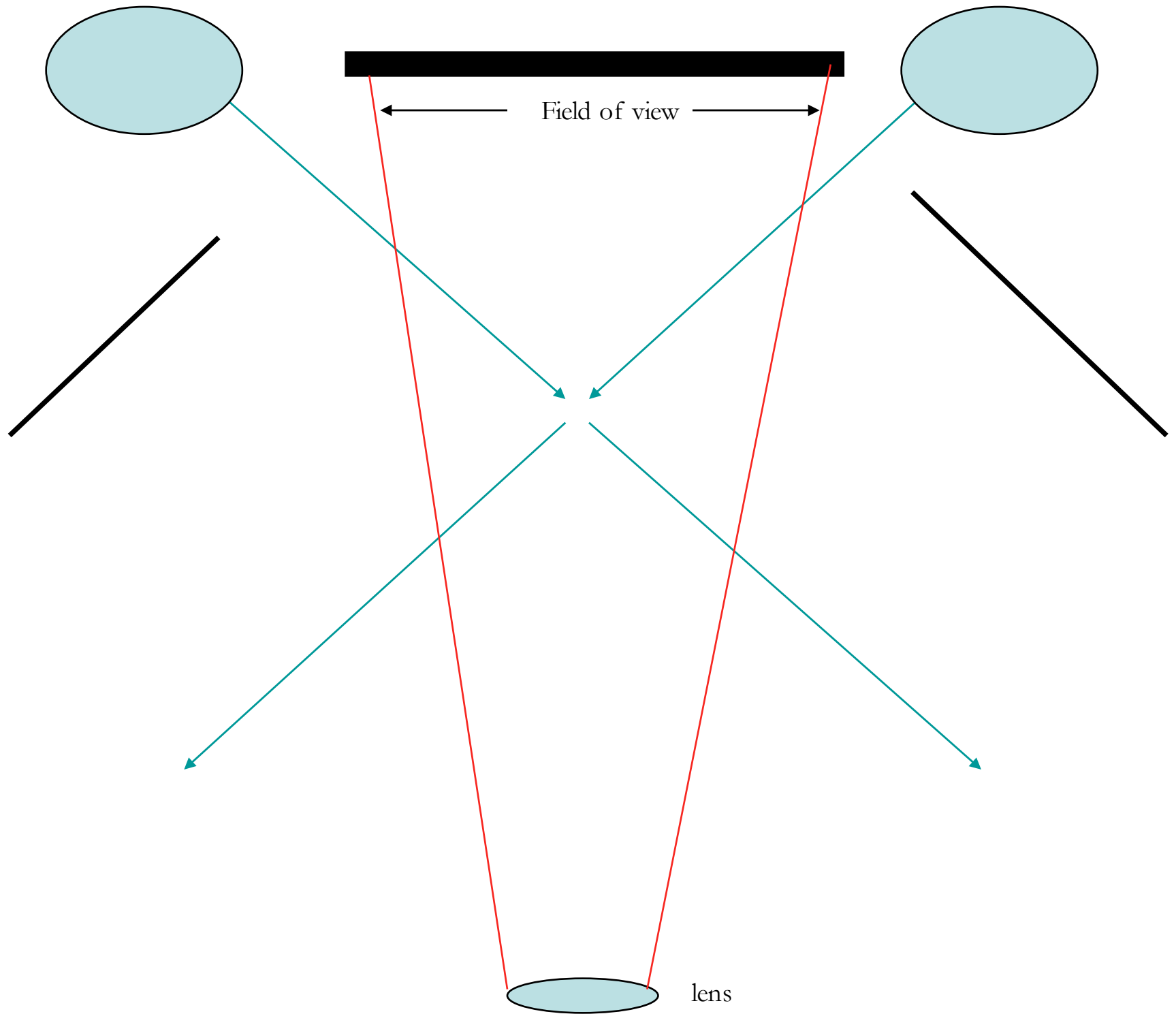


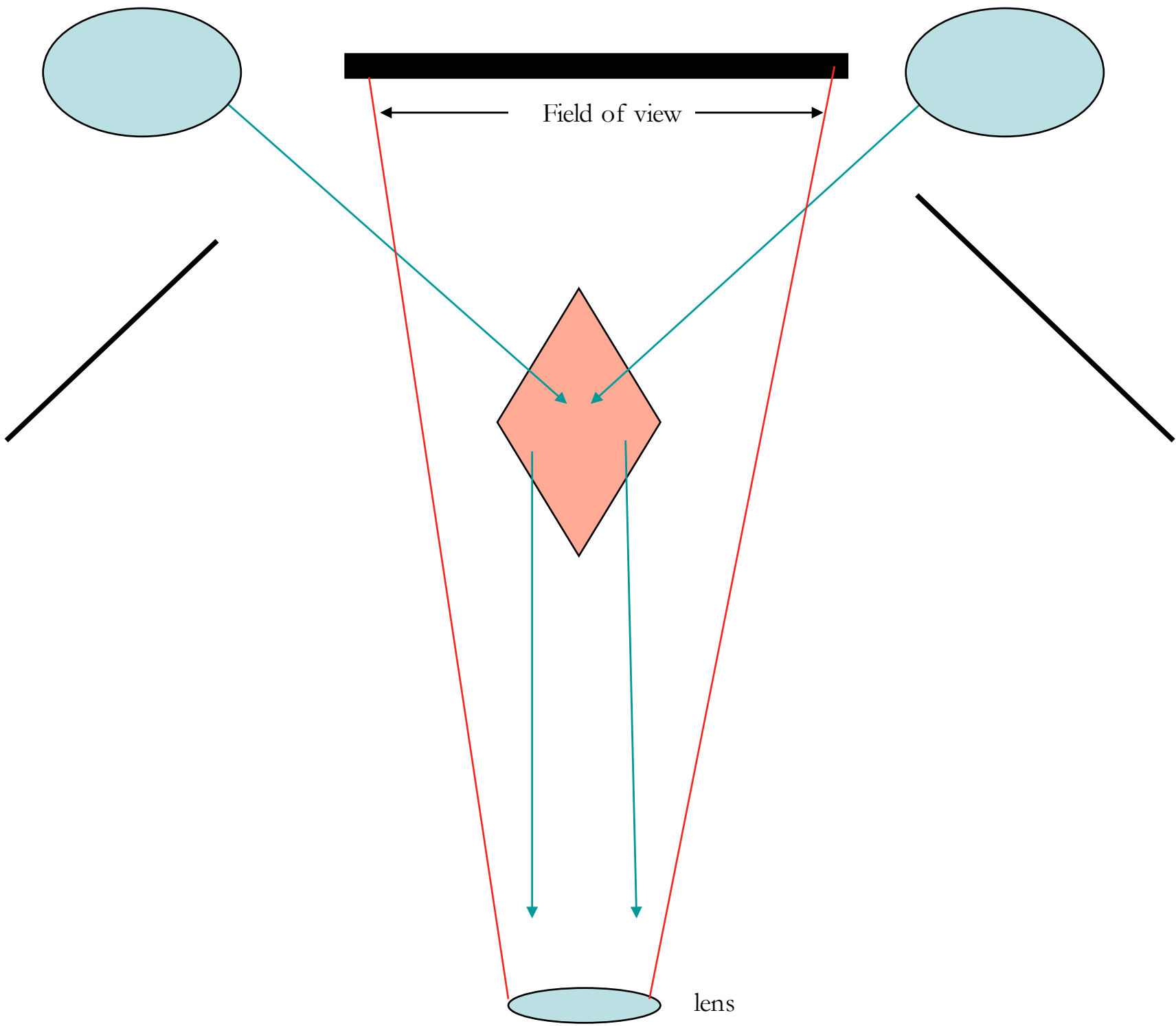
Darkfield Illumination
&
Creating Image Contrast





Oblique angle lighting



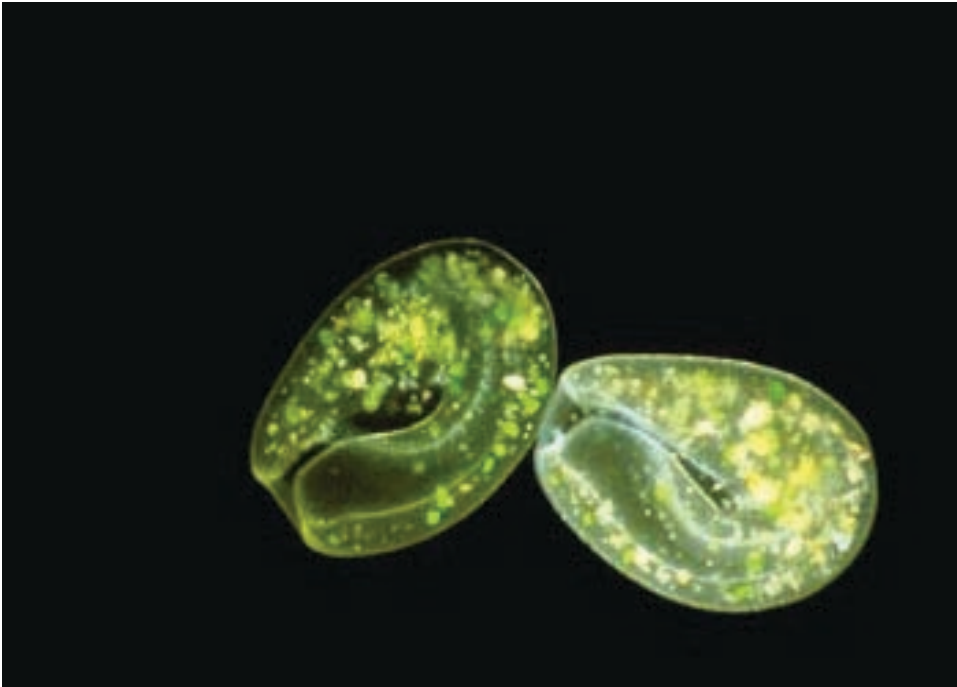
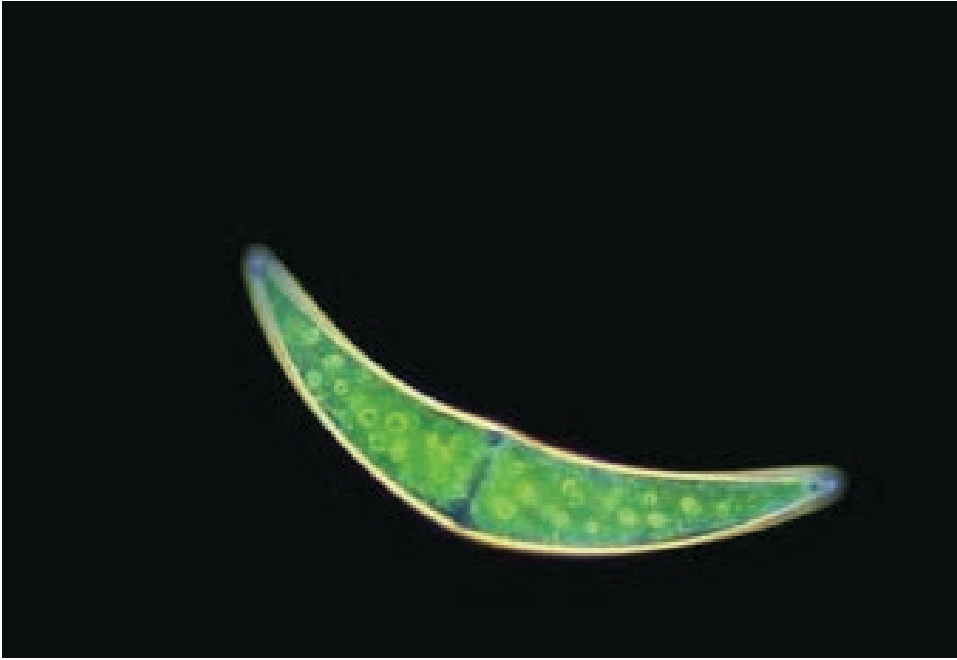


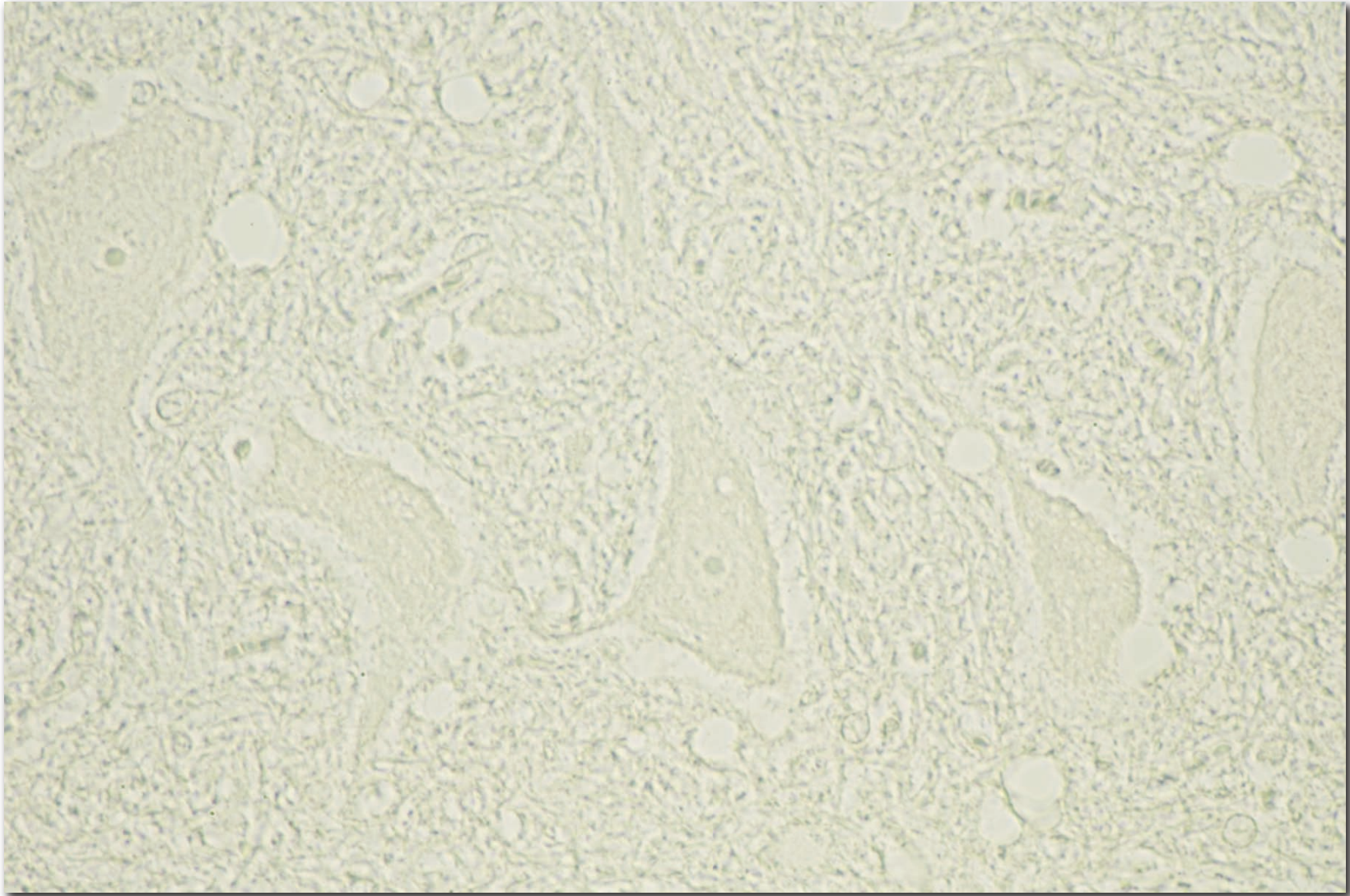
Useful for not stainable living
semi-transparent subjects



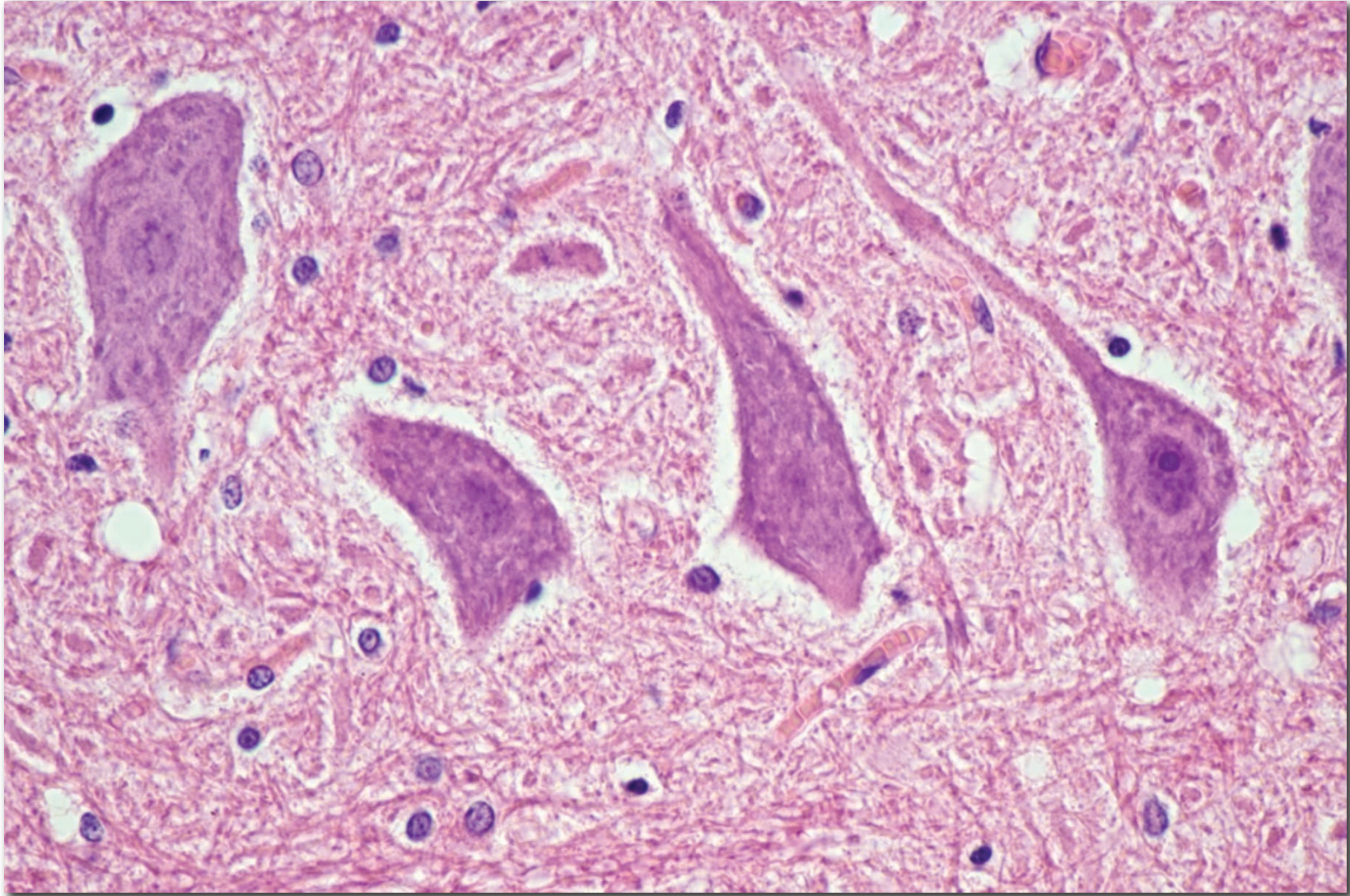
Reveals internal structure while
delineating edges





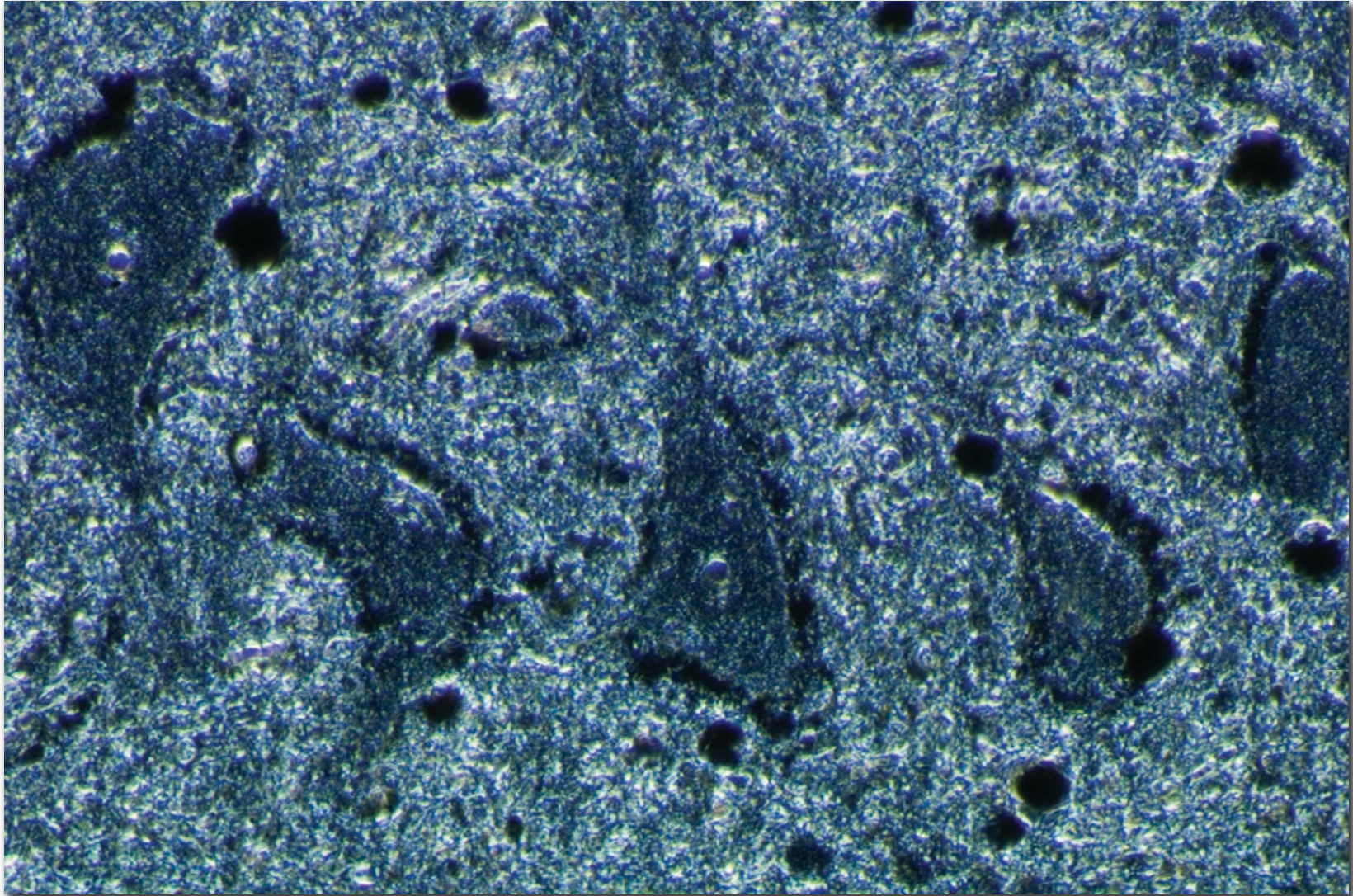


Human Nerve cells in Cerebellum
Unstained - brightfield illumination

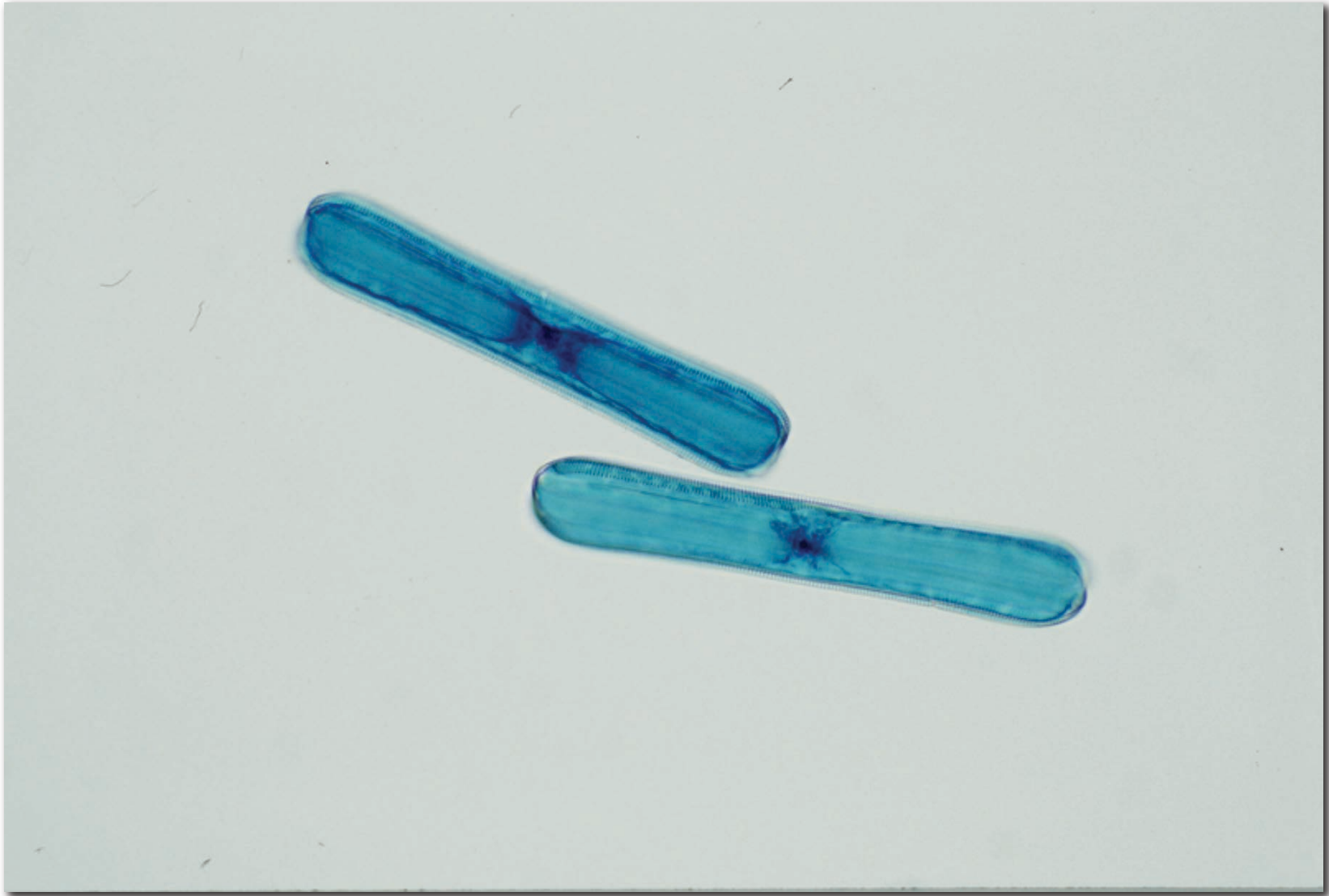


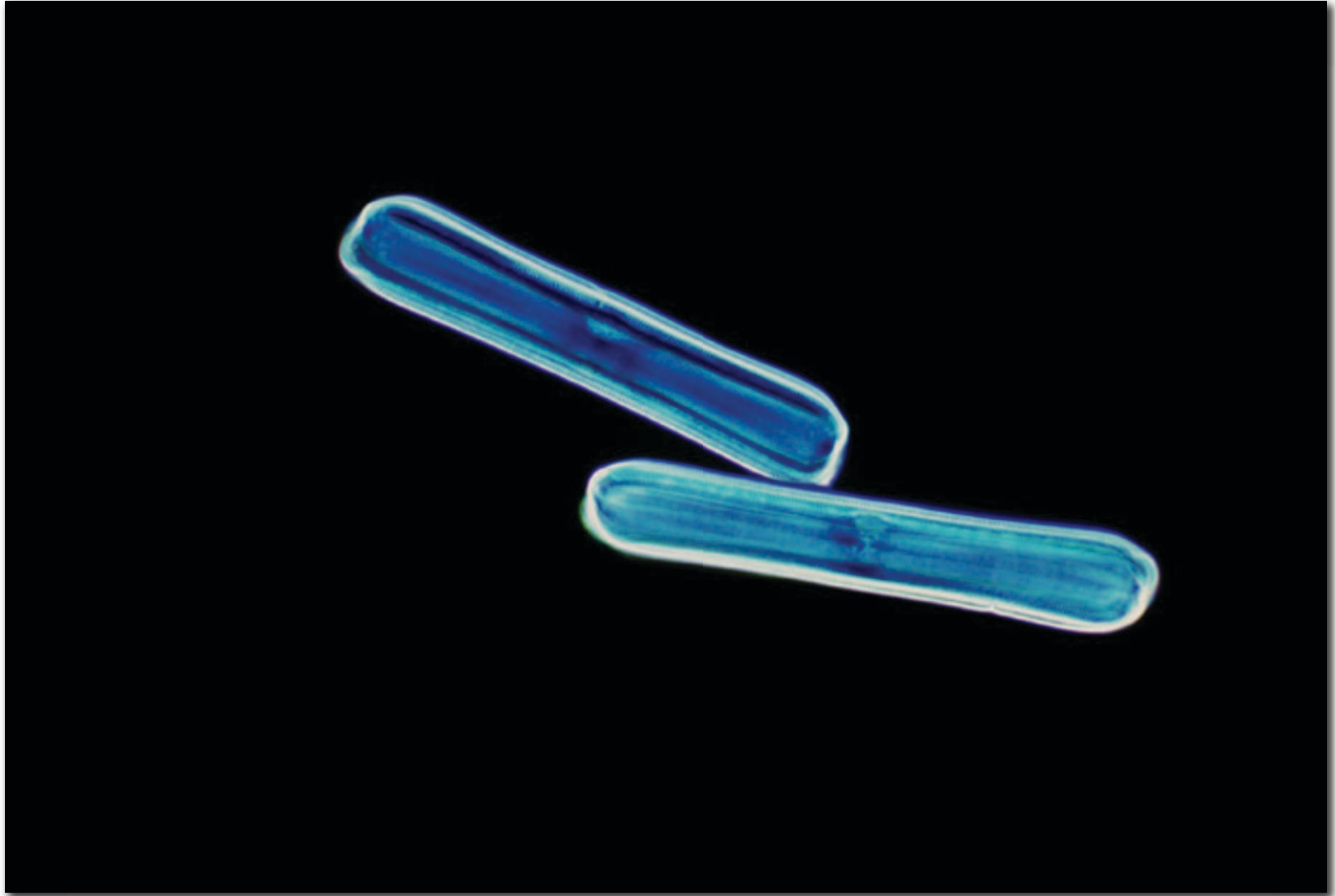
Human Nerve cells in Cerebellum

Stained with Hematoxlin & Eosin- brightfield illumination



Human Nerve cells in Cerebellum
Unstained - darkfield illumination





Equipment & Optics

Brightfield Objectives

Darkfield Condenser

Darkfield Theory

Remove zero order of illumination

∴

Disc stop equal to the NA of objective
at the

Location of Aperture Diaphragm

Exit Pupil of the Objective

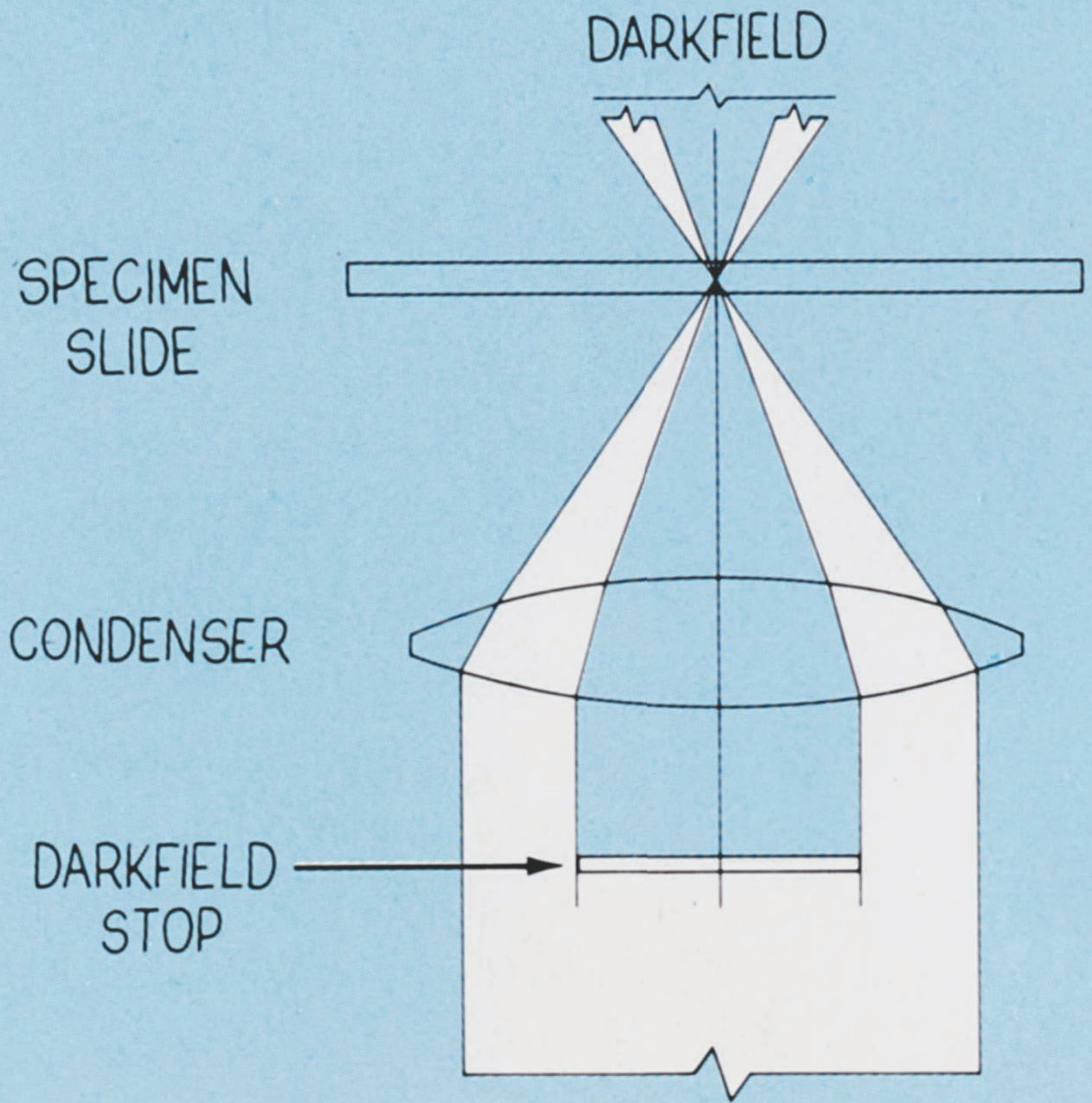
Set Up

No Kohler

Open field stop completely

Illumination is defocused

Raise and lower substage condenser until apex
of illumination reaches sample plane



DARKFIELD

SPECIMEN
SLIDE

CONDENSER

DARKFIELD
STOP

NA of Condenser
must be **greater** than
NA of Objective

Diffraction Rays

Illuminate sample

Sample *refracts* illumination
to imaging system

Types of Condensers

Dry

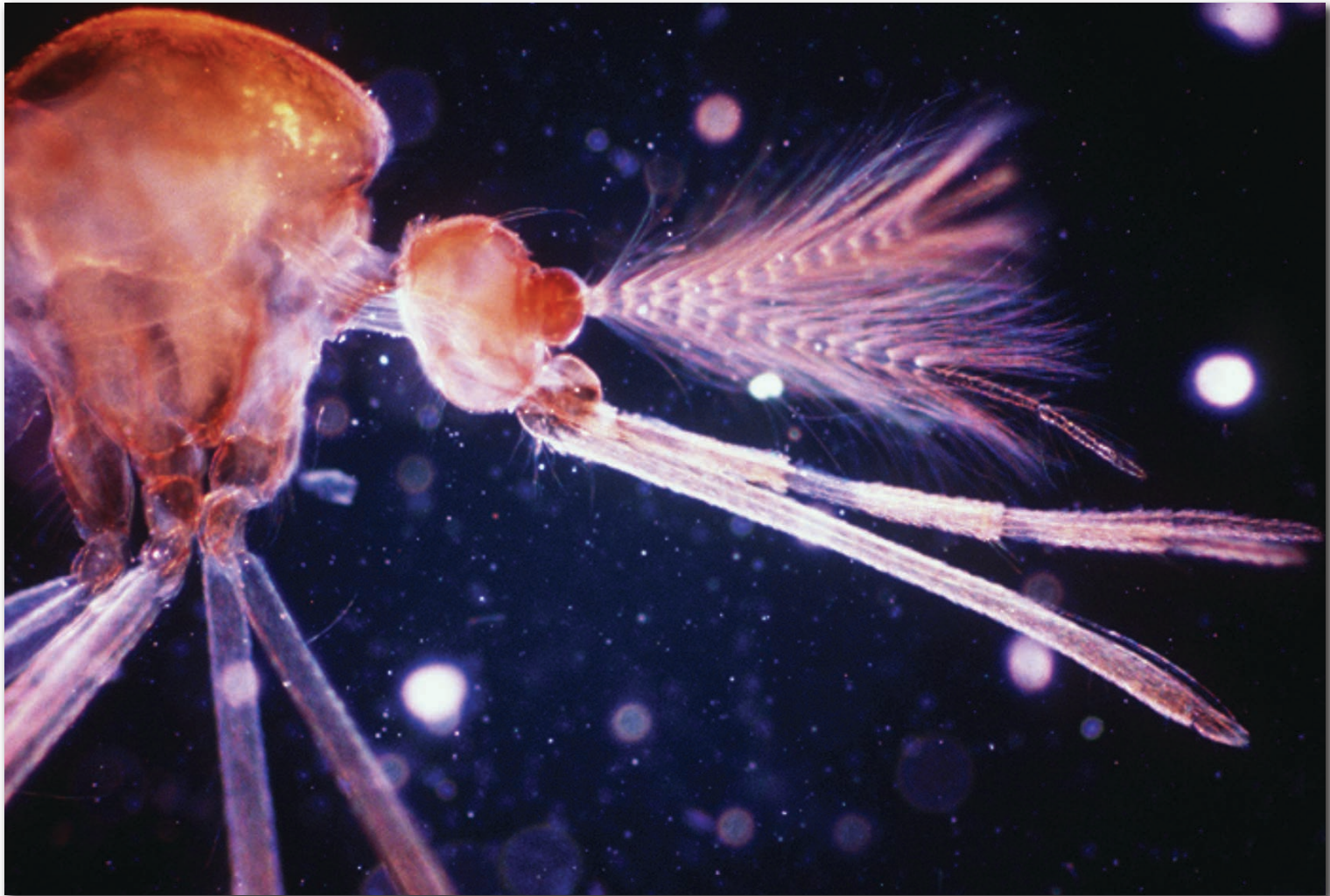
10x-20x achro objectives

Oil

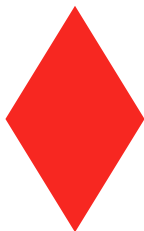
40x -60x dry achro objectives

Low Power - difficult

Imaging Problems



Exposure Placement



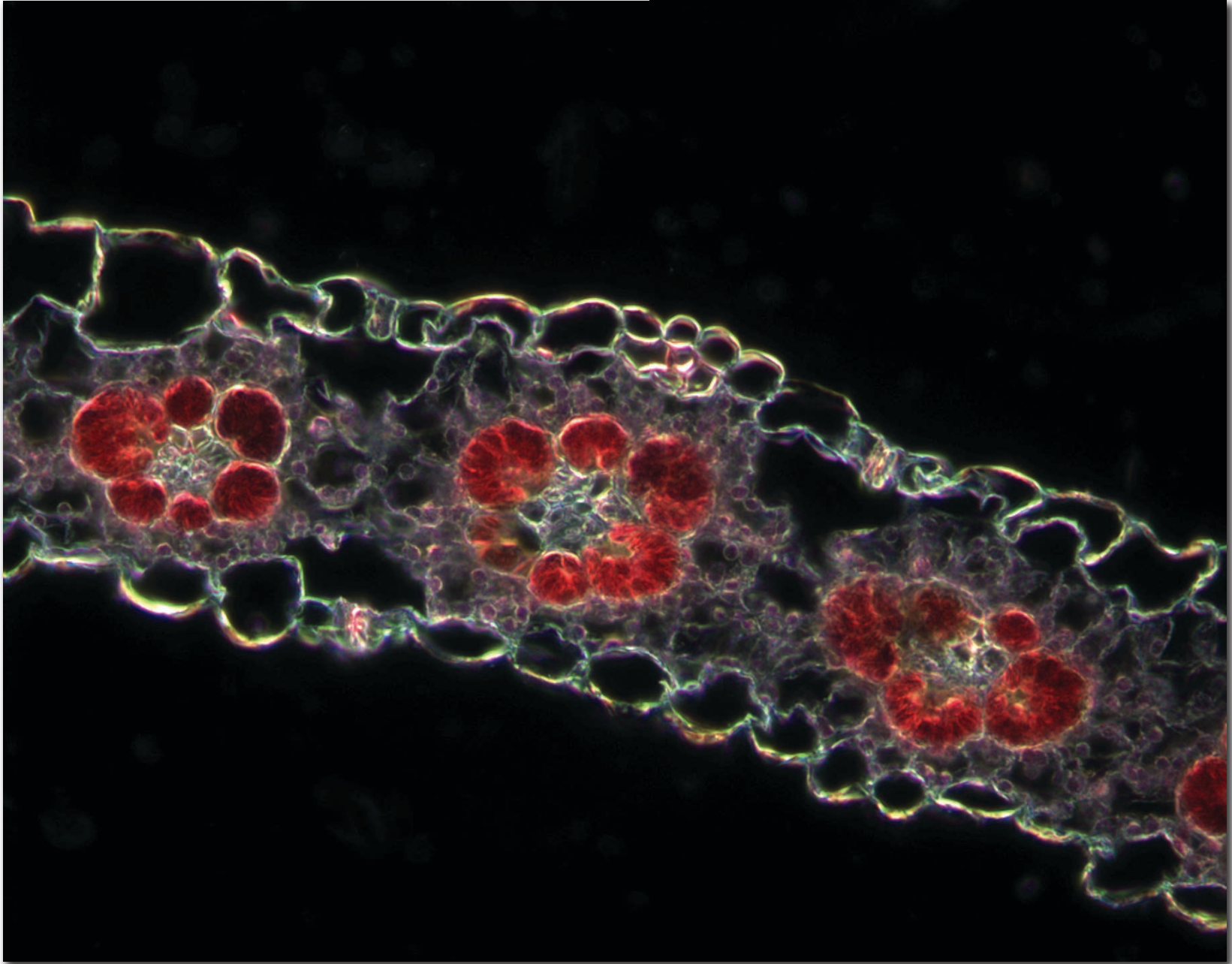
DF
- 2

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BF





Megan Miller





