The creation of three-dimensional (3D) models is a valuable tool for exploring a wide range of questions in biological research. The choice of technique used to generate 3D models in a particular study is important for the resolution quality, the investment of time, and production cost. Modeling intricate plant structures generally involves extracting two-dimensional (2D) images of a desired structure, interpreting those shapes, and then recreating the morphology in 3D. High-resolution X-ray computed tomography has been used to construct virtual 3D models of plant structures (Dhondt et al., 2010) and even fossilized plants (Gee, 2013) by extracting 2D slices linearly through a structure, and then reconstructing a 3D surface from those images. Another method, optical photogrammetry (Eulitz and Reiss, 2015), accomplishes the same task by rotating the object to collect 2D images from multiple angles. The method that we describe here operates similarly to X-ray computed tomography, but uses refined microscopy techniques to produce conventional anatomical slides containing serial sections. Imaging those serial sections with standard light microscopy effectively replaces extracting layers with X-rays. Virtual 3D models are then constructed by manually designing individual splines to create the architecture of the plant structure. The advantages of the 3D modeling technique described in this paper as compared to X-ray computed tomography are cost efficiency, simultaneous production of microscope slides for anatomical study, and the creation of an easily editable virtual model before the creation of the surface mesh. An advantage over photogrammetry is the ability to accurately recreate internal structures with a high level of detail.

An example of the utility of this method is our work on the flowers of the grass family. Poaceae is the fifth most diverse flowering plant family and one of the most ubiquitous in terrestrial distribution (Stevens, 2001 onwards; Kellogg, 2015). It is the largest plant family to rely primarily on wind pollination and is thus unique among the five most diverse families. The small size, concealing architecture of the floral units (spikelets), and compound arrangement of parts makes grass synflorescences notoriously difficult to study, especially with respect to pollination biology. We have developed a protocol to model the spikelets in 3D using computer-assisted design (CAD) software. The models enable visual exploration of spikelets through virtual dissection. The same models can be subjected to simulations to test a suite of new hypotheses regarding mechanical properties...
and aspects of pollination biology. These digital models facilitate scientific research, explanatory taxonomic videos, and public outreach with the same virtual object. The spikelet of the unquestionably important and often taxonomically feared Poaceae is an ideal structure to demonstrate this new method.

The grass spikelet is a compact structure of bracts (glumes, lemmas, paleas) that envelop each other and the developing grain. Understanding the positions and shapes of the bracts is the principal method of taxonomic identification in the grass family. These structures are traditionally represented by 2D illustrations; however, it is challenging to accurately represent 3D structures and their concealing arrangement with conventional botanical illustration. Static illustrations cannot permit adjustable transparency of parts to allow the visualization of the intricate interiors of botanical structures, nor can they be used for downstream analyses. The dynamic 3D models produced by our method exist in a gradient between classical botanical illustration and virtual reality. If morphometric integrity is retained in three dimensions, the same model can be used to visualize the composition of a spikelet in a plant systematics class or provide surfaces for geometric morphometric studies (Adams, 2014). Video animations that rotate spikelets, virtually dissect spikelets or parts of the grass synflorescence, and animate taxon-specific morphology may be used as helpful online resources to accompany taxonomic keys or botany courses.

Anatomically accurate, digital 3D models of plant structures can be used in a variety of research applications. For example, we plan to import these 3D spikelet models into computational fluid dynamics simulations to analyze wind flow around reproductive structures. Modifications can be made to the virtually modeled object relatively easily in order to experimentally test how individual parts of the spikelet, such as glumes or awns, contribute to the aerodynamics of the system. These 3D models could be used for ancestral state reconstructions and provide a means to explore observed and unobserved areas of morphospace (Runions et al., 2017). The frontiers of computer technology have been integrated with biology to produce major advances in genomics and bioinformatics; we aim to apply the same computational edge to studies of morphology.

METHODS AND RESULTS

This procedure is useful for the 3D modeling of any small, compartmentalized plant material, but our description of the method will refer specifically to the example of grass spikelets. Multiple spikelets are removed from living plants by cutting the pedicel 2 mm below the lowest glume at anthesis or stigma exsertion (Fig. 1A). Multiple spikelets are used to increase the available material in later phases of the process to minimize the risk of damaging all material. Redundancy of material also allows the final modeling to incorporate an average of structures instead of relying on a single sample. Spikelets are separated into three groups for three different degrees of dissection (Fig. 1B) before fixation, dehydration, paraffin embedding, and sectioning (Appendix 1) to permit alternate views of the structure that are necessary for accurate 3D modeling. These groups are as follows:

1. A set of spikelets is processed whole. This batch can produce the most complete set of continuous serial sections, but is often hindered by air bubbles trapped within the spikelet compartments. Gently forcing air bubbles out with a blunt dissection probe

FIGURE 1. A flow diagram illustrating the dissection and microscopy techniques applied to spikelets in order to obtain images of cross sections. The procedure includes (A) collecting fresh material and vouchering the specimen, (B) dissecting some spikelets while taking photographs, (C) fixing the material in gFAA and then dehydrating in a graded series of ethanol and xylene, (D) embedding in paraffin wax, (E) serial sectioning at 10 μm thickness with a microtome, mounting sections on a slide, and photographing to produce (F) images that will be used as references for 3D modeling.
facilitates a more complete infiltration of fixatives and subsequent fluids.

2. A set of spikelets is cut transversely at a point above all bract insertions to expose the interior of the spikelet to increase infiltration of fluids, allowing more complete embedding and smoother sectioning. This set allows for serial sections, which progress along the rachilla, to be made, and provides a reference for each bract’s location and attachment. If working with a taxon with an elongated spikelet with more than two florets (e.g., *Poa L.* or *Eragrostis Wolf*), it will be necessary to cut the spikelets into segments along the length of the rachilla.

3. A third set of spikelets is dissected completely in order to remove individual bracts. Several spikelets contribute their individual parts, such that separate vials each consist exclusively of lower glumes, upper glumes, fertile lemmas, etc., and are labeled accordingly. This set allows sections to be made of each bract without other material interfering or dulling blades. Sectioning individual bracts provides the clearest anatomical view and informs the modeling of each bract, but only in combination with the other preparations can the bract placements and overall spikelet structure be recreated.

Material was taken from *Danthoniopsis dinteri* (Pilg.) C. E. Hubb., *Panicum virgatum* L., and *Poa pratensis* L., all grown in a greenhouse, during the development of this procedure. Herbarium material may also be used, if spikelets were not deformed during the pressing and drying processes, by soaking in Pohl’s solution (Pohl, 1965) prior to fixation. The spikelets (whole and cut) or dissected bracts are placed in scintillation vials and then fixed in gFAA (1% glutaraldehyde added to formaldehyde–acetic acid [Sass, 1958]) for a minimum of 24 h before further processing (Appendix 1). The liquid contents of the vials are then carefully removed and replaced with ethanol (ETOH). We have found that progressing from gFAA to 70% ETOH, 90% ETOH, 95% ETOH, 100% ETOH, and ending with a second round of 100% ETOH, in hourly steps allows timely dehydration, but at a rate slow enough to prevent cells from lysing, which could cause the shape of the organs to become distorted. Placing the vials in a rotator, or using an aspirator, may assist complete infiltration. The samples are gradually transferred from pure ETOH to xylene prior to the addition of paraffin wax. The vial is placed in a 60°C oven, and paraffin wax is added. The vial lid is unscrewed to allow the xylene to evaporate, leaving the plant material in melted paraffin wax.

Paraffin wax is poured into a mold before the desired plant material is removed from the vial and placed in an upright position at the center of the mold with the pedicel touching the bottom of the mold (Fig. 1D). After the paraffin has hardened, material is sectioned at 10 μm using a rotary microtome (Fig. 1E). Serial sections are placed on a glass slide with Haupt’s solution (Bissing, 1974), stained so that they can be imaged, and then sealed with Permound and a coverslip (Sass, 1958). Images are taken at appropriate magnifications, which may vary depending on species and the size of spikelets, using a light microscope and saved as TIF files. These images provide a framework for accurately sculpting the shape of the spikelet in the CAD software. Complete spikelets under a dissecting scope are imaged to provide additional reference material.

For 3D modeling (Video S1), we use two CAD programs with alternate advantages. Modeling begins in a program called Cinema 4D (MAXON Computer GmbH, Friedrichsdorf, Germany). The microscopy cross-section TIF files are imported into the modeling environment and oriented parallel to the X–Z plane. The shape of each bract in cross section is traced from the image using a Bezier spline drawing tool. Reference images of the whole spikelet from a side view are imported, oriented in the correct direction, and then traced (Appendix 1). The cross-section images are moved to the proper height by vertically transposing the splines until they intersect the correct points in the side-view splines. The splines are organized as sets of sequential splines and placed in a Loft object, which creates a continuous surface between splines. The separate Loft objects are then oriented together to form the entire spikelet. Cinema 4D produces beautiful rendered images and includes a wide range of textures, lighting, and animation techniques. However, as Cinema 4D is an artistically oriented software, it lacks the technical capacities of true nonuniform rational basis splines (referred to as NURBS). The object is therefore transferred to another program called Rhinoceros 5 (Rhinoceros 5 SR13; Robert McNeel & Associates, Seattle, Washington, USA) and the plugin Grasshopper (version 27 August 2014; Robert McNeel & Associates), which are scripts-based, engineering-grade CAD software. The transfer consists of removing splines from their Loft object in Cinema 4D and exporting them in a format compatible with both programs (.dxf), importing them into Rhinoceros 5, converting the Bezier splines into Basic splines, and then re-lofting them using a Grasshopper script. The Rhinoceros 5 version of the spikelet model provides a surface mesh for computational fluid dynamics simulations to calculate aerodynamic properties.

Previous methods for analyzing aerodynamic properties of grass spikelets relied on conventional wind tunnels (Niklas, 1985) or measurements taken in the field (Friedman and Harder, 2004). Virtual simulation of computational fluid dynamics allows for the calculation of the Navier–Stokes equation (Temam, 1984), which provides air pressure and speed vectors for points within the simulated space. This is an enormous resource for testing hypotheses pertaining to structures and their relationship to the surrounding air currents. We know of one instance in the literature of computational fluid dynamics simulated around a virtual grass synflorescence (Cresswell et al., 2010). This study avoided modeling the intricate bract shapes by substituting all parts with half spheres. Although all models are approximations, our method of modeling the shape of each bract is an improvement over this study because it recreates the structure of spikelets more accurately. Virtual models produced by X-ray computed tomography are sample-specific and may contain abnormalities of the limited (possibly singular) sample size or exhibit deformations from noise during the acquisition of 2D images. If the purpose of a model is to provide a surface for simulating computational fluid dynamics, the intricacies of the botanical morphology should be approximated in the virtual model to accurately portray the biological inspiration.

As an educational tool, virtual models are an improvement upon 2D line drawing by providing the ability to rotate an object in 3D, visually zoom into regions of interest, and dissect the object to show how structures are oriented internally. The 3D models of grass spikelets we create with this procedure are uploaded to the 3D model repository website Sketchfab (https://sketchfab.com/pklahs) for public viewing. Because these models are constructed with points and splines in 3D space, they can be used in geometric morphometric analyses; ancestral reconstruction of 3D form; or for calculations of surface area, volume, and other 3D descriptors of shape and size. Morphometric studies require precision as to which landmarks are chosen to represent the nature of a structure's shape.
The virtual models consist of many recorded points from which sets of informative points can be selected and provide a setting in which very precise measurements can be taken.

CONCLUSIONS

This method produces 3D models that can be used for geometric morphometric analysis and in silico simulation studies on the structural properties of plants and their interaction with environmental forces. For example, we intend to use the grass spikelet models generated in this study to assess their properties under different computational fluid dynamics models to address hypotheses of spikelet aerodynamics. Structural modifications to spikelets, ranging from impractical to impossible in vivo, can be made to the virtual model relatively easily to test hypotheses of structural influence of individual parts on the entire system. For example, awns can be added or removed from bracts, or individual bracts scaled in size, before conducting the aerodynamic simulations to explore their potential effects on wind pollination. Exploring the interactions of these structures will provide insights into the evolution of reproductive structures in the grass family. Application of this procedure is not limited to grass spikelets, and is immediately applicable to other types of flowers or even vegetative parts. Virtual models created with this method can be used to address a wide range of structural questions, all while presenting the beauty of botanical morphology in a novel way.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

VIDEO S1. An animation of the steps required for 3D modeling of spikelets using Cinema 4D software. This video is an MP4 file and is available under the Supporting Information section at the end of the article, or can be viewed from the Botanical Society of America’s YouTube channel.

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APPENDIX 1. Methods for microscopy preparation and 3D modeling of spikelets.

Spikelet collection, dissection, fixation, dehydration, and paraffin infiltration protocol

I. Collection—Cut the pedicel of the spikelet 2 mm below the lowest glume. This will take up the smallest space in the scintillation vial and later the paraffin block, but will ensure that the spikelet stays intact. Collect multiple spikelets to increase the probability of usable material.

II. Dissection—Spikelets are prepared for future steps in three different ways. Each set should include multiple spikelets or bracts. Images should be taken during this process. See section X: Imaging, below.

1. Set of whole spikelets: A set of spikelets can continue to the next step exactly as they were removed from the plant. In later steps after fixation, air bubbles may persist inside the overlapping bracts. Massaging these bubbles out with a probe will be necessary to ensure proper infiltration and complete paraffin embedding.

2. Set of intact spikelets cut transversely: Take a set of spikelets and cut them with a razor blade transversely at different heights along the rachilla to remove apical portions of the spikelet. Be mindful of the depth of the paraffin block mold you will be using, especially if spikelets are large. In some cases, removing entire florets and cutting a floret transversely may be desirable. Cut some spikelets very close to the base, but leaving parts of the glumes so that the serial section through that material may show the vasculature and the proper heights of attachment for the basal bracts.

3. Sets of bracts: Carefully remove all bracts from several spikelets and make sure to keep them grouped according to position, such that all the lower glumes from these spikelets are placed together, etc.
III. Fixation—Place each set in an appropriately labeled 20-mL scintillation vial with enough gFAA to cover material. In some cases, the plant material will float because of trapped air bubbles. Gently spinning the vials in a rotator or placing the vial without a lid in a vacuum may help. Plant material should be allowed to fix in gFAA for at least 24 h. The samples can remain in this solution for temporary storage. (Plastic containers may be used for temporary storage, from weeks to months.) For long-term storage, transfer material to 70% ETOH and use glass containers. Always label containers with an identification number, scientific name, type of bract or portion of spikelet, and date. Record this information in a lab journal.

IV. Dehydration—The following steps involve decanting, that is, draining the previous liquid, and replacing with a new solution without losing plant material. Each step should be allowed to soak for an hour unless otherwise noted. Gently spinning in a rotator greatly assists each step of this process. See section XII: Recipes, for an hour of spinning in a rotator and are similar to the previous steps. See section XII: Recipes, below.
1. [gFAA] This is the initial solution and after soaking for 24 h the dehydration may begin.
2. [70% ETOH] The material can remain here indefinitely.
3. [90% ETOH]
4. [95% ETOH]
5. [100% ETOH]
6. [100% ETOH]

V. Paraffin infiltration—The first three steps of this process require an hour of spinning in a rotator and are similar to the previous steps.

1. [0.5% safranin in 1:1 100% ETOH:xylene] This step stains the material to increase its visibility in later steps.
2. [Xylene]
3. [Xylene]

Add an equal volume of warm paraffin to the vial. Allow paraffin to flow along the side of the vial so that it forms a cap over the xylene. Place in 60°C oven for 12 h.
Pour off 1/2 the volume and replace with an equal volume of paraffin. Place in 60°C oven for not more than 4 h.
Repeat the previous step, and pour off 1/2 the volume and replace with an equal volume of paraffin. Place in a 60°C oven for ≥12 h with caps off to allow xylene to evaporate.
Proceed to embedding or cool to room temperature for long-term storage.

VI. Tissue embedding—
1. Pre-label embedding molds with identification number, scientific name, type of bract or portion of spikelet, and date.
2. Fill the embedding mold with new melted paraffin. The lower portion of mold will start to cool first. This base layer of solidifying paraffin will make it easier to transfer plant material and hold it to prevent it floating around while trying to orient it.
3. Using forceps, carefully transfer the desired plant material to the center of the mold and gently force it down into the solidifying base layer. The mold is oriented upside down during this process, so the material will be sectioned from the bottom up. Also realize that only material beyond the plastic container within the paraffin of the mold will be accessible for sectioning without further re-melting and repositioning. Once the plant material is in place, you may gently nudge it to align it perpendicular to the base. During sectioning, the blade will cut parallel to the bottom surface of the mold.
4. Add extra melted paraffin to completely fill the mold if parts of the plant material are still exposed to air.
5. Store at room temperature and allow to cool and/or harden ≥12 h before sectioning. Vials may be placed back in a 60°C oven if more molds will be made to ensure they do not harden; if that set is completed, vials may be allowed to cool for room temperature storage. Vials and paraffin molds can be kept at room temperature indefinitely.

VI. Tissue embedding—
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VII. Microtome sectioning—After the paraffin block containing plant material has hardened, it may be prepared for sectioning.
1. Remove the plastic mold tray on the bottom and carefully trim the paraffin exposed from the mold. A razor blade can be used to cut down the sides and the microtome can be used to cut the surface low enough to expose the plant material.
2. Soak the block with the sample face down in 10:3:90 aerosol OT:glycerine:distilled water solution. Soak for a minimum of 48 h. This solution softens the material for easier sectioning. Some samples will improve after 72 or 96 h.
3. Prepare a fresh razor blade and a clean piece of paper to lay the samples on. Be safe! Handle the sharp razor blades very carefully. Position the block in the microtome holder such that the plant material is perpendicular to the edge of the razor blade, or the surface of the paraffin is parallel to the edge of the razor blade. Section at 8–10 μm at a slow, consistent rate while gently helping the ribbon to stay flat by carrying the weight with a small-tipped paintbrush. Slowly move the tip of the paintbrush away as the ribbon gets longer with more sectioning.
4. Lay the ribbons on the clean piece of paper and mark which end is the beginning. Cut the ribbon into appropriate lengths depending on the size of coverslips you will be using later.

VIII. Ribbon mounting—
1. Clean slides with 70% ethanol and allow to dry.
2. With the rough label surface facing up, place one drop of Haupt’s adhesive onto a clean slide. Smear the adhesive solution uniformly across with a gloved finger, limiting air bubbles.
3. Using a pencil, label the slide with an identification number and record that number along with the material that will be placed on that slide in a lab journal.
4. Flood the slide with 3% tissue section adhesive in dH2O.
5. Float the wax ribbon containing the sectioned plant material on the surface of the dH2O/tissue section adhesive with the shiny side of the ribbon facing down. Place multiple ribbons on the slide in this manner with the beginning ends lining up such that the serial sections are similar to reading a paragraph.
Allow enough space around the edges to accommodate the coverslip.

6. Drain some excess tissue section adhesive fluid by dabbing the corners of the slide with a Kimwipe (Kimberly-Clark Corporation, Irving, Texas, USA). Be careful that the sections do not float off the slide.

7. Place the slide on a 47°C slide warmer, and center the ribbons if they have moved. Allow the slide to dry for approximately 2 min.

8. Remove any excess tissue section adhesive fluid that still may be on the slide, and then allow slides to sit for 4–24 h on the slide warmer.

IX. Staining—To prepare the slides for staining, place them in a 60°C oven for 10 min to melt the paraffin wax. After the paraffin has melted, the slides will undergo a series of treatments in different solutions. They may be placed on a slide-holding rack that can be individually treated. Make sure that the entire slide, especially the region containing the plant material, is submerged in the solution.

1. [Xylene] for 5 min
2. [Xylene] for 5 min (submerge in a different container of xylene)
3. [Xylene] for 5 min (submerge in a different container of xylene)
4. [100% ethanol] 1 min
5. [95% ethanol] 1 min
6. [70% ethanol] 1 min
7. [50% ethanol] 1 min
8. [0.5% safranin O stain] for 1–12 h, see section XII: Recipes, below. (The duration of this step may change depending on the properties of the material and the desired results for imaging.)
9. [70% ETOH] 5 dips
10. [95% ETOH] 5 dips
11. [100% ETOH] 5 dips
12. [1% Fast Green in ethanol] 4 fast dips, no more than 10 s total, see section XII: Recipes, below. (If you are staining a large quantity of material, regularly filter the stain solution through a coffee filter prior to use.)
13. [Clove oil/xylene/100% ETOH] 10 dips or until cleared of excess stain
14. [1 : 1 100% ETOH : xylene] 5 dips (regularly filter this through a coffee filter prior to use)
15. [Xylene] for 5 min
16. [Xylene] for 5 min (submerge in a different container of xylene)
17. [Xylene] for 5 min (submerge in a different container of xylene)

Coverslips may now be added to slides with Permount (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Allow slides to sit 48 h before handling.

Spikelet imaging and 3D modeling

X. Imaging—Photographs capturing the shape of spikelet bracts in an unambiguous orientation are key to being able to accurately model the spikelet with CAD software. There are two phases of imaging: while dissecting the spikelet and after serial sections are made.

Dissection images—Spikelets should be photographed intact and before removal from the plant. These photos provide a record of complete synflorescence structure, as well as color for accurate rendering later, and can be used in presentations. After removing an ideally shaped spikelet, support the pedicle in a small ball of clay (this is easily done if a longer portion of the pedicle is left below the glumes and later trimmed to the 2 mm recommended in section I: Collection, above) and place under a dissecting scope. Make sure lighting provides clear edges to the spikelet parts. A dark background color may provide good results.

Photograph perpendicular to the distichous axis of the spikelet with the pedicle at the lower edge of the image and the apical portion at the top. Rotate the clay ball to orient the spikelet, enabling photography at different angles in this plane. After you are satisfied with the rotational photographs, turn the clay ball and spikelet such that you are looking straight down on the spikelet.

Begin dissecting the spikelet by removing the glumes. Repeat the photographs in the rotational plane and from a top-down perspective. These photos will provide a clearer view to the florets as they are positioned along the rachilla.

Remove a floret. Repeat the photographs in the rotational plane and from a top-down perspective. If the floret is too large or awns extend out of view, multiple photos may be taken and later spliced together to create a complete image.

Dissect another spikelet such that all that remains attached to the pedicle are the glumes. Repeat the photographs in the rotational plane and from a top-down perspective.

Photograph a bare rachilla.

Section images—Microscope slides will contain cross sections of spikelets or bracts in a serial progression. Images may be taken using microscopes with photographing capabilities. The extent of magnification may change depending on the size of the material or the purpose of images. Higher magnification may provide better anatomical details, but requires splicing many images together to create a complete cross section of the spikelet. Magnification should be recorded so the size of bracts in relation to other parts may be understood.

XI. 3D modeling—

1. Import reference images that will be helpful for modeling the structure of the spikelet. You will likely not use all of the photos you have taken.
2. Position those photos in the correct planes. For example, the top-down photos of the spikelet and the microscopy cross-section images should be placed parallel to the X–Z plane.
3. Begin tracing the cross section of a bract by positioning the CAD software camera view to be looking straight down at the images and using a spline drawing tool (orange lines in Video S1). Be mindful of the number of points you are using to create that spline and where you position them. If you were to use these cross-section splines to create your mesh or Loft NURBS, you would want the same number of points in each trace. Work through all the cross-section images by changing the layer visibilities of your reference images and tracing the same bract.
4. Change your CAD software camera view to a side perspective and make a reference image of the bract you are working on visible in
this orientation. Move each traced spline vertically so that they are positioned at the correct height (see Video S1 for an example).

5. Trace the vascular bundles from a side view position (yellow lines in Video S1) starting with the midrib. Trace the edge of the bract; depending on how many vascular bundles there are, you may need to add some lines between vascular bundles to accurately portray the shape.

6. Overlay the two sets of splines for each bract to allow a visual guide for positioning each point in the proper X–Y–Z spot.

7. Group sets of splines that will be used to create a Loft NURBS or mesh. Place this set into a NURBS object.

8. Repeat steps 3–7 for all bracts.

9. Position all bracts in their correct orientation to create the entire spikelet. Make modifications where virtual bracts may be overlapping by moving points in the appropriate splines. Place all objects into a single group that is named after the species.

10. Export as DXF file to allow import into Rhinoceros (Robert McNeel & Associates, Seattle, Washington, USA).

XII. Recipes—

Recipe for gFAA:
- 500 mL 100% ethanol
- 20 mL 50% glutaraldehyde
- 50 mL glacial acetic acid
- 50 mL formaldehyde
- 380 mL distilled water

Recipe for 0.5% Safranin O:
- 200 mL 50% ethanol
- 1 g Safranin O

Allow to mix for 48 h
Filter through a coffee filter prior to use

Recipe for 1% Fast Green:
- 200 mL 95% ethanol
- 2 g Fast Green

Allow to mix for 24 h
Filter through a coffee filter prior to use