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Dynamical speckles patterns of action potential transmission effects in squid giant axon membrane.

A tribute 50 years later to the memory of the Hodgkin and Huxley Nobel Prize, 1, 2013, Portugal.

Dynamical speckles patterns of action potential transmission effects in squid giant axon membrane. A tribute 50 years later to the memory of the Hodgkin and Huxley Nobel Prize.

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ABSTRACT

Undoubtedly the most important result of the investigations in physiology and biophysics was the discovery of the electrochemical mechanism of propagation of the action potential in nerves that was made by Hodgkin and Huxley during the first half of the past century. Since some decades ago diverse experiments about the electro optical properties of the axon membrane there was published using the most diverse optical experimental procedures[6-10]. In this paper some results of a dynamical speckle technique applied for obtaining microscopic images of a section of a squid giant axon membrane during the activation by electrical impulses and his digital process are presented.

Keywords: Speckle patterns, squid giant axon, H-H theory, action potential, membrane potential, sodium channels.

1. INTRODUCTION

One of the most important results of the investigations in physiology and biophysics was the discovery of the electrochemical mechanism of propagation of the action potential in nerves made by Hodgkin and Huxley during the first half of the past century using the giant axon of squid[1-5].

Since that moment many investigations were developed in order to reveal the specific mechanism of transmission of the action potential because this phenomenon is in the base of the nervous transmission in all de life organism and the success of an optical relation between the birefringence properties of the phospholipid than conform the giant squid axon and the action potential transmitted was a very interesting success[6-10].

The use of speckle patterns techniques has been little reported[11] however this technique complemented with digital image process is a good alternative to study some effects of the action potential in the optical properties of the giant squid axon.

In this paper results of recorded and processed images of the speckle patterns of the giant squid axon activated using electrical impulses are presented.

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2. METHODOLOGY

2.1. Experimental design:

In order to observe the giant axon of squid, the speckle pattern technique was implemented. When the laser light was reflected or transmitted in a non-homogeneous surface a spatial and granular pattern of interference is formed, this is the speckle pattern[12]. The experimental system was used as the one that is shown in the figure 1.

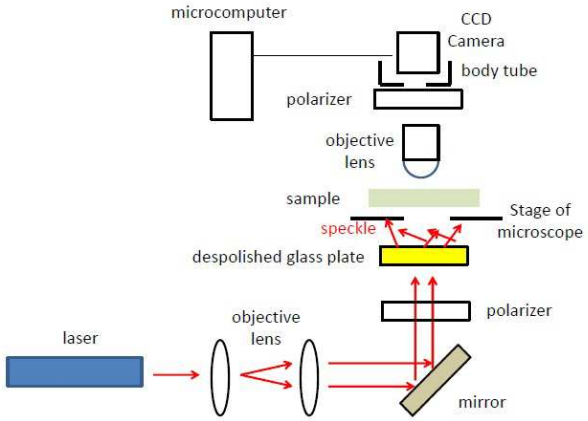

Fig 1. Experimental setup scheme

A He-Ne laser 632 nm wavelength and 6,0 mW was used. The laser light was expanded by lens and deviated by a mirror. Then part pass through a polarizer plate and scattered by an unpolished glass plate used to form a speckle pattern that illuminate the sample mounted over the stage of an optical microscope (OPTICOM, XSZ-107T) with magnification 64 X. The image was captured using a CCD camera with 1027 x 850 pixel and frame rate of 30 fps connected to the microcomputer. The experimental setup was completed with a signals generator and an oscilloscope in order to produce and visualize the signal of square pulses used to activate the axon and to see the signal response in the oscilloscope. See fig. 2 and 3. The polarization direction of the second polarizer, after the objective lens, could be aligned or not to the polarization direction of the first polarizer.

Fig.2. Two views of the experimental setup.

Fig. 3 Giant axon with electrodes in the Petri dish mounted on the plate of the microscope

The giant axon used was extracted by surgery of a big specimen (30 cm long) of squid (*loligo saopauliensis*) and imbibed in a saline solution of Na Cl 300 mM in a Petri dish.

In order to process digitally the obtained images some softwares were specifically developed using MATLAB 7.9 (R2009b) (MatWork Inc. USA) and Imagen J for Windows XP (Professional) operational system. And was used the software Tracker, version 2.54. to process the videos.

2.2. Capture and process of images method.

The axon was stimulated with a signal of square pulse of 75 mV maximal voltage varing the frequency from 20 Hz to 100 Hz.

The process of activation was recorded with the CCD camera that was focused over the surface of the saline solution and the axon interface is visualized. All videos have 500 frames (≈17 s).

Before the capture of the spreading process an image of the reference speckle pattern obtained in the saline solution at rest was captured in each case. This reference pattern was subtracted of each frame of the video. Using our software to modify the format of color RGB to white and black.

To obtain some information about the birefringence properties, the images were registered using crossed polarizers. In this condition no light is transmitted after the second polarizer, unless that the sample changes the polarization direction of the transmitted light. This light allows to visualize the displacement of the electrical impulse along the border of the axon.

Finally each frame was processed using algorithms of the techniques Laser Speckle Contrast Analysis (LASCA) and Laser Speckle Temporal Contrast Analysis (LASTCA)[11,13] to increase the contrast of image in the border of axon.

3. EXPERIMENTAL DATA

The code colors associated to the figures are the blue corresponds to the lower contrasts and the red one to the biggest contrast. The giant axon was excited for different conditions and frequencies, the results are presented in the following.

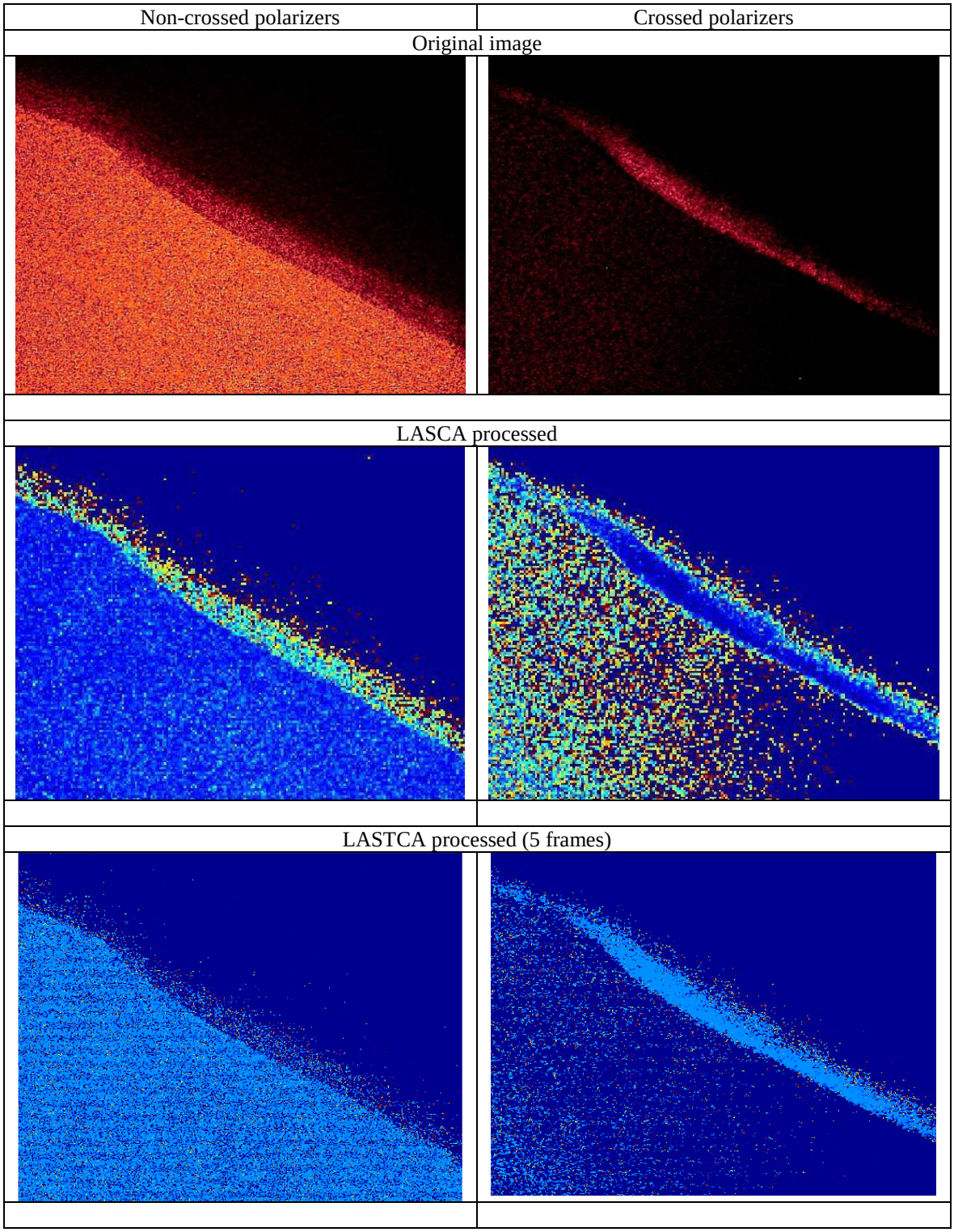

Fig. 4. Comparing images of axon border stimulated at the frequency of 20 Hz

4. RESULTS AND DISCUSSION.

In fig. 4 the original image of the speckle pattern of the border of axon is well defined and the effect of birefringence in this border is observed with crossed polarizers. The image processed by LASCA shows good contrast just in the border and low contrast inside the axon that indicates the electric signal is propagating along the border as in previous investigations[1,3,6] it was demonstrated. On the other hand for the crossed polarizers the border shows the smallest contrast compared with the external region, probably that in the external vicinity occurred a process of the interchange of mass between the axon and the saline solution. In the images processed by the technique LASTCA show the change of contrast when comparing the crossed polarizers case with the other case. In any image the internal side of the axon not reveals an appreciable optic activity

In the images processed by LASTCA the change of contrast is revealed too comparing the non-crossed polarizers image with the crossed polarizers images. In any image the internal side of the axon not reveals an appreciable optic activity.

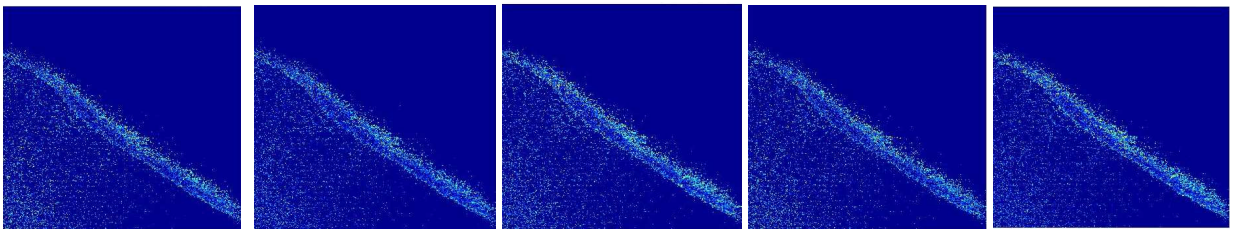

Fig.5. A sequence of images processed by the technique LASTCA of the border of axon activated with a periodic square pulse of 100 Hz of frequency.

In fig. 5 a sequence of images of the axon activated with a periodic square pulse of 100 Hz of frequency captured with crossed polarizers and processed by LASTCA is presented. It is possible to observe a slight quasi-periodical change of the values of the contrast pattern in the center of image. This behavior can have relationship with the rhythm[8,9] with which the action potential is transmitted and it deserves a study of frequency later on.

The figure 6 shows the framework of the software Tracker, at the left is the image and at the rigth at top the graphic of intensity oscilations in function of the time. In this graphic it is possible to visualize the periodical oscillations of values of contrast in the center of the border of the axon at the frequency of 20 Hz.

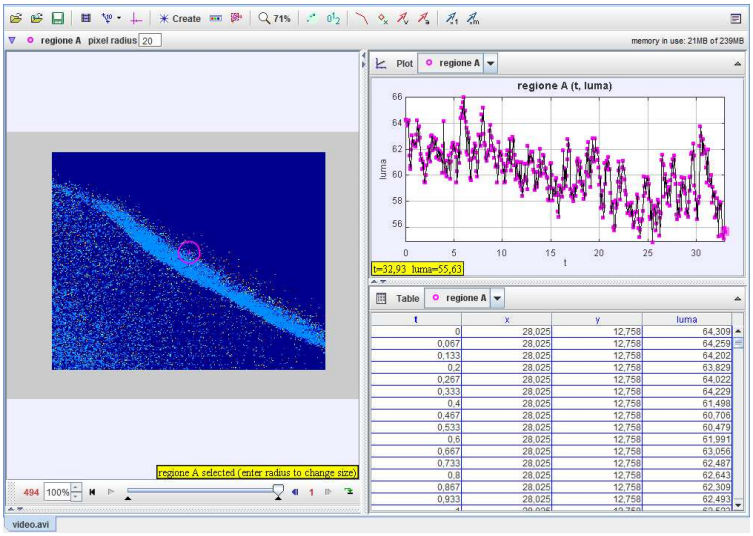

Fig. 6. Quasi periodical oscilations of intensity pattern in the center of the border of axon stimulated with a periodic square pulse of 20 Hz of frequency.

The results of the images processed digitally reveal the possibilities to use speckle methods to study the dynamics of the nervous impulse in the axon.

5. CONCLUSIONS.

Using speckle pattern techniques and digital processing of images is possible to obtain some regularities of the transmission of action potential in the giant axon.

The technique allows visualizing stages and characteristic of the transmission of the action potential process that are not visible using the traditional optic methods with non-coherent light.

The processed images reveal behaviors according with the literature reported.

ACKNOWLEDGEMENT

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