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Brush them away: a simple method to remove trichomes and visualize stomata

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We use this protocol and it's working

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Abstract

This protocol outlines a simple method for removing trichomes from heavily covered poplar (*Populus* sp.) leaves and creating an imprint of the leaf epidermis. The method involves using a paintbrush to gently remove trichomes, applying clear nail polish to create an imprint, and then viewing the imprint under a microscope to observe structures such as stomata. The expected results include clear leaf imprints that allow for detailed observation and counting of stomata.

Image Attribution

Sarah Wodtke

Materials

Dried leaves (dried at 80°C for 24-48 h), clear adhesive tape, waterproof pen, smooth surfaces that are not damaged by alcohol (e.g., glass), soft paintbrush (approx. 5 mm wide), microscope, razor blade, ethanol (70%), microscope slides, scissors, double-sided adhesive tape (ideally with one protected side), clear nail polish



Safety warnings

❗ **Handling Chemicals:** Ensure proper handling and disposal of ethanol (70%) as it is flammable and can cause skin and eye irritation. Use appropriate personal protective equipment (PPE) such as gloves and safety goggles.

Using Sharp Tools: Be cautious when handling razor blades and scissors to avoid accidental cuts.

Adhesive Tape: Be mindful of the adhesive nature of the tape used. Avoid contamination by handling tape strips only at the ends.

Ethics statement

This protocol does not involve the use of animals or human subjects. It focuses on the manipulation and observation of plant material, specifically leaves.

Preparation

- 1 Clearly label microscope slides with a waterproof pen, preferably in the upper right corner, with sample IDs or experiment names (Figure 1).

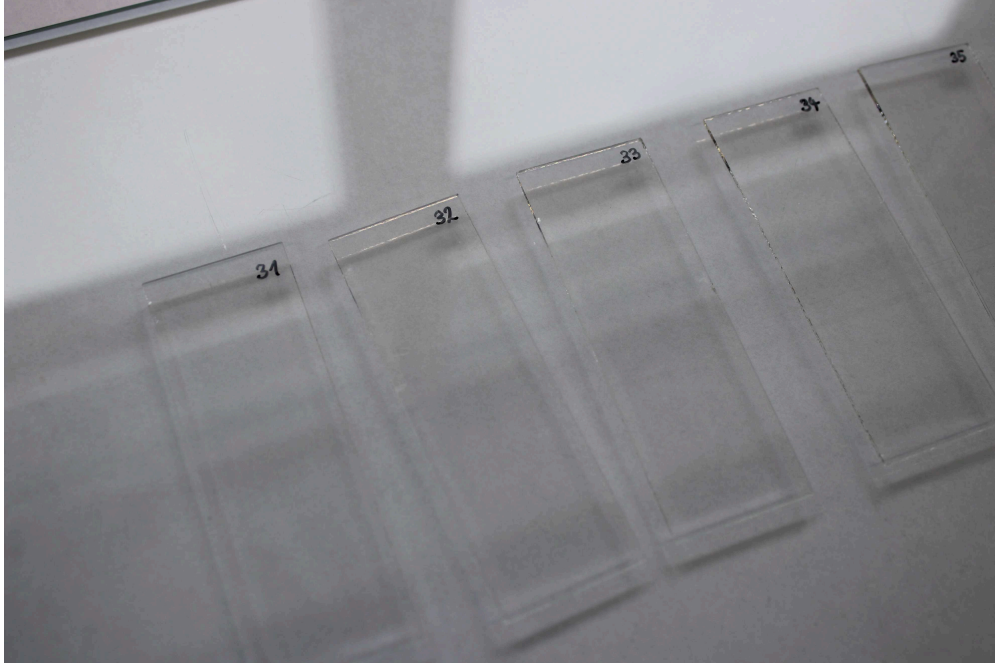


Figure 1: Microscope slides labeled with numbers.

- 2 Prepare a clean, smooth surface (e.g., a glass plate).

Apply double-sided adhesive tape

- 3 Apply several approximately 10 cm long strips of double-sided adhesive tape to the surface, depending on the number of leaves to be processed (Figure 2).

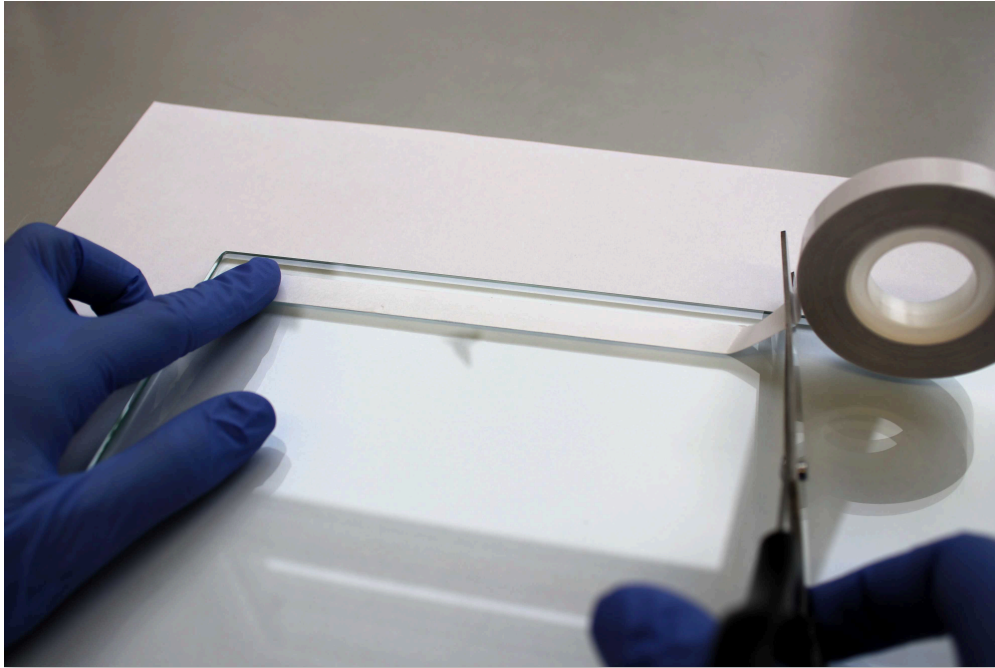


Figure 2: An approximately 10 cm long piece of double-sided adhesive tape applied to the surface.

- 4 Write identification numbers or names next to the tape strips (Figure 3).

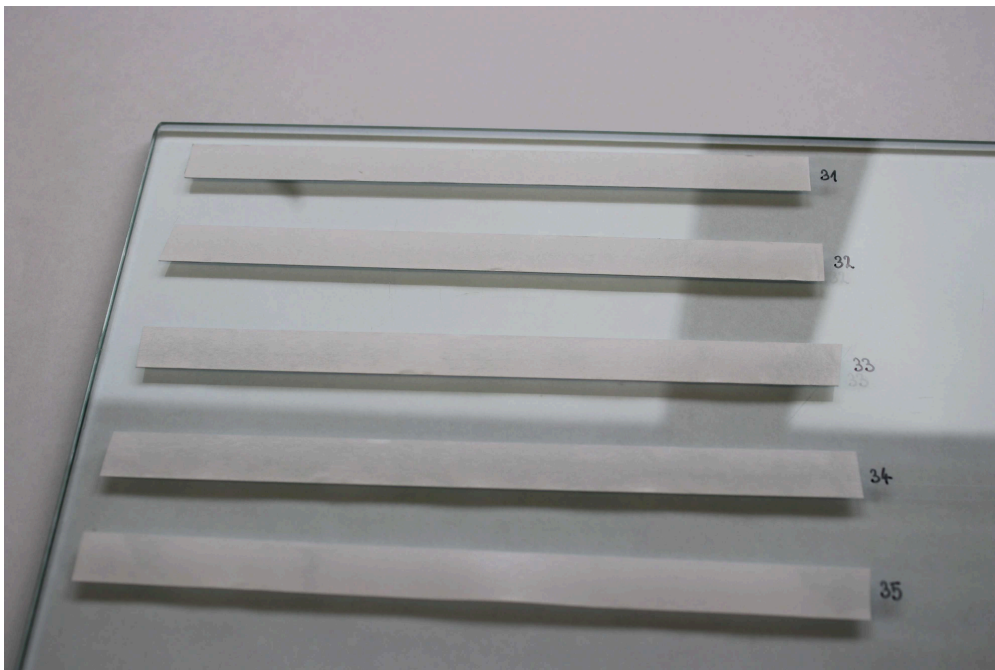


Figure 3: Label the adhesive tape strips like the microscope slides.

Cut dried leaf

- 5 Place the dried leaf upside down and cut it along the central rib into two halves (Figure 4). Take one half and cut it into three rectangular pieces. Steps 6 - 8 describe in detail how the leaf half is processed with the razor blade to obtain one rectangular piece.



Figure 4: Divide the leaf into two halves to remove the central rib.

- 6 From one half, cut a narrow strip whose width corresponds to the width of the double-sided adhesive tape (Figures 5 and 6).

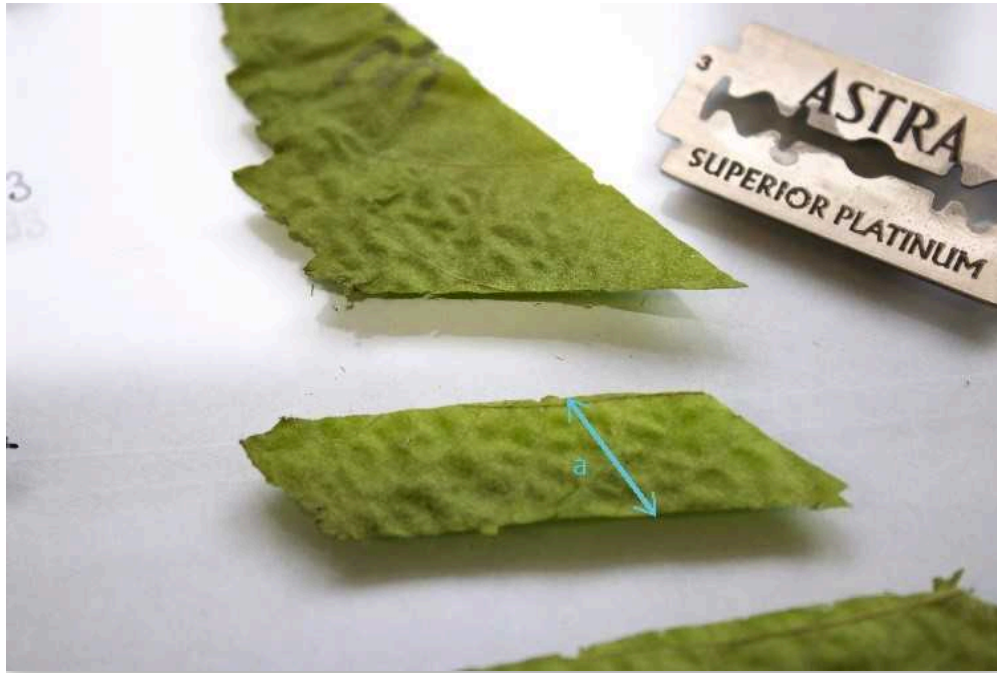


Figure 5: Piece of leaf corresponding to the width of the adhesive tape.

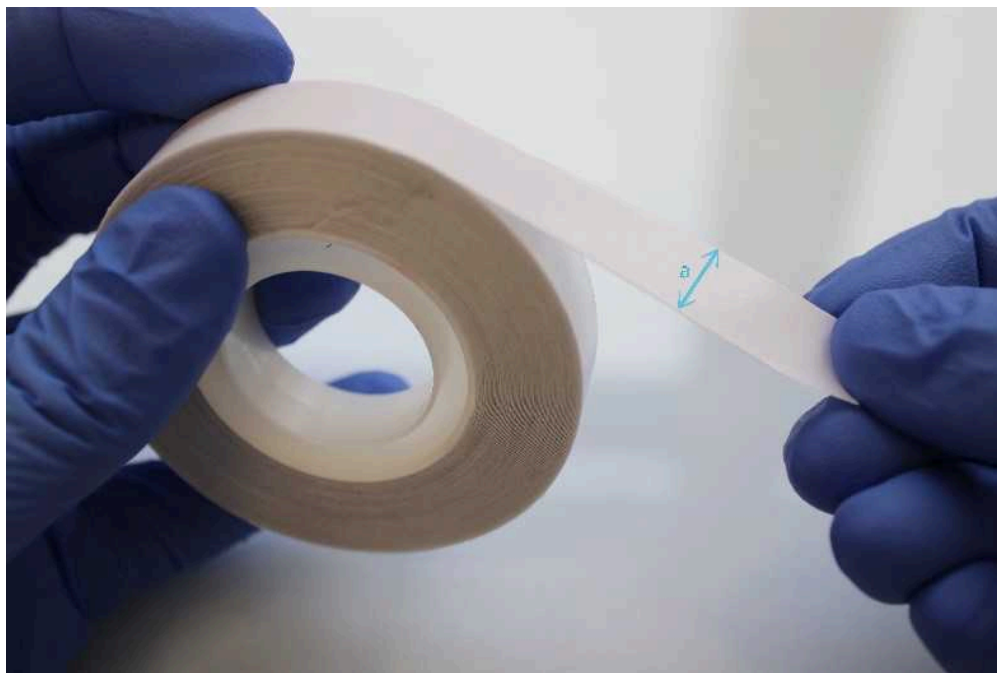


Figure 6: Width of the double-sided adhesive tape.

- 7 Choose a spot with minimal and small leaf veins, and avoid damaged spots.

- 8 Shorten the leaf strip at both ends to create a rectangle whose width approximately matches the width of the clear adhesive tape (Figure 7).

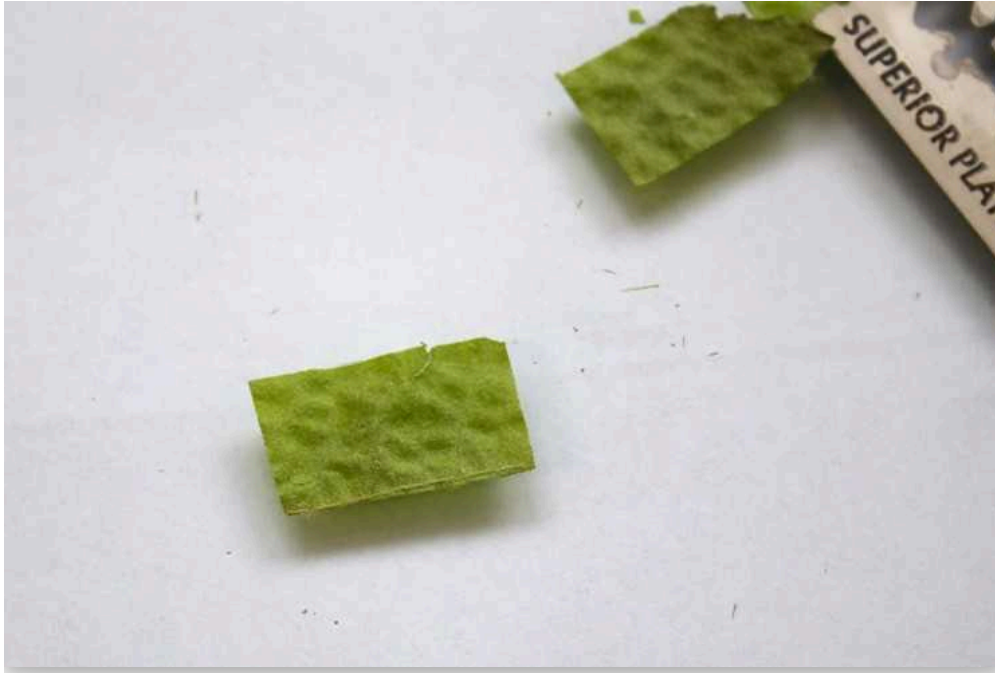


Figure 7: A rectangular piece of leaf corresponding to the width of the clear adhesive tape.

Prepare leaf pieces on tape

- 9 Remove the protective film from the double-sided adhesive tape (Figure 8).

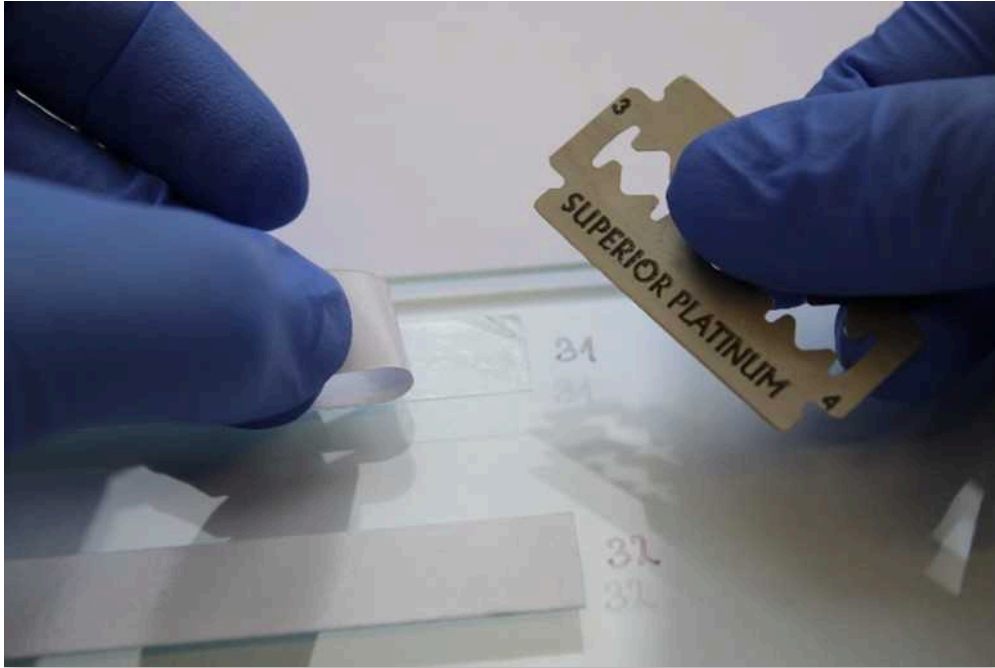


Figure 8: Removal of the protective film from the double-sided adhesive tape.

- 10 Place the cut leaf piece with the underside facing up on the double-sided adhesive tape and press down (Figure 9).

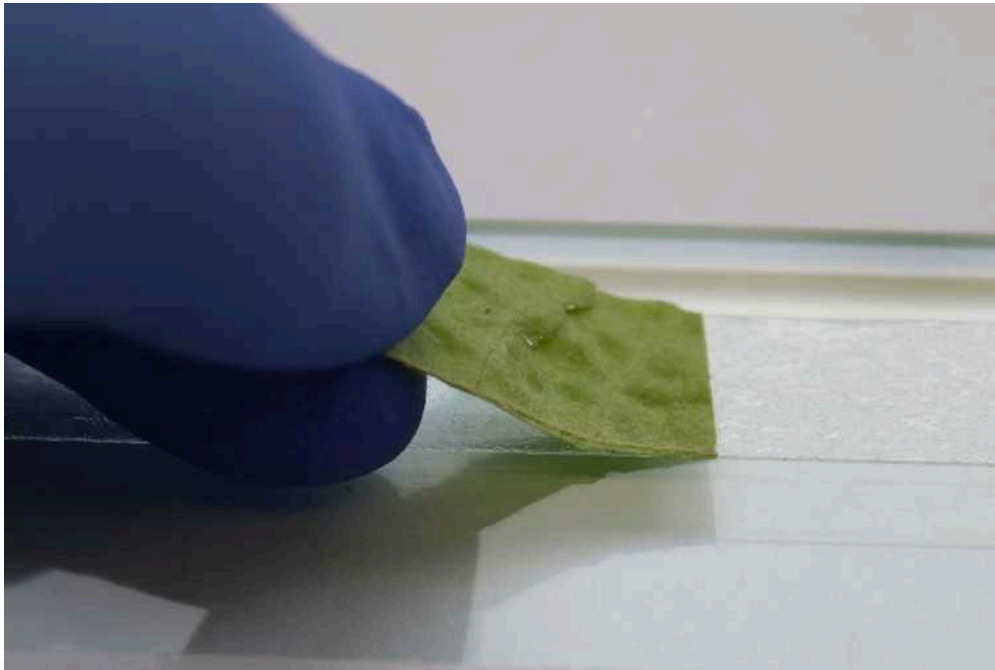


Figure 9: Preparation of the leaf piece on the double-sided adhesive tape.

- 11 Cut and remove any protruding leaf tissue with the razor blade if necessary (Figure 10).

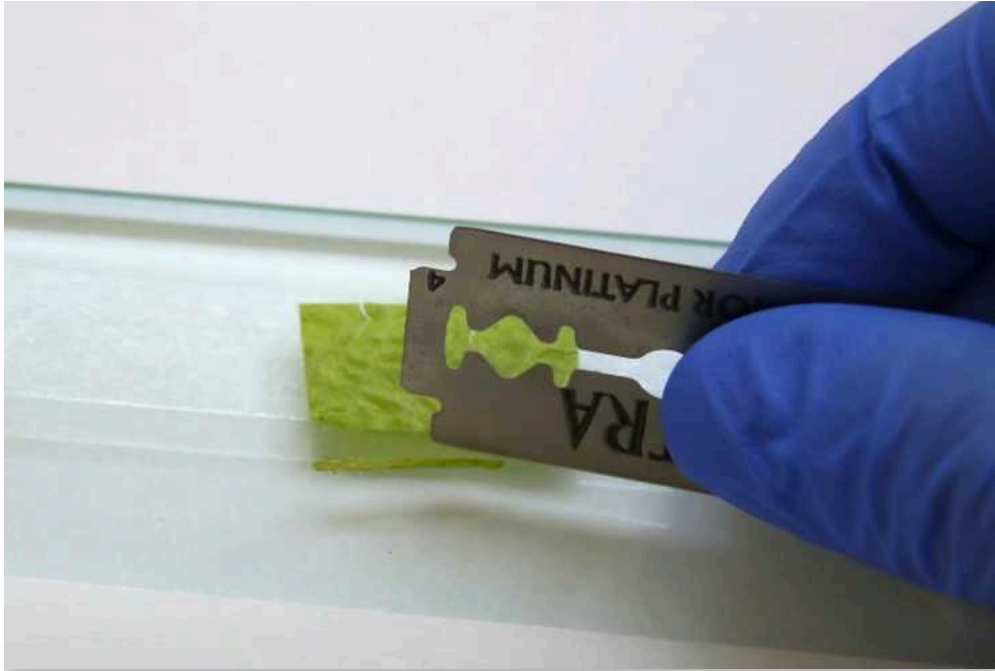


Figure 10: Removal of protruding leaf tissue.

- 12 Place all of the three pieces on the double-sided adhesive tape, leaving about 2 cm space between the pieces (Figure 11).

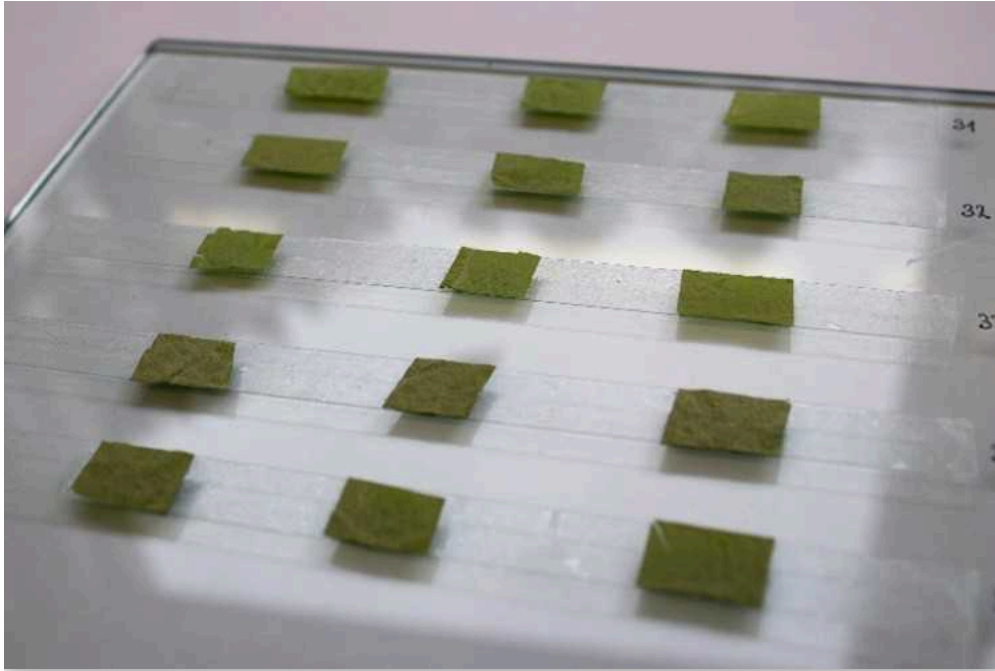


Figure 11: Three leaf pieces per leaf with approximately 2 cm spacing on the adhesive tape.

Remove trichomes

- 13 Carefully remove the trichomes from the leaf pieces using a soft synthetic paintbrush (Figure 12).



Figure 12: Removal of the trichomes with a paintbrush.

- 14 The trichomes will collect in small clusters that can be brushed off onto the double-sided adhesive tape (Figure 13).



Figure 13: Brushing off the trichome clusters onto the adhesive tape.

- 15 Depending on the leaf, the trichomes may be more or less easy to remove. For harder-to-remove trichomes, work with the brush in circular motions from the center of the leaf piece outward.

Apply clear nail polish

- 16 Apply a layer of clear nail polish to each leaf piece. The nail polish layer should be continuous, not too thick, and not too thin (Figure 14).

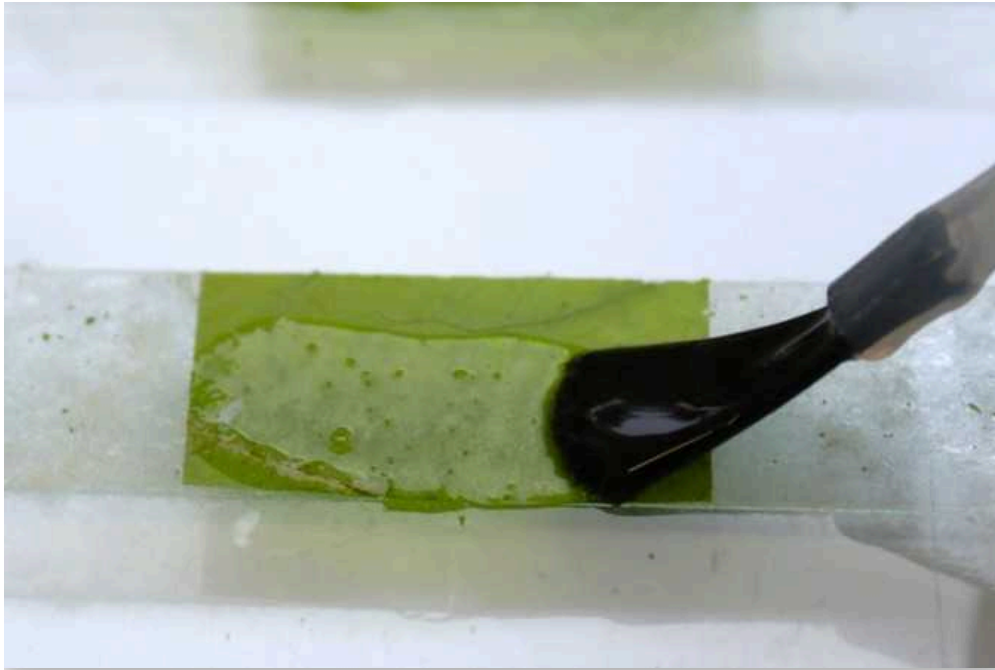


Figure 14: Application of a layer of nail polish to the leaf pieces.

- 17 Allow the nail polish to dry for at least 15 minutes.

Create leaf imprint

- 18 Once the nail polish is dry, cut a 4 to 5 cm long strip of clear adhesive tape.
- 19 Hold the adhesive tape strip only at the ends to avoid contamination.

- 20 Attach the adhesive strip rotated 90° to the double-sided adhesive tape onto the first leaf piece so that some adhesive tape protrudes on both sides (Figure 15).

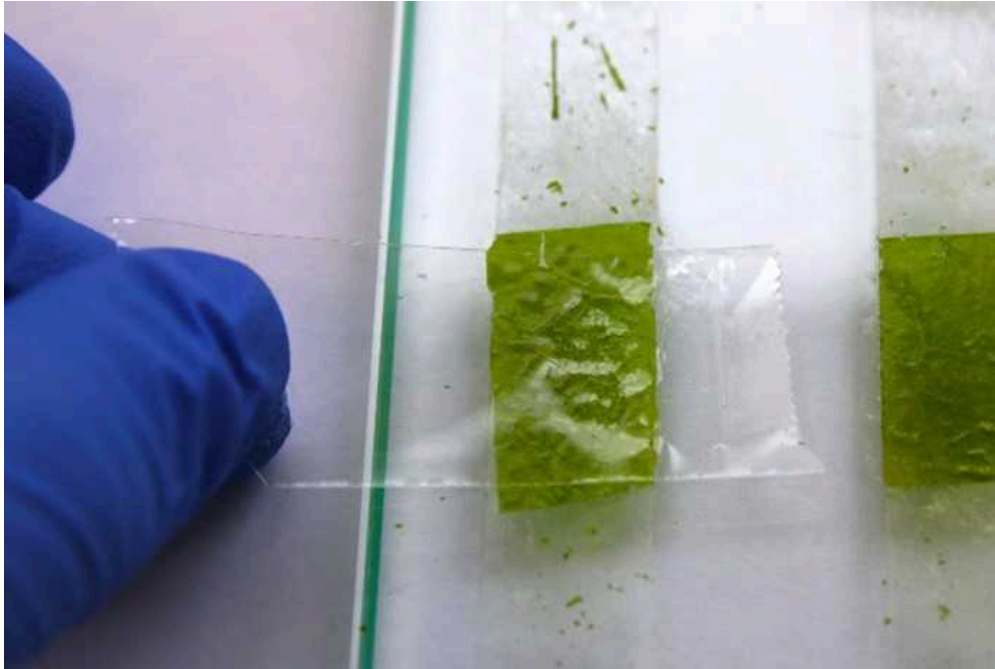


Figure 15: Attachment of the adhesive tape to the leaf piece.

- 21 Press the adhesive tape down firmly.
- 22 Peel off the adhesive strip along with the leaf imprint (Figures 16 and 17). Depending on the condition of the leaf, green leaf tissue may occasionally remain on the nail polish imprint.

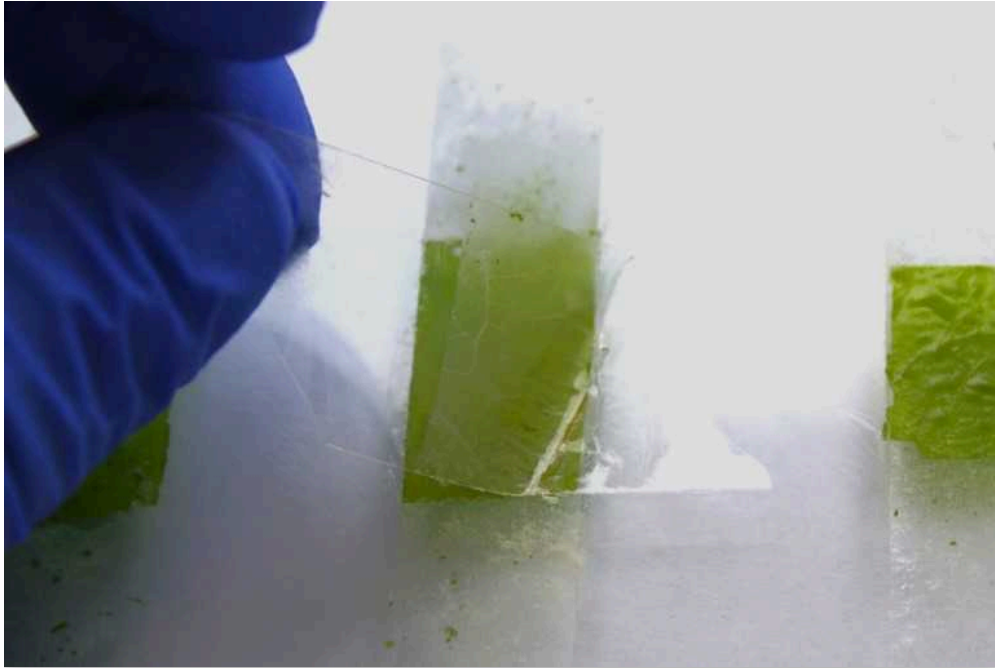


Figure 16: Removal of the adhesive tape from the leaf piece.

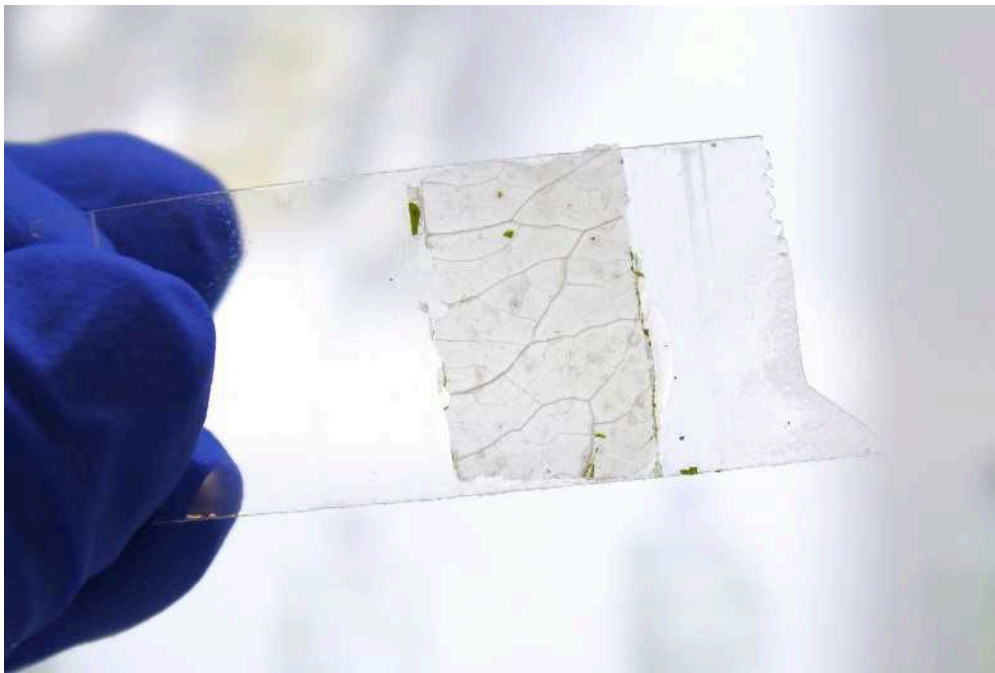


Figure 17: The leaf imprint adheres to the adhesive tape.

Mount leaf Imprint on slide and microscopy

- 23 Attach the leaf imprint with the adhesive tape to the corresponding microscope slide.
- 24 Attach the other two leaf pieces from the same leaf and trim the protruding adhesive tape ends with scissors (Figure 18).

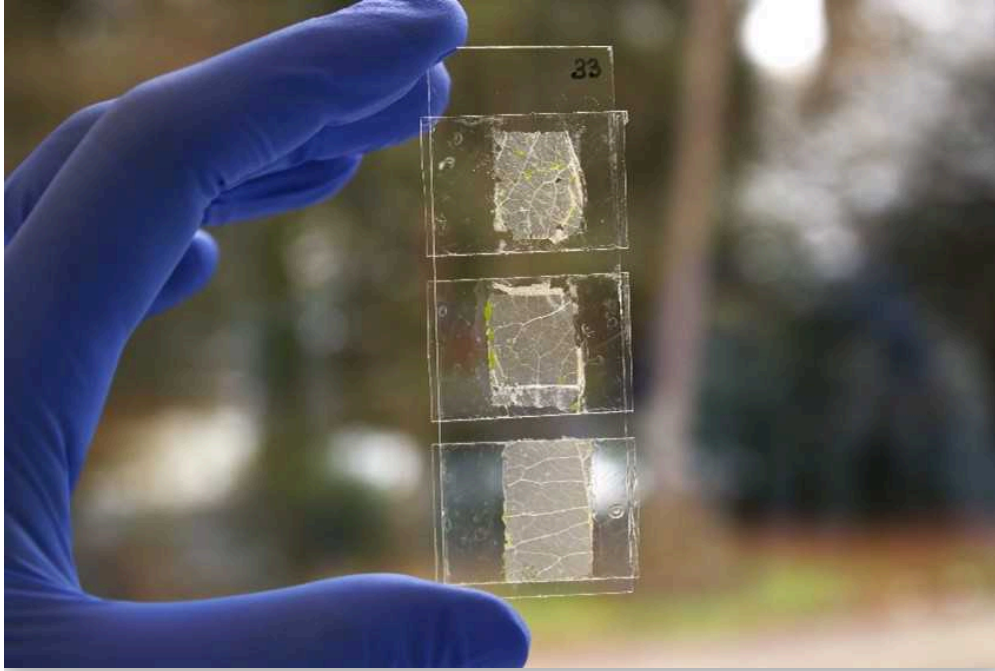


Figure 18: Three leaf pieces per leaf are attached to a microscope slide.

- 25 The imprints can be viewed under a light microscope (Figure 19).

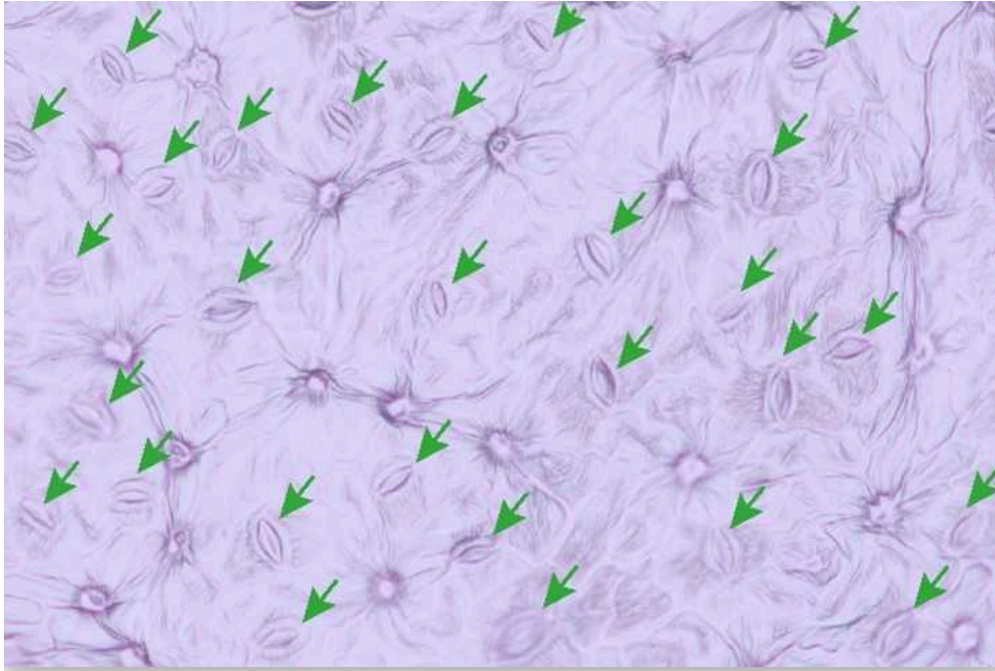


Figure 19: Microscopic view of the leaf imprint (20× magnification). The stomata are indicated with green arrows.