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Micromanipulation of Mitotic Chromosomes in PTK₂ Cells Using Laser-Induced Optical Forces ("Optical Tweezers")

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To study the potential use of optical forces to manipulate chromosome movement, we have used a Nd:YAG laser at a wavelength of 1.06 μm focused into a phase contrast microscope. Metaphase and anaphase chromosomes were exposed while being monitored by video microscopy. The results indicated that when optical forces were applied to late-moving metaphase chromosomes on the side closest to the nearest spindle pole, the trapped chromosomes initiated movement to the metaphase plate. The chromosome velocities were two to eight times the normal rate depending on the chromosome size, geometry, and trapping site. At the initiation of anaphase, a pair of chromatids could be held by the optical trap and kept motionless throughout anaphase while the other pairs of chromatids separated and moved to opposite spindle poles. As a result, the trapped chromosome either was incorporated into one of the daughter cells or was lost in the cleavage furrow, or the two chromatids eventually separated and moved to their respective daughter cells. If the trap was removed at the beginning of anaphase B, the chromosome moved back to the poles. Our experiments demonstrate that the laser-induced optical force trap is a potential new technique to study noninvasively the mitotic spindle of living cells. © 1991 Academic Press, Inc.

INTRODUCTION

Enormous progress has been made toward understanding the cell division process in a wide variety of organisms and cell types using the methods of molecular biology as well as light and electron microscopy. To date, the cell structures and molecules involved in mitosis have been extensively characterized [8]. However, how the molecular constituents of the spindle and the cell organelles function in an integrated fashion to achieve cell division is still inadequately understood. One of the main reasons for this gap in our knowledge is

the difficulty of performing actual experiments in living cells to study the forces involved. It was Nicklas' ingenious study [11], observing the deflection of a microneedle attached to chromosomes in dividing grasshopper spermatocytes, that resulted in the first actual measurements of forces in a mitotic spindle of a living cell. Unfortunately, measurement of spindle forces in cultured mammalian cells may not be valid due to the greater resistance offered by their membranes [12]. In addition, the invasiveness of this method, combined with the high skill needed to place the microneedle, limits the speed by which cells can be evaluated.

By using a highly focused laser beam as a single-beam optical trap [2], forces comparable to that exerted by the mitotic spindle can be applied to subcellular objects. An object is drawn into the focused laser beam spot as a result of the change in momentum to the light that interacts with the object. Thus, the object is confined by the trap along all three axes solely by the optical forces, without physical contact. Ashkin first proposed and demonstrated the use of these optical traps for biological objects such as *Escherichia Coli*, lymphocytes, red blood cells, and macrophages [1-3]. Subsequently, the use of radiation pressure and optical trapping has been extended to cell manipulation and cell motility [5, 6, 14, 15]. In 1989, Berns *et al.* [4] first reported the manipulation of chromosomes in mitotic cells *in vitro* by optical trapping. In this paper, we report on the continued use of optical traps to alter chromosome movement during mitosis, furthering their application to cell biology and cell genetics. For example, by holding chromatids motionless during anaphase, it may be possible to measure more accurately the maximum force the mitotic spindle can exert on a single chromosome [11]. In cell genetics, one can create monosomic and trisomic cells by holding a chromosome from anaphase to cytokinesis with the optical trap and keeping it in one of the daughter cells.

MATERIALS AND METHODS

Cell culture. All experiments were performed on PTK₂ cells, an epithelial cell line from the kidney of the male rat kangaroo (*Potorous tridactylis*). These cells are thin and remain relatively flat throughout

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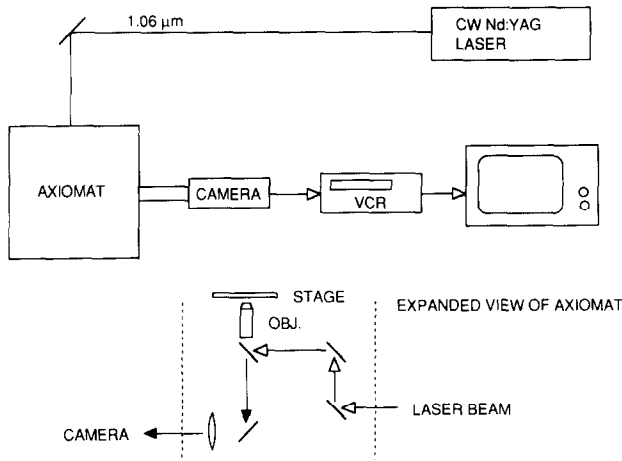


FIG. 1. Simplified diagram of the single-beam gradient force optical trap.

the cell cycle, permitting clear visualization of the chromosomes. Cells were grown as monolayer cultures in T_{25} flasks in minimal essential medium (GIBCO Laboratories, Grand Island, NY), containing 10% fetal calf serum and 1% 200 mM glutamine. Cultures were maintained at 37°C in a 5–7.5% CO_2 incubator. Cells were subcultured once a week using a viokase solution for digestion. Two or three days before each experiment, cells collected from the stock flasks were seeded into Rose chambers [13] by injection.

Laser instrumentation. The instrumental setup for the optical trap is shown diagrammatically in Fig. 1. The optical trap was created using a neodymium:YAG laser (Quantronix 116, Smithtown, NY) operating at 1.06 μm , continuous wave, in the TEM_{00} mode. The laser was directed into a Zeiss Axiomat photomicroscope and focused by a Neofluar X100 phase-contrast objective (Zeiss) with a numerical aperture of 1.3. The laser power was checked at the objective with a Coherent (Palo Alto, CA) Model 210 power meter. For the experiments described herein, the power varied from 60–200 mW. A dichroic mirror inside the microscope was used to reflect the infrared laser beam to the objective while visible light for image formation could pass through the mirror to the camera. A video camera was coupled to one of the observation ports of the Axiomat. The video image was recorded by a half-inch time lapse VCR (Panasonic) and displayed on a monochrome monitor. In all the experiments, the target chromosomes were exposed to the optical trap while continually being monitored by video microscopy. Measurement of the chromosome velocity was made from the recorded image displayed on a video monitor. In addition, all of the micrographs presented herein were reproduced from the stored videotape image.

RESULTS

Optical Trapping of Late Moving Chromosomes

In this experiment, we selected mitotic cells with a late moving chromosome during metaphase. This is a chromosome located between the metaphase plate and one mitotic pole at a time when all the other chromosomes have reached the equatorial plate. The optical trap, with a power of ~ 120 mW, was applied to the chromosome on the side closest to the nearest spindle pole for 1–2 min. Each cell was observed for approximately 5 min. Out of 204 cells observed, 63 chromo-

somes moved rapidly out of the trap toward the metaphase plate within a few seconds after application of the trapping force (three examples, Fig. 2). The movement stopped when the chromosome reached the metaphase plate. Of the 63 cells that responded to the optical trap, chromosome velocities ranged from 4.6 to 16.7 $\mu\text{m}/\text{min}$, with the average being 9.5 $\mu\text{m}/\text{min}$. The normal rate of chromosome movement for this cell is approximately 2 $\mu\text{m}/\text{min}$ [9].

We observed that the velocity of the trapped chromosome was related to its visible surface area. Therefore, trapped chromosomes were classified into the following three groups according to their velocity: (1) slow movement group, 4.5–8.0 $\mu\text{m}/\text{s}$; (2) moderate movement group, 8.5–12.5 $\mu\text{m}/\text{s}$; and (3) fast movement group, over 12.5 $\mu\text{m}/\text{s}$. The response of the cell was such that the chromosome either moved at a velocity greater than 4.5 $\mu\text{m}/\text{s}$ or did not move. As indicated in Table 1, the velocity of the trapped chromosomes was inversely related to the chromosome size. The differences between the three movement groups were all significant (Table 2), with P values less than 5%, as determined by one-way ANOVA tests.

In cells where the chromosomes did not initiate movement out of the trap, we occasionally saw a different response, such as a rotation of the chromosome to a different orientation.

To determine the survival rate following optical trapping of the chromosome, 22 metaphase cells from the group of 204 cells were observed until they divided. Of the 22 cells, 19 divided successfully to daughter cells, giving a survival rate of 86% (Table 3).

We tested the hypothesis that the induced chromosome movement is in the opposite direction from that of the trapping force when the trapping force is applied on the chromosome side closest to the nearest spindle pole. Chromosomes moving normally toward the metaphase plate (not late-moving chromosomes) were initially trapped for 1 min on the side closest to the metaphase plate. The trap was turned off for 2 min and then repositioned over the side of the chromosome facing the spindle pole. The trap was then switched back on. Three different chromosome responses were observed: (1) in 17 cells the chromosome moved toward the metaphase plate only when the trap was positioned on the side closest to the spindle pole—the chromosome did not move when the force was applied on the side closest to the metaphase plate; (2) in 15 cells the chromosomes did not respond to the trapping force regardless of the side of application of the trapping force; (3) in only two cells the chromosomes moved toward the metaphase plate regardless of the side of the chromosome that the trap was applied. Thus, of the 19 cells that exhibited chromosome movement in response to the trapping force, 17 (89%) moved in the direction opposite to the trapping

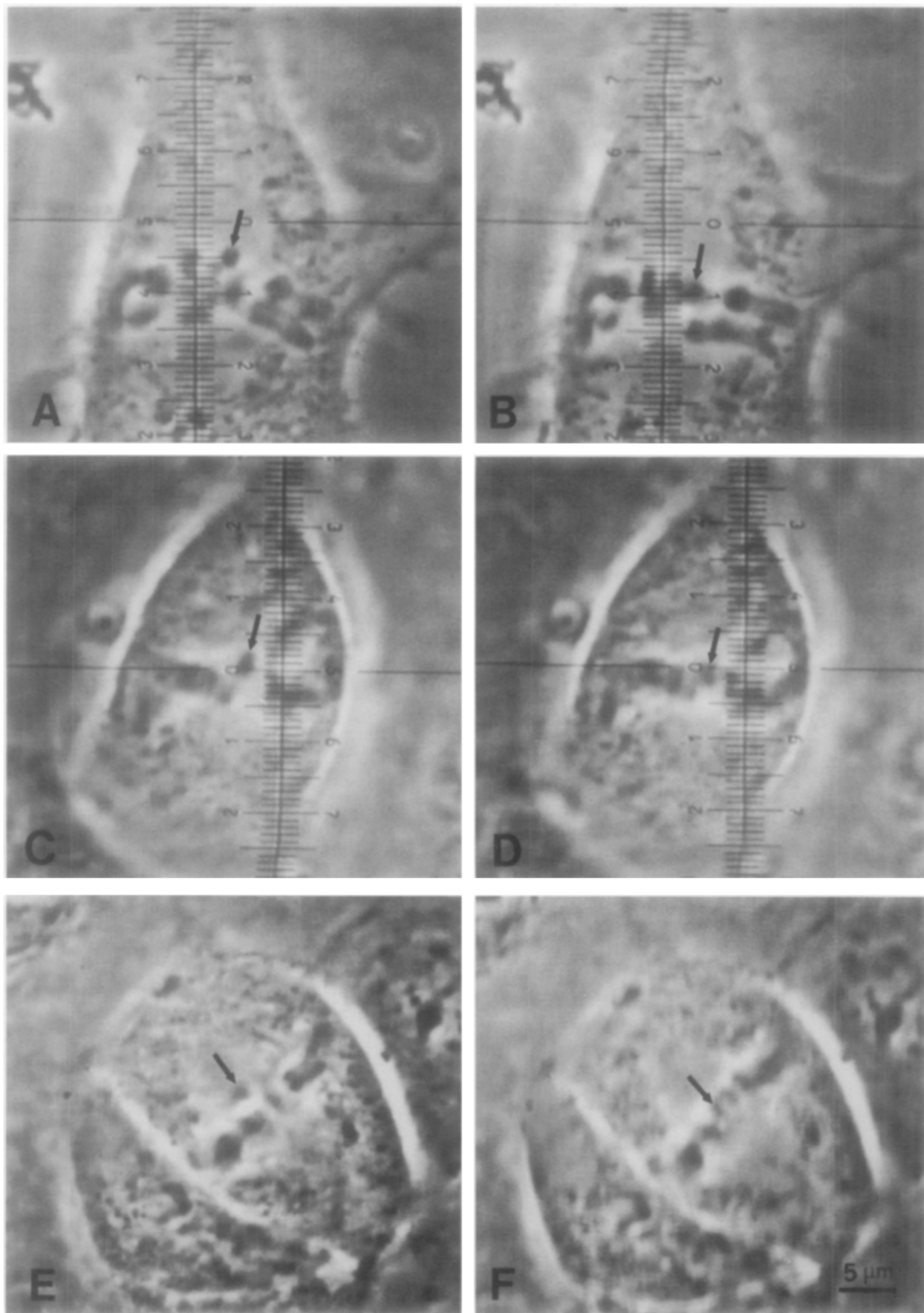


FIG. 2. Optical trapping of late moving metaphase chromosomes. Three paired examples are shown (A-B, C-D, E-F) before and after optical trapping. Arrows indicate the trapped chromosome.

TABLE 1
Movement Velocities of Trapping Chromosome
Relevant to Chromosome Size in PTK2

| Group | No. of samples | Mean visible chromosome surface area (μm^2) | SE |
|---|----------------|--|-------|
| Slow (4.5–8.0 $\mu\text{m/s}$) | 29 | 5.676 | 0.422 |
| Moderate (8.5–12.5 $\mu\text{m/s}$) | 22 | 4.293 | 0.33 |
| Fast (over 12.5 $\mu\text{m/s}$) | 12 | 2.14 | 0.257 |

(pulling) force. It is not clear why 15 cells did not respond at all to the trapping force.

Optical Trapping of Anaphase Chromosomes

Initially, we studied the response of the anaphase cell to the length of time the optical trap, at constant power, was used to hold a pair of chromatids. We observed that a laser power of 60 mW, representing the minimum power needed to hold an anaphase chromosome, was optimal. When the optimal trap force was applied to the end of the paired chromatid arms at the initiation of anaphase, the trapped chromosome was held and kept motionless while the other chromatids separated and moved to their respective spindle poles. If the trap was removed at anaphase B, the trapped chromatids separated after a few minutes and moved to their respective poles (Fig. 3). However, if the optimal trap power was applied to a pair of chromatids throughout anaphase, the trapped chromosomes were then held at the equatorial plate of the cell throughout anaphase. As a result, the trapped chromosome was either incorporated into one of the daughter cells (Figs. 4–6), lost in the cleavage furrow (Fig. 7), or the two chromatids eventually separated and moved to their respective daughter cells (Fig. 8). In Figs. 7 and 8, the ability to hold the two chromatids stationary in the optical trap while the other chromosomes move is particularly clear. If the optimal trap power was used to hold a pair of chromatids from anaphase through telophase, a cleavage furrow did not form

TABLE 2
Comparison between Groups

| Comparison | Mean diff. | Fisher PLSD | Scheffe <i>F</i> test |
|------------------|------------|-------------|-----------------------|
| Slow vs moderate | 1.382 | 1.042* | 3.519* |
| Slow vs fast | 3.536 | 1.266* | 15.617* |
| Moderate vs fast | 2.153 | 1.323* | 5.299* |

* Significant at 95%.

TABLE 3
Survival Rate of Cells following Optical Trapping
of Metaphase Chromosomes in PTK2

| Number of cells attempted | Cells divided successfully to daughter cells | | Cells failed to undergo further division | |
|---------------------------|--|------|--|------|
| | No. | % | No. | % |
| 22 | 19 | 86.4 | 3 | 13.6 |

on the side of the cell where the trap was applied. As a result, the two daughter cells were fused, forming a giant cell with two nuclei. Finally, with respect to Figs. 4–8, the position of the trapped chromosomes throughout mitosis were carefully followed by continual time lapse video. This ensured that the phase dark material indicated by the arrows at the end of the process could be correlated with the trapped chromosome.

An increase in the prolongation of mitosis was observed as the trapping interval increased, especially when the optical trap was applied throughout the remainder of mitosis. The effect of prolongation varied from minutes to hours depending on the cell. During chromatid movement in anaphase A, we observed that chromatids on the side of the cell where the optical trap was applied were lagging with respect to the others. Subsequently, a cleavage furrow appeared only on the side of the cell away from the optical trap.

In general, when the optimal power level was used, optical trapping was very successful in retarding the movement of individually trapped anaphase chromosomes in PTK₂ cells. In 32 out of 39 cells (82%), we were successful in holding a pair of chromatids motionless, while the other chromosomes continued through normal anaphase movement (Table 4). At higher power levels, not only was the trapped chromosome held motionless, the separation and movement of all other pairs of chromosomes was prevented as well. At least on two occasions, at these power levels, we observed chromosome movement toward the side of the cell where the trap was applied, rather than to the poles, as expected (Fig. 9). Finally, the cell appeared sick, indicating that viability was impaired at the higher power levels concomitant with trapping intervals of 10–20 min.

We observed that the time when optical trapping was initiated was critical for success in holding the anaphase chromosome motionless. The optical force should be applied just at the time of initiation of anaphase. When the trap force was applied before anaphase, the separation of all chromatid pairs were blocked; conversely, when the trap force was applied after the initiation of anaphase, the movement and separation of the trapped chromosome could not be prevented.

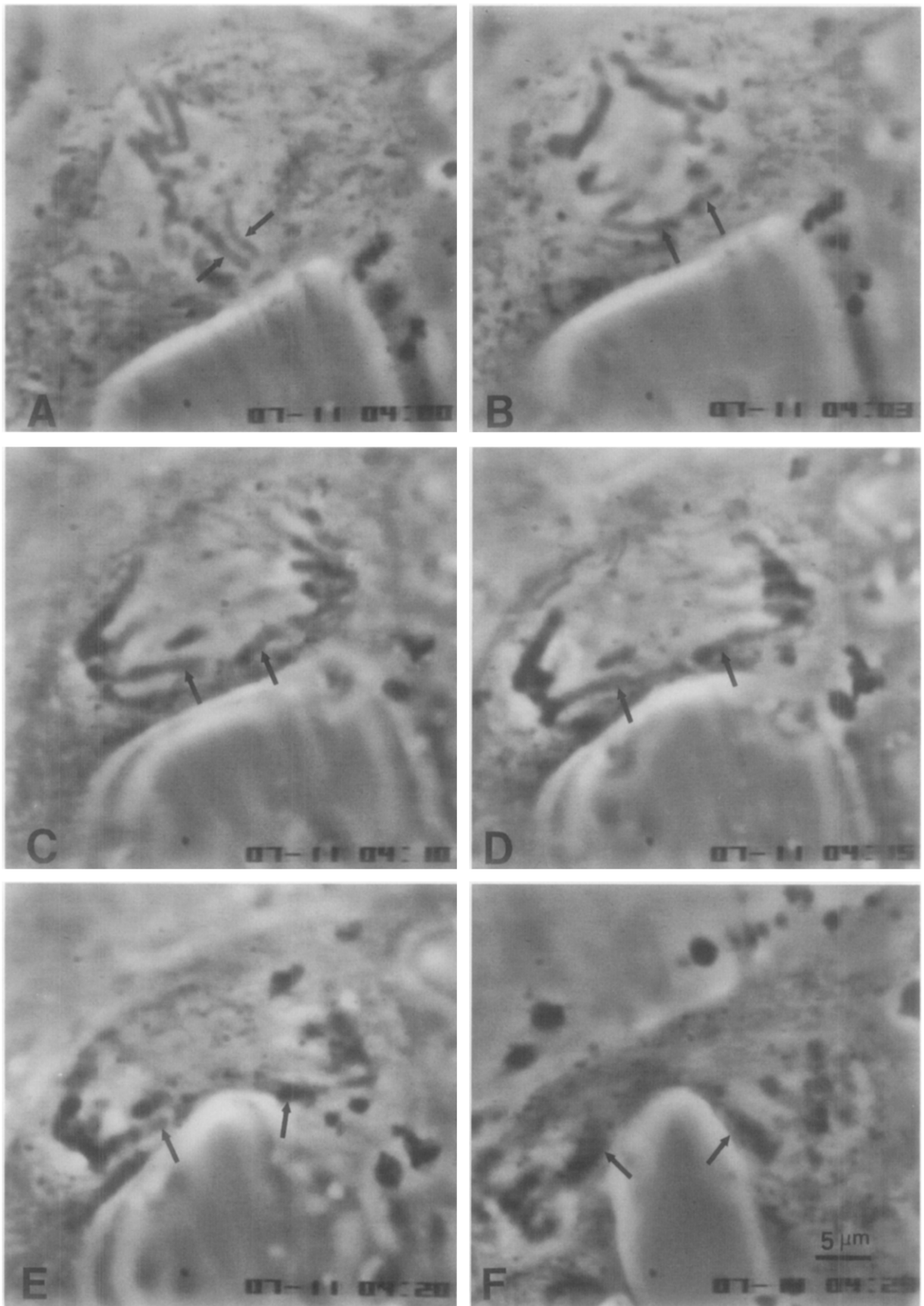


FIG. 3. Optical trapping of a pair of chromatids at anaphase A. The trap was switched on at time zero and turned off 10 min later. Elapsed time is indicated in the lower right of the micrograph. Arrows indicate the trapped chromosome.

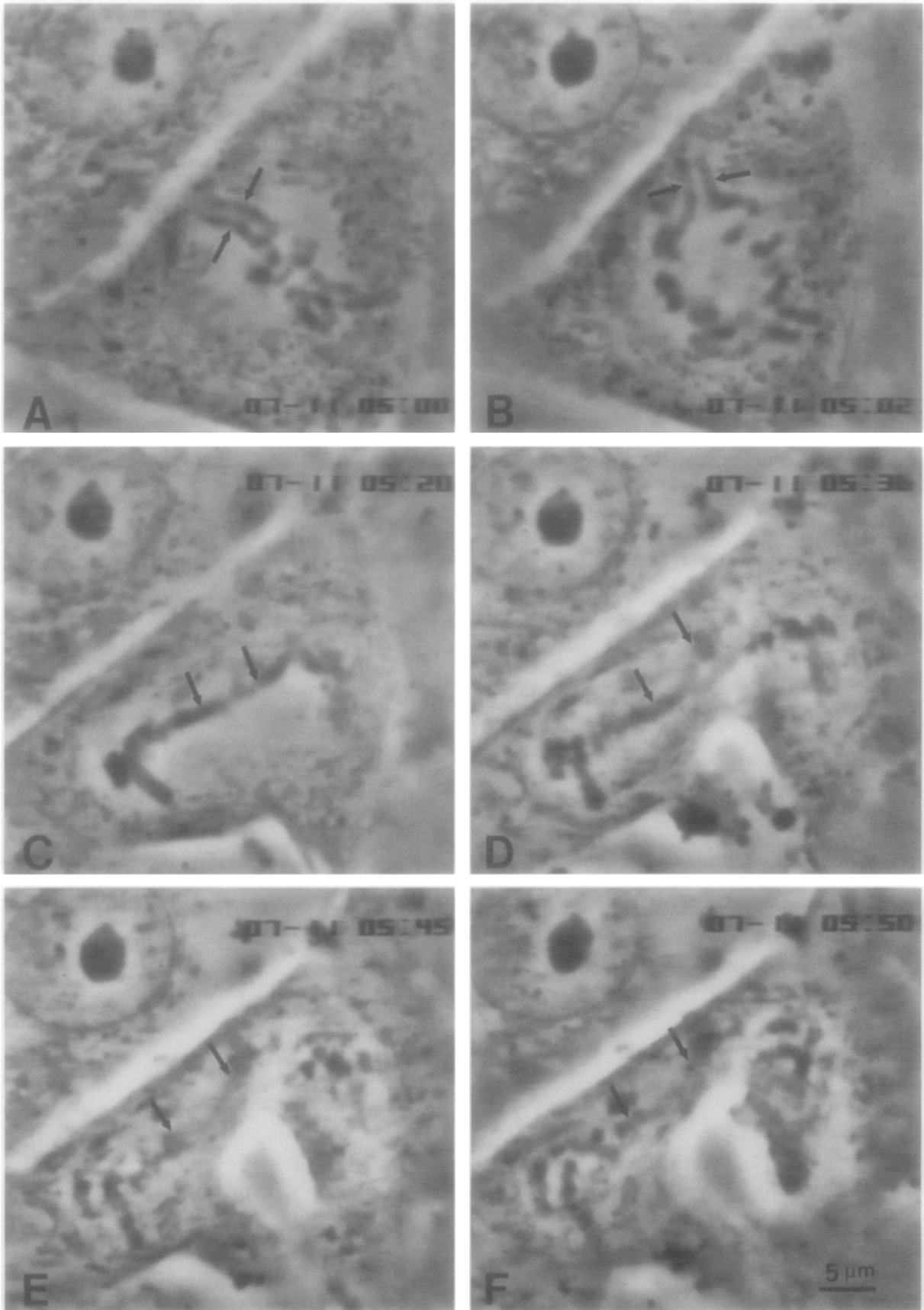


FIG. 4. An example of a pair of chromatids that were incorporated into one daughter cell following optical trapping. Arrows indicate the trapped chromosome.

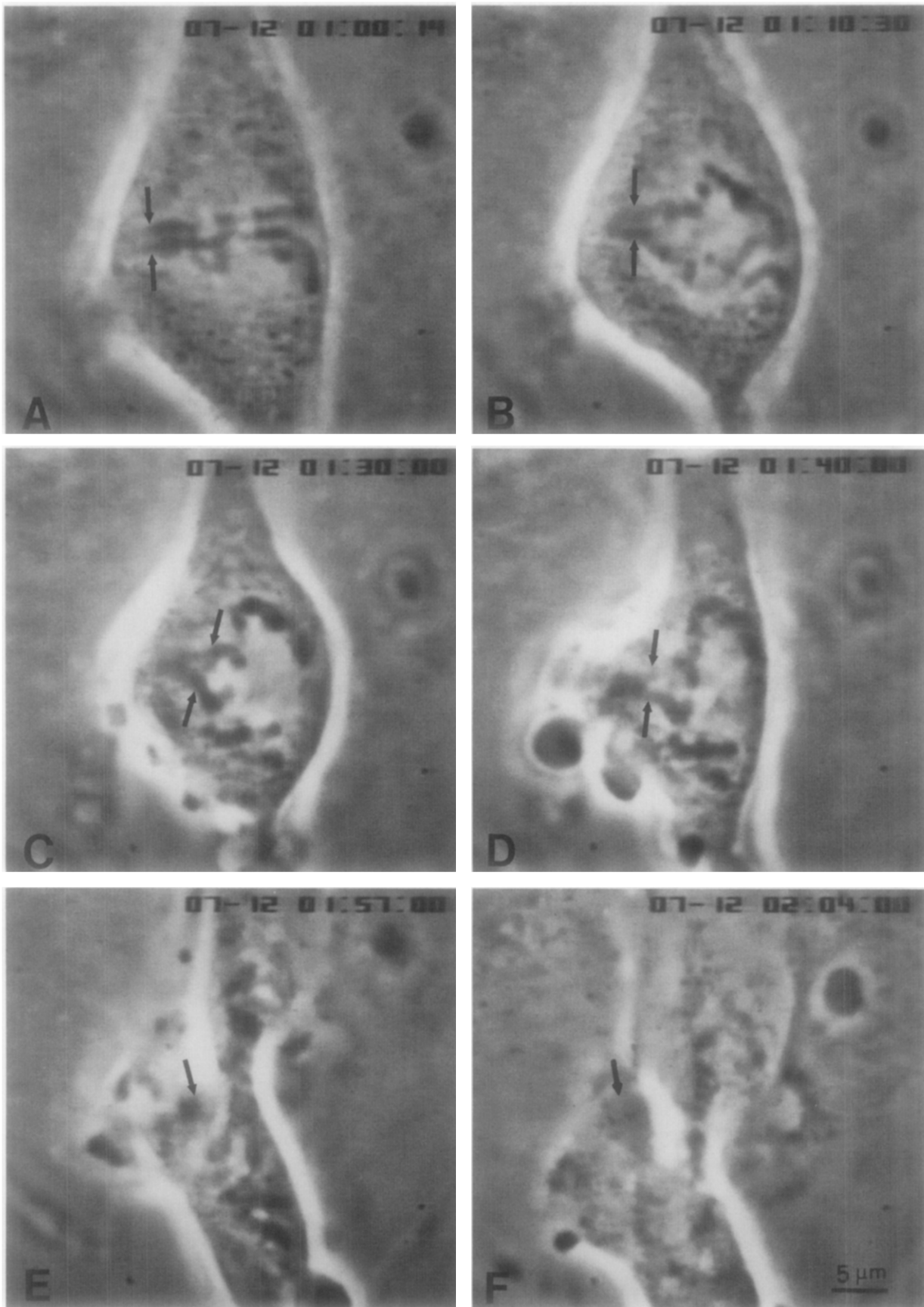


FIG. 5. Same as Fig. 4 for a different cell.

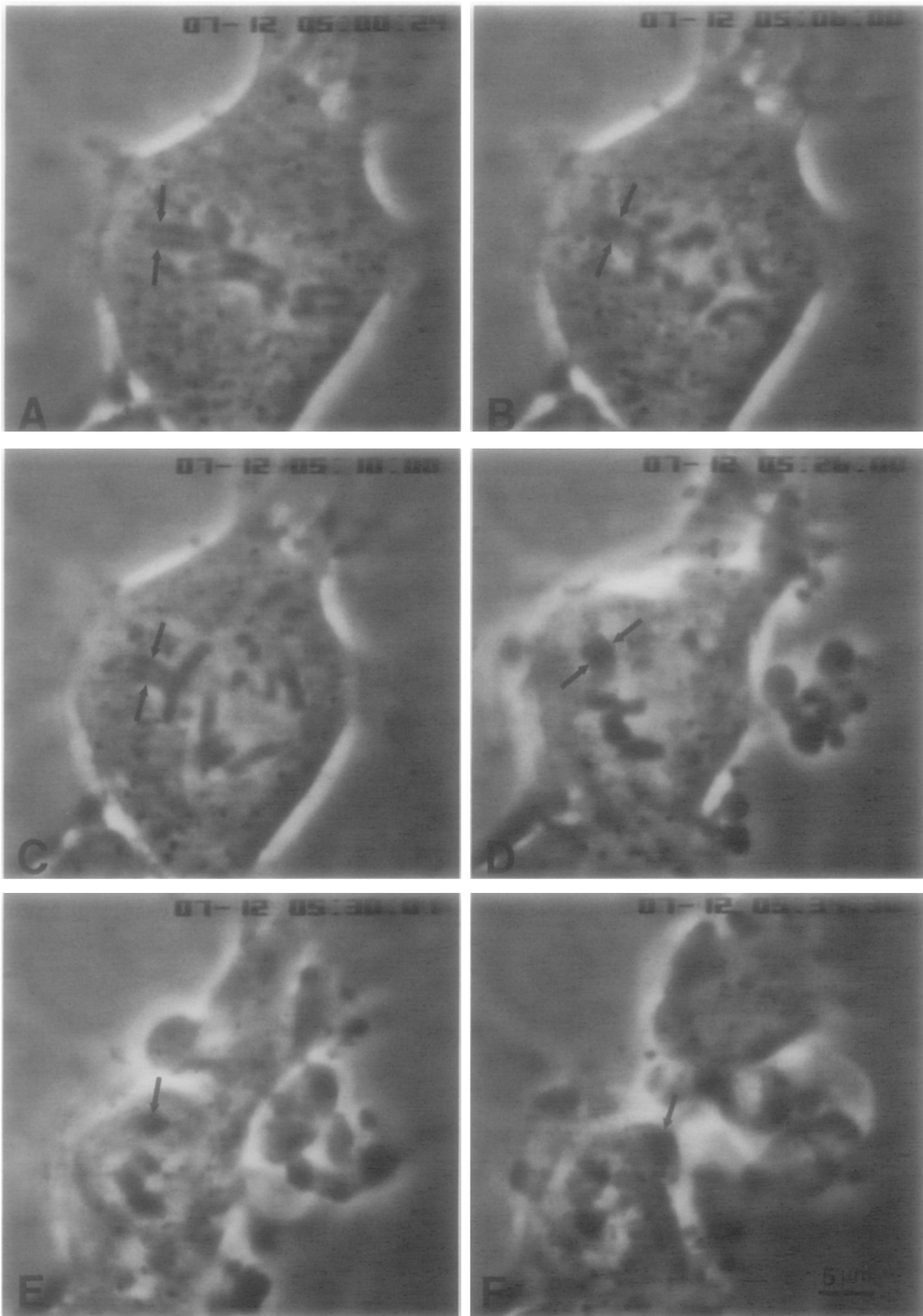


FIG. 6. Same as Fig. 4 for a different cell.

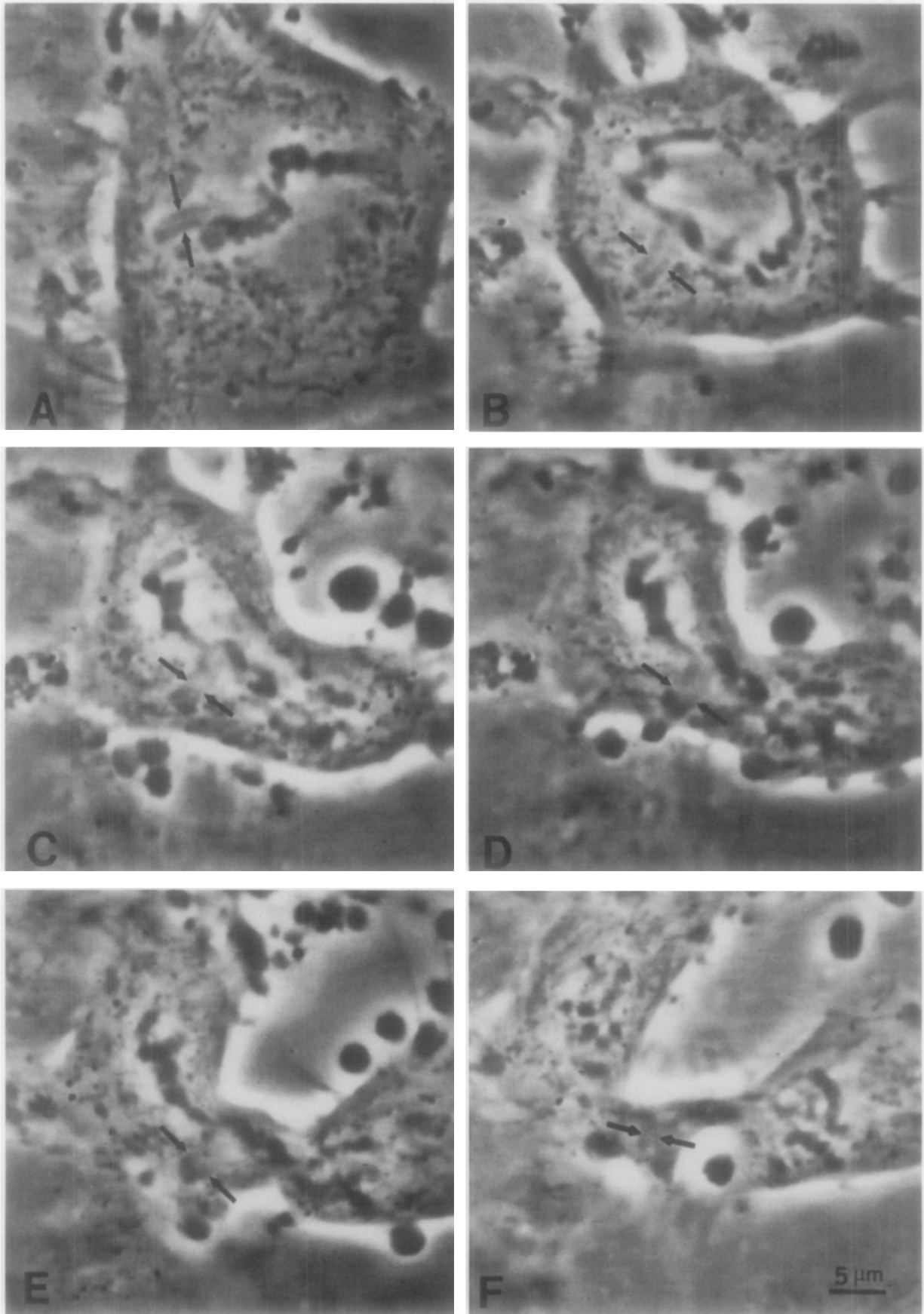


FIG. 7. An example of optically trapped chromatids that were lost in the cleavage furrow. Arrows indicate the trapped chromosome.

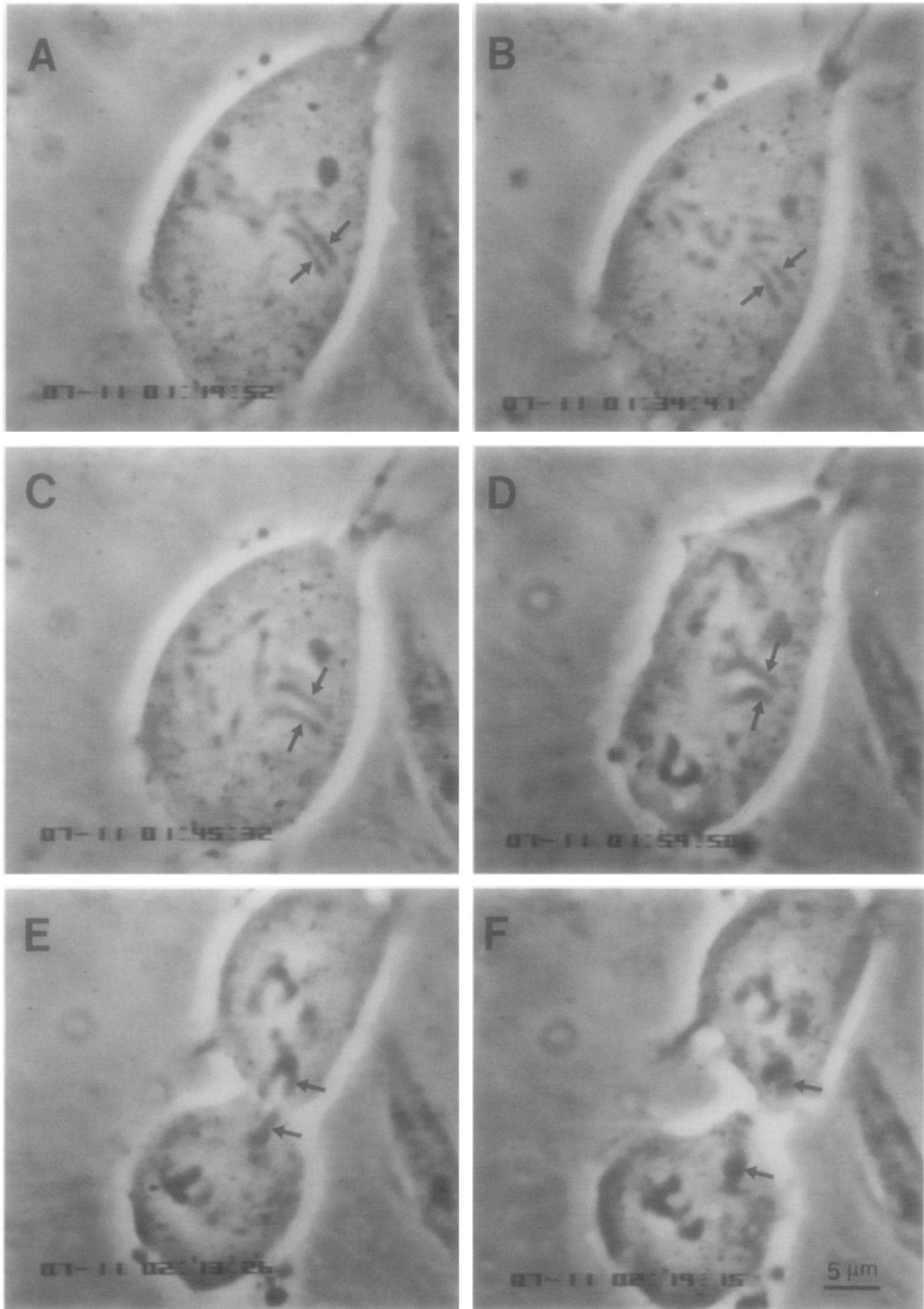


FIG. 8. An example of optically trapped chromatids that eventually separated and moved into their respective daughter cells. Arrows indicate the trapped chromosome.

TABLE 4
Effectiveness of Optical Trapping in Holding
Anaphase Chromosomes in PTK2

| Number of cells attempted | Number of cells where a pair of chromatids were held motionless | | Number of cells where the chromatids were not held motionless | |
|------------------------------|--|------|---|------|
| | No. | % | No. | % |
| 39 | 32 | 82.1 | 7 | 17.9 |

We noticed, on occasion, that when the optical force was applied to the chromosome located either in the center or on the side of the equatorial plate, two or three additional pairs of chromosomes which were closest to the trapped chromosome were also kept motionless (Figs. 10 and 11).

The survival rate of cells was high, regardless of the prolongation of mitosis as a result of the trapping of anaphase chromosomes. Out of 27 cells studied, 23 were successful in dividing into two daughter cells (85%) (Table 5). The other 4 cells failed to undergo further division. These results demonstrate that anaphase chromosomes can be held motionless repeatably using optical traps and that the survival rate of cells following optical trapping is essentially as good as those for metaphase chromosomes.

DISCUSSION

The experimental results from a large number of cells corroborate the observation [4] that laser-induced optical gradient forces can be used to accelerate the metaphase chromosomes. The average chromosome velocity was $9.5 \mu\text{m}/\text{min}$, five times the normal rate [9]. When the chromosome has an index of refraction higher than the cytoplasm, the direction of the net force will be back toward the laser focal point [2]; i.e., the chromosome will be pulled into the laser beam. This was clearly demonstrated using free chromosomes from lysed cells [4]. In response to the trapping force, the cell attempts to pull the chromosome in the opposite direction, out of the trapping beam. Consequently, the laser-induced pulling force may induce the polymerization and depolymerization of microtubules at the kinetochore, causing the spindle to pull the chromosome out of the trap and toward the metaphase plate.

The observations of the present study are consistent with our earlier study [4] in which the applied optical force induced the movement of the late moving chromosomes toward the metaphase plate. The chromosomes stopped moving when they reached the metaphase plate. This indicates that when the late moving chromo-

somes reached the metaphase plate and once a normal bipolar microtubule-chromosome linkage was established, the cell underwent normal division. The high success rate of cells containing optically trapped chromosomes that underwent mitosis shows that, at the trapping power used, the short-term detrimental effects are minimal.

The trap can also be used to hold anaphase chromosomes motionless, reported here for the first time. When the cell is in anaphase, it is possible that when an optical pulling force was exerted on a pair of chromatids at the initiation of their separation (i.e., the initiation of the depolymerization of microtubules between the chromosomes and the poles) and if this restoring force is greater than or equal to the force the cell exerts to separate the pair of chromatids, the depolymerization of microtubules at the kinetochore [7] might be prevented. As a result, the separation of this trapped pair of chromatids would be blocked and kept motionless as long as they were held by the optical force. However, once the optical force is removed, the normal depolymerization of microtubules between the chromosome and the pole would be reestablished, permitting the separation of chromatids and the movement toward opposing poles.

In the metaphase experiments, we cannot rule out the possibility that the optical trap is heating the kinetochore, though it is clear that we are able to induce chromosome movement toward the metaphase plate only when the trap is applied to the side of the chromosome facing the spindle pole. If the induced chromosome movement was in response to an increase in temperature rather than a pulling force (optically induced), there should be no relationship between the side of the chromosome that the laser is applied to and the direction of chromosome movement. The chromosome is small enough in diameter so that the size of the focused laser spot constituting the optical trap ($>1 \mu\text{m}$), combined with the high thermal conductivity of water, should result in the same temperature rise on both sides of the chromosome. Other studies [10] have shown that heating the kinetochore increases the chromosomal speed relative to unheated kinetochores during anaphase A; however, the increase was not as great as expected based on the estimated thermal gradient.

When a trapping force greater than necessary was used, the exerted tension force may have been strong enough to prevent the separation of all pairs of chromosomes or to block the depolymerization of chromosome to pole microtubules. Heating of the cell as a result of the increased laser power required to generate the higher forces, combined with the length of time the trap was applied, may be responsible for the adverse changes in cell health and occasional cell death. These results strongly support the assumption that the chromosome will be seized by the laser beam when the optical force is applied.

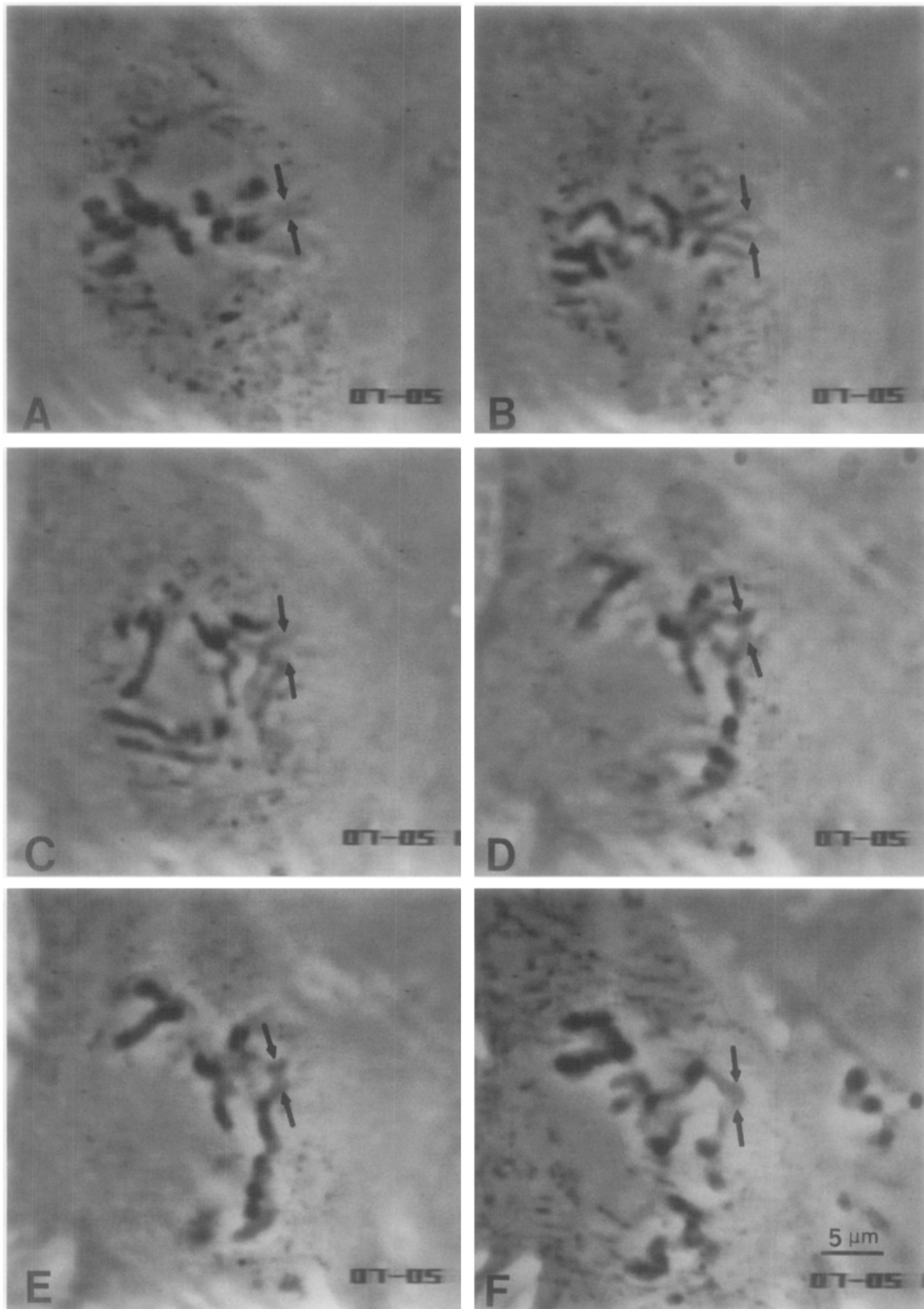


FIG. 9. Anaphase chromatids trapped at higher power levels than necessary.

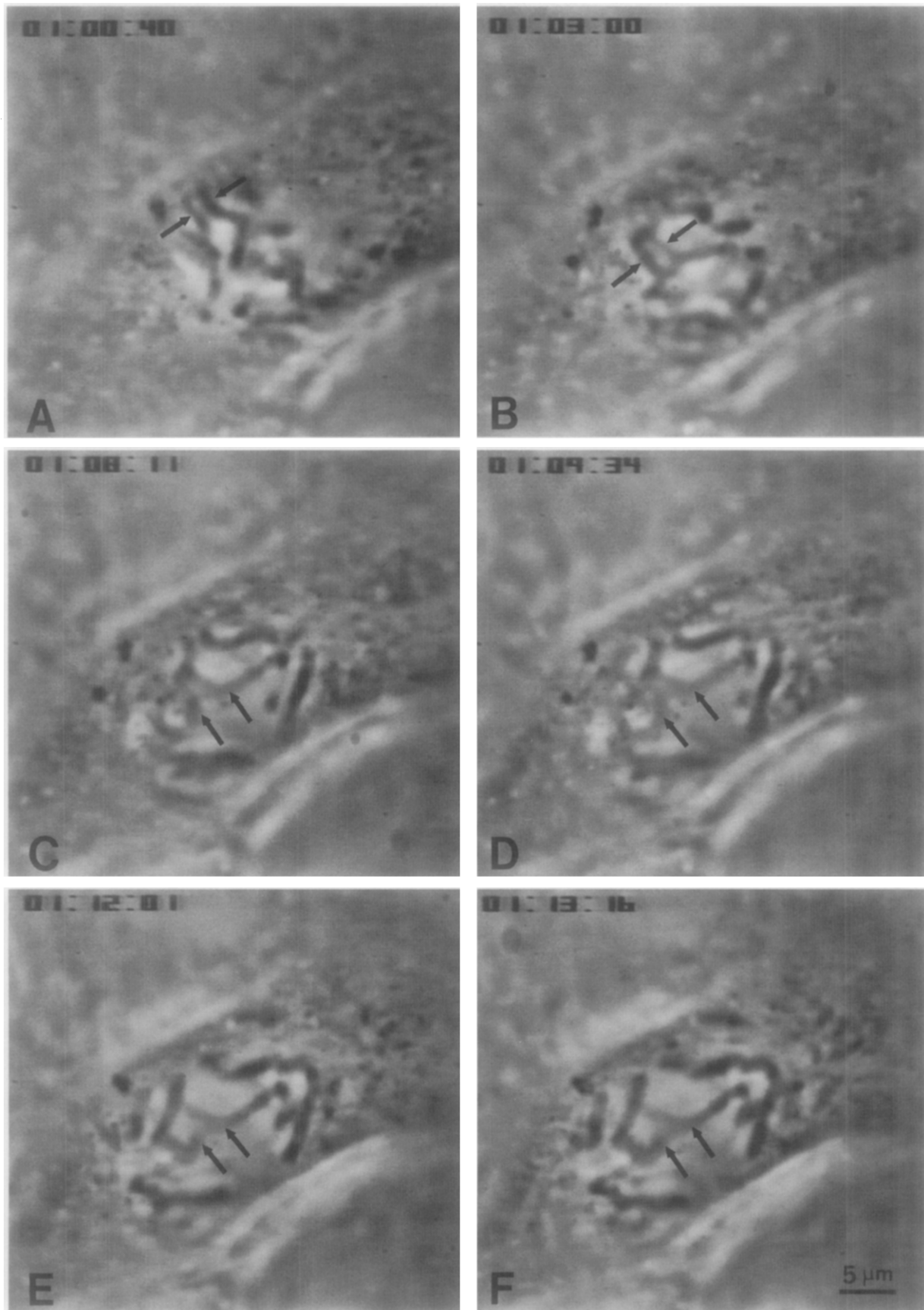


FIG. 10. A series of micrographs showing that chromosomes adjacent to the trapped chromosome in anaphase are affected when the trapping is done in the center of the cell. Arrows indicate the trapped chromosome.

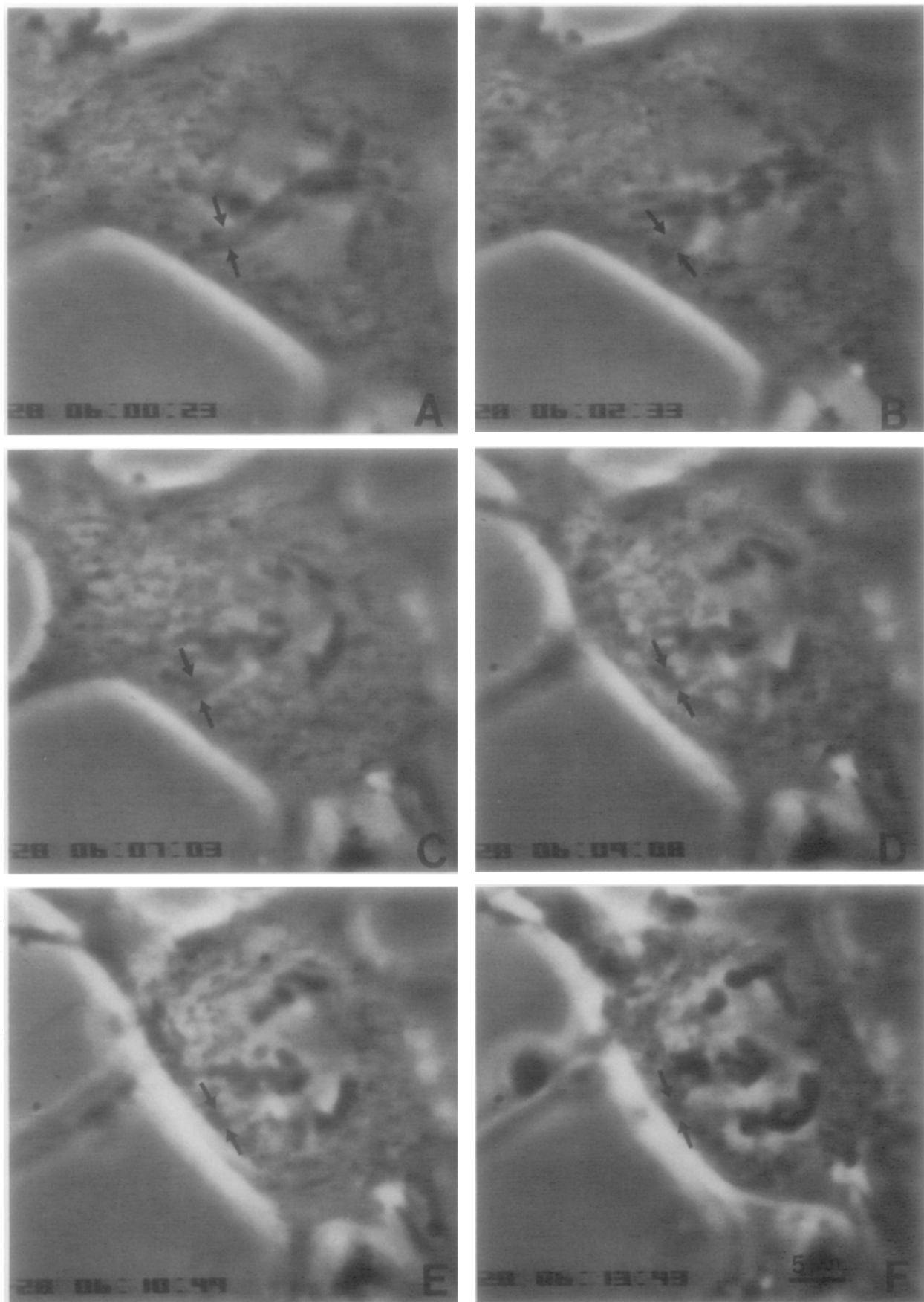


FIG. 11. Similar to Fig. 10 except that now the trap is applied to the side of the cell, as indicated by the arrow.

TABLE 5
Survival Rate of Cells following Optical Trapping of Chromosomes throughout Anaphase in PTK2

| Number of cells attempted | Cells divided successfully to daughter cells | | Cells failed to undergo further division | |
|---------------------------|--|------|--|------|
| | No. | % | No. | % |
| 27 | 23 | 85.2 | 4 | 14.8 |

Quantitative measurement of the forces acting on the chromosomes are essential in order to clearly understand the process of mitosis. Physical transducers, such as calibrated microneedles [11], can be used to measure mitotic forces. The optical trap provides a noninvasive means of applying localized forces to the chromosome. The actual amount of force exerted on the chromosome, however, depends on the physical properties of the chromosome (shape, size, composition) as well as the laser beam. This requires that the optical trap be calibrated beforehand by measuring the critical drag force of chromosomes through the cytoplasm, by measuring the light scattered by the trapped chromosome and solving an inverse scattering problem [17], or by calculating the expected force on an idealized chromosome using ray-optics [16] or light-scattering calculations. Though preliminary experiments have been conducted using 10- μ m-diameter polystyrene microspheres [16], similar studies must be done on chromosomes in order to fully exploit the optical trap in mitosis.

Of course, the mechanism of assembly and disassembly of microtubules in response to optical forces applied to the chromosome should be studied by electron microscopy and immunofluorescence. These studies are under way.

The success rate for holding anaphase chromosomes was much higher than that for the acceleration of metaphase chromosomes. We were successful in holding a pair of anaphase chromatids motionless with the optical trap in 82% of the cells observed. For metaphase cells, however, only 63 out of 204 cells (31%) responded to the trapping force. One of the main reasons for this difference may be that the biological parameters, such as the orientation, shape, size, and geometry of the trapped chromosome during metaphase differs from cell to cell. Also, the possibility can not be ruled out that the time for sensing the opposing force due to the optical trap may be critical and specific. All these factors may affect the experimental results. However, studies involving the holding of anaphase chromosomes were performed under conditions that were more consistent. The parameters listed above were more uniform from cell to cell.

When the optimal trapping force was applied to a pair of chromatids throughout anaphase, we noticed that the trapped chromosome was occasionally lost in the cleavage furrow or incorporated into one of the daughter cells. This implies that the optical trap force may be used as a potential micromanipulator in cell genetics to induce monosomic and/or trisomic cells that could be studied further by cloning the daughter cells.

In conclusion, these studies are the first to demonstrate the potential of light-induced optical forces ("optical tweezers") for the study of cell division and genetics. Considerable work must now be done to elucidate the structural and molecular aspects of optical trap manipulations and to further characterize and quantify the actual forces themselves.

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