Oil-immersion objective design and use

Introduction

Oil-immersion objectives are designed to be used with a coverslipped sample. This is usually a 25 x 75 x 1 mm thick glass slide, with the sample mounted between the slide and a thin glass coverslip. The sample is often thinly sliced fixed tissue that has been stained with a dye or treated with fluorescently labeled antibodies for visualization of cellular details. The slide is oriented with the coverslip facing the objective, and a small drop of oil is placed between the front lens of the objective and the coverslip. The oil "links" the two glass surfaces with a similar refractive index, and this optical link greatly improves the amount of light and detail obtained in the image. Immersion oil should only be used with objectives labeled Oil – if used on other (i.e., "dry") objectives it may harm them.

Live cell imaging using an oil-immersion objective is slightly different from fixed-tissue imaging. For live-cell experiments, cells are commonly grown on a coverslip and then viewed in a chamber containing a cell culture medium or buffered saline. The coverslip forms the bottom of the chamber, and an inverted microscope is used to focus through the coverslip and see the cells. Microtiter plates with coverslip-thick glass bottoms are also available for high-resolution imaging of live cells. When viewed with an oil-immersion objective, cultured live cells can be seen with improved resolution and detail compared to a "dry" coverslip-corrected objective.

For best results, coverslip-corrected objectives are designed to be used with a "#1.5" coverslip. This corresponds to a glass thickness of 0.170 mm, or 170 micrometers.

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The working distance of a coverslip-corrected objective is the distance from the front lens of the objective to the first glass surface (i.e., the coverslip) when the sample is in focus and can be seen (Figure 1).

How far an objective can theoretically view into a sample is determined by its working distance. As the objective approaches the coverslip, the focal plane moves further into the sample. If the sample is thick (for example, a brain slice), the working distance dictates how deep into the sample the objective can image before touching the coverslip*. Once the coverslip is in contact with the objective lens, it isn't possible to image any deeper into the tissue. Working distances for high-magnification objectives are usually relatively short, and it's important to keep this in mind when working with particular objectives and sample types. High-magnification "dry" objectives have a working distance of less than 1 mm; oil-immersion objectives usually have a working distance of less than 300 µm. Oil-immersion objectives should be used with coverslip-thick glass (or optically equivalent plastic) to achieve their best imaging performance. In fact, oil-immersion objectives are limited to use with coverslip-prepared samples, since they don't have enough working distance to focus through the 1 mm or thicker plastic wall of a typical cell culture vessel.

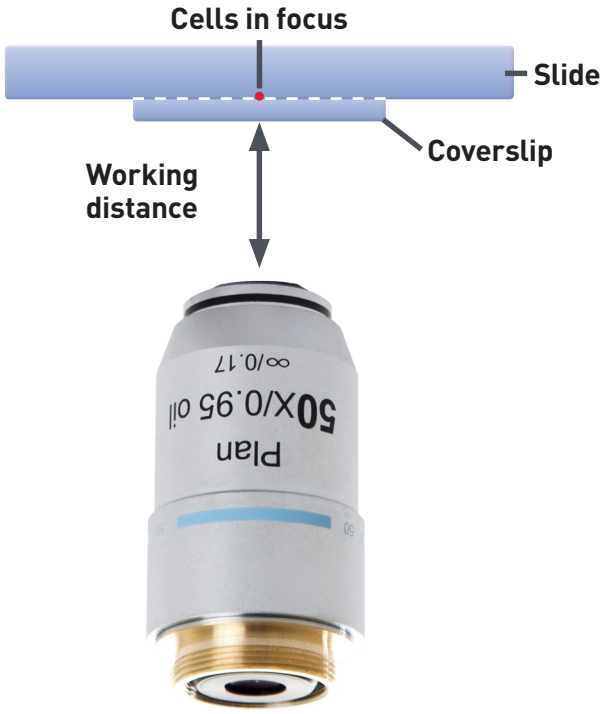

Figure 1. Working distance of a coverslip-corrected objective.

NOTE: It is physically impossible for a coverslip-corrected objective to focus into a cell culture vessel having a wall thicker than its working distance. Attempting to do so by securing the sample to the stage and driving the objective further into the specimen can severely damage the objective.

Long working distance (LWD) objectives are designed to focus through the relatively thick walls of culture vessels and image live cells. They are not designed for oil immersion. Culture vessels (T-flasks), microtiter plates with plastic bottoms, Petri dishes, and other cell culture plasticware are made with optically clear plastic walls 1.0–1.5 mm thick, and LWD objectives work best with these types of samples. LWD objectives can also be used with coverslipped samples when viewing through a glass or plastic substrate of similar thickness. This can be easily done by turning the glass slide over and viewing the cells through the 1-mm thick slide, with the coverslip facing away from the objective. In this orientation the optical performance of the LWD objective will be slightly compromised compared to that of a coverslip-corrected objective, but there is also greater versatility since LWD objectives can be used with cell culture vessels, whereas coverslip-corrected objectives cannot. LWD objectives can also be used to image through coverslip-thick glass at medium to low magnifications (20x or less) without significant loss of image quality. At 40x and higher, LWD objective images are usually not as sharp as those of coverslip-corrected objectives. Their primary benefit lies in being able to image cells at high magnification in culture vessels, which isn't possible with coverslip-corrected objectives.

*Note that optical conditions usually limit the imaging depth to a much smaller value than the objective working distance.

Methods

1. Place the slide coverslip-down on the stage.
2. Focus the specimen with the 10x objective using either fluorescence or transmitted light, and center a desired feature in the field of view.
3. Lower the objective, remove the slide, and rotate the turret to the 100x objective.
4. Gently place a small drop of immersion oil (10–20 µL) on the front lens of the objective.
5. Replace the slide in the same position and turn on the appropriate fluorescence illumination for the sample.
6. While looking at the slide on the stage (not at the LCD screen), carefully refocus the objective until the drop of oil makes contact with the coverslip. You will see a sudden flash of light when contact occurs.
7. Continue to raise the objective slowly until the specimen comes into focus. Fluorescent details will become brighter and sharper as they approach the focal plane.

Discussion

Be careful not to introduce any air bubbles into the oil—this will result in poor images. If the image can't be focused well or if it shifts laterally during focusing, you probably have one or more air bubbles. Clear them by using the edge of a Kimwipe to tease them out of the oil, then carefully replenish the oil and try again. It is important to clean the oil objective at the end of the day's use. Take a piece of lens tissue (NOT facial tissue or abrasive eyeglass tissue) and gently blot the oil off the front of the objective. Moisten (do NOT bathe) another piece of lens tissue in a lens cleaning solution and gently draw the tissue across the front lens of the objective. Chloroform or 90% ethanol may be substituted for lens cleaning solution. Take care not to rub the front lens, to avoid effacing the lens coating. Dry the front lens using a simple air blower, such as a child's bulb ear syringe. Do NOT use canned compressed air blowers.

NOTE: Do not use an oil objective without oil immersion; the image will be poor.

Sample type	Long working distance	"Dry" coverslip-corrected	Oil immersion
Glass slide with coverslip	Fair to excellent image quality — better with lower magnification	Good to excellent image quality	Excellent image quality
Coverslip-bottom live cell chamber	Fair to excellent image quality — better with lower magnification	Good to excellent image quality	Excellent image quality
Glass bottom (coverslip-thick) microtiter plate	Fair to excellent image quality — better with lower magnification	Good to excellent image quality	Excellent image quality. However, oil immersion has to be applied for each well
MatTek Petri dish (coverslip bottom)	Fair to excellent image quality — better with lower magnification	Good to excellent image quality	Excellent image quality
Petri dish (approx. 1 mm thick)	Good to excellent image quality	May not be possible, depending on working distance limitation	Not possible due to working distance limitation
Plastic microtiter plate (approx. 1.2–1.6 mm thick)	Good to excellent image quality	May not be possible, depending on working distance limitation	Not possible due to working distance limitation
T-flask (approx. 1.3 mm thick)	Good to excellent image quality	May not be possible, depending on working distance limitation	Not possible due to working distance limitation

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