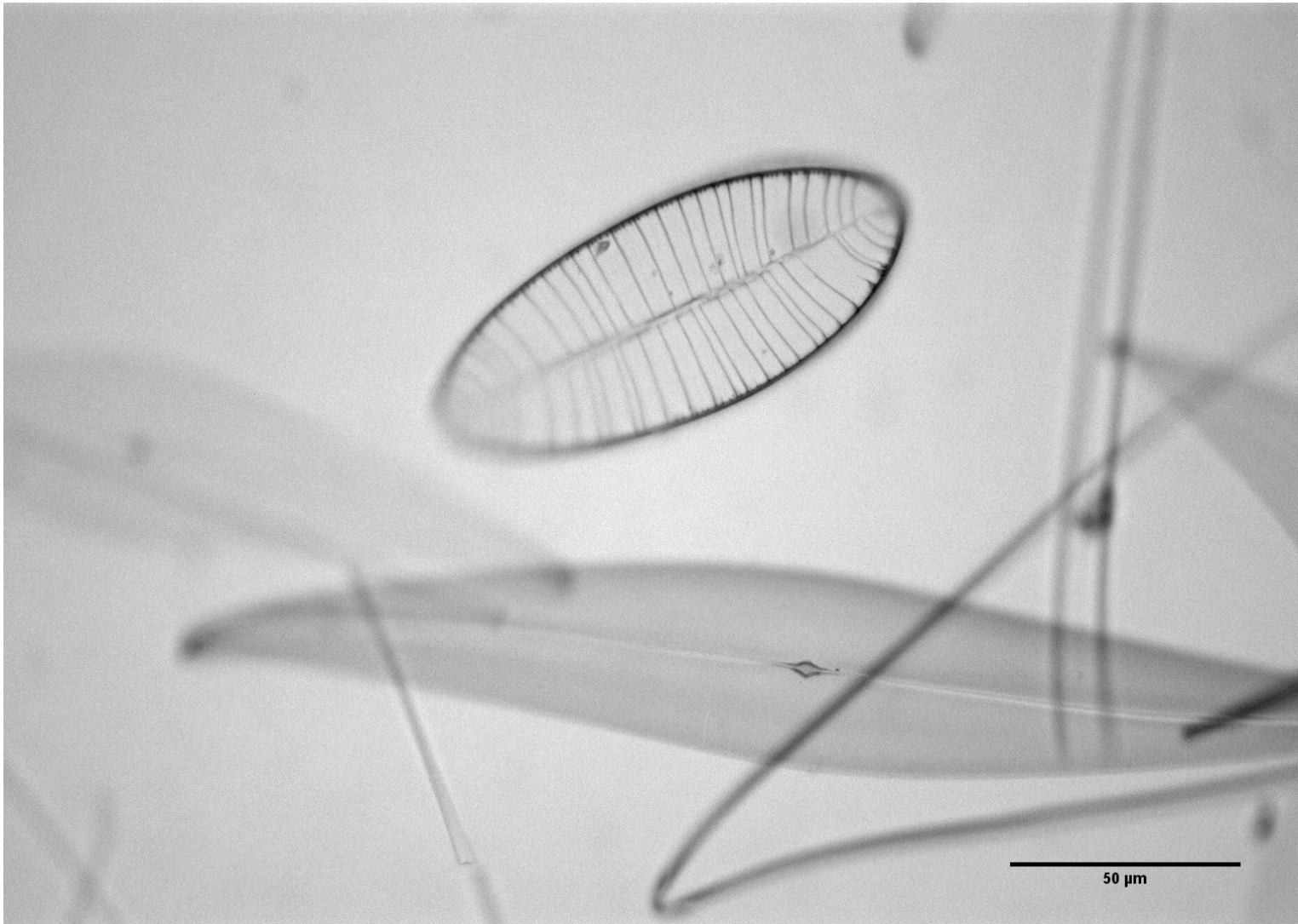


Dark field microscopy
or
Dark ground microscopy

How do we improve contrast of unstained specimen ?

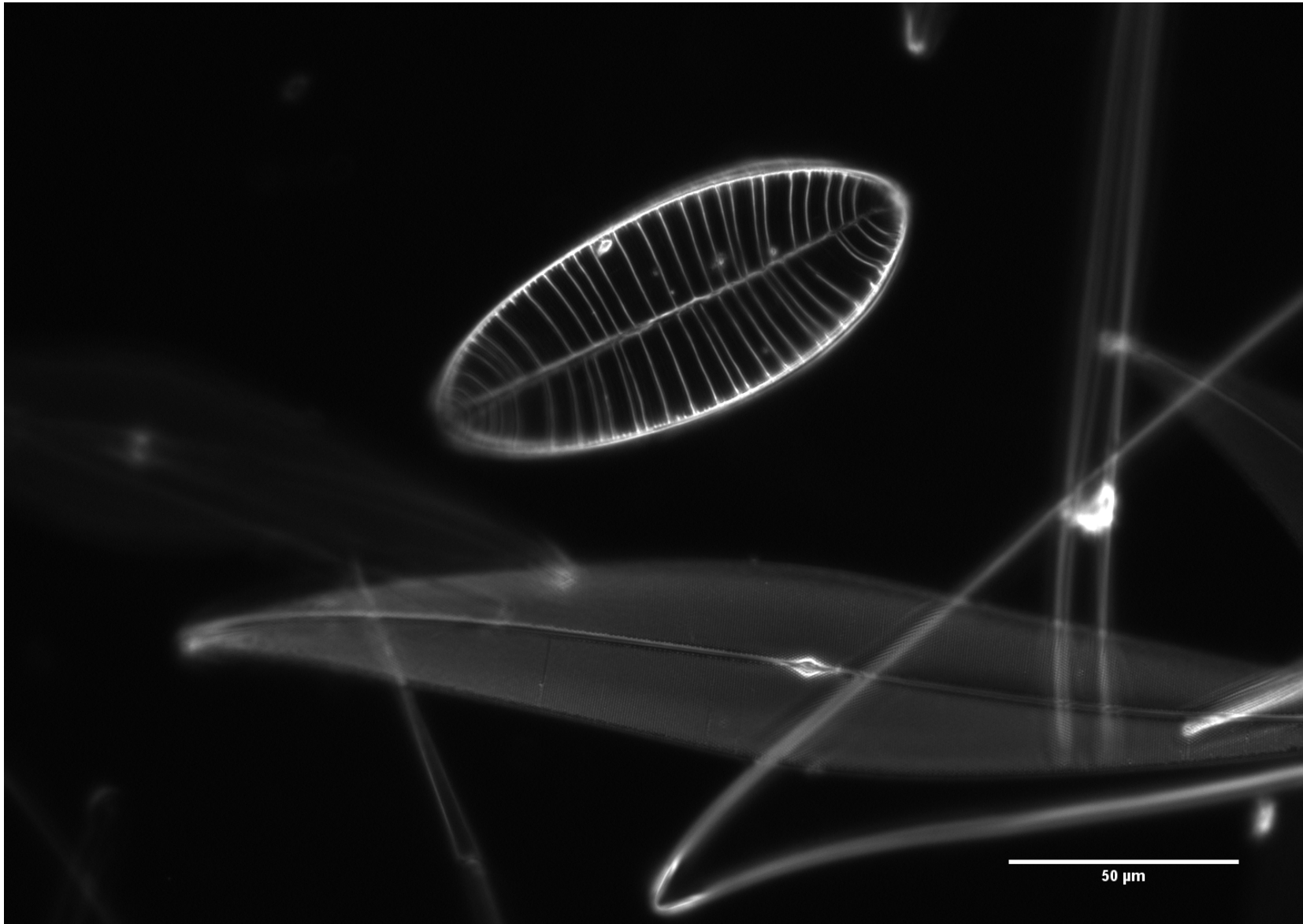


Diatoms

objective Plan Neofluar 40x/0,75, bright field, illuminating aperture diaphragm 80% open



Diatoms
objective Plan Neofluar 40x/0,75, bright field, illuminating aperture diaphragm 30% open

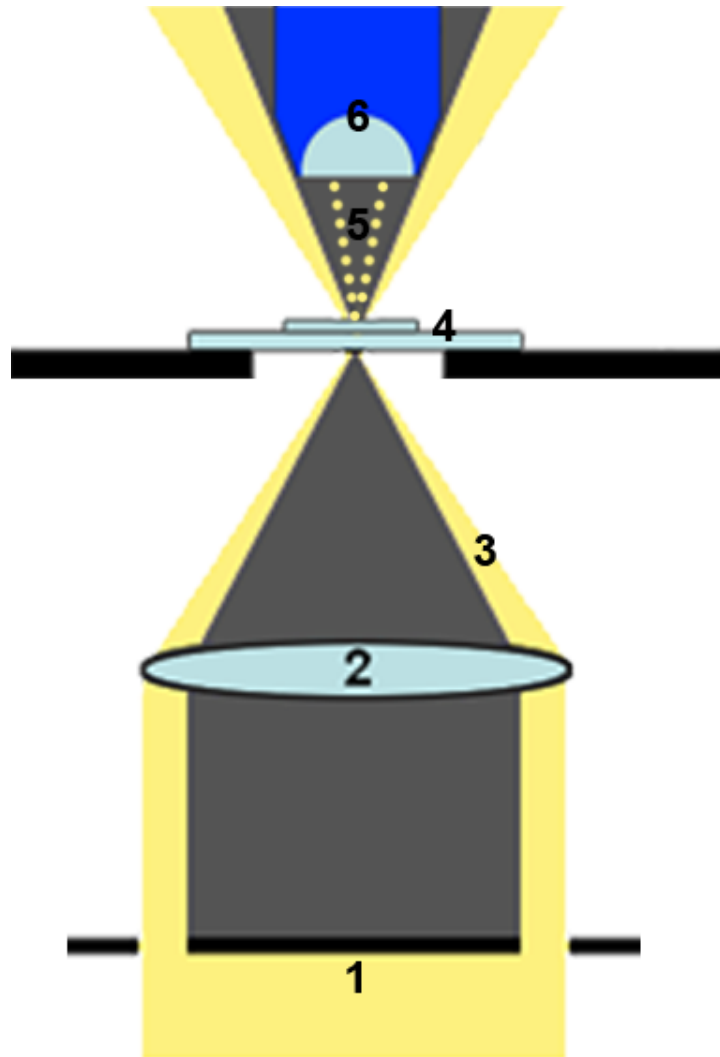


Diatoms, objective Plan Neofluar 40x/0,75, dark field

Principle

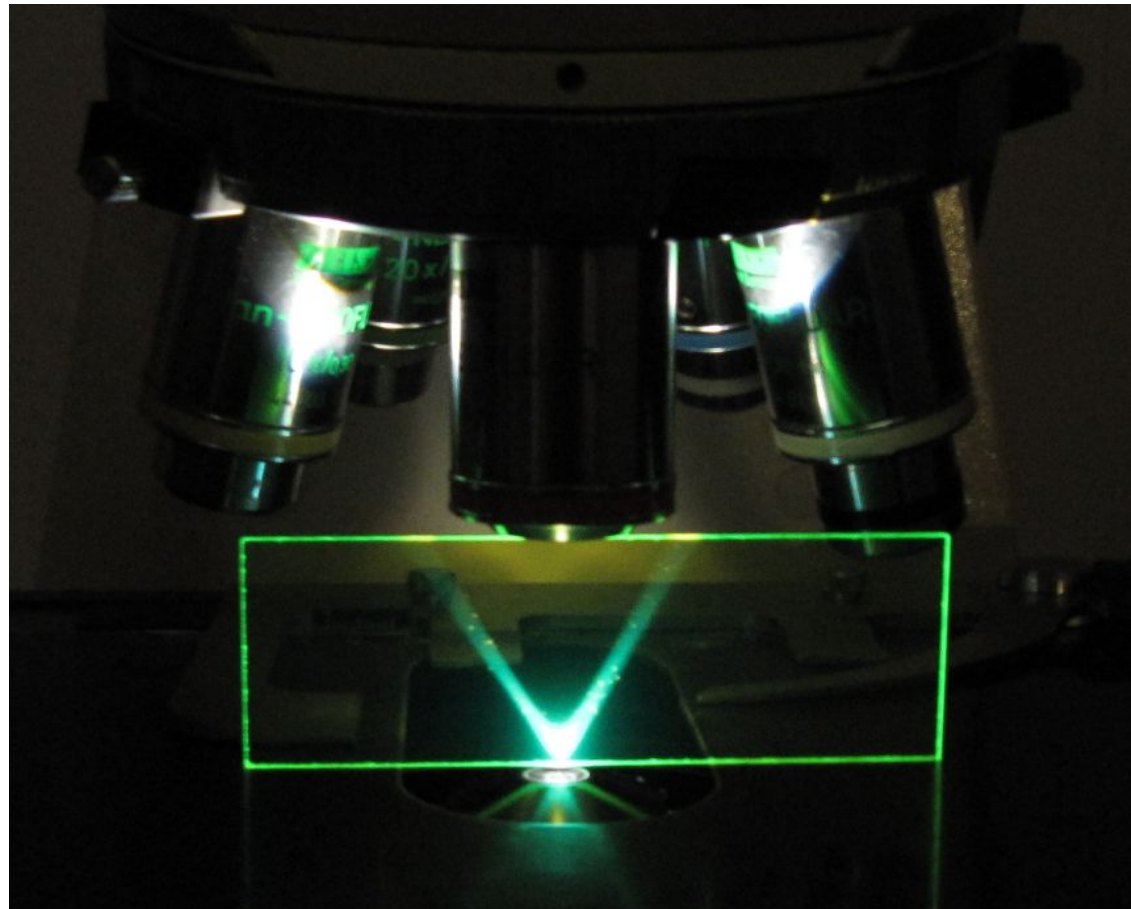
- Remove (block), or do not collect zero order beam that does not diffract on specimen
- This provides improved contrast of unstained samples: small features and edges of the samples shine with white light on black background

Dark field scheme



6. Objective lens
5. Diffracted light
4. Sample
3. Illuminating light
2. Condenser lens
1. Dark field "patch" stop

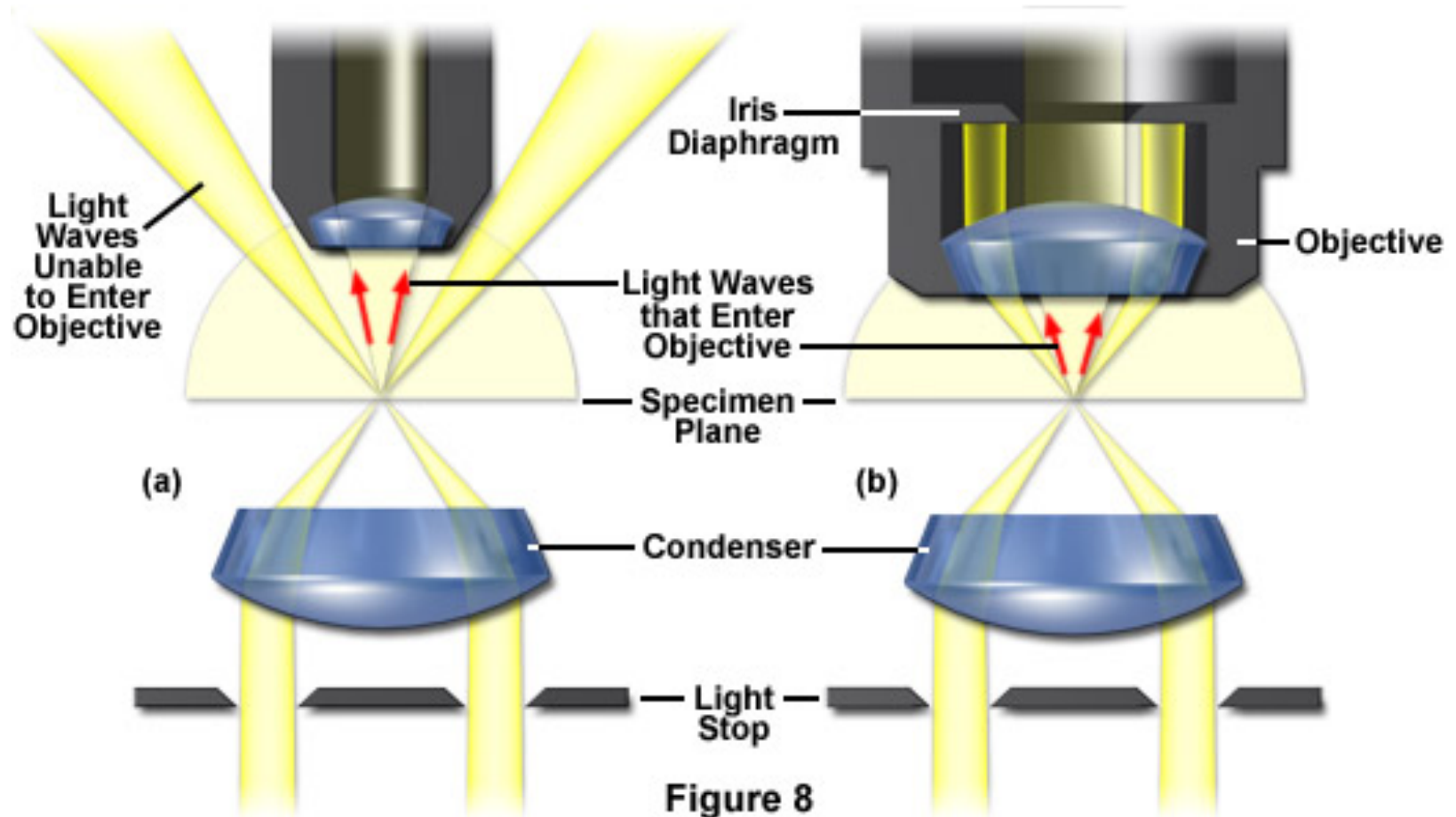
Dark field illumination cone



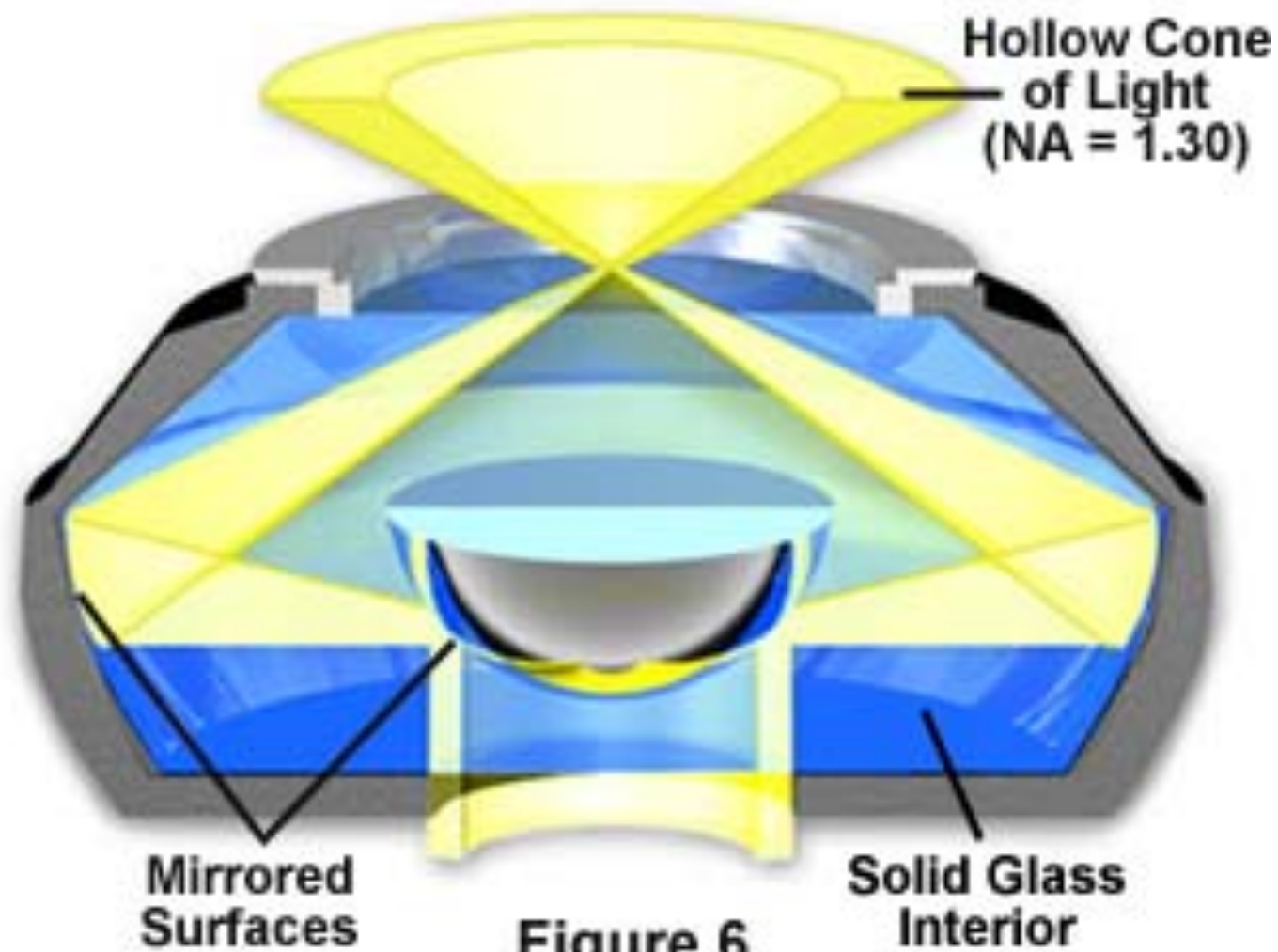
How to set it up

- condenser must have higher N.A. than objective
- condenser must have specially sized disc so called “patch stop” that blocks some light of the light source leaving an outer ring of illumination
- objective can have iris that allows for making N.A. smaller than is N.A. of the condenser

Darkfield Microscope Optical Configurations



Bispheric Double Reflecting Condenser



What is it good for

- Imaging of samples that are several microns thick, close to cover glass
 - Blood cells
 - Sperm cells
 - Ciliated cells (paramecium, chamydomonas)
- Imaging of nanoparticles

Example :

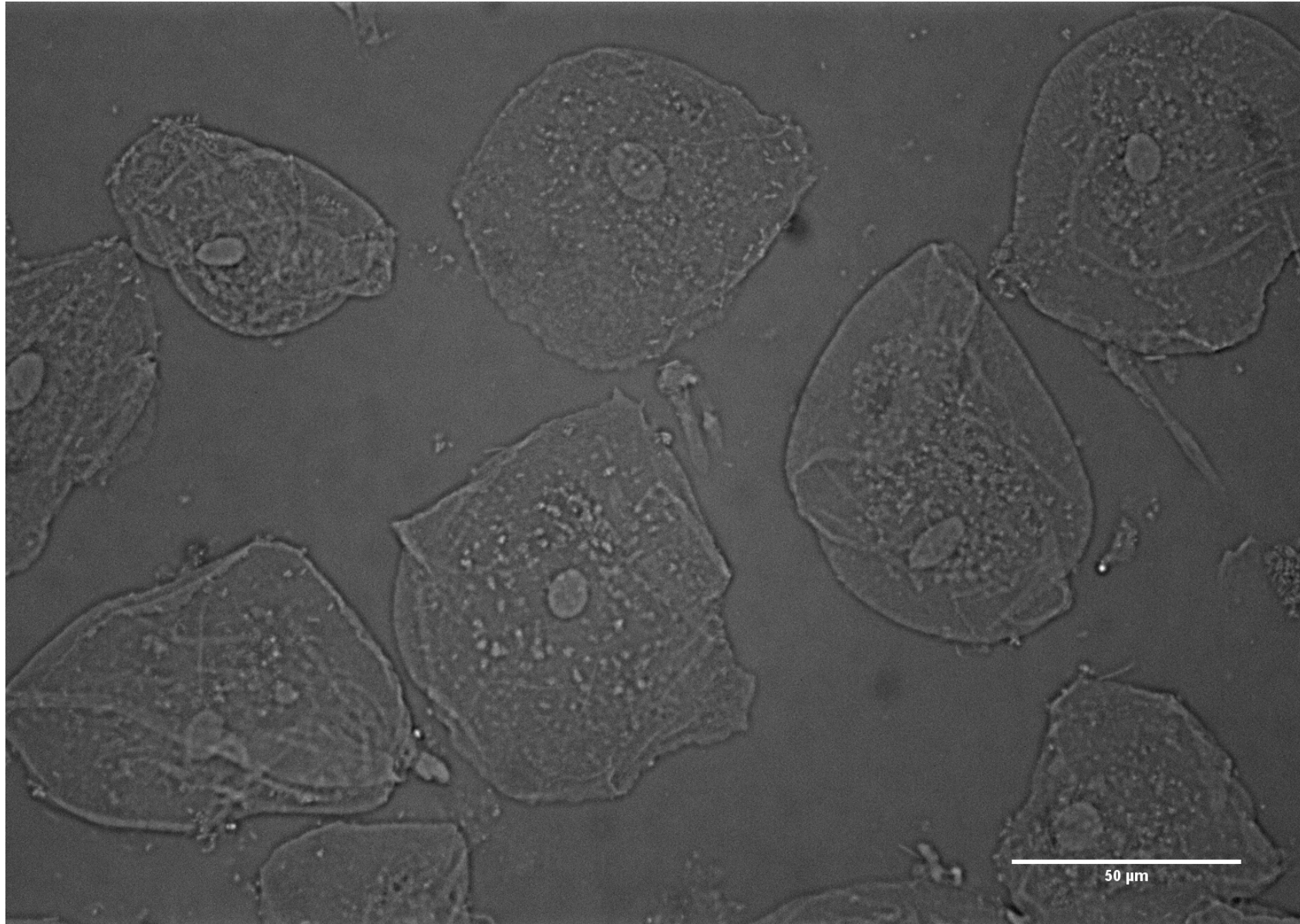
Dark field microscopy of blood cells

https://www.youtube.com/watch?v=Mg21897IW_4

Example :
Dark field microscopy of
Chamydomonas (Gaia Piginio lab)

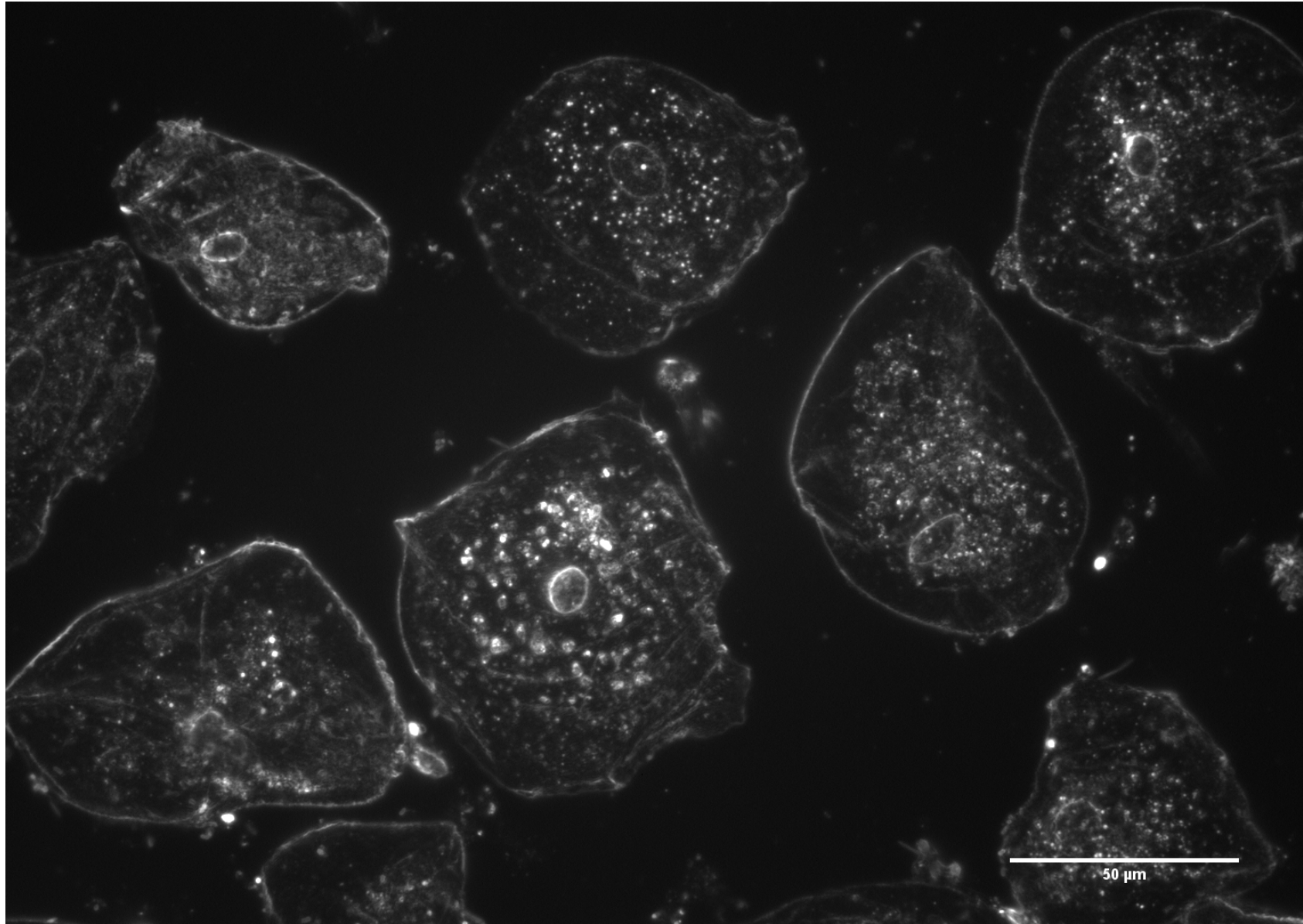
Practice

- condenser must have higher N.A. than objective
- condenser must have specially sized disc so called “patch stop” that blocks some light of the light source leaving an outer ring of illumination
- on inverted microscopes sometimes illuminating phase annulus for phase contrast is sufficient for low N.A. objective
- objective can have iris that allows for making N.A. smaller than is N.A. of the condenser



Buccal cells

objective Plan Neofluar 40x/0,75, bright field, illuminating aperture diaphragm 30% open



Buccal cells, objective Plan Neofluar 40x/0,75, dark field