Photon-HDF5 Documentation

Release 0.2

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Version 0.3

Photon-HDF5 is a file format for freely diffusing single-molecule spectroscopy experiments such as single-molecule FRET (smFRET) (with or without lifetime), Fluorescence Correlation Spectroscopy (FCS) and related techniques.

Any dataset containing photon timestamps and other per-photon data can be stored in Photon-HDF5 files. Photon-HDF5 is designed for long-term preservation and data sharing and can therefore store experimental details and metadata such as setup configurations, sample information, authorship and provenance.

The present document contains the reference documentation for the Photon-HDF5 format.

Other related resources are:

- Examples of reading Photon-HDF5 files in multiple languages
- phconvert: reference library to create and convert Photon-HDF5 files
- Why an HDF5-based smFRET file format
- Photon-HDF5: an open format for single-molecule spectroscopy using photon-counting devices (*coming soon*).

Note: This document describes **Photon-HDF5 version 0.3**. The latest version of this document can be found at http://photon-hdf5.readthedocs.org. To send correction or getting involved in the file format development please see the *contributing* section.

CHAPTER 1

Table of contents

1.1 Introduction

1.1.1 Overview

This document contains specifications of the Photon-HDF5 format. This format allows saving single-molecule spectroscopy experiment data in which at least one stream of photon timestamps is present. It has been designed as a standard container format for a broad range of experiments involving confocal microscopy. Examples are confocal smFRET experiments performed with a single or multiple excitation spots. Both μs-ALEX and ns-ALEX data are supported.

1.1.2 What problems are we trying to solve?

- Ensuring long term data persistence.
- A disk space and read speed efficient file format for repeated use as well as for archiving.
- Facilitating data sharing and interoperability between analysis programs and research groups.

1.1.3 Features of HDF5

- Open-standard: language and platform independent, self-describing format with open source implementations.
- Efficient: the HDF5 format is a binary format that allows compression and fast read/write operations.
- Flexible: data arrays can be stored in "groups" (hierarchical format). Metadata can be attached to each data entry (attributes).
- No limit in data size.
- Support for a variety of numeric and non-numeric data types.

1.1.4 Photon-HDF5: Design principles

The main design principles are:

- Simplicity
- Flexibility
- Compatibility

We aim to define a format that has a minimal set of specifications and therefore is easy to implement. At the same time, it is important that the format can be expanded to accommodate new use cases while maintaining backward compatibility.

To achieve simplicity, the only required file characteristics are a general file layout and the presence of a few basic attributes and parameters. The remaining (small set of) fields defined in this document will be present only when needed by a particular measurement.

We retain flexibility by allowing the user to save any arbitrary data outside the specs of this document. To assure that future versions of this format will not conflict with user-defined fields, we require that all user-defined fields be contained in groups called `user`.

1.2 Photon-HDF5 format definition

1.2.1 Overview

A Photon-HDF5 is a HDF5 file with a predefined structure for timestamp-based data.

A screen-shot of a typical Photon-HDF5 file opened in HDFView is shown here:

The previous figure shows the 5 main groups contained in a Photon-HDF5 file. Of these, */photon_data* and */setup* contains the raw data and all the information needed for the analysis. A schematic overview is shown in the next figure:

The remaining 3 groups (`/sample`, `/identity`, `/provenance`) provides additional metadata that make the Photon-HDF5 files self-contained and suitable long-term on-line archival. An overview of there groups is shown below:

As a quick reference, here we list links to the documentation for each group:

- */photon_data*: RAW data and measurement type specifications.
- */setup*: Description of the experimental setup.
- */identity*: Information about the data file.
- */provenance*: Information about the original data file (when the Photon-HDF5 file has been converted from another format).
- */sample*: Description of the measured sample.

The following sections describe the Photon-HDF5 groups and fields.

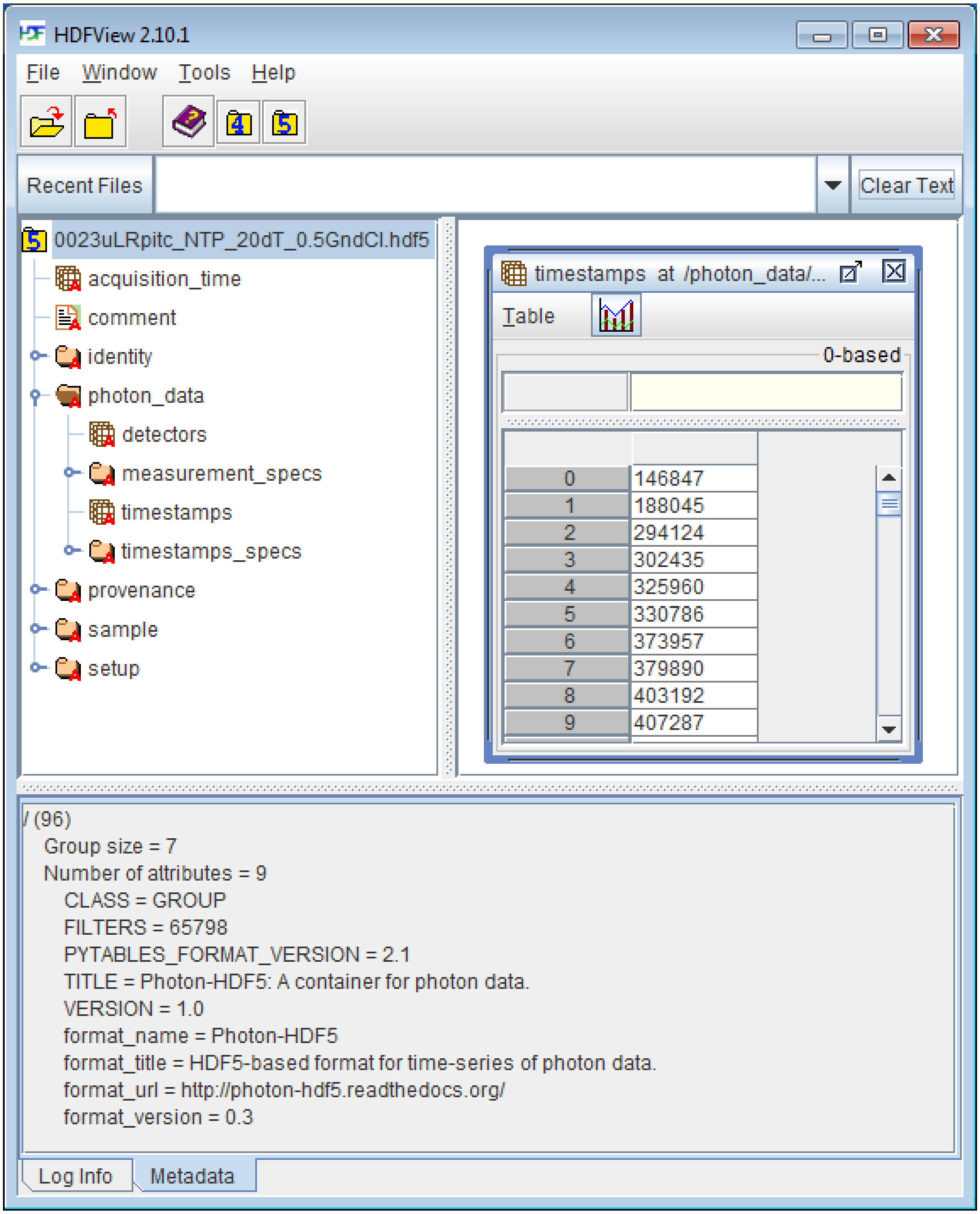
1.2.2 Root-level parameters

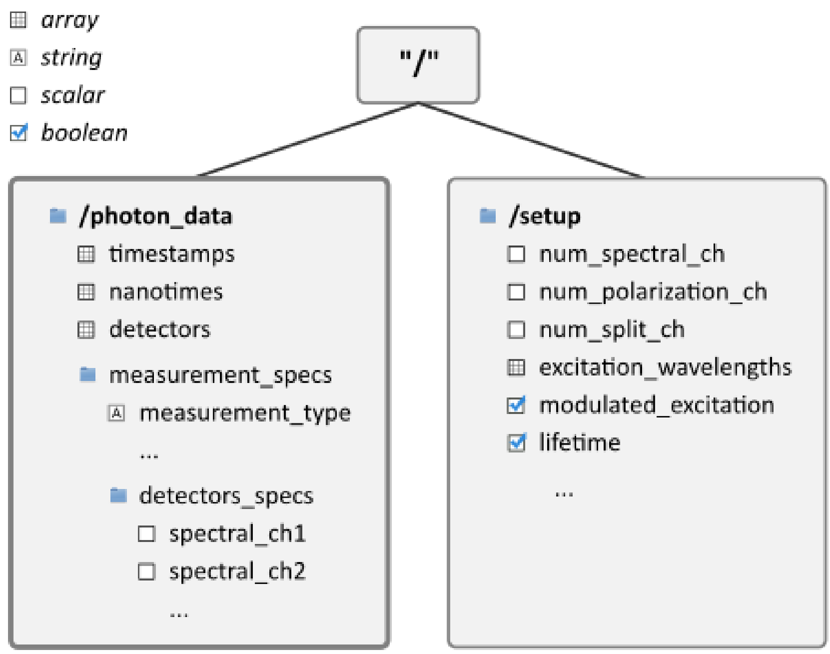
The root node ("/") in a Photon-HDF5 file contains the following fields:

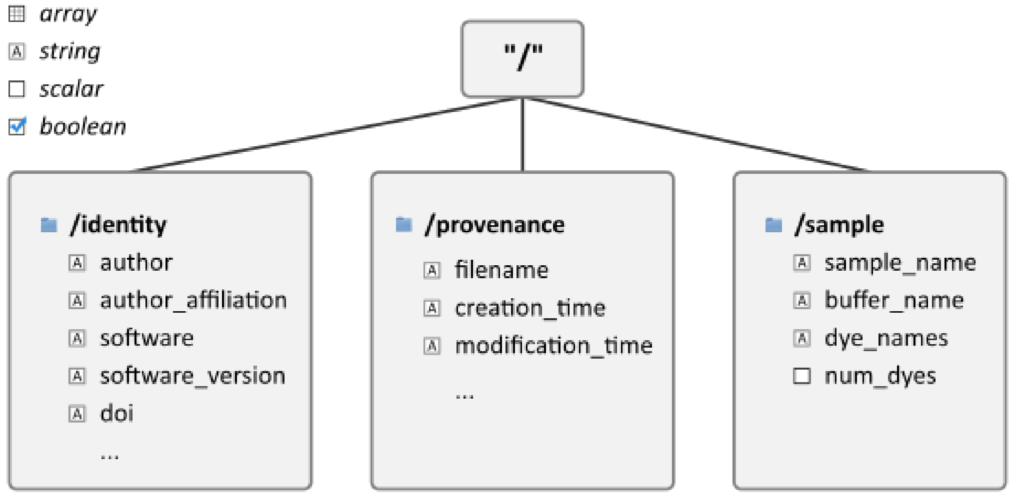
- **/acquisition_time**: (float) the measurement duration in seconds.
- **/comment**: (string) a user-defined comment.

In addition, the root node has the following attributes that can be used to distinguish Photon-HDF5 files from other HDF5 files:

- **format_name**: must contain the string "Photon-HDF5"
- **format_version**: (string) the Photon-HDF5 format version.


HDFView 2.10.1
File Window Tools Help
Recent Files
Clear Text
0023uLRpitc_NTP_20dT_0.5GndCl.hdf5
acquisition_time
comment
identity
photon_data
detectors
measurement_specs
timestamps
timestamps_specs
provenance
sample
setup
timestamps at /photon_data/...
Table
0-based
0 146847
1 188045
2 294124
3 302435
4 325960
5 330786
6 373957
7 379890
8 403192
9 407287
/ (96)
Group size = 7
Number of attributes = 9
CLASS = GROUP
FILTERS = 65798
PYTABLES_FORMAT_VERSION = 2.1
TITLE = Photon-HDF5: A container for photon data.
VERSION = 1.0
format_name = Photon-HDF5
format_title = HDF5-based format for time-series of photon data.
format_url = http://photon-hdf5.readthedocs.org/
format_version = 0.3
Log Info
Metadata


array
string
scalar
boolean
"/"
/photon_data
timestamps
nanotimes
detectors
measurement_specs
measurement_type
...
detectors_specs
spectral_ch1
spectral_ch2
...
/setup
num_spectral_ch
num_polarization_ch
num_split_ch
excitation_wavelengths
modulated_excitation
lifetime
...


array
string
scalar
boolean
"/"
/identity
author
author_affiliation
software
software_version
doi
...
/provenance
filename
creation_time
modification_time
...
/sample
sample_name
buffer_name
dye_names
num_dyes

1.2.3 Photon-data group

This section describes the layout and fields in the **/photon_data** group. Note that only the kind of data is specified (i.e. scalar, integer array, float array), but no data type size is mandated. For arrays, the most commonly used data-type is indicated.

Mandatory fields:

- **timestamps**: (array) photon timestamps. Typical data-type int64.
- **timestamps_specs/**
  - **timestamps_unit**: (float) timestamp units in *seconds*.

Optional if there is only 1 detector, otherwise mandatory:

- **detectors**: (array of integers) detector ID for each timestamp. Typical data-type uint8.

When the dataset contains nanotime information (i.e. arrival time of each photon with respect to a laser pulse), the following fields must be present:

- **nanotimes**:(array of integers) TCSPC nanotimes. Typical data-type uint16.
- **nanotimes_specs/**
  - **tcspc_unit**: (float) TAC/TDC bin size (in seconds).
  - **tcspc_range**:(float) full-scale range of the TAC/TDC (in seconds).
  - **tcspc_num_bins**: (integer) number of TAC/TDC bins.
  - **time_reversed**: (boolean) *True* if nanotimes contains the time elapsed between a photon and the next laser pulse. *False* if it contains the time elapsed between a laser pulse and a photon.

Finally, if the data come from a simulation, `/photon_data` may contain:

- **particles**: (array of integers) a particle ID (integer) for each timestamp. Typical data-type uint8.

Measurement specs

The optional **/photon_data/measurement_specs** group contains additional information allowing unambiguous interpretation of the data for each specific type of measurement.

- **measurement_type**: (string) the type of the measurements. Valid names are:
  - "smFRET" (1 excitation color, 2 detection colors)
  - "smFRET-usALEX" (2 excitation colors, 2 detection colors)
  - "smFRET-usALEX-3c" (3 excitation colors, 3 detection colors)
  - "smFRET-nsALEX" (2 excitation colors, 2 detection colors)

  New names can be created for different kind of measurements and we encourage users to submit new name requests.

The *measurement_type* field describes the type of measurement saved within the file. It is an important field allowing software packages reading and saving Photon-HDF5 files to perform consistency checks (see also *Measurement type*).

For μs-ALEX, 2, 3 or N colors:

- **alex_period**: (integer or float) duration of one complete excitation alternation period expressed in timestamp units. The alternation period is equal to `alex_period * timestamps_unit`.

For ns-ALEX (or lifetime with no alternation):

- **laser_pulse_rate**: (float) excitation laser pulse repetition rate in *Hertz*.

For 2-color (or more) μs-ALEX and ns-ALEX (optional):

- **alex_period_spectral_ch1**: (array with an even-number of integer elements) start and stop values identifying the *spectral_ch1* (i.e. *donor* for smFRET measurements) emission period (see note below for more details).
- **alex_period_spectral_ch2**: (array with an even-number of integer elements) start and stop values identifying the *spectral_ch2* (i.e. *acceptor* for smFRET measurements) emission period (see note below for more details).
- etc...

Note: For μs-ALEX, both *alex_period_spectral_ch1* and *alex_period_spectral_ch2* are 2-element arrays. In this case, these values are expressed in *timestamps_units*. For ns-ALEX (also known as PIE), they are arrays with an even-number of elements, comprising as many start-stop nanotime pairs as there are excitation periods within the TAC/TDC range. In this case these values are expressed in *nanotimes_units*.

For more details see *Definition of alternation periods*.

Detectors specs

Within **measurement_specs**, the **detectors_specs/** sub-group contains all the *detector ID*–detection channel associations, i.e. spectral bands, polarizations or *beam-split channels*.

When a measurement records more than 1 spectral band, the fields:

- **spectral_ch1**
- **spectral_ch2**
- etc...

specify which detector is employed in each spectral band. When the measurement records only 1 spectral band these fields may be omitted. The spectral bands are strictly ordered for increasing wavelengths. For example, for 2-color smFRET measurements `spectral_ch1` and `spectral_ch2` represent the *donor* and *acceptor* channel respectively.

If a measurement records more than 1 polarization states, the fields:

- **polarization_ch1**
- **polarization_ch2**

specify which detector is used for each polarization. When the measurement records only one polarization, these fields may be omitted.

When the detection light is split into 2 channels using a non-polarizing beam-splitter the fields:

- **split_ch1**
- **split_ch2**

specify which detector is used in each of the “beam-split” channels.

All previous fields are arrays containing one or more detector IDs. For example, a 2-color smFRET measurement will have only one value in `spectral_ch1` (donor) and one value in `spectral_ch2` (acceptor). A 2-color smFRET measurement with polarization (4 detectors) will have 2 values in each of the `spectral_chX` and `polarization_chX` fields (where X=1 or 2). For a multispot smFRET measurement, `spectral_chX` will contain the list of donor/acceptor detectors (see *Multi-spot measurements*).

Finally, a label (string) can be associated to each detector using the optional *labels* field:

- **labels**: (optional) table with 2 columns: detector ID (integer) and detector label (string).

For 2-color smFRET measurements, it is recommended to use the “donor” and “acceptor” labels for the respective detectors. Note, however, that these labels only represent an additional user-defined metadata and are not necessary for the interpretation of the measurement. When detector ID is a *n*-tuple, `labels` has *n+1* columns (*n* for the ID and 1 for the labels).

1.2.4 Setup group

The **/setup** group contains information about the measurement setup:

- **num_pixels**: (integer) total number of detector pixels. For example, for a single-spot 2-color smFRET measurement using 2 single-pixel SPADs as detectors this field is 2.
- **num_spots**: (integer) the number of excitation (or detection) “spots” in the sample. This field is 1 for all the measurements using a single confocal excitation volume. When not applicable, for example under widefield illumination with 2-D imaging detectors, this field is omitted.
- **num_spectral_ch**: (integer) number of distinct detection spectral channels. For example, in a 2-color smFRET experiment there are 2 detection spectral channels (donor and acceptor), therefore its value is 2. When there is a single detection channel or all channels detect the same spectral band, its value is 1.
- **num_polarization_ch**: (integer) number of distinct detection polarization channels. For example, in polarization anisotropy measurements, its value is 2. When there is a single detection channel or all channels detect the same polarization (including when no polarization selection is performed) its value is 1.
- **num_split_ch**: (integer) number of distinct detection channels detecting the same spectral band **and** polarization state. For example, when a non-polarizing beam-splitter is employed in the detection path, its value is 2. When no splitting is performed, its value is 1.
- **modulated_excitation**: (boolean) *True* (or 1) if there is any form of excitation modulation either in the wavelength space (as in μs-ALEX or PAX) or in the polarization space. This field is also *True* for pulse-interleaved excitation (PIE) or ns-ALEX measurements.
- **lifetime**: (boolean) *True* (or 1) if the measurements includes a *nanotimes* array of (usually sub-ns resolution) photon arrival times with respect to a laser pulse (as in TCSPC measurements).
- **excitation_wavelengths**: (array of floats) list of excitation wavelengths (center wavelength if broad-band) in increasing order (unit: *meter*).
- **excitation_cw**: (array of booleans) for each excitation source, this field indicates whether excitation is continuous wave (CW), *True*, or pulsed, *False*. The order of excitation sources is the same as that in `excitation_wavelengths` and is in increasing order of wavelengths.

The following fields are optional and not necessarily relevant for all experiments. If the associated information is irrelevant or not available, these fields are omitted.

- **excitation_polarizations**: (arrays of floats) list of polarization angles (in degrees) for each excitation source. The order of excitation sources is the same as in `excitation_wavelengths` and is in increasing order of wavelengths.
- **excitation_input_powers**: (array of floats) excitation power in *Watts* for each excitation source. This is the excitation power entering the optical system.
- **excitation_intensity**: (array of floats) excitation intensity in the sample for each excitation source (units: *Watts/meters*^{2}). In the case of confocal excitation this is the peak PSF intensity.
- **detection_wavelengths**: (arrays of floats) reference wavelengths (in *meters*) for each detection spectral band. This array is ordered in increasing order of wavelengths. The first element refers to `detectors_specs/spectral_ch1`, the second to `detectors_specs/spectral_ch2` and so on.
- **detection_polarizations**: (arrays of floats) polarization angles for each detection polarization band. The first element refers to `detectors_specs/polarization_ch1`, the second to

`detectors_specs/polarization_ch2` and so on. This field is not relevant if no polarization selection is performed.

- **detection_split_ch_ratios**: (array of floats) power fraction detected by each "beam-split" channel (i.e. independent detection channels obtained through a non-polarizing beam splitter). For 2 beam-split channels that receive the same power this array should be `[0.5, 0.5]`. The first element refers to `detectors_specs/split_ch1`, the second to `detectors_specs/split_ch2` and so on. This field is not relevant when no polarization- and spectral-insensitive splitting is performed.

1.2.5 Identity group

The **identity/** group contains information about the specific Photon-HDF5 file. If some information is not available the relative field may be omitted.

- **author**: (string) the author of the measurement (or simulation).
- **author_affiliation**: (string) the company or institution the *author* is affiliated with.
- **creator**: (string) the Photon-HDF5 file creator. Used when the data was previously stored in another format and the conversion is performed by a different person than the author.
- **creator_affiliation**: (string) the company or institution the *creator* is affiliated with.
- **url**: (string) URL that allow to download the Photon-HDF5 data file.
- **doi**: (string) Digital Object Identifier (DOI) for the Photon-HDF5 data file.
- **filename**: (string) Photon-HDF5 file name at creation time. This field saves the original file name even if the file is later on renamed on disk.
- **filename_full**: (string) Photon-HDF5 file name (including the full path) at creation time.
- **creation_time**: (string) the Photon-HDF5 file creation time with the following format: "YYYY-MM-DD HH:MM:SS".
- **software**: (string) name of the software used to create the Photon-HDF5 file.
- **software_version**: (string) version of the software used to create the Photon-HDF5 file.
- **format_name**: (string) this must always be "Photon-HDF5"
- **format_version**: (string) for the current version it must be "0.3"
- **format_url**: (string) A URL pointing to the Photon-HDF5 specification document.

1.2.6 Provenance group

The **provenance/** group contains info about the original file that has been converted into a Photon-HDF5 file. If some information is not available the relative field may be omitted.

- **filename**: (string)
- **filename_full**: (string)
- **creation_time**: (string)
- **modification_time**: (string)
- **software**: (string)
- **software_version**: (string)

1.2.7 Sample group

The **/sample** group contains information related to the measured sample. This group is optional.

- **num_dyes**: (integer) number of different dyes present in the samples.
- **dye_names**: (array of string) list of dye names (for example: `['ATTO550', 'ATTO647N']`)
- **buffer_name**: (string) a user defined description for the buffer.
- **sample_name**: (string) a user defined description for the sample.

1.2.8 Additional notes and definitions

Detector IDs

A detector ID is the "name" of each pixels and is typically a single integer (when all the pixels are numbered with a progressive index). In some case (when using detector arrays) the detector ID can be a *n*-tuple of integers. This allow to specify, for each pixel, the module number and the X, Y location, for example. Therefore, an array of detector IDs can be either a 1-D column array or a 2-D array. In either cases, each row identifies a detector.

Beam-split channels

When the emitted light path is split in 2 or more detection paths by using a non-polarizing beam splitter the measurement has so called beam-split channels. The fields *split_ch1* and *split_ch2* contains the list of detector IDs for each beam-split channel (see *Detectors specs*).

Definition of alternation periods

Note for μs-ALEX

The *alex_period_spectral_ch1* and *alex_period_spectral_ch2* fields allow defining photons detected during donor or acceptor excitation. As an example, let's define the array

`A = timestamps` *MODULO* `alex_period`

as the array of timestamps modulo the μs-ALEX alternation period. Photons emitted during the donor period (respectively, acceptor period) are obtained by applying one of these two conditions:

- `(A > start) and (A < stop)` when `start < stop` (*internal range*)
- `(A > start) or (A < stop)` when `start > stop` (*external range*).

Measurement type

Each *measurement_type* has an associated set of mandatory fields which must be present to ensure that all information needed to unambiguously interpret the data is present. For example, for a 2-color smFRET measurement, a software package creating a file should check that the association between detector and donor or acceptor channel is present. If some necessary field is absent, the software package should warn the user in order that this information is added before saving the file.

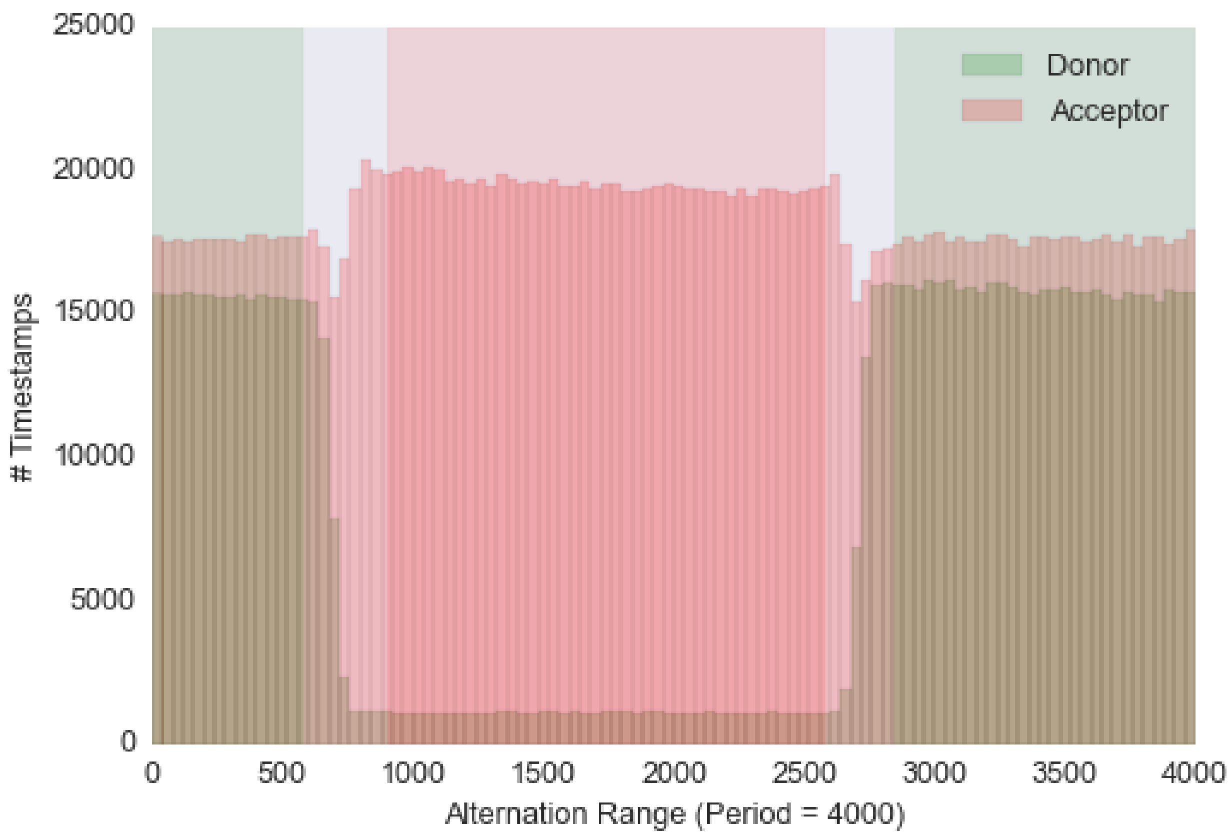


Fig. 1.1: Alternation histogram showing selection for the donor and acceptor periods. In this case the donor period is defined as an "external range" (2850, 580) while the acceptor period is defined as an "internal range" (900, 2580). This situation is due to the ALEX period being out of phase with respect to the time stamping clock.

Multi-spot measurements

Multi-spot measurements are simply handled by having multiple `photon_data` groups, one for each excitation spot. The naming convention is the following:

```
photon_data0
photon_data1
...
photon_data10
...
photon_data100
```

Note that the enumeration starts from zero and there is no zero filling.

1.3 Known Limitations

In this section, we list some features that are not currently supported. If you think that some of these should be included in the specifications, please contact us.

1.3.1 Timestamps with rollover

In Photon-HDF5 timestamps are always signed 64 bit integers. Thanks to compression, there is no size penalty compared to 32 bit integers. Most timestamping hardware produce a timestamp with 24 or 32 bits and a rollover flag in order to compute the full "unwrapped" timestamp. Saving timestamps with a separate rollover information is not currently supported, therefore the rollover correction must be computed before saving data in a Photon-HDF5 file.

Timestamps with rollover may be supported in a future version of Photon-HDF5.

1.4 Collaborating

The success of a file format is only determined by the extent of its adoption. For this reason we greatly welcome any feedback and contribution from interested users.

The Photon-HDF5 project consist of these parts:

1. Reference Documentation (i.e. this document).
2. Examples on reading Photon-HDF5 in multiple languages.
3. phconvert: reference python library for writing and converting Photon-HDF5 files.

All the sources (including for the documentation) are hosted on GitHub and we encourage to open *GitHub Issues* in the documentation repository to discuss any topic related Photon-HDF5. You can also contact us by email, but we prefer to use GitHub in order to keep any discussion public.

Contributions (such as fixes or enhancements) can be sent using *GitHub Pull Requests* (PR). You can find guides on how to send a PR on the GitHub website. If have have any doubts, please contact us and we will be glad to help you getting started.

There are several ways you can get involved:

- **Sending feedback**: if you use or plan to use Photon-HDF5 and have any comment or suggestion, please send it to us! Even if you don't have any problem we would like to hear back about your use case. For this topic please open an issue in the documentation repository.

- **Documentation contributions**: if you feel that some section of this document should be expanded or enhanced in any way, please feel free to open an issue or send a PR (see note above) on the documentation repository.
- **Contributing examples**: you can send a new example on reading Photon-HDF5 files in a new language or for a different measurement type. Or simply send a fix for the current examples.
- **Contributing to phconvert**: you can open issues to report bugs, discuss usage or propose enhancements. You are also more than welcome to send PR for fixes or enhancements to the library. The official repository is this one.

1.4.1 Contributions Acknowledgement

Any contributions to this document will be listed in the front page, just below the authors.

Contributions to the examples repository need to be under the MIT license and will retain the copyright notice of the original contributor.

Contributions to phconvert, currently, need to be under the GNU GPL v2 license. We are discussing to relicense phconvert to MIT license, so stay tuned.