BIOLOGICAL MICROIRRADIATION

Classical and Laser Sources

MICHAEL W. BERNS

BIOLOGICAL MICROIRRADIATION

Classical and Laser Sources

Michael W. Berns

University of California
Irvine, California

Prentice-Hall, Inc., Englewood Cliffs, N. J.

Library of Congress Cataloging in Publication Data

Berns, Michael W

Biological microirradiation.

(Biological techniques series)

Bibliography: p.

1. Radiobiology—Technique. 2. Irradiation.

I. Title. DNLM: 1. Cells—Radiation effects.

2. Lasers. 3. Radiobiology. QH652 B531b 1974

QH652.B46 574.1'915 73-18329

ISBN 0-13-077032-9

PRENTICE-HALL INTERNATIONAL, INC., *London*
PRENTICE-HALL OF AUSTRALIA, PTY. LTD., *Sydney*
PRENTICE-HALL OF CANADA, LTD., *Toronto*
PRENTICE-HALL OF INDIA PRIVATE LIMITED, *New Delhi*
PRENTICE-HALL OF JAPAN, INC., *Toyko*

10 9 8 7 6 5 4 3 2 1

Printed in the United States of America

PREFACE

The motivating factor in writing this book was the general misconception that exists with respect to microbeams and their application. It is not uncommon for investigators to be openly pessimistic about the use of microbeams in the quest for answers to their problems. On the other hand, it is not uncommon to find investigators who believe that the microbeam can answer all of their questions. Both conceptions are extremes and result from a general misunderstanding about the technique. This can stem from a poor understanding of radiological principles, naiveté with respect to the complexity *and* simplicity of the apparatus, a lack of familiarity with the past procedures, or even a poor understanding of the biological system being investigated.

This book is an attempt to place all of these factors in perspective. The initial chapters present some basic principles of radiation biology, equipment design and operation, and inherent limitations of the technique. Subsequent chapters treat biological microirradiation in both a historical and current context. A wide variety of experiments are discussed in order to provide the reader with enough background so that he/she can judge for himself/herself whether or not the microbeam approach stands a reasonable chance of being successful.

This book is designed so as to provide all the information necessary for the investigator who is considering using the approach in the laboratory. In addition it provides all the basic principles involved in microirradiation as well as a rather extensive review of the literature. This book

should serve to introduce the student and the researcher to a biological approach that has been used since 1912 and which was, and still is, contributing to scientific progress in a large number of biological disciplines.

I am deeply indebted to those colleagues who personally encouraged and motivated me towards an academic career. Specifically, William T. Keeton and Lowell D. Uhler provided me with the impetus and support necessary to establish and pursue my goals. I am particularly grateful to Donald E. Rounds for providing an environment in which I could learn cell culture procedures and further develop my interests in employing lasers for partial cell irradiation. Particular thanks are given to Drs. Marcel Bessis, Giuiliana Moreno, and Christian Salet for the generous supply of photographs and illustrations that are contained in this manuscript. In addition I would like to thank Drs. Raymond Zirkle, Henry Harris, Robert Perry, Philip Dendy, Norman Saks, Joe Griffin, R. Storb, and J. Daniel for kindly providing original copies of illustrations.

MICHAEL W. BERNS

Irvine, California

CONTENTS

Introduction

ONE

In 1912 the Russian Tchakhotine built the first microirradiation device for the purpose of destroying small regions of single cells, organisms, or embryos. From the years 1912 to 1959 this prolific investigator employed his ultraviolet microbeam to irradiate organisms such as sea urchin eggs, bivalve molluscs, numerous types of protozoans, spermatozoa, algae, etc. The types of biological problems studied varied from basic developmental biology (fertilization, determination, and parthenogenesis) to problems of cell physiology (such as contractility and cell motility) and cell divison. It is fair to say that if there ever were a founder of a science or a particular scientific method of approach, Tchakhotine would have to be designated the father of microirradiation.

From his early work employing ultraviolet microirradiation for the study of basic biological processes, several sub-applications were derived. Microirradiation, for example, has been employed using numerous radiation sources, generally with three purposes in mind: (1) to elucidate cell or organism function by altering or destroying a partial region of the target system (this was the major approach as originally employed by Tchakhotine); (2) for defining the radiosensitivity of various subcellular regions and structures (an approach primarily of a radiobiological nature); and (3), strictly as an

analytical tool for reading-out quantitative and qualitative cellular information.

When one consults the four major biological reviews of microirradiation (Zirkle, 1957; Moreno, Lutz, and Bessis, 1969; Smith, 1964; and Berns and Salet, 1972), it becomes obvious that in terms of published research and, presumably, interest, the vast majority of investigators have been employing microirradiation to study biological function rather than radiobiology, or as an analytical biological tool. However, this statement may be somewhat misleading, because literally hundreds of studies have been performed employing microspectrophotometry and microfluorimetry with focused ultraviolet radiation. In these studies ultraviolet microirradiation is employed in an analytical way to generate quantitative and qualitative information about cells and cell structures. In these studies the cells are often dead, therefore, cell survival for even a short time is not of interest (Caspersson, 1968; Glubrecht, 1958, 1960, 1963).

Similarly, Glick (1969) has employed a focused laser to read-out the low levels of elements within single cells by flash spectroscopy. By analyzing the spectrum of the incandescent light emitted from a vaporized sample, it has been possible to make elemental determinations as small as 10^{-9} g. The fact that the people who have written the reviews on microbeams (myself included) employ microirradiation to study biological function is probably why *analytical* microirradiation has been generally ignored. In addition, the analytical microirradiation systems, such as a microspectrophotometer, have evolved to such a refined level of sophistication and automation, that an investigator need only place his specimen under the system and make the appropriate measurements. Reviewing the hundreds of studies employing these techniques would be quite impossible. Consequently, in this volume I will follow the convention of treating biological microirradiation as an approach to study biological function by *partial irradiation* and also as a method to study radiosensitivity of biological systems, while at least acknowledging the existence of other microirradiation-like systems.

I should like to address myself for a moment to terminology. As the title of this book implies, the subject is really *partial irradiation*. The use of the phrases *partial cell irradiation* (PCI) and

microbeams has been purposely avoided. Indeed numerous studies involving partial irradiation of whole multicellular organisms, embryos, and tissues have been performed. Truly, these are not studies in partial *cell* irradiation. Similarly, numerous partial irradiation devices are not microbeams. Large macrobeams that employ partial shielding of the target are not microbeams. The microsource of polonium alpha particles (Munro, 1957) is not a microbeam. Therefore, the more general, all-inclusive phrases *partial irradiation of biological systems* and *biological microirradiation* are employed extensively in this book.

In writing a volume on a biological technique, one can devote much space to a description of methodology. However, for such a work to be useful to the large number of diverse scientists, one must deal with the kinds of problems that one might encounter and the types of questions that can be answered. I have devoted approximately half of this book to a description of methodology, equipment, and basic radiation biology, and the remaining half to a discussion of the biological questions and answers approached by the technique. As a result, numerous studies are not mentioned at all, some studies are discussed only briefly, and others are discussed in great detail. I have attempted to select a very diverse group of investigations for discussion. By doing this, it is my hope that any biologist picking up this book can rapidly determine whether or not his questions can be answered.

Finally, as a biologist, I find it particularly difficult to write a volume dealing only with a technique. Therefore, I have attempted throughout the second half of this volume to deal at length with those microirradiation studies that have contributed to the solution, or partial understanding, of significant biological questions.

Instrumentation
TWO

There are as many different microbeam devices as there are investigators using them. As a general rule each microbeamist designs and builds an instrument suited to his or her particular needs. These needs are reflected in the type of radiation employed, the nature of the material irradiated, and the kinds of questions being asked. A discussion of the variety of devices would take an entire book. However, the various methods of PCI can be lumped into four general categories, of which all employ a radiation source external to the cell or organism.

A. MACROBEAM FOCUSED TO A MICROBEAM

One of the most widely employed methods of microbeam irradiation is to take a polychromatic source of UV light, separate the desired wavelength, and focus it to a small spot within the target specimen. This was the first method of PCI reported by the Russian Tchakhotine in 1912 and subsequently refined and described by him in 1935. This system is diagrammed in Fig. 2-1. Light from a magnesium spark is collected by a quartz lens and directed into two quartz prisms arranged to refract the 2800 Å light down the optical

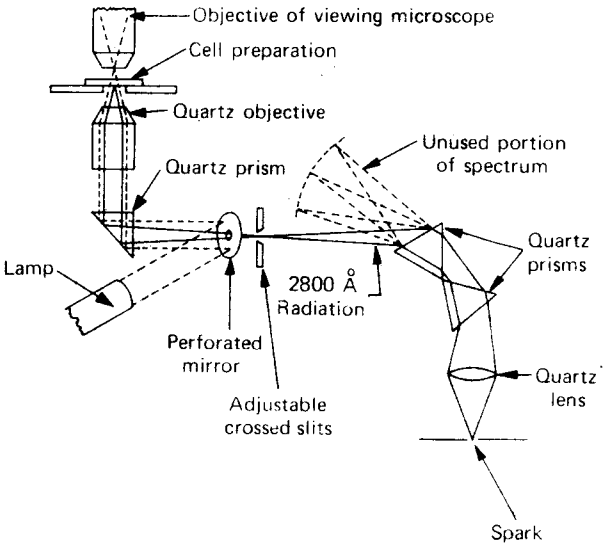

Fig. 2-1. Tchakhotine's 2800 Å microbeam device. The 2800 Å light is designated by an unbroken line; the unused portion of the spark spectrum and the visible viewing light is designated by a broken line. (From Zirkle, 1957.)

system. The radiation passes through a rectangular aperture formed by two adjustable slits and through a perforated mirror. It is next reflected by a quartz prism into the quartz objective that focuses the beam to a small spot within the cell. The cell is viewed with a regular compound microscope using visible substage transmitted illumination that is reflected off the perforated mirror, quartz prism, and through the quartz objective, which acts as the condenser. Aiming of the microbeam is accomplished by focusing the UV light into a drop of fluorescent dye (fluorescein) that is placed on a microscope slide. The tip of a movable pointer in the ocular is located at the center of the *hot spot* of fluorescence. The fluorescent specimen is removed and replaced with the target cell, which is moved until the part selected for irradiation is located under the tip of the pointer.

Tchakhotine's method of focused UV microirradiation was the primary microbeam technique until 1954 when Uretz and co-workers at the University of Chicago developed the reflecting objective. Their system is diagrammed in Fig. 2-2. The reflecting objective is

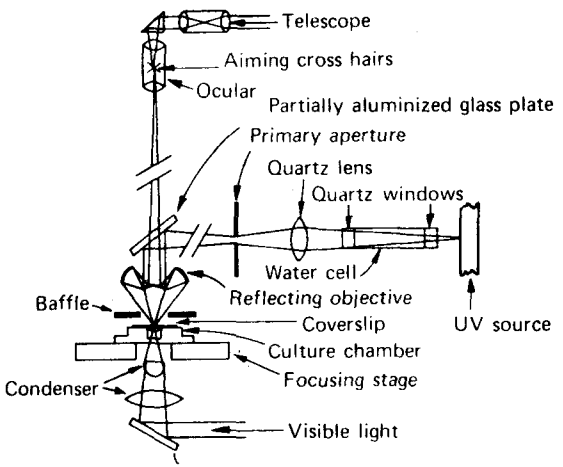

Fig. 2-2. Reflecting objective ultraviolet microbeam. (From Zirkle, 1957.)

utilized both for focusing the incident radiation and as the observation objective. A standard substage condenser and light source provide illumination. The UV light is collected by a quartz lens after traveling through a water cell and is directed into an adjustable aperture. It then passes through the aperture and is reflected into the objective by a partially aluminized mirror (coated to transmit visible light; e.g., the specimen image). The UV image of the aperture (microimage) is focused onto the same plane as the viewing microscope by adjusting the distance between the aperture and the objective. The microspot of UV light can be located directly under the cross hair in the ocular by moving the microaperture in a plane perpendicular to the beam axis. The specimen is placed under the microscope and the area to be irradiated is moved under the crosshair. The opinion expressed by Zirkle in his 1957 review was that the reflecting objective provides an apparatus that "is simpler, much less expensive, more accurately aimed, more flexible in use, wider in application, and much less demanding of operational labor than Tchakhotine's."[1] However, it must be pointed out that the reflecting objective does have a small numerical aperture; it is difficult, but possible, to incorporate phase into the system; and it

[1] R. E. Zirkle, "Partial Cell Irradiation," *Adv. Biol. Med. Phys.*, 5 (1957), 122.

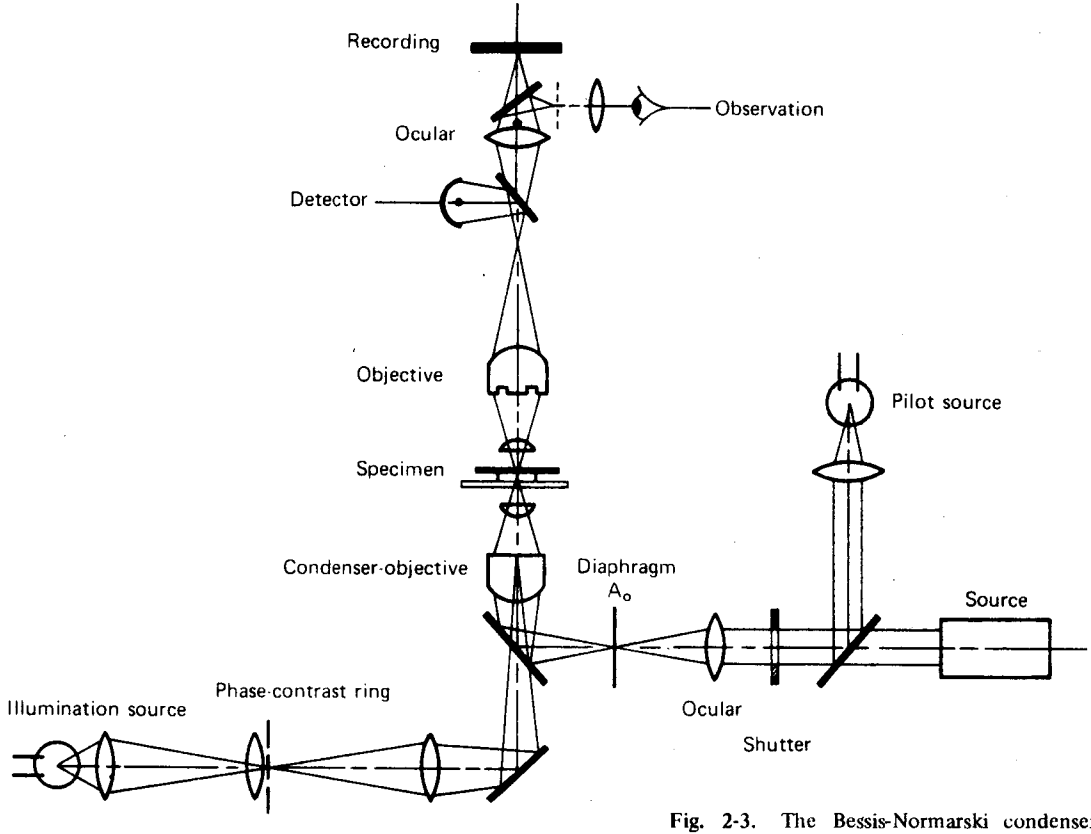

Fig. 2-3. The Bessis-Normarski condenser-objective UV microbeam. (Bessis and Nomarski, 1959.)

cannot focus light to a spot less than several micrometers (2-10μm) in diameter.

The next major advance in UV microbeam irradiation was the development of the achromatically corrected (2310-7000 Å) Zeiss Ultrafluar objective. The development of this objective permitted the return to transmission phase objectives of high magnification and large numerical aperture. Bessis and Nomarski (1959) developed one of the first systems (Fig. 2-3). An image of the aperture (A_0 is projected through a substage ultrafluar objective-condenser onto the specimen. It is claimed that by changing the diameter of the aperture, focal spots varying in diameter between 0.2 and 10μm can be produced. The design of the Bessis-Nomarski system returns to

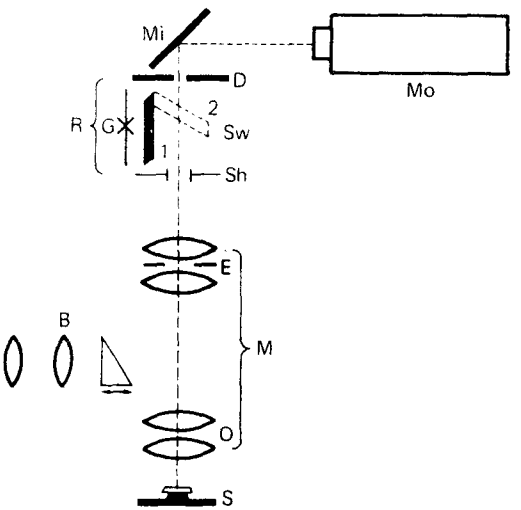

Fig. 2-4. The UV optical path of the reflex camera microbeam. When the swinging mirror (*Sw*) of the reflex camera (*R*) is in position 1, UV radiation from the monochromator (*Mo*) is projected onto the specimen (*S*) which is covered with a quartz coverslip. The instrument is aimed and focused with visible light from a substage condenser by moving the swinging mirror into position 2 so that an image of the specimen is projected on the ground glass screen (*G*) and brought into coincidence with the cross. First surface aluminized mirror. *Mi*; field diaphragm, *D* with an aperture which defines the size of the microbeam; shutter, *Sh*; microscope, *M*; ultrafluar eyepiece, *E*; ultra objective, *O*; binocular eyepieces with slide-in prism, *B*. (From Rustad, 1968).

the original two microscope system of Tchakhotine; one for viewing and one for focusing. The ultrafluar objective has permitted construction of several different microbeam systems that utilize the same objective both for viewing and focusing. One of the most recent systems is diagrammed in Fig. 2-4. This unique system utilized a reflex camera in conjunction with a microscope equipped with ultrafluar optics.

In addition to the development of the ultafluar objective, Montgomery and Hundly (1961) developed a unique UV microbeam utilizing *flying-spot* television microscopy (Fig. 2-5). The image of the UV-emitting scanning tube is focused on the object plane using a UV microscope in reverse. This causes a reduction in size. By feeding the UV-emitting tube with a *brightening* pulse so that only a

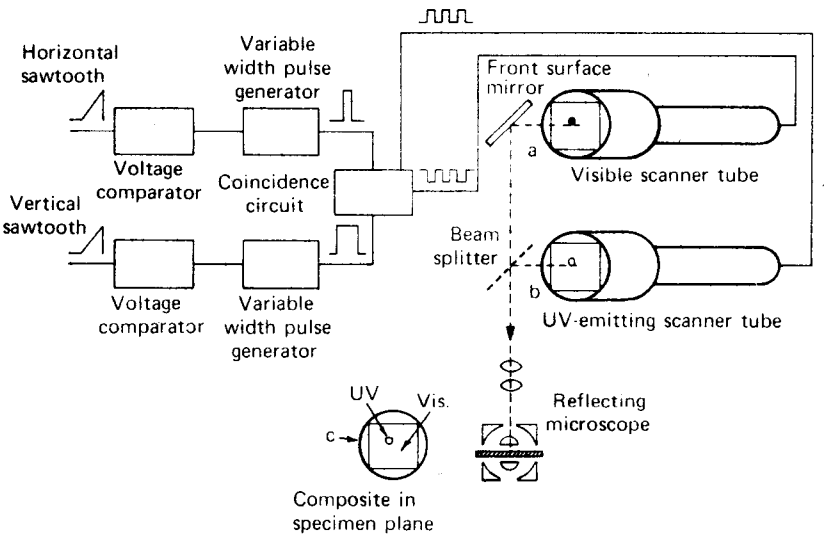

Fig. 2-5. Montgomery's flying-spot television UV microbeam. (From Montgomery and Hundley, 1961.)

small area of its surface emits UV light, the area of the emission may be only a couple of microns in diameter. The low intensity of the emission requires exposure times of several hours.

The impression given so far is that all the macrobeams that are focused to microbeams use UV light. This is not true. Laser light is

routinely focused by objectives. Laser emission can occur anywhere between the far UV and the infrared portions of the electromagnetic spectrum. However, the majority of the laser microbeams have employed emissions in the visible region of the spectrum. Consequently, conventional light microscope optics can be employed readily.

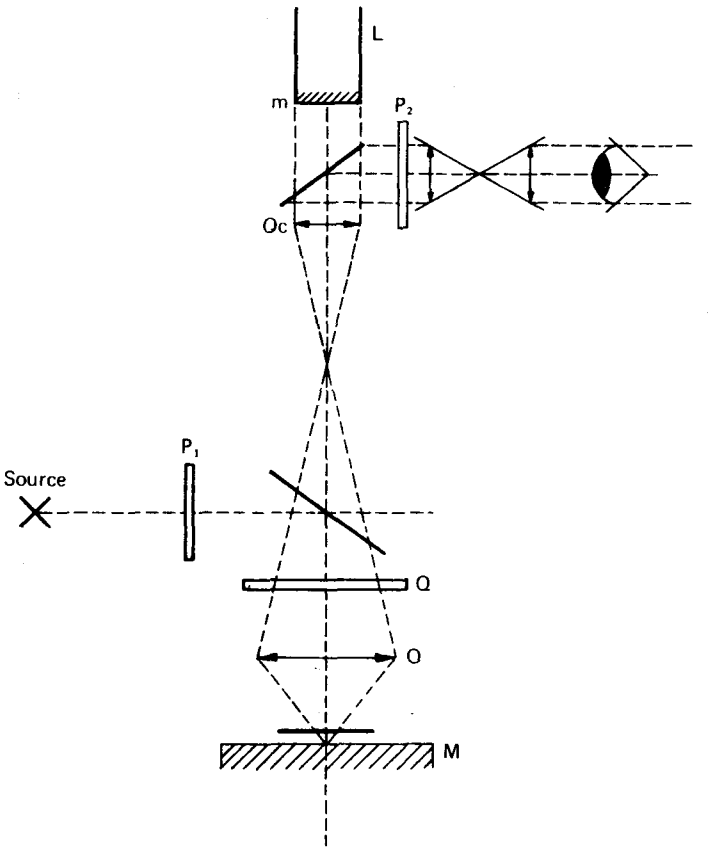

Fig. 2-6. The Bessis et al. ruby laser microbeam: *L*, laser; *M*, dielectric mirror; *M*, exit surface of laser rod; *Oc*, ocular; *O*, objective; *Q*, quarter wave plate; P_1P_2, polarizers. (After Bessis, Gires, Mayer, and Nomarski, 1962.)

The first laser microbeam developed by Bessis and co-workers in 1962 (Fig. 2-6) used ruby rods 3 mm wide and 50 mm long excited by a xenon flash lamp. The laser was mounted above a microscope