

AN ALTERNATIVE STAGE MICROMETER FOR USE AT LIGHT MICROSCOPE

Régua micrométrica alternativa para uso ao microscópio de luz

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Stage micrometer is an indispensable instrument for light microscopy laboratory as a tool for micrographs and eyepiece scale calibration (LAWSON, 1972). This make possible to carry out measurements, as placing scale bars on micrographs and calibrating software image analyzers. It consist of a small scale, generally 1mm long, graduated in 10 μ m, accurately stamped on a histological slide. Although simple, it is a relatively expensive item, often inaccessible to laboratories in the early stages of structuring or with limited resources.

Electron microscopy grid, or specimen grid, consists of a small riddled or perforated disk, usually measuring 3.05mm in diameter, which accommodates sections or liquid suspensions for observation at the transmitting electron microscope. It is extremely variable in mesh designs and mash sizes (bars per inch). The electron microscopy grid manufacturing uses electrolytic process, resulting in a extremely precise product (BOZZOLA & RUSSELL, 1991). Because it has a reticulated and precise structure, the mesh of the microscopy grid can be calibrated, thus replacing a stage micrometer.

There are several examples of inexpensive alternative laboratory equipment and techniques established to be effective (JORDÃO & TAKAKI, 1986; TAKAKI, 1987; PAIVA *et al.*, 2006; LEITÃO & CORTELAZZO, 2008; MARINHO & LEITÃO, 2014; LEITÃO, 2015; MARINHO *et al.*, 2016). Thus, the present work aims to develop an alternative stage micrometer, using a electron microscopy grid as graduation.

To make the alternative stage micrometer were employed a virgin electron microscopy grid 300 mesh standard square, a cleaned histological slide, a cover glass 24x24mm and Canada balsam. The electron microscopy grid, the slide and the cover glass were dipped in xylene and then immediately assembled according to usual methodology for mounting of permanent histological slides (RUZIN, 1999), with due care to avoid air bubbles formation. The mounted slide was then kept in a hot plate at 58°C (LEITÃO, 2015) for three days, with a weight on the cover glass, for hardening of the mounting medium. After this period, the slide was removed from the hot plate for cooling, for later removal of excess Canada balsam with a razor blade. The finished alternative stage micrometer is shown in figure 1A.

When viewed under a microscope, it is seen that the electron microscopy grid is extremely accurate (Fig. 1C). Its mesh is formed by bars of regular thickness and spacing, which delimits square holes equally regular. One bar plus one hole equals one pitch (Fig. 1D).

The calibration of the alternative stage micrometer was performed by obtaining micrographs of it in a Leica DM750 microscope with plan objectives, equipped with Leica ICC50 HD digital photographic system. Subsequently, micrographs of an Olympus commercial stage micrometer (Fig. 1B) graduated in $10\mu\text{m}$ were obtained using the same photomicroscope under identical magnification conditions.

The digital micrographs of the alternative and commercial stage micrometers were compared using Power Point software. For this purpose, a line was drawn on the micrograph of the electron microscopy grid of the alternative stage micrometer, starting to the left of the bar of the first pitch and ending to the left of the bar of the last one (Fig. 1E). The same was done on the commercial stage micrometer micrograph in the same magnification, with the line starting to the left of the first graduation and ending to the left of the last one (Fig. 1F).

The measurements in centimeters of the drawn lines (Figs. 1E-F) provided by the Power Point were used to calculate the values in micrometers of the line drawn in the electron microscopy grid micrograph. This value is called "X" here (Fig. 1G). To obtain the measure in micrometers of each pitch, the result "X" of the line length drawn on the electron microscopy grid micrograph was divided by the number of pitches covered by this line, being this result denominated "Z" in this work (Fig. 1H).

With the measurements and calculations made here, the results was $X = 1,264.26\mu\text{m}$ (Fig. 1G) for the line covering 15 pitches in the electron microscopy grid (Fig. 1E), and $Z = 84.28\mu\text{m}$ (Fig. 1H) for each pitch (Fig. 1D). Therefore, the alternative stage micrometer produced in the present work is graduated in $84.28\mu\text{m}$. This calibration procedure is the same used to make measurements in micrographs as well as the calculation of the scale bars.

It is worth noting the importance of using a photomicroscope equipped with plan objectives in the alternative stage micrometer calibration, as well as in all the research activities. Plan objectives have correction for spherical aberration, which guarantees images without distortion, that is, reliable for measurements and calibrations (LAWSON, 1972).

The alternative stage micrometer proved to be easy to make and operate, since the electron microscopy grid bars are precise, allowing accurate measurements. It is important to emphasize the need to use new electron microscopy grid, of good quality and in perfect condition, since the intention is to build a reliable measuring instrument. However, it must be considered that the alternative stage micrometer was calibrated from another stage micrometer, which necessarily entails a small imperfection of the value found for its graduation. Notwithstanding, by performing the calibration with the utmost care, the imprecision of the stage micrometer develop here is expected to be insignificant. Thus, it is an inexpensive solution to overcome the lack of a commercial stage micrometer.

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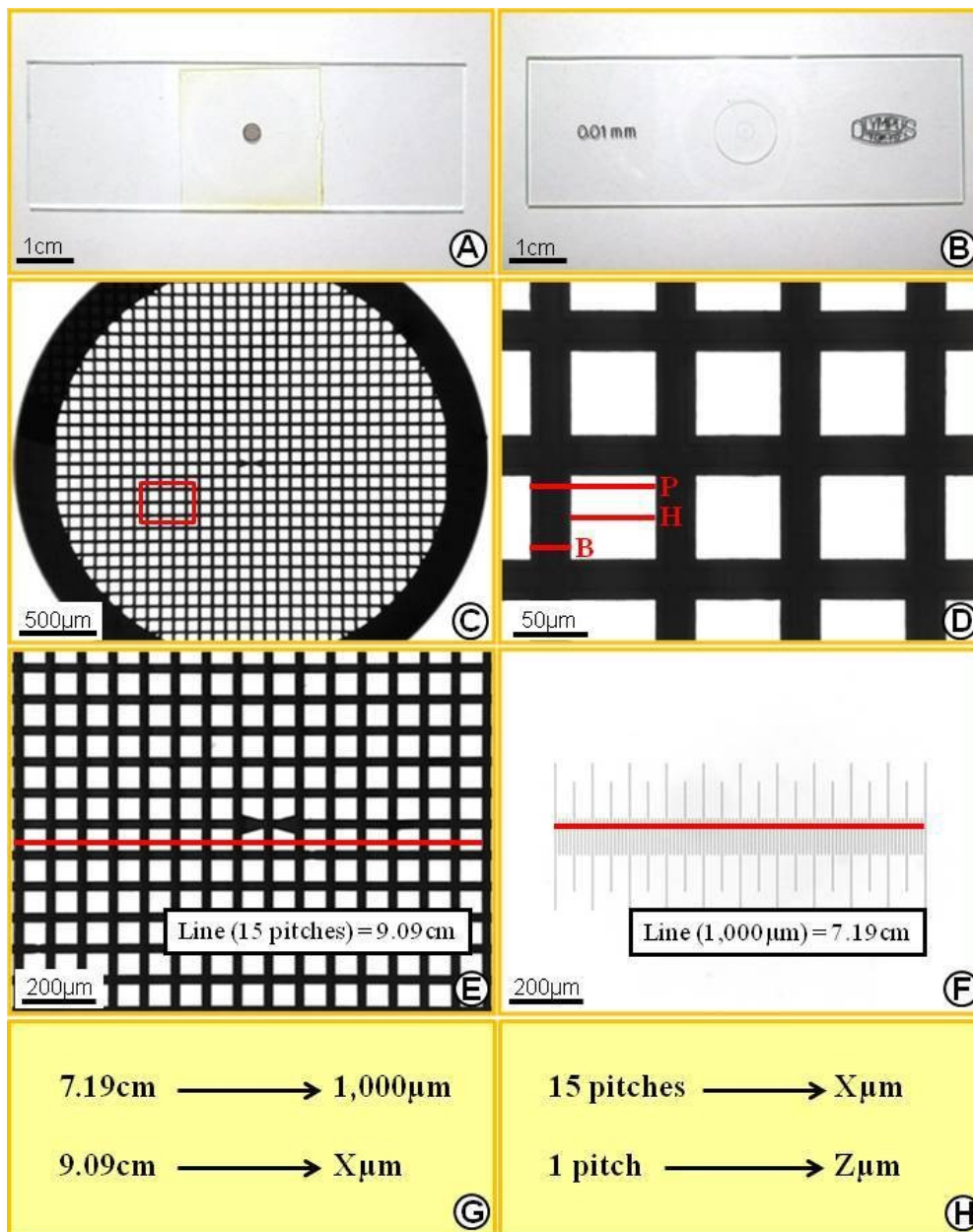


Figure 1. Alternative and commercial stage micrometers and procedures to calibration. **A.** Alternative stage micrometer. **B.** Commercial stage micrometer brand Olympus. **C.** General view of the electron microscopy grid in the alternative stage micrometer. The rectangle corresponds to the magnification in **D**. **D.** Parts of grid mesh. **E.** Measurement of the greatest possible number of the pitches of the alternative stage micrometer for calibration. Magnification at objective lens 10x. **F.** Measurement of the greatest possible number of graduations of the commercial stage micrometer for calibration. Magnification at objective lens 10x. **G.** Proportion calculation for the size, in micrometers, of the red line in **E**. **F.** Proportion calculation for the size of a pitch. Legends: B - bar, H - hole, P - pitch.

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