

2020 – A first year of SPA in Dahlem

We can now look back on the first year of productive use of the 300 kV transmission electron microscope at FUB as part of the new “Berlin Cryo-EM” facility. As intended in the joint 91b application for the two “Titan Krios” in Buch (Charité) and Dahlem (FUB), the microscope in Dahlem was used exclusively to obtain high-quality data for single particle analysis (SPA).

First and foremost, we would like to thank the Department of Physics for hosting our Titan Krios until its final move into the new SupraFAB building. After a short testing period in December 2019, we started productive work in the first week of 2020. In parallel, we have implemented our SPA workflow into the OpenIRIS booking system (see right box) to allow users request for beam time and track the progress of their requests.

Despite lockdown and annual closing the Krios was acquiring data more than 53% of the time in 2020. We still have capacity for samples to be measured and would be happy to advice interested groups with regard to sample requirements and post-measurement data analysis.

Aside the great scientific results - a first structure was published already in December - we are happy to introduce Dr. Kai Ludwig as the new head of the FZEM, succeeding retired Dr. Christoph Böttcher.



Contact SPA data acquisition

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How-to SPA requests

SPA data collection can be requested by any of the applicants of the 91b proposal. We have implemented three services at *fub.openiris.io* to manage the requests:

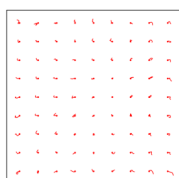
- **Participate in SPA data acquisition** (to sign up a working group and group members for the SPA service)
- **Register to SPA** (to authorize further group members to request for beam time)
- **SPA data acquisition – Titan Krios** (to request beam time for their samples)

The system automatically forwards each SPA request to the group head for definitive approval and acceptance of the costs.

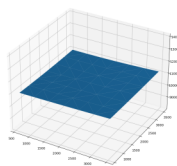
More in-depth instructions will be provided on the FZEM's soon-to-be-launched new web-site at *fzem.fu-berlin.de*.

On-the-fly processing

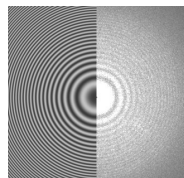
We have now implemented cryoSPARC™ live⁽¹⁾ and cryoFLARE⁽²⁾ into our productive workflow for on-the-fly processing. This allows direct assessment of image and sample quality **immediately** during data collection generating near-atomic resolution reconstructions within hours.



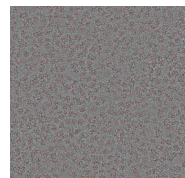
Local motion



Patch CTF



2D CTF



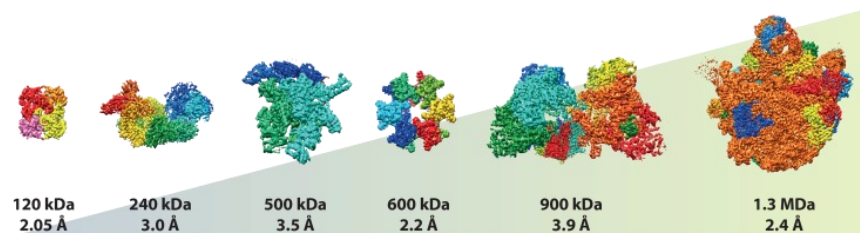
Picking

(1) Punjani *et al.* (2017), *Nat Methods* **14**, 290-296. doi: 10.1038/nmeth.4169

(2) Schenk *et al.* (2017), *J Chem Inf Model* **60**(5), 2561-69. doi: 10.1021/acs.jcim.9b01102

Structures solved

Meanwhile, structures of various samples ranging from <100 kDa to >1 MDa were solved achieving near-atomic resolution without using the VPP.



Figures

- 102 requests
- 101 measurements
- 4,700 hrs beam-time
- 53% run-time
- 206 hrs performance validation
- 195 hrs down-time

Publication(s)

Pei, Hilal, Chen *et al.* The δ subunit and NTPase HeID institute a two-pronged mechanism for RNA polymerase recycling. *Nat Commun.* 2020 Dec 18;11(1):6418. doi: 10.1038/s41467-020-20159-3.

FZEM

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SPA Team - SupraFAB

Benedikt Kirmayer (left), Dr. Tarek Hilal (middle), and Dr. Boris Schade (right).

