

A simple, magnet-assisted bryophyte cross sectioning tool for beginners and for teaching in bryology

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I describe the production and the application of a low-tech, easy-to-handle sectioning aid, which might be helpful for everyone who struggles with cross sectioning bryophytes. Technical plans and an instructional video are provided as supplementary material.

High-quality cross sections of bryophyte leaves, stems or thalli are important for the identification of species and the study of bryophyte morphology (Fig. 1). While experienced bryologists section bryophytes easily, fast and routinely, those new to the field often struggle with this task, which involves, simultaneously, fixing a tiny object under the dissection microscope with one hand while precisely cutting it with the other (Nishimura 1997, Pilkington 2013). Moreover, newcomers to bryology are frequently also beginners in microscopy. Thus, failure in appropriate sectioning (e.g. too thick sections) of bryophytes is common and often leads to frustration. Worst of all, it could dampen students' enthusiasm of working with bryophytes, leading them to give up the discipline. In order to enhance beginners' sectioning success, I developed a low-tech, easy-to-handle sectioning aid, whose production and application is described in the present article.

Among the large variety of sectioning methods applied by bryologists, technical sectioning aids such as microtomes, pith and wax embedding (O'Brien and McCully 1981) are rarely used due to their time consuming application (Glime and Wagner 2013). Hence, also the present method is kept simple and fast: it basically is an extension of the widely applied "double slide sectioning technique" (Glime and Wagner 2013) with a magnetic support plate. This allows a specimen to be fixed on a slide aided by a second slide which is placed at 90 degrees to the first slide and which is pressed down by means of magnets

attached to it (Fig. 2). This set-up fixes the specimen into position without the involvement of the hands and therefore allows the cutting of sections with a razor blade that is guided by both hands. The latter substantially facilitates sectioning.

Production of the two involved components, a metallic support plate and a magnetic slide, is straightforward. The support plate is made of a $120 \times 96 \times 1$ mm ferromagnetic rust-proof steel sheet type EN 1.4016 (AISI 430) tailored by an industrial laser cutter. This type of steel is widely used for domestic appliances, and many companies that sell steel sheet have this in stock and are able to cut it using a laser. By sending the technical plans from the supplementary material (PDF plan and corresponding CAD file) to the respective company, a set of 20 support plates can be produced for less than €10 per piece (mainly processing costs). The template is set up for standard 76×26 mm microscope slides (DIN ISO 8037-1), whereas for slides with other dimensions, this has to be modified as specified in the PDF plan. After production, the eight laser-cut tongues are manually bent upwards (e.g. with the help of a slotted screwdriver), so that their underneath edges just reach the surface of the plate. The two lateral tongues are used to tightly align a non-magnetic (e.g. aluminium, wood or plastic) rectangular strip of ca. $120 \times 10 \times 4$ mm, which is glued into its position, ideally with a two-part adhesive glue. The magnetic slide is a standard microscope slide with cut, not bevelled, edges. On each narrow side, a neodymium magnet (ferritic magnets are generally too weak) is aligned to the edges and glued to the slide. Commercially available self-adhesive $20 \times 10 \times 1$ mm neodymium magnets (<€0.50 per piece; <www.supermagnete.com>) have proven suitable for this application, but other neodymium magnets can also be used (e.g. fixed with double-sided tape).

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Figure 1. A leaf cross section showing the leaf anatomy of Polytrichaceae with lamellae and the forked terminal cells, which are diagnostic for *Polytrichum commune*. This section is the best result out of three trials with the magnetic sectioning aid by someone who had never sectioned a bryophyte before. In all three trials, sections with clearly recognisable diagnostic features were present.

Application of the method is demonstrated in an instructional video in the supplementary material, which may help to harness the magnetic aid for teaching and personal use. Sectioning starts with the positioning of a standard microscope slide on the support plate in the area between the six central tongues. This prevents the slide from gliding away during sectioning and correctly aligns it with the lateral non-magnetic strip. On this lower slide, a

specimen is placed and then fixed with the magnetic slide. Fixation starts by directly aligning one narrow side of the magnetic slide against the inner edge of the non-magnetic strip (magnets orientated upwards). Then, the magnetic slide is gently laid down on the specimen so that it is partly covered. Slight pressure on the middle of the magnetic slide will tilt it to a horizontal position. Now, the magnetic slide fixes the specimen by means of the lateral magnets, which symmetrically apply pressure on the orthogonally and centrally orientated lower slide. The support plate with the fixed specimen can now be placed under the dissecting microscope (in case the above steps have not already been conducted there). The part of the specimen that protrudes from the magnetic slide is set on focus and cut into sections, as thinly as possible, with a razor blade that is controlled with both hands. After cutting, the magnetic slide is removed and sections that remain stuck on the blade and/or the lower slide are pushed into a drop of water placed on the lower slide. Then, a cover slip is laid on the sections and the slide is grasped through semicircle slots for examination under the light microscope.

This method can be varied depending on the type of specimen involved and on personal preferences. Some of the possible variations are also shown in the instructional video. For extra stability or in case of thick specimens, the magnetic slide can be placed with downwards orientated magnets, which will prevent it from tilting. For this option, the magnets have to be equal in height to the slides being used. In order to increase the force by which the specimen is pressed down, additional magnets can be placed on the magnets attached to the magnetic slide. This also enhances the stability of the system and might be useful for fine specimens that need to be pressed down more powerfully for proper fixation. Notably, if the applied magnets are too strong, the magnetic slide will bend and therefore loose pressure. Further, reorientation of the specimen might be achieved by actively tilting and shifting the magnetic slide or by pushing it slightly backwards while sectioning, which will gradually expose the specimen. Also, orientation of the support plate, guidance and type of the razor blade, amount of specimen material and of water involved etc. can be varied according to individual preferences. I encourage users to find their own best setting, which will most probably vary for different types of specimen.

The method has been informally assessed in the Bachelor course 'Biology of Bryophytes and Ferns' 2016, which is taught at Swiss Federal Institute of Technology in Zurich (ETH Zurich). After several practical days, in which students sectioned bryophytes in a traditional way (i.e. fixing of specimen with forceps), they were introduced to the present method. The students readily applied the magnetic aid and in the subsequent oral evaluation they (n = 19) concordantly agreed that it substantially enhanced their success in preparing cross-sections of bryophyte leaves and stems. Hence, in future courses the magnetic aid will be incorporated from the beginning.

Everyone who struggles with cross sectioning bryophytes, especially people new to bryology and microscopy, might profit from the presented method. Low priced production makes the aid an affordable method for use during teaching

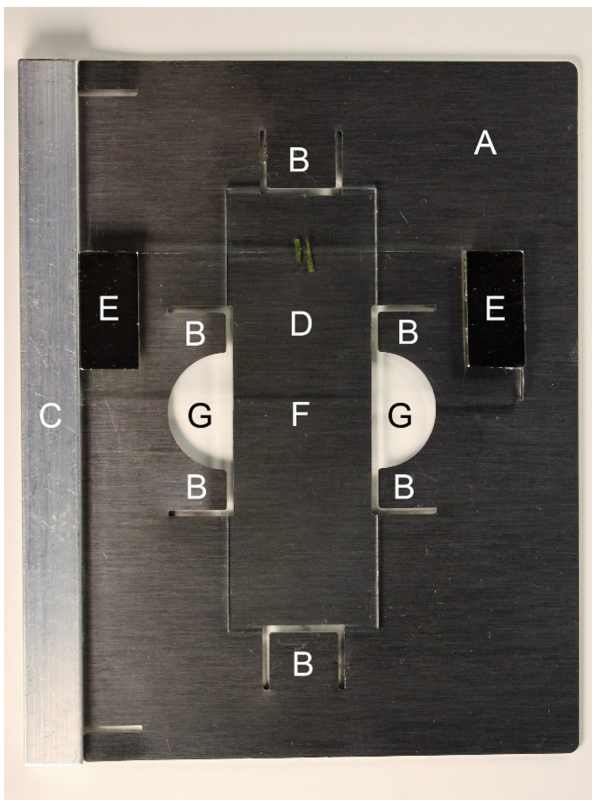


Figure 2. The magnetic sectioning aid with fixed *Polytrichum* leaves ready for sectioning. The support plate (A) consists of a magnetic, rust-free steel sheet with laser cut tongues (B) which are slightly bent upwards, and a non-magnetic strip (C) which helps to align the magnetic slide (D) with its attached neodymium magnets (E) centrally on the lower slide (F) in order to fix the specimen for sectioning. Sections are cut under a dissecting microscope with a razor blade that is guided with both hands. The semicircle slots (G) help to place and grasp the lower slide.

in class, and it might well be applied for sectioning materials other than bryophytes.

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Caution note – The method involves sharp, fragile and magnetic objects. Their handling might cause injury from which the author declines any liability.

Supplementary material:

Instructional video: <<https://doi.org/10.6084/m9.figshare.4781254.v1>>
PDF and CAD plans of the support plate: <<https://doi.org/10.6084/m9.figshare.4785490.v1>>

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