A Simple Modification of the Stage Insert for Nikon Inverted Microscopes

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This article describes a simple, economical modification of the standard stage insert used on the Nikon TMD, Diaphot and E800 models of inverted microscopes to support slide securely without lateral motion or sample compression. The central portion of the stage on these popular microscopes consists of a removable insert that provides sample support and demarks of the imaging area. The insert may be replaced with holders for a variety of culture chambers and other specialized sample supports. This unique design contributes to the adaptability of these microscopes for a wide range of imaging applications such as confocal microscopy, calcium imaging and electrophysiology.

The standard insert is actually constructed of two pieces, an outer ring and a central plate (Figure 1A). The central plate is removed from the modified ring, which is then used in the inverted position (Figures 1B, 3, 4). The outer ring is made of 2 mm thick nickel-plated brass and has an outer diameter of 108 mm and an inner diameter of 72 mm (Figure 2B). The under side of the intact ring tapers toward the center to provide objective clearance. The inner margin of the ring has a 4 mm wide circular ledge depressed by 0.3 mm (Figure 2A). The 0.3 mm thick central plate normally sits in the 4 mm ledge, held in place with a brittle adhesive, creating a flat surface across the stage.

The central plate can be removed from the outer ring by simply positioning the insert upside down (beveled surface facing upwards) on a bench with several blocks of wood supporting the outer ring. The central plate should pop out when struck with a hammer. In practice, the central plates tend to fall out with heavy usage without need for percussive assistance.

A milling machine was used to cut two ledges extending into the tapered portion of the outer ring, at opposite sides of its central opening. The ledges extend 2 mm into the ring along the central axis of the slide. The ledges may be seen in Figure 1B and are shown schematically in Figure 2C. Two high magnification views of the ledges show their relationship to the tapered surface of the stage ring (Figure 3). When the modified ring is inserted into the microscope stage in its inverted position, slides can be supported by their ends resting on the two ledges (Figure 4). Slides may now be imaged without sample compression since the weight of the slide is no longer borne by the coverslip, so long as it is applied in such a manner as to not encroach upon either end of the slide. The ledges created for our stage ring allow 1 mm clearance over the nominal 25 mm by 75 mm microscope slide dimensions. This clearance makes it easier to change slides in the dark, and accommodates slight variations in slide size. Although stage clips are generally sufficient to hold slides in place for most imaging requirements, small bits of clay pressed into any two corners of the new ledges will further immobilize a slide. If the slide holder is turned perpendicularly to the rotation of objectives, immersion oil may be applied to the objective without disturbing the slide or using syringes, as shown in Figure 4.

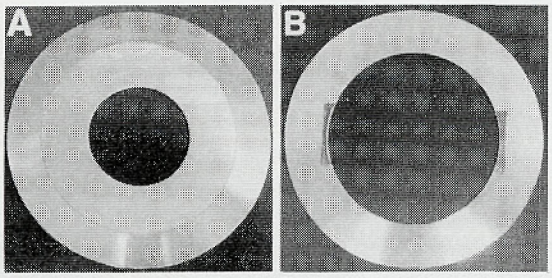

Figure 1. A) Intact standard stage insert. The central plate can be readily distinguished from the outer ring. B) The inverted ring after modification.

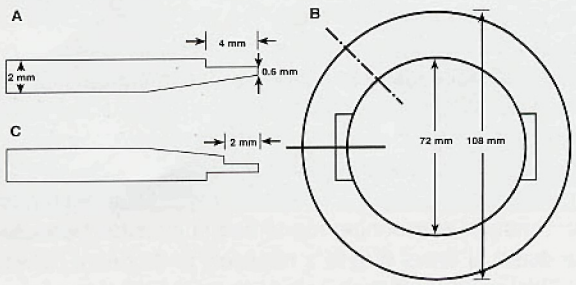

Figure 2. A) Cross-section through the standard stage ring as it would appear if cut through the dashed line. B) Overall dimensions of the outer ring of the Nikon stage insert. C) Cross-section taken through the modified portion of the ring (solid line) showing the new ledge extending 2 mm from the inner edge of the ring, along the centerline of the slide axis. Note that 2A shows the orientation of the intact ring, whereas 2C is the inverted position used after modification.

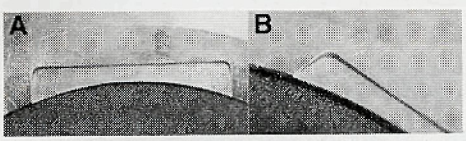

Figure 3. High magnification views of a modified ledge are shown in orthogonal (A) and oblique (B) views.

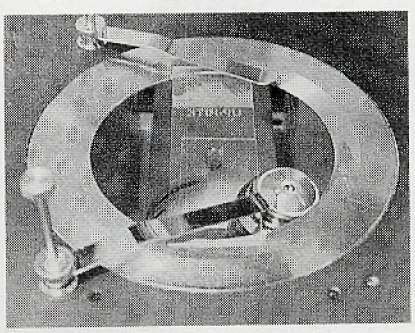

Figure 4. The modified ring is shown on a Nikon Diaphot 300 microscope attached to a Bio-Rad MRC-1024UV confocal microscope. The slide has been oriented such that oil may be applied to immersion objectives without removing the slide or using syringes. (The orange hue in this picture is due to the UV enclosure surrounding the microscope.)