HANDBOOK OF BIOLOGICAL CONFOCAL MICROSCOPY

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Edited by
James B. Pawley

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Preface

In 1987 the Electron Microscopy Society of America (EMSA) under the leadership of J. P. Revel (Cal Tech) initiated a major program to present a discussion of recent advances in light microscopy as part of the annual meeting. The result was three special LM sessions at the Milwaukee meeting in August 1988: The LM Forum, organized by me, and Symposia on Confocal LM, organized by G. Schatten (Madison), and on Integrated Acoustic/LM/EM organized by C. Rieder (Albany). In addition, there was an optical micro-analysis session emphasizing Raman techniques, organized by the Microbeam Analysis Society, for a total of 40 invited and 30 contributed papers on optical techniques.

Following this successful meeting, discussions among the participants revealed support for a slightly more focussed approach at the next meeting. The benefits of confocal techniques were now felt to be widely appreciated and it seemed time to really evaluate the actual performance of the various instruments and to compare this with theoretical benchmarks and so produce a consensus on where major improvements were likely to be possible in the future. It was felt important to shift from the assertion that "Confocal Works" to the matter of how to make it work better.

Because of the rapid pace of development in the field, we recognized that we were unlikely to be able to be totally definitive on all matters affecting the confocal microscope, but we also felt that the field would benefit from access to a good list of questions and as many answers as time permitted. To do this, it was decided to try to elicit a series of talks in which each one covered a single instrumentational feature unique to confocal microscopy (particularly biological confocal microscopy). The initial list included 12 topics ranging from laser and conventional sources through scanning systems, objective lenses, chromophors, "the pinhole", photon detectors and 3D data display, as well as three overviews on the genesis of the confocal approach, its fundamental limitations and its quantitative capabilities.

In parallel with these developments at EMSA, Drs. J. Wooley and S. Pierce of the Instrumentation and Instrument Development Program at the National Science Foundation had followed a similar path. The Instrumentation and Instrument Development Program is responsible for supporting the purchase of major items of multi-user instrumentation for the conduct of basic research in the life sciences, particularly that which is supported by the NSF Divisions of Behavioral and Neural sciences, Cellular Biosciences, Molecular Biosciences, and BIOTIC Systems and Resources. For the past five years, the Program had emphasized three areas of activity: 1) New instruments that either extend current sensitivity or resolution, or provide new techniques for detection, quantification or observation of biological phenomena. 2) New computer software to enhance current or new instrumentation, and 3) Sponsored workshops in emerging areas of instrumentation or instrument development. They believed that it was clear that confocal microscopy and other new microscopical instrumentation was going to drive important scientific discoveries across wide areas of physiology, cellular biology and neurobiology. They had been looking for a forum in which they could advance the state of the art of confocal microscopy, alert manufacturers to the limitations of current instruments, and catalyze progress toward new directions in confocal instrument development.

These goals were so close to those of the EMSA project that the two groups decided to join forces with EMSA to provide the organization and the venue for a Confocal Workshop and NSF to provide the financial support for the speakers expenses and for the publication of extended abstracts.

The abstracts were initially envisioned as each being about 10–15 pages of camera-ready manuscript but, because of the generous and enthusiastic response of the many leaders of the confocal LM community who agreed to participate, the manuscripts actually submitted were up to fifty pages in length. In addition, scissions and additions increased the list of the topics covered to a total of 19, plus an annotated bibliography.

As the aim of the volume was to discuss the instrument rather than to describe specific applications, the biological emphasis emerges in most chapters as the need to use photons efficiently at every stage of the imaging process and thereby reduce the effects of bleaching and photo-damage to the specimen. In this context, several chapters in this volume emphasize for the first time limitations imposed by everything from fluorescence saturation and sub-optimal signal digitization to specimen preparation.

On a more general level, chapters were added on related instrumentation and on the often unrecognized limitations imposed by the process of pixelating the data contained in digitally recorded images. Several months of frenzied activity got the final mock-ups to the printer in early July.

The nineteen papers were presented at a two day workshop on August 8–9, 1989 at the EMSA Meeting in Houston, TX where the first, soft-cover edition of the Handbook was distributed at that time under the convenient but largely fanciful imprimatur of the IMR Press.

The response was so enthusiastic that it was decided to produce a second, hard-cover edition with an established publisher. This would permit wider distribution and would allow us to correct the errors associated with the short preparation time of the first edition. In addition, extra paragraphs and figures were added to fill gaps in the original or to take note of recent developments.

Taken as a whole, I believe these papers constitute the most complete consideration on the topic available at this time. I am sure that all of the other authors join me in the hope that it will prove to be a catalyst in the development of yet better instrumentation and techniques in the field of biological confocal microscopy. Indeed, improvements evident in the design of the Biorad MRC-600 and of the Leitz CLSM show some evidence of this trend.

Many people have contributed to the production of this volume starting with Drs. Pierce and Wooley and all of the authors.

In addition, I should like to single out R. and C. Moen and K. Hamele for their editorial assistance and C. Thomas, C. Ewing, K. Morgan and P. Henderson for help in retyping some of the manuscripts, A. Freidman and L. Moberly of University of Wisconsin-Madison Publications and W. Kasdorf and N. MacMiller of Impressions for their patience with the typesetting. Special thanks are also due to G. Benham of the Biorad Corporation, and V. Argiro of Vital Images who, when rising costs threatened to delay publication of the first edition, stepped in to fund the printing of the colored cover. This gesture is noted here because, due to a printing mix-up, no mention of the source of the cover images or of the support was included in that edition.

My heartfelt thanks to you all.

James Pawley
Editor
12/89

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