Enabling Science through European Electron Microscopy

Report on automation of experimental workflows for quantitative TEM, with applications to the measurement of weak signals, high speed and/or in situ experiments

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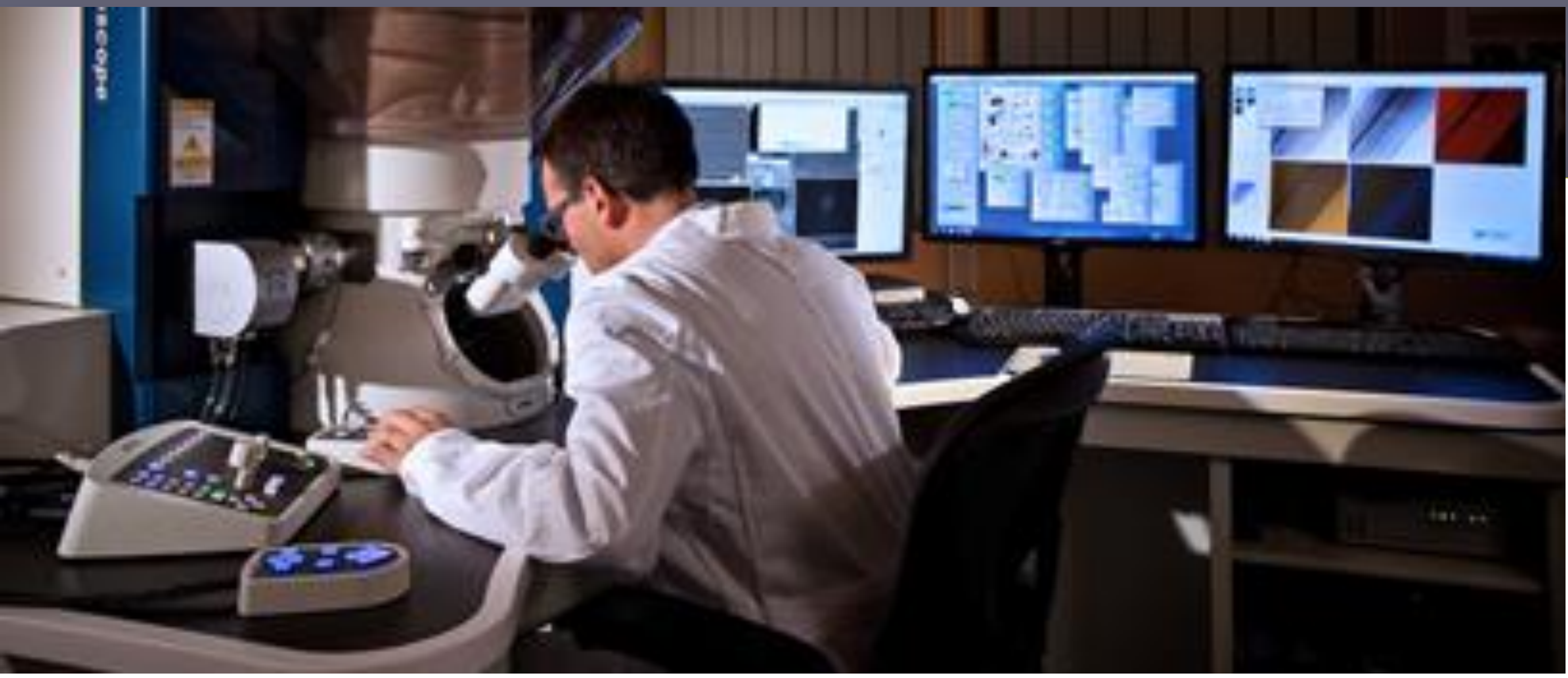

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Revision history log

Version number	Date of release	Author	Summary of changes
V0.1			First draft of deliverable
V0.2			Feedback from partners
V.03	30.11.2021	P. van Aken	Minor amendments and approval
V.04	30.11.2021	Aude Garsès	General review
V.05	06.12.2021	Dieter Weber	Minor amendments
V.1	06.12.2021	Aude Garsès	Final review

Introduction

This document is a follow-up to reports D11.1 and D11.2. Since automation, quantitative interpretation, measurement of weak signals, high speed and in-situ experiments are closely intertwined with the topics that were covered previously, it focuses on new developments since that report and aspects that are specific to automation.

Tasks

A recap of the tasks related to this deliverable:

- The development of solutions and workflows for automated instrument control, measurement of TEM and specimen parameters and stability, adjustment of TEM alignment (auto-tuning) and data acquisition for quantitative TEM (WP4), incorporating feedback loops based on measurements and automated data analysis
- The development of acquisition and analysis software and interfaces for quantitative TEM, in order to allow user-friendly, practical data acquisition, management, access, cloud storage, processing and visualisation
- The implementation of dose- and time-optimised data acquisition schemes, including control interfaces, machine learning, high speed data acquisition using direct electron detectors, fast shutters and pulsed electron sources, smart acquisition and applications to dynamic and in situ experiments (WP6)

These tasks are closely related and are therefore reported on together.

Automated instrument control, acquisition and analysis software, dose- and time-optimised data acquisition schemes

Interfaces to automate instrument control have improved substantially since ESTEEM3 started. A large number of devices and software have added Python application programming interfaces (APIs) that allow implementation of complex control and acquisition workflows. Examples were already included in the previous report D11.2. Since then, improved interfaces to CEOS hardware and DENSsolutions specimen holders were made available, as an example.

LiberTEM

Automated tuning of the QSORT device was implemented by using LiberTEM-live to feed data from a Gatan K2 camera into a neuronal network[1]. The output of the network (

[1] https://doi.org/10.1016/j.ultramic.2021.113338

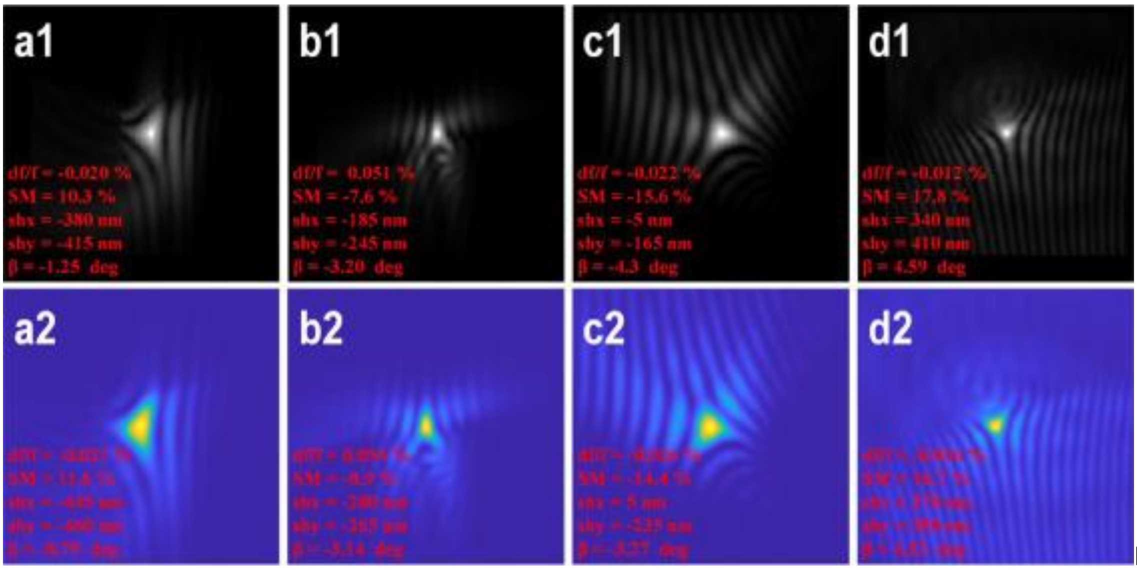

Figure 1) is used to calculate control inputs to adjust the device, and these commands are then applied to the instrument in a closed loop (JUL, with QSORT project, submitted for publication).

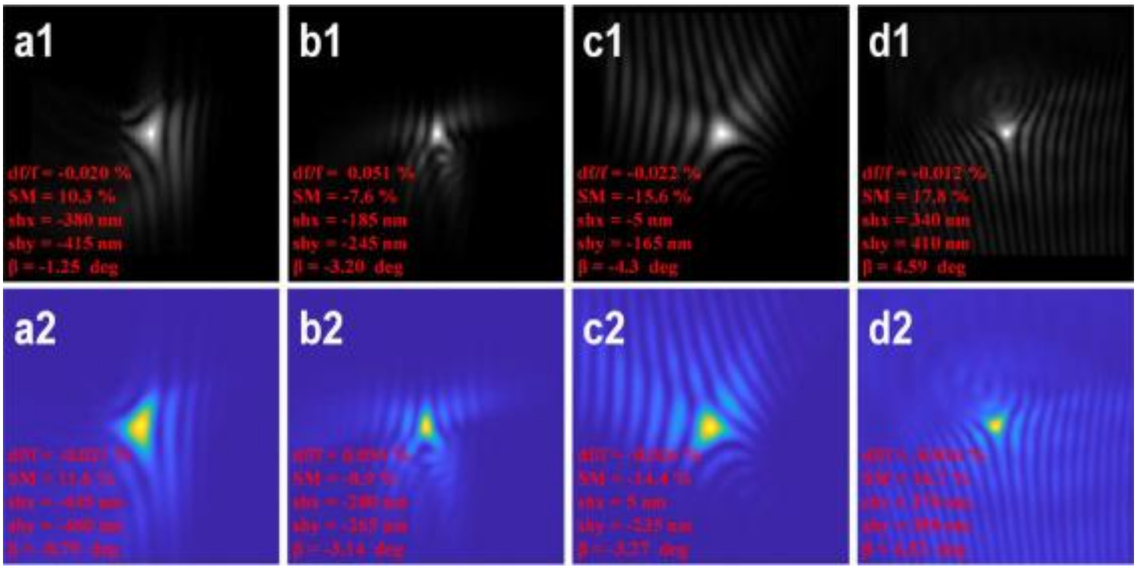

Figure 2: Adjustment parameters for the QSORT device derived from detector images using a neuronal network

Furthermore, the live processing capability in LiberTEM-live that was described in the previous report was used successfully for live 4D STEM data processing at OXF in collaboration with JUL.

Live ptychography that was described in previous deliverable D11.2 was published as a JRA success story on the ESTEEM3 website:

https://www.esteem3.eu/news?backRef=215&news=JRA_Success_Stories_LiberTEM

Similar to controlling the QSORT device, a simulation-based prototype for live data processing in combination with controlling an in-situ experiment was developed by JUL in collaboration with in-situ holder manufacturer DENSsolutions based on their recently introduced Python API. This is to be tested in the real world when access to the required instruments is available.

Integration and data exchange between Hyperspy, Pyxem and LiberTEM made significant progress. Prototypes are already working well, and this feature will likely be included in the next release of LiberTEM. This creates the opportunity to use the same file readers and the same implementations for data processing algorithms within these projects, working towards the goals of FAIR data and open, interoperable and user-friendly software for electron microscopy.

RoboTEM

Note: RoboTEM is currently not publicly available.

Automation is also about ensuring stable operating conditions during experiments. Indeed, this is an essential requirement for most quantitative techniques as changes in microscope conditions and/or specimen position complicates the analysis and introduces uncertainty. Data acquired during in-situ and operando experiments, for example, need to be compared across different specimen environments, such as temperature or electrical bias. The RoboTEM project at TOU aims to address this problem through dynamic automation of the microscope and specimen stage[2].

The initial implementation of the scheme is shown in Figure 2 on the I2TEM (Hitachi 3300-C) microscope for in-situ off-axis holography experiments. Images from the OneView camera (Gatan) are analysed continuously and in real-time using dedicated scripts running in the image-processing package Digital Micrograph (Gatan). Two processes run in parallel to control the specimen position and the hologram fringes independently.

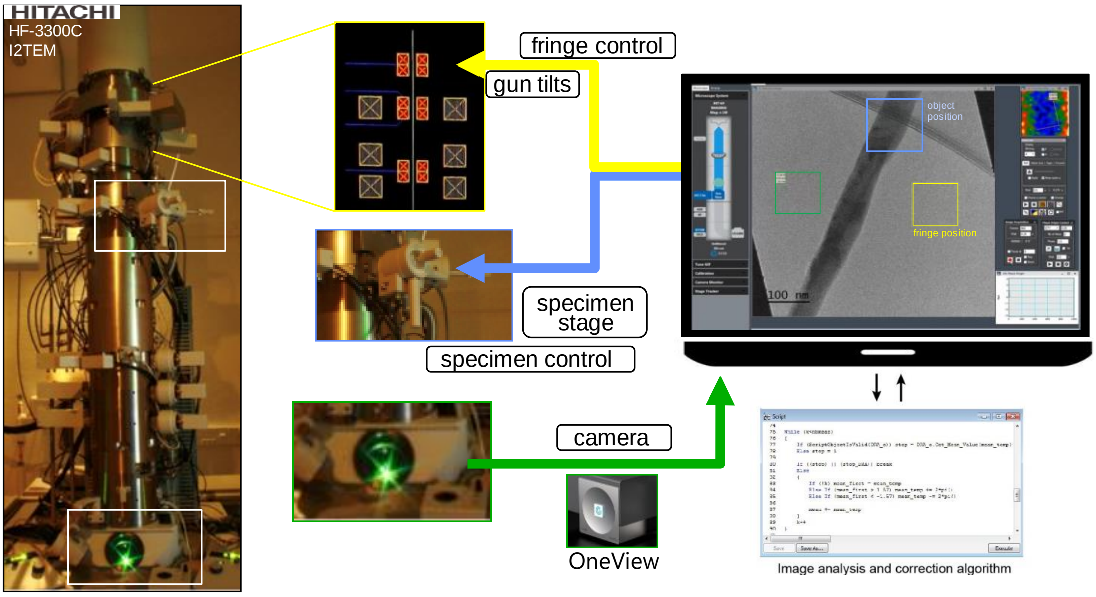

Figure 2: Dynamic automation of the I2TEM microscope (TOU). Images are analysed continuously to determine specimen position and hologram phase. Feedback loops to specimen stage and gun-tilt coils ensure stability of experimental conditions.

An interface allows the user to select a region of interest (ROI) for the specimen (in blue) which has distinctive features, and a region of uniform hologram fringes (in yellow). The quality of the hologram fringes is monitored continuously in another user-defined ROI (in green). Once the conditions are satisfactory, the user can activate the stabilisation. An essential part of the implementation was indeed

[2] C. Gatel, J. Dupuy, and M. J. Hÿtch, Microsc. Microanal. 27 (S1), 248-250 (2021). Dynamic automation in transmission electron microscopy: application to electron holography. doi:10.1017/S1431927621001471

the development of the user-friendly interface. Otherwise, experiments would be too cumbersome to be used on a regular basis.

The stabilisation of the specimen position is achieved by determining the specimen drift through cross-correlation routines. The information is then feedback to the specimen stage to supply the necessary adjustments. In this way, the specimen could be maintained (Figure 3a). Similar control is achieved for the hologram fringes by stabilising their phase relative to the image coordinates by feedback to the gun-tilt coils (Figure 3b). This allows holograms to be acquired over very long exposure times for improved calibration routines (as reported in WP4, D4.4). An additional benefit of the fringe control is that sophisticated reconstruction methods can be implemented that are impractical without automation.

The automation routines were tested successfully on the Titan-Holo (ThermoFisher) microscope in JUL equipped with a K2 direct detection device (Gatan) for both specimen and fringe stabilisation. The beam tilt coils were used for the hologram fringe stabilisation, since the gun-tilt coils were not accessible by remote-control. With this setup, feedback loops were able to apply corrections every 0.5s.

Such dynamic automation experiments were only possible under two important conditions. Firstly, the microscope and stage must be accessible by remote control from third party software. In the case of the I2TEM microscope, Hitachi supplied the communication protocols, thus giving access to all the lens currents, apertures, bi-prisms and specimen stage controls. The Titan microscope (ThermoFisher) was controlled through commands available in Digital Micrograph (Gatan) but for a more limited number of elements. Secondly, images must be processed in real time, several times a second, in order to correct the instabilities before the drift becomes too important. In the case of RoboTEM, images were acquired with rapid Gatan cameras, either in TOU (OneView) or JUL (K2). The images needed to be processed within Digital Micrograph: direct access to the camera is not provided by the manufacturer and exporting the images is too slow.

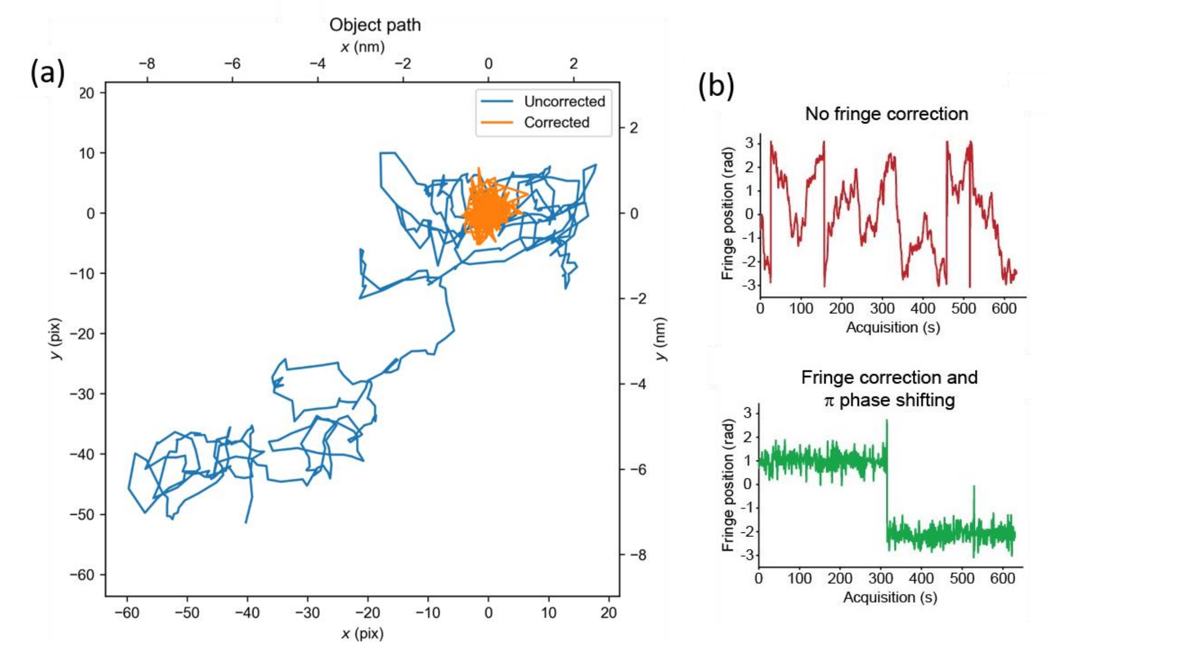

Figure 3. Stabilisation of specimen position (a) with and without automated correction (orange, blue), and phase of hologram fringes (b) with and without correction (green, red). Hologram phase

was programmed to change by exactly π radians after 300 s of exposure time to allow use of the phase-shift reconstruction algorithm.

Future experiments are planned on a JEOL microscope (UOX) to test the routines on a third platform. In particular, the fringe control will be tested for high-resolution TEM experiments under low-dose conditions. The specimen control will also be tested for dark-field and bright-field imaging during in-situ experiments, where thermal drift is an important issue. Finally, stabilisation routines have been designed in such a way that they can be integrated into step-by-step automated workflows.

HyperSpy and pyXem

Hyperspy, pyXem and LiberTEM contributors discussed the possibility of dedicated Hyperspy and/or pyXem maintainers in order to help with maintenance and continued development of this important Open Source infrastructure for data processing in electron microscopy. With the departure of Phillip Crout, pyxem is now very short of developers meaning there would be plenty of work for a research software developer. Projects like ESTEEM3 could potentially help fund and coordinate such activities.

Stabilisation plans are starting to appear for pyxem. This is deemed important to avoid the risks of developer churn.

Work on improving HyperSpy's handling of very large datasets for non-live datasets progressed at TRD is ongoing: both plotting and visualization (https://github.com/hyperspy/hyperspy/pull/2568), and generic "very large dataset" processing functionality (https://github.com/hyperspy/hyperspy/pull/2703). Both these will be a part of the next release. Especially the latter is important "backend" work for pyXem, as the "very large dataset" methods can all be used via the same Python library (dask).

Overarching activities

Activities to coordinate metadata for electron microscopy are ongoing in various settings:

- JUL: FAIRmat project Area B, Helmholtz Metadata Collaboration, Ptychography 4.0

Furthermore, a working group for software is established within the e-DREAM initiative for sustainability and long-term consolidation of these activities.