Adjust layer thickness

The layer thickness is a decisive parameter in order to be able to utilize the maximum resolution of an objective. When examining living organisms, the choice of the correct layer thickness depends on many parameters and must be adjusted individually. In the case of mobile and deformable organisms (e.g. ciliates or rotifers), one should start with a large layer thickness in order to be able to study the natural movement and shape of the organisms. This also prevents premature denaturation of the organisms. In the case of immobile and less sensitive organisms such as algae, the preparation can start with a lower layer thickness, adapted to the size of the organisms.

In order to document all the details and features of the organisms, it is necessary to fix and squash them by reducing the layer thickness. In the course of this, continuously higher magnifications can then be applied. In my routine workflow, adjusting the layer thickness is therefore a frequently repeated routine.

For very sensitive organisms (e.g. heliozoans), it is advisable to reduce the layer thickness by normal evaporation, which is very slow and takes a long time. For most other organisms, however, the layer thickness can be reduced more quickly, also to prevent motile organisms from swimming to the edge of the coverslip. For this purpose, I use filter paper to suck up water at the edge of the coverslip.

The use of conventional coffee filters has proven to be particularly suitable and very cost-effective for this purpose.



Fig. 1: Use of common coffee filters für adjustment of the layer thickness.

For my purpose I cut these coffee filters into strips about 1 cm wide and cut them in half again (s. fig. 2 a-b).

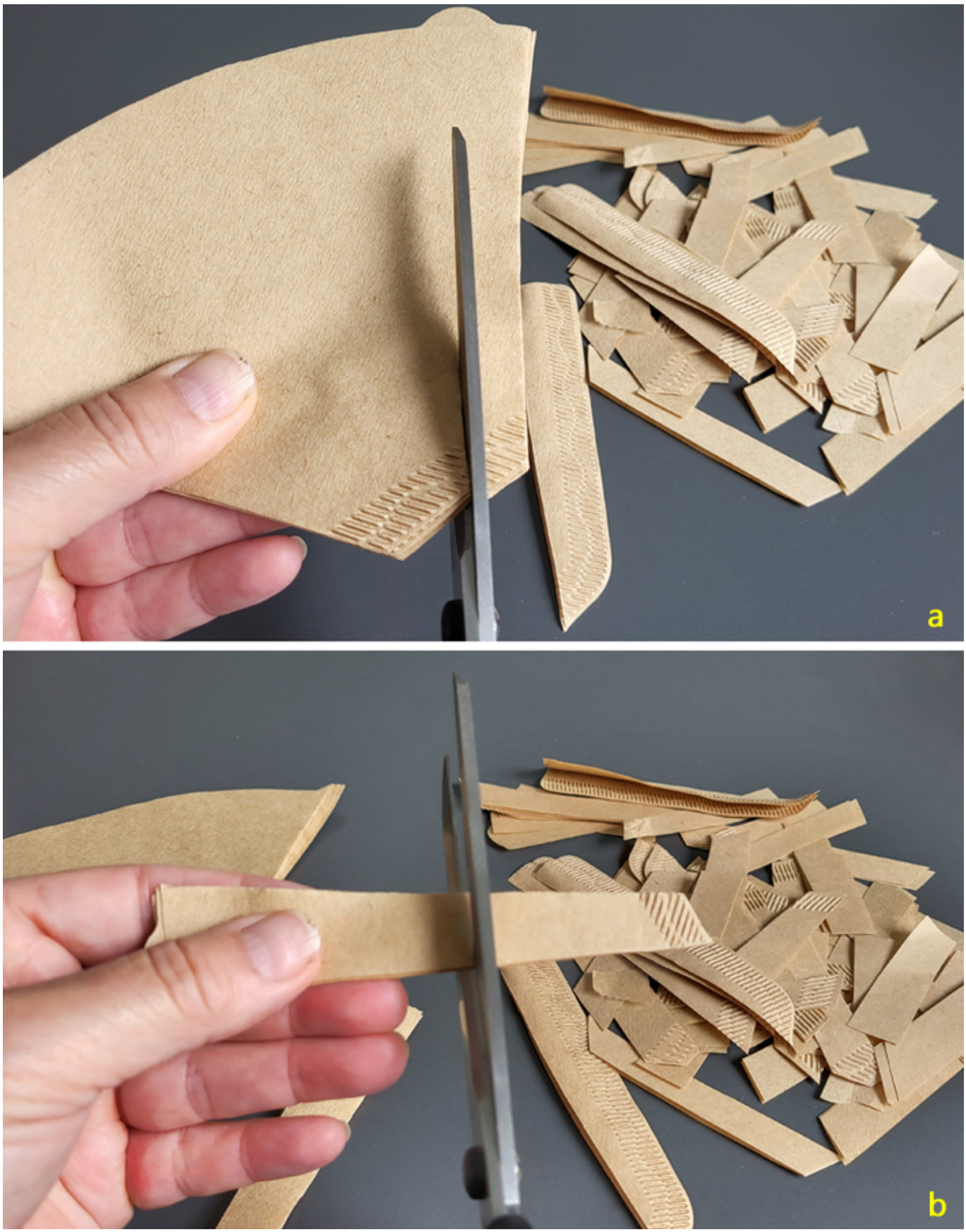


Fig. 2 a-b: Cut the coffee filters in short strips.

The strips can now be used to reduce the layer thickness very easily and in a

controlled manner by bringing the strip at the edge of the coverslip into contact with the water film. This also works if there is only little space between the objectives (s. fig. 3 a-d). The effect on the objects can be observed simultaneously through the eyepieces or on a monitor.

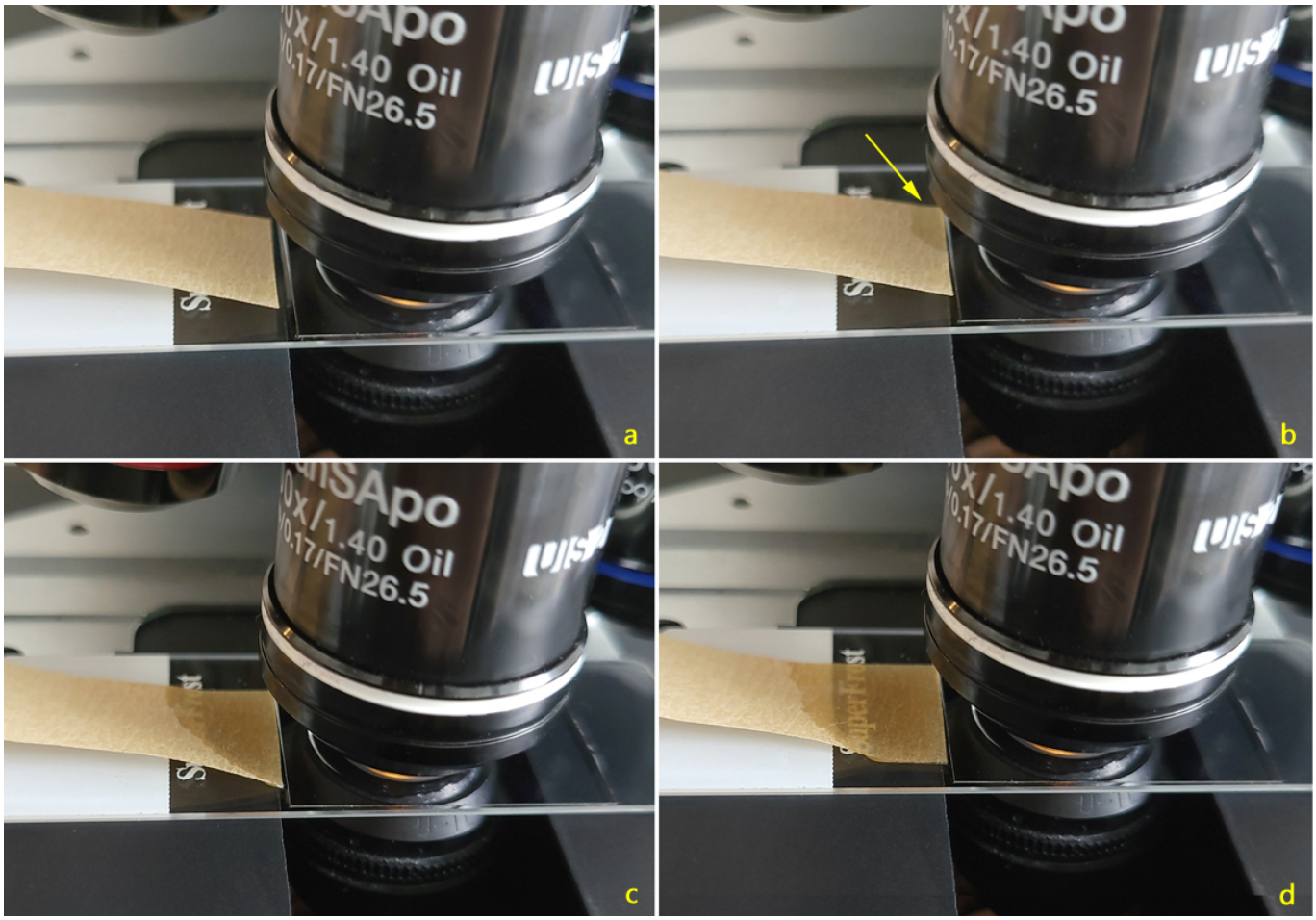


Fig. 3 a-d: Once the filter strip has come into contact with the water film, suction begins by capillary forces (b, arrow).

For the documentation of many features, such as extrusomes, micronuclei, cell wall structures or dorsal brushes, it is necessary to reduce the layer thickness to less than 10 µm. On the one hand, these features only then become visible in a focal plane and on the other hand, the 100 X objective only then reaches its full resolution. The layer thickness then becomes so small that you can no longer make contact with the water film on the edge of the coverslip with the filter paper strips. In these cases, I use a little trick and tear the filter strips (s. fig. 4).

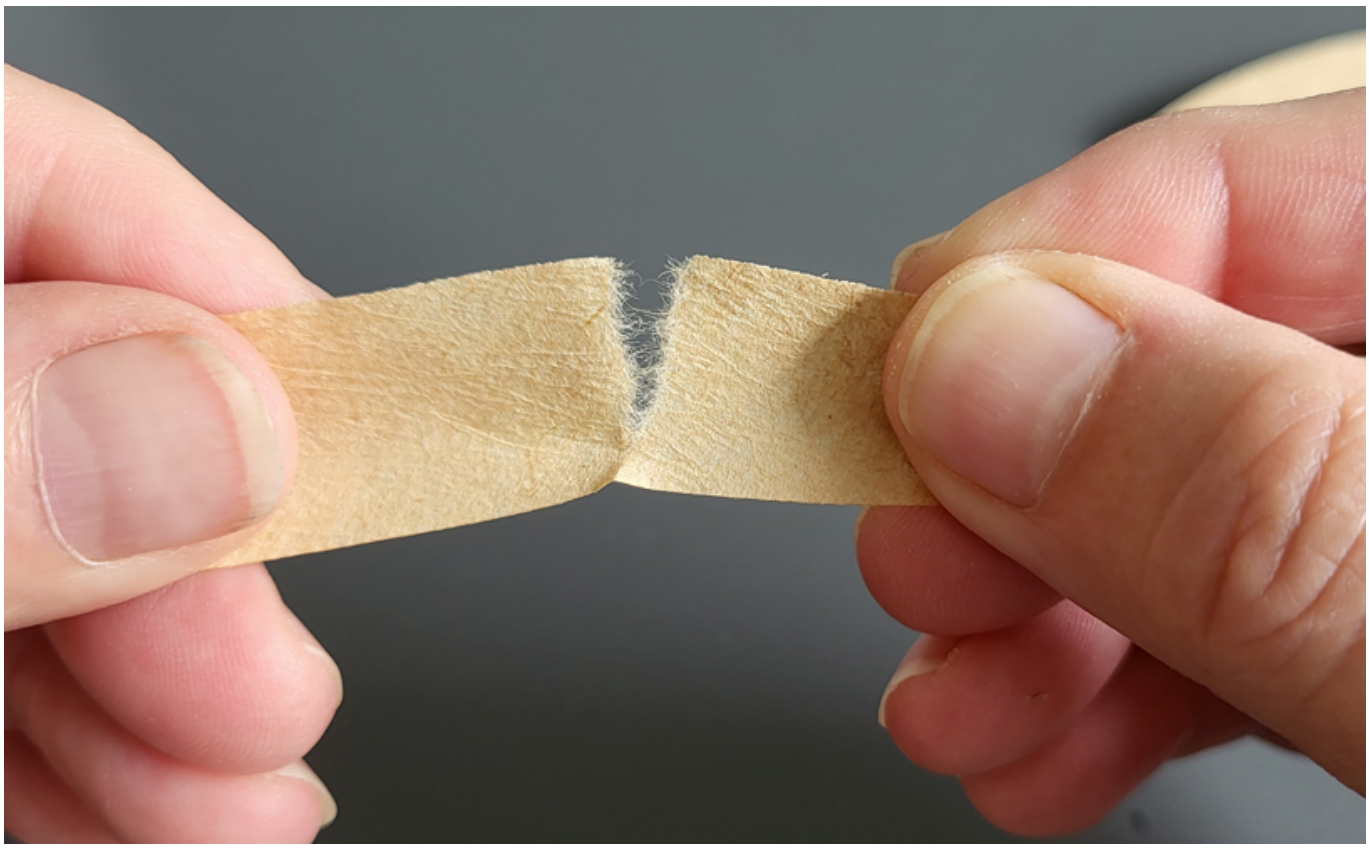


Fig. 4: To achieve the smallest layer thicknesses, it is necessary to tear the filter strips.

The coffee filters are made of a very long-fibered material. The protruding fibers of the tear-off edge are now thin enough to reach the water film under the cover glass. You may have to wait a few seconds for the capillary forces to take effect (s. fig. 5 a-c).



Fig. 5 a-c: The fine fibers of the torn strips extend to the water film under the coverslip. It may take a few seconds for the capillary effect to become visible (b,

arrow).

This technique can be used to reduce the layer thickness quickly and, above all, in a controlled manner. However, it must be clear that there must be no large objects such as grains of sand, detritus or air bubbles under the coverslip in order to make optimum use of the filter paper strips. This means that it is essential to work cleanly when making the preparation.