Chapter 6: Microscopic Imaging and Organelles

Outline:

Microscopy
Introduction to various Microscopy techniques
Cell Fractionation
Cell Organelles
Cell Specific Markers

Overview:

• Cellular imaging is achieved by microscopy technologies. Light microscopy technologies include phase contrast, confocal microscopy, and immunofluorescence. Electron microscopy can achieve resolution on the nanometer scale.

• Sub-cellular fractionation allows for the isolation of cellular components for further study. This technique utilizes centrifugation gradients to segregate whole cell lysates.

• Contained within the cell are numerous organelles. Each organelle carries out specific functions vital to cellular homeostasis.

• Cell Marker technologies allow for the visualization of specific target cellular components.
Imaging:
The light microscope: an instrument that enables the human eye, by means of a lens or combinations of lenses, to observe enlarged images of tiny objects. It made visible the fascinating details of worlds within worlds.

Anton van Leeuwenhoek (1632-1723)
The father of microscopy, Anton Van Leeuwenhoek of Holland, started as an apprentice in a dry goods store where magnifying glasses were used to count the threads in cloth. Van Leeuwenhoek was inspired to take up microscopy by Hooke's works and in 1668 learned to grind lenses to make simple microscope. These microscopes were extremely simple devices using only one lens mounted in a tiny hole in a brass plate. The specimen was mounted on the sharp point in front of the lens, and its position and focus was adjusted by turning the two screws. He taught himself new methods for grinding and polishing tiny lenses of great curvature which gave magnifications up to 270 diameters, the finest known at that time. These led to the building of his microscopes and the biological discoveries for which he is famous. He was the first to see and describe bacteria, yeast plants, the teeming life in a drop of water, and the circulation of blood corpuscles in capillaries. During a long life he used his lenses to make pioneer studies on an extraordinary variety of things, both living and non living, and reported his findings in over a hundred letters to the Royal Society of England and the French Academy.

Light Microscopy

Microscopy means seeing a large image of something small. The light microscope, so called because it employs visible light to detect small objects, is probably the most well-known and well-used research tool in biology. The light microscope employs visible light to detect small objects. Object shows up because pigments absorb light differentially, or because they are thick enough to absorb a significant amount of light despite being colorless. Resolution is defined as the ability to distinguish two very small and
closely-spaced objects as separate entities. Resolution is calculated the wavelength of light, the light-gathering power of the objective, condenser lenses and defines the smallest distance (dmin) separating the two very small objects.

\[ d_{\text{min}} = \frac{1.22 \times \text{wavelength}}{\text{N.A. objective}} + \text{N.A. condenser} \]

This equation represents the theoretical resolving power of a light microscope per a given wavelength. However in practice, specimen quality usually affects dmin resulting in values greater than the theoretical lower limit.

**Bright Field Microscopy**

With a conventional bright field microscope, light from an incandescent source is aimed toward a lens beneath the stage called the condenser, through the specimen, through an objective lens, and to the eye through a second magnifying lens, the ocular or eyepiece. We see objects in the light path because natural pigmentation or stains absorb light differentially, or because they are thick enough to absorb a significant amount of light despite being colorless. A *Paramecium* should show up fairly well in a bright field microscope, although it will not be easy to see cilia or most organelles. Living bacteria won't show up at all unless the viewer hits the focal plane by luck and distorts the image by using maximum contrast.

A good quality microscope has a built-in illuminator, adjustable condenser with aperture diaphragm (contrast) control, mechanical stage, and binocular eyepiece tube. The condenser is used to focus light on the specimen through an opening in the stage. After passing through the specimen, the light is displayed to the eye with an apparent field that is much larger than the area illuminated. The magnification of the image is simply the objective lens magnification (usually stamped on the lens body) times the ocular magnification.

![Figure 2: Optical Path in a Light Microscope](image)

Most biological specimens do not absorb much light to produce contrast, but they diffract the light and cause a phase shift of the transmitted wave. Overall cell shape, membrane, nucleus and large organelles. Most of the internal structures of the cell are invisible. The possible solution for the problem is to use-- transmitted light-based techniques for improving contrast (Phase, Darkfield, Polarization, DIC)
Bright field microscopy is best suited to viewing stained or naturally pigmented specimens such as stained prepared slides of tissue sections or living photosynthetic organisms. It is useless for living specimens of bacteria, and inferior for non-photosynthetic protists or metazoans, or unstained cell suspensions or tissue sections. Here is a not-so-complete list of specimens that might be observed using bright-field microscopy, and appropriate magnifications (preferred final magnifications are emphasized).

Proper specimen illumination is required to achieve optimal images. The light should be uniform across the field of view. This is facilitated by the use of a condenser lens. The condenser lens is located beneath the specimen and is used to focus the light to the exact plane of the specimen. When properly aligned the focal point of the condenser lens is in the same plan as the focal point of the objective and ocular lens.

Prepared slides, stained bacteria (1000x), thick tissue sections (100x, 400x), thin sections with condensed chromosomes or specially stained organelles (1000x), large protists or metazoans (100x).

- Smears, stained blood (400x, 1000x), negative stained bacteria (400x, 1000x).
- Living preparations (wet mounts, unstained) - pond water (40x, 100x, 400x), living protists or metazoans (40x, 100x, 400x occasionally), algae and other microscopic plant material (40x, 100x, 400x). Smaller specimens will be difficult to observe without distortion, especially if they have no pigmentation.

Phase Contrast Microscopy
This method described by the Dutchman Frits Zernike in 1934 not only earned its discoverer the Nobel prize for physics in 1953, but also revolutionized biomedical basic research of living – i.e. unstained – cells. Phase contrast is ideal for thin unstained objects, for example culture cells on glass, which are approx. 5 bis 10 μm “thick” above the cell nucleus, but less than 1 μm “thick” at the periphery, and which barely exhibit any light absorption in the visible part of the spectrum. The eye can scarcely see them in brightfield and darkfield. However, very small differences exist between the refractive indices of the cells and the surrounding aqueous solutions (A) and within the cells between the cytoplasm (B) and the cell nucleus (C).
Phase contrast makes these tiny differences visible by the use of optical devices – i.e. it translates them into differences in intensity. The optical effect used consists of a shift of phase in the light ray. During their journey through cell nuclei, cytoplasm or water, the light waves are shifted by small degrees, since these media have slightly different refractive indices. The higher the refractive index of a medium, the smaller the speed or velocity of light in the medium. As a result, a light wave which has passed through a cell nucleus, lags behind the light waves which only had to pass through water. The amount of “lag” is called phase shift.

Before their entry into the sample, the waves are still “in phase”, but this is no longer the case when they have passed through the various materials. The amount of the phase shift behind the sample depends on what media (refractive indices) the waves had to pass through on their paths and how long the paths were in these media.

The human eye cannot see these phase shifts in the microscope image. It can only distinguish between different intensities and colors. Therefore, the phase contrast technique uses optical tricks to translate phase shifts into “grey values”.

Much like darkfield, the aperture diaphragm is replaced by a phase stop (1) which illuminates the sample (3) via the condenser optics (2). However, here the entire light bundle enters the objective (4) and an image of the phase stop (1) is created in the objective pupil (5). A “phase ring” is attached to the objective pupil (5) which does two things: firstly, it attenuates – like a grey filter – the pronounced bright light coming from the phase stop of the condenser, and secondly, it adds a constant phase shift to this light. If the specimen contains objects such as cells and their nuclei, they guide the light from the direct ray to new paths (7). This light will not pass through the phase ring in the objective, i.e. it will neither be attenuated nor will it be “retarded”. All the partial rays are fused to form the intermediate image (9) by the tube lens (8). The partial rays which have all been “retarded” to varying degrees are superimposed in the intermediate image, where they amplify or attenuate each other, depending on the phase position. Since the direct ray was strongly attenuated by the phase ring in the objective, the much weaker, diffracted light can become effective. The result of these interference processes in the intermediate image are bright and dark spots without which the cell to be examined would not be visible to the eye. Optimum contrast is created by selecting the right retardation and attenuation for the light waves in the phase ring of the objective.

A concomitant of phase contrast is the haloes of light which appear on the structure borders. They are caused by the optical principle and may result – especially in the case of thick specimens – in “illegibility” of the image, since the haloes are superimposed many times over. Therefore, phase contrast is a method which is only recommended for very thin objects where several structures are not physically lying on top of each other. In a thick specimen, details may be blended into an image which, in the final analysis, is then no longer “legible”.

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**Diagram Notes:**

1. Phase stop
2. Condenser optics
3. Sample
4. Objective
5. Objective pupil
6. Image of phase stop
7. Partial rays
8. Tube lens
9. Intermediate image

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Darkfield Contrast Microscopy

In order to increase the contrast of the images, the non diffracted light can be gotten rid of. This is achieved with the help of dark field microscopy. It is good for imaging unstained microorganisms, sub-resolution objects such as flagella (20nm diameter) visible with darkfield. This is not recommended for internal structure.

Light enters the microscope for illumination of the sample. A specially sized disc, the patch stop blocks some light from the light source, leaving an outer ring of illumination. The condenser lens focuses the light towards the sample. The light enters the sample. Most is directly transmitted, while some is scattered from the sample. The scattered light enters the objective lens, while the directly transmitted light simply misses the lens and is not collected due to a direct illumination block (see figure).

Only the scattered light goes on to produce the image, while the directly transmitted light is omitted. Everything is visible regardless of color, usually bright white against a dark background. Pigmented objects are often seen in “false colors,” that is, the reflected light is of a color different than the color of the object. Better resolution can be obtained using dark field as opposed to bright field viewing. Dark field illumination is most readily set up at low magnifications (up to 100x), although it can be used with any dry objective lens. Any time you wish to view everything in a liquid sample, debris and all, dark field is
best. Even tiny dust particles are obvious. Dark field is especially useful for finding cells in suspension. Dark field makes it easy to obtain the correct focal plane at low magnification for small, low contrast specimens.

Use dark field for initial examination of suspensions of cells such as yeast, bacteria, small protists, or cell and tissue fractions including cheek epithelial cells, chloroplasts, mitochondria, even blood cells (small diameter of pigmented cells makes it tricky to find them sometimes despite the color).

- Initial survey and observation at low powers of pond water samples, hay or soil infusions, purchased protist or metazoan cultures.
- Examination of lightly stained prepared slides. Initial location of any specimen of very small size for later viewing at higher power.
- Determination of motility in cultures

The main limitation of dark field microscopy is the low light levels seen in the final image. This means the sample must be very strongly illuminated, which can cause damage to the sample.

**Figure 7: Blood sample under dark field microscope**

**Fluorescence Microscopy**

In fluorescence microscopy, the specimens are treated with special reagents. Their individual molecules are able to absorb light for an extremely short time – usually billionths of a second – and then to emit it again. However, the emitted light features a wavelength which is slightly shifted “towards red”. If, for example, blue light is absorbed, green light will be emitted immediately afterwards. Green is changed to yellow, yellow to reddish orange.

**Figure 8: Fluorescent microscopic images**
and invisible UV light to visible light. This shift is termed Stokes shift after its discoverer. In fluorescence, the wavelength of the emitted light is about 20 to 50 nanometers longer than absorbed exciting light. Fluorescence molecules can only absorb light of a certain wavelength. Each of the various fluorochromes exhibits its own, very specific absorption spectrum, depending on the internal structure of the fluorescence molecules and sometimes also on their surroundings. Furthermore, not every photon is absorbed, but only a part of their radiating light. The absorbed photons are not emitted again in their entirety either. Good fluorescence markers feature a high “quantum yield” – a term describing the ratio of the emitted to the absorbed photons.

This effect is very useful for microscopy: a specimen marked in this way is illuminated with pure, filtered blue light and viewed using a barrier filter which is completely opaque to blue light, but which transmits long-wave green, yellow and red light. The structures marked with fluorescence molecules – e.g. parts of a cytoskeleton – then light up green against a black background. When microfluorescence was initially introduced, the specimens were usually dyed non-specifically with fluorochromes. This type of marking usually looks bright, since many fluorescence molecules are bonded everywhere. Nowadays, however, fluorescence methods are much more specific. This has been made possible in particular by the permanent coupling of the fluorescence molecules with biological substances, e.g. antibodies (in this case, it is no longer the dye which determines the bonding position, but the biologically active molecule). Normally, this results in weak fluorescence images in the microscope because much less dye is bonded. However, the information obtained, e.g. in the diagnosis of illnesses, is becoming much more exact.
Epi Fluorescence Microscopy is a method of fluorescence microscopy that is widely used in life sciences. The excitatory light is passed from above (or, for inverted microscopes, from below), through the objective lens and then onto the specimen instead of passing it first through the specimen. The fluorescence in the specimen gives rise to emitted light which is focused to the detector by the same objective that is used for the excitation. Since most of the excitatory light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light and this method therefore gives an improved signal to noise ratio. An additionally filter between the objective and the detector can filter out the remaining excitation light from fluorescent light. A common use in biology is to apply fluorescent or fluorochrome stains to the specimen in order to image distributions of proteins or other molecules of interest. The filters and the dichroic are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen. In this manner, a single fluorophore (color) is imaged at a time. Multi-color images of several fluorophores must be composed by combining several single-color images.

Multifluorescence is now being increasingly used for the marking of specimens. This method makes different structures light up in different colors which can be viewed separately –
each on its own. The reflector slider can be used to change between the images. However, it is easier to use special filter sets which permit the simultaneous viewing of two or three markers in one image.

**Confocal Microscope**

The major advantage is the ability to collect high-resolution serial optical sections from thick specimens. The resolution of the images collected with widefield optical microscopes is limited by out-of-focus light. In the confocal microscope, a beam of intensive light (1) is transferred to the specimen (3) via the objective (2). The scanners (4), with which the light ray scans the specimen (3), are included in the beam path. The fluorescence light generated there travels back via the scanners – i.e. the ray motion is neutralized again – passes through the beam splitter (5) and is focused on the pinhole (6). Behind the pinhole arrier filters (7) and is continuously measured by the light detector (8). The computer composes an electronic image from the measured values. The decisive factor is that only fluorescent light from the focal plane of the objective (3) can pass through the pinhole (6) in the spatial filter (A). Light from other planes of the specimen (e.g. 3a) is blocked out very effectively by the pinhole (6). This permits individual planes of the specimen to be viewed. 3D images are generated with computer support.

The illumination is achieved by scanning one or more focused beams of light, usually from a laser, across the specimen. The images produced by scanning the specimen in this way are called optical sections. This terminology refers to the noninvasive method by which the instrument collects images, using focused light rather than physical means to section the specimen.

**Photomultiplier tubes** (photomultipliers or PMTs for short), members of the class of vacuum tubes, and more specifically phototubes, are extremely sensitive detectors of light in the Ultraviolet, visible, and near infrared ranges of the electromagnetic spectrum. These detectors multiply the current produced by incident light by as much as 100 million times enabling individual photons to be detected when the incident flux of light is very low.

**Practical hints on fluorescence microscopy:**

- **The work environment:**
  If fluorescence is weak, you had better work in a dark environment. Make sure that you do not have to enter a bright area during work or even have to look at lamps or bright windows.

- **Bleaching of specimens:**
  When the specimen is not being viewed or photographed, block out the excitation light using the filter slider in the fluorescence illuminator in order to avoid undesired bleaching by the excitation light.

- **Fluorescence-free immersion oil:**
  If autofluorescent immersion media are used, the background brightens and the image contrast is reduced.

- **The heat-protection filter:**
  Fluorescence filters are sensitive to the heat emitted by the illuminator. Please never remove the integrated heat-protection filter.

- **Adjustment of the lamp:**
  The illuminator must be readjusted occasionally – in all cases after lamp replacement. Please see the operating instructions for further information and remember to observe the safety regulations (see page 26).

- **Specimen preparation:**
  The unbonded fluorochromes should be removed from the specimen, e.g. by washing them out. The "contrast" in the fluorescence image is generated by the dark background only, which – in turn – is unnecessarily brightened.
Figure 12: Highly specific multiple fluorescence: Various fluorochromes mark exactly defined structures in the cytoskeleton of individual cells.

Laser Scanning Confocal Design

Mirrors in scanning head steer the laser beam to sample the entire specimen area for each focal plane. This is the slow step in image formation.
Transmission electron microscopy (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. The resolution of light microscopy is limited by visible light’s wavelength size (0.6 microns). TEM uses electrons as the “light source”. Because the wavelength of electrons are much smaller than those of visible light it possible to get resolution a thousand times better than with a light microscope. In an electron microscope a “light source” at the top of the microscope emits electrons that travel through a vacuum in the column of the microscope. The microscope uses electromagnetic lenses to focus the electrons into a very thin beam. The electron beam then travels through the specimen. Depending on the density of the material present, some of the electrons are scattered and disappear from the beam. At the bottom of the microscope the unscattered electrons hit a fluorescent screen, which gives rise to a “shadow image” of the specimen with its different parts displayed in varied darkness according to their density. The image can be studied directly by the operator or photographed with a camera.
Different Types of Electron Microscope

**Transmission electron microscope (TEM):**

It operates in vacuum, for specimen that are usually fixed, embedded, sectioned, and stained with an electron-dense material, resolution at 1 nm. A beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen.
**Scanning Electron Microscope (SEM):**

It is used to visualize surfaces of tissues, cells, isolated cell parts. Specimen is fixed and coated with thin layer of heavy metal. This microscope images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties.

**Cell Organelles**

**Sub Cellular Fractionation**

Cell is the basic structural and functional unit of life, or the building block of life. Certain organisms such as most Bacteria are unicellular, unlike humans who are multicellular (100 trillion cells). Size of a typical cell 10 µm and typical cell mass is 1 Nanogram. The composition of a single cell: 70%H2O, 7% small molecules, 20% proteins, 2% RNA, 1%DNA by mass. The two major kinds of organisms are eukaryotic and prokaryotic. The prokaryote cell is simpler and smaller, than a eukaryote cell, lacking a nucleus and most of the other membrane bound organelles.

In order to study these organelles or to obtain a specific protein to work and characterize its functions and structure, we need to isolate the specific organelle. This is done in various ways, by fractionating cells. A schematic for fractionating cells is shown in the adjoining figure with the help of density centrifugation.

**Figure 16: Schematic of Sub cellular Fractionation**

**Figure 17: EM (nuclei, mitochondria, rough ER, Golgi, plasma membrane, peroxisome)**
Lysosomes

Lysosomes are spherical organelles that contain enzymes (acid hydrolases). They break up food so it is easier to digest. They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at the 4.5 pH they require. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are created by the addition of hydrolytic enzymes to early endosomes from the Golgi apparatus. The name *lysosome* derives from the Greek words *lysis*, which means dissolution or destruction, and *soma*, which means body. They are frequently nicknamed "suicide-bags" or "suicide-sacs" by cell biologists due to their role in autolysis. Lysosomes were discovered by the Belgian cytologist Christian de Duve in 1949.

Figure 18: Lysosomes

The size of lysosomes varies from 0.1–1.2 μm. At pH 4.8, the interior of the lysosomes is acidic compared to the slightly alkaline cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons (H⁺ ions) from the cytosol across the membrane via proton pumps and chloride ion channels. The lysosomal membrane protects the cytosol, and therefore the rest of the cell, from the degradative enzymes within the lysosome. The cell is additionally protected from any lysosomal acid hydrolases that leak into the cytosol as these enzymes are pH-sensitive and function less well in the alkaline environment of the cytosol.

Mitochondria

In cell biology, a mitochondrion (plural mitochondria) is a membrane-enclosed organelle found in most eukaryotic cells. These organelles range from 0.5 to 10 micrometers (μm) in diameter. Mitochondria are sometimes described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling.

Figure 19: Mitochondria
cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process.

Several characteristics make mitochondria unique. The number of mitochondria in a cell varies widely by organism and tissue type. Many cells have only a single mitochondrion, whereas others can contain several thousand mitochondria. The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the inner membrane, the intermembrane space, the mitochondrial matrix, and the cristae. Mitochondrial proteins vary depending on the tissue and the species. The mitochondrial proteome is thought to be dynamically regulated. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome. Further, its DNA shows substantial similarity to bacterial genomes.

A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins. The two membranes, however, have different properties. Because of this double-membraned organization, there are five distinct compartments within the mitochondrion. There is the outer mitochondrial membrane, the intermembrane space (the space between the outer and inner membranes), the inner mitochondrial membrane, the cristae space (formed by infoldings of the inner membrane), and the matrix (space within the inner membrane).

"These are complex, energy producing organelles which are found in all eukaryotic cells. (Both plant and animal). These organelles are the site of "cellular respiration" (ATP synthesis). (Energy conversion from "foods" (glucose). Certain cells which requires large amounts of energy contain large numbers of mitochondria. Mitochondria are oval elongated organelles. They have two membranes. An outer membrane and an inner (highly folded) membrane. We will return to mitochondria when we examine cell exegetics".

"A dominant role for the mitochondria is the production of ATP as reflected by the large number of proteins in the inner membrane for this task. This is done by oxidising the major products of glycolysis: pyruvate and NADH that are produced in the cytosol. This process of cellular respiration, also known as aerobic respiration, is dependent on the presence of oxygen. When oxygen is limited the glycolytic products will be metabolised by anaerobic respiration a process that is independent of the mitochondria. The production of ATP from glucose has an approximately 15 fold higher yield during aerobic respiration compared to anaerobic respiration"
Chloroplasts are organelles found in plant cells and other eukaryotic organisms that conduct photosynthesis. Chloroplasts capture light energy to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis. Photosynthesis occurs on thylakoid membranes. It contains two functionally and spatially distinct photosystems (protein complexes, PS). Cyclic electron flow in PSI generates ATP but no NADPH and PSII oxidizes water to give O2. The two photosystems absorb light energy through proteins containing pigments, such as chlorophyll.

The light-dependent reactions begin in photosystem II. When a special chlorophyll molecule of PSII absorbs a photon, an electron in this molecule attains a higher energy level. Because this state of an electron is very unstable, the electron is transferred from one to another molecule creating a chain of redox reactions, called an electron transport chain (ETC). The electron flow goes from PSII to cytochrome b6f to PSI. In PSI the electron gets the energy from another photon. The final electron acceptor is NADP. In oxygenic photosynthesis, the first electron donor is water, creating oxygen as a waste product.

Cytochrome b6f and ATP synthase are working together to create ATP. This process is called photophosphorylation, which occurs in two different ways. In non-cyclic photophosphorylation, cytochrome b6f uses the energy of electrons from PSII to pump protons from the stroma to the lumen. The proton gradient across the thylakoid membrane creates a proton-motive force, used by ATP synthase to form ATP. In cyclic photophosphorylation, cytochrome b6f uses the energy of electrons from not only PSII, but also PSI to create more ATP and to stop the production of NADPH. Cyclic phosphorylation is important to create ATP and maintain NADPH in the right proportion for the light-independent reactions.
Endoplasmic Reticulum

The endoplasmic reticulum (ER) is an eukaryotic organelle that forms an interconnected network of tubules, vesicles, and cisternae within cells. Rough endoplasmic reticulums synthesize proteins, while smooth endoplasmic reticulums synthesize lipids and steroids, metabolize carbohydrates and steroids, and regulate calcium concentration, drug detoxification, and attachment of receptors on cell membrane proteins. The lacy membranes of the endoplasmic reticulum were first seen by Keith R. Porter, Albert Claude, and Ernest F. Fullam in 1945.

There are two basic kinds of endoplasmic reticulum morphologies: rough and smooth. The rough endoplasmic reticulum is involved mainly with the production and processing of proteins that will be exported, or secreted, from the cell. Smooth endoplasmic reticulum is chiefly involved with the production of lipids (fats), building blocks for carbohydrate metabolism, and the detoxification of drugs and poisons. Smooth endoplasmic reticulum also
plays a role in various cellular activities through its storage of calcium and involvement in calcium metabolism.

A ribosome only binds to the ER once it begins to synthesize a protein destined for the secretory pathway. Here, a ribosome in the cytosol will not begin synthesizing a protein until a signal recognition particle recognizes the pre-piece of 5-15 hydrophobic amino acids preceded by a positively charged amino acid. This signal sequence allows the recognition particle to bind to the ribosome, causing the ribosome to bind to the RER and pass the new protein through the ER membrane. The pre-piece is then cleaved off within the lumen of the ER and the ribosome released back into the cytosol.

The membrane of the RER is continuous with the outer layer of the nuclear envelope. membrane-bound vesicles shuttle proteins between these two compartments. Vesicles are surrounded by coating proteins called COPI and COPII. COPII targets vesicles to the golgi and COPI marks them to be brought back to the RER. The RER works in concert with the Golgi complex to target new proteins to their proper destinations.

The smooth endoplasmic reticulum (SER) has functions in several metabolic processes, including synthesis of lipids and steroids, metabolism of carbohydrates, regulation of calcium concentration, drug detoxification, attachment of receptors on cell membrane proteins, and steroid metabolism.

The smooth endoplasmic reticulum is known for its storage of calcium ions in muscle cells.

Calnexin and calreticulin assist the folding of glycoproteins in the endoplasmic reticulum (ER) (for simplicity, only calreticulin is depicted). After transfer of the core oligosaccharide (Glc$_3$Man$_9$GlcNAc$_2$, where Glc is glucose (red circles), Man is mannose (blue circles) and GlcNAc is N-acetylglucosamine) to the nascent chain of the protein, two glucoses are removed by glucosidases I and II. This generates a monoglucosylated (Glc$_1$Man$_9$GlcNAc$_2$) glycoprotein that can interact with calnexin and calreticulin. Both chaperones associate with the thiol-disulphide oxidoreductase ERp57 through an extended arm-like domain. During the catalysis of disulphide-bond formation, ERp57 forms interchain disulphide bonds (S–S) with

![Figure 23: Calnexin/Calreticulin cycle: Quality Control [1]](image-url)
bound glycoproteins. Cleavage of the remaining glucose by glucosidase II terminates the interaction with calnexin and calreticulin. On their release, correctly folded glycoproteins can exit the ER. By contrast, non-native glycoproteins are substrates for the UDP-glucose:glycoprotein glucosyltransferase, which places a single glucose back on the glycan and thereby promotes a renewed association with calnexin and calreticulin. If the protein is permanently misfolded, the mannose residue in the middle branch of the oligosaccharide is removed by ER x1,2-mannosidase I. This leads to recognition by the ER degradation-enhancing 1,2-mannosidase-like protein (EDEM), which probably targets glycoproteins for ER-associated degradation (ERAD). Please note that, for simplicity, only some of the sugar moieties of the oligosaccharide core structure are depicted fully in the figure.

**Golgi Complex**

The primary function of the Golgi apparatus is to process and package macromolecules, such as proteins and lipids, after their synthesis and before they make their way to their destination; it is particularly important in the processing of proteins for secretion. It primarily modifies proteins delivered from the rough endoplasmic reticulum but is also involved in the transport of lipids around the cell, and the creation of lysosomes. In this respect it can be thought of as similar to a post office; it packages and labels items which it then sends to different parts of the cell.

**Nucleus**

It contains most of the cell's genetic material, is organized as multiple long linear DNA molecules in complex with a large variety of proteins. The genes within these chromosomes are the cell's nuclear genome. It also helps maintain the integrity of these genes and to control the activities of the cell by regulating gene expression.
Small particles (< 30 kDa) are able to pass through the nuclear pore complex by passive diffusion. Larger particles are also able to pass through the large diameter of the pore but at almost negligible rates. Efficient passage through the complex requires several protein factors. Any cargo with NLS will be transported efficiently across the pore. And import into the nucleus.

The classical scheme of NLS-protein importation begins with Importin-α first binding to the NLS sequence, and acts as a bridge for Importin-β to attach. The importinβ—importinα—cargo complex is then directed towards the nuclear pore and diffuses through it. Once the complex is in the nucleus, RanGTP binds to Importin-β and displaces it from the complex. Then the cellular apoptosis susceptibility protein (CAS), an exportin which in the nucleus is bound to RanGTP, displaces Importin-α from the cargo. The NLS-protein is thus free in the nucleoplasm. The Importinβ-RanGTP and Importina-CAS-RanGTP complex diffuses back to the cytoplasm where GTPs are hydrolyzed to GDP leading to the release of Importinβ and Importina which become available for a new NLS-protein import round.

Although cargo passes through the pore with the assistance of chaperone proteins, the translocation through the pore itself is not energy dependent. However, the whole import cycle needs the hydrolysis of 2 GTPs and is thus energy dependent and has to be considered as active transport. The import cycle is powered by the nucleo-cytoplasmic RanGTP gradient. This gradient arises from the exclusive nuclear localization of RanGEFs, proteins that exchange GDP to GTP on Ran molecules. Thus there is an elevated RanGTP concentration in the nucleus compared to the cytoplasm.

**Figure 26: Schematic of Nuclear transport**

**Figure 27: Nuclear Pore**
Cytosol

“The cytosol is the main component of the cytoplasm, the fluid that fills the inside of the cell. The cytoplasm is everything in the cell except for the cytoskeleton and membrane-bound organelles. The cytosol performs structural functions within a cell. The cytosol often comprises more than 50% of a cell's volume. Beyond providing structural support, the cytosol is the site of protein synthesis and site of the centrosomes and centrioles”
**Cell Markers**

![Western Blots](image)

**Figure 29:** Organelle specific antibodies: Western blots of mitochondrial preparations probed with antibodies specific for organelle-/cell-compartment specific marker proteins. PM: plasma membrane; ER: endoplasmic reticulum
Figure 30: Four-panel composite image of mouse fibroblasts that were incubated with MitoTracker Red CMXRos, and then formaldehyde-fixed, acetone-permeabilized and stained with the F-actin–specific probe, BODIPY FL phallacidin and with DAPI. Images were obtained by taking single and multiple exposures through bandpass optical filter sets appropriate for fluorescein, the Texas Red dye and DAPI.
References

2.