negative gustatory reinforcement. This procedure was repeated 10 times. The learning takes longer than 20 min since the 1 min conditioning does not include the time for transferring the larvae. Transferring the larvae occurred as rapidly as possible (~10 seconds). For the visual preference test, the larvae are recorded every 30 sec as being either on a dark side or on a light side for a total of 5 min. The % of larvae before and after training on the various dark/light locations are then compared for significant difference in learning.

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Iontophoretic dye injections into *Drosophila* cells.

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Abstract

Computer managed iontophoretic device was constructed and successfully applied in regeneration study. General scheme of the device, the method of dye injection, and associated software is discussed.

The Device and Results

Iontophoresis can be used as a method to mark a living cell and its progeny cells with a vital charged dye or to deliver active substances to a cell. Of particular interest are the injections of antisence oligonucleotide probes, giving an opportunity to make a single gene silent (Aramaki *et al.*, 2003). In *Drosophila* research this method was used for marking cells from the wound edges of imaginal discs in regeneration study and to test the cell communications through the newly formed cell contacts (Bryant and Fraser, 1988). The aim of the present communication is to decribe the iontophoretic device according to current technology and to show its use.

As it was mentioned in Bryant and Fraser (1988), the positioning of the iontophoretic capillary within a cell is a critically important factor for successful injection. The correct position can be determined by measuring the membrane potential of the cell. Special scheme was constructed to

register a membrane potential of the cell, as well as to inject a fluorescent dye into this cell. The scheme is presented in Figure 1. The scheme contains the microelectrode, preamplifier, ADC/DAC board L-761, voltage controlled depended current source and reed relay. Physically the preamplifier, relay and current source are positioned onto the same board and shielded. While membrane potential polarizes the microelectrode, voltage produced is delivered to the input terminal of the preamplifier. Preamplifier is assembled on operational amplifier chip LPC-662 manufactured by National Semiconductor Corp. It has a high input resistance >1 Tera Ω and ultra low leakage current 2 fA. The preamplifier is intended to transfer signals from the microelectrode to ADC without any distortion as well as to hamper leakage current occurrence. To reduce the leakage current value, a special chip assembling on the printed-circuit board surface was employed. The output signal is digitized by PCI ADC/DAC board L-761 (L-Card Company). This board also supplied with double-channel DAC. The first channel is employed to control the relay, commutating the microelectrode to the preamplifier input and to the current source output. The second channel controls the current source. The current source is intended to deliver alternate current to microelectrode. Typically current represents a sequence of 200 ms pulses with amplitude of 4 nA and porosity of 2. Such current causes fluorescent dye injection into cell.

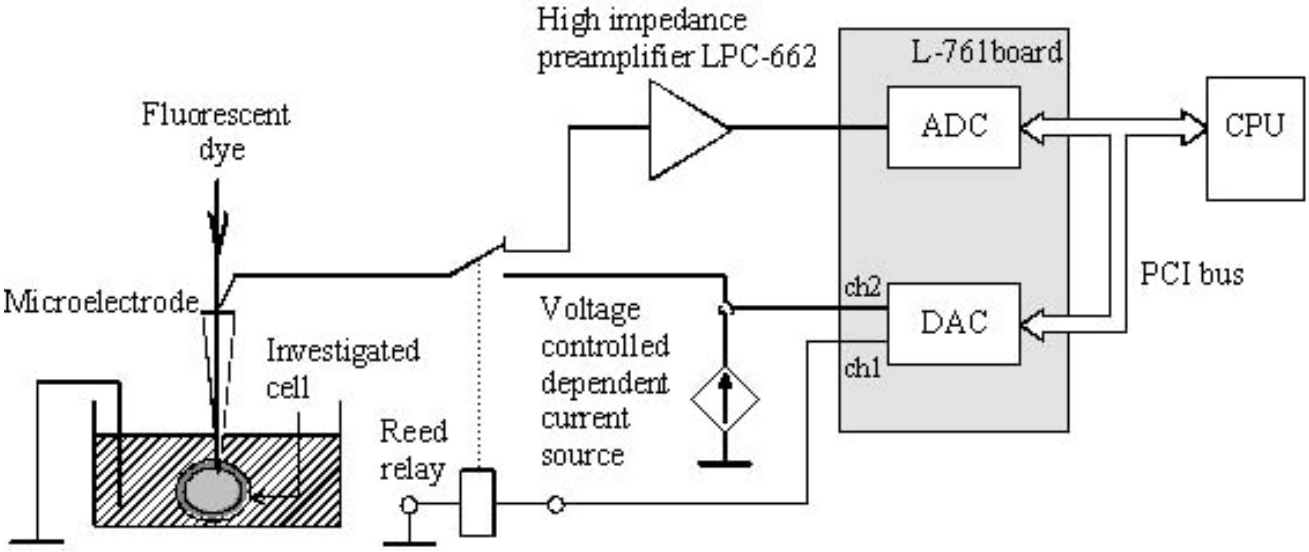

Figure 1. Connection layout of the iontophoretic device. Microelectrode is denoted as a triangle, the cell as a grey oval.

With use of LabVIEW 6.1 package the program to control L-761 board was developed. This program realizes virtual oscilloscope and voltmeter to register a membrane potential that depends on microelectrode penetration depth into cell. This program has the following features.

- Microelectrode signal registration and its shape examination on screen.
- Relay commutation control.
- Delivering of current signal of any given shape to the microelectrode to inject dye into cell.

The iontophoretic capillaries with the tip diameter less than 1 micrometer were filled first by the 200mg/ml rhodamine-conjugated dextran 40S (Sigma) and second by 1.2M LiCl. The fluorescent dye used is positively charged and was injected by pulses of the positive polarity. The capillary was

mounted on Narishige micromanipullator MWO-202 attached to Axiovert-200 Carl Zeiss inverted microscope. Transmitted and fluorescent light images (10× and 20× objectives) were taken by Axiocam MBC digital camera attached to the microscope. Injections of imaginal discs into adult female abdomen were done similarly to those already described (Bryant and Fraser, 1988).

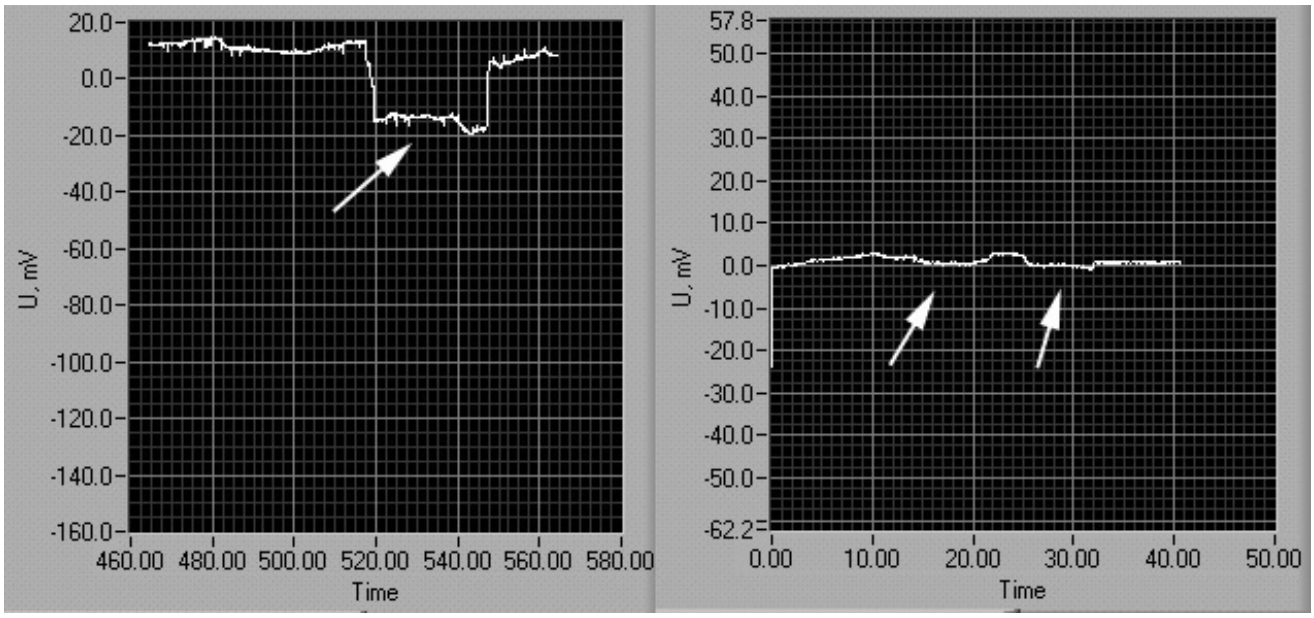

Figure 2. Membrane potential measurement. Oscillogram showing a polarization of microelectrode during introduction and moving off the capillary into the salivary gland polytene cell (right, one rectangular impulse marked with white arrow) and into the diploid imaginal disc cells (left, two negative impulses marked with white arrows). A window of LabVIEW software is shown.

Figure 2 illustrates the oscillogram after introduction and moving off the capillary into the salivary gland polytene cell (right, one rectangular impulse marked with white arrow) and into the imaginal disc cells (left, two negative impulses marked with white arrows). Figure 3 shows the phase contrast image of fragment of salivary gland containing target polytene cell (left, white arrow) and fluorescent image of inotophoretically injected cell (right, position where the capillary was introduced is marked by yellow by the remainder of capillary staying in the cell wall). It can be seen that the rhodamine label does not penetrate into nuclei (denoted by white arrow), but is localized in the cytoplasm. The example of iontophoretic injections into diploid *Drosophila* cells of the wing imaginal disc are given in Figure 4 (merged fluorescent and phase-contarast images). The most left photo - freshly injected cells of imaginal disc. At the next photo the same disc after culturing for 1 day in adult female abdomen is represented. The right photo – the same disc after 2 days of cultivation, note the right rhodamine spot was subdivided into two parts as a result of intercalary proliferation of nearby non-labeled cells or by allocation and proliferation of marked cells. Therefore, the injections made are stable through several cell generations. The application of the system for the study of *D. melanogaster* imaginal discs regeneration was published earlier (Mattila *et al*., 2004).

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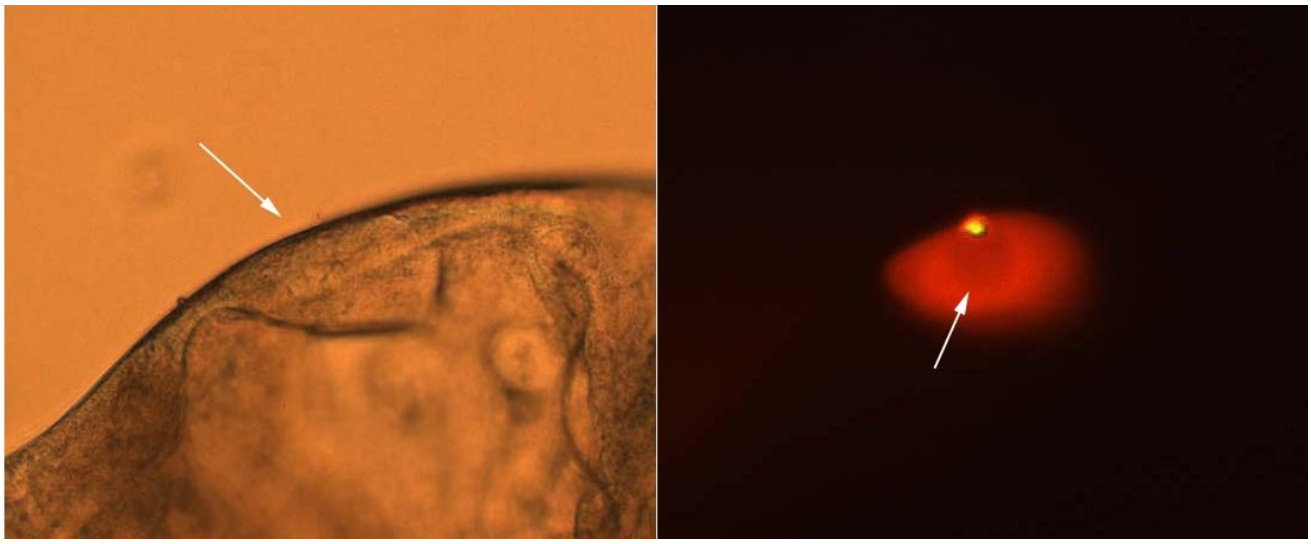

Figure 3. Injections into polytene cell of the larval salivary gland. Left: phase contrast image of fragment of salivary gland, containing target polytene cell (white arrow). Right: fluorescent image (red) of iontophoretically injected target cell (position where the capillary was introduced is marked by yellow by the remainder of capillary staying in the cell wall).

Figure 4. Diploid cells of the wing imaginal disc injections. Merged fluorescent (red) and phase-contrast images are shown. Left: freshly injected cells of imaginal disc. Middle: the same disc after culturing for 1 day in adult female abdomen is represented. Right: the same disc after 2 days of cultivation.

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Comparison of somatic clones of the eye in the analysis of cell growth.

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Introduction

Cell proliferation and increases in cell size are the processes that contribute to cell growth. Organs or tissues have a tendency to develop within the constraints of a normal final size and vary from a large number of small cells to a small number of large cells (Day and Lawrence, 2000). One important pathway in the control of cellular growth is the insulin receptor signaling pathway, which is highly conserved among invertebrates and mammals (Brogiolo *et al.*, 2001). When manipulated, several components of this pathway can alter growth rates of *Drosophila melanogaster*. The *Drosophila* eye normally consists of 700 to 800 ommatidia that develop in a highly regulated manner (Baker, 2001) and is ideal for the study of cell growth and cell survival. Mosaic eye clones can be created via the yeast site-specific recombination FLP/FRT system (Theodosiou and Xu, 1998), which depends upon the presence of *FRT* sites and expression of the enzyme FLP directed in the developing eye tissue. In the presence of FLP, homologous chromosomes undergo mitotic recombination between the *FRT* sites located on chromosome pairs. Heterozygous parent cells produce homozygous tissue within a heterozygous organism. In this paper, two methods of generating somatic clones of the eye are compared in the study of cell growth.

Drosophila Strains and Culture

Experiments were carried out on standard media containing cornmeal, molasses, yeast, agar, and water at 25°C. The control line *w; +/+; P[FRT ;w^{+}]^{2A} P[ry^{+} neo^{R} FRT]^{82B} akt1^{+}* (*w; FRT^{2A} FRT^{82B} akt1^{+}*) was obtained from Dr. Norbert Perrimon, Harvard University (Perrimon *et al.*, 1996). The P-element insertion line *akt1^{04226}* was obtained from the Bloomington *Drosophila* Stock Center. This line contains a P-element inserted within the 5' untranslated region of the *akt1* gene on the third chromosome (Perrimon *et al.*, 1996; Spradling *et al.*, 1999). The novel derivative of *akt1^{0422}*, *akt^{PR52}*, was generated by imprecise excision. Both *akt1^{04226}* and *akt1^{PR52}* were then recombined with the third chromosome *FRT*'s by standard means, and, therefore, have the genotype of *w; +/+; P[FRT ; w^{+}]^{2A} P[ry^{+} neo^{R} FRT]^{82B} akt1^{04226}(or^{PR52})/TM6B* (*w; FRT^{2A} FRT^{82B} akt1^{04226} (or^{PR52})*). The stocks required for creation of somatic clones of the eye were received from the Bloomington *Drosophila* Stock Center. The full genotype of *Drosophila* stock containing *eyeless-FLP* is *y^{d2} w^{1118}; P{ry$^{+t7.2}$=ey-FLP.N}^{2}, P{GMR-lacZ.C(38.1)}^{TPN1}; P{ry$^{+t7.2}$=neoFRT}^{82B}, P{w^{+t*} ry^{+t*}=white-un1}^{90E}, l(3)cl-R3^{1}/TM6B, P{y$^{+t7.7}$ ry $^{+t7.2}$=Car20y}^{TPN1}, Tb^{1}* for method one (Newsome *et al.*, 2000), hereby termed the *ey-FLP* method. The genotype *y w; P{w^{+m}=GAL4-ey.H}$^{3-8}$, P{w^{+mC}=UAS-FLP1.D}^{JD1};*