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D15.2

Image Registration Procedures

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Image registration procedure for correlative Nano-Spectroscopy and Nano-Diffraction experiments. Detailed description of the python-based GUI script and validation using different modalities of microscopic imaging. Automated identification of isolated nanoparticles for analysis at selected nano-lab instruments and focused X-ray beamlines.

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NATURE

- R - Report
- P - Prototype
- DEC - Websites, Patent filing, Press & media actions, Videos, etc
- O - Other

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INTRODUCTION

This deliverable report 15.2 “Image registration procedures” of working package WP15 of NFFA--Europe|PILOT (NEP) project aims to establish a unified and harmonized GUI platform for correlative experiments utilizing image registration and hierarchical markers for one-to-one and simultaneous microscopic and spectroscopic imaging. With this deliverable, DESY has developed a python-based GUI image registration software for correlative nano-spectroscopy and nano-diffraction that incorporates the nano-object transfer and positioning developed within the previous NFFA-Europe project, and additionally integrates image registration tools for further automatization.

The most significant challenge is the identification and re-localization of nanoscale objects when they are pre-selected at lab-based instruments and transferred to X-ray beamlines at analytical large-scale facilities (ALSFs). This is a barrier for both ex-situ and, more significantly, in-situ studies, where sample chambers could obstruct a direct visualization with an optical microscope. Image registration tools assist in identifying the pre-selected and marked nano-object by adjusting the motor positions of the sample stage.

Fundamentally, image registration is the process of mapping and geometrically aligning two or more images of similar or different modalities. This image registration software facilitates three essential features, including (a) “align images” to adjust the dimensions of images, (b) “add fiducial marker” to register the images using two sets of alignment marks, and (c) “Discrete Fourier transform (DFT) position refinement” to identify and localize a statistically relevant number of distinct nanoscale objects of different lab-based and X-ray modalities.

Within the previous NFFA-Europe project deliverable and milestone reports, a MATLAB-based nano-positioning software has already been developed for the transfer and positioning of nano-objects, primarily based on high contrast, hierarchically arranged markers created by the ion-/electron beam induced deposition (IBID/EBID) process (Abuin et al., ACS Appl. Nano Mater. 2019, 2, 8, 4818–4824). This enables a re-localization of nanoscale regions of interest at different nano-instruments. Recently, a python-based, automated image registration software has been designed for correlative nano-spectroscopy and diffraction that offers an accessible platform for a routine nano-transfer between nanoscience instruments and focused X-ray beamlines. Additionally, it is proficient in positioning a statistically relevant number of nano-objects using the DFT position refinement feature. The python-based GUI script will be integrated at selected X-ray beamlines.

Protocol of python-based image registration software

We already described before the MATLAB implemented GUI in the earlier JRA5 deliverable reports D10.1 “Software routine for coordinate definition and relocation of single nano-objects”, D10.5 “Demonstration of relocating nano-objects based on in-situ deposited fluorescence”, D10.6 “Test experiment of transfer and positioning system to a soft X-ray microscopy setup” and the JRA5 milestone report MS13 “Design and protocols accepted at nanocenters and ALSF”, for the Advanced Nano-Object Transfer and Positioning protocol within the previous NFFA-Europe project.

A new platform “Image registration tool” is currently being developed within NEP project written in python language because python offers a user-friendly interface with the comprehensive standard library and work on various platforms (Windows, Linux, etc.). As such, it offers to use the python scripts at any PC. The python program was scripted in collaboration with beamline scientists by Stijn Van Malderen at PETRA III, DESY (Stijn Van Malderen, Jan Garrevoet, Ken Vidar Falch). A detailed



overview of the python GUI script, and the algorithms and steps of operation of the image registration program is given in the following. Figure 1 shows a screen-shot of the image registration software, which offers three options in the menu bar:

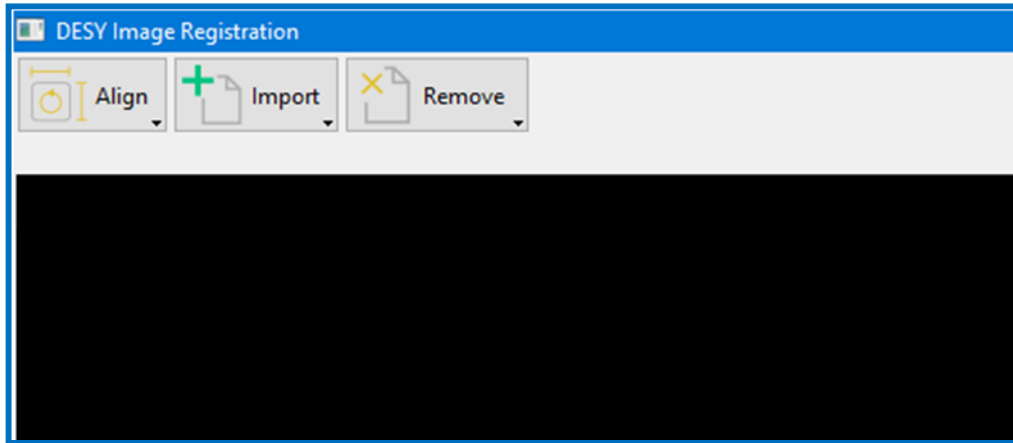


Figure 1: Screen shot of the image registration program.

Algorithms

Multiple algorithms are used in the python script to functionalize this program. Specific features are listed in the following:

1. **Import module:** To upload the images by filename. The module calls the appropriate load or open function. Currently supported file types are .png, .jpg, .cvs, .dat, .txt, .gif, .tiff, etc.
2. **Geometry unit:** This module contains a tool to change the position and rotation of the image in the workspace and permits to receive information on the particle's coordinates.
3. **Field area tool:** This tool can be used for implementing many types of selection boxes with rotate/translate/scale handles. ROIs can be customized to have a variety of shapes (by subclassing or using any of the built-in subclasses) and any combination of draggable handles that allow the user to manipulate the ROI.
4. **Field fiducial markers:** To select the two set of points for image registration.
5. **Field ORB registration:** Oriented FAST and rotated BRIEF (ORB) detector used to find key-points and similar features on the image.
6. **Field DFT registration:** This module helps in image registration based on discrete Fourier transforms DFT-based input which rescale the target frame to the resolution of the reference frame and create a new frame with registered images.
7. **Export module:** To export the files.



Operation of the program

Combining the above algorithms, the GUI is being operated manually and able to localize the coordinates of the nanoparticles. Automated feature analysis will be implemented with a high level of precision and integrated to the selected focused X-ray based beamlines. The following alignment steps (Figure 2) must be implemented in order to obtain a one-to-one correlation via image registration. Furthermore, Figure 3 provides a step-by-step illustration of the software for easy comprehension.



Figure 2: Process to receive the single coordinate image via image registration software.

Step 1: Using this “Import” menu, users may upload images from different modalities with the appropriate resolution and given contrast.

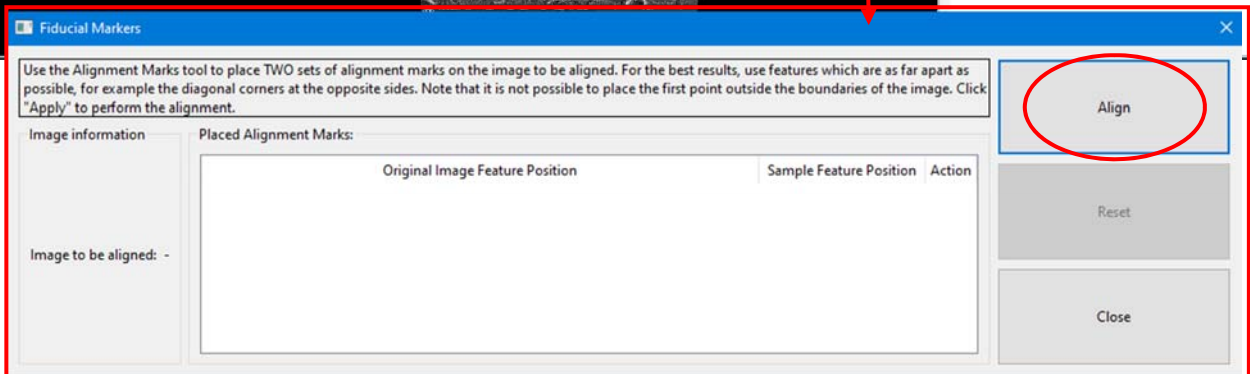
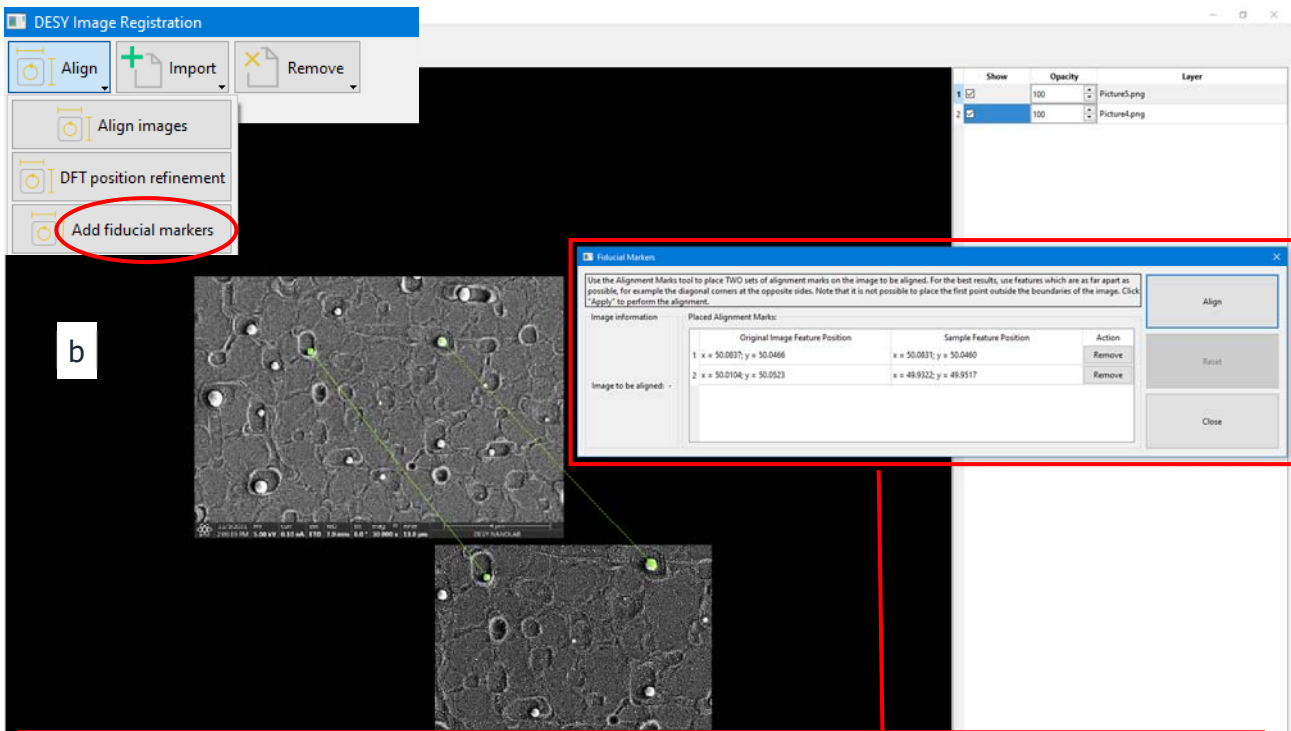
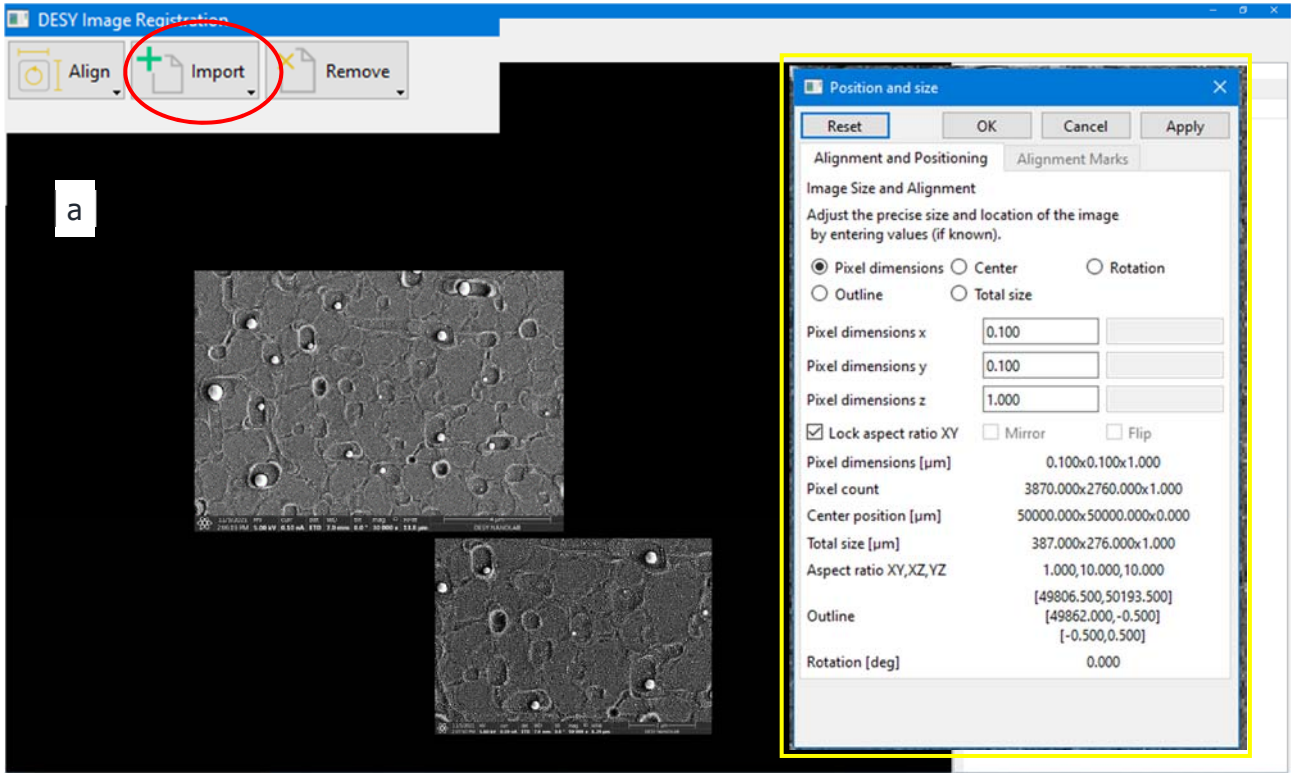
For the test experiments, two different-resolution SEM images were loaded (Figure 3(a)). After importing the images, the position and size menu (in the yellow box) can be used to adjust the dimensions of the images such as pixel dimension, rotation, and centre. Images maybe manually resized, rotated and dragged to an alternate position in the workspace. One image serves as the reference image, while the other serves as the transferred image.

Step2: This is the most important tool as it permits to create two sets of alignment marks on transferred and reference images with the same feature.

Figure 3(b) illustrates two sets of lines generated manually using "add fiducial marker", by selecting the two points on the transferred image and the other two on the reference image. The set of features on both the images should correspond.

Step 3: Click “Align” to process the alignment and receive a new frame with the registered image.

Figure 3(c) represents the registered images in the workspace after clicking the “Align”. To verify how well the images register, one may vary the opacity (red boxes) of both the images.



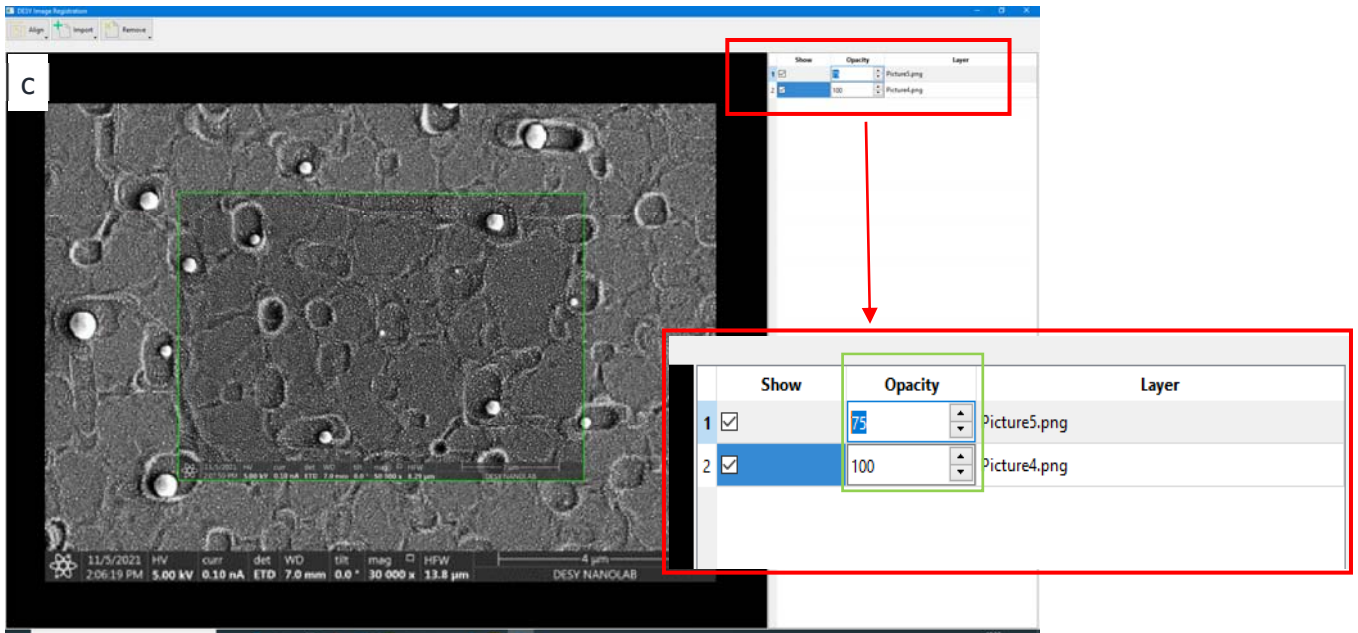


Figure 3: a-c) Screenshot of the various steps of the image registration process using two different resolution SEM images that users have to follow while using this software.

Authentication of one-to-one nanoparticle correlation using image registration

As seen in the preceding segment, the script is functioning properly and has correctly registered the two SEM images. Authenticity of the python-based GUI has been verified several times using microscopic imaging at DESY NanoLab. For this test experiments, PtRh alloy nanoparticles were prepared on a niobium-doped strontium titanate (STO) single crystal by dewetting a thin Pt film during annealing and subsequent overgrowth with Rh in UHV. Two different microscopic modalities such as atomic force microscopy (AFM) and scanning electron microscopy (SEM) were utilized for this study. Scanning electron imaging was used to determine the lateral size and distance between the nanoparticles. Using a marker-based correlative imaging approach, the region pre-selected in the SEM was relocated in the atomic force microscopy to obtain the nanoparticle height information. Figure 4 (a, b) displays corresponding microscopic images of different size/resolution. The scale size of the SEM is 4 μm , while the size of the AFM is 2 μm . Moreover, the resolution of both modalities is quite different. This program, which includes a DFT registration refinement module, assists in rescaling the target frame to complement the resolution of the reference frame. The red box in Figure 4 represents the region of interest that we used for the image registration. Then, Figure 5 illustrates the resulting overlay SEM/AFM image of the PtRh alloy nanoparticles sample created with the image registration software described above. For high contrast images, the estimated precision of image registration is a few nm and depends on the overall image-settings and -resolution.

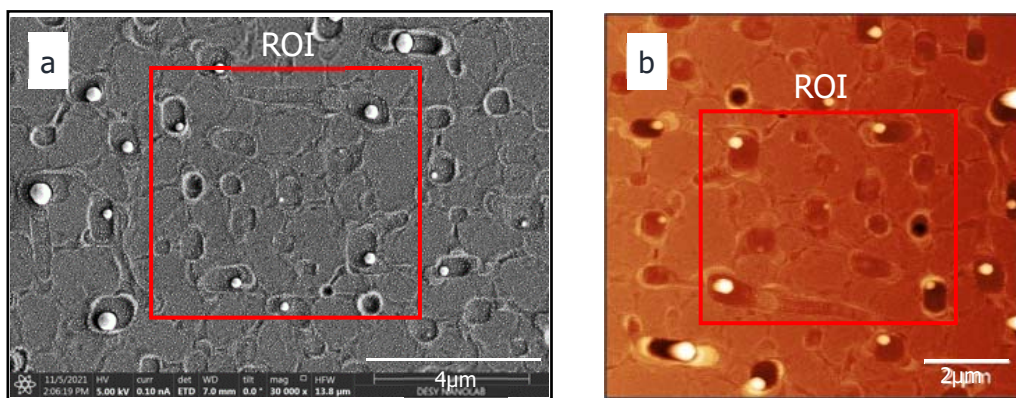


Figure 4: (a) High-resolution scanning electron microscopy (SEM) image and (b) atomic force microscopy (AFM) image.

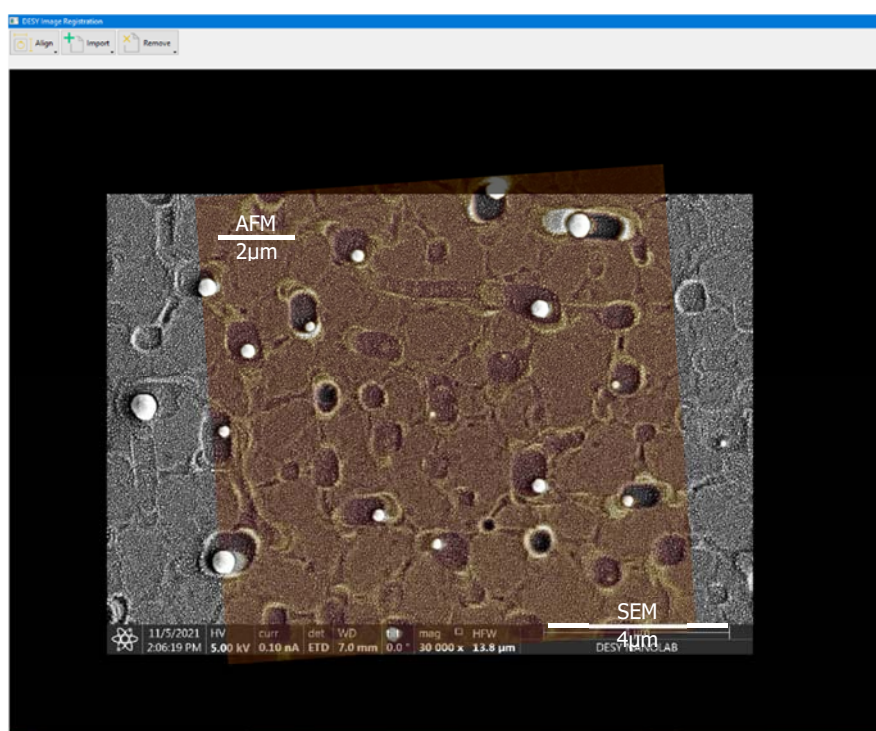


Figure 5: SEM/AFM overlay using the python-based image registration program.

Additionally, the positions of isolated nanoparticles of the sample may be identified using a precision thresholding algorithm within the GUI script. Figure 6 demonstrates the determination and identification of a statistically relevant number of isolated nanoparticles even of smaller dimensions (130 isolated nanoparticles). In the submitted milestone MS9 of the previous NFFA-Europe project, the developed templates and deposition protocols were discussed in order to prevent nanoparticle accumulation and illuminate a single nanoparticle only, while being analysed at a focused X-ray beamline to achieve real one-to-one structure-property correlations. The foot-print of a focused x-ray beam typically ranges from a few $10 \text{ nm} \times 10 \text{ nm}$ to a few $10 \mu\text{m} \times 10 \mu\text{m}$, depending on various parameters such as the X-ray energy and the tilt angle between the X-ray source and the sample surface.

Isolation of the nano-objects is required to ensure that signals are collected from only single nanoparticles at the same time. The above sample consisting of sufficiently isolated nanoparticles is appropriate for an X-ray analysis with their respective lateral position coordinates. Furthermore, the

pre-selection of nanoparticles with known/pre-calculated position coordinates serve to minimize the scan time during an X-ray beamline experiment. So far, the majority of beamline experiments rely on time-consuming lateral scans of the entire sample region, and subsequently recognizing the desired nano-objects (Figure 7(a)) during data analysis. Certain beamlines specify the shape of the scanning area in an effort to accelerate scanning and locate the desired nanoparticles. (Figure 7(b)). Instead, the automatically determined position of the isolated nanoparticle can in the future reduce the amount of time needed for scanning during the x-ray analysis by only collecting X-ray data on the pre-determined nanoparticle sites (Figure 7(c)).

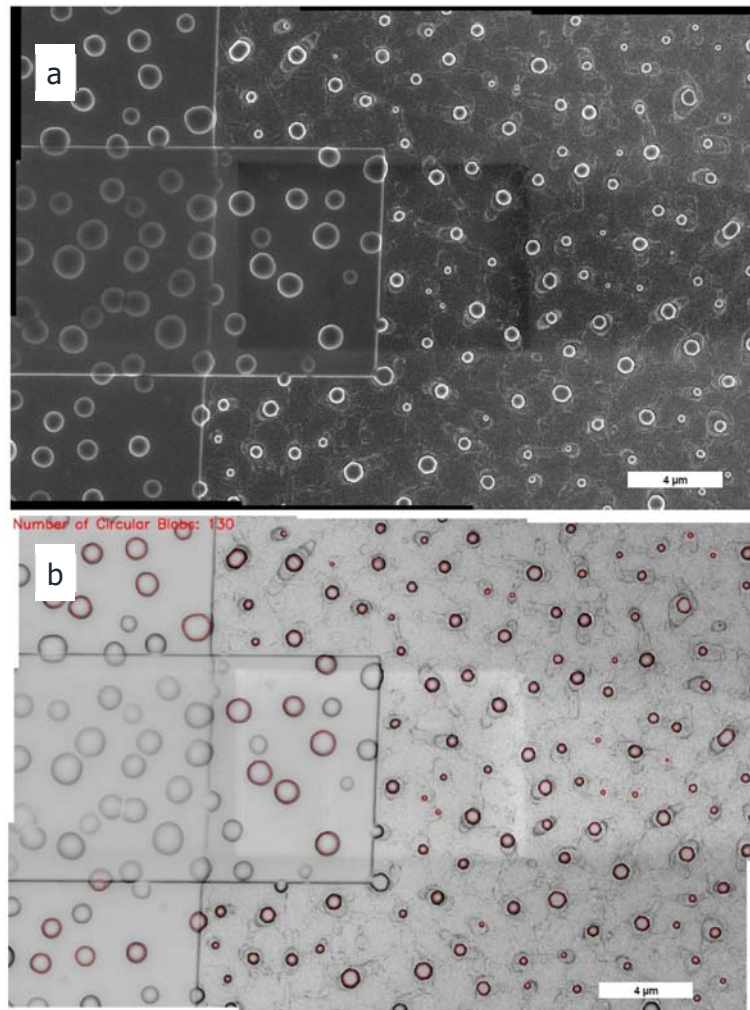


Figure 6: a) High resolution composite stitched SEM image and b) outcome of an automated nanoparticle detection displayed as an overlay on the SEM in a). 130 circular blobs were identified by the python script in the registered image and encircled in red.

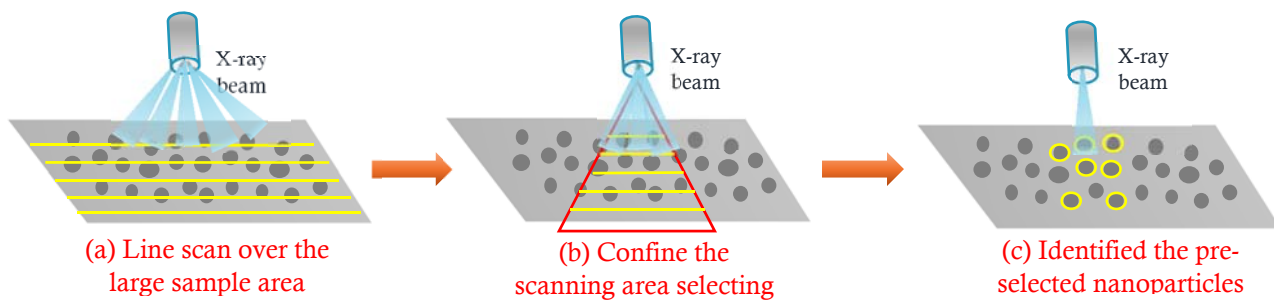


Figure 7: Representation of scanning strategies during x-ray analysis at beamlines a) full range, b) confined range, c) nanoparticle sites only.

CONCLUSION AND OUTLOOK

An automated image registration software protocol is currently being developed and implemented at DESY that permits correlative nano-spectroscopy and nano-diffraction experiments. The python-based script was validated using nanoscience modalities and will be incorporated and implemented for use at dedicated focused x-ray beamlines starting at beamline P06 at PETRA III at DESY. This GUI provides a new platform to the NFFA and regular PETRA III users to identify and re-localize the nano-objects based on image registration and image recognition. The image registration software will facilitate one-to-one correlation studies utilizing spectroscopic and microscopic micro- and nanoscale imaging at nano-labs and ALSFs. Here, we employed two nanoscience tools, such as SEM and AFM to test and document the program on a scientifically relevant sample system with isolated PtRh alloy nanoparticles. The next step is to collect a correlative data set for a nanoscience study and complement the lab-based approach with a correlative X-ray experiment after the implementation at an X-ray beamline, utilizing different contrasts such as Bragg diffraction, X-ray absorption spectroscopy (XAS), etc. The script could also be utilized in the future to reduce the scanning time during an X-ray experiment by confining the scanning region to the relevant nano-objects containing regions or sites. Particular focus will be the user-friendliness for future NFFA and also PETRA III users.