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Liquid level detection (LLD) in high-throughput screening applications

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Accurate and reliable liquid level detection (LLD) is a key feature of pipetting robots suitable for performing highly repetitive pipetting tasks in high-throughput screening and in vitro diagnostic applications.[1,2] Today, the state-of-the-art technique for pipetting robots to detect liquid surfaces is the measurement of a combination of capacitance and conductivity while the pipette is moving toward the liquid surface. Once the surface is reached, a signal change is measured, indicating the position of the surface. However, capacitive LLD is restricted to conductive, or more precisely, polar liquids. Different suppliers of laboratory equipment apply different solutions for detecting non-conductive liquids. Here, the author presents measurements of pressure and capacitance to detect liquid surfaces as implemented in the Microlab® series of pipetting robots **(Hamilton Bonaduz AG,** Bonaduz, Switzerland).

Fig. 7 Microlab STAR pipetting workstation.

Experimental

The results were obtained using a Microlab *STAR* -Sequential Transfer and Aliquoting Robot *(Figure* 7). The multichannel pipetting device works on the basis of a pure air displacement pipetting principle, like a handheld pipette, without any system liquid. This opens up the possibility of building a pressure and a temperature sensor into each of the eight independent and freely spreadable pipetting channels of the robot. The pressure sensor can then be used for pressure-based LLD.

To obtain reliable pressure measurements, tight coupling of the pipetting tips to the pipetting head is of crucial importance. The unit is equipped with CO-RE Technology (Compression-induced O-Ring Expansion) pipette tips (patents pending). As shown in *figure 2,* a motor moves a cylinder at the lower end of the pipetting head to compress the O-ring. The O-ring expands and holds the tip, which has a cylindrical connector instead of the widely used conical one. This technique allows the tip/head connection to be sealed very accurately without applying high forces, and thus enables real-time pressure measurement. In addition, this feature allows for high positional accuracy of the tip and an aerosol-free tip release.

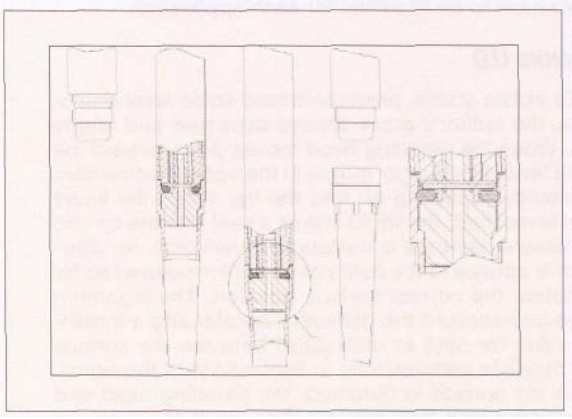

Fig. 2 The CO-RE technology to tightly couple disposable tips or reusable, disposable steel needles to the pipetting head of the Microlab STAR. The O-ring is painted black. This technology allows for high positional accuracy of the tip and an aerosol-free tip release.

Results & Discussion

Capacitive/conductive LLD

As already pointed out, the measurement of a combination of capacitance and conductivity to detect surfaces of conductive liquids is the state of the art for pipetting robots. On approaching the liquid surface

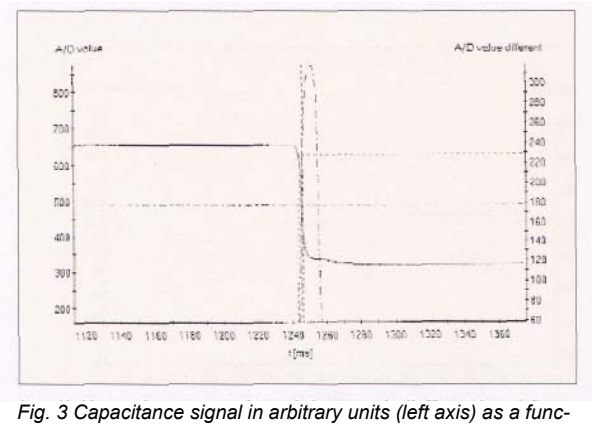

Fig. 3 Capacitance signal in arbitrary units (left axis) as a function of time (solid line). The surface is detected 5 msec after the signal started to change (approx. 1250 msec after the channel started to move toward the surface). This is indicated by the perpendicular broken line. After the detection, the pipetting head stops moving. In addition, the threshold capacitance C is plotted as a horizontal broken line (- - -). A gradient (right axis, -.-.-) and the mean value before and after surface detection (- _ - _ -) are given as well. The liquid is distilled water with 0.1% (weight) of KCl added.

with the pipetting head using a conductive tip, a decrease in the capacitance signal AC(t) is measured, due to the conductive connection between the instrument deck and the pipetting head. This sharp decrease during 10 msec is shown in *figure 3* for the detection of a water surface. Even for liquids with low conductivity such as distilled water or dimethylsulfoxide (DMSO), a change in C can be measured so as to detect the surface. However, AC and At also depend on the size of the reagent container because stray capacitance also contributes to the signal change. Therefore, sensitivity levels have to be specified for each application.

Pressure LLD

To obtain stable, pressure-based liquid level detections, the author's group always uses new and empty tips. When the pipetting head moves down toward the liquid level, the plunger moves in the opposite direction, constantly aspirating air into the tip. When the liquid level is reached, the liquid makes a seal with the tip and *a* pressure decrease is measured in real time. An algorithm is applied to the data points $p_n(()$ measured so far to detect the correct surface position. The algorithm takes into account the derivative Ap(!)/At and a threshold value for Ap(r) to distinguish between the surface and possible perturbations or fluctuations of the signal. Once the surface is detected, the pipetting head and the plunger stop their motions. The result of a pressure LLD measurement on the Microlab *STAR* is shown in *figure 4.* After an initial decrease of pressure, a minimum is reached at *t*= 1290 msec. Then, the pressure starts to increase again to reach a plateau at *t* > 1400 msec. This plateau is, compared to the initial pressure (at least on the time scale of this experiment), reduced by the hydrostatic pressure p/-/of the liquid that is aspirated into the tip. 3~5

***p(t* > 1400 msec) = *p(t* < 610 msec) -**

Both capacitance and pressure LLD detect the surface in less than 10 msec after the start of the signal change (or at approx. 1250 ± 5 msec as measured from

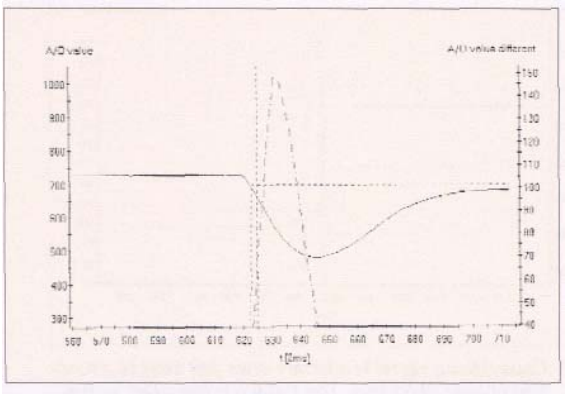

Fig. 4 Pressure signal (left axis) in arbitrary units as a function of time (solid line). The surface is detected 10 msec after the signal started to change (approx. 1250 msec after the channel started to move toward the surface). This is indicated by the perpendicular broken line. After the detection, the pipetting head stops moving. In addition, the threshold pressure is plotted as a horizontal broken line (- - -). A gradient (right axis, -.-.-) is given as well. The liquid is distilled water with 0.1% (weight) ofKCI added.

the beginning of the movement of the pipetting head), although the exact value depends on the chosen sensitivity settings. Comparing the derivatives AC/At and Ap/At for the same liquid, the capacitance changes almost instantly (within 10 msec) whereas the pressure reaches the minimum value more smoothly within 100 msec. This is because the pressure signal p(!) depends on the plunger speed, the relatively slow flow rate of the liquid from the bulk of the vessel into the tip and the density of the liquid.

In contrast to the capacitance measurement, the Ap signal is independent of the vessel size and thus has a potential for LLD even within 1536-well microplates.

Capacitive & Pressure LLD

For difficult cases such as the detection of slightly inhomogeneous liquid surfaces (e.g., bubbles), one may wish to use a combination of both LLD types. This dual LLD feature is incorporated into the Microlab *STAR*. In addition to the sensitivities for pressure and capacitive LLD, an additional parameter has to be defined, giving the maximum height difference between where the pLLD and the cLLD responds. This parameter can be adjusted to meet the needs of a given application. Applying a speed of 100 mm/sec to the pipetting head to move toward the liquid surface, a time difference of 5 msec as measured for the pLLD/cLLD response, gives a height difference of approx. 0.5 mm, which is about the size of a bubble on the surface.

Conclusion

Both LLD types — pressure and capacitive — have different advantages in different situations. Whereas capacitive LLD can also be used in dispensing with full tips, pressure LLD can be applied to any given liquid. With the capability to use both types during the same assay, more flexibility is gained and a higher level of process security is reached in pharmaceutical and genetics applications, such as DNA sequencing and high throughput drug screening.

References

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