Depth of sharpness of optical microscope

What is the definition ?

Depth of Sharpness of the Microscope studies how an objective can be focused without loose the sharpness of the image. Therefore we can state that the depth of field is the ability of a microscope to produce a sharp image from a surface (non flat surfaces). Many people have studied this subject and confirm that by closing the aperture of the lens objective. Nonetheless, when the aperture is closed the optical resolution will decrease as well as a loss in illumination.

Ambiguity of the term - Depth of field or depth of focus

Normally both terms are not well understood, and the definitions are quite different. Yet, in this piece of paper we will use the term Depth of field because it clearly defines the specific distance a person have in front of the objective and checks if the image stays sharp in all directions to the object being imaged.

One of the objectives of the depth of field numerical variable is that it makes unequal surfaces in a sharp contrast and a better quality of image. On the other hand, we can also single layers of of tissues (ex: cells) without a significant interference of both superior and inferior tissue layers.

How to increase the depth field ?

After our analysis about way the depth of field is extremely important for image quality and sharpness, this leads us to the willing of the discovery to increase the Depth of field. This could be important in the ability of observing even more smaller particules, at a size of nanometers. Therefore increasing the depth of field will promote an increasing in the sharpening of nanometer objects. Nevertheless this cannot be accomplished with conventional microscopes since they can not handle the two different variables - depth of field/sharpness and resolution- without decreasing one of them.

If we have an object, and we increase the depth of field this will increase the sharpness of the layers and structures of the object with 3 dimensional view allowing for a better understanding of each object under the microscope. Mathematically, the total depth of field is given by,

$$dtot = \lambda n/NA2 + (n/M \bullet NA)e$$

This is the sum of the geometrical and wave related optical depths, where λ is the wavelength of illumination, n is the refractive index of the medium where the image is placed, NA is the numerical value of the aperture, M is the lateral magnification of the objective and the e variable is the smallest distance that can be eventually seen by the detector placed in the same plane of image of the objective.

The first variable placed on the right-hand side of the equation decreases inversely proportional with the square of the numerical aperture. On the other hand, the lateral limit of resolution decreases with the first power of the value of aperture (numerical value).

Practical values for visual depth of field

In DIN/ISO standards, the depth of field is defined as the depth in the axial plane of the space, present on both sides of the object quadrant being studied within it can be replaced without any considerable reduction of sharpness in the image viewed. Besides this, the image plane and objective continue the same.

Pratically, one knows that at low magnifications, the value of the depth of field can be substantially increased while there is a reduction in the value of numerical aperture. However, if there is a smaller numerical aperture value, therefore will exit a lower lateral resolution. Thus, it is rather important to find a perfect equilibrium between these two concepts, the depth of field and the resolution of the microscope.

Links

Related articles

External links

Bibliography

http://www.grayfieldoptical.com/history.html
http://www.matter.org.uk/tem/depth_of_field.htm
http://en.wikipedia.org/wiki/Focus_stacking
http://www.leica-microsystems.com/science-lab/how-sharp-images-are-formed-depth-of-field-in-microscopy/