Ref. Ares(2024)6169641 - 31/08/2024

nffa.eu PILOT 2021 2026

DELIVERABLE REPORT

WP15 JA5 - Correlative Nano-Spectroscopy and Nano-Diffraction

# D15.5

# Implementation of user offer “correlative platform”

Due date
M42

This initiative has received funding from the EU’s H2020 framework program for research and innovation under grant agreement n. 101007417, NFFA-Europe Pilot Project

## PROJECT DETAILS

| PROJECT ACRONYM | PROJECT TITLE |
|---|---|
| NEP | Nanoscience Foundries and Fine Analysis - Europe\|PILOT |

| GRANT AGREEMENT NO: | FUNDING SCHEME |
|---|---|
| 101007417 | RIA - Research and Innovation action |

START DATE

01/03/2021

## WORK PACKAGE DETAILS

| WORK PACKAGE ID | WORK PACKAGE TITLE |
|---|---|
| WP15 | JA5-Correlative Nano-Spectroscopy and Nano-Diffraction |

WORK PACKAGE LEADER

Dr. Thomas F. Keller (DESY)

## DELIVERABLE DETAILS

| DELIVERABLE ID | DELIVERABLE TITLE |
|---|---|
| D15.5 | Implementation of user offer "correlative platform" |

DELIVERABLE DESCRIPTION

In this deliverable report 15.5, we introduce the "SMART" software platform, designed to provide an image registration-based tool for nanoparticle identification and re-localization. This advanced tool integrates Discrete Fourier Transform (DFT) algorithms with real-time camera streaming for continuous monitoring, and incorporates precise image registration and position refinement features. Tested at the X-ray beamline P06 at PETRA III, DESY, SMART aims to improve the interoperability between microscopy, spectroscopy, and imaging systems across lab-based and X-ray beamline facilities for users, and to permit correlative work flows.

| DUE DATE | ACTUAL SUBMISSION DATE |
|---|---|
| M42 31/08/2024 | 31/08/2024 |

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This initiative has received funding from the EU's H2020 framework program for research and innovation under grant agreement n. 101007417, NFFA-Europe Pilot Project

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## NATURE

☒ R - Report

☐ P - Prototype

☐ DEC - Websites, Patent filing, Press & media actions, Videos, etc

☐ O - Other

## DISSEMINATION LEVEL

x P - Public

☐ PP - Restricted to other programme participants & EC: (Specify)

☐ RE - Restricted to a group (Specify)

☐ CO - Confidential, only for members of the consortium

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## REPORT **DETAILS**

| ACTUAL SUBMISSION DATE | NUMBER OF PAGES |
|---|---|
| 31/08/2024 | 18 |

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| VERSION | DATE | AUTHOR(S) | DESCRIPTION / REASON FOR MODIFICATION | STATUS |
|---|---|---|---|---|
| 1 | 20/08/2024 | Dr. Jagrati Dwivedi | First draft | Draft |
| 2 | 26/08/2024 | Dr. Thomas F. Keller | Revision and submission for internal approval | Version submitted for internal approval |
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# INTRODUCTION

The work package 15 - Joint Action JA5 - Correlative Nano-Spectroscopy and Nano-Diffraction aims to develop, implement and test a correlative user platform connecting dedicated focused X-ray beamlines at analytical large-scale facilities (ALSFs) to complementary lab-based nanoscience instruments such as Scanning-Force (AFM) and Scanning Electron (SEM) Microscopy. One of the objectives of this correlative platform is to link the microscopy, spectroscopy and imaging infrastructures already in place at the nanoscience foundries and at the X-ray beamlines and to ensure the mutual compatibility and interoperability. The deliverable report D15.5, Implementation of user offer "correlative platform", describes a software-based correlative work flow permitting to conduct correlative experiments, that is now available as offer to nanoscience users, e.g., transnational users of the European NFFA Pilot access program.

To enable a correlative one-to-one identification of region of interests (ROIs), the Python- based Smart Multi-Application Running Toolkit (SMART) was developed at DESY. This software provides a tool for automated particle identification and re-localization of nanoscale objects after pre-selection with lab-based microscopes and sample transfer to X-ray beamlines at ALSFs. As a reference experiment we describe the correlative workflow utilizing the SMART software platform.

To document the implementation of the SMART software at the X-ray beamlines, a test sample with $CeO_2$ nanocubes with average particle size of 50 nm on SiN membrane was prepared by drop casting. This method enabled a precise placement and optimal sample preparation, ensuring sufficient isolation of individual particles. Isolation of nano-objects is essential to ensure that signals are collected from a single nanoparticle at a time.

Previously, NFFA deliverables and milestone reports introduced a MATLAB-scripted nano-positioning software designed for the transfer and positioning of nano-objects. This software primarily relied on high-contrast, hierarchically arranged markers created through the ion- /electron beam-induced deposition (IBID/EBID) process, as detailed by Abuin et al. (ACS Appl. Nano Mater. 2019, 2, 8, 4818–4824). This nano-positioning software is part of the NFFA user offer "Advanced Nano-Object Transfer and Positioning protocol".

Within NFFA Pilot, an image registration option was introduced with a Python-based Graphical User Interface (GUI), as described in the deliverable Report D15.2., which enabled manual image registration using fiducial markers. This tool is particularly important as it permits to create two sets of alignment marks on the transferred and reference images with the same feature.

In the current deliverable report, the advanced software tool "SMART", specifically designed for an automated identification and re-localization of a statistically relevant number of nano-objects, is described. As a reference, the application of SMART is documented for the chemical composition analysis of $CeO_2$ nanocubes within a correlative experiment.

The SMART software utilizes advanced algorithms, including the Discrete Fourier Transform (DFT) image registration tool to permit and enhance the accuracy of nanoparticle identification and tracking. One of the key features of SMART is its integration of a real-time camera stream, which allows for a continuous online monitoring of the nanoparticle behaviour. Additionally, the software includes powerful image registration and position refinement functions, ensuring precise alignment and positioning of nanoparticles during experiments. The correlative work flow was tested at beamline P06, PETRA III, DESY and the microscopes at the DESY NanoLab in the framework of several experiments.

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# SMART: A correlative platform

Building on our previous work, we have upgraded the Python GUI script "Image Registration Tool" into the more advanced software Smart Multi-purpose Application Running Toolkit SMART. This upgraded toolkit is highly customizable and user-friendly, now featuring integrated beamline control and automated particle detection capabilities. The development of the correlative work flow is a collaborative effort between PETRA III, DESY and NFFA-Pilot. SMART is designed to offer a comprehensive correlative platform that supports various microscopic as well as spectroscopic modalities, significantly enhancing the efficiency and precision of experiments. Figure 1 presents a screenshot of the SMART software interface, highlighting its user-friendly design and advanced functionalities. Through SMART, users can achieve higher accuracy in particle detection and positioning, facilitating more detailed and reliable experimental outcomes.

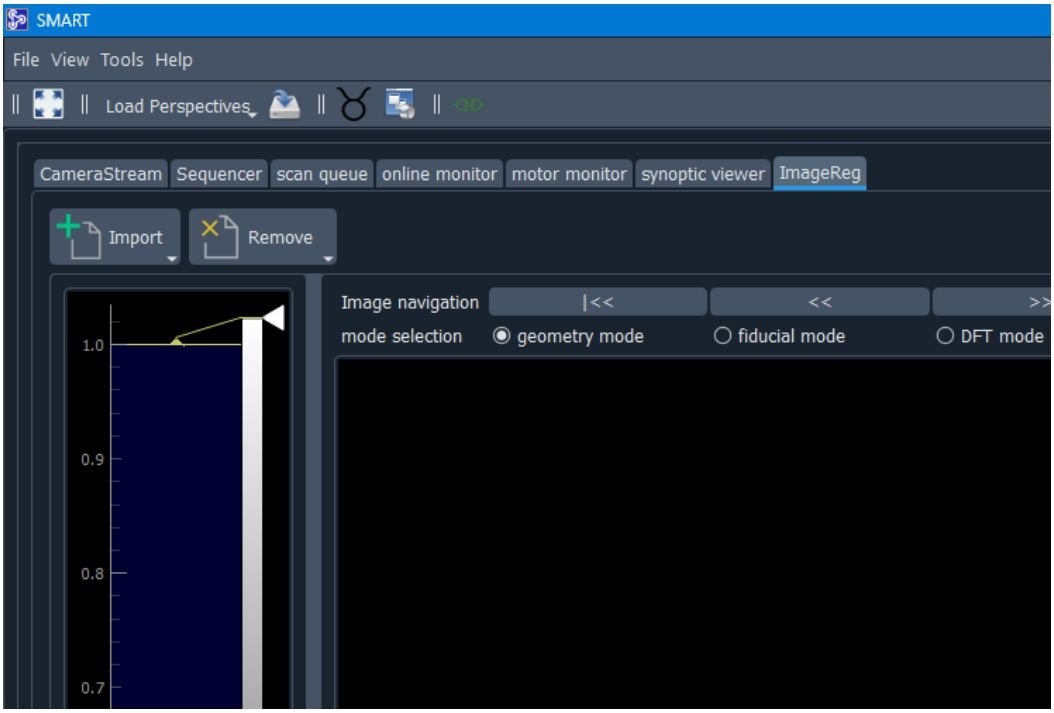


Figure 1: Screen shot of the SMART software interface.

# Implementation and application of SMART

## Sample preparation as a reference for the correlative platform

As a reference sample to document the implementation, functionalities and capabilities of the SMART software at PETRA III and the DESY NanoLab, $CeO_2$ cube powder was dispersed by drop-casting a solution of 10% ethanol in ultra-pure water on a SiN membrane substrate. Drop-casting ensured an even distribution of the nanoscale $CeO_2$ particles with a nominal average particle size of 50 nm across the substrate surface. Following this recipe enabled a precise placement and optimal preparation of the samples, ensuring the existence of isolated individual particles. Figure 2(a) represents the preparation method of $CeO_2$ nanocubes on SiN membrane, and they are clearly visible in the high-resolution SEM image in Figure 2(b).

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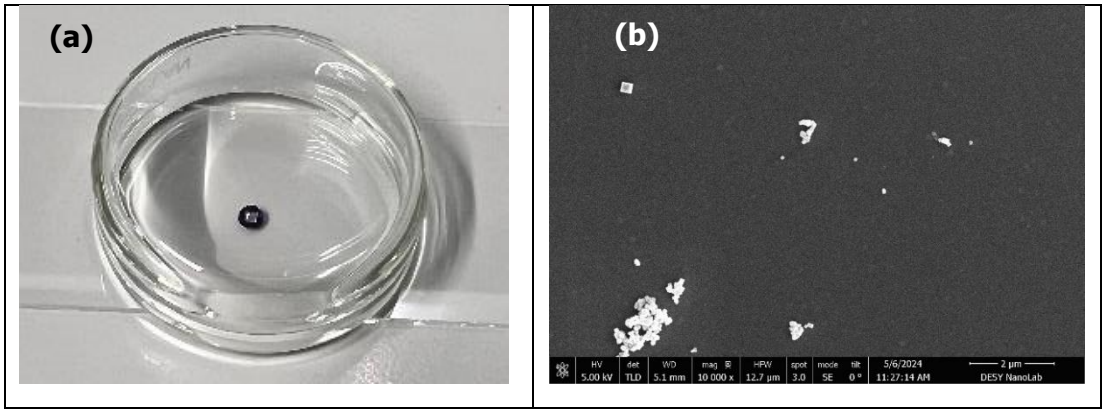


Figure 2: (a) Sample preparation. SiN membrane with drop casted $CeO_2$ nanocubes on a microscope slide, covered by a Petri dish. (b) SEM of isolated and agglomerated nanocubes.

We employed Pt markers using ion- and electron-beam induced deposition (IBID and EBID) techniques as part of the NFFA Nano-Object Transfer & Positioning offer to accurately relocate regions of interest across different imaging modalities. Figure 3(a) presents a high-resolution SEM image that includes Pt markers and clearly shows clusters of $CeO_2$ nanocubes, with a scale bar of 20 µm. Utilizing a marker-based correlative imaging approach, the region identified in the SEM was precisely relocated for X-ray fluorescence (XRF) measurements at the P06 beamline at PETRA III, DESY. This allowed us to obtain spatially resolved elemental distribution and composition of the sample, with an estimated spot size of 100 x 100 nm² at an energy of 12 keV. The XRF energy survey spectrum, shown in Figure 4(a), confirms the presence of cerium (Ce) and platinum (Pt) through distinct peaks corresponding to their characteristic X-ray energies. Furthermore, the XRF images in Figure 4(b), recorded at the $L_\alpha$-edge energies for Pt and Ce over a scan area of 120 µm × 120 µm, reveal the precise spatial distribution of these elements within the sample. The Pt $L_\alpha$ map highlights regions with high Pt concentration, particularly on the marker areas, while the Ce $L_\alpha$ image shows the distribution of Ce within the $CeO_2$ clusters, indicating areas of enrichment or depletion for both elements. Furthermore, the SEM-based energy dispersive X-ray spectroscopy (EDX) mapping of Pt and Ce confirm the distribution of Pt and Ce within the sample. The scan size of the map is 80 µm x 80 µm and were taken with a resolution of 4096 pixels at an electron energy of 10 keV. The resulting maps provide a colour-coded representation, where each colour corresponds to a specific element. The intensity of the colour reflects the relative concentration of that element, with brighter areas indicating higher concentrations. For instance, in the provided maps (Figure 3(b)), green represents Pt in the upper image, whereas yellow represents the Ce in the lower image. The EDX spectra, shown in Figure 3(c, d, e), were extracted from the Pt marker, SiN membrane, and $CeO_2$ cluster regions as marked in Ce map of Figure 3(b). The Ce signal is distinctly present only in regions with $CeO_2$ particles or clusters, while the Pt signal is only seen in the marker region. Note, that the Pt markers remain visible in the Ce EDX map due to the automated, non-optimized fit of the EDX spectra that also lead to an enhanced Ce background. Moreover, the resolution of specific imaging modalities is in general not identical and needs to be adjusted by the SMART software.

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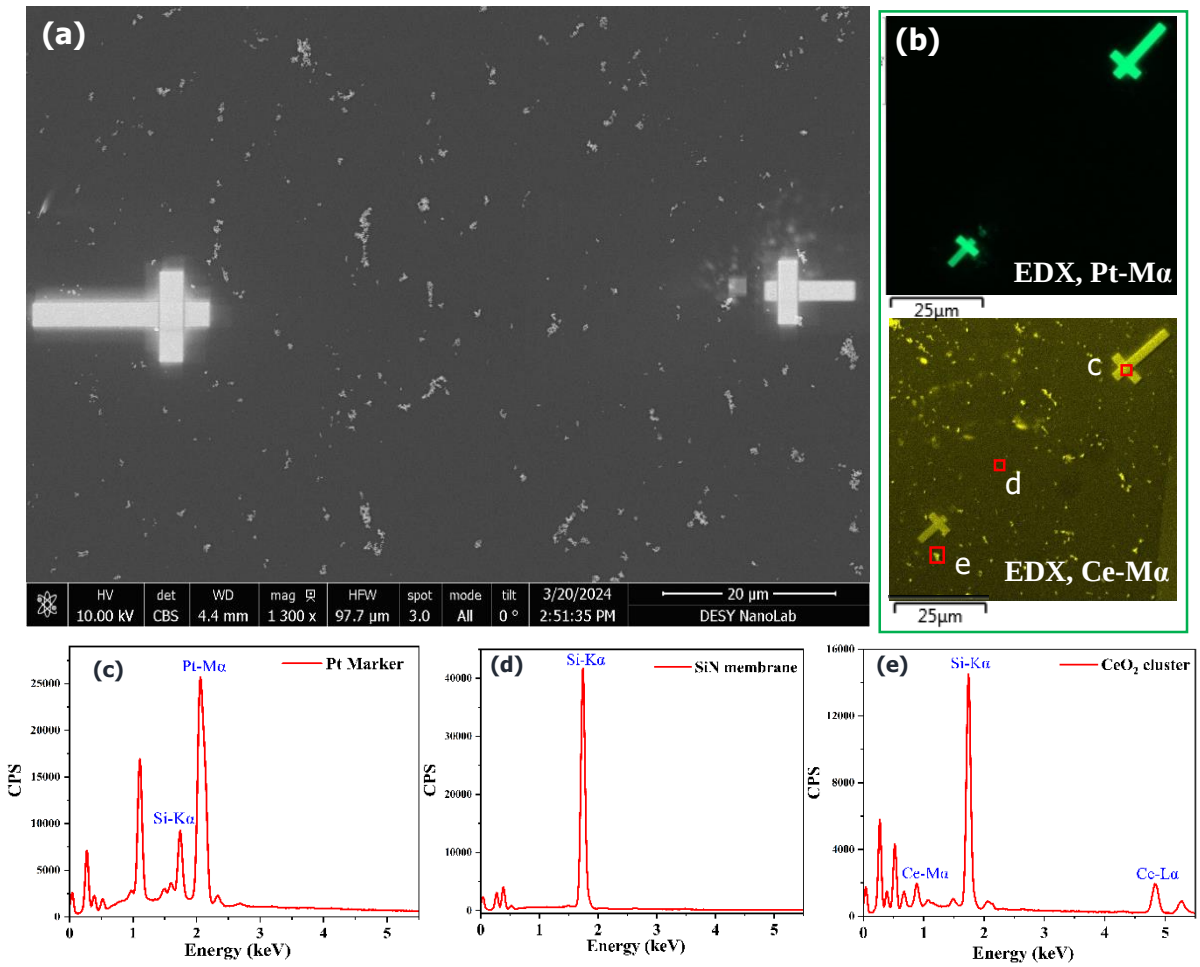


Figure 3: (a) SEM image showing $CeO_2$ nanocubes with a Pt guiding marker. (b) High-resolution energy dispersive X-ray (EDX) mapping of Pt and Ce at the Mα edge. The EDX spectra belong to the (c) Pt marker, (d) SiN membrane, and (e) $CeO_2$ cluster region as marked in the Ce-Mα map.

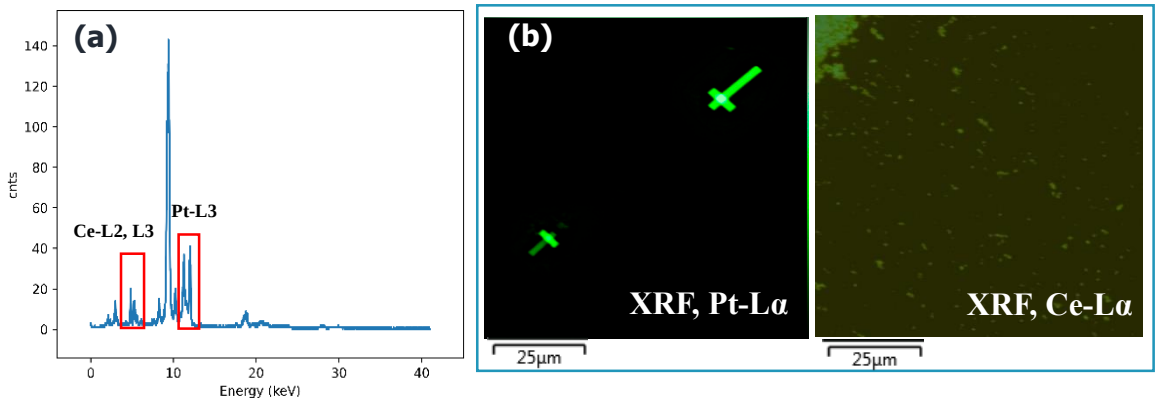


Figure 4: ALSF X-ray experiment at beamline P06 at PETRA III at DESY. (a) X-ray fluorescence spectra and (b) X-ray fluorescence scanning images of the Pt-L and Ce-L edge collected from the identical region during a single scan.

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## Beamline control

SMART comes with a PyQt-based GUI, a Python-plugin of a platform independent graphical user interface toolkit, which can be configured to monitor the whole beamline in terms of motor positions, detector status, scan status and even more. The SMART software is designed to map the relationship between the viewport, i.e. the field of view for the current imaging modality, and the motor stage coordinate systems with high precision. This capability is essential for seamless integration of different imaging modalities. The process begins with capturing a microscopy image, such as AFM or SEM, mounting the sample on the sample stage at the X-ray beamline, and then acquiring a live optical microscopy image with the inline optical microscope for a course alignment. After loading the AFM or SEM image, the live image is registered to the latter using Discrete Fourier Transform (DFT) techniques, ensuring accurate alignment of features across the different images. For registration, a region of interest (ROI) is selected on the AFM image, for example around a marker region, to obtain its viewport coordinates. A corresponding feature is then identified in the live image, and its viewport coordinates are recorded. The SMART software then converts these viewport coordinates into motor stage coordinates, enabling precise positioning for subsequent scans or the creation of a macro. The strategy used for mapping the position relationships between the viewport and motor stage coordinate system is illustrated in Figure 5. In a second iteration, the alignment may be refined by using a higher resolution SEM and AFM image and relocalisation features visible in X-ray scanning or full-field images, taken with one of several possible contrasts, like e.g., X-ray absorption, XRF, Bragg, etc.) for a fine alignment.

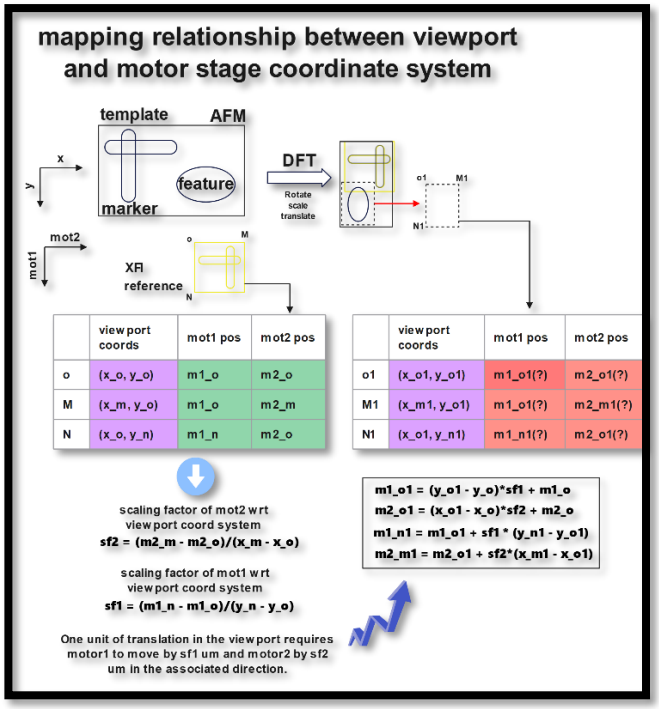


Figure 5: Sketch of the methodology to implement the mapping relationships between viewport and motor stage coordinate systems in the SMART software.

Additionally, the SMART software includes a camera stage control (see Figure 6), which allows users to adjust the imaging stage in real-time to fine-tune the alignment and focus on the sample. The software also features a scan macro generator option (see Figure 6), which simplifies the process of defining and executing scan sequences. This option allows users to create customized scan paths

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based on the mapped coordinates, ensuring that the X-ray beam or other analytical tools target specific areas of interest with precision. By automating these processes, the SMART software significantly enhances the efficiency and accuracy of a nanoscale analysis, making it a powerful tool for correlative microscopy and spectroscopy.

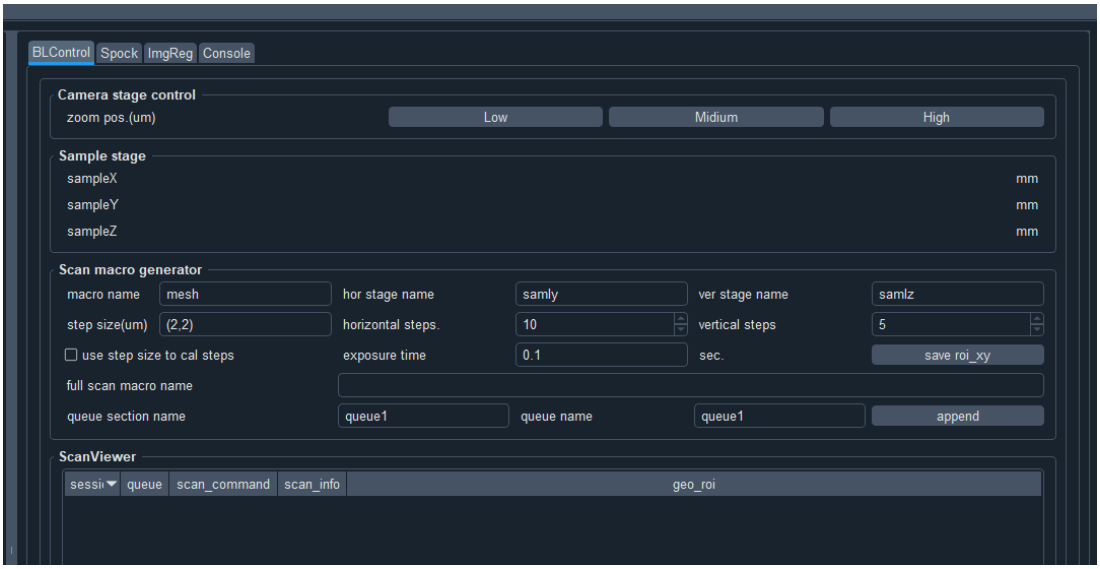


Figure 6: Screenshot of beamline (BL) control window in the SMART software.

## Image registration using DFT

In the previous deliverable report D15.2, we thoroughly discussed the various algorithms and features utilized in the Python script to functionalize this program, including the import module, geometry, and more. Additionally, we employed two sets of manually generated lines using the "fiducial marker" for image registration. Now, the SMART software, provides an advanced automated feature analysis implementation with a high level of precision, which can be integrated at selected focused X-ray based beamlines. Notably, two new features have been included in the SMART software: DFT - Discrete Fourier Transform - registration and Automated Particle Detection.

Figure 7 provides a step-by-step illustration of the DFT registration process. Users can upload images from different imaging modalities with appropriate resolution via the "Import" menu located at the top left corner of the software (see Figure 7-1). They can also adjust dimensions and drag the images into the workspace using the "geometry" menu (highlighted in yellow). The DFT registration tool allows the selection of two corresponding features on both images. One image is selected as the reference, while the other is referred to as the target image (Figure 7-2, 3). Users can then draw a box (see the green box in Figure 7-4) around corresponding features on both, the transferred and the reference images and save the selected regions using the "select feature" option indicated by the red circle in Figure 7-4. By clicking "register", the software generates a new frame with the registered images. The green region in Figure 7-5 highlights the registered area. In this example, we utilized two SEM images of $CeO_2$ nanocubes on a SiN membrane with different spatial resolutions. Similarly, SEM and XRF images were registered using the procedure described above, as shown in Figure 8.

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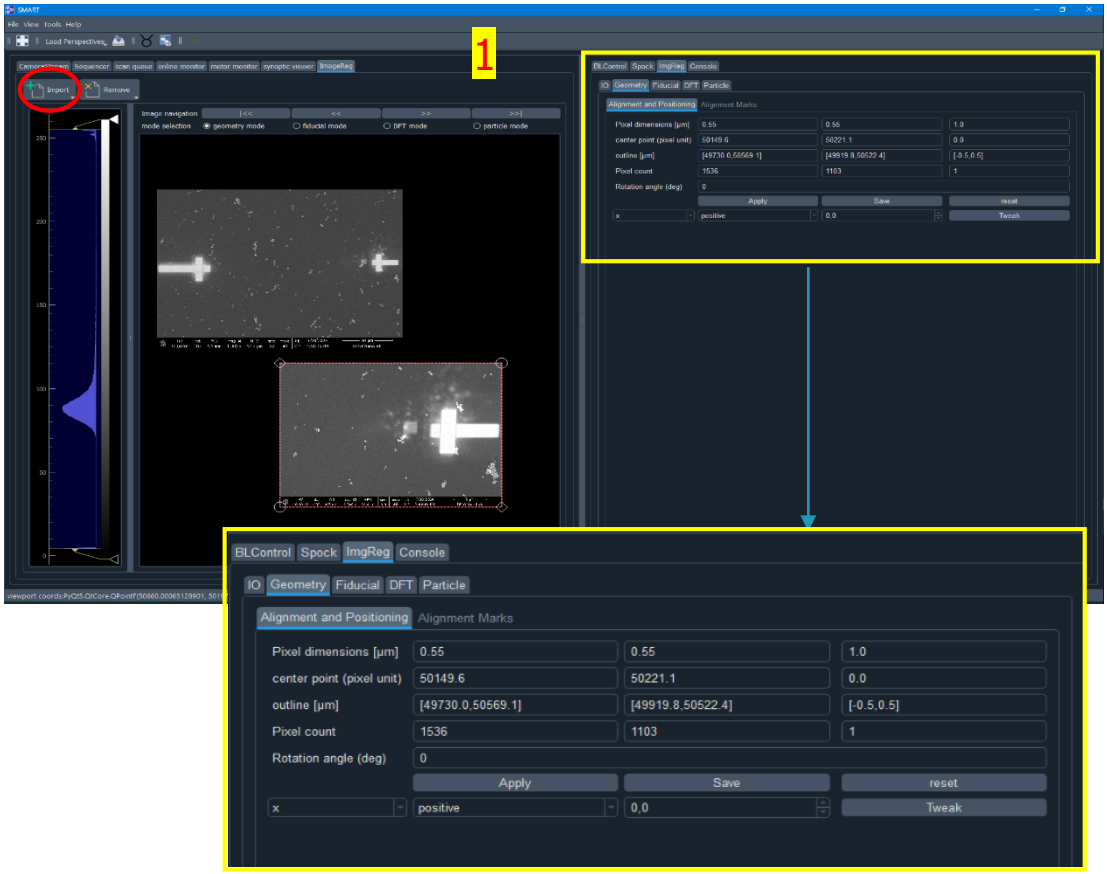


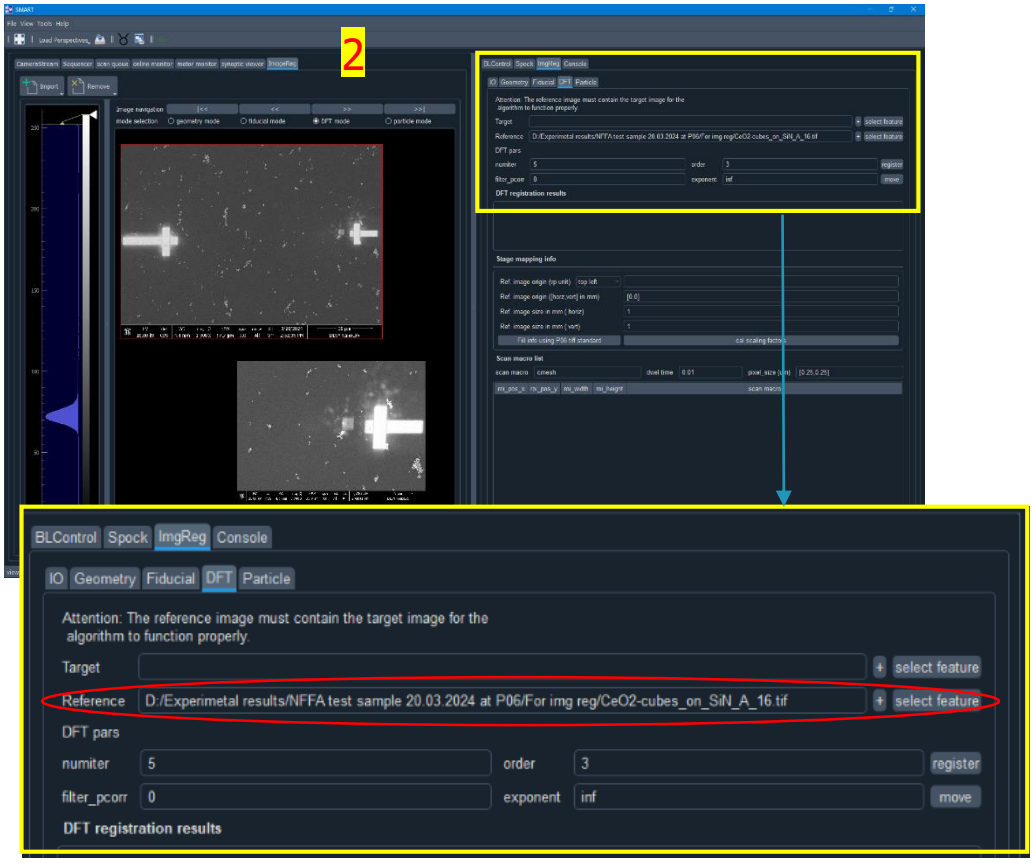


Figure 7: to be continued

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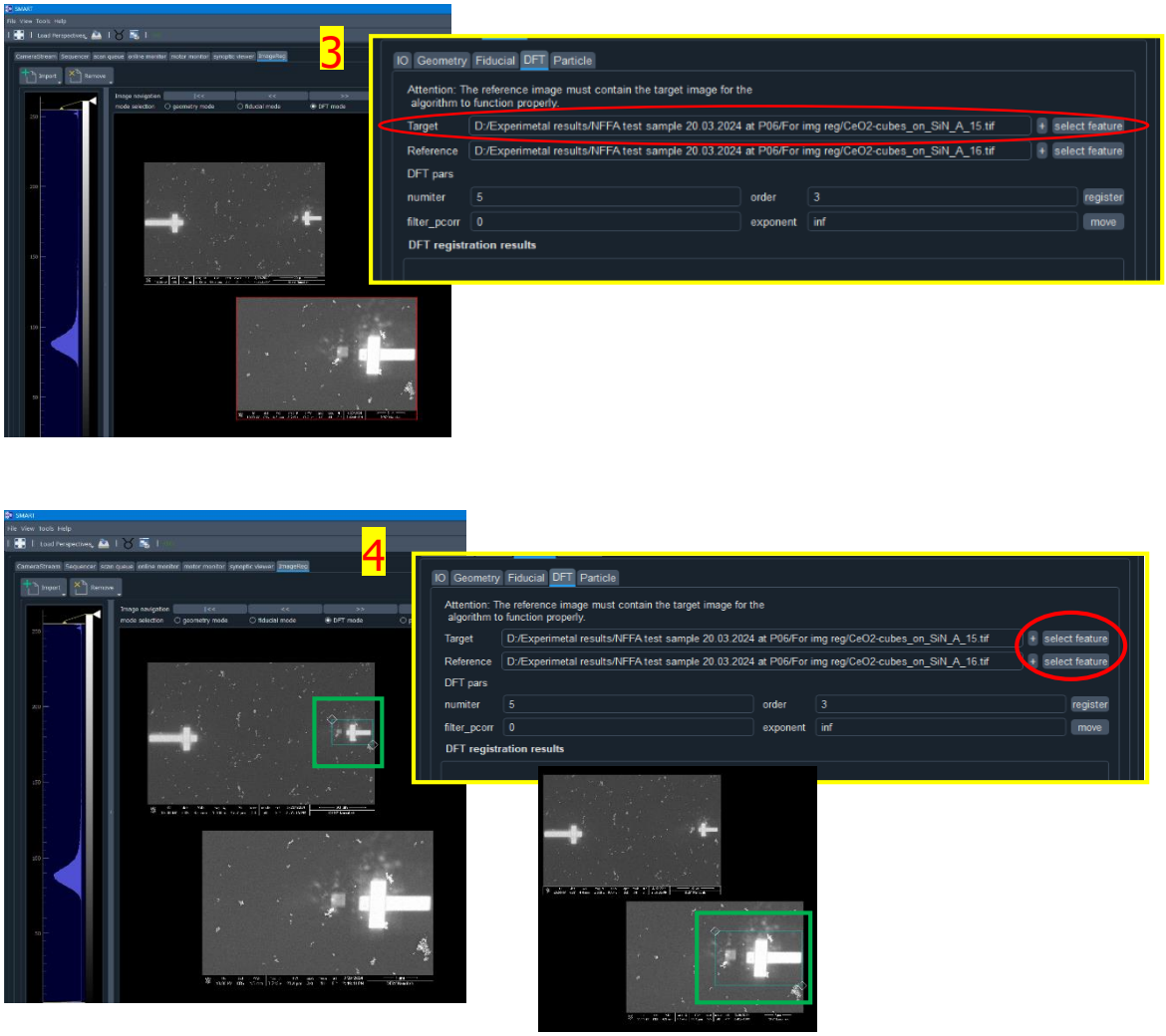


Figure 7: (1-5) Screenshots of the various steps of the DFT image registration process using two different resolution SEM images that users have to follow while using this software.

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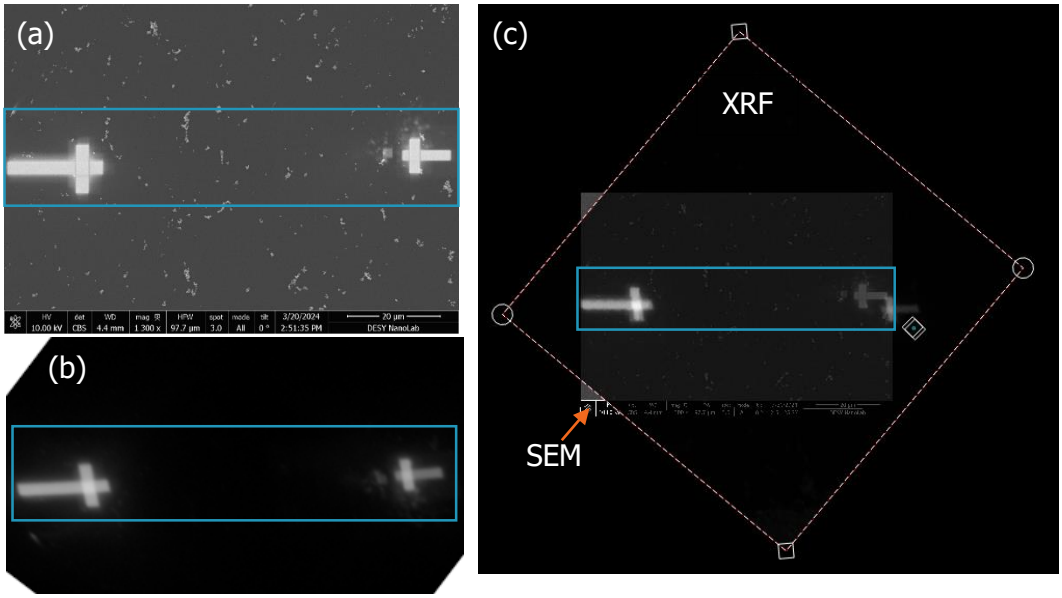


Figure 8: (a) SEM image, (b) image-registered XRF images. (c) SEM/XRF overlay using the SMART software. The blue frame indicates the common image region.

## Particles auto detection

The SMART software also integrates a precision Trackpy algorithm for the identification and localization of isolated nanoparticles, enhancing its ability to identify and track particles in 2D and 3D with high resolution. This functionality is crucial for the analysis of a statistically relevant number of isolated nano-objects. It also requires sample preparation protocols, see., e.g., the deliverable report D15.3, fabrication of nanoparticle pattern templates, to ensure a sufficient nanoparticle isolation. By these means it can be ensured that the footprint of the focused X-ray beam, typically ranging from tens of nanometres to several micrometres and influenced by factors such as the X-ray energy and the sample's tilt angle. illuminates only single nanoparticles. Then, data could be collected from single nanoparticles only, a process that so far requires lengthy lateral scans and manual identification during data analysis. By automating nanoparticle localization, the SMART software could reduce the time needed for future X-ray analysis by focusing only on pre-determined nanoparticle sites. Overall, the particle tracking capability permits to achieve one-to-one structure-property correlations from ALSF X-ray beamlines and microscopes at Nano labs. Figure 9 demonstrates a screenshot of the particle tracking window.

The particle tracking window offers several key features to help identify and analyse particles. One can set the diameter in pixels to estimate feature sizes within work frame. The size must be an odd integer. The software also allows to adjust the "mass" parameter and use the "invert" option to handle images with dark features. The threshold setting helps in differentiating actual particles from noise by evaluating their total brightness. Additionally, one can specify the minimum mass to filter out unwanted particles. The results are displayed both as annotated particles on the image and in a tabular format with details such as pixel coordinates and size etc. as shown in Figure 10.

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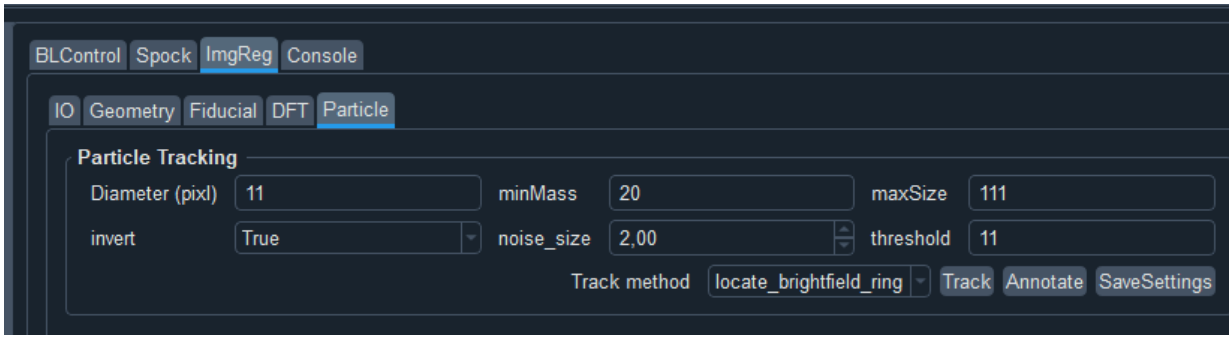
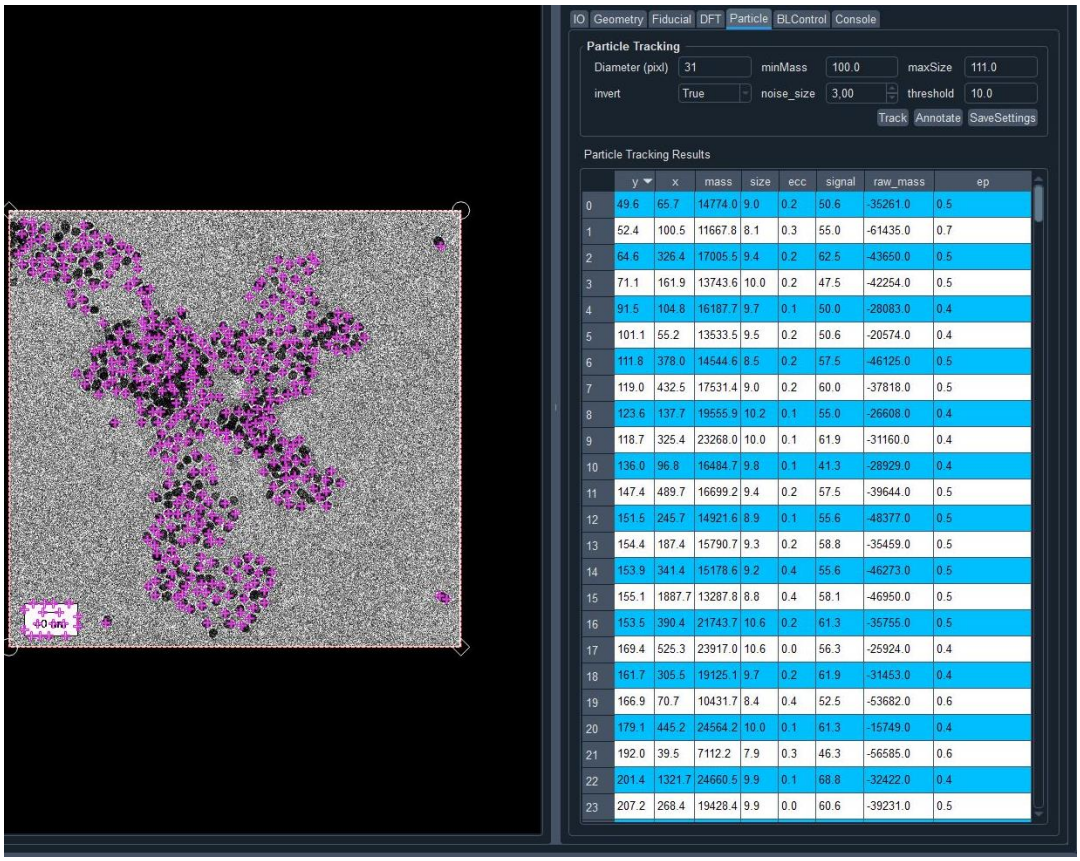



Figure 9: Screenshot of particle tracking window within the SMART software.

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| 23 | 207.2 | 268.4 | 19428.4 | 9.9 | 0.0 | 60.6 | -39231.0 | 0.5 |

Figure 10: Particle detection and annotation using particle tracking feature in SMART software.

## Open source information

SMART is an open-source project. The source code can be downloaded from GitHub: https://github.com/jackey-qiu/smart-line

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# Developments at contributing institutions

Integrated concepts for correlative work flows are also developed at the institutions participating in the work package 15 - Joint Action JA5 - Correlative Nano-Spectroscopy and Nano-Diffraction, e.g. at the synchrotrons SOLEIL and the ESRF.

As one example, at SOLEIL, the correlative measurements including scanning transmission X-ray microscopy (STXM), spectro-ptychography, and scanning transmission electron microscopy (STEM) were performed to demonstrate enhanced photoelectrochemical performances by simply annealing Ti-doped hematite photoanodes grown by aqueous chemical growth under nitrogen compared to the commonly used air annealing (shown in Figure 11). The same nanoparticles were analysed using STXM (Figure 11(a)), spectro-ptychography (Figure 11(b)), and STEM in EDXS (energy dispersive X-ray spectrometry) and HAADF (high angle annular dark field) modes (Figure 11(c,d), respectively). This correlative approach was possible using a relocation approach that utilizes special hole-tagged SiN membranes which are compatible with multiple microscopy techniques. Yellow arrows in the Figures 11(a-d) guide the viewer to identical sample positions across microscopic images obtained from different techniques. Using STXM, the full NEXAFS spectral region across the Ti $L_{2,3}$ absorption edge was measured with high spectral resolution, achieving better than 0.1 eV accuracy. The RGB color-coded map (Figure 11(a)) was obtained using the Singular Value Decomposition (SVD) method in the aXis2000 software, following drift correction. At the HERMES beamline, spectro-ptychography was seamlessly integrated by replacing the standard photomultiplier 0D detector tube with a 2D sCMOS camera, without altering the sample setup. Spectro-ptychography data were recorded from the same region of interest (ROI) as STXM (Figure 11(b)), generating an RGB map across the Ti L3 t2g transition using SVD, although fewer energy points were measured due to the extensive data size. The spectro-ptychography reconstructed image revealed surface clusters ranging from approximately 10 to 100 nm, with a pixel size of 8.5 nm. Furthermore, analytical STEM-EDX (Figure 11(c)) on the same nanoparticles confirmed the presence of Ti-rich areas at the surface of the Ti-doped hematite particles, with a corresponding HAADF image providing additional structural context (Figure 11(d)). Figure 11(e) shows the Overlay of spectro-ptychography and STEM signals.

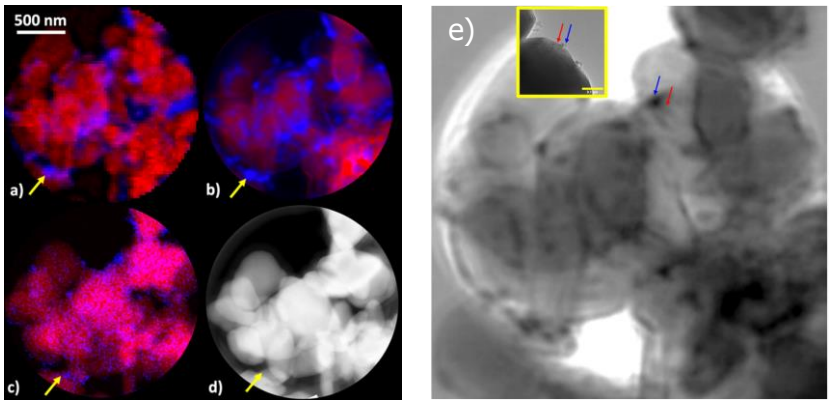


Figure 11: a) Overview of STXM, b) spectro-ptychography, and c,d) STEM EDXS and HAADF results obtained from the same regions. e) Overlay of spectro-ptychography and STEM signals. Reprinted with permission from ACS Appl. Mater. Interfaces 2023, 15, 22, 26593–26605. Copyright 2023 American Chemical Society.

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At the ID21 beamline of the ESRF, $Si_3N_4$ membranes with Pt markers written by ion- and electron-beam induced deposition (IBID and EBID) techniques will be used as part of the NFFA activities for transferring and positioning nano-objects. These Pt markers, with a thickness of 100 nm will enable precise alignment in correlative analyses with X-ray fluorescence and X-ray absorption spectroscopy techniques at ID21.

Initially, three membranes will be analysed to conduct preliminary tests. These tests are designed to evaluate the quality of the Pt markers, and based on the results, adjustments may be made to the fabrication process before producing more membranes.

The Pt markers were placed in three of the four corners of each $Si_3N_4$ membrane. Three membranes (Figure 12, left) have been produced with these markers. The markers are located 200 µm from the edge of the membrane, ensuring their correct placement in the corners. The SEM image in Figure 12 on the left shows the arrangement and proportions of the markers on the $Si_3N_4$ membrane.

The markers consist of a $2 \times 2$ µm$^2$ square and L-shaped elements measuring $6 \times 3$ µm$^2$, which are visible under a 10× objective. These elements are contained within a 10x10 µm frame, located $200 \times 200$ µm$^2$ from the membrane corner. Figure 12 on the right shows an SEM image of one of the $Si_3N_4$ membranes with the deposited Pt markers, confirming their correct deposition and positioning.

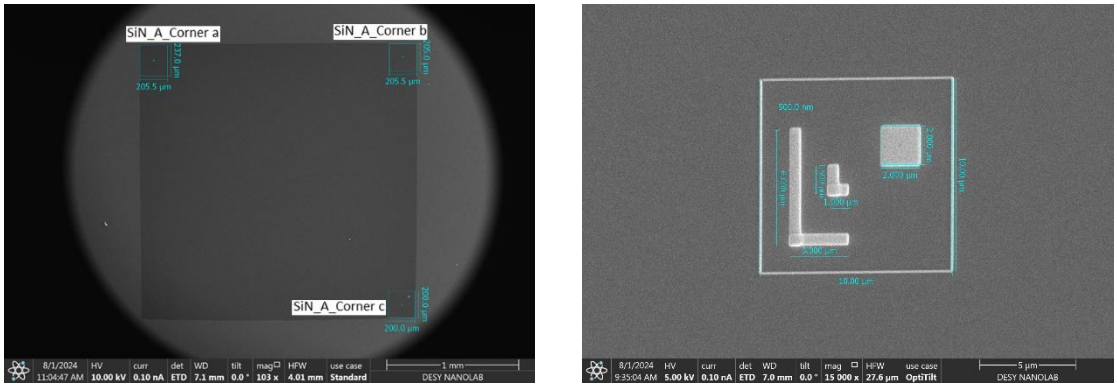


Figure 12: left, SEM image showing the positioning of the markers at the corners of the $Si_3N_4$ membranes, right, SEM detail of the geometric structure of the Pt markers on a $Si_3N_4$ membrane.

At ID21, the Daiquiri interface (Fisher et al., J. Synchrotron Radiat., 28(6), 1996-2002, 2021) is used for sample navigation and registration. This tool allows marking regions and collecting 2D µXRF maps, as well as multispectral µXRF maps in regions of interest, while µXAS is performed on points of interest. Therefore, to have the correlative studies, the external images obtained from scanning electron microscopes (SEM) or visible microscope are imported in Daiquiri to locate regions of interest for X-ray analysis.

Once the images are imported, reference points or fiducials are selected on the image. These points must be easily recognizable and characteristic, as they will be used to align the sample in the microscope. At least three fiducials are required to create a well-defined area of interest. Subsequently, the fiducial points are linked to the corresponding positions in the microscope image, allowing for precise alignment of the sample on the stage and ensuring that the areas of interest are correctly located for further analyses. Additionally, the Daiquiri interface enables multiple images to be added to the canvas for large samples, facilitating detailed correlative analysis in different areas of the sample.

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# CONCLUSION AND OUTLOOK

The work package JA5 focuses on implementing a correlative platform that bridges microscopy, spectroscopy, and imaging infrastructures at nanoscience foundries and X-ray beamlines. The SMART software developed, implemented and tested partly within deliverable report 15.5 automates the identification and re-localization of nanoscale objects when transferred from lab-based instruments to large-scale X-ray facilities. Utilizing advanced algorithms, including DFT for precise alignment, SMART also incorporates a real-time camera stream for continuous nanoparticle monitoring during live experiments and for image registration and position refinement functions. The software was implemented and tested at the P06 beamline, PETRA III, DESY, using $CeO_2$ nanocube samples prepared in such a way to ensure the existence of isolated, individual nanoparticles. By employing Pt markers, imaging registration tools and correlative imaging approaches, the software facilitated accurate relocation and elemental analysis of the nanoparticles using X-ray fluorescence (XRF), as directly compared to SEM-EDX mappings. These features, combined with the high-resolution capabilities of the SMART software, mark a significant advancement in nanoscale research, allowing for precise structure-property correlations and improving the efficiency of beamline experiments. This work advances the correlative platform to include automated feature analysis and integration with focused X-ray beamlines, aiming to enable one-to-one correlative analysis of a statistically relevant number of nanoparticles. The platform is now ready to link the existing microscopy, spectroscopy, and imaging infrastructures at nanoscience foundries and X-ray beamlines, ensuring the mutual compatibility and interoperability needed to offer comprehensive translational access to users.

## Acknowledgements

SMART is part of the PETRA III beamline P25 project, which is a joint project between DESY's Industry and Technology Transfer (ITT) group and Photon Science, under the umbrella of the DESY Innovation Factory. $CeO_2$ cubes where provided from Lachlan Caulfield and Dr. Eric Sauter from the Institute of Functional Interfaces (IFG), Karlsruhe Institute of Technology, Karlsruhe, Germany.

This initiative has received funding from the EU's H2020 framework program for research and innovation under grant agreement n. 101007417, NFFA-Europe Pilot Project