CD MICROFLUIDICS FOR MOLECULAR DIAGNOSTICS

MS. MARY AMASIA

DR. MARC MADOU

BIOMEMS LAB

UNIVERSITY OF CALIFORNIA, IRVINE

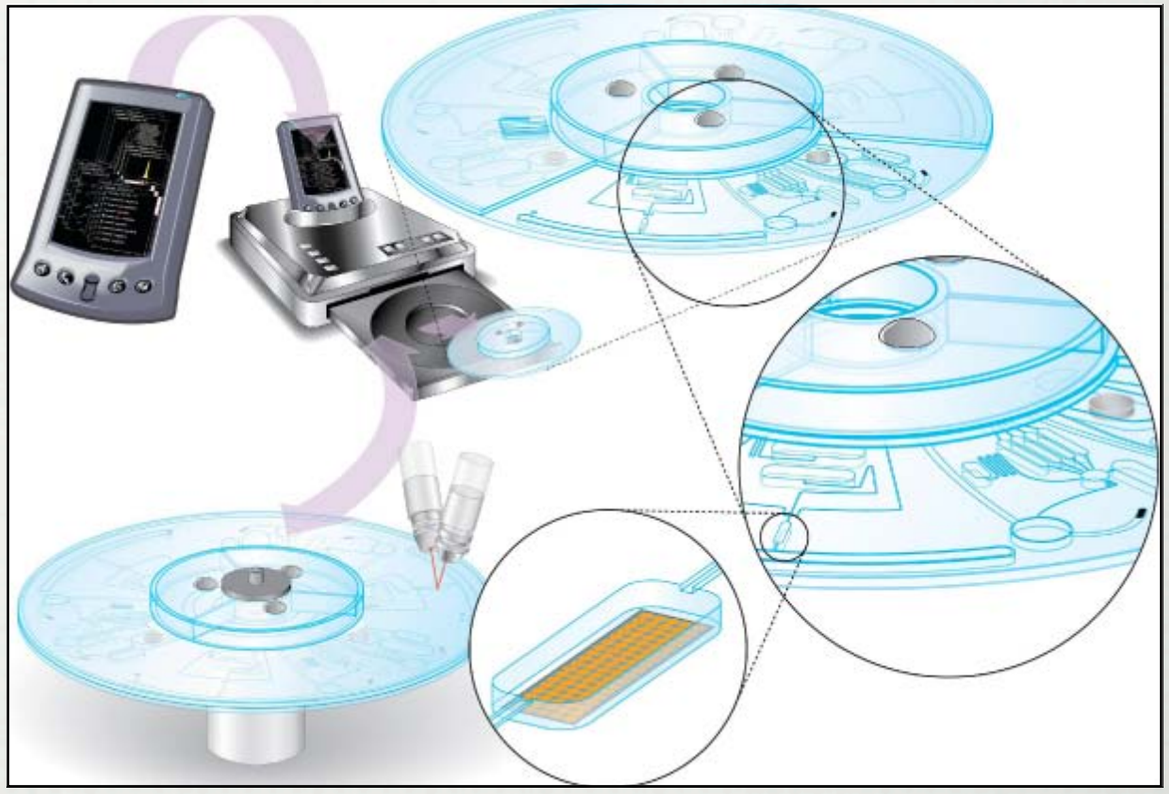
MARCH 16, 2010

UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

OUTLINE

- ☐ CD AS A DIAGNOSTIC PLATFORM
- ☐ CD MICROFLUIDICS FUNDAMENTALS
- ☐ CELL LYSIS
- ☐ DNA ARRAY HYBRIDIZATION
- ☐ NEXT STEPS TOWARDS SAMPLE-TO-ANSWER SYSTEMS



SIEGRIST, ET AL, 2009

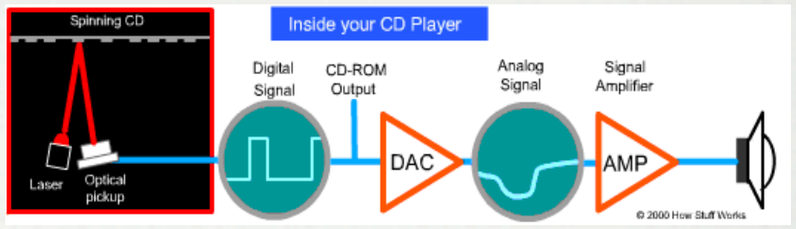
UCIRVINE | UNIVERSITY of CALIFORNIA

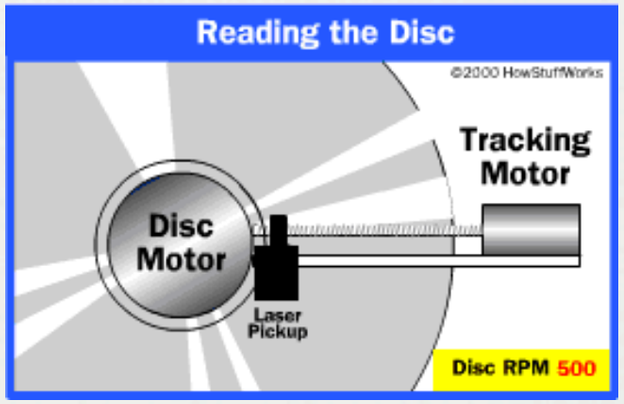
BIOMEMS Research Group @ UC Irvine

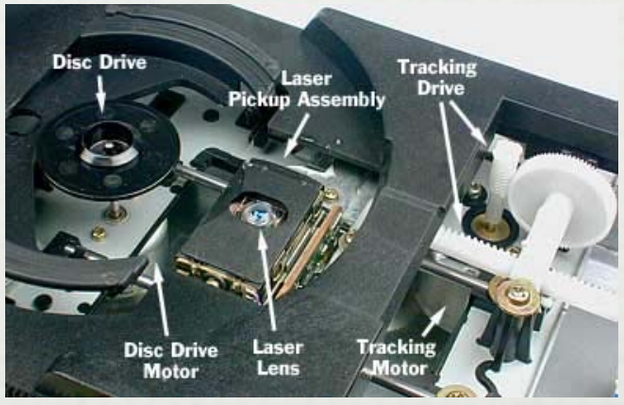
HOW DOES A CD PLAYER WORK?

- THE FUNDAMENTAL JOB OF THE CD PLAYER IS TO FOCUS THE LASER ON A TRACK OF BUMPS. THE LASER BEAM PASSES THROUGH THE POLYCARBONATE CD MATERIAL AND REFLECTS OFF AN ALUMINUM LAYER AND HITS AN OPTO-ELECTRONIC DEVICE THAT DETECTS CHANGES IN LIGHT. THE BUMPS REFLECT LIGHT DIFFERENTLY THAN THE "LANDS" (THE REST OF THE ALUMINUM LAYER), AND THE OPTO-ELECTRONIC SENSOR DETECTS THAT CHANGE IN REFLECTIVITY.

- A TRACKING MECHANISM MOVES THE LASER ASSEMBLY SO THAT THE LASER'S BEAM CAN FOLLOW THE SPIRAL TRACK. THE TRACKING SYSTEM HAS TO BE ABLE TO MOVE THE LASER AT MICRON RESOLUTIONS.



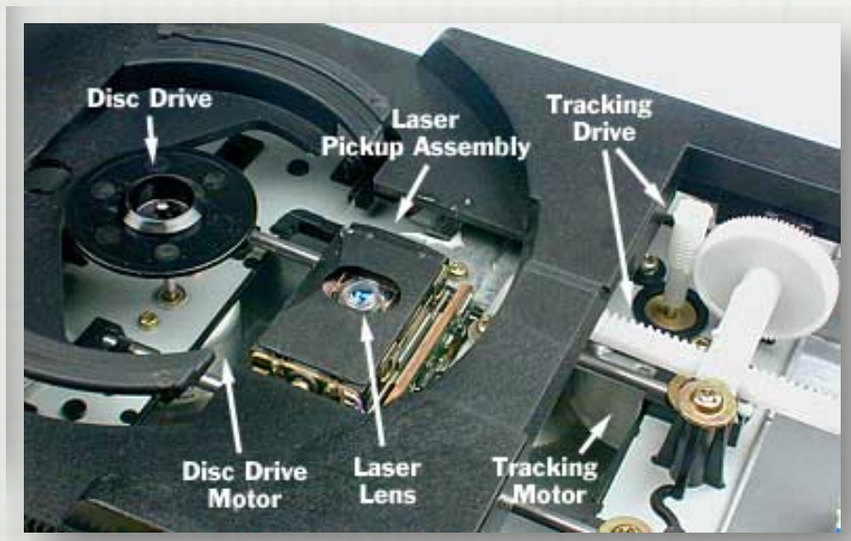




H. KIDO, J. ZOVAL

CD AS A MICROSCOPE

BY MODIFYING THE CD DRIVE BY ADDING A PHOTOSENSOR AND SIGNAL CONVERTERS, WE ARE ABLE TO SCAN FEATURES ON SURFACE OF A DISK



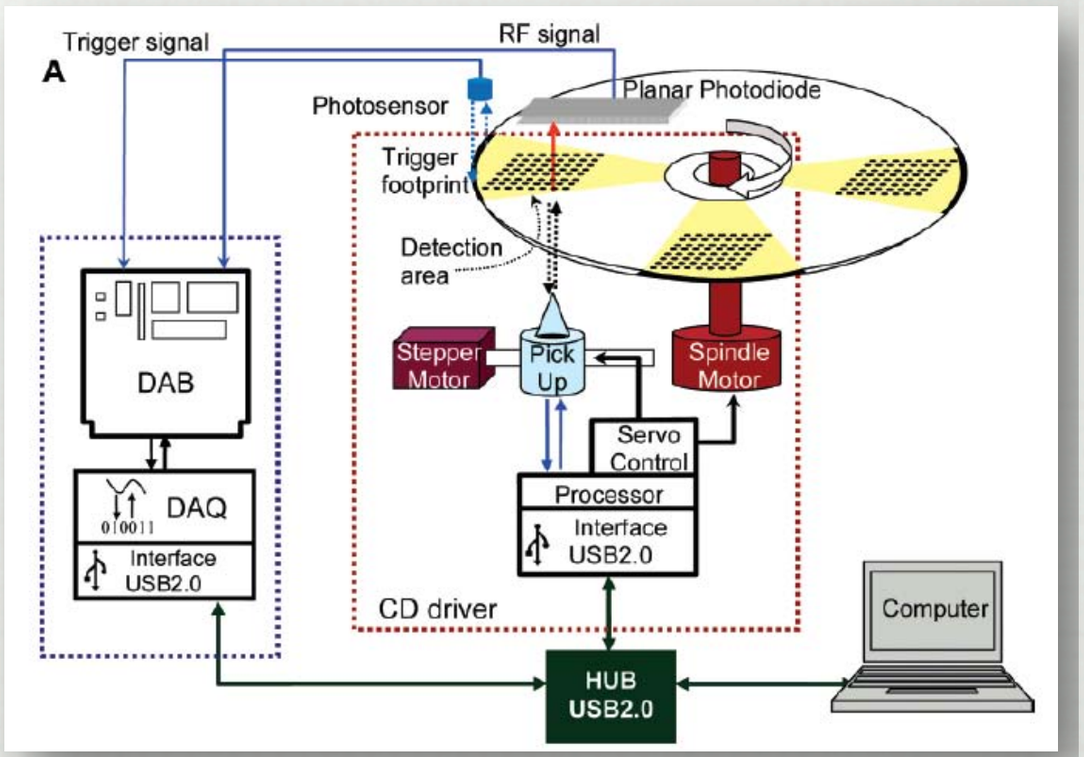
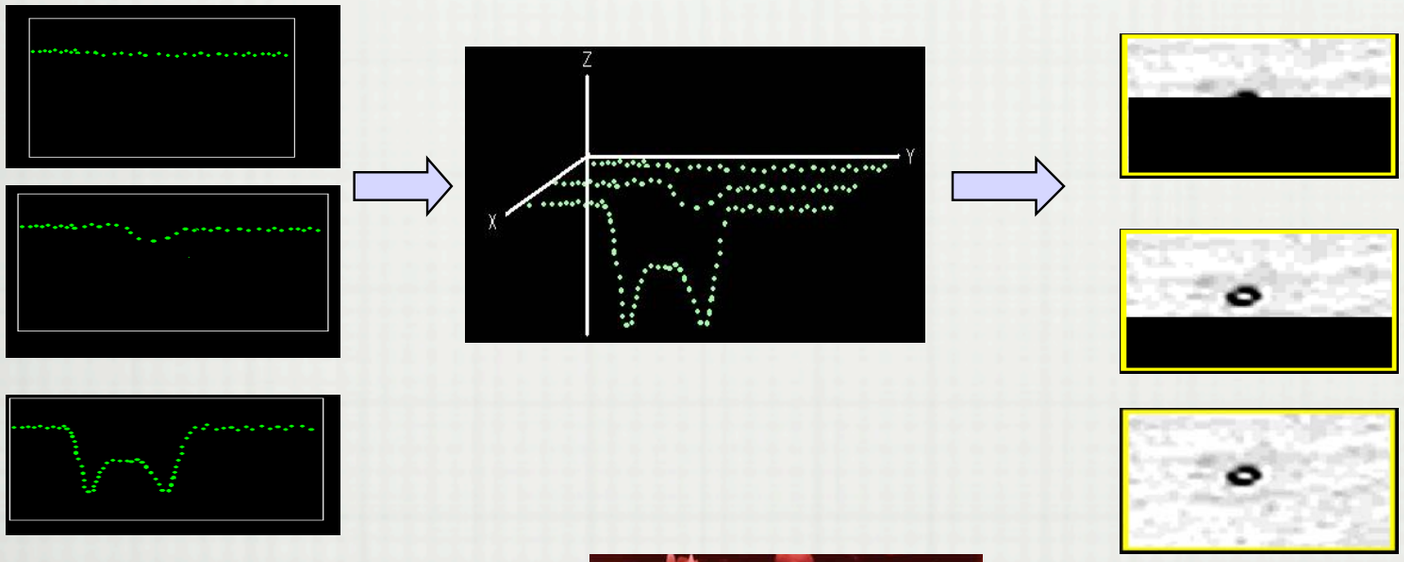


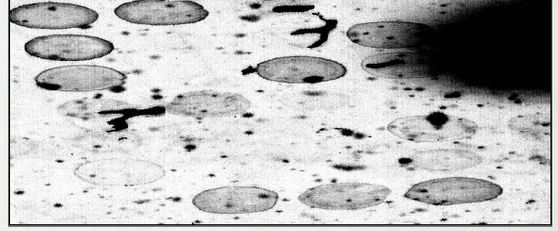
Image Courtesy of H Kido

CD AS A MICROSCOPE

VOLTAGES READ BY PHOTO DETECTOR ARE RECONSTRUCTED INTO IMAGES WITH A/D CONVERTERS AND GRAPHICS SOFTWARE



DIPS= AREAS OF LESS TRANSMITTED LIGHT



IMMUNOASSAYS

DNA ARRAYS

WHITE BLOOD CELLS

GNAT'S WING

IMAGES COURTESY OF H.KIDO

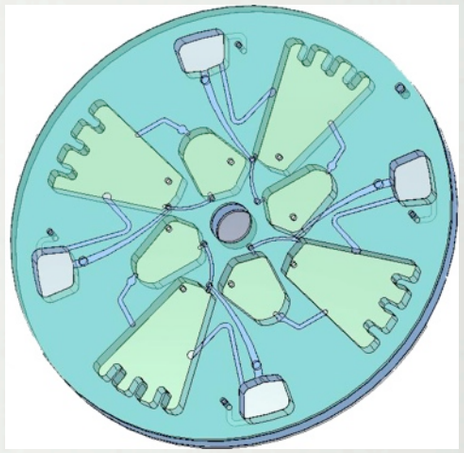
UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

CD AS A DIAGNOSTIC SYSTEM

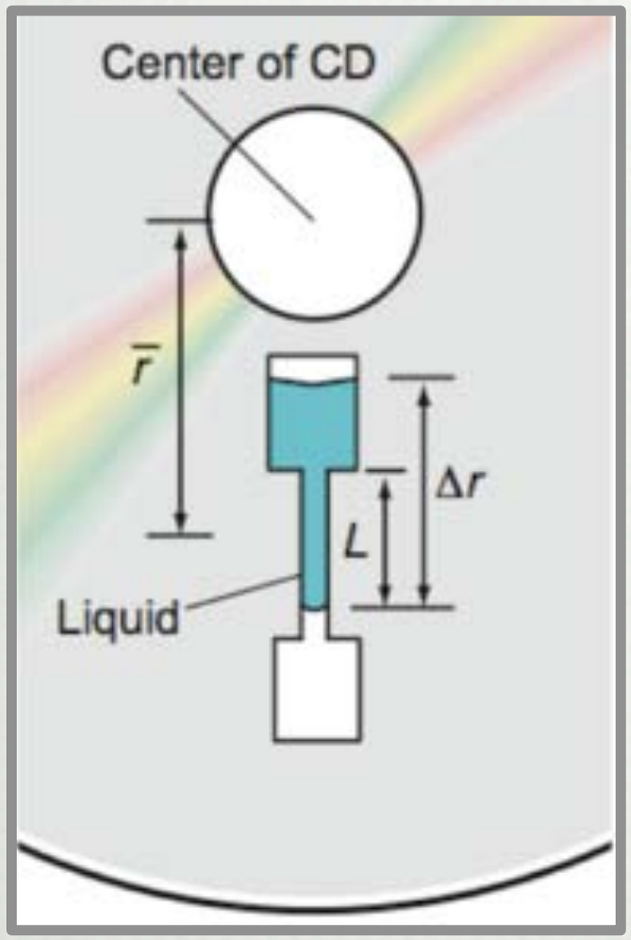
ADVANTAGES OF CENTRIFUGAL MICROFLUIDICS

- ☐ PROGRAMMABLE CENTRIFUGE - AUTOMATION OF SEVERAL PROCESSES ONTO SINGLE DISC
- ☐ COMPATIBLE WITH A WIDE RANGE OF SAMPLES
- ☐ NO FLUID CONTACT WITH PUMPING MECHANISM
- ☐ LIQUID AND VAPOR VALVING SOLUTIONS
- ☐ MULTIPLEXED DESIGNS FOR HIGH THROUGHPUT
- ☐ RAPID PROTOTYPING
- ☐ CHEAP, DISPOSABLE PLASTIC DEVICE
- ☐ MASS PRODUCIBLE - INJECTION MOLDING, HOT EMBOSSING



M. AMASIA AND J.SIEGRIST

CD MICROFLUIDICS FUNDAMENTALS



MADOU, ET AL. 2006

CD FLUID PROPULSION IS ACHIEVED THROUGH CENTRIFUGALLY INDUCED PRESSURE AND DEPENDS ON ROTATION RATE, GEOMETRY AND LOCATION OF CHANNELS AND RESERVOIRS, AND FLUID PROPERTIES.

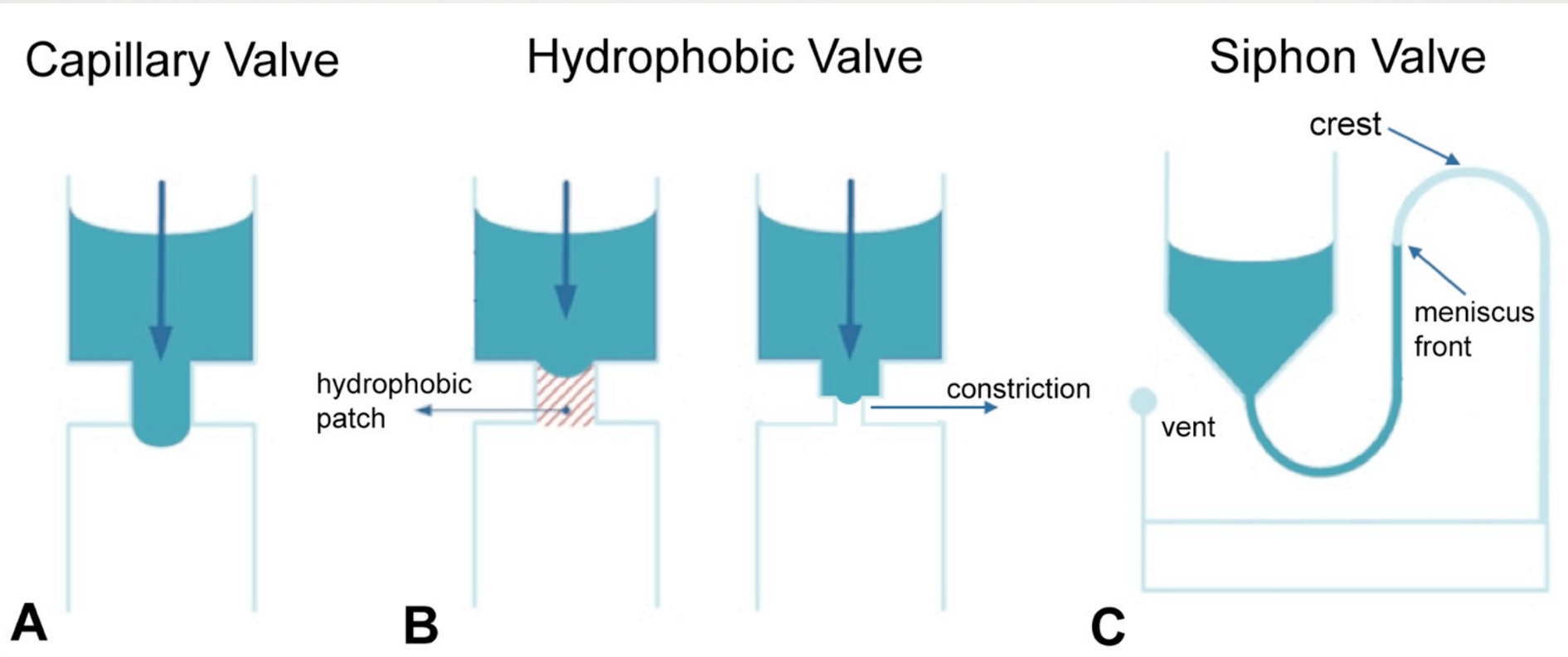
FLOW RATE, Q, IN A MICROFLUIDIC CD IS DESCRIBED BY:

$$Q = AD_h^{\,2}\rho\omega^2\bar{r}\Delta r / 32\mu L$$

A is channel cross-sectional area
D_h is hydraulic diameter
ρ is liquid density
ω is angular velocity of the spinning CD
r is the average distance of the liquid from the CD center
Δr is radial extent of the fluid
μ is fluid viscosity
L is the length of liquid in the channel

CD MICROFLUIDICS FUNDAMENTALS

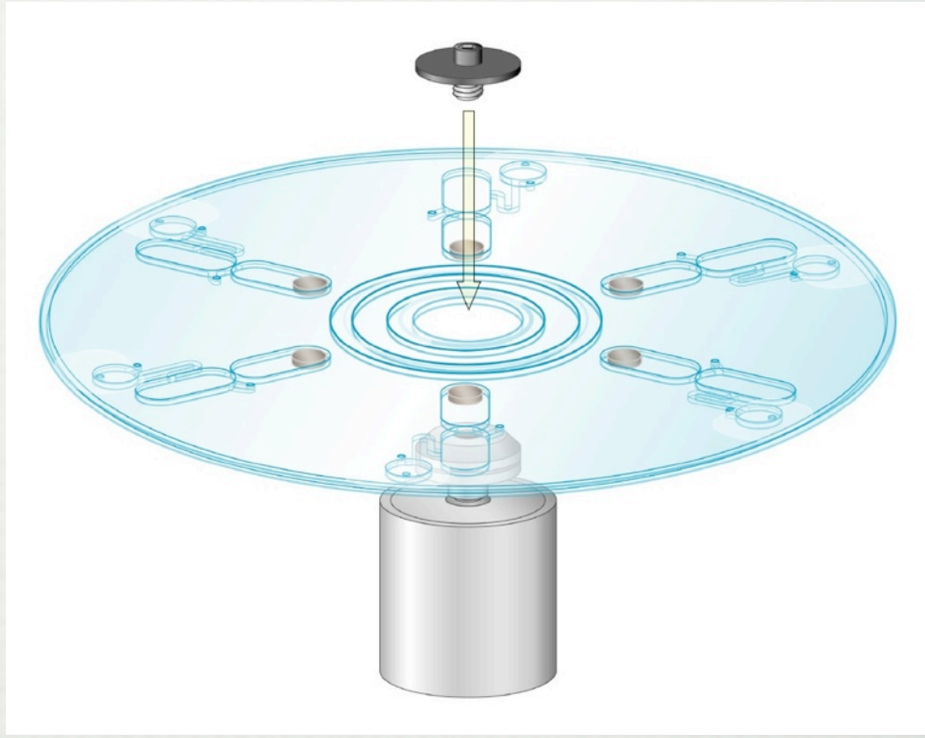
LIQUID VALVING MECHANISMS FOR THE CD PLATFORM:



CAPILLARY VALVES AND SIPHON VALVES DEPEND ON SURFACE TENSION FORCES DUE TO HYDROPHILIC SURFACE (CONTACT ANGLES <90°)

CELL LYSIS ON CD

- LYSIS IS IMPORTANT STEP IN SAMPLE PREPARATION, NEED TO GET GENETIC MATERIAL OUT OF CELL/SPORE FOR DOWNSTREAM NUCLEIC ACID ANALYSIS STEPS
- MANY DIFFERENT KINDS OF LYSIS ARE USED: MECHANICAL, CHEMICAL, THERMAL, ETC
- KIDO, ET AL UTILIZED MECHANICAL BEAD-BEATING LYSIS BY USING MAGNETIC DISCS WITHIN A CD DEVICE TO CREATE SHEER FORCES THAT LYSE THE CELLS
  - RAPID-- LYSIS OCCURS WITHIN 3-5 MIN
  - NO RESIDUAL CHEMICALS THAT CAN INHIBIT DOWNSTREAM PROCESSING STEPS



H. KIDO

CELL LYSIS ON CD

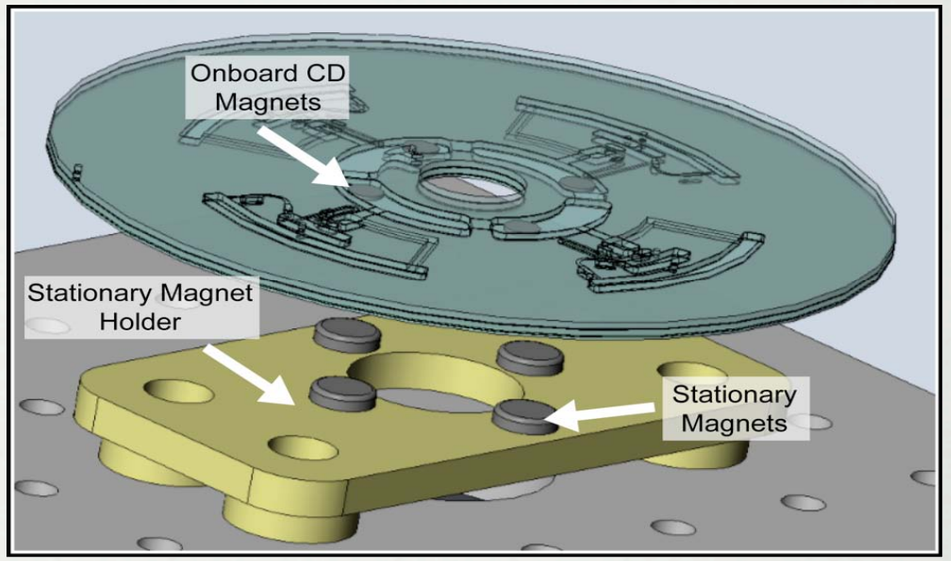
STEP	RPM	Seconds
Lysis	100	120
Transfer to clarification chamber	6000	30
Priming of siphon	100	10
Transfer to collection chamber	1500	10

CELL LYSIS ON CD

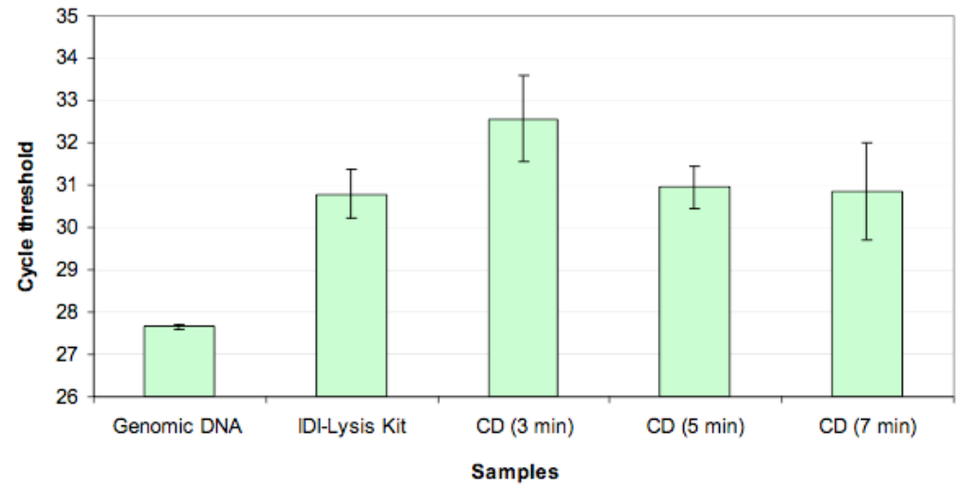
- IN THIS SYSTEM, A STATIONARY PLATFORM EMBEDDED WITH PERMANENT MAGNETS WAS ADDED BELOW THE SPINNING CD

- AS THE DISC SPINS, ON-CD MAGNETIC DISCS WERE ACTUATED, CAUSING GREATER IMPACTION THAN SUSPENDED BEADS ALONE. THE SYSTEM ALSO PERFORMED CLARIFICATION, WHEREBY LYSIS BY-PRODUCTS WERE CENTRIFUGED IN A CHAMBER, AND THE SUPERNATANT (CONTAINING THE RELEASED DNA) IS THEN SIPHONED INTO A SEPARATE COLLECTION CHAMBER.

- LYSIS BOF B. GLOBIGII SPORES OCCURS FULLY AND RAPIDLY WHEN COMPARED TO PURIFIED GENOMIC DNA AND IDI LYSIS KIT



SIEGRIST, ET AL 2009



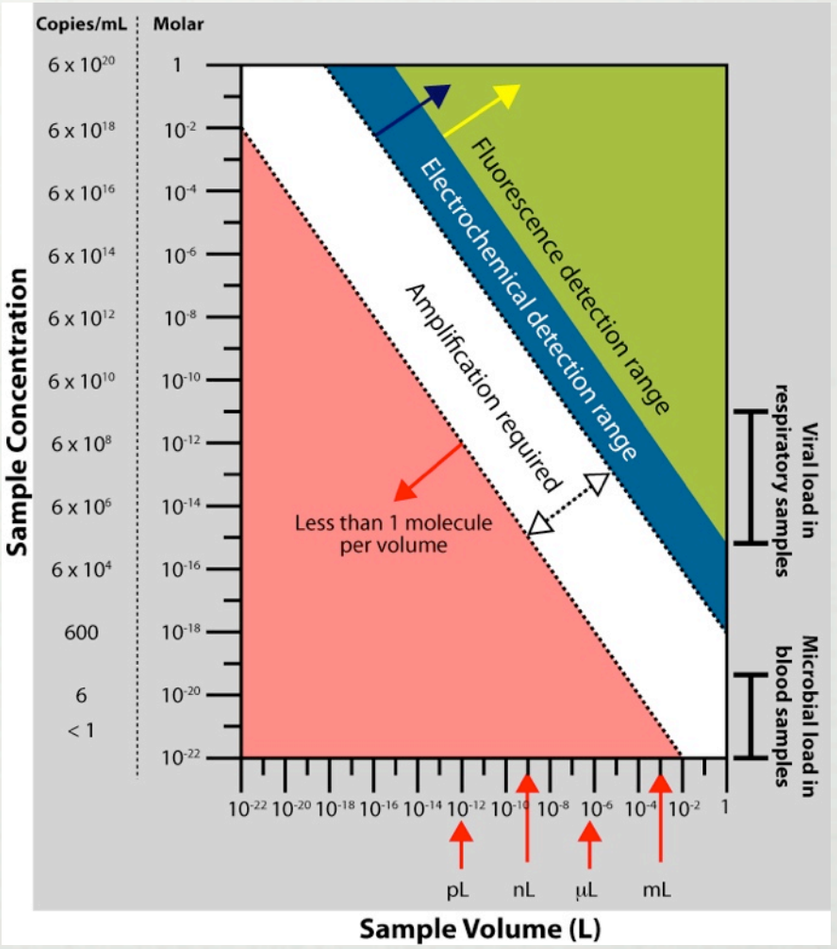
KIDO, ET AL 2006

UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

WORKING WITH LOW ANALYTE CONCENTRATIONS

- ANALYTE CONCENTRATION DICTATES WHAT TYPE OF DETECTION METHOD TO USE
- IN NA DIAGNOSTIC SYSTEMS, THE ANALYTE IS OFTEN PRESENT IN VERY LOW CONCENTRATIONS (<10 COPIES/ML) AS FOR THE CASE OF SEPSIS INFECTIONS
- ARRAY HYBRIDIZATION IS A HIGHLY SENSITIVE AND SELECTIVE METHOD FOR VIRIAL OR MICROBIAL DETECTION FOR SAMPLES WITH LOW ANALYTE CONCENTRATIONS



SIEGRIST, ET AL 2009

DNA ARRAY HYBRIDIZATION

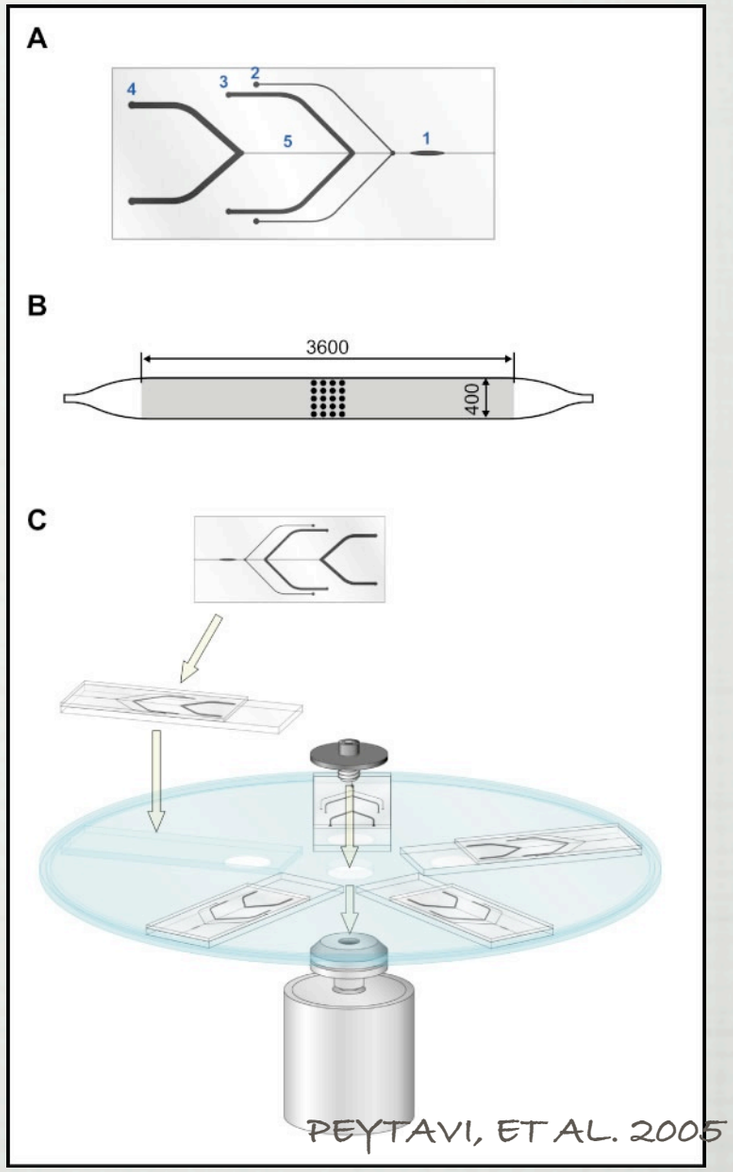
- IN POC DIAGNOSTICS, A SPEEDY ANALYTICAL RESULT IS IMPORTANT, BUT PASSIVE (DIFFUSION-LIMITED) DNA HYBRIDIZATION MAY TAKE MANY HOURS

- THE EQUATION DESCRIBING THE CAPTURE EFFICIENCY OF THE ARRAY IS:

$$CapE = \frac{C_{out}}{C_{in}} = 1 - \frac{h - \sqrt{2Dt}}{h}$$

WHERE COUT IS THE AVERAGE OUTPUT CONCENTRATION, CIN IS INPUT CONCENTRATION, H IS THE HEIGHT OF THE HYBRIDIZATION CHAMBER (50 MICRONS), T IS TIME AND IS CALCULATED FROM THE LINEAR VELOCITY AND THE SIZE OF THE ARRAY SPOT, AND D IS THE DIFFUSION COEFFICIENT OF DNA.

- BASICALLY, WE WANT TO USE A MICROFLUIDIC FLOW CHAMBER TO SHORTEN THE DIFFUSION TIME OF THE TARGET MOLECULE TO ITS BINDING PARTNER

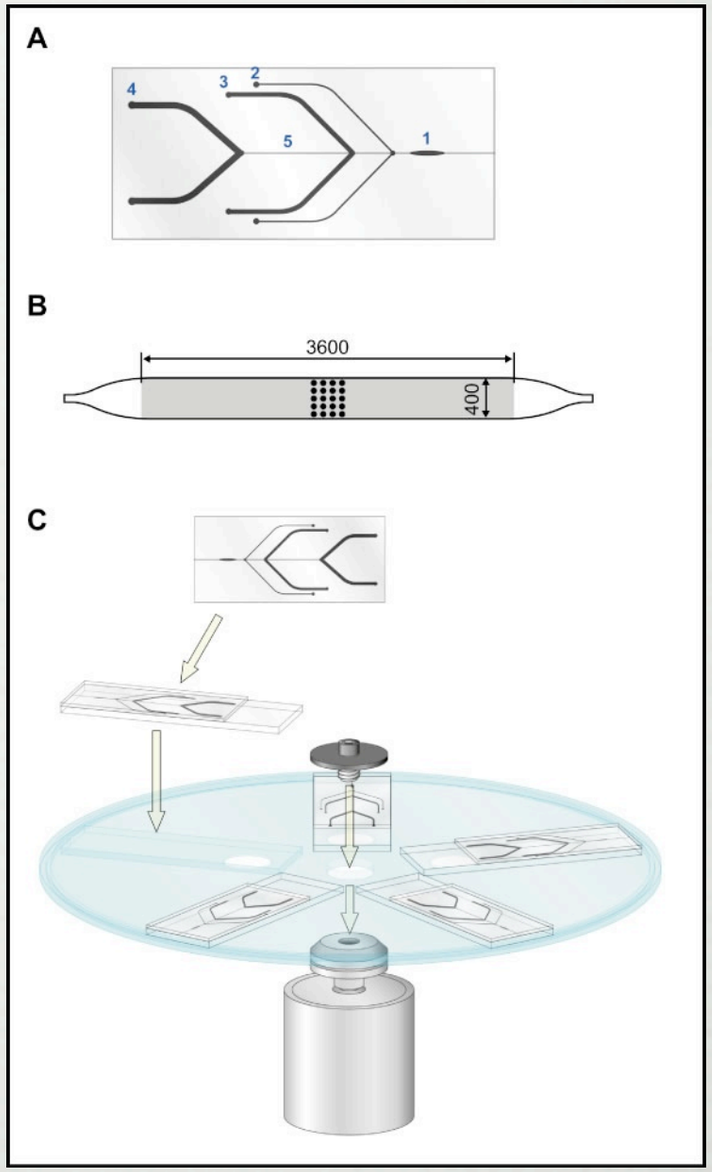


UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

DNA ARRAY HYBRIDIZATION

- WE DEVELOPED A SIMPLE MICROFLUIDIC SYSTEM MOLDED INTO A POLYDIMETHYLSILOXANE SUBSTRATE (PDMS) (PEYTAVI ET AL., 2005).
  - PDMS IS A LOW-COST MATERIAL THAT CAN BE EASILY PROTOTYPED AND CAN MAKE REVERSIBLE WATERTIGHT SEALS WITH GLASS SLIDES.
- THESE UNITS ARE PLACED ON AN ENGRAVED CD SUPPORT FIXED ON A ROTATIONAL DEVICE. IT COMPRISES A LINEAR HYBRIDIZATION CHAMBER COVERING THE MICROARRAY, 2 BUFFER RESERVOIRS, A SAMPLE RESERVOIR, A WASTE OUTLET, AND THE CONNECTING CHANNELS. WASTE LIQUIDS ARE COLLECTED INTO A CIRCULAR CHAMBER MACHINED AROUND THE PLASTIC SUPPORT.
- CENTRIFUGAL FORCES DRIVE THE SAMPLE AND BUFFERS DIRECTLY ONTO THE MICROARRAY SURFACE.
  - LOW DENSITY DNA PROBES MICROARRAY ARE PRINTED ON A STANDARD GLASS SLIDE MODIFIED WITH OUR NEW SURFACE CHEMISTRY, TO FORM A FUNCTIONAL MICROFLUIDIC UNIT.
  - UP TO 6 (150 SPOTS)-MICROARRAY SLIDES CAN BE HELD IN A SLIDE HOLDER CD

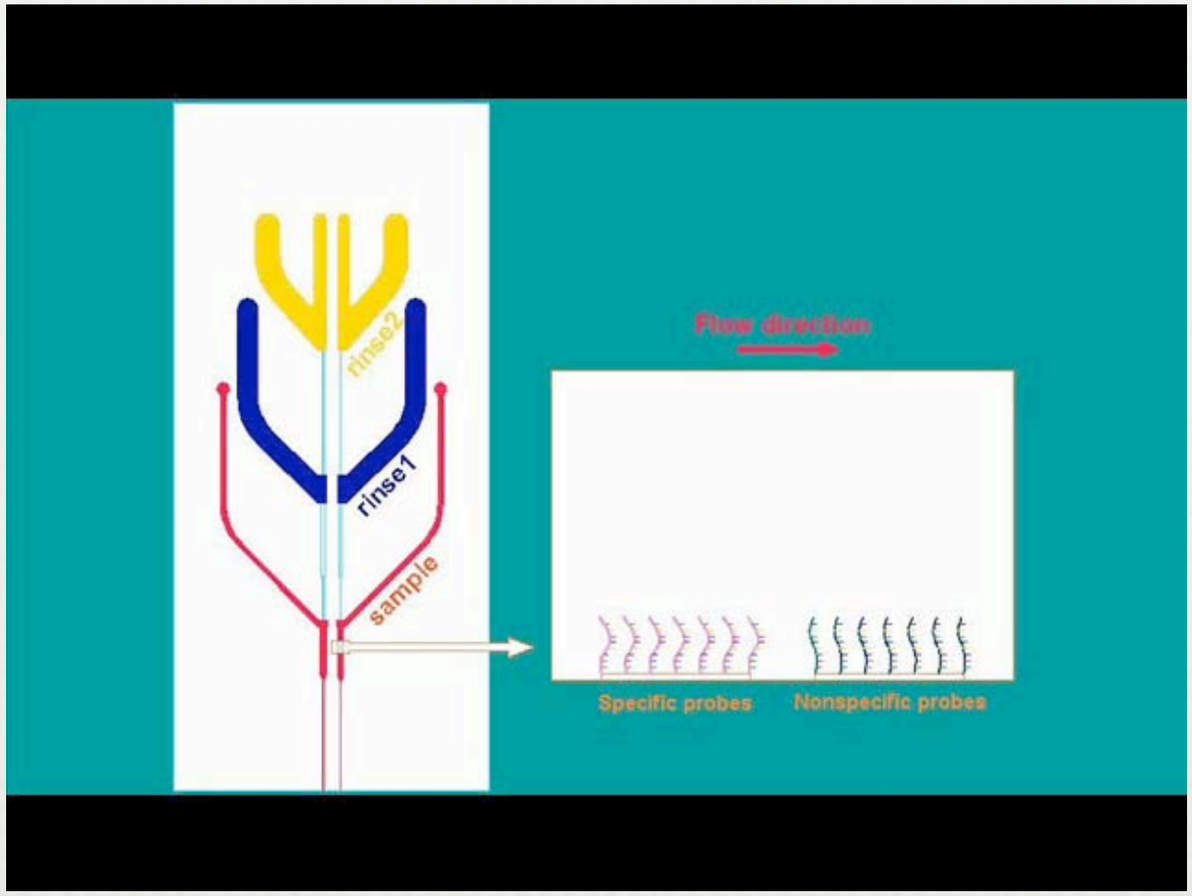


PEYTAVI, ET AL. 2005

UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

DNA ARRAY HYBRIDIZATION



VIDEO BY J. ZOVAL

DNA ARRAY HYBRIDIZATION

CAPILLARY VALVES BURST AT:
450 RPM SAMPLE LOADING,
700 WASHING BUFFER,
850 RPM RINSE

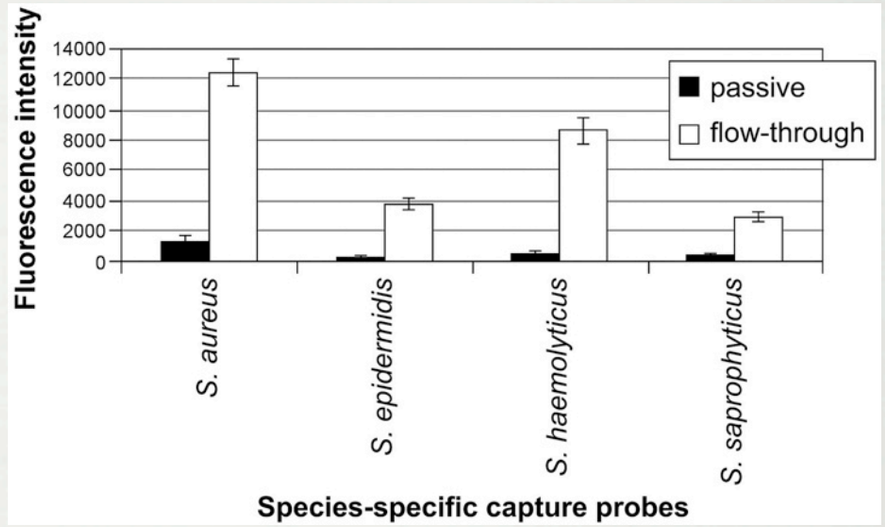
UCIrvine | University of California

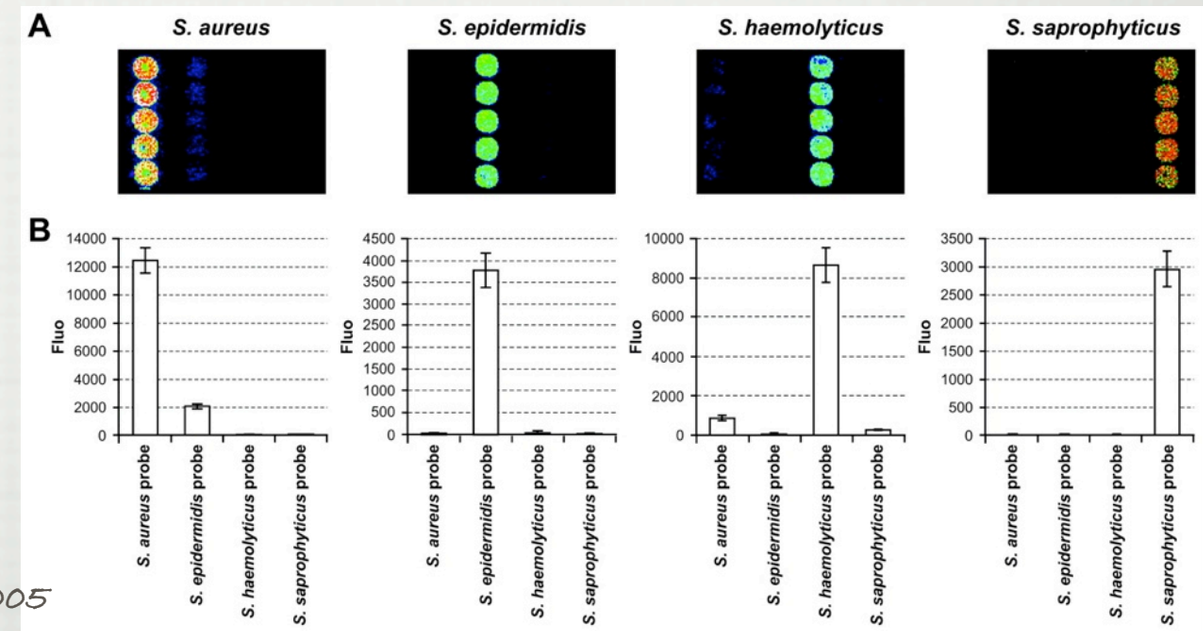
BioMEMS Research Group @ UC Irvine

DNA ARRAY HYBRIDIZATION

- REDUCED THE ARRAY HYBRIDIZATION TIME DOWN TO 15 MINUTES
- THIS MICROFLUIDIC SYSTEM ALLOWS A DRASTIC REDUCTION IN THE VOLUME OF REAGENTS NEEDED FOR HYBRIDIZATIONS AND DOES NOT REQUIRE A PCR AMPLICON PURIFICATION STEP.
- OUR MICROFLUIDIC SYSTEM ALLOWED DETECTION OF PCR AMPLICONS GENERATED FROM 10 BACTERIAL GENOME COPIES, WHICH IS AT LEAST 1000 TIMES MORE SENSITIVE THAN RESULTS OBTAINED BY OTHER GROUPS USING MICROARRAY HYBRIDIZATION ON MICROFLUIDIC DEVICES (LENIGK ET AL., 2002).
- IN TERMS OF SPECIFICITY, THIS SIMPLE SYSTEM DIFFERENTIATED 4 DIFFERENT STAPHYLOCOCCUS SPECIES WITH A POST-PCR HYBRIDIZATION PROTOCOL OF ONLY 15 MIN (PEYTAVI ET AL., 2005B

PEYTAVI, ET AL. 2005





UCIRVINE | UNIVERSITY of CALIFORNIA

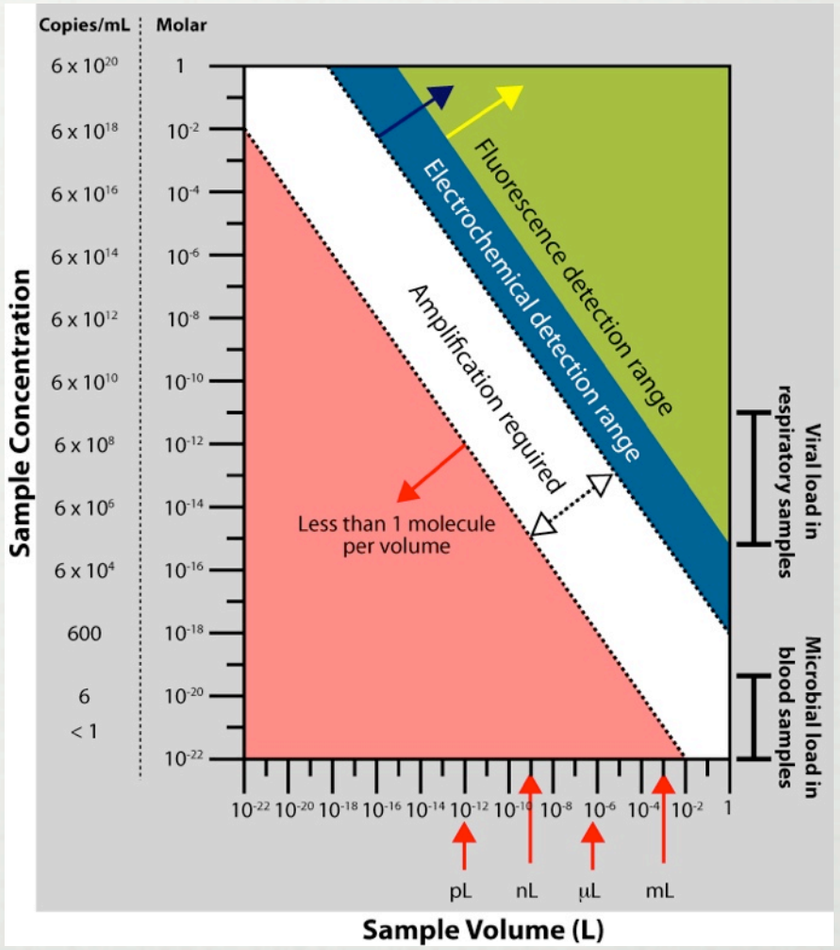
BIOMEMS Research Group @ UC Irvine

WORKING WITH LOW ANALYTE CONCENTRATIONS

- IN NA DIAGNOSTIC SYSTEMS, THE ANALYTE IS OFTEN PRESENT IN VERY LOW CONCENTRATIONS (<10 COPIES/ML) AS FOR THE CASE OF SEPSIS INFECTIONS

- IF AN ASSAY REQUIRES 10 COPIES FOR NA DETECTION, THEN THE ASSAY REQUIRES 1 ML OF BLOOD SAMPLE

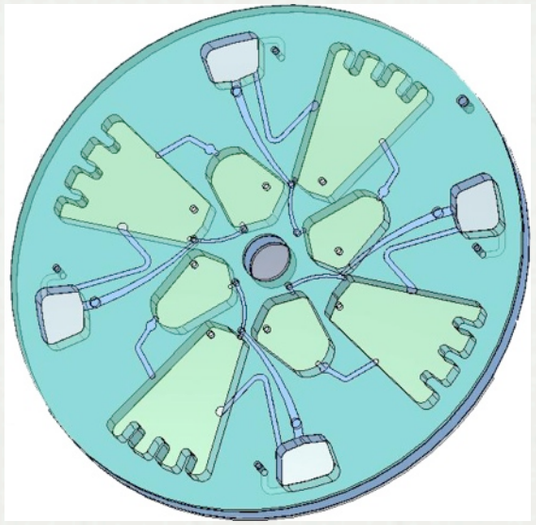
- TO ACHIEVE DIAGNOSIS, THE DEVICE MUST BE ABLE TO PROCESS THESE LARGE SAMPLES, WHILE STILL ALLOWING FOR INTEGRATION OF DOWNSTREAM STEPS (CONCENTRATION, LYSIS, PCR, DETECTION)

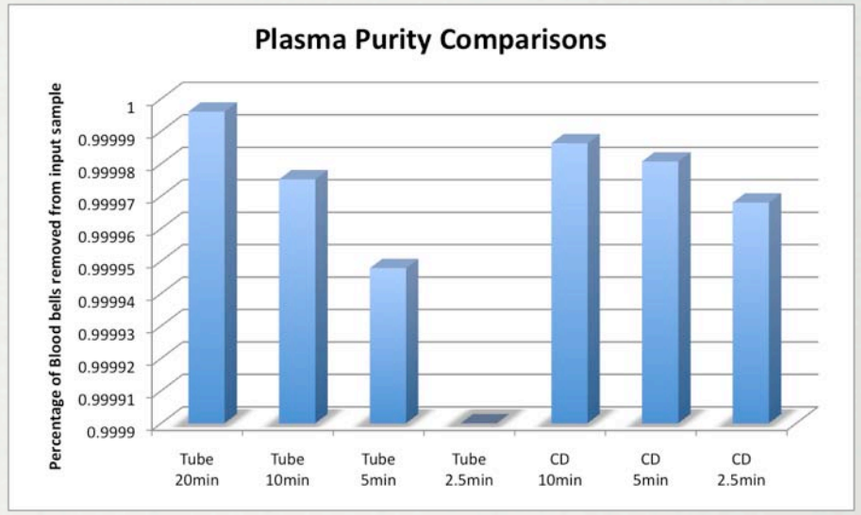


SIEGRIST, ET AL 2009

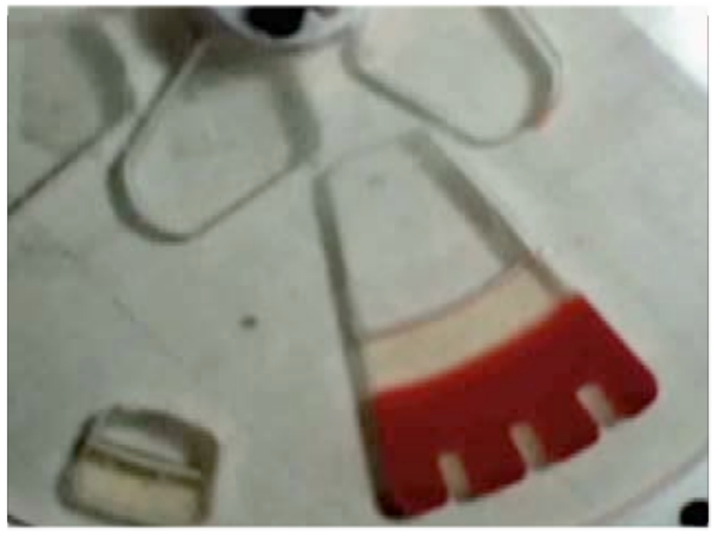
TOWARDS SAMPLE-TO-ANSWER SYSTEMS

WE HAVE DEVELOPED A LARGE-VOLUME BLOOD SAMPLE PREPARATION CD TO PROCESS THESE LARGER SAMPLE SIZES





- PLASMA SEPARATION FROM 2 MLS OF WHOLE BLOOD
- EQUIVALENT PURITY FROM CD SEPARATION @ 2.5 MIN COMPARED TO GOLD-STANDARD TUBE SEPARATION 10 MINUTES



M. AMASIA

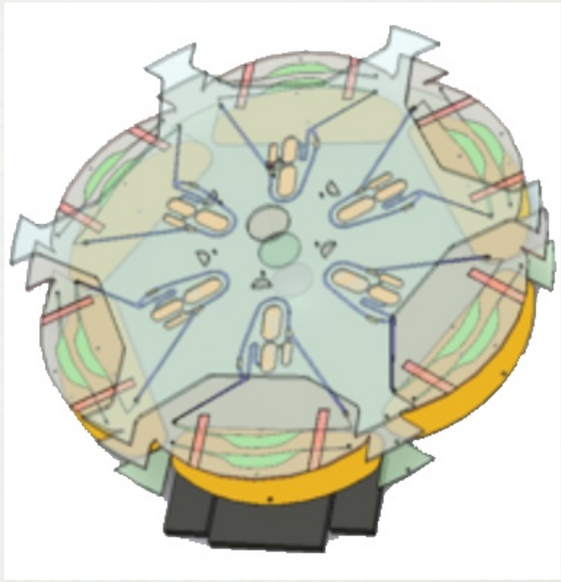
UCIRVINE | UNIVERSITY of CALIFORNIA

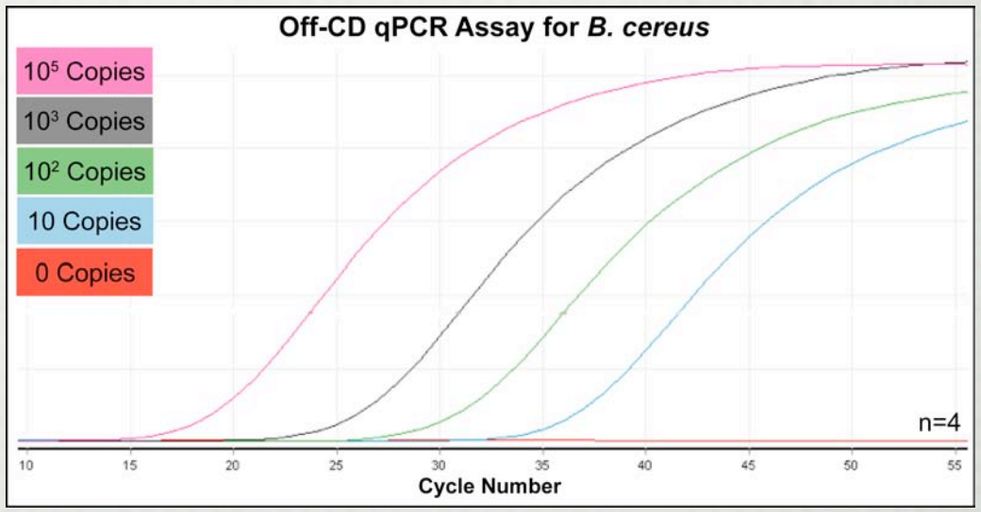
BIOMEMS Research Group @ UC Irvine

SAMPLE-TO-ANSWER SYSTEMS

DEVELOPING SYSTEM FOR THE RAPID DETECTION OF B.ANTHRACIS BY REAL-TIME PCR.

- ☐ SAMPLE PREPARATION (LYSIS AND CLARIFICATION, METERING)
- ☐ PCR AMPLIFICATION
- ☐ REAL-TIME PCR DETECTION



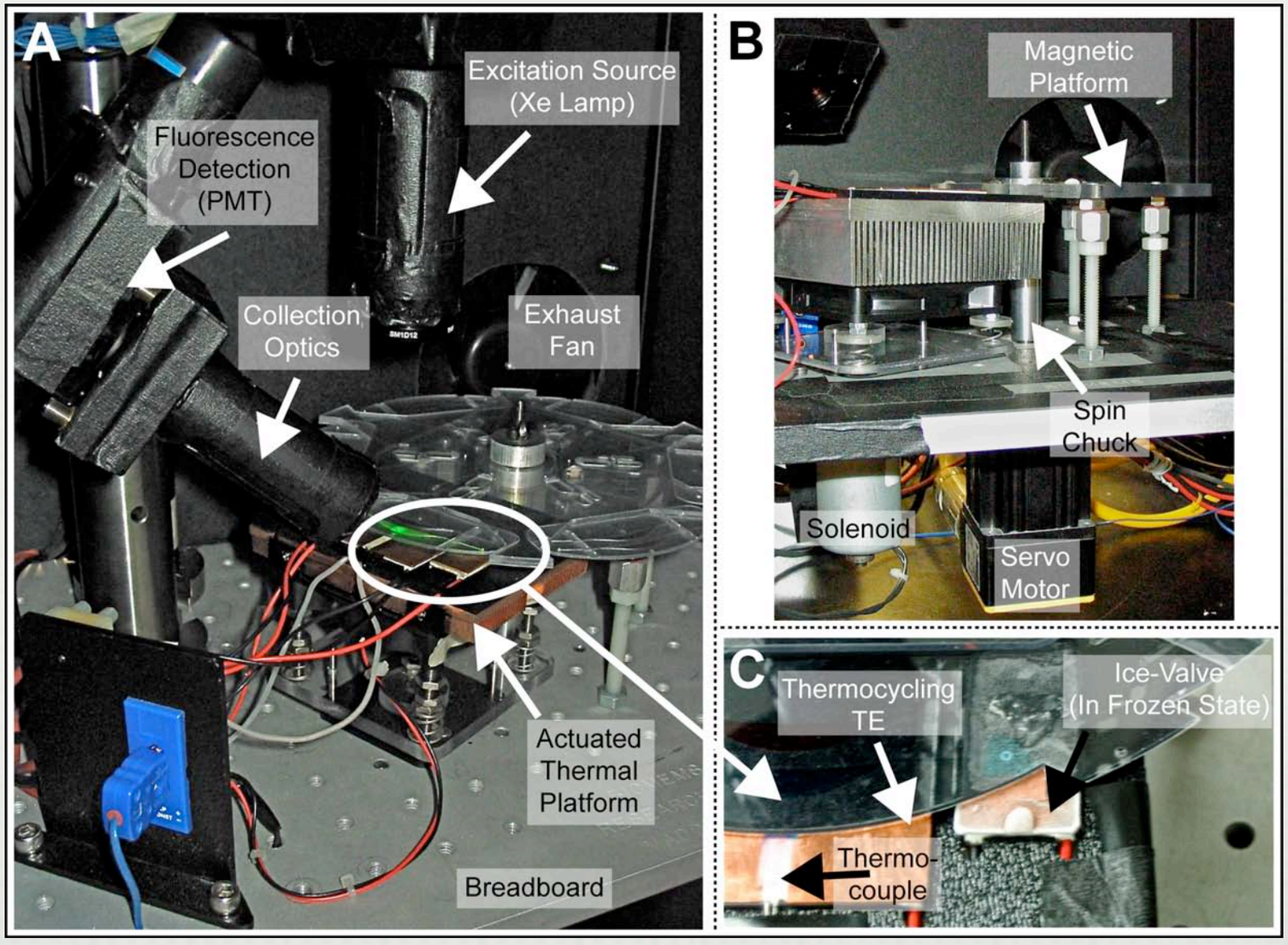


M. AMASIA AND J. SIEGRIST

UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

SAMPLE-TO-ANSWER SYSTEMS



J.SIEGRIST AND M.AMASIA

UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

SAMPLE-TO-ANSWER SYSTEMS

Virus	NA Composition	Common Illness
Influenza A (various subtypes)	RNA	Common flu
Influenza B (various subtypes)	RNA	Common flu
H5N1 (Influenza A subtype)	RNA	Avian flu
H1N1 (Influenza A subtype)	RNA	Swine flu
SARS-CoV	RNA	SARS
HPIV (various subtypes)	RNA	Parainfluenza
Adenoviruses (various subtypes)	DNA	Various ailments

J. SIEGRIST

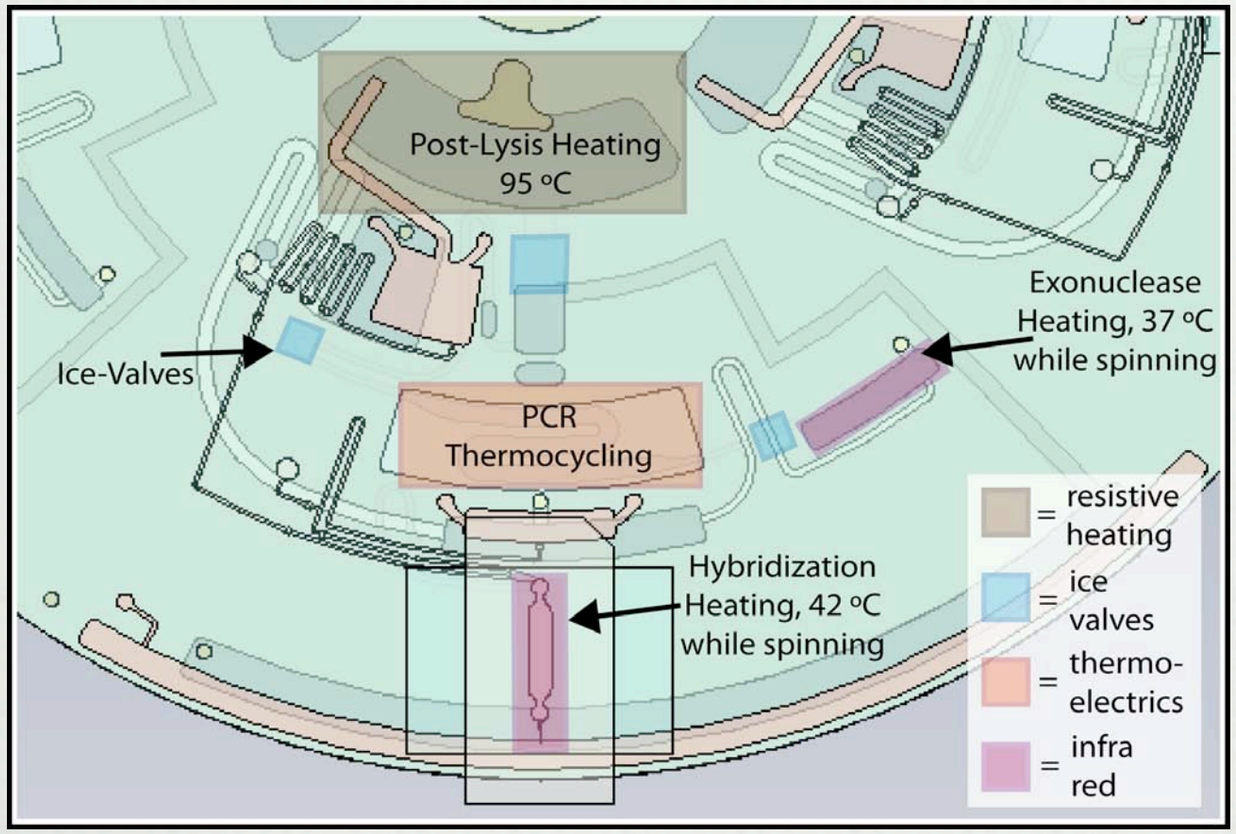
WORKING WITH M. BERGERON'S GROUP AT UNIVERSITY OF LAVAL TO DEVELOP A SYSTEM FOR RAPID RESPIRATORY VIRUS DETECTION THAT INTEGRATES:

- ☐ SAMPLE PREPARATION (LYSIS AND CLARIFICATION, METERING)
- ☐ PCR AMPLIFICATION
- ☐ DNA ARRAY DETECTION

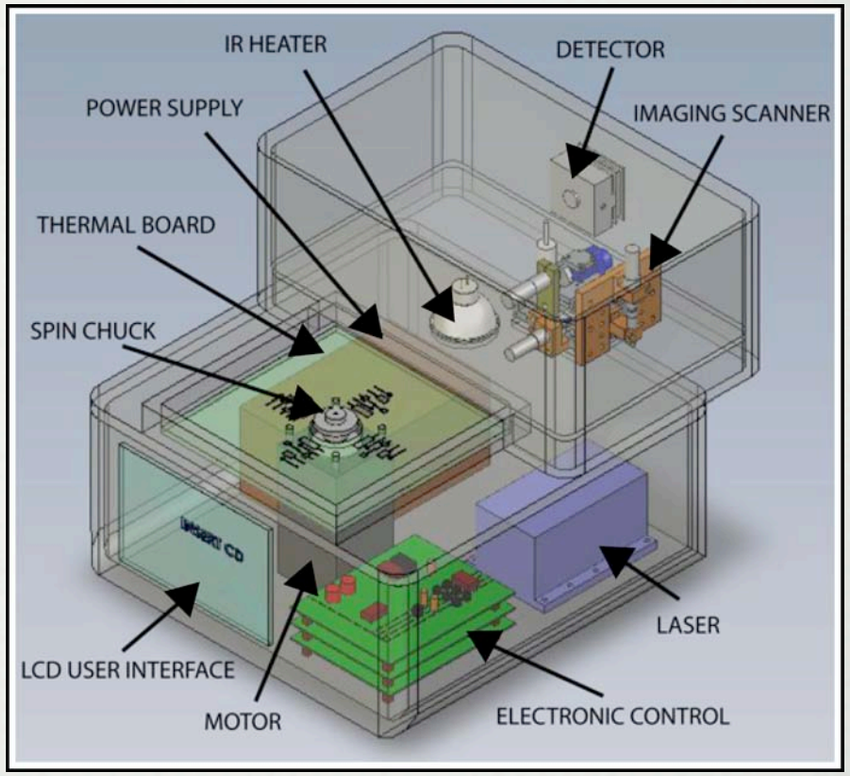
UCIRVINE | UNIVERSITY of CALIFORNIA

BIOMEMS Research Group @ UC Irvine

SAMPLE-TO-ANSWER SYSTEMS



J. SIEGRIST



R. GORKIN

THANK YOU!

UCIrvine | University of California

Research Group @ UC Irvine