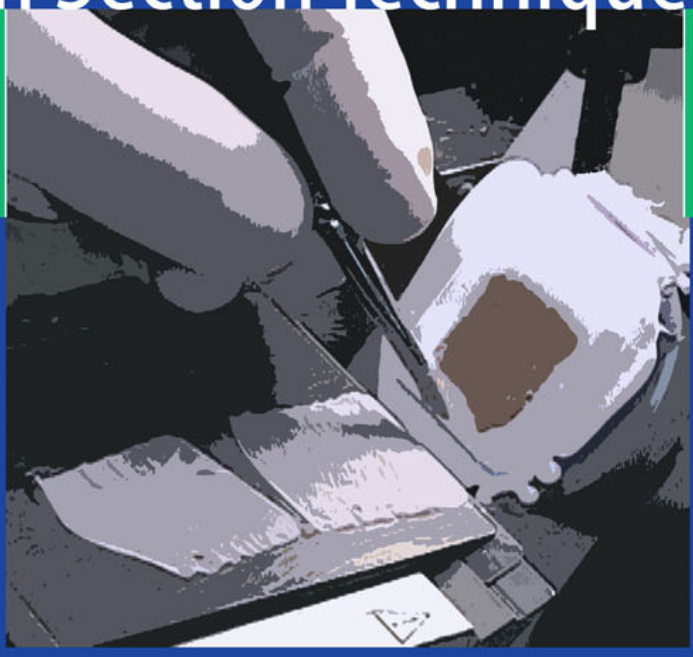
Stephen R. Peters *Editor*

A Practical Guide to Frozen Section Technique



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Preface

Frozen section technique is a valuable tool used to rapidly prepare slides from tissue for microscopic interpretation. Frozen section technique is used in a myriad of clinical and research settings. In surgical pathology, frozen sections are routinely used for rapid intra-operative diagnosis, providing guidance for our surgical colleagues. In Mohs Micrographic Surgery, the surgeon relies entirely on the frozen sections to determine the extent of the excision needed to eradicate a skin tumor. Numerous research applications rely on the frozen section technique to prepare microscopic slides utilizing a host of sophisticated morphologic, immunohistochemical and molecular methods.

Preparation of frozen section slides is a complex technical process requiring development of refined technical skills, as well as an understanding of the histology, microanatomy and pathology of the tissues being examined. Whether used for intra-operative consultation or in research, the results will hinge on our ability to achieve a high quality preparation.

The training in frozen section can vary considerably among the various subsets of practitioners. The subject is part of the curriculum in formal histology and pathologist assistant programs although much of the hands on technique is passed along at the work bench. Likewise in many pathology residency programs and research applications, training is accomplished entirely on the job sometimes with little discussion of the myriad of variables and difficulties the operator will experience along the way.

I like many pathology residents received training on the job with little more than a brief introduction to the operation of the cryostat, simple face up embedding, and to cut frozen sections using a brush. My teacher was a resident in his third year of training. From that point on it seemed that every specimen had its own set of properties. Some cut easily; some with more difficulty; some tissues would fall off the slide; and the function of our cryostat seemed to change from day to day. It also became painfully obvious that using the simple embedding methods available, I was unable to get satisfactory results in a many difficult situations. In the early days I lived in fear of exhausting precious minute samples. Over the years, through observation, experimentation and trial and error, a variety of parameters and approaches emerged which have played a significant role in my ability to prepare quality sections.

This book is intended to provide a simple yet comprehensive guide to learning frozen section technique. The authors hope to share what knowledge they have gained over years of practicing these techniques so that the newcomers will reach their goal more quickly than those of us who struggled blindly in the past.

My contributions I have written from the view point of the surgical pathologist and cover all of the steps in preparing the frozen section slide from grossing to cover slipping. The information consists of a set of methods and the details of that have proved valuable in my practice. I have tried to detail the many parameters which influence the quality of our preparations and examples of many of the aberrations that may arise.

Hoping this book will find its way into the hands of Pathology residents, I have included a discussion on interpretation of the microscopic slides in Chap. 7. The chapter discusses an approach to reading microscopic slides through careful examination, concentration and an organized plan for each specimen type. I have shared key observations about the ability to visually process information and maintaining focus and concentration. The chapter offers suggestions on dealing with difficult cases and making the most of what we have learned.

In an attempt to make this text a comprehensive guide to frozen section technique, I enlisted experts in areas outside of my experience. I am grateful for the contributions of Philip Hyam, who has spent his career in the cryostat industry for sharing his expertise and helping our readers to better understand the cryostat; Barbara Beck HT/HTL (ASCP) for sharing her expertise developed over years of practicing and teaching the histotechnology of Mohs Micrographic surgery; Charles W. Scouten, Ph.D. a noted expert in the field of neuroscience research for sharing his knowledge and expertise in frozen section technique as it applies to the animal research setting; and for the help of Catherine Susan Delia, BS.,HT. ASCP a highly experienced and knowledgeable histotechnologist for her guidance and in the preparation of our chapter on fixatives and staining.

The techniques and experiences shared in this book are those used successfully by the authors in their practice. As most of the information we have to share is derived from lifelong experience as such there are relatively few references to offer. In no way can we hope to know and cover the many different approaches used by our colleagues around the world. As so many of us have arrived at our own individual techniques and observations as a means of survival, I am certain that there are many with successful methods and ideas that differ from what we can offer. We all evolved in our own environment, taking what skills we have learned and improving on them where they were suboptimal. There may be some to take exception to what we have written. So many clever techniques are passed along at the lab bench but never find their way into our literature. I am hoping this text may encourage others to share their ideas and techniques. Together we hope to provide a body of information on frozen section technique to guide for those who find themselves immersed in this challenging field.

I would like to thank my dear friends Claudia Dorenkamp, Jan Minshew, George Kennedy and their colleagues at Leica Microsystems for their keen foresight in recognizing the valuable new technology and their help and supporting my mission

to bring these techniques to our colleagues. I am grateful to my loving wife and partner Jeannine for her unwavering support and tolerance in all of my endeavors and for rolling up her sleeves to share in the arduous task of running our little company Pathology Innovations, LLC; whose sole mission is to share better ways to help our colleagues and their patients. I would also like to thank the numerous bright young residents of the University of Medicine and Dentistry of New Jersey that I have had the privilege to help train. I could not have understood the process of learning without observing each of their unique examples. Their love and support, is more valuable to me than any reward I have known in my career. I would also like to acknowledge the dedicated and hard working histotechnologists and pathologist's assistants around the world. My pathologist colleagues and I could not begin to practice our profession without them. They are both scientists and artisans and are all too often under rewarded for the important job they perform and stresses we put them through.

I would like to dedicate this book to my late father George J. Peters. He was a man of limited education but of unlimited ingenuity. He taught me how to use tools; to make this out of that; and to *live* outside the box. Without his example, I doubt I could have gathered the information offered in this book.

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Contents

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Chapter 1
Understanding and Maintaining the Cryostat

Philip Hyam

Abstract The chapter presents a user-friendly review of the main components of a routine cryostat and their function in the preparation of frozen sections of mammalian tissue. Topics covered include sectioning hints and tips and proper methods for disinfection and cryostat maintenance.

Keywords Cryostat • Microtome • Knife holder • Chamber temperature • Object temperature • Freezing shelf • Peltier element • Disinfection • Routine maintenance

Frozen sections, quick sections, in clinical terminology, intraoperative consultations, are prepared using a cryostat.

A cryostat is a cooled chamber, or cabinet that houses an instrument to section frozen samples; a rotary microtome and knife (or blade) holder, and a means to freeze samples.

Several types of cryostats are commercially available and can be categorized as follows:

- Single compressor (chamber cooling only)
- Double compressor (chamber and object cooling)
- Manual sectioning
- Motorized sectioning

These are free-standing instruments that are insulated to very high standards to ensure that selected temperatures are easily maintained. Access to the chamber is via a heated sliding window. The normal working chamber temperature is from 0°C to −35°C, the limiting factor being the type of compressor and refrigerant used. Cryosectioning at temperatures lower than −35°C requires the use of a cryogen such as liquid nitrogen.

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