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Monitored air displacement pipetting

Monitored air displacement technology has been used to design a pipetting robot which handles all automated liquid-handling tasks with more safety, positional accuracy, flexibility and speed than previously possible.

Dr Christoph Mauracher, Hamilton Bonaduz AC

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In the past, we have all tipped our pipette into a beaker and - through a slight lapse of attention - the tip has broken the surface and we have aspirated air. Through routine visual inspection of the tip, we have realised the error and repeated the step, and the experiment has been saved. Liquid-handling robots do not have the luxury of intelligent visual inspection, nor do they have the time to inspect the volumes, as the aim is to accomplish the pipetting of 384- or even 1536-well plates; nevertheless, the process safety of accurate pipetting, as well as detecting insufficient sample or clogged tips, remains a central demand for liquid-handlers.

At Hamilton, we have radically redesigned the entire liquid-handling process, and have arrived at an instrument which handles all automated liquid-handling tasks with more safety, positional accuracy, flexibility and speed than previously possible. The following data will illustrate some of the new technologies incorporated into this novel instrument.

Instrumentation

The newly introduced liquid-handling robot (the Microlab[8] STAR) is intended to perform medium- to high-throughput pipetting tasks in the laboratory (Figure 1). On its large workspace, 4, 8 or 16 individual channels can perform independent pipetting tasks. The individual channels are mounted on a moveable axis, on which they can move in an independent fashion. Each channel is a self-controlled pipette moving in an independent up and down direction (z axis), and performing its own pipetting task (Figure 2).

Figure 1. Front view of the Microlab® STAR.

The instrument uses air displacement, rather than the more common column movement of liquid via syringe drives. The departure from this more conventional technology allowed the design of a more flexible instrument. Each individual channel contains two important sensors - one for temperature and one for

Figure 2. View of the individual pipetting channels.

Temperature sensor

As air is a medium which significantly changes volume in relation to temperature, the channel's temperature sensor allows the correction of the pipette's stroke height. This ensures the accurate performance of the pipette throughout the changing temperature of the working day.

Pressure sensor

Semiconductor-based pressure sensors are connected directly to the air-space of die pipetting chamber. As liquid is aspirated, the pressure within the chamber drops and is filled with aspirated liquid; during the end of pipetting, the pressure returns to the original pressure, minus the pressure exerted by the liquid column now present in the tip. The pressure development is monitored continuously during the pipetting process in all channels.

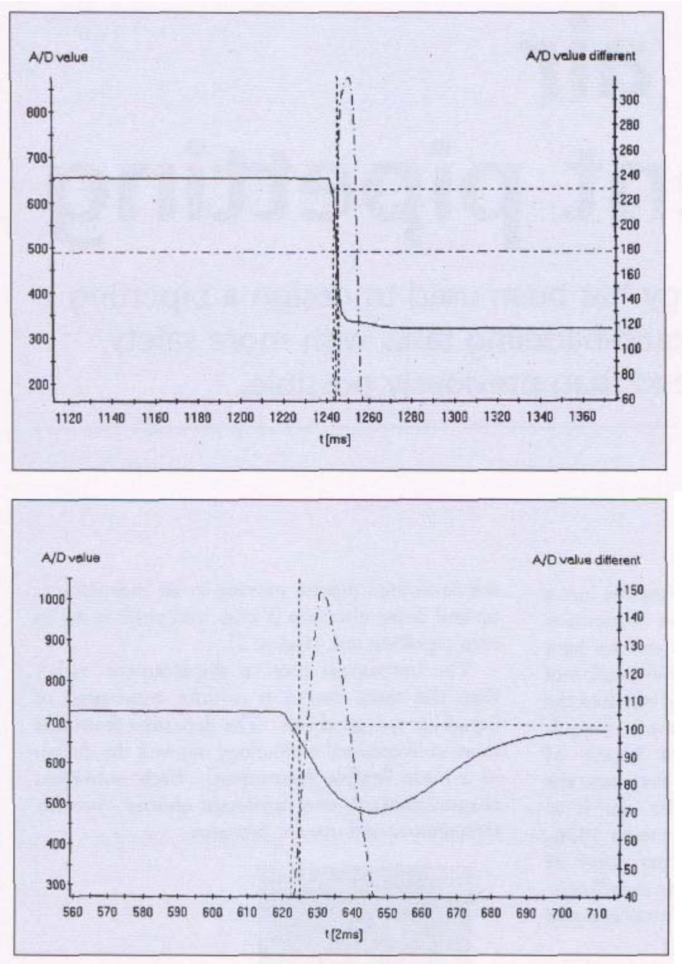

Figure 3. A typical capacitance (top) and pressure (bottom) measurement during liquid aspiration.

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Dual liquid level detection (LLD)

With the inclusion of pressure-monitoring, the process safety of pipetting has taken a significant step forward. Today, the state-of-the-art detection of liquid surfaces involves a combination of the measurement of capacity and conductivity while the pipette is approaching the liquid surface. Once the surface is reached, a signal change is measured indicating the level of the surface. However, two significant limitations are still encountered using this method:

- non-polar liquids cannot be detected, and
- bubbles and foam can result in false signals leading to air aspiration.

To provide a solution to this limitation, we have combined the capacitance liquid level detection (LLD) with the pressure LLD. As the pipette reaches the liquid surface, the capacitance change is detected and the pressure drop within the pipette chamber is also measured, as liquid is aspirated (Figure *3).*

The absence of a pressure change when using aqueous liquids indicates a wrong capacitance change due to foam on the surface, and corrective responses can be programmed into the system (for example, abort, pipette until pressure LLD is positive, and so on). Another novelty is the facility to provide a liquid level detection for non-conductive organic liquids. By introducing the combination of two LLD methods, we have now arrived at an unparallelled process which allows for:

- Increased safety of the pipetting process,
- Avoidance of errors due to foam or bubbles, and
- Liquid level detection of non-conductive liquids.

The pipetting step

The pressure detection within the channels is extended one step further with the Microlab® STAR. The entire aspiration and dispensing process is monitored; this allows the following important factors to be checked:

- Tip clogging,
- Sufficient sample, and
- Correct volume aspiration.

In Figure 4, we examine the pipetting of homogenised tissue samples used in the new Prionics ELISA assay for the diagnosis of BSE (bovine spongiform encephalitis) in cow brain. Three examples are shown during the pipetting step, with the pressure within the pipetting channel (p (t)) plotted versus time (t). As the system aspirates, the pressure within the channel drops (blue line); as liquid enters the tip, the pressure remains at a constant level and then returns back to the starting level (minus the pressure exerted by the liquid column in the tip). The blue profile is indicative of a normal pipetting step. The green pressure line indicates a sample with too little liquid, with the pressure prematurely returning to its original level. By programming the expected pressure behaviour over time, abnormal pressure curves and low sample errors can be detected. The third curve indicates a blocked tip (red line). During the aspiration step, the tip is clogged with tissue fragments; consequently, the pressure within the channels decreases continuously as no liquid fills the tip.

The instrument may either recover by initiating an automated repeat cycle, or it may simply be programmed to flag the sample and continue with other samples.

The importance of the pipette tip

With the introduction of pressure-monitoring in the chamber, one last technical difficulty needed to be solved - that of a constant seal between the tip and the pipette head. Hamilton approached this task through a complete redesign of the tip coupling - as the standard approach of a conical head pressed on the pipette is not sufficiently reliable. The patented CO-RE tip technology was found to provide a solution to the problem (Figure 5). The tip is mounted on a cylindrical pipette tip, and an O-Ring is compressed to hold the tip. In its expanded form, the O-Ring sits tightly in a groove of the pipette tip; this ensures a defined seal and perfect fit. Upon relaxation of the O-Ring, the tip can be gently released from the pipetting channel. This revolution in tip design allowed us not only to have a pressure-tight tip coupling, but also provided a solution to the following important requirements:

- Positional accuracy is guaranteed by the cylinder shape of the pipette head and by the accurate z-height definition through a shoulder in the tip,
- Tip release is gentle and does not cause aerosol contamination, as is commonly observed during normal tip ejection,
- Disposable tips and steel needles can be coupled in an interchangeable manner,
- Three tip sizes can be chosen and used interchangeably - low volume (0.5 - 10ul),medium volume (5 - 300ul) and high volume (10- 1200ul), and
- Pipetting accuracy with individual tips has been validated to be 3%.

Conclusion

Hamilton has chosen to pioneer a novel pipetting concept and has introduced these on its Microlab STAR pipetting workstation. Through the introduction of monitored air displacement technology, we have arrived at a pipetting robot with unrivalled flexibility, and work and process safety, incorporating features such as:

- Positional accuracy of < 0.05 mm with tips or needles,
- Independent movement of 4, 8 or 16 pipetting channels,

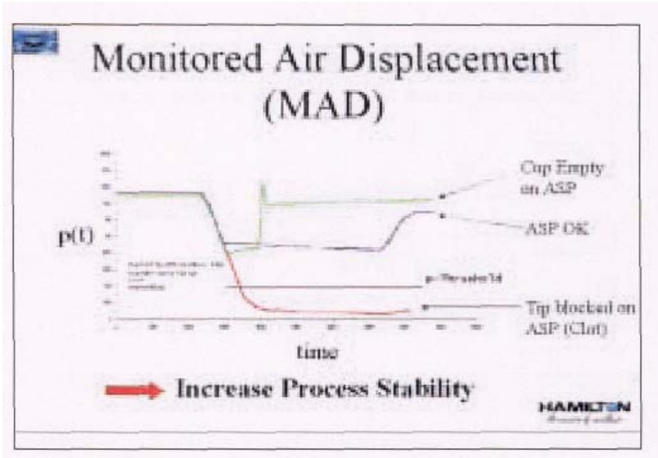

Figure 4. Monitored air displacement during the pipetting of liquids.

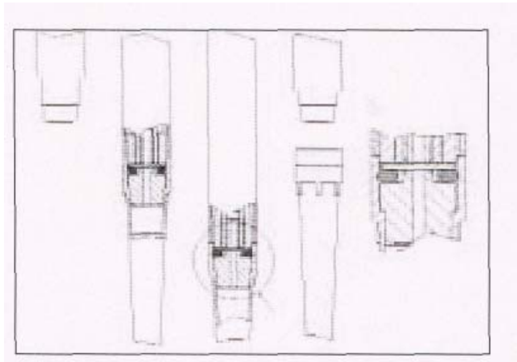

Figure *S*. CO-RE Tip Technology. Tip coupling through an expandable O-Ring.

- Dual liquid level detection,
- Highest process security through monitored air displacement features, and
- No aerosol or system liquid contamination.

For laboratories in which these features are important in routine applications, then we believe we have succeeded in providing an attractive solution.

Dr Christoph Maumcher is the International Sales Director of Hamilton Bonaduz AG, Switzerland. Following his training in Pathology at the University of British Columbia, he joined Roche as a Research Scientist and later as a Business Director. At Hamilton, his focus has been on optimising the sales and service organisation, as well as ensuring the customer-oriented development of Hamilton's range of instruments. The Microlab STAR is the most recent example of how the company incorporates technological advances into products developed

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