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Targeted Sequencing for All on Ion GeneStudio™ S5 Sequencers: GPUs Make it happen

Mohit Gupta

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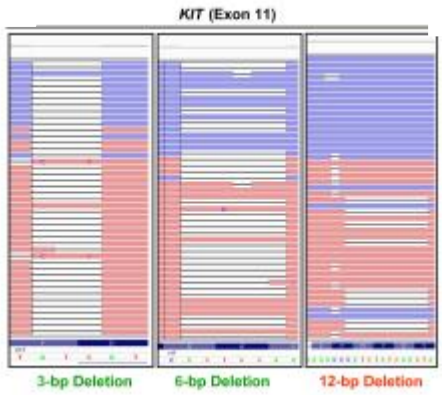
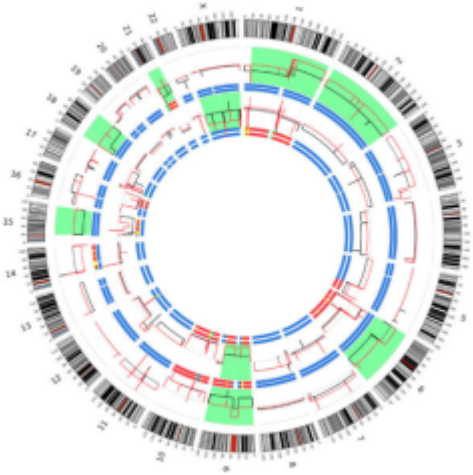
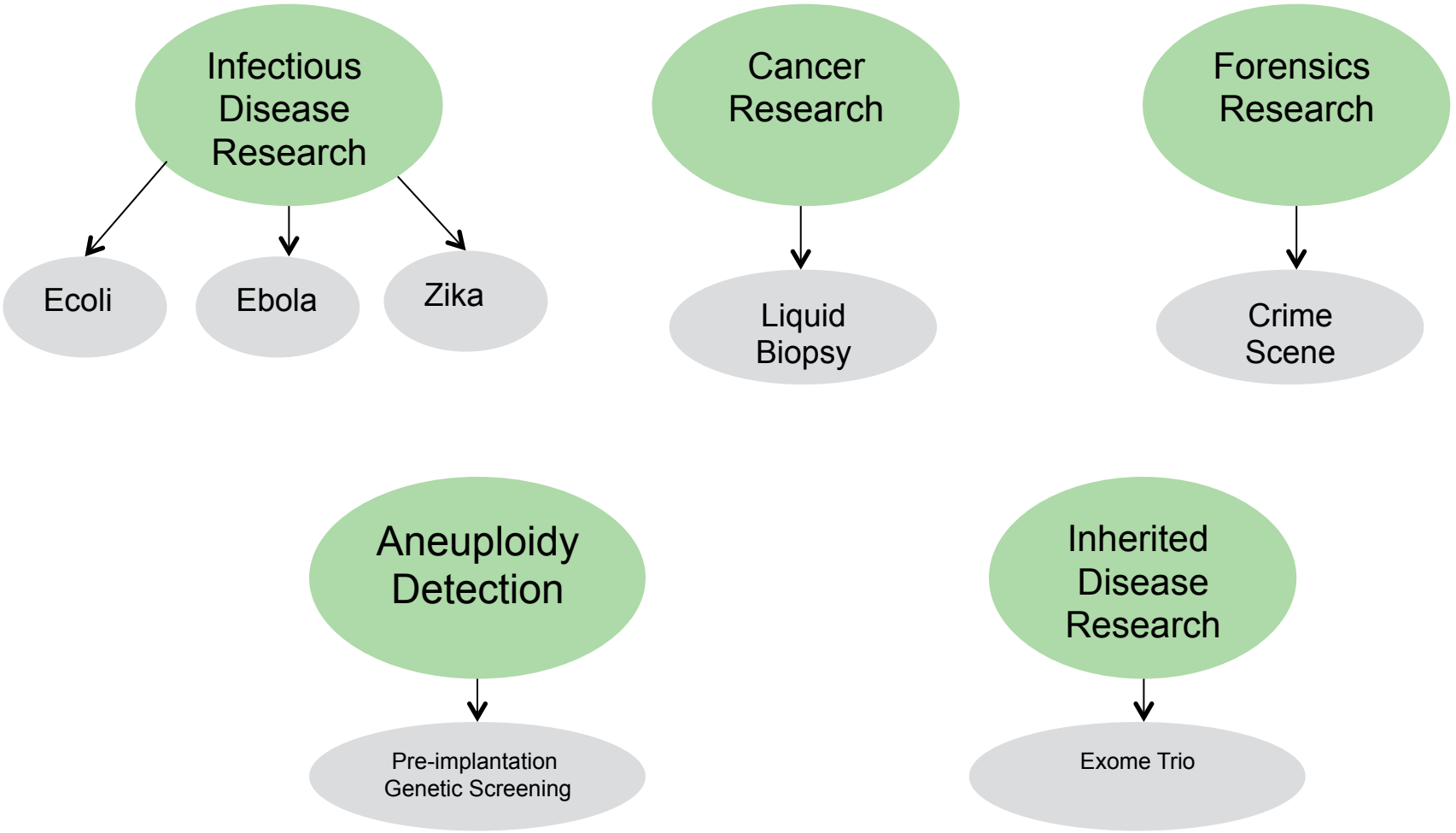
The world leader in serving science

Why Targeted Sequencing
More cost effective, more time efficient and simpler to analyze

	Targeted Sequencing	Whole Exome	Whole Genome
			
Variants generated per run	**10s to 100s**	**~50,000**	**~3,000,000**
Likely number of variants for follow-up	**1-10s**	**1-10s**	**1-10s**
Time to analyze	**Hours to Days**	**Days to Weeks**	**Weeks to Months**
Total cost including analysis	**$**	**$$**	**$$$**

Sequencing turnaround time is highly critical



NGS Oncology Research Solutions: Targeted Sequencing with quick turnaround time

Routine research → **Translational**

Oncology research specimen

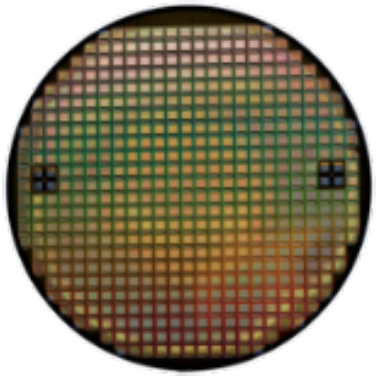
FFPE-based solutions	Heme-oncology solutions	Liquid biopsy solutions	Immuno-oncology solutions
Oncomine™ Comprehensive Assay	Oncomine™ Myeloid Research Assay	Oncomine™ cfDNA Assays for Lung, Breast, Colon	Oncomine™ Immune Response Research Assay
Oncomine™ Focus Assay	New Heme Assay (in development)	Oncomine™ Lung cfTNA Research Assay	Ion AmpliSeq™ Immune Repertoire Assay Plus, TCR β
Oncomine™ BRCA Research Assay		Oncomine™ Breast cfDNA Research Assay v2	Oncomine™ Tumor Mutation Load Assay
		Oncomine™ Pan-Cancer Cell-Free Assay	

Full characterization of oncology Research samples

- **Immuno-oncology solutions**
- **Liquid biopsy solutions**
- **Heme-oncology solutions**
- **FFPE-based solutions**

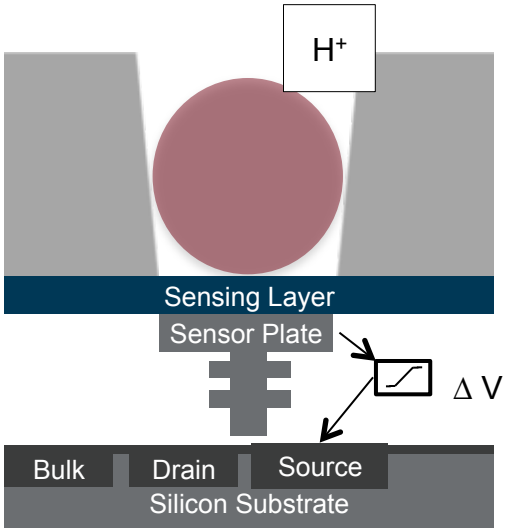
The content provided herein may relate to products that have not been officially released and are subject to change without notice.

Low Cost, Simple, Scalable, Real Time Sequencing



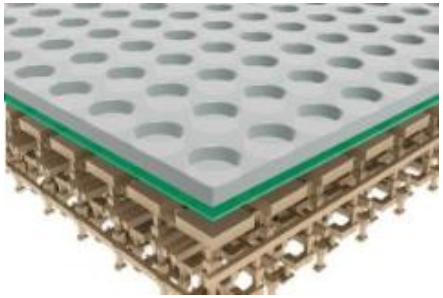
Wafer
Semiconductor Manufacturing

Chip
Semiconductor Packaging



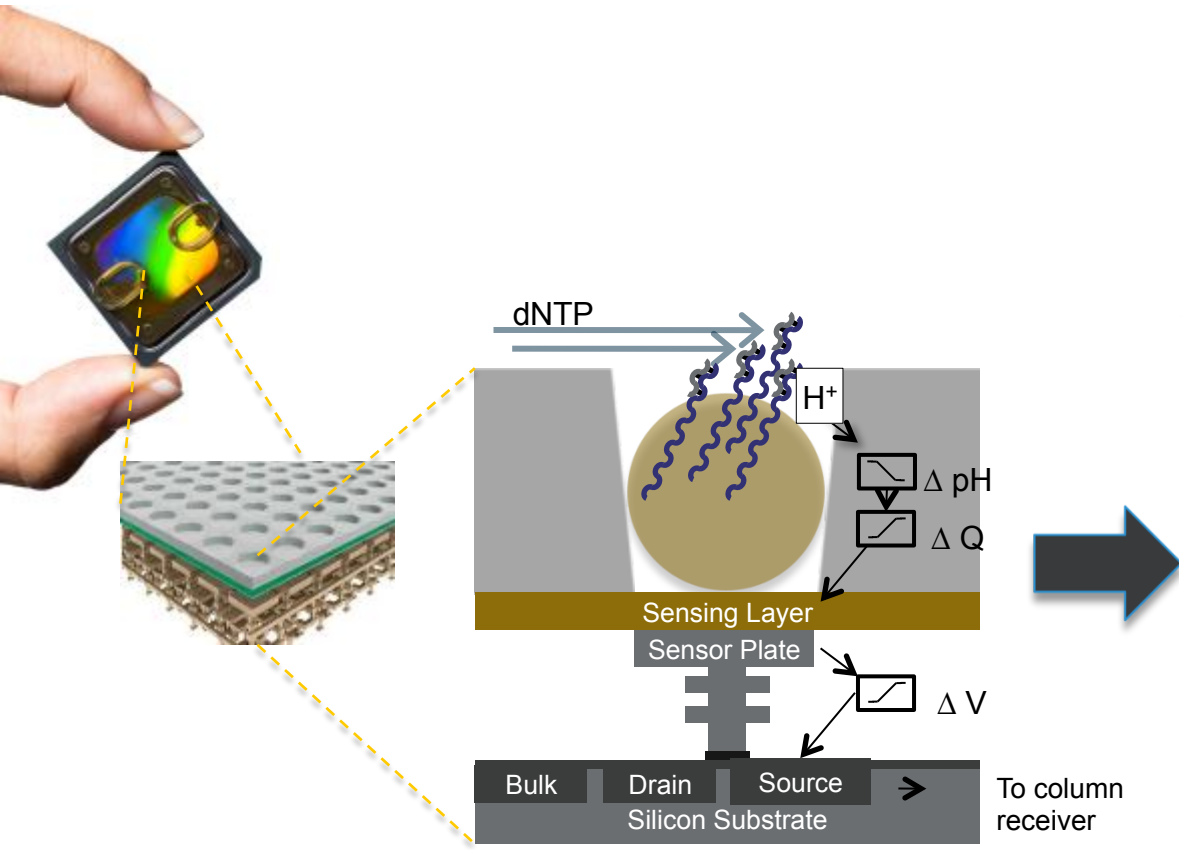
TCGTACC...

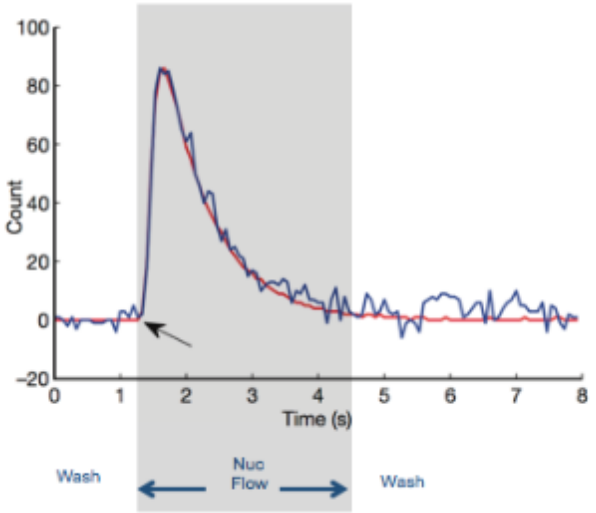
Single Sensor
Chemical to Digital Sequence



Millions of Sensors
Semiconductor Design

Transistor as a pH meter

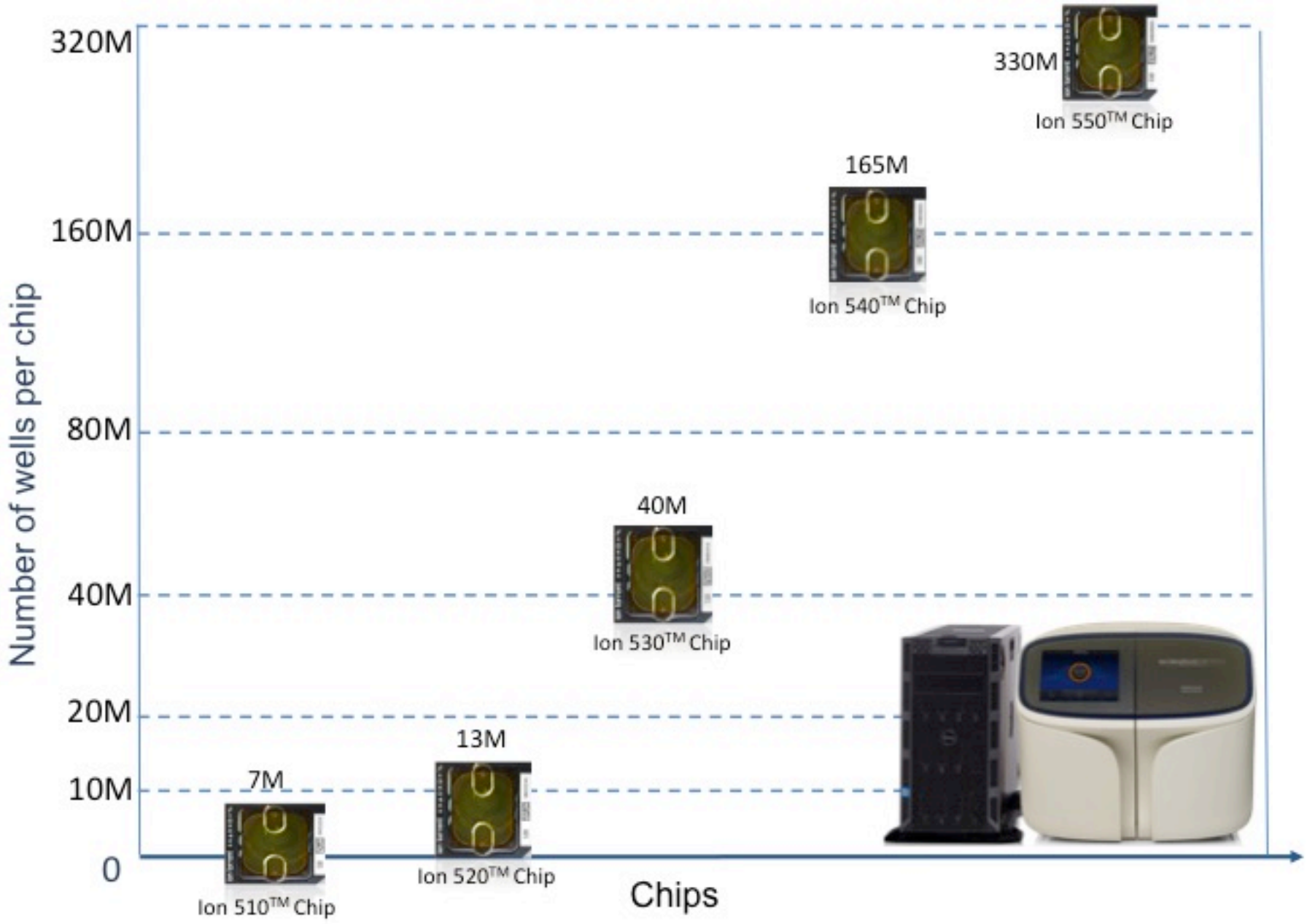




Rothberg J.M. *et al Nature doi:10.1038/nature10242*

Compute Intensive signal processing

Scaling Well Density with Ion Chip Generations



Ion NGS Evolution

Hard to Use

Easy to Use

Ion PGM

Ion Proton

Ion Chef

Ion S5™ XL System

Ion S5™

Ion GeneStudio™ S5 Series

2010 2012 2014 2016 2018

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Ion GeneStudio™ S5 Series

Ion GeneStudio™ S5

Fast.

Ion GeneStudio™ S5 Plus

Flexible.

Ion GeneStudio™ S5 Prime

Powerful.

Ion 510™ Chip
2–3 M reads
Up to 400 bp

Ion 520™ Chip
3–6 M reads
Up to 600 bp

Ion 530™ Chip
15–20 M reads
Up to 600 bp

Ion 540™ Chip
60–80 M reads
Up to 200 bp

Ion 550™ Chip
100–130 M reads
Up to 200 bp

* Throughputs based on 200bp sequencing

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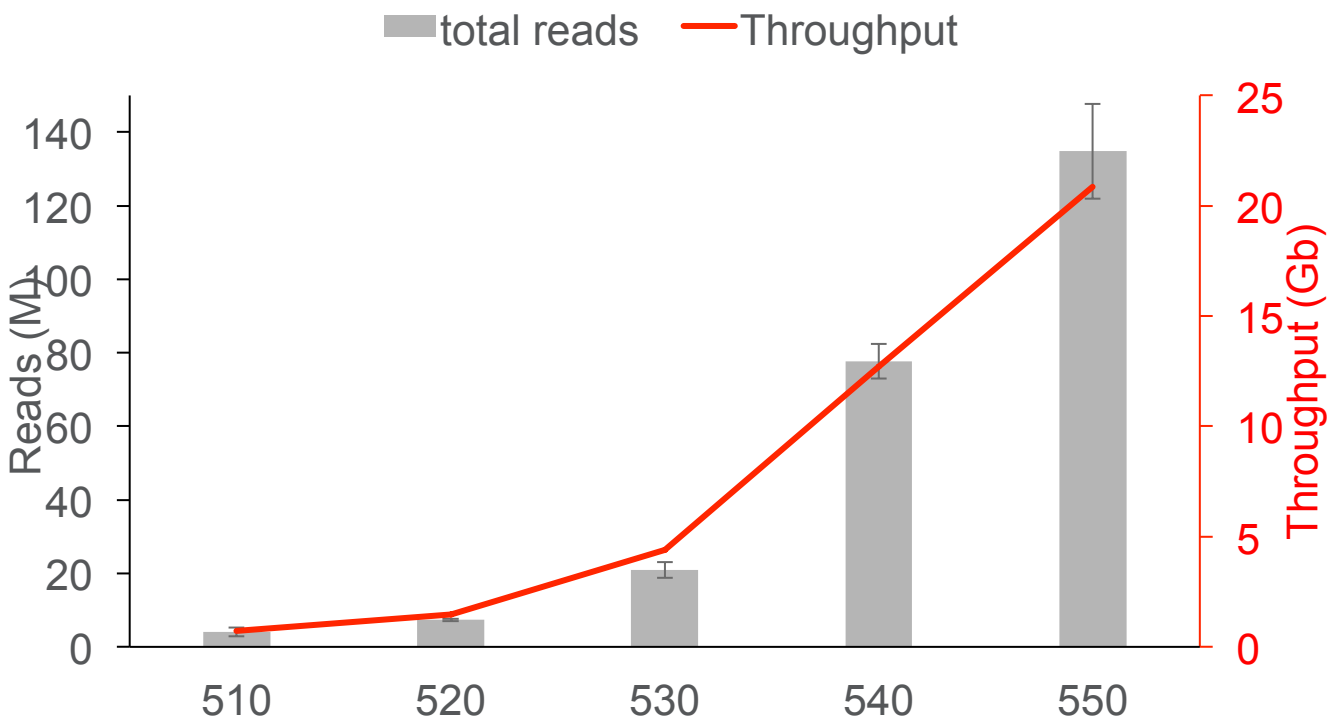
Internal Data Shows Reliable Output from 2 to 130 M Reads



Number of runs	8	8	5	6	9
Mean read length	203	203	203	181	169
Raw read accuracy	99.5%	99.5%	99.5%	99.02%	99.1%

*Ion 510, 520 and 530 Chips sequenced 1/6th of AmpliSeq™ Exome pool
**Ion 540 and 550 sequenced complete AmpliSeq Exome pool

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Maximum Sequencing Output Per Day and Turn-Around Time

	Ion GeneStudio™ S5	Ion GeneStudio™ S5 Plus	Ion GeneStudio™ S5 Prime
Speed*	19 hrs	10 hrs	6.5 hrs
Output (max/day):	15 Gb/80 M	30 Gb/160 M	50 Gb/260 M
Chips (max/day)**:**	1 x 540	**2 x 540** or 1 x 550	2 x 550

* Based off 540 chip – sequencing (2.5 hours) and analysis (varies) time

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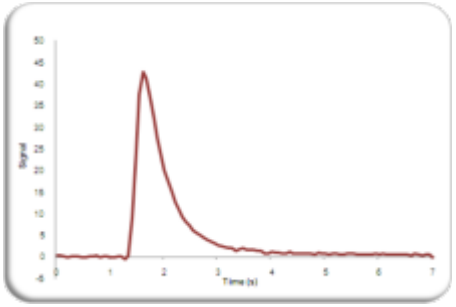
Data Processing Pipeline

20 TB
(Ion 550™ chip)



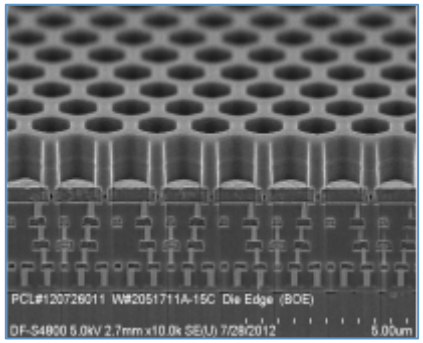
Data acquisition and compression

1 TB

Signal Processing

300 GB

BaseCalling, Alignment and Variant Calling



TGTATACCTCTTACTTTACAGATAGGTTCATTATCT
||||||||||||||||||||||||||||||||||||
TGTATACCTCTTACTTTACAGATAGGTTCATTATCT
10 20 30

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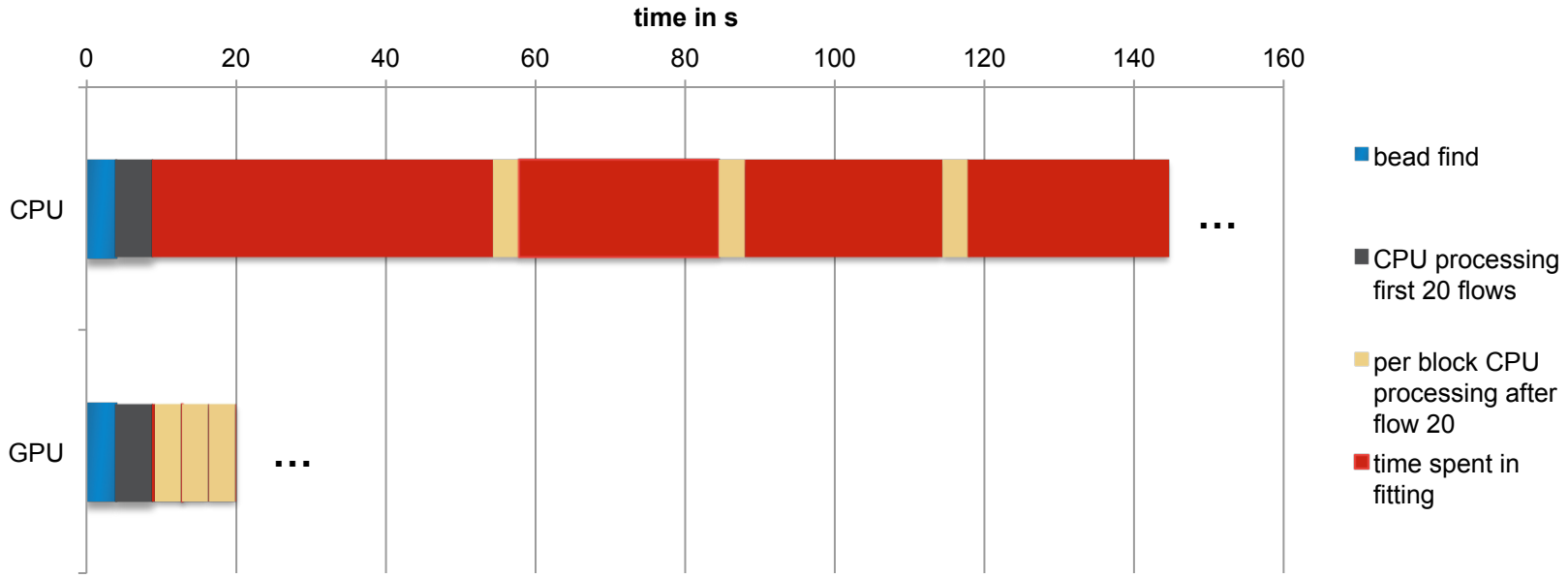
GPU to the rescue

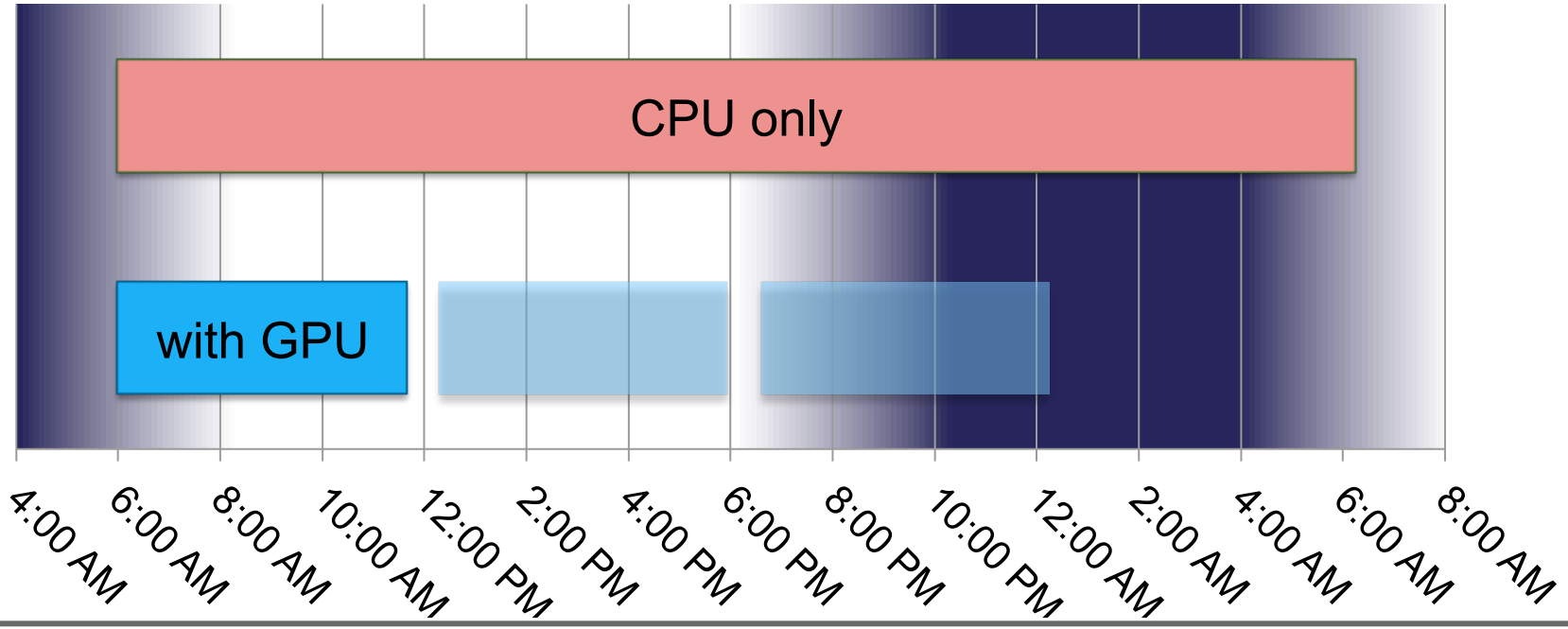
- Removed main hotspot in signal processing pipeline
- **Speedups of 270x over a CPU core!**



GPU's Impact

- Multiple sequencing runs a day generating
  - **260M 200bp reads with Ion 550™ chip (Nvidia Quadro P5000)**
- Swift pace of Research and Development
- Accelerated product innovation
- Lower cost per sample for applications like Liquid Biopsy and NIPT

On Instrument Analysis Time with and without GPU



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Signal Processing

Signal Processing Flow

Reading flow data

Raw Data Processing

Regional Parameter Estimation (Common to all wells)

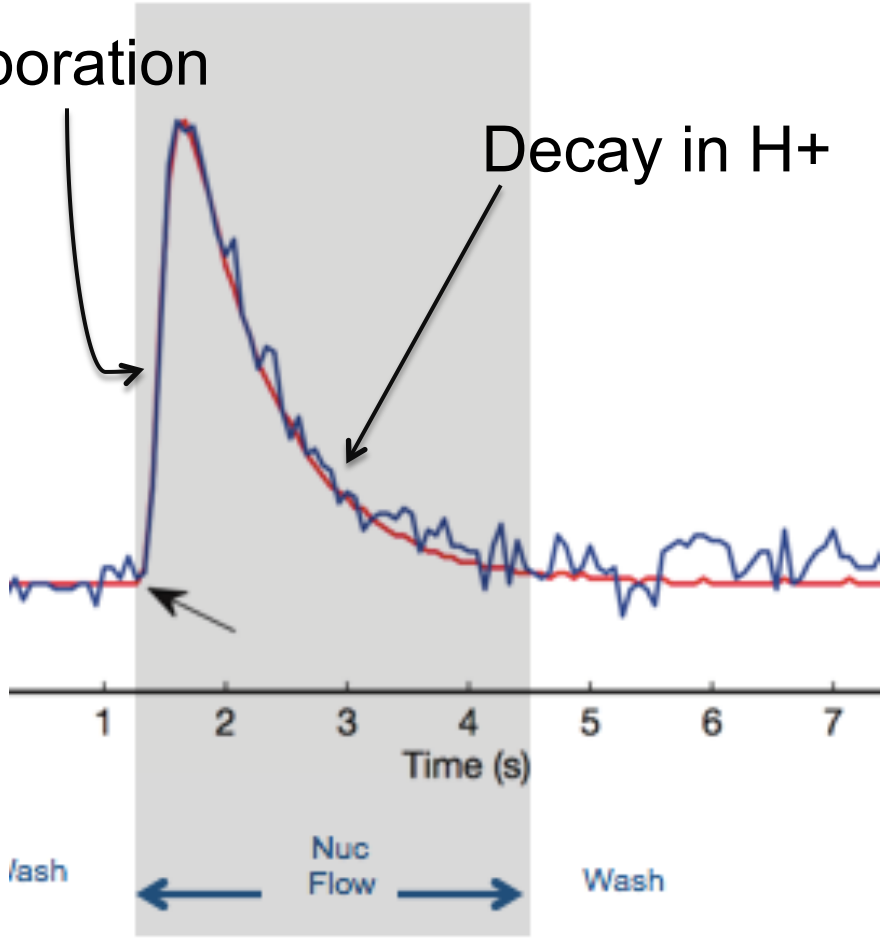
Parameter Estimation unique to each well

Post Fit Processing

Writing signal values

Mathematical model

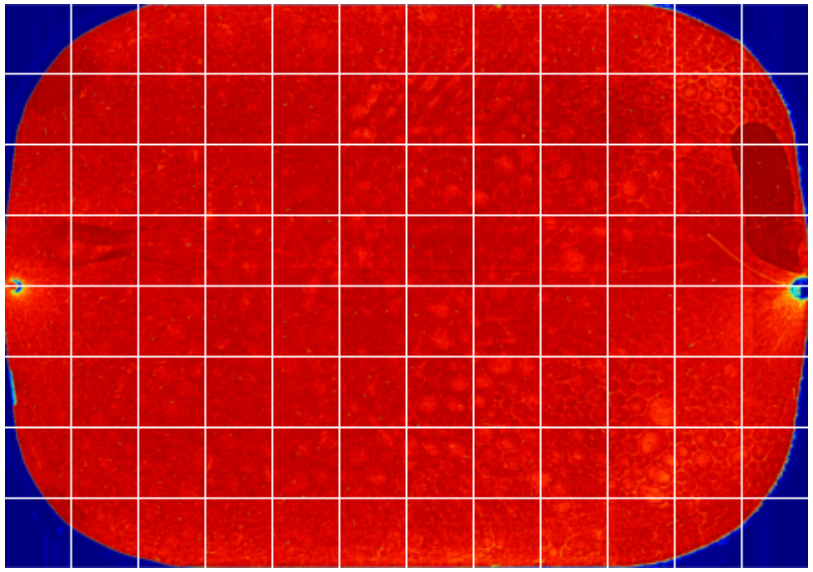
- Sophisticated model
  - Background correction
  - Incorporation
  - Buffering
- Regional Parameters
  - Enzyme kinetics, nucleotide rise, diffusion etc.
- Well Parameters
  - Hydrogen ions generated, buffering, DNA copies etc.

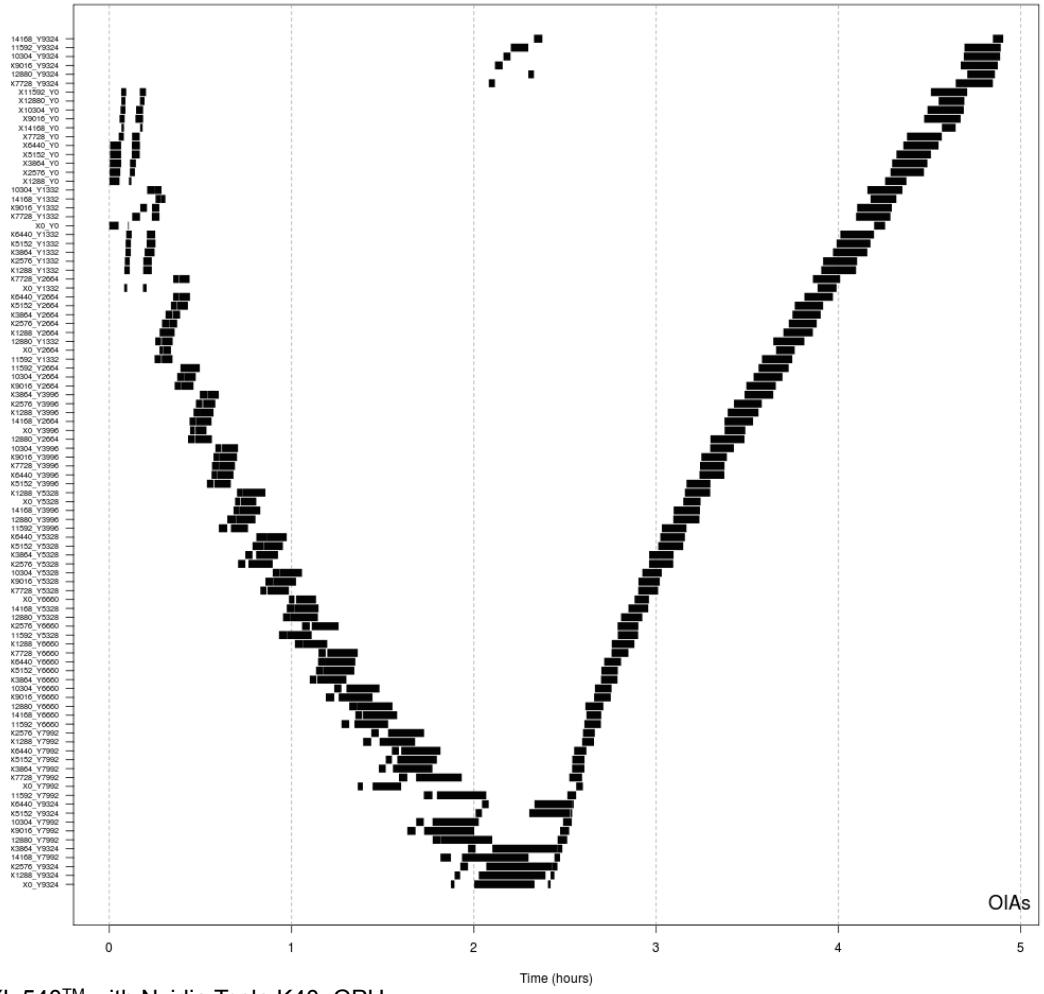


GPU Acceleration

Pipeline Execution Model

- Multiple Concurrent Processes

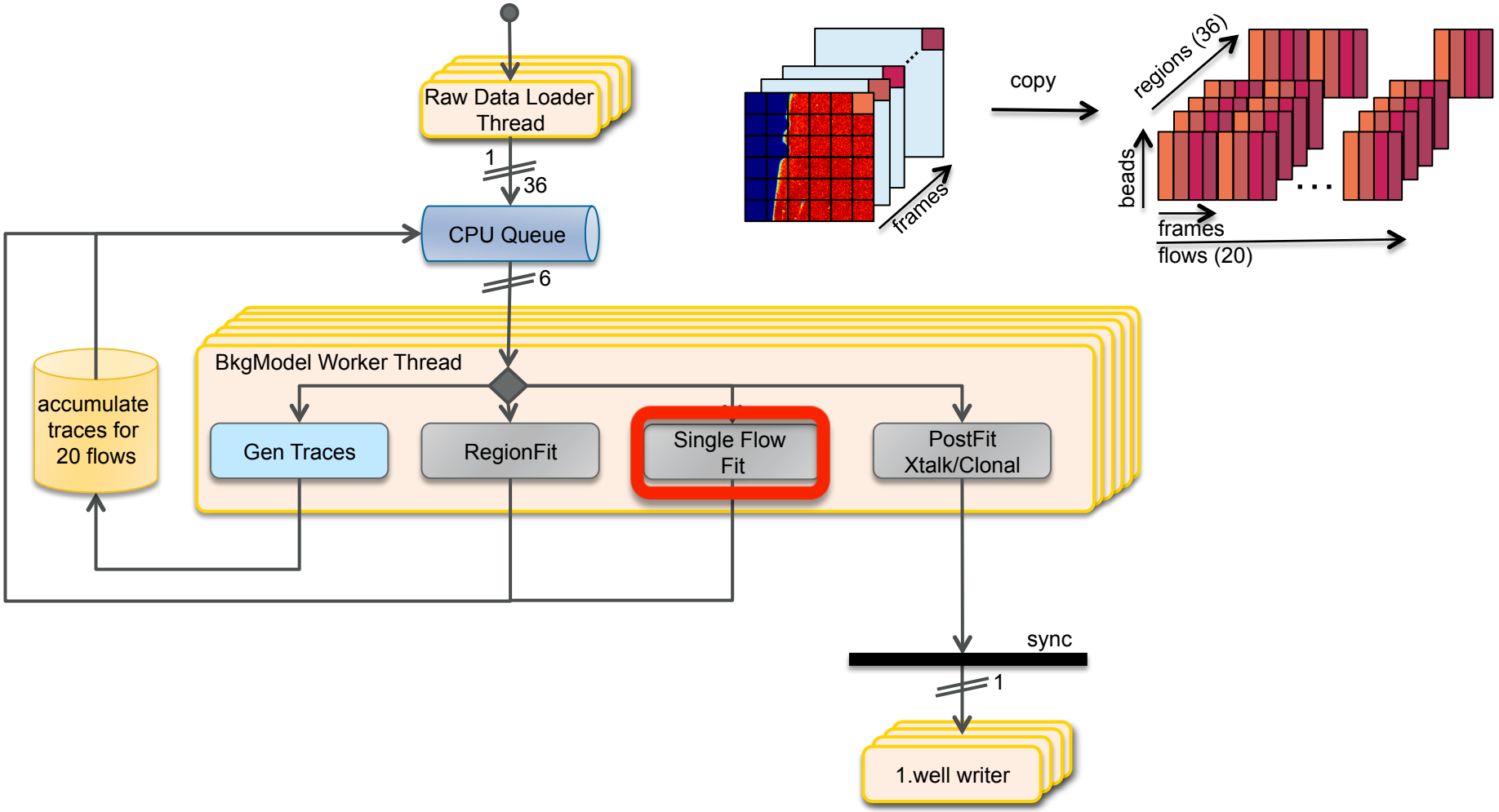




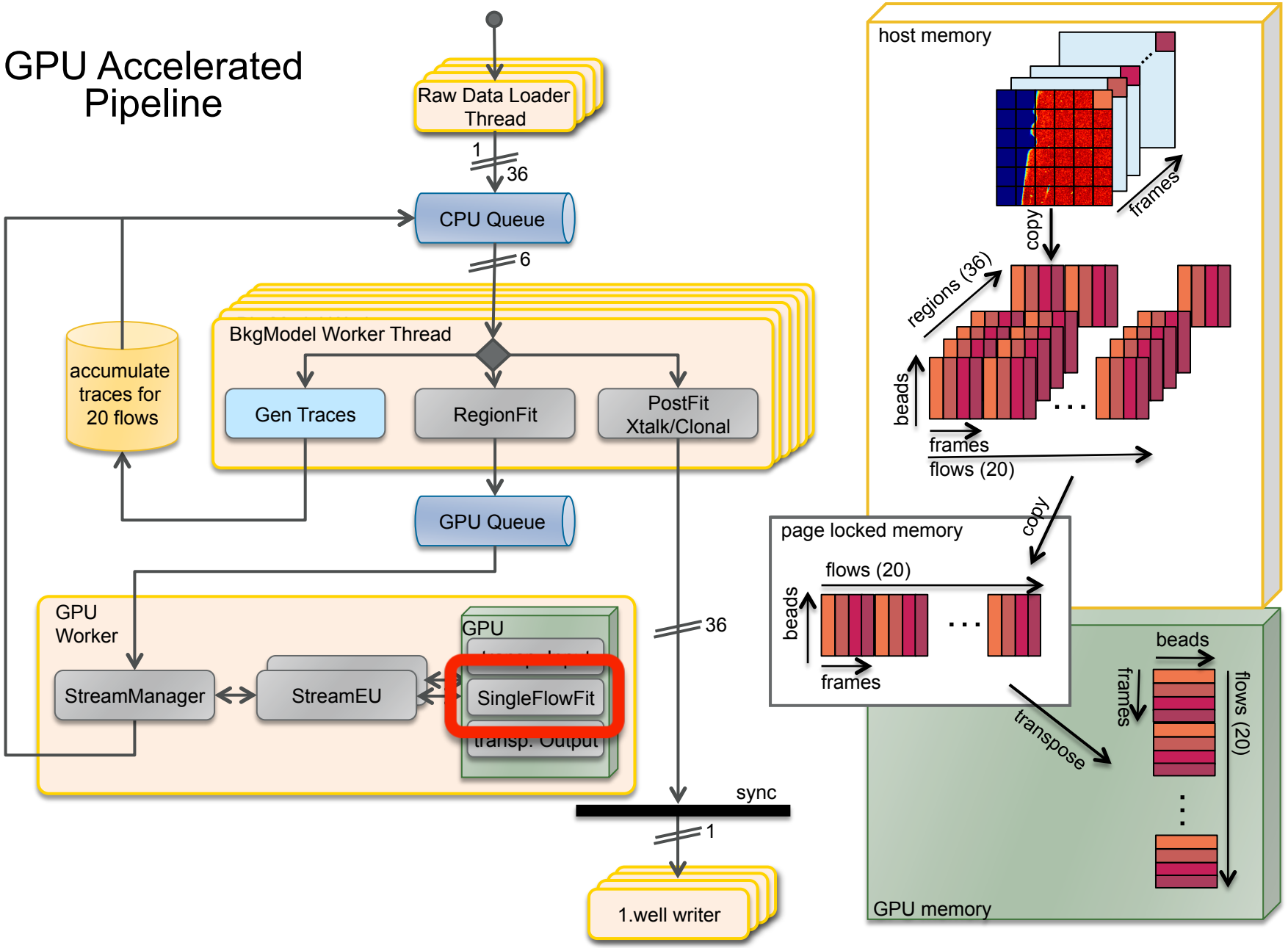
- 96 blocks
- depending on hardware 4 to 6 processes in parallel
- work on available data during experiment

*Heat-map and timing from a S5™ XL 540™ with Nvidia Tesla K40 GPU

CPU Execution Model

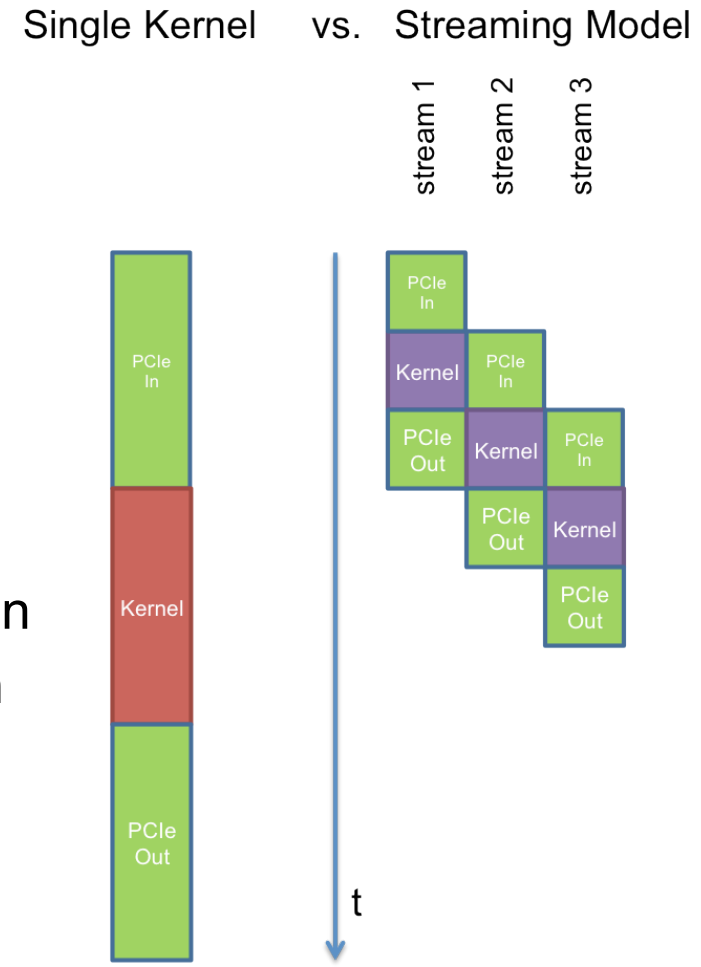


GPU Accelerated Pipeline

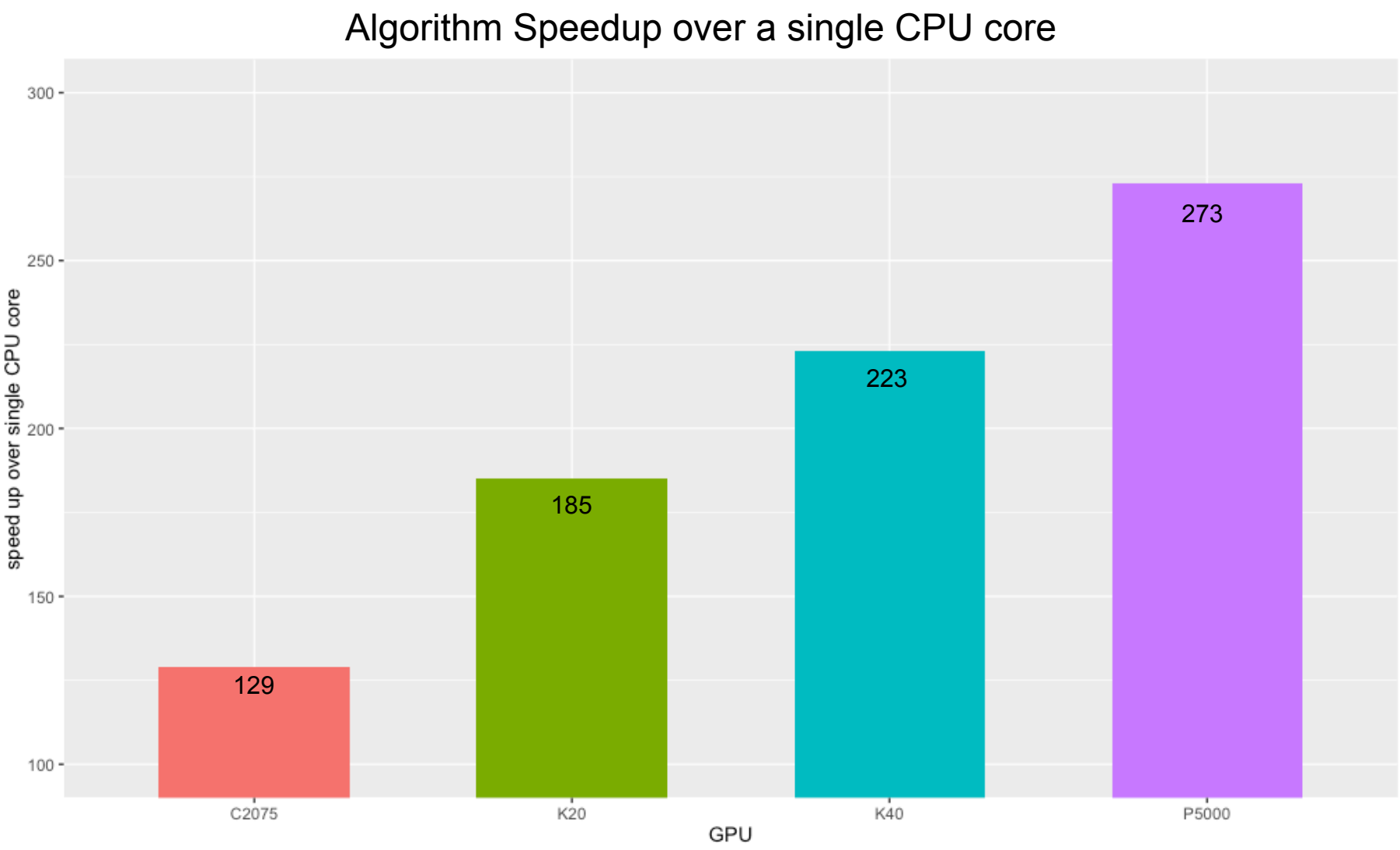


GPU Execution Model

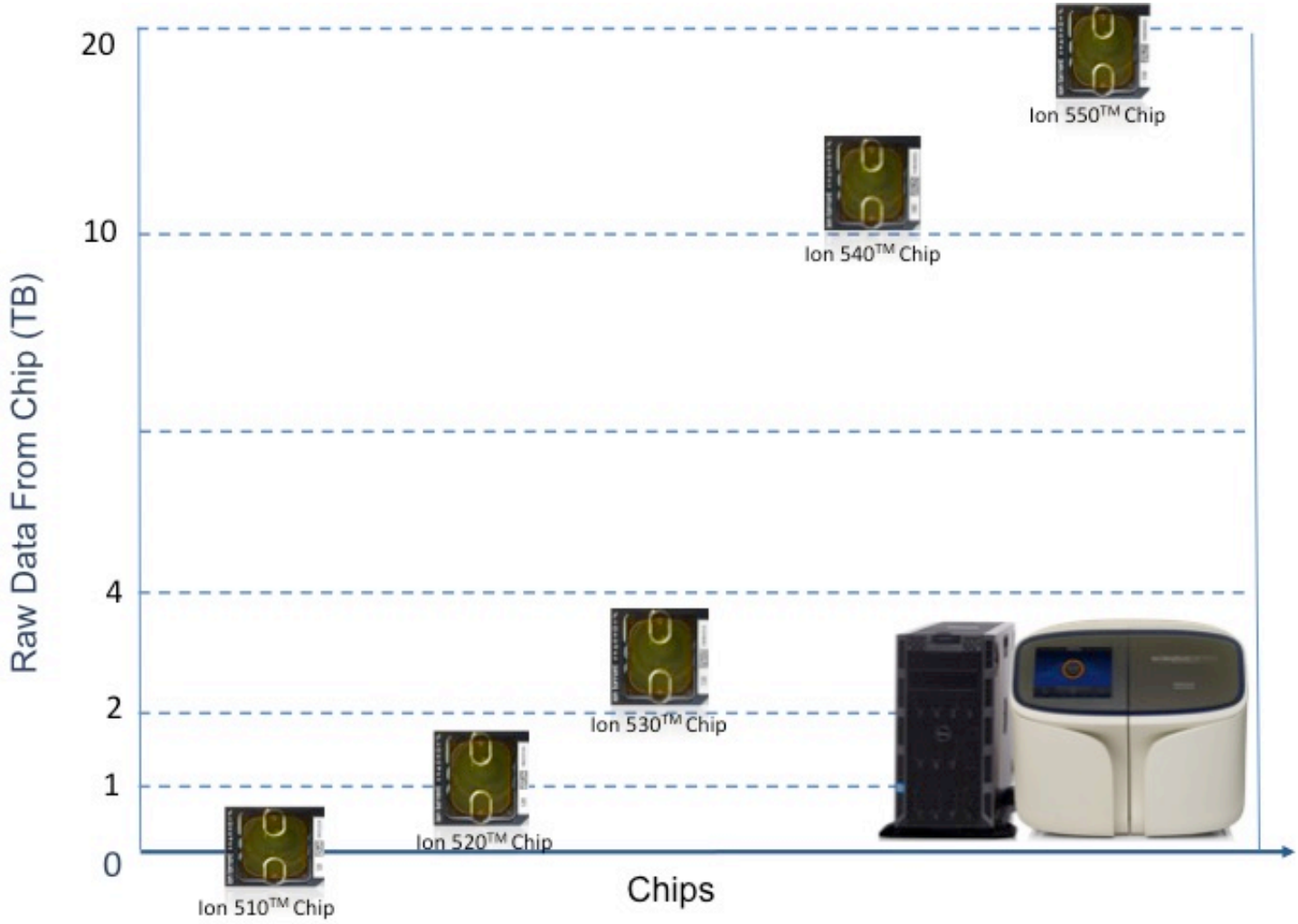
- Stream based execution allows for overlap of
  - Kernel execution
  - PCIe transfers
  - host side data reorganization
  - CPU pre and post processing
- Queue system for heterogeneous execution
  - Balances execution of different tasks between CPU and GPU



GPU Speedup with different architectures − Fitting Algorithm



Raw Data Explosion with Ion Chip Generations



- **Limited On-Instrument storage**
- **Disk writes become huge bottlenecks**

*Based on 500 flow run acquired at 15fps for 7s

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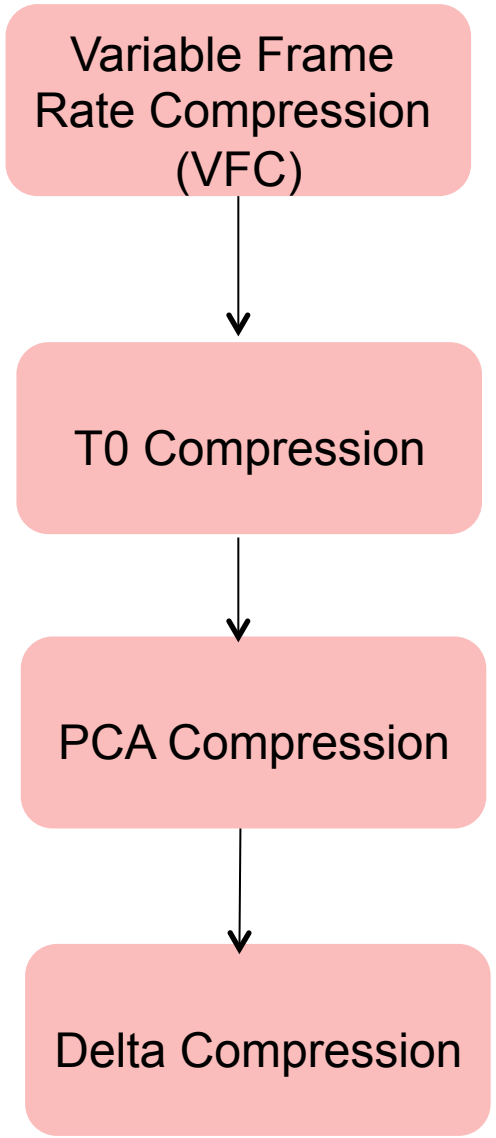
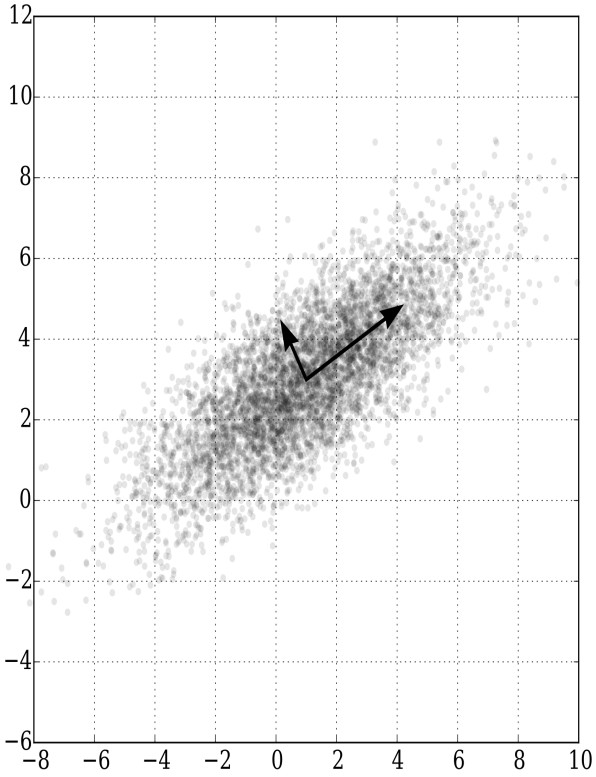
Raw Data Compression

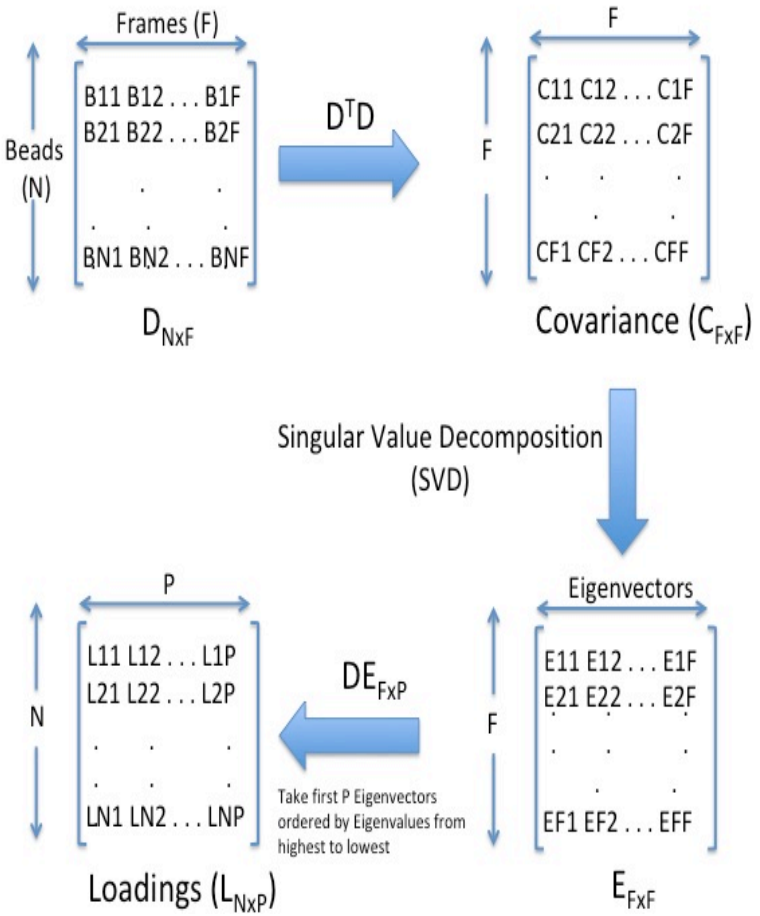
- Sequencing raw data undergoes several compression stages before it hits the disk
  - Variable Frame Rate: Heavy averaging before nucleotide hits the well and during wash step
  - T0: Adaptive change after VFC to tightly compress data around the timepoint nucleotide hits the well
  - PCA: Reduced basis using orthogonal transformation to capture maximum variance in the data
  - Delta: Store the difference from the previous frame



PCA Compression on Ion 550™ raw data

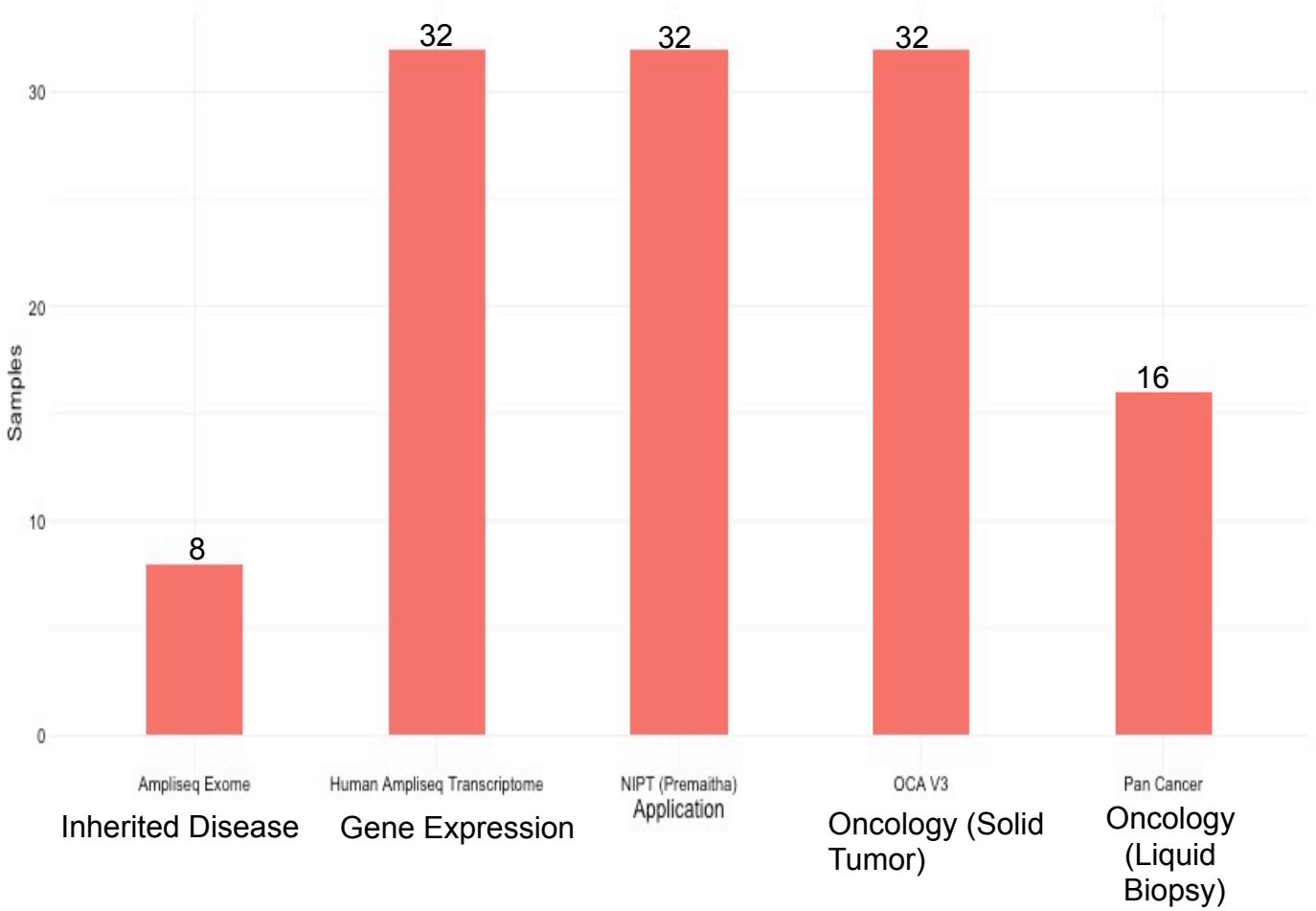
Statistical Procedure that uses an orthogonal transformation to convert set of linearly correlated observations into a set of linearly uncorrelated variables





PCA Compression flow for well traces. The final loadings are used to get back the traces in chosen eigenvector space.

Sample Throughput for different applications on Ion 550™ Chip



- **Four-fold (4TB -> 1TB)** reduction in data allows to double sample throughput for different applications
- Potentially sample throughput can be quadrupled
  - Instrument has to be re-initialized after two runs (Reagents run out)
  - Turnaround time might be more than 24 hrs if not connected to compute cluster

Future Directions

- We have peaked the timing improvement from GPU acceleration for current algorithms
  - Out of the box thinking in algorithms
  - GPU Friendly algorithms
- Variant Calling
  - Core Algorithm to evaluate every candidate variant can be GPU accelerated
  - Will further shorten the sample to variant workflow

Thank You

NVIDIA
specifically the DevTech Team

My supervisor Eugene Ingerman
and
The entire Ion Torrent R&D team

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