Localization and Tracking Toolbox for Ultrasound Superresolution

User Guide

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Academic references to be cited

Details of the code published in 2020 article by Heiles, Chavignon, Hingot, Lopez, Teston, and Couture. Open Platform for Ultrasound Localization Microscopy: performance assessment of localization algorithms

1. General information

Aim

LOTUS is software for ultrasound localization microscopy. Individual and isolated microbubbles are localized with sub-pixel precision. Then a tracking algorithm pairs microbubbles' positions into long trajectories. It enables microvascular imaging in various organs.

Minimum requirements

LOTUS software integrates MATLAB Runtime R2021a (9.9) (MathWorks). We recommend using Microsoft Windows 10 (version 1803 or higher), with 4 GB RAM minimum, and 5 GB of free space.

Installation instructions

1. Download LOTUS from Github repository
2. Unpack the archive in a preferred directory.
3. Launch the installer LOTUS_v1_3_installer.exe
4. Follow the instructions
5. Restart your computer
6. LOTUS should be now available for use in the start-up menu

Complementary scripts

This GUI is part of a larger platform called PALA available on https://github.com/AChavignon/PALA/ This repository contains all the scripts run by PALA and we strongly recommend the users wanting to devise their own scripts or understand the algorithms called by LOTUS to visit the page. For all the variables defined in this user guide, we have added the name of their respective counterpart in the scripts in italic : "*See value XX in the PALA_scripts*". The user can read and run the script PALA_InVivoULM_example.m as a starting point.

Input data type

LOTUS software accepts a list of images or a list of folders containing these files.The images can be given in the .mat format, in which case they are organized in a [space, space, time] matrix, or in a .bin format in which case the user is prompted to give the size of the matrix (more details in section 2 o this user guide). Images can be complex or real, without log compression. In the .bin format, the user can choose whether to recompose complex IQ data according to this operation:

```
size2=size(fileRead,2);
IQ=fileRead(:,1:size2/2,:)+1i*fileRead(:,size2/2+(1:size2/2),:);
```

For long acquisition, data should be split into multiple individual files of a maximum of 2000 images to prevent time-consuming filtering (max 200 MB per file). The name of the variable containing the images is assumed to be IQ by default. In the case where LOTUS can not clearly identify the name of the variable, it will ask the user to define a name in a pop-up dialog box.

Image visualization

Images will be saved in the specified folder. We recommend using ImageJ software to visualize tiff images and adjust brightness/contrast/color balance. (https://imagej.nih.gov/ij/)

Any questions should be addressed to the authors via the Github repository by opening an issue.

Disclaimer

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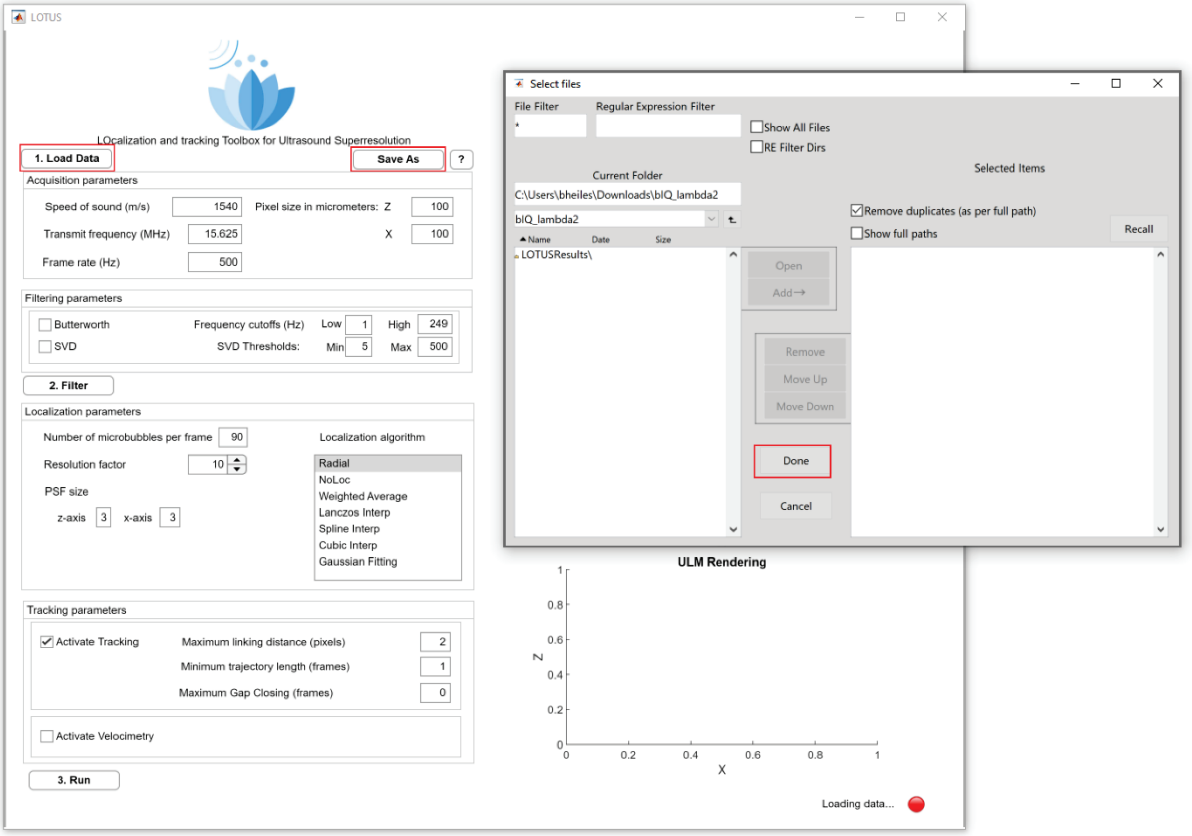
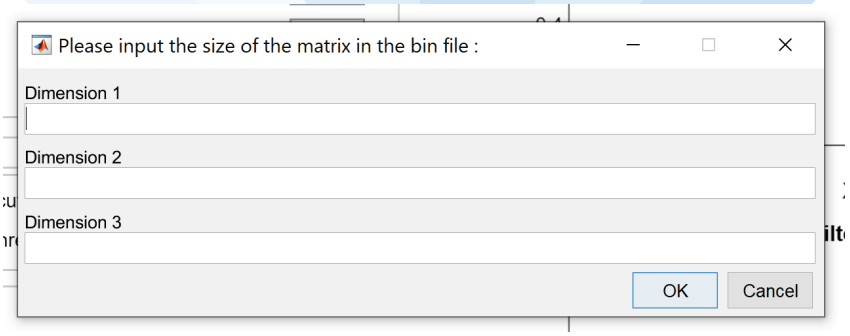
2. Open LOTUS and load data

Click on "Load Data" to import stacks of images. For .mat files, images must be stored in a variable *IQ(n_x, n_z, n_t)*. Pixel size must be provided in micrometers.

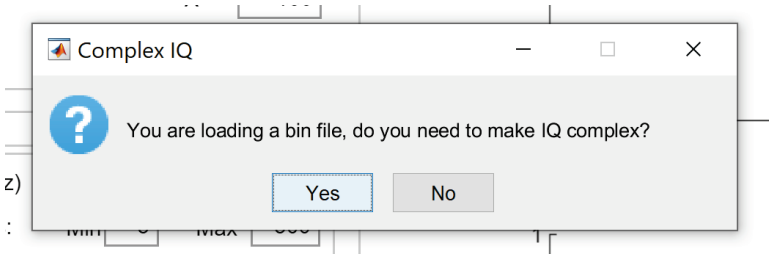
You can add both folders and files, LOTUS will take all ".mat" files including in the folder containing the files. WARNING: if you add files and there are other ".mat" files in the folder containing these files, LOTUS will load all of them. You should keep your images in a folder containing images only.



When loading .bin files, the user will be prompted with a dialog box asking to input the size of the matrix data to be recomposed. It is assumed that the matrix loaded is a 3D matrix of size Dimension 1 x Dimension 2 x Dimension 3.



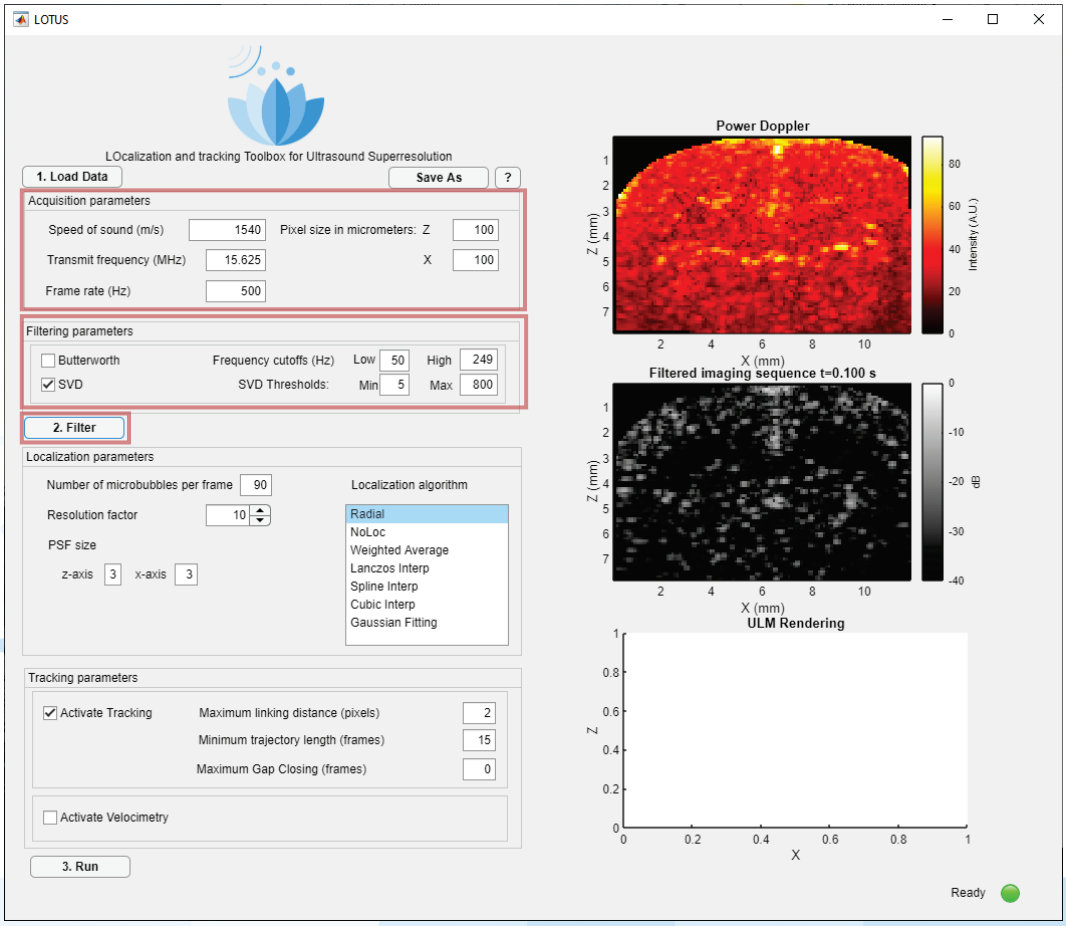
Following reshaping of the matrix, another dialog box pops up asking if LOTUS needs to recompose the complex IQ data.



The operation done if the user clicks yes is:

```
size2=size(fileRead,2);
IQ=fileRead(:,1:size2/2,:)+1i*fileRead(:,size2/2+(1:size2/2),:);
```

3. Select acquisition and filtering parameters



Acquisition parameters

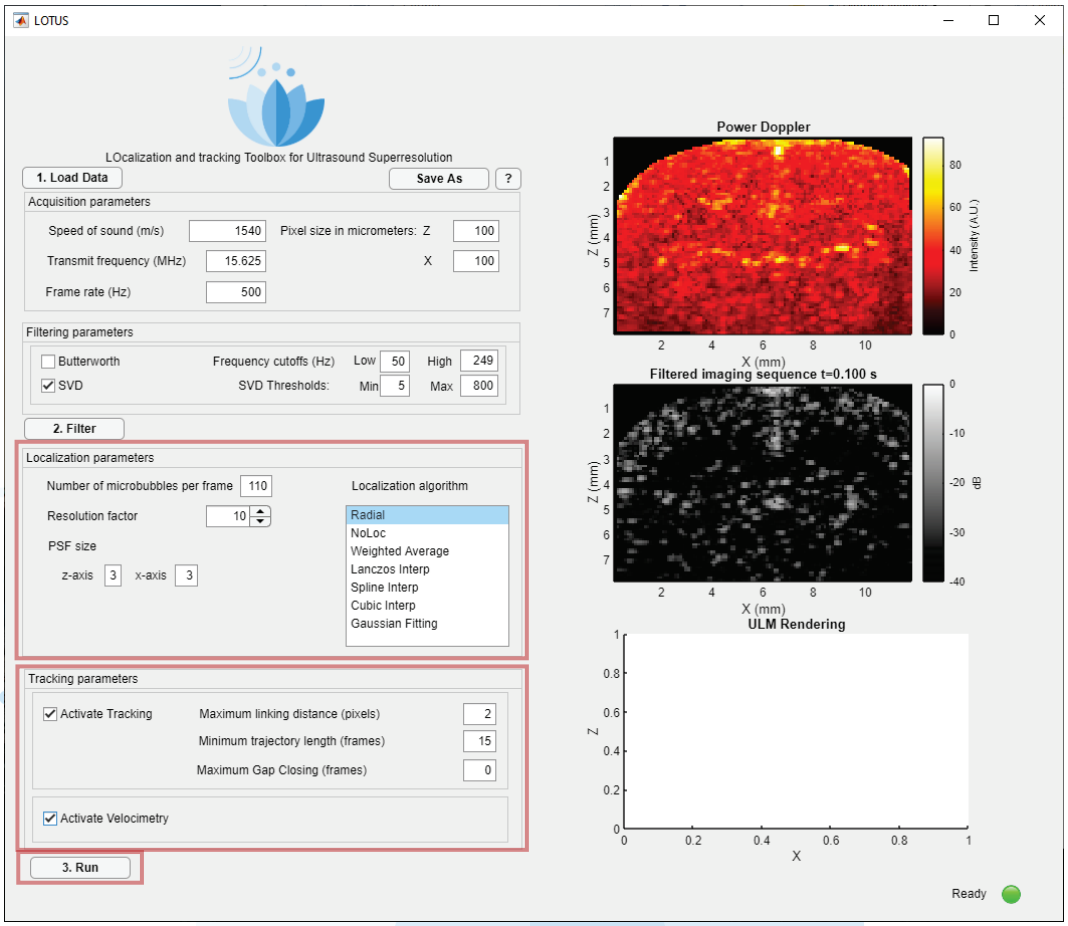
- Speed of sound: media speed of sound in meters per second
- Transmit frequency: in MHz, for wavelength estimation *See value UF.TwFreq in the PALA_scripts (available on Github* https://github.com/AChavignon/PALA*)*
- Framerate: compounded frame rate of the images fed to LOTUS in Hz *See value framerate in the PALA_scripts*
- Pixel size in micrometers: height [z] and width [x] of pixels in micrometers *See value ScaleOfPixel in the PALA_scripts*

Filtering parameters

- Singular Value Decomposition: adjust the minimum and maximum kept eigenvalues *See value ULM.SVD_cutoff in the PALA_scripts*
- Butterworth: adjust the range of the bandpass filter in Hz *See value ULM.ButterCuttofFreq in the PALA_scripts*

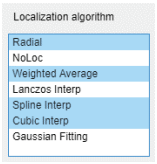
Note: You can click on “2. Filter” to visualize the result of the filtering in the middle figure. It is highly recommended to try a few parameters and then run the entire algorithm to avoid unnecessary calculations.

Select localization and tracking parameters



Localization parameters:

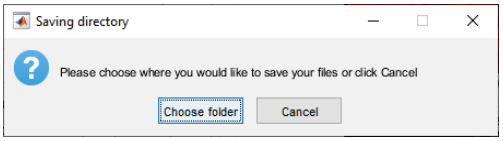
- Number of microbubbles per frame: estimated number of microbubbles in a unique frame (in 2D, a dozen to a couple of hundreds depending on the dataset) *See value ULM.numberOfParticles in the PALA_scripts*
- Resolution factor: the resolution factor determines by how much the original pixel size in micrometers will be multiplied by (ex: an original 100x100 micrometers pixel size with a resolution factor of 10 will result in a final image with a pixel size of 10x10 micrometers) *See value ULM.res in the PALA_scripts*
- PSF size: the estimated size of the Full Width at Half Maximum of the microbubbles in pixel (usually the size of the microbubble PSF is a few wavelengths in each axis). *See value ULM.fwhm in the PALA_scripts*
- Localization algorithm: select one or multiple localization kernels *See value ULM.LocMethod in the PALA_scripts*



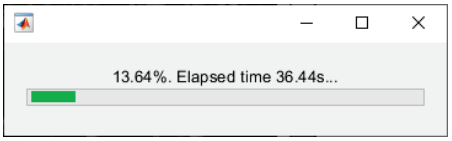
Tracking parameters

- Maximum linking distance: the maximal distance between two microbubbles that can be paired in pixels *See value ULM.max_linking_distance in the PALA_scripts*
- Minimum trajectory length: minimum length of a trajectory to be kept in frames *See value ULM.min_length in the PALA_scripts*
- Maximum Gap Closing: allowed gap between frames to link microbubbles together *See value ULM.max_gap_closing in the PALA_scripts*

Press "Run" to launch LOTUS. If no saving directory has been define, you will be asked to select a saving directory.



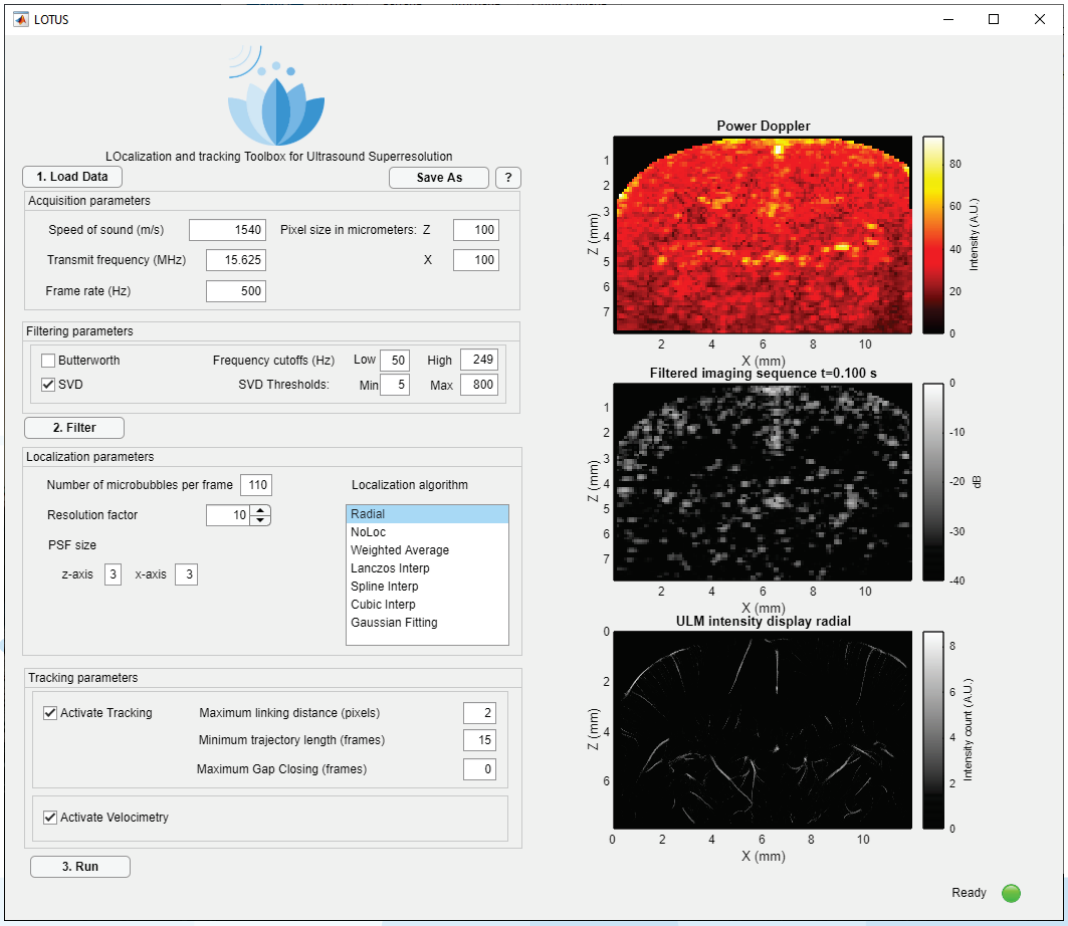
LOTUS will then start ULM processing.



Parallel execution:

The software will try to execute the process on many parallel workers at once. This way, the calculation time is reduced as much as possible. If it can not run in parallel, it will run normally, executing one task after the other. The downside of parallel execution is that there is no way it can be stopped once it is launched so we recommend the user to start testing its parameters on a reduced dataset before executing the whole process.

4. Display results

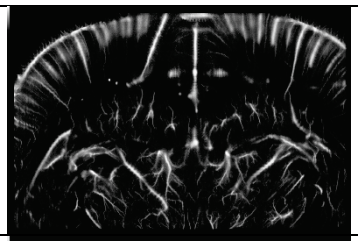
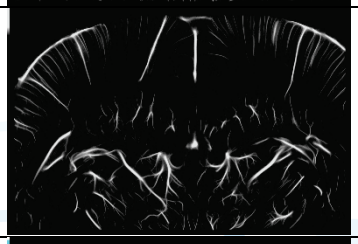
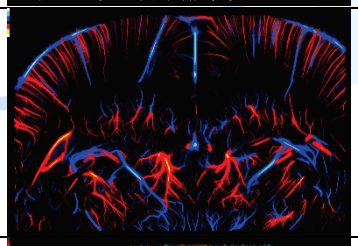
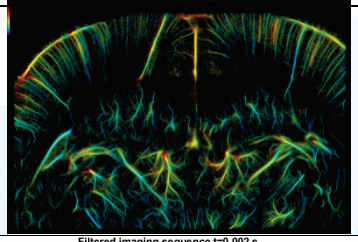
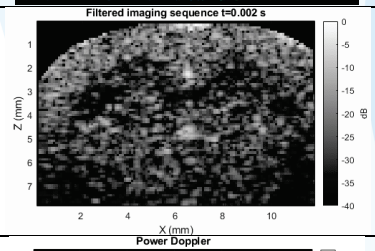
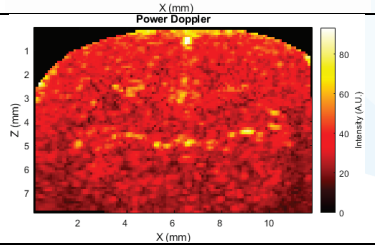


When the processing ends, the intensity rendering is displayed in the GUI.

Additional renderings are directly saved in the saving directory given that one was chosen.

Saved files

LOTUS software provides different rendering based on intensity, velocity, and direction of microbubbles' trajectories, saved in the folder called LOTUSResults_XXXXXX in a folder containing the name of the algorithm used, for example : "./LOTUSResults_2022_02_16_11_44_54/radial/"

	***LOTUS_MatrixLoc_xxxx_locAlgo.tif*** Intensity rendering with localization of microbubbles
	***LOTUS_MatrixTrack_xxxx_locAlgo.tif*** Intensity rendering with tracking of microbubbles and interpolation of trajectories
	***LOTUS_MatrixZdir_xxxx_locAlgo.tif*** Intensity rendering with upward trajectories in red, and downward trajectories in blue
	***LOTUS_MatrixVeLNORM_xxxx_locAlgo.tif*** Velocity rendering with average velocity norm
	***LOTUS_filtered_xxxx.gif*** Movie of the filtered images
	***LOTUS_PDoppler_xxxx.tif*** Power Doppler extracted from the filtered images

Raw data (localization, tracks, and rendering matrices) are saved in MatLab files:

- The file LOTUS_Localizations_All contains two variables
  1. ULM: A structure regrouping all the parameters of the ULM process

Field	Description
numberOfParticles	Number of particles to be localized
size	Size of the input image (z,x,time)
scale	Scale of the pixel in mm and time between frames
lambda	Wavelength value in mm
framerate	Framerate iin Hz
res	Resolution factor
seuil	Thresholds of the SVD filter
ButterCutoffFreq	Butterworth filter values
max_linking_distance	Maximum distance allowed to pair two particles
min_length	Minimum length of a trajectory (in number of particles)
fwhm	Full Width at Half Maximum of the PSF of a particle
max_gap_closing	Trajectories can be linked in between frames by setting this number to a non-zero
interp_factor	Interpolation time factor on which trajectories are interpolated (time between each points of the trajectory will be time between frames times this factor)
Tracking	Logical value (1 if tracking is required)
Velocimetry	Logical value (1 if velocimetry is required)
isSVD	Logical value (1 if SVD filtering is used)
isButter	Logical value (1 if low-pass Butterworth ffiltering is used)
SRscale	Scale of the final pixel in the superresolved image
SRsize	Size of the superresolved image in pixels
LocMethod	Localization method
MethodName	List of localization methods selected
InterpMethod	Interpolation method (only if an interpolation based method is used)

2. Localizations: A number of cells matching the number of files input into LOTUS containing:

Localizations{1, 1}

	1	2	3	4
1	2.8279e+06	0.0022	0.0022	1
2	3.3051e+06	0.0047	0.0047	1
3	3.2622e+06	0.0019	0.0019	2
4	2.6758e+06	0.0022	0.0022	2
5	3.8631e+06	0.0031	0.0035	2
6	3.5597e+06	0.0042	0.0046	2
	Pixel intensity	Axial and lateral coordinates (meters)		Frame index

- LOTUS_TrajectoriesAll:
  1. ULM: A structure regrouping all the parameters of the ULM process (same as table above)
  2. an ensemble of cells containing all trajectories computed and concatenated. If opened:

Trajectories{1, 1}

	1	2	3	4	5
1	0.0022	0.0022	0.0150	0.0100	0
2	0.0022	0.0022	0.0150	0.0100	8.0000e-05
3	0.0022	0.0022	0.0150	0.0100	1.6000e-04
4	0.0022	0.0022	0.0150	0.0100	2.4000e-04
5	0.0022	0.0022	0.0150	0.0100	3.2000e-04
6	0.0022	0.0022	0.0150	0.0100	4.0000e-04
	Axial and lateral coordinates (meters)		Axial and lateral velocities (meters/second)		Time sampling (seconds)

5. Error logs

In case of errors, a log file is created in the following folder C:\Users*username*\AppData\Roaming\MathWorks\MATLAB Add-Ons\PersistentFolder\Logs with a time stamp. If you have a question regarding an issue, please send us this log file and the file and steps done to reproduce the error.