Metrological Methods for Low Volume Liquid Handling in Drug Delivery and In-Vitro Diagnostics

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Outline

Introduction

Measurement methods

- "**Off**line" methods
- "**On**line" methods

Quantitative Benchmark

Conclusions

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- "Offline" methods
- "Online" methods

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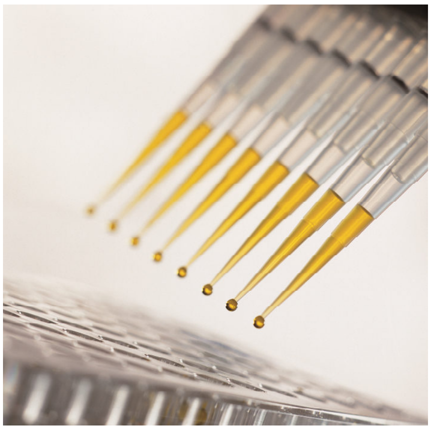
Liquid Handling

Important task for biochemical assays preparation

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



[Source: Bernd Müller]

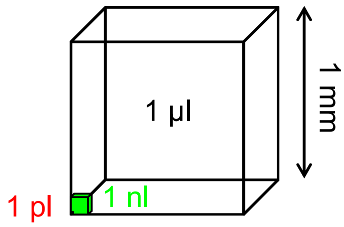
Automation by workstations

- IVD turnkey systems
- Pipetting robots
- …

[Source: Roche Diagnostics GmbH]

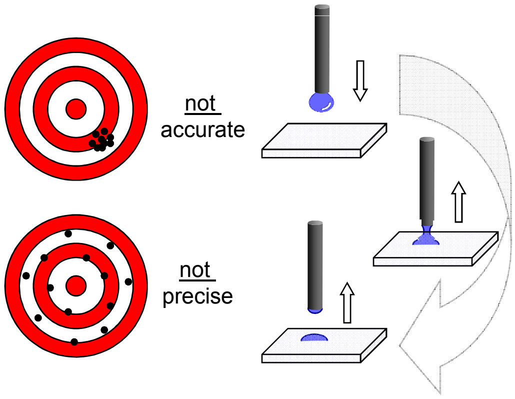
Low Volume Liquid Handling

Low volumes are usually below 1µl



General Requirements

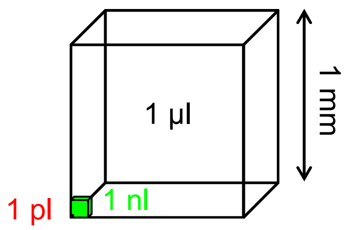
- Non-contact micro dosage
  - Prevent volumetric errors due to adhesion
  - Avoid cross contamination
- High accuracy & precision in delivery
- *"Online*" verification of delivered volume is desirable



Precise volumetric measurement (no standards yet!)

Low Volume Liquid Handling

Low volumes are usually below 1µl



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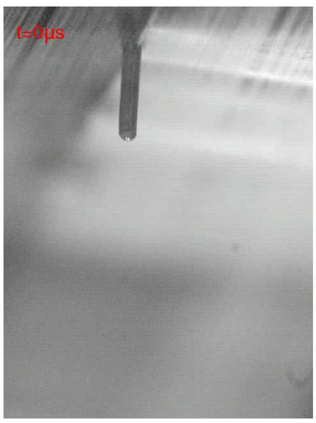


Volumetric measurement standards (no available yet!)

Low Volume Measurement Approaches

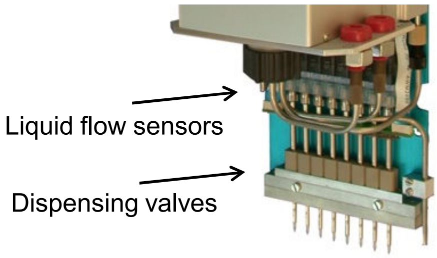
Volumetric measurement (offline)

- Determine volume of liquid aliquot
- **Liquid volume is consumed** by the measurement e.g. by gravimetric method

[Source: Mettler Toledo, Missisauga, Canada]

Flow / droplet measurement (online)

- Determine transient flow rate / droplet volume during delilvery
- **Liquid volume can still be used** for the given purpose e.g. by measurement through flow sensor

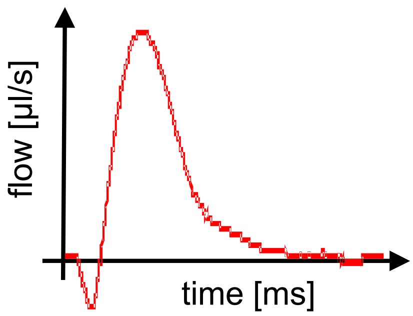


[Source: Seyonic, SA, Switzerland]

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

[Source: Seyonic, SA, Switzerland]

Challenges for Flow Rate Measurement

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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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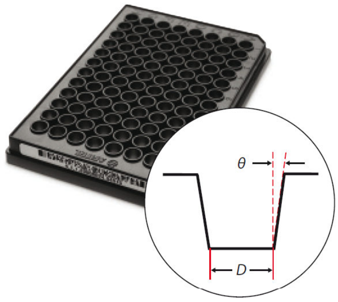
Quantitative Benchmark

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)



[Source: Artel Inc., Westbrook, ME, USA]

Reference method

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

Limitations

- Works only with special calibrated solution

Artel MVS measurement system

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

[Source: Mettler Toledo, Missisauga, Canada]

Reference method

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

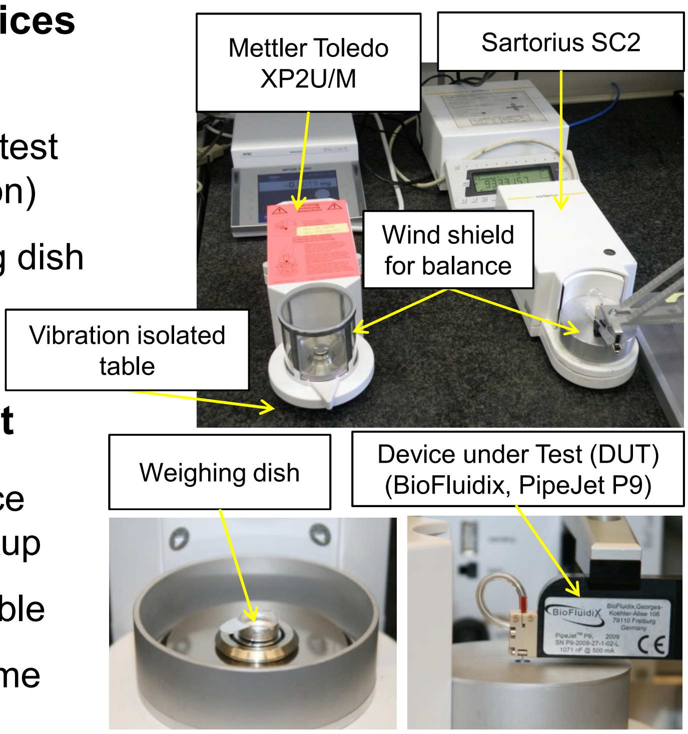
Gravimetric Regression Method (GRM)

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

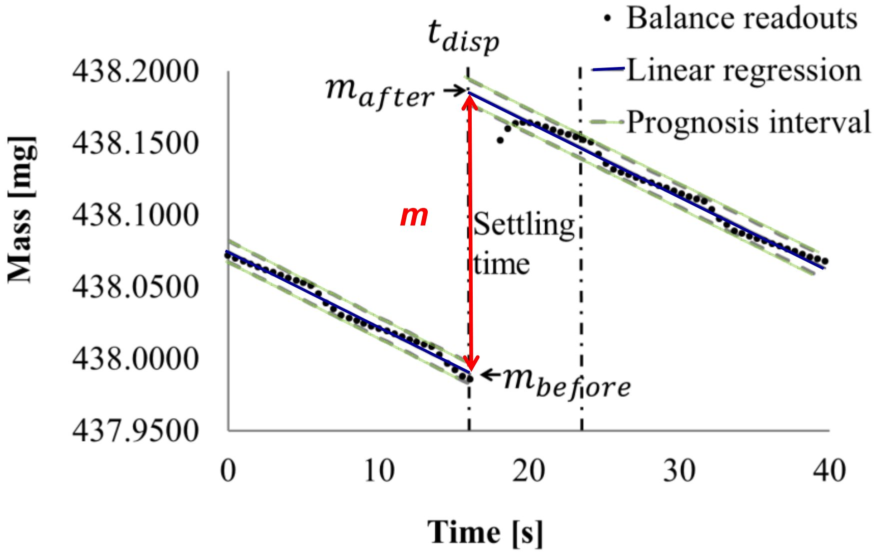
- Wind shield cover for balance and in addition for whole setup
- Vibration isolating granite table
- Non-contact dosage of volume



GRM working principle

Basic approach

- Linear regression on balance readings to account for evaporation



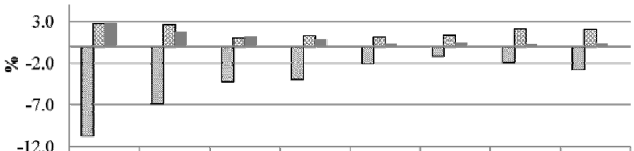
GRM Benchmark with Artel MVS

Both methods are in good agreement

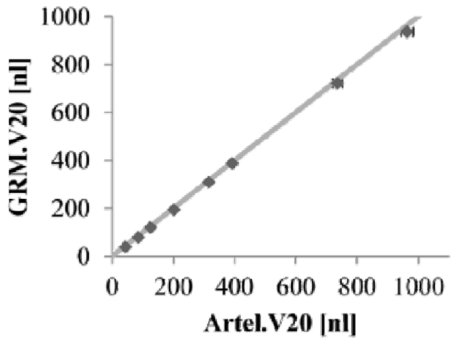
GRM provides almost constant measurement uncertainty U(V20) ≈ 6nl, k=2

- With silicon oil layer to reduce evaporation uncertainty can be improved to U(V20) ≈ 6nl, k=2

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



	~40 nl	~80 nl	~120 nl	~200 nl	~320 nl	~400 nl	~760 nl	~1µl
Bias [nl]	-4.7	-5.8	-5.3	-8.0	-6.4	-4.5	-14.1	-26.5
Bias %	-10.7	-6.9	-4.2	-4.0	-2.0	-1.1	-1.9	-2.8
CV(Artel.V20) %	2.8	2.6	1.0	1.3	1.1	1.3	2.1	2.0
CV(GRM.V20) %	2.8	1.7	1.2	0.8	0.3	0.5	0.3	0.4
U(Artel.V20),k=2 [nl]	1.0	1.8	2.3	4.6	6.5	7.6	16.5	21.0
U(GRM.V20),k=2 [nl]	12.5	12.5	12.1	12.6	13.0	12.2	12.1	12.0



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Stroboscopic Imaging

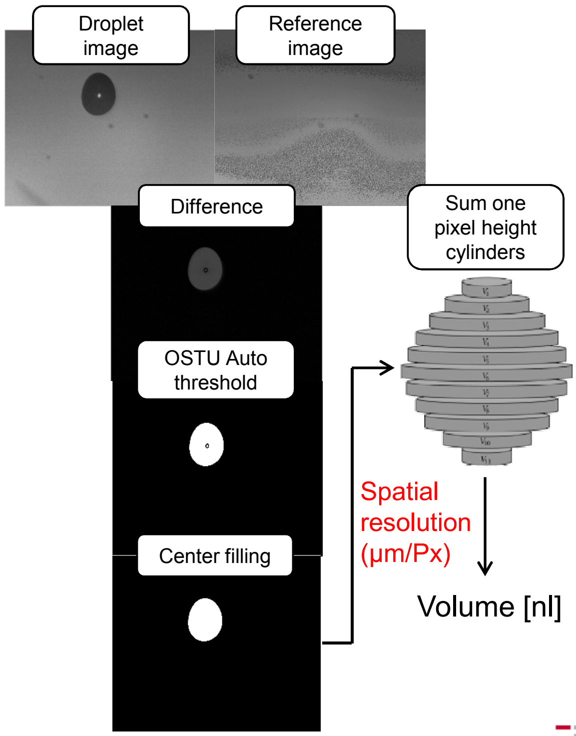
Based on stroboscopic image of free flying droplets

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

Established method for inkjet droplet characterization

Limitation

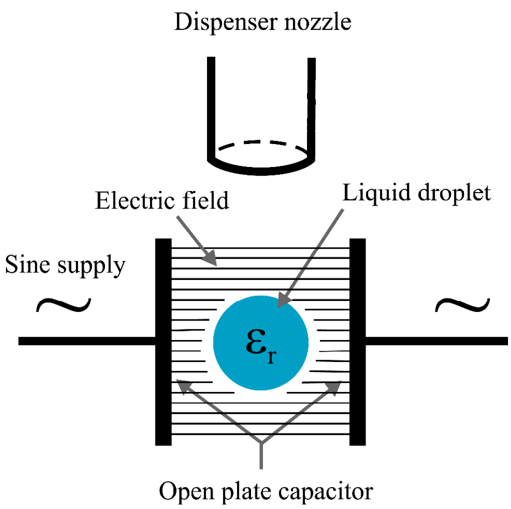
- Works only on small droplets (< 100nl)



Capacitive droplet sensor

Based on interaction of liquid droplet with electric field

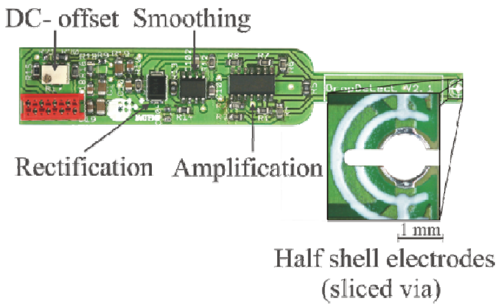
- Open capacitor as transducer
- Droplet passes through electric field
  - causes change in capacity
- Capacitance increase
  - dielectric constant
  - droplet volume
  - $V_{drop} < 100$ nl → $\Delta C < 3$ fF
- Analog amplification/ readout on board by adapted electronic circuit

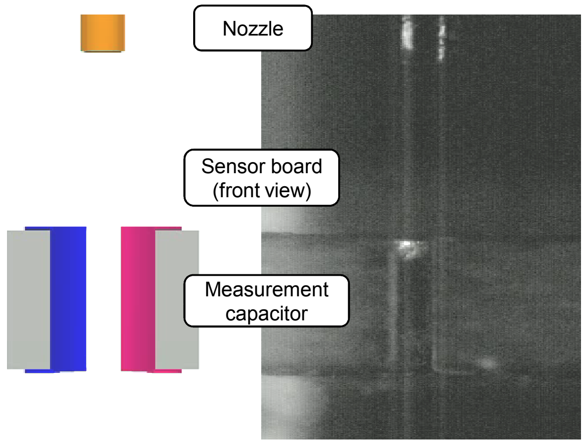


Capacitive measurement principle

Capacitive droplet sensor

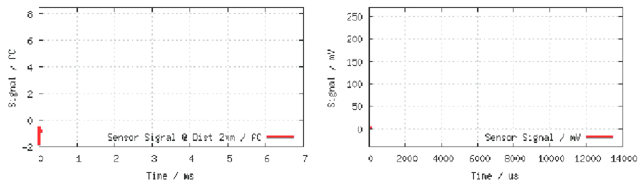
Sensor prototype developed in PCB technology





Sensor Signal investigated

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

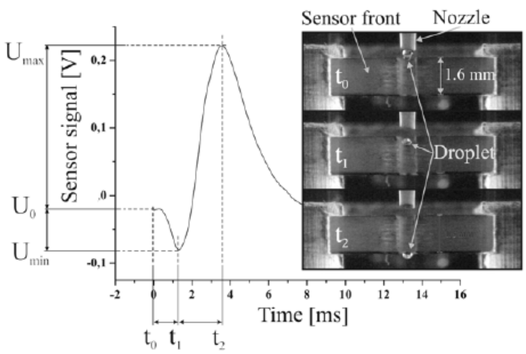


droplet volume ≈ 30 nl

Capacitive droplet sensor

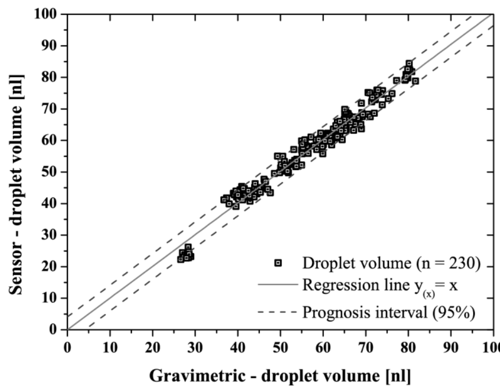
Quantitative measurement

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



Consistence with gravimetric reference

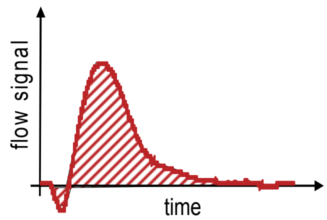
- Prognosis interval (95%) @ ± 3nl



[Ernst, A., et al. "A capacitive sensor for non-contact nanoliter droplet detection." *Sensors and Actuators A: Physical* 153.1 (2009): 57-63., DOI: 10.1016/j.sna.2009.04.023]
http://www.sciencedirect.com/science/article/pii/S0924424709002209

Air Flow Sensor Method

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

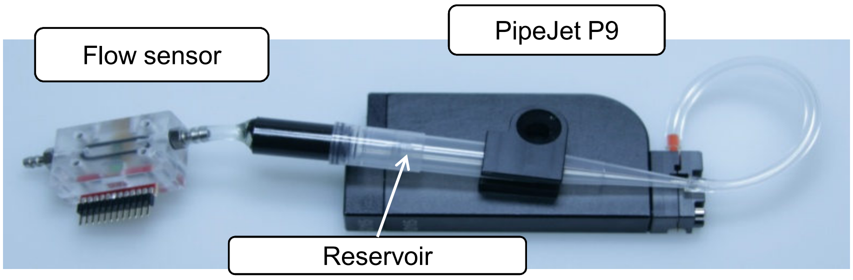


- Integral of air flow equals liquid volume

Advantage of measuring air instead of liquid

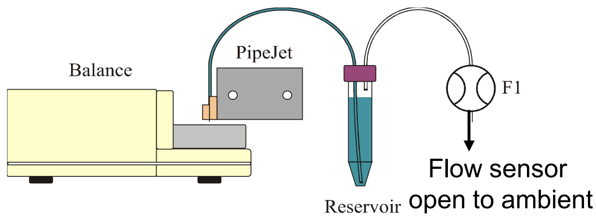
- Method is independent of liquid properties
- No cross contamination of liquids

[Streule, W., et al. "Kontaktfreie und medienunabhangige Volumenbestimmung in Nanoliter Dispensern." *Mikrosystemtechnik Kongress 2005*. VDE VERLAG GmbH, 2005.]



Air Flow Sensor Method Validation

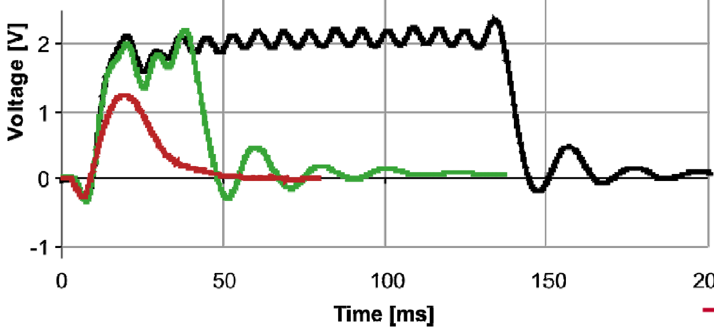
Time resolved sensor signal measured in parallel with gravimetric method



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

	Number of droplets	Gravimetric reference	Sensor signal
	1	53 nl (±0.6%)	54 nl (±5%)
	3	132 nl (±0.2%)	125 nl (±4%)
	15	576 nl (±0.5%)	574 nl (±2.3%)

Volume from flow integral matches well with gravimetric reference volume

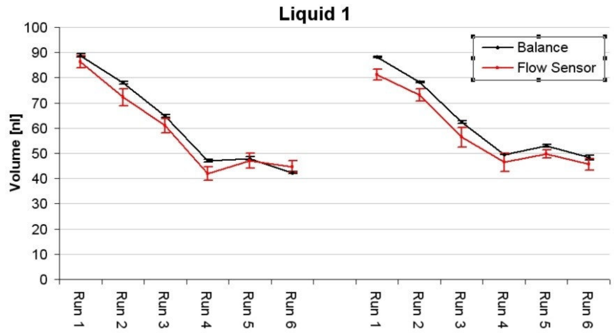


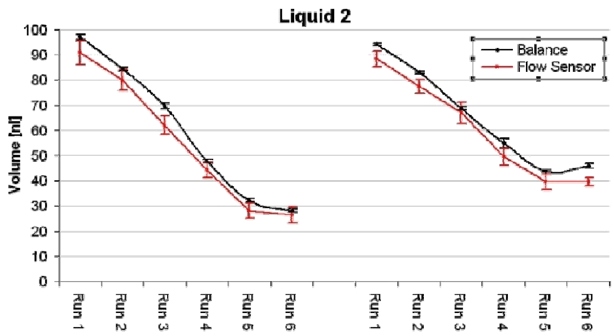
Air Flow Sensor Method Validation

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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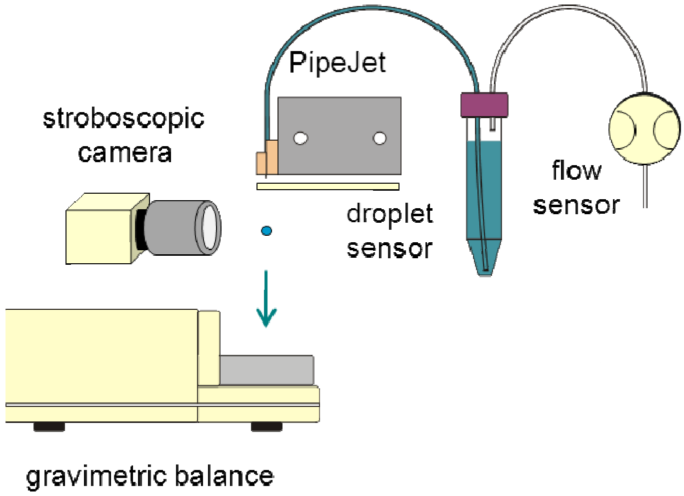
Multi Principle Measurement Setup

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)

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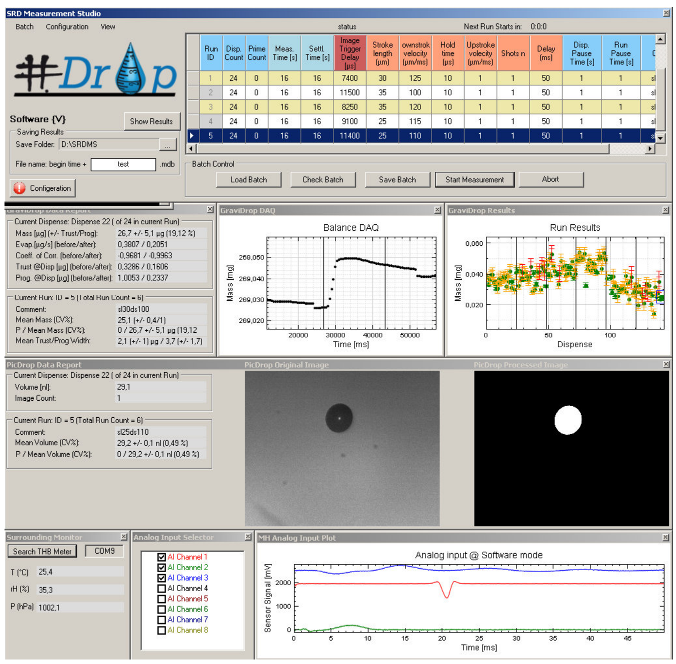

Investigated methods

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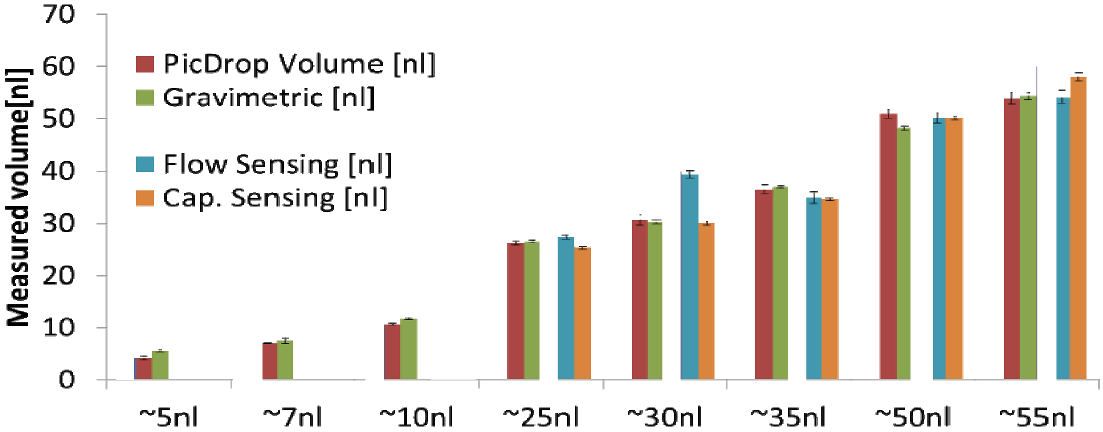
Benchmark Results

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!