A New Single-control Micromanipulator

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With one Plate

INTRODUCTION

SEVERAL types of micromanipulator have been described (for reviews see Peterfi, 1928; Chambers and Kopac, 1937; Seifriz, 1936). The growing importance of micrurgical techniques in bacteriology, electrophysiology, embryology, plant physiology, and experimental cytology have focused attention on the problem of micromanipulator design. The question has assumed a new importance with the recent development of two revolutionary advances in microscopy. The first of these is the high-performance reflecting microscope of Dr. C. R. Burch (see Burch, 1947; Barer, 1948*a*). The instrument in use at Oxford has a working distance of 13 mm. when used at N.A. 0·65. This long working distance is exceptionally useful for microdissection, as it enables the usual type of moist chamber and hanging drop preparation to be dispensed with. It is now also possible to perform micromanipulation on the surfaces of intact organs *in situ*, e.g. the liver, spleen, kidney, or brain of anaesthetized animals (Barer, unpublished results). The second outstanding advance in microscopy has been Zernike's phase-contrast method (Zernike, 1942; Burch and Stock, 1942; Barer, 1947*b*, 1948*b*). This enables living unstained cells to be studied under optical conditions vastly superior to those hitherto available. There is no doubt that much early work will now have to be repeated and extended by the use of this method. The technique of microdissection by phase-contrast illumination is far from easy, but the results amply repay the trouble.

FEATURES DESIRABLE IN A MICROMANIPULATOR

1. *Single Control*

Most micromanipulators, e.g. those of Chambers and Peterfi, depend on three main controls, one for each direction in space. These usually take the form of screws which operate rack-and-pinion mechanisms or cause the spreading-apart or sliding motion of metal plates. While such methods of control may give very precise linear movements, the latter are rather limited and unnatural, since in order to go from point A to point B, one can only travel via X, Y, and Z. This would not be a very convenient way of writing one's name, nor is it the ideal method for microdissection. The ideal micromanipulator should enable the operator to move an instrument smoothly and rapidly along any desired path between two points in space. So far as we are aware this ideal condition has yet to be achieved, and the mechanical problems involved are very formidable. Fortunately an approach can be made to the problem if we are prepared to sacrifice complete smoothness of control in three dimensions, substituting instead smooth movement in one plane combined with an independent movement in a direction perpendicular to this plane. Simple micromanipulators based on this principle have been described by Buchthal and Persson (1936) and by Schuster (Barer, 1947*a*). These instruments allow any desired movement in a vertico-lateral plane, with a screw control for antero-posterior movements. Their great advantage is that all movements are operated from a single control, which remains in the hand during the entire operation, but their inherent weakness lies in the fact that for most high-power work the greatest precision of movement is required in the *horizontal* plane, i.e. the plane in focus under the microscope. Thus, although such micromanipulators are quite satisfactory for use at medium magnifications (up to about 500 times), their use at higher powers demands considerable skill. Of the instruments which allow complete freedom of movement in a horizontal plane perhaps the best known is that of de Fonbrune (1932, 1937). This outstanding high-precision design has been developed over a period of many years and the present model must rank as one of the finest available. It is unusual in that it works on pneumatic principles. Air pressure from three mutually perpendicular pistons is transmitted through three rubber tubes, on to three tambours, resembling aneroid barometers, which are connected to a lever holding the micro-instrument. The pressure in the pistons is controlled by a single handle. Movement of the handle in a horizontal plane moves one or both of the horizontal pistons, and vertical movement is obtained by a screwing motion of the handle. With practice something very near to complete freedom of movement in three dimensions can be achieved. The de Fonbrune micromanipulator incorporates a number of other important features which will be referred to below.

2. *Freedom from Vibration*

The elimination of vibration is essential in micrurgy. Factors which may help in this respect are (1) massive construction; (2) clamping the micromanipulator and microscope to a common base-plate; (3) remote control. Most commercial micromanipulators rely on factors (1) and (2), either alone or together. Massive construction is usually no disadvantage in high-power cytological work, but it may be an encumbrance if the instrument is to be used for other types of work, where it may require to be poised in mid-air at an angle. The same may sometimes be said of factor (2). Some micromanipulators are only designed for use for one specific purpose and a more or less fixed base-plate assembly is provided. This greatly limits the versatility of the instrument and in general it is preferable to have an instrument which can be made quite independent of the microscope if required. A common base-plate with clamps can always be added as an accessory. The Schuster micromanipulator is unusually adaptable in this respect. Although usually mounted on a heavy base, the effective part of the instrument can be detached and mounted in any position or orientation on a clamp or sliding bar. This makes it particularly useful for accurate positioning of electrodes. Remote control, i.e. absence of rigid connexion between the control screws or handle and the micro-instrument holder itself, is obviously a valuable feature in reducing vibration, and accidental jarring of the controls may not be transmitted to the micro-instrument itself. Although remote control was introduced into a form of the Chambers micromanipulator, the de Fonbrune is the remote-control instrument *par excellence*. Here the single-control handle and the instrument proper are built as two independent units, connected only by a considerable length of flexible rubber tubing. The instrument holder is comparatively light and delicate but freedom from vibration and accidental jolts is assured by this independence, which also enables the instrument to be used to some extent for other than cytological work. For maximum stability and robustness there is no doubt that massive construction is an advantage. On the other hand, remote control is often very convenient for ease of manipulation and may enable a lighter type of construction to be adopted.

3. *Freedom from Play* (*backlash or lost motion*)

It is essential that the instrument holder should respond without delay to any movement of the controls, and that there should not be any further movement or 'creep' on sudden removal of the hand. This 'dead-beat' condition can only be achieved by careful attention to details of design. If differential screw feeds or rack-and-pinion movements are used they must be made with a considerable degree of precision. Sliding surfaces and bearing points must be of suitable material and should be so designed that the effects of wear are eliminated. Multiple levers and cam mechanisms are on the whole to be avoided as they are rarely free from play. This fault makes the design of micromanipulators on the usual principles of a pantograph rather impracticable.

4. *Variable Sensitivity of Control*

It is extremely useful to be able to vary the sensitivity of control according to the magnification of the microscope. If this cannot be done it may be found that work under low powers is too slow, especially with screw controls, as it may take a long time to traverse the field of a low-power objective. Again with screw controls a large field can as a rule only be traversed by a series of turns of the screw, thus leading to intermittence and jerkiness of operation. Some degree of variation of sensitivity can be introduced into the Chambers and rack-and-pinion instruments by attaching special levers on to the operating screws, but this profusion of rods jutting out in various directions of space is clumsy and inconvenient. One of the most successful methods of achieving smooth and *continuous* variability of sensitivity is that adopted by de Fonbrune. A sliding collar is fitted around the vertical control handle. Rods run from this collar to each of the two pistons which control movements in a horizontal plane. A movement of the sliding collar alters the angle of movement of these piston levers and thus the range and sensitivity of motion in the horizontal plane. The vertical movement is unaffected, but variation of this is relatively unimportant for most work. This mechanism is exceedingly valuable, but three disadvantages should be noted. In the first place the transition from rather coarse to very fine movement is not linear but tends to be rather abrupt—most of the reduction takes place in the final few millimetres of travel of the sliding collar. This is not a very serious fault since the variation is continuous. Secondly, the range of movement diminishes with increasing sensitivity; for example, if the sensitivity is doubled the range of movement is halved. This is inconvenient if very fine work has to be done over a very large field, although this requirement is rare in practice. In principle a screw mechanism could be made free from this fault. The third disadvantage is rather more serious. A movement of the sliding collar not only alters the angle of movement of the horizontal piston levers but moves the pistons bodily by a small amount, thus shifting the micro-instrument. The latter thus has to be recentred and may even move out of the field of view altogether.

5. *Limitation of Movement to the Field of View*

In most micromanipulators no provision is made for this. Thus if by chance the micro-instrument should wander out of the field of view it may be very difficult to find it again. This is particularly so when working at high magnifications, when the only practicable plan may be to return to an objective of lower power. In the de Fonbrune instrument the movement of the control handle is limited by a metal circle. The range of movement can be made to correspond exactly with the field of view by adjustment of the sliding collar mentioned above.

6. *Rapid Centration*

Some form of coarse movement to enable rapid centration of the micro-instrument in the field of view is highly desirable. This may not be so important in instruments made to clamp in a fixed position relative to the microscope, and when micro-instruments of a standard length are used, but it is almost essential in other cases. A coarse vertical motion is also desirable.

7. *Robustness, Price, &c.*

This is to some extent connected with the question of massive construction. In choosing between two instruments of roughly equal performance it is natural to decide in favour of the more robust and less easily damaged. It is also important that any accidental damage should be capable of easy repair, preferably by any competent workshop technician, and that spare parts should be available at low cost. This puts delicate instruments and those involving high-class precision engineering at a disadvantage. It is often possible to cut down the price of an instrument very considerably by employing methods of design not involving high-precision fits. Price and robustness are important considerations in a micromanipulator, since for most work a pair of instruments is required.

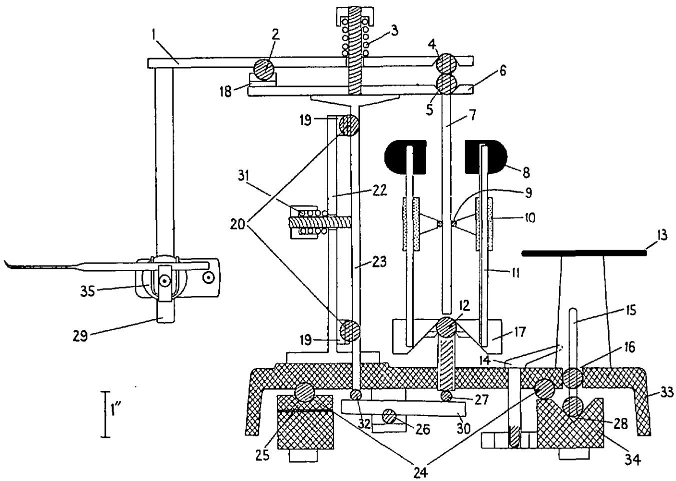
The New Micromanipulator

Although the resemblance is at first difficult to find, the present instrument began as a simple modification of the Schuster micromanipulator. As we have seen, the chief drawback of the latter is the fact that smoothest motion occurs in a vertical plane, whereas we should like it in the horizontal plane. A fairly obvious modification was thus to turn the instrument through 90°, with the control handle vertical, and to attach the micro-instrument holder to the upper sliding plane. The first rough models gave surprising results. The action in a horizontal plane was beautifully smooth and controllable, and by attaching the control handle to a heavy block of metal which could slide on a polished surface it was possible to trace most complicated patterns with extreme rapidity. Unfortunately, in order to obtain perfect uniplanar motion the vertical movement had been sacrificed. Experiments were made with a simple tilting vertical motion by means of a screw fixed through the base of the instrument, but this did not prove sufficiently good for high-power work. At the same time the first results seemed so promising that it was felt justifiable to build a much more ambitious model incorporating several of the desirable features described above.

The construction of the latest version of the new instrument will be seen from the diagram (Text-fig. 1) and photograph (Pl. I). The steel ball-bearings (2) of the horizontal movable brass plate (1) rest in a groove and a flat (18) on the horizontal fixed brass plate (6), clamped by the adjustable spring (3). The double-ball-ended lever (4, 5, 7) locates in two cones in the plates (1) and (6) and is gripped by three small steel balls (9) mounted on a slider (10) which moves on three rods (11) fixed to the plastic handle (8) and the coned base (17). Various types of bearing points, including jewel tips, were tried at (9), but the steel balls were found to be most satisfactory and did not score the lever (7).

The coned base (17) is clamped to the screw-mounted steel ball (12), the lower end of the screw making contact with a steel ball (27) fixed to the lever (30), which moves about the ball fulcrum (26). The balls (20, 20), fixed to the vertical movable plate (23), rest in V-grooves and a flat (19, 19) of the vertical fixed plate (22). These two plates are held together by the adjustable spring (31). The lower end of the vertical plate (23) is fixed to a ball (32) resting on the lever (30). The balls (24, 24) of the heavy base (33) rest in grooves (25) and flats on the undercarriage (34). The base (33) and undercarriage (34) can be fixed together by the clamp (14). The short double-ball-ended lever (15) locates in a hole (16) in the base (33) and in a cone (28) in the undercarriage (34).

The micro-instrument holder (35) is clamped to the tool post (29) and is adjustable vertically, horizontally, and angularly before locking.



TEXT-FIG. 1. Diagram of micromanipulator. For explanation of the numbers see text.

OPERATION

The micromanipulator is placed at a convenient distance from the microscope, and if desired it can be clamped to a common base. (The three supports on the undercarriage can be conveniently sunk in three slots.) The micro-instrument holder is adjusted to a suitable height and the micro-instrument inserted. It should be noted that the type of holder we have found best is a small version of the Singer clamp (Registered Design No. 847073). This holder will accommodate anything from a very fine needle to a tube 9 mm. in diameter, thus allowing the use of non-polarizable micro-electrodes of wide bore. The micro-instrument is held in place by pressure from a metal leaf against two V slots. The micro-instrument does not have to be held horizontally as is the case with many other types of holders, but can be used at any desired angle. The desirability of having a rack-and-pinion movement of the holder on the post (29) was considered, but it was felt to be an unnecessary refinement for most work.

The clamp (14) is now loosened and the tip of the micro-instrument is centred rapidly in the field of view by means of the handle (15) which causes the base (33) to slide on the undercarriage (34). This movement of the base on the undercarriage is of wide range and can even be used for very low-power micromanipulation. As soon as the micro-instrument is centred the clamp (14) is tightened, preventing any further movement of the base.

The hand of the operator rests on the plastic hand-rest (13). The fingers grasp the handle (8). Movement of the handle produces reduced motion through the balls (9) working on the ball (12) to the lever (7). This moves the plate (1) to which the tool post (29) is fixed. The plate (1) can only move in a horizontal plane. Thus a rocking movement of the handle (8) enables any desired curve to be traced in a horizontal plane, at any desired speed. Twisting of the handle (8) rotates the screw-mounted ball (12). This moves the lever (30) about its ball fulcrum (26), and allows the plate (23) to be raised or lowered against the fixed plate (22). The two horizontal plates (1) and (6) and with them the tool post (29) are thus moved vertically.

The slider (10) enables the sensitivity of the instrument to be varied through a wide range. When at its highest position, close to the handle (8), a fairly coarse movement of wide range is obtained, suitable for low-power work. In the lowest position, close to the coned base (17), the movement is very delicate, but reduced in range. At the same time it will be noted that the movement of the handle is automatically limited by its circumference coming into contact with the lever (7). This restricts the range of movement of the micro-instrument tip. In practice it will be found most convenient to move the slider (10) until a position is found at which the range of movement of the micro-instrument tip corresponds with, and is limited to, the field of view of the microscope in use at the time. The sensitivity of the instrument can be altered at will without any undesirable movement of the micro-instrument.

Remarks

We are now in a position to consider how far the present instrument meets the requirements (1) to (7) discussed above.

1. Although we have not achieved perfect control in three dimensions we have virtually perfect control in the two dimensions of a horizontal plane, combined with vertical movement controlled by the *same single handle*. The majority of cytological work can be carried out in a horizontal plane with only occasional use of the vertical movement. One minor fault must be pointed out here. It would be desirable to be able to carry out a vertical movement without risk of a slight horizontal displacement. This is sensibly so at low and medium sensitivities. At very high sensitivities, however, when the slide (10) is close to the coned base (17), the effective leverage is so great that the horizontal movements are carried out against appreciably less resistance, making it more difficult to twist the handle (8) without producing some slight horizontal displacement, though this can be done with practice. However, this tendency can be reduced to a large extent by tightening the screw spring (3), which increases the resistance to horizontal movement. The tension of the same spring can be adjusted for maximum ease of manipulation at any sensitivity.

2. The instrument will be found to be remarkably free from vibration. Even if the handle (8) is struck against the lever (7) with considerable force the vibration of the micro-instrument is relatively slight and rapidly damped. Movements of the hand on the hand-rest are usually without obvious effect. This feature has been achieved largely by massive construction.

3. Despite numerous trials no appreciable play has been noticed. This very important result has been achieved entirely without any fine-limit precision-engineering methods, but mainly by attention to the design of the bearing surfaces. Any tendency to the development of play as a result of wear can be taken up by adjustment of the tensions of the two springs (3) and (31). The design is such that the bearing surfaces would merely 'bed' into one another as wear occurs.

4. Sensitivity can be varied quickly and easily over a wide range, and without moving the micro-instrument. The variability is continuous and sensibly linear, with no sudden transition from coarse to fine movement. It will be noted, however, that the range is automatically reduced as the sensitivity is increased.

5. The range of movement can be limited at will to the field of view.

6. Rapid centring is achieved by movement of the base (33) on the undercarriage (34).

7. The instrument is exceptionally robust. There are no delicate components whatsoever, and no high-precision work is involved. Should repairs ever be necessary they can be carried out by any competent workshop technician. It may perhaps be stated that the instrument has been subjected to considerable mechanical violence on a number of occasions in order to test its strength, and on no occasion has damage ensued. The same robustness is evident in the very versatile micro-instrument holder. The lack of any high-precision work enables the instrument to be made relatively cheaply, bringing a *pair* of micromanipulators within the reach of most investigators. The possibility that the instrument might be used at marine biological stations has been considered, in deciding the best type of corrosion-resisting finish. Corrosion of metals by sea-water is a very difficult problem, but it is felt that chromium plating combined with reasonable care offers the most practical solution.

In conclusion it may be mentioned that the instrument has been tried out for a number of purposes, including microdissection by phase-contrast illumination, and has proved very successful in every way.

We wish to thank all those, too numerous to mention, who have aided us by their discussion, criticism, and advice. In particular Mr. P. J. Peade and Mr. J. Parkinson have been most helpful. We also wish to place on record our indebtedness to certain principles of design laid down by the late Dr. W. N. Bond.

R. BARER AND A. E. SAUNDERS SINGER—PLATE I

Summary

New developments in microscopy and electrophysiology have brought a renewal of interest in methods of micromanipulation.

A number of features desirable in the design of a micromanipulator is discussed.

A new micromanipulator is described. Its outstanding features are (1) single control; (2) massive construction with freedom from vibration; (3) freedom from play; (4) continuous variability of sensitivity; (5) limitation of range of movement to the field of view; (6) rapid low-power centring; (7) unusual robustness combined with delicacy of movement achieved without any high-precision methods. The performance of the instrument is adequate for high-power cytological work and for microdissection by phase-contrast illumination.

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