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**INFORMATION TECHNOLOGY AND
ELECTRICAL ENGINEERING -
DEVICES AND SYSTEMS,
MATERIALS AND TECHNOLOGIES
FOR THE FUTURE**

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Setup for colorimetric measurements of aqueous micro- and nanoliter droplets

The analysis of liquids represents one of the most important fields in clinical and pharmaceutical laboratory everyday work and research. Common analysis systems utilize cuvettes with a typical volume of a few 100 μl or are based on closed fluidic systems (lab-on-a-chip). With regard to cost- and analysis efficiency the reduction of sample volume is of great interest [1].

We present a setup for colorimetric measurements in an open fluidic system for micro- and nanoliter droplets. The setup is part of a novel integrated GaN-based system for physical, biological and chemical analysis. Combined with a transparent AlGaIn/GaN-based pH-sensor [2] it allows simultaneous electronic and optical analysis of droplets.

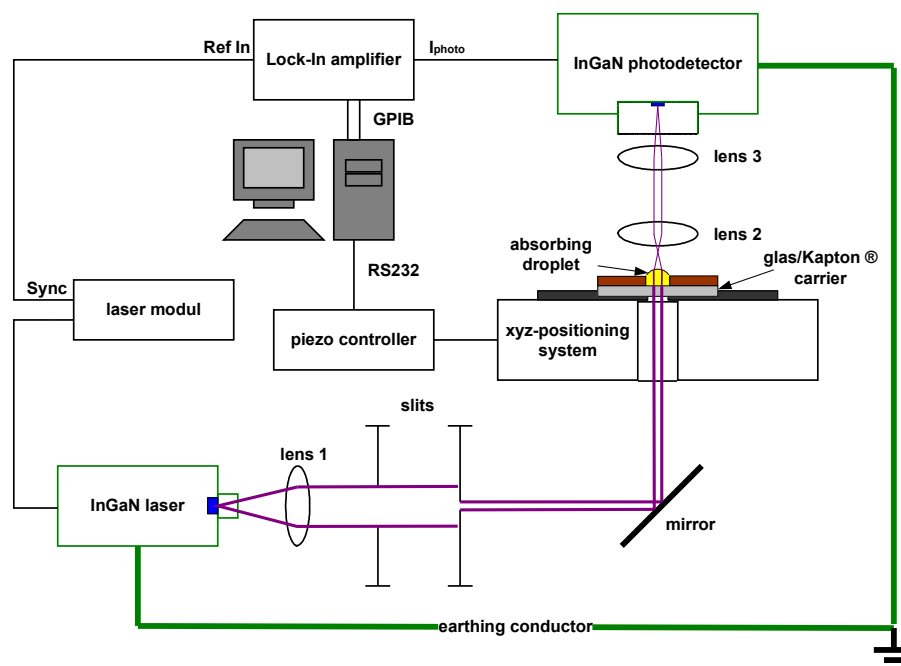


Figure 1: Experimental setup for optical analysis of water based droplets.

A combination of an InGaN laser diode and a novel selective sensitive InGaN photodetector [3] is used to measure optical absorbance of droplets (Fig. 1). The sample droplet is pipetted and positioned manually in the collimated laser beam. To account for the influence of the droplet geometry on the path of light a model for describing the focal length as a function of droplet volume and radius is derived (Fig. 2). The detector optics were adjusted accordingly to focus the transmitted light onto the detector.

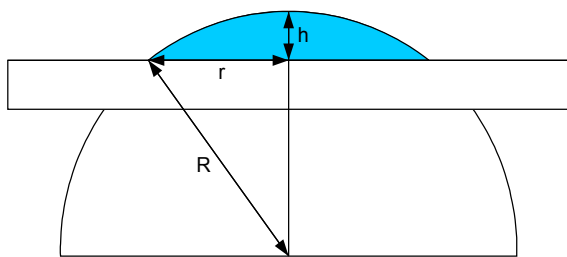


Figure 2: Model of droplet geometry.

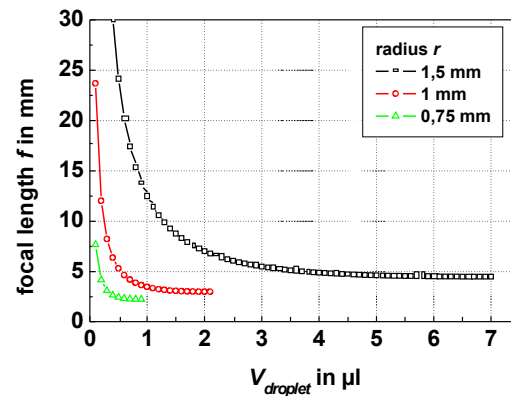


Figure 3: Calculated focal length for different droplet volumes $V_{droplet}$ and radius r .

Positioning accuracy, droplet evaporation and the regulation of humidity as well as the influence of light, temperature and electromagnetic fields limit the accuracy and sensitivity of the setup. To guarantee reproducibly formed droplets a polyimide film with a thickness of 125 μm was structured onto a glass carrier (Fig. 4). The positioning of the droplet with respect to the laser beam was performed by a piezo-driven xyz-positioning system. Droplet evaporation was limited by installing the setup into a glove-box and increasing the humidity to 70 %rH. Ambient light had no influence on the detector signal due to bandpass like sensitivity of the employed InGaN photodetector. Changes in ambient temperature resulted in fluctuations of the measured signal as the laser diode was not stabilized. They were minimized by the temperature control of the glove-box. High frequency electromagnetic interference from the pulsed laser diode could only be suppressed partially and turned out to be the limiting factor for measuring absorbance greater than 1. The temporal stability of the detector signal was measured for different droplet volumes over several minutes and was determined to be ± 1 % (Fig. 5).

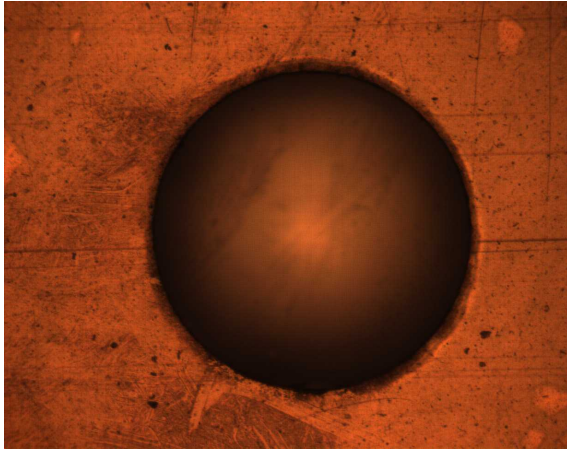


Figure 4: Structured polyimid film with a 5 μ l droplet of water.

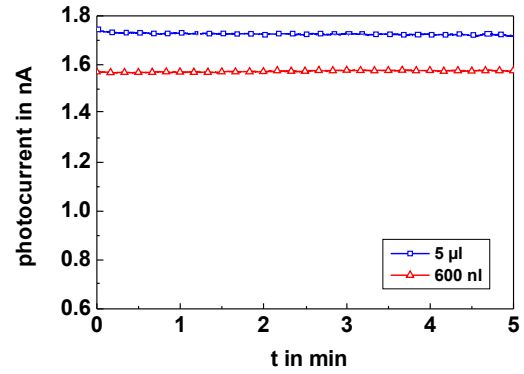


Figure 5: Stability of detector signal for different droplet volumes.

Paranitrophenol (Sigma-Aldrich) was used as a model system for colorimetric measurements. This dye is released in various enzymatic assays and can also be used as a pH indicator. Measurements for different concentrations show that the error in calculated concentration increases dramatically for absorbance lower than 0.1 (Fig. 6). Therefore the working range of this experimental setup with an error of less than 10 % is from 0.1 to 1 absorbance. It could be noticed that in this range the measurements agreed very well with values obtained by a state-of-the-art spectrophotometer (Fig. 7).

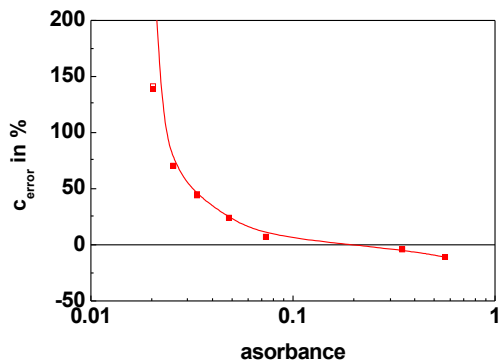


Figure 6: Error in measured concentration with regard to droplet absorbance.

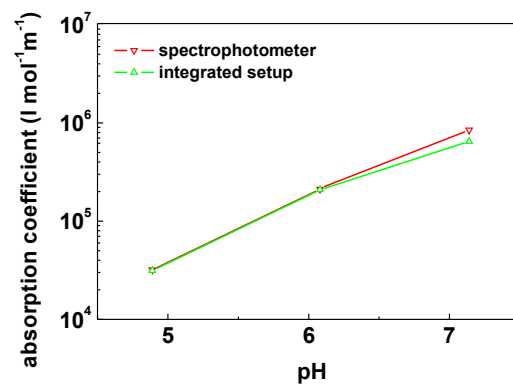


Figure 7: Measured absorption coefficient of paranitrophenol for different pH-values of the droplets.

The setup was sufficiently stable to observe dynamic chemical reactions. The release of paranitrophenol in an enzymatic assay was measured and the reactivity of the enzyme was determined (Fig. 8).

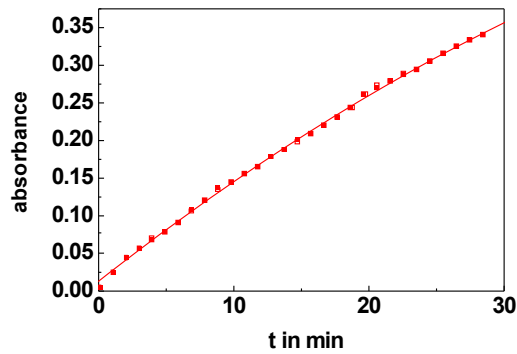


Figure 8: Increase of absorbance due to release of paranitrophenol during enzymatic conversion of acetylparanitrophenylphosphat.

The developed fluidic setup is capable of measuring absorbance in μl - and sub- μl droplets. Stable and reproducible measurements of dye concentration were made between 0.1 and 1 absorbance. Dynamic chemical reactions in μl reaction volumes could be measured.

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