MICROMANIPULATION BY LIGHT IN BIOLOGY & MEDICINE

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Micromanipulation by Light in Biology and Medicine

The Laser Microbeam and Optical Tweezers

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Preface

There are probably few people who do not dream of the good old times, when doing science often meant fascination, excitement, even adventure. In our time, doing science involves often technology and, perhaps, even business. But there are still niches where curiosity and fascination have their place. The subject of this book, technological as its title may sound, is one of the fortunate examples. It will report on lasers generating the coldest places in the Universe, and on table top laser microtools which can produce a heat "inferno" as it prevails in the interior of the Sun, or simulate, for specific plant cells, microgravity of the space around our planet Earth. There will be some real surprises for the reader. The applications range from basic studies of the driving forces of cell division (and thus life) via genetic modification of cells (for example, for plant breeding) to medical applications such as blood cell analysis and finally *in vitro* fertilization.

What are these instruments: laser microbeams and optical tweezers? Both are lasers coupled with a fluorescence microscope. The laser microbeam uses a pulsed ultraviolet laser. Light is focused, as well as possible, in space and time, in order to obtain extremely high light intensities – high enough to generate, for a very short instant, extremely hot spots which can be used to cut, fuse or perforate biological material. Laser microbeams have evolved from microbeams with classical light sources which have been used in biology since the beginning of this century. Optical tweezers, on the other hand, use infrared lasers of moderate intensity and involve only little interaction with biological tissue. Their main purpose is to hold microscopic particles solely with the force of light or to measure microscopic forces with incredible precision. In some sense optical tweezers are an extended version of laser cooling of atoms and molecules.

Due to their different working principles, laser microbeams and optical tweezers so far have often been treated separately in the scientific literature. Reports on both microtools in combination are rare. Thus, this book is an attempt to bridge this gap and to use the synergy of both techniques. Interestingly, they are probably the

only tools which allow one to work in the interiors of unopened objects – a truly exciting aspect.

This interdisciplinary book is intended to bridge a second gap: the gap between those who are new to the field (or who just want to learn more about it out of curiosity) and the specialists at the cutting edge of this field of research. Hopefully, the wide range of subjects presented will excite the interest of readers from many branches of science: physicists or chemists who want to learn how the dramatic effects of these tools can be used in biology, and biologists and physicians, who want to learn how the physical effects are generated. Certainly, the readers of this book will be of very different backgrounds. Therefore it is written on different levels. The main text should give the reader an overview. Details, hard stuff and data are presented in boxes, which the reader will wish or need to study in detail only if he or she wants a deeper understanding of the subject. For non-biologists, three sections called «intermezzos» are inserted which give some basic biological or biomedical information. Certainly these sections can not replace good textbooks, but they may give at least a foretaste of the biology and biomedicine described in subsequent chapters and sections. Finally, the information in the Appendix seeks to reduce the number of textbooks required by those who really want to build their own laser microbeam and optical tweezers.

I would like to thank all colleagues who have developed the techniques and have ingeniously used them to solve important questions of science. I hope that I have acknowledged their work properly by citing representative publications. Particularly I would like to thank Alla Margolina Litvin and Steve Block for numerous constructive critical comments.

Jena, 1 August 1998 Karl Otto Greulich

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Introduction: The history of using light as a working tool

Certainly the earliest pioneers of optics realized that sunlight focused through a piece of curved glass can be used to perforate or slice thin pieces of wood or similar materials. Since then it has been known that light of a high power density can be used much like mechanical tools such as knives or scissors. At the beginning of this century, microscopists learned that a powerful conventional light source focused into a microscope could be used to manipulate biological objects. A conventional light source could be focused down to a spot size of a few micrometers. It was immediately clear that the "Strahlenstich", as it was called, was a tool for biologists. Probably the first work using highly focused light to manipulate biological material was that of S. Tschachotin (1912). Fig. 1 is a facsimile of the first page of Tschachotin' s paper.

Die mikroskopische Strahlenstichmethode,
eine Zelloperationsmethode.

Von Dr. Sergeï Tschachotin.

(Vorläufige Mitteilung.)

(Aus der parasitologischen Abteilung (Vorstand: Prof. Dr. Th. v. Wasielewski) des Instituts für Krebsforschung in Heidelberg, Direktor: Prof. Dr. V. Czerny, Exz., und aus dem pharmakologischen Institut der Universität Genua. Vorstand: Prof. Dr. A. Benedicenti.)

Manche Gründe bewegen mich, einer Serie von Arbeiten, die noch nicht ganz abgeschlossen sind, diese vorläufige Mitteilung voranzuschicken.

Den Anstoß zu den zu erwähnenden Untersuchungen gab die sich allmählich immer dringender einstellende Überzeugung, dass wir unsere erfolgreiche experimentelle Methodik, die in den letzten Jahrzehnten zu einem ungeahnten Fortschritt auf allen Gebieten der biologischen Forschung geführt hat, dimensional verfeinern und auf die kleinsten materiellen Einheiten des Lebens, auf die Zelle als Individuum, auszudehnen versuchen müssten.

Wie wir beim Experiment in größerem Maßstabe praktisch-methodisch einen eingreifenden und einen registrierenden, beobachtenden Teil unterscheiden, so würde auch beim Mikroexperiment unser Augenmerk auf die Ausarbeitung erstens der Mikroläsions- und zweitens der Mikroobservationstechnik zu richten sein. Letztere kann bekanntlich subjektiv und objektiv (z. B. Mikrophotographie

Fig. 1: First page of Tschachotin' s paper on the «Strahlenstich» (which, today, could be translated as "microbeam".

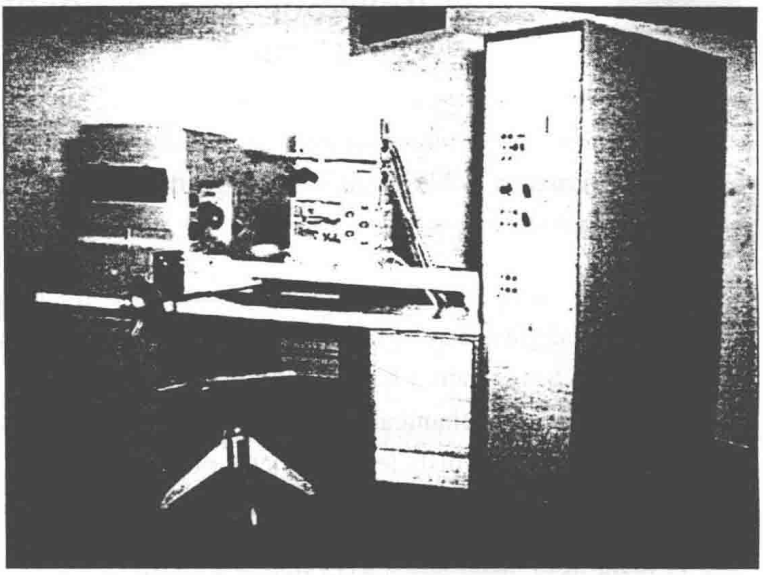

Fig. 2: *LMA 1, the first commercially available microbeam apparatus (Foto: V. Meinel and M. Ludwig, Jena).*

In spite of its age, the "Strahlenstich" is far from being old-fashioned. Whenever accuracies of a few micrometers and moderate power densities are sufficient the classical microbeam is as useful as the laser microbeam.

The latter, using a red ruby laser, became available in 1962 (Bessis et al., 1962), i.e. less than two years after the laser had been invented by Maiman (1960). In 1965, during an industry fair in Leipzig, Carl Zeiss, Jena, presented the LMA1 (laser microbeam analyzer) based on a ruby laser with microsecond pulses of a few millijoules each. Its spot size was approximately 2 micrometers.

In the early years, several groups had sporadically tried using the laser microbeam but then moved on to other topics. Since 1969 on, there has been a continuity in the use of laser microbeams. Michael Berns of the University of California at Irvine, published first papers on the use of laser microbeams in cell biology (Berns et al., 1969). He has continued this work with ever-increasing sophistication. A number of chapters of this book will be devoted to his studies.

Before the laser microbeam came of age, its application was split into two different directions. One direction was governed by work performed by Berns and his group (see, for example, Berns, 1974 or Berns et al., 1981). The second direction developed into molecular analysis: Laser ablation was combined with mass spec-

trometry and finally developed into laser mass analysis (LAMMA) and matrix assisted laser desorption (MALDI) (Hillenkamp et al. 1975). A variant of this is fluorescence recovery after photobleaching (FRAP, Peters, 1986). Here, fluorescent molecules on the surface of cells are bleached by highly focused laser pulses and the flow of molecules on the cell membrane is observed via fluorescence recovery. Today, the field of applications of laser microablation is so wide that not all aspects can be treated in a text the size of the present book. Therefore, reluctantly, its scope has to be limited, and LAMMA, MALDI and FRAP will not be covered in spite of the enormous impact they have.

The development of the optical tweezers is uniquely connected to the work of Arthur Ashkin from the AT&T Bell Labs in Holmdel, N J. (Ashkin, 1970). In the early seventies it was realized that the speed of an atom or a molecule can be reduced when a laser of a suitable color is directed into the direction of motion of the molecule. This phenomenon has been termed laser cooling since the speed of a molecule is related to its temperature, and it gained the 1997 Nobel prize in physics for S. Chu, W. Phillips and C. Cohen Tannoudji. Later it was shown that particles of the size of one micrometer, such as polystyrene beads, could also be manipulated by laser light. In that experiment, a focused laser was required and the particles were balanced on a focused laser beam, similar to a ping pong ball balanced on a jet of water. A significant step was the use of gradient forces which, in contrast to light pressure, pull dielectric particles into the focus of a laser, i.e. they can also be moved against the direction light propagation. The set up for such an experiment was named "single beam optical trap" or "optical tweezers" (Ashkin et al., 1986). Even today, both expressions are used in the scientific community and will be used interchangeably throughout this book.

Until 1987 the single beam gradient trap was used successfully only with nonliving material. Then, Ashkin's group published two papers changing the field dramatically. The breakthrough for the use of the optical trap in biology came when viruses and bacteria were first trapped with a green argon ion laser (Ashkin and Dziedzic, 1987) and subsequently an infrared NdYAG laser was used for manipulation of whole cells (Ashkin et al., 1987). This change from green to infrared was an important step since infrared light with a wavelength of about one micrometer is only weakly absorbed by biological material. Fragile mammalian cells survived this laser treatment. This was the beginning of optical trapping in cell biology. The end of the decade was the time of pioneers in optical trapping, most notably among

them were the groups of Todor Buican (Buican 1987) and Steven Block (Block 1989) and of course, Arthur Ashkin.

In 1989 the microbeam field and the optical trapping field were merged by the first combination of laser microbeams and optical tweezers (Greulich et al., 1989, Greulich et al 1990). From then on complete micromanipulation by light was possible with one single piece of equipment. The last decade of this millenium is now witnessing a dramatic expansion of the field. Micromanipulation by light may be on the way to becoming a standard tool for many fields of science.

Box 1: Milestones in the development of laser microbeams and optical tweezers

1912	S. Tschachotin publishes the first work on the microbeam using a thermal light source
1917	A. Einstein develops the theoretical foundations of lasers
1961	M. Maiman presents the first laser
1962	Bessis present the first laser microbeam
1969	Berns develops microbeams into a standard tool for many fields of biology
1968/70	Letokhov and Ashkin publish the principles of using light pressure to manipulate atoms, molecules and microscopic particles
1986	The single beam gradient trap (optical tweezers)
1987	Ashkin's papers on the use of *infrared* gradient traps with living objects
1989	First quantitative force measurements with optical tweezers by the groups of Ashkin and Block
1989	Combination of the laser microbeam and the optical tweezers
1989–now	Complete micromanipulation by light
1997	Physics Nobel Prize for cooling atoms and molecules by light

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