

Experiment: The microscope

2.1 Aim of the experiment

The aim of this experiment is to understand the fundamentals of the compound microscope. Therefore, the main magnitudes of the microscope are measured, such as the lateral magnification of the objective lens, the visual field or the depth of focus.

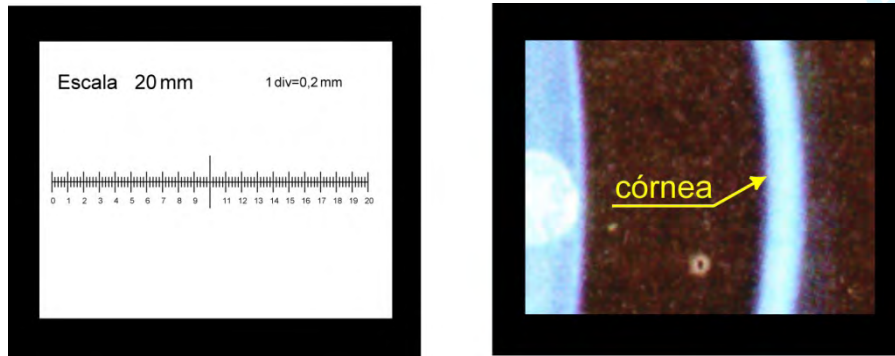


Figure 2.1: The photo on the left shows an image of the ruler used in this experiment as the test object and the reticle. The photo on the right shows a transversal section of an eye. On the last part of the experiment, the microscope is used to measure the thickness of the cornea.

2.2 Instructions

Note that it's mandatory that the optic system is perfectly aligned to avoid aberrations. In order to do so, it's useful to use the light source as a reference to align and establish the height of the rest of the elements of the experiment.

We propose to build two different microscope configurations. Therefore, all the experiments have to be done with the first configuration and, after that, repeat all of them with the second configuration:

- (a) $f'_{ob} = 50 \text{ mm}$ y $\phi_{ob} = 10 \text{ mm}$, $f'_{oc} = 50 \text{ mm}$ y $\phi_{oc} = 21 \text{ mm}$, y $t = 160 \text{ mm}$.
- (b) $f'_{ob} = 40 \text{ mm}$ y $\phi_{ob} = 6 \text{ mm}$, $f'_{oc} = 50 \text{ mm}$ y $\phi_{oc} = 21 \text{ mm}$, y $t = 160 \text{ mm}$.

The microscope is a system composed by two lenses, the objective lens and the eye lens. First, we place at the optical bench the corresponding objective lens and the eye lens, see Fig. 2.2. Note that the objective and the eye lens are separated a distance $e = f'_{ob} + t + f'_{oc}$.

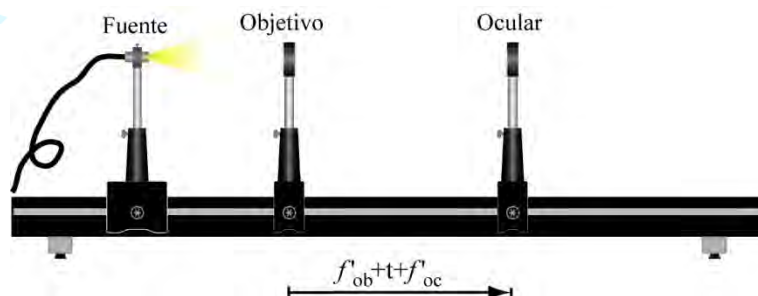


Figure 2.2: To build the microscope, the objective lens and the eye lens are placed on the optical bench. Note that both need to be aligned with the light source.

2.2.1 Exit pupil size and position.

It's very important to accurately obtain the exit pupil position because, in order to observe the object with the maximum quality, the observer's eye pupil must be placed at the exit pupil of the system.

Therefore, to establish the position and size of the exit pupil we just need to obtain the image, made by the eye lens, of the objective lens mount. In order to do so, the objective is illuminated by placing in front of it a diffuser paper, as shown in Fig. 2.3. Next, we place the screen behind the eye lens and shift its position until the image of the mount of the objective lens appears sharply on it. Due to the transparent paper, it's better to observe the image via transmission. Note that the objective lens is the aperture stop of the system.

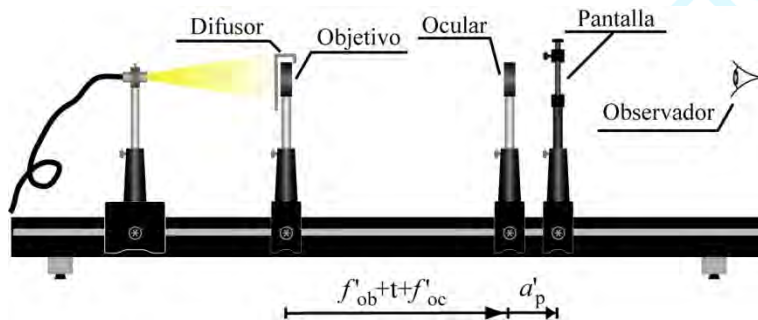


Figure 2.3: Diagram to measure the position and size of the exit pupil.

The diameter of the exit pupil, ϕ_{PS} , is measured using the screen ruler. The position of the exit pupil, a'_p , is measured using the ruler on the optical bench. Note that when performing an experiment it is mandatory to make, at least, three measures.

a'_p	\bar{a}'_p (mm)	ϕ_{PS} (mm)	$\bar{\phi}_{PS}$ (mm)

2.2.2 Measure of the objective lens lateral magnification.

The microscope provides magnified images in a two-step process. On the first step, the objective lens images a real and reversed intermediate image of the object on the object focal plane of the eye lens. This image has a magnification of

$$\beta_{ob} = -\frac{t}{f'_{ob}} \quad (2.1)$$

Now, the intermediate image acts as the object for the eye lens. Then, when the eye lens images the intermediate image, increases the size of the image by a factor

$$\Gamma_{oc} = \frac{250}{f'_{oc}} \quad (2.2)$$

The visual magnification of the microscope is the product of both factors

$$\Gamma = \beta_{ob} \Gamma_{oc} = -\frac{250 t}{f'_{ob} f'_{oc}} \quad (2.3)$$

The negative sign indicates that the final image is reversed.

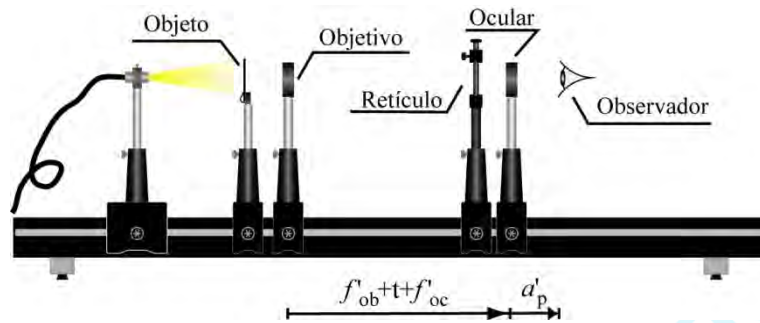


Figure 2.4: Diagram of the configuration used to measure the lateral magnification of the objective lens. This configuration also serves to measure the visual field.

As we now understand the two-step imaging process of the microscope, we can proceed to measure the lateral magnification of the objective. For that, we follow the next steps. First, we place the test object in front of the objective, approximately at a distance $2f'_{ob}$ away from it. Remember that the observer's eye is placed on the exit pupil of the microscope, as it's shown in Fig. 2.4. Next, we slowly move the object, bringing it closer to the objective, until the first position where the image is seen sharply (see Fig. 2.5 (a)).

Immediately after that, we add the transparent reticle to the system. Note that it needs to be properly aligned and placed on the object focal plane of the eye lens, f'_{oc} . With the observer eye placed on the exit pupil we need to check if the reticle and the object images are sharp. If this is not the case, we can move the test object and the reticle until both images appears sharp.

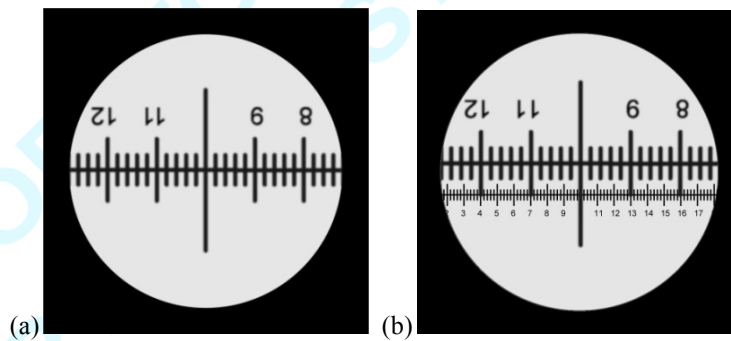


Figure 2.5: (a) Image seen by the observer when the object is in focus; (b) Image seen by the observer when the object and the reticle are properly placed. In this example, the intermediate image is 3 times bigger than the ruler printed in the reticle. Therefore, the objective lateral magnification is $\beta_{ob} = -3.0$. Note that the intermediate image is reversed.

Now, when we look through the microscope we will see the sharp images of the object and the reticle overlapping each other, as seen in Fig. 2.5(b).

Comparing the two ruler it is possible to evaluate directly the value of the lateral magnification.

$y(mm)$	$y'(mm)$	$\beta_{ob} = y'/y$	$\bar{\beta}_{ob}$
1.0mm			
1.6mm			

2.0 mm			
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An additional and interesting experiment is to check what happens if we place our eye a few centimeters away from the exit pupil. The visual field is very small and it gets bigger when we get closer to the exit pupil plane. If you want to check this, it's better to remove the reticle from the optical bench. Note: when you look through the microscope placing your eye on the exit pupil plane, your laboratory partner can see the image of the mount of the lens on your eye!

2.2.3 Visual field

A parameter of great importance is the visual field. It's defined as the part of the object plane visible through the instrument. For the microscope, the value of the diameter of the visual field is

$$2\rho_m = \frac{250 \phi_{oc}}{|\Gamma| a'_p} , \quad (2.4)$$

where

$$a'_p = f'_{oc} + \frac{f'^2_{oc}}{f'_{ob} + t} . \quad (2.5)$$

We can measure the visual field without changing the experimental setup. We only need to evaluate the part of the test object which is visible through the microscope (in the example in Fig. 2.5 the visual field is $2\rho_m = 5.6 \text{ mm}$). We note the visual field value on the table in section 2.3.7.

2.2.4 Measuring objects.

Now, as we know the lateral magnification of the microscope, we can measure objects (for example, the thickness of the cornea in the transversal section of an eye photo). In order to do so, we just need to measure, using the reticle as we did before, the size of the intermediate image, y' . Then, the size of the object is obtained by $y = y' / |\beta_{ob}|$. Note that the object now is not the test object but the photo of a transversal section of an eye.

y'	\bar{y}'	$\bar{y} = \bar{y}' / \beta_{ob} $

2.2.5 Depth of focus.

Theoretically, the depth of focus of a microscope is

$$\Delta_e = \left(\frac{0.25}{\Gamma} \right)^2 A_m . \quad (2.6)$$

For the configurations we are employing and for a usual person of 20 years ($A_m = 10 \text{ D}$), the depth of focus is approximately $\Delta_e = 2 \text{ mm}$. To check this, we remove the reticle and use again the test object. Next, we slowly shift the object until we find the first position where the image is blurred. The distance where the image is still sharp is the depth of focus Δ_e .

2.2.6 Field stop.

To check the differences between the aperture and the field stops we are going to make a little experiment. We replace the reticle with an iris aperture and observe what happens when the aperture is opened or closed (the aperture works as the field stop, so the visual field should change) Next, we shift

the aperture from the object focal plane of the eye lens to a plane next to the objective lens. And then, we open and close the aperture again (the aperture works as the aperture stop, so the illumination changes)

2.2.7 Double eye lens.

If we want to increase the visual field we can add a second lens, called field lens, to the eye lens. The field lens is placed at the object focal plane of the eye lens. Together, the field lens (L_F) and the eye lens (L_E), form a double eye lens. Note that the field lens doesn't change the visual field nor the size of the exit pupil but modifies the positions of the exit pupil. Now, the exit pupil position is

$$\bar{a}'_p = a'_p - \frac{f'^2_E}{f'_F}, \text{ donde } a'_p = f'_E + \frac{f'^2_E}{f'_{ob} + t} \quad (2.7)$$

In addition, the visual field is increased and now its value is

$$2\rho_m = \frac{250 \phi_E}{|\Gamma| \bar{a}'_p}. \quad (2.8)$$

In order to check this experimentally, we place a field lens with focal $f'_F = 100 \text{ mm}$ at the object focal plane of the eye lens, see Fig. 2.6. But, before looking through the microscope we need to obtain the new position of the exit pupil to correctly place the observer's eye. Next, we measure the field view and note it on the table. We can compare the values of the visual field with and without the field lens.

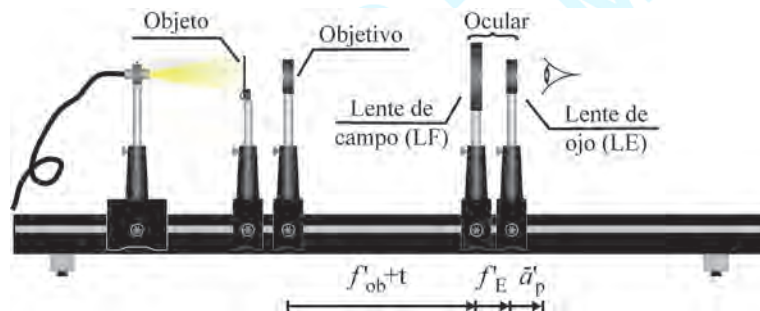


Figure 2.6: The field lens is placed on the intermediate image plane. Our double eye lens is composed by a field lens with $f'_F = 100 \text{ mm}$ and an eye lens with focal $f'_E = 50 \text{ mm}$.

Simple eye lens		Double eye lens	
$2\rho_m$	$2\bar{\rho}_m$	$2\rho_m$	$2\bar{\rho}_m$