EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structural studies of intermediate filaments.	**Experiment number**: MX2249
Beamline: CM01	**Date of experiment**: from: 03 Jul 2020 to: 05 Jul 2020	**Date of report**:
Shifts: 6	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Anastasia Lilina*
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Report:

Allocated beamtime was used as followed: 2 hours for preparing grid atlases, 3 hours for grid screening and selecting the best grid, the remaining time was used for data collection. There were network problems at the facility, so the start of the data collection was postponed for a couple of hours, but the session was extended in order to compensate for the lost time. As a result, we were able to collect a dataset of 6278 movies for a large complex formed by the intermediate filament protein.

In total 7 grids with frozen sample were loaded into the microscope. However only grids #2 and #5 were showing good ice quality and decent particle distribution. In total 21 grid squares were selected on grid#2. Examples of a good grid square and a grid hole with particles are shown in figure 1A and 1B. The data were collected with the following parameters: magnification 105k, pixel size 1.35 Å/pixel, dose per movie 40.0 $e^-/Å^2$, number of frames per movie 40, defocus range: -1.0 – -2.4 µm in 0.2 µm step . The overall statistics for resolution and astigmatism distribution is shown in figure 1C.

Processed and raw data are being copied from ESRF server using rsync command. During the download, we are giving priority to the pre-processed images that we can use directly for CTF estimation and particle picking. Data processing is being carried at KU Leuven using CryoSparc v 2.15.

Using the 2D templates from previous data collection we picked more than 120k particles from ~5000 micrographs. After several rounds of 2D classifcation, we had 40k particles suitable for 3D reconstruction

(figure 2A and 2B). We managed to refine the structure up to 20Å (figure 2C). However, this is not sufficient. Further processing is necessary in order to improve resolution.

During the initial inspection of the micrographs, we observed that template-based particle picking selected a lot of aggregated particles. Additional work must be done in order to optimize particle-picking strategy using the deep learning models (Topaz or Cryolo) as we can see that single particles are present in the micrographs (figure 1B).

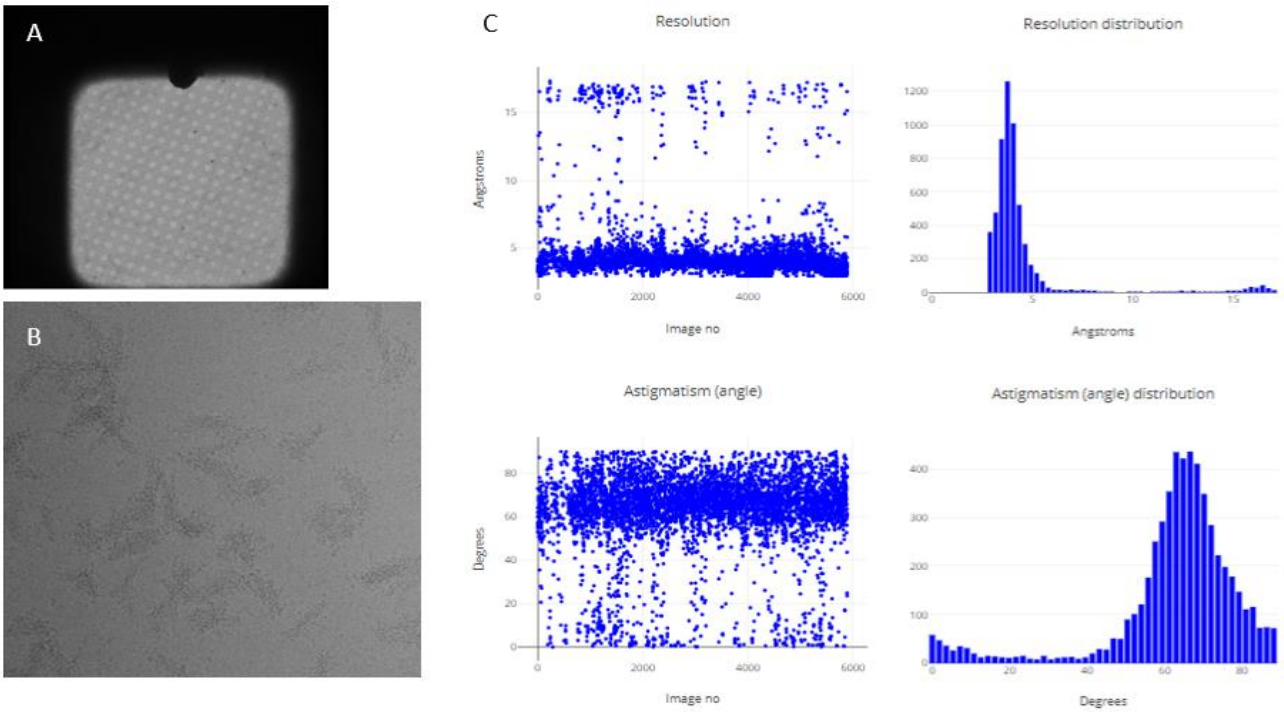

Figure 1. A. Example of selected squares for data collection. B. Carbon hole with graphene oxide containing vitrified sample of the protein complex. C. Data collection statistics.

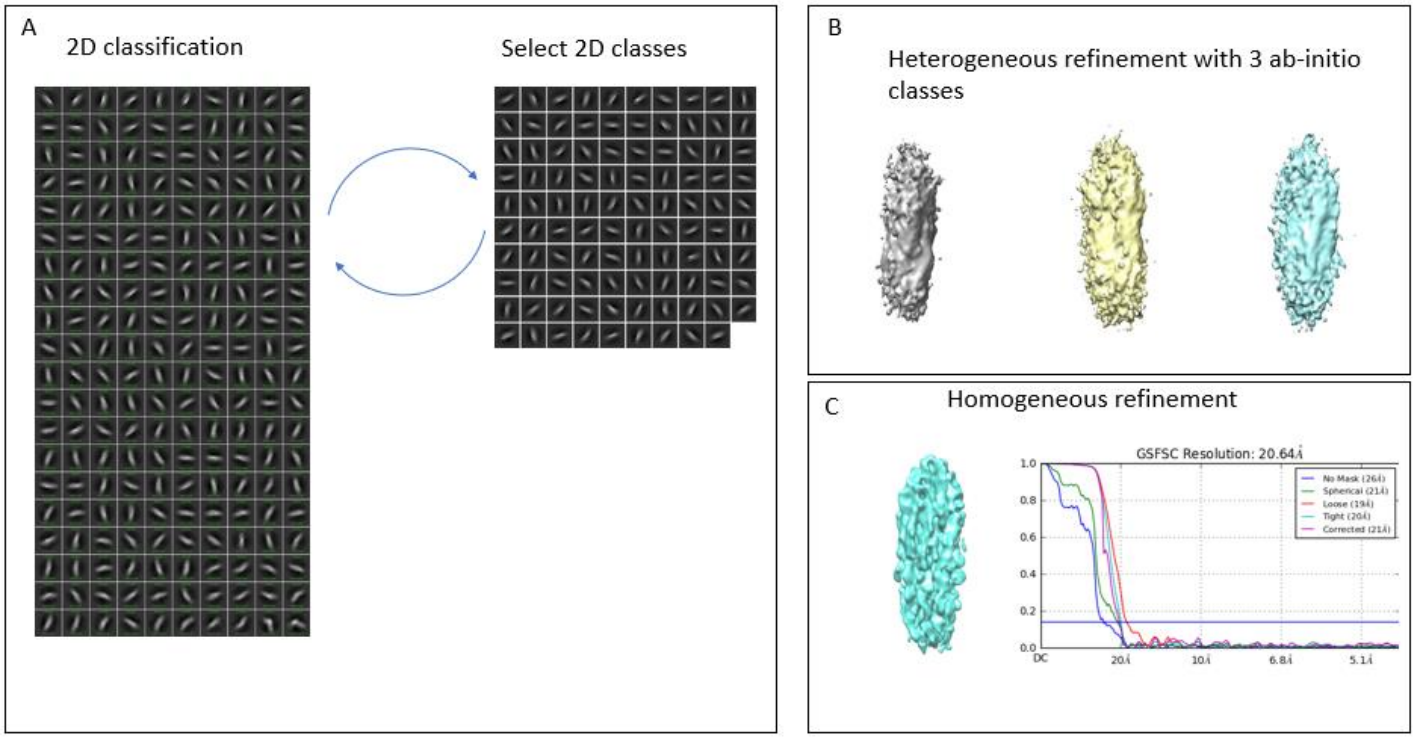

Figure 2. A. 2D classification and selected 2D classes for ab-initio reconstruction. B. 3D classes obtained after ab-initio reconstruction and refinement. C. Homegeneous refinement of one of the classes obtained during ab-initio reconstruction.