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A user-friendly tool to convert photon counting data to the open-source Photon-HDF5 file format

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ABSTRACT

Photon-HDF5 is an open-source and open file format for storing photon-counting data from single molecule microscopy experiments, introduced to simplify data exchange and increase the reproducibility of data analysis. Part of the Photon-HDF5 ecosystem, is **phconvert**, an extensible python library that allows converting proprietary formats into Photon-HDF5 files. However, its use requires some proficiency with command line instructions, the python programming language, and the YAML markup format. This creates a significant barrier for potential users without that expertise, but who want to benefit from the advantages of releasing their files in an open format. In this work, we present a GUI that lowers this barrier, thus simplifying the use of Photon-HDF5. This tool uses the **phconvert** python library to convert data files originally saved in proprietary data formats to Photon-HDF5 files, without users having to write a single line of code. Because reproducible analyses depend on essential experimental information, such as laser power or sample description, the GUI also includes (currently limited) functionality to associate valid metadata with the converted file, without having to write any YAML. Finally, the GUI includes several productivity-enhancing features such as whole-directory batch conversion and the ability to re-run a failed batch, only converting the files that could not be converted in the previous run.

Keywords: photon-counting, single-molecule, fluorescence, open-source, Photon-HDF5, phconvert, GUI, Python

1. INTRODUCTION

1.1 Photon-HDF5 File Format

The single-molecule fluorescence spectroscopy (SMFS) community has recently promoted some level of standardization in measurement practices and analysis pipelines.¹ In order to enable reproducibility of data analysis and thus strengthen trust in scientific results, it is important for data to be freely and easily available, for example by depositing it in online repositories. Moreover, data should be readable and well-documented, which is best done using standardized file formats and abundant, human-readable metadata.

Photon-counting SMFS methods implement widely varying setup geometries and hardware. Common photon-counting techniques include single-molecule Förster Resonance Energy Transfer (smFRET), fluorescence correlation and cross-correlation spectroscopy (FCS and FCCS), and fluorescence lifetime spectroscopy. In the case of smFRET, setups may have a variety of different features, for example, multi-color detection schemes, nano or microsecond alternative laser excitation (ns- or μ s-ALEX), or polarization anisotropy. In each of these cases, parameters change between measurements such as the number and wavelength of light sources, the laser period in setups using alternation, number of detectors, or the presence of polarizers, etc.

In addition to the wide variety of hardware, there is a wide variety of acquisition software and file formats. Data is often recorded using custom-built binary file formats, either supplied by an equipment manufacturer or designed in-house by the laboratory in which the experiment was performed. Oftentimes, the former are preferable to the latter, as manufacturers have a vetted interest in long-term support of their customers, while

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graduate students and postdocs follow each other in a lab, with variable degree of overlap. However, manufacturers understandably develop their own formats adapted to their hardware and market niche, with little concern for compatibility with other products. The result is a slew of file formats, some well-documented, some not at all, which leads to loss of effective access to the data, even when the files remain available. Unifying common data storage formats helps to alleviate the issues associated with proprietary file formats, and as the number and variety of techniques continues to grow, it becomes increasingly important to standardize an open and accessible format for the burgeoning SMFS community.

A few years ago, we introduced a open and standardized photon-counting file format, Photon-HDF5, based on the HDF5 open source file format.² The HDF5 organization is a vibrant community supporting multiple operating systems, and provides a free HDFView software for these platforms, enabling users to open, read, and copy data from these files without knowing any programming, making it an ideal vehicle for universal data storage.^{3,4} Based on these foundations, the Photon-HDF5 file format formalizes the organization of SMFS data obtained with photon-counting hardware, by logically storing raw data along with metadata for the sample and measurement. Storing data and metadata together is extremely important for long-term reproducibility, see Photon-HDF5.org or <http://photon-hdf5.github.io/> for a complete documentation.

By virtue of the HDF5 format's built-in features, Photon-HDF5 incorporates efficient data compression, and can be easily read within computing environment programming languages such as Python or MATLAB. Furthermore, accompanying the format's detailed description, code snippets for MATLAB and LabVIEW are provided as examples, as well as a python based file converter called **phconvert** (<http://photon-hdf5.github.io/phconvert/>).⁵ The format is supported by an increasing number of SMFS data processing packages, such as **FRETbursts** (python)⁶ and **ALiX** (LabVIEW).⁷

1.2 phconvert

phconvert is a helper library which was released together with Photon-HDF5, designed to convert some common proprietary file formats to the Photon-HDF5 format. Currently supported raw data files include PicoQuant's .HT3/.PT3/.PTU/.T3R, Becker & Hickl's .SPC/.SET, and the .SM format used by our lab and others for μ s-ALEX smFRET. As mentioned earlier, one advantage of such a conversion is that files can be viewed in human readable forms using HDFView,⁴ and most importantly, when done properly, the data is stored with relevant metadata, helping to make sense of the experimental and measurement parameters.

Using **phconvert** requires basic familiarity with Python, something which is becoming increasingly common in the community, but remains a potential obstacle to the adoption of this simple action: converting ones' data files into Photon-HDF5 formatted files for deposition into a public online repository. Additionally, some understanding of the different metadata fields of Photon-HDF5 (and more generally, an understanding of the general logic of the format organization) is required to generate fully documented files. Finally, the metadata to be provided to **phconvert** needs to be prepared in a YAML file, which requires some knowledge of this simple yet specific data serialization language (YAML Ain't Markup Language™).⁸

In order to lower the barrier to adoption of Photon-HDF5 for sharing photon-counting data generated in SMFS experiments, we have developed a graphical user interface (GUI) for the **phconvert** library that significantly reduces the need for the specialized technical skills currently required for file conversion. In this paper, we will describe the current state of the GUI, detailing some of the ways it can currently be used, its limitations, and future improvements that could be made.

2. SOFTWARE DESIGN

2.1 Features

The goals set for this tool were to:

Simplify installation by packaging all dependencies To obtain **phconvert**, users have several options, all of which involve technical knowledge. They can download the package from GitHub, taking care to properly install it so that it plays well with the system's Python installation, or they can use a package manager such as **pip** or **conda**. We wanted to improve on this experience for users without technical experience.

Most users have likely not heard of these tools, let alone feel comfortable using them. So installation of the GUI should be as straight-forward as possible, and not require mucking about with language package managers or stuff like the `PYTHON_PATH` variable.

Provide a user-friendly conversion tool Minimal prior knowledge should be presumed of the user. Especially no technical knowledge regarding programming, the command line, YAML, etc. Common operations should be obvious, so that the user is not left hunting for what they want. The correct thing to do should be made as easy as possible, and there should be no hidden surprises that sabotage users.

Support batch conversion of multiple files Users may have more than one file that they wish to convert, and it can be convenient to deal with a large directory all at once. To streamline the process of converting many files at a time, there should be batch conversion mode.

Provide helpful feedback during and after conversion As a minimum requirement, there should be a way for the users to know whether the conversion process was successful. Users should also be able to find out what when wrong in the case of a failure, so that they can fix the situation and try again.

2.1.1 Additional nice-to-have features

YAML-free metadata specification Many users won't know what YAML is, nor the syntax for it, or even how to create a YAML file in the first place. A method for users to provide the metadata for the data file(s) being converted to Photon-HDF5 without having to use YAML directly would definitely be useful.

Support for multiple platforms For widest adoption, the GUI should be available on all the common platforms so that most researchers who want to use it can.

Seamless upgrades to future phconvert versions The user should be insulated from worrying about differences in the underlying library version, and the GUI should be developed in such a way that the maintainer(s) have as smooth a time as possible when the time comes to update.

2.2 Implementation

To make things truly point-and-click, a thin wrapper around `phconvert` was needed that provided a single entry point for conversion. To make the interface as simple and discoverable as possible, a minimum amount of graphical elements were added, and a focus was placed on trying to make the things that should be easy, as easy as possible. The button to convert a file or batch is the biggest, most visible button. There is a status bar for each conversion task that lets the user know if conversion succeeded, and the status bar for batches tries to be informative regarding both the current attempt and the overall status of the batch.

Implementing the resume feature for batch conversions required keeping track of the files that had been converted already, and to not abort the whole conversion process when an error is encountered. To deal with both of these, a basic logging system was implemented. When a directory is selected for batch conversion, a log file is created inside it. As the software goes through the directory trying to convert (sensible) files, it writes to the log whether each file was successful or not, and the reason for failure. If not all files are successful, this log can be used by the GUI to reconstruct the list of files it already converted successfully, saving time for the user by only converting the files in the batch that failed, and which were presumably fixed. It is also intended to be useful to users in finding out why a conversion failed, and logs printed representations of all errors encountered.

2.3 Tools used

2.3.1 GUI design

The GUI is built using Python's Tkinter toolkit.⁹ The primary reason this was chosen was because of all the graphical user interface toolkits available to Python, Tkinter appeared to support the most interactivity during development. A high degree of interactivity can improve development productivity by reducing the time between writing code and seeing the results, and in the ideal case allows updates on the fly so that bugs can be fixed literally seconds after identification. Interactivity becomes even more valuable when there is significant

uncertainty in the development process, such as when developing a novel graphical user interface for a workflow previously based on the command line and Python programming. To this end, a prototype of the GUI was developed with the Common Lisp language and the Common Lisp Omnificent GUI (CLOG) toolkit.¹⁰ when the code was changing quickly, CLOG was a fast and convenient way to develop the GUI. After the initial development steps, the GUI was ported to the target language and toolkit, Python and Tkinter.

2.3.2 GUI distribution

Once the GUI was ready to be distributed to others for testing, a new set of tools for deployment were required. Since we are writing this GUI for users who are not expected to manually install a Python package and dependencies with a package manager like `pip` or `conda`, the GUI should be distributed as a self-contained executable that the user need only download and double click to get started. To achieve this, we used `pyinstaller` to package up the GUI code along with all of its dependencies, including the Python interpreter, with the exception of things such as `glibc` which are provided by the operating system. At this early stage, the GUI is packaged as an executable within a zipped up directory structure that contains all the mentioned dependencies. This permits easier debugging. In later stages, a single self-contained executable, rather than the executable plus dependencies folder used for development, will be created and released on Github, all that is needed being a command flag in the build process.

2.3.3 Build automation

In order to provide multi-platform releases (at least on the most popular ones in the SMSF community, namely Linux, Mac, and Windows) without the burden of supporting dedicated machines for the builds, the AppVeyor online platform was used.¹¹ As part of getting software into the hands of testers, a continuous integration (CI) pipeline connected to the GUI's Github repository was developed using AppVeyor, starting from the configuration used by `phconvert`. Each time a commit is pushed, the pipeline builds the executable bundles on both Linux and Windows, then uploads them to the Github releases page. Initially, we intended to release an executable running on macOS, but unresolved issues related to Apple's security policies have led to us to postpone it. Contributions towards resolving these problems are welcome.

While developing the CI pipeline, the requirement that the code snippets in the configuration file be runnable on every platform being targeted, except if flagged otherwise, initially caused some issues. The crux of the matter was that each platform had a different shell. Windows had `batch/cmd.com`, Linux had `Bash`, and Mac had `Zsh`. At the face of it, this meant that large chunks of code would need to be copied between the different platforms' build processes, each slightly tweaked for the specific platform. This is bad from a maintainability standpoint, since it multiplies the amount of work necessary to make a single change and the repetitiveness of the code provides a place for bugs to hide and the opportunity for things to be forgotten to be changed. What was wanted was a way to write the build process code in the AppVeyor configuration file such that it would be valid on all platforms, with as much reuse as possible so that only the things which absolutely needed to be different (such as installation of software on Linux and Mac that was already present on Windows) were different. Luckily, one common denominator across all the AppVeyor images used is the installation of the `Powershell` or `Powershell Core (pwsh)` shell. Additionally, AppVeyor provides a means in the configuration file to mark code as `pwsh` snippets/blocks to be run. This provided the necessary common language between platforms. As of now, all code in the AppVeyor configuration file with the exception of one special case is written in `pwsh`, for better maintainability.

3. SOFTWARE DEMONSTRATION

In the following subsections, we will demonstrate the GUI's basic features by illustrating how files can be converted to Photon-HDF5.

3.1 Installation

The current installation process for the GUI is as follows:

1. Go to <https://github.com/ramenbytes/phui>, which is the working repository for the GUI right now. PHUI stands for **Ph**convert **U**ser **I**nterface.

2. Click on **Releases**.
3. Download the zip file of your choice corresponding to your platform and chosen Python version. For Linux, choose an Ubuntu-labeled release, and for Windows, choose a Visual Studio one.
4. Extract the downloaded zip file.
5. Inside the zip folder, descend into the `dist/gui` folder.
6. Double-click the `gui` executable to run the GUI.

The only system requirements are that the user be on a platform at least as up-to-date as the Appveyor image used to build the executable. Currently, that is Ubuntu 18.04.4 LTS (Bionic Beaver) and Windows 10. They do not need to be on the *same* system, just one whose system libraries (such as `glibc` on Linux) are contemporary to the build image's. The rest of the dependencies are taken care of by the build process and are stashed in the distributed folder containing the executable which the user downloads.

3.2 Single-File Conversion

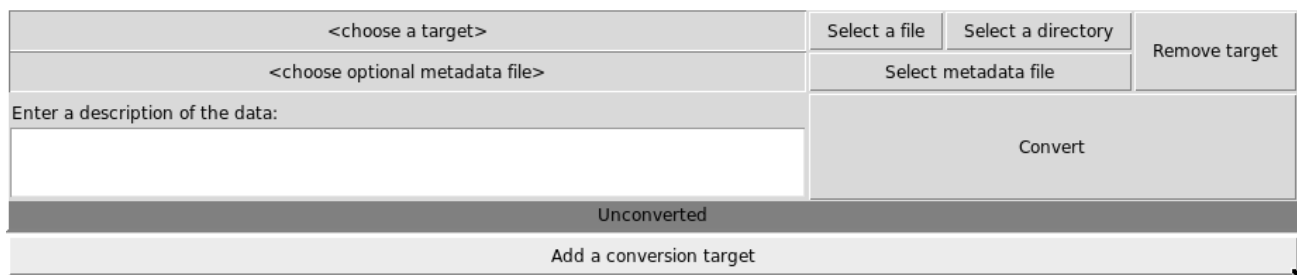


Figure 1. Initial window shown to users upon starting the GUI.

The simplest use case for the GUI is the conversion of a single file. Upon starting the GUI, the user is greeted by the window in Figure 1. Starting in the top left, the initially empty **choose a target** field displays the filename of the data file the user wants to convert. To select a file for conversion, the user clicks on the **Select a file** button to the immediate right of the field. The user is presented with a file dialog which they can use to navigate to and select the desired file. After a file is chosen, metadata needs to be associated with it. There are currently several methods of doing so, and more planned.

- First, the user can provide a description in the white textbox near the bottom of the window. The Photon-HDF5 format requires a description, so the conversion process will fail if one isn't provided.
- Second, `phconvert` provides default values for certain metadata fields. Absent user input, these values will be used. To override these default values or to provide values for other fields, users currently need to use the third method,
- Third, clicking the `bfSelect metadata file` button to choose a YAML file containing the relevant info. This is intended as a stopgap solution until a graphical metadata editor is developed, freeing the users from worrying about YAML files. However, in its current state, the conversion process is not entirely YAML-free.

After a data file has been chosen, and metadata associated with it, users will need to press the **Convert** button. If the conversion is successful (no errors are thrown during the process), then the status bar near the bottom of the window (grey, with the label **Unconverted** in Fig. 1) will change to green, as shown in Fig. 2.

If an error occurs during the conversion process, the status bar changes to red with a message noting the failure, and a dialog window with a printed description of the error is presented to the user as seen in Fig. 3.

The conversion can be retried by closing the error dialog, and pressing **Convert** again. If the user fixes the error before trying again, the conversion should succeed this time around.

/home/vir/weisslab/phui/data/022rde11_T_minus15_acc_NT_plus10_don_1.sm	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data: Hello world!		Convert	
Converted			
Add a conversion target			

Figure 2. A successful conversion


/home/vir/weisslab/phui/data/022rde11_T_minus15_acc_NT_plus10_don_1.sm	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data:		Convert	
<div style="border: 1px solid black; padding: 5px; text-align: center;">  Invalid_PhotonHDF5('Missing field "description" in "/. ') OK </div>		Failed Conversion	
		Add a conversion target	

Figure 3. An unsuccessful conversion

3.3 Batch Conversion

Directly to the right of the **Select a file** button is the **Select a directory** button, as seen in Fig. 1. This button allows a user to select an entire directory as a candidate for conversion, as opposed to a single file. When the user attempts to convert the directory, a subroutine will gather a list of all the files in the provided directory that can be converted, based on the filename extensions. Everything else is excluded from the conversion attempt. Then, one by one, the program tries to convert each of these files, recording in a log whether a file conversion was successful, and if not, what the error was.

A user can try re-running the batch by clicking **Convert** again; this time, only the files marked as failures in the log will be tried again. This is designed such that long batch conversions are not repeated needlessly. At the end of each attempt, the status bar shows the fraction of unconverted files that were successfully converted on this attempt, how many were converted in previous attempts, and where to find the log file. See Fig. 4 for an example.

/home/vir/weisslab/phui/data	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data: hi		Convert	
Converted 0 out of 1 unconverted files. 9 file(s) already converted before this attempt. Progress log: /home/vir/weisslab/phui/data/phui_conversion_progress.log			
Add a conversion target			

Figure 4. Status from a partial batch conversion. At the start of this attempt, one file was unconverted. Nine were logged as previously converted. This attempt failed to convert the single unconverted file. The progress log at the provided path lists the error which resulted in the failure.

The metadata for batch conversions is obtained in the same way as for single file conversions, however it will be applied to *all* files in the batch. That means that currently, batch conversions are only suitable for groups of files that have identical metadata. For example, data from several runs of the same experiment. Offering more flexibility in different scenarios is a top priority for future development of the GUI.

3.4 Multiple Targets

The last two buttons of interest are **Remove target** in the top right, and **Add a conversion target** on the bottom of Fig. 1. These allow the addition of additional individual conversion “tasks” for the GUI to perform. Adding targets allows the user to add files or batches of files, each of which is handled individually with its own associated metadata, status bar, and button for conversion. See Fig. 5 for an example.

/home/vir/weisslab/phui/data/022rde11_T_minus15_acc_NT_plus10_don_1.sm	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data: This file should succeed	Convert		
Converted			
/home/vir/weisslab/phui/data	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data: Here is a partially successful batch	Convert		
Converted 0 out of 1 unconverted files. 9 file(s) already converted before this attempt. Progress log: /home/vir/weisslab/phui/data/phui_conversion_progress.log			
/home/vir/weisslab/phui/data/trace_T2_300s_1_coincidence.ptu	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data: This file should fail	Convert		
Failed Conversion			
<choose a target>	Select a file	Select a directory	Remove target
<choose optional metadata file>	Select metadata file		
Enter a description of the data:	Convert		
Unconverted			
Add a conversion target			

Figure 5. Multiple targets for conversion. One successful file (top), one partially successful batch (second from top), one failed file (second from bottom), and one target that hasn’t been attempted yet and does not have any data or metadata (bottom).

Using the **Remove target** button, users can remove files or batches of files from the GUI’s display, for whatever the reason may be.

4. CONCLUSION

We have developed a GUI which lowers, but does not yet eliminate, the barriers faced by users in the adoption of Photon-HDF5. Absolutely no Python or command line experience is necessary to use the GUI, which is certainly an improvement. The primary remaining barriers are in metadata storage and discoverability.

4.1 Metadata

The GUI allows users to associate metadata with the data sets being converted, but in a very limited manner. Anything different than the defaults provided by `phconvert` must be provided via the fallback approach, i.e., manually entering the metadata into a YAML file using a text editor. Unfortunately, because of the variety of different experimental setups in the photon-counting community, most data sets will not perfectly match the defaults provided and the YAML file option will need to be used to correct the default values.

At this stage, it is also not immediately apparent to the user that there are default values for fields, or even which fields exist. As of now, nothing is displayed in the GUI that indicates the presence of these fields and that the defaults from `phconvert` are being used. This means that if a user is not familiar with the GUI, it is very easy for them to be fooled into thinking that they have successfully converted a file to Photon-HDF5, when in fact that is only partially true. While their experimental data may have successfully been converted, it is now associated with the default values provided by `phconvert`, which are not guaranteed to make sense in the user's specific case.

However, this temporary limitation can easily be avoided since most users will typically generate similar files with the hardware at their disposal. Therefore, proper use of the GUI will be guaranteed if the users use the same YAML file, adapted to their setup, simply modifying the few fields identifying each experiment before running the GUI.

Since the GUI allows entering a new data description for each new converted file, we believe that this functionality of the software will help a number of laboratories to convert their files, once they are in possession of an appropriate template YAML file. We are planning to provide a few examples of such YAML files with the first release, and encourage users to publicize theirs, together with a detailed description of their setups, so that the community can benefit from best practices from the leading labs.

Future developments of the GUI will explore the possibility of implementing a basic metadata editor in the GUI, which presents all the fields to the user and allows them to enter values into them.

A related issue is that for batch conversions, there is currently no way of specifying metadata on a per-file basis. This restricts the set of data files which can be usefully and correctly batch converted to those which are differing minimally or not at all. If even one metadata field is different between two files, they will have to be processed in separate batches or individually. Ideally, users would be able to have a fine-grained control over the metadata for each file in a batch, perhaps being able to set defaults for the entire batch and overriding or supplementing them on a per-file basis.

Finally, even if users can easily associate any metadata they want with a file and/or batch, they still need to know what fields are required in the first place. The Photon-HDF5 specification is quite rich, and learning what metadata needs to be associated with the data from which experimental setup is challenging for a first time user. Beyond the suggestion made above of using a standardized YAML file for a given setup or set of experiments, one possible (partial) solution would be to add a workflow in the GUI that walks the user through the process, guiding them towards the fields they need to include.

4.2 Documentation

A definite possibility for future improvements would be documentation for the GUI, complete with clear instructions displayed in the repository README file detailing how to install the GUI on each of the supported platforms and the system requirements for each.

4.3 Releases and Contributions

This project is hosted on Github at <https://github.com/ramenbytes/phui>, and the executables and source code can be found there. During this stage of development, all contributions such as pull requests or issues should be opened against this repository. However, it is hoped that in the future PHUI will be merged with the `phconvert` repository, in which case a notice will be posted to the above repository directing users to the new location.

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