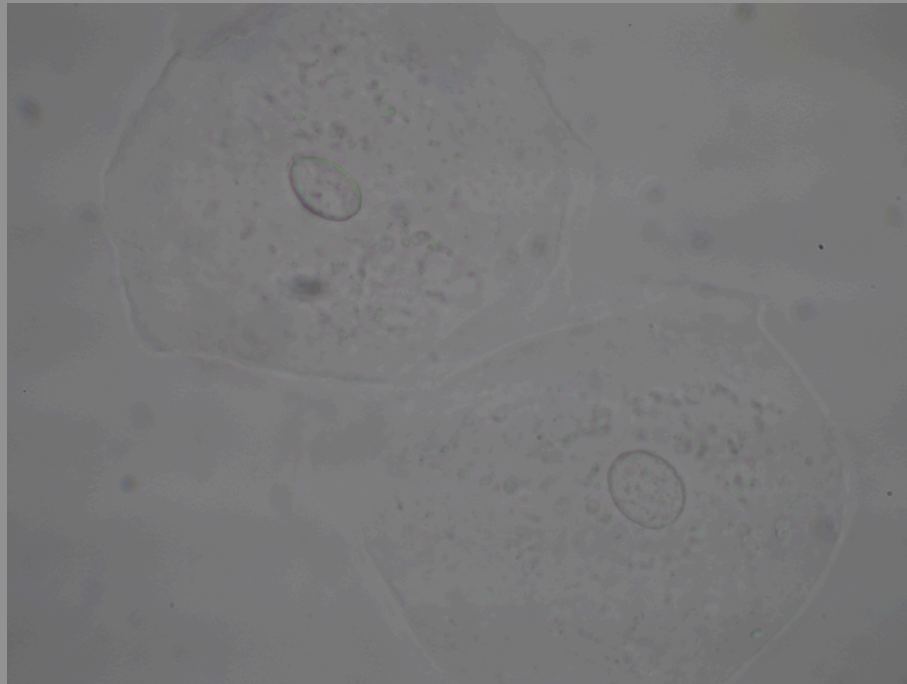
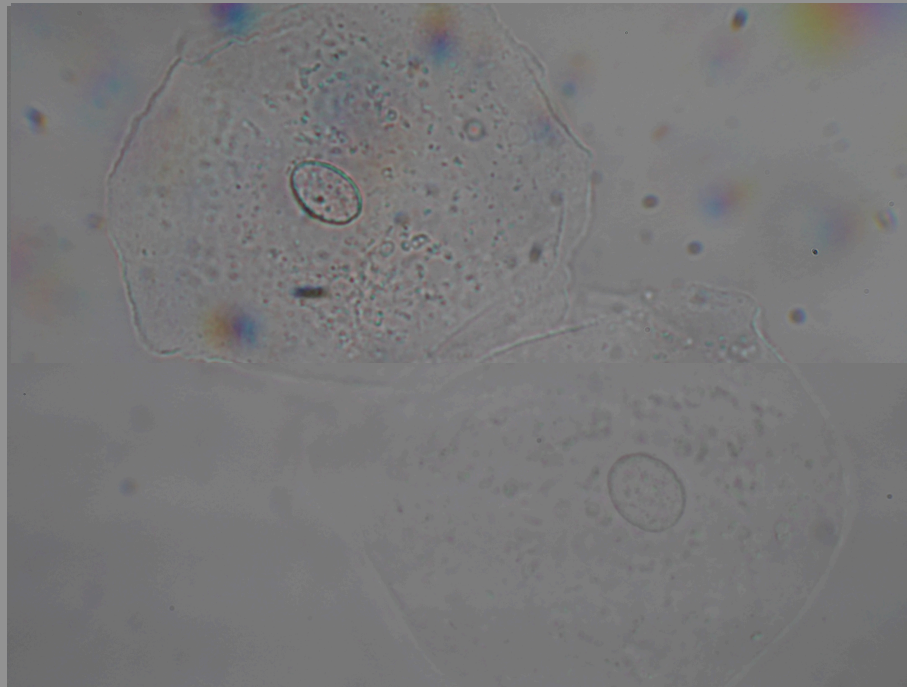

Resolution is nothing without contrast

Contrast characterises the
relative difference between
signal and background

**For thin samples contrast in brightfield microscopy
is diminished by lots of bright background light**



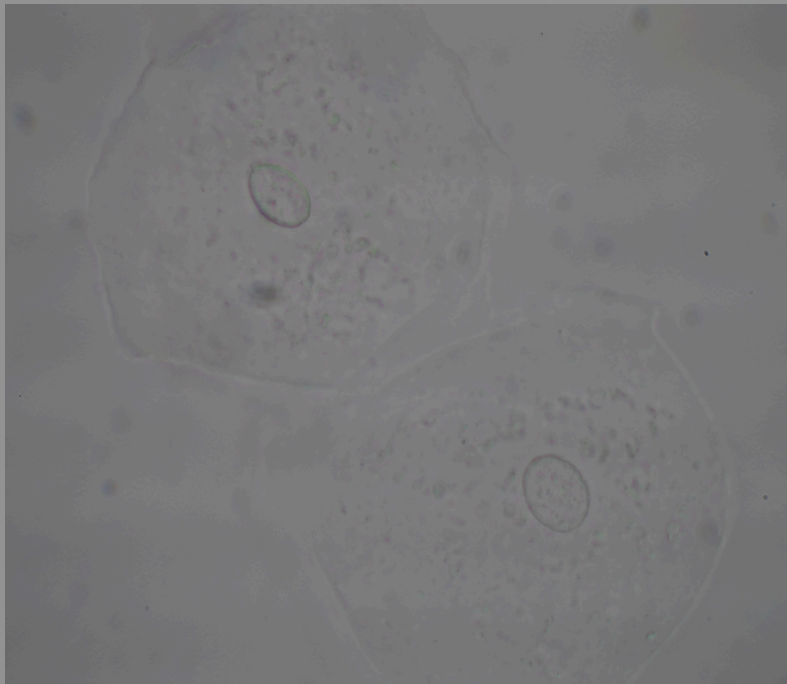
Closing the aperture diaphragm can increase contrast at the cost of introducing artifacts



Images from Peter Evennett

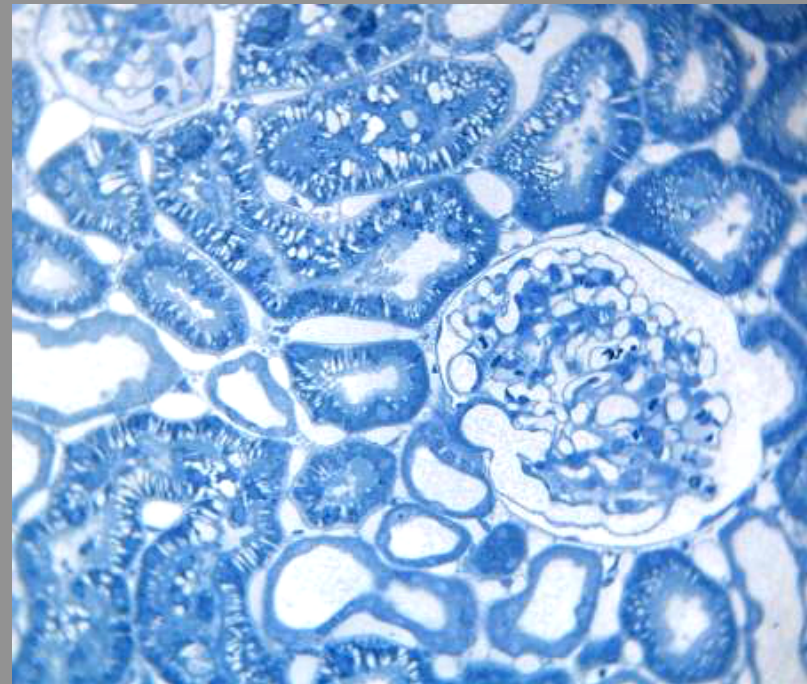
Our eyes can only perceive contrast based on
brightness or colour

Brightness



Buccal cells

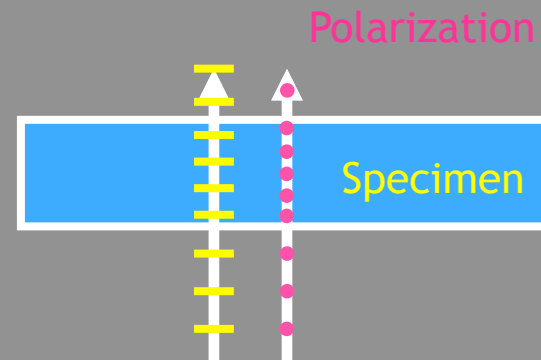
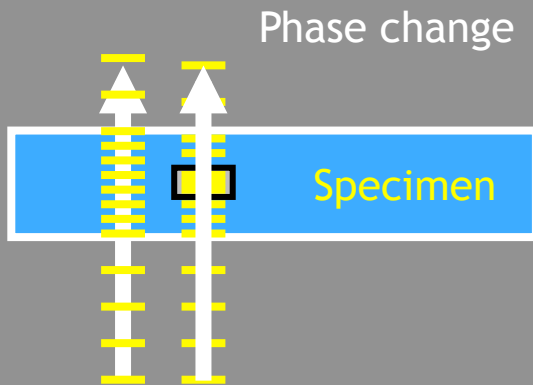
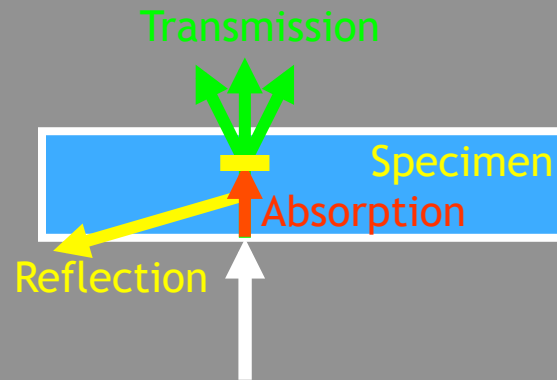
Colour



Kidney section

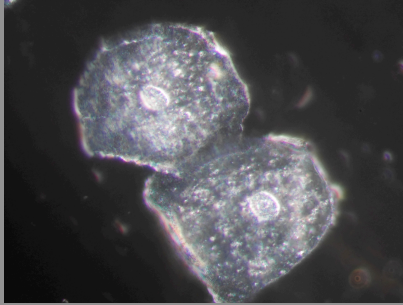
Images from Peter Evennett

Light and specimen can interact in several visible and invisible ways

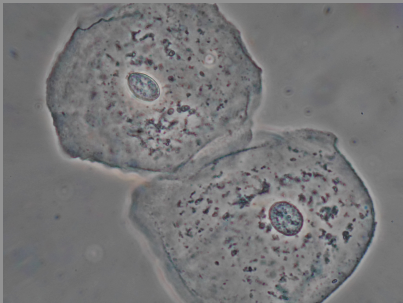


Adapted from Peter Evennett

The following contrast techniques for transmitted light imaging will be introduced



Dark Field



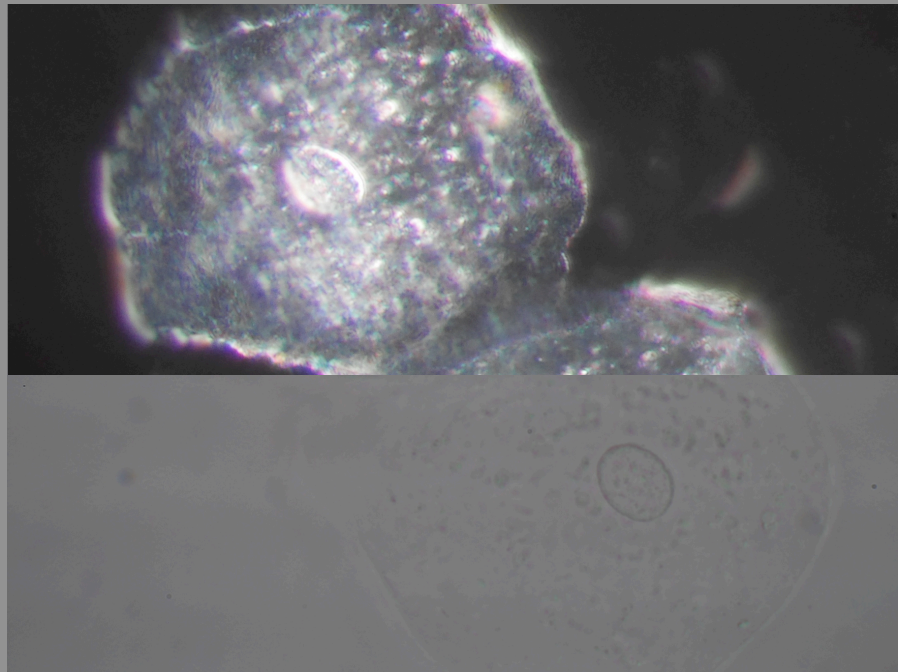
Phase Contrast



Differential Interference Contrast

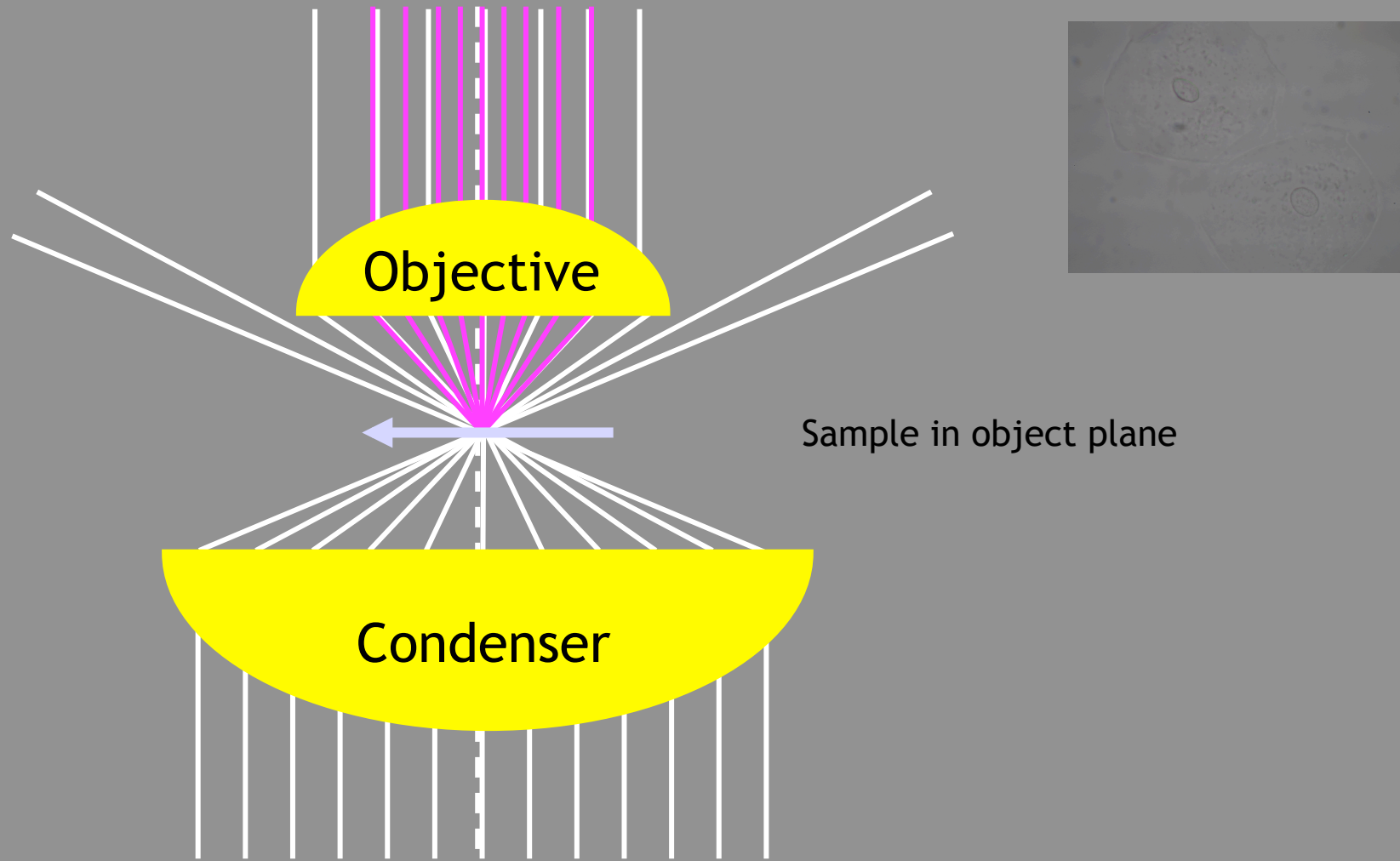
Images from Peter Evennett

Dark field illumination removes the bright background of light that does not interact with the sample

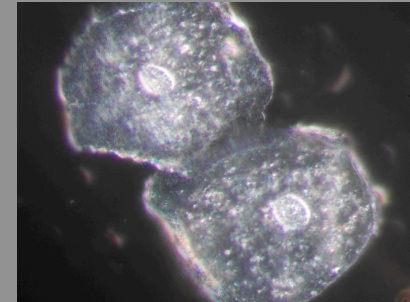
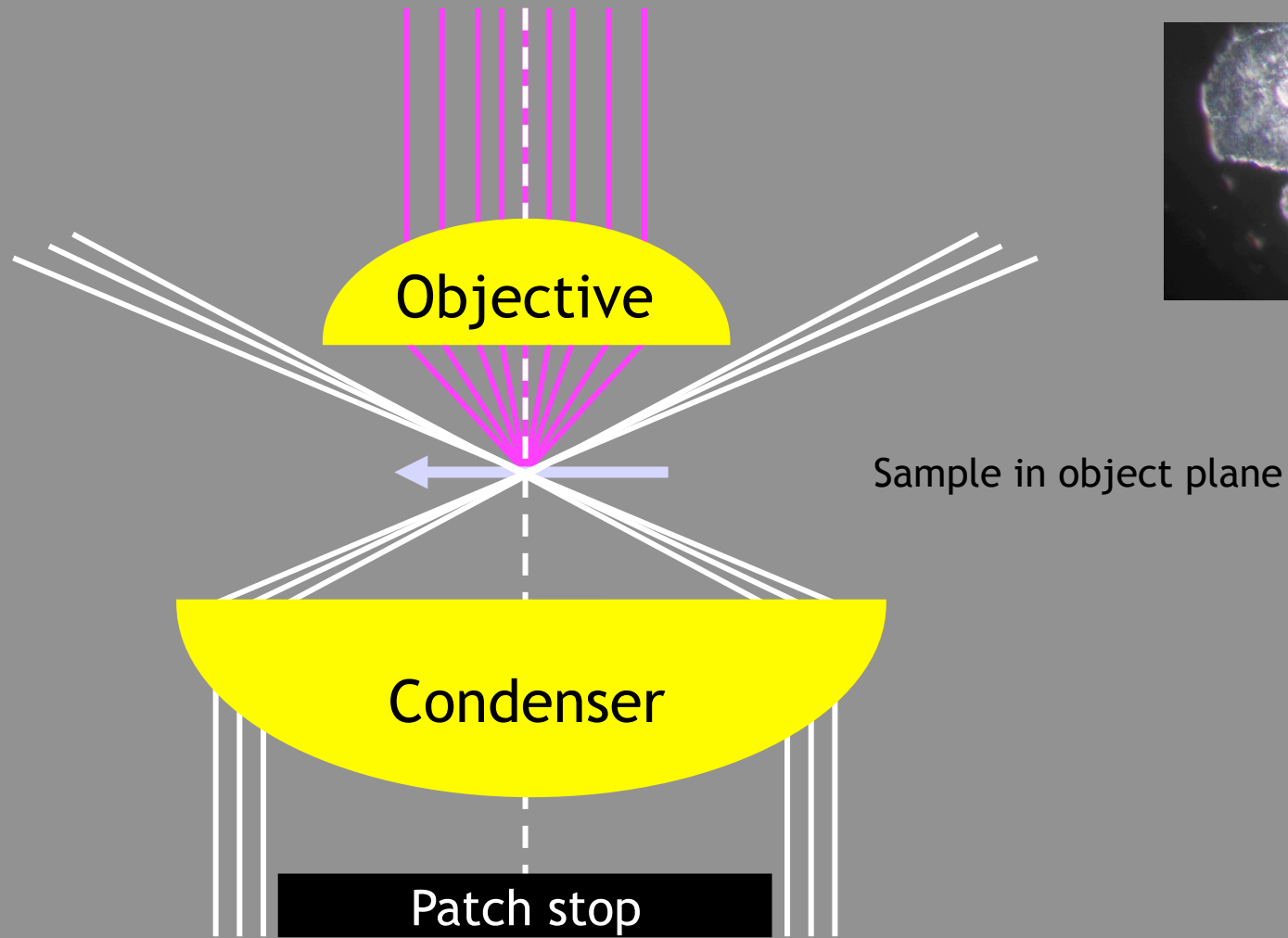


Images from Peter Evennett

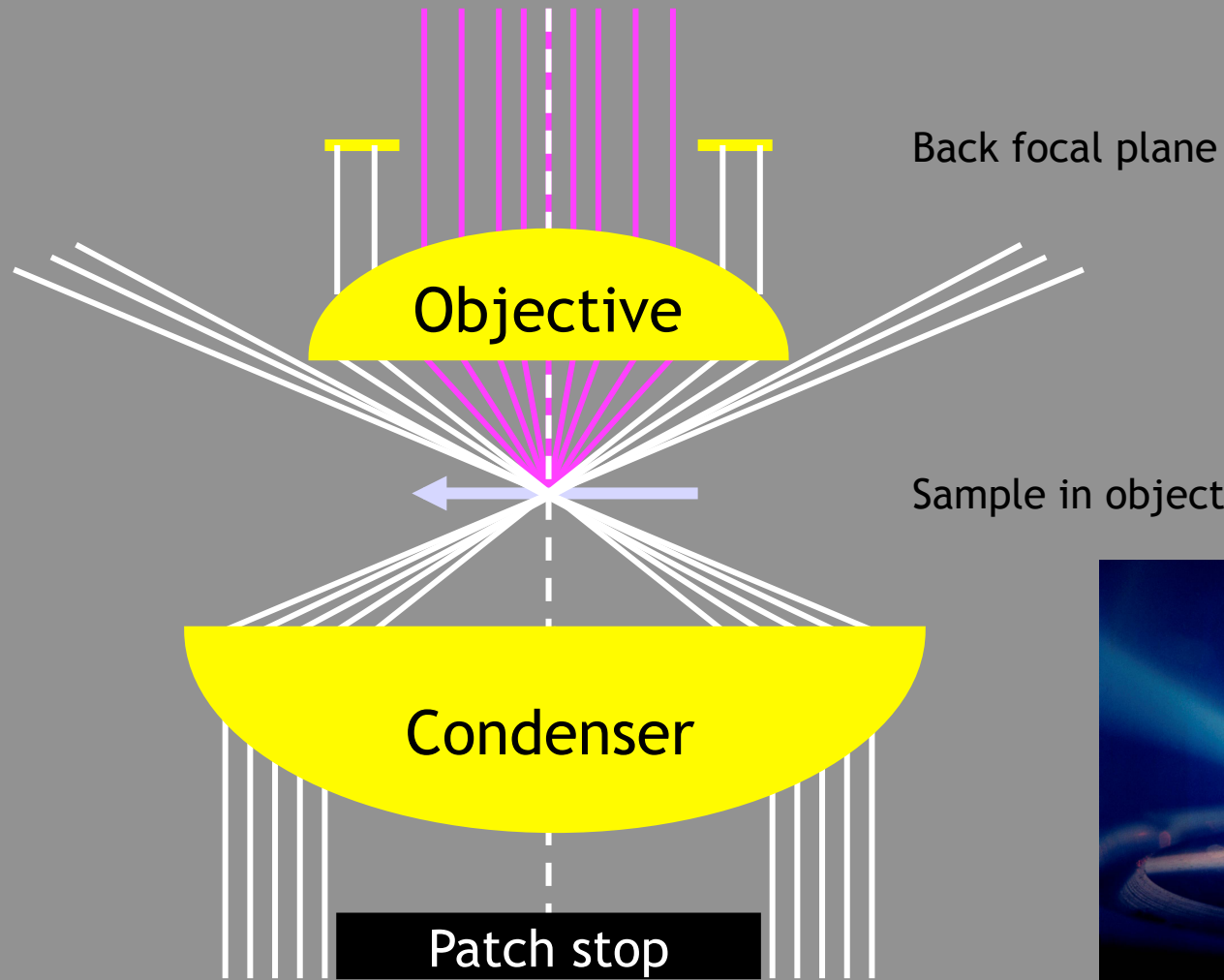
Bright field illumination can suffer from excessive background obscuring the actual image information



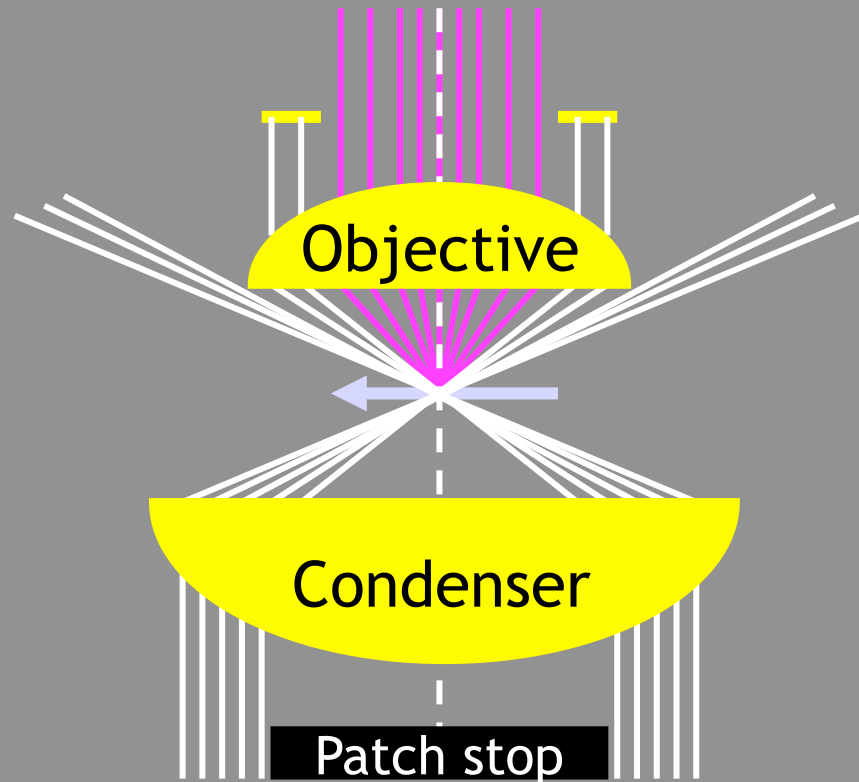
Dark field illumination removes the bright background of light that does not interact with the sample



Dark field using objective with iris diaphragm



Three steps to adjust dark field illumination

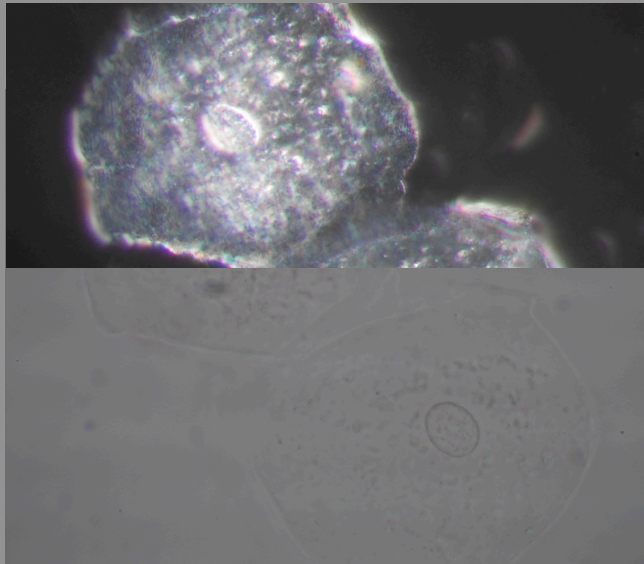


1. Set up Köhler illumination

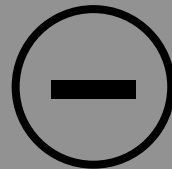
2. Swing in patch stop
(position "D" in condenser)

3. Adjust iris diaphragm to make NA of
objective smaller than NA of condenser

Pros and cons of dark field illumination



- High contrast for small, thin specimen
- Easy and cheap to set up for low NA (dry objectives)



- Not for thicker specimens
 - Requires very clean light path
 - Delicate to set up for high resolution applications
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