

Fiber Optic Microarray Detection of Freshwater Cyanobacteria

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Why Detection Matters

- The release of cyanotoxins into waters used for drinking and recreational activities
- Cyanotoxins: neurotoxins, cytotoxins or hepatoxins
- The LD₅₀ for such toxins respectively is 20ug/kg and 50ug/kg compared to Cobra venom which has an LD₅₀ of 185ug/kg in murine studies
- WHO drinking water guidance value for safe consumption is 1µg/L
- The potential toxicity of these organisms demonstrates the need to safely and effectively monitor influxes of large amounts of cyanobacteria in freshwater systems for public health measures and environmental integrity

Cyanobacteria

- In collaboration with WHOI, Tufts Chemistry Department and Ahura Technologies ...the overall project goal is to adapt and validate a rapid and accurate optical fiber-based technology for CyanoHAB cell detection and enumeration in both laboratory and field settings.



Capture & Signal Probes

Base pair mismatches for capture probe development are highlighted in yellow

Microcystis probe #1

S. elongates

C. raciborskii

A. flos-aquae

C	A	C	C	G	A	T	G	T	T	C	T	T	C	C	C	A	A	T	C
C	G	C	T	G	G	T	G	T	T	C	T	T	C	A	G	A	A	T	A
C	A	G	A	C	C	C	T	T	T	A	C	G	C	C	C	A	A	T	C
C	G	G	A	C	C	C	T	T	T	A	C	G	C	C	C	A	A	T	C

Cylindro probe #2

A. cylindrica

M. aeruginosa

A. flos-aquae

C	A	G	C	A	G	A	C	T	T	T	C	A	G	T	T	C	C
C	A	G	C	A	G	A	C	T	T	A	C	A	T	G	G	C	C
C	A	G	C	C	A	C	A	C	C	T	T	C	C	G	G	T	A
C	A	G	C	A	G	A	C	T	T	A	C	A	A	T	G	C	C

Signal probe #1

M. aeruginosa

C. raciborskii

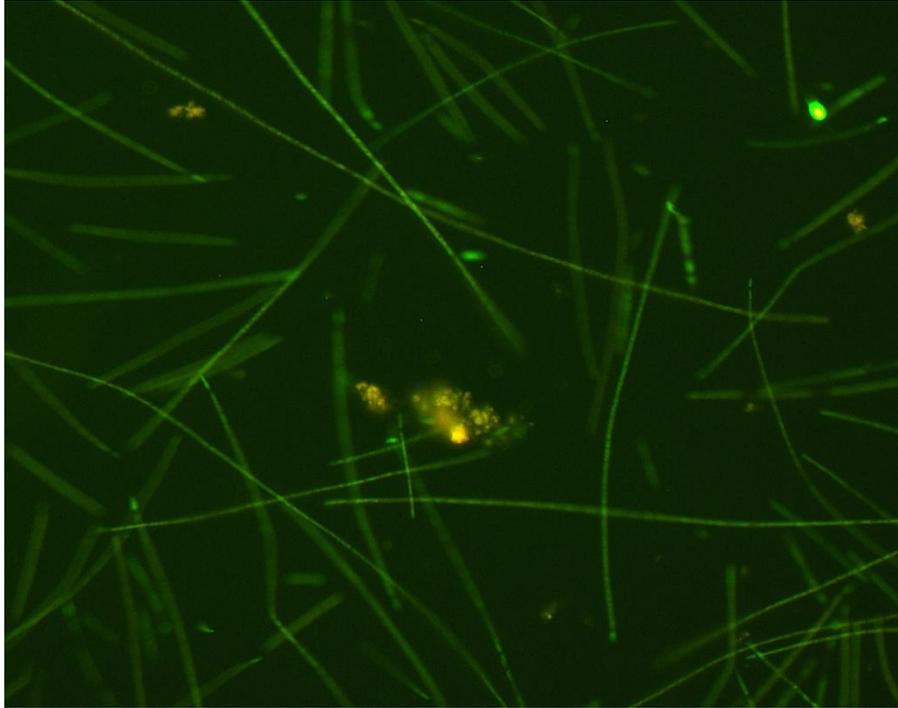
A. flos-aquae

C	T	G	A	G	A	C	A	C	G	G	C	C	C	A	G	A	C
C	T	G	A	G	A	C	A	C	G	G	C	C	C	A	G	A	C
C	T	G	A	G	A	C	A	C	G	G	C	C	C	A	G	A	C
C	T	G	A	G	A	C	A	C	G	G	C	C	C	A	G	A	C

Fluorescent *In Situ* Hybridization

Cylindrospermopsis

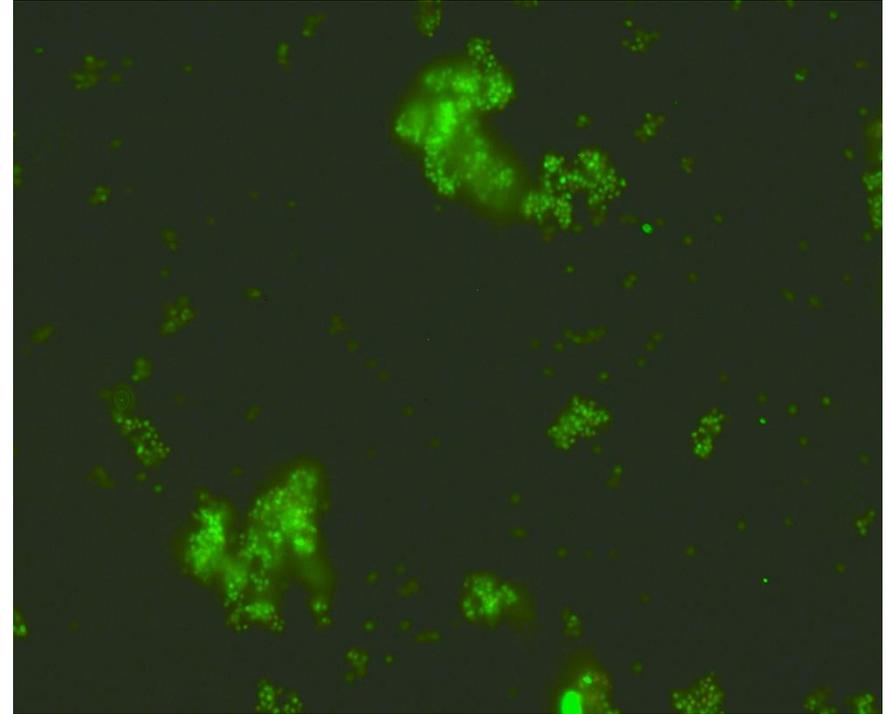
Cylindro probe 1



20x: Few Dozen

Microcystis

Micro probe 11



20x: ~100

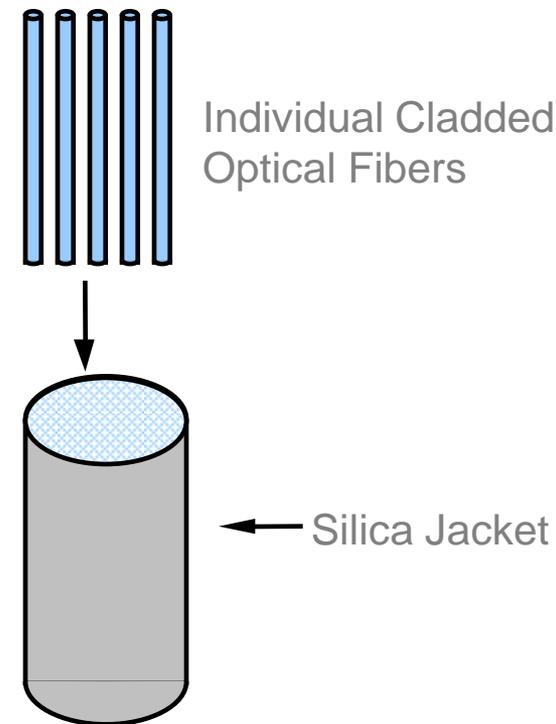
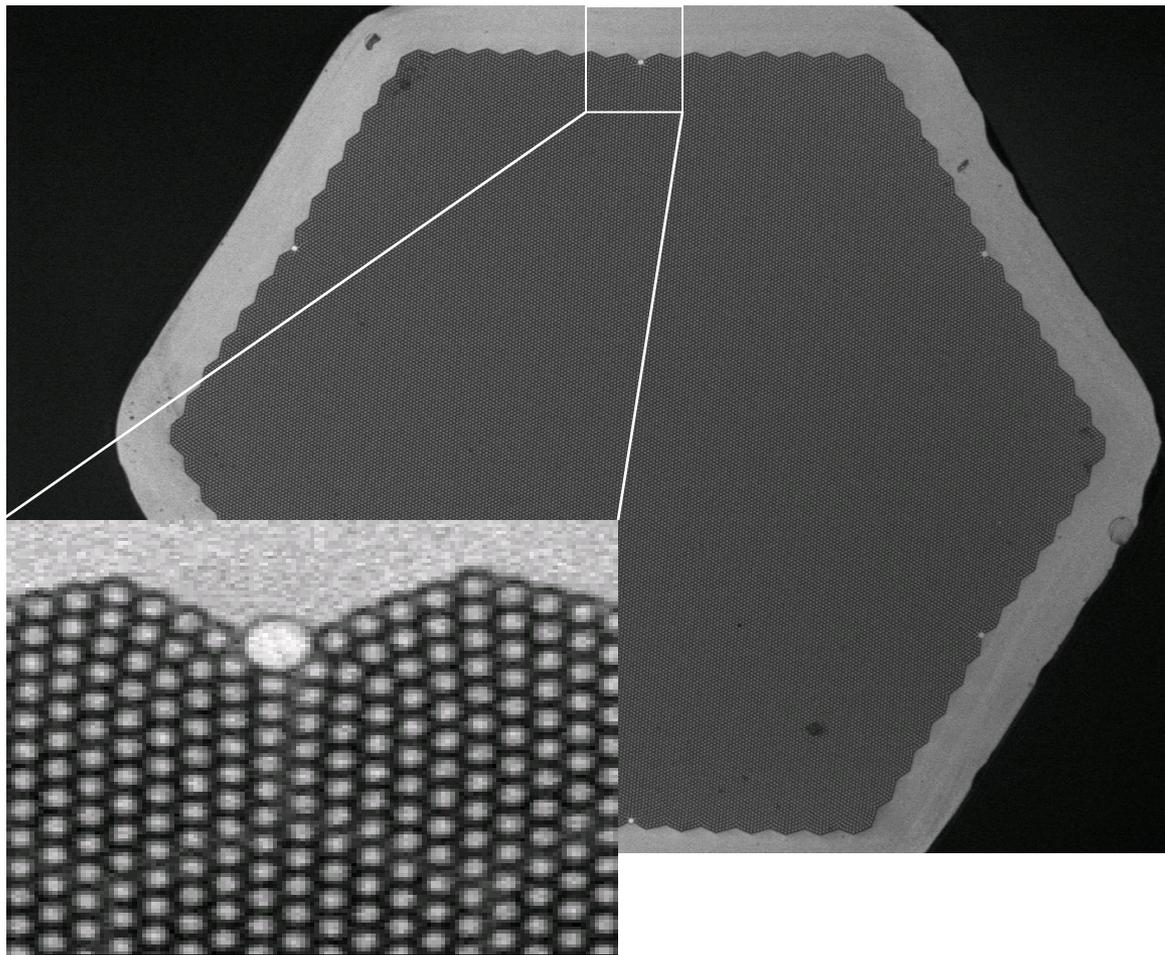
FISH → Microarray

Probe Number	CYL 1	CYL 2	CYL 3	CYL 11	CYL 12
Length (bp)	18	20	21	22	29
Tm (°C)	50.6	54.9	58.8	57.1	58.4
<i>M. aeruginosa</i> LB 2664				+	+
<i>M. aeruginosa</i> LB 2061	-	-			-
<i>M. aeruginosa</i> LE-3	-	-		-	
<i>M. flos-aquae</i> 2673	-	-			-
<i>C. raciborskii</i> AWT 205	+	+			
<i>C. raciborskii</i> LB 2897	+	+	+		-
<i>C. raciborskii</i> THAI	+	+			-
<i>C. raciborskii</i> AWT				+	+
<i>A. flos-aquae</i> LB 2557	-	-			
<i>A. flos-aquae</i> LB 2558	-	-			
<i>A. flos-aquae</i> NH-5	-	-			
<i>A. flos-aquae</i> UTEX 2391	-	-			
<i>Anabaena variabilis</i> B377				-	
<i>Aphan. flos-aquae</i>	-	-			
<i>A. cylindrica</i> LB 1611				-	
<i>A. cylindrica</i> UTEX B 629	-	-			
<i>A. bergii</i> AZ-73	-	-			
<i>Anabaenopsis</i> sp. AZ-16	-	+			
<i>Nostoc muscorum</i> UTEX 1037	-	-			
<i>Nodularia</i> sp.	-	-			
<i>Planktothrix</i> PCC 7811	-	-			
<i>Cyanophora paradoxa</i> LB 555					+
<i>Calothrix parietina</i> LB 1952				-	+
<i>Cylindrospermum</i> sp. LB 942				-	+
<i>Microcoleus</i> LB 2220					-
<i>Synechococcus</i> sp. LB 2537					-
<i>Synechococcus</i> sp. BO8807	-	-			

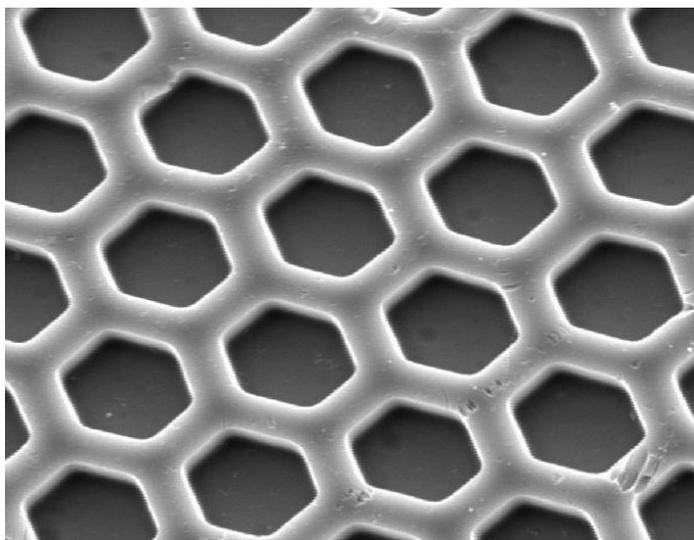
+ = false positives

- = false negatives

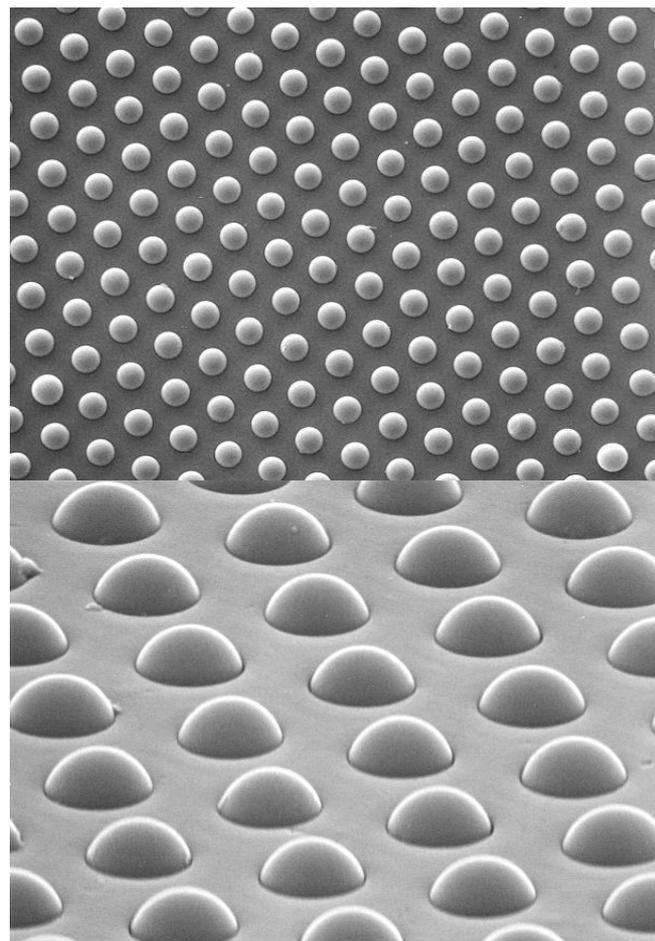
Fiber Optic Bundles



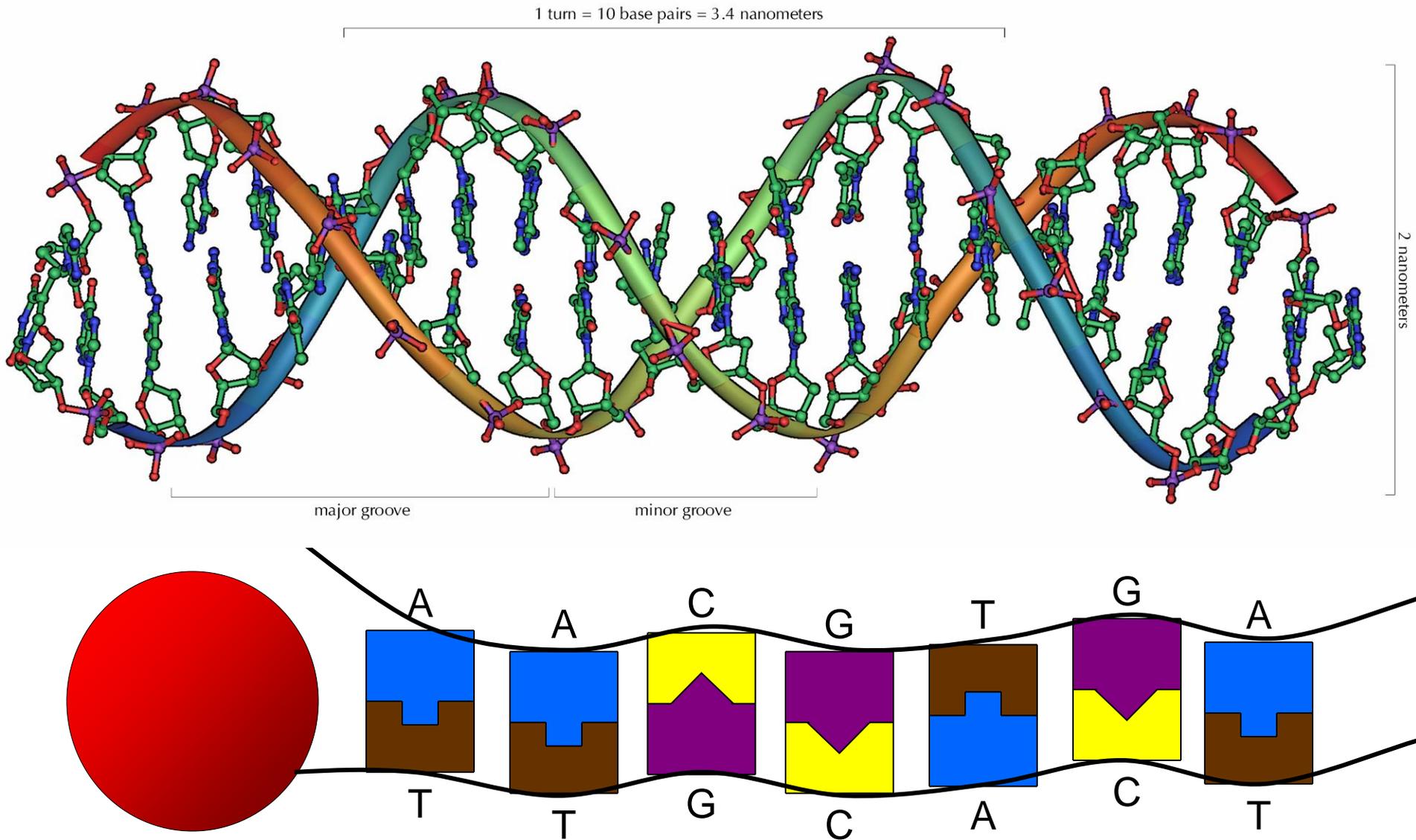
Microspheres in Etched Wells



Wet etching with HCl

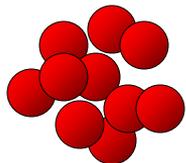


Base Pairing

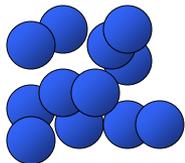


Microsphere Array

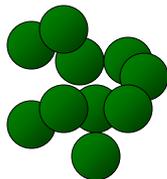
Probe A



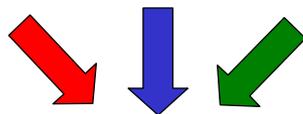
Probe B



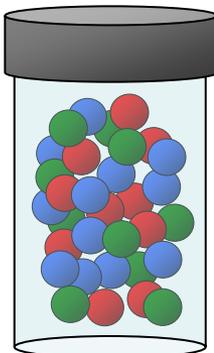
Probe C



1) Different DNA probes are first attached to beads

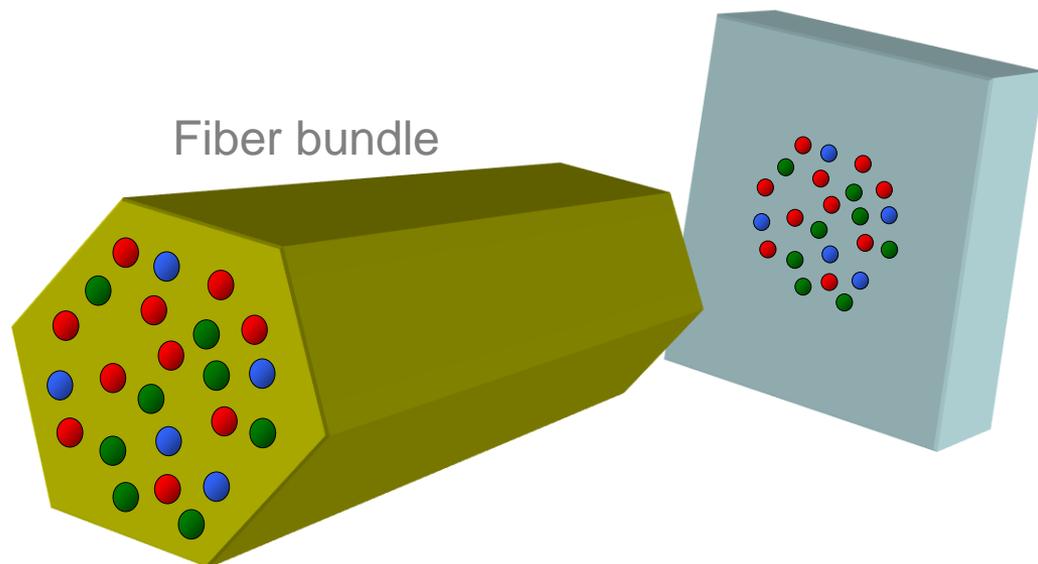


2) Probes are combined into a library

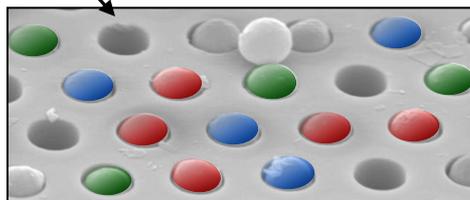
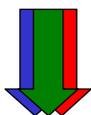


CCD chip

Fiber bundle



Microwells etched on distal fiber face



3) Probe bead library is distributed randomly into wells on fiber array surface

Microarray Assay

Encoding



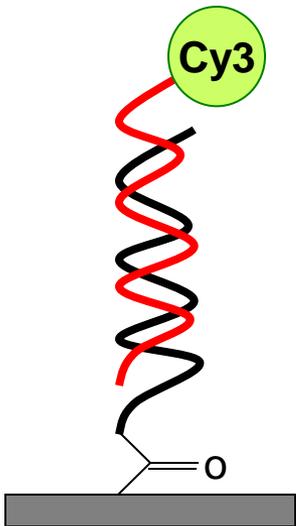
Sandwich
hybridization
assay



Background



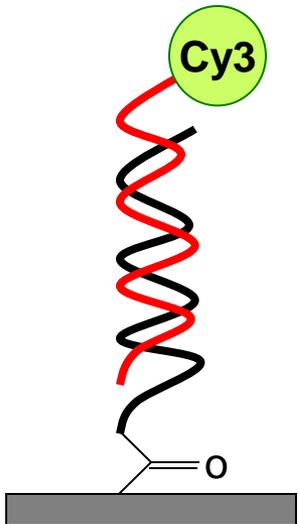
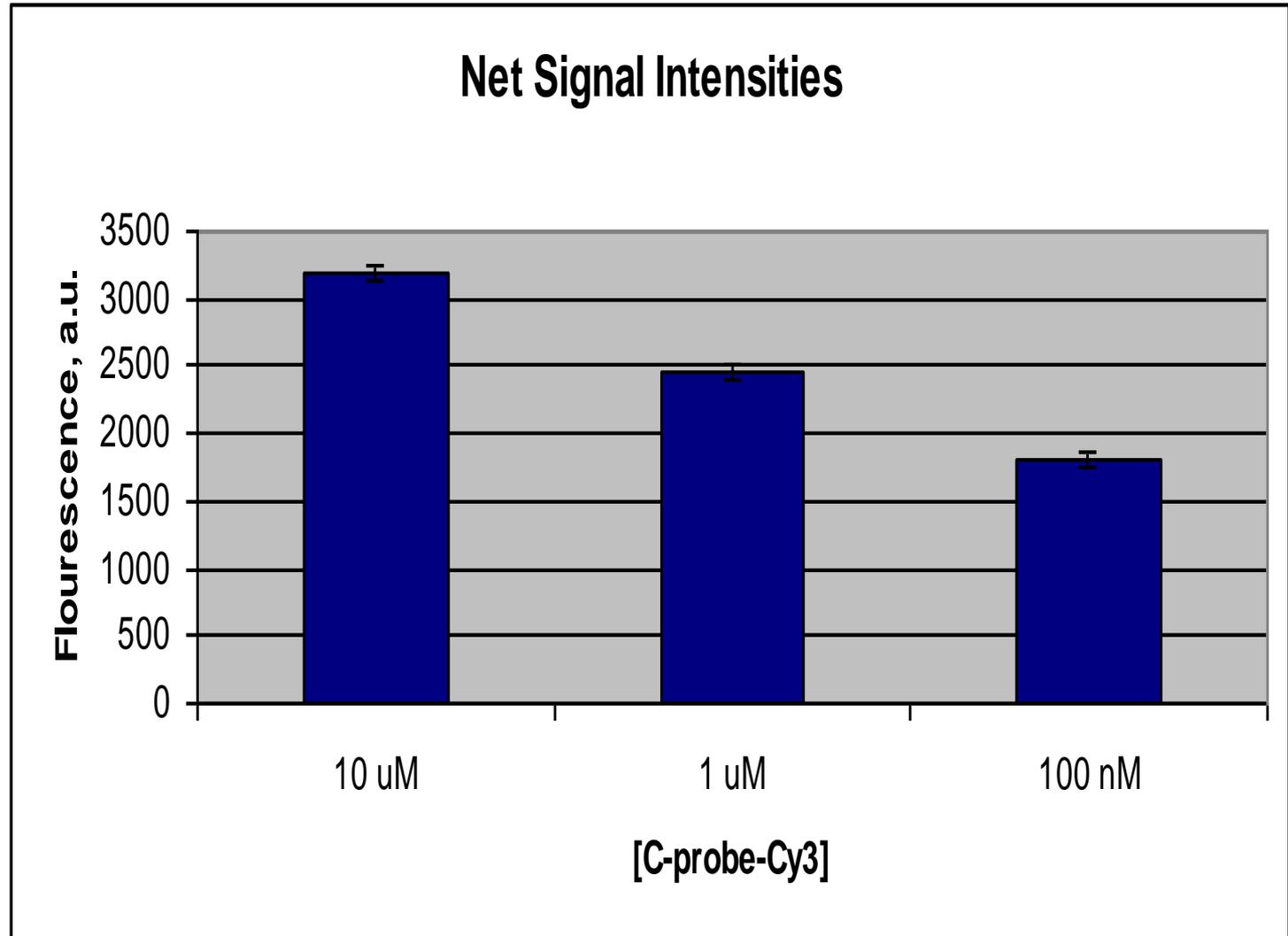
Direct hybridization



Direct hybridization



Cylindrospermopsis

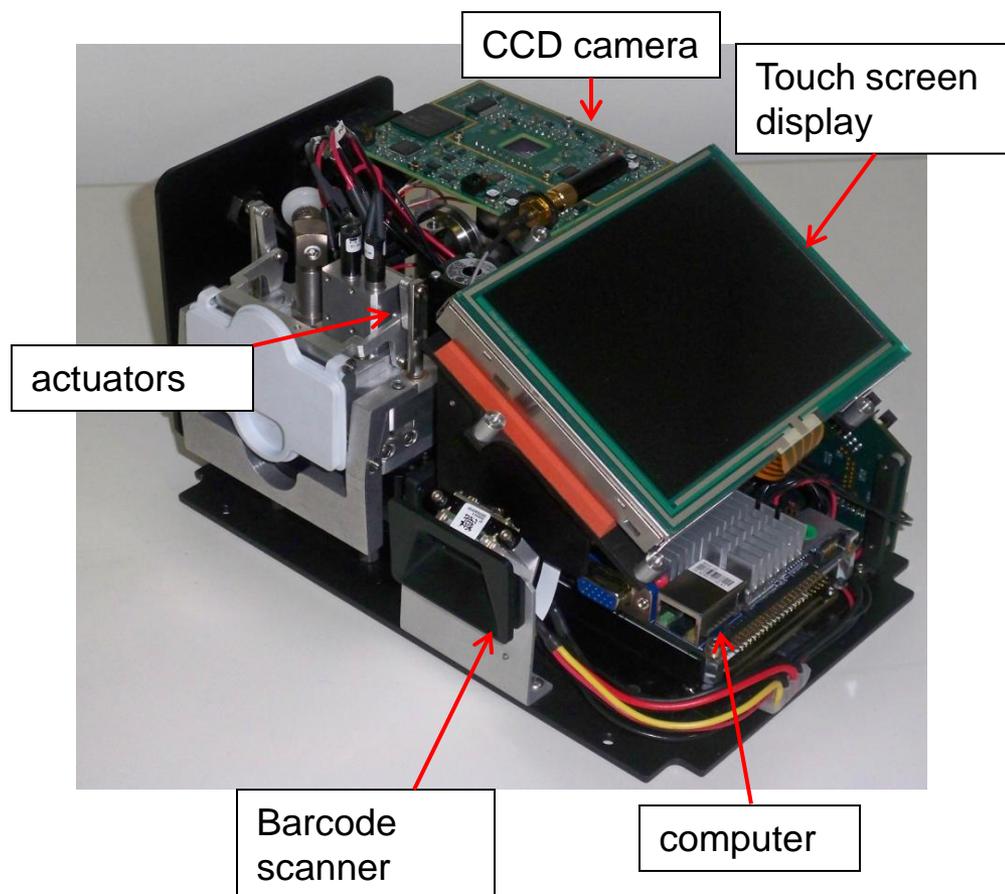


- Arrays of both Microcystis and Cylindrospermopsis are able to detect 1 nM of target within 10 minutes and are currently being tested for detection of pM target concentrations.
- Microarray platforms are capable of detecting multiple analytes simultaneously.
- Goal is to provide an early warning system utilizing laboratory-based flow-through systems, or remote, moored instruments capable of detecting and providing early warning of organisms that threaten public and ecosystem health.

Ahura



- 28x Magnifications
- Field of view: 350 x 280 micron
 - Over 2000 three- μm beads in field of view
- NA: 0.55
- Depth of focus: ~ 1 micron
- Two-color measurement, Cy3 and Cy5 dyes
- Folded to reduce height and width



Acknowledgements

SAC Committee

Dr. Saul Tzipori

Dr. David Walt

Dr. Dan Milner

Dr. Don Anderson

Groups

Walt Lab

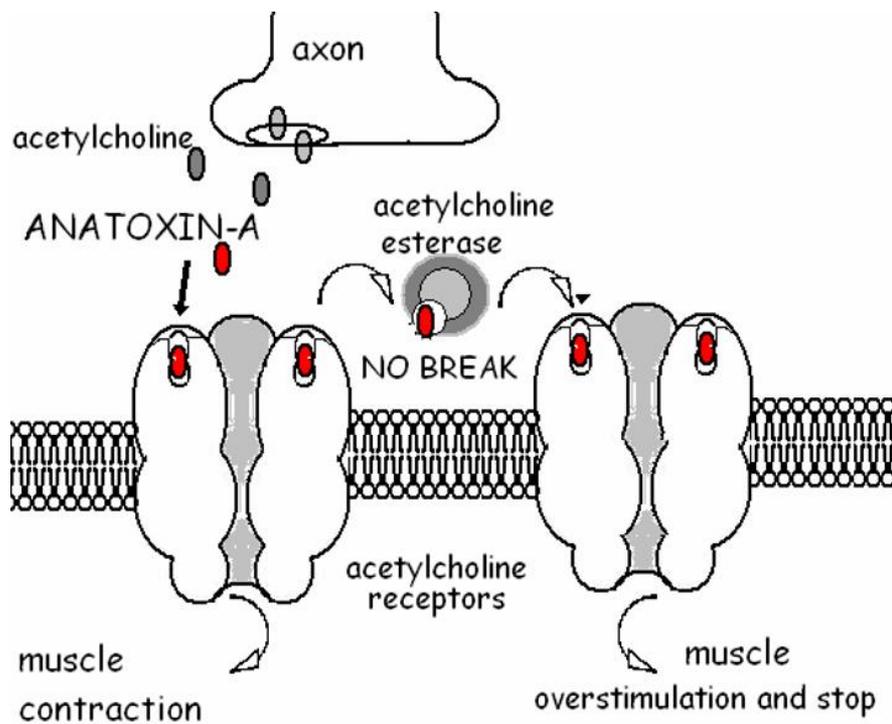
WSSS Program

Funding

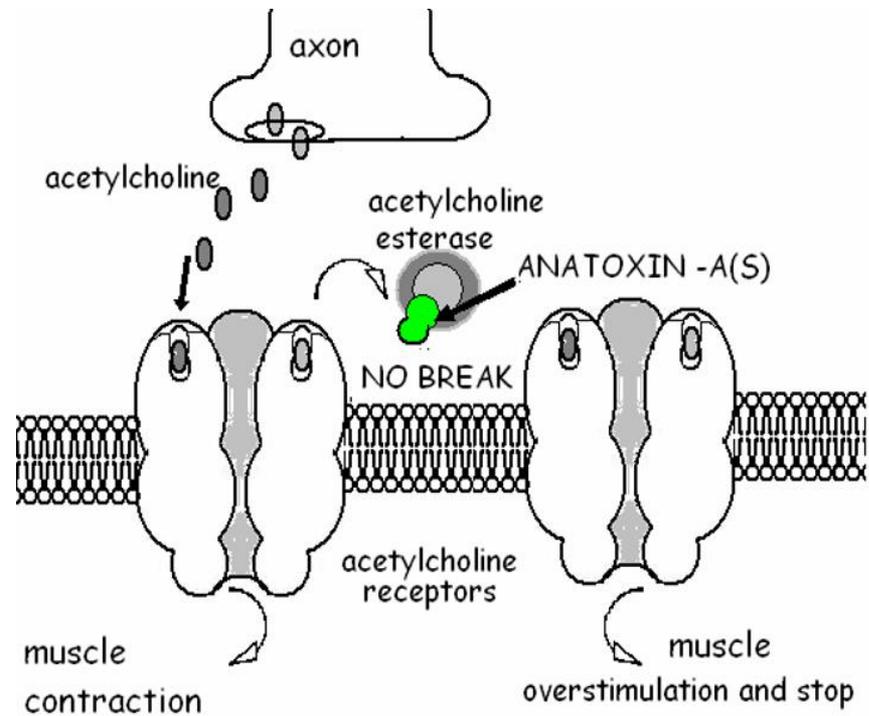
NIH

EPA STAR

Anatoxin-a



Anatoxin-a(s)

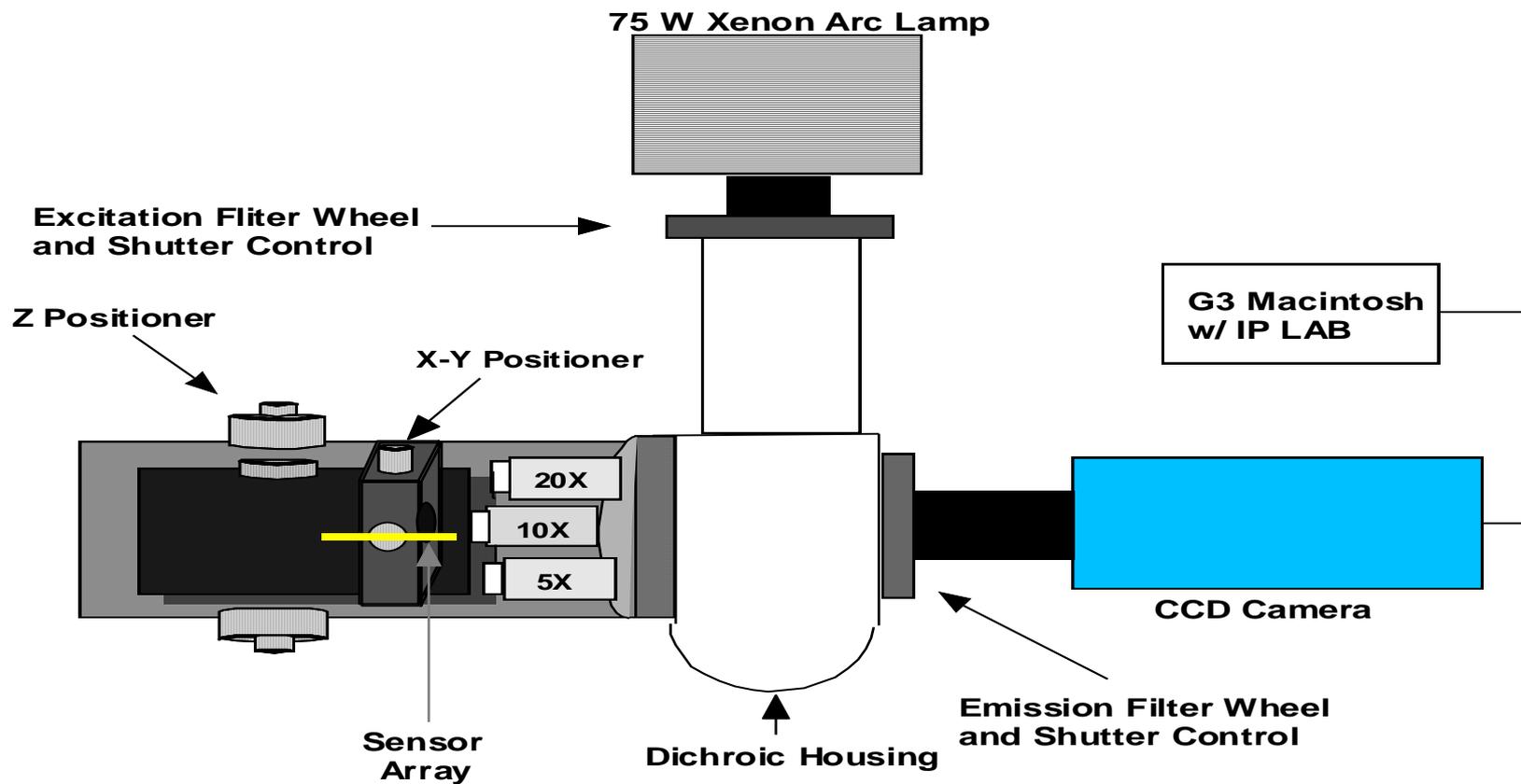


Microarray Probes

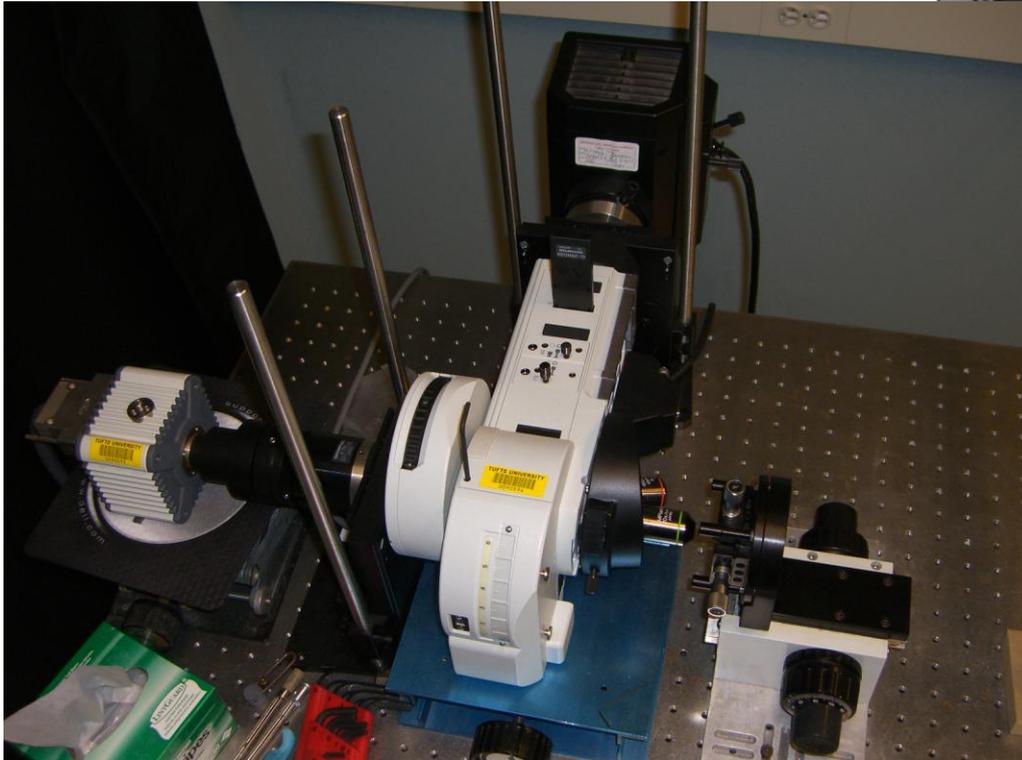
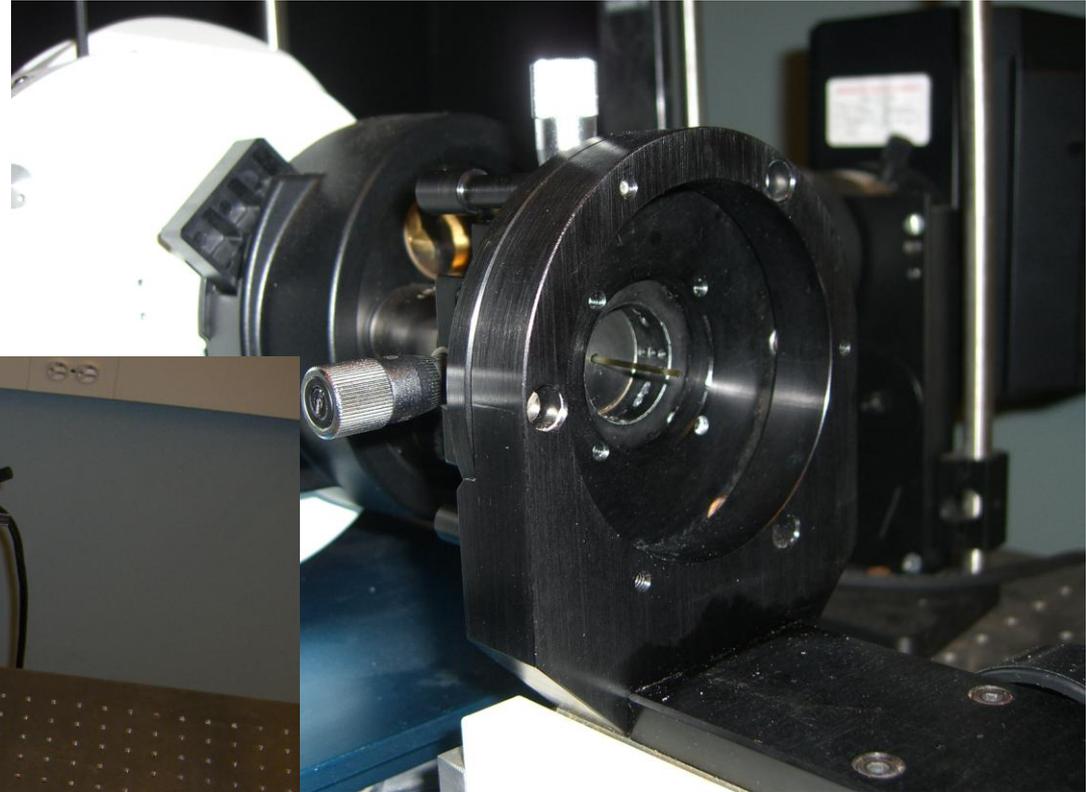
- Probes successfully tested for cross-reactivity are then transitioned to fiber-optic microarray format and tested against synthetic target and cell lysates from target species.

Probe Type	Target Organism	Length (bp)	Sequence (5' → 3')
Capture Probe	Cylindrospermopsis	18	CAG CAG ACT TTC AGT TCC
Synthetic Target Cy3 Labeled	Cylindrospermopsis	18	GGA ACT GAA AGT CTG CTG
Capture Probe	Microcystis	24	CCG CCT TTA GGT CGT TAA GC
Synthetic Target Cy3 Labeled	Microcystis	24	GGT TGC TTA ACG ACC TAA AGG C

Instrumentation



Imaging System



Analytical Measurements

- Using the software in IPLab overlay the ROI obtained from the Eu image onto the background and hybridization images
- Using the Measurements/ROI option measure the mean intensities of the beads and the standard deviation of the signals

