DROPLET DEP AND BIODEVICES

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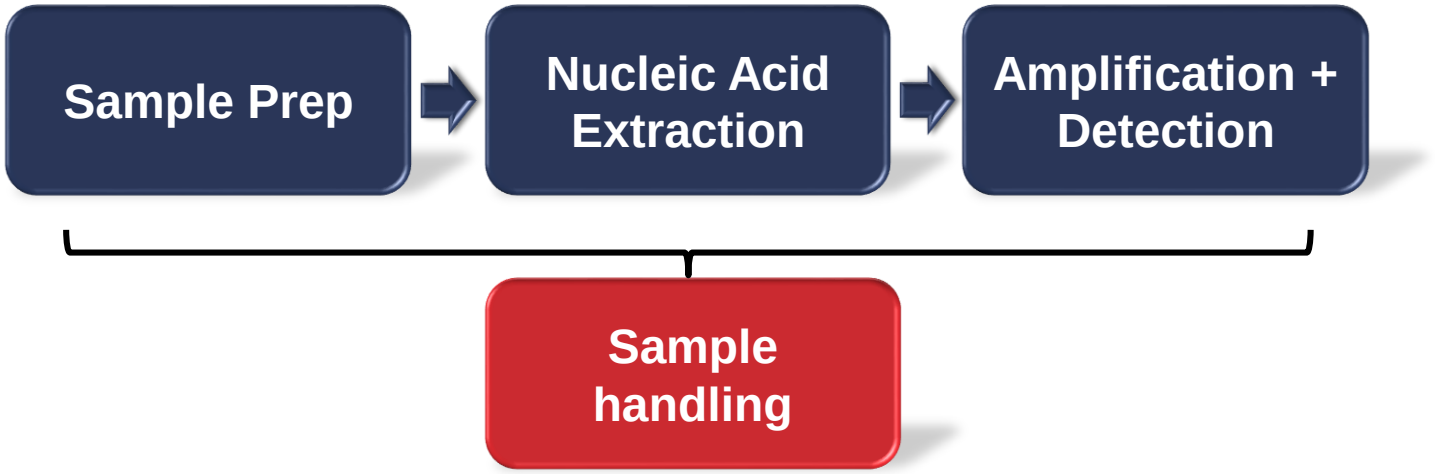
Presentation Outline

- **Droplet microfluidic nucleic acid diagnostic system requirements**
  - Sample preparation; Nucleic acid extraction; Sample/reagent handling; Nucleic acid amplification and detection
  - Droplet microfluidics (DMF) and different schemes for electro-actuation of droplets;
  - Dielectrophoresis (DEP) for sample dispensing and handling;
- **Anatomy of Micro/Nano fabricated DMF devices**
- **DMF measurement system**
- **Validation of DMF based nucleic acid diagnostic platform**
  - Illustration of DEP based sample preparation using yeast cell samples, functionalized beads
  - qRT-PCR detection of Influenza A virus in a clinical blind panel
  - Detection of multiple respiratory panel viruses (Influenza A, Influenza B) in a clinical blind panel
  - On-chip extraction and qRT-PCR amplification of different MS2 bacteriophage samples
- **Summary of current capabilities and future goals**

Sample-to-Detection In Nucleic Acid Based Diagnostics

- The four components of nucleic acid based point-of-care diagnostic system;
- Droplet Microfluidic approach towards miniaturized sample handling and integration of the different components on a single microfluidic device.



- Sample and reagent dispensing;
- Mixing and separation of different samples/reagents;
- DEP based handling of liquid samples, multiphase media and particle samples for biological applications

Droplet Microfluidics (DMF) for Sample Handling

Droplet based Microfluidics:

- Droplet based open channel/surface microfluidic technology (consist of patterned micro-electrodes/insulated top surfaces)
- Controlled dispensing of aqueous sample/reagent droplets (μL-pL volume)
- Aqueous sample dispensing, manipulation (transportation, mixing/splitting, thermal cycling) achievable in parallel, automated fashion
- Suitable for low-volume, parallel and multiplexed bio-diagnostic assays

Salient features of DMF:

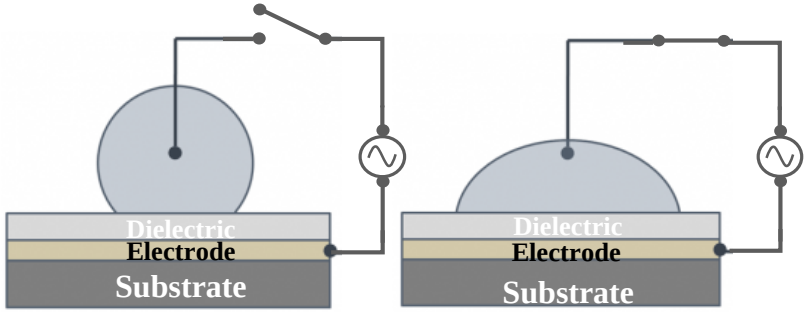
- Reduced volume of bio- samples/reagents
- Rapid testing and high throughput screening
- Custom micro-fabrication of devices tailored for target application
- Ease of integration of sensor technology (waveguides, CMOS/CCD sensors)

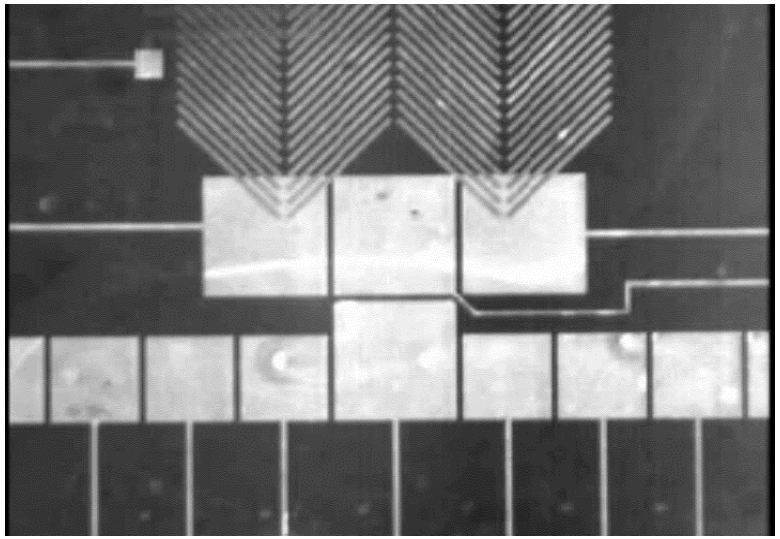
Electro-actuation Of Droplets

- Utilizes suitably energized electrode architectures to facilitate one or more microfluidic applications (sample dispensing, transport, mixing/splitting etc...)

Droplet Manipulation: EW and DEP

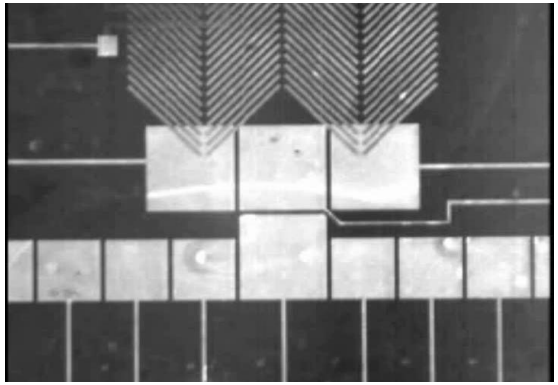
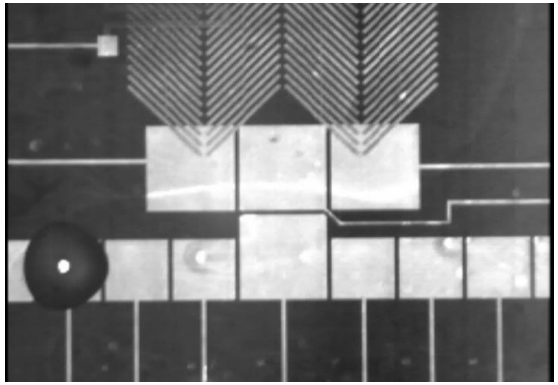
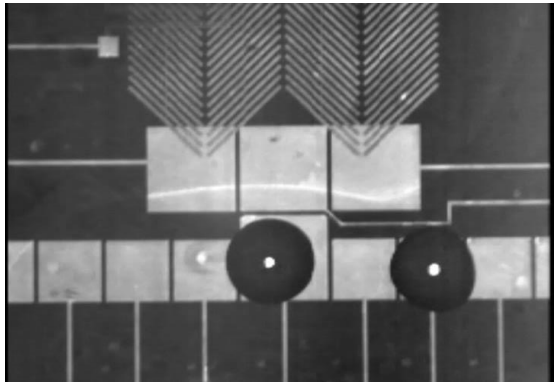
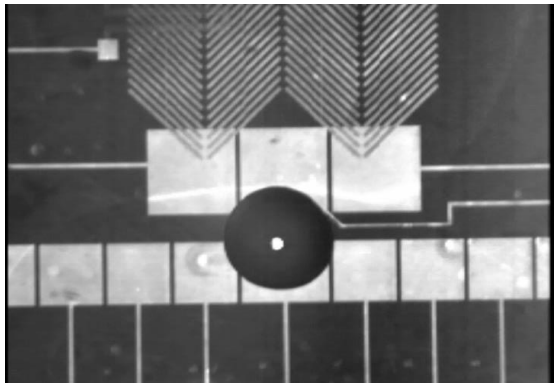
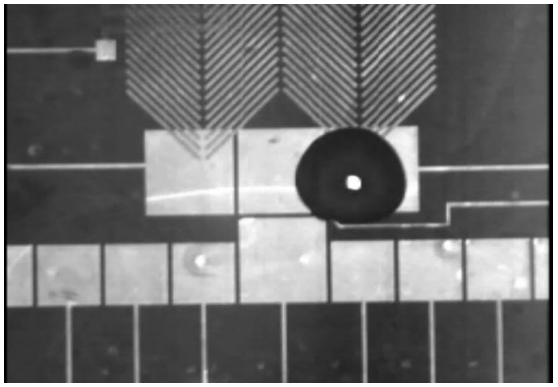
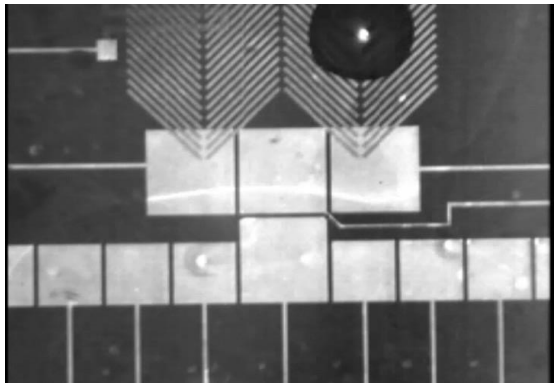
- Electrowetting (EW) modulates the liquid contact angle using an applied voltage
- Single surface EW can be achieved using a patterned, dielectrically passivated electrode arrays and superhydrophobic surfaces
- EW droplet transport is produced as a result of active electrode switching





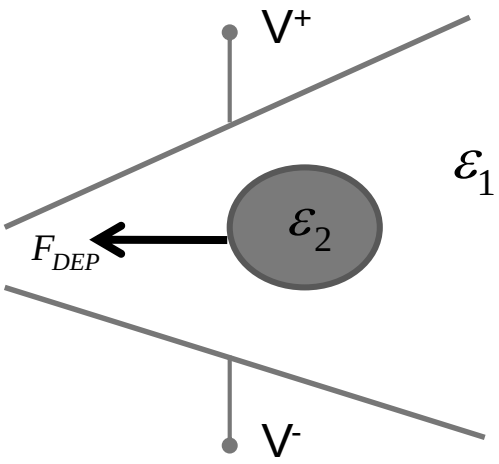
A single surface Electrowetting DMF device

SNAPSHOTS OF EW AND DEP DROPLET ACTUATION

DIELECTROPHORESIS (DEP)

An electromechanical effect that results from the interaction of a nonuniform electric field with polarizable matter (dielectric particles, biological cells, fluid media, etc…)



$$\vec{F}_{DEP} = \vec{P} \bullet \nabla \vec{E}$$

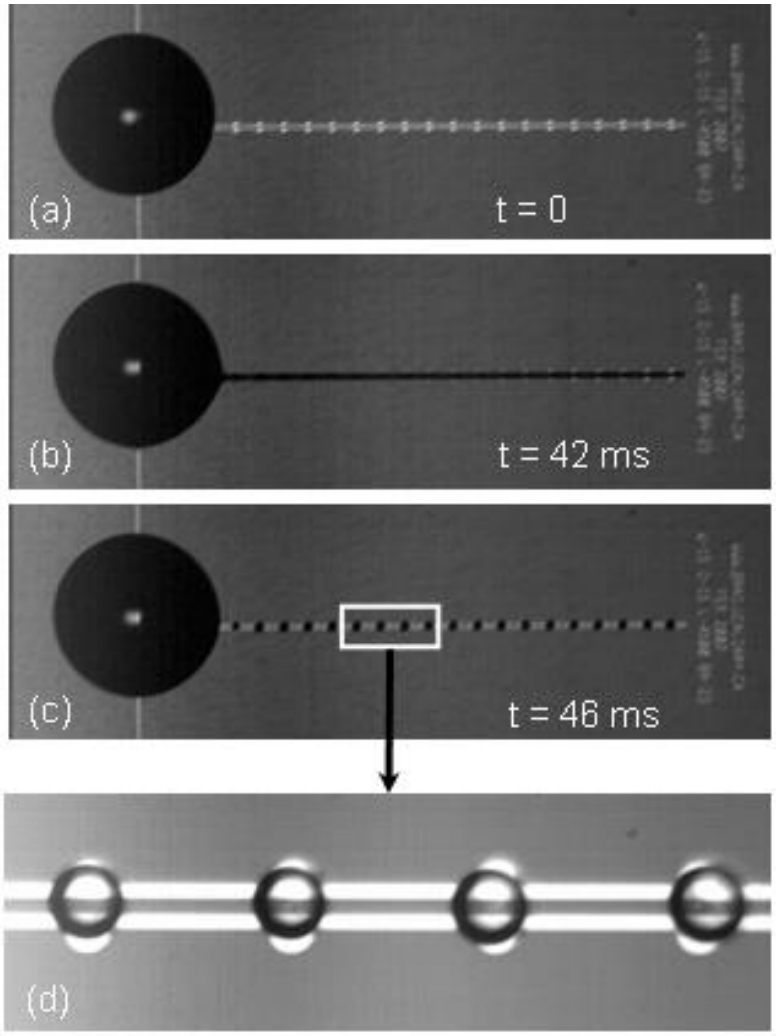
$$\vec{F}_{DEP} \propto \overrightarrow{\nabla E^2}$$

Positive DEP ($\varepsilon_2 > \varepsilon_1$)
Body force impels it into a region field of intensity maxima.

Negative DEP ($\varepsilon_2 < \varepsilon_1$)
Body force impels it into a region of field intensity minima

DEP For Sample Dispensing And Handling

- **Liquid DEP (LDEP):** Manipulation of dielectric fluids using spatially non-uniform electric field



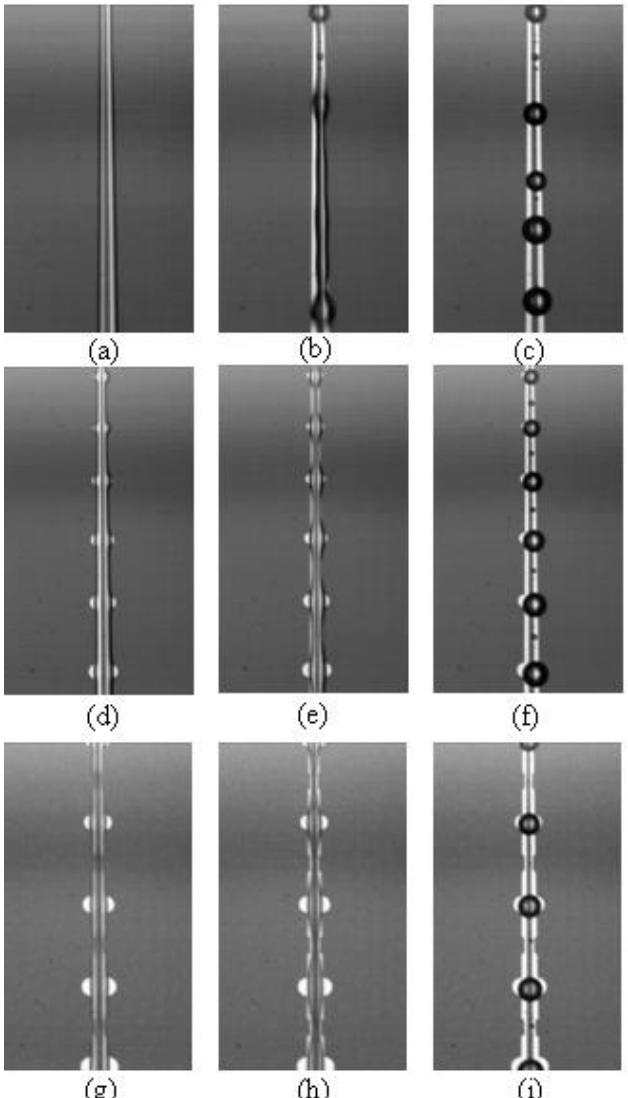
(a-c)A liquid rivulet originating from the parent droplet (DI water), conveyed along the full electrode length (average actuation speed ~6cm/sec

liquid rivulet breakup into individual droplets when the voltage is removed (electrode width (*w)* = 15μm, electrode spacing *(s)* = 15μm, actuation voltage = 210V_{RMS} at 100 kHz);

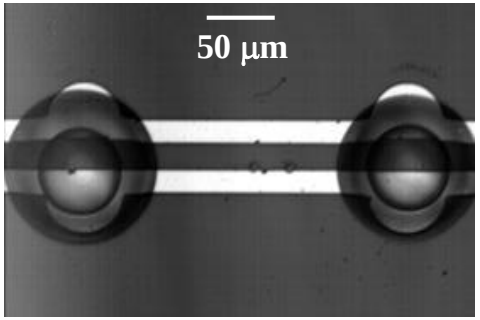
liquid rivulet breakup into individual droplets when the voltage is removed (electrode width (*w)* = 15μm, electrode spacing *(s)* = 15μm, actuation voltage = 281V_{RMS} at 100 kHz);

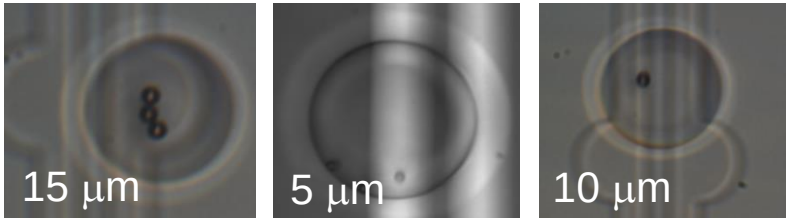
DEP FLUIDIC SAMPLE DISPENSING EXAMPLES

Variable volume dispensing schemes



Multiphase vesicle dispensing





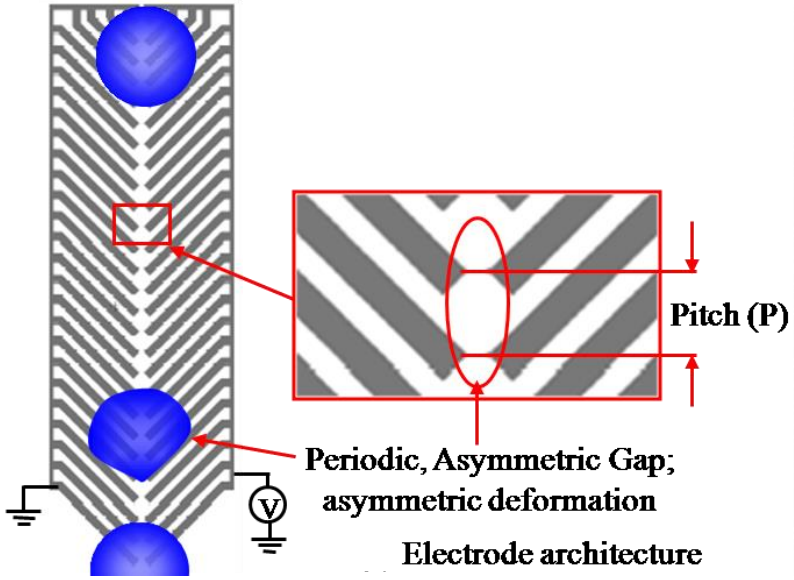
Functionalized microbeads of various sizes dispensed in ultra-low conc.

DEP For droplet Handling

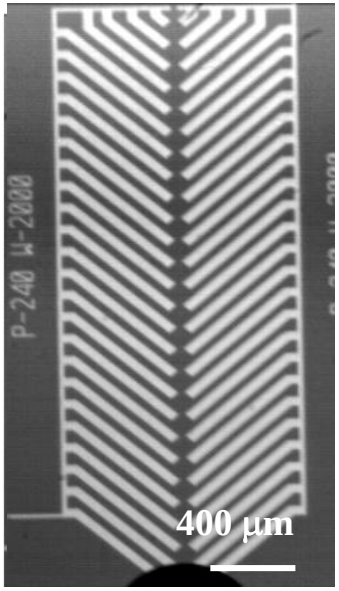
DEP based droplet transport and mixing

Electrostatic droplet actuation (or, D-DEP):

- Utilizes herringbone shaped electrode to transport and mix sample droplets;
- Low voltage (<100 Vpp), low frequency (<90 Hz) electrostatic actuation (low electrical power requirement)
- Suitable for droplet transport, mixing and thermal cycling

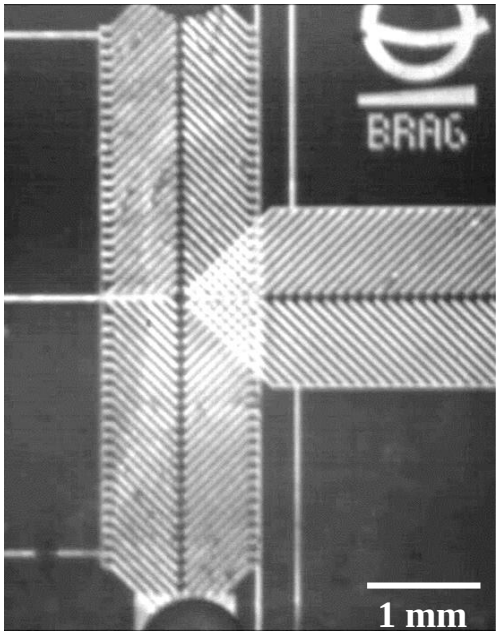


Sample transport



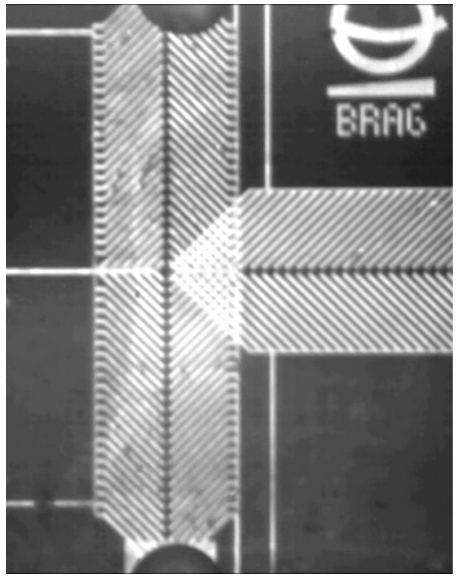
Actuated droplet volume: 1.0 μL

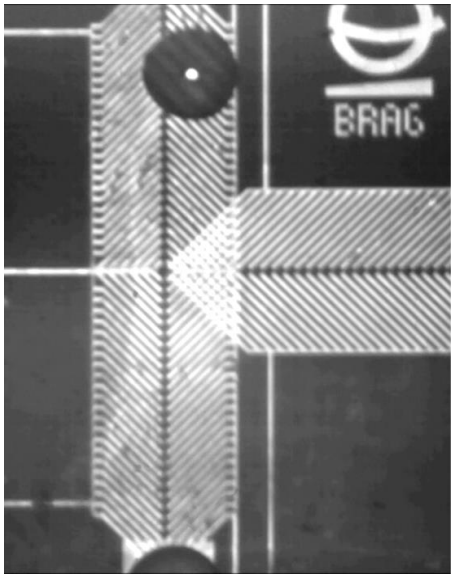
Sample/reagent mixing

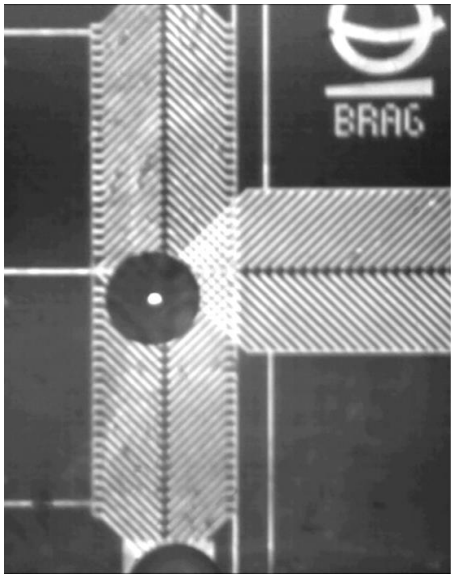


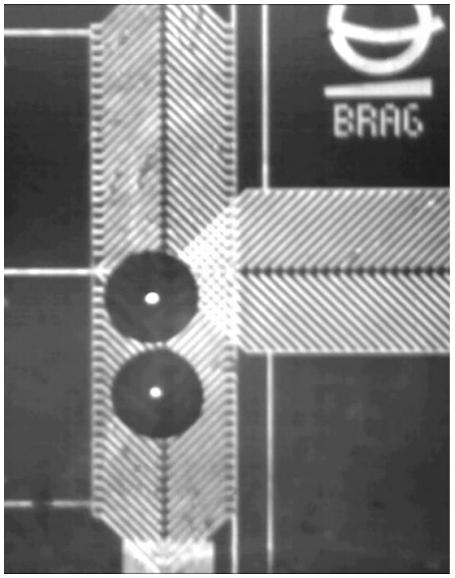
Actuated droplet volume: 5, 10 μL

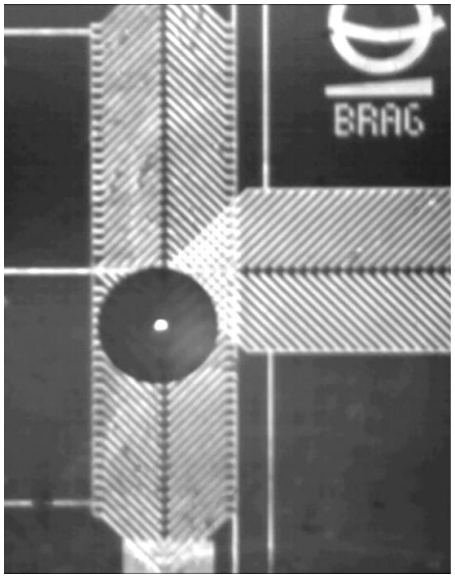
DEP For Sample Dispensing and Handling Snapshots

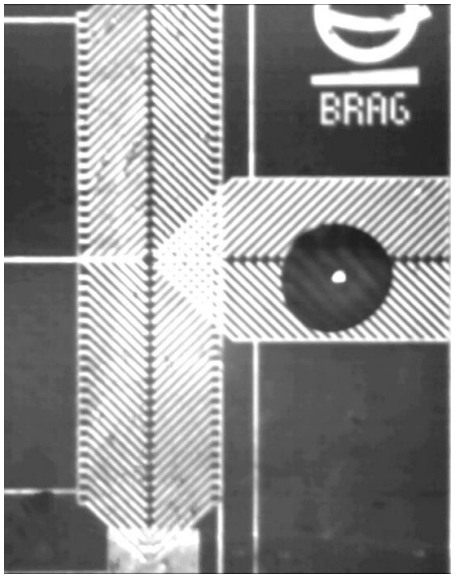








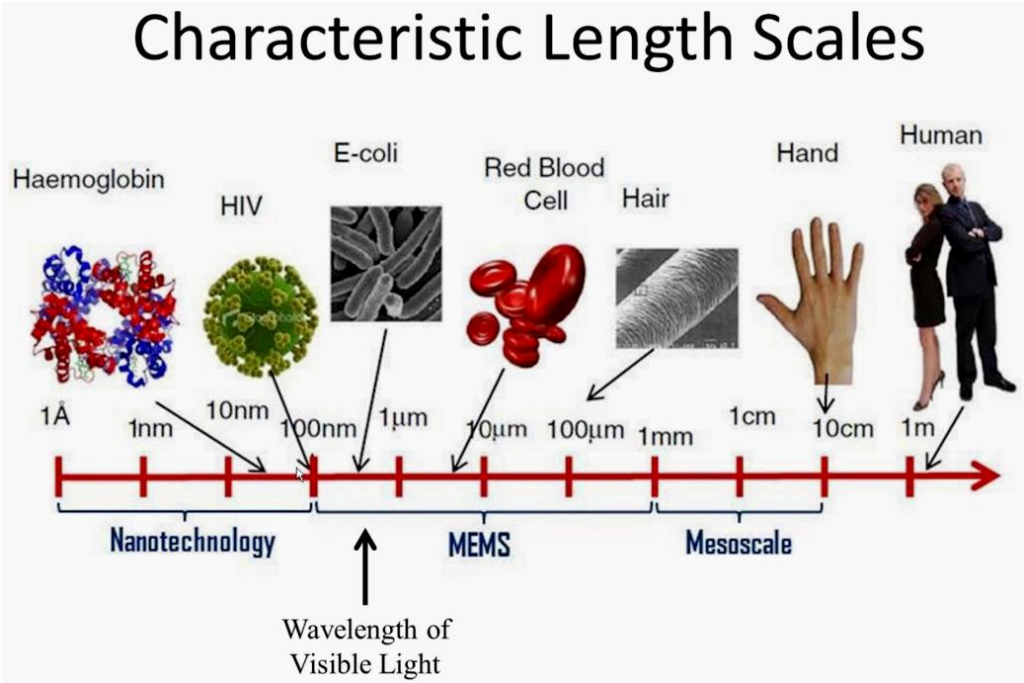




Chip Based Sample-to-Detection For Nucleic Acid Based Diagnostics

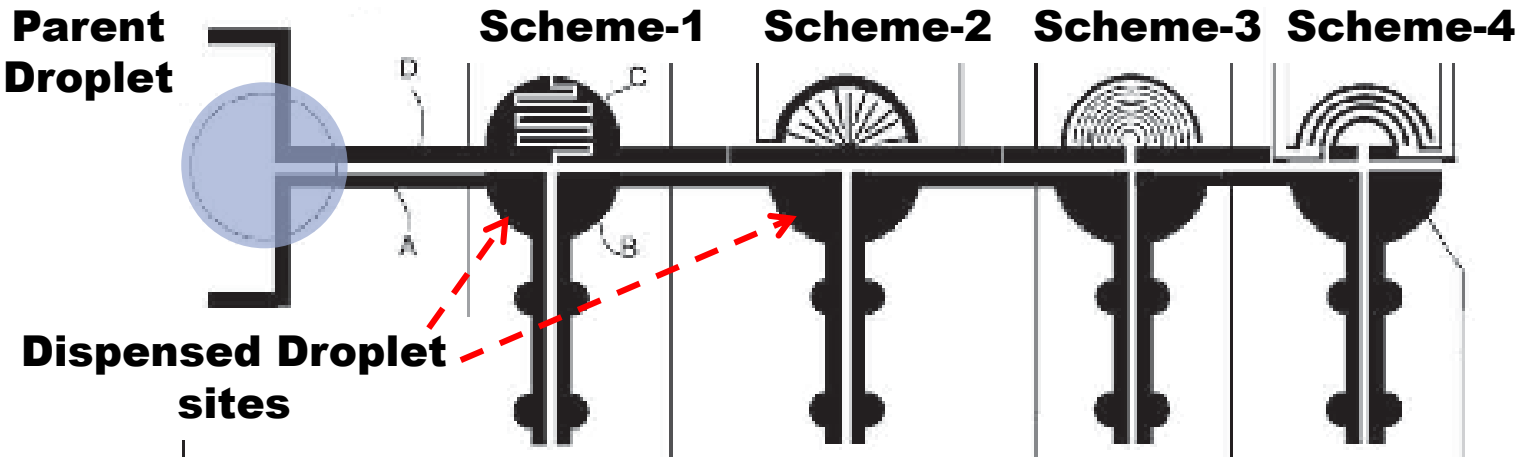
- Road-map for the development of a nucleic acid based diagnostic microsystem;
- Integration of the existing and newly developed droplet technologies on a microsystem platform

- Sample preservation in suitable transport medium (UTM);
- Chemical/electro-chemical dissolution of fiber and complex protein matrix;
- DEP based cell sorting/separation as a method for pre-concentration in the raw clinical samples



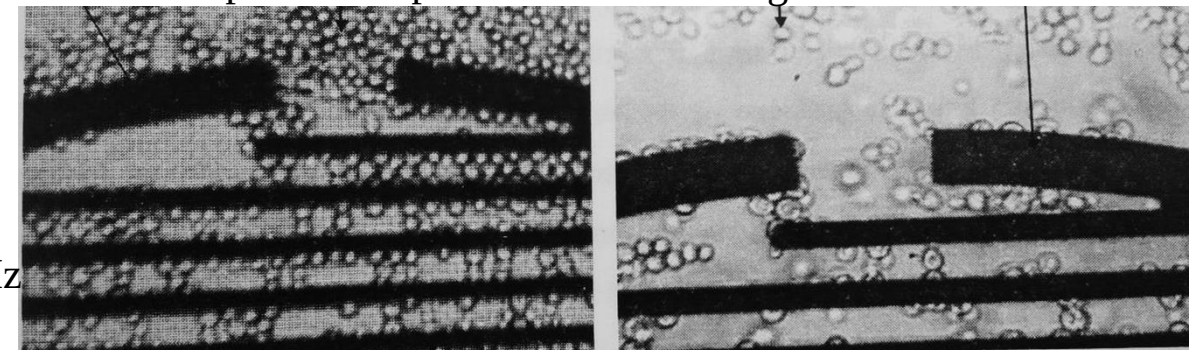
DEP Based Sample Preparation: Cell And Micro-Particles

- Multi-frequency, travelling wave DEP (TW-DEP) electrode schemes can be used for particle/cell sorting during sample preparation
- Schemes 1-4 utilize interdigitated DEP electrodes for sorting/separation of target cells in dispensed biological sample droplets



Positive-DEP capture of yeast cells in dispensed droplets

Yeast cells in dispensed droplets under negative-DEP effect



Interdigitated DEP electrode scheme-1

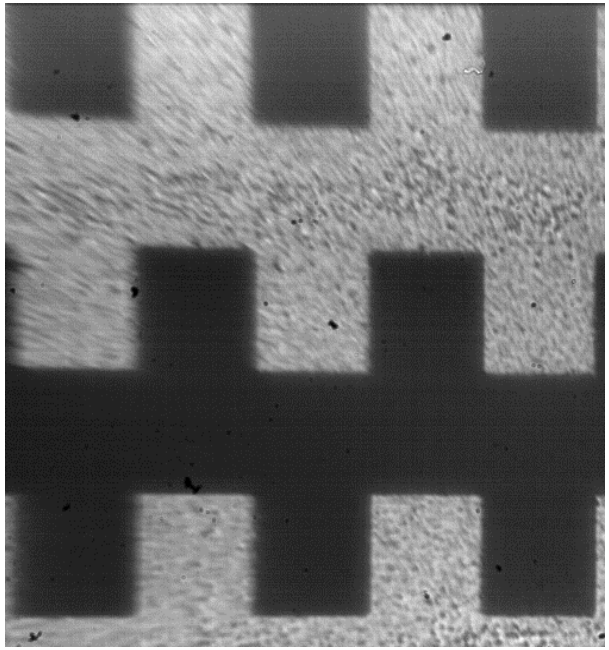
+ve DEP capture: 2.5 Vrms at 600 kHz

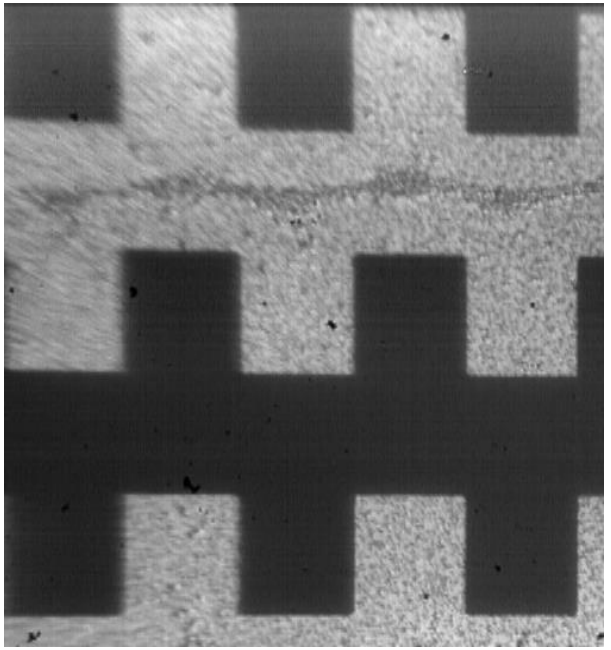
-ve DEP capture: 2.5 Vrms at 10 MHz

*Electrowetting 2006

DEP Based Sample Preparation: Illustrations for RNA-binding Bead Capture

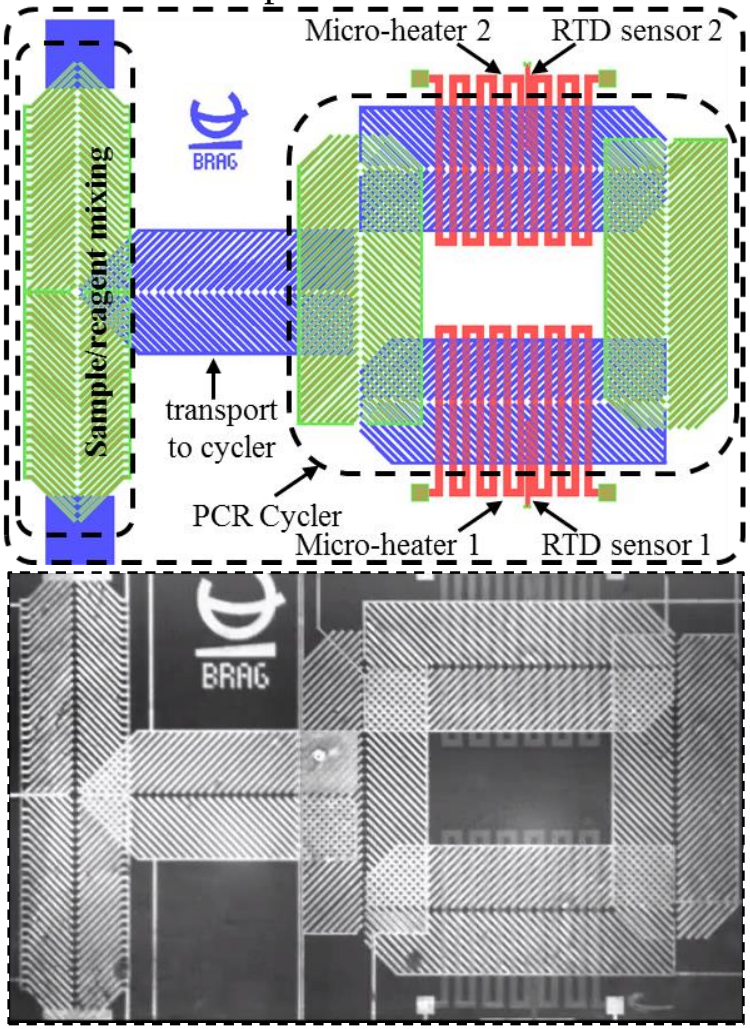
- Example of bead capture on other DEP electrode structure (w = g = 50 μm)
- Higher actuation voltage requirement (Vpp = 120 V)
- Bead re-collection in field minima (null regions) using negative-DEP
  - Capture freq.: ~550 kHz for capture over electrode; along electrode length



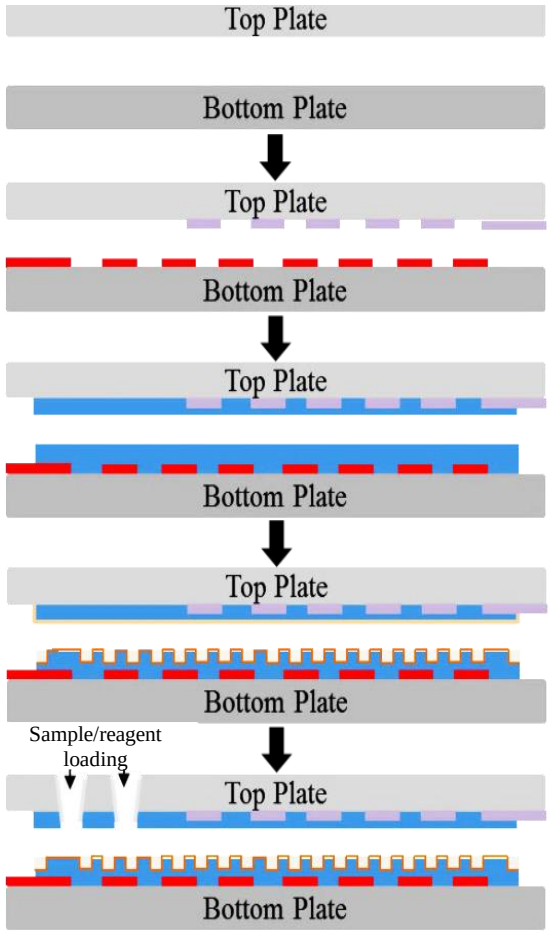


Anatomy Of Micro/nano Fabricated DMF Devices*

Schematic and video illustration of a single PCR micro-chip



Process flow diagram for a typical DMF micro-chip fabrication



ITO coated glass substrate (Top)

Glass substrate (Bottom)

Photolithography patterning of ITO electrodes for cell sorting (sample preparation)
Sputter deposition and lithography patterning (metal electrodes: 200 nm)

PECVD deposition and RIE patterning of Si_3N_4 layer (500 nm) for dielectric passivation of electrodes (both top and bottom plates)

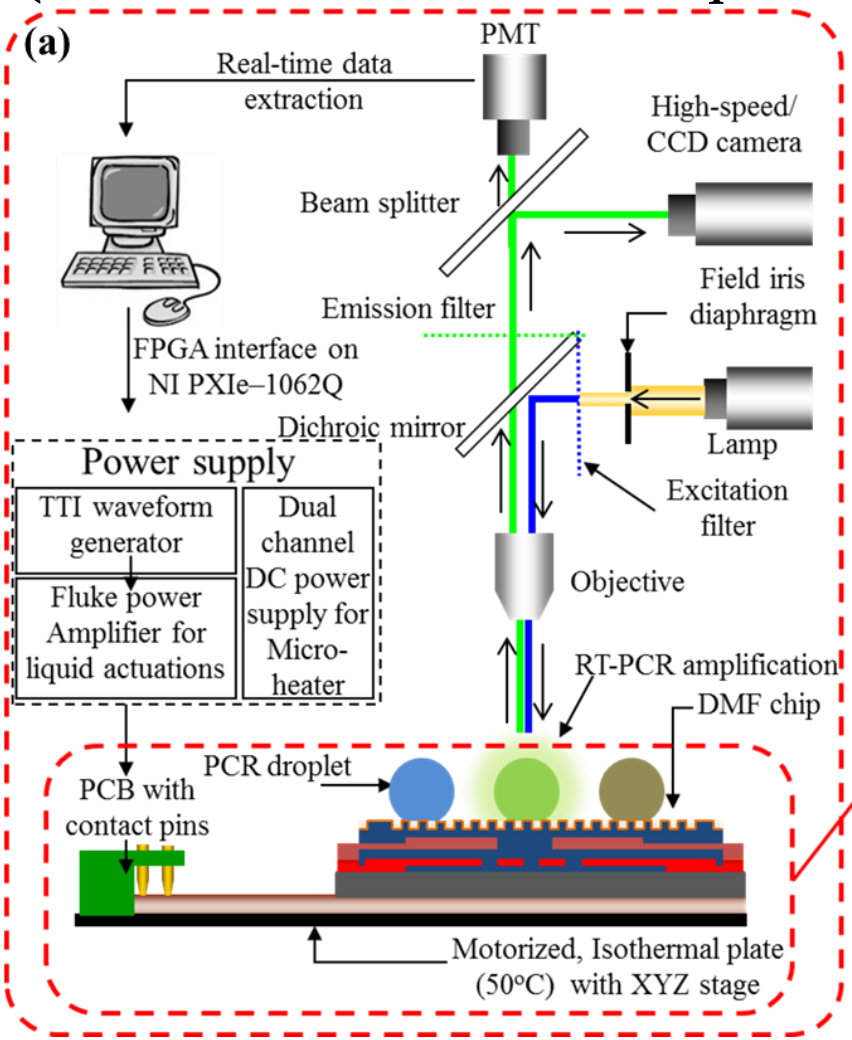
Colloidal lithography, plasma etching based nano-texturing and superhydrophobic rendering (contact angle ~ 155°)

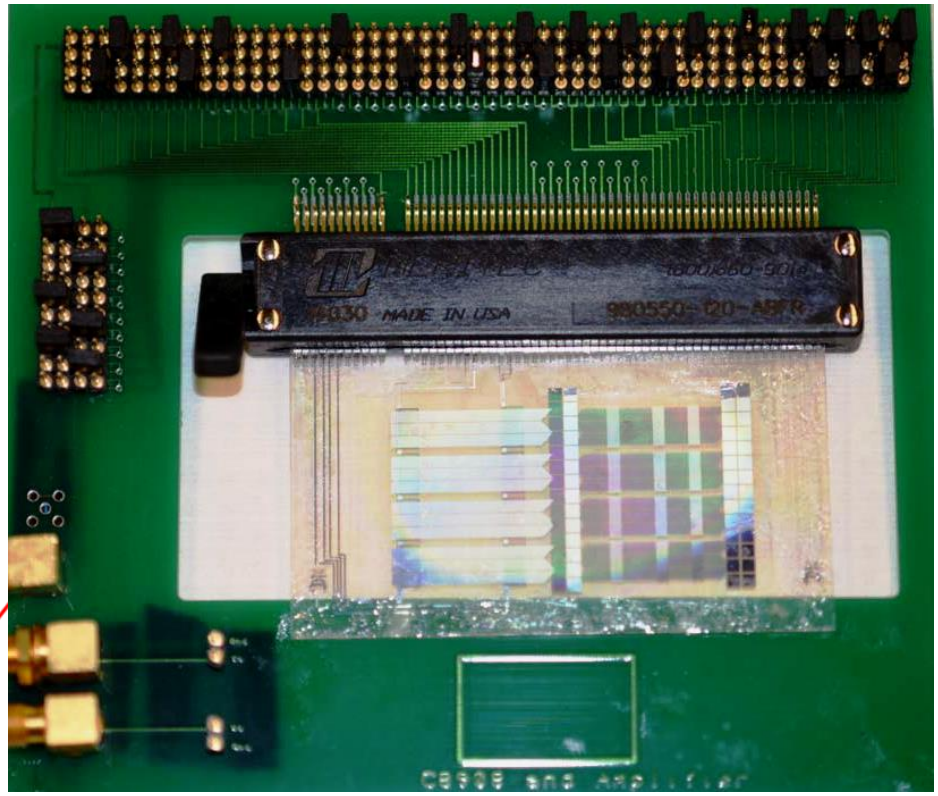
Through holes on top plate for sample and reagent loading using hanging droplets; Composite fluorocarbon deposition using plasma deposition and spin coating process

*J. Elec. Soc. 2014

DMF Measurement System

Schematic of the microscope based measurement system (for fluorescent based quantitative analysis)



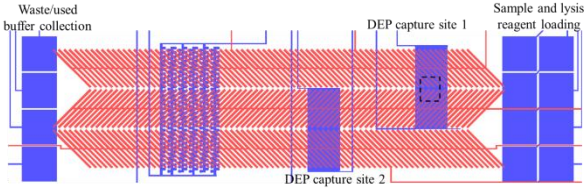


Sample-to-Detection In Nucleic Acid Based Diagnostics

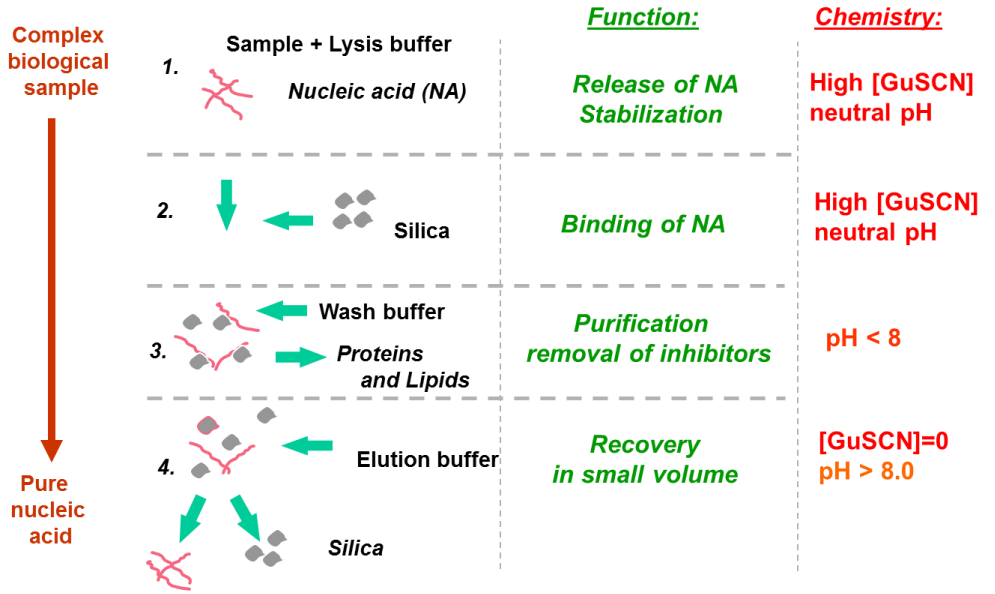
Sample Prep → **Nucleic Acid Extraction** → **Amplification + Detection**

- Chemical and electro-chemical disruption of cells and virions;
- DEP based washing, capture and purification of the extracted nucleic acids; (next slide)
- Low reagent volume requirements and multiplexed sample extraction capabilities;

On-chip extraction



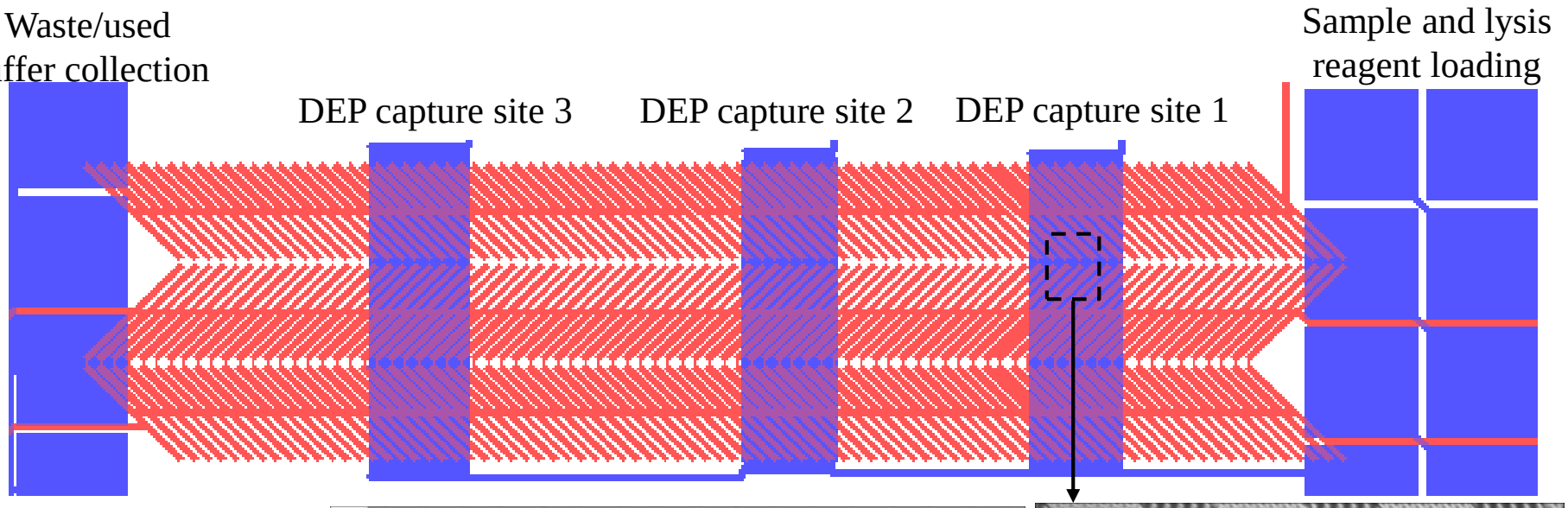
Boom™ Chemical Extraction Method



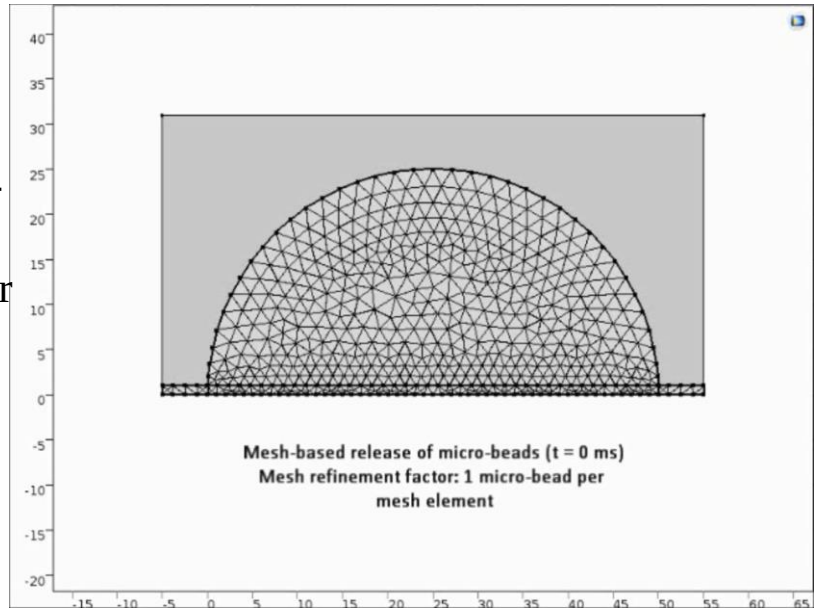
Components of a conventional extraction set-up

1. NucliSens mini MAG
2. Thermo shaker (200 – 2000 rpm; Eppendorf)
3. Vortex

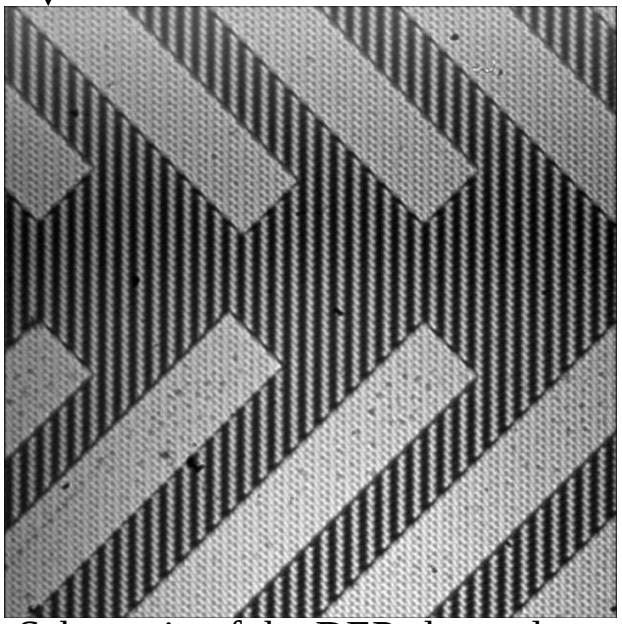
DEP Electrodes For Chip Based Extraction



Video: Capture of RNA-binding beads from a 20 μL sample-in-lysis buffer
Capture time: 90 sec
Capture freq.: 800 kHz
AC voltage (Vpp): 80 V



COMSOL simulation showing negative-DEP capture of RNA-binding micro-beads

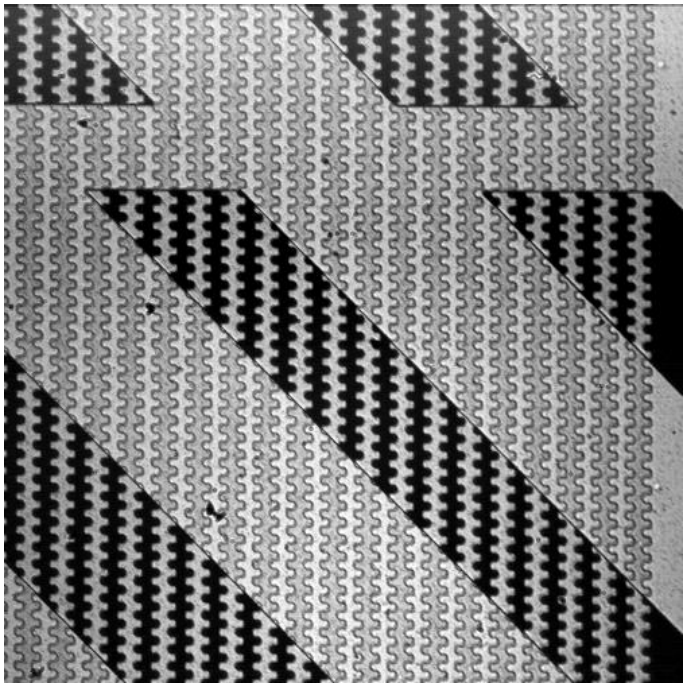


Schematic of the DEP electrode structure (w = g = 5 μm)

Video Illustrations Of DEP Based Micro-bead Capture and Re-collection

- Bi-directional droplet actuation using D-DEP (10-20 μL)
- Mag-bind® bead (2.0 μm) capture from lysis-sample mixture (20 μL) (Step 1)
- Bead re-collection during VHB and SPR buffer wash step (20 μL)
- Elution of purified nucleic acid into RNAse free PCR water (~ 12 μL)

Micro-bead capture (Step 1)

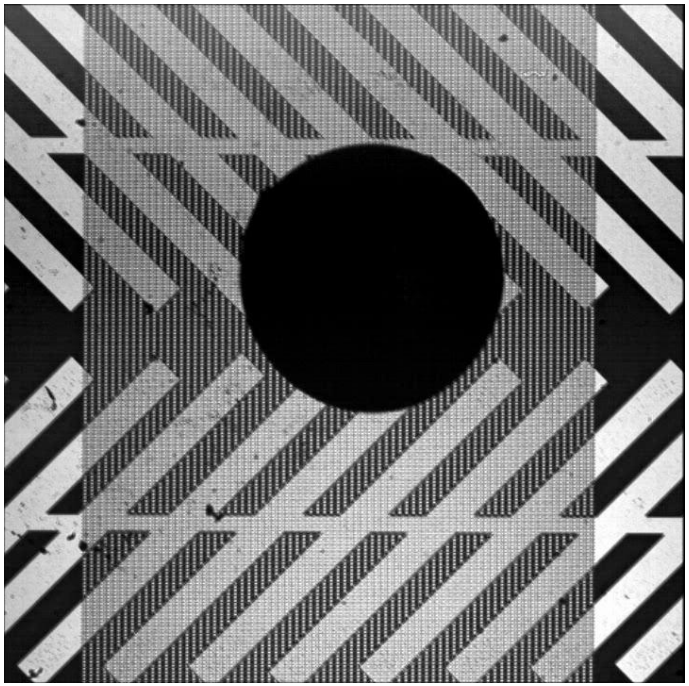


Capture time: 90 sec

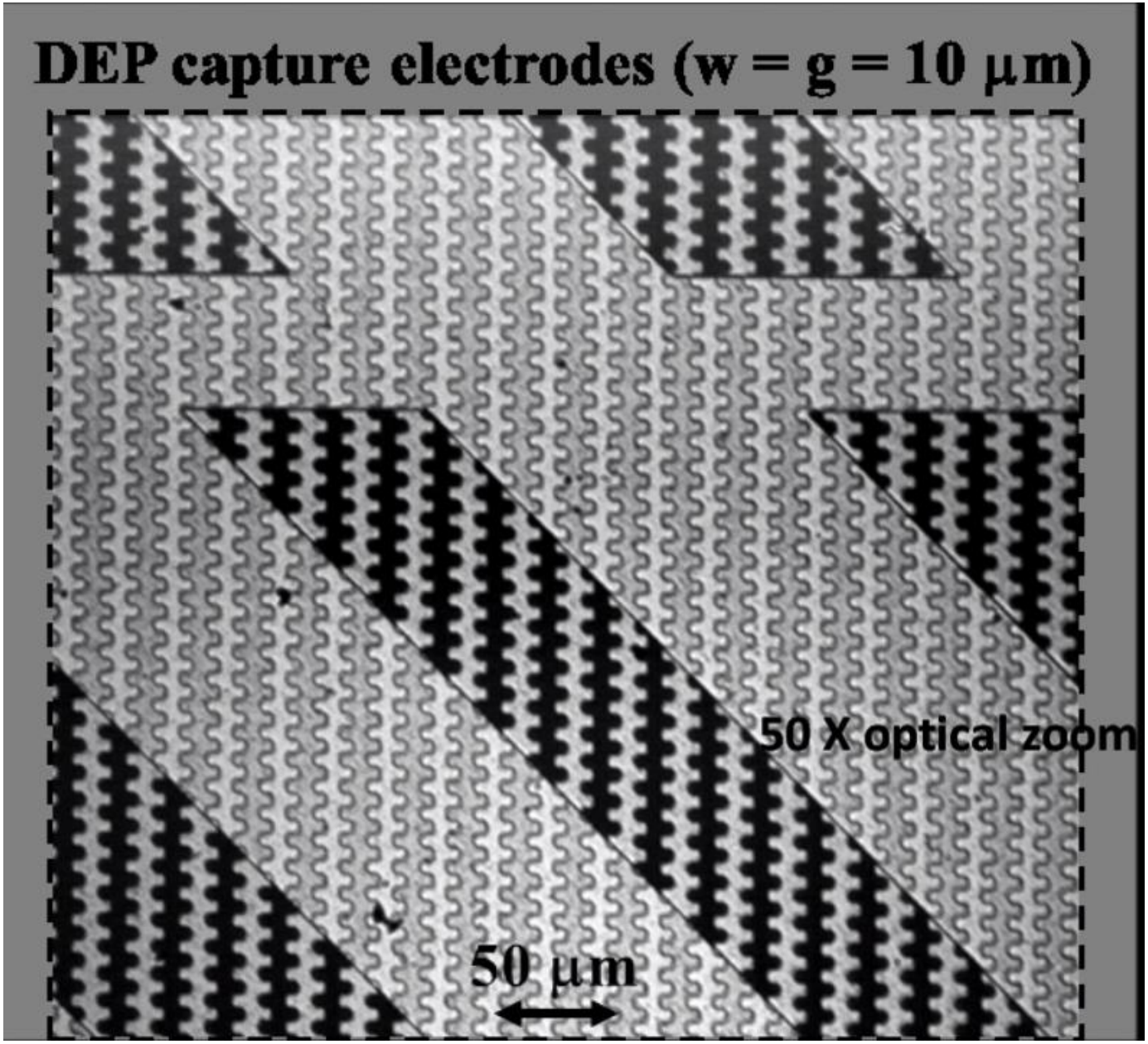
AC frequency: ~800 kHz, voltage: 60Vpp for negative DEP capture;

Re-collection/ washing time: 90 sec

Micro-bead washing (Step 2)



DEP Based Nucleic Acid Extraction (Video)



Clinical Validation Of the Chip Based Nucleic Acid Extraction and Purification (Panel 1)

- Blind panel of Nasal Swab samples (Clinical)
- Four sample anonymized clinical panel subjected to the on-chip Nucleic acid extraction and purification assay; samples run in triplets to test reproducibility of the chip based extraction assay;
- MS2 (conc.:10^{-3}) was used as internal control in all the on-chip and easyMag™ extraction experiments.
- MS2 is a bacteriophage with an RNA genome, suitable to characterize viral extractions

Sample Number	Chip C_t (Flu A)	ProvLab C_t (Flu A)	Chip C_t (MS2)	ProvLab C_t (MS2)
1a	Neg.	Neg.	26.95	27.5
1b	Neg.			
1c	Neg.			
2a	21.09	20.50/21.20	26.87	27.5
2b	20.91			
2c	20.88			
3a	30.43	29.80/29.80	27.35	27.50
3b	29.40			
3c	29.55			
4a	Neg.	Neg.	26.40	27.50
4b	Neg.			
4c	Neg.			

Repeatability of the on-chip nucleic acid extraction and purification

RT-PCR efficiency of chip extracted nucleic acids: ~ 94-95 %

Clinical Validation Of the Chip Based Nucleic Acid Extraction and Purification(panel 2)

- Blind panel of co-infected nasal swab samples (Clinical)
- Eight sample anonymized panel (clinical samples), reflecting co-infections (three respiratory viruses, FluA, FluB and RSV with different viral loads categorized as Hi and Lo) to the on-chip extraction and assay; samples run in triplets to test reproducibility of the chip based extraction;
- Each panel sample was extracted in duplex to yield a combined extracted volume of 25 µL, suitable for a four-panel RT-PCR analysis for the three targets and the internal control (MS2).
- Successful extractions from wide ranged co-infection samples illustrate the robustness of the sample preparation chip.

Sample Name	Targets	ProvLab	CHIP	ProvLab	CHIP	ProvLab	CHIP
		Flu A	Flu A	Flu B	Flu B	RSV	RSV
3.1	FluA-Hi, FluB-Lo, RSV-Hi	17.44	18.79	23.60	21.75	21.45	22.01
3.2	FluA-Hi, FluB-Hi, RSV-Lo	17.44	18.83	18.59	20.09	31.64	30.04
3.3	FluA-Lo, FluB-Lo, RSV-Hi	27.54	27.82	23.65	21.05	21.45	20.54
3.4	FluA-Lo, FluB-Hi, RSV-Lo	27.54	28.01	18.59	19.62	31.64	29.85
3.5	FluA-Hi, FluB-Hi, RSV-Hi	17.44	18.71	18.59	19.69	21.45	20.92
3.6	FluA-Lo, FluB-Lo, RSV-Lo	27.54	28.25	23.65	20.96	31.64	29.25
3.7	FluA-Hi, FluB-Lo, RSV-Lo	17.54	18.50	23.65	20.96	31.64	29.76
3.8	FluA-Lo, FluB-Hi, RSV-Hi	27.54	27.08	18.59	18.75	21.45	20.35

RT-PCR efficiency of all chip extracted nucleic acid samples lie within ~ 94-95 % (bench-top RT-PCR efficiency ~ 97-98 %)

Clinical Validation Of the Chip Based Nucleic Acid Extraction and Purification (Panel 3)

- Blind panel of Blood plasma samples (Clinical)
- Two anonymized clinical samples (plasma samples) subjected to the on-chip Nucleic acid extraction and purification assay; samples run in duplex to test reproducibility of the chip based extraction assay;
- MS2 (conc.:10^{-3}) used as internal control in all the on-chip and EasyMag™ extraction experiments.

Sample ID	Sample type	ProvLab Ct	Chip Ct	Chip Ct for MS2 (Internal Control)
1 (a, b)	Plasma (Echo18)	33.50	34.08	25.80
2 (a, b)	Plasma (CoxA6)	27.68	28.96	26.10
3	Neg. (MS2)	26.50	25.44	25.44

- Overall proficiency table for the performance of the DEP nucleic acid extraction and purification chip

	Chip Based Extraction			
Gold Standard (EasyMag™)	No. of Tests	Positive	Negative	
	Positive	40	0	No loss of sensitivity
	Negative	0	10	
		No loss of Specificity		

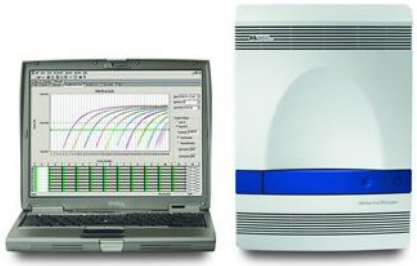
Sample-to-Detection In Nucleic Acid Based Diagnostics

- Optical and/or electro-chemical read-out during PCR amplification and detection;
- Real-time PCR or, end-point PCR read-out.

- Real-time PCR curves obtained using a scanning Photomultiplier tube;
- qPCR and standard quantification of target DNA template;
- qRT-PCR assays for 1-step amplification and quantification of RNA samples (includes a reverse transcription stage to convert RNA to λ-DNA;
- Spatially and spectrally multiplexed PCR reactions for panel PCR assays.

PCR And The Current State Of Technology

- qPCR: A real-time, quantitative PCR assay where detection and quantification of the amplified template occurs in real-time, during the PCR thermal cycling.
- Existing bench-top set-ups can achieve up to six parallel real-time PCR assays in ~ 30-40 minutes
- Existing commercial equipment range from $40,000 - $100,000 USD



ABI 7500 Fast (Applied Biosystems)

QuantStudio™ Flex rt-PCR System (Applied Biosystems)

Micro-systems and Miniaturization of qPCR and real-time PCR Technology

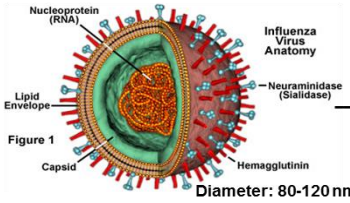
- Recent attempts to minimize the sample volume requirements by incorporating microfluidic technology
- E.g.: The Droplet Digital PCR System (Bio-Rad) and Quantstudio Flex real-time/digital PCR system (Applied Biosystems) illustrate application of a microfluidic system for controlled sample preparation and dispensing of multitude of PCR droplet arrays.

Rotor-Gene 6000 (Corbett)

Mx3000P (Agilent)

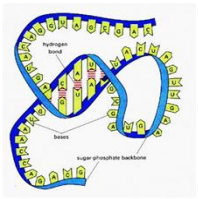
QX200™ Droplet Digital PCR System (Bio-Rad)

qRT-PCR Detection Of Influenza Viruses



Structure of Influenza virus (Microbiology 2009)

RNA extraction



Structure of extracted RNA

Off-chip sample preparation

(On-chip extraction has now been validated)

Off-chip preparation of sample and RT-PCR reagents

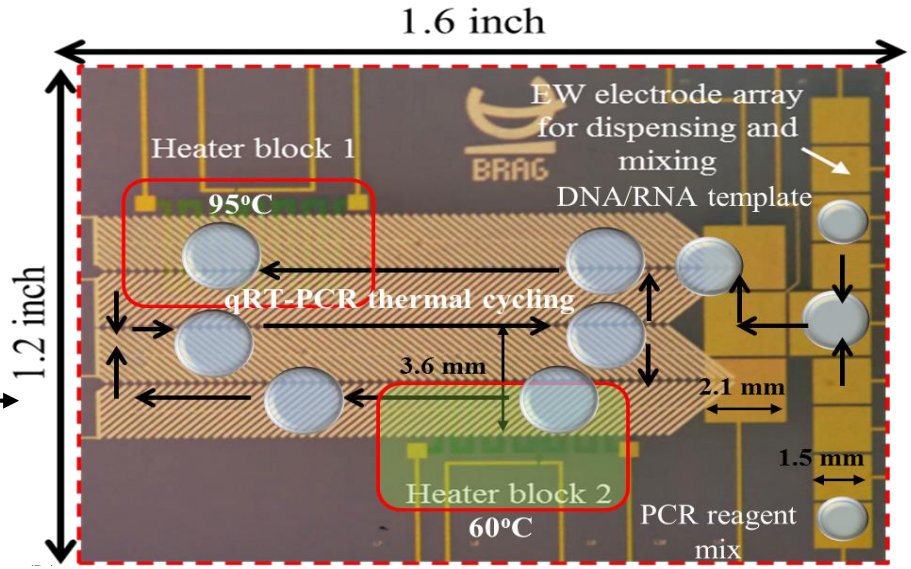
Manual sample/reagent loading

Sample and reagent droplet mixing/transport

Thermal cycling and real-time RT-PCR detection

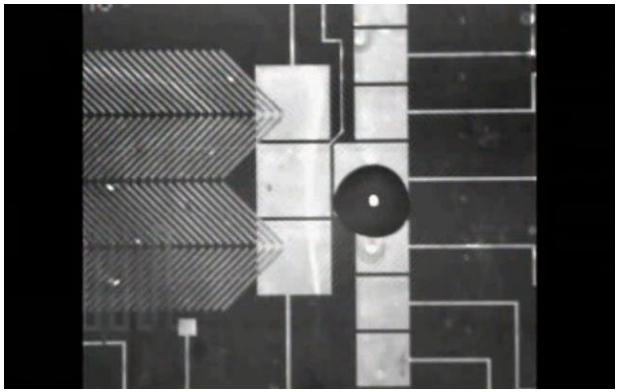
- RNA to cDNA (RT reaction; 50°C)
- Enzyme activation (95°C)
- RT-PCR Amplification cycles
  - Denaturation (95°C)
  - Annealing and read-out (60°C)

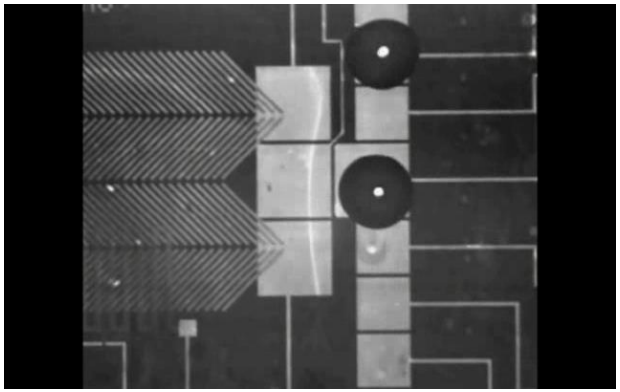
On-chip qRT-PCR

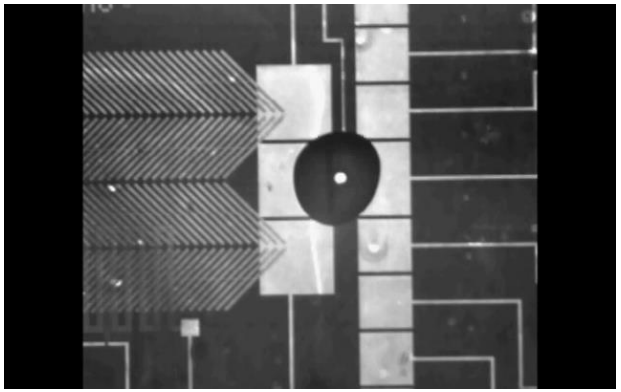


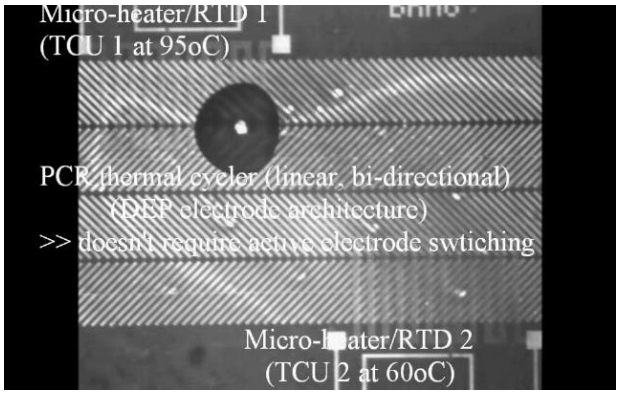
*J. Elec. Soc. 2014

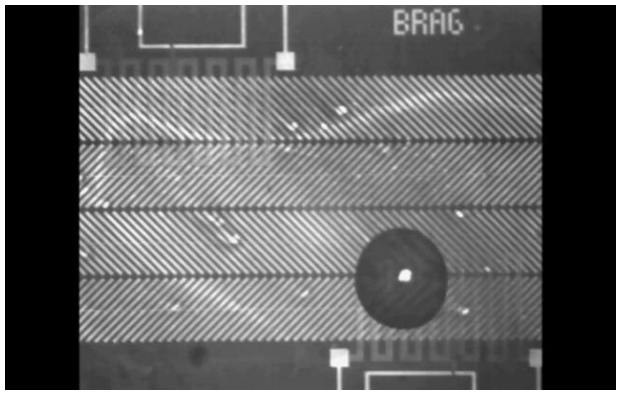
qRT-PCR Detection on DMF device

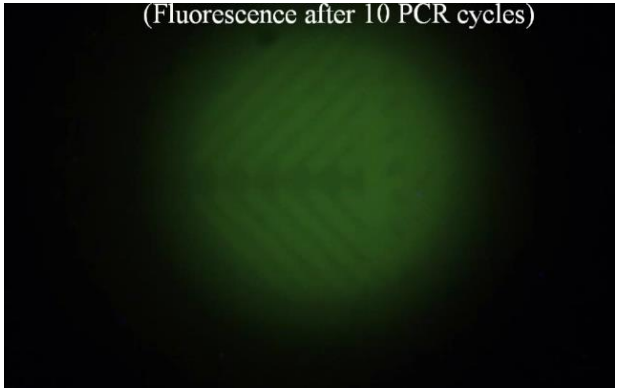


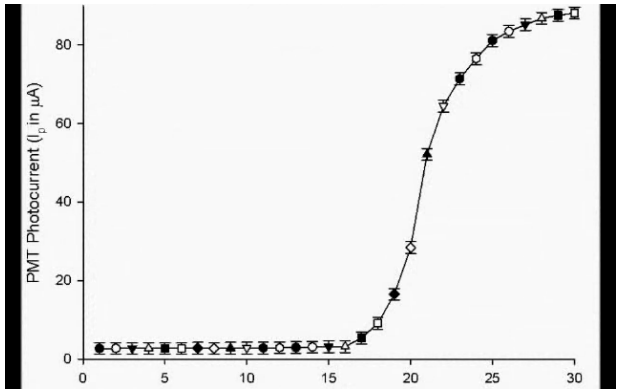






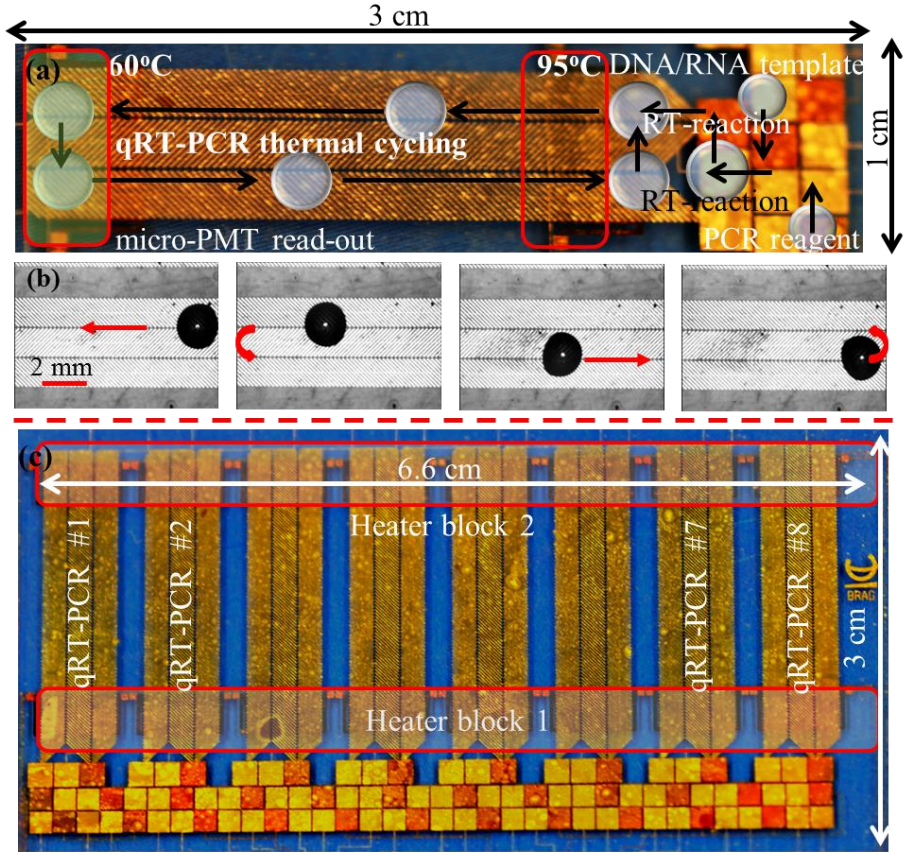


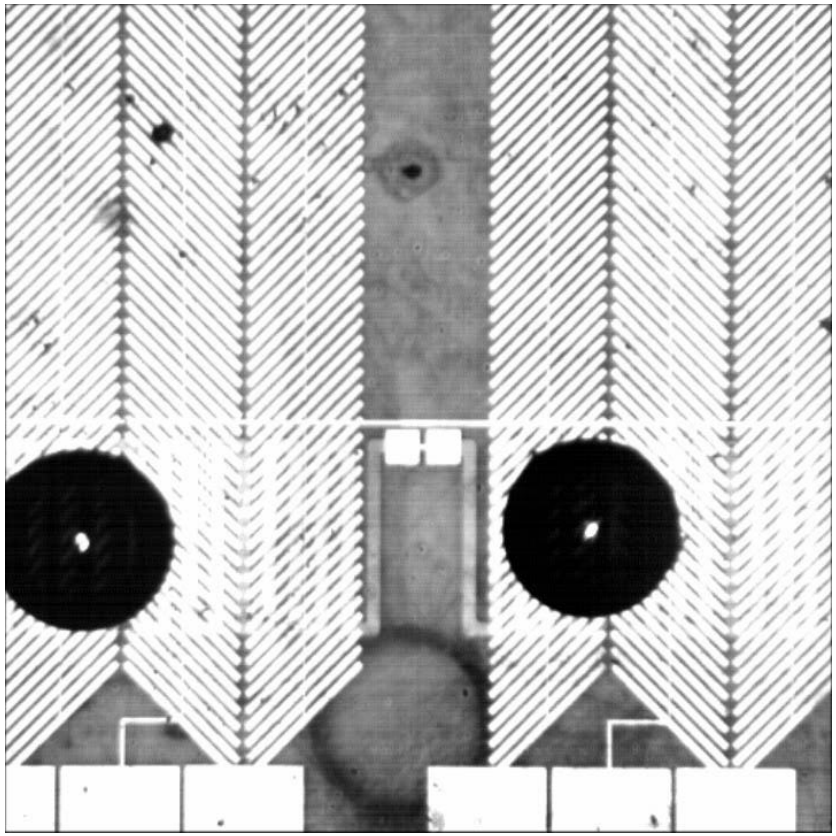




Multiplexed qRT-PCR Detection Of Influenza Viruses*

- PCR reaction volume: 10 µL
- Integration of droplet electro-actuation and resistive heater blocks for up to eight parallel, automated qPCR assays
- Reaction time: ~ 40 minutes for 36 cycles
- PMT scanning set-up for extraction of quantitative curve during the multiplexed qPCR reactions
- Video illustrates two adjacent PCR droplets during a multiplexed qRT-PCR assay



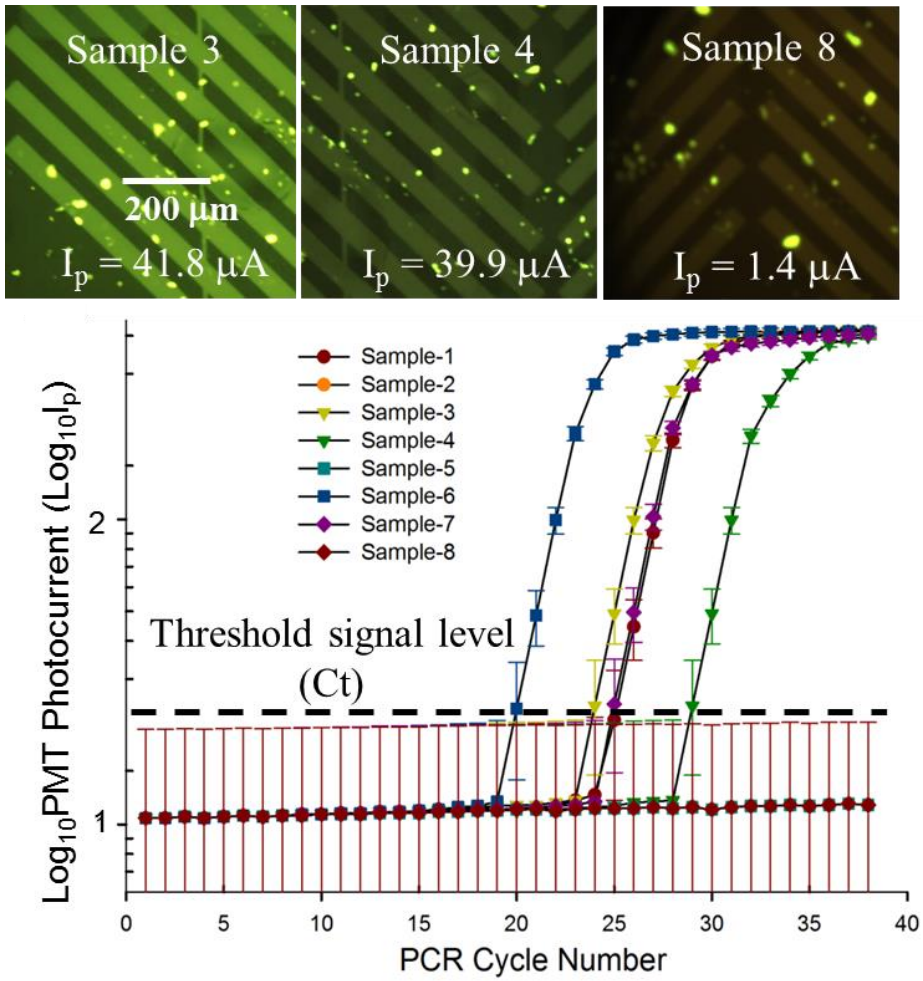


*J. micromachines 2015

Multiplexed qRT-PCR Detection Of Influenza A Blind Panel*

- **Six sample Influenza A blind panel**

Sample No.	Target	CDC-M Ct	pdm09 Ct	Chip Ct
1	FluA; pdm09	29.53	26.82	25
2	Resp neg	Neg	Neg	Neg
3	FluA; pdm09	29.85	25.71	24
4	FluA; pdm09	31.75	32.89	30
5	Resp neg	Neg	Neg	Neg
6	FluA; pdm09	24.12	21.02	20
(+ve control)	FluA; H3	~29	n/a	26
(-ve control)	----------	Neg	Neg	Neg

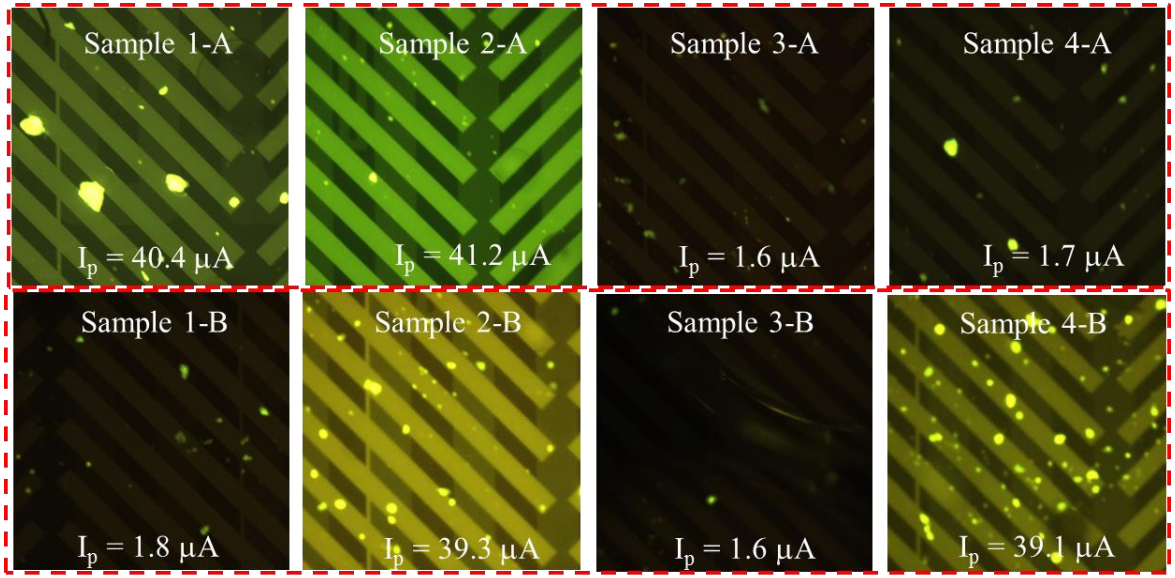


- Spatially multiplexed qRT-PCR micro-device was used to conduct the Influenza A blind panel test
- Real-time, continuous-mode PMT read-out for five Influenza A +ve samples and the -ve control sample.

*J. micromachines 2015

Multiplexed qRT-PCR Detection Of Influenza A, Influenza B Mixed Blind Panel*

- **Four sample Influenza A, Influenza B blind panel**



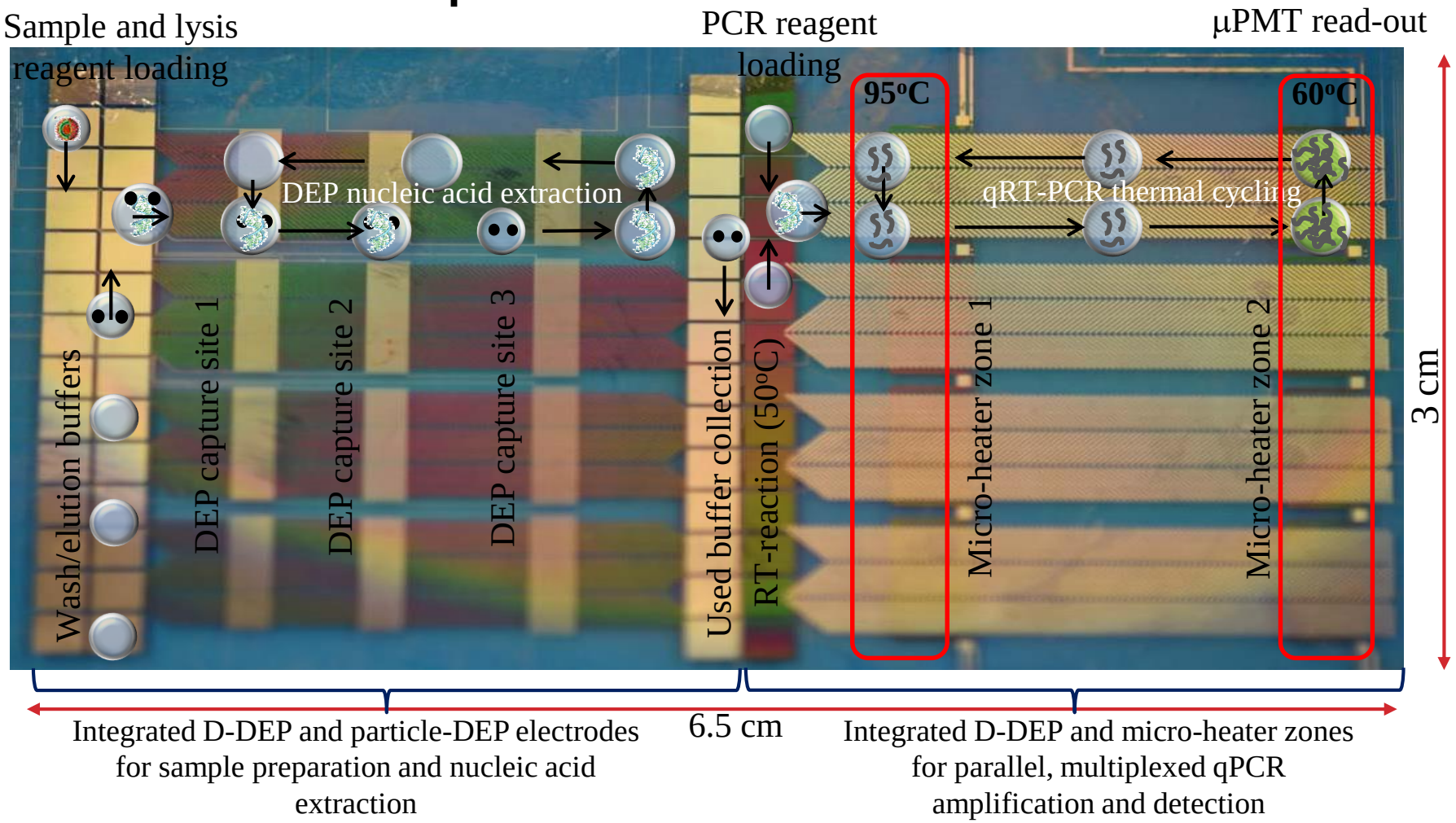
FAM™ as Fluorophore for Influenza A

VIC™ as Fluorophore for Influenza B

Panel Sample No.	Sample Type	Target	CDC Ct	Chip Ct
1-A	Nasopharyngeal Swab	FluA	29.18	27
1-B	Nasopharyngeal Swab	FluB	Neg	Neg
2-A	Nasopharyngeal Swab	FluA	27.29	24
2-B	Nasopharyngeal Swab	FluB	27.6	25
3-A	Nasopharyngeal Swab	FluA	Neg	Neg
3-B	Nasopharyngeal Swab	FluB	Neg	Neg
4-A	Nasopharyngeal Swab	FluA	Neg	Neg
4-B	Nasopharyngeal Swab	FluB	30.42	28

*J. micromachines 2015

Integrated Sample-to-detection Droplet Microfluidic Chip



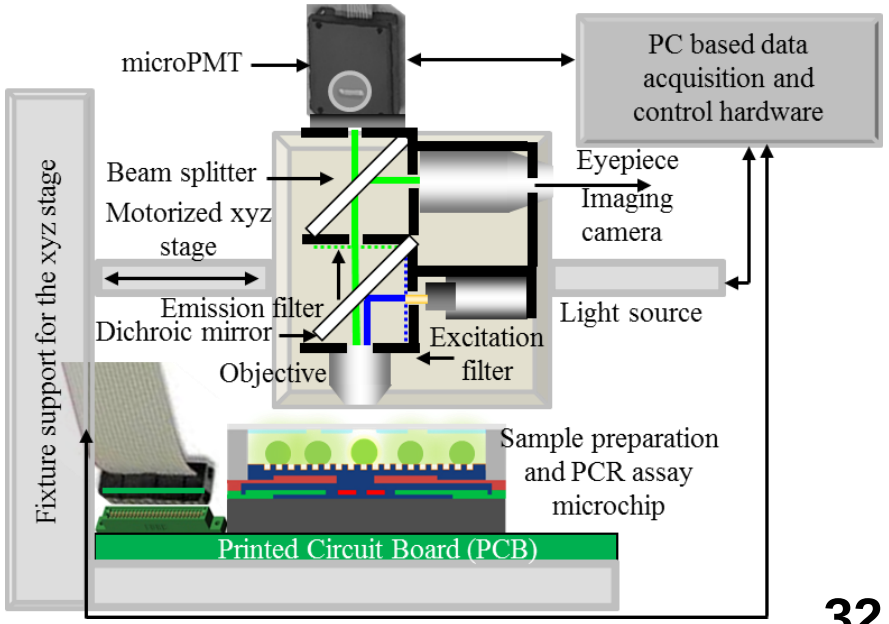
- Panel of clinical samples (up to four samples) can be prepared;
- Whole nucleic acid from the samples extracted and purified;
- Parallel, RT-PCR amplification and quantification of pathogenic RNA/DNA;
- Sample-to-detection achieved in up to four hours; in four clinical samples

Current Capabilities and Future developments

- Nano-textured micro-devices for parallel, multiplexed RT-PCR assays
- Nano-textured micro-devices for electro-chemical disruption/lysis of viral and bacterial pathogens in clinical samples
- Integrated micro-device for rapid, parallel sample preparation (nucleic acid extraction, purification) and multiplexed RT-PCR amplification
- A miniaturized fluidic microsystem platform for sample-to-detection assay
- Polymer MFD for disposable micro-devices (lower cost platform)
- Disposable MFD for other pathogenic targets (Dengue, Zika, West Nile)
- Extending the microsystem towards post-amplification applications (sub-typing, sequencing, deep sequencing

Other Capabilities:

- Vesicles for drug delivery, cell-on-chip;
- Multiplexed combinatorial bio-assays;
- Molecular beacon™ based DNA quantification and detection assay;



Schematic of the proposed integrated microsystem platform for point-of-care diagnostic application

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Thank you