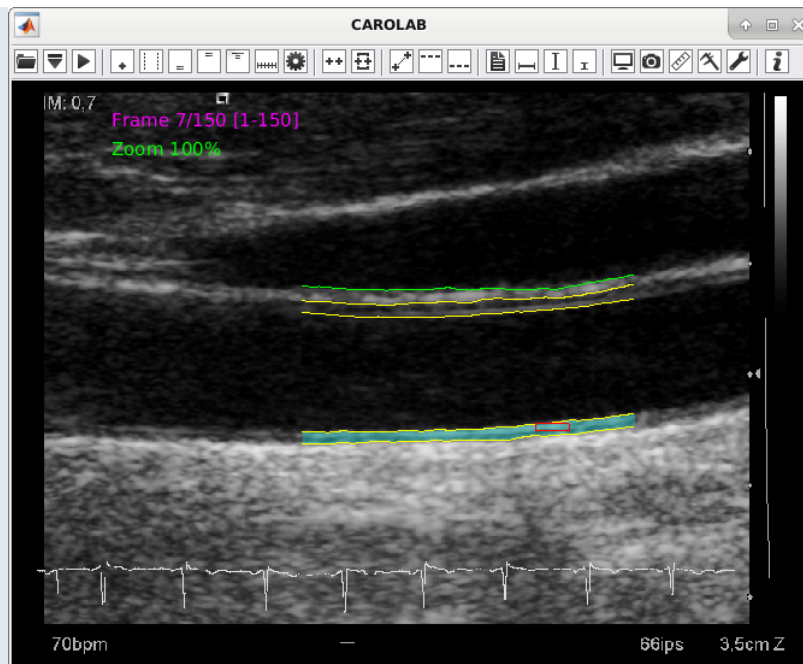


## CAROLAB v5.0 – User manual (2021/04/10)



**Guillaume Zahnd<sup>1</sup>, Maciej Orkisz<sup>2</sup>, Eduardo Enrique Dávila Serrano<sup>2</sup>, Didier Vray<sup>2</sup>**

<sup>1</sup>Computer Aided Medical Procedures, Technische Universität München,  
Boltzmannstraße 3, 85748 Garching bei München, Germany  
g.zahnd@tum.de

<sup>2</sup>Univ Lyon, INSA-Lyon, Université Claude Bernard Lyon 1, UJM-Saint Etienne, CNRS, Inserm,  
CREATIS UMR 5220, U1206, 7 avenue Jean Capelle, 69621 Villeurbanne cedex, France  
maciej.orkisz@creatis.insa-lyon.fr, eduardo.davila@creatis.insa-lyon.fr, didier.vray@creatis.insa-lyon.fr

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## Get CAROLAB

CAROLAB software can be downloaded at: [www.creatis.insa-lyon.fr/carolab/](http://www.creatis.insa-lyon.fr/carolab/)

## Troubleshooting

*“Help! CAROLAB won’t install on my computer! I don’t understand how it works! It does not read my image data! I get weird results! I need this and that features for my project but CAROLAB can’t handle it!”*

Contact us and we’ll do our best to find a solution ;)

## How to cite CAROLAB

Thank you for using CAROLAB! This software is protected by an APP deposit (IDDN.FR.001.080024.000.S.P.2016.000.10000). If you use CAROLAB anywhere we would appreciate if you cite the following references:

- Please always cite [1].
- Please also cite [2] if CAROLAB was used for contour segmentation.
- Please also cite [3] if CAROLAB was used for single-point motion tracking.
- Please also cite [4] if CAROLAB was used for dense-field motion tracking.

We would also appreciate if you could send us the references of your publications where CAROLAB is cited, so as to let us add them to the list on [www.creatis.insa-lyon.fr/carolab/](http://www.creatis.insa-lyon.fr/carolab/).

## Main features

CAROLAB is dedicated to simple and accurate analysis of ultrasound carotid artery images.

- Visualize B-mode ultrasound DICOM image sequences (movie clips) of the carotid artery
- Automatically measure the diameter variation during time [2]
- Automatically measure the IMT compression-decompression during time [2]
- Automatically track the wall motion during the cardiac cycle (LOKI) [3–8]
- Assess the extra-media thickness (EMT) [9]
- Perform manual measurements if required
- And more

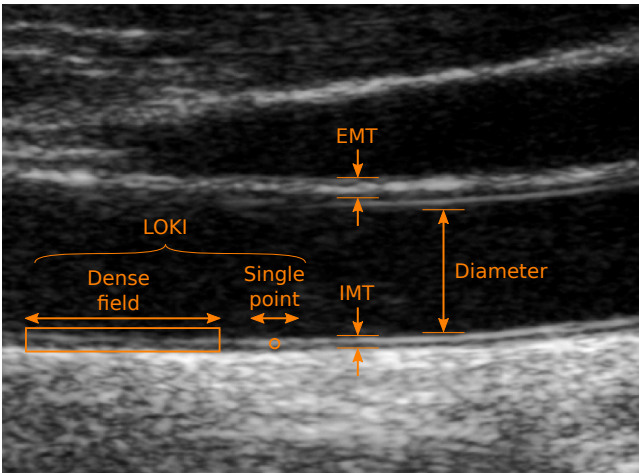


Figure 1: Schematic representation of the different types of measurements performed by CAROLAB.

## Basic commands

- **Left click** – Validate the action
- **Right click** – Cancel, stop, or reset the action (in most of the cases)
- **Mouse wheel** – Zoom in/out by scrolling up/down
- **Left/Right arrow** – Go to the previous/next frame of the clip
- **Held right click + horizontal mouse motion** – Quickly go to any frame of the clip
- **Space** – Mark/Unmark the current frame as a specific ECG time-point (Fig. 3)

## Interface buttons

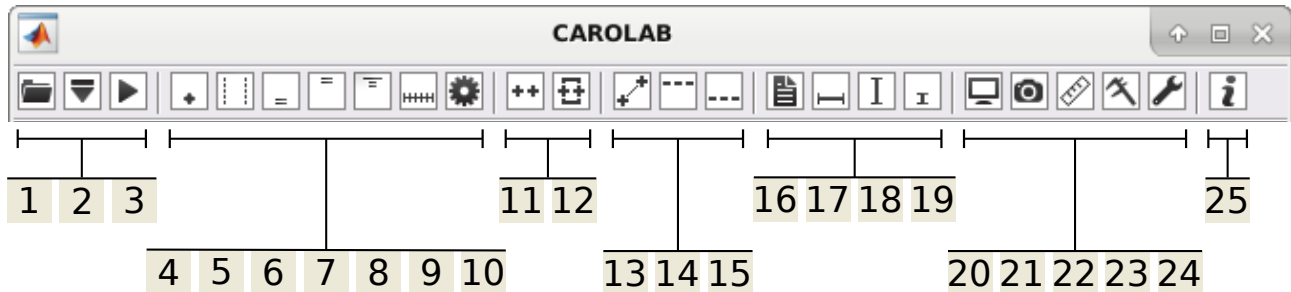



























Figure 2: Taskbar

1.  **Load clip** – Open a clip (image data) to be analyzed.
2.  **Load results** – Open the results of an already analyzed clip. In the first dialog box, select the result file. In the second dialog box, select the corresponding clip.
3.  **Play/Pause** – Click once to view the frames as a movie clip. Click again to pause the clip.
4.  **Set tracking point** – Indicate the location of the point whose 2D trajectory is to be estimated during the duration of the clip. It is recommended to zoom in and select, within the intima-media complex of the far wall, a salient echo scatterer with a well contrasted speckle pattern, as displayed in Figure 5 [3].
5.  **Set segmentation margins** – Indicate the left and right borders of the region where the contours will be segmented. Please note that if the aim is to assess the temporal variation of the IMT during the

cardiac cycle [2], it is recommended to set the width of the analyzed region to 3 mm (which is the default value) for improved accuracy.

6.  **Set far wall** – Indicate the approximate location of the far wall (*cf* Fig. 4).
7.  **Set near wall** – Indicate the approximate location of the near wall (*cf* Fig. 4).
8.  **Set EMT** – Toggle on/off the EMT evaluation [2,9].
9.  **Set dense field** – Toggle on/off the Dense Field evaluation [4].
10.  **Run analysis** – Launch the processing task, using the initialization parameters and settings previously determined by the user. This operation may take a couple of minutes. After the process, the results are automatically saved in the “res” folder.
11.  **Store batch** – Store the initialization settings for the current image in the “batch” folder.
12.  **Process batch** – Process all the files stored in the “batch” folder. All the clips are iteratively loaded, the process is automatically performed with the corresponding initialization settings previously stored.
13.  **Manual annotations: single point tracking** – Construct and store a golden standard reference corresponding to the temporal trajectory of a specific point via manual annotations.
14.  **Manual annotations: segmentation of contour #1** – Construct and store a golden standard reference corresponding to the segmentation of an anatomical interface via manual annotations.
15.  **Manual annotations: segmentation of contour #2** – Construct and store a golden standard reference corresponding to the segmentation of another anatomical interface via manual annotations.
16.  **Show results** – Display the results of all processed tasks (*i.e.*, LOKI, diameter, IMT).
17.  **Get longitudinal measurements** – Measure the LOKI amplitude  $\Delta X$ , *i.e.*, the peak-to-peak amplitude of the tissue motion in the direction parallel to the blood flow [3]. In order to compensate for a potential drift in the trajectory (*viz.*: a slight contraction of the subject’s neck muscles, creating an additional motion of the entire image w.r.t. the probe), three points must be placed to perform a measurement, as depicted in Figure 6.
18.  **Get diameter measurements** – Measure the amplitude  $\Delta D$  of the variation of the cross sectional diameter during the cardiac cycle, as well as the maximal diameter  $D_{\max}$ , as depicted in Figure 8.
19.  **Get IMT measurements** – Measure the amplitude  $\Delta \text{IMT}$  of the variation of the IMT during the cardiac cycle, as well as the maximal thickness value  $\text{IMT}_{\max}$ , as depicted in Figure 9.
20.  **Hide/Show** – Click once to hide all drawings and text from the image. Click again to restore the annotations.
21.  **Screenshot** – Take a screenshot of the current frame and save the image in the “screenshots” folder.
22.  **Ruler** – Measure the distance (in  $\mu\text{m}$ ) between two given points.

23.  **Caliper** – Used to determine the spatial resolution (*i.e.*, the actual size of one pixel in micrometers), which is required for the analysis. This mode is not used when analyzing DICOM clips, since information about spatial resolution are natively stored in the DICOM format. However, this mode is mandatory when analyzing video clips such as AVI files, since the pixel size is unknown. To set up the caliper, use the the scale marks of the image to draw a line that is 1 cm long, as displayed in Figure 10.
24.  **Settings** – Display a dialog box to edit the parameter settings. It is recommended to keep the default settings, but feel free to try your own brew.
25.  **About CAROLAB** – Display information about CAROLAB.

## Filesystem tree

A series of folders are automatically created in the user home repository. It is possible to copy, move, or delete the files generated by CAROLAB for backup of cleanup purposes, however the location of the working folder shall remain the same (*i.e.*, user home repository).

- `CAROLAB.RESULTS\`: Top folder, contains all the subfolders listed below.
- `batches\`: Contains the `.mat` initialization settings for the collection of image clips to be batch-processed, stored one-by-one via **button 11**, executed all at once via **button 12**.
- `results\`: Contains the `.mat` files that describe all the parameters and all the results corresponding to each processed clip. These documents can be loaded with MATLAB for further analysis and post-processing. Also contains the `.txt` files that describe all the parameters and all the results corresponding to each processed clip. These document can be loaded without MATLAB, via any table editor, for further analysis and post-processing.
- `res_print\`: Contains the `.png` images that provide, for each processed clip, a visual overview of the results for quick inspection.
- `screenshots\`: Contains all the `.png` images corresponding to the screenshots taken by the user.
- `references\`: Contains all the `.mat` and `.txt` files corresponding to the manual reference annotations performed by the user.

## Exploiting the results

**Result files** After the analysis is complete, all results are automatically saved in the “`results`” folder, under the name “`results_<SUBJECT_NAME>.mat`”. Such result files can then be re-opened (**button 2**), along with the corresponding image clip, to visualize them again and perform manual measurements (**buttons 17-19**).

**Results in formatted text** Additionally, the measurements are also automatically written in a text file named “`results_<SUBJECT_NAME>.txt`” in the “`results`” folder. Such results are devised to be copied and pasted in a spreadsheet, in the objective to gather the results from multiple subjects. An example of formatted text is provided below:

```
Subject:  John Doe ; deltaX AVG = 318 ; deltaX STD = 95 ; sigmaX = 142 e-3 ; deltaD = 664 ; Dmax
= 6064 ; deltaIMT = 45 ; IMTmax = 630
```

Here, `deltaX AVG` is the average LOKI amplitude ( $\pm$  STD) (Fig. 6), `sigmaX` is the inhomogeneity index (Fig. 7, [4]), `deltaD` the amplitude of the temporal diameter change (Fig. 8), `Dmax` the maximal diameter (Fig. 8), `deltaIMT` the amplitude of the temporal IMT change (Fig. 9), and `IMTmax` the maximal IMT (Fig. 9). All values are in micrometers, except `sigmaX` which is unitless.

**Results for the graphs** Data corresponding to the graphs (Fig. 6, 8, 9) is saved in another text file named “results\_<SUBJECT\_NAME>\_graph.txt” in the “results” folder. It is thus possible to copy-paste these data in a spreadsheet and re-plot the graphs. The text format is coma-separated, with one line per time-point (*i.e.*, frame number), as detailed in the example below:

Frame number	Key frame (ECG mark)	IMT near ( $\mu\text{m}$ )	IMT far ( $\mu\text{m}$ )	Diameter ( $\mu\text{m}$ )	LOKI ( $\mu\text{m}$ )	EMT ( $\mu\text{m}$ )
1	1	543	512	4752	184	555
2	0	530	506	4745	153	512
3	0	518	524	4739	124	471
...	...	...	...	...	...	...

**EMT results in formatted text** Moreover, results from the EMT analysis are also written in another text file named “results\_<SUBJECT\_NAME>\_emt.txt” in the “results” folder. An example of formatted text is provided below:

```
Subject: John Doe ; EMT mean: 528 um ; EMT std: 15 um ;
EMT median: 529 um ; EMT min: 461 um ; EMT max: 591 um ;
EMT(int) mean: 97 ; EMT(int) std: 31 ; EMT(int) median: 101 ;
EMT(int) min: 12 ; EMT(int) max: 176 ; IMT mean: 563 um ;
IMT std: 41 um ; IMT median: 556 um ; IMT min: 459 um ;
IMT max: 693 um ; IMT(int) mean: 66 ; IMT(int) std: 20 ;
IMT(int) median: 64 ; IMT(int) min: 8 ; IMT(int) max: 123 ;
```

Here, EMT mean, std, median, min and max correspond to the average, standard deviation, median, min and max value of the EMT, over the segmented region of all analyzed frames, respectively. All these values are in micrometers. Additionally to the tissue thickness, echo-lucency (*i.e.*, image intensity) is also characterized: EMT(int) mean, std, median, min and max correspond to the average, standard deviation, median, min and max value of the gray level of all the pixels inside of the segmented region of all analyzed frames. The same information is also provided for the IMT of the near wall, for comparison purpose. The intensity range of the image is [0, 255].

## Manual annotations

### Manual annotations: About

This functionality enables the user to manually perform annotations in order to construct golden standard references. Such references correspond to *i)* the temporal 2D trajectory of a single point during the cine-loop and *ii)* the contour segmentation of up to 2 different anatomical interfaces in any frame of the cine-loop (let us define contour #1 and #2 as the lumen-intima and media-adventitia interfaces, respectively). These references can be further exploited either to assess the accuracy of tracking or segmentation methods, or to directly measure indices on the analyzed ultrasound data. Here, the term “*ground truth*” should preferentially not be used since references are only as accurate as the user performance and therefore do not correspond to absolutely exact quantifications. For this reason, evaluating the inter- and intra-user reproducibility is also encouraged.

### Manual annotations: Single point tracking

This action is performed via **button 13**. The commands are:

- Click on **button 13** to activate the “*Manual tracking*” functionality
- Zoom in using the mouse wheel to precisely select a target point to be manually tracked
- Left click on the image to indicate the  $(x, y)$  coordinates of the target point
- Navigate to the next frame using the right arrow key
- Left click on the image to indicate the new  $(x, y)$  coordinates of the target point in the next frame
- Repeat until the trajectory of the point has been generated during the entire cine-loop
- Click on **button 13** to exit the “*Manual tracking*” functionality

To correct erroneous annotations, the commands are:

- Navigate to the previous frame using the left arrow key
- Left click in the neighborhood of an existing point to move it to a new location

## Manual annotations: Contour segmentation

This action is performed via **buttons 14** (for contour #1) and **15** (for contour #2). The commands are:

- Click on **button 14** (or **15**) to activate the “*Manual contouring*” functionality
- Zoom in using the mouse wheel to precisely localize a target anatomical interface to be manually segmented
- Left click on the image to place a series of control points, defining a smooth contour
- Zoom in and out to focus on different regions of the image and place control points
- Up to two contours (#1 and #2) can be specified in each frame of the cine-loop
- Click on **button 14** (or **15**) to exit the “*Manual contouring*” functionality

To correct erroneous annotations, the commands are:

- Navigate to the desired frame
- Right click in the neighborhood of an existing control point to delete it
- Left click in the neighborhood of an existing control point to move it to a new location

## Manual annotations: Results storing and exploitation

All manual annotations are automatically saved in the folder `references\` under the following organization:

- “<SUBJECT\_NAME>\_ref.mat”: MATLAB file containing all the annotations (single point tracking, contours #1 and #2).
- “<SUBJECT\_NAME>\_ref\_tracking.txt”: Text file containing the annotations about single point tracking information, which can be used for table import. Columns and rows corresponds to  $(x, y)$  coordinates in the image and frames of the sequence, respectively.
- “<SUBJECT\_NAME>\_ref\_seg1.txt”: Text file containing the annotations about contour #1, which can be used for table import. Columns and rows corresponds to  $x$ -coordinates in the image and frames of the sequence, respectively.
- “<SUBJECT\_NAME>\_ref\_seg2.txt”: Text file containing the annotations about contour #2, which can be used for table import. Columns and rows corresponds to  $x$ -coordinates in the image and frames of the sequence, respectively.

Each individual file contains the information for all frames of the movie clip. The files are automatically saved after each modification, and automatically loaded with the opening of an already annotated sequence. To perform inter- or intra-user variability analysis, it is recommended to rename each file accordingly.

## Supported image formats

CAROLAB supports DICOM and AVI video clips. However, you should be aware of the following pitfalls:

- Although DICOM is the best suited format (mainly due to an overall better image quality and embedded metadata), this format is not completely universal between different scanner manufacturers. In rare situations, it can happen that a DICOM clip does not contain certain data fields that are required by CAROLAB, and therefore can not be analyzed.
- Due to image compression and down sampling, video clips (*e.g.*, AVI) are most of the time sub-optimal w.r.t. the original DICOM version. More specifically, the image quality is often poorer (*i.e.*, smaller and more blurry), and the actual frame rate is slightly different. Therefore, to obtain more accurate results, it is recommended to use DICOM files rather than the equivalent video files whenever possible.

If your image files can not be processed with CAROLAB, please do contact us, and we will do our best to make CAROLAB compatible with your data.

## Appendix 1: Detail of some interface buttons

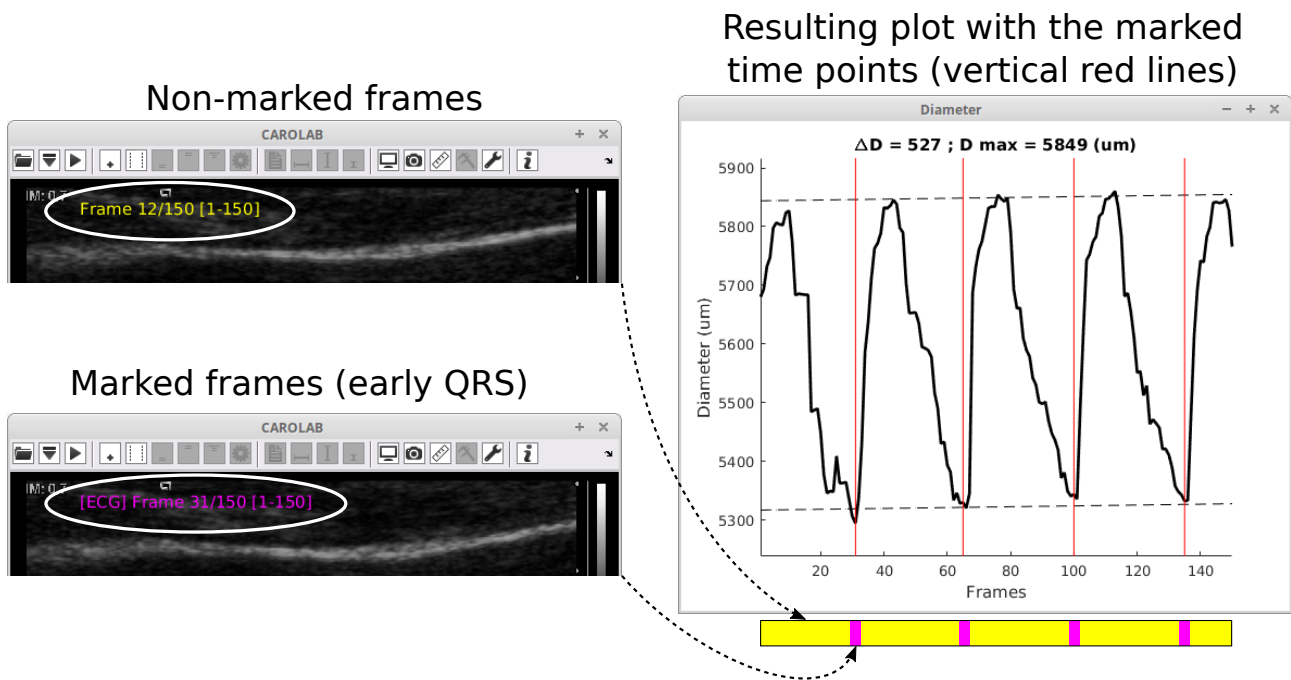


Figure 3: Example of frame-marking using the space key to indicate some time-points related to the ECG.

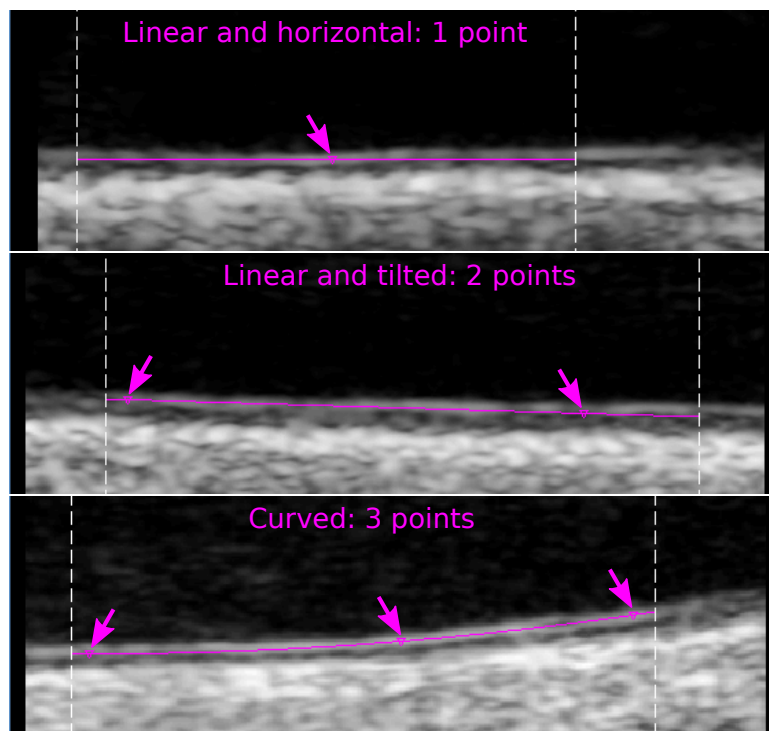


Figure 4: Setting up the approximate location of the far wall (**button 6**).



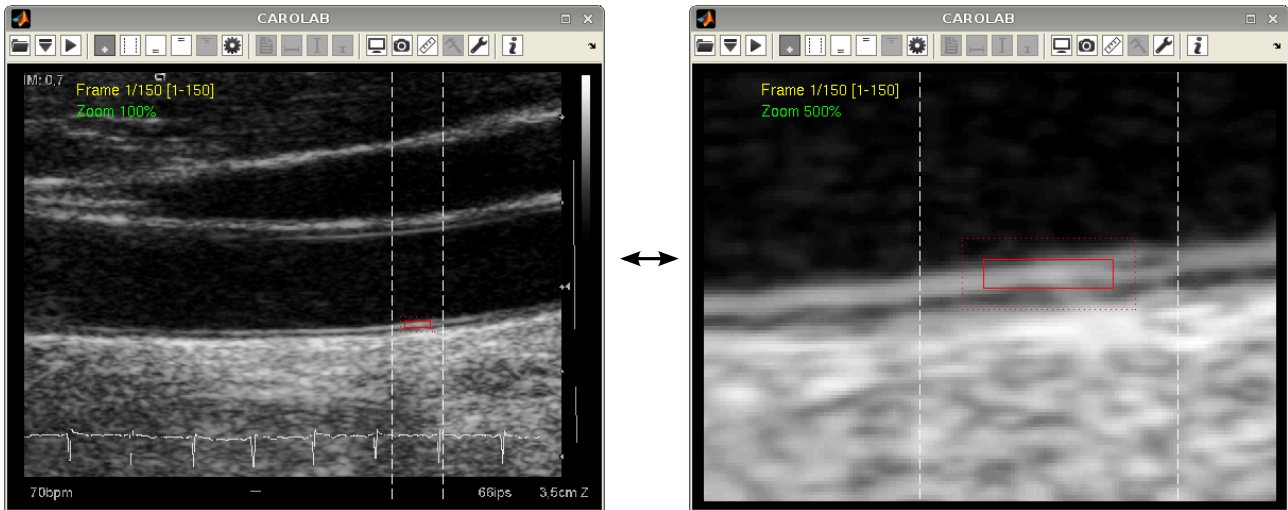
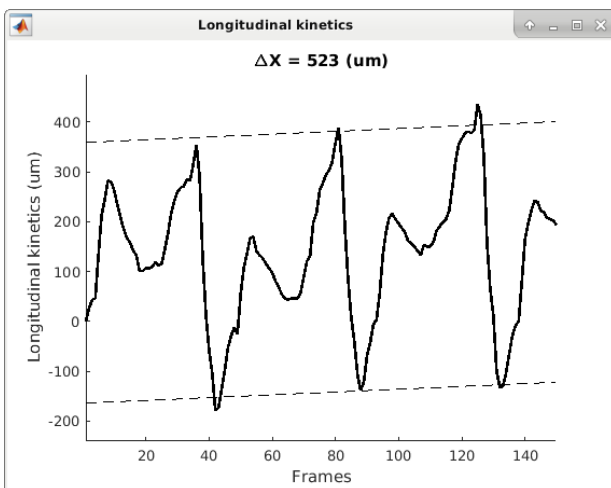
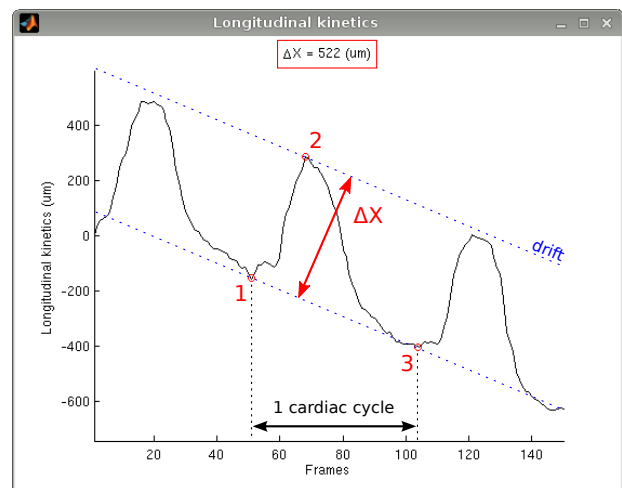


Figure 5: Setting up the point to be tracked during the clip, centered on a bright and well perceptible spot (**button 4**).



Automatic amplitude



Manual annotation/correction process

Figure 6: Measuring LOKI amplitude  $\Delta X$  (**button 11**), on two different clips. To automatically compensate for a potential drift, three points must be indicated following the order indicated in the right panel.

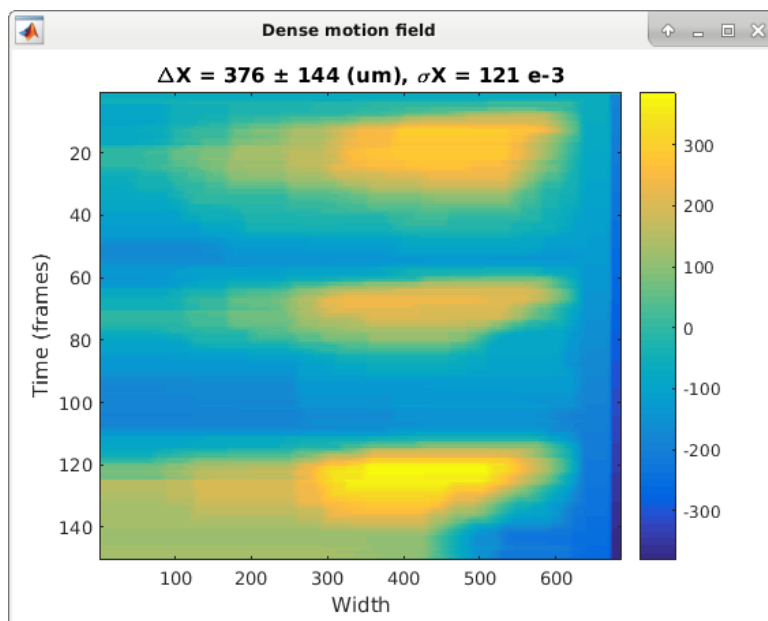
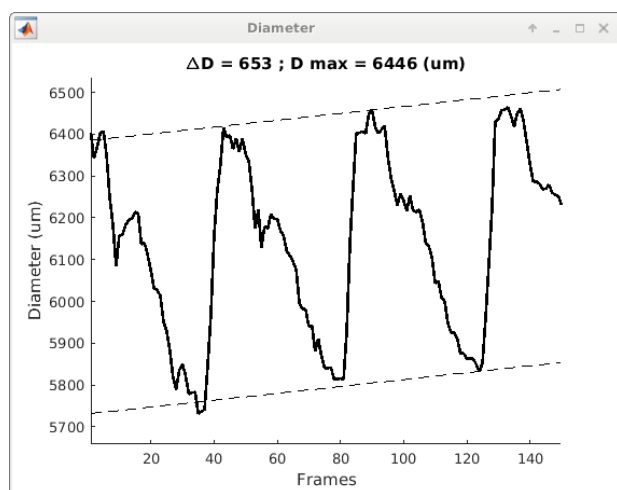
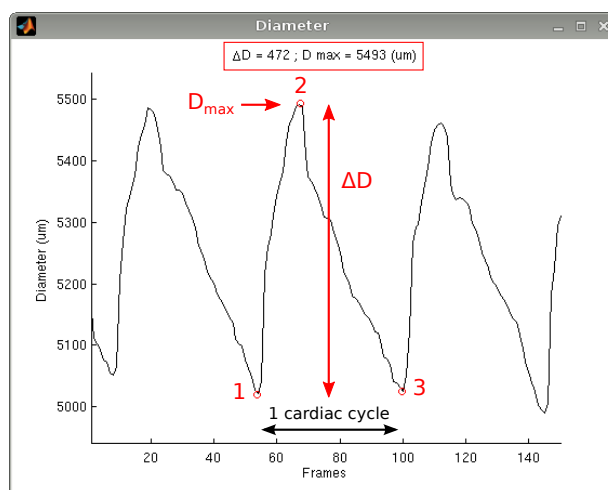


Figure 7: LOKI, Dense motion field (**button 9**). The average ( $\pm$  STD) motion amplitude  $\Delta X$  as well as the inhomogeneity index  $\sigma_x$  are automatically calculated.

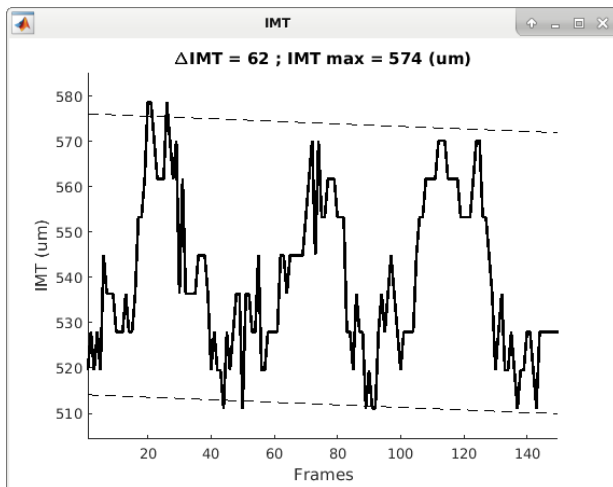


Automatic amplitude

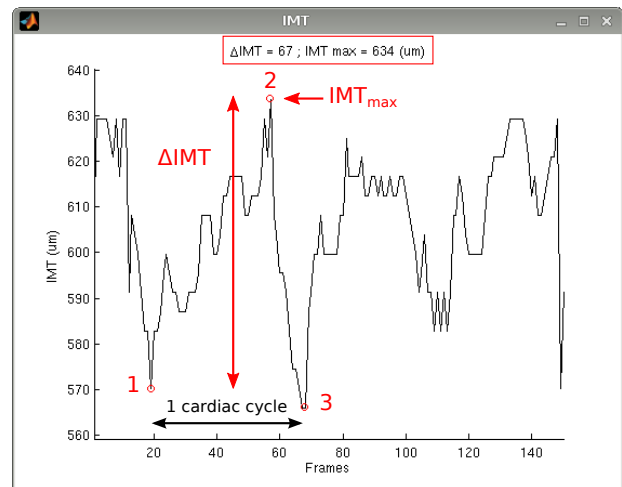


Manual annotation/correction process

Figure 8: Measuring the amplitude  $\Delta D$  of the variation of the cross sectional diameter during the cardiac cycle, as well as the maximal diameter  $D_{\max}$  (**button 15**), on two different clips. To automatically compensate for a potential drift, three points must be indicated following the order indicated in the right panel.



Automatic amplitude



Manual annotation/correction process

Figure 9: Measuring the amplitude  $\Delta\text{IMT}$  of the variation of the IMT during the cardiac cycle, as well as the maximal thickness value  $\text{IMT}_{\text{max}}$  (**button 16**), on two different clips. To automatically compensate for a potential drift, three points must be indicated following the order indicated in the right panel.

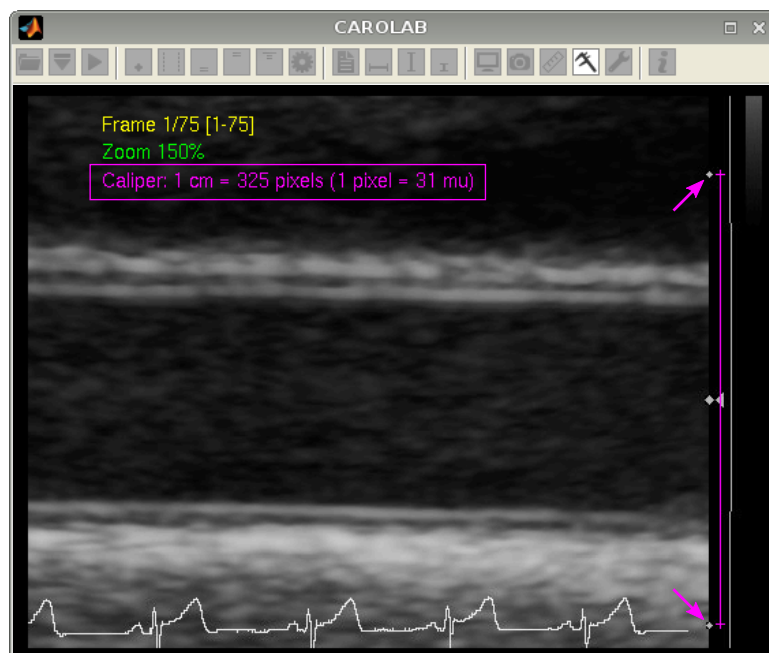


Figure 10: Setting up the spatial resolution using the caliper (**button 20**).

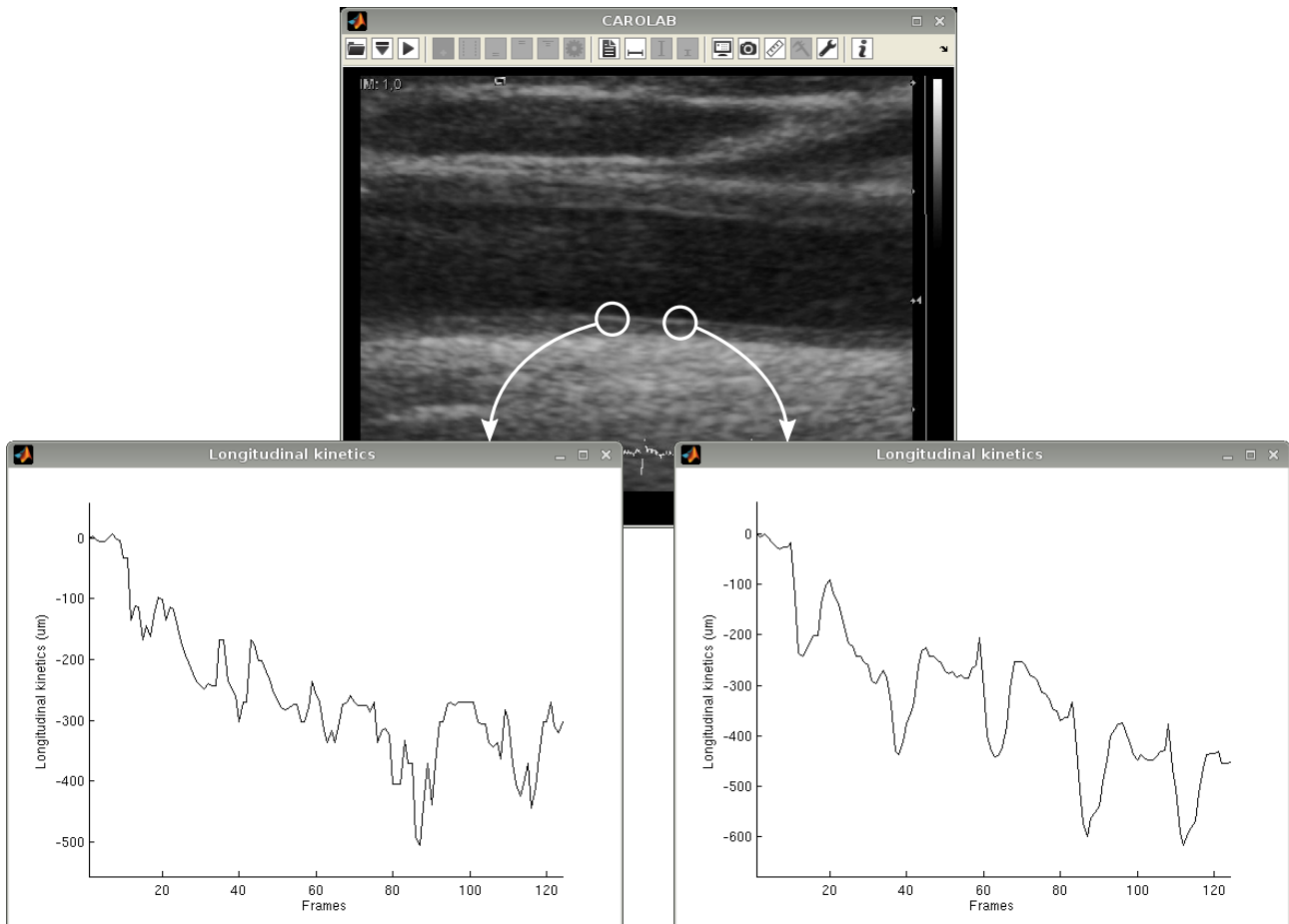


Figure 11: LOKI estimation on two different locations, showing that the choice of the region where LOKI is measured is paramount. The image quality in the left region is slightly worse, and the resulting curve is very noisy. The image quality on the right region is better, and the resulting curve is reproducible. A video example is available at: <https://vimeo.com/130765720>

## Appendix 2: Guidelines for optimal LOKI acquisition

Longitudinal kinetics (LOKI, Fig. 12) is the elastic deformation of the arterial wall tissues in the direction parallel to the blood flow during the cardiac cycle, characterized by a reproducible shearing motion of the intima-media complex with respect to the tunica adventitia [3,6–8].

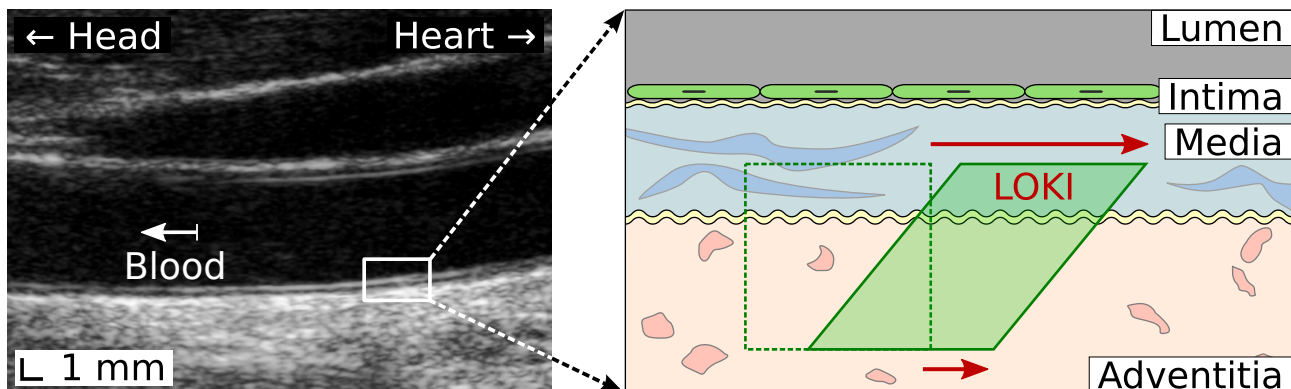


Figure 12: Longitudinal kinetics (LOKI). Image from [8]. Video example: <https://vimeo.com/130765720>.

Since LOKI amplitude  $\Delta X$  is rather small (*i.e.*, approximately 0.5 mm, or 15 pixels), specific care must be taken during ultrasound image acquisition, in the aim to obtain accurate results from the analysis. LOKI can sometimes be tricky to measure in noisy images, as depicted in Figure 11. Guidelines for optimal LOKI acquisition have been extensively described in a dedicated review [5], and can be summarized as follow:

- The subject must be relaxed and perfectly still (lying on the back).
- The subject must perform breath-hold.
- The subject must not swallow saliva.
- The probe should be positioned parallel to the vessel axis, in such way that there is no out-of-plane motion.
- The probe must be held perfectly still.
- The focal zone must be centered on the far wall.
- The zoom must be set to that the image is centered around the lumen axis, including both near and far wall.
- The contrast and brightness must be configured so that the intima-media complex is clearly visible: *i)* the lumen-intima and media-adventitia interfaces must be clearly perceptible as neat lines, and *ii)* the tissues of the intima-media complex must present a grainy texture and not be homogeneously saturated with white.
- At least one entire cardiac cycle must be acquired (five or more is best).
- LOKI must be measured on a well-perceptible and contrasted speckle pattern that must remain visible through the entire duration of the clip. If the resulting curve is not a periodic signal, it is advised to measure LOKI at a different location of the wall where the image quality is best (*cf* Fig. 11).

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