LLS - Liquid Level Sensing methods in qPCR set-up

By

Dr. Rudolf Walser

Managing Director, DORNIER-LTF GmbH

RickenbacherStrasse 107, 88131 Lindau; Germany

Introduction

One of the main goals of all liquid level sensing techniques in automated pipetting systems is to obtain most precise control over actual pipetting performance, which largely depends on ensuring optimum liquid level (meniscus) in the well and precise immersion of the pipette tip in the liquid.

Various techniques for liquid level sensing are reported in literature. All of them have some advantages and limitations. Some of the techniques are used for checking the proper setup of a workstation deck prior to starting a run. Other so-called ‘real time’ level sensing devices are able to track the meniscus in each well at every pipetting step. Real time level sensing systems give the additional benefit of being able to monitor the whole pipetting procedure and provide for a consistently pipetting protocol after each run.

Tracking the meniscus in each well at every pipetting step enables the pipette tip to be positioned near the liquid surface. To avoid wetting on the outside of the tip the robot calculates the minimal submersion depth necessary for pipetting the set volume. By these actions both hydrostatic pressure of the liquid and wetting of the pipette tip are minimized. Therefore accuracy in pipetting smaller volumes is enhanced as well as aerosol formation due to droplet transfer at the outside of the tip is minimized. This kind of real time level sensing gives maximum safety control for the precision and safety of all performed pipetting steps.

Depending on the field of application only a few of these methods provide adequate performance for setting up polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR) applications.

Four main techniques are applied:

- Pressure based solutions,
- Capacitive liquid level monitoring techniques,
- Ultra sonic measurements and
- Optical methods

1. Pressure based liquid level sensing techniques

Pressure-based liquid level sensing (LLS) relies on highly accurate monitoring of pressure changes during the pipetting processes.

By use of the so-called air-displacement pipettors (1) a slight airflow is produced by moving the piston inside the air displacement channel slowly downwards. A pressure sensor measures the air pressure inside the barrel during pipetting. The data from this sensor changes as the tip approaches the liquid surface, touches the surface and drives below. This data can be used to control pipetting in real-time. Pressure level sensing is an appropriate way to determine the level also of non-ionic liquids.

Pressure data can be continuously recorded during pipetting. When the pressure goes outside the pressure limits prescribed for different times during the aspiration or dispense cycles, an error event is registered and communicated.

To handle these events the internal software can be optimized for various responses. Ignore certain signals, stop the run, ask for user intervention or automatically attempt to deal with the error without user intervention.

Fig.1. Level-sensing in a 0, 2 ml PCR vessel

For example, in the event of liquid missing the system, this can either pause the run asking for user intervention or proceed without stopping for a later check-up and providing a note about this event (e.g. in subsequent report after the finishing of the run). Even clogging or unusually low pressure events during aspiration, can be detected by this method. Modern software packages provide cleaning procedures like evacuating the tip or needle followed by a repeated aspiration. Such kind of intelligent work around tool can also be programmed to move the tip slightly to avoid a swab or foam layer in a tube or to make several aspiration attempts before requesting user intervention or move on to the next sample. Those kinds of events will be highlighted in reports that are provided after completion of the run.

The respective events (e.g. missing sample(s)) are flagged as an error.

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Additional benefits provided by pressure based LLS

Two additional benefits from pressure-based LLS method can be identified:

a) Error detection on aspiration

Any clogging material (e.g., particles or clusters) present in the liquid can cause blocking of the tip opening. In this case the pressure drops below the minimum set values and a tip blockage is detected. The minimum set values need to be predetermined by test runs as they vary with aspiration/dispense time. To minimize false errors the limits can be adjusted. Insufficient sample (e.g., air bubbles in the liquid) or incorrect aspiration can also be detected when the pressure changes dramatically during aspiration.

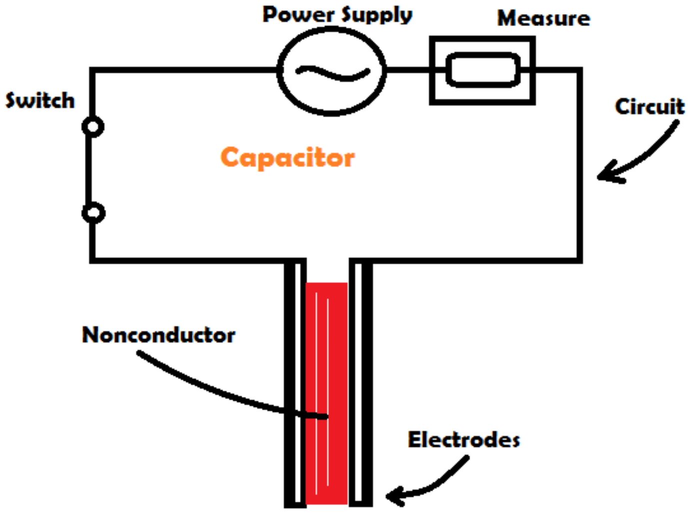
b) Error detection on dispensing

Pressure-based LLS also makes it possible to detect any kind of blockage in the tip. A blocked tip will be detected if the pressure increase above the maximum pressure limit during the dispense phase. Clogging may appear in case some particles or plugging material is sticking at the tip opening or in case of the tip is touching the bottom of the vessel. Those events might lead to a poor pipetting result. Such kind of tip blockings can be detected and indicated in the error messages. When the pressure decreases below the minimum pressure limit during dispense, leaking seals can be detected as well.

2. The capacitive liquid level sensing method

Capacitive sensing is based on the electrical potential between the pipetting channel and the lab ware container.

Fig. 2. Principle of a Capacitive Liquid Level Sensor



Capacitive liquid level sensing was one of the first techniques of performance monitoring for workstations and is still used. The principle is based on measuring the capacitive potential between two conductive surfaces that are in close proximity and electrically isolated by a nonconductor.

When a voltage or potential is applied to the circuit the two conductors are at different potentials (voltages) and the system is capable of storing an electric charge. Thus conductive materials (tips and an ionic liquid) are mandatory for this kind of applications.

Fig. 3a: Conductive robotic tips (Ritter Black Knights) 1000µl (racked)

Fig. 3b: Conductive robotic tips (Ritter Black Knights) 200µl (single)

Fig. 3c: Conductive robotic tips (Ritter Black Knights) 50µl (single)

The above shown kinds of Liquid Level Sensing tips are made from carbon black conductive polypropylene and are designed to work in automated liquid-handling systems. When using conductive tips the shift in capacitance between tip and an ionic liquid is measured. From this data the software can determine the level of the liquid surface and take appropriate action.

For reliable liquid level sensing, the capacitive change upon tip touching the liquid surface must be significantly greater than the summation of all other non-contact changes. The higher the difference between the dielectric constants of the two environments is, the easier the measurement will be. The dielectric constant of a material can change due to variations in temperature, moisture and humidity. Polar compounds will have higher dielectric constants. The following are some common dielectric constants at 20°C, 1 Atmosphere (unless otherwise stated).

Table 1: Dielectric constants of different materials

Material	**Dielectric Constant** 20°C, 1 ATM unless stated
Vacuum	1.0
Air	1.00059
Hexane	1.9
Cyclohexane	2.0
Mineral Oil	2.1
Teflon	2.1
Benzene	2.284
Polyethylene	2.25
Polystyrene	2.6
Polyvinyl chloride	3.18
Glass	3.7
Ethanol	24.3 (25°C)
Liquid ammonia	25 (-78°C)
Methanol	33.1
Glycerin	42.5
DMSO	48.0
Water	80.4

Reference: LabAutopedia.org

As most of the plastic lab ware that is mainly used in automated liquid handling robots tends to build up static loading, has sometimes been difficult to distinguish from the capacitive change caused by the dielectric constant of the surrounding lab ware and the liquid level, particularly in dry air environments. Obviously, this also eliminates using capacitive sensing to determine the presence of non-polar solvents or mineral oil.

The benefits of using the capacitive LLS are firmly based in the durability of this technology as the sensor contains no moving parts, is rugged, simple to use and easy to clean. Capacitive sensors in liquid handling have a variety of uses but are limited to conductive liquids.

3. Ultrasonic liquid level sensing methods

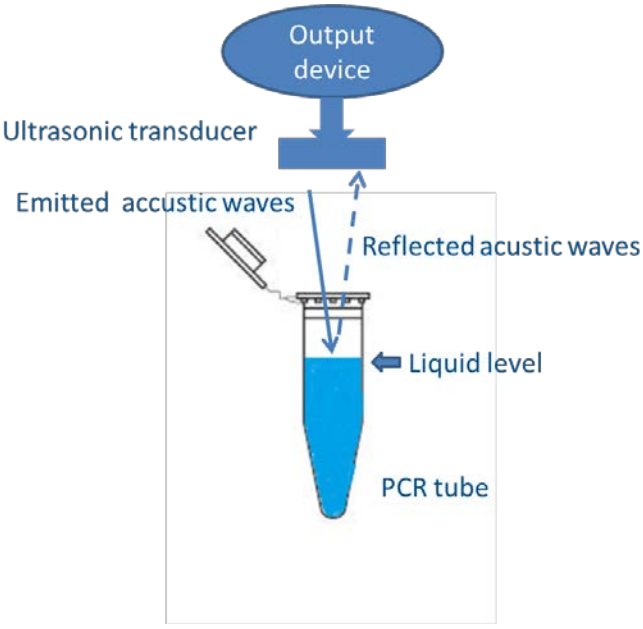
Ultrasonic-based liquid level sensing has recently appeared in the laboratory workstation environment.

New developments in piezoelectric crystal technology have enabled miniature sensor fittings into robotic liquid handling pipetting heads.

The ultrasonic approach uses high-voltage pulses (vibrations) from a piezoelectric crystal. These vibrations are reflected back off the nearest surface and generate a voltage that is monitored in the piezoelectric crystal.

The return time together with the known velocity of the signals in the air allows calculation of the distance to the encountered surface. Thus the liquid level in a vessel can be determined. Level sensors based on ultra sonic technology are frequently used for non-contact level sensing of highly viscous liquids. As ultrasonic liquid level sensing is affected by the changing speed of sound, the accuracy of the measurements can be strongly influenced by variations in moisture, temperature, and pressure. Correction factors need to be applied to the level measurement to improve the accuracy of measurement.

Fig. 4: Principle of ultra-sonic level sensing



One of the advantages of Ultrasonic liquid level sensing is the non-contact measurement that enables a large number of measurements to be executed in a very short time.

As PCR and qPCR methods tend to become increasingly miniaturizes to meet demands for higher throughput and cost effectiveness, the lab ware used in robotic liquid handlers has also turned to smaller vessel diameters. This has led to some applicative limitations for the currently available ultrasonic sensor modules. Thus ultrasonic liquid level sensing mostly goes beyond just liquid level sensing, as it can be used just like radar or sonar to map the working deck of a workstation. Such capability could be used to confirm the proper setup of a workstation deck prior to starting a run. It could also be used to eliminate manual mechanical teaching of deck fixture locations and/or for fine tuning positions over time.

4. Optical liquid level sensing methods

A furtherapproach for the determination of the liquid levels in microplates and vessels is based on optical techniques, mostly performed by CCD cameras.

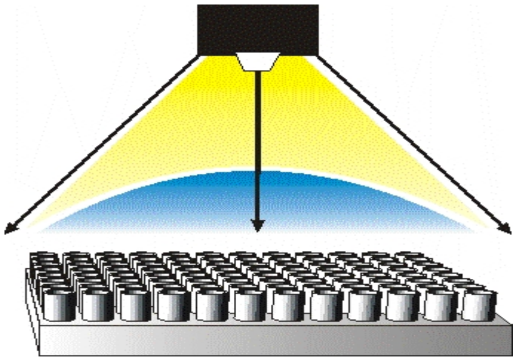
A CCD-camera (Charge Coupled Device) is in principle an analog device that is composed of an array of photosensitive diodes that create a small electrical charge in each photo sensor when light strikes the chip.

The charges of each pixel are converted to voltage at a time and read from the chip at a time. The voltage is converted into digital information by an additional circuitry in the camera.

The measurement of the liquid levels using CCD cameras is also combined with special algorithms that evaluate and visualize the measured data. Camera systems are fast (less than one second to take a picture) but are also very sensitive to ambient light.

Taking a picture of the whole 96-well plate needs to be optimized and adapted to ambient light as well as to the form and position of droplets in the vessel. Badly positioned droplets; the potential for quantitative volume estimation from two-dimensional droplet projections, fluid properties, and the shape of the vessels are influencing the measurement. Also the light paths for each well do have an influence to the quality of the signal. The distance traveled by light is shorter to middle wells than corner wells on bigger (e.g. 96 well) blocks. This can be compensated with optics but is never 100%. Thus the speed benefit of optical liquid level sensing is weakened by the drawbacks for intensive image processing.

Fig. 5: Light intensity across a 96 well plate – a parabolic effect can be observed



In addition to fill-level detector systems (capacitive and pressure based systems), the CCD-detector technology could theoretically provide a way of monitoring individual pipetting steps, but the time taken for such kind of an approach would be considerably longer as level detection and pipetting steps need to be performed sequentially. Such capability is very useful to confirm the proper setup of a workstation deck prior to starting a run. It could also be used to eliminate manual mechanical teaching of deck fixture locations and/or for fine tuning positions over time.

As optical sensors detect contactless the presence or intensity of light and convert that to an electrical signal, they offer a substantially larger detection range compared to inductive or capacitive sensors. But they are also more complex, expensive and prone to their own unique failure modes.

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