Biomedical Shape Measurement for Neurotoxicity Testing

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Abstract
In this paper, we present the results of the new image-based morphometric approach used to estimate toxic damage to the ganglia in alternative in vitro test. The main part of this research is devoted to multiscale and multiresolution approaches of biomedical planar shape description using continuous and discrete wavelet transform.

Keywords: neurotoxicity, shape similarity, wavelet transform

1 Introduction

Neurotoxicity is one of the most serious toxicological events. Some years ago, we developed a sensitive method to study the neurotoxic effects of chemicals in vitro - organotypic cultures of chick embryonic dorsal root ganglia (DRGs) maintained in a semi-solid culture medium [3]. The changes of morphological characteristics and growth parameters of neurites growing out from cultured dorsal root ganglia are used for evaluation of the reaction of ganglia to toxic agents. The described method was introduced as a promising new toxicity test that can be used as an alternative to tests carried out on laboratory animals [2].

Alternative in-vitro testing methods are very prospective, but reliable quantitative evaluation methods are necessary for their broader acceptance and use [5]. Today, visual and semi-quantitative evaluation are the most used methods, but they suffer from the lack of reproducibility and reliability. The well-known methods for image-based evaluation of the cells offer tools for description of blob-like objects. They are unusable for characterization of organotypic cultures of dorsal root ganglia.

In this paper, we present the first results of the new image-based morphometric approach used to estimate toxic damage to the ganglia in our alternative in vitro test. Porphyrine derivatives already tested in our laboratory [1] were used as the model toxic substances in this study.

2 Subject and Methods

The evaluated scene consists of the background (represented by culture medium), the ganglion and the region of neurites growing out from the ganglion (see Fig. 1). The cultivation medium as an image background is non-homogeneous and often includes the artifacts of a size up to one half of ganglion diameter. Due to the large contrast variation in the area of neurite outgrowth, ranges of pixel values of background and neurites often overlap. From a local view (3x3-pixel neighborhood) one is unable to determine whether an examined pixel belongs to the image background or to neurites. As the background is formed after the embedding of ganglia, it is impossible to use the subtraction for background removal.

Our automatic segmentation method tries to avoid the reduction of information content before the last stage of preprocessing [4]. To achieve that, we use the gray-scale and binary image morphology extensively, especially for neurite removal and extraction. Due to high ratio between neurite width and ganglion diameter, we cope with low signal-to-noise ratio, low contrast and near critically sampled images.

The first developed method is based on radial length distribution. Radial length is computed as a difference between inner and outer contour distances (Fig. 1). The contour distances are measured from the centroid of the ganglion in range of 0-360 degrees. For subjective evaluation of ganglion growth, the radial length distribution can be visualized by histogram (see Fig. 1). More detailed information offers the statistical description using the mean, the standard deviation and the skewness.

The mean radial length characterizes the overall growth of neurites. Mean radial length generally decreases with increasing toxic effect. As a global descriptor gives no information about growth abnormalities. The standard deviation specifies the non-uniformity of neurite lengths, for the comparison should be normalized using the mean radial length.

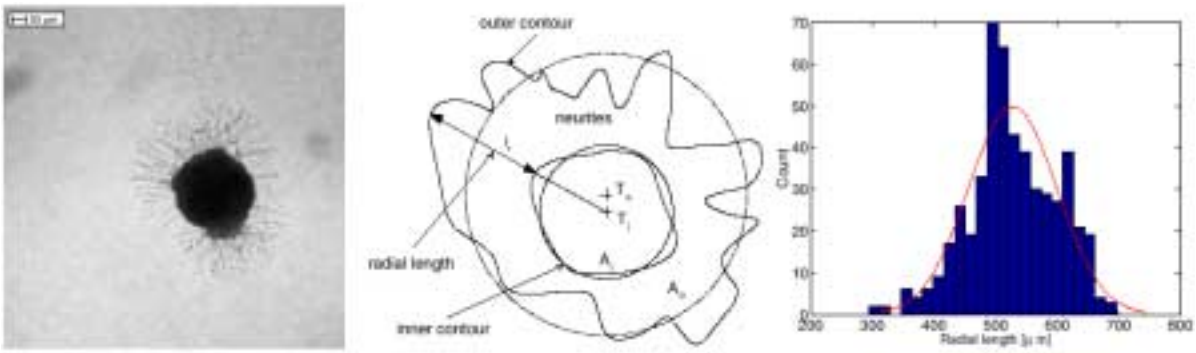
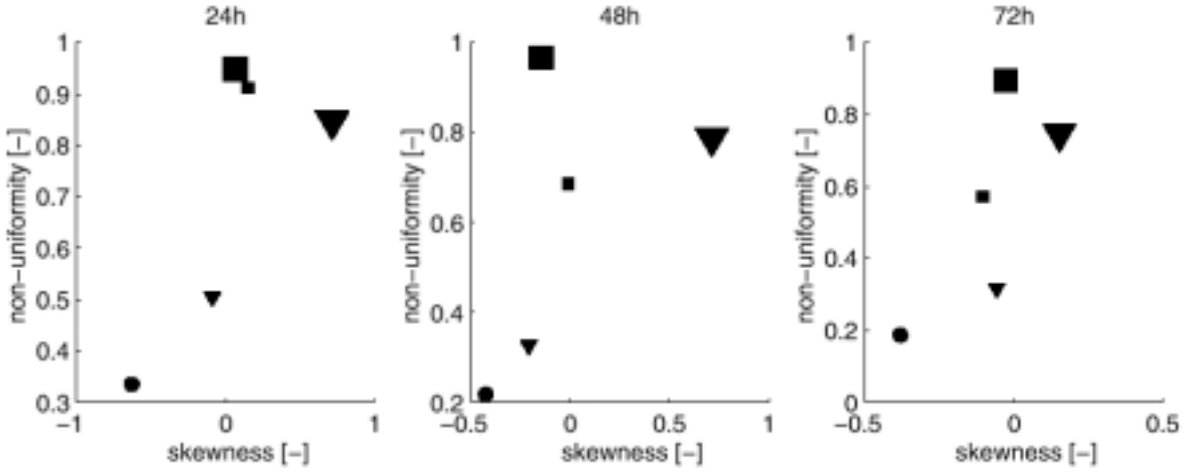


Figure 1: Image of ganglion (left), radial length as a difference between inner and outer contour distances (middle), typical histogram of radial length distribution (right)



circle . . . control, triangle . . Photosan, square . . . $TPPS_4$,
small symbol . . . 60μg/ml, large symbol . . . 120μg/ml

Figure 2: Scatter diagram of skewness and non-uniformity parameters

Skewness is a measure of the asymmetry of the data (the radial length in our case) around the sample mean. If skewness is negative, the data are spread out more to the left of the mean than to the right. As the toxic effect increases, neurites growth slows down. However, in certain cases there are neurites that grow abnormally and cause changes in asymmetry of the radial length distribution.

The main part of this project is devoted to multiscale and multiresolution approaches of planar shape description. Wavelet transforms, both continuous (CWT) and discrete (DWT), were used for searching of shape dissimilarities between reference and damaged ganglia. Dominant points of the shape are detected using local maxima lines in the coefficients of CWT.

$$CDc(\tau, s) = \sum_{k=1}^{N} \int_{k}^{k+1} c(k) \frac{1}{\sqrt{s}} \psi \left(\frac{t-\tau}{s} \right) dt \tag{1}$$

where *CDc* is the wavelet map of measured contour *c*, *N* the length of *c*, *s* the scale and τ the position. The endpoints of the maxima lines serve as a representation for shape comparison. Shape similarity is evaluated using a sum of Euclidean distances between the appropriate points of two shape representations.

DWT descriptor uses the multiresolution decomposition for non-redundant shape representation. Weighted distance between two DWT descriptors represents the shape similarity criterion d_{DWT}.

$$d_{DWT} = \sum_{n=1}^{N} 2^{-P+p} \, |DDc1(n) - DDc2(n)|, \tag{2}$$

Thresholded maxima in the autocorrelation scale-space carry the information about scale and period of the periodic components.

$$WR_{xx}(t, s) = \frac{1}{N} \sum_{n=0}^{N-1} Wf(n \bmod N, s) \, Wf((n+t) \bmod N, s) \tag{3}$$

3 Results

The results of the first method are demonstrated in Figure 2. In this experiment different concentrations of model toxic chemicals (porphyrins) were used. Neurites growing out from ganglia in control cultures (without porphyrins) were radially arranged around the perimeter of the ganglia. Toxic effect of both chemicals was expressed by the reduction of the number of neurites growing out from the ganglia as well as by shortening of the length of neurites. General shortening of the length of neurites results in lower mean radial length.

Detailed analysis showed also the differences in non-uniformity of neurite outgrowth, which was prominent in heavily damaged ganglia. Higher values of skewness reflect the irregularity of the length of the neurites in damaged ganglia. Skewness smaller then -0.3 indicates the normal growth, majority of neurites grows uniformly, few neurites are longer. Damaged ganglia are characterized by values of the skewness larger then -0.3 (increasing number of abnormally long neurites). The mean radial length and index of areas are very close to results obtained in the previous manually evaluated study [1].

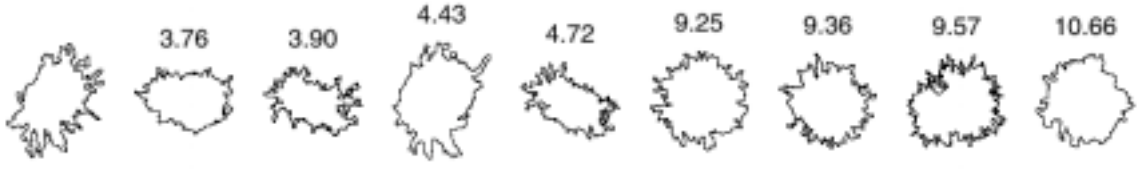


Figure 3: Shape similarity

Characterization of contour similarity is demonstrated in Fig. 3.

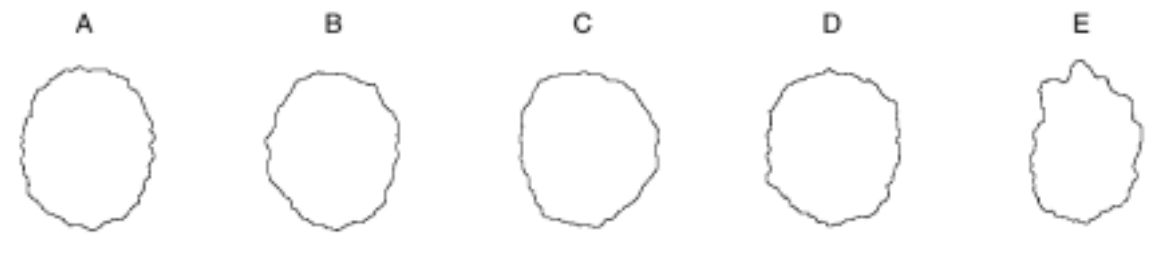


Figure 4: Averaged contours (A. . . control, B,C. . . Photosan D,E . . . $TPPS_4$, 60µg/ml, 120µg/ml)

Shape averaging using discrete wavelet transform served for obtaining the model shape of ganglia classes for future classification (Fig. 4).

4 Discussion

Dorsal root ganglia cultured in-vitro represent a highly sensitive system that can be used for neurotoxicity testing [3]. Changes in morphology and growth parameters of neurites growing out from dorsal root ganglia in our model serve for the quantification of toxic effect of tested chemicals. The described approach enables not only more precise evaluation of the intensity of toxic damage to the ganglia, but also can contribute to the elucidation of the toxic action of the tested chemical agent. The main part of this research was devoted to multiscale and multiresolution approaches of planar shape description. Wavelet transforms, both continuous (CWT) and discrete (DWT), were used for searching of shape dissimilarities between reference and damaged ganglia.

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