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Low Energy Electron Microscopy (LEEM) Imaging of a Neuron Network

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Abstract

The feasibility of using the LEEM technique in neurobiology was tested with experiments on a rat cerebellar granule cell culture. A series of good-quality images, showing fine details such as axons and dendrites of the neuron network, demonstrate that the test was successful.

A new series of microscopy techniques is being developed, with complementary techniques and considerable advantages in life-science applications [1/4]. For example, they are progressively eliminating the need for sample preparation procedures such as coating and staining. Furthermore, most of them can be implemented in a spectroscopic mode, delivering microchemical information [1–4].

We present here the first test of one such technique in neurobiology: Low Energy Electron Microscopy or LEEM [1/3]. This is the latest member of a family of microscopies that utilize the immersion-lens geometry, and is described in detail in Refs [1–3]. This is the latest member of a family of microscopies that utilize the immersion-lens geometry, and is described in detail in Refs [1–3]. In essence, electrons produced by a high-energy electron source are decelerated to very low energies right before interacting with the specimen; after reflection by the specimen, the electrons are accelerated again and form images on a phosphor screen. The accelerating and decelerating portions of the instrument are symmetric.

In order to understand the peculiarities of LEEM, it is interesting to make a direct comparison with the low-voltage scanning electron microscope (SEM) [2]. The SEM forms images with a sequential construction point-by-point using relatively high-energy electrons, whereas the LEEM is based on parallel imaging and uses low-energy electrons. The LEEM is also related to the PEEM (photoelectron emission microscope) [4], with the main difference that in this last instrument the specimen itself becomes the source of electrons.

A detailed comparison of LEEM and other surface macroscopies can be found in Ref. [2]. As far as future developments are concerned, the biological applications could greatly profit from the introduction of energy filtering – and the possibility to perform spectroscopic microchemical analysis.

The potential advantages of the LEEM in the life science have already stimulated a series of feasibility tests by Griffith *et al.* [2], performed on fibroblasts and their cytoskeleton. Life-science tests have also been performed with the related technique of mirror electron microscopy (MEM) [5]. We believed that it was important to extend such feasibility tests to neuron cells, for two reasons.

First of all, these cells have recently been extensively investigated with photoelectron microscopy [6, 7], and therefore offer a good opportunity for comparing the two techniques. Second, photoelectron spectromicroscopy of brain cell cultures has produced some exciting results on the selectivity of metal uptake by different types of cells [6]. The availability of a different technique to perform similar but complementary experiments would therefore be quite interesting.

The LEEM tests were performed on a granule cell neuron culture prepared in the following way [6]. Primary granule cells were obtained by enzymatic and mechanical dissociation of 8-day-old rat cerebellum. Cerebella were sliced, suspended in Krebs-Ringer solution and incubated with trypsin, centrifuged, resuspended, treated with DNAse and finely triturated. Dissociated cells were recovered by centrifugation and resuspended in Basal Eagle's Medium with Earle's salts containing fetal calf serum.

The cells were plated and allowed to grow on poly-L-lysine treated gold-coated stainless steel plates. Then, the specimens were fixed with 4% para-formaldehyde in PBSS (phosphate buffer saline solution) and dehydrated.

Note that the preparation procedure does not involve any type of coating, labeling or staining. The same specimens were used for experiments with photoelectron spectromicroscopy, offering the possibility of a direct comparison of the two techniques.

Figures 1 and 2 show two results of our tests. The first figure is the image of a granule cell: at the center the neuron's body is clearly visible; the fine structures originating from it are axons and dendrites. The estimated resolution is at least 10 nm. This image is one of a series of approximately 70 of similarly good quality.

Figure 2 is a patchwork combination of 14 partially overlapping images, showing an extended portion of the network. Note in particular the long connections between cell bodies and the good contrast with respect to the gold-coated substrate.

The success of these tests is important for several reasons. First, it corroborates the conclusion of Griffith *et al.* [2] that this class of microscopies, in principle developed for surface studies, find indeed interesting applications in the

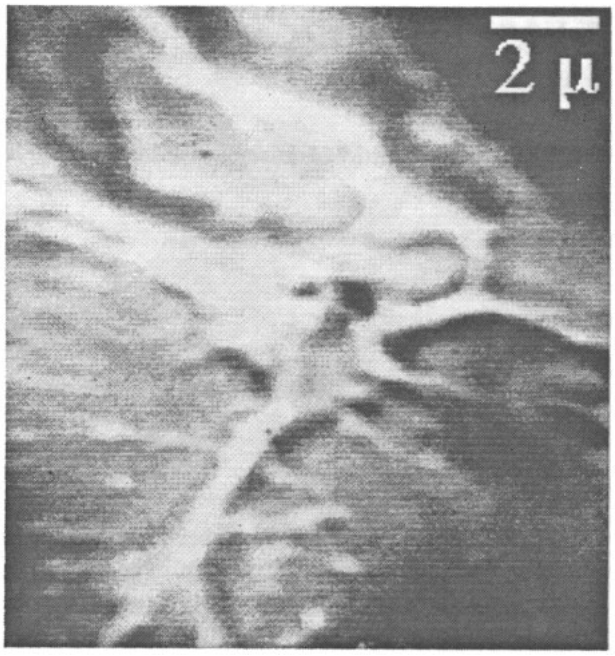

Fig. 1. LEEM microimage of a portion of a granule cell culture, showing a neuron cell body and neurites (axons and dendrites) departing from it.

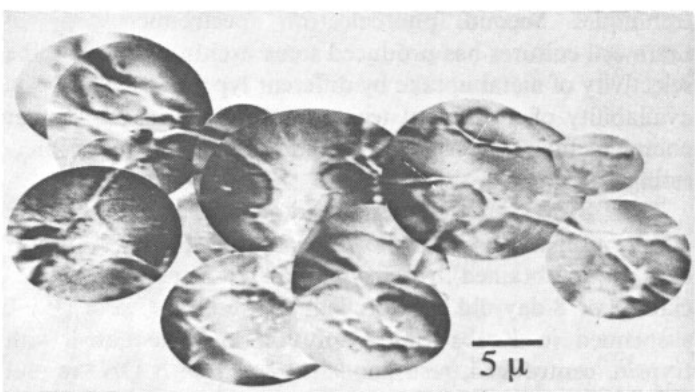

Fig. 2. Patchwork of 14 differently shaped LEEM microimages from the same specimen as Fig. 1.

life sciences as well. Second, the technical solutions adopted for the LEEM instrument used in these tests can also be exported to other microscopies, notably photoelectron microscopy [4, 6, 7]. enhancing for example their aberration correction. Third, there is the aforementioned possibility to transform LEEM into a spectromicroscopy, by means of an electron energy analyzer – which would provide a powerful new tool to study the fundamental problem of the uptake of toxic metals like aluminum.

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