Optical trapping for chromosome manipulation: a wavelength dependence of induced chromosome bridges

Ivan A. Vorobjev,* Hong Liang, William H. Wright, and Michael W. Berns

Beckman Laser Institute and Medical Clinic, University of California, Irvine, California 92715, USA; and A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia

ABSTRACT Using a tunable titanium-sapphire laser, we have compared different wavelengths (from 700 to 840 nm) for their utility in optical trapping of chromosomes in mitotic rat kangaroo *Potorous tridactylus* (PtK_2) cells. It was found that irradiation with a near-infrared light induces the sticking together of chromosome shoulders. The attached chromatids failed to separate, or separated with significant delay and formed a chromosome bridge during anaphase. Using this bridge (and induced c-mitosis) as a reference, we compared the action of different wavelengths (from 700 to 840 nm). Chromosomes were irradiated at metaphase and the cells were observed until the end of cytokinesis. Chromosomes were irradiated for different periods of time, using 130 mW of power at the objective focal plane. The biological responses observed after optical trapping were: (1) normal cell division, (2) formation of a temporary chromosome bridge, (3) formation of a permanent chromosome bridge, (4) complete blockage of chromosome separation (c-mitosis). The chromosomes were found to have a maximal sensitivity to 760–765 nm light and minimal sensitivity to 700 and 800–820 nm light. Cells with chromosomes irradiated for a long time, using wavelength 760–765 nm, generally were incapable of going through anaphase and remained in c-mitosis. We conclude that the optimal wavelengths for optical trapping are 700 and 800–820 nm.

INTRODUCTION

Laser microbeams have been used for intracellular manipulations for over 20 years (for reviews see references 3 and 5). Recently this application has been expanded by the introduction of optical trapping "tweezers" (1, 2) (for reviews see references 5 and 7). The first optical tweezers employed the blue-green argon ion laser (1). Subsequent systems used the infrared neodymium YAG laser at 1.06 μm wavelength (2–4), because higher absorption by natural chromophores at the blue-green wavelength was detrimental.

With the help of a force-generating near-infrared laser beam it was possible to move chromosomes in lysed cells (4–5) and sometimes affect their motion in living cells (see reference 8). However, even in that study, some limitations of the optical trapping caused by water absorption at 1.06 μm were found. The tunable titanium–sapphire laser (700–1000 nm) seems to have some advantages in optical trapping, because it emits wavelengths in a range in between that of the blue-green argon and infrared YAG (6). If optical tweezers are going to have a major role as a tool in experimental cell biology, their limitations must clearly be defined, and the optimal wavelengths and energy parameters must be determined (7).

The goal of the present study is to understand the biological limitations of different wavelengths of optical tweezers on chromosome manipulations using the tunable titanium–sapphire (Ti/sapphire) laser. Mitotic chromosomes were chosen as the in vitro model, because their large dimensions make them easy to observe and manipulate (8), and because mitotic cells are extremely sensitive to any kind of perturbation.

Address correspondence to Dr. Michael W. Berns, Beckman Laser Institute and Medical Clinic, Department of Surgery, University of California, 1002 Health Sciences Road East, Irvine, CA 92715, USA.

MATERIALS AND METHODS

Cells of the rat kangaroo (*Potorous tridactylus*) (PtK_2) were grown on glass coverslips (0.17 mm thickness) mounted in Rose tissue culture chambers as described previously (4).

Optical trapping experiments were carried out on upright Zeiss photomicroscope equipped with a Neofluar 100/1.3 oil immersion objective lens (Carl Zeiss, Inc., Thornwood, NY). Constant temperature at 37°C on the microscopic stage was maintained with an air-curtain incubator. Data were recorded using a Newvicon camera (DAGE-MTI, Inc., Michigan City, IN) mounted on a trinocular photographic port of the microscope and interfaced with a time-lapse video-cassette recorder (Model 6030; Panasonic Corp., Secaucus, NJ). Photography utilized Kodak Plus-X film (Eastman Kodak Co., Rochester, NY) and the internal camera of the microscope.

The laser beam from a titanium–sapphire tunable laser pumped with an argon ion laser (Model 899; Coherent Inc., Palo Alto, CA) was reflected into the microscope off a dichroic beamsplitter ($R > 95\%$ at $\lambda > 700$ nm; $T > 90\%$ at $\lambda < 650$ nm) as described previously (6–8). The laser beam was focused to a spot with a diameter near the diffraction limit (approx. 0.5 μm) and beam power was measured in the focal plane of the objective, using a Coherent power meter (Model 210) detector that was placed directly under the objective (6). Flat mitotic cells were selected and moved to the position in the field of view where the trapping beam would be when activated. Chromosomes for irradiation were chosen when two chromatids were lying side by side in the same focal plane, as described in the next section.

RESULTS

Morphological observations

All the cells reported in this study were exposed to the optical trap while in metaphase. The chromosomes were aligned along a well organized metaphase plate, and both spindle poles were clearly evident. In all cases reported here, the beam was focused on two chromatids of one of the large chromosomes by positioning the target chromosome on the TV screen under a crosshair that corresponds to the focal point of the trapping beam. The chro-

0006-3495/93/02/533/06 $2.00

TABLE 1 **Results of chromosome irradiation with different wavelengths**

Exposure time:	Results of chromosome trapping:	Wavelength (*nm*)													
		700	720	740	745	750	755	760	765	770	775	780	800	820	840
1–5 min	C-mitosis							2							
	Chromosome bridge	25	5								4	5	19	6	5
	Normal division	15										1	9	4	
5–30 s	C-mitosis							9	3						
	Chromosome bridge		4	12	12	9	11	8	2	6	6	6	1	1	7
	Normal division	1	5	1	3	3	2			1	2	4	2	2	5
0.3–2	Chromosome bridge				1	1	4	21	10	7					
	Normal division			4	4	2	5		3	3	3				
Subtotal number of irradiated cells		41	14	17	20	15	22	40	18	17	15	16	31	13	17
% of any abnormalities		61	64	71	65	67	68	100	83	76	67	69	65	58	71

mosome was exposed to the trapping beam distal to the kinetochore approximately half-way down the long arm. The trap was turned on for a given time period (see Table 1), and then turned off in order to observe subsequent chromosome movements and cytokinesis. In control cells, a region of the spindle between the metaphase plate and the pole was exposed to the optical trap. All of the control cells underwent normal anaphase and cytokinesis.

The morphological/behavioral results of cells following chromosome trapping fell into four categories: (1) normal anaphase and cytokinesis, (2) formation of a temporary chromosome bridge followed by normal anaphase movements and cytokinesis (Fig. 1), (3) formation of a permanent chromosome bridge, with nontrapped chromosomes undergoing normal separation, separation of spindle poles, and cytokinesis (Fig. 2), and (4) complete blockage of chromosome separation (c-mitosis) (Fig. 3). In the last case all chromosomes remained in the central part of the cell, without separation of chromatids. Long-term observations on several cells showed that they remain rounded, with collapsed chromosomes in the center (c-mitosis) for more than 6 h. In the case of chromosome separation with the formation of a chromosome bridge, the attachment site of the chromatids to form the bridge always occurred at the precise point on the chromatids where the optical trap was applied. The chromosomes appeared stretched between the kinetochore and the trapping point.

Wavelength dependence

Using the four morphological criteria, the effectiveness of optical trapping was studied as a function of (1) wavelength and (2) time in the trap. The wavelength range was 700–840 nm, and the time in the trap was 0.3 s–5 min. The power at the objective focal plane was kept constant at 130 mW because, in previous studies (2), it was determined that power levels of at least 60–150 mW were necessary to hold chromosomes.

The data for 288 cells are presented in Table 1. Cell response versus wavelength is plotted in Fig. 4. The data were grouped and plotted as the percentage of cells exhibiting abnormal behavior/morphology for each wavelength. "Abnormal" is defined as any cell exhibiting a permanent or temporary chromosome bridge, or c-mitosis. It is interesting to note that there is a distinct wavelength dependence of abnormal cell responses to the trapping beam. It appears that there are two optimal trapping wavelength regions: 700 nm and 800–820 nm. Since the laser system does not go below 700 nm it is not possible to determine the bandwidth for this spectral region. However, it appears that 800–820 nm is the best wavelength range for optical trapping of mitotic chromosomes. The wavelength peak at 760 nm for cell damage is unequivocal: 760 and 765 nm were the only wavelengths that induced c-mitosis (see Table 1).

With respect to the length of time that the 130 mW trap can be applied before significant damage is produced, only the 700 and 800–820 nm wavelengths permitted trapping for longer than 1 min (see Table 1). For trapping periods of less than 30 s, several other wavelengths could be used. It is also important to note that at 760 nm, as little as 0.5–1.0 s in the trap was sufficient to induce abnormal behavior.

DISCUSSION

This study examines the wavelength dependence of optical trapping in the near-infrared region of the spectrum. This study does not approach the question of the rela-

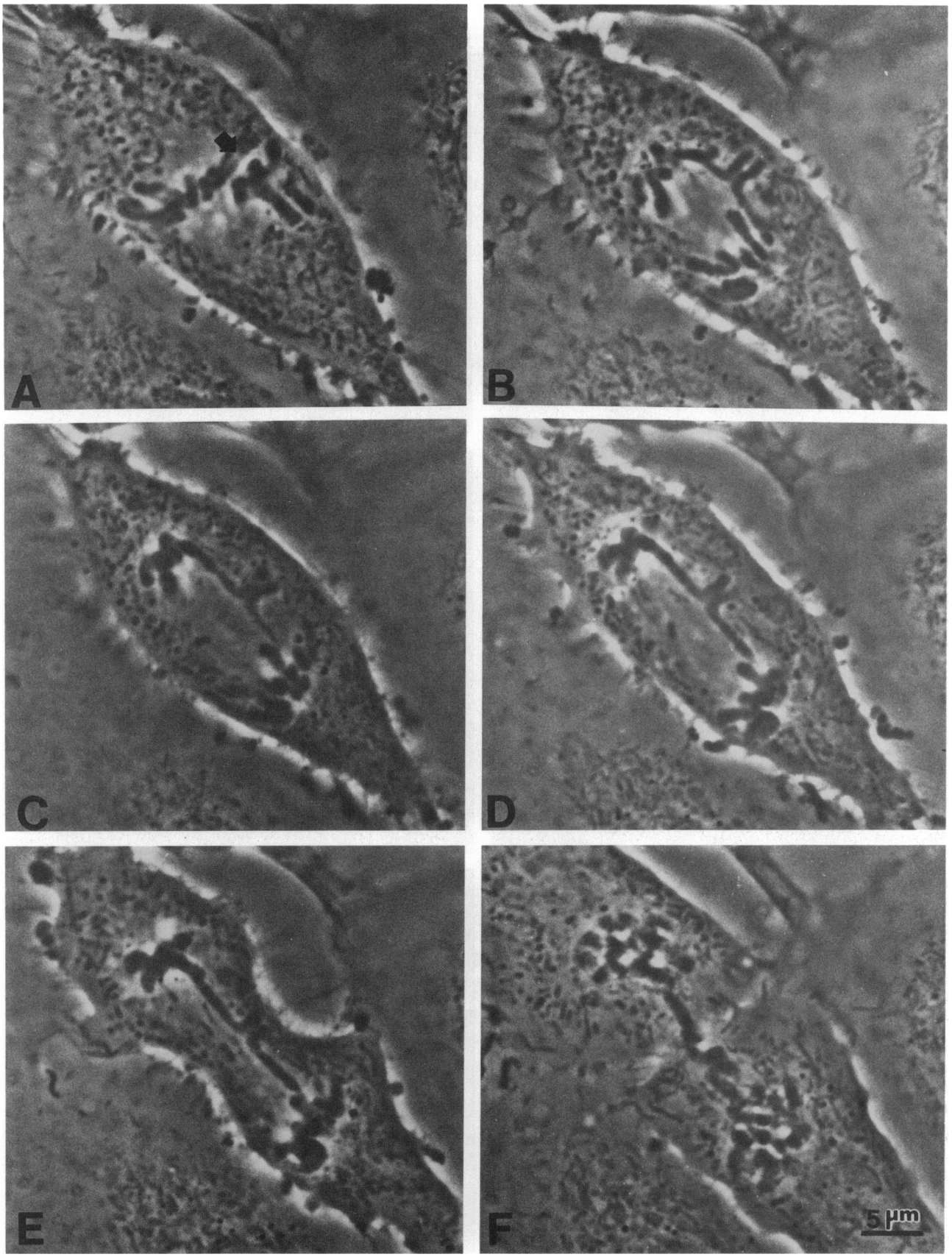

FIGURE 1 Permanent chromosome bridge after 1.0 s. irradiation (λ = 760 nm). Arrow points to the irradiated chromosome region. (*A*) (before irradiation)—12.56:10 (h:min:s); (*B*) (immediately after irradiation)—12.56:55; (*C*)—13.05:40; (*D*)—13.08:35; (*E*)—13.10:31; (*F*)—13.20:06. Bar 5 μm.

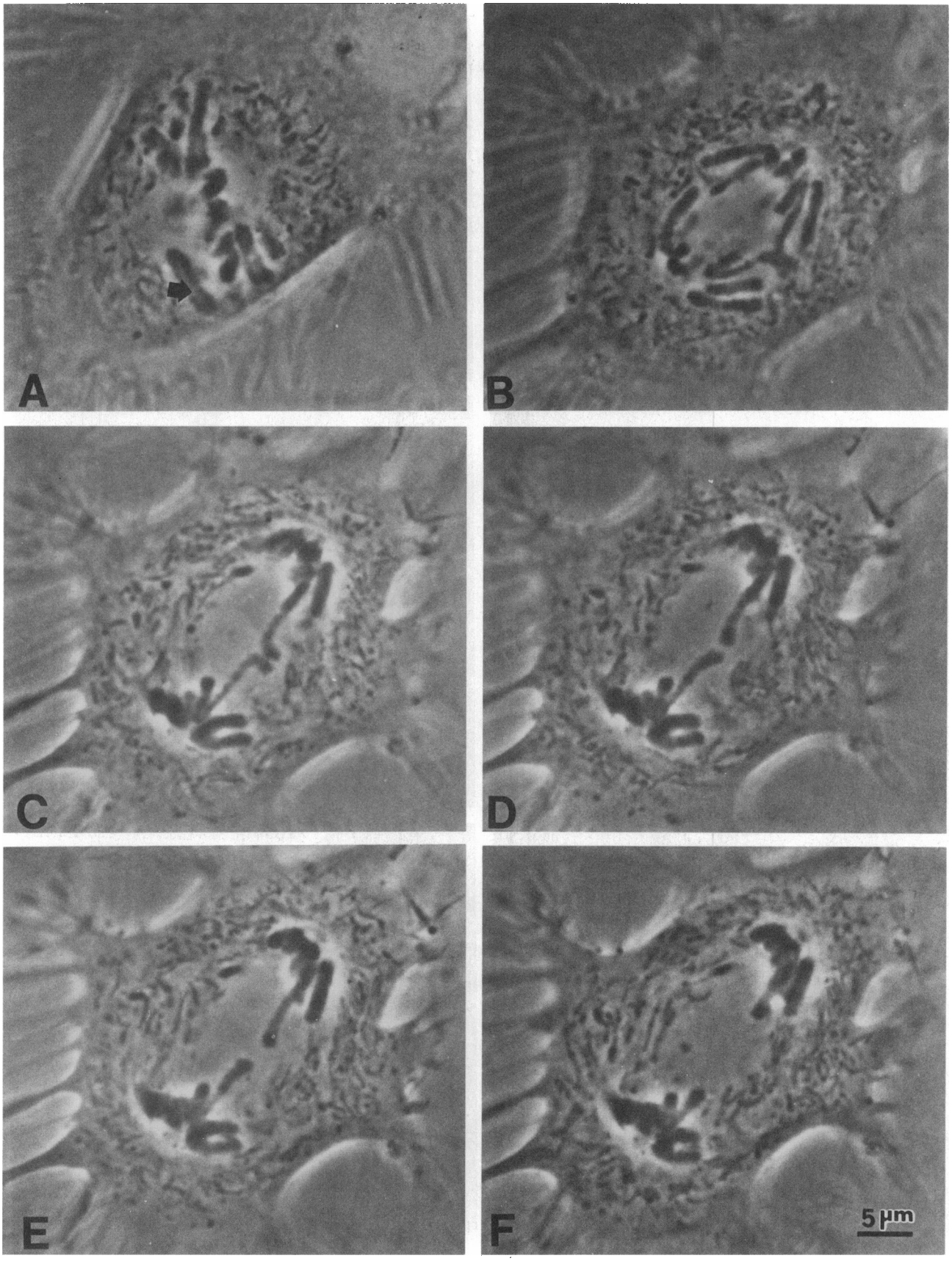

FIGURE 2 Temporary chromosome bridge after 0.6 s. irradiation (λ = 760 nm). Arrow points to the irradiated chromosome region. (*A*) (immediately after irradiation)—20.33:40 (h:min:s); (*B*)—20.51:55; (*C*)—20.55:44; (*D*)—20.56:25; (*E*)—20.56:49; (*F*)—20.58:04. Bar 5 μm.

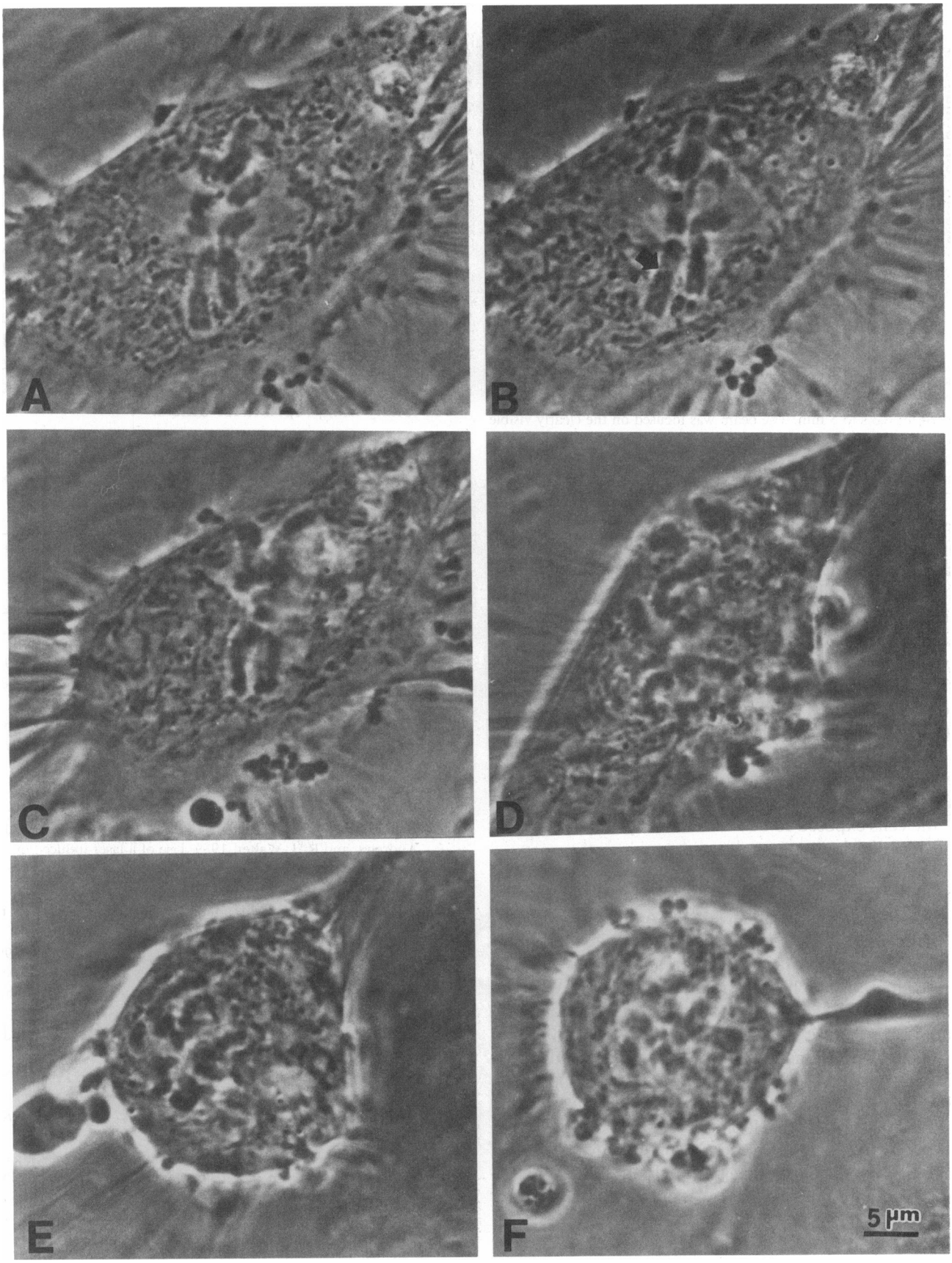

FIGURE 3 C-mitosis after 20 s. irradiation (λ = 760 nm). Arrow points to the irradiated chromosome region. (*A*) (before irradiation)—17:43:23 (h:min:s); (*B*) (immediately after irradiation)—17:44:10; (*C*)—18:01:22; (*D*)—18:21:13; (*E*)—18:28:29; (*F*)—20:19:06. Bar 5 μm.

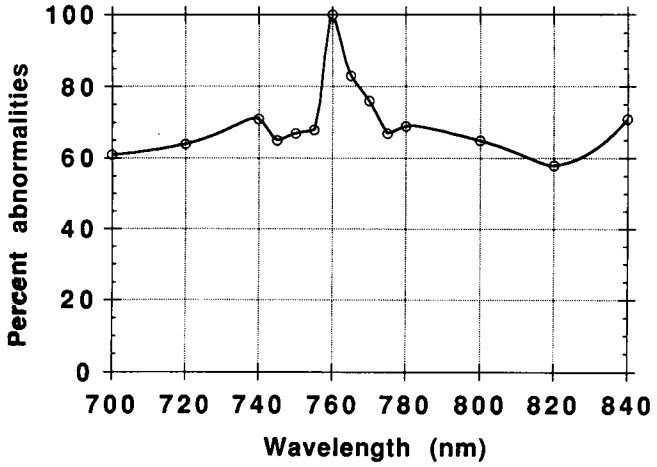

FIGURE 4 Plot of the percent of abnormal mitoses induced with different wavelengths under constant power of 130 mW. All cells were exposed for 0.3 s to 5 min. The beam was focused on the clearly visible shoulder of a large chromosome. Abnormal mitoses were classified as: (*a*) chromosome bridge during anaphase, (*b*) chromosomes failing to separate.

tionship between optical trapping force and the biological response, but rather, it examines the damage potential of the trapping wavelength due to absorption. The distinct maximum damage potential of 760 nm should discourage the use of this wavelength region for trapping experiments in cells, and especially in mitotic cells. On the other hand, it would appear that 130 mW of laser power can be used to hold chromosomes at either 700 or 800 nm for as long as 1–2 min. However, even at these wavelengths, anywhere from 30–60% of the cells may exhibit temporary chromosome bridges. On the positive side, for trapping times of less than 30 s, more than 50% of the cells exposed to 800 or 820 nm will undergo normal cell division.

The nature of the absorption and subsequent effect on the cells is unknown. There is very little information on the absorption characteristics of DNA and protein in the near infrared region of the spectrum. This is probably because the small amount of contaminants would obscure any signal. Whether the small amount of absorbed energy leads to a localized temperature rise or to a photochemical response is not known.

The ability to generate chromosome bridges with consistency may be useful in studying the forces applied on chromosomes (especially the kinetochore), during anaphase A, anaphase B, telophase, and cytokinesis.

It would also be of interest to investigate the fate of chromosome bridges during late telophase and the beginning of interphase. The results presented in this paper demonstrate that sometimes the attached chromosomes will separate during late cytokinesis, and at other times they may remain attached to each other in the interphase nucleus. Finally, in combination with selective laser ablation of kinetochores (9), it should be possible to detach one chromatid from its pole, and by creating a chromosome bridge, "direct" an extra chromatid into one of the daughter nuclei. This offers the possibility to produce cells without any preselected chromosome and could have direct application in cytogenetics.

In conclusion, this study demonstrates optimal wavelengths for optical trapping of chromosomes. It also points out the need to be cautious with respect to potential applications of this new technique. It would seem important to characterize the wavelength specificity for optical trapping for each biological system being studied. Optical "tweezers" might be good for one type of study, but totally inappropriate for others.

This work was supported by funds from the NIH (RR 01192), the Office of Naval Research (ONR N00014-91-C-0134) and the Department of Energy (DE-FGO3-91ER61227).

Received for publication 29 June 1992 and in final form 9 September 1992.

REFERENCES

1. Ashkin, A., J. M. Dziedzic, J. E. Bjorkholm, and S. Chu. 1986. Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* 11:288–290.
2. Ashkin, A., J. M. Dziedzic, and T. Yamane. 1987. Optical trapping and manipulation of single cells using infrared beams. *Nature (Lond.).* 330:769–771.
3. Berns, M. W. 1974. Biological Microirradiation. Prentice Hall Series on Biological Techniques, Englewood Cliffs, NJ. 152 pp.
4. Berns, M. W., W. H. Wright, B. J. Tromberg, G. A. Profeta, J. J. Andrews, and R. J. Walter. 1989. Use of a laser-induced optical force trap to study chromosome movement in the mitotic spindle. *Proc. Natl. Acad. Sci. USA* 86:4539–4543.
5. Berns, M. W., W. H. Wright, and R. M. Steubing. 1991. Laser microbeam as a tool in cell biology. *Int. Rev. Cytol.* 129:1–44.
6. Berns, M. W., J. R. Aist, W. H. Wright, and Hong Liang. 1992. Optical trapping in animal and plant cells using a tunable, near-infrared titanium-sapphire laser. *Exp. Cell Res.* 198:375–378.
7. Block, S. M. 1990. Optical tweezers—A new tool for biophysics. *In* Noninvasive Techniques in Cell Biology. J. T. Foskett and S. Grinstein, editors. Wiley-Liss, New York. 375–402.
8. Liang, H., W. H. Wright, H. Wei, and M. W. Berns. 1991. Micromanipulation of mitotic chromosomes in PtK_2 cells using laser-induced optical forces ("optical tweezers"). *Exp. Cell Res.* 197:21–35.
9. McNeill, P. A., and M. W. Berns. 1981. Chromosome behavior after laser microirradiation of a single kinetochore in mitotic PtK_2 cells. *J. Cell Biol.* 88:543–553.