International Conference on
Parasitology
Philadelphia, USA Aug 24-26, 2015

MCR- Movable computer ruler:
A new method for measuring the dimensions of parasite, particles and other objects, using optical microscope and the digital images

By
Yehia OTIFY (PhD)
Prof. PARASITOLOGY
Faculty of VETERINARY MEDICINE,
Alexandria University, EGYPT



Measuring the dimensions is important in a microscopical identification of parasites and other particles.

Methods for measuring the dimensions are:

1. Ocular micrometer

2. Screw micrometer eyepiece

3. Electronic micrometer eyepiece

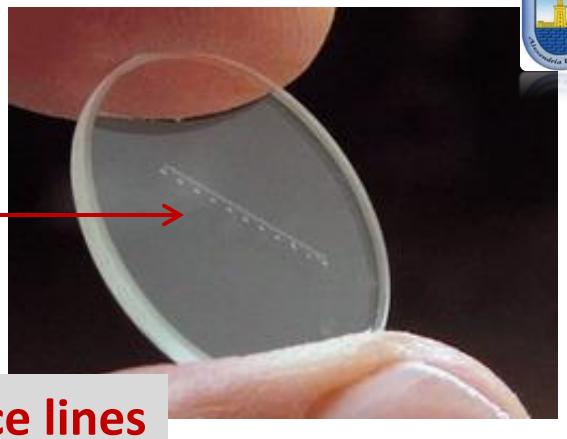
4. Image analysis system

. These 4 methods are applied while using the optical microscope.

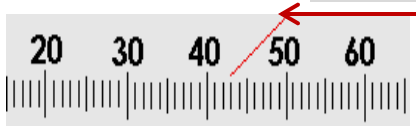
Traditional Methods

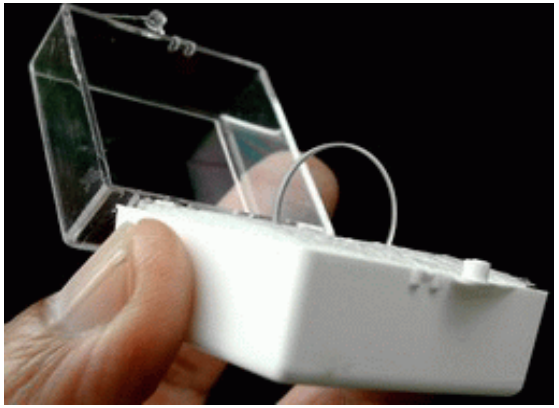
1. Ocular micrometer





Eyepiece lines









Micrometer slide

2. Screw micrometer eyepiece







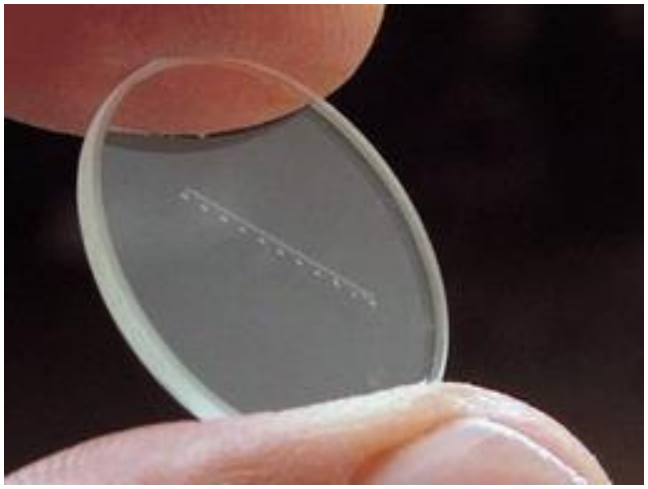
. Remove one eyepiece of the microscope

. Put the screw Micrometer eyepiece.





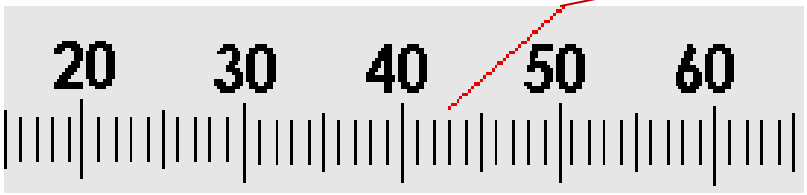
1. Ocular micrometer

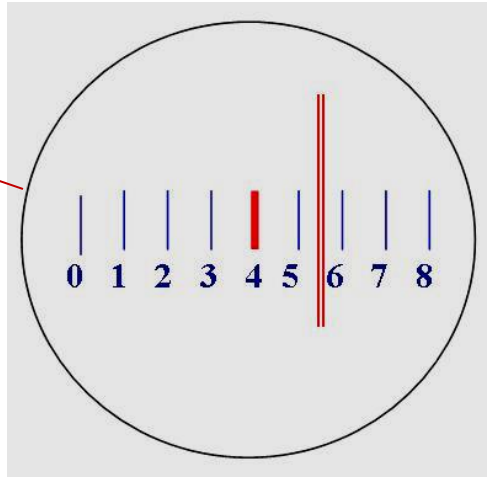


2. Screw micrometer eyepiece



Eyepiece lines





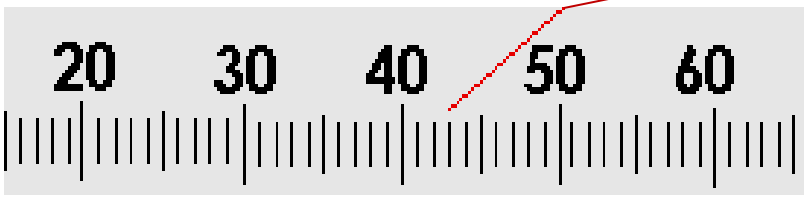
. **Adjust the eyepiece lines** using calibration scale.

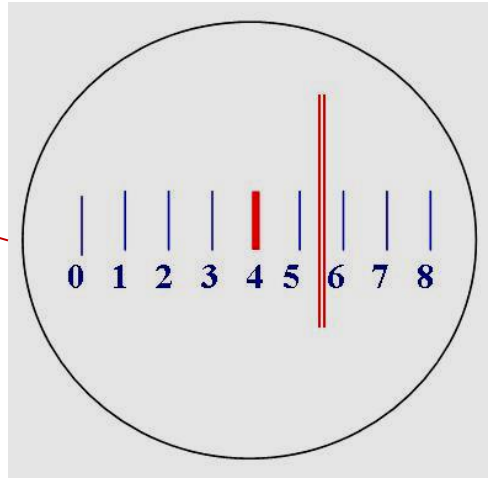
. **Factors** depending on power of lens (4x, 10x & 40x…).



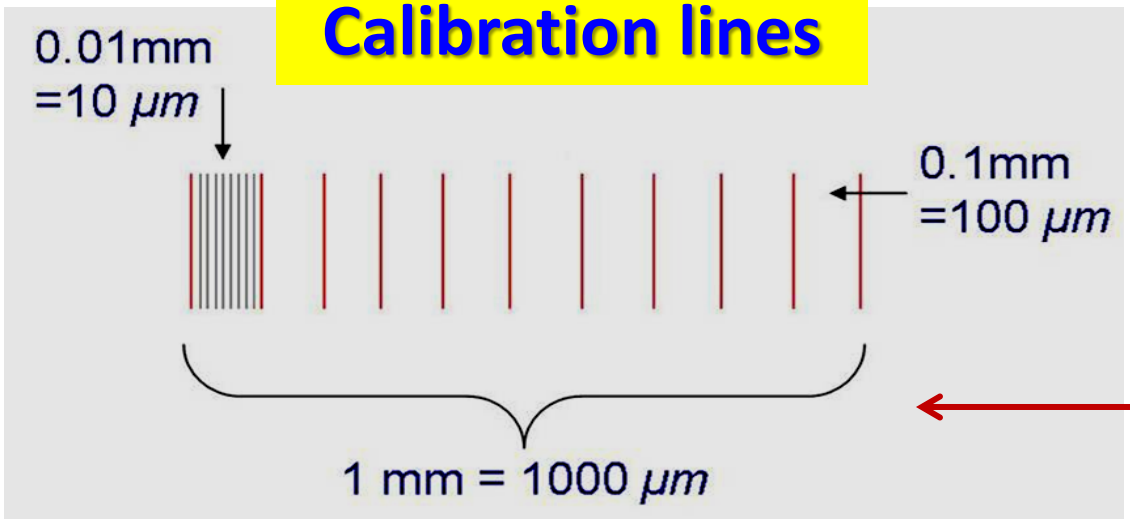
Stage micrometer (Calibration scale)

Eyepiece lines





Calibration lines





Calibration factor for each objective power Lens

4x, 10x, 16x, 20x, 40x, 100x

. Takes more efforts and stress eyes

. Consumes more time and energy.

Movable Computer Ruler (MCR)

 # Applied on digital image using MS PowerPoint

and MS Picture Manager programs.

The new Method

Movable computer ruler (MCR)



Materials

1. Optical microscope
2. Digital fixed camera attached with PC or laptop + MS PP & MS Picture Manager programs
3. Slide of parasite or any particle specimens
4. Stage micrometer (Calibration slide)

Methods

1. Image processing of parasites
2. Image processing of stage micrometer
3. Designing the movable computer ruler (MCR)
4. Application of MCR method
5. Measurements of parasites using other methods.

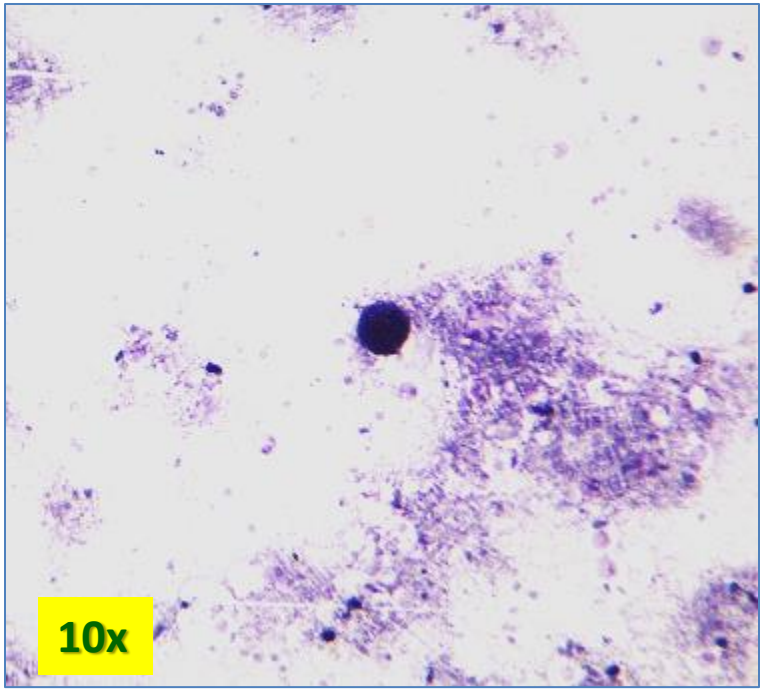


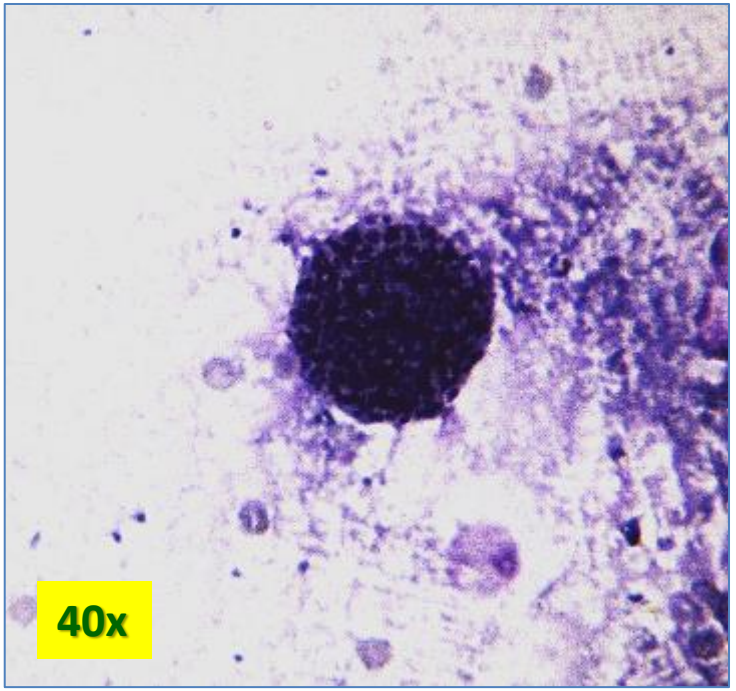
1

Photography and image processing of parasites

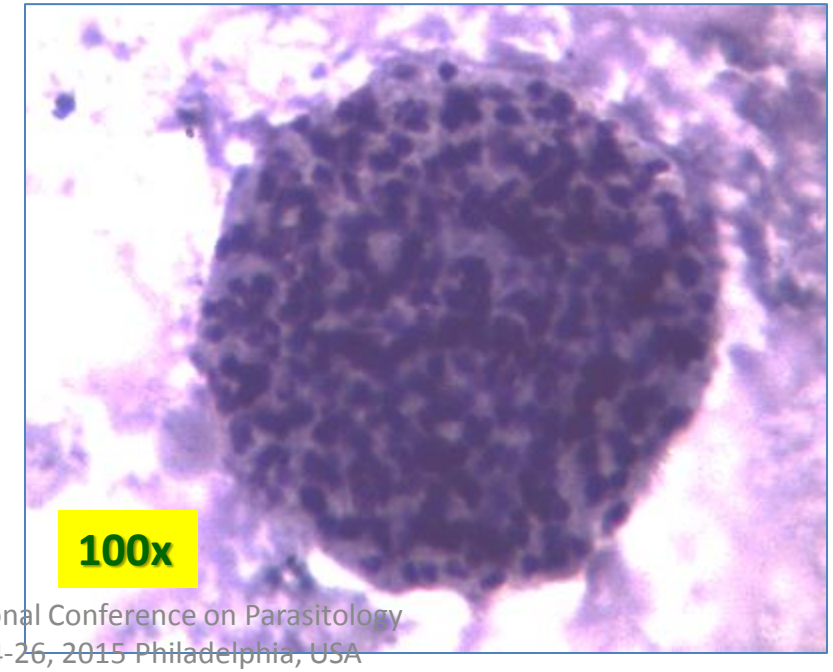


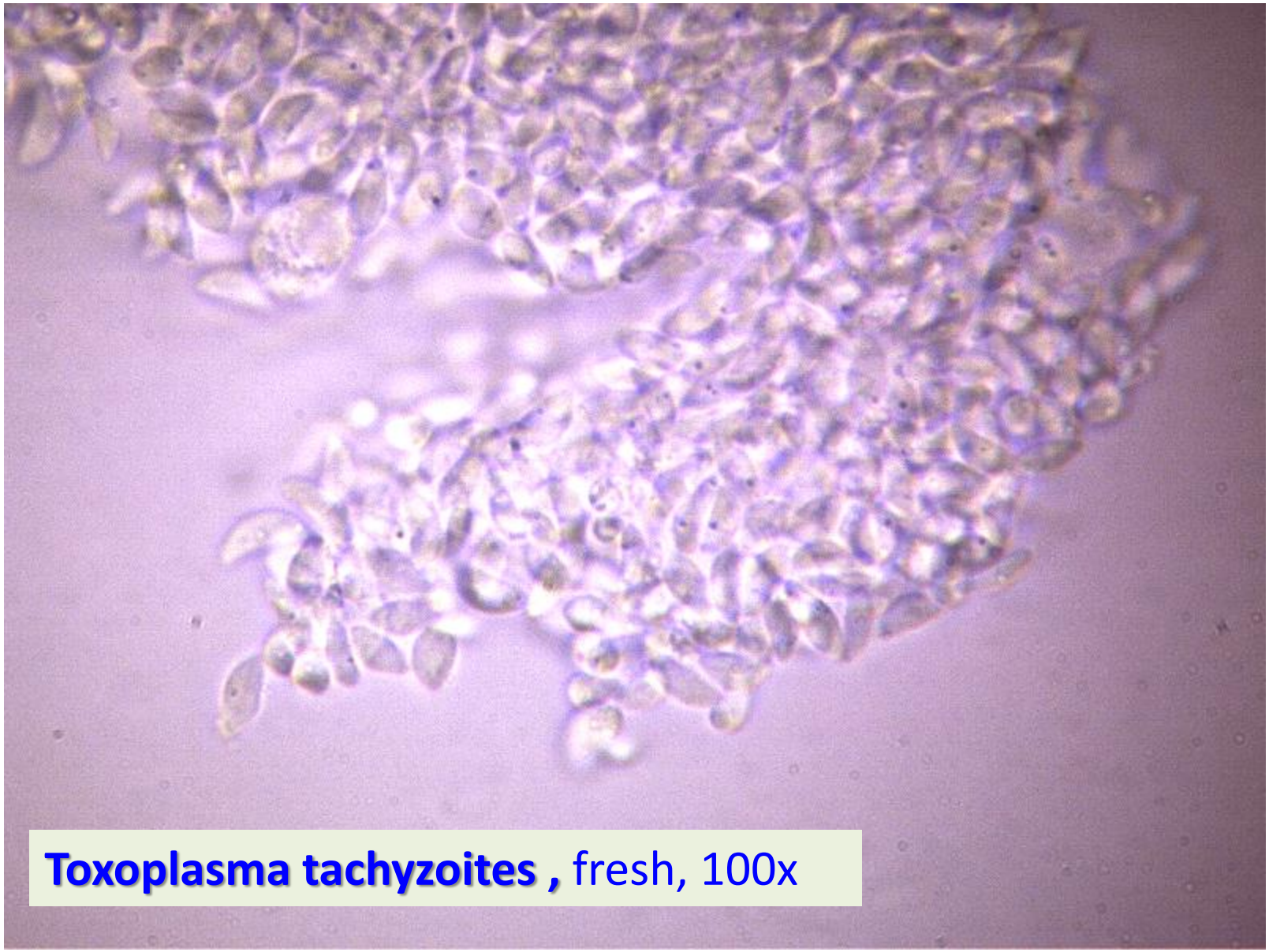
Taking the images of Selected parasites



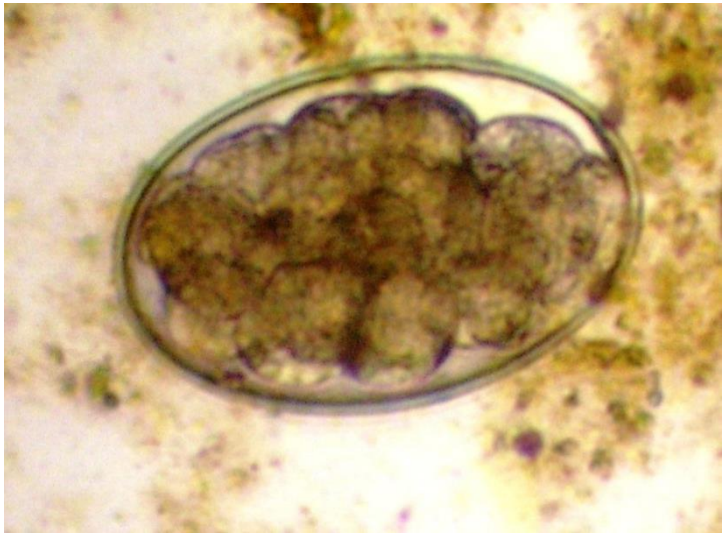


Toxoplasma cysts ,
Giemsa stained.
Images taken with 10x & 40x & 100x





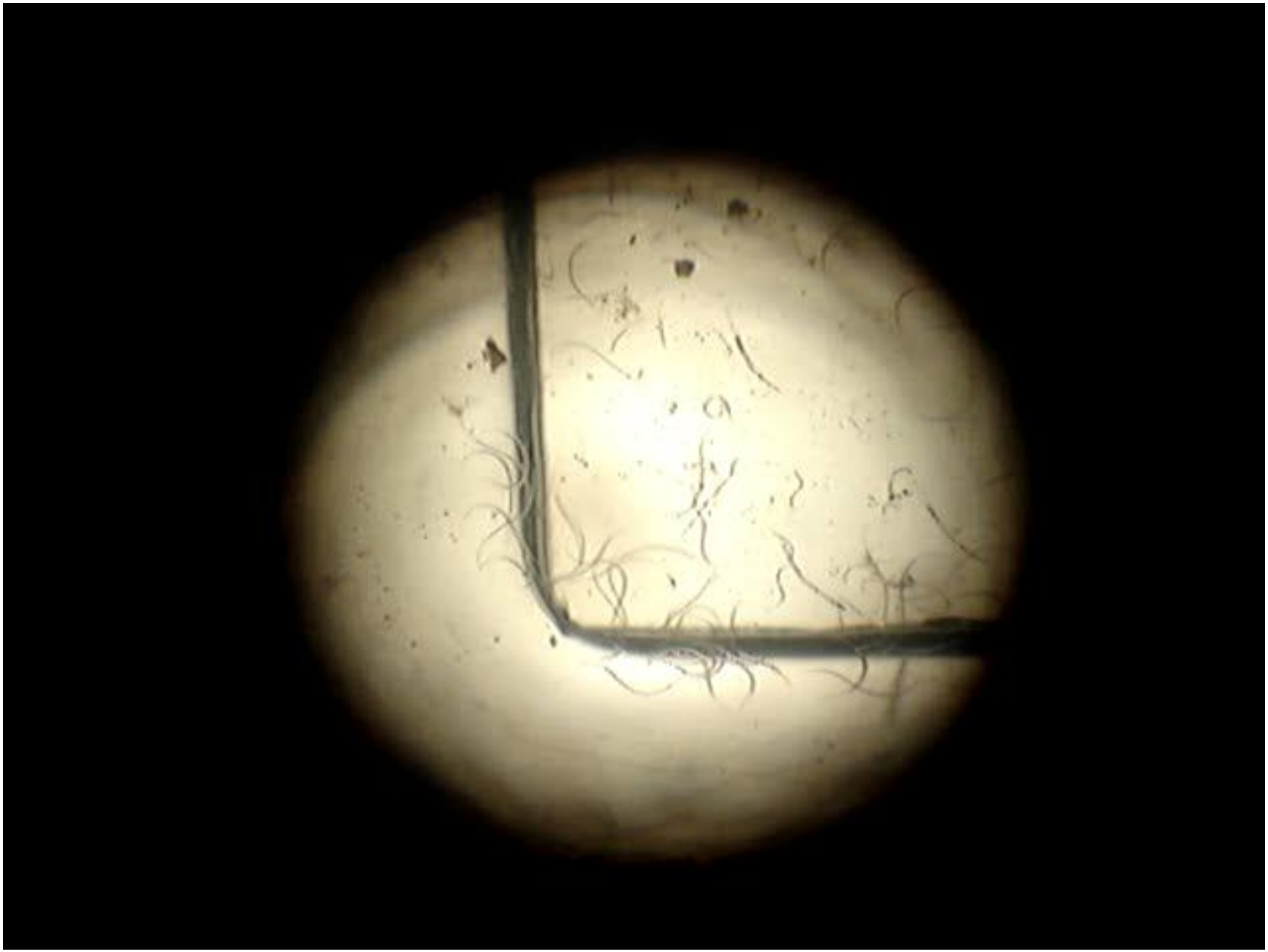
Toxoplasma tachyzoites , fresh, 100x







Eggs of gastrointestinal nematodes, 40x

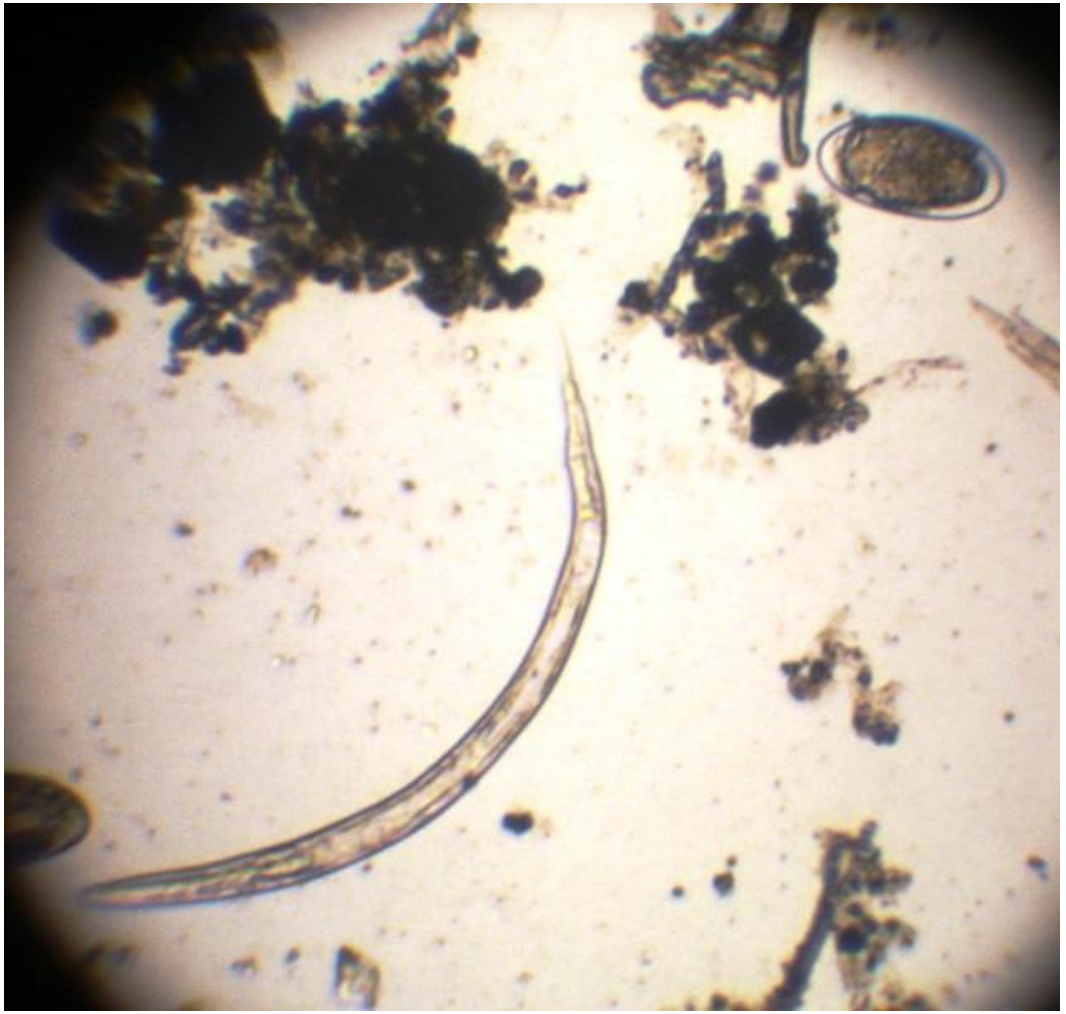


Nematode larvae, fresh, 10x



Nematode larvae, fresh, 40 x



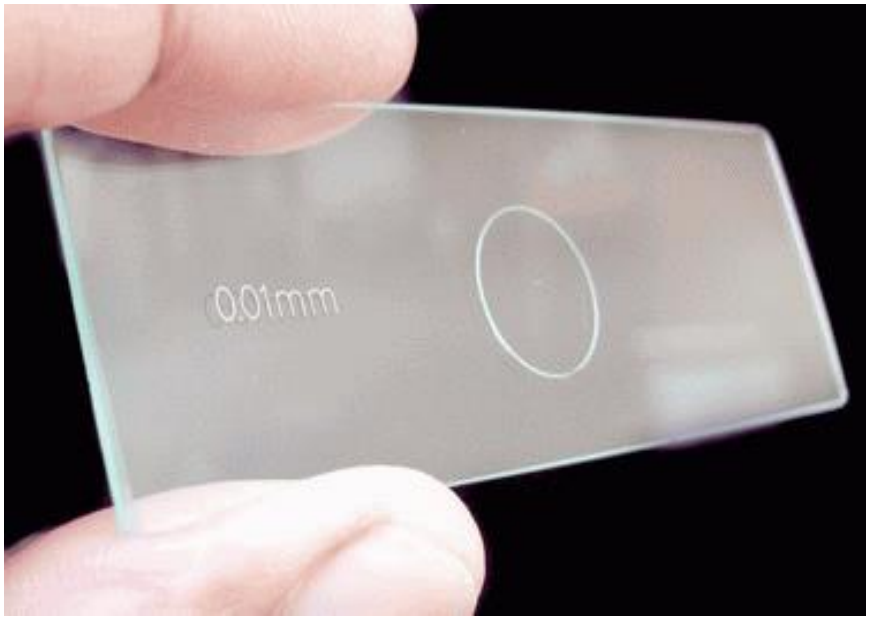


Larvae and egg of nematode, fixed and fresh, 40 x

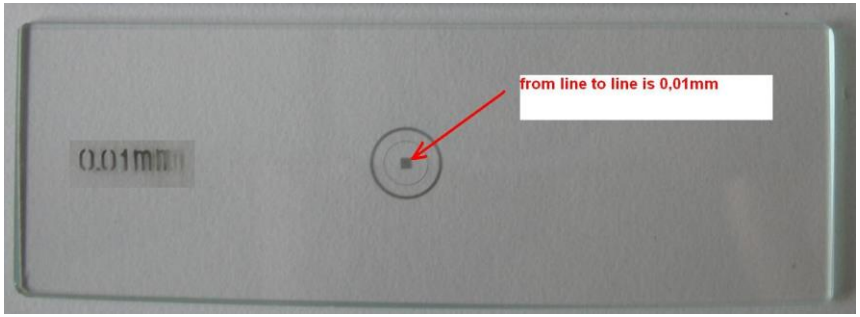
2

Photography and image processing of stage micrometer (Calibration scale)

Stage Micrometer (Calibration glass)





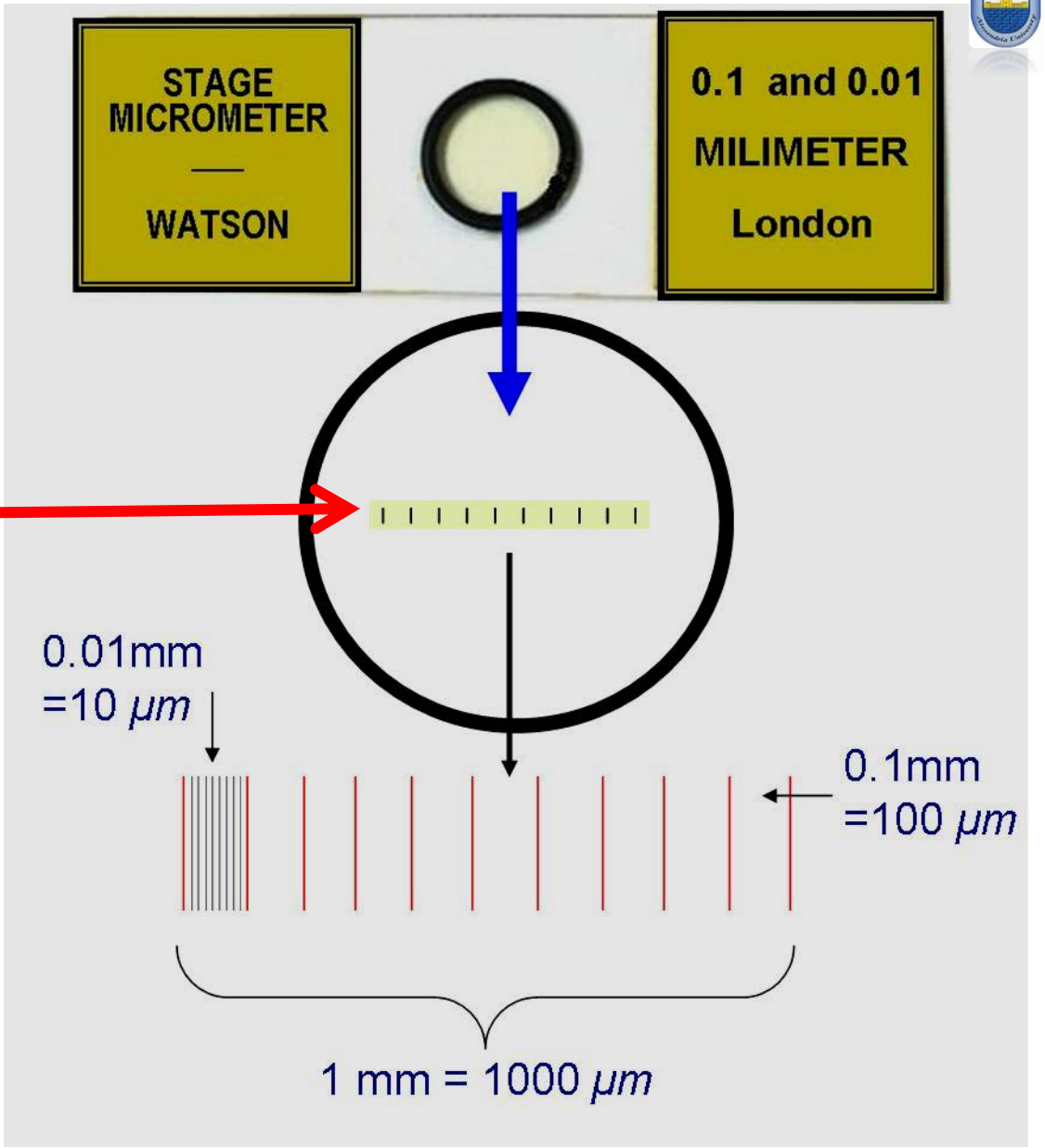


Different types of the calibration glass

Stage micrometer (Calibration scale)

the 1 mm scale

Note: The unit division patterns into 100 µm and subdivision 10 µm.



Objective Lens

4 x

10 x

16 x

20 x

25 x

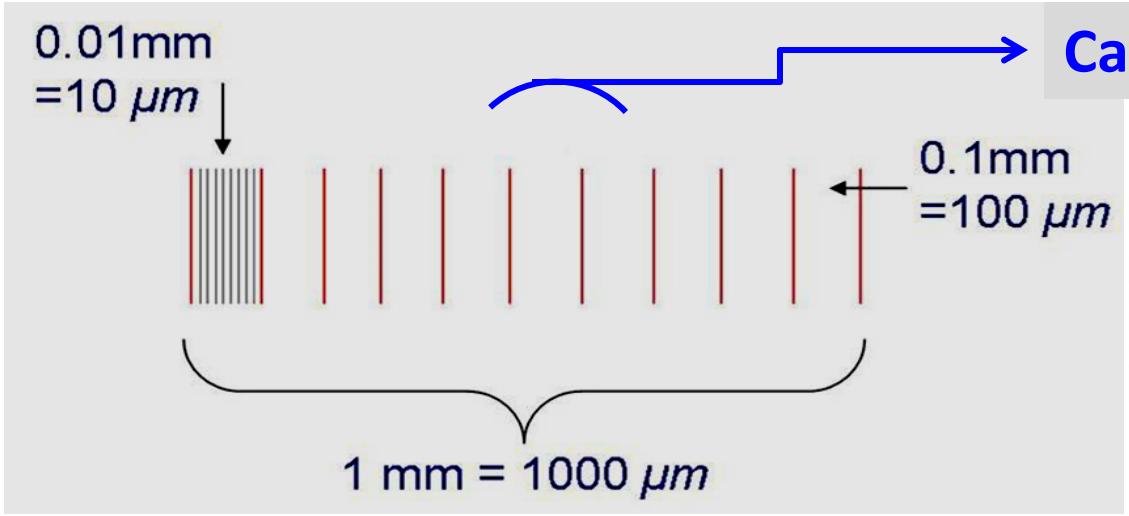
40 x

100 x



Calibration factor for each objective power Lens

4x, 10x, 16x, 20x, 40x, 100x



Calibration lines





The fixed conditions for the images of both parasite and the calibration scale :

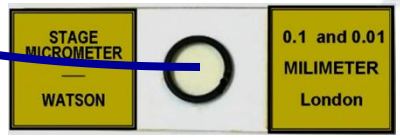
1. Use same microscope and objective lens

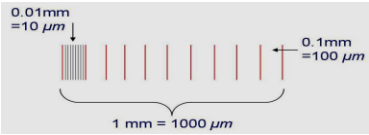
2. Use same digital camera

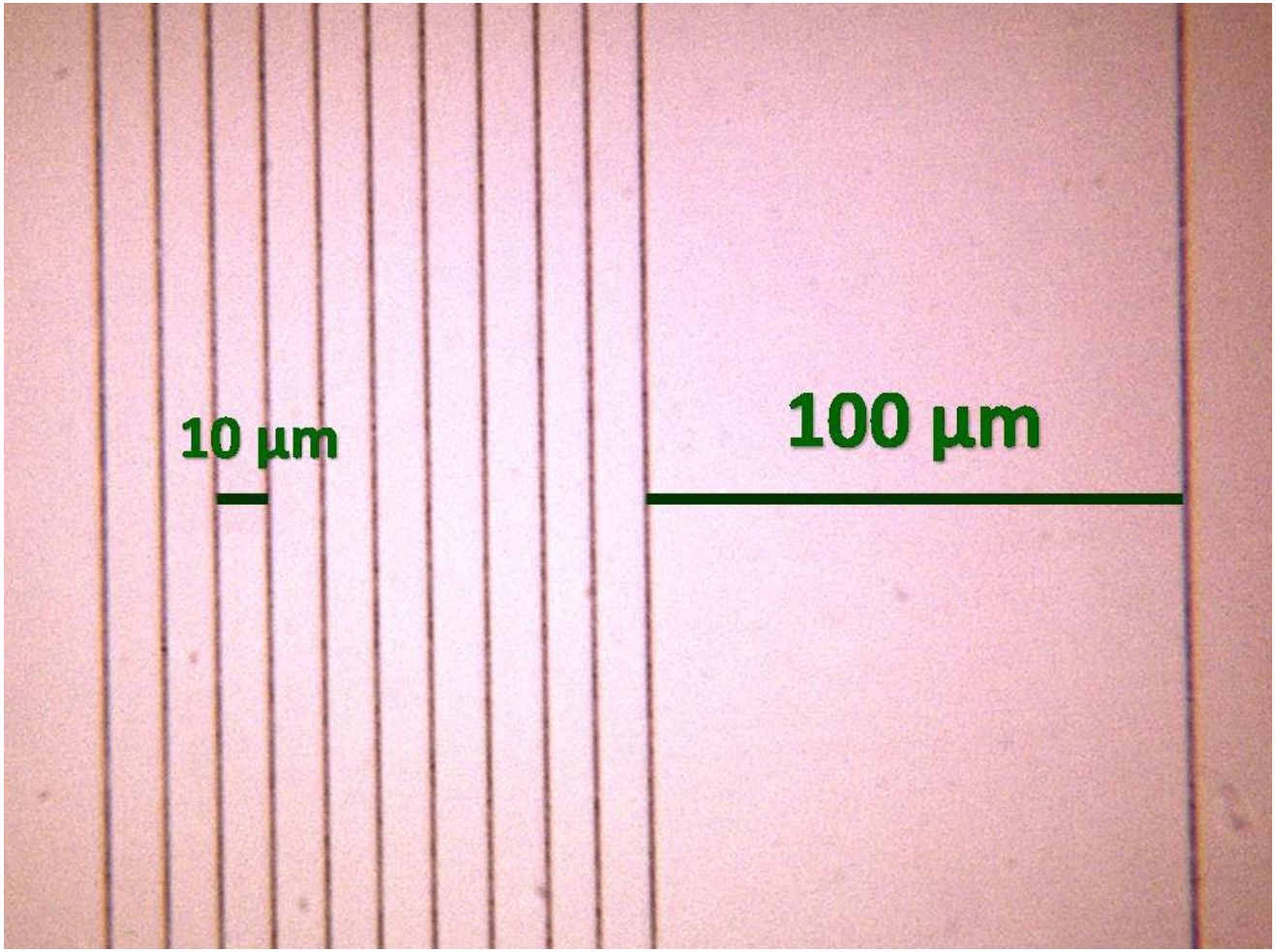
The fixed conditions for the images of both parasites and the calibration scale :

3. Same width, height, pixel number and density

4. Images should not be processed in other programs before taken the measurements









40 x

Image of the Stage micrometer (Calibration lines) with objective lens 40 x, insert on Power point slide.



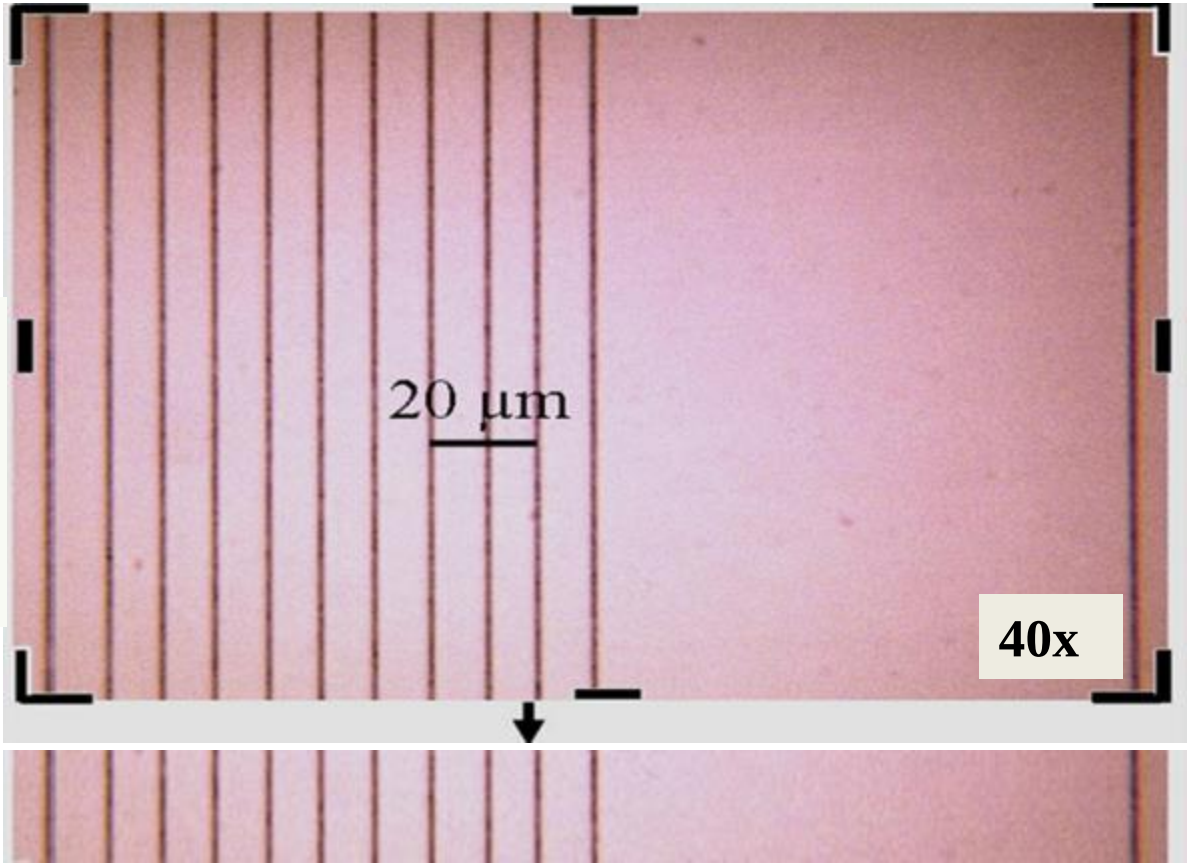
Designing the movable computer ruler (MCR)

3

Designing the movable computer ruler (MCR)

Click on the image.
In toolbar, go to
Format - Crop

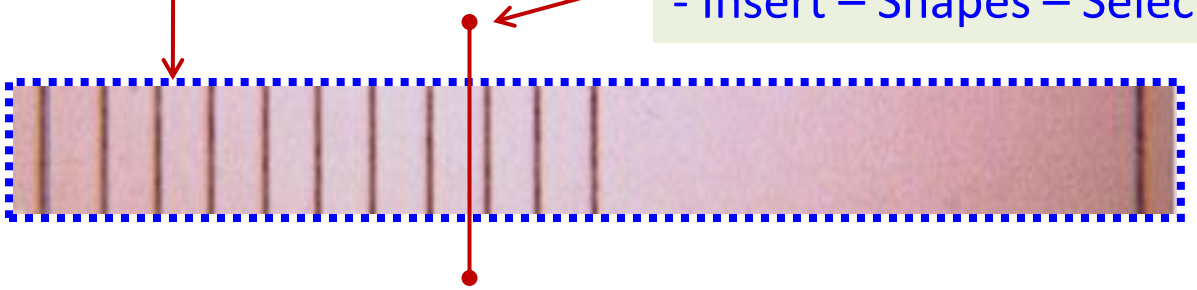
Crop the picture to remove unwanted part, (leave part up to 1/7 of the original picture)



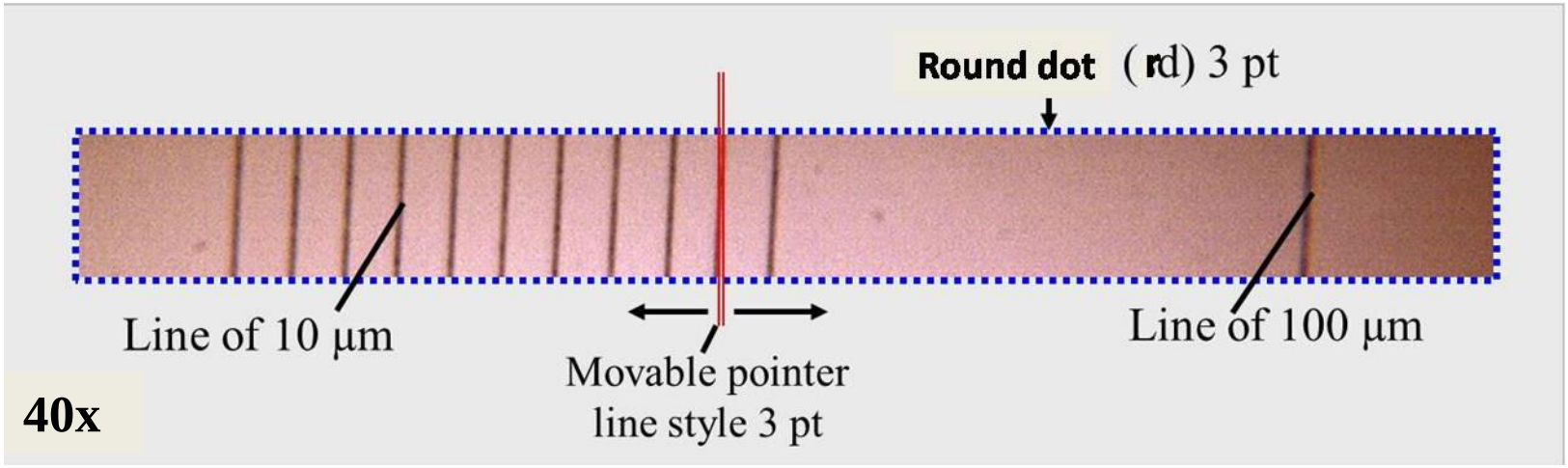
Making the raw ruler to be used in the movable computer ruler (MCR) calibration.

Round dots (3 pt)
- Home - Shape Outline
- Weight Select 3pt
- Dashes Select Round dots

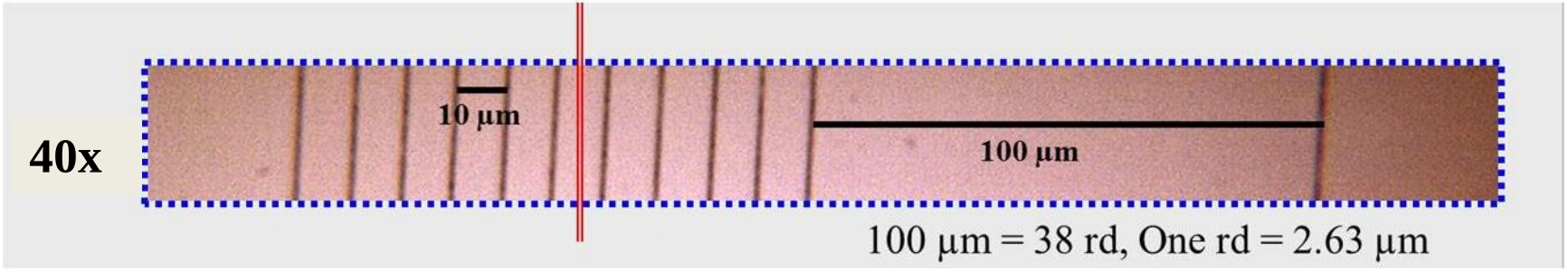
Movable pointer
- Insert – Shapes – Select Line



Insert round dots (3 pt) and movable pointer to making the movable computer ruler (MCR 40)

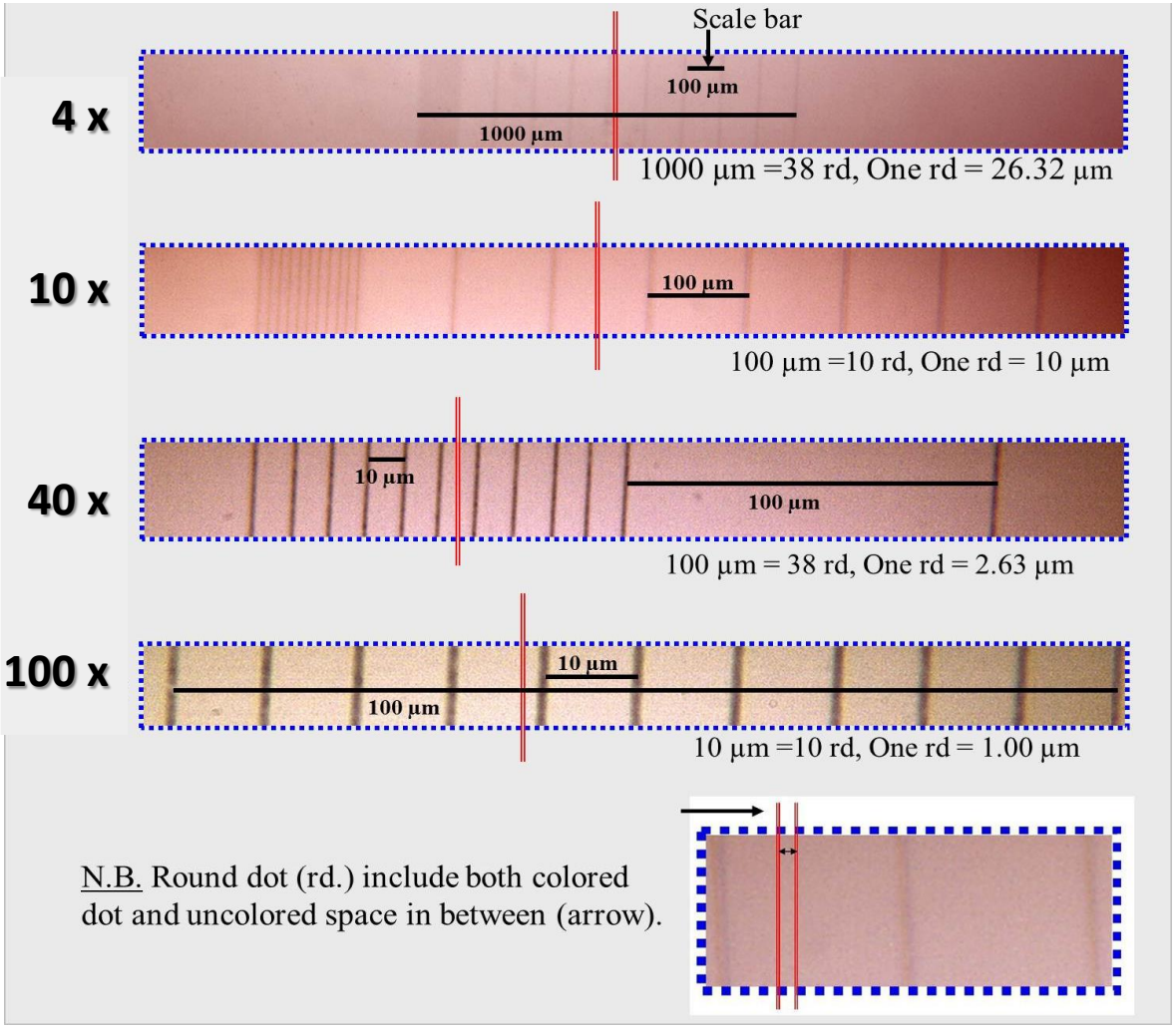


Calculation the size of round dot to microns



MCR 40

100 / 38 = 2.63



Making a movable computer ruler (MCR) for images acquired by various magnifications **(MCR 4, MCR 10, MCR 40 and MCR 100)**.
Note the formats of MCR, calculation of round dot size to micron, and scale bars.

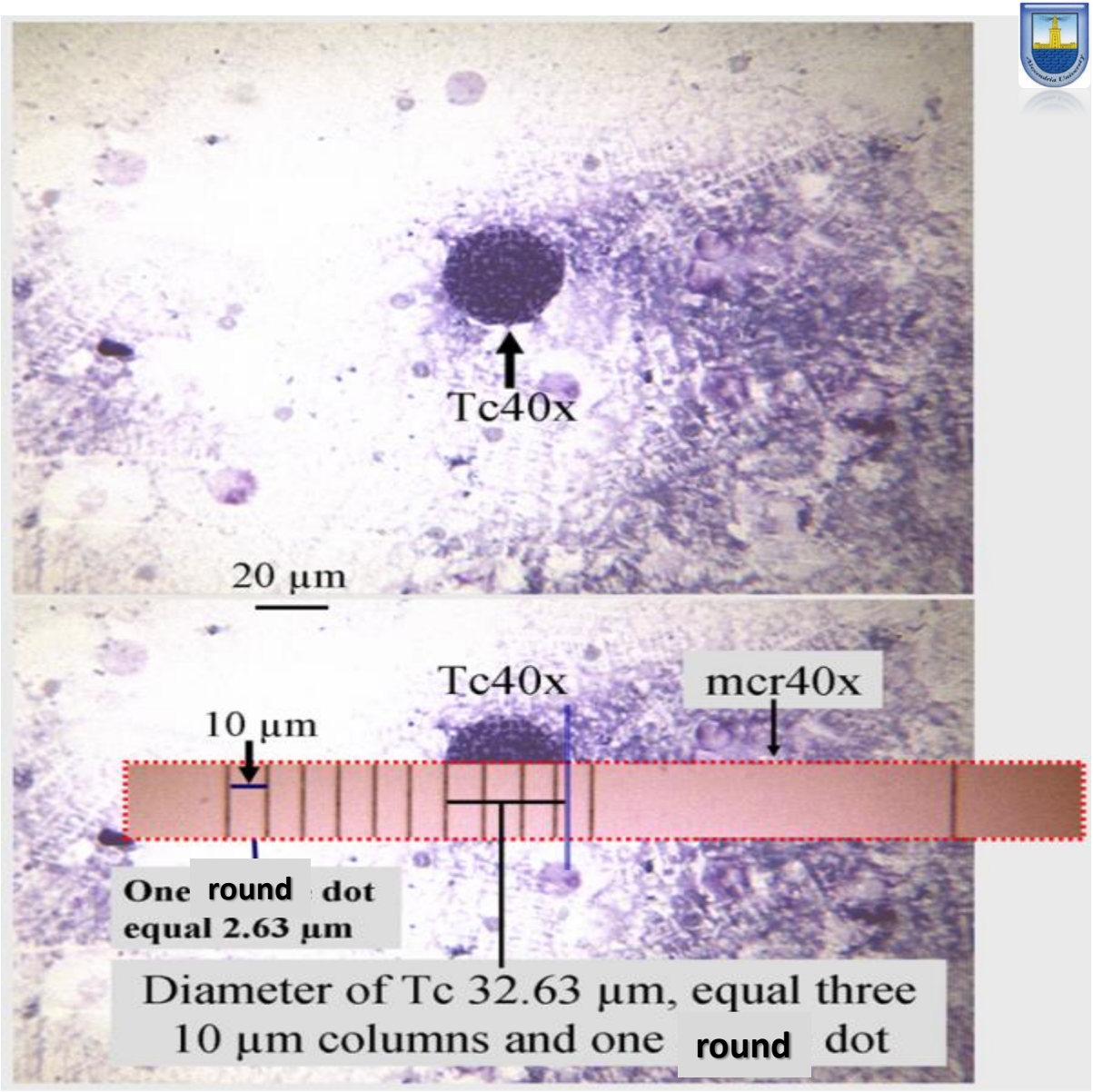
4

Application of MCR method

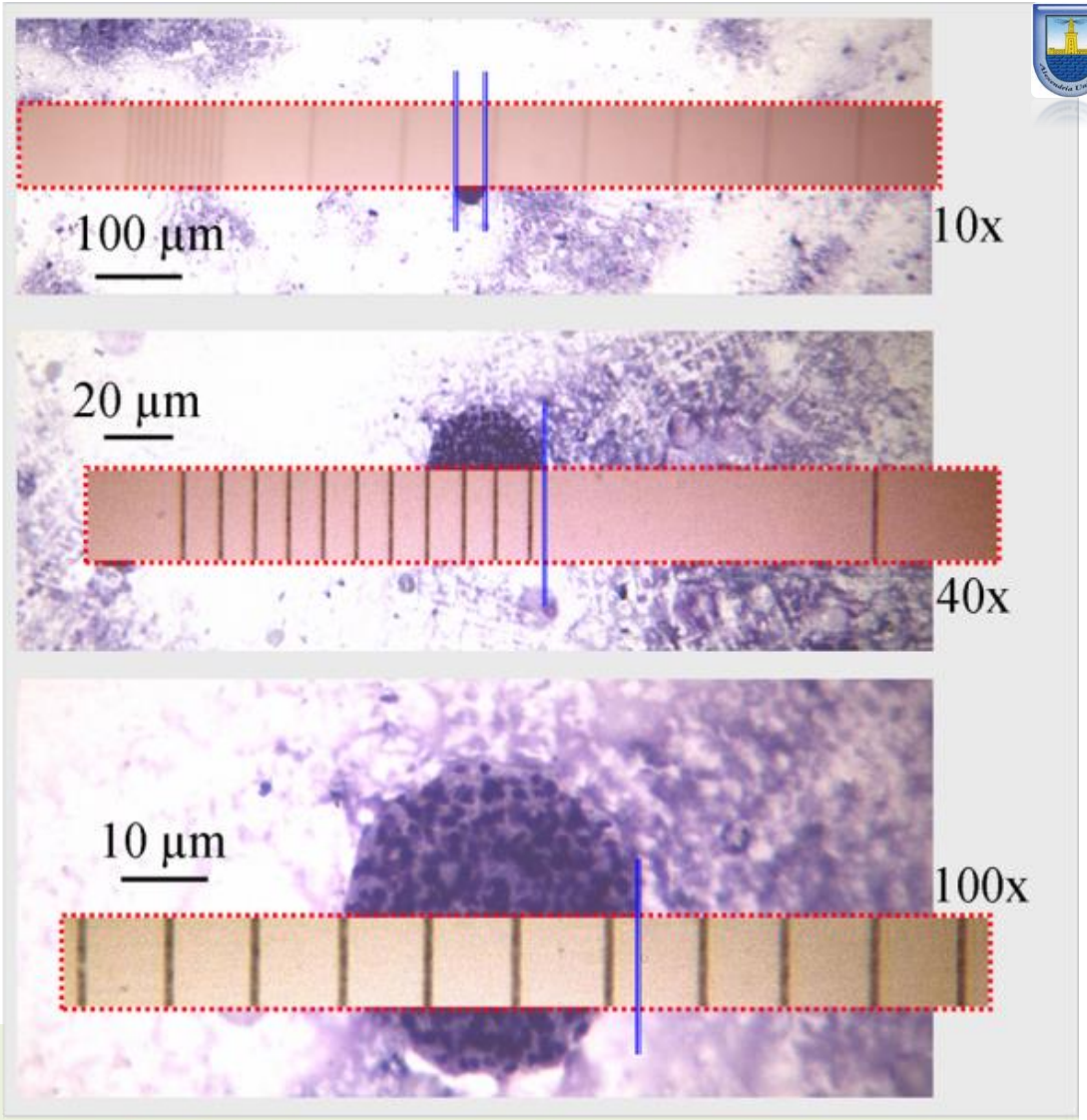
Application of **MCR** for measurement of **Toxoplasma cyst** in scrape from mouse brain (Giemsa stain).

Upper, the cyst image was captured by objective lens 40x (Tc40).

Lower, the cyst image after superimposition on MCR 40.

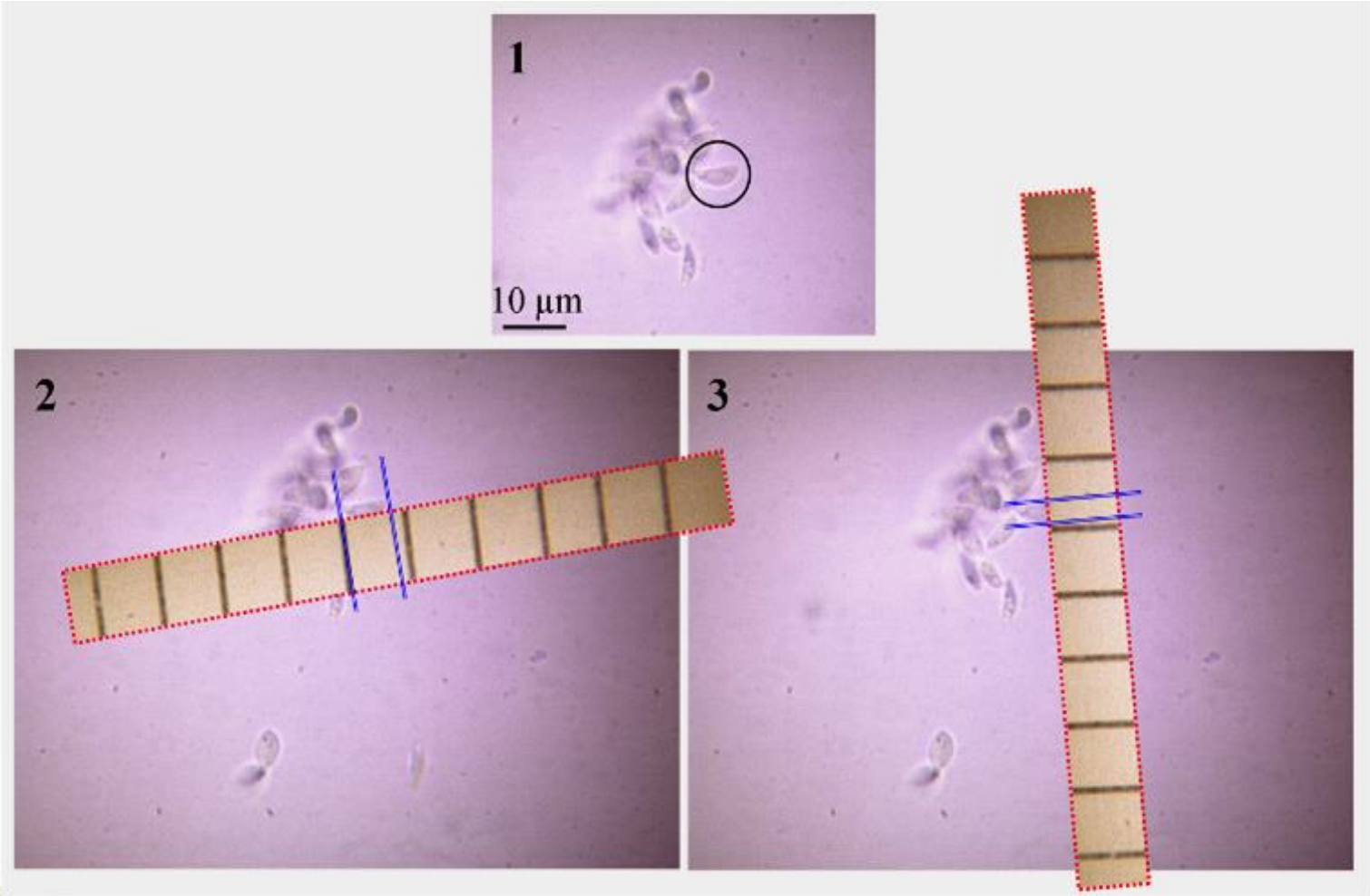


MCR of different magnifications (MCR 10, MCR 40, MCR 100) used to measure the size of Toxoplasma cyst obtained using various microscope magnification powers (Tc10, Tc40, Tc100).



Diameter of Tc

Use of 10x = 30µm & 40x = 32.63µm & 100x = 32.5µm

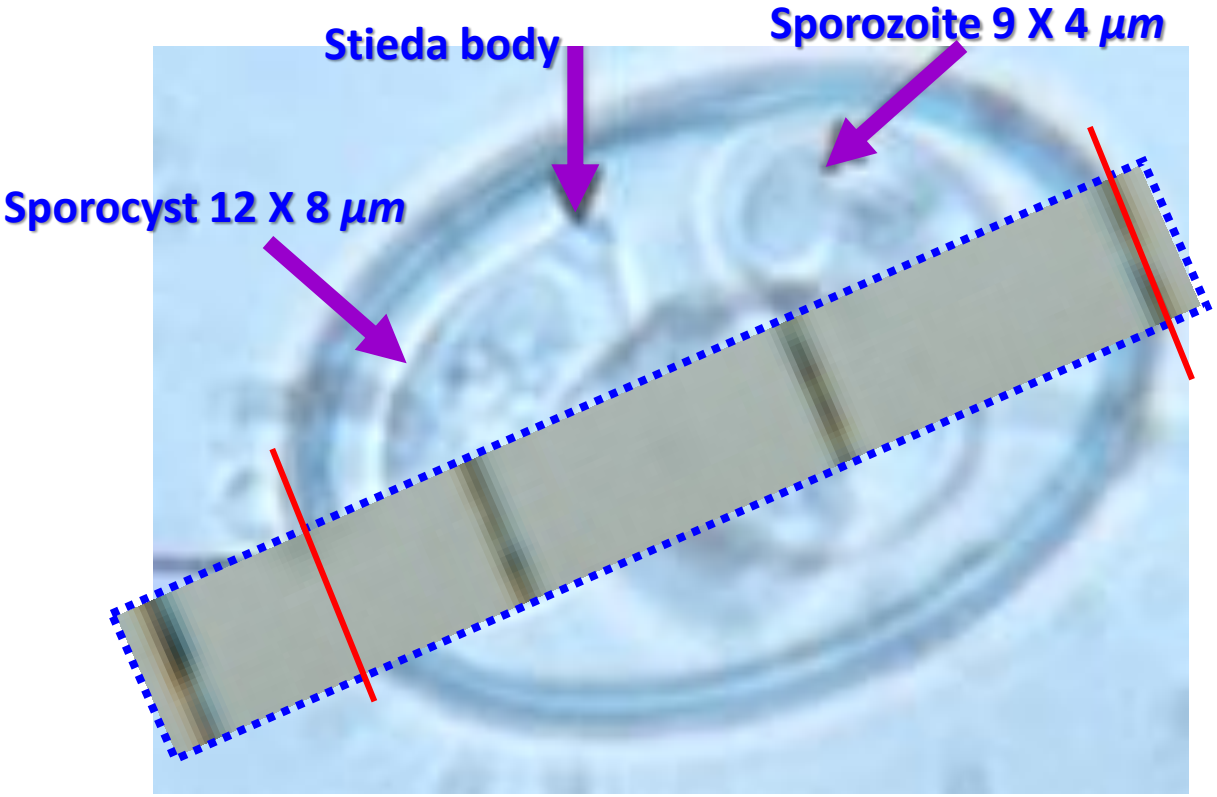


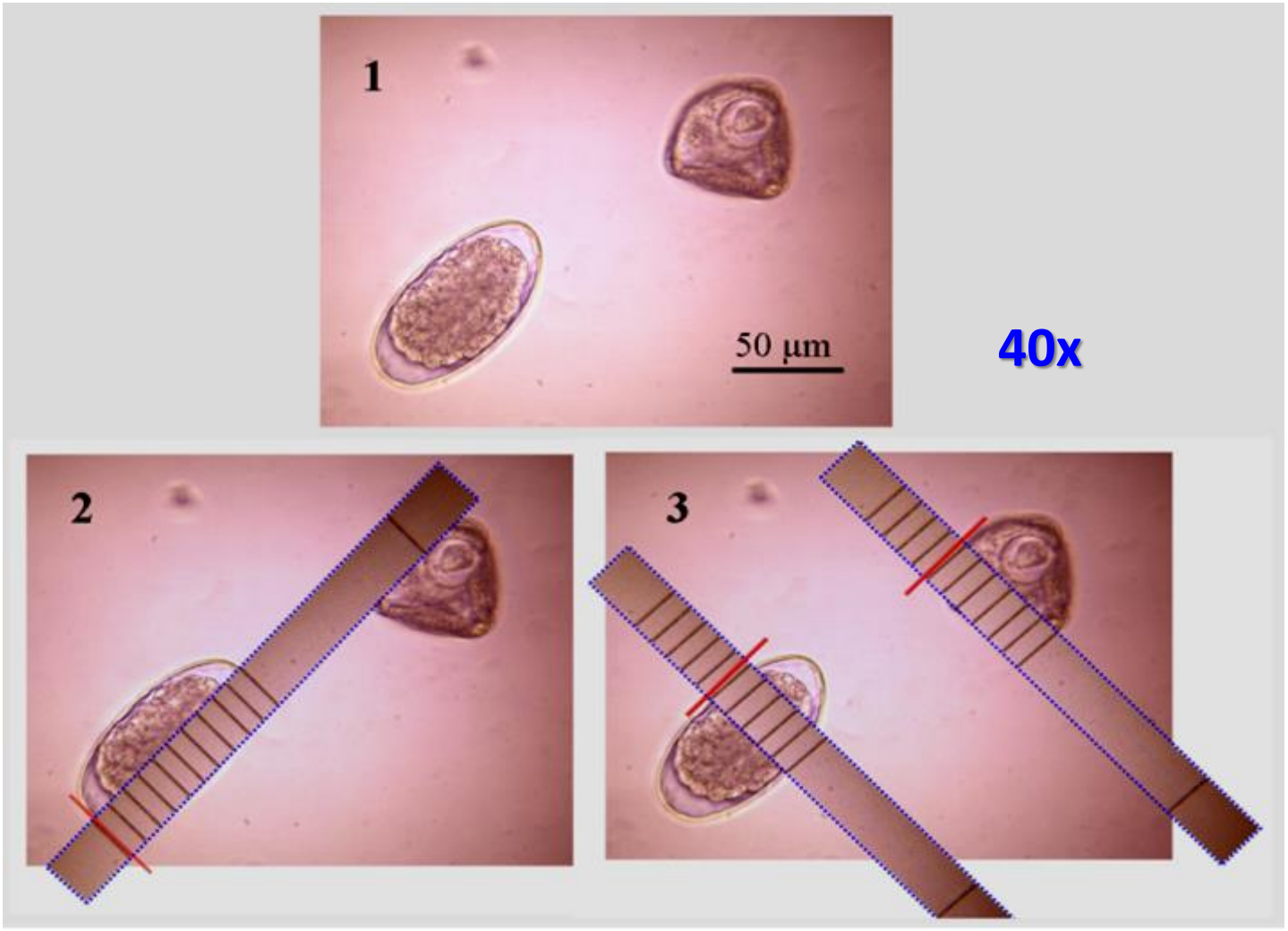
Measuring the size of Toxoplasma tachyzoite (Tz) at 100x

One rd = 1 µm, the length is 7.5 rd (equals 7.5 µm),
width is 2.5 rd (equals 2.5 µm).

The Tz size is 7.5 X 2.5 µm.

Measurements of different structures of sporulated Eimeria oocyst use MCR 100




1
50 µm
40x
2
3



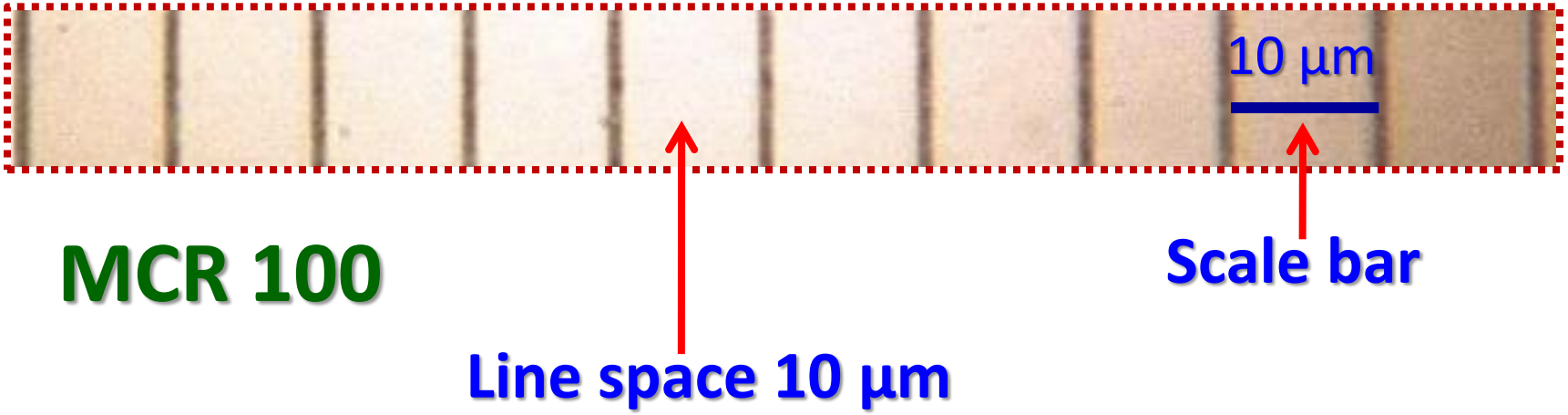
***Trichostrongylus sp* egg, 40x.**

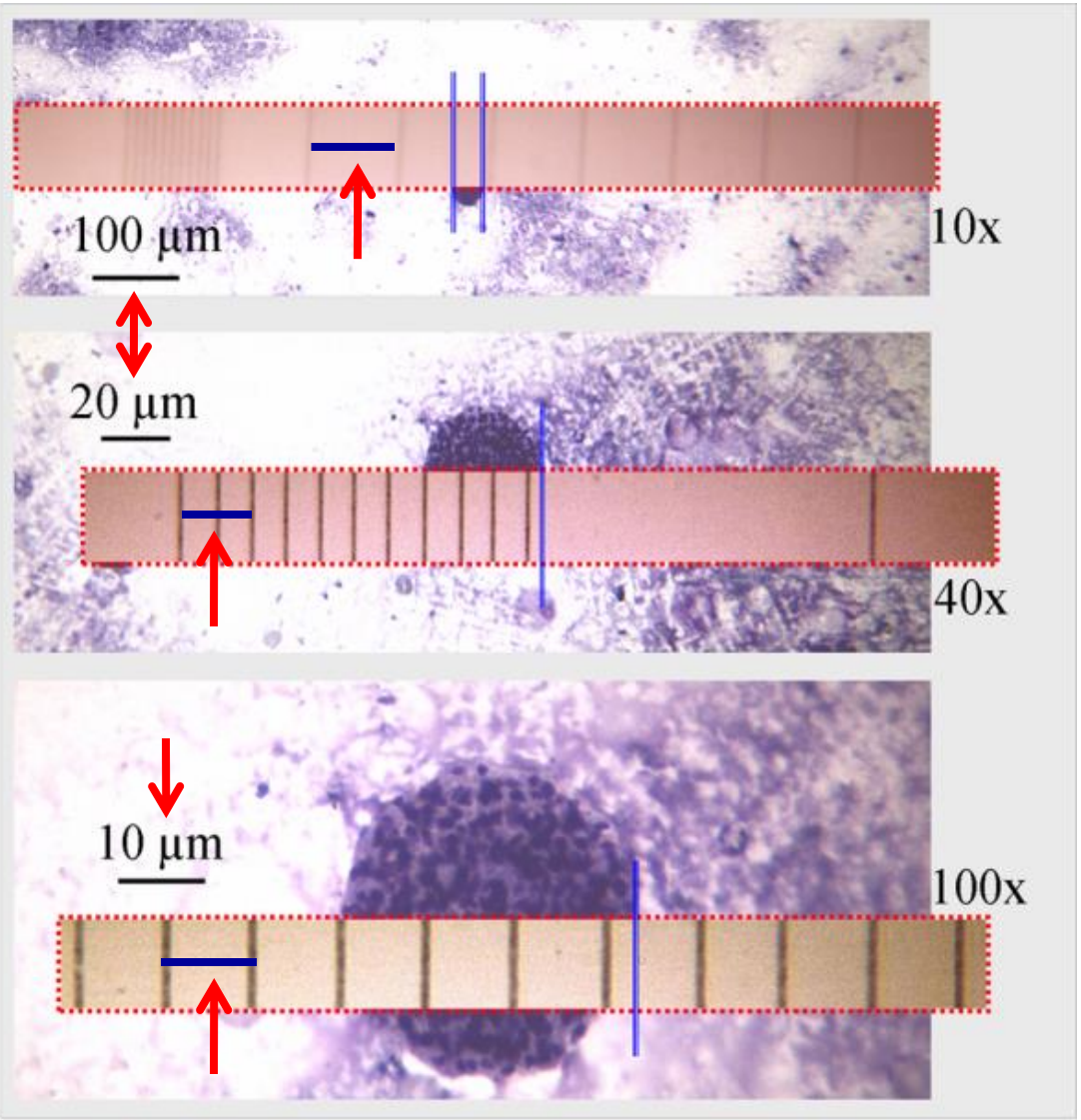
100 µm equal 38 rd at 40X, each rd = 2.63 µm

Length = 37.5 rd X 2.63 = 98.63 µm.

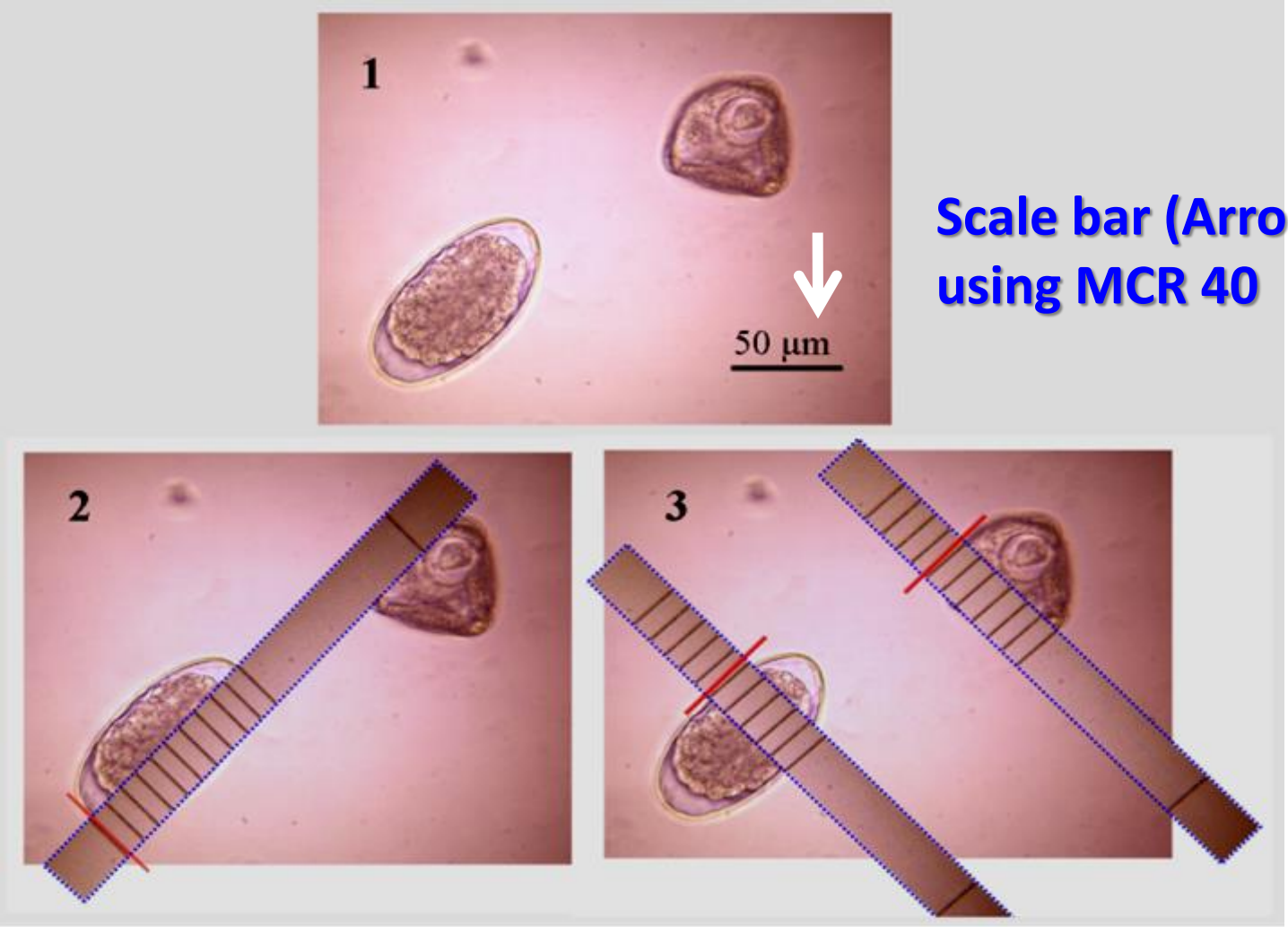
Creation of Scale bar

By formation of **MCR**, scale bar is easily created and attach to the image of parasite



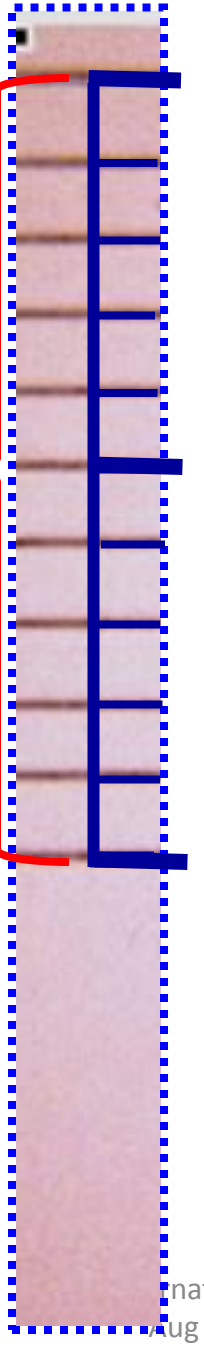


Scale bars (Arrows),
using MCR 10, MCR 40 & MCR 100



Scale bar (Arrow), using MCR 40

Creation of graduated scale bar using MCR 40

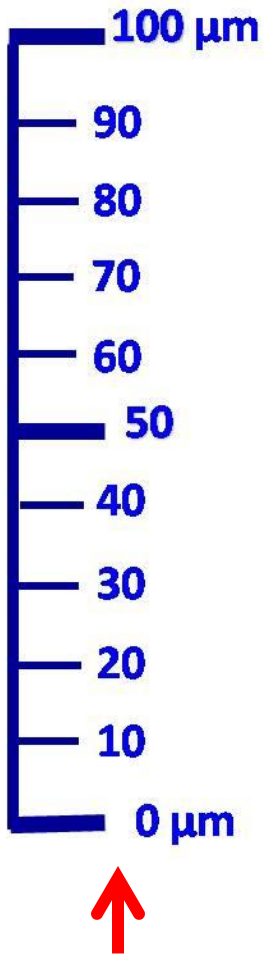


MCR 40



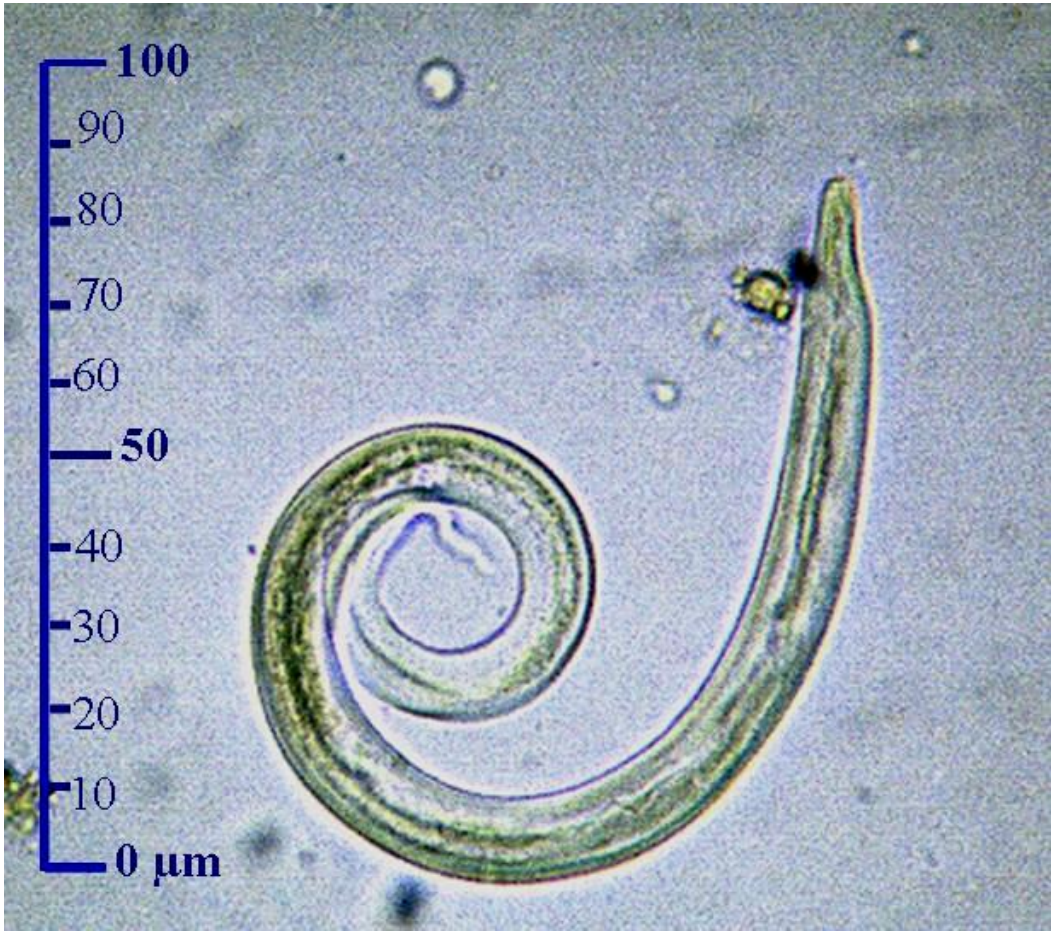
***Protostrongylus sp.* (Sheep), first larva, 40x**



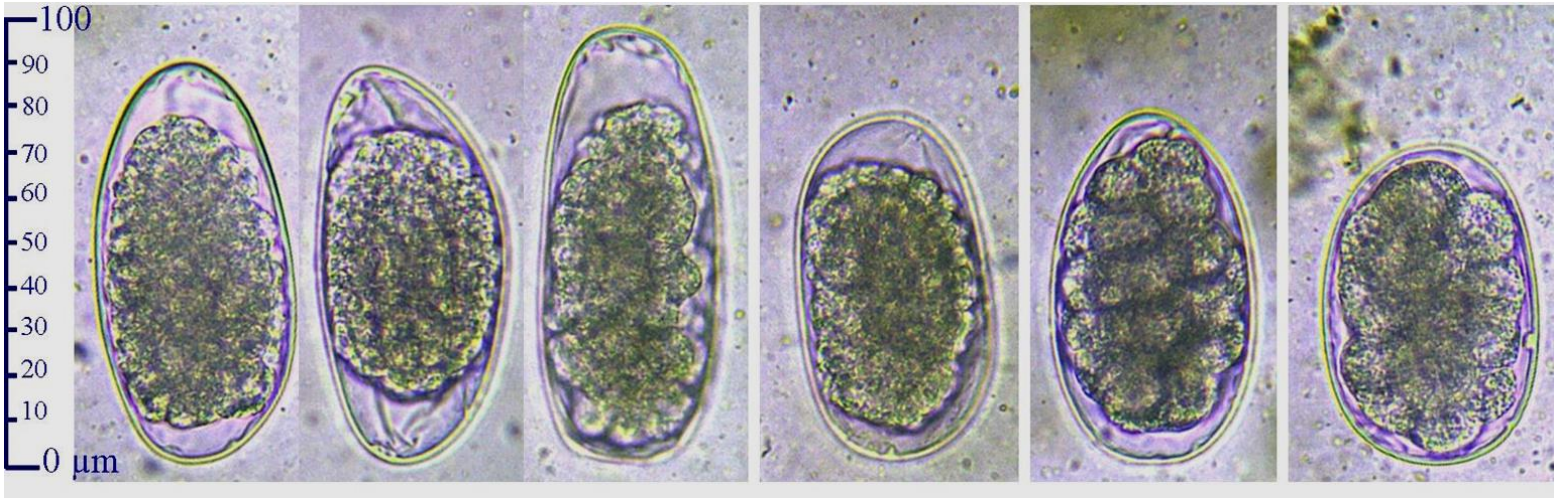


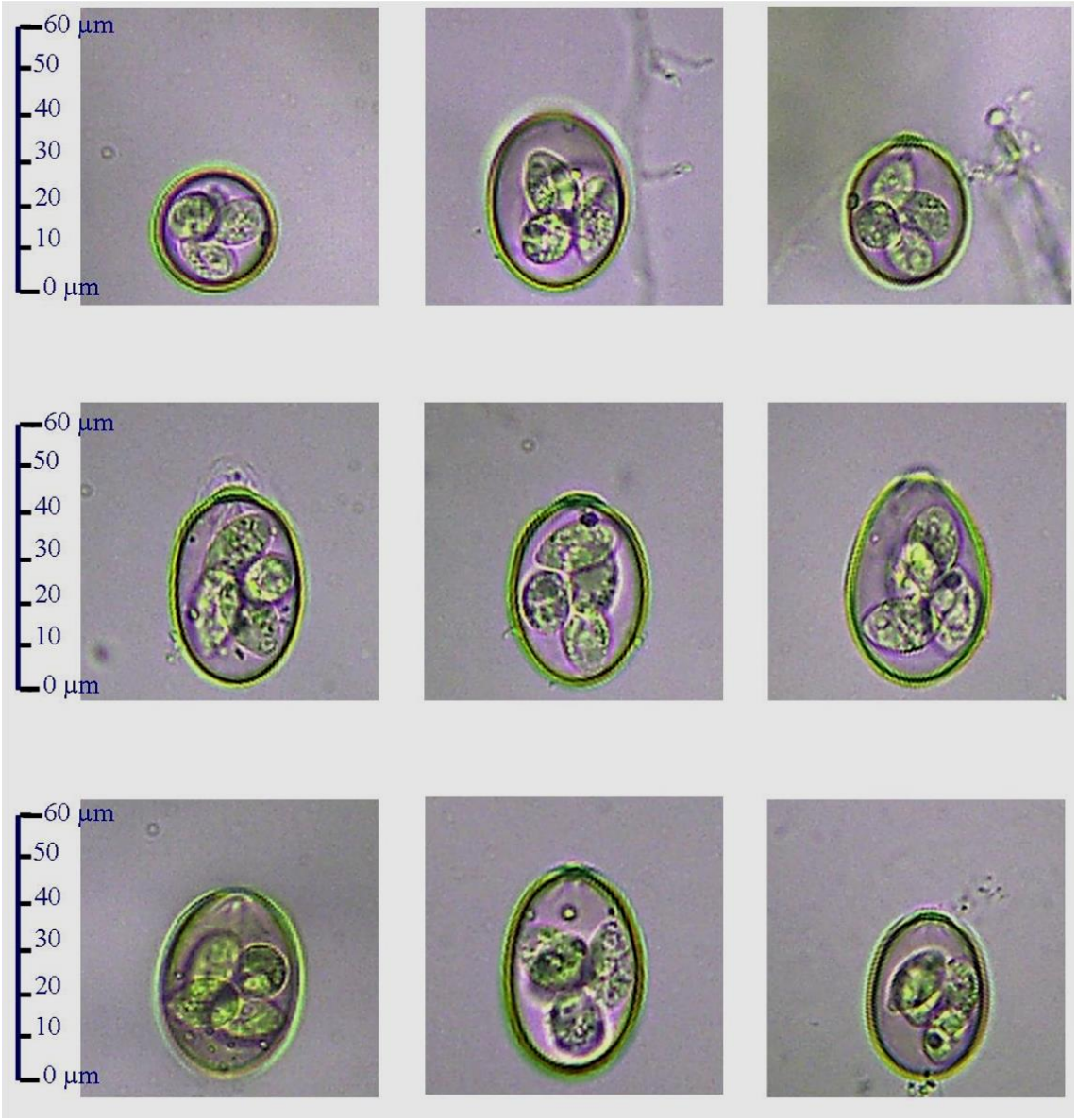


Graduated scale bar (Arrow),



***Protostrongylus sp.* (Sheep),** first larva, 40x


100
90
80
70
60
50
40
30
20
10
0 µm


60 µm
50
40
30
20
10
0 µm
60 µm
50
40
30
20
10
0 µm
60 µm
50
40
30
20
10
0 µm

Research Summary

- MCR is a new method for measuring the size of parasites and other objects.
- MCR its design is derived from digital images of a stage micrometer.
- MCR can be superimposed on images of parasites to measure their size.
- Width, height, pixel number and density are fixed for every image.
- MCR method saves time and efforts.

Conclusion

- **MCR have been tested using selected parasites.**
- **It has been shown to be as accurate as the traditional methods.**

. The images must be:

- From well-prepared specimen
- Good focus and good quality

- The images should **not be edited** in other program prior taking the measurements.

- **MCR method is less complex and more precise.**
- **Applicable even in laboratories with limited resources.**

- **MCR saves effort and cost.**
- **Easy and fast in application.**
- **Can be used every time you need.**

- **MCR is more efficient technique.**
- **Scale bar, also will be easily created and attach to the image.**

. Reference for more details is published:

Exp. Parasitology 130 (2012) 1–5.



Faculty of Veterinary Medicine, Alexandria University, EGYPT
Since 1975



Alexandria, EGYPT, Mediterranean sea and lagoon of Bibliotheca Alexandrina



Thank you شكرا