Methods used in study of cells and tissues

Microscopes use lenses to magnify images created by photons or electrons.

Light Microscopy

Bright-field

- Visible light is directed at a sample from below - condenser focuses light onto sample
- Light is transmitted through the tissue, enlarged and projected by the objective lens
- Eyepiece further magnifies image and directs toward viewer
- Immersion oil is used at higher magnifications (1000X) to increase the resolving power; it keeps excess light waves from reflecting
- Most simple and common method

Fluorescence Microscopy

- *UV* light is directed at sample
- Light emitted from tissue is within visible spectrum
- Used to localize particular macromolecules within cell (ex: DNA, antibodies)

Parts of a Light Microscope: 1. Eyepiece (10X magnification) 2. Revolving objective head 3. Objectives 4. Coarse adjustment knob 5. Fine adjustment knob 6. Stage 7. Light Source 8. Condenser with diaphragm 9. Slide shift

Phase contrast

- Light passing through different structures in the sample changes speed accordingly
- Lens system visualizes these changes as lighter/darker areas
- Advantage: does not require fixation/staining, so it can be used to view living cells

Confocal microscopy

- Aligns the point light source, focal point of lens, and pinpoint aperture of detector in one focal plane
- Reduces stray light beams normally present in bright-field microscopy
- Image is sharper and of higher resolution

Polarizing Microscopy

- Recall that visible light has electric and magnetic components that are perpendicular, but can be polarized (https://courses.lumenlearning.com/physics/chapter/27-8-polarization/) by crystalline substances
- The sample is placed between two perpendicular filters; this normally cancels out all transmitted light
- However, if the sample also contains substances of a periodic structure (ex: collagen), they will rotate the axis of light after it passes through the first filter, and it will no longer be perpendicular to the second filter
- The image formed shows the substance on a black background

Electron Microscopy

Transmission Electron Microscopy (TEM)

- Electrons pass through sample and are focused via electromagnetic "lenses"
- Denser substances absorb/deflect electrons better and appear darker
- Image contrast improves with the use of heavy metal ions
- Microstructural details are better observed with cryofracture/freeze etching
- Typical magnification is up to **120,000 X**

Scanning Electron Microscopy (SEM)

- Sample is coated with a heavy metal
- Electrons reflect off of sample at various angles
- Image appears 3D with shadows and highlights

Other methods

Autoradiography

- Radioactive monomers are offered to a culture
- As cells synthesize polymers, they aggregate and become visible

- Allows one to localize synthesis of biopolymers (often DNA or proteins)

Enzyme histochemistry

- Enzyme substrate is offered to a tissue section
- Enzymatic reaction produces product, which in turn reacts with marker compound
- Marker compound precipitates in sites of high activity
- Ex: used to study phosphatases, dehydrogenases, peroxidases

Immunohistochemistry

- Antibodies (tagged for visibility) are added to tissue
- Antibody binds only to specific protein; shows location after rinsing
- Can be used to diagnose specific tumors or viral infections

References

- Mescher, A. and Junqueira, L., 2018. *Junqueira's basic histology*. New York: McGraw-Hill, pp. 4-13.