Diversions with an Inverted Leitz Diavert

by Brian Matsumoto and Carol Roullard

Getting a microscope is the start of an adventure. It begins by learning to handle and operate the instrument. Every microscope has unique features, and the owner becomes experienced with exploiting its strengths and overcome its weaknesses. Often this requires research, reading, and, we must admit, trial and error. After becoming familiar with the instrument, you use it to explore your favorite subjects to determine if its newly discovered features helps you to discern new structures or events unobservable with your previous scope.

I recently started my adventure by obtaining an Inverted Leitz Diavert. For me, it is an unusual acquisition for as a hobbyist; I am more familiar and comfortable with an upright stand whose objectives are above the stage and whose condenser is beneath it. For studies requiring the highest resolution, this design has several advantages. First, it facilitates changing magnification. It is easy to grasp the rim of the nosepiece and rotate a new lens into the optical path. If one needs to upgrade an objective, it is easy to grab its barrel to unscrew it. A replacement objective is easily screwed in the vacated opening. Other tasks for high resolution work such as adjusting an objective lens correct collar or its iris or applying a drop of oil between the lens and the coverglass are readily accomplished.

An inverted microscope is a contrarian design whose objective lenses lie beneath the stage and its illuminator is held above it (Figure 1). Adding a drop of oil or adjusting a correction collar is made difficult by the cramped access between the nosepiece and the stage. The configuration is so restricted as to make it difficult to rotate a new lens into the optical path. However, its design is justified by making it a convenient tool for exploring live samples. Rather than thinking of the microscope for working with static structures at the highest resolution, it should be regarded as an instrument whose strength is studying the dynamics of living organisms at low to medium powers.

Since the Diavert Inverted Microscope had not been described in this periodical, a description of how I outfitted this microscope may interest the reader. The microscope is used at lower to moderate magnifications. Today, it has a 2.5x, 4x, 10x, 20x, and 32x objective. So with a 10x eyepiece, I have a magnification range from 25x to 320x. I use 25x and 40x for viewing large fields within a 60 mm diameter culture dish containing my pond plants and animals. If I find something interesting, I increase the magnification to study the organism in greater detail. For most of my videography and photography, the 32x objective is adequate. If I need higher magnifications, I transfer the specimen to a regular glass slide with a fine pipet, adding a cover glass, and use my upright microscope. The open stage of an inverted microscope provides easy access for selecting the organism with a pipet.

The Diavert has a handy feature that makes it easy to upgrade objective lenses: its nosepiece can be rotated and slid out from the microscope (Figure 2). When freed from the stand, you can unscrew its objective lens for replacement. An invaluable feature of this microscope is it can be equipped with a trinocular head for attaching a camera. I use a Panasonic GH5 and its mounting tube fits on both my upright Leitz Ortholux and my Inverted Leitz Diavert. The binocular viewing tubes are at a height that makes it is easy to view through the oculars while resting one's forearms on the tabletop to adjust the focus or move the specimen. The design makes it comfortable to use for long periods of study. Additionally, its design is suitable for fine manipulation of the specimen. I can place my elbows on the desktop, and by bracing my arms, I can hold and position tools over the sample. The increased stability provided by posting one's elbow onto the tabletop makes it easier to precisely place the pipet tip over the specimen to be collected.

I view my samples through the bottom of the culture dish. The dish's thickness is such that two conditions must be met to obtain sharp views. First, one works with lower power objectives with a smaller numerical aperture. Such lenses are not as adversely affected by viewing through an increased thickness of material between the specimen and the objective lens. Secondly, for higher power work, one need use objectives designed to view through thicker material than a coverglass. In the case of the Leitz inverted Diavert, its 25x and 32x are so intended. Unlike many modern inverted microscope objectives, these lenses do not have a correction collar for working with varying thicknesses in the culture dish. Most of the Leitz objectives are designed to work through a coverglass of 0.17 mm thickness. The Inverted Diavert 20x and 32x objectives were designed to operate through thicker material. In order to work with higher resolution objectives, I will modify my plastic culture dishes by cutting a hole in their bottom and sealing it with a coverglass. This will serve as a viewing window, and it will allow me to use lenses with a larger numerical aperture and a shorter working distance. I will also have to modify the standard illuminator.

The illuminator/condenser for the Inverted Diavert is elegant in its simplicity. It is fixed in position and is usable with all my objective lenses. One does not need to focus, size, or center the field diaphragm. It has a long working distance (60 mm or 2.4 inches), and I can examine the contents of a 2 oz plastic bottle 2 inches in height and 2 inches in diameter. The bottle size is perfect as it serves both as a collection and an observation bottle. Whenever I go fishing, I carry a couple to collect water samples. When I return, I can uncap the jar, allow the contents to settle, and then view the contents directly with the microscope.

To select specimens for detailed studies, I use a 2-step process. The first step is to use a 10x-40x stereo microscope to study the bottle’s contents. The upright configuration of the stereo microscope allows me to explore the collection jar by looking down into it. The second step uses the inverted Diavert. I look up studying the bottom half of the jar. It surprises me how much I miss by limiting myself to just using the stereomicroscope; however, the 3D view of a vibrant collection of tiny organisms is too beautiful to pass up. The second step has the advantage that delicate specimens can be studied in situ. Amoeba can be frequently found it my ponds and their activities can be studied without removing them from the jar.

The illuminator is a combination of light source and condenser (Figure 3). The condenser has a small numerical aperture; it is only 0.25. However, considering the Leitz Diavert highest supplied objective is a 32x N.A. 0.4, the condenser is adequate for transmitted light work at 320x. The Leitz long working distance Phaco (Phase Contrast) objectives have a standardized phase plate, so a single annular ring is used with all three objectives (10x, 20x, 32x). The annular ring is supplied as a "lollipop" filter that can slip into a slot for the light source. Two thumbscrews are used to center the phase ring to the phase annulus. Aligning one phase objective ensures correct alignment for the remainder. So it is a simple matter to change the magnification by rotating a new objective in the light path. The phase ring generates darkfield illumination when used with either the 2.5x or 4x objective.

My only complaint is the standard light source is a 6V 15W tungsten bulb. In my opinion, this does not provide enough light for taking pictures or videos with either darkfield or phase contrast. By replacing the light source with a RetroDiode LED fixture, the illuminator's output is almost on the level of a 12V 100W metal halide bulb. The light's intensity is adjustable, and there is no flicker when recording with a video camera. A potential benefit of using a LED is its reduced heat output. Hopefully, this will aid my long-term video experiments by reducing heat damage.

When working at 25x to 100x, I can move a slide or culture dish with my fingers. However, when working at 200x or 320x, fine adjustment becomes difficult. Precise framing of the specimen within the camera's field of view is imprecise. Worse, stalked organisms such as *Vorticella* or *Stentor* suffer"panic" attacks at abrupt slide movement. These organisms contract when a slide is moved with fingers. A highly desirable accessory is a mechanical stage (Figure 4). Leitz's accessory is easily attached to the microscope, and even for those who are mechanically challenged, such as myself, it is a simple matter to join it to the microscope. One need only tighten two screws, and it is firmly attached. An additional stage platform is connected via two spring-loaded clips. It has a 60 mm aperture to hold a small culture dish. Also, there is a cutout for accepting a glass slide. If I need to observe a larger 100 mm diameter Petri dish, I can remove the plate easily.

The microscope was not an eBay purchase. Instead, it was purchased from a microscope vendor (Bunton Instruments Co) with Jim Averill's help. Admittedly, this is a more expensive approach than buying it from an online auction site; however, using a reputable vendor ensured the instrument was aligned and cleaned. Moreover, Jim's expertise was invaluable in that he recommended equipping the microscope with the LED light source from RetroDiode and getting the mechanical stage. He knows my interest in videography so the illumination system, mechanical stage controls makes this microscope an ideal recording tool. So my upcoming adventure is to explore the videography of live microorganisms. You can view an example of my early experiment at this link.

The link above shows the last stages of cell division of a heliozoan. It is a time-shifted movie sequence that speeds up cell division 20x.

Reference

Diavert Inverted Microscope manual,

https://www.micromagus.net/Microdocs/diavert_manual.pdf

Figures

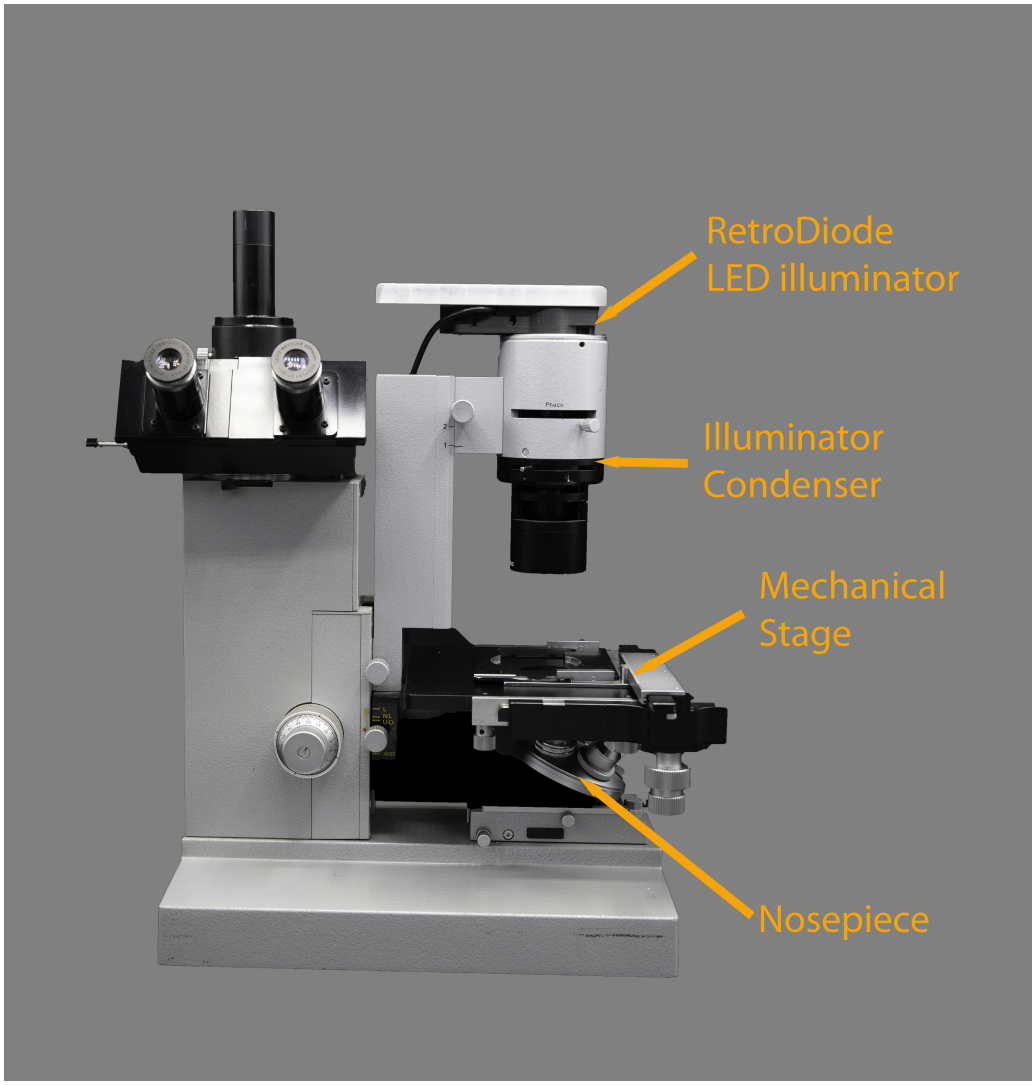

Figure 1. The Inverted Leitz Diavert, showing its principal parts.

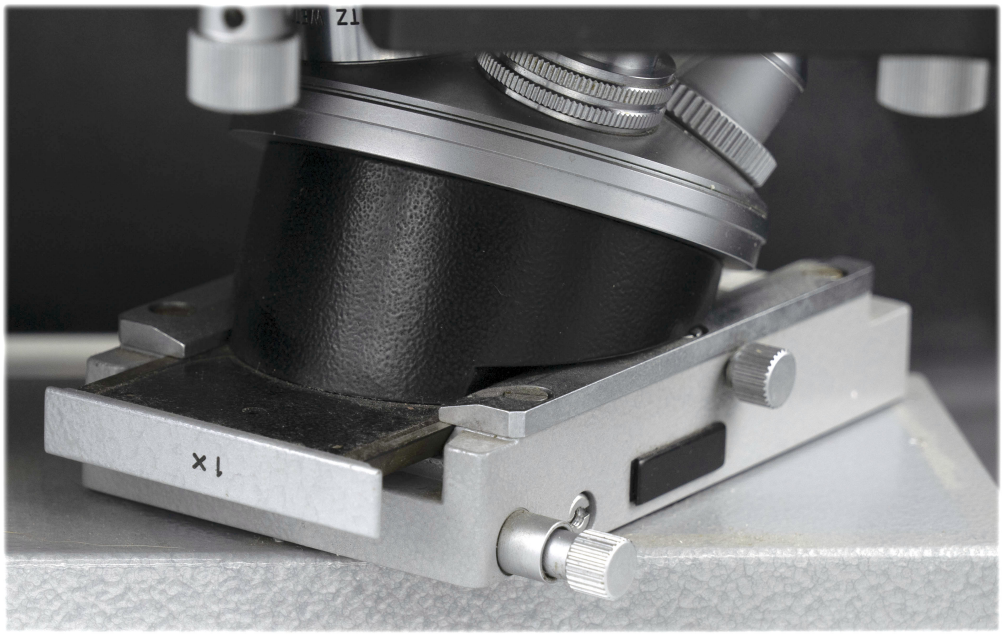

Figure 2. The nosepiece of the microscope, partially turned out for removal.

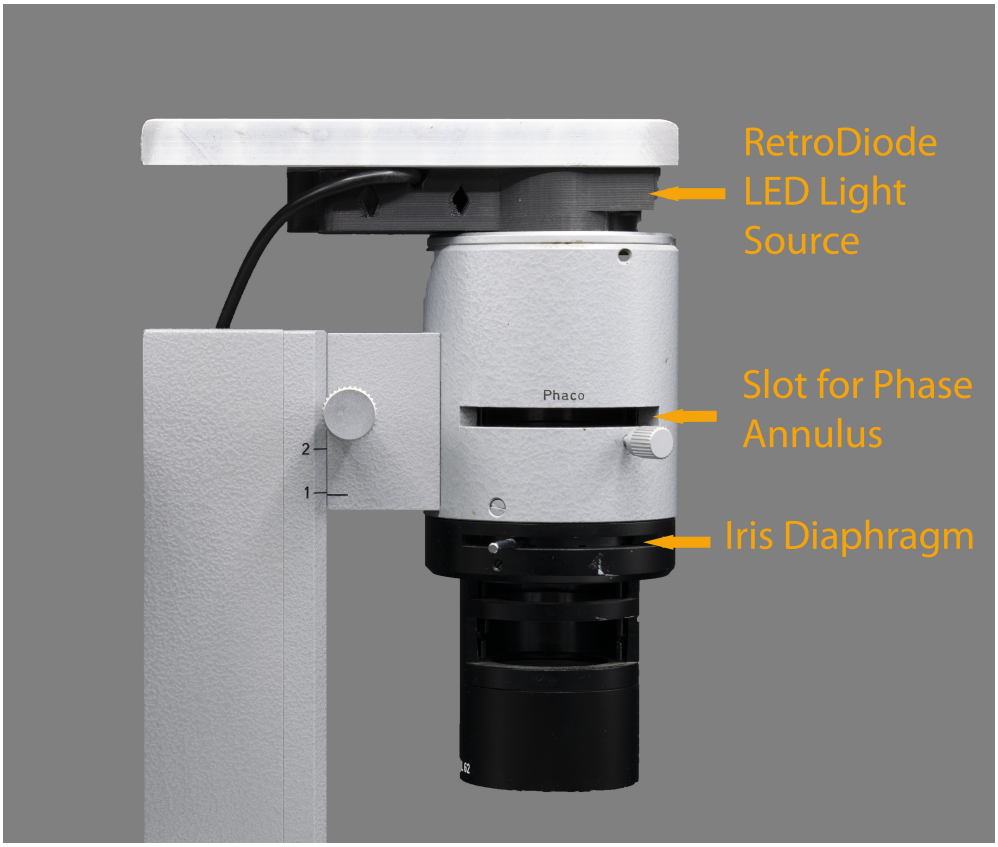

Figure 3. The illuminator and condenser are equipped with a RetroDiode LED illuminator.

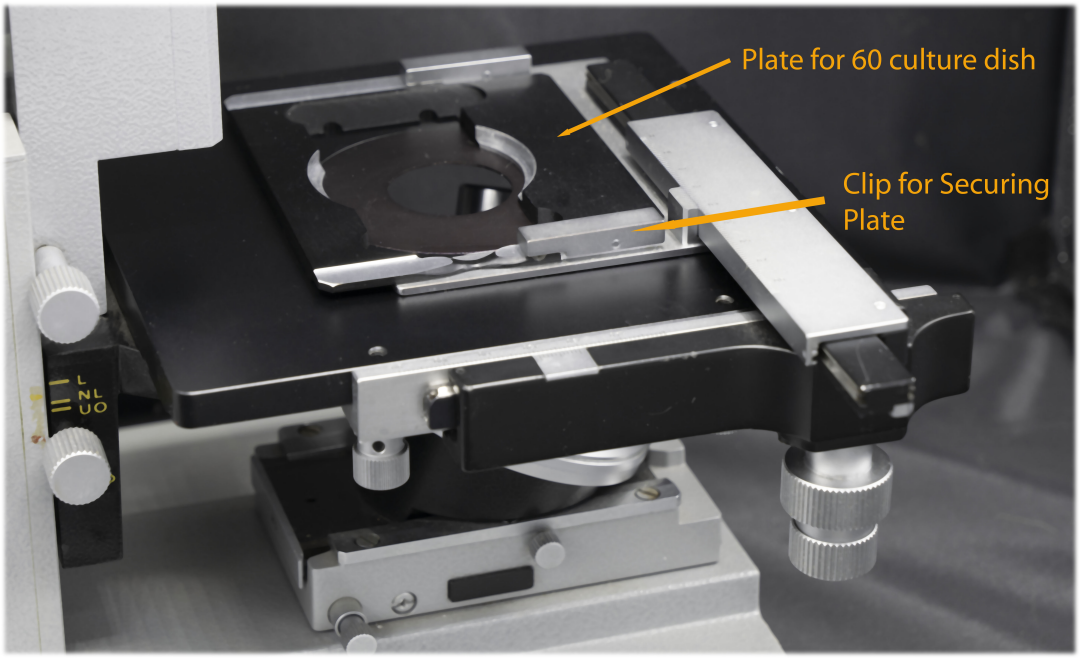

Figure 4 The mechanical stage of the microscope.

Comments to the authors are welcomed, email sum_of_all_parts AT mac DOT com

Published in the May 2021 edition of *Micscape* magzine.

www.micscape.org