A CMOS Label-free DNA Microarray

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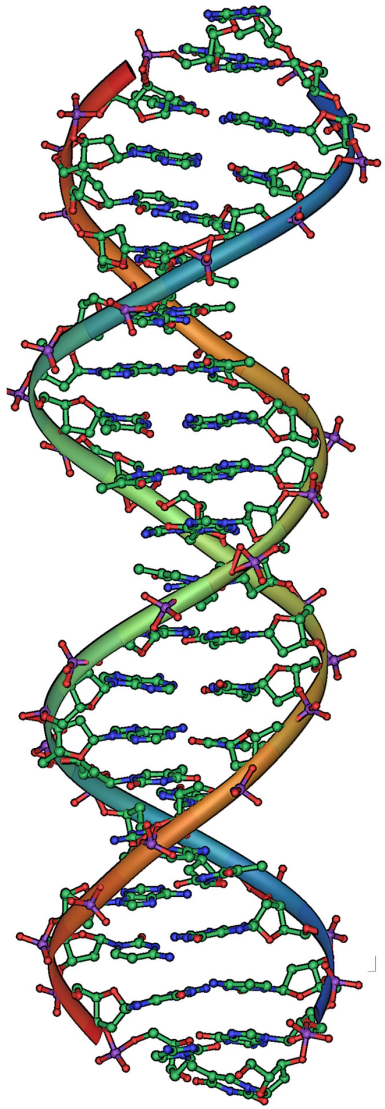
I2MTC 2008

Motivation

- Affymetrix + Agilent alone had $2.4 billion (USD) in revenue in 2007 for bio-analytic measurements
  - Drug discovery
  - Diagnostics
  - Research
  - Forensic testing
- Growing interest in personalized medicine
  - Therapeutics tailored to your genetic profile
- Conventional microarrays are expensive, big bulky systems (optics, lasers, reagents)
  - Can we leverage integrated circuit fabrication techniques for a low-cost approach?

www.dnavision.be

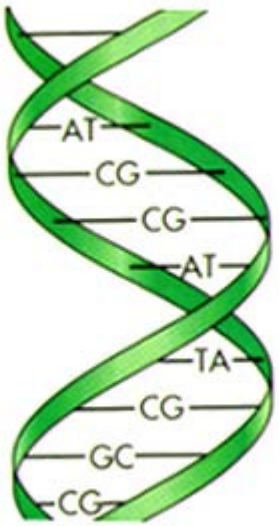


Outline

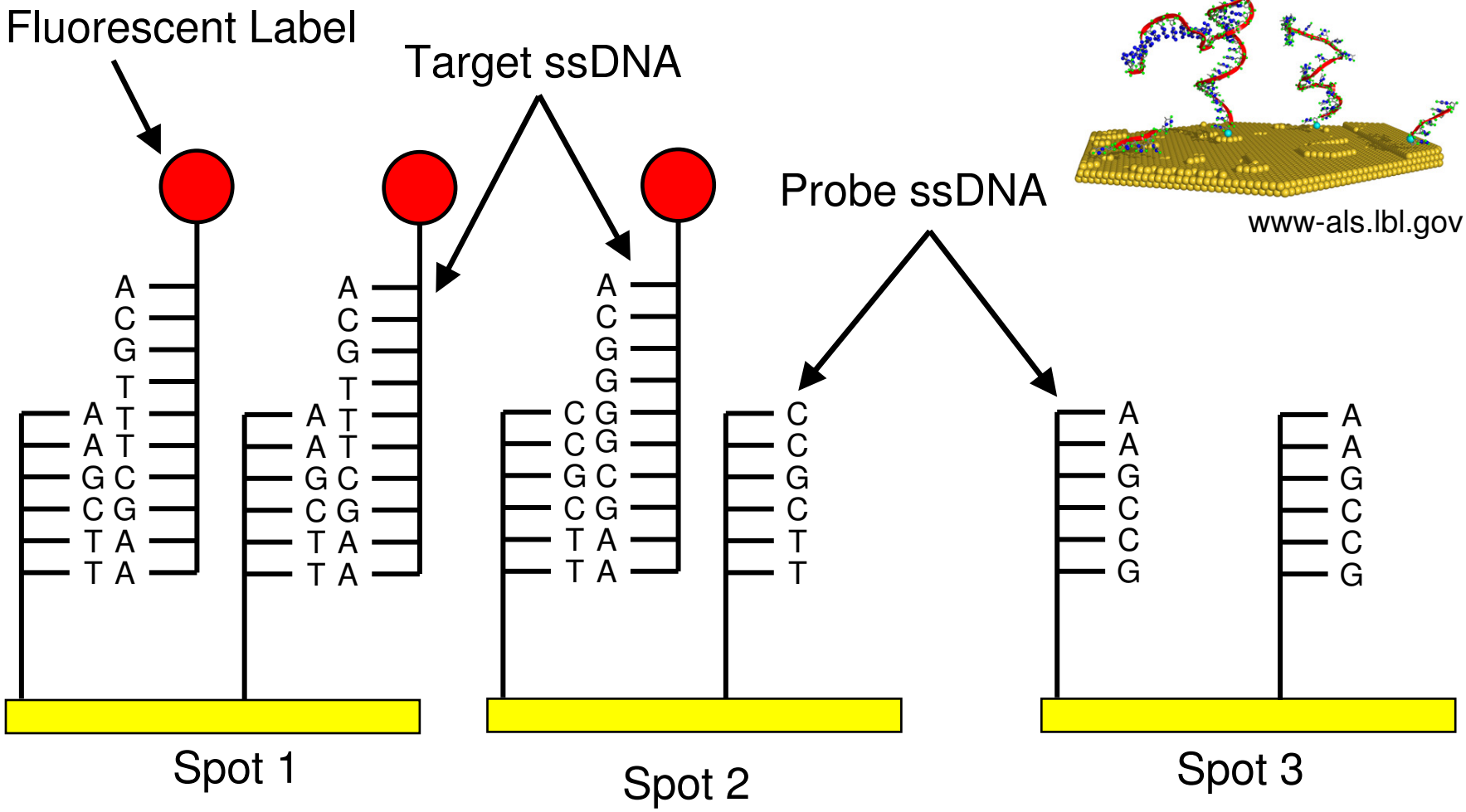
- Motivation
- Background
- Charge sensing of DNA polymerization
- CMOS sensor
- Conclusions

DNA

- Contains genetic instructions to construct and regulate cellular components
- Consists of 4 nucleotides
  - Adenine (A), Thymine (T), Cytosine (C), Guanine (G)
- Usually found double-stranded, but single-stranded version exists too
- A only binds with T, C only binds with G

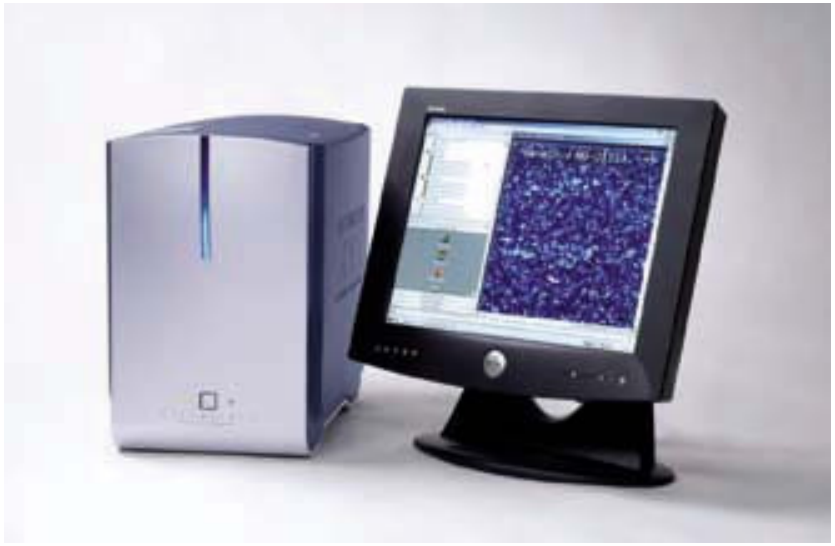


Microarray Basics I

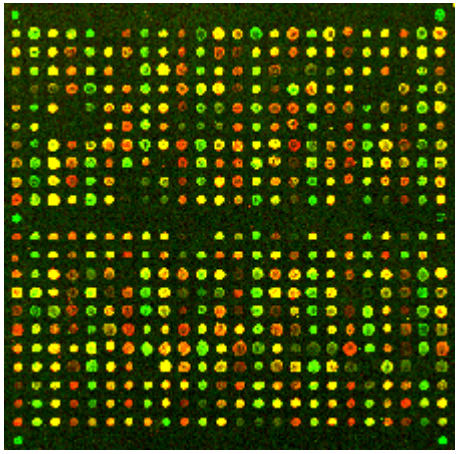


Microarray Basics II

Affymetrix Gene Chip



Microarray Scanner –
Cost: ~$200k



Gene Chip
Image

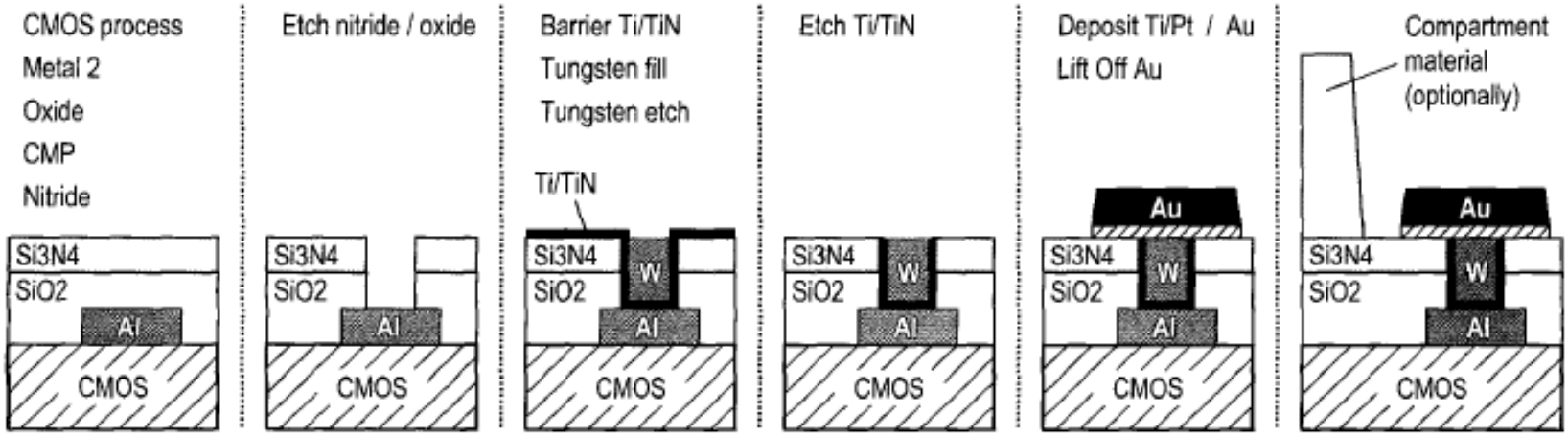
- Light from a grid location indicates the presence of the corresponding target in a sample
- Limitations: Expensive and not portable

Images courtesy of Affymetrix

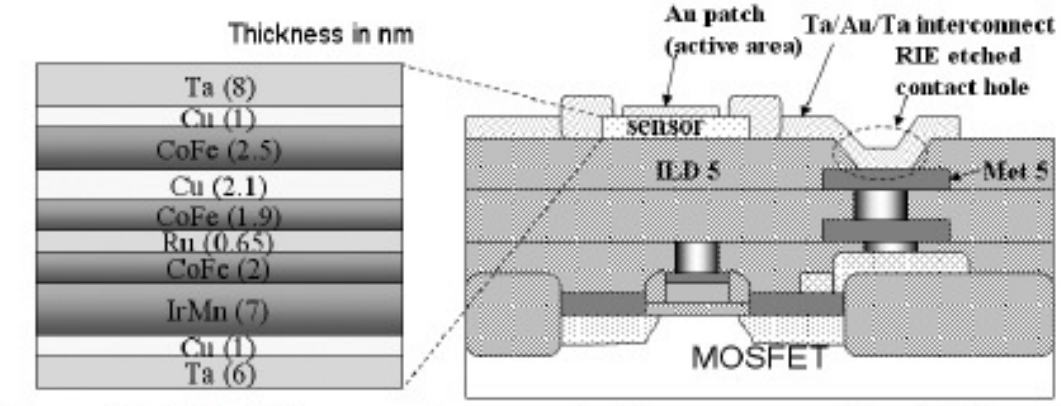
TAG4 Example from SGTC

- TAG4 = yeast genome used with optical scanners
- Run time
  - DNA Extraction 2 hr
  - PCR & labeling 2 hr
  - Hybridization preparation 0.5 hr
  - Hybridization 6-16 hr
  - Wash & Stain 3 hr
  - Scan of chip 0.25 hr
- Cost per chip (“Academic Prices”)
  - Chip $150-300
  - Reagents $50-150
- 100,000 features or “spots” which are 8 µm x 8 µm
- Probes are 20 nucleotides in length
- Targets range from 100-200 nucleotides
  - 10-100 ng/mL amplified (PCR) to concentrations of 1 µg/mL
- Works well when you are interested in massively parallel detection
  - Suitable for point-of-care applications?

Post Processing Challenges



Thewes et al. ISSCC 2002.

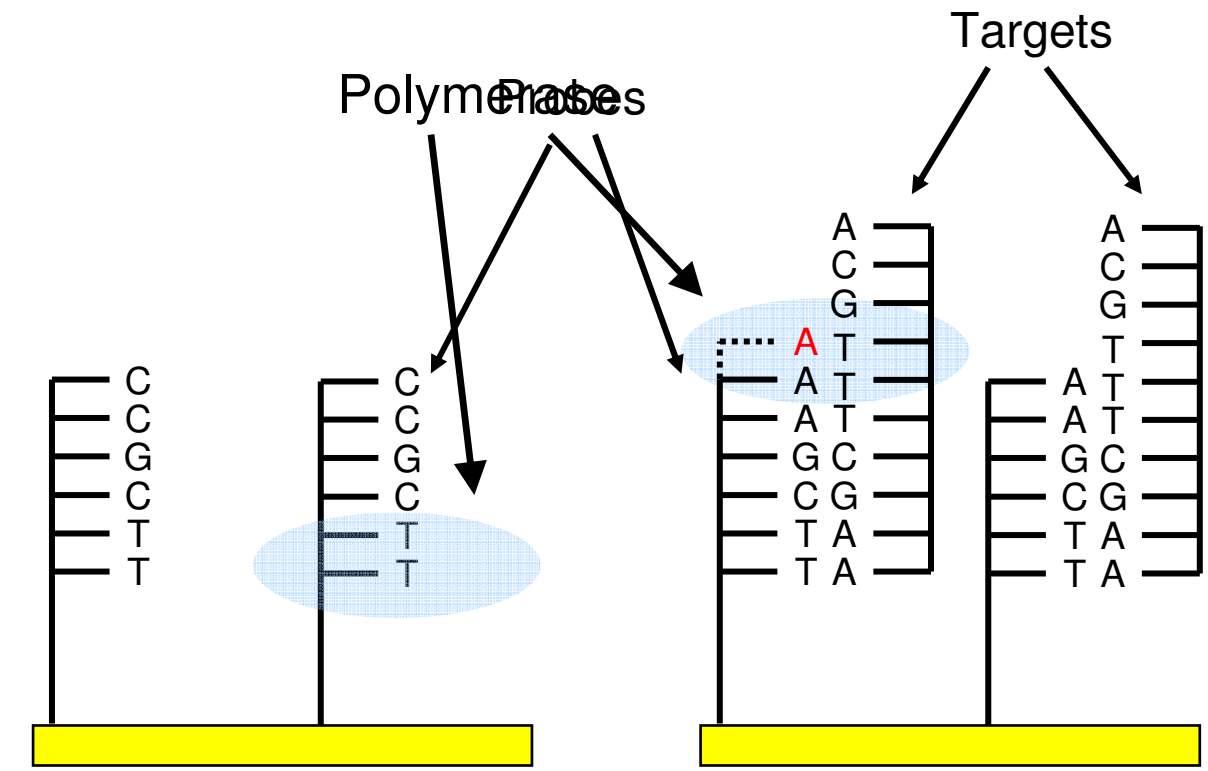


Han et al. ISSCC 2007.

System Requirements

- Suitable for point-of-care applications
  - Leverage IC fab technology for low-cost approach
  - Label-free
  - Easy post-processing
  - Integrate microarray with the “readout”
  - Reduced number of features from conventional optical techniques – goal is 25
- Detects targets at 10 µg/mL

DNA Polymerization

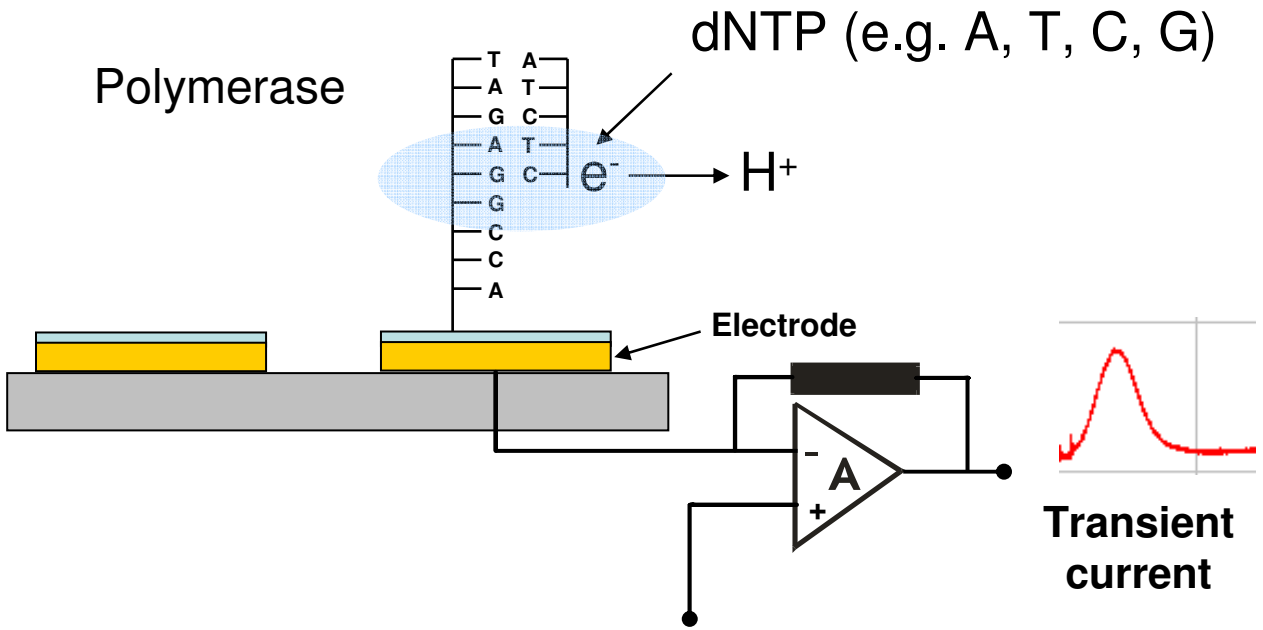


Second strand **CANNOT** be synthesized

Second strand **CAN** be synthesized

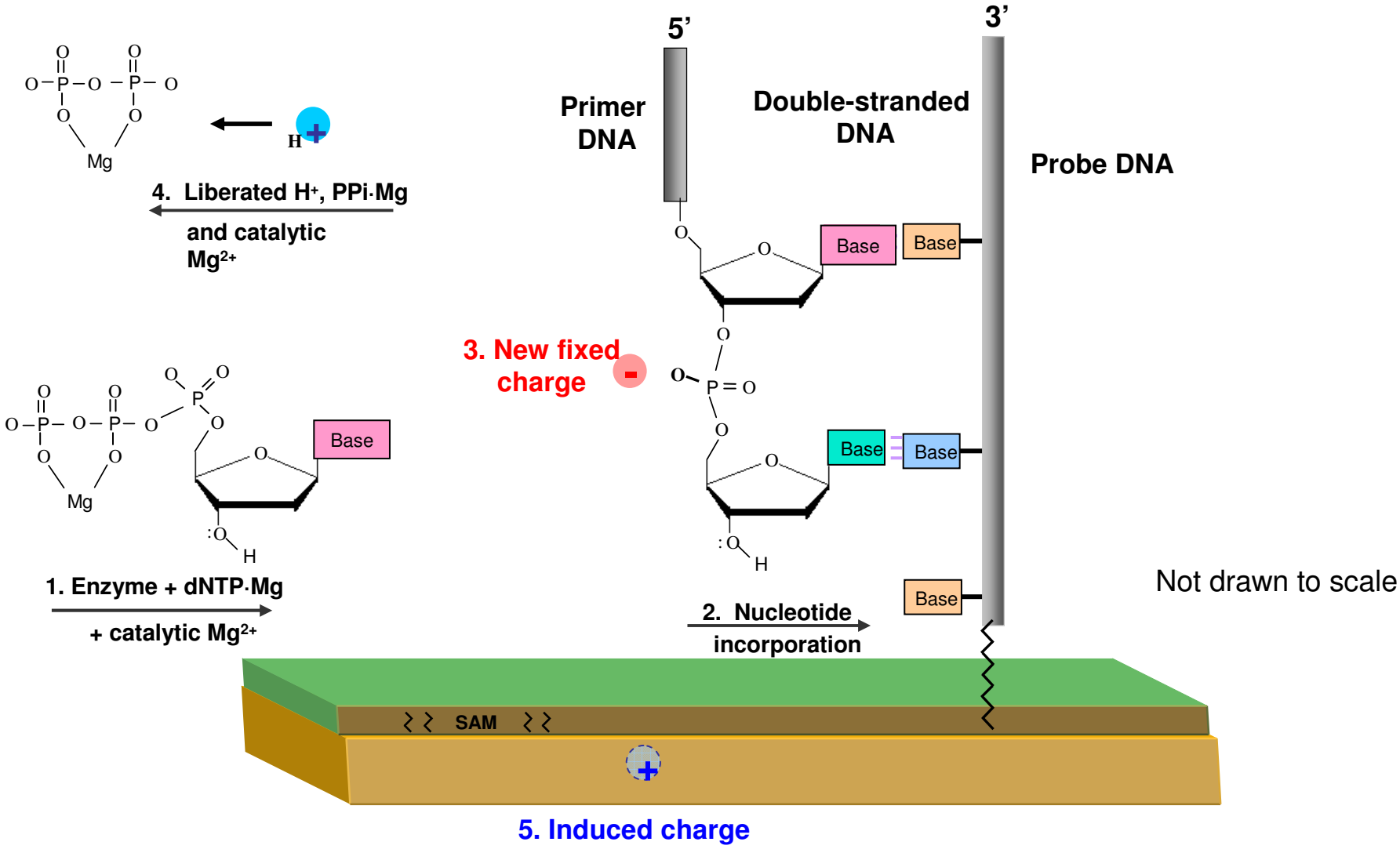
Polymerase works at double-strand / single-strand junctions

Principle of Detection



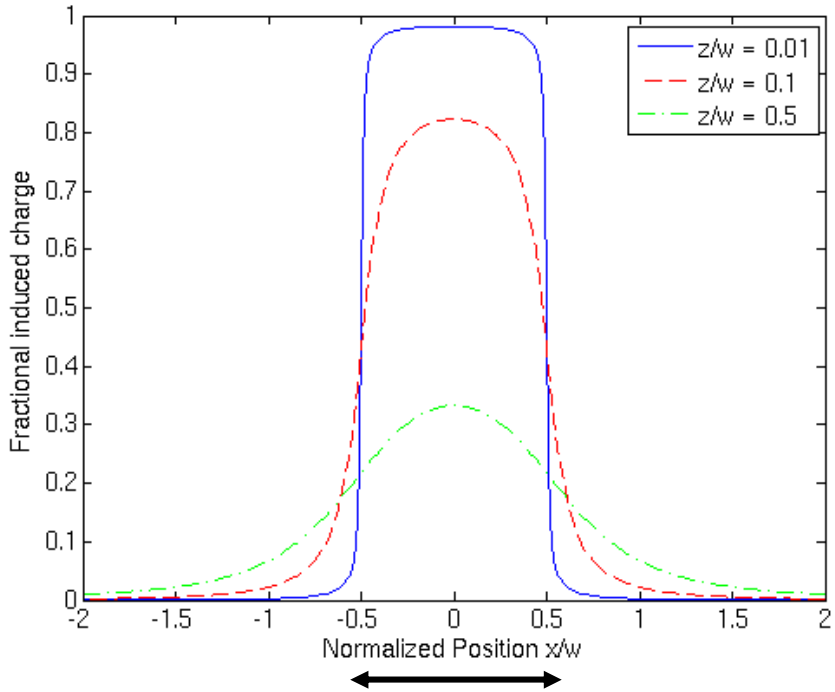
- System detects a NON-equilibrium charge distribution

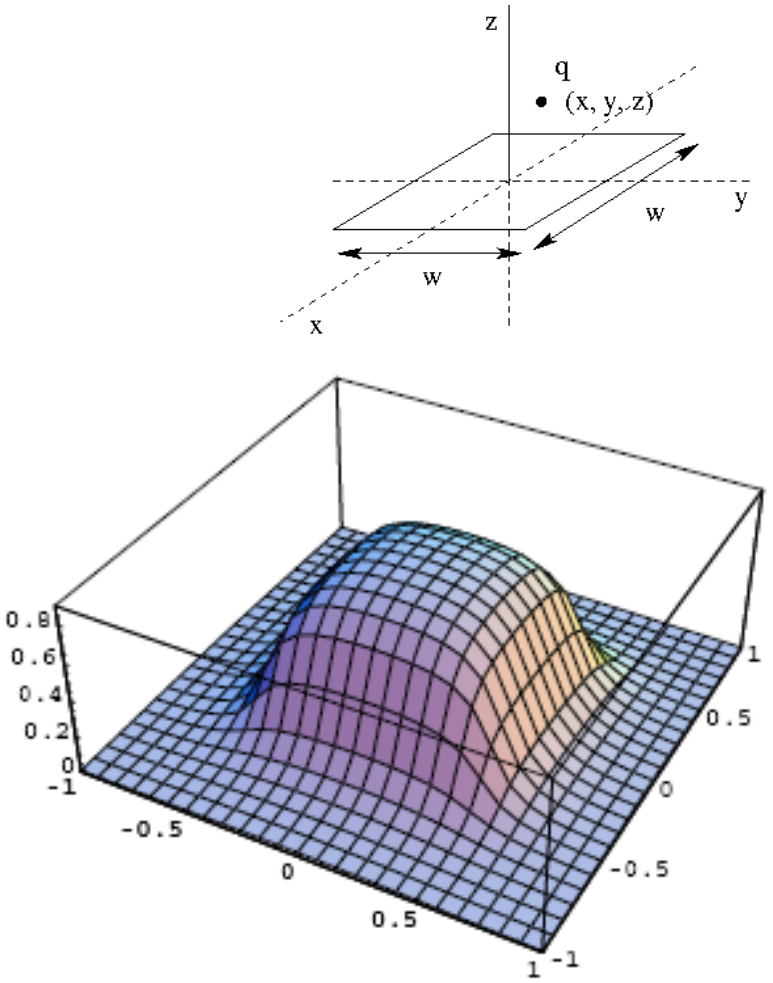
Polymerization Chemical Reaction



Induced Charge

What fraction of a charge is induced on a nearby electrode?





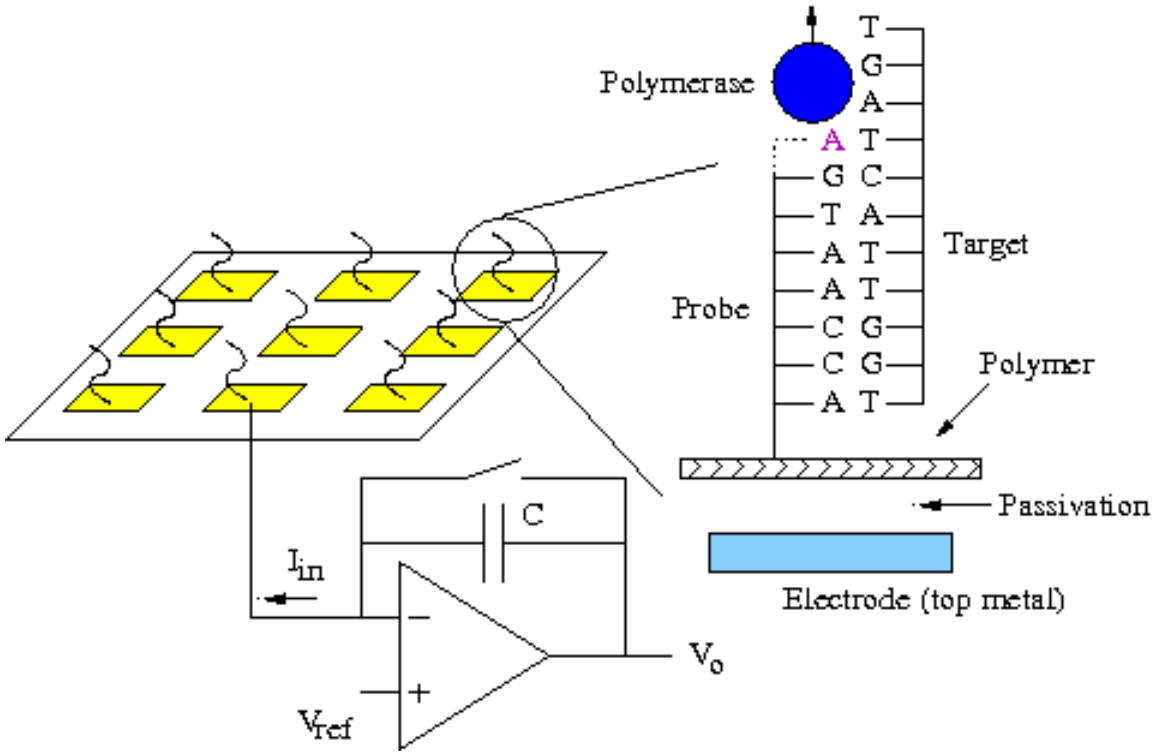
Immobilize DNA close to electrode to maximize induced charge

Charge is 0.1 electrode-widths above electrode

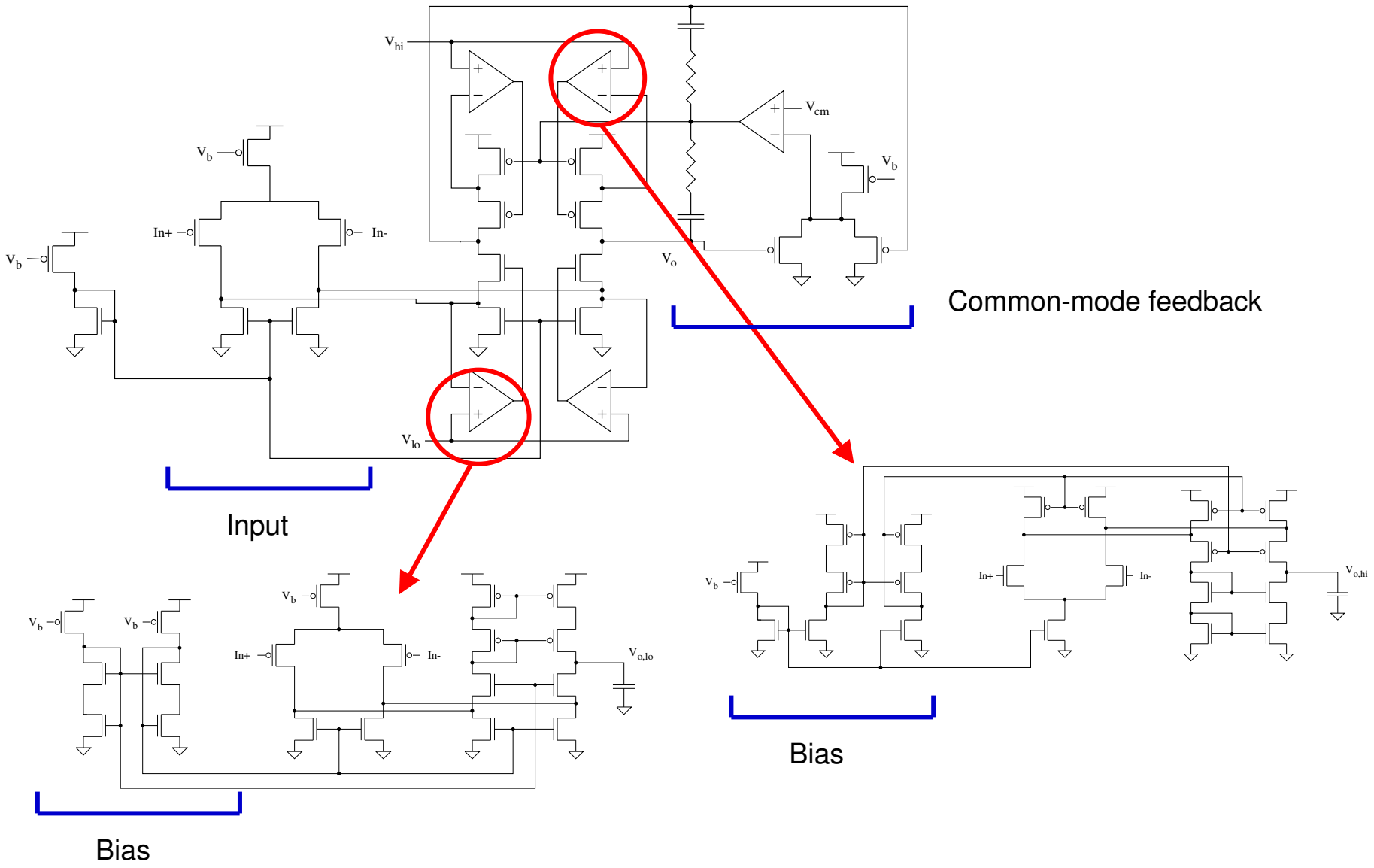
CMOS System Requirements

- Linear, monotonic signal response
- “Low power” (back-of-envelope estimate, ≤ 42 mW)
  - Die surface temperature should not rise more than 1 ℃ above ambient over 5 minutes
- “Low noise”
  - Amplifier noise ≤ other system noise contributions
- Electrode area large enough for spotting DNA onto electrodes (≥ 100 – 200 square μm)
- Easy post-processing
- ±1 V swing at output (use thick gate-oxide devices)

CMOS Architecture



OTA

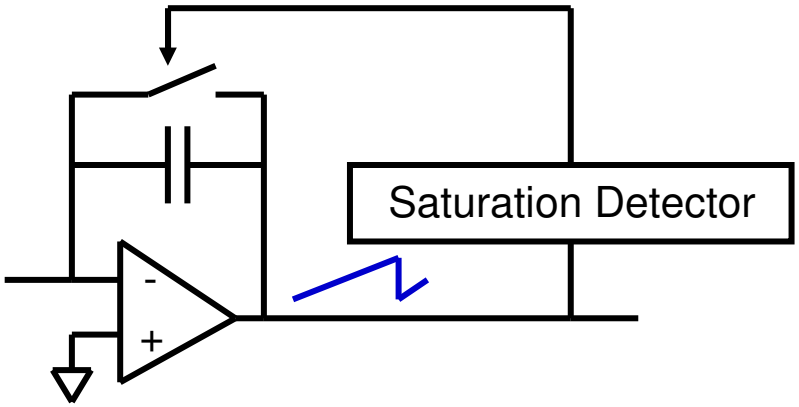

Vhi
Vcm
Vb
Vo
In+
In-
Vlo
Common-mode feedback
Input
Vo,lo
Vo,hi
Bias
Bias

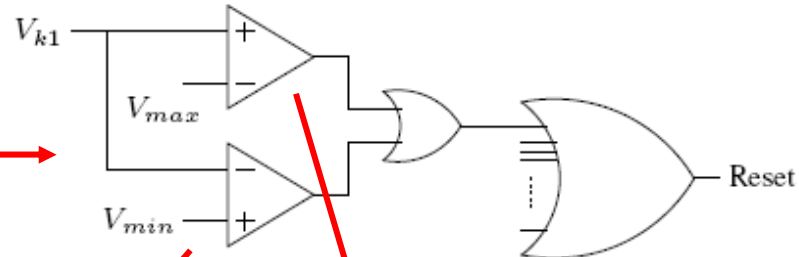
OTA Specs

Technology	0.18 µm CMOS (3.3V devices)
Gain	110 dB
Gain(Vo = 1V)	63 dB
Gain(Vo = -1V)	82 dB
Phase Margin	75°
CMRR	110 dB
PSRR+	70 dB
PSRR-	110 dB
Unity Gain	250 kHz
Power per pixel	1.7 mW

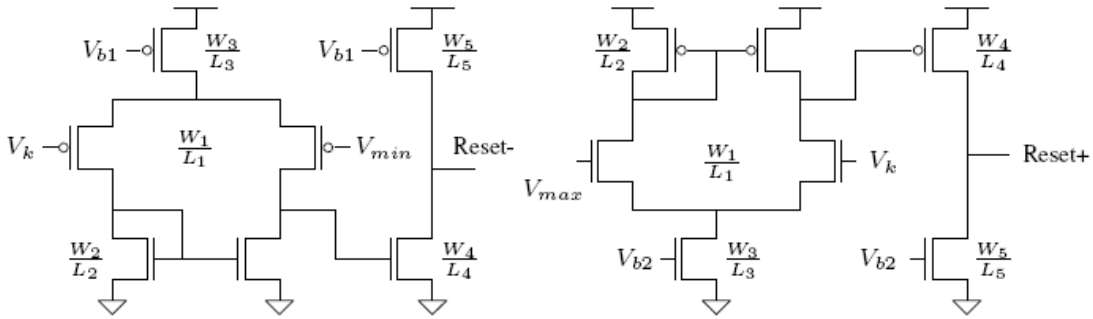
Simulated for typical corner at 75 ℃

Reset Logic

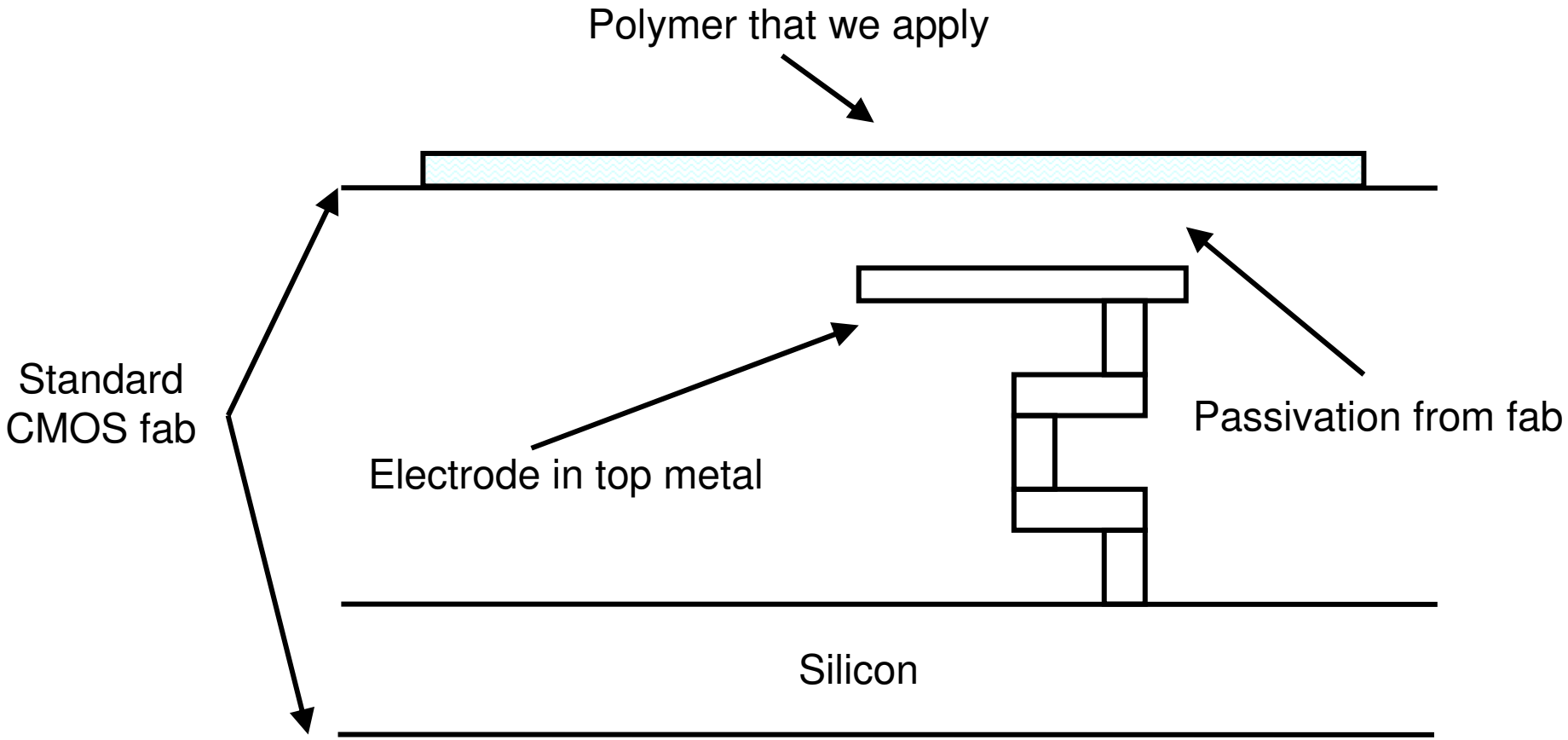




Used to extend dynamic range

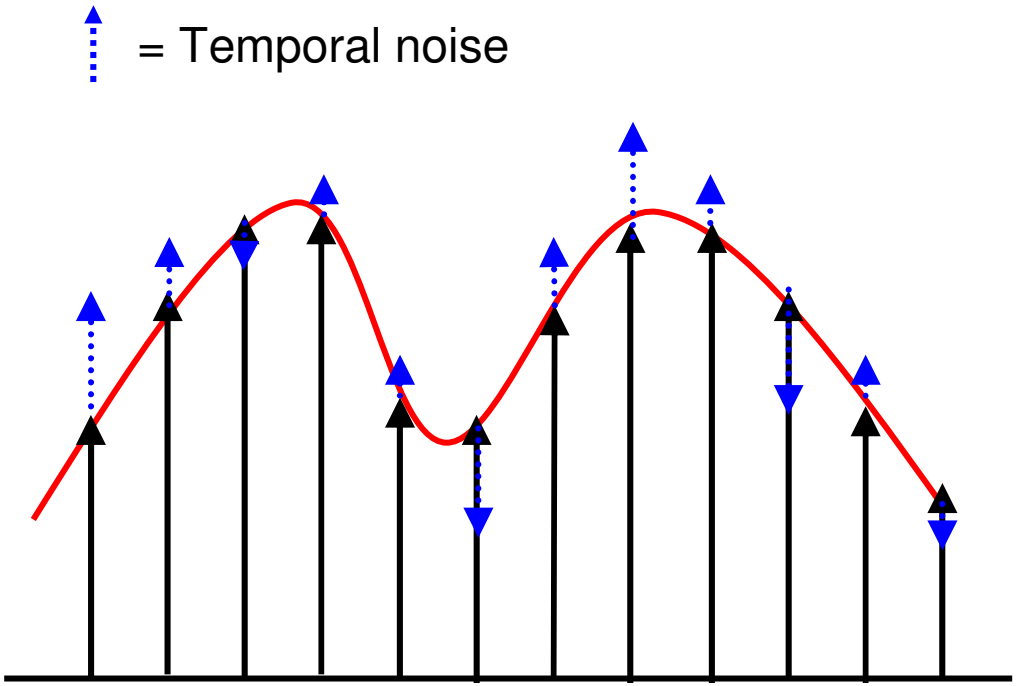


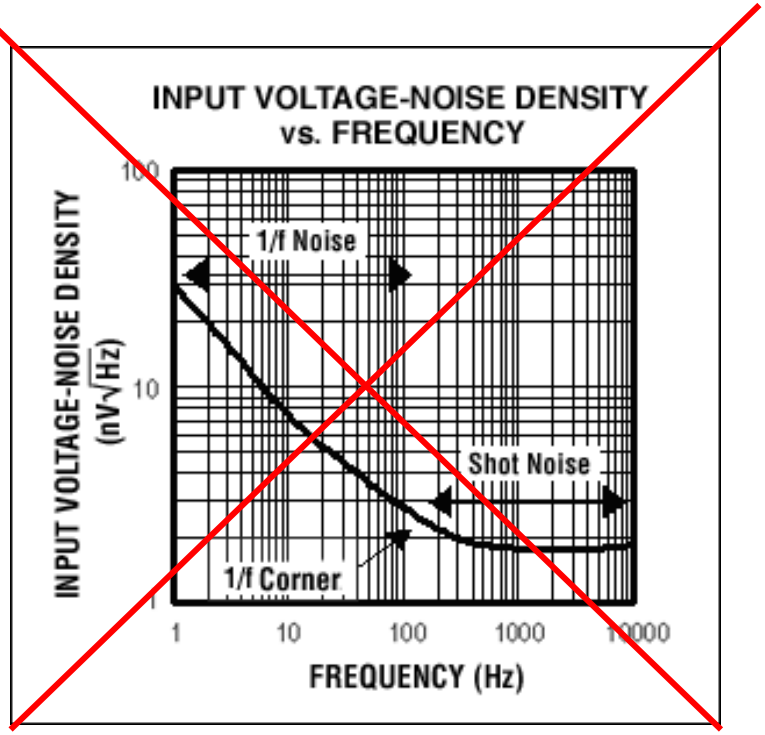
Easy Post-Processing



Temporal Noise

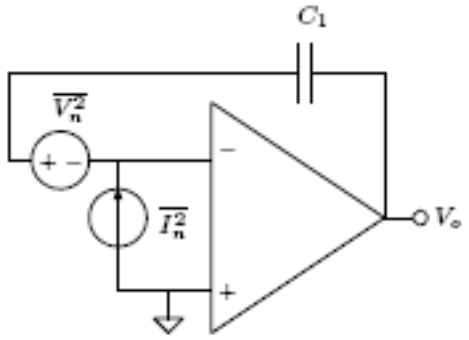
- Noise spectral density is not the right analysis
- Signal is observed in time → want time domain noise
- Temporal noise = variance of noise at a particular instant in time



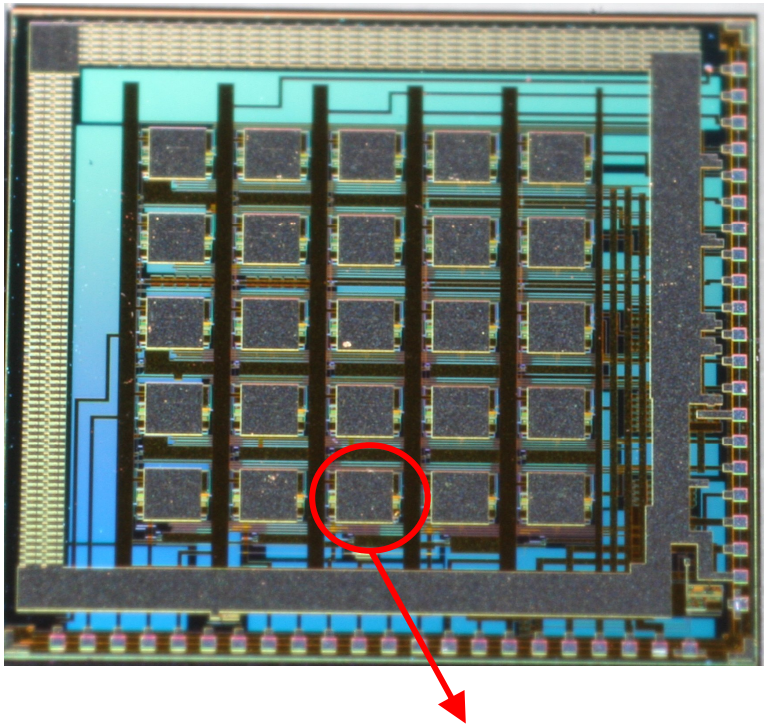


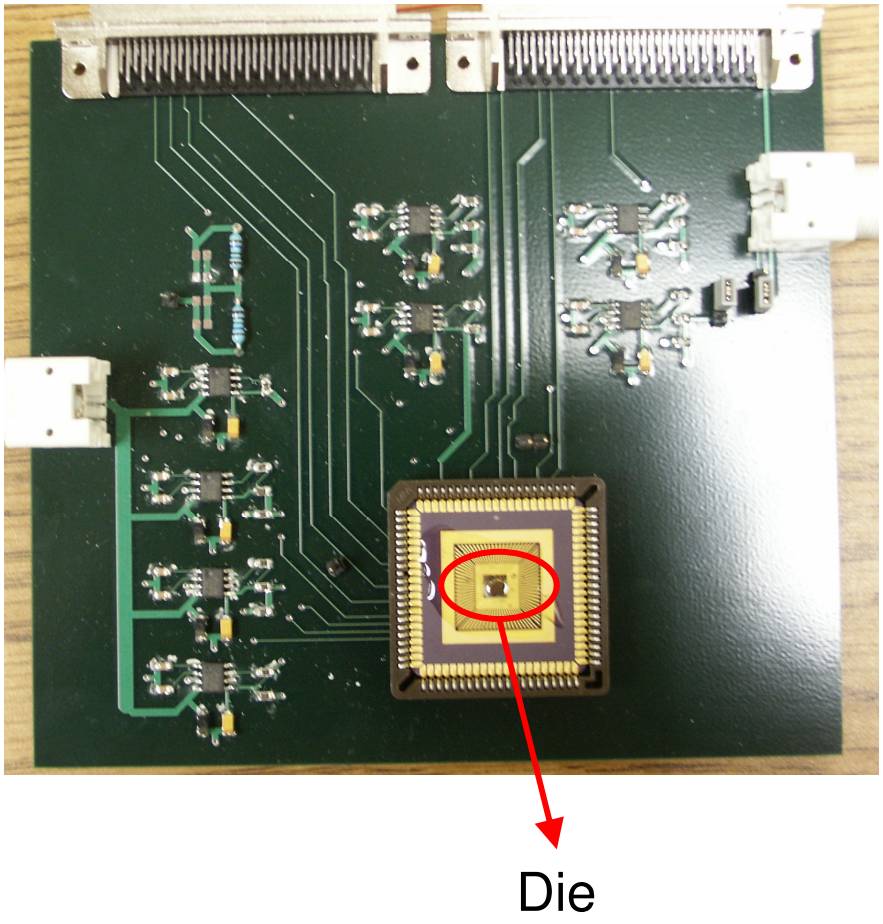
Electronic Noise Contributions

	Equation	Value @1 sec	Comments
Flicker	$\sqrt{2K_f \ln(\frac{\omega_{max}}{\omega_{min}})}$	244 μV	$K_f = 2.4\times10^{-10}\,V^2 \quad f_{max} = 250\,kHz \quad f_{min} = 1\,Hz$
Thermal Voltage	$\sqrt{\frac{A_o \omega_o \overline{V_n^2}}{2}}$	19.5 μV	$GBW = 250\,kHz \quad \overline{V_n^2} = 22\,\frac{nV}{\sqrt{Hz}}$
Thermal Current	$\sqrt{\frac{\overline{I_n^2} t_{int}}{C_1^2}}$	10.5 μV	$C_1 = 30\,pF \quad \overline{I_n^2} = 1\,\frac{fA}{\sqrt{Hz}}$
Cap. Reset	$\sqrt{\frac{kT}{C_1}}$	11.7 μV	$T = 300\,K \quad C_1 = 30\,pF$
Shot	$\sqrt{\frac{2qI_{avg}t_{int}}{C_1^2}}$	6.0 μV	$I_{avg} = 1\,pA \quad C_1 = 30\,pF$



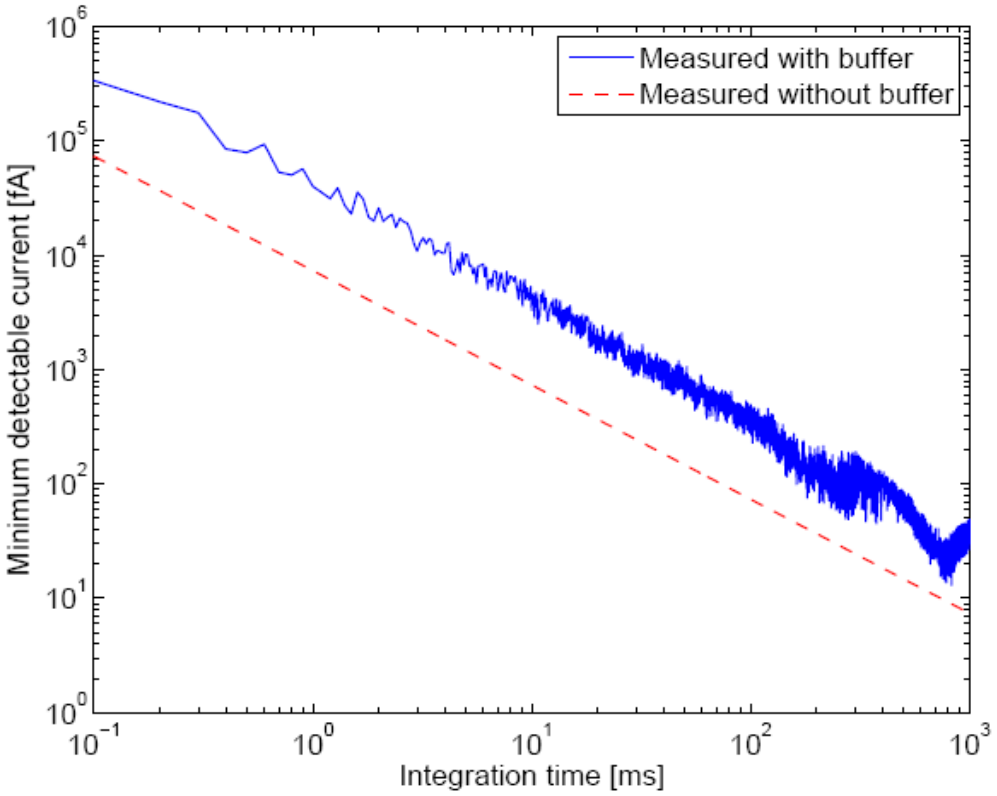
Die Photo + Test Board





Bondwires encapsulated in epoxy

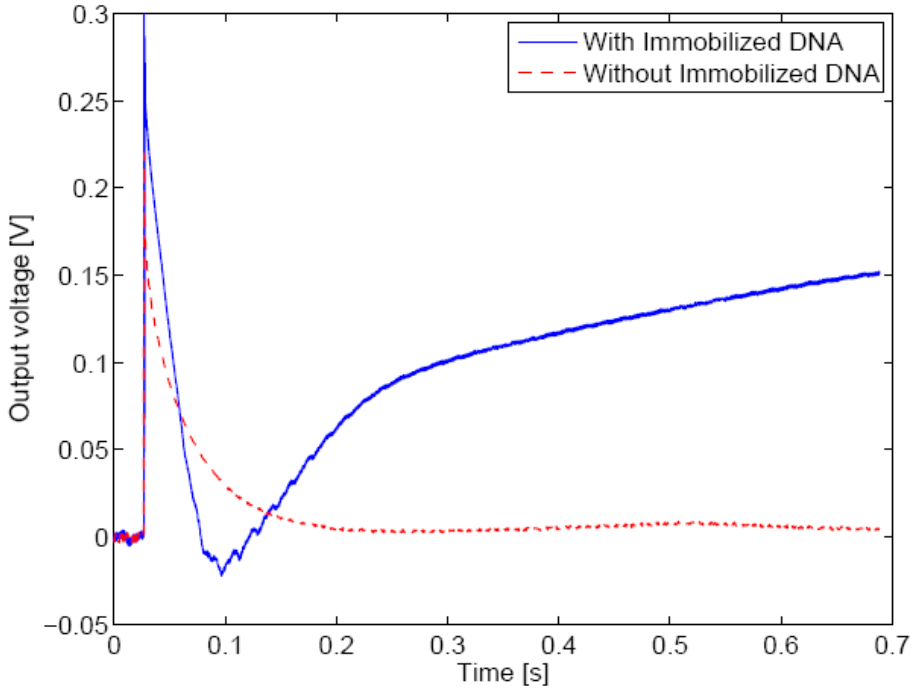
Minimum Detectable Current



- Enzymatic buffer noise is constant w.r.t. integration time ~830 μV RMS
- Limit of detection with buffer is 25 fC
  - Corresponds to biological limit of detection of 8 ng/mL (worst case)
- Crosstalk dominated by system noise → not measurable

Noise from enzymatic buffer dominates electronic noise

Measured Signal from CMOS Chip



Probe: GTG CCA AGT ACA TAT GAC CCT ACT

CAC GGT TCA TGT ATA CTG GGA TGA CCA TAC CTG TAC GAC TCG AGT GAC GAG ACG GCG TA

Exposed segment

- Target concentration 10 µg/mL

Conclusions

- Designed first CMOS DNA polymerization sensor
  - Targeted to low-cost, point-of-care applications
  - Demonstrated sensor could detect useful concentrations

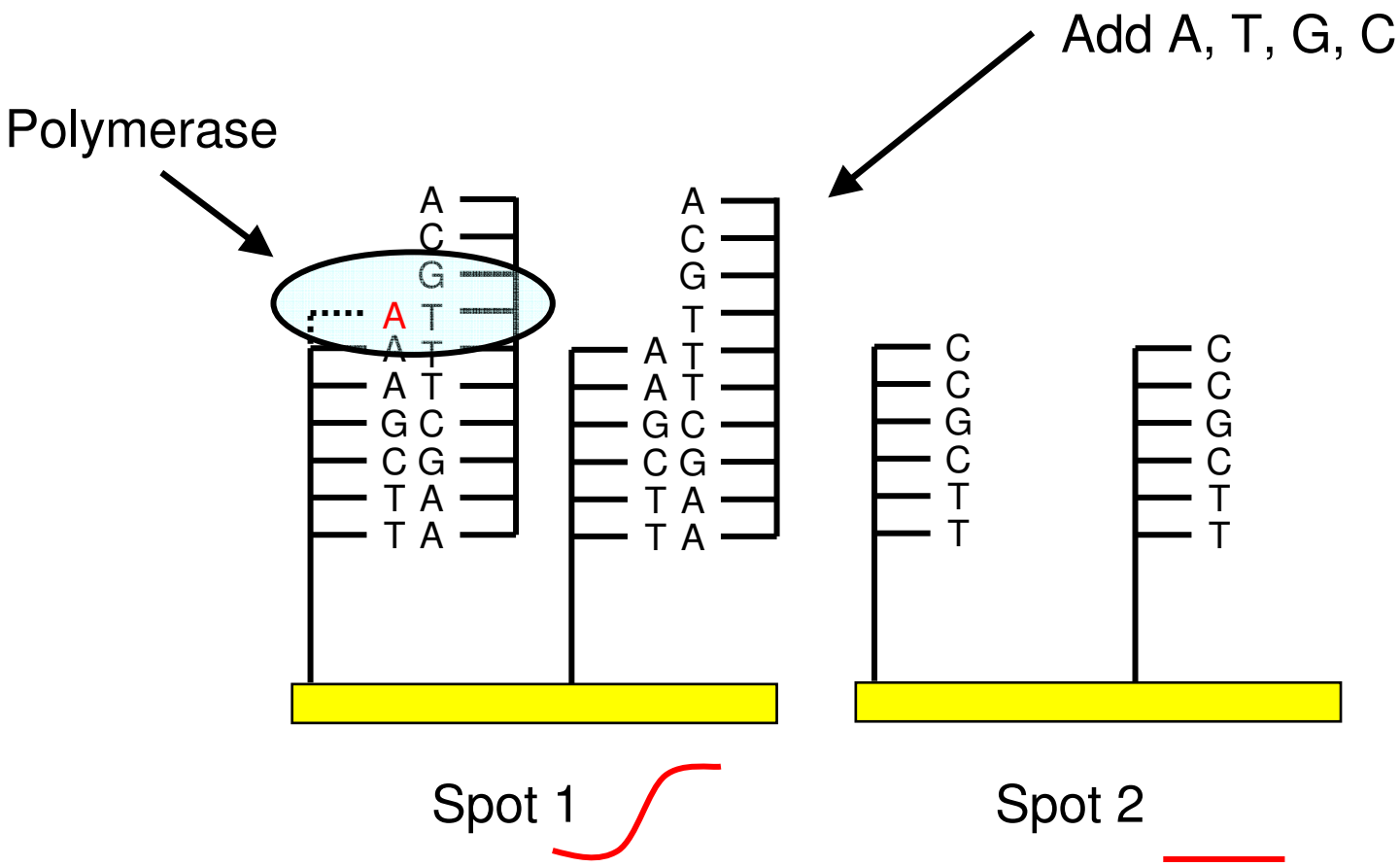
Supplemental

- Following slides are supplemental

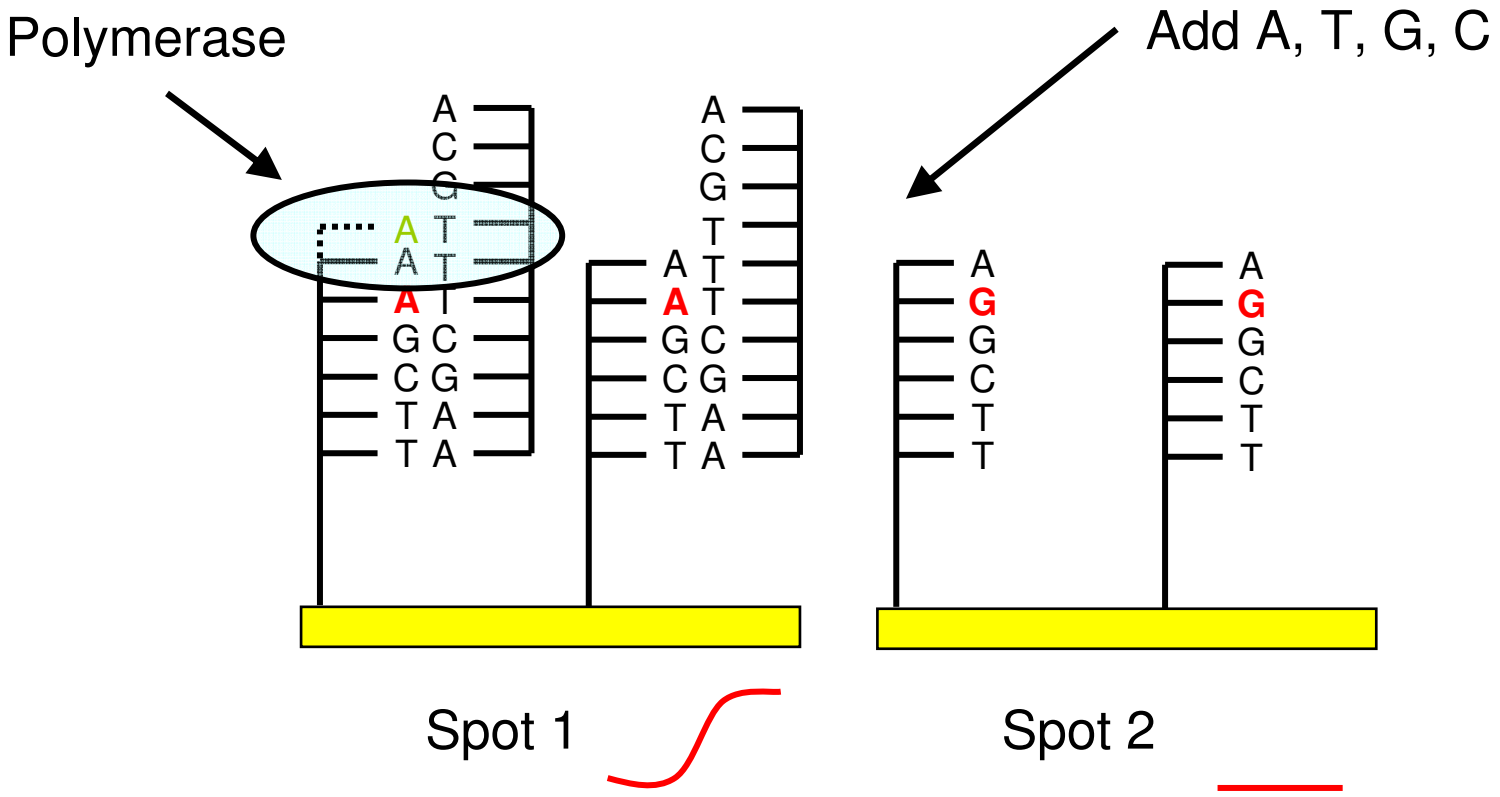
Future Applications

- Clinical, point-of-care diagnostics
- Personalized medicine
  - Enabled by low cost fab techniques
- Pathogen detection
- Short segment DNA sequencing
  - Sequentially add nucleotides and observe the signal
- Simple Nucleotide Polymorphism (SNP) Detection
  - SNP = an alteration in a few nucleotides, e.g. AAAA vs. ATAA
  - SNPs form 99.77% of all genetic variation

Pathogen Detection

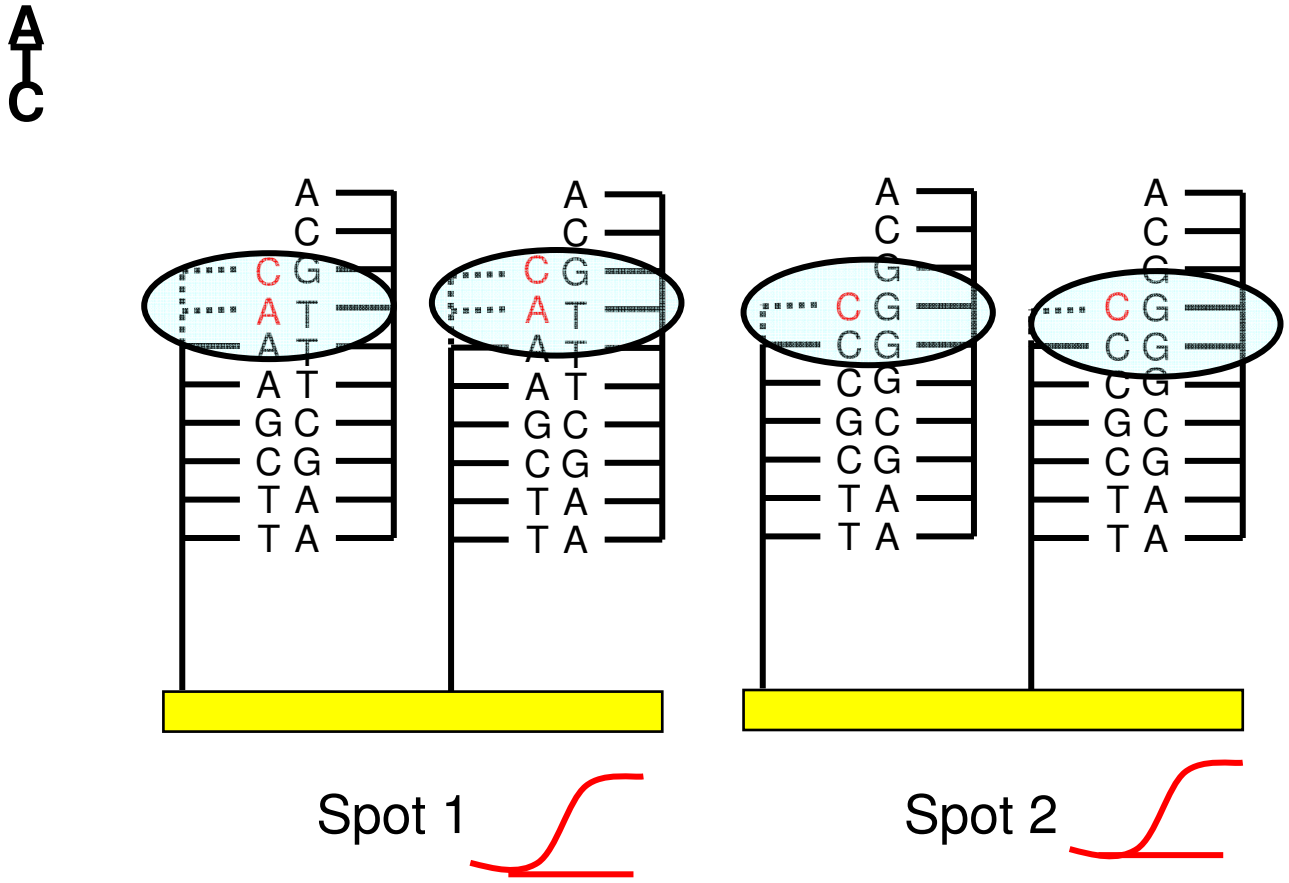


SNP Detection



- Trick is to make the mutation part of the target
- Add nucleotides and read out a signal at spots where SNP is present

Short Segment Sequencing



- Sequentially add bases
- Wash away unused bases between additions

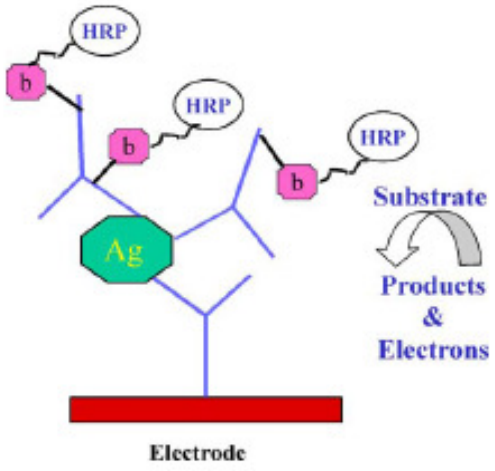
Thermal Issues

- Does the power dissipation adversely affect operation?
- Must keep surface heating low enough so that…
  - chemical reaction can still occur
  - buffer does not evaporate
- DNA denaturing not a problem, i.e. double strand → 2 single strands
  - Denaturing occurs at high temperatures, ~ 55 ℃ or higher
- Enzyme "activity" is affected by temperature
  - "activity" = rate of enzyme performing its function
- 43 mW → 0.5 ℃ change measured over 5 minutes, buffer still present

Power dissipation not a problem
→ no change necessary

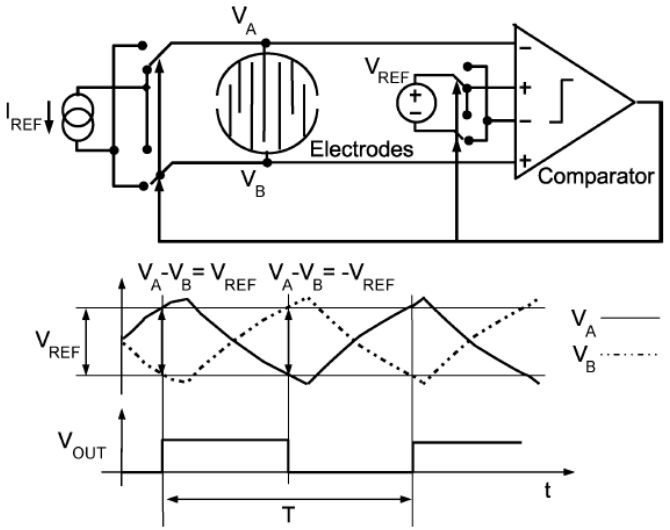
CMOS heat source

Some Electronic Microarrays



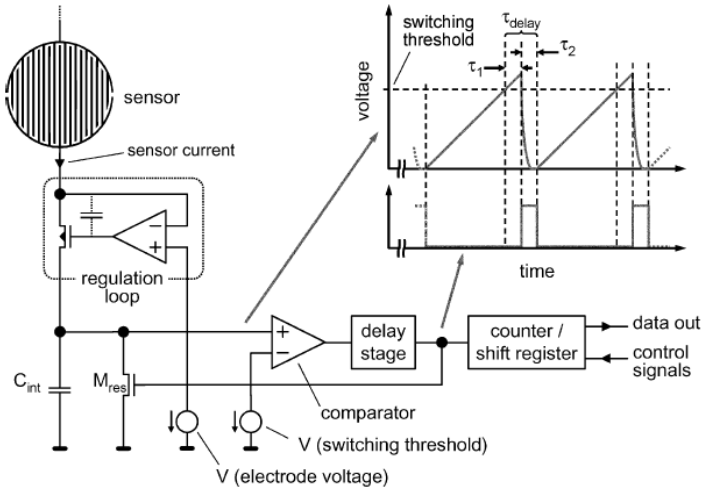
An Electroactive enzyme (HRP) generates a current flow into the electrode.

Dill, Biosensor & Bioelectronics, 2004.



Capacitance measurement. Stagni, JSSC 2006.

- Electronic approaches integrate the microarray chip with the “reader”

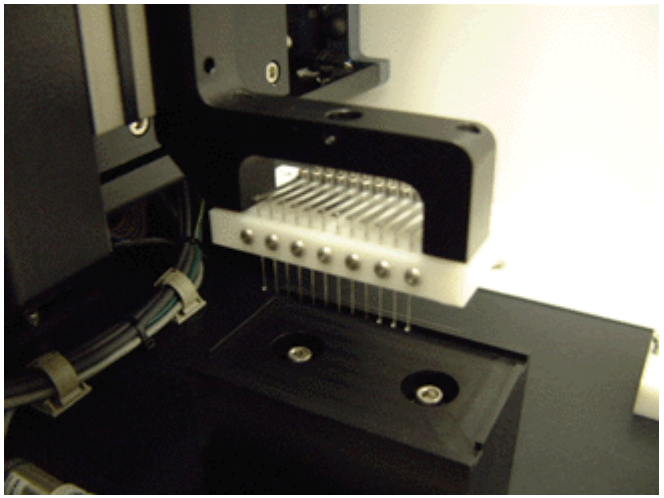


Redox cycling generates current. Schienle, JSSC 2004

Immobilizing DNA

2 Ways

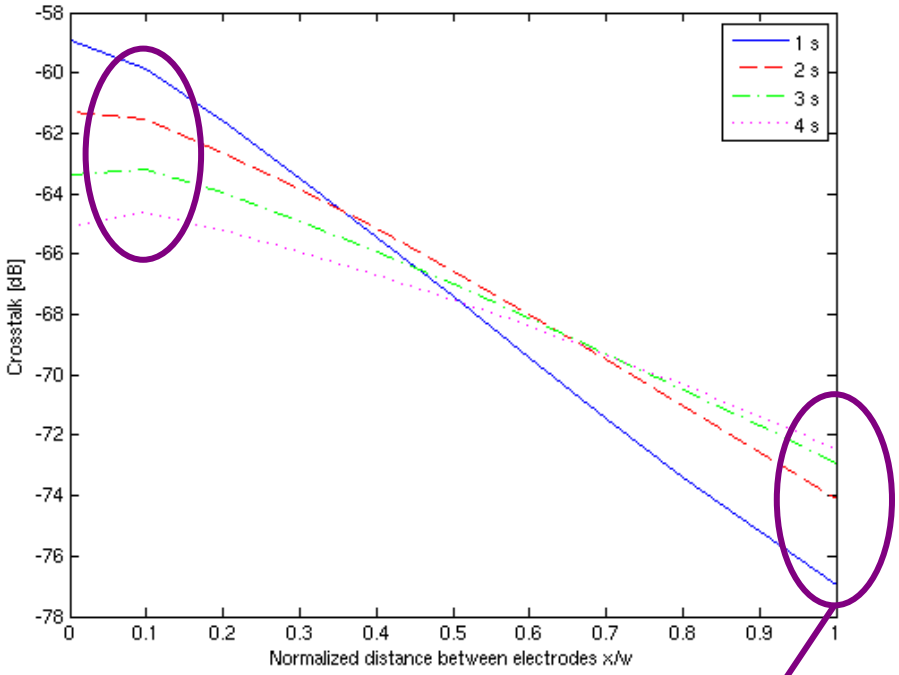
1. Build up ssDNA nucleotide-by-nucleotide using photolithography and chemistry
   - Requires ~4n masks, n = sequence length (25-mer → 100 masks)
2. "Spot" DNA onto location by depositing a droplet of liquid

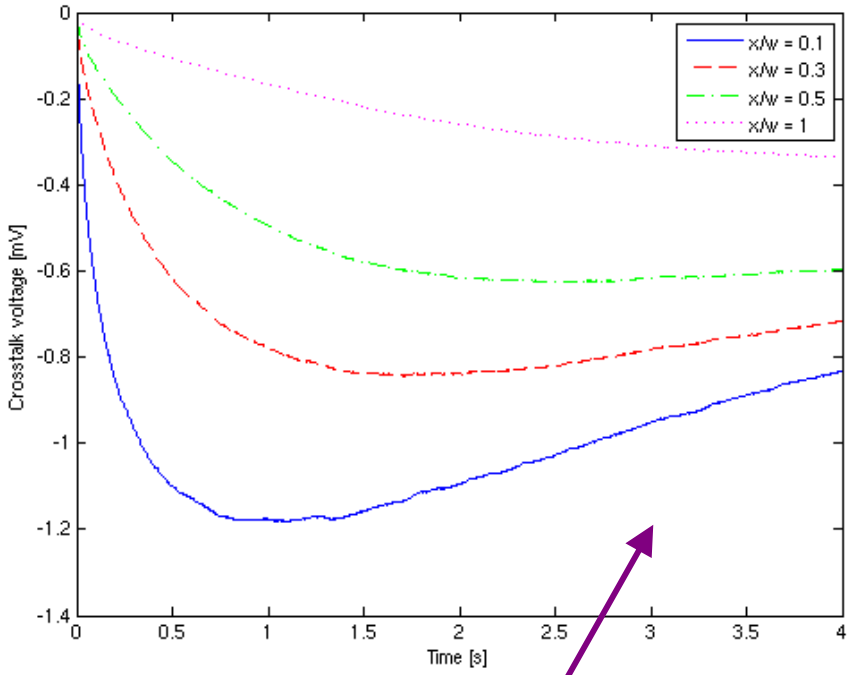


Surface Chemistry

1. Wash surface with acetone and iso-propanol for 1 minute each
2. 3 minute exposure to UV-ozone
3. Surface immersed in 5% (w/w) (chloromethyl)phenylethyltrimethoxysilane in ethanol solution with gentle shaking for 12 hours
4. Rinsed with ethanol 3 times and dried in air
5. 100 μM solution of probe oligonucleotides in phosphate buffer saline at pH 7.4 (0.01M sodium phosphate, 1.0 M NaCl) was manually spotted onto the microchips and kept in a humidifier overnight, immobilizing the probes above the electrodes
6. Unattached probes washed away in DI water
7. Chips blocked with 50 mM ethanolamine solution for 2 hours at room temperature

Diffusion-based Crosstalk





- Why does crosstalk increase with time at larger separations when it decreases with smaller separations?
- Look at the induced voltage. Smaller separations are affected by a reflecting boundary.

On Pulse Shapes

- Possible causes of variation in height and width
  - DNA crowding
  - Spots not identical
  - dNTPs diffuse to each spot – varying distance
  - Distribution of polymerase

Quantization Noise

$$\text{Noise Power} = \frac{\Delta^2}{12}$$

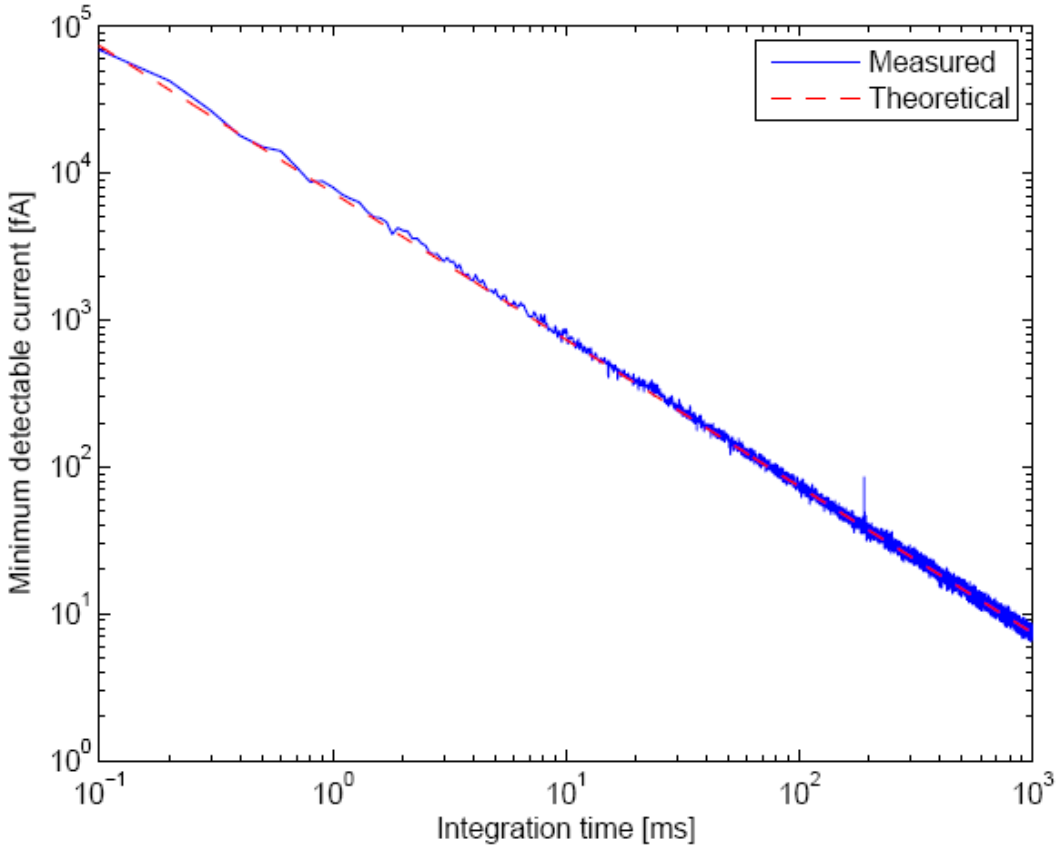
For a 5V range

- 12 bits → 352 μV
- 16 bits → 22 μV
- 24 bits → 86 nV

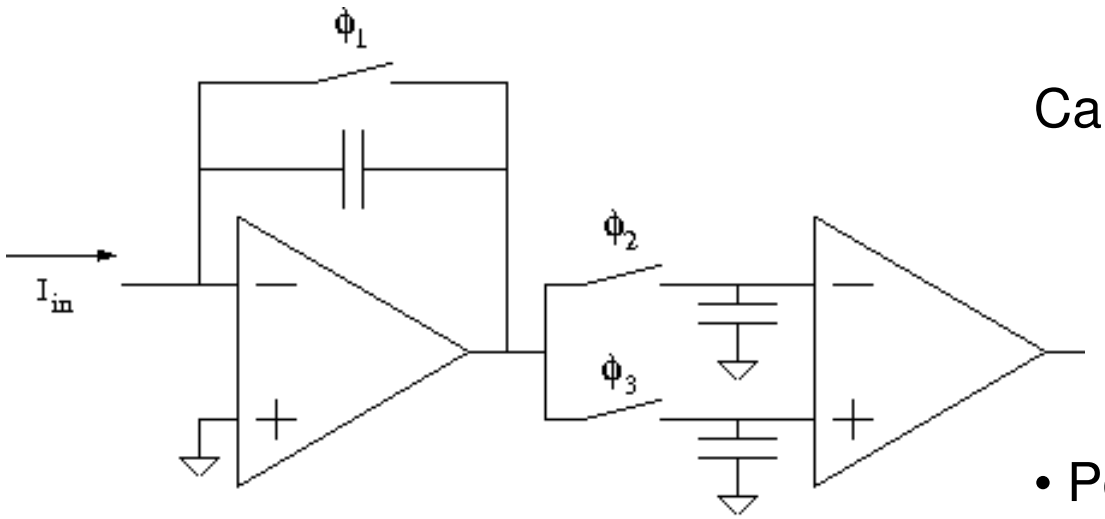
For a 3.3V range

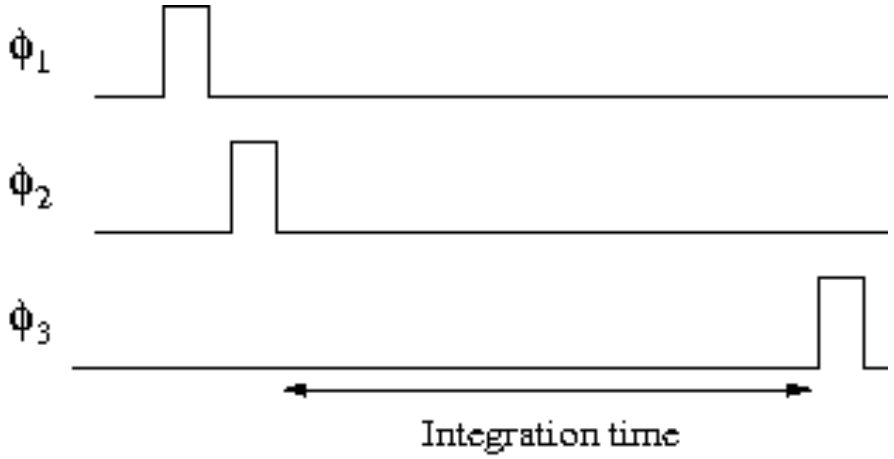
- 12 bits → 232 μV
- 16 bits → 15 μV
- 24 bits → 57 nV

Electronic Noise (Theoretical vs. Measured)



Correlated Double Sampling

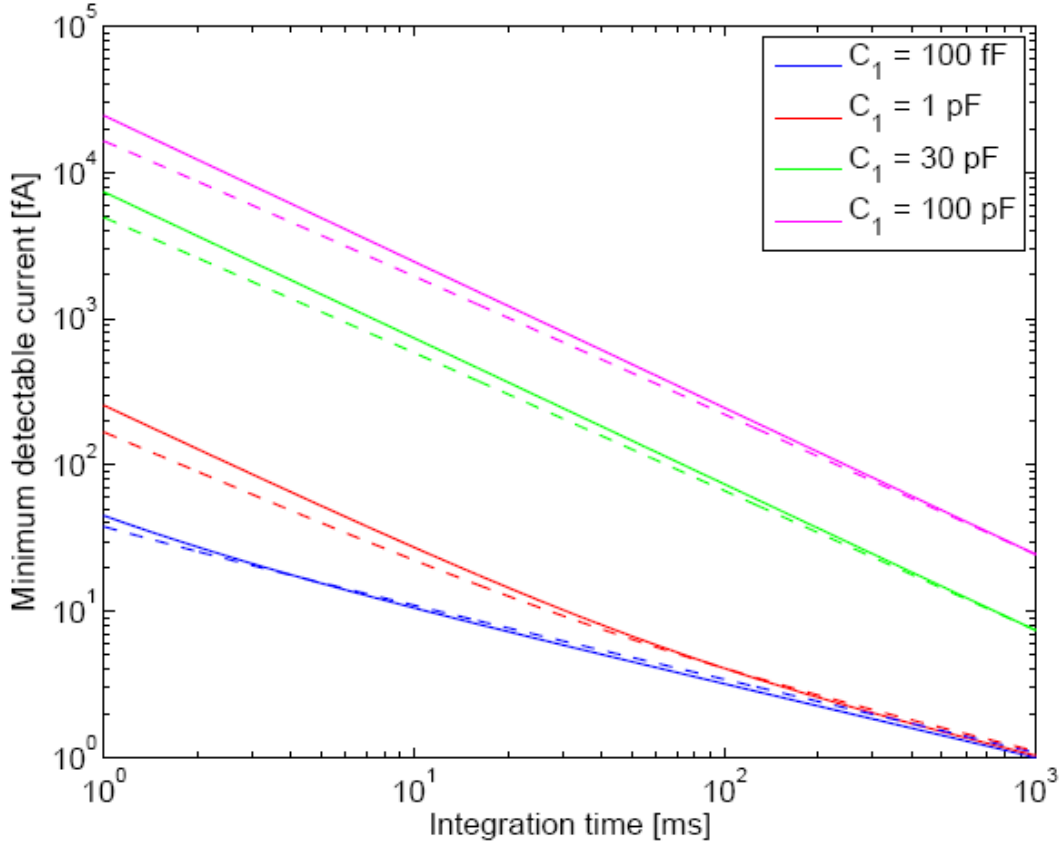




Can CDS reduce noise?

- Popular technique in image sensor read out circuits
- Sample integrator output at beginning and end of integration period and subtract
- Cancels thermal reset noise of integration capacitor and partially cancels 1/f noise

Correlated Double Sampling?



Dotted = with CDS

Solid = without CDS

- Correlated double sampling does not have much of an affect above 100 ms.
- Assumes no noise added by CDS

Number of Probes

- $10^{11} – 10^{12}$ probes / cm^2
- 90 – 900 Million probes in 300 μm square area
- Probes occupy between 0.4 – 4% of surface area

Detection Limit & Noise

- Q_n/q “noise” charges at electrode
- Each “real” charge induces f “noise” charges → $Q_n/(fq)$ “real” charges
- Assume 1 signal charge per probe (could get multiple)
- For P probes on the electrode → $\theta = Q_n/(fqP)$ is the required fraction of “bound” probes
- From Langmuir-Isotherm theory, $\theta = [target]_{bulk}/([target]_{bulk} + K_d)$ where K_d = ratio of forward and reverse rate constants
- $[target]_{bulk} = K_d\, Q_n /(fqP)$
- P = 90 – 900 million, q = 1.6e-19, Q_n = 25 fC, K_d = 10pM-10nM (depends on many factors, e.g. probe length, target length)
- $[target]_{bulk}$= 8 ng/mL (K_d = 10nM, P = 90 million, f = 0.5) worst case
- $[target]_{bulk}$= 40 fg/mL (K_d = 10pM, P = 900 million, f = 1) best case

Comparison with other Work

- Look at other CMOS DNA chips
- [1] Detected 31 µg/mL at SNR=3*
- [2] not reported
- [3] Detected ?? at SNR=1.5*
- This work 10 µg/mL at SNR=180*

[1] Stagni et al., IEEE Sensors, 2007.

[2] Schienle et al., JSSC 2006

[3] Stagni et al., JSSC 2006

* = label-free

Diffusion Coefficients

- H^+ 9000 $\mu m^2/s$
- K^+,Cl^- 2000 $\mu m^2/s$
- Mg^{2+} 1400 $\mu m^2/s$

- Source: Kovacs Micromachined Transducers, CRC Table