Optical Microscopes

Lexicon

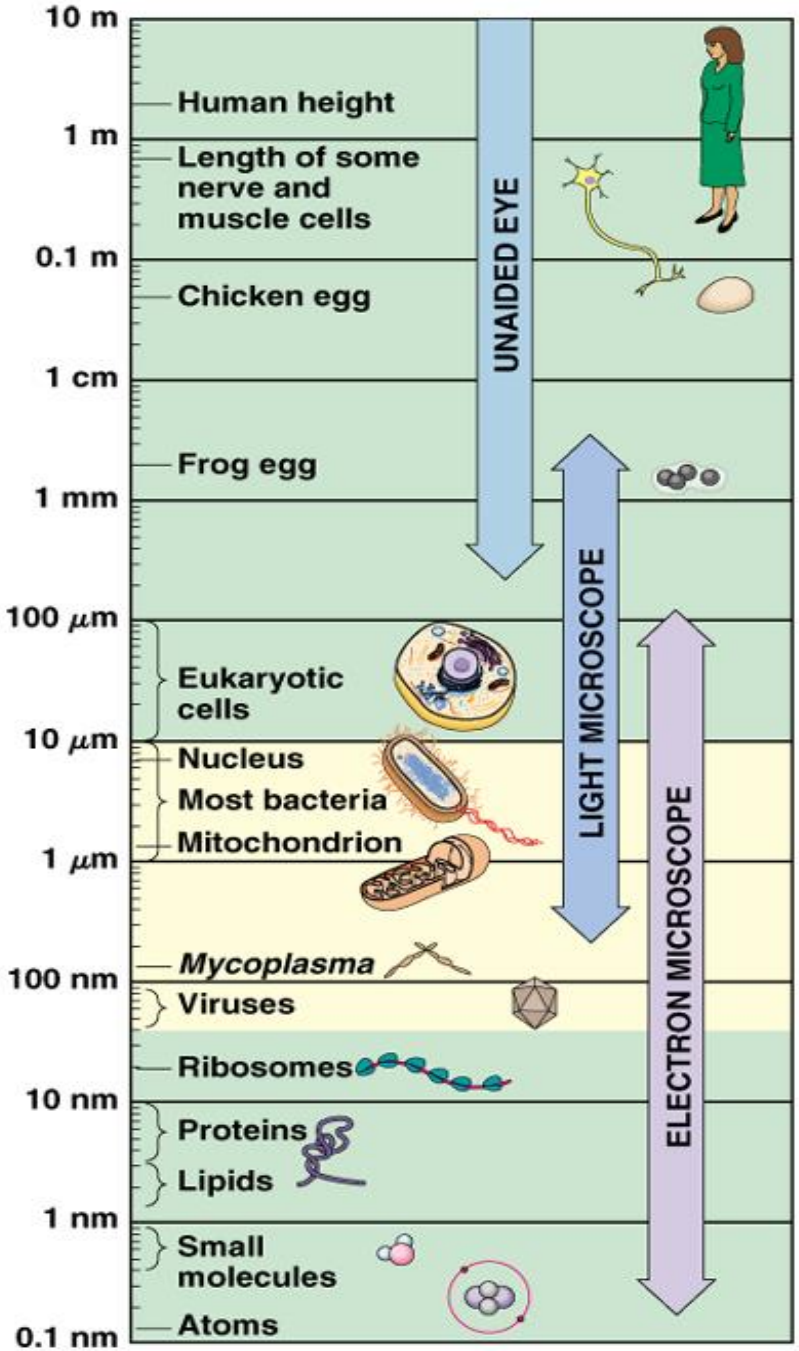
- **Micro**: very small

  microgram, micrometer, microsecond, micron, microdot, microbiology, microorganism, microsurgery, microeconomics, microelectronics, microwave, microchip, microcomputer, microprocessor, microphone, microfilm, ……

- **Microscope**: an instrument for magnifying very small objects

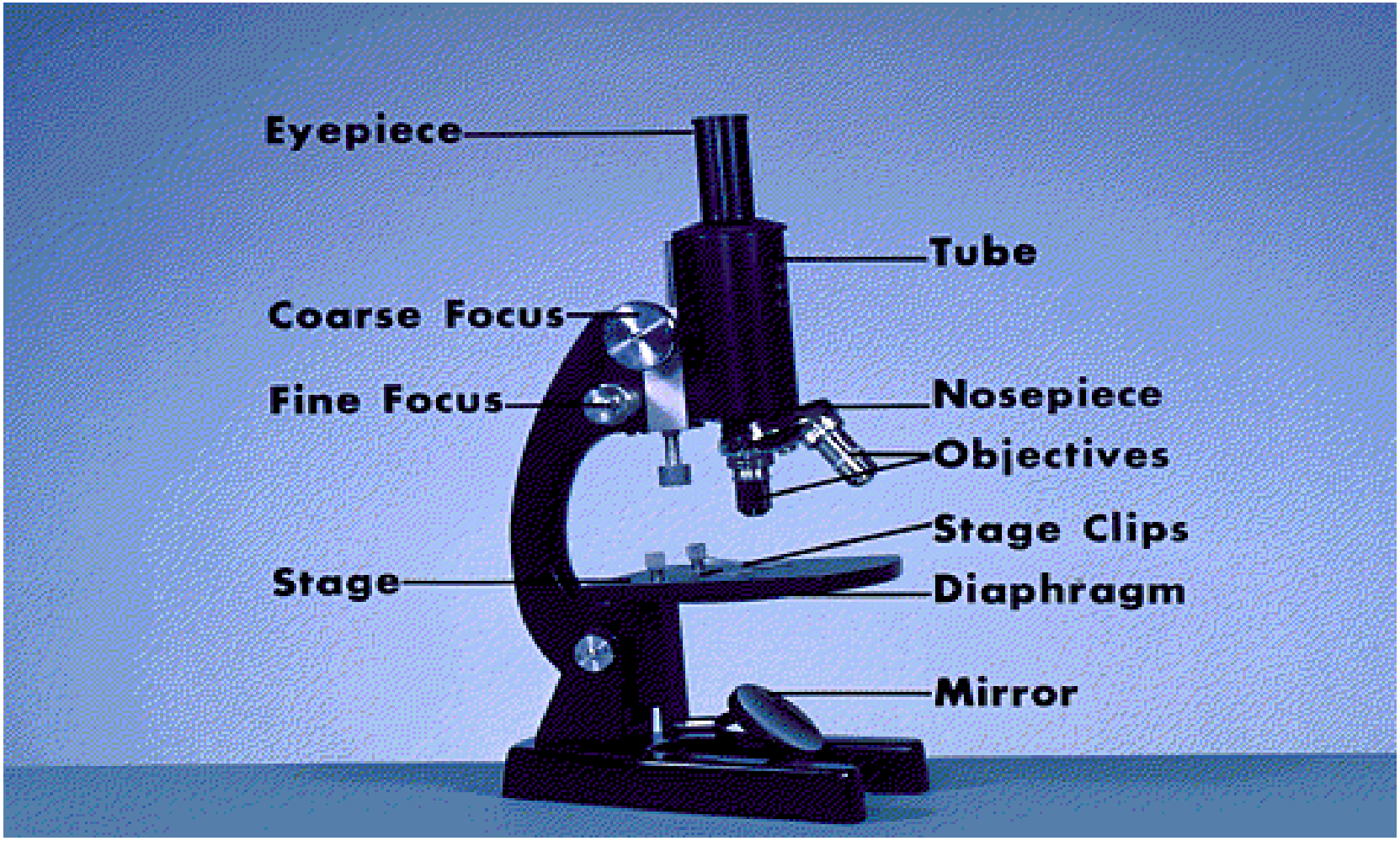
- **Microscopic**: so small as to be visible only with a microscope

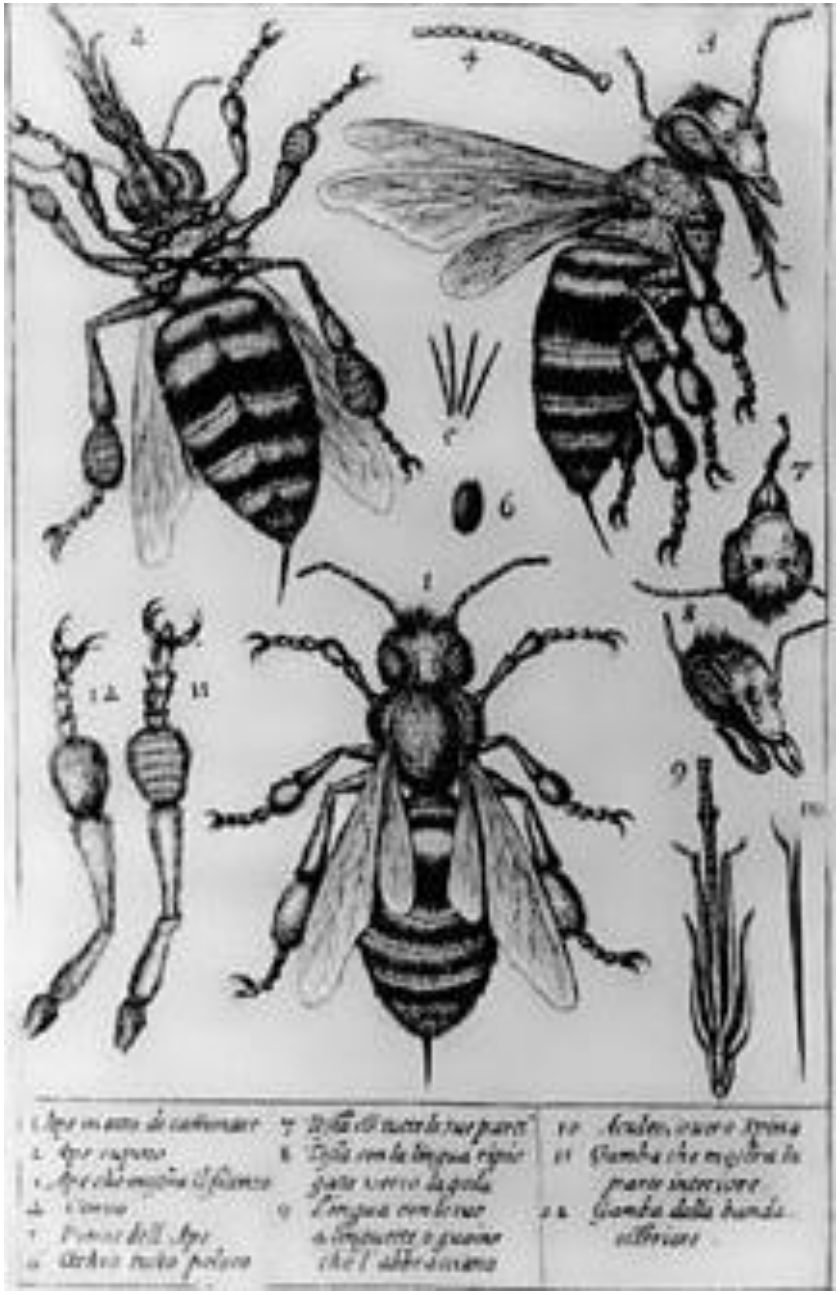
- **Microscopy**: the use of a microscope



Microscope

One or more lens that makes an enlarged image of an object.





Oldest published image known to have been made with a microscope: Bees by **Francesco Stelluti**, 1630.

- Optical microscope, often referred to as "**light microscope**", is a type of microscope which **uses visible light** & a system of lenses to magnify images of small samples.
- Optical microscopes are the oldest design of microscope & were possibly designed in their present compound form in 17th century.
- Aim: to improve **RESOLUTION** & **CONTRAST**.
- Microscopes which do not use visible light are:

  Scanning Electron Microscope (SEM)

  Transmission Electron Microscope (TEM)

  Atomic Force Microscope (AFM)

- **Inventor of microscope: Galileo Galilei**

  Galileo developed a compound microscope with a convex & concave lens in 1609.

- **Giovanni Faber coined the name "microscope". Greek words:**

  micron meaning "small"

  skopein meaning "to look at"

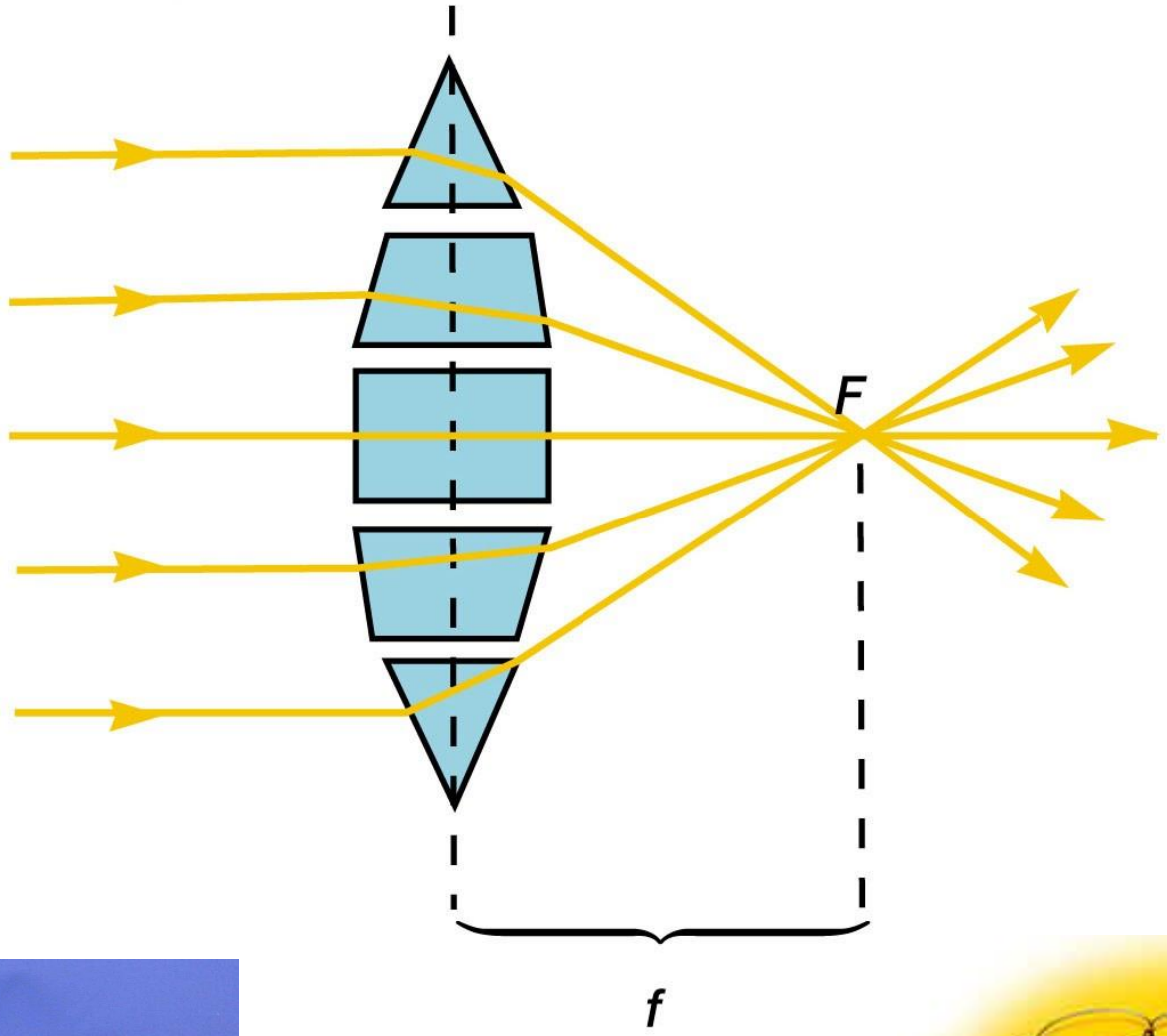
- Optical & electron microscopy involve **diffraction**, **reflection**, or **refraction** of electromagnetic radiation/electron beams interacting with specimen, & subsequent collection of this scattered radiation or another signal in order to create an image.

- This process may be carried out by wide-field irradiation of sample (e.g., standard **light microscopy** & **transmission electron microscopy**) or by scanning of a fine beam over sample (e.g., **confocal laser scanning microscopy** & **scanning electron microscopy**).

- **Scanning probe microscopy** involves interaction of a scanning probe with surface of object of interest.

Lenses & Bending of Light

- **Lenses focus light rays at a specific place, called focal point.**
- **Strength of lens is related to focal length.**
  Short focal length → more magnification
- **Light is refracted (bent) when passing from one medium to another.**
- **Refractive index: a measure of how greatly a substance slows velocity of light.**
- **Direction & magnitude of bending is determined by refractive indices between the two media forming the interface.**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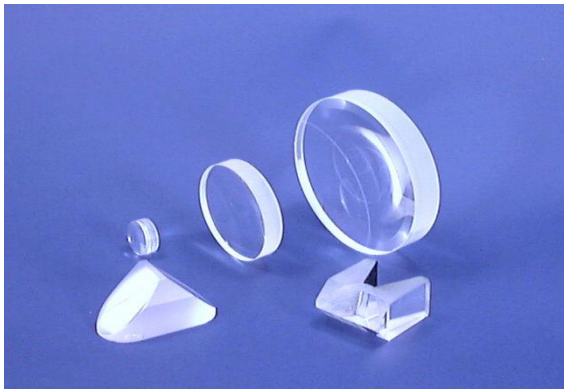


Eyepiece Lens

Usually has a power of 10 X.

Eyepiece Lens × Objective Lens = Total Magnification

Objective Lens: **Low power = 4x**
Medium power = 10x
High power = 40x

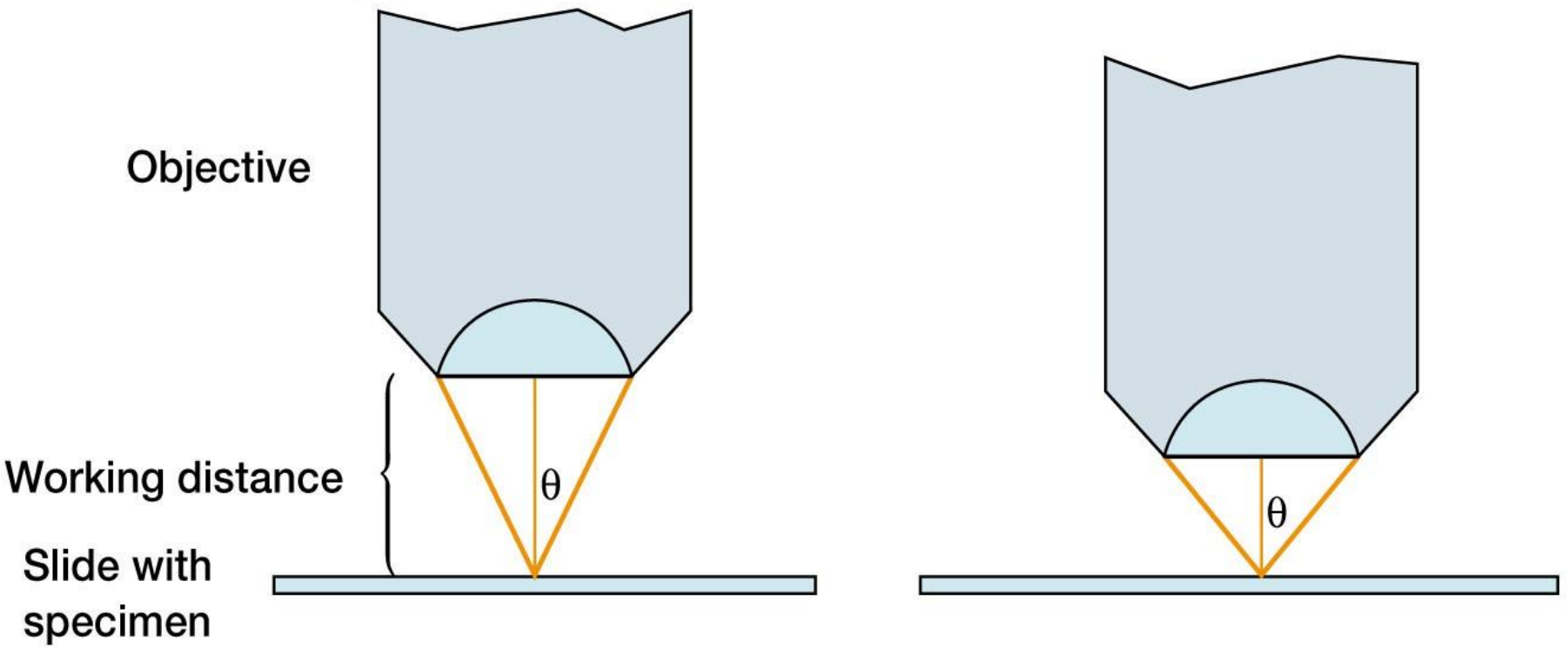
Microscope Resolution

- **Ability of a lens to separate or distinguish small objects that are close together.**
- **Wavelength of light used is major factor in resolution**

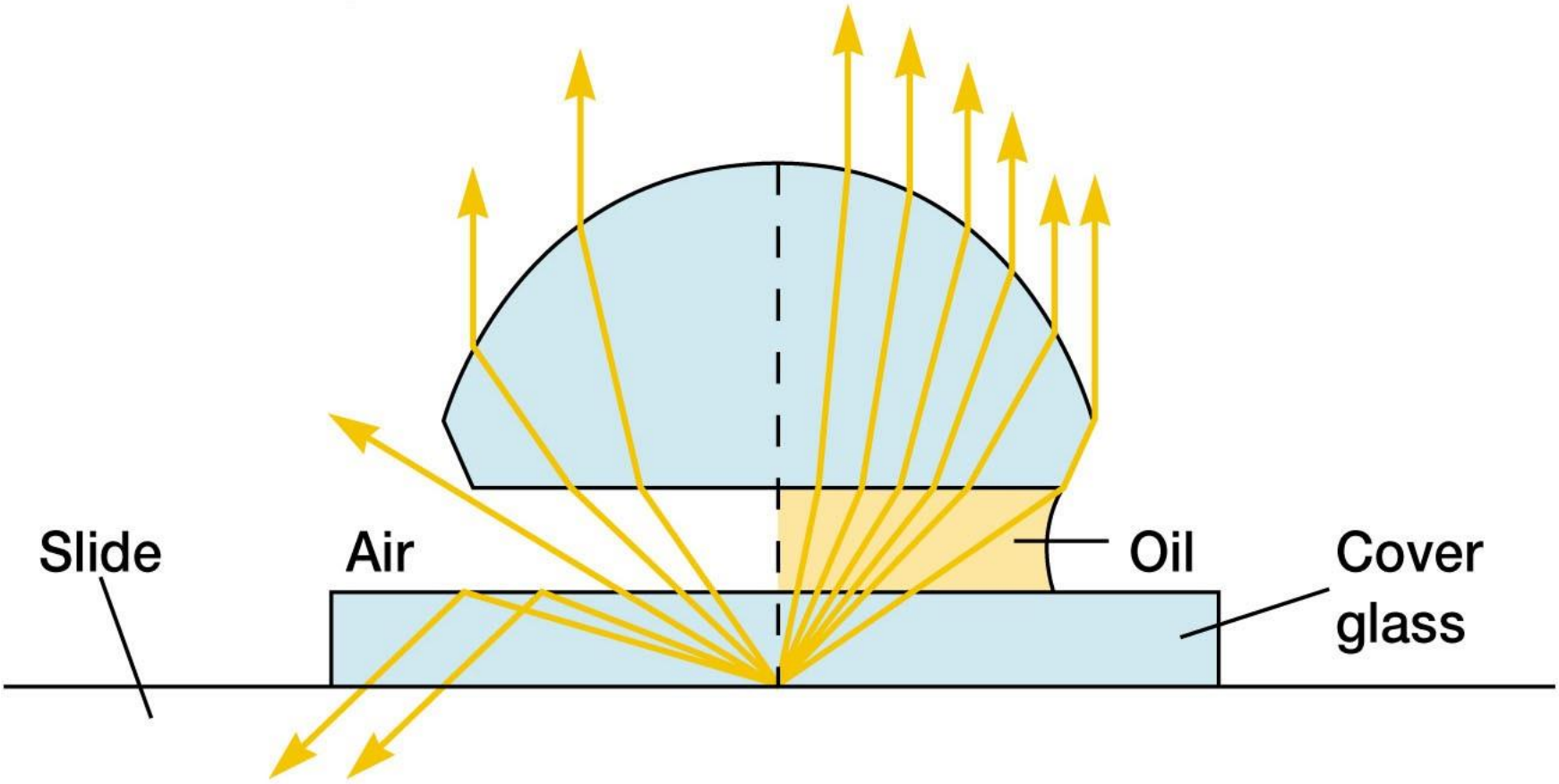
Shorter wavelength → Greater resolution

Properties of Microscope Objectives

	Objective			
Property	**Scanning**	**Low Power**	**High Power**	**Oil Immersion**
Magnification	4×	10×	40–45×	90–100×
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length (*f*)	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 µm	0.9 µm	0.35 µm	0.18 µm


Objective
Working distance
Slide with specimen
θ
θ

Objective Lens



Types of Microscope

- Simple microscope
- Compound microscope
- Stereoscopic microscope
- Electron microscope
- Phase-Contrast microscope
- Digital holographic microscope

Simple Microscope

Similar to magnifying glass & has only one lens.

Compound Microscope

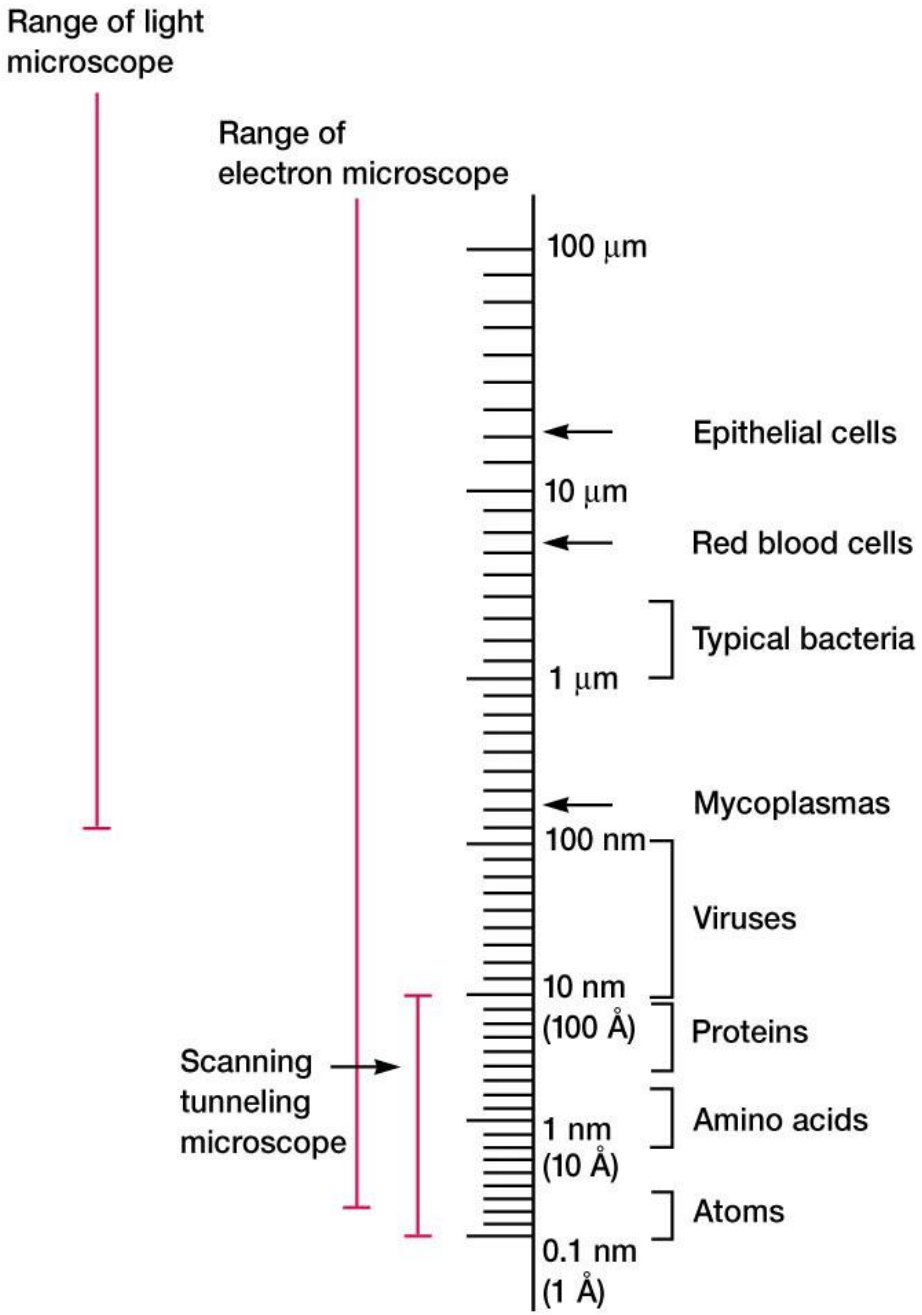
Lets light pass through an object & then through two or more lenses.

Stereoscopic Microscope

Gives a three-dimensional view of an object.
(Ex. Insects & leaves).

Electron Microscope

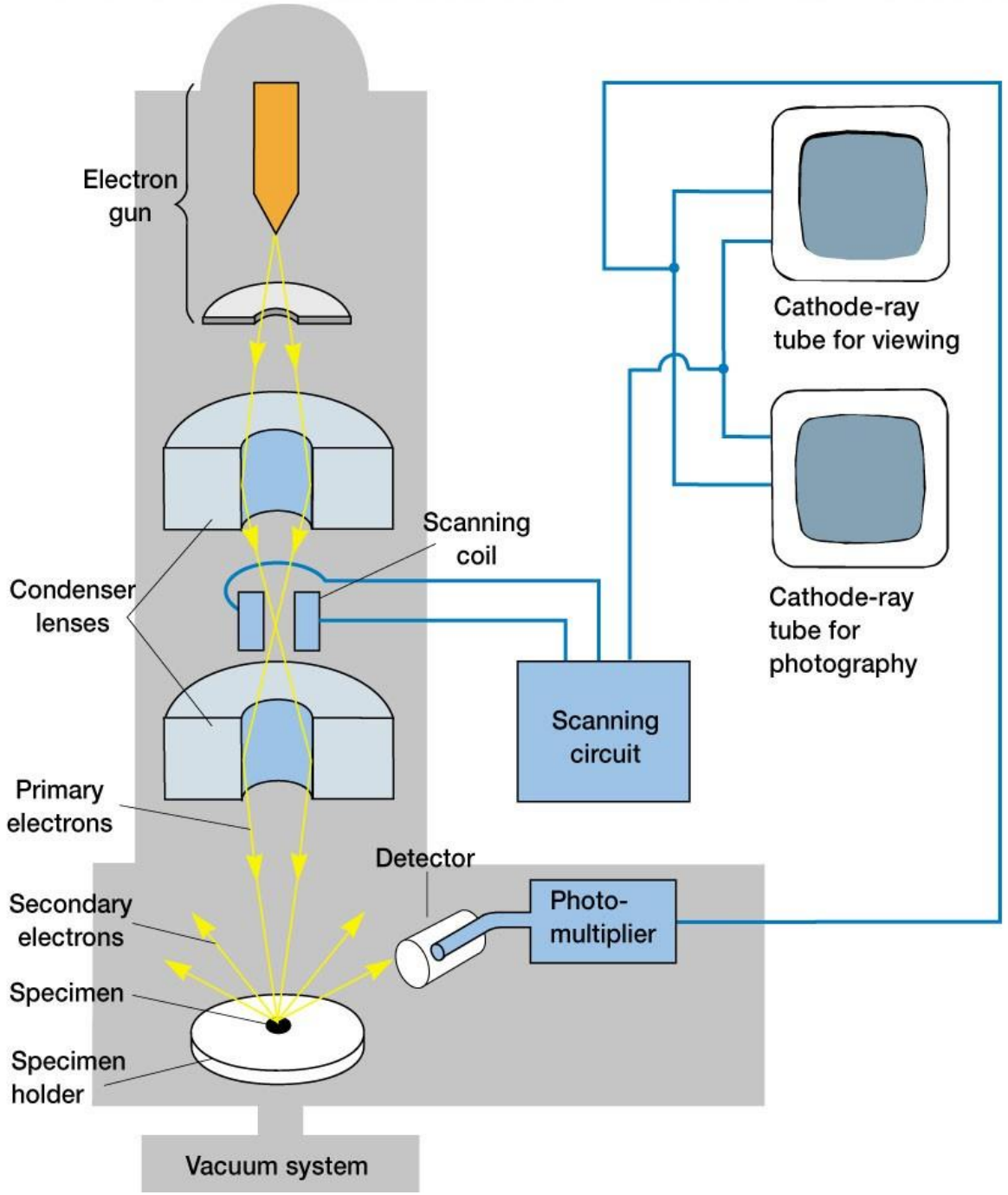
- Uses a magnetic field to bend beams of electrons; instead of using lenses to bend beams of light.
- Wavelength of electron beam is much shorter than light, resulting in much higher resolution.


Range of light microscope
Range of electron microscope
100 μm
Epithelial cells
10 μm
Red blood cells
Typical bacteria
1 μm
Mycoplasmas
100 nm
Viruses
10 nm
(100 Å)
Proteins
Scanning tunneling microscope
1 nm
(10 Å)
Amino acids
Atoms
0.1 nm
(1 Å)

Scanning Electron Microscope

- SEM uses electrons reflected from surface of a specimen to create image.
- Sample is scanned with a beam of electrons in a raster scan pattern.
- Electrons interact with atoms that make up the sample producing signals that contain information about sample's surface topography, composition, & other properties such as electrical conductivity.

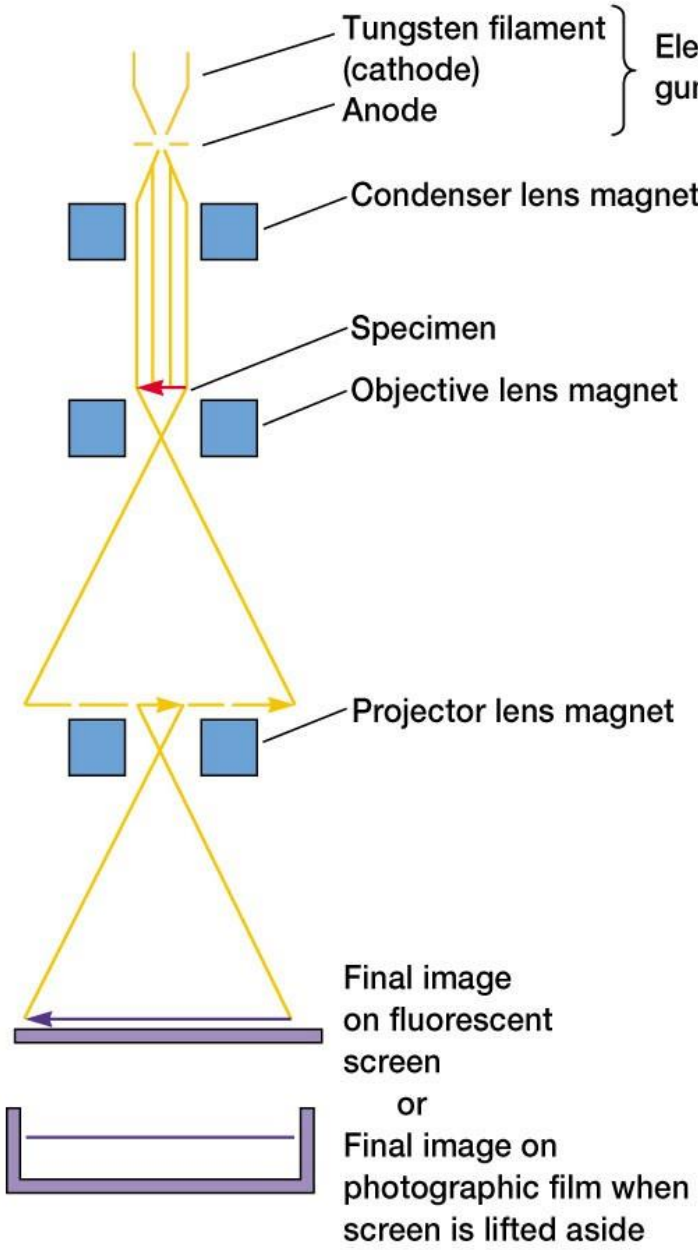
SEM


Electron gun
Cathode-ray tube for viewing
Cathode-ray tube for photography
Scanning coil
Condenser lenses
Scanning circuit
Primary electrons
Detector
Photo-multiplier
Secondary electrons
Specimen
Specimen holder
Vacuum system

Transmission Electron Microscope

- Electrons scatter when they pass through thin sections of a specimen.
- Transmitted electrons (those that do not scatter) are used to produce image.
- Denser regions in specimen, scatter more electrons & appear darker.

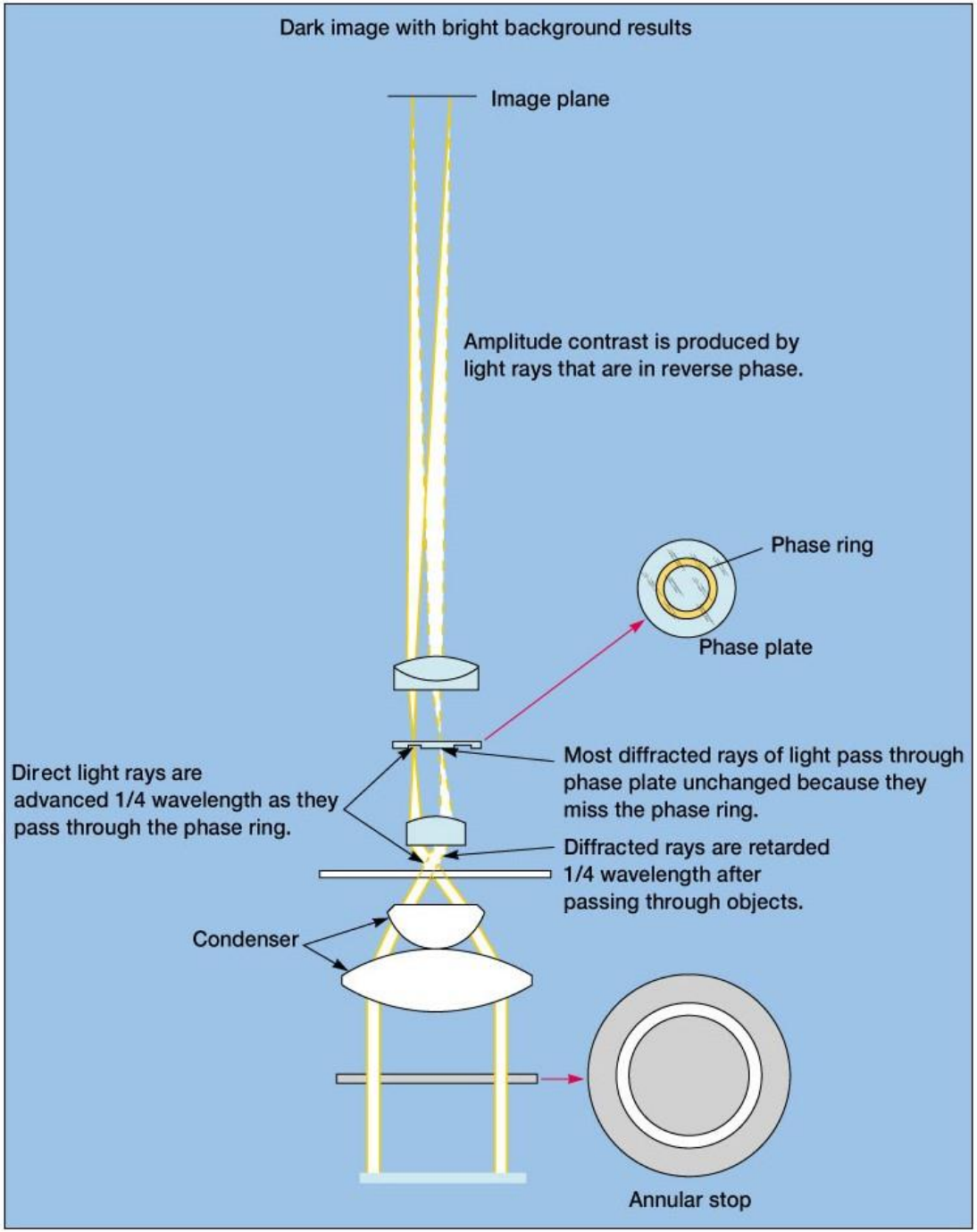
Transmission electron microscope



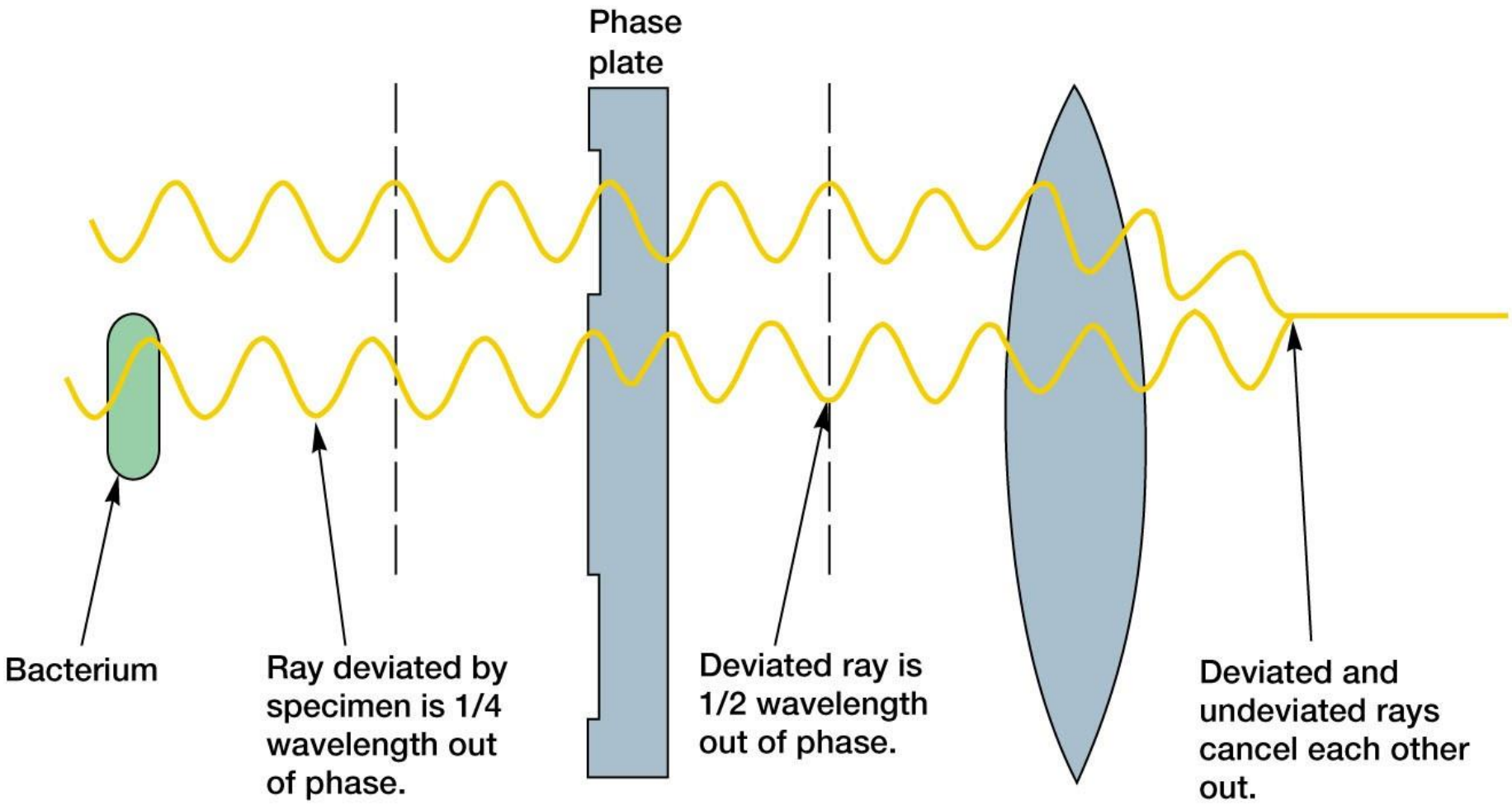
Phase-Contrast Microscope

- Enhances contrast between intracellular structures having slight differences in refractive index.
- Excellent way to observe living cells.

Phase-contrast microscope



Phase-contrast microscope



Bright-Field Microscope

- **Produces a dark image against a brighter background.**
- **It uses several objective lenses – parfocal microscopes remain in focus when objectives are changed.**
- **Total magnification:**

  product of magnifications of ocular lens & objective lens

Dark-Field Microscope

- **Produces a bright image of object against a dark background**.
- It is used to observe living, unstained preparations.

Digital Holography

Digital Holographic Microscope

- Holography was invented by Dennis Gabor to improve electron microscope.
- Basic concept of DHM is to magnify hologram image by adopting an optical lens system so that microscope fringes can be resolved.
- DHM, unlike other microscopy, doesn't record projected image of object, rather light wavefront information originating from object is digitally recorded as a hologram.
- Imaging lens in traditional microscopy is replaced by a computer algorithm.

Applications of DHM

DHM has capability of non-invasively visualizing & quantifying biological tissues.

Biomedical applications of DHM:

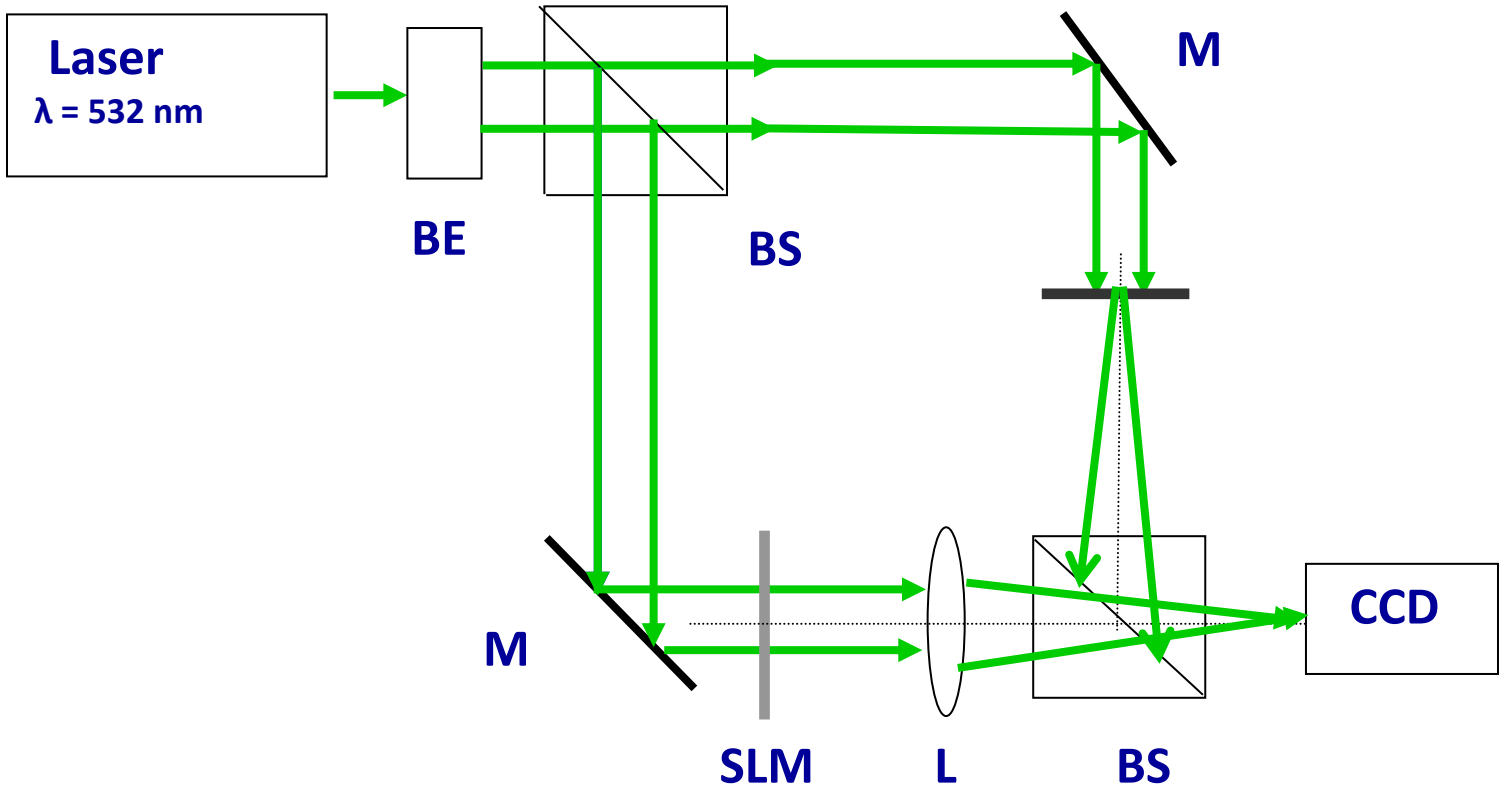
- To perform cell counting & to measure cell viability directly in cell culture chamber.
- To study apoptotic process (programmed cell death) in different cell types. Refractive index changes taking place during apoptotic process are easily measured with DHM.
- **Cell cycle analysis**: Phase shift induced by cells has been shown to be correlated to cell dry mass, which can be combined with other parameters obtainable by DH, such as, cell volume & refractive index, to provide a better understanding of cell cycle.

- **Morphology analysis of cells:** to study cell morphology using neither staining nor labeling.
- DHM is used for automated **plant stem cell** monitoring.
- To study undisturbed processes in **nerve cells** as no labeling is required. Swelling & shape changing of nerve cells caused by cellular imbalance is easily studied.
- To measure **3-D motion of human red blood cells** moving in a microtube flow. Phase shift images are used to study red blood cell dynamics.
- Red blood cell volume & hemoglobin concentration are measured by combining information from absorption & phase shift images to facilitate **complete blood cell count**.
- By combining several images calculated from same hologram, but at different focal planes, an **increased depth of field** is obtained.

Advantages

- **Simplicity of microscope**: It requires a laser, a pinhole, & a CCD camera, but no lenses at all (no aberration correction required).
- **Simplicity of sample preparation in biology**: no sectioning or staining are required, so that living cells can be viewed.
- **Maximum information**: a single hologram contains all information about 3-D structure of object.
- **Speed**: changes in specimen can ultimately be followed at capture video rate of CCD chip.
- **Maximum resolution** of order of λh of laser can easily be obtained, & can be further improved by at least a factor of two or three with setup of immersion holography.
- Compared to OCT, DHM requires only a pair of particle hologram images to get complete 3D flow information.

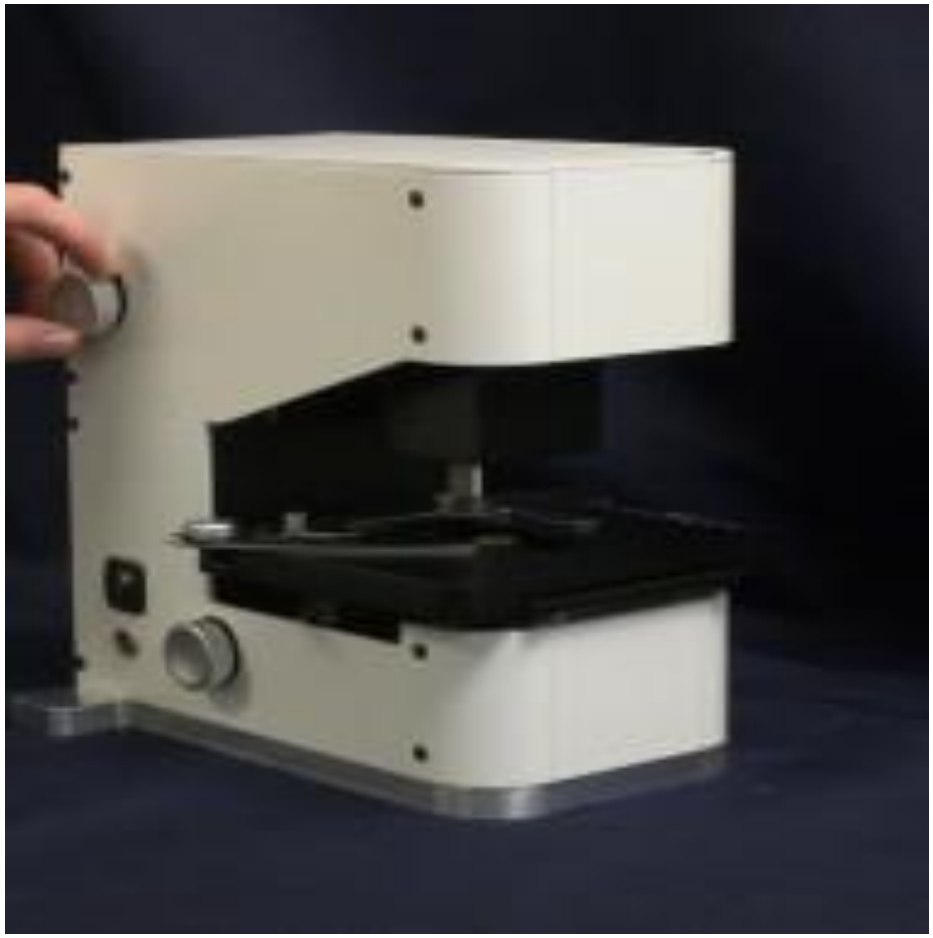
Digital Holography



BE: beam expander, BSs: beam splitters, SLM: spatial light modulator, RPM: random phase mask, CCD: charge coupled device, L: lens

Phase Holographic Imaging's The Holomonitor™ M3 (Sweden)

www.phiab.se



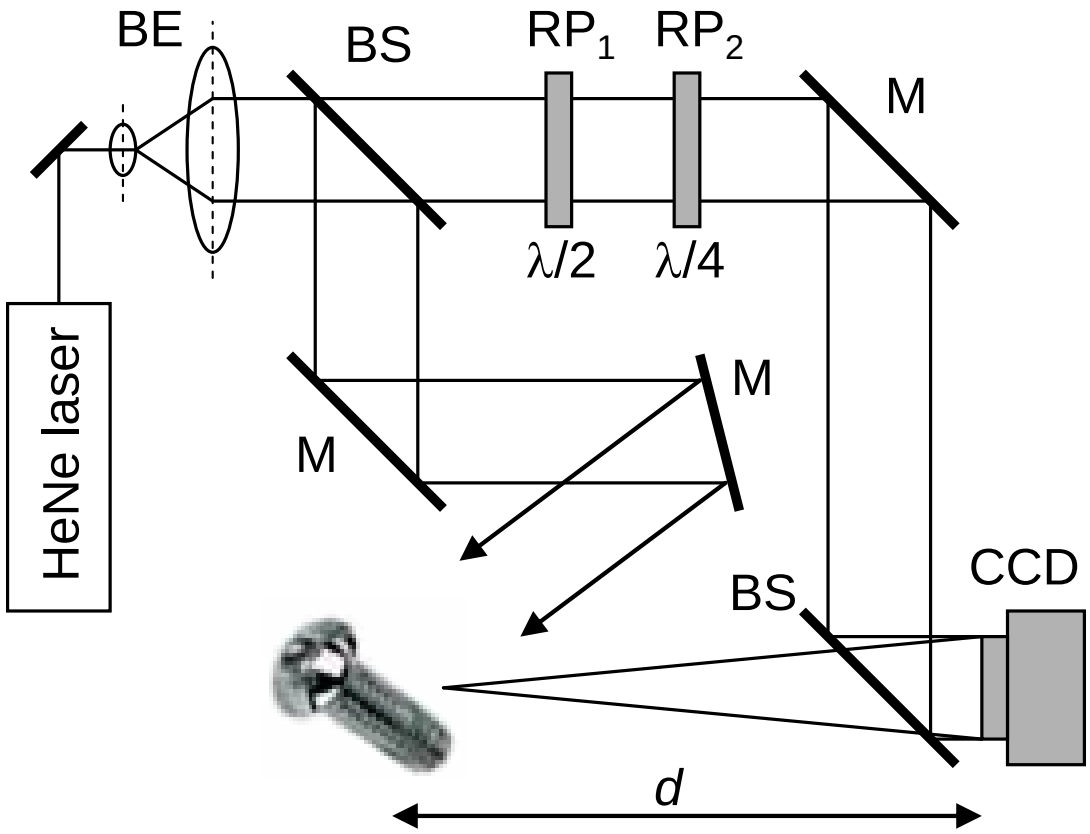
Resolutions Optics's Desktop System (Canada)
www.resolutionoptics.com

Submersible system is a product with all functionality of 3D imaging technology **encased in a waterproof housing**. It allows quickly & easily observation of micro-organisms & particles up to a **depth of 5 kilometers**.

Resolutions Optics's Submersible System (Canada)
www.resolutionoptics.com

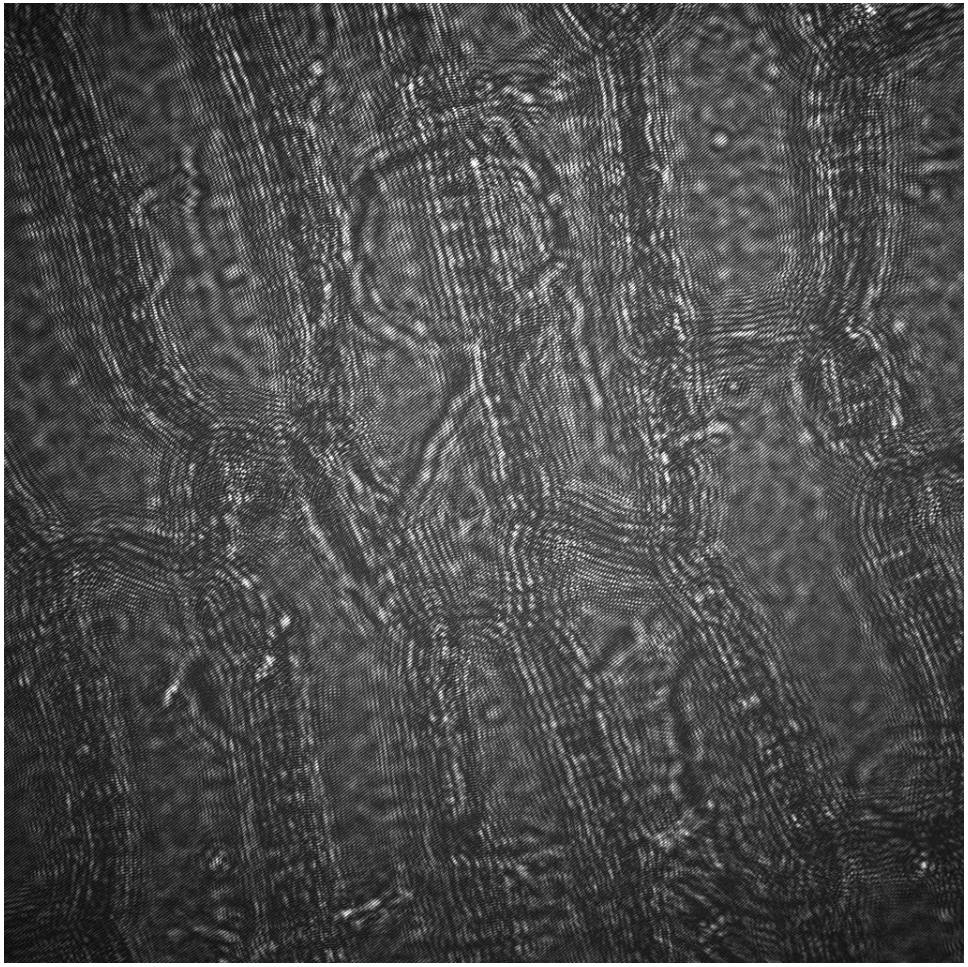
Digital Holographic Microscope DHMT1000 [Lyncee tec, Switzerland]

www.lynceetec.com

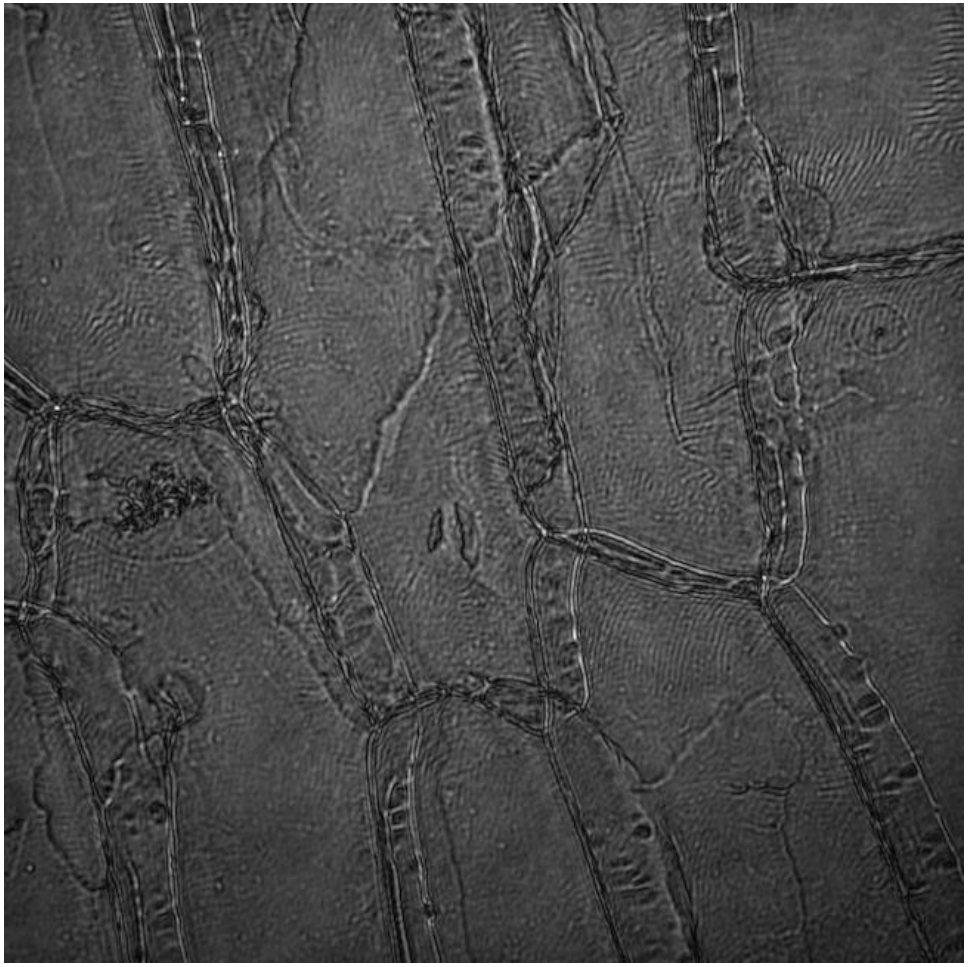


Phase-shifting digital holography. BE: beam expander, BS: beam splitter, RP: retardation plate, M: mirror, CCD: charge-coupled device.

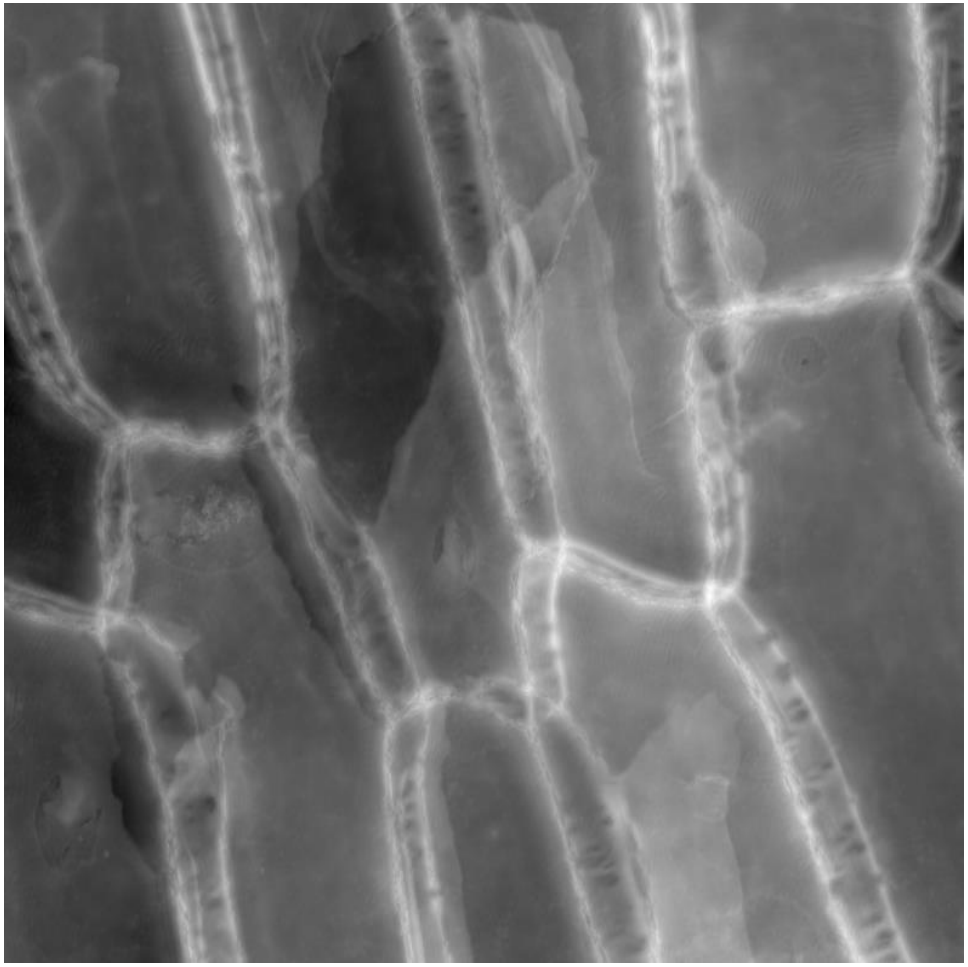
Digital Hologram of Onion Peel (10X)



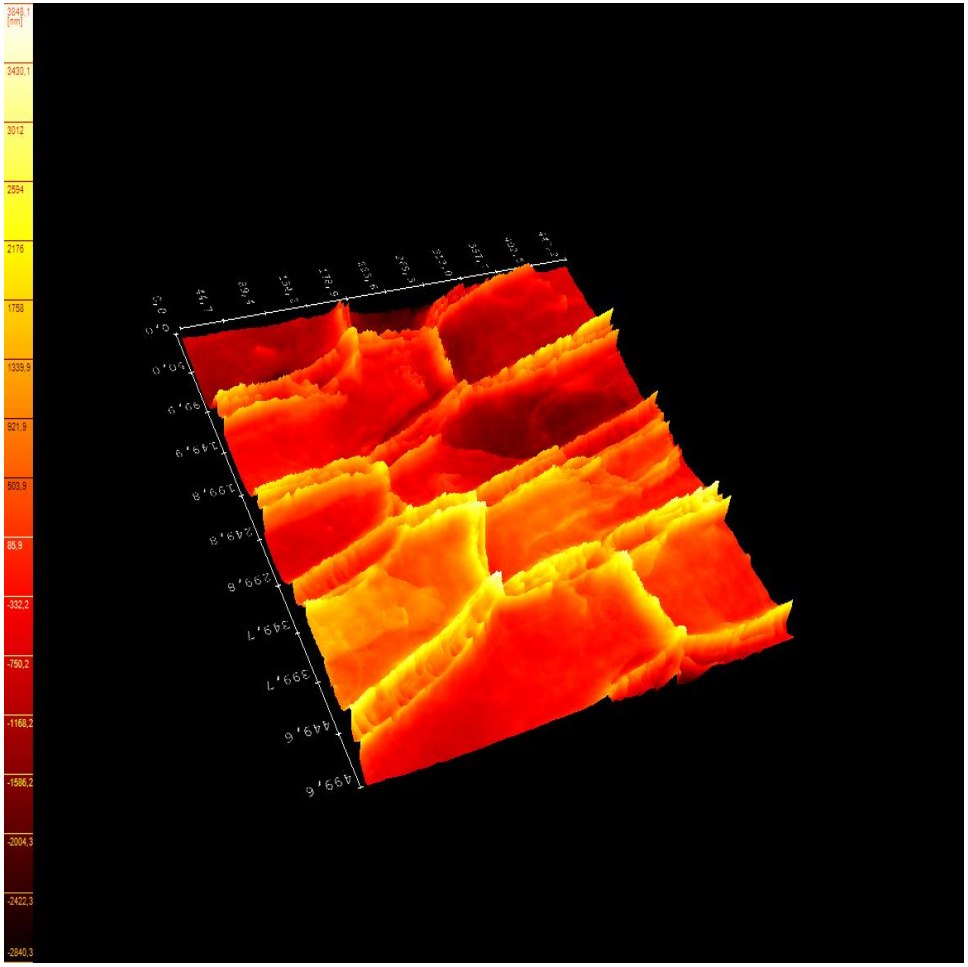
Intensity of Numerical Reconstruction with DH Onion Peel (10X)



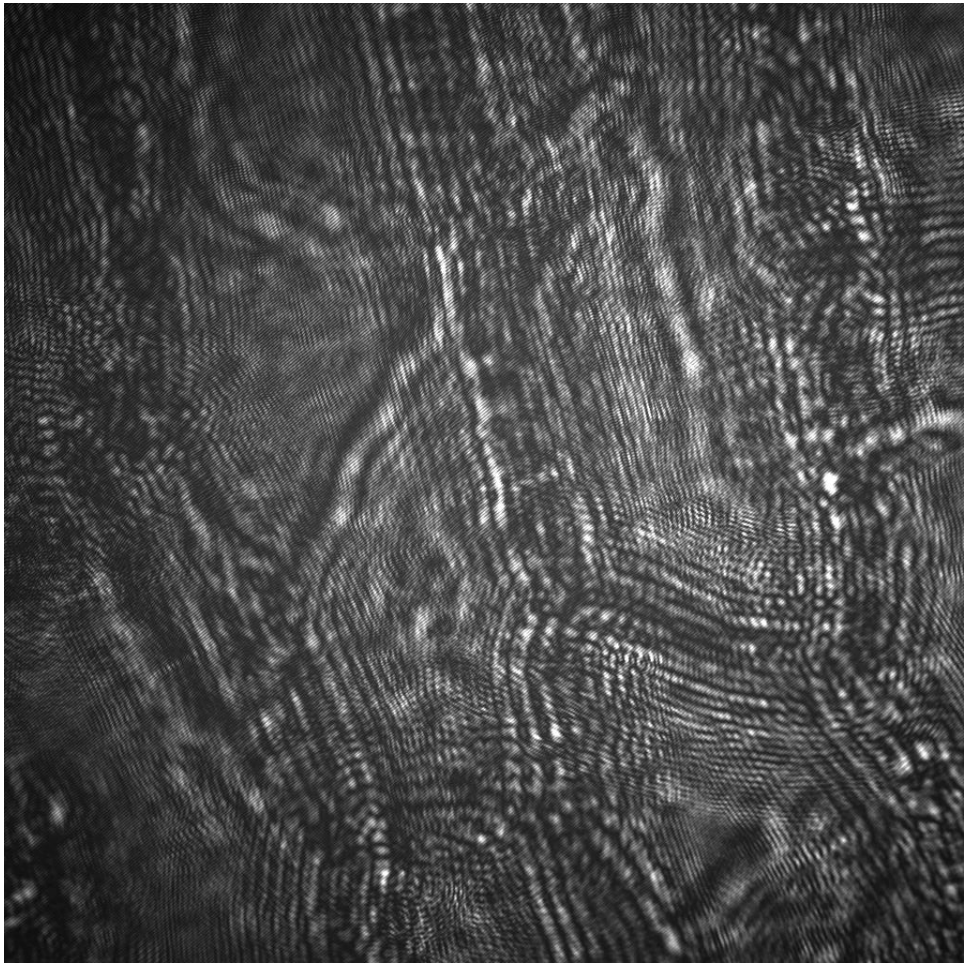
Phase of Numerical Reconstruction with DH Onion (10X)



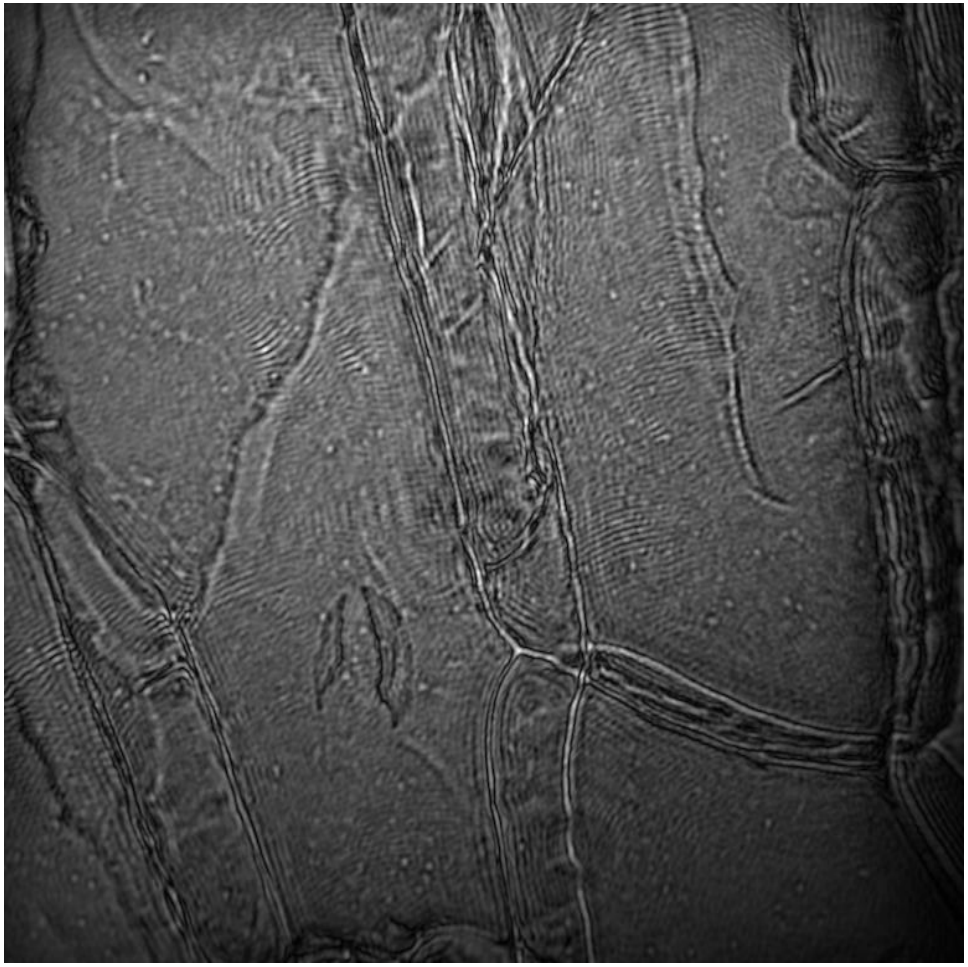
3-D presentation of Numerical Reconstruction's Phase with DH of Onion (10X)



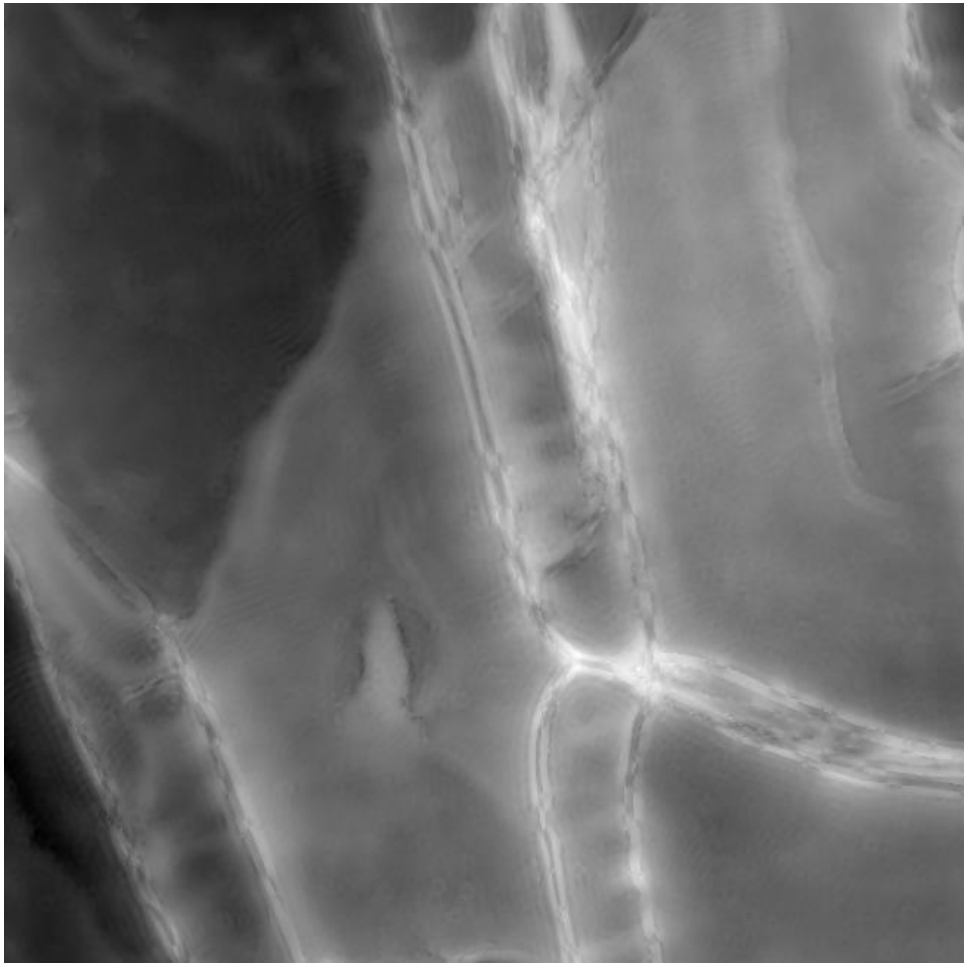
Digital Hologram of Onion Peel (20X)



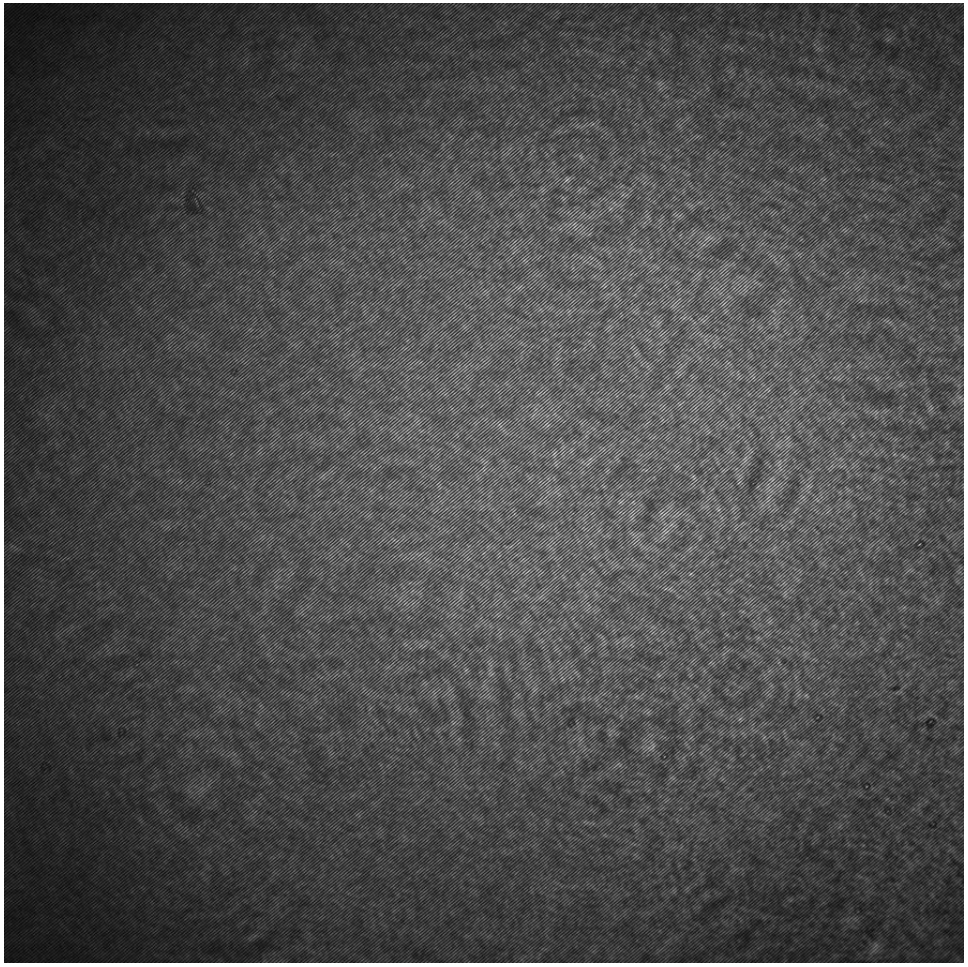
Intensity of Numerical Reconstruction with DH of Onion Peel (20X)



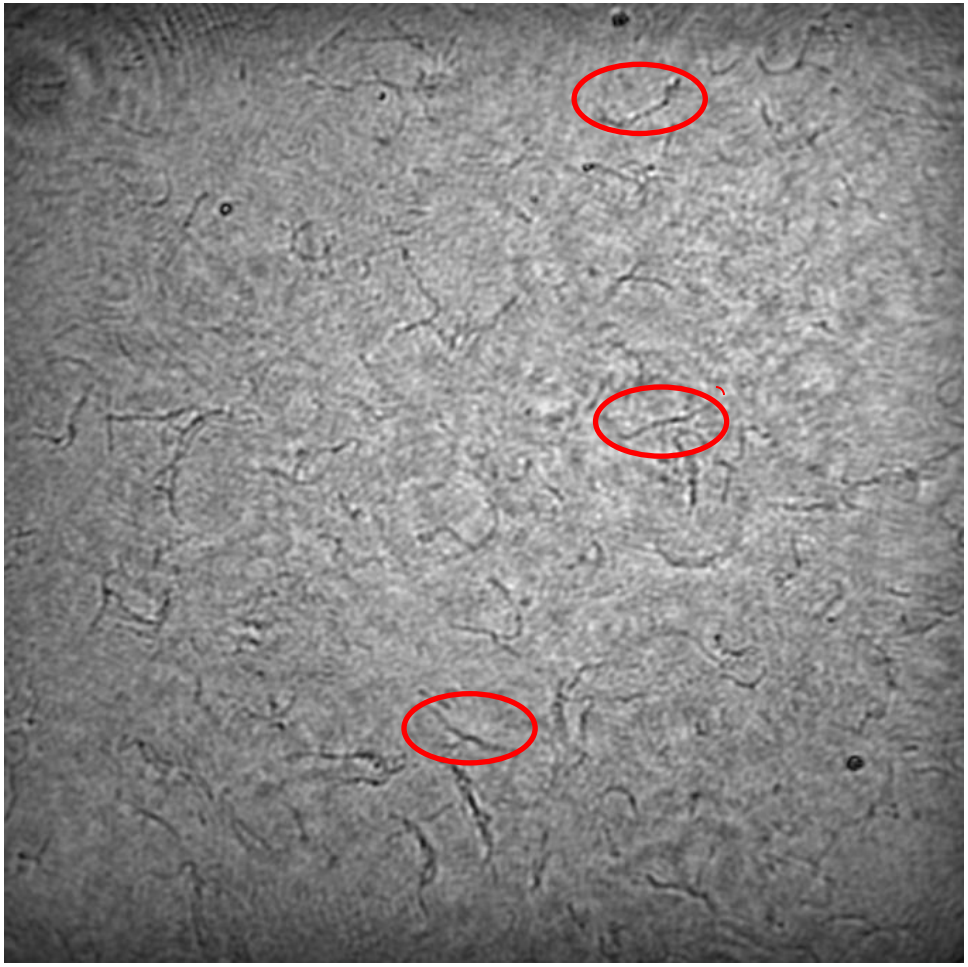
Phase of Numerical Reconstruction with DH of Onion (20X)



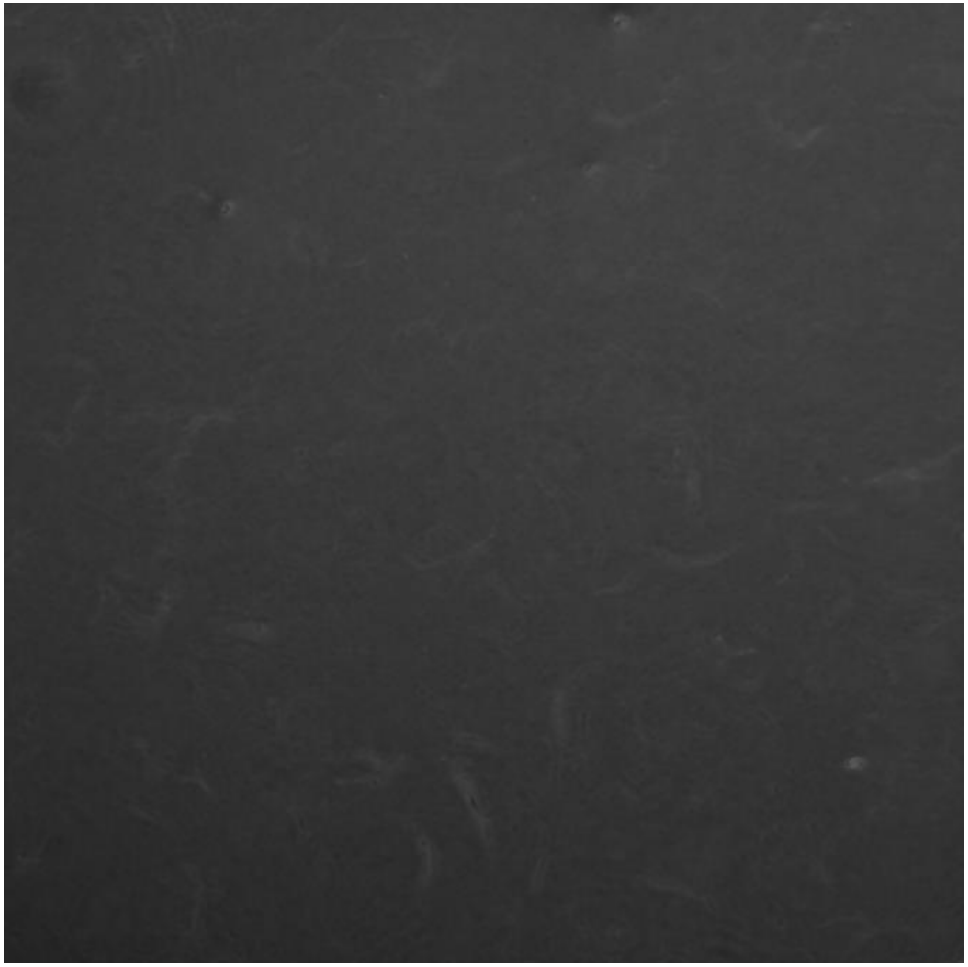
Digital Hologram of *E.coli* (20X)



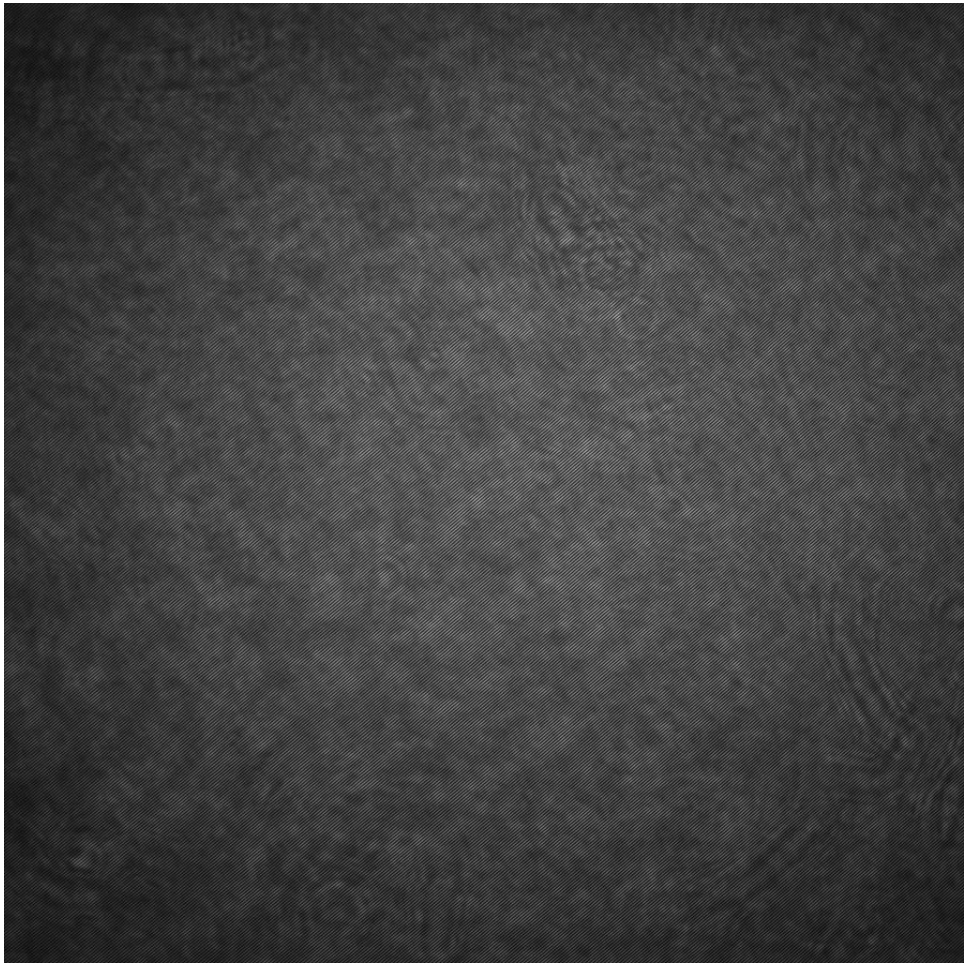
Intensity of Numerical Reconstruction with DH of *E. coli* (20X)



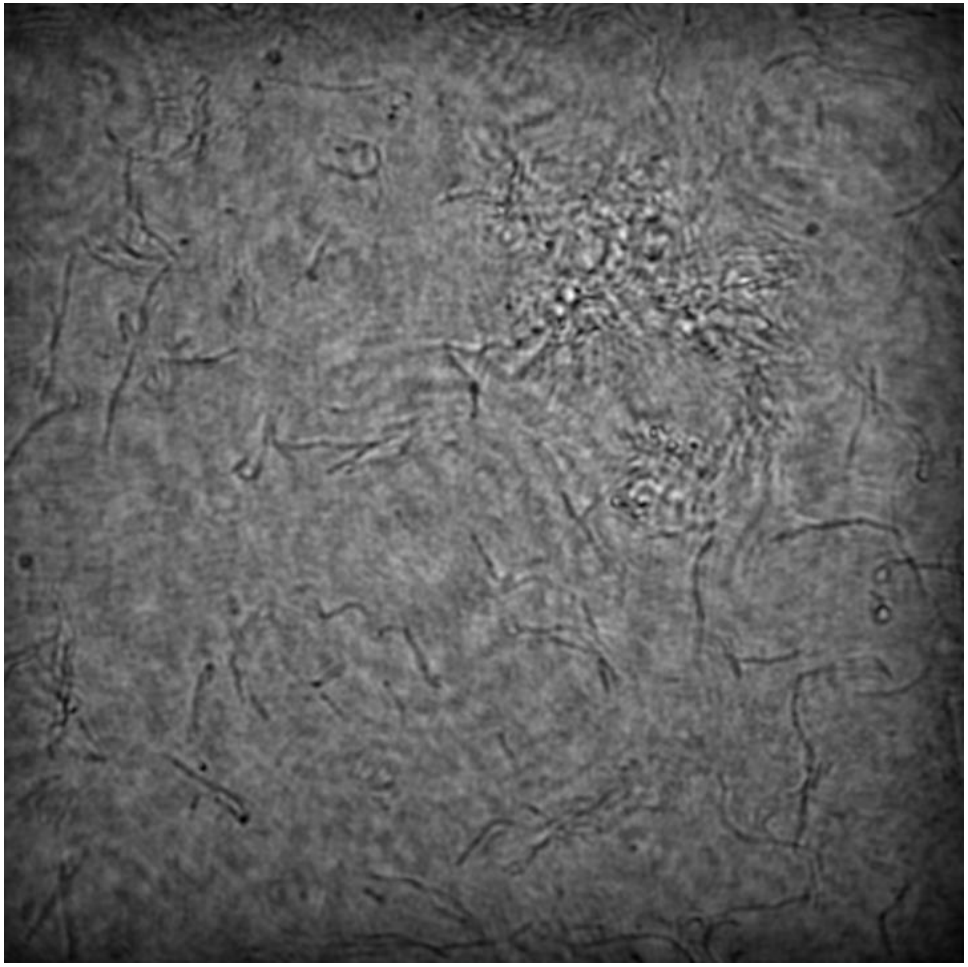
Phase of Numerical Reconstruction with DH of *E. coli* (20X)



Digital Hologram of *E.coli* (40X)



Intensity of Numerical Reconstruction with DH of *E. coli* (40X)



Phase of Numerical Reconstruction with DH of *E. coli* (40X)