

# 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

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

- Light up the *specimen* **uniformly**
  - over a **controllable** *area*
- Illuminate the *objective aperture* **uniformly**
  - over a **controllable** *angle*

# Microscope Illumination

Two basic methods of illumination:

Source-focused (or 'Critical') Illumination:

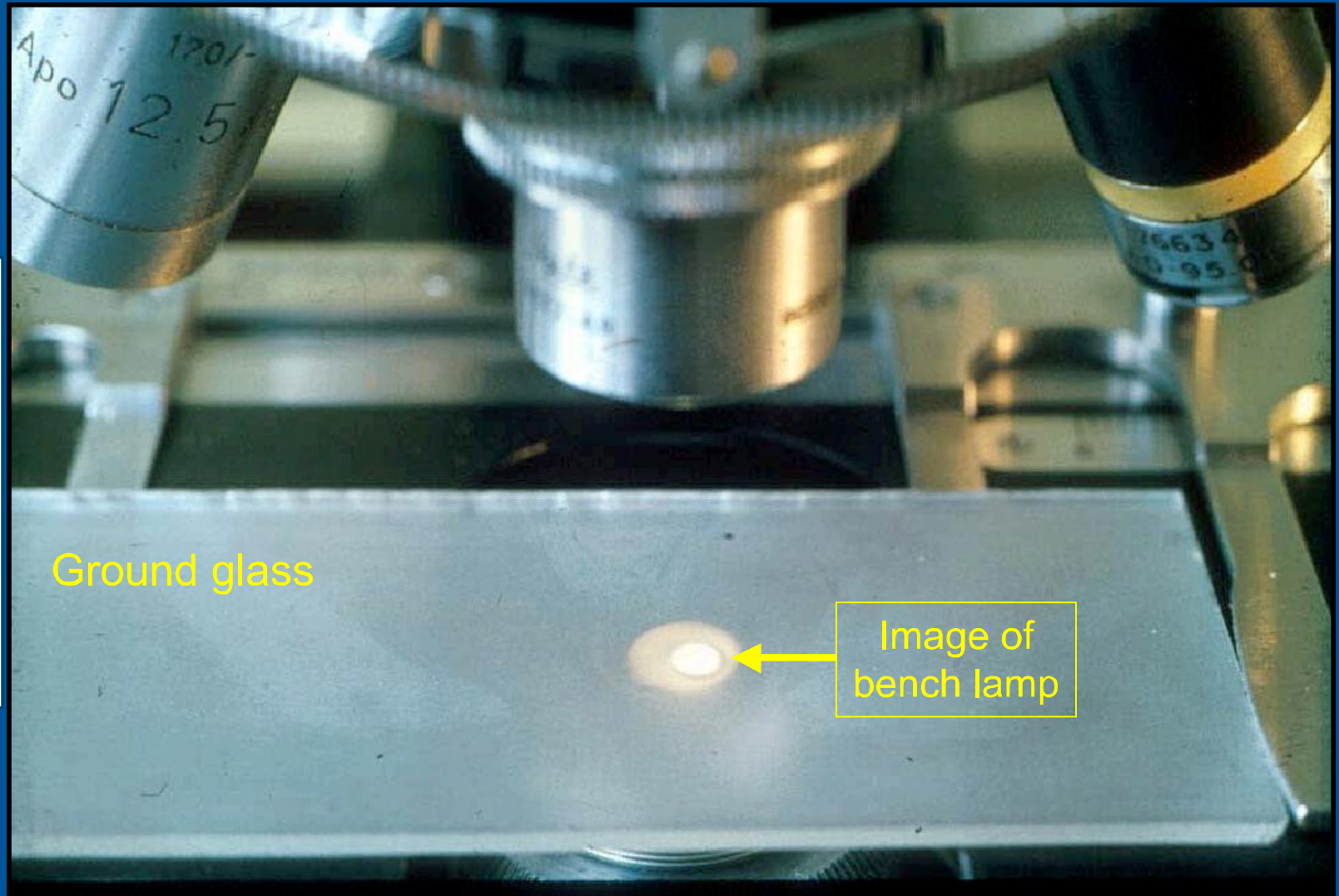
Light-source imaged on to specimen

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

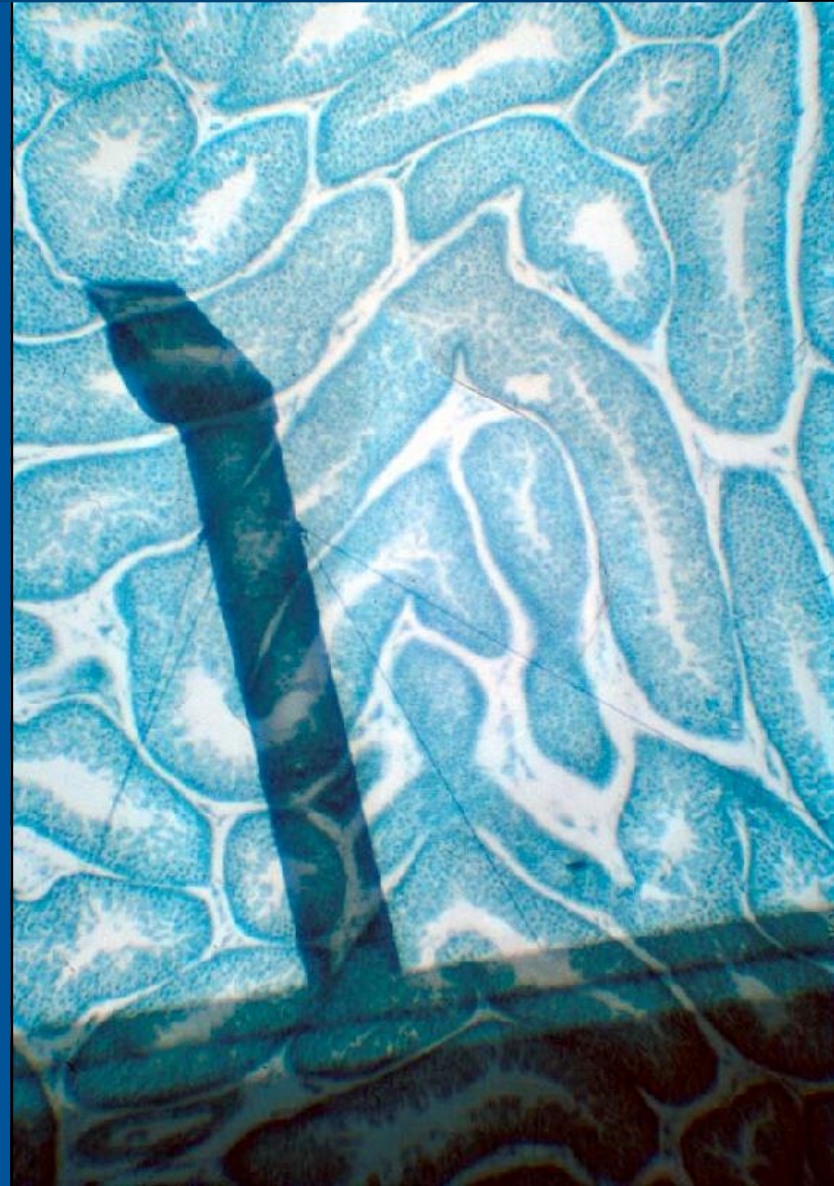
- throws an image of source on to underside of slide

# 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*



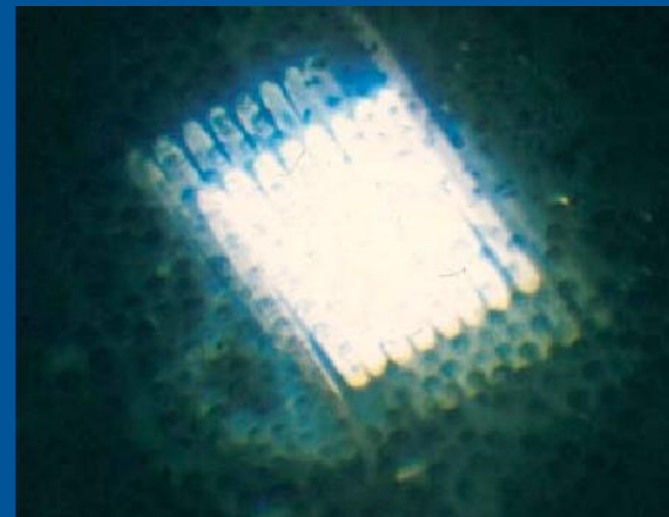


# Source-focused Illumination

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

Köhler Illumination solves this problem

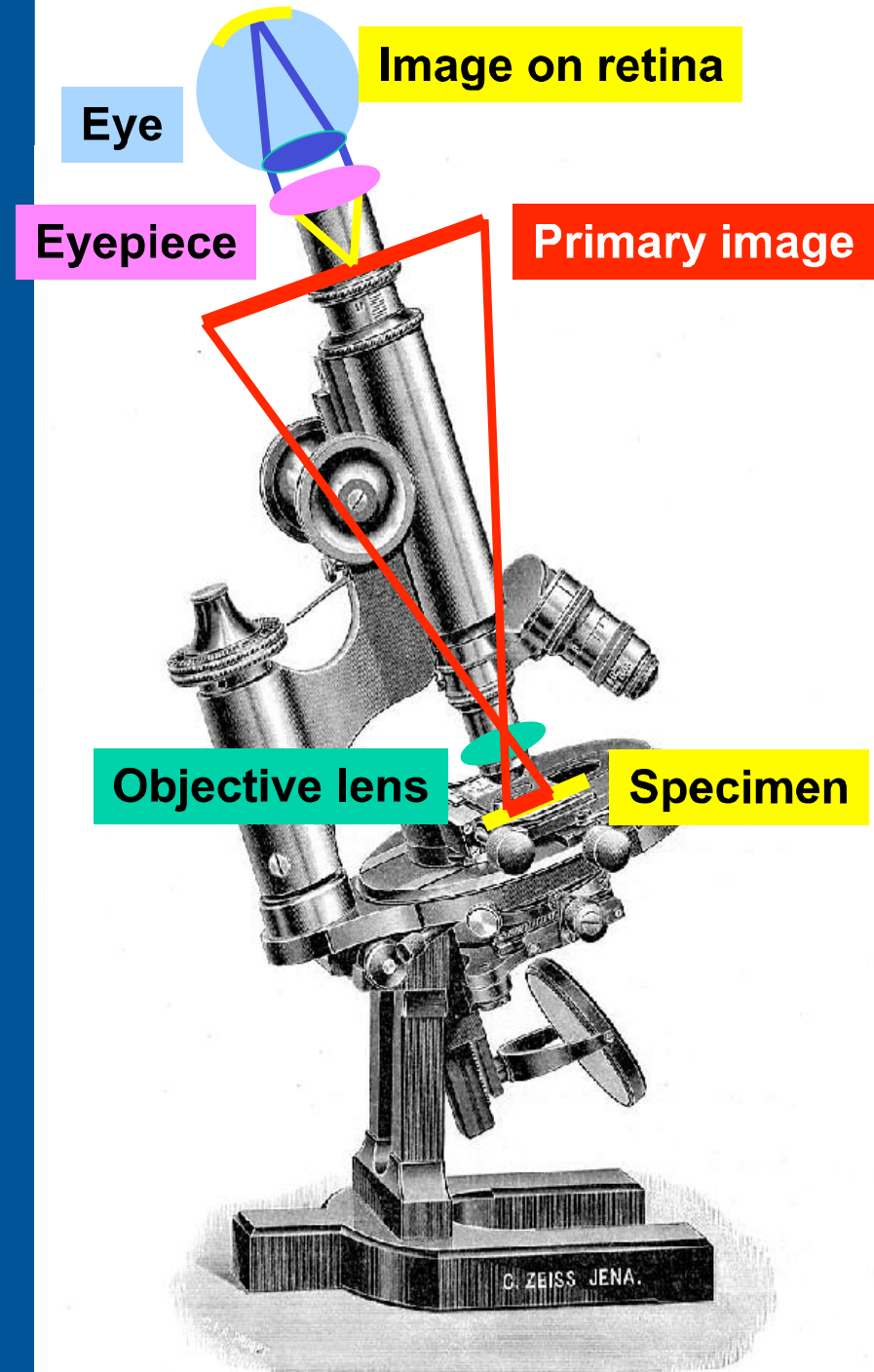
...and a modern halogen lamp is even worse



# 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.





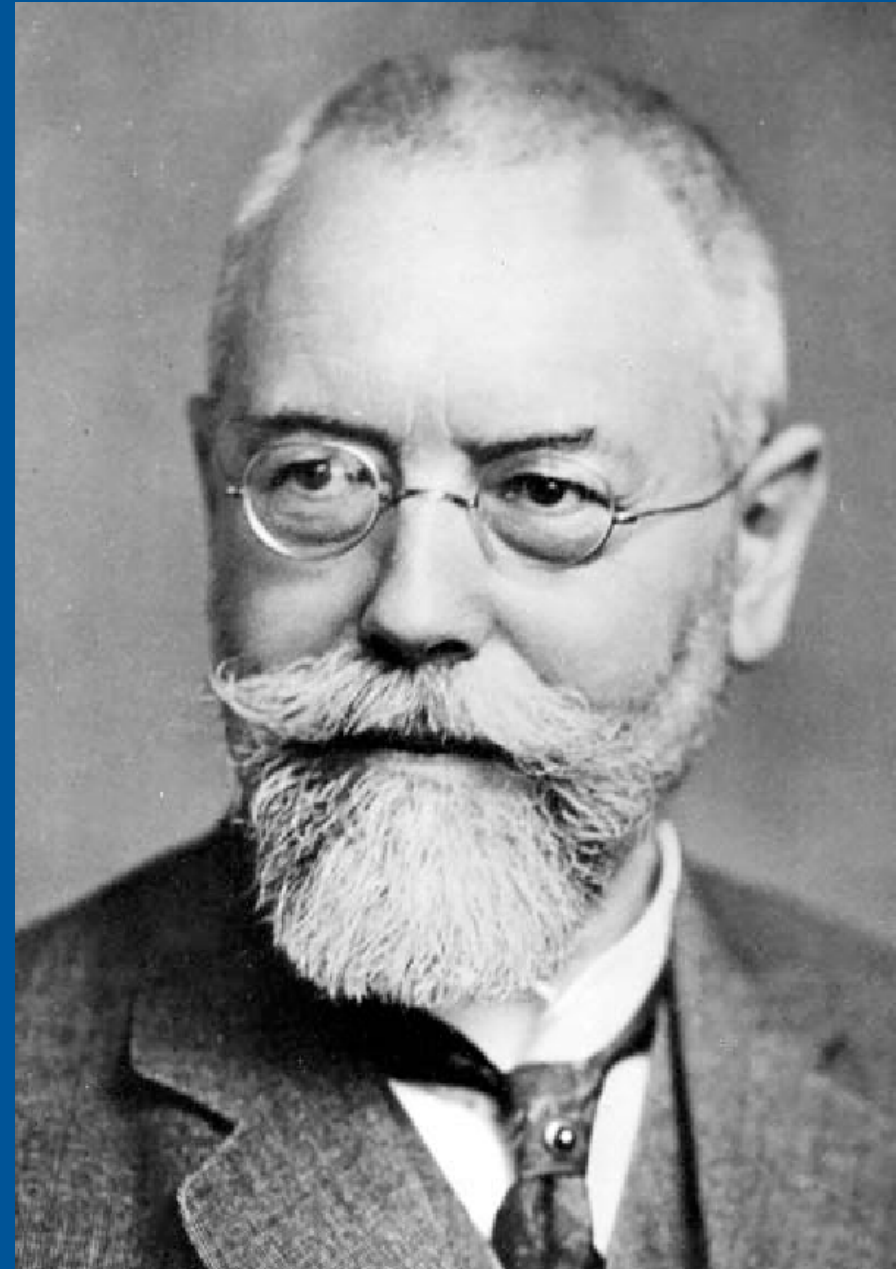
# August Köhler

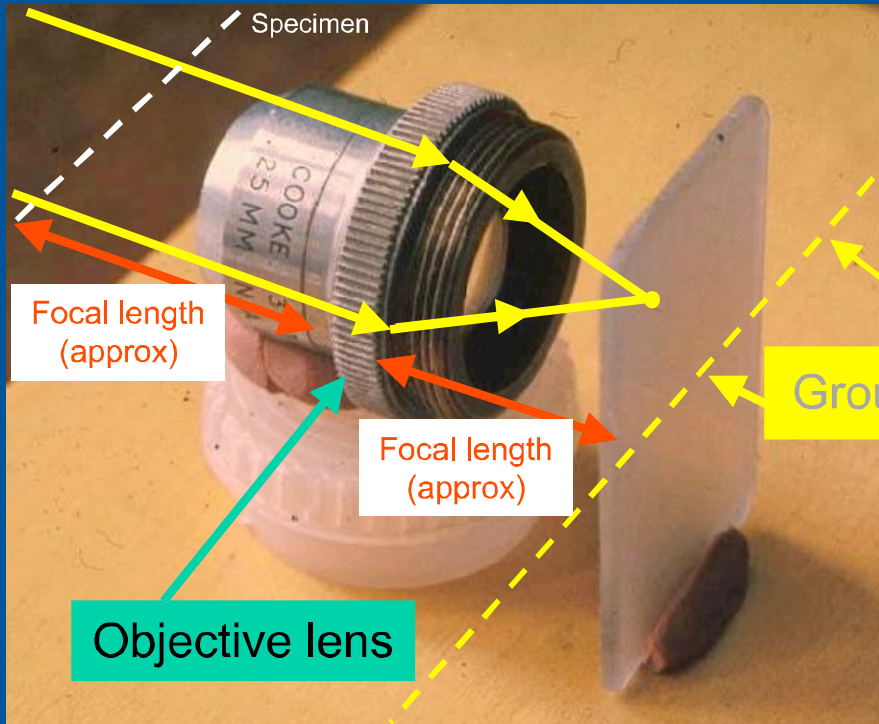
1866 - 1948

published

*A new system of  
illumination for  
photomicrographic  
purposes*

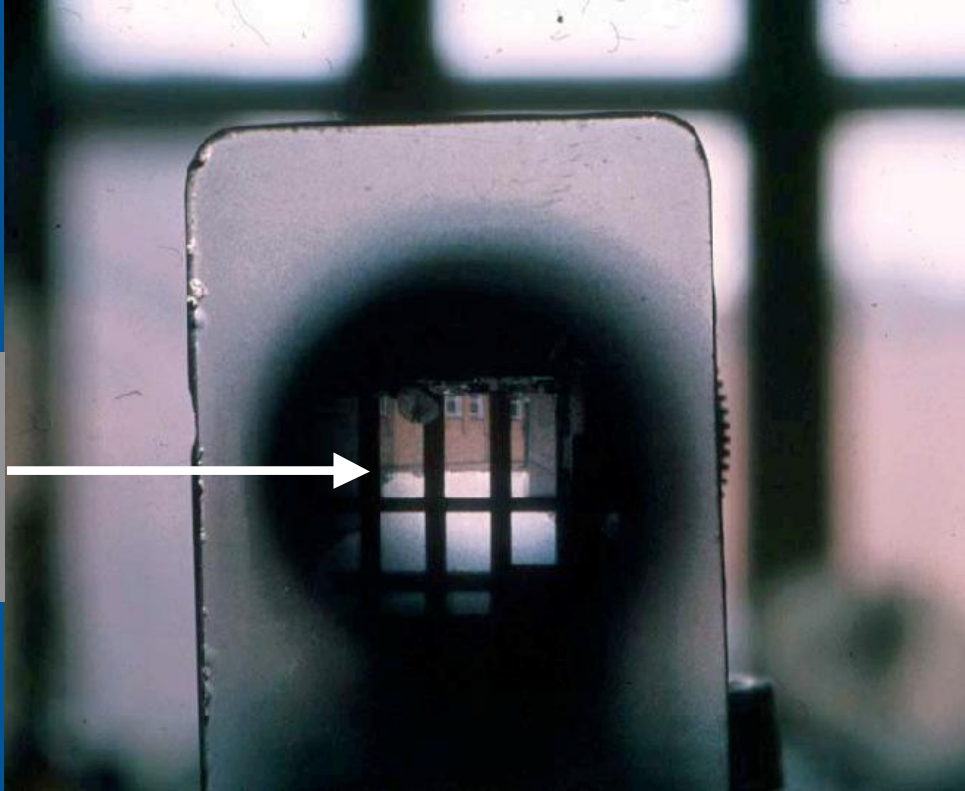
*(in German) in 1893.*





The back focal plane of the objective

Image of objects at 'infinity' in back focal plane of objective



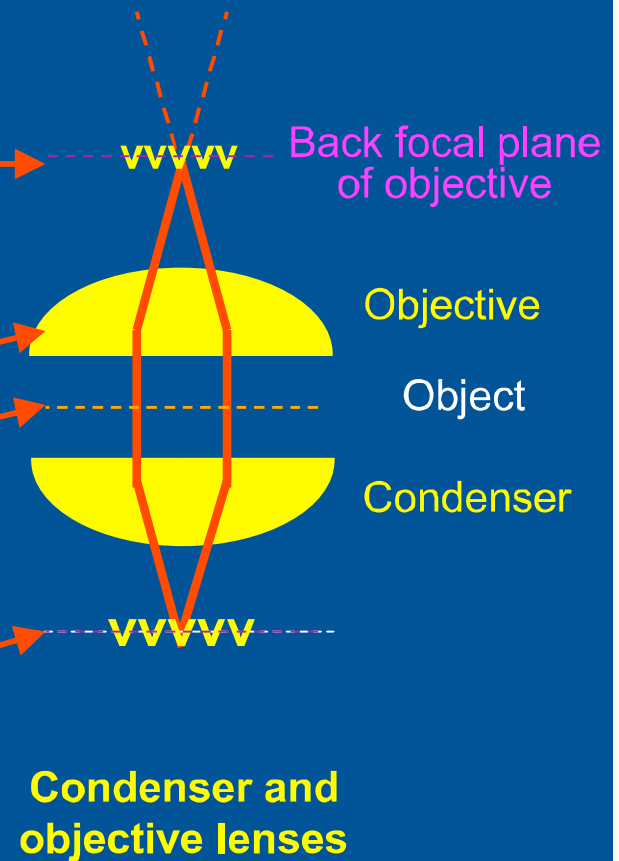
# How do we light up the specimen *uniformly*?

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

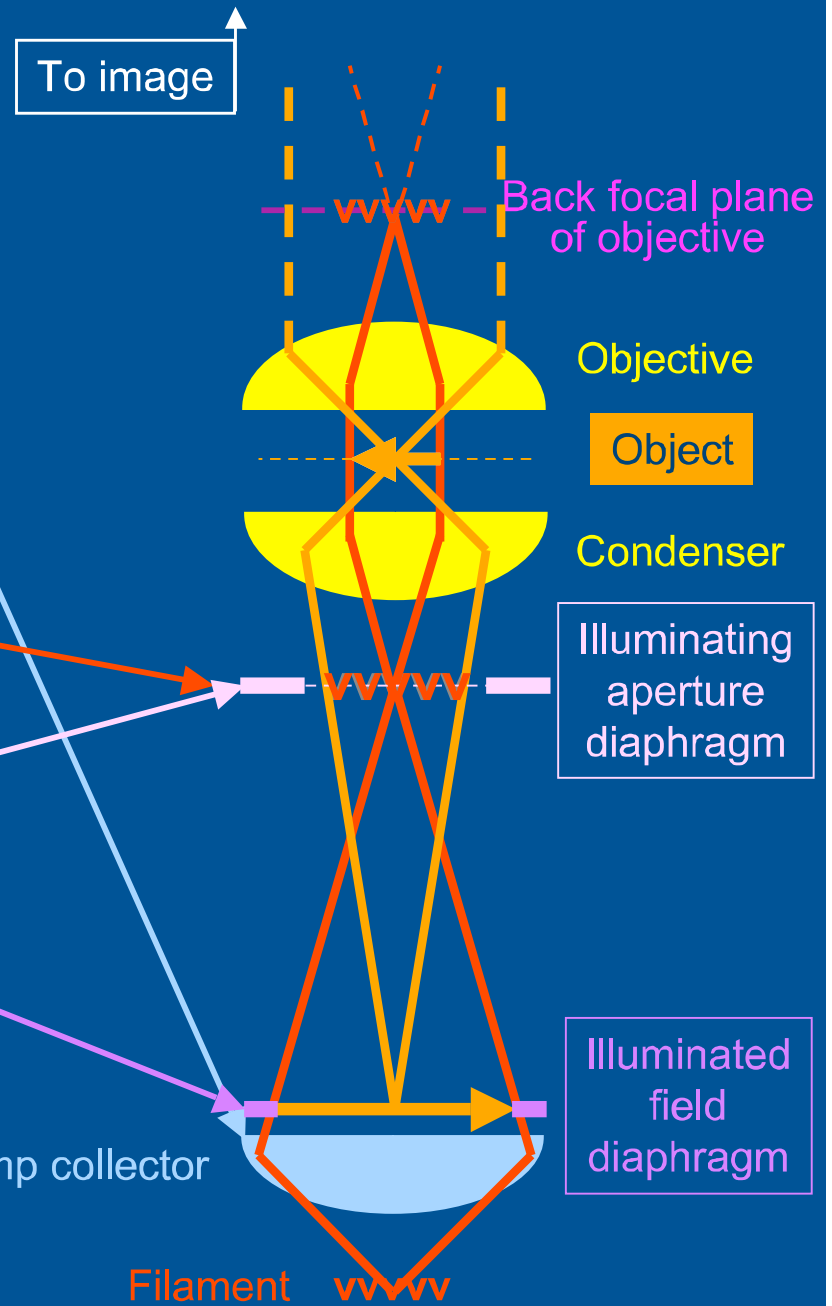


# How do we light up the specimen *uniformly*?

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.



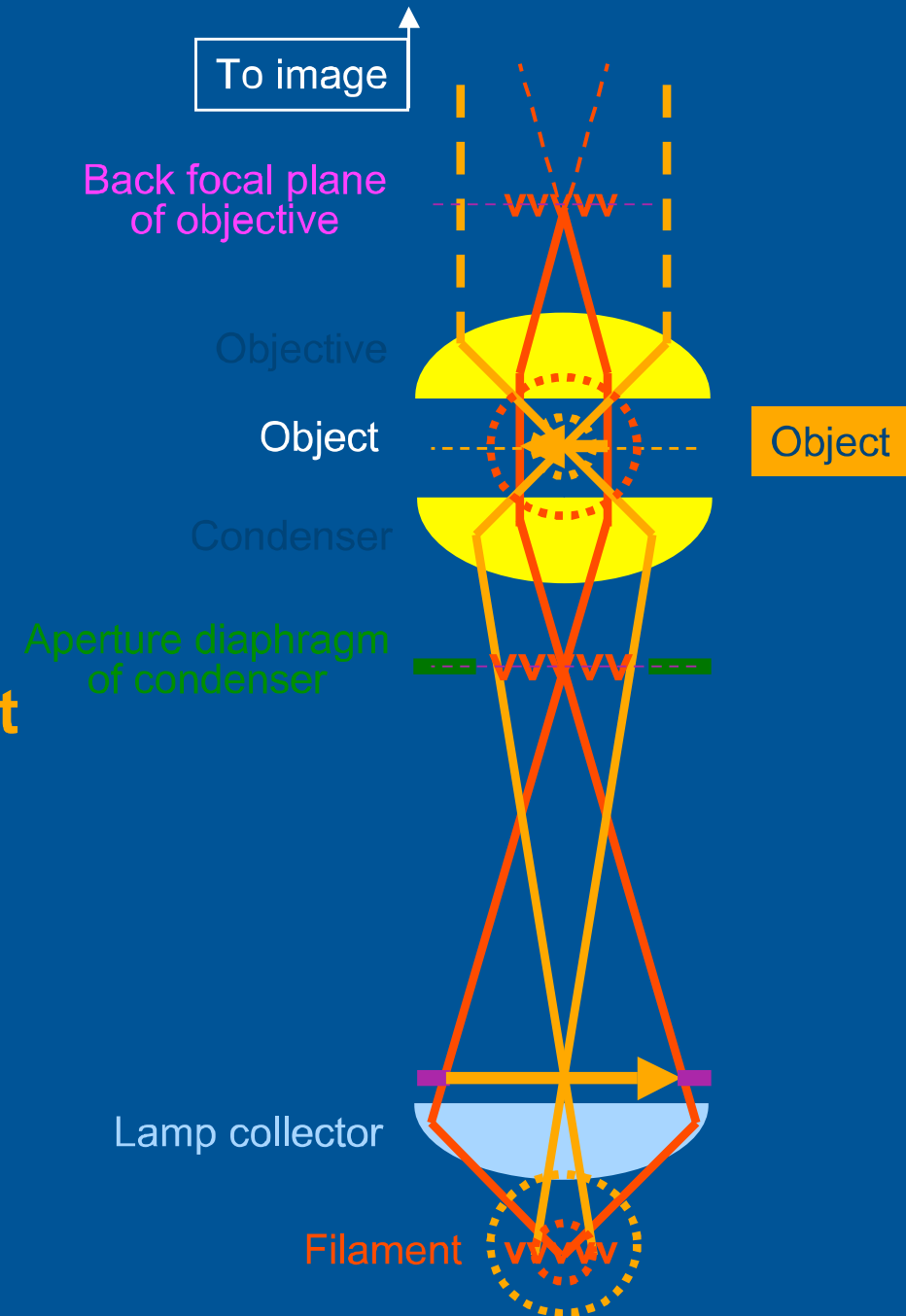
# How do we light up the specimen *uniformly*?

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...

Light up the specimen uniformly over a **controllable area**?

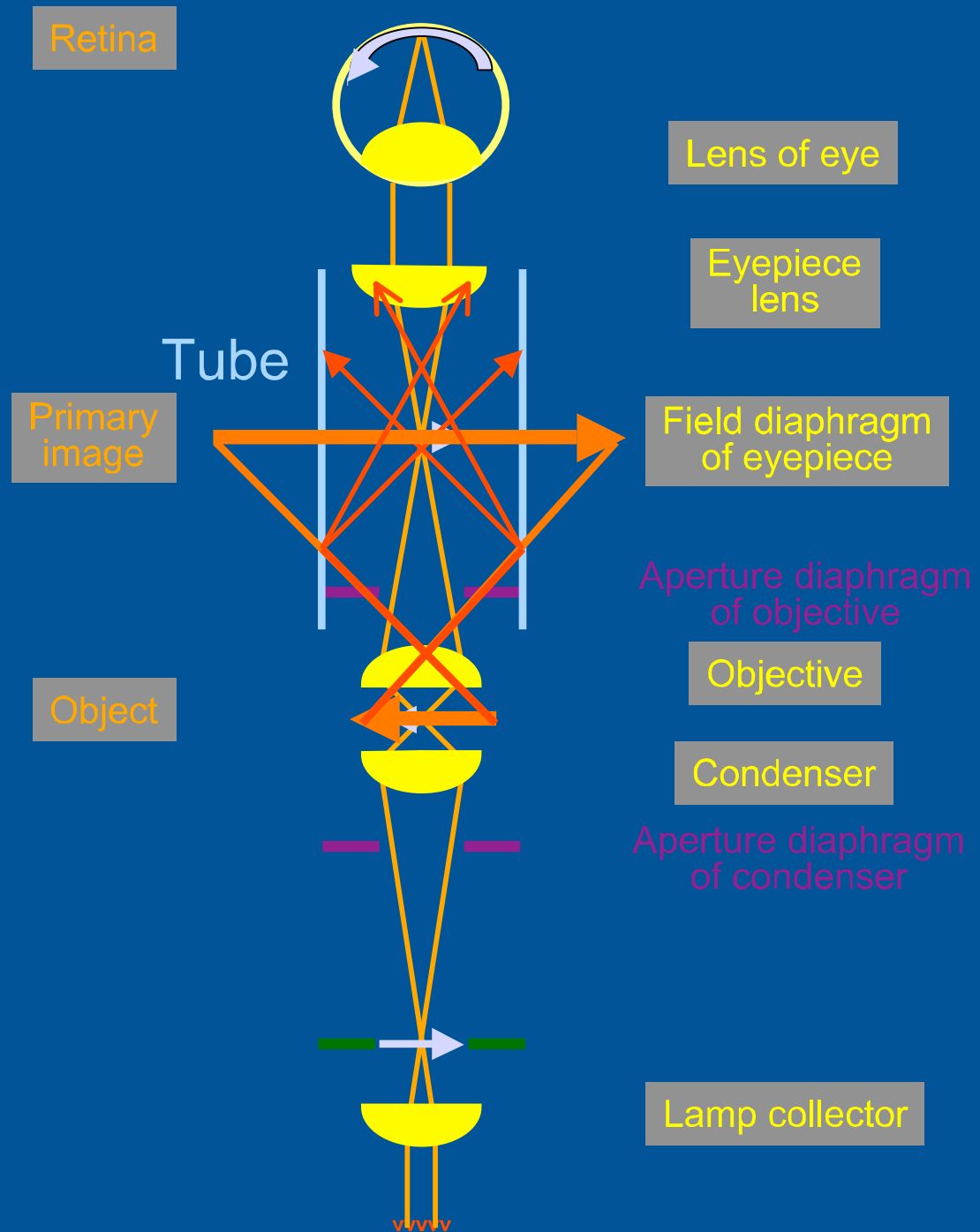
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It is unnecessary, and often detrimental, to illuminate parts of the specimen outside the field of view

- 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** control 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

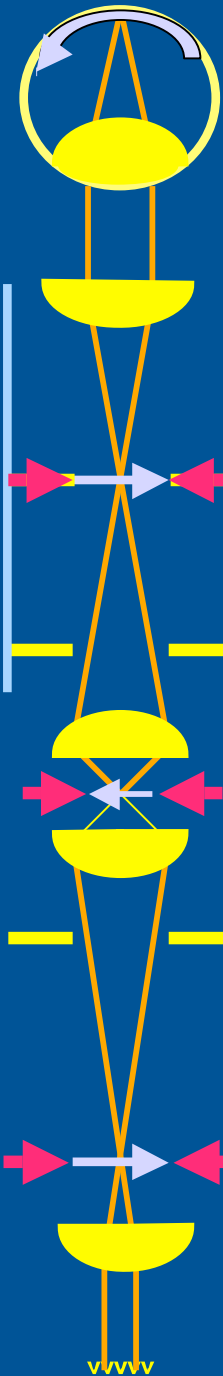
## How do we control the *area illuminated* ?

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**

Tube



Lens of eye

Eyepiece lens

Field diaphragm of eyepiece

Aperture diaphragm of objective

Objective

Condenser

Aperture diaphragm of condenser

Illuminated Field Diaphragm

Lamp collector

# Why is it necessary to...

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

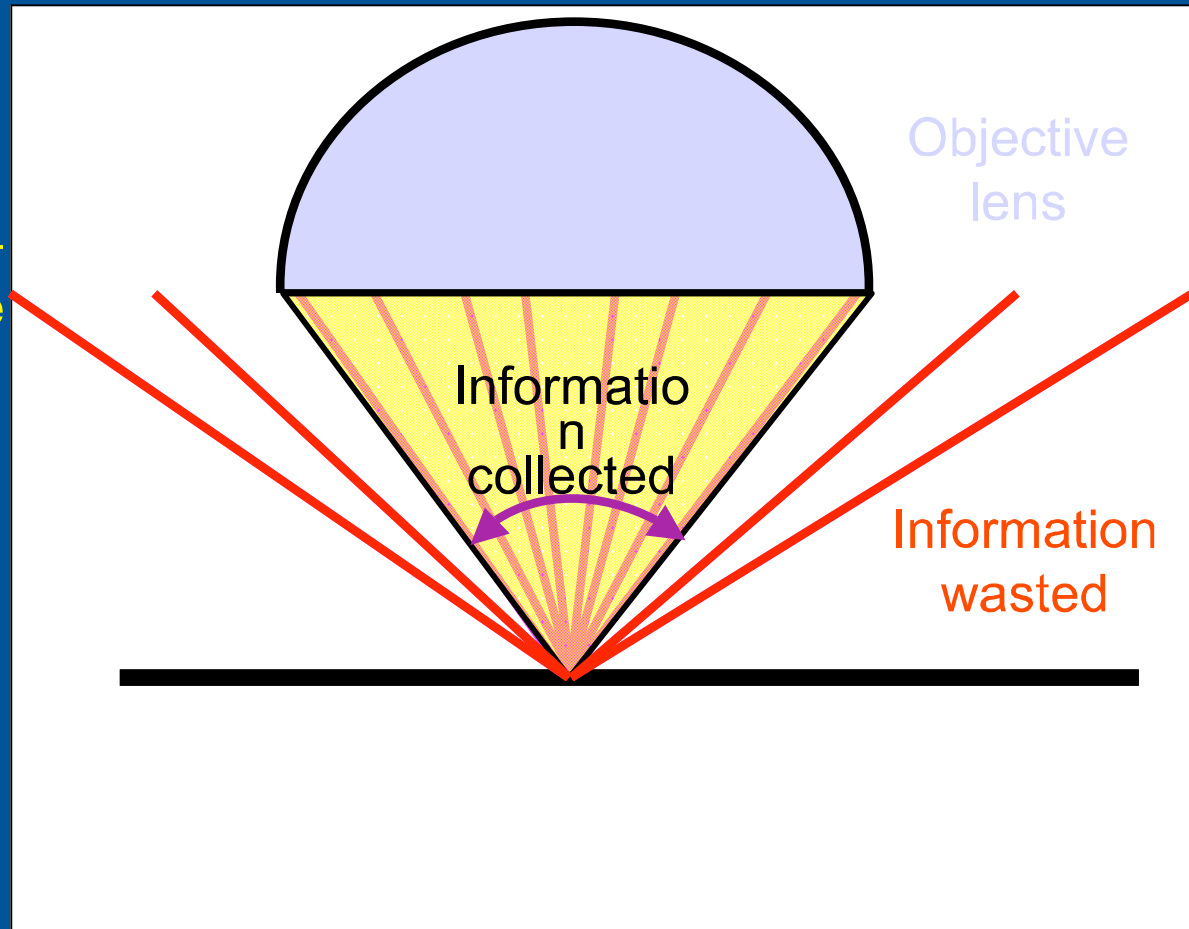
Consider that every ray leaving the object carries some information about fine detail in the object  
*Some of these rays*

– and *some of the information* – will be collected by the objective

and some rays

– and some information – will *NOT* be collected, and will be wasted.

Resolution will thus depend on the *angular aperture* of the objective - the larger the aperture the higher the resolution



# Why is it necessary to...

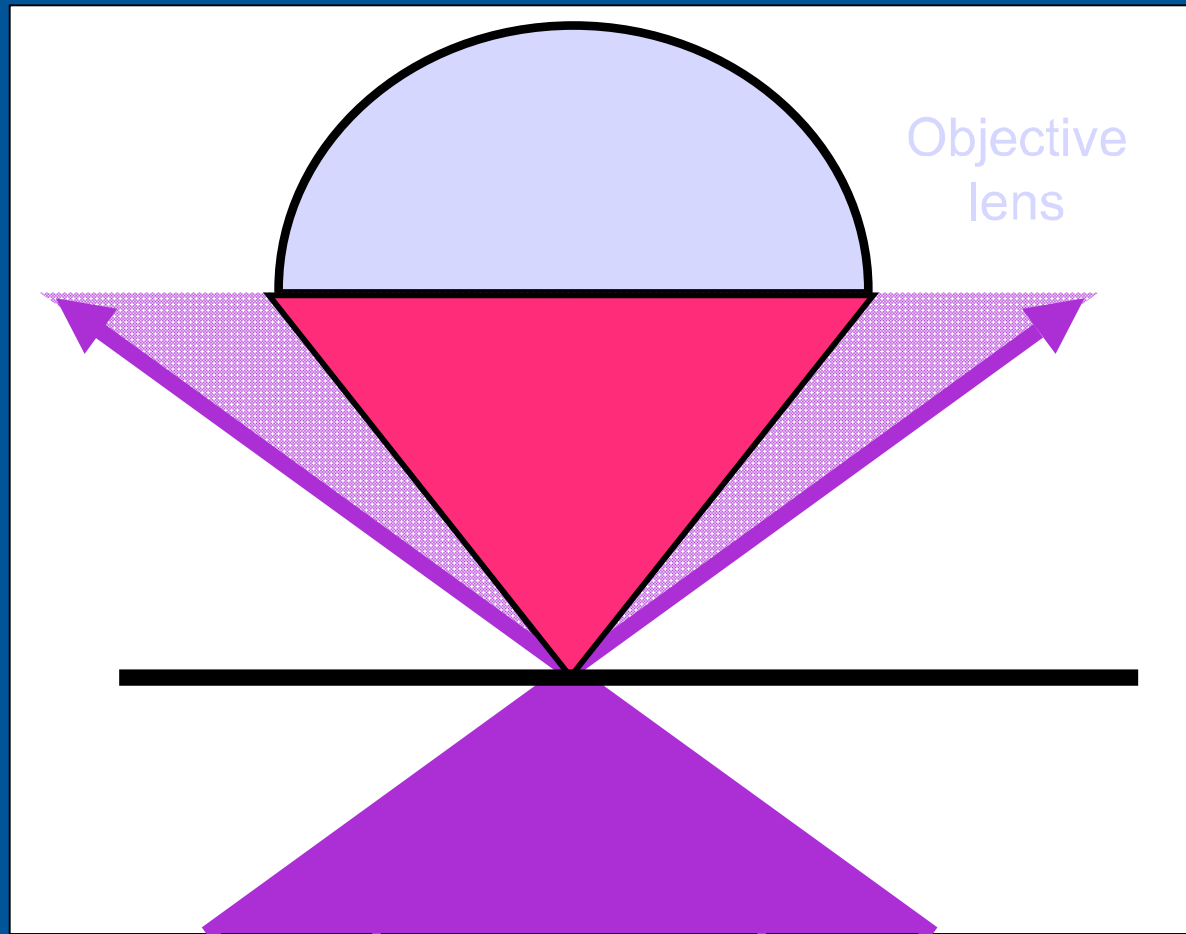
Illuminate the *objective aperture uniformly* over a *controllable angle*?

'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* ?

Why not *all* ?

Why not a *very wide* cone of light ?

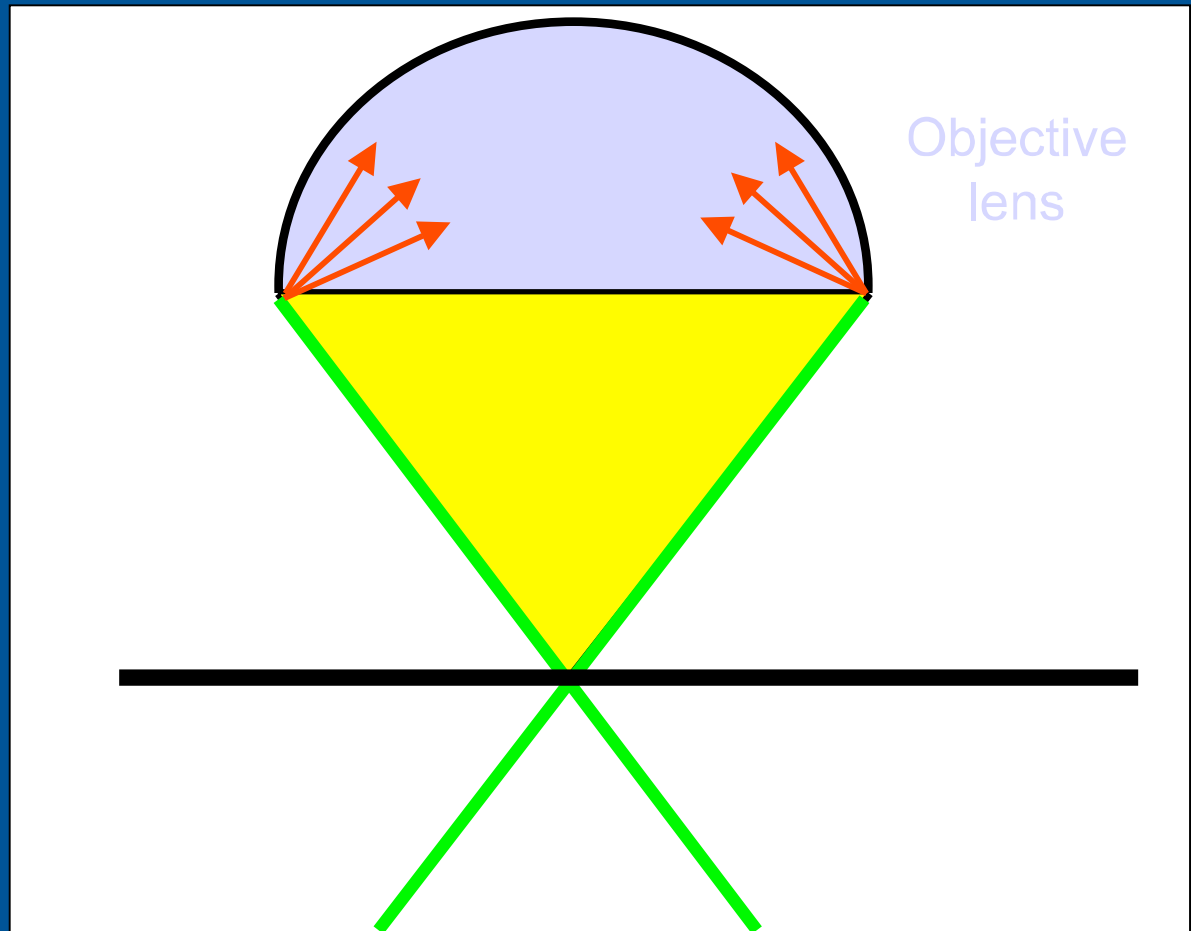




# Why is it necessary to...

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.

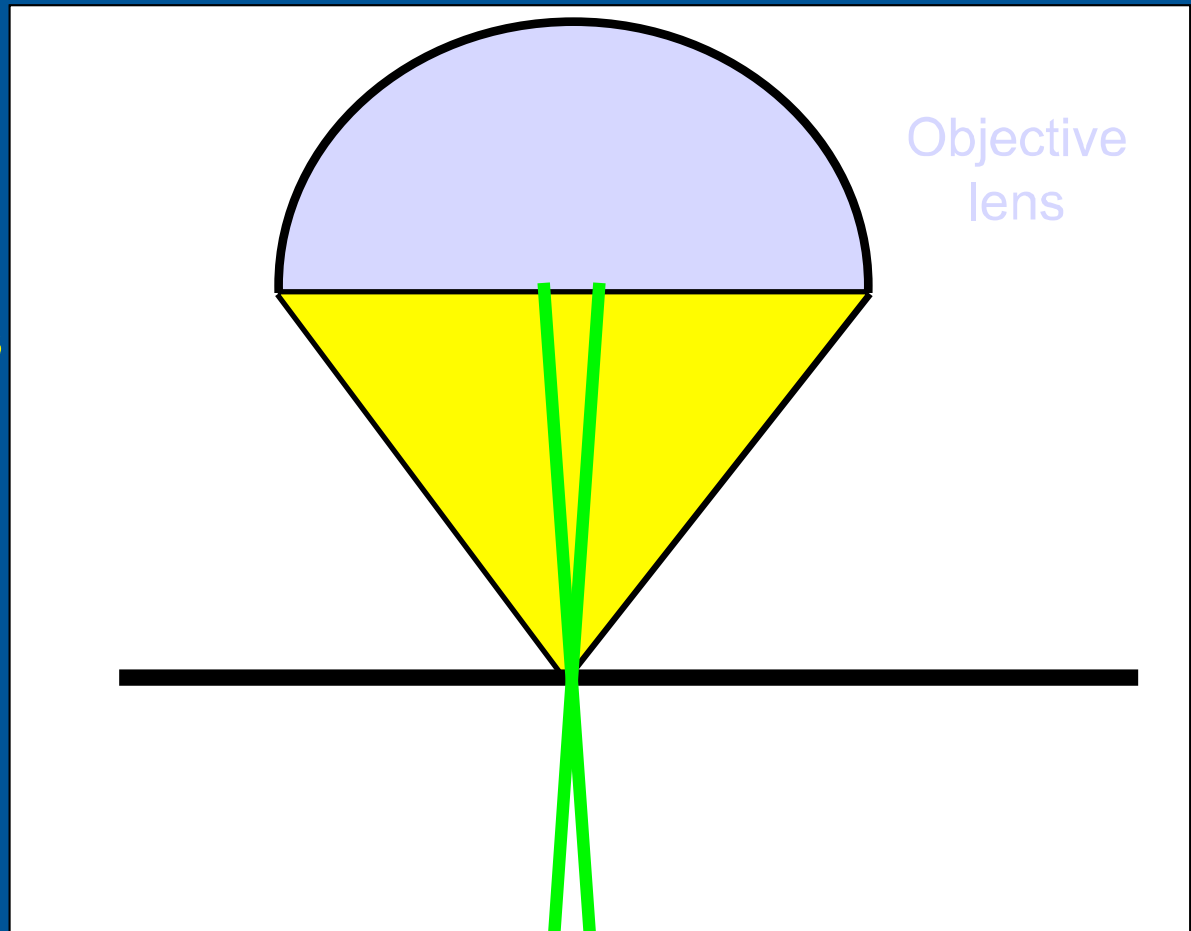


# Why is it necessary to...

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.

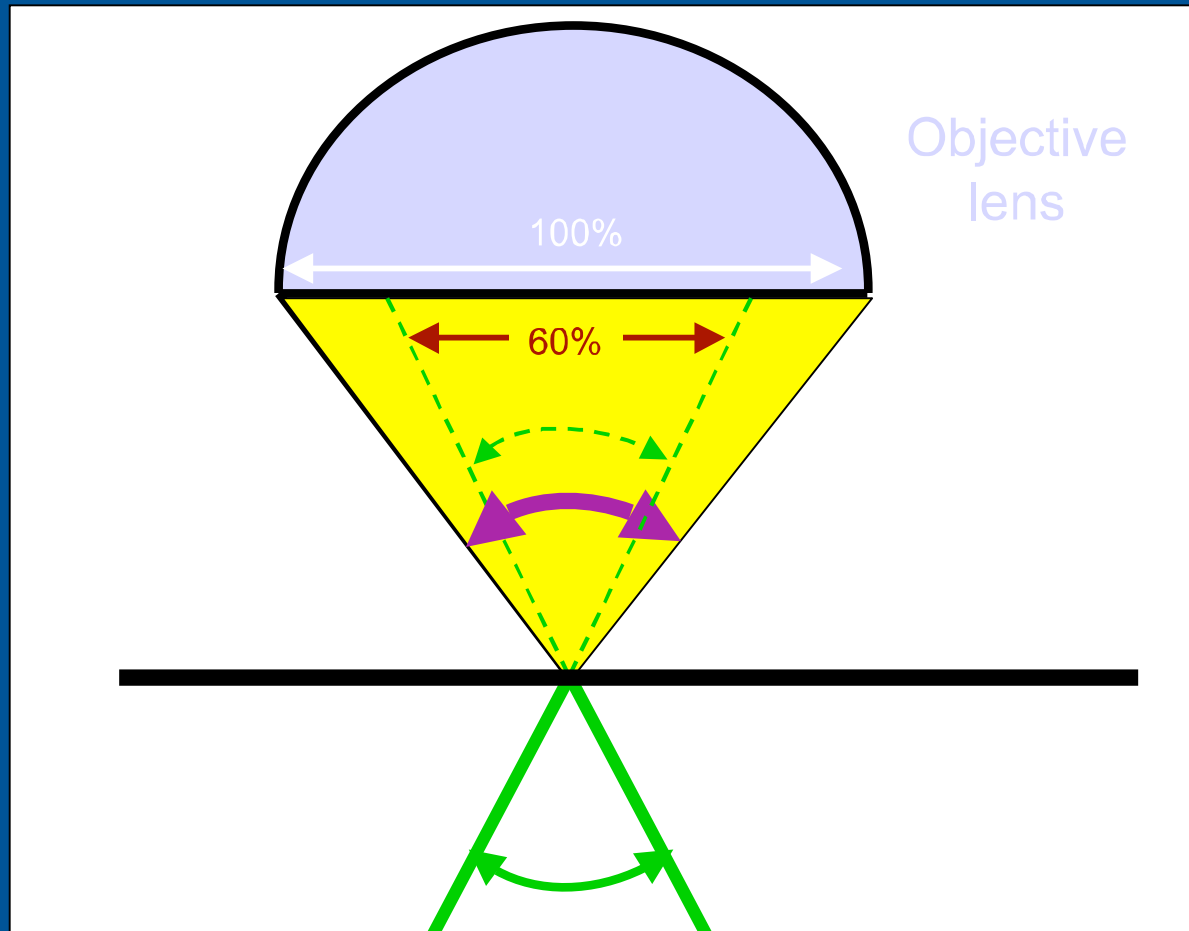


# Why is it necessary to...

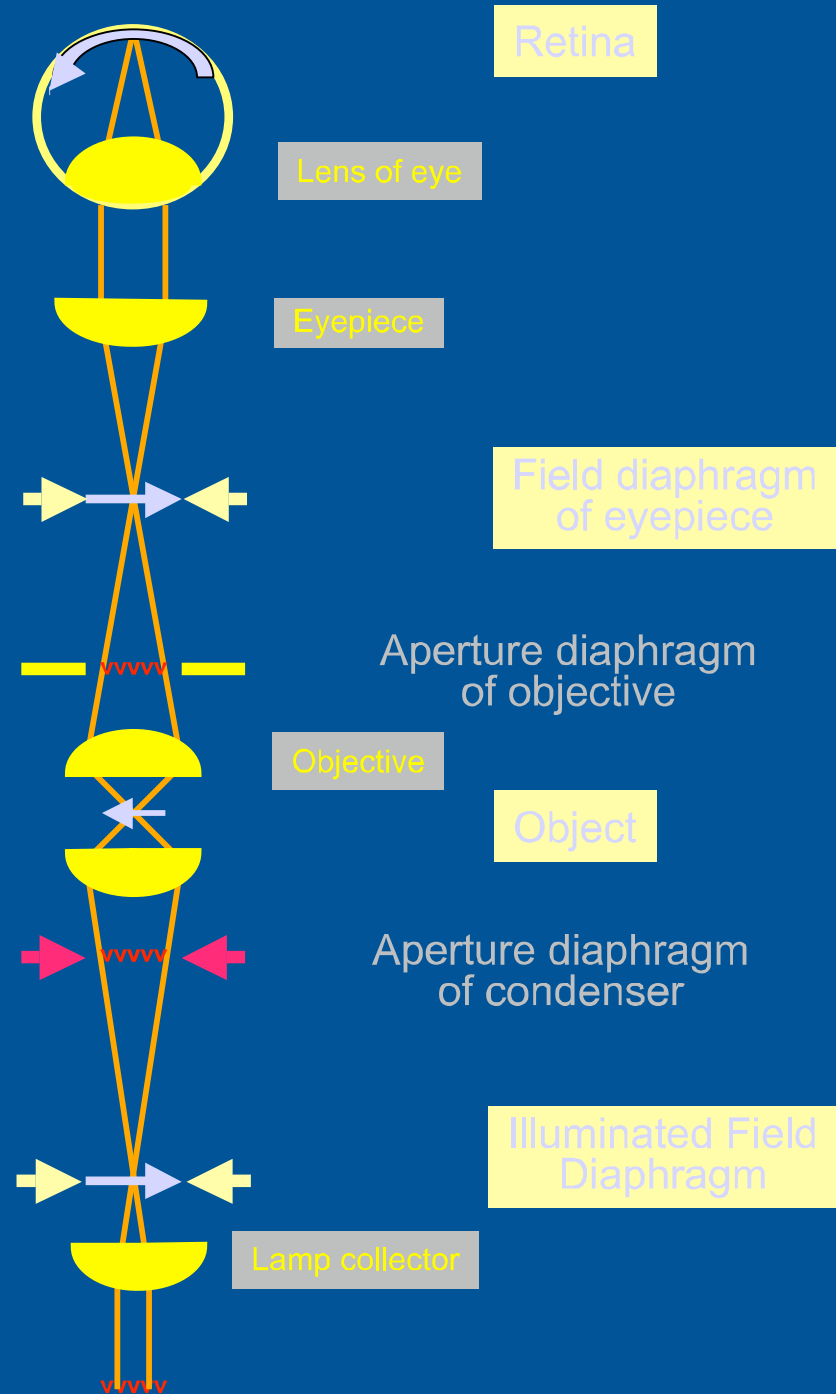
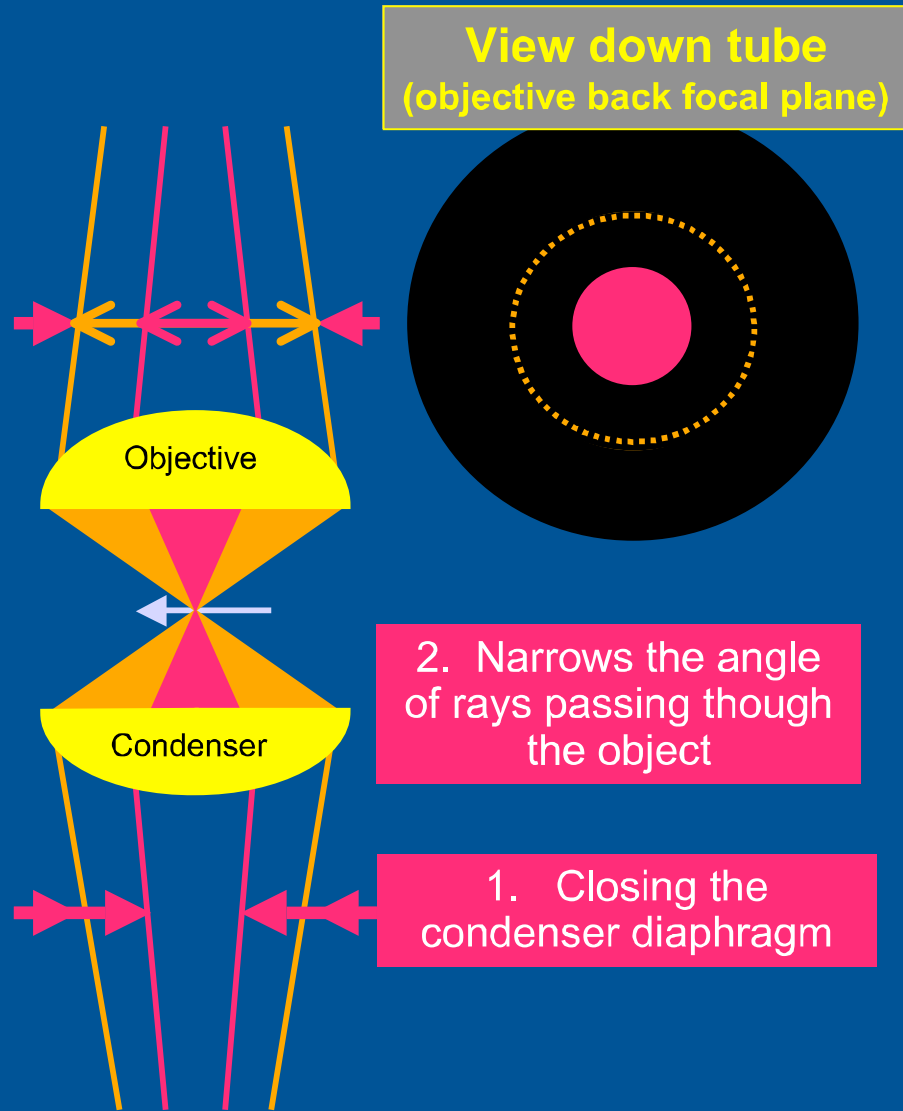
Illuminate the *objective aperture uniformly* over a *controllable angle*?

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

60 to 75% is often  
recommended

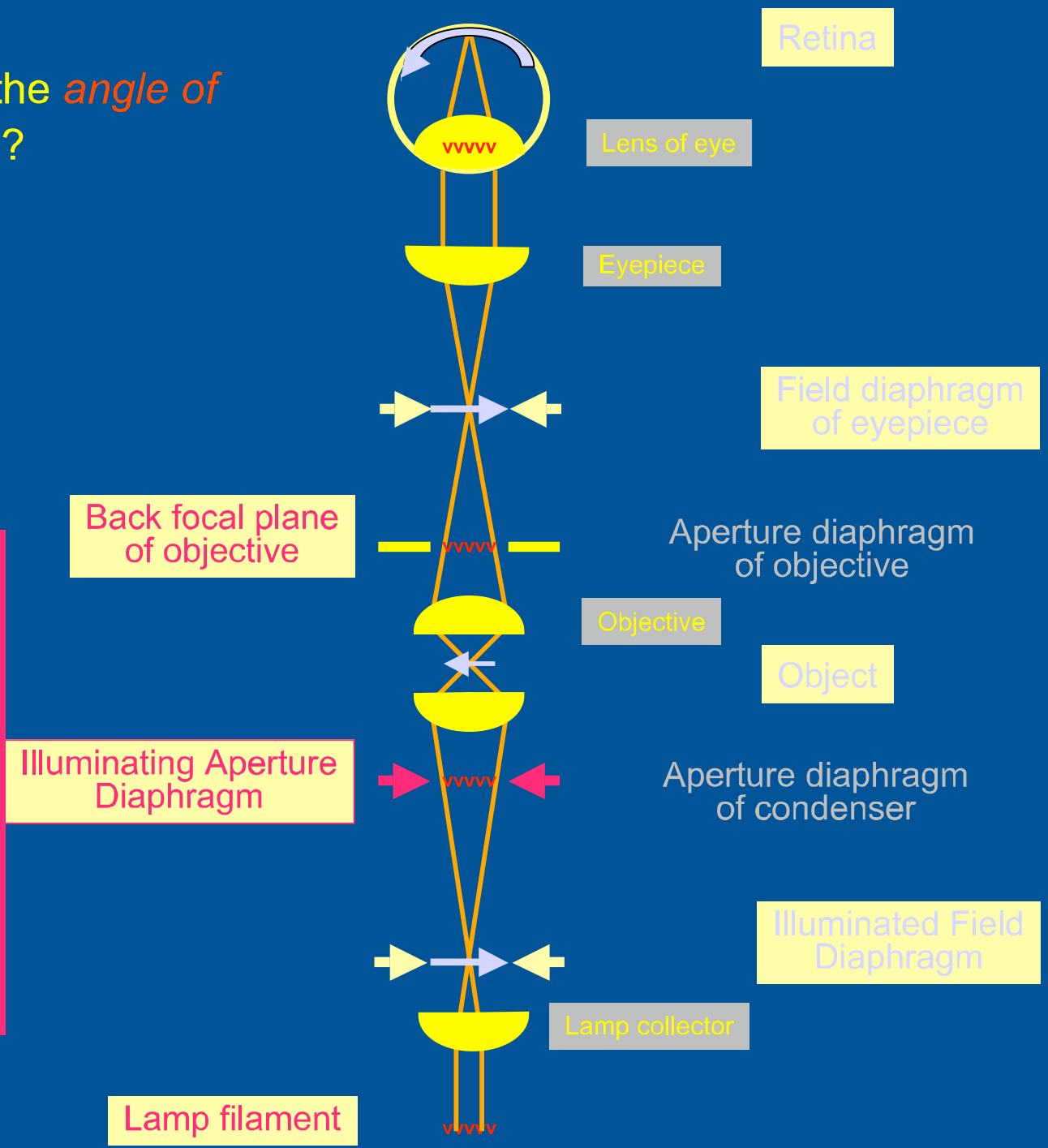


# How do we control the *angle of illumination* ?



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

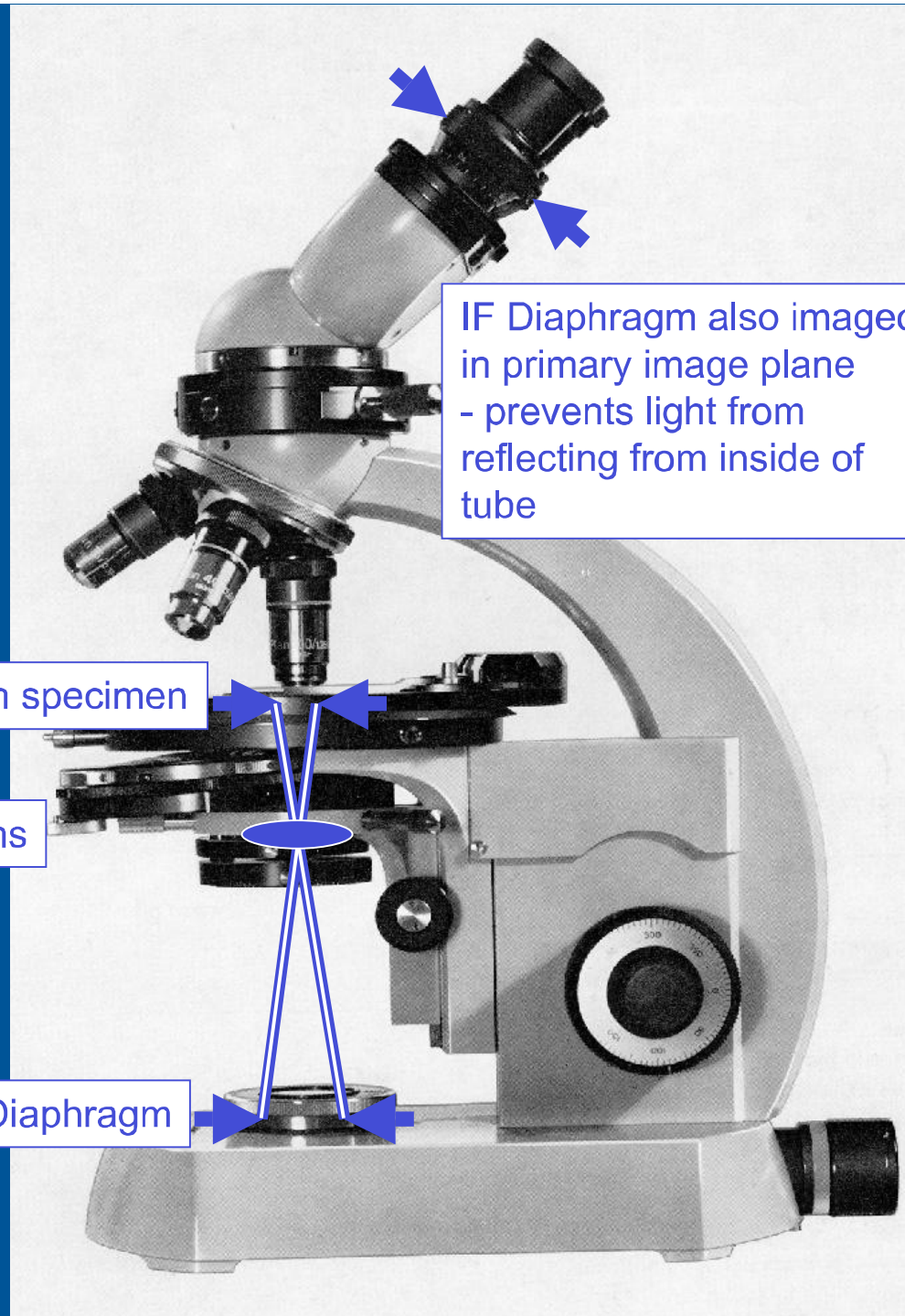
The aperture diaphragm of the condenser thus acts as the *Illuminating Aperture Diaphragm* – so called because it is the diaphragm which regulates the *Illuminating Aperture*







# What are the diaphragms for?



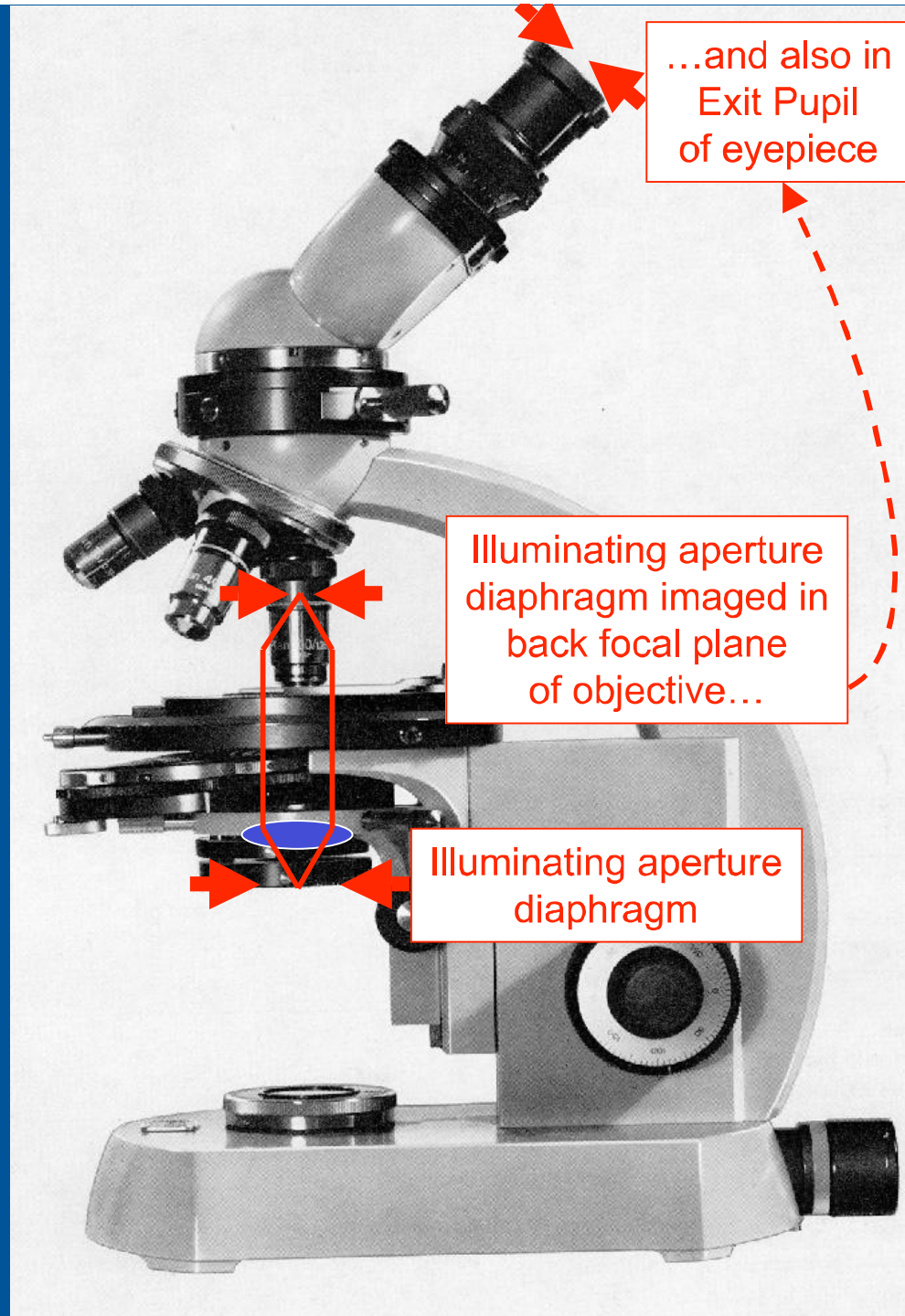
IF Diaphragm also imaged in primary image plane - prevents light from reflecting from inside of tube

Image of IF Diaphragm on specimen

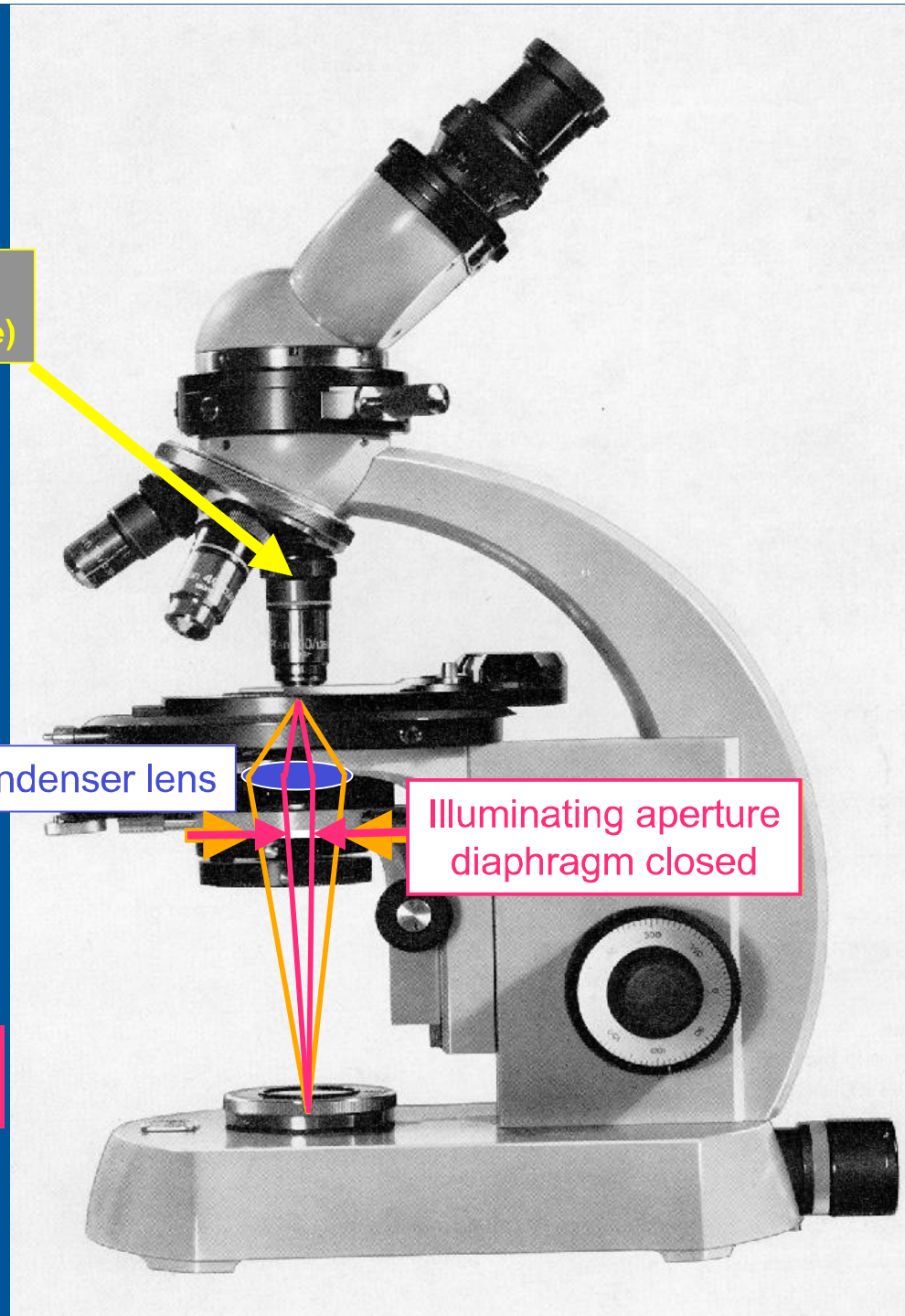
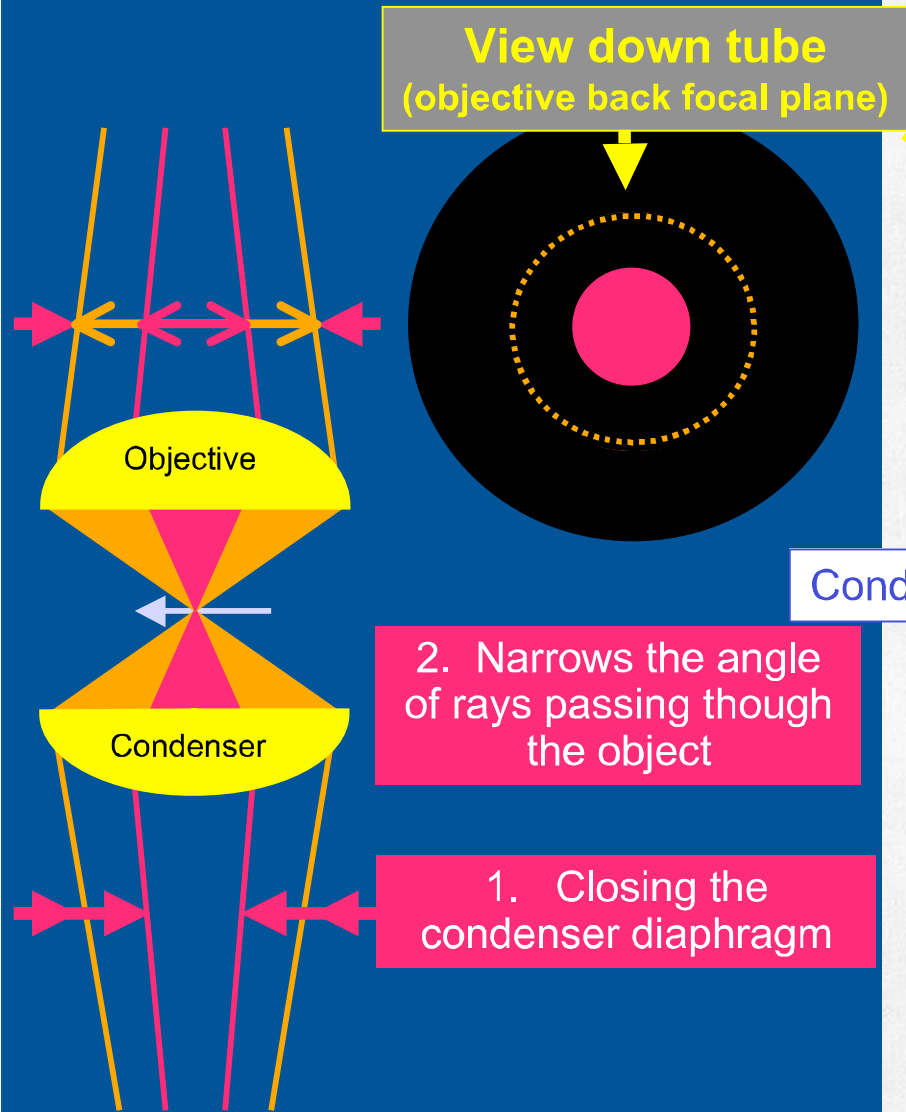
Condenser lens

Illuminated Field Diaphragm

# What are the diaphragms for?



# What are the diaphragms for?





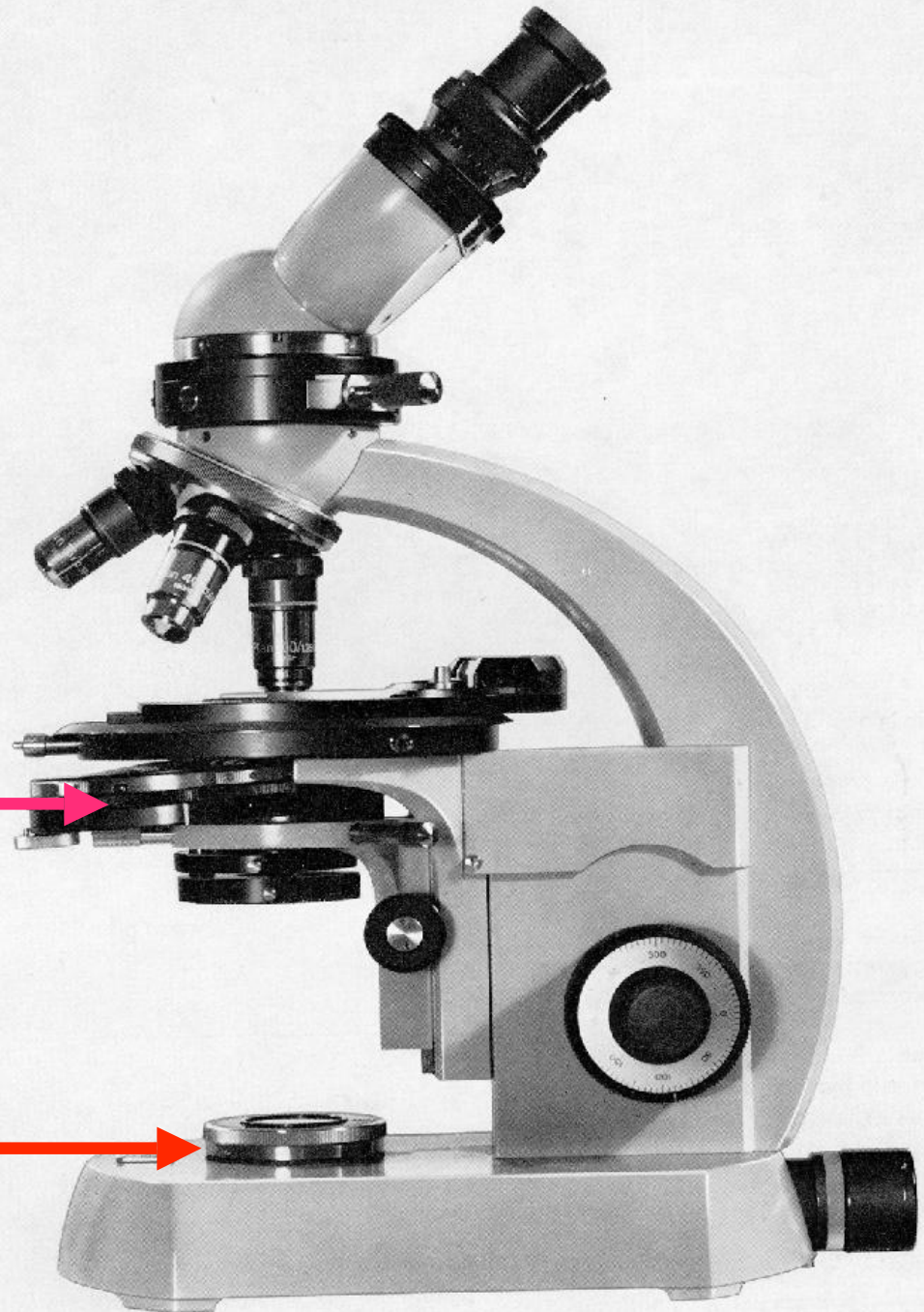
## What are the diaphragms for?

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

The

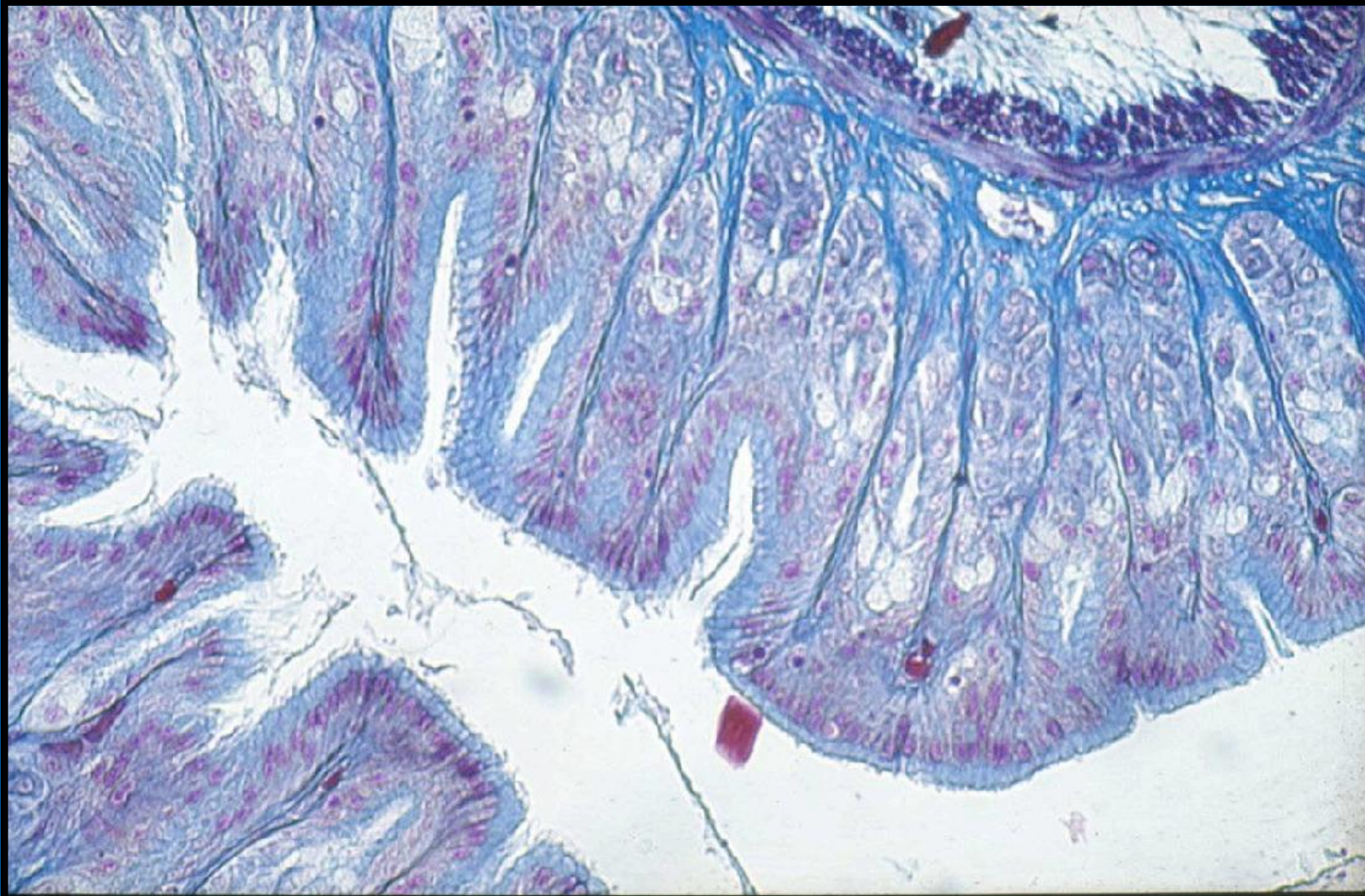
*Illuminating Aperture Diaphragm* sets the **angle** of the cone of light illuminating the specimen, and is adjusted according to **objective NA**

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



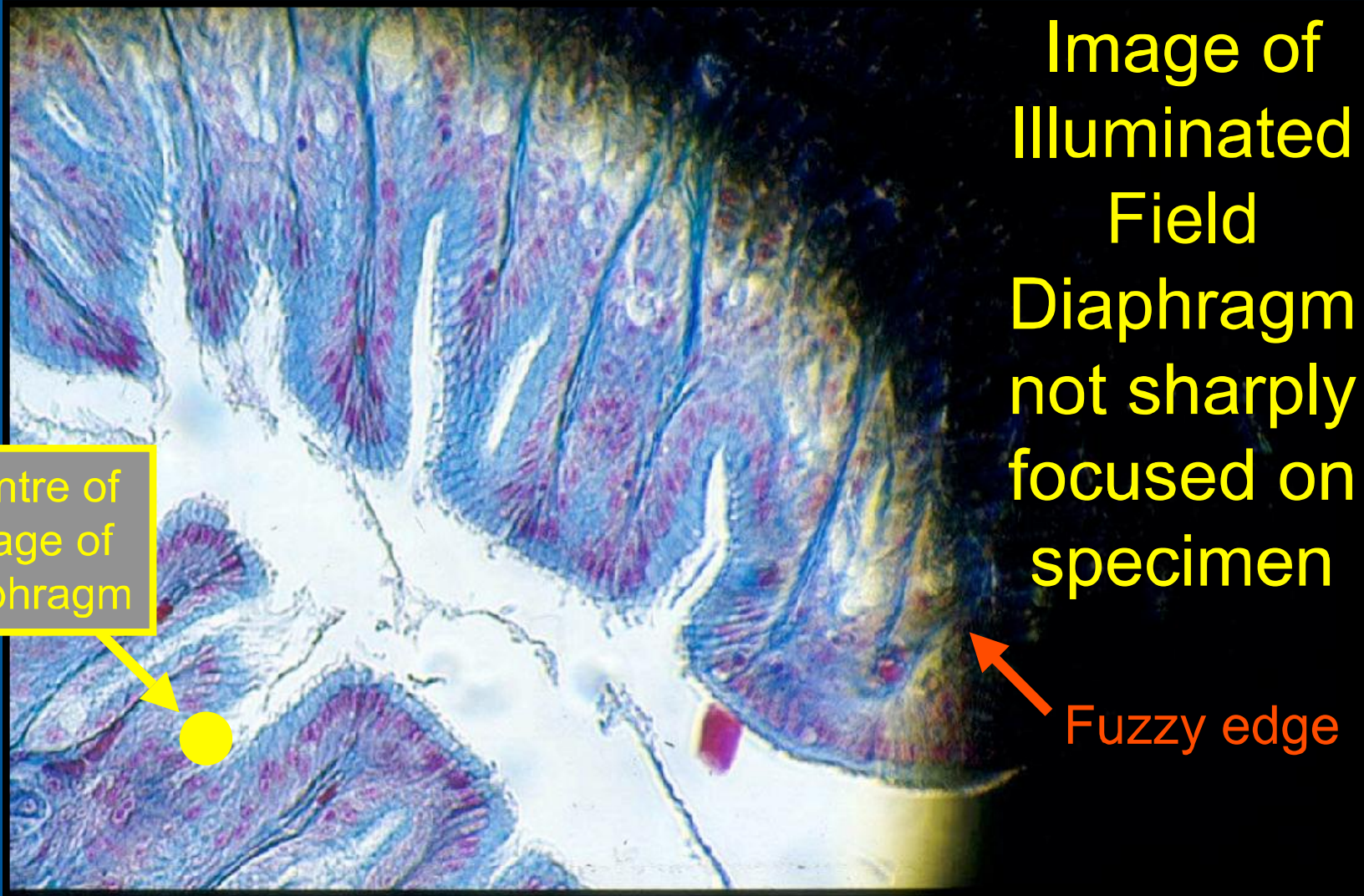


# Illuminating system completely out of adjustment





Illuminated Field Diaphragm closed;  
its image is not centred on field of view



Centre of  
image of  
diaphragm

Image of  
Illuminated  
Field  
Diaphragm  
not sharply  
focused on  
specimen

Fuzzy edge

# Condenser focus adjusted

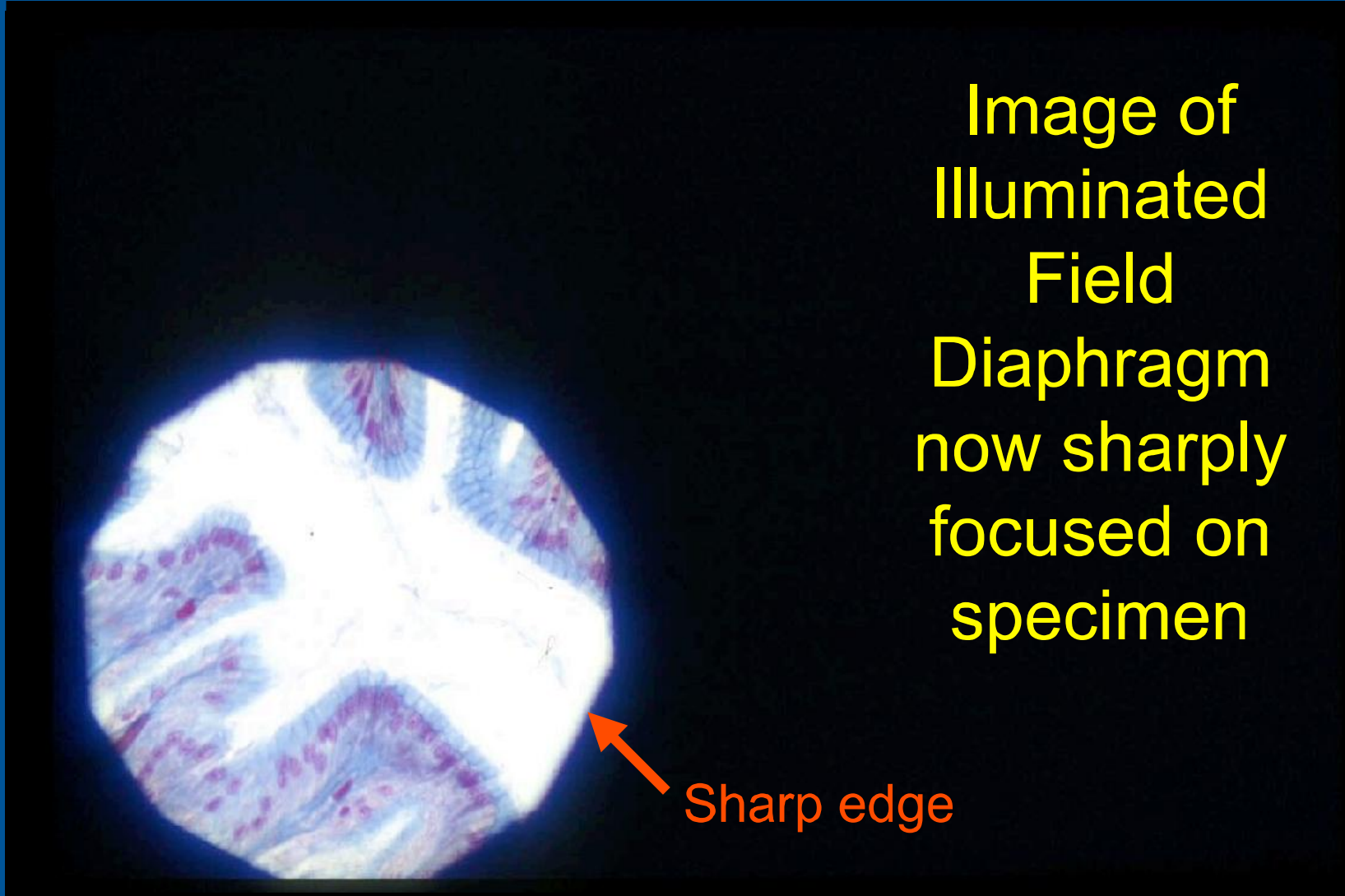
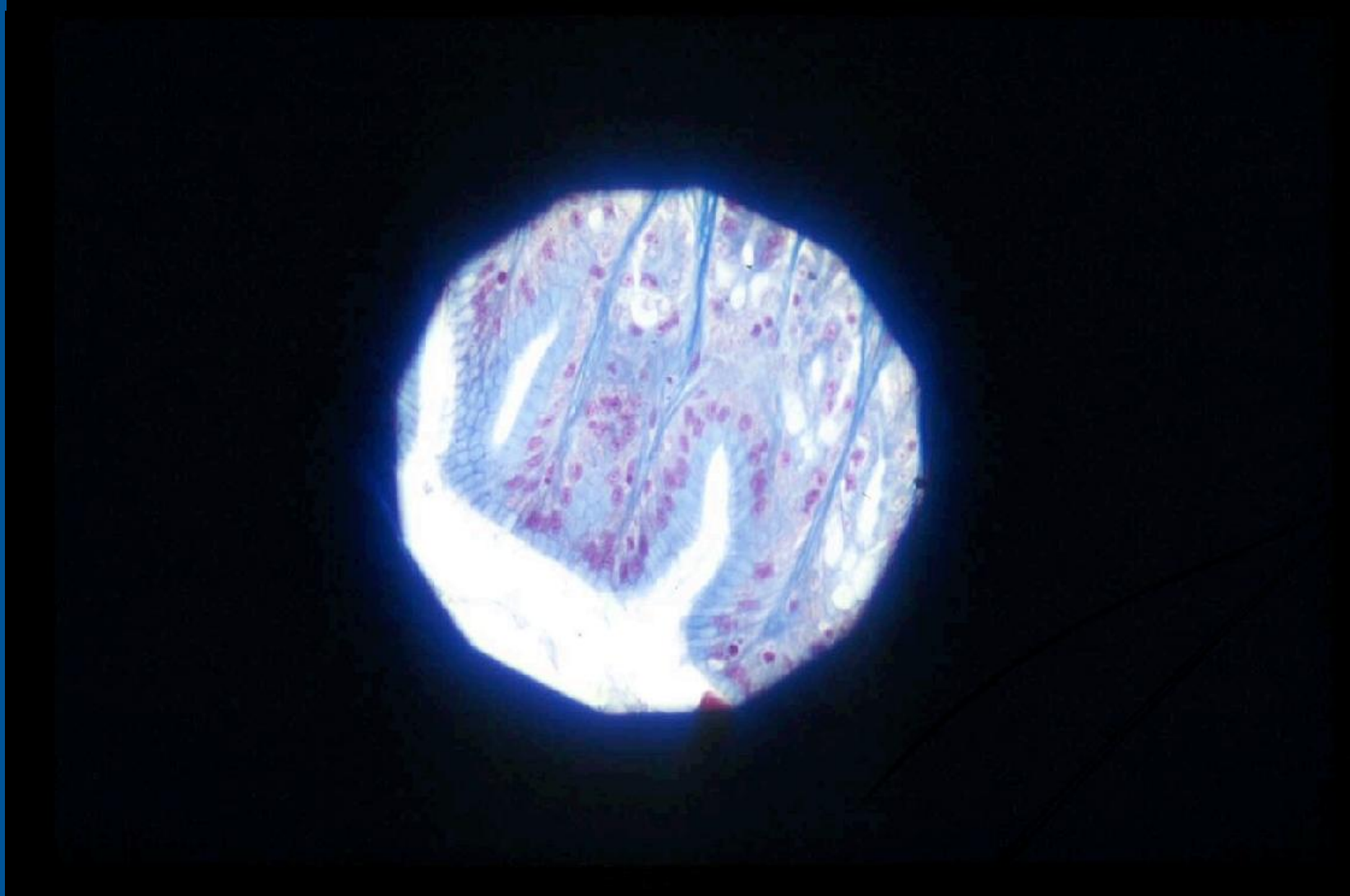


Image of  
Illuminated  
Field  
Diaphragm  
now sharply  
focused on  
specimen

Sharp edge

# Image of Illuminated Field Diaphragm now centred on field of view



# Image of Illuminated Field

## Diaphragm centred on field of view

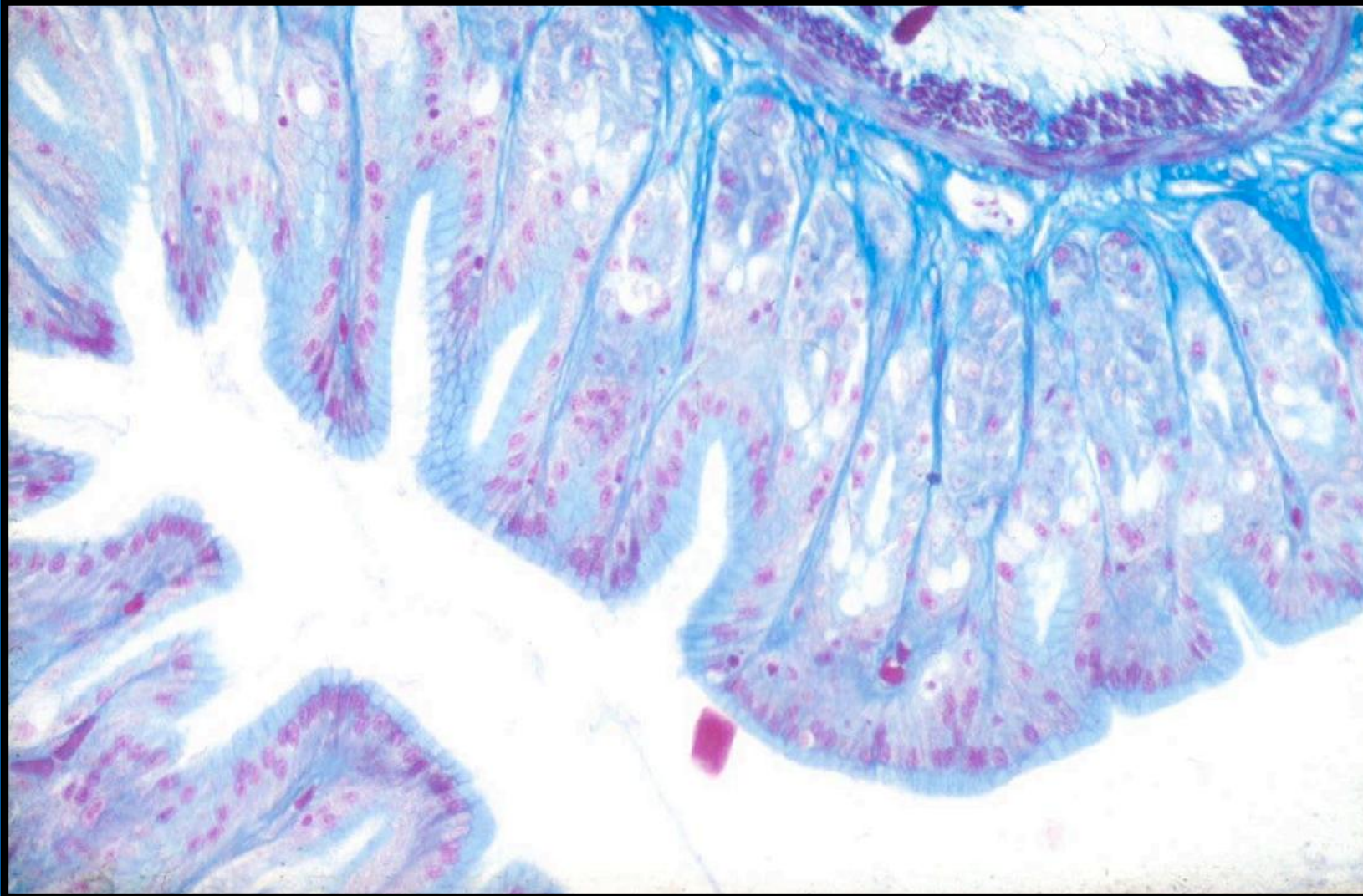
### How?

- moving the condenser lens in x-y
- moving the Illuminated Field Diaphragm in x-y
- waggling the microscope mirror
- moving the entire lamp about on the bench
- centring the objective lens

**Most common  
system**



# Illuminated Field Diaphragm opened to illuminate full field of view



# Back Focal Plane of objective



Lamp not  
centred  
with  
collector  
lens

# Back Focal Plane of objective



Lamp  
centred  
with  
collector  
lens

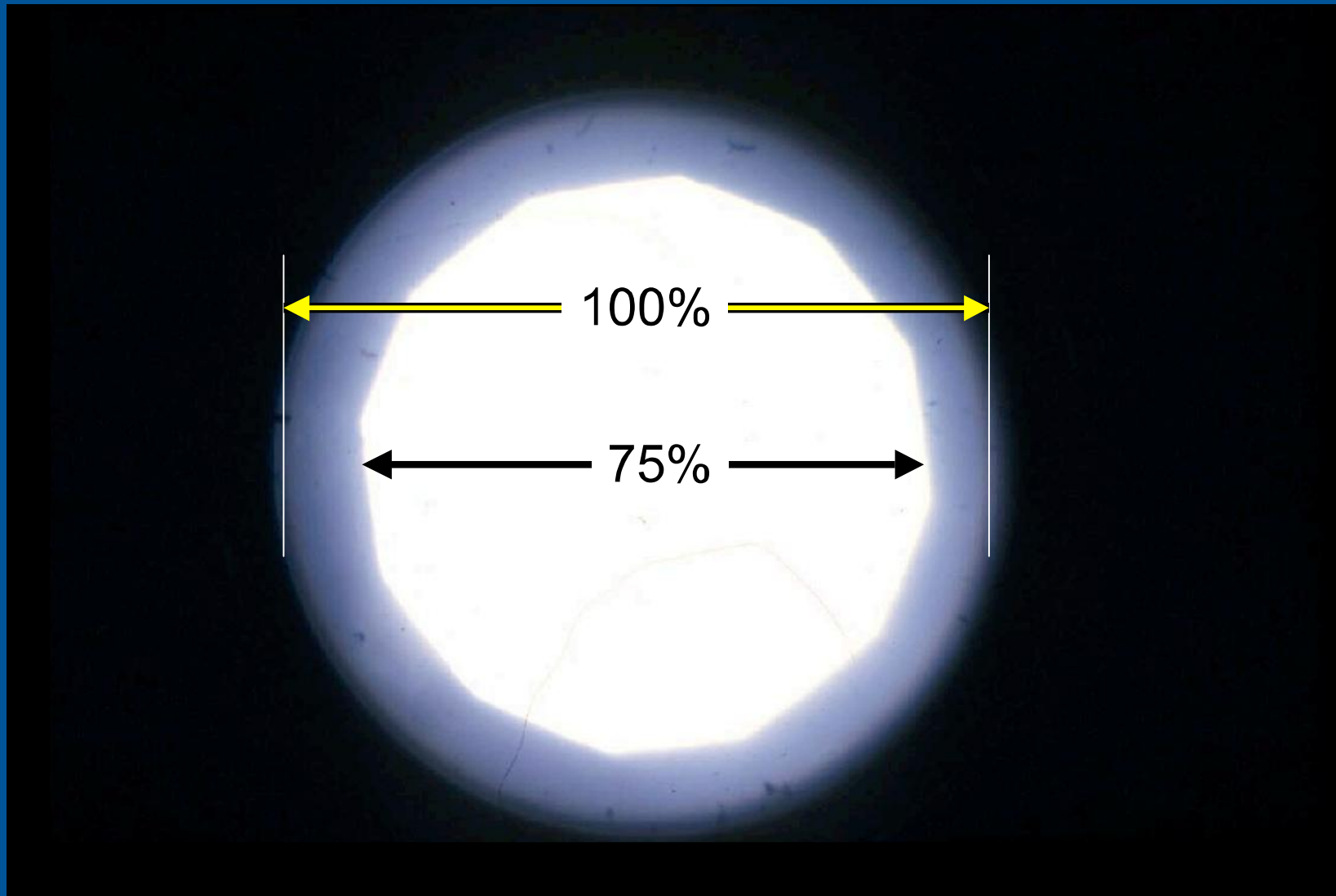


# Diffuser inserted between lamp and collector lens





# Illuminating Aperture Diaphragm closed to c75% of objective aperture



# Illuminating Aperture too large



Image hazy and 'washed out'



# Illuminating Aperture correct



Image contrast optimal



# Illuminating Aperture too small

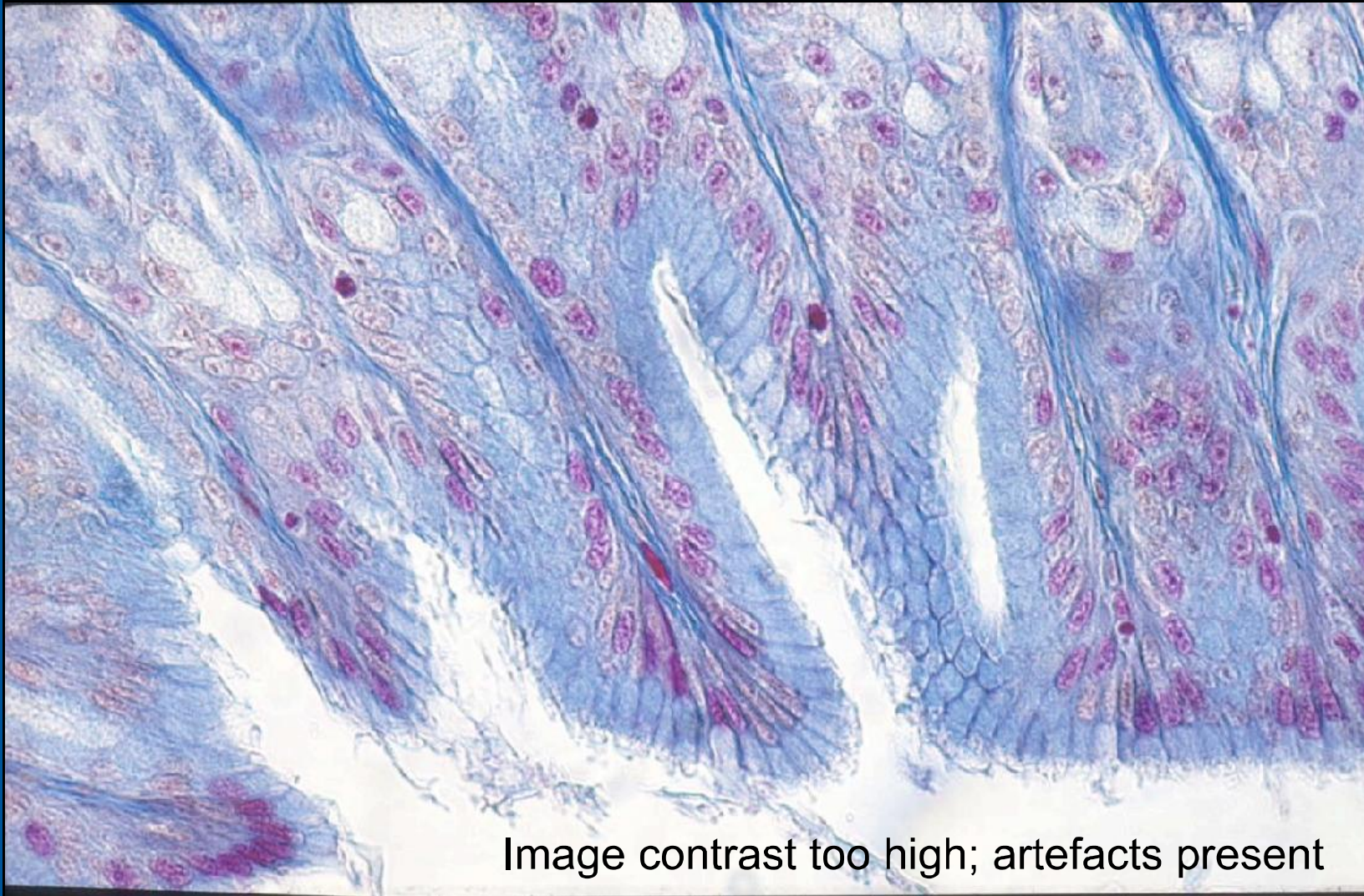
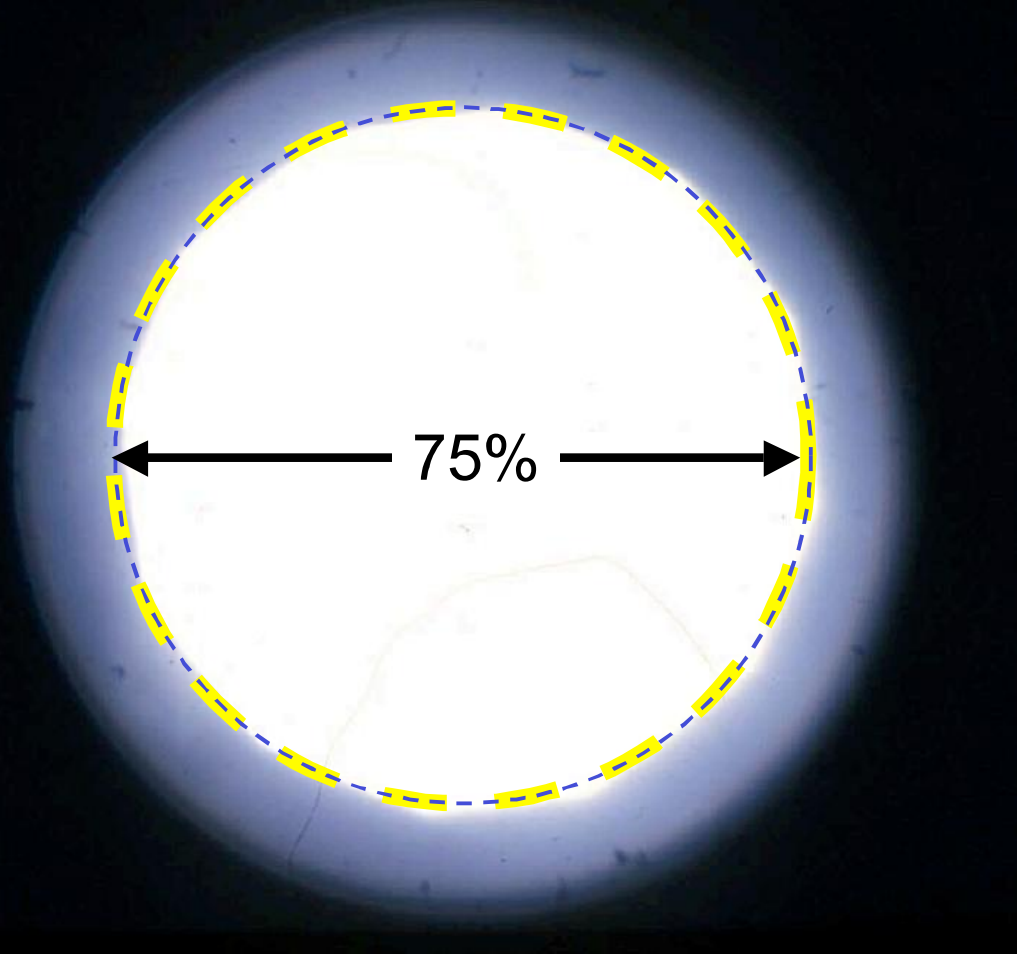


Image contrast too high; artefacts present

# Illuminating Aperture

Correct

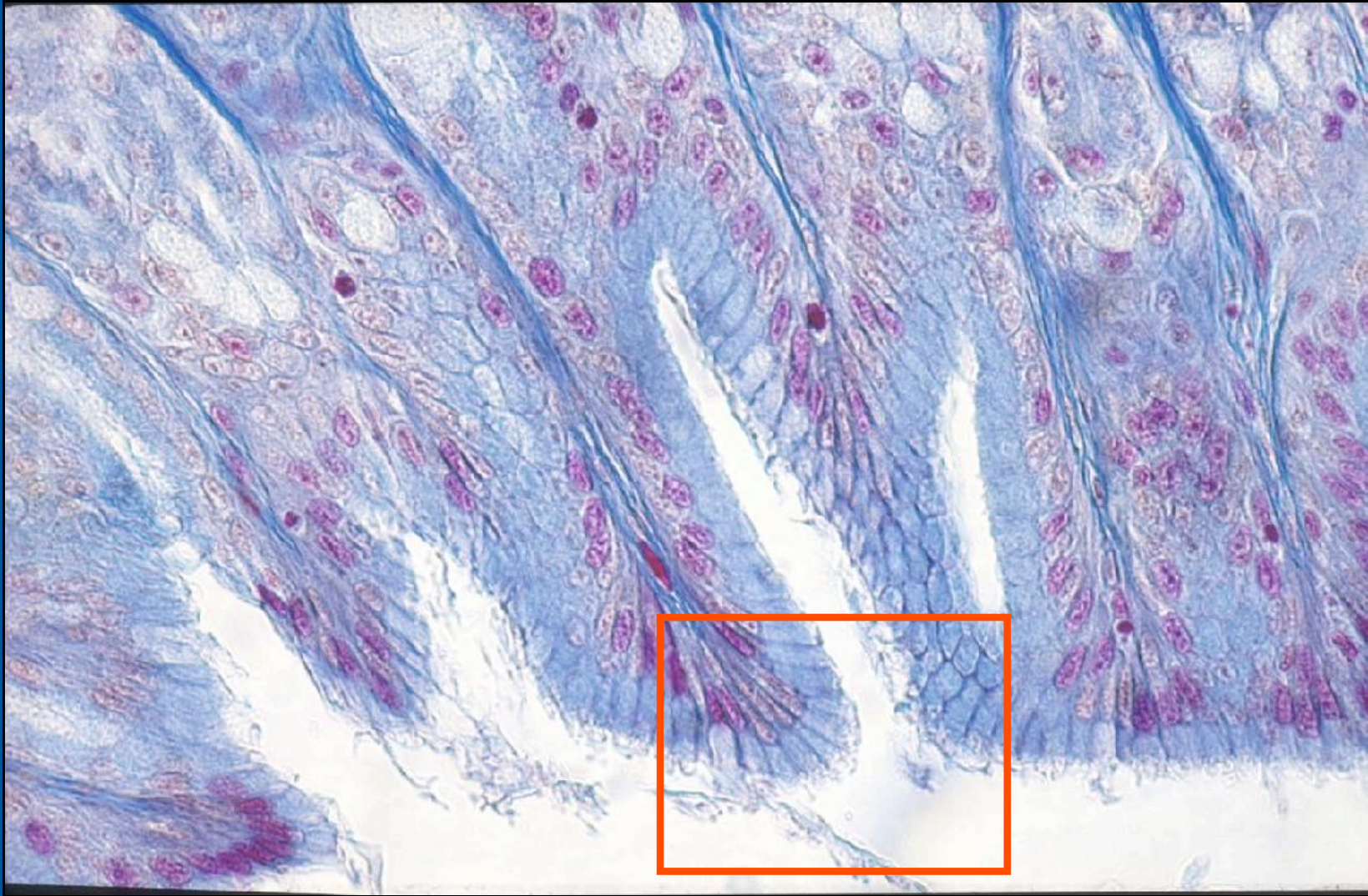


Too small

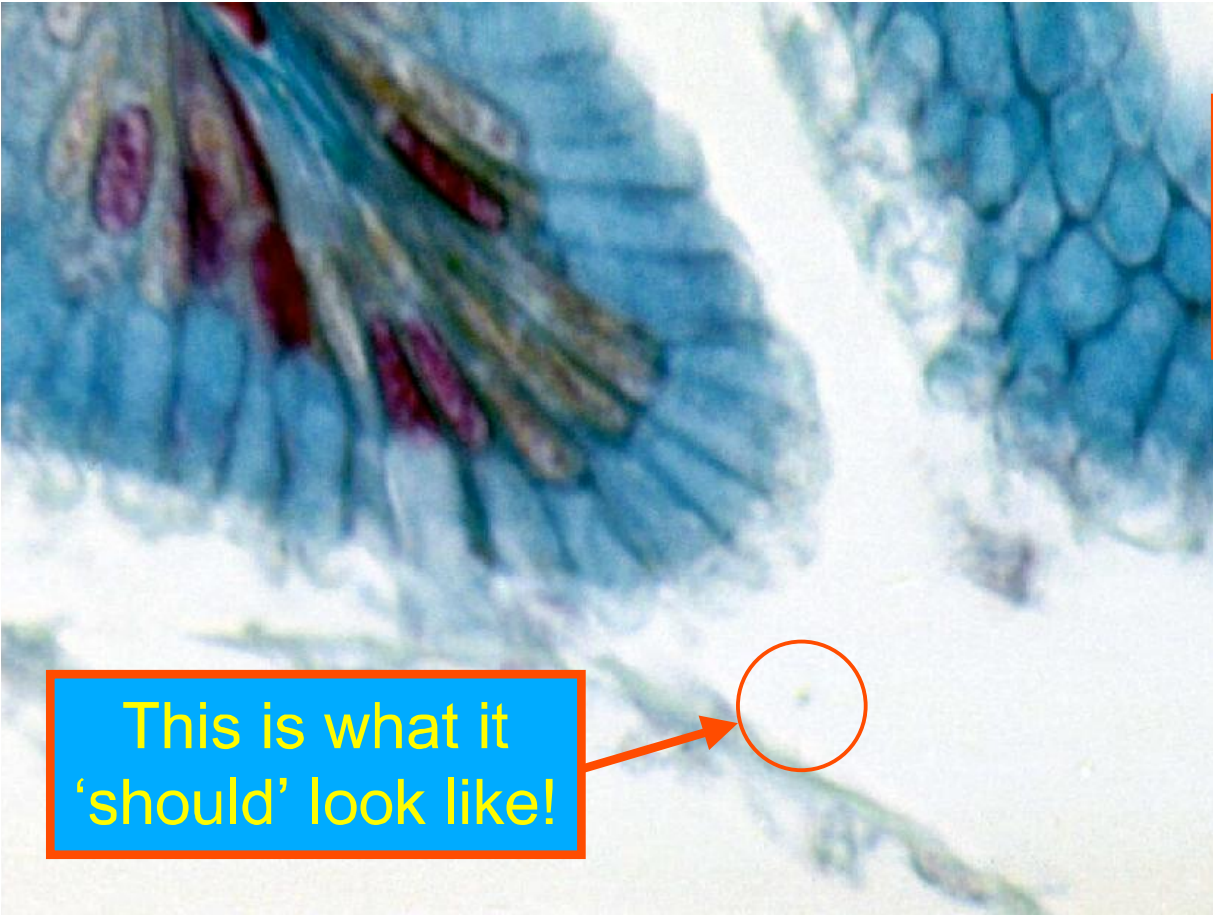




# Illuminating Aperture too small







Illuminating  
aperture  
correct

This image shows a micrograph of plant tissue, likely a leaf cross-section, stained with a blue dye. The cells are clearly visible, and the overall appearance is sharp and well-defined. A small, circular object is circled in red, and an arrow points to it from the text box below.

This is what it  
'should' look like!

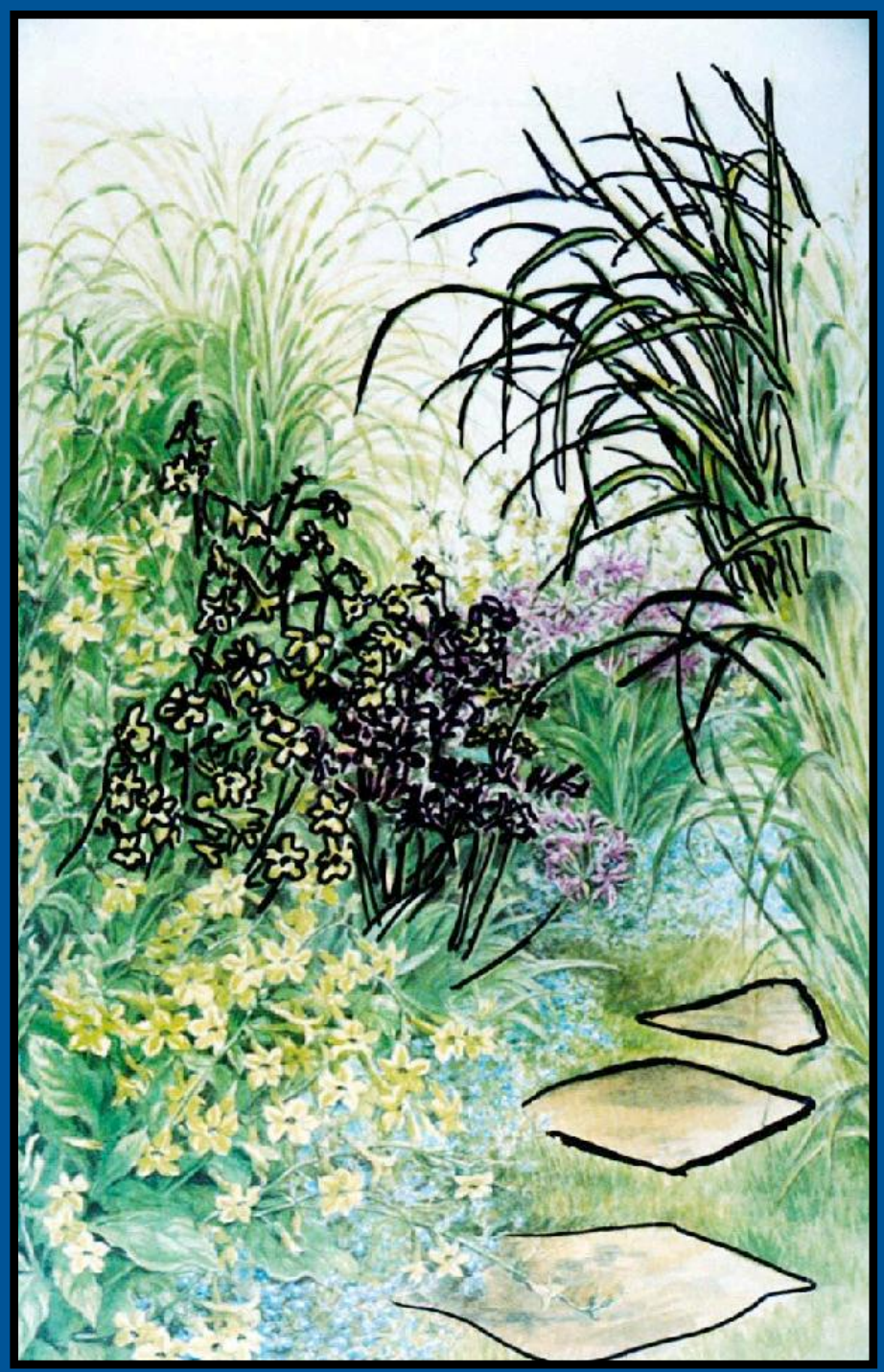
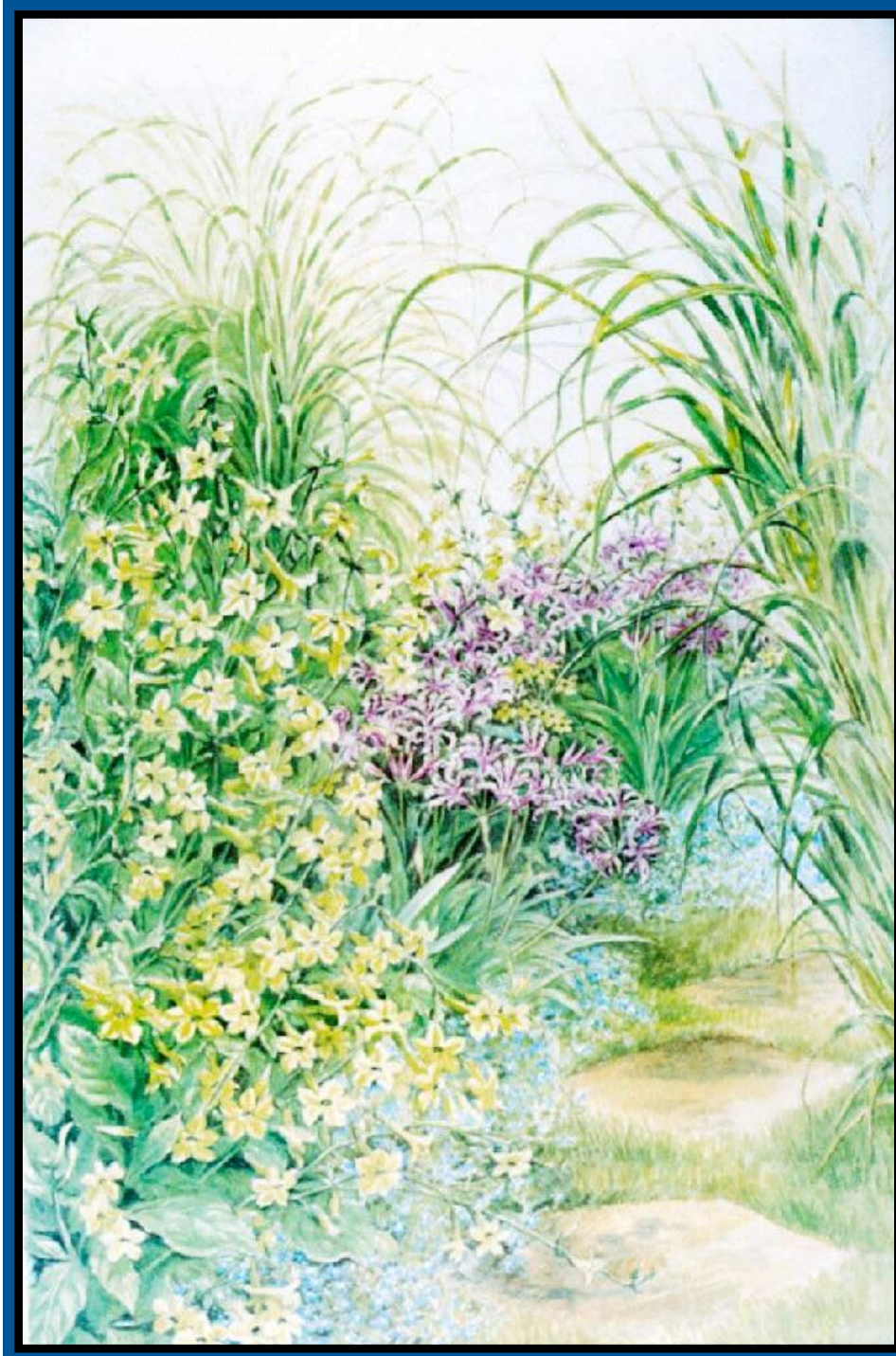


Illuminating  
aperture  
too small

This image shows the same micrograph of plant tissue as above, but with a smaller illuminating aperture. The overall appearance is much less sharp, and the details are significantly blurred. A small, circular object is circled in red, and an arrow points to it from the text box below.

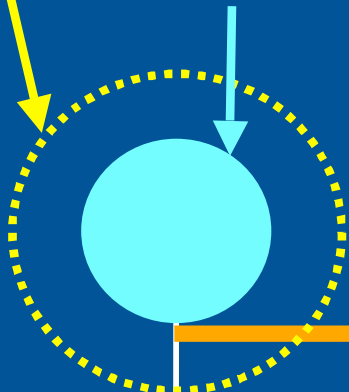
Note this object



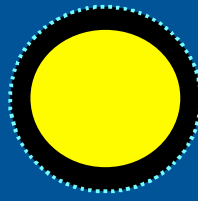
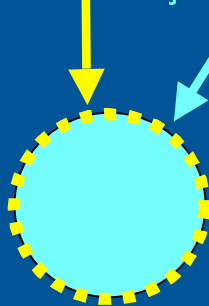




Illuminating aperture wide open:  
much larger than  
objective aperture



Gradually close illuminating  
aperture diaphragm.  
Illuminating aperture now equal to  
objective aperture

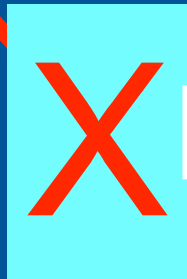


Setting the Illuminating  
aperture diaphragm –  
a simple way

**Optimum setting:**  
Stray light removed but  
still have adequate  
illuminating aperture

Removing stray light

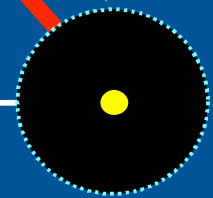
Image  
brightness

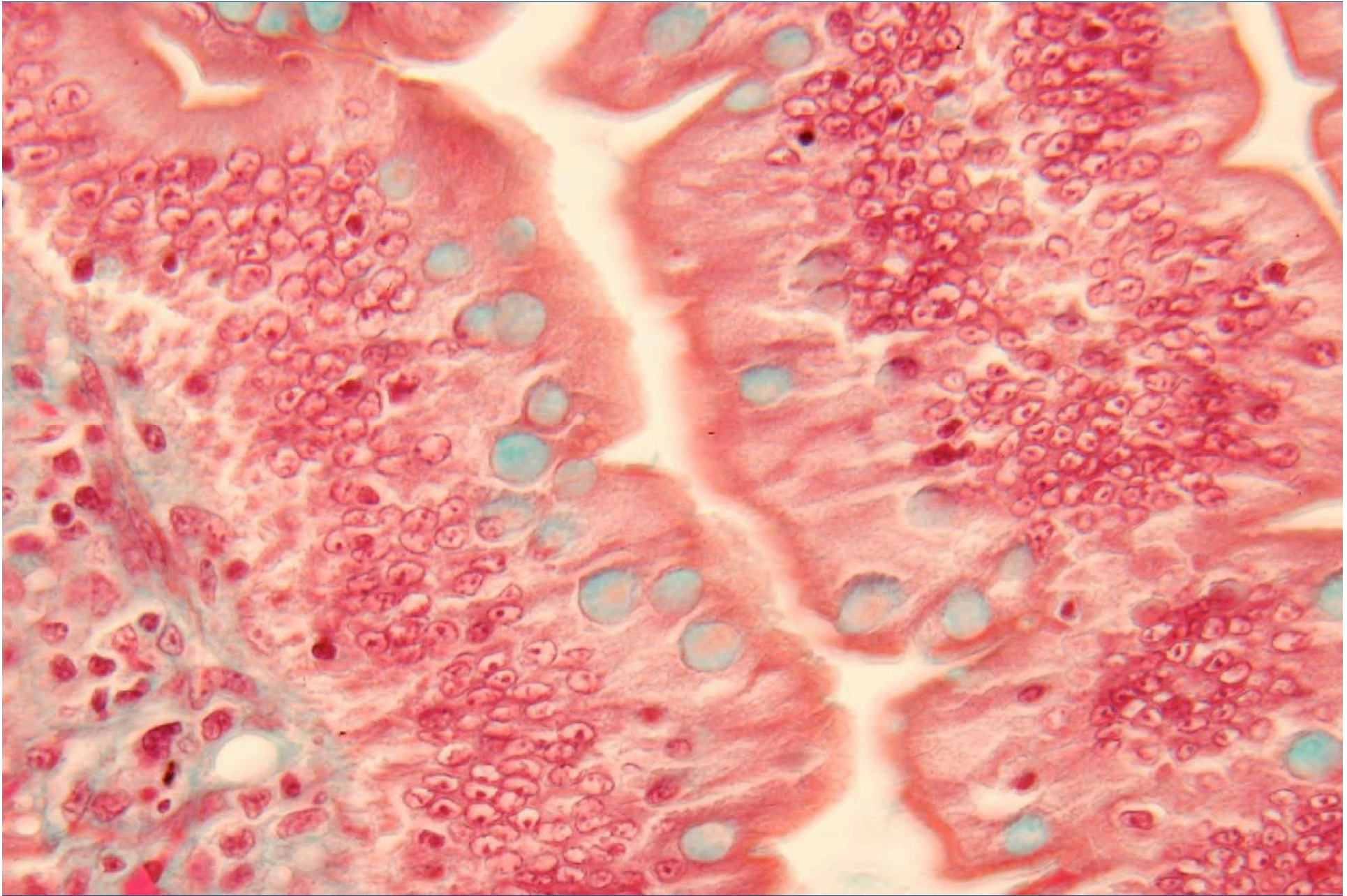


**Poor image!**

Illuminating aperture  
much smaller than  
objective aperture

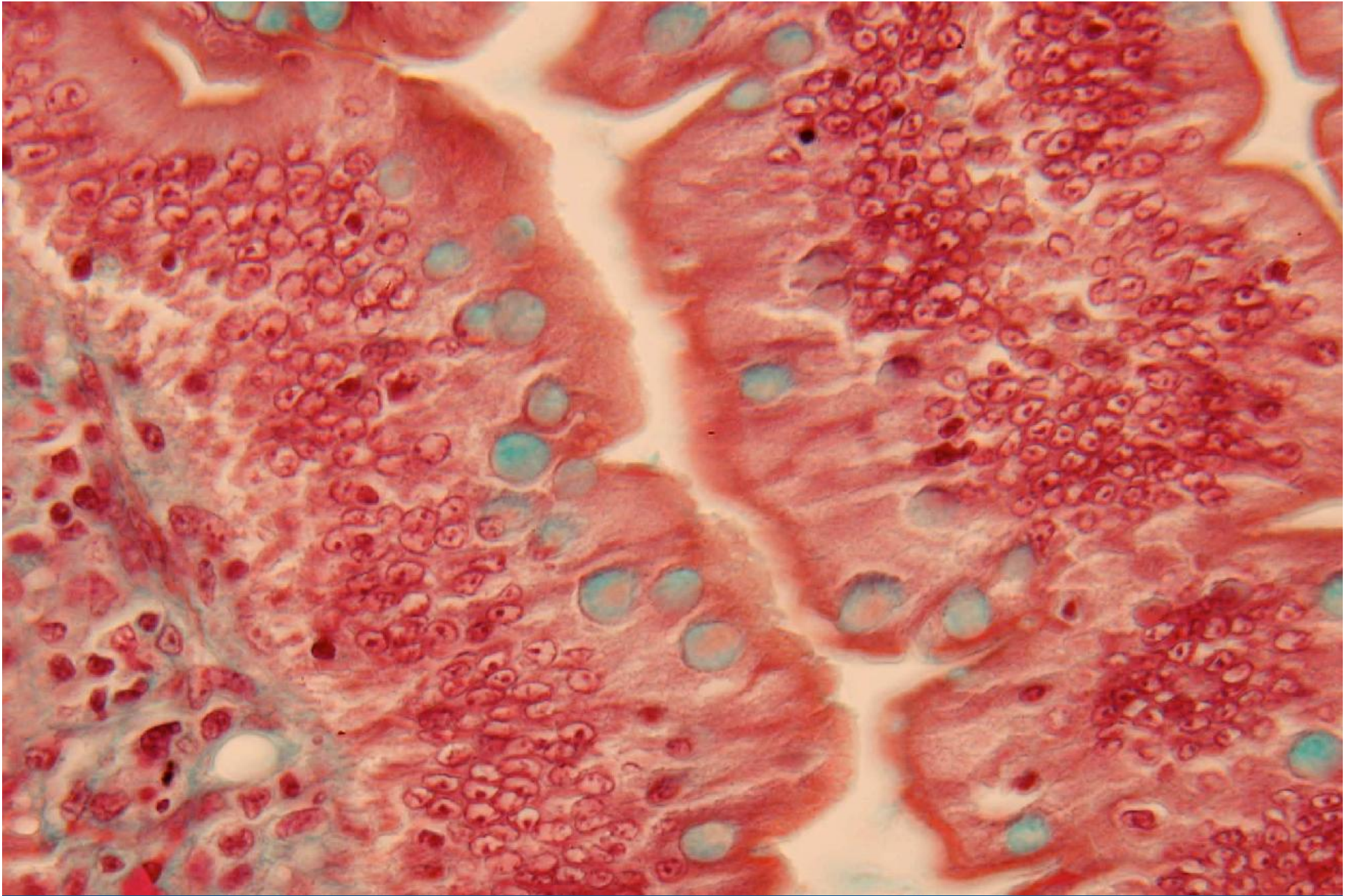
Reducing illuminating aperture





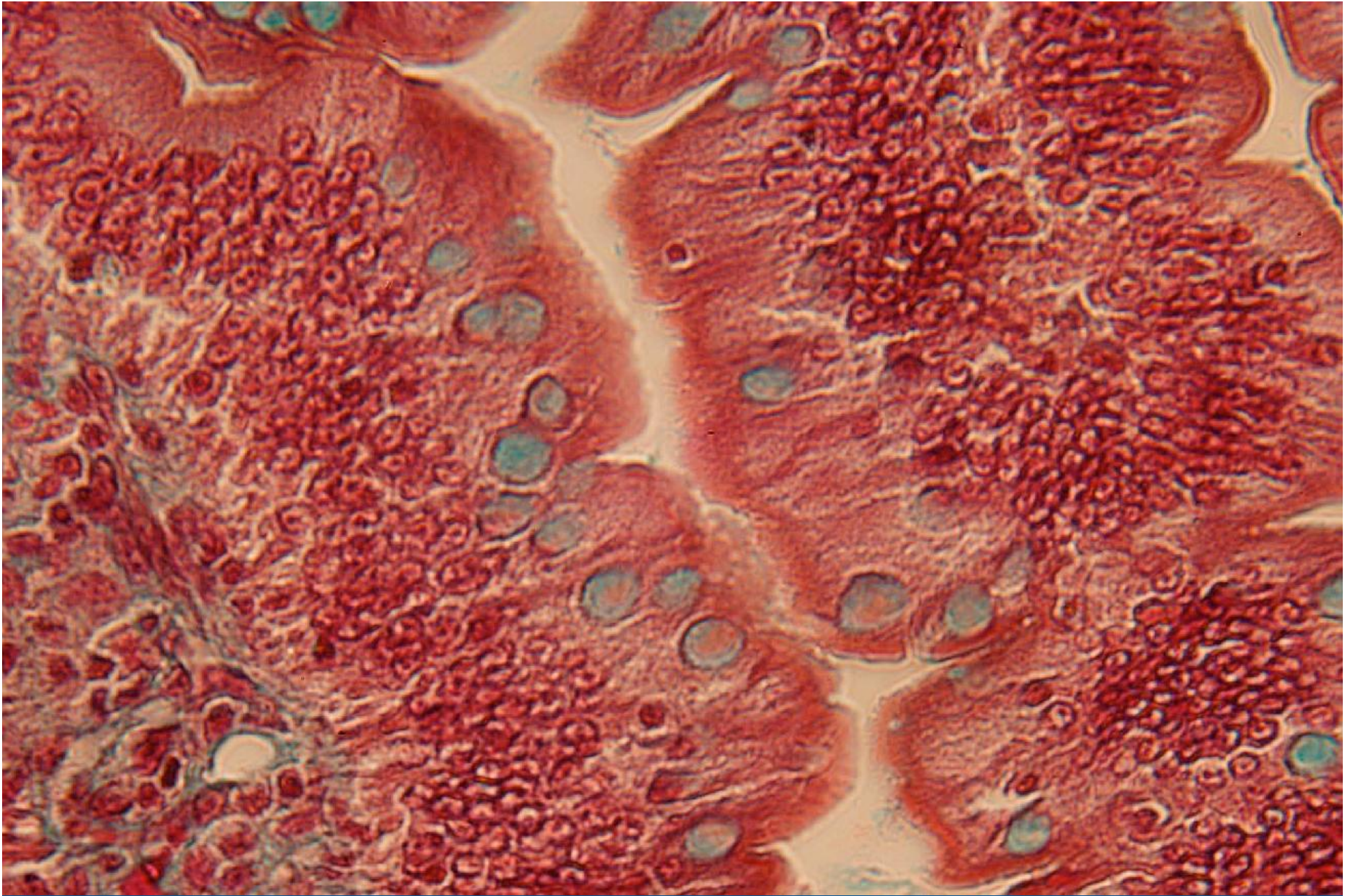
**Illuminating aperture too large**





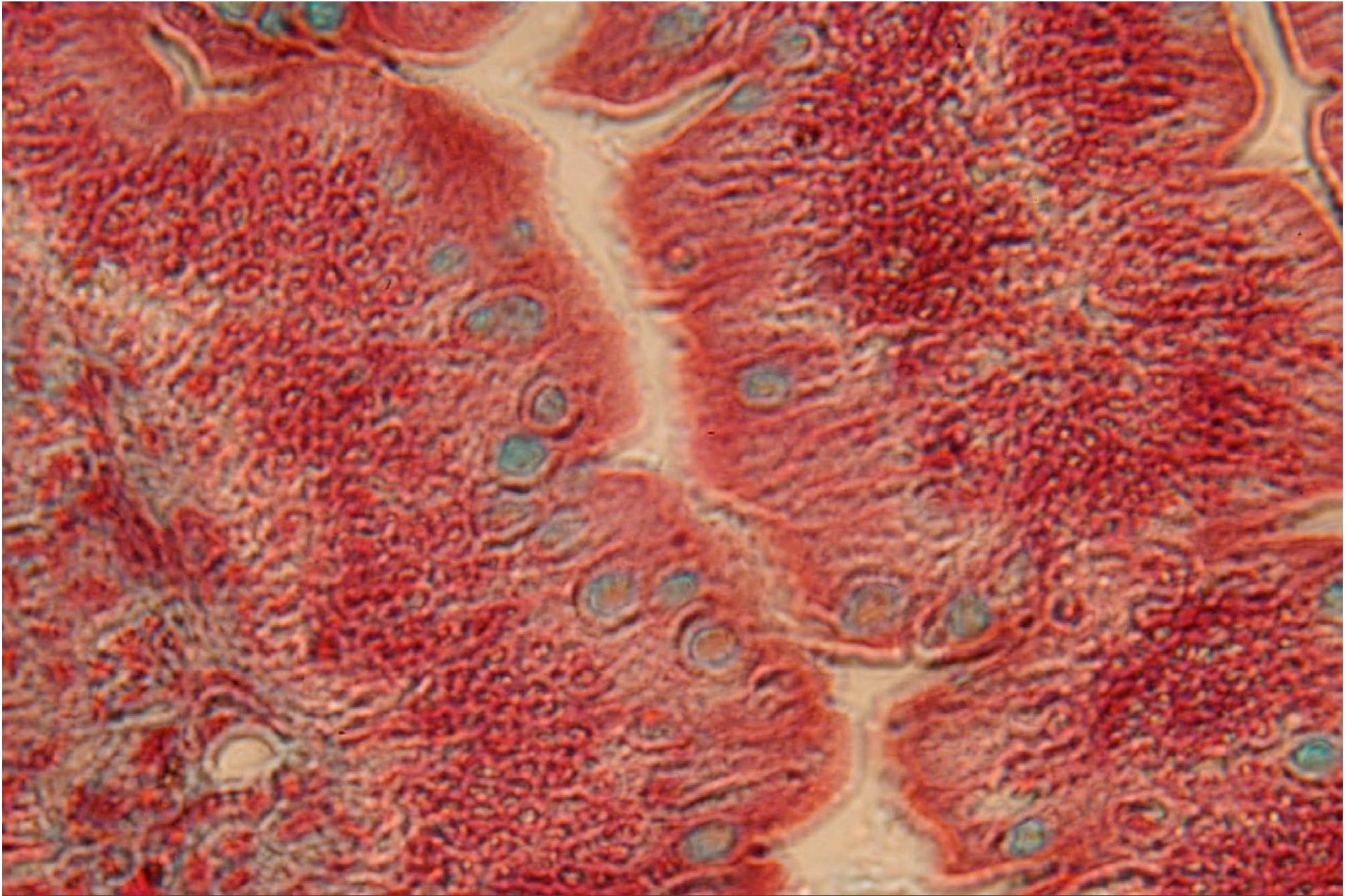
**Illuminating aperture correct**





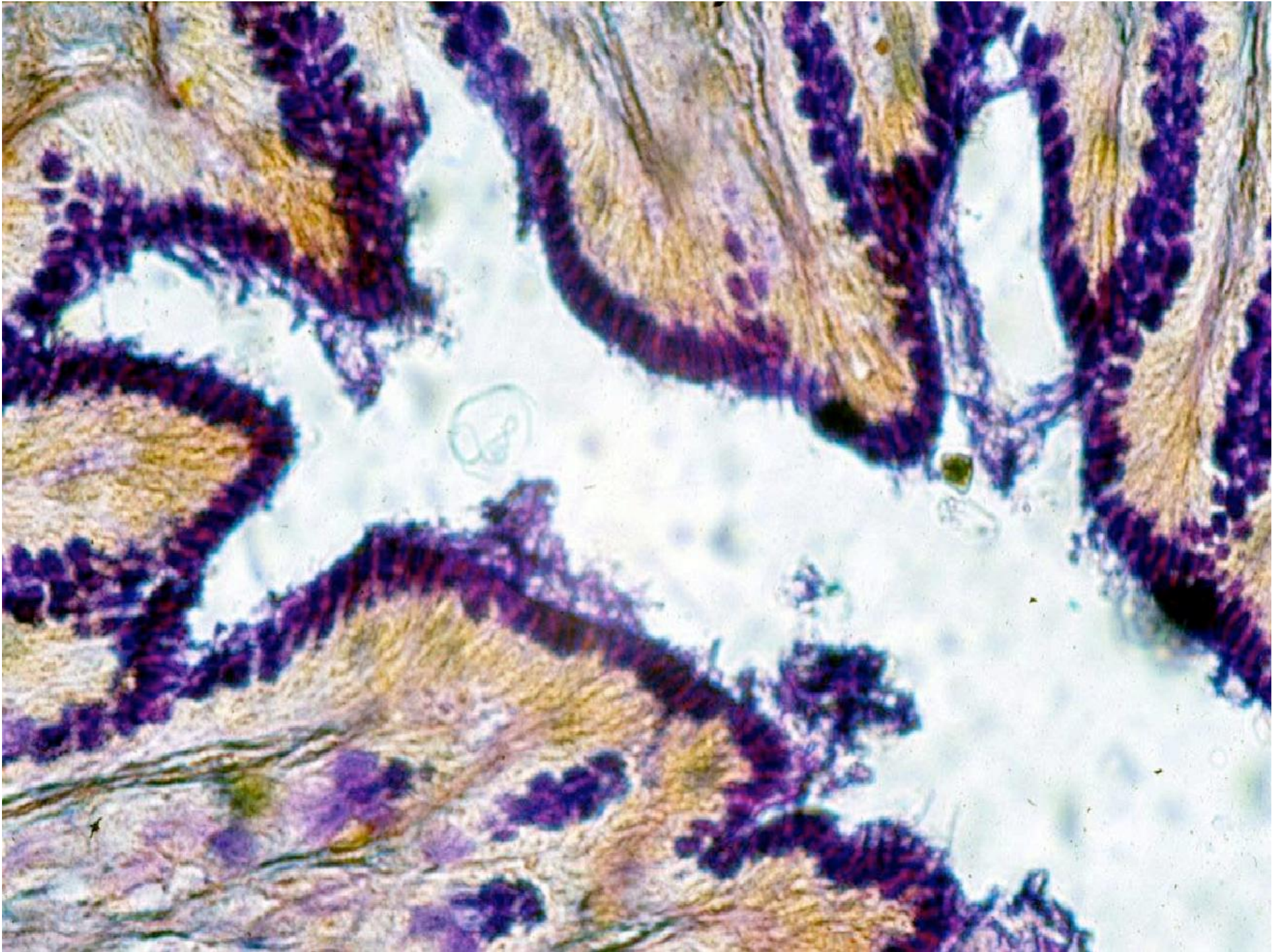
**Illuminating aperture too small**





**Illuminating aperture much too small – an extreme example**

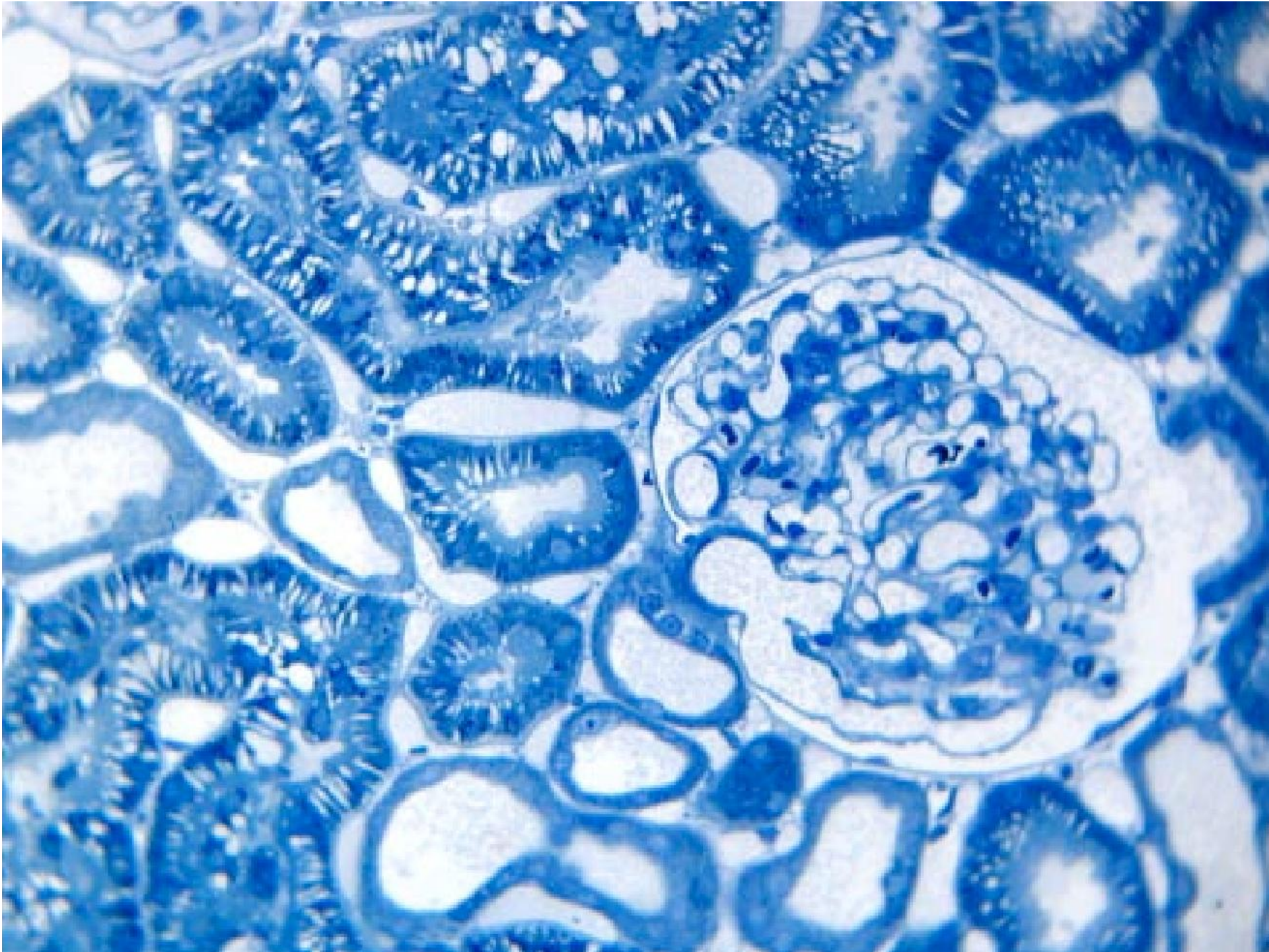












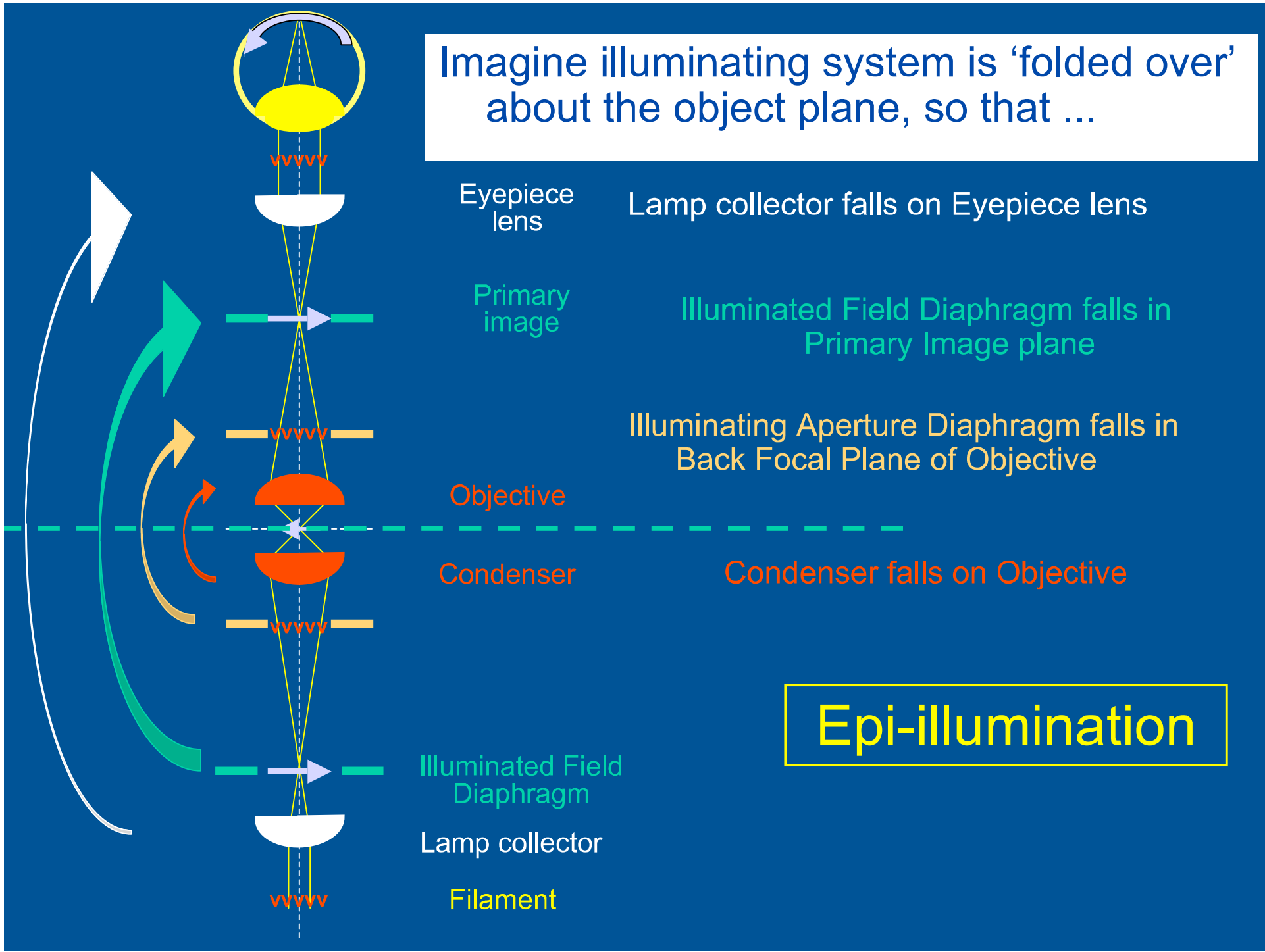
# Köhler Illumination provides

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Control of **Area** illuminated by the  
**Illuminated Field Diaphragm**,  
which is adjusted according to **magnification**.

Control of **Angle** of illumination by the  
**Illuminating Aperture Diaphragm**  
(the condenser diaphragm),  
which is adjusted according to objective **aperture**.

Imagine illuminating system is 'folded over' about the object plane, so that ...



Eyepiece lens

Lamp collector falls on Eyepiece lens

Primary image

Illuminated Field Diaphragm falls in Primary Image plane

Objective

Illuminating Aperture Diaphragm falls in Back Focal Plane of Objective

Condenser

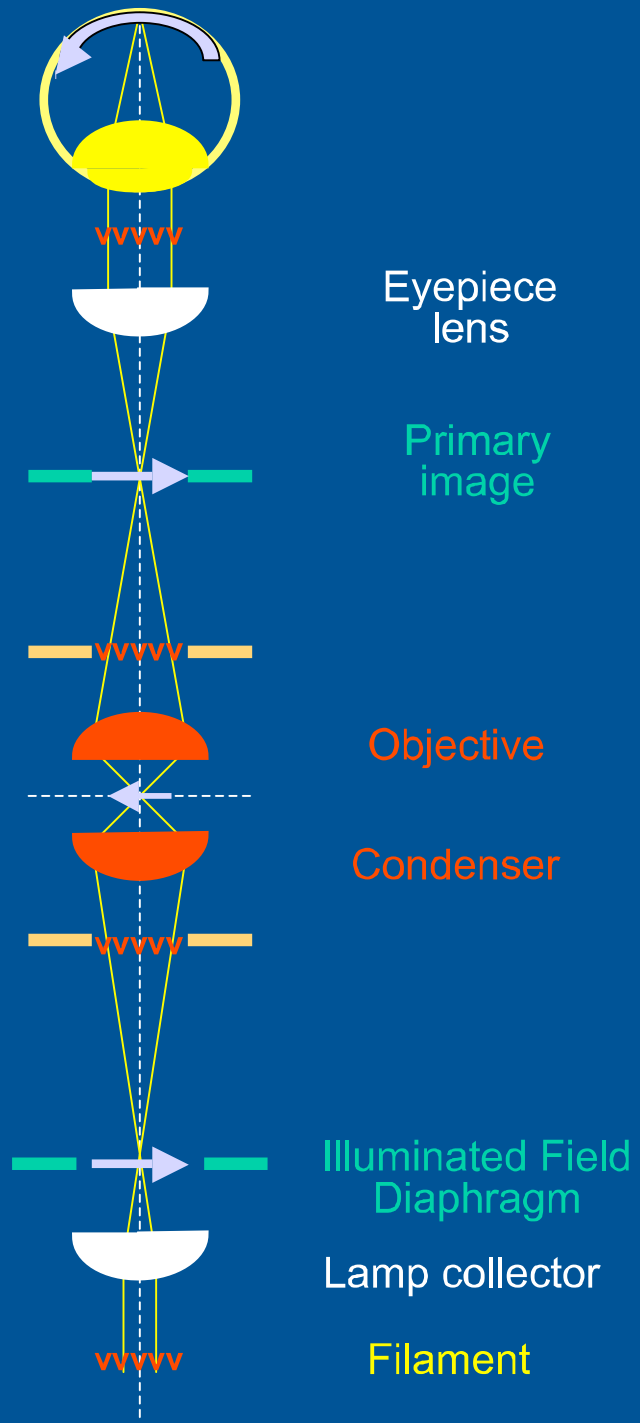
Condenser falls on Objective

Illuminated Field Diaphragm

Lamp collector

Filament

Epi-illumination



Eyepiece lens

Primary image

Objective

Condenser

Illuminated Field Diaphragm

Lamp collector

Filament

