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Sample Preparation Handbook for Transmission Electron Microscopy

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Foreword by Ron Anderson

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TEM image of freezing defects in a frozen thin film, showing clusters of segregated crystals along the holes of the carbon membrane. (Baconnais S., CNRS-UMR8126, Villejuif, FR).

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The gift that microscopy brings us, beyond the beauty of the images, is that it gives us access to the Art of Matter and brings us to the heart of the mechanisms, "from the structure of inert matter to the complexity of the living."

There, where the infinitely small and the infinitely large come together...

It is a lesson of life and humility.

Jeanne Ayache

Foreword

Successful transmission electron microscopy in all of its manifestations depends on the quality of the specimens examined. Biological specimen preparation protocols have usually been more rigorous and time consuming than those in the physical sciences. For this reason, there has been a wealth of scientific literature detailing specific preparation steps and numerous excellent books on the preparation of biological thin specimens. This does not mean to imply that physical science specimen preparation is trivial. For the most part, most physical science thin specimen preparation protocols can be executed in a matter of a few hours using straightforward steps. Over the years, there has been a steady stream of papers written on various aspects of preparing thin specimens from bulk materials. However, aside from several seminal textbooks and a series of book compilations produced by the Material Research Society in the 1990s, no recent comprehensive books on thin specimen preparation have appeared until this present work, first in French and now in English.

Everyone knows that the data needed to solve a problem quickly are more important than ever. A modern TEM laboratory with supporting SEMs, light microscopes, analytical spectrometers, computers, and specimen preparation equipment is an investment of several million US dollars. Fifty years ago, electropolishing, chemical polishing, and replication methods were the principal specimen preparation methods. Ion milling, tripod polishing, and focused ion beam (FIB) tool methods were yet to be introduced. Today, a modern ion milling tool can cost tens of thousands of dollars and a fully outfitted FIB tool can easily cost a million dollars. With investments of this magnitude – made necessary by the demands placed on modern TEM analysis – it is paramount that the staff preparing TEM specimens have all of the training and resources possible to carry out their duties. This is where the book in your hands comes in!

But thin specimen preparation is more than just laboratory hardware and excellent protocols for thinning a specimen to electron transparency. Successful thin specimen preparation also requires knowledge of the information required from the TEM analysis. The question determines the method! For example, there may be several methods that could be used to produce specimens of the same material, but without a clear idea of the information required, even successfully thinned specimens may be only marginally useful. Thus, considerable thought should go into understanding the problem. In some cases, information from light microscopy, SEM, powder X-ray diffraction, and a trip to the library (or at least to the Internet) will solve the problem without even making a TEM thin specimen. In other cases, such information will be helpful not only in the TEM analysis itself but also in preparing appropriate thin specimens for such analysis. Unlike analytical methods that routinely deal with completely unknown specimens, say powder XRD, successful TEM requires the analyst to bring considerable knowledge to the microscope – and even to the specimen preparation! It is more important to bring knowledge to the specimen prep. Wrong prep, and the scope time is useless. Thus, we should set some realistic goals for our thin specimens and bring as much intelligence to the table as possible. Here are three goals to consider no matter what material is to be thinned:

Goal 1: *To produce an electron transparent specimen representative of the bulk material in both structure and composition.* To meet this requirement, the researcher must have a comprehensive knowledge of the structure of the material system to be studied. It might be possible to produce an electron transparent specimen by beating your material with a hammer and collecting the thinnest shards for observation. However, it is likely that the resulting specimen would not have any relationship to the structure of the material before it was so "processed." The writer is certain that there are researchers working with silicon semiconductor specimens who think that the microstructure of single crystal Si contains numerous "salt and pepper" small defects that are actually ion milling artifacts. A good rule of thumb to follow is to prepare TEM specimens by more than one method if possible. Comparing ion-milled Si with chemically polished Si thin sections will immediately establish the true microstructure of Si, for example. The well-prepared analyst should know that as-grown single-crystal Si should be featureless and that an Al–Cu alloy will contain a family of precipitates as a function of the specimen's thermal history. Facts like these on any system to be studied may be found in the literature or learned in discussion with colleagues. This handbook provides clear instruction on the many specimen preparation methods by which it should be possible to produce alternative studies of a given material – with the advantages, disadvantages, and artifact risks of each – so that an analyst can deduce the true microstructure of their specimen.

Goal 2: *To provide easy access to the required specimen information.* This would not be a problem if the specimen preparation protocol always yielded "ideal" specimens. What is an "ideal" specimen? First, the transmission electron microscopy specimen must be thin. How thin? Optimum thickness varies with the microscopy application and the information desired. The optimum thickness for dislocation density measurements may be 100 nm or greater, but the optimum thickness for electron energy loss spectrometry measurements is often less than 10 nm. The ideal specimen should maintain an ideal thickness over a large area. Second, an ideal specimen should be flat, strong, homogenous, and stable under the electron beam for hours and in the laboratory environment for years. Finally, an ideal specimen should be clean, conducting, and non-magnetic. The reader may well conclude that there is no such thing as an ideal specimen. Compromises have to be made. Perhaps no single specimen preparation method is perfect. Given a thin film alloy containing precipitates, for example, electropolishing might thin the alloy matrix but leave the precipitates too thick to analyze, whereas, ion milling might thin the precipitates but induce objectionable artifacts in the film matrix. Specimen preparation may also be limited by external factors. In the example just given, a focused ion beam (FIB) tool could prepare a satisfactory thin specimen exhibiting both the precipitates and the matrix. However, such a tool can be very expensive, and the analyst's laboratory may not have access to one. Thus, less-expensive methods must be found. Expertise in as many thin specimen preparation protocols as possible is a great advantage in any laboratory, hence the utility of the present handbook.

Goal 3: *To produce a thin specimen that enables the microstructure of the material to be accurately studied and convincingly illustrated in reports and peer-reviewed publications.* The end goal of thin specimen preparation is the production of new knowledge displayed as micrographs in publications. Correct, artifact-free exposition of the specimen microstructure is all that matters in the final analysis and will probably be the only thing recognized by the scientific community. That community, and the analyst's management, really will not care which or how many preparation protocols are employed. It is the artistic skill and the knowledge of the specimen preparer that counts, hence the value of the present handbook.

This book provides the novice with a grounding in the major specimen preparation methods in use today, assessing their merits, and identifying those modalities that are most likely to yield success. Experienced specimen preparers can use these protocols to find alternative ways to prepare their standard specimens. In addition, new requirements may become necessary, such as high-spatial resolution in the prepared thin specimen itself, where the locations of specific predetermined sites are required to be within 100 nm. Moreover, now it is often required to prepare thin specimens in much shorter times than a decade ago.

For the most part, this handbook serves the physical science community. However, there has been a trend in recent years for performing materials science analysis in biological laboratories – especially with the increase in work on biomaterials and biomimetics. So what do biologists do with materials samples? Where do they turn for specimen preparation help? I am suggesting that this book and web site are the place.

The authors have chosen a unique format for publishing their work. They originally considered a book in two volumes with a companion CD. This static approach, where readers would wait between editions to learn new content, was abandoned in favor of a handbook with a companion dynamic web site, where the content can be updated as soon as new material appears. As fully explained in this handbook, the researcher is provided with web-based guides containing both a database of materials and an "automated route" to lead to the most appropriate specimen preparation technique based on sample properties and the choice of microscopy technique. The web content is extended via links to international microscopy centers and databases. The short files on the web site are augmented by the extensive treatment each topic receives in the book. You, the reader, can be part of this novel pedagogical approach;

there are facilities whereby you may add updates and new content to the web site as you develop them. Manufacturers making specimen preparation tools and supplies may also contribute to the project. This remarkable work will remain current and provide continually increasing value to the specimen preparation community.

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Fellow of the Microscopy Society of America and Past President
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September 2009

Preface to the English Edition

It was a real adventure for our special club of five. Jeanne Ayache selected four collaborators for our supposed expertise in different areas of sample preparation and our belief that we really owed this "little job" the preparation of a guide to sample preparation, to our young and new colleagues. It is always attractive to share the experience of a career, and anyway the project (we thought!) could be completed within a year. Five microscopy specialists, each working in a different discipline and having a long-standing practice of teaching courses in this field, constituted a one-of-a-kind team.

With 5-times-20 years of experience, which, as they say in finance, comes to 100 years in accumulated surplus, our collaboration could not be reduced to a little 200-page manual. As the meetings went by, the program took shape, not without pains, resulting in a web site (in French and English) and the volume that we offer you today.

The first difficulty of this project was the language. Although we all speak French, we very quickly came up against our personal jargon: the "dialects" of a lab or of a scientific community (physicist, biologist, chemist, etc.). The richness of the French language is such that translations from French into English are different from one field to another, and habits are thrown in. For example, physicists talk about microstructures down to the scale of the nanometer, while biologists talk about ultrastructures and often stop at the scale of a tenth of a micron. Biologists who practically perform nothing but ultramicrotomy talk of "cuts," while physicists prepare "thin slices," even when they are making cuts! It almost felt like being in the tower of Babel. In short, we first had to create a glossary with a definition that provides exactly the meaning ascribed to the word used. This was a task that called for many debates and all our energy during long meetings. Once this primordial step in any interdisciplinary or cross-disciplinary undertaking was completed, everyone drafted the sections on techniques they practice frequently and know well.

The second unique aspect of the project was the collective reading of the various techniques, always with an "uninitiated" member in the group who knew nothing about the field being introduced. How should you explain an electrochemical manipulation to a biologist and an immunolabeling to a metallurgist, for example? The result is a selection of expressions accessible to all, including the non-specialist, at the expense of a super-precise aspect, of course. The techniques that we present here are written so that they may be understood by those who have never practiced them. We not only give you the outlines that make it possible to understand their implementation, their limits, and their artifacts, but also often present the details that enable their success. However, it seems difficult, for some techniques at least, to head to the workbench for an initial test, regardless of how complete the description is. Implementing a technique is not an "intellectual" task, but rather a technical task that can only be well learned in a practical training course. Our descriptions must enable you to choose which training course will be best adapted to the problem presented on the given material. Thanks to our shared experiences, we have listed the limits and imperfections of the techniques discussed for many types of materials. However, we do not claim to present all the variations and adaptations of techniques that may have been developed here or there with success.

Everyone knows the techniques most commonly used in their field, but do they know the ones used in other disciplines? Curiously, we realize that the process leading to the selection of the technique is the same in all disciplines: knowledge of one's material, the methods of action of the techniques considered, and the requirements of the mode of observation planned. We also realize that a technique considered classic in one discipline may be poorly known in other scientific areas. Ultramicrotomy is probably the best example of a technique that had been bringing joy to biologists for the past 50 years before materials researchers became aware of its strengths as well as its limitations. By knowing the actions coming into play in each type of technique, we invite you to think about what is going on during preparation. This will enable us to predict whether or not our material will be damaged by preparation. We thus train our critical minds by improving the recognition of artifacts and refining the interpretation of our results. Technique is just like cooking, but scientifically reasoned cooking has a much greater chance of being effective and reproducible.

Today there are still too few interdisciplinary bridges due to a lack of relationships, communication difficulties, and/or hyper-specialization. But these bridges are essential to resolve the problems of materials that grow more and more complex and often involve mixed and composite materials. This work is aimed at the latest generation of microscopists, the researchers in emerging disciplines who need to characterize their new materials, and industrial researchers who are often confronted with never-before-seen problems that are sometimes far removed from their base training. In this compilation, they will find the ideas that are indispensable to understanding their problems and the means for solving them. This work might also be of great service to those who make it their calling to be open to all, such as technical platforms and joint imaging and analysis centers.

Yes, this was an adventure that carried us through 5 years of work in spite of ourselves. From being highly professional, our meetings also became very friendly, with bitter and heated debates to be sure, but always in the spirit of serving science rather than some personal flattery. Oh, how many things we learned in the course of those 5 years! First, in the disciplines that we were not familiar with, in the strictness of expression striving for a more universal language, and last, in the art of using all of the resources of a computer, including those for maintaining long-distance relationships between the various partners. Many times we had to go back

to the drawing board, or to colleagues, to confirm an idea or illustrate a proposal. We would like to thank them wholeheartedly for their diligent and effective assistance. It was a lovely undertaking and a truly shared one, with each bringing their skills to the service of the common cause. It was a marvelous human adventure that will leave its mark on our professional relationships.

We would like to thank our various supervisors for agreeing to give us the time to do this and for the two retirees, thanks goes to their families for understanding the worthiness of this commitment. We also thank those who helped us technically speaking, including Michel Charles and the CNRS-Formation department in the creation of the web site preparation guide, Frédéric Lebiet for setting up the web site, Avigaël Perez for creating the diagrams, Bernard Lang for translating the web site sheets into English, Aurelien Supot and Michael Healey from Atenao Company for the translation of the French version of the books into English, and Joseph McKeown (Arizona State University, Tempe, USA) for the review of the final English manuscript.

Our gratitude most especially goes out to our colleagues of the LM2C laboratory of CNRS UMR 8126 at IGR and the CIME of EPFL of Lausanne, for their moral support, their help, and their precious advice on the creation of this collective work. We would like to thank those who supported us morally and financially in our undertaking: CNRS-Formation and the French Microscopy Society. Last, Gérard Lelièvre, Director of the MRCT of the CNRS, deserves special recognition. He supported us very early on in our approach and gave us the material means for this creation. We owe the publication of this book to him.

Villejuif, France — Jeanne Ayache
Paris, France — Luc Beaunier
Villeurbanne, France — Jacqueline Boumendil
Strasbourg, France — Gabrielle Ehret
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Jeanne Ayache is a CNRS physicist and microscopist researcher. Since she joined the CNRS in 1977, her research activities have been focused on studying the structure of materials belonging to the interdisciplinary fields of materials and earth sciences. She especially studied the structure of natural and industrial carbon-based nanomaterials, superconducting ceramics, oxide-based thin film, and heterostructures, down to the atomic or molecular scale. She is now working in the life science research field, at the Cancer Institute Gustave Roussy UMR 8126 of CNRS in Villejuif, France, where she is developing the aspects of electron microscopy in cell biology.

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Luc Beaunier is a CNRS researcher in physics in the Electrochemical Interfaces and Systems Laboratory at the Université Pierre et Marie Curie, Paris, France. His research activities in the physical metallurgy fields are related to corrosion phenomena induced by chemical and physical defects in metals. His last research interest is surface-alloyed metals by light energy laser treatment. All these materials are characterized by electron microscopy and spectrometry analysis (TEM, SEM-FEG, EDS, PEELS).

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Jacqueline Boumendil was technical director of the Center for Applied Electronic Microscopy in Biology and Geology CMEABG at the Université Claude Bernard-Lyon, Villeurbanne, France, and is now retired. The 37 years she spent in this center led her to study many normal and pathological biological samples, as well as structure of new polymeric materials. She has set up training in electron microscopy sample preparation techniques that she taught for over 20 years. She has been in charge of the development of these techniques and particularly the cryotechniques.

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