Phasor Analysis for TCSPC Data: Manual for the MATLAB Application

Chyi Wei Chung (Molecular Neuroscience Group)
Please send any errors or suggestions to cwc53@cam.ac.uk

May 14, 2020

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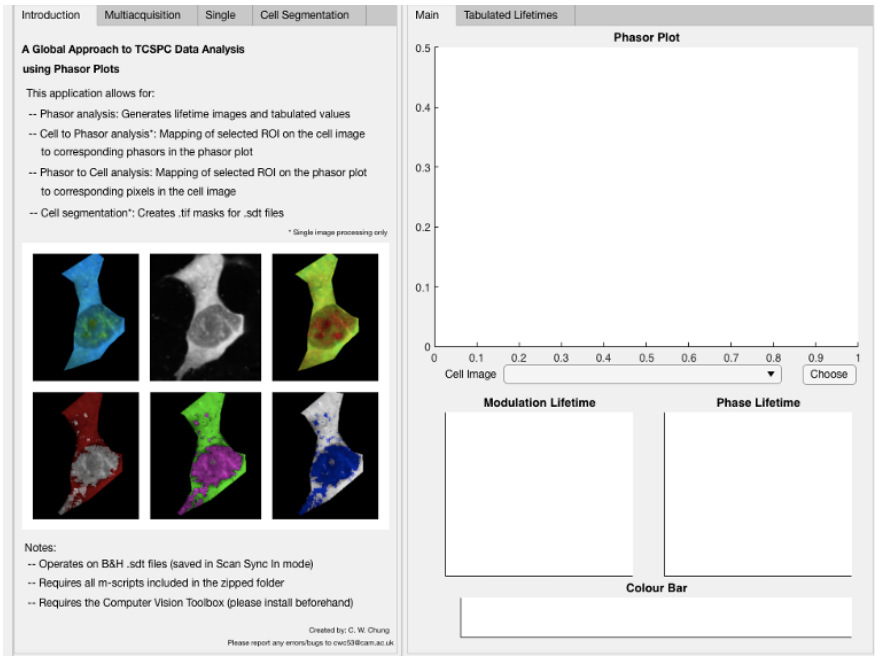

Figure 1: *Main page of application*

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 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 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**

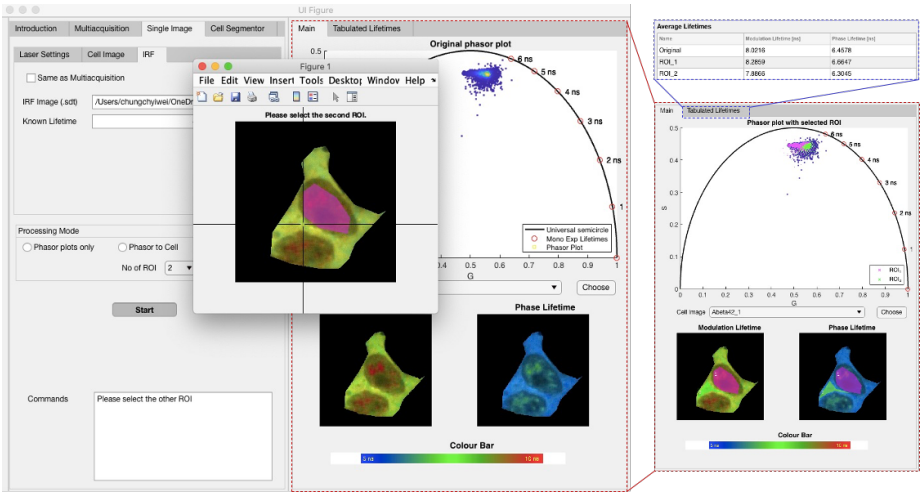

Figure 2: *Cell to Phasor analysis – The user is prompted to select a ROI as desired, upon which the phasor plot and lifetime images (red inset), as well as tabulated lifetime values (blue inset) are updated to reflect the selection.*

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