Phasor Analysis for TCSPC Data: Manual for the MATLAB Application

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Please send any errors or suggestions to cwc53@cam.ac.uk
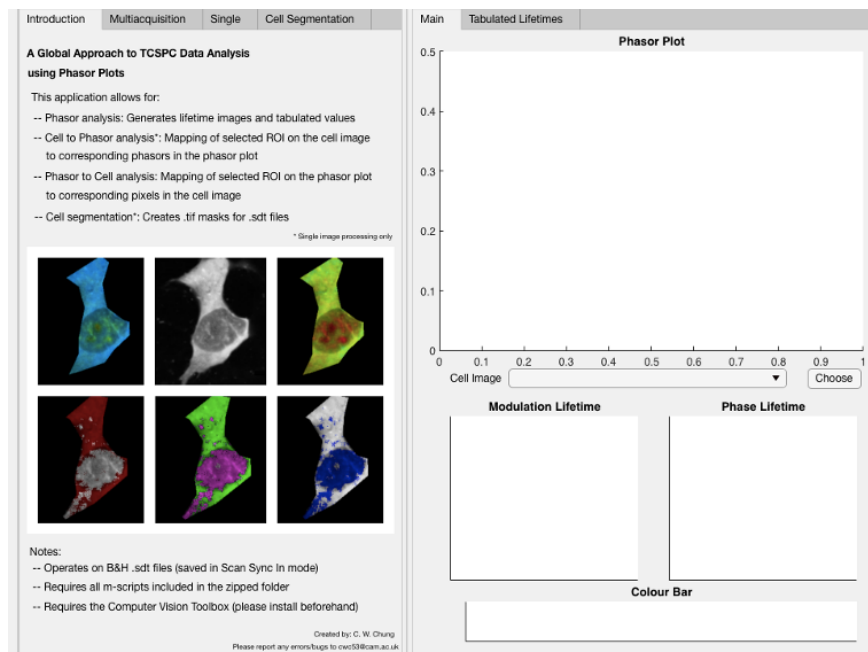
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Contents

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).

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**: 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
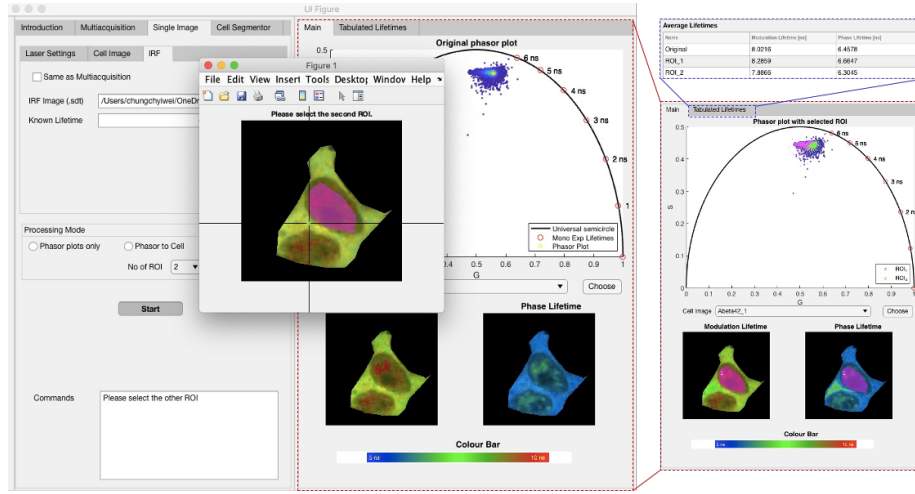   - Outputs are similarly located as for **Phasor plots only**

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