



PACBIO®

SMRT Sequencing: Opportunities & Challenges for Integrated Photonics

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2022 EPIC World Photonics Technology Summit

1

Founded in 2004



ENABLING THE PROMISE OF GENOMICS TO BETTER HUMAN HEALTH

We create some of the world's most advanced sequencing technologies.

2

Design, develop and manufacture
Instruments and Consumables for
DNA sequencing



3

~ 700 employees,
Preliminary annual 2021 revenue ~\$130M



DNA - Cell's Instruction Set



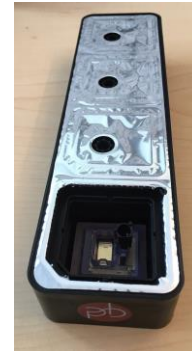
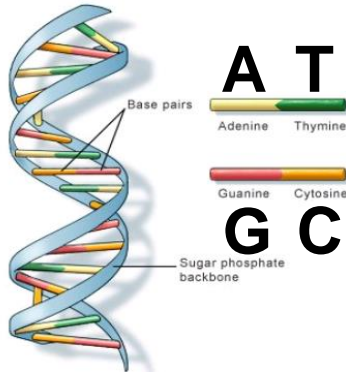
DNA is a long paired-polymer chain within each living cell that stores its “genetic information”

4 types of monomers: **A, T, C, G**

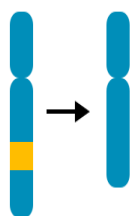
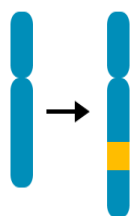
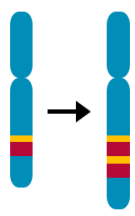
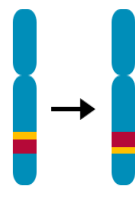
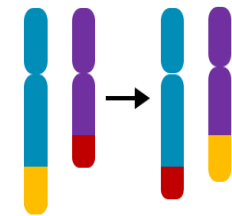



100-1000 monomers

→ DNA segment (gene)

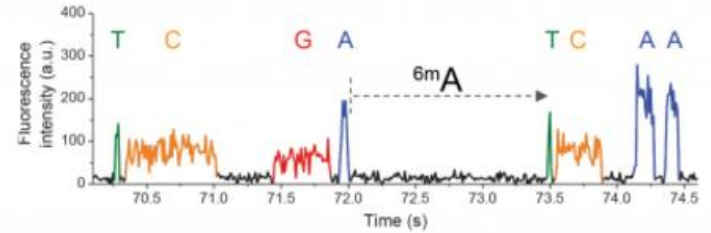
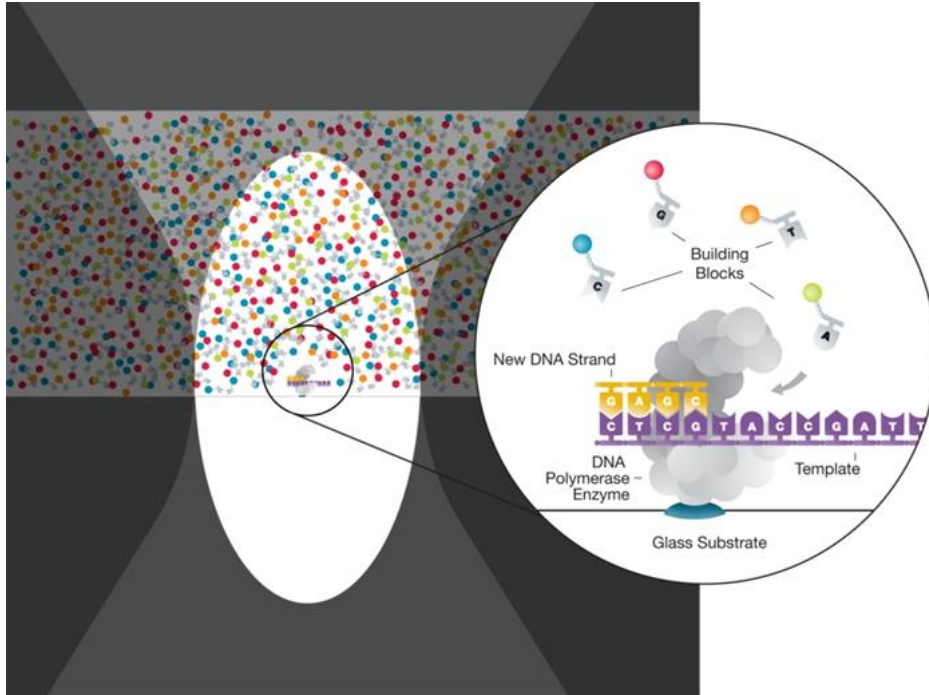
→ (Instructions for) 1 protein



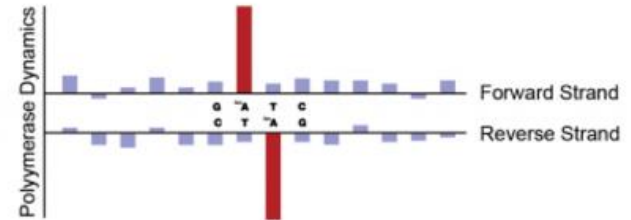
Assays to Detect Structural Variants

	deletion	insertion	duplication	inversion	translocation
					
					
			Cytogenetics	Microarray	Sequencing
technologies			karyotype, FISH	oligo aCGH, SNP aCGH	short read, PacBio long read
resolution			>5 Mb, >100 kb	>50 kb	basepair
limitation			marker density, microscope resolution	probe density	read length (mappability and spanning), systematic bias

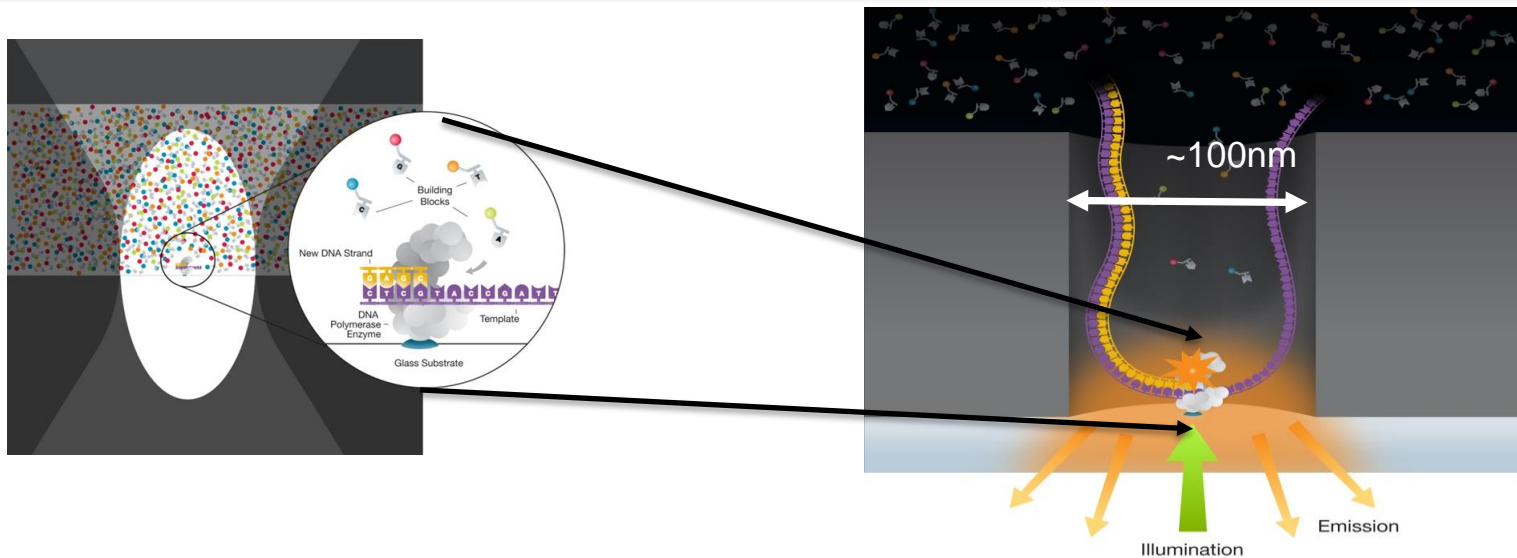
PacBio Long-Read Sequencing: Single Molecule Real-Time(SMRT) Sequencing



Analysis of Polymerase Kinetics



The Zero-Mode Waveguide *Enables* Single Molecule Detection



How do we efficiently illuminate and detect
100k-1M+ ZMWs simultaneously?

PacBio SMRT Sequencing Systems

Prototype

RS

Sequel[®]

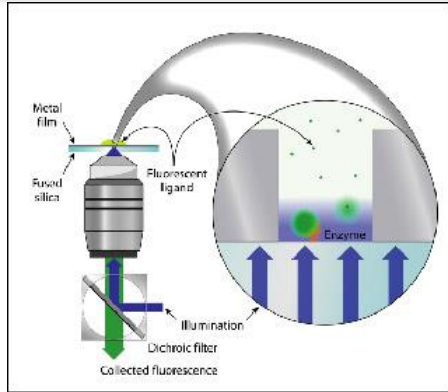


Off-the-shelf hardware
Low wafer-level
integration

Custom bulk optics
Moderate wafer-level
integration

Minimal bulk optics
High wafer-level
integration

Prototype Instrument– ZMW cell with free-space optics



Fabrication Challenges:

Pattern a uniform array of ~100nm Vias

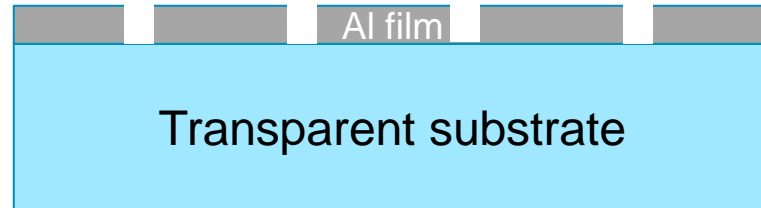
E-Beam Lithography → Metal Lift-off

193nm Photolithography → Metal Etch

Inline Metrology to monitor key parameters

Instrument: modified
inverted high NA microscope

Multiplex limited to ~3,000ZMW



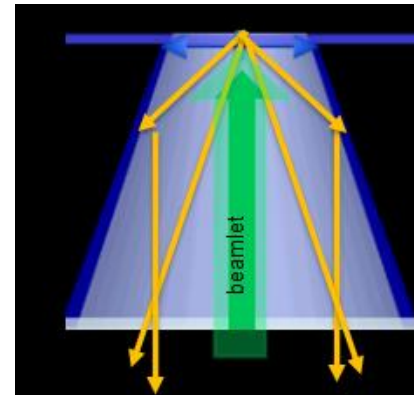
PACBIO RS System

RS Instrument built with custom optics
capable of imaging 150,000 ZMW

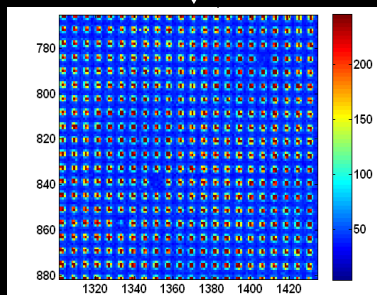
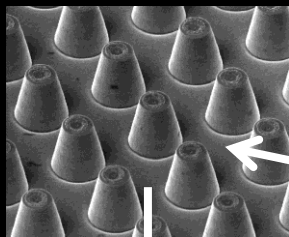
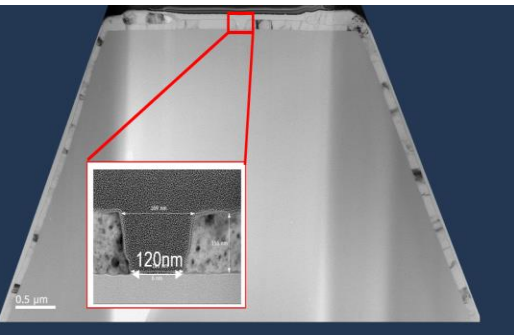
enabled by integrated collimating optics with ZMW



SMRT CELL



Collection Optics Integrated on RS System SMRT Cell



CAMERA IMAGE

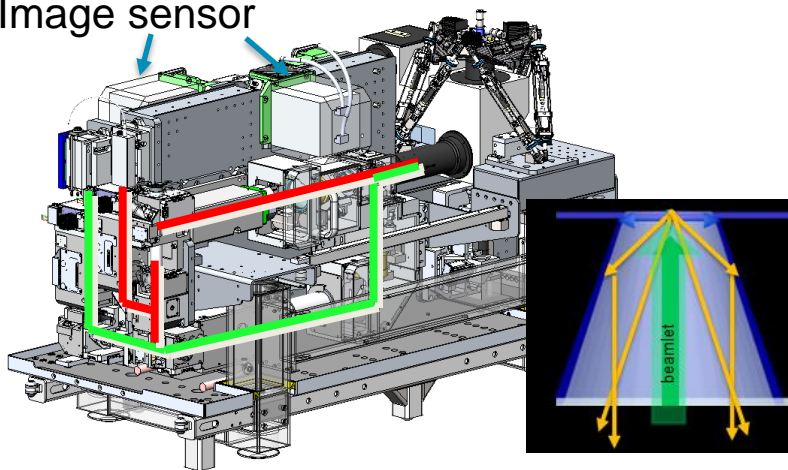


SMRT Cell

PacBio Sequel System: 1 M integrated ZMW



Image sensor

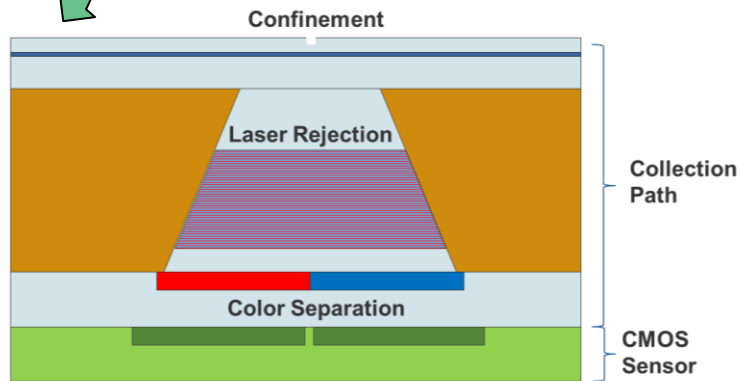
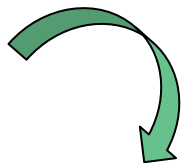


RS optical train

RS ZMW



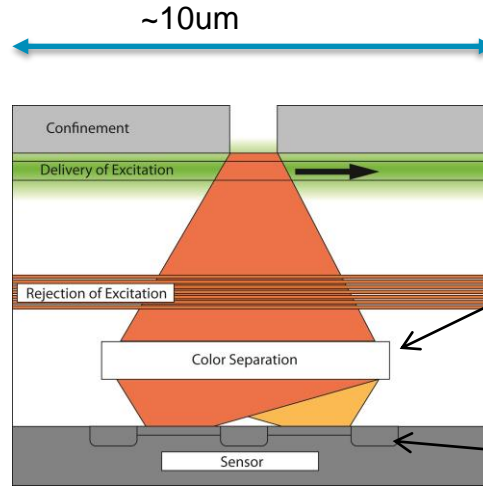
7x increase in ZMW #
6x reduction in instrument size
Reduction in instrument cost



Sequel concept

EPDA Tools: Designing the Integrated Sequel SMRT Cell

Rigorous EM simulator



Si CMOS sensor



Thin film optimization

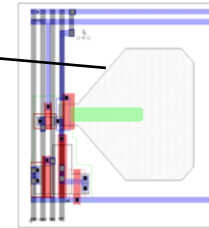
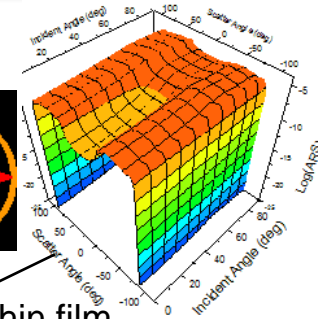
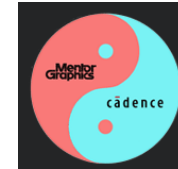


Figure 5. Typical pixel layout

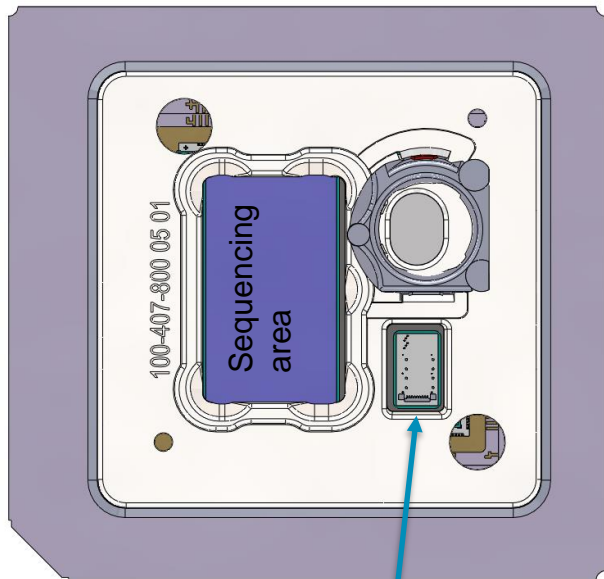
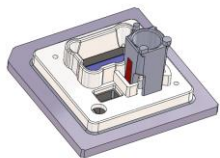
Detector pixel circuit



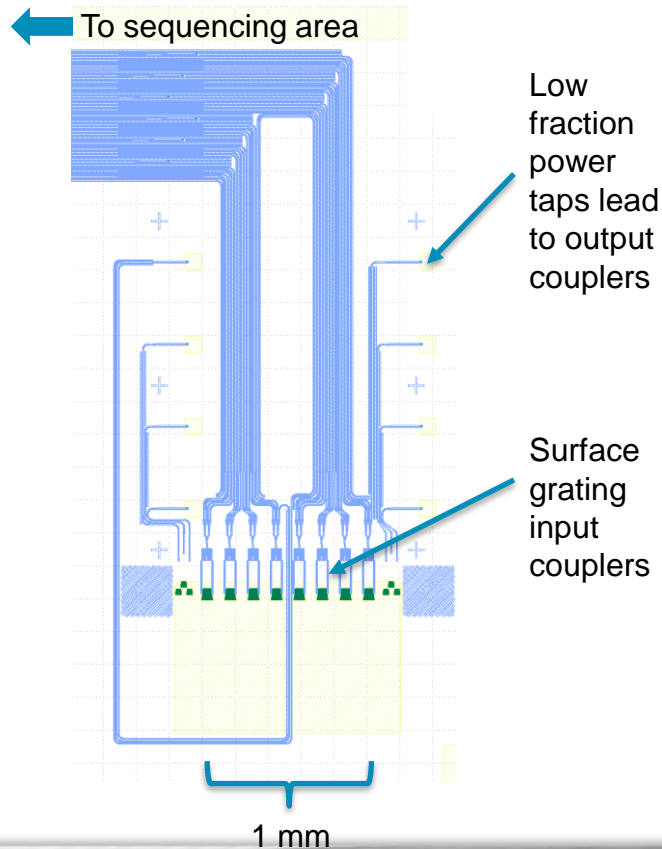
Signal: Dye spectrum → filter requirements → collection optics

Illumination: Scattering → rejection filter → collection optics

Sequel System SMRT Cell



Optics port

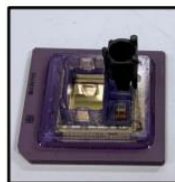


Advantages of Integration

Increased Throughput Capacity With the Sequel II System

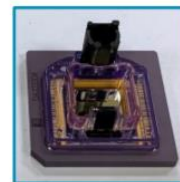


Sequel System



1 million ZMWs
SMRT Cell 1M

Sequel II System



8 million ZMWs
SMRT Cell 8M

- ~8-fold increase in data yield
- Reduced project time
- Lower project cost
- Equivalent performance

Key Challenges: Module development and integration

→ Linking materials, processes, metrology & design

- operate in visible light range
- co-package photonics, electronics, liquids
- nonstandard thermal budget constraints

Opportunity: lowers cost of DNA sequencing and access



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