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DELIVERABLE REPORT

WP14 JA4 - A safe-by-design platform for nanomaterials

D14.4

Integration and characterization of the space correlation functionality on the complete setup

Due date

M51

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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D14.4	Integration and characterization of the space correlation functionality on the complete setup

DELIVERABLE DESCRIPTION

Preliminary results from a pilot study using Si_3N_4 membranes with Pt markers to enable correlative imaging techniques, including optical microscopy, SEM, and synchrotron-based µXRF and µXANES.

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NATURE

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☐ P - Prototype

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

☐ O - Other

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

☐ RE - Restricted to a group (Specify)

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1. INTRODUCTION

This report presents an experimental workflow designed to perform correlative measurements as part of the NEP-NFFA project, using silicon nitride (Si_3N_4) membranes equipped with platinum (Pt) markers. These membranes were developed through a collaboration between DESY NanoLab and ESRF-ID21. DESY NanoLab was responsible for the Pt deposition on the Si_3N_4 membranes, while ESRF-ID21 carried out the correlative measurements. The purpose of the Pt markers on the membranes is to act as fiducial points that help to precisely locate specific regions or points of interest (ROI/POI) with micrometric or nanometric accuracy. This is essential to analyze the same point of the sample using different techniques, and to collect complementary data for a better understanding of the sample.

The proposed workflow involves complementary techniques such as optical microscopy, scanning electron microscopy (SEM), and synchrotron-based techniques like micro X-ray fluorescence (µXRF) performed at the nano-X-ray microscope (nano-SXM) at beamline ID21 of the Softhis report describes the initial design of the membranes with the markers, as well as the optimized version, based on results obtained during the first tests with nano-SXM. In addition, it shows how the Pt markers enable accurate correlation through the web-based graphical interface Daiquiri, linking the morphological information from optical microscopy with the chemical information obtained from µXRF. The technical feasibility of this approach has been confirmed, showing that the process is reproducible and potentially applicable to similar studies. This is possible due to the standardized Pt deposition process and the use of nano-SXM for sample localization and data acquisition.

2. METHODOLOGY

2.1 Fabrication of markers on Si_3N_4 membranes

The Pt markers were deposited using a Dual-beam Focused Ion Beam (FIB) instrument via ion beam induced deposition (IBID) of a Pt containing precursor gas, dosed through a gas injection needle (DESY NanoLab - 2016)[1]. A Ga ion source operated at an acceleration voltage of 30 kV and ion currents of 10 pA and 1.6 pA were used. Before writing the markers, a carbon coating of about 5 nm thickness was deposited on the membranes using a sputter coater in order to improve the electrical conductivity needed for better FIB writing and proper observation in the SEM.

2.2 Correlative measurement workflow

The experimental process begins by placing the sample on the membrane that includes the Pt markers as described above. Then, initial images are taken using optical microscopy (Zeiss Axioplan 2), with the goal of identifying specific areas of interest. Depending on the technique used, different key features of the sample can be visualized. These images show precisely the position of the sample in relation to the Pt markers. Subsequently, the sample is transferred to the nano-SXM microscope.

[1] Stierle, A., Keller, T. F., Noei, H., Vonk, V., & Roehlsberger, R. (2016). Desy nanolab. Journal of large-scale research facilities JLSRF, 2, A76-A76.

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In this instrument, sample positioning and navigation are controlled with the Daiquiri interface[2]. The optical-microscopy images taken before are loaded into Daiquiri to align the sample on the nano-SXM stage, and at least three fiducial points defined by the Si_3N_4 layout, the sample, and the Pt markers are chosen to transfer the coordinates accurately. After the experiment, the same data can be viewed and processed in Mimosa, a user-friendly web portal based on Daiquiri for post-treatment of ID21 data.

The selected points in the previous images are linked to their corresponding positions in the nano-SXM images, allowing accurate coordinate transfer. This step is essential to align the region of interest and ensure that the same site is analyzed using µXRF. Finally, the ROIs are defined to acquire 2D µXRF maps, revealing the chemical states and elemental environments at the local scale.

The test sample consisted of thin section of an alfalfa plant leaf, previously embedded in epoxy resin. The thin section (6 µm thick) was placed directly on membrane F, centered around the Pt marker. Ethanol was used to help the fragment adhere; after evaporation the resin block remained fixed to the Si_3N_4 surface. The goal was to detect elemental signals of manganese (Mn, from salt deposits), calcium (Ca, structural component of the plant cell wall), and Pt (fiducial marker) by µXRF, while using the marker itself for accurate coordinate transfer. The plant material covered only the area near the marker, not the entire area of the membrane F was covered with the sample

3. RESULTS

3.1 First set of membranes

3.1.1 Design

The first set of membranes developed by DESY NanoLab consisted of three units, named SiN_A, SiN_B, and SiN_C. This set was characterized by including the marker design shown in **Figure 1**.

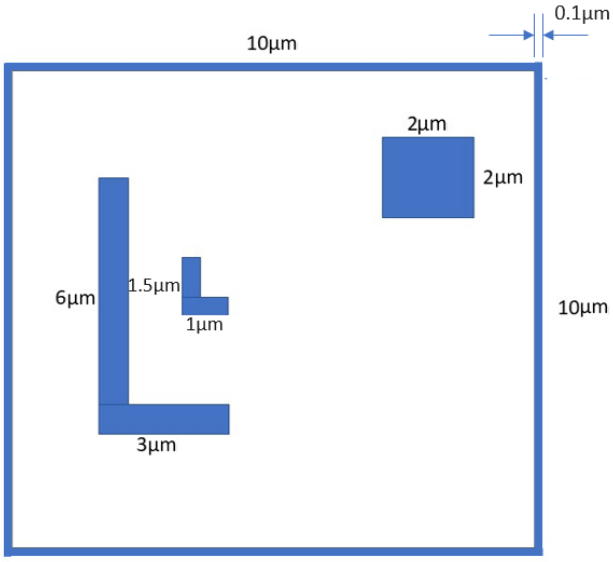

Figure 1. Pt marker design for the first membrane set

[2] FISHER, Stuart, et al. Daiquiri: A web-based user interface framework for beamline control and data acquisition. *Synchrotron Radiation*, 2021, vol. 28, no 6, p. 1996-2002.

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In the initial design, shown in **Figure 1**, a square outer frame of 10 µm × 10 µm was included, with a line thickness of about 100 nm. Additionally, internal markers were added, consisting of:

- A large "L" shape with arms of 6 µm (vertical) and 3 µm (horizontal), both with a line width of 0.5 µm.
- A small "L" shape with arms of 1.5 µm (vertical) and 1 µm (horizontal), also with a line width of 0.5 µm.
- A solid square marker of 2 µm × 2 µm, located near the top-right corner of the frame.

In this design, each membrane (SiN_A, SiN_B, SiN_C) was defined to include three fiducial markers, placed in three out of the four corners. Each marker (**Figure 1**) was positioned at a distance of 200 µm in both horizontal and vertical directions from the corresponding vertex.

3.1.2 Fabrication of the Pt markers

The first Pt markers on Si_3N_4 membranes were successfully deposited. **Figure 2** shows an SEM image of one of them, showing the result of the deposition process using the Dual-beam Focused Ion Beam instrument. The image clearly shows the precision and quality of the Pt deposition. The dimensions of each marker element matched the specifications of the initial design.

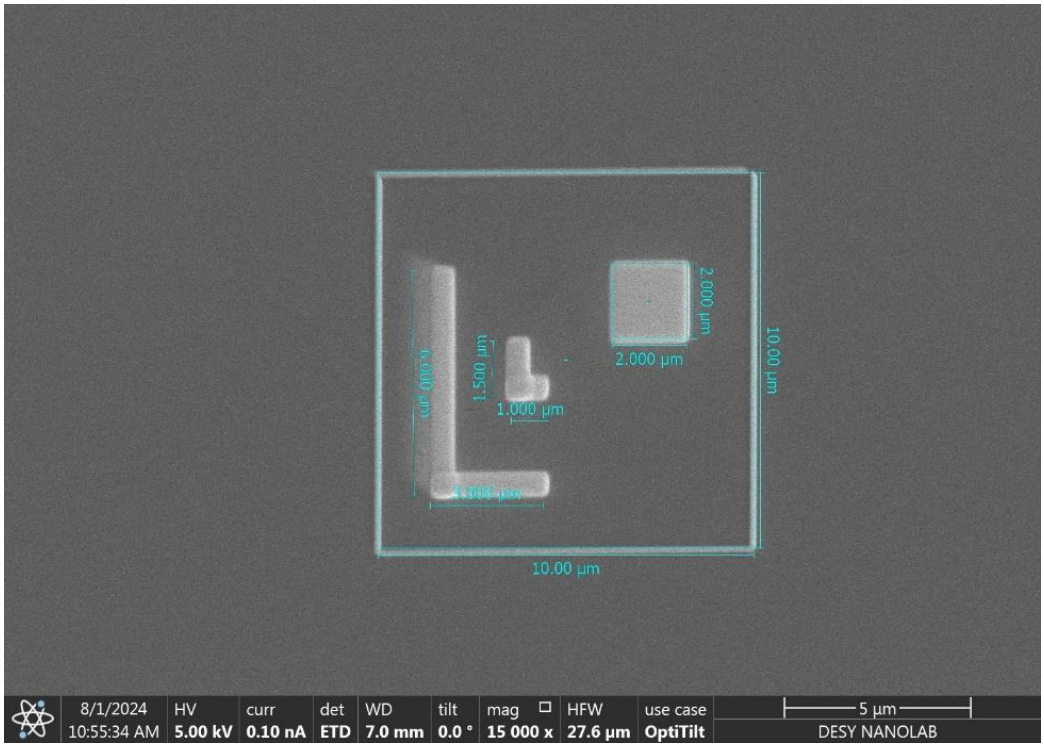

Figure 2. SEM image at 15000× magnification of one of the Pt fiducial markers deposited on one of the three membranes.

Figure 3 shows an overview SEM image of the general layout of the fiducial markers located at three corners of the membrane. Each set of square markers was experimentally placed at a distance of approximately 200–205 µm from the edge of the Si_3N_4 window.

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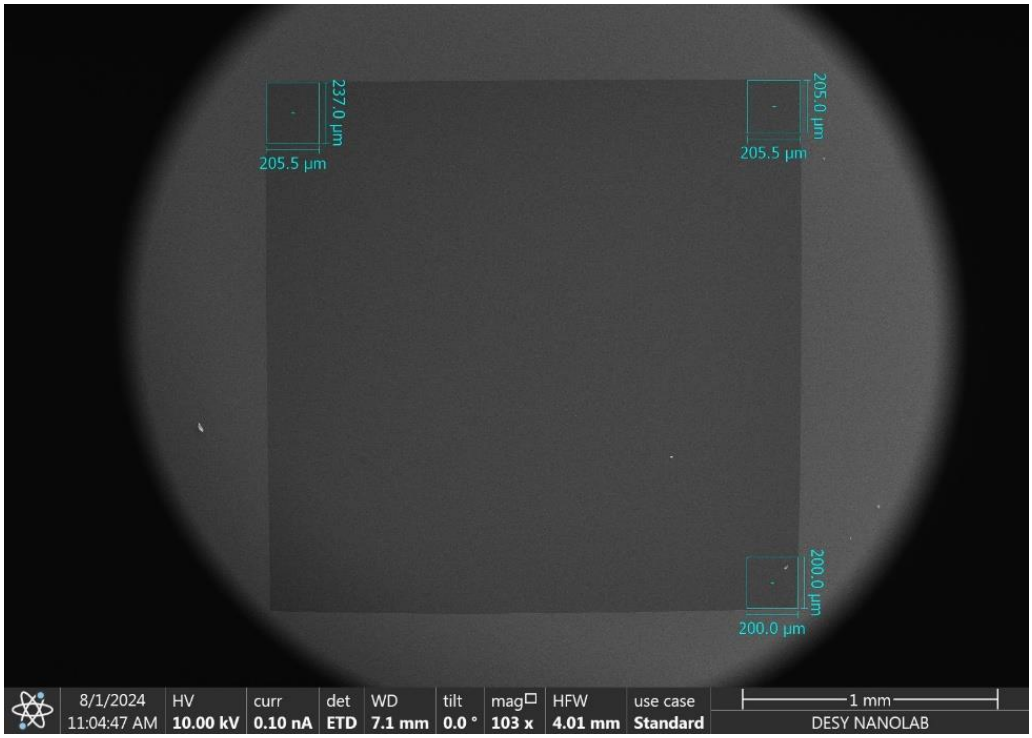

Figure 3. SEM image at 103× magnification of one Si_3N_4 membrane after Pt marker deposition. The light blue lines indicate the positions of the markers at three corners.

3.1.3 Use of the first membrane set with Pt markers at ID21

The first Si_3N_4 membranes with Pt markers were sent to ID21 for evaluation using the nano-SXM microscope. Initially, the functionality and visibility of the markers were tested by comparing SEM images with µXRF maps (**Figure 4**). This correlative imaging confirmed that the Pt markers were clearly visible and effective as fiducial references.

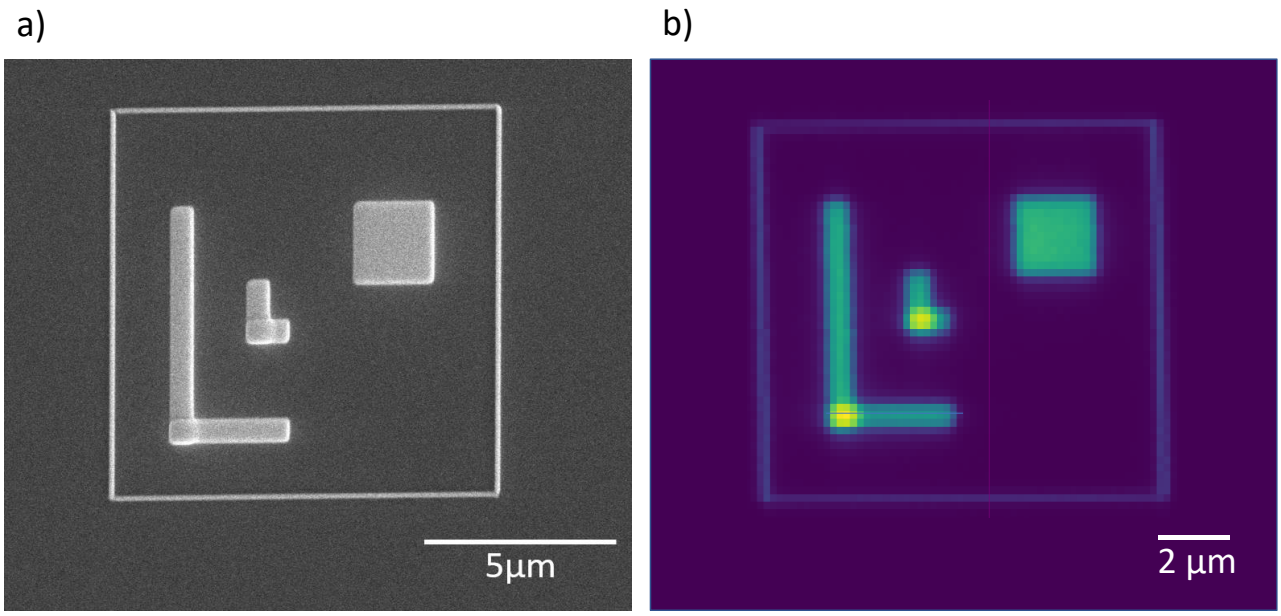

Figure 4. Correlative imaging of a Pt fiducial marker: SEM (left) and µXRF map (right) showing the same structure.

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However, during these tests, it became clear that the small "L"-shaped marker did not provide any practical benefits, so its removal was recommended. It was also found that a complete square frame around the marker was unnecessary, and a simplified frame (partial inverse "L" shape) would be sufficient. On the other hand, to improve the transfer of the coordinates, it was recommended to add an extra marker at the center of each Si_3N_4 membrane, in addition to the markers placed in the corners. This adjustment reduces the distance between the fiducials, enhancing the alignment of any sample on the membrane. Additionally, an estimate of the beam size was made using the thinnest deposited Pt line. However, since the beam size was in the same range as the line thickness, it was concluded that the line width of the inverse "L" frame should be less than 100 nm. This modification would enable more precise measurements of the beam size in future evaluations.

3.2 Second set of membranes

3.2.1 Design

The second set of membranes developed by DESY NanoLab consisted of three units, named SiN_D, SiN_E, and SiN_F. This set included the marker design shown in **Figure 5**.

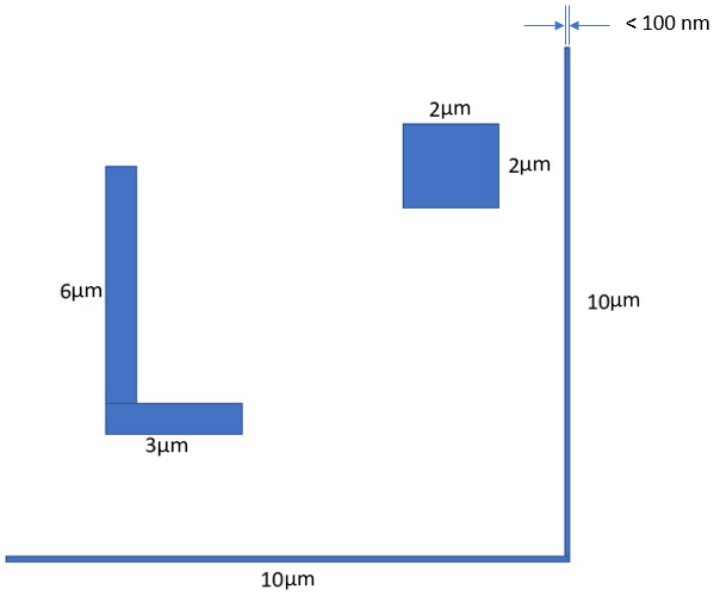

Figure 5. Pt marker design for the second membrane set

This membrane set included a simplified marker (**Figure 5**) with an open frame in the shape of an inverted "L", made of two lines of 10 µm each (one horizontal and one vertical). Since the goal was also to use the membranes to estimate the beam size, the design included thinner lines, with a thickness of less than 100 nm (ideally around 50 nm). Also, in this version, the small "L" from the design in Figure 1 was removed.

3.2.2 Fabrication of the Pt markers

Figure 6 shows an example of the second Pt marker design, deposited on one of the Si_3N_4 membranes. The image illustrates also the precision and quality of the deposition. Thanks to the use of smaller apertures in the Dual-beam Focused Ion Beam system, it was possible to achieve Pt lines as thin as 70 nm.

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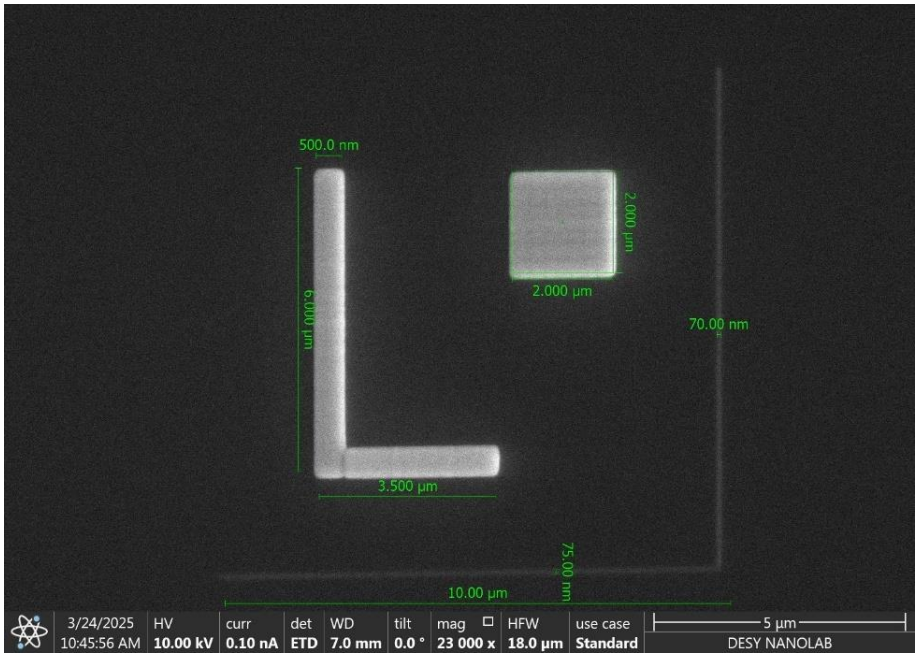

Figure 6. SEM image at 23000× magnification of one of the Pt fiducial markers deposited on one of the three membranes.

On the other hand, **Figure 7** shows an image of the upper-left corner of one of the membranes. At 1200× magnification, part of the marker is visible, showing both the "L" structure and the solid Pt square.

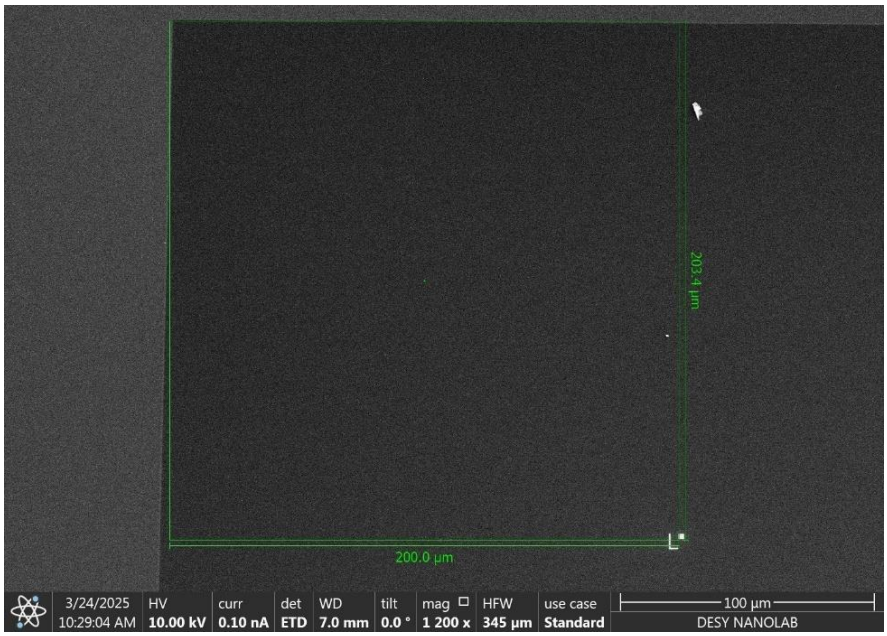

Figure 7. SEM image at 1200× magnification of the upper-left corner of one Si_3N_4 membrane.

Finally, **Figure 8** clearly shows the general position of the fiducial markers in three corners, located approximately 200 µm from the edge of the Si_3N_4 window. Although the central marker is not highlighted with a green rectangle, its presence is confirmed.

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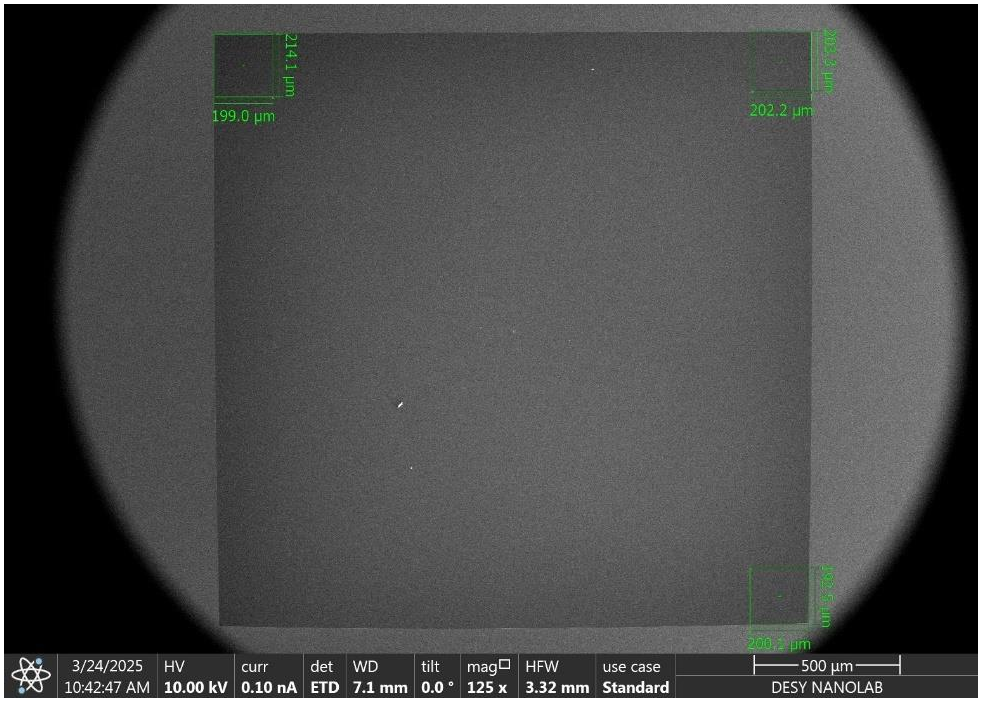

Figure 8. SEM image at 125× magnification of one Si_3N_4 membrane after Pt marker deposition. The green lines show the positions of the markers in three corners. The central marker is not indicated.

3.2.3 Use of the second membrane set with Pt markers at ID21

The second group of Si_3N_4 membranes with Pt markers was used at ID21. **Figure 9** shows an example of an external optical microscopy (OM) image (Zeiss Axioplan 2) taken from membrane F. The enlarged area (100× magnification) shows clearly the platinum marker, including the large "L" and the solid square. Small Mn salt particles, coming from the sample fixation, are also visible. As an observation, slightly bigger markers might be helpful to make them easier to see with the optical microscope, especially if the sample placed on the membrane is thick.

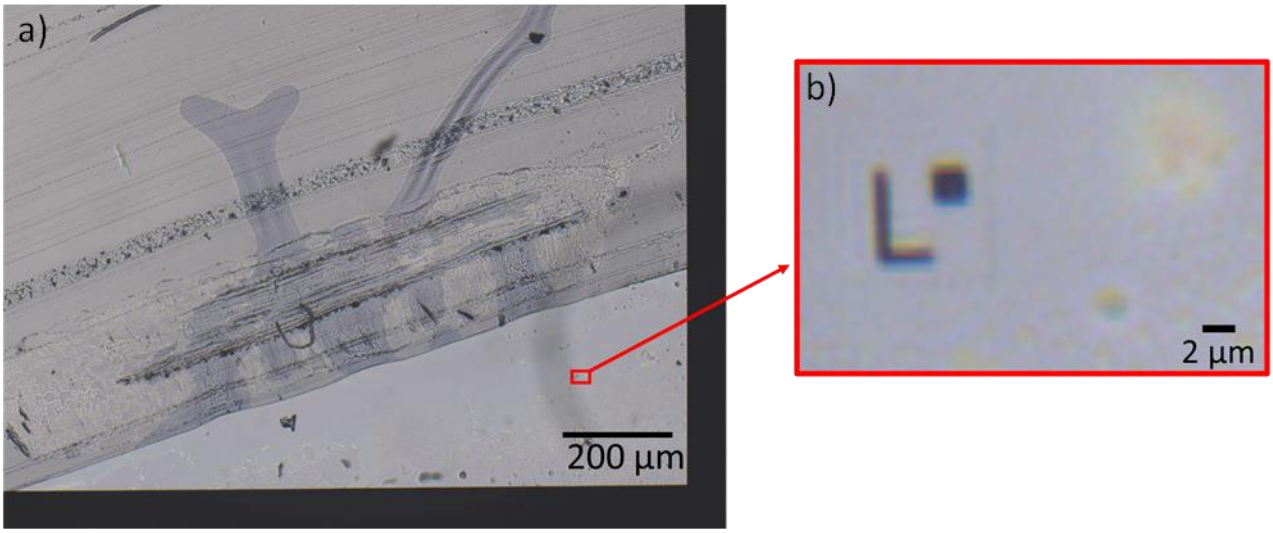

Figure 9. External OM image (Zeiss Axioplan 2) of membrane F with Pt marker (scale: a = 200 µm, b = 2 µm).

To verify the coordinate transfer between microscopes, **figure 10a** shows the optical image taken with the internal microscope of the nano-SXM, while **figure 10b** displays the external OM image

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after registration in Daiquiri. During this transformation the rectangular external OM frame became a slight trapezoid/parallelogram, because the corner angles are no longer 90 °. Four fiducial points (labelled 1–4 in the figure) were used for the alignment: points 1 and 2 correspond to the square and the vertex of the Pt "L", point 3 marks a corner of the Si_3N_4 membrane, and point 4 indicates a clear feature on the sample. Using these points, Daiquiri converts the pixel space of the external OM image into nano-SXM motor positions, ensuring that exactly the same region can be analyzed with both microscopes.

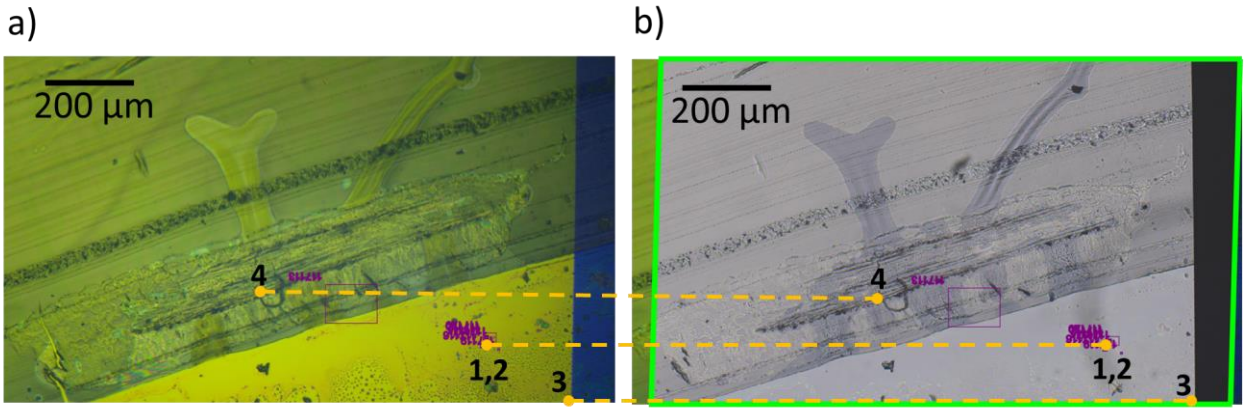

Figure 10. Comparison of internal nano-SXM optical image (a) and external OM image (b) after coordinate alignment using Daiquiri.

To confirm that the alignment works correctly, **Figure 11** compares µXRF maps taken at nano-SXM with the external OM image. In **figure 11a**, the red dashed line shows the area containing Mn salt particles, and the green dashed line shows the Pt marker area. **Figure 11b** compares fluorescence signals: the red dashed area shows Mn-Kα fluorescence, and the green dashed area shows Pt-M fluorescence. Using nano-SXM and Pt markers, it becomes possible to detect chemical details that the external OM cannot show clearly.

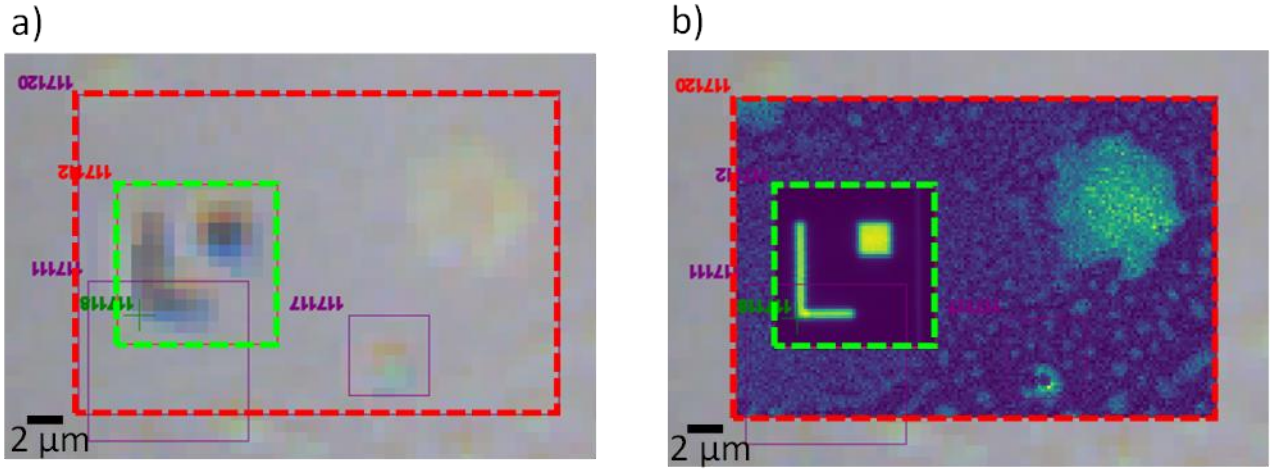

Figure 11. Verification of coordinate alignment: external OM image (a) and nano-SXM µXRF maps of Mn-Kα (red) and Pt-M (green) emission (b).

Finally, **Figure 12** shows an example of using this correlative method to study bigger areas. It shows how µXRF maps (in this case, Ca fluorescence) from nano-SXM can be overlaid directly onto external OM images. The result confirms that the coordinate transfer method works effectively even for large sample regions, making it easy to study the same cells or areas with multiple microscopes.

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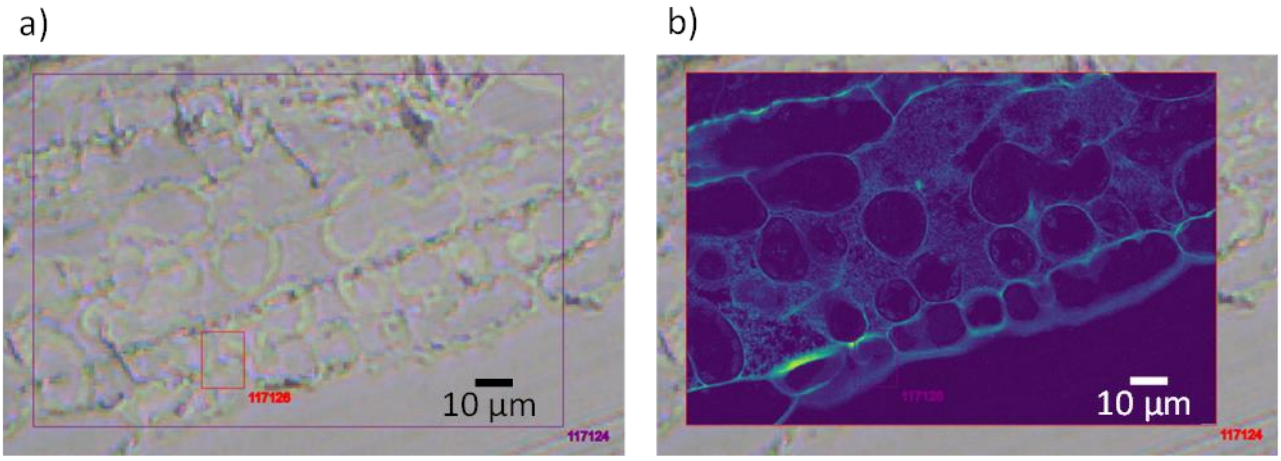

Figure 12. Overlay of Ca µXRF map (nano-SXM) on external OM image in bigger areas

However, all the previous results were obtained using only one of the four markers on the Si_3N_4 membrane, specifically the marker located in corner C. The central marker could not be reached because the nano-SXM vertical stage was close to its range limit. Therefore, an additional test is planned to use the central marker on the membrane. The goal is to validate the use of a second marker in order to reduce the minor distortions observed when µXRF maps are overlaid on the nano-SXM optical image.

4. CONCLUSIONS

This report demonstrates that the developed Si_3N_4 membranes with Pt markers effectively enable accurate correlative measurements between optical microscopy and µXRF. The marker design, initially tested and later optimized through collaborative work between DESY NanoLab and ESRF-ID21, proved successful in precisely locating ROI. Using the Daiquiri interface, coordinate transfers from external optical microscopy to the nano-SXM were precise, confirming the technical feasibility and reproducibility of this workflow. Additionally, µXRF mapping with nano-SXM revealed chemical details not visible with standard microscopy, highlighting the complementary strengths of this correlative approach. These results confirm that this standardized marker-based strategy is practical, reliable, and suitable for future correlative experiments across various laboratories and research fields.

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