TSC SP8 FALCON File Format Description

LAS X Version 3.5.0

1. LAS X File Formats

The data from FALCON acquisitions are stored in files with *Leica Image File Format (*.lif)* by default.

A user can decide to store the data in *Extended Leica File Format (*.xlef*) by selecting the corresponding *Save as type* in the file save dialog. When a user enables the *Auto-Save* option in LAS X the data are stored automatically during acquisition or manipulation in *Extended Leica File Format*.

A *Project* in the LAS X project tree can contain multiple *Nodes*. *Nodes* can be an *Image*, a *FCS data set* or a *Collection* of *Nodes*.

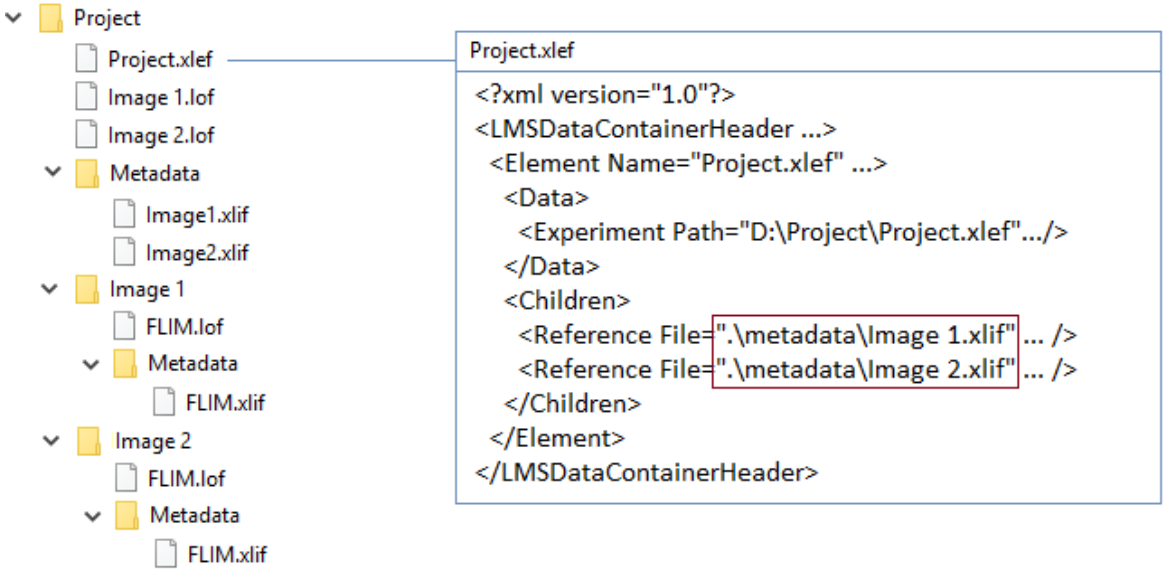
1.1. Leica Image File Format

In *Leica Image File Format* all *Nodes* of a *Project* are stored in one single lif-file. A lif-file is a binary file with multiple *Blocks*.

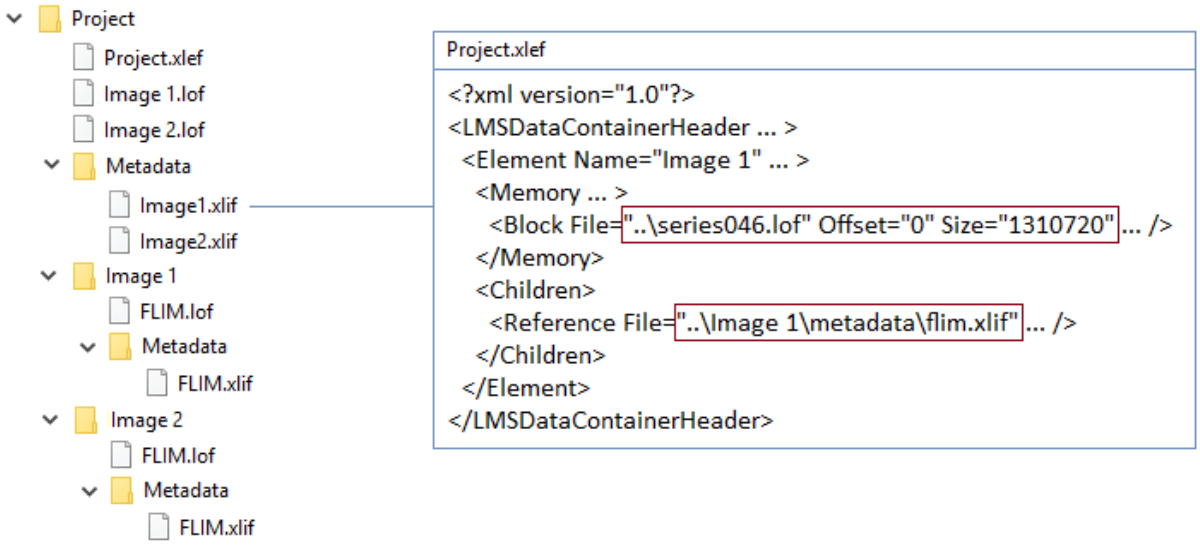
1.2. Extended Leica File Format

In *Extended Leica File Format* the *Nodes* of a *Project* are stored in multiple files. The *Project* hierarchy is projected to directories in the file system.

The *Extended Leica File* (*.xlef) with UTF-8 XML content contains the references to the metadata files of the child *Nodes*.



Metadata are stored as UTF-8 XML content in *Extended Leica Image Files* (*.xlif). The file contains a reference to the binary files and to the metadata files of the child nodes.



Binary data are stored in *Leica Object Files* (*.lof). In addition, the *Leica Object Files* contain the corresponding metadata for the *Node*.

1.3. Block Structure in *Leica Image Files* and *Leica Object Files*

Leica Image Files (*.lif) and *Leica Object Files* (*.lof) are binary files with successive *Blocks*.

Each *Block* starts with a *Common Block Header* followed by a *Metadata Block Header* or a *Binary Block Header.*

A *Leica Image File* always starts with a *Metadata Block* followed by multiple *LIF Binary Blocks.*

Leica Image File:	*Metadata Block*	*LIF Binary Block*	*LIF Binary Block*	…	*LIF Binary Block*

A *Leica Object File* starts with a *Binary Block* followed by a single *Metadata Block.*

Leica Object File:	*LOF Binary Block*	*Metadata Block*

1.3.1. Meta Data Block

A *Metadata Block* starts with a *Common Block Header* followed by a *Metadata Block Header* followed by the metadata in XML UTF-16 format.

Common Block Header	*Metadata Block Header*	*Metadata*

1.3.2. LIF Binary Block

A *LIF Binary Block* starts with a *Common Block Header* followed by a *LIF Binary Block Header*. Then follow the Binary data.

Common Block Header	*LIF Binary Block Header*	*Binary Data*

1.3.3. LOF Binary Block

A *LIF Binary Block* starts with a *Common Block Header* followed by a *LOF Binary Block Header*. Then follow the Binary data.

Common Block Header	*LOF Binary Block Header*	*Binary Data*

1.3.4. Block Headers

All binary values in the headers are stored in little endian byte order.

Common Block Header

Field	Format	Description
Identifier	32-bit integer	always 70 hex
Common Block Header size	32-bit integer	Number of bytes in the *Metadata Block Header* or *Binary Block Header* that follows

Metadata Block Header

Field	Format	Description
Identifier	8-bit integer	always 2A hex
Number Characters	32-bit integer	Number of UTF-16 characters of *Metadata* that follow

LIF Binary Block Header

Field	Format	Description
Identifier 1	8-bit integer	always 2A hex
Binary Data Size	64-bit integer	Number of bytes in the *Binary Data* section
Identifier 2	8-bit integer	always 2A hex
Binary Data Identifier Size	32-bit integer	Number of UTF-16 characters in the *Binary Data Identifier* field
Binary Data Identifier	UTF-16 characters	A character string that identifies the *Binary Data*.

LOF Binary Block Header

Field	Format	Description
Identifier 1	8-bit integer	always 2A hex
Binary Data Identifier Size	32-bit integer	Number of UTF-16 characters in the *Binary Data Identifier* field
Binary Data Identifier	UTF-16 characters	A character string that identifies the *Binary Data*.
Identifier 2	8-bit integer	always 2A hex
Format version major	32-bit integer	Major part of the binary data format version
Identifier 3	8-bit integer	always 2A hex
Format version minor	32-bit integer	Minor part of the binary data format version
Identifier 4	8-bit integer	always 2A hex
Binary Data Size	64-bit integer	Number of bytes in the *Binary Data* section

1.4. *Binary Data* in *Leica Image Files*

In *Leica Image Files* the *Metadata* section contains text identifiers for the binary data in the *MemoryBlockID* attributes of the *Memory* XML elements.

```
<LMSDataContainerHeader Version="2">
  <Element Name="Project" ...>
    ...
    <Children>
      <Element Name="Image 1" ...>
        <Data ...>
          ...
        </Data>
        <Memory Size="1310720" MemoryBlockID="MemBlock_1"/>
        <Children>
          <Element Name="FLIM" ...>
            <Data>
              <SingleMoleculeDetection ...>
                ...
              </SingleMoleculeDetection>
            </Data>
            <Memory Size="60407558" MemoryBlockID="MemBlock_2"/>
          </Element>
        </Children>
      </Element>
    </Children>
  </Element>
</LMSDataContainerHeader>
```

The *Binary Block* with a *Binary Data Identifier* with this character string contains the binary data for the *Node*.

1.5. FLIM and FCS data in *Leica Files*

The metadata are stored in XML format in the sub-element *Data/SingleMoleculeDetection* of *Element*. For FLIM data the *Element* node is a child of the *Element* node of the corresponding image. For FCS data the *Element* node is a child of the parent collection *Element* node. The *Memory* XML sub-element of *Element* contains the reference to the raw data.

```
<LMSDataContainerHeader Version="2">
  <Element Name="Project" ...>
    ...
    <Children>
      <Element Name="Image 1" ...>
        <Data ...>
          ...
        </Data>
        <Memory Size="1310720" MemoryBlockID="MemBlock_1"/>
        <Children>
          <Element Name="FLIM" ...>
            <Data>
              <SingleMoleculeDetection ...>
                ...
              </SingleMoleculeDetection>
            </Data>
            <Memory Size="60407558" MemoryBlockID="MemBlock_2"/>
          </Element>
        </Children>
      </Element>
    </Children>
  </Element>
</LMSDataContainerHeader>
```

2. FLIM and FCS metadata

Attributes of the *SingleMoleculeDetection* XML element

Field	Description
IsImage	Text *true* for FLIM and text *false* for FCS
IsAnalysisResult	Text *true* if the node has been created to store the results of an analysis. If the value is *true* there is no raw data block present for this node.

Values in the *SingleMoleculeDetection/Dataset/RawData* XML element

Name	Description
Format	A text that identifies the raw data format. "LMSRAW" identifies data acquired with TCS SP8 FALCON. Other values are used for imported data.
VoxelSizeX VoxelSizeY VoxelSizeZ	The image stack voxel size in meter.
ClockPeriod	The FLIM base clock in seconds.
SyncronizationMarkerPeriod	The clock period of the FCS counter in seconds.
FrameRepetitionsMarked	If *true* the data have been acquired where frames are completed when a specified number of photons have been detected in the brightest image pixel. In this case the number of frame repetitions is variable. The line start- and end markers contain a flag B (see 3.1. FLIM Raw Data Format). This bit is inverted after the frame repetitions completed.
PixelTime	The pixel dwell time in seconds
BiDirectional	The value *true* indicates that bi-directional scan was used. The pixels in the backward line have reverse order.
SequentialMode	The identifier of the sequential scan mode: *Simultaneous* - non-sequential scan *SequentialLine* - sequential line by line *SequentialFrame* - sequential frame by frame *SequentialStack* - sequential stack by stack
Dimensions	The list of image dimensions that have been used to acquire the data.

Values in the *SingleMoleculeDetection/Dataset/RawData/Dimensions/Dimension* XML element

Name	Description
DimensionIdentifier	The identifier of the dimension: X - x Y - y Z - z (stack) C - channels T - time M - rotation in a SPIM experiment (not used for FLIM/FCS data) WlEm - emission in a spectral-scan

	WlEx - excitation in a spectral-scan S - tiles in a mosaic scan or stage positions is a mark&find scan L - loops in a live data mode experiment.
Size	The number of pixels for the dimension

Values in the *SingleMoleculeDetection/Dataset/RawData/Channels/Channel* XML element

Name	Description
Color	A 32-bit unsigned integer value with the color of the channel. The lower 8 bits contain the red value. Then follow 8 bits green and 8 bits blue value. The highest 8 bits contain the alpha value.
Name	A text with the name of the channel
Detectors	A list with references to the detectors that have been used to generate the data for the channel. The assignment of detectors to channels can be changed by the LAS X FLIM/FCS software after acquisition.
Sequence	A list which specifies, how the raw data have been acquired. For sequential scan there is one element *SequenceItem* per sequential scan setting. For non-sequential scan there is only one element *SequenceItem.*

Values in the *SingleMoleculeDetection/Dataset/Sequence/SequenceItem* XML element

Name	Description
FrameRepetitions	The number of frame repetitions used to acquire the raw data.
LineRepetitions	The number of line repetitions used to acquire the raw data.
Detectors	A list with the properties of the detectors which have been used to acquire the raw data.

Values in the *SingleMoleculeDetection/Dataset/Sequence/SequenceItem/Detectors/Detector* XML element

Name	Description
DataType	An identifier for the type of raw data stored in the binary block. *Unknown* – Imported data *RisingEdge* – Timestamps have been generated for the rising edge of the photon pulse only. *PulseVersion1* *PulseVersion2* – Timestamps have been generated for rising and falling edge of the photon. See section *4. FLIM Pulse Detection* for details.
LaserPulseFrequency	The laser repetition rate in Hz
DeadTime	The detector dead time in seconds
Name	The name of the detector

3. FLIM and FCS Raw Data

3.1. FLIM Raw Data Format

TCS SP8 FALCON FLIM raw data consist of a series of big endian 16 bit *records*.

On computers with Intel CPU the lower and upper bytes of the *records* have to be exchanged to get 16 bit CPU words.

A *line* is a series of *records*. The whole raw data block contains a series of lines.

Line 0	Line 1	Line 2	…	Line n

In case of line repetitions or sequential scan multiple *lines* represent an image line.

The correct combination of the data from several *lines* to image lines has to consider the metadata information and as described in section *3.2. Assignment of FLIM raw data to image pixels.*

A line starts with two start marker records *S1* and *S2*. Then follow a sequence of the photon records *P* and pixel marker records *Px* for the individual pixels. The line ends with two end marker records *E1* and *E2*.

S1	S2	P	P	Px1	Px2	P	P	P	Px1	Px2	…	E1	E2

Line Start Marker Record S1

First record for start of a line

MSB LSB

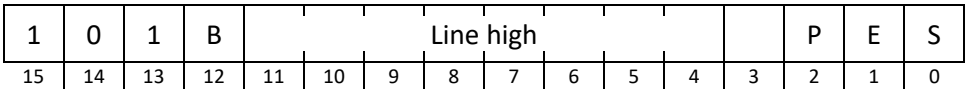
1	0	1	Line low					Line mux					P	E	S
15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0

S – Line start (bit is 1 for start marker)
E – Line end (bit is 0 for start marker)
P – Pixel (bit is 0 for start marker)
Line mux – Line multiplex index for sequential scan line by line
Line low – Lower 5 bits of the image line index

Line Start Marker Record S2

Second record for start of a line

MSB LSB



S – Line start (bit is 1 for start marker)
E – Line end (bit is 0 for start marker)
P – Pixel (bit is 0 for start marker)
Line high – The upper 8 bits of the image line index
B – When the frame complete condition is set to a user specified number of photons, the bit is inverted when a frame has completed (number of photons reached).

Photon Record P

A photon has been detected

MSB LSB

Det – index of the active detector channel (0 to 3). The value corresponds to the index in the *SingleMoleculeDetection/Dataset/Sequence/SequenceItem/Detectors* list (see 2. FLIM and FCS metadata).
F – 1 – first detected photon after a laser pulse, otherwise 0
Arrival time – number of base clocks since the previous laser pulse (0-4095)

Pixel Record Px1

First record marking the end of a pixel

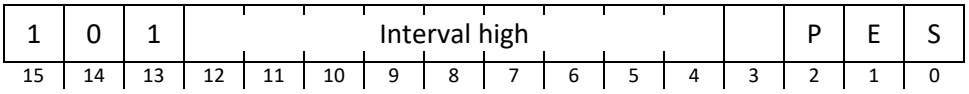
MSB LSB

S – Line start (bit is 0 for pixel marker)
E – Line end (bit is 0 for pixel marker)
P – Pixel (bit is 1 for pixel marker)
Line mux – Line multiplex index for sequential scan line by line
Interval low – Lower 5 bits of the number of 80 MHz clocks the finished pixel lasted.

Pixel Record Px2

Second record marking the end of a pixel

MSB LSB



S – Line start (bit is 0 for pixel marker)
E – Line end (bit is 0 for pixel marker)
P – Pixel (bit is 1 for pixel marker)
Interval high – The upper 9 bits of the number of 80 MHz clocks the finished pixel lasted.

Line End Marker Record S1

First record for start of a line

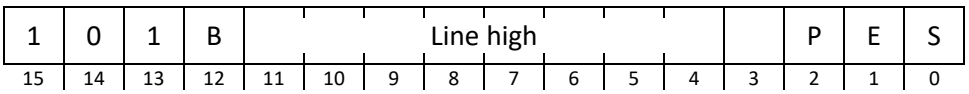
MSB LSB

S – Line start (bit is 0 for end marker)
E – Line end (bit is 1 for end marker)
P – Pixel (bit is 0 for end t marker)
Line mux – Line multiplex index for sequential scan line by line
Line low – Lower 5 bits of the image line index

Line End Marker Record S2

Second record for start of a line

MSB LSB



S – Line start (bit is 0 for end marker)
E – Line end (bit is 1 for end marker)
P – Pixel (bit is 0 for end marker)
Line high – The upper 8 bits of the image line index
B – When the frame complete condition is set to a user specified number of photons, the bit is inverted when a frame has completed (number of photons reached).

3.2. Assignment of FLIM raw data to image pixels

The FLIM data are recorded in the order they arrive. For the assignment to image pixels the information from the meta data has to be considered. The relevant information consists of:

Dimension Size – The number of pixels in the corresponding dimension. The information can be found in the *SingleMoleculeDetection/Dataset/RawData/Dimensions/Dimension/Size* elements.

Line Repetitions – The number of line repetitions affect the assignment for all dimensions except the lowest (dimension x). The information is in the value in the SingleMoleculeDetection/Dataset/Sequence/SequenceItem/LineRepetitions element.

Frame Repetitions – The number of frame repetitions affect the assignment to upper dimensions. The information is in the SingleMoleculeDetection/Dataset/Sequence/SequenceItem/FrameRepetitions element. For acquisitions with enabled option “Acquire until n Photons” the frame repetitions can vary. Then the value in the *FrameRepetitions* element is irrelevant. This mode was active when the value in the SingleMoleculeDetection/Dataset/RawData/FrameRepetitionsMarked element is true. The actual number of frames for *Frame Repetitions* can be derived from the markers B in the *Line Start Marker Record S2***.** Each time the bit toggles a new frame was started.

Sequential Mode – The sequential mode (simultaneous, line, frame, stack) affects the assignment for all dimensions except the lowest (dimension x). The sequential mode can be found in the SingleMoleculeDetection/Dataset/RawData/SequentialMode element.

Sequence Items – In a sequential scan the beam path setting is changed. For each such setting there is an entry in the list *SingleMoleculeDetection/Dataset/Sequence* with information about the used detectors.

Bi-Directional – In a bi-directional scan the pixels in some of the lines have to be assigned in reverse order. It was a bi-directional scan if *SingleMoleculeDetection/Dataset/RawData/BiDirectional* is true. The first line of an image frame starts in forward direction.
In *Simultaneous* and *Sequential Line* mode for each group of 2 * *Line Repetitions* * *Sequence Items* the lines are scanned with alternating direction.
In *Sequential Frame* and *Sequential Stack* mode for each group of 2 * *Line Repetitions* the lines are scanned with alternating direction.

For the assignment to the image dimensions consider the order in which the raw data lines are scanned. The order is different for different scan and sequential modes.

Sequential Mode	Simultaneous or Sequential Line	Sequential Frame	Sequential Stack
Order in x-y-scan Size Y > 1	Line Repetitions Sequence Items Y Frame Repetitions Z WlEx WlEm S T L	Line Repetitions Y Frame Repetitions Sequence Items Z WlEx WlEm S T L	Line Repetitions Y Frame Repetitions Z Sequence Items WlEx WlEm S T L
Order in x-z-scan Size Z > 1 and Size Y = 1	Line Repetitions Sequence Items Z Frame Repetitions WlEx WlEm T L	Line Repetitions Z Frame Repetitions Sequence Items WlEx WlEm T L	Line Repetitions Z Frame Repetitions Sequence Items WlEx WlEm T L
Order in x-t-scan Size Y = 1 and Size Z = 1	X Line Repetitions Sequence Items T L		

3.3. FCS Raw Data Format

FCS data are recorded with a 312,5Mhz counter. The counter values are registered when a photon is detected and *FCS Time Record* (F) with the lower 12 bits of the counter is appended to the raw data stream.
When there is an overflow of the lower 12 bits of the counter two FCS Overflow Records (O1 and O2) with the upper bits of the counter are appended to the raw data stream.

F	F	…	O1	O2	F	F	…	O1	O2	…

In case of pulsed laser excitation an *Arrival Time Record (A)* follows each *FCS Time Record* (F).

F	A	F	A	…	O1	O2	F	A	F	A	…	O1	O2	…

FCS Time Record F

A photon has been detected

MSB LSB

Det – index of the active detector channel (0 to 3). The value corresponds to the index in the *SingleMoleculeDetection/Dataset/Sequence/SequenceItem/Detectors* list (see 2. FLIM and FCS metadata).

FCS time – lower 12 bits of the number FCS clocks since the start of the acquisition

Arrival Time Record A

A photon has been detected

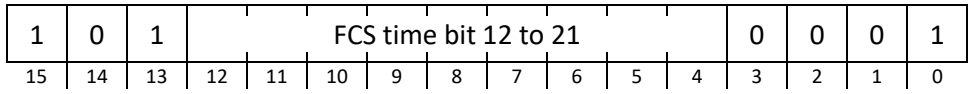
MSB LSB

Chan – number of the detection channel (0 to 3)

Arrival time – number of base clocks since the previous laser pulse (0-4095)

FCS Overflow Record O1

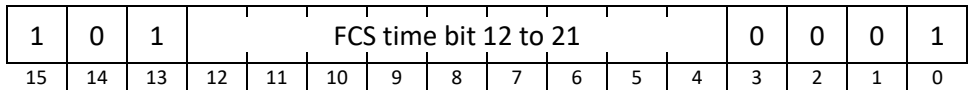
MSB LSB



FCS time bit 12 to 21 – medium 9 bits of the FCS time counter

FCS Overflow Record O2

MSB LSB



FCS time bit 22 to 31 – upper 9 bits of the FCS time counter

4. FLIM Pulse Detection

The first detected photon after the laser pulse is marked by the *F* flag in the raw data *Photon Record P*. When only these records are used classical TCSPC analysis methods can be applied when the count rate was below 0.1 photons per laser pulse. For higher count rates advanced analysis methods should be applied and additional records have to be considered.

Note that some of the advanced FLIM detection and analysis methods are considered Leica intellectual property. Granted patents exists and pending patent applications have been filed. Please contact your Leica representative regarding commercial and non-commercial use of Leica intellectual property.

To generate a correct overall decay at high count rates only the laser pulses can be used where exactly one single photon has been detected. When a record is marked as first photon by the *F* flag and the next record for this detector is also marked then the current record represents such a single photon.

For the image fit with high count rate data a correction according M. Patting et.al, Dead-time effects in TCSPC data analysis, Proc. of SPIE Vol. 6583, 658307, (2007) is possible. The fit model function is replaced by a modified model function $f_m(t)$

$$f_m(t) = f(t) \cdot \prod_{y=t-t_d}^{t} \left\{1 - \frac{1}{P} \cdot f(y)\right\}$$

where the unmodified model function *f (t)* is multiplied by a correction term which is the probability that within the dead time no photon has been detected. *P* is the number of laser pulses and t_d is the dead time of the system. The dead time is stored in the *SingleMoleculeDetection/Dataset/Sequence/SequenceItem/Detectors/Detector/DeadTime* element.

The ***Leica HyD Imaging*** detectors detect the rising edge of the photon signal. The *DataType* value in *SingleMoleculeDetection/Dataset/Sequence/SequenceItem/Detectors/Detector* is *RisingEdge*.

For the image fit with dead time correction all records should be considered.

The ***Leica HyD SMD*** detectors detect rising and falling edge of the photon signal.

The *DataType* value in *SingleMoleculeDetection/Dataset/Sequence/SequenceItem/Detectors/Detector* is *PulseVersion1* or *PulseVersion2*. A record where the F flag is not set represents either a rising edge or a falling edge.

If the pulse is wider than a single photon pulse, the arriving time of the photon that caused falling edge is also reported as a *Photon Record P*.

For *PulseVersion1* consider two adjacent records where the F flag in the second record is not set. If the *Arrival time* value t2 in the second record is smaller than *Arrival time* value t1 in the first record then and only then the second record represents a falling edge. The fact that the second value is lower but should be higher was a mistake in *PulseVersion1*. The true arrival time of a falling edge photon is 2*t1 – t2 (mirrored at t1).

In *PulseVersion2* the falling edge is identified when the time difference between the reported arrival times is smaller than the dead time.

For image fit with dead time correction and *PulseVersion1* or *PulseVersion2* data the falling edge photons should be excluded unless a more sophisticated correction model is used. The falling edge photon can always be used for intensity calculations since it represents a true photon.

Data in the format *PulseVersion1* have been generated during the TCS SP8 FALCON beta test. All release versions of the FALCON software generate data in the *PulseVersion2* format instead.