

Three-dimensional imaging of structural development in the fruiting body of field-collected true slime molds

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Categories and Subject Descriptors

J.3 [Computer Applications]: Life and Medical Science, Biology and Genetics

Keywords

Myxomycetes, TEM, 3D LM, fruiting body formation

True slime molds are unicellular, multinucleate eukaryote amoebae. *Physarum polycephalum* Schwein., is the most well-known and unique species of true slime molds, and it has recently been in the spotlight again for its use in computational and physical research. Although such studies are focused exclusively on the plasmodium, true slime molds also have another interesting characteristic as an organism; the single cell produces their complicated fruiting body structures with non-cellular materials. This unique and remarkable characteristic has been described since the 19th century [1]. However, how their structure is made from a single cell remains largely unknown because true slime molds are mostly unculturable, and we are therefore still only at the early stage of discovering the principles that underlie the nature of this organism. Here we report methodological advances that extend the conventional dissecting approach for broad visual field imaging of the immature fruiting body in three dimensions with high-resolution 2D images. We have developed a protocol that enables generation of 3D image stacks of the entire plastic-embedded fruiting body by light microscopy (LM) and high-resolution 2D images by transmission electron microscopy (TEM) on the same fruiting body. This protocol will be useful for investigating mechanisms underlying the formation of the sporangial type fruiting bodies of unculturable true slime molds.

In the present study, we focused on the sporangial type fruiting body, a relatively small and fairly uniform shape for a particular species, to observe the inner differentiation of fruiting body formation. Plasmodia or immature fruiting bodies were collected from fields with substrata. The plasmodium or developing sporophore picked off the substratum, or just with a small piece of substratum, was chemically fixed, dehydrated, and embedded in resin. Serial thick sections were made on an ultramicrotome with a histo-jumbo diamond knife (Diatome, Switzerland) for 3D LM

and with an ultra diamond knife for 2D TEM. Pictures were taken by LM with a microscope camera, and 3D reconstruction was performed by IMOD (Colorado State University, USA).

True slime molds have disadvantages for scientific research in terms of ecology: 1. difficult to culture, and 2. fruiting body morphology: varies in size, generally too small to observe by the naked eye and to dissect under binoculars, too thick and opaque to observe by a light microscope, and too large to observe by a transmission electron microscope. Some studies on slime mold morphogenesis have been performed, but most of those studies were performed before 1990, and terms used in those studies do not completely match modern biological terms, and sometimes certain characters were missing due to technical difficulties in methods of observation. Although fruiting body structures are used as taxonomic characters, there is no agreement on definition and terminology for the structures [2]. These facts cause widespread speculation, and unreliable information on true slime molds is becoming prevalent in science. To clarify the nature of true slime molds, accumulation of the results of detailed studies on the inner differentiation of various species is needed. Our protocol was tested on several species of developing sporangial type fruiting bodies of unculturable true slime molds. To use our protocol, information on the exact location of each section was obtained. The inner architecture of the developing fruiting body was clearly shown, and detailed information on the development of its structures, i.e., hypothallus, stalk, columella, capillitium, peridium and spore wall, in relatively chronological order was obtained. In summary, our protocol has the following advantages: (1) it is applicable to unculturable field-collected species, (2) both broad visual field images by 3D LM and high-resolution images by 2D TEM of immature fruiting body can be obtained from a single fruiting body, (3) it requires no expensive modern equipment; only a traditional ultramicrotome, LM and TEM for images and a general-use computer for reconstruction are required, and (4) there is no need for special sample preparation, i.e., it is applicable to stored normal resin-embedded fruiting bodies. This extended abstract summarizes a contributed presentation delivered at PhysNet 2015.

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