User Guide for climeLoc

Overview:

Channel-linked multi-emitter localization (climeLoc) is a software for single-molecule localization microscopy (SMLM). It can be opted between the single-emitter fitter for regular SMLM data and the multi-emitter fitter for SMLM data at high density. It can be used for single-channel SMLM data as well as for multi-channel data for which the localization is correlatively performed across different channels.

The procedure of running climeLoc includes (See appendix for each step):

1. **PSF calibration**. A stack of beads that was acquired in a range of ±1000 nm with respect to the coverslip with 10~50 nm spacing in z will be used to calibration the PSF. The calibration will align, normalize, and average the beads to generate the PSF model. The PSF model is characterized by Gaussian (if available) and interpolated by Cubic Splines. This step generates the calibration files such as the camera settings (Camera_settings.pkl) and the PSF information (PSFCalibration_unlinked.pkl). These files are saved in the directory where the stack of beads is and will be used for single-molecule localizations.
2. **Channel calibration (optional)**. If the stacks of beads were acquired in a multi-channel configuration, the bead image will be also used to calibrate the polynomial transformation across the channels. in this case, at least d^2 beads are needed for the calibration, where d is the degree of the polynomial warping.
3. **SMLM localization**. The main body of climeLoc. It needs the calibration files generated during PSF calibration. It will make a subdirectory named smlm_result in the directory where the SMLM data is saved, and will generate the localization data (XXX_rawlocsnm.pkl) saved in the smlm_result folder.
4. **Post Process**. Post processing of the localization data, including customized filtering, redundant cross-correlation for drift correction, in-frame merging (if it was multi-emitter fitting), and grouping the localizations emitting photons in consecutive frames. It reads the XXX_rawlocsnm.pkl localization file and saves a new localization file XXX_locsnm.pkl saved in the same directory after all the post processes.
5. **Fluorophore register (optional)**. For ratiometric data, a step to register each localization to a type of fluorophores. It directly modifies the XXX_locsnm.pkl file.
6. **Visualization**. This render the localizations into super-resolution images. It reads the XXX_locsnm.pkl localization file and saves the reconstructed images in the same directory.

Requirements:

1. Microsoft windows 10 or newer, 64-bit
2. CUDA capable graphics card.
3. CUDA 11 compatible graphics driver.
4. The cuda codes were compiled with minimum Compute Capability 7.5

Run climeLoc:

Use windows PowerShell to get into the directory where the codes are unzipped.
Use command line `python -m GUI_calibration` to perform the PSF calibration and channel calibration, following the instructions (Appendix).
Use command line `python -m GUI_smlmloc` to perform SMLM localization, following the instructions (Appendix).
Use command line `python -m GUI_smlmpp` to perform all the post processing and rendering, following the instructions (Appendix).

Appendix

Calibration -> PSF Calibration

Beads Info

- `modality`: imaging modalities.
    a. `2D`: for bead stacks acquired at a series of z-positions on a common 2D setup.
    b. `2Dfrm`: for a single frame bead image acquired at the focus plane on a common 2D setup.
    c. `AS`: for bead stacks acquired at a series of z-positions on an astigmatic 3D setup.
    d. `BP`: for bead stacks acquired at a series of z-positions on a bi-plane 2D setup.
    e. `DH`: for bead stacks acquired at a series of z-positions on a double-helix 3D setup.
- `bead fname`: absolute file name of the bead stack. Calibration results will be saved in the same directory, including camera settings `Camera_settings.pkl`, PSF model `PSFCalibration_unlinked.pkl`, etc.

Camera Info

- `from file`: check if the camera specifications are saved in a python pickle file as a dictionary.
- `cam_spec`: absolute file name of the camera specification file, ignored if from file is not checked.
- `chip size`: chipszx and chipszy of the camera chip, disabled if from file is not checked.

- `cam_offset`: offset of the camera (ADU), pixel-unspecific, disabled if from file is not checked.
- `cam_var`: the read-out noise of the camera (e⁻), pixel-unspecific, disabled if from file is not checked.
- `cam_A4D`: the analog-to-digital conversion (e⁻/ADU), pixel-unspecific, disabled if from file is not checked.
- `cam_emgain`: the EMgain of the camera if an EMCCD is used and the EM Gain is applied, pixel-unspecific. Set as 1.0 for sCMOS cameras. disabled if from file is not checked.

Camera Plus

- `view_type`: the channel configurations.
  a. `fullview`: for single-channel configuration.
  b. `dualview`: for left-right 2-channel configuration.
- `scmos_var`: check if pixel-specific characteristics of the sCMOS camera is provided in the cam_spec file. If the pixel-specific characteristics of the sCMOS camera is provided but scmos_var is not checked, averaged value will be used for the camera settings. Ignored if no pixel-specific characteristics file is provided.
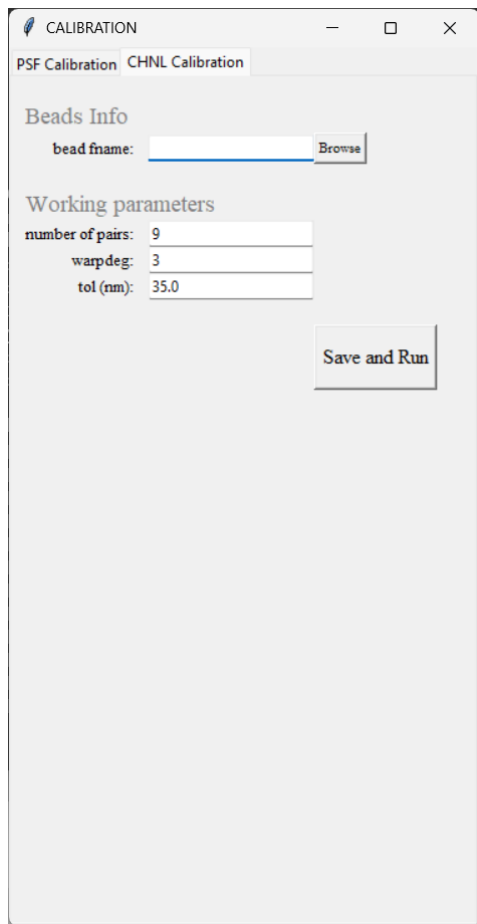- `px size (nm)`: pixel size in the x and y axes in nanometer.

Axial Info

- `zstepsz(nm)`: size of the z step as the bead step was acquired. Ignored if the modality is `2Dfrm`.
- `zfocus(slice)`: the index of the slice at the focus for the two channels. It could be different if modality is BP. Make the two the same if it is a `fullview` (single-channel) configuration.
- `zrange(nm)`: axial range for bead calibration, must be not greater than the thickness of the bead stack.

Working Parameters

- `box size`: cubic spline will be calibrated within a radius of the box size (in pixel) around the center of the aligned and averaged bead.
- `beads number`: number of the beads to manually pick for alignment and average.

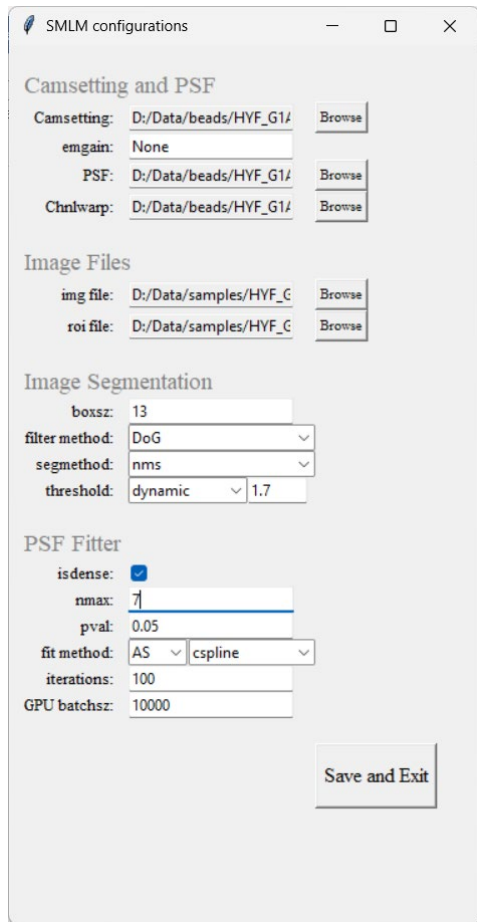# Calibration -> CHNL Calibration (optional)

Beads Info

- `bead fname`: absolute file name of the bead stack. Calibration results will be saved in the same directory. The camera settings `Camera_settings.pkl` and the PSF model `PSFCalibration_unlinked.pkl` in the same directory generated during `PSF Calibration` will be used.

Working Parameters

- `number of pairs`: number of the bead pairs for manual-picking of the beads. On the promoted bead image, click on a bead in the 0-th (left) channel, and click the same bead in the 1-th (right) channel, and then click the second bead in the 0-th channel, and the same bead in the 1-th channel… until all the `number of pairs` beads are clicked. The clicked position will automatically move to the center of a bead adjacent to the mouse click. The manually picked pairs are used to initialize the transformation for finer mapping in the later procedures.
- `Warp deg`: degree of the polynomial transformation.
- `tol(nm)`: the tolerance distance to match two beads into a pair.

SMLM Localization

Camsetting and PSF

- `Camsetting`: the camera setting file `Camera_settings.pkl` generated during `PSF calibration` in the directory where the bead stack is saved.
- `emgain`: The EM Gain for SMLM imaging, leave it `None` if the EM Gain is set the same as the acquired bead stack.
- `PSF`: the PSF model `PSFCalibration_unlinked.pkl` generated during `PSF calibration` in the directory where the bead stack is saved.
- `Chnlwarp`: the calibrated channel warping `chnlwarp_coeff.pkl` generated during `CHNL Calibration` in the directory where the bead stack is saved.

Image Files

- `img file`: the directory where the SMLM tiff images are saved. A `smlm_result` subdirectory will be made (if not exists) in this directory and the localization results will be saved as a python dictionary in a pickle file named as `XXX_rowlocsnm.pkl`. This file will be used for post processing and render in the later procedures.
- `Roi file`: the absolute name of a single .roi file or .zip file for multiple rois. The rois should be drawn by ImageJ. For `dualview` configuration, the rois should be drawn in the 0-th (left) channel.

Image Segmentation

- `boxsz`: the size of the box to segment each single-molecule peak, in pixel.
- `filter method`: the filtering method for peak detection.
  a. `Gauss`: the background is extracted and then images are filtered by a Gaussian kernel.
  b. `DoG`: difference-of-Gaussian.
  c. `DoA`: difference-of-Atrous.
  d. `PSF`: the background is extracted and then images are filtered by the central slice of the PSF model.
  e. `MIP`: the background is extracted. Images are then filtered by each slice of the PSF model and return the maximum projection.
- `segmethod`: segmentation method.
  a. `nms`: local maxima will be filtered via non-maximum-suppression.
  b. `grid`: Images are segmented into grids. Grids without local maxima are discarded.
  c. `None`: Images are segmented around each local maximum without nms.
  d. `threshold`: threshold method and corresponding values to filter the local maxima.
     i. `dynamic`: a dynamic threshold for each frame according to the quantiles of the local maxima.
     ii. `pval`: pixel values of the images are transferred from the Poisson distribution to a normal distribution and thresholded by a corresponding p-value.
     iii. `std`: folds of the standard deviation of the difference images filtered by `DoG` or `DoA`.
     iv. `const`: constant threshold in e⁻.

PSF Fitter

- `isdense`: check for multi-emitter fitting.
- `nmax`: maximum number of emitters for multi-emitter fitting, ignored if `isdense` is not checked.
- `pval`: the p-value to determine if the fitting should be processed from the N-emitter to $(N + 1)$-emitter model.
- `fit method`: modalities (needs to be agreed with the modality chosen for PSF Calibration) and the PSF model, either `cspline` for Cubic Spline interpolated experimental PSF model or `gauss` for Gaussian approximated PSF model.
- `iterations`: number of L-M iterations for PSF fitting.
- `GPU batchsz`: number of boxes sent to GPU at the same time for PSF fitting.

SMLM Post Process -> Post Process

raw localization files

- `_rawlocsnm.pkl`: the raw `XXX_rawlocsnm.pkl` localization pickle file generated by SMLM localization. A `XXX_locsnm.pkl` localization pickle file will be saved in the same directory after following procedures.

Qc filters (quality control filters)

- `p-value`: the p-value to reject a PSF fitting. The p-value is calculated by the $2\chi^2_{\text{MLE}}$ and the degree of freedom.
- `frm_rng`: the starting and ending frame index.
- `min_photon`: photon numbers less than `min_photon` will be rejected.
- `zrange(nm)`: the lower and upper z positions.
- `prec(nm)`: the $\sqrt{\text{CRLB}}$ threshold in x, y, and z axis. Precision larger than these values are rejected.
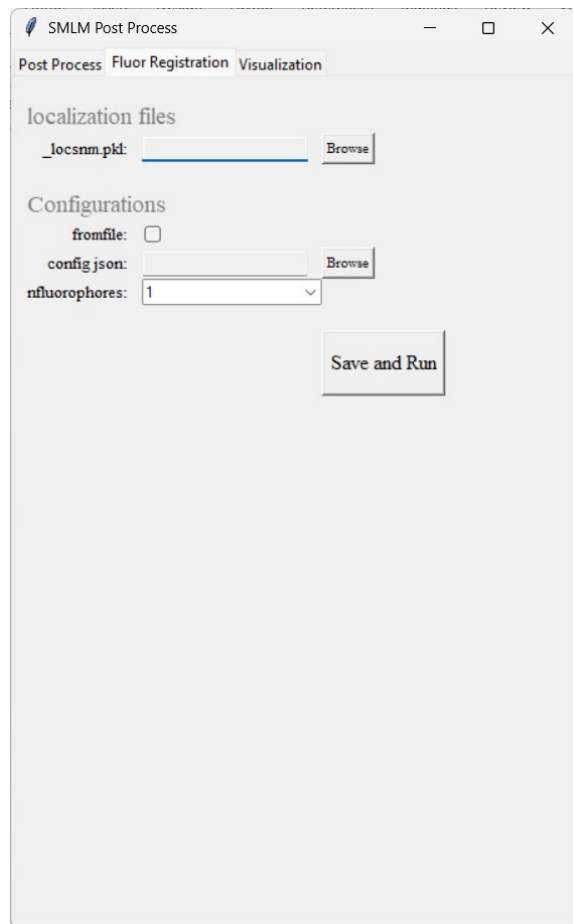
RCC drift correction

- `rcc`: check if to perform rcc drift correction on the data.
- `tar_pxsz`: the localizations are rendered to pixelized images with pixel size of `tar_pxsz` in nm to calculate the correlation.
- `frmbinsz`: number of consecutive frames to consider as one time point for rcc.

- `ccorrsz(nm)`: the window size in x, y, and z axis around the center of the correlation map to search for the correlation maximum.
- `fitsz(nm)`: the window size around the correlation maximum to finer fit the correlation maximum with a Gaussian model.

In-Frame merge and Consecutive Group

- `merge`: check for in-frame merging.
- `maxR(nm)`: the radius (lateral) for merging.
- `maxZ(nm)`: the radius (axial) for merging.
- `group`: check for consecutive group.
- `maxR(nm)`: the radius (lateral) for grouping.
- `maxZ(nm)`: the radius (axial) for grouping.
- `gap frame_tol`: number of gap frames allowed for consecutive group.

SMLM Post Process -> Fluor Registration (optional)

localization files

- **_locsnm.pkl**: the `XXX_locsnm.pkl` localization pickle file generated after `Post Process`. The localization pickle file will be modified after `Fluor Registration`.

Configurations

- `fromfile`: check if a ratiometric configuration json file is available.
- `config json`: the absolute file name of the ratiometric configuration json file. Disabled if `fromfile` is not checked.
- `nfluorophores`: number of fluorophores in the ratiometric data.

SMLM Post Process -> Visualization

localization files

- `_locsnm.pkl`: the `XXX_locsnm.pkl` localization pickle file generated by `Post Process` and modified after `Fluor Registration`. A `XXX_locsnm.pkl` localization pickle file will be saved in the same directory after following procedures.

Visualization

- `tar_pxsz`: size (in nm) of the pixel of the image to render.
- `frm_range`: the starting and ending frame index to render.
- `non-singlet`: check if isolated localizations need to be excluded. A localization is considered as a singlet localization if no more than 2 neighbors can be found within a radius of the input number.
- `crlb blur`: the sigma of a Gaussian when convolving a localization with a Gaussian kernel. If the input is negative, the convolution sigma of each localization is its $\sqrt{CRLB}$.
- `Intensity`: the amplitude of a Gaussian when convolving a localization with a Gaussian kernel.
  a. `blink`: the amplitude is 1 for each localization.
  b. `photon`: the amplitude for each localization is its photon numbers.
- `alpha`: the transparency when overlay a new localization onto the rendered image. A new localization is directly added onto the image if `alpha` < 0.

RGB Options

- `RGB disp`: check if to render images into RGB.
- `feature`: the feature to encode into the colormap for RGB rendering.
- `zmin/zmax`: the lower and upper bound in nm of the z positions.
- `nfluorophores`: number of fluorophores to render.
- each row following is the `name of fluorophore`, `minimum quantile`, `maximum quantile`, `colormap`, respectively, for rendering.