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## DIACHROMIC MLCROSCOPE CONDENSER

FIELD OF THE INVENTION

The present invention relates to light microscopy, and more particularly concerns a substage condenser allowing to illuminate in a certain fashion the very small objects observed (Appendix 1).

We recall, the specimen in light microscopy must be illuminated by a visible light source. Visibility of the objects (or particles) depends on three factors; the object's absorption power, its refraction and its diffraction.

The absorption power defines the spectrum of light that is stopped by the object, and can be wavelength sensitive, which can influence the object's apparent color.

Refraction refers to the amount of light that is deviated by refraction from its trajectory when going through the object. if this refraction is weak, which is often the case with very small objects (or particles), the deviated light is too weak to be immediately perceptible.

As for diffraction, it generates luminous circles around small objects which become less defined. With very small objects, the resulting image becomes a small and diffuse spot which does not become any clearer if the microscope's magnifying power is increased (more and more magnification gives a blur). In addition, when outside of normal range of the resolving power of the light microscopes, (appendix 2) the diffuse spot simply becomes invisible. (Resolution power being independent of the wavelength of the light source used, that is visible light in this case, and the numerical aperture of the objectives used).

Light sources in light microscopy generally consist of a beam of coherent white light projected from under the sample to be observed. Different substage condensers offer various
techniques of microscopy according to specific needs : (Example : bright field illumination, dark field illumination, phase contrast illumination, and differential interference illumination). Neither of these previous techniques however allow to maximize the visibility of the very small sample (objects or particles).

## SUMMARY OF THE INVENTION

The present invention therefore provides a substage condenser for any kind of light microscopes, a condenser that maximizes the visibility of the observed sample. Specifically, this is realized by generating a two-colored contrast in the light illuminating the sample : this is the bichromic contrast.

This new concept can be explained as follow: The incoming white visible light source (Figure 1 - portion 22) is separated into two different beams.

THE FIRST BEAM : A first white beam (Figure 1 - portion 24) (wavelength X - Ref. : Figure 4) deflected from its original trajectory to impinge on the sample from its sides, and achieved with aluminized surfaces.

A SECOND BEAM : A second straight forward beam travelling in a vertical light path (Figure 1 - portion 26) (wavelength $Y$ - Ref.: Figure 6) passing through a light filter that lets only a portion of the original spectrum through and impinging directly on the sample, originating from underneath.

SUMMARY: The concept behind this invention can be summarized by saying that it is the result of a certain design of the condenser's lens that permits the overlapping of two different light frequencies both emerging from one unique light source.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 : Schematic view of the invention (piece 1-2-3 combined)
FIGURE 2 : Piece \#1 (1 out of 3)
FIGURE 3 : Piece \#2 (2 out of 3)
FIGURE 4 : Light spectrum of the acrylic material used for piece 1 and 2
FIGURE 5 : Piece \#3
FIGURE 6 : Light spectrum of the Hesalite material used for piece 3
FIGURE 7 : Critical angles of refraction of piece \#1
FIGURE 8 : Schematic design and drawing of the universal support

## DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

Referring to FIG. 1, there is shown the lens portion \#10 of the condenser (piece no 1,2,3 purposely assembled) according to a preferred embodiment of the invention. It includes an acrylic piece \#12 (Figure 2) having its inside walls aluminized (14). Inside the acrylic piece \#12 is inserted no \#16 (Figure 3), its wider end being inside the acrylic glass piece \#12.

Assembly of the three separate components of the lens (portion \#12, 16, 20) creates an inverted flare-shaped channel when the lens is in the upright position. The outside walls of this flare-shaped channel are also aluminized. At the widest end of the channel, we find a light filter (20), as described in figure 5. The light filter 20 is made of Hesalite H.T. is transmissive only in the higher portion of the spectrum of visible light. The other pieces \#12, 16 (figure 2, figure 3) are transmissive to the full spectrum of the visible light.

The present innovation is made possible by the contrast created by the overlapping of these two variations of transmissive qualities of the assembled pieces designed according to the preferred embodiment.

Said differently, the assembly of piece 12,16 and 20 (len portion \#10 of the assembled condenser) because of their respective design, when assembled according to the referred embodiment creates an inverted flare-shaped channel, thus permitting the light path of the second light beam to arrive directly on the light filter (20) to light up the specimen at the focal point $\# 28$.

In use, the lens (10) is put into the trajectory of a beam of white light (22) so that the radius of said beam corresponds to the radius of the wider end of the flare-shaped channel. In this manner, an outer ring portion 24 of the beam 22 is reflected on the walls 18 . Whereas the center portion 26 of the beam 22, enters the flare-shaped channel and goes in a vertical path through the
light filter 20. The critical angle of the outside walls 18 of the channel, and the shape of the inside walls 14 of the acrylic piece \#12 is calculated so that the outer ring portion 24 of the beam impinges on the outside walls 18 at the angle of maximum reflection, being then also totally reflected on the inside walls 14 of the acrylic piece \#12 and finally focused on a single point 28 , with which the sample should be aligned. The center portion 26 of the beam 22 following a direct trajectory, it will also impinge on the point 28 . The sample will therefore be illuminated from different angles by two beams of two different colors, originating from one light source.

Advantageously, for any light microscope, the Diachromic condenser according to the present invention, will provide a better contrast of the very small objects or particles, and will virtually eliminate the diffraction effects normally blurring the image.

In a second preferred embodiment, the actual light filter (20) is replaced by another type created with polarized material rather than the Hesalite (same design different material). This modification in the material used for the manufacturing process of the light filter, when used in combination with a commercial polarizer on the light source generates a phase difference between beams 24 and 26 . By simply rotating the polarizer on the light source, the phase differences can easily be adjusted to any desired value. Producing the second embodiment has the advantage of having various types of illumination, without the need to remove the substage condenser. A specimen therefore can be observed in a specific field permitting either type of illumination (the darkfield, the phase contrast and the brightfield, etc.) depending on one's need. This feature is mostly requested in observation of live biological material.

## THE SUPPORT

The Diachromic condenser is called "universal" because it can be easily adapted to all existing brands of light microscopes. The design of the support is found at Figure 8.

# MANUFACTURER'S GUIDE AND TECHNICAL DATA 

Object : Naessens" DIACHROMIC "Condenser



The assembled Naessens "Diachromic" Condenser, in its upright position. Because the Naessens Condenser can be easily adapted to the major brands of light microscopes, it is considered a "universal condenser".

## MANUFACTURER'S GUIDE AND TECHNICAL DATA

## Object : Naessens" Diachromic "Condenser

## 1. COMPONENTS : Optical and mechanical parts

The Naessens Diachromic Condenser is composed of three different individual parts:


## 2. OPTICAL CONCEPT : BICHROMICAL CONTRAST

The Nacssens Diachromic condenser is based upon to the concept of bichromic contrast that expresses in variations of luminance the overlapping of different luminous frequencies emerging from one light source. This condenser was specifically designed to enhance the overall performance of light microscopes used in the observation of live biological material. The Naessens Diachromic Condenser can also find many applications in the medical fields whenever a diagnosis is required.

Although the Naessens Diachromic condenser does not enhance the resolution power of the light microscope, it does make very small particles appear clearly as brilliant objects on a magenta background. (unique feature of the Nacssens design). Morphological details are seen with extreme sharpness, if the condenser is properly adjusted according to the manufacturer's guidelines.

## 3. APPLICATIONS

Other than the observation of live material, the Naessens Diachromic Condenser can be used:

- In various fields of scientific research, mainly in biological sciences, (ex: bacteriology, virology, etc...)
- In clinical microbiology and/or hematology
- For industrial quality control
- For all applications (medical or other) requiring the bichromic contrast


## 4. REQUIREMENTS FOR MAXIMUM PERFORMANCE

A) For optimal performance, the Naessens Diachromic Condenser needs to be used with a light microscope equipped with the following :
i) A 100 Watt halogen lamp as the prime light source (not less)
ii) A 100 X oil immersion objective (with an iris)
iii) A 4X scanning objective for the centering of the condenser
B) For optimal performance, the Naessens Diachromic Condenser must be used with appropriate microscopic material:
i) Immersion oil

Type : Low viscosity, low fluorescence immersion oil
Refractive index : $\mathrm{nD}=1.516$ at $23^{\circ}$ Centigrade (if working with glass specimen slides and cover glass)
ii) Specimen slide

Dimension (standard): 3 inches X 1 inch ( 75 millimeters X 25 millimeters)
Thickness : .035 inch to .040 inch ( $<1$ millimeter)
iii) Cover glass

Dimension : 700 inch X .700 inch (no bigger) ( 18 millimeters X 18 millimeters)
Thickness : .006 inch ( 0.152 millimeter) (Number 1)
C) For optimal performance, when using the Naessens Diachromic Condenser, the double oil immersion technique is required. This technique can be summarized as follows :
i) Prepare the specimen using the recommended specimen slide and cover glass.
ii) Once ready for microscopic observation, take the specimen slide in your hand then turn the specimen slide upside down and place a drop of immersion oil in the center area of the slide where the cover glass is located. When done, quickly turn the slide right side up.
iii)Place a drop of immersion oil in the center area of the cover glass. Place the specimen on the stage.

Note : The oil on the condenser will make immediate contact with the oil on the bottom part of the slide. The oil on the upper part of the slide will make contact with the 100X objective when the stage is moved slightly upward. When all contacts occur simultaneously, the specimen to be observed will be illuminated.

## 5. ADJUSTMENT OF THE NAESSENS DIACHROMIC CONDENSER : STEP BY STEP INSTRUCTIONS

The adjustment of the condenser consists of three different steps:
A) Inserting the condenser
B) Finding the proper height of your condenser
C) Centering the condenser
A) To properly insert the condenser

## Step 1



Make sure that the adapter ring provided with the condenser is compatible
 with your brand of microscope. (specify when ordering)


Step 2
Remove the condenser from its case or box. Place the condenser in the upright position with the lens facing upward (the N.A. scale indicator should face the front of the microscope). The condenser is ready to be inserted in its mounting support.


Step 3
Slightly loosen the adapter ring (turn counter-clockwise).


Step 4
Insert the condenser into the mounting support just below the stage. It should slide in easily. (If not, kecp unscrewing the adapter ring until the condenser slides in without resistance.)

Step 5
When fully inserted, tighten the adapter ring by turning it clockwise. The condenser should be well inserted and the adapter ring tightly secured in place.



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Once the condenser is properly inserted (fully inserted on the horizontal axis) you can lock the condenser in this position by using the clamping screw usually located on the right side of the condenser's support.

Leave the condenser at the lowest possible
 vertical position. Then you can proceed to the next adjustment.

## B) To adjust the height of the condenser

The condenser needs to be perfectly located in terms of height in order to create a perfect focal point of the light path on the specimen, not below, nor above the specimen, otherwise the image will be out of focus (as for example, approximately 61 mm from bottom to top of condenser, in the vertical position for the Olympus microscopes).

Even though the condenser has been adjusted in terms of height (according to the brand of microscope) prior the packaging, it is recommended to verify this setting before using the condenser. It may have been displaced during shipping procedures, making the height shorter than requested.

As a bench mark, the condenser when inserted, raised to its optimal position, should make very light contact with the slide placed on the stage.

Here are the steps to follow in order to adjust properly the height of the condenser.
Step 7 目
Place a clean slide on the stage.


## Step 8

Now, raise the condenser to its highest position. At that position, the lens of the condenser should slightly touch the slide, just enough to insert a thin paper between the slide and the stage.

## Step 9 G

If, at the highest position, the condenser does not touch the slide, your condenser needs to be lengthened. Remove the condenser from the support and gently unscrew the tightening ring at the base of the lens tube and adjust accordingly (counter-elockwisc). Tighten the condenser at the sclected height, again using the tightening ring (clockwise). Repeat procedures from step 4 until the condenser slightly raises the slide at the highest position. When done, you may proceed with the centering process.


## C) To center the condenser

The final adjustment required is to make sure the light path reaching the lens of the condenser is centered properly. An uncentered condenser would bring a constant white shadow on one side of the slide material. It could impair the optimal optic performance expected. Here is the procedure, step by step, on how to center the condenser.

## Preliminaries

- Turn the microscope ON.
- Turn the light to the maximum intensity.
- Open the light diaphragm to the maximum position.
- Select the 4 X scanning objective on the microscope.
- Make sure there is no slide on the stage and that the condenser is at the lowest possible position.


## Centering of the condenser

1. Look in the cyepieces making sure they are properly adjusted to your eycsight.
2. Start raising the condenser slowly towards its highest position.
3. Check carefully, a magenta colored disk will appear in the center area of a bright clear field. Focus on that colored inner circle.
4. Bring this inner circle as close as possible to
 the perfect center position in the field of view. This can be done with the two condenser centering knobs located at the front part of the condenser's support (right and left knobs). These knobs are very sensilive and require only slight adjustments.
5. Once the colored circle is perfectly centered, raise the condenser slowly to its upward position.
6. As the condenser progressively rises, the image of the colored circle will take more and more space, until it perfectly overlaps the full field of view. You can still adjust the center position of the colored circle (centering knobs), although it is easier to do when the circle is at its smallest dimension.

The condenser is now perfectly centered and ready to use. If the microscope is permanently stationed, the condenser will remain centered. Regular adjustment is needed if the microscope is moved frequently.

## Please note before using the condenser

Once these adjustments are made, before using the microscope for specimen observation, bring the condenser at the maximum upright position and put a coating of immersion oil on the Condenser's lens. This is necessary to perform the double oil immersion technique.

## 6. CARE AND MAINTENANCE

Proper care of the Naessens Diachromic Condenser is recommended for optimal observation of slide material.

Periodically, the condenser needs to be cleaned thoroughly: either as a regular maintenance program or for storage in its wooden box.

To remove excessive oil on the lens surface, always use lens paper since this material will not scratch the lens (cotton tissuc could leave fibers on the lens). To thoroughly remove the oil (once or twice a month) the lens paper can be soaked with a small amount of $94 \%$ alcohol. Use very sparingly. Always wipe off the lens very carefully.

Never use xylene, ether, or acetone directly on the condenser : (same restriction as for any optical accessory). It could dissolve the mounting cements.

Never leave the condenser's lens (or any other optical accessory) directly soaking in a cleaning solution.

When using the Naessens Diachromic Condenser on a daily basis, you don't need to remove excess oil every time you stop using the microscope. All that is needed is to place an unused specimen slide on the stage in order to keep a permanent contact with the condenser's lens. This can be done by putting a generous coating of immersion oil on the condenser's lens. This technique will preserve the life span of your condenser.

## 7. GUARANTEE

When operation and maintenance of the Naessens Diachromic Condenser are performed according to these guidelines, including the use of adequate microscopy material and proper light source, the Naessens Diachromic Condenser is fully guaranteed against any defect for a 3 year period calculated from the date of purchase.

## 8. MANUFACTURER'S DISCLAIMER

The Naessens Diachromic Condenser is an optical accessory. Owning such a condenser does not automatically confer the required skills to perform reliable live blood testing.

CERBE inc., manufacturer of the Naessens Diachromic Condenser declines any and all responsibilities associated with blood interpretation or any diagnosis performed with the use of the condenser.

## 9. MANUFACTURER'S RECOMMENDATIONS

It is recommended that you carefully read the instruction manual of your microscope.
To explore the numerous possibilities of this condenser, it is recommended to be familiar with the basic notions of microscopy*. (See Appendix I) including minimal experience handing light microscopes.

## FIGURE 1 : Schematic view of the invention (piece 1-2-3 combined)



FIGURE 2 : Piece \#1 (1 out of 3)


## FIGURE 3 : Piece \#2 (2 out of 3)




FIGURE 4 : Light spectrum of the acrylic material used for

## FIGURE 5 : Piece \#3



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& \text { FIGURE } 6 \text { : Light spectrum of the Hesalite material used } \\
& \text { for piece \#3 }
\end{aligned}
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FIGURE 7 : Critical angles of refraction of piece \#1


FIGURE 8 : Schematic design and drawing of the universal support


