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Ultramicroscopy – imaging a whole animal or a whole brain with micron resolution

Randolf Menzel*

Institut für Biologie – Neurobiologie, Freie Universität Berlin, Berlin, Germany
**Correspondence: menzel@neurobiologie.fu-berlin.de*

A commentary on

Three-dimensional reconstruction and segmentation of intact *Drosophila* by ultramicroscopy
by Nina Jährling, Klaus Becker, Cornelia Schönbauer, Frank Schnorrer and Hans-Ulrich Dodt (2010). Front. Syst. Neurosci. 4:1. doi: 10.3389/neuro.06.001.2010

Drosophila is the species of choice for a large range of questions that can be addressed using molecular genetic approaches. Its small size and translucent body also makes it a most suitable species for whole-body imaging with the light microscope. Hans-Ulrich Dodt and co-workers have developed an "ultramicroscope" that allows fast fluorescence imaging of *Drosophila* for high-throughput phenotyping (Jahrling et al., 2010). But the ultramicroscope provides us with even more options: high resolution imaging of rather large pieces of tissue such as the whole brain of a mouse (Dodt et al., 2007).

Whole animal imaging with micrometer resolution is a challenging task. Micro-CT (also: synchrotron radiation-based computer microtomography, SRμCT) and infrared tomography may offer a solution but do not allow the detection of selected tissues or cells as marked by molecular genetic methodology using fluorescence probes. Confocal and multiphoton microscopy and their various combinations with other optical tricks (e.g., stimulated emission depletion microscopy, STED[1]) or stochastic optical reconstruction microscopy (STORM[2]: Zhuang, 2009) go beyond the diffraction limits of light but the high magnifying objectives make whole animal imaging either impossible or a complicated and time consuming task. The ideal system should detect fluorescence signals because the respective signaling molecules can be expressed in selected tissues or even single cells. It should allow fast imaging of the whole animal for high-throughput analyses, and the resulting imaging stacks should permit fast processing with conventional imaging software. The ultramicroscope developed by Hans-Ulrich Dodt at the Technical University of Vienna comes close to such an ideal system. It takes advantage of the rather transparent body of white-eyed *Drosophila* mutants and the rich repertoire of molecular genetic tools to express particular fluorophores in selected tissues and cells. The method produces impressive images of the whole interior of the *Drosophila* body (including nervous system, brain, and muscles) even when just the auto fluorescence signal of the tissues induced by glutaraldehyde fixative is imaged (see the figures in Jahrling et al., 2010).

Dodt's imaging device is based on the ultramicroscope developed in 1913 by Henry Siedtopf from the Zeiss Company and Richard Zsigmondy, professor of inorganic chemistry at the University of Göttingen, who received the Nobel Prize in 1926 for his work on colloid material. They developed a dark field microscope that allowed the detection of nanoparticles well below the diffraction limit of the light microscope. As with the fluorescence microscope the light is emitted by the substrate and not imaged by absorption or diffraction of the transmitted light. In Dodt's ultramicroscope (Dodt et al., 2007; Becker et al., 2008) the light produced by a 200-mW diode laser (488 nm) illuminates the object from both sides by two colocalized thin sheets of light and induces fluorescence signals in a horizontal layer of the tissue that are picked up by a 10× objective (NA 0.3, take Figure 1 in Dodt et al., 2007). The two colocalized sheets of light penetrate the tissue from two opposing sides compensating for the absorption gradient within the tissue. The quality of images also depends on the thickness of this light-sheet. One can make it very thin near to the focal point of the cylinder lens, but its Rayleigh range (which is a measure of the depth of focus) is limited and a smaller aperture leads to loss of power, a limiting factor for more strongly absorbing tissue. In a recent improvement of the light-sheet-generation optics, longer Rayleigh ranges were reached, and higher resolution achieved (Saghafi et al., 2010).

The material to be imaged needs to be as transparent as possible. Dodt applies a method published in 1914 by Spalteholz (1914) that makes animal and human tissue almost transparent. Werner Spalteholz was an anatomy professor at the University of Leipzig and became famous for his Handatlas der Anatomie des Menschen (with three volumes published between 1895 and 1903, and translations into English) still considered to be one of the most elegantly illustrated anatomical atlases (Williams, 1999). A mixture of benzyl benzoate and benzyl alcohol (often also only methyl benzoate) is used after the tissue has been dehydrated. This medium has the same refractive index as the fixed tissue so that light passing through the medium is not scattered by the different refractive index of the tissue. The cuticle of insects is usually rather dark, but the melanin can be bleached with KOH or potassium permanganate (Wigglesworth, 1972).

The whole animal or block of tissue is positioned in a bath of the clearing solution as close to the objective as possible. Stacks of images are produced by stepping the bath with the material with 1.66 μm vertical spacing. Image stacks are processed in conventional ways. In the case of *Drosophila* the whole procedure takes less than half an hour including image processing. An attractive feature of the set-up is that instrumentation efforts are rather moderate and do not require a specialist in the physics of imaging with light. A commercial ultramicroscope is now available (LaVision Biotec[3]).

[1]For a nice explanation of STED go to http://genetik.bcp.fu-berlin.de/

[2]For a nice explanation of STORM go to http://zhuang.harvard.edu/research.html

[3]http://www.lavisionbiotec.com/en/microscopy-products/ultramicroscope/

Ultramicroscopy allows resolution <10 μm. Immunolabeling, lectin staining, and GFP expression in single or groups of neurons give impressive images of the *Drosophila* brain. The whole mouse brain can be imaged providing excellent images of neurons from mice that have been genetically engineered to express fluorophores in neurons[4,5]. Even spines can be resolved[6].

As with any experimental procedure the ultramicroscope also has its limitations. The working distance of the objectives and their moderate apertures (0.7 for the 10× objective used in Dodt's studies) is a major limitation for selection of the material. Higher resolution by objectives with larger apertures would be most beneficial, but then the working distance would be compromised. Animals with dark surfaces which cannot be bleached or cleared are less suitable. An improvement was reported recently by combining the principle of confocal microscopy (using a slowly rotating Nipkow disk) with ultramicroscopy (Kalchmair et al., 2010). It was found that confocality minimizes background noise and improves the signal-to-noise ratio. It is also important to keep in mind that the dehydration process necessary for the clearing leads to substantial shrinking effects that may not be isotropic across the animal or the block of tissue (Bucher et al., 2000). Thus size geometry measures may not reflect *in vivo* conditions.

[4]http://www.tuwien.ac.at/flash_video/09dodt_gehirn_ganz/

[5]http://www.tuwien.ac.at/flash_video/09dodt_cortex_hippocampus/

[6]http://www.nature.com/nmeth/journal/v4/n4/suppinfo/nmeth1036_S1.html

REFERENCES

Becker, K., Jahrling, N., Kramer, E. R., Schnorrer, F., and Dodt, H. U. (2008). Ultramicroscopy: 3D reconstruction of large microscopical specimens. *J. Biophotonics* 1, 36–42.

Bucher, D., Scholz, M., Stetter, M., Obermayer, K., and Pfluger, H. J. (2000). Correction methods for three-dimensional reconstructions from confocal images: I. Tissue shrinking and axial scaling. *J. Neurosci. Methods* 100, 135–143.

Dodt, H. U., Leischner, U., Schierloh, A., Jahrling, N., Mauch, C. P., Deininger, K., Deussing, J. M., Eder, M., Zieglgansberger, W., and Becker, K. (2007). Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. *Nat. Methods* 4, 331–336.

Jahrling, N., Becker, K., Schonbauer, C., Schnorrer, F., and Dodt, H. U. (2010). Three-dimensional reconstruction and segmentation of intact *Drosophila* by ultramicroscopy. *Front. Syst. Neurosci.* 4:1. doi: 10.3389/neuro.06.001.2010

Kalchmair, S., Jahrling, N., Becker, K., and Dodt, H. U. (2010). Image contrast enhancement in confocal ultramicroscopy. *Opt. Lett.* 35, 79–81.

Saghafi, S., Becker, K., Jahrling, N., Richter, M., Kramer, E. R., and Dodt, H. U. (2010). Image enhancement in ultramicroscopy by improved laser light sheets. *J. Biophotonics* 3, 686–695.

Spalteholz, W. (1914). *Über das Durchsichtigmachen von menschlichen und tierischen Präparaten*. Leipzig: Hirzel.

Wigglesworth, V. B. (1972). *The Principles of Insect Physiology*, 7th Edn. London: Chapman and Hall, 827.

Williams, D. J. (1999). The history of Werner Spalteholz's Handatlas der Anatomie des Menschen. *J. Vis. Commun. Med.* 22, 164–170.

Zhuang, X. (2009). Nano-imaging with STORM. *Nat. Photonics* 3, 365–367.

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