doi:10.1017/S1431927618014022
Microsc. Microanal. 24 (Suppl 2), 2018

Biological Laboratory X-ray Microscopy

Mikael Kördel[1a,*], Emelie Fogelqvist[1a], Valentina Carannante[2], Björn Önfelt[1b], Hemanth K. N. Reddy[3], Kenta Okamoto[3], Martin Svenda[3], Jonas A. Sellberg[1a] and Hans M. Hertz[1a]

[1. a.] Biomedical and X-Ray Physics, [b.] Biophysics, Department of Applied Physics, AlbaNova University Center, KTH Royal Institute of Technology, 10691 Stockholm, Sweden.
[2.] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 17177 Solna, Sweden.
[3.] Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, 75124 Uppsala, Sweden.
* Corresponding author, mikael.kordel@biox.kth.se

Laboratory x-ray microscopy is an important complement to the accelerator-based techniques for imaging intact cells in their native or near-native state. Not only does it provide accessibility for a wider community of biological researchers, but also allows for more time-consuming and complex studies where the biology, including the sample preparation, is not trivial.

Here we present recent results from the Stockholm laboratory x-ray microscope which is based on a liquid-nitrogen-jet laser-plasma source powered by a 100 W 2 kHz 600 ps diode-pumped Nd:YAG slab laser. The $\lambda = 2.48$ nm line emission from the laser-plasma is collected by a normal incidence 500 double-layer Cr/V condenser mirror and focused onto the sample, which is positioned in a TEM-type cryo sample stage. A 30-nm outer zone width 200-µm diameter Ni zone plate finally images the transmitted x-rays onto a cooled back-illuminated CCD detector.

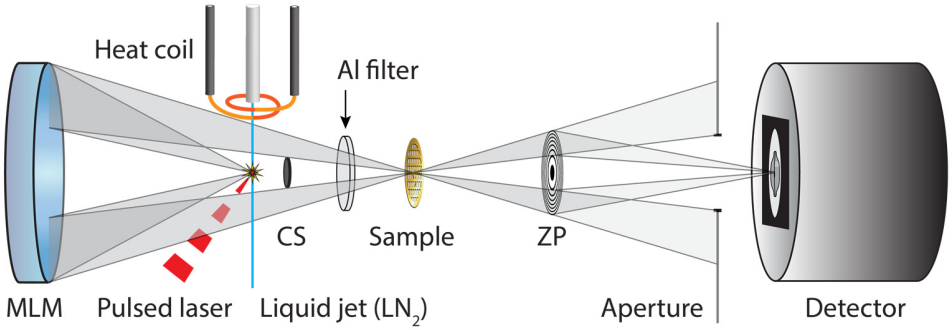

Figure 1. The setup of the Stockholm laboratory x-ray microscope.

The Stockholm laboratory x-ray microscope has undergone some major improvements in the last few years resulting in an increased x-ray flux at the sample, but perhaps more importantly an improved stability and reliability. The system now allows, for the first time, extensive studies of relevant biological samples, including 3D cryo-tomography [1]. In brief, the major upgrades to the setup consist of an improved liquid jet stability by introducing a radiative heating element that counteracts initial freezing as the jet is ejected into vacuum [2], and a new condenser mirror with a factor >7 higher reflectivity at $\lambda = 2.48$ nm.

The improved performance of the Stockholm laboratory x-ray microscope was first demonstrated in 2017 by performing two biological studies [1]. In the first study the development and distribution of carbon-dense vesicles was monitored in starving human embryonic kidney cells (HEK293T). Nutrient starvation in cells causes stress and may induce autophagy, which involves the formation of µm and sub-µm carbon-dense vesicles in the cytoplasm [3]. The second study further demonstrates the microscope's capabilities

by imaging the interaction between natural killer (NK) cells and HEK293T target cells. NK cells constitute an important part of the innate immune system. As the name suggests, they are able to kill cells expressing certain signals of stress [4]. The killing process starts with the NK cell adhering to the target cell.

Figure 2 shows a 20-s exposure of a cryofixed starving HEK293T cell. Most clearly noticeable in the image are the dark carbon-dense vesicles (typically 200-400 nm in diameter) and the lamellipodia along the edges of the cell, showing that the cell is still adhered to the holey carbon film. In this study starvation was induced simply by not adding any nutrients to the cell medium. After 2-3 days a majority of the imaged cells showed structural changes such as the formation of carbon-dense vesicles, withdrawal of lamellipodia and a rounded cell shape. The cells in this image were starved for approximately two days.

Figure 3 shows a 20-s exposure of three NK cells in different stages of adhesion to a HEK293T target cell. Most striking here is how the smaller NK-cells stretch out toward the larger HEK293T cell, possibly to form the NK-cell-target-cell immune synapse.

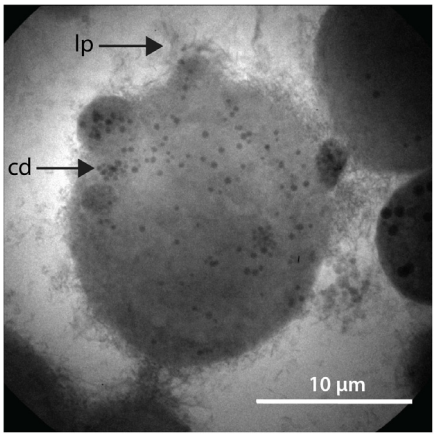

Figure. 2. A 20-s exposure of a starving HEK293T cell. Arrows indicate lamellipodia (lp) and the carbon-dense vesicles (cd).

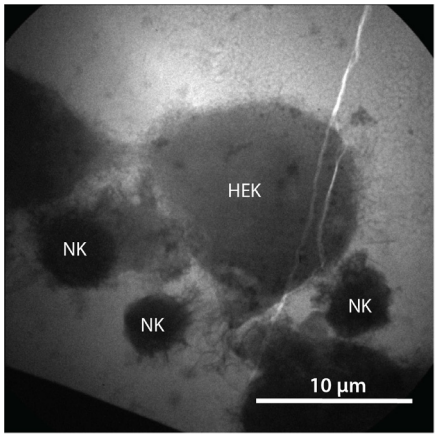

Figure. 3. A 20-s exposure of three NK-cells (NK) adhering to a HEK293T cell (HEK).

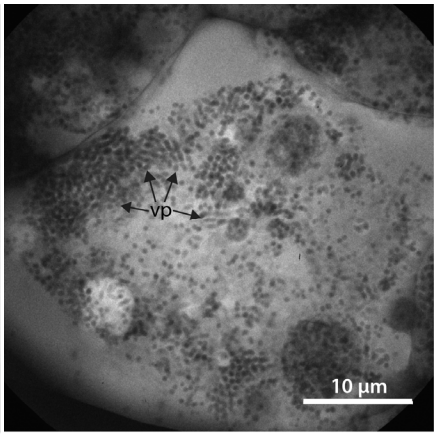

Figure. 4. A 60-s exposure of an infected amoeba containing numerous viral particles (vp).

In a more recent project the laboratory x-ray microscope was used to study a newly discovered, so called, giant DNA virus [5] and the process of viral replication inside a host amoeba (A. castellanii). An example is shown in Fig. 4. The infected amoebae were cryofixed and imaged at a range of different times post-infection, up to three days. Preliminary results indicate a rapid production of viral particles during this time, to the point where the particles fill a large part of the host cell volume. In conclusion we note that the demonstrated experiments benefit from the possibility of 3D high-resolution imaging of intact cells and the long-term access to the laboratory x-ray microscope for optimizing the sample preparation.

References:

[1] Fogelqvist, E., et al., Sci. Rep. **7** (2017) 13433.
[2] Fogelqvist, E., et al., J. Appl. Phys. **118** (2015) 174902.
[3] Shibutani, S.T. and T. Yoshimori, Cell Res, **24** (2014) 58.
[4] Orange, J.S., Nat. Rev. Immunol. **8** (2008) 713.
[5] Abergel, C., M. Legendre, and J.M. Claverie,. FEMS Microbiol. Rev. **39** (2015) 779.

https://doi.org/10.1017/S1431927618014022 Published online by Cambridge University Press