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Integrated Geometric and Mechanical Analysis of an Image-Based Lymphatic Valve

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Abstract

Lymphatic valves facilitate the lymphatic system's role in maintaining fluid homeostasis. Malformed valves are found in several forms of primary lymphoedema resulting in incurable swelling of the tissues and immune dysfunction. Their experimental study is complicated by their small size and operation in low pressure and low Reynolds number environments. Mathematical models of these structures can give insight and complement experimentation. In this work, we present the first valve geometry reconstructed from confocal imagery and used in the construction of a subject-specific model in a closing mode. An FEA study was performed to identify the significance of the shear modulus, the consequences of smoothing the leaflet surface and the effect of wall motion on valve behaviour. Smoothing is inherent to any analysis from imagery. The resolution and SNR of the image, segmentation and meshing all cause attenuation of high-frequency features. Smoothing not only causes loss of area but also the loss of high-frequency geometric features which may reduce geometric stiffness. This work aimed to consider these effects and inform studies by taking a manual reconstruction and through manifold harmonic analysis, attenuate higher frequency features to replicate lower resolution images or lower degree of freedom reconstructions. Two metrics were devised: pressure required to close the valve and the retrograde volume displacement during closing. Both are possible mechanisms of insufficiency; however, retrograde volume displacement has not previously been considered in an experimental analysis of lymphatic valves. In the case of the image-specific reconstructed valve the consequences of smoothing the valve surface included a 40 percent change in the trans-valvular pressure required to close the valve

Keywords

lymphatic; valve; harmonic manifold analysis; segmentation; FEA

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Author Contributions

DW and RvL performed the finite element analysis and manifold harmonic analysis. IS developed and performed the image processing, segmentation and meshing. DZ provided the image set and guidance on its interpretation. JM, DZ and RvL designed the study. All authors read and approved the final manuscript.

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1. Background

The lymphatic system is responsible for maintaining fluid balance within the tissues [1]; by collecting excess fluid from the interstitial tissues and transporting it through valved contractile tubular structures, into and through the lymph nodes, before emptying into the venous system [2]. The valve behaviour is poorly understood and essential for efficient fluid transport against gravity. Insufficient transport can lead to lymphoedema i.e. an accumulation of lymph fluid and inflammation in the tissues. Resulting in swelling and later fibrous tissue formation and compromised immune function [3][4]. Several authors have commented on the potential benefit of a lumped model of the lymphatic system and attempts to that end have been made [5] [6] [7]. Sensitivity analysis of lumped models revealed that valve resistance is a determinant of lymphatic pumping function [8] as is the trans-valvular pressure required to close the valves [9].

As far as the authors are aware the only finite element analysis currently published about lymphatic valve closure, is flow around a two-dimensional valve [7]. Studies have looked at the opening behaviour of three-dimensional parametric valves [10].

However, reconstruction from confocal imagery [11] reveals several difficult to idealise features present on the valve, some of which have not been included in previous parametric models [10]. Reconstruction, spectral analysis and finite element analysis of these structures may elucidate their role and allow their inclusion in parametric models.

Confocal scans of lymphatic valves allow the reconstruction of lymphatic geometries. This process is intensive, and an idealised representative model would allow large parametric studies to find the relationships necessary to construct a lumped model of the lymphatic system. The presence of wavy features on the surface of the valve poses a problem as they can only be captured with higher order geometric models which require more information. The idealised geometries currently considered have all been smooth, and the sensitivity of lymphatic models to the smoothing inherent to imaging and meshing has not been established.

This work aimed to provide the first image-specific lymphatic valve geometry and to use finite element analysis to study the closure of these compliant valves. The sensitivity of this model is assessed against smoothing, the shear modulus of the leaflet and transmural pressure.

2. Methods**2.1. Image Processing and Segmentation**

The image set used was produced by confocal imagery of a lymphangion isolated from the mesentery of a rat. A section of lymphatic vessel was extracted and placed in a calcium-free

solution to prevent contraction. The vessel was cannulated and loaded intra-luminally with Cell Tracker Green. The lymphangion was pressurised to a trans-mural pressure of 5cmH₂O and scanned with a Leica AOBs SP2 confocal-multiphoton microscope with an U APO 40.0×1.15 W CORR objective a 100mW 488nm laser was attenuated with an acousto-optical modulator, and acousto-optical beam-splitters were used to select the wavelengths from the emission spectrum between 510–525µm. A single *x-y* confocal slice was acquired then the focal plane was advanced in the *z*-axis before acquiring the next *x-y* image. The resolution in *x* and *y* is the same and dependent on the lens, scanning characteristics, confocal pinhole and staining intensity. However, the resolution in *z* is lower and is dependent on the step size and confocal pinhole size. The scans represent a pack of 195 2D slices containing 512-by-512 pixels at a resolution of 0.6-by-0.6 µm. The distance between consecutive images is 1 µm. A 307 micron length of wall was segmented from the image set containing a valve. The valves length was 230 microns from the middle of the bases to the middle of the commissures. The diameter at the commissures is 160 microns, and the diameter at the base was 90 microns.

As can be seen in Figure 2.A, the images are very noisy, the objects blurred and the intensity is non-uniform. Intensity decays exponentially from the surface to the depth of the object due to adsorption. These issues complicate the reconstruction of the leaflets, which was ultimately performed manually. The image was converted to an intensity map which was then subjected to a median filter. An overview of the lymphatic valve geometry is shown in Figure 1.A and B.

2.1.1. Segmentation of the Wall—The wall was segmented by an automatic active contour method. In order to achieve this, we introduce the Cartesian coordinates: *x* and *y* in horizontal and vertical directions, respectively, in every slice and the *z*-axis, is the depth of a slice. Then wall contours are arranged in every *z-y* slice, see Figure 2.B. The initial contour in the first *z-y* slice is an ellipse set manually by four points on the image in the middle of the wall. The contour displaces along its normal, moving up intensity gradients and stopping when it reaches the maximal intensity. In all subsequent slices, the final contour of the previous slice is taken as the initial. Thus the procedure is practically automatic except the setting of the first four points. As a result, the wall medial surface is recovered and represented as a cloud of points. After finding the medial wall surface, its local thickness is determined by analysing the decay of the image intensity from the medial surface. It is also smoothed along the surface to remove noise effects, shown in blue in Figure 2.C.

2.1.2. Reconstruction of the leaflets—First, a separation line is drawn between the two leaflets on each slice, Figure 2.A. This line can then be used to identify the two leaflets. A *z-y* and *x-y* slice with the two leaflets coloured red and blue is shown in Figure 2.B and C respectively. The intensity for each coloured leaflet is summed in the *z* direction for both sides of the separation line creating a 2D image that reveals the outline of the leaflets, shown in Figure 2.D. The trailing edges of the valve leaflets are traced and used as a guide for manual reconstruction of the leaflets. A vector is constructed such that it is roughly perpendicular to the valve in the *z-y* plane, the guidelines are parallel to this vector, as can be seen in Figure 2.C. For each slice, the user places a number of vertices resulting in a

polygonal chain that describes the shape of the leaflet, see Figure 2.D. These points can then be post-processed and meshed to obtain the final geometry, see Figure 2.C.

2.2. Material Properties

Whilst the composition of lymphangions has been established; with the leaflet consisting of elastin and the wall a collagen-elastin composite[11]. Existing experimentation on rats has found a highly non-linear pressure-diameter relationship for lymphangions, Rahbar et. al. fitted a pressure-diameter relationship of the form [11]:

$$P_{mural}(D) = P_0 \left(\exp \left[S_p \left(\frac{D}{D_0} - 1 \right) \right] - 0.001 \left(\frac{D}{D_0} \right)^{-3} + 0.05 \right) \quad (1)$$

Where P_{mural} represents the transmural pressure in cmH_2O , D represents the vessel diameter in μm , S_p the sharpness of the curve and D_0 the normalising diameter measured at $P_{mural} = P_0$. For lymphangion sections taken upstream of valves the mean values for P_0 , S_p and D_0 were 18.0, 20.4 and 157.4 respectively. It is important to note that the normalising pressure, P_0 , and diameter, D_0 , are the largest considered. Ideally, study toward a constitutive model would take into account fibre orientation and quantifications of the composition of the three distinct layers of the lymphangion wall, as has previously been performed for the arterial system[14]. However, experimentation is complicated by the small size preventing classical measurements and the collapse of the wall in a stress-free condition. Taking the assumption that the wall is dominant over the leaflets a method by which representative wall motion could be achieved was attained through an elasto-plastic model whereby the wall was considered very stiff elastically, and artificial plastic deformation was used to model the non-linear component of the behaviour described in Eq 1. This model allows representative wall motion for use in leaflet study. Firstly the wall is considered to be a homogenous thin walled cylinder described in $\{\Theta, R\}$ as $0 < \Theta < 2\pi$, $R = r(P_{mural})$ with associated thickness, $t(P_{mural})$; where r is the radius of the wall at pressure P_{mural} . The assumption of symmetry allows only displacement u in R . The assumption of incompressibility suggests that cross-sectional area is conserved.

$$\pi \left[r(P_{mural}) + \frac{t(P_{mural})}{2} \right]^2 - \pi \left[r(P_{mural}) - \frac{t(P_{mural})}{2} \right]^2 = Const \quad (2)$$

The principle strains can be written as:

$$\varepsilon_{\Theta} = \frac{u}{r(P_{mural})}, \varepsilon_R = \frac{t - t(P_{mural})}{t(P_{mural})} \quad (3)$$

The principle stresses can be written as:

$$\sigma_{\Theta} = \frac{r(P_{mural})P_{mural}}{t(P_{mural})}, \sigma_R = \frac{-P_{mural}}{2} \quad (4)$$

This allows the creation of a von-Mises equivalent stress-strain relationship. The pre-stress of the cylinder was modelled by the addition of the existing stress; where P_1 is the transmural pressure at imaging.

$$\sigma_{vM}(P_{mural}) = \sigma_{vM}(P_1) + \sigma_{vM}(P_{mural} - P_1) \quad (5)$$

This relationship was decomposed into a linear-elastic component representing the gradient at $P_{mural} = P_0$, in this case, a modulus of 413 *KPa* and the remaining strain through artificial plastic deformation. This model was implemented in *ANSYS* workbench, and the wall was pressurised to 18 *cmH₂O*. Shown below in Figure 3 is the fit described in Eq 1 compared to the mean D/D_0 for 10 equally sized bands through the wall geometry. As can be seen, the reconstructed wall is slightly more compliant than the fit. In the absence of collagen an incompressible neo-Hookean model was used for the leaflets with a shear modulus of 50kPa for the smoothing study. This value has been used in other studies of lymphangions but is based on experimental data from arterial elastin[10]. The sensitivity of this value was assessed in the material properties study.

2.3. FEA model set-up

ANSYS Workbench was used to solve the FEA problems. Meshes of varying densities were originally created for the reconstructed geometry. For the smoothing study, these meshes were then converged to a mean 1 percent relative error by the Euclidean norm of the entire displacement solution field, at a meshing density of 1 node per 3 μm^2 . The results of the manifold harmonic analysis were re-meshed, to preserve mesh quality, at a finer level of 1 node per μm^2 . For the flexible wall studies an initial mesh of 1 node per 5 μm^2 was refined until the pressure required to close the valve and the retrograde displacement of volume had a relative error of less than one percent. For every mechanical model, the leaflet was represented as a series of linear quadrilateral shells each with an associated thickness of 5 microns. Contact between the leaflets was modelled by an augmented Lagrange algorithm; the normal stiffness was relaxed to 0.5 of the default setting to allow convergence and an under-relaxation between equilibrium iterations of -0.5 the time derivative of displacement was used. The leaflets were considered rough, and slipping was discouraged through a penalty scheme. The effects of large displacement were modelled through the ramped application of load in up to 50 steps, during the static analysis. Both leaflets were pressurised with a trans-valvular pressure difference of 5 *cmH₂O* in order to close the valve. For the smoothing study, the annulus was fixed in space. For the flexible wall studies the afferent and efferent ends of the wall were held in-plane, and one node on the afferent end of the wall was fixed in space. The leaflets were bound to the wall by coupling displacements

to nearest nodes on the wall. Rotations were also coupled in order to represent collagen buttressing of the leaflet previously observed[11].

2.4. Smoothing Method

The leaflets were subjected to manifold harmonic analysis and low-pass filtered in order to provide: 1) a clear quantification of the leaflet geometries for further geometric analysis, 2) controlled removal of sinusoidal features from the reconstructed geometry. For detail on the manifold harmonic transform see Appendix Appendix A. Filters can be applied to the transformed geometry, much like any Fourier transform of an image. By simply truncating the transform prior to taking the inverse transform we achieve a low-pass filter [13]. By removing high-frequency features from the valve surface, it allows us to differentiate features that affect valve behaviour. To reduce filtering artefacts, a high roll-off low-pass Gaussian filter was used with a defined cut-off frequency, ω_{cut} . From the eigenvalue matrix, we can define a frequency vector, $\omega_i = \sqrt{\Omega_{ii}}$. The filter kernel $f(\omega)$, can now be constructed as below.

$$f(\omega_i) = \begin{cases} 1 & \text{for } \omega_i < \omega_{cut} \\ e^{-r(\omega_i - \omega_{cut})^2} & \text{for } \omega_i \geq \omega_{cut} \end{cases} \quad (6)$$

with r being a constant related to the roll-off (in this case $r = 10^5$ was used).

The new set of vertices, $\mathbf{Y}$, can now be defined as below.

$$\mathbf{Y} = (f(\omega) \otimes \Phi)^T \langle \mathbf{V}, \Phi \rangle \quad (7)$$

Using harmonic analysis, the transform of the reconstructed geometry was found and eight filters placed exponentially through the frequency space. In the case of each filter, the transform was inverted creating a series of smoothed geometries with frequency features removed. As shown in Figure 4 the filter points are superimposed on a magnitude-frequency spectrum of both leaflets. The solid lines represent the apex of the Gaussian filter, and the dashed lines represent the half-power points. The full cases can be seen in Figure B.10.

3. Results

Three phases of displacement can be identified during the application of an increasing trans-valvular pressure gradient. The mean of the peak axial displacement of the trailing edge for both leaflets is shown in Figure 5. Firstly, the unimpeded motion of the trailing edges toward each other; secondly, a transition as the trailing edges start to co-ap together, and finally the leaflets co-ap and the valve seal develops. As shown in Figure 5.D these regions can be delineated by the first trans-valvular pressure at which initial contact between the two

leaflets occurs and closure of the valve, defined as the first load step for which a contiguous area spanned a leaflet which was within half a micron of the other.

3.1. Leaflet Material Properties Sensitivity Study

A study was performed to assess the sensitivity of the model to changes in the leaflet shear modulus. The wall was included in this study though its material properties were kept constant as they were chosen to give representative motion. The shear modulus for the leaflets was varied from 10KPa to 60KPa. A trans-valvular pressure of $5\text{ cmH}_2\text{O}$ was applied to the valve in a closing manner. Shown below in Figure 8 are the effects of leaflet shear modulus on the displacement of the leaflet. There is a clear relationship between decreasing stiffness and a reduced pressure required to close the valve. Comparing this to the experimental data for a comparable valve it would appear the shear modulus lies somewhere between 10 and 20 *KPa*. After closure of the valve, the leaflets continue to displace axially under the applied trans-valvular pressure which results in retrograde displacement of the fluid within the lymphangion. This volume was estimated by comparing integration between the two leaflets in the imaged state and then after the application of a $5\text{ cmH}_2\text{O}$ trans-valvular pressure difference in a closing manner. The change in retrograde volume displaced at $5\text{ cmH}_2\text{O}$ is shown in Figure 7. As can be expected the retrograde volume displaced is a function of the trans-valvular pressure required to close, but it also takes into account the compliance of the geometry.

3.2. Leaflet Smoothing Sensitivity Study

In a static analysis the load was steadily increased until closure had occurred. The wall was considered rigid, as only the behaviour of the leaflets due to smoothing was considered. Shown in Figure 6 are the trans-valvular pressures required to close the valve for various cut-off frequencies. As can be seen, some filters reduce the pressure required to close and some filters increase the pressure required. With no apparent pattern this implies that it is the geometric nature of the smoothing rather than the loss of area which affects valve sufficiency.

4. Discussion

Considering the retrograde volume displaced during valve closure of between $7.2 \times 10^{-2}\text{ nL}$ and $2.2 \times 10^{-2}\text{ nL}$ in the context of observed average in-situ rat mesenteric lymph flow rates of 3.88 nL/s [18]. Whilst a small albeit measurable quantity, as lymphatic pressure increases during insufficiency so does the rate of valve contraction [17] similarly greater distention of the lymphangion at higher pressures may increase the hysteresis, and thus the volume displaced. This quantity may warrant further investigation in understanding valve insufficiency.

The confocal images are sufficient to allow an image-specific reconstruction of the leaflets. The absolute relative error between the seventh filter and the original geometry is within four percent. Suggesting critical features maybe those not attenuated by the 8th filter i.e., those with a wavelength of less than 4.094 microns. These features could still be discerned at a resolution as low as the Nyquist interval for that filter of approximately 2 microns per

pixel, which is half of the z resolution of the images used in this study and three-tenths of the resolution of the other axes. Lowering the resolution may be beneficial to the study of real geometries if a coarser image had a higher signal to noise ratio.

There are several limitations to this study. The contractile nature of the wall was not included in any of the mechanical models. Secondly, the material properties of the wall in this study were designed only to produce representative motion, the creation of a constitutive model based on the interaction between collagen and elastin at various strain-states within the wall would allow for a greater consideration of its behaviour. The estimated shear modulus for the leaflet of 50KPa based on arterial studies of elastin and similar to previously used values appears to be an over-estimation[10]. The properties describing the elastin network in lymphatic leaflets are still unknown and might affect valve behaviour. Experimental determination of these properties is very challenging given the small size of the leaflets. Hence, future work could address these limitations by performing a material characterisation of the valves imaged in two positions and a two-way fluid-structure interaction study.

The potential significance of retrograde displacement of lymph has been identified further study of this phenomenon could influence modelling of lymphatic sufficiency. As far as the authors are aware this work represents the first image-based values on the retrograde displacement of lymph. The consequences of smoothing have been revealed to be non-trivial, and further work should seek to address questions of the existence of such features and methods of incorporating them into idealised models. These preliminary values could be included in lumped models currently being developed. Further work to overcome the above limitations and the inclusion of more datasets could allow the definition of a more representative idealised valve.

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Appendix A. Smoothing Algorithm

G. Taubin [19] first constructed analogues of common image processing functions by analysing the discrete Laplacian of graphs. B. Lévy [20] then used the eigenvectors of the Laplace-Beltrami operator as bases for spectral filtering of triangulations. Consider any triangulation with n_v vertices $\mathbf{V}$ and an associated edge list $\mathbf{E}$. Now a geometric weight matrix $\mathbf{W}$ of size n_v can be defined as,

$$\mathbf{W}_{ij} = \begin{cases} -\frac{\cot(\beta(ij)) + \cot(\hat{\beta}(ij))}{2} & \text{for } (i, j) \in \mathbf{E} \\ 0 & \text{else} \end{cases} \quad (\text{A.1})$$

$$\mathbf{W}_{ii} = -\sum(\mathbf{W}_i) \quad (\text{A.2})$$

where $\beta(ij)$ and $\hat{\beta}(ij)$ are the measures of the angles opposing the edge (i, j) . A diagonal matrix, $\mathbf{D}$, can be defined where $\mathbf{D}_{ii}$ equals the finite Voronoi area of the i^{th} member of $\mathbf{V}$, calculated using the method described by Meyer et al. [21]. The Laplace-Beltrami operator can now be defined as $\Delta = \mathbf{D}^{-1}\mathbf{W}$. However, it may not be symmetric thus the following symmetric generalised eigenvector problem is solved $\mathbf{W}\Phi = \mathbf{\Omega}\Phi\mathbf{D}$ [22]. Which yields a n_v -by- n_v matrix of eigenvectors Φ , and the eigenvalue matrix, $\mathbf{\Omega}$, whose diagonal, $\mathbf{\Omega}_{ii}$, corresponds to the square of the fundamental frequencies those bases represent. We can thereby transform $\mathbf{V}$ to give $\hat{\mathbf{V}} = \langle \mathbf{V}, \Phi \rangle$ with $\hat{\mathbf{V}}$ the spectral representation and with $\langle \mathbf{A}, \mathbf{B} \rangle$ as the $\mathbf{D}$ normalised inner product of $\mathbf{A}$ and $\mathbf{B}$, i.e. $\mathbf{A}^T \mathbf{D} \mathbf{B}$. Upon the boundaries, only one angle opposing the edge is found. Hence, $\cot(\hat{\beta}(ij))$ does not exist, and only the finite Voronoi region is considered thus creating a Neumann boundary condition [20]. The use of a Dirichlet boundary condition was considered, but as the wavy features are also present on the boundary, the smoothing of the domain would create transitional features between the wavy boundary and the smoothed domain. This was considered unrepresentative of the problem. However, the use of Neumann boundary conditions makes the issue of area loss more acute.

Figure A.9.

Geometries produced by filtering the reconstructed leaflets. Top: leaflet 1. Bottom: leaflet 2. Frequencies in radm^{-1}

Appendix B. Meshing

The polygonal chains describing the centre of the leaflets were processed to form a triangulated mesh for use in finite element analysis. First, the edge wall nodes are moved to the lumen wall surface by the shortest path, or an additional point is inserted on the wall surface. If the closest leaflet point is located at a large distance from the wall; then the angular positions of the wall edge points, relative to the vessel centreline, are smoothed along the vessel axis. The free edge line is smoothed by a method previously described [12].

After that, the leaflet lines in every z - y slice are smoothed by the same method with the weighting coefficients proportional to the length of each segment. This modification is necessary to reduce variations in segment length. The new points are then uniformly distributed along every line, approximated by a cubic spline.

Note that point positions have essential variation from slice to slice, i.e. along the vessel axis (Figure 5.A). A mesh has to be built before the application of the smoothing technique. As the points are organised along lines, the part of the surface between two subsequent lines - a strip - can be easily triangulated to form a Delaunay triangulation for each strip. Thus the total mesh is not necessarily Delaunay compliant. The boundary nodes, i.e. nodes along the

free edge and the wall edge of the leaflet are located non-uniformly in some places. They are substituted by uniformly distributed points, and the mesh is re-triangulated locally. The mesh needs anisotropic smoothing to preserve the reconstruction in the y and x -axis whilst sufficiently attenuating variation from slice to slice in the z -axis. Therefore a modification of the Laplace smoothing method is proposed in which contiguous nodes placed in the same line are disregarded. The result of such a method is presented in Figure ???. The initial leaflet mesh is too fine: it has voxel size elements and contains 35000 nodes and 64000 elements. The initial mesh is used as a benchmark and for fast re-gridding of the mesh to obtain a range of element sizes necessary to assess convergence of the finite element scheme. To aid this process, every node of the coarser meshes exactly coincides with one of the nodes of the initial fine mesh. In the re-gridding procedure, the fact that mesh nodes are arranged along lines is actively used.

Figure B.10.

Smoothing manual reconstruction. Variation in colour represents variation in the surface normal direction. A) The meshed leaflet before smoothing. B) The meshed leaflet after smoothing.

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Figure 1.

An overview of the lymphatic valve configuration and nomenclature. The wall was not included in this study and is shown for illustrative purposes only. A) Nomenclature for the valve leaflets. B) The configuration of the leaflets to form the valve. C) Shows the Segmented structures of a subject-specific valve. Leaflets are shown in red and green, and the wall is shown in blue.

Figure 2.

A series of z-y slices illustrating a step by step overview of the segmentation process. A) A z-y image slice. B) A segmentation of the wall (yellow). C) The guidelines of leaflet edges used in reconstruction (cyan). D) Manual reconstruction of the leaflet structures (red and blue).

Figure 3. A plot of the mean D/D_0 for ten equally spaced bands throughout the geometry during pressurisation; also shown is the fit for experimental data found by Rahbar et al.

Figure 4. Magnitude-frequency spectrum with filter cut-off frequencies superimposed solid lines indicate filter apex, dashed lines indicate half power points. Red and blue indicate the two leaflets.

Figure 5.

A plot of the peak axial displacement of the trailing edge against trans-valvular pressure. Also shown is the original valve at three different trans-valvular pressures with the Euclidean displacement field coloured. A marks the first contact between the leaflets. B marks the point of minimal orifice area and C is the maximum applied trans-valvular pressure.

Figure 6.

Trans-valvular pressure required to close the valve for differing leaflet shear moduli. The black lines represent experimental data for a lymphatic valve without tone and a trans-mural pressure of 5 cmH₂O[17]

Figure 7.
Retrograde volume displaced for differing leaflet shear moduli.

Figure 8. Trans-valvular pressure required to close the valve for differing filter cut-off frequencies. Cases coloured as in Figure 4.