

# Chapter 6

## Artifacts in Transmission Electron Microscopy

### 1 Introduction

An artifact is damage caused by a preparation technique and can easily be confused with the sample's microstructure. Artifacts can be due to mechanical, chemical, ionic, or physical action. During TEM observation, especially in a TEM/STEM, other artifacts may be produced due to irradiation under the electron beam.

Therefore, there are two families of artifacts: those formed during the preliminary preparation steps and sample thinning and those formed under the effect of the electron beam during observation. Materials will be more or less sensitive to the formation of artifacts depending on their type and the nature of the chemical bonds.

### 2 Preparation-Induced Artifacts

Included among the possible artifacts are primary damage, formed by the preparation techniques, and secondary thermal damage, caused by a rise in the temperature during preparation or during observation under the electron beam. Table 6.1 shows all of the possible artifacts.

The artifacts and secondary thermal damage have been classified based on either the type of action (mechanical, ionic, chemical, and physical) involved in the preparation of the thin slices or their formation during the electronic radiation in the microscope.

The definition of each artifact is provided, as well as the preparation techniques from which they may be derived.

Lastly, this chapter presents illustrations of the artifacts most commonly encountered in the mechanical, ionic, chemical, and physical thinning techniques. It also provides illustrations of artifacts derived from the combination of several techniques in the fields of solid-state physics and biology.

**Table 6.1** Table summarizing the various artifacts formed by the preparation techniques or during TEM observation

| Mechanical preparation-induced artifacts | Ionic preparation-induced artifacts | Chemical preparation-induced artifacts | Physical preparation-induced artifacts | Artifacts induced during observation |
|------------------------------------------|-------------------------------------|----------------------------------------|----------------------------------------|--------------------------------------|
| Deformation                              | Redeposition                        | Material displacement                  | Deformation                            | Dehydration                          |
| Material displacement                    | Implantation                        | Selective dissolution                  | Microstructure change                  | Charge effects                       |
| Material tearing                         | Vacancies                           | Composition change                     | Segregation of liquid phases           | Destruction                          |
| Cracks                                   | Dislocation loops                   | Structural change                      |                                        | Contamination                        |
| Fractures                                | Cavities                            | Microstructure change                  |                                        |                                      |
| Inclusion of abrasive grains             | Fractures                           | Change of molecular bonds              |                                        |                                      |
| Dislocations                             | Roughness                           | Change of natural contrast             |                                        |                                      |
| Glide planes                             | Selective abrasion                  | Protein reticulation                   |                                        |                                      |
| Twinning                                 | Structural change                   | Residues                               |                                        |                                      |
| Strain hardening                         | Microstructure change               |                                        |                                        |                                      |
| Selective abrasion                       |                                     |                                        |                                        |                                      |
| Particle aggregation                     |                                     |                                        |                                        |                                      |
| Roughness                                |                                     |                                        |                                        |                                      |
| Structural change                        |                                     |                                        |                                        |                                      |
| Microstructure change                    |                                     |                                        |                                        |                                      |
| Crystal-network change                   |                                     |                                        |                                        |                                      |
| Composition change                       |                                     |                                        |                                        |                                      |
| Residues                                 |                                     |                                        |                                        |                                      |
| <i>Secondary thermal damage</i>          |                                     |                                        |                                        |                                      |
| Fusion                                   | Fusion                              | Microstructure change                  | Deformation                            | Demixing                             |
| Phase transformation                     | Phase transformation                | Changes in distribution in the phases  | Particle aggregation                   | Amorphization                        |
| Loss of chemical elements                | Loss of chemical elements           |                                        | Fusion                                 | Phase transformation                 |
| Amorphization                            | Amorphization                       |                                        | Phase transformation                   | Loss of chemical elements            |
|                                          | Demixing                            |                                        | Frost                                  | Migration                            |
|                                          |                                     |                                        |                                        | Fusion                               |

## 2.1 Mechanical Preparation-Induced Artifacts

**Deformation:** Change in shape caused by the compression, stretching, or tearing of all or part of a sample's volume. This type of artifact can generate a change in matter conformation and distribution.

*Techniques involved: sawing, mechanical polishing, dimpling, crushing, ultramicrotomy, cryo-ultramicrotomy, freeze fracture, and extractive replica.*

**Matter displacement:** Transport by creeping or stretching of either matter on the sample surface or a particular phase in a multiphase material, caused by the friction of abrasive grains on the sample or by the cutting action.

*Techniques involved: sawing, mechanical polishing, dimpling, tripod polishing, ultramicrotomy, and cryo-ultramicrotomy.*

**Tearing of matter:** Localized loss of matter or of a particular phase in a multiphase material, caused either by the friction of abrasive grains on the sample or by the cutting action.

*Techniques involved: sawing, mechanical polishing, dimpling, tripod polishing, ultramicrotomy, and cryo-ultramicrotomy.*

**Cracks:** Crevices on the surface of the material, caused by the friction of abrasive grains on the sample, by the application of pressure (e.g., during cutting) or by thermal effects.

*Techniques involved: sawing, mechanical polishing, dimpling, grinding, tripod polishing, ultramicrotomy, and cryo-ultramicrotomy.*

**Fractures:** Splitting or separation of regions of the sample. The fracture can be caused by the rubbing of the sample on abrasive grains, the cutting action, the application of either pressure or tension, or thermal effects.

*Techniques involved: sawing, mechanical polishing, dimpling, grinding, wedge cleavage, tripod polishing, ultramicrotomy, cryo-ultramicrotomy, and freeze fracture.*

**Inclusion of abrasive grains:** The implantation by mechanical action of abrasive grains used during the technique, or even residues derived from contaminated polishing wheels.

*Techniques involved: mechanical polishing, dimpling, and tripod polishing.*

**Dislocation:** Linear structural defect (1D) resulting in an additional plane in an atomic stack. Dislocations are formed under the effect of a mechanical stress, e.g., impacts under the surface or tearing from abrasive grains on brittle materials.

*Techniques involved: sawing, ultrasonic cutting, mechanical polishing, dimpling, grinding, wedge cleavage, tripod polishing, and ultramicrotomy.*

**Glide planes:** Plane defect (2D) corresponding to the dense atomic plane of a crystal where dislocations propagate under the effect of a stress. This strong pressure on the crystal causes movements of whole atomic planes.

*Techniques involved: sawing, ultrasonic cutting, mechanical polishing, dimpling, tripod polishing, and ultramicrotomy.*

**Particle aggregation:** Agglomerate of particles formed by poor dispersion of isolated particles, or by migration of particles in solution during the dessication process. This is due to attractive forces between particles.

*Techniques involved: fine particles dispersion technique.*

**Twinning:** Plane defect (2D) corresponding to a joint or perfect mirror interface at the atomic scale. The atomic plane of the twin is common to the crystal networks of two grains. Their formation can be caused by a thermal phase transformation or mechanical stresses, a violent impact can cause twinning in brittle materials.

*Techniques involved: sawing, ultrasonic cutting, mechanical polishing, dimpling, tripod polishing, and ultramicrotomy.*

**Strain hardening:** Change in microstructure due to mechanical stresses, which induces a set of disturbances in the material involving dislocations and glide planes.

*Techniques involved: sawing, ultrasonic cutting, mechanical polishing, dimpling, tripod polishing, and ultramicrotomy.*

**Selective abrasion:** Thinning of matter at different rates by mechanical polishing. It can reveal a phase or lead to its disappearance through the faster abrasion of one phase over another. It can create surface roughness.

*Techniques involved: mechanical polishing, dimpling, and tripod polishing.*

**Roughness:** State of the non-plane surface revealing either scratches caused by material tearing or variations in thin slice thickness caused by selective abrasion or the tearing of matter.

*Techniques involved: sawing, ultrasonic cutting, mechanical polishing, dimpling, tripod polishing, ultramicrotomy and cryo-ultramicrotomy.*

**Structural change:** Partial or total change of the crystallographic organization caused by mechanical stresses. It can lead to a change in form, amorphization of the crystal network, a change in network, crystallization, or re-crystallization.

*Techniques involved: mechanical polishing, dimpling, and tripod polishing.*

**Microstructural change:** Partial or total change in the structural organization of microstructural components that can be caused by mechanical effects. It can result in changes in morphology, redistributions of phases, changes in crystal structure, precipitation, chemical gradients, formation of new phases, etc.

*Techniques involved: mechanical polishing, dimpling, tripod polishing, and ultramicrotomy.*

**Crystal-network change:** Change in the geometry of the crystalline structure and/or the crystalline motif. Any mechanical action can cause additional changes to the crystal network, such as dislocations, glide planes, twinning, and strain hardening.

*Techniques involved: sawing, ultrasonic cutting, mechanical polishing, dimpling, tripod polishing, and ultramicrotomy.*

**Composition change:** Change in the elemental composition of the sample due to a loss or dissolution of chemical elements during preparation.

*Techniques involved: ultramicrotomy.*

**Residues:** Deposits of matter extrinsic to the sample that may be polishing or sectioning residues resulting from preparation. They are superimposed on the thin slice and impede its observation. Residues are produced by mechanical actions during preliminary preparation or during final thinning.

*Techniques involved: mechanical polishing, grinding, tripod polishing, ultramicrotomy, and cryo-ultramicrotomy.*

### 2.1.1 Secondary Thermal Damage Induced During Mechanical Preparation

**Fusion:** Change of the solid–liquid physical state of a solid sample. It can be caused by an increase in temperature during mechanical preparation.

*Techniques involved: mechanical polishing and tripod polishing.*

**Phase transformation:** Total or partial transformation of the crystallographic and/or chemical structure, which can be caused by thermal effects during mechanical techniques.

*Techniques involved: sawing, mechanical polishing, dimpling, and tripod polishing.*

**Loss of chemical elements:** Selective loss of a chemical element, which may be caused by secondary thermal effects during mechanical preparation.

*Techniques involved: sawing, mechanical polishing, dimpling, and tripod polishing.*

**Amorphization:** Partial or total degradation of the chemical bonds of a crystalline sample, resulting in its destruction. This type of defect is caused by thermal effects during the mechanical action of preparation.

*Techniques involved: mechanical polishing and tripod polishing.*

## 2.2 Ionic Preparation-Induced Artifacts

**Redeposition:** Deposition of material previously milled by an ion beam, either intrinsic or extrinsic to the sample, which redeposits on the specimen surface during thinning. This is a form of contamination.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Implantation:** Accelerated ions from the beam penetrate the sample and remain trapped there, creating a new chemical type.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Vacancies:** Point defects (0D) corresponding to the loss of an atom in the crystal network. Accelerated ions can remove atoms or ions from the matrix, creating vacancies in the crystal network or creating defects (grain boundaries, twin boundaries, etc.). Accumulation of vacancies can form cavities.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Dislocation loops:** Partial dislocations that border an atomic-plane defect and close on themselves creating a dislocation loop.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Cavities:** Area free of matter inside a material. It is caused by the accumulation of vacancies and corresponds to volume defects (3D) that form an intra- or intergranular cavity.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Fractures:** Splitting or separation of regions of the sample. The fracture is caused by the relaxation of stresses due to the increase of temperature during ion thinning.

*Techniques involved: sawing, mechanical polishing, dimpling, grinding, wedge cleavage, tripod polishing, cryo-ultramicrotomy, freeze fracture and focused ion beam thinning FIB.*

**Roughness:** Variation of surface relief caused by selective abrasion.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Selective abrasion:** The ion beam can reveal phases if one of the phases abrades more slowly than the other. Selective abrasion can create an undulating surface, cause roughness by revealing dense atomic phases, or form peaks if there are precipitates or segregation zones (grain boundary, dislocation, etc.). These types of defects are also preferentially torn and leave cones of material around crystal defects. These are clouds of impurities that delay the abrasion of the zone, particularly around screw dislocations.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Structural change:** Partial or total change in the crystallographic organization of a sample, caused by ionic effects. It can lead to a change in form,

amorphization of the crystal network, a lattice change, crystallization, or re-crystallization.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Microstructural change:** Partial or total change of the structural organization of the components of a microstructure that can be caused by ionic effects. It can result in changes in morphology, redistributions of phases, changes in crystal structure, precipitation, chemical gradients, formation of new phases, etc.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

### 2.2.1 Secondary Thermal Damage Induced During Ionic Preparation

**Fusion:** Change of solid–liquid physical state of a solid sample. It can be caused by an increase in temperature during ionic preparation.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Phase transformation:** Total or partial transformation of the crystallographic and/or chemical structure of a sample, which can be caused by thermal effects during ionic preparation.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Loss of chemical elements:** Selective loss of a chemical element of the sample, which may be caused by secondary thermal effects during ionic preparation.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Amorphization:** Partial or total degradation of the chemical bonds of a crystalline sample, leading to its destruction, caused by thermal effects during the ionic preparation.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

**Demixing:** Separation of partially miscible phases of a mixture or alloy caused by thermal effects, leading to the exceeding of the solubility limit of one or more compounds.

*Techniques involved: ion milling and focused ion beam thinning (FIB).*

## 2.3 Chemical Preparation-Induced Artifacts

**Matter displacement:** Transport by creeping of either matter on the sample surface or a particular phase of a multiphase material, caused by chemical action.

*Techniques involved: electrolytic polishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, full-bath chemical thinning, and extractive replica.*

**Selective dissolution:** Chemical or electrolytic polishing or etching can reveal phases if one of them dissolves more slowly than another. Selective dissolution can create an undulating surface if the flow of the dissolution layer or bath agitation are not correctly ensured. It can cause roughness if the bath reveals dense atomic planes and form peaks if precipitates or segregation zones (grain boundaries, dislocations, etc.) are sensitive to the bath.

*Techniques involved: electropolishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, full-bath chemical thinning, and extractive replica.*

**Composition change:** Change in the elemental chemical composition of the sample due to a loss or addition of chemical elements tied to contamination or dissolution during preparation.

*Techniques involved: electropolishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, full-bath chemical thinning, and extractive replica.*

**Structural change:** Partial or total change of the crystallographic and chemical organization of a sample, caused by chemical effects. It can result in a change in form, amorphization of the crystal network, a change in network, or crystallization or re-crystallization.

*Techniques involved: electropolishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, full-bath chemical thinning, and extractive replica.*

**Microstructural change:** Partial or total change of the structural organization of microstructure components, caused by ionic effects. It can result in morphology changes, phase distribution, changes in crystal structure, precipitation, chemical gradients, formation of new phases, etc.

*Techniques involved: electropolishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, full-bath chemical thinning, and extractive replica.*

### 2.3.1 Changes Specific to Biological Materials

**Structural change:** Alterations of structure at different scales, leading to the observation of a sample that is different from the living material.

- Volume change
- Denaturing of components leading to textural changes
- Transformation of the protein gel into a reticulated structure

*Techniques involved: chemical fixation.*

- Transformation of membrane phospholipids into unbroken lines

*Techniques involved: osmic fixation.*

**Change in the natural contrast:** Change induced by the addition of a heavy metal.

*Techniques involved: chemical fixation using osmium and “positive-staining” contrast.*

**Change in the molecular bonds:** Creation of new bonds between macromolecules, leading to interpretation errors of reactive sites after labeling.

*Techniques involved: chemical fixation using glutaraldehyde.*

**Protein cross-linking and changes in their spatial conformation:** Connection of macromolecular chains by the creation of bridges or chemical bonds with an exogenous compound, i.e., fixative. This leads to the formation of a network with physical–chemical properties different from the initial material. Cross-linking is an irreversible process that constitutes an obligatory artifact resulting from chemical fixation, but which is indispensable to being able to carry out dehydration. Cross-linking leads to a change or loss of reactivity of the primordial macromolecules. It also causes the formation of bonds between molecules that were originally independent.

*Techniques involved: chemical fixation and substitution–infiltration–embedding at room temperature.*

**Change in chemical composition:** Loss of compounds: saturated lipids, simple sugars, ions, salts, loss or addition of chemical elements.

*Techniques involved: chemical fixation, substitution–infiltration–embedding at room temperature, and “positive-staining” contrast.*

**Residues:** Deposits of matter extrinsic to the sample, which are chemical residues resulting from preparation. They are superimposed on the thin slice and impede observation. Residues are produced by chemical additions during preliminary preparation or during thinning.

*Techniques involved: electropolishing, chemical polishing, continuous support film, holey support film, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, full-bath chemical thinning, extractive replica, chemical fixation, freeze fracture, “positive-staining” contrast, and immunolabeling.*

### 2.3.2 Secondary Thermal Damage Induced During Chemical Preparation

**Microstructural change:** Partial or total change of the structural organization of microstructure components that can be caused by thermal effects during chemical preparation. It can result in changes in morphology, phase redistribution, crystal-structure changes, precipitation, chemical gradients, formation of new phases, etc.

*Techniques involved: electropolishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, and full-bath chemical thinning.*

**Changes in phase distribution:** Aggregation of chemical elements caused by thermal diffusion or aggregation of several elements of a microstructure that might lead to the disappearance of the initial phases.

*Techniques involved: electropolishing, chemical polishing, twin-jet electrolytic thinning, full-bath electrolytic thinning, twin-jet chemical thinning, and full-bath chemical thinning.*

## 2.4 Physical Preparation-Induced Artifacts

**Deformation:** Change in shape caused by the formation of ice crystals in a sample that should contain only vitreous ice.

This artifact can generate a change in the conformation and distribution of the matter.

*Techniques involved: cryo-fixation and frozen hydrated film*

**Microstructural changes:** Partial or total change in the structural organization of the components of a microstructure, caused by thermal effects (low temperatures). It can result in changes in morphology, phase redistribution, changes in crystal structure, precipitation, chemical gradients, formation of new phases, etc.

*Techniques involved: cryo-fixation, substitution–infiltration–embedding in cryogenic mode, cryo-ultramicrotomy, and frozen hydrated film.*

**Segregation of liquid phases:** Migration of a solution in a solvent during a change of physical state that occurs too slow.

*Techniques involved: fine particle dispersion Technique, cryo-fixation.*

### 2.4.1 Secondary Thermal Damage Induced During Physical Preparation

**Deformation:** Change in shape due to heating during physical preparation, leading to the re-crystallization of vitreous ice. This artifact can generate a change in matter conformation and distribution.

*Techniques involved: cryo-fixation, substitution–infiltration–embedding in cryogenic mode, cryo-ultramicrotomy, frozen suspension film.*

**Particle aggregation:** Agglomeration of particles caused by the mobility of metallic particles deposited on the sample surface, which is created by a thermal effect or charge effect between ionic particle interaction. This leads to the creation of larger particles through coalescence.

*Techniques involved: direct replica, indirect replica, freeze fracture, and decoration shadowing, “positive-staining” contrast.*

**Fusion:** Change in solid–liquid physical state of a solid sample.

It can be caused by an increase in temperature during cryogenic preparation.

*Techniques involved: cryo-fixation, substitution–infiltration–embedding in cryogenic mode, and frozen hydrated film.*

**Phase transformation:** Total or partial transformation of the crystallographic and/or chemical structure of a sample, which can be caused by thermal effects during physical deposition.

*Techniques involved: direct replica, indirect replica, decoration-shadowing, and “negative-staining” contrast.*

**Frost:** Condensation of atmospheric water vapor on a very cold thin slice.

*Techniques involved: cryo-ultramicrotomy and frozen hydrated film.*

### 3 Artifacts Induced During TEM Observation

The investigation of microstructure using electron microscopy involves the interaction of the electron beam with the sample. This interaction irradiates the sample, creates charges in the slice, and can produce heat. The result is physical or chemical changes or the destruction of the material. All of these effects are a function of the acceleration voltage, the dose of electrons received, the size of the zone irradiated, and of course, the nature of the sample. The different types of degradation include artifacts caused by observation and secondary damage caused by thermal effects during observation.

#### 3.1 Artifacts Not Linked to Thermal Damages

**Dehydration:** The principal effect of the vacuum in the microscope is the instantaneous dehydration of materials in liquid solution or hydrated materials. We cannot work directly on these samples; they must either be immobilized or the water must be extracted.

On samples that are more lightly hydrated and chemically unstable (polymers, biological materials, non-stoichiometric oxides, etc.), the vacuum combined with irradiation can lead to the loss of light elements in a structure. This subsequently leads to phase transformations, resulting in significant changes in shape, which may ultimately result in the complete loss of structure.

*Charging:* If the charge dissipation is insufficient, the electrons and ions produced under the electron beam cause the appearance of a localized current proportionate to the intensity of the irradiation. This effect results in the instability of the sample under the beam.

The effects are even greater if the beam is focused and the material is insulating, i.e., if it does not allow the flow of electronic charges on the material via the support grid and the specimen holder.

One way to reduce this effect is to deposit a carbon (or metal) film on the surface of the sample.

*Destruction:* Sample destruction can be caused by the combined effects of irradiation and temperature rise during observation.

Irradiation results in the degradation of the sample, the appearance of stresses causing sample contraction, and the formation of cracks that eventually destroy the sample.

The electron–matter interaction produces heat by plasmon deexcitation. The temperature rise is proportionate to the current density of the beam and is inversely proportionate to the thermal conductivity of the sample.

These effects can result in the amorphization of the material, crystallization, phase demixing, and sample destruction. These effects can be reduced or eliminated by the use of a liquid-nitrogen-cooled specimen holder.

During electronic irradiation, atomic displacement reduces when the incident kinetic energy increases, i.e., when the TEM acceleration voltage increases. Therefore, at high energy, the effective cross section of electron interaction with the matter is greater. The resulting elastic diffusion only leads to weak atomic displacement. For most elements, the probability of atomic displacement is 100 times less than the probability of all elastic interactions. Voltages ranging between 100 and 200 keV represent the best practical compromise for all materials in order to limit irradiation damage.

The contrast of samples composed of light elements is even lower if the acceleration voltage is high. Voltages ranging between 75 and 120 keV represent the best practical compromise for organic materials.

*Contamination:* Contamination occurs under the action of the electron beam and is caused by the combustion of hydrocarbons present in the TEM column or on the surface of the sample. Hydrocarbons essentially come from oil vapors from the distribution pumps. The hydrocarbon deposits formed are composed of carbon and are more significant when working under a focused probe and an intense probe. Contamination is greatest in a STEM. It results in the formation of dark zones that cover the irradiated portion. Contamination is reduced by an anti-contamination cold trap placed near the sample, in the air gap of the objective lens. This contamination is irreversible and introduces a carbon signal into the chemical analysis. It increases when the TEM vacuum decreases.

In cryomicroscopy, the specimen is very cold and can be contaminated very quickly. In this case, the anti-contamination trap must be placed very close to the specimen.

### 3.2 Secondary Thermal Damage

*Amorphization:* Partial or total degradation of the chemical bonds of a crystalline sample, resulting in its destruction. It is caused by thermal effects during the electronic irradiation.

The main effect of irradiation is amorphization of the sample by the breaking of bonds and the loss of elements. It is greater in thin slices prepared by ion milling, which leads to the diffusion of light atoms. In the case of organic materials, electronic irradiation produces changes in the chemical composition by reticulation and the preferential degradation of particular bonds (C–H, C–C, COOH, C–NH<sub>2</sub>). The loss of mass with the formation of volatile compounds is often a consequence with polymer materials.

Another effect is annealing by the electron beam, resulting in an atomic surface rearrangement. This chemical disorder of the surface is initiated by diffusion phenomena during ion milling. It is even more significant if the material has a complex phase diagram.

*Phase transformation:* Total or partial transformation of the crystallographic and/or chemical structure of a sample, which can be caused by thermal effects during electronic irradiation.

*Loss of chemical elements:* Selective loss of a sample's chemical element, which may be caused by secondary thermal effects during electronic irradiation.

*Migration:* Collective displacement of atoms that can lead to a migration of phases or interfaces, etc.

*Fusion (or sublimation):* Change of physical state (solid–liquid) of a solid sample. It can be caused by an increase in temperature during TEM observation.

## 4 Examples of Artifacts

### 4.1 Artifacts Induced by the Tripod Polishing Technique

**Fig. 6.1** Bright-field TEM image of an MgO single crystal (ceramic material), prepared by the tripod polishing technique, showing cracks due to cleavages in the sample (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

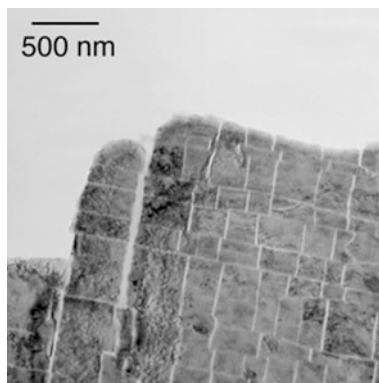


**Fig. 6.2** Bright-field TEM image of a germanium cross-section sample (semiconductor) prepared by the tripod polishing technique, showing a tearing of matter due to directional mechanical polishing (white arrows) (C. Dieker, EPFL-CIME, Lausanne)

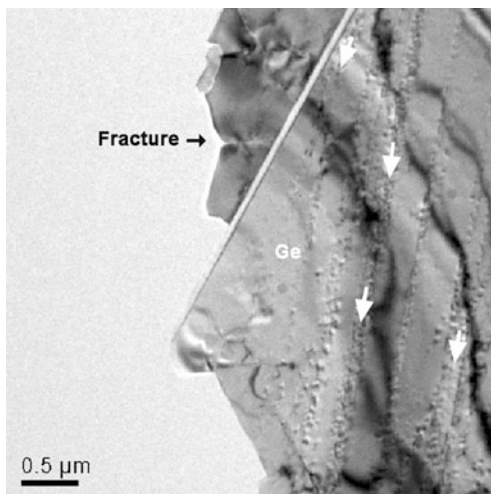


**Fig. 6.3** Bright-field TEM image of a cross-section of a SiO<sub>2</sub>/Au multilayer on a Si substrate (mixed-composite material) prepared by the tripod polishing technique, showing: (1) displacement of gold particles in the resin; (2) residues of silicon used for final polishing (S. de Chambrier, A. Schüller, EPFL-LESO, Lausanne)

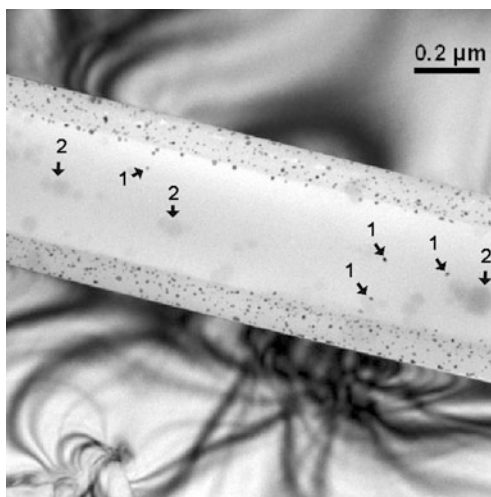


**Fig. 6.4** Same sample as in Fig. 6.3, at a higher magnification (*S. de Chambrier, A. Schüler, EPFL-LESO, Lausanne*)

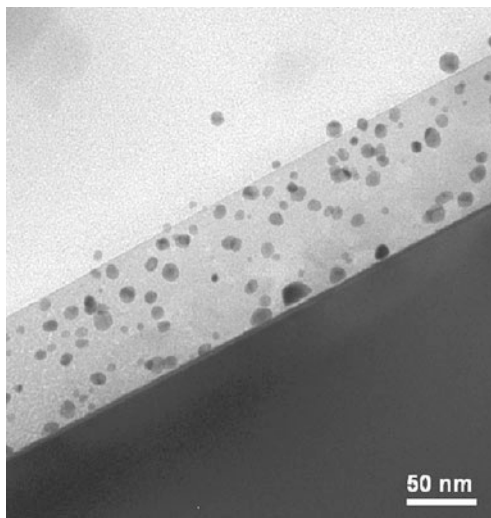


**Fig. 6.5** Dark-field TEM image of  $\text{YBa}_2\text{Cu}_3\text{O}_7/\text{SrTiO}_3$  (composite ceramic cross section) prepared by the tripod polishing technique, showing: (1) tearing of matter in the multilayer and (2) dislocations in the substrate, induced by mechanical polishing (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

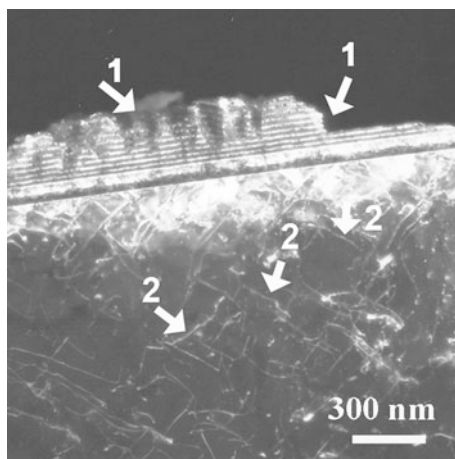
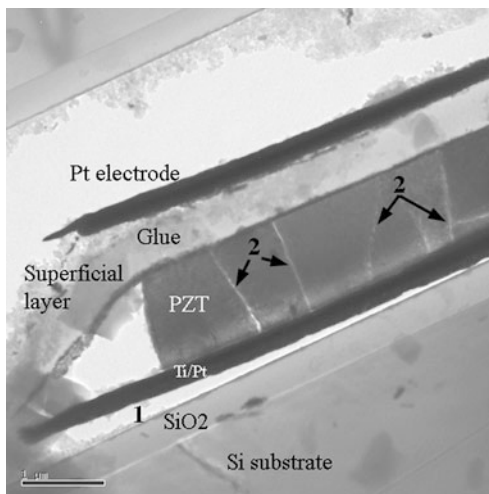


**Fig. 6.6** Bright-field TEM image of a cross-section of Si/SiO<sub>2</sub>/Ti/Pt/PZT/Pt material prepared by the tripod polishing technique, showing: (1) fracturing of the metal-ceramic SiO<sub>2</sub>/Ti/Pt interface of the multilayer material and (2) tearing of matter in the PZT layer, caused by mechanical polishing (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)



## 4.2 Artifacts Induced by the Ultramicrotomy Technique

**Fig. 6.7** Biphasic polymer prepared using ultramicrotomy, showing vibrations and morphology change perpendicular to the sectioning direction (*arrow*) due to compression (*A. Rivoire, EZUS-UCB Lyon 1*)

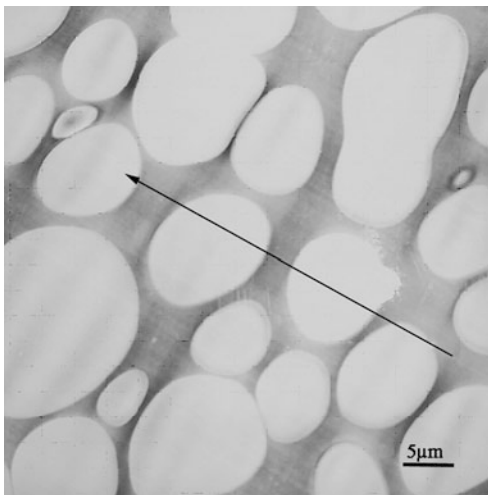


**Fig. 6.8** Bright-field TEM image of another biphasic polymer (polymer–composite) section prepared using ultramicrotomy, showing folds on a nodule in the matrix, which is of a different hardness. The folds (1) are found on the softer polymer (A. Rivoire, Ezus-UCB Lyon 1)

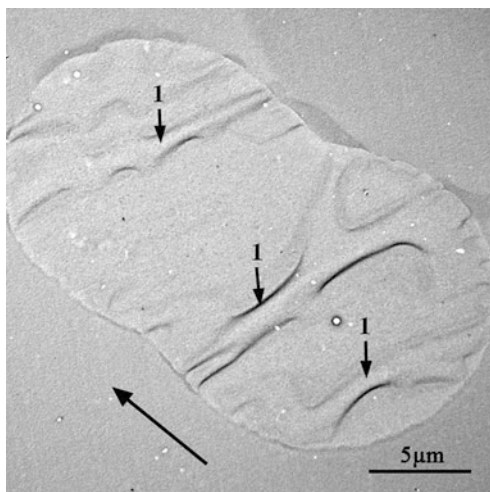


**Fig. 6.9** Bright-field TEM image of a diatom section prepared using ultramicrotomy after embedding in resin, showing (1) folds and (2) tears of the embedding resin. This is an alga in which only the mineralized shell has been preserved. This shell is ornamented with fine ribs presented in the form of regularly spaced thicknesses of  $\text{SiO}_2$  (small black masses) separated by pores (D. Badaut, EOST Strasbourg)

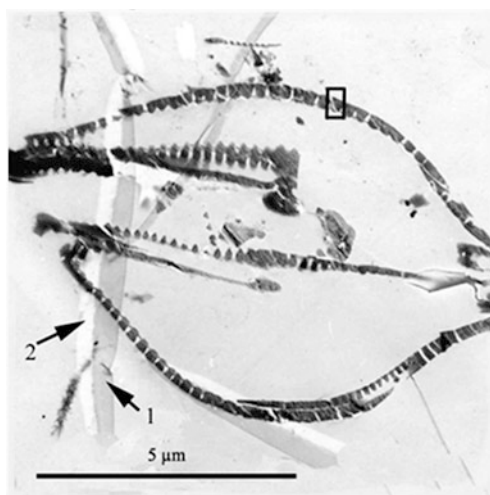


**Fig. 6.10** Higher magnification of one area of the previous image, showing fractures and detachments in a side of the siliceous wall of the diatom. Silica is a hard and brittle material that is difficult to section (*D. Badaut, EOST Strasbourg*)

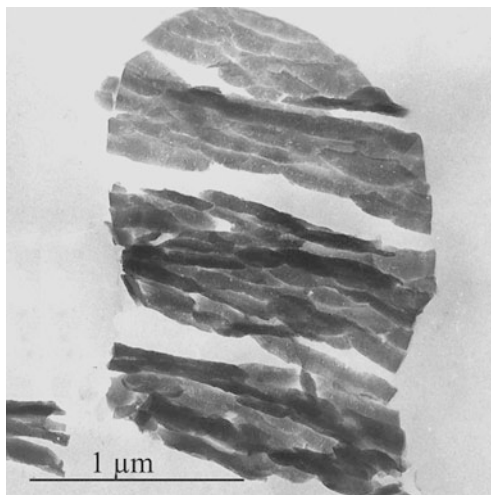


**Fig. 6.11** Bright-field TEM image of a cross-section of SiO<sub>2</sub> fibers (ceramic material) embedded in a resin prepared using ultramicrotomy, showing: tearing of two fibers that were partially ejected by the diamond blade in cutting because of their difference in hardness with the embedding resin (*D. Laub, EPFL-CIME, Lausanne*)

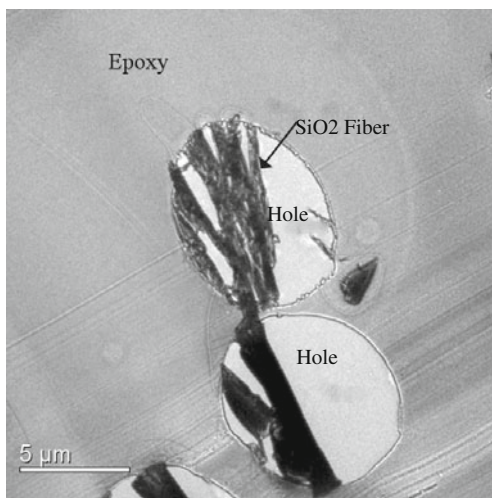


**Fig. 6.12** Bright-field TEM image of isolated carbon particles (semi-crystalline) embedded in a resin prepared using ultramicrotomy, showing: (1) tearing due to the difference in hardness of the spheres with the embedding resin, (2) microfissures in the spheres, caused by the compression due to the blade followed by a rupture of the material, (3) a large knife ridge perpendicular to the cracks (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

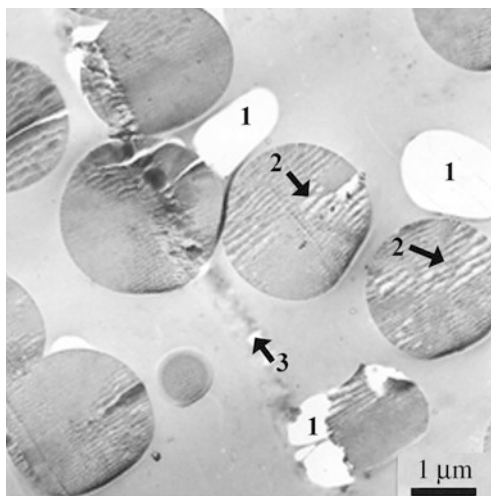


**Fig. 6.13** Bright-field TEM image of a tin (*metal*) particle, embedded and prepared by ultramicrotomy, showing: (1) parallel knife ridges in the cutting direction (*arrow*), (2) compression and strain hardening of the material highlighted by the micro-undulations perpendicular to the sectioning direction (*arrow*) (*D. Laub, EPFL – CIME Lausanne*)

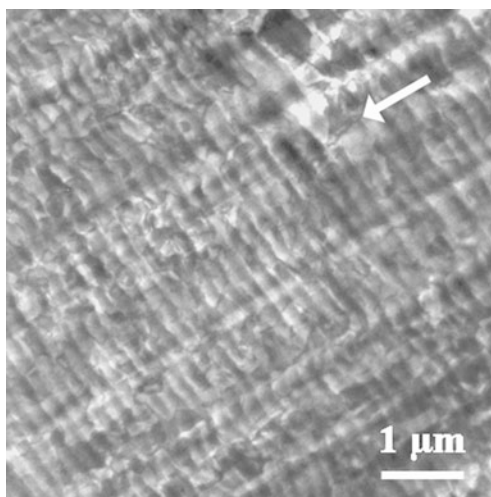


**Fig. 6.14** Bright-field TEM image of a Si-Fe/SiO<sub>2</sub> (mixed-composite) particle sample prepared using ultramicrotomy, showing fractures in the SiO<sub>2</sub> perpendicular to the cutting direction. However, observation of the Fe-Si and the Fe-Si/SiO<sub>2</sub> interface is possible (*D. Laub, EPFL – CIME Lausanne*)

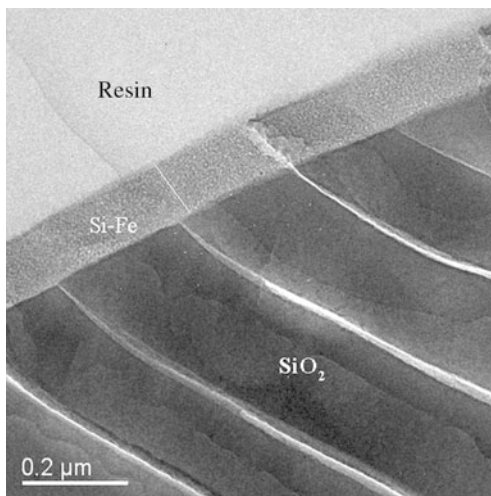


**Fig. 6.15** Bright-field TEM image of an aluminum-based alloy (metallic material) prepared without embedding on the bulk material using ultramicrotomy, showing tearing of matter due to the heterogeneity in hardness of phases (2) and (3) with the strain-hardened matrix (1). More significant strain hardening can be seen in phase (3) (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

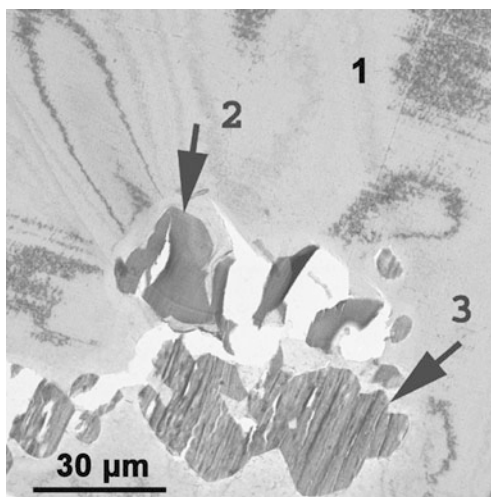


**Fig. 6.16** Bright-field TEM image of a cross-section of a multilayer DLC/Ti/Si<sub>3</sub>N<sub>4</sub>/Si (mixed-composite) material embedded in an epoxy resin and prepared by ultramicrotomy, principally showing fractures in the Si<sub>3</sub>N<sub>4</sub> layer (3) and Si (4) perpendicular to the cutting direction (*arrow*) and undulations of compression visible in the DLC (1) and Ti (2) (*H. Gnaegi, Diatome, Bienne*)

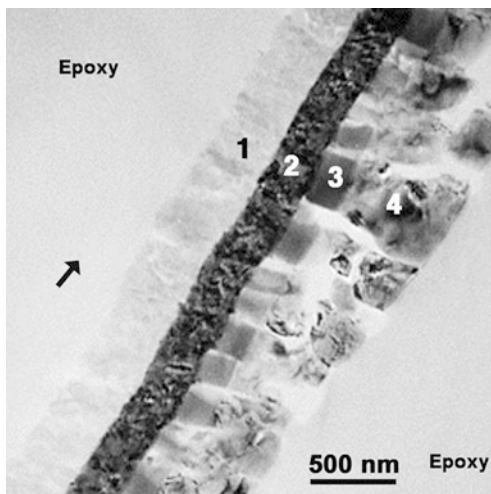


**Fig. 6.17** Bright-field TEM image of a cell prepared using chemical fixation, embedding in epoxy resin, and ultramicrotomy, showing cutting residues (*large arrow*) which are superimposed over the structures to be observed. Saturated lipids form white spheres (*small arrows*) (*J. Boumendil CMEABG, UCB Lyon I*)

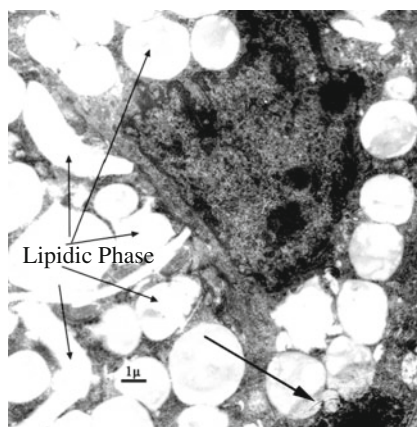


**Fig. 6.18** Bright-field TEM image of a liver cell prepared using chemical fixation, embedding in epoxy resin, and ultramicrotomy, showing a triglyceride phase (*arrow*) that has undergone compression phenomena: ridges and matter displacement, resulting in holes followed by folds in the sectioning direction (indicated by the *arrow direction*). From this we conclude that the softer phase is poorly embedded (*J. Boumendil, CMEABG UCB Lyon 1*)

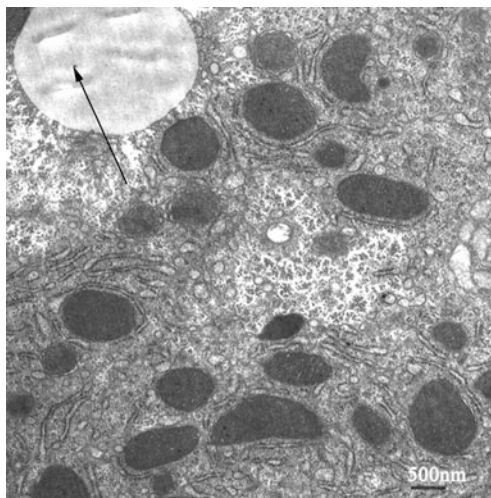


**Fig. 6.19** Bright-field TEM image of a slice of a protozoan embedded in epoxy resin and prepared by ultramicrotomy showing vibrations (*arrows*) and folds (*arrow heads*) (*E. Delain, CNRS-UMR8126-IGR, Villejuif*)

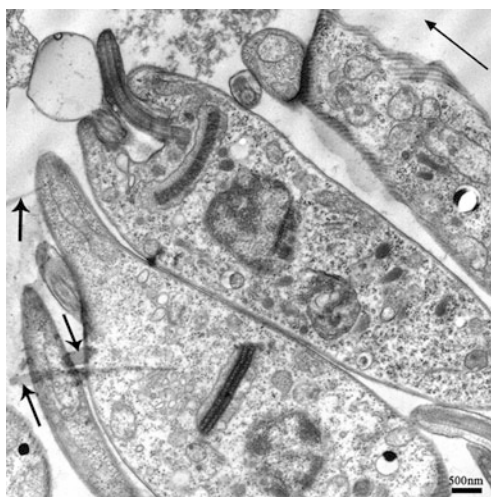
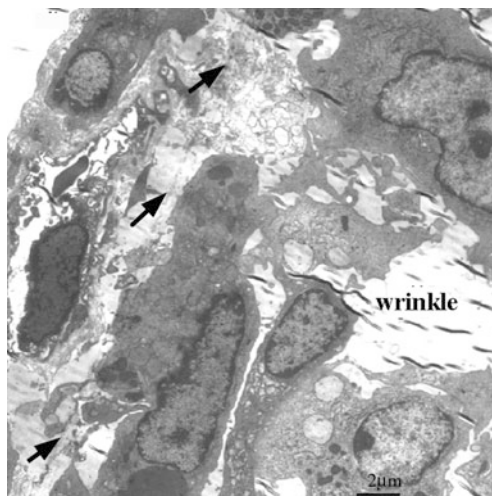


**Fig. 6.20** Bright-field TEM image of a slice of placenta prepared by ultramicrotomy showing folds perpendicular to the cut and a long ridge (marked by *arrows*) in the cutting direction  
(*J. Boumendil, CMEABG UCB Lyon 1*)



### 4.3 Artifacts Induced by the Freeze-Fracture Technique

**Fig. 6.21** Bright-field TEM image of a liver cell replica, prepared using the freeze-fracture technique, showing a rupture of the replica produced when the replica was washed and the presence of carbon residues (white arrow heads)  
(*A. Rivoire, EZUS-UCB Lyon 1*)

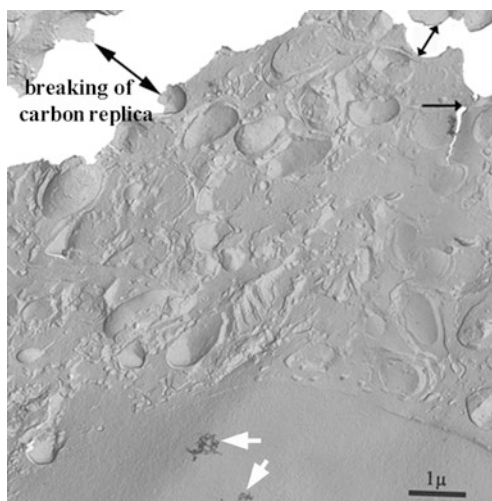
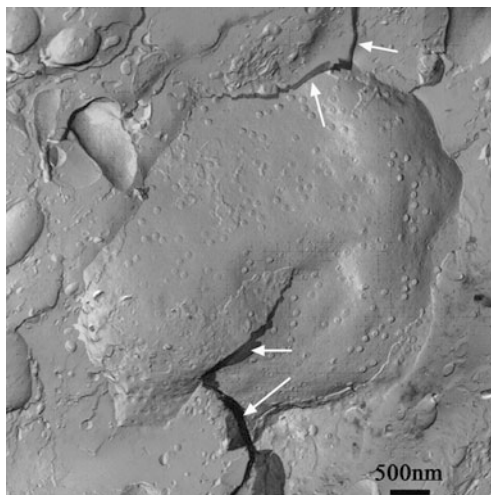


**Fig. 6.22** Bright-field TEM image of a replica of a cell showing the nucleus prepared using the freeze-fracture technique. Folds of the replica (white arrows) are evident in the areas of greatest relief of the sample (A. Rivoire, EZUS-UCB Lyon 1)



#### 4.4 Artifacts Induced by Ion Milling or FIB

**Fig. 6.23** Bright-field TEM image of a polycrystalline ceramic,  $\text{YBa}_2\text{Cu}_3\text{O}_7$ , prepared using the ion milling technique, showing an intergranular crack (arrow) (J. Ayache, CNRS-UMR8126-IGR, Villejuif)

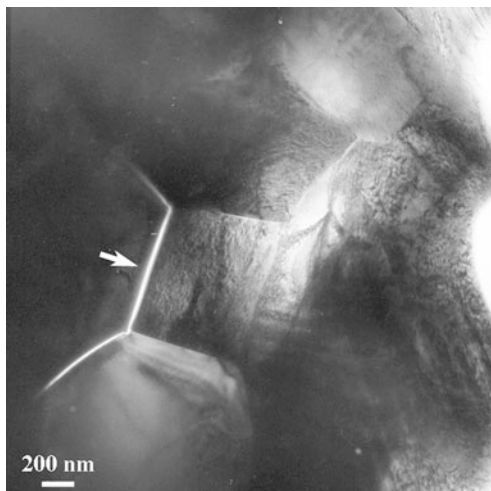


**Fig. 6.24** Bright-field TEM image of an MgO bicrystal grain boundary (ceramic), prepared by the ion milling technique, showing a preferential thinning at the grain boundary (*arrows*) (*J. Ayache, CNRS-UMR8126-IGR Villejuif*)

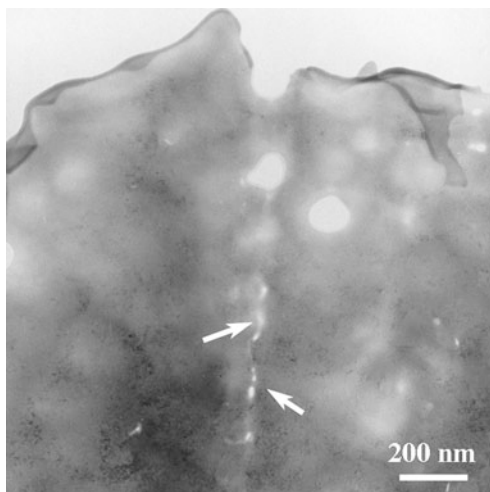


**Fig. 6.25** Bright-field TEM image of a polycrystalline ceramic,  $\text{YBa}_2\text{Cu}_3\text{O}_7$ , prepared using ion milling, showing differential ionic abrasion of the grains due to differences in crystallographic orientation (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

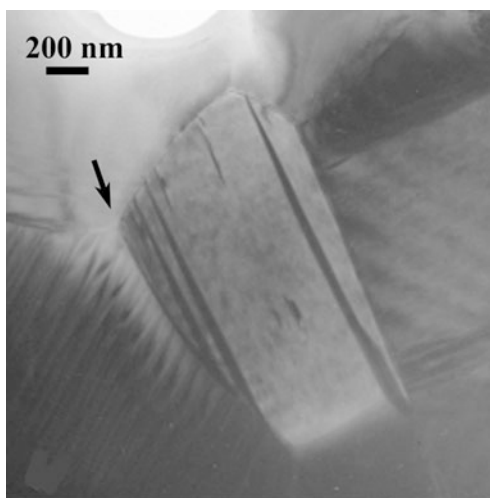


**Fig. 6.26** Triple-grain boundary from Fig. 6.25 (arrow) at a higher magnification. The difference in thickness between grains does not enable high-resolution observation and quantitative chemical analysis of the interfaces (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

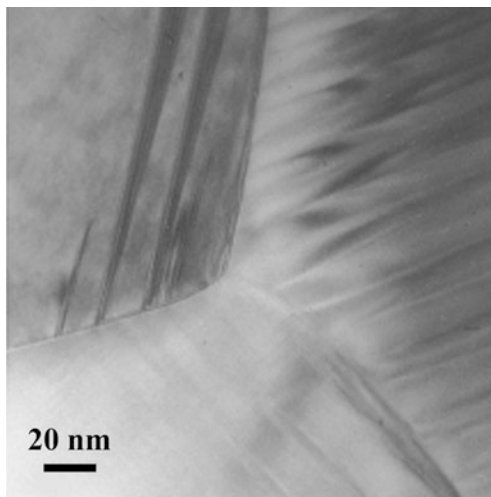


**Fig. 6.27** HRTEM image in cross-section of a multilayer Ru/Zr (2) on a  $\text{SrTiO}_3$  substrate, prepared using the tripod polishing method followed by final ion milling. The external Ru/Zr layers (1) were destroyed by ion milling (*D. Laub, EPFL-CIME, Lausanne*)

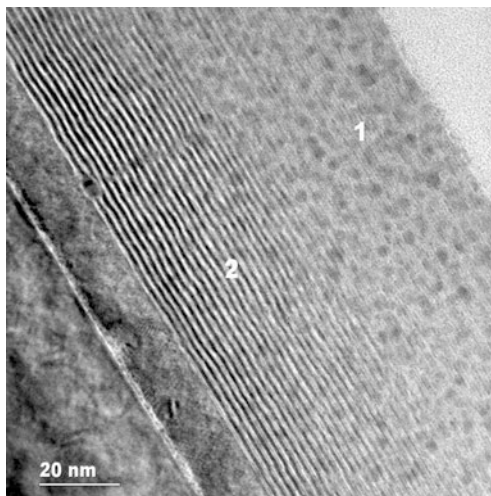


**Fig. 6.28** Bright-field TEM image. This is the same sample as shown in Fig. 6.27, but instead focuses on another area where all of the layers were damaged and covered with redeposited particles. Dark particles on the layers have been abraded and redeposited on the material during ion milling. They make observation difficult (*D. Laub, EPFL-CIME, Lausanne*)

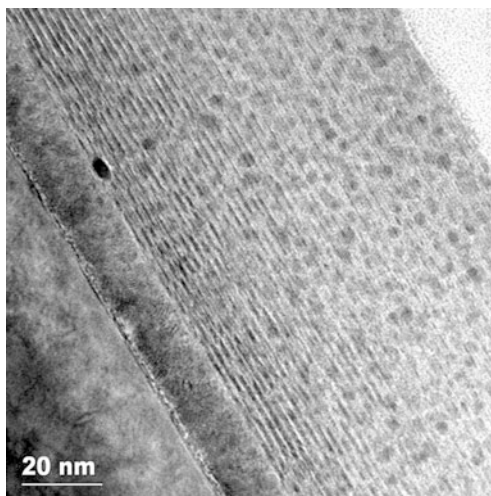


**Fig. 6.29** Bright-field TEM image of a  $\text{YBa}_2\text{Cu}_3\text{O}_7$  ceramic, prepared using the ion milling technique, showing: (1) amorphization of the material at the edges of the twin walls in the very thin zones, (2) precipitates (arrows) due to the demixing of  $\text{YBa}_2\text{Cu}_3\text{O}_7$ , first under the action of the ion beam and then the electron beam (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

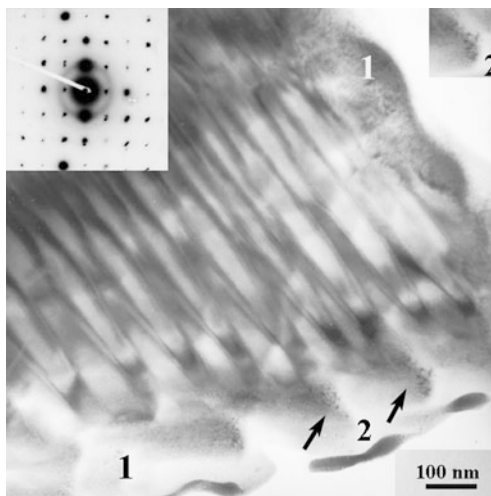


**Fig. 6.30** Bright-field TEM image of a multifilament  $\text{Nb}_3\text{Sn}$  sample in a bronze matrix prepared using the FIB method, showing a hole in the slice due to differential thickness (“curtain effect”) (1) and fractures in the filaments due to stresses to the material during preparation (*arrows*) (*M. Cantoni, EPFL-CIME, Lausanne*)

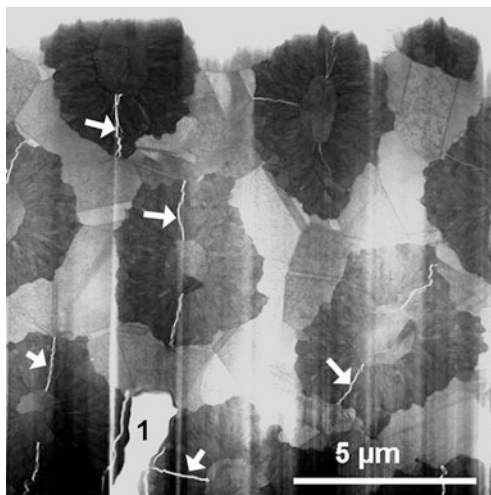


**Fig. 6.31** HRTEM image of  $\text{YBa}_2\text{Cu}_3\text{O}_7$  ceramic prepared using the ion milling technique, showing amorphization of the material (1) in the very thin areas, and irradiation damage (*arrows*) due to the ion beam and electron beam (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

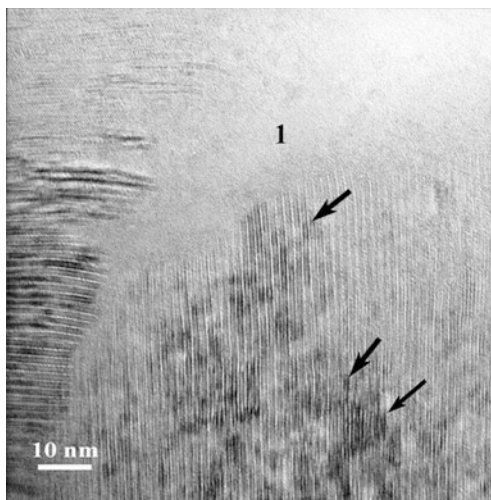


**Fig. 6.32** HRTEM image of the polycrystalline  $\text{YBa}_2\text{Cu}_3\text{O}_7$  prepared using the ion milling technique, showing (1) a selective thinning at a grain boundary, where the grains present a strong disorientation and contain an intergranular phase, and (2) damage from irradiation under the electron beam (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

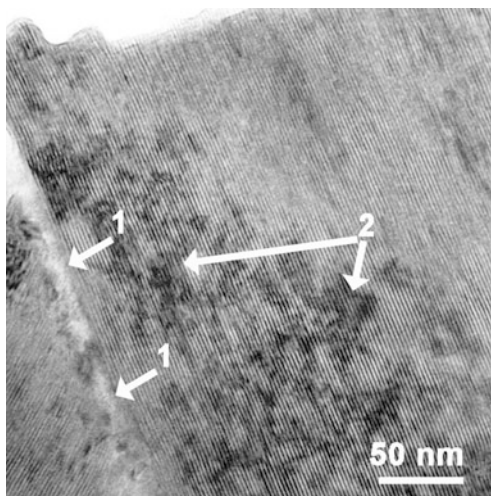
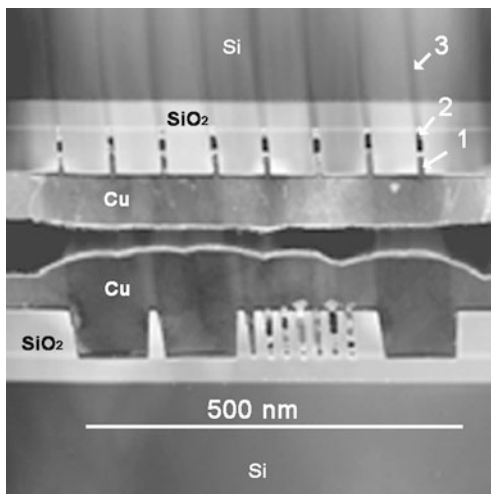
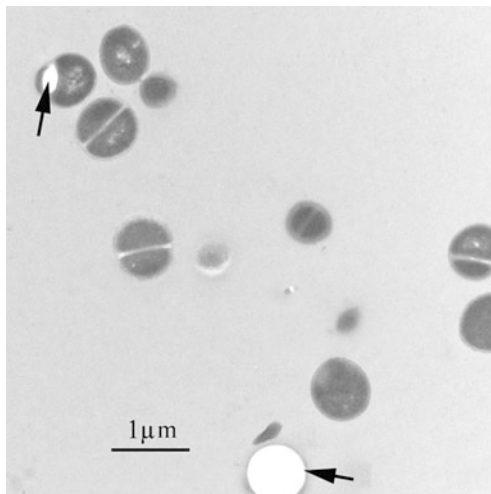


**Fig. 6.33** Bright-field TEM image of a  $\text{Cu}/\text{SiO}_2/\text{Si}$  sample prepared cross-sectionally using the tripod polishing technique, followed by ion bombardment in single sectorial rotation. Copper (1) is not found in the bottom of the gaps (2). The dark lines behind the gaps (3) are thicker regions, resulting from the abrasion redeposition of Cu (*D. Laub, F. Cosandey, Rutgers University, Piscataway, NJ, USA*)



### 4.5 Artifacts Induced by the Substitution–Infiltration–Embedding Technique

**Fig. 6.34** Bright-field TEM image of a slice of *Staphylococcus aureus* embedded first in agar and then in epoxy resin. This image shows holes in the resin due to incomplete dehydration and embedding (arrows) (J. Boumendil, CMEABG UCB Lyon 1)



### 4.6 Artifacts Induced by Chemical Fixation

**Fig. 6.35** Bright-field TEM image of an adipocyte cell, showing a loss of lipids (white holes with asterisks) located in the cytoplasm. The lipids disappeared during chemical fixation. As sectioning was possible, the resin has filled the residual holes, but does not show any contrast (J. Boumendil, CMEABG UCB Lyon 1)

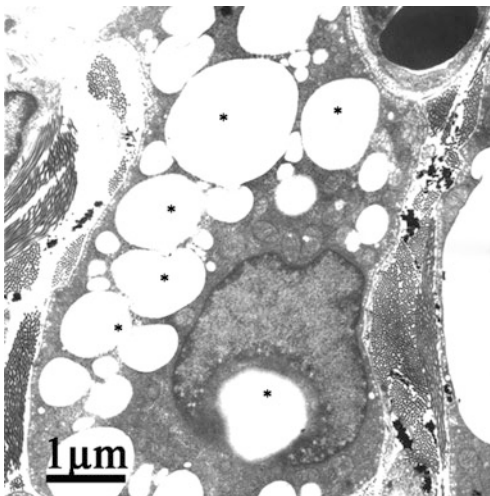
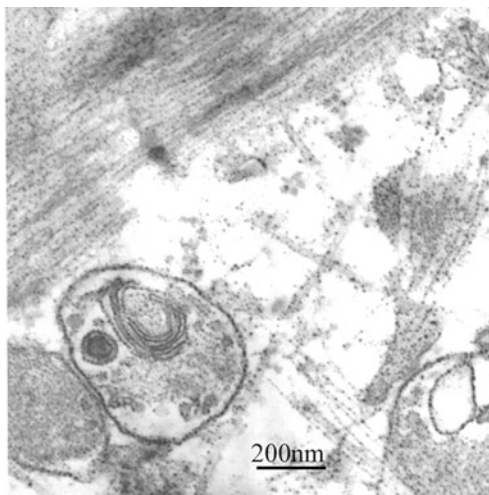
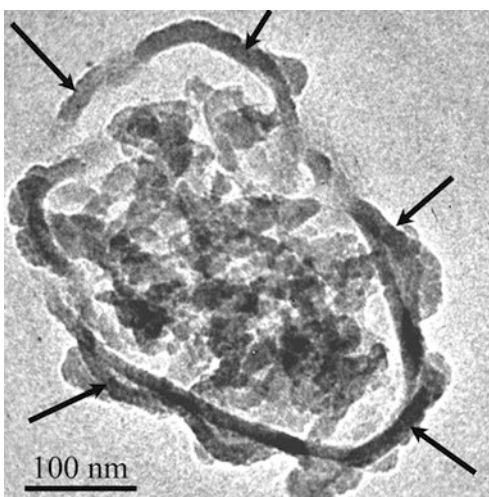


**Fig. 6.36** Bright-field TEM image showing a portion of a cell that has undergone a double chemical fixation, embedding in epoxy resin and positive staining. The small dark grains visible everywhere are precipitates of metallic osmium, which are not bound to the structures and constitute an artifact (*J. Boumendil, CMEABG, UCB Lyon 1*)



#### 4.7 Artifacts Induced by the Extractive-Replica Technique

**Fig. 6.37** Bright-field TEM image of a catalyst containing 0.2% particles of platinum on an alumina substrate ( $\text{Pt}/\text{Al}_2\text{O}_3$ ), prepared using the extractive-replica technique. The final chemical attack with fluorhydric acid was not sufficient and, consequently, the interior of the alumina substrate was not dissolved. The outside edges of the alumina substrate are indicated by the arrows (*G. Ehret, IPCMS, Strasbourg*)



### 4.8 Artifacts Induced by the Shadowing Technique

**Fig. 6.38** Bright-field TEM image of DNA molecules prepared by the shadowing technique, showing defects in the homogeneity of the support film (*E. Delain, CNRS-UMR8126-IGR, Villejuif*)

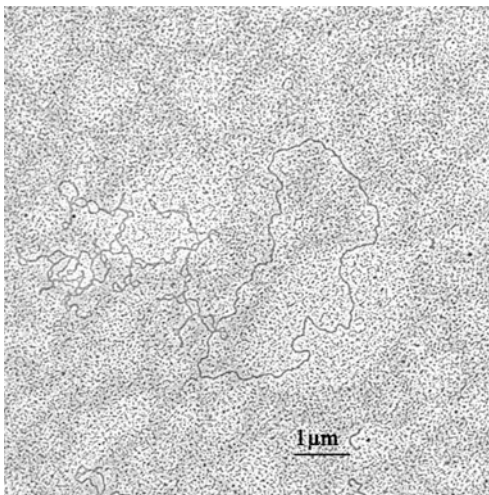
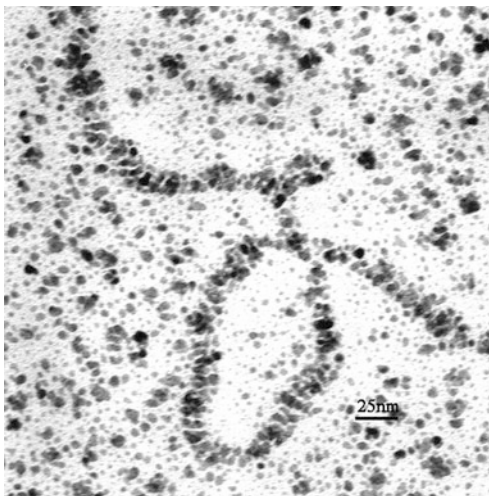


**Fig. 6.39** Bright-field TEM image of a fragment of DNA molecules prepared by the shadowing technique, showing an aggregation of the platinum shadowing particles (*E. Delain, CNRS-UMR8126-IGR, Villejuif*)



### 4.9 Artifacts Induced by the “Positive-Staining” Contrast Technique

**Fig. 6.40** Bright-field TEM image of a slice of embryonic cells from a seedling showing needle-shaped uranyl precipitates (*J. Boumendil CMEABG, UCB Lyon 1*)

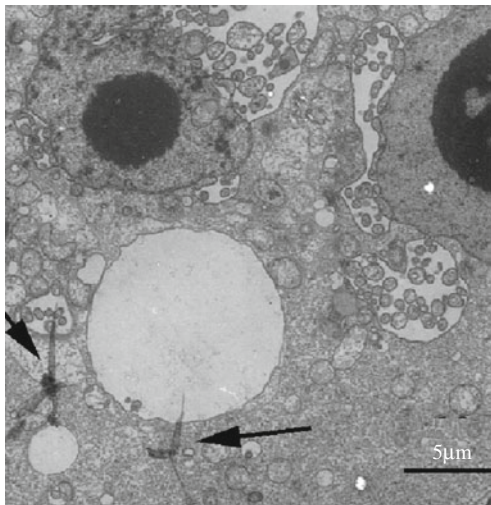
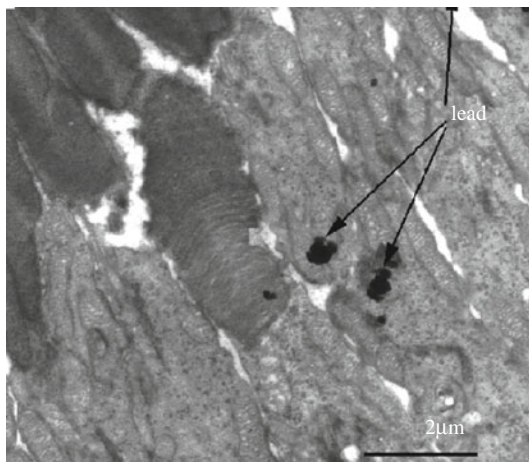


**Fig. 6.41** Bright-field TEM image of a slice of a cell showing spherical lead precipitates (*J. Boumendil CMEABG, UCB Lyon 1*)



### 4.10 Artifacts Induced by the Cryofixation Technique

**Fig. 6.42** Bright-field TEM image of a rat liver cell slice cryofixed using a projection on cooled copper at 20  $\mu\text{m}$  around the freezing front, then freeze substitution in the presence of osmium and embedding at low temperatures in resin. It shows microstructural changes due to the presence of ice crystals throughout, which disturb the interpretation of the image. The freezing was too slow (*J. Boumendil, CMEABG – UCB Lyon 1*)

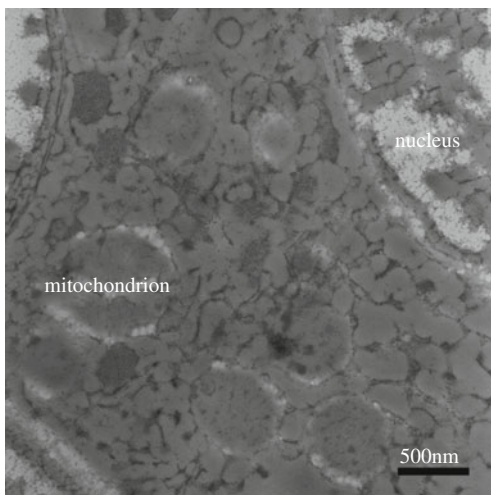


**Fig. 6.43** Bright-field TEM image of a rat liver cell slice cryofixed using a projection on cooled copper and at about 10  $\mu\text{m}$  around the freezing front, then freeze substitution in the presence of osmium and embedding at low temperatures in Lowicryl resin. Note the presence of very small crystals of cubic ice (*black arrows*) in the nucleus. The microstructure has been modified; however, the image can still be interpreted (*J. Boumendil, CMEABG – UCB Lyon 1*)

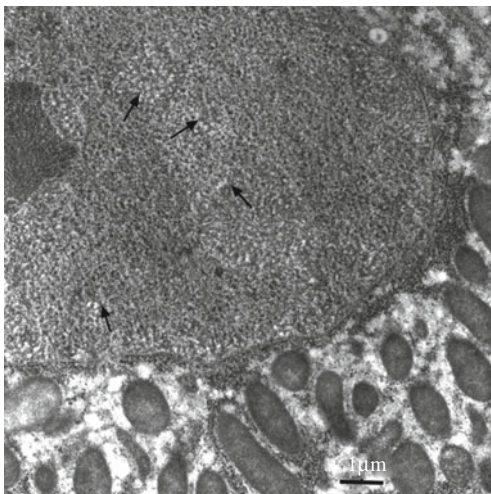
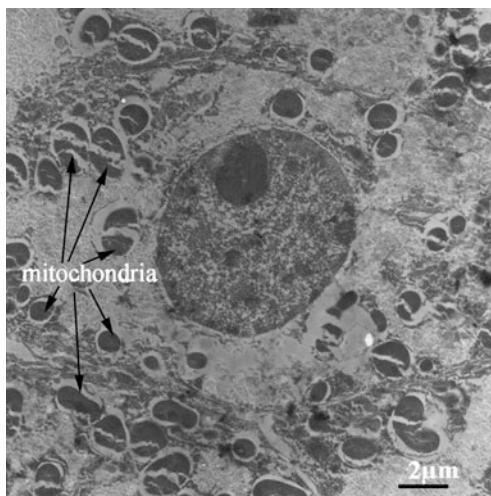


**Fig. 6.44** Bright-field TEM image of a slice of liver frozen under high pressure, cryo-dehydrated without  $\text{OsO}_4$ , cryo-embedded and contrasted using uranyl acetate/lead. This image shows fractured mitochondria, either from the high pressure or due to mechanical tearing when recovering the sample after freezing (*J. Boumendil, CMEABG – UCB Lyon 1*)



#### ***4.11 Artifacts Induced by the Fine Particle Dispersion Technique***

**Fig. 6.45** Bright-field TEM image of Pt/Ru/Sn (metal) powder dispersed on a carbon film grid. The particles were dissolved in ethanol, then agitated under ultrasound waves before a drop of supernatant was deposited on the support grid. The particles remain agglomerated (*D. Laub, EPFL-CIME, Lausanne*)

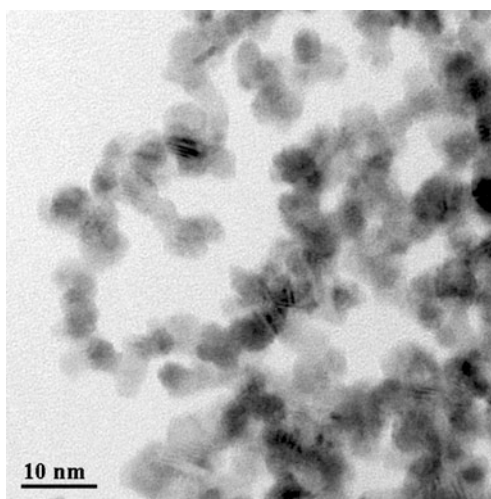


**Fig. 6.46** Enlargement of the TEM image from Fig. 6.45. The agglomeration of the particles makes it impossible to precisely measure particle size (*D. Laub, EPFL-CIME, Lausanne*)

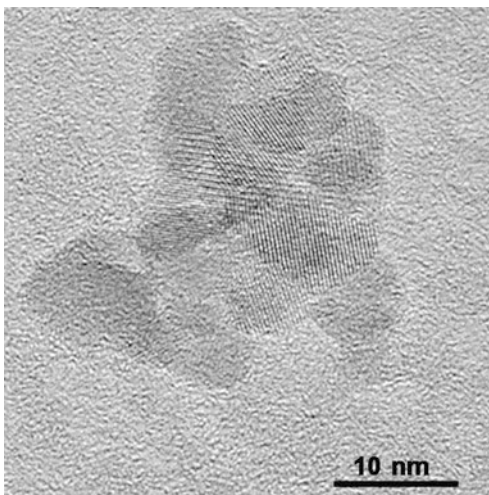


**Fig. 6.47** Bright-field TEM image of a dispersion of carbon nanotubes (polymer) obtained by the variant of the crushing technique (i.e., dry crushing between two glass slides). The nanotubes are then transferred to a holey-carbon grid. The tangling of the nanotubes, because of their flexibility, makes their observation difficult at low magnifications (*C.S. Cojocaru, IPCMS, Strasbourg*)

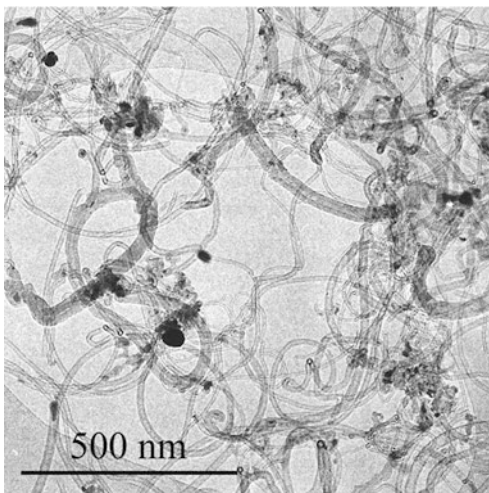
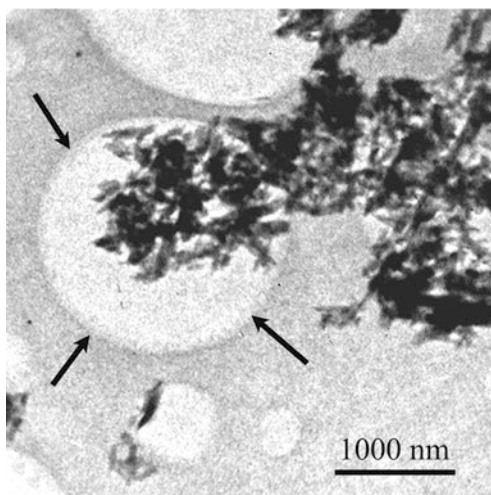


**Fig. 6.48** Bright-field TEM image of ZnO (ceramic) particles obtained by dispersion in water and deposited on a holey-carbon grid; the particles are agglomerated in a hole, indicated by the *arrows*, making observation difficult (*K. Lattaud, IPCMS, Strasbourg*)



#### ***4.12 Artifacts Induced by the Frozen-Hydrated-Film Technique***

**Fig. 6.49** Bright-field TEM image of a frozen hydrated film showing crystals of cubic and hexagonal ice on the carbon holes without a frozen hydrated film (*G. Pehau-Arnaudet, Institut Pasteur-CNRS, Paris*)

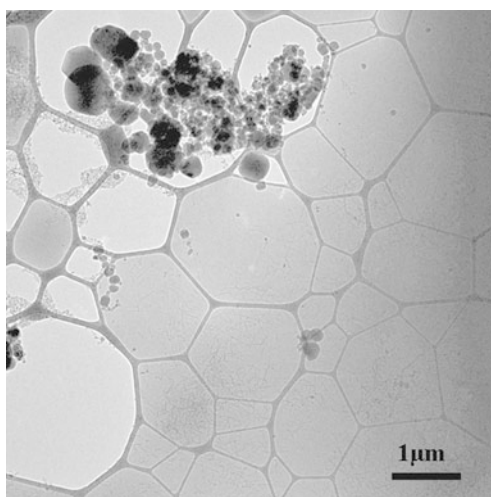


**Fig. 6.50** Bright-field TEM image of a frozen hydrated film showing clusters of agglomerated crystals on the edges of a hole on a holey carbon film support. This is due to poor freezing  
(*S. Baconnais, CNRS-UMR8126-IGR, Villejuif*)

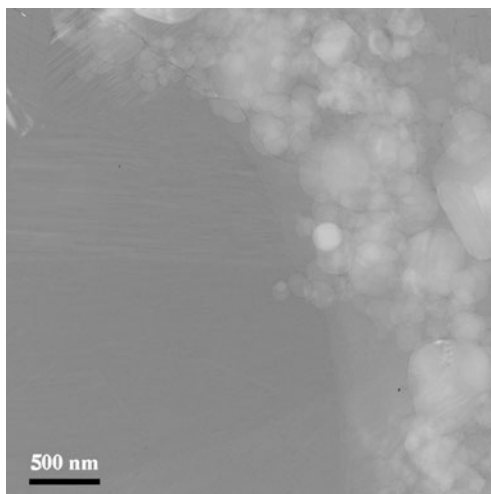


**Fig. 6.51** Bright-field TEM image of a continuous frozen hydrated film presenting local small crystals of hexagonal ice. Higher magnification of an ice crystal is shown in the upper right-hand corner  
(*S. Baconnais, CNRS-UMR8126-IGR, Villejuif*)

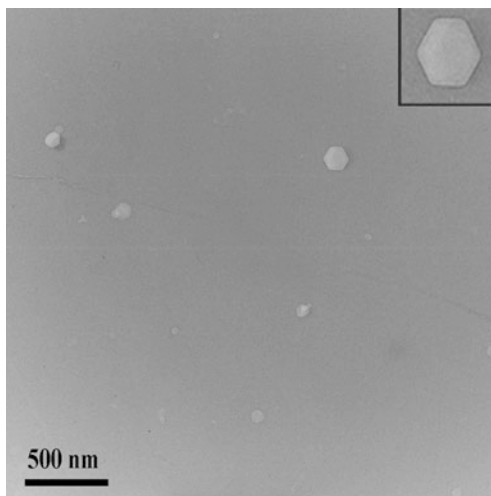
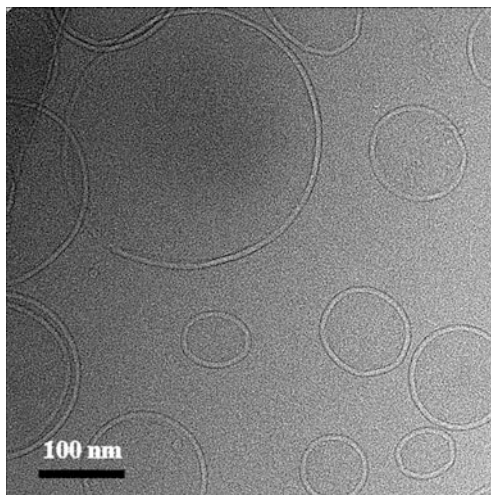


**Fig. 6.52** Bright-field TEM image of vitrified liposomes in a frozen hydrated film, showing a phase-contrast inversion between the ice and the liposome which is due to the absence of osmium or heavy metal (Ur or Pb) commonly used for biological preparation  
(G. Pehau-Arnaudet, Institut Pasteur-CNRS, Paris)



#### 4.13 Artifacts Induced by the “Negative-Staining” Contrast Technique

**Fig. 6.53** Bright-field TEM image of a liposome suspension under negative staining using uranyl acetate. This image shows residues of the dispersion agent (*white spots*), which can be observed around the liposome and are sometimes superimposed over the liposome structure. They come from a component of the suspension liquid that was not eliminated (J. Boumendil, CMEABG – UCB Lyon 1)

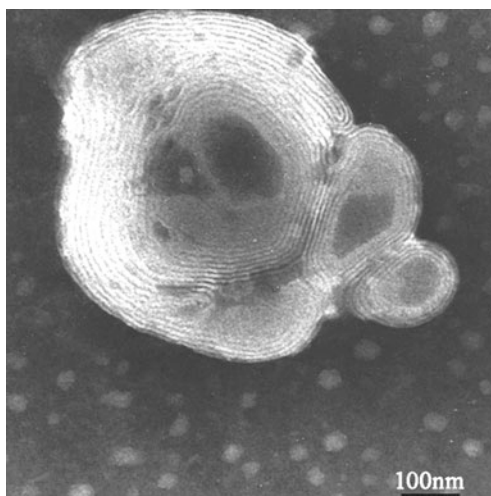
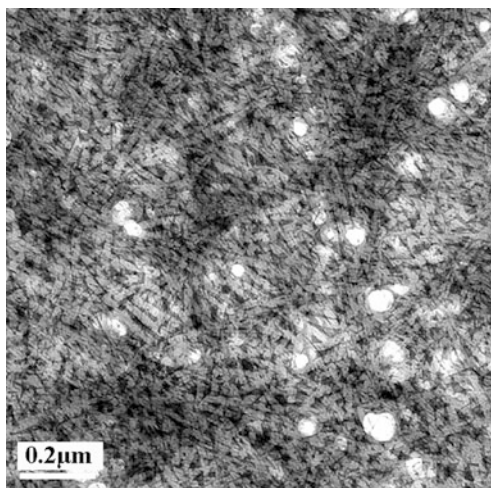


**Fig. 6.54** Bright-field TEM image of a suspension of the tobacco mosaic virus (TMV) in 5% uranyl acetate, showing a strong concentration of TMV, which prevents the observation of isolated particles (*P.Y. Sizaret, Université Tours*)



#### ***4.14 Artifacts Induced by the Electron Beam***

**Fig. 6.55** Bright-field TEM image of negative staining of viral pseudo-particles using uranyl acetate, showing the presence of irradiation damage formed under the electron beam, resulting in sublimation of the staining agent that becomes small white dots (*P.Y. Sizaret, Université Tours*)

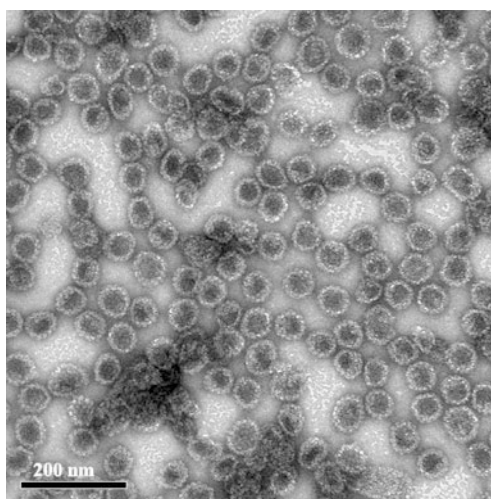


**Fig. 6.56** Bright-field TEM image of a superconducting ceramic,  $\text{YBa}_2\text{Cu}_3\text{O}_7$ , after EDS chemical analysis on the walls of the twins. We can see the traces of carbon contamination (*arrows*) due to the electron beam (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

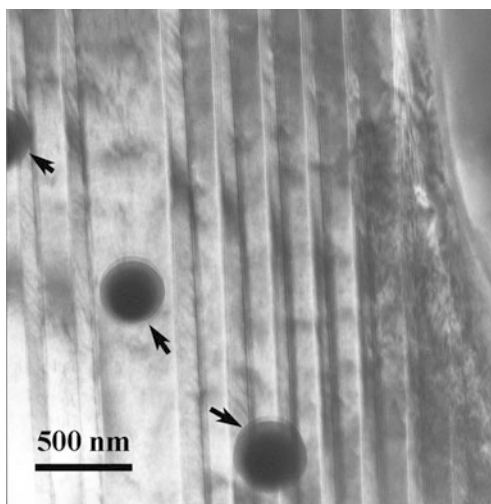


**Fig. 6.57** Bright-field TEM image of a superconducting ceramic,  $\text{YBa}_2\text{Cu}_3\text{O}_7$ , showing irradiation damage under the electron beam. They appear in the form of pores (*arrows*). This damage is encouraged by the strong tendency toward decomposition, due to the non-stoichiometry of these perovskite oxides (*J. Ayache, CNRS-UMR8126-IGR, Villejuif*)

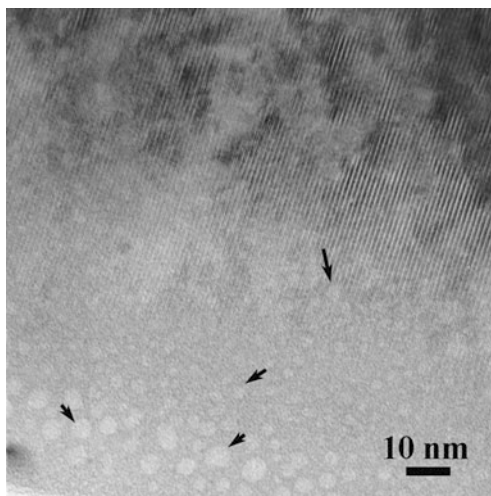


**Fig. 6.58** Bright-field TEM image of an interface of Si-Fe/SiO<sub>2</sub> prepared using ultramicrotomy, showing the structure of the SiO<sub>2</sub> after a short exposure to the electron beam (*D. Laub, EPFL-CIME, Lausanne*)

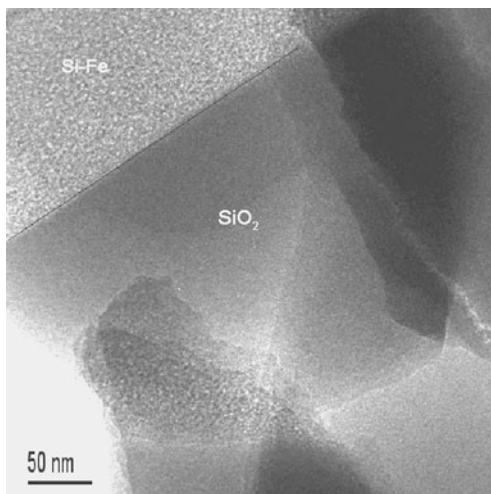


**Fig. 6.59** Same region as shown in Fig. 6.58. This image shows the structure of SiO<sub>2</sub> after an approximately 1-min exposure under the electron beam. A porous structure has formed (*D. Laub, EPFL-CIME, Lausanne*)

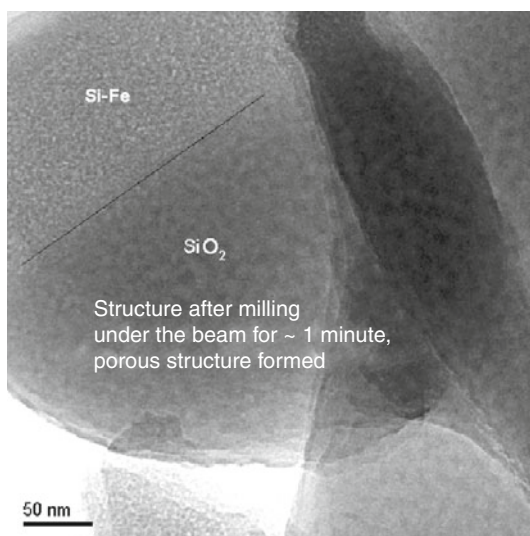


**Fig. 6.60** Bright-field TEM image of a frozen hydrated film, showing bubbles from ice melting under the electron beam (*S. Baconnais, CNRS-UMR8126-IGR, Villejuif*)

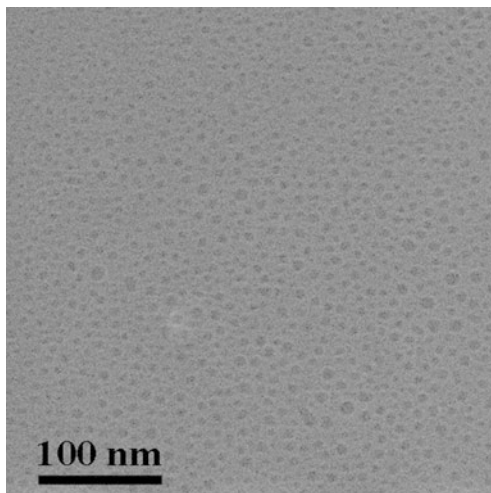
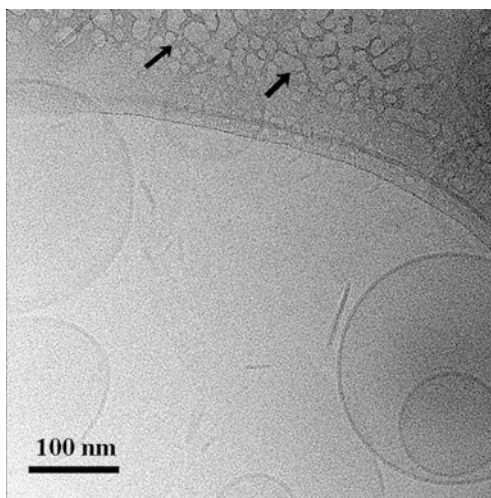


**Fig. 6.61** Bright-field TEM image of vitrified liposomes in a frozen hydrated film, showing the effects of irradiation on the ice film (arrows) (*G. Pehau-Arnaudet, Institut Pasteur, Paris*)



5 Summary Tables

Two summary tables are presented below (Tables 6.2 and 6.3). These tables list the artifacts caused by preliminary preparation techniques, chemical- and ionic-thinning techniques, mechanical-preparation techniques, replica techniques, techniques specific to divided materials, contrast enhancement techniques, and immunolabeling techniques.

**Table 6.2** Table summarizing the various artifacts formed by the preliminary preparation techniques

|                                                         | Artifacts induced by preparation |       |          |          | Thermal damage due to preparation |
|---------------------------------------------------------|----------------------------------|-------|----------|----------|-----------------------------------|
| Technique                                               | Mechanical                       | Ionic | Chemical | Physical |                                   |
| <i>Preliminary preparation techniques</i>               |                                  |       |          |          |                                   |
| Sawing                                                  | +                                |       |          |          |                                   |
| Ultrasonic cutting                                      |                                  |       |          |          |                                   |
| Mechanical polishing                                    | +                                |       |          |          | +                                 |
| Dimpling                                                | +                                |       |          |          |                                   |
| Electropolishing                                        |                                  |       | +        |          |                                   |
| Chemical polishing                                      |                                  |       | +        |          |                                   |
| Sandwich technique                                      |                                  |       |          |          |                                   |
| Embedding                                               |                                  |       | +        |          | +                                 |
| Substitution–infiltration–embedding at room temperature |                                  |       | +        |          | +                                 |
| Substitution–infiltration–embedding in cryogenic mode   |                                  |       | +        | +        |                                   |
| Chemical fixation                                       |                                  |       | +        |          |                                   |
| Physical fixation: Cryo-fixation                        |                                  |       |          | +        |                                   |
| Continuous support films                                |                                  |       | +        | +        |                                   |
| Holey support films                                     |                                  |       | +        | +        |                                   |

**Table 6.3** Table summarizing the various artifacts formed by the techniques

| Preparation Techniques                              |                  |       |          |          |                                   |
|-----------------------------------------------------|------------------|-------|----------|----------|-----------------------------------|
| Technique                                           | Type of Artifact |       |          |          | Thermal Damage due to Preparation |
|                                                     | Mechanical       | Ionic | Chemical | Physical |                                   |
| <i>Chemical- and ionic-thinning techniques</i>      |                  |       |          |          |                                   |
| Twin-jet electrolytic thinning                      |                  |       | +        |          | +                                 |
| Full-bath electrolytic thinning                     |                  |       | +        |          | +                                 |
| Twin-jet chemical thinning                          |                  |       | +        |          | +                                 |
| Full-bath chemical thinning                         |                  |       | +        |          | +                                 |
| Ion milling                                         |                  | +     |          |          | +                                 |
| Focused ion beam (FIB)                              |                  | +     |          |          | +                                 |
| <i>Mechanical-preparation techniques</i>            |                  |       |          |          |                                   |
| Grinding                                            | +                |       |          |          |                                   |
| Wedge cleavage                                      | +                |       |          |          |                                   |
| Tripod polishing                                    | +                |       |          |          | +                                 |
| Ultramicrotomy                                      | +                |       |          |          |                                   |
| Cryo-ultramicrotomy                                 | +                |       |          | +        | +                                 |
| <i>Replica techniques</i>                           |                  |       |          |          |                                   |
| Direct replica                                      |                  |       |          | +        |                                   |
| Indirect replica                                    |                  |       |          | +        |                                   |
| Extractive replica                                  |                  |       | +        |          |                                   |
| Freeze fracture                                     | +                |       |          | +        |                                   |
| <i>Techniques specific to divided materials</i>     |                  |       |          |          |                                   |
| Fine particle dispersion                            |                  |       |          | +        |                                   |
| Frozen hydrated film                                |                  |       |          | +        |                                   |
| <i>Contrast-enhancement and labeling techniques</i> |                  |       |          |          |                                   |
| Decoration shadowing                                |                  |       |          | +        | +                                 |
| “Negative-staining” contrast                        |                  |       |          | +        |                                   |
| “Positive-staining” contrast                        |                  |       | +        |          |                                   |
| Immunolabeling                                      |                  |       | +        |          |                                   |

## Bibliography

- Ayache, J. and Albarede, P.H. (1994). *New Application of Klepeis Ion Mill Free Sample Preparation to YBCO System Thin Films and Bulk Ceramics*, P. H. ICEM 13, 1. Les Editions de Physique, Paris, 1023–1024.
- Ayache, J. and Albarede, P.H. (1995). TEM cross section preparation by ionless tripod polisher method applied to YBCO superconducting multilayer thin films and bulk ceramics. *Ultramicroscopy*, **60**, 195–206.
- Ayache, J., Thorel, A., Lesueur, J., and Dahmen, U. (1998). Characterization of a three-dimensional grain boundary topography in a YBa<sub>2</sub>Cu<sub>3</sub>O<sub>7</sub> thin film bicrystal grown on a SrTiO<sub>3</sub> substrate. *J. Appl. Phys.*, **84**(9), 4921–4928.
- Ayache, J., Kisielowski, C., Kilaas, R., Passerieux, G., and Lartigue-Korinek, S. (2005). Determination of the atomic structure of a  $\Sigma$ 13 SrTiO<sub>3</sub> grain boundary. *J. Mater. Sci.*, **40**, 3091–3100.
- Böhler, S. (1970). *Artefacts and Specimen preparation faults in freeze etch technology*, Balzers AG. Liechtenstein.
- Bouchet, D. and Colliex, C. (2003). Experimental study of ELNES at grain boundaries in alumina: Intergranular radiation damage effects on Al–L<sub>23</sub> and O–K edges. *Ultramicroscopy*, **96**, 139–152.
- Bouleau, D. (1974). La préparation des répliques destinées à l’observation des échantillons d’acier en MET-IRSID, 1996. Dalmay-Imelik G., Leclercq Ch., Mutin J., *J. de Microscopie*, **20**, 123.
- Cavalier, A., Spehner, D., and Humbel, B.M. (eds.). (2008). *Handbook of Cryo-Preparation Methods for Electron Microscopy*. CRC Press, Boca Raton.
- Crang, R.F.E. and Klomparens, K.L. (1988). *Artefacts in Biological Electron Microscopy*, Plenum Press, New York.
- Kato, N.I., Kohno, Y., and Saka, H. (1999). Side-wall damage in a transmission electron microscopy specimen of crystalline Si prepared by focused ion beam etching. *J. Vac. Sci. Technol. A.*, **17**(4), 1201–1204.
- McCaffrey, J.P., Phaneuf, M.W., Madsen, L.D. (2001). Surface damage formation during ion-beam thinning of samples for transmission electron microscopy. *Ultramicroscopy*, **87**, 97–104.