Super-resolution ultrasound imaging software

This document gives brief descriptions about software components. More details will be given in a future paper. Questions and feedbacks can be posted on Github Issues or sent to Jipeng Yan (j.yan19@imperial.ac.uk; jipeng_yan@163.com).

1 Introduction

The software is designed for generating super-resolution ultrasound (SRUS) images based on low-frame-rate data acquired from commercial ultrasound (US) systems. The software is also compatible with high-frame-rate data. The algorithms embedded in this software **won the Ultra-SR Challenge in Localisation and Tracking category. Some codes used for the challenge are revised for easy and general purpose use. We are open to collaborate if you have specific requirements and please feel free to contact us at:** j.yan19@imperial.ac.uk, mengxing.tang@imperial.ac.uk.

The software contains eight components: loading data, quick run, motion correction, background removal, super-localisation, tracking, plotting, and post-processing. In the top Menu bar, the current processing parameters can be saved, or previous processing parameters can be loaded. The software can save the results of each step and load data for re-processing without going through each step from the beginning. Graphical guides can be found in the User Guide.

2 Before using

Current Version 2.3

Components	Version (Main. Minor)
GUI	**2.3**
Quick Run	**2.0**
Motion correction	**2.0**
Background removal	**2.0**
Localisation	**3.1**
Tracking	**2.0**
Plotting	**2.2**
Parameter Calculation	**2.1**

The user should note the version used for processing data and use the same main version for all the data to be analysed and compared.

2.1 Installation

The software was tested using the following system hardware/software:

- Windows 10
- CPU: AMD Ryzen 9 5900 Processor
- GPU: Nvidia Geforce RTX3080
- RAM: 128 Gb

Different configurations can be used but have not been tested so will not necessarily run as expected. To install the software:

- Download ULIS_SRUS(version).exe file.
- Run above .exe file as administrator.
- If first time installing, select folder for R2021a Matlab Runtime installation otherwise skip this step.
- Choose path for installation.

Please make sure your computer has enough RAM when you process long-acquisitions If you have issues with this you can also split the frames into parts and contact us to get codes for merging the data.

If you have any questions/issues regarding installation, please email us.

2.2 Authorisation

This software is totally free for research purpose, but the usage of the software still needs our authorisation to help us know who is using the software. We also expect feedbacks from every user to help us improve the software performance or make the software easier to use. **The user can use the software without authorisation before the end of 2023. Some functions will be limited after that day without authorisation.** It is easy to get the authorisation by below steps.

1. Click authorisation and then system info to generate an identical file for your PC.
2. Send the system info file to us.
3. We will send you back the key as soon as possible.
4. Click authorisation and then activate and select the key we sent to you.

Please do not delete the key file after activation. When you move the key to another folder, you need to activate the software again by selecting the key in the new folder. The key is valid for one year and you can renew it by following instructions above again.

2.3 Key tips

- Folder names in the path of the data should be without spaces or any special characters. Otherwise, the software cannot create folders for the results. As we noticed, following characters are allowed to be used for the folder and file names: a-z, A-z, 0-9, _ .
- Pixel resolution of the input data cannot be too low. According to our practice, each dimension of the Point Spread Function (PSF) should contain at least 5 pixels. The software needs to estimate PSF from the data. If the pixel resolution of the input data is too low, the PSF cannot be estimated, and the software won't work.
- RAM of PC should be large enough (at least 20 times of input data size). Videos input from DICOM or AVI/MP4 files usually becomes much larger than the original file in the software and many intermediate variables need to be kept during the processing.
- 'Out of memory' error in localization. Deconvolution uses lots of memory for data with high bubble concentrations; Cross-correlation (before **Localisation** version 2.1) used lots of memory for data with large pixel map and high SR ratio. Using multi-thread can speed up the computation time but has the risk to lead to out of memory error. If the error happens, the user should disable multi-thread and click 'Localisation (All).'
- 'Out of memory' error in tracking. Tracking processing can also result in 'out of memory' error if there are too many bubbles in three adjacent frames. User can reduce the risk of this problem when they test parameters of localisation by using a high background removal threshold or discard frames with too high bubble concentrations after motion correction. Automatic algorithms to deal with this error will be provided in the future version of software.
- The user can make the 'Save Mat Files' button unpressed when tuning processing parameters to save time and is suggest making the button pressed after fixing processing parameters.

3 Components and Methods

3.1 Load Data

3.1.1 Open File

Video data in DICOM, AVI and MP4 format can be loaded by the software. As names of variables vary in the DICOM files of different systems, we have specified the functions for loading DICOM data generated by three machines three brands, 'Canon', 'Mindray', and 'Philips'. If the user meets problems when loading the DICOM file, it is better to send us your file and we add a new function (button) accordingly. User can do motion correction and remove noise using their own code, and then load Mat file generated by Matlab to the software for localisation and tracking. User needs to make sure the variable names in the file follow below instruction.

Mandatory variables:

C, or **contrast_image**, or **registered_contrastimage** – CEUS sequence, 3D matrix (depth x lateral x frame)

Optional variables:

x_res - pixel size along lateral resolution. If not provided, user needs to input in the 'Data Information'

z_res - pixel size along depth resolution. If not provided, user needs to input in the 'Data Information'

frame_rate – frame rate of the input data

B_mode_image – B-mode sequence, 3D matrix (depth x lateral x frame). If not provided, motion correction won't work.

3.1.2 Set Data Information

Pixel resolution and frame rate can be read from DICOM and mat file if the variable names in the files are known by the software. Otherwise, the user needs to measure the pixel resolution on the image by clicking the 'Measure' button and sets the frame rate manually. Sometimes, there is only a scale along the depth direction in the image. In this case, the pixel resolutions along the depth and lateral directions are generally same. The user can push the 'Square Pixel' button down, only measure the pixel resolution along the depth and then get two resolutions.

The user should check if the frame rate is right. Frame rate read from the DICOM file might be different from the value used in acquisition. Some machines downsample the frame rate when saving the data. It is the frame rate of saved data that should be used. Frame rate read from MP4 and AVI files is the value of the saved video, which might also differ from that of data.

3.1.3 Crop the Data

The user can select frames used for the processing through the two sliders.

It is better to crop the image to reduce the computation and make motion correction easier. For purpose of motion correction, you need to input two-column data, consisting of B-mode and CUES images. For two-column data, the user needs to tell the software the boundary between CEUS and B-mode images by clicking the 'Get Midline' button. When choosing the midline, the user can use the mouse to enlarge the image to improve the accuracy of midline.

Then, the user should click the 'Crop Data' button and draw region on the B-mode Image. For the data that only contains CEUS sequence, the user should use the 'Data Column' button to switch to the 'Single' side. The settings about motion correction will disappear and the user is then required to draw region to be used on the maximum projection of the CEUS sequence displayed.

The region selected via cropping the data should be a bit larger than the region of interest, as bubble images at the edges of region are cropped reducing the accuracy of localisation.

3.2 Quick Run

Three buttons are provided for simple operation by using pre-set parameters without going through each step. Pre-set parameters for motion correction, localisation, and tracking are automatically loaded from the 'Setting Parameters' file in the created configure folder. If there is not that file in the folder, default parameters will be used. The user can save setting parameters when all the processing has finished and load setting parameters from any folder.

3.3 Motion Correction

The software provides three selections for motion type, rigid, affine and non-rigid. Pushing the nonrigid button down will run the correction in two-stages: doing rigid or affine transformation first and then the non-rigid transformation. The non-rigid transformation is done using the 'imregdemons' function. Generally speaking, using larger smoothing value gives weaker effect but less distortion of the algorithm; using larger pyramid levels gives better correction performance but consumes more time.

After motion correction, the user can check the video and discard frames with large motions. If the user enters 20:25,41,51 in the space provided and click the button, frame 21,22,23,24,25, 41 and 51 will not be used in the following processing.

The user can contact us with your data to obtain specific motion correction codes, but we cannot promise we are able to deal with all kinds of tissue motions.

3.4 Background Removal

Two kinds of background removal method are provided. After clicking the 'Remove' button, the images are subtracted by the minimum value in the sequence, normalised by the maximum, denoised by

2D Wiener and median filters, and enhanced by histogram equalisation algorithm. If the switch is turned to 'Global', signals weaker than the threshold will be removed; if the switch is turned to 'Local', signals are removed by adaptive thresholding. To simplify the usage of software, only a value for setting threshold is adjustable by the user. More signals can be discarded with larger thresholds. The definition of the threshold is empirical and might affect SR images much sometimes. To avoid or reduce the effect of the threshold selection on SR images, the user is suggested to acquire data with same protocol and process the data with a same threshold.

According to experience, the local one is more suitable than the global one when there are a lot of bubble signals in the data.

3.5 Localisation

3.5.1 Super-resolution map generation

The 'SR Ratio' is used to define the pixel size ratio of input image and the SR image. According to our experience, the pixel size of the SR image should be smaller than the quarter of wavelength to make the SR image smooth enough. Otherwise, the localisation accuracy can be limited by the pixel resolution.

Localisation uncertainty is the Full Width of Half Maximum (FWHM) of a Gaussian distribution to describe the precision of localisation. Many factors, such as accuracy of motion correction, ultrasound frequency and signal to noise ratio (SNR), can affect the localisation uncertainty. The localisation uncertainty of an imaging system can be measured by an in vitro experiment where a wire target is fixed and imaged by the system. Localisation uncertainty of bubbles in vivo is generally larger than the estimated value in the in vitro experiment for tissue motions and lower SNR, etc. Localisation uncertainty should be less than half of wavelength. Otherwise, SR imaging cannot be achieved.

3.5.2 PSF estimation

Point Spread Functions (PSF) are estimated from the data by letting the user select ten isolated bubbles from the image sequence. If the user thinks the PSF varies much in the different regions, the user can increase the number of sub-regions; the image can be divided into sub-regions equally by the number; a PSF can be estimated for each sub-region. Deconvolution will use each PSF for bubble images in each region. Cross-correlation in current version will use the average of all the PSFs for the whole frame.

3.5.3 Drop Down: Deconvolution or Cross-correlation

Sparse recovery is used to do the deconvolution and normalised cross-correlation is also provided as an alternative.

1) **Deconvolution**.

'Sparsity Coefficient' and 'Region Map Ratio' are adjustable parameters. Sparsity coefficient is in a range larger than 0 to infinity. Currently, it is an empirical parameter. Generally, increasing its value from zero generates more localised events from clustered bubbles and less events from weak signals. 'Region Map Ratio' is the ratio of pixel size of original map to that of map to save image after deconvolution. This ratio should be no less than 1 and no more than SR Ratio. Larger the values, less griding effect and lower computation speed. 'Region Map Ratio' can be empirically set as 4.

2) **Cross-correlation**.

Normalised cross-correlation coefficient threshold is a user-defined parameter to discard wrong localisations. Higher values generate less localisations.

Compared to Deconvolution, normalized cross-correlation usually provides a faster speed in computation but a lower precision in bubble isolation when bubble concentration is high. It is suitable when the user does not care about flow in the large vessels where bubble concentration is much higher than that in micro-vessels. If so, the user should mask the large vessel out when calculating the SR metrics.

In some cases, the cross-correlation gives better isolation than Deconvolution. For example, there are multiple peaks in single bubble images when the SNR are still low after denoising; some image reconstruction methods in the commercial machine can generate unsmoothed bubble images at boundaries of sub-rectangle or sub-sector regions; PSF varies too much for different bubbles that can easily happen for low frequency ultrasound.

3.5.4 Test Parameter

After clicking the button, localisation will be done on the frame with most bubble signal intensity. The user can adjust background removal threshold and localisation parameters according to localised results.

3.5.5 Localisation (ALL)

After clicking the button, localisation will be done on all the frames.

3.5.6 Multi-thread

This is a state button. If pressed down, localisation on all the frames will be done in parallel. Number of frames processed at the same time is limited by the available threads of the PC CPU. The user cannot see the processing progress. Sometimes, software might be out of the computer memory. If so, the user can press the button up and only one frame will be processed at the same time.

3.6 Tracking

Tracking is done with by 2D graph-based assignment combined with feature difference and Kalman motion model. Kalman state vector for each new bubble and Kalman motion model are both initialised by our proposed method, of which details are presented in the supplementary methods of the second reference to be cited.

3.6.1 Max blood flow speed

This defines the maximum blood flow speed the software can track. It is better to set the value according to the existing knowledge. The tracking accuracy is affected by the ratio of bubble moving distance between frames to the distance between bubbles in one frame. If bubble concentration is too high or the frame rate is too low, it is better to reduce this value when the user finds too much noise in the tracked results.

3.6.2 Drop Down: Reference Image

The user can select images that can be used to improve tracking accuracy.

None: No image is used for tracking.

CEUS MIP: Maximum intensity projection (MIP) of CEUS sequence is used in tracking.

SR Density Map: Blurred SR density map is used in tracking.

If vasculature in SR density map is smooth with enough localisation events, user can select SR density map; If localisation events are not enough but vasculature in CEUS MIP is smooth, user can select CEUS MIP; If bubble signals are very sparse and vasculature in CEUS MIP are not smooth, user should select None.

3.6.3 Track bubbles

After clicking this button, Kalman motion model will be initialised first, and bubbles will be tracked frame by frame. If it takes a very long time to process one frame, there might be something wrong in setting parameters, such as, too large max blood flow speed or wrong pixel resolution. The user can cancel the processing, check parameters and re-run the tracking again.

3.6.4 Filter tracks

Tracking result can be filtered by either the distance travelled by tracked bubbles or from the length of the tracks. Using the former filter can help remove static or semi-static bubbles that are trapped on the vessel wall; using the latter filter will discard bubbles that appear in too few frames. Higher both values are, higher tracking precision is but lower tracking recall is. Empirically, the former can be set as 0 and the latter can be set as 3 or 4.

3.7 Draw SR Images

3.7.1 Start and End frames

The user can select the interested period of in the whole sequence by these two slides.

3.7.2 Interp tracks

This is a state button. When it is pressed down, localised and tracked bubbles will be linked by lines when plotting SR images, which can enhance the saturation of reconstructed vessels. If it is not pressed down, SR images are only plotted using localised bubbles.

3.7.3 Colour Limits

Density and speed colour limits are used to set the dynamic range used when showing the vasculature and flow speed. The user can adjust the density colour limit by clicking "Preview" button and visually evaluating the image. Speed colour limit can be set as the max blood flow speed but can be reduced if most blood flow slow.

3.7.4 Image Blurring Factor

This is used to adjust the size of Gaussian function for blurring the image. The FWHM of the Gaussian function is set as localisation uncertainty divided by the number. The smaller the factor is, the more blurred the image will be.

3.7.5 Image type

Density and flow maps are mandatory if the user wants to obtain all the metrics in the post processing.

SR Density: Localised events (or linking lines) are accumulated in the map and blurred by Gaussian filtering.

SR Separated Density: Localised events (or linking lines) are separated into two directions by their flowing direction (up or down), accumulated in two maps and blurred by Gaussian filtering, respectively.

SR Density with direction: The above two density maps are combined into one image via Doppler colour. The dynamic range is set as half of the user defined density colour limit.

SR Flow: Three maps are provided: a speed-mean velocity magnitude map, a vector-mean velocity magnitude map and a direction map. These images are plotted with a two-dimensional colour map. The hue demonstrates magnitude/angle, and the brightness demonstrates bubble density.

MIP/Stacked CEUS: Maximum Intensity Projection (MIP) CESU image is plotted by using the highest intensity of each pixel across the sequence after thresholding. Stacked CEUS image is plotted by averaging pixel intensity across the sequence after thresholding.

3.8 Post-processing

3.8.1 Draw ROI for parameter calculation

Accuracy of localisation and tracking at the boundary of the image is usually not high enough for the cropped bubble images. The software still needs the user to crop the boundary out. Besides, the user might not be interested in the whole image. To get the region of interest:

1. the user can choose a low-resolution image (CUES MIP/B-mode) and some SR images (Density/direction(if plotted)) and then overlap them to help the user draw ROI with the low-resolution image acting as a reference. Sometimes, the SR images and the reference images may not be aligned. It is generally because the estimated PSF is not symmetric along the centre of the saved patch.

It would not be a problem for SR metric calculation and the user should crop the image according to the SR image.

2. the user Clicks 'Draw Mask (In)' and draws a polygon for the region of interest.
3. the user Clicks 'Draw Mask (Out)' and draws a polygon for the region of no interest, such as large vessels. This step can be jumped or done multiple times.
4. the user can change the shape of regions by dragging the points of polygon(s) or adding new vertex to a polygon by right mouse click. The region of interest for parameter calculation can be updated by click 'Draw Mask (Adjust)'.

3.8.2 Parameter Calculation

Metrics of the SR images will be calculated by clicking the 'Calculate Parameters' buttons. Metrics includes:

Vessel Density: SR density map is binarized by 0.9 (empirical). The vessel density is the ratio of non-zeros pixel number to all the pixel number in the ROI. Software gives a curve of time-varying density and a number of the final density.

Vessel Diameter: Diameter is measured by the double distance between the centre line vessels, detected by Matlab ‘bwmorph’ function with ‘skel’ property, and nearest boundary of binarized SR density map. Software gives the distribution, average and standard deviation of diameters corresponding to all the pixels on the centre lines.

Fractal Dimension: It is calculated using the “box count” method and needs the user to select a flat range of the curve by drawing a line from the start to the end of the range. Software gives average and standard deviations of the numbers in user-selected range. This metric is not trusted enough when the vessel saturation is low.

Tortuosity: It is calculated for each tracked bubble by the ratio of the length of its trajectory to the distance between the start and the ends of the trajectory. Software gives the distribution, average and standard deviation of tortuosity of all tracked bubbles.

Velocity: It is calculated for non-zero values in the velocity magnitude map. Software gives the distribution, average and standard deviation of tortuosity of all the non-zero pixels.

If you have any requirement for additional parameters, please email us.