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Enabling Science through European Electron Microscopy

Report on protocols for sample preparation techniques of materials for transport

Deliverable D10.4 – version 1.1

Estimated delivery date:	April 30th
Actual delivery date:	April 3rd
Lead beneficiary:	AGH-UST, NTNU
Person responsible:	Adam Kruk, Randi Holmestad
Deliverable type:	☒ R ☐ DEM ☐ DEC ☐ OTHER ☐ ETHICS ☐ ORDP
Dissemination level:	☒ PU ☐ CO ☐ EU-RES ☐ EU-CON ☐ EU-SEC

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRANT AGREEMENT NO **823717**

Grant Agreement No:	823717
Funding Instrument:	Research and Innovation Actions (RIA)
Funded under:	H2020-INFRAIA-2018-1: Integrating Activities for Advanced Communities
Starting date:	01.01.2019
Duration:	54 months

Table of contents

Revision history log

Version number	Date of release	Author	Summary of changes
V0.1	31.03.2023	Adam Kruk Grzegorz Cempura	
V1.0	03.04.2023	Peter A. van Aken	Minor amendments and approval
V1.1	03.04.2023	Aude Garsès	General review

1. Report on protocols for sample preparation techniques of materials for transport - AGH-UST

1.1. Specimen Preparation from Materials for Transport

This task intends to outline effective methods and techniques for sample preparation for transmission electron microscope testing. This is particularly relevant for "difficult" structural materials, such as nickel-base superalloys, Advanced High-Strength Steels (AHSS), titanium-, aluminium-, and magnesium-based alloys. The method that should be used to study materials using transmission electron microscopy methods depends on what we are studying and the physical characteristics of the material. For example, if it is soft or hard, ductile or brittle, etc. We must use mechanical processing methods to get a thin sample from a more significant piece. This can add stress or deformation, changing our test results or making them impossible to do.
The following techniques will be explored:

- Focused Ion Beam (FIB) to create thin, artefact-free lamellae followed by post-processing.
- Preparation of samples for In-situ TEM investigation.
- Preparation of samples for Atom Probe Tomography.
- Preparation of samples for Electron Tomography.
- Preparation of samples from fibrous and powdered materials.
- Gentle ion milling and electropolishing techniques.

When preparing a specimen for transmission electron microscopy (TEM), it is crucial to ensure that the specimen is electron transparent and represents the material being studied accurately. Moreover, several preparation steps should not change its initial microstructure composition and properties.

In order to perform high-resolution research on the atomic scale requires the specimen to be as thin as possible and stable under the electron beam and in the laboratory environment. However, these conditions can often be challenging to meet, mainly when dealing with structural materials, such as steel, which are ferritic, non-conductive or ceramic materials.

Producing high-quality, electron-transparent thin specimens is essential for state-of-the-art TEM/STEM investigations, especially when examining the structure of materials at the atomic scale. Optimizing existing sample preparation procedures and techniques such as tripod polishing, ion milling, FIB techniques, electropolishing, and ultramicrotomy is crucial for high-quality investigations.

Tripod polishing is typically used for preparing bulk specimens, whereas ion milling is better suited for preparing thin specimens. FIB techniques provide a versatile means of producing thin, electron-transparent specimens from larger samples. These techniques involve using a focused ion beam to create a small hole on the sample's surface, which is then precisely milled, allowing a thin specimen to be extracted or imaged directly. Electropolishing is another helpful technique used to create electron-

transparent specimens for TEM/STEM, particularly for metal alloys that are difficult or impossible to polish.

When preparing a thin sample, several mechanical processing methods are used, each with its unique effect on the material. Some methods, such as FIB or ion milling, have minimal impact on the material, while others, like tripod polishing, may induce mechanical stress or plastic deformation in the sample. These mechanical changes can significantly impact the resulting test results or even make it impossible to carry out the analysis on a sample prepared in this way.

As a result, the choice of preparation method must be done judiciously, taking into account the nature of the material and the specific analysis being conducted (**Fig. 1**). This requires a deep understanding of the sample preparation techniques available to researchers and how each preparation method can alter the properties of the material being studied. By applying the most appropriate technique, scientists and researchers can limit the impact of mechanical processing on the sample and more accurately analyze the structural and chemical properties of the material.

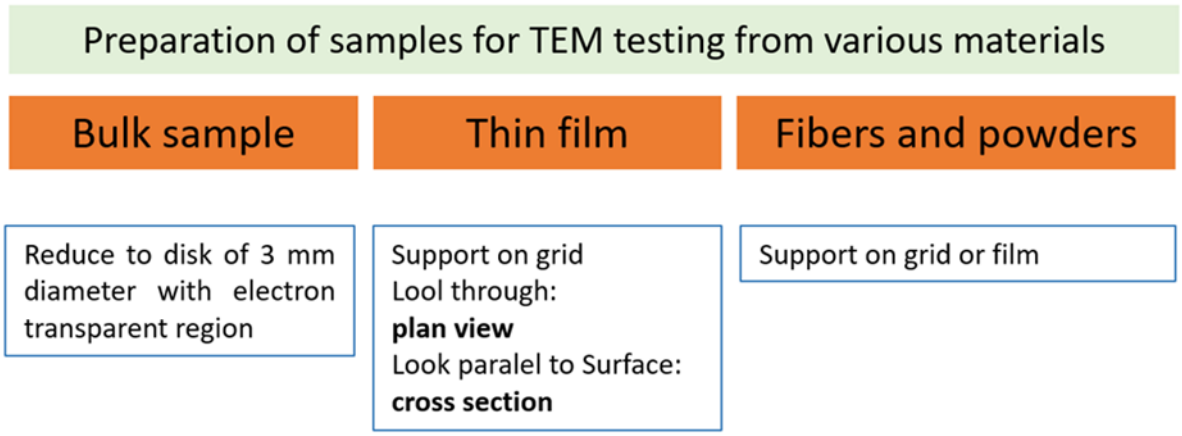


Fig. 1. Preparation of samples from a different form of materials for TEM investigations

1.2. Thin foil technique

1.2.1. Preparing a self-supporting disk for final thinning

The process generally involves several steps (**Fig. 2**):

- The first step involves cutting the sample from a larger piece of the material.
- The second step involves an initial thinning process that produces a slice of material of a thickness of around 200 µm.
- In the third step, a 3 mm disk is cut from the slice produced in step two.
- Thinning from one or both faces of the disk is carried out in the final step to reduce the thickness to a few micrometres (usually around 80 um). This step is crucial in preparing the specimen for further processing and analysis.

Materials can exhibit varying mechanical properties, making them susceptible to different processing methods. Ductile metals and alloys require specific consideration to avoid mechanical damage. In such cases, a string saw, a wafering saw with a soft metal blade, or spark erosion using electro-discharge machining can be used to obtain thin slices of about 1 mm without mechanical damage. Brittle ceramic materials require special consideration during the thinning process to prevent fractures.

Brittle materials such as ceramics:

- A first case, when the introduction of mechanical damage is not possible, a second case where mechanical damage is negated in electron microscopy investigations involves materials such as Si, GaAs, NaCl, and MgO, among others, with a well-defined cleavage plane, which can be repeatedly cleaved with a razor blade to obtain electron-transparency. Ultramicrotome cutting is an alternative, allowing direct examination of skinny slices of soft materials. Limitations of sawing include the destruction of samples, especially in hard materials. Vibrations introduced during mechanical processing, such as cutting, can lead to chipping of the outer layer on steel surfaces, making it necessary to cut lamellas using a Focused Ion Beam (FIB) to produce high-quality, electron-transparent specimens for TEM analysis. The FIB technique is versatile, providing a means of producing thin, electron-transparent specimens from hard materials, such as steel and metal alloys and fibrous and powdered materials. By selecting the appropriate method, researchers can ensure high-quality specimens and accurate analysis of the studied materials.

1.2.2. Cutting the disk

For reasonably ductile materials, disks with a 3 mm diameter can be cut using a mechanical punch with minimal damage. However, this process can induce internal stresses. Spark erosion is recommended for brittle materials that require high precision.

Sawing a slice of about 1 mm thickness

Grinding and polishing to a slice of < 0.1 mm thickness

Punching of 3mm discs

Sample ready for dimpling or final thinning

Fig. 2. Preparing a self-supporting disk for final thinning

1.2.3. Pre-thinning the disk - dimpling

This process aims to thin the disk's centre while minimizing surface damage. This stage is commonly referred to as 'dimpling.' The method of preparing one-sided and two-sided dimpling is presented in **Figure 3**. Any damage created at this point must be removed in the final thinning process. Most commercial mechanical dimplers on the market use a small radius wheel to grind and polish the disk to a fixed radius of curvature in the centre. Advantages of this method include being able to control the load used, precisely determine removed material thickness (i.e. depth of dimple), quickly change polishing tools, and pause processing for closer sample examination before continuing.

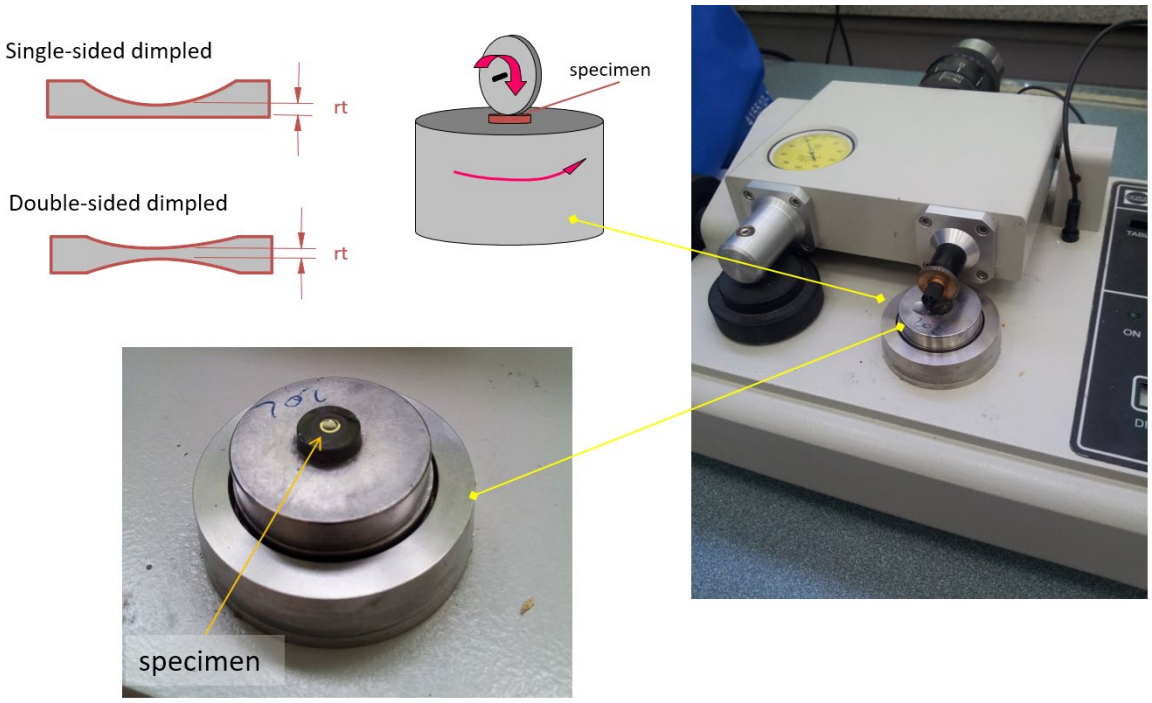


Fig. 3. TEM specimen preparation, dimpling

1.2.4. Final thinning of the disks

a. Electropolishing

Electropolishing is an effective method for the final TEM sample preparation of metals and alloys. The process can be relatively quick (a few minutes) and produce foils without mechanical damage. However, it should be noted that electropolishing can modify the surface chemistry of the specimen, as some precipitates may be removed due to having a different reaction with the electrolyte than the alloy's matrix. The principle behind this technique is that a specific applied voltage results in the anodic dissolution of the specimen, creating a polished surface instead of etching or pitting. It is, therefore, essential to select an appropriate electrolyte for a given type of material along with suitable current-voltage conditions to achieve desired effects on the final sample. The device for the final thinning of samples using electropolishing is shown in Fig. 4.

Electropolishing solution contains:
Oxidising agent + solvent for oxidation products (+ viscosity controller)

input		*output*
acidic solution	e.g mixture from per-chloric and acidic acid	polishing rate
temperature	-50 – 0°C	quality of thinning
flow rate	low as possible	Smooth surface Large transparent area
voltage	20 – 30 V	

Fig. 4 TEM specimen preparation final thinning, electropolishing

b. Ion milling

Ion milling involves bombarding the specimen with energetic ions, which sputter material from the sample until it is thin enough to be studied in the TEM. A schematic diagram and a commercial model are shown in **Figures 5-7**. The variables that can be controlled include voltage, temperature of the

specimen (eg. cold milling with liquid nitrogen) and the geometry, such as the angle of incidence. An accelerating voltage in the range of a few kV is usually used.

The ion beam will always penetrate the specimen to some extent, so we minimize this by inclining the incident ion beam to the surface of the specimen. It is a very versatile process, which can be used for ceramics, composites, polyphase semiconductors and alloys, as well as many cross section specimens. Additionally, fibers and powders - which constitute a wide range of materials - can also be thinned through ion milling.

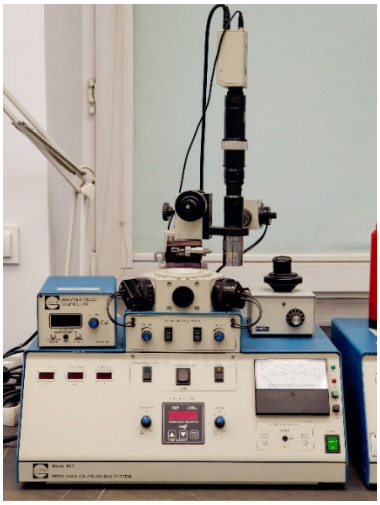


Fig. 5. Precision Ion Polishing Systems, PIPS (Gatan) -equipped with a cooling stage and CCD camera for monitoring of the thinning process and low energy ion polishing system

Fig. 6. NanoMill 1040 (Fischione) - ultra-low energy, concentrated ion beam device for producing the highest quality samples for TEM, free from preparation-induced artefacts (post-FIB processing)

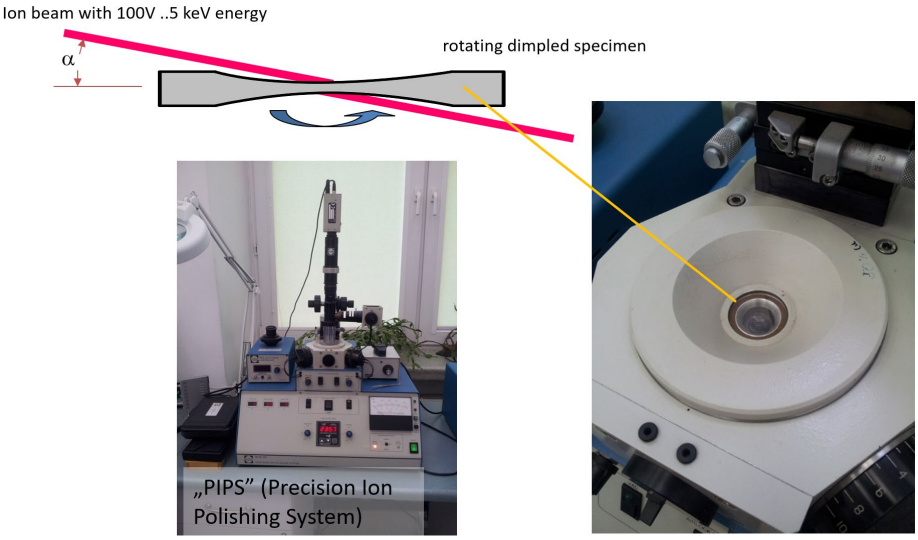


Fig.7. TEM specimen preparation final thinning, ion milling

c. Some final remarks

In the case of multiphase materials, the thinning process may vary due to differences in chemistry, physical and mechanical properties. The operator of the ion miller should run a test specimen periodically with nominally the same conditions to make sure that the machine is still operating correctly. When using an ion miller, it is important not to start with a thick sample; the surface should always be smooth before the ion thinning process begins. The beam current, angle of incidence, rotation rate and kV should also be logged for record-keeping purposes. It should also be noted that ion milling will form a layer on one or both surfaces, which may contain amorphous, highly damaged, and implanted material.

1.3. Cross-section specimens - Conventional TEM preparation techniques

Conventional preparation techniques for cross-sectional TEM samples combine mechanical grinding polishing and ion milling. The cross-section specimen is a particular type of self-supporting disk. This is an essential preparation technique if we are studying interfaces. It has been highlighted that the TEM has limited sensitivity to variations in the structure and chemical composition of the specimen along the direction of the electron beam - making this preparation technique especially important.

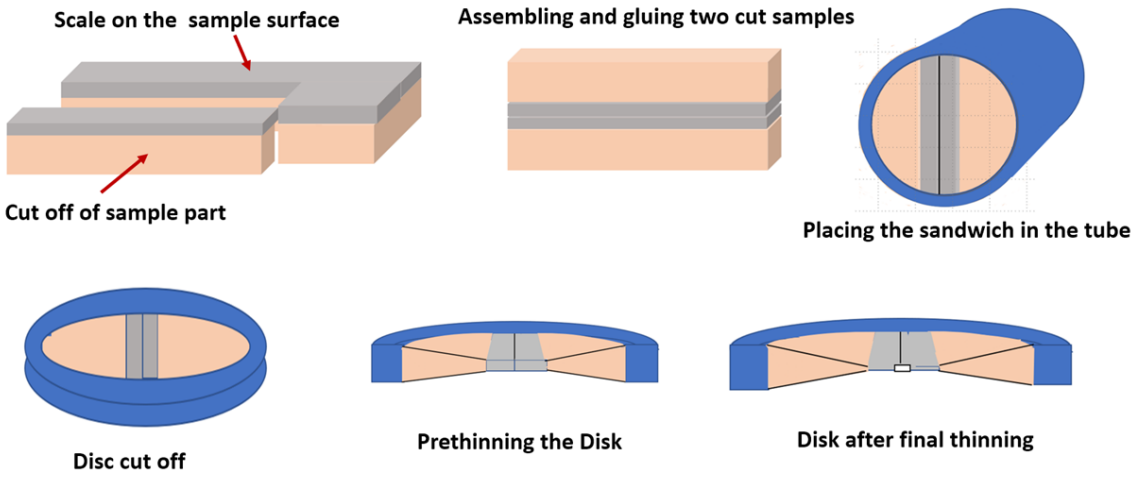


Fig. 8. Cross-section sample preparation for TEM investigation

Trying to thin only one interface may cause the spallation of outer layers. Such a case occurs during the microscopic examination of oxide scales formed on structural steels after service at high temperatures. Rather than trying to thin one interface only, the sample can be cut and glued together to produce several layers, rather like a sandwich. Then, the sandwich is sectioned to see the layers, as shown schematically in **Figure 8**. A critical step in this process is the glueing of the sections to form the sandwich. Several epoxies are available that cure at low temperatures so that we will not heat treat the specimen inadvertently. The thickness of the epoxy layer must be such that it is thick enough for good adhesion but not so thick that it is wholly thinned away during final ion milling. We can cut

the glued sections into 3-mm rods using an ultrasonic drill. Alternatively, we can cut the samples smaller and encase them in a 3-mm thin-walled tube. Section the filled tube into disks, which we can then ion thin. The advantage of this method is that the final specimen has a thick ring of tube metal around it, which gives it mechanical stability. With multiple interfaces, the final thinning is almost always guaranteed to produce electron transparency at a useful region.

1.3.1. Matrix replica technique

Direct replication (matrix replicas) methods are among the oldest TEM specimen preparation techniques. We usually use this method to study fracture surfaces or surface topography of samples damaged due to fatigue load. The successive stages of making a replica are shown in **Figure 9**. A thin plastic film (triafol) plasticized in a suitable solvent (acetone), pressing it on the surface, and allowing it to harden after drying adheres well to the analyzed surface. After removing the plastic film from the analyzed surface, we vaporize it with carbon. The next step is to dissolve the plastic film in acetone and float off the carbon onto distilled water before picking it up on a grid. The last step is to put on fragments of the carbon film on a grid for observation (**Fig. 10**). After picking up on a grid, coating the replica obliquely with heavy metal may be helpful to enhance any topographic (thickness) contrast. Then we receive a sample that shows enhanced mass-thickness contrast.

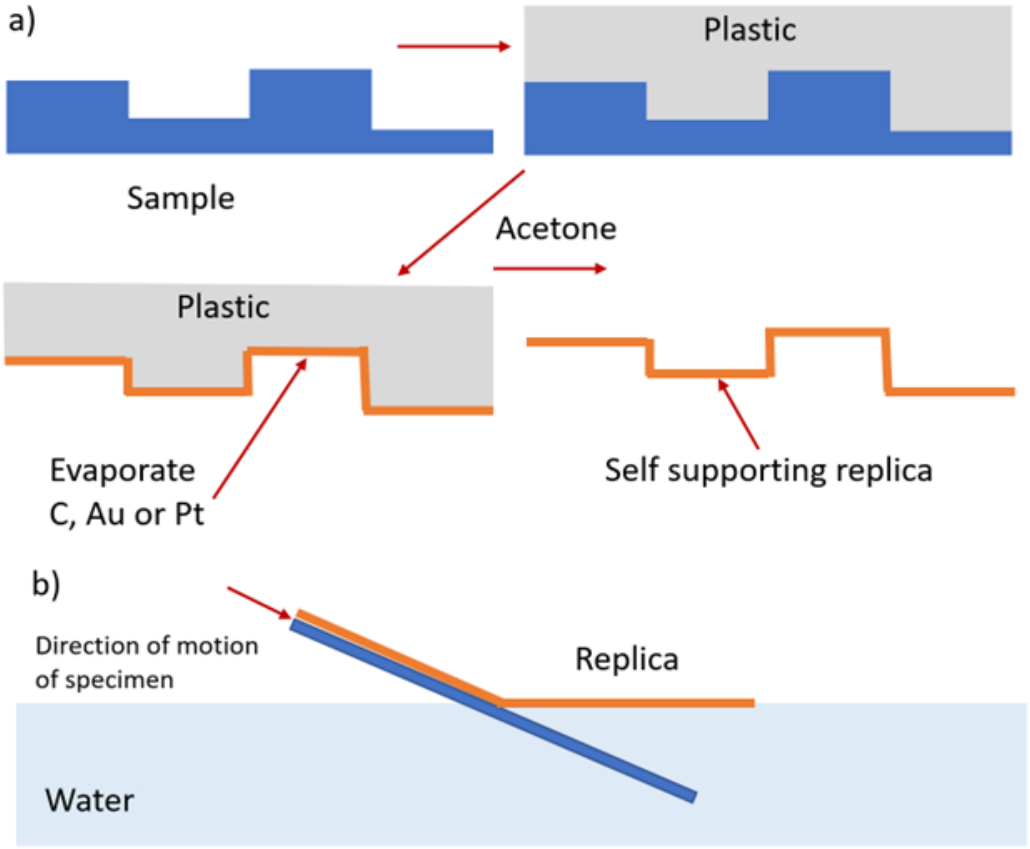
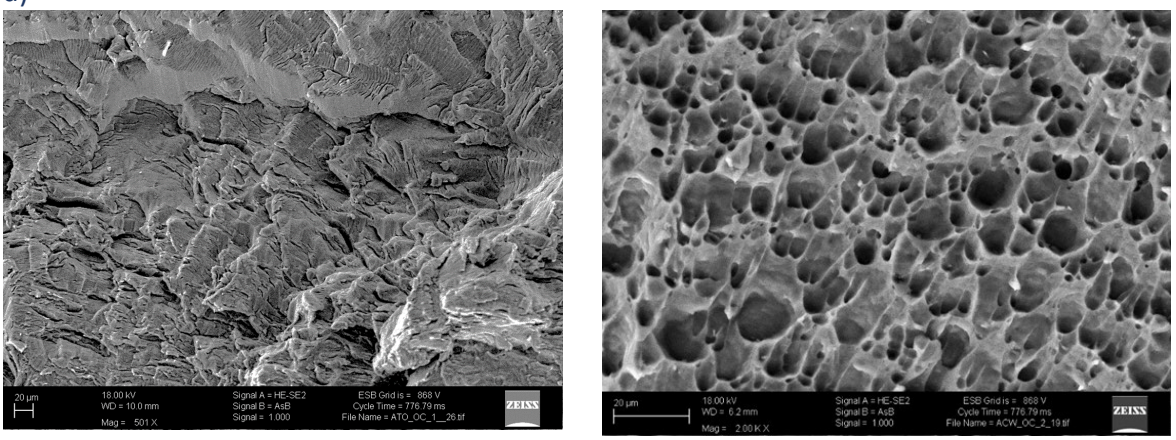


Fig. 9. Following steps in preparation of matrix replica

a)



b)

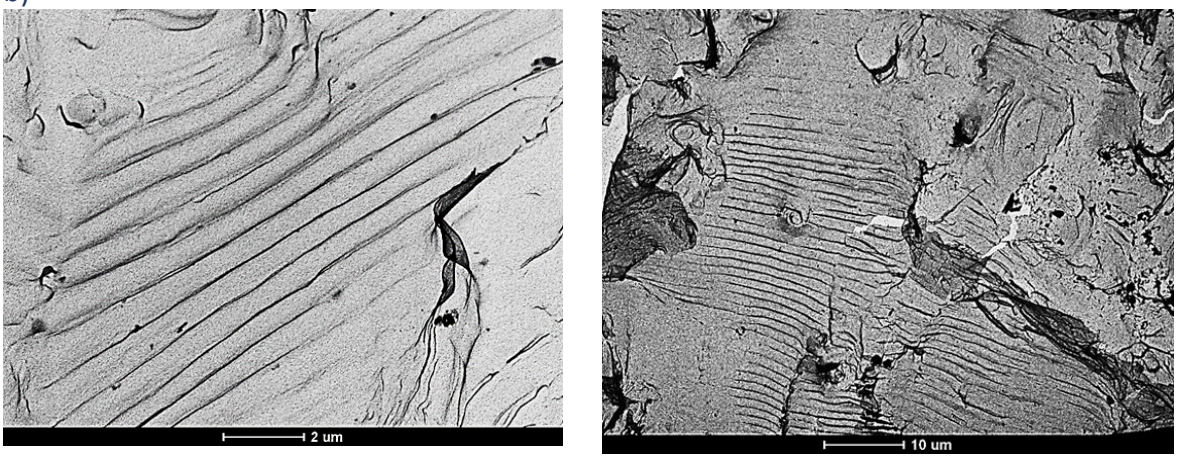


Fig.10. Fatigue fractures of steel samples-a). Example matrix replicas from the fatigue fracture area of a steel sample-b).

1.3.2. Extraction replica technique

The extraction replication method allows us to extract a particle from its surrounding matrix, thus allowing us to analyze that phase alone without interference from electron scattering into the matrix. This is particularly important in the chemical composition or diffraction analysis of small precipitates due to eliminating the influence of the chemical composition and crystal structure of the matrix from the analysis. The various steps for preparation of extraction replicas are shown in **Figure 11**. Initially, the sample is polished metallographically to expose the particles on the surface. An appropriate etching process removes the matrix so that the particles protrude on the surface. A thin carbon film is evaporated onto the surface and scored into 2 mm squares. Then the etching process is continued. As the matrix dissolves, the squares of the carbon film rise to the surface, taking the precipitate particles with them. Placing such a fragment of the film on the grid gives a ready sample for TEM observation. Exemplary extraction replicas made of ODS alloy strengthened with yttrium oxides and P92 structural steel strengthened with Laves phase particles are presented in **Figure 12**.

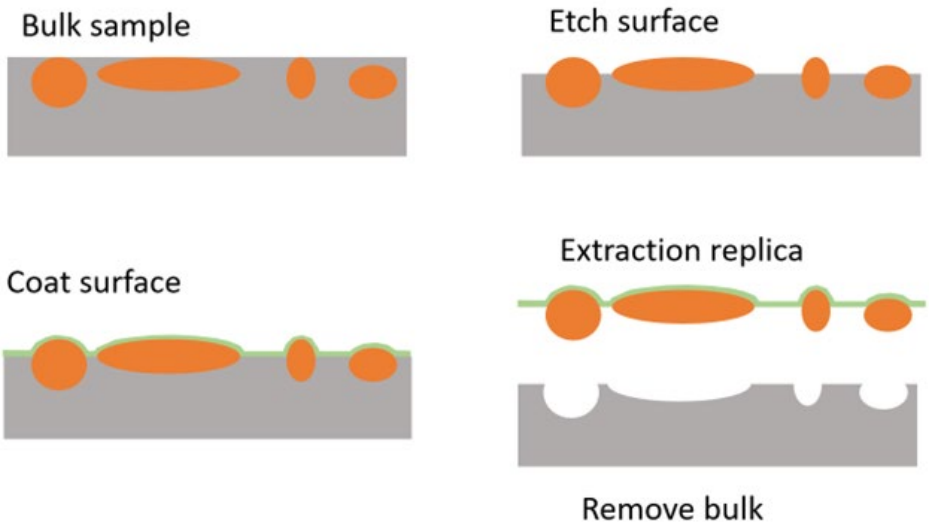


Fig. 11. Following steps in preparation of extraction replica

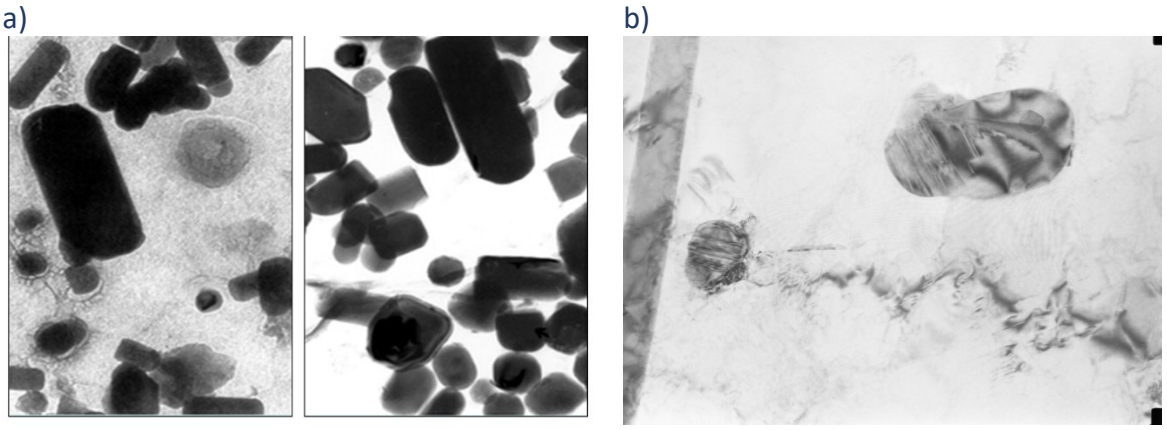


Fig. 12. Exemplary extraction replicas made of ODS alloy strengthened with yttrium oxides -a) and P92 structural steel strengthened with Laves phase particles

1.4. Correlative multiscale electron microscopy, 3D FIB-SEM tomography with detailed TEM investigation – Targeted Sample Preparation

A novel methodology for combining FIB-SEM 3D tomography with the preparation of cross-section lamellae has been developed. FIB-SEM tomography is an imaging technique that combines a focused ion beam (FIB) with a scanning electron microscope (SEM) to obtain information on the internal structure of specimens. In a typical FIB-SEM tomography experiment, the specimen is situated at the coincident point of the FIB and SEM beams. After finding an area of interest (AOI) in the microstructure and depositing the platinum protective layer (IBID – ion-induced deposition), a trench is first carved on the upper surface of the sample with the FIB (**Fig. 13**). The trench helps prevent the adverse effects of material redeposition on the process. Line marks are made for further stack alignment and slice thickness calibration.

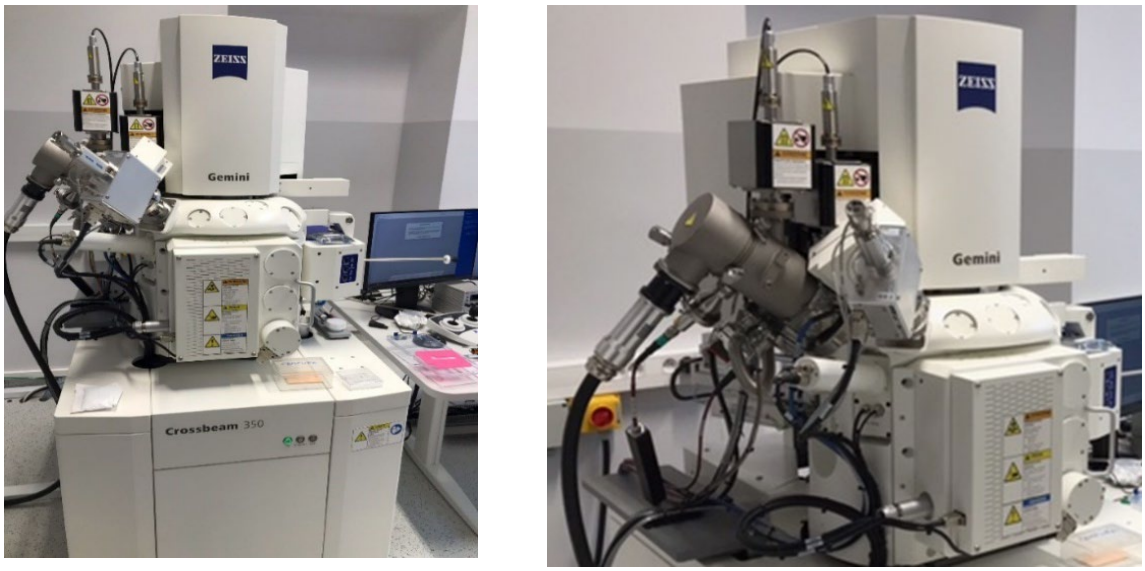


Fig. 13. Example FIB-SEM Crossbeam 350 dual-beam device (ZEISS)

Then dozens or hundreds of cross-sections of the internal structure of the specimen are made with the FIB and consecutively imaged with the SEM-BSE. The outcome is a stack of SEM images of every cross-section, which, after alignment and further image processing, reconstructs the investigated volume in three dimensions (**Figs. 14, 15**). The ion beam is blanked after each slice to not interfere with imaging and unblanked for further milling. It is necessary to find the parameters of the ion and electron beams so that the cutting and imaging cycle of one slice is as short as possible. In addition, equilibrium needs to be established between the sample, the milling beam and the imaging beam. During the slicing process, it is possible to live image every cross-section (**Fig. 15**).

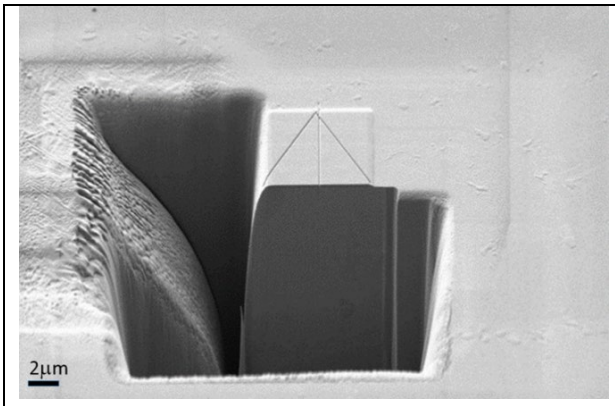


Fig. 14. Platinum layer deposited IBID method with the carved trench.

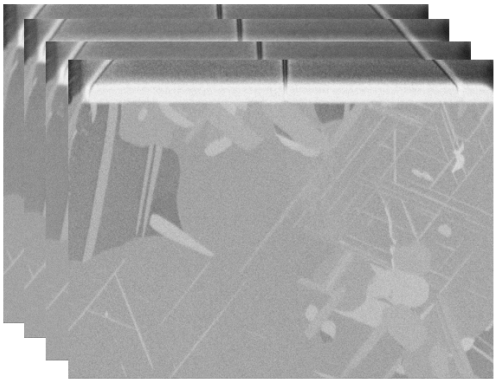


Fig. 15. Stack of SEM-BSE ATI 718 plus microstructure images.

When we spot an extraordinary part of microstructure, which can't be visible on the surface of our material, we can stop the slicing process and start FIB lamellae preparation for further TEM microstructure investigation.

1.5. TEM Sample Preparation using the CrossBeam® technique

When preparing TEM specimens, we consider the FIB as an SEM with a built-in ion gun. The single ion gun produces a well-controlled beam of Ga ions (rather than Ar used in the ion mill). In the most straightforward (cheaper) design, the ion beam also acts as the electron beam of the SEM, with the secondary electrons being used to form the 'SEM' image of the sample.

Common FIB-induced artefacts include (**Fig. 16**):

1. "curtain effect": due to different materials with variable milling rates, that can be reduced by FIB deposited protective pad,
2. low melting point, gallium phase formation on the sample surface,
3. beam damage on FIB-prepared TEM specimens,
4. beam-induced grain growth in nano-crystalline materials,
5. beam damage to most of the HCP materials,
6. materials redeposition during milling,
7. surface amorphization.

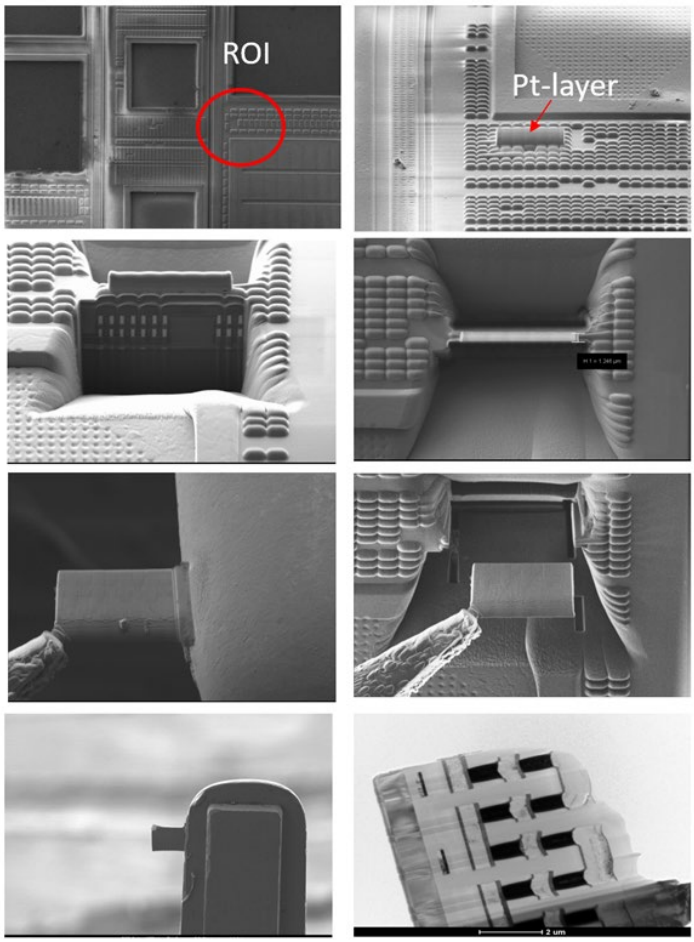


Fig.16. TEM sample preparation using the CrossBeam® technique

Preparing a FIB lamella for TEM usually starts with depositing a protective layer Pt layer (**Fig. 17**). Such a thin layer is mandatory to protect the sample from any damages that could occur with a thick layer (several µm) made by ion-induced deposition (IBID). When preparing a sample for transmission microscopy, we want the lamellas to contain microstructural features that are crucial from the analysis point of view (**Fig. 18**). We suggest combining FIB-SEM tomographic image acquisition used for 3D visualization with lamella cutting to make this happen. Such a procedure results in a Targeted Sample Preparation (TSP).

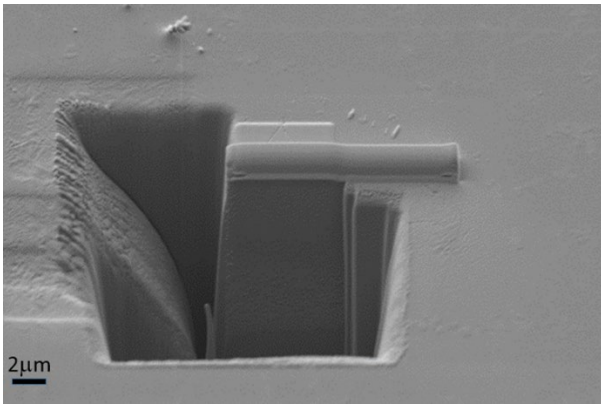


Fig. 17. Platinum layer deposited IBID method for FIB lamella preparation

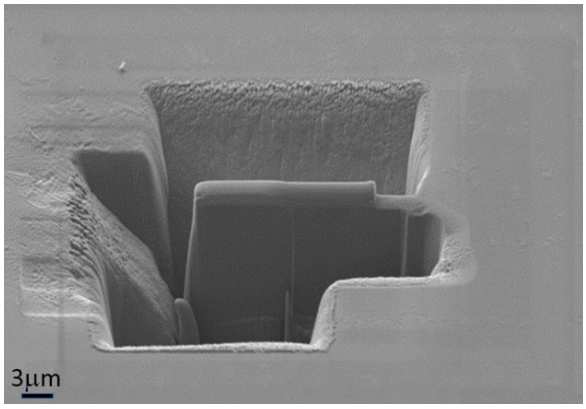


Fig. 18. Lamellae prepared for an omniprobe TEM holder

The next step was cutting a second trench with Ga+-focused ions. The accelerating voltage with a value of 30kV and a beam current of 30nA were used. The lamella was thinned with an energy beam 30kV 1.5nA and prepared for transfer to the omniprobe TEM holder.
The transferred lamella was thinned with an energy beam in the following steps 30kV 300-100-50pA, polished with 10kV 15pA and cleaned with 2kV 10pA. The polishing process is complete when the lamellae are transparent for electrons, observed in the SEM with 5kV of voltage. (**Fig. 19**.). **Figures 20, 21** presents the results of the investigations of sample, 3D visualization (**Fig. 20**) and results of STEM-EDS elemental map (**Fig. 21**). The combination of 3D FIB-SEM tomography with targeted sample removal from the analyzed sample volume for TEM studies -Targeted Sample Preparation (TSP) was presented in **Figure 22**.

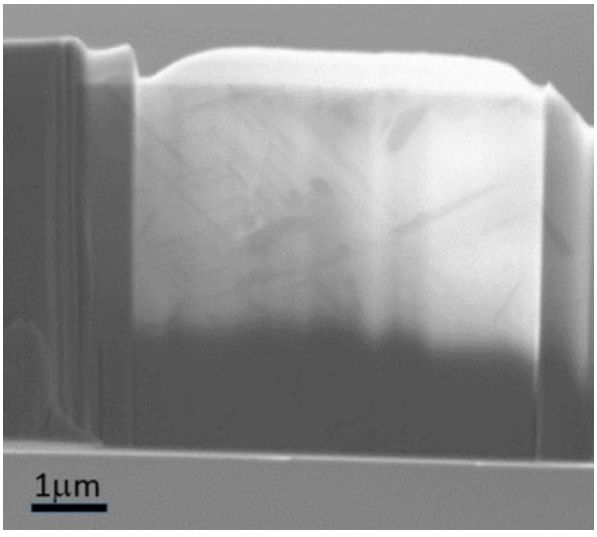


Fig. 19. Polished FIB lamellae of Inconel 718 plus

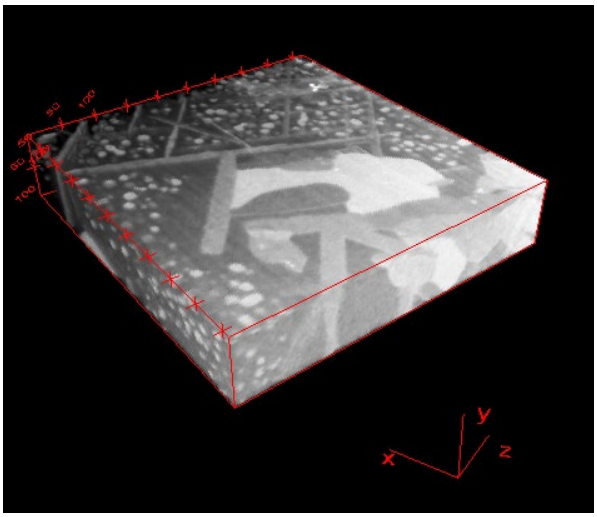


Fig 20. 3D visualization of the investigated volume. Result from FIB-SEM tomography.

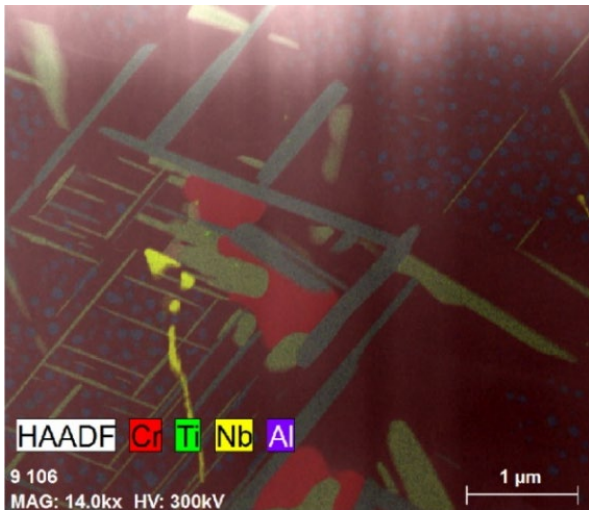


Fig. 21. Superposition of selected STEM-EDS elemental maps. TEM lamella.

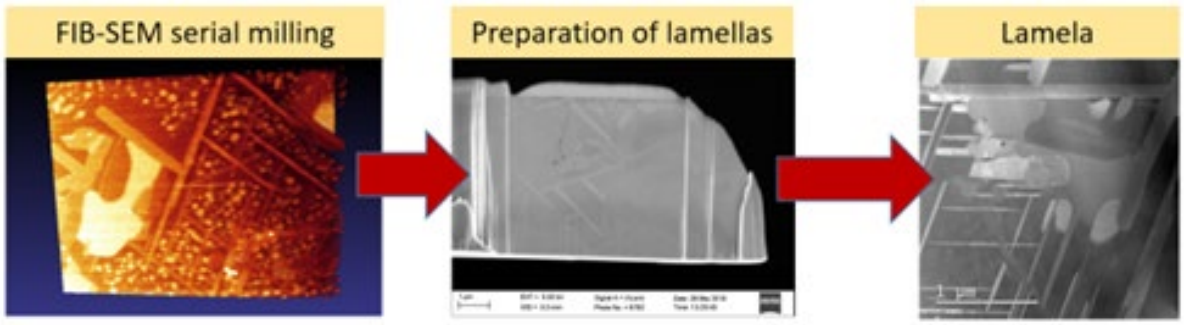


Fig. 22. The combination of 3D FIB-SEM tomography with targeted sample removal from the analyzed volume of sample for TEM studies -Targeted Sample Preparation (TSP)

1.6. Sample preparation for in-situ heating in TEM

1.6.1. Sample : age-hardening aluminium alloy

Lamellae of age-hardening aluminium alloys on DENS chips for in-situ heating in TEM were prepared using electropolishing plus FIB techniques to get the best quality samples in the correct orientations (**Fig. 23**). The procedure is as follows:

1. Electropolishing (standard preparation using a Struers Tenupol with an electrolyte consisting of 1/3 HNO_3 and 2/3 Methanol).
2. Study the electropolished disk in TEM to locate thin grains close to [001] zone axis.
3. FIB lift-out:
    - mill out [001] grain,
    - attach the lift-out needle to the thicker part of the grain with C welding,
    - attach the lamella to the DENS chip using C welding, and
    - cut off the lift-out needle.

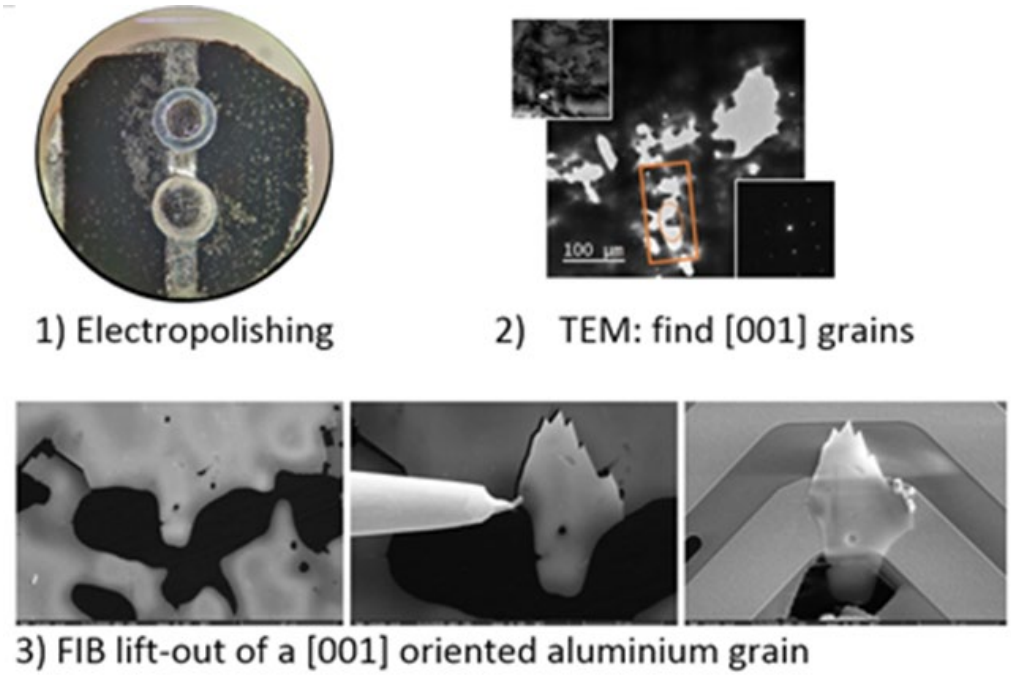


Fig. 23. Overview of the aluminium lamellae preparation for in-situ heating in TEM. Results stem from NTNU TRO.

The quality gets best by never exposing the region of interest (ROI) to the ion beam. Limiting redeposition on the ROI by performing all milling and welding more than ~20 µm from the ROI is crucial. In this way, achieving close to electropolished surface quality is possible by combining electropolishing and FIB lift-out to get a site-specific area in the correct zone axis (**Fig. 24**).

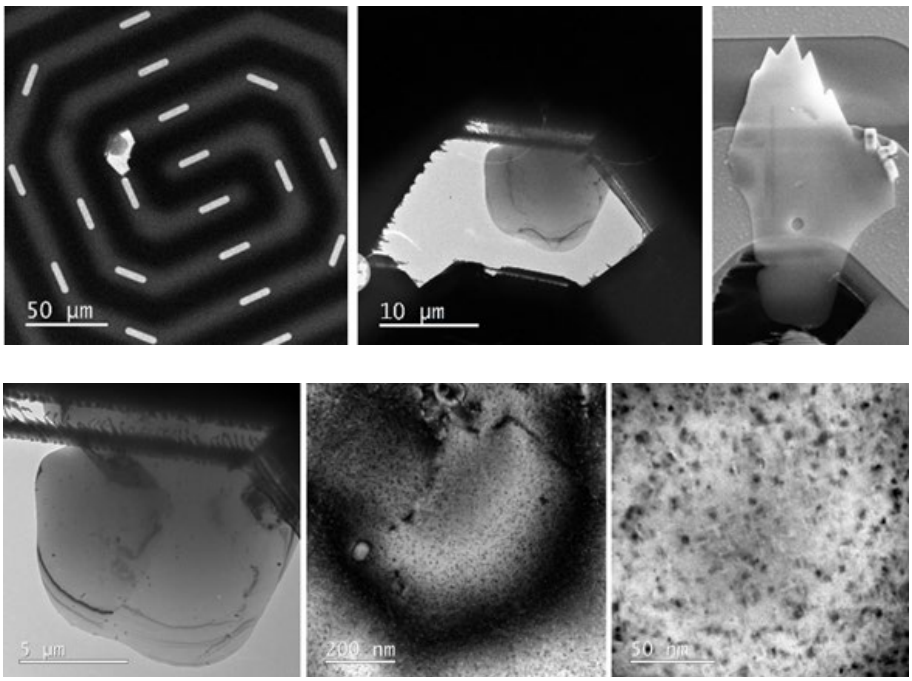
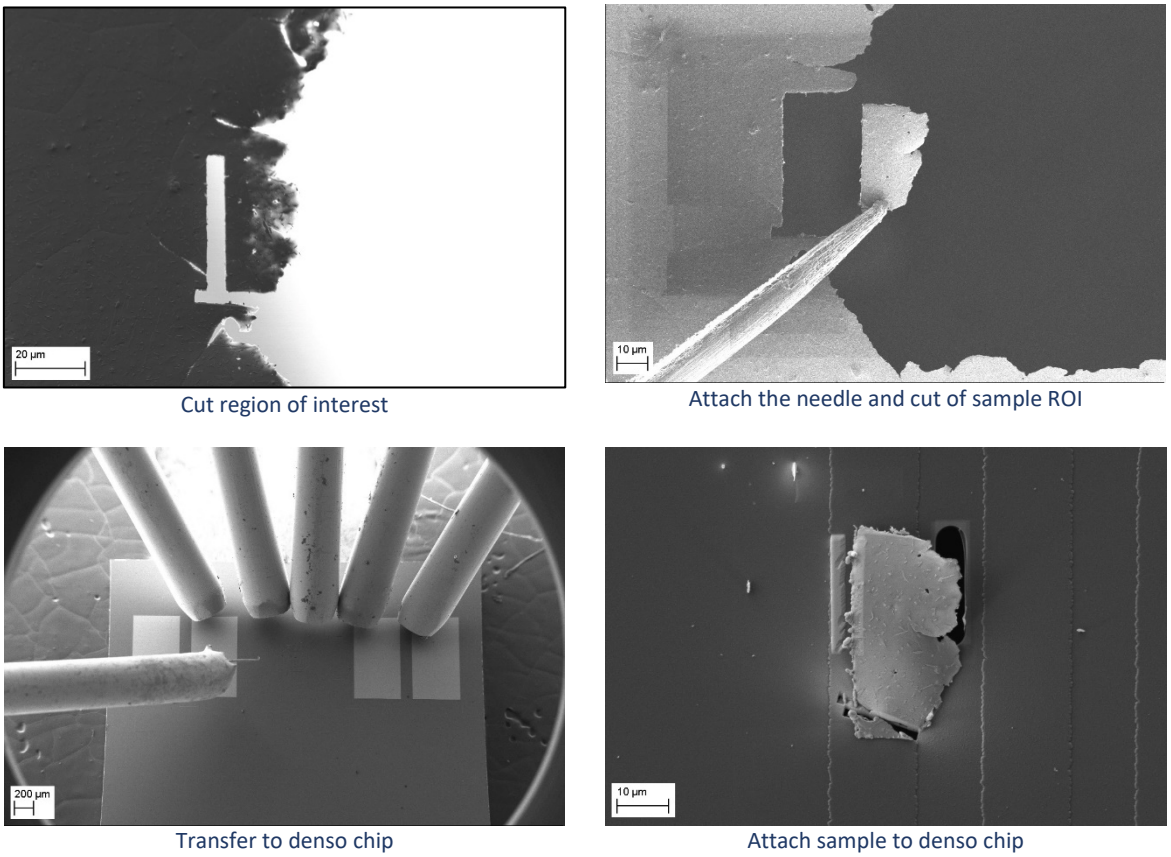


Fig.24. BF-TEM images and an SEM image (top right) of an aluminium lamella attached to a DENS chip for in-situ heating in TEM. Results stem from NTNU TRO.

1.6.2. Sample : Sanicro25 steel

Lamellae of **Sanicro 25** alloy on DENS chips for in-situ heating in TEM were prepared using electropolishing plus FIB techniques to get the best quality (**Fig. 25**). The procedure is as follows:

1. TEM sample was prepared with a standard twin-jet electrochemical electron polishing using a solution of 95% ethanol and 5% perchloric acid at 40 V.
2. Study the electropolished disc in TEM to locate the region of interest (ROI).
3. FIB lift-out:
   - Mill out an interesting part of the sample,
   - attach the lift-out needle to the thicker part of the sample with Pt deposition,
   - attach the lamella to the DENS chip using Pt deposition, and
   - cut off the lift-out needle.



Cut region of interest | Attach the needle and cut of sample ROI

Transfer to denso chip | Attach sample to denso chip

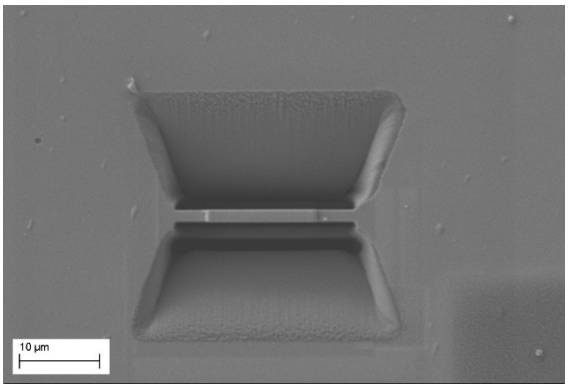
Fig. 25. Overview of the Sanicro 25 lamellae preparation for in-situ heating in TEM.

The quality gets best by never exposing the region of interest (ROI) to the ion beam. Limiting redeposition on the ROI by performing all milling and welding as far as possible from the ROI is crucial. In this way, achieving close to electropolished surface quality is possible by combining electropolishing and FIB lift-out to get a site-specific area in the correct zone axis.

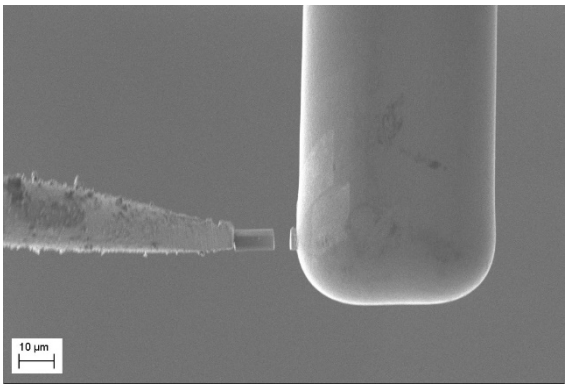
1.6.3. Sample: Ge-Ag-Ge layered films

FIB SEM preparation of Lamellae of Ge-Ag-Ge layered films on DENS chips for in-situ STEM observation of the kinetics of phase transformations (**Fig. 26**.). The procedure is as follows:

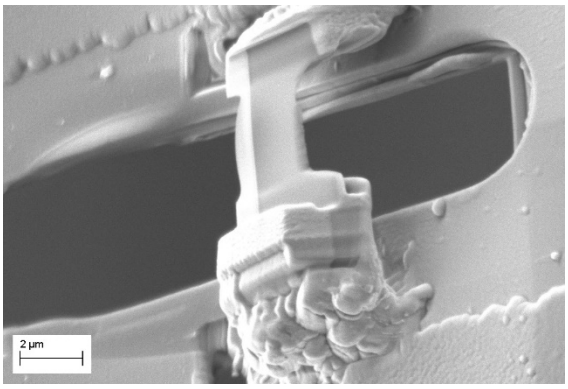
1. Classic FIB-SEM lamellae preparation of Ge-Ag-GE layered film on Si substrate.
2. Attach lamellae to the needle, and rotate the sample to achieve a 10-15 degree angle between the sample and denso chip.
3. Attach lamellae to chip with Pt deposition and cut out the needle.
4. Polish attached lamellae with ion beam until it starts to be transparent for electrons.



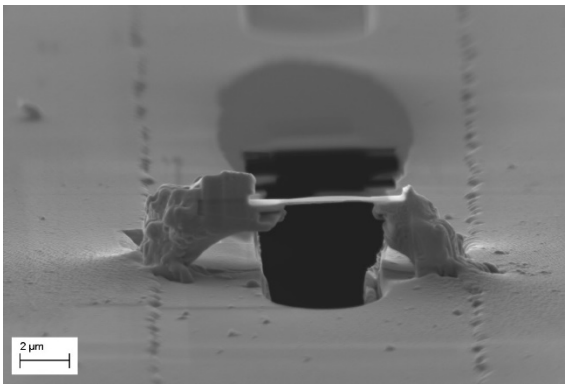
FIB-SEM lamellae preparation



Attach to needle and rotate



Attach lamellae to denso chip



Thined and polished lamellae

Fig. 26. Overview of the Ge-Ag-Ge layered film lamellae preparation for in-situ heating in TEM

If the built-in micromanipulator does not have the function of rotating the needle along its axis, transfer the sample to the grid, rotate the grid by the appropriate angle, reattach the sample to the needle and then transfer it to denso chip.

1.7. Conclusions

- It is necessary to remember that good TEM specimen preparation is the basis of good qualitative and quantitative TEM analysis.
- The selection of the preparation method is dependent on the kind of material and the methods and techniques used in TEM analysis.
- To observe the layers or interfaces of coated materials, specimens could be prepared by the sandwich or comparable other methods.
- TEM specimen preparation by FIB is an all-purpose method for most materials and types of TEM analysis.
- Post-FIB processing is required when conducting high-resolution TEM studies (HRSTEM and HRTEM).
- Correlative multiscale electron microscopy combines two techniques, which are 3D FIB-SEM tomography and detailed TEM investigation – Targeted Sample Preparation gives new possibilities in the qualitative and quantitative phase analysis of the microstructure elements of the structural materials.