Outline

Introduction

Measurement methods
- “Offline” methods
- “Online” methods

Quantitative Benchmark

Conclusions
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Liquid Handling

Important task for biochemical assays preparation

- In-vitro diagnostic (IVD) tests
- Pharmaceutical research / production
- Biotechnology
- ...

Automation by workstations

- IVD turnkey systems
- Pipetting robots
- ...

[ Source: Roche Diagnostics GmbH ]
Low Volume Liquid Handling

Low volumes are usually below 1µl

General Requirements

- Non-contact micro dosage
  - Prevent volumetric errors due to adhesion
  - Avoid cross contamination
- High accuracy & precision in delivery
- “Online“ verification of delivered volume is desirable

Precise volumetric measurement (no standards yet!)


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Volumetric measurement standards (no available yet!)


Peter Koltay
Low Volume Measurement Approaches

Volumetric measurement (offline)
- Determine volume of liquid aliquot
- Liquid volume is consumed by the measurement e.g. by gravimetric method

Flow / droplet measurement (online)
- Determine transient flow rate / droplet volume during delivery
- Liquid volume can still be used for the given purpose e.g. by measurement through flow sensor

Challenges for Flow Rate Measurement

Transient flow rate during dispensing
- Short duration of flow
- Relatively high flow rate (~μl/s)

High accuracy & precision
- Independence of viscosity / temperature

Integration into automation
- Frequent automatic exchange of liquids
- No cross contamination between liquids
Challenges for Flow Rate Measurement

Transient flow rate during dispensing

- Short duration of flow
- Relatively high flow rate (~µl/s)
  
  Fast response time (~ 1 ms)

High accuracy & precision

- Independence of viscosity / temperature
  
  Absolute calibration & traceability

Integration into automation

- Frequent automatic exchange of liquids
- No cross contamination between liquids
  
  Large number of sensors (automatic exchange)

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Traceability & Standardization
Dual-dye ratiometric photometry

Based on optical absorption
- Complete measurement system commercialized by Artel Inc. (USA)
- Artel MVS system has traceability to NIST in the range 30nl to 200μl
- Carried out in microtiter plates (MTP)

Reference method
- Standard measurement method for manual pipette calibration (ISO 8655-7)

Limitations
- Works only with special calibrated solution

Gravimetry

Based on weight of liquid mass
- Various vendors of ultra-precision balances exist
- Traceability of most sensitive balances in the range 0.1μg to 2mg
- Carried out on balance tray

Reference method
- Standard measurement method for manual pipette calibration (ISO 8655-7 / ASTM E542)
- Pharmaceutical research
- Liquid handling companies
Gravimetric Regression Method (GRM)

Primary measurement devices is ultra-microbalance
- Weighing dish prefilled with test liquid (~ constant evaporation)
- Silicon oil on top of weighing dish (~ hardly any evaporation)

Isolation from environment
- Wind shield cover for balance and in addition for whole setup
- Vibration isolating granite table
- Non-contact dosage of volume

GRM working principle

Basic approach
- Linear regression on balance readings to account for evaporation
GRM Benchmark with Artel MVS

Both methods are in good agreement

GRM provides almost constant measurement uncertainty
\( U(V20) \approx 6\text{nl}, k=2 \)

- With silicon oil layer to reduce evaporation uncertainty can be improved to \( U(V20) \approx 6\text{nl}, k=2 \)

Artel MVS provides smaller uncertainty in range below approx. 500 nl

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**Stroboscopic Imaging**

Based on stroboscopic image of free flying droplets

- Stroboscopic image acquisition
- Image processing by computer algorithms
- Volume reconstruction from 2D image by rotation

Established method for inkjet droplet characterization

**Limitation**

- Works only on small droplets (< 100nl)

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**Capacitive droplet sensor**

Based on interaction of liquid droplet with electric field

- Open capacitor as transducer
- Droplet passes through electric field
  - causes change in capacity
- Capacitance increase
  - dielectric constant
  - droplet volume
  - \( V_{\text{drop}} < 100 \text{ nl} \rightarrow \Delta C < 3 \text{ fF} \)
- Analog amplification/ readout on board by adapted electronic circuit

Capacitive measurement principle
Capacitive droplet sensor

Sensor prototype developed in PCB technology

Sensor Signal investigated
- Sensor signal depends on volume, velocity & position
- CFD simulation model established to study effects

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Quantitative measurement
- Evaluation algorithm needed to account for velocity dependence of signal peak ($U_{\text{max}}$)
- Using droplet velocity determined from the zeros of the signal and $U_{\text{max}}$ the volume can be calculated

Consistency with gravimetric reference
- Prognosis interval (95%) @ ± 3nl

Air Flow Sensor Method

Thermal flow sensor mounted in back of reservoir to sense air flow replacing the dispensed volume

- Integral of air flow equals liquid volume

Advantage of measuring air instead of liquid

- Method is independent of liquid properties
- No cross contamination of liquids


Air Flow Sensor Method Validation

Time resolved sensor signal measured in parallel with gravimetric method

- Individual droplets can be identified in the flow signal
- Total volume calculated based on calibrated sensor

<table>
<thead>
<tr>
<th>Number of droplets</th>
<th>Gravimetric reference</th>
<th>Sensor signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83 nl (10.8%)</td>
<td>84 nl (10.5%)</td>
</tr>
<tr>
<td>3</td>
<td>132 nl (40.2%)</td>
<td>135 nl (44.6%)</td>
</tr>
<tr>
<td>10</td>
<td>579 nl (60.1%)</td>
<td>574 nl (52.3%)</td>
</tr>
</tbody>
</table>

Volume from flow integral matches well with gravimetric reference volume
Air Flow Sensor Method Validation

Method works well with different liquid types even at small volumes

Quantitative measurements with good reproducibility need

- Fast & sensitive flow sensor (small SNR)
- Absolute calibration of sensor
- Correction for air density / property variations due to
  - Pressure
  - Temperature
  - Humidity

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Multi Principle Measurement Setup

Multiple measurement methods executed on the same individual droplet

- Quantitative benchmark of the individual methods
- Under identical conditions

Investigated methods

- Gravimetric (reference)
- Stroboscopic
- Capacitive droplet sensor
- Flow sensor (air replacement)
Benchmark Results

All methods are in very good agreement

- Gravimetric and stroboscopic method are very robust
- Flow sensor is sensitive to environment (sound / convection)
- Capacitive sensor is sensitive to misalignment of the nozzle

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Precise quantitative measurement of low volumes can be achieved by various methods (online / offline) consistently

- Flow sensing (air replacement) is one promising online method
- Requirements are different than for low stationary flows
  - Fast response times & relatively high flow rates (media independent!)
  - Accurate & traceable measurements require absolute media independent calibration which is challenging (viscosity, temperature, convection, …)

Which measurement method is most suitable for a given application depends on many parameters

- Volume range & liquid properties
- Prevention of cross contamination & online measurements

We want to thank the partners of the Smart Reagent Dosing project:

Thank you very much for your attention!