Journal of Biomechanics 47 (2014) 1157–1163

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Vision-based force measurement using neural networks for biological cell microinjection

Fatemeh Karimirad [a,*], Sunita Chauhan [a], Bijan Shirinzadeh [b]

[a] Department of Mechanical and Aerospace Engineering, Monash University, Building 31, VIC 3800, Australia
[b] Department of Mechanical and Aerospace Engineering, Robotics and Mechatronics Research Laboratory (RMRL), Monash University, VIC 3800, Australia

ARTICLE INFO

Article history:
Accepted 6 December 2013

Keywords:
Micro-injection
Machine vision
Vision-based force measurement
Biomechanics modelling

ABSTRACT

This paper presents a vision-based force measurement method using an artificial neural network model. The proposed model is used for measuring the applied load to a spherical biological cell during micromanipulation process. The devised vision-based method is most useful when force measurement capability is required, but it is very challenging or even infeasible to use a force sensor. Artificial neural networks in conjunction with image processing techniques have been used to estimate the applied load to a cell. A bio-micromanipulation system capable of force measurement has also been established in order to collect the training data required for the proposed neural network model. The geometric characterization of zebrafish embryos membranes has been performed during the penetration of the micropipette prior to piercing. The geometric features are extracted from images using image processing techniques. These features have been used to describe the shape and quantify the deformation of the cell at different indentation depths. The neural network is trained by taking the visual data as the input and the measured corresponding force as the output. Once the neural network is trained with sufficient number of data, it can be used as a precise sensor in bio-micromanipulation setups. However, the proposed neural network model is applicable for indentation of any other spherical elastic object. The results demonstrate the capability of the proposed method. The outcomes of this study could be useful for measuring force in biological cell micromanipulation processes such as injection of the mouse oocyte/embryo.

1. Introduction

Biological cells are stimulated through micromanipulation processes, and they respond accordingly by changing geometrically (Lu et al., 2009; Diaz et al., 2010). A biological cell senses forces of the order of μN–mN through injection process (Sun et al., 2003; Kim et al., 2004a; Xie et al., 2010). It has been reported that simultaneous vision and force feedbacks have a higher degree of success in injecting the desired material into the cell in comparison with that of the vision feedback alone (Pillarisetti et al., 2007). Excessive force may significantly damage the cell and directly influence the success rate of microinjection. Force sensing capability helps by supplying the haptic device with force signal; consequently the operator feels once the cell membrane is ruptured. As a result, force feedback enables minimally invasive injection which reduces physical damage to the cell. Moreover, in some particular cases such as individual cell based diagnosis or pharmaceutical test, acquiring force information is the main achievement of the application (Kim et al., 2005; Kawakami et al., 2011). For example, the thickness of zebrafih embryos have been studied at different embryonic stages (Kim et al., 2005; Kawakami et al., 2011). Characterization of this mechanical property of zebrafish embryos was conducted by measuring the required force to penetrate the egg envelope at each developmental stage. Thus, force feedback can also provide a basis to characterize the mechanical properties of the cell and model the behavior of the biomembrane. However, relatively few research efforts have addressed measurement and control of the applied force in the cell micromanipulation processes (Sun et al., 2003; Kim et al., 2004b; Murayama et al., 2004; Pillarisetti et al., 2007; Lu et al., 2009; Xie et al., 2010). Thus far, the realization of force feedback in biological cell micromanipulation systems has been achieved by integrating micro force sensors into micromanipulation setups. There are mainly four types of micro force sensors used. These include piezoresistive, piezoelectric, capacitive, and optical sensor (Lu et al., 2006). PVDF (polyvinylidene fluoride) film as a piezoelectric sensor has been used to fabricate the most widely used piezoelectric force sensors for measuring the cell injection forces (Kim et al., 2004a; Murayama et al., 2004; Nelson et al., 2005; Pillarisetti et al., 2006; Wejinya et al., 2006; Xie et al., 2010). PVDF has excellent sensitivity, high compliance and high signal to noise

* Corresponding author. Tel.: +61 3 9905 3545; fax: +61 3 9905 1825.
E-mail address: Fatemeh.Karimirad@monash.edu (F. Karimirad).

0021-9290/$ - see front matter
http://dx.doi.org/10.1016/j.jbiomech.2013.12.007

ratio (Incorporated, 1993; Fung et al., 2001, 2002). However, it is acoustically sensitive and also susceptible to changes in temperature, implying that the experiments must be undertaken in a constant temperature environment. Piezoresistive sensors, also called strain sensors or strain gauges, have the property that their resistance varies when pressure is applied. It has been reported that the strain gauge is less sensitive than the piezo film, thus it cannot display the true force in the μN–mN range (Fukuda and Arai, 2000; Lin et al., 2001; Cho and Shim, 2004). Moreover, their resistance and the gauge factor change with temperature. On the other hand, there have been a series of high sensitivity piezoresistive sensors which have been modified for use in the micromanipulation systems (Lu et al., 2007). The main limitation with their integration is that the micropipette is directly bonded to the sensor. Thus, it is not applicable in the microinjection setup where the micropipette is connected to the injector via a pipette holder. The technique of optical beam deflection is of excellent potential for its high resolution (down to nN) (Nelson et al., 1998) and the electromagnetic immunity. Moreover, optical sensor is an effective method for non-contact force measurement. One of the important applications of this technique is in the atomic force microscopy (AFM). However, the cantilever-based optical sensor measuring forces in the range of nN (Zhang et al., 2004), requires a complex transmitter-receiver setup and the photodiode that can detect only a small range of deflection. Furthermore, the force measurement is inaccurate due to reflection and refraction of the transmitted light through aqueous medium where the biological cells survive. In capacitive force sensors, the capacitance between two metal plates changes due to the applied force. Compared to strain gauges and piezoelectric force sensors, capacitive force sensors are more stable and sensitive, and exhibit no hysteresis, but they have a limited measurement range (Fahlbusch and Fatikow, 1998). Sun et al. developed a two-axis capacitive sensor for sensing the force applied to the mouse oocyte (Sun et al., 2003). However, a motion constraint in displacement of the pipette reveals in cell micromanipulation applications where a pipette is usually attached to the probe tip of the sensor. An alternative for force measurement in biological cell micromanipulation is a vision-based approach, referred to as vision-based force measurement (VBFM) (Greminger and Nelson, 2003). This technique estimates the applied force using visual data. It has been shown that having an accurate model of an elastic object, the applied force to the object can be measured by computer vision (Wang et al., 2001; Greminger et al., 2002). However, there are some cases in which having such a model is infeasible including for materials of nonlinear elastic properties, materials without available model, and finally objects with available model but with unknown parameters to define the model. An artificial neural network model was presented for such elastic objects to avoid the limitations of the aforementioned methods (Greminger and Nelson, 2003). Their neural network elastic model inputs a point (x, y) from within the object and the load F applied to the object, and returns the deformed location (x_0, y_0) of the input point. Unlike the previous work, we use neural networks to accurately model a precision loadcell for measuring the applied load based on the geometric features of the injecting cell. The desired geometric features including deformation, orientation and size of the cell are extracted from a series of images using well-established image processing algorithms. The images are acquired under various known loading conditions measured by a microrobotic force sensing system. After training the neural network with adequate data, the neural network can be used as a precision loadcell to estimate force for new images. This paper is organized as follows. Section 2 presents the architecture of the proposed neural network. Section 3 describes the method of tracking and characterizing cell deformation, introduces a microrobotic biomanipulation system with force measurement capability used for collecting the training data and explains the training algorithm. Section 4 provides the experiment design including the experimental setup and the results, and finally Section 5 draws the conclusion.

2. Neural network design

Sending many inputs causes the neural networks perform inefficiently. Thus, it is desirable to feed the proposed VBFM neural network with the necessary data that optimally describe the deformation of the cell through the injection process. To achieve this end, discrete cosine transform (DCT) of the image has been used for the application of face recognition (Pan et al., 2000) and VBFM for a cantilever beam (Greminger, 2002). The displacement of the points within an elastic body also has been used for this purpose (Greminger and Nelson, 2003). Investigations have been conducted to select the most informative feature(s) describing a circular biological cell deformation under the load of injection process. These data have been used to propose a theoretical model to study and characterize the mechanical properties of biological cells, and to find the relationship between the deformation of a cell and the associated force (Sun et al., 2003; Tan et al., 2008; Lu et al., 2009). A method has been proposed in our previous study to describe the geometry of a circular cell at different indentation depths (Karimirad et al., 2013). Using this methodology, deformation of a spherical cell is quantified using a parameter referred to as the dimple angle. The dimple angle is the angle within the dimple which is created when a micropipette exerts a uniaxial indentation force to a circular embryo. An example of a dimple angle is illustrated in Fig. 1. Assuming that an embryo is a perfect circle, the proposed parameter truly satisfies the requirement for characterization of the deformation of an embryo. However, embryos are often elliptic. An ellipse can be represented by three parameters as illustrated in Fig. 2: the major radius, the minor radius and the orientation angle. It has been observed that the range of average radius of zebrafish embryos is very small (Karimirad et al., 2013). Thus, the direct inclusion of the radii is not justifiable. Instead, the proportion of the minor radius to the major radius of an embryo can be considered as a parameter describing the elliptical shape of an un-deformed embryo. Area of the embryo is also fed to the neural network as one of the inputs

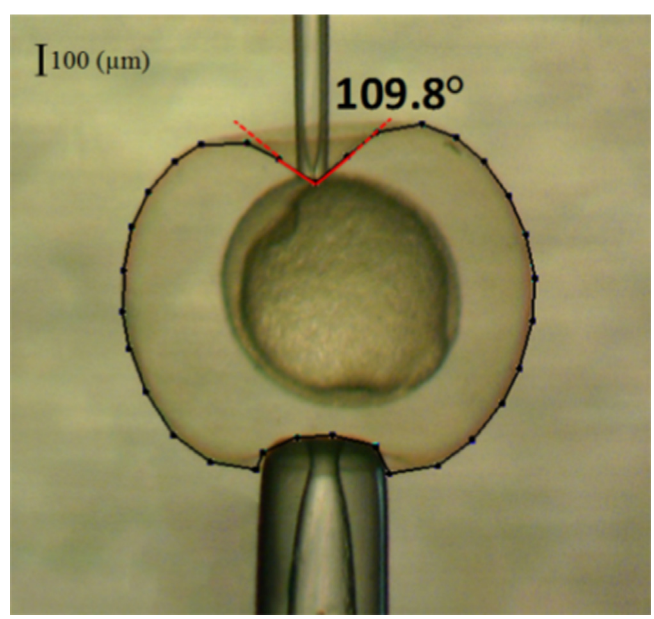

Fig. 1. An example illustrating the dimple angle within a deformed cell (Karimirad et al., 2013).