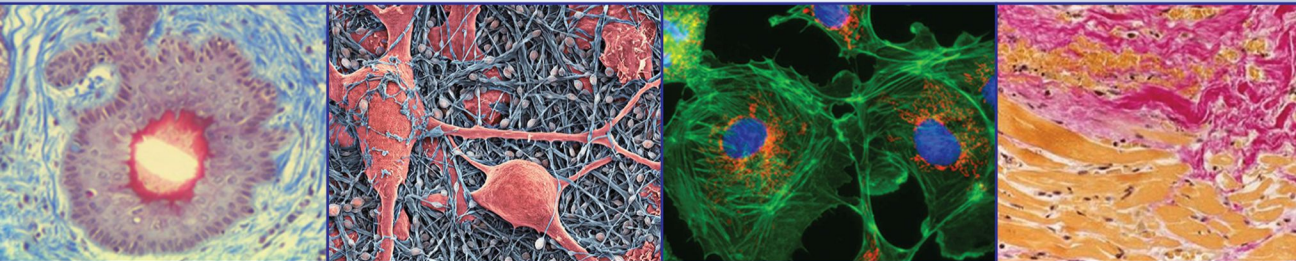


**Second
Edition**

Principles and Techniques in

HISTOLOGY,
MICROSCOPY and
PHOTOMICROGRAPHY



DR Singh



CBS *Dedicated to Education*
CBS Publishers & Distributors Pvt Ltd

Principles and Techniques in

HISTOLOGY
MICROSCOPY

and

PHOTOMICROGRAPHY

SECOND EDITION

This Page is Intentionally Left Blank

Principles and Techniques in

HISTOLOGY
MICROSCOPY
and
PHOTOMICROGRAPHY

SECOND EDITION

DR Singh MBBS MS PhD

Emeritus Professor
Department of Anatomy
King George's Medical University
Lucknow, UP

Former

Professor and Head, Department of Anatomy
King George's Medical College, Lucknow University, Lucknow

Professor and Head, Department of Anatomy
BP Koirala Institute of Health Sciences, Dharan, Nepal

Seema Dental College, Rishikesh, Uttarakhand
Nepalgunj Medical College, Kathmandu University, Nepal



CBS Publishers & Distributors Pvt Ltd

New Delhi • Bengaluru • Chennai • Kochi • Kolkata • Mumbai
Hyderabad • Jharkhand • Nagpur • Patna • Pune • Uttarakhand

Disclaimer

Science and technology are constantly changing fields. New research and experience broaden the scope of information and knowledge. The authors have tried their best in giving information available to them while preparing the material for this book. Although, all efforts have been made to ensure optimum accuracy of the material, yet it is quite possible some errors might have been left uncorrected. The publisher, the printer and the authors will not be held responsible for any inadvertent errors, omissions or inaccuracies.

eISBN: 978-93-881-7825-9

Copyright © Authors and Publisher

Second eBook Edition: 2018

All rights reserved. No part of this eBook may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system without permission, in writing, from the authors and the publisher.

Published by Satish Kumar Jain and produced by Varun Jain for
CBS Publishers & Distributors Pvt. Ltd.

Corporate Office: 204 FIE, Industrial Area, Patparganj, New Delhi-110092

Ph: +91-11-49344934; Fax: +91-11-49344935; Website: www.cbspd.com; www.eduport-global.com;

E-mail: eresources@cbspd.com; marketing@eduport-global.com

Head Office: CBS PLAZA, 4819/XI Prahlad Street, 24 Ansari Road, Daryaganj, New Delhi-110002, India.

Ph: +91-11-23289259, 23266861, 23266867; Fax: 011-23243014; Website: www.cbspd.com;

E-mail: publishing@cbspd.com; eduportglobal@gmail.com.

Branches

-
- **Bengaluru:** Seema House 2975, 17th Cross, K.R. Road, Banasankari 2nd Stage, Bengaluru - 560070, Karnataka Ph: +91-80-26771678/79; Fax: +91-80-26771680; E-mail: bangalore@cbspd.com
 - **Chennai:** No.7, Subbaraya Street Shenoy Nagar Chennai - 600030, Tamil Nadu Ph: +91-44-26680620, 26681266; E-mail: chennai@cbspd.com
 - **Kochi:** 36/14 Kalluvilakam, Lissie Hospital Road, Kochi - 682018, Kerala Ph: +91-484-4059061-65; Fax: +91-484-4059065; E-mail: kochi@cbspd.com
 - **Mumbai:** 83-C, 1st floor, Dr. E. Moses Road, Worli, Mumbai - 400018, Maharashtra Ph: +91-22-24902340 - 41; Fax: +91-22-24902342; E-mail: mumbai@cbspd.com
 - **Kolkata:** No. 6/B, Ground Floor, Rameswar Shaw Road, Kolkata - 700014 Ph: +91-33-22891126 - 28; E-mail: kolkata@cbspd.com

Representatives

-
- **Hyderabad**
 - **Pune**
 - **Nagpur**
 - **Manipal**
 - **Vijayawada**
 - **Patna**

to

*my wife, Rekha, for her constant encouragement;
my children, for their heartedly inspiration;
my revered teachers, for their helpful guidance
my professional colleagues, for their critical suggestions
and
my past and present students, for their love and affection*

— this book is for you

This Page is Intentionally Left Blank

Foreword

The dawn of the twenty-first century heralds the coming age of molecular medicine and the era of predictive medicine. Physical examination (resolution 0.2 mm) alone no longer suffices for clinching a diagnosis and the light microscopy (resolution 0.2 μm) needs to be strengthened by ultrastructural analysis (up to 0.2 nm) for the evaluation of disorders at the molecular level. The greater use of the manifold techniques of light microscopy as well as electron microscopy entails a thorough in-depth knowledge and understanding of the equipment and methods basic to the aforementioned tools. This book is primarily addressed to the fulfilment of the objectives listed above. It is common knowledge that a thorough grasp of the tools of the trade equips one with capabilities to maximize their skillful utilization with finesse. Kudos to Prof Dhanraj Singh for his timely realization of this dire need and for painstaking following it up with singular drive and devotion towards the creation of this reservoir of technical details which has grown to almost encyclopedic proportions. The author has superbly integrated his artistic acumen and skills of photography with the recently acquired mastery of computer graphics for creating this *vade mecum* of microscopy not only to address undergraduates and postgraduates of anatomy and pathology but also to histology and histopathology technicians, oral pathologist, veterinarians, paramedics in general.



(1936–2013)

Padmashree Prof (Dr) Mahdi Hasan

MBBS MS (Hons) PhD DSc FAMS FICS FRMS FNAsc FNA

Recipient, Dr BC Roy National Award

Founder Director of Interdisciplinary Brain Research Centre

Ex-Professor and Chairman, Department of Anatomy, and

Principal and Dean, JN Medical College

Aligarh Muslim University, Aligarh

Founder Fellow, Indian Academy of Neurosciences

Adviser, WHO

This Page is Intentionally Left Blank

Preface to the Second Edition

The publication of the second edition of *Principles and Techniques in Histology, Microscopy, and Photomicrography* was a great feeling since I received many comments and suggestions from the readers. While revising the book I have considered all these and it has greatly helped me in further developing the book.

In the second edition I have attempted to provide students with the most contemporary and useful text possible and in a more clear and concise manner without compromising on the information required by the postgraduate students. To facilitate understanding, I have restructured the contents to build a logical flow of information at the end of all main topics. Also a summary has been provided at the end of each chapter.

New tables have been added in the 'Techniques in Histology' section, which also includes now colour pictures of histologic slides on important components. The text in chapters on the general guidelines for photographic processes and technical details of photomicrography (Part 3 in the first edition) though has become old-fashioned, it has been retained for dual reasons: *First* to maintain the originality of the book; and *second* because of the fact that not all the laboratories concerning photomicrography are provided with the modern sophisticated equipment including digital SLR cameras needed for photomicrography. A new chapter on 'Digital Photomicrography' has been added to update the relevant information required for better equipped postgraduate students. Common questions asked during the *viva voce* of practical examination of histological techniques (in almost all university examinations) with the answers have been provided for the benefit of the students and also to make the book examination-friendly. The book is likely to help the students succeed in postgraduate examination.

A clear understanding of the subject, its retention and reproducibility, and usefulness for examination, the second edition has evolved into a truly integrated, and student-friendly textbook for histology students. It can also serve as a useful companion for medical undergraduates pursuing MBBS.

I would appreciate any comments or suggestions concerning any of the sections in the book. You may contact me at dr.dr Singh40@gmail.com.



DR Singh

Preface to the First Edition

Histology provides a basis for understanding the internal structure of body organs. With increasing importance of histology in the anatomy curriculum, it becomes necessary for histologists to facilitate understanding of the basic principles in examination of a stained section with an optical light microscope. There is some diversity of opinion as to what features of histology should be taught, and to what extent. The purpose of this book is to give the student a concise account of the relevant details which have been brought up to date. The present book departs from other books in that it has combined animal tissue preparation for obtaining stained paraffin sections, proper use of the light microscope, and finally to get the images photomicrographed. Techniques in histology, microscopy, and photomicrography have been so overwhelming that the medical student often has difficulty in grasping the basic steps about the actual procedures. Since photomicrography has become of great practical value, both in teaching and research, Part 3 of this book is provided with various formulas for the final processing of photographic emulsions for the desired reproduction of results to large audiences; and also for scientific publications. The book is freely illustrated to enable the reader visualize summarized concepts and processes. While writing this book, an attempt was to keep in mind the often-quoted Chinese proverb, "A little picture is worth a million words". It is hoped, however, that detail has not been neglected too much and that the text is sufficiently readable although precise. In summary, the book is bound to satisfy the students' need in learning and, despite its brevity, create sufficient confidence to the self-dependent in the way of learning histological techniques.

DR Singh

Acknowledgements

The assistance, cooperation, and professionalism of the editorial staff of the CBS Publishers & Distributors, New Delhi, is greatly acknowledged. A special appreciation is extended to Mr Satish Jain, CMD, who encouraged me to get my book revised for the second edition; the first edition of which was published in 2003. The challenging task was initiated with my first meeting with Mr YN Arjuna, Senior Vice President—Publishing, Editorial, and Publicity, who deserves my special thanks and sincere regards for his many helpful suggestions and much consideration and cooperation throughout the completion of this edition. I also appreciate Mr Prasenjit Paul, Mrs Sunita Rautela and Mr Rohan Ram who worked extremely hard to organize and finalize the illustrations and the proofs.

I am greatly indebted to my faculty colleagues and postgraduate students of my alma mater—Department of Anatomy, King George's Medical College, Lucknow, and postgraduate students at Nepalgunj Medical College, Chisapani, Nepal, as well, for their suggestions to bring out the long-awaited second edition of this book.

I put on record my full appreciation and sincere regards to my revered teachers in anatomy—(late) Prof Dharam Narayan, (late) Prof AC Das, and (late) Prof Mahdi Hasan at King George's Medical College, Lucknow (now upgraded as KG's Medical University), who taught me anatomy during 1959–1961. For the long association with my alma mater for more than five decades, I owe all appreciation for the Department of Anatomy, where actually I learned the subject of anatomy. Prof A Halim, *ex*-Professor and Head, Department of Anatomy, has been one of the best teachers during my undergraduate medical career and later as my role model in teaching when I joined as faculty in the Department of Anatomy.

Finally, I owe much to my wife Rekha and children who always encouraged me in all my academic pursuits. It is impossible to put into words the sum total of my gratitude to them.

DR Singh

This Page is Intentionally Left Blank

Contents

<i>Foreword by Prof (Dr) Mahdi Hasan</i>	<i>vii</i>
<i>Preface to the Second Edition</i>	<i>ix</i>
<i>Preface to the First Edition</i>	<i>x</i>

Part 1 Techniques in Histology

1. Overview of Histology and Approaches to its Study	3
2. Some Basic Things to Know	17
3. Grossing of Specimens and Fixation of Biological Samples	35
4. Decalcification of Bone Samples	49
5. Dehydration and Clearing	60
6. Impregnation (or Infiltration) and Embedding of Biological Samples	67
7. Paraffin Waxes and Paraffin Block Making	74
8. Microtomes and Microtome Knives	81
9. Microtomy: Technique of Sectioning	103
10. Stains and Staining	125
11. Special Staining of Tissue Sections and Preparation of Blood Film	141
12. Mounting, Covering, and Labeling of Sections	157
13. Specialized Techniques in Anatomy	165
<i>Annexure: Basic Histological Techniques</i>	<i>185</i>

Part 2 Microscopy and Micrometry

14. Microscope and Microscopy: Overview and Historical Account	193
15. Aberrations in Microscope Lenses and their Correction	204
16. Parts of Compound Light Microscope	210

17. Best Performance of Light Microscope	239
18. Special Types of Microscopes and the Principles for their Use	252
19. Different Devices and Methods used for Micrometry	281
<i>Annexure: Basic Microscopy and Micrometry</i>	<i>294</i>

Part 3**Photomicrography**

20. General Principles of Photography and Photomicrography	297
21. Photomicrography: Introduction, Methods and Techniques	305
22. Photographic Emulsions and Processing Solutions	317
23. An Overview of Techniques in Photography	325
24. Digital Photomicrography	335
<i>Annexure: Photomicrography</i>	<i>350</i>
<i>Index</i>	<i>351</i>