

FLUORESCENT ANTIBODY TECHNIQUES AND THEIR APPLICATIONS

Second Edition

**Edited by
Akiyoshi Kawamura, Jr.**

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Foreword to the Second Edition

Even in the field of medical science the development of research resources including systematized techniques is often a matter of *sine qua non*, resulting in rapid and steady progress in the field. In the Institute of Medical Science at the University of Tokyo, formerly named the Institute for Infectious Diseases, many of our senior scientists have consecutively revised a book, 細菌学実習提要, (Manuals in Bacteriology, written in Japanese) through their own experiences, and the volume is constantly consulted in our laboratories.

This revised edition of *Fluorescent Antibody Techniques and Their Applications* was written similarly by specified scientists of this Institute under the leadership of Prof. Akiyoshi Kawamura, Jr., now chief investigator in the Department of Immunology of this Institute. Prof. Kawamura was originally a rickettsiologist and the son of a famous pathologist, Prof. Rinya Kawamura, and has long been engaged in the fundamental immunofluorescence technique first developed in 1950 by Prof. Albert H. Coons.

The beauties of nature should be revived artistically. Antigens or antibodies in sectioned tissues should be visualized beautifully. We very much hope the use of this volume as a reference book in laboratories contributes many new approaches in the field of medical science.

January, 1977

Tadashi Yamamoto, M.D.
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Foreword

First developed by Professor A.H. Coons, fluorescent antibody techniques and applications have now reached their highest level of achievement. In the Institute of Medical Science at the University of Tokyo, under the leadership of Associate Professor Akiyoshi Kawamura, Jr., the authors of this volume began about sixteen years ago to study the basic fluorescent antibody techniques in their respective fields, maintaining close contact with each other concerning the results of their work.

A number of monographs and reviews have been published introducing the principles and methods, but they have been too concise to give a complete picture of the technique. The authors of this book had only published their results in Japanese when they first had the opportunity to demonstrate their method in the West at a symposium for standardization of fluorescent antibody techniques in London under the sponsorship of the IAMS and at a training course for fluorescent antibody techniques in Copenhagen, sponsored by WHO. It was at these two conferences that they received recognition for the superior level of their techniques and were encouraged to publish a fuller explanation in English in book form.

Each section of *Fluorescent Antibody Techniques and Their Applications* is written by the specific scientists involved in the mastery of the technique discussed, thus ensuring the most comprehensive explanation.

This book is not a general introduction but rather describes the specific applications of the fluorescent antibody method. It is hoped that the numerous color pictures will assure the readers that the same results will be obtained if the procedures outlined are followed.

We hope that this volume will contribute significantly to the existing literature on fluorescent antibody techniques and applications.

March, 1969

Ayao Yamamoto
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Preface to the Second Edition

More than a quarter century has elapsed since immunofluorescence (fluorescent antibody techniques) was developed by Prof. Albert H. Coons in 1950. Since then the techniques have been increasing in applicability to fundamental research in immunology, microbiology, pathology and clinical diagnostics. In the Institute of Medical Science, the University of Tokyo, a research group was organized under the leadership of the editor to standardize immunofluorescence and to elucidate its fundamental technical problems. The group has been devoted to solving the problems involved, and contributed to the brilliant successes which were evaluated as top level at three international symposia: Training course for fluorescent antibody techniques (Copenhagen, 1965), Symposium for standardization of fluorescent antibody techniques (London, 1968) and Conference on defined immunofluorescent staining (Stockholm, 1970). In particular, the methods developed and improved by the group were recognized to be overwhelmingly superior to those of other groups at the conference where the results of international field trials concerning the standardization of conjugates were presented.

The first edition of *Fluorescent Antibody Techniques and Their Applications* was published in 1969 on the basis of the successes described above. Since that time substantial progress has been made: (a) improvement in observation, e.g. incident illumination system, (b) availability of a purified antibody class and its active fragment for direct method and class-specific antibodies for indirect method and (c) general recognition of the importance of pretreatment. In addition to these advances, there was general demand for another printing of the book. Considering this background, it was necessary, the editor felt personally, to revise the book, and the revision was actually already planned three years ago. The revised edition is being published so late, despite the completion of the majority of chapters describing fundamental methodology, because of delay in some other parts. Hence this edition is not the second one but actually the third one.

Considering the general trend toward internationalization of various techniques, it was expected that the book would be reviewed in the first-run specialized journals shortly after its publication. It is regrettable, however, that most of

the criticism made was rather superficial and missed the point. The reviews could be summarized as making the following points: (a) the book did not contain extensive references as a textbook on applied aspects of immunofluorescence should; (b) the book was not suitable for beginners because specialized terms, e.g. F/P molar ratio, were used without much explanation; and (c) the book was written in poor English (or American) and many errors were found. Concerning point (a), I am fully convinced that the criticism is unacceptable. We intended to describe not the general method like a textbook but the standard methods we actually followed and the results obtained which had a great impact on the international stage. As most books on immunofluorescence are so incomplete as manuals for performing the technique, I dare say here that our descriptions are very instructive in performing the techniques. In fact a number of people around the world have been carrying out immunofluorescence according to the methods described in the book and have obtained satisfactory results. On point (b), explanation of the terms was rather brief because we intended primarily to describe important points in performing the technique. As for the language, the book was published from a non-English speaking country and contained many mistakes, which were corrected on the occasion of the reprinting of the first edition.

This book was originally entitled *Fluorescent Antibody Techniques and Their Applications*, and the same title is used in the second edition since alteration of the title of the book was not allowed. Recently, however, 'immunofluorescence' has become more common than the term 'fluorescent antibody techniques,' based on the principle of this technique. In the text of this edition, the term 'immunofluorescence' is used instead of 'fluorescent antibody techniques,' according to international usage. Therefore, in a practical sense, fluorescent antibody technique as a term refers to one of the representative methods in immunofluorescence.

General parts of the first edition were descriptions by the core of our research group, mainly by the editor. In the second edition, these (Chapters 1-6) are further supplemented by Dr. H. Nakamura on immunochemical aspects, by Mr. K. Wada on optic systems and also generally by Drs. S. Fujiwara, K. Hayashi and F. Shimizu. In addition, Prof. Dr. Ernest H. Beutner contributed a paper on the determination of antibody protein (pp. 63-65). Chapter 6 contains a new description for better understanding and performance of the exact procedures of immunofluorescence and standardization of reagents employed, giving practical illustrations. Chapter 7 on applications (Part II) was taken charge of by Dr. Aoyama's group (Dr. Y. Aoyama, Dr. K. Hayashi and Dr. T. Kurata) and by myself. It was an unexpected pleasure that many new results obtained in application of our methods were added to this edition. Now I am fully confident that the contents of this edition, though published from a corner of the Orient, are at the top level in the world and hope that this edition will contribute to the real standardization

of immunofluorescence. Last but not least I would like to express sincere thanks for the dedicated labor of Mr. M. Murata, Mr. K. Hamajima and Ms. E. Hamao during the course of the publication of this book.

January, 1977

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Preface

Almost two decades have passed since Dr. Albert H. Coons and his co-workers first established the principles of the fluorescent antibody technique. This revolutionary technique, based on more than ten years of investigation, made it possible, by using the specific reactivity of labeled antibody, to locate precisely the corresponding antigen in tissue. Subsequently a number of improvements and refinements were devised, giving this simple and reproducible technique a degree of specificity and sensitivity which has led to its increasing application in a variety of fields, including immunology, microbiology, pathology, histology and clinical diagnostics.

A number of monographs and articles dealing with the theoretical and practical aspects of this technique have been published to date, but most of these have dealt with specific applications rather than the general methodology of the technique itself. Our fluorescent antibody (FA) study group at the Institute of Medical Science began work on synthesizing a fluorescent dye, fluorescein isocyanate, more than ten years ago. When Riggs and others succeeded in synthesizing the dye and it had become readily available to us, we directed our efforts to eliminating nonspecific fluorescence—the most troublesome problem encountered in using the fluorescent antibody technique. The problem, both in terms of antigen and antibody, could be approached only on the basis of the extensive knowledge accumulated over the years by the members of the Institute and through their multi-disciplinary cooperation. A number of important improvements in the optical system were also achieved by the physicists of our staff. The total number of contributors to these studies was well over 180. The improved technique which resulted from these investigations can now be used to achieve fluorescent antibody staining with a minimum of nonspecific fluorescence and a maximum of clarity and excellence.

The details of our method were presented at a meeting of the Training Course of the FA Technique, organized by WHO in Copenhagen in 1965, and have subsequently been discussed with many investigators here and abroad. We have at present determined to publish them in the hope that they will be of value to other workers and that they will stimulate further research on methodology.

The present book is divided into two sections: the first includes theories and

the general method, the second describes specific applications. The first section was written by the core of our study group, namely the editor, Assoc. Prof. Kawamura, and Prof. Matsuhashi, Drs. Kawashima (immunology) and Nakamura (immunochemistry), Prof. Kusano, Assoc. Prof. Aoyama and Dr. Hayashi (pathology), and Mr. Wada (optics). The second section was also written by members of our group, all leading authorities in their fields in Japan, and is based on actual experiments and methods which we devised. We have used as many color photographs as possible to illustrate particular points in these chapters.

Some sections of the book were originally written in Japanese and were translated by Assoc. Prof. Watanabe with the cooperation of Dr. Ebisawa. The entire manuscript has been revised by Mrs. Annik L. Chamberlain, the second section by Prof. Ishikawa, and part of the first by Dr. Colin. Their dedicated labor is greatly appreciated. Much valuable advice and a number of useful suggestions were obtained on the general plan and various aspects of this book from Profs. Yamamoto and Tsunematsu; Mr. Noda kindly provided assistance with the photographic techniques. I would also like to express my cordial thanks to Mr. Shigeo Minowa for his help in furthering the publication of this book.

March, 1969

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PART I

