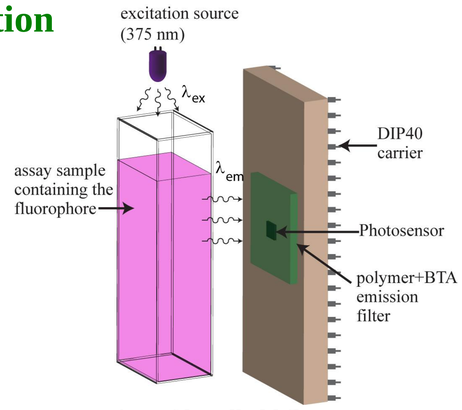
Microfluorometry

Nicole Nelson∗, David Sander∗, Marc Dandin†, Anshu Sarje∗, Somashekar Prakash∗, Pamela Abshire∗
∗Department of Electrical and Computer Engineering, Institute for Systems Research
†Fischell Department of Bioengineering, University of Maryland, College Park

Integrated Biomorphic Information Systems

Introduction

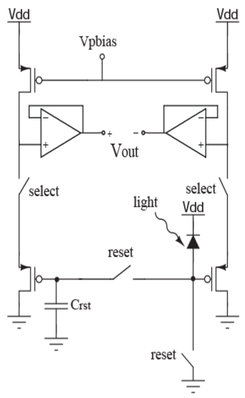
- **Fluorescence sensing is a powerful tool in modern biology and is used in many applications including DNA analysis, pathogen detection, drug delivery, cell imaging and counting and monitoring ion concentration**
- **Fluorescence measurements require excitation light, emission filter and a detector**
- **Platform for microscale fluorometry. Everything except the light source fits inside 1mm³.**



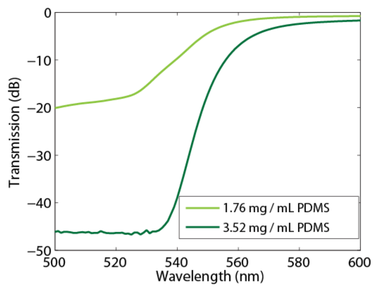
Overview of handheld fluorometer

Fluorometer = custom filter + detector + LED

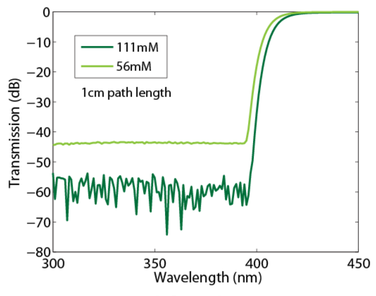
- **Fluorometer consists of a CMOS active pixel sensor with in-pixel CDS, coupled with a custom chromophore-polymer emission filter and a UV LED as the excitation source.**
- **Detector is differential active pixel sensor**
  - **Differential readout minimizes correlated noise sources**
  - **Fabricated in 0.5 μm 2-poly, 3-metal CMOS process**
- **Filter is a chromophore in polymer matrix cast onto die.**
- **Two filters fabricated with long pass characteristics (cut on @ 400nm and 540nm)**
- **Both filters proven to be biocompatible**



Differential active pixel sensor



Sudan II in PDMS



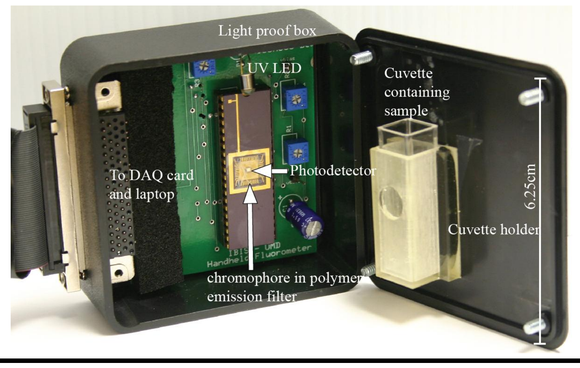
BTA in toluene

SUMMARY OF DIFFERENTIAL ACTIVE PIXEL SENSOR CHARACTERISTICS

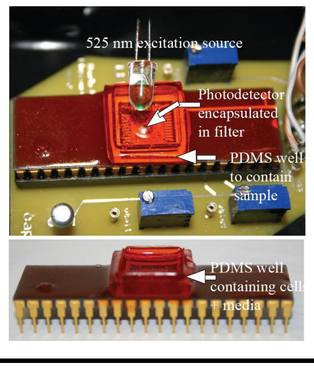
Readout noise	175.3 μV
Reset noise	360 μV
Supply voltage	5 V
Power consumption	68 μW
Dynamic range	59 dB
Maximum signal	3.5 V
Dark signal	4.1 mV/s
Conversion gain	530 nV/e$^-$
Detection limit	2.2× 10^8 photons/cm^2

Experimental Configuration

- **Configuration for excitation light provided by external LED and standard cuvette as sample holder**

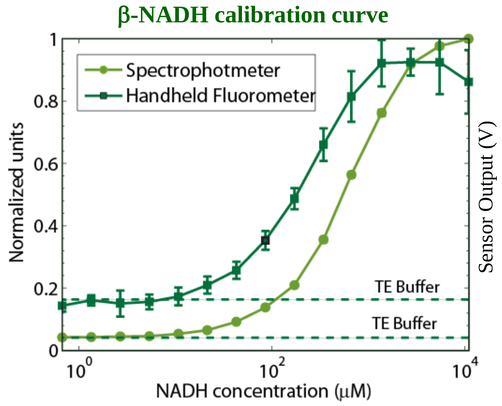


- **Configuration for excitation light provided by external LED and custom well as sample holder**



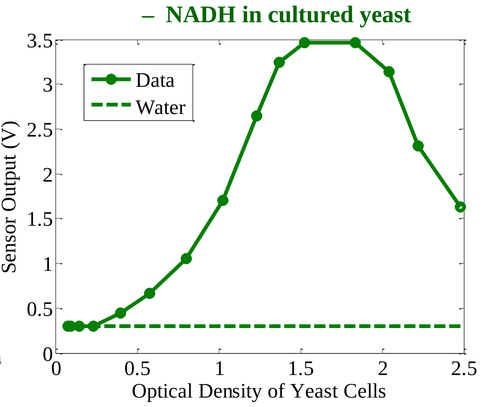
Experimental Results

Metabolic Activity Assay I – β-NADH calibration curve



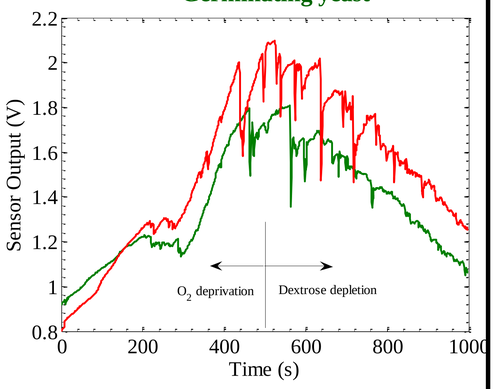
- **Excited with 375nm, peak emission @ 460nm (blue). BTA is used in emission filter**
- **Serial dilution of β-NADH from 17.62mM to 1.08μM**
- **β-NADH calibration compared with measurements from a standard spectrophotometer**

Metabolic Activity Assay I – NADH in cultured yeast



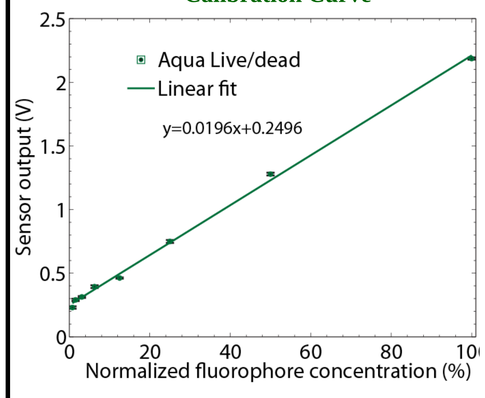
- **Yeast cells cultured for 2 days @ 37ºC**
- **Yeast concentration determined using optical density measured in a standard spectrophotometer**
- **Optical density ∝ cell concentration**
- **Sensor V ∝ NADH concentration**

Metabolic Activity Assay I – Germinating yeast



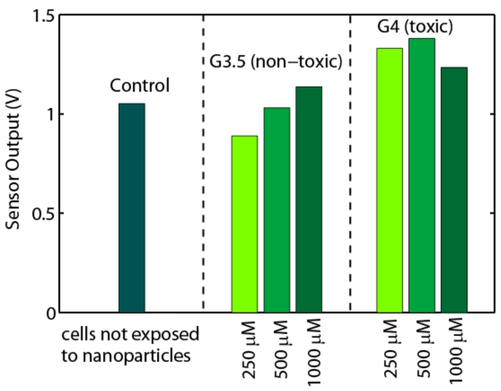
- **Dry yeast added to warm dextrose solution**
- **As O2 supply ↓ NADH level ↑**
- **As dextrose ↓ NADH level ↓**

Cytotoxicity Assay – Calibration Curve



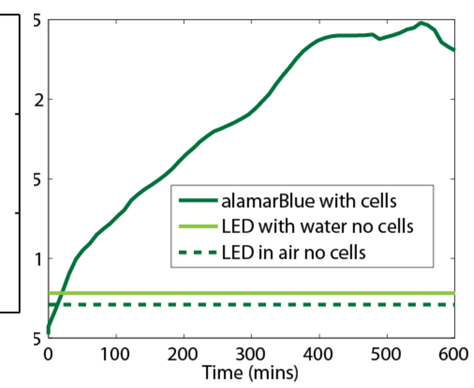
- **Excited with 375nm, peak emission @ 526nm (green). BTA is used in emission filter**
- **Serial dilution of Aqua live/dead reagent from 100% to 12% of recommended dosage shows good linear fit.**

Cytoxicity Assay



- **Cells exposed to two different PAMAM dendrimers (nano-particles), control not exposed to any nano-particles**
- **Compromised cells produce greater fluorescence intensity**

Metabolic Activity Assay II – Alamar Blue



- **Excited with green light, peak emission @ 595nm Sudan II is used in emission filter**
- **BAOSMC cells cultured in PDMS well on top of chip overnight in 37°C, 5%CO_2.**
- **Metabolically active cells reduce non-fluorescent form to fluorescent form.**

Summary

- **Experimental results shown for three biochemical assays**
- **System is portable, easy to use and can be fabricated using standard processes and micro-fabrication steps.**
- **Miniaturization of costly laboratory equipment → useful for lab-on-chip applications.**
- **Can serve as a platform for future applications in microfluorometry.**

Acknowledgements

- **MOSIS service for chip fabrication. This chip was used in teaching an undergraduate VLSI course**
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