

The SIBMAR consortium is developing a technique for three-dimensional holographic imaging of biomolecules with atomic-level resolution using low-energy electrons for their 'light'. With the requisite expertise unavailable in any single country, the consortium combines the research strengths in biochemistry, biophysics, molecular biology and mesoscopic physics of leading labs in Switzerland, Germany and the UK.

Atomic resolutions

X-ray crystallography and nuclear magnetic resonance (NMR) – the most common imaging techniques for structural analysis of biomolecules – have their limitations. They require homogenous samples, which must be crystalline in the case of X-ray crystallography, or in the case of routine NMR must be in a liquid solution. They also require comparatively large quantities of the molecule to be analysed. The resulting gaps in the range of imageable biomolecules are motivating efforts to develop new ways to probe the structure of molecules.

Holographic microscopy was first proposed more than half a century ago to improve the resolution of electron microscopy. In this method, a coherent electron beam is directed at an object, which scatters some of the electrons, while the remainder pass by unaffected. A two-dimensional image – a hologram – of the object forms on a detector placed some distance away, due to interference between the scattered and unscattered electrons. In principle, the hologram contains all the phase and amplitude information necessary to provide a three-dimensional image of the object.

Lens-free microscopy

When bright coherent laser sources became available, optical holography was one application they were quickly put to. In similar fashion, it has taken the invention of bright coherent electron-beam sources to make holography a viable proposition in electron microscopy. In low-energy electron point-source (LEEPS) microscopy, a coherent source of low-energy electrons is used to illuminate the object. The SIBMAR partners aim to develop a LEEPS microscope suitable for visualising molecular structure down to 2 Å. And they plan to test existing LEEPS microscopes along the way to help them avoid reproducing any of their defects when they design their prototype.

Single molecule preparation

In addition to the prototype microscope, the team will be working on various methodologies to facilitate examination of biomolecules by LEEPS microscopy. One concerns the way samples can best be held in the beam of electrons. Two options are to be tested – one based on filamentous phage scaffolds; the other on atomically thin films made from two-dimensional crystals such as graphene.





SIBMAR NEST ADVENTURE

Converting a two-dimensional image to three-dimensions requires mathematical transformation using amplitude and phase of object wave.

AT A GLANCE

Official title

Obtaining Atomically Resolved Structural Information on Individual Bio-Molecules Using Electron Holography

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For their biological samples the team are using two groups of proteins that are extremely important from a pharmaceutical point of view. They are the MAP kinases and G protein-coupled receptors, important components of signal-transduction pathways. Although these are already the object of much interest in drug development, structural knowledge about both is very limited. Novel ways of attaching these proteins to the two types of supports are therefore prominent on the SIBMAR agenda.

Converting the two-dimensional hologram into a three-dimensional representation of the original object can be done by a mathematical transformation of the amplitude and phase information contained in the hologram, although this is not without its problems. Together with the in-focus image, an out-of-focus twin image is created. The SIBMAR researchers are devising numerical methods to cancel this artefact and are also intent on improving the signal-to-noise ratio of the holograms.

The consortium believes the LEEPS microscope they build will have a positive impact on the European biotechnology industry. It could serve, for example, in the development of plant-defence proteins to tackle plant diseases. In enzyme kinetics, it could help in the engineering of enzymes to operate over a certain pH range and at specific temperatures. But it is in the pharmaceutical industry that the LEEPS microscope is likely to shine most brightly. In particular, it could fill in important gaps in our knowledge of the structure of membrane-bound receptors, an area of enormous interest in drug development. It could also accelerate the design of enzyme inhibitors through studies of the ligand-protein binding sites in enzymes at the atomic level.

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SIXTH FRAMEWORK PROGRAMME