CMOS Biochips for Point-of-Care Molecular Diagnostics

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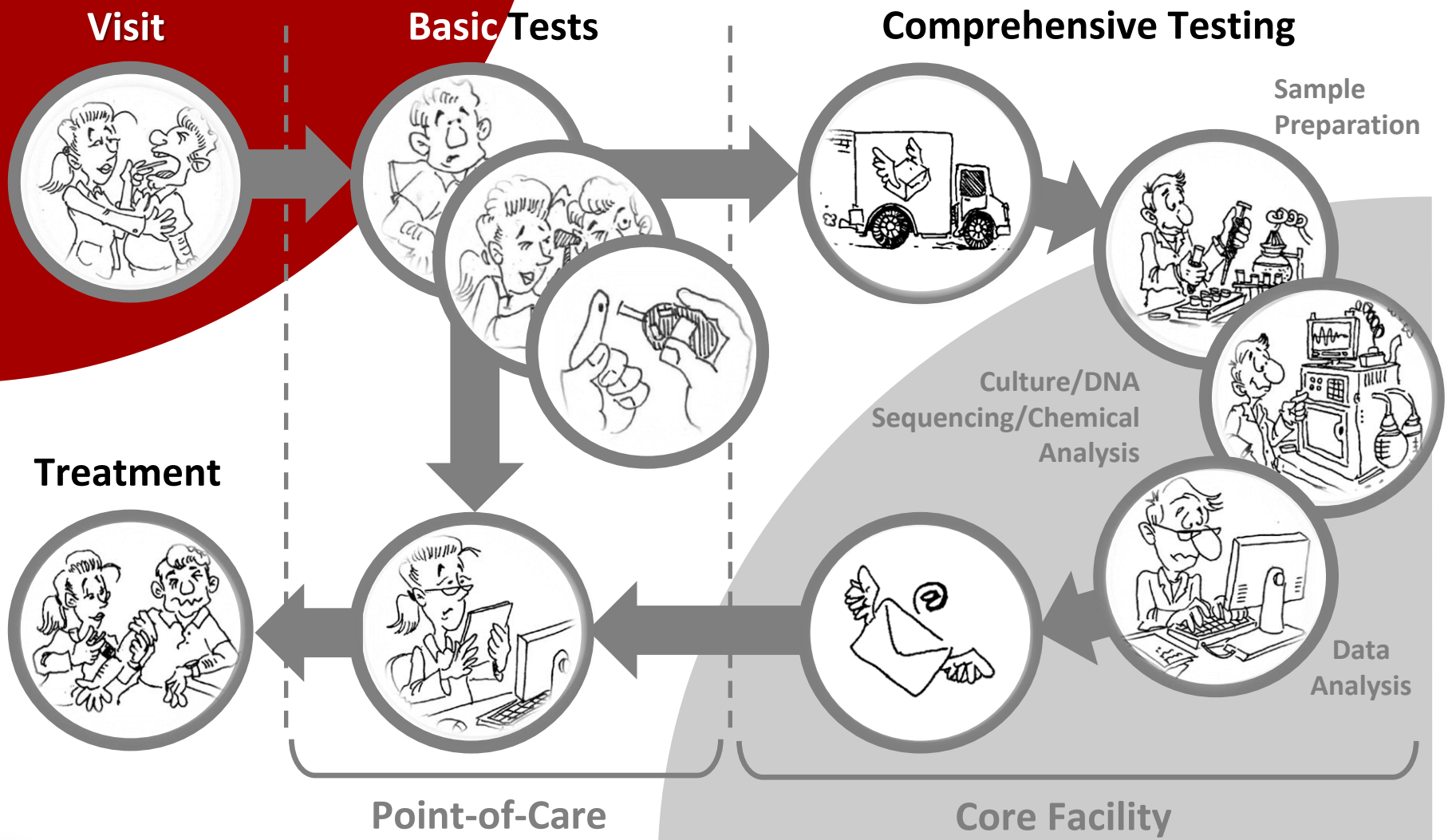
OVERVIEW

Molecular Diagnostics (MDx) and Stuff…

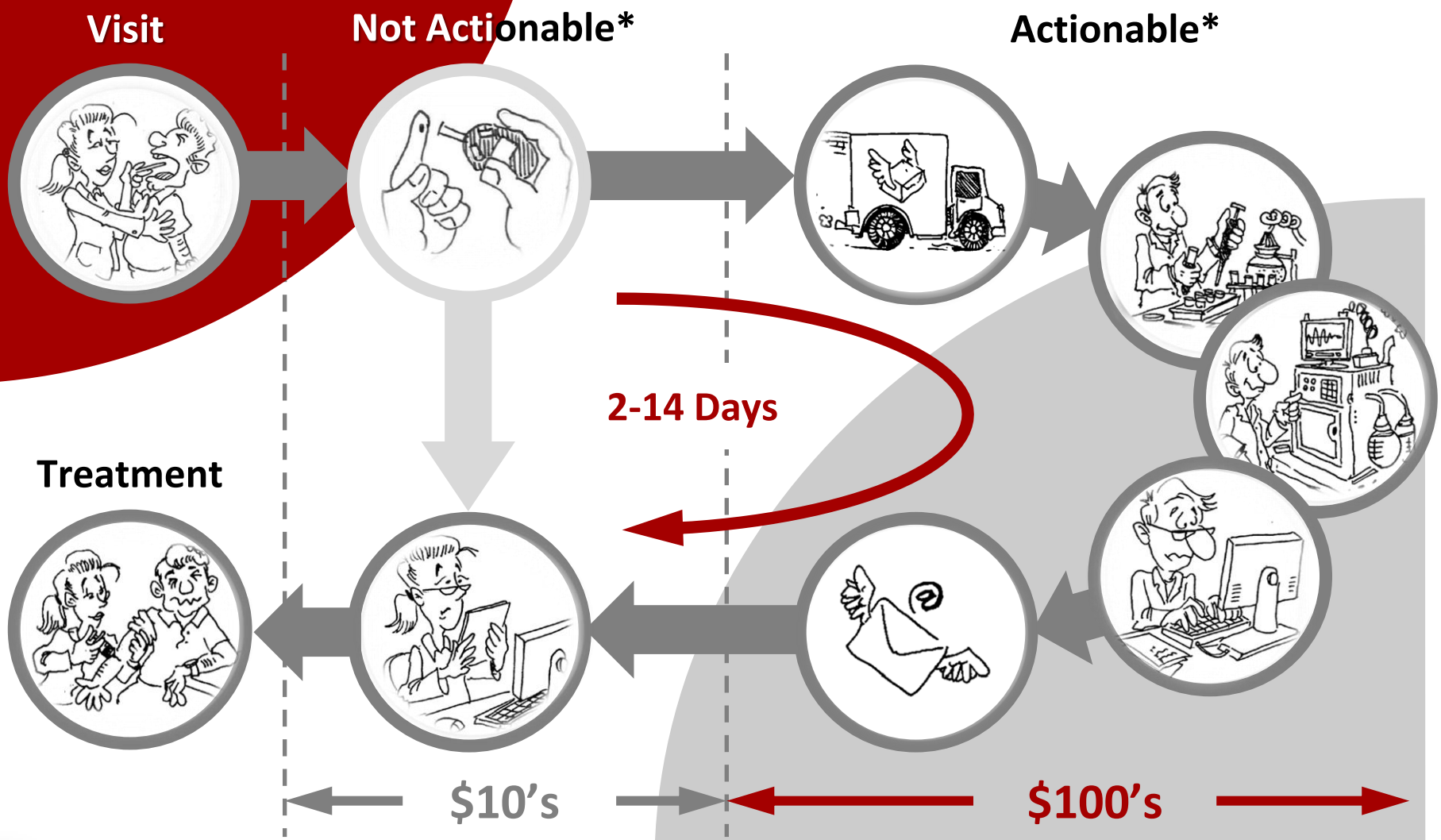
Diagnostics Information Flow (1)

Treatment options are based on diagnostics results



Diagnostics Information Flow (2)

Actionable tests* take too long and are too expensive



* A conclusive test that requires no further testing

Molecular Diagnostics (MDx)

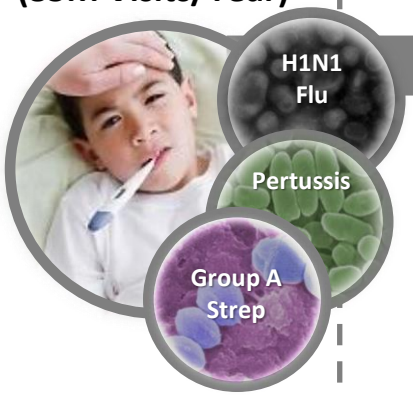
Highly actionable, but not mass-deployable

Urinary Tract Infection
(7M Visits/Year)

MDx

0.5 Hour	2 Days	14 Days
Dipstick Test ($30)	Culture ($40-$100)	DNA Analysis (>$500)
Infection (67% Accuracy)	E-Coli Present (+99% Accuracy)	E-Coli Present Antibiotic Resistant Strain (99% Accuracy)

Respiratory Infection
(85M Visits/Year)

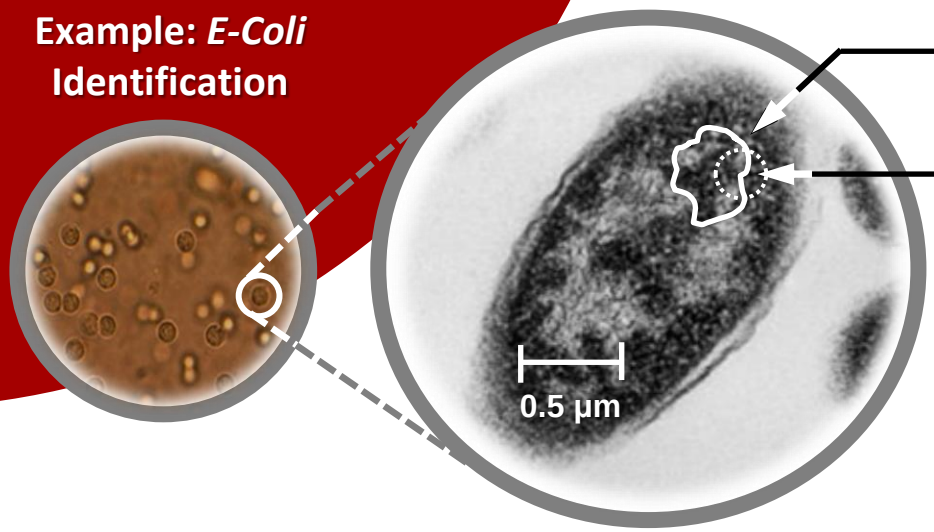


0.5 Hour	2 Days	14 Days
Throat Swab ($40)	Culture ($40-$100)	DNA Analysis (>$500)
Group A Strep (73% Accurate)	Group A Strep Present (99% Accurate)	Group A Strep, H1N1 Flu, Pertussis, … (99% Accuracy)

MDx Problem Statement (1)

Identify molecular structures (*e.g.*, DNA sequences) in presence of similar structures in a "dirty" biological environment

Example: *E-Coli* Identification



***E-Coli* Genome (47 Million bases)**

....CGGCGTTCGGCGGGGTTTTCCCGAG
GTTCGGGGCGCCAAAAAACGTTCTTCA
AAAAGTAAGGTAACGTTTACGTTTCGA
TTCAGGTTTCAGGTTTTGGTTTTAAAA
GGTACCGTTTACAAAGGTATGGAAA...

A unique sequence found only in pathogenic E-Coli

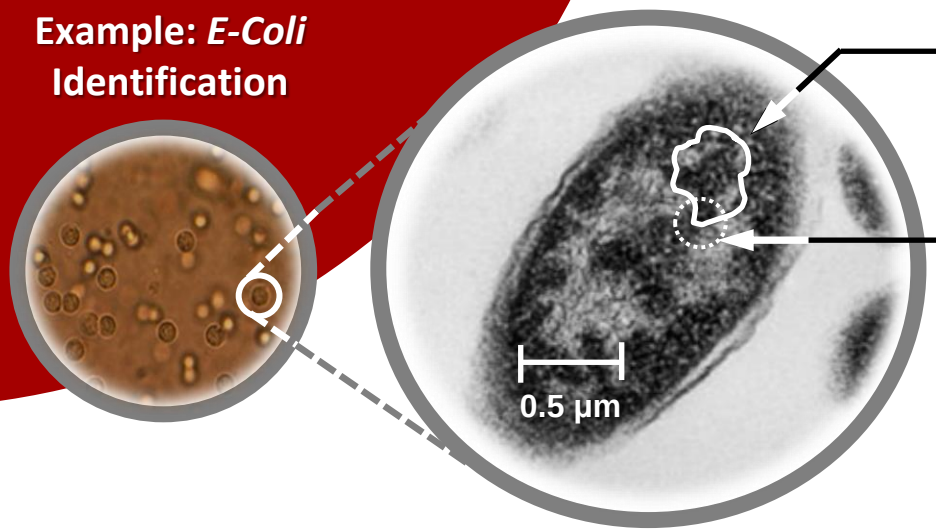
GTTTCGATTCAGGTTTCAGGTTTTGGTT (28 bases)

$$\text{Prob}\{\text{Random Occurrence}\} = \left(\frac{1}{4}\right)^{N}\Bigg|_{N=28} \approx 1.388 \times 10^{-17}$$

MDx Problem Statement (2)

Check specific DNA sequence for specific mutations that result in functional changes in the behavior of the organism

Example: *E-Coli* Identification



E-Coli Genome (47 Million bases)

…. GTCTTAGGCAGTTAAA
CGTTTACGATAAACACGGT
ACGACGATTTTACAGGG…

Resistant to Antibiotic (Super Bug)

…. GTCTTAGGCAGTTAAA
CGTTTACGATGAACACGGT
ACGACGATTTTACAGGG…

Sensitive to Antibiotic (Normal Bug)

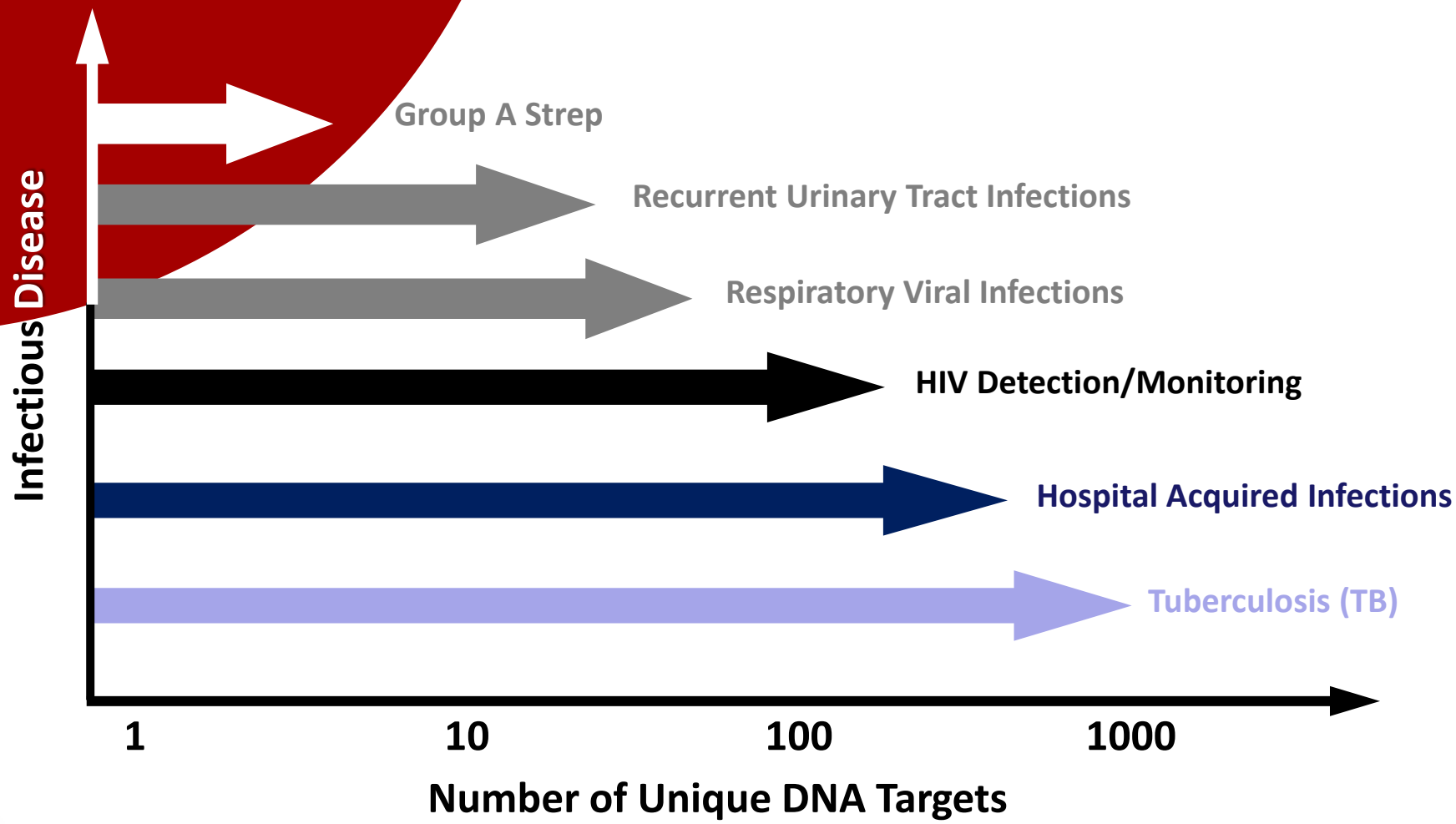
…. GTCTTAGGCAGTTAAA
CGTTTACGATAAACACGGT
ACGACGATTTTACAGGG…

TGA ← TAA

Mutation

MDx Problem Statement (3)

For actionable infectious diseases MDx, 10's to 100's of unique DNA sequences and/or mutations should be detected



Current State-of-the-Art

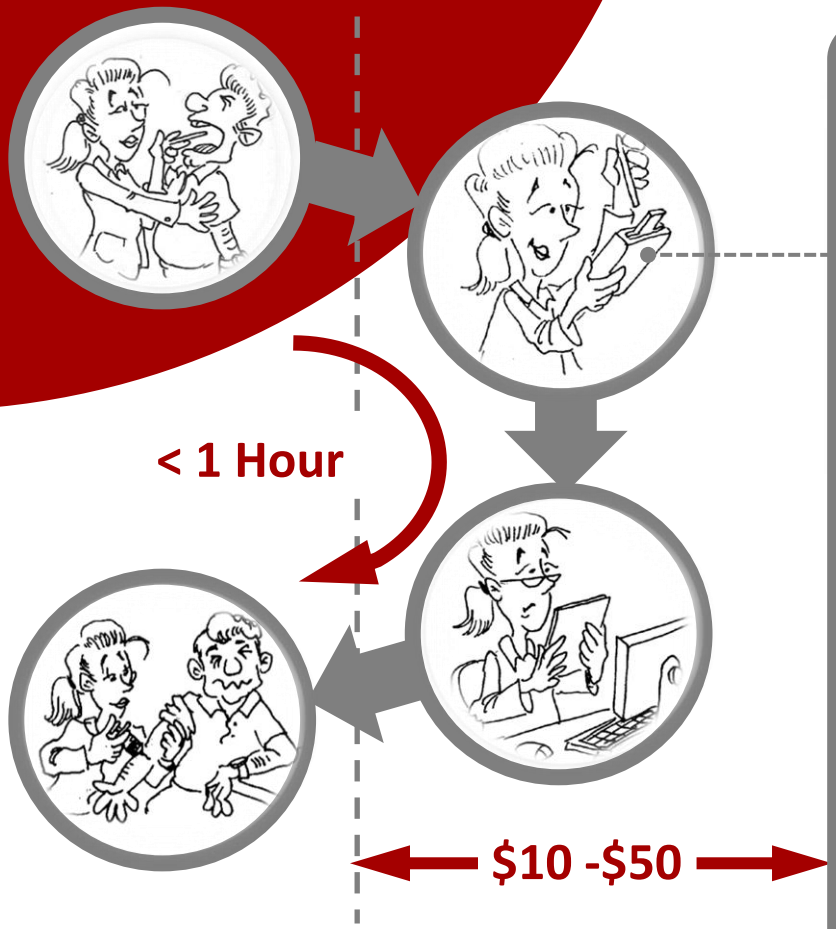
TECHNOLOGY	PCR[1]	DNA ARRAYS	DNA SEQUENCING	MDx NEED
Instrument (Setup) Price	$20K -50K	$25K-75K	$100K-$700K	< $1000
Price per Test[2]	$80-$400	$200-$1000	$2.5K-$10K	< $50
Max DNA Targets	6-20	20-1000	+1M	1000
Detection Accuracy	High	Low	Medium	High
Test Time	2-3 hours	6-12 Hours	> 2 Day	1 Hour
Fully-Automated	Yes	No	No	Yes
Portable	No	No	No	Yes
PoC Compatible	Yes	No	No	Yes

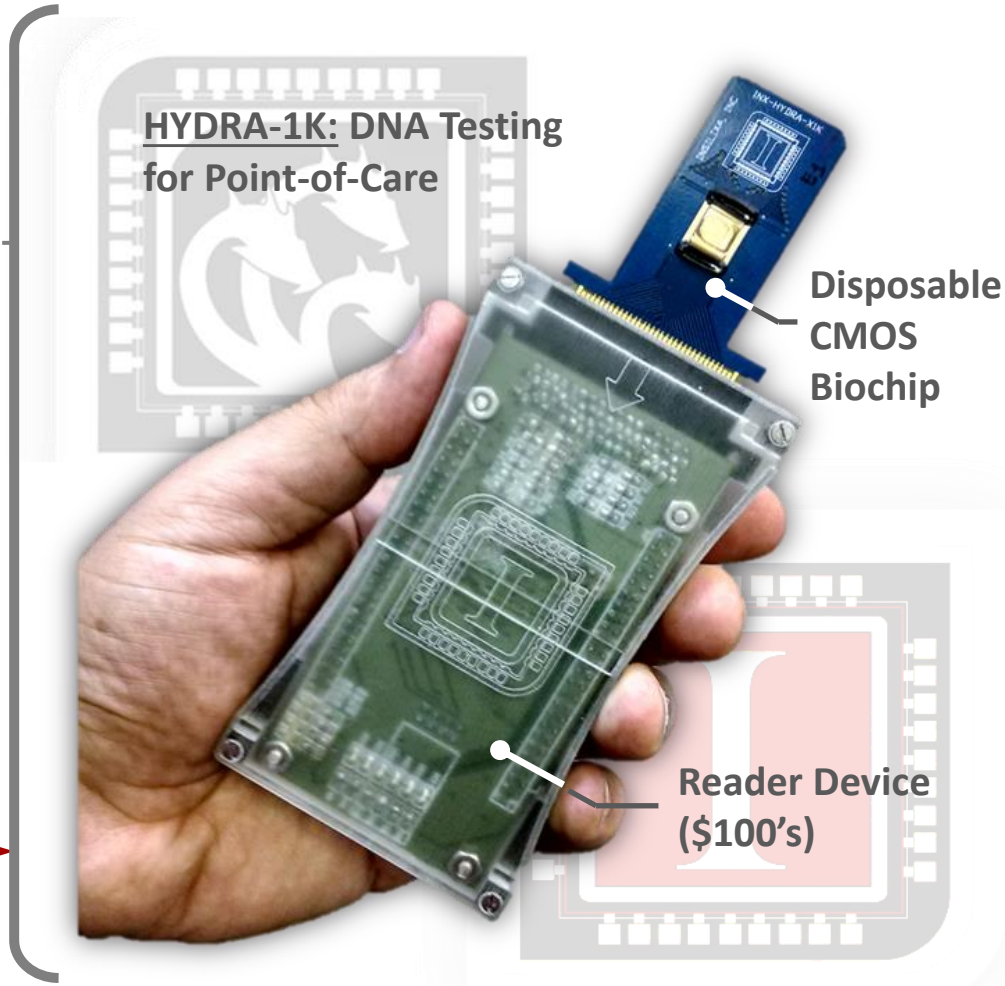
[1] Polymerase chain reaction

[2] Excluding any service charge

Solution: InSilixa's HYDRA-1K

Rapid (< 1 hr), low-cost ($10's), and simple (sample-to-answer) MDx to detect up to 1000 unique DNA sequences/targets





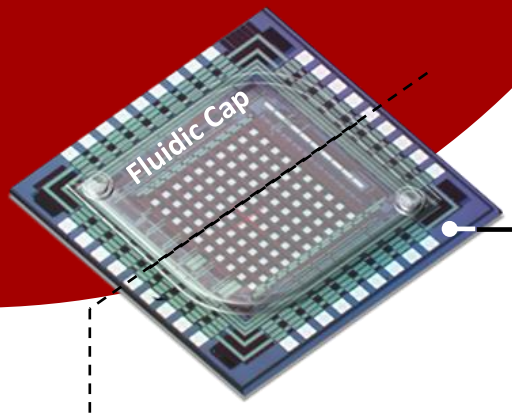
TECHNOLOGY

HYDRA-1K System

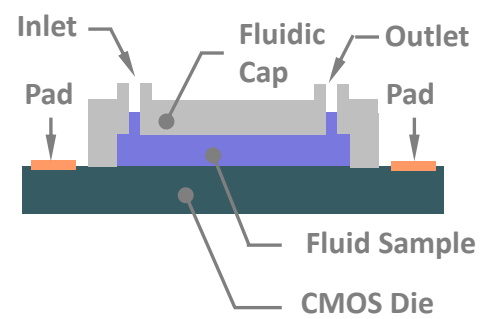
CMOS Biochips

CMOS ICs that are enhanced and specifically packaged to function as high-performance molecular sensors

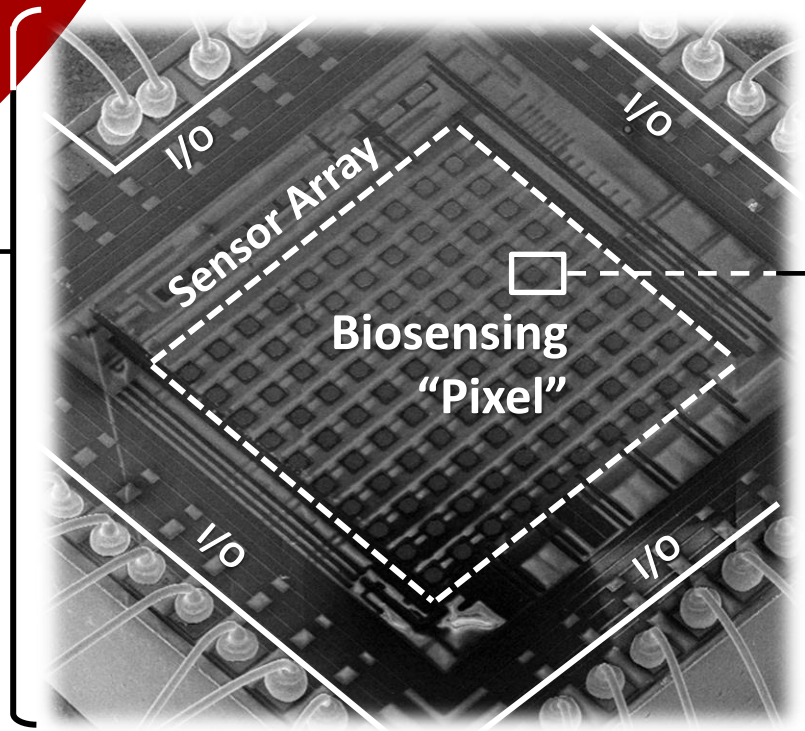
Biochip Module



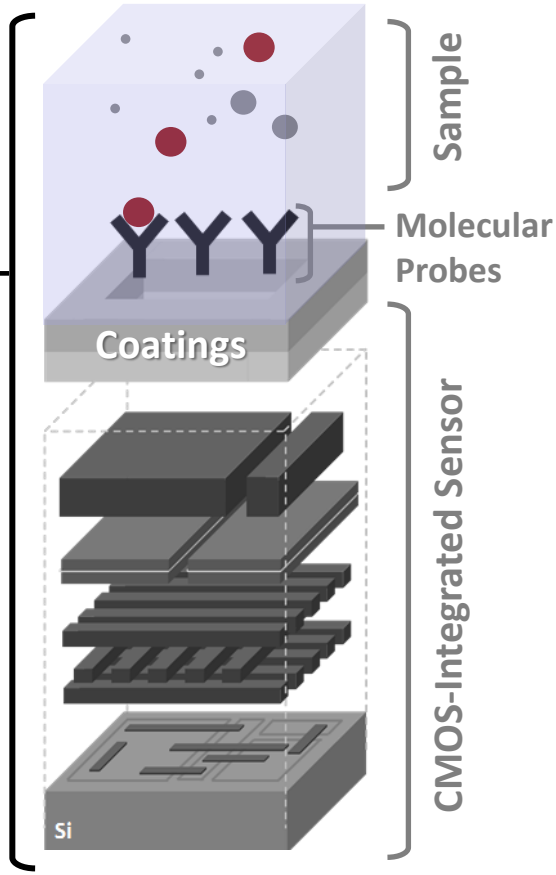
Cross-Section



CMOS Die



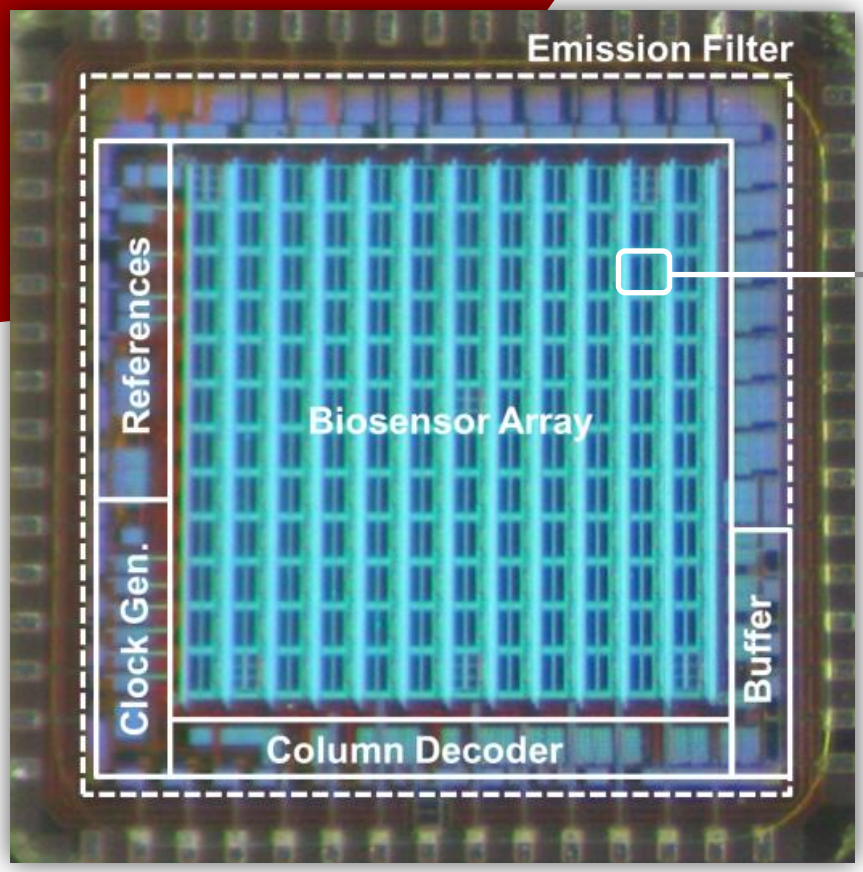
Biosensing Pixel

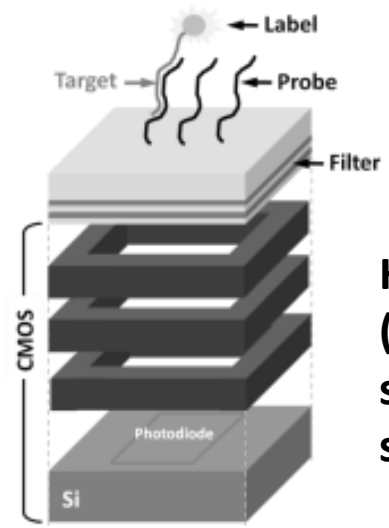


Biosensing Pixels

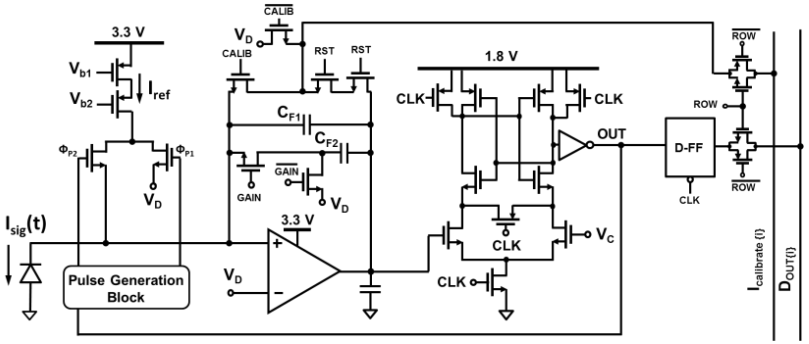
Depending on the adopted chemistry, various detection modalities can be implemented in the pixels

Fluorescent Detection Arrays*





High dynamic range (HDR) wavelength-specific ΔΣ photo-sensor in every pixel

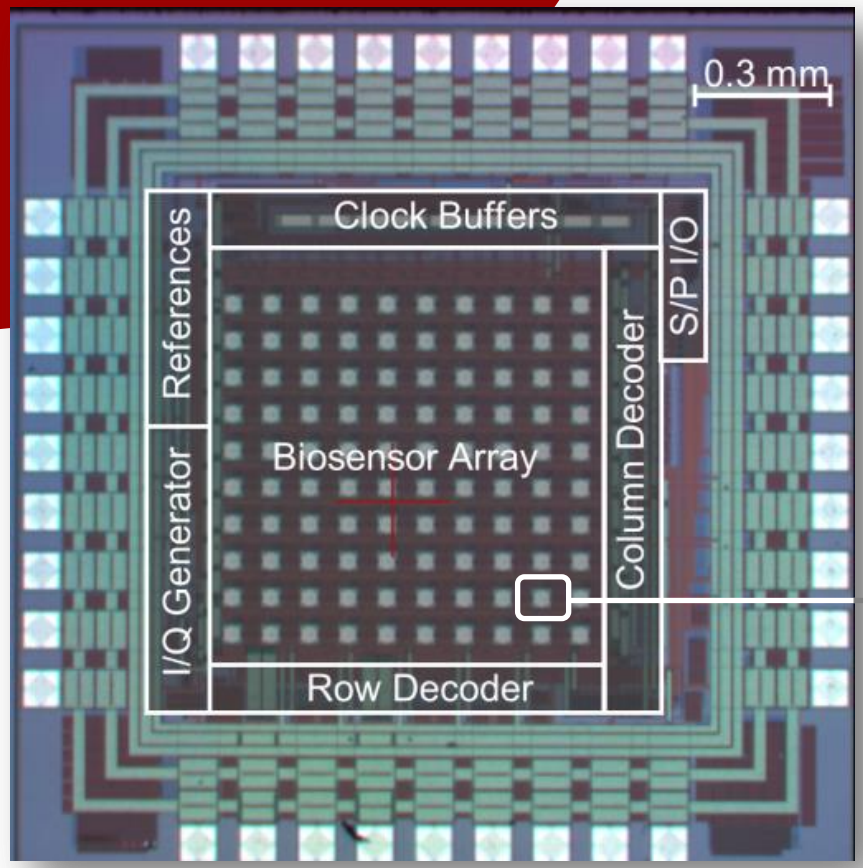


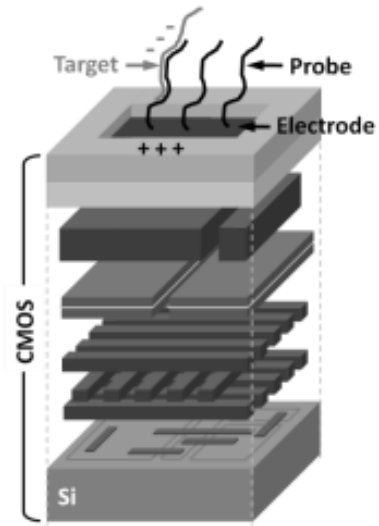
*Singh *et al.*, VLSI Symp. (2011)

Biosensing Pixels

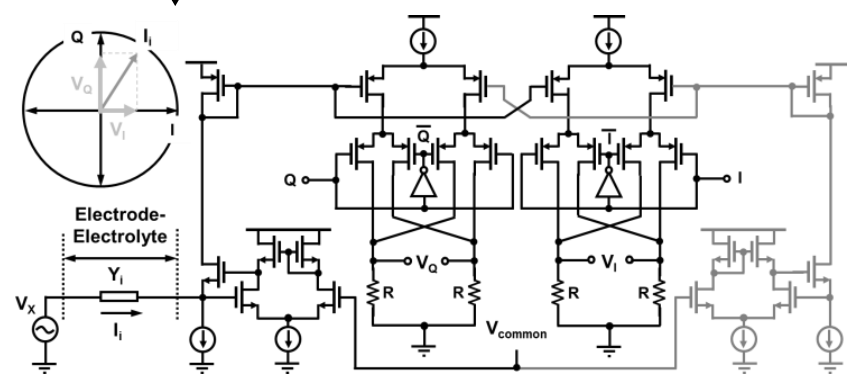
Depending on the adopted chemistry, various detection modalities can be implemented in the pixels

Impedance Spectroscopy Arrays*





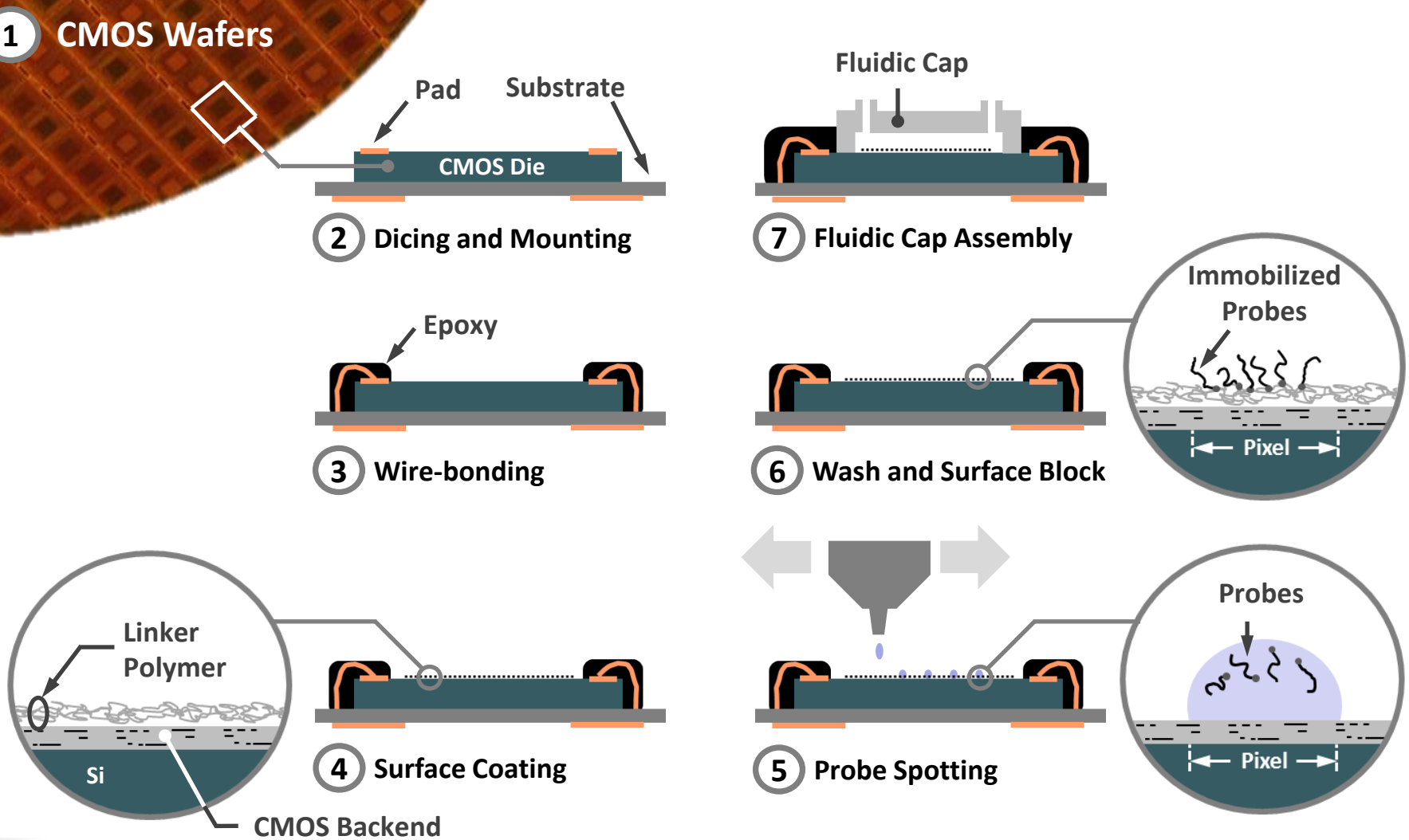
Lock-in amplifier-based impedance measurement in every pixel



* Manickam *et al.*, ISSCC (2010)

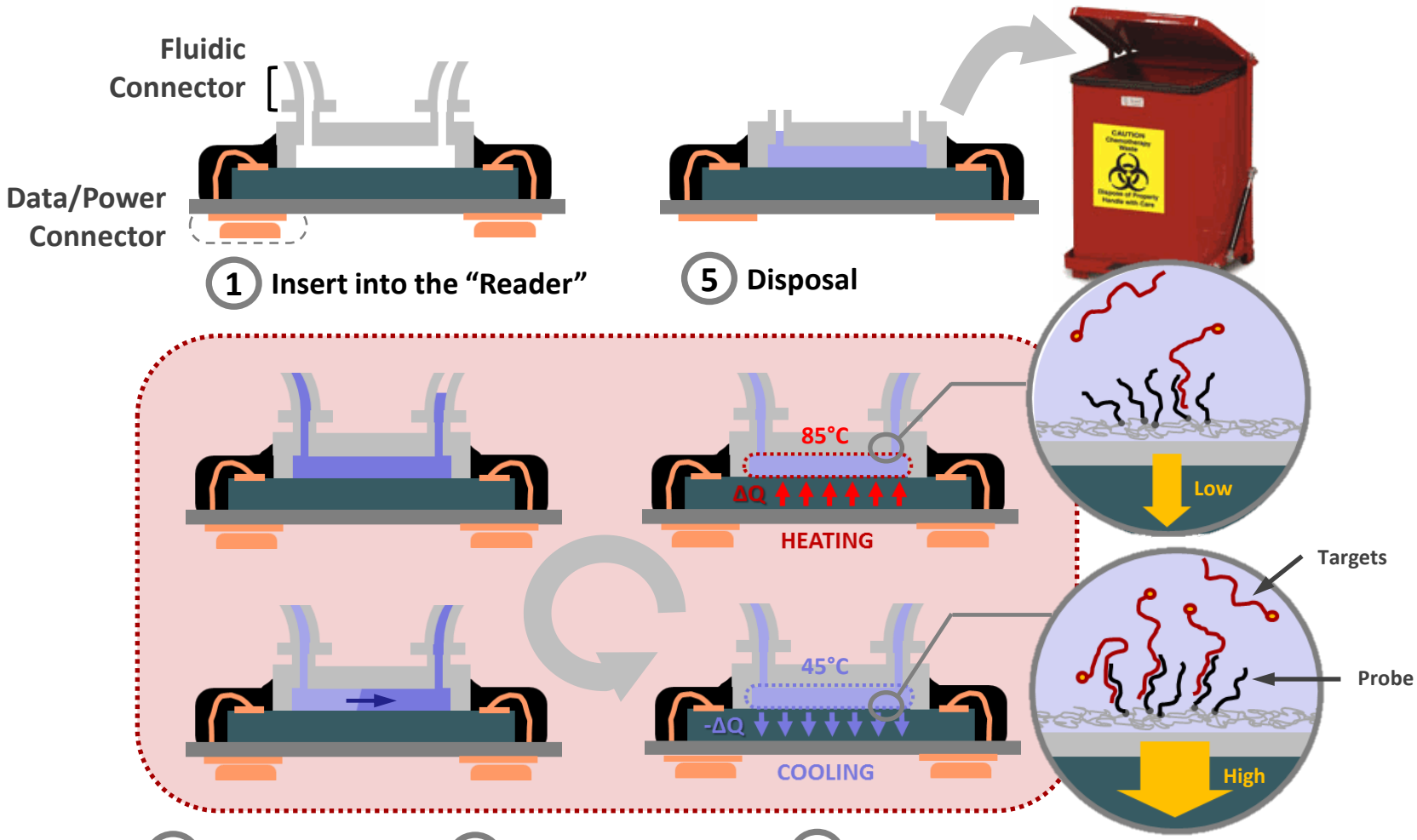
Manufacturing Process

Biochips require a complex packaging/assembly process



Detection Process (1)

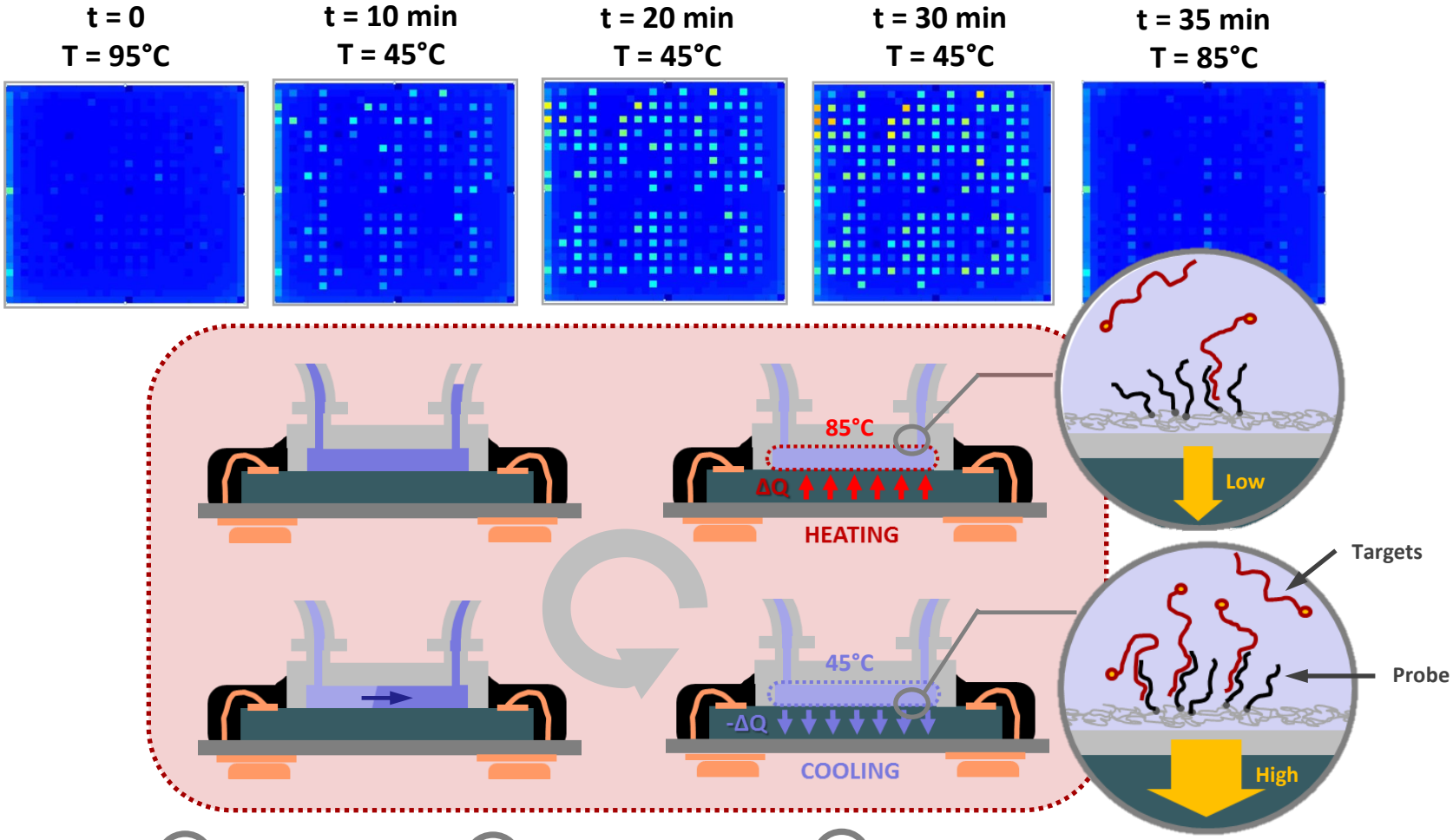
Chip (and the pixels) are exposed to the sample containing the targets



2 Sample Insertion, 3 Thermal Cycling, and 4 Data Acquisition

Detection Process (2)

Output data reports the DNA capturing events, as a function of time and temperature, at every biosensing pixel

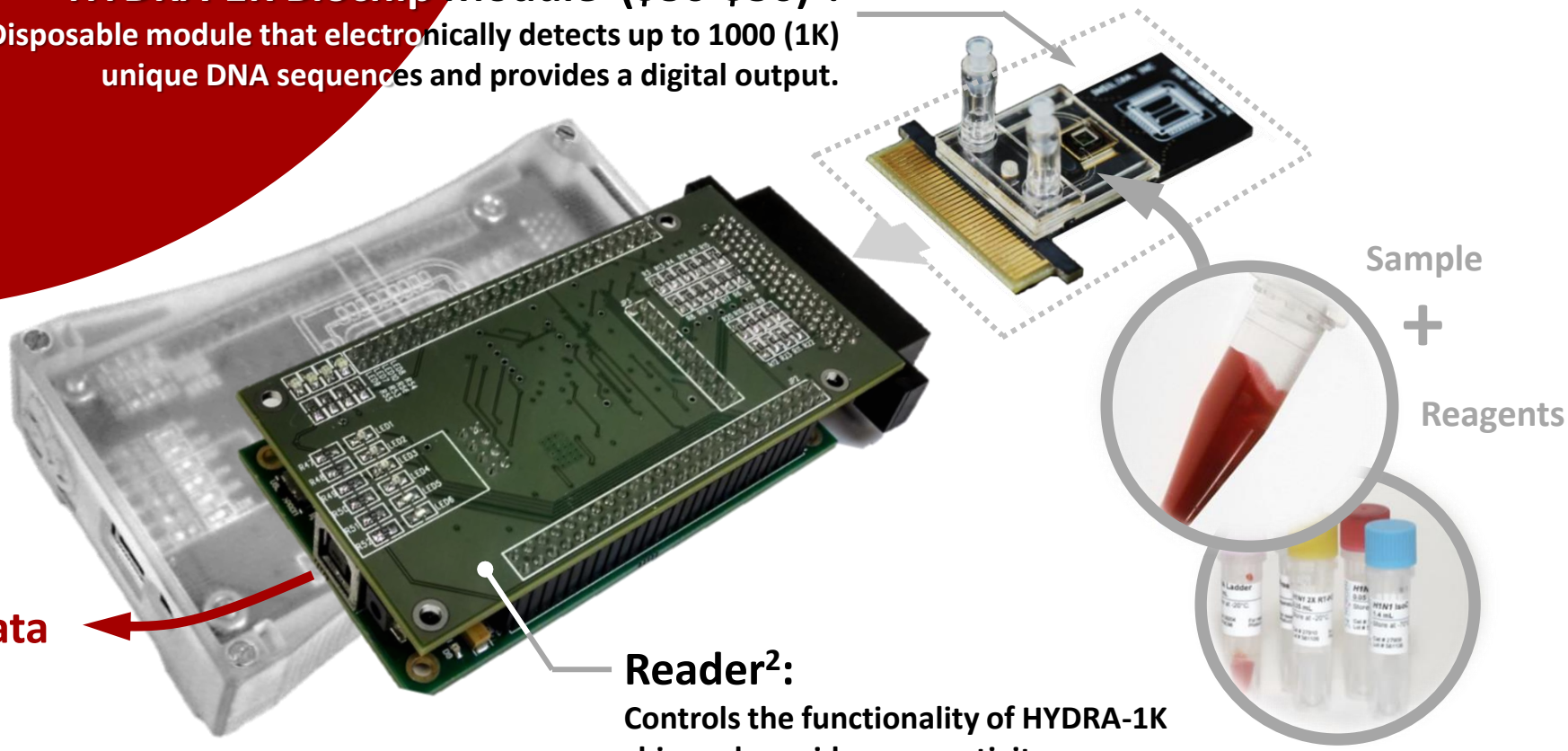


2 Sample Insertion, 3 Thermal Cycling, and 4 Data Acquisition

HYDRA-1K Platform

A CMOS biochip system for point-of-care DNA analysis

HYDRA-1K Biochip Module ($30-$50)[1]:
Disposable module that electronically detects up to 1000 (1K) unique DNA sequences and provides a digital output.



Reader[2]:
Controls the functionality of HYDRA-1K chip and provides connectivity

[1] Cost varies depending on the application

[2] Not the final design

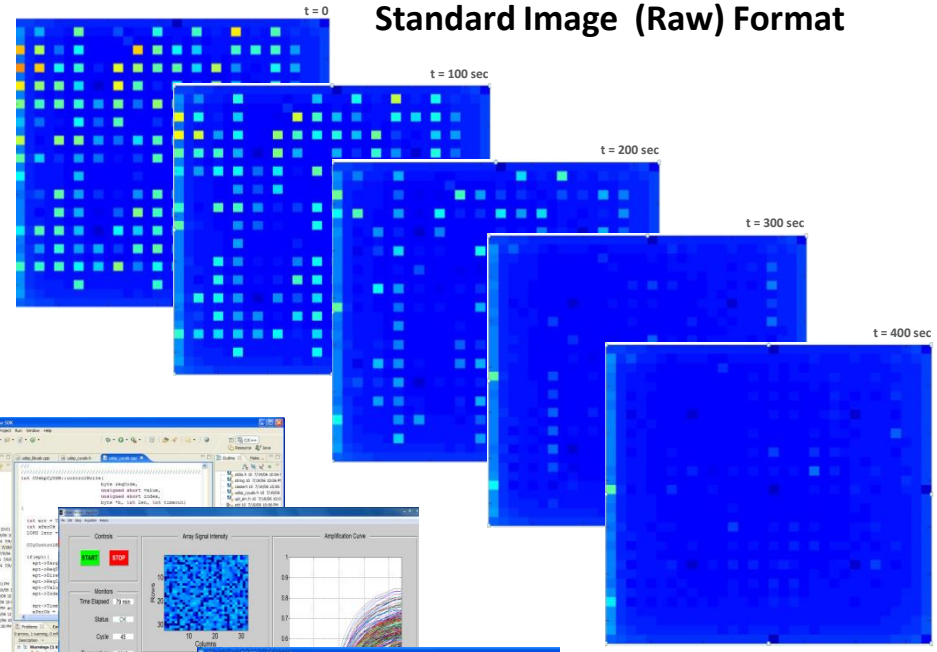
HYDRA-1K: Open Platform for MDx

HYDRA-1K reagents, hardware, and software are designed to enable flexible application development

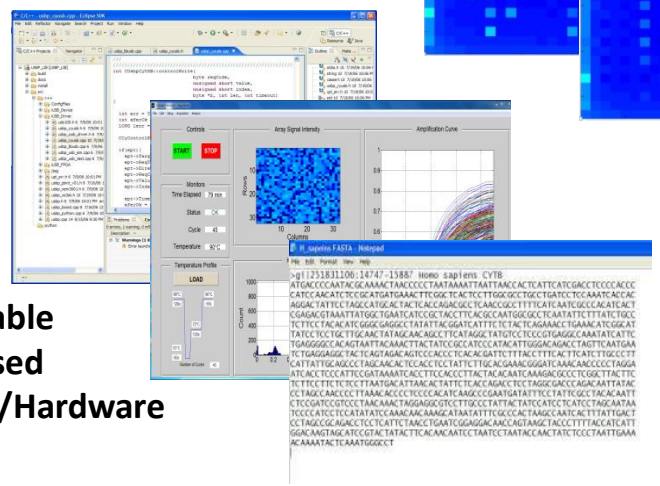
CMOS Biochip

Standard Reagents and DNA Probes

Configurable FPGA-based Software/Hardware



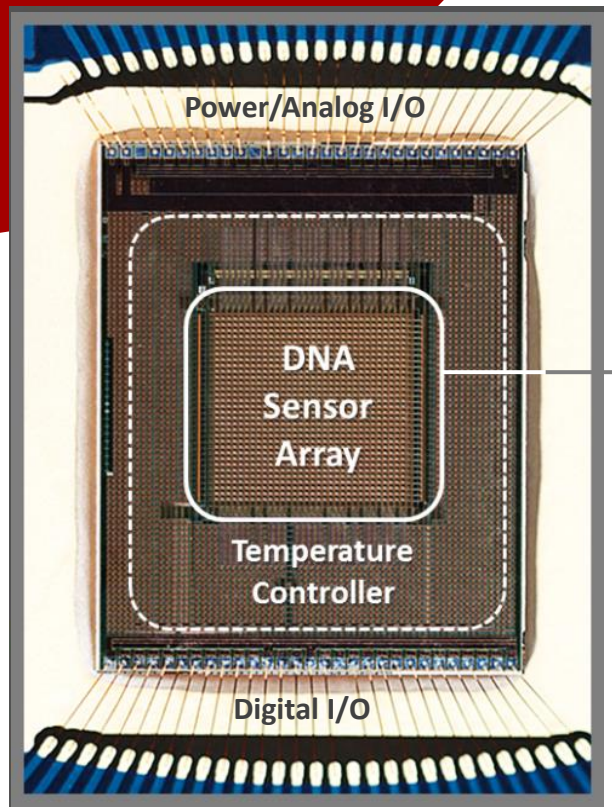
Standard Image (Raw) Format

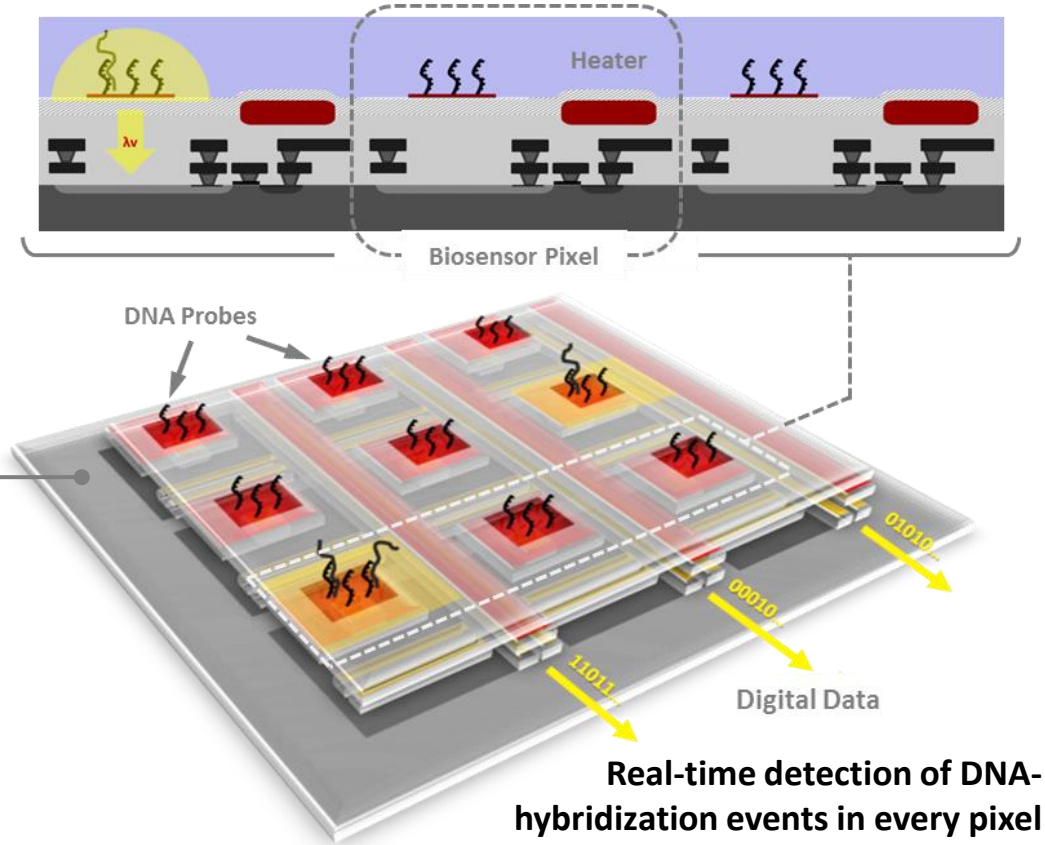


Standard (FASTA) Output File

HYDRA-1K: DNA Sensor Array

DNA sequence identification enabled by pixel-level DNA capturing and optical detection



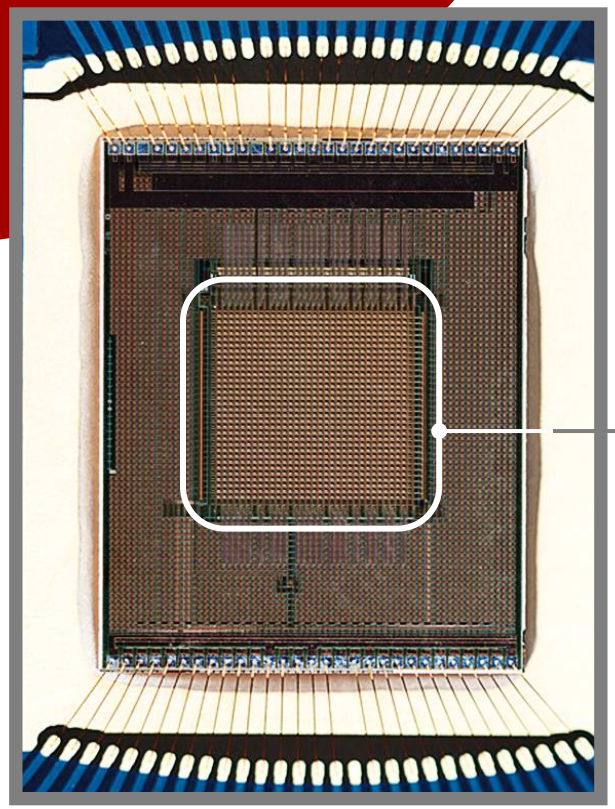


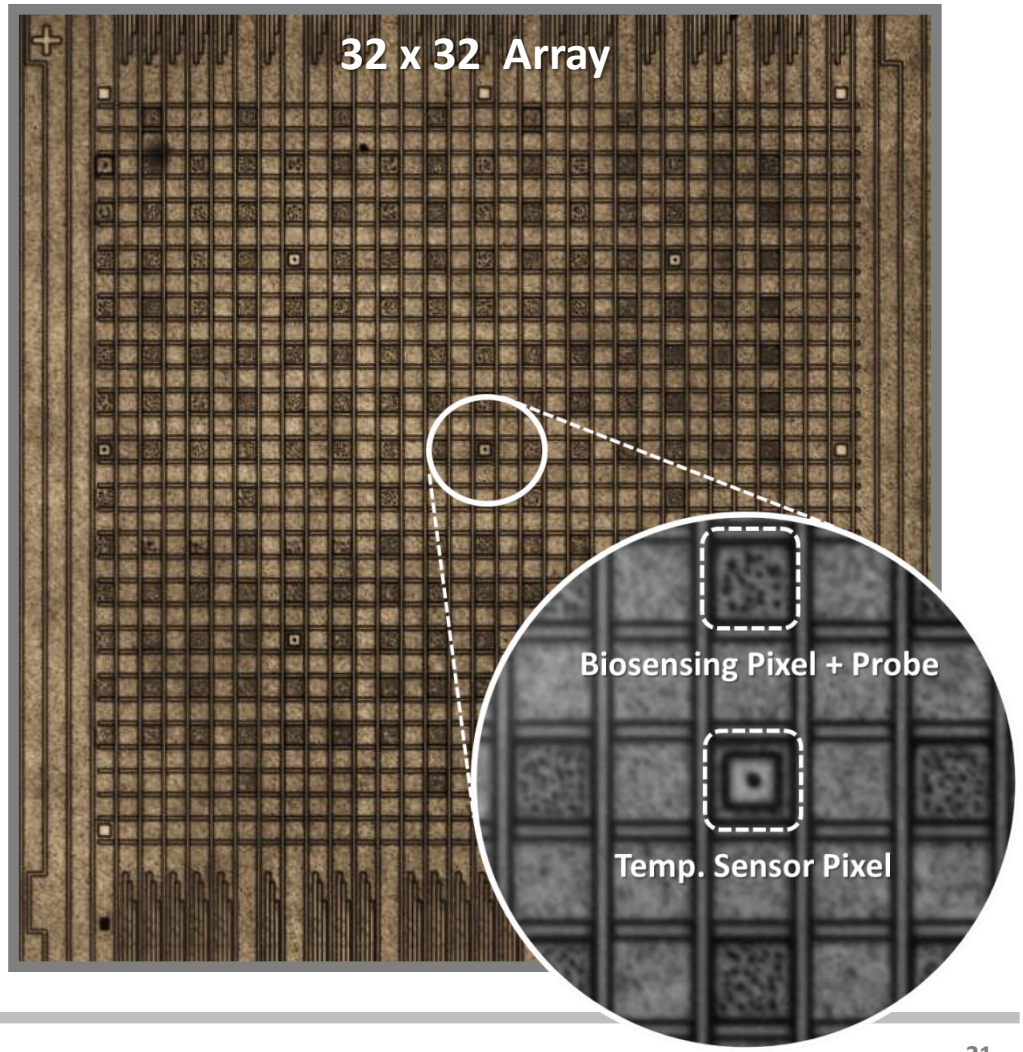
Real-time detection of DNA-hybridization events in every pixel

HYDRA-1K: CMOS Biosensor Array

DNA sequence identification enabled by pixel-level DNA capturing and optical detection

CMOS Biochip

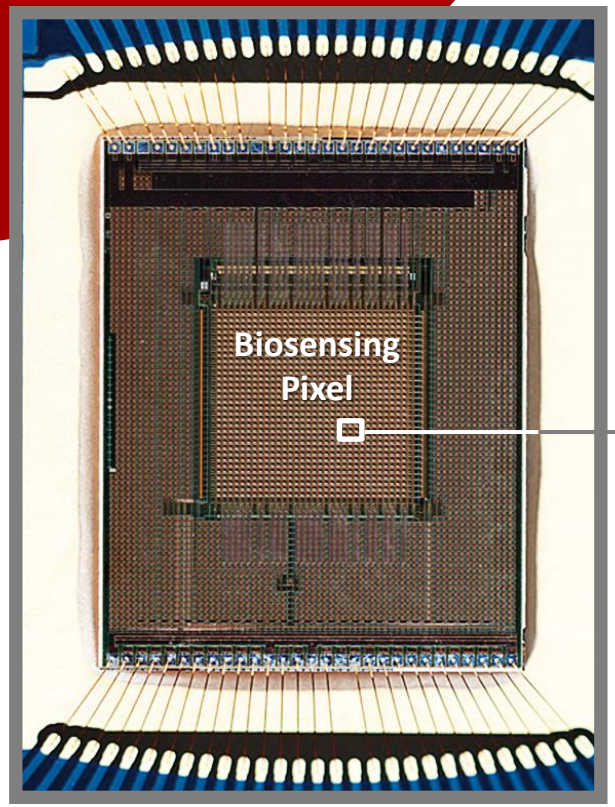


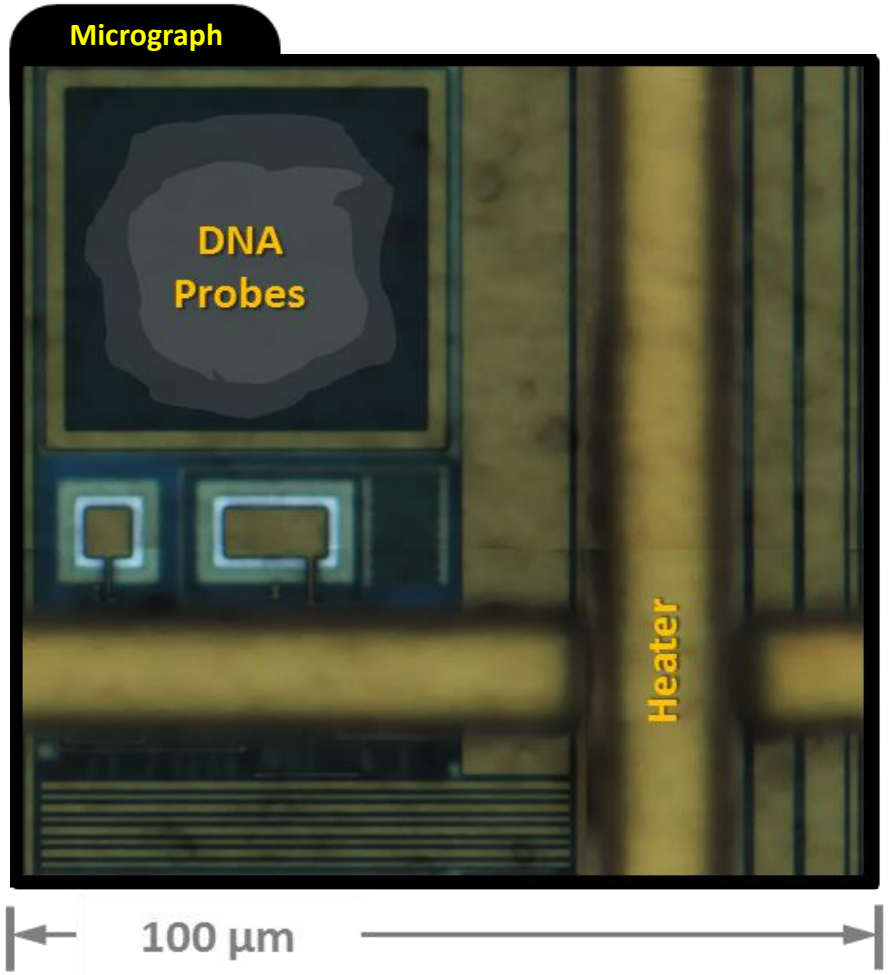


HYDRA-1K: Integrated Pixels

Programmable +120 dB dynamic range photo-sensor and thermo-cycler are integrated in every pixel

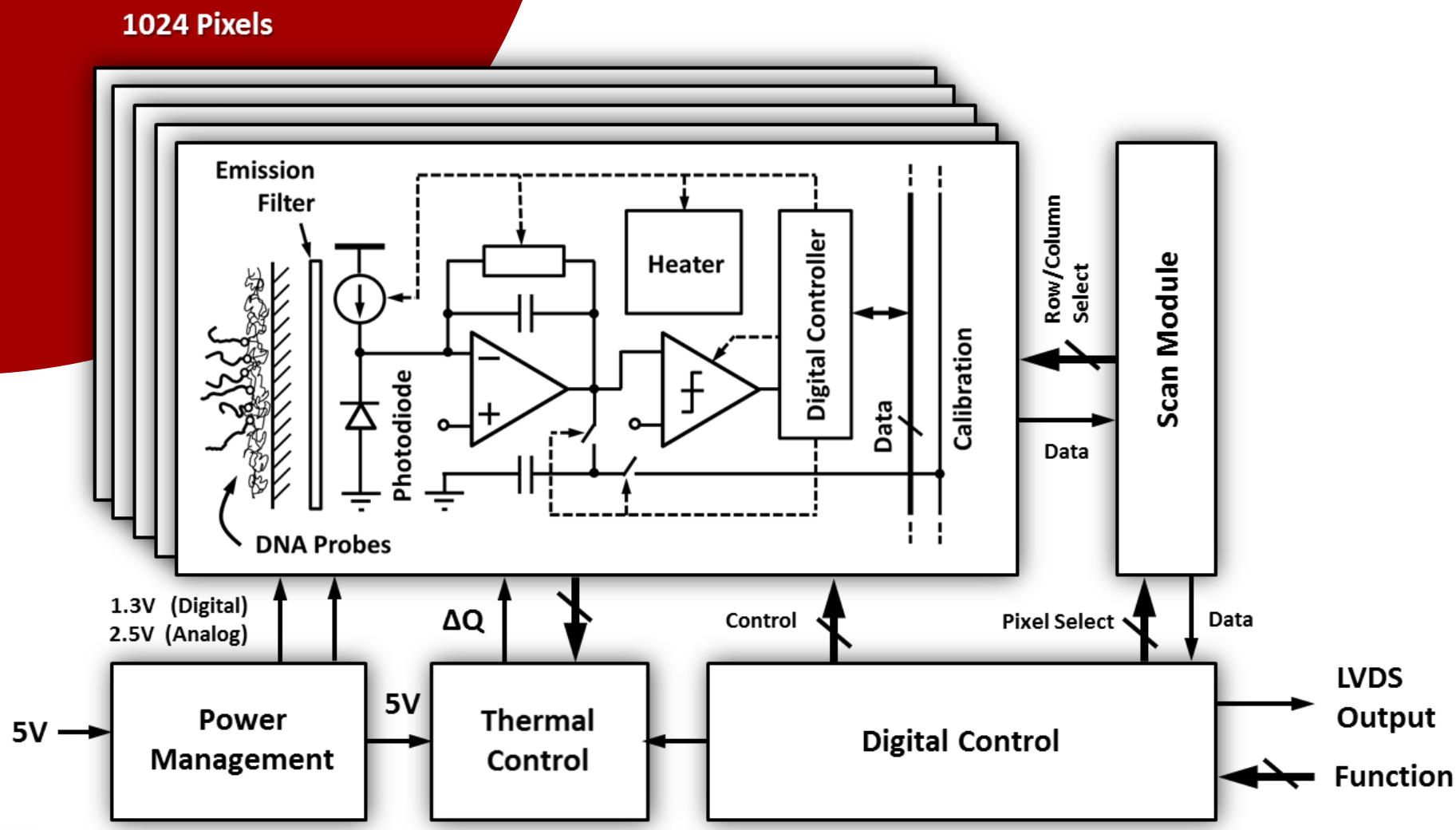
CMOS Biochip





HYDRA-1K: Chip Architecture

The biochip includes the 1024-element biosensor array, a 24-bit ΣΔ data converter, on-chip thermal controller, and power management system



Chip Performance/ Characteristics

TECHNOLOGY	IBM 6RF (L_{min} = 0.25 µm, 1P, 4M, 2.5V/5V)
ARRAY	32 x 32 (1008 Biosens. + 16 Temp. Sens.)
BIOSENSING PIXELS	Fluorescence ($\lambda \sim$ [450 nm, 700 nm])
DETECTION DYNAMIC RANGE	145 dB ($I_{ph} \sim$ [0.5fA, 10nA])
SPEED	0.1-50 Frames/Sec (Programmable)
RESOLUTION	>24 Bit
POWER CONSUMPTION	112 mW (I_C = 45 mA @ 2.5V)
HEATING/COOLING RATES	(+4/-4)°C/sec
TEMPERATURE CONTROL ACCURACY	0.25°C/sec
COST (INCLUDING ASSEMBLY)	$30-$50*

*Varies depending on the application

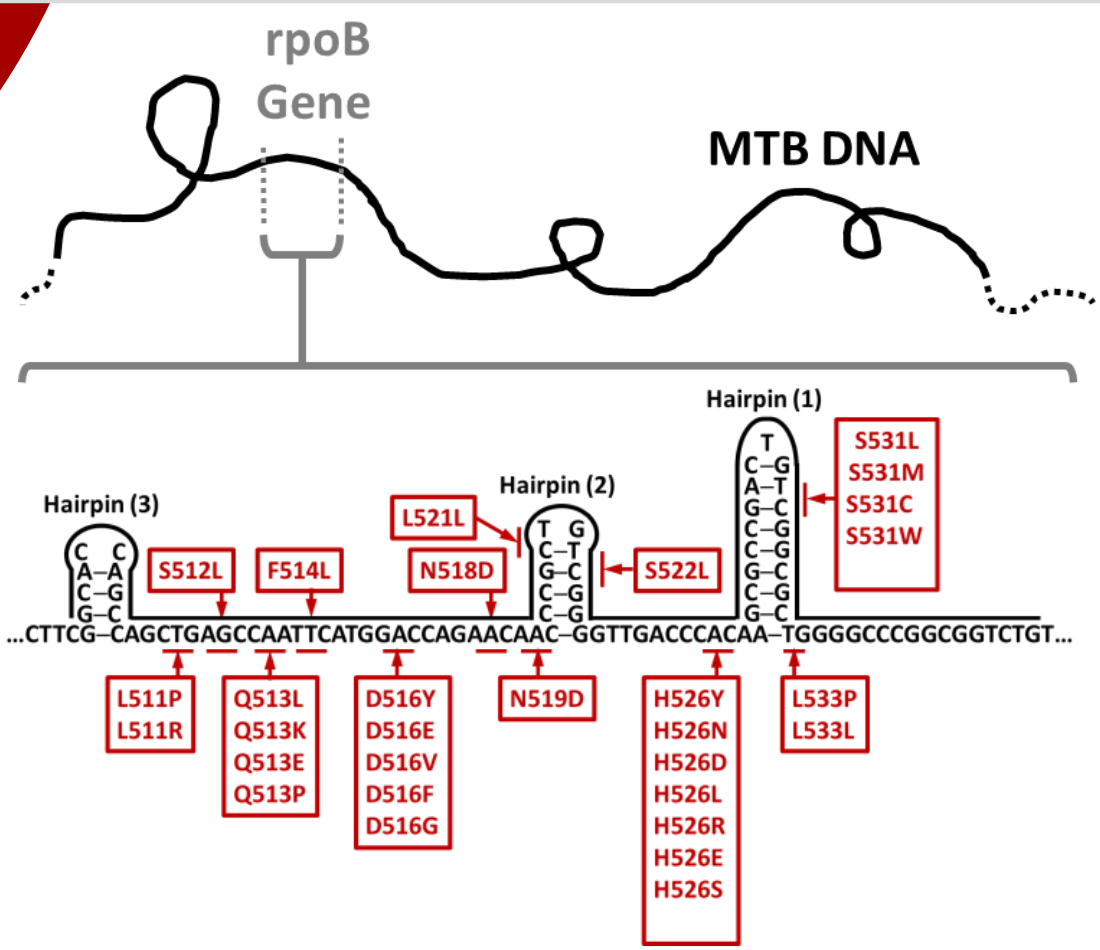
EXAMPLE

Mycobacterium Tuberculosis **(MTB) Detection**

Detecting Drug Resistant TB

Mycobacterium Tuberculosis (MTB)

TB bacterium has a very specific DNA region with possible mutations that result in resistance to antibiotics

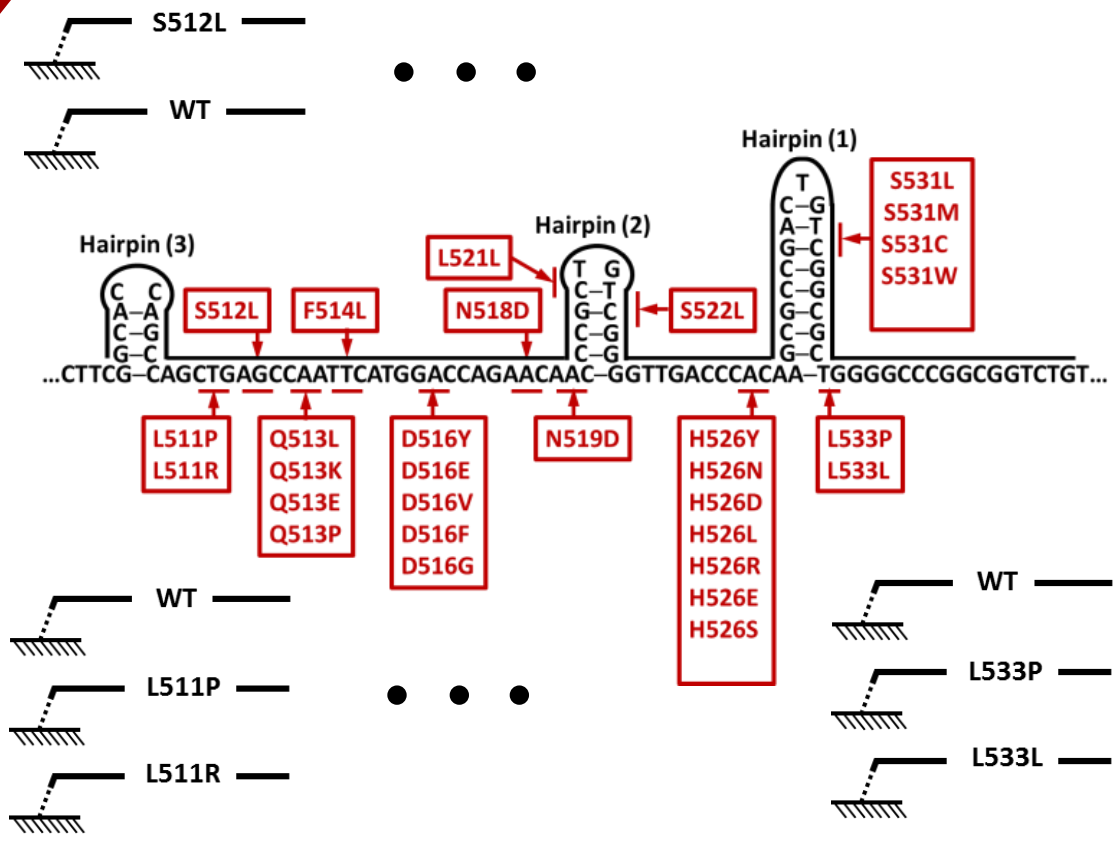


30 mutations within the *rpoB* gene that cause resistivity to Rifampicin

Probe Design Procedure

DNA probes are design to capture the wild-type and the mutant strains for every possible mutation

Mycobacterium Tuberculosis (MTB)



Probe Location within the Array

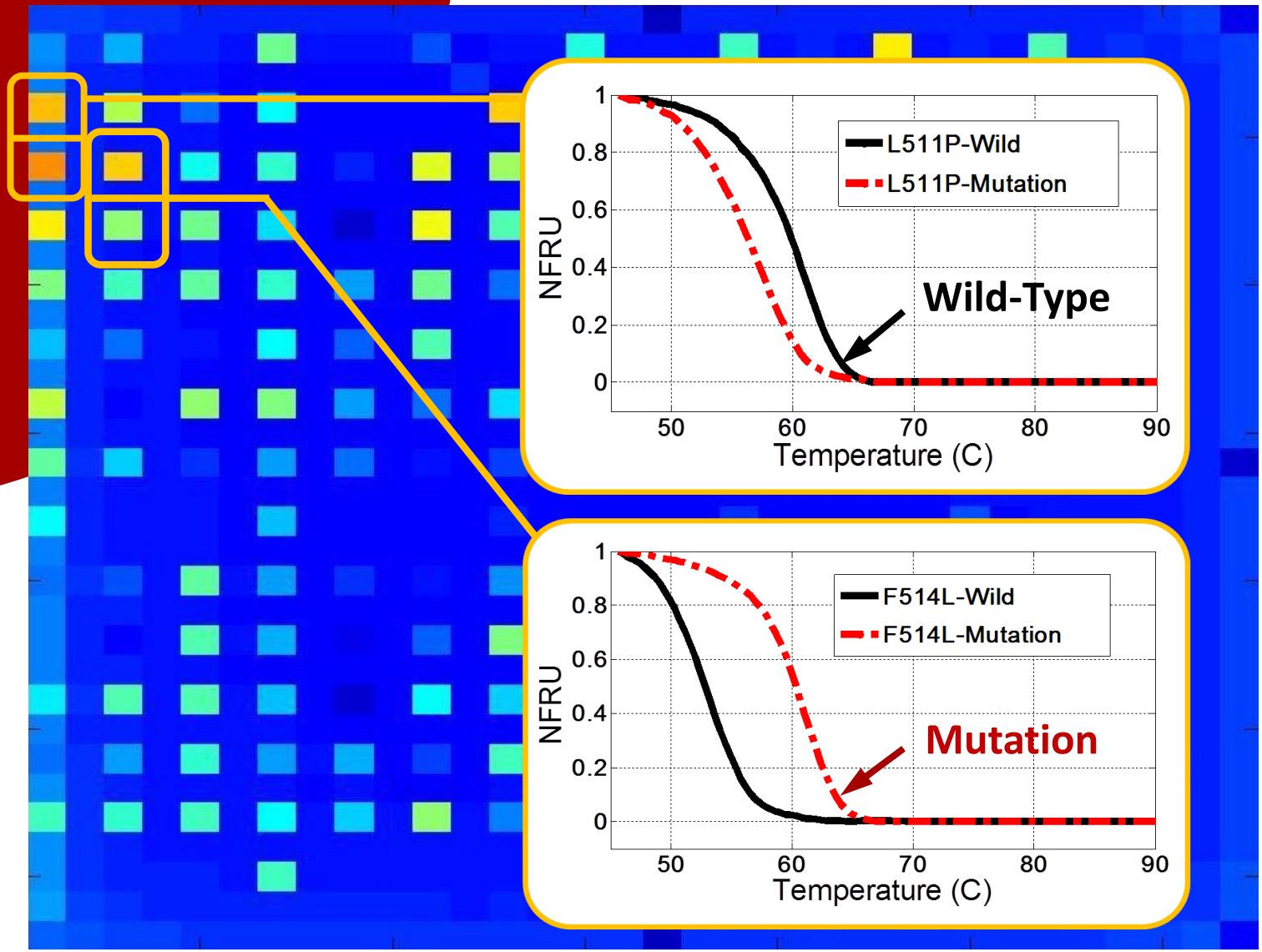
L511P (W)	Control (2)	N519D (W)	H526D (M1)	H526L (M-L)	L533P (W)	S512L (W)	N518D (W)	H526N (M1)	H526N (M-L)	S531L (W)	Control (1)	D516Y (W)	Control (3-H)	H526L (M)	Control (2-Cy)
L511P (M)	F514L (W)	N519D (M)	H526D (M2)	H526R (M-L)	L533P (M)	S512L (M)	N518D (M)	H526N (M2)	H526D (M-L)	S531L (M1)	Q513L (W)	D516Y (M1)	L533P (W)	H526R (W)	H526E (M-L)
L511R (M)	F514L (M)	L521L (W)	H526L (M)	H526E (M-L)	L533L (M)	F514L (W)	N519D (W)	H526D (M1)	H526L (M-L)	S531L (M2)	Q513L (M)	D516Y (M2)	L533P (M)	H526R (M)	H526S (M-L)
S512L (W)	D516Y (W)	L521L (M)	H526R (W)	H526S (M-L)	Control (3-H)	F514L (M)	N519D (M)	H526D (M2)	H526R (M-L)	S531M (W)	Q513K (M)	D516E (M)	L533L (M)	H526E (W)	S531L (W)
S512L (M)	D516Y (M1)	S522L (W)	H526R (M)	S531L (W)	L511P (W)	D516Y (W)	L521L (W)	H526L (M)	H526E (M-L)	S531M (M)	Q513E (M)	D516V (M)	H526Y (W)	H526E (M)	S531L (M1)
Q513L (W)	D516Y (M2)	S522L (M)	H526E (W)	S531L (M1)	L511P (M)	D516Y (M1)	L521L (M)	H526R (W)	H526S (M-L)	S531C (M)	Q513P (M)	D516F (M)	H526Y (M1)	H526S (M)	S531L (M2)
Q513L (M)	D516E (M)	H526Y (W)	H526E (M)	S531L (M2)	L511R (M)	D516Y (M2)	L522L (W)	H526R (M)	L533P (W)	S531W (M1)	F514L (W)	D516G (M)	H526Y (M2)	H526Y (W-L)	S531M (W)
Q513K (M)	D516V (M)	H526Y (M1)	H526S (M)	S531M (W)	Q513L (W)	D516E (M)	S522L (M)	H526E (W)	L533P (M)	S531W (M2)	F514L (M)	N519D (W)	H526Y (M3)	H526Y (M-L)	S531M (M)
Q513E (M)	D516F (M)	H526Y (M2)	H526Y (W-L)	S531M (M)	Q513L (M)	D516V (M)	S526Y (W)	H526E (M)	L533L (M)	L511P (W)	N518D (W)	N519D (M)	H526N (M1)	H526N (M-L)	S531C (M)
Q513P (M)	D516G (M)	H526Y (M3)	H526Y (M-L)	S531C (M)	Q513K (M)	D516F (M)	H526Y (M1)	H526S (M)	S512L (W)	L511P (M)	N518D (M)	S522L (W)	H526N (M2)	H526D (M-L)	S531W (M1)
Control (1)	N518D (W)	H526N (M1)	H526N (M-L)	S531W (M1)	Q513E (M)	D516G (M)	H526Y (M2)	H526Y (W-L)	S512L (M)	L511R (M)	L521L (W)	S522L (M)	H526D (M1)	H526L (M-L)	S531W (M2)
Control (1-Cy)	N518D (M)	H526N (M2)	H526D (M-L)	S531W (M2)	Q513P (M)	Control (2)	H526Y (M3)	H526Y (M-L)	Control (1)	Control (3-H)	L521L (M)	Control (2)	H526D (M2)	H526R (M-L)	Control (1-Cy)

Wild-Type Probe

Mutated Strain Probe

Controls

Capturing and DNA Melt-Curve Analysis



Example Results

Mutation	Wild-Type TB		Strain-1		Strain-2		Strain-3	
	Actual	Meas.	Actual	Meas.	Actual	Meas.	Actual	Meas.
L511P	CTG	CTG	CCG	CCG	CTG	CTG	CTG	CTG
Q513L	CAA	CAA	CAA	CAA	CTA	CTA	CAA	CAA
F514L	TTC	TTC	TTC	TTC	TTC	TTC	CTC	CTC
D516F	GAC	GAC	TTC	TTC	GAC	GAC	GAC	GAC
N518D	AAC	AAC	AAC	No Call	GAC	GAC	AAC	AAC
L521L	CTG	CTG	CTG	CTG	CTG	CTG	TTG	TTG
H526Y	CAC	CAC	TAC	TAC	CAC	CAC	CAC	CAC
S531L	TCG	TCG	TCG	TCG	TTG	TTG	TCG	TCG
L533P	CTG	CTG	CTG	CTG	CTG	CTG	TTG	TTG

Sensitivity > 50 Copies-per-Sample

Strain Detection Success Rate= 97.22%

Comparison

TECHNOLOGY	PCR[1]	DNA ARRAYS	DNA SEQUENCING	HYDRA-1K
Instrument (Setup) Price	$20K -50K	$25K-75K	$100K-$700K	~$250
Price per Test[2]	$80-$400	$200-$1000	$2.5K-$10K	$30-$50
Max DNA Targets	6-20	20-1000	+1M	1000
Detection Accuracy	High	Low	Medium	High
Test Time	2-3 hours	6-12 Hours	> 1 Day	1 Hour
Fully-Automated	Yes	No	No	Yes
Portable	No	No	No	Yes
PoC Compatible	Yes	No	No	Yes

[1] Polymerase chain reaction

[2] Excluding any service charge

Small DNA Differences Matter

Albert Einstein
(1879-1955)

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Bobo the Chimp
(1995-Now)

= 1.5% DNA Difference