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A novel method for analyzing images of live nerve cells

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ABSTRACT

Analysis of images from live-cell experiments is a central activity to studying the effects of stimulation on neuronal behavior. Image analysis techniques currently used to study these effects rely for the most part on the salience of the neuronal structures within the image. In both fluorescent and electron microscopy, neuronal structures are enhanced and therefore easy to distinguish in an image. Unlike images obtained via fluorescent or electron microscopy, however, images produced via transmission microscopy (e.g., bright field, phase contrast, DIC) are significantly more difficult to analyze because there is little contrast between the object-of-interest and the image background. This difficulty is amplified when a time-dependent sequence of images are to be analyzed, because of the corresponding large data sets. To address this problem, we introduce a novel approach to the analysis of images of live cells captured via transmission microscopy that takes advantage of commercially available software and the Fourier transform. Specifically, our approach utilizes several morphological functions in MATLAB to enhance the contrast of the cells with respect to the background, which is followed by 2-D Fourier analysis to generate a spectrum from which the orientation and alignment of cells and their processes can be measured. We show that this method can be used to simplify the interpretation of complex structure in images of live neurons obtained via transmission microscopy and consequently, discover trends in neurite development following different types of stimulation. This approach provides a consistent and reliable tool for analyzing changes in cell structure that occurs during live-cell experiments.

1. Introduction

Scientists and engineers have stimulated neurons using chemical (Hammarback et al., 1988; Snow and Letourneau, 1992; Clark et al., 1993; Song et al., 2006; Kim et al., 2010), electrical (Jaffe and Poo, 1979; Patel and Poo, 1982; Borgens et al., 1994; Erskine and McCaig, 1997; Schmidt et al., 1997) topographical (Rajnicek et al., 1997; Bruder et al., 2007; Gomez et al., 2007), and mechanical (Georges et al., 2006) cues to trigger nerve regeneration in the nervous system. Image analysis has been the central tool used to study changes in the movement or development of neurons following these various forms of stimulation. In most studies of neuronal development, images of fixed cells (e.g., fluorescent images, electron microscopy images) are analyzed. The analysis of images of fixed neurons, however, presents several challenges. For example, in cases where electrical stimulation was used, it was found that neurites lose their directionality only a few hours after the electric field (E-field) was removed (Patel and Poo, 1982; Erskine and McCaig, 1997). In a second example, the process of fixation and immunostaining increases the probability that neurites will be damaged or lost prior to imaging (Debbage et al., 1980). Based on these challenges alone, the development of a method for analyzing a large data set of images obtained from live-cell experiments is critical to gaining a deeper understanding of neuronal development in the presence of external stimulation.

While several studies have introduced methods for analyzing images of living neurons, these methods have been limited to the analysis of a single neurite or neurites with simple structures (Patel and Poo, 1982; Borgens et al., 1994; Diefenbach et al., 2000) and thus provide little help when dealing with images of neurons with complex structures such as neurite branching or multiple connections between adjacent neurons. Extracting the lengths and angles of neurites in images of branched or highly interconnected neurons is a difficult task typically done by hand, which can introduce significant errors that lead to ambiguous or contradictory results.

The aim of this study was to develop an automated method that gives reliable and consistent results from the analysis of images of live neurons produced via transmission microscopy. In order to enhance the contrast in these types of images, several morphological operations were applied to the original images in MATLAB.

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Subsequently, discrete Fourier transform (DFT) was used to detect any change in neurite location or length following stimulation. DFT is computed using the fast Fourier transform (FFT) algorithm in MATLAB and so from here on the term FFT will be used to mean the DFT calculation. FFT has been tried in previous studies for enhanced image analysis (Wu et al., 1997; Alexander et al., 2006; Corey et al., 2007; Ayres et al., 2008). Among these studies, FFT analysis has been used almost exclusively on images of immunostained cells (Alexander et al., 2006; Corey et al., 2007). In contrast, this study uses FFT to extract changes in cell geometry (e.g., rate of neurite elongation) and direction (e.g., guidance) from transmission microscopy images collected during live-cell experiments of neurons. By simplifying the analysis of images of live cells, the challenges imposed by images of fixed cells can be avoided.

2. Materials and methods

2.1. Stamping of laminin (LN) stripes onto glass cover slips (chemically guided neurons)

A PDMS stamp was fabricated by a previously described method (Kumar and Whitesides, 1993). The procedure for stamping LN stripes onto a glass slide is shown in Fig. 1 and has been reported elsewhere (Bernard et al., 1998; Bruder et al., 2007). Briefly, a PDMS stamp was soaked in Hank's balanced salt solution (HBSS) containing 50 μg/ml of natural mouse laminin (Invitrogen) for 30 min. Subsequently, the stamp was dried under sterile nitrogen gas and then placed on a plasma-activated glass cover slip for 10 min. The stamp was removed from the glass and the LN patterns were allowed to cure for 10 min in a sterile environment. The LN-patterned glass was rinse with PBS three times prior to cell seeding.

2.2. Fabrication of a platform for electrically stimulating neurons

Based on previous studies (Poo et al., 1978; Borgens et al., 1994; McCaig et al., 2005), a special platform for applying electrical stimulation to neurons was designed with two parallel chambers to allow for the simultaneous study of neurons with and without electrical stimulation. The sides of the chambers were molded out of poly(dimethyl siloxane) (PDMS) whereas the floors of the chambers were glass cover slips. The glass cover slips were subjected to plasma etching prior to attachment to the PDMS to ensure a good seal. Small wells distal to the chambers were included in the platform to prevent contamination of the cell culture by possible chemistry at the electrodes. Platinum wire electrodes were placed in the wells associated with the chamber to be electrically stimulated (Fig. 2a). Salt bridges between wells and chambers were made of U-shaped glass tubes (diameter: 5 mm) and filled with a 2% agar gel soaked with Dulbecco's modified eagle's medium (DMEM). Glass cover slips that functioned as the floors of the chambers were prepared as follows: (1) 30 s plasma etch, (2) rinsing with a mixture of HCl and methanol (1:1 (v/v)) to increase the number of reactive SiOH groups (Cras et al., 1999), (3) coating with LN (50 μg/mL in Hank's balanced salt solution (HBSS)), (4) agitated on a shake table at 30 rpm for 1 h at room temperature, and (5) rinsing with DI-H_2O prior to cell plating.

To prevent evaporation and contamination of the media, a transparent culture foil was affixed to the PDMS mold with aluminum

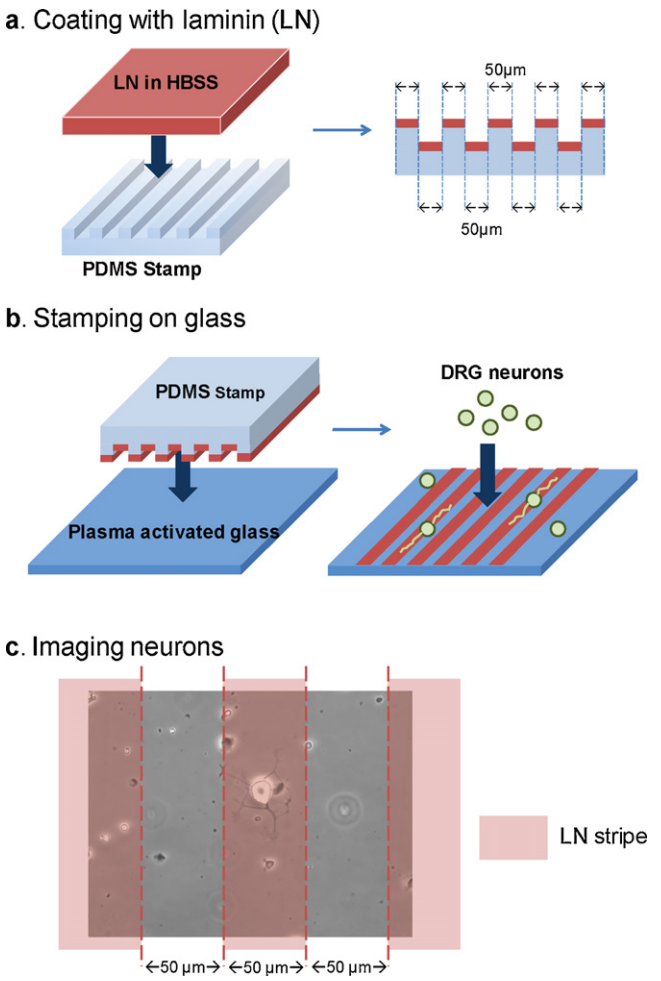

Fig. 1. Procedure for micro-stamping LN stripes onto a glass slide to chemically guide DRG neurons.

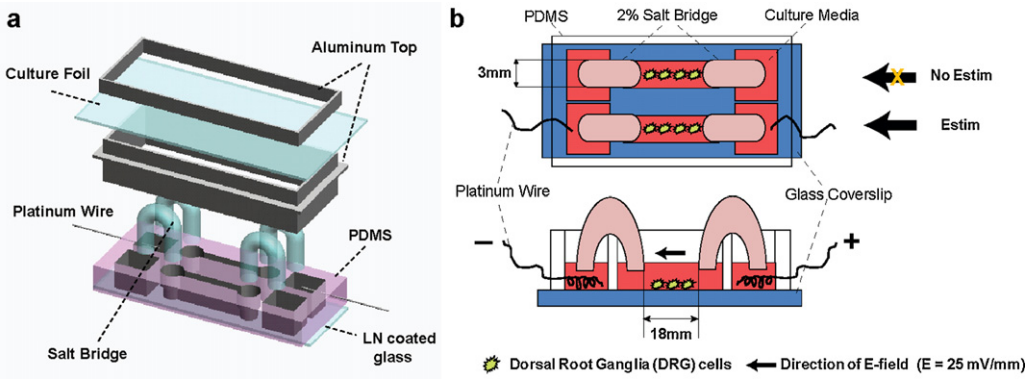

Fig. 2. Device used for electrically stimulating cultured neurons. (a) Schematic of the device including culture foil to protect cells. (b) Neurons were stimulated electrically by application of an electric field (25 mV/mm) through the experimental chamber for 10 min.