Experts push the boundaries of 3-D microscopy

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Two newly developed methods will help researchers to study the 3-D structure of complex surfaces and of individual neurons better than ever before. Sebastian Munck and Natalia Gunko, two expert technologists at VIB-KU Leuven, report new imaging protocols that will advance neuroscience and (bio)imaging in general.

The biotech R&D sector is thriving in Flanders, and this is in no small part due to presence of a lot of tech development and know-how, enabling scientists to carve out a path towards new insights and therapies. This month, two colleagues at VIB and KU Leuven report on new ways to study 3-D surfaces and the 3-D ultrastructure of brain cells.

From Lego to flies: ALMOST allows unprecedented 3-D surface imaging

Recent developments in 3-D microscopy have revolutionized biomedical research by enabling the imaging of whole model organisms such as zebrafish and fruit fly larvae as well as cleared mouse embryos and organs. In many cases, however, this requires making a sample transparent using chemical 'clearing' methods, says light microscopy expert Sebastian Munck (VIB-KU Leuven): "Clearing methods are time intensive and can't be applied to every type of sample. Moreover, if you want to study surface morphology or color, optically clearing is counterproductive."

That is why Munck and his team developed ALMOST, an optical method for 3-D surface imaging of reflective opaque objects. Munck: "ALMOST stands for 'a label-free multicolor optical surface tomography' method. It provides a 3-D surface reconstruction of non-transparent samples, including information on its color and reflective properties."

Munck believes that many research fields will benefit from this straightforward way of documenting and quantifying 3-D surfaces, as ALMOST can be applied to both biological and non-biological samples: "The ability to record the surface of a medium-sized object in 3-D opens perspectives for digital repositories of zoological and botanical collections and enables a link to 3-D printing of these objects. From pigment analysis to virtual reality, or even art, the possibilities are endless."The scientists neatly illustrate this by imaging not only biological samples such as fruit flies and seed cones, but also Lego figurines.

From silver to gold: optimizing a century-old method to study neurons in more detail

In the late 19th century, Camillo Golgi developed a method to stain the long protrusions of individual brain cells in what he called "the black reaction." Now referred to as the Golgi method, the protocol has been refined over the years and proved instrumental for many groundbreaking advances in neurobiology. Nevertheless, it also has some important drawbacks, according to Natalia Gunko (VIB-KU Leuven): "Golgi staining techniques are still widely used in research and clinical diagnostics, but they are incompatible with further studies of the subcellular architecture of neurons with electron microscopy due to the formation of large, electron-dense silver deposits that mask ultrastructural details."

To solve this problem, Gunko and her team adapted the Golgi method for electron microscopy by replacing silver salts with gold salts, resulting in far smaller particles that are often deposited at the periphery of neurons.

"It's the first successful use of a Golgi-based staining technique for tracing neurons over their entire length, preserving the ultrastructural details," says Gunko, who immediately applied the technique to study neuronal ultrastructure in an Alzheimer's disease model.

"We combined the Golgi staining with fluorescent labeling and tissue clearing to visualize spatial relationships between entire neurons and amyloid plaques in brain samples of an Alzheimer's mouse model." This is but one example of the use of the new method in fundamental neuroscience and the study of neuronal morphology in brain disease.

More information: A Label-free Multicolor Optical Surface Tomography (ALMOST) imaging 1 method for nontransparent 3D samples, Kerstens et al. 2019 *BMC Biology*

Modernization of Golgi staining techniques for high-resolution, 3-dimensional imaging of individual neurons, Vints et al. 2019 *Scientific Reports*

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