

## Automatic Turgor Pressure Recording in Plant Cells

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### Turgor Pressure

An automatically-regulated pressure probe is described, which facilitates the direct and continuous measurement of turgor pressure and water relation parameters in plant cells. An optoelectronic sensor drives a feed back system so that the position of the oil/cell sap interface in the measuring capillary remains constant. The sensor is easily applied to most measurement set-ups by mounting it on a focusing screen connected to the microscope used for observation. The position of the oil/cell sap boundary is sensed by its movement along the image of the capillary on the screen and can be set to any appropriate position. The optical coupling of the regulation signal is advantageous because it leaves the experimenter free to measure the electrical or other parameters of the cell. Experiments on cells of *Eremosphaera viridis* have shown that the device allows much faster and more exact measurements of water relation parameters, and over longer periods, than the earlier method.

Water relation parameters in cells of solitary algae or higher plants were measured by use of the pressure probe developed by Zimmermann *et al.* [1] almost 20 years ago. The method has been described in numerous publications [2–4] and can be used to determine – besides the actual turgor pressure of the cell – the hydraulic conductivity  $L_p$  of the cell membranes and the elastic parameters of the cell wall, both of which control the water exchange between cell and medium. The method is based on a compensation principle *i.e.* the pressure in the cell is transmitted *via* an oil filled capillary to a pressure transducer and transformed into a proportional voltage.

It is evident that manual regulation of the meniscus between oil and cell sap in the capillary tip causes disturbances and subsequent leakage of the cells so that measurements lasting longer than several minutes are very rare. A second difficulty arises from the extremely short half times of water transport in higher plant cells which make it very hard to keep the meniscus in a constant place.

An elegant way out of these problems is an electronic control that is optically coupled to the movement of the highly refractant interface between oil and cell sap. For this purpose a focusing screen was connected to the microscope. This is possible with every microscope that has a photographic tube. The movement of the oil/cell sap boundary is monitored by means of a small sensor containing eight phototransistors in a row. The sensor is positioned on the surface of the screen along the image of the oil filled capillary by means of an adjustable support (Fig. 1).

The eight phototransistors are used to detect the position, the direction of the movement, and the speed of the movement of the oil/cell sap boundary, as well as the basic luminance of the focusing screen. These signals are fed into an electronic circuit (Fig. 2), connected to a small motor, which regulates the position of the oil/cell sap boundary in the capillary by displacement of a metal rod in the pressure chamber. Depending on the position of the meniscus different flip flops are set when the appropriate sensor element recognizes the movement of the meniscus. As each of the flip flops represents different speed steps or directions for the motor regulation, the speed of the motor increases the more the meniscus

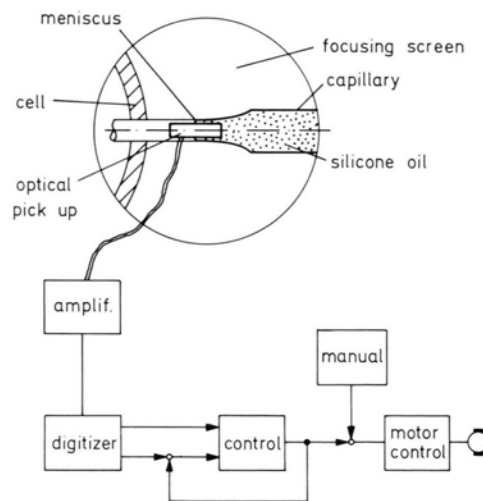


Fig. 1. An optical sensor – mounted on a flat screen along the image of the oil filled capillary – is used to monitor the movement of the meniscus between oil and cell sap. The signal from the sensor is fed into an electronic circuit which regulates the position of the meniscus by driving a metal rod into or out of the pressure chamber (not shown). For further information see text.

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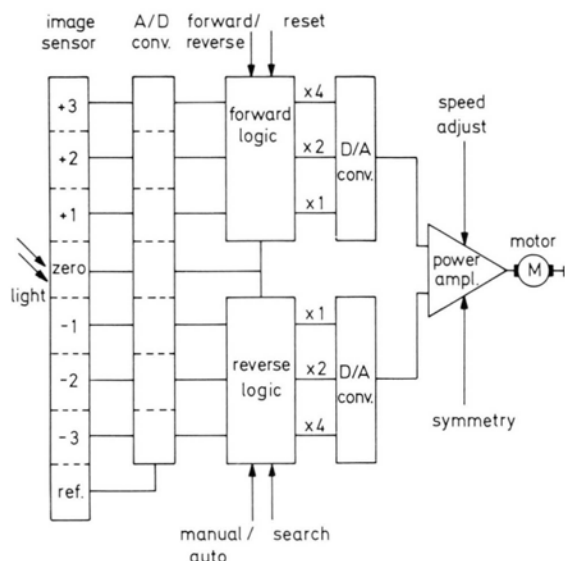


Fig. 2. Schematic diagram of the electronic circuit for the regulation of the meniscus position. Signals from eight photo sensors are digitized, fed into a forward/backward control logic and then converted back into analog signals. These are used to control the movement of the oil/cell sap boundary via the volume regulation in the pressure chamber. The detailed circuit diagram is available on request from the authors.

cus drifts away from its nominal position. When the phototransistor at the nominal position is crossed by the meniscus image it resets all flip flops so that the motor movement comes to a stop instead of constantly regulating to and fro.

In this way the position of the meniscus can be kept constant up to a maximum deviation of  $6\text{ }\mu\text{m}$  corresponding to a volume change of little more than  $0.05\text{ pl}$ . Of course, the control can be done manually as before. A search model is also included in which one only has to determine whether the meniscus has to be moved forward or backward to be trapped and fixed at the desired position. The device can easily be connected to the recently developed modified probe for pressure clamp experiments [5].

Fig. 3 shows a turgor pressure recording of the peat-bog alga *Eremosphaera viridis*. The water flux has been induced osmotically by addition of sucrose to the external medium. As can be calculated from the trace shown in the inset, the half time for the water flux is on the order of 9 seconds. The control system is fast enough to cope with the speed of the meniscus movement, at even shorter half times. Moreover measurements could be carried out on a single cell for longer than an hour during which up to ten experiments at different turgor pressures were performed by changing the external medium.

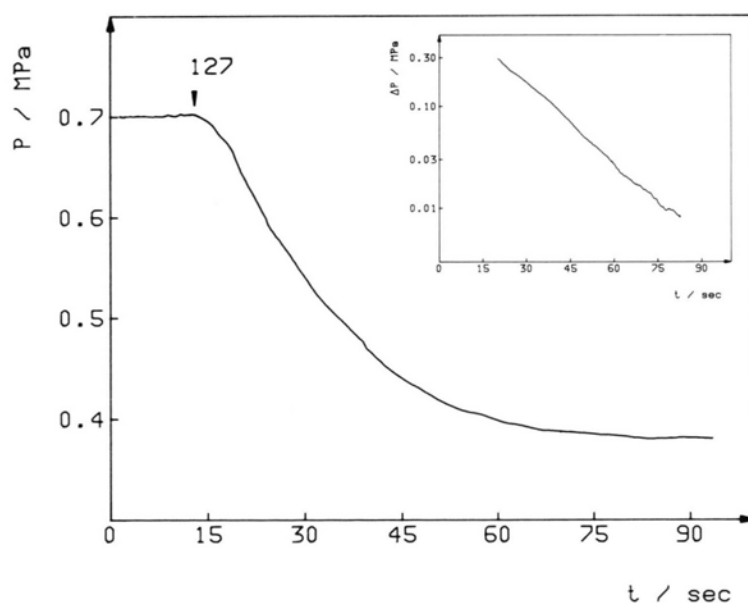


Fig. 3. Turgor pressure regulation of a cell of *Eremosphaera viridis*. The stationary turgor pressure of the cell was  $0.7\text{ MPa}$ . After addition of a solution containing  $127\text{ mOsm/l}$  sucrose the pressure dropped exponentially and achieved a final value of  $0.4\text{ MPa}$ . As can be seen from the semilogarithmic plot shown in the inset, the pressure decay was purely exponential exhibiting a half time for the water flux of 9 seconds.

We feel that this inexpensive and easy to build device provides great help for certain kinds of measurements, which up to now required an enormous amount of skillfulness and exercise on the experimenter side. Besides that the use of the automated system allows simultaneous measurements on differ-

ent sites of the plant for the determination of water pathways and turgor pressure gradients in whole plants.

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