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DEPC-MPR 059**

**Measurement of flow induced
strain in a mechanically
active bioreactor**

**J LAM, D PENN and
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Measurement of flow induced strain in a mechanically active bioreactor

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ABSTRACT

It is becoming increasingly apparent that the cells being cultured *in vitro* to form replacement viable tissues for use in medicine require some degree of mechanical stimulation to maintain their phenotype. Ideally this should mimic the conditions found *in vivo*. New designs of bioreactor are being designed to be able to provide mechanical stimulation to cell-seeded scaffolds but exactly how this is transferred to specimens has not been widely studied. In this report we compare two approaches for mapping flow induced dilation of a tubular structure in a biodynamic chamber designed for cardiovascular tissue engineering. These are digital image correlation and laser micrometry.

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1 INTRODUCTION

‘Conventional’ tissue engineering research has tended to focus on the production of scaffold structures made from a range of materials and understanding the culture conditions required to create new viable tissues. Mechanical stimulation of cell-seeded scaffolds during the culture period has not been widely addressed although it has been established that it is essential to prevent dedifferentiation in, for example, chondrocytes and its role in culturing vascular conduit is of paramount importance.

Early tissue engineered vascular structures (Weinberg and Bell 1986) were produced using combinations of endothelial cells, smooth muscle cells and fibroblast and although histologically similar to natural blood vessels lacked strength and required support from a dacron mesh. Subsequently Niklason (Niklason, Gao et al. 1999) showed that exposing the *in vitro* cultured tissue to a pulsatile flow that was sufficiently low so as not to induce significant radial expansion of the tube could enhance the strength of cultured conduits. This requires the development of a mechanically active bioreactor that is able to mimic the physiological environments that exist within arterial and vascular conduits. This is achieved by applying physiological relevant waveforms and pulsatile pressures to a cell-seeded scaffold during culture. Various designs have been proposed that have some synergy in the way in which the pulsatile flow is induced (Thompson, Colon-Hernandez et al. 2002). The key parameters that need to be controlled are the tidal flow, pressure and pulse frequency. Fluid flow through the vessel generates a shear stress or tangential drag force at the wall of the vessel. The magnitude of this stress is directly proportional to the viscosity and flow rate of the blood and inversely proportional to the cube of the vessel radius.

Flow of fluid through a tube, if it is sufficiently compliant, will cause it to dilate by an amount related to the pressure of the fluid, i.e. generate a hoop or circumferential strain. Modulating the flow to mimic the pulsatile nature of the circulatory system will be mirrored by the hoop strain in the wall of the tube by an amount that depends on its dimensions, its viscoelastic properties and degree of porosity. Endothelial cells lining the inner surface of a scaffold will therefore be subjected to both a dynamic wall shear stress and hoop strain. In some designs e.g. Niklason (Niklason, Gao et al. 1999), the flow rate and tube geometry is controlled to minimize the amount of hoop strain produced, whereas in other designs, local sample strain is apparently not monitored (McCulloch, Harris et al. 2004; Buttafoco, Engbers-Buijtenhuijs et al. 2006).

Table 1 lists some materials that have been used in tissue engineering of blood vessels, which are both synthetic and natural and are mostly biodegradable. These have been fabricated to form a range of tube geometries that have significant differences in wall thickness and in the tube length to diameter ratio. The latter is especially important in understanding the role that edge effects have on the mechanical response of the tube. Many of the materials used in tissue engineering are both porous and biodegradable, as shown in Table 1, hence the compliance of the tube will change with time. The pressure drop per unit length of the tube will also change as the tube becomes thinner and pore sizes increase changing the distribution of hoop strain within the tube. These time dependent changes are not easily predictable, especially for a cell-seeded scaffold where the number of cells and their associated extra cellular matrix is constantly changing affecting both the stiffness of the conduit and the kinetics of the degradation

process. Changes in dynamic strain behaviour are not usually monitored, nor is the strain field over the conduit mapped.

In this paper we compare two non-contacting methods for determining dynamic strain in a tubular structure mounted in a mechanically active bioreactor in both air and in a fluid environment.

Table 1: Examples of materials and tubes geometries that have been used to tissue engineer blood vessels. NA = information not available.

Reference	Materials	Length (mm)	Diameter (mm)	Wall thickness (mm)	Porosity (%)	Pore size (µm)	Flow conditions
(Buttafoco, Kolkman et al. 2006; Engbers-Buijtenhuijs, Buttafoco et al. 2006)	Collagen/elastain 1:1 (wt)	40	6 (OD) 3 (ID)	1.5	90	130-150	Pulsatile flow (mean flow rate of 9.6ml/min) at 2Hz
(Narita, Hata et al. 2004)	Polylactic acid (MW 56 kDa)	80	20	1	>95	NA	Pulsatile flow (mean flow rate of 500ml/min) at 2Hz
(Nasseri, Pomerantseva et al. 2003)	Polyglycolic acid / poly-4-hydroxybutyrate coated with collagen	20	5 (ID)	1 (PGA)	NA	NA	NA
(Yost, Baicu et al. 2004)	Collagen	12.5	5 (OD)	0.5	NA	1-20	NA
(Williams and Wick 2004)	Polyglycolic acid (non-woven felts)	50	4.5 (ID)	4.51	97	NA	Pulsatile flow (mean flow rate of 40ml/min) at 1.5Hz
(Doi, Nakayama et al. 1996)	Polyurethanes	20	2 (ID)	0.1	90	100-300	NA
(Boccaccini, Blaker et al. 2005)	PLGA (75/25), PLGA/Bioglass (1% wt.)	20	3 (ID)	1.5 - 3	NA	100 and 10-50	NA
(Guan, Fujimoto et al. 2005)	Polyurethaneureas	NA	5 (ID)	1	80-97	80-120	NA
(McCulloch, Harris et al. 2004)	Actifoam	50	20 (ID)	6	NA	NA	Pulsatile flow (mean flow rate of 282 ml/min) at 1Hz
(Madhally and Matthew 1999)	Chitosan (x-linked)	NA	3.5 (ID)	1-1.2	NA	40-250	NA

2 THE BIODYNAMIC CHAMBER

Figure 1 shows a biodynamic chamber, the Bose-Enduratec BioDynamic cardiovascular model. This design provides a mechanism for creating a controllable wall shear stress for cell-seeded tubular scaffolds during culture. The principle of the apparatus is schematically shown in Figure 2.

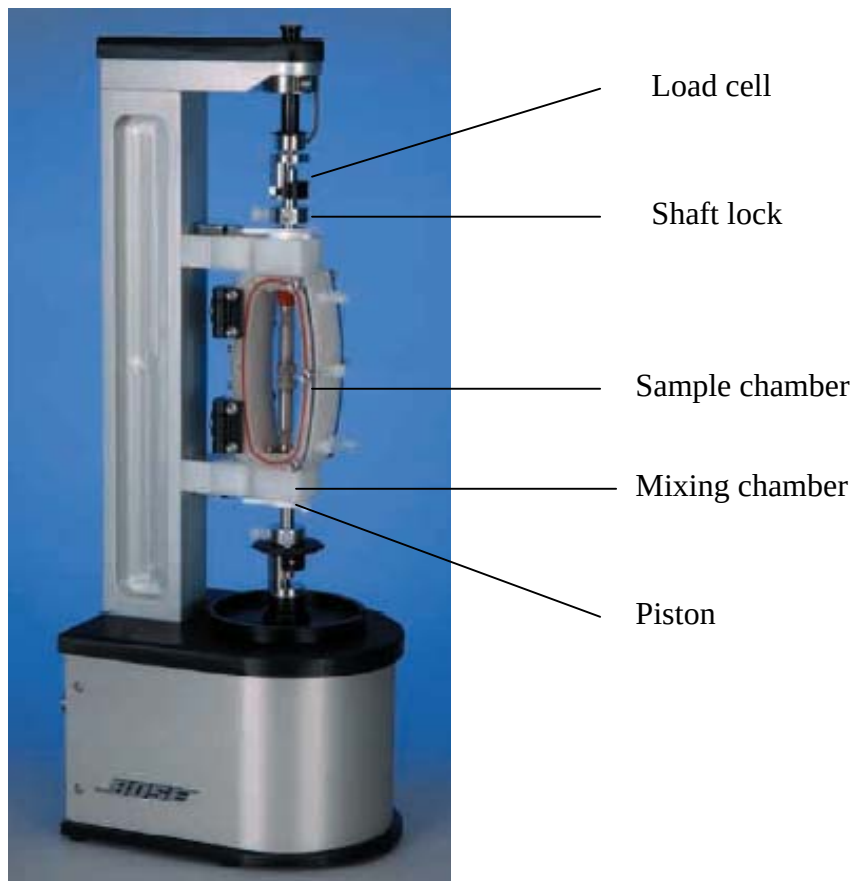


Figure 1: The Bose-Enduratec biodynamic chamber

Fluid pumped from the reservoir enters a mixing chamber, the volume of which is controlled by the stroke of a piston. This enables the mean flow rate to be modulated producing pulsatile flow at a set frequency, amplitude and waveform. A sensor sited within the mixing chamber records changes in pressure. This sensor is positioned in a conduit within the wall of the mixing chamber and as such has minimal impact on flow behaviour. The position of the piston is also logged via a displacement transducer. After exiting the mixing chamber the fluid passes through the sample tube, which, since it is contained in a chamber can be surrounded by air or fluid and returns to the reservoir via a tube open to the atmosphere.

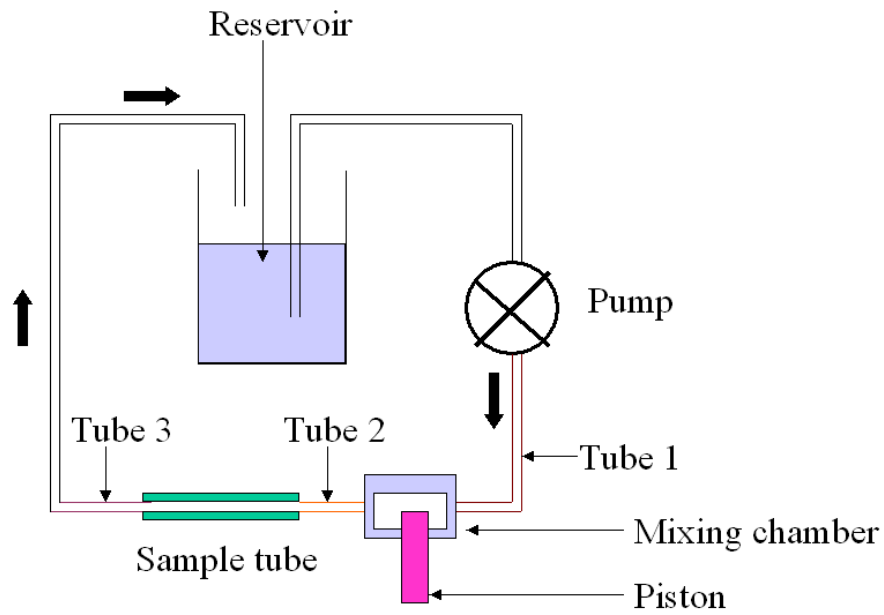


Figure 2: A schematic representation of a biodynamic chamber.

Both the mean flow rate and pulsatile volume can cause the tube that connects the sample to the mixing chamber to dilate as well as the sample tube itself. The connector tube (tube 2 in Figure 3) has to be relatively long (~80 cm) due to the design of the mixing chamber. Therefore much of the pulsatile energy can be ‘lost’ through flow mediated dilation of the walls of this tube before it reaches the mixing chamber. The actual amount of this energy loss depends on the diameter of tube 2 and its wall thickness and mechanical properties. It can be minimized by using a ‘stiff’ tube as used in high pressure or vacuum systems.

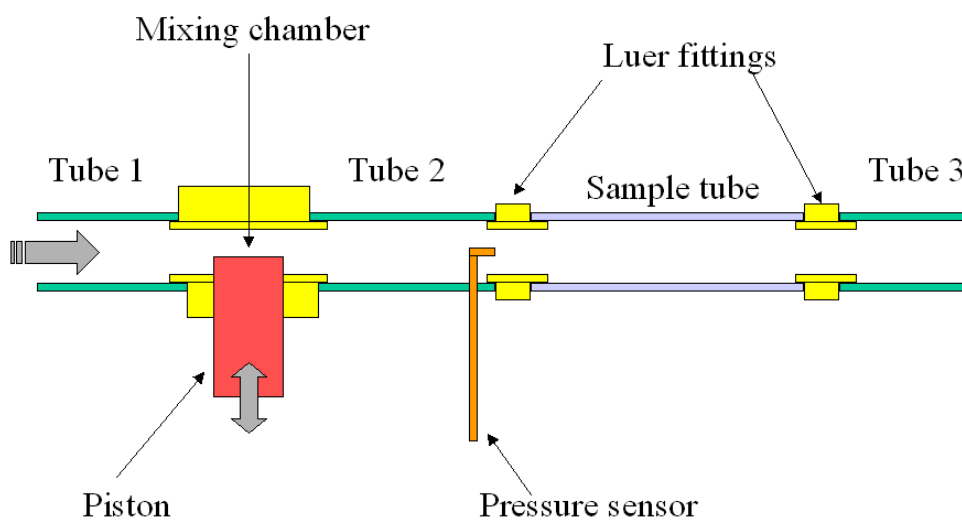


Figure 3: Details of the biodynamic chamber.

A further significant pressure drop occurs as fluid flows through the Luer fittings that are used to position the sample within the rig. The extent of this pressure drop depending on the inner diameter of the Luer fitting connector. In this investigation 6.25 mm internal diameter fittings were used.

3 DESIGN OF EXPERIMENTS

3.1 MATERIALS AND SPECIMEN GEOMETRY

Comparisons of strain behaviour using different techniques and experimental conditions rely on using a reproducible material and geometry. For this reason commercially available silicone tubing (Masterflex 96400-36) with an internal diameter of 6.3 mm and a wall thickness of 1.6 mm was used. The tubing was cut into 80mm lengths. Specimens were mounted on 6mm diameter Luer fittings (Kynar).

3.2 EXPERIMENTAL CONDITIONS

The geometry of the tube was chosen as being representative of that commonly used in tissue engineering applications. Similarly, the variations in flow were also selected on the basis of their physiological relevance in terms of the calculated wall shear stress. The key variables that can be controlled within the biodynamic chamber are:

- The mean flow rate
- The dynamic flow rate
- The pulse frequency

All of which have an impact on tube dilation and the wall shear stress.

Steady state flow of a fluid through a straight tube can be described by Poiseuille's law¹

$$\Delta p = \frac{8\eta L}{\pi a^4} Q \quad (1)$$

which states that there will be a pressure drop, Δp along the length of the tube, L . The pressure drop is directly proportional to the flow rate Q (ml/s) and the viscosity of the fluid, η (Pa.s) and inversely proportional to the fourth power of the tube radius

A wall shear stress, σ_w is defined according to:

$$\sigma_w = \frac{4Q\eta}{\pi a^3} \quad (2)$$

that is inversely proportional to the cube of the tube radius.

¹ Poiseuille's law applies to Newtonian fluids such as water and becomes an approximation for fluids such as blood that are non-newtonian.

If the flow is pulsatile then in principle Poiseuille's law is no longer strictly valid and the Womersley function should be used instead but this is beyond the scope of this study. For steady state flow conditions flow of pressurized fluid through the sample tube will cause dilation that can be measured at different positions along its length. The wall shear stress and pressure gradient within the tube is constant for a given flow rate and are listed in Table 2.

Table 2: Steady state flow conditions and associated wall shear stress.

Flow rate (ml/min)	Wall shear stress (Pa)
250	0.17
500	0.34
750	0.51
1000	0.68
1250	0.85
1500	1.02

Under dynamic conditions the wall shear stress depends on the dynamic flow behaviour of the fluid. The conditions required to produce a given dynamic wall shear stress need to be determined knowing the mean and dynamic flow rates as well as the pulsatile volume. The mean flow rate, Q (ml/min) is obtained by averaging the peak dynamic flow rate (A_1) and minimum dynamic flow rate (A_2) according to

$$Q = \frac{(A_1 + A_2)}{2}$$

The corresponding dynamic flow amplitude, A (ml/min) is then given by:

$$A = \frac{(A_1 - A_2)}{2}$$

and the time dependent pulsatile volume, P_v is:

$$P_v = \frac{A}{2\pi f} \cos(2\pi ft) .$$

The experimental conditions listed in Table 3 are primarily designed to examine the effect that pulse frequency, mean flow rate and hence pulsatile volume have on the dynamic strain behaviour of the silicone tube. In some cases the mean pump flow rate is kept constant for different dynamic flows and or pulse frequencies (Experiments 4 and 14 and experiments 21 and 22), whilst in others the difference in dynamic flow is maintained for different mean pump flow rates and frequencies (experiments 4 and 14 and experiments 4 and 21). The conditions used in experiments 2 and 12 were selected for investigating the effect of low flow rates and those of experiments 4, 7, 14, 17, 21 and 22 for high flow rates.

The wall shear stresses are also listed in Table 3.

Table 3: An experimental programme designed to investigate the influence that different flow conditions have on tube dilation.

Exp.	Minimum dynamic flow value (A_2) (ml/min)	Maximum dynamic flow value (A_1) (ml/min)	Frequency (Hz)	Mean pump flow rate (ml/min)	Dynamic flow amplitude (ml/min)	Wall shear stress (Pa)
2	0	500	1	250	250	0 ± 0.17
3	0	1000	1	500	500	0 ± 0.34
4	0	2000	1	1000	1000	0 ± 0.68
8	500	1000	1	750	250	0.34 ± 0.17
10	1000	2000	1	1500	500	0.68 ± 0.34
21	500	2500	1	1500	1000	0.34 ± 0.68
12	0	500	2	250	250	0 ± 0.17
13	0	1000	2	500	500	0 ± 0.34
14	0	2000	2	1000	1000	0 ± 0.68
18	500	1000	2	750	250	0.34 ± 0.17
20	1000	2000	2	1500	500	0.68 ± 0.34
22	500	2500	2	1500	1000	0.34 ± 0.68

3.3 REPEATABILITY OF RESULTS

Whilst there may be some differences in the mechanical responsiveness of the different regions of an extruded silicone tube these are considered to be insignificant. Therefore, in practice, differences between repeat measurements are much more likely to be due to the way in which the tube is placed within the chamber. Small differences in wall tension can occur when positioning the tube over the Luer fittings so that the tube may not be under uniform tension. Such differences can manifest themselves as slight bending of the tube. Practically the effect can be minimized by ensuring that the tube is 'straight' and minimizing the tensile load applied to the tube as registered by the load cell cited above the specimen (as shown in Figure 1). Despite these precautions repeatedly mounting the tube within the bioreactor is a source of uncertainty. For this reason three repeat measurements were made on the same tube after three insertions.

4 NON-CONTACTING METHODS FOR DETERMINING STRAIN

A variety of non-contacting techniques are available for measuring the dynamic strain of compliant materials. These can be broadly grouped into methods that capture and analyse images and those that are able to detect and monitor changes in sample dimensions via the interface between it and its surrounding medium. Examples of the former are digital image correlation and video extensometry. Methods for detecting interfaces include acoustic microscopy that is sensitive to differences in acoustic impedance and optical coherence tomography that is sensitive to differences in refractive index. In this paper we report strain measurements derived from laser micrometer data and from analysis of digital image correlations. The former is a technique that can be used to record changes in the dimensions of an artefact at a particular point, whilst the latter provides 2-D data within a user defined sample area.

4.1 DIGITAL IMAGE CORRELATION (DIC)

The principle of this technique is to capture a series of images of a tube as it is being dynamically stimulated using a CCD camera. These images are analysed using an appropriate software package (LA Vision, Davis Strainmaster) to track the displacement of surface features present on the surface of the artefact. In practice the surface ‘features’ are speckles of paint. This is applied in a contrasting colour over a light base coat, typically black speckles lying on top of a white base layer, Figure 4.

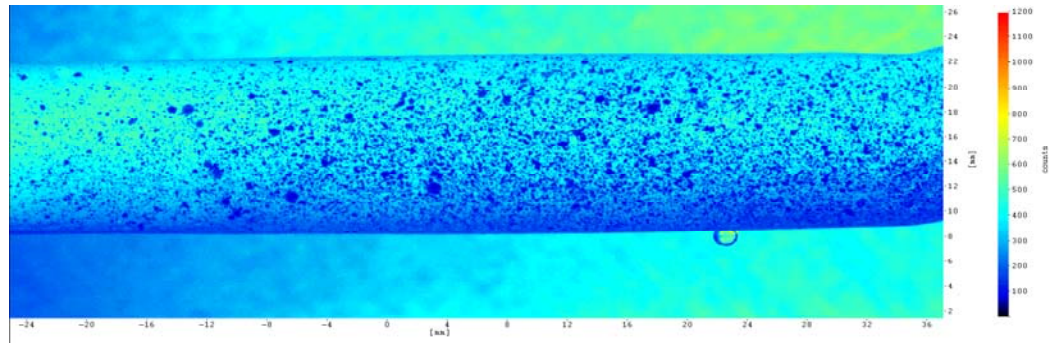


Figure 4. Diagram showing the spray-painted sample of a water filled tube surrounded by water

Corrections need to be made to the data to accommodate the curvature of the tube. These are obtained by masking the tube with an array of regularly spaced dots that will appear distorted in a 2D image captured by the camera, i.e. the spacing between adjacent dots decreases with increasing tube curvature, Figure 5. This effect can be corrected by the software package to correctly calculate displacements in this region of the image. Any corrections for any rotational misalignment of the tube should be made before curvature corrections are applied. This can be done by rotating the image.

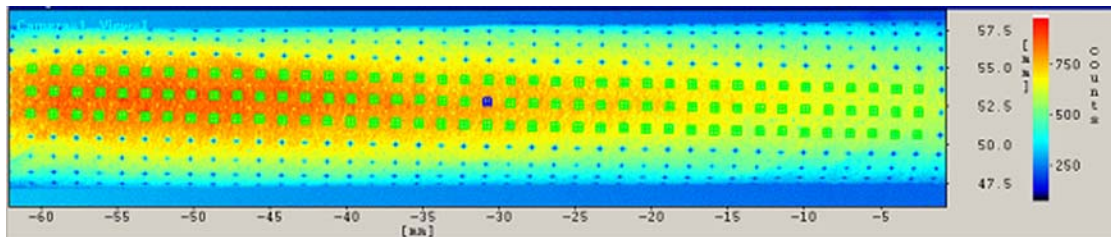


Figure 5: An image of a uniform array of points can be used to calculate the degree of curvature of a surface.

Data, such as that shown in Figure 6, are analysed by first grouping adjacent pixels to find the mean intensity at a particular point. The choice of how many pixels to select in the averaging process is decided by the user and is a balance between resolution, noise reduction and processing time. A window size of 12×12 pixels was used to produce a map of the resultant displacements in the longitudinal and transverse directions for the region shown in Figure 6. An overlap of 50% was used for both window sizes to increase the number of data points whilst minimizing smoothing. The effective window used for tracking movement is therefore 6x6 pixels where each pixel corresponds to an

area of $47\text{ }\mu\text{m} \times 47\text{ }\mu\text{m}$. Data can be analysed as a vector map representing the resultant of the transverse and longitudinal displacements over the entire tube or as displacements about a particular point.

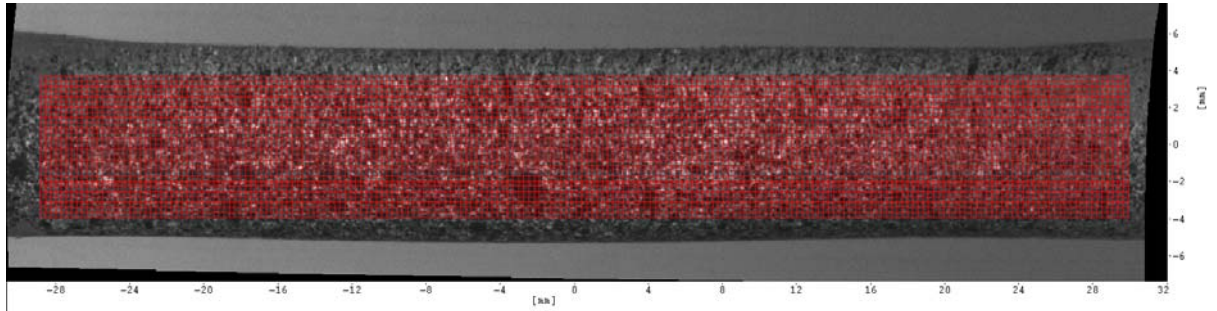


Figure 6. Details of a speckle pattern and the masked area used for analysis. The tube swelling seen at either end of the speckle pattern is due to the Luer fittings.

To avoid problems of correcting for excessive curvature of the tube, only the masked area shown in Figure 6 was analysed. The dimensions of the mask are 8mm x 60mm. Both contour plots of displacement and vector maps can easily be generated from this region of the speckle pattern. These plots provide a global picture of the changes in tube dimensions that occur during successive cycles of mechanical stimulation. However in order to compare DIC results with those obtained by the laser micrometer, data needs to be analysed at specific points along the tube. These are shown in Figure 7.

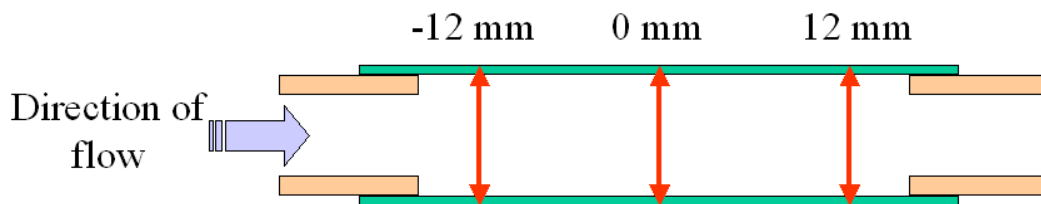


Figure 7: Transverse and longitudinal displacements can be analysed at specific points along its length.

The midpoint of the length of the tube is defined as 0 mm, measurements made away from this datum are then recorded as positive or negative depending on whether they are closer to the flow inlet or further away from it. Defining the datum at the midpoint of the tube overcomes the problem of the knowing exactly where the Luer fittings lie within the tube. Changes in the width of the mask were determined by averaging the calculated displacements over 4 windows, i.e. 24 pixels about the measurement point.

Capturing data from a dynamically stimulated artefact at sufficient speed and resolution requires a high speed camera and appropriate computer processing power. For this particular investigation images were captured at a rate of 8 frames per second by using a software mask to reduce the amount of superfluous information recorded. Four complete cycles, i.e. 32 frames were captured together with pressure sensor data in order to relate the displacement maps to the phase of each pulse. The relationship between frame number and the pulse waveform is shown in Figure 8.

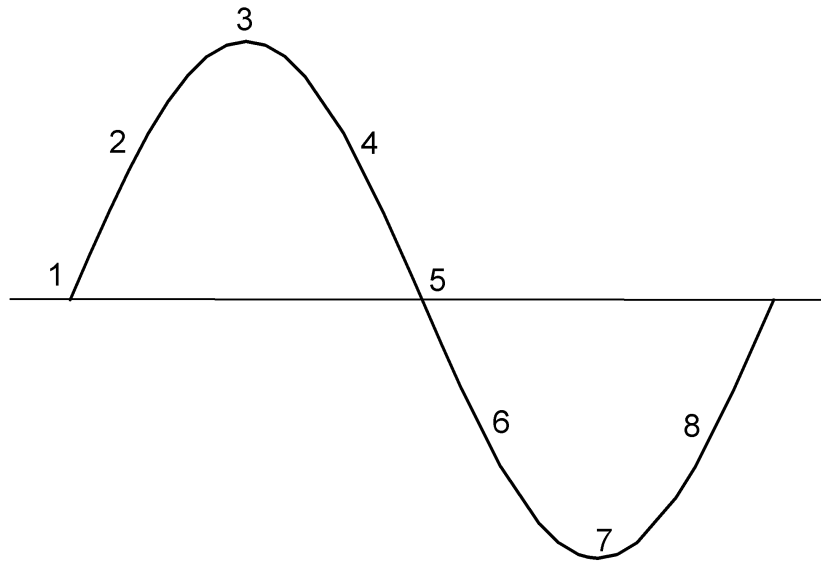


Figure 8. The relationship between image frame number and the pulse waveform at 1Hz as used for digital image correlation.

4.2 LASER MICROMETRY

A laser micrometer (Mitutoyo LSM 301)) can be configured as a simple shadowgraph detector as shown schematically in Figure 9. The tube, when placed perpendicular in a parallel beam of light produces a shadow on the detector that can be detected and logged.

The device can be positioned to record changes in the diameter of a tube at the same specific locations as used in the DIC analysis shown in Figure 7. Data from the laser micrometer as well as the pressure sensor located within the mixing chamber were logged using a Labview based programme. Data were captured at a rate of 10 measurements per second at 16 bit resolution. The pressure sensor data was used to synchronise measurements of the tube diameter with the pulsatile flow generated by the biodynamic chamber. The laser micrometer has a resolution of $\pm 0.1 \mu\text{m}$.

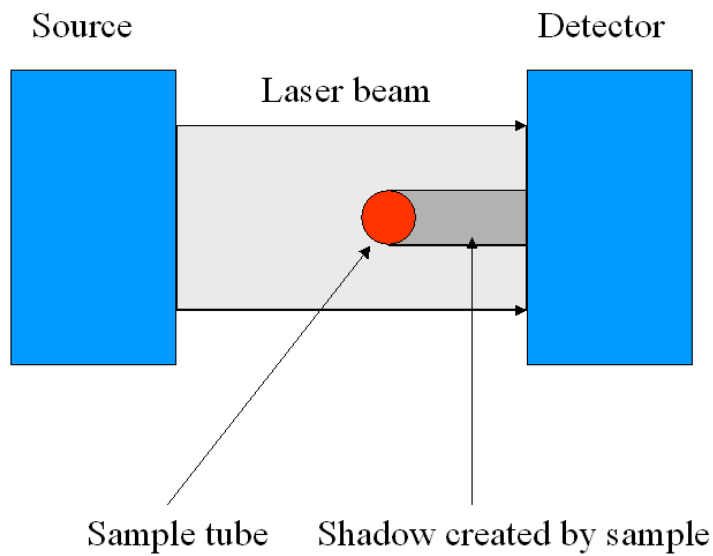


Figure 9: Schematic representation of a laser micrometer. The sample tube is viewed from above.

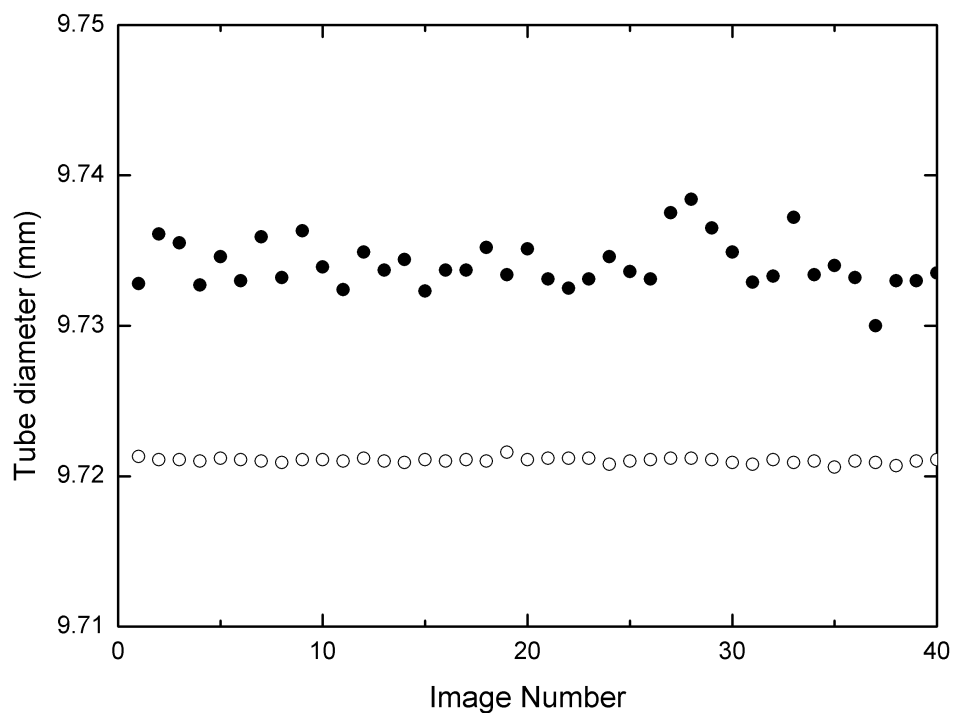


Figure 10: A comparison of measurements made of flow induced tube dilation (flow rate 750 ml/min) when surrounded by fluid (●) and in air (○).

5 RESULTS

5.1 LASER MICROMETER

5.1.1 The influence of air and water on steady state measurements

Figure 10 shows a comparison of the tube diameter measured under continuous flow conditions when surrounded by fluid (water) and air. In both situations measurements were made through the two glass doors that form two sides of the chamber. From this figure it is apparent that the presence of water around the tube results in increased noise within the data compared with measurements made in air. However the uncertainty in measurement is insignificant when compared to the diameter changes induced by constant or dynamic flow of fluid through the tube (section 5.1.2). Subsequent measurements were made in air for convenience. Table 4 lists the mean and standard deviations for repeat measurements.

Table 4: Fluid mediated changes in mean tube diameter for a tube made in air and surrounded by fluid based on laser micrometer measurements.

Medium surrounding the sample tube	Flow rate (ml/min)	Average diameter (mm)	Standard deviation ($\pm$ mm)
AIR	0	9.7453	0.0009
Air	250	9.7493	0.0012
Air	500	9.7501	0.0004
Air	750	9.7544	0.0004
Air	1000	9.7609	0.0005
Air	1250	9.7691	0.0004
Air	1500	9.7797	0.0005
Water	0	9.7425	0.0009
Water	250	9.7443	0.0008
Water	500	9.7480	0.0009
Water	750	9.7539	0.001
Water	1000	9.7623	0.0014
Water	1250	9.7722	0.0015
Water	1500	9.7855	0.0047

5.1.2 Flow induced tube dilation.

The data listed in Table 4 are plotted in Figure 11. The agreement between measurements made with either air or water surrounding the tube in the sample chamber is very good. Although at the highest flow levels measurements of the tube diameter when it is surrounded by water can become noisy as reflected in the standard deviations. The origin of this noise is due to light scattering from small bubbles and particulate matter contained within the circulating water of the sample chamber. This can be reduced simply by reconfiguring the apparatus so that the water jacket is not disturbed during measurements or by de-gassing the water. The relationship between tube dilation and flow rate is non-linear.

The amount of tube dilation depends on the position of the measurement along the tube length and the flow rate as shown in Figure 12. These data suggest that the amount of tube dilation decreases with increasing distance from the point at which fluid is pumped into the tube, an expected result as the pulse will attenuate in a flexible material.

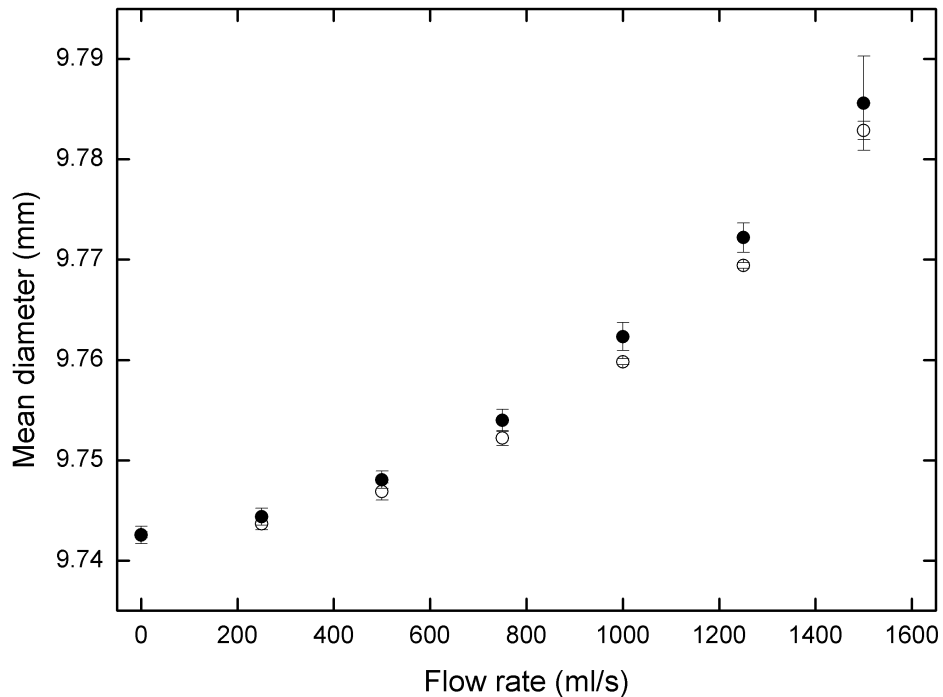


Figure 11: Flow mediated dilation of the tube as a function of flow rate. Measurements were made in fluid (●) and in air (○) at the zero point on the sample tube (Figure 7).

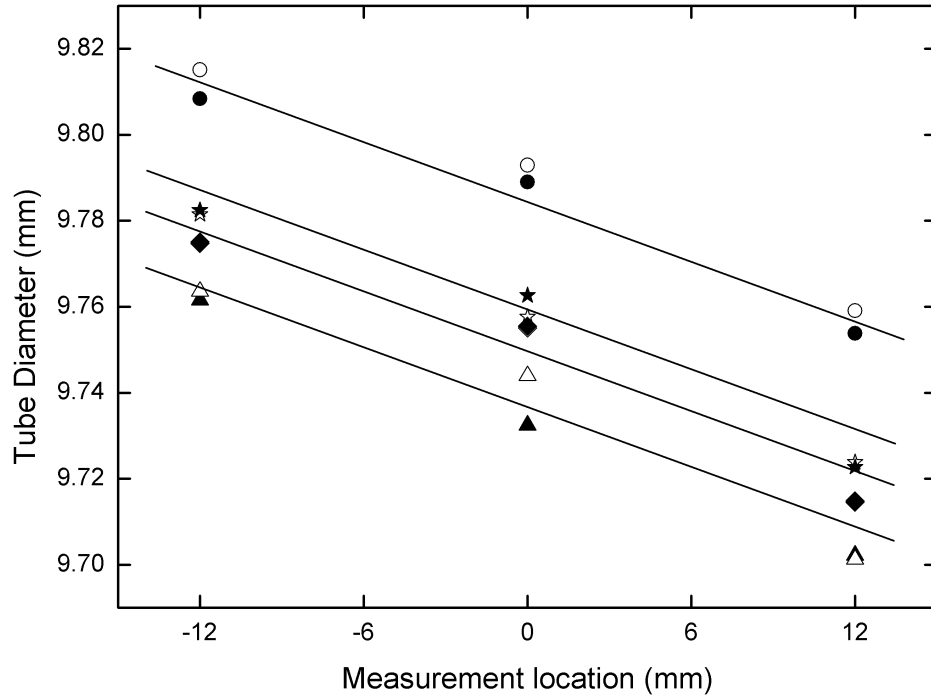


Figure 12: The influence of different continuous flow rates on the tube diameter measured at different points along its length. ● = 250 ml/min, ★ = 750 ml/min, ◆ = 1000 ml/min and ▲ = 1500 ml/min measured for the tube surrounded by water. Open symbols correspond to measurements made in air at equivalent flow rates. The lines are for indication only.

A plot of the pressure measured in the mixing chamber is and induced strain², ε in the tube as defined by,

$$\varepsilon = \frac{\Delta \text{diameter}}{\text{diameter}}$$

is shown in Figure 13. In principle these data could be used to calculate a value of Young's modulus. However such calculations depend on the constraints imposed on the tube by mounting it within the biodynamic chamber.

² Converting displacements to a dimensionless quantity facilitates comparisons of data obtained from different techniques. The quantity, ε is the hoop or circumferential strain.

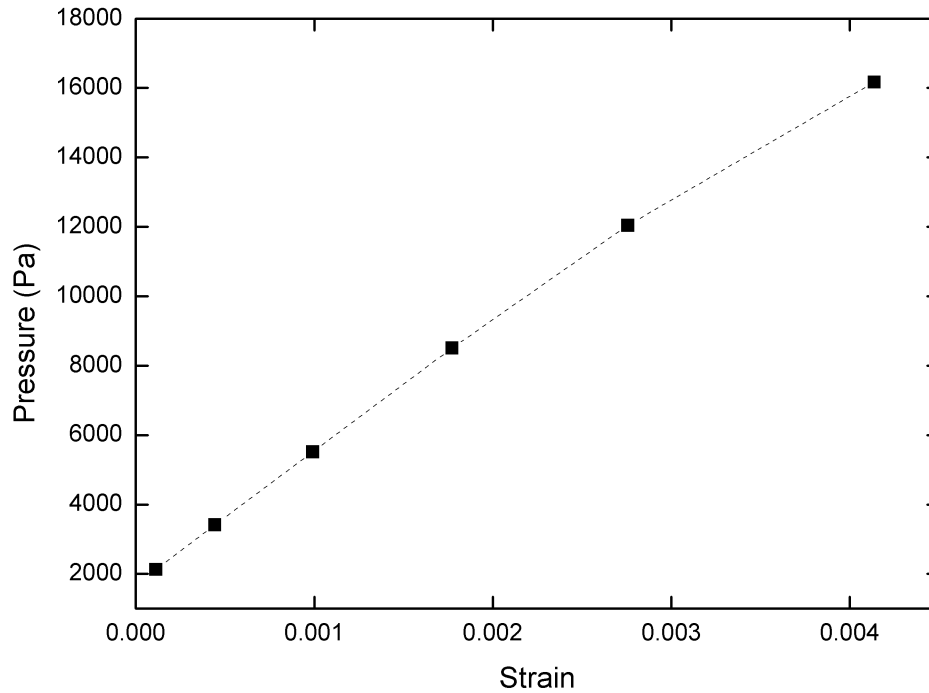


Figure 13: A plot of tube pressure versus strain for continuous flow.

5.1.3 Pulsatile flow

Figure 14 shows the changes in tube diameter as measured at three points along its length with the conditions listed in Table 3 for experiment 4. From this figure it is apparent that the laser micrometer measurements are able to adequately monitor oscillatory tube dilation at 1 Hz. These data can be used to generate the mean displacement amplitude (MDA) according to:

$$\text{Mean displacement amplitude: } MDA = \frac{D_{\max} - D_{\min}}{2}$$

where $D_{\max}$ and $D_{\min}$ are the averages of the maximum and minimum tube diameters respectively. The mean displacement amplitude can be used as a parameter on its own or to calculate strain.

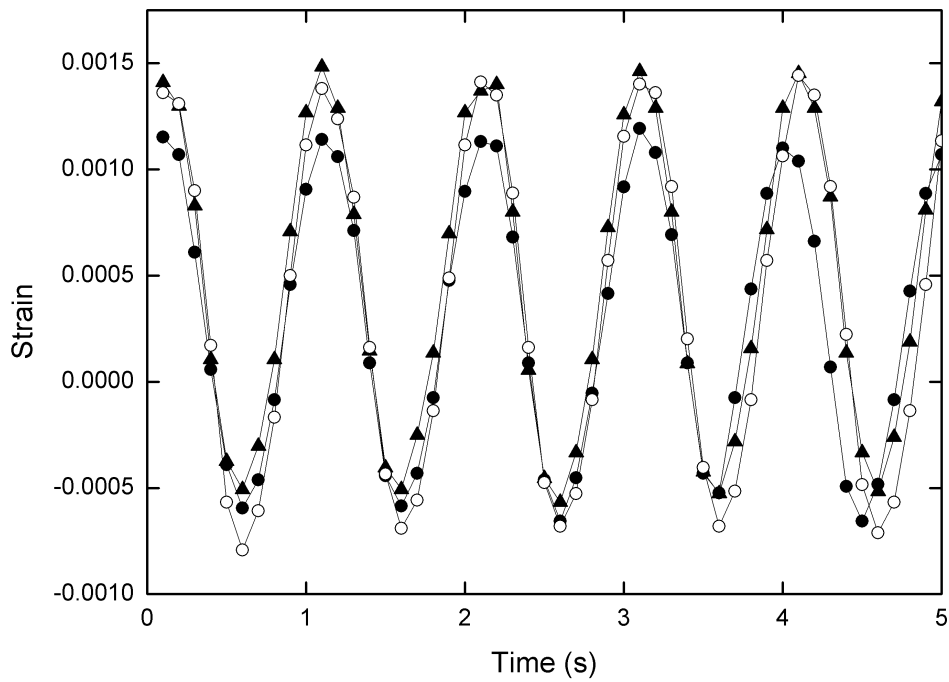


Figure 14: Variation in the tube diameter at different points along its length ○ = 12mm, ● = 0 mm and ▲ = -12mm.

Figure 15 shows the mean displacement amplitude for some of the experiments listed in Table 3. From this figure it is evident that there is a clear difference in the MDA between 1 Hz and 2 Hz data. The figure also shows that there is no clear trend in the amount of tube dilation that occurs during pulsatile stimulation with measurement position.

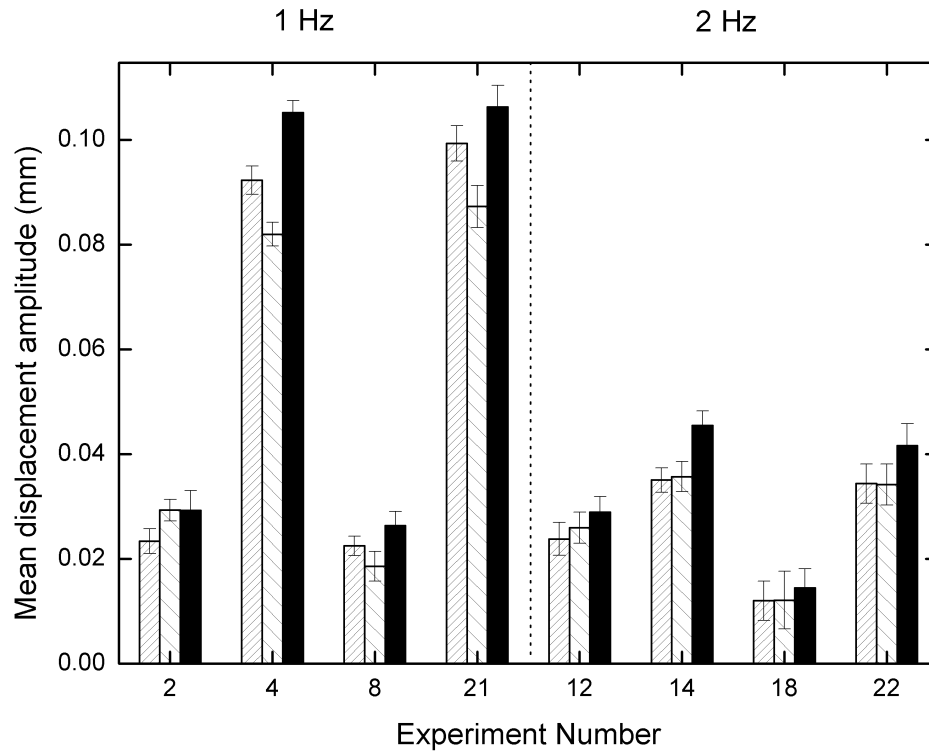


Figure 15. The variation in mean displacement amplitude determined at different points along the sample tube, -12, 0 and 12 mm respectively, for different experimental conditions (Table 3).

Ignoring the differences in frequency, the data in Figure 15 differ only in the mean flow and dynamic flow rates, therefore the measured tube dilation should correlate with data obtained at different continuous flow rates. This hypothesis appears to be confirmed in Figure 16 that shows an excellent agreement between tube dilation at different mean flow rates (pulsatile flow) and continuous flow rates as shown in Figure 11. This agreement that is independent of the pulse frequency.

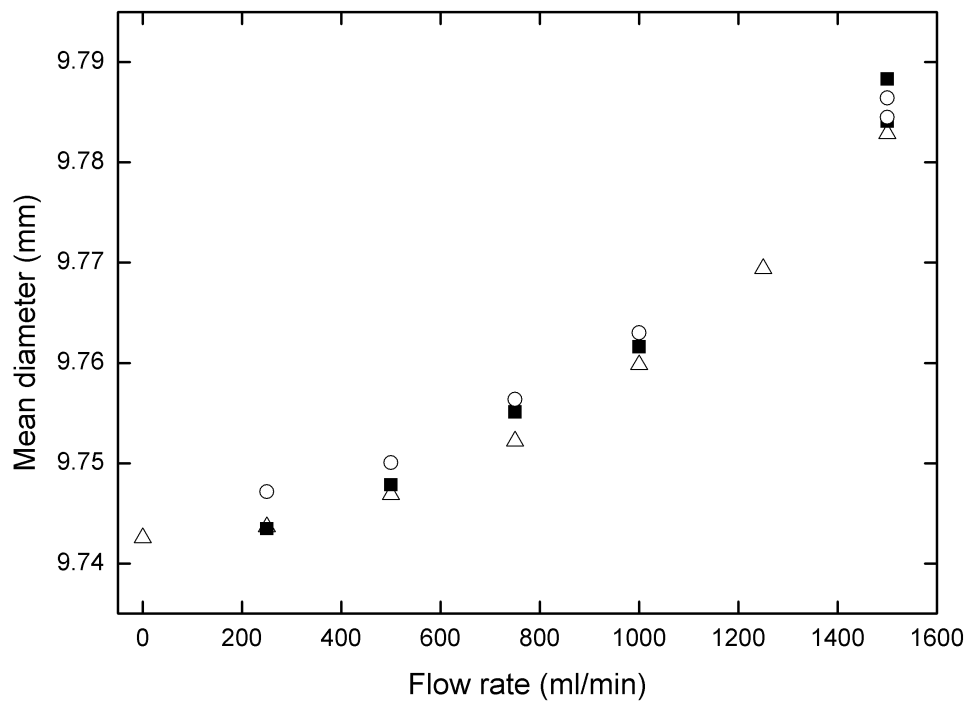


Figure 16: Comparison of flow induced tube dilation produced by continuous flow and the mean flow rate of a dynamically stimulated tube. Δ = continuous flow at 1 Hz, ○ = pulsatile flow at 1 Hz, and ■ = pulsatile flow at 2 Hz.

5.2 DIGITAL IMAGE CORRELATION (DIC)

5.2.1 Static results using DIC

The DIC method detects movement of a speckle pattern and is therefore well suited to monitoring the tubes response to dynamic flow conditions. Monitoring flow induced tube dilation under continuous flow conditions is practically more difficult as the reference image to which subsequent images refer should be a water filled tube under no flow conditions. Whilst in principle such an experiment can be done, in practice the act of inducing flow through the tube will cause it to move due to compliance within the rig. Hence the measured displacements will be a mix of movement due to rig compliance and flow induced dilation. For this reason such measurements were not made.

5.2.2 Pulsatile flow

Figure 17 shows the changes in both longitudinal and transverse displacement that occur during a single pulse cycle as a series of contour maps. From this figure it is apparent that there is a cyclic longitudinal displacement that is substantially greater than the transverse displacement which remains effectively constant. This is shown in Figure 18 for experiments 4 and 14. The flow conditions for both of these experiments are the same with the exception of the pulse frequency.

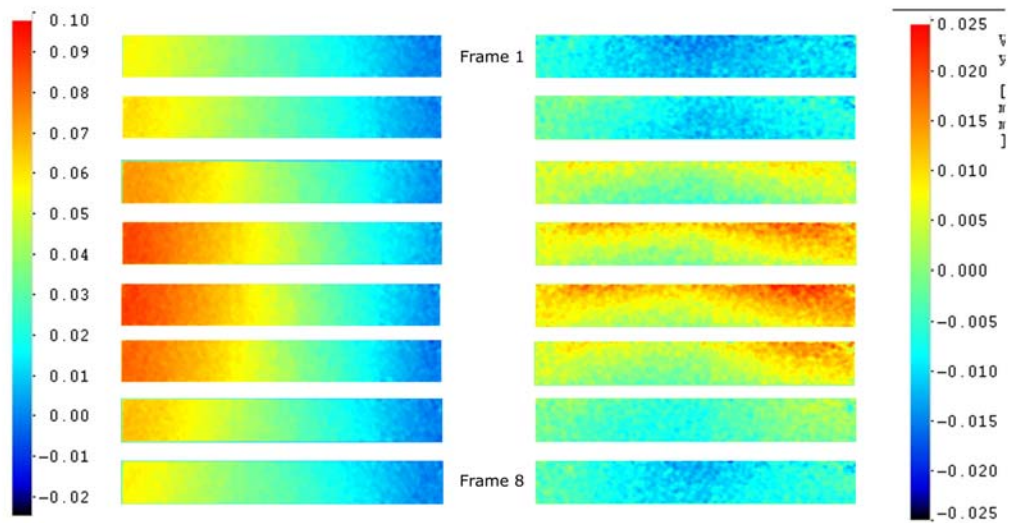


Figure 17. A sequence of eight displacement maps that show the longitudinal and lateral displacement of the tube that occurs during one pulse cycle. The tube was surrounded by air (experiment 4)

Explaining the observed longitudinal displacements in terms of fluid mediated dilation is difficult, especially given the constraints imposed on the tube by mounting it within the biodynamic chamber. However such a displacement pattern could be caused by transmission of the piston movement through the dynamic chamber itself. Movement of the structure is minimized through innovative design of the chamber. However tracking the thread position of the Luer fittings using a laser micrometer shows that the sample is moving longitudinally in synchrony with the piston used to create the pulsatile flow, Figure 18. The effect is significantly more pronounced in the leur fitting closest to the mixing chamber although it is also observed in the upper fitting.

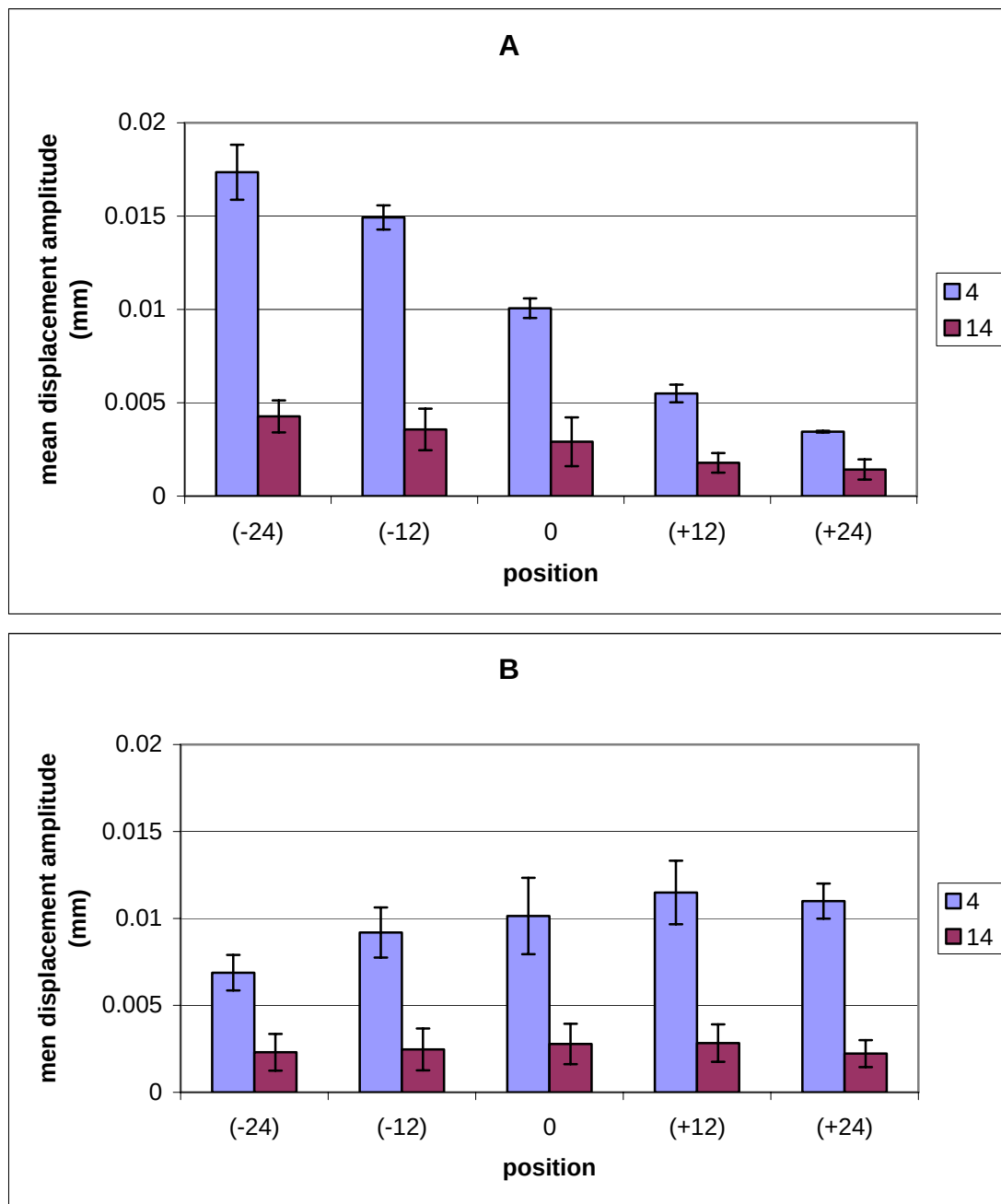


Figure 18.

A) Longitudinal displacement of the tube as a function of measurement position decreases with increasing distance from the point of fluid entry into the tube. The legend identifies the experiment (Table 3).

B) Transverse displacement as a function of measurement position is effectively constant.

Measurements of the effective diameter of a screw thread to which the Luer fittings are attached are very sensitive to vertical movement of the sample chamber as indicated in Figure 20. The origins of this movement have yet to be investigated but the effect can be substantially reduced by locking the upper shaft (the shaft lock is shown in Figure 1).

The transverse displacements can be analyzed following the procedure used for laser micrometry. However, plots of displacement versus time for the majority of measurements were far too noisy to be analyzed, indeed reliable data could only be obtained for experiments 4 and 21.

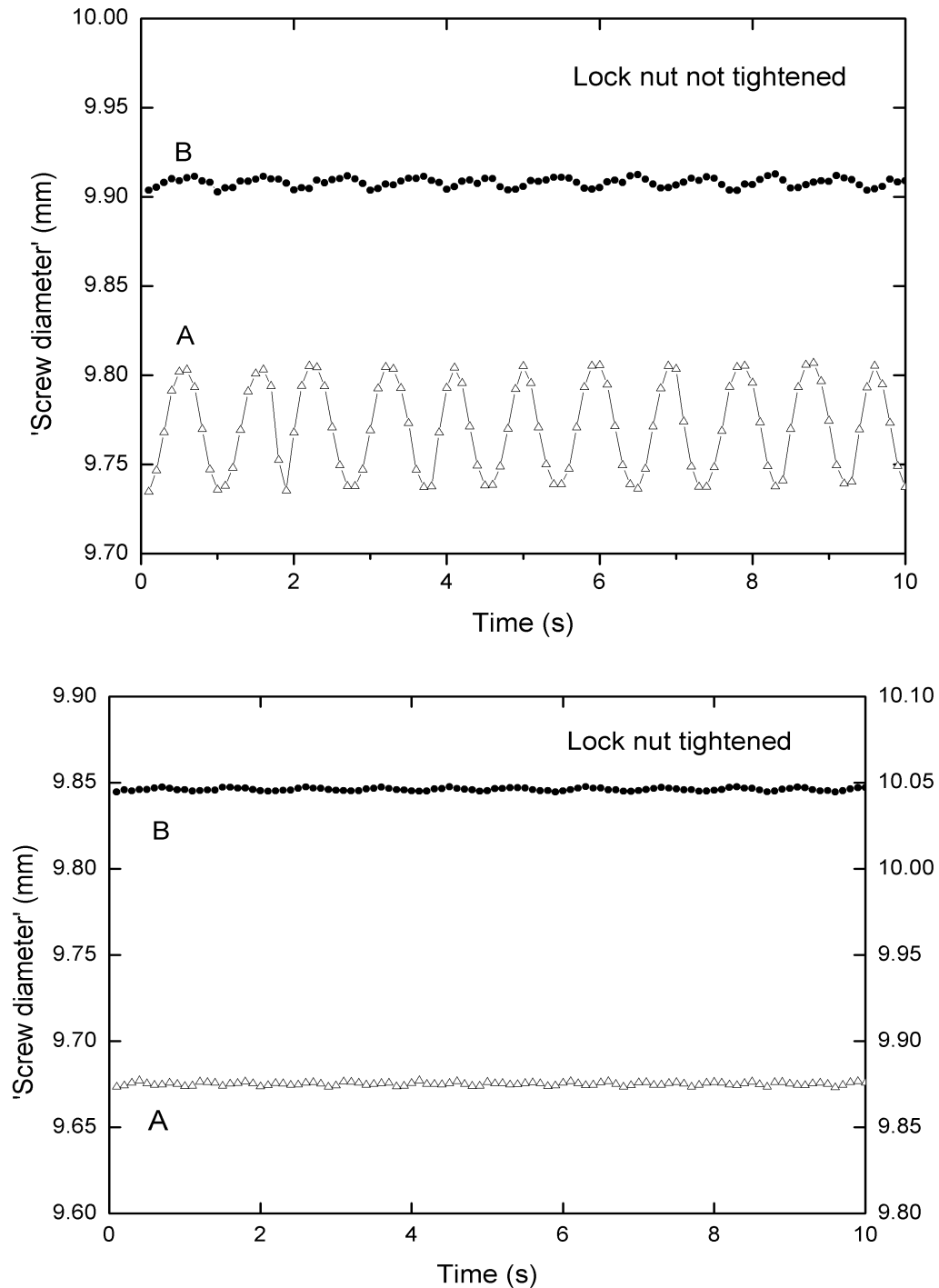


Figure 19. When the lock nut is not tightened movement of the piston is strongly detected in the Luer fitting closest to the mixing chamber (A – see Figure 20)) and observable in the upper Luer fitting (B). This effect can be damped by increasing the stiffness of the rig (lock nut tightened).

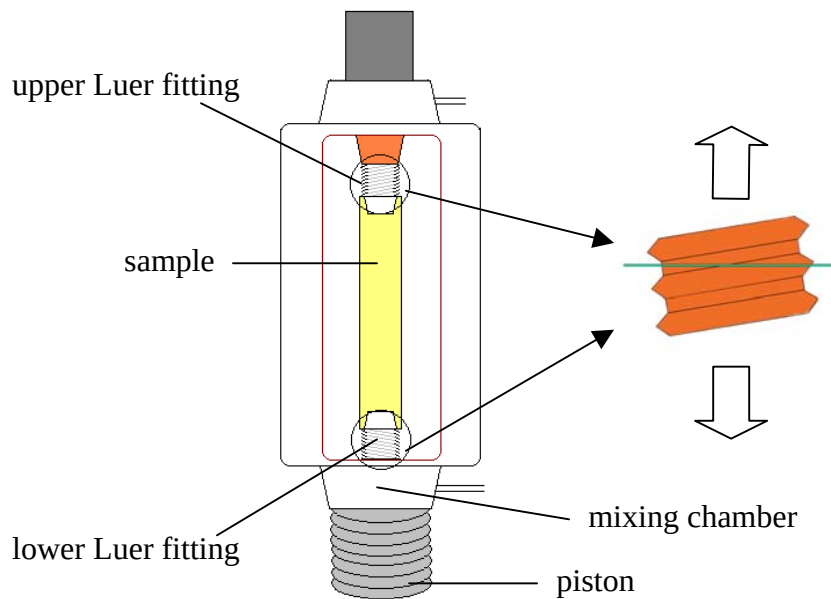


Figure 20. A laser micrometer can be used to monitor vertical movement of the sample tube by detecting changes in the effective diameter of a screw thread.

6 DISCUSSION

Both the laser micrometer and digital image correlation strain mapping can be used to monitor fluid-mediated tube dilation, both under continuous flow and dynamic flow conditions. The former is able to provide a measure of changes in the transverse dimensions of the tube at user specified points. The latter provides a detailed map of displacement that reveals both longitudinal and transverse movement of the tube. Being able to generate a displacement map offers the obvious advantage of more information – the longitudinal movement of the tube due to piston motion would not have been detected using the laser micrometer alone. However, such measurements are ‘noisier’ than those made by laser micrometry and require a more complex calibration to account for tube curvature. The choice of pixel grouping to define windows for analysing data and details of window translation are operator choices and are therefore subject to an uncertainty.

For this investigation most of the DIC data was too noisy to analyse. The origins of this noise could be attributed to the relatively low image capture rate (8Hz) but comparable data captured at a slightly higher frequency (10Hz) by the laser micrometer showed no such problems. A more probable explanation seems to be that the displacements resulting from pulsatile flow are approaching the resolution limit of the DIC equipment used in this study.

Table 5 provides a comparison of DIC and laser micrometry.

Table 5. A comparison of digital image correlation and laser micrometer methods for measuring flow induced tube dilation.

Technique	Advantages	Disadvantages
Digital image correlation	Provides a global map of longitudinal and transverse displacements	Requires corrections for tube curvature that become increasingly important for small diameter tubes.
	Simultaneous data capture over the entire sample area.	Requires a contrasting speckle pattern on the surface of the sample that may need to be created using spray paint.
		Lower resolution than the laser micrometer.
Laser micrometer	Sample requires no preparation	Requires measurements to be made at a specific location.
	High resolution available (1×10^{-4} mm)	Measurements cannot be made at a number of points simultaneously.
	Tube geometry minimizes any errors due to horizontal misalignment of the micrometer	Measurement uncertainties are sensitive to vertical misalignment of the micrometer.

7 FURTHER WORK

The measurements reported here relate to changes in the external dimensions of sample tubes. These will be mirrored by changes in the wall thickness. Such changes can be measured by techniques such as optical coherence tomography and scanning acoustic microscopy, methods that are able to detect changes in refractive index or acoustic impedance within the sample. These techniques will be used to further understand the local strain environment that exists within a potential tissue scaffold materials that are subjected to pulsatile flow.

The actual pulsatile flow rates through the biodynamic chamber depend on the compliance, geometry and spatial position of the tubing and the inner diameters of any connecting blocks, all of which affect the pressure drop through the system. Methods for measuring this flow and determining its sensitivity to different experimental conditions are being explored.

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