How to place a coverslip

The supposedly simple process of placing the coverslip often leads to air bubbles or dust getting under the coverslip, which prevents the subsequent reduction of the layer thickness. This is very annoying when it comes to rare objects or objects that are difficult to insulate.

I would like to present my personal approach here. First of all, I would like to say that I have been using only 24 x 32 mm coverslips around 1990. This has two advantages. Firstly, with larger coverslips there is a greater chance that the object will not come to rest at the edge after the coverslip has been placed on it. This is problematic if you want to examine the object later with oil immersion (which is almost always the case for me). On the other hand, the constant coverslip size allows to gain experience over the years as to how large the amount of water needs to be in order to achieve an optimum layer thickness without crushing the object.

I usually use coverslips of the manufacturer Marienfeld (s. fig. 1), 24 X 32 mm type no. 1. The specification of these coverslips provided by Marienfeld:

- made of chemically resistant borosilicate glass
- hydrolytic class, absolutely colourless, perfectly clear
- suitable for fluorescence microscopy
- thickness no. 1 (0.13 to 0.16 mm)

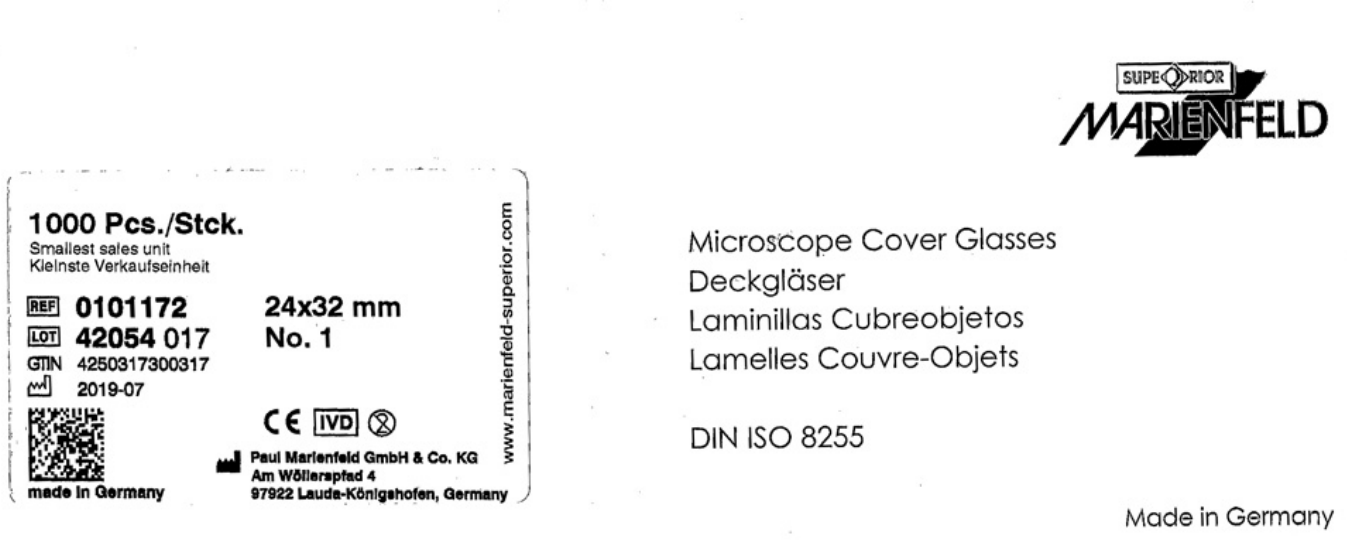

Fig. 1: The label from the coverslips 24 X 32 mm, type no. 1, provided by the manufacturer Marienfeld.

In the following, I show how to place a coverslip without bubbles and, if possible, without dust. I clean the slide beforehand with a commercially available glass cleaner (discount store) and a microfiber cloth. Then I place the water drop (with the isolated object) on the slide. I clean the coverslip with a cosmetic brush to remove adhering glass dust (from the cutting process) and other particles. Only then do I place the coverslip on top without trapping an air bubble (s. fig. 1 a-f).

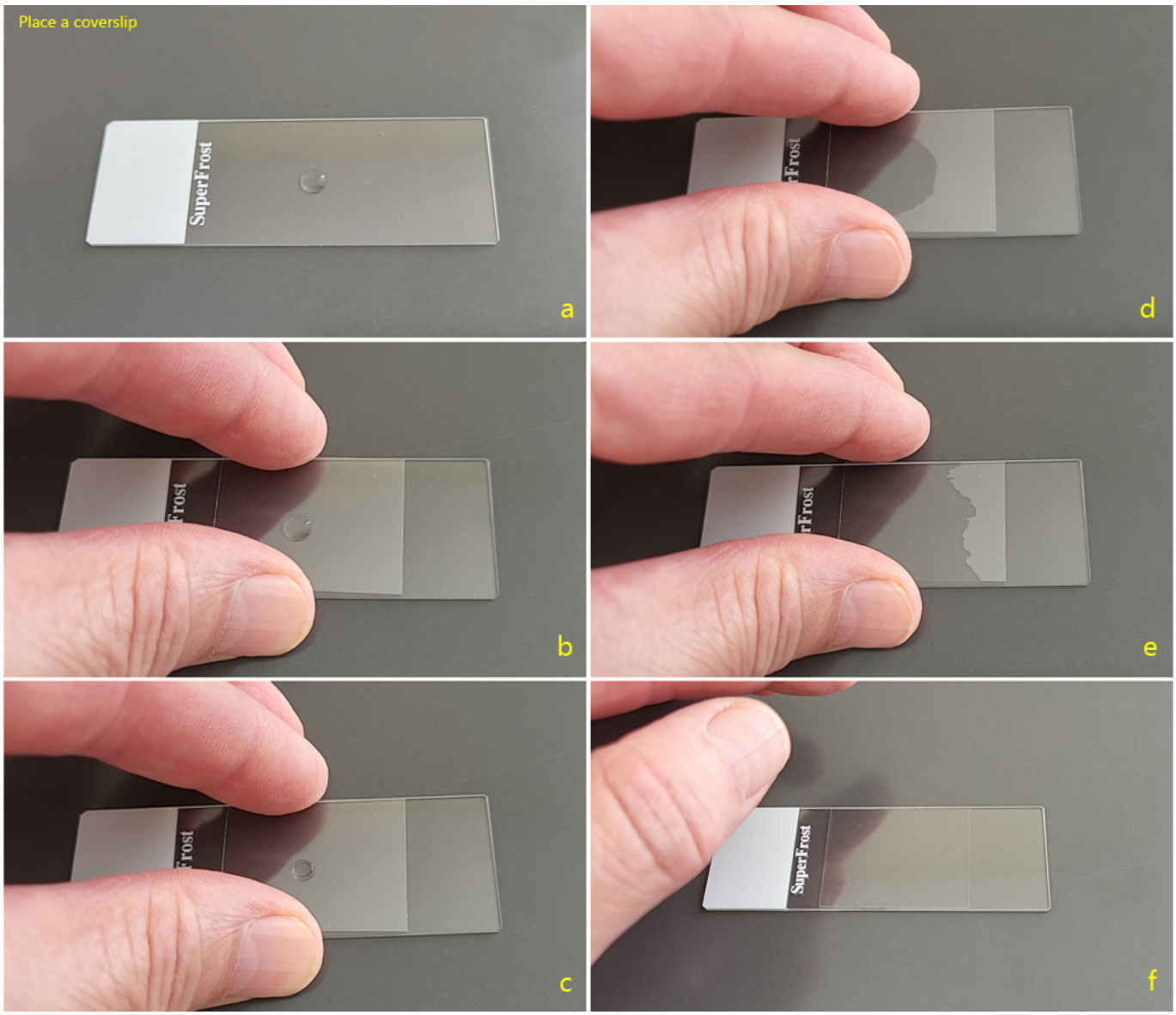

Fig. 2 a-f: Place the coverslip on a volume of water of approx. 20 µl.

Viewed from above, it looks as if I drop the coverslip onto the drop of water. In this case, the probability of trapping an air bubble would be very high. Instead, I use my fingertips as a buffer to ensure that the coverslip is placed slowly and evenly. This is the only way to give the water film enough time to spread out under the coverslip without trapping any air bubbles. In fig. 3 a-d you can clearly see from the side how I press my fingertips onto the table and thus carry out the placement in a controlled and slow manner.

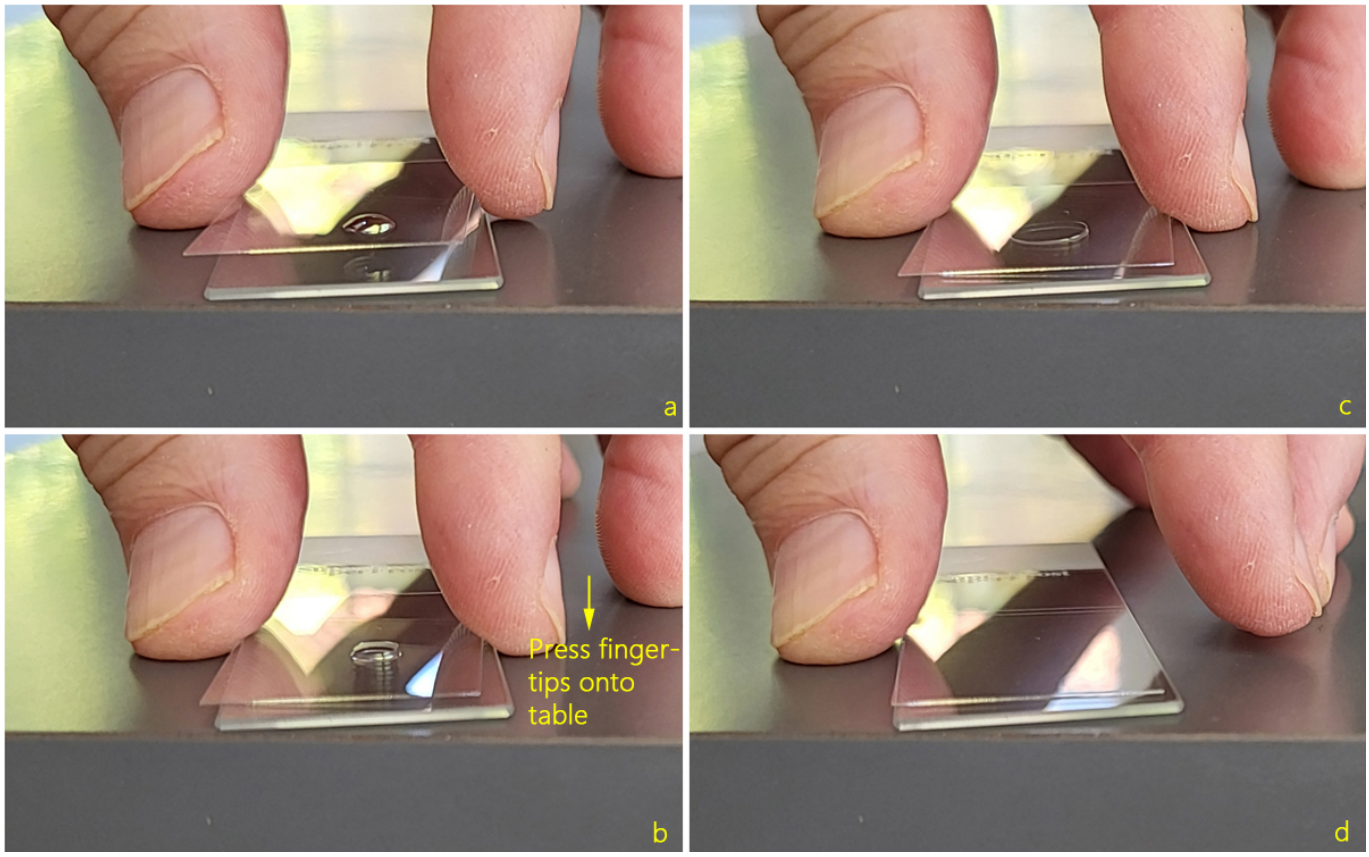

Fig. 3 a-d: Placing the coverslip viewed from the side. The fingertips are pressed onto the tabletop and serve as a buffer for controlled and slow placement of the coverslip.

This controlled application of the coverslip also means less mechanical stress for the insulated objects as many of which are sensitive to coverslip pressure.

It takes some experience to determine the correct amount of water under the coverslip. For sensitive objects, it is better to use too much water than too little. For very small objects, I use very little water. A volume of 10 µl leads to a layer thickness of 13 µm under a 24 x 32 mm coverslip (768 mm²). This can be used as a rule of thumb to estimate the required volume depending on the object to be examined.