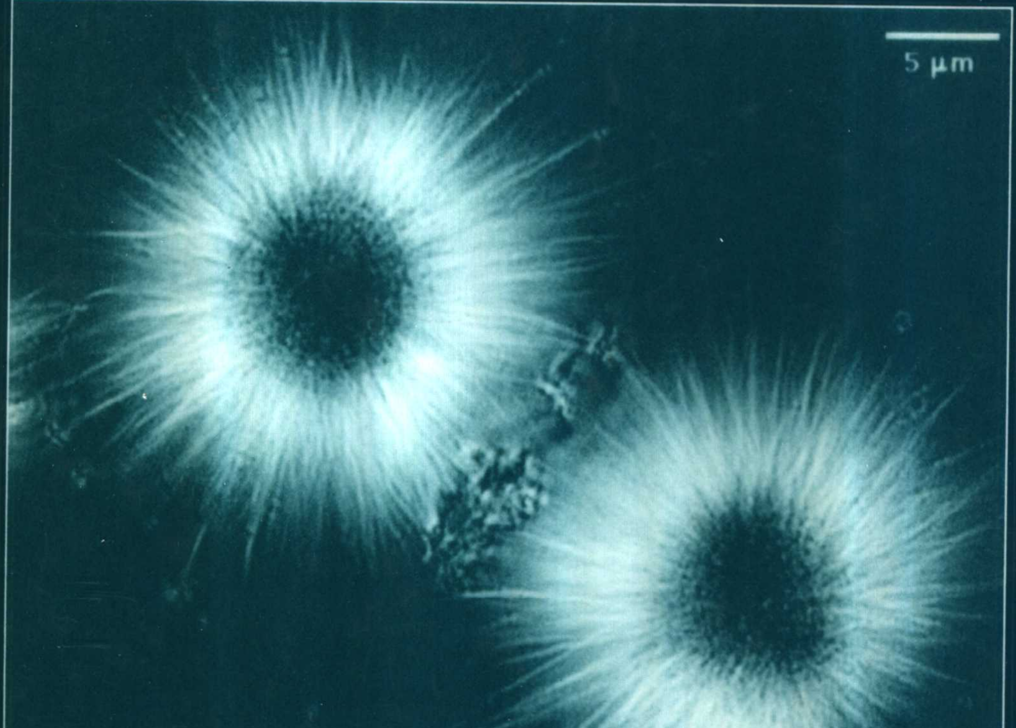
SECOND EDITION

VIDEO MICROSCOPY

The Fundamentals



SHINYA INOUÉ
AND
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Video Microscopy SECOND EDITION

The Fundamentals

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Preface

The current edition of *Video Microscopy* has been totally revised to reflect the advances in the tools for electronic imaging, processing, recording, and analysis, as well as applications that are being made in video microscopy and related modes of electronic imaging. The less spiral organization of the revised edition reflects an audience expected to be more experienced in video and computer image processing than in the earlier days when coupling of video equipment and computers to the light microscope was still considered to be a novelty.

Nevertheless, we have emphasized the how-tos, as well as the fundamental principles, involved in imaging and processing in the light microscope, the human visual system, video and related electronic imaging, and the digital image processor in the hope that the reader will develop enough understanding, not only to apply rationally what is available, but also to contribute actively to the development of this evolving field. In the text, the terms appearing in the Glossary are printed in bold type, generally, at first appearance. Italics are used for emphasis.

As in the first edition, Chapter 1 reviews the history of video and briefly summarizes present applications and developments in video microscopy. A vastly expanded and updated Chapter 2 reviews the physical optics and basics of microscope image formation, including point spread functions, contrast transfer functions, major modes of contrast generation, and scanning microscopy. Chapter 3 covers practical aspects of microscopy, including selection, use, and care of the components. Chapter 4 characterizes the eye as a detector and covers the fundamentals of color vision. Chapter 5 deals with the fundamentals of the video signal as well as current video standards. Chapter 6 introduces a new section on the electronic detection of light and covers vidicon tube cameras.

Chapters 7–9, which are completely new, deal with solid-state detectors, both video rate and slow scan (Chapter 7); image intensification (Chapter 8); and the color video signal and color video cameras (Chapter 9). An updated and expanded Chapter 10 covers video monitors, projectors, and printers. Chapter 11 reviews devices for recording the video signal by both analog and digital means and includes advice on creating, editing, and presenting video data. An overview of digital image processing relevant to microscopy is presented in Chapter 12. The last chapter, Chapter 13, deals with system integration, including component selection, setup, troubleshooting, and computer interfacing. Principles of perfusion chamber design and temperature regulation have also been included. The chapter ends with selected examples of complex video microscopy systems that push performance to the present-day limits of the field. Updated appendixes include a Glossary and a List of Manufacturers.

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Kenneth R. Spring

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The rapid development of video microscopy would not have been possible without the willing and helpful participation by many commercial organizations, many of whom are listed above. Although trademarks are not designated in the text, it should be pointed out that the following are registered trademarks or copyrights of the indicated companies: Chalnicon, Toshiba; Image-I, MetaMorph, and MetaFluor, Universal Imaging Corporation; Kimwipe and Kleenex, Kimberly-Clark; Newvicon, Matsushita; Plumbicon, Philips-Amperex; Saticon, Hitachi; Scotch Cover-Up Tape, 3M Company; SIT, ST Vidicon, Ultricon, and Ultricon II, RCA; Videotherm, ISI Group.

We are grateful to these companies, and others, for permission to reproduce illustrations from their publications and produce specification sheets. In this regard, extensive use has been made of the RCA *Electro-Optics Handbook* (now *Burle/Electro-Optics Handbook*) and the Conrac *Raster Graphics Handbook.*

We are grateful to Dr. Gordon W. Ellis of the University of Pennsylvania, Dr. Rudolf

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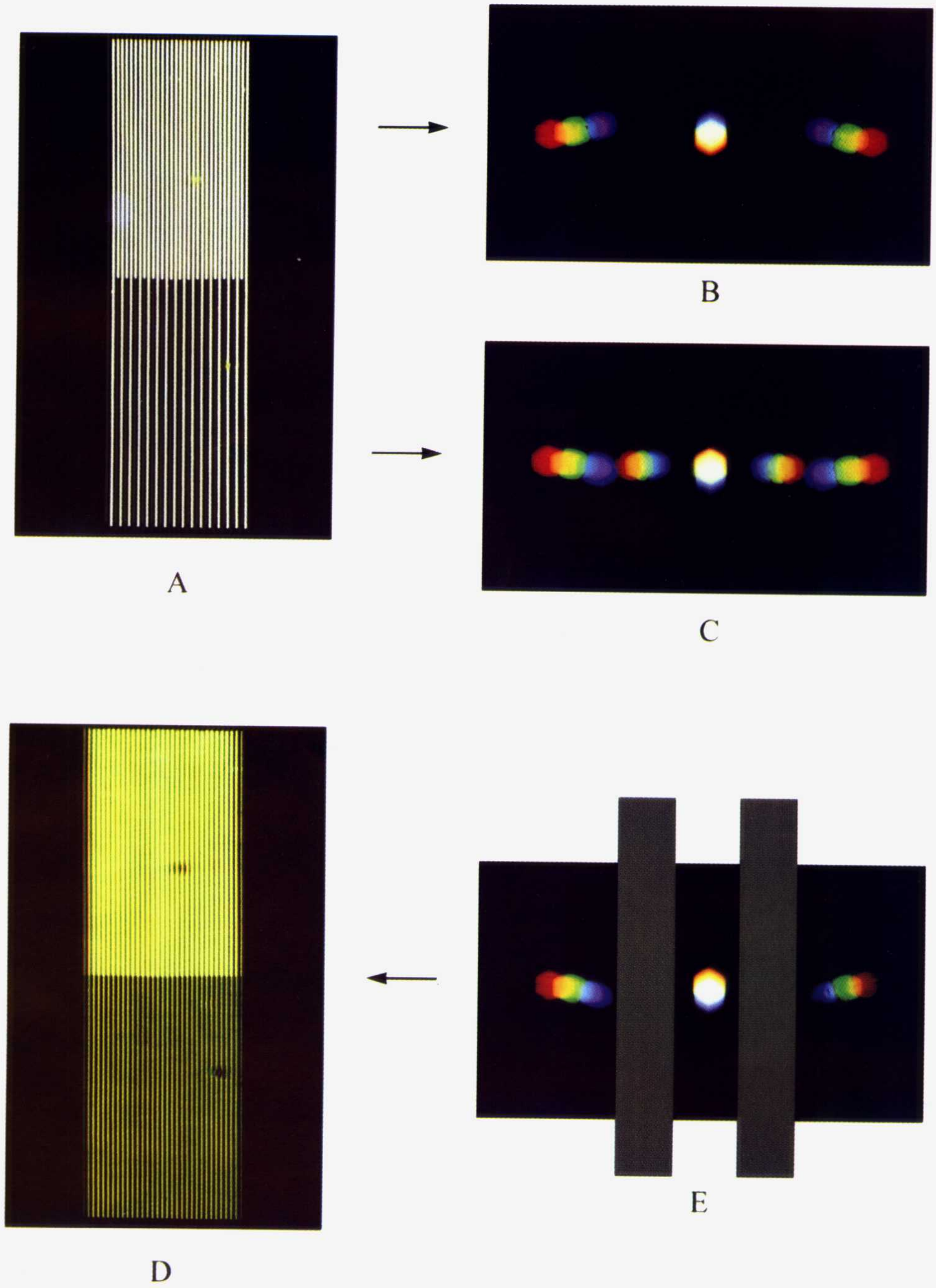
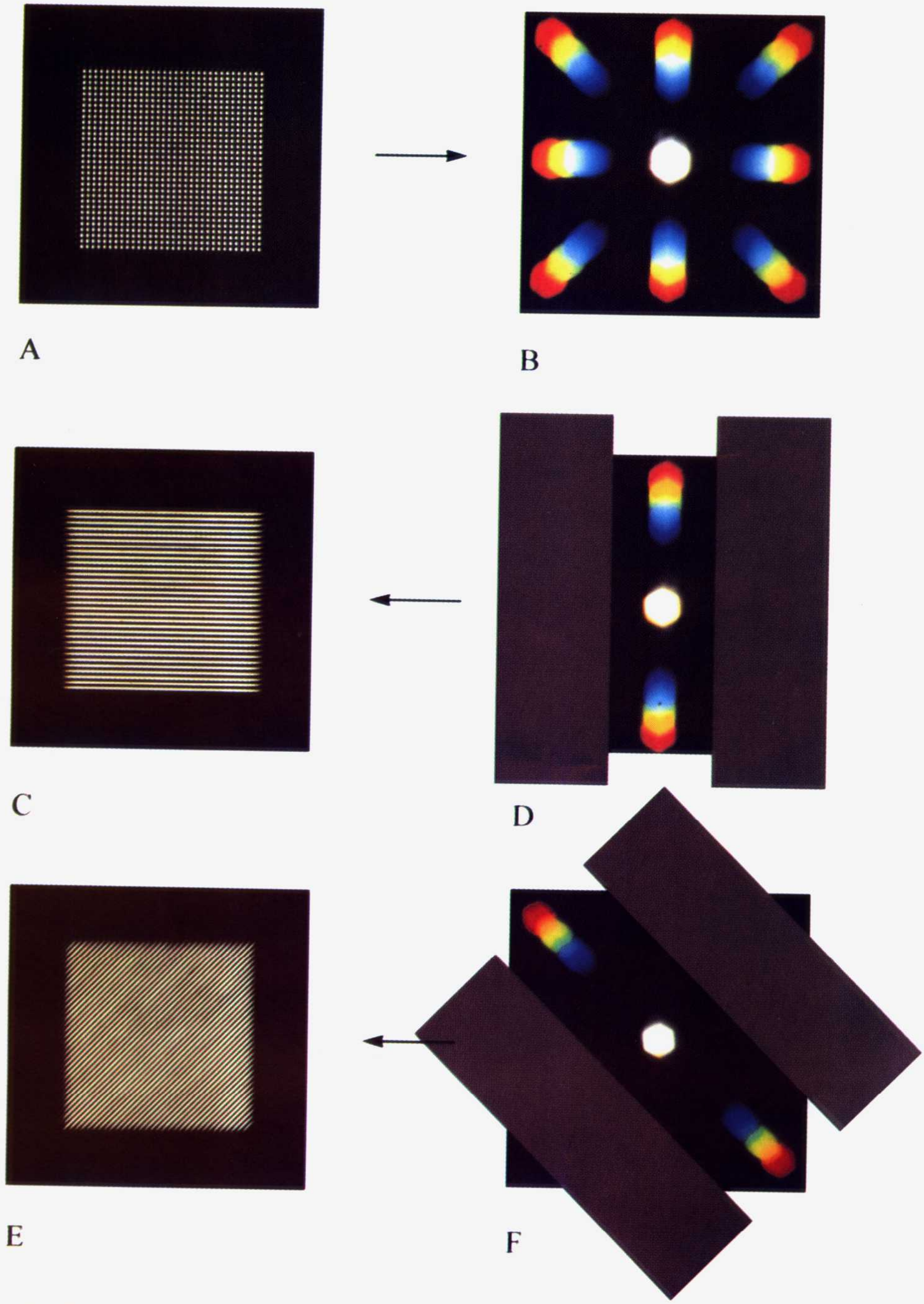
S.I., K.R.S.

*Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not reflect the views of the National Science Foundation or the National Institutes of Health.

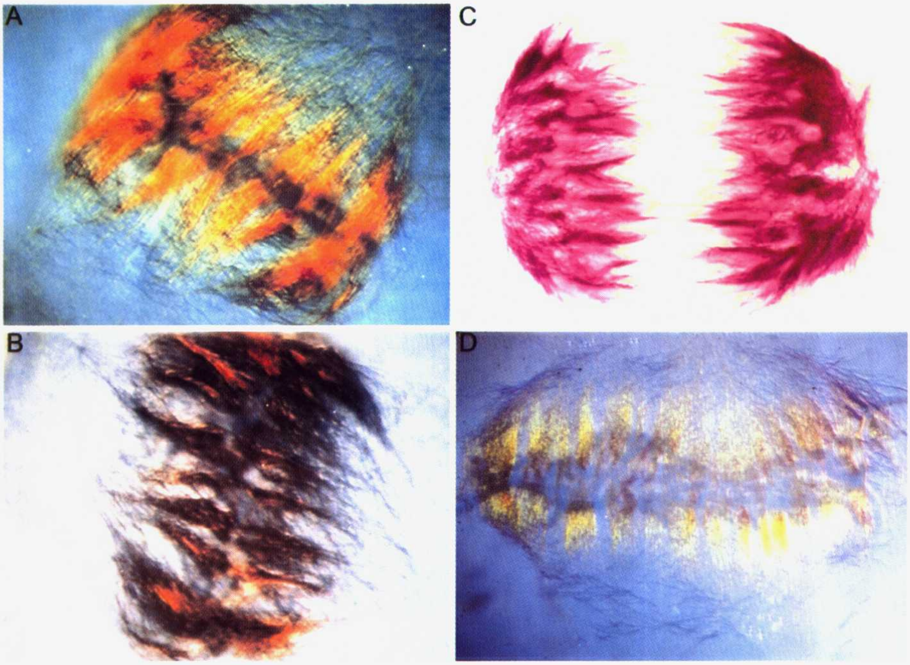
Color Plates



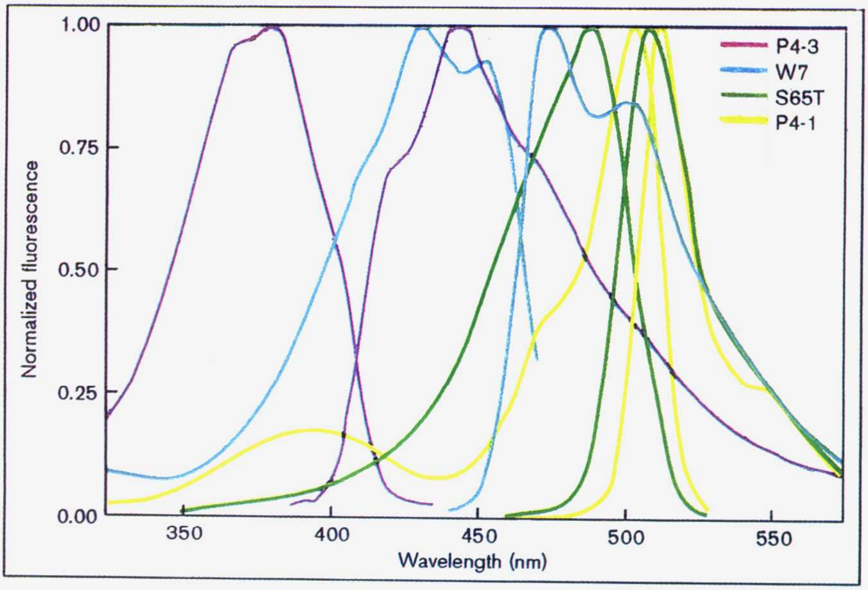
COLOR PLATE I (Figure 2-22). Orthoscopic and conoscopic images (actual photographs) of periodic gratings. (A) Orthoscopic image of fine grating (*top*) and grating with half the spatial frequency (*bottom*). (B, C) Corresponding diffraction patterns seen conoscopically at the back aperture of the objective lens. (D) Same test specimen as in panel A, but viewed through objective lens with masks (spatial filters, panel E) that eliminated the first-order diffraction patterns shown in panel C. Note that the coarser grating now appears as though it has twice the spatial frequency of the specimen itself. (See also Color Plate II and Fig. 2-24.) (Modified after slides kindly loaned by Ernst Keller of Carl Zeiss, Inc.)

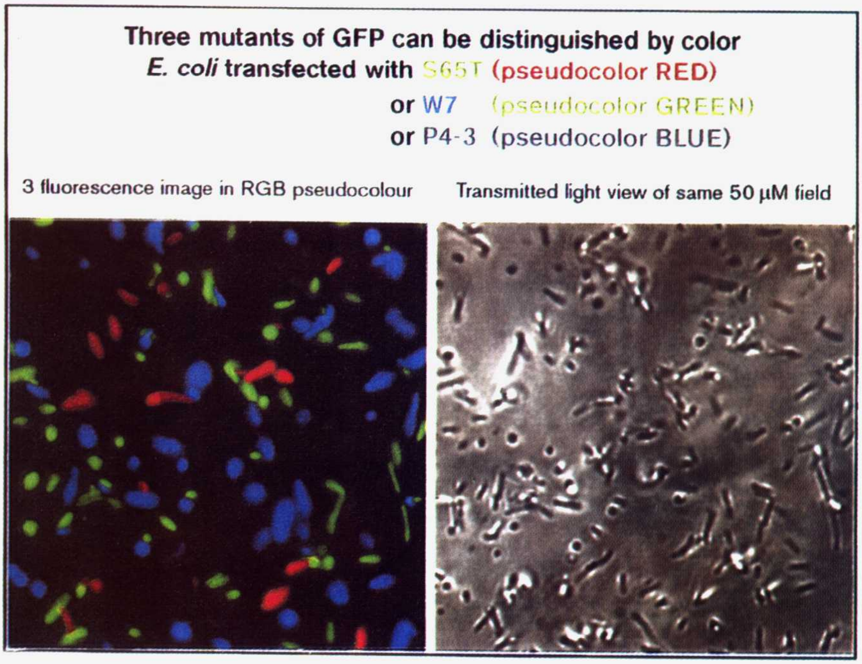


COLOR PLATE II (Figure 2-24). Orthoscopic and conoscopic images (actual photographs) of test target with orthogonally arranged dots. (A) Orthoscopic image viewed through the full aperture of the objective lens. (B) Corresponding diffraction pattern seen conoscopically at the back aperture of the objective lens. (C, E) Same test specimen as in panel A but viewed through objective lens with masks that eliminated selected diffraction patterns as shown in panels D and F. (See also Color Plate I and Fig. 2-22.) (Modified after slides kindly loaned by Ernst Keller of Carl Zeiss, Inc.)

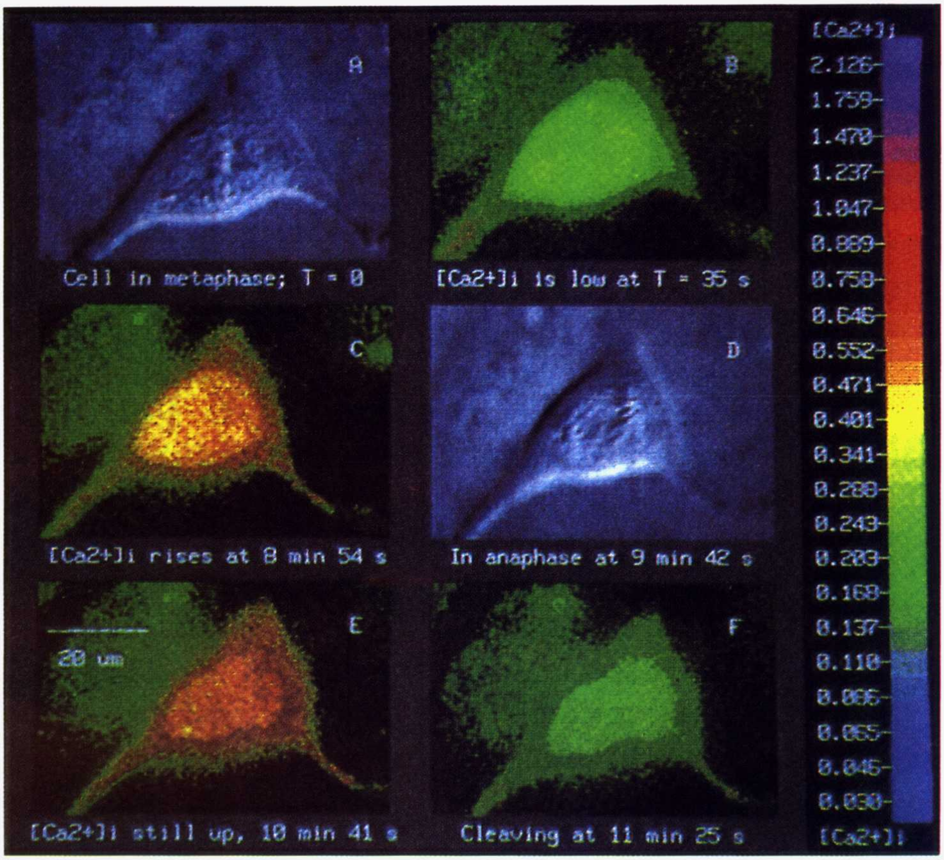


COLOR PLATE III. Polarization color of microtubules in dividing *Haemanthus* endosperm cell. The microtubules were decorated with 5-nm-diameter immunogold. (A, B) Cell in metaphase. Orientation of the stage of the polarizing microscope (illuminated with a quartz halogen bulb) was changed, while the settings of the λ/18 compensator, and the polarizer and analyzer (which were off-crossed by a few degrees), were kept constant. (C) A cell in late anaphase in bright-field microscopy. The microtubules appear in the brick red color characteristic of colloidal gold. (D) A flatter cell in anaphase at somewhat different compensator and polar settings. Depending on orientation, microtubules are visible in different colors, ranging from gold to purple. See comments at the end of Section 2.6.5.

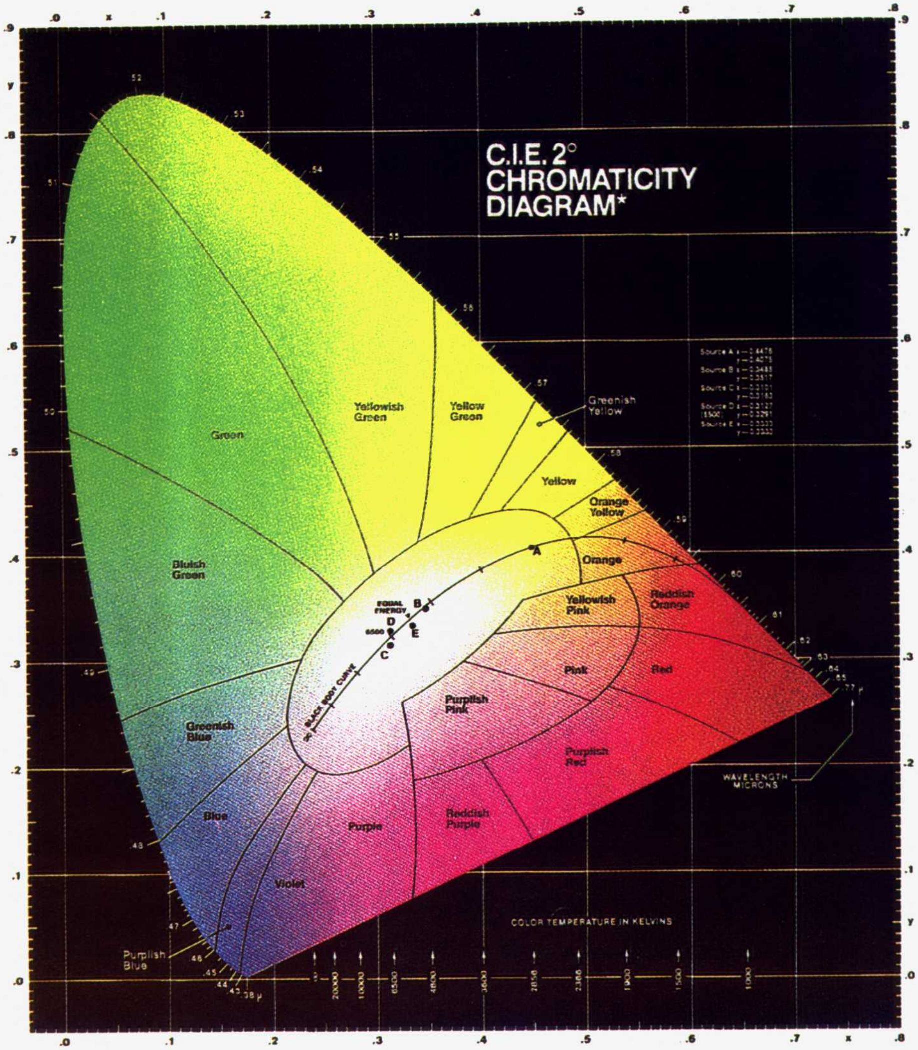




COLOR PLATE IV (Figure 2-67). *Top*: Excitation and emission spectra of different green fluorescent protein (GFP) mutants. *Lower left*: Mixed *E. coli* populations, each transfected with a cDNA encoding the P4-3, W7, or S65T mutant. The three mutants of GFP can be clearly distinguished by their pseudocolor. The red, green, and blue pseudocolors were applied to the fluorescence images acquired with three different filter sets, after which the images were superimposed. *Lower right*: The same 50-μm square area in bright-field microscopy. (From Heim and Tsien, 1996; photos courtesy of Dr. Roger Tsien.)



COLOR PLATE V. Fluctuation of $[Ca^{2+}]$ (cytoplasm-free calcium ion concentration) observed by ratio imaging at mitosis in a PtK_1 cell (Section 2.8.6). (A, D) DIC images in metaphase and anaphase. In panel B (taken 35 sec after panel A) and panel F (as the cell enters cleavage), $[Ca^{2+}]_i$ is uniformly low throughout the cell. (C) The rise, and (E) the (usually prolonged) decay of $[Ca^{2+}]_i$. The ratio-imaging dye, Fura-2, was applied as a membrane permeant ester that becomes hydrolyzed by the esterases inside the cell. To the right is a pseudocolor scale for $[Ca^{2+}]_i$ concentration. (From Tsien and Poenie, 1986.)



COLOR PLATE VI (Small inset in Fig. 4-19). The 1931 CIE Chromaticity Diagram shown schematically in Fig. 4-18. The 1976 version (the larger chromaticity diagram shown in Fig. 4-19) better reflects the responses of the average human visual system to light of different hues. (See Miller, 1985.) (Courtesy of Photo Research, Burbank, California.)

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