Equipment for coupling a digital camera to a optical microscopy

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In this work has been described a instrument for coupling photographic or movie cameras to optical microscope. This equipment allows to obtain microscopic photos or movies of materials over a appropriate glass sheet."

Keywords: optical microscopy, photo micrography .

Introduction

The visualization of images in microscope has extreme importance in several professional activities. It is not known accurately who invented the microscope, however after its invention (XVII century), our perception of the invisible small world became very different. The microscope invention is attributed to it the Galileo, however Leeuwenhoek improved it and used in the of beings livings creature. The first microscopes used to have only one glass lens, allowing increases of up to 300 times with reasonable clearness, thus a invisible world became visible to our eyes.

The simple microscope of Leeuwenhoek, was improved by Hooke, earning plus a lens, getting itself bigger increases still[1]. The modern optic microscopes are much more powerful than the ones used by XVII century scientists beginning. They are endowed with two systems of crystal lenses (ocular and objective), that can allow magnifications between 100 and 1000 times.

In the common microscopes, the objective lens produces an real image inside of the pipe next to the ocular lens and, the ocular is used to examine the image already increased, as it was to look at an object. Both, objective and ocular contribute for the total increase of the microscope. Therefore the ocular is a lens that serves to increase the image produced for the objective lens, for example with the ocular of 10 times, conjugated with an objective of 30 times, the total increase will be 300 times the original size of the object[2].

The electron microscope appeared in 1932, being quickly perfected, nowadays they allow increases from 5 thousand to 500 a thousand times. The difference between the microscopes optic and electronic is that in this last one instead of the light a beam of electrons is used. The crystal lenses were replaced by bobbins, called electromagnetic lenses. The enlarged image is generated by the passage of the electron beam through the sample and is project in a black and white monitor. It is not possible to observe living material. The samples used in this in this type of microscope[3] has to be dried and covered with very hard special resins to allow ultra thin cuts.

The optical instruments possess the limitation of the wave length of the visible light. A scanning electron microscope (SEM) capable to work in nanometric scale ($1nm = 10^{-9}m$) needs high vacuum with destructive effects for biological samples. Moreover, they do not give good information on depth. The scanning probe microscope (Scanning Probe Microscope) is composed basically by a piezoelectric ceramics system (piezoeletric ceramic whose dimension varies in function of the difference of potential applied). The image is obtained by the movement of the probe over the sample. The movement is controlled by PID (proportional differential integral) feedback system. During the scanning process the

informations obtained from the control system and the probe position detector are used to form the sample image. It has many types of SPM, for example: STM (Scanning Tunneling Microscope)[4] and, AFM (Atomic Force Microscope) and SNOM (Scanning Near-Field Optical Microscope). The most used SPM is the AFM whose probe is formed by a connecting rod with a needle in its inferior part (joint rod-needle is called cantilever). For the STM a platinum wire is used. These equipments are capable to obtain nuclear dimension images. Resolutions up to 0,1 angstrom has been reported[5].

Equipment

The instrument for coupling photo or movie camera to the optic microscope, showed in figure 1, is attached on the chamber of the ocular lens of the microscope. This make possible to take microscopical photos or record movies of of materials over the glass slides (Fig 1). The optic microscopes increase the image in 100, 200, 400 or 1,000 times. With the coupling of a digital camera, the zoom adjustment can be used to increased image size ten or twenty times or more, improving the visualization and the diagnosis. Also it makes possible to connect de microscope to a projector or TV monitor, allowing the image visualization to other people. This can be very useful for didactic processes.

The coupler of cameras is constituted of a metallic ring with internal diameter of 35 mm and 15 mm height. The wall has thickness of 6 mm and is fixed to the ocular lens with three equidistant 5 mm screws (Fig. 2)

Connected to the ring there is a 6mm screw (Fig. 2/2), has one arm with dimensions of 20 mm by 40 mm, with 3 mm thickness. This has an oblong hole with 25 mm length and diameter of 6 mm which holds another arm with a 6mm screw (Fig. 2/4), this allows to align the height of the chamber to the center of the ocular of the microscope. This arm is metallic with 3mm of thickness, 200 mm of length and 20 mm of width and contains an oblong hole 6mm diameter by 25 mm length (Fig. 2/5). The screw 6 (Fig. 2/6) is used to fix the camera to system. Figure 3 shows the complete device.

Figure 1. Coupling of digital camera to the microscope.

Results

A result can be observed in the figure 4, where the image made in optic microscope with increase of 100 times and 40 times camera zoom, with a total of 4.000 times. Thus details of the study material can be better observed, facilitating the diagnosis.

With the assistance of a swab was collected material of the nasal mucosa of a cooled person. The material was fixed using methylic alcohol and taken into the microscope with 400X increase an 20X camera zoom (Fig. 5). We used a AFM to get a similar image with the same magnification (Fig. 6) from the same slide. To confirm the magnitude of these these images, it was obtained an image of the calibration grating of AFM, with the AFM and the microscope. The results are in the figures 7 and 8.

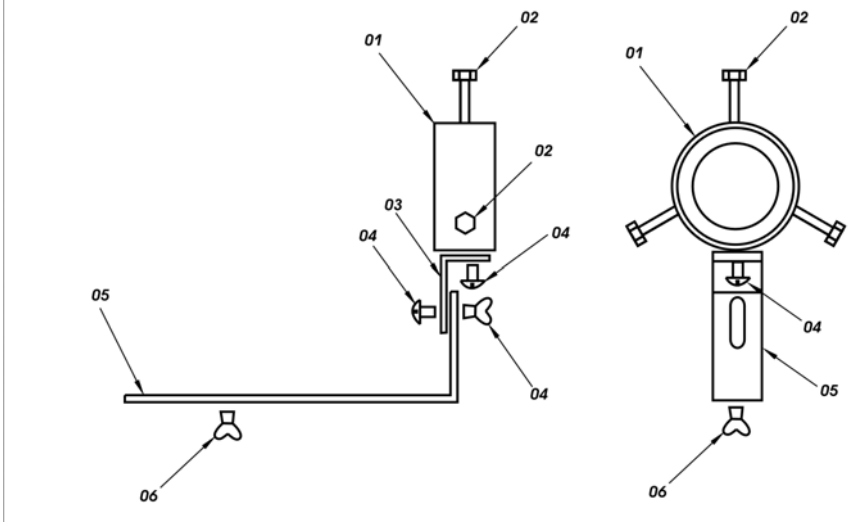

Figure 2. Diagram of the camera microscope coupling device.

Figure 3. Photo of the camera microscope coupling device.

Conclusion

The equipment for coupling digital camera to the optic microscope allows to take images with high magnification as can be observed in figures 4 and 5. It allows, for example, to get images of microphylarias of Stephanofilirias spp of peripheral human blood, only observed with increase of 4.000 times (Fig. 4)[8]. This technique can be useful in the diagnosis of several kinds of infections, including viral ones. This equipment allows the examination of not translucent materials, with the assistance of the Epi-illumination, and differs from the technique developed per Piper[9] that it uses adapted lenses, and does not raise the magnitude of an optic microscope five or twenty thousand times, as occur with the technique used in this work

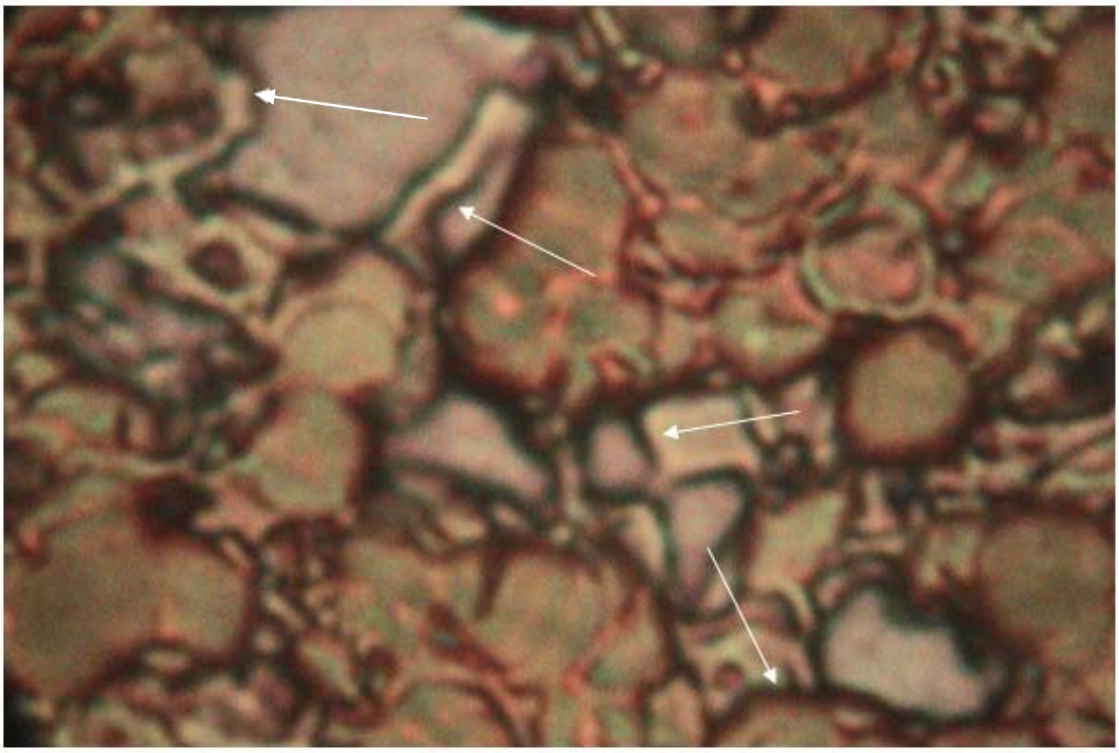

Figure 4. Photomicrography of peripheral human blood deposited over glass slide. The arrows point the microphylairas of Stephanofilaria spp. The 4000 times magnification is obtained by coupling the zoom camera to the optical microscope.

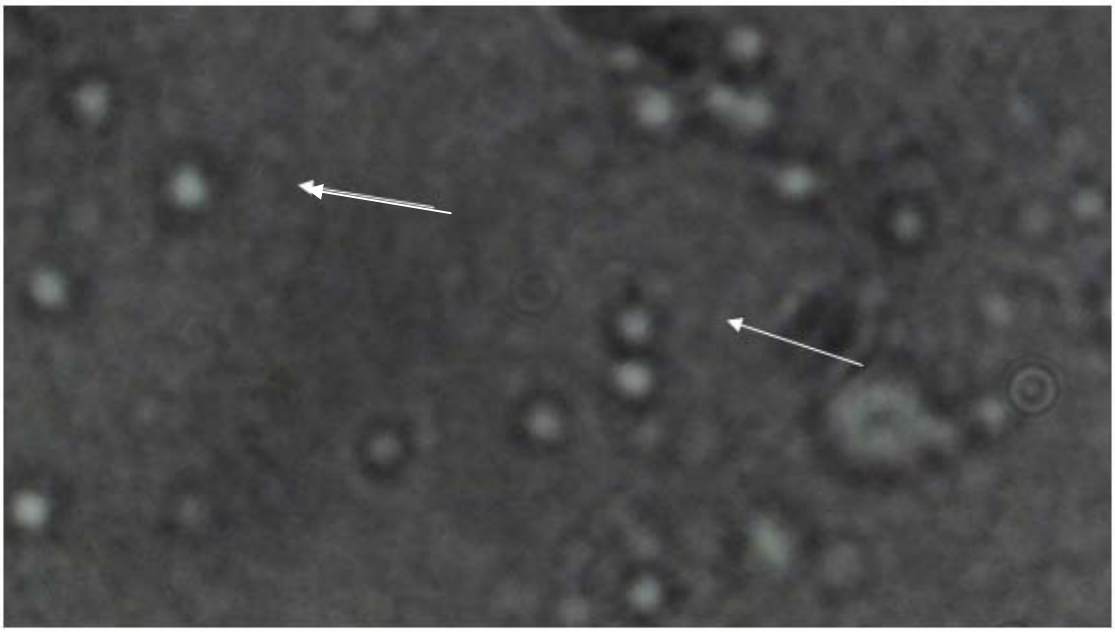

Figure 5. Photomicrography of the influenza virus obtained in optical microscope with zoom camera.

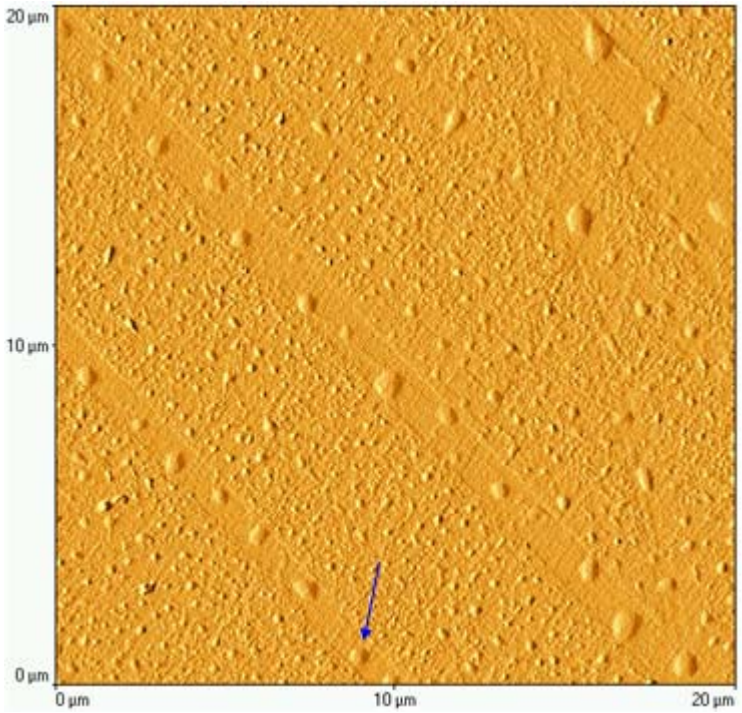

Figure 6. AFM image of influenza virus from the same slide used in figure 5. The magnification is 10.000 times.

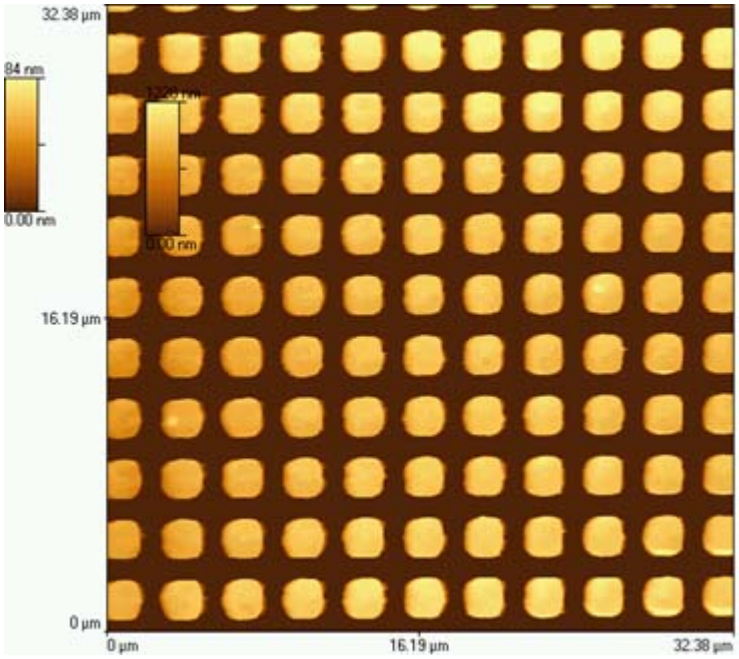

Figure 7. AFM image of the calibration grid with 8.000 times magnification.

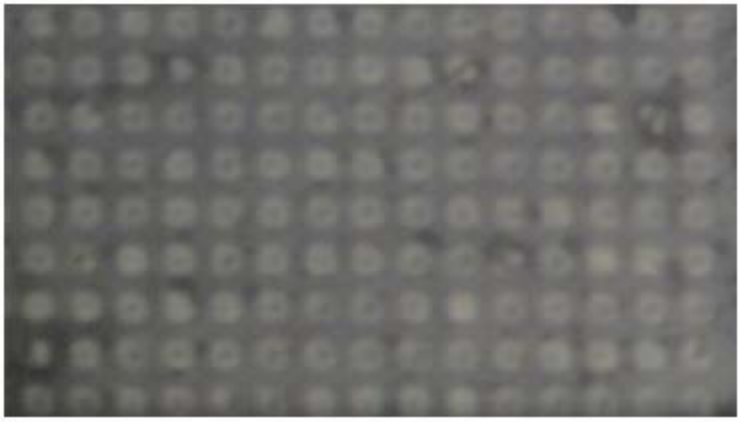

Figure 8 – Photomicrography of the AFM calibration grid obtained with the optical microscope and zoom camera. The epi-illumination was used and the magnification was 8000 times.

Bibliographical references

[1] SYNGE, E. H. A suggested method for extending microscopic resolution into the ultra-microscopic region. Philosophical Magazine, London, v. 6, p. 356–362, 1928.

[2] BISCEGLI, C. I.; RABELLO, L. M.; CRUVINEL, P. E.; HERRMANN JUNIOR, P. S. de P.; FERREIRA, W. S. Manutenção de instrumentos laboratoriais na pesquisa agropecuária. Brasília, DF: EMBRAPA-SPI; Jaguariuna: EMBRAPA-CNPDIA, 1997. 273 p.

[3] POHL, D.W.; DENK. W.; LANZ, M. Optical stethoscopy – Image recording with resolution Lambda/20. Applied physics letters, New York, v. 44, n. 7, p. 651-653, 1984.

[4] BINNING, G.; ROHRER, H.; GERBER, C.; WEIBEL. E. Surface studies by scanning tunneling microscopy. Physical review letters, New York, v. 49, n. 1, p. 57-61, 1982.

[5] BINNIG, G.;.QUATE, C. F.; GERBER, C. H. AtomicForce. Microscope. Physical review letters, New York, v. 56, n. 9, 1986.
[6] HERRMANN, P. S. P.; SILVA, M. A. P. da.; BERNARDES FILHO, R.; JOB. A. E.; COLNAGO, L. A.; FROMMER, J. E.; MATTOSO, L. H. C. Microscopia de varredura por força: uma ferramenta poderosa no estudo de polímeros. Polímeros: ciência e tecnologia, São Carlos, SP, v. 7, n. 4, p. 51-61, 1997.
[7] LEITE, F. L.; HERRMANN JUNIOR, P. S. P. Application of atomic force spectroscopy (AFS) to studies of adhesion phenomena: a review. Journal of Adhesion Science and Technology, Utrecht, v. 19, p. 365-405, 2005.
[8] NOVAES, A. P.; MIYASHIDA,Y. A. Estefanofilariose uma zoonose vetores e mecanismo de transmissão: uma nota preliminar. São Carlos, SP: Embrapa Instrumentação Agropecuária, 2006. 12 p. (Embrapa Instrumentação Agropecuária. Série Documentos, 22).
[9] PIPER, JÖRG. Adapting consumer digital câmeras for photomicrography: technical aspects. Microscopy and analysis, issue, 86, S5-S8, 2007.