# Microscope Illumination

Once you have bought the objective lenses, there is little you can be done to *improve* resolution…

…but it can easily be made worse by poor illumination of the specimen

# Microscope Illumination

**What** are we trying to do when illuminating a microscopical specimen?

**Why** is it necessary?

**How** do we do it?

# **What** are we trying to do when illuminating a microscopical specimen??

- Light up the ***specimen*** **uniformly**
  - over an **adjustable** ***area***
- Illuminate the ***objective aperture*** **uniformly**
  - over an **adjustable** ***angle***

# Microscope Illumination

Two basic methods of illumination:

Source-focused (or 'Critical') Illumination:

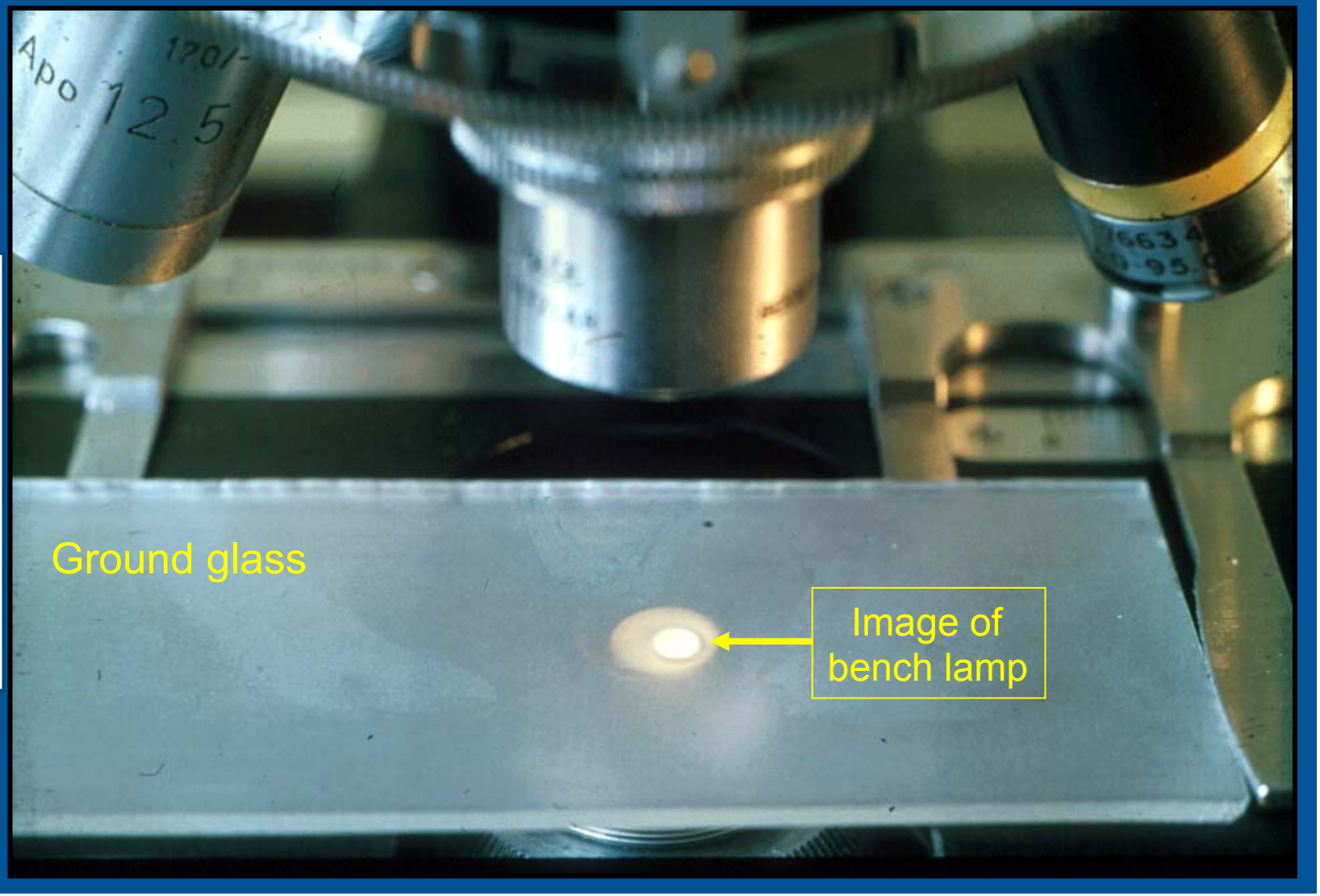
Light-source imaged on to specimen

Köhler Illumination:

Light-source imaged in the aperture of the condenser

# Source-focused Illumination

Bench lamp imaged on ground glass on stage by condenser lens



# Light sources suitable for source-focused illumination:

Uniformly-illuminated sky *

Flame of oil-lamp

Surface of opal light bulb *

Uniformly-illuminated white paper or ground glass*

*note that these are really 'secondary sources'

Condenser lens acts like a camera lens

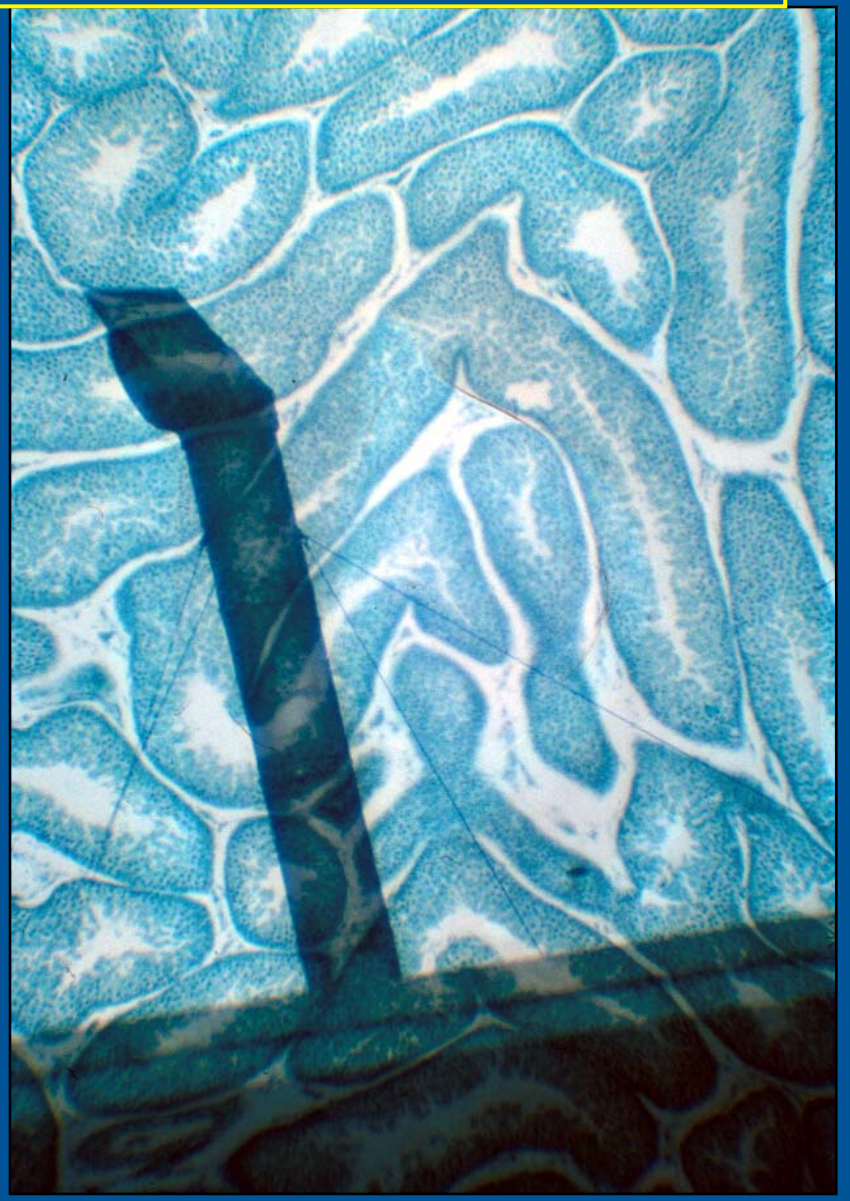
- throws an image of source on to underside of slide

# Source-focused Illumination

But looking for a region of uniformly illuminated sky in Leeds…

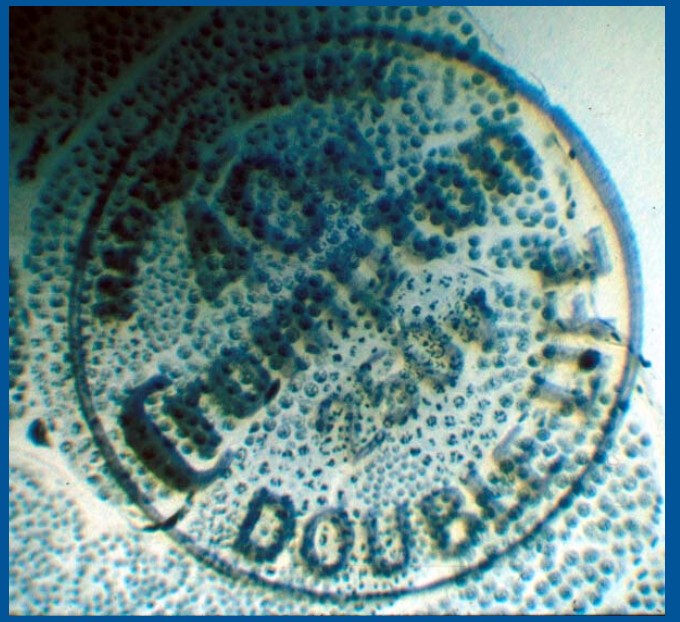
gave an image of the stink-pipe on the Chemistry Building

…when the microscope was set up *correctly*



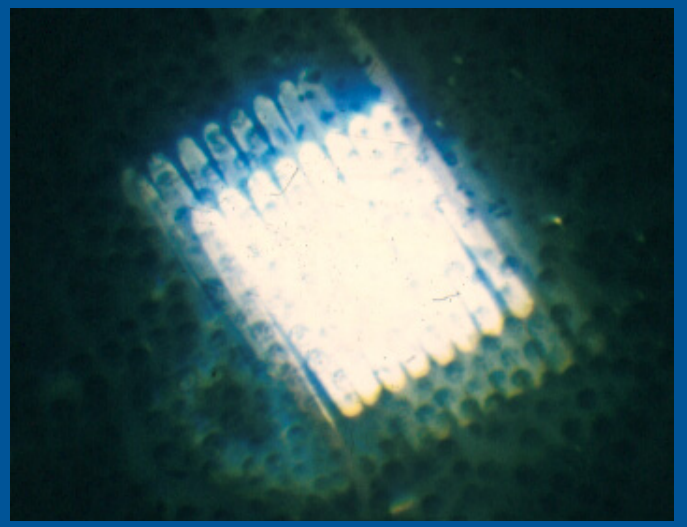
# Source-focused Illumination

Using a normal electric lamp gives an image of the writing on the end of the bulb



Köhler Illumination solves this problem

…and a modern halogen lamp is even worse



# Conjugate planes

An image of the object

forms the primary image

and this is transferred

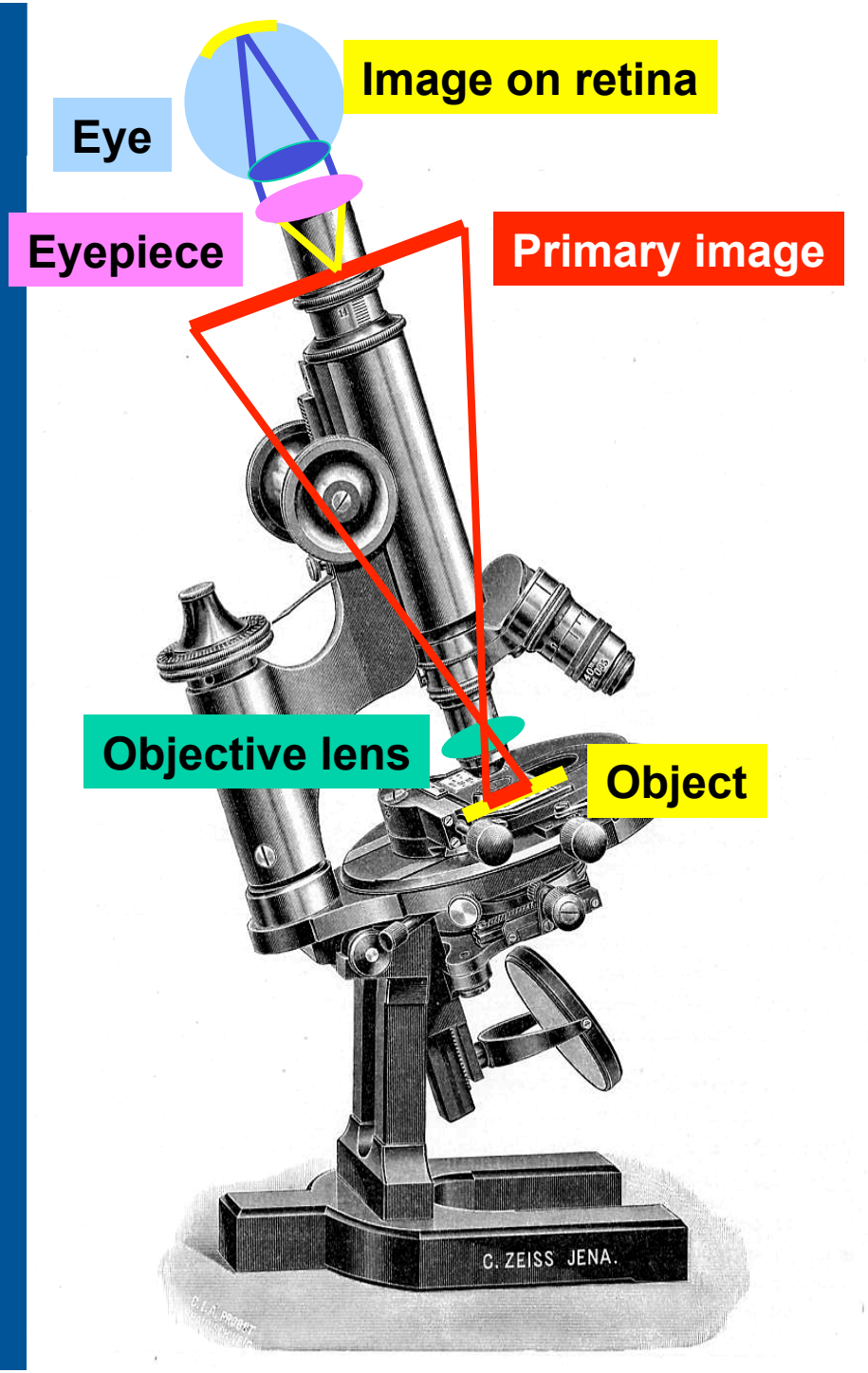
to the retina

These are three

conjugate planes

- successive images of one another

… and there are more.



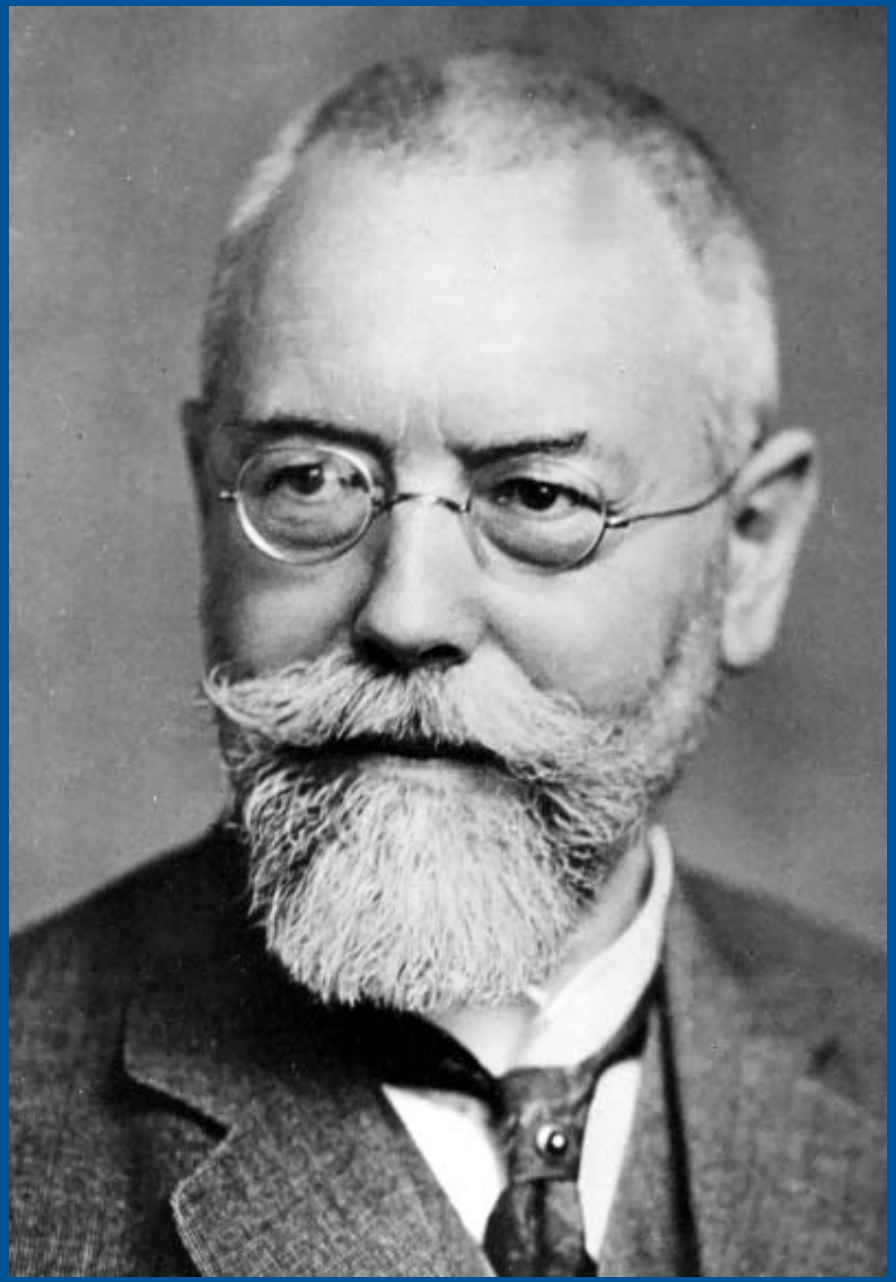
# August Köhler

1866 - 1948

published

*A new system of illumination for photomicrographic purposes*

*(in German) in 1893.*



# The back focal plane of the objective

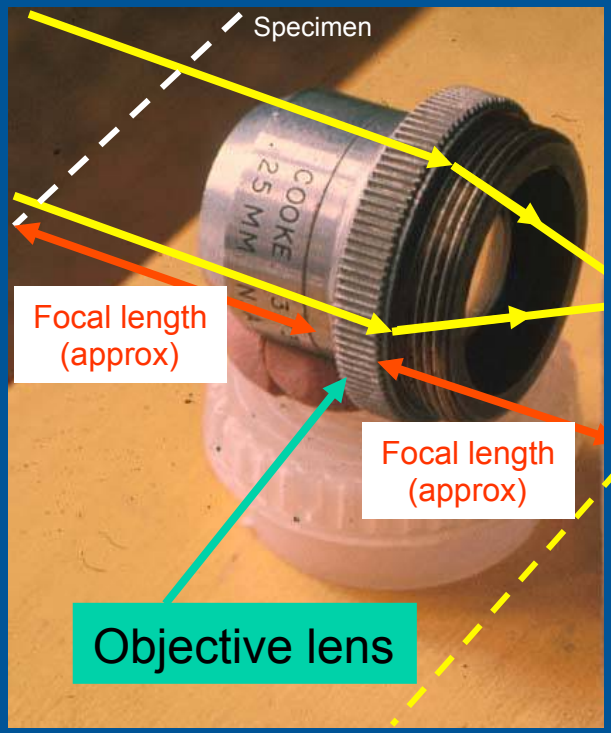
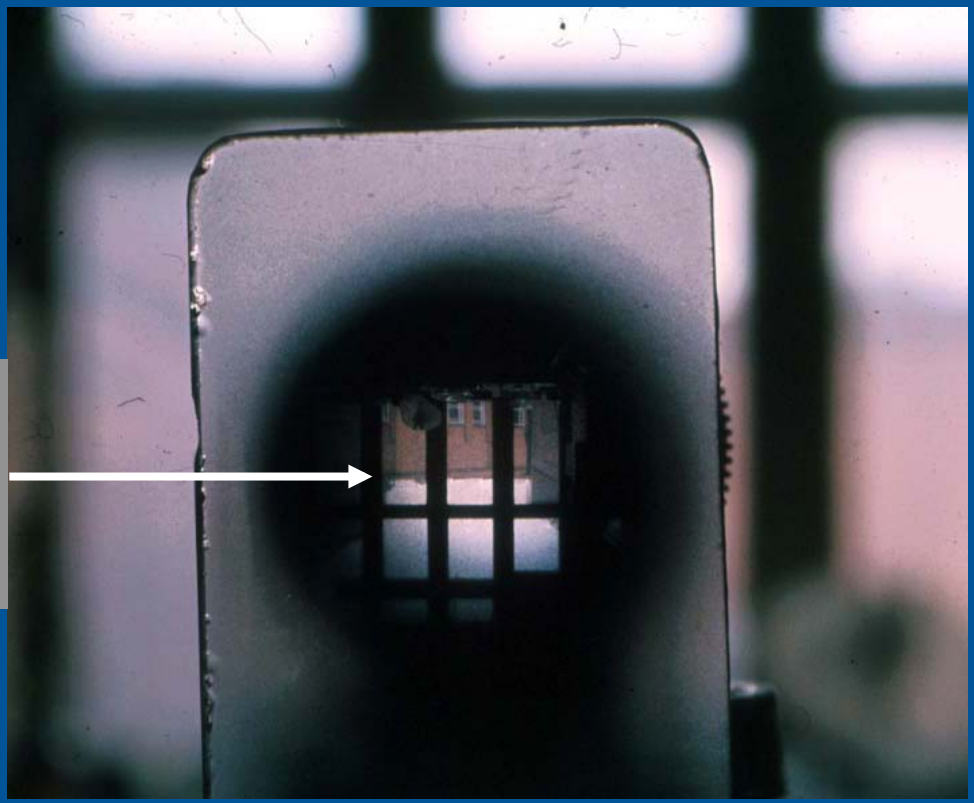



Image of objects at ‘infinity’ in back focal plane of objective



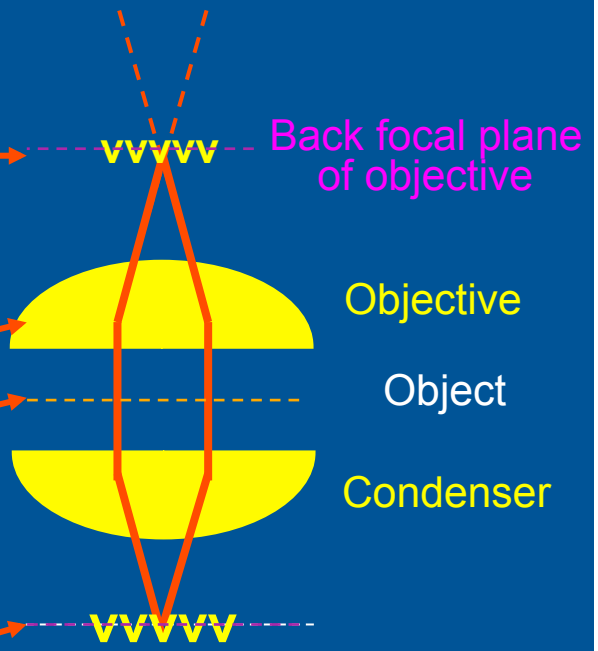
# **How** do we light up the specimen ***uniformly***?

And be brought into focus in the back focal plane of the objective

Into the objective

Light will pass *parallel* through the object

Imagine a light source in the first focal plane of the condenser



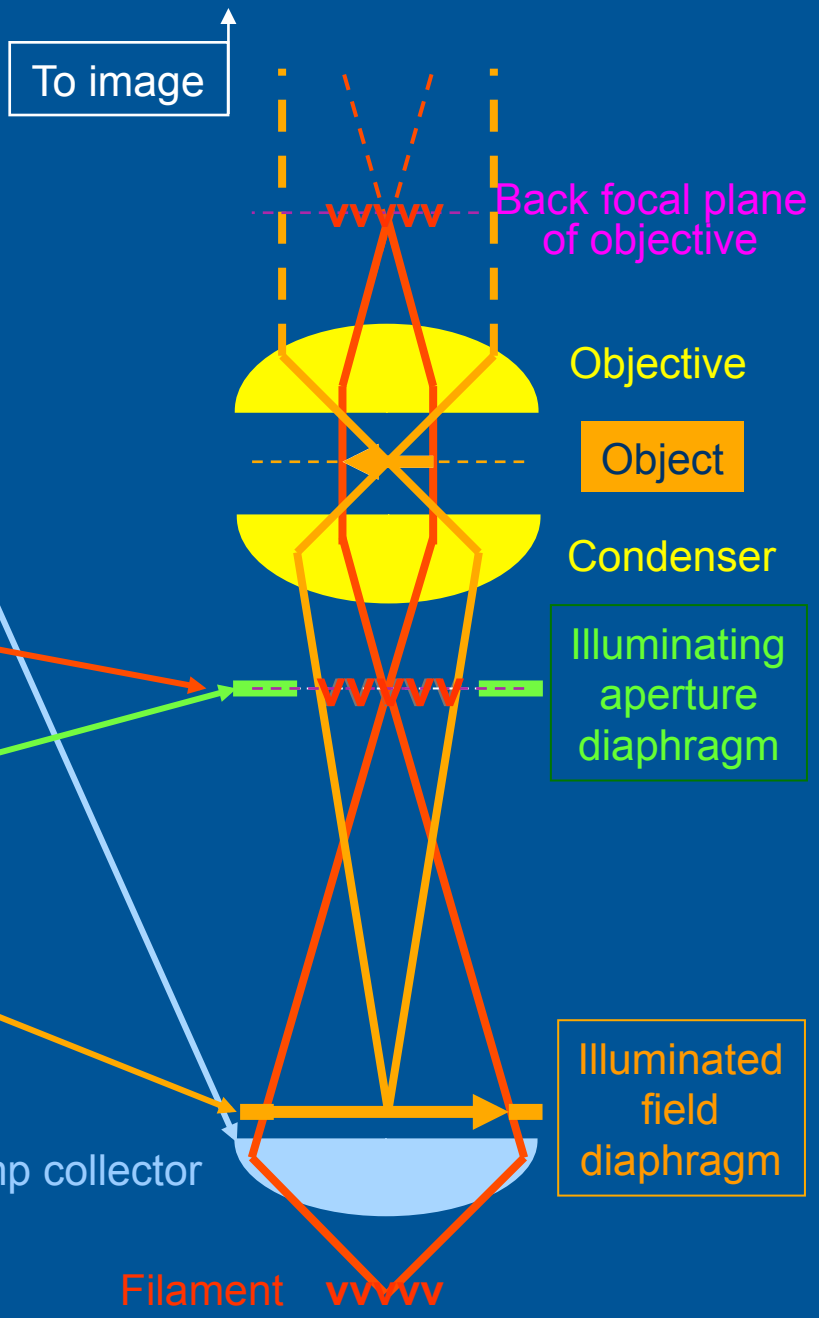
# **How** do we light up the specimen ***uniformly***?

In Köhler Illumination an extra lens, the Lamp collector lens throws an *image* of the filament into the first focal plane of the condenser

This image of the filament falls also on the aperture diaphragm of the condenser, the Illuminating aperture diaphragm

The Illuminated field diaphragm fitted just after the lamp collector is imaged on to the object by the condenser lens

In this situation the lamp collector lens appears to be uniformly filled with light



# **Why** is it necessary to…

Light up the specimen uniformly over an ***adjustable area***?

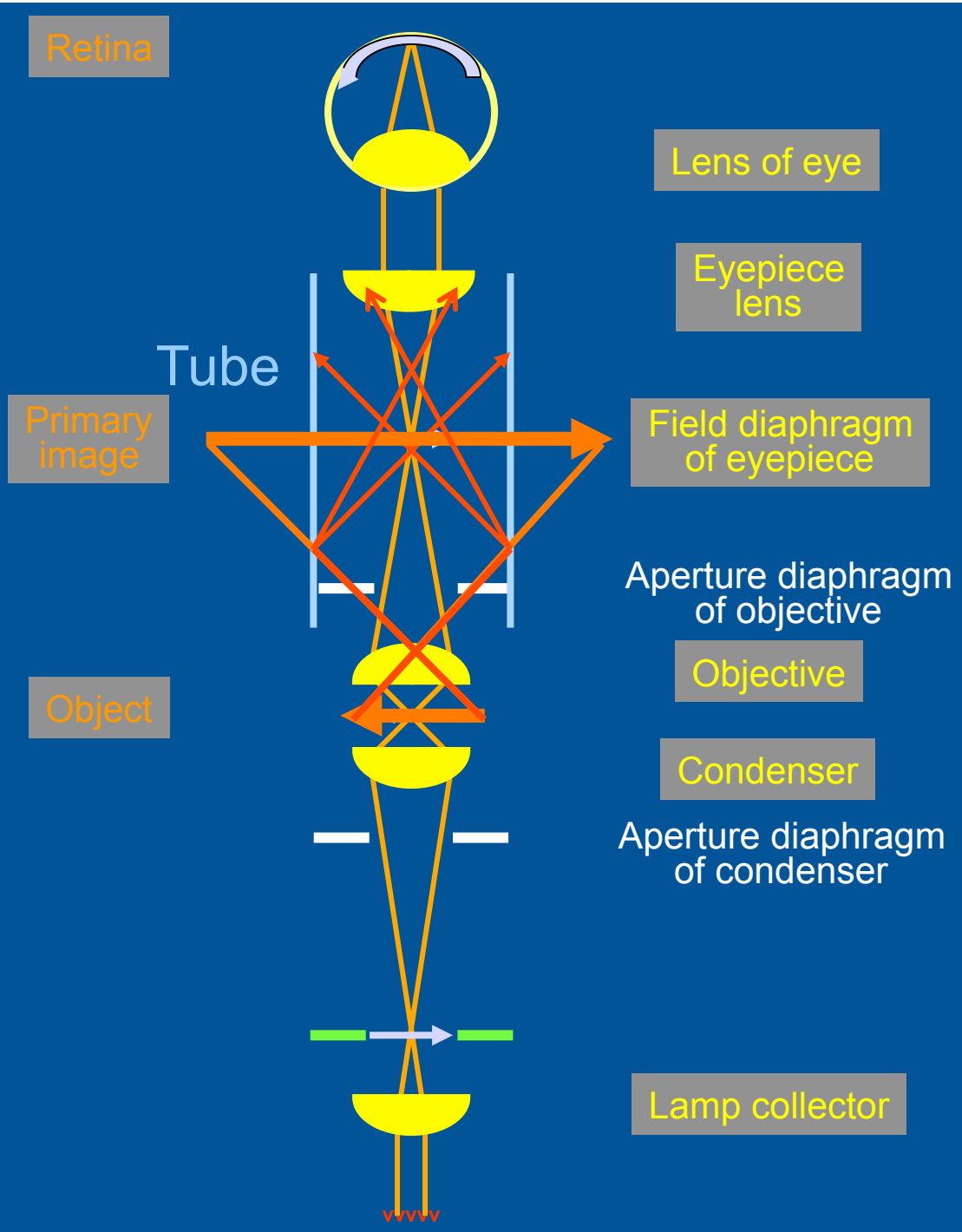
---

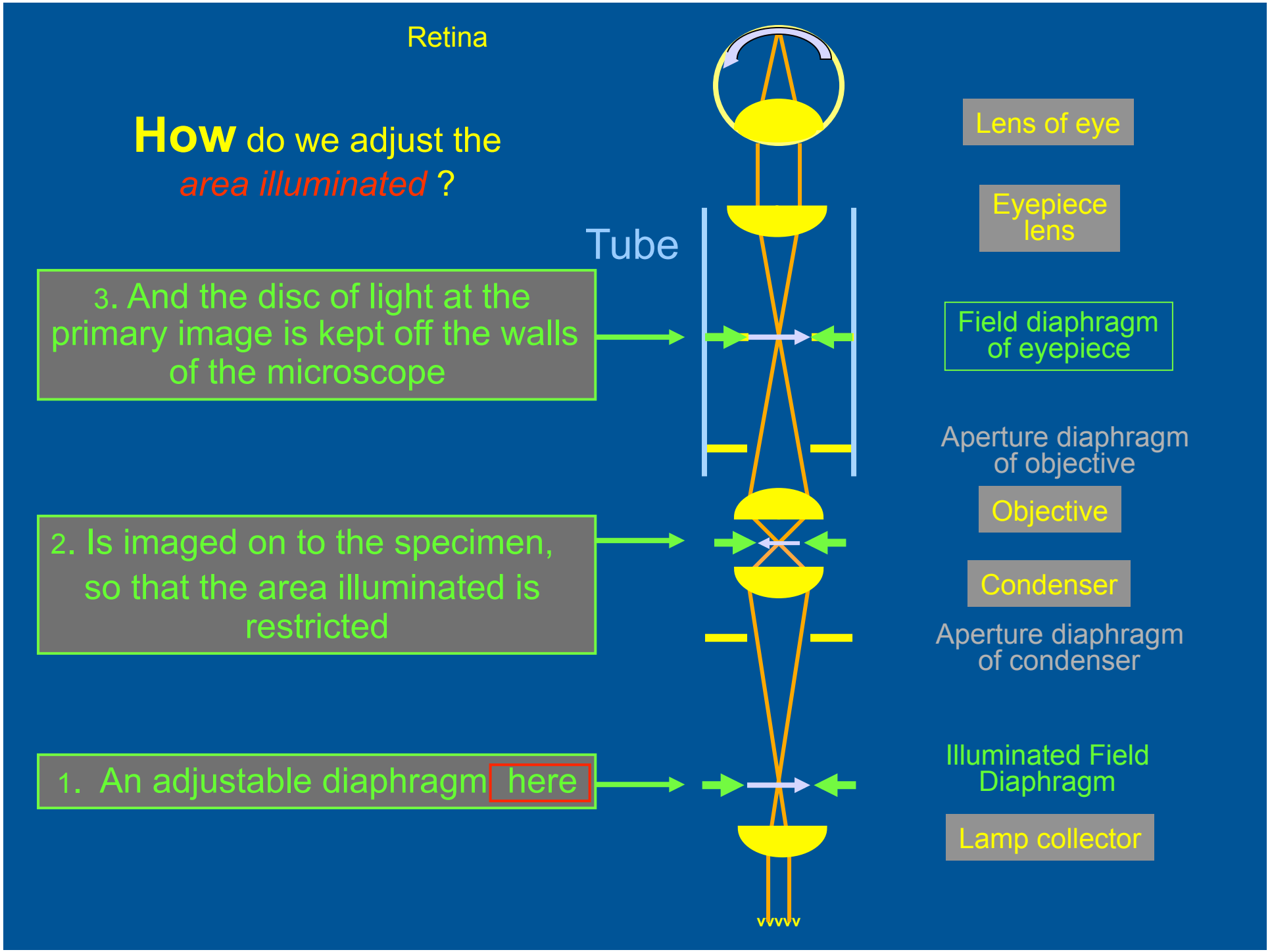
It is unnecessary, and often detrimental, to illuminate parts of the specimen outside the field of view

- some specimens are light-sensitive, and could be damaged
- light can be scattered into field of view from outside this area
- illuminating a large area of specimen produces a large primary image, and light can reflect from internal walls of microscope, reducing contrast in the image

**Why** adjust the *area illuminated* ?

Large area of object illuminated provides large disc of light at primary image causing reflections from walls of microscope and reduction in contrast




Retina
How do we adjust the area illuminated ?
Tube
3. And the disc of light at the primary image is kept off the walls of the microscope
2. Is imaged on to the specimen, so that the area illuminated is restricted
1. An adjustable diaphragm here
Lens of eye
Eyepiece lens
Field diaphragm of eyepiece
Aperture diaphragm of objective
Objective
Condenser
Aperture diaphragm of condenser
Illuminated Field Diaphragm
Lamp collector
vvvvv

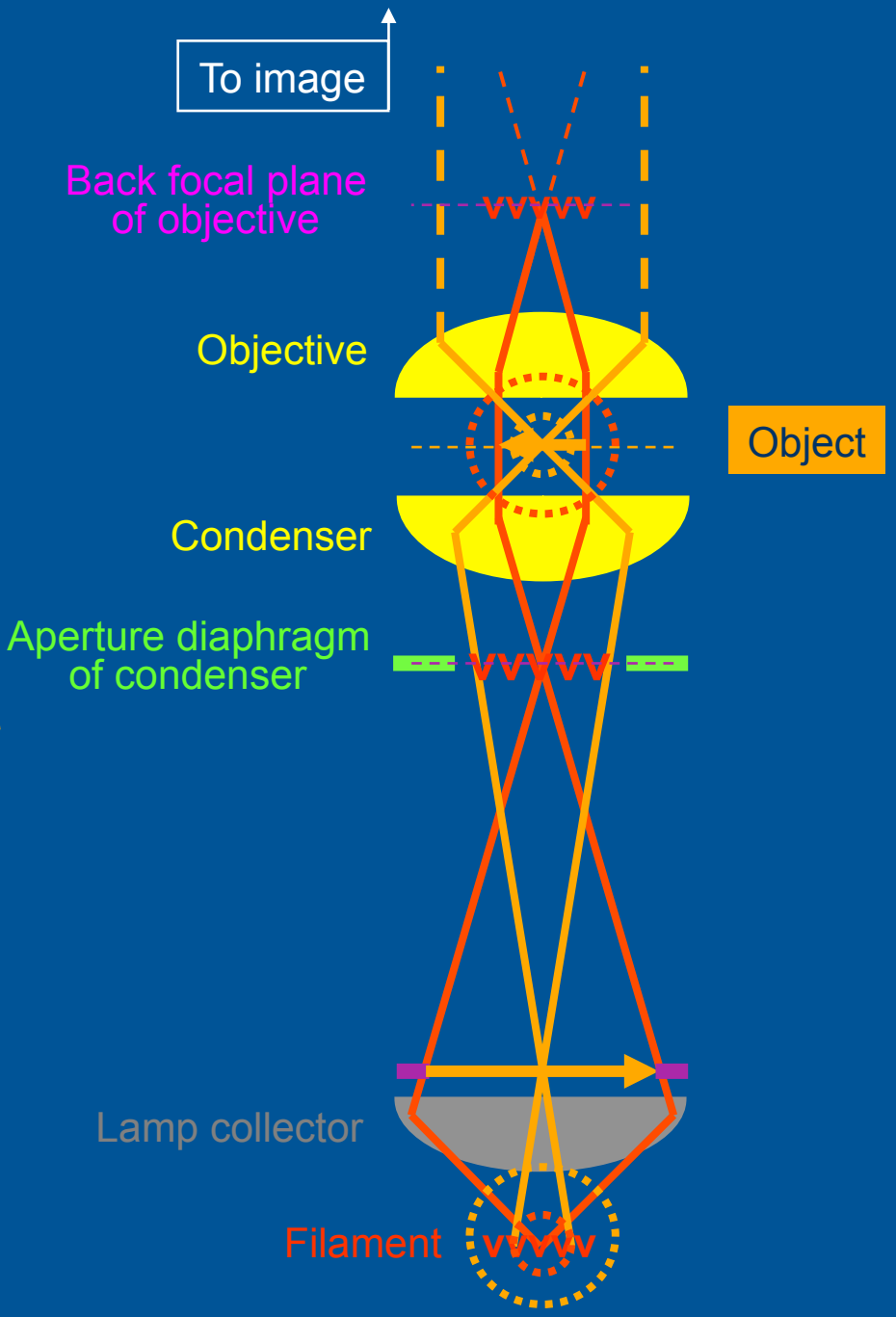
**How** do we light up the specimen ***uniformly***?

Note that

- ***Each*** point in the **object** receives light from ***many*** points on the **filament**

and that

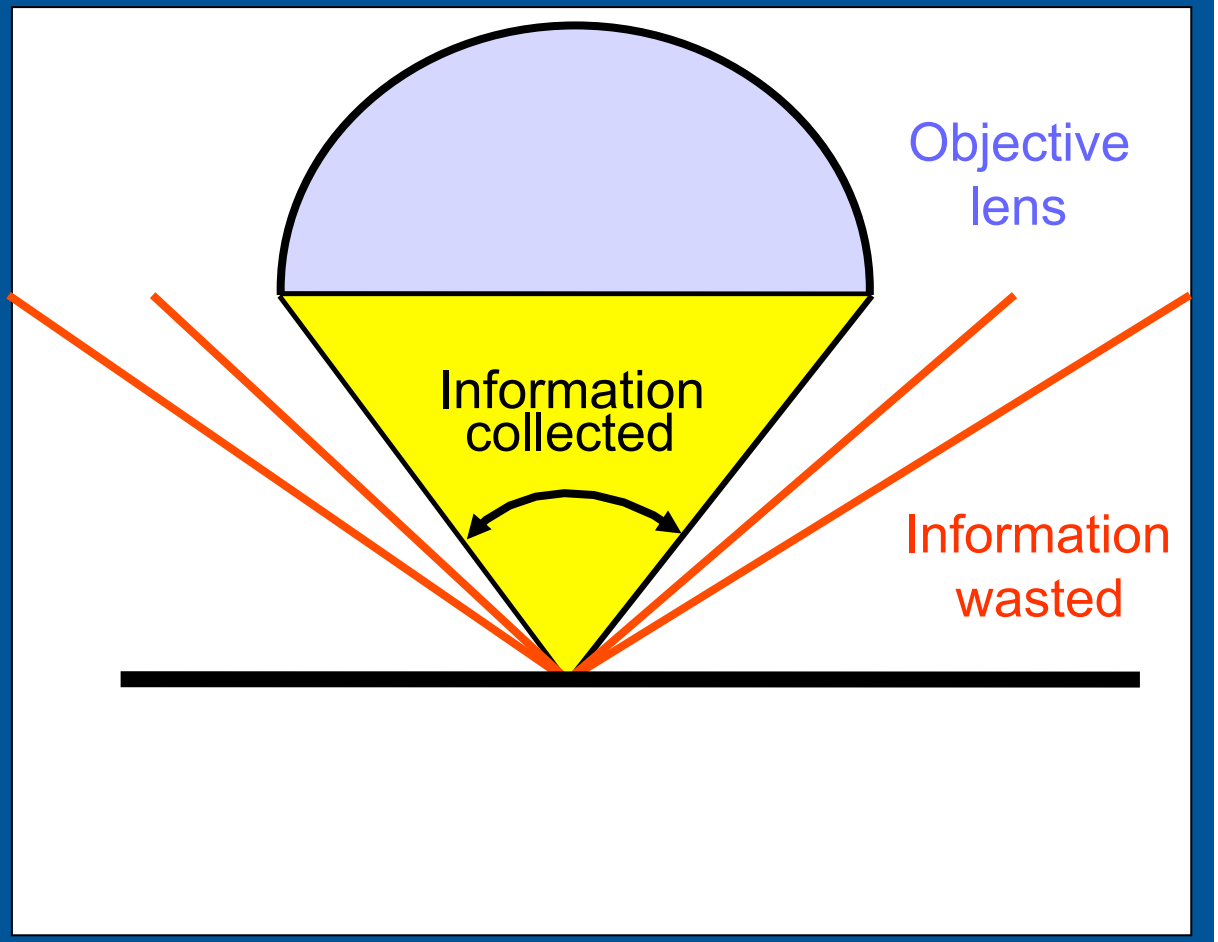
- ***Each*** point of the **filament** provides light to ***many*** points on the object



# **Why** is it necessary to…

Illuminate the *objective aperture uniformly* over a *controllable angle*?

Resolution depends on the angular aperture of the objective. The larger the imaging aperture the higher the resolution



# **Why** is it necessary to…

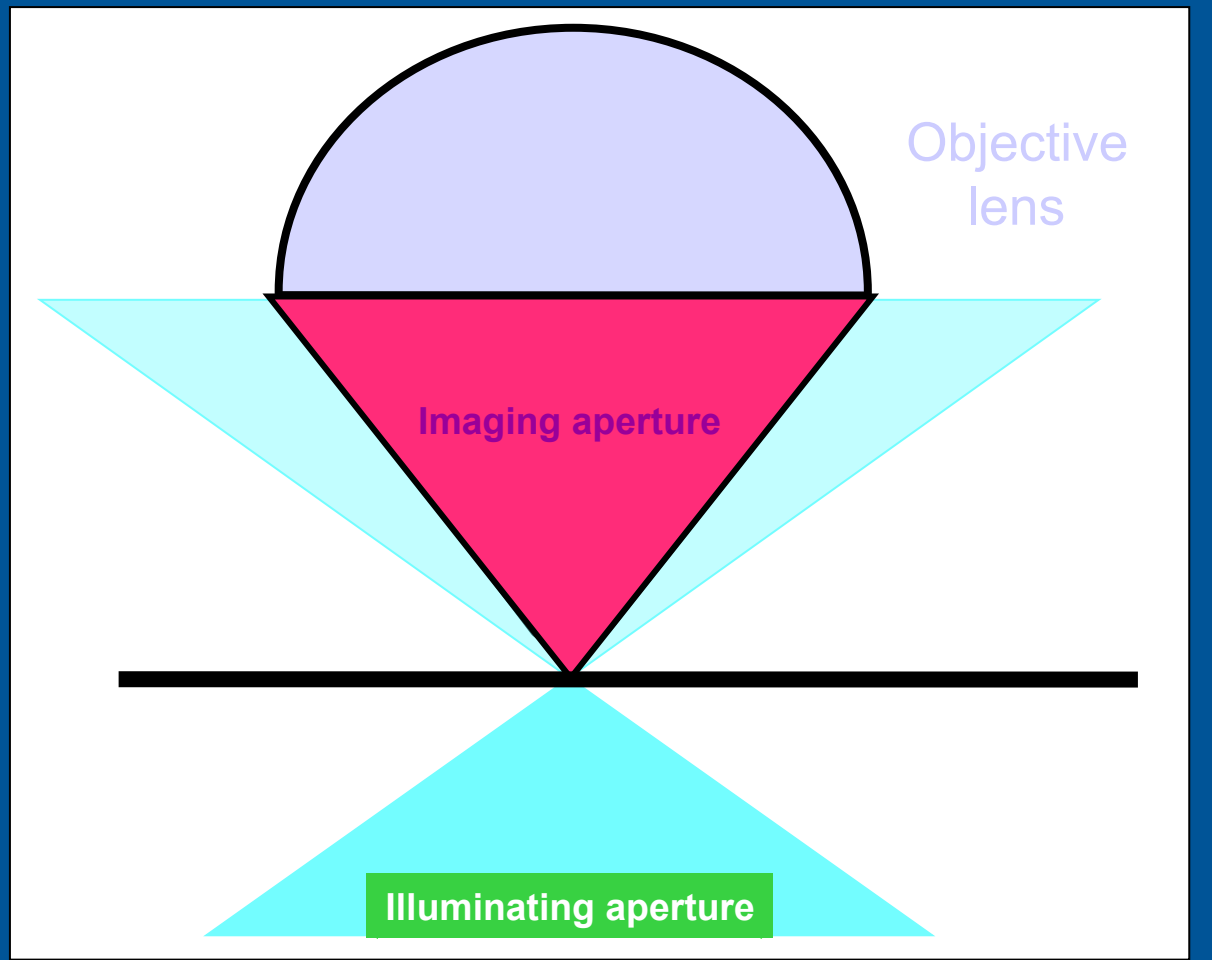
Illuminate the *objective aperture uniformly* over a *controllable angle*?

'Common sense' suggests that if we expect to receive light over a large angle, it is important for good resolution that *most* of the objective aperture should be illuminated

But why just *most* of the aperture ?

Why not ***all*** *of the aperture*?

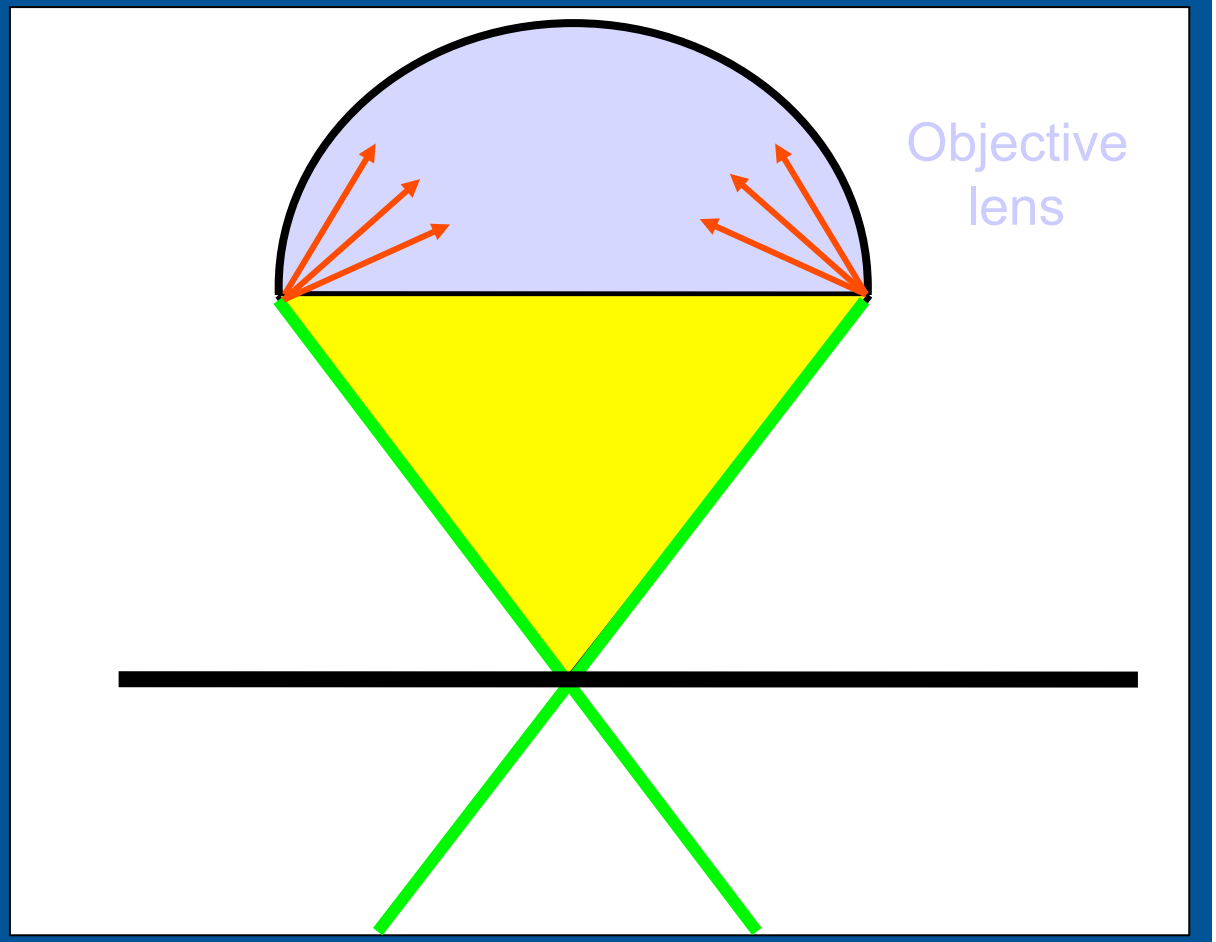
or even a ***very wide*** cone of light ?



# **Why** is it necessary to…

Illuminate the *objective aperture uniformly* over a *controllable angle*?

If the illuminating aperture is too ***large***, light will be scattered from the edges of the objective lens, thus reducing contrast.



# **Why** is it necessary to…

Illuminate the *objective aperture uniformly* over a *controllable angle*?

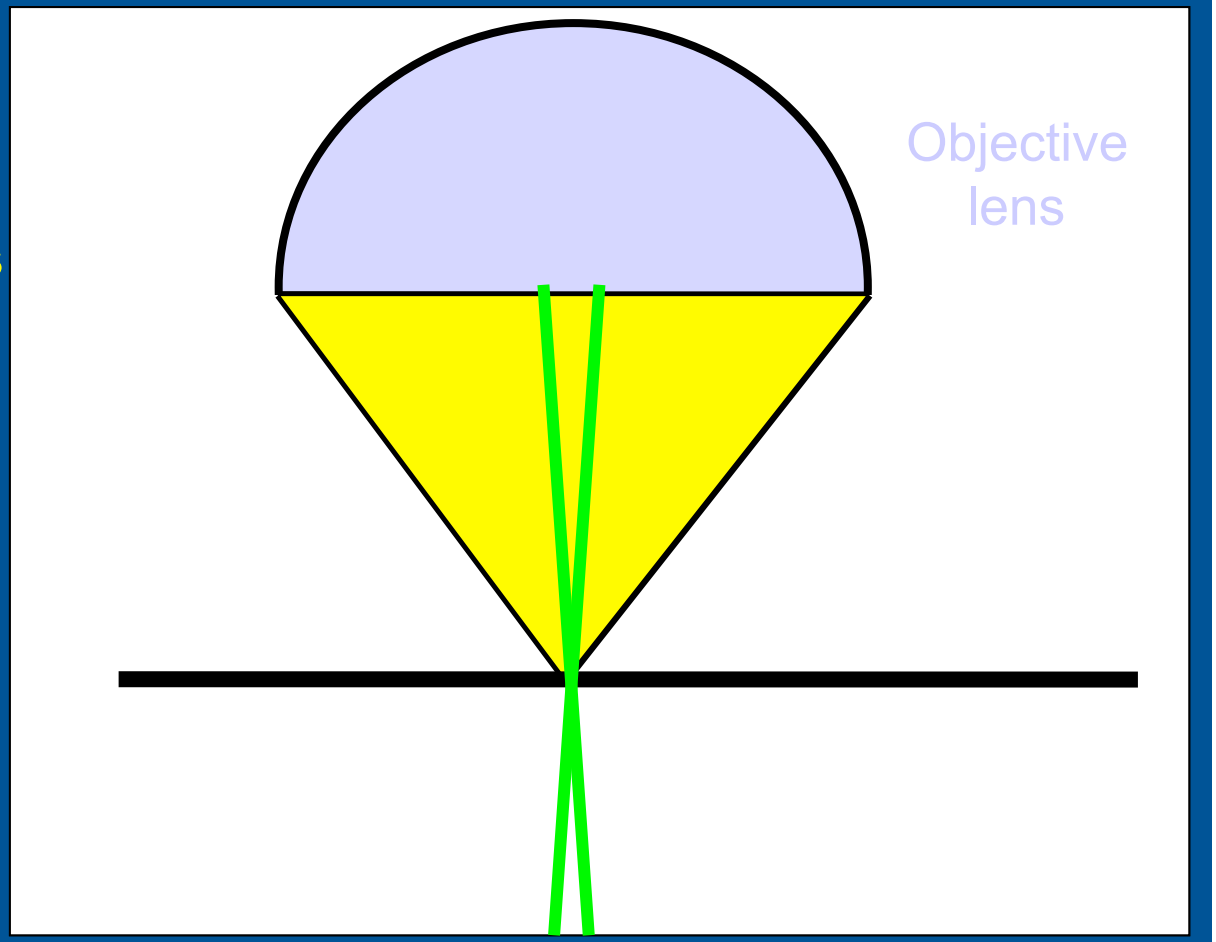
**Worse**

If the illuminating aperture is too ***small***,

resolution will be reduced and

image quality will be impaired

though contrast will be increased.

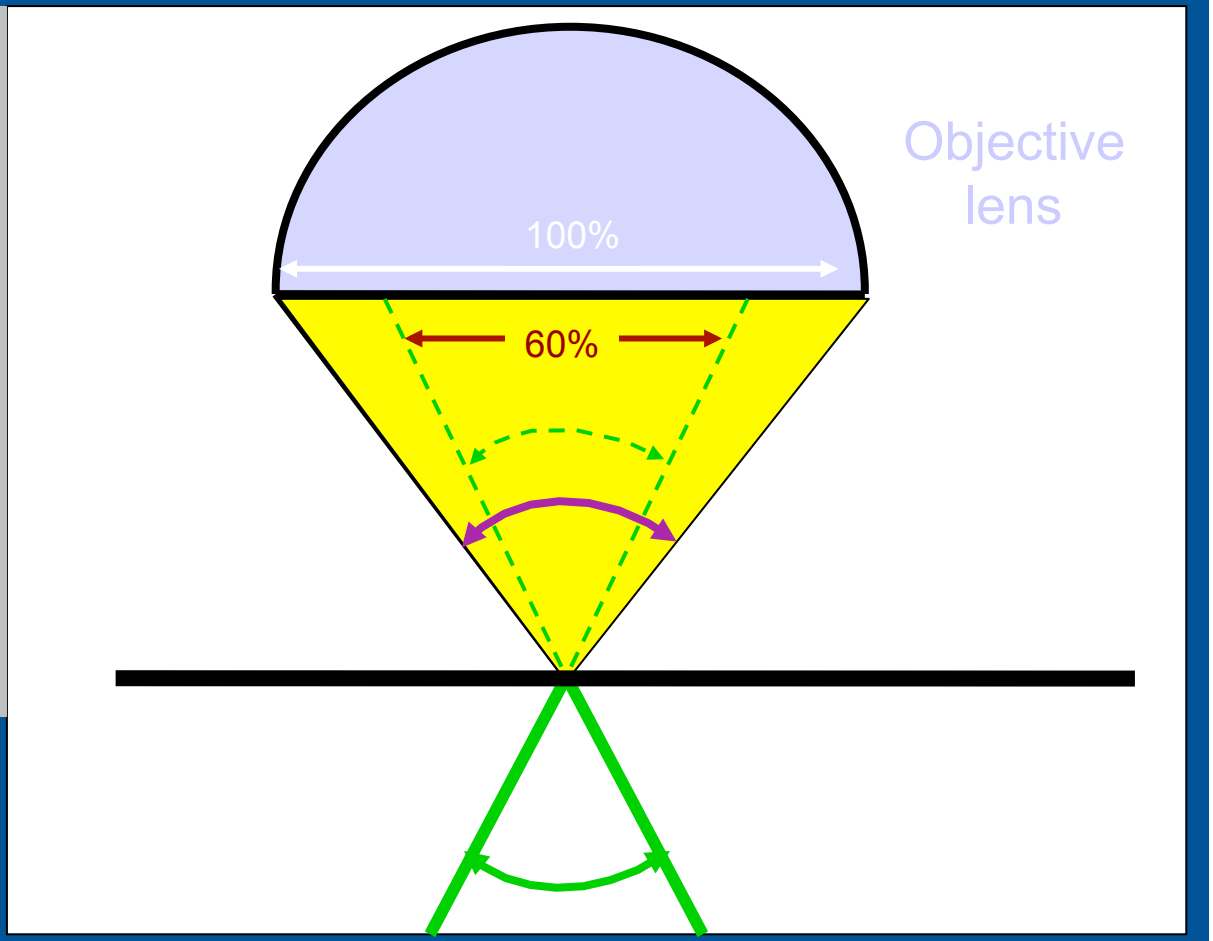


# **Why** is it necessary to…

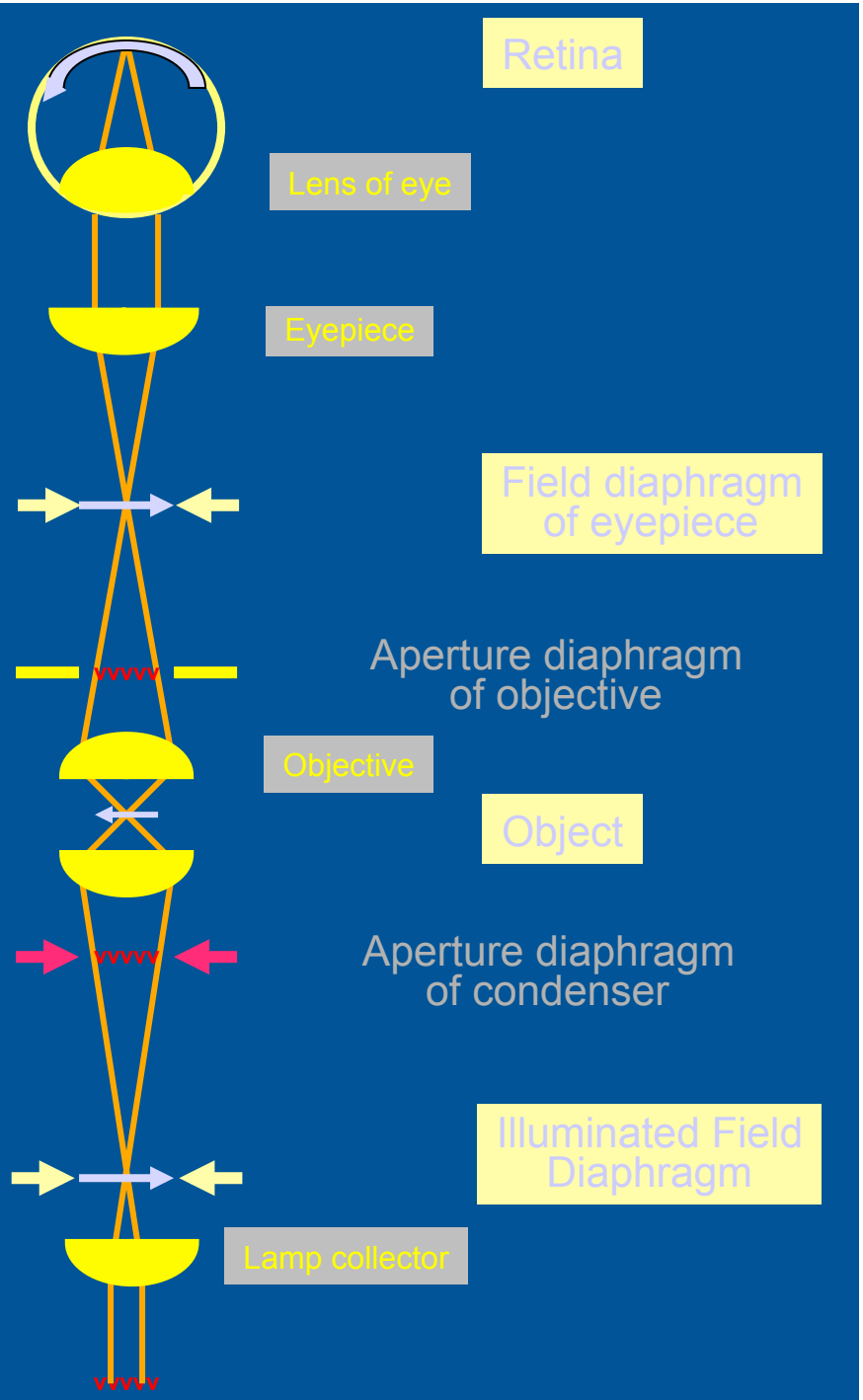
Illuminate the *objective aperture uniformly* over a *controllable angle*?

So for best resolution the illuminating aperture should approach the imaging (objective) aperture.

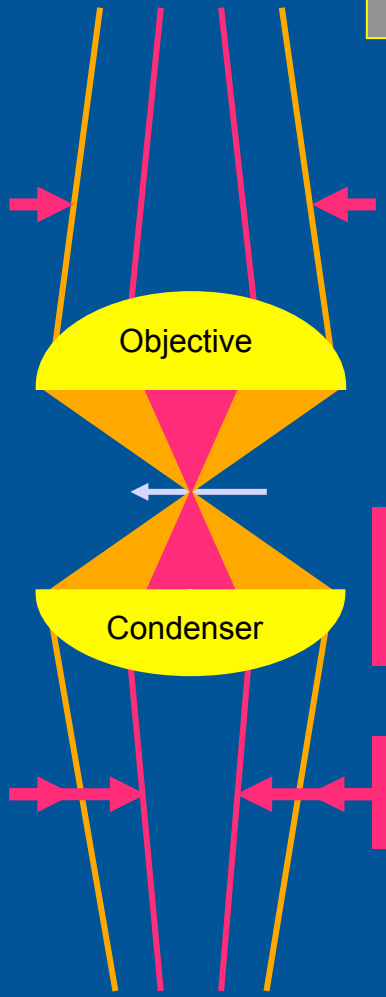
**60 to 75% is often recommended**



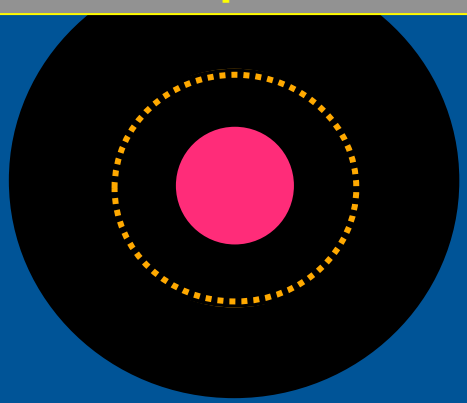
# **How** do we adjust the *angle of illumination* ?



# **How** do we adjust the *angle of illumination* ?

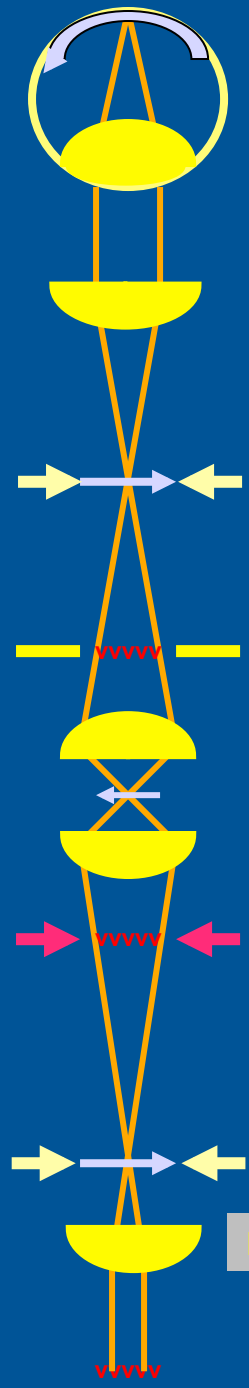



**View of bfp down tube**



2. Narrows the angle of rays passing though the object

1. Closing the condenser diaphragm



Retina

Lens of eye

Eyepiece

Field diaphragm of eyepiece

Aperture diaphragm of objective

Objective

Object

Aperture diaphragm of condenser

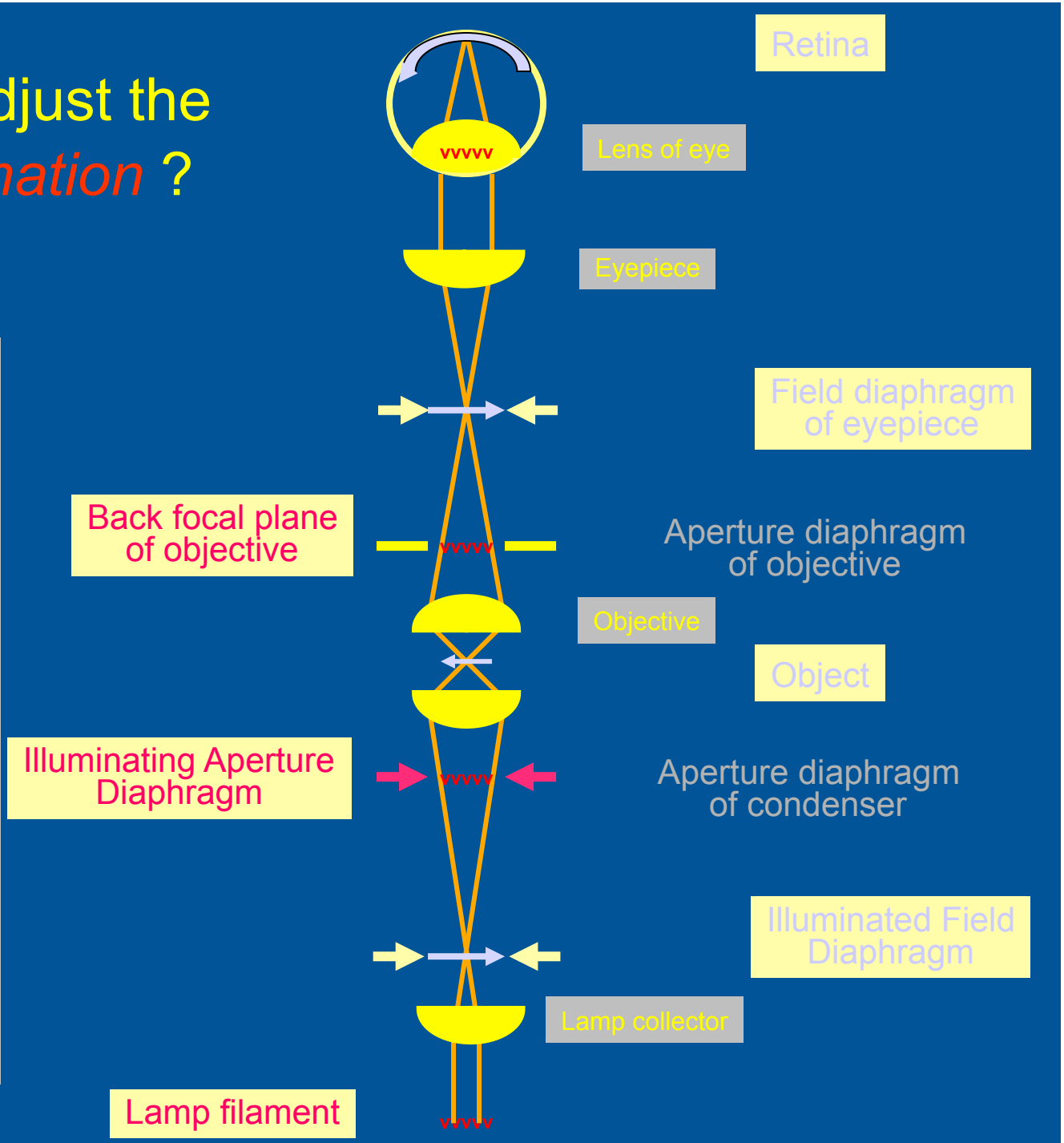
Illuminated Field Diaphragm

Lamp collector

# **How** do we adjust the *angle of illumination* ?

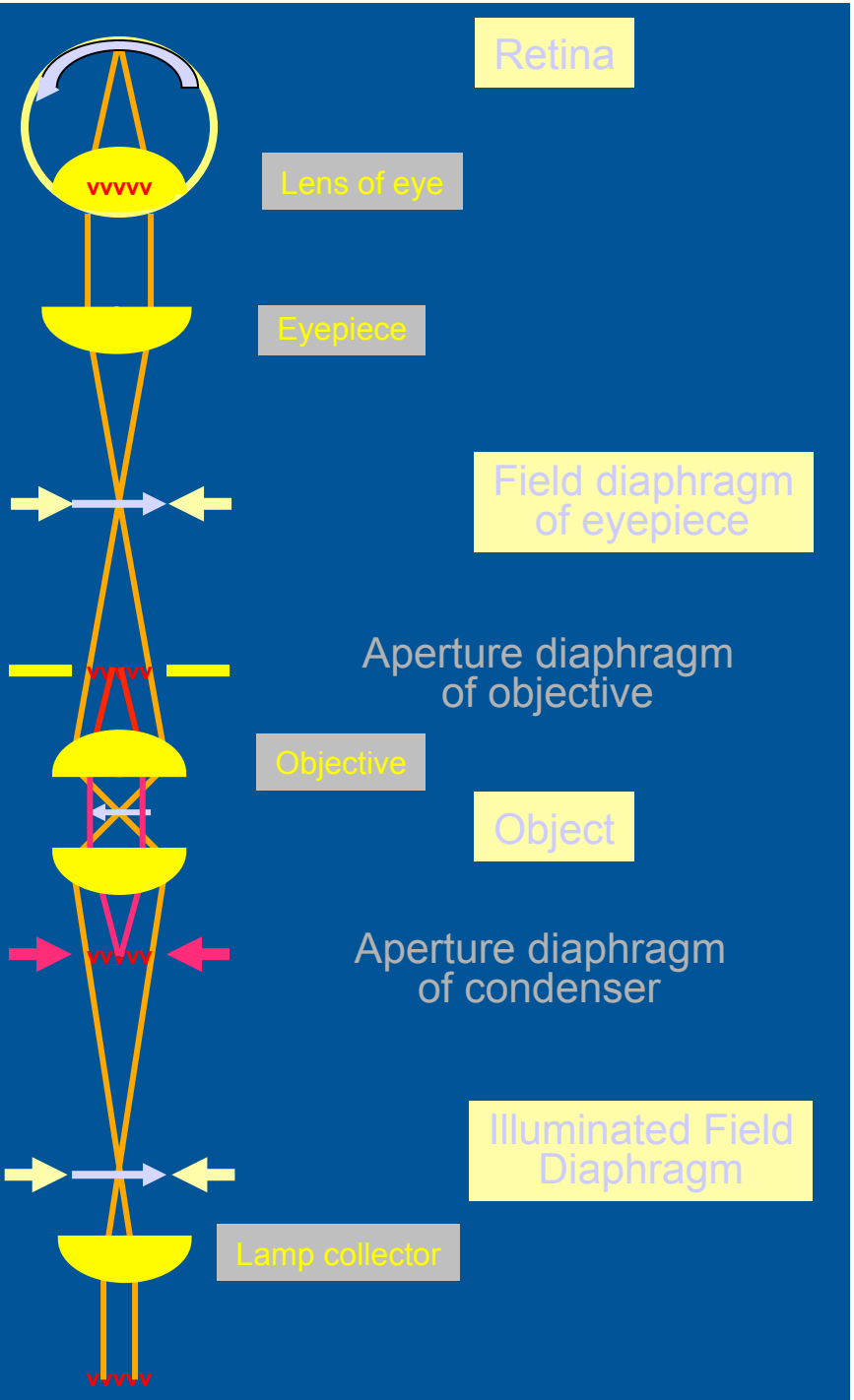
The aperture diaphragm of the condenser thus acts as the *Illuminating Aperture Diaphragm*

– so called because it is the diaphragm which regulates the Illuminating Aperture



# **How** do we adjust the *angle of illumination* ?

An image of the filament is formed in the Back Focal Plane of the Objective

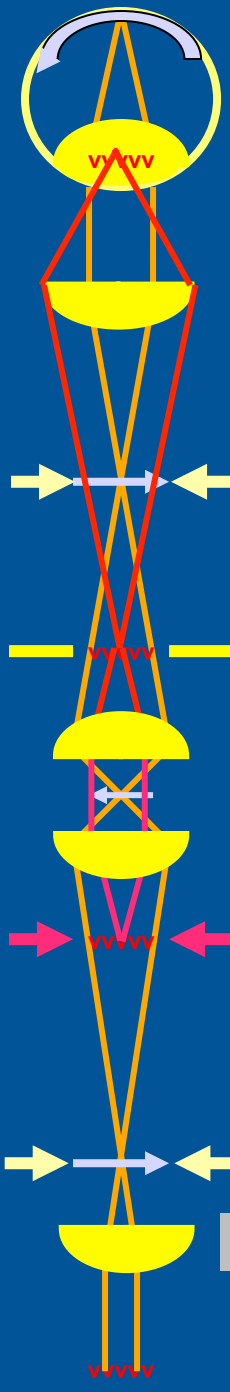


# **How** do we adjust the *angle of illumination* ?

A further image of the filament is formed just above the eyepiece

-the *exit pupil* of the eyepiece.

This should fall on the lens of the eye



Exit pupil

Retina

Lens of eye

Eyepiece

Field diaphragm of eyepiece

Back focal plane of objective

Aperture diaphragm of objective

Objective

Object

Illuminating Aperture Diaphragm

Aperture diaphragm of condenser

Illuminated Field Diaphragm

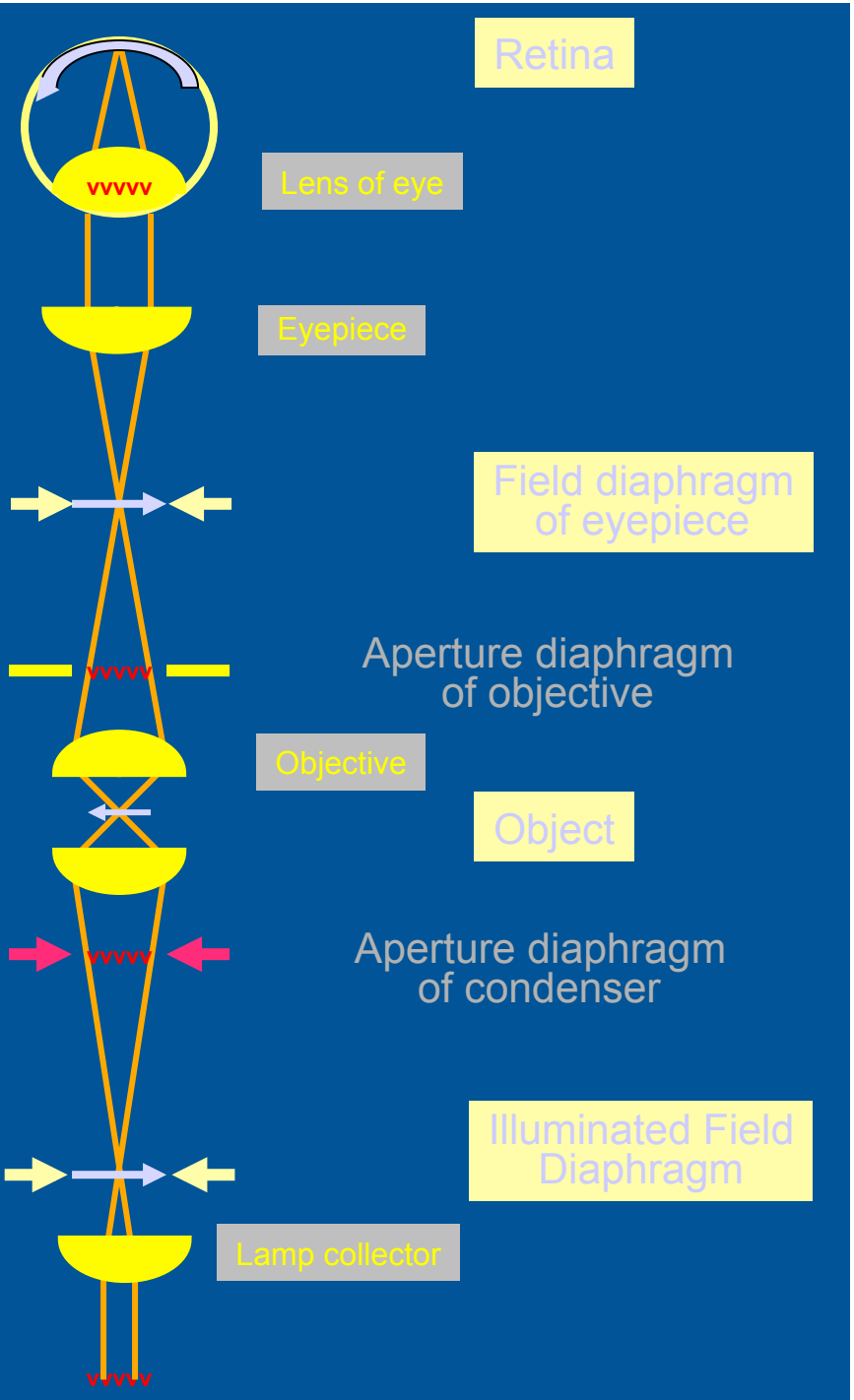
Lamp collector

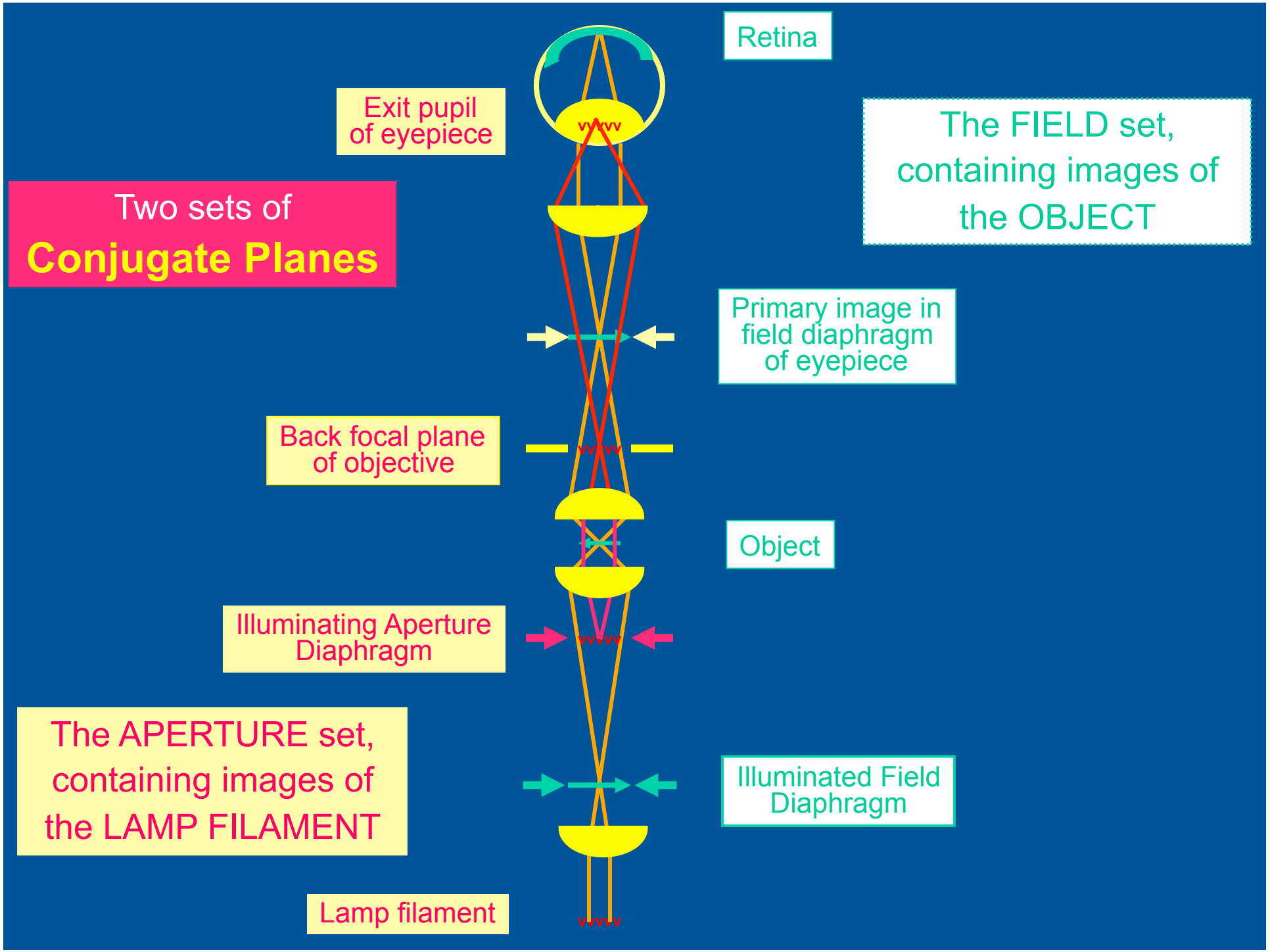
Lamp filament

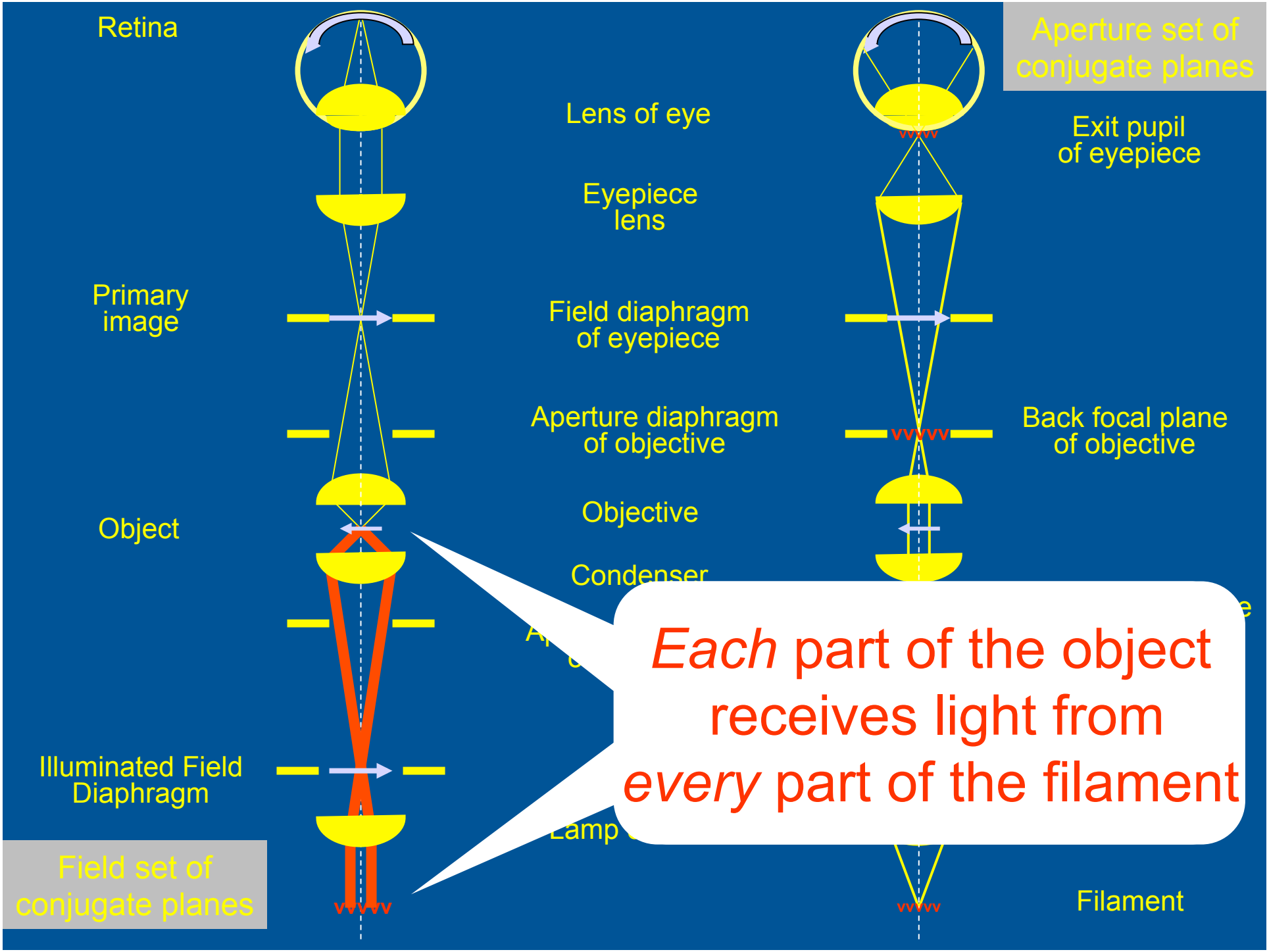
**How** do we adjust the *angle of illumination* ?

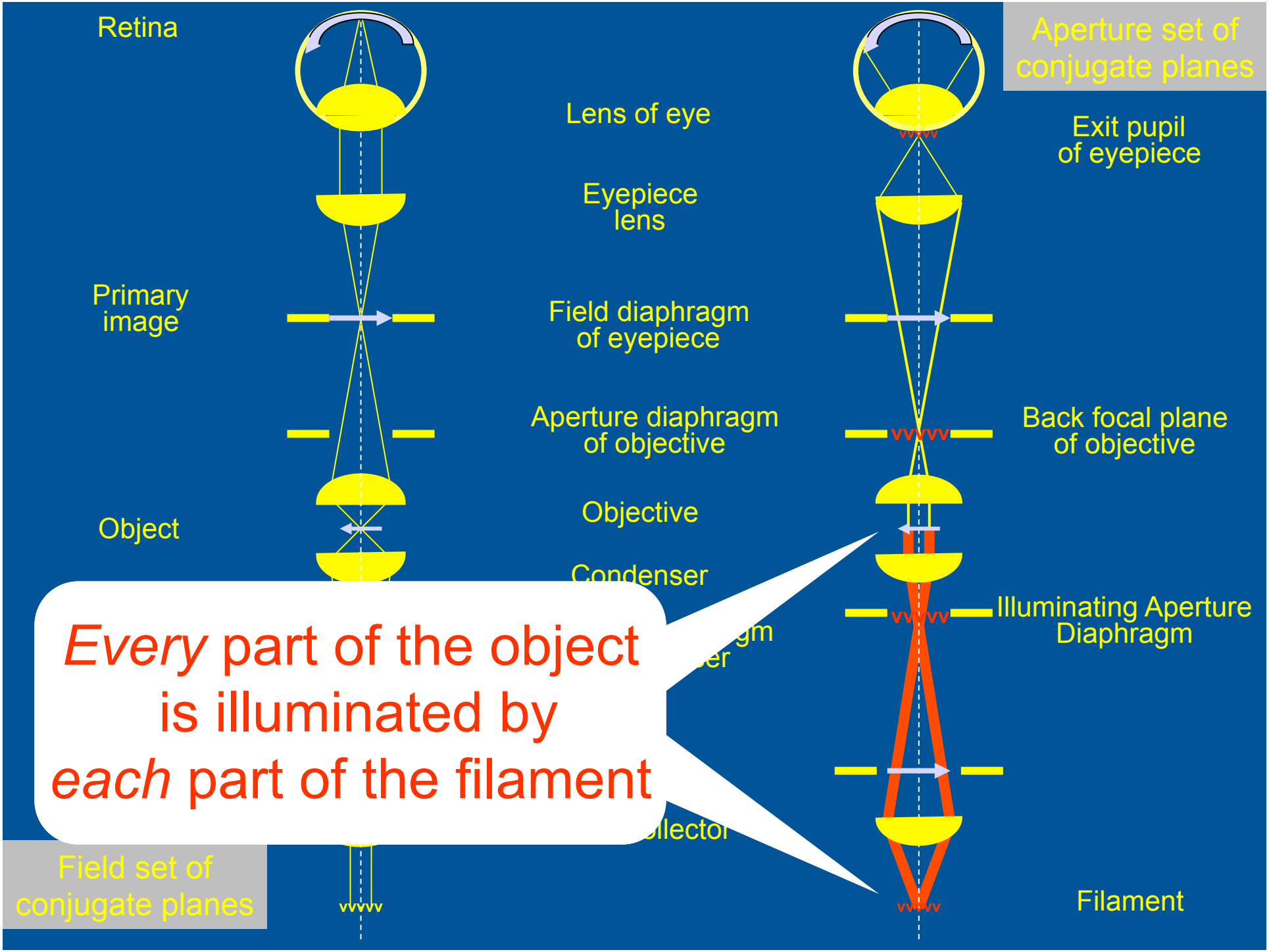
The aperture diaphragm of the condenser thus acts as the *Illuminating Aperture Diaphragm*

*– so called because it is the diaphragm which regulates the Illuminating Aperture*




Retina
Exit pupil of eyepiece
The FIELD set, containing images of the OBJECT
Two sets of
Conjugate Planes
Primary image in field diaphragm of eyepiece
Back focal plane of objective
Object
Illuminating Aperture Diaphragm
The APERTURE set, containing images of the LAMP FILAMENT
Illuminated Field Diaphragm
Lamp filament

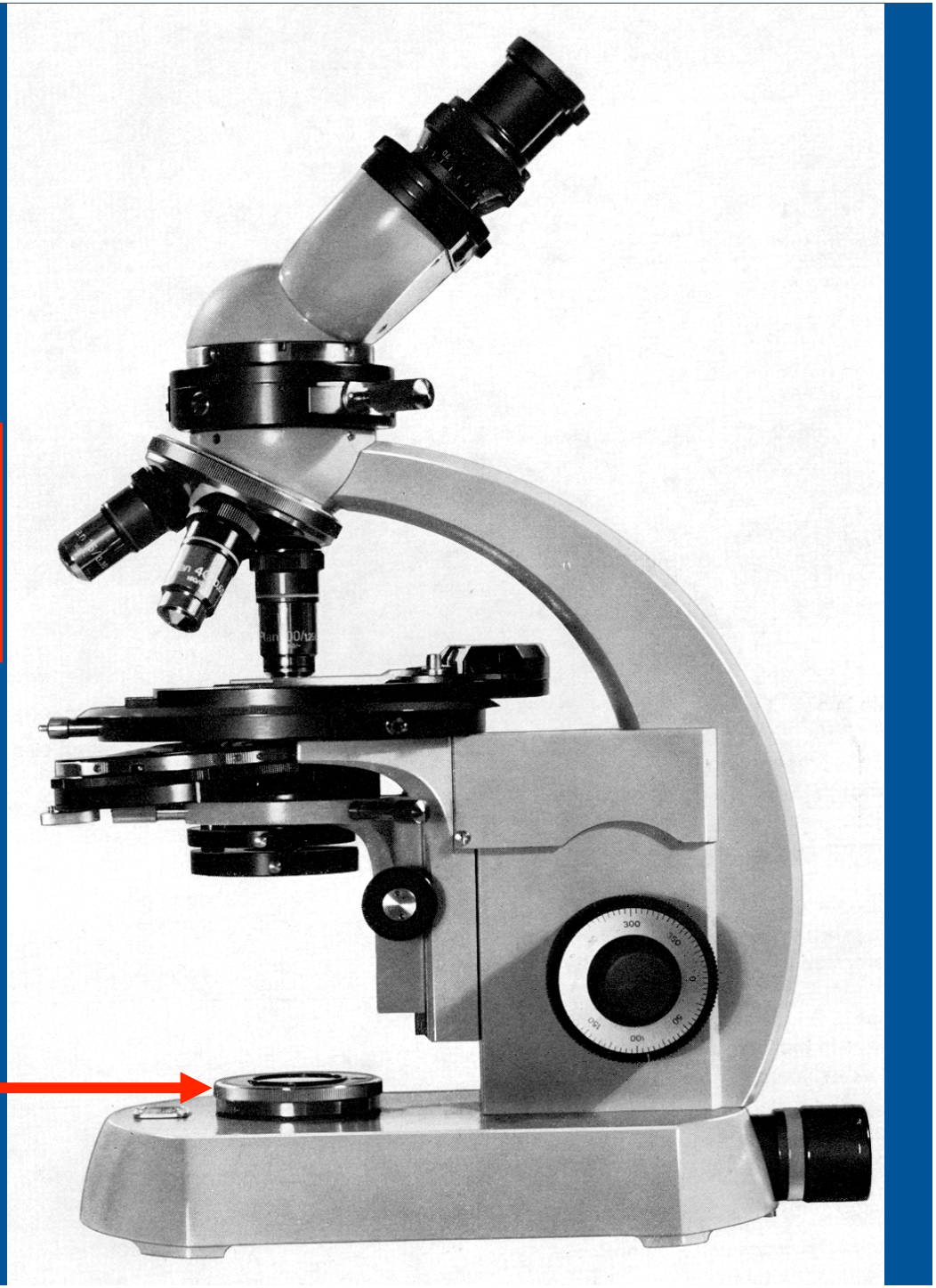

Retina
Lens of eye
Eyepiece lens
Primary image
Field diaphragm of eyepiece
Aperture diaphragm of objective
Object
Objective
Condenser
Illuminated Field Diaphragm
Lamp
Field set of conjugate planes
Aperture set of conjugate planes
Exit pupil of eyepiece
Back focal plane of objective
Filament
Each part of the object receives light from every part of the filament


Retina
Lens of eye
Eyepiece lens
Primary image
Field diaphragm of eyepiece
Aperture diaphragm of objective
Objective
Object
Condenser
Field set of conjugate planes
Aperture set of conjugate planes
Exit pupil of eyepiece
Back focal plane of objective
Illuminating Aperture Diaphragm
Filament
Every part of the object is illuminated by each part of the filament

# What are the diaphragms for?

The diaphragms are NOT intended for adjusting the brightness of the image

The *Illuminated Field Diaphragm* sets the ***area*** of specimen illuminated, and is adjusted according to ***magnification***

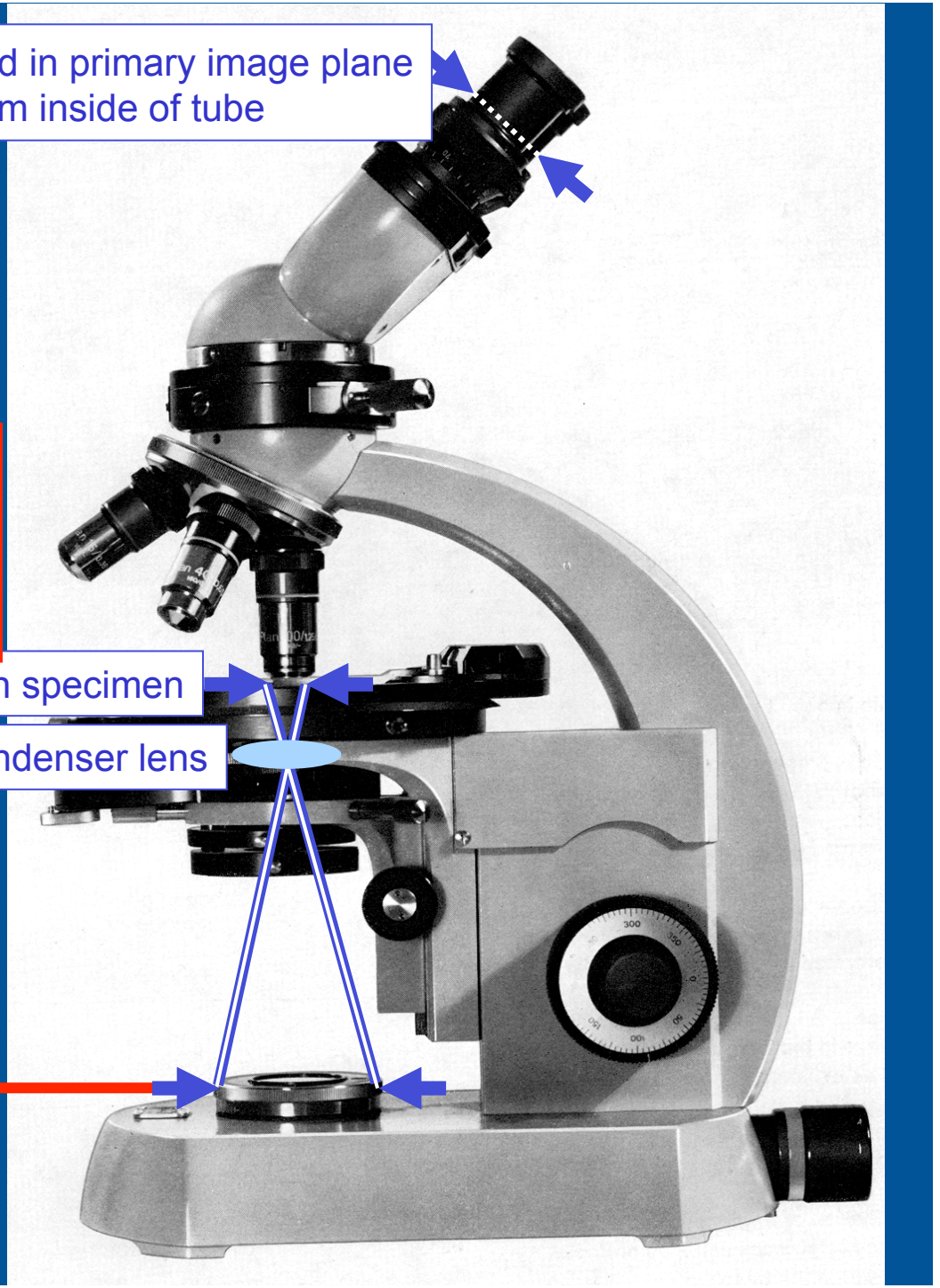


Illuminated Field Diaphragm also imaged in primary image plane
- prevents light from reflecting from inside of tube

The diaphragms are NOT intended for adjusting the brightness of the image

Image of Illuminated Field Diaphragm on specimen

Condenser lens



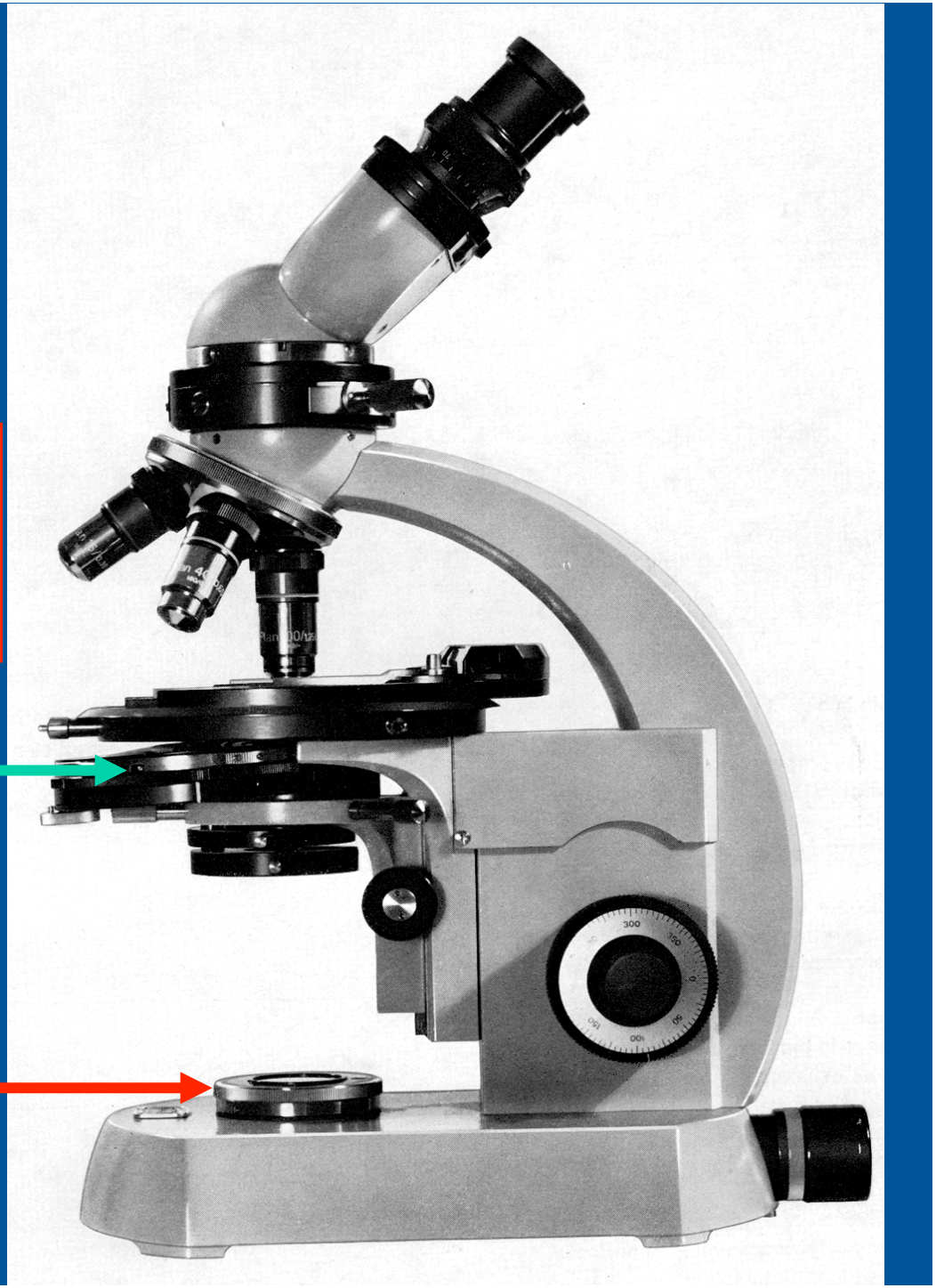
The *Illuminated Field Diaphragm* sets the ***area*** of specimen illuminated, and is adjusted according to ***magnification***

# What are the diaphragms for?

The diaphragms are NOT intended for adjusting the brightness of the image

The *Illuminating Aperture Diaphragm* sets the ***angle*** of the cone of light illuminating the specimen, and is adjusted according to ***objective NA***

The *Illuminated Field Diaphragm* sets the ***area*** of specimen illuminated, and is adjusted according to ***magnification***



# Image of lamp filament seen in back focal plane of objective