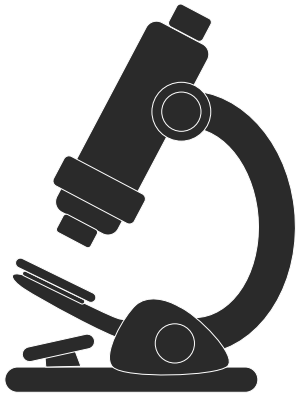
Bi9393:
Analytical cytometry

Microscopy techniques



practical handout

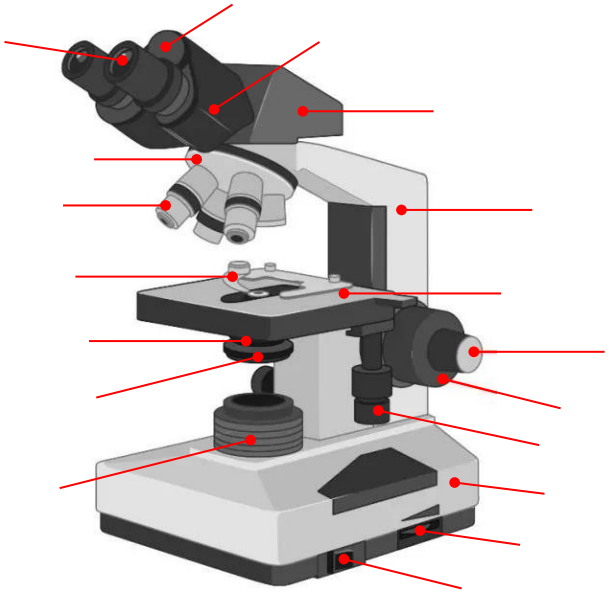
History of Microscopy

Do you remember important milestones of in life sciences and microscopy? Work in the group and arrange cards with important discoveries in chronological order.

Insert picture of the timeline.

Do you know microscope parts?

Describe parts of the microscope and highlight components containing lenses.



Magnification vs. Resolution

1) During cell tissue culture, you produced a beautiful image of human cells, and your supervisor asked you to provide a total magnification of the picture. Your senior colleague told you the characteristics of the used microscope (Olympus CKX53, objective 4x, eyepiece 10x).

What is the overall magnification? _____

2) You have provided the overall magnification, but your supervisor asked you about the microscope's resolution. You went back to the cell culture lab and found the following information on the microscope (4x objective N.A. 0.13, condenser N.A. 0.3).

How will you calculate the resolution of the microscope? Write down the formula:

What is the resolution of the CKX53 microscope with a 4x objective?

3) In the lab, you are approached by a colleague who is asking for advice on imaging. Your colleague wants to obtain images with the highest possible resolution (as you want to see fine actin filament structures) on the local confocal microscope with the following objectives:

A) 40x objective, N.A. 0.95, dry
B) 60x objective, N.A. 1.45, oil
C) 100x objective, N.A., 1.45, oil

Which objective do you recommend and why?

Do you know the parameters of the objective?

1) Describe the parameters of the objectives:

What is a mechanical correction collar? Based on what it needs to be adjusted?

2) In the lab, you are planning an experiment during which you want image cells stained for four different fluorophores and have the highest possible resolution. On the local microscope, you have the following objectives:

A) Plan Apo 20x objective, N.A. 0.8, dry
B) FL 40x objective, N.A. 0.95, dry
C) Plan Ach 60x objective, N.A. 1.45, oil

Which objective will you choose and why?

2) In the lab, you are planning an experiment during which you want image cells stained for four different fluorophores, want to have good resolution, but also want to capture many cells. On the local microscope, you have the following objectives:

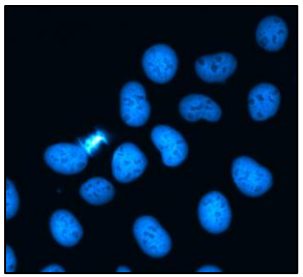
A) Plan Apo 20x objective, N.A. 0.8, dry
B) Plan Apo 40x objective, N.A. 0.95, dry
C) Plan FL 60x objective, N.A. 1.45, oil

Which objective will you choose and why?

3) In the lab, you are approached by a colleague who is asking for advice on imaging. Your colleague needs to image a survival screen in a 96-well plate (10 plates in total) containing human cells stained with one color DAPI (dye to stain cell nuclei).

96-well plate

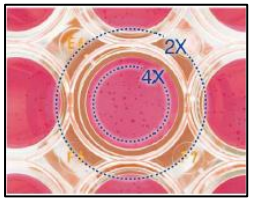
Human cells stained with DAPI



In the local widefield microscope, you have a following objective options:

A) Plan Ach 2x objective, N.A. 0.06, dry
B) Plan Apo, 4x objective N.A. 0.16, dry
C) Plan Apo 40 x objective, N.A., 0.95, dry

Which objective do you recommend and why?



Microscopy techniques

1) Thanks to the microscopy crash course, you now have a good overview of available microscopy techniques ☺. One day, your supervisor approaches you to ask for advice on which routine microscope to buy for the cell culture lab where you are cultivating transparent human cells. The options are the following:

A) Brightfield microscope
B) Darkfield microscope
C) Phase contrast microscope
D) DIC microscope

Which microscope do you recommend and why?

2) You spent a day in the lab preparing your sample for confocal imaging (human cells with four fluorescent colors). You want to book a confocal microscope in the facility, but you are not sure whether your staining worked (maybe you forgot to add DAPI).

What you will do?

3) You spent a day working at the confocal microscope and have a beautifully resolved image of active filaments on the computer screen. When a new colleague (who is just learning about microscopy) comes, you show him your sample in the microscope. Your colleague is confused; in the eyepiece, she/he cannot see such fine resolution of the actin structures you have on screen. Why?

Explain the principle of confocal microscope.

4) In the lab, your colleague wants to resolve the structure of actin filaments to the highest possible resolution with conventional fluorophores. In the microscopy facility, there are available following super-resolution microscopes:

A) Photo-activated localization microscope
B) Stimulated emission depletion microscope
C) Structural illuminated microscope

Which technique would you recommend and why?

5) In the lab, your colleague wants to resolve the nuclear pore complex to the highest possible resolution and specific dyes for super-resolution techniques are also available in the lab. In the microscopy facility, there are available following super-resolution microscopes:

A) Photo-activated localization microscope
B) Stimulated emission depletion microscope
C) Structural illuminated microscope

Which technique would you recommend and why?

6) You are planning the imaging experiment during which you want to capture cell division of human cells without any dyes. In the facility, they offer time-lapse imaging for the following systems:

A) Brightfield microscope
B) Fluorescent microscope with phase contrast
C) Fluorescent microscope with DIC

Which technique will you choose and why?

7) You are planning the imaging experiment during which you want to capture your favorite GFP-tagged protein of interest using time-lapse imaging. In the facility, they offer time-lapse Imaging for the following systems:

A) Confocal laser-scanning fluorescent microscope
B) Widefield fluorescent microscope with phase contrast
C) Confocal spinning-disk microscope with DIC

Which technique will you choose and why?

8) In the lab, your colleague approaches you for advice regarding her projects. He/she wants to study protein dynamics in living cells.

Which method will you recommend for the project and why?

9) Based on your previous suggestion, your colleague performed FRAP using a laser-scanning fluorescent microscope. He/she is presenting her data during the lab meeting and shows that control cells showed the same dynamic of GFP-tagged protein of interest as treated cells. This data surprises you because there should be a visible difference in protein diffusion in control vs. treated cells.

Why your colleage cannot see any difference? Could you suggest any changes in image acquisition?

Preparation of samples for imaging

1) For your PhD project, you need to stain the protein of your interest with an antibody. Unfortunately, there are no antibodies against your protein of interest available in your lab. Your supervisor agrees to buy a new antibody but asks you to find a suitable antibody.

Shortly describe all aspects that you will consider.

2) Your antibody arrived, and you are planning to test its specificity against the protein of your interest.

Shortly describe or draw how you would test the specificity of the antibody using a fluorescent microscope.

3) Your colleague is planning an imaging experiment, during which she/he needs to visualize the localization of a protein of interest in fixed cells.

Using which techniques she/he can visualize the protein of interest?

What will be your further suggestions (pros & cons) about these techniques?

4) In the lab, your colleague approaches you as he/she wants to visualize a protein of interest using fluorescent microscopy, and she is considering two fluorophores:

A) Alexa Fluor 488
B) FITC

Consider the properties of the fluorophores. Which one would you recommend and why?

FluoroFinder

FluoroFinder

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References of used images:

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