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Electrophysiology Tool Construction

David Ide¹

¹National Institutes of Health, Section on Instrumentation, NIMH, Bethesda Maryland

Abstract

This protocol documents the construction of a custom microscope stage system currently in widespread use by a wide variety of investigators. The current design and construction of this stage is the result of multiple iterations, integrating input from a number of electrophysiologists working with a variety of preparations. Thus, this tool is a generally applicable solution, suitable for a wide array of end-user requirements; its flexible design facilitates rapid and easy configuration, making it useful for multi-user microscopes, as individual researchers can reconfigure the stage system or have their own readily replaceable stage plates. Furthermore, the stage can be manufactured using equipment typically found in small research machine shops, and by keeping the various parts on hand, machinists can quickly satisfy new requests and/or modifications for a wide variety of applications.

Keywords

physiology apparatus; microscope stage; slice chamber holding

INTRODUCTION

It is generally desirable to mount micromanipulators holding electrodes in a stable relationship with the preparation (prep) being studied. A standard microscope-mounted stage for the prep, using electrodes held in manipulators mounted to the air table upon which the microscope sits, permits no movement whatsoever of the stage during recording. The standard configuration is to mount the stage and manipulators to the air table and reposition the microscope. One way to achieve this is with a large stable platform. Unfortunately, large platforms use up a lot of the working space and are inflexible in their configuration. Only some manipulators can be used. Changing the height of the platform is extremely time-consuming. Another configuration is to use a cantilevered stage. There are several cantilevered stages available commercially. However, none of them are as flexible as the stage system described here. The system described below takes up very little room on the air table, leaving more space for optimal positioning of the microscope. It can be mounted wherever there is room for it, as the amount of cantilever is adjustable. This is an advantage over some commercial systems that must be mounted at a fixed distance from the microscope optical axis. The system described here (Fig. 6.26.1) also conveniently allows attaching pipets, electrodes, and other tools. The stage can be easily mounted on a small x - y or x - y and z translator allowing standard stage motion, or in conjunction with a translating microscope, independent motion of the microscope and the stage. As there is no large

platform in the way, it is easy to achieve the minimum possible approach angle for electrodes or pipettes.

Most of the figures in this protocol are static screen exports from CAD renderings. They are provided only as a convenient visual reference and should NOT be used for construction. To view the CAD renderings, please use a suitable (freely available) viewing program (e.g., SolidWorks eDrawings). Again, most machine shops will know how to use the CAD files for construction purposes.

There are three supplemental files (file 1: bread_board_and_mouse_holder.EASM; file 2: stage and breadboard.EASM; file 3: stage_plate_with_dovetail_clamp.EASM) for this unit, which can be accessed at <http://www.currentprotocols.com/protocol/ns0626>.

BASIC PROTOCOL: CONSTRUCTION OF CUSTOM MICROSCOPE STAGE SYSTEM

The instructions below, combined with the 3-D CAD renderings, should provide a machinist with all required information for construction. This protocol is atypical in that the overwhelming majority of scientists are not expected to have machining expertise. Some may have access to a machine shop and wish to construct their own device. While there are no particularly difficult machining operations, attention needs to be given to some details for the system to work properly. Clearly there is a great deal of latitude in the details of each component, as well as methods of fabrication. The dimensions shown in the 3-D solid model have been found to work well for most users.

This protocol is organized in paragraph format intentionally. The CAD renderings are meant for machinist use, and the order of construction varies. The text below highlights important aspects for construction; these points should be communicated to the machinist(s).

Materials

Due to the degree of customization, it is impossible to provide a traditional materials list. Please have the machinist(s) refer to the CAD renderings. Pay particular attention to material type requirements. Except where noted the parts are made from 6061 aluminum, anodized after fabrication to resist corrosion from saline solutions.

Base (Fig. 6.26.2)—The base is made from 3-in. diameter aluminum bar stock. A 1.001-in. diameter hole is bored through and a side slit made to allow clamping of the 1-in. column. A hole is drilled, tapped, and counter bored for a 1/4–20 socket head cap screw at right angles to the slit to allow additional clamping of the column. Clearance holes are drilled through the face to accommodate screws appropriate for the intended air table. The hole for the column should be made precisely. In most cases, after the slot is cut, the hole will close up a little and it will be impossible to insert the column. A piece of metal can be inserted in the slot and a screw threaded into the threaded side of the clamping hole to open the base enough to allow insertion of the column. This procedure, while slightly cumbersome, results in very secure clamping. Alternatively, the hole for the column can be

made slightly larger, but care must be taken not to exceed the capability of the locking screw to compress the clamp.

Column (Fig. 6.26.3)—The column can be made of aluminum or stainless steel. In either case, it is preferable to use precision shafting so that other components will fit smoothly. Anodized and ceramic-coated 1-in. diameter shafting and precision stainless shaft material are available from McMaster-Carr. While standard 1-in. diameter bar could be used, the use of precision shafting is strongly recommended so that when several systems are constructed the components will remain interchangeable. If aluminum shaft is used, it should be anodized, both to resist corrosion and abrasion by the rotation of the stage holding block.

Stage holding block (Fig. 6.26.4)—The stage holding block is made from 1.5 in. × 3 in. 6061 bar stock, cut 3 3/8-in. long. A 1.001-in. hole is drilled and reamed 3-in. deep for a smooth sliding fit with the column. Two rows of 1/4–20 tapped holes are machined 1/2-in. deep in the top. Excess material is milled or sawn off to reduce the weight of the block and to reduce the space the block takes up near the microscope stage. The block can be easily split and holes drilled and tapped for locking screws. The locking mechanism, using a clamping wedge, shown in the drawing, works better as it tightens quite securely with a single screw or knob but when loosened slightly, allows the block to easily rotate; it is worth the extra trouble in fabrication.

Clamp (Fig. 6.26.5)—A clamp should be installed under the stage holding block to preserve height adjustment while allowing the block with the stage plate attached to be easily rotated to access the stage. The design shown in Figure 6.26.5 works well. Here, the clamp is machined from aluminum and then anodized; its thin profile results in very little screw force being needed to lock it quickly and securely to the column.

Stage plate (Fig. 6.26.6)—The lollipop-shaped stage plate is made from 1/4-in. thick 6061 aluminum plate, anodized after machining. Particular care should be taken to the fabrication of the large end of the lollipop. The center hole and the array of 4/40 tapped holes should be closely concentric to the outer diameter for the system to work properly. It is recommended that the dimensions shown in the CAD drawing be followed. If concentricity is not maintained, there will be interference between the chamber holding plate and the ball holders, and the ball holders may not fit. It is preferable to have the magnets (which will hold the chamber plate, see below) in hand before machining the holes into which they will be pressed. A very light press fit is desired to avoid deforming the stage plate. If the press is too tight, the plate will need to be re-machined after the magnet is inserted. An alternative construction is to make the hole oversized and secure the magnet with a set screw or glue.

Ball holders (Fig. 6.26.7)—The ball holders allow tremendous flexibility in attaching electrodes, perfusion pipets, and other tools securely and in close proximity to the preparation being studied. The ball holder bases are machined from aluminum. Several versions are shown in the CAD solid model; one is mounted to the top of the stage, another to the bottom. There is a round hole all the way through the base to receive the round ball holder post, and a square recess (with over cut corners) to receive a square ball holder. The corners of the bases are drilled and tapped for 4–40 socket head cap screws to secure the

posts. The round posts have a large degree of adjustability in height. The square posts can be removed and re-positioned repeatedly in the same position and orientation. The ¼-in. diameter brass balls are held in Delrin plastic blocks into which a cavity has been milled with a ¼-in. diameter ball end mill to slightly less than 1/2 the diameter of the balls, to allow clamping. 2–56 socket head cap screws are used to secure the two halves together. The clamping friction can be adjusted to allow movement or to rigidly secure the balls.

It is best to tap the lower holder all the way through so that different length screws can be used without running out of thread. The balls are drilled through to fit hypo tubing. 15-GA is handy as it allows passage of commonly used pipets. The hypo tube can be secured by soldering or with a set screw. A slot can be cut in the end of the hypo tube and the ends slightly compressed to hold another tube or pipet with spring action. There is considerable room for customization of the ball holders. The round holders are the easiest to construct and will satisfy most users.

Chamber plate (Fig. 6.26.8)—The chamber plate is made from carbon steel and then nickel plated to resist corrosion. It needs to be magnetic so that it will be held by the magnets inserted in the stage plate (see above). The plate shown will serve as a general guide—individual users will have to machine different-sized recesses to fit their particular chambers. Users of larger chambers may choose not to use the chamber plate at all but attach their chambers directly to the stage plate; the addition of a thin steel ring to a larger chamber will allow it to be held by the magnets in the stage plate. Alternatively, clamps can be devised to hold the chamber utilizing the many tapped holes available on the stage plate.

COMMENTARY

Advantages of this System

This system is superior to commercially available systems in several respects. It takes up very little of the scarce space on the air table and can be mounted almost anywhere, leaving room for other equipment. It is very compact around the preparation and under the microscope, leaving room for hands and other apparatuses. Unlike some commercial stages, the amount of cantilever is adjustable, as is the height of the stage plate. It provides many options for securing perfusion tubes, electrodes, wires, and whatever else may be desired. The ball holders are sufficiently low in profile to allow unobstructed access to the preparation with recording electrodes. Unlike the square opening in some commercial stages, the round opening in this design makes it possible to orient the preparation as desired. The diameter of this stage plate opening accommodates many different bath chambers, including the most popular commercially available ones. The modular design of this system makes it easy for two people to share a microscope, each user with their own stage plate, which can be rapidly exchanged, sharing the same column, or by having two complete systems which can be easily exchanged.

Adaptation for holding mouse (Fig. 6.26.9)—The stage system can be configured to hold a mouse for in-vivo work under the microscope. Due to the height of this type of preparation the stage plate must be lowered. This is accomplished with a different holding block that is bored all the way through so that it can slide down the column. A clamping

mechanism attached to the “joshplate” permits easy installation and removal of the mouse holding plate with a mouse held stereotactically. The mouseplate can be rotated. Fore and aft movement is achieved by moving the “joshplate.” Switching between an in-vivo mouse preparation and a cultured cell or slice preparation is quickly performed by switching holding blocks, as both use the same column.

Adaptation with dovetail clamp (Fig. 6.26.10)—The stage can be built using a dovetail clamp to hold the stage plate. This is preferable for those who wish to move the stage plate repeatedly and easily fore and aft. The clamp shown was purchased from Really Right Stuff (cat. no. B2-Pro/L).

Critical Parameters and Troubleshooting

Notes on machining—Most machine shops are prepared to work with Solidworks (sldasm and prt) files. Many CAM systems used to control CNC machine tools are capable of reading the geometric and dimensional data directly from the (sldasm and prt) files included in this protocol. Alternatively, these files can be used within Solidworks to produce working drawings of each component. Tolerances are pretty relaxed but attention should be paid to functionality. Easy sliding fits should be maintained between the column and the clamps. Concentricity should be maintained between the inside opening of the stage plate, the outside, and the tapped holes so that the ball holders can be positioned anywhere. All aluminum parts should be anodized to resist corrosion.

Anticipated Results

Provided the machine shop can work with Solidworks files (as discussed in the paragraph above), and any customizations are clearly communicated beforehand [by the customer to the machinist(s)], this protocol along with the CAD renderings should allow a machine shop to recreate the custom microscope stage system(s) without issue.

Time Considerations

Construction time varies depending on machine shop workload, availability of materials, and degree of customization requested. Allow several weeks for construction. Most of the expense is set up time so several systems can be built in almost the same time as one.

Figure 6.26.1.

(A) Actual microscope stage plate in use. Note that ball clamps are in top-mount orientation in this setup. (B) Static rendering of stage plate as described in the Basic Protocol.

Figure 6.26.2.

Close-up of the base. Central hole permits insertion of the column while the top holes allow attachment to the air table. Note the central slit running down the side, which facilitates insertion and removal of the column. Holes at the right angle of the base along the central slit permit secure attachment of the central column.

Figure 6.26.3.
Static rendering of the column inserted into base. For clarity, the base has been made transparent.

Figure 6.26.4.

(**A**) Oblique view of the holding block (solid) with clamping wedge (solid, gold) and securing screw (solid), attached to the column and the base (both transparent). (**B**) Oblique view of the holding block (transparent) with clamping wedge (solid) and securing screw (solid). (**C**) Rear view of panel B. (**D**) Oblique bottom view of the holding block, clamping wedge, and securing screw. (**E**) Oblique top view of the holding block, clamping wedge, and securing screw. Note the holes tapped along the top of the holding block. *NOTE: The column clamp is not shown in any of these images.*

Figure 6.26.5.

(A) Oblique view of the clamp attached to the column, directly under the holding block. (B) Rear view of panel A.

Figure 6.26.6.

(**A**) Top view of the stage plate attached to the holding block. In this view, the chamber plate and diamond chamber are visible at the circular end of the plate. (**B**) Bottom view of panel A. From this perspective, the two magnets attached to the chamber plate are visible. (**C**) Oblique view of the stage plate assembly. Note the slight elevation of the chamber plate and the diamond chamber. (**D**) Opposite oblique view of panel C. (**E**) Oblique top view of the stage plate for comparison to panel (**F**), where the chamber plate has been made transparent to provide a clearer view of the magnets that attach the chamber plate to the stage plate.

Figure 6.26.7.

(A) Rear top view of the stage plate, with focus on the ball holders. Five complete ball holders are visible here: starting clockwise from the 7 o'clock position is a square ball holder, followed by another square ball holder, a short round ball holder, a tall round ball holder, and another square ball holder. All holders use an undermount attachment mechanism in this rendering. (B) Oblique bottom view of panel A. Note attachment mechanism, as well as differences between square and round ball holders.

Figure 6.26.8.

(A) Top oblique view of the chamber plate installed in the stage plate, with diamond chamber. Magnets that secure the chamber plate to the stage plate are not visible in this view. (B) Bottom oblique view of the chamber plate; here, magnets are visible. Also, note the ridged construction of the stage plate. The small ridge allows the stage plate to sit in the hole in the stage plate. (C) Top oblique view of the chamber plate only. This provides a clear view of the chamber plate insertion area, with four screw holes to secure the diamond chamber to the stage plate.

Figure 6.26.9.

(A) Adaptation of the stage system for ACUC-approved in vivo mouse work. Several key differences include a modified, fully bored-through holding block and a different stage equipped with specialized apparatuses for securing mice. (B) Rear view of panel A without air table, showing the same wedge lock mechanism+clamp to secure the holding block. As mentioned in the manuscript, this modified tool shares the same base, column, and stage plate as the microscope-chamber plate setup. Please use CAD files for construction and additional information.

Figure 6.26.10.

(A) Rear oblique view of an alternate dovetail clamp adjustment mechanism for fore and aft movement of the stage plate. Rather than using screws to attach the stage plate to the various holes in the rear of the holding block, the dovetail mechanism uses a knob to permit easier and finer adjustment along the x axis. (B) Slightly more oblique view of panel A, with the holding block and a portion of the stage plate made transparent to highlight the differences in mechanism. Note the separate circular end of the stage plate, which is secured by screws from below. The holding block is unchanged, and the dovetail clamp acts as an intermediary between the holding block and stage plate.