STRESS AND STRAIN IN BIOLOGICAL TISSUES – MATHEMATICAL DEFINITION AND PHYSICAL CONCEPTS

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Abstract. The work represents a new approach that is designed to integrate different stages of cell analysis – from image processing to global analysis of cell dynamics combined with an interface to continuum mechanics based computational analysis. Initially an apparatus for cell reconstruction and global geometric analysis have been developed. Subsequently methods for data filtering and surface mesh generation have been added. The method possess significant advantages if used in biologic applications as it could process data directly from optical or general image devices such as microscopes and scans. Real time on-line reconstruction and relevant computational analysis could be performed due to the rapid computational speed which in turn provides a good opportunity for the development of an integrated medical diagnostics technology. It is further discuss the role of global reconstruction for geometric analysis of cells in order to establish a reference state as there is still an unresolved issue as to what exactly is the non-deformed shape of a cell and is it feasible to implement the classic continuum mechanics concepts of stress and strain to the analysis of biological cells and is this is a concept issue related to multiscaling.

1 Introduction

The role of mechanical deformations in cell response is known as mechanotransduction [5,8]. However, it is still not clear as how exactly do cells react to external loads. One of the important issues is that the transfer of continuum mechanics concepts to microbiological level has not been entirely successful. There are several reasons for this but one is the lack of analytical methods for analysis of cells due to the dominance of digital equipment that in effect imposes discrete image representation. Without a doubt cells react, adapt, respond and get damaged by mechanical loads [7,8,9,10]. As a result their mechanical properties change at both whole cell and sub-cellular level [1,2,6,7].

Dabnichki [3] and Dabnichki and Zhivkov [4] developed a method to integrate different stages of cell analysis – from image processing to global analysis of cell dynamics combined with an interface to continuum mechanics based computational analysis. However, the main focus of the current work is to establish a method of identifying the non-deformed cell status or more precisely a method for identifying a group of equivalent reference state that could be used to analyse cell response to loading. It is argued that at micro-level the classic continuum mechanics definition of stain and stress is not applicable as cell shape is affected by the functionality. Hence the homeostatic state is not necessarily the equivalent of stress free state but a reference state. This work shows a 3D method for cell reconstruction that allows reconstructing their geometry from noisy data and establishing equivalent states of the cell and hence referencing state for non-deformed shape.

2 Modelling of the shape of the cells

Recent years have seen big advancements in cell imaging and tremendous progress in this area. However, the primary driver for this development is production of realistic images that allow medical specialists to quantitatively assess the change in cell shape and not the production of accurate data that could be directly utilised in computational analysis. The nature of most techniques is that three dimensional reconstructions are obtained from two-dimensional images. These images produce contours of the cells and due to noise in the data those contours are normally smoothed. It is important to note the use of the word smoothed as it indicates that this process is rather arbitrary and not based on rigorous mathematical criteria. Hence the first step is to introduce rigorous procedures in reconstruction of cell contours. Such procedures are described below where an approach that allows the reconstruction of the entire contour in a single step is presented.

2.1 Definition of theta Functions

The four theta functions as defined below by their Fourier series are used in this work to describe the geometry of different objects.

$$\begin{aligned}
\theta_0(z,q) &= 1 + 2q\cos(2z) + 2q^4\cos(4z) + 2q^9\cos(6z) + 2q^{16}\cos(8z) + \ldots \\
\theta_1(z,q) &= 1 - 2q\sin(2z) + 2q^4\sin(4z) - 2q^9\sin(6z) + 2q^{16}\sin(8z) + \ldots \\
\theta_2(z,q) &= 1 + 2q^{1/4}\cos(z) + 2q^{9/4}\cos(3z) + 2q^{25/4}\cos(5z) + 2q^{49/4}\cos(7z) + \ldots \\
\theta_3(z,q) &= 1 - 2q^{1/4}\sin(z) + 2q^{9/4}\sin(3z) - 2q^{25/4}\sin(5z) + 2q^{49/4}\sin(7z) + \ldots
\end{aligned} \tag{1}$$

Each function θ_i depends on the complex arguments z and q, |q|<1. In order to ensure that the series represent real-valued theta functions, the following constrains are additionally imposed:

$$q \in \Re, \qquad 0 \le q < e^{-\pi/4}, \quad z = u \in \Re \text{ or } z = iv \in i\Re \qquad i = \sqrt{-1} \tag{2}$$

The additional condition which does not affect generality $|q|<0.455$ ensures exponentially fast convergence of all theta and any order of their derivatives.

More important for geometric reconstruction are the actual ratios of the theta functions as presented below

$$\begin{aligned}
\Theta_1(u,v,q) &= \frac{\theta_1(u,q)\theta_1(iv,q)}{\theta_0(u,q)\theta_0(iv,q)} \\
\Theta_2(u,v,q) &= \frac{\theta_2(u,q)\theta_2(iv,q)}{\theta_0(u,q)\theta_0(iv,q)} \\
\Theta_3(u,v,q) &= \frac{\theta_3(u,q)\theta_3(iv,q)}{i\theta_0(u,q)\theta_0(iv,q)}
\end{aligned} \tag{3}$$

These functions are real-valued if u,v and q are real. In effect they represent a set of analytical coordinates for any point on the sphere:

$$\Theta_1(u,v,q)^2 + \Theta_2(u,v,q)^2 + \Theta_3(u,v,q)^2 = 1 \tag{4}$$

They have two independent semi-periods as can be seen below:
Up to some non-significant constants, $\Theta_i(u,v,q)$ can be represented as

$$\begin{aligned}
\Theta_1(u,v,q) &= sn(u,q)sn(iv,q) \\
\Theta_2(u,v,q) &= cn(u,q)cn(iv,q) \\
\Theta_3(u,v,q) &= dn(u,q)dn(iv,q)
\end{aligned} \tag{5}$$

where *sn, cn* and *dn* stand for the classical Jacobi's elliptic functions.

The following relations are very important as they provide a fast and accurate method for calculation of their derivatives.

$$\begin{aligned}
\frac{d}{dz}\frac{\theta_1(z,q)}{\theta_0(z,q)} &= \theta_2(0,q)^2\,\frac{\theta_2(z,q)}{\theta_0(z,q)}\frac{\theta_3(z,q)}{\theta_0(z,q)} \\
\frac{d}{dz}\frac{\theta_2(z,q)}{\theta_0(z,q)} &= -\theta_1(0,q)^2\,\frac{\theta_3(z,q)}{\theta_0(z,q)}\frac{\theta_1(z,q)}{\theta_0(z,q)} \\
\frac{d}{dz}\frac{\theta_3(z,q)}{\theta_0(z,q)} &= \theta_0(0,q)^2\,\frac{\theta_1(z,q)}{\theta_0(z,q)}\frac{\theta_2(z,q)}{\theta_0(z,q)}
\end{aligned} \tag{7}$$

It should be noted that the above formula could be used repeatedly to obtain any order derivatives. The so defined thetas possess natural properties that allow their use in accurate reconstruction of biological objects. A sensible first step in the reconstruction in the reconstruction of any closed shape image is the use of ellipsoid. Let us assume that we are given a set of points lying on the cell surface that are derived from microscopic images.

It is obvious from the above that the minimum set of points to define the ellipsoid is 10. However one will normally need a lot more in order to obtain a good reconstruction. This two dimensional method allows a reconstruction with any given accuracy but also allows to remove the noise in the data as illustrated in the results section. However, there are limitations to its application, it is effective when a "mild" level of deformation is applied to the ellipsoid, Meaning that the cell need to be a pseudo-ellipsoid – a main cross section rotated about one of the main inertia axes.

2.2 Direct three dimensional reconstruction

The equation of the ellipsoid in this coordinate system is

$$E_0 : A_1x_1^2 + A_2x_2^2 + A_3x_3^2 + A_4x_1x_2 + A_5x_1x_3 + A_6x_2x_3 + A_7x_1 + A_8x_2 + A_9x_3 + A_{10} = 0 \tag{8}$$

This shape is used for the initial approximation of the cell shape. Then the next steps allow through exponential functions with a compact carrier to reconstruct the entire ellipsoid

$$E_1 : \quad E_0 + A_{11}e^{(x-x_0)^2+(y-y_0)^2+(z-z_0)^2} = 0 \tag{9}$$

$$E_{i+1} : \quad E_i + A_{10+i}e^{(x-x_0)^2+(y-y_0)^2+(z-z_0)^2} = 0 \tag{10}$$

This procedure combined with minimisation of the quadratic Euclidean error in the 3D space allows a very accurate reconstruction and furthermore analysis of physically admissible state.

3 Results and discussion

The results presented below are based on confocal images of cell for the two dimensional reconstruction. As can be seen the data is quite noisy that makes the "edge detection" very cumbersome. We approximated the image using the theta apparatus and the the error is illustrated in figure. 2. The reconstruction with a single ellipse yielded an accuracy of over 99% indicating clearly – that this is a sufficiently accurate approximation. In order to obtain all the points bar one, 108 "harmonics" were needed. This shows quite clearly that the microscopic images are processed although raw data are claimed. Almost identical situation occurred for the reconstruction of a "deformed" cell in fig. 3b (researcher reported and analysed the deformed shape in published. Figure 2a represents the cell prior to mechanical loading being applied. The entire construct was subjected to prolonged shear strain (10%). The biologists believed that the cells from the culture deformed but after the reconstruction that the sample cells remained 99.9% identical after deformation of 5% and 10%. However, some chemical response was detected. The example illustrates how reference state based on the reconstruction functions could be established.

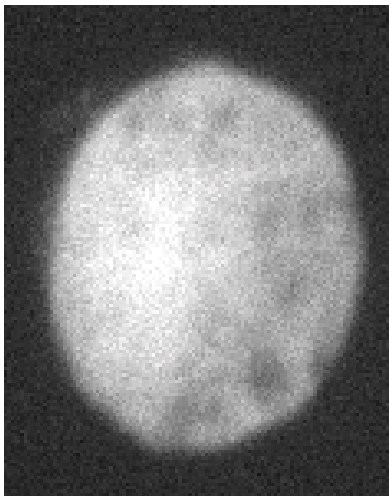


Figure 1. Confocal cell image.

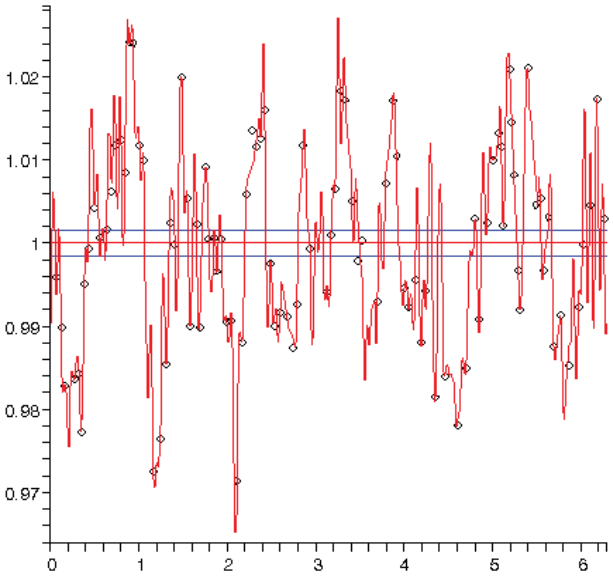


Figure 2.Eror in reconstruction using a single ellipse.

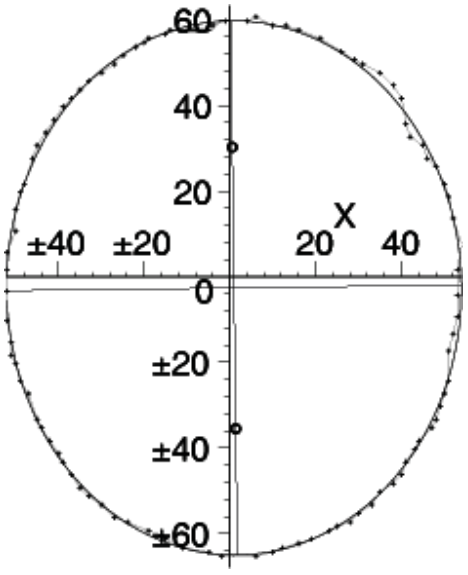
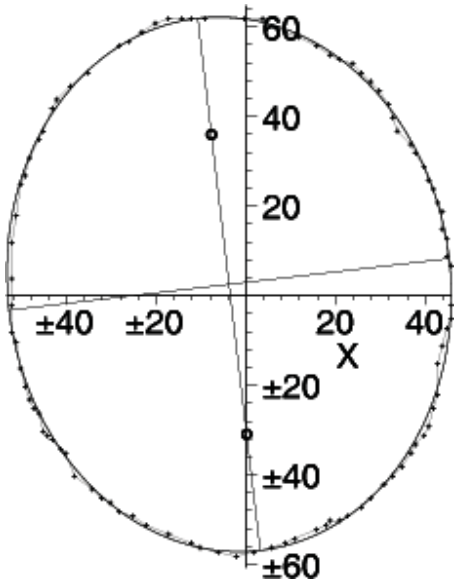


Figure 3 a. Approximation of load free cell and



3b. Approximation of a deformed cell

Figure 4 below represents the deformed shape of the cell based on data from scaled up and mechanically deformed cell model. The reason for using a model rather than microscopic data was that 3D data are not properly scaled and the images represent graphical visualisation rather than proper 3D reconstruction.

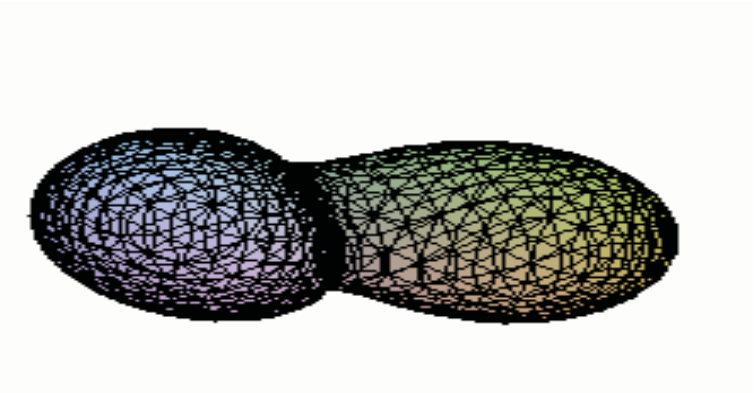


Figure 4. Deformed cell shape obtained through the 3D algorithm

The 3D algorithm allows reconstruction and again based on the functions used to asses identical states in terms of same volume, surface, Gaussian curvature and other geometric invariants. However, there is one important difference to the 2D one. While in the 2D case indefinite number of elliptic functions could be added without compromising the closed contour, this is not the case in 3D. If the degree of approximation is exceeded the reconstruction could result in a non-closed shape as the one shown in figure 5.

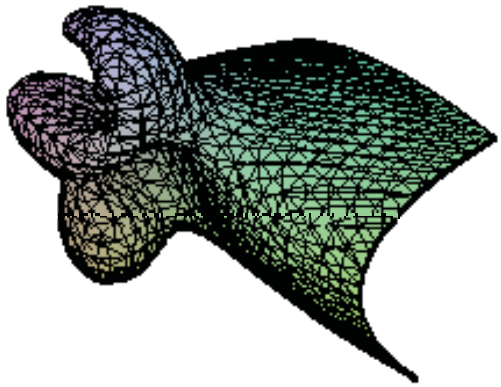


Figure 5. Non-connected 3 dimensional shape as a result of excessive approximation.

Based on the experience of cell reconstructions the following observations were made

- Proper three dimensional images reconstructed to a scale cannot be obtained at the moment to the best of our knowledge. We approached the British Microscopy Society and they could not provide us with such.
- Hence all modelling work is conducted on simple curves such as circles and spheres hence never validated.
- Robust methods for establishing reference state and subsequently constitutive equations are not developed yet and rigorous mathematical based definition is needed.

4 Conclusion

The developed method was tested on specially designed experiments where mechanically instrumented cells were artificially deformed and their state digitised. The method successfully reconstructed the deformed cells beyond biologically admissible level. The proposed technique allows identifying a family of equivalent shapes and hence establishing a reference state which is not a mathematical but biological problem. The achieved accuracy is less than 2% of the greatest radius. Unfortunately to date the method could not be properly tested on microscopic images as they are processed and approximated by ellipsoids as reported in earlier works

Outstanding theoretical issues that the proposed technique is applicable to include

- mass exchange between red blood cells and endothelial cells [6]
- analytical method for establishment the homeostatic shape of cells undergoing mechanical loads [1,2,9]
- non-isochoric interactions of the endothelial cell and capillaries.

We believe that the proposed method offers the potential for rigorous analysis of mechanical deformation of cells as it allows to differentiate the changes at global level and this way assess the real cell response rather than noise in the data. Furthermore it allows to recognise that cells are dynamic systems and they do not have a fixed reference shape.

5 References

[1] Barbee, K.A., Mundel, T., Lal, R. & Davies, P.F. *Subcellular-distribution of shear-stress at the surface of flow-aligned and nonaligned endothelial monolayers.* Am. J. Physiol. Heart Circ. Physiol. 37 (1995), H1765–H1772.

[2] Caille, N.; Thoumine O.; Tardy and Meister, J.J.: C*ontribution of the nucleus to the mechanical properties of the endothelial cells.* Journal of Biomechanics, 35 (2002), 177-187.

[3] Dabnichki P.: *Mathematical and computational model of global cell mechanical responses*. Recent Research Developments in Biomechanics, 3 (2006), 1-13

[4] Dabnichki, P. and Zhivkov A.: *Analytical Approach to cell geometry reconstruction*. Computer, Materials and Continua, 2 (2005), 97-104.

[5] Davies, P.F. 1995 *Flow-mediated endothelial mechanotransduction.* Physiological Reviews, 75, 519–560.

[6] Dong, C. and Lei, X.X.: *Biomechanics of cell rolling: shear flow, cell surface adhesion and cell deformability.* Journal of Biomechanics (2000), 33, pp. 45-51.

[7] Guilack, F. Compression-induced changes in the shape and volume of the chondrocyte nucleus. *Journal of Biomechanics*, 28 (1995), 1529-1541.

[8] Huang, H.D., Kamm, R.D. & Lee, R.T. *Cell mechanics and mechanotransduction: pathways, probes, and physiology.* Am. J. Physiol. Cell Physiol. 287(2004), C1–C11

[9] Kaspar, D.; Seidl, W.; Neidlinger-Wilke, C.; Ignatius, A. and Claes, L. Alterations in the Young *Modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage.* Journal of Biomechanics, 33 (2000), 45-51.

[10] Voorhees A., Nackman G.B. and Wei T. *Experiments show importance of flow-induced pressure on endothelial cell shape and alignment*, Proceedings of the Royal Society A, 463 (2007), 1409-1419