A method for relocation of specified regions in tissue culture dishes

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Summary. An apparatus and method are described which facilitate the rapid and accurate visual relocation of specified areas within ordinary, circular, plastic petri-dishes.

During the long term culture of cells and tissues it is frequently necessary to relocate specific cells or colonies for repeated examination or time-lapse photography. The apparatus and method described facilitate the rapid relocation of specified areas of interest within ordinary, circular, plastic petri-dishes of various diameters, using any microscope fitted with a mechanical positioning stage, the precision being limited by the accuracy of the stage scale.

The apparatus shown was designed for use with the Gillett and Sibert Inverted Conference Microscope, fitted with standard mechanical stage, scales and verniers. It is made from 3 mm Perspex sheet and consists of a spring-loaded jig which accomodates dishes of diameter 30–60 mm (figure 1). The base section is shaped to make 2 points of contact with the dish and is cut to fit between the microscope stage clamps. (In an alternative design 2 pins set in the base engage with sockets drilled in the microscope stage). 1 of the contact points bears a reference mark [M], for positioning the dish. A third contact point is on a spring-loaded finger bevelled on its upper surface so as to slide beneath the dish lid.

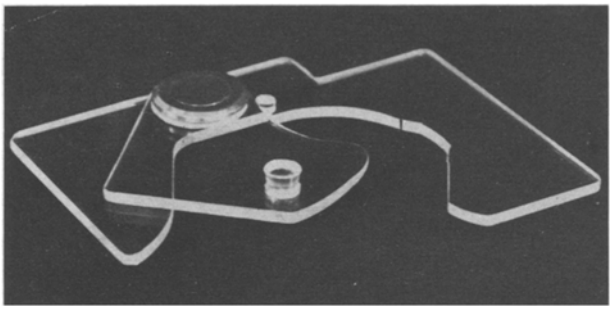

Fig. 1. The dish holder, showing reference mark M at one fixed contact point.

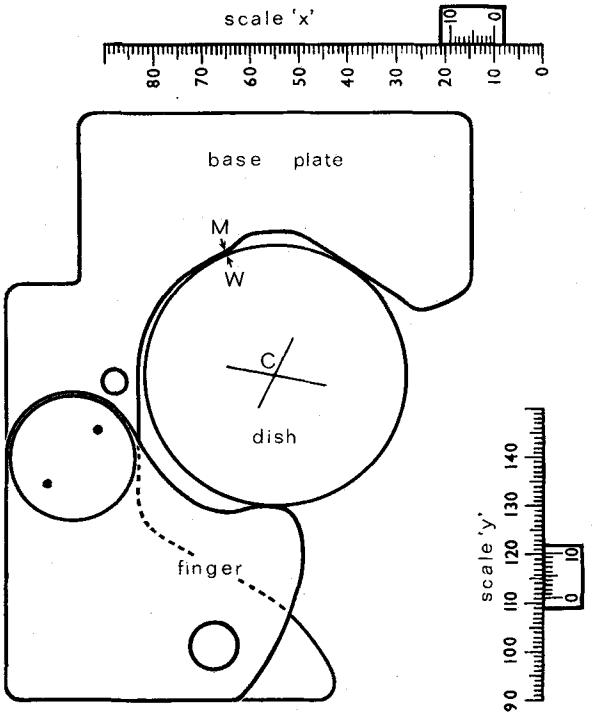

Fig. 2. Plan diagram of the holder with a marked dish in position.

In addition a means of marking a reference point at or near the centre of the dish is required, the most convenient being a needle and an engineer's centre square. The holder is clamped in position on the microscope stage and 2 reference points or crosses, are scratched on the outside surface of the dish containing the culture, 1 (C) at the centre of the base of the dish, the other (W) on the wall of the dish about 3 mm above its base. The dish is then placed in the holder and rotated until the reference point W is located over the reference mark M on the holder base plate (figure 2). The stage positioning knobs are adjusted until reference point C is centrally situated in the field of view and the microscope stage scale readings are recorded as accurately as possible (xC_1; yC_1). The stage positioning knobs are then adjusted to bring the area of interest (I) into the field of view and the scale readings again recorded (xI_1; yI_1).

When the area of interest is to be relocated, the clamps are replaced in any position on the stage and the dish inserted as above. The centre reference point C is again located in the field of view and a second set of scale readings (xC_2; yC_2) obtained. A pair of correction factors is then derived from the difference between the 2 sets of readings for the centre (xC_1–xC_2; yC_1–yC_2). The area of interest can then be relocated by moving the stage so that the scale readings correspond to a pair of values (xI_2; yI_2) calculated as the sum of the initial readings for this area and their respective correction factors:

$$(xI_2 = xI_1 + xC_1 - xC_2;\ yI_2 = yI_1 + yC_1 - yC_2)$$

In conjunction with the Gillett and Sibert Inverted Conference microscope fitted with standard accessories, the apparatus can be used to relocate culture dish areas with a mean positioning accuracy of 20 μm.

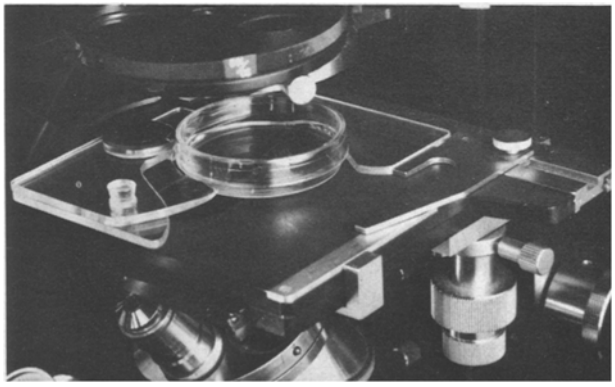

Fig. 3. The dish holder in use.