# **Correction Methods for Probe Misalignment**

# What is probe misalignment?

Accurate measurements of sap flow and stem water content are highly dependent on knowing the exact distance between the heater probe and the temperature sensors. By design, the temperature sensors are 0.6 centimetres (cm) distance (i.e. x = 0.6) on the Implexx Sap Flow Sensor Gen1 model and 0.8 cm (i.e. x = 0.8) for the Implexx Sap Flow Sensor Gen2 model.



Implexx Sap Flow Sensor Gen1 model includes the grey box and the probe spacing is 0.6 cm.



Implexx Sap Flow Sensor Gen2 model with the white sensor only and the probe spacing is 0.8 cm.

In practice, the exact distance is almost never 0.6 cm or 0.8 cm. When the distance is not 0.6 cm or 0.8 cm, this is known as probe misalignment. For extremely precise measurements of sap flow and stem water content, it is important to correct for probe misalignment.

In our experience, the distance between temperature sensors and the heater ranges between 0.5 and 0.7 cm for the Gen1 model and 0.7 and 0.9 cm for the Gen2 model. Most commonly, it is within  $\pm 0.05$  cm – that is only half a millimetre offset in the correct placement of the probe which is only a small distance. Regardless, where precision measurements of sap flow and stem water content are required this offset must be considered.



Note that the probe misalignment in the following schematic has been over-emphasised. The schematic is a representation of various probe misalignment scenarios.



Figure 1. A schematic of various scenarios of probe misalignment. Note that these diagrams have been exaggerated for demonstration purposes.

# What causes probe misalignment?

During the installation process, it is common for the temperature and/or heater probes to be unintentionally installed at a slight angle relative to the other. Despite drill guides, it is almost impossible to install probes exactly parallel. Unfortunately, we are not machines or robots and it is difficult for any user to precisely install every probe.

Probe misalignment is most common when an installation is rushed. It is extremely important to slow down and carefully install the probes in the stem. Probe misalignment is also common when the installer is tired and is common towards the end of a long day in the field.

Even when extreme care is taken during the drilling and the installation of probes, it is still possible for the probes to be misaligned because of xylem anatomy. The assumption is that the xylem conduits, such as tracheids and vessel elements, are perfectly perpendicular to the probes of the sap flow sensor. However, it is impossible to know prior to an installation the distribution and arrangement of xylem elements. Therefore, even when the probes of the sap flow sensor are perfectly parallel, the tracheids and vessel elements may be offset on a slight angle which subsequently alters the distance between the heater and temperature sensors. This problem is commonly observed in woody vines, such as grapevines, where xylem anatomy may be tortuous. It



may also occur if a sensor is installed near an old branch, or bole, or where a trunk or stem is on an unusual angle.

It is, therefore, difficult to always install every sap flow sensor perfectly. A correction to the data is inevitable and various methods of correction are outlined below.



Figure 2. Probe misalignment can occur even when extreme care is taken with drilling and the installation process. Grapevines, such as seen here, can be difficult as well as installing a sensor where the trunk is at an unusual angle.

# How do I know if my probes are misaligned?

The best method to determine probe misalignment is to examine heat velocity in the outer and inner measurement positions. Night-time heat velocity, when relative humidity is ~100 %, and soil moisture is saturated or at field capacity, should be 0 cm/hr. Under these saturated conditions, there is no energy driving sap flow in xylem which is why heat velocity should be zero. If heat velocity is not zero, then it is likely there is probe misalignment.

Probe misalignment is most obvious when there are several nights of observations. For example, Figure 3 shows seven nights of heat velocity data. The solid red line along the x-axis is where heat velocity equals 0 cm/hr. However, the blue line, which is observed heat velocity, is negative during the night. This is an indication of probe misalignment. When night-time data, which should be zero, is either negative or positive then this is probe misalignment.

Importantly, it must be a flat line at night. This is best demonstrated on Night 5 in Figure 3 where heat velocity is a flat line all night. This is clearly a period of zero sap flow when values should be 0 cm/hr. Yet, the heat velocity on Night 5 is approximately -0.15 cm/hr indicating probe misalignment.





Figure 3. An example seven-day data set showing probe misalignment. In this case, night-time values are approximately -0.15 cm/hr when the value should be 0 cm/hr. The difference is due to probe misalignment.

# When should the sensor be reinstalled because of probe misalignment?

If the night-time values are more extreme than -5 cm/hr or +5 cm/hr, then it is likely that the installation of the sap flow sensor is poor. In this scenario, correcting for the probe misalignment, with procedures outlined below, is unreliable. It is strongly recommended to remove the sensor and reinstall in freshly drilled holes.

# Methods to correct for probe misalignment

#### Over-length method

The over-length method can be used in the field immediately following the drilling of holes into the stem. This method is ideal to quickly check whether your drilling is adequate or whether a new set of holes must be drilled.

It is possible to assess the accuracy of drilling by inserting dummy probes into the drill holes prior to the insertion of Implexx Sap Flow Sensor into the stem. The drill bits that are used to create the holes for the sap flow sensor can be used as the dummy probes.

A visual inspection of the dummy probes, and how closely they are aligned in the axial and tangential axes, will minimise errors associated with probe misalignment. Where dummy probes are clearly misaligned, the installation site should be abandoned, and a set of new holes should be drilled.



The dummy probes can also be used to measure the angle and distance between probes and, with trigonometric equations, the true distances between temperature and heater probes can be calculated (Dye et al 1991, Hatton et al 1995, Forster 2020).

#### Cut-stem method

Zero flow conditions can be artificially induced by the severing, or cutting, of the sap stream, below the Implexx Sap Flow Sensor (Figure 4). This approach is known as the "cut-stem method" but it can only be used at the end of a measurement campaign and on a stem that can be damaged. Therefore, it is not a practical method and certainly should not be used on stems or trees that cannot be damaged. Regardless, it is a favoured method for numerous scientific publications (e.g. Burgess et al 2000, Forster 2012, Roddy and Dawson 2012, Zeppel et al 2010). Deciduous species can be checked for zero flow during the season when leaves are not present (e.g. Do and Rocheteau 2002).



Figure 4. An example data set showing heat velocity following the stem being cut beneath the sap flow sensor. The stem was severed on day 2 and heat velocity immediately ceased. The subsequent flat line data is the probe misalignment which can be corrected.

#### The saturation method

As mentioned above, there are certain periods when sap flow and heat velocity must be zero. In plants, sap flow (and by inference heat velocity) is driven by some form of energy. That is, plants cannot actively move sap in xylem. Sap flow is a passive process driven by gradients in energy. During the day, this energy is provided by solar radiation, temperature, and low relative humidity (that is, high vapour pressure deficit, VPD). During the night, sap flow is also driven by VPD, and even wind. The process of hydraulic redistribution means sap flow can also move if there is a gradient of water potential. For example, sap can flow from the roots to the soil if the soil is extremely dry.

When there is no energy in the system to drive sap flow, then sap flow must be zero. A period of no (or, at least, extremely low) energy is during the night, when VPD is ~0 kPa (or relative humidity is ~100 %), and when soil moisture is saturated (i.e. soil water potential is ~0 to -10 kPa). This is known as a saturated period. That is, the soil, plant and atmosphere are completely saturated and there is nowhere for sap to flow. Under these conditions, it is extremely probable that sap flow and heat velocity are zero.



As already shown from Figure 3, Night 5 on Figure 5 is an excellent example of zero flow under saturated conditions. The heat velocity on Night 5 was -0.15 cm/hr.



Figure 5. Night 5 is an excellent example of zero flow caused by saturated environmental conditions.

To correct for probe misalignment, simply add 0.15 to the entire data set. This will shift, or offset, the heat velocity data corrected to zero (see Hogg and Hurdle, 1997, for a reference):



Figure 6. The data set from Figure 5 has been shifted upwards by 0.15 cm/hr. This is known as the zero-flow offset or zero-flow correction. The data shown in this figure has now been corrected for probe misalignment.



Care must be exercised with the saturation method because sap flow can naturally be positive or negative during the night. Do not rely on a single night's data; but rely on several periods of saturation. For example, Figure 7 shows six days and nights of heat velocity data. A quick examination of the data on nights 1 and 2 suggests that there is a positive offset and probe misalignment of ~2 cm/hr. Night 3, however, is unusual and may suggest reverse flow and hydraulic redistribution. On night 4, and days 5 and 6, there was heavy rainfall and the system is now under saturated conditions. Heat velocity on nights 4, 5 and 6 is already ~0 cm/hr or, at worst, slightly negative. In this installation, there is little probe misalignment and the data should not be adjusted.



*Figure 7. An example data set where probe misalignment is not evident or very minimal. The positive values on nights 1 and 2 are nocturnal sap flow which is common in plants.* 

#### The cut-stem or saturation method?

The saturation method is preferable to the cut-stem method because it does not require destructive harvesting or physical damage to the plant. Additionally, the saturation method can be applied at any time during a measurement campaign once there has been some periods of saturated conditions. Various studies have relied on the saturation technique to determine zero flow conditions but typically only relying on periods when VPD was close to zero (e.g. Hogg and Hurdle 1997, Looker et al 2016, Roddy and Dawson 2012, Zeppel et al 2010) or periods of zero VPD following periods of extended rainfall (e.g. Doronila and Forster 2015, Pfautsch et al 2011).

#### The Tmax method and the Implexx Sap Flow Sensor

The Implexx Sap Flow Sensor is ideally designed for the Tmax method because of its high temporal resolution (0.2 seconds) and advanced, digital electronics. The Tmax signal from the Implexx Sap Flow Sensor is very stable and therefore it can be used to determine zero flow conditions and probe distance.

The following equation can be used to determine thermal diffusivity with Tmax data (Kluitenberg and Ham, 2004):



$$k = \frac{x^2}{4t'_m} \frac{t_0}{(t'_m - t_0)} \left[ \ln\left(\frac{t'_m}{t'_m - t_0}\right) \right]^{-1}$$
 (Equation 1)

where k is thermal diffusivity (cm<sup>2</sup> s<sup>-1</sup>), x is distance between the heater and temperature sensor (cm), and  $t'_m$  is the time to maximum temperature (seconds) following a heat pulse under zero flow conditions,  $t_0$  is the heat pulse duration (seconds). The equation can be rearranged to solve for x:

$$x = \sqrt{\frac{k4t'_{m}\ln(\frac{t'_{m}}{t'_{m}-t_{0}})(t'_{m}-t_{0})}{t_{0}}}$$
(Equation 2)

Therefore, x can be solved if k,  $t'_m$  and  $t_0$  are known. See the application note on how to measure k or see references Looker et al (2016) and Forster (2019). The  $t'_m$  value can be found from the Implexx Sap Flow Sensor which outputs  $t_m$  in all four temperature positions: outer downstream, outer upstream, inner downstream and inner upstream. The default  $t_0$  value is 3 seconds. Therefore, x can be found for all four measurement positions using data collected by the Implexx Sap Flow Sensor.

To do this, firstly collect and download the  $t_m$  data (also called the Tmax raw data) from the Implexx Sap Flow Sensor's data acquisition unit. Secondly, find several night-time periods when there is a stable reading of the  $t_m$  data. It is best to use the saturation method (see above) to determine these periods as  $t_m$  will likely be most stable under zero sap flow conditions. The best time of the night to observe the data is between 2 AM and 4 AM. It is possible to average the  $t_m$  data over this time period to minimise signal noise. This value can then be used as  $t'_m$  in Equation 2 to find x.

Figure 8 shows a seven-day data set of the raw Tmax data ( $t_m$ , seconds). The  $t_m$  values are lowest in the middle of the day because it takes a shorter time for the heat pulse to reach a maximum temperature. At night, heat moves more slowly in the stem therefore the highest values indicate slow flow and night-time periods. In this data set, there was a rainy period, or a saturation period, across nights 4 and 5. The data show a consistent reading with values on nights 1, 2, 3 and 6. The average  $t'_m$  between 2 AM and 4 AM on these nights was 50.6 seconds.





Figure 8. An example data set of Tmax raw data. During a saturated period, there is zero sap flow and the  $t_m$  values can be used for  $t'_m$ .

Thermal diffusivity, k, was measured as 0.002113 cm<sup>2</sup> s<sup>-1</sup>. Therefore, Equation 2 becomes:

$$x = \sqrt{\frac{0.002113 \times 4 \times 50.6 \ln(\frac{50.6}{50.6-3})(50.6-3)}{3}}$$
$$x = 0.644 \text{ cm}$$

Therefore, the actual distance between the heater probe and temperature sensor is 0.644 cm.

### How to configure SDI-12 commands to collect probe misalignment data

Several parameters inside the Implexx Sap Flow Sensor can be collected that are useful for correcting probe misalignment. These include the raw data from the Tmax measurements which can be found under the M! and M5! commands.

The M! command also outputs alpha\_outer and alpha\_inner which are the raw data required for the slow rates of flow method (SRFM) also known as the heat ratio method (HRM). The alpha values are useful for the recalculation of heat velocity once the correct distance between the heater and temperature sensors is known (see next section, "Recalculating heat velocity following probe misalignment correction").



The beta\_outer and beta\_inner parameters should also be collected. The beta parameters can be found under the M! command. The beta parameters are used for the Dual Method Approach (DMA) to determine heat velocity.

**Table 1.** The parameters, and their SDI-12 command, that should be collected from the Implexx Sap Flow Sensor for probe misalignment correction and subsequent recalculation of heat velocity.

Parameter	SDI-12 Command	Description
tMaxTouter	M! or C!	The time to maximum temperature in the downstream,
		outer sensor.
tMaxTinner	M! or C!	The time to maximum temperature in the downstream,
		inner sensor.
tMaxTusOuter	M5! or C5!	The time to maximum temperature in the upstream,
		outer sensor.
tMaxTusInner	M5! or C5!	The time to maximum temperature in the upstream,
		inner sensor.
alpha_outer	M! or C!	The ratio of temperature increase 60 to 80 seconds
		following the heat pulse to baseline, pre-heat pulse
		temperature in the outer position.
alpha_inner	M! or C!	The ratio of temperature increase 60 to 80 seconds
		following the heat pulse to baseline, pre-heat pulse
		temperature in the inner position.
beta_outer	M! or C!	The ratio of maximum temperature following the heat
		pulse to baseline, pre-heat pulse temperature in the
		outer position.
beta_inner	M! or C!	The ratio of maximum temperature following the heat
		pulse to baseline, pre-heat pulse temperature in the
		inner position.



### Recalculating heat velocity following probe misalignment correction

The Implexx Sap Flow Sensor outputs heat velocity in the outer and inner positions: VhOuter and VhInner. However, these data are calculated on the assumption that x (probe distance) is 0.6 cm. Where x has been determined with the Tmax method to correct for probe misalignment, it is recommended to recalculate VhOuter and VhInner. To undertake these calculations, it is recommended to collect all the parameters listed in Table 1. It is also recommended to understand how heat velocity is calculated via the Dual Method Approach (DMA<sub>Péclet</sub>) outlined in detail by Forster (2020).

The following equation is the spatially explicit form of the SRFM or HRM equation (Forster, 2020) and is used for reverse, zero and slow velocities:

$$V_h = \frac{2k\alpha}{x_d + x_u} + \frac{x_d - x_u}{2(t - \left(\frac{t_0}{2}\right))}, \beta \le 1$$
(Equation 3)

where  $V_h$  is heat velocity (cm s<sup>-1</sup>), k is thermal diffusivity (cm<sup>2</sup> s<sup>-1</sup>),  $\alpha$  is the ratio of temperature increase post to pre heat pulse,  $x_d$  and  $x_u$  (cm) are the corrected distances to the downstream and upstream temperature sensor, respectively, t is the time when temperature measurements are made (60 seconds),  $t_0$  is the heat pulse duration (3 seconds), and  $\beta$  is the ratio of maximum temperature post heat pulse to pre-heat pulse temperature.

The following equation is the spatially explicit form of the Tmax equation (Forster, 2020) and is used for faster velocities:

$$V_{h} = \sqrt{\frac{4k}{t_{0}} ln\left(1 - \frac{t_{0}}{t_{m}}\right)} + \frac{{x_{d}}^{2}}{t_{m}(t_{m} - t_{0})}, \beta > 1$$
 (Equation 4)

where  $t_m$  is the time to maximum temperature (seconds) in the downstream temperature probe following the heat pulse.

These equations are included in the freely available Excel worksheet "Dual Method Approach Sap Flow Calculations".

For detailed information, and for methods to convert heat velocity to sap flux density and volumetric sap flow, see Forster (2020), the Theory Section in the Implexx Sap Flow Sensor Manual, or the "Quick Start Guide – Excel SF Software".



### An example of improving data accuracy with probe misalignment correction

An Implexx Sap Flow Sensor was installed into a 2 m tall mulberry tree with a trunk diameter of approximately 3 cm. Data were recorded via the M! and M5! SDI-12 commands in the Implexx Sap Flow Sensor. Figure 9 displays the heat velocity data, in the outer and inner positions, downloaded directly from the ES-SYS data logging system. Figure 9 is an eight-day data set where there was a saturated period, of heavy rainfall and saturated soils, on Day 6.



*Figure 9. The heat velocity data downloaded directly from the ES-SYS data logging system measured from an Implexx Sap Flow Sensor.* 

On a quick visual inspection, it is apparent that the heat velocity data in Figure 9 shows probe misalignment. On Day 6, during a saturated period, heat velocity does not reach zero and is ~1.6 cm/hr in the outer position and ~0.8 cm/hr in the inner position. Therefore, the data were adjusted to zero, using a zero-offset of -1.6 and -0.8 for the outer and inner position. The results are shown in Figure 10.



Figure 10. Heat velocity corrected to zero using the saturation method.



The Tmax method can also be used to correct for probe misalignment. The ES-SYS data logger also recorded the raw Tmax values from the downstream outer and inner temperature sensors (M! SDI-12 command) and the upstream outer and inner temperature sensors (M5! SDI-12 command). Figure 11 displays the raw Tmax data from the mulberry measurements.



Figure 11. The raw Tmax data (seconds) in the outer and inner positions.

The average values, between 2 AM and 4 AM, on night 5 and 6 were used to find  $t'_m$  to calculate the distance between the heater and temperature sensors. These values were 51.6 and 55.5 seconds for the downstream and upstream outer temperature sensors; and 48.8 and 50.5 seconds for the downstream and upstream inner temperature sensors. Thermal diffusivity was measured following methods outlined by Forster (2019) and the result was 0.002252 cm<sup>2</sup> s<sup>-1</sup>. The distance, or *x*, was calculated as (via Equation 2): 0.672 cm (outer downstream), 0.697 cm (outer upstream), 0.653 cm (inner downstream), and 0.664 cm (inner upstream). Equations 3 and 4 were then used to recalculate heat velocity with the results displayed in Figure 12.



*Figure 12. Heat velocity data corrected for probe misalignment using the Tmax method.* 



# The Tmax or saturation method?

Comparing Figure 10 and Figure 12, the results appear similar as both techniques shifted the zeroflow data towards zero. There does appear to be a qualitative difference between the outer position in Figure 10 and Figure 12. And the overall data in Figure 12 is slightly lower than Figure 10.

It is recommended that the Tmax method for probe misalignment correction is adopted where possible. The Tmax method is a quantitative method based on theoretical equations and it is embedded in the theory of conduction and convection of heat in porous media. The saturated method, on the other hand, relies on the qualitative judgement of the user. The results from the saturated method may differ depending on different users.

### References

Burgess et al (2010), Ann. Bot. 85: 215-224. doi: 10.1006/anbo.1999.1019 Cohen et al (1981), Plant Cell Environ., 4: 391-397. doi: 10.1111/j.1365-3040.1981.tb02117.x Do & Rocheteau (2002). Tree Physiol., 22: 641–648. doi: 10.1093/treephys/22.9.641 Doronila & Forster (2015). Int. J Phytoremed., 17: 101-108. doi: 10.1080/15226514.2013.850466 Dye et al (1991). J Exp. Bot., 42: 867-870. doi: 10.1093/jxb/42.7.867 Forster (2012). Fungal Ecology, 5: 702-709. doi: 10.1016/j.funeco.2012.06.005 Forster (2019). Forests, 10, 46. doi: 10.3390/f10010046 Forster (2020). Tree Physiol. doi: 10.1093/treephys/tpaa009 Hatton et al (1995). Tree Physiol., 15: 219-227. doi: 10.1093/treephys/15.4.219 Hogg & Hurdle (1997). Tree Physiol., 17: 501-509. doi: 10.1093/treephys/17.8-9.501 Kluitenberg & Ham (2004). Agri. For. Meteorol., 126: 169-173. doi: 10.1016/j.agrformet.2004.05.008 Looker et al (2016). Agric. For. Meteorol., 223: 60-71. doi: 10.1016/j.agrformet.2016.03.014 Pfautsch et al (2011). Tree Physiol., 31: 1041-1051. doi: 10.1093/treephys/tpr082 Roddy & Dawson (2012). Acta Hort. 951: 47-54. Zeppel et al (2010). Tree Physiol., 30: 988-1000. doi: 10.1093/treephys/tpq053

