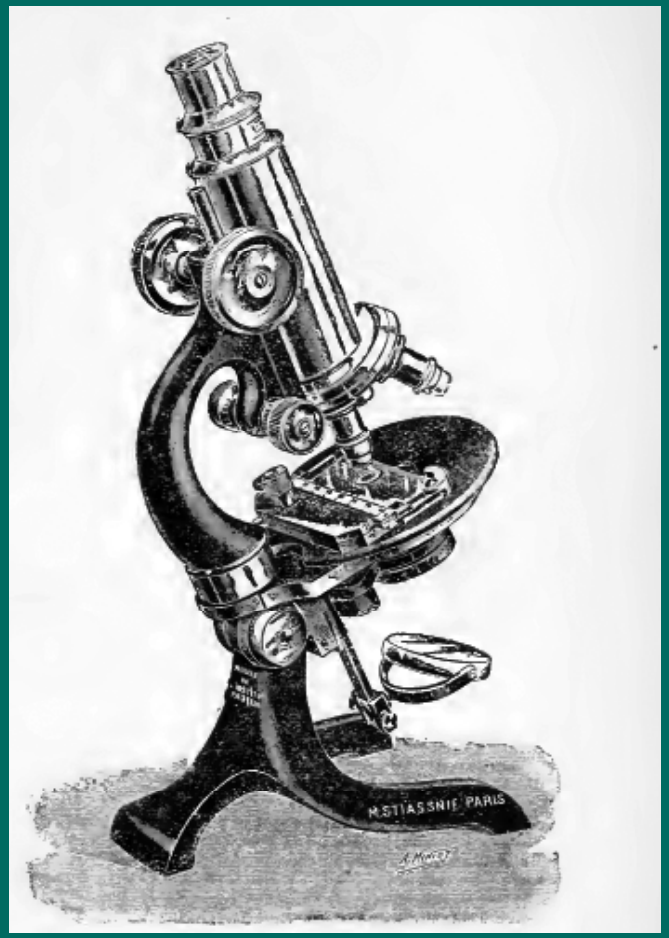


Le Microscope

Marc Moreau Catherine Leclerc
Centre de Biologie du développement
UMR 5547 et GDR E n° 731 Toulouse

BC02 mars 2013

Pourquoi utiliser un microscope ?

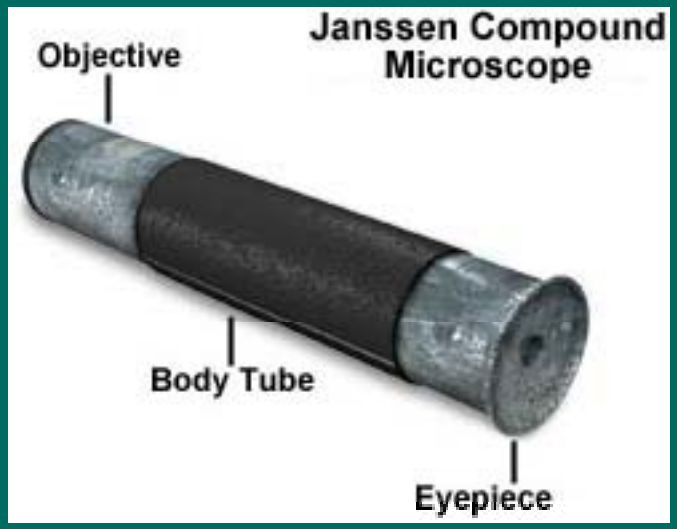
La limite de résolution de l'oeil est due à la structure de la rétine:
L'écartement minimal de deux récepteurs étant de 2,2 µm, la limite de résolution est en moyenne d'environ 3.10^{-4} radian = 1' d'arc soit 0,2 mm à une distance de 25 cm.

Le microscope est destiné à donner une image grandie d'un objet transparent

Hans & Zacharias Janssen

1590

H. et Z. Janssen construisent empiriquement le premier microscope composé.
Gallilée s'en est inspiré pour construire sa lunette.



MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

MAGNIFYING GLASSES

WITH

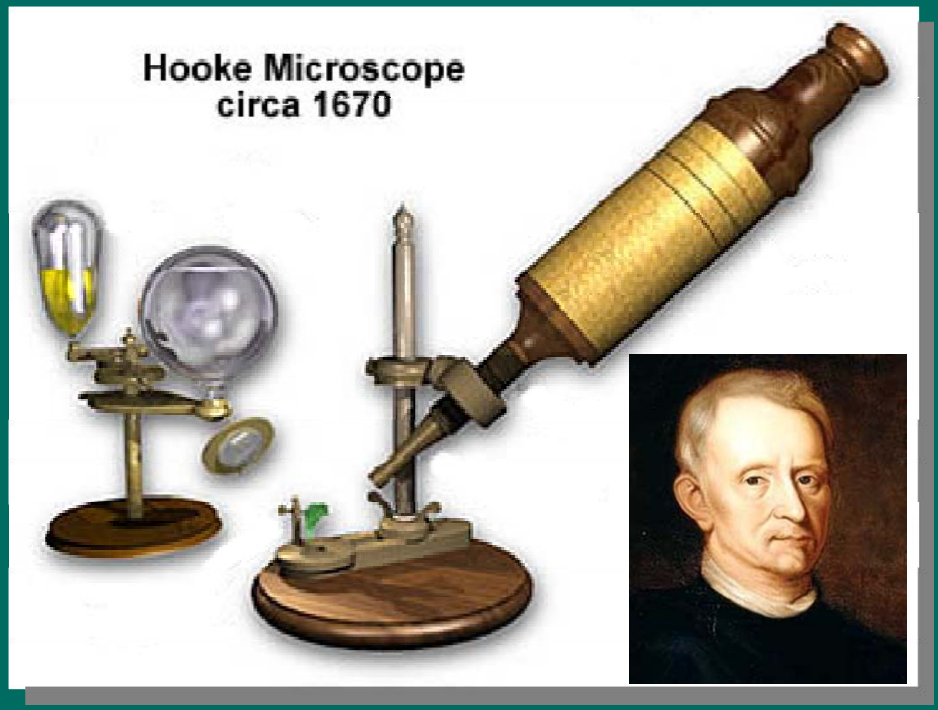
OBSERVATIONS and INQUIRIES thereupon.

By *R. HOOKE*, Fellow of the ROYAL SOCIETY.

Non possis oculo quantum contendere Lynceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

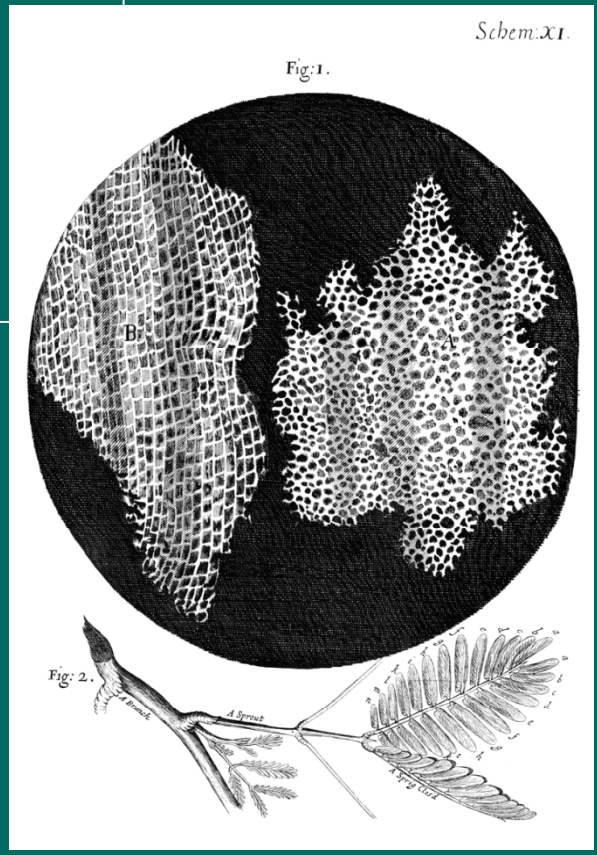
LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the *Bell* in S. *Paul's* Church-yard. M DC LX V.

Robert Hooke (1665)



". . . I could exceedingly plainly perceive it to be all perforated and porous. . . these pores, or cells, . . . were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this."

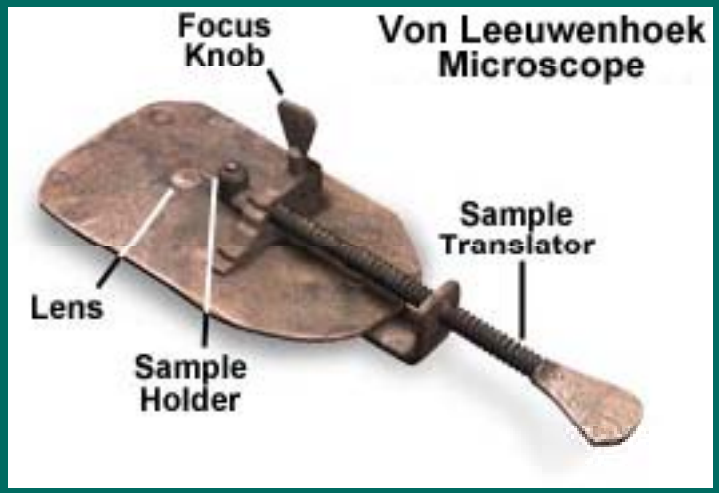
Robert Hooke (Micrographia, 1665)



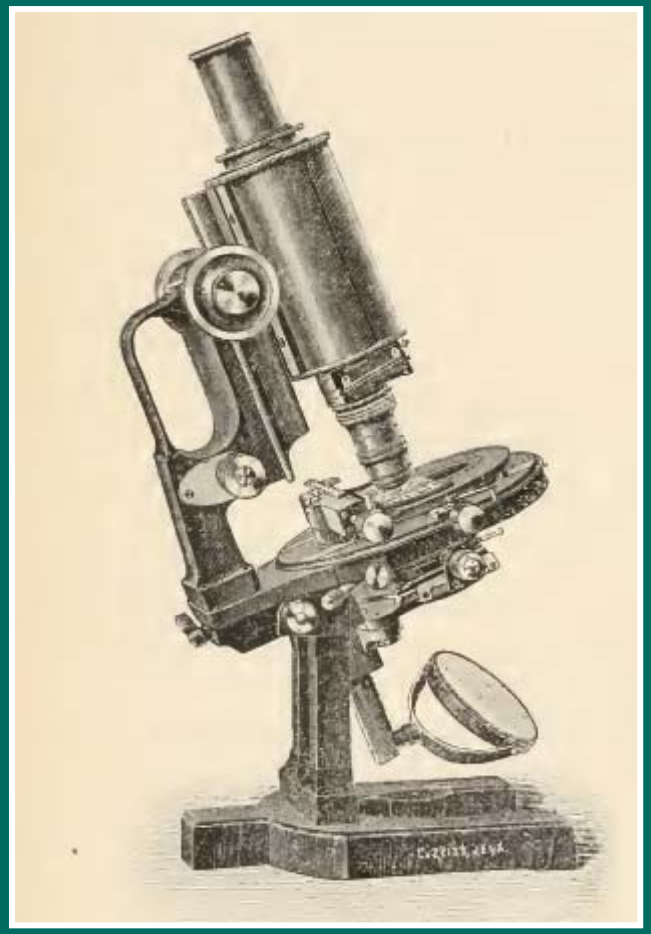
Antioni van Leeuwenhoek (1632-1723)

.1673
création d'un microscope simple. Leeuwenhoek n'est pas l'inventeur du microscope, il a construit un microscope simple avec un grossissement d'environ 275x.

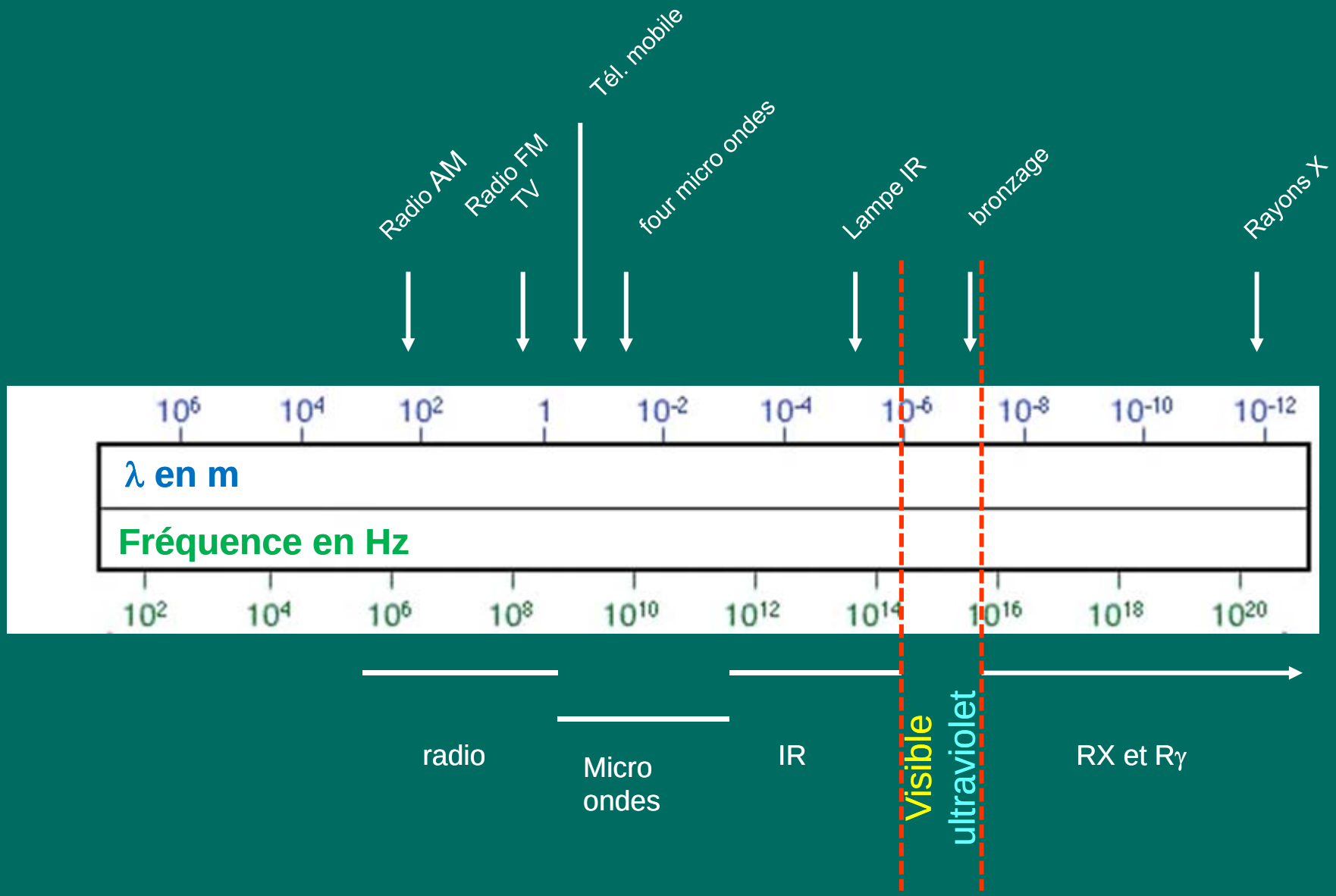
• Il a publié en 1683 un recueil de dessins concernant de nombreux micro-organismes.



Quelques définitions...



Le spectre des ondes électromagnétiques



indice de réfraction

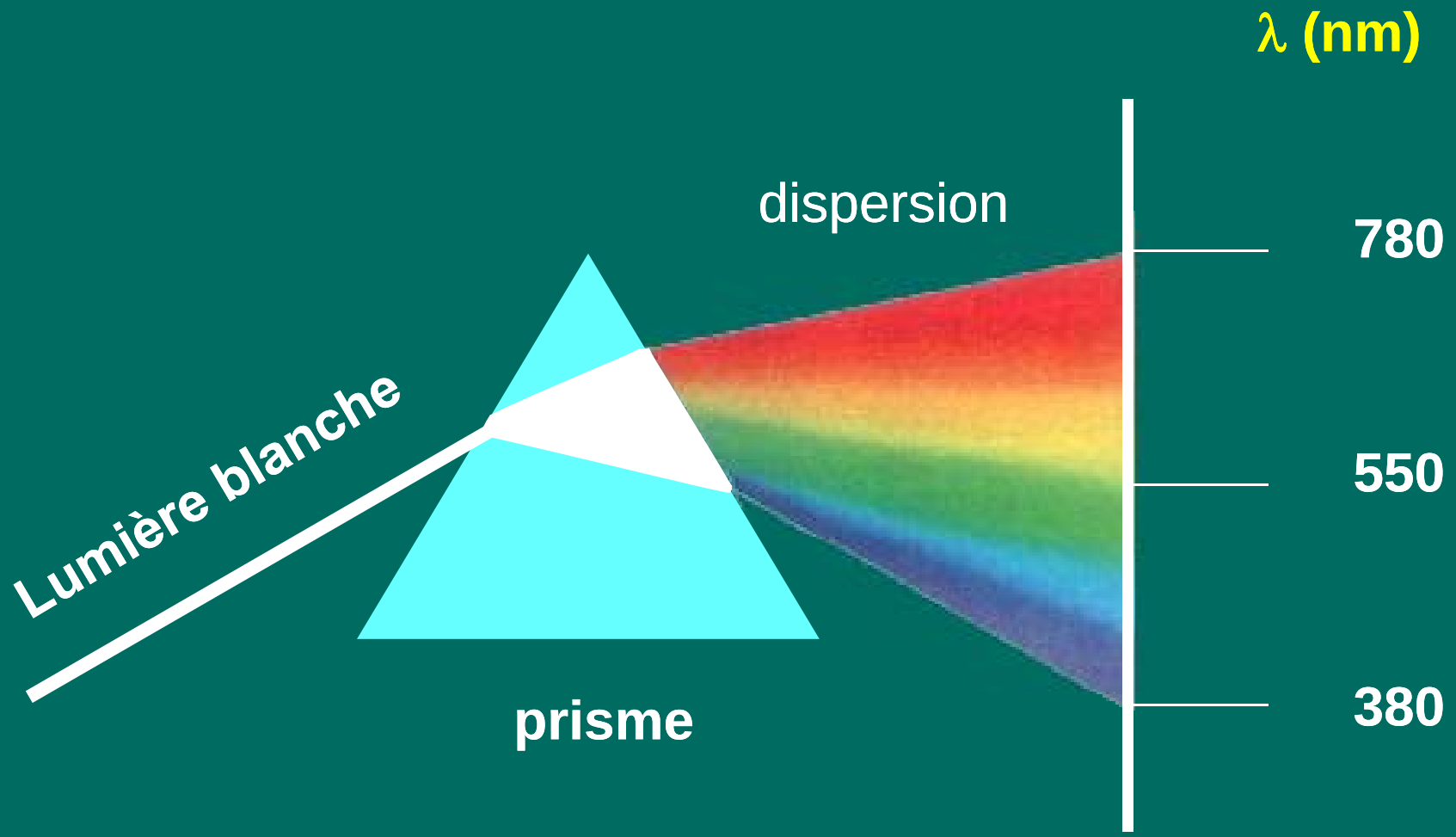
l’indice de réfraction d ’une substance transparente est le rapport de la vitesse de la lumière dans le vide à la vitesse de la lumière dans le milieu.

$$N = c/v$$

L’indice de réfraction n ’a pas d ’unité, il est toujours > à 1

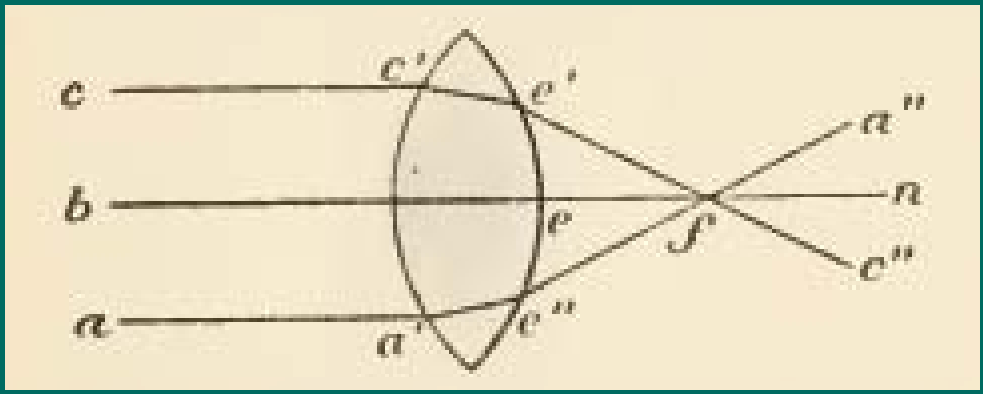
Exemple :

eau :	1.33
quartz :	1.51
air :	1.00029



L’indice de réfraction dépend de la longueur d’onde

Application aux lentilles



Foyer
Distance focale

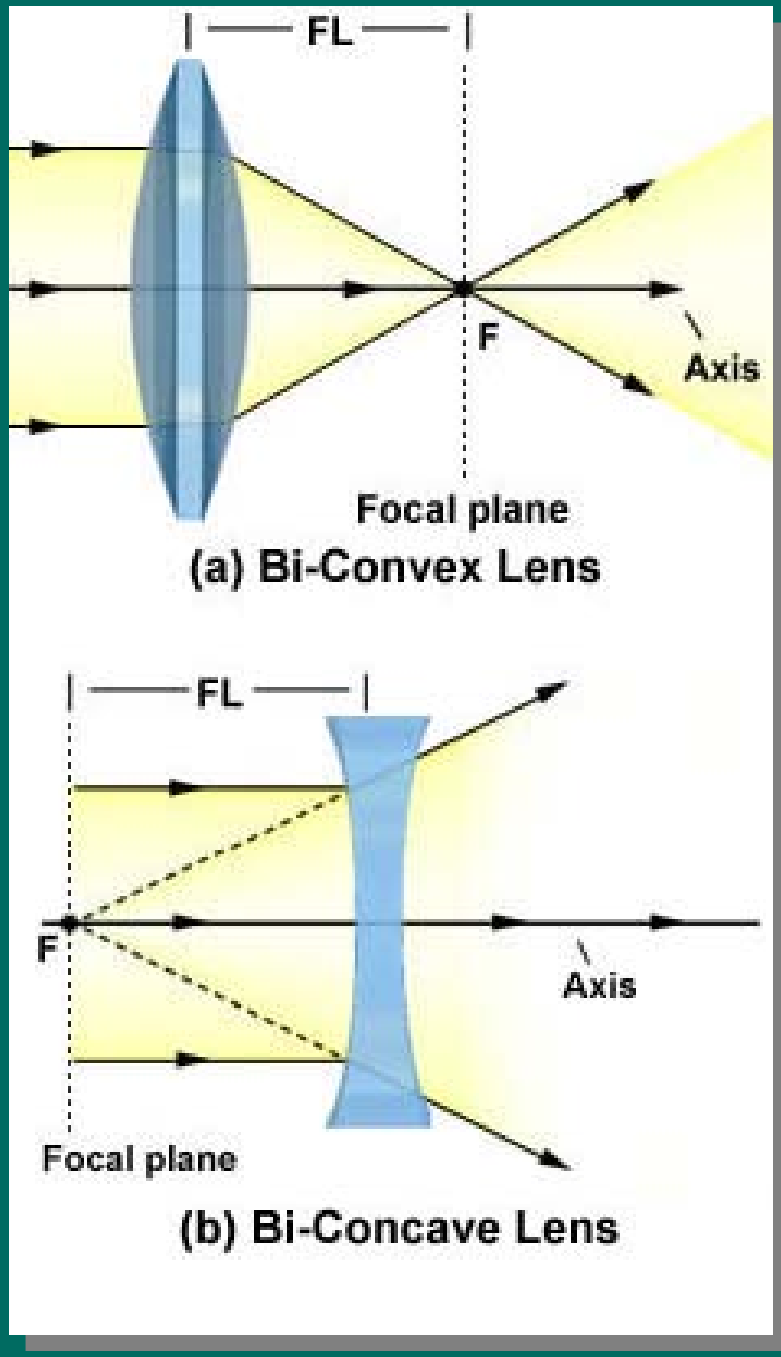

FL
F
Axis
Focal plane
(a) Bi-Convex Lens
FL
F
Axis
Focal plane
(b) Bi-Concave Lens

Image réelle :

Elle peut être recueillie sur un écran et est inversée
Ex : l ’image fournie par le vidéoprojecteur
Elle est fournie par des rayons convergents

Image virtuelle :

Elle ne peut pas être recueillie sur un écran, elle semble se situer derrière l ’objet. Seul un observateur peut la voir.
Ex : l ’image d’un objet par la loupe.
Elle est fournie par des rayons divergents

oculaires

objectifs

Platine + porte objet

Condenseur
+diaphragme

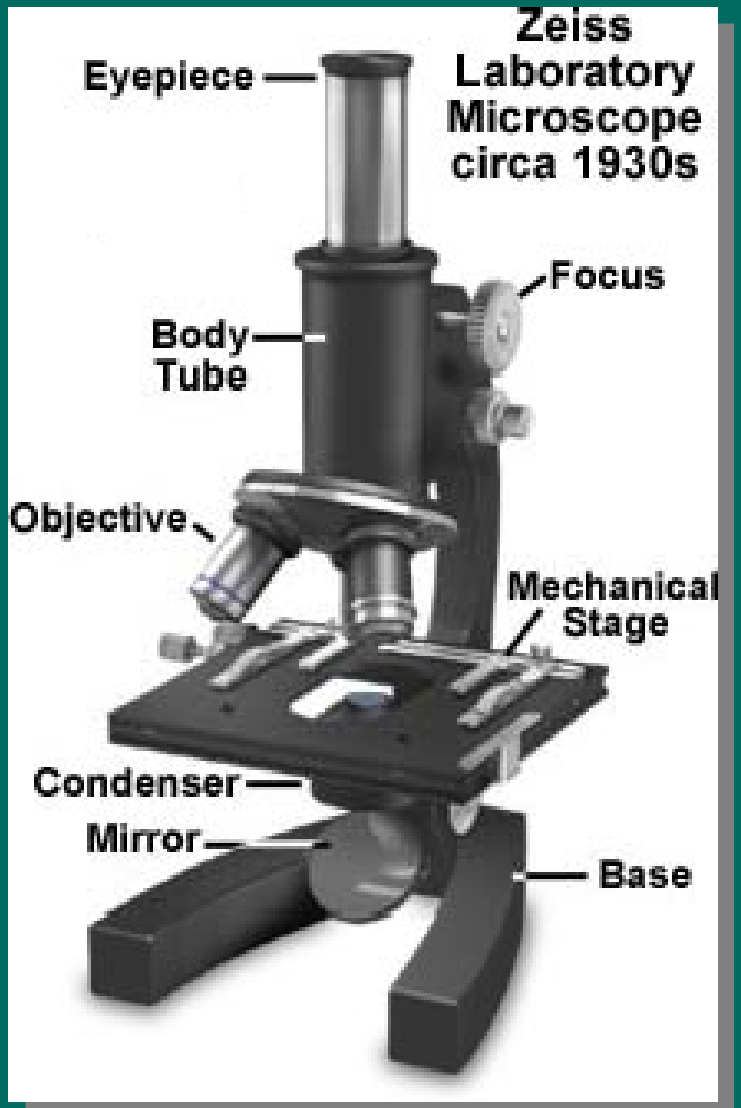
Diaphragme
de champ

Base (avec lampe)

Mise au point

Déplacement Y

Déplacement X


Zeiss
Laboratory
Microscope
circa 1930s
Eyepiece
Focus
Body
Tube
Objective
Mechanical
Stage
Condenser
Mirror
Base

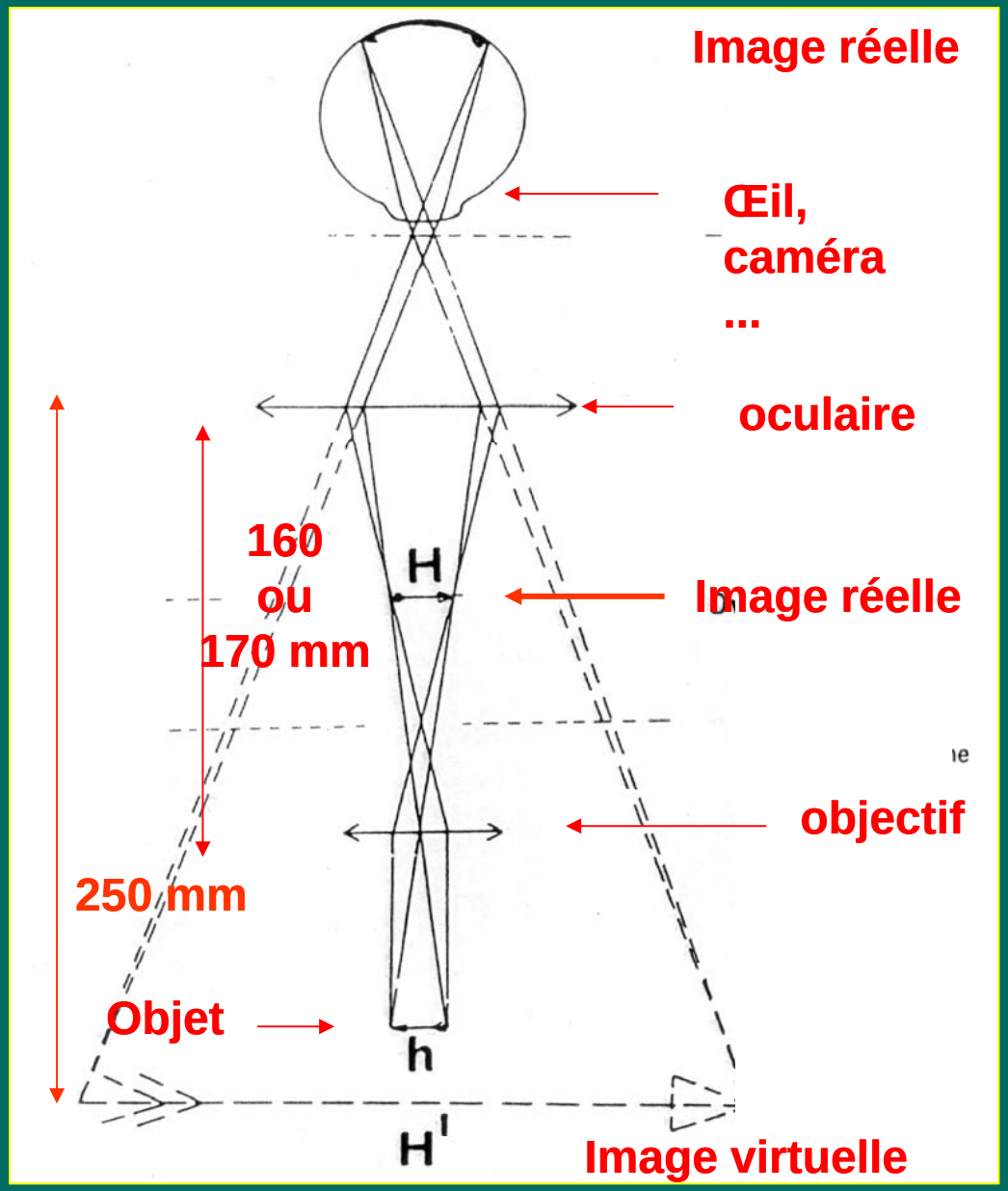
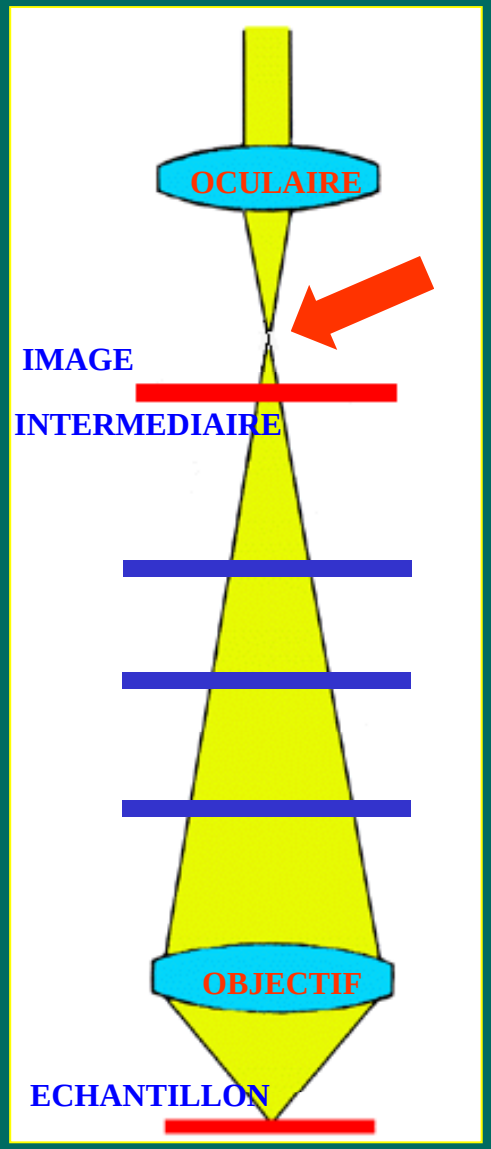


Image réelle
Œil,
caméra
...
oculaire
160
ou
170 mm
H
Image réelle
objectif
250 mm
Objet
h
H'
Image virtuelle

Les microscope les plus récents ont des optiques dites à l'infini.

Conséquence : la longueur des tubes est variable


OCULAIRE
IMAGE
INTERMEDIAIRE
OBJECTIF
ECHANTILLON

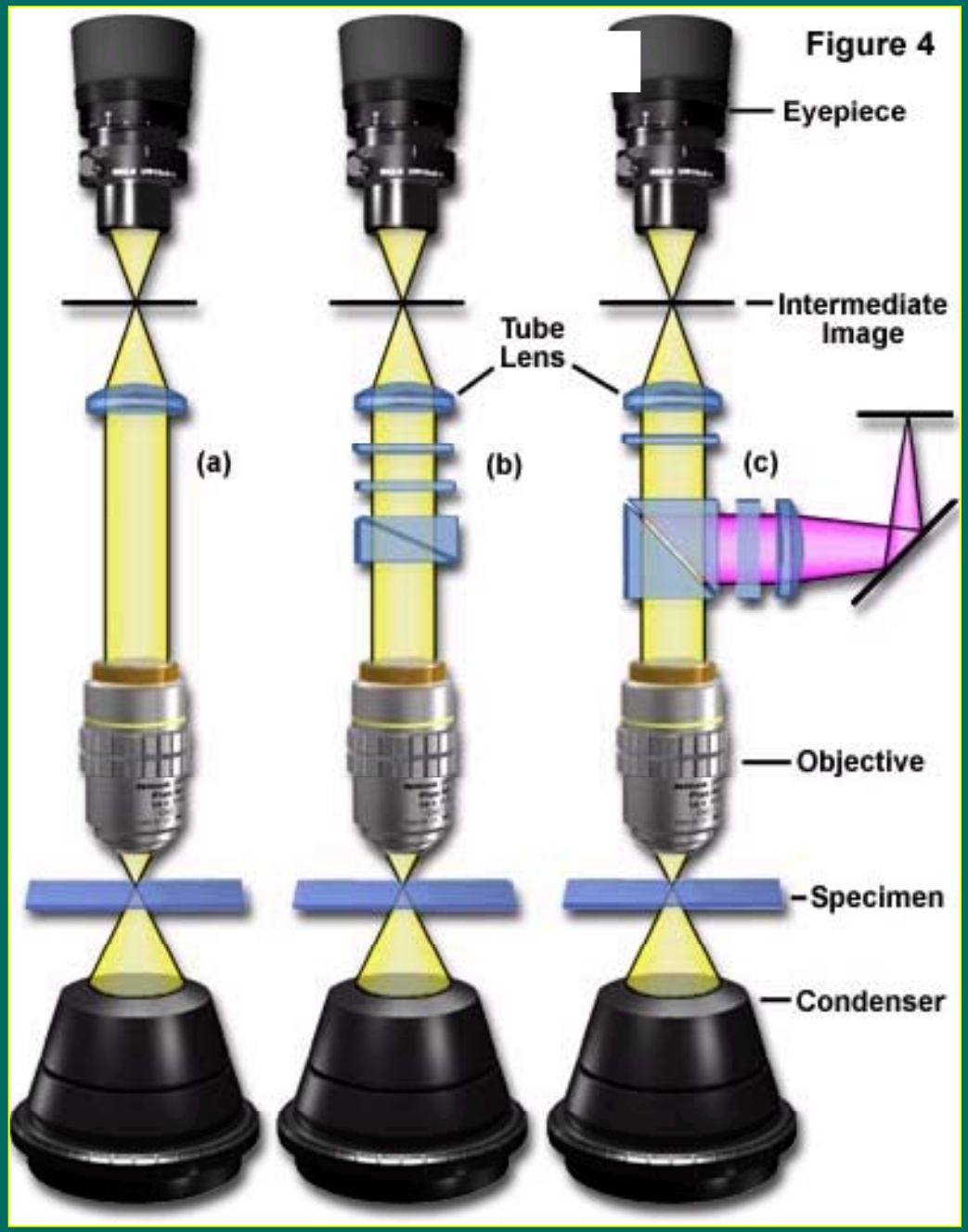
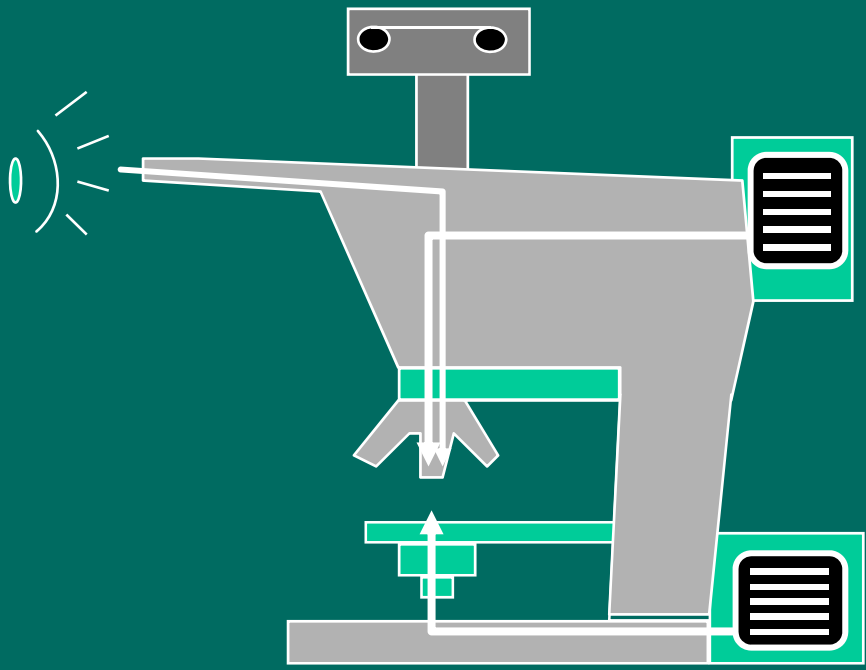
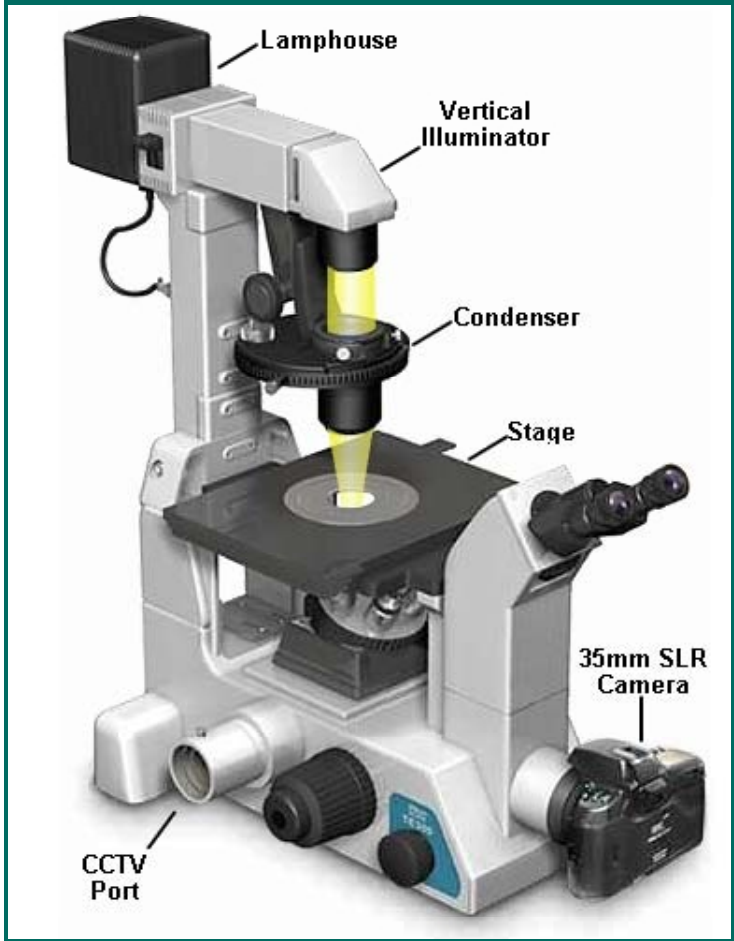


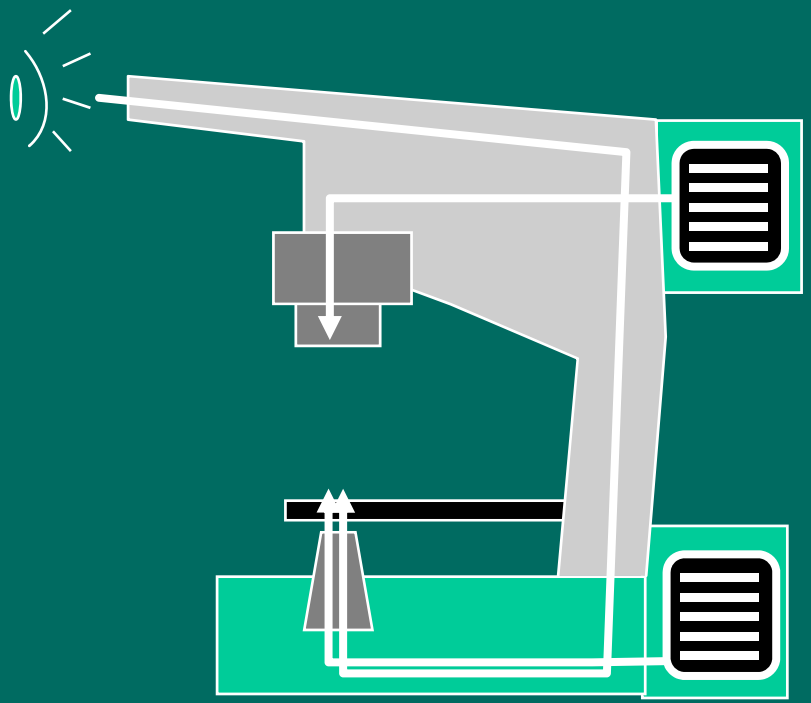
Figure 4
Eyepiece
Intermediate Image
Tube Lens
(a)
(b)
(c)
Objective
Specimen
Condenser

Microscope droit



Microscope inversé

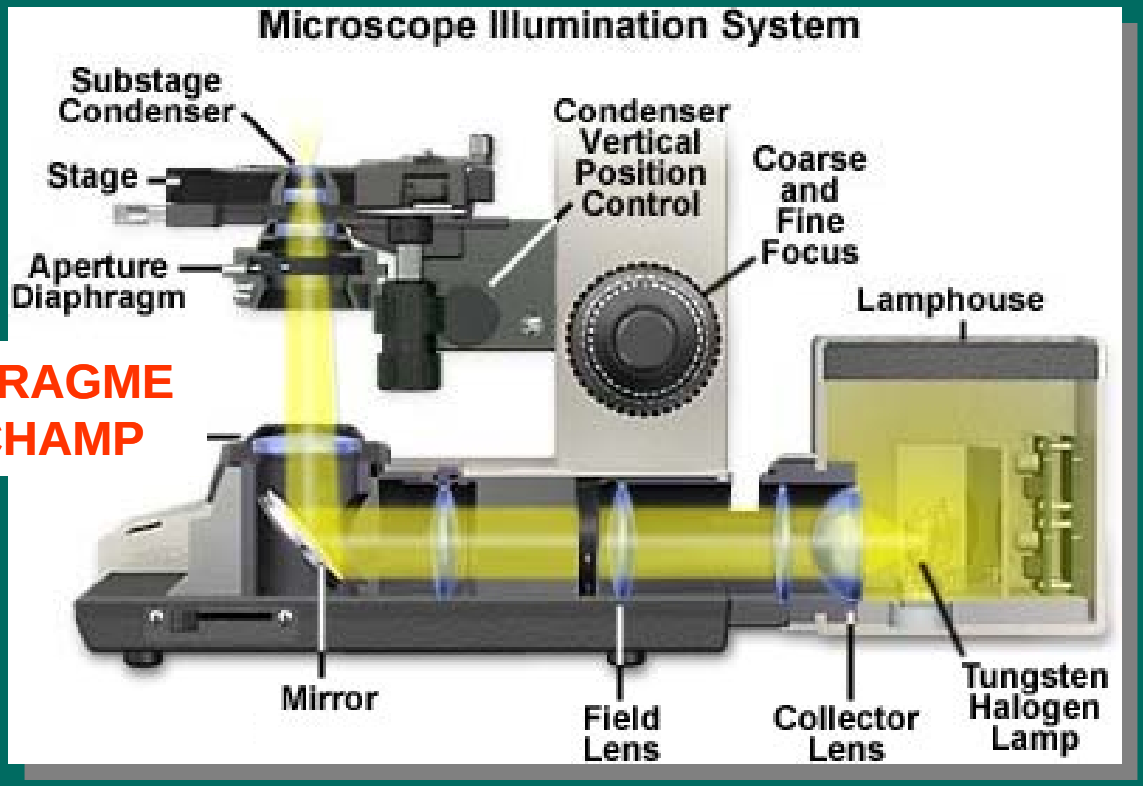


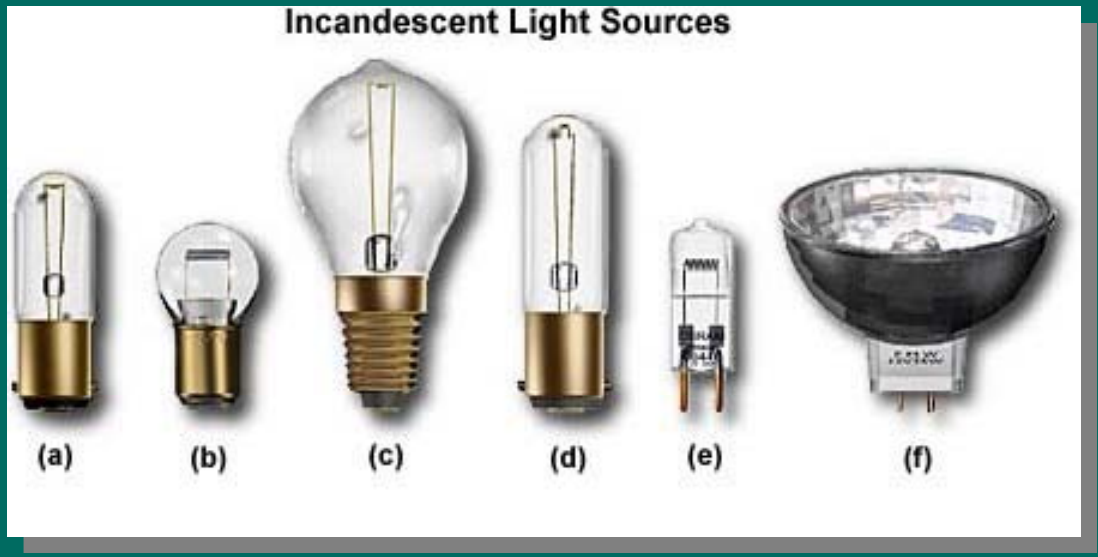


transmission

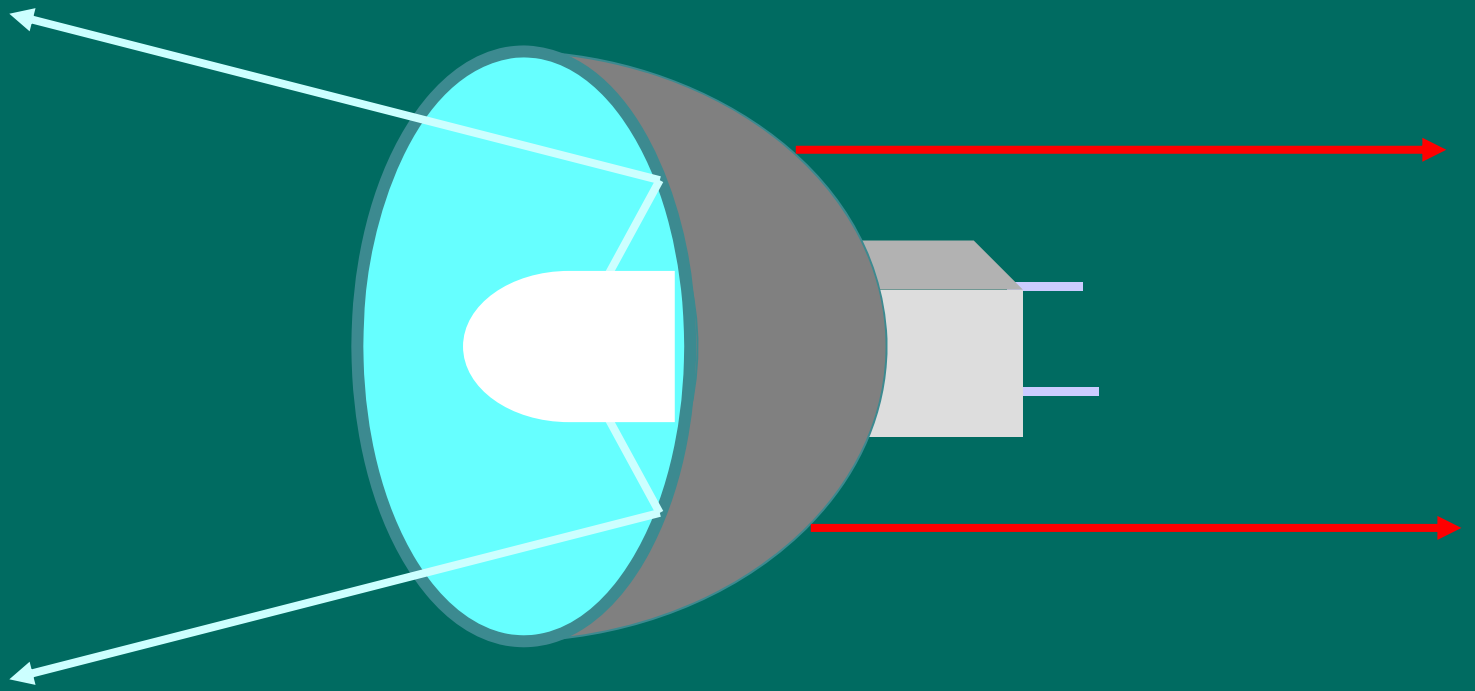
Epi illumination

DIAPHRAGME DE CHAMP





Ampoule à miroir dichroïque

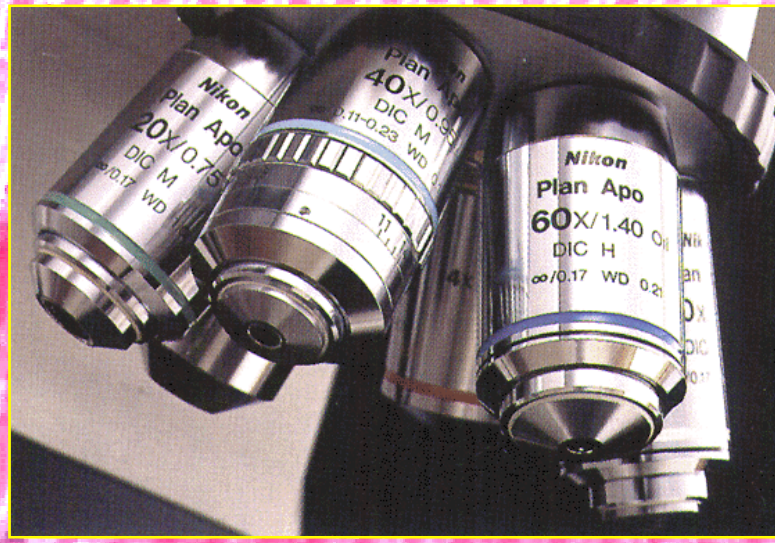


Les infrarouges sont transmis

Les courtes longueurs d’ondes sont réfléchies

OBJECTIFS

Les objectifs sont constitués de systèmes de lentilles qui fournissent la première image agrandie de l'échantillon.

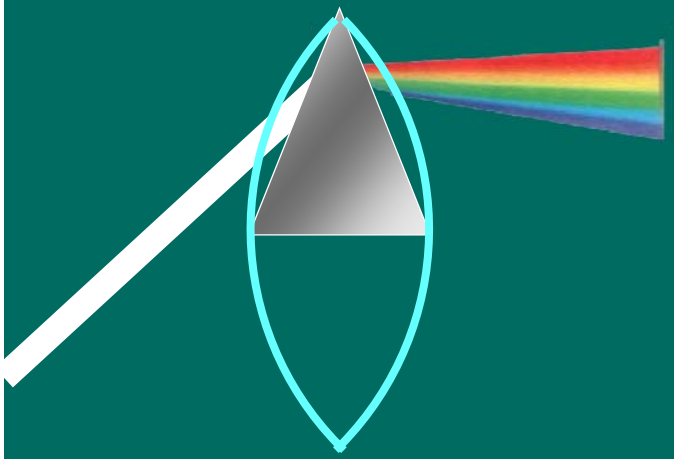


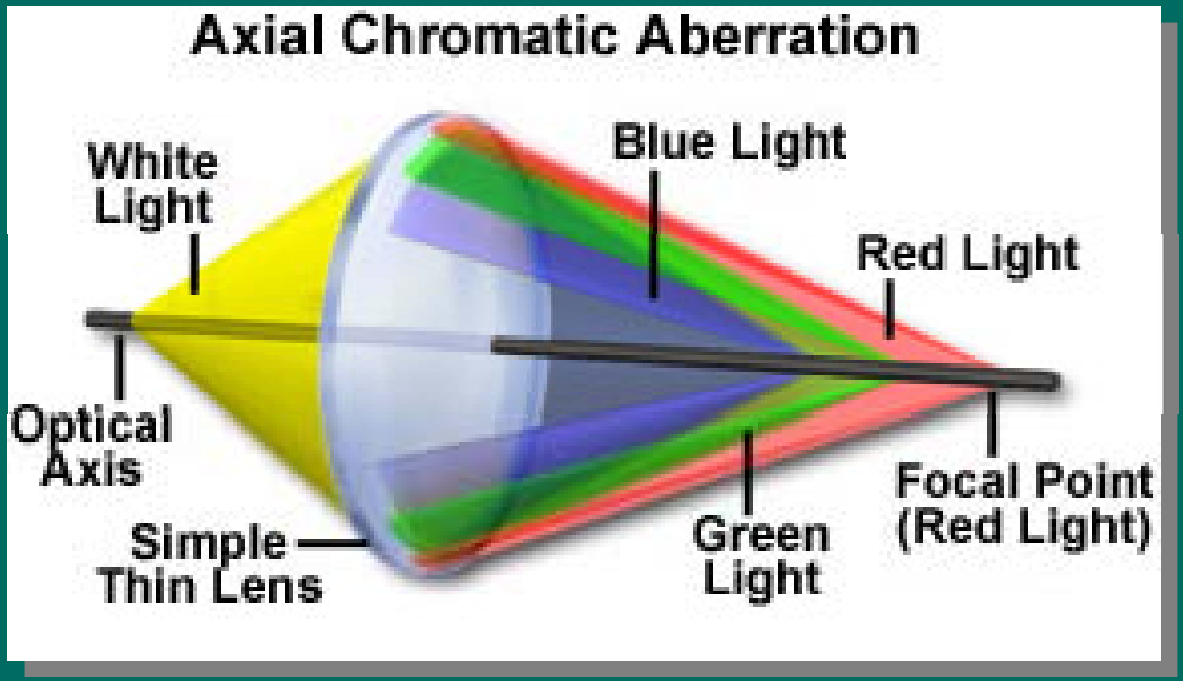
Ils sont placés dans une tourelle porte-objectifs ou tourelle revolver qui comporte six ou sept objectifs avec des grossissements différents.

Longueur du tube


25 mm
20.32 mm
Pitch = 0.706
Fluor
Immersion
Objective
Parfocal
Distance
(45 mm)
Immersion
Medium
Color Code
Immersion Oil
Parfocal
Distance
(60 mm)

ABERRATION CHROMATIQUE



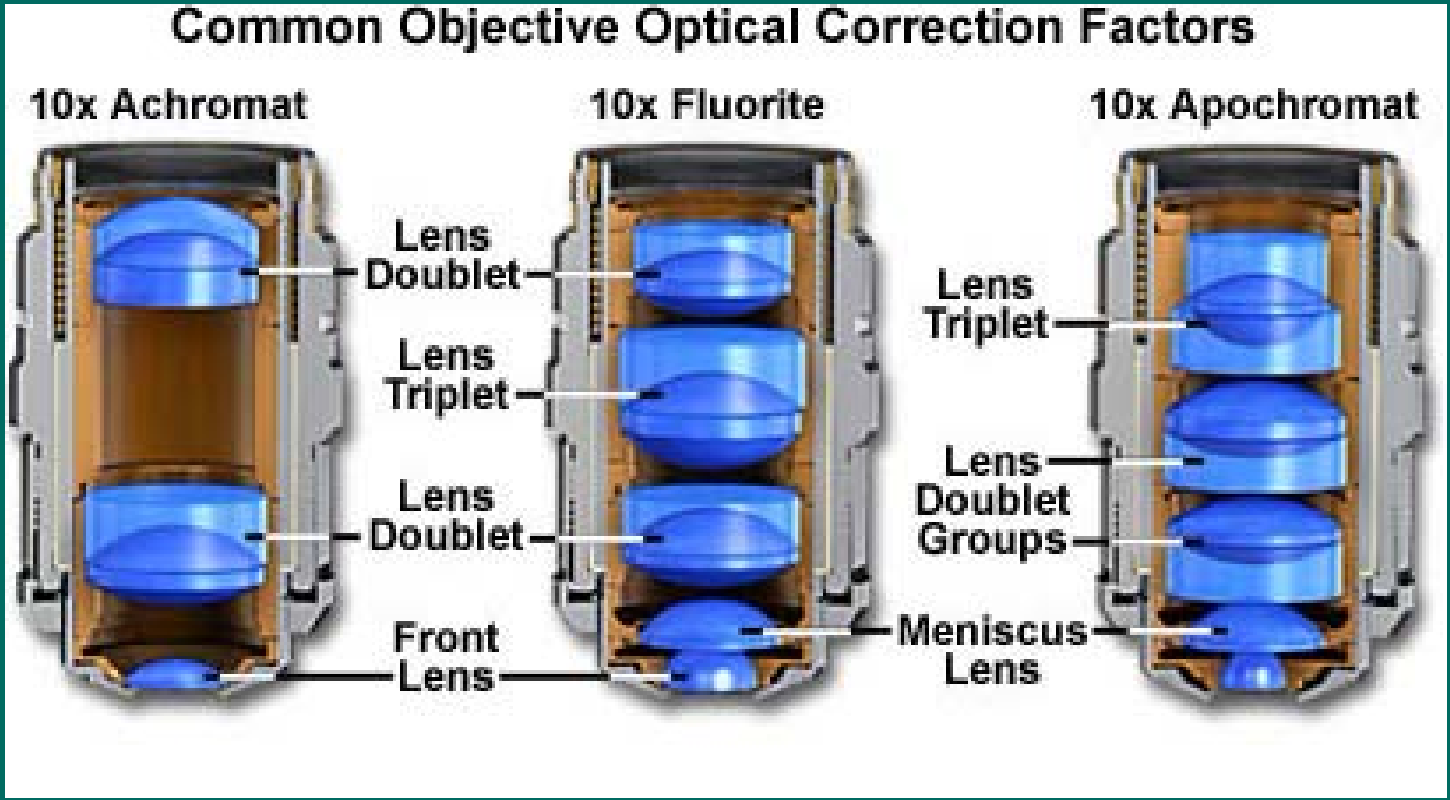


les rayons
ne se focalisent pas tous
sur un point commun.

• objectif **ACHROMATIQUE** : le vert et le rouge, sont focalisées.

• objectif en **FLUORITE** permet une meilleure correction, rapprochant de plus en plus les points de focalisation des rayons avec diverses couleurs

• objectif **APOCHROMATIQUE** : le vert, le rouge et le bleu sont focalisés.


Common Objective Optical Correction Factors
10x Achromat
10x Fluorite
10x Apochromat
Lens Doublet
Lens Triplet
Lens Doublet
Front Lens
Lens Triplet
Lens Doublet Groups
Meniscus Lens

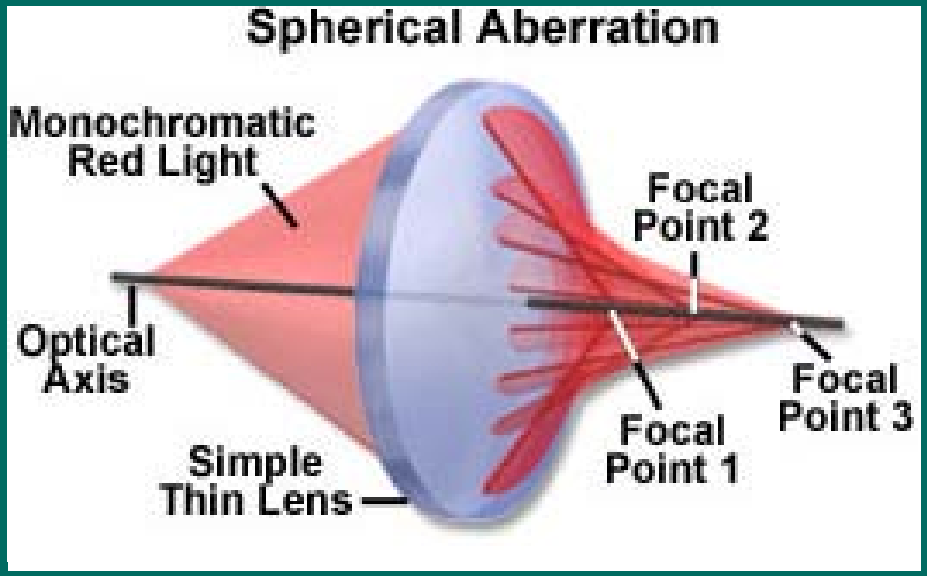
278 €

897 €

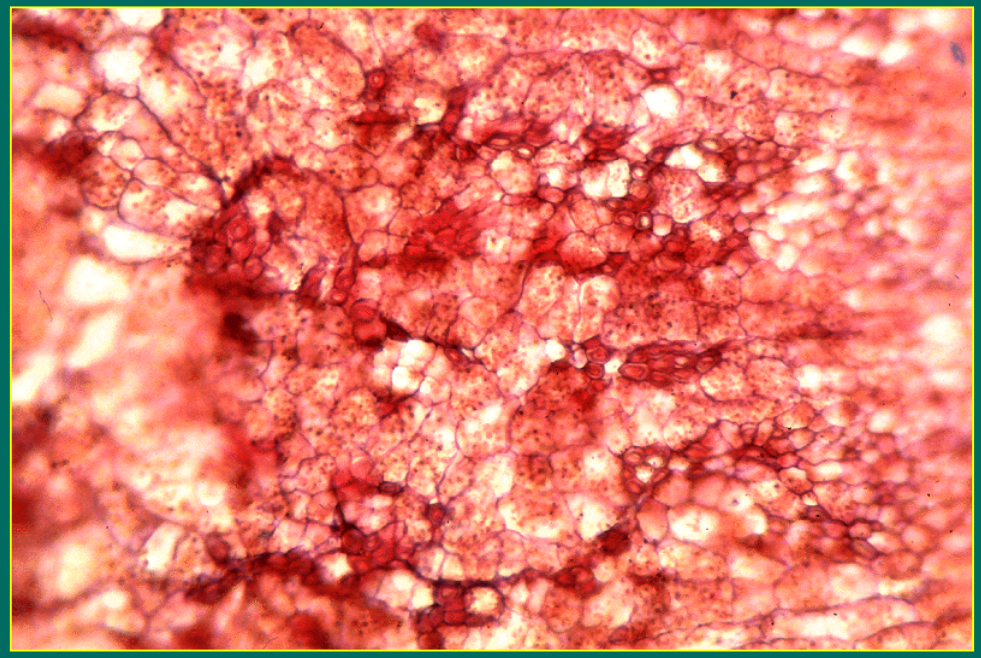
2076 €

L'ABERRATION SPHERIQUE

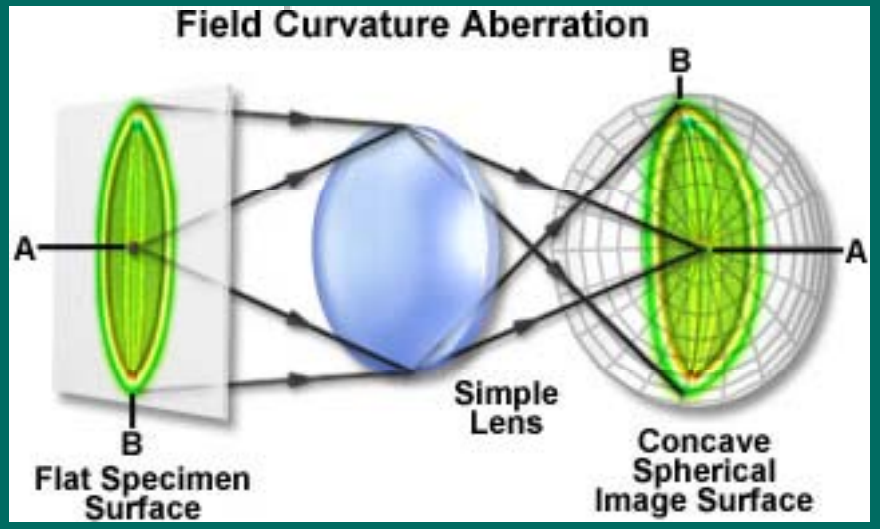
apparaît lorsqu'une lentille concentre des rayons marginaux dans des plans qui sont plus proches de la lentille que les rayons centraux.

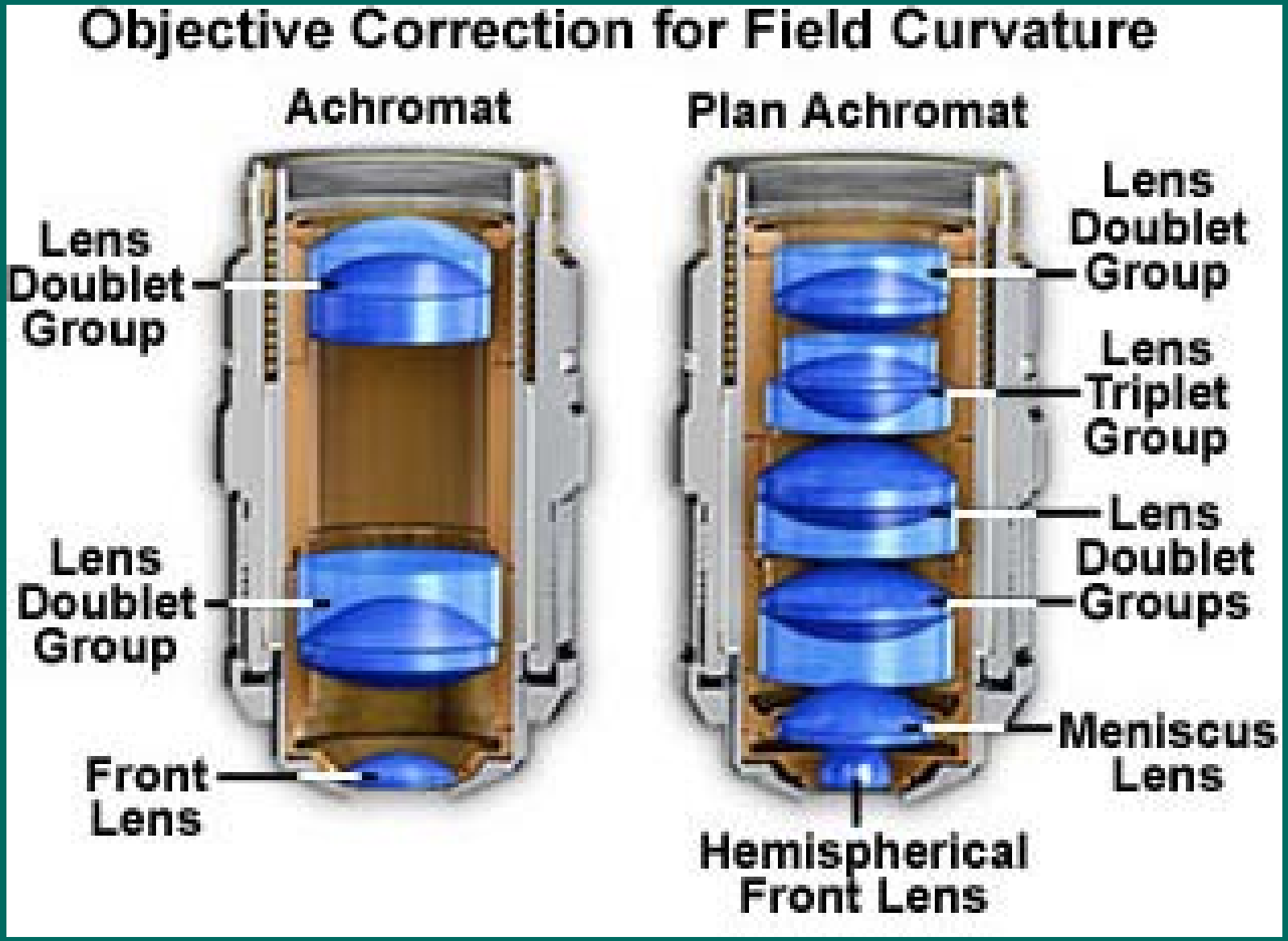


COURBURE DE CHAMP



Objectifs : **PLAN**

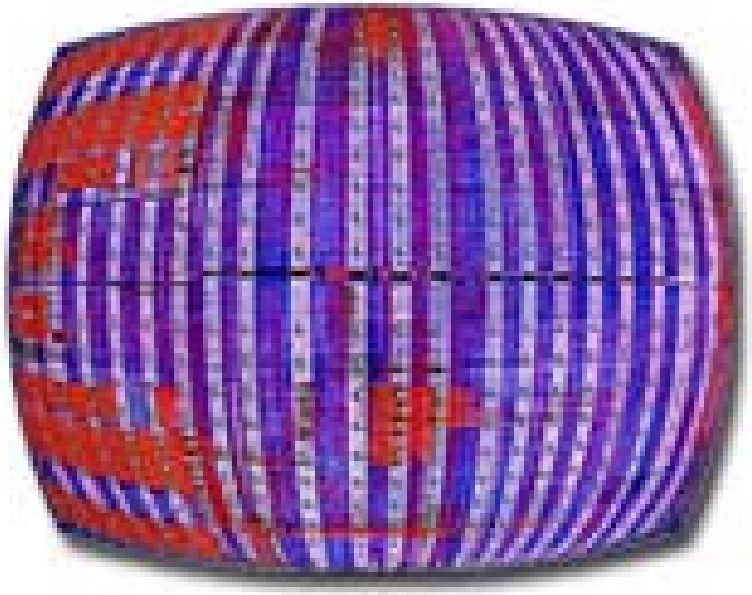



Objective Correction for Field Curvature
Achromat
Plan Achromat
Lens Doublet Group
Lens Doublet Group
Front Lens
Lens Doublet Group
Lens Triplet Group
Lens Doublet Groups
Meniscus Lens
Hemispherical Front Lens

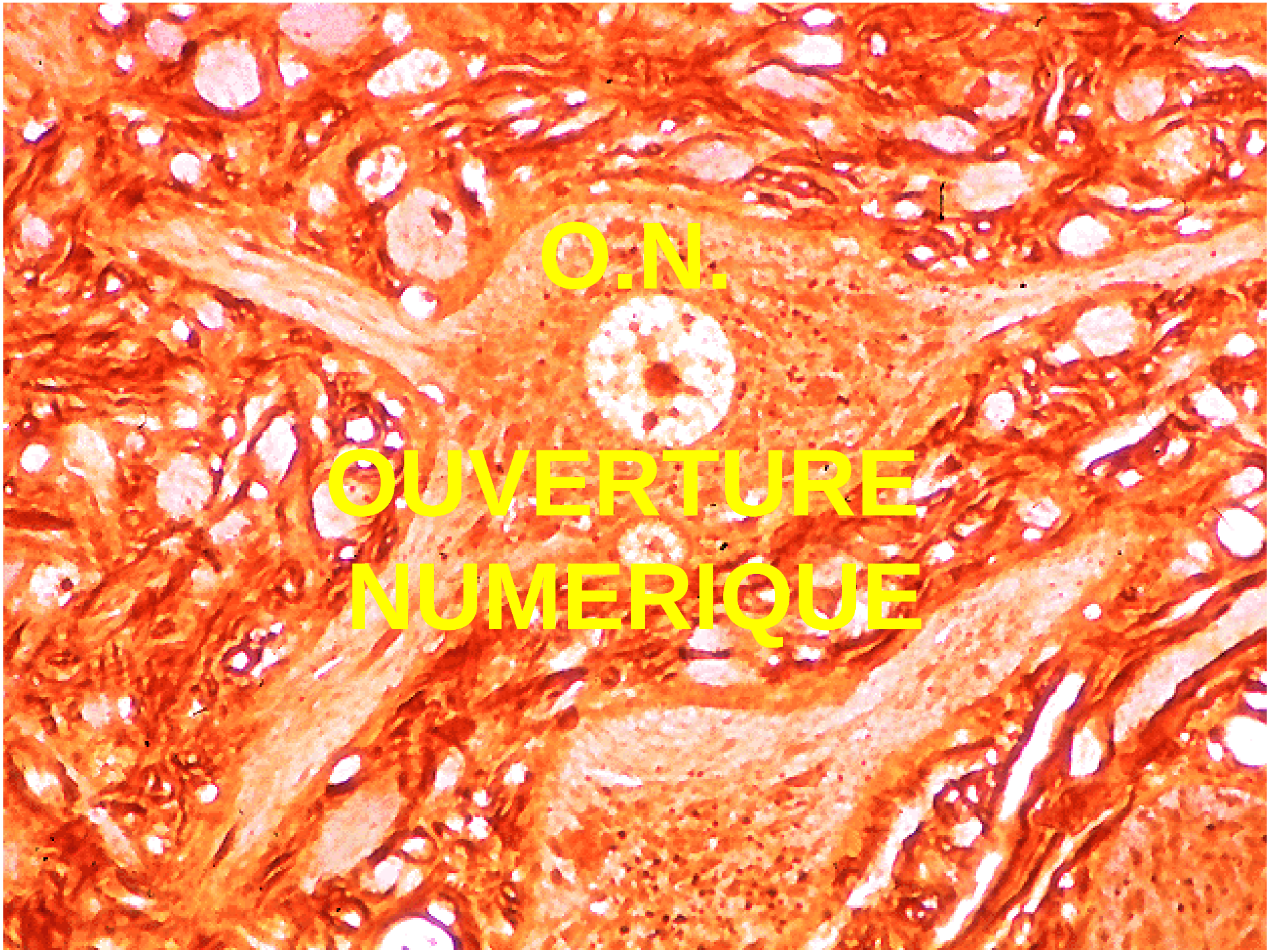
5000 à 15000 €

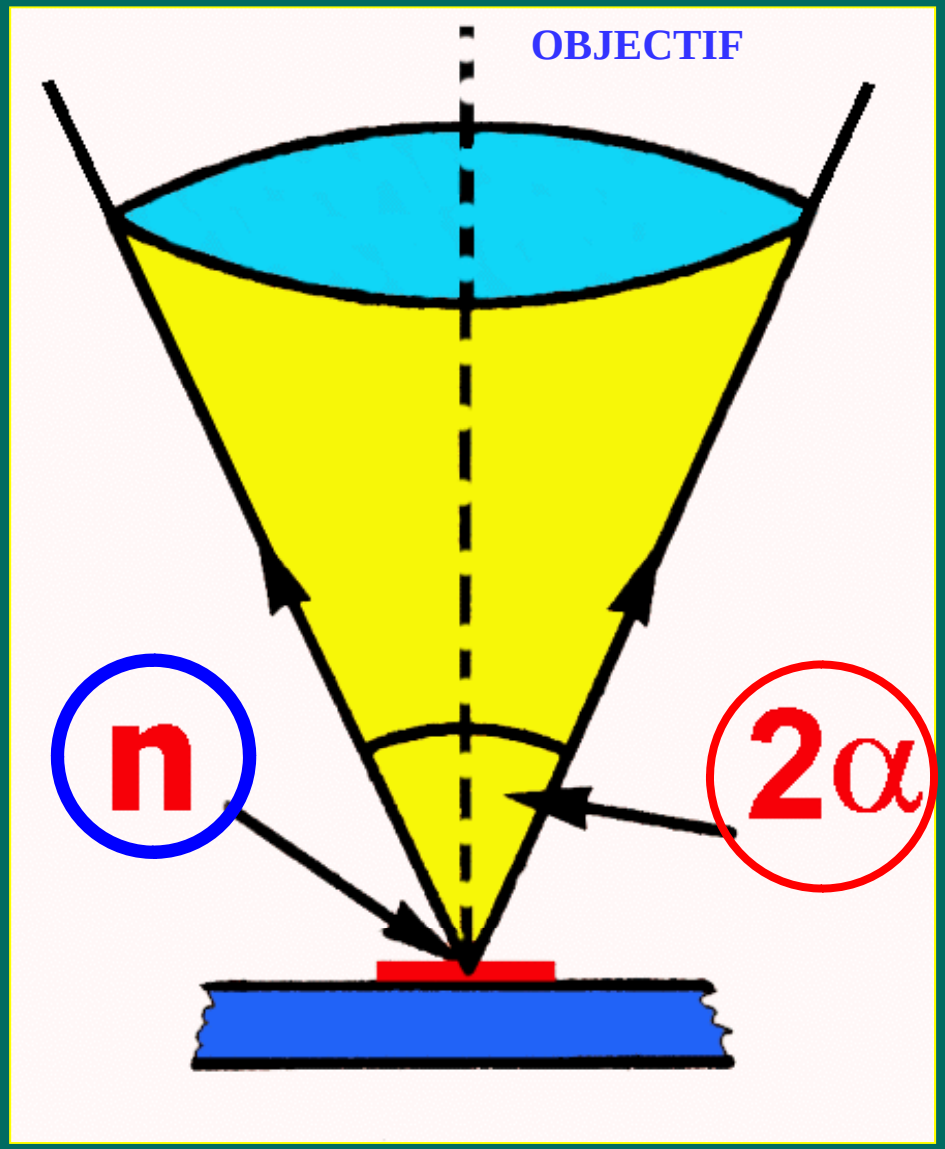
Geometric Distortion

Pincushion

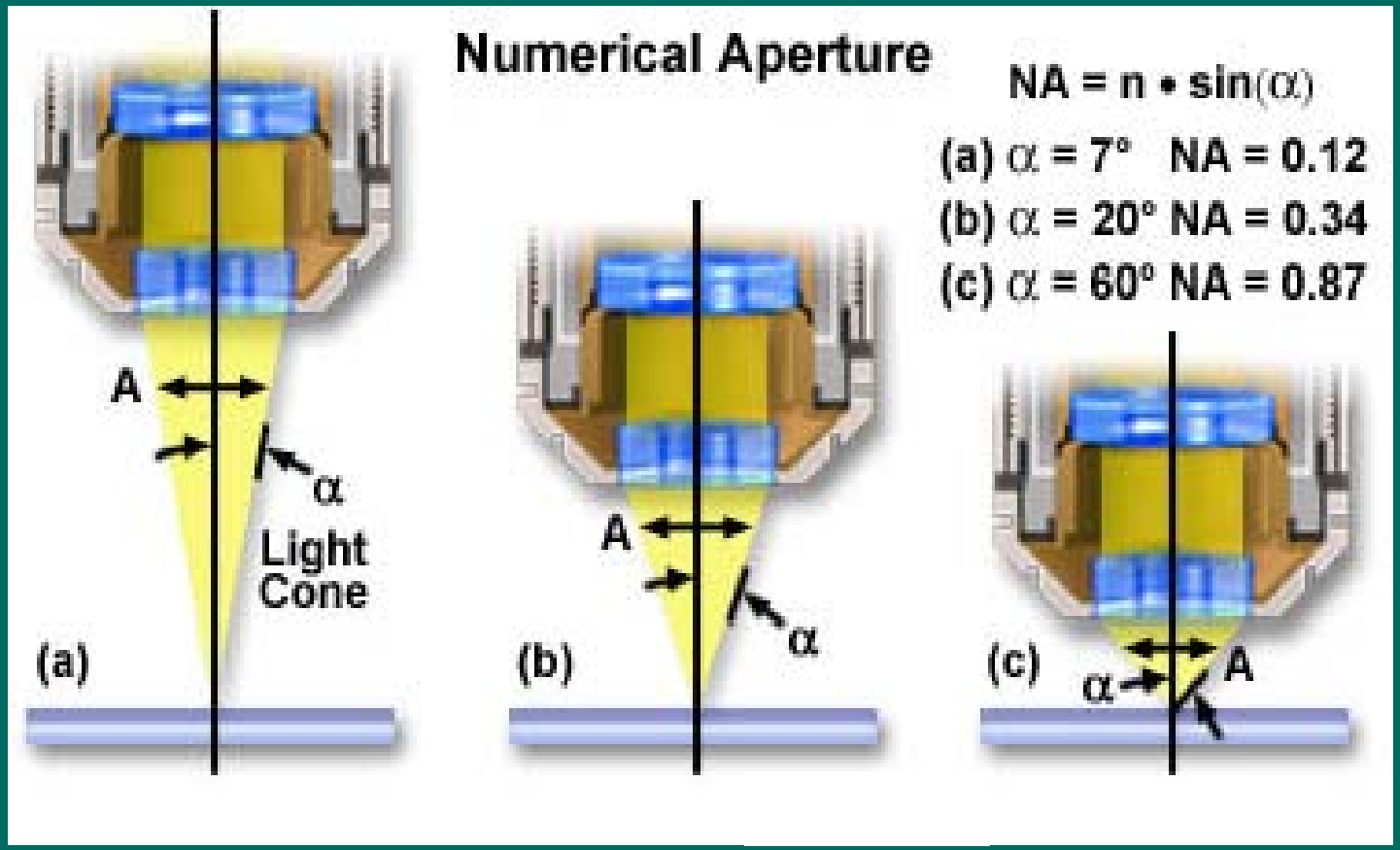


Barrel


O.N.
OUVERTURE
NUMERIQUE



$$O.N = n \sin \alpha$$



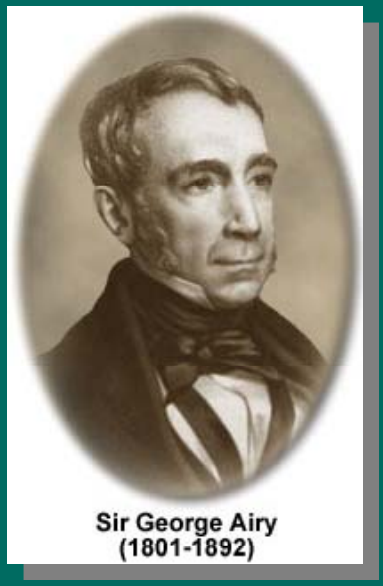
Les objectifs à forte ouverture numérique
ont des distances de travail faibles.

Pouvoir séparateur

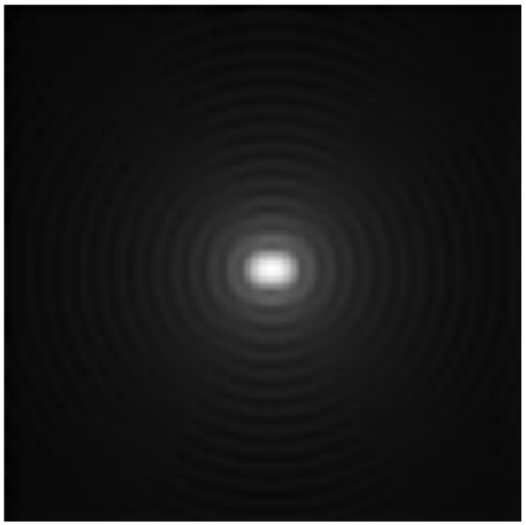
pouvoir séparateur = capacité de distinguer deux points adjacents comme distincts

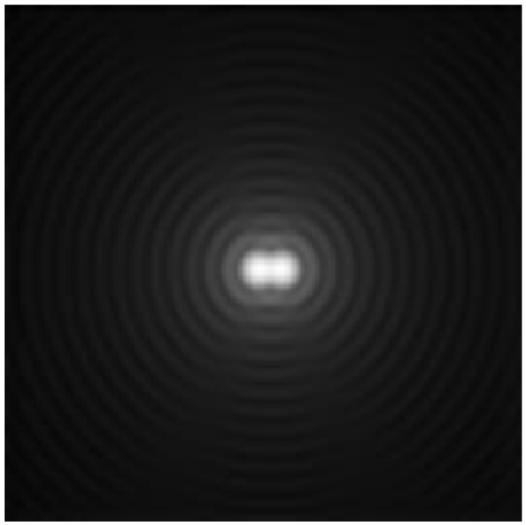
Dans un système optique l'image d'un point est donnée par une série d'anneaux de diffraction : disque d'Airy

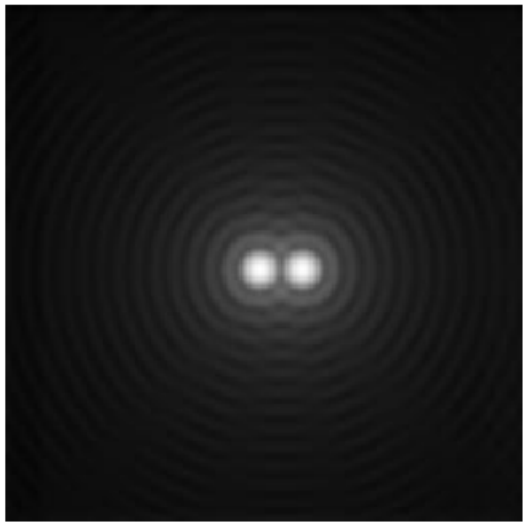
$$R_{airy} = 1.22\ \lambda/N.A.$$



Conséquences

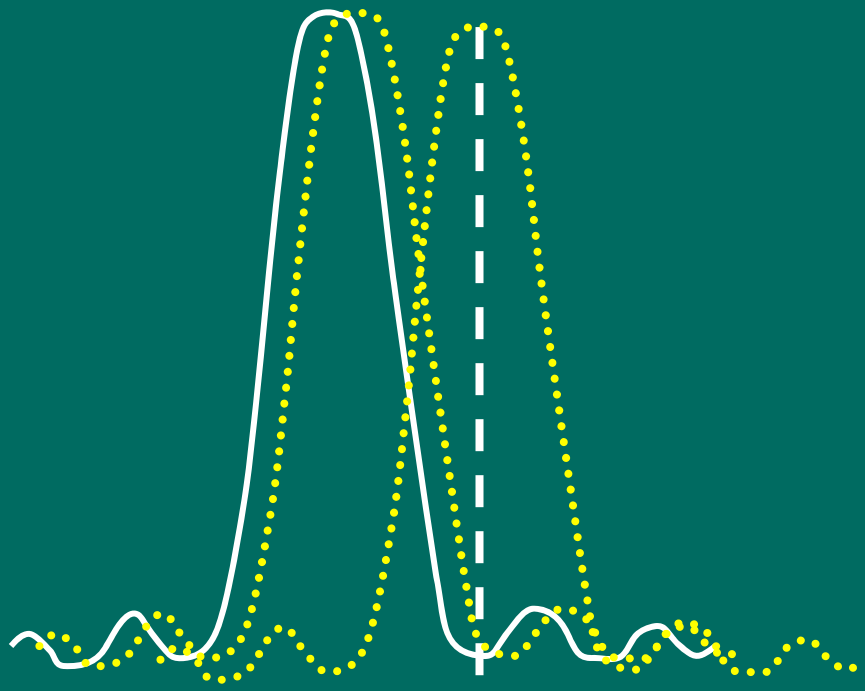






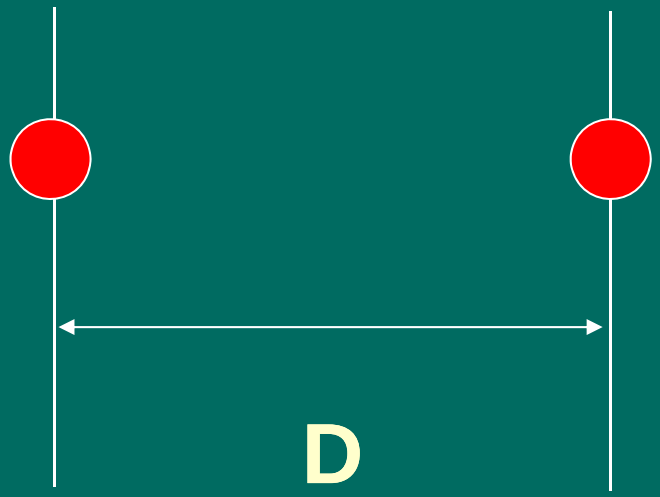
Pouvoir séparateur

Non résolu



résolu

2 points lumineux sont résolus si le maximum de la figure d ’Airy de l’un est au delà du premier minimum de l ’autre.



$D = 0.61\ \lambda / n\sin\alpha$

$n\sin\alpha = NA$

Ex : $\alpha = 70°$ $\sin\alpha = 0.94$

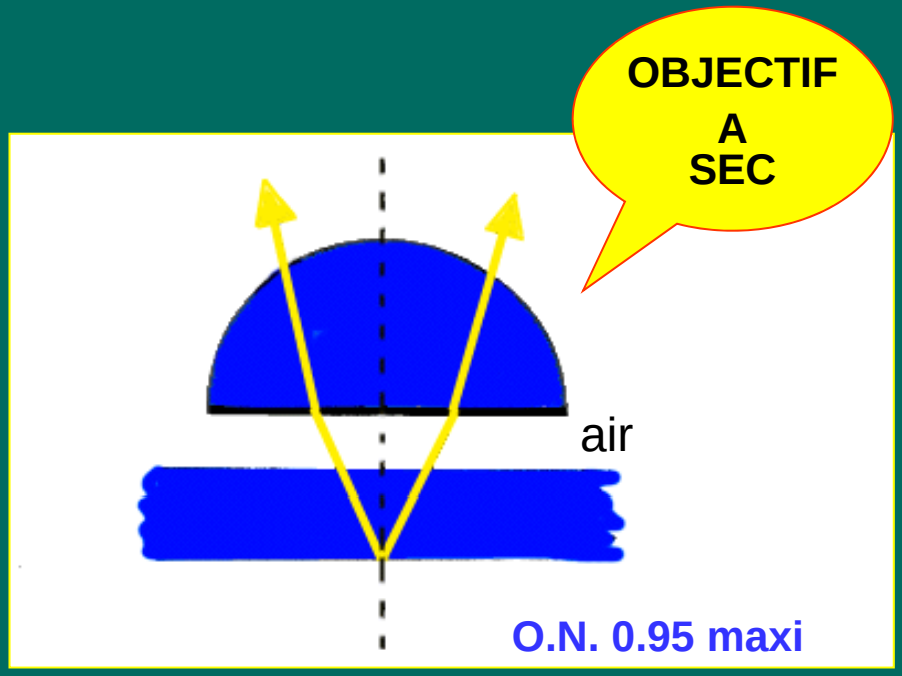
$\lambda = 450$ nm, N = 1 (air)

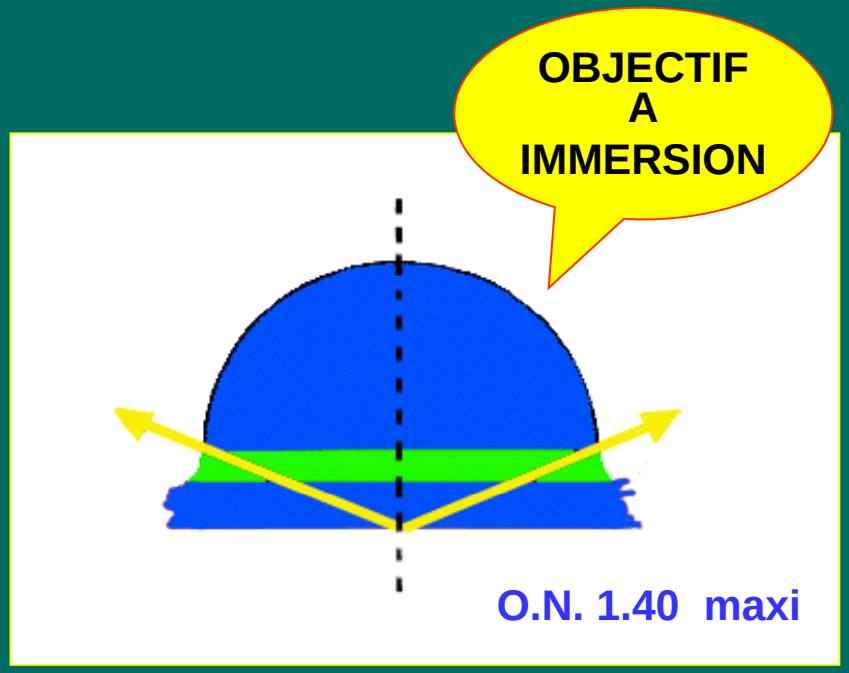
D = 0.292 µm

si N = 1.5 (huile)

D = 0.194 µm

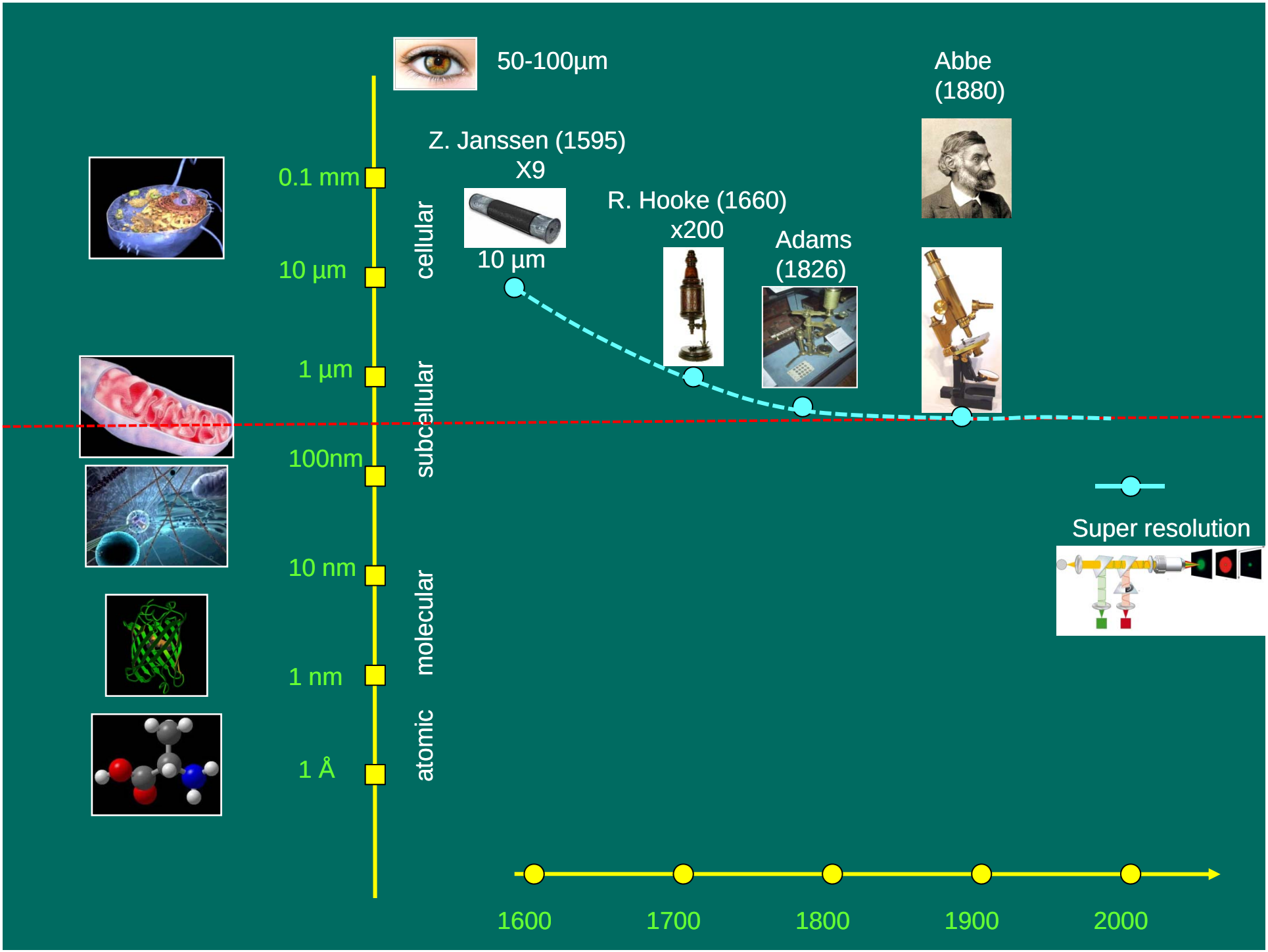
Ernst Abbe



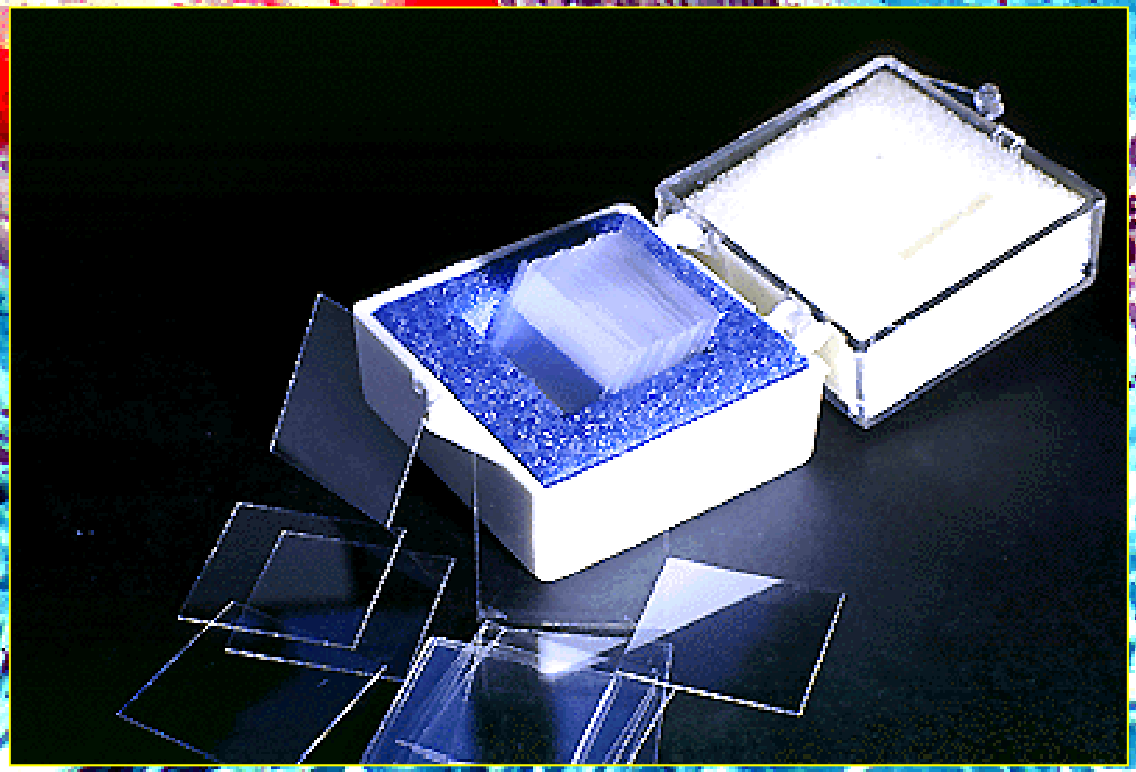


N.A. = n sinα

Air	n = 1.00
Eau distillée	n = 1.334
Glycérine	n = 1.450
Essence de cèdre	n = 1.515
Huile synthétique	n = 1.518


50-100µm
Abbe
(1880)
Z. Janssen (1595)
X9
R. Hooke (1660)
x200
Adams
(1826)
10 µm
0.1 mm
10 µm
1 µm
100nm
10 nm
1 nm
1 Å
cellular
subcellular
molecular
atomic
Super resolution
1600
1700
1800
1900
2000

LE COUVRE-OBJET



Épaisseur de la lamelle couvre objet = 0.17 mm

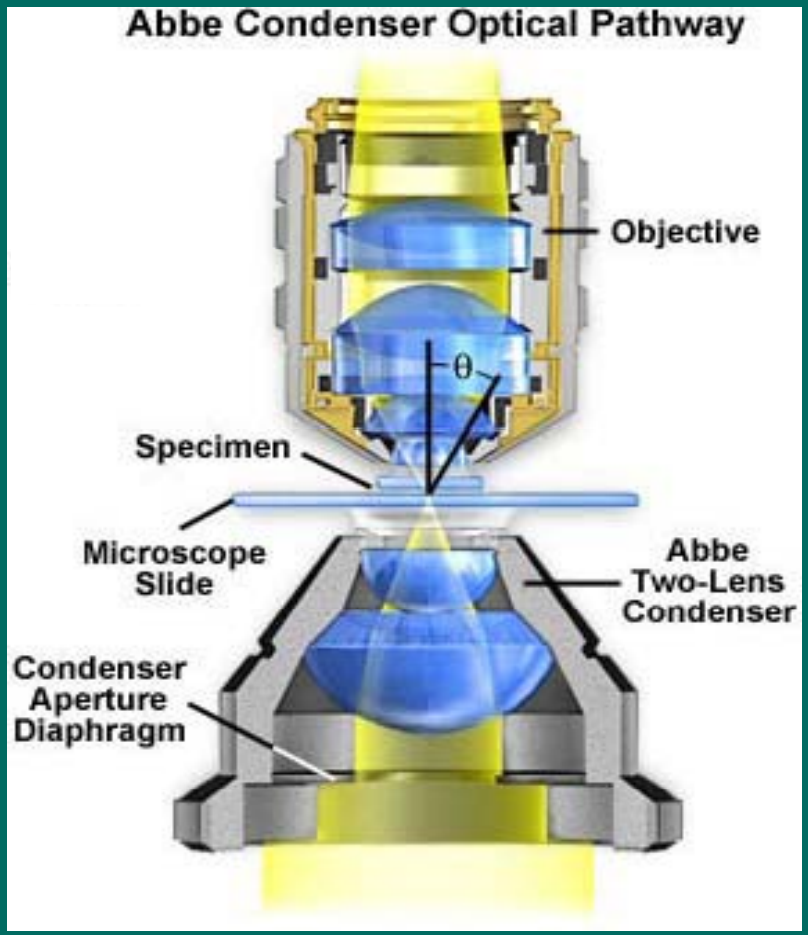
Rapport entre l'O. N. de l'objectif et l'épaisseur du couvre-objet

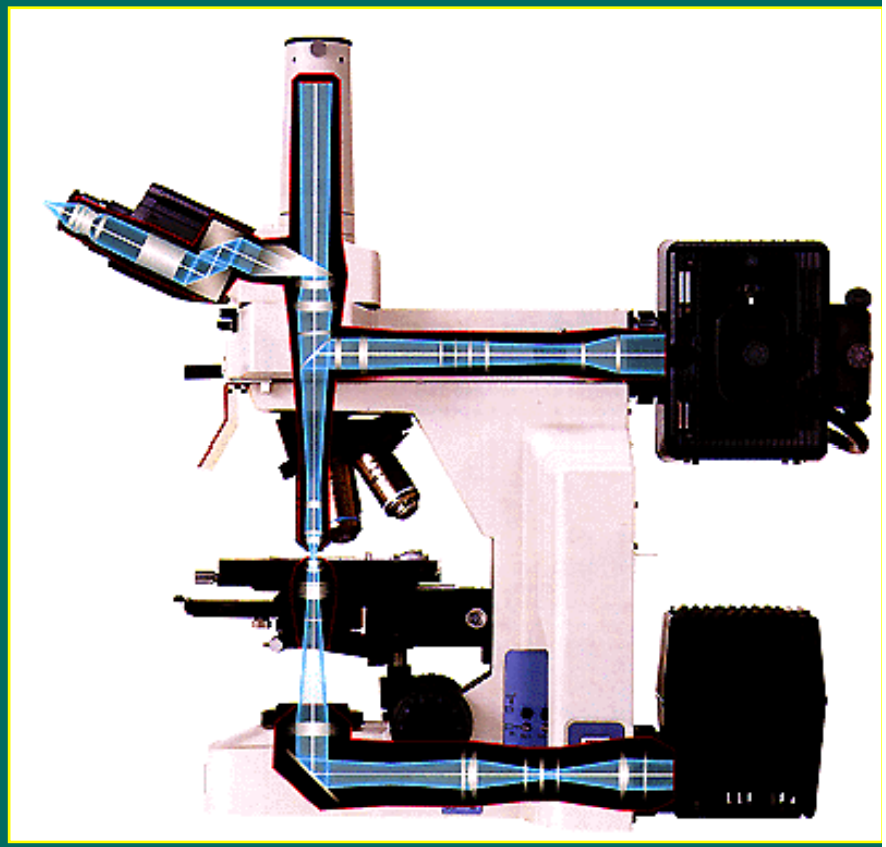
O.N.	Tolérance réf.: 0,17	Plage du couvre-objet
0,08-0,30	--	0 - 0,3
0,30-0,45	+/- 0,07	0,1 - 0,24
0,45-0,55	+/- 0,05	0,12 - 0,22
0,55-0,65	+/- 0,03	0,14 - 0,20
0,65-0,75	+/- 0,02	0,15 - 0,19
0,75-0,85	+/- 0,01	0,16 - 0,18
0,85-0,95	+/- 0,005	0,165 - 0,175

condenseur

Le condenseur est situé sous la platine du microscope.

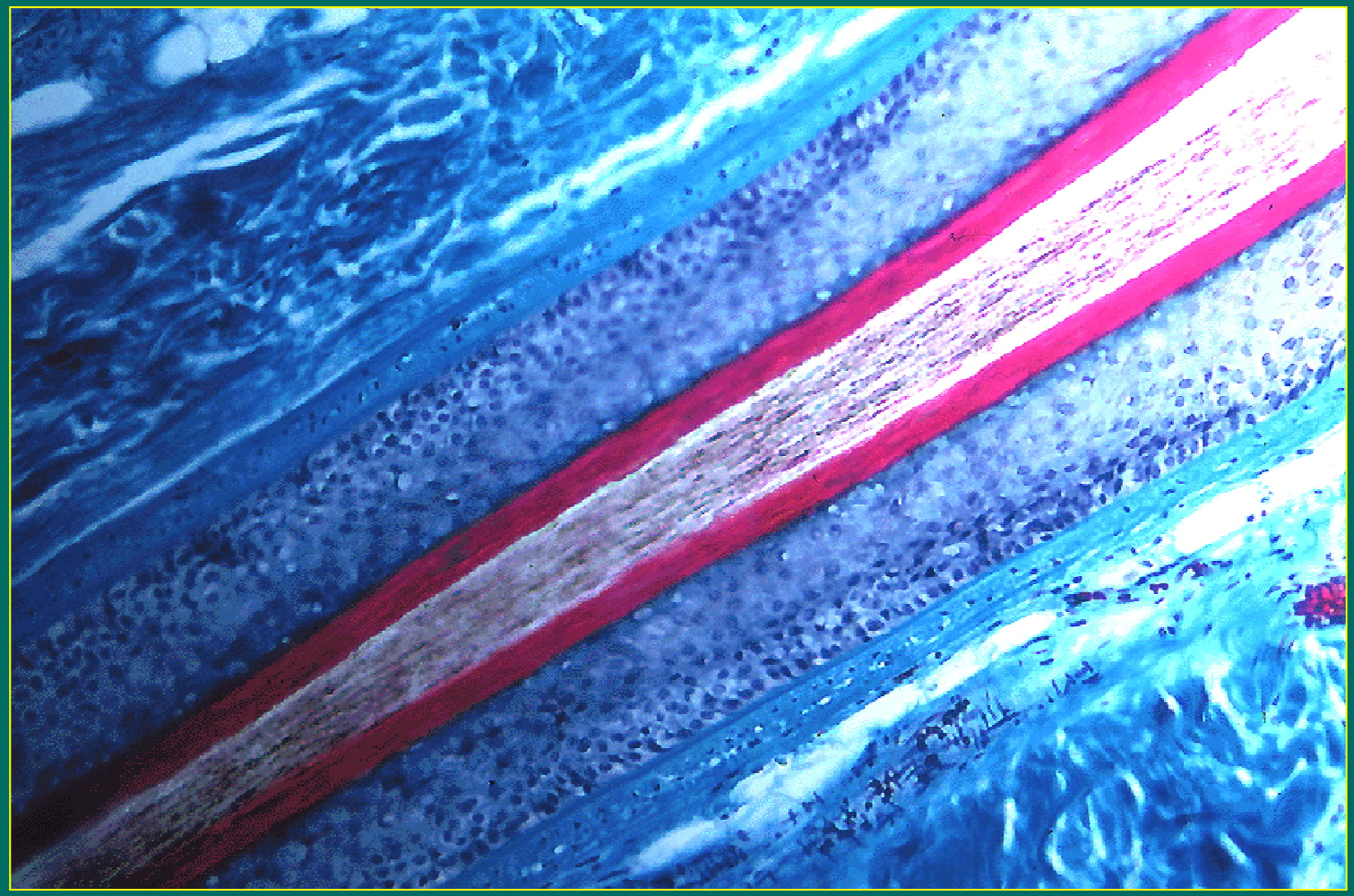
Il doit être réglable et doit être centré.



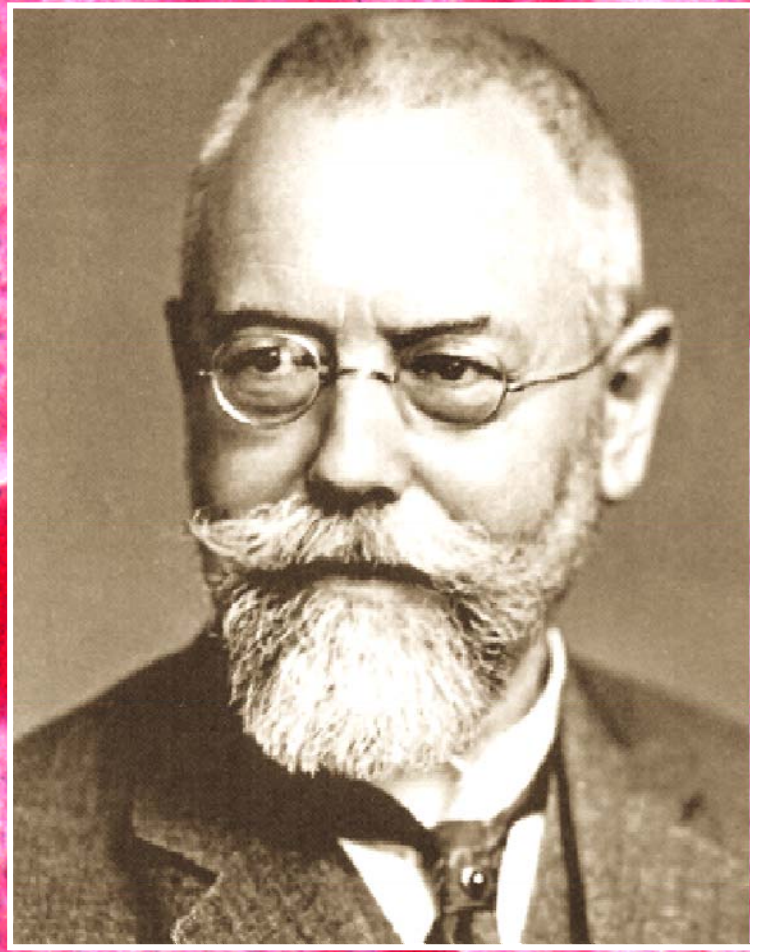


L’ouverture numérique du condenseur

Le condenseur doit être centré avec exactitude, sinon l'éclairage du champ n'est pas homogène.



ECLAIRAGE DE KÖHLER



Patented Nov. 15, 1927. **1,649,068**

UNITED STATES PATENT OFFICE.

AUGUST KÖHLER, OF JENA, GERMANY, ASSIGNOR TO THE FIRM CARL ZEISS, OF JENA, GERMANY.

MICROSCOPE.

Application filed March 31, 1925, Serial No. 19,697, and in Germany April 16, 1924.

It is customary in microscopes, which are provided with a tube, containing the optical parts, and a stage for the reception of the object, to render the whole tube displaceable relatively to the stage, in order to enable the observer to adjust with objectives having a different free working distance to sharp images of the surface, facing the objective, of a more or less thick object. If it be desired to examine objects of a thickness varying within practically wide limits, it is suitable to also render the stage itself movable in the same direction. However, on the one hand, it is always desirable to support the object as firmly as possible, e. g. on a fixed stage and on the other hand, an adjustment of the tube, possible within very wide limits, has the drawback that the observer will be obliged in one case, whilst in a sedentary position, to bend down his head, in another case to work in standing in an uncomfortable position in order to be able to keep his eye-surface may take place either indirectly or directly. As a surface destined for the direct reception may serve, provided the objects are of a shape and size fit for laying-on, for instance the surface of a fixed stage, or the surface can be determined by the supports of the microscope foot if the microscope be adapted to be placed upon the object to be examined. However, the case of indirect reception of the object to be examined exists if one uses a special device supported on a fixed surface in order to impart to the object certain definite possibilities of displacement (e. g. revolving stage, mechanical stage, spherical stage etc.). In this sense may, for instance, also be understood the bearing surface of a pivot, which perpendicularly intersects the optical axis of the microscope and about which a tiltable stage can be turned, as a surface destined for the indirect reception of the object.

Nov. 15, 1927. **1,649,068**

A. KÖHLER

MICROSCOPE

Filed March 31 1925

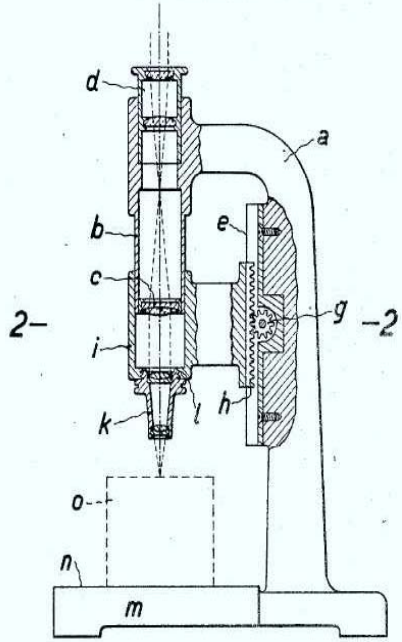


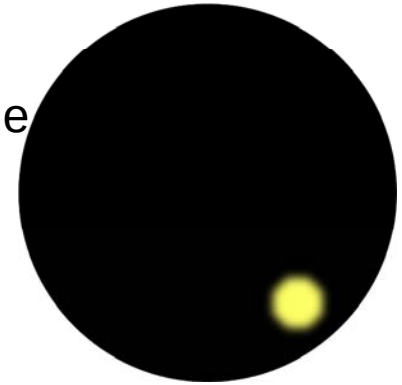
Fig. 1

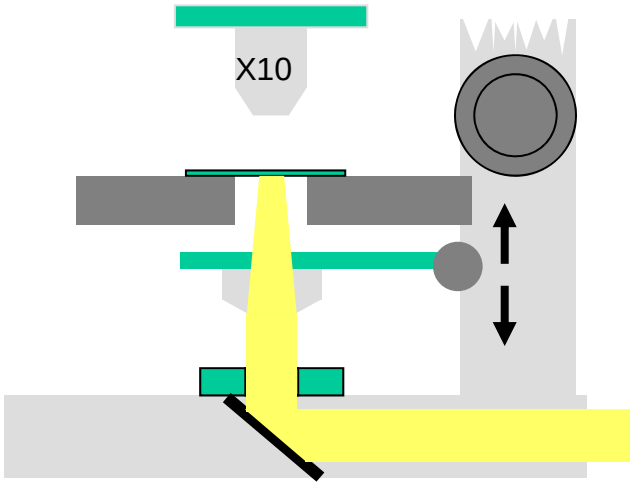
méthode de Köhler

1. Reproductibilité des conditions d'observation;
2. Répartition homogène de la lumière dans le champ
3. Exploitation maximale de l'O. N. de l'objectif;
4. Elimination à la fois des rayons marginaux et de la lumière diffusée qui entraînent une réduction de la qualité de l'image.

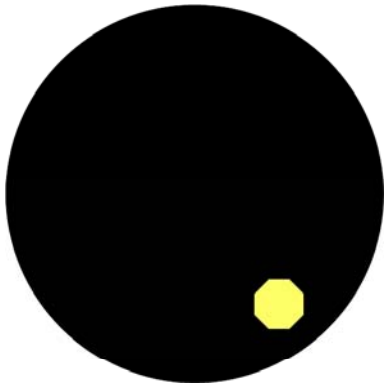
ECLAIRAGE DE KÖHLER

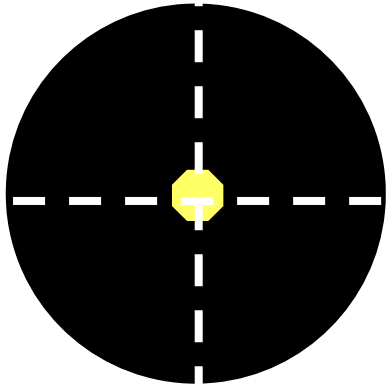
Utiliser un faible grossissement
Fermer le diaphragme de champ



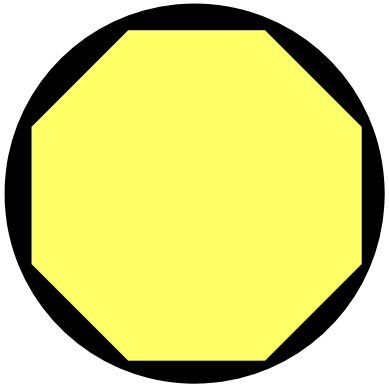


Mettre au point l'image du diaphragme avec la molette du condenseur





centrer



Ouvrir le diaphragme

Les oculaires

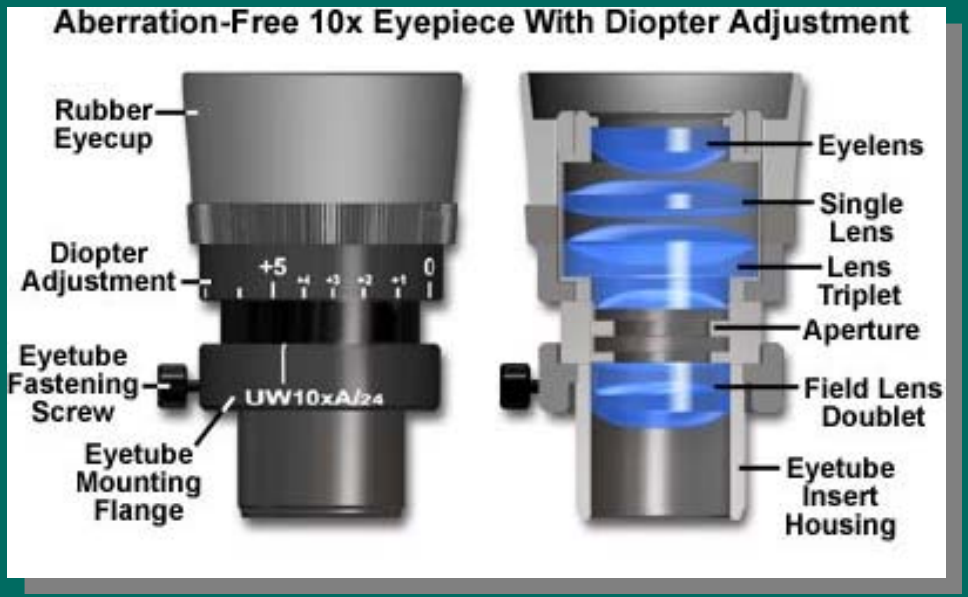
Sur l'oculaire est inscrit un nombre qui indique le facteur de grossissement.

$$G_v = Gross_{oc} \times Gross_{obj}$$

Attention aux échelles sur les photos !

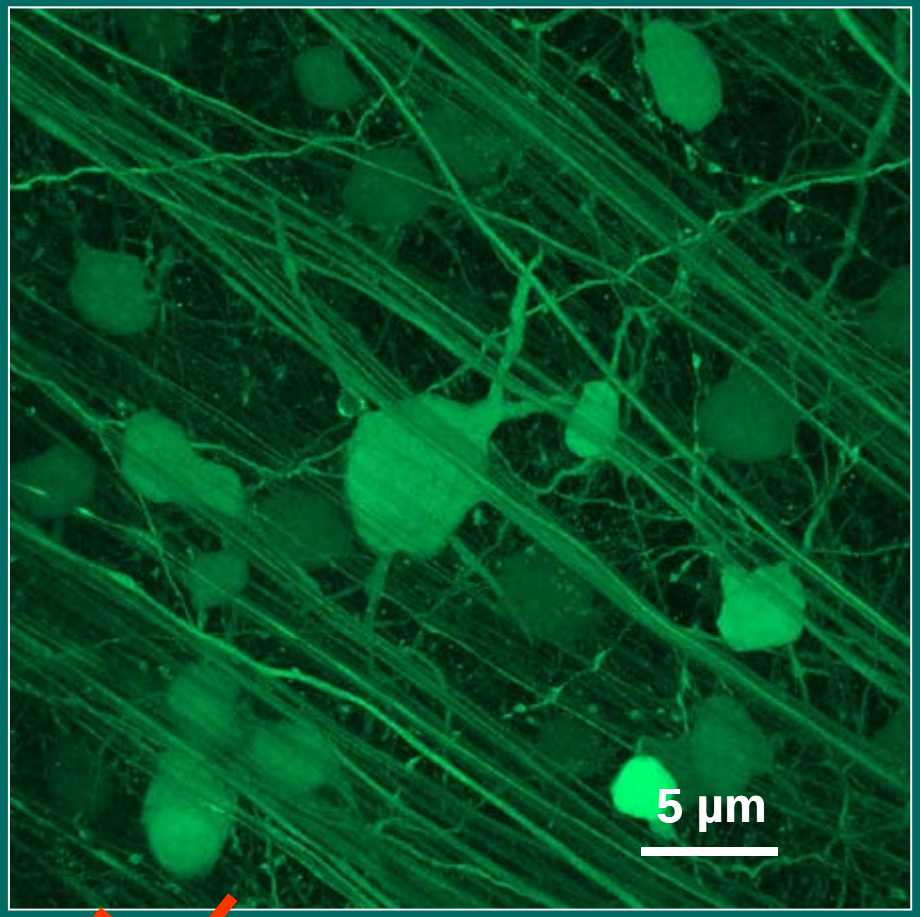
Il faut :

- Régler à sa vue
- Régler l'écartement à ses yeux
- Nettoyer la lentille frontale des oculaires avec du papier photo.



indice de champ qui correspond au diamètre de la zone circulaire observable sur la préparation

Cherchez l’erreur !



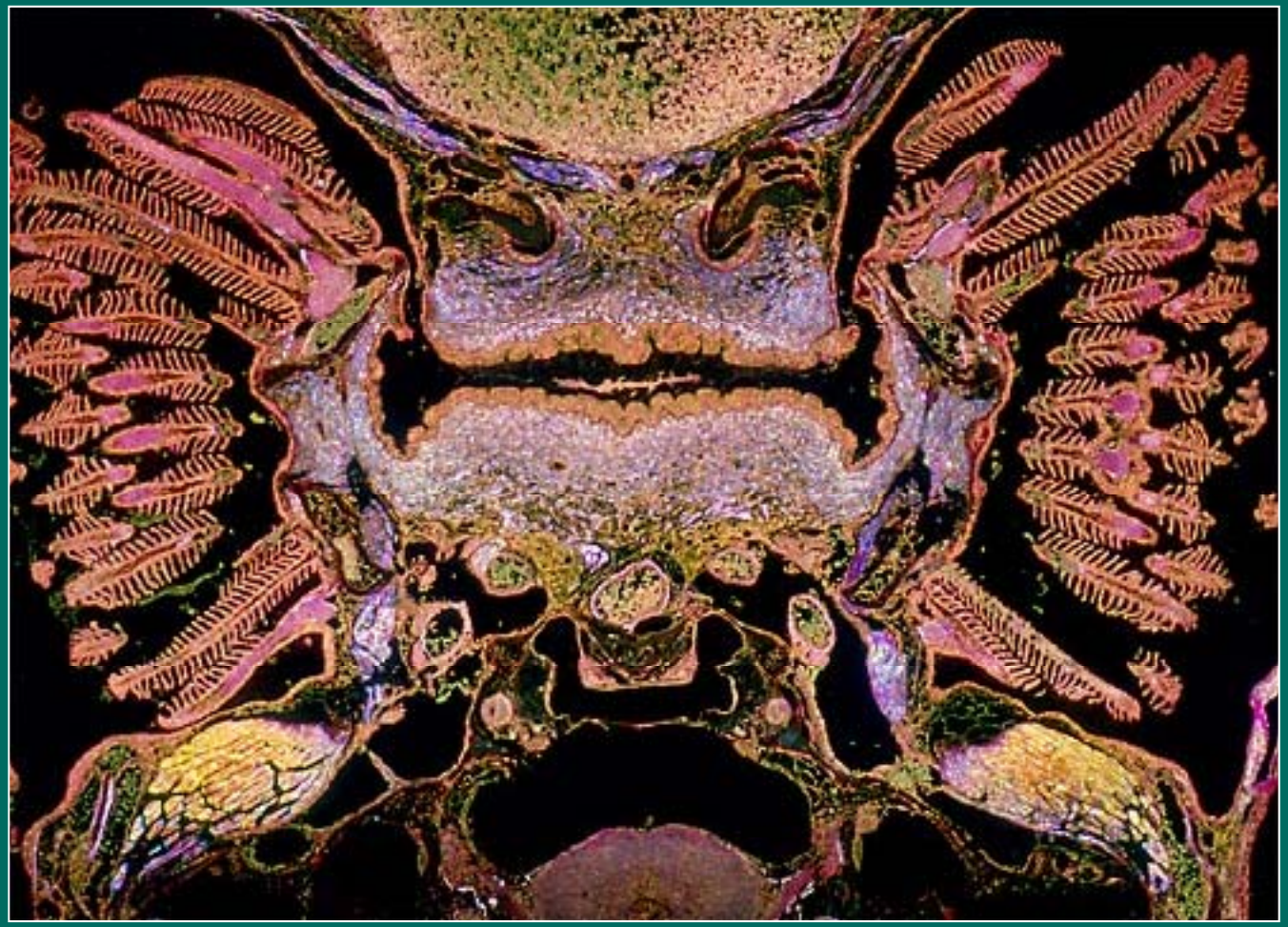
~~X 400~~

METHODES DES CONTRASTES

- Microscopie à fond noir
- Microscopie à contraste de phase
- Microscopie à contraste interférentiel

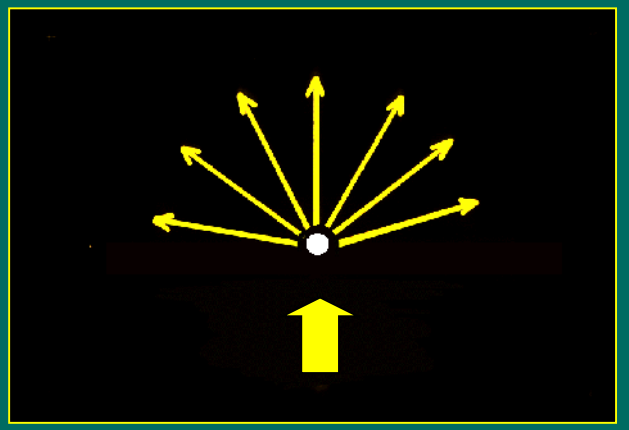
MICROSCOPIE A FOND NOIR

La microscopie à fond noir révèle la présence, mais pas la structure, d'échantillons.

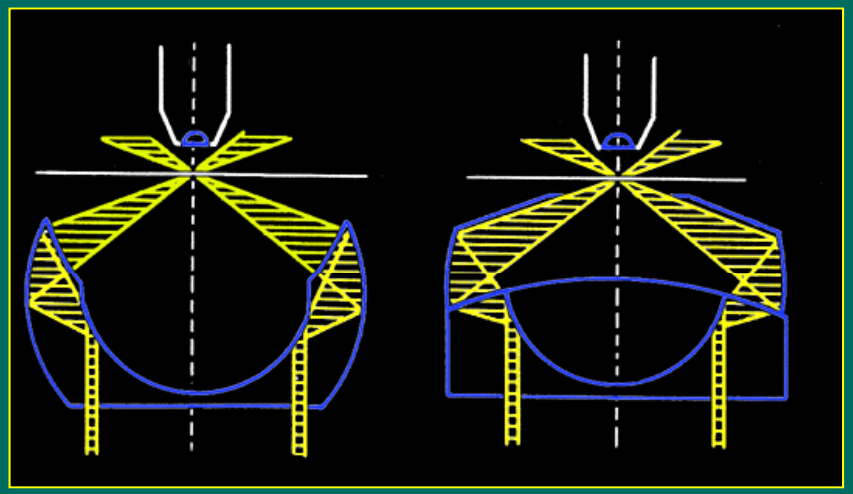


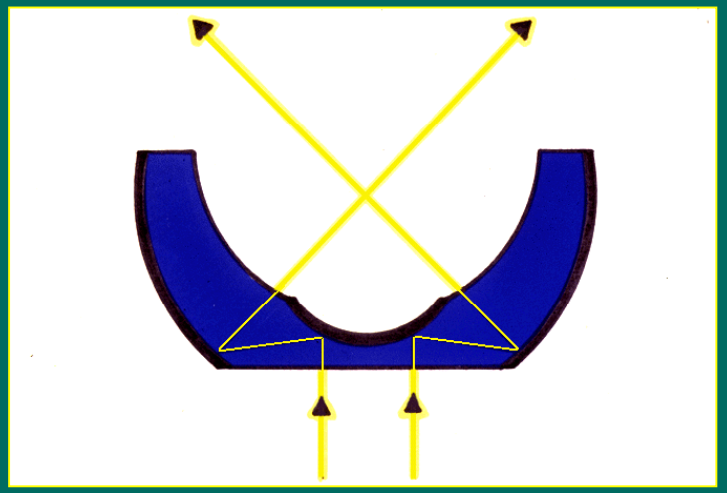
Branchies de Zebrafish

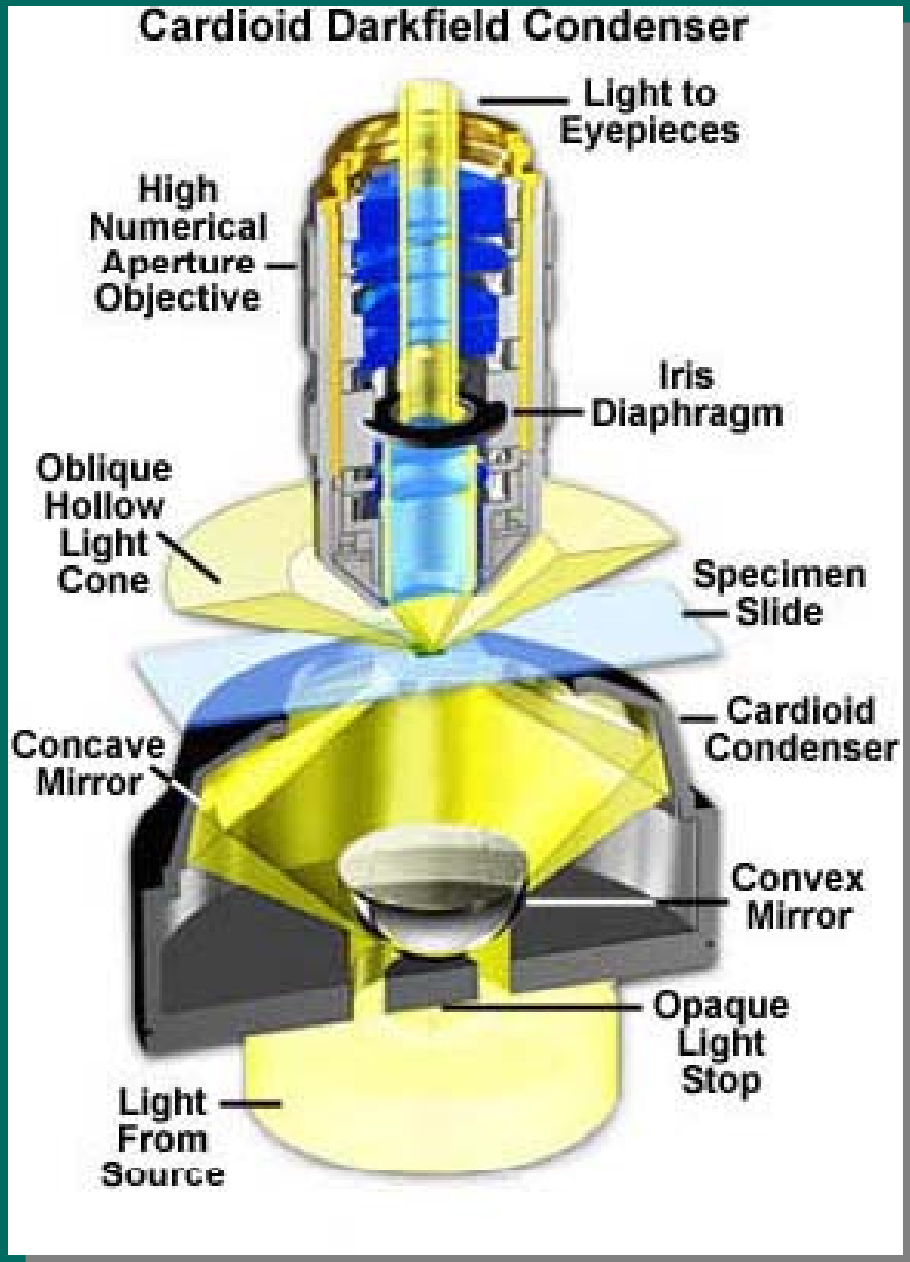
Les particules apparaissent parce qu'elles diffractent la lumière incidente alors que l'environnement est sombre.



En microscopie à fond noir, on utilise des condenseurs à miroir qui envoient une lumière très oblique sur l'échantillon.



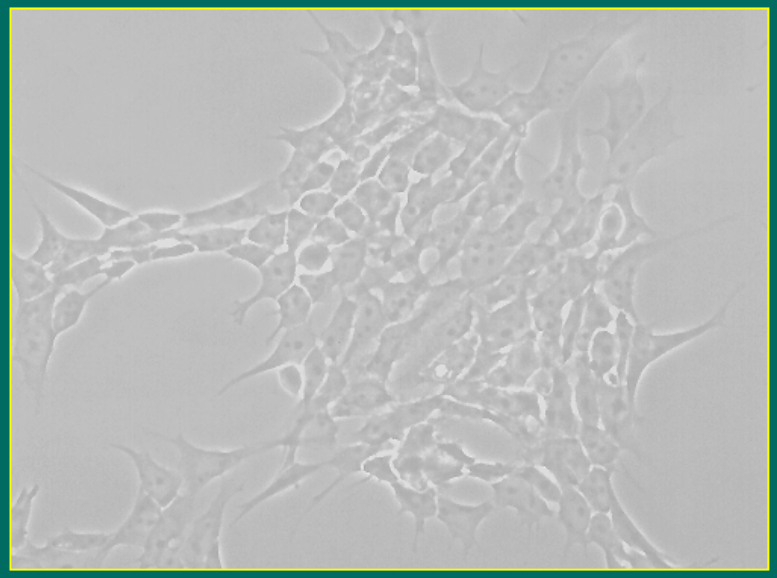



Cardioid Darkfield Condenser
Light to Eyepieces
High Numerical Aperture Objective
Iris Diaphragm
Oblique Hollow Light Cone
Specimen Slide
Cardioid Condenser
Concave Mirror
Convex Mirror
Opaque Light Stop
Light From Source

MICROSCOPIE
A
CONTRASTE DE PHASE

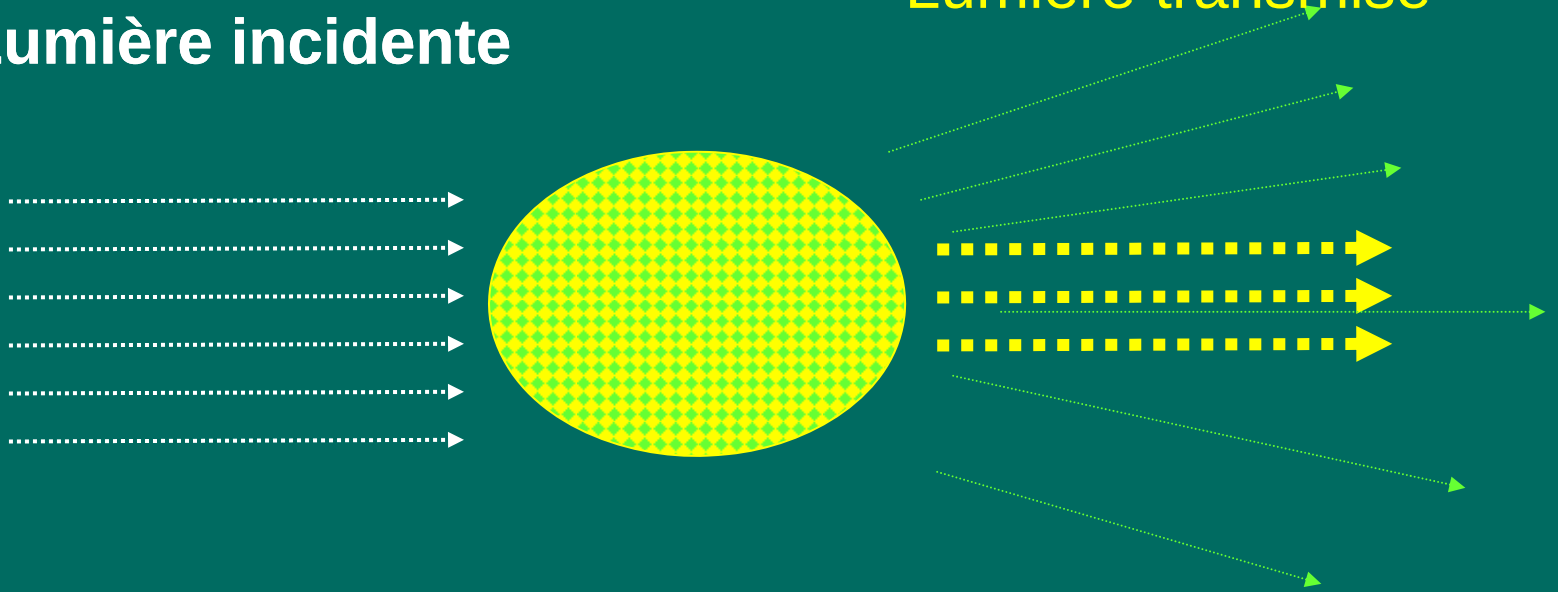
Les objets sont très fins et transparents et souvent invisibles.

même si l ’objet est est invisible à l'œil nu, la lumière qui est renvoyée contient son image.



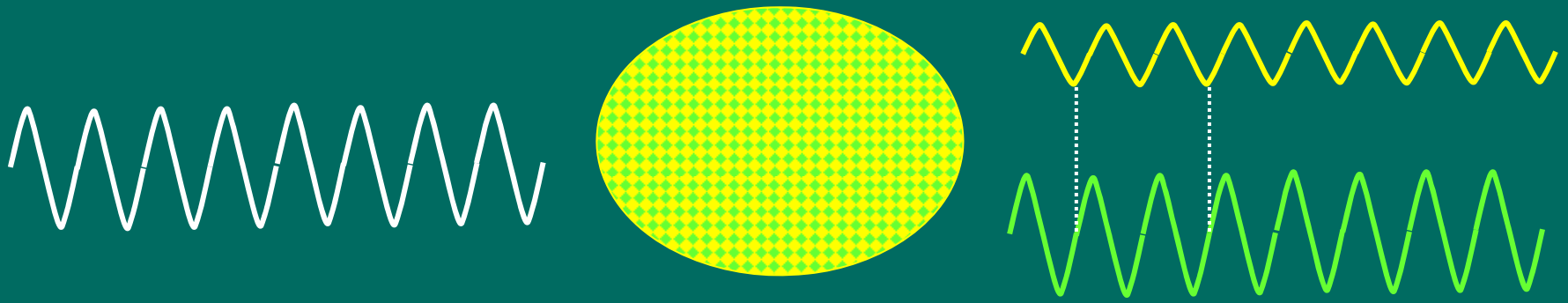
Lumière incidente

Lumière transmise

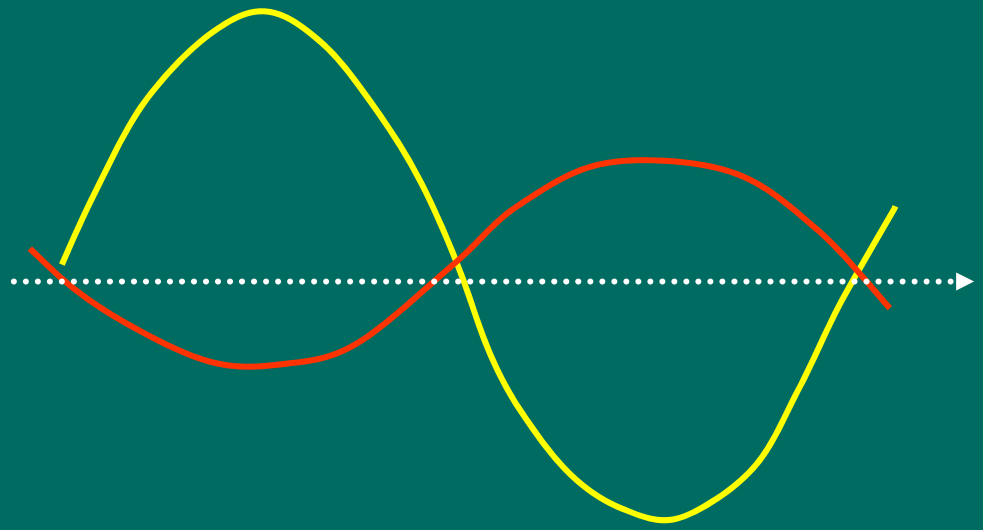


Lumière diffractée

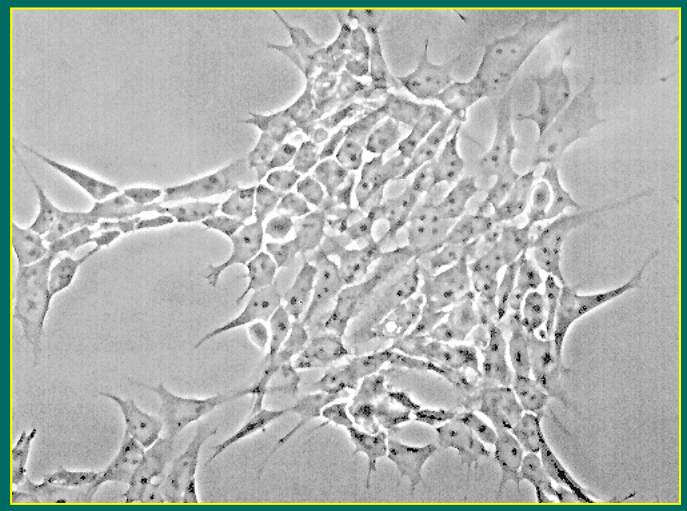
La formation de l'image est le résultat de l'interférence de l'éclairage direct avec la lumière diffractée.



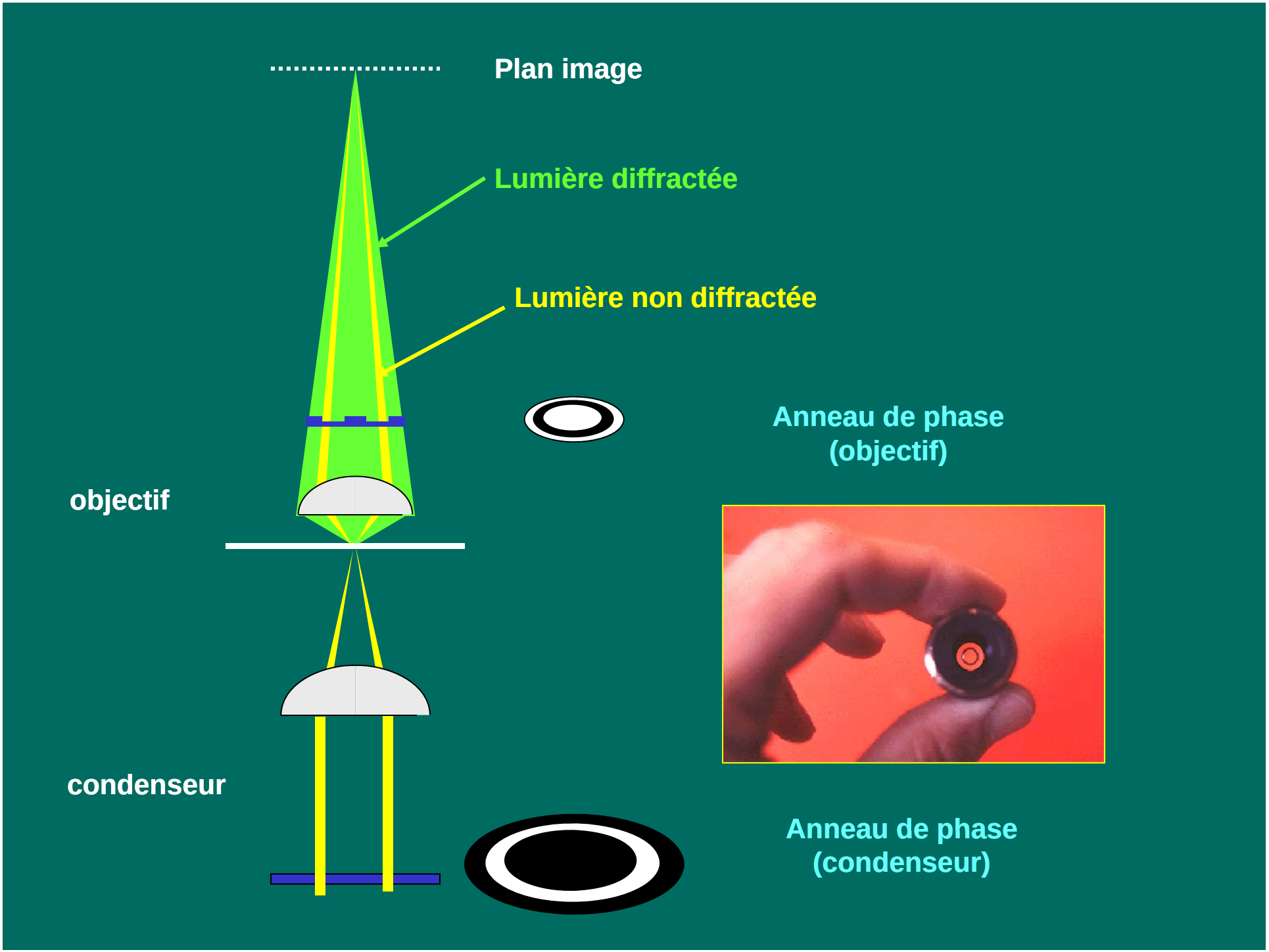
Si la lumière diffractée est déphasée d'une demi-longueur d'onde, par rapport à la lumière transmise, la différence de luminosité et de couleur entre ses différentes parties permet de voir l'échantillon.

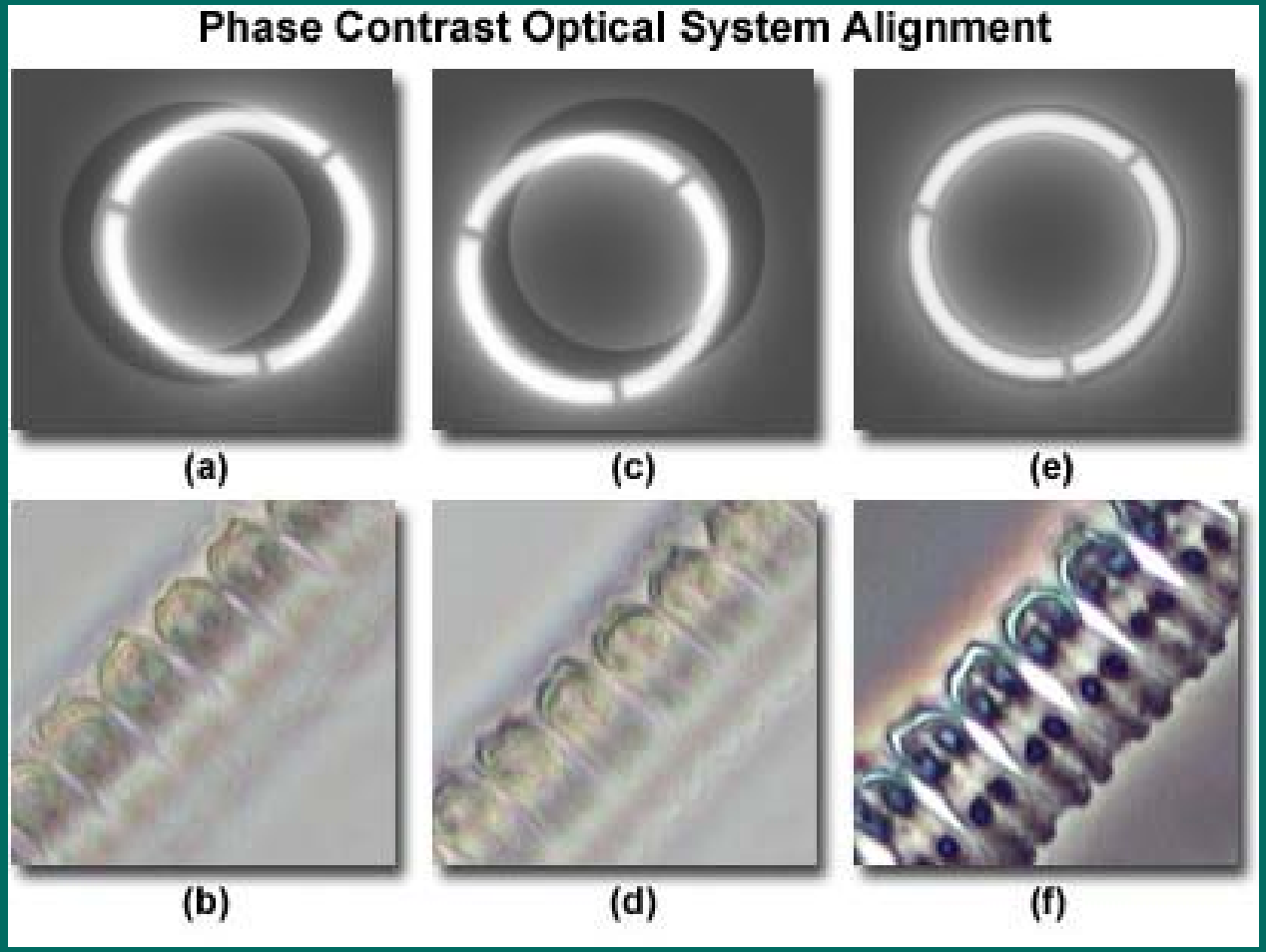


1/2 λ



C'est aux Pays-Bas en 1934 que FRITZ ZERNIKE créa le premier microscope à contraste de phase et cela lui a valu le Prix Nobel de physique en 1953.


Plan image
Lumière diffractée
Lumière non diffractée
objectif
condenseur
Anneau de phase
(objectif)
Anneau de phase
(condenseur)


Phase Contrast Optical System Alignment
(a)
(c)
(e)
(b)
(d)
(f)

MICROSCOPIE A CONTRASTE INTERFERENTIEL

Georges (Jerzy) Nomarski
(1919-1997)

1952

Georges Nomarski (1919-1997)

United States Patent Office

2,924,142

Patented Feb. 9, 1960

2,924,142

INTERFERENTIAL POLARIZING DEVICE FOR STUDY OF PHASE OBJECTS

Georges Nomarski, Paris, France, assignor to Etablissement Public dit: Centre National de la Recherche Scientifique, Paris, France

Application May 11, 1953, Serial No. 354,362

Claims priority, application France May 14, 1952

13 Claims. (Cl. 88—14)

There exist a large number of optical instruments which utilize diffraction and interference phenomena, making it possible to reproduce more or less faithfully, the real contour of a reflecting surface or to determine the distribution of refraction indices combined with thickness variations in a transparent body. The present invention concerns a device which makes visible the optical structure of an object which does not modify substantially the intensity of the transmitted or reflected beam. Such an object is called a "phase object," as it acts on the phase of the incident wave and not on the amplitude thereof. Now the difference in phase being not perceptible to the eye, it is thus necessary to have amplitude variations corresponding to the phase variations.

The device according to the present invention, mainly provided for studying transparent bodies or reflecting surfaces and which do merely have an action on the phase of the light waves, essentially comprises a light source giving a coherent illumination, a first optical system giving a real image of the source, which is located after the object in the direction of light beam, an interferential polarizing system having at least one main blade or birefringent wedge placed perpendicularly to said beam after the object, the fringes caused by said system being localized at the point of the image of the source, and a second optical system or ocular system, for observing or recording the effective image of the phase object, the pupil of said second system being at a small distance from said main blade.

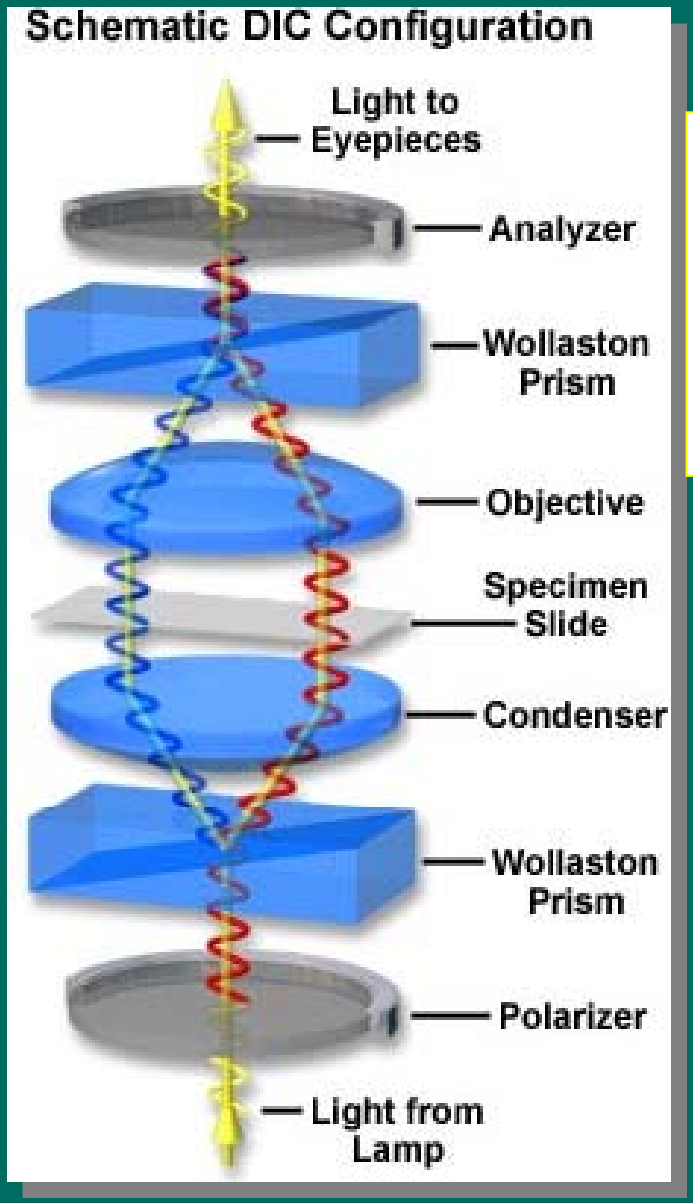
The interferential polarizing system is for instance a compound of two polarisers of any suitable type and opti-

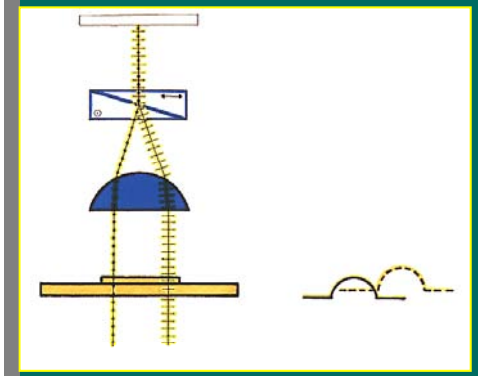
Let us take a bi-refringent crystal blade cut along any plane but at an angle between 0° and 45° to the axis. If the blade is not of uniform thickness, it is well known that there may be observed, in polarized light, a system of interference fringes, visible in monochromatic light. Such a blade, slightly prismatic for instance, in placed in the pupil of an optical instrument set for a finite distance. The object, for instance a transparent microscope preparation, being placed in position, the image of a slot limiting the aperture of an optical lighting system, is projected through the latter, on the system of interference fringes which forms on the prismatic blade itself or at a small distance therefrom. Before reaching the blade or bi-refringent wedge, the light from the slot source goes through the condenser, the object examined and perhaps one or more optical elements of the associated instrument.

In order to be able to use white light, there is placed on the path, for instance close to the slot source, another crystal blade, plane and parallel faced for example, with such a thickness and orientation that its bi-refringence compensates substantially that of the prismatic blade at the point where the image of the light source is projected on it. If the two blades are between two crossed polarizers, the object is observed on a black or gray background, according to whether the image of the slot source coincides exactly or not with the black interference fringe of the so-called "main" prismatic blade, placed in the vicinity of one pupil in the observation instrument. The first polarizer is oriented at 45° to the neutral lines of the first crystal blade, for giving identical amplitudes to the polarized waves, emerging at right angle from said blade. Since, by hypothesis, the auxiliarily blade compensates exactly the bi-refringence of the main blade, the light which arrives on the image is destroyed by interference, owing to the second polarizer crossed with the first one, the phase difference between the two polarized waves being equal to π. This is strictly true only in the absence of any object.

The phase structure of said object causes in the beam small local deviations which have the effect of spreading the pupil image of the slot. Now the transmission of light amplitudes (complex) varies rapidly in a direction perpendicular to the interference fringes in polarized light, i.e. perpendicularly to the edge of the dihedron formed by the faces of said blade or birefringent wedge.

Dans l'objectif, un second prisme de Wollaston unit les deux faisceaux en un seul dans lequel coexistent les deux plans polarisés.



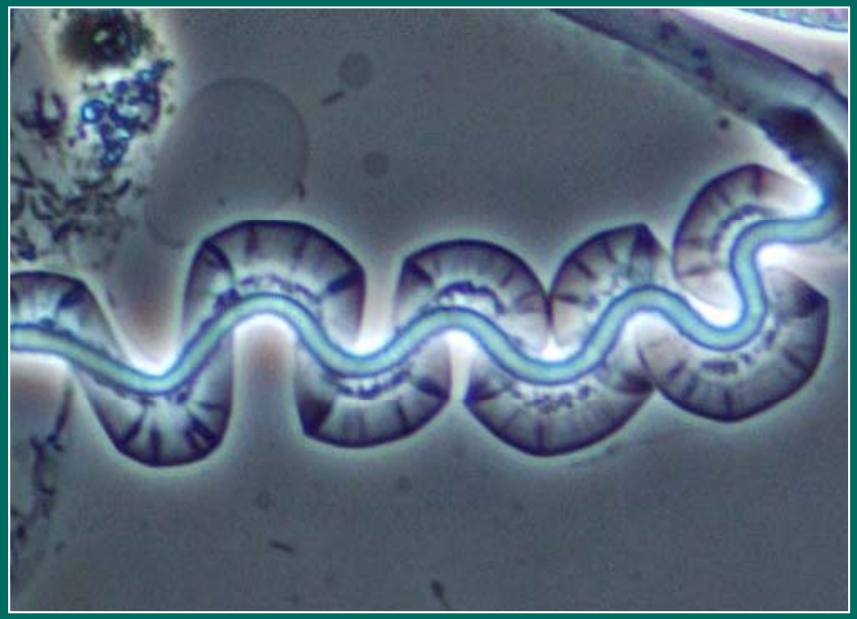


deux rayons lumineux traversent l'échantillon et pénètrent dans l'objectif en véhiculant une image objet avec un déphasage.

le PRISME DE WOLLASTON, divise le faisceau de lumière en deux parties, polarisées sur des plans perpendiculaires.

Les deux images véhiculées par les ondes sont légèrement décalées, l'image finale aura un « ombrage »

Comparaison contraste de phase/contraste interférentiel



Contraste de phase

Contraste interférentiel

Spirulina

1665

Hooke

1673

Leeuwenhoek

1670

Campani

1720

Culpeper

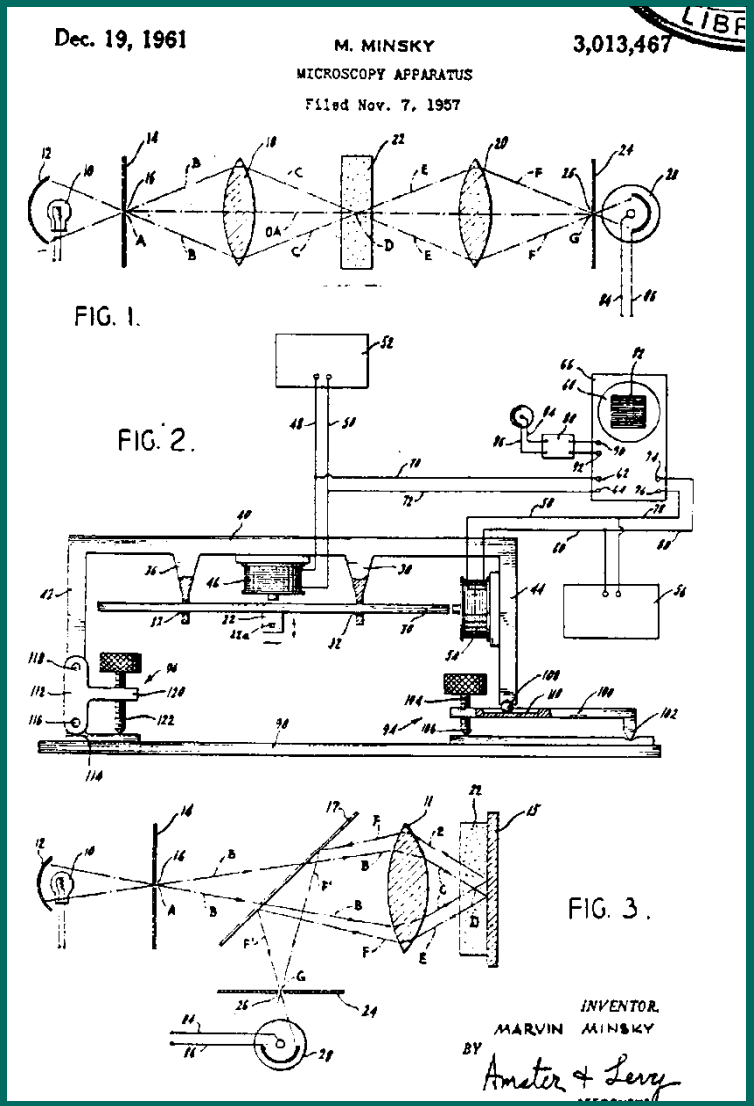
1790

Adams

1813

Amici

Marvin Lee Minsky (1953)
Principe du microscope confocal



Dec. 19, 1961 M. MINSKY 3,013,467

MICROSCOPY APPARATUS

Filed Nov. 7, 1957

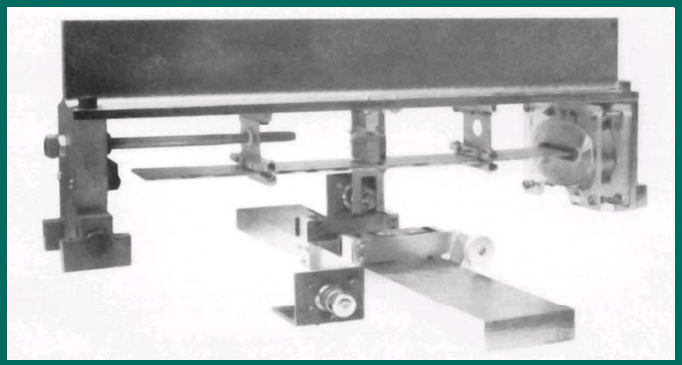
FIG. 1.

FIG. 2.

FIG. 3.

INVENTOR.
MARVIN MINSKY
BY Ameter & Levy

A. The optical system as described has the novel feature that (all light rays originate at a point of the optical axis of the instrument, and only rays which terminate at another point of the optic axis (i.e., at 5a) are accepted by the photosensitive element. Thus only rays of axial origin the optical elements of the system deal only with what may be called "axial cones of light", i.e., families of rays which originate or terminate on a single point of the optical axis. The following are among the very important consequences of this fact



Johan Sebastian Ploem


Johan Sebastiaan Ploem
(1927-Present)

1965

Met au point pour Leitz la microscopie de fluorescence Epi-illumination

Leitz PLOEMOPAK illuminator

W.B. Amos (1986)
Réalisation du premier microscope confocal

MRC Laboratory of Molecular Biology in Cambridge

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biology of the Cell 95 (2003) 335–342
Biology of the Cell
www.elsevier.com/locate/bicell

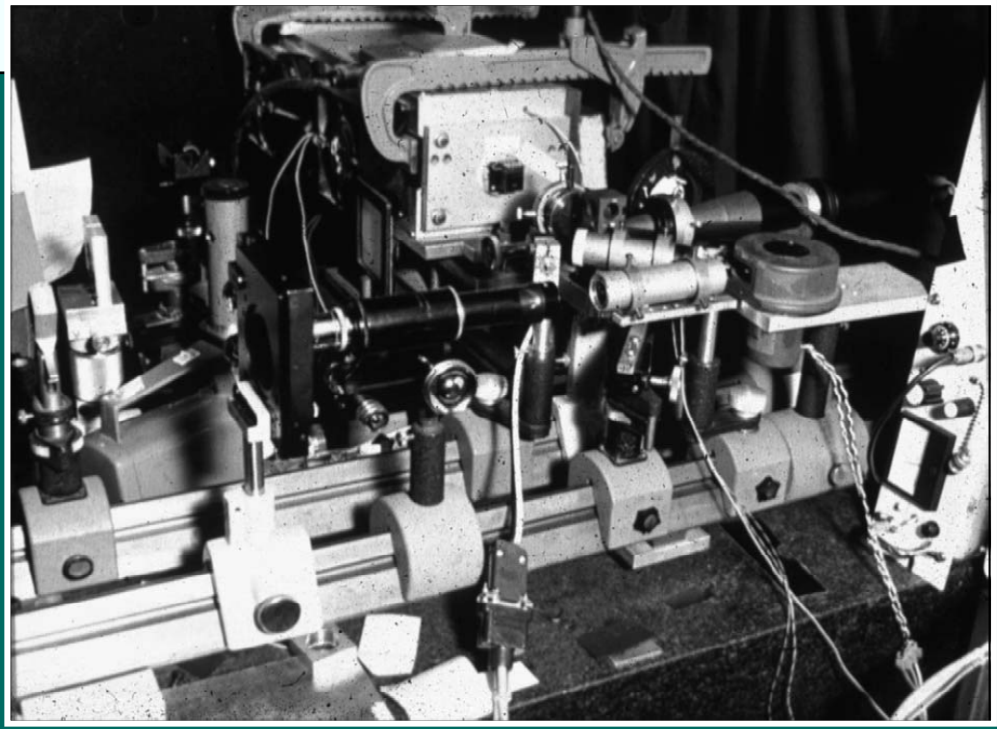
Scientiae Forum / History of biology of the cell

How the Confocal Laser Scanning Microscope entered Biological Research

W. B. Amos [a,*], J.G. White [b]

[a] *MRC Laboratory of Molecular Biology, Cambridge, UK*
[b] *Laboratory of Molecular Biology, University of Wisconsin, Madison, USA*

Received 21 May 2003; accepted 27 May 2003

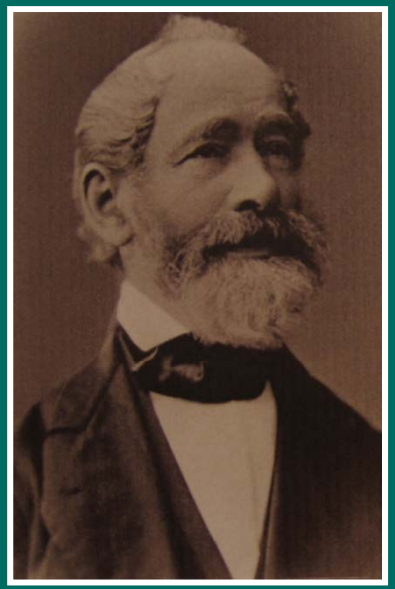


1730

Chester More Hall corrige les aberrations chromatiques dans l' objectif. C'est le premier objectif achromatique.

Mais ce n'est qu'en 1827 que **Giovanni Battista Amici**, construisit le premier microscope composé achromatique.
En 1813, il avait montré que l'immersion de l'objectif dans l'eau permettait d'avoir une meilleure résolution

Carl Zeiss



En 1846 Carl Zeiss ouvre un atelier à Jena en Allemagne pour la fabrication des verres de lunettes et des microscopes pour l'Université.
Avec Ernest Abbe, ils développent l'immersion à huile et construisent des objectifs ayant une ouverture numérique de 1.4

A la même époque Leitz ouvre une manufacture de microscopes.

Otto Schott

Otto Schott, s’associe à Zeiss et Abbe pour fabriquer un alliage spécial de verre pour les lentilles (1884)

En 1886 il crée l’objectif , apochromatique à la suite des travaux d’Abbe.