Cell, Vol. 14, 477-488, July 1978, Copyright © 1978 by MIT

Stereo Immunofluorescence Microscopy: I. Three-Dimensional Arrangement of Microfilaments, Microtubules and Tonofilaments

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Summary

An easy manipulation of the commercial fluorescence microscope allows stereo pairs of pictures to be taken, which when examined with a stereo viewer, give a strong three-dimensional impression. The procedure is described in detail. Its use allows the documentation by immunofluorescence microscopy of the three-dimensional display and organization of microfilament bundles, microtubules and tonofilaments in some well characterized tissue culture cell lines.

Introduction

Microfilaments, microtubules and intermediate filaments are among the components forming the cytoskeletons of cells of higher organisms. The distribution and organization of these fibrous systems in cells in tissue culture have been studied extensively by electron microscopy (see, for instance, Buckley and Porter, 1967; Goldman and Knipe, 1972) and by high voltage electron microscopy (Buckley, 1975; Wolosewick and Porter, 1976). These fibrous systems can be viewed also in immunofluorescence microscopy. Thus the intracellular organization of microfilament bundles has been viewed by antibody to actin (Lazarides and Weber, 1974; Goldman et al., 1975), and that of cytoplasmic microtubules by antibody to tubulin (Weber, Pollack and Bibring, 1975a; Brinkley, Fuller and Highfield, 1975; Osborn and Weber, 1976a, 1976b, 1977; Frankel, 1976). The organization of prekeratin-like tonofilaments has been demonstrated in one cell line (Osborn, Franke and Weber, 1977), and the display of 10 nm filaments has been demonstrated in other cell lines (Hynes and Destree, 1978).

In immunofluorescence microscopy, only the fibrous system of choice is viewed. Other features of the technique which may be of advantage under particular circumstances include the overview provided of the entire cell and the opportunity to observe numerous cells simultaneously. Thus the common features of the intracellular organization of any antigen which is in the form of a supramolecular structure can be readily discerned. For example, in the case of microfilament bundles, immunofluorescence microscopy has shown that these bundles are not just F actin in a storage form, but contain several accessory proteins. The biochemical anatomy of these microfilament bundles (reviewed by Weber, 1976) has revealed the presence of myosin (Weber and Groeschel-Stewart, 1974), tropomyosin (Lazarides, 1975) and α-actinin (Lazarides and Burridge, 1975), as well as filamin (Wang, Ash and Singer, 1975), indicating that they could act as cytomuscular structures. Immunofluorescence microscopy has also been used to show that drugs such as the cytochalasins and the actin-specific drug phalloidin cause dramatic rearrangements of the actin present in the cell (Weber et al., 1976b; Pollack and Rifkin, 1976; Rathke et al., 1977; Wehland, Osborn and Weber, 1977). In the case of cytoplasmic microtubules, immunofluorescence microscopy has documented convincingly that these structures traverse the cytoplasm for long distances between the perinuclear area and the edge of the cell. After depolymerization, cytoplasmic microtubules appear to regrow in a unidirectional manner from the cytocenter to the plasma membrane (Osborn and Weber, 1976a, 1976b; Frankel, 1976). In the case of intermediate filaments, immunofluorescence microscopy is helping to define the different subclasses and to document the location of these filaments within cells (see, for example, Osborn et al., 1977; Hynes and Destree, 1978).

In spite of these insights into the cellular anatomy, however, current immunofluorescence microscopy even with excellent micrographs has lacked the three-dimensional informational content which must be present in its specimens. Here we report a simple manipulation of the fluorescence microscope which permits stereo pairs of pictures to be obtained. These pictures, when examined with a stereo viewer, give a strong three-dimensional impression. We have used this approach to visualize, in several commonly used tissue culture cell lines, the three-dimensional display and organization of microfilaments, microtubules and tonofilaments.

Results and Discussion

Stereo Microscopy

Binocular light microscopes equipped with epifluorescence optics and a recording camera are currently used widely in high resolution immunofluorescence microscopy. Although not generally appreciated by the manufacturers, these microscopes can be adapted to obtain stereo pairs of pictures of a specimen. The principle relies on known optical rules for the light microscope, and the extremely few changes on the microscope which it demands are also freely reversible. It is based on the use of a

half-aperture which is inserted appropriately into the light path of the microscope before taking the first micrograph. The position of the half-aperture is then held at the same level in the light path but rotated through 180°, and the second micrograph is taken. Details of the necessary modifications are given in Experimental Procedures. The pictures provided in this paper are best viewed with a stereo viewer, although some observers are able to fuse the two images with the unaided eye. Two lens stereo viewers are available from several commercial sources.

The impression obtained with stereo pairs depends in part upon the relative placement of each member of the stereo pair. As illustrated below, the same stereo pair can be mounted so that the cell appears as though it is viewed either from above or from underneath, although once the micrographs are mounted, only one view is possible. We initially thought also that the stereo impression might be influenced by the choice of direction from which the cell is photographed. Normally since the cells are grown on coverslips, which are later mounted on glass slides and viewed by epifluorescence, we photograph the cells from underneath; a "flip-flop" technique, however, can also by used to photograph the cells from above (see Experimental Procedures). Experiments with the three fibrous systems studied here showed that stereo micrographs of cells which are photographed from underneath and then mounted "in reverse"—that is, so the cell appears as though it is viewed from above—give a three-dimensional impression similar to stereo micrographs of cells photographed from above and then mounted normally so the cell is viewed from above. Since the cells are easier to photograph when viewed from underneath because less light is absorbed by the mounting medium, most of the stereo pairs presented below are of cells photographed from underneath and then mounted in reverse.

Actin-Containing Structures

Stereo pairs of some actin-containing structures are shown in Figures 1–8. In tissue culture cells, actin is detected by fluorescence microscopy in microfilament bundles and in submembraneous surface protrusions such as microvilli, microspikes and blebs. Diffuse cytoplasmic fluorescence is also seen. In general, the distribution of actin-containing structures seems to be related to cell shape. Thus large flat cells will often have well developed microfilament bundles, whereas smaller, more rounded cells are more likely not to contain thick bundles of microfilaments, but often display a wealth of strongly staining surface protrusions. Processes and ruffles are strongly stained by the antibody, as are certain specialized structures such as polygonal nets.

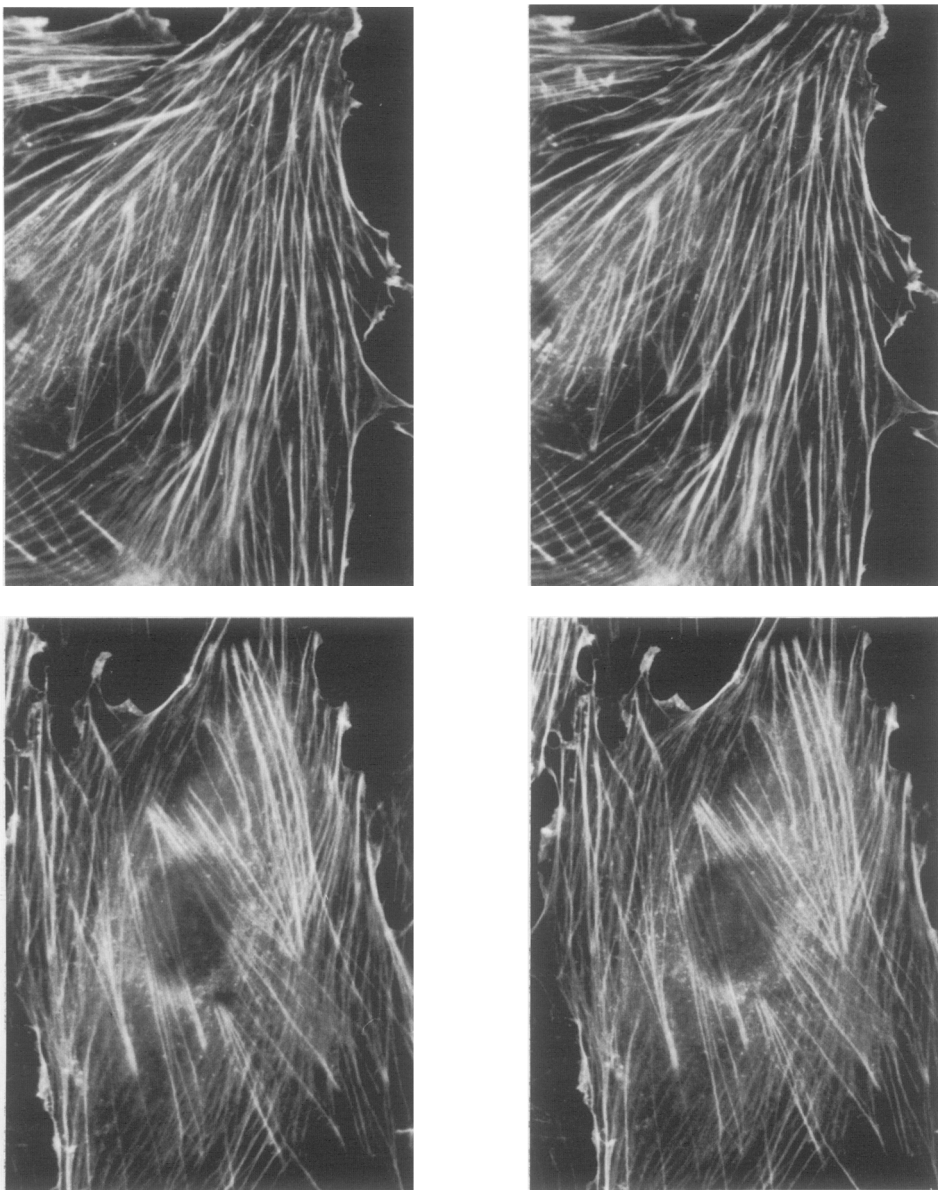
Microfilament Bundles

In mouse 3T3 cells, rat mammary cells or mouse embryo fibroblasts, immunofluorescence microscopy with actin antibody shows that microfilaments are organized into a system of bundles of various diameters which run parallel to each other for long distances and sometimes converge to focal points (Lazarides and Weber, 1974). These "actin cables" are predominantly found as submembraneous bundles of microfilaments on the adhesive side of the cell and correspond to the stress fibers observed by phase-contrast microscopy in living cells and by electron microscopy (Buckley and Porter, 1967; Goldman et al., 1975). The actin cables terminate in nonruffling areas of the cell close to the cell margin, but in ruffling areas, they seem to terminate prior to the ruffle.

Inspection of the stereo micrographs shown in Figures 1–4 confirms these impressions and, in addition, shows several additional features. First, in cells such as 3T3 cells (Figures 1 and 2), the actin cables present on the cytoplasmic side of the lower plasma membrane are not present in exactly the same focal plane, but can be seen to cross over and under each other. Second, in many types of cells including 3T3 cells and rat mammary cells, a second set of microfilament bundles is present in some cells. This fiber set is displayed above the nucleus, and thus the nucleus is contained within the two sets of microfilament bundles. As shown in Figures 1 and 3, these microfilament bundles lie above the nucleus and often run from one end of the cell to the other, underneath the upper plasma membrane. These arch-like actin cables insert at an acute angle into the much more strongly developed system of actin cables, lying parallel to the lower surface. Third, the insertion points of these arches, as well as the points where microfilament bundles present on the cytoplasmic side of the lower membrane intersect [see, for example, Figure 2, which can be compared with the electron micrograph in Figure 12 of Goldman et al. (1975)], probably represent the focal points described by Lazarides and Weber (1974). Fourth, in contrast to the cells in Figures 1–3, PtK2 cells, which are probably of epitheloidal origin and grow in flat sheets, do not seem to show microfilament bundles running above the nucleus. The actin cables are found within a very narrow focal plane and often run in several different directions within this plane (Figure 4). Strong staining of peripheral microfilament bundles and of the lamellipodia-like extensions of these cells is also often seen.

Polygonal Nets of Ordered Microfilament Bundles

Geodesic dome-like structures of highly ordered



Figures 1 and 2. Stereo Micrographs of 3T3 Cells Treated with Antibody to Actin and Viewed in Indirect Immunofluorescence Microscopy

The fixation conditions for this and subsequent figures are given in Experimental Procedures. Cells were photographed from below (except for Figure 7), and either mounted in reverse (Figure 1 and all subsequent figures, except for Figures 2 and 11B) or mounted so that the cell is viewed from below (Figures 2 and 11B) (see text). In Figure 1, the microfilament bundles appear to cross over and under each other; in Figures 1 and 2, a second set of microfilament bundles above the nucleus is also visible. Focal points are present both on the lower surface (Figure 2) and where the upper set of fibers inserts into the lower set (Figure 1). Magnification 620×.

microfilament bundles have been described in spreading rat embryo cells (Lazarides and Burridge, 1975; Lazarides, 1976). These investigators have emphasized that these nets can occur above the nucleus, as well as in other cytoplasmic regions. The stereo pairs in Figures 5 and 6 show these nets in an established line of epidermal origin in which they occur with a frequency of ~15%. In Figure 5, in which the cell was treated with actin antibody, a polygonal net occurs above the nucleus, and connecting microfilament bundles cascade arch-like into the lower set of submembraneous actin cables on the adhesive side of the cell. In other cells, these polygonal nets can be found close to the cellular margin and in lower parts of the cell body. This is illustrated in Figure 6, where myosin antibody was used. The finding that myosin is located along connecting microfilaments but absent in the vertices of the nets continues the protein chemical anatomy of these structures. Ear-

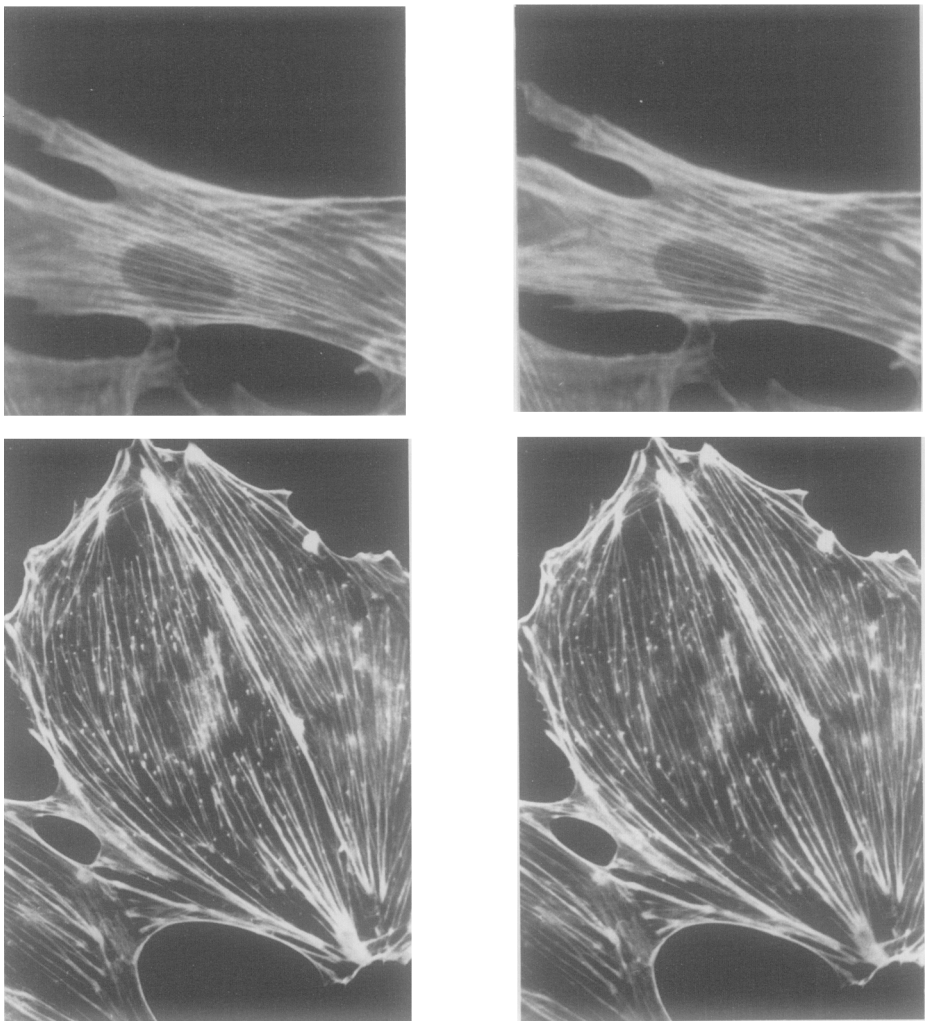


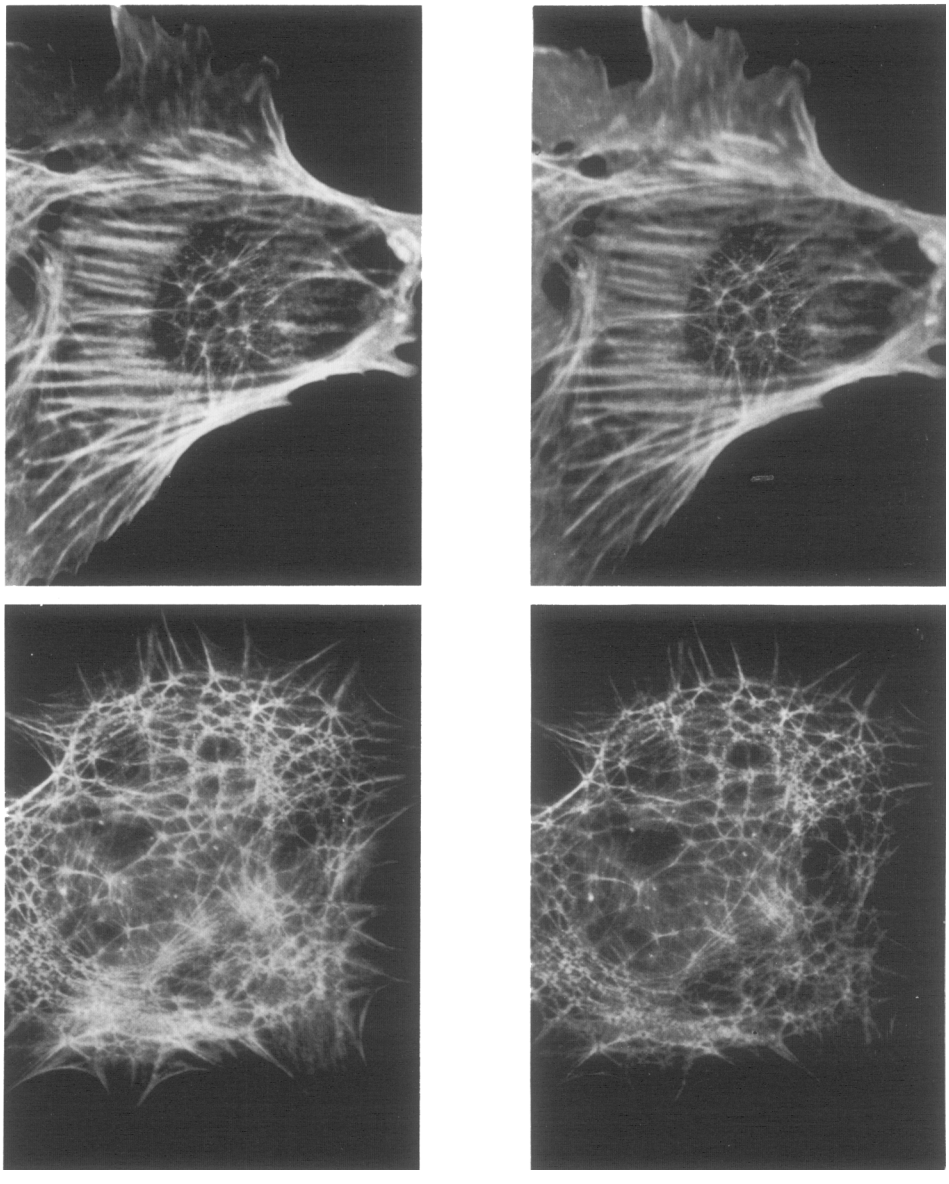
Figure 3. Stereo Immunofluorescence Micrograph of Rat Mammary Cells Treated with Antibody against Actin
Microfilament bundles are present above the nucleus. Magnification 620×.

Figure 4. Stereo Immunofluorescence Micrograph of Rat Kangaroo PtK2 Cells Treated with Antibody against Actin
The microfilament bundles seem to be present in a very narrow focal plane. The dots on the upper surface may correspond to microvilli (see text). Magnification 600×.

lier studies (Lazarides and Burridge, 1975; Lazarides, 1976) have shown actin to be present both in the connecting microfilament bundles and in the vertices, whereas α-actinin and tropomyosin were arranged in a mutually exclusive fashion, α-actinin being present in the vertices but not in the connecting microfilaments, and tropomyosin showing the reverse distribution. It remains to be seen whether the geodesic dome-like structures really represent intermediate arranagements in cell spreading as suggested by Lazarides, or whether they are a special arrangement forming a supporting net underneath the membrane typical of only certain cells. Irrespective of their function, however, they are a beautiful example of highly ordered structures which can occur at a variety of places in the cytoplasm, including the area above the nucleus.

Other Actin-Containing Structures: Microvilli, Ruffles and Cell Processes

Our actin antibody stains the microvilli in amphibian oocytes (Franke et al., 1976). Microvilli in tissue culture cells should be decorated by the antibody since these structures contain ordered arrangements of microfilaments (Follet and Goldman, 1970). The stereo pair of HeLa cells in Figure 7 shows numerous thin surface protrusions which may correspond to microvilli since scanning electron microscopy of these cells shows abundant microvilli (Porter, Fonte and Weiss, 1974). The dot-like structures present on the upper surface of a variety of cell types (see, for example, Figure 4)



Figures 5 and 6. Stereo Views of Polygonal Nets Visualized in an Established Cell Line of Epidermal Origin

In Figure 5, the polygonal net lies above the nucleus and has been stained with antibody to actin. In Figure 6, the polygonal net lies around the cell periphery and has been stained with antibody to myosin. Actin appears to be present in the vertices of the networks while myosin is not (see text). Magnification 600×.

could also correspond to microvilli, although a direct comparison between electron microscopic methods and fluorescence microscopy on the same specimen is necessary to prove this. The stereo pair in Figure 7 shows that retraction fibers also stain strongly with the actin antibody.

The three-dimensional nature of cell ruffles and cell processes has been beautifully demonstrated in numerous scanning electron microscopic studies, as well as in movies of living cells. Since these structures contain actin, the three-dimensional nature of the actin arrangement is also apparent in stereo immunofluorescence microscopy. Figure 8 shows a stereo pair of a neurite-like process of a neuroblastoma cell in which, as expected (Ludueña and Wessells, 1973), microspikes are strongly stained.

The cells shown in Figures 1–8 show some general cytoplasmic fluorescence, as do ordinary immunofluorescence micrographs of many types of cells stained with actin antibodies. Quantitation of the proportion of actin present in the different structures is not yet possible, since the affinity of the antibody for the different actin polymers pres-

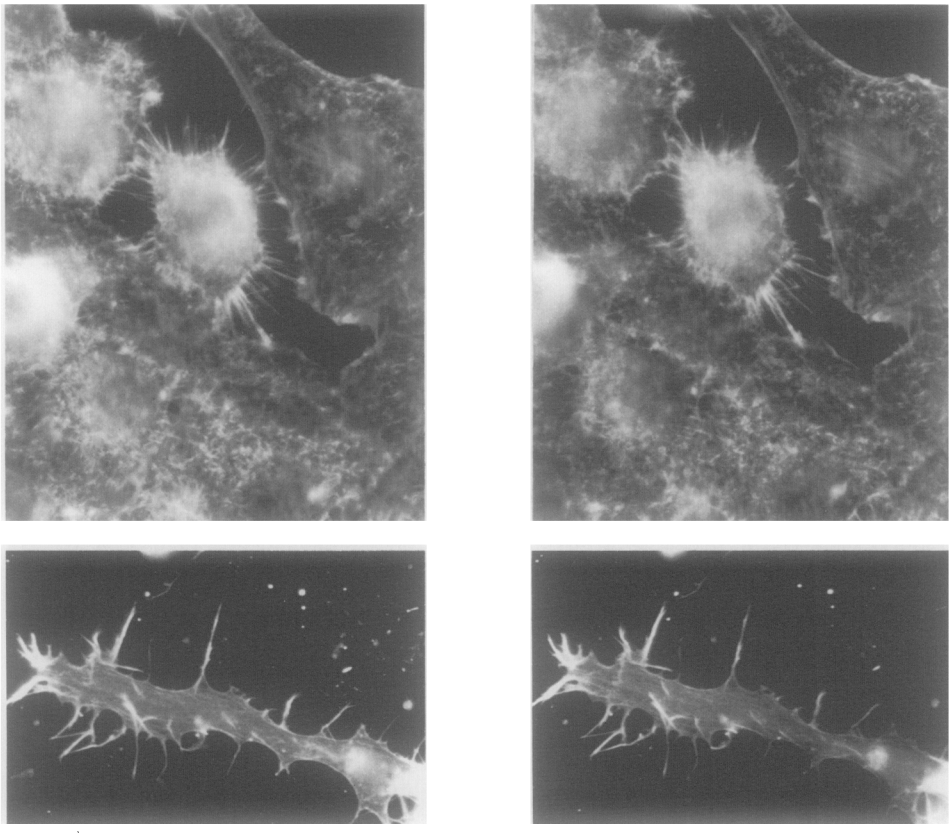


Figure 7. Stereo Views of Hela Cells Treated with Actin Antibody

Surface protrusions and the retraction fibers of the mitotic cell are strongly stained. Magnification 600×.

Figure 8. Stereo View of a Neurite-Like Extension of a Neuroblastoma Cell (Clone N-18) Fixed with Glutaraldehyde and Stained with Antibody to Actin

The surface protrusions stain strongly. This process was photographed from above. Magnification 620×.

ent in the cell, and for soluble actin, has not been determined. In addition, it still remains to be shown whether any of the actin forms are preferentially extracted or destroyed during the fixation procedures, and what the effects of the different fixation procedures are on the cell surface.

Stereo pairs have been taken for a variety of cell types stained with the known accessory proteins of the microfilament bundle—that is, myosin, α-actinin, tropomyosin and filamin. These will be documented elsewhere.

Tubulin-Containing Structures

Stereo pairs of tubulin-containing structures are shown in Figures 9–11. In cells in tissue culture, tubulin is detected by fluorescence microscopy in cytoplasmic microtubules, mitotic spindles, cilia and vinblastine-induced paracrystals.

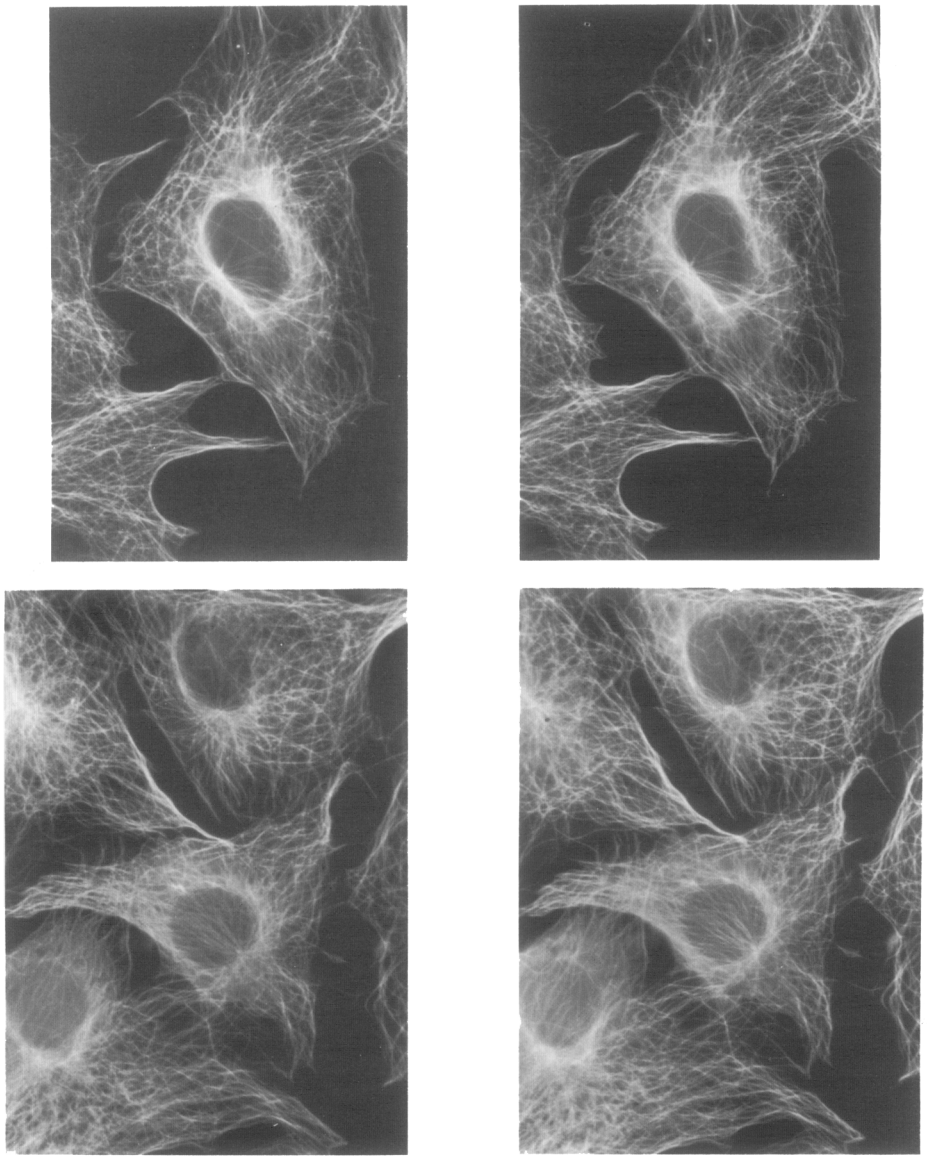
Cytoplasmic Microtubules

Cytoplasmic microtubules can be visualized by antibodies to tubulin (Weber et al., 1975a), and their distribution in a wide variety of tissue culture cells has been documented (see, for example, Brinkley et al., 1975; Frankel, 1976; Osborn and Weber, 1976a, 1976b, 1977). They seem to be present both in normal and in transformed cells (Osborn and Weber, 1977; however, see also Brinkley et al., 1975). In addition, glutaraldehyde can be used as a fixative in immunofluorescence microscopy either with antibodies which display a strong preference for glutaraldehyde-fixed tubulin (Eckert and Snyder, 1978) or with normal tubulin antibodies (Weber, Rathke and Osborn, 1978). The patterns obtained seem equivalent to those obtained in the earlier studies using formaldehyde or organic solvents, although a better ultrastructural preservation is achieved with glutaraldehyde.

Immunofluorescence microscopy with monospecific tubulin antibody has described the following features of cytoplasmic microtubules in well spread fibroblastic cells (above references). Cytoplasmic microtubules traverse the cytoplasm for very long distances and can often be followed for the whole distance between the perinuclear space

and the edge of the cell. Microtubules seem in general to conform with cellular morphology and to enter most cellular processes. Sometimes microtubules seem to bend gently and then to follow the cell margin. Microtubules do not show as strong a parallel orientation with the lower adhesive side of the cell as the actin fibers. They often seem to originate from an area close to the nucleus which acts as a microtubular organizing center. Recently, in a direct comparison of fluorescence microscopy and electron microscopy on the same specimen, we have been able to show that individual fluorescent fibers correspond to single microtubules, at least in well spread areas of the cell (Osborn, Webster and Weber, 1978).

Stereo microscopy emphasizes the three-dimensional display of cytoplasmic microtubules. Selected micrographs of cytoplasmic microtubules in mouse 3T3 cells after treatment with glutaraldehyde are shown in Figures 9–11. Examination of these figures suggests the following features. First, many of the microtubules seem to originate in the perinuclear area. These microtubules then cascade at steep angles from the perinuclear space through



Figures 9 and 10. Stereo View of Mouse 3T3 Cells Fixed with Glutaraldehyde and Treated with Monospecific Antibody against Tubulin
Many microtubules can be traced from the perinuclear area to the plasma membrane and appear able to bend. They are present at all levels in the cytoplasm, including both above and below the nucleus. In some cells, the impression of a unifocal origin of microtubules seems strong. Figure 9 magnification 550×; Figure 10 magnification 600×.

the endoplasm. After this rather precipitous fall, they reach the well spread ectoplasm of the cell and are in the majority now displayed in the same focal plane. These microtubules enter the different cell processes where they can appear as bundles or as individual fibers. Second, it is very often possible to follow individual microtubules from the perinuclear space to the edge of the cell—that is, for distance $\geq 50\ \mu$. Third, on reaching the membrane, microtubules can stop approximately at right angles to it (for example, Figure 10) or can run for long distances parallel to the membrane surface (for example, Figure 11). Fourth, microtubules are not restricted to the lower part of the cell. They are also clearly present, although in restricted number, above the nucleus. Fifth, bends in microtubules are frequently seen (for example, the microtubules cascading into the ectoplasm in Figure 11B, and the microtubules running parallel to the lower surface in Figure 11A). Sixth, the complex three-dimensional microtubular pattern very often obscures the documentation of the cytocenter and

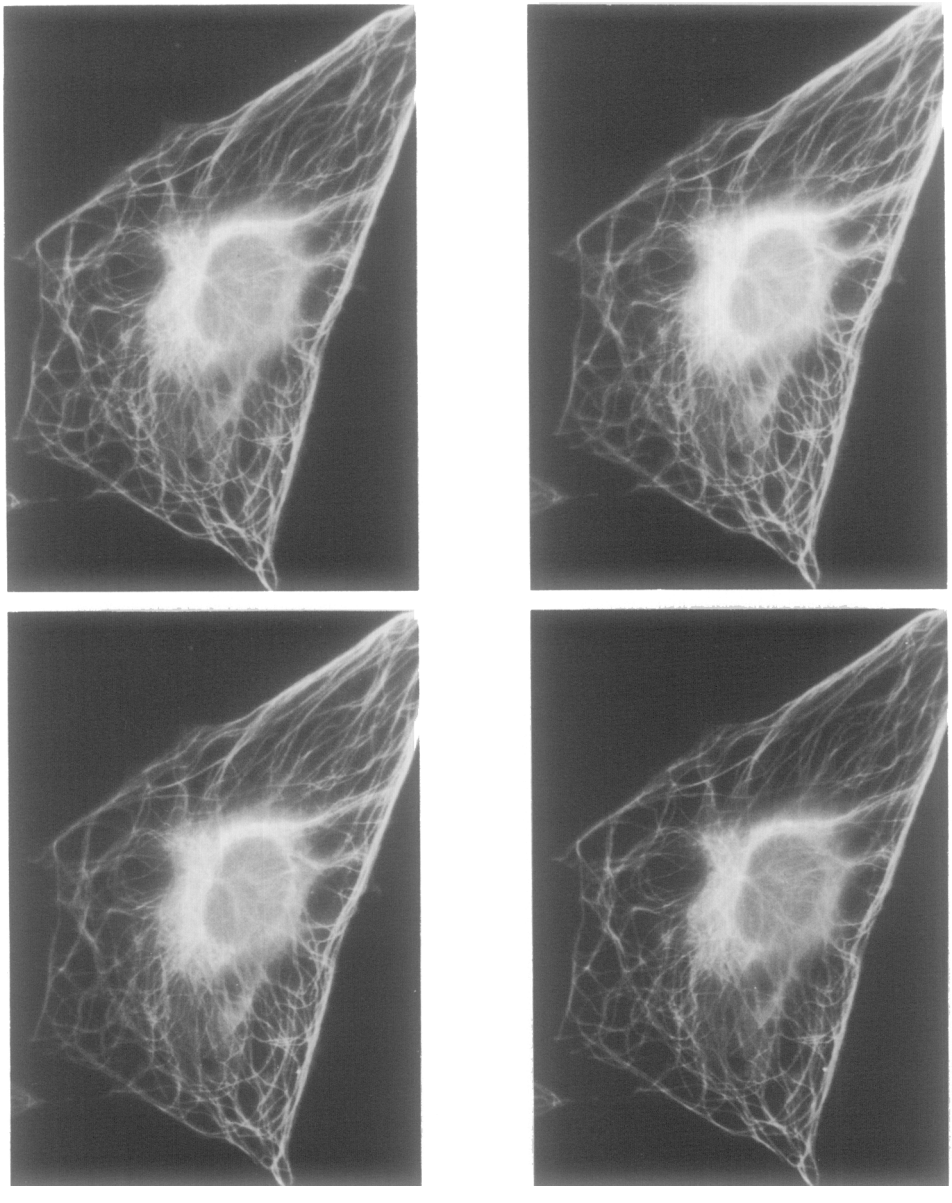
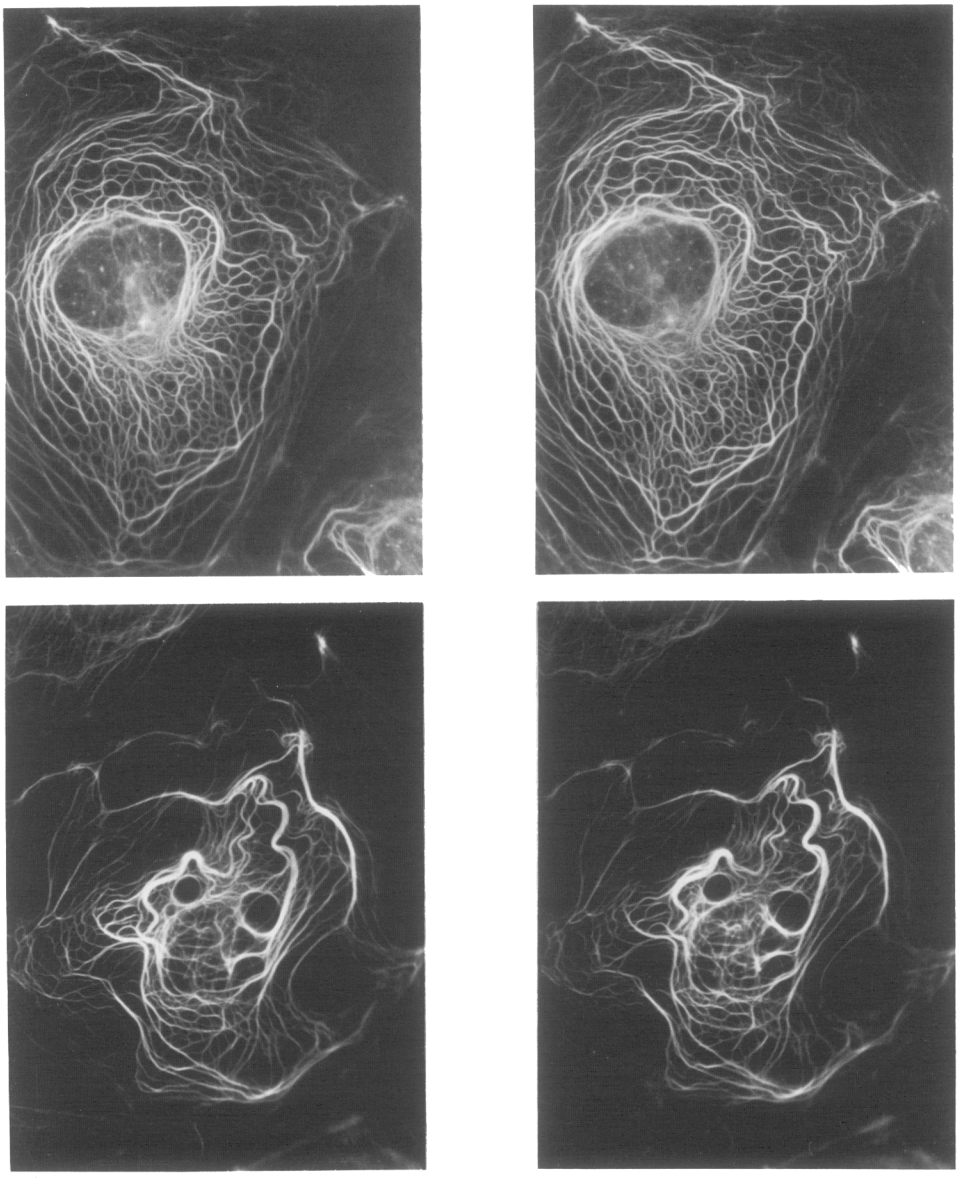


Figure 11. Cytoplasmic Microtubules in a 3T3 Cell Photographed from Below

The same stereo micrographs are shown mounted either in reverse so that cell is viewed from above (A) or directly so that the cell is viewed from below (B). Note the different impressions obtained and the presence of microtubules parallel to the lower membrane in (B). Magnification 710×.

the primary cilium identified previously in these cells (Osborn and Weber, 1976a), although it can be distinguished in some of the stereo pairs (for example, Figure 10). Other cell types including PtK2 cells and macrophages show a unifocal origin of microtubules more clearly (see also Frankel, 1976). Seventh, when microtubules cross over each other, an increased light intensity is observed (for example, Figures 9 and 11). As discussed elsewhere (Osborn et al., 1978), if microtubules are closer together than ~2000 Å, they will not be resolved as individual fluorescent fibers, but an increased light intensity will be observed. Finally, Figure 11 illustrates the different impressions obtained of cytoplasmic microtubules when the same stereo pair is mounted so that the cell is viewed either from above (Figure 11A) or from below (Figure 11B). Microtubules displayed in the region of the cell above and to either side of the nucleus are more clearly seen in Figure 11A (that is, in the cell viewed from above), whereas microtubules parallel to the lower adhesive side of the cell are better displayed in Figure 11B (that is, in the cell viewed from below).



Figures 12 and 13. Stereo View of the Bundles of Tonofilament-Like Fibers Present in Rat Kangaroo PtK2 Cells

The fibers surround the nucleus, and are found both above and below the nucleus (Figure 13). The two prominent "holes" in the center of Figure 13 contained nuclear material which was separated from the rest of the nuclear material by tonofilaments. Magnification 610×.

Tonofilament-Like bundles

The established rat kangaroo cell lines PtK1 and PtK2 contain a complex system of wavy fibers which are different from microfilaments and microtubules, and which can be detected either with certain rabbit autoimmune sera (Osborn et al., 1977) or with monospecific antibodies against prekeratin (Franke et al., 1978). In immunofluorescence microscopy, these fibers are revealed as bundles of variable diameters. They are especially strong around the nucleus, but are also found in the other areas of the cell, where some of them connect to points at the cell boundary resembling desmosomes of a fascia adherens-like organization. In electron microscopic analysis, these fibers appear as bundles of individual filaments (7–10 nm thick) which appear flexible and wavy and show a variety of bizarre branchings and interconnections, resembling the tonofilament or prekeratin fibrils present in certain epithelial and epidermal cells (Osborn et al., 1977). It is not yet clear whether a normal 10 nm system of the type described by electron microscopy for other cell types (Goldman and Knipe, 1972) is present in PtK2 cells in addition to the tonofilament bundles.

Stereo microscopy confirms these observations and gives strong three-dimensional images of the spatial organization of these tonofilament-like bundles (Figures 12 and 13). They swirl around the nucleus, often forming a perinuclear cage. Bundles can be distinguished both above and below the nucleus. They cascade down from the perinuclear space into the more flattened cytoplasm, where they seem to connect to finer fibers of the ground plasm. Sometimes the bundles bend and turn toward the upper side of the cell before sloping once again to the outer margins of the cell. The overall impression is that of a network, which is particularly well developed in the perinuclear cytoplasm, and which upon reaching the lower levels of the cell is present at different levels before it inserts into desmosome-related structures at the cell periphery. Clearly the majority of the tonofilament-like bundles lie above the focal plane of the actin cables, which in PtK2 cells are found close to the adhesive side of the cell (see Figure 4).

Outlook

Stereo microscopy with a normal commercial fluorescence microscope is an easy procedure. It avoids the stage-tilting principle used to obtain stereo pictures in the electron microscope, a procedure which seems not to be suitable for fluorescence microscopy because of the limited depth of the available high resolution objectives. Instead it uses a half-aperture diaphragm and a tube extension which are available commercially, and which can be easily and reversibly incorporated into the instrument. The three-dimensional impression is excellent, and the procedure is obviously not restricted to fluorescence microscopy, but can also be used for other light microscopic techniques.

The stereo impressions coupled with the high resolution and sensitivity of the fluorescence microscope, as well as the possibility of using fixation procedures which avoid drying of the specimen, should aid in the further understanding of static and dynamic aspects of the cellular cytoskeleton. An especially attractive feature is the possibility of viewing the underside of the cell. Thus the results can be compared directly with those obtained by reflection-contrast microscopy, a procedure which emphasizes cell-substratum contacts (see, for example, Izzard and Lockner, 1976). In the future, it will also be interesting to compare views of the same specimen obtained by stereo immunofluorescence microscopy with views obtained by the more established techniques of scanning electron microscopy and of stereo electron microscopy of whole mounts.

Experimental Procedures

Cells and Immunofluorescence Microscopy

Growth conditions for mouse 3T3 cells (Osborn and Weber, 1976a), rat mammary cells (Rathke et al., 1977), rat kangaroo PtK2 cells (Osborn et al., 1977), neuroblastoma cells, clone N-18 (Marchisio, Osborn and Weber, 1978), and HeLa cells (Osborn and Weber, 1977) have been described.

Cells were grown on round 12 mm glass coverslips and fixed for immunofluorescence microscopy by one of the following procedures. All steps unless otherwise noted were at room temperature. First, 3.7% formaldehyde in phosphate-buffered saline (PBS) for 10 min, methanol at −10°C for 6 min, acetone at −10°C for 30 sec, air-dry (Weber et al., 1975b) (Figures 1–5). Second, Procedure as above, but with the omission of the formaldehyde step (Osborn et al., 1977) (Figures 6 and 13). Third, methanol at −10°C for 6 min, PBS wash (Osborn and Weber, 1977) (Figure 12). Fourth, 1% glutaraldehyde in PBS for 15 min, followed by organic solvents (procedure II of Weber et al., 1978) (Figures 8–11). Fifth, 3.7% formaldehyde in PBS for 10 min, 0.2% Triton X-100 in PBS for 2 min (Heggeness, Wang and Singer, 1977) (Figure 7). With most actin-containing structures, the selection of a particular fixation procedure seems not to be critical. For microtubules, the best stereo impression is obtained with the fourth procedure, although stereo pictures have also been taken after procedures one and three. For tonofilament bundles, procedures two and three were used.

The procedures for indirect immunofluorescence microscopy have been described (Weber, Bibring and Osborn, 1975b). The first antibody, elicited in rabbits, was applied for 45 min at 37°C. After washing with PBS, the second antibody—fluorescein-labeled goat anti-rabbit IgGs—was applied at ~0.5 mg/ml. After incubation at 37°C for a further 30–45 min, the coverslips were again washed well with PBS and mounted in Moviol on microscope slides. Alternatively, if the cells were to be viewed from the top (for example, Figure 8), the coverslip was mounted on a second coverslip which in turn was mounted on the microscope slide (Schliwa, Osborn and Weber, 1978). Slides were photographed with a Zeiss photomicroscope equipped with epifluorescence optics and modified as described for stereo microscopy.

Antibodies

The actin antibody was elicited in rabbits against denatured chicken gizzard actin (Weber et al., 1976b). Its properties are similar to those of our original actin antibody (Lazarides and Weber, 1974). The antibody was made monospecific by affinity chromatography on homogeneous monomeric pig brain actin covalently bound to Sepharose 4B. The myosin antibody was elicited in rabbits against denatured myosin heavy chains from gizzard myosin (Weber, 1976). Its properties are similar to those of our original anti-gizzard myosin antibody (Weber and Groeschel-Stewart, 1974). The antibody was made monospecific by affinity chromatography on homogeneous gizzard myosin covalently bound to Sepharose 4B. The tubulin antibody was raised in rabbits against homogeneous 6S porcine cerebrum tubulin free of microtubule-associated proteins and made monospecific by affinity chromatography on homogeneous tubulin covalently bound to Sepharose 4B (Weber, Wehland and Herzog, 1976a). The arguments for the tubulin specificity of such antibodies have been summarized (Osborn and Weber, 1977). In addition, such antibodies can be used to measure tubulin quantitatively in a radioimmunoassay (G. Hiller and K. Weber, manuscript in preparation). Rabbit sera-decorating tonofilaments in PtK2 cells have been described (Osborn et al., 1977). In this work, antibody against tonofilaments was used either after purification (Figure 12) or after passage over prekeratin bound to Sepharose 4B (Figure 13). The patterns obtained with these sera on PtK2 cells are very similar to those obtained with the autoimmune sera, and to those obtained with antibodies raised against prekeratin and then made monospecific (Franke et al., 1978).

Stereo Microscopy

The parts required for stereo microscopy can be made in the workshop or assembled after modifications are made to two attachments available from the manufacturers for other purposes. These parts consist of an intermediate ring fitted with a lens to compensate for the increased tube length (part number 474465, Carl Zeiss, Oberkochen, FRG) and a differential interference contrast slider (part number 474571, minus the prism, also from Carl Zeiss). The metal pins in the intermediate ring are removed so that the slider can be moved easily in and out of the ring. A metal diaphragm is welded into the slider to cover approximately half the aperture to form the "stereo insert." Optical principles suggest that the stereo insert should be as close to the exit pupil of the objective as possible. This is achieved by screwing the intermediate ring into the normal revolving nosepiece and attaching one of the objectives used for fluorescence microscopy to it. The stereo insert can now be slid into the intermediate ring to obscure one half of the field. After taking the first picture of the stereo pair, the stereo insert is removed, inverted and replaced, so that the opposite half of the field is blocked. The second picture of the pair is then taken.

Photographs were taken with the automatic camera of the Zeiss photomicroscope on Tri-X film using ASA settings between 800 and 1100.

The following points seem important for obtaining good stereo micrographs: sensitive films should be used to take two exposures of the same specimen without too much bleaching; the three-dimensional impression will be increased if the plane of focus is chosen not at the bottom of the cell with its wealth of microfilament bundles and microtubules, but slightly above it; the focal plane should not be changed between the first and the second exposure, and the exposures should be made quickly so that the plane of focus does not drift because of temperature of mechanical changes.

Acknowledgments

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Received March 22, 1978; revised May 1, 1978

References

Brinkley, B. R., Fuller, G. M. and Highfield, D. P. (1975). Cytoplasmic microtubules in normal and transformed cells in culture: analysis by tubulin antibody immunofluorescence. Proc. Nat. Acad. Sci. USA *72*, 4981-4985.

Buckley, I. K. (1975). Three dimensional fine structure of cultured cells: possible implications for subcellular mobility. Tissue Cell *7*, 51-72.

Buckley, I. K. and Porter, K. R. (1967). Cytoplasmic fibrils in living cultured cells. Protoplasma *64*, 349-380.

Eckert, B. S. and Snyder, J. A. (1978). Combined immunofluorescence and high voltage electron microscopy of cultured mammalian cells using an antibody that binds to glutaraldehyde-treated tubulin. Proc. Nat. Acad. Sci. USA *75*, 334-338.

Follet, E. A. C. and Goldman, R. D. (1970). The occurrence of microvilli during spreading and regrowth of BHK-21/C13 fibroblasts. Exp. Cell Res. *59*, 124-136.

Franke, W. W., Rathke, P. C., Seib, E., Trendelenburg, M. F., Osborn, M. and Weber, K. (1976). Distribution and mode of arrangement of microfilamentous structures and actin in the cortex of the amphibian oocyte. Cytobiolobie *14*, 111-130.

Franke, W. W., Weber, K., Osborn, M., Schmid, E. and Freudenstein, C. (1978). Antibody to prekeratin: decoration of tonofilament arrays in various cells of epitheloid character. Exp. Cell Res., in press.

Frankel, F. R. (1976). Organization and energy-dependent growth of microtubules in cells. Proc. Nat. Acad. Sci. USA *73*, 2798-2802.

Goldman, R. D. and Knipe, D. M. (1972). Functions of cytoplasmic fibers in non-muscle cells. Cold Spring Habor Symp. Quant. Biol. *37*, 523-534.

Goldman, R. D., Lazarides, E., Pollack, R. and Weber, K. (1975). The distribution of actin in non-muscle cells: the use of actin antibody in the localization of actin within the microfilament bundles of mouse 3T3 cells. Exp. Cell Res. *90*, 333-344.

Heggeness, M. H., Wang, K. and Singer, S. J. (1977). Intracellular distribution of mechanochemical proteins in cultured fibroblasts. Proc. Nat. Acad. Sci USA *74*, 3883-3887.

Hynes, R. O. and Destree, A. T. (1978). 10 nm filaments in normal and transformed cells. Cell *13*, 151-163.

Izzard, C. S. and Lockner, L. R. (1976). Cell to substrate contacts in living fibroblasts. An interference reflexion study with an evaluation of the technique. J. Cell Sci. *21*, 129-160.

Lazarides, E. (1975). Tropomyosin antibody: the specific localization of tropomyosin in non-muscle cells. J. Cell Biol. *65*, 549-561.

Lazarides, E. (1976). Actin, α-actinin and tropomyosin interaction in the structural organization of actin filaments in non-muscle cells. J. Cell Biol. *68*, 202-219.

Lazarides, E. and Burridge, K. (1975). α-Actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cells. Cell *6*, 289-298.

Lazarides, E. and Weber, K. (1974). Actin antibody: the specific visualization of actin filaments in non-muscle cells. Proc. Nat. Acad. Sci. USA *71*, 2268-2272.

Ludueña, M. A. and Wessells, N. K. (1973). Cell locomotion, nerve elongation and microfilaments. Dev. Biol. *30*, 427-440.

Marchisio, P. C., Osborn, M. and Weber, K. (1978). Changes in intracellular organization of tubulin and actin in N-18 neuroblastoma cells during the process of axon extension induced by serum deprivation. Brain Res., in press.

Osborn, M. and Weber, K. (1976a). Cytoplasmic microtubules in tissue culture cells appear to grow from an organizing structure towards the plasma membrane. Proc. Nat. Acad. Sci. USA *73*, 867-871.

Osborn, M. and Weber, K. (1976b). Tubulin-specific antibody and the expression of microtubules in 3T3 cells after attachment to a substratum. Exp. Cell Res. *103*, 331-340.

Osborn, M. and Weber, K. (1977). The display of microtubules in transformed cells. Cell *12*, 561-571.

Osborn, M., Franke, W. W. and Weber, K. (1977). Visualization of a system of filaments 7-10 nm thick in cultured cells of an epitheloid line (PtK2) by immunofluorescence microscopy. Proc. Nat. Acad. Sci. USA *74*, 2490-2494.

Osborn, M., Webster, R. E. and Weber, K. (1978). Individual microtubules viewed by immunofluorescence and electron microscopy in the same PtK2 cell. J. Cell Biol., in press.

Pollack, R. and Rifkin, D. B. (1976). Modification of mammalian cell shape: redistribution of intracellular actin by SV40 virus, proteases, cytochalasin B and dimethylsulfoxide. In Cell Motility, R. D. Goldman, T. Pollard and J. Rosenbaum, eds. (New York: Cold Spring Harbor Laboratory), pp. 403-417.

Porter, K. R., Fonte, V. and Weiss, G. (1974). A scanning electron microscope study of the topography of Hela cells. Cancer Res. *34*, 1385-1394.

Rathke, P. C., Seib, E., Weber, K., Osborn, M. and Franke, W. W. (1977). Rod-like elements from actin-containing microfilament bundles observed in cultured cells after treatment with cytochalasin A(CA). Exp. Cell Res. *105*, 253-262.

Schliwa, M., Osborn, M. and Weber, K. (1978). The microtubules system of isolated fish melanophores as revealed by immunofluorescence microscopy. J. Cell Biol. *76*, 229-236.

Wang, K., Ash, J. F. and Singer, S. J. (1975). Filamin, a new high-molecular weight protein found in smooth muscle and non-muscle cells. Proc. Nat. Acad. Sci. USA *72*, 4483-4486.

Weber, K. (1976). Biochemical anatomy of microfilaments in cells in tissue culture using immunofluorescence microscopy. In Contractile Systems in Non-muscle Tissues, S. V. Perry, A. Margreth and R. S. Adelstein, eds. (Amsterdam: North-Holland), pp. 51-66.

Weber, K. and Groeschel-Stewart, U. (1974). Antibody to myosin: the specific visualization of myosin-containing filaments in non-muscle cells. Proc. Nat. Acad. Sci. USA *71*, 4561-4564.

Weber, K., Pollack, R. and Bibring, T. (1975a). Antibody against tubulin: the specific visualization of cytoplasmic microtubules in tissue culture cells. Proc. Nat. Acad. Sci. USA *72*, 459-463.

Weber, K., Bibring, T. and Osborn, M. (1975b). Specific visualization of tubulin containing structures in tissue culture cells by immunofluorescence. Cytoplasmic microtubules, vinblastine induced paracrystals and mitotic figures. Exp. Cell Res. *95*, 111-120.

Weber, K., Wehland, J. and Herzog, W. (1976a). Griseofulvin interacts with microtubules both *in vivo* and *in vitro*. J. Mol. Biol. *102*, 817-829.

Weber, K., Rathke, P. C., Osborn, M. and Franke, W. W. (1976b). Distribution of actin and tubulin in cells and in glycerinated cell models after treatment with cytochalasin B. Exp. Cell Res. *102*, 285-297.

Weber, K., Rathke, P. C. and Osborn, M. (1978). Cytoplasmic microtubular images in glutaraldehyde-fixed tissue culture cells viewed by electron microscopy and by immunofluorescence microscopy. Proc. Nat. Acad. Sci. USA *75*, 1820-1824.

Wehland, J., Osborn, M. and Weber, K. (1977). Phalloidin induced actin polymerization in the cytoplasm of cultured cells interferes with cell locomotion and growth. Proc. Nat. Acad. Sci. USA *74*, 5613-5617.

Wolosewick, J. and Porter, K. (1976). Stereo high-voltage electron microscopy of whole cells of the human diploid line W1-38. Am. J. Anat. *147*, 303-323.