Detection of MCLR by Capacitance Measurement

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GROUP MAY14-11

The Problem

The problem: Microcystin

- Cyanobacteria (a blue green algae) is found in freshwater sources worldwide
- When in bloom, the Microcystin-LR (MCLR) toxin can be produced
- MCLR can be harmful to aquatic life, humans, and livestock

Photo from
Lamiot in the Wikipedia Commons

The Problem: Microcystin

Many unknowns:

- Toxin release mechanism
- Livestock tolerance
- Effects of secondary consumption

The ability to test for the presence and concentration of this toxin can help to further the current understanding of MCLR.

Photo from Lake Champlain International

The Problem: Detection

Current measurement methods exist

- Sample is taken when bloom is suspected
- Mailed to lab for analysis
- ~7 day waiting period
- Within waiting time, bloom period may have passed—results are no longer useful
- Cost, size, and complexity of machinery eliminate possibility for in-field testing

Our Solution

Our Solution: Circuitry

Design measurement device for MCLR detection

- Small, convenient size
- Simple, non-technical user-interface
- Economically feasible
- Highly accurate for concentrations between
  1 ng/L – 2 ug/L
- Fast measurement

Our Solution: Electrodes

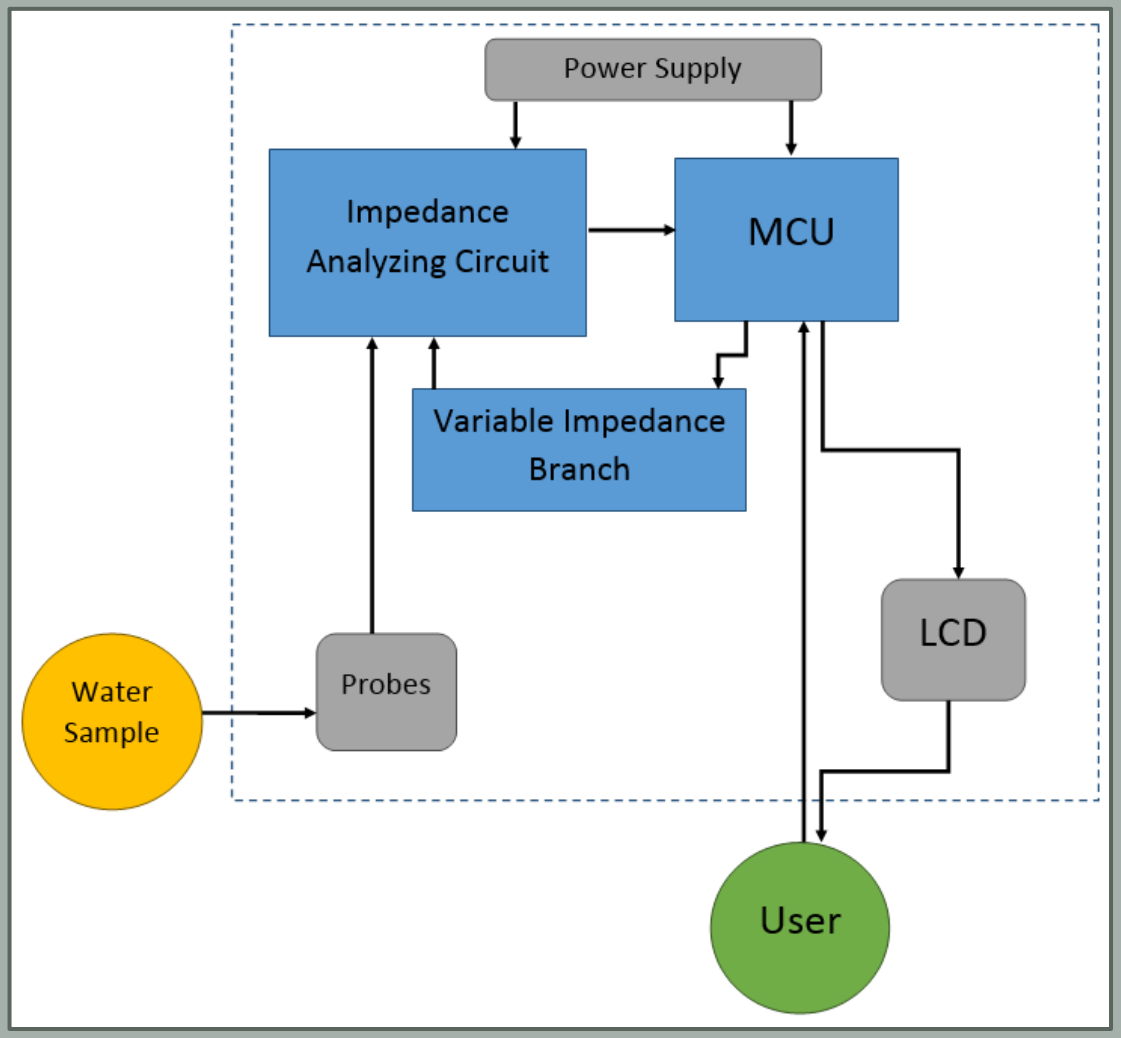
Acquire and prepare special electrodes to detect MCLR

- Select manufacturer
- Apply surface chemistry
- Obtain experimental data

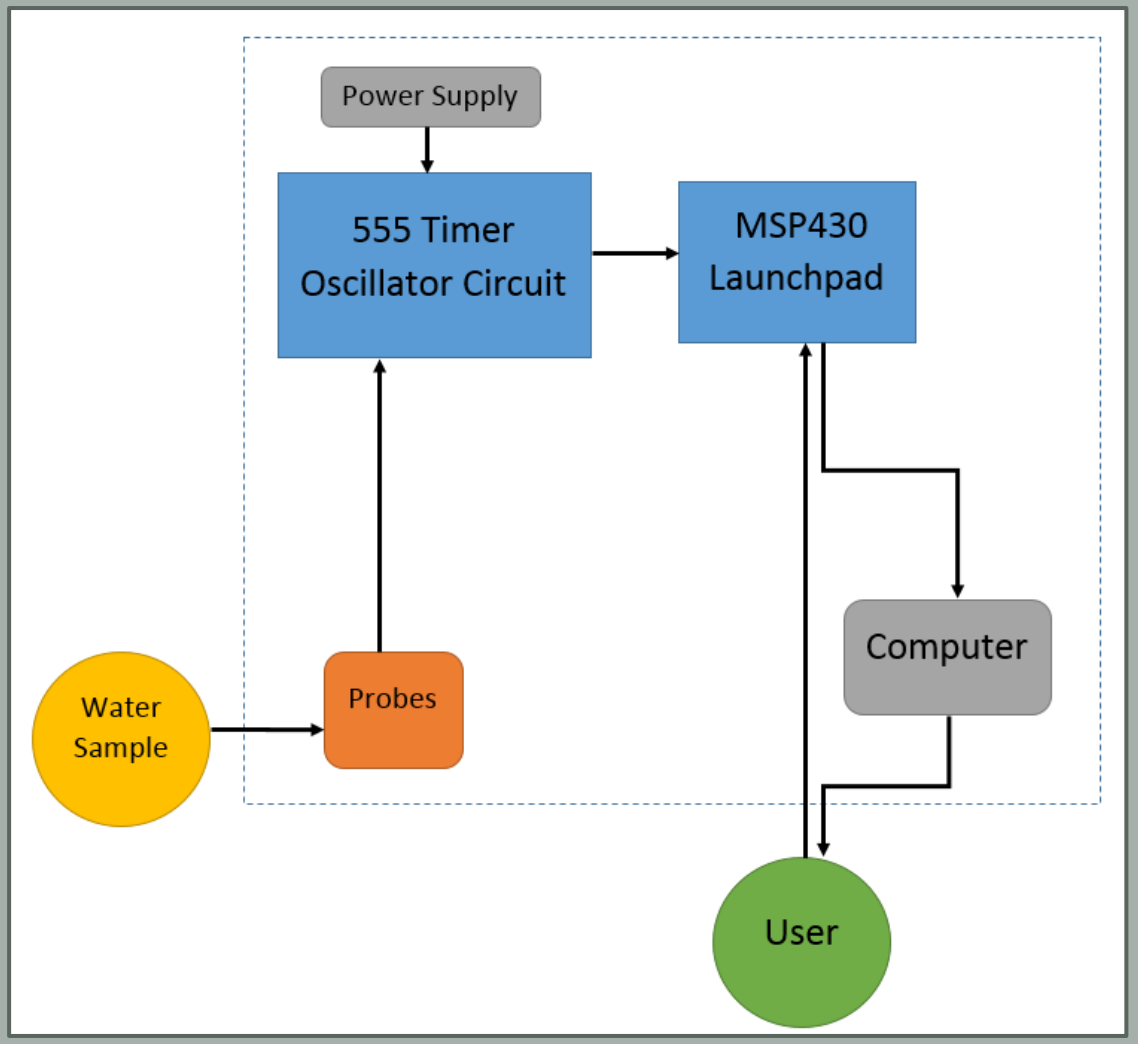
Circuitry

For Capacitance Change Detection

Initial Design

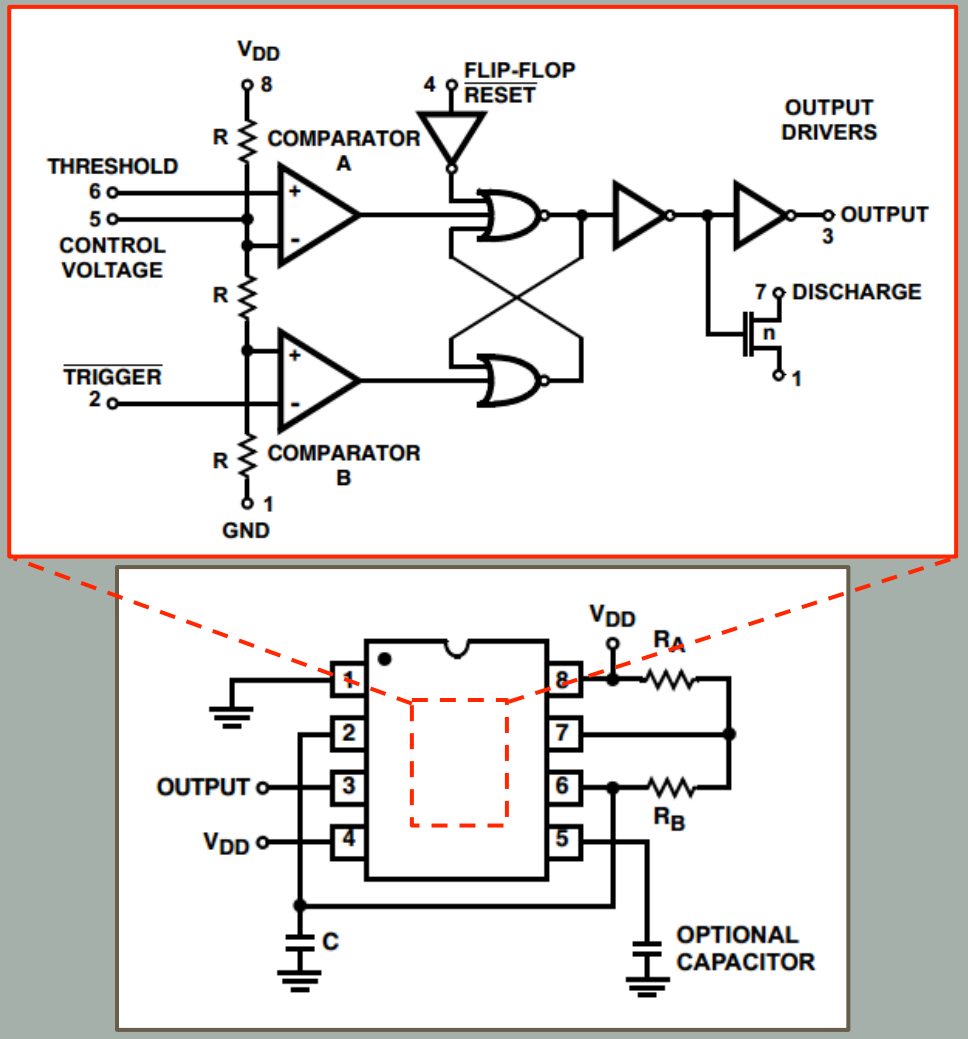


New Design


Power Supply
555 Timer Oscillator Circuit
MSP430 Launchpad
Computer
Water Sample
Probes
User

Capacitance Measurement Method

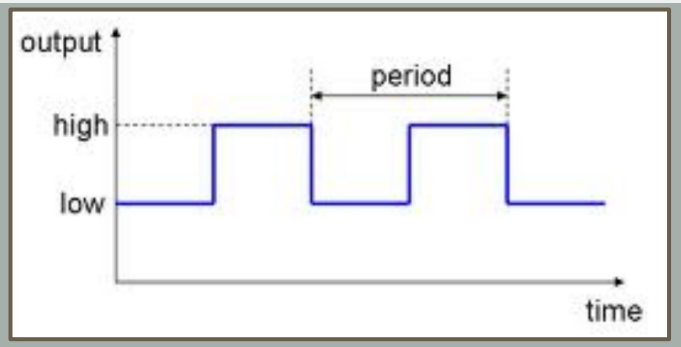
- Utilize the 555 timer as an oscillator
- Output frequency changes based on C, R_A, R_B
- Use the ICM7555 from Intersil



Images from
Intersil ICM7555 Datasheet

Capacitance Measurement Method

- Output is square wave, period changes with C:
  - $T = 0.693*(R_A+2*R_B)*C$

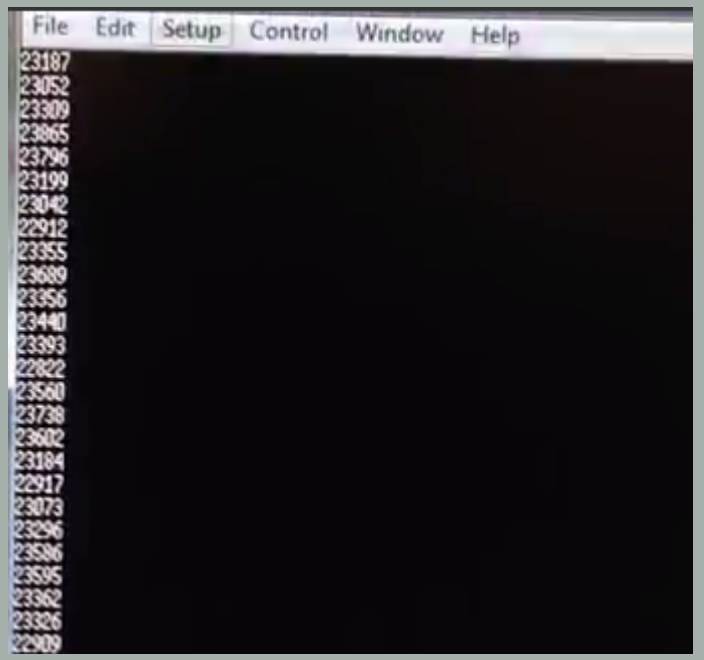


- Connected as input to MSP430 Launchpad
  - A timer continuously counts
  - Captures on each rising edge
  - Records number of counts in a period
- Relate change in capacitance to change in the counted number

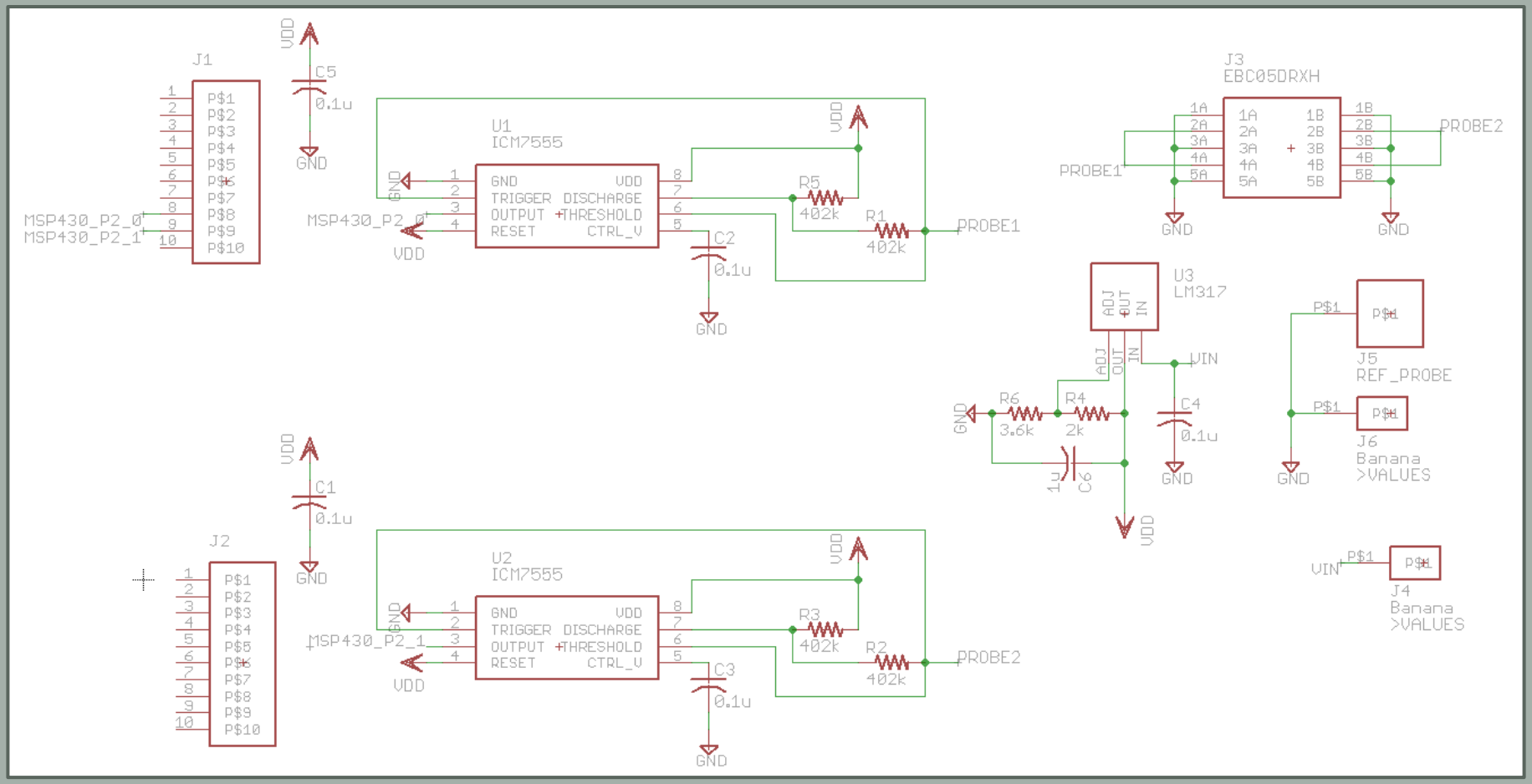
Image from
bacalfa.zxq.net

Capacitance Measurement Method

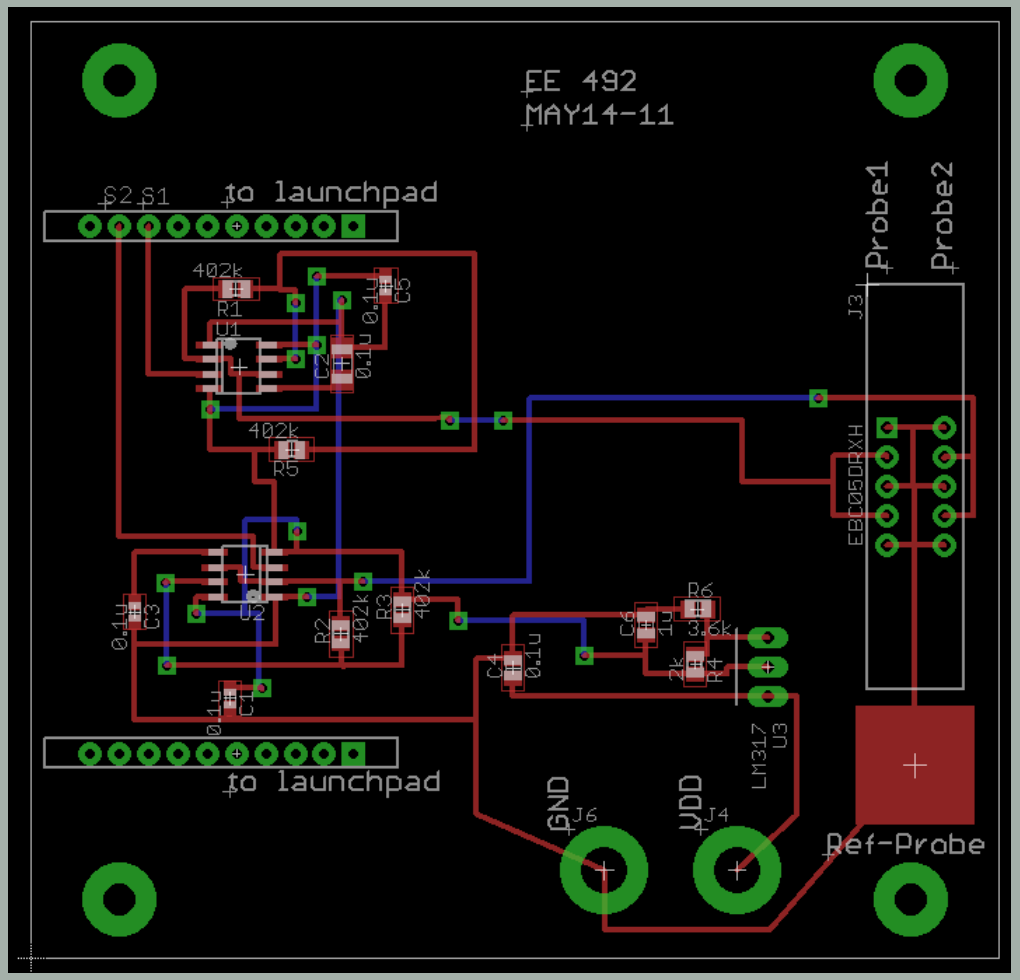
- Program the MCU via UART
- Does 50 captures in succession
- MCU sends the counted numbers to the computer terminal
- Numbers are averaged



Board Schematic

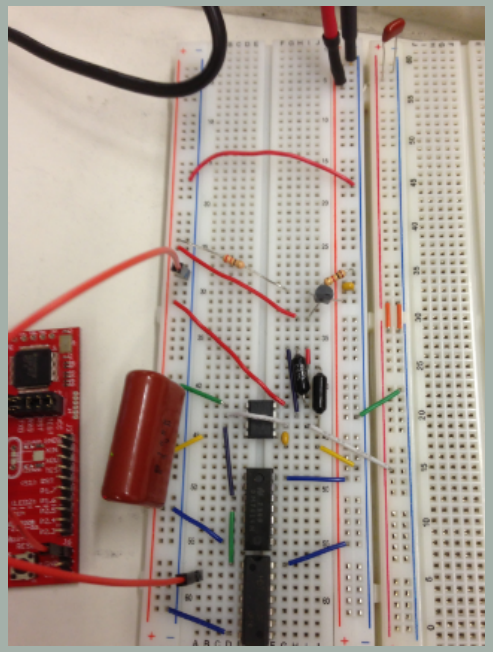


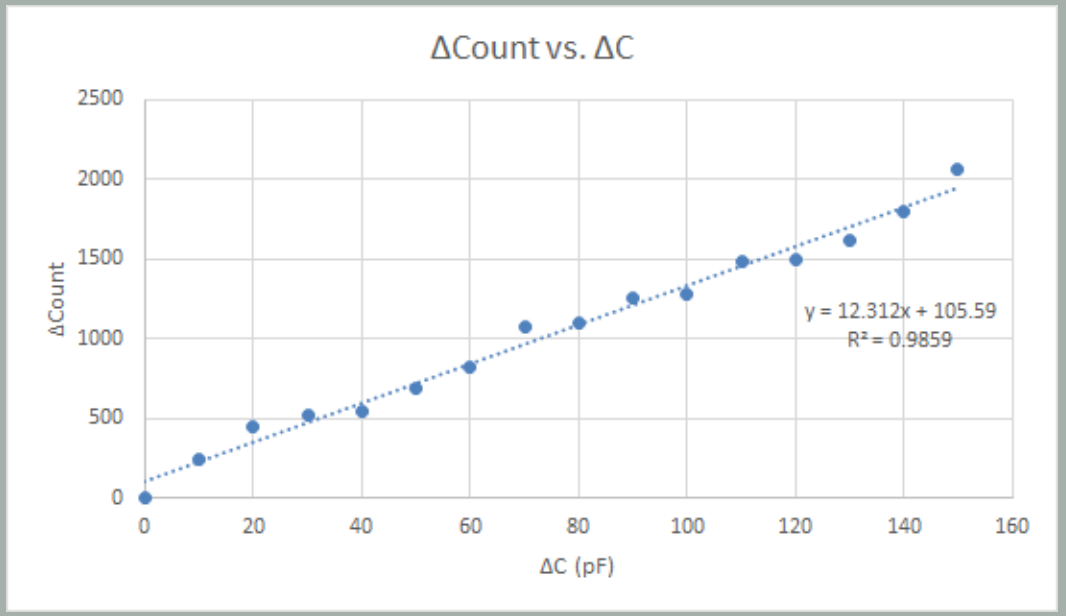
Board Layout



Circuit Testing: Breadboard

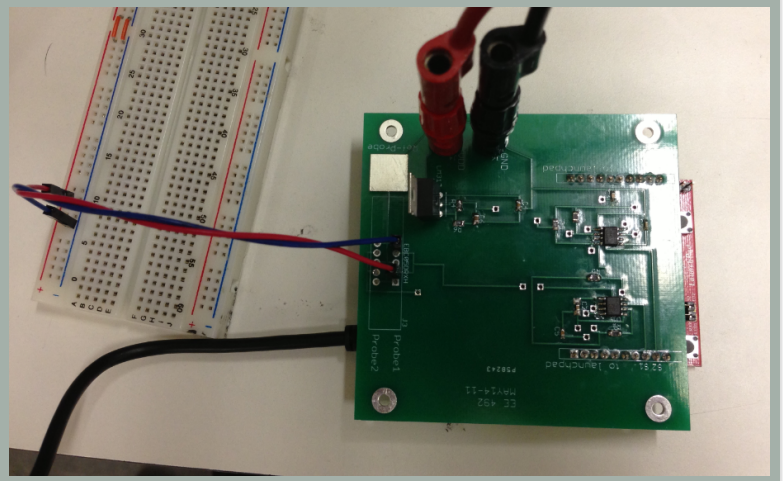
- Initial circuit setup was built on a breadboard
- Individual capacitors plugged in /unplugged
- Some errors introduced

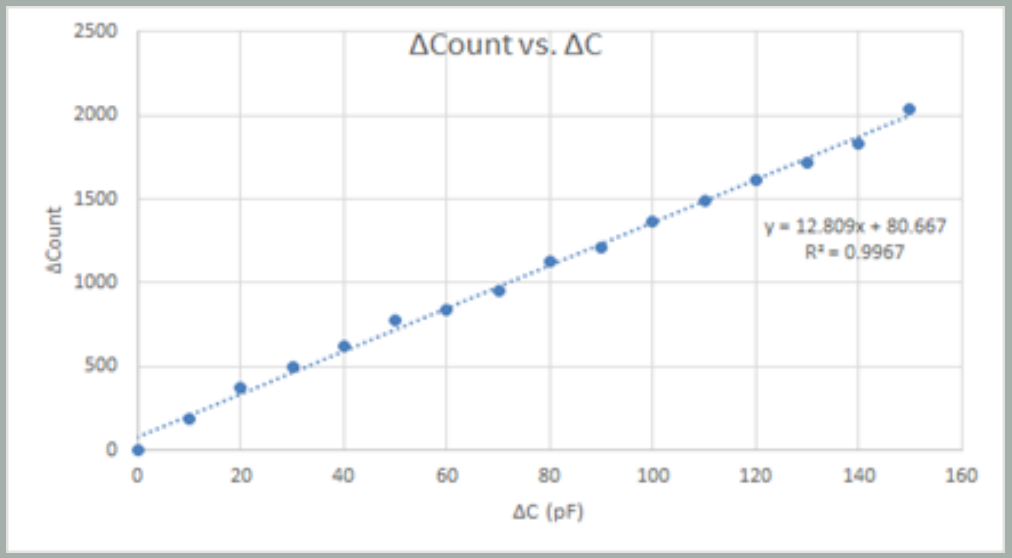




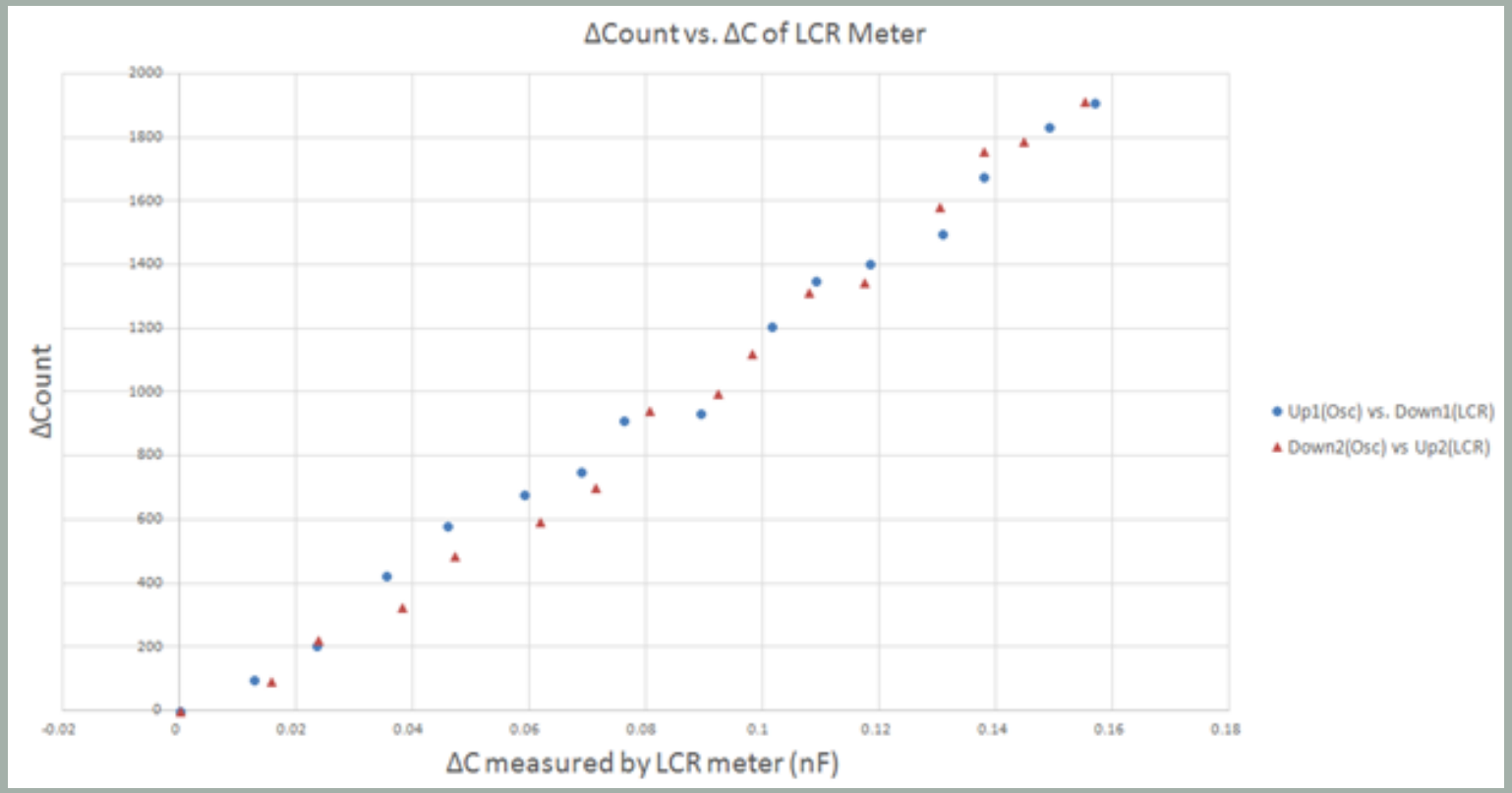
Circuit Testing: PCB

- Subsequent tests done with PCB
- Errors lessened to an extent
- Still have some variation with plugging/ unplugging individual capacitors



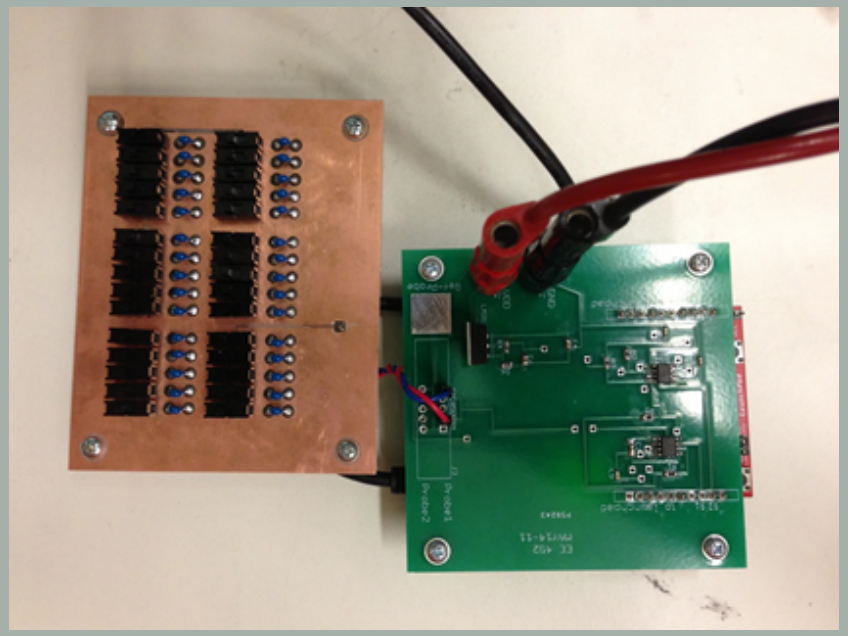


Circuit Testing: PCB



Results compared with LCR meter readings

Circuit Testing: PCB with Array



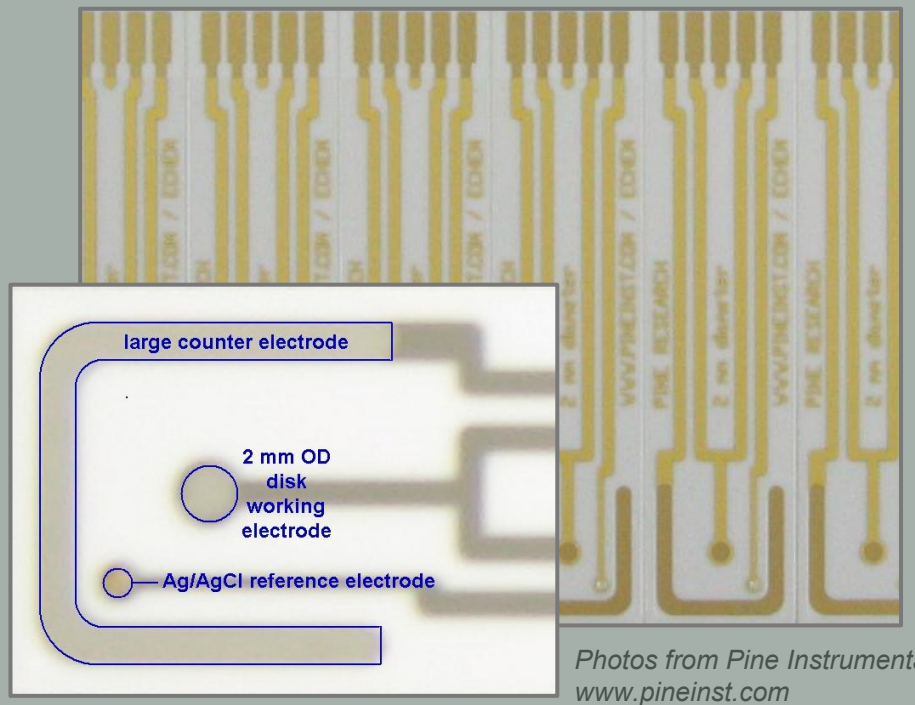
- Switched capacitor array fabricated in-house
- Issues with reliability exist - no viable data as yet

Electrode Fabrication and Testing

Selection, Preparation, and Experimentation

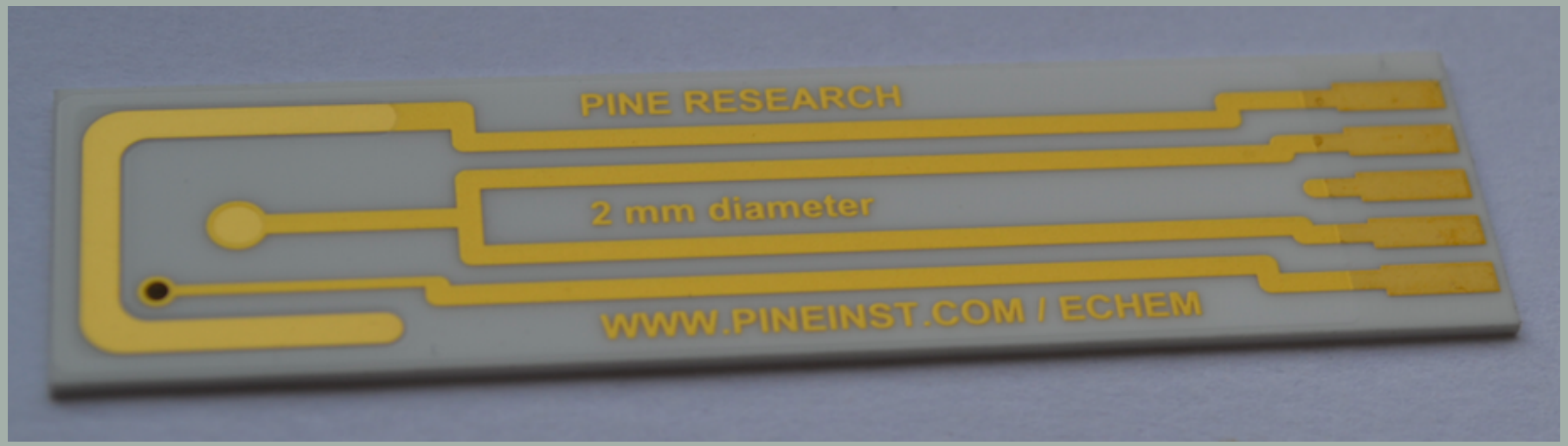
Electrode Selection

- Pine Research Instrumentation
- Gold Screen Printed Electrode
- Designed for electrochemical applications
- Counter, reference, working electrodes



Photos from Pine Instrumentation
www.pineinst.com

Surface Chemistry



Surface Chemistry

- Collaborated with Smith group in Chemistry
- Surface chemistry was applied to all purchased electrodes
- Three electrodes were painted with MCLR antibody for experimentation

Photos from
http://group.chem.iastate.edu/Smith/group_information.html

Note - IBC Approval

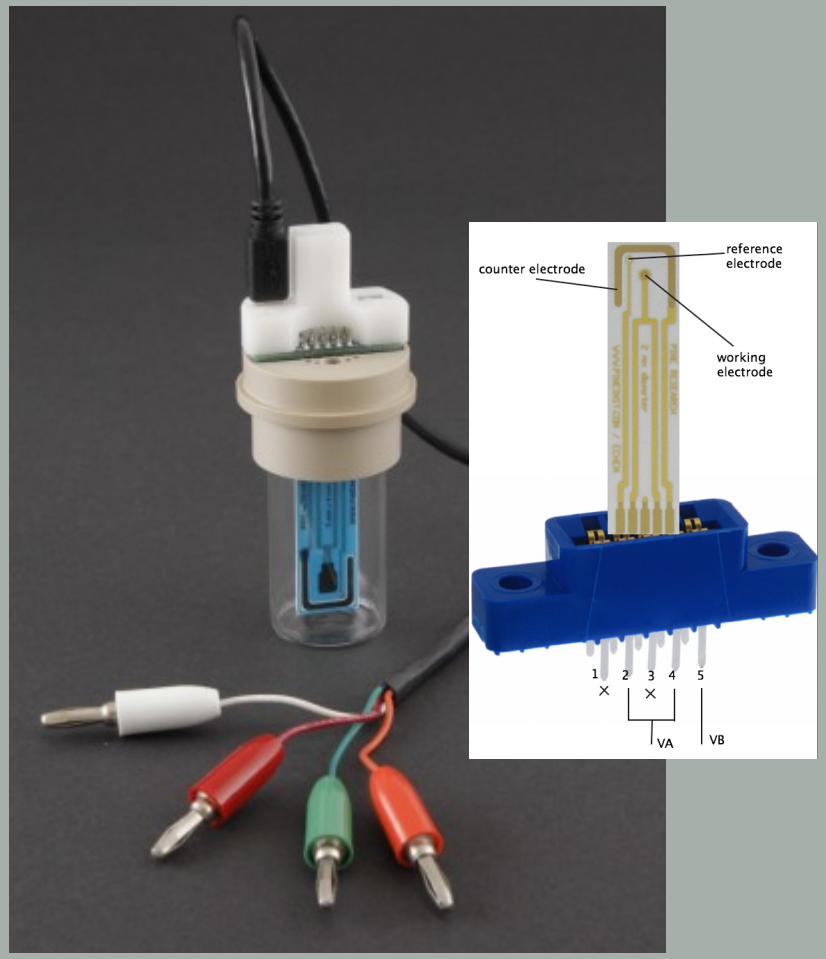
In order to work with the MCLR toxin, all group members applied for and received IBC approval for working with the substance.

Approval also allowed for future dilutions to be prepared in Coover Hall.

Dilution Preparation

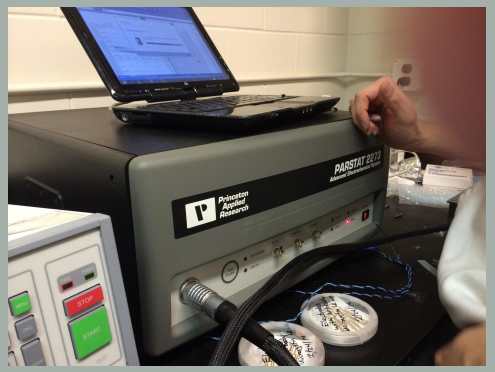
- Worked with Dahai Shao and Dwayne Schrunk in the VetMed Diagnostic Laboratory here at ISU
- Created and followed a systematic dilution process
- Prepared 5 solutions of MCLR of varying concentrations ranging from 4ug/L to ~0.01ug/L

Electrode Testing



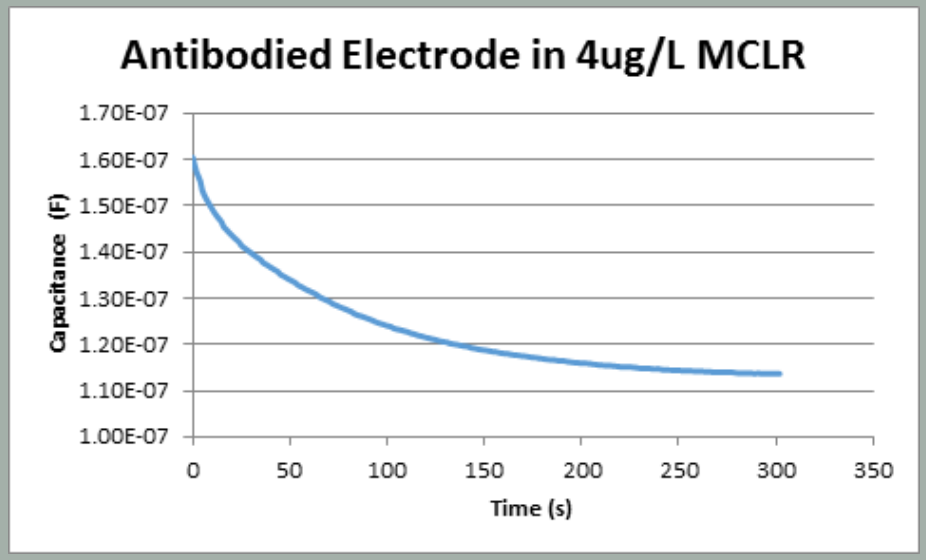
^Photos from pineinst.com

- Worked with John Carr at Microelectronics Research Center (ISU)
- Performed 2 separate experiments
- Used PARSTAT 2273 capacitance analyzer



IN 4UG/L MCLR SOLUTION

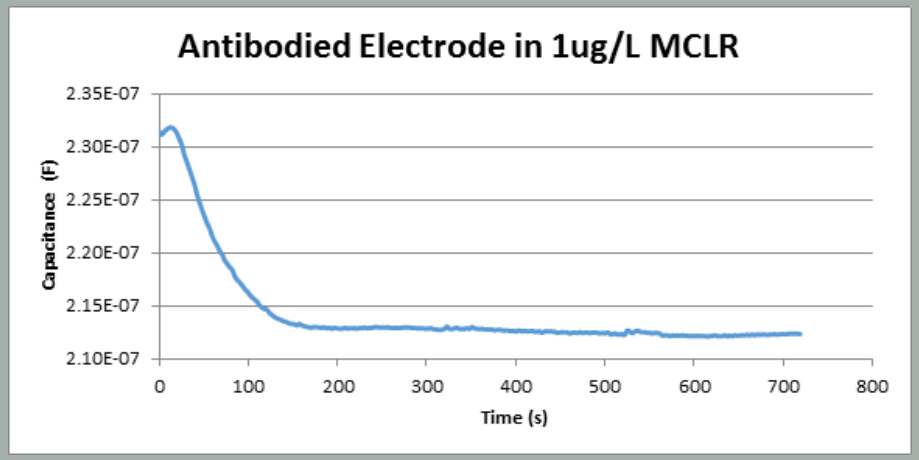
- Measured antibodied electrode
  - dry
  - in DI water
  - in 4ug/L MCLR solution



Capacitance vs. time results for an antibodied electrode in 4ug/L MCLR solution. The capacitance drop seen was 46.8nF.

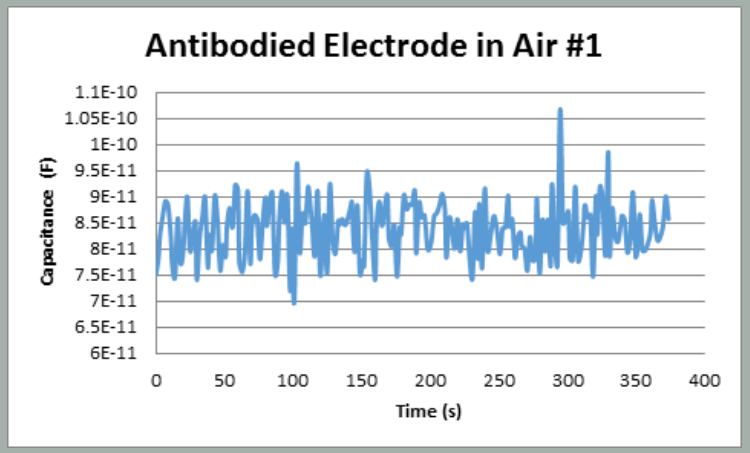
IN 1UG/L MCLR SOLUTION

- Measured antibodied electrode
  - dry
  - in DI water
  - in 1ug/L MCLR solution
- Measured non-antibodied electrode
  - in 1ug/L MCLR solution

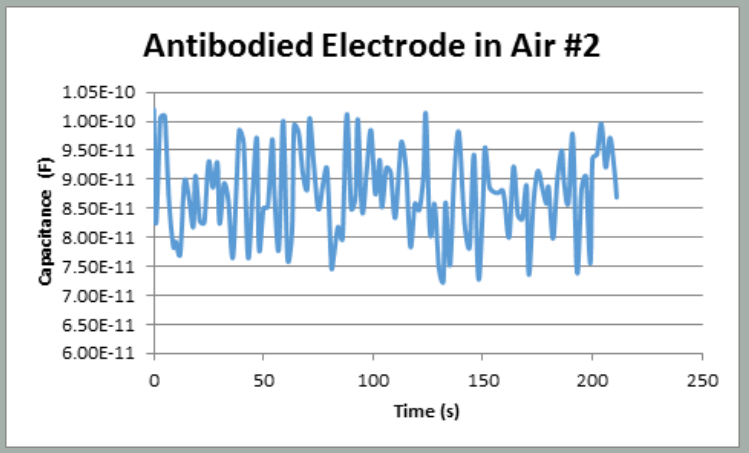


Capacitance vs. time results for an antibodied electrode in 1ug/L MCLR solution. The capacitance drop seen was 18.9 nF.

In Air

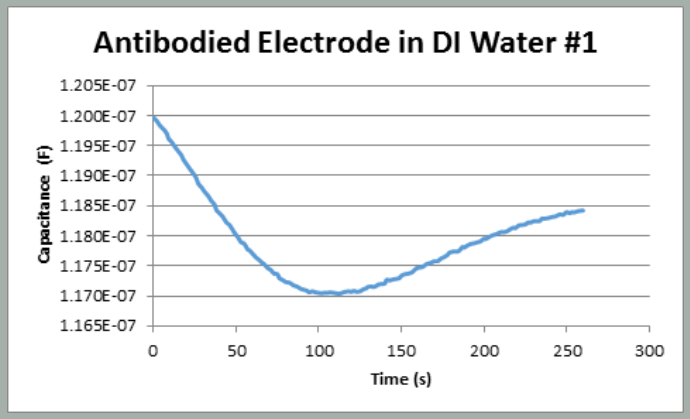


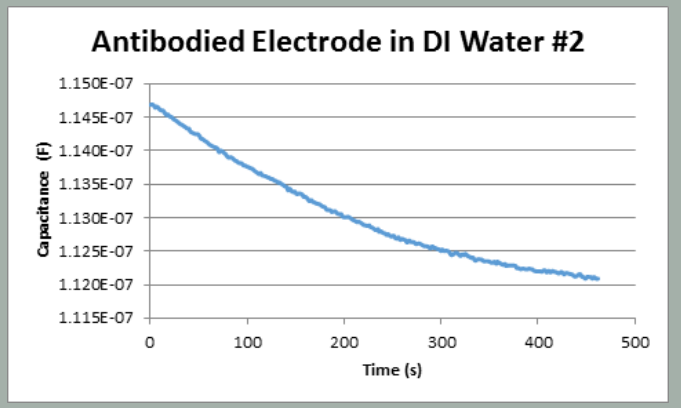
Capacitance vs. time for antibodied electrode in air, before water tests.

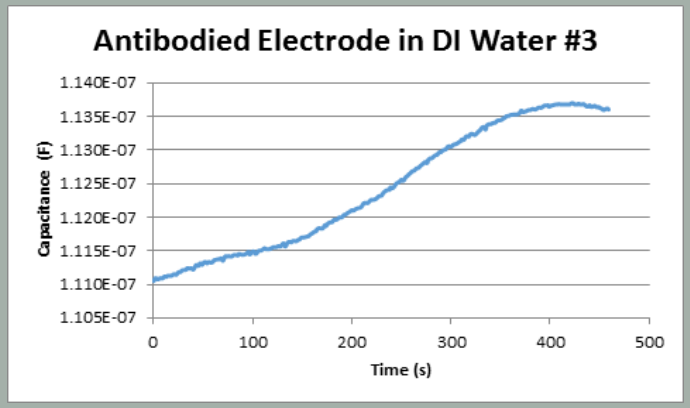


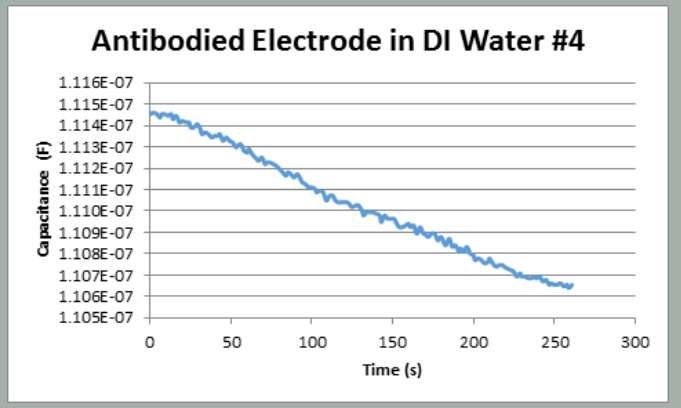
Capacitance vs. time for antibodied electrode in air, after 4 measurements in DI water.

IN DI WATER

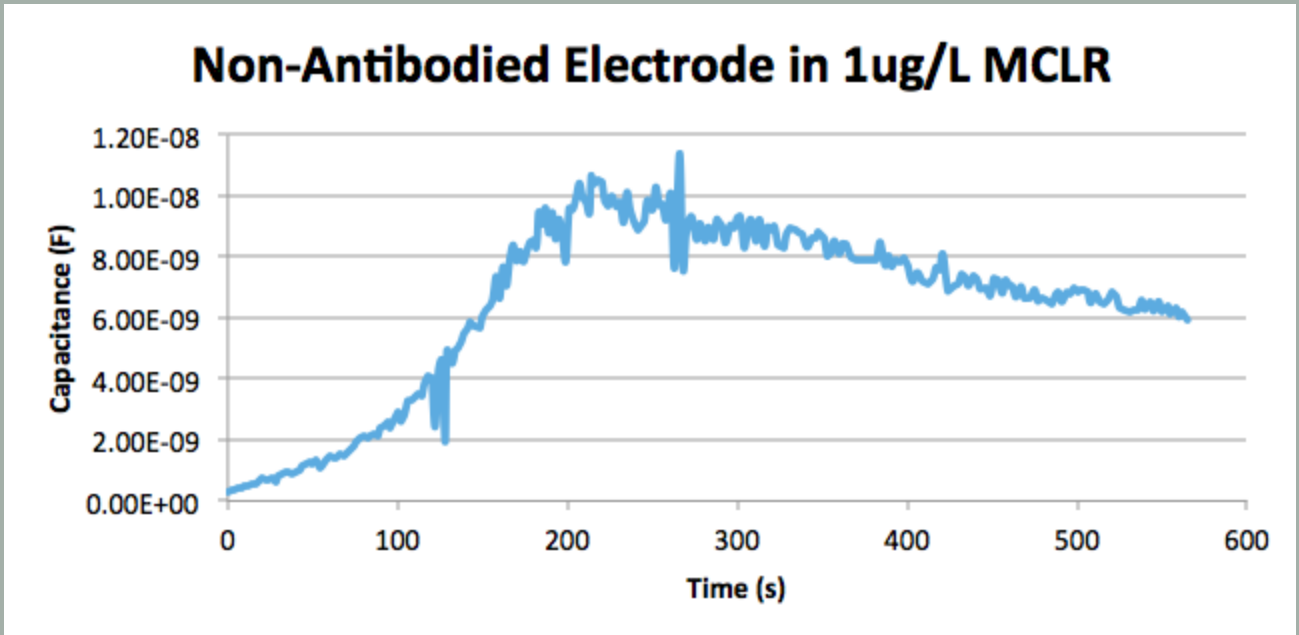

Antibodied Electrode in DI Water #1
Capacitance (F)
1.205E-07
1.200E-07
1.195E-07
1.190E-07
1.185E-07
1.180E-07
1.175E-07
1.170E-07
1.165E-07
0
50
100
150
200
250
300
Time (s)


Antibodied Electrode in DI Water #2
Capacitance (F)
1.150E-07
1.145E-07
1.140E-07
1.135E-07
1.130E-07
1.125E-07
1.120E-07
1.115E-07
0
100
200
300
400
500
Time (s)


Antibodied Electrode in DI Water #3
Capacitance (F)
1.140E-07
1.135E-07
1.130E-07
1.125E-07
1.120E-07
1.115E-07
1.110E-07
1.105E-07
0
100
200
300
400
500
Time (s)


Antibodied Electrode in DI Water #4
Capacitance (F)
1.116E-07
1.115E-07
1.114E-07
1.113E-07
1.112E-07
1.111E-07
1.110E-07
1.109E-07
1.108E-07
1.107E-07
1.106E-07
1.105E-07
0
50
100
150
200
250
300
Time (s)

Electrode without Antibody



Experiment Conclusions

- Electrode sees capacitance drop in 4ug/L solution greater than in DI water
- The change in capacitance seen by the electrode in 1ug/L MCLR < 4ug/L MCLR solution
- In order for an electrode to be re-used, an antibody regeneration scheme needs to be developed

Experiment Conclusions

- Each electrode may have a different base capacitance due to fabrication variation
- More investigations on behavior in DI water should be performed
- Non-antibodied electrode was not measured extensively, and collected data may not be useful

Turnover Details

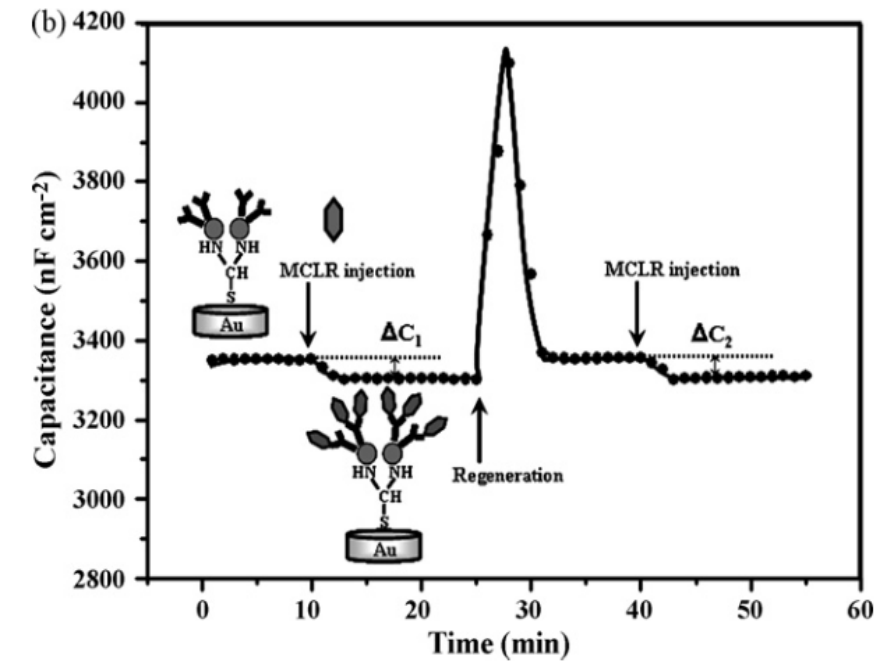
Due to the long-term nature of this project, may details have been left for an additional team to complete in the future.

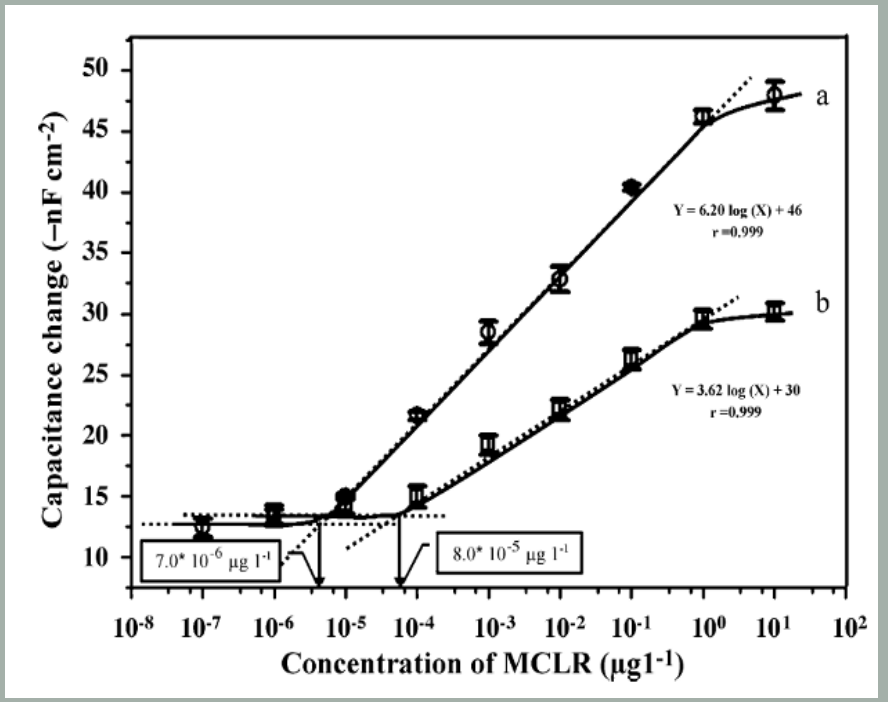
- Electrode Fabrication
- Electrode Testing
- Bridge Circuit
- Prior Work

Questions?

Thank you for your time!

CONCENTRATION OF MCLR





Label-free capacitive immunosensor for microcystin-LR using self-assembled thiourea monolayer incorporated with Ag nanoparticles on gold electrode

Suchera Loyprasert[a,b], Panote Thavarungkul[a,c], Punnee Asawatreratanakul[a,d], Booncharoen Wongkittisuksa[a,e], Chusak Limsakul[a,e], Proespichaya Kanatharana[a,b,*]

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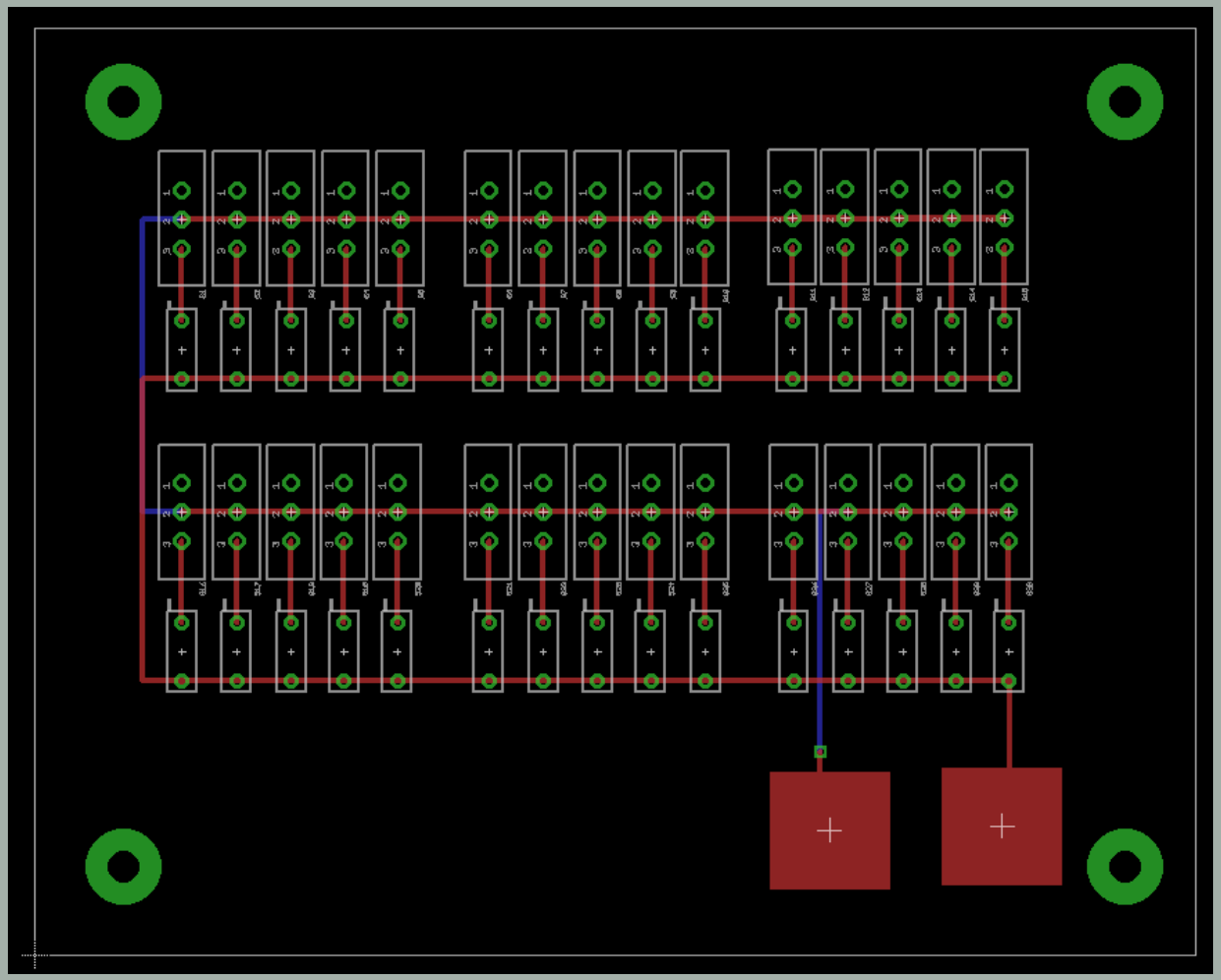
Capacitor Bank



Binding Theory

If you have a liquid with assumed uniform distribution, when electrode is put in, it has vacancies (open binding sites) on the antibody. For every bound site there is a probability that it will unbind (dissassociating).
P(binding) > P(disassociating).

After time, P(binding) ~= P(disassociating). Equilibrium point is dependent on concentration. So, time to reach equilibrium is about the same, but equilibrium point is different. Capacitance changes by changing separation distance of plates.