Quick Study Guide: Essential Principles of Microscopy

Microscopy is a cornerstone of biology, enabling scientists and students to study microbes, cells, and tissues too small to see with the naked eye. This guide summarizes the essential principles of microscopy for quick revision.

1. Fundamentals of Microscopy

1. Refraction: Bending of light as it passes between media (e.g., air to glass). Basis of magnification.
2. Convex lenses: Focus parallel rays to a focal point to create magnified virtual images.
3. Immersion oil: Reduces refraction and increases resolution with the 100x lens.
4. Units of measurement: mm (tissues), µm (bacteria), nm (viruses).

2. Bright-Field Microscope

1. Ocular lens: 10x magnification.
2. Objectives: 4x, 10x, 40x, 100x (oil immersion).
3. Stage: Holds slide in place.
4. Condenser: Focuses light on specimen.
5. Iris diaphragm: Adjusts contrast and brightness.
6. Illuminator: Provides light source.
7. Focus knobs: Coarse and fine adjustments.

3. Magnification

Total Magnification = Objective Power × Ocular Power (e.g., 40x objective × 10x ocular = 400x).

4. Resolution and Numerical Aperture (NA)

1. Resolution = ability to distinguish two close points.
2. Formula: $d = 0.5\lambda / NA$.
3. $NA = n \sin\theta$ (n = refractive index, θ = half-angle of light cone).
4. Oil immersion increases NA and resolution (~0.2 µm).
5. Shorter wavelengths (blue light) improve resolution.

5. Specimen Preparation

1. Fixation: Heat or chemical methods preserve cells.
2. Staining: Simple (one dye), Differential (e.g., Gram stain), Special (e.g., spore, flagella).
3. Smears: Thin, even films for clear observation.
4. Wet mounts: For live specimens (lower detail).
5. Pitfalls: Over-staining, uneven smears, poor fixation.

6. Practical Tips

1. Start with the lowest objective (4x) to locate specimen.

2 Use coarse focus at low power, fine focus at high power.
3 Adjust iris diaphragm for optimal contrast.
4 Only use immersion oil with 100x lens.
5 Bright-field works best for stained, non-living specimens >0.2 µm.

Conclusion

Microscopy combines physics and biology to explore cells and microbes. Mastering light principles, resolution, and specimen preparation builds strong laboratory skills.

Reference

Willey, J. M., Sherwood, L., & Woolverton, C. J. (2011). Prescott's Microbiology (7th ed.). McGraw-Hill.