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Measuring the hydraulic conductivity of plant samples

Materials

= Tygon 3601 tubing (1/4 in ID, 1/16 in wall) [this composes the majority of the tubing in the system]


= Tygon 3603 tubing (1/2 in ID, 1/8 in wall) [this composes the wider tubing that samples are loaded into with the aid of latex tubing grommets]


= 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)

Plastic tubing connectors 5-1 Connectors (58018) – these are used in the system to connect the places in the conductivity and flushing manifolds where samples are usually loaded. When the system is being bleached, these connectors close the openings and allow for all of the tubing and connections to be bleached.

In-line filter Calyx Capsule Nylon, 0.1 micron, 3/8 in barb, DCN010006

Some miscellaneous sizes of latex tubing may be needed to make grommets that will fit a wide range of sample diameters.

Extra hose clamp sizes are probably needed in some parts of the system (i.e. connected to the captive air tank and the filter)—there are sets available that contain a few pieces of each of many different sizes.

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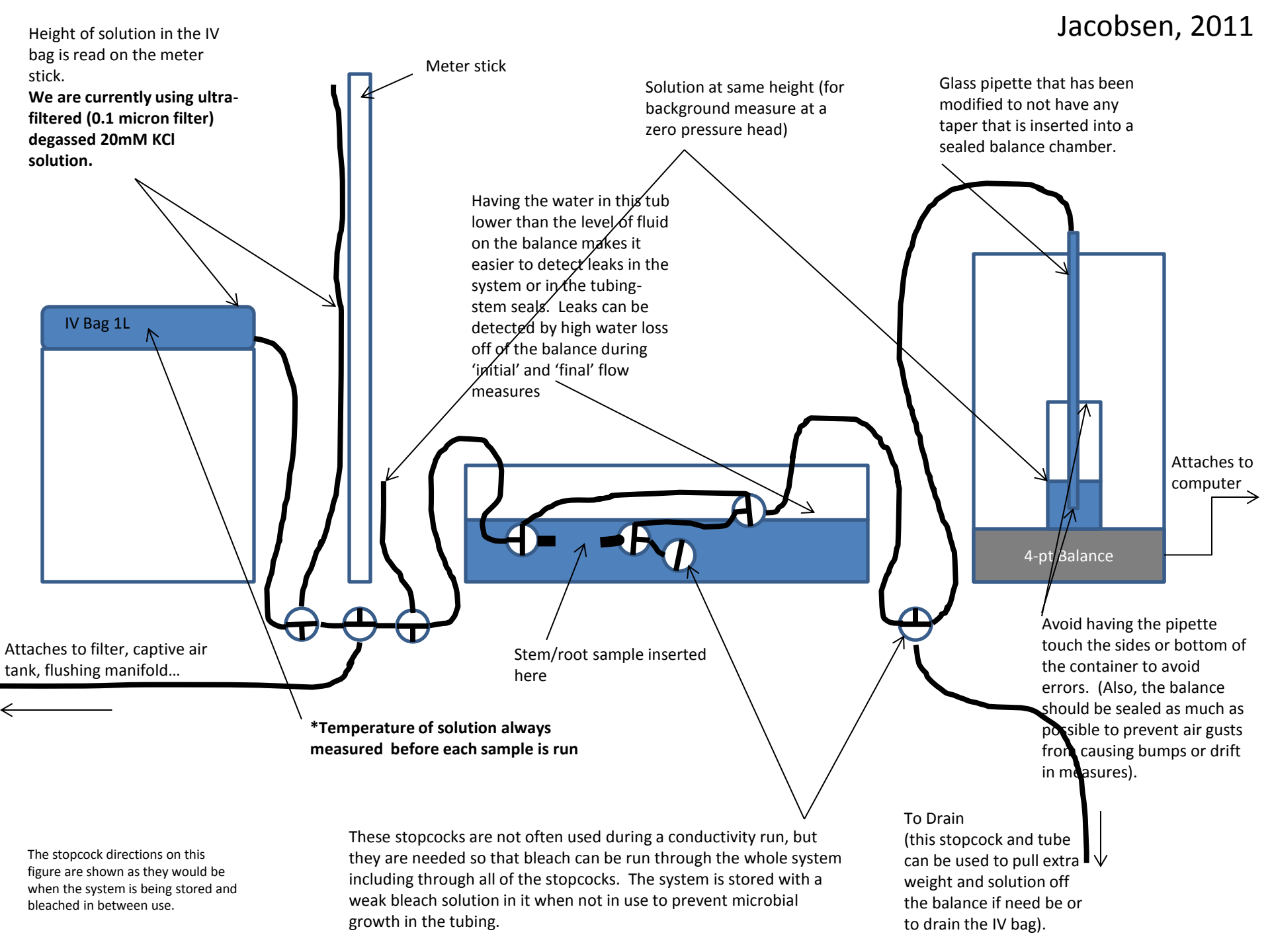
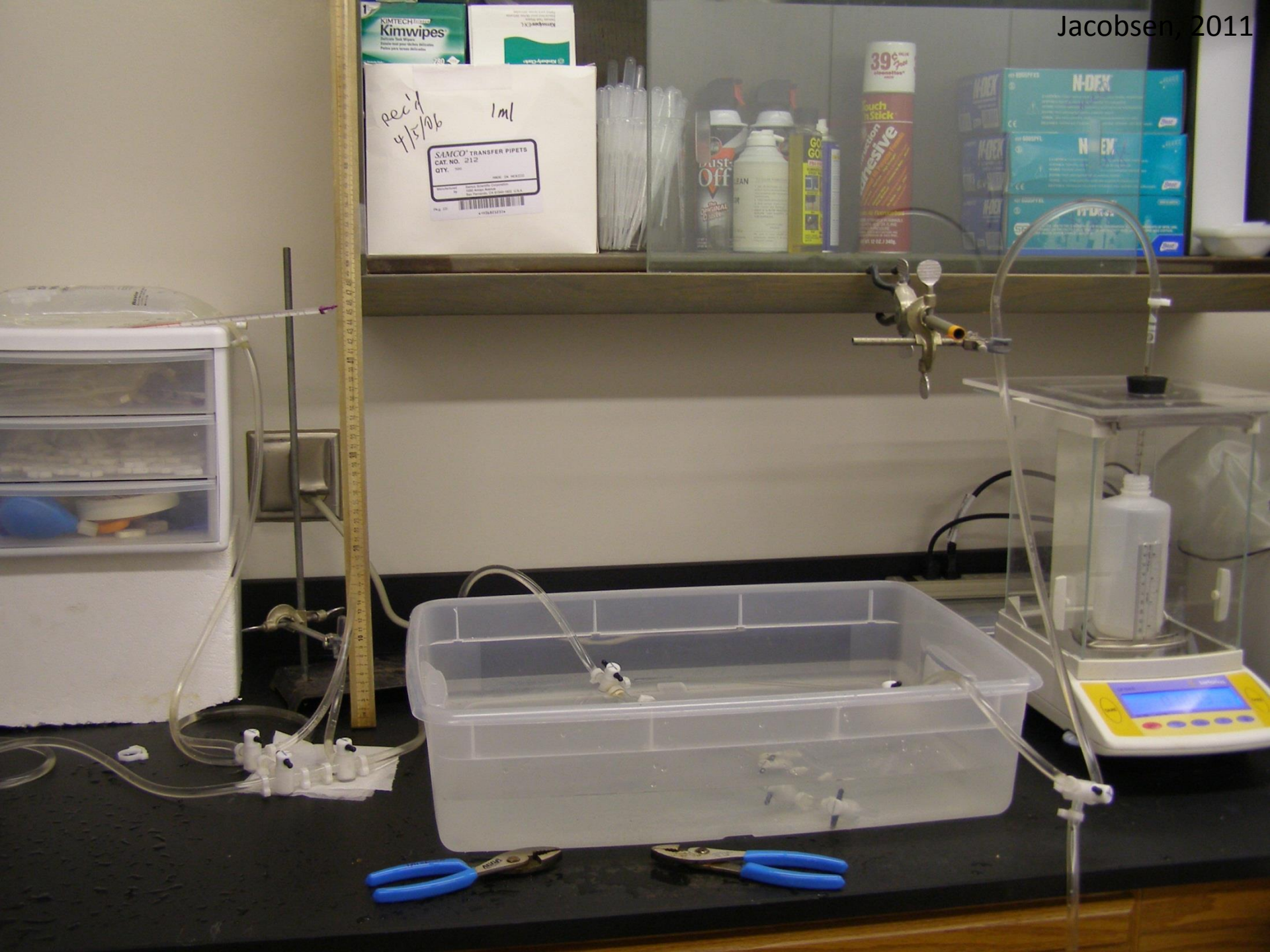


These stopcocks are not often used during a conductivity run, but they are needed so that bleach can be run through the whole system including through all of the stopcocks. The system is stored with a weak bleach solution in it when not in use to prevent microbial growth in the tubing.

The stopcock directions on this figure are shown as they would be when the system is being stored and bleached in between use.



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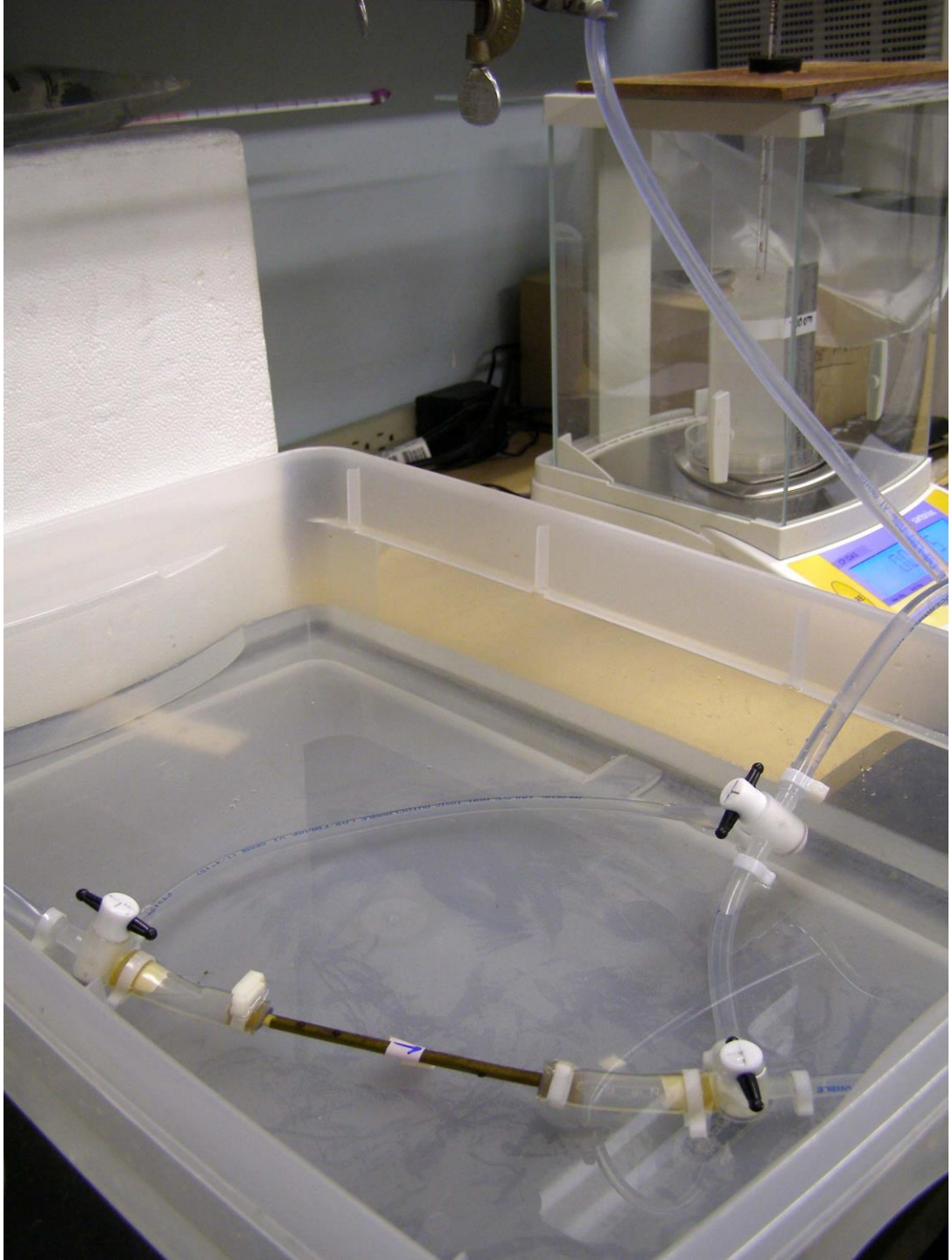
Kimwipes
Kimwipes
N-DEX
CAUTION



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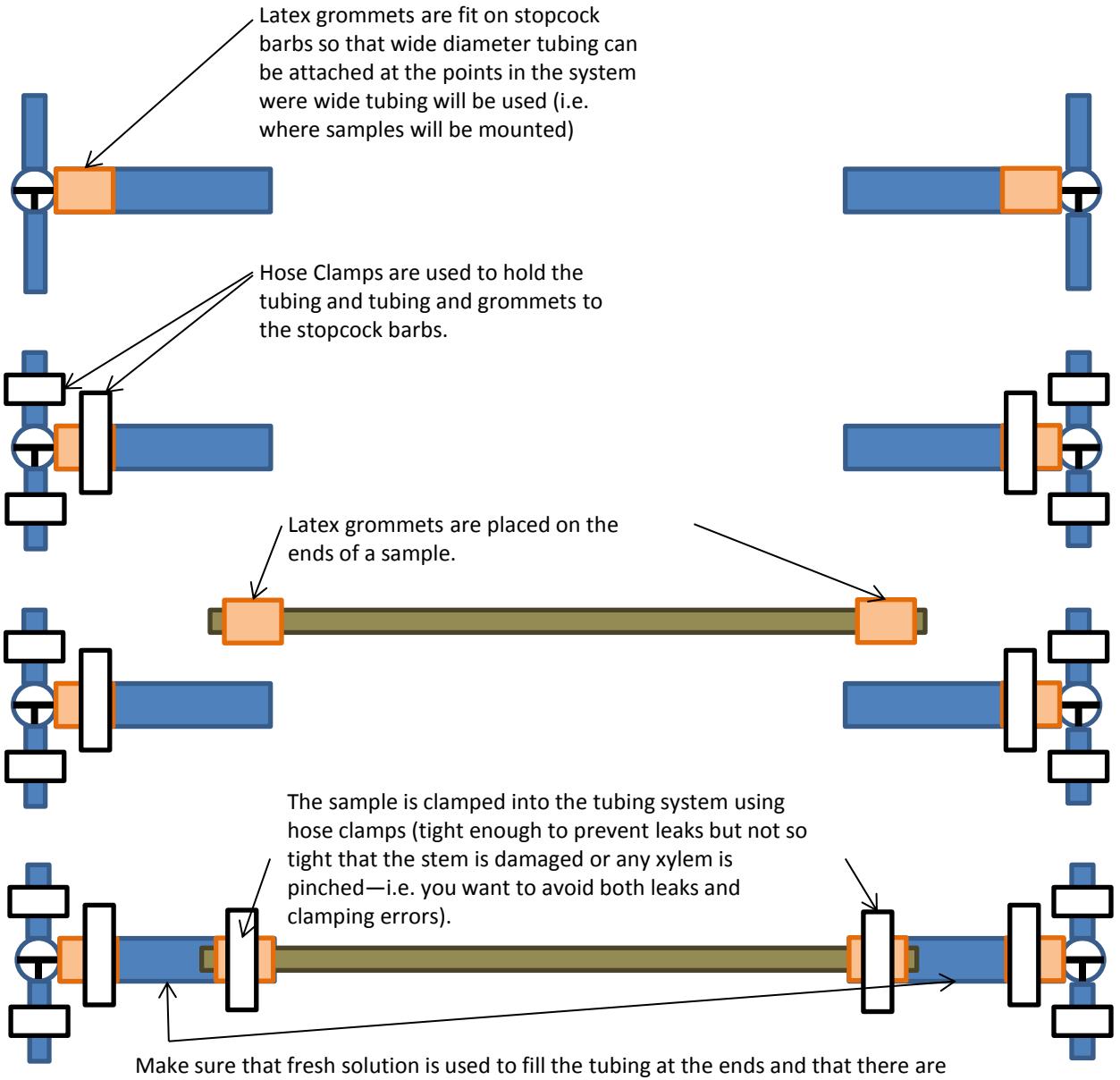
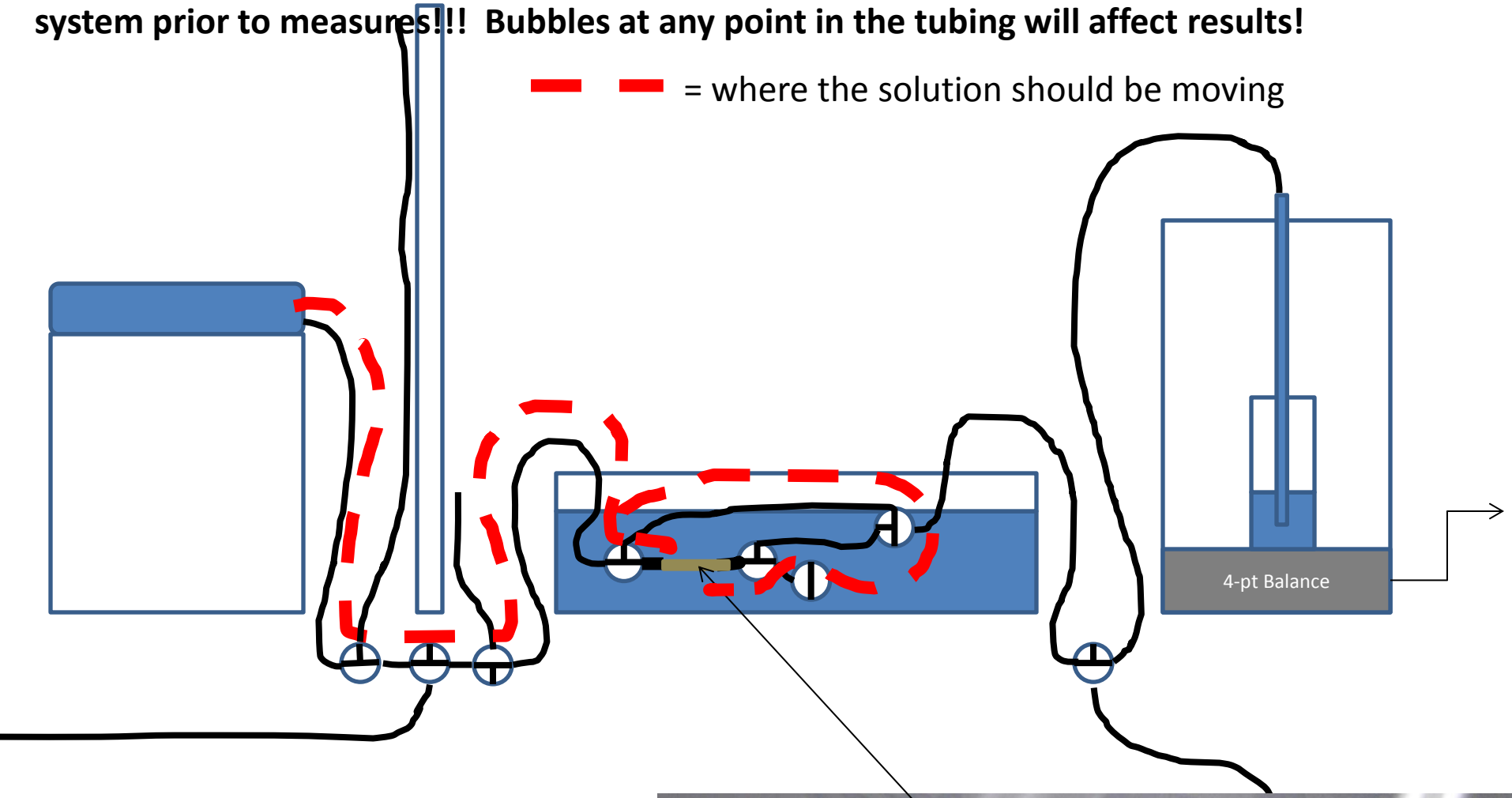
Assembling the system and loading samples:

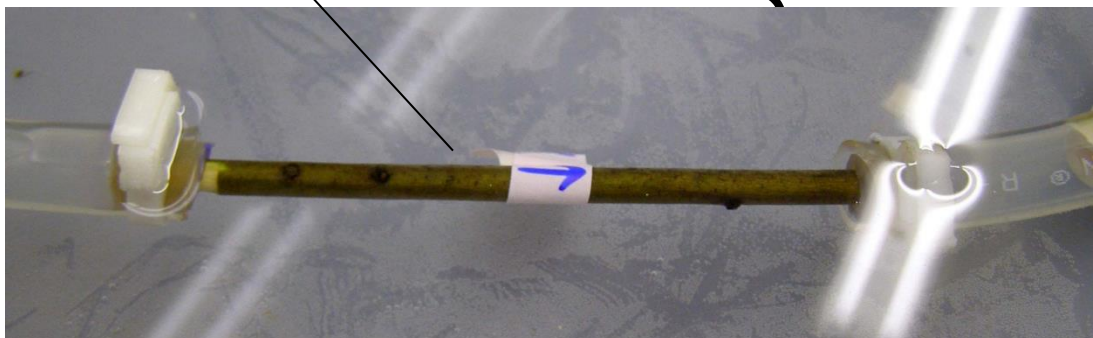


Direction of stopcocks to load a stem—**NOTE: All bubbles must be removed from the tubing system prior to measures!!! Bubbles at any point in the tubing will affect results!**

= where the solution should be moving



Stem is held in place with latex grommets (latex tubing of many different diameters is cut into small pieces so that samples of any size --usually 6-8 mm in diameter--can be fit into the tubing).
Samples are held in place using teflon hose clamps.
NOTE: Solution is always moved through the stem in the same direction as flow would be occurring in the intact plant. See the arrow placed on the stem to the right to indicate the direction of flow.



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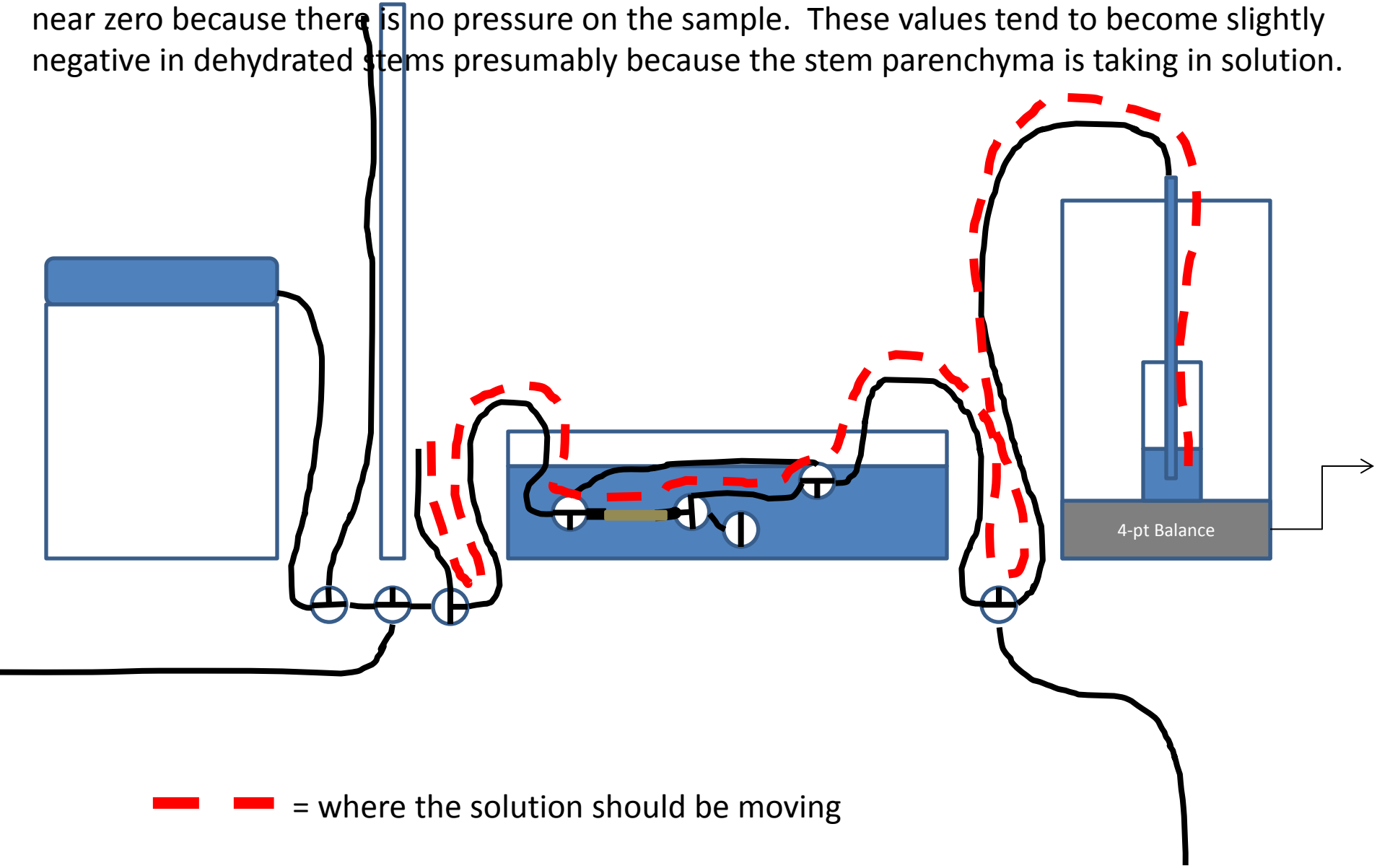
Direction of stopcocks run initial and final (background) flows—these flow measures should be near zero because there is no pressure on the sample. These values tend to become slightly negative in dehydrated stems presumably because the stem parenchyma is taking in solution.



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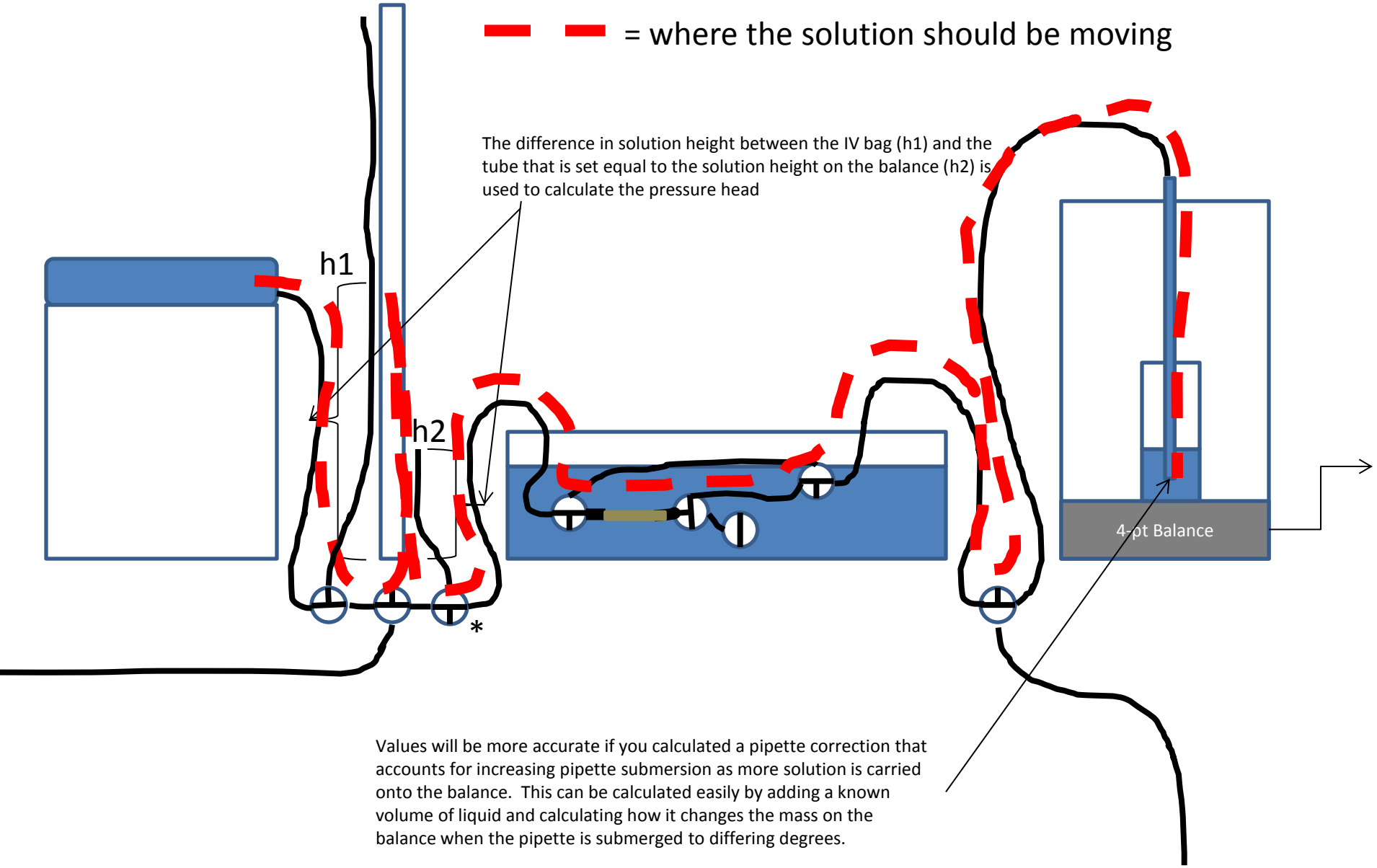
Direction of stopcocks to measure flow through a stem with pressure



Values will be more accurate if you calculated a pipette correction that accounts for increasing pipette submersion as more solution is carried onto the balance. This can be calculated easily by adding a known volume of liquid and calculating how it changes the mass on the balance when the pipette is submerged to differing degrees.

A note on initial and final flow measures—

I have received several emails asking questions about why we measure initial and final flows through the system with a pressure head of zero. The most common questions seem to revolve around whether taking these measures "saves" you from potential leaks in the system (mostly stopcocks that might leak) or from leaks around where the sample fits into the tubing system.

The short answer to this question is "no."

The water level in the tub containing the sample should always be kept lower than the "zero" level so that you can easily detect leaks in the system as large negative initial and final measures. A leak in the system will prevent you from being able to accurately measure flow through the stem (because you won't know how much solution you are losing through the leak that is not ending up on the balance and the leak most likely will be more extreme when the system is under pressure). If you detect a leak in the system, the most common location for a leak is usually around the sample and you should try taking the sample out and reloading it into the system to see if that fixes the leak. If that doesn't work, slowly work your way through the system, testing that each stopcock is able to prevent flow and is not leaking. Replace any leaking stopcocks (they tend to last for several months, but stopcocks definitely have a "life span" in the system).

At low flows and especially as stems become more dry, initial and final measures (which are usually slightly negative) are a way to account for the small amount of solution that is being taking in by tissues within a sample during the course of the measurement. They are also a way to account for small fluxes that may occur within the system. Initial and final measures may not be as important when you are measuring things with very high flow rates, but they become very important when samples have low flow rates such as we find in many desert plants and some chaparral shrubs. Finally, opening the system to the "zero" pressure heads is a way to equalize the system after pressure has developed in some of the tubing sections during the loading of samples into the system (during which time the pressure head is used to flush the tubing with fresh solution, especially in the segments of the tubing on either side of the sample).

Flow is calculated as:

Fstem – (Finitial + Ffinal)

(this is to take into account the influence of background flows on measures)
(these values should already be pipette corrected)
(also, remember to include density changes in the solution for different temperatures—this is why the solution temperature is always measured)

Hydraulic conductance, K_h, (m^4 MPa^{-1} s^{-1}) can be calculated using the following equation:

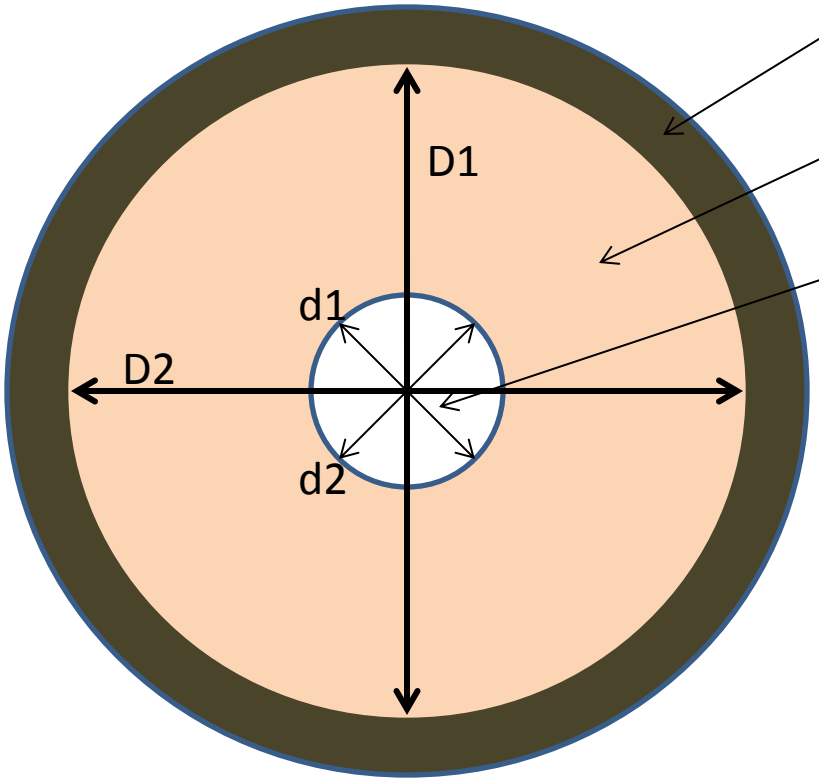
$$\mathbf{K_h = q\ (dp/dx)^{-1}}$$

Where *q* is the rate of flow (m^3 s^{-1}) and (*dp/dx*) is the pressure gradient (MPa m^{-1}; calculated from the pressure head height relative to the height of solution on the balance and the length of the sample).

K_h is also reported as kg m MPa^{-1} s^{-1}

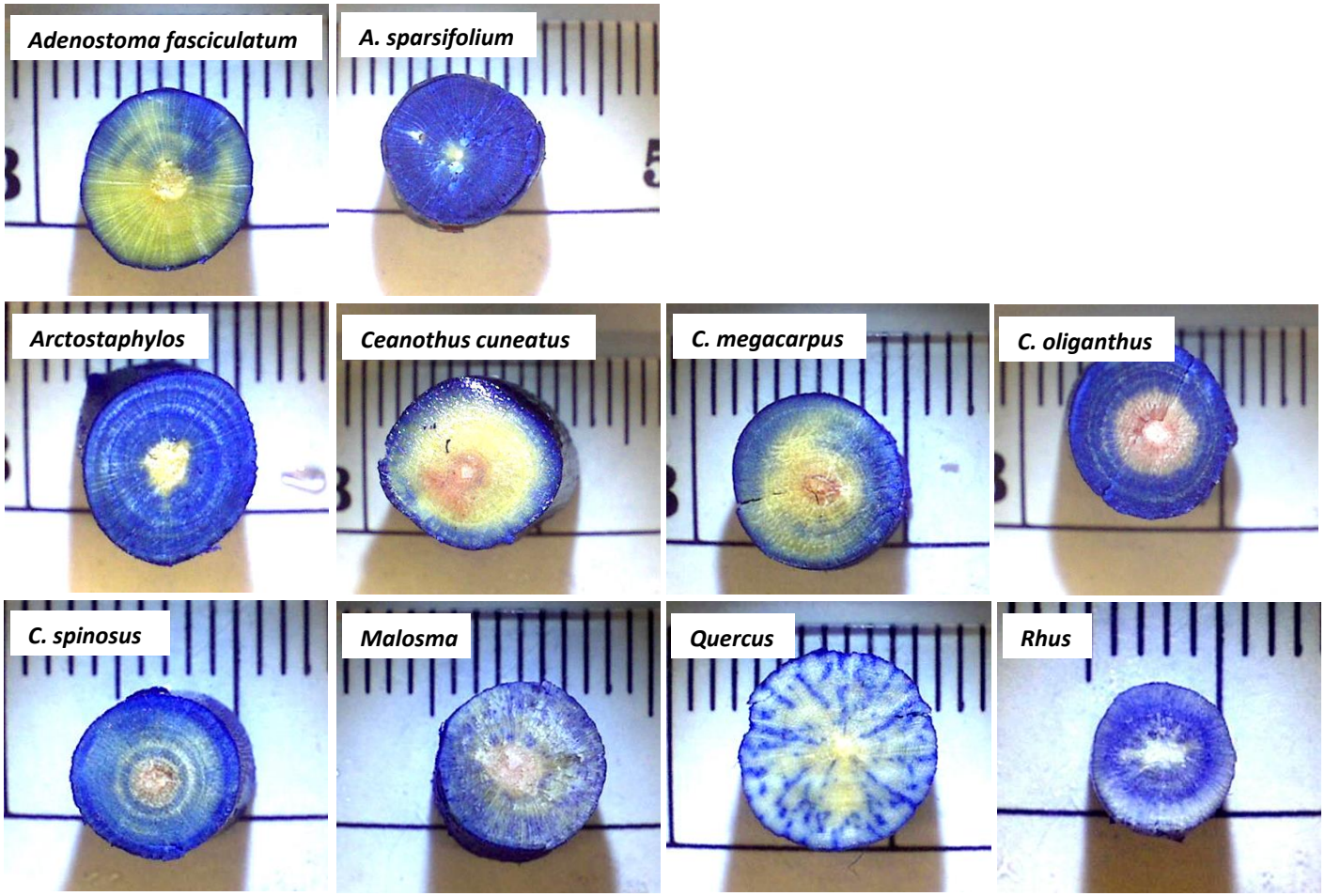
Xylem specific conductivity (K_s) is K_h divided by the cross sectional xylem area and is typically reported in kg m^{-1} MPa^{-1} s^{-1}).
This can be calculated two different ways.

1) Most commonly, the cross sectional xylem area at the distal end of a sample is measured by determining the area of the xylem from xylem diameter and then subtracting the area of the pith.

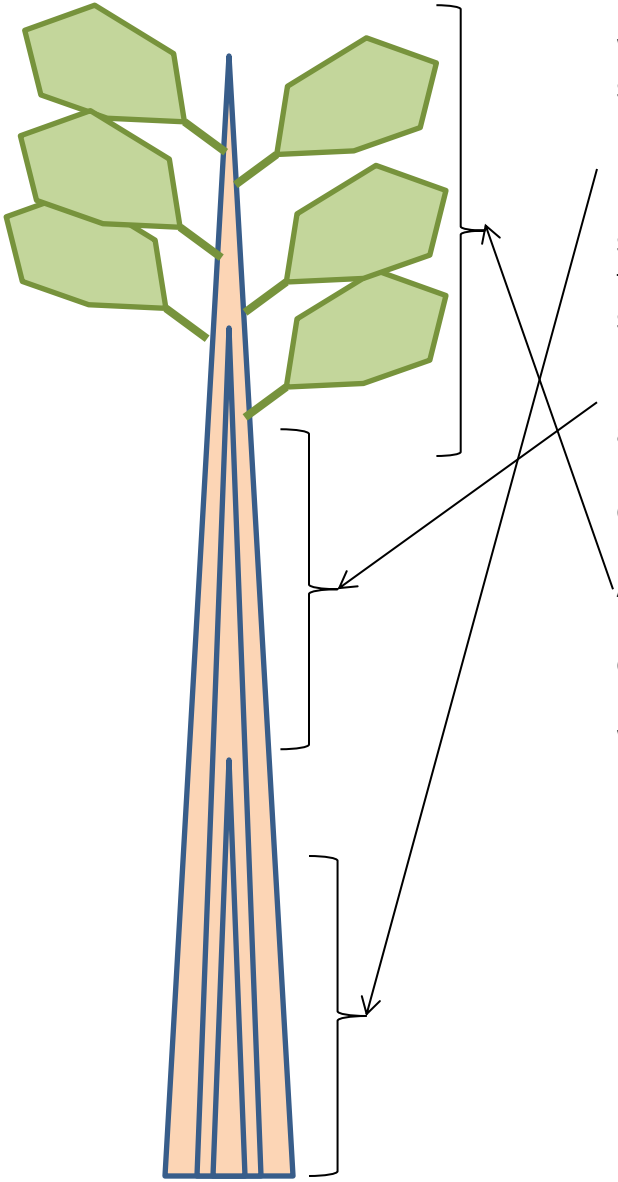


Xylem area (m^2) =
[(D1/2) * (D2/2) * pi] – [(d1/2) * (d2/2) *pi]

2) Alternatively, cross sectional area can be measured as the active xylem area determined by staining (staining methods are posted in a separate file). As you can see, the xylem through which sap is being moved is often not the entire xylem area, even for relatively young samples. For stained xylem samples, the active xylem area can be determined through measurement of the entire stained area or, more commonly, measurement of the xylem area of active growth rings (i.e. an entire ring is included in the "active" xylem area if any part of it stains).



Leaf specific conductivity (K_l) is K_h divided by the leaf area supplied by the measured xylem segment and is typically reported in kg m^{-1} MPa^{-1} s^{-1}).



When measuring a xylem segment for K_l, care should be taken in choosing a stem segment that most captures the xylem that is supplying the measured leaf area.

For instance, a segment measured far basally from measured leaves may produce a Kl that is much higher than is actually available to leaves. We have found this for some of our desert plants (especially those that produce vascular parenchyma at the end of a growth ring). This issue is familiar to those who have had to select stem samples for allometry and huber value measures.

In general, it is best to select distal stem segments that are near where leaves are attached to stems and which have relatively few growth rings (leaves for most plants will be connected to only the outer most growth ring or rings—this can be examined by pushing dye into stems through a cut petiole).

All of the downstream leaves are collected and the leaf area measured in m^2. Only leaves that are attached to a sample beyond its distal end are measured (because only the solution that travels all of the way through a sample is measured using our hydraulic conductivity method). The measured K_h is then divided by this leaf area. We use a Li-Cor 3100 Area Meter to measure leaf area.

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See the following reference for where measuring hydraulic conductivity was first described:

Plant, Cell and Environment (1988) **11**, 35–40

A method for measuring hydraulic conductivity and embolism in xylem

J. S. SPERRY, J. R. DONNELLY & M. T. TYREE Department of Botany, University of Vermont, Burlington, Vermont, U.S.A.

Maintaining your hydraulic conductivity system:

Overtime, microbes are prone to building up within the tubing of the hydraulic conductivity system. This may lead to problems with sample clogging. We always sterilize our system at least once a week, using a dilute (1:3) bleach solution. The system is drained and the bleach solution introduced to the tubing. We let the system sit overnight with the bleach solution in the tubing before draining it, flushing it with deionized water, and then flushing it again with our degassed conductivity solution.

Check your system regularly to make sure that stopcocks are intact, latex grommets have not oxidized, and all of the hose clamps are intact and properly closed. All of these parts and sections of tubing should be replaced overtime as they break or become damaged. It is particularly important to check the stopcocks for leaks. This is most easily done by closing the system and looking for negative flow off the balance, indicating that solution is leaking somewhere. You can route solution through different parts of the tubing apparatus to isolate the source of solution loss.