

# **Proposal for MOSIS Educational Research Program**

## **Integrated optical detection of fluorescent probes**

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## Research proposal for integrated optical detection of fluorescence

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### Abstract:

We are developing a CMOS based optical interface to be integrated with MEMS structures as part of an effort to build a microarray of Biolabs-on-chip for monitoring and measuring individual cells. This optical interface can be used as a specimen plane imager (contact imager), fluorescence sensor, or luminescence sensor depending on the specific application.

The availability of a large variety of fluorescent probes and advanced imaging and analysis techniques allow chemical and molecular details of individual cells to be assessed. This enables biologists to gain unprecedented insight into single cells' structure and behavior which can not be deduced from results averaged over many cells. However, most existing fluorescence-based biological instruments are complicated, bulky, expensive, and require the infrastructure and resources of a biochemistry lab in order to operate. With continuous advances of CMOS and MEMS technologies, it's appealing and realistic to develop a low-cost, portable, low power, high throughput integrated fluorescence sensor for single cell analysis. Though relaxed spatial resolution is expected due to the absence of a sophisticated optical microscope, the proposed integrated fluorescence sensor will address many important applications in cell biology, biomedicine, environmental science, and homeland security such as cell sorting, high throughput screening, and toxin detection.

This development encompasses several stages including: characterization of quantum efficiency and spectral selectivity of integrated photodiodes, post-processing integration of additional optical films as needed to enhance selectivity, the development of real time imagers for contact fluorescence measurements and cell monitoring, and experimental characterization of the sensors on the bench and with biological samples. In previous work, we characterized spectral sensitivity and responsivity of integrated photodectors fabricated using TSMC 0.35 $\mu\text{m}$  technology. A few prototype CMOS contact imager chips have been designed and fabricated using AMI05 process as class projects through MOSIS Educational Program. In order to take full advantage of advanced CMOS technology, we plan to use TSMC 0.18 $\mu\text{m}$  process (CM018) for our next CMOS contact imager and fluorescence sensor design. We choose this process mainly because of its small feature size and stacked contacts and vias. The small feature size will enable the sensor to achieve the highest spatial resolution possible. Given a fixed pixel area, each pixel can afford more transistors and therefore more options for pixel level signal processing. Furthermore, the silicide block layer (unsilicided active region) and thick gate oxide (3.3V operation) help to enhance the optical sensitivity and dynamic range of sensors respectively. Simulation will be performed using SpectraS and measurement will be performed using standard electronic equipment and techniques as described in further detail below.

### Project description:

Fluorophores (or fluorescent dyes) are molecules which fluoresce after being illuminated. A fluorescent probe is a fluorophore specially designed to localize certain type of components within a biological

specimen or to respond to a specific stimulus. When these molecules come into contact with targeted functional components in cells, fluorescent probes undergo changes in optical properties. The three typical changes are: 1) an increase or decrease in the quantum yield with little change in either absorbance or fluorescence spectra; 2) a shift of the fluorescence excitation spectrum to shorter wavelengths with little shift in the emission peak; 3) a shift in both excitation and emission spectra to shorter wavelengths. These changes make it possible to characterize cell properties using fluorescent probes.

Modern fluorescent probes can sensitively detect many important properties of cell function, including transmembrane voltage, ionic concentration, and pH. Such measurements routinely performed in labs throughout the world typically require large, expensive equipment and instrumentation such as fluorescence microscopes. Efforts at contact fluorescence imaging have been reported, although the associated instrumentation remains peripheral to the system. It is possible and practical to integrate all of the elementary components in this measurement, and we propose to do exactly that: develop an integrated optical interface to characterize single cells using fluorescent probes. This integration requires circuit design, process characterization, and development of post-processing steps as necessary to enhance performance. This work will enable future advances in biosensing and bio-opto-electronic interfaces with cells.

The proposed contact imaging and fluorescence detection project will implement a new design for a fully differential pixel structure to reduce noise, thereby increasing both signal to noise ratio and dynamic range of the sensor. Since fluorescence is very weak, typically in the range of  $10^{-3} \sim 10^{-7}$  lux ( $10^{-5}$  lux corresponds to 4.6 photons/100  $\mu\text{m}^2/\text{s}$ ), the sensor area for fluorescence sensing needs to be large enough to collect several hundred photons per second. On the other hand, the pixel size needs to be comparable to the size of cells in order to perform real time cell monitoring. Therefore, we plan to investigate new imager architecture in order to implement pixel pooling size variation in order to integrate both contact imager and fluorescence sensor into one system.

### **Estimated project size:**

The proposed design includes the pixel array, digital logic for random access to the pixel array, and analog signal processing circuits for noise suppression. The layout size of the proposed design will be confined within a 3mm by 3mm area including bonding pads.

### **Simulation plans:**

All components described above will be designed and simulated using SpectraS (Cadence<sup>TM</sup> tool). We will use SPICE model parameters provided by MOSIS for the TSMC 0.18  $\mu\text{m}$  technology. The layout will be created using the Virtuoso Environment of Cadence<sup>TM</sup>. DRC (design rule checking), LVS (layout versus schematics), and post-layout simulation including the parasitics due to layout will be performed to ensure that the layout design satisfies the desired specifications. The ultimate performance of the system will also depend on the optical responsivity of pixel sensors.

## Test plans:

Independent test structures for design components, including single pixel, logic components, column level readout circuitry, will be included in the design and will be characterized first. The proper bias and clock conditions will be determined from these initial measurements. The whole system will be tested on the bench as a regular CMOS image sensor under normal illumination conditions. Standard performance characteristics for CMOS image sensor, including signal noise ratio (SNR), dynamic range (DR), frame rate, spatial and temporal noise, will be determined at this stage. These performance characteristics will be determined for varying spatial resolution as well.

After the imager has been successfully tested on the bench top, we will package the chip in order to test it as a contact imager. Microbeads will be placed directly on the chip surface and imaged under a variety of lighting conditions. Thus biologically compatible packaging is not required for this stage. Fluorescence detection will be performed as well using the calcium sensitive, UV excited fluorescent probe Fura-2. We will use the standard calcium solutions of calibrated concentration as the test samples.

Finally, we will test the chip with cells. Cells will be kept alive in appropriate culture medium in a biologically compatible package built on top of the chip.

Both optical and electronic instruments will be used to characterize the performance of the image sensor. A list of the main test equipment is given in the following table.

Table 1: A list of equipment for chip characterization

Optics	Electronics
UV LED (Nichia)	Measurement Computing™ data acquisition cards
Tungsten light source	Ubicom™ microcontroller
Mini monochromator	Tektronix™ arbitrary function generator
Integrating sphere	Newport™ Optical power meter
Lenses	HP™ 54503A digital oscilloscope
Filters (neutral density, fluorescence)	

The experimental configuration will be adjusted for each characterization described above.