

The light microscope is increasingly used as analytical tool for recording signals from living cells and tissues. Cells are injected, impaled, or otherwise manipulated with probes that are mounted on a mechanical translator (micromanipulator). The following text deals with the choice of the essential components of a micromanipulation system and explains how products from Vestavia Scientific may fit these needs.

## The Microscope Stage

Most light microscopes feature a mechanical object stage that moves the specimen relative to the optical axis in the X- and Y-direction. Either the entire surface of the stage moves around or the specimen slides on a fixed stage, driven by a mechanical object guide. Some stages also incorporate a Z-drive for focusing which, however, makes them less stable and, therefore, less suitable for micromanipulations.

A particularly stable stage design can be found in top research microscopes, such as the Zeiss Axiovert™ and the Nikon Diaphot 300/200™ where the object stage is mounted like a bridge between two support columns. Compared to conventional microscopes, inverted microscopes - with the condenser on top and the objective below the stage - are much better suited for manipulations since the access to the specimen is less restricted.

## Mounting the Manipulator

In the simplest case, the manipulator is placed on the bench, next to the microscope. This type of mount is very unstable since any movement of the microscope caused by touching the controls or pressing against the eye piece will result in movements of the manipulator relative to the specimen.

A much better stability is obtained when the manipulator is mounted directly to the microscope. As illustrated in Fig. 1, this can be done in two different ways. In the *fixed* mount (left) the manipulator is attached to the body of the microscope or a fixed part of the object stage. In the *moveable* mount (right)

the manipulator is connected to a moving part of the stage or the object guide.

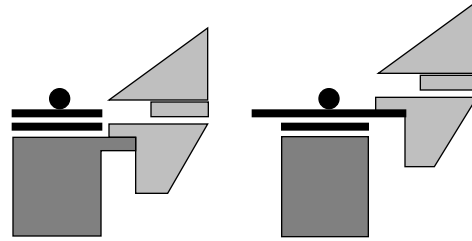


Fig. 1

The choice of mounts depends largely on the application. With a fixed mount, a probe connected to the manipulator, e.g. a glass pipette, stays in the same spot within the field of view while the specimen is moved around. This may be of advantage when injecting and probing a large number of cells.

In the movable mount, the manipulator moves together with the specimen. Thus, the pipette tip may stay inside a cell while the stage is re-positioned to inspect other parts of the tissue. Applications that employ more than one manipulator, e.g. the perfusion of isolated kidney tubules, generally demand a movable mount.

A second aspect in selecting a mount is the stability of the probe. When viewed through the microscope, a fixed mount appears to be more stable than a moveable mount since there are fewer mechanical interfaces between probe and microscope (Fig. 1). On the other hand, the geometry of specimen and probe is less secure as there are additional interfaces between them. For the micromanipulation of cells and tissues, the stability of the probe relative to the specimen is often the more important consideration and, therefore, a moveable mount is generally preferred.

## The Vestavia Stage Plate

The Vestavia stage plate features a quick-release mount for a specimen incubation chamber and provides for the attachment of up to two XYZ-manipulators. The plate simply rests on top of the microscope stage or can be bolted down by clamps. On light microscopes with fixed stages, the plate has

<i>Make</i>	Zeiss	Olympus	Nikon	Nikon	Zeiss
<i>Model</i>	Axiovert 100 Axiovert 135 IM35	IMT2	Diaphot	Diaphot 200 Diaphot 300	Axiovert 100 Axiovert 135
<i>Stage type</i>	fixed	moving	moving	moving	moving
<i>Stage width (mm)</i>	211 (238 w/guide)	230	225	272	
<i>Mounting holes (mm)</i>		4x M4 100 x 120	4x M3 60 x 110	4x M3 60 x 110	3x M4 (to be made)
<i>Catalog #</i>	01-30	01-31	01-31	01-32	
<i>Bracket</i>	02-40	02-40	02-40	02-42	02-41
<i>Clamps</i>		rectangular	round	round	
<i>Remarks</i>	attached to object guide				only 1 manipulator

to be connected to the object guide.

By providing a common platform for specimen and manipulator, the stage plate offers all the advantages of a moveable mount on a fixed stage. It also supplies excellent stability relative to the optical system since, when the stage plate is not moving, light microscope and manipulator essentially form a single unit (Fig. 2). As a fixed stage is often more compact than a moving stage, the manipulator can be positioned closer to the specimen, further enhancing the stability of the probe.

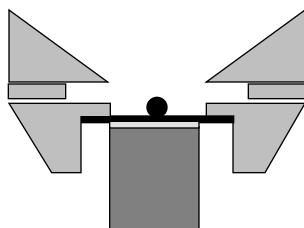


Fig. 2

Combined with a moving stage, the stage plate provides the convenience that the microscope does not need to be modified for accepting a specimen chamber and manipulator. Also, the manipulation system can be put on and off as one piece, without the need for a permanent attachment.

The plate can be fitted to a variety of inverted light microscopes (see Table). One version is designed for the mechanical stage with low drive on the Zeiss Axiovert™ or

IM35™. Another plate is for microscopes with moving stages, such as the Olympus IMT2™ and Nikon Diaphot™. A third plate fits the Nikon Diaphot 300/200™.

A compact version of the stage plate is intended for fixed manipulator mounts or applications that do not involve the use of a manipulator. Due to its small size, the plate fits most light microscopes, including conventional upright microscopes. The plate can be inserted into holders and cutouts for standard microwell plates.

### The Vestavia XYZ-Manipulator

Larger manipulators are generally more stable than small manipulators; however, this advantage may be offset by a higher weight and the difficulty to mount the manipulator close to the specimen. A critical feature that is often overlooked is the quality of the connection between manipulator and probe.

The Vestavia manipulator is a compact, three-stage mechanical translator with precision ball-bearing slides. It attaches to the stage plate in a hanging fashion which brings the controls within convenient reach of the operator's hand. The drives are backlash-free. For extremely delicate applications, the manipulator can be motorized.

Brackets for adaptation of the manipulator to a variety of microscopes are available (see

Table). Though primarily designed for the Vestavia stage plate, the manipulator can be attached directly to the stage of most inverted and upright microscopes.

The top stage of the manipulator accepts a wedge with dove-tail guide for connecting a pipette holder or the V-track of the Vestavia microperfusion system. The dove-tail mount combines high stability with the convenience of easy attachment and removal of the probe.

### **The Vestavia Perfusion Chamber**

The Vestavia perfusion chamber is designed for the incubation of wet biological specimens on the microscope stage. Its low-profile design provides free access from all sides. The chamber mounts to the stage plate via a quick-release connection. It slides-in from the front, facilitating the exchange of the specimen during the experiment and rapid removal after the experiment.

The bottom of the chamber is formed by a thin glass window that is available in thicknesses down to #00, allowing the use of an objective lens with short working distance and high numerical aperture.

The Vestavia stage plate contains five tapped holes for mounting various chamber accessories, such as tubing connectors, a bath electrode, and a temperature probe. The chamber can be ordered with built-in heating coil and custom temperature controller, featuring a low-noise DC power supply. For applications requiring a rapid bath exchange a smaller version of the chamber is available.

*vers. 10/00*