formats are available.

### Save to \*.sdt

In [ClassicMarker Mode](#) you can create a \*.sdt file readable by Spcm.exe and SPCImage.exe. It contains the decay curves of every pixel in the image and can be used in our Becker&Hickl advanced FLIM analysis software SPCImage for detailed and precise lifetime calculation.

Note, that the file size and decay curve detail depends on the setting for [ImageSize](#) and [SW Resolution](#) and might exceed your PC's capabilities.

After a successful measurement press the  button and a file select box will open to ask you for a saving location and file name.

All three channels are written to the file.

A new [Start Measurement](#) will clear the decay curves from memory.

### Save to \*.png

In [1<sup>st</sup> Moment Mode](#) you can create \*.png files readable by almost all image capable applications.

Without the checkmark in  pressing the  button will open a file select box to ask you for a saving location and file name for the currently visible images in the **Weighted 1stMom** windows. The [channel name](#) is automatically appended to each file.

When placing a checkmark in the **all Frames** box you will be asked for a saving location and base file name for all following images and the **Save PNG** button will be armed.

With every fully recorded frame a new file will be created in the same folder with the base name, [channel name](#) and an incrementing number suffix.

Press the armed Save PNG button to stop recording.

Note, that only channels selected in the [Menu>Save Channels](#) will be saved to files.

### Save to \*.avi

In [1<sup>st</sup> Moment Mode](#) you can create \*.avi video files displayable by a wide range of video capable application. The used codec is H264. As video frame rate the **Frame Time** from the GVD controller is used.

When pressing the  button a file select box will open to ask you for a saving location and file name. Each channel will be saved to a separate file appended with the [channel name](#).

With every fully recorded FLIM frame a new image will be added to the \*.avi files.

The Save AVI button will display as . Press this to stop recording.

**Stop Measurement** will also stop recording the \*.avi and finalize it to disk.

You can start and stop recording anytime during, before or after [Start Measurement](#).

Note, that only channels selected in the [Menu>Save Channels](#) will be saved to files.

### Save to \*.spc

In both **Marker Modes** you can save the photon event raw [data](#) as received from the SPC-QC-104 to a \*.spc file useful for further analysis in post-processing.

In [ClassicMarker Mode](#) this comprises of single events for every received photon and events created by markers. These files are readable by Spcm.exe

In [1stMoment Mode](#) the raw data includes information about the 1<sup>st</sup> moment and photon count in each pixel alongside the events created by markers. The format of this stream is currently not compatible with the SPCM software.

\*.spc files include data from all four channels.

Note, that \*.spc files can become quite large even if you don't see any photons. This is because even than the marker events will pile up.



The **Save SPC** button is disabled by default, until you change the **Keep Data** option from **OFF** to **ON**. This is to preserve memory space, since usually the raw data is processed during measurement and not further needed for displaying purposes. It is recommended to turn Keep Data ON only if you need explicitly to save the raw data.

After a successful measurement with the Keep Data option to ON press the Save SPC button and a file select box will open to ask you for a saving location and file name. All the raw [data](#) will be saved to file.

A new [Start Measurement](#) will clear the raw data from memory.

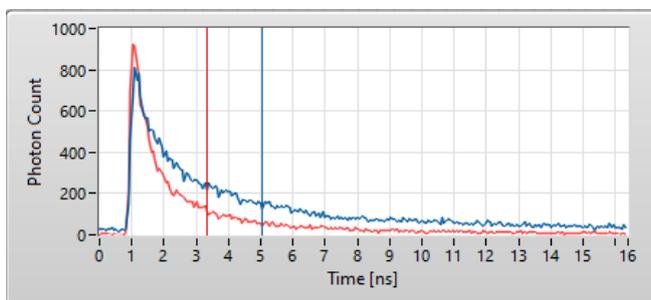
## 1<sup>st</sup> Moment Algorithm

In order to reach the throughput necessary to create lifetime imaging in real 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 real time analysis. For post-processing on the other hand the Becker&Hickl **SPCImage** application is an advanced tool, that offers that sort of calculations.

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.

In our single photon counting cards like the SPC-QC-104, decay curves are recorded. See the examples in the next image. You see the red decay curve emitted from a pixel 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.

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

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

We call this the 1<sup>st</sup> moment and ExpressFLIM is based on this 1<sup>st</sup> moment approach to lifetime analysis.

Of course we still need to subtract the start position of the decay curve. ExpressFLIM 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 ExpressFLIM

ExpressFLIM 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.

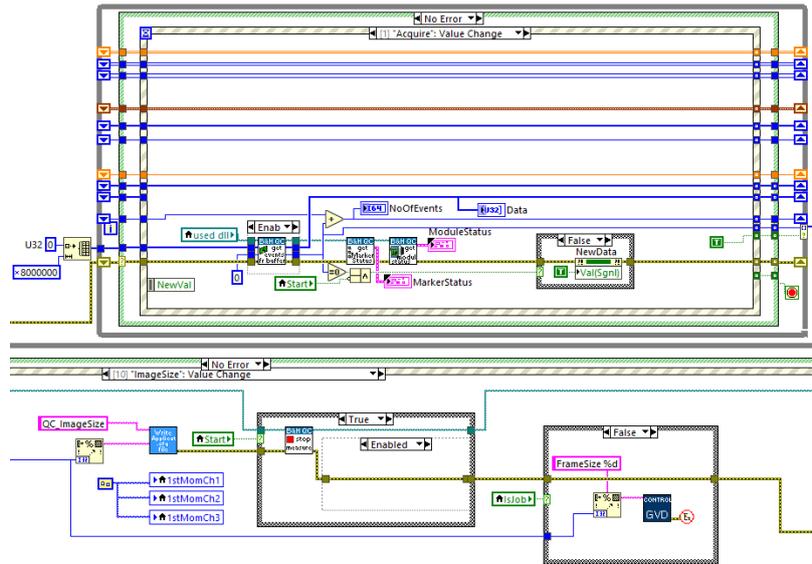
Similar interfaces are in place for the [DCC-Control](#) and [GVD-Control](#).

## Source Code Distribution

For developers a simple LabVIEW 2017 example is available, showing how to initialize and use the SPC-QC-104 module using the spc-qc-104.dll

This code is available for free from Becker&Hickl.

The complete LabVIEW 2017 source code (Virtual Instruments) of the ExpressFLIM 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 <a href="#">Message</a> window for error messages
The Message displays: Error: hardware driver could not be initialized	<ul style="list-style-type: none"> <li>• There is no SPC-QC-104 module inserted in the PC</li> <li>• Has the ExpressFLIM installer successfully copied all files to your disk?</li> </ul>
Error: File Not Found Call Library Function Node in QC_init.vi- >ExpressFLIM.vi C:\..\spsc_qc_104.dll-	The mentioned dll was not found at its default location
Error: Please choose different Names for each Channel	You have named <a href="#">Channel Names</a> equally. The default names have been already inserted
Error: You should have selected at least one laser in the "use laser" field for successful measurement	In the <a href="#">use laser</a> field no laser has been selected. No laser ON command can be send automatically to the GVD
The <a href="#">Photon Count Rates</a> window shows no Sync signal	<ul style="list-style-type: none"> <li>• The trigger signal from the laser is not connected to the QC-SPC-104</li> <li>• The laser is not turned on in the GVD controller</li> </ul>
The <a href="#">Photon Count Rates</a> window shows no signal for certain channels	<ul style="list-style-type: none"> <li>• Check the DCC-controller: It might have gone into overload shut-down – press the Reset button and continue with lower Gain</li> <li>• The laser power might be too low or the beam path blocked</li> <li>• Check the <a href="#">User Input</a> fields for feasible values</li> </ul>
I can't see the peak of the decay curve in the <a href="#">Decay</a> window	Use the <a href="#">delay</a> values to shift the curves in the window
I started a <a href="#">measurement</a> , but the images are not updating	<ul style="list-style-type: none"> <li>• Have you selected <a href="#">Trigger</a>, but no TTL level is reaching the SPC-QC-104?</li> <li>• Are <a href="#">pixel, line and frame markers</a> enabled</li> </ul>
The images seem skewed	Check <a href="#">Trigger Polarity</a> and <a href="#">Marker Polarity</a>
The 1stMoment images are mainly in grey-scale	Too low photon count rate doesn't allow for <a href="#">1stMoment calculation</a> . Try raising laser power, DCC gain, <a href="#">Sample Frames</a> or lowering <a href="#">Photon Threshold</a>
The 1stMoment images are too red / blue	Try setting the Lifetime Colour Bar scale to lower / higher values or select <a href="#">Lifetime Autoscaling</a>
I started the app, but most <a href="#">User Input</a> values are zero or otherwise not feasible	The default configuration file is corrupt. If you don't have <a href="#">saved any settings</a> to file that you are able to reload, try closing the app and deleting the default configuration file at C:\Users\ <user&gt;\appdata\local\bh\expressflim.cfg </user&gt;\appdata\local\bh\expressflim.cfg  On restart the default values will be inserted

I can't <a href="#">save *.avi</a> videos	Are the avi dlls in the <a href="#">data folder</a> ?
I want to save a <a href="#">*.png</a> / <a href="#">*.avi</a> but the image displays "not recording"	Use the <a href="#">Menu&gt;Save Channels</a> to enable saving for this channel
I checked, but the <a href="#">*.avi</a> won't play in my player	<ul style="list-style-type: none"><li>• Is there a H264 codec installed on your PC</li><li>• The *.avi file is finalized only after Stop Rec or <a href="#">Stop Measurement</a> is pressed</li></ul>
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 ExpressFLIM with <a href="#">Menu&gt;Hide Debug Tabs</a> unchecked

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