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Array tomography vs. confocal microscopy for studying the nervous system of the smallest insect

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Confocal microscopy produced a revolution in the study of the structure, physiological processes, development, and many other aspects of research on a wide range of biological objects. Currently it remains one of the most widely used methods of microscopy. But the application of this approach to the study of insects has not resulted in a breakthrough similar to that it produced in studies of many other objects, because the majority of insects have non-transparent chitinous exoskeletons impermeable to large molecules. Of course, studies of particular organs and body parts of insects yield excellent results, but a number of challenges require working with intact specimens, or making preparations that cannot be made (for instance, because of the extremely small size of the object). It is usually impossible to produce sections of insects by freezing or in embedding media, because the insect exoskeleton is too hard for these methods. The array tomography method, developed a few years ago [1, 2] allows applying to insects the whole spectrum of immunofluorescence methods used in Confocal microscopy. Hard embedding medium (LR White) allows readily producing complete series of sections of any thickness. Working with sections solves the problem of the non-transparency of the exoskeleton and permeation of antibodies. This method can be excellently combined with the correlative light and electron microscopy. The algorithm of section digitalization and subsequent computer processing proposed by the authors allows obtaining a complete 3D picture of the object. Array tomography can be successfully used for studying insects, but no studies by this method have been performed to date on this group of objects. To compare confocal microscopy and array tomography, we selected one of the objects most difficult to study: the smallest insects, which have a body length of at most 250 µm. In one of such insects, a member of the genus *Megaphragma* (Hymenoptera: Trichogrammatidae), a unique phenomenon was recently described: almost all cells of the central nervous system in adult insects are anucleate [3], but all basic functions of the central nervous system are retained. This phenomenon, previously unknown in any animals, requires further studies, which would have been impossible without immunolabeling. To estimate the usefulness of confocal microscopy for studying this object, we tried to solve the antibody permeability problem by dividing the body of the insect into separate tagmata and treating them with Triton X-100; to solve the problem with exoskeleton transparency, we used various methods of clearing (including Murray's Clear); and to confront the autofluorescence of the cuticle, we worked in various parts of the light spectrum. Nevertheless, results of the needed quality have not been obtained. On the other hand, array tomography does solve all above mentioned problems, but the principal difficulty of this method is that antibodies label only those antigens that are located on the surface of the section, and in most cases the resulting fluorescence is extremely weak. This problem can largely be solved by pretreatment of sections with Triton X-100 [4] and by heat-introduced antigen retrieval [5], two techniques that strongly enhance the immunolabeling of sections. An important advantage of array tomography is the fact that it allows obtaining complete 3D models of entire organisms with either permeable or impermeable exoskeletons. Another advantage is the resolution, which is considerably higher than in confocal microscopy. The possibility to use sections employed in array tomography in studies using scanning electron microscopes allows simultaneously performing also ultrastructural studies, including immunnogold labeling. As a result, particular cells can be studied even in the smallest known animals.

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