OVERVIEW OF SAMPLES PREPARATIONS METHODS
FOR TRANSMISSION ELECTRON MICROSCOPY (TEM)

Why is the sample preparation so important?
Because no good sample preparation, no good TEM observation !!!
Why is the sample preparation so important?

INTRODUCTION

Size and thickness of the sample

Diameter: 3 mm

1) Reduce size of large sample
2) Use 3 mm grid support for small sample

Thickness: between 10 and 200 nm depending on the material and the kind of observation to be done

1) depend on chemical composition
2) high resolution observation, EELS analysis or not
INTRODUCTION

Electrons transparent area, yes, but how, where ???

Sample as also to be:

- electrically conductive
- stable under vacum
- free of hydrocarbures contamination
- should not contain artefacts that could conduct to wrong analyse

The sample for TEM observation must be representative of the true nature and morphology of the material.

It will be impossible to prepare a sample without any artefact, so the good method as to be choosen depending on the type of analyse needed and the type of artefacts induced by one or the other technique.
INTRODUCTION

How to choose the preparation technique in relation with the material and the analysis to be done

<table>
<thead>
<tr>
<th>Material</th>
<th>Geometry</th>
<th>Physical structure</th>
<th>Chemical phases</th>
<th>Physical properties</th>
<th>Electrical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal</td>
<td>Bulk</td>
<td>Compact</td>
<td>Monophased</td>
<td>Hard</td>
<td>Conductive</td>
</tr>
<tr>
<td>Semiconductor</td>
<td>Multilayer</td>
<td>Porous</td>
<td>Multiphased</td>
<td>Soft</td>
<td>Insulating</td>
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<tr>
<td>Ceramics</td>
<td></td>
<td>Liquid phase</td>
<td></td>
<td>Fractile</td>
<td></td>
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<tr>
<td>Mineral</td>
<td></td>
<td></td>
<td></td>
<td>Ductile</td>
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<tr>
<td>Biological</td>
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<tr>
<td>Polymer</td>
<td></td>
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<td></td>
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<tr>
<td>Composite</td>
<td></td>
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</tbody>
</table>

Choice of technique depending on analysis to be done and/or induced

Sample orientation
- Any
- Particular

TEM Samples preparation, D. Laub, 2017

ARTEFACT

Ru/Zr/SrTiO₃
Preparation: tripod planar polish followed by Ion milling

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How to observe the true structure of a material?

Diamond grains used for grinding penetrate into the sample.
How to observe the true structure of a material?

After the PG, the traces caused by the PG are still visible.

No thermal damages!
INTRODUCTION

DIFFERENT TYPES OF PREPARATIONS:

- Mechanical polishing down to electron transparency
- Clivage
- Ultramicrotomy
- Crushing
- Nanoparticles dispersion

- Grinding, (dimpling), ion milling
- FIB

- Electro-chemical polishing
- Chemical polishing or etching

- Replica (direct or double)
- Thin film deposition
- ...

TYPE OF MATERIALS

- Semiconductors
- Metals and alloys
- Polymers
- Minerals
- Cements
- Ceramics
- Wood (paper)
- Etc.

THE OBSERVATION DIRECTIONS

- Planar view
- Transversal view (cross section)
- Anisotropes materials = planar or tranversal view
The Ion Milling

Using electric discharge, Ar\(^+\) ions of some kV are generated and focused on the sample. The goal is the crystal lattice destruction at the surface followed by ejection of superficial atoms.

**DRAWBACK and ARTEFACT:**
- Surface roughness
- Creation of amorphous layer on both surfaces
- Ions implantation
- Creation of dislocations
- Modification of stoichiometry
- Differential thinning rates on different compounds or phases
- Heating

**Diagram of an ion milling chamber**

**Cross-section after ion milling**

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TECHNIQUES

Dispersing Electron transparent Nanoparticles for TEM

Observation: random direction

Observations:

• Particles size and shape
• HRTEM
• Diffraction
• EDX analysis
• …

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Dispersion de Nanoparticules pour la Microscopie Electronique en Transmission

Particles well dispersed

Clean particles, well dispersed

Goal

Clean particles, well dispersed on the adequate support

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Working with nanoparticles or nanofibers
Safety rule n° 1: preparation under fume hood!

Absolutely not needed
Not needed but up to you!
Absolutely needed

Selection of suitable support grid

- Standard carbon coated grid (C thickness 25–40 nm) 1
- Ultrathin carbon film (4-5 nm) 2
- Holey carbon film with ultrathin carbon windows (5 nm) 3
- Holey carbon film 3
- Lacey carbon film 4
- ...

Depends on the analysis to be done and the particles size

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The use of solvent to disperse particles

**Polar, non polar or does not matter?**

**Ethanol:** polar

**Toluene:** non polar

Dispersion time needed: from 1 minute to several hours

Ultrasonic Device to Disperse Nanomaterial

Centrifuging to wash and concentrate the nanomaterials

- To wash the particles if in dirty or inadequate solvent
- To concentrate the particles if too few

**Hielscher Ultrasound**

Heating may help

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Dispersion

Dilution

Concentration

Ultrasound

Ready to pick up the droplet

TEM Samples preparation, D. Laub, 2017

Dispersion

One droplet on the grid: 3 ways

With the perfect loop

Dry under infra-red or standard desk lamp

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Dispersion via Spin coating

Particles + solvent

Splash protection

TEM Samples preparation, D. Laub, 2017

A few examples of dispersion

Before TEM observation

At least 1 hour under infra-red or standard desk lamp = removal of some hydrocarbons

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**Conclusion**

- Polar or non polar?
- Particles size
- Choose the support
- Type of analysis?
- Choose the support
- Sample contains Cu?
- Energy peak overlap with element to be analyzed?
- Select other grid support (Au, Mo, ...)
- Try both
- Plain C, holey C, ...

**TECHNIQUES**

**THE PLANAR VIEW**

*Observation parallel to the growing axis or to the preferential axis of the material*

**Observations:**
- Crystalline defects
- Linear defects (dislocations, ...)
- Planar defects (twins, ...)
- Study of structure and granular interfaces
- Précipitation
- ...
Super conducting wire

Preparation: wedge mechanical polishing + ion milling 20 min

Sample + TEM observation: N. Merck

Planar view of a supra-conducting wire
TEM image, bright field

Advantage: large thin area

Drawback: no information about different positions along the observation axis

SrTiO$_3$ Grains boundary
(J. Ayache)

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Materials:
- All kind

Method:

Possible defects
- Dislocations
- Irradiation
- Amorphisation of surface layers
- Modification of chemical composition

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Observation perpendicular to the growing axis or to the preferential axis of the material

**Advantage:** observation of anisotropy along the growing axis  
**Drawback:** small thin area  
**Observations:**  
- characterization of multilayer materials  
- layers thickness measurement  
- layers and interfaces structure analysis

CROSS-SECTION

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THE TRIPOD METHOD

Mechanical thinning, in a wedge configuration, down to electron transparency or to a thickness that requires very short ion milling time.

TiO2 / Silicon, Optical microscope, reflected light
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The Tripod tool

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Preparation for the first side polishing

First trick: choose good diamond lapping films

Deeplness of abrasion depending on grain size

<table>
<thead>
<tr>
<th>Grain size (µm)</th>
<th>True rotation speed (t/min)</th>
<th>Minimal depth of abrasion (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>50-75</td>
<td>Until the sample is good on the whole surface</td>
</tr>
<tr>
<td>15</td>
<td>20-30</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>10-12</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>Minimum speed</td>
<td>18</td>
</tr>
<tr>
<td>1</td>
<td>Minimum speed</td>
<td>9</td>
</tr>
<tr>
<td>0.5</td>
<td>Without rotation, make straight lines</td>
<td>Remove the front micrometer screw</td>
</tr>
<tr>
<td>Colloidal silica or other 0.03</td>
<td>100</td>
<td>To no more scratches</td>
</tr>
</tbody>
</table>

Defects can be introduced to a depth corresponding to three times the grain size used previously
Checking the sample under optical microscope

If no inverted microscope available, a modified microscope table can be used

Direction of motion: parallel (generally) to the glue line

Final mechanical-chemical polishing with colloidal silica or other medium

Careful removal of the colloidal silica

Use hand to remove silica suspension from the pad

TEM Samples preparation, D. Laub, 2017
Surface after final polishing

Optical microscope, bright field image

Dark field will show the best information about surface quality

Second side polishing

Polishing from back side

Area of interest

max. 2.4 mm

3 mm

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Result after final polishing for a Si substrate sample

Si₃N₄/ Si, optical microscope, transmitted light

TiO₂/ Si, optical microscope, reflected light

Planar polishing using Tripod polisher

- 4 areas to observe
- Easy to manipulate
- Needs longer ion milling time
SOME EXAMPLES

Example 1: InP/GaAs cross-section

After final polishing.
The narrow shows the glue line.

InP/GaAs interface: TEM, bright field
Image L. Sagalowicz, EPFL.

Sample after ion milling: 1h at 5 keV, 10 min at 2 keV, 16° angle, 2 guns.
Experimental conditions
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TEM observation

Cu/SiO2/Si cross-section.
Artefact or not?

Thicker area

Sample preparation D. Laub, TEM analysis F. Costeley, Rutgers University, USA
TEM Samples preparation, D. Laub, 2017

Near hole area.
GaN on sapphire substrat

Additional ion milling: 15 minute, 3 and 2kV, 2 guns, sectorial rotation, 5° angle

TEM image, dark field
TEM image, bright field

Au particles /TiO2, cross-section, planar grinded

Ion milled at low incidence angle, sectorial rotation for 15 minutes

TEM images F. Cosandey, Rutgers University

TEM Samples preparation, D. Laub, 2017
TEM observation

Si sample doped with He (cavity) TEM image, bright field

No ion milling

J. Werkmann, IPCMS, Strasbourg
TEM Samples preparation, D. Laub, 2017

TEM observation

Au/SiO₂ layer on Si Substrate. No ion milling

Optical microscope, reflected light, mag. 1000x

Artefact !!!

TEM bright field image

A. Schüler, S.de Chambrier, EPFL, Lausanne
TEM Samples preparation, D. Laub, 2017
TEM observation

Thin PbLaTiO₃ ferroelectric film on SrTiO₃ substrate

Tripod no ion milling

Dimpler+ions

J. Ayache, CSNSM-CNRS-IN2P3, 91405 Orsay

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THE FOCUSED ION BEAM (FIB) METHOD

Gaz: usually Galium

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The FIB (Focused Ion Beam)

For:
- Planar view
- Cross-section
- Any orientation

TEM Samples preparation, D. Laub, 2017
Comparison between techniques: Tripod and FIB

Nb₃Sn multifilament in a bronze matrix. Tripod method + Ion milling low angle (5°). Only the filaments edges and the matrix are electrons transparent. M. Cantoni, EPFL-Lausanne.

Some sample prepared by FIB. The lamella has a constant thickness and the entire filaments + matrix are electrons transparent. M. Cantoni, EPFL-Lausanne.

FIB (Focused Ion Beam)

« Internal Lift out »

« External Lift Out »

E. Bobard, M. Cantoni, CIME-EPFL
TEM Samples preparation, D. Laub, 2017
Comparison between techniques: FIB and Tripod

**Low magnification picture of a HA/TiN/Ti FIB lamella.**

**Some sample at higher magnification; Dark field image.**

This FIB lamella is not thin enough to allow HRTEM.

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**Some sample prepared with the Tripod technique.** (the Ti substrate is not visible here) A part of the HA layer has been removed during grinding and polishing. Despite that and the irradiation defects, HRTEM observation is possible.

*J. Ayache & al.*

**TEM Samples preparation, D.Laub, 2017**

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**Comparison between techniques: FIB and Tripod**

**Tripod = Ion milling**

**FIB thinning**

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**TEM Samples preparation, D.Laub, 2017**
THE CLIVED WEDGE METHOD

The cleaved wedge is a monocrystalline substrate (+ layers), dimension about 0.6/0.6 mm, obtained by 2 or 3 cleavages along designed atomic planes that give a perfect edge.

Cleavage: make use of the fact that crystals may be split along planes which are weakly bonded.

**GaAs wafer e.g**

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**Origin of the contrast:**

- The observed contrast is linked to the sample thickness and its chemical composition.
- As for a cleaved wedge, the sample thickness is accurately known, the chemical composition can be deduced from the thickness fringes profile.
- The electron beam is parallel to the layer interfaces.
- The layer interfaces are put forward by a discontinuity of the fringes (perpendicularly to the wedge edge).

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TEM Samples preparation, D. Laub, 2017.
How to cleave?

- Partially scribe the sample with a fine diamond scriber onto layers using a plastic rule and fingers.

Orientation of cleavage planes in a [001] GaAs wafer:

TEM Samples preparation, D. Laub, 2017
How to cleave?

- Turn it over, roll on the cylinder on the full sample
- Small samples must be turned over very carefully

Select good wedges using optical microscope

Positioning the wedge cleaved sample on the grid support

Take care of:
1) Eucentricity
2) Left and right
3) Sample orientation should be about 45° with respect to the direction of the electron beam
Few examples

AlGaAs/ GaAs

Calculations (JEMS) can be done to interpret the thickness fringes profile in a semi-quantitative way.

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AlGaAs/GaAs

Chemical composition measurements for AlGaAs/GaAs interfaces

500 nm

AlGaAs 35% GaAs
AlGaAs 90% GaAs
AlGaAs 70% GaAs
AlGaAs 50% GaAs
AlGaAs 30% GaAs
AlGaAs 10% GaAs

Georgia 100 nm

Few examples

AlGaAs/ GaAs

Quantum wells degradation in AlGaAs by Zinc diffusion from the surface

J.D. Ganière, EPFL

Tem samples preparation, D. Laub, 2017

T = 0° / t = 0 h.

T = 579° / t = 1 h.

T = 579° / t = 2 h.

T = 579° / t = 3 h.

100 nm
Comparison between a cleaved wedge and a "usual" cross-section of quantum wells AlGaAs/GaAs AlGaAs/GaAs

P.A. Buffat, J.D. Ganière
EPFL

TEM Samples preparation, D. Laub, 2017

Few examples
WTEM Grinsch

P.A. Buffat, EPFL-CIME, Lausanne

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Si substrate

! Cleaving planes are different from the one of GaAs

THE ULTRAMICROTOMY

Slicing of the sample to a constant thickness of 20-200 nm, using a diamond knife, carried out at room temperature

THE CRYO-ULTRAMICROTOMY

Slicing of the sample to a constant thickness of 50-200 nm, using a diamond knife, carried out at low temperature
THE ULTRAMICROTOMY, CRYO-ULTRAMICROTOMY

Observations
- Statistic of particles size
- EDX chemical analysis, EELS chemical analysis (needs thin constant thickness)
- Material microstructure
- Cross-section or plan view of materials that cannot be ion milled, mechanically or electrolytically thinned
- Heterogeneous materials, multilayer
- Small diameter fibres or tips
- Powders (metallic or not)

Materials
- Polymer /polymers with additional compounds
- Catalyst
- Geological
- Biomaterial
- Wood
- Metal

Drawback:
- Deformation of the sample due to compression or/and cracks
- Dislocations
- Shape modification
- …

TEM Samples preparation_D.Laub_2017

The ultramicrotome
- Cutting speed control
- Thickness selection

The knives
- Ultra diamond knives 35° and 45° angles
- PS spheres in Epon cut with a 15° knife
- Cut with a 45° knife

TEM Samples preparation_D.Laub_2017

TEM Samples preparation_D.Laub_2017
Method

- Reduce the sample size if needed
- Embed the sample if needed

Important: the embedding resin should have the same hardness/softness as the sample

For porous material: embedding under vacuum or infiltration-embedding

TEM Samples preparation, D. Laub, 2017

<table>
<thead>
<tr>
<th>Resin</th>
<th>Knife angle</th>
<th>Compression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowicryl K4M</td>
<td>45°</td>
<td>24%</td>
</tr>
<tr>
<td></td>
<td>35°</td>
<td>12%</td>
</tr>
<tr>
<td>EM-Bed</td>
<td>45°</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>35°</td>
<td>14%</td>
</tr>
<tr>
<td>Spurr's (hard grade)</td>
<td>45°</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>35°</td>
<td>10%</td>
</tr>
<tr>
<td>LR White (hard grade)</td>
<td>45°</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>35°</td>
<td>8%</td>
</tr>
<tr>
<td>Epofix</td>
<td>45°</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>35°</td>
<td>6%</td>
</tr>
</tbody>
</table>

TEM Samples preparation, D. Laub, 2017
Two step trimming

First trimming step

Second trimming step by ultramicrotomy (500nm sections)

Diatome, Helmut Gnaegi presentation
TEM Samples preparation, D.Laub, 2017

TEM Samples preparation, D.Laub, 2017
The color of the resin gives information such as:
- Homogeneity of the section thickness
- Thickness of the section

Isabelle Pignot-Paintrand, UPR-CNRS 5301
Cutting the sample to the desired (or possible) thickness

- Section thickness 40 - 50nm
- Sectioning speed 0.2mm/sec

Induced damages

Thin slices are done!
Now we have to fish them!!!
Sections collection

Results

Optical microscope, transmitted light

TEM, bright field image
Some examples

Mica sample

Mechanical thinning followed by ion milling
did not give a suitable result

50 nm

Hole

Some sample, prepared by ultramicrotomy

50 nm

Final thinning by ion milling,
optimized for high speed abrasion

TEM Samples preparation, D. Laub, 2017
Synthetic hydroxyapatite needle

Sectioned perpendicular to its length (C axis)

Diatome, Helmut Gnaegi presentation

J. Hemmerlé, INSERM U 424, Strasbourg

TEM Samples preparation, D. Laub, 2017

Amorphous Si/Si

 Ion beam deposited amorphous Si+ film (B) on a Si substrate (A)

Drawback

Si-Fe/SiO₂ particles Embedded in epoxy

TEM bright field image

J. Ayache, UMR-CNRS-IGR, Villejuif

TEM Samples preparation, D. Laub, 2017

Carbon particles in epoxy resin

TEM, bright field image
ELECTRO-CHEMICAL POLISHING (JET POLISHING)

Effect of electrolytical polishing is due to anodic dissolution of a pre-polished surface in an electrolyte bath

- A bath for the electrolyte
- A continuous current source
- An anode (the sample)
- A cathode

Observations:
- dislocations (orientation)
- Twins (macles)
- Grain boundaries
- precipitates and phases
- ...

TEM Samples preparation, D. Laub, 2017

Principle

Electrolytic bath:
- acid or alkaline solution
- viscous solution
- ionisable liquid

Current density is proportional to the concentration gradient: lower in crevasses, stronger on projections = lavelling of surface roughness.

TEM Samples preparation, D. Laub, 2017
Material must be an electrical conductor

- Metal and alloys, one or more phases
- Carbides
- Graphite
- Some oxides
- Some composite materials with metallic matrix and fine particles

**Advantage:** non destructive method

**Drawback:** may cause preferential etching, dissolution of interface or some phases

Possible damages: eventually residual oxidation layer at the sample surface

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**CHEMICAL POLISHING**

Some principle as electro-polishing but more difficult to control

The solutions are more reactive and used at higher temperature

**Observations:**
- Similar to the planar view or cross section

**Materials:**
- Metals
- Semiconductors
- oxides
- glass
- ...

**Method:**
- Cutting and/or cross section procedure
- Polishing onto soft tissue, specific for chemical addition
- Chemical thinning until hole

**Advantage:** possible for non conductive materials

**Drawback:** dislocations, etching (etch pits)

Possible damages: residual oxidation layer at the sample surface
THE REPLICA METHOD

The replica is the reproduction of the sample surface topography. It is done by polymer, carbon or oxide film deposition onto the surface sample, which is then removed from the sample and observed into TEM.

Observations
- Multiphase materials
- Surface topography
- Second phase particles analysis obtained by the extraction replica method
- Radiation sensitive samples

Method
- Film deposition, either « soft » polymer or in a solvant solution
- Carbon film deposition for non conductive samples
- Pulling away the film from the sample by its immersion into solvant, by pulling out or by chemical etching of the sample.
- Mounting the replica onto a 2.3 mm or 3 mm support grid
Comparison between techniques:
Fine particles dispersion - Cleaved wedge and Tripod method

Au particles          SiO$_2$/Au particles on Si substrate

Four techniques for one sample !!!!!!
Ru on C particles

- Embedded with G1 (Epoteck) resin
- Tripod polished (wedge)
- Glued on a grid
- Embedded in Araldite Epoxy
- Sliced with an Ultramicrotome
Don’t forget that sample preparation is also...

...like cooking!!!...