

1     **Motion synchronisation patterns of the carotid atheromatous**  
2                             **plaque from B-mode ultrasound**

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20 Asynchronous movement of the carotid atheromatous plaque from B-mode  
21 ultrasound has been previously reported, and associated with higher risk of stroke,  
22 but not quantitatively estimated. Based on the hypothesis that asynchronous  
23 plaque motion is associated with vulnerable plaque, in this study, synchronisation  
24 patterns of different tissue areas were estimated using cross-correlations of  
25 displacement waveforms. In 135 plaques (77 subjects), plaque radial deformation  
26 was synchronised by approximately 50% with the arterial diameter, and the mean  
27 phase shift was 0.4 s. Within the plaque, the mean phase shifts between the  
28 displacements of the top and bottom surfaces were 0.2 s and 0.3 s, in the radial  
29 and longitudinal directions, respectively, and the synchronisation about 80% in  
30 both directions. Classification of phase-shift-based features using Random Forests  
31 yielded Area-Under-the-Curve scores of 0.81, 0.79, 0.89 and 0.90 for echogenicity,  
32 symptomaticity, stenosis degree and plaque risk, respectively. Statistical analysis  
33 showed that echolucent, high-stenosis and high-risk plaques exhibited higher  
34 phase shifts between the radial displacements of their top and bottom surfaces.  
35 These findings are useful in the study of plaque kinematics.

36

## 37    **Introduction**

38    The carotid atheromatous plaque is a lesion of the carotid artery wall and typically  
39    consists of a fibrous cap (mostly smooth muscle cells, collagen and elastic fibers) of  
40    varying thickness and a lipid core (mostly cholesterol and cellular debris). In cases of  
41    advanced degeneration, plaque lesions present a more complicated structure,  
42    including calcification, intraplaque hemorrhage and ulceration<sup>1</sup> and narrow the  
43    arterial lumen, obstructing blood flow and oxygen supply to the brain. More severe  
44    damage may be caused by vulnerable plaques, i.e. plaques prone to rupture. These  
45    are strongly associated with the formation of blood clots and the release of plaque  
46    fragments into the systemic circulation, which may cause a cerebrovascular event,  
47    such as stroke or transient ischemic attack (TIA)<sup>2</sup>. Given the substantial burden of  
48    stroke (15 million people worldwide suffer a stroke annually, of whom 5 million die  
49    and 5 million are left permanently disabled<sup>3</sup>, investigating the behaviour of carotid  
50    plaque towards improving stroke prevention is of utmost importance.

51        Ultrasound imaging is the preferred imaging modality for the diagnosis of carotid  
52    atheromatous plaque, owing to a number of advantages, including noninvasiveness,  
53    bedside availability, short examination times, lack of radiation exposure, and low  
54    cost<sup>4</sup>. Currently, clinical management of carotid plaque is based on the degree of  
55    stenosis, i.e., the percentage of lumen area occupied by atheromatous material, and  
56    the prior occurrence of symptoms<sup>5</sup>. Although the degree of stenosis is a validated  
57    marker for management of carotid plaques, some studies have indicated that a high  
58    degree of stenosis is not necessarily related to a high risk of a cerebrovascular  
59    event<sup>6,7</sup>. These facts indicate that there is room for improving the current clinical

60 scheme for assessing plaque vulnerability, possibly through the identification of  
61 noninvasive, low-cost and reliable imaging markers for predicting strokes<sup>8</sup>.

62 For instance, carotid motion analysis estimated with ultrasound image sequences  
63 has gained increasing attention as a potential index of plaque vulnerability<sup>9-12</sup>.

64 Motion analysis can be defined as the estimation of arterial tissue displacement  
65 during one or more cardiac cycles. It has been shown that carotid atheromatous  
66 plaque performs a complex, multidirectional, often periodic, motion during the  
67 cardiac cycle<sup>13</sup>. Despite the technical challenges, such as the low image resolution in  
68 ultrasound imaging and the complexity of the local tissue geometry and mechanics,  
69 several studies have suggested a number of kinematic and strain indices associated  
70 with plaque rupture risk<sup>14</sup>.

71 A number of efforts have focused on motion of non-atheromatous segments of  
72 the arterial wall in normal<sup>15,16</sup> and pathological conditions, such as hypertension,  
73 diabetes and coronary artery disease<sup>17,18</sup>, as well as the motion of the wall adjacent  
74 to carotid plaque<sup>12,16,19,20</sup>. These studies have studied the expected cyclical motion in  
75 the radial direction and have also identified a longitudinal component of wall  
76 motion. It has also been observed that decreased longitudinal movement of the  
77 common carotid artery is associated with higher plaque burden<sup>20</sup>. Significantly lower  
78 amplitudes of both radial and longitudinal displacements have been found in older  
79 diabetic subjects, compared to healthy young adults<sup>18</sup>. Recently, the feasibility of  
80 assessing tissue motion inhomogeneities was demonstrated along with their  
81 association with the presence of coronary artery disease<sup>21</sup>. Blood pressure has been  
82 positively correlated with common carotid artery displacement<sup>17</sup>. Other studies have  
83 suggested that the severity of carotid stenosis is associated to axial wall stresses and

84 accelerations<sup>19</sup>, as well as to the presence of an anterograde component in the  
85 longitudinal direction of wall motion<sup>12</sup>.

86 Related studies have proposed various metrics to quantify plaque motion  
87 patterns, including statistical measures of velocities, motion amplitudes and diastole-  
88 to-systole displacements of the entire plaque area during the cardiac cycle<sup>11,13</sup>,  
89 maximal (discrepant) surface velocities<sup>9,22</sup> and displacement vector maps<sup>23</sup>. A group  
90 of studies have also qualitatively described the so called “jellyfish sign”  
91 phenomenon, according to which the carotid plaque surface rises and falls in a  
92 manner inconsistent with arterial pulsatile wall motion<sup>24-26</sup>. Other similar  
93 phenomena include motion of intraplaque contents<sup>27</sup>, mobility at the edge of the  
94 plaque, mobility in all parts of the plaque and mobility at the bottom of an ulcer on  
95 the plaque<sup>26</sup>. Studies have also investigated tissue strain, i.e. the change of  
96 displacement with respect to some initial reference status<sup>10,28-32</sup>. These studies have  
97 converged to the general conclusion that softer, echolucent plaques undergoing  
98 higher strains tend to be more prone to rupture and they are associated with poorer  
99 patient cognition. The concept of concordant and discordant motion was recently  
100 introduced to describe the spread of motion of different plaque areas<sup>33</sup>.

101 Among the investigated phenomena, relative motion between the plaque and  
102 the adjacent wall<sup>13,24-26</sup>, as well as within the plaque itself<sup>26,27</sup> has been reported in  
103 some studies. The patterns of synchronisation of such relative movements have only  
104 been estimated qualitatively in a few studies<sup>24-26</sup> and have shown that asynchronous  
105 motion of the plaque relative to the adjacent wall is associated with plaque  
106 instability and stroke recurrence.

107 To the best of our knowledge, there is no study focused on investigating  
108 synchronisation patterns of carotid plaque motion in an automated and quantitative  
109 way. Therefore, the purpose of this study was to quantify synchronisation patterns  
110 of the carotid plaque, in relation to its adjacent wall and within itself, and investigate  
111 potential associations of these synchronisation patterns with different plaque  
112 phenotypes, including echogenicity, stenosis degree, patient symptoms and plaque  
113 risk. The major contributions of this work are to (a) suggest a systematic approach  
114 for assessing such patterns, (b) provide specific numerical indices (measured in  
115 seconds) for the related phenomena, i.e. the phase shifts between plaque and wall,  
116 and within plaque in radial and longitudinal directions, and (c) evaluate the derived  
117 indices in different plaque phenotypes, based on the hypothesis that asynchronous  
118 plaque motion is associated with phenotypes characterising vulnerable plaque,  
119 namely echolucency, symptomaticity, high stenosis degree and high risk. These  
120 contributions will provide new knowledge about plaque biomechanics, which is  
121 important and necessary for future studies, including prognostic follow-up  
122 assessments.

123

## 124 **Materials and Methods**

### 125 *Dataset*

126 Seventy seven consecutive patients (59 men, 18 women) with carotid  
127 atherosclerosis were included in the study, free from comorbidities, including heart  
128 failure, liver dysfunction, cancer, chronic diseases etc. Subjects were on statin-based,  
129 anti-platelet and lipid-lowering medication. The dataset included 18 symptomatic  
130 patients (31 plaques, degrees of stenosis  $66\% \pm 29\%$ ), 57 asymptomatic patients (98

131 plaques, degrees of stenosis  $73\% \pm 22\%$ ) and 2 patients (6 plaques) whose  
132 symptomaticity or stenosis degree was unknown; the latter were only included in  
133 the association-with-echogenicity study. The symptomatic subjects, for whom only  
134 the ipsilateral artery was studied, had experienced a stroke or a TIA, within 6 months  
135 prior to the examination. A number of asymptomatic subjects had plaque in both the  
136 right and left carotids and in both types of subjects more than one plaque may be  
137 present in an artery (tandem lesions); tandem lesions were treated as separate  
138 plaques. The patients' ages were  $70 \pm 9$  years (range 43-85 years), and their stenosis  
139 degrees  $75\% \pm 17\%$  (range 20-99%), based on Doppler ultrasound measurements.

140 B- mode ultrasound images were acquired in longitudinal section using a LOGIQ  
141 Book (GE Medical Systems, Milwaukee, WI, USA) scanner and a linear array 4-10  
142 MHz transducer. Subjects were examined in a supine position, with a slight backward  
143 inclination of the head, towards the opposite side of the carotid under examination.  
144 Patients rested for at least 5 minutes before the examination, to stabilise their heart  
145 rate and blood pressure. To minimise movements due to factors other than  
146 haemodynamic forces, the operator held the transducer as stable as possible,  
147 exerting minimal pressure, and the patients were asked to breath-hold during  
148 recordings. Scanner and transducer settings included a high dynamic range (60 or 75  
149 dB) and zero persistence, and 10 MHz centre frequency. At least three cardiac cycles  
150 were recorded at a rate of 25 frames/s. Image resolution was 12 pixels/mm in the  
151 radial and longitudinal directions. The room temperature was kept constant at  $26^{\circ}\text{C}$ .

152 All ultrasound examinations were performed by 4 experienced physicians in the  
153 Vascular Surgery Department of the University Hospital "ATTIKON", Athens, Greece.  
154 Data collection was approved by the ATTIKON hospital institutional review board and

155 all subjects included in the study gave their informed consent to the scientific use of  
156 the data. The methods were carried out in accordance with the relevant guidelines  
157 and regulations.

158

#### 159 *Estimation of plaque motion synchronisation patterns*

160 Plaque motion synchronisation patterns relative to the adjacent normal wall as  
161 well as within the plaque were estimated through cross-correlations of pairs of  
162 waveforms representing displacements of plaque and wall tissue.

163 *1) Basic principles of cross-correlation.* Cross-correlation  $r_d$  is a measure of  
164 similarity of two signals in the form of time series,  $x(i)$  and  $y(i)$ , where  $i =$   
165  $1, 2, \dots, N$  denotes time points, as a function of the displacement  $d$  (also known as  
166 lag) of one relative to the other<sup>34</sup>. If cross-correlation is calculated for all lags  
167  $d = 0, 1, \dots, N - 1$ , then the resulting cross-correlation sequence is twice as long as  
168 that of the correlated series. The following formula for cross-correlation was used:

169

$$r_d = \frac{\sum_i [(x(i) - m_x)(y(i - d) - m_y)]}{\sqrt{\sum_i (x(i) - m_x)^2} \sqrt{\sum_i (y(i) - m_y)^2}}$$

170

171 where  $m_x$  and  $m_y$  are the mean values of signals  $x(i)$  and  $y(i)$ , respectively. The  
172 denominator in this formula serves to normalise the correlation coefficients, so that  
173 the cross-correlation is 1, for lag equal to 0. The subtraction of the mean values  
174  $m_x$  and  $m_y$  from the signals allows signals from different subjects to be comparable.  
175 The length  $N$  of the signals coincides with the maximum duration of the ultrasound  
176 recording in each case.



177 If the peaks (or the troughs) of two time-varying signals coincide in time, their  
178 cross-correlation has a high positive value. These signals are considered  
179 synchronous, or in-phase, or with a  $0^\circ$  phase shift. If the peaks of one signal coincide  
180 in time with the troughs of the other signal, their cross-correlation has a high  
181 negative value. These signals are considered asynchronous, or out-of-phase, or with  
182 a  $180^\circ$  phase shift. A cross-correlation value equal to 0 indicates uncorrelated signals.

183 *2) Description of methodology.* The main steps of the methodology are described  
184 below and illustrated in Fig.1.

185 *A - Selection of regions of interest (ROIs).* For each plaque image sequence  
186 (video), an experienced physician marked manually in the first frame the following  
187 four ROIs: the posterior and anterior wall-lumen interfaces (PWL and AWL,  
188 respectively), and the plaque top and bottom surfaces (PTS and PBS, respectively)  
189 (Fig.1a). PWL and AWL were selected on the normal, i.e. non-atheromatous, arterial  
190 wall, adjacent to the plaque.

191 *B - Motion estimation of selected ROIs.* The radial and longitudinal positions of all  
192 pixels included in the selected ROIs were estimated across all frames with an  
193 adaptive block-matching algorithm, which incorporates Kalman filtering<sup>35</sup>. This  
194 algorithm was evaluated in an *in silico* framework consisting of 13 simulated  
195 sequences, and has been shown to be accurate and robust in motion tracking of the  
196 arterial wall from B-mode ultrasound images<sup>13</sup>. For each ROI,  $1.6 \times 1 \text{ mm}^2$  reference  
197 blocks were selected in the first frame, centred at ROI pixels. Fig.1b shows examples  
198 of selected ROIs (AWL, PWL, PTS, PBS) for a diastolic, an intermediate and a systolic  
199 frame and of the sequence.

200        *C - Waveforms extracted from motion analysis.* ROI positions were used to  
201        estimate six sets of waveforms for each plaque:  
202        (i) wall diameter, which was selected as the most representative waveform, i.e. the  
203        one in which the most clear cyclic motion was observed, among the distances of  
204        vertical pairs of AWL and PWL pixels,  
205        (ii) radial displacements of all PTS pixels, namely their radial positions along  
206        consecutive frames,  
207        (iii) longitudinal displacements of all PTS pixels, namely their longitudinal positions  
208        along consecutive frames,  
209        (iv) radial displacements of all PBS pixels,  
210        (v) longitudinal displacements of all PBS pixels, and  
211        (vi) radial distances of PTS and PBS pixel pairs, defined as the absolute differences of  
212        waveforms (ii), (iv) across vertical pixel pairs.

213        Twenty five pixels from the right and 25 from the left edge of the plaque PTS and  
214        PBS were removed to ensure that only plaque pixels, and no normal (non-plaque)  
215        wall area, were included in the analysis. The number of removed pixels (25) was  
216        heuristically determined, following visual inspection and testing. Fig.1c shows  
217        examples of interrogated waveforms.

218        A high-pass 4<sup>th</sup> order Butterworth filter with a cutoff frequency of 0.6 Hz was  
219        applied to the displacement waveforms<sup>36</sup>, so as to remove unwanted offsets or  
220        abrupt fluctuations present in the low-frequency band. The cutoff value was selected  
221        to ensure that heart rates above approximately 40 beats per minute remain  
222        unaffected after filtering. Independent component analysis (ICA) demonstrated that

the suggested methodology is robust against external motion (Supplementary methods).

*D - Calculation of cross-correlations.* Three types of cross-correlations were calculated using the previously described waveforms:

- a) Cross-correlation 1 (CC1): Radial deformation of the plaque with wall diameter, i.e. waveforms (i) and (vi),
- b) Cross-correlation 2 (CC2): Radial displacements of plaque top and bottom surfaces, i.e. waveforms (ii) and (iv), and
- c) Cross-correlation 3 (CC3): Longitudinal displacements of plaque top and bottom surfaces, i.e. waveforms (iii) and (v).

CC2 and CC3 describe intra-plaque kinematics, whereas CC1 was considered, so as to provide a measure with respect to a well-known arterial parameter.

Fig.1d shows examples of interrogated pairs of waveforms ((a)-(c), above) and their corresponding cross-correlations.

Signals to be correlated were confined within an average cycle window, estimated from the dominant frequency of the wall diameter waveform.

From each cross-correlation waveform, two types of measurements were obtained: (a) the sign corresponding to the maximum absolute cross-correlation, and (b) the corresponding lag  $d_{max}$ , in seconds (Fig.1d). For each plaque, cross-correlation waveforms were produced for all PTS-PBS pairs, and the following indices were then extracted:

- The synchronisation percentage, defined as the percentage of the positive values present in the entire set of maximum signed cross-correlation values, derived from all PTS-PBS pairs of the plaque. According to the principles of

247 cross-correlation described previously, this percentage represents the  
248 proportion of plaque pairs that exhibit synchronous motion patterns for a  
249 given type of cross-correlation.

- 250 • Seven statistical (histogram-based) measures (maximum-, minimum-, mean-,  
251 median-value, standard deviation, skewness, and kurtosis) of the lags  $d_{max}$   
252 extracted from all PTS-PBS pairs of the plaque.

253

254 Therefore a total of 24 features were extracted for each plaque, namely 8  
255 features (synchronisation percentage and 7 statistical indices) for each of the 3  
256 cross-correlation types.

257

#### 258 *Grayscale normalisation and estimation of plaque echogenicity*

259 To normalise ultrasound images according to widely accepted procedures<sup>37</sup>, the  
260 physician selected a region in the blood and one in the adventitia, and the median  
261 pixel values of these regions ( $GSM_{blood}$  and  $GSM_{adv}$ , respectively) were set as the  
262 lowest (black) and the highest (white) values in the image, respectively. Then, the  
263 image grayscale intensities were linearly adjusted so that  $GSM_{blood}$  was 0, and  $GSM_{adv}$   
264 was 190<sup>37</sup>.

265 An echolucent plaque is a dark appearing plaque in the ultrasound recording,  
266 while an echogenic plaque is a bright appearing one<sup>38</sup>. Plaque echogenicity was  
267 estimated as follows: the plaque was located automatically in each frame of the  
268 sequence after the first frame, using motion analysis of PBS and PTS areas, and the  
269 corresponding grayscale median (GSM) values were calculated. Plaque GSM was

270 defined as the mean value of the GSMs of all frames. Echolucent plaques were  
271 considered those with a  $GSM < 25^{39}$  and echogenic those with  $GSM \geq 25$ .

272

### 273 *Variability study*

274 Intra and inter-observer variability were assessed by means of phase shift  
275 measurements performed for plaque boundaries displaced by 0-2 pixels with respect  
276 to the original (expert-annotated) ones. This experiment was designed based on the  
277 assumption that different observers, or the same observer at different times,  
278 produce different tissue outlines, which are displaced versions of a given contour.  
279 The range of the displacements (0-2 pixels, including subpixel values) was selected  
280 heuristically, based on observations that tissue outlines derived by different experts  
281 were not more than 2 pixels apart. Differences between original and displaced  
282 versions in all cases were assessed statistically.

283

### 284 *Classification & statistical analysis*

285 The four associations investigated were validated through classification schemes  
286 using supervised machine learning. The purpose of classification was to evaluate the  
287 overall potential of the extracted features, which, can alternatively be considered as  
288 a “motion synchronisation signature”, through their association with the four clinical  
289 phenotypes. Subsequently, statistical analysis was performed, to identify the  
290 features with the highest discriminatory ability.

291 Feature selection was applied using Principal Component Analysis (PCA),  
292 whereby the initial feature set is converted into a reduced set of linearly  
293 uncorrelated features, orthogonal to each other (principal components), which

294 retains most of the initial set's variance, namely, its information content<sup>40</sup>. For this  
295 study, as many principal components as necessary were retained to cover 95% of the  
296 initial set's variance.

297 Classification models for each association were implemented using the Weka  
298 workbench version 3.6 (Machine Learning Group at the University of Waikato,  
299 Hamilton, New Zealand)<sup>41</sup>. Among the algorithms available in Weka, the Random  
300 Forest (RF) algorithm was used, due to its superior performance and its robustness  
301 to overfitting<sup>42</sup>. The RF algorithm uses a number of parameters that need to be  
302 tuned properly, before training, to avoid overfitting or underfitting. The two  
303 parameters that were tuned included the number of features to be used in random  
304 selection (range: 2-number of features, with a step of 1), and the number of trees to  
305 be generated (range: 100-900, with a step of 200). For parameter tuning, 10-fold  
306 cross-validation was used. The parameters that were tuned included the number of  
307 data points, the number of features of each tree of the forest, and the number of the  
308 trees that we build for the forest.

309 To address the problem of class imbalance that is present in our data, the  
310 ADASYN algorithm<sup>43</sup> was applied to create synthetic samples for the minority class,  
311 i.e. the class with the lowest number of cases. Of note, these synthetic samples were  
312 used only for training the model, not for testing.

313 For the evaluation of each model, leave-one-out cross-validation (LOOCV) was  
314 chosen, because the medium size of our dataset indicated it as the optimal choice in  
315 terms of computational cost, as well as bias-variance trade-off<sup>44</sup>.

316 To evaluate the performance of the classification models, a set of metrics was  
317 calculated, including accuracy (ACC), sensitivity (SENS), specificity (SPEC), precision

318 (PREC), negative predictive value (NPV), F1 score (F1SC) and the area under the  
319 Receiver Operating Characteristics (ROC) curve (AUC)<sup>45</sup>.

320 Statistical analysis was performed using the non-parametric Wilcoxon rank sum  
321 test and statistical significance was considered for a p-value equal to or lower than  
322 0.05.

323 All analyses were performed using Matlab R2016a (MathWorks, Natick, MA, USA) and a  
324 computer with an Intel Core i5 220 GHz CPU.

325

## 326   **Results**

327       Table 1 shows the performance of the RF classifier, for the four associations  
328   interrogated, in terms of the evaluation metrics described in the previous section.  
329   This corresponds to the overall performance of all interrogated PCA-selected  
330   features.

331       Regarding the variability study, all indices were similar between the original and  
332   the displaced versions. As an example, the p-values for the mean phase shifts were  
333   0.46 for CC1 and CC2 and 0.39 for CC3.

334       In the following subsections detailed results are presented for the statistical  
335   analysis of the entire dataset, for each of the investigated scenarios. Tables showing  
336   statistical analysis results present values for synchronisation percentages and mean  
337   phase shifts, even if they were not found statistically different, so as to provide a feel  
338   for these measures, given they are reported for the first time.

339

### 340   *Association with plaque echogenicity*

341       Of the 135 plaques of the dataset, 37 were echolucent ( $GSM < 25$ ) and 98 were  
342   echogenic ( $GSM \geq 25$ ). The stenosis degrees and ages were not statistically different in  
343   the two groups (p-values=0.17 and 0.24, respectively).

344       The application of PCA identified 13 features as the principal components  
345   satisfying the 95% variance coverage criterion for this association.

346       Table 2 shows the mean values and corresponding p-values of the  
347   synchronisation percentages, mean phase shift values, and statistically significant  
348   features for the three cross-correlation types, in echogenic and echolucent plaques.  
349   As we can see, in echolucent plaques, the top plaque surface moves less



350 synchronously (with a higher phase shift) relative to the bottom surface, than in  
351 echogenic plaques, in the radial direction (higher  $\text{mean}_{\text{CC2}}$  and  $\text{median}_{\text{CC2}}$ ). Also, the  
352 mean phase shifts between top and bottom surfaces of both echogenic and  
353 echolucent plaques were significantly higher in the longitudinal direction, compared  
354 to the radial direction.

355

#### 356 *Association with symptomaticity*

357 Of the 124 plaques used in this substudy, 93 caused a degree of stenosis higher  
358 than or equal to 70%. Of these 93 high-stenosis plaques, 71 were asymptomatic and  
359 22 were symptomatic. The stenosis degrees and ages were not statistically different  
360 in the two groups ( $p\text{-values}=0.15$  and  $0.35$ , respectively).

361 The application of PCA identified 11 features as the principal components  
362 satisfying the 95% variance coverage criterion for this association.

363 Table 3 shows the mean values and corresponding  $p\text{-values}$  of the  
364 synchronisation percentages, mean phase shift values, and statistically significant  
365 features for the three cross-correlation types, for asymptomatic and symptomatic  
366 plaques. As we can see, there was no difference between symptomatic and  
367 asymptomatic cases (except for 3 histogram-based features). Also, the mean phase  
368 shifts between top and bottom surfaces of asymptomatic plaques were significantly  
369 higher in the longitudinal direction, compared to the radial direction. Symptomatic  
370 plaques did not show such difference.

371

372 *Association with stenosis degree*

373       Of the 124 plaques used in this substudy, 97 were asymptomatic. Of these 97  
374 asymptomatic plaques, 26 caused a low degree of stenosis ( $<70\%$ ) and 71 caused a  
375 high degree of stenosis ( $\geq 70\%$ ). The ages of the patients were not statistically  
376 different in the two groups ( $p\text{-value}=0.16$ ). By definition, the high-stenosis group in  
377 this study is the same as the asymptomatic group in the previous study.

378       The application of PCA identified 13 features as the principal components  
379 satisfying the 95% variance coverage criterion, for this association.

380       Table 4 shows the mean values and corresponding  $p$ -values of the  
381 synchronisation percentages, mean phase shift values, and statistically significant  
382 features for the three cross-correlation types, for low- and high-stenosis plaques. As  
383 we can see, in high-stenosis plaques, the top plaque surface moves less  
384 synchronously (higher  $\max_{CC2}$ , higher  $\text{mean}_{CC2}$ ) and less uniformly (higher  $\text{stdev}_{CC2}$ )  
385 relative to the bottom surface, than in low-stenosis plaques, in the radial direction.  
386 Also, the mean phase shifts between top and bottom surfaces of both low- and high-  
387 stenosis plaques were significantly higher in the longitudinal direction, compared to  
388 the radial direction.

389

390 *Association with plaque risk*

391       Of the 124 plaques used in this substudy, 26 were low-risk and 98 were high-risk.  
392 The ages of the patients were not statistically different in the two groups ( $p$ -  
393 value=0.25). According to the current clinical decision-making scheme, high-risk  
394 subjects are symptomatic ones with stenosis degrees  $\geq 50\%$ <sup>46</sup> and asymptomatic  
395 subjects with stenosis degrees  $\geq 70\%$ <sup>47</sup>; otherwise subjects are considered low-risk<sup>5</sup>.

396 The application of PCA identified 12 features as the principal components  
397 satisfying the 95% variance coverage criterion for this association.

398 Table 5 shows the mean values and corresponding p-values of the  
399 synchronisation percentages, mean phase shift values, and statistically significant  
400 features for the three cross-correlation types, for low- and high-risk plaques. As we  
401 can see, in high-risk plaques, the top plaque surface moves less synchronously  
402 (higher  $\text{mean}_{\text{CC2}}$ ) and less uniformly (higher  $\text{stdev}_{\text{CC2}}$ ) relative to the bottom surface,  
403 than in low-risk plaques, in the radial direction. In addition to this, most of the  
404 significantly different features (3 out of 4) were derived from cross-correlation type  
405 2, namely between radial motion of top and bottom plaque surfaces. Also, the mean  
406 phase shifts between top and bottom surfaces of both low-risk and high-risk plaques  
407 were significantly higher in the longitudinal direction, compared to the radial  
408 direction.

409

410 As it can be observed, a few of the features in the previous Tables 2, 4 and 5  
411 present high standard deviations, sometimes even higher than the corresponding  
412 mean values ( $\text{median}_{\text{CC2}}$  in Table 2, and  $\text{min}_{\text{CC1}}$  in Tables 4 and 5), indicating a high  
413 inter-plaque variability, probably due to differences between subjects.

414

### 415 *Representative examples of cross-correlation distributions*

416        Figures 2 and 3 illustrate examples of distributions of cross-correlations of the  
417 three types of cross-correlations for an echogenic, asymptomatic, low-stenosis case  
418 and an echolucent, symptomatic, high-stenosis case, respectively. Cross-correlation  
419 values correspond to pixels along the manually extracted plaque contour in the first  
420 frame of the sequence. Videos 1 and 2 show the displacements of the interrogated  
421 ROIs (AWL, PWL, PTS and PBS) in each case. Synchronisation percentages were 38%,  
422 100% and 100% for CC1, CC2 and CC3, respectively, in the asymptomatic case and  
423 95%, 72% and 53% for CC1, CC2 and CC3, respectively, in the symptomatic case.  
424 Mean phase shifts were 0.45 s, 0.00 s and 0.04 s for CC1, CC2 and CC3, respectively,  
425 in the asymptomatic case and 0.79 s, 0.33 s and 0.48 s for CC1, CC2 and CC3,  
426 respectively, in the symptomatic case.

427

### 428 **Discussion**

429        This study showed that the synchronisation percentages in our dataset were  
430 approximately 50%, 80% and 80%, for CC1, CC2 and CC3, respectively, and the mean  
431 phase shifts were 0.4 s, 0.2 s and 0.3 s, respectively. To the best of our knowledge,  
432 such features characterising phase shifts and synchronisation percentages of the  
433 motion of carotid atheromatous plaque from B-mode ultrasound have not been  
434 previously quantified. The RF algorithm yielded AUC scores of 0.81, 0.79, 0.89 and  
435 0.90, for the association with echogenicity, symptomaticity, stenosis degree and  
436 plaque risk, respectively. It was also observed that echolucent, high-stenosis and  
437 high-risk plaques had significantly higher phase shifts between the radial

438 displacements of their top and bottom surfaces (0.23-0.26 s on average), compared  
439 to echogenic, low-stenosis and low-risk plaques (0.16-0.20 s on average).

440 The interrogated phenotypes were selected on the grounds of their associations  
441 with plaque vulnerability and selection of treatment. Specifically, echogenicity has  
442 been associated with increased vulnerability. Symptomatic and asymptomatic  
443 plaques with stenosis degrees higher than 70% are currently offered carotid  
444 revascularisation<sup>5</sup>. Asymptomatic subjects with low- and high-stenoses are offered  
445 different treatments; conservative treatment with medication for the former, while  
446 carotid revascularisation for the latter<sup>5</sup>.

447 Feature selection identified the same set of features for most association  
448 scenarios (3 out of 4, with a small differentiation for the symptomaticity scenario).  
449 Also, in all association studies, NPV had the lowest value among all evaluation  
450 metrics. This is expected, because the “negative” class was the minority class,  
451 namely it was outnumbered by the “positive” class, therefore, this metric reflects the  
452 inferiority of the “negative” class in terms of sample size. It is pointed out that 3  
453 additional classifiers, besides RF, were benchmarked on the same dataset, namely  
454 Multilayer Perceptron, Nearest Neighbours and Support Vector Machines (SVMs).  
455 These algorithms perform supervised machine learning, i.e. their inputs and outputs  
456 are known; see<sup>48</sup> for more information. The performances of these classifiers were  
457 inferior compared to the RF algorithm.

458 Although feature selection identified 12-13 features for each association  
459 scenario, statistical analysis yielded fewer features, namely 2-4 depending on the  
460 scenario. This indicates that despite the relatively low number of statistically  
461 significant features in a specific association, there is additional, potentially

462 discriminatory, information which is uniformly distributed among the entire set of  
463 the 24 features, and is revealed with classification. The good performance of the  
464 classifiers, ranging from 79% to 90%, indicates that there is sufficient information  
465 present in the datasets of all association scenarios.

466 Most of the previous studies have used statistical tests to validate their  
467 results<sup>9,13,22,25-27</sup>, while machine learning methodologies have been introduced in  
468 fewer cases<sup>11,30,32 24</sup>. Specifically, Gastounioti et al.<sup>11</sup> compared multiple classifiers  
469 and feature selection methods, as well as combinations of them, and concluded that  
470 the SVM classifier combined with the Fisher Discriminant Ratio for feature selection  
471 were optimal in discriminating symptomatic and asymptomatic patients. Meshram et  
472 al.<sup>30</sup> and Wang et al.<sup>32</sup> implemented a logistic regression classifier and ROC analysis,  
473 towards correlation of plaque strain indices with patient cognitive function. Finally,  
474 Ichinose et al.<sup>24</sup> implemented a multiple linear regression analysis (stepwise analysis  
475 and partial least squares analysis), followed by a machine learning analysis using an  
476 Artificial Neural Network based on the Log-Linearised Gaussian Mixture Network, to  
477 correlate the “jellyfish sign” of motion with the presence of new lesions, detected by  
478 diffusion-weighted imaging. The generation of these lesions is the most common  
479 complication caused by carotid artery stenting. Machine learning is appropriate for  
480 the study of complex relations, whereas statistical tests are limited to simpler cases.  
481 The combination of both machine learning and statistical analysis methodologies,  
482 which is implemented in the current study, allows the design of a robust, multi-level  
483 validation scheme and, thus, the extraction of reliable results about the complex  
484 phenomenon of plaque motion synchronisation.

485 Echolucent, high-stenosis and high-risk plaques presented significantly higher  
486 phase shifts between the radial displacements of their top and bottom surfaces,  
487 compared to echogenic, low-stenosis and low-risk plaques. A potential implication of  
488 these findings is that asynchronous motion patterns are associated with higher  
489 plaque vulnerability, given their association with its determinants, including  
490 echolucency, high-stenosis and presumed high risk. These results and related  
491 implications should be confirmed in follow-up studies. In contrast, statistical analysis  
492 between symptomatic and asymptomatic plaques did not reveal any differences.  
493 This finding may imply that echogenicity and stenosis degree hold more information  
494 and, thus, are more crucial clinical parameters, than symptomaticity, as far as plaque  
495 kinematics are concerned. Moreover, the significantly higher phase shifts in the  
496 longitudinal direction, in the majority of interrogated groups (7 out of 8), indicate  
497 more asynchronous intra-plaque motion in the longitudinal direction, than in the  
498 radial direction.

499 The main findings of this research, namely that echolucent, high-stenosis and  
500 high-risk plaques are characterised by higher phase shifts and, thus, less synchronous  
501 motion patterns between the radial motion of their top and bottom surfaces than  
502 echogenic, low-stenosis and low-risk plaques, qualitatively agree with other studies  
503 on plaque kinematics. Gastounioti et al.<sup>13</sup> reported that symptomatic plaques  
504 presented 37% higher radial motion range of PTS and 50% higher relative movement  
505 between PTS and PBS. Moreover, Kume et al.<sup>25</sup>, Ogata et al.<sup>26</sup> and Ichinose et al.<sup>24</sup>  
506 showed that the jellyfish sign, a pattern that characterises the asynchronous motion  
507 of the plaque relative to the adjacent wall, is associated with plaque vulnerability  
508 and stroke recurrence. Gastounioti et al.<sup>49</sup> found that echolucent plaque segments

509 moved more intensely in the radial direction, compared to echogenic plaque  
510 segments. Finally, Tat et al.<sup>12</sup> reported that patients with severe plaque stenosis  
511 presented greater longitudinal anterograde wall motion than those with moderate  
512 stenosis. In combination with our finding that high-stenosis plaques had significantly  
513 higher and more dispersed phase shifts between the radial displacement of their top  
514 and bottom surfaces, this suggests that irregular wall dynamics characterising high-  
515 stenosis cases may be reflected not only within plaque but also in relative movement  
516 with the adjacent wall.

517       This work is one of the studies demonstrating the ability to extract features  
518 characterising tissue kinematics from B-mode ultrasound images. Although  
519 radiofrequency ultrasound is being widely used for tissue motion and strain  
520 estimation<sup>23,31,32</sup>, B-mode has also been used for motion measurements<sup>22,24,26,29</sup>. In  
521 this work, only B-mode data were available in the commercial scanning device that  
522 was used. It has been shown that radiofrequency ultrasound outperforms B-mode,  
523 due to its reduced variability in cardiac strain estimation<sup>50</sup>. A more recent study  
524 however showed that local arterial characteristics can be assessed equally reliably  
525 and accurately with B-mode technology<sup>51</sup>. Advantages of B-mode include relatively  
526 low-cost and widespread use in clinical practice, while radiofrequency devices are  
527 higher-cost and mostly used for research purposes. It is therefore important to be  
528 able to extract as much information as possible from the widely available B-mode  
529 devices allowing to address a wider range of clinical applications. B-mode-  
530 ultrasound-based tissue kinematics could be further combined with other plaque  
531 properties, such as neovascularisation and elasticity, assessed using contrast-



532 enhanced ultrasound and elastography, respectively, towards providing an overall  
533 valid plaque characterisation<sup>52</sup>.

534 Motion of the arterial wall and plaque during the cardiac cycle is a particularly  
535 complex phenomenon, resulting from the combined effect of a number of different  
536 forces/stresses, including translation, rotation, shear, tethering, etc. Taking into  
537 account the complexity of this phenomenon, in this study we selected to address  
538 representative plaque motion patterns, namely in relation to adjacent wall as well as  
539 in the radial and longitudinal directions within itself.

540 The limitations of this study include the medium size and the heterogeneity of  
541 the dataset. Compared to previous studies on ultrasound-based carotid plaque  
542 kinematics, in which dataset sizes ranged from 11 to 165 patients, our 77-patient  
543 (135-plaque) dataset was considered adequate for benchmarking our methodology.  
544 Dataset heterogeneity consists in including subjects of both gender and with lesions  
545 located in both the left and right carotids. Although larger and more homogeneous  
546 datasets are always desirable to reach safer conclusions, we believe that the medium  
547 size of our dataset and the grouping into smaller, somewhat more homogeneous,  
548 datasets has allowed us to make some reliable and interesting observations.

549 The findings presented in this study are promising for further in-depth study of  
550 carotid plaque kinematics from B-mode ultrasound. Future work in this area might  
551 focus on the combination of phase-shift features with other ultrasound-based  
552 kinematic features towards extracting valuable information about plaque mechanics.  
553 The application of the proposed classification model to substantially larger datasets,  
554 including follow-up patient data, will allow the identification of potential novel  
555 markers for improved risk stratification.

556 In conclusion, this study quantified synchronisation patterns of the carotid  
557 atheromatous plaque from B-mode ultrasound, and associated them with  
558 echogenicity, symptomaticity, stenosis degree and plaque risk. Synchronisation  
559 percentages in our dataset were approximately 50%, 80% and 80% and the mean  
560 phase shifts 0.4 s, 0.2 s and 0.3 s, for cross-correlation types 1, 2 and 3, respectively.  
561 The RF algorithm, combined with PCA, achieved very good performance in the  
562 benchmarking procedures, yielding AUC scores of 0.81, 0.79, 0.89 and 0.90, for the  
563 association with echogenicity, symptomaticity, stenosis degree and plaque risk,  
564 respectively. Statistical analysis showed that echolucent, high-stenosis and high-risk  
565 plaques exhibited higher phase shifts between the radial displacements of their top  
566 and bottom surfaces. These findings are promising for further in-depth study of  
567 ultrasound-based carotid plaque kinematics, towards improving risk stratification.

568

## 569 **Data availability**

570 The datasets generated and analysed during the current study are available from the  
571 corresponding author on reasonable request.

572

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718

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725

## 726 **Author contributions**

727       S.G., E.P., A.G. and K.S.N. designed the experiments. E.P. performed the  
728       experiments. E.P., A.G., I.A. and S.G. analysed the data. C.L. overviewed recruitment  
729       of subjects and collection of clinical data. S.G. and E.P. wrote the main manuscript.  
730       All authors reviewed the manuscript.

731

732

## 733 **Additional Information**

734       **Competing Interests:** the authors declare no competing interests.

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## 737 **Figure Legends**

738       Fig.1. Examples of interrogated ROIs and corresponding waveforms, illustrating  
739       the different steps of the methodology. A – Plaque and wall ROIs in frame 1. Vertical  
740       yellow dotted lines indicate the boundaries of the investigated area. The top and  
741       bottom edges of the vertical yellow solid line correspond to the ROI pair for which

742 waveforms are illustrated. B – Plaque and wall ROI pixel positions at three different  
743 frames representing different phases of the cardiac cycle, and corresponding time  
744 points, obtained after motion analysis. Pixel positions at diastole are superimposed  
745 on intermediate and systolic frames as dashed lines. C – Radial displacements of  
746 selected pixels of PTS, PBS and their difference (left column), and radial  
747 displacements of selected pixels of AWL, PWL and their difference, which represents  
748 the arterial wall diameter (right column). D – Displacement pairs for estimation of  
749 cross-correlation (top row), the corresponding cross-correlation waveforms (middle  
750 row) and the selected cross-correlation segment for calculation of features (bottom  
751 row). RDis: radial displacement, PRDef: plaque radial deformation, WD: wall  
752 diameter, LDis: longitudinal displacement.

753 Fig. 2. Example of an echogenic (GSM=30) low-stenosis (60%) asymptomatic  
754 plaque, with (a) contours superimposed on the B-mode image, illustrating the  
755 distribution of CC1 values on PTS and PBS (top row), and displacement waveforms of  
756 the central pixel pair and the corresponding cross-correlation waveform (bottom  
757 row), (b) contours superimposed on the B-mode image, illustrating the distribution  
758 of CC2 values on PTS and PBS (top row), and displacement waveforms of the central  
759 pixel pair and the corresponding cross-correlation waveform (bottom row) and (c)  
760 contours superimposed on the B-mode image, illustrating the distribution of CC3  
761 values on PTS and PBS (top row), and displacement waveforms of the central pixel  
762 pair and the corresponding cross-correlation waveform (bottom row). RDis: radial  
763 displacement, PRDef: plaque radial deformation, WD: wall diameter, LDis:  
764 longitudinal displacement.

765        Fig. 3. Examples of an echolucent (GSM=15) high-stenosis (70%) symptomatic  
766        plaque, with (a) contours superimposed on the B-mode image, illustrating the  
767        distribution of CC1 values on PTS and PBS (top row), and displacement waveforms of  
768        the central pixel pair and the corresponding cross-correlation waveform (bottom  
769        row), (b) contours superimposed on the B-mode image, illustrating the distribution  
770        of CC2 values on PTS and PBS (top row), and displacement waveforms of the central  
771        pixel pair and the corresponding cross-correlation waveform (bottom row) and (c)  
772        contours superimposed on the B-mode image, illustrating the distribution of CC3  
773        values on PTS and PBS (top row), and displacement waveforms of the central pixel  
774        pair and the corresponding cross-correlation waveform (bottom row). RDis: radial  
775        displacement, PRDef: plaque radial deformation, WD: wall diameter, LDis:  
776        longitudinal displacement.  
777  
778



Table 1. Values of evaluation metrics for the four associations investigated, corresponding to the overall performance of all interrogated PCA-selected features.

	<b>ACC</b>	<b>SENS</b>	<b>SPEC</b>	<b>PREC</b>	<b>NPV</b>	<b>F1SC</b>	<b>AUC</b>
<i>Echogenicity</i>	0.73	0.73	0.73	0.88	0.51	0.80	0.81
<i>Symptomaticity</i>	0.69	0.69	0.68	0.88	0.41	0.77	0.79
<i>Stenosis degree</i>	0.85	0.86	0.81	0.92	0.68	0.89	0.89
<i>Plaque risk</i>	0.84	0.83	0.88	0.96	0.58	0.89	0.90

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Table 2. Mean  $\pm$  standard deviation values and corresponding p-values of the synchronisation percentages, mean phase shift values, and statistically significant features for the three cross-correlation types, for echogenic and echolucent plaques.

	Echogenic	Echolucent	p-value
$sp_{CC1}$	$52\% \pm 24\%$	$60\% \pm 23\%$	0.11
$sp_{CC2}$	$82\% \pm 17\%$	$81\% \pm 15\%$	0.47
$sp_{CC3}$	$77\% \pm 18\%$	$74\% \pm 16\%$	0.22
$mean_{CC1}$ (s)	$0.42 \pm 0.20$	$0.40 \pm 0.19$	0.83
$mean_{CC2}$ (s)	$0.20 \pm 0.15$	$0.26 \pm 0.15$	<b>0.05</b>
$mean_{CC3}$ (s)	$0.30 \pm 0.18^*$	$0.34 \pm 0.15^*$	0.09
$median_{CC2}$ (s)	$0.09 \pm 0.17$	$0.11 \pm 0.13$	<b>0.05</b>

sp: synchronisation percentage

\* indicates significant difference (p-value<0.05) with respect to  $mean_{CC2}$

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Table 3. Mean  $\pm$  standard deviation values and corresponding p-values of the synchronisation percentages, mean phase shift values, and statistically significant features for the three cross-correlation types, for asymptomatic and symptomatic plaques of high stenosis degrees.

	Asymptomatic	Symptomatic	p-value
$sp_{CC1}$	$57\% \pm 23\%$	$53\% \pm 21\%$	0.38
$sp_{CC2}$	$82\% \pm 15\%$	$79\% \pm 14\%$	0.34
$sp_{CC3}$	$80\% \pm 14\%$	$76\% \pm 19\%$	0.36
$mean_{CC1}$ (s)	$0.40 \pm 0.20$	$0.47 \pm 0.18$	0.13
$mean_{CC2}$ (s)	$0.23 \pm 0.14$	$0.24 \pm 0.13$	0.51
$mean_{CC3}$ (s)	$0.29 \pm 0.15^*$	$0.32 \pm 0.18$	0.64
$max_{CC1}$ (s)	$1.02 \pm 0.20$	$1.14 \pm 0.13$	<b>0.01</b>
$stdev_{CC1}$ (s)	$0.33 \pm 0.11$	$0.39 \pm 0.09$	<b>0.05</b>
$max_{CC3}$ (s)	$0.96 \pm 0.27$	$1.06 \pm 0.24$	<b>0.05</b>

sp: synchronisation percentage, stdev: standard deviation

\* indicates significant difference (p-value<0.05) with respect to  $mean_{CC2}$

783

784

Table 4. Mean  $\pm$  standard deviation values and corresponding p-values of the synchronisation percentages, mean phase shift values, and statistically significant features for the three cross-correlation types, for low-stenosis and high-stenosis plaques.

	Low-stenosis	High-stenosis	p-value
$sp_{CC1}$	50% $\pm$ 27%	57% $\pm$ 23%	0.27
$sp_{CC2}$	85% $\pm$ 16%	82% $\pm$ 15%	0.14
$sp_{CC3}$	75% $\pm$ 18%	80% $\pm$ 14%	0.23
$mean_{CC1}$ (s)	0.41 $\pm$ 0.20	0.40 $\pm$ 0.20	0.98
$mean_{CC2}$ (s)	0.16 $\pm$ 0.15	0.23 $\pm$ 0.14	<b>0.03</b>
$mean_{CC3}$ (s)	0.27 $\pm$ 0.15*	0.29 $\pm$ 0.15*	0.49
$min_{CC1}$ (s)	0.03 $\pm$ 0.07	0.02 $\pm$ 0.08	<b>0.03</b>
$max_{CC2}$ (s)	0.66 $\pm$ 0.41	0.94 $\pm$ 0.26	<b>0.00</b>
$stdev_{CC2}$ (s)	0.19 $\pm$ 0.13	0.29 $\pm$ 0.12	<b>0.00</b>

sp: synchronisation percentage, stdev: standard deviation

\* indicates significant difference (p-value<0.05) with respect to  $mean_{CC2}$

785

786

Table 5. Mean  $\pm$  standard deviation values and corresponding p-values of the synchronisation percentages, mean values and statistically significant features for the three cross-correlation types, for low-risk and high-risk plaques.

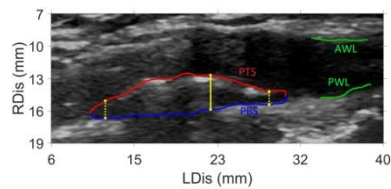
	Low-risk	High-risk	p-value
$sp_{CC1}$	50% $\pm$ 27%	56% $\pm$ 22%	0.34
$sp_{CC2}$	85% $\pm$ 16%	81% $\pm$ 15%	0.08
$sp_{CC3}$	75% $\pm$ 18%	79% $\pm$ 16%	0.36
$mean_{CC1}$ (s)	0.41 $\pm$ 0.20	0.41 $\pm$ 0.20	0.79
$mean_{CC2}$ (s)	0.16 $\pm$ 0.15	0.23 $\pm$ 0.14	<b>0.02</b>
$mean_{CC3}$ (s)	0.27 $\pm$ 0.15*	0.30 $\pm$ 0.16*	0.35
$min_{CC1}$ (s)	0.03 $\pm$ 0.07	0.02 $\pm$ 0.07	<b>0.04</b>
$max_{CC2}$ (s)	0.66 $\pm$ 0.41	0.94 $\pm$ 0.27	<b>0.00</b>
$stdev_{CC2}$ (s)	0.19 $\pm$ 0.13	0.29 $\pm$ 0.12	<b>0.00</b>

sp: synchronisation percentage, stdev: standard deviation

\* indicates significant difference (p-value<0.05) with respect to  $mean_{CC2}$

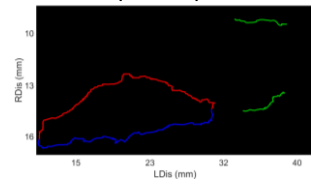
**A - Selection of ROIs**

Frame 1

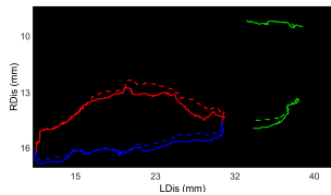


**B - Motion estimation of selected ROIs**

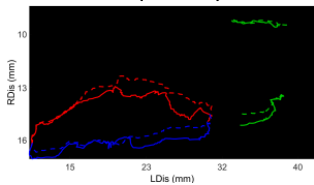
Diastolic frame  
(5.32 s)



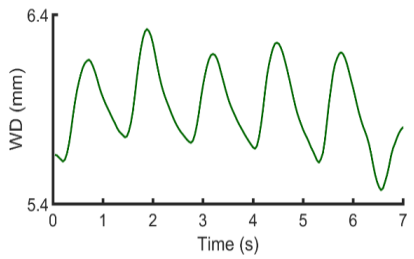
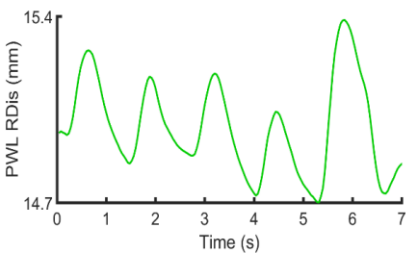
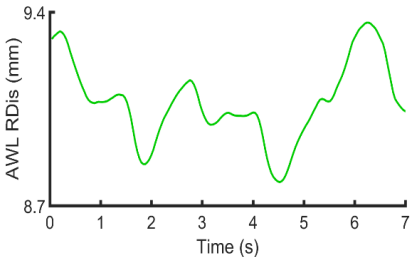
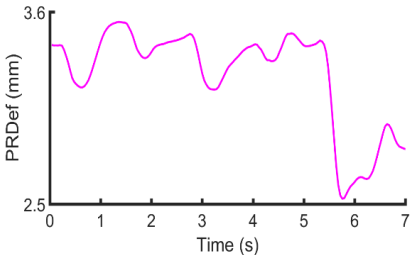
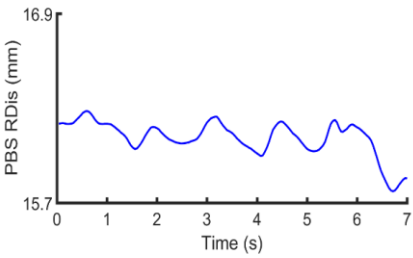
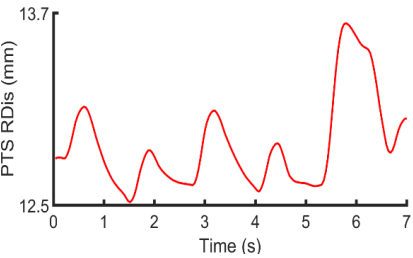
Intermediate frame  
(5.56 s)



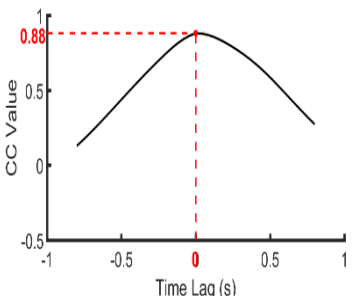
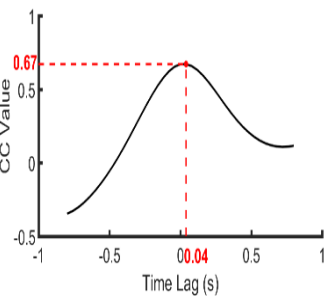
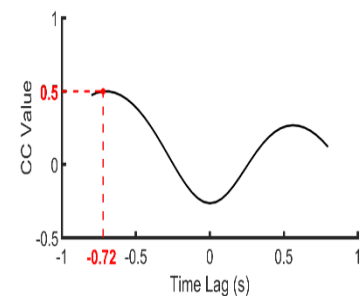
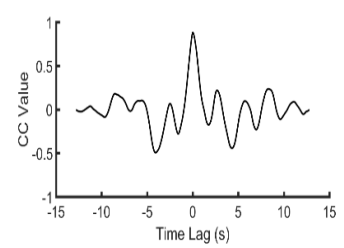
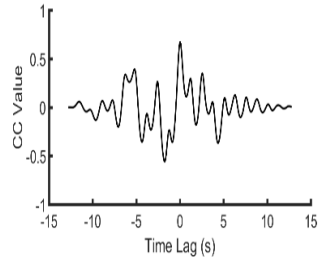
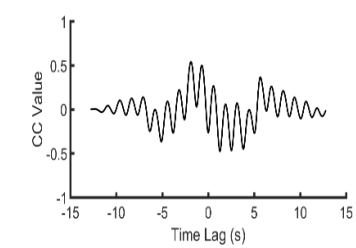
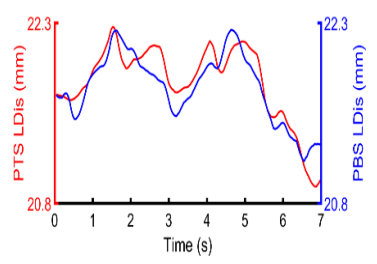
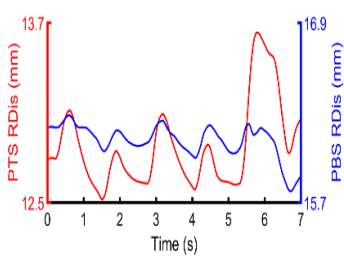
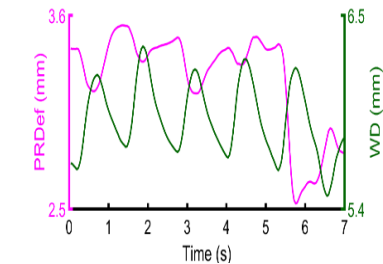
Systolic frame  
(5.76 s)

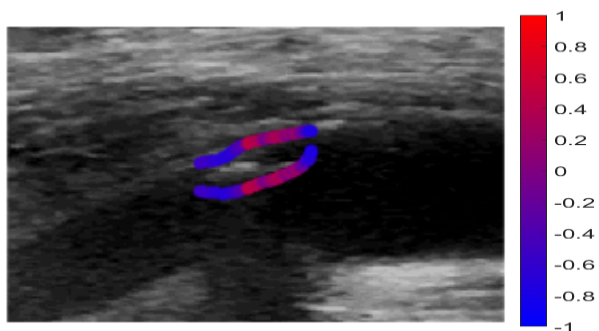


**C - Examples of interrogated waveforms**

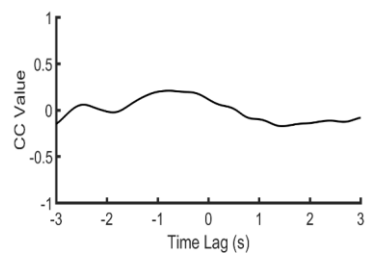
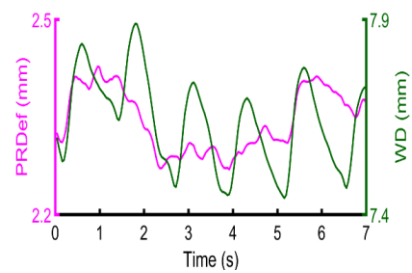


**D - Examples of cross-correlation waveforms**

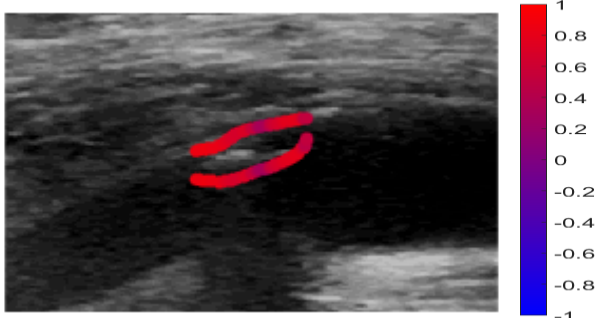
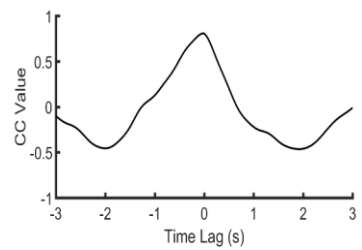
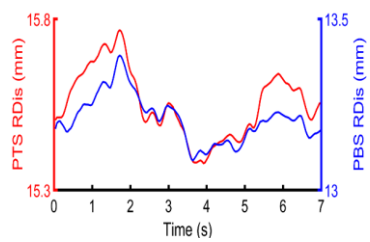




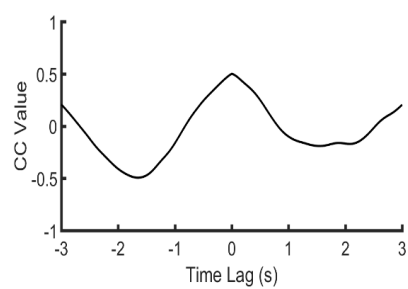
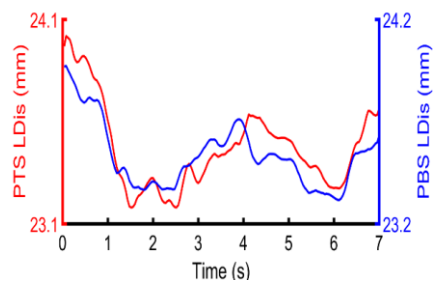
(a)

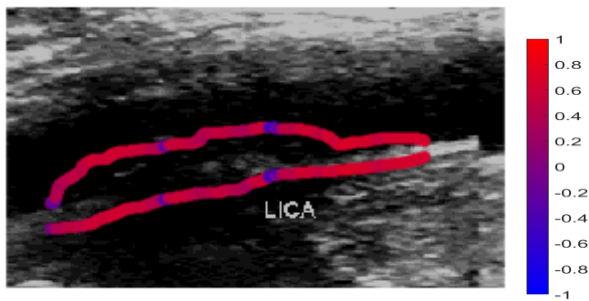


(b)

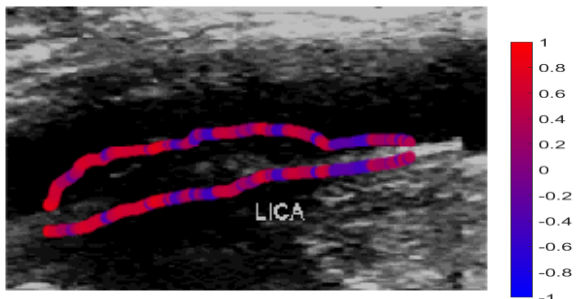
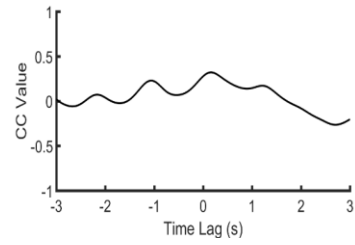
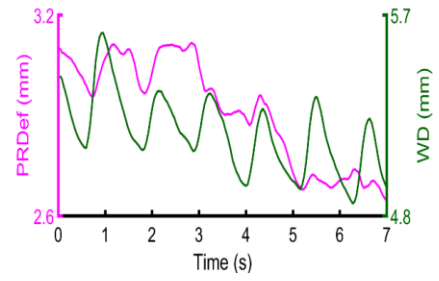


(c)

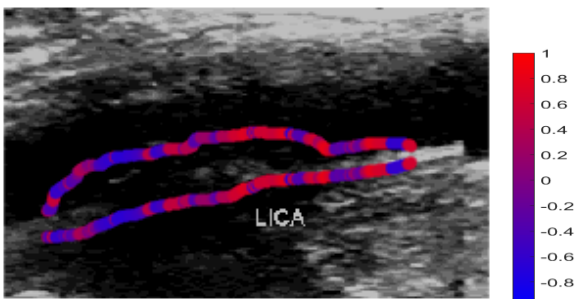
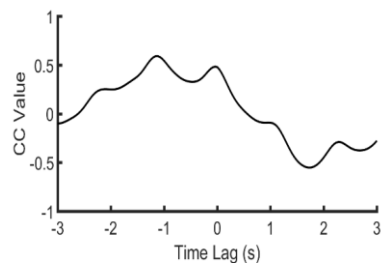
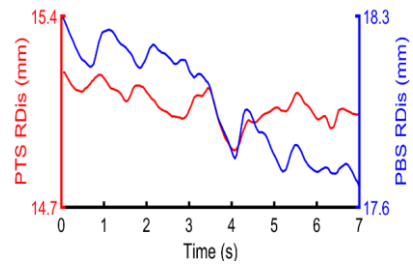




(a)



(b)



(c)

