Objectives:
By completing this laboratory session you will be able to:

1. Learn the parts of the compound light microscope and understand the role of each.
2. Understand the image formation of the compound light microscope.
3. Employ Köehler illumination for optimal alignment of the microscope.
4. Make leaf peels to prepare fresh epidermal specimens for observation.
5. Prepare replicas of surface structures.

Materials:
Letter “e” prepared slide
*Elodea* leaves
*Monstera* leaves
*Coleus* plants
*Zebrina* plants
Light microscopes
Duco® cement
Tooth picks
*Toluidine Blue-O*
Onion bulbs
*Apium* stalks
mm ruler

Laboratory Exercises:
Use of the Light Microscope –
Obtain an Olympus compound light microscope and record the number with the TA. In the future, use only the same microscope and keep it in good operating condition. Always transport the instrument with two hands holding one hand beneath the instrument and grasping firmly the “arm” of the microscope with the other hand.

With your microscope, fill in the information in the table below. Make approx. distance measurements with a mm ruler.

<table>
<thead>
<tr>
<th>Direct magnification of obj. lenses</th>
<th>N.A. for each lens</th>
<th>Total magnification of virtual image with oculars</th>
<th>Working distance of each lens</th>
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Explain why the N.A. changes with magnification. What is the relationship of N.A. with working distance for each lens?
Observe prepared slide of newsprint
Obtain slide of letter “e” and observe using a low magnification objective lens of the microscope. How does the orientation of the letter look different on the stage vs. the observed image? Why?

Note the texture of the paper. Of what material is the paper composed?

*Fresh specimen observations*
Celery: Peel the surface layer of cells (epidermis) from innermost portion of the stalk and quickly place in a small drop of water on a microscope slide. Add a small drop of the IKI stain and then gently place a glass cover slip over the preparation so as to not trap air bubbles.

What results are seen? What material is stained the darkest?

Onion and Zebrina: Make a peel of the epidermis and observe both fresh samples with the light microscope (no staining—just in a drop of water with cover slip) and duplicate specimens stained with toluidine blue-O. Make a drawing below showing how your best preparation looks and identify as many of the structures as you can by extending a line to a label.
Monstera leaf: Make a leaf peel and observe with 10× and 20× objectives. Mount the epidermis as indicated above without staining or with light staining using toluidine blue-O. Now also smear a small drop of Duco® cement over a small area (e.g. ~5 × 5 mm) of the Monstera leaf using a toothpick. Avoid getting air bubbles in the thin smear. Allow to dry for 5-10 min. and then gently peel the cement off of the leaf surface. Place the cement replica on a glass microscope slide, add a drop of water and a cover slip. Observe as with the fresh epidermal peels of Monstera.

Compare the two preparations. Try to identify different cell types.

What is different in the prep of the replica from that of the peel? __________________________
______________________________________________________________________________
______________________________________________________________________________

Make sketches below of each preparation from observations at the same magnification, and label structures that you can identify.

Fresh epidermal peel  Replica of leaf surface

When finished with the above exercises, clean your lab desk space and carefully return the microscope to its cabinet space. Discard slides and cover slips in the “sharps” container, and other materials into the general waste container. Follow this protocol at the end of each lab in the course.