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## Particle Image Velocimetry to Evaluate Pulse Wave Velocity in Aorta Phantom with the lnD–U Method

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1 **Title: Particle image velocimetry to evaluate pulse wave velocity in aorta phantom with the InD-U method**

2  
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10  
11 **Abstract (251 words)**

12 *Purpose* - Pulse Wave Velocity (PWV) is an indicator of arterial stiffness used in the prediction of cardiovascular  
13 disease such as atherosclerosis. Non-invasive methods performed with ultrasound probes allow one to compute PWV  
14 and aortic stiffness through the measurement of the aortic diameter (D) and blood flow velocity (U) with the InD-U  
15 method. This technique based on *in vivo* acquisitions lacks validation since the aortic elasticity modulus cannot be  
16 verified with mechanical strength tests. *Method* - In the present study, an alternative validation is carried out on an  
17 aorta phantom hosted in an aortic flow simulator which mimics pulsatile inflow conditions. This *in vitro* setup  
18 included a Particle Image Velocimetry device to visualize flow in a 2D longitudinal section of the phantom, compute  
19 velocity fields (U), and track wall displacements in the aorta phantom to measure the apparent diameter (AD)  
20 variations throughout cycles. *Results* - The InD-U method was then applied to evaluate PWV ( $5.92 \pm 0.32$  m/s) and  
21 calculate the Young's modulus of the aorta phantom ( $0.66 \pm 0.08$  MPa). This last value was compared to the  
22 elasticity modulus ( $0.53 \pm 0.07$  MPa) evaluated with tensile strength tests on samples cut from the silicone phantom.  
23 *Conclusion* - Considering the uncertainties from the two methods, the measured elasticities are consistent and close  
24 to a 50-60 years old male aortic behavior. A comparison with *in vivo* data shows that the choice of silicone for the  
25 phantom material is a relevant and promising option to mimic the human aorta on *in vitro* systems.

26  
27 **Keywords:** Pulse Wave Velocity, aorta, phantom, circulatory mock loop, Particle Image Velocimetry

29 **1. Introduction**

30 In vivo, aortic stiffness is associated with some pathologies such as atherosclerosis which is known to reduce  
31 arterial wall elasticity [1, 2]. Different techniques have been developed to investigate arterial stiffness, identify risky  
32 regions and predict the outbreak of cardiovascular diseases [3-5]. One of the most common method to evaluate arterial  
33 stiffness is the measurement of the pulse wave velocity (PWV). PWV is the velocity at which the blood pressure pulse  
34 travels through a vessel. This quantity depends on blood and aortic wall properties and can thus, provide information  
35 on those properties such as aortic stiffness which could indicate the development of a plaque. The measurement of  
36 pulse wave velocity (PWV) can be achieved with non-invasive techniques such as the two points Transits-Time (TT)  
37 method [6,7], the one-point PU-loop (pressure-velocity loop) method [8], and the similar QA (flowrate-area loop)  
38 method [9] and lnD-U (logarithm of the diameter and velocity) derived methods [10-12]. These methods are based on  
39 the study of the relationship between the flow velocity or flowrate in the aorta and aortic walls movements which can  
40 be investigated through pressure, aortic section area; and diameter changes at one location (one-point methods). “Two  
41 points” methods consist in measuring the pressure pulse in two locations of the arterial tree. Knowing the distance  
42 between these two points and measuring the time required for the pulse to travel between them, PWV can be  
43 calculated. This method can be difficult to implement since invasive tools are often needed, the distance measurement  
44 is not trivial, and a perfect synchronization of the two devices that measure the pressure pulse is required [13]. 4D-  
45 MRI (Magnetic Resonance Imaging) can be used to image the whole aorta but involve much more complex and  
46 expensive equipment [14] with a strong impact of temporal resolution on computed PWV [15].

47 Conversely with “two points” assessments, the lnD-U technique provides local stiffness information as a “one-  
48 point” measurement. It can be performed with a routine US-Doppler (UltraSound) exam and only requires computing  
49 the aortic diameter change and blood velocity. However, the main limitation in the lnD-U method is the lack of in-  
50 vivo validation [11,12]. The method is mainly based on the Bramwell-Hill equation [16] which states that PWV is  
51 proportional to the aorta pressure variation and inversely proportional to aortic area variation throughout a cardiac  
52 cycle. From this PWV value, the arterial stiffness can be estimated through the Moens-Korteweg equation which  
53 shows a relationship between PWV, blood density, aortic wall thickness, Young’s modulus, and radius [17]. In vivo,  
54 directly deducing the arterial stiffness from PWV measurement is questionable since the actual Young’s modulus is

55 difficult to verified with traditional mechanical characterization tests on the subject to validate the theory. Alternative  
56 experimental validations are proposed by comparing PWV evaluation through other methods such as TT-method [18]  
57 shear wave elastography or fluid-structure interaction simulations which showed good agreements [19,20,21]. It  
58 would be desirable to be able to validate the computed material elasticity from this *in vivo* method with classical  
59 mechanical testing such as tensile tests on the same material. Since this comparison is not achievable from *in vivo* to  
60 *ex vivo* testing on a living patient, an equivalent method could be implemented with *in vitro* models and aorta  
61 phantoms. This comparison was achieved in Zimmermann et al., 2021[15] with aorta phantoms, a circulatory mock  
62 loop, 4D-flow MRI intra-luminal pressure measurements, transit-time like PWV estimation and tensile tests. This last  
63 study provided a volumetric information on the aorta behavior thanks to 4D-MRI and shows large discrepancies in  
64 the Young's modulus based tensile tests and PWV estimation. The pressure amplitude inflow conditions were very  
65 wide (105-40 mmHg) compared to *in vivo* conditions with a strong diameter variation along the aorta compared to  
66 *in-vivo* conditions. These conditions impacted the constant aortic diameter approximation used in the Moens-  
67 Korteweg equation to calculate the Young's modulus from PWV which was not adapted in this case. In the current  
68 study, we will confine ourselves to 2D in-plane evaluation which can be related to traditional US-Doppler PWV  
69 measurement methodology in the way that we use 2D imaging in one location of the aorta where velocity and diameter  
70 change are computed [12, 11]. The main purpose of this article is to provide a methodology to measure PWV and  
71 phantom stiffness on an *in vitro* simulator. Future experiments could be conducted by introducing the bench in an  
72 MRI [15] or using US-Doppler on the phantom to mature the present technique and compare the limitations of each  
73 methods.

74 An *in vitro* aortic flow simulator was developed in Moravia et al., 2022 [21] to reproduce physiological pulsatile  
75 flow rates and pressures in an aorta phantom made up of silicone with a patient-specific geometry and realistic  
76 elasticity. A Particle Image Velocimetry (PIV) setup was implemented on the mock loop to visualize the flow and  
77 track the aortic wall displacements. PIV was used to capture instantaneous velocity fields in a flow in a plane which  
78 crossed the flow domain in a chosen location. Prior to the experiment, the fluid was seeded with particles that acted  
79 as tracers. 2D-2C PIV (2 dimensions – 2 components PIV) consisted in illuminating a 2D section in the flow with a  
80 laser sheet to enlighten the particles and take snapshots of their position to track their displacements and deduce the  
81 flow velocity field at any point of the plane. The PIV setup is used to simultaneously measure flow velocity and aorta  
82 apparent diameter (AD) variations along a cardiac cycle. This AD refers to an “in-plane” diameter observed at the

83 intersection between the PIV laser sheet and the aorta phantom in the longitudinal direction. The lnD-U method can  
84 then be applied to compute PWV and estimate the aorta phantom stiffness based on this AD and other phantom  
85 characteristics. The great advantage of this *in vitro* system is that the aorta phantom Young's modulus can then be  
86 measured with mechanical tensile tests [22] and compared to the previous results.

87 In the current paper, we provide tools and a methodology to perform those two measurements and to investigate  
88 the stiffness evaluated from PWV with the lnD-U method and mechanically measured elasticity modulus with tensile  
89 tests in this *in vitro* context with an aorta phantom. The measured PWV and Young's modulus were also compared  
90 to *in vivo* data from the literature to determine if the silicone material is a relevant choice to mimic aorta with  
91 phantoms. The present experimental simulator tried to reach a relevant degree of biofidelity to mimic aortic flow in  
92 an *in vitro* context knowing that a perfect portrayal is impossible. We believe to have reached a significant mimicking  
93 of important aortic flow features among which the Young's modulus of the phantom is fundamental for such  
94 fluid/solid interaction system. Note that all along the paper the equations are given without units, all the indicated  
95 quantities are in SI units.

## 96 **2. Methods**

### 97 *2.1 The lnD-U method*

98

99 In routine exams, the lnD-U method can be used to evaluate the local stiffness of the aorta. Conversely with the  
100 Transit-Time method which requires to image pulse wave at two distant locations (and thus averages the PWV on this  
101 whole distance), the lnD-U method can be performed in a single section of the aorta as a local evaluation. This  
102 technique relies on the study of the AD variation of the aorta in relation to the blood velocity at the same location  
103 throughout the cardiac cycle [11,12]. This relation result from the Bramwell-Hill equation [16] and the water-hammer  
104 relationship between the PWV, pressure, and flow velocity [10].

$$105 \quad PWV = \frac{1}{2} \frac{dU}{d \ln(D)} \quad (1)$$

106 where  $U$  is the velocity of blood flow in the investigated section,  $D$  is the diameter of the aorta in the same section  
107 and the derivative is the time variation of  $U$  for  $dU$  and  $\ln(D)$  for  $d \ln(D)$ . This relation (equation 1) is only valid in  
108 unidirectional wave periods along the cardiac cycle. If reflective waves appear, the relation can no longer be applied.  
109 Methods to identify reflection-free periods in the cardiac cycle exist [11]. They are based on wavefront analysis of

110 the blood pressure or velocity waveform in the aorta. The study of the rate of pressure (here converted to diameter  
111 change  $\frac{dD}{dt}$ ) provides information on the contribution of forward and backward wavefronts. As expressed in [11], wave  
112 intensity (WI) can be calculated with velocity and diameter time derivation to investigate the contribution of each  
113 wavefront direction (equation 2). Forward wavefronts have a positive contribution on the WI while backward  
114 wavefronts have a negative contribution. Locating positive peak in WI is an indicator of a predominant forward  
115 wavefront [11].

$$116 \quad WI = \frac{dD}{dt} \frac{dU}{dt} \quad (2)$$

117 Studies have shown that backward waves have negligible effects on diameter and velocity variations at the two  
118 positive peaks of WI [11, 23, 24]. In most cases, these peaks (which indicate reflection-less periods) correspond to  
119 early and late systole but the boundary conditions can affect them. The wavefront analysis has to be conducted on the  
120 experimental simulator with its own boundary conditions to identify those reflection-less. Locating one or the two  
121 phases allows determining where backward wave contributions are negligible to apply equation 1 to the measured  
122 flow velocity and phantom diameter change. Finally, the Moens-Korteweg equation [17] sets the relationship between  
123 the Young's modulus and the PWV (equation 3).

$$124 \quad PWV = \sqrt{\frac{Eh}{\rho D}} \quad (3)$$

125 where E is the Young's modulus of the aorta, h is the thickness of the aorta,  $\rho$  is the density of the fluid and D is the  
126 aorta diameter. Note that E, h,  $\rho$  and D are mean values on the region of interest (ROI) in the aorta where the PWV  
127 was calculated. In the present experiment, this region covered a length of 63.57 mm in the aorta along which these  
128 mean values are averaged. Regarding equation 3, the most variable parameter is the aortic diameter which is supposed  
129 to be constant but can actually strongly vary along a real aorta. The ROI was chosen to obtain the flow velocity field  
130 with PIV.

131 Finally, applying the lnD-U method requires for synchronized measurements of the velocity and the diameter  
132 change which can be difficult to achieve where two devices are used to measure these quantities. In the present study  
133 PIV was used to calculate both on the same set of data (the same images) which solves the synchronization issue.

## 134 135 *2.2 Experimental setup*

137 The experimental setup consists of a circulatory mock loop which mimics aortic circulation (figure 1). This  
138 aortic flow simulator generates realistic pulsatile flow rate and pressures in the aorta phantom. A compliant silicone  
139 aorta phantom based on a patient-specific geometry and a blood mimicking fluid (BMF) with realistic shear-thinning  
140 properties [25] were designed for the experiment. The BMF is a mixture of water (55.6% by weight), glycerol (37%  
141 by weight), xanthan gum (200 ppm), and NaCl (7.4% by weight) and was designed to match the refractive index (RI)  
142 of the phantom (RI = 1.4) to minimize optical distortion for the PIV measurements. The fluid had a density of 1146  
143 kg.m<sup>-3</sup>. The aorta geometry was extracted from a patient's CT-Scan (Computed Tomography) provided by the  
144 Hospices Civils de Lyon and approved by the hospital's ethic committee. The aorta phantom was designed by Segula  
145 Technologies with a molding technique and silicone injection. The exact type of silicone could not be communicated.  
146 Inflow conditions are shown in figure 2. Cheng et al., 2003 [26] was taken as the reference for human abdominal aorta  
147 flowrates at rest. The Reynolds number at the systolic peak and Womersley numbers were set to 1171 and 16.3  
148 respectively thanks to the mock loop controlling system. Moravia et al., 2022 [21] provides the details on the design  
149 and properties of the phantom, the BMF, the circulatory mock loop, and the pulsatile inflow conditions. Additional  
150 details on the setup are provided in the supplementary material 1. The technique of Particle Image Velocimetry (PIV)  
151 is used to compute velocity fields in different sections of the aorta phantom. The PIV system, configuration, and  
152 particles choice are described in Moravia et al., 2022 [21] and summarized in table 1. In the current experiment, a  
153 region-of-interest (ROI) was defined as the cross-section between the laser sheet and the inner part of aorta phantom  
154 (where the fluid flows). This ROI is located in the aorta longitudinal direction and in the straightest region of the  
155 phantom to minimize out-of-plane flow for the application of the InD-U method. The laser sheet was placed to meet  
156 the centreline of the aorta model at the location where the higher diameter was detected. This region was chosen as the  
157 one where both the flow and the wall movement could be imaged in a 2D-plane. In other regions the tortuosity of the  
158 model limited the use of this 2D imaging technique.

### 160 *2.3 PIV measurements and post-processing*

161  
162 The mock loop imposed a pulsatile flow rate and pressure in the aorta phantom [21] while a series of PIV  
163 measurements were performed on the ROI (indicated on figure 1). The measurements consisted of pairs of images to  
164 track the particles displacements between the 2 images of each pair. The imposed cardiac cycle period was of  $T = 804$

165 ms. The camera acquired a total of 1000 pairs of images at a framerate of 10Hz with a time-lapse  $\Delta t = 4$  ms between  
166 the two images of each pair. This method allowed one to generate a batch of images that described different instants  
167 throughout the imposed cardiac cycle. Moreover, knowing the cycle period and imaging frequency (10 Hz), all the  
168 images can also be located on the imposed cardiac cycle. The designed system enabled to trigger the PIV imaging at  
169 chosen instants in the cardiac cycle which allows to locate each measurement in the imposed cardiac cycle. This was  
170 achieved thanks to a synchronized pulse waveform generation and PIV triggering in the in-house LabVIEW program  
171 that controlled the simulator. For the cross-correlation computation, a unique  $\Delta t = 4$ ms was chosen to maintain a  
172 consistent particle displacement (5 to 20 pixels) between two images of a pair all along a cardiac cycle. Indeed, the  
173 flow velocity strongly varied along a cardiac cycle which involved a wide range a particle displacement from systole  
174 to diastole. Even though  $\Delta t$  could be shorter (down to 1ms) during the systolic phase, the main limitation came from  
175 the slow-motion diastolic phase with risks of capturing a zero displacement if  $\Delta t$  was too short. Thanks to preliminary  
176 experiments,  $\Delta t = 4$  ms was found to suit all the cycle phases. The PIV setup and imaging parameters are summed up  
177 in Table 1. A total of 125 successive cycle were needed to acquire those 1000 pairs of images. To achieve those 1000  
178 measurements, the work was divided into 5 series of dataset with 200 pairs of images each because of camera RAM  
179 limitations with such a high framerate. The inflow conditions and parameters were kept the same for each dataset to  
180 ensure reproducibility.

181 For each of the PIV images, the aorta apparent diameter and velocity fields were computed (figure 3). The  
182 outer diameter (D) of the aorta was estimated thanks to a MATLAB in-house program [21] thanks to aorta phantom  
183 upper and lower wall detection with digital image treatments and analyses (based on grey scale transitions on the  
184 images). Additional details on the algorithm are provided in the supplementary material 2. The velocity fields were  
185 calculated with the software Davis 10 (LaVision). With Davis 10, the image cross-correlation was conducted with a  
186 multipass method in the ROI to calculate the velocity field (figure 3). The first pass was performed with 32x32 pixels  
187 interrogation windows with an ellipsoid weight with the long axis in the main flow direction x and a 0% overlap. the  
188 second pass was reduced to 16x16 pixels interrogation windows with a round Gaussian weight and an overlap of 50%.  
189 For each velocity field, a 2<sup>nd</sup> order polynomial interpolation algorithm was applied to fill up missing vectors (less than  
190 1% of vectors) and was followed by a 3x3 smoothing filtering. The 16x16 pixels interrogation windows have an  
191 equivalent resolution of 0.40x0.40 mm<sup>2</sup> knowing that the mean aorta diameter is about 32.1 mm. This mean value on  
192 the ROI and throughout the cardiac cycle was calculated as an “in-plane” diameter with the PIV images (intersection



193 between the laser sheet and the phantom). The signal to noise ratio is limited by the value chosen as classically for  
194 PIV analysis.

#### 195 2.4 Tensile strength tests

196 The silicone phantom elasticity is measured through uniaxial tensile strength tests performed on 8 samples cut from  
197 a sacrificial aorta phantom. This sacrificial phantom is the same model as the one used in the mock loop (same  
198 geometry, silicone and manufacturing technique). The pre-condition for each sample was set to 10N and the loading  
199 was achieved with 1mm/s jaw displacements to keep conditions. From those samples, the phantom wall thickness was  
200 measured as  $h = 2.19 \pm 0.42$  mm (mean and standard deviation over the 8 samples). The tests were performed on a  
201 Lloyd LF-Plus machine and combined with a Digital Image Correlation measurement [22,27]. A random speckle  
202 pattern was applied on the sample and a camera captured their displacement with the tensile machine gripped jaws  
203 movement. The cross-correlation on the speckle displacement was computed with the software VIC-3D (Correlated  
204 Solution). Knowing the displacement and the force applied for the jaw displacements, the elastic modulus could be  
205 calculated with the longitudinal Lagrangian strain [22,28, 29].

### 206 3. Results

207 The mean D, along the aorta and the mean velocity (U) on the whole ROI were calculated for each pair of  
208 images (equation 4 and 5). The mean velocity was integrated on the volume based on the 2D measured velocity in the  
209 ROI and with a cylindrical symmetry approximation.

$$210 \quad D = \frac{1}{n_y} \sum_i^{ROI} D_i \quad (4)$$

$$211 \quad U = \left( \sum_{i,j}^{ROI} u_{i,j} \cdot r_j \right) \cdot \frac{ds}{L \cdot \left(\frac{D}{2}\right)^2} \quad (5)$$

212 where  $D_i$  was the AD computed at each of the  $n_y$  vertical line of the ROI with index  $i$  along the  $x$ -axis,  $n_y$  is the  
213 total number of vertical lines,  $u_{i,j}$  was the velocity on each interrogation windows inside the ROI (figure 3) at a  
214 corresponding radius  $r_j$  in the phantom,  $ds$  is the surface of a pixel and  $L$  is the length of the ROI. D and U waveform  
215 are shown in figure 4.a on a cardiac cycle timeline. To rescale the D and U data on a cardiac cycle timeline ( $T = 804$   
216 ms), the datasets were rescaled knowing the 10Hz imaging frequency and location of the first image on the imposed  
217 cardiac cycle. The corresponding wave intensity (WI) was then computed and plotted in figure 4.b to detect local  
218 maxima. The early systole maximum was identified as the first peak [23] and indicated in red on the graph. This

219 period was thus considered to be a reflection-free one where the diameter / velocity relationship could be analyzed to  
220 calculate PWV.

221 The lnD-U loop graph is provided in figure 5.a for the full set of 1000 pairs of images. The cycle showed a similar  
222 shape as in Di Lascio et al., 2014 [11] where the experiment was conducted on mice with ultrasound measurements.  
223 To compute PWV, the data corresponding to the early systole (reflection-free period identified with the WI analysis)  
224 were isolated and presented in figure 5.b. The linear regression was performed on those selected data and the slope  
225 gives the  $\frac{d \ln D}{dU}$  factor. From equation 1, the calculated PWV was  $5.79 \pm 0.33$  m/s. The uncertainty was evaluated from  
226 linear regression standard error on the slope parameter.

227 Thanks to the Moens-Korteweg equation (equation 3), the Young's modulus was computed knowing that the aorta  
228 wall thickness was  $h = 2.19 \pm 0.42$  mm, the blood mimicking fluid's density was  $\rho = 1146$  kg.m<sup>-3</sup> and the aorta mean  
229 AD = 32.1 mm. This diameter was set as the mean diameter in the ROI (equation 4) which was then averaged on the  
230 whole cardiac cycle. As a result, the computed Young's modulus was  $E_{\ln D-U} = 0.56 \pm 0.12$  MPa. The uncertainty  
231 was evaluated with the uncertainty propagation formulation on equation 3 that took into account the error from the  
232 PWV calculation (standard error of the linear regression coefficient) and the wall thickness  $h$  (refer to supplementary  
233 material 3).

234 The Young's modulus calculated from the tensile strength tests was equal to  $E_{tensile} = 0.53 \pm 0.07$  MPa (the error is  
235 the standard deviation between the 8 measured samples). Considering the uncertainties of each method, the two values  
236 ( $E_{\ln D-U}$  and  $E_{tensile}$ ) were consistent with each other. Uncertainties for the present method,  $E_{\ln D-U}$ , came from PIV  
237 measurements and from the silicone manufactured walls. These last ones are common to the ones used for  $E_{tensile}$ .  
238 The tensile technique has its own uncertainties. It is thus interesting to note that the use of tensile technic or PIV  
239 technic provides similar uncertainties which is cumulative with the ones coming from silicone manufacturing. The  
240 ideal solution would be to first conduct PIV measurements at one location and second, to cut some sample from the  
241 wall at the exact same location to perform the tensile tests. However, the price of silicone phantom was sufficiently  
242 high not to sacrifice all our phantoms in tensile tests. Another point is that the tensile method supposed anisotropy.  
243 Such hypothesis has to be tested in the future and some departure could be responsible of some differences.

#### 4. Discussion

In the current study, PWV and aorta phantom Young's modulus were measured with the local InD-U loop method applied on flow velocity and diameter variations data obtained with PIV. For PWV and both Young's modulus computations, the results were compared to typical data from human aortas. Concerning PWV, the value of  $5.79 \pm 0.33$  m/s was in the range of an abdominal aorta for a normal subject of about 50 years old [20, 30]. The young's modulus was also in the range of measured elasticities of human aortas according to Lang et al., 1994 [31] with typical values ranging from 0.25 to 1.7 MPa. Moreover, under the realistic pulsatile conditions imposed by the mock loop, the phantom diameter variation was around  $\Delta D = 0.8$  mm which was in accordance of a 53-69 years old male  $\Delta D$  [32]. In the field of *in vitro* simulator where rigid glass [33], 3D-printed [15] and silicone [34] models are used, the aorta phantom made up of silicone appeared as a relevant choice to mimic those aorta phantom mechanical responses to pulsatile flow.

Regarding the  $E_{InD-U}$  and  $E_{tensile}$  comparison, the values were in accordance regarding the uncertainty factors. The percentage difference between the two values was 5.6%. As noted above uncertainties were various and some were inherent to the different techniques. To provide an order of magnitude, when calcification occurs in the aorta and atherosclerosis develops, the Young's modulus of the arterial wall can double and even become ten times higher in sever calcification cases compared to a healthy aorta [34]. In the current study, the difference in the measured E values were provided large uncertainties but remains in the range of realistic human aorta stiffness. Moreover, the measurements were based on 'in-plane' values giving flow velocity components. The out-of-plane flow is source of a bias error, linked to the plane thickness and responsible for an increase of particle pattern differences from one frame to the other. That is limited via the time step recording between two frames and acceptable signal to noise ratio fixed during cross correlation analysis. To go further, the technique of 3D-PTV (particle tracking velocimetry) can be used to calculate the velocity with three velocity component determination in our region-of-interest but in a volume [36]. This method involves multiple cameras and the use of a laser volume instead of a laser sheet to illuminate the targeted region which could be adapted to the existing experimental bench but with a rather more complex data analysis. Global diameter and velocity change on the whole volume could provide a more precise PWV evaluation [15]. Finally, changing the boundary conditions to modify wave reflections and inflow conditions could help testing the limits of this InD-U technique to evaluate elastic properties and test the robustness of this method with the help of the WI calculation to identify reflection-less phases. In any case, the same PIV measurements can be applied to

274 simultaneously compute the flow velocity and diameter change on multiple planes and/or other phantoms with the  
275 current aortic flow simulator and the imaging methodology.

276 The main limitation with InD-U method applied with our PIV system comes from the AD detection. As  
277 explained previously, each point presented on the InD-U loop corresponded to a single pair of images. The statistics  
278 relied on large series of single pair of images shot randomly throughout the cardiac cycle. As a consequence, in the  
279 present experiments, we count on a large amount of random data on the period of interest (early systole) to guaranty  
280 a reliable linear regression to compute the  $\frac{d \ln D}{dU}$  slope parameter. On a pair of images, the most difficult value to  
281 compute is the AD, since punctual wall detection defects can occur due to the presence of foreign body and light  
282 reflection in the field of view (mostly in the background). The in-house algorithm is based on grey scale value  
283 differences between the dark background and the lighter phantom. Strong disturbance on the background or micro-  
284 bubble deposit on the phantom surface can cause incorrect localization of the phantom's walls (refer to supplementary  
285 material 2). A way to improve the technique would be to optimize the wall detection algorithm.

286 In the current set of data, 1000 pairs of images were shot but only 109 of them were in the early systole (reflexion-  
287 less) period (10.9%). However, a complete cycle imaging was necessary to conduct the WI analysis and identify this  
288 reflexion-less period. The PIV setup parameters or the implementation of a fast PIV device could help focusing the  
289 imaging in this particular period.

290 Finally, the current patient-specific aorta phantom was designed to reach a constant wall thickness while the human  
291 aorta has a non-uniform wall thickness [37]. Specific protocol should be developed to extract the corresponding  
292 patient's aorta thickness and to implement it on the manufacturing technique. It could provide more relevant models  
293 and influence the results. The current observations, calculations and conclusions are all based on the uniform wall-  
294 thickness hypothesis which differs from real patients and our aorta phantom. The Moens-Korteweg is based on this  
295 hypothesis which can limit the accuracy of the resulting Young's modulus.

## 296 **5. Conclusion**

297 An *in vitro* investigation of the InD-U method was achieved on a circulatory mock loop which replicates pulsatile  
298 flow rates and pressure conditions in an abdominal aorta phantom made up of silicone. The InD-U method often lacks  
299 validation with traditional tensile test to measure material elasticity. The current experiment on the aorta phantom is  
300 used to compare the InD-U method to compute Young's modulus and traditional tensile test evaluation. Consistent  
301 Young's modulus values were found between the InD-U and the tensile tests methods with a 5.6% difference but not

302 sufficient to validate the theory on the relation between PWV and arterial stiffness. Errors can emerge from out-of-  
303 plane flow, wall thickness approximation and diameter change evaluation. Further investigation conducted on other  
304 phantom sections or other phantom geometries with the same silicone material are necessary to validate this InD-U  
305 method with larger statistics than this single case. However, the current benchtop simulator and imaging methodology  
306 can be implemented for further investigation of the InD-U technique and test the robustness of this method by varying  
307 boundary conditions (connectors, inflow conditions, peripheral resistance with valves, etc.). The same methodology  
308 can be implemented on those future cases and the system could also be used for other method comparisons such as  
309 the TT or PU-loop method.

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313 tests were performed by Marine Menut et Laurie Portero in LaMCoS (INSA de Lyon).

### 315 **Conflict of interest**

316 Anaïs Moravia, Serge Simoëns, Mahmoud El Hajem, Benyebka Bou-Saïd, Marine Menut, Pascale Kulisa, Patrick  
317 Lermusiaux and Nellie Della-Schiava declare that they have no conflicts of interest.

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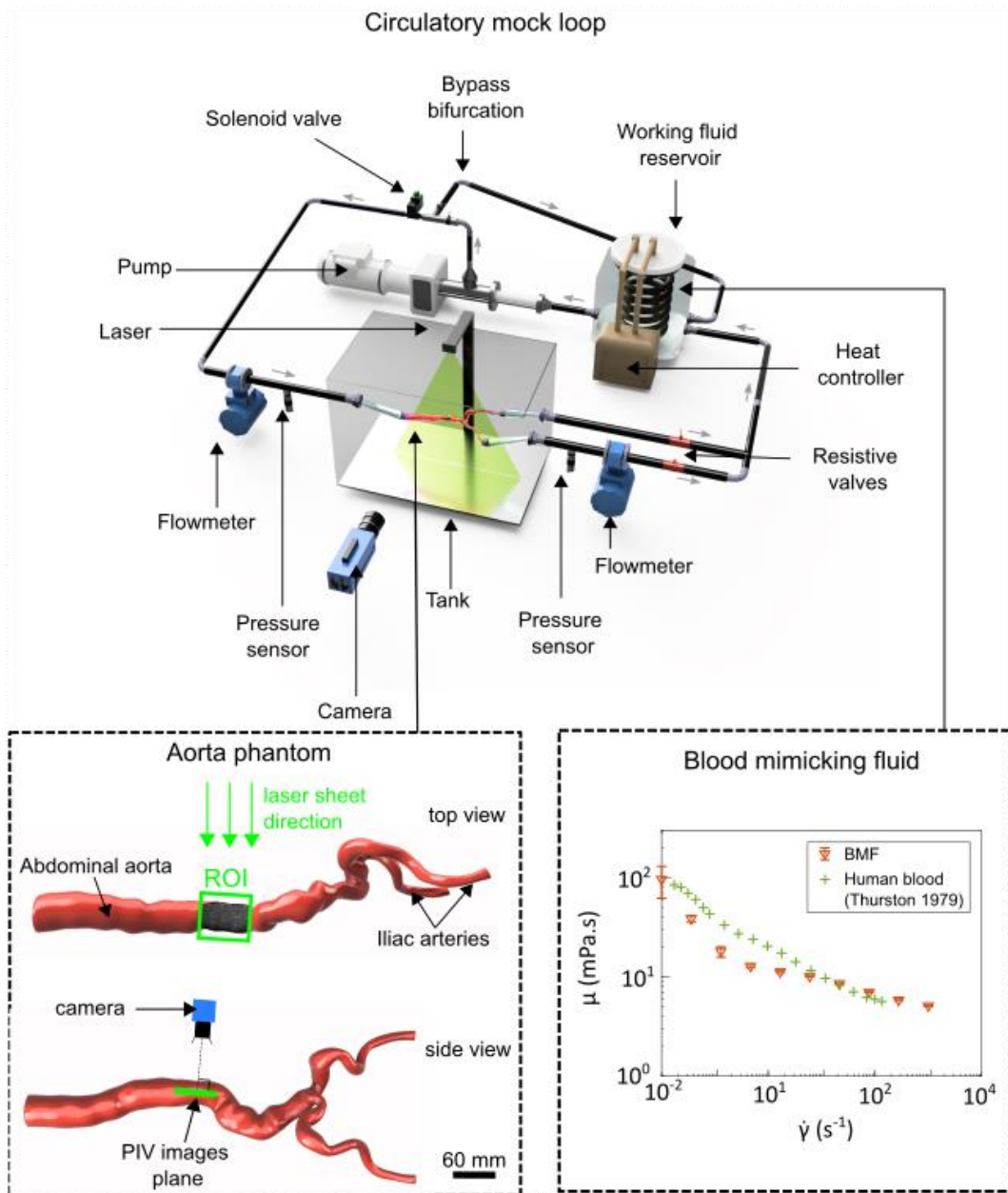
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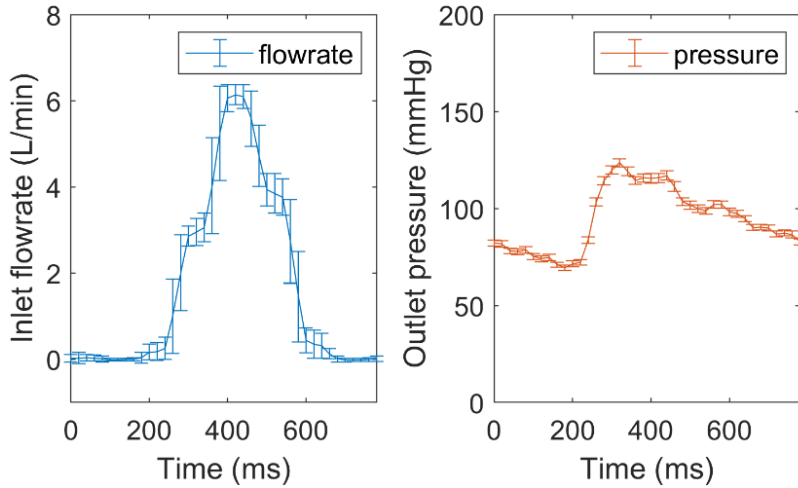
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419

420 **Fig.1** Experimental setup with the circulatory mock loop hosting the aorta phantom and conveying the blood  
 421 mimicking fluid (BMF). The Regions of interest (ROI) corresponds to the imaged PIV plane with the camera and is  
 422 located in the longitudinal direction of the phantom at the maximum of diameter. The BMF viscosity measurements  
 423 are provided for different shear rates [18] (with human blood reference [22])

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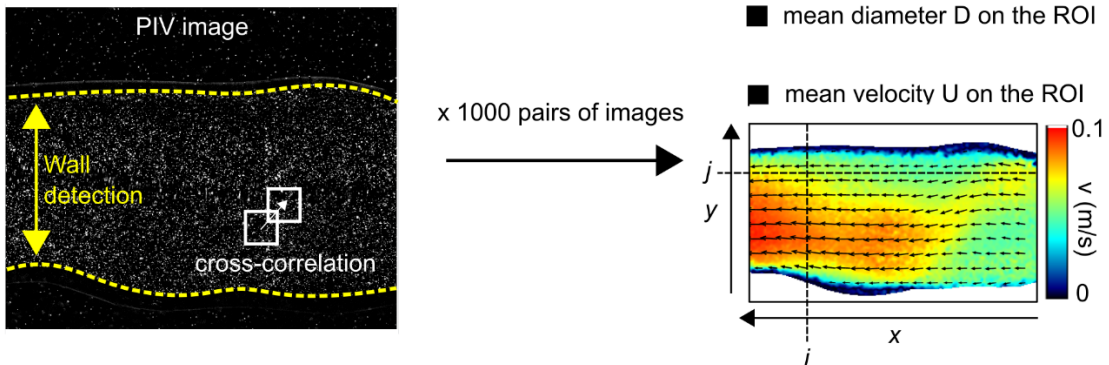
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Figure 2: Inlet flowrate and outlet pressure on the experimental setup. The flowrate amplitude was set to meet the data from Cheng et al. 2003 (abdominal aorta flowrate measurements). Note that the inlet flowrate is measured in a rigid pipe upward from the compliant phantom and can only detect flow in the main direction (toward the phantom). A dampening effect on the curve and backflows are observed in the phantom but cannot be captured by this flowmeter. The standard deviation is indicated for the 125 successive cycles needed for the experiment.



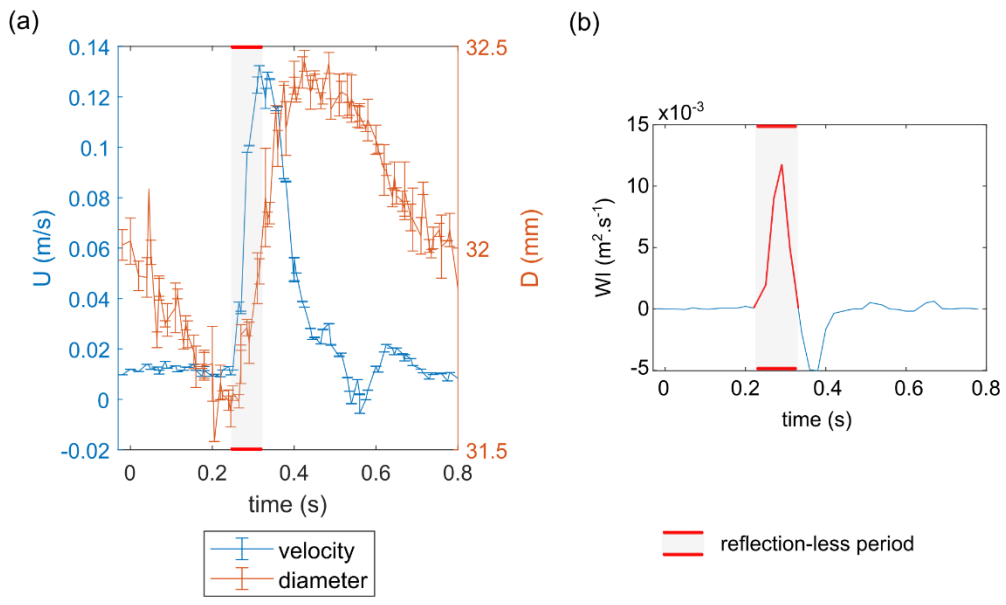
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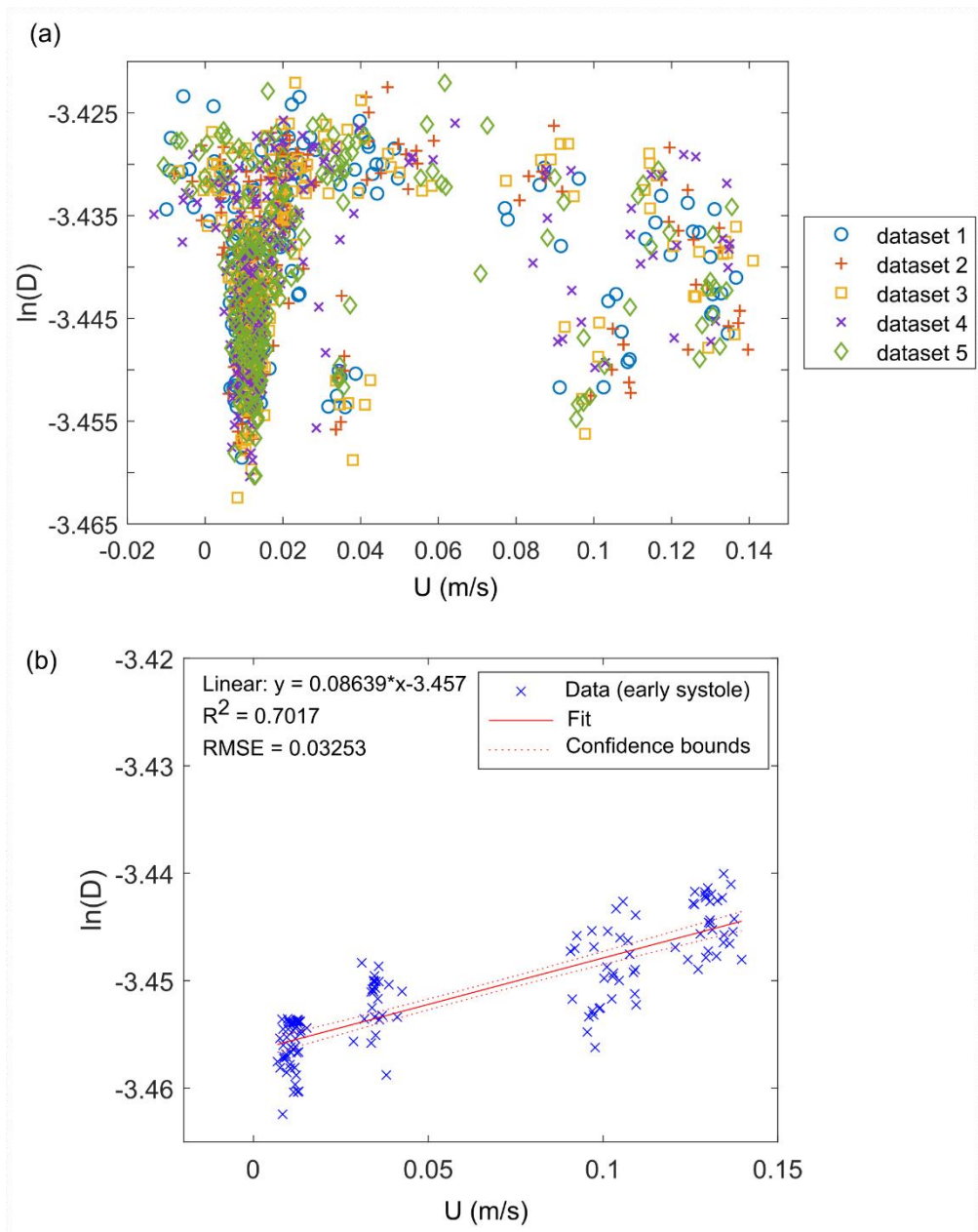
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**Fig.3** Illustration of the lnD-U method applied on the PIV images thanks to mean diameter and mean velocity computation on the ROI. Refer to the bottom left box on figure 1 for the orientation of the camera an image compared to the aorta position and flow main direction (from the abdominal aorta toward the iliac arteries)



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**Fig.4** (a) Mean diameter and velocity waveform in the ROI throughout time rescaled on a cardiac cycle period, (b) WI was calculated and a local maximum was identified (red part from  $t = 0.22$  to  $0.33$  ms is the cardiac cycle timelapse). The two other visible maxima will not be considered in this analysis.



445

446 **Fig.5** (a)  $\ln D$ - $U$  loop graph for 5 datasets of 200 images each (total of 1000 pairs of images). Each dataset corresponds

447 to PIV measurements in the ROI, shot with the same imaging parameters. ( b) Curve fit on data corresponding to the

448 increase in systole (109 images) where WI showed a local maximum

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<b>Setup element</b>	<b>Reference</b>
<b>Laser type</b>	Nd:YAGnanoPIV, $\lambda = 532$ nm, Litron laser
<b>Camera</b>	sCMOS, 2560 x 2160 pixels, LaVision
<b>Lens</b>	AF Micro-Nikkor 60mm f/2.8D, Nikon
<b>Particles</b>	PMMA-RhB, $d = 1.190$ $\mu\text{m}$ , LaVision
<b>Parameters</b>	
<b>Laser sheet thickness (mm)</b>	1
<b>PIV framerate (Hz)</b>	10
<b><math>\Delta t</math> between pulses (ms)</b>	4
<b>Field-of-view (mm x mm)</b>	63.57 x 53.37
<b>Interrogation window (pixel x pixel)</b>	16 x 16
<b>Interrogation window (mm x mm)</b>	0.40 x 0.40

453

454 Table 1: PIV set up and parameters

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456

## **Supplementary material 1: Experimental setup**

The main element of the experimental simulator was the aorta phantom with is a scale model of a patient's aorta geometry. The phantom was immersed in a tank containing a fluid with the same refractive index than the working fluid (BMF). The fact that the tank was filled up with a fluid had two purposes: (1) to minimize optical distortions with an homogenised refractive index media (BMF, phantom and tank fluid) and (2) to apply a certain pressure on the aorta phantom to mimic its surrounding environment. When immersed in the tank, the aorta phantom was lying on a support that mimics the shape of a backbone and to stabilize the model when subjected to pulses.

In the tank the aorta is connected to the main loop thanks to rigid hose connector. Note that any change in the boundary conditions, rigidity of the connector or configuration of the mock loop pipe with influence the way the pulse waveform reflects in the loop. The InD-U method is based on the hypothesis of unidirectional wavefront analysis as specified in the article. Therefore, a new WI analysis should be conducted if the mock loop and inflow conditions are modified to locate the reflection-less phases.

The PIV setup configuration allowed to generate a 2D laser sheet emitted from the top of the tank which enlighten a section visible from the side of the tank as indicated in figure 1 from the main text. When imaging flows in "tubes" with such tortuosity the main problem with 2D-PIV is to find positions where a 2D plane can pass and where most of the mass flux is captured.

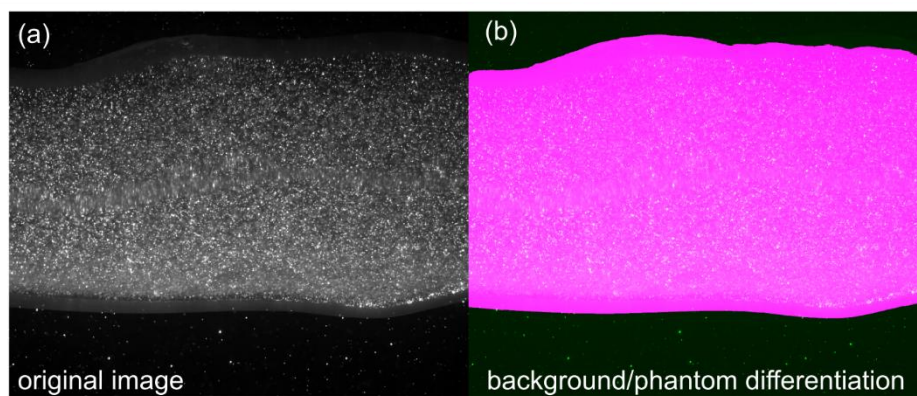
In the hypothesis of equation (5), converting 2D n-plane to 3D flow could become a source of large bias when the transverse velocity (out-of-plane) is not negligible compared to the longitudinal one (in plane). 3D-PTV studies would allow to quantify this bias. In the current study, this bias was indirectly checked by comparing the mass flux computed with PIV and the one provided by the inlet flowmeter which integrated flow on the whole section. We observed a 13% underestimation of mass flux with the PIV compared to the inlet flowmeter. The conclusion was that the in-plane flow is not sufficient to estimate the whole flow in the aorta phantom and is only an approximation.

## **Supplementary material 2: Wall detection algorithm**

485 The wall detection algorithm allowed to detect the position of the outer wall of the aorta phantom based on greyscale  
486 difference between the dark background and the lighter wall. The results of this algorithm were first used in Moravia  
487 et al., 2022 to track wall displacement in the same aorta phantom but in another region of the model.

488 The phantom walls were compliant and moved with a periodic pattern when pulsatile flowrate and pressure  
489 was imposed in the circuit. The detection was conducted with a in-house MATLAB program using a threshold to  
490 differentiate the background from the wall. A Morphological closing filter is than applied on the image to deal with  
491 foreign elements that can be present in the background. Indeed, the phantom is immersed in a tank and some bubbles,  
492 fibres of PIV particle can fly in the background. These elements visible in figure S2 result from small leakages or the  
493 presence of air in the mock loop when installing the phantom prior to the experiment.

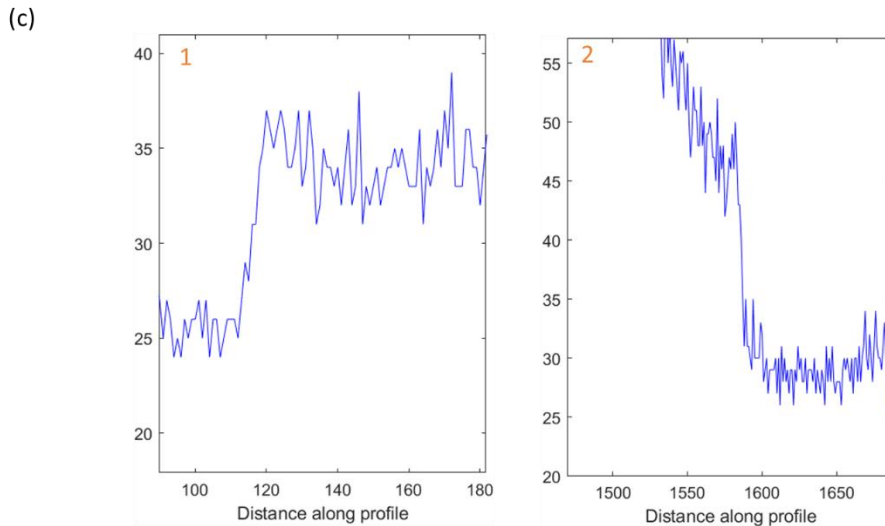
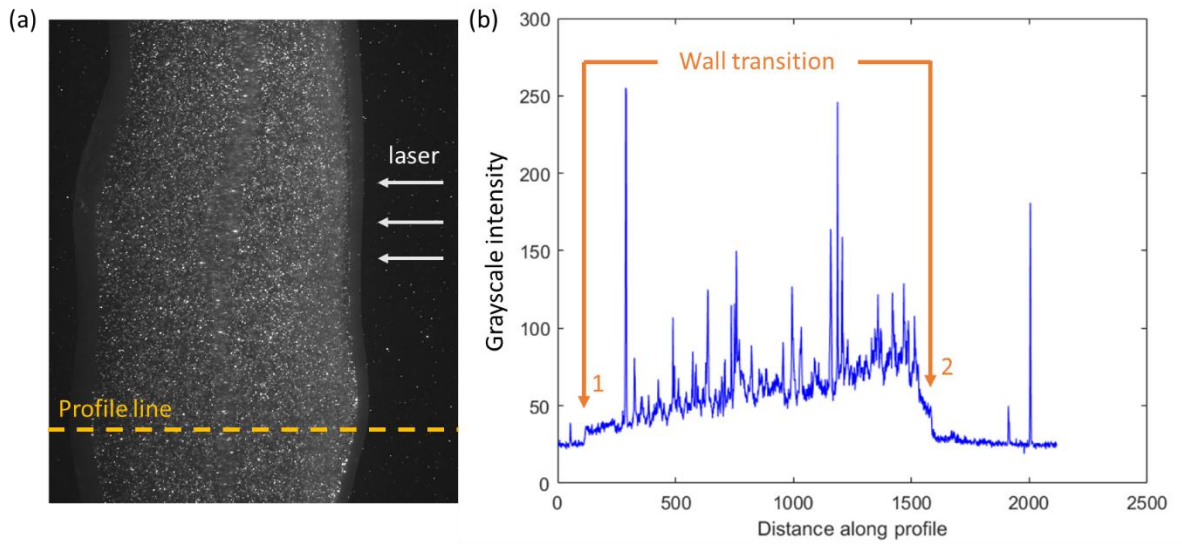
494 In our case the threshold was sat to 555 greyscale level for our 64 bits images and the closing algorithm was  
495 operated rectangular patch of 5x5 pixels. These values can vary depending on the lightening and the resolution of the  
496 images.



497  
498 Figure S2-1: Example of an outer wall detection with the threshold algorithm. (a) shows the original PIV  
499 image and (b) shows the separated phantom region from the background. This computation in conducted on the whole  
500 set of images to follow the wall displacement throughout the cardiac cycle.

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 503 Figure S2-2: Example an intensity profile line on a PIV image. The wall transition indicated when the  
 504 background ends and the wall begin. To separate the phantom from the background, a threshold algorithm was used  
 505 isolate grayscale over this transition.

506 Figure S2-2 shows an intensity profile on the aorta phantom. Depending on the location on the phantom, the  
 507 intensity transition is typically observed in 2 to 3 pixels along the profile which corresponds to 0.050 to 0.075 mm ( $\mu$ ).  
 508 On the opposite side of the laser (lower light), the transition takes 3 to 5 pixels along the profile (0.075 to 0.12 mm)  
 509 knowing that the diameter variation is about 0.8 mm.

510 A mentioned in the discussion, some particles or air bubbles punctually deposit on the outer wall. PIV  
511 particles size are between 0.020 and 0.50 mm which will affect the near wall light intensity transition and add extra  
512 error on the diameter estimation.

513 .  
514 Not that the program also allowed to detect small vibrations of the system. When running the mock loop  
515 small vibrations are observed in the circuit and in the aorta phantom because of the pump running. Thanks to multiple  
516 imaging in different regions of the phantom the vibration were quantified as displacements up to 3 pixels in the  
517 phantom. This quantity is provided for the current article field-of-view size. The program can differentiate contraction  
518 (upper and lower get closer), dilatation (upper and lower border diverge) and vibration (shift of the upper and lower  
519 wall in the same direction in a range of 3 pixels) of the phantom.

### 522 **Supplementary material 3: Uncertainty calculation**

523 The Moens-Korteweg equation gives a relationship between the PWV and the Young's modulus E such that:

$$524 E = \frac{PWV^2 \rho D}{h}$$

525  
526 This equation is a power and product type where letters Q, x, y and z represent physical quantities and  $\Delta Q$   
527  $\Delta x$ ,  $\Delta y$  and  $\Delta z$  their uncertainties:

$$528 Q = x^m y^n \dots z^p$$

529 The propagation uncertainty formulation takes into account product and power factors thanks to the  
530 following formula:

$$531 \frac{\Delta Q}{|Q|} = \sqrt{\left(|m| \frac{\Delta x}{|x|}\right)^2 + \left(|n| \frac{\Delta y}{|y|}\right)^2 \dots + \left(|p| \frac{\Delta z}{|z|}\right)^2}$$

532 Such formulation was used to compute the uncertainties for the Young's modulus computation based on  
533 the known uncertainties of the other physical quantities.