

Movable computer ruler (MCR): A new method for measuring the size of *Toxoplasma gondii* cysts, tachyzoites and other selected parasites

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ABSTRACT

A new method for measuring the size of parasites and other objects using optical microscopy was developed using a specifically designed movable computer ruler (MCR) derived from digital images of a stage micrometer. Subsequently, MCR can be superimposed on images of parasites to measure their size. MCR derived from the stage micrometer under a particular objective lens can be used to measure the size of an object acquired by the same lens/microscope/camera system. The conditions are fixed for every superimposed image including width, height, pixel number and density. The MCR was tested using selected parasites, and shown to be as accurate as the ocular micrometer disk, screw micrometer eyepiece and image analysis software. The lower technical complexity of the MCR method makes it applicable even in laboratories with limited resources.

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1. Introduction

Measuring the diagnostic stages of morphologically indistinguishable parasites or non-fixed living parasites such as nematode larvae, trypanosomes and ciliates on fresh smears can be difficult.

Several methods have been described that differ in their application and accuracy, and many of them require time, effort and experience (Gonzalez-Ruiz and Bendall, 1995; Cavanahac, 2000; Dioni, 2002; Zajac and Conboy, 2006). The most widely used techniques for measuring parasites by light microscopy are the ocular micrometer disk, screw micrometer eyepiece, and stage micrometer. A combination of these methods is used to calibrate the ocular scale unit with different objective lenses to determine the calibration factor for each lens. In another approach, a stage micrometer can be used on its own, measuring printed images of both the parasite under the study and the stage micrometer acquired by the same objective lens (Chantangsi, 2002).

Finally, some of the newer digital cameras attached to microscopes are provided with distance measurement software, which can be used, after calibration, for measurement of parasites. Here, a new computerized method for measuring parasites or other

objects is described which depends on digital images of both the parasite and stage micrometer.

2. Materials and methods

2.1. Parasite specimens

Images of selected parasites were chosen for measurement and to demonstrate the use of this new method. *Toxoplasma gondii* cysts in a fixed scrape from a mouse brain 30 days post infection, and *Toxoplasma* tachyzoites in fresh smears from peritoneal fluid of a mouse 7 days post infection were mainly used. Additionally, eggs of the nematode *Trichostrongylus sp.*, the cestode *Moniezia expansa*, and the oocyst of *Eimeria bateri* were obtained from fecal smears of their corresponding host (see Supplementary materials, S1).

2.2. Photography and image processing of parasites

Digital images of *Toxoplasma*, *Trichostrongylus* and *Moniezia* eggs were obtained using a 3.2 megapixel digital camera attached to a light microscope (OPTIKA digital biological microscope, DM-20& Eyepiece: WF10×/20 mm& Objectives: 4×, 10×, 40×, 100×). Images of *Eimeria* oocysts were obtained using 35 mm camera fitted with Kodak photographic film (Wild Photoautomat MPS 45, Eyepiece:

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10×) attached to an ordinary light microscope. The 35 mm photographs were then scanned to digitize the image. Microsoft PowerPoint and MS Picture Manager were used for processing and editing of the parasite (P) images as both programs are readily available in most laboratories. (Supplementary materials, S2).

2.3. Photography and image processing of stage micrometer

The 0.1 and 0.01 mm stage micrometers (W. Watson & Sons Ltd., London) were used throughout (Fig. 1). Images of the stage micrometer were obtained using the 4×, 10×, 40×, 100× objective lenses (Fig. 2). File images were named according to the lens power used, e.g. SM4× for stage micrometer image taken by a 4× objective lens. SM images were then processed using MS Picture Manager (Supplementary materials, S2).

2.4. Designing the movable computer ruler (MCR) for digital images

To producing the MCR the following steps were performed:

1. Import a specified slide into a blank PowerPoint slide.
2. Highlight the image and crop to cut down the image to nearly 1/7 of its original size to form the raw ruler (Fig. 3).
3. A frame and a pointer for the raw ruler are then made, [click on the raw ruler, choose 3 pt from line style 'weight' and the square dot from 'dashes' for the frame. For a pointer, click on 'line', drag to draw a line twice the width of the raw ruler, then highlight this line and click on 'weight, choose 3 pt (double line)']. The pointer is movable to right or left by highlighting the pointer and clicking on right or left arrows on the keyboard (Fig. 4). Two pointers can be made, one fixed at the edge of parasite while the second pointer can be moved to other edge. To make rulers for all SM images, the same process is repeated for each magnification (MCR4×a, MCR10×a, MCR40×a and MCR100×a).
4. Calibrate the size of square dot (sd) to microns (μm): the number of 3 pt square dots per 100 μm were counted (Fig. 4). The size (in μm) from the edge of one dot to the corresponding side of the adjacent dot was derived by $100/\text{number of dots in } 100 \mu\text{m}$. This was repeated for every raw ruler obtained at different magnifications (e.g. square dot size of $4\times = 26.32 \mu\text{m}$, $10\times = 10 \mu\text{m}$, $40\times = 2.63 \mu\text{m}$ and $100\times = 1 \mu\text{m}$). The calibration factors were only obtained once and were applied every time the MCR was used (Fig. 5).

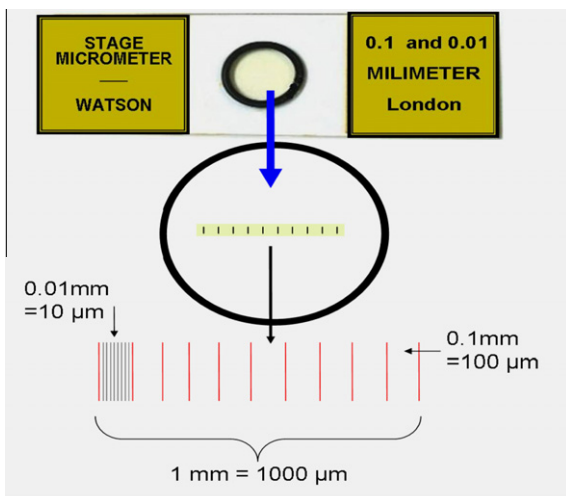


Fig. 1. The stage micrometer (W. Watson & Sons Ltd., London). Note the unit division patterns into 0.1 mm (100 μm) and 0.01 mm (10 μm).

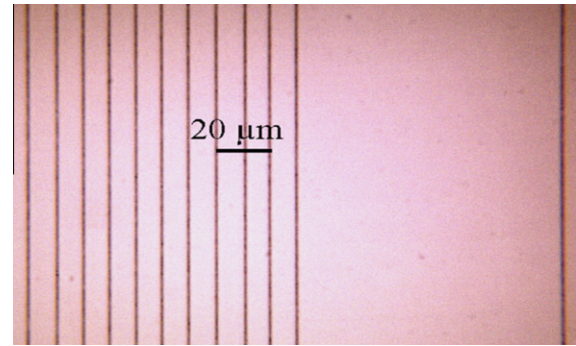


Fig. 2. Image of the stage micrometer with objective lens 40 (SM40×). Note the two unit division which equal 20 μm .

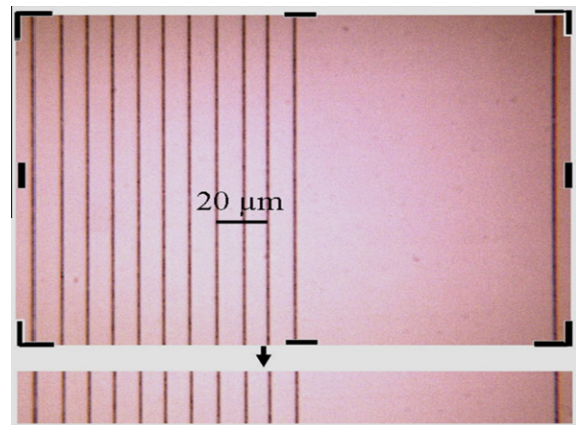


Fig. 3. Making the raw ruler to be used in the movable computer ruler (MCR) calibration.

2.5. Designing an MCR for photographs

For non-digital photographs obtained with a 35 mm film camera, a different strategy was followed (see Supplementary materials, S3, Fig. S5).

2.6. Measurements of parasites using image analysis software

(Optika Micro Image Analysis Software, OPMIAS), (see Supplementary materials, S4).

3. Results

3.1. Measurements of size using movable computer ruler (MCR)

3.1.1. Measuring the diameter of *Toxoplasma* cysts (Tc) acquired by different lens powers

The diameter of the same Tc depended on the objective lens used (Figs. 5 and 6). However, the diameter measured using 100× (32.5 μm) was the most accurate as shown in Table 1.

3.1.2. Measuring different sizes of *Toxoplasma* cysts, with same lens power

Different sizes of ten Tc measured in the same brain scrape (Fig. S1). Their diameters were 32.5, 37.5, 48.0, 48.5, 51.0, 52.0, 54.0, 54.0, 60.5 and 63.0 μm , with a mean of 50.10 μm using a 100× power lens.

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