

Phasor Analysis for TCSPC Data: Manual for the MATLAB Application

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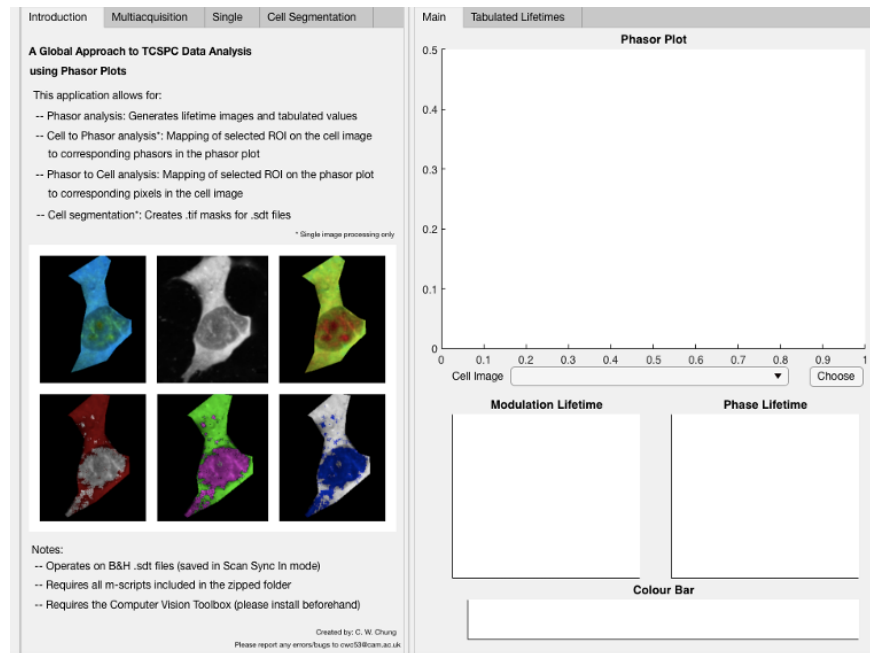


Figure 1: Main page of application

1 Notes on start up

Files are shared at @LAG-MNG-CambridgeUniversity on GitHub. The Computer Vision Toolbox will require installation on MATLAB beforehand, and a MATLAB runtime of 9.7 (i.e. MATLAB 2019B) would be needed. Default parameters given in this manual is based on sample data (i.e. within Datafiles folder).

Starting the MATLAB application

- On a PC with MATLAB runtime of 9.7 or MATLAB 2019B (and the Computer Vision Toolbox) installed, and navigate the folder containing the downloaded files.
- Double click on PhasorPlot_TCSPCFLIM_20200517_GitHub.mlapp to run the programme.
- An UI Figure window should appear showing the main page of the application (Fig.1).

2 Multiacquisition

Laser settings

- Input **Laser Repetition Rate**: 20 MHz

Cell Image

- Input **Lifetime Range**: 5 to 10 ns
- Click Browse on **Cell Image Folder**: Open Abeta42 folder under Datafiles folder
 - Abeta42 should appear automatically as **Image Name**
- Click **No. of Image**: 3 should automatically appear (total images in folder as default, change otherwise as desired).
- For **Masking**, either (change radio button accordingly):
 - Click Browse on **Mask (.tif)**: Open Cell Mask - Whole folder under Abeta42 folder
 - Intensity: 10 to 1E5 photons/px.

IRF

- Click Browse on **IRF Image (.sdt)**: Choose Rhod6G.sdt under IRF folder
- Input **Known Lifetime**: 4 ns

Processing Mode

- **Phasor plots only:** Outputs phasor plots, lifetime images, and tabulated lifetime values
- **Phasor to Cell:** Choose **No. of ROI** and **Points/ROI** as desired

Save directory for output files

- Click Browse on **Save in:** Choose folder into which output figure will be saved into (otherwise these are saved into current directory as default).

Click Start

- **Phasor plots only:**
 - Phasor plot: **Main** tab
 - Phase and modulation lifetime images: Pop out images and toggle selection **Main** tab
 - Lifetime values: **Tabulated Lifetimes** tab
- **Phasor to Cell analysis:**
 - Define ROI in pop out phasor when prompted
 - Outputs are similarly located as for **Phasor plots only**

Note on file names

For multiacquisition of the same dataset, the series of .sdt files should be named according to their folder name (bold), with a numerical appendant in the suffix (italics):

Dataset1 (Name of folder)

1. **Dataset1_1**.sdt (First image)
2. **Dataset1_2**.sdt (Second image)
3. and so forth.

Accordingly, names of .tif masks should match their corresponding .sdt files, with a space and then 'segmentation' (bold italics), as follows:

Dataset1 - masks

1. **Dataset1_1 *segmentation***.sdt (First image)
2. **Dataset1_2 *segmentation***.sdt (Second image)
3. and so forth.

3 Single Image

Laser settings

- Input **Laser Repetition Rate**: 20 MHz

Cell Image

- Input **Lifetime Range**: 5 to 10 ns
- Click Browse on **Cell Image (.sdt)**: Open Abeta42_1.sdt folder under Abeta42 folder in Datafiles folder
 - Abeta42_1 should appear automatically as **File Name (.sdt)**
- For **Masking**, either (change radio button accordingly):
 - Click Browse on **Mask (.tif)**: Select Abeta42_1 segmentation.tif under Cell Mask - Whole folder in Abeta42 folder
 - Intensity: 10 to 1E5 photons/px.

IRF

- Click Browse on **IRF Image (.sdt)**: Choose Rhod6G.sdt under IRF folder
- Input **Known Lifetime**: 4 ns

Processing Mode

- **Phasor plots only**: Outputs phasor plots, lifetime images, and tabulated lifetime values
- **Phasor to Cell or Cell to Phasor (Fig.2)**: Choose **No. of ROI and Points/ROI** as desired

Save directory for output files

- Click Browse on **Save in**: Choose folder into which output figure will be saved into (otherwise these are saved into current directory as default).

Click Start

- **Phasor plots only**:
 - Phasor plot: **Main** tab
 - Phase and modulation lifetime images: Pop out images and toggle selection **Main** tab
 - Lifetime values: **Tabulated Lifetimes** tab
- **Phasor to Cell or Cell to Phasor analysis**:
 - Define ROI in pop out phasor when prompted
 - Outputs are similarly located as for **Phasor plots only**

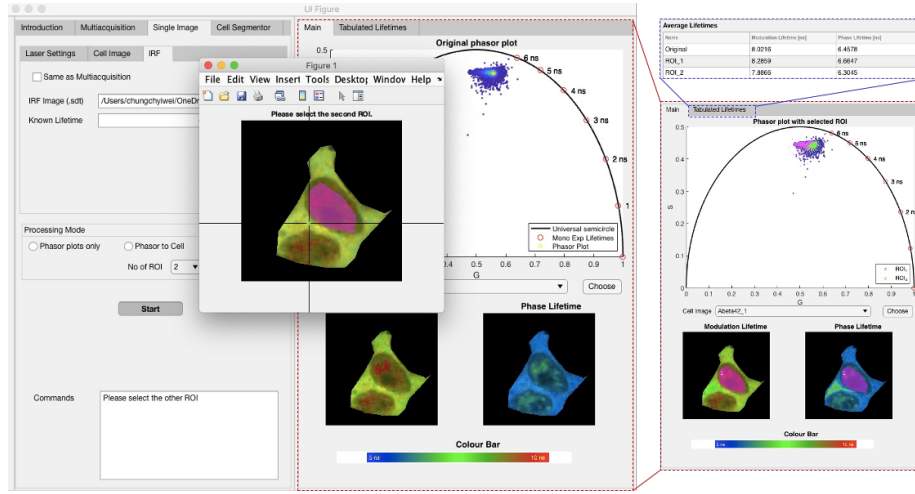


Figure 2: Cell to Phasor analysis – The user is prompted to select a ROI as desired, upon which the phasor plot and lifetime images (red inset), as well as tabulated lifetime values (blue inset) are updated to reflect the selection.

Note on file names

Names of .tif masks should match their corresponding .sdt files, with a space and then ‘segmentation’ (bold italics), as follows:

Dataset1 - masks

1. **Dataset1_1 segmentation.sdt** (First image mask)

4 Cell Segmentation

Cell Segmentation

- Click Browse on **Cell Image (.sdt)**: Open Abeta42_1.sdt folder under Abeta42 folder in Datafiles folder
 - Abeta42_1 should appear automatically as **File Name (.sdt)**
- Input **Points/Mask**

Save directory for output files

- Click Browse on **Save in**: Choose folder into which output mask will be saved into (otherwise these are saved into current directory as default).

Click Start

- Define mask on pop out cell image

Note on file names

Names of .tif masks automatically save as corresponding .sdt filename, with a space and then ‘segmentation’ (bold italics), as follows:

Dataset1 - masks

1. **Dataset1_1 *segmentation*.sdt**