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Under snow and ice, a dense bloom of the huge ciliate *Spirostomum* was encountered in November 2016. Right at the collection site, off-white and slender 3.0 mm long threads displayed active swimming patterns to unaided eyes. Please share this encounter, which the dogs and I enjoyed.

The bloom was found in standing waters of a patch of broad leaf cat tails (*Typha latifolia*). The 3.0 mm size of these protozoa has me speculating: who would eat these huge single cell protozoans? These ciliates graze on bacteria in their winter aquatic community, thus these are bactivores, or bactivorus members of their food web.

*Spirostomum* are thus secondary consumers in the cat tail patch under snow and ice. In pure fancy I wonder if hydra species, or small fish, or flatworms would consider these huge protozoans as food.

At the microscope bench these elegant cells are as easy to place on a glass slide as coconut shavings! With the 4X objective, I observe these cetacean like prowling protists.

Each cell in the swarm of cells, randomly and abruptly contracts to half its body length. These contractions occur repeatedly and are not triggered by any stimulus I might impart to the drop of water they swarm

Figure 1: Cat tail patch one week before recent snow falls.

Figure 2: Cat tail patch a week after snow.
I wonder if these contractions assist in mass transport of substances within such large cells, perhaps these strong contractions assist in movements under a sludge layer of their environment?

I have enjoyed observation of Spirostomum over the years, this is my first encounter of a Spirostomum bloom. In late 2012, a bloom of Spirostomum panicked a sewage treatment operator. The TPO (treatment plant operator) called in an emergency consultant when the treatment plants secondary clarifier effluent “turned a milky white.”

The sewage plant cleared their Spirostomum bloom by an “increase in dissolved oxygen and other adjustments”. In the trade journal ”TPO treatment plant operator” of February 2012, this Spirostomum bloom was showcased in an article titled "Attack of the Free Swimming Ciliates".

The Natural History Museum, London, produced an online document and interactive tool for TPOs after 1992, an atlas of sewage plant ciliates! This fantastic tool lists a large collection of protozoa line drawings and a key to protozoa. This key suggests a procedure for using protozoan populations at each stage of sewage treatment to bioassay proper sewage treatment. Spirostomum ciliates are in this schema.

These 3.0 mm sized active and periodically contracting cells posed a challenge for me to image capture their entire body forms, yet capture crisp details of their spiral ciliature and inner organelles. No one “image stacks a free swimming whale” - yet crisp body features are image captured of whales. I simply cannot image capture entire Spirostomum bodies, yet obtain crisp details of these large and active protozoa. Perhaps hand illustrations, such as the delightful 1853 line drawing (Figure 8) included in this report, are the best method of total body image capture of live Spirostomum?

Protozoa along with meiofauna, and slightly larger metazoas (snails, aquatic insects, flatworms, amphipod crustaceans, small fish) all play key roles in food webs of any season.
Federico Buonanno reported in August 2011, that the flatworm *Stenostomum sphagnetorum* change their predatory behavior towards *Spirostomum* after one encounter with these large ciliates. The flatworms regurgitated any ciliates they ingested and avoid feeding on these ciliates after that experience! *Spirostomum* species have a chemical defense. *Spirostomum* and other ciliates produce and accumulate low molecular weight toxic compounds, place these toxins within specialized ejectable organelles. These organelles are termed extrusomes.
Like a Monarch Butterfly’s defense by chemicals against corvid bird ingestion (the birds must learn by an encounter and regurgitation of an ingested Monarch!), *Spirostomum* ciliates have developed a chemical defense with their extrusomes.

What conditions favored my encounter of a *Spirostomum* bloom under snow and ice, so many other protozoa in that cat tail patch food web? Who eats these large *Spirostomum* ciliates? Thank you for sharing this encounter with a *Spirostomum* bloom.

**Sources**

Comparative Protozoology, O.Roger Anderson, 1988


Biologia, August 2011, vol 66, issue 4, pp648-653, Federico Buonanno

TPO, February 2012, "Attack of the Free Swimming Ciliates"


"Inhabitants of a Drop of Water", Harpers New Monthly Magazine, May 1853, vol 51, No 351, pp 797-800
I thought it might be both interesting and illuminating to carry out a virtual build of a 160 mm tube system microscope from one of the “Big 4” based on the best or at least the more reasonable actual prices (1) items sold for on Ebay.

Since many of the outrageous prices for microscopes and parts advertised on Ebay are placed by unscrupulous (2) sellers hoping to take advantage of newbies by capitalising on their recognition of an established name in microscopy - I thought it high time that those contemplating such a venture have a clearer idea of what is involved and how much a build of this nature is likely to cost if one avoids the worst excesses on Ebay.

I have chosen a Zeiss Standard WL as the virtual stand for two reasons. Firstly, it is the top stand of the Zeiss Standard 160 mm system line (I have omitted the Photoscope, Ultraphot and Universal stands which also use the 160 mm tube length due to their substantial size and weight), and secondly because it is the instrument that I actually have, and so I am familiar with all the bits and pieces that conform it as well as what constitutes a reasonable price (in as much as there is such a thing as a reasonable price in the amateur microscope market of one of the “Big 4”).

The Standard WL differs from the other standards in several ways, firstly in the focus block, which has a fine focus range of 2 mm and a 2 μm adjustment (as opposed to the full range fine focus and 5 μm adjustment of the other standards), secondly in the use of quick release stage and condenser carriers and thirdly in having a wider and longer base. It shares the removable nosepiece/turret of the Standard 18.

The use of a removable nosepiece along with the quick release stage and condenser carriers make the WL a pleasure to work with. Cleaning up after using an oil objective is about as painless as it is ever likely to be, as remov-

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1. The prices items actually sell for on eBay (as opposed to what sellers would like you to pay) can be found by pressing the Advanced feature to the right of the blue search button, adding the search terms, ticking Sold listings under the Search including heading and lastly pressing the blue search button. The prices items have sold for appear in green.

2. The term unscrupulous and worse is richly deserved by those who not only attempt to charge grossly inflated prices but also omit mentioning known problems with equipment by either claiming ignorance of the field or by the use of a minimal description while hiding behind such phrases as sold as is.

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**Figure 1: The basic stand:**

A. removable nosepiece  
B. quick release stage carrier  
C. quick release condenser carrier  
D. focusing block  
E. external lamp house collection tube.
ing both the nosepiece and condenser to allow unrestricted access to them takes just seconds. Similarly, being able to remove the carriers makes it much easier to make many adjustments as it unclutters the work area. The stand itself can be further disassembled by the removal of the 4 bolts that hold the arm on the base and further yet by the removal of the 6 bolts that hold the coarse and fine focus to the arm. In fact the WL is designed to be completely disassembled in minutes.

Even though the Zeiss Standard WL as a top of the range modular stand is capable of hosting a large array of illumination methods, this exercise is not an attempt to convince anyone who decides to attempt a similar build to necessarily equip their instrument with every conceivable adaptation. Both the budget and the present needs of each individual microscopist should set the extent of the build.

The idea is to arrive at a working instrument that is a pleasure to use and which is capable of growing with us at each step of the way.

**Pricing the build**

All the virtual items used in this build were sold during the previous 60 days on Ebay, that is the time period that sold items remain visible in the sold item section. A little more patience and better prices yet are potentially available.

Patience is the key to better prices, that and avoiding sellers that skimp on images, descriptions, claim large discounts and use sold as is as a matter of course.

A little research in the sold items section will give one a fair idea of what the average price is. Ask the seller questions, ask for more or better images. The more you ask the better the chance of a successful transaction.

Best offers are also a good way to obtain a better price. One should not offer too little however, that is as bad as sellers asking too much. One can do worse than be guided by the average price and a sense of fairness when making offers.

The condition of an item should be clearly stated - if an item is not as described, as in it has a flaw that renders its use or the price paid void, then it should be returned or the money refunded. Do not be afraid of giving negative feedback if the seller has deliberately glossed over flaws.

Many unscrupulous sellers use the artificial discount technique to entice unwary buyers. The seller starts by offering an item at much more than it is actually worth, any sales made during this period to the unwary/impatient is considered a bonus. The seller then reduces the vastly inflated price to as
lightly less outrageous one while claiming a large discount has been made. Do not obsess on fantastic bargains - if something is too good to be true it generally is. Con men would have a much harder time if most people were not permanently on the lookout for a bargain or out to double their money.

**The Stand**

I will start the virtual purchase with a pretty basic bare stand, the one sold on Ebay consisting solely of the base, field diaphragm housing, 6V 15W collector tube, arm, focus block and nosepiece. Missing and needed to get the microscope up and running are:

- microscope head
- eyepieces
- objectives
- condenser carrier
- stage carrier
- condenser
- stage collection tube (46 70 50)

- 12V 60W lamp house
- power supply

The first step is to complete the basic stand with the addition of the missing condenser carrier and stage carrier and the replacement of the 6V 15W collection tube (46 70 50) with the much more versatile (46 70 40) that will allow us to connect either the 12V 60W external tungsten lamp house or the 12V 100W external halogen lamp house.

Prices of the quick release carriers (best offer accepted) were probably in the $25 - $35 range. There was no connector tube of type (46 70 40) sold during this period, however it is not unreasonable to assume that one could be obtained for about $20 - $25 dollars.

The next step is to obtain a head. There are several different types of Zeiss heads which can be used, though ideally, if one plans on taking photographs and most of us do, then a trinocular head is our goal.

Fortunately for the purposes of this build, one was sold at a very reasonable price during this time period by a responsible and reputable seller. A pair of 12.5X eyepieces complements this head nicely. We now need to locate a stage. Again we were lucky.

A rotating stage is very useful if one is not able to rotate one’s camera as it allows one to take either landscape or portrait images as needed. It is also very useful when using both oblique illumination and DIC as varying the angle of the subject can improve its visualization.

There are various types of Zeiss condensers that could be used on this stand, as there were several sold during this period, I shall show a selection. The first one shown is, along with the 0.9, the most basic of the Zeiss Flip-Top condensers that will work on the WL - brightfield only. The second one, an Achromat Aplanic 1.4 NA is perhaps the most interesting, as apart from the default brightfield it has phase 1, 2 and

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**Figure 3: Various condensers.**
3 and a darkfield stop that when oiled allows one to achieve darkfield with higher magnification objectives. The third condenser has brightfield (the default illumination of all these condensers) but lacks phase 1 and the extra darkfield stop. It has phase 2 and 3 and of course adds DIC. Phase 1 basically allows the use of the 10X objective, but as phase 2 starts at 16x, the lack of ph1 is not critical.

To complete the illumination we need a lamp house and a power supply. That just leaves the objectives. In this case the best plan (as our aim is to get up and running) is to go with phase, as phase objectives work well in brightfield and hence oblique and darkfield, as the slight theoretical image loss is in practice barely perceptible.

**The Sums**

<table>
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<tr>
<th>Item</th>
<th>Price</th>
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</thead>
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<tr>
<td>The stand</td>
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<tr>
<td>Stage carrier</td>
<td>$30</td>
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<td>Condenser carrier</td>
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<td>Connector tube</td>
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<tr>
<td>Trinocular head</td>
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<tr>
<td>12.5x eyepieces</td>
<td>$35</td>
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<tr>
<td>Rotating stage</td>
<td>$160</td>
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<tr>
<td>Phase contrast/DIC condenser</td>
<td>$402</td>
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<tr>
<td>12V 60W Lamp House</td>
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<tr>
<td>12V 100W Power Supply</td>
<td>$65</td>
</tr>
<tr>
<td>6.3x/0.16 objective</td>
<td>$59</td>
</tr>
<tr>
<td>16x/0.35 ph2</td>
<td>$79</td>
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The prices paid for this virtual build will of course vary for any one attempting such a build in reality. Just the postage for so many individual items will come to a not insubstantial sum. It is hard to say by how much or in which direction - whether it would come to more or less - as there are a couple of good bargains in this virtual exercise and though patience is a virtue it is sometimes hard to put into practice.

There were also choices made that might not have been made by the reader, such as the choice of condenser for example. I did consider the possibility of choosing the darkfield condenser with 3 phase ports and recommending that more time be spent in searching for a DIC condenser with a higher NA.

There was no attempt to include more parts for the DIC system, this was partly because there were no additional parts sold during this time and partly because there are 4 DIC systems available for the Zeiss WL (see references). Which of these would turn out to be more practical or economical by the time one has all the parts or as many of them as possible is hard to say.

The price paid so far for this microscope compares very favourably with many of the generic budget Chinese microscopes that go for over a thousand dollars for the darkfield and phase versions.

Yet we are not really comparing prices here as one can only really do that when comparing like with like and the WL is as unlike any generic budget Chinese microscope as one is likely to get.

For at the end of the day, due to the simplicity and beauty of its modular design as well as its excellent build quality, the Standard WL is not only a pleasure to use, but is also eminently expandable, such that it will be able to keep up with our interest as it grows and develops. It will in all likelihood out live you, and with care, will probably give the next generation a run for their money.

**References**

Figure 5: Various objectives.
Revisiting the slide guide card

By Andy Chick

Early slide preparers treated slides like works of art which are still prized to this day, whereas modern slides are often prepared in a more utilitarian manor, this paper aims to re-examine and demonstrate how to make one of the tools used by early microscopists.

The modern methods of producing slides often lack the finesse of some of the classically prepared slides. Modern methods are often aimed at simplicity and preparing bulk numbers of slides quickly and economically. A specimen is placed roughly in the middle of the slide in a suitable media, covered with a square or rectangular cover slip and if ringed, a rough coat of nail varnish or enamel model paint is used. While this is a perfectly serviceable way to prepare a slide and certainly useable for permanent archival slides most would agree that such slides lack the artistic beauty of one of the Victorian slides that are sort after by collectors.

Using a ringing table and circular cover slips improves the look of slides as many will attest, but the extra time and expense for little practical improvement means such techniques are not often employed. Another old technique used by early microscopists is that of a guide card as recommended by Gage (1917), a simple device use to centre a specimen on a slide (Figure 1)

The website vade mecum microscope (2016) presents a method for fabricating a guide card (referred to as a centring card) from index cards which, while perfectly serviceable, lack the durability that some microscopists might prefer. The aim of this paper is to show the current authors slide guide card and the process of replicating it if desired.

Figure 1: the guide card as presented in Gage (1917).

Figure 2: Materials required.
Materials and methods

The following materials were used to assemble the slide guide:

- Pigment ink drawing pen (Figure 2A), the ink with stands most of the common solvents used in microscopy.
- Microscope slide (Figure 2B).
- Tile spacers (Figure 2C).
- Scrap of plastic (Figure 2D).
- Bristol board/card (Figure 2E).
- Super glue (not shown).
- (optional) matt black paint (not shown).

The method of fabrication is as follows:

- The tile spacers a cut diagonally in a similar manner described by Chick (2011).
- Using a slide glue the cut title spacers to the plastic sheet to form a frame (Figure 3).
- Cut the card to the size of a slide
- Using the drawing pen connect the diagonal corners to find the centre of the card.
- Measure and mark up the most commonly used types of coverslip on the card from the either side of the centre (Figure 4).
- Optional: paint the plastic sheet
- Add the card to the plastic template (Figure 5).

Discussion

The slide guide is rather simple to fabricate from common materials, the author left some scrap plastic behind the tile spacers rather than cutting it flush to allow the guide to be anchored down using the stage clips of a stereo microscope, if the microscopist is mounting small specimens. While not essential to the production of a slide (like a ringing table) the slide guide allows the microscopist to make their slides look more standardised and thus more aesthetically pleasing.

References


Gage, S.H., 1917 The microscope: an introduction to microscopic methods and to histology. 12th ed. Comstock publishing company.

http://www.biodiversitylibrary.org/item/71844#page/340/mode/1up accessed 22/10/2016

Vade mecum microscope 2016

Most permanent and semi-permanent mounting media benefit from gentle warming to speed up the drying process. This paper shows how a simple bench top slide oven can be easily made with minimal DIY knowledge.

When making slides using either resinous mounting media such as Canada balsam, Euparal or Damar gum or any of the non-gelatine type water based media such as Farrants, Hoyers, Apathys or Dionis, gentle warming of the slide aids the evaporation of the solvent drying the setting process; reducing the possibility of the specimen moving or being damaged, and allowing the microscopist to ring and examine the slide in a more timely manner.

In the professional laboratory a microscopist would use either a purpose built slide warmer or a hot plate to evaporate the excess solvent, the amateur microscopist might use the radiator in their home laboratory or an airing cupboard or the lucky one might have the same equipment as a professional laboratory. A slide must be protected during its drying from dust and sudden movement, as some of the options discussed above are not ideal methodologies. With this in mind the current author attempted to make a small warming oven that would not take up much space in the home laboratory the fruits of his work are shown below.

Figure 1. The desk top slide oven.
The slide oven

The basic oven was made from a wine box (Figure 1) that held two bottles this provides a small shelf when placed on its side. The box was then lined with tin foil to reflect heat from the heat source (Figure 2). A vivarium heat mat was used to provide heat to the oven as suggested by Chick (2009). As the box was used on its side some small feet were added to allow the oven to be opened without tipping (Figure 3) as a final touch a small electric vivarium thermometer was added so that the oven’s temperature could be seen externally. The oven heats to between 30 and 40°C depending on how long it has been on for while this is not very hot it provides enough heat to speed up the drying time of Gum Dammar and Gum Arabic based mounts similar to “airing cupboard” and “radiator” times, with the added benefit of protecting the specimens.

References


Figure 2. The inside of the oven.
Figure 3: The legs added to the oven.
Figure 4: An electric vivarium thermometer mounted in the oven.
Cladocera, possibly *Alona sp.*

Plan 40x/0.65, 470μm, DIC, 20 stacks stitched in Photoshop

Images by Forbes Pettigrew
Comparison of stomata of different vegetables.
60X obj. and stained with red food coloring.

Top left: Iceberg Lettuce
Top right: Romain Lettuce
Bottom left: Broccoli
Bottom right: Brussel Sprout

Images by Jim Tallon
Bryophytes are small plants known to inhabit humid places and often called mosses. Conversely, these plants have different morphologies and evolutionary histories that allowed the bryologists to classify them in three phyla: Anthocerotophyta (hornworts), Bryophyta (mosses) and Marchantiophyta (liverworts), totaling about 18,700 species. Moreover, these plants display a very diverse set of sexual systems and means for reproducing, sexually or asexually.

First, the life cycle of bryophytes fall into two distinct phases: gametophytic (haploid), and sporophytic (diploid). In the diploid sporophyte, through meiosis, spores are generated; these will be launched in the environment and generate a female or male gametophyte. The gametophyte is the dominant phase that will generates the female (egg) and the male gametes (sperm). And when the sexual reproduction is not possible, the gametophyte produces asexual structures for the establishment in new habitats. Bryophyte sperm need water to reach the egg inside the female gametangium (that is why the importance of the water presence), and after the fertilization the zygote develops into a sporophyte, restarting the cycle.

Sexual systems in plants are basically divided into two main groups: monoecious (male and female structures in the same individual), and dioecious (female and male structures present in distinct individuals). Among vascular plants (i.e., ferns and seed plants), only 6% are dioecious; while in bryophytes, these values vary around 60%. The dioecious species can generate an intersexual relation of competition for the space that may or may not result in a spatial separation.

This diversity of sexual systems and means of reproduction in bryophytes seem to be evolved in response to the water dependence for reproduction in these plants, in addition to the common spatial separation of male and female plants. Bryophyte species found different ways to overcome these obstacles, ensuring efficient dispersal and colonization. In addition, reproductive costs influence in the sex-specific differences, sex ratios, and reproductive strategies present in the species.

We investigated two dioecious species of Brazilian mosses from the genus Schlotheimia Brid. regarding their curious sexual system. In Schlotheimia rugifolia (Hook.) Schwägr. the males are 10 times smaller than the females, and in Scho-
Schlotheimia tecta Hook F. & Wilson. The males are 32 times smaller than females. They are often found on the female leaves, being a tiny gametophyte represented by some leaves and 1-3 male gametangia. Because they live in association with females, plants are also considered pseudoautoicous (dwarf males functionate as male branches on females). Dwarf males may be optional or mandatory. For instance, in some species when the male has contact with female plant it grows into a reduced size; however when withdrawn and cultivated at the female absence, a male grows into a larger size.

In general, in dioecious species the sex ratios are female biased, suggesting that this can be related to differential dynamics during spore germination and gametophyte development. There is the suggestion that male spores have higher abortion rates compared to female ones. But sex expression may be linked to environmental conditions or plant maturity. So, to consider the presence of the sexes is a broader issue that may involve several variables.

Apparently, dwarf males are an important adaptation for reducing fertilization distances in pseudoautoicous bryophytes, meaning that the size and sex-specific differences occur for increasing the likelihood of successful sexual reproduction, and also decrease intersexual competition. On the other hand, dwarf males have shortcomings and are more sensitive to the environment; that is, water remains an important factor for the reproduction in these species.

Despite this, all these dwarf males are successful in sexual reproduction, where the dwarf male samples are frequently found with sporophytes. From the first moment that dwarf male comes in contact with a female population, it fertilizes nearby female gametangia, and the resulting sporophyte may produce spores that will develop into new generations of fe-

**Glossary**

**Anteridium:** Male sexual organ responsible for producing sperm.

**Archegonium:** Female sexual organ that houses the egg.

**Dioecious:** Classification given to the species with the female and male sexual organs present in different plants.

**Diploid:** When the cell, individual or generation has two chromosomal groups (2n).

**Gametangia:** Organs containing gametes.

**Gametophytic:** Haploid sexual generation producing gametes. Haploid When the cell, individual or generation has only one chromosomal group (n).

**Meiosis:** Meiosis is a process of cell division that produces haploid gametes.

**Monoecious:** Classification given to the species plant with the male and female sexual organs present on the same plant.

**Pseudoautoicous:** Classification given to the bryophyte species with dwarf male epiphyting the female plant.

**Figure 2:** Female shoots of **Schlotheimia rugifolia**.

**Figure 3:** Dwarf males of **Schlotheimia rugifolia** associated to a female leaf.
males and dwarf males. To investigate the sexual systems and the plant architecture allows us to understand the strategies and adaptation for reproduction in bryophytes. For this reason, all this information is important to understand the life history of species and promote the correct conservation of different populations.

References:


Figure 4: This image shows female gametangia (archegonia) of Schlotheimia rugifolia.

Figure 5: Male gametangia (antheridia) of Schlotheimia rugifolia.
Figure 6: This image shows a female plant with sporophyte (arrow) beside a dwarf male (inside a circle) of *Scholothemia tecta*.

Figure 7: Dwarf male of *Scholothemia tecta*.

Figure 8: Female gametangium (archegonium) of *Scholothemia tecta*.