Revised on 14 May 2015 by L. Sofen

SFS Sap Flux - Granier Sapflow Sensor Protocol
Templer Lab at Boston University

Note: A very useful reference and the basis for this protocol is Nathan Phillips' file Nathan's Sacred Sap Flow Package.pdf which is in the following folder: C:\Lab Files\Projects\Soil Frost Study\SFS Sap Flux

I. Materials:

1. Becton-Dickenson 19G 1.5 stainless steel hypodermic needles (2 per sensor)
2. Copper-constantan thermocouple wire (www.omega.com catalog # TT-T-36) (100 ft. makes 203 thermocouples)
3. Constantan heating wire (www.omega.com catalog # TFCP005-500) (100 feet makes 203 thermocouples, 60 heating wires)
4. Lead-free solder
5. Krazy glue ("Original", the runny kind) (0.07 oz. is enough for ~150 probes)
6. Safety goggles
7. Aluminum tubing (Vendor: Small Parts Inc.; Part #: TTRA-2-36) (21mm per sensor)
8. Heat-conducting paste (1 tube is enough for thousands of sensors)
9. 4-wire aluminum foil-shielded cable (2-pair 22 gauge with 24 gauge ground, Belden catalog # 8723)
10. Electrical tape
11. Water shields (large plastic cups or 20 oz soda bottles, cut in half, one half per sensor)
12. Reflective shields (Reflectix bubble insulation)
13. Silicon sealant (acetic acid-free only as acetic acid degrades the tree's bark; approx. 1 tube per 15 sensors)
14. Gorilla Glue
15. Staples for staple gun (5-10 staples per sensor)
16. Butane for soldering iron for field use
17. Flagging or sewing elastic to hold water shield in place for caulking

II. Tools:

1. Electric rotary dremel tool
2. One electric soldering iron (for lab use), and one butane-powered (for field use)
3. Razor blade
4. Multimeter with settings for ohms, volts, and amps
5. Ruler (cm)
6. Wire clippers
7. Electric drill
8. 2.5 mm diameter drill bit (3/32") (+ extra)
9. 2.8 mm drill bit (7/64") (+ extra)
10. 2 mm drill bit (1/16") (+ extra)
11. Small chisel
12. 'Pointy tool' (as found in most soldering accessory kits)
13. Small (datalogger-size) screwdriver
14. Wire stripper (22 gauge and 26 gauge)
15. Staple gun

16. Caulk gun
17. Lighter
18. Pliers
19. Tool belt, tool box, trash bag and mats to minimize impact on work site

NOTE: *Probe* = either an individual reference probe or an individual heated probe.
Sensor = the reference and heated probe together make one sap flow sensor.

Granier Sensor Construction

A Granier sap flow sensor consists of one heated probe placed in the sap wood of the study species 10-cm above one reference probe. Due to the vertical temperature gradient that exists in trees, Goulden and Field (1994) developed a modification to the traditional Granier sensor that consists of two reference probes installed in the tree which corrects for the natural temperature gradient. If you choose to use Goulden modified sap flow sensors, you can construct the probes according to the instructions below for reference probes (II.,A.). For more details see:

Goulden, M.L. and C.B. Field. 1994. Three Methods for Monitoring the Gas Exchange of Individual tree Canopies: Ventilated- Chamber, Sap-Flow and Penman-Monteith Measurements on Evergreen Oaks. Functional Ecology 8: 125-135.

Note: Granier sensors are fragile and are prone to breaking during installation and throughout the growing season. **Make at least 20% more sensors than you plan to use**.

I. Preparation:

A: Needles

1. Decide which length of probes you will be using based on the characteristic depth of sap wood in your study species. In general, diffuse-porous species like sugar maple require 20 mm long probes, while ring-porous species like oak require 10 mm probes. Depending on your goals, you may choose to use a combination of the two (if for example you wanted to measure a gradient of flow through the sap wood area).
2. Using an electric rotary cutter set to 4-6, and **wearing safety goggles**, cut needles to appropriate length (21 mm for 20 mm long sensors, 11 mm for 10 mm long sensors). Use a board with the needle point on the right so that 21mm is resting on the wood; line up the needle with the mark on the board.
    a. Goggles are necessary to prevent sharp ends of needles from flying into your eyes!!
3. Very carefully cut a small hole in the center of the needle to make a ‘window’ into the needle shaft. Don’t cut too deep as this weakens the sensor. Ideally, cut until just a film of metal is there that can be poked out with an uncut needle.

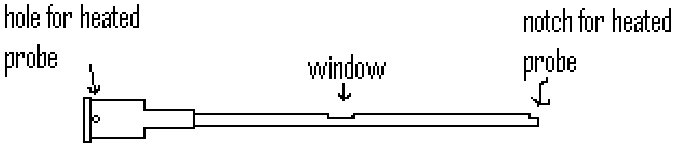


Figure 1

4. **For heated sensors only:**
    - **a.** Cut a ~0.5 mm notch in the cut tip of the needle. This will provide a spot to wrap the heated wire around later.
    - **b.** Poke a hole in the plastic base of the needle, near the very end, with an uncut needle.
5. Clean out needles by running a small metal wire through the sensors repeatedly, removing any burrs or sharp points. This helps when you need to thread wires through the needles later and prevents sharp metal from damaging wires.

B: Thermocouples (for both probes)

1. The thermocouple is the temperature sensor that will go into both the heated probe and the reference probe.
2. Use wire cutters to cut ~15 cm long pieces of blue copper wire and red constantan wire, one pair per probe. Spools of wire are in a cardboard box with the wires threading out through holes. If you have trouble stripping the wires without breaking them, cut the pieces slightly longer than 15cm so that after stripping you are left with 15cm pieces.
3. Strip off ~5 mm of the outer plastic coating on each side of each wire, exposing the bare metal. Be very careful not to nick the wire, as it will easily break. Small wire clippers work well with some practice.
    - **a.** Test that you did not nick the wire by bending the stripped section. If you nicked it, it will break.
4. Make 3-4 tight symmetric twists of the bare metal between a blue and red wire. Do not wrap one wire around the other, but rather wrap them evenly together.
5. Solder the twists.
    - **a.** When soldering, hold the connection to be soldered and the solder itself to the tip of the soldering iron.
    - **b.** With small wires like these, you need to pass the twists through the point where the solder and solder iron tip meet *quickly* to achieve a thin but complete coat. Don’t leave any blobs of solder. You should no longer see any copper color in your twist, just shiny silver from the coat of solder.
    - **c.** If some bare wire remains above or below the soldered point, that’s ok. You will trim the extra from the top later. The soldered twist should be ~ 3 mm. You now have a thermojunction! It should look like Fig 2 below.
6. Use a multimeter to check the resistance between the two leads of the thermojuction.
    - **a.** Electrical resistance, in layman’s terms, is the resistance that a current meets when traveling through a wire, like debris in a flowing river or plaque in an artery. So a correctly made junction has low resistance (smooth flow), and a badly-connected junction has high resistance (dam in the river, or stroke). The length of the wire also increases resistance.

b. The thermojunction should have a resistance of 3-50 ohms. Out of this range means that the junction was not successfully made.

Multimeter used to measure resistance:

Ω = ohms

"m" in bottom right corner = mega-ohms

1. Set multimeter to ohms.
2. Hold bare leads of thermocouple to bare leads of multimeter.
3. In this case, polarity does not matter.

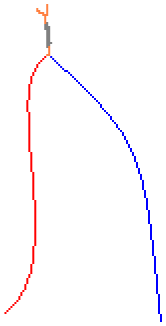


Figure 2

C: Constantan heating wire (for heated probes only)

1. Cut 50 cm of the red constantan wire for 20 mm sensors, 30 cm for the 10 mm sensors.
2. Stretch the plastic coating of the wire by running a finger nail along its length so that the coating is thinned as evenly as possible along the wire.
    a. This is **a critical step** so than when the wire is wrapped around the needle shaft, it will fit into the aluminum sleeve it ultimately lives in.
    b. It should be a tight fit, stretching the coating too much will cause a connection to be made between the thermocouple and the heating wire and the probe will be unable to be used (see IV. C. below).
3. Strip ~ 3 mm of coating off of either end to expose the bare metal.

II. Probe Assembly:

A: Reference Probes:

1. Insert both the blue and red thermocouple wires through the cut end of the needle and gently push until they come out the needle base.
2. Slowly pull the thermocouple wire through the needle until you see the soldered end show up in the 'window' in the middle of the shaft.
3. Apply very small droplets of superglue to the hole and wait until they are sucked in. Repeat until you see glue at the cut end of the needle and no more glue is taken in. It is best to apply small droplets that contact only the region of the hole – if you start covering the outside of the needle, the capillary action of the hole is relatively weaker.
4. Leave out to dry overnight. Test that the glue is dry by gently tugging on the thermocouple wires. They should not move within the needle.
5. Fill the plastic base of the probe with Gorilla glue and leave inverted until dry.

B: Heated Probe:

1. Insert the blue and red thermocouple wires along with one end of the heating wire into the cut end of the needle and gently push until they come out the needle base.
2. Slowly pull the wires through the needle until you see the soldered end of the thermojunction in the 'window' in the middle of the shaft.

   - **a.** Make sure that the heating wire end coming out of the needle base is shorter than the thermocouple wires. This allows you to distinguish between the wires later.

3. Apply very small droplets of superglue to the hole and wait until they are sucked in. Repeat until you see glue at the cut end of the needle and no more glue is taken in.
   - **a.** It is best to apply small droplets that contact only the region of the hole – if you start covering the outside of the needle, the capillary action of the hole is relatively weaker.

4. If the glue is still wet, you can start wrapping the loose end of the heating wire around the notch, and continue down the needle shaft.
   - **a.** Keep a steady, but not too extreme, tension on the heating wire as you wrap, and make sure the wraps are as close together as possible and do not overlap.
   - **b.** When you come to the center hole, the wraps will depress into the hole, which is fine.
   - **c.** Wrap until you have covered the metal portion of the needle (ignore the white coating at the base of the needle). Then, while holding the coil steady with one hand, take the loose end and insert it into the hole in the plastic base and pull out. Give a little tug and bend to the heating wire so that it stays in shape and doesn't uncoil.
   - **d.** If you let the superglue dry before wrapping, make sure to first scrape off any dry glue residue from the outside of the needle shaft with a razor blade. This avoids bulges in the sensor that will make it impossible to insert into the aluminum sleeve.

5. Trim the ends of the heating wire so that both leads extend ~5cm from the base of the needle and are slightly shorter than the thermocouple wires. Strip off ~1cm of insulation from all leads if you have not already done so.

6. Fill the base of the probe with Gorilla glue and leave inverted until dry.

III. Aluminum sleeves:

Score aluminum tubing with a sharp razor blade at 21 mm or 11 mm lengths (depending on the length of your heated probe) by rolling back and forth across the tubing. The tubing should snap off easily. Remove any sharp burrs and ream out the end with a pointy tool. Only the heated probes go into sleeves, so you need one sleeve per sensor plus ~20% extra in case of breaking or bending on installation.

IV. Checking Sensor Function

A. Use a multimeter to measure the resistance between the two leads of the heating wire. This should be less than 20 ohms. If the resistance of any heating wire is greater than 20 ohms it will inhibit the ability of the power card to discharge sufficient current to heat the sensors.

1. If it is very high, such as if the multimeter is reading in Mega-ohms, then the heating wire is severed. Discard this sensor.
2. If it is slightly higher than 20 ohms, you may not have shortened the leads enough. Make the leads of the heating wire shorter and test the resistance again.

B. Check the thermocouple resistance as well, to make sure it was not damaged while you assembled the sensor. It should have a resistance of 3-50 ohms. Out of range means that the junction was damaged.

C. In the heated probe, measure the resistance across one of the thermocouple leads and one of the heating wire leads. It should be in the hundreds of kilo-ohms or mega ohms, meaning that there is infinite resistance. If the resistance is in ohms, it means there is a connection between the thermocouple and the heating wire. The probe may be used as a reference probe in this case but not as a heated probe. You may also try using liquid saran, which coats all the surfaces, to isolate the wires from one another and recover the sensor.

1. If probes fail this test, it may indicate that you are stretching the heating wire coating too much, stretch it less to ensure that it is well covered. It should be a tight fit in the aluminum sleeve.

Power Cards

I. General information about power cards

The power card is designed to take the incoming direct current (DC) from a power source (in our case a marine grade battery or a DC power source connected to alternating current (AC) power) and regulate the output current to the necessary amperage. For sap flow sensors, the output current needed to generate the desired amount of heat is 0.124 amps. This amperage is achieved by tightening or loosening the screw on the variable resistor (also called a “trimpot,” see diagram below).

Each terminal block in the power card is preceded by a single, incomplete circuit. The circuit is completed by the heating wires of the Granier sensors. The terminal block we use each has six ports, and each heated probe uses two of these ports (one port for each end of the heating wire). Therefore each six port terminal block supplies heat to three probes. A power card that has four circuits can therefore supply heat to a maximum of twelve sensors.

You can construct your power card with as many or as few circuits as suits your needs. Note that each circuit functions independently, so that if one circuit in a power card doesn’t work or is unused, the remaining three circuits will still function. The diagram below shows a power card with 4 circuits, as well as a single circuit with the components labeled.

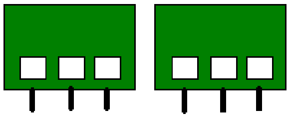
II. Laying out parts

A. You will need all the parts listed in the table below to make one power card with four circuits. Note that the part may look slightly different if you are using one of Nathan Phillips’ old cards as a reference, but the writing on the parts should be the same.

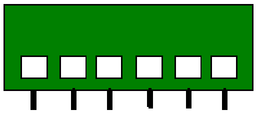
B. The best way to lay out a power card is to use an existing power card as a reference, and build your new power card by copying the layout of the reference, or by using the diagram below (Figure 4).

1. Insert the leads of each part through the perfboard and bend the leads flat so that the part does not fall out when you flip the card. Laying out the parts in exactly the same way as your reference card will aid you in making the connections later.
2. The spacing on some parts is especially important, such as that between the fuse holders, so that they hold the fuse correctly. You can count holes in the perfboard to help you achieve this.

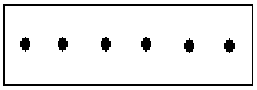
3. The six port terminal block is actually composed of two 3- port terminal blocks that slide together. In order to complete the circuit, you must create a connection between the second and third ports in the terminal block, and the fourth and fifth ports. You can do this by soldering a piece of copper wire between the leads of these ports on the bottom of the perfboard (see figure 3).



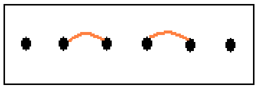
1). Two 3-port terminal blocks, side view. One has a groove and the other has a notch on the side. Slide them together to form one 6-port terminal block.



2). One 6- port terminal block, side view.



3) One 6- port terminal block as seen from the bottom, black dots represent the leads which poke through the perf board.



4) One 6- port terminal block with connections made between the appropriate leads using copper wire.

Figure 3

4. The **capacitor** and **diodes** are polarized (ie, there is a negative and positive lead). Take care that these parts are laid in the same orientation as in your reference card and the diagram.

  a. The diodes have one silver tip and one black tip; the silver tips should face each other.
  b. The capacitor's negative side (with a stripe) should face the heat sink.

III. Connect the parts

A. To make the connections on the back of the perfboard, you must use an existing power card as a reference. The way you laid out the pieces will help you figure out which leads go where, but take care and check that each connection is made between the same pieces and polarities as on your reference.

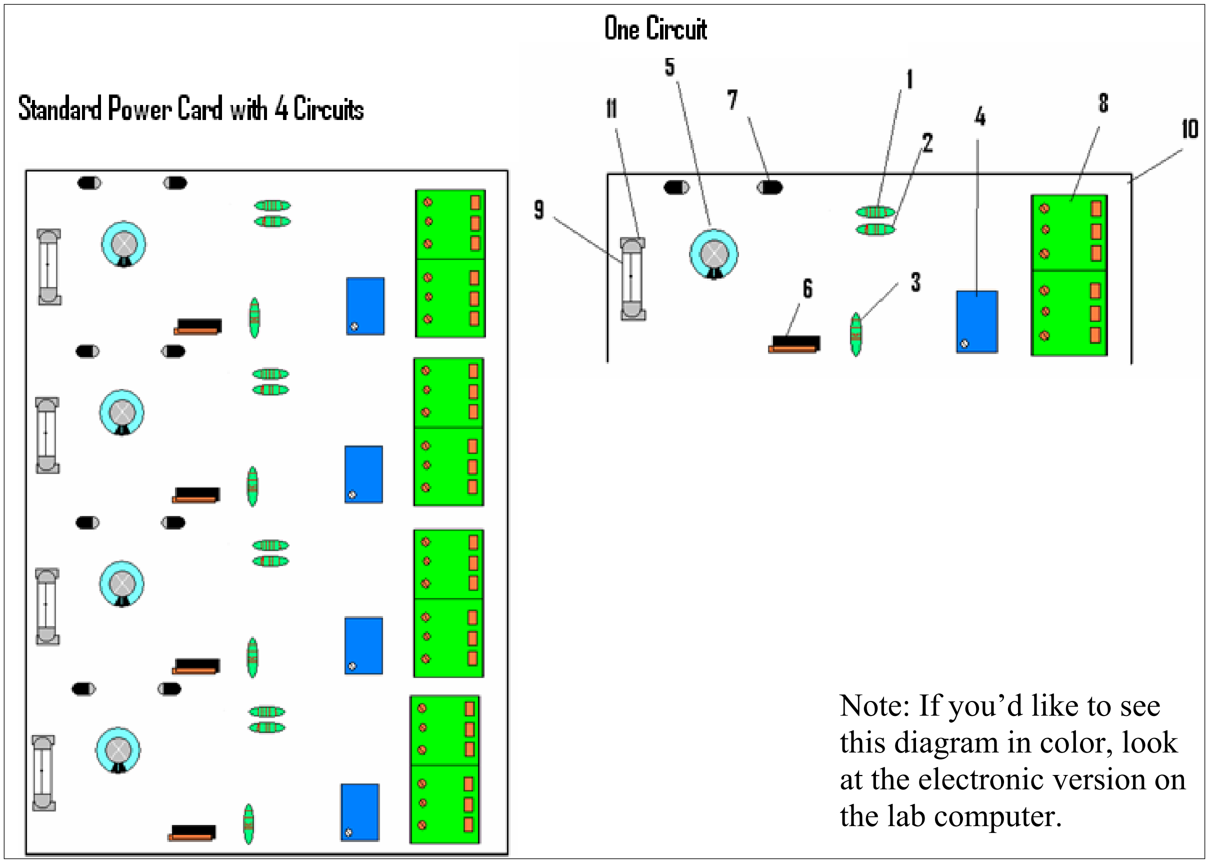
B. Work on one connection at a time, checking and double checking that the connection matches your reference before you solder it together. There are two types of connections: those that connect within the circuit, and those that connect the circuits together. Work within one circuit at a time, and then connect all the circuits last.

1. Identify a connection you need to make and double check it. It helps to wrap the leads together so that they remain close together and close to the perfboard without you needing to hold them, so that you have two hands free to solder.
2. Good soldering is essential here – make sure that soldered connections are secure and that your soldering job connects only the pieces you want connected (ie, you haven't inadvertently made an extra connection by letting the soldering blob get out of control).
3. If leads won't reach each other, use a piece of connecting wire to bridge leads together. Lots of things can be used as connecting wire, but a copper, large-gauge (ie, thin) wire works well.
4. Check that your soldered connections are secure by gently tugging the leads of each part. They should not pull apart.

IV. To check that it is working properly, hook the power card to a DC power source and follow the steps in the **Sensor Installation** section, **B., III., 5.,** below to check and adjust the current.

A. It is a good idea to adjust all the currents in this manner while in the comfort of the lab, as well as to check that the power card works before you bring it to the field. However, you will need to do this again in the field to make sure the current going to the heated probes is correct.

Figure 4: Power Card configuration



Note: If you'd like to see this diagram in color, look at the electronic version on the lab computer.

Table 1: Power card components

Part # corresponding to diagram	Part #	Price	Description	Quantity for 1 power card with 4 circuits
1		0.04	Fixed resistor, low precision (12Ω, 15%) (1/2 wat)	4
2		0.04	Fixed resistor, low precision (47Ω, 15%) (1/2 wat)	4
3		0.04	Fixed resistor, low precision (180Ω, 15%) (1/2 wat)	4
4	38-143	1.35	1 kΩ trimpot (variable resistor - upright)	4
5	15-770	0.15	220uF polarized capacitor (radial)	4
6	26-370	0.2	LM317T heat sinks	4
7	1N4001	0.03	Garden variety diode - many work	8
8	74-483	0.41	3 position terminal blocks	8
9	31-325	0.75	1A, 250V fuses - or 0.5 A fuse works too	4
10	276-1396	4.49	IC-spacing perfboard, pre-punched .042"-diameter holes, 0.1x0.1" grid pattern. Dimensions are 6x8".	1
11	27F773	0.133	Phenolic laminate Fuse Holder (1/4 x 1-1/4 ")	8
NOTE: These part numbers were provided by Nathan Phillips, they are for Hosfelt Electronics, Inc. 2700 Sunset Boulevard, Steubenville OH 43952 Phone: 800-524-6464. See appendix 1 for additional supplier information.				

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Sensor Installation

Field Supplies:

- Sensors
- Aluminum sleeves, cut to size of heated probe (11-mm or 21-mm)
- Heat conducting paste
- Belden cable (2 pair 22 AWG with 24 AWG ground, catalog #8723)
- Lead-free solder
- Electrical tape
- Sharpies, pencils, field book, protocol, etc.
- Water shield (Plastic cups)
- Flagging or sewing elastic
- Silicone or latex caulk (acetic acid free only)
- Solar radiation shield (Reflectix bubble insulation)
- Staple gun staples
- Trash bag

Tools:

- Staple gun
- Wire stripper
- Wire cutter
- Chisel
- Drill and drill bits (1/16”, 3/32”, including extras)
- Solder tool (pointed tool to insert Al sleeve into tree)
- Soldering iron, with butane and lighter
- Caulk gun
- Measuring tape
- Pliers
- Scissors
- Toolbelt and toolbox
- Multimeter
- Small (datalogger-sized) screwdriver
- Screwdriver to open junction boxes for wiring

I. Lay Out Cables

A. Each sensor requires one 4-wire aluminum foil-shielded cable connecting the sensor to the datalogger. (Each sensor also occupies one high and one low channel in a multiplexer or datalogger, and two ports in a power card.)

B. Lay out the cables so that they run flat along the ground, up to the tree, flat along the tree trunk, and end approximately between the heated and reference probes of each sensor. Use a staple gun to secure the cable to the tree in two or three places. If you have slack, make a few big loops of the cable at the tree trunk so that if someone trips over the cable, it will pull on these loops rather than pulling the sensor out of the tree. For the portion of cable laying on the ground, thread the cable into wire loom for added protection.

C. At the tree end, strip approximately 10 cm of the outer sleeve of the cable and approximately 1.5 cm of each wire within the cable. Cut off the bare shield wire (which is the grounding wire) and remove the aluminum foil.

1. Do the same at the datalogger end, but do not cut the grounding wire.

2. You may need to strip more of the outer sleeve of the cable for the wires to reach their ports, depending on how you positioned the multiplexer and power card.

D. Using a sharpie, mark each cable with the sensor ID on both ends of the cable.

II. Install Sensors in the Tree

****Re-test sensor function in the field immediately before installing in the tree to make sure sensors did not get damaged in transit.**

A. Using a chisel, remove a small section of bark from the tree at approximately breast height (1.4 m from the ground; at CCASE, 1.2m from the ground; if on a slope, measure the height from the highest elevation side of the tree), and another one 10 cm directly above it. On the lower opening, remove as little bark as possible- just enough to expose a few centimeters of the cambium layer. The upper opening can cut deeper into the bark until you see sapwood.

1. It is helpful to practice on a few trees outside the study area. The cambium layer is the first 'wetter' wood you find right beneath the inner bark layer (see figure 5). Its depth varies by tree and by species. Take care not to chisel into the cambium layer, as this will damage the trees' living tissue.

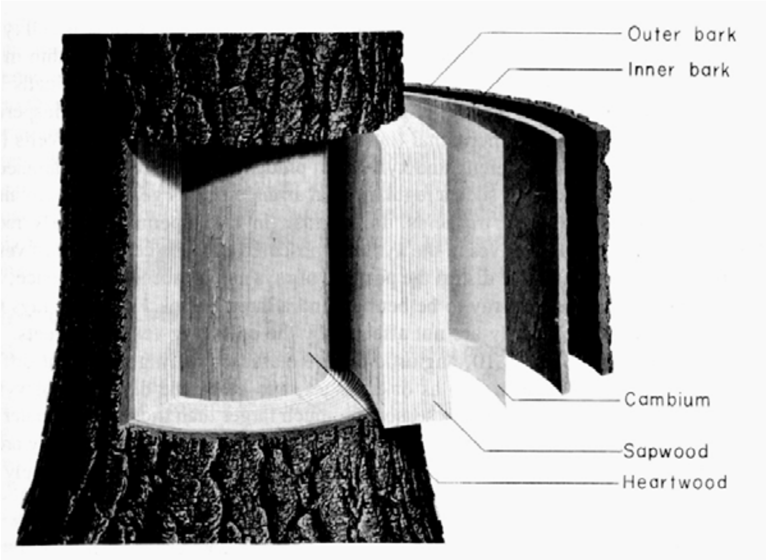

Figure 5: http://z.about.com/d/forestry/1/0/8/3/tree_bark.jpg

2. Try to choose an area of the tree free of wounding or knotting since the sensors rely on sap flow between the upper and lower probe.

B. Using a 2.5 mm diameter drill bit (3/32" drill bit), drill a 21 mm-deep hole into the center of the upper section of exposed cambium for 20 mm sensors. For 10 mm sensors, drill to a depth of 11 mm.

1. Before you start, measure 21 or 11 mm on the length of the drill bit and mark this with a piece of tape or a sharpie. This makes it easy to drill to the correct depth.
2. Take care to drill straight in and out of the tree to create a straight hole. Keeping the bit level also prevents putting weight on the bit – these are thin bits and break easily!
3. Never stop the drill bit from spinning while in the tree. This prevents the bit from getting stuck in the tree. It is not usually necessary to reverse the drill direction in order to pull the bit out, just keep it moving.

4. In between drilling holes, clean out the wood in the grooves of the drill bit. This helps prevent the drill bit from getting stuck in the tree.
5. Depending on the tree species and the weather conditions that day, it may be necessary to use a slightly bigger drill bit (2.8 mm, 7/64") to create a hole big enough to fit the aluminum sleeve. Figure out which bit works best by testing a tree outside the study area (see below for the method of inserting the aluminum sleeve). Sometimes the ideal hole size is in between the two drill bit sizes. If this is the case, you may be able to get the right size by using the 3/32" drill bit, and carefully reaming out the hole to slightly widen it. You will also find it easier to insert the aluminum sleeve if you do so immediately after drilling, before the tree wood has time to swell and close the hole.

C. Position the aluminum sleeve on a pointy tool and firmly insert the sleeve into the drilled hole, making sure to be very straight, until the end of the sleeve is flush with the exposed cambium, not sticking out at all. Be careful not to push too hard, this will widen the aluminum sleeve and reduce its contact with the heating wire.

D. Dip a heated probe in some heat conducting paste and then insert it very carefully into the sleeve until the base of the sensor is flush with the sleeve.

1. As you insert the probe, rotate it so that the heat conducting paste coats the probe evenly and gets in between the threads of the heated wire.
2. This is a very delicate step that can easily result in probe breakage, so be careful. DO NOT force the probe in. If the probe was made correctly, it should slip into the sleeve fairly easily. If the sensor will not go in easily, the most likely problem is that the heating wire's plastic coating was not stretched sufficiently when the probe was made. Go home and make new probes.
3. Wipe off any excess heat conducting paste.

E. Using a 2 mm diameter drill bit (1/16" drill bit), drill a 21 or 11 mm-deep hole in the lower exposed cambium. You may need to use the 2.5mm drill bit. Use the same precautions noted above.

F. Insert a reference probe into the lower drilled hole.

G. Make sure that all of the sensor wires are just long enough to reach each other, and no longer. Making connections between wires that are excessively long will create too much electrical resistance and affect the current supplied to the sensors. Trim and strip any wires that are too long before proceeding.

H. Connect the sensor wires to the cable wires by wrapping the bare leads of the sensor wires 3-4 times around the bare leads of the cable wires:

1. Connect the **blue**-coated (copper) wire from the upper probe to the **white** cable wire.
2. Connect the **blue**-coated (copper) wire from the lower probe to the **green** cable wire.
3. Connect the two (2) **red**-coated heating wires from the upper probe to the **black** and **red** cable wires.

I. Twist the red-coated thermocouple wires from the upper and lower probes together, making 3-4 symmetrical twists.

J. **Do before soldering** Check the connections just made using a multimeter on the ohms setting. It is important to check the sensor at this point in time, because it may have been damaged during installation, and to insure that everything is working before you solder the connections.

1. **Method 1**: Check the resistance between the various connections at the tree:
    - **a.** Check the thermocouple connection of the upper probe by holding the multimeter leads to the bare leads of the blue wire and red wires. This should have a resistance of less than 20 ohms (make sure you are reading ohms Ω and not megaohms MΩ).
    - **b**. Check the thermocouple connection of the lower probe by continuing to hold one multimeter lead to the red wires, and moving the other multimeter lead to the lower probe's blue wire lead.
    - **c**. Check the heated wire connection by holding the multimeter leads to each of the heated wire leads. This should have a resistance less than 50 ohms.
    - **d.** Check that no connection was made between the thermocouple and the heated wire by holding one multimeter lead to where the red thermocouple wires are twisted together, and the other multimeter lead to one of the heated wire leads. This should have a very high resistance (in the mega-ohms).

2. **Method 2:** Check the resistance between the various connections at the datalogger, this ensures that the cables are also intact:
    - **a.** Test the resistance between the green and white wires; it should be between 3-50 ohms.
    - **b.** Test the resistance between either the green or the white wire, and either the red or the black wire. This tests the connection between the heating wire and the thermocouple, and should read in the hundreds of kilo-ohms or in mega ohms which indicates infinite resistance and no electrical connection.
    - **c.** Test the resistance between the red and black wires, this tests the resistance of the heating wire and should be less than 20 ohms.

K. Solder all connections. Wear safety gloves! This doesn't have to be a very neat soldering job – just assure that there is a good blob of solder covering where you wrapped the wires. You should not be able to separate the wires if you tug gently on them.

L. Insulate all connections with electrical tape.

III. Modifications to the Granier Sensor

1) **Goulden modified sensors** measure the naturally occurring vertical temperature gradient of the tree.
    a) Instead of using one heated probe and one reference probe, install two reference probes, one ten centimeters above the other in the same manner as the traditional Granier sensor.

- b) The Goulden sensor should be installed 10-cm laterally from a traditional Granier sensor so that the vertical temperature gradient data can be used to correct the Granier sensor data.
- c) To connect the sensor wires to the cable wires:
    - i) Wire the blue copper wire from the upper probe to the white wire in the cable and wire the blue copper wire from the lower probe to the green wire in the cable (as for the traditional Granier sensor).
    - ii) Clip off the red and black wires at both the tree and in the datalogger box.
    - iii) Twist together the two constantan wires from the thermocouples.
- d) Connect the green and white wires to the multiplexer; these probes do not use power cards as they are unheated.

2) **At-depth sensors** are installed deeper in the sap wood to explore the profile of the sap flow.
    - a) In diffuse porous species, which include maples, there is substantial sap flow occurring beyond the 21-mm probe. This isn't true of ring porous species such as oaks.
    - b) The probes which are installed at-depth are exactly the same as the traditional Granier probes (ie. 21-mm length, both heated and reference).
    - c) To install the probes:
        - **i)** Use the chisel as described above to reach the sap wood.
        - **ii)** Next use a boring bit to drill to the desired depth and install the probe at that depth in the same manner as described above for the traditional Granier sensor.
    - d) Wire the sensor as described for the traditional Granier sensor.

IV. Connect Sensors to Datalogger and Power Source

NOTE: The wiring required depends on the type of datalogger and/or multiplexer and what channels are available. The wiring and configuration required to connect sensors to a power source depends largely on what power source is used. These are basic instructions, which can be amended depending on your unique set-up.

Types of Power: These sap flow sensors rely on power in the form of 12 volts direct current. There are various sources that supply this type of power including a DC power supply connected to AC power as used in Harvard Forest, and deep cycle marine batteries as used in Hubbard Brook. Other batteries and options also exist, such as the UB1240 batteries that fit inside the datalogger box.

General Instructions:

A. Connect sensors to datalogger or multiplexer.

1. **1.** Position your cables so that all the green and white wires can reach the datalogger/multiplexer channels, and all the red and black wires can reach the power card ports.
    - a. It will greatly behoove you to do all possible to reduce the 'spaghetti factor' of the wires in your project box. Some tips:
        - i. Position the datalogger, multiplexer, power card(s), battery(s), etc., inside your enclosure so that wires don't cross unnecessarily or block places you'll need to access like other channels or serial connection ports.

  - ii. Tie together the green and white wire from each sensor with a small knot. Do the same for the red and black wire. This will prevent like-colored wires from different sensors getting mixed up.

2. Hook the white wire of the first sensor to the 'High' port of the first datalogger/multiplexer channel, and the green wire of the first sensor to the 'Low' port of the same channel. Hook the grounding wire into the ground port of the same channel. Hook in the remaining sensors in the same way.

  a. Make sure you record which sensor is going to which channel, so that you can match individual sensors to the data you will get later!

  b. Tug gently on each wire to make sure it is secure in the port.

B. Connect sensors to power card:

1. Hook the red and black wires of your first sensor into the first two ports of the power card. Hook in the remaining sensors down the power card in the same way, recording what goes where.

2. Make sure that each terminal block in the power card is full. The power card controls the current of each of its circuits simultaneously. The sensors which are wired into the terminal block complete the circuit and the ports in the terminal block therefore cannot be left empty. Each six port terminal block holds 3 sensors when full.

3. If you don't have enough sensors to fill a terminal block, you can still complete the circuit. If you have only two sensors, hook the second heating wire of the second sensor into the last port of the set. If you have only one sensor, hook each end of the heating wire into the ports on each end of the terminal block. The diagram shows three wiring schemes that achieve a complete circuit (see figure 6).

4. Tug gently on each wire to make sure it is secure in the port.

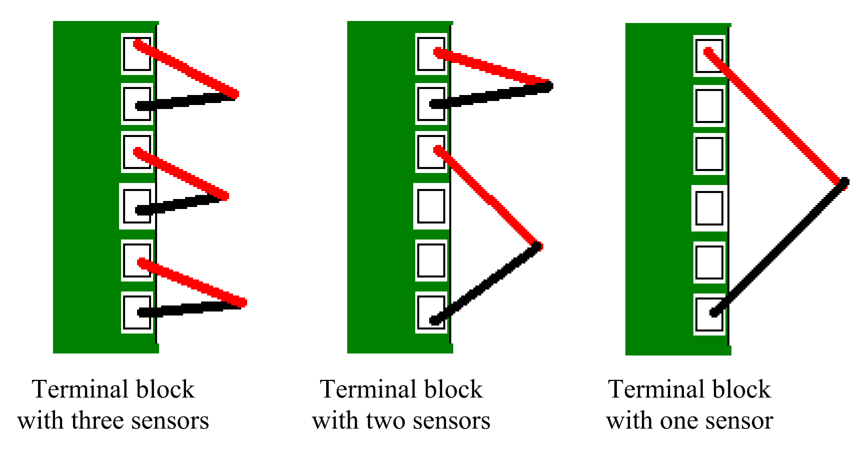

Figure 6

5. Once the power card is connected to the power source, you must adjust the current of each set of ports to 0.124 amps.

  a. Set the multimeter to the amps setting and connect the red multimeter lead into the '10 Amp Max' port on the left side of the multimeter.

b. Unhook the last sensor wire in the first set of ports. Stick one lead of the multimeter into the port and make sure it makes good contact with the metal inside the port.
c. Hold the other multimeter lead to the bare lead of the loose sensor wire. It is very helpful here to have multimeter clips to hold the connection.
d. Alternatively, you can leave the sensors completely wired and test the current by placing one multimeter lead on the screw of the first port in the set of ports and the other multimeter lead on the screw of the last port in that set.
e. The multimeter should read approximately 0.124 amps if you have already adjusted the current in the lab.
f. Adjust the current by slowly turning the screw on the variable resistor (or "trimpot"), which is in front of the terminal block, until the multimeter reads 0.124 amps. Turning the screw to the right increases the output current.
g. If you cannot achieve 0.124 amps, your sensor leads (on the tree-side) may be too long, creating too much resistance.
h. Re-connect the loose sensor wire into the power card port.
i. Continue down the power card so that each channel is adjusted to 0.124 amps. Note that a quick test of a power card channel is to test the heat sink; if the channel is working, the heat sink will be warm or hot. If it is not working, it will be cold.

HBEF CCASE Wiring notes

At HBEF CCASE, the cables from the plots are wired through a box on the ground at the base of each datalogger box for the treatment plots and at the base of the Campbell Scientific box housing the power cards for the reference plots. The plot cables are connected using Wago clip connectors to wires that then connect to the dataloggers and the power card. Wiring diagrams are posted in the Campbell boxes housing the dataloggers as well as in the binder in the CCASE shed.

V. Sensor Protection

A. Once everything is hooked up and functioning properly, the sensors must be protected from the elements.

B. Water protection:

1. Many common objects work well to create a water shield, such as large plastic cups or 20 oz soda bottles. Whatever you have available, it must be long and wide enough when cut in half to cover both probes. It is also important that it be open at the bottom so that it does not create an hermetic environment around the sensor.
2. Place the shield over the sensor and secure it in place so that it is as flush as possible with the trunk. A piece of sewing elastic or flagging works well to tie it to the trunk, or a few staples from a staple gun.
3. Acetic acid degrades bark; therefore use caulk (silicone or latex) which is acetic acid-free. You can detect acetic acid in caulk because it will smell of vinegar. Caulk around the edge of the shield to seal it to the tree and prevent water from entering.

  a. Read the instructions on the sealant to make sure you provide the correct drying time before rain or freezing temperatures are expected.

4. Apply to all sensors.

C. Solar protection:

1. Solar protection is important because these sensors work by temperature gradient. If a sun fleck were to fall on the sensor, it could affect the sensor reading.
2. Wrap a reflective material around the tree to cover all sensors and staple in place. Reflectix silvery bubble insulation works very well. Staple only the top of the Reflectix to prevent light from reaching the sensor while still allowing as much air flow as possible below the shield. Monitor throughout the season to make sure the shield does not get blown off by strong winds.

VI. Removing Sensors from Trees

A. Pull off Reflectix and water protection cups. Use pliers to remove staples from Reflectix and wire. Pull off all caulk from trees; a chisel may be helpful.
B. With wirecutters, cut the cable below the soldered connections. Cut as close to the connection as possible so that more cable is left to use in the future.
C. Pull the sensor out of the tree using your fingers or pliers. Remove the aluminum sleeve with pliers or pointy tool. Discard the sensors and aluminum sleeves.
D. If leaving the cables in the field overwinter, coil the cables so that they will not be disturbed.

VII. Troubleshooting in the Field

There are multiple problems that arise in the field, and it is very common to have to replace probes or fix some other aspect of the set up during routine field visits. The best diagnostic tools are the Archer Field PC, which will allow you to see your data while at the plot, and a multimeter. It is necessary to have extra probes and your installation tools for each field visit.

1) Typical data and indications of problems

Once connected to the datalogger, the Archer Field PC allows you to see the newest set of averages recorded by the datalogger as well as the results of the datalogger's last scan. By evaluating the reported values, you can identify sensors that aren't reading correctly. The problem may be with the sensor itself or with some other aspect of the set up, such as the cables or the powercard.

a) Typical data from Granier sensors will be between 0.3 – 0.9 mV.
b) Data from the Goulden modified sensor will be close to 0 mV.
c) If the data point is recorded as -6999, -9999 or NAN it indicates that the sensor is no longer functioning. This could be caused by:
  i) A severed cable
  ii) A sensor/probe that needs to be replaced due to a severed wire or thermocouple.
d) If the data from a Granier sensor is reading the voltage close to zero, the sensor is not being heated correctly. This could be caused by various issues, see #2 below.

2) **Diagnostic steps for heated probes**
    a) A quick test of whether or not a power card channel is working is to touch the heat sink.
        i) If the channel is working, it will be warm to the touch, in some cases very hot.
        ii) A cool heat sink indicates that the power card channel isn't working. This may be due to a problem in the power card itself or to a heated sensor that has broken (see "b" below).

    b) To determine whether the problem is in the power card or one of the sensors, put the multimeter in the amps setting and touch the red lead to one of the screws at the end of the terminal block and the black lead to the screw at the other end. In doing this, you are bypassing the sensors by using the multimeter to complete the circuit.
        i) If the power card channel is broken, the output current will be zero (see "c" below).
        ii) If the channel is alright, the output current will be 0.124 amps (see "d" below).
        iii) **Note**: if the heating wire of any sensor is broken, it breaks the entire circuit and no current will be discharged to any of the sensors on that power card channel.

    c) If the power card channel itself isn't working:
        i) First check the fuse: ensure that the filament is intact and that the fuse is making good contact with the fuse holder. A loose fuse may shift and break the circuit. A blown fuse needs to be replaced.
        ii) Second, check the connections at the back of the power card.
            (1) If a connection has broken or the solder has fallen off the back, it will need to be re-soldered in the field.
            (2) Ensure that there aren't any accidental connections being made between the bare metal leads and solder on the back of the power card.
        iii) In general there are few problems with power cards once the initial field set up has been established.

    d) If the power card channel is functioning, the problem is either with the cables or the heated sensors.
        i) Disconnect each sensor from the power card and test the resistance between the red and black wires; it should be less than 20 ohms.
            (1) When any one sensor breaks, it breaks the circuit and no current will be discharged to any of the sensors on that power card channel, meaning that you will often have 3 sensors logging bad data but only one that needs to be fixed.
            (2) Resistance greater than 20 ohms indicates either a broken heating wire or a severed cable.
                (a) Check the cable between the datalogger and the sensor; if it is severed you can splice the wires to solve the problem.
                (b) If the cable is alright, the problem is with the probe itself and the probe, or possibly the entire sensor, will need to be replaced.
        ii) The datalogger will also record low voltages if there is an electrical connection between the thermocouple and the heating wire.

        (a) For a given sensor, test the resistance between either the green or the white wire (which is hooked into the multiplexer) and either the red or the black wire (hooked into the power card).
            (i) This tests the connection between the heating wire and the thermocouple.
            (ii) Should read in the hundreds of kilo-ohms or in mega ohms. If not, the sensor must be replaced.

e) If all of the sensors on a power card terminal show similar problems, another step is to run the sensor for a few days without power to verify that the unpowered sensors only measure low background thermal gradients.
    i) Remove power from the heated sensors, either by removing the fuse from the power card circuit or disconnecting one wire from the power card terminal to break the circuit.
    ii) The sensor values should be ~0 mV when unpowered. The sensor may record minor diurnal temperature gradients but recall that 0.04 mV ~ 1°C so larger gradients indicate that the sensor is measuring something besides background thermal gradients.

3) Diagnostic steps for reference probes

a) When the cable is severed or the thermocouple in one of the probes breaks, the datalogger will record -6999, -9999 or NAN, depending on the datalogger.
    (1) Check the cable between the datalogger and the sensor; if it is severed you can splice the wires together to solve the problem.
    (2) If the cable is alright, the problem is with the sensor itself and one or both probes will need to be replaced.

4) Notes on replacing sensors

The most common repair to a sap flow set up is replacing broken sensors. After you have gone through the diagnostic steps and decided that the sensor must be replaced, follow these steps:

a) Remove the solar shield (reflectix bubble tape) and the water shield (plastic cup) from the sensor along with all staples and caulk left on the tree.
b) Clip the ends of the wire in the Belden cable from the sensor wires and strip ~1-cm of the wire.
c) Remove the probes from the tree (the heated probe is often difficult to remove- you may need pliers).
d) Clean out the aluminum sleeve; the heat sink compound hardens when it dries and it will prevent you from installing a new probe.
    i) This is best achieved using a small drill bit (1/16”) and will take some probing to do.
    ii) This step is critical; if you do not do this you will very likely break your new probe when you try to insert it.
    iii) If you need to remove the aluminum sleeve as well, use a slightly larger drill bit to drill out the sleeve. Alternatively, you may be able to pull out the sleeve with needle-nosed pliers, but this usually breaks the sleeve. Once you remove the sleeve, you may need to re-drill the hole slightly before you can insert a new sleeve in the same hole. Be careful not to drill the hole too large.
e) Install the new sensor into the same hole following the instructions in this protocol.

f) Check that the output amperage of the power card channel is still 0.124A. (This could potentially change when you change sensors since the resistance of the new sensor is different than the old, and changes the resistance of the circuit.)
g) Verify that the new sensor is reading correctly by connecting the Archer Field PC to the datalogger and checking the results of the latest scans.
h) Once you have checked that the new probe is functioning, reattach the water shield and solar shield to the tree.

IV. Data Analysis

NOTE: *Baseliner comes with a thorough manual in the Word document 'help.rtf' in the program file for Baseliner. Below is the Baseliner protocol as it has been used thus far for our work. Refer to the manual for how to use Baseliner's additional functions.*

A. Prep data from datalogger for input into Baseliner

1. Datalogger files have a .dat extension. Open the datalogger file by right clicking with the mouse and choosing "open in Excel."
2. The datalogger file will have all the data from each time point saved in one cell. In order to separate them into different columns, select the first column (by clicking on the "A" column header in excel) then in the "Data" menu choose "Text to columns."
    a. Convert text to columns wizard, step 1 of 3: choose delimited
    b. Convert text to columns wizard, step 2 of 3: select tab and comma, then click on "Finish." The text will then be separated into individual columns.
3. Row 1 should have the column headers. Delete any extra rows before the column headers.
4. Save the file as a CSV file (which is a .csv extension in excel). The CSV format is one of the formats that Baseliner can work with.
5. Also copy the raw data into your sap flow master Excel file, into a page for raw data for that plot, so that you also have it in xls-format.
6. Your sensor data is given in ΔmV (difference in milli-volts). This is the difference in temperature between the heated and reference probe, where 0.04 mV = 1 °C.
7. Take note of any bad data. Typical good sensor data is between 0.3 – 0.9 mV. Lower than 0.3 mV indicates that the sensor was not receiving power. Data points of -6999 (or 999999 for some dataloggers) indicate that the sensor is broken or otherwise disconnected from the datalogger/multiplexer.
8. **Calculate VPD = Vapor Pressure Deficit**

a. Use the following data from the master datalogger files: RH_temp (=average temperature in degrees C) and RH (observed relative humidity).

b. For CCASE, average the temperature and RH data from the reference plots and from the treatment plots (measured at the shed) for every time point. Use these averaged values as the temp and RH for all 6 plots.

c. Use the following equations to calculate VPD:

i. Saturated Vapor Pressure = 613.75*EXP((17.502*ATEA)/(240.97+ATEA))
ii. Actual Vapor Pressure = (Saturated VP)*(OBS RHC)/100
iii. Vapor Pressure Deficit (Pa) = Saturated VP (Pa) – Actual VP (Pa)
iv. Vapor Pressure Deficit (Kpa) = VP(Pa)*(1KPa/1000Pa)

B. Convert Data to Flux in Baseliner

1. Open the csv file created in step A in Baseliner.
2. A window will pop up asking what columns you want to leave unconverted. Check all columns except for the columns that your sap flow data are in. Also check the box to save the column headers in the output file, and uncheck the VPD pre-process option
3. In the top window, display your first sensor using the pull-down window in the upper left corner. You should see some sort of diurnal-looking pattern displayed.
    a. The Y-axis is in ΔmV (difference in milli-volts). This is the difference in temperature between the heated and reference probe, where 0.04 mV = 1 °C.
    b. The X-axis is time.
4. In the bottom window, display the vapor pressure deficit (VPD) which will guide your determination of the zero flow condition (ΔT_{max}) in the trees.
5. Use the “Ref” box on the y-axis to draw a line at a particular point in the lower display window.
    a. For VPD, set the reference point at 0.1 kPa.
        1. When the VPD is less than this, it is more likely that the trees are not transpiring and it is therefore a useful reference line for comparison to the sap flow curves.
6. The plots are auto-scaling by default. You can turn that off by entering the desired low and high value in the boxes to the left of each pane.
7. Before you convert the data, you need to establish the baseline. The baseline is a straight line connecting the first and the last point in the selection. You establish the baseline by identifying two points of zero sap flow (ΔT_{max}, where the temperature difference between the heated and reference probe in the tree is greatest) and highlighting the sap flow curves between those two points.
    a. For Granier sensors, the baseline is drawn between high points on the curve so that you need to make your selection from one high point to the next.
    b. The lowest point of the cycle should be somewhere around mid-day, or whenever on that day the conditions were most conducive to transpiration.
    c. Environmental factors that impact stem water content cause variation in ΔT_{max} over time, so the absolute value of the baseline changes throughout the growing season.
    d. The zero flow condition will not necessarily occur nightly, as there is evidence that some trees transpire at night. Zero flow conditions are identified by the following:
        1. The temperature gradient between the reference and heated probe stabilizes for at least 2 hours, which causes the curve in baseliner to be flat.
        2. The ambient vapor pressure deficit is calculated to be less than 0.1 kPa.
    e. The baseline should be set for periods no longer than 10 days, due to the changeable nature of the baseline (see point c above).
    f. An explanation of the shape of sap flow curves:
    The sensors do not record sap flow directly. They are recording the difference between the temperature (in mVs) of the heated and reference probe. When no sap is flowing, the heated probe has a much higher temperature than the reference probe. The temperature difference between the probes is therefore the greatest. When sap is flowing, the heat of the heated probe is dissipated, and its temperature is more similar to the reference probe. The temperature difference between the probes is therefore less. The purpose of Baseliner is essentially to invert these peaks, by converting them from the temperature differences into the flux of sap flow.

   g. Why is the point of lowest sap flow so variable??
      1. It is normal for the point of lowest sap flow to occur anywhere from the end of the day to into the next morning. It is your job in Baseliner to find this point, defining each diurnal cycle of sap flow (so that we can know the sap flux for each day). It is variable because the point of lowest sap flow will naturally occur at a different time each day, depending on conditions. The same can be said for the point of highest sap flow.
      2. You may also notice that the point of lowest sap flow is a little higher or lower each day. Small fluctuations are normal, due to the tree's sap flow settling slightly differently each night. If the point of low sap flow is obviously not reaching anywhere near the point that it has reached before, you may be suspicious of sap flow occurring at night.

8. Using the guidelines in 6.d. above, determine the first point on the curve where ΔT_{max} occurred. Holding down the mouse's left-click button, drag the cursor from that point until the next point where ΔT_{max} occurred. The section you drag across should be highlighted in red.
   a. Do not include more than 10 days in the highlighted selection. Due to the changing nature of the baseline in sap flow, it is unlikely that the baseline will be the same for long periods of time.
   b. Changes in stem water content will be noticeable in the data due to the general angle of the curves over several days. Take these trends into account when converting the data so that you are not either overestimating or underestimating sap flow by imposing an incorrect baseline.
9. Click the 'Convert' button at the top of the window. The red highlight will jump ahead, and automatically highlight the same length section as you just converted. Adjust the highlighted section according to the guidelines above for determining ΔT_{max} and convert. Repeat this for the entire data set.
   a. If you have sections of bad or missing data that you do not want to use, you may skip over these sections, and they will not be converted. Do not, otherwise, skip over sections.
10. When you are all done defining your baseline, hit the 'View' button above the window. This will show you a plot of the converted data. You have gone from ΔmV to Sap Flux!
    a. You can toggle back and forth between the unconverted and converted data by using the View button (or by pressing V on the keyboard).
    b. Sap Flux is in (g_{H2O} / m^2_{SWA}/ second), where g_{H2O} is grams of water and m^2_{SWA} is meters squared of sap wood area.
11. Save the file with the "Save As" function. Save in the "Baseline Converted Data" folder, including the words 'Converted SapFlow' in the file name, along with the days the data is from, the location the data is from, your initials, the date the data was worked on in baseliner, and A, B, C… depending on whether you converted this data set more than once that day. For example: *Converted SapFlow Stn7 day166-180 (AW090730C).* The data will be imported into a new csv file under this title.
    a. If you plan to continue to work on the data file, save the unconverted and baseline data as well as the converted data (check these selections in the "save as" dialog box).
    b. If Baseliner has an error it is likely to shut down and in that case you will lose any unsaved data. Save the file frequently to avoid this.
12. Open your file of converted data and copy the data into your sap flow master, which should

be in xls format.

a. Record on the data page what csv file(s) the data in your master is from.
b. Areas that you skipped in the converting process are represented with periods in the cell. You can replace them with zeros if needed for your data analysis using Excel's "Find and Replace" function. Make sure if you do this that you check the "Match Entire Cell Contents" box, or all your decimal points will be replaced too!

D. Convert output from Baseliner from (g H_2O/m^2 sap wood/sec)

1. **Convert (g H_2O/m^2 sap wood/sec) to (mol H_2O/m^2 sap wood/sec)**
   Multiply (g H_2O/m^2 sap wood/sec) * (mol H_2O/18.01 g H_2O) → (mol H_2O/m^2 sap wood/sec)
2. **Convert (mol H_2O/m^2 sap wood/sec) to (mol H_2O/cm^2 sap wood/sec):**
   Multiply (mol H_2O/m^2 sap wood/sec) * (m^2/100cm*100cm) → (mol H_2O/cm^2 sap wood/sec)
3. **Calculate Crown Gas Exchange = Transpiration = E.**
   This equation incorporates ratio of 2.65 cm^2 sapwood/m^2 leaf area (from Tang et al. 2006 – specific to Sugar Maple):
   (mol H_2O/cm^2 sap wood/sec) * (2.65cm^2 sapwood/m^2 leaf area) → E = (mol H_2O/m^2 leaf area/sec)
4. **Convert E to (mol H_2O/m^2 ground/sec).**
   This equation incorporates ratio of LAI = 5.21 from Tang et al. 2006 (specific to sugar maple).
   (mol H_2O/m^2 leaf area/sec) * (5.21 m^2 leaf/m^2 ground) → (mol H_2O/m^2 ground/sec)
5. **Calculate Crown Average Stomatal Conductance = g**
   This equation incorporates atmospheric pressure of 101.3kPa
   (mol H_2O/m^2 ground/sec) *101.3kPa/VPD (kPa) → g = (mol H_2O/m2 ground/sec)
6. **Calculate Canopy Net Photosynthesis** = A, using this equation: A = (0.3)*(Ca*g)
   Ca=atmospheric CO_2 = 385ppm = 385mol/1,000,000mol (should look up actual current atmospheric concentration of CO_2)
   Multiply (mol H_2O/m^2 ground/sec) * (1000*1000umolH_2O /mol H_2O) * (385mol CO_2/1,000,000mol)*g (from step 6) → A = umol CO_2/m^2 ground/sec

Note: For example of calculations, see file entitled "Conversion Practice (Sapflow to C exchange).xls"