Active xylem staining

The technique described in this file was reported in brief in the following studies:

Jacobsen et al. 2007 Ecological monographs 77: 99-115

Jacobsen & Pratt 2012 New Phytologist 194: 982-990

And is described in more detailed in:

Jacobsen et al., in press (pdf will be posted soon)

Materials

For the staining manifold:
Tygon 3601 tubing (1/4 in ID, 1/16 in wall)
Tygon 3603 tubing (1/2 in ID, 1/8 in wall)
Nalgene Three-way stopcock PP/TFE 6470-0004 4mm size
Nalgene Two-way stopcock PP/TFE 6460-0004 4mm size
Hose clamps:
For narrow diameter tubing: Cole-Parmer Snp-4 hose clamp (06832-04)
For wider diameter tubing (areas of the system where samples are loaded): Cole-Parmer Snp-12 hose clamp (06832-12)
Some miscellaneous sizes of latex tubing may be needed to make grommets that will fit a wide range of sample diameters.
Test tubes, test tube rack.

For making the dye solution:
Crystal violet
HCl
pH meter
Stir plate

For filtering the dye solution:
90mm Glass Microanalysis Holder and Glass Frit Support
Osmonics, Inc. MAGNA Nylon Supported Plain 0.1 micron, 90mm filters (Catalog: R01AP09025)
Faucet aspirator
Erlenmeyer flask with upper side arm
Some additional miscellaneous glassware may be required for mixing or storing the dye solution.

Making the dye solution

Start with 1 L deionized water in a glass beaker

Adjust the water to a pH of 2.0 using HCl

Add enough powdered crystal violet stain to make a 0.1% (w/v) solution:

i.e. for 1 L of water add 1 g of crystal violet

(1 g / 1000 mL = 0.001 = 0.1% w/v)

(Note: the powder will appear metallic green when dry but will become very dark purple when in solution)

Place the solution on a stir plate and allow it to mix on medium speed for ~ 2 hr

The dye solution may be stored for several months if placed into a sealed glass container and refrigerated.

<u>The dye solution must be filtered before each use (see the next page)…</u>

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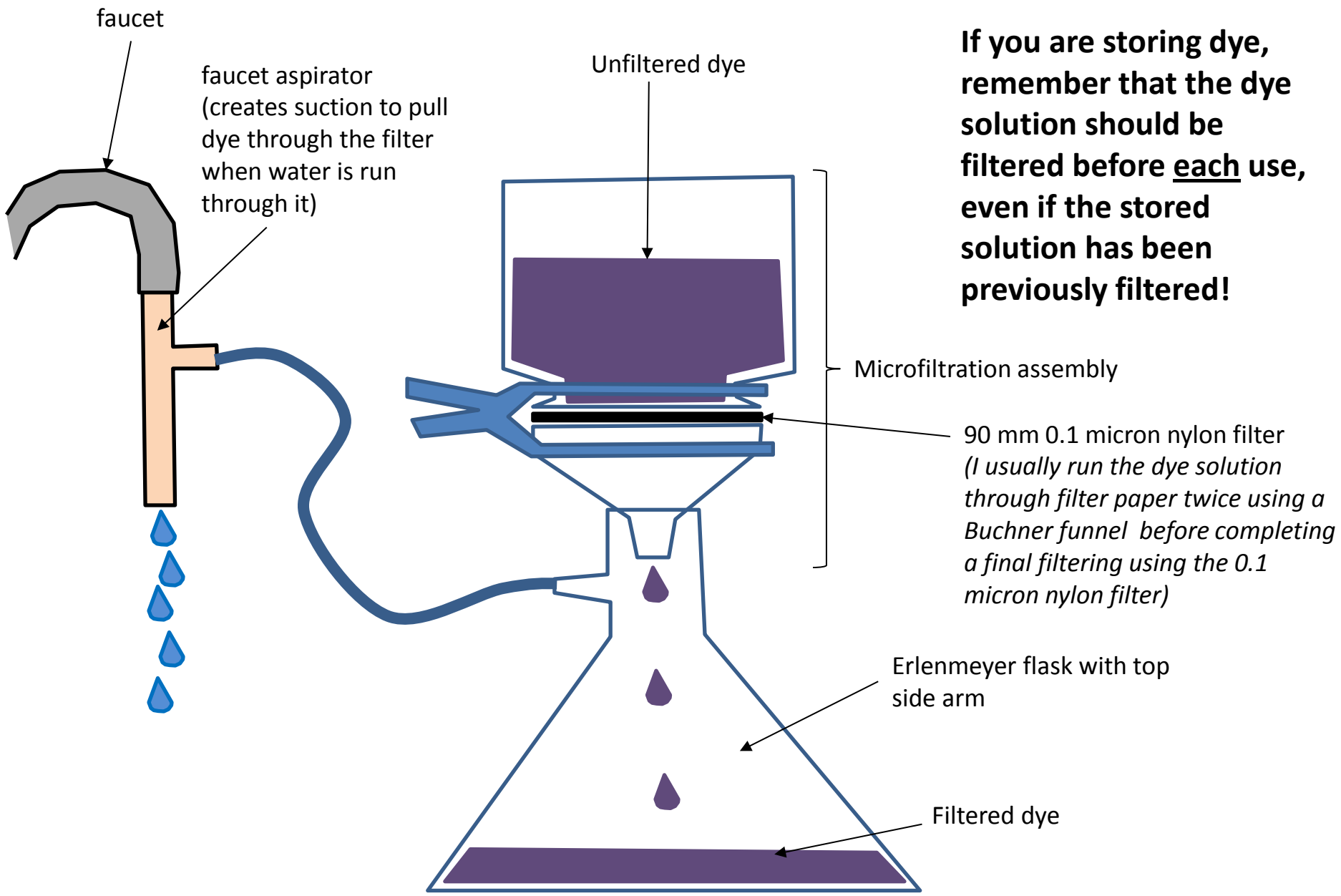
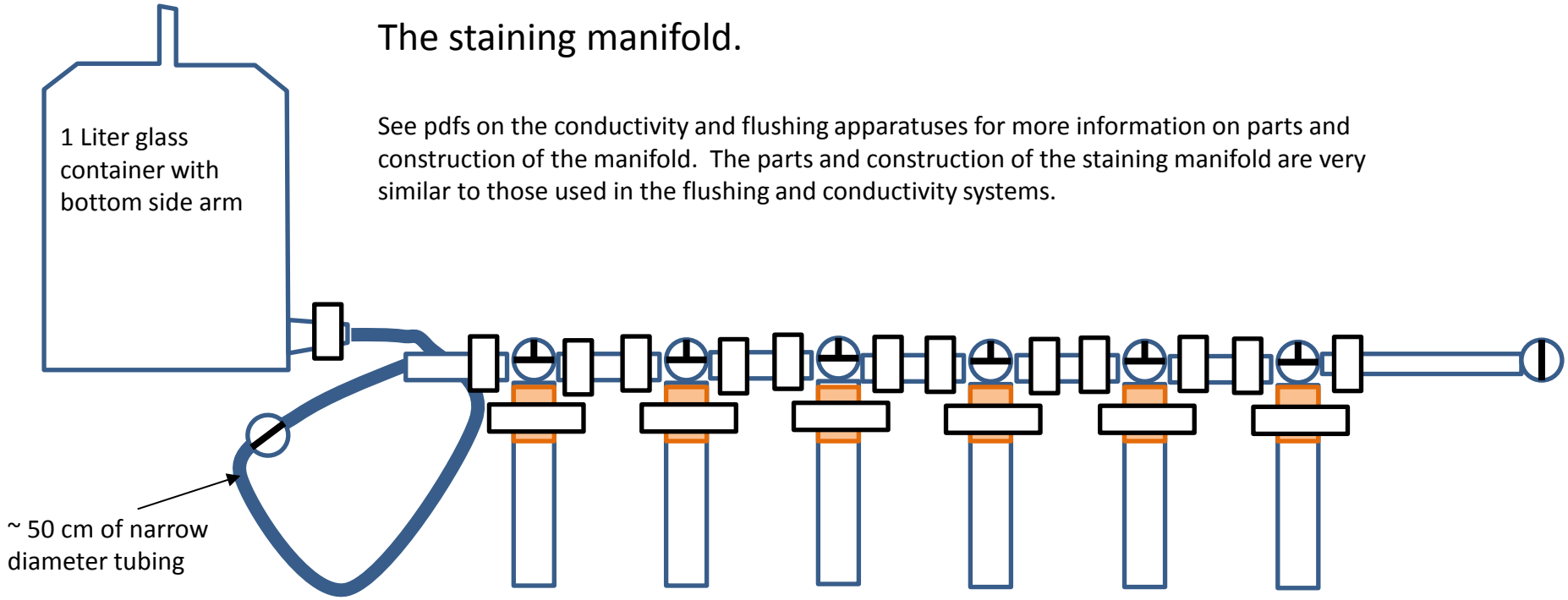
Filtering the dye solution



If you are storing dye, remember that the dye solution should be filtered before <u>each</u> use, even if the stored solution has been previously filtered!

The staining manifold.

See pdfs on the conductivity and flushing apparatuses for more information on parts and construction of the manifold. The parts and construction of the staining manifold are very similar to those used in the flushing and conductivity systems.



Depending on what you are trying to stain for, you may want to flush your samples prior to staining them. If so, please refer to the flushing methods pdf on my webpage for more information. (http://www.csub.edu/~ajacobsen/Flushing%20Methods.pdf)

Note: If you are trying to identify living vs. non-living vessels, flushing can disrupt the perforation partitions of still living vessels precluding their identification. For these samples, I flush 20 cm segments and then excise only the central portion for staining.

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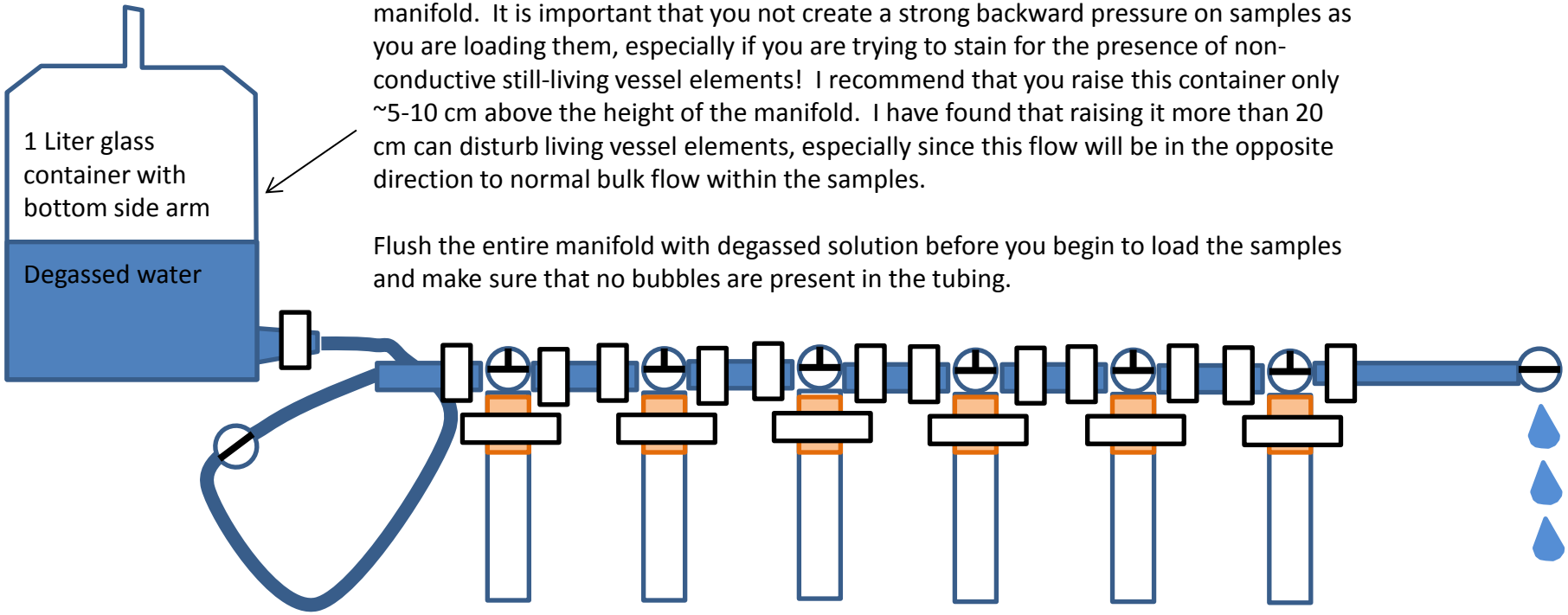
When loading samples…



Raise the beaker with degassed solution slightly above the height of the staining manifold. It is important that you not create a strong backward pressure on samples as you are loading them, especially if you are trying to stain for the presence of non-conductive still-living vessel elements! I recommend that you raise this container only ~5-10 cm above the height of the manifold. I have found that raising it more than 20 cm can disturb living vessel elements, especially since this flow will be in the opposite direction to normal bulk flow within the samples.

Flush the entire manifold with degassed solution before you begin to load the samples and make sure that no bubbles are present in the tubing.

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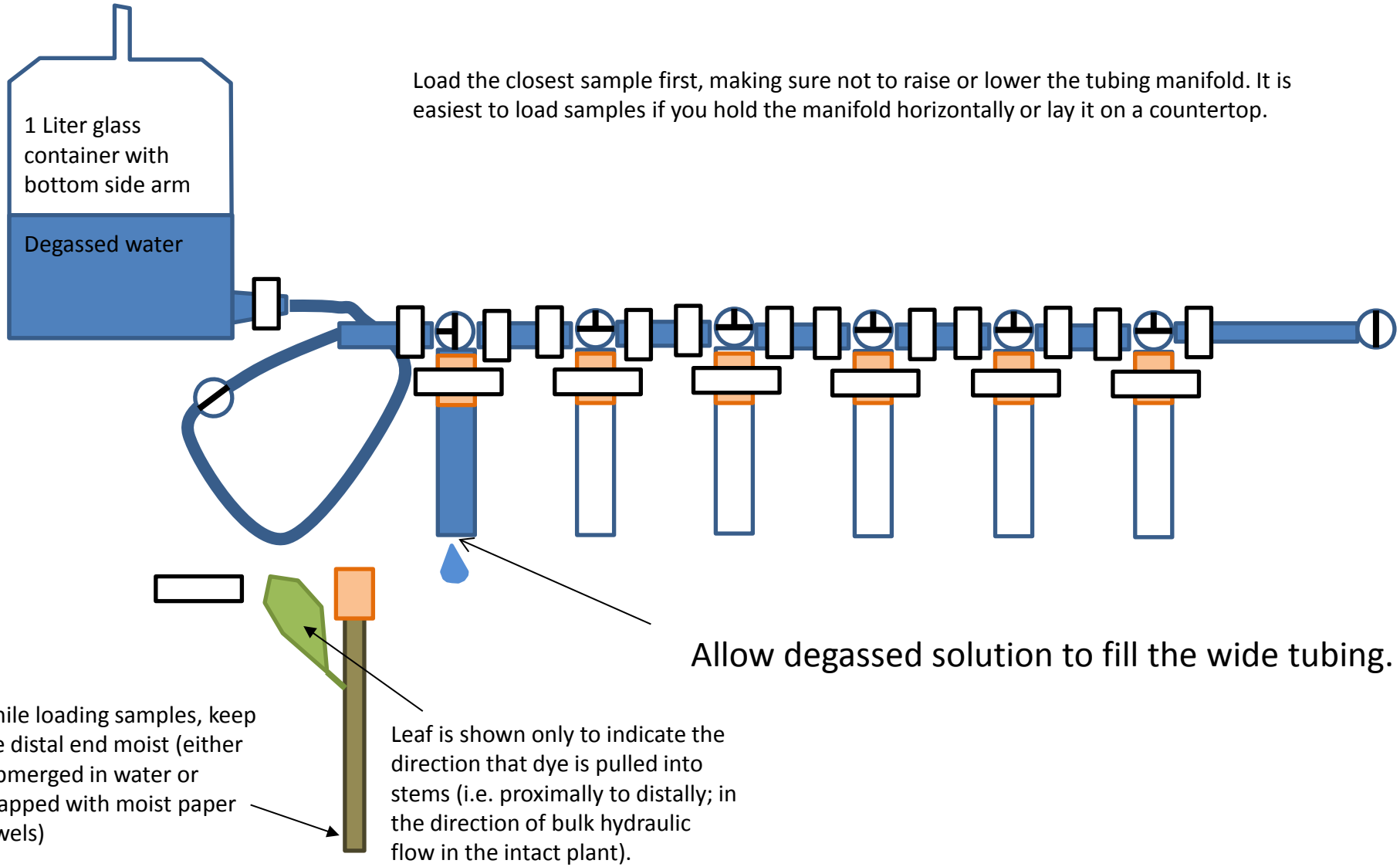
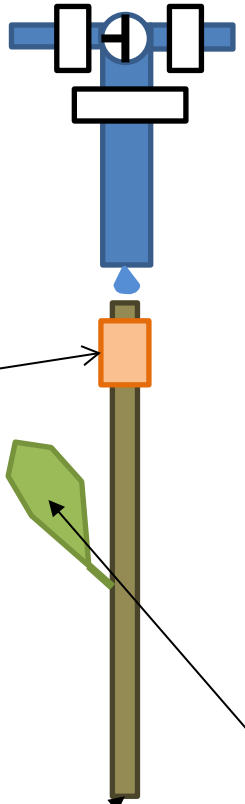
When loading samples…



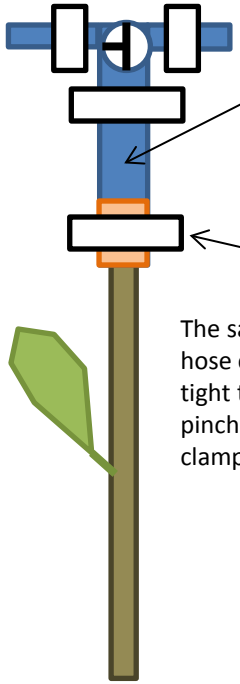
Loading samples:



Latex grommets are placed on the ends of a sample.

While loading samples, keep the distal ends of all samples moist (either submerged in water or wrapped with moist paper towels)

Leaf is shown only to indicate the direction that dye is pulled into stems (i.e. proximally to distally; in the direction of bulk hydraulic flow in the intact plant).



Make sure that there are no bubbles in the tubing or the "pull" of the dye will be interrupted.

The sample is clamped into the tubing system using hose clamps (tight enough to prevent leaks but not so tight that the stem is damaged or any xylem is pinched—i.e. you want to avoid both leaks and clamping damage).

I usually stain samples that are 10-14 cm in length. Staining times and the tension used to pull dye may need to be altered if you use longer samples or if you are working with species with very narrow vessels.

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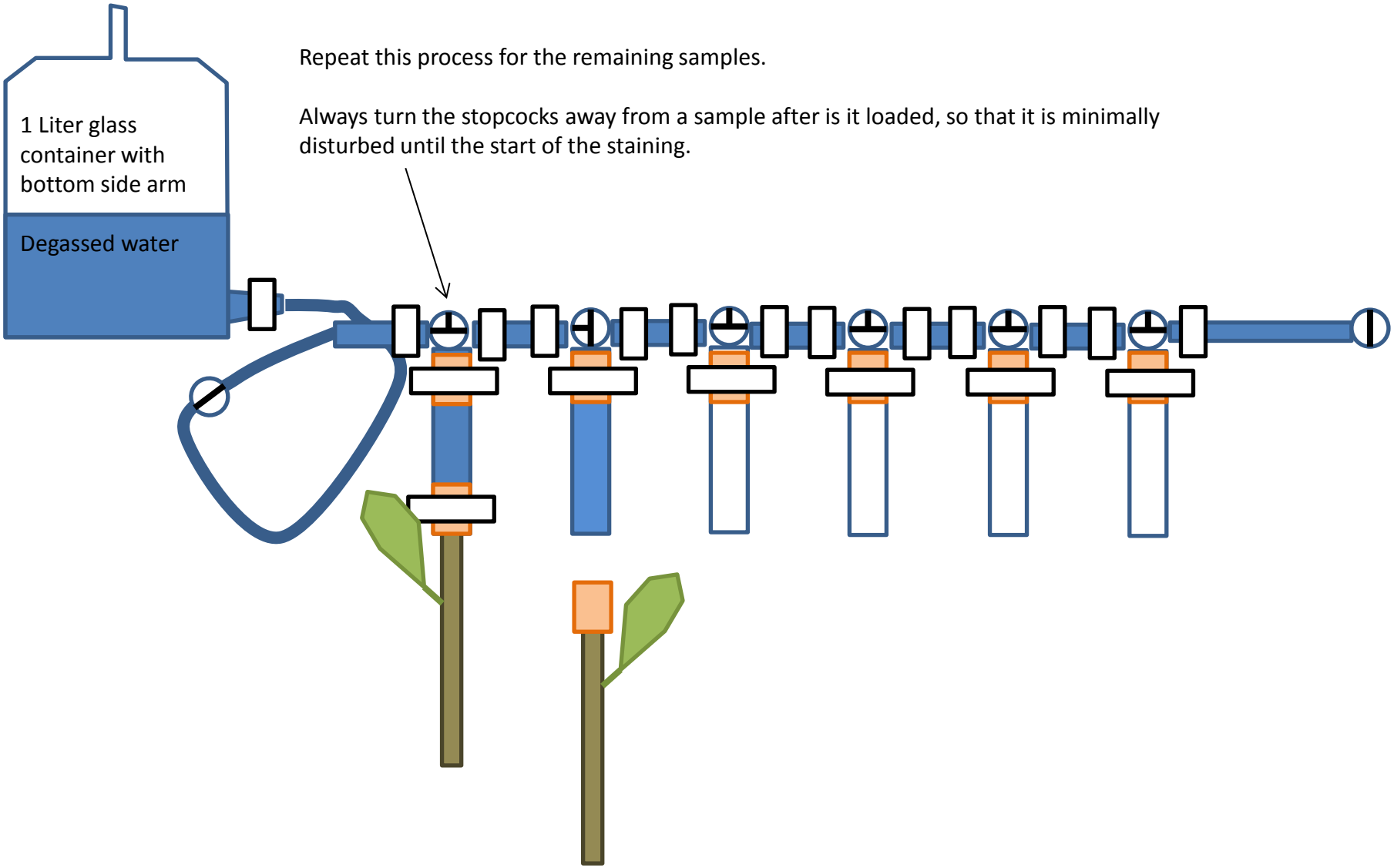
When loading samples…



Repeat this process for the remaining samples.

Always turn the stopcocks away from a sample after is it loaded, so that it is minimally disturbed until the start of the staining.

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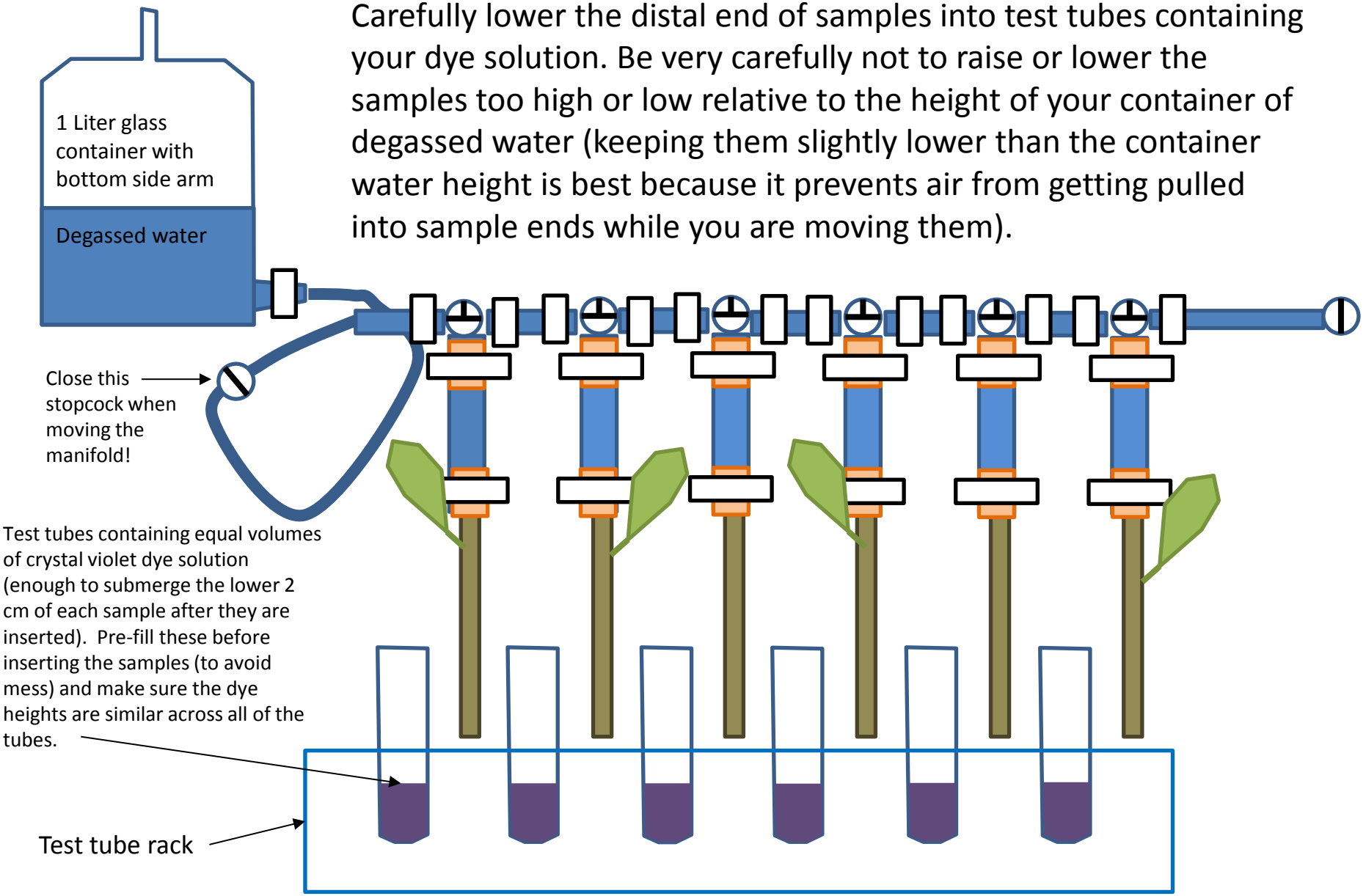
When loading samples…

Carefully lower the distal end of samples into test tubes containing your dye solution. Be very carefully not to raise or lower the samples too high or low relative to the height of your container of degassed water (keeping them slightly lower than the container water height is best because it prevents air from getting pulled into sample ends while you are moving them).



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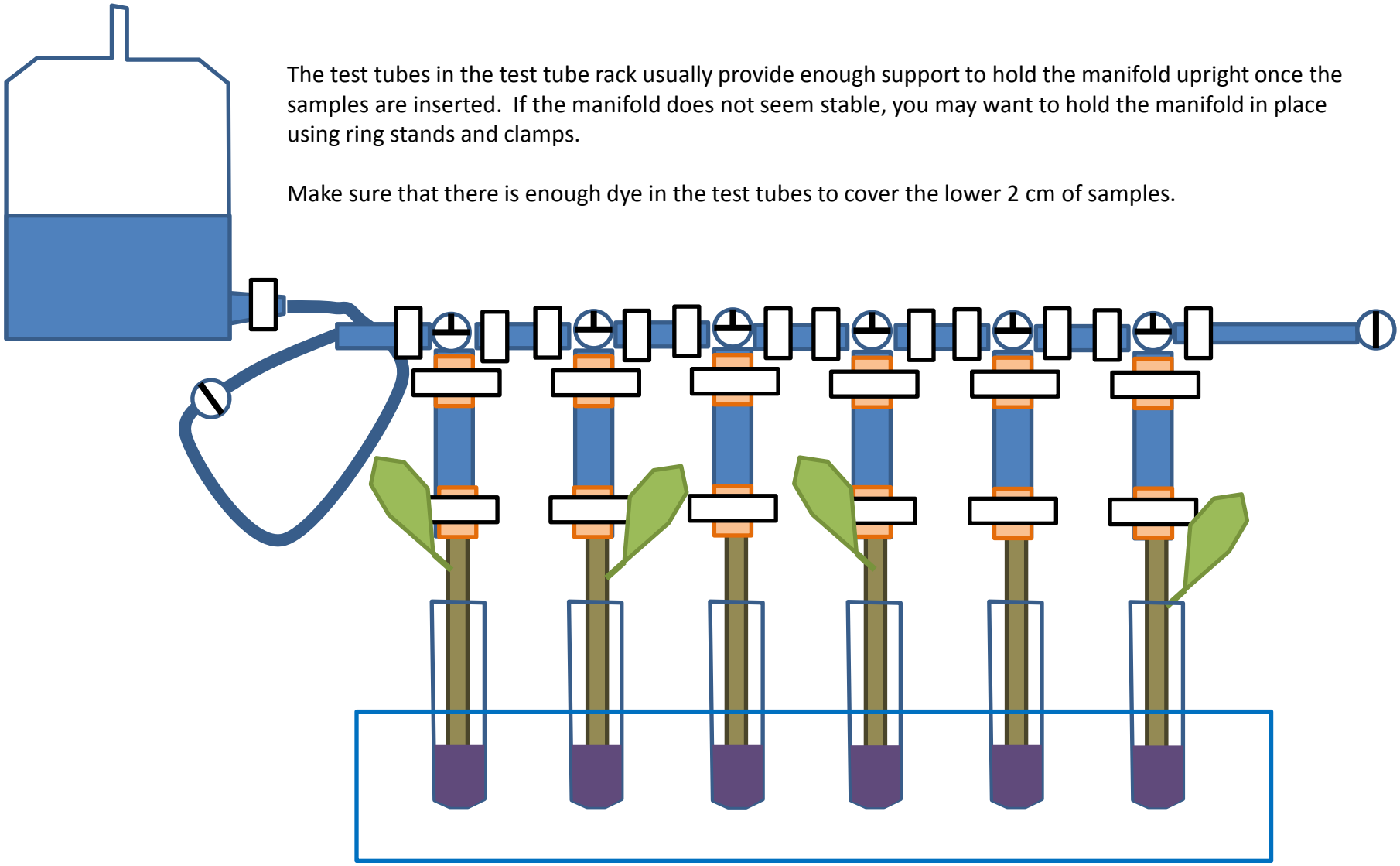
When loading samples…

The test tubes in the test tube rack usually provide enough support to hold the manifold upright once the samples are inserted. If the manifold does not seem stable, you may want to hold the manifold in place using ring stands and clamps.

Make sure that there is enough dye in the test tubes to cover the lower 2 cm of samples.



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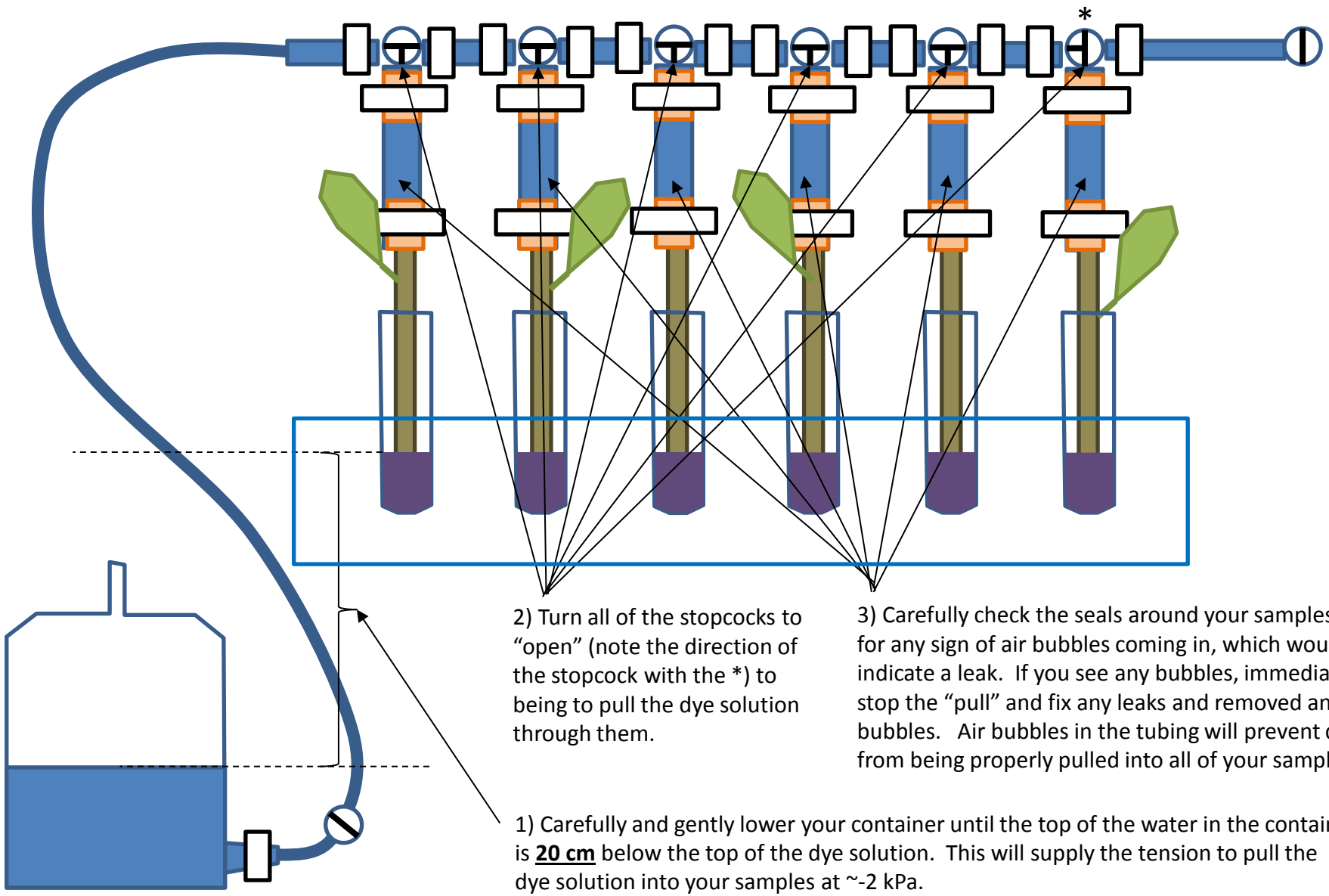
To begin pulling dye into your samples…



2) Turn all of the stopcocks to "open" (note the direction of the stopcock with the *) to being to pull the dye solution through them.

3) Carefully check the seals around your samples for any sign of air bubbles coming in, which would indicate a leak. If you see any bubbles, immediately stop the "pull" and fix any leaks and removed any bubbles. Air bubbles in the tubing will prevent dye from being properly pulled into all of your samples!

1 Liter glass container with bottom side arm
Degassed water

1) Carefully and gently lower your container until the top of the water in the contain is **<u>20 cm</u>** below the top of the dye solution. This will supply the tension to pull the dye solution into your samples at ~-2 kPa.
(I keep the samples on the countertop and put the water container on a box (adjusted to be at the proper height) located in a pulled out drawer below the counter height).

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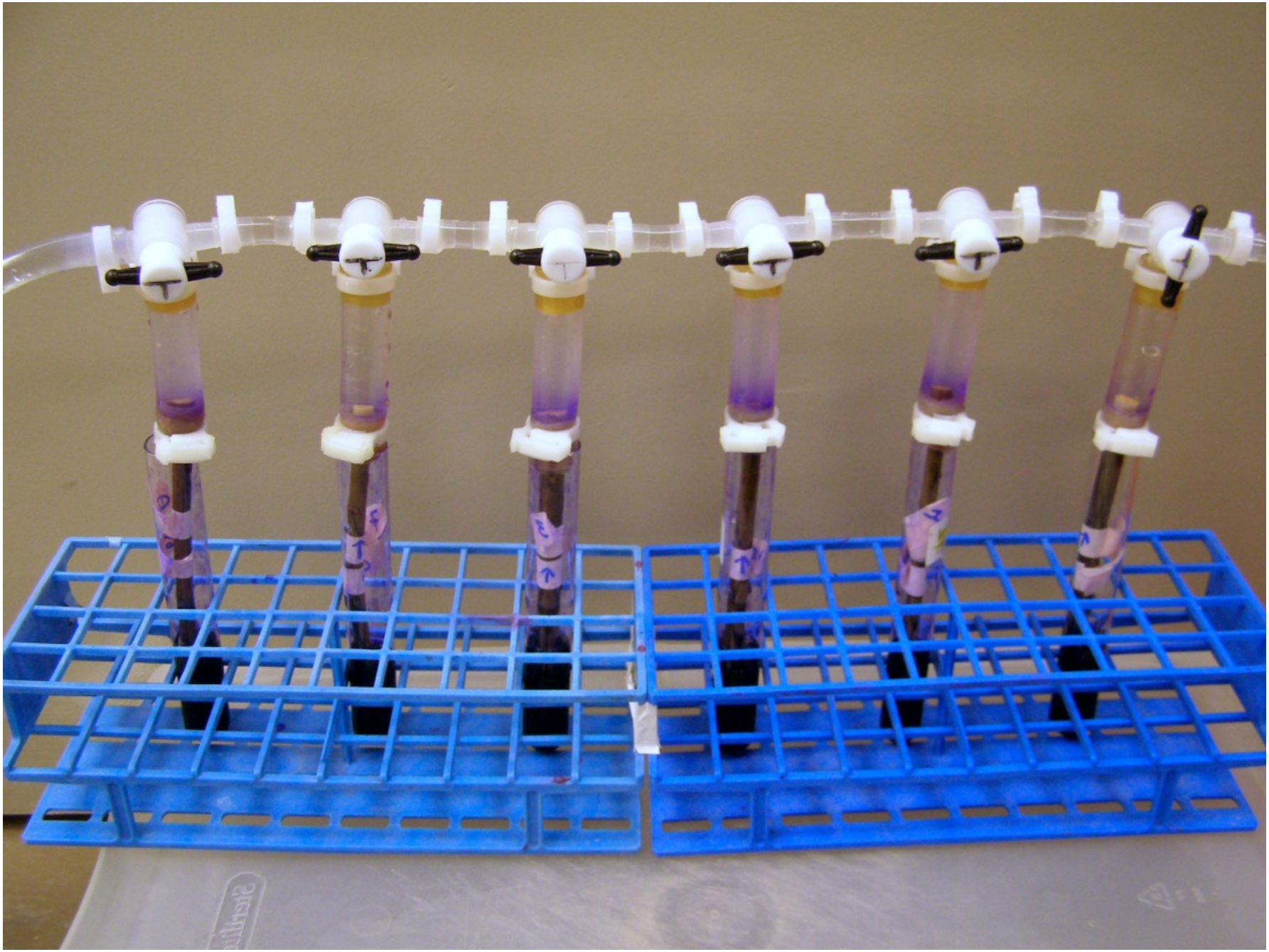

Stain for ~20-30 min, until dye can be seem emerging from the tops of samples.
(You may need to stain for longer for species with narrow vessels, tracheids, or for longer samples)

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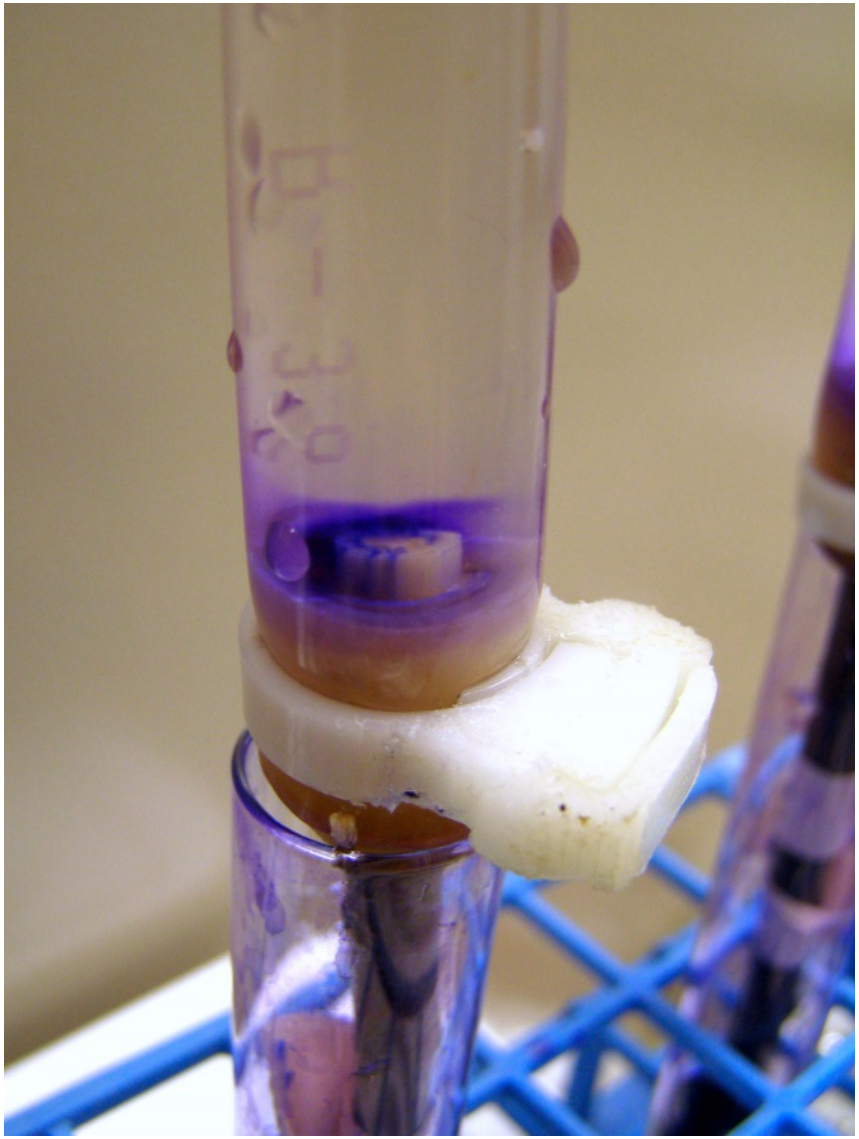
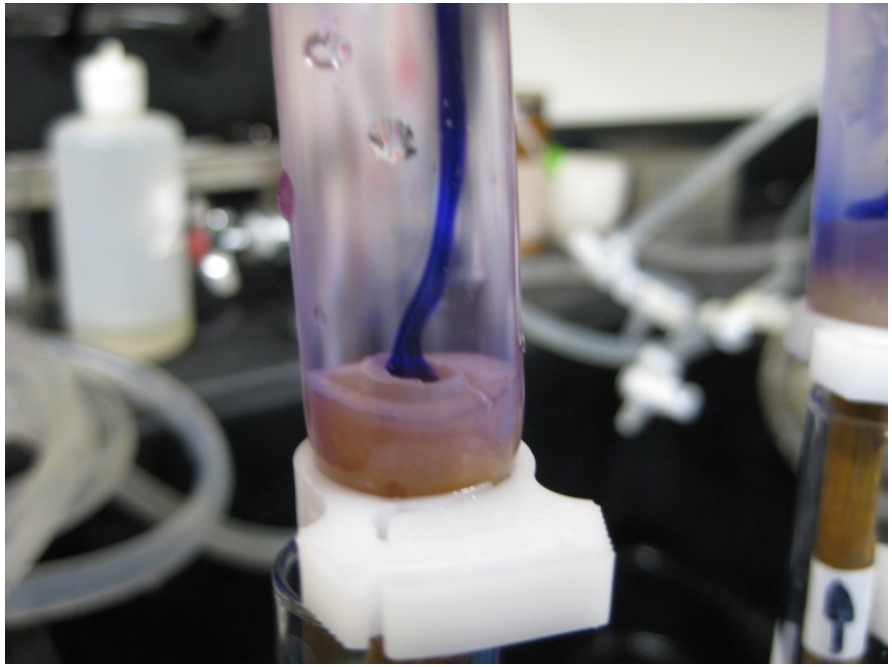
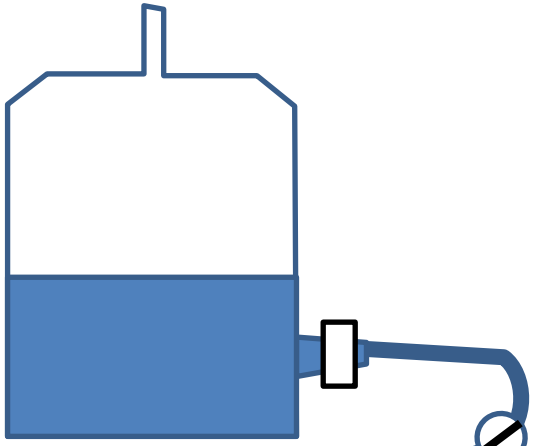


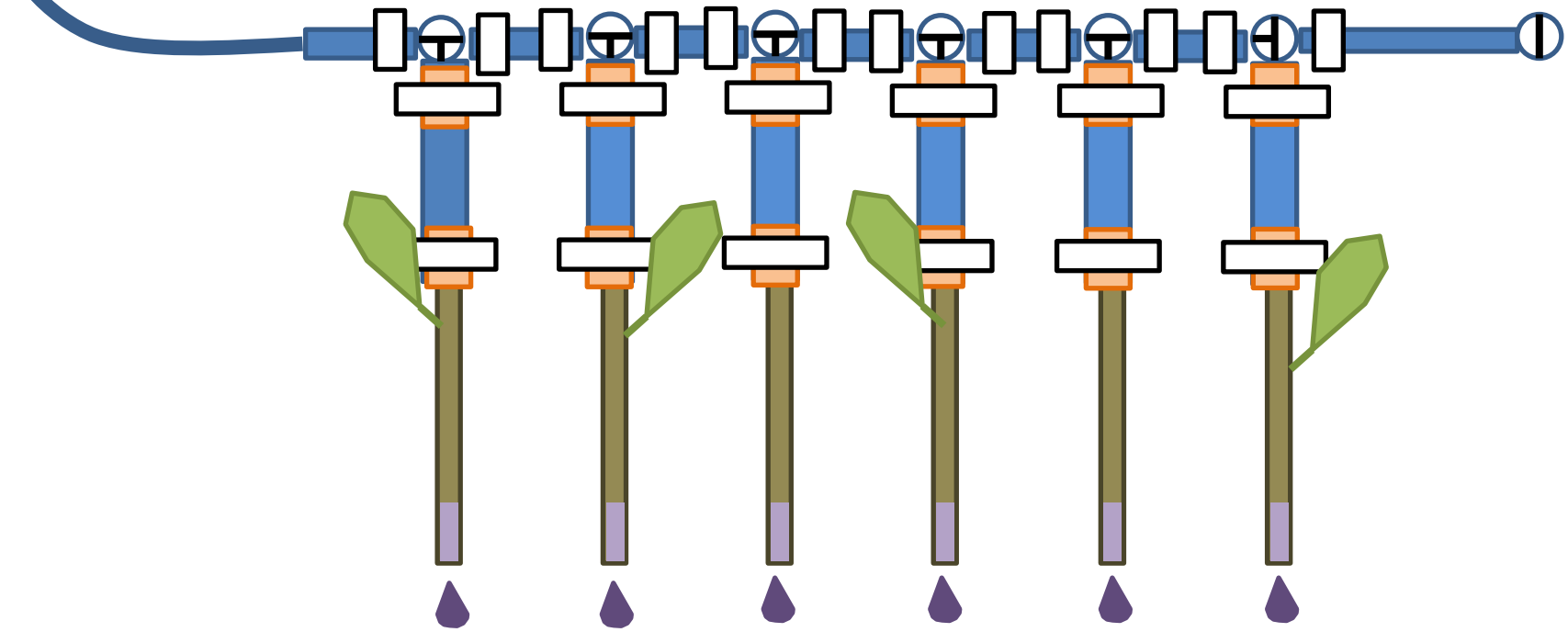




After your dye has been pulled through, carefully remove your samples from the test tubes containing dye solution (again, being careful to avoid big changes in height between the samples and the height of water in the container).

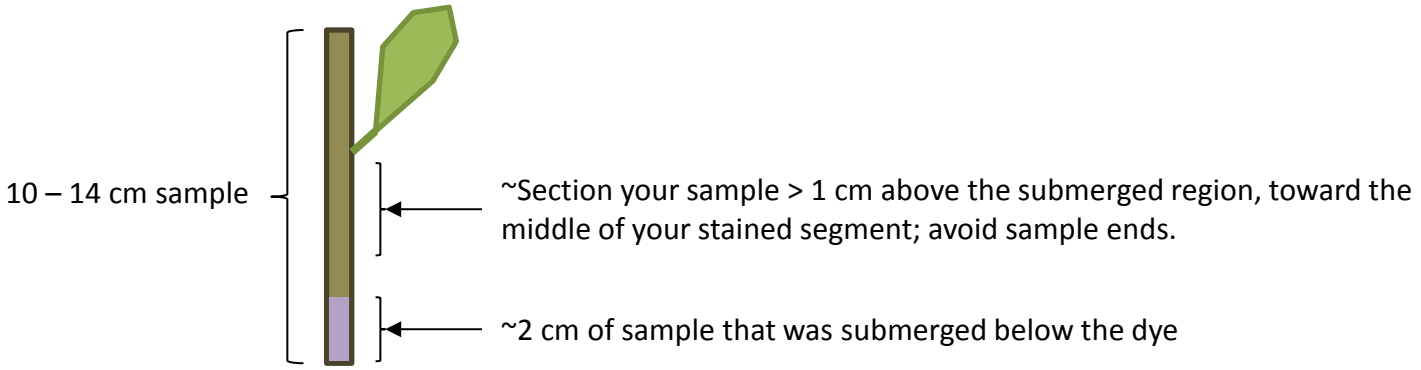
Lower your samples to 20 cm <u>below</u> the height of the container solution and allow dye to flush out (in the opposite direction to the direction it was pulled it) for ~ 10-15 min. This will remove some of the dye solution from vessel lumens and will minimize “bleeding” of dye into cells adjacent to the xylem and cross-contamination when you section later.

(I usually place the water container on the counter to remove the samples from the dye (this is the same height that the samples are at) and then I lower the samples into a sink and lay them flat during this stem. I let them drip down the drain and occasionally rinse them with tap water to remove excess dye from the ends).



Sectioning samples…

The dye will start to bleed into adjacent tissues as soon as you are done staining. You must either section and photograph your samples immediately or freeze your samples until you are ready to section them. If you freeze them, it is best to then section later while they are still frozen. Waiting for them to thaw will allow the dye to spread.



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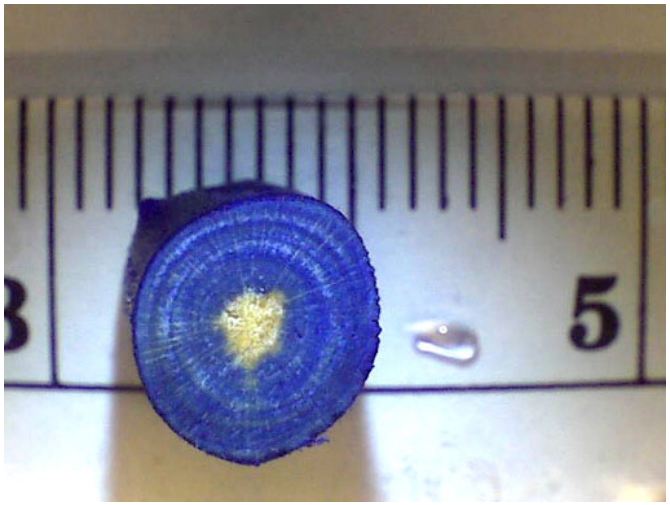
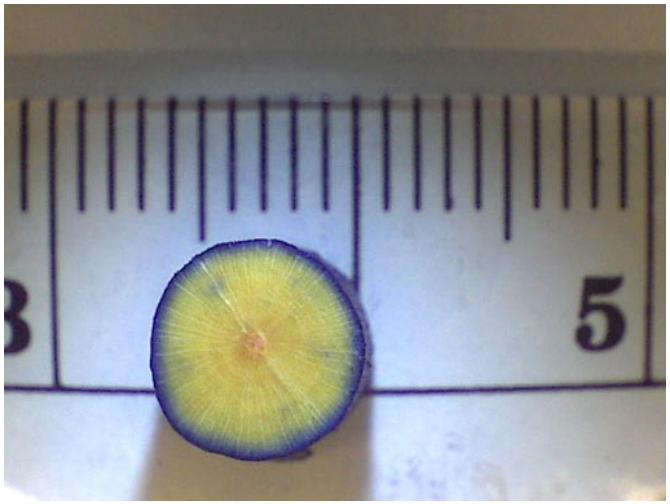
Examples of stained samples used to determine…

Sapwood area (purple area is conductive):





Presence of conductive vs. non-conductive and/or living vessels (purple vessels are conductive):