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Master's thesis

Speeding up Genotyping through GPU Acceleration

Exploring the effects of GPU accelerating Python-based genotyping software

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Abstract

In the last few decades, high-throughput DNA sequencing has steadily become more cost effective and accessible. With the potential for millions of genomes being sequenced in the coming years, tools for analyzing the large amounts of genetic sequence data produced will become increasingly important. Genotyping - the process of determining the genetic sequence variants present in the chromosomes of a genome - is a core application for such genetic sequence data. Work in alignment-free genotyping, a new method that forgoes aligning each sequenced snippet to a reference genome sequence, has recently revealed that statistical models on analysis of *k*mers can yield competitive accuracies while being significantly faster compared to more traditional alignment-based methods. A recently published genotyping tool, KAGE, showed that an alignment-free genotyper implemented in Python could yield competitive accuracies while being more than 10 times faster than any other known method. This thesis explores how parts of KAGE, that is implemented in Python and deal with large matrix- and array-operations, can be GPU accelerated using a number of different methods with different advantages and shortcomings. We then finally present GKAGE, a GPU accelerated version of KAGE. GKAGE achieves up to 10 times speedup compared to KAGE and is able to genotype a human individual in only a few minutes on consumer grade hardware - significantly faster than any other known genotyping tool today. We believe that the results achieved indicate that existing bioinformatics tools can benefit greatly from GPU acceleration.

Acknowledgements

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Contents

1 Introduction

A central problem in biology is to effectively uncover and characterize genetic sequence variations in humans. By understanding where and how the human genome varies from individual to individual, we can vastly improve our understanding of how an individual's genetic makeup affects its observable traits - its phenotype. To realize this goal, it is inescapable that we need fast and reliable methods to analyze how genetic variations manifest in the population, in order to gather the vast amounts of data necessary to explore links between genotypes and phenotypes adequately. Today, these methods are commonly referred to as *genotyping* - the process of determining which genetic variants are present in both of the chromosomes of an individual.

As the price of high-throughput sequencing has steadily become cheaper over the last few decades [1], whole genome sequencing of human genomes has become more accessible than ever before. Today, we can relatively cheaply sequence a whole human genome and expect to receive hundreds of millions of short polynucleotide reads (often of length ~ 150) [2]. From just such reads, where we neither know where the read originates from in the sequence's genome or where it may be erroneous, we want to perform the difficult task of accurately genotyping the individual, and to perform this analysis as quickly as possible.

The traditional and more established methods for genotyping a human individual have involved aligning all sequenced reads to a reference genome to examine where the reads differ from the reference, noting the genotypes supported. Such *alignment-based* methods are computationally expensive and time consuming, with established genotypers such as GATK [3] requiring tens of gigabytes of memory and several hours to run [4]. In recent years, new *alignment-free* strategies for genotyping human individuals have emerged. Because of work such as the 1000 Genomes Project [5], we today have access to vast knowledge about human genetic variation and genotype information. Alignment-free genotyping methods leverage such knowledge to skip the demanding alignment step altogether in favour of genotyping individuals directly based on analysis of $k$mers - short

substrings of the read sequences - and previous knowledge of known genetic variation [4, 6]. Recently, a new genotyping tool, KAGE, showed that by deploying an alignment-free method, it could achieve competitive genotype prediction accuracies while being an order of magnitude faster than any other known genotyper [4].

In recent years, with the introduction of the *general purpose graphical processing unit* (GPGPU), the *graphical processing unit* (GPU) has become increasingly popular for solving problems that require heavy amounts of compute, with many such problems existing in the space of scientific computing. Common for all traditional genotyping tools is that they are implemented to run on the *central processing unit* (CPU). This likely stems from the fact that memory usage commonly reaches tens, and sometimes hundreds of gigabytes when running these tools [4], and that not all problems are fit to run on the GPU architecture. However, the currently fastest known genotyping tool, KAGE, requires significantly less memory compared to its competitors. Additionally, KAGE is implemented in Python, and performs a significant amount of large array operations - a type of operation well suited for the GPU architecture - using the Python library NumPy [7]. Because of this, KAGE seemingly stands to benefit from GPU acceleration.

In this thesis, we will explore whether we can speed up (alignment-free) genotyping in any significant way by utilizing the GPU. We will start with KAGE as a base genotyper, and explore different avenues for implementing GPU acceleration in KAGE to assess whether they results in significant speedup. Since KAGE is implemented in Python and consists of several steps that can in effect be considered independent, several possible avenues for GPU acceleration are possible. We will explore a subset of them, and assess what options developers may have to GPU accelerate existing work in Python, and evaluate the advantages and drawbacks of each method.

1.1 Thesis Goals

This thesis has two main goals. One of the goals is to explore whether state-of-the-art genotyping can be sped up in any significant way by utilizing GPUs.

More specifically, this thesis will investigate whether alignment-free genotyping, which presently is significantly faster compared to alignment-based genotyping, can be sped up by utilizing the GPU. To investigate this, we will attempt to integrate GPU accelerated functionality into a base alignment-free genotyper, KAGE, the fastest known genotyping tool that also yields competitive results. KAGE, is implemented in Python and relies heavily on the array-library NumPy for performance. Thus, there are several possible avenues for integrating GPU support. Low level implementations in C++ using the CUDA framework provides great granularity and control, but require deep knowledge and understanding of hardware, the C++ programming language, and CUDA. Alternative methods may be to use existing Python packages that provide out-of-the-box GPU accelerated functionality. This leads us to the second goal of this thesis - to investigate and experiment with different ways of GPU accelerating existing Python code that relies on array-programming libraries such as NumPy, and to reveal advantages and drawbacks of each method.

2 Background

In this section we will cover preliminary information for the further topics presented and discussed in this thesis. Firstly, we will introduce necessary concepts and terms relating to biology, bioinformatics, and graphical processing units. Then, we will cover implementation tools and software libraries used throughout this project, and finally, give an introduction to KAGE [4], a central tool for the work presented in this thesis.

2.1 Biology

2.1.1 DNA, Chromosomes and Genomes

DNA, or *deoxyribonucleic acid*, is a type of molecule that contains all the genetic material found in the cells of all known living organisms [8]. The molecule is composed of two complementary strands of *nucleotides* that are twisted together to form a double helix structure, connected by bonds formed between complementary nucleotides. The two strands are, in turn, composed of the four nucleotide bases: adenine (A), guanine (G), cytosine (C) and thymine (T), where A and T, and C and G are complementary bases [9, p.15]. Furthermore, the two complementary strands of nucleotide bases encode the same precise information. This is because with knowledge of just one of the strands' nucleotide sequence, say $strand_1$, we can determine the sequence of the other strand, $strand_2$, by exchanging each nucleotide in $strand_1$ with their complements and finally reversing the strand to determine what $strand_2$'s sequence is.

Figure 1: A conceptual representation of a DNA molecule made up of two strands. The strands are composed of nucleotides forming base pairs where A (adenine) and T (thymine), and C (cytosine) and G (guanine) are complements of each other.

Relatively small differences in these DNA sequences are what differentiates individuals within the same species from one other. It is therefore interesting to study these sequences of nucleotides encoding organisms' genetic information, as the encoded information can reveal details about both associated physical traits and diseases. In human cells, these DNA strands are estimated to be roughly $3 * 10^9$ bases long [9, p.13].

DNA is organized into structures called *chromosomes*. Humans have 23 chromosome pairs, making up a total of 46 chromosomes. Each of the pairs include one version of the chromosome inherited from the male parent, and one version inherited from the female parent [10].

The term *genome* can be used to refer to the complete genetic material of an organism. In practice, however, the genome of an organism often simply refers to the complete DNA nucleotide sequence of one set of chromosomes for that organism [9, p.13]. Commonly in bioinformatics, one can also encounter the term *reference genome*, referring to a theoretical reconstruction of an organism's genome created by scientists. Such genome reconstructions are commonly used when examining new DNA sequences, often by aligning new DNA sequences to the reference in order to see at which positions their nucleotides differ and what differences are present at those positions [3].

2.1.2 High-Throughput DNA Sequencing

High-throughput sequencing (HTS), also known as *next-generation sequencing* (NGS), refers to an assortment of recently developed technologies that parallelize the sequencing of DNA fragments to provide unprecedented amounts of genomic sequence data in short amounts of time. While several such technologies with varying details exist today, they commonly follow a general paradigm. This paradigm consists of performing a template preparation, clonal amplification where pieces of DNA are cloned in order to sequence the clones in parallel, and finally, cyclical rounds of massively parallel sequencing [11]. The resulting DNA sequences produced by HTS technologies are usually referred to simply as (DNA) *reads*. Such reads are commonly stored as plain text in FASTA or FASTQ files,

which can later be used for different kinds of analysis such as genotyping. Depending on which HTS technology is used, one can expect read lengths ranging from as low as 150 bases using *Illumina* technologies, referred to as *short reads* [2], all the way up to 15-20 thousand bases using *Pacific Biosciences* (PacBio) technologies, referred to as *long reads* [12]. There are three important factors to consider when determining which HTS technology to use for a given purpose: 1) the read lengths produced, 2) the average probability for each base being erroneous, usually referred to as the error rate, and 3) the cost of sequencing given the technology, which can potentially limit how much data one may be able to produce.

example.fa

```
>read 0
ACGTATGCGGCGGGGCGCGATTATTCGTTGCGTATGC
>read 1
ACACGTCGTGCGTAGCGTGTCAGTCACAGTAAACAAA
>read 2
CGTTGCCATCAACGGCTGTGCACGATTGGGGGCGCGC
...
```

Figure 2: An illustration of how sequenced DNA reads are stored as plain text in a FASTA (.fa) file. Before each read, a header file beginning with the character ">" may provide information about the read.

When performing DNA sequencing, we have no knowledge of where a read originates from in the original genome. This issue, in addition to the fact that some bases are erroneously sequenced, introduces uncertainties when trying to predict features about an individual's genome based on the sequenced reads. Many techniques rely on *aligning* (or *mapping*) sequenced reads to a reference genome sequence in order to better predict where in the genome the read originates from. To gain better confidence and local support along these alignments, it is common to sequence an individual's genome at higher coverages, so that aligned reads may overlap and correspondance can be asserted. The term sequencing *coverage* is used as an indication of how many times each nucleotide in a genome has been sequenced on average. E.g. 15x coverage means that every nucleotide in the

genome has been sequenced, on average, 15 times.

2.1.3 Variants and Variant Calling

When examining the genome of several individuals within the same species, one will find locations along the genome where the nucleotides differ for the different individuals. These distinct nucleotide manifestations are commonly referred to as *variants*. The term *variant calling* refers to the process of determining which variants are present in an individual's genome. In practice, however, the term variant often refers to a segment of an individual's genome that differs from a reference genome sequence. Thus, variant calling is often, in practice, referred to as the process of determining where and how an individual's genomic sequence differs from a reference genome sequence. The traditional way of performing variant calling is to use an alignment-based method, which consists of the following three steps: 1) sequence the genome of interest to get DNA reads (described in section 2.1.2), 2) align the reads to the reference genome by finding where along the reference genome sequence each read fits best, typically by using a comparison heuristic to determining which location the read originates from, and 3) examine the alignments and note where and how the reference and the individual's sequences differ to determine the variants present in the individual's genome [13]. A popular and well-established alignment-based method for variant calling is to use the GATK [3] toolkit.

Variants found in the genomes of individuals of the same species can manifest in several different forms. Three common types of variants are:

- A *single nucleotide polymorphism* (SNP) is a variant in which only a single nucleotide differs in the sequenced genome when compared to a reference genome at a location of interest.
- An *indel* refers to one of two types of variants: *insertion* - a sequence of nucleotides, not present in the reference genome, that has been introduced in the sequenced genome, and *deletion* - a sequence of nucleotides, present in the reference genome, are missing in the sequenced genome.
- A *structural variant* is a larger-scale genomic variation referring to inser-

tion, deletion or inversion events affecting more than 50 bases [14].

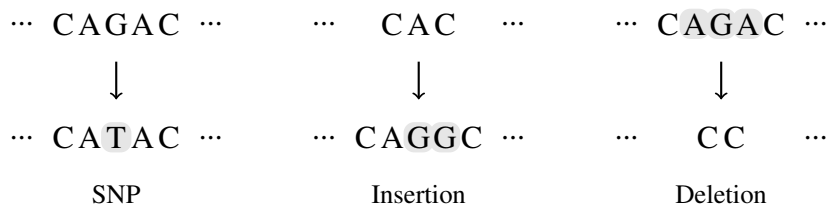


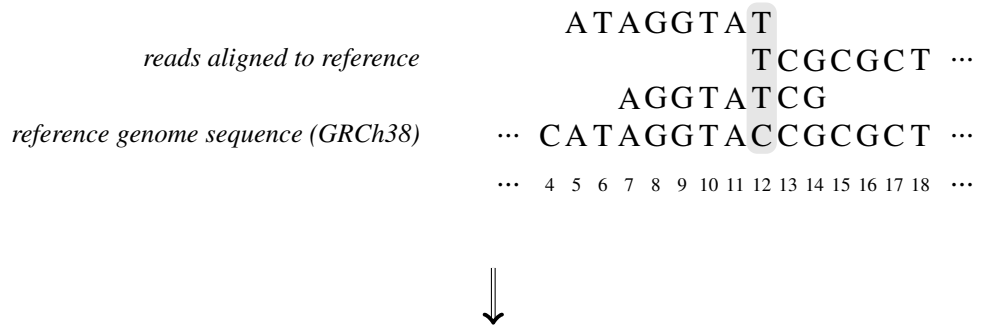
Figure 3: An illustration demonstrating how three types of variants can manifest when aligning sequenced reads to a reference genome. From left to right: a single nucleotide polymorphism (SNP), an insertion (indel), and a deletion (indel).

The software tool KAGE, described in section 2.6, focuses particularly on genotyping SNPs and indels.

A common way to represent genome sequence variations is to encode them according to the *Variant Call Format* (VCF) file format. The VCF file format encodes a single variant per line, and each line contains a number of columns where each column encodes a particular piece of information about the associated variant, such as [15]:

1. CHROM: an identifier for the reference sequence used, i.e. the sequence against which the sequenced reads varies.
2. POS: the position along the reference sequence where it varies against the sequenced reads.
3. ID: an identifier for the variant.
4. REF: the reference base (or bases) found at the POS position in the reference sequence.
5. ALT: a list of the alternative base (or bases) found at this POS position.

While more columns are usually present, this encapsulated the necessary knowledge about variants and their representation needed for this thesis.



example.vcf

```
##fileformat=VCFv4.2
##reference=GRCh38
#CHROM POS     ID  REF  ALT  QUAL FILTER INFO
1      12      .   C    T    30   PASS   NS=3;DP=3
...
```

Figure 4: An illustration of how sequenced reads can be aligned against a reference genome sequence in order to call variants present in the genome of the sequenced individual. The called variant in the illustration is then stored in a variant call format (VCF) file where the chromosome identifier, 1-indexed position along the chromosome reference sequence, an identifier for the variant, the allele or alleles present in the reference sequence, the alternative variant allele or alleles, along with a number of other parameters are used to represent each variant.

2.1.4 Genotypes and Genotyping

Much like variant, the term *genotype* is used slightly ambiguously in the world of bioinformatics. The term genotype may be used to refer to an individual's total genetic makeup. It may also refer to the variants an individual carries in its chromosomes at a particular location along the genome. Throughout this thesis, we assume the latter definition. For humans, who have two copies of each chromosome, we may have the same or two different variations present at a particular location in the genome, making up the genotype.

Genotyping an individual refers to the process of determining which genotypes an individual carries. For humans, this would entail determining the set of variants present at each variant site. In most genotyping software tools today, genotypes are given in a format that specifies whether a particular variant is present in none,

one or both of a human's chromosomes. For instance, given a reference genome sequence where a variant site is known to could manifest an A at a particular allele where the reference sequence contains a G, a human individual's genotype for this variant could either be referred to as $0/0$, meaning that the variant allele A is present in neither of the chromosomes, $0/1$, meaning that it is present in one of the chromosomes, or $1/1$, meaning that it is present in both chromosomes.

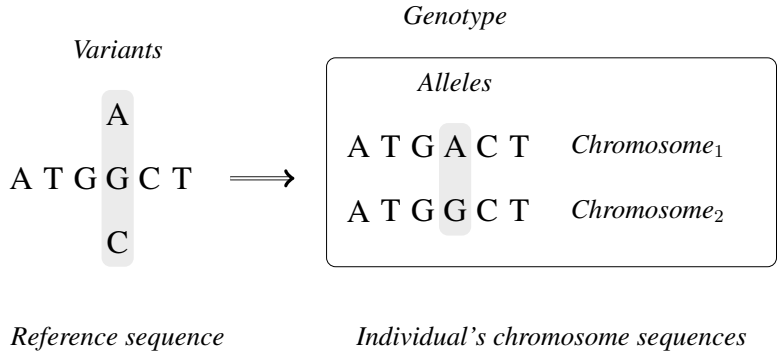


Figure 5: In humans, who carry two copies of every chromosome, a genotype constitutes a set of two alleles or variants - one in each chromosome at the variant site. Along the reference sequence on the left, several possible variants may be known to occur at a specific site. After examining the sequence of an individual, we try to determine the individual's genotype by scoring which variants are present in each chromosome at the location of interest. In this illustration, the individual's genotype may be described as two scores: $0/1$ for the SNP variant where the G is replaced by an A in chromosome$_1$, and $0/1$ for the G remaining the same as in the reference in chromosome$_2$.

Today, the most established ways to genotype individuals are based on *alignment-based* methods. Such methods rely on aligning sequenced DNA reads to a reference genome and performing variant calling before calling genotypes by assessing which genotypes are most probable based on locally supported variants [3]. However, given the vast amount of reads provided by high-throughput sequencing technologies today, aligning each read to a $3 * 10^9$ long reference sequence is very compute, memory and time consuming. As a result, a set of new prominent methods have emerged in recent years to help alleviate these issues. *Alignment-free* genotyping methods, where the alignment of reads and variant calling step are skipped altogether, have yielded promising results and significant speedup compared to their alignment-based counterparts. In such methods, small parts of the sequenced reads called *k*mers are analyzed, and statistical models are used to

compute genotype-probabilities supported by the results from the *k*mer analysis and previous knowledge of variants and genotypes that have been accumulated over years of research [4, 6, 5]. One such alignment-free genotyper, KAGE, has recently shown that it can genotype a human individual more than 10 times faster than any other known genotyper tool, while still providing competitive accuracy scores for its genotype calls [4].

2.2 Nucleotide Binary Encoding

DNA nucleotide sequences (described in section 2.1.1) inside computer software are commonly represented simply by a sequence of the 8-bit characters A, C, T, and G (or alternatively the lowercase a, c, t, and g). This representation, however, is cumbersome to operate on and requires large amounts of memory to store. To circumvent these issues, a widely adopted technique is to encode the nucleotides into binary form, often referred to as *2-bit encoding*. This encoding leads to much quicker processing of nucleotide sequences and reduces the memory usage needed to store the sequences by 75%. This is achieved by realizing that only 2 bits, giving $2^2 = 4$ possible unique states, is enough to represent all of the four DNA nucleotides A, C, G and T. The binary encoding can be extended further to represent whole nucleotide sequences in binary arrays. For example, an integer array, if interpreted 2 consecutive bits at a time, can represent such a sequence.

Nucleotide	A	C	G	T
2-bit encoding	00	01	10	11

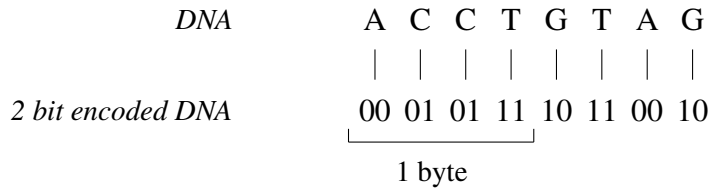


Figure 6: A lookup table showing how nucleotides can be encoded using 2 bits and a DNA nucleotide sequence represented both as plain characters as well as its 2 bit encoded representation. Recall that computers use 8 bits to represent a single nucleotide with a character, whilst the 2 bit encoding only needs 2 bits to represent a nucleotide.

2.3 *k*mers and the *k*mer Counting Problem

A *k*mer is a substring of *k* consecutive nucleotides that occur in a DNA (or RNA) sequence. Because of how single nucleotides can be represented in a computer's memory using only 2 bits 2.2, and how when we sequence an individual's genome we can not know which strand our sequenced read comes from, a popular choice of value for *k* is 31 - the default value used in KAGE 2.6. The value 31 is used in KAGE for two reasons: 1) having an odd value for *k* ensures that no *k*mer is equal to its reverse complement, and 2) having *k* equals 31 means we need $31 * 2 = 62$ bits to represent the *k*mer in a computer's memory, thus the *k*mer will fit inside a single 64-bit integer.

A common problem in various bioinformatics applications is to count the number of times each valid *k*mer in a set of nucleotide sequences occurs in those sequences. This problem is commonly referred to as *k*mer counting.

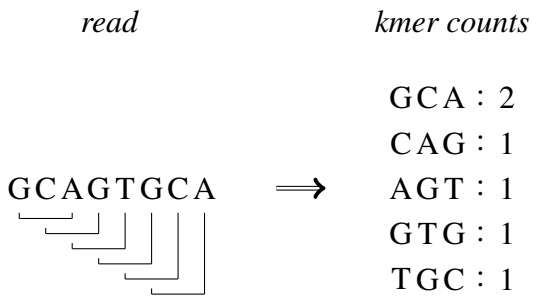


Figure 7: *Full k*mer counting where we count the observed frequency of every valid 3-mer in our read set.

The genotyping software tool KAGE, detailed in section 2.6, contains *k*mer counting as one of its core steps in its genotyping pipeline. However, the *k*mer counting process in KAGE is slightly different from the process commonly referred to by the term *k*mer counting. Rather than counting the observed frequency of every valid *k*mer in a set of input reads, KAGE only counts the observed frequencies of every *k*mer in a predefined set. Given how many valid *k*mers one can observe in a set of hundreds of millions of reads, which is typical when sequencing and genotyping a human genome, not needing to store each with an associated count

value makes this new *k*mer counting variant significantly less memory and time consuming.

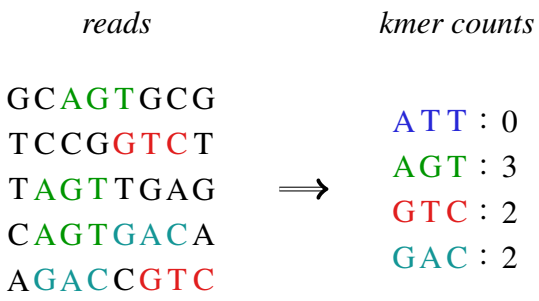


Figure 8: *Partial* *k*mer counting where we only count the observed frequencies of 3-mers present in a predefined set. In this example, our set of predefined 3-mers is {ATT, AGT, GTC, GAC}. During counting, 3-mers not present in this set are skipped.

Henceforth in this thesis, we will in the favour of brevity refer to the former *k*mer counting process where we count every valid *k*mer's occurrence as *full* *k*mer counting, and to the latter process where we only count the occurrences of *k*mers in a predefined set as *partial* *k*mer counting.

While several *k*mer counting software tools have been developed in previous work, with at least one, Gerbil [16], supporting GPU acceleration [17], these tools are designed to solve the *full* *k*mer counting problem.

2.4 Graphical Processing Units

Graphical Processing Units (GPUs) are massively parallel processing units designed for high-throughput parallel computations. *Central Processing Units*, on the other hand, are designed to perform many serial computations quickly, reserving more transistors to caching and predicting how programs will branch in the future. GPUs were originally developed to accelerate computations performed on images, a highly parallel task where it is commonplace to have millions of relatively small independent computations that must be performed quickly in a single memory buffer. Although GPUs have mainly been used for graphical computations, they have in recent years been adopted in other areas with the introduction

of the *General Purpose Graphical Processing Unit* (GPGPU). The concept of the GPGPU is to use a GPU to accelerate computations in other domains where CPUs have traditionally been used. Fields such as artificial intelligence and the broader scientific computing community have enjoyed great utility from GPUs, using them to accelerate embarrassingly parallel problems, e.g., matrix operations. Despite being comparable in power consumption, a GPU can provide much higher instruction throughput and memory bandwidth compared to its CPU competitors. These capability advantages exist in GPUs because they were specifically designed to perform well with regards to these dimensions.

While several distinct brands of GPUs exist, the work presented in this thesis only leverages GPUs produced by Nvidia, one of the leading accelerated computing manufacturers for scientific computing today [18].

2.4.1 GPUs in Computers

Two main computer GPU setups are prominent today: *integrated* graphical processing units (iGPUs), and *discrete* graphical processing units (dGPUs). iGPUs are GPUs integrated onto the same die as a computer's CPU, where the two share the same physical *Random Access Memory* (RAM) unit. dGPUs are dedicated GPU devices that are physically distinct from the host computer's CPU and RAM, and have their own physical RAM. dGPUs are significantly more powerful in terms of compute throughput when compared to iGPUs. However, having their own physical RAM introduces an overhead; Memory buffers with input data must be copied to the dGPU's RAM before processing, and results copied back from the dGPU's RAM to the host RAM.

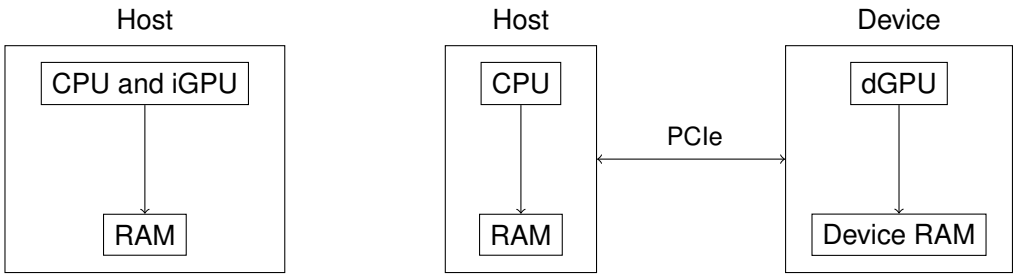


Figure 9: **Left**: A computer setup with a CPU and an iGPU sharing the same die and the same physical RAM. **Right**: A computer setup where a dGPU is connected over PCIe and the dGPU has its own physical RAM, adding the overhead of copying data both to and from the host computer when utilizing the GPU.

For the work presented in this thesis, only dGPUs were utilized, as we did not have access to any iGPU systems. Therefore, the term GPU will from here on out be referring to a dGPU and not an iGPU. This means that all GPU implementations discussed in this thesis will include copying memory back and forth from the *host* (CPU) RAM and the *device* (GPU) RAM. It is also possible for a single computer to have several connected GPUs, allowing for further parallelization of both memory transfers and compute, however this was not utilized in this thesis' work.

2.4.2 CUDA

CUDA [19] is Nvidia's general purpose parallel computing platform and programming model that allows software engineers to leverage the parallel compute engines in Nvidia GPUs. Although the CUDA software environment can be imported in several supported programming languages, development of GPU accelerated programs have traditionally been done in C++ with CUDA functionality imported through header files [19].

The CUDA programming model is designed to be utilized to solve parallel problems where the GPU is used. To programmers, the programming model comprises a hierarchy of three units of different granularity: *threads*, *blocks*, and *grids*.

2.4.2.1 Threads, Blocks and Grids

CUDA threads are the most granular units of parallelism in the CUDA programming model. Thread blocks are collections of threads that can be one-, two- or three-dimensional. Grids are at the top of the hierarchy, as collections of thread blocks. Both thread blocks and the grids can be one-, two- or three-dimensional. Thus, their dimensions are referred to as x for width, y for height and z for depth. In the CUDA model, each thread can be distinctly identified by its index in a thread block, and the thread block's index in a grid. When developing parallel programs, each thread has direct access to its (up to) three-dimensional index inside its thread block, and its thread block's (up to) three-dimensional index in its grid. If a thread resides in a three-dimensional thread block of dimensions (Dx_b, Dy_b, Dz_b) at index (x_b, y_b, z_b), its unique index within said block can be computed using:

$$i_b = (x_b + y_b Dx_b + z_b Dx_b Dy_b) \tag{1}$$

Then, we can compute the thread's unique grid-wide index. If the thread's thread block resides in a grid of dimensions (Dx_g, Dy_g, Dz_g) at grid-index (x_g, y_g, z_g), the thread's unique grid-wide index can be computed using:

$$i_g = (x_g + y_g Dx_g + z_g Dx_g Dy_g)(Dx_b Dy_b Dz_b) + i_b \tag{2}$$

This thread-hierarchy provides a natural way to perform computations over elements in e.g. vectors, matrices or tensors. All threads in a thread block must reside on the same *stream multiprocessor core* in the GPU. Therefore, a size limit exists on how large thread blocks can be. The two types of Nvidia GPUs used in this thesis, the Nvidia Tesla V100 and the Nvidia GTX 1660 SUPER, have limits of 2048 and 1024 threads per thread block respectively.

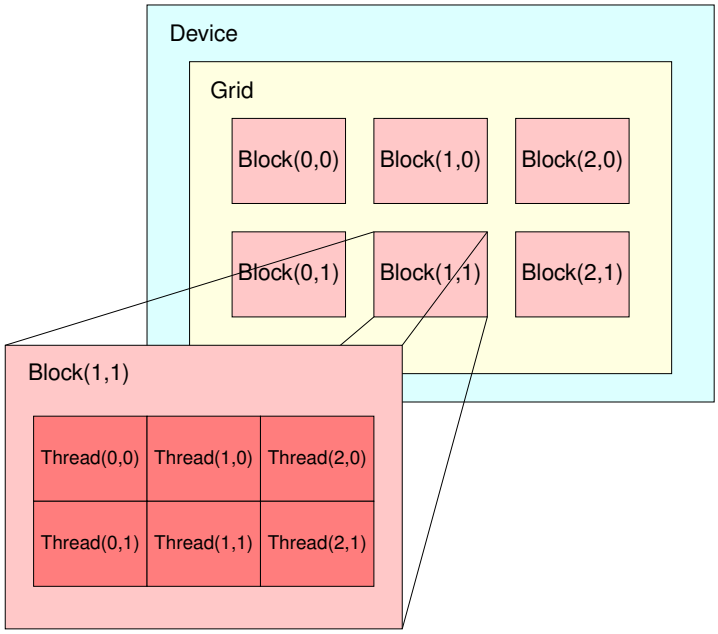


Figure 10: An overview of the CUDA programming model. A single two-dimensional grid contains two-dimensional thread blocks.

2.4.2.2 Kernels

When writing parallel programs for the GPU using CUDA, *kernel* functions define how the GPU should solve problems in a parallel fashion using the thread-hierarchy. Kernels are functions that, when called, launch once for each thread in the thread-hierarchy configurations, all in parallel. Thus, the code that resides in the kernel definition is executed in parallel by every distinct CUDA thread. Inside the kernel, each CUDA thread has access to its unique index within its thread block as well as a unique thread block index within its grid. The thread blocks' dimensions and number of threads are typically determined by the problem and limited by the max number of threads per streaming multiprocessor core on the system. For the grid, the size is generally determined by the size of the problem, and computed as a result of how many thread blocks must be launched to complete the necessary computations fully.

Below is a simple example program where a kernel is implemented to increment every integer in a vector by one.

main.cu

```
// Kernel definition
__global__ void increment_kernel(int *array, size_t N) {
  int i = (threadIdx.x * blockDim.x) + threadIdx.x;
  array[i] = array[i] + 1;
}

int main() {
  size_t N = 1048576;
  // Allocate and initialize array on the GPU
  int *array = ...

  // Determine grid- and block-dimensions and then launch
     kernels
  dim3 block_dim(1, 1, 1028);
  dim3 grid_dim(1, 1, N / 1028);
  increment_kernel<<<grid_dim, block_dim>>>(array, N)
  ...
}
```

Figure 11: A simple example program showing how the CUDA thread-hierarchy is utilized in a kernel to implement GPU acceleration for incrementing every value in an integer array.

The example program above is implemented in C++. Certain keywords used are CUDA specific, and are only supported by including the necessary CUDA header files beforehand. `__global__` is used to declare that a function is a kernel. The number of CUDA threads launched to run a kernel is specified in the `<<<...>>>` part of the kernel call.

2.4.2.3 Memory Hierarchy

Each CUDA thread has access to several distinct memory areas. Every distinct CUDA thread has its own set of registers and local memory. Typically, all the relevant data for a single CUDA thread is contained within its set of registers. If, however, a thread runs out of registers or otherwise uses too much memory, it will turn to its local memory. A CUDA thread's local memory is a piece of the GPU's global memory allocated for the thread. While accessing memory from registers is swift, memory requests to and from global memory are slow in comparison. It

is therefore desirable to avoid spilling memory from registers into local memory for individual threads.

Each thread block has a shared memory region accessible by all threads contained in the block. This memory region shares its lifetime with its associated block and is also very fast compared to global memory.

The GPU's global RAM is the largest memory region in the GPU, typically ranging from a few to several gigabytes. The global memory is several orders of magnitude slower than the previously mentioned memory regions, so effective memory access patterns to minimize the number of memory requests and maximize bandwidth usage are paramount for performance.

2.4.2.4 Programming and Considerations

Nvidia describes the GPU to be a *Single Instruction Multiple Threads* (SIMT) machine [19]. Although CUDA threads are described as threads, modern GPUs can in effect be considered to be massive *Single Instruction Multiple Data* (SIMD) machines. Strict flow control is therefore important; The same set of instructions should run in the same order for maximum utilization of the GPU's capability. Therefore, it is important to keep in mind that code branching may damage performance. Furthermore, because of the massive amounts of transistors dedicated to processing in the GPU, the bottlenecks in parallel programs on the GPU is typically memory bandwidth. Particularly global memory requests are expensive and should be avoided when possible. Ideally, consecutive CUDA threads should access consecutive data from the GPU's global RAM memory. This allows for the GPU to make larger and fewer memory requests, resulting in significantly better memory bandwidth and overall performance.

2.5 Implementation Tools and Libraries

This section will provide a brief introduction to some core programming languages and software libraries that were used in the work presented in this thesis.

2.5.1 C and C++

C and C++ are compiled general purpose programming languages. C++ is a superset of C, and can in effect be considered to be C with classes among some other high-level functionality. C and C++ are popular choices for implementing optimized and performant code. They offer granular control over hardware and memory, where data such as arrays must be manually allocated and de-allocated.

2.5.2 Python

Python is an interpreted high-level general purpose programming language that has gained significant traction, including in the scientific computing community, in recent years [20, 21]. Since it is an interpreted language, Python in and of itself is not performant, and can in many instances be measured to be several orders of magnitude slower than similar implementations in performant compiled languages such as C and C++. The Python interpreter is written in C, and thus, the Python programming language can naturally be extended with additional C and C++ code. Because of its high-level and interpreted nature, it is well suited for quick software prototyping. However, because of its ability to be extended with additional C and C++ code, many packages exist today that supplement Python with high-performance libraries, making Python a serious language candidate for scientific computing software today, albeit through calling upon optimized and performant C and C++ code.

2.5.3 NumPy

NumPy is a scientific computing library for Python that provides support for fast multi-dimensional arrays along with a multitude of mathematical and other types of functions to operate on arrays efficiently [7]. NumPy works as a Python interface to fast C and C++ code that implements the underlying functionalities. This underlying code relies on vectorization and SIMD instructions to perform array operations fast. While NumPy's standard functionality is designed to run efficiently on a single CPU core, multithreading can be utilized to parallelize on the local data (SIMD), as well as the total work level (multithreading) at the same time. Its flexible and easy-to-use interface along with its highly performant solu-

tions that support a wide range of hardware have made it a popular choice for any array-based scientific computing in Python.

2.5.4 CuPy

CuPy is GPU accelerated NumPy [7] and SciPy [22] compatible array library that, much like NumPy [7], provides a multi-dimensional array object as well as mathematical functions and routines to operate on these arrays. In fact, CuPy's interface is designed to closely follow that of NumPy, meaning that most array-based code written in NumPy can seamlessly be replaced with CuPy to GPU accelerate the array operations. CuPy, unlike NumPy, will store array data in GPU memory, and all array routine and function calls made using CuPy arrays will be run on the GPU.

CuPy also offers some access to CUDA functionality such as CUDA streams used to copy memory to and from the GPU's memory while simultaneously processing data, and device synchronization to halt the CPU until a process started on the GPU has completed. Additionally CuPy supports creating custom kernels that can operate on GPU allocated arrays, directly in Python. These kernels are then JIT (just-in-time) compiled when the program first encounters the kernel. Thus, CuPy provides a useful module where GPU accelerated implementations can be made directly with a NumPy-like array interface, also supporting more granular custom kernels that can operate on the data in these arrays directly inside Python.

2.5.5 npstructures

npstructures (NumPy Structures) is a Python package built on top of NumPy that provides data structures with NumPy-like features to augment the NumPy library [23]. This is achieved by building these new data structures using NumPy's underlying multi-dimensional array object and fast array routines.

Some of npstructures' data structures have been central in work done in this thesis. Those data structures will therefore be detailed in this section.

2.5.5.1 Ragged Array

A central feature in npstructures is its ragged array object, a two-dimensional array data structure with differing column lengths that provides NumPy-like behaviour and performance. The ragged array object works as a drop in alternative to NumPy's multi-dimensional array object where one needs an array structure where the column lengths can vary, supporting many of the common NumPy functionalities such as multi-dimensional indexing, slicing, universal functions and a subset of the function interface.

```
>>> import numpy as np
>>> from npstructures import RaggedArray
>>> data = np.array([0, 1, 2, 3, 4, 5, 6, 7, 8])
>>> column_lengths = np.array([4, 2, 3])
>>> ra = RaggedArray(data, column_lengths)
>>> ra
ragged_array([0, 1, 2, 3]
[4, 5]
[6, 7, 8])
>>> ra.ravel()
array([0, 1, 2, 3, 4, 5, 6, 7, 8])
>>> type(ra.ravel())
<class 'numpy.ndarray'>
>>> np.sum(ra)
36
```

Figure 12: A simple illustration of how npstructures' data structures can be used directly in Python as drop-in augmentations to the NumPy library.

2.5.5.2 Hash Table and Counter

npstructures also provides a memory efficient hash table built on top of the ragged array data structure. This is designed to give dictionary-like behaviour for NumPy-arrays where chunks of key-value pairs can be operated on at once using fast NumPy array routines. The hash table object is the base for a counter object that allows for counting of occurrences of a predefined set of keys.

The counter (built on top of the hash table) achieves its memory efficiency by implementing a type of bucketed hash table where a ragged array is used to repre-

sent the table. The number of rows of the array is equal to the number of buckets in the table, and the varying column lengths are equal to the bucket sizes. Upon initialization, the hash table hashes every key in the static key-set provided and computes how many keys hash to each row in the ragged array, thereby determining the column lengths (bucket sizes) for each row.

2.5.6 BioNumPy

BioNumPy is a Python library built on top of NumPy that allows for easy and efficient representation and analysis of biological data [24]. This includes functionality for efficiently and correctly reading a multitude of different file types that are commonly used for storing biological data directly into NumPy arrays, fast encoding from character arrays representing biological sequences into 2-bit encoding for faster processing and better memory efficiency, and *k*mer analysis support.

2.5.7 pybind11

pybind11 [25] is a C++ library that provides easy-to-use macros and tools for creating bindings between Python and C++. Its main use case is to create Python bindings for existing C++ code. Using pybind11, this can be achieved seamlessly by using C++ macros to expose C++ functions, classes and their methods to Python. In addition, pybind11 also allows for direct use of certain Python types such as lists, tuples and dicts in C++, supporting both receiving and returning references to such Python objects in C++. NumPy is also supported, making it easy to send NumPy array references to C++ functions where the C++ function is allowed the freedom to both read and write to the array's data. While the work in this thesis revolves around GPU accelerating parts of a software tool written in Python, some of the solution implementations presented in this thesis were written partly in C++ for fast performance. pybind11 was, in these cases, crucial in order to create the bindings from these C++ implementations, making them usable directly in Python.

2.5.8 Snakemake

Snakemake [26] is a workflow management system built on top of Python that allows for the creation of reproducible and scalable data analyses. This is achieved through creating *workflows*, using a human readable, Python based scripting language. Such workflows are created as sets of rules, where each rule define how to produce one or more output files from one or more input files, for example by running a piece of software. Once a workflow has been defined, Snakemake determines the order of which rules should be executed in order to produced the desired output. Snakemake also ensures that workflows are reproducible by tracking input files, output files, and parameters used for executing rules, and parallelization of rule execution.

2.6 KAGE

KAGE [4] is an alignment-free genotyping tool for SNPs and short indels. Unlike alignment-based genotyping tools, KAGE uses an alignment-free method that does not align sequenced reads to a reference genome. Instead, KAGE counts the occurrences of a predefined set of *k*mers in the input reads to then directly compute the likelihood of every possible genotype ($0/0$, $0/1$ or $1/1$) being present for each variant in an input set of known variants. These probabilities are based on the observed *k*mer counts in the reads and the expected *k*mer counts for an individual who carries the particular genotype. The expected *k*mer counts for each genotype are estimated beforehand based on large catalogues of genotype data accumulated over many years of research from projects such as the 1000 Genomes Project [5]. Indices of estimated expected *k*mer counts are created once and then re-used for many consecutive genotyping runs. KAGE recently showed (2022) that its accuracy was on par with the best existing alignment-free genotyping tools while being an order of magnitude faster [4].

KAGE is implemented in Python and relies extensively on the NumPy library for performance. Its genotyping pipeline is split into two distinct programs: *kmer_mapper*, solving the *partial k*mer counting problem of counting *k*mer frequencies for a predefined set of *k*mers in the input reads, and *kage*, which

finally computes the genotype-probabilities based on the *k*mer counts provided by *kmer_mapper*. We will use KAGE to refer to the full KAGE genotyping pipeline, and *kage* to refer to the piece of software the performs the genotype-probability computations, which is one component of the total pipeline.

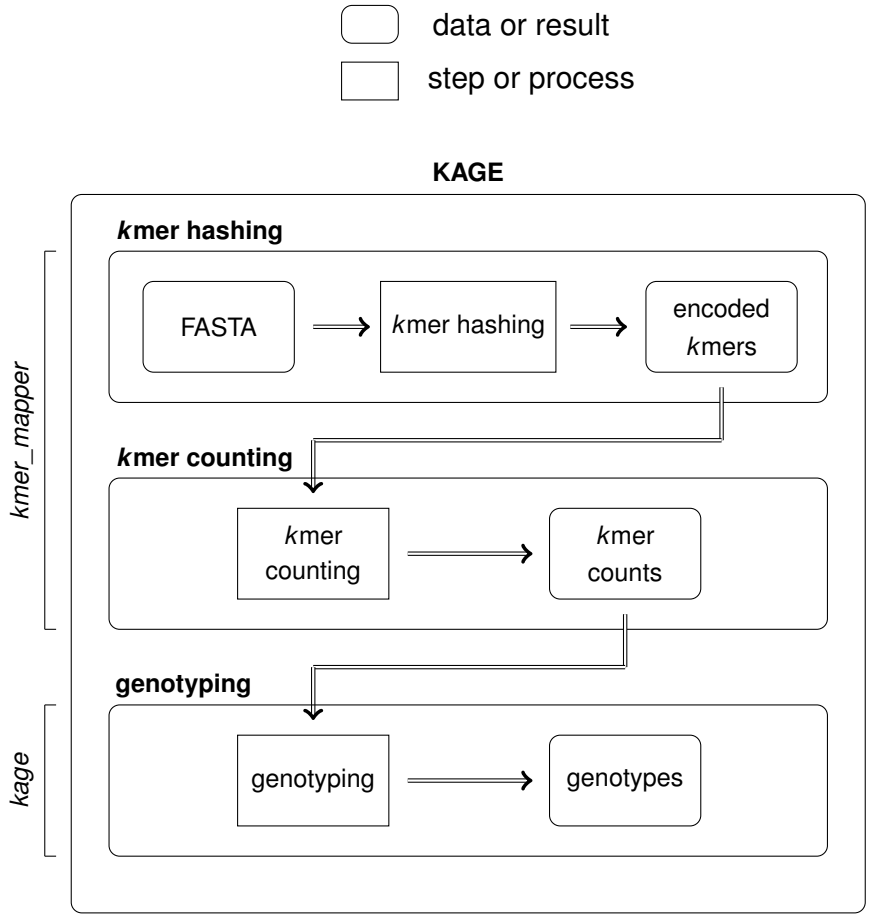


Figure 13: A simplified overview of the KAGE genotyping pipeline. Three important parts of the pipeline are shown as distinct processes in the illustration: 1) ***k*mer hashing**, which refers to the process of 2-bit encoding input reads from the fasta file and extracting - *hashing* - all valid *k*mers from those reads, 2) ***k*mer counting**, which refers to the process of *partial k*mer counting - counting the observed frequencies of a predefined set of *k*mers in the input reads, and 3) **genotyping**, which refers to the process of computing the final genotype-probabilities supported by the observed *k*mer counts. The *k*mer hashing and counting and the genotyping are implemented in two pieces of software respectively: *kmer_mapper* and *kage*.

3 Methods

In this section we cover the development process of GKAGE - a version of KAGE where parts of the pipeline are GPU accelerated for improved performance.

Firstly, we give an account of how we determined which parts to focus on GPU accelerating. Secondly, we describe a testing strategy that allowed us to GPU accelerate existing NumPy code directly in Python to effectively assess whether significant runtime improvements was plausible. Then, we cover how we developed GPU acceleration for three different components of KAGE by testing several different GPU acceleration methods, and for each component include an assessment showing the effects of GPU acceleration compared to the previous CPU runtimes. These preliminary benchmarking results for the GPU acceleration methods are included because they were used to guide development. Finally, we describe how we selected which GPU acceleration methods to integrate into KAGE, and how we then set up a Snakemake [26] pipeline to benchmark GKAGE's performance against KAGE.

3.1 Determining which Components to GPU Accelerate

When developing GKAGE, we started with KAGE as a baseline and analyzed the pipeline to find the most pronounced bottlenecks in terms of runtime. We then examined whether these bottlenecks could benefit from GPU acceleration by considering the type of computations performed, whether they would be suitable for the GPU's architecture, and how much of the overall runtime the component constituted. We would then intuit whether the component in question would be worthwhile trying to GPU accelerate given the project's time constraints and estimated difficulty.

The KAGE genotyping pipeline, which we described in section 2.6, is split into two distinct processes (or programs): 1) counting the *k*mer frequencies observed in the DNA reads of the individual being genotyped, and 2) genotyping the individual using a statistical model based on the observed and expected *k*mer frequencies. Initial benchmarking on a consumer desktop revealed that the *k*mer counting

step constituted nearly 97% of KAGE's total runtime, with the *k*mer counting step taking 2080 seconds and the genotyping step taking 68 seconds for a total of 2148 seconds (35 minutes and 48 seconds) to genotype a whole human genome with 15x coverage. Thus, the *k*mer counting step, having such a clear margin for runtime improvement over the genotyping step, was the main focus for GPU acceleration for potential runtime speedup.

3.2 Preliminary Testing

Before delving into the GPU accelerated methods used to produce the final GKAGE product, we will here describe an initial test we performed to assess two things. Firstly, we wanted to assess whether we could effectively GPU accelerate existing NumPy solutions using CuPy, all while staying within the comfort of Python. And secondly, whether such an implementation would result in significant runtime improvement over a CPU solution or at least provide insight into the plausibility of realizing a runtime improvement. For this, we focused on the *partial k*mer counting problem, detailed in section 2.3.

As detailed in section 2.5.5, npstructures is a Python library that enhances the NumPy library by providing additional data structures with NumPy-like behaviour and performance. Additionally, in section 2.5.5, we detailed a subset of npstructures' data structures, one of which was a counter object that efficiently counts occurrences of a predefined keys-set in a sample. This counter object had previously been utilized to count a predefined set of *k*mer's frequencies, albeit on the CPU.

Since npstructures' data structures are all heavily reliant upon NumPy's array routines for fast performance, they are designed around utilizing the CPU's vectorization and data parallelism. Large array operations where data parallelism matters are ideal for the GPU architecture, which often can provide orders of magnitude more parallelism this way. In addition, we know that most, if not all of the functionality used from NumPy, will be supported with GPU acceleration by CuPy through a nearly identical interface. Thus, by replacing the NumPy functionality used in npstructures' data structures with CuPy's equivalent GPU accelerated

functionality, we can GPU accelerate the necessary data structures in npstructures without having to leave Python. With this strategy we GPU accelerated npstructures' counter object using CuPy.

3.2.1 Using CuPy as a Drop-in Replacement for NumPy

Rather than creating a standalone package version of npstructures with GPU acceleration, we instead opted to add the possibility for GPU acceleration to the already existing package. This added a new self imposed requirement; we did not want CuPy to be a npstructures dependency since many users may wish to use it simply for the NumPy implementations. Additionally, we still needed a way to redirect NumPy function calls to their equivalent CuPy functions. We fulfilled both of these requirements by exploiting Python's module system.

Consider the following Python package example where our package, *mypackage*, contains two modules, *my_classes* and *my_funcs*, both relying on NumPy for their implementations:

mypackage.my_funcs.py

```
1 import numpy as np
2
3 def some_func_using_numpy():
4   return np.zeros(10)
```

mypackage.my_classes.py

```
1 import numpy as np
2
3 class SomeClassUsingNumPy:
4   def __init__(self):
5     self.data = np.zeros(10)
6
7   def get_data(self):
8     return self.data
```

Our package's initialization file imports our function and class from their respective modules, and all functionality is usable without needing to import CuPy in either module or initialization file. Pay attention to the initialization file's *set_backend* function which takes a library as a parameter and reassigns the np variable in both package modules from the NumPy to the provided library.

mypackage.__init__.py

```
from .my_funcs import some_func_using_numpy
from .my_classes import SomeClassUsingNumPy

# Swaps NumPy with lib (presumably CuPy)
def set_backend(lib):
  from . import my_funcs
  my_funcs.np = lib

  from . import my_classes
  my_classes.np = lib
```

In our program, where we will import our package, *mypackage*, we can either directly use our package's implementation with NumPy, or do as the following example shows and import CuPy and set the backend in the entire package to use CuPy functionality instead of NumPy.

program.py

```
import cupy as cp

import mypackage
mypackage.set_backend(cp)

array = mypackage.some_func_using_numpy()
type(array) # cupy.ndarray
```

Exploiting Python's module system this way has the benefits of not making CuPy

a dependency for npstructures, and it also allows for gradual GPU support by way of only updating the backend in modules where the existing implementations are ready to be ported as is to CuPy.

3.2.2 Resolving Unsupported or Poorly Performing Functionality

Two issues can arise when utilizing this method of GPU accelerating NumPy code. Firstly, some NumPy solutions may be effective on the CPU's architecture but ineffective on the GPU's, thus, resulting in poor performance. This will often be the case when significant portions of the code needs to be run in a serial fashion, as opposed to parallel, or when array sizes are too small to mask the overhead of copying data to and from the GPU memory. Secondly, certain NumPy functions are simply not supported by CuPy. In the case of the former issue, one might want to create an alternative solution that better utilizes the GPU's strengths, such as its massive parallelism. For the latter issue, there is no other practical option than to create a custom solution that achieves the desired behaviour by using what CuPy functionality is available. We circumvented both of these issues by creating custom implementations where we had the freedom to use the full extent of CuPy's functionality to reproduce the desired behaviour. For classes, this can be achieved by subclassing and overriding methods where we wish to create our new custom implementations. To demonstrate this, we will have to edit our example package, *mypackage*.

Consider if our package's class definition had instead been the following:

mypackage.my_classes.py

```
1 import numpy as np
2
3 class SomeClassUsingNumPy:
4   def __init__(self):
5     self.data = np.zeros(10)
6
7   def pad_with_ones(self):
8     arr = self.data
```

```
9     self.data = np.insert(arr, [0, len(arr)], 1)
```

In the code above, we use NumPy's insert function, which as of April 2023 is not supported by CuPy. To circumvent this issue, we can subclass our *SomeClassUsingNumPy* class and create our own custom implementation of *pad_with_ones*. We will create a new file where we implement our CuPy compatible solution, and our subclass will override the *pad_with_ones* method.

mypackage.cp_my_classes.py

```
1 import numpy as np
2 import cupy as cp
3
4 from .my_classes import SomeClassUsingNumPy
5
6 class CPSomeClassUsingNumPy(SomeClassUsingNumPy):
7   def pad_with_ones(self):
8     arr = self.data
9     self.data = cp.pad(arr, (1, 1), 'constant',
        constant_values=1)
```

In the above example we override the *pad_with_ones* method with our CuPy implementation. Our alternative CuPy implementation uses a different CuPy function to achieve the same behaviour as NumPy's insert, the *pad* function. Now, our two different *pad_with_ones* implementations will behave equivalently, although our custom implementation will leverage CuPy and be GPU accelerated.

Finally, we need to make one small change to our package's initialization file so that users of our package will import our CuPy compatible subclass of *SomeClassUsingNumPy* after setting the backend to CuPy:

mypackage.__init__.py

```
1 from .my_funcs import some_func_using_numpy
2 from .my_classes import SomeClassUsingNumPy
```

```
3
4  # Swaps NumPy with lib (presumably CuPy)
5  def set_backend(lib):
6    from . import my_funcs
7    my_funcs.np = lib
8
9    from . import my_classes
10   my_classes.np = lib
11
12   # Use CuPy compatible version of SomeClassUsingNumPy
13   global SomeClassUsingNumPy
14   from .cp_my_classes import CPSomeClassUsingNumPy
15   SomeClassUsingNumPy = CPSomeClassUsingNumPy
```

By utilizing this method, we implemented partial GPU support for the npstructures library, including enabling GPU support for the counter object used to count occurrences of a predefined key-set in a sample.

3.2.3 Assessment

To assess the effect GPU accelerating npstructures by using CuPy as a drop-in replacement for NumPy, we benchmarked the GPU accelerated npstructures counter object by counting the occurrences of a predefined set of 50 million unique *k*mers in a set of 20 million DNA reads, each of length 150. This benchmark used BioNumPy to read the FASTA file in chunks of 10 million bytes, 2-bit encode the reads and hash the valid *k*mers before counting. The time spent preparing each chunk was not included in the final measured runtime, since we were only interested in seeing the effects of GPU accelerating npstructures' counter object. It is important to note that while this benchmark provided a good assessment of the speedup gained from GPU accelerating our counter, it omits the overhead of transferring each chunk of *k*mers to the GPU in order to be counted by our GPU accelerated counter. We justified the omission of this overhead because of two reasons. Firstly, during our initial testing we were only interested in assessing the runtime differences during processing on the GPU versus the CPU when using

CuPy as a drop-in replacement for NumPy. Secondly, we only GPU accelerated one component of a much larger pipeline, and in such a case, the true effects of GPU acceleration may be overshadowed. We intended to GPU accelerate several consecutive components in a way where only a single copy step would need to be performed before several consecutive processing steps could benefit from GPU acceleration, minimizing the effects of the CPU to GPU copy overhead.

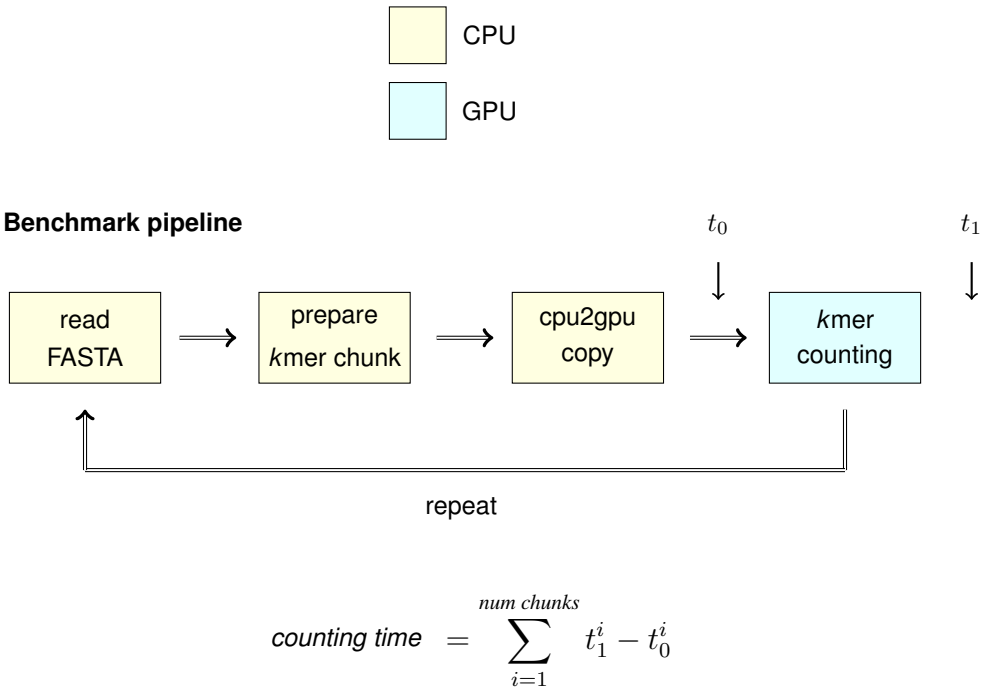


Figure 14: The pipeline used to benchmark the CuPy-based against the NumPy-based npstructures counter object. Only the calls made to the counter object to count chunks of *k*mers are timed, and these times are finally summed to get the total runtime spent on counting *k*mers. The difference between the two runtimes are then used to infer the effects of GPU accelerating npstructures' counter object using our initial testing method.

We ran the *k*mer counting pipeline and measured the time spent counting *k*mers for both the NumPy-based CPU version of the counter and the CuPy-based GPU version. The total time spent counting *k*mers was measured by timing each call to the counter object and summing these runtimes in order to get the total counting time. For the GPU version, we synchronized the GPU to the CPU between each call, since kernel calls made from the CPU to the GPU are asynchronous

otherwise. This benchmark yielded the following results:

Method	Counting time (seconds)
NumPy	234.7
CuPy	24.31

Table 1: The total time spent counting *k*mers using npstructures' counter object, one with NumPy as the backend array-library, running on the CPU using one thread, and the other using CuPy as the backend, running on the GPU.

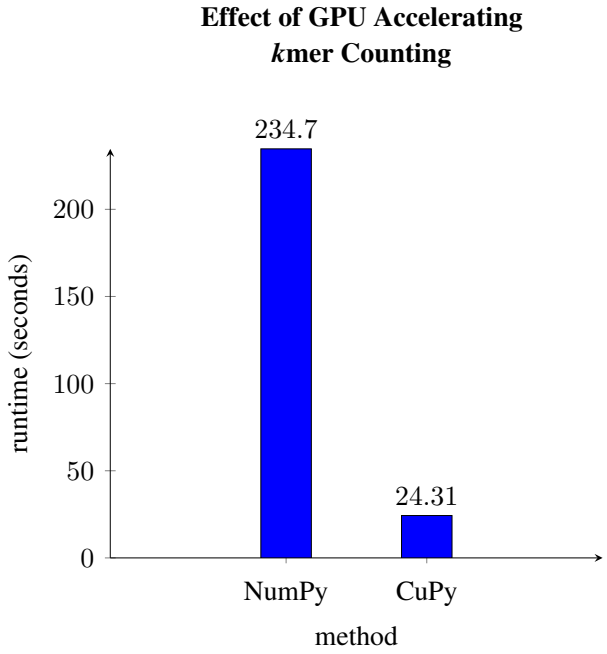


Figure 15: Elapsed time counting the occurrences of 50 million unique *k*mers in a set of 20 million reads, each of 150 bases. Both methods use BioNumPy to read, 2-bit encode and hash chunks of *k*mers, reading 10 million byte chunks from the FASTA at a time. Both methods also use npstructures' NumPy-based counter object to count the *k*mer frequencies, although the CuPy-based version replaces NumPy with CuPy to achieve GPU acceleration.

As can be seen in table 1, simply using CuPy as a drop-in replacement for NumPy in npstructures to GPU accelerate its counter object yielded a nearly 10X speedup in counting efficiency, without having to otherwise change the implementation or even leave Python.

3.3 GPU Accelerating *k*mer Counting

While several *k*mer counting solutions have been developed in previous work, with at least one having support for GPU acceleration [17], most such tools are designed to solve the *full k*mer counting problem, which we described in section 2.3. Additionally in section 2.3, we described how KAGE's *k*mer counting step is a less compute and memory demanding problem which we defined as *partial k*mer counting. As a reminder: we defined *partial k*mer counting as the process of only counting the observed occurrences of a predefined set of *k*mers in a sample. Repurposing *k*mer counting tools designed to solve the *full k*mer counting problem is futile if the goal is speedup. Thus, we opted to implement our own GPU accelerated *k*mer counting tool where its design is considered in the context of the *partial k*mer counting problem.

Although we succeeded in GPU accelerating a hash table in npstructures using CuPy, we decided also to attempt to implement a hash table directly in C++ using CUDA, Nvidia's programming platform. Moving to C++ and CUDA would allow for a more granular implementation as we would no longer be constrained to a solution originally designed for NumPy array functions.

3.3.1 GPU Hash Table Implemented using CUDA

The hash table's interface needed to support three main operations:

- ***insert*** - insert each *k*mer found in an input array of *k*mers (although only once upon initialization),
- ***count*** - increment the value associated with each *k*mer found in an input array of *k*mers, and
- ***query*** - fetch the values associated with each *k*mer found in an input array of *k*mers.

Common for all three operations mentioned above is that they need to adhere to an addressing and probing scheme. Since our hash table resides on the GPU, certain common paradigms such as open hashing - where each address in the table

contains a pointer to a linked-list or tree-type data structure for placing values - are immediately disqualified. This is because GPUs, which architectures are designed for massive data parallelism, perform poorly when needing to dereference pointers and make many strided memory accesses. Thus, we used a simple scheme using open addressing and linear probing.

Two arrays of equal size comprise the data structure where one array stores the table's keys (*k*mers) and the other array stores its values (counts). The keys array is an array of 64-bit unsigned integers. The choice of using 64 bits for the keys, as opposed to e.g. 32, was to accommodate larger *k*mer values since 32 bits would only allow for *k* up to $(32/2) - 1 = 15$. Using 64-bit keys increases our maximum *k* size to 31, which is the default value for KAGE. This choice also reveals a limitation of our hash table, which is that it can not support *k*mers where $k > 31$ in its current form. The reason why 64 bits does not allow for *k* up to 32, considering a 2-bit encoded 64-mer can be stored in a single 64-bit integer, is because the max 64-bit integer value is used as an indicator that a slot is empty in the hash table. The values array is an array of 32-bit unsigned integers. While 16-bit unsigned integers would suffice for our purpose of counting *k*mers, the choice of using 32-bit unsigned integers instead was made because the CUDA framework does not have a readily usable implementation of the atomicAdd function for 16-bit integers.

The probing scheme we used - linear probing - describes how we solve collisions in the hash table. This scheme is shared for all three operations supported by the hash table: insert, count, and query. A murmur hash [27] is used to hash and compute the first search index for a *k*mer. The initial probe index p_0 and every consecutive probe index p_i for a *k*mer k can be found using:

$$p_0 = hash(k) \bmod c \qquad\qquad p_i = (p_{i-1} + 1) \bmod c \tag{3}$$

where $hash$ is the murmur hash function and c is the capacity of the hash table (the length of the arrays).

For example, in a hash table of capacity c, we query a *k*mer k using the following

algorithm:

```
input: uint64[] keys, uint32[] values, int c, uint64 k
begin
  h ← murmur(k) mod c
  while true do
    if keys[h] = k then
      return values[h]
    else if keys[h] = empty then
      return 0
    else
      h ← (h + 1) mod c
    end if
  end while
end
```

Figure 16: The algorithm used for querying keys (*k*mers) in the parallel GPU hash table implemented in C++ using CUDA. When querying an array of N *k*mers, N CUDA threads are launched and each thread is assigned a *k*mer. The threads then perform this algorithm in a parallel fashion to query many *k*mers in parallel. The probing scheme used in this algorithm is the same used in both insertions and updating of values.

When computing the initial probe position in the hash table, using a murmur hash function results in more uniform placements across the table as opposed to just using the *k*mers integer value instead. This, in turn, results in less clustering when inserting *k*mers, meaning we need to deal with fewer collisions.

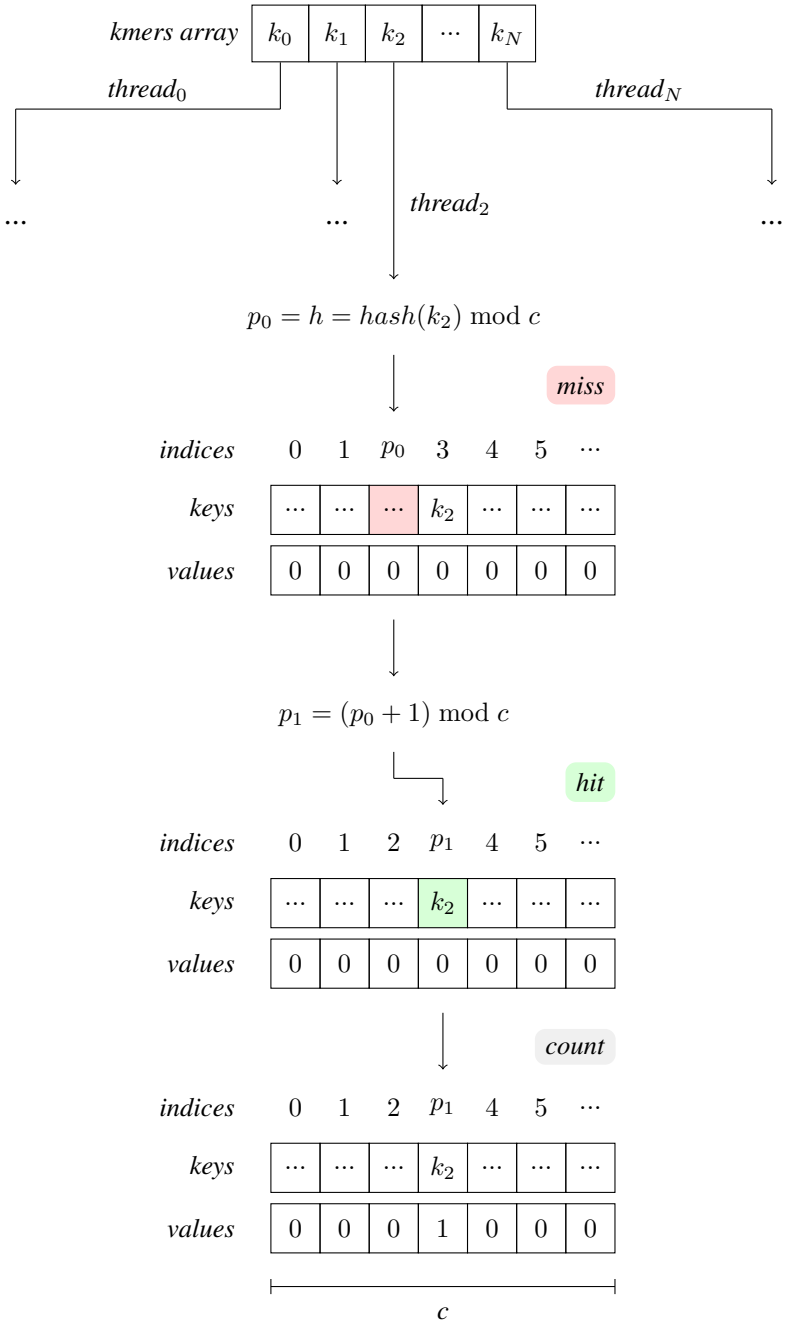


Figure 17: As an array of N 64-bit integer encoded kmers are counted by the hash table, N CUDA threads will launch and each will compute the first probe position p_0 for its assigned kmer k. Then, if the key at slot p_0 does not contain k, it will continue probing by linearly moving up to the next consecutive slot until either an empty key or k is observed. If an empty key is observed, the thread terminates without changing any of the hash table's values. If k is observed, the current slot's value is incremented.

3.3.1.1 Python Integration

In order to integrate the hash table implemented in C++ using CUDA to Python so that it would be compatible with KAGE, we used pybind11 (introduced in section 2.5.7) to create Python bindings for the C++ class and then wrap the implementation in a Python class. pybind11 provides easy-to-use macros for this, allowing us to add bindings by simply creating a bindings C++ file and then compiling a module where our C++ functionality is contained.

The produced module is finally wrapped around a Python class that takes care of any transitional details between usage and the C++ interface, such as determining whether to call upon a version of a function that expects NumPy arrays with data allocated in the host's RAM or a CuPy array with data allocated in the GPU's RAM.

The final implementation of this CUDA accelerated hash table can be found and installed at https://github.com/kage-genotyper/cucounter.

bindings.cpp

```cpp
#include <pybind11/pybind11.h>
namespace py = pybind11;

int my_function(int a, int b) {
  return a + b;
}

class MyClass {
public:
  MyClass(int number) : number(number) {}
  int get_number() const { return number; }
private:
  int number;
};

// Use pybind11's PYBIND11_MODULE macro to create Python
   bindings for our simple function and class
PYBIND11_MODULE(my_module, m) {
  m.doc() = "Documentation for my module";

  m.def("my_function", &my_function);

  py::class_<MyClass>(m, "MyClass")
    .def(py::init<int>())
    .def("get_number", &MyClass::get_number);
}
```

Figure 18: An illustration of how pybind11 can be used to create Python bindings for C++ functions and classes using a simple C++ macro: PYBIND11_MODULE. The C++ project can then be compiled to produce the module that can be imported directly into Python. pybind11 takes care of translating common data types automatically, including some data structures such as tuples and lists. In addition, pybind11 has specific support for NumPy, making the usage of NumPy arrays seamless.

3.3.2 Assessment

To assess our new CUDA hash table implementation, we ran the benchmark described in section 3.2.3, but using the CUDA hash table with Python bindings for *k*mer counting instead of npstructures' counter object. The benchmark yielded the following results:

Method	**Counting time (seconds)**
NumPy	234.7
CuPy	24.31
CUDA	2.94

Table 2: The total time spent counting *k*mers using the CUDA hash table implemented in C++ with Python bindings. NumPy and CuPy refers to the previously found runtimes using npstructures' counter object with NumPy- and CuPy-backends respectively.

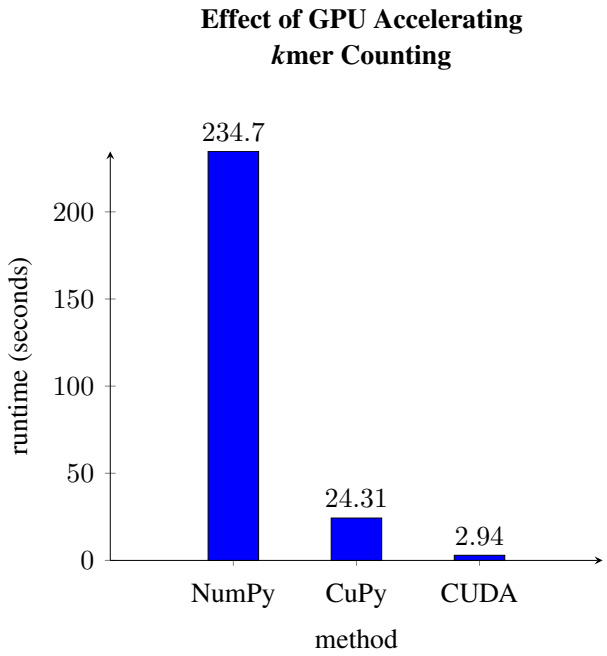


Figure 19: Elapsed time counting the occurrences of 50 million unique *k*mers in a set of 20 million reads, each of 150 bases.

As shown in table 2, the CUDA hash table implemented in C++ with Python

bindings provided significantly better counting efficiency compared to the CuPy-based npstructures counter, with nearly 80X speedup compared to the original NumPy-based counter and more than 8X speedup compared to the CuPy-based counter.

3.3.3 Re-Implementing the Hash Table Directly in Python

While the CUDA hash table we implemented in the previous section yielded significant speedup when *k*mer counting and much better results than the GPU accelerated version of npstructures' hash table, it required us to delve into C++, using CUDA's programming framework and leaving the comforts of Python behind in order to implement. We therefore explored another possible avenue for implementing such a hash table, this time directly in Python. CuPy offers more than just a GPU accelerated subset of NumPy's array interface. Additionally, CuPy allows for custom kernels written directly in Python, to be *jit* (just-in-time) compiled. By exploiting this, we were able to re-implement our parallel hash table directly in Python using CuPy's jit functionality. This implementation can be found online at https://github.com/jorgenwh/cupycounter.

3.3.4 Assessment

Again, we used the same benchmark used to assess the CuPy-based npstructures counter and the CUDA hash table (section 3.2.3) also to assess the CuPy JIT (just-in-time) compiled implementation where we wrote custom kernels to get near-identical behaviour and performance as the CUDA hash table, all directly in Python. The benchmark yielded the following results:

Method	Counting time (seconds)
NumPy	234.7
CuPy	24.31
CUDA	2.94
CuPy JIT	2.99

Table 3: The total time spent counting *k*mers using the just-in-time compiled kernels written directly in Python using CuPy's custom kernel support. NumPy, CuPy and CUDA refers to the previous benchmark results from section 3.2.3 and 3.3.2.

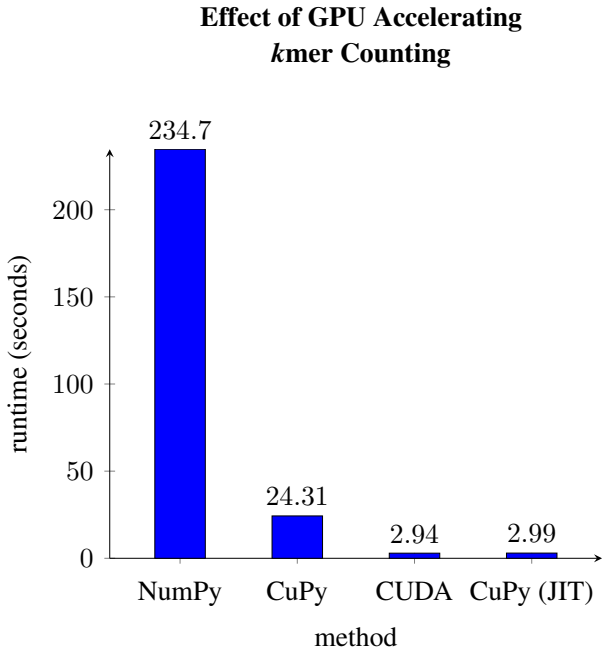


Figure 20: Elapsed time counting the occurrences of 50 million unique *k*mers in a set of 20 million reads, each of 150 bases.

As shown in table 3, the CuPy just-in-time compiled method of GPU accelerating *k*mer counting resulted in (close to) identical performance as the CUDA hash table implementation, all in spite of being implemented directly in Python using CuPy's custom kernel support. The memory usage using the CuPy just-in-time compiled solution was also practically identical to that of the CUDA hash table.

3.4 GPU Accelerating *k*mer Hashing

The *k*mer hashing component of KAGE is responsible for reading genomic reads from FASTA, FASTQ or other file types, encoding the reads as 2-bit encoded data and finally hashing all valid *k*mers from the 2-bit encoded reads. In KAGE, this component's output is a 64-bit unsigned integer array where each element is a 2-bit encoded 31-mer represented in the right-most 62 bits of the integer. Since the number of valid *k*mers in a typical FASTA or FASTQ from sequencing a human genome is extremely vast, the file is usually read, encoded and hashed in chunks to alleviate the required amount of memory. Functionality for this exists in the BioNumPy Python package. BioNumPy implements this using NumPy and some parts of npstructures, resulting in an efficient solution that relies on NumPy's fast array operations for its performance.

3.4.1 Replacing NumPy with CuPy

The Python package BioNumPy, which implements *k*mer encoding and hashing directly in Python, is built on top of NumPy and npstructures. We utilized the method described in section 3.2 and used CuPy as a drop-in replacement for NumPy to add further GPU acceleration support to npstructures, and partial GPU support to BioNumPy. The resulting pipeline remained the same, but with two notable differences.

1. The raw byte chunk read from the FASTA files would be copied directly to the GPU.
2. The chunk of data received on the GPU would go through the same pipeline of array operations to 2-bit encode and hash the *k*mers, but on the GPU as opposed to the CPU.

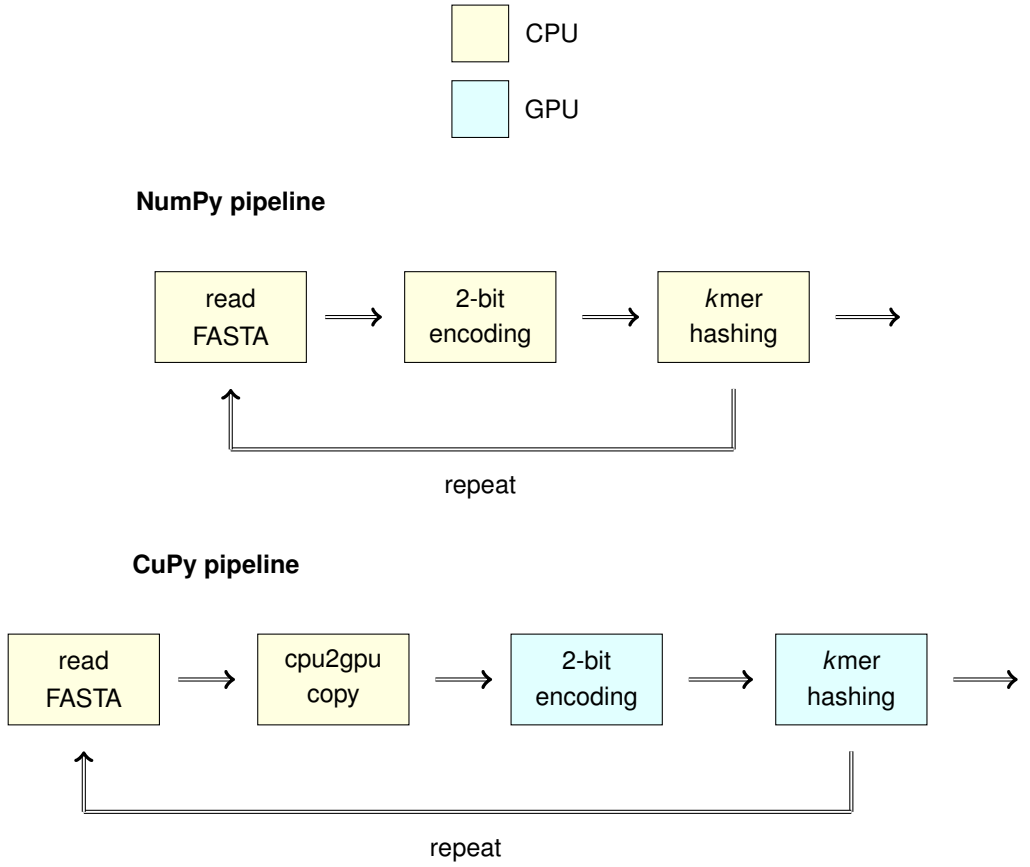


Figure 21: By using CuPy as a drop-in replacement to add GPU acceleration to KAGE's *k*mer hashing step, we effectively introduced a new step in the pipeline: copying the raw text data read from the FASTA file directly to the GPU to allow for all of BioNumPy's array operations that constitute 2-bit encoding and hashing the *k*mers to be performed on the GPU.

Upon completing the 2-bit encoding and *k*mer hashing of the chunk, the resulting *k*mer array already resides in GPU memory for the counting step. Previously, when the 2-bit encoding and *k*mer hashing were performed using only the CPU, the resulting *k*mer array would need to be copied to the GPU for counting when deploying the GPU accelerated *k*mer counting functionality detailed in section 3.3.

3.4.2 Assessment

To assess the effects of GPU accelerating *k*mer hashing, we measured the total runtime of reading, 2-bit encoding and hashing every valid *k*mer from a FASTA file containing 20 million reads, each of length 150. The FASTA was read in chunks of 10 million bytes. We benchmarked this runtime using BioNumPy with the standard NumPy backend, and again using CuPy as the backend to compare. The benchmarking yielded the following results:

Method	**Hashing time (seconds)**
NumPy	62.46
CuPy	5.1

Table 4: The runtimes found by hashing all valid *k*mers from a set of 20 million DNA reads, each of length 150. BioNumPy was used to read the FASTA file in chunks of 10 million bytes, then 2-bit encode and hash each chunk. We ran the benchmark both using NumPy as the backend-library for BioNumPy, meaning the hashing was performed entirely on the CPU using a single thread, and using CuPy as the backend-library for BioNumPy, meaning the raw data read from the FASTA file was copied to the GPU and then 2-bit encoded and hashed there.

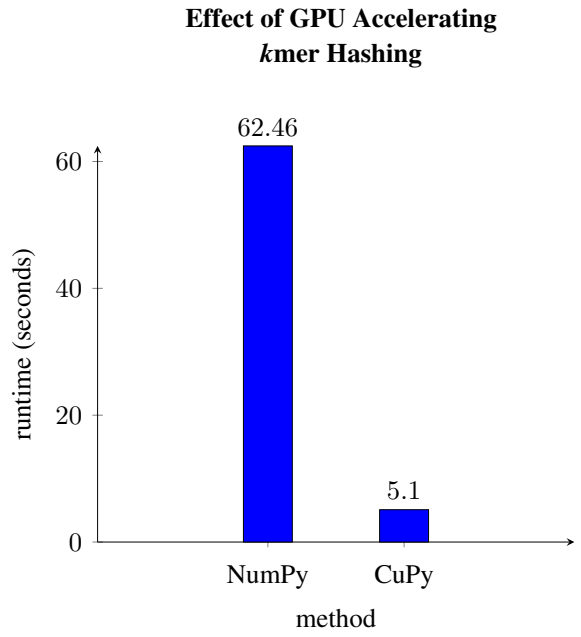


Figure 22: Elapsed time spent reading, 2-bit encoding and hashing all valid *k*mers from 20 million reads in 10 million byte chunks using both NumPy as CuPy to perform the processing on the CPU and GPU respectively.

As shown in table 4, GPU accelerating BioNumPy's *k*mer hashing functionality, simply by using CuPy as a drop-in replacement for NumPy, yielded a more than 12X speedup when hashing every valid *k*mer in a set of 20 million reads of length 150. This dramatic increase in efficiency is achieved despite having to copy each chunk of raw data read from the FASTA file to the GPU memory before processing can begin. Recall that in section 3.2.3, we justified only benchmarking the time spent counting *k*mers on the GPU without including the overhead of copying the *k*mer chunks to the GPU memory. Combined with the *k*mer hashing component's GPU acceleration, which yields a dramatic increase in efficiency despite including this overhead, *k*mer chunks that are hashed on the GPU will already reside in the GPU's memory for counting.

3.5 GPU Accelerating Genotyping

The genotyping step of KAGE (introduced in section 2.6) computes genotype-probabilities supported by the *k*mer counts found during the *k*mer counting portion. A central part of this step is computing the *logarithm of the probability mass function* (LOGPMF) a large number of times on large arrays. Because the LOGPMF computations took up most of the runtime in KAGE's genotyping step and its implementation relied on computing many parallel mathematical expressions for large arrays, we chose to focus on LOGPMF to GPU accelerate the genotyping step.

The logarithm of the probability mass function at k is computed using:

$$o = k * \log(\mu) - \mu - \ln(|\Gamma(k+1)|) \tag{4}$$

where Γ is the gamma function. In KAGE, this formula is computed for every element at every index in two equal-sized arrays k and μ. The original implementation utilizes NumPy's efficient array-operations to compute LOGPMF efficiently using data-parallelism on the CPU.

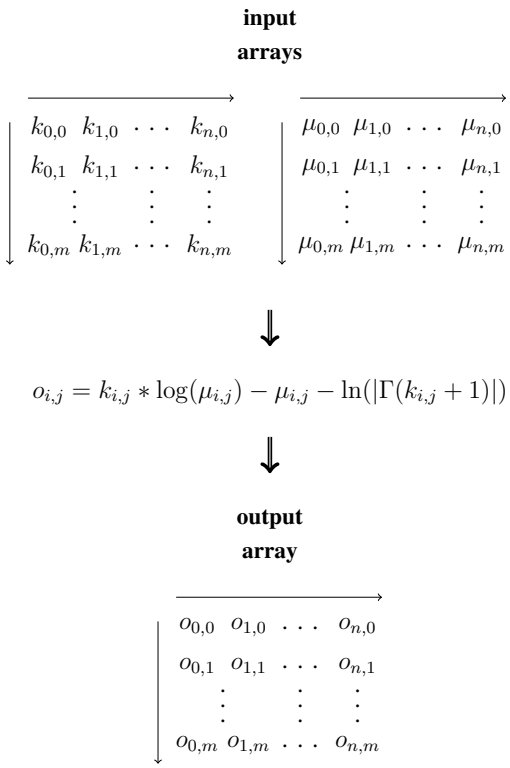


Figure 23: The LOGPMF computation in KAGE is performed on large arrays, relying on NumPy's efficient and data-parallel solutions to run efficiently on the CPU.

3.5.1 Replacing NumPy with CuPy

Since the original KAGE solution for computing LOGPMF was implemented using NumPy, we first attempted to GPU accelerate the LOGPMF using the method described in section 3.2.1, where we used CuPy as a drop-in replacement for NumPy. The NumPy solution from KAGE relies on SciPy [22] to compute the natural logarithm of the gamma function: $\ln(|\Gamma(x)|)$. For our CuPy solution, we instead used CuPy's cupyx.scipy's implementation.

logpmf.py

```
1  import numpy as np
2  import cupy as cp
3
4  # Original function used in KAGE. Assumes k and r are
       NumPy arrays
5  def numpy_poisson_logpmf(k, r):
6    return k * np.log(r) - r - scipy.special.gammaln(k+1)
7
8  # Assumes k and r are CuPy arrays
9  def cupy_poisson_logpmf(k, r):
10   return k * cp.log(r) - r -
         cupyx.scipy.special.gammaln(k+1)
```

Figure 24: The original NumPy-based solution used in KAGE for computing LOGPMF, and a CuPy-based version for GPU acceleration. The CuPy-based GPU accelerated version assumes both input arrays, k and r, are residing in GPU memory.

3.5.2 Assessment

For the purpose of assessing the effect of GPU accelerating KAGE's LOGPMF function by using CuPy, we benchmarked both the original NumPy-based and the GPU accelerated CuPy-based functions by computing LOGPMF for input arrays k and r. The k array was initialized with 40 million 32-bit integers, randomly set to values between 1 and 100. r was initialized with 40 million 64-bit floating point numbers, randomly set to values between 1 and 100.

The CuPy-based GPU accelerated function presented in figure 24 requires both input arrays, k and r, to be allocated in GPU memory. Benchmarking only the function calls as presented therefore ignores the overhead of copying both k and r to GPU memory before the function call can be made. Thus, for completion, we did not only benchmark both the NumPy and CuPy solutions. We additionally benchmarked the CuPy solution where k and r start off in RAM and are copied to GPU memory before processing and the result array is copied back to RAM afterwards. This way, our benchmarking accounted for the overhead of copying data to and from the GPU.

Our benchmarking yielded the following results:

Method	**Time (milliseconds)**
NumPy	606.94
CuPy (with copy)	201.9
CuPy (without copy)	82.49

Table 5: Time spent computing the logarithm of the probability mass function for 40 million elements. **NumPy** uses the NumPy-based solution with CPU data-parallelism and a single core. **CuPy (with copy)** uses the CuPy-based solution with GPU acceleration, but k and r are both copied from RAM to GPU memory before processing begins, and finally the result array is copied from GPU memory back to RAM. **CuPy (without copy)** uses the CuPy-based solution with GPU acceleration, but k and r are both already residing in GPU memory, and the result array is not copied back to RAM. In other words, we only measure the processing time of computing the 40 million LOGPMF values. Runtimes are the mean of 100 runs.

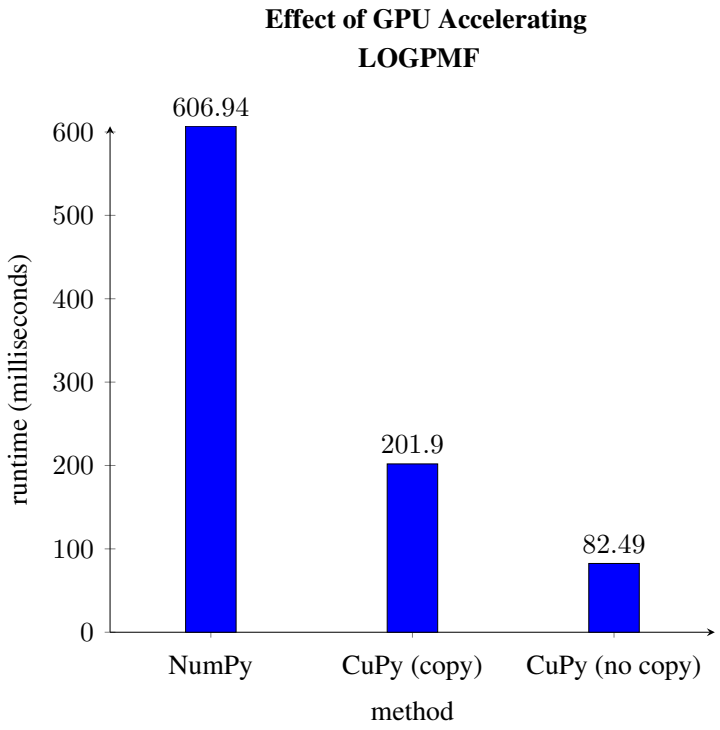


Figure 25: Time (milliseconds) spent computing the LOGPMF for input arrays of 40 million elements.

3.5.3 Implementing LOGPMF using CUDA

While the method of using CuPy as a drop-in replacement for NumPy provided a significant speedup when computing the LOGPMF, it reveals a problem that arises when using NumPy and CuPy in Python. When computing a nested expression, such as when computing the LOGPMF shown in equation 4, the expression will be computed and evaluated following Python's evaluation rules, subsequently creating several temporary arrays. As a result, array allocations, de-allocations, memory writes, and reads are happening needlessly while evaluating the Python expression.

By moving into C++ and using CUDA, we can create our own CUDA kernel to compute the LOGPMF for large arrays, avoiding these redundant operations. We implemented a simple kernel where we avoid needlessly storing sub-expressions in temporary arrays.

logpmf_kernel.cu

```
__global__ void logpmf_kernel(
    const int *k, const double *r, double *out, const int
        size) {
  int i = blockIdx.x * blockDim.x + threadIdx.x;
  if (i < size) {
    out[i] = k[i] * logf(r[i]) - r[i] - lgammaf(k[i] + 1);
  }
}
```

Figure 26: The CUDA kernel implemented for computing the LOGPMF for arrays on the GPU.

3.5.4 Assessment

To benchmark the CUDA kernel implementation we used the same benchmark as for the CuPy solution described in section 3.5.2. Our benchmarking yielded the following results:

Method	Time (milliseconds)
NumPy	606.94
CuPy (with copy)	201.9
CuPy (without copy)	82.49
CUDA (with copy)	68.33
CUDA (without copy)	4.88

Table 6: Time spent computing the logarithm of the probability mass function for 40 million elements. **NumPy**, **CuPy (with copy)** and **CuPy (without copy)** are the benchmark results from table 5. **CUDA (with copy)** uses the CUDA kernel implementation, but k and r are both copied from RAM to GPU memory before the kernel is launched, and the result array is copied from GPU memory back to RAM. **CUDA (without copy)** uses the CUDA kernel implementation, but k and r are both already residing in GPU memory and the result array is not copied from GPU memory to RAM. Runtimes are the mean of 100 runs.

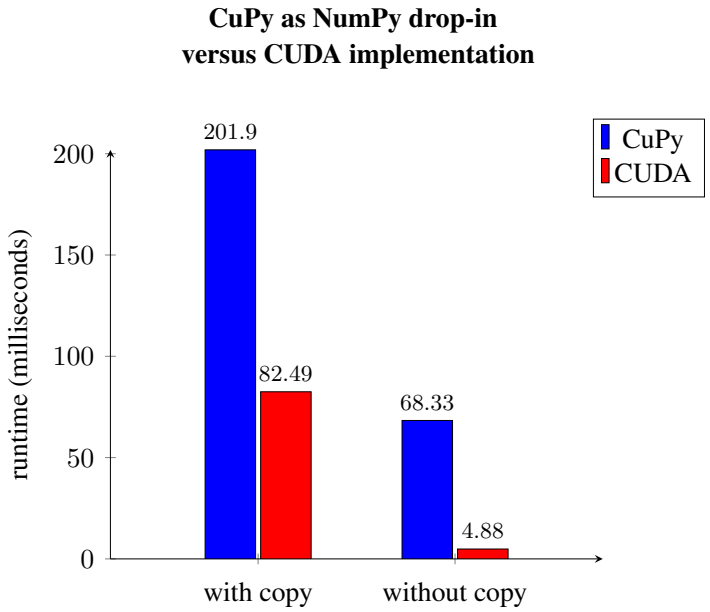


Figure 27: Time (milliseconds) spent computing the LOGPMF for input arrays of 40 million elements.

As shown in table 6, implementing our own CUDA solution, alleviating the wasteful array allocations and memory reads and writes, resulted in a significant speedup.

3.5.5 Extending the LOGPMF Implementation

Despite already having achieved significant speedup using CuPy and CUDA to GPU accelerate LOGPMF, further examination of KAGE revealed that more of the context around the LOGPMF function call was eligible to be GPU accelerated. The original part of KAGE that made calls to the LOGPMF function is presented below:

kage/sampling_combo_model.py

```
1 def extended_logpmf(observed_counts, counts):
2     sums = np.sum(counts, axis=-1)[:, None]
3     frequencies = np.log(counts / sums)
4     poisson_lambda = (np.arange(counts.shape[1])[None, :] +
          ERROR_RATE) * BASE_LAMBDA
5     prob = logpmf(observed_counts[:, None], poisson_lambda)
          # LOGPMF call is made here
6     prob = logsumexp(frequencies + prob, axis=-1)
7     return prob
```

Figure 28: The function inside the KAGE genotyper implementation that uses the LOGPMF function. The outer function also relies on NumPy array operations for performance.

We used two previously described methods to implement the outer function from figure 28 with GPU acceleration: we used CuPy as a drop-in replacement for NumPy, and we implemented a CUDA kernel in C++ where we again were able to optimize away unnecessary array allocations and de-allocations, as well as RAM writes and reads. The implementations can be found (along with the GPU accelerated LOGPMF function) online at https://github.com/jorgenwh/custats.

3.5.6 Assessment

To assess the extended LOGPMF implementation, we benchmarked the NumPy-based version found in KAGE, the CuPy drop-in replacement for NumPy version, and the CUDA kernel implementation. For both GPU accelerated methods, namely the CuPy and CUDA methods, we additionally benchmarked their performances when inputs are copied from RAM to GPU memory beforehand and results are copied from GPU memory to RAM.

To benchmark, we initialized the input arrays *observed_counts* and *counts* (the model counts) to be randomly generated numbers. *observed_counts*, a one-dimensional array, contained 5 million 32-bit integers. *counts*, a two-dimensional array, contained 5 million rows of length 15, totalling 75 million 16-bit floating point numbers, except when running the CUDA version since we did not implement support for half-precision.

Our benchmarking yielded the following results:

Method	**Time (milliseconds)**
NumPy	2242.9
CuPy (with copy)	328.44
CuPy (without copy)	293.24
CUDA (with copy)	56.59
CUDA (without copy)	5.92

Table 7: Benchmarking results for the extended LOGPMF function. The results were determined from averaging the runtime of the NumPy implementation in KAGE, the same implementation with CuPy as backend array-library, both with and without including the time spent copying input arrays to the GPU and the result array from the GPU back to RAM, and the CUDA solution we implemented, both with and without copy times. The NumPy solution running on the CPU does not need to account for copying data to and from the GPU. Thus, no value is present for the 'with copy' bar for NumPy.

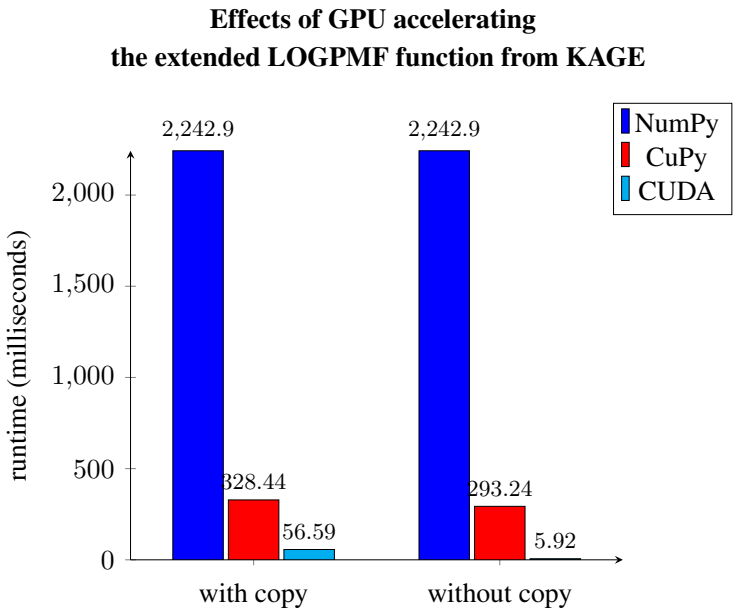


Figure 29: Benchmarking results for the extended LOGPMF function. The NumPy solution running on the CPU does not need to account for copying data to and from the GPU, thus, their runtimes are identical.

As can be seen in table 7, both the methods we tried for GPU accelerating the extended LOGPMF function found in KAGE yielded significant runtime speedup. Clearly, our CUDA implementation performs significantly better than simply using CuPy as a drop-in replacement for NumPy.

3.6 Integration into KAGE

Previously in section 3, we described how we implemented GPU acceleration for three components of KAGE: the *k*mer hashing, *k*mer counting, and genotyping components. Several different methods were explored with different yields in terms of runtime efficiency. Common for each GPU accelerated component is that they were GPU accelerated in isolation to keep their modular behaviour identical to the non-GPU accelerated versions. Thus, each accelerated component could be used as drop-in substitutes for their non-GPU counterparts in KAGE.

For each component, we selected the GPU accelerated version using the method

that yielded the best runtime and integrated it into KAGE. The selected methods can be seen in table 8 below.

Component	**Method**
*k*mer hashing	CuPy as a NumPy drop-in replacement
*k*mer counting	custom CUDA hash table
LOGPMF (genotyping)	custom CUDA kernel

Table 8: The implementations chosen to be integrated into KAGE for GPU acceleration. The GPU accelerated *k*mer hashing and *k*mer counting components were integrated into the *kmer_mapper* program, while the GPU accelerated log of the probability mass function was integrated into the *kage* program.

Recall from section 2.6 that KAGE is composed of two separate pieces of software: *kmer_mapper* - performing the *k*mer hashing and counting, and *kage* - performing the genotype-probability computations supported by the *k*mer counts provided by *kmer_mapper*. In close collaboration with the KAGE developers, we substituted *kmer_mapper*'s *k*mer hashing and *k*mer counting components, and *kage*'s LOGPMF function, with our GPU accelerated versions. Additionally, in the final GPU accelerated version of *kmer_mapper*, the *k*mer hashing component delivered the resulting *k*mer arrays directly to the *k*mer counting hash table, thus, avoiding needless data copying between the host and the GPU. As a result, *kmer_mapper* only encounters the overhead of copying data between the host and the GPU once, copying the raw input buffer read from the FASTA file to the GPU. Then, 2-bit encoding, *k*mer hashing and *k*mer counting are all performed directly on the GPU.

The final GPU accelerated version of KAGE, composed of the GPU accelerated versions of *kmer_mapper* and *kage*, was ultimately named GKAGE (GPU KAGE). GKAGE was directly integrated into KAGE, and when installing KAGE from the web today, it comes with the GPU accelerated functionality out-of-the-box.

3.7 Benchmarking Setup

In order to benchmark the effectiveness of the added GPU acceleration in GKAGE, we decided to benchmark GKAGE against KAGE to account for the speedup. The choice of only benchmarking GKAGE against KAGE was made because of the fact that KAGE recently showed (2022) that it was an order of magnitude faster than any other known genotyper [4].

In section 2.3 we mentioned that another *k*mer counting tool, Gerbil [16], has support for GPU acceleration, but that Gerbil is designed to solve the *full k*mer counting problem (for *partial* and *full k*mer counting, refer to section 2.3). Therefore, for completeness, we also ran Gerbil (with GPU acceleration enabled) to compare its runtime against GKAGE's (*kmer_mapper*'s) *k*mer counting runtime where only the *partial k*mer counting problem is solved.

3.7.1 Snakemake Pipeline

In order to adequately benchmark GKAGE, we set up a Snakemake pipeline that runs KAGE and GKAGE on a full human genome with 15x sequencing coverage. Additionally, the Snakemake pipeline records the runtimes and asserts that the results of both processes are identical. The Snakemake pipeline will automatically download all necessary input files from the web. Then, the full KAGE genotyping pipeline, consisting of *kmer_mapper* and *kage*, is performed with and without GPU acceleration enabled. Additionally, the pipeline runs Gerbil with GPU acceleration enabled for *k*mer counting. All runtimes and results are cached and a HTML table is produced with the final runtimes. This Snakemake pipeline is available online at https://github.com/kage-genotyper/GKAGE-benchmarking.

3.7.2 Systems

We benchmarked GKAGE against KAGE on two different systems. **System 1**: a high-end compute server with a 64-core AMD EPYC 7742 CPU and two Nvidia Tesla V100 GPUs. **System 2**: a consumer grade desktop with a 6-core Intel Core i5-11400F CPU and a Nvidia GTX 1660 SUPER GPU.

System	CPU	GPU
1: High-end server	AMD EPYC 7742	2x Nvidia Tesla V100
2: Consumer desktop	Intel Core i5-11400F	Nvidia GTX 1660 SUPER

Table 9: The two systems used to benchmark GKAGE against KAGE. **System 1** is a high-end compute server with top-of-the-line hardware. **System 2** is a consumer grade desktop gaming computer.

Benchmarking on these two systems allowed us to benchmark GKAGE and compare its performance against KAGE and Gerbil on both a very as well as a less performant system to examine the effects of GPU acceleration in both instances. A caveat with the high-end server system (system 1) is that it is a high-end compute server available to both staff and students at the Biomedical Informatics research group at the University of Oslo. Therefore, others frequently ran jobs on the server, occupying CPU and GPU processing cores and memory. To the best of our ability, we conducted our benchmarkings during periods of lower activity.

4 Results

In the following section we present the benchmark results found by running the Snakemake pipeline introduced in section 3.7.1, the final GPU accelerated version of KAGE - GKAGE, as well as some additional results yielded by the work presented in this thesis.

4.1 Benchmarking

We here present our benchmarking results that demonstrate the effect of GPU accelerating the KAGE pipeline. While our initial benchmarks yielded positive results, one component of KAGE was replaced by an optimized solution by the KAGE developers in the time between our implementations were completed and GKAGE was released. As a result, the GPU accelerated version of the componet - the log of the probability mass function in the genotype-probability computation - was rendered redunant as it no longer provided sufficient speedup to be worthwhile using over the new solution introduced by the KAGE developers. Thus, we opted to only include the GPU accelerated components in *kmer_mapper*, enabling GPU acceleration for *k*mer hashing and counting, while using KAGE's new solution in the *kage* software for the best possible performance. For completeness, we still include the initial runtimes achieved, including the GPU accelerated LOGPMF function, to demonstrate the effects of GPU accelerating each component. Then, we show the final performance of GKAGE against KAGE.

4.1.1 Initial Runtimes

After incorporating the GPU accelerated components into *kmer_mapper* and *kage*, we benchmarked both programs to assess their respective runtime improvements. Additionally, we ran Gerbil [16] to compare the GPU accelerated *kmer_mapper*'s *k*mer counting against an existing *k*mer counting tool with GPU acceleration.

Program	**CPU**	**GPU**
kmer_mapper	484 sec	61 sec
Gerbil	-	156 sec
kage	68 sec	54 sec

Table 10: Benchmarking results from integrating the GPU accelerated components into *kmer_mapper* and *kage*. *kmer_mapper* and *kage* together constitute the KAGE genotyping pipeline. This benchmark was ran on a high-end compute server with an AMD EPYC 7742 64-Core CPU and two Nvidia Tesla V100 GPUs. Both *kmer_mapper* and *kage* were allowed 16 cores and the GPU versions were allowed one Nvidia Tesla V100 GPU. Gerbil was allowed 16 cores as well as one Nvidia Tesla V100 GPU.

As can be seen in table 10, the GPU accelerated *k*mer hashing and counting components yielded significant runtime improvements to the *k*mer counting portion of KAGE. Similarly, GPU acceleration yielded a faster runtime for the genotype-probabilities computation portion of KAGE, although a less dramatic improvement. Additionally, when enabling GPU acceleration for both *kmer_mapper* and Gerbil, *kmer_mapper* outperforms Gerbil for *k*mer counting by a large margin.

While the GPU accelerated functionality in the *kage* software resulted in some runtime decrease, an optimized solution, provided by the KAGE team after we introduced our implementation, yielded significantly better runtime results (on the CPU). This new solution cut the runtime of *kage* down to just 44 seconds on the same system used to find the runtimes presented in table 10. Thus, we opted not to include our GPU accelerated solution for *kage* in our final version of GKAGE.

4.1.2 Final Runtimes

After replacing our GPU accelerated version of the LOGPMF computation in *kage* with the new and more performant solution made by the KAGE developers, we achieved our final version of GKAGE.

We benchmarked GKAGE against KAGE on both the high-end compute server (system 1) and the consumer grade desktop computer (system 2) that we introduced in section 3.7.2. When running on system 1, we allowed KAGE and GK-

AGE 16 threads and GKAGE one Nvidia Tesla V100 GPU. When running on system 2, we allowed KAGE and GKAGE 6 threads and GKAGE the Nvidia GTX 1660 SUPER GPU. Gerbil, which only performed *full* *k*mer counting, was allowed 16 and 6 threads on system 1 and 2 respectively. We ran the Snakemake pipeline on both systems, benchmarking GKAGE against KAGE and running Gerbil. From this we found the following results:

System	KAGE	GKAGE	Gerbil (only *k*mer counting)
1	510 sec	94 sec	156 sec
2	1993 sec	178 sec	464 sec

Table 11: The runtimes (in seconds) found by benchmarking GKAGE against KAGE and running Gerbil for *k*mer counting on a high-end server and a consumer desktop computer

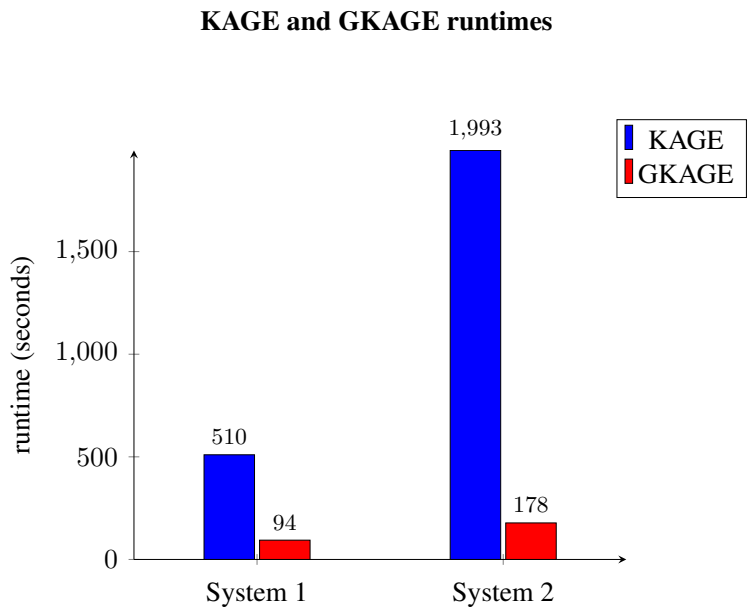


Figure 30: Benchmarking GKAGE against KAGE on both system 1 (high-end server) and system 2 (consumer desktop) revealed that GKAGE could genotype a full human genome more than 5 times faster than KAGE on a high-end server, and more than 11 times faster on a consumer desktop computer.

The results from the benchmarking revealed that GKAGE achieves significant speedup over KAGE when genotyping a full human genome. As seen in table 11

and figure 30, GKAGE achieves more than 5X speedup over KAGE on a high-end compute server and more than 11X speedup on a consumer grade desktop computer. Additionally, GKAGE's runtime when performing the full genotyping pipeline is smaller than that of Gerbil with GPU acceleration, despite Gerbil only performing *k*mer counting.

Since KAGE recently showed that it was an order of magnitude faster than any other known genotyping tool [4], GKAGE's significant speedup leads us to conclude that it is, to the best of our knowledge, now clearly the fastest method for genotyping an individual, allowing for the genotyping of a human genome in just 3 minutes on consumer hardware and 1.5 minutes on a high-end compute server.

4.2 GKAGE

As part of this master's project, we also present GKAGE: a new and improved version of KAGE that utilizes CUDA enabled GPUs to reduce the runtime of genotyping significantly. As shown in section 4.1.2, GKAGE outperforms KAGE up to 5X on a high-end compute server and 11X on a consumer grade desktop computer in terms of runtime, while producing identical outputs. Since KAGE recently showed that it was an order of magnitude faster than any other known genotyping tool, to the best of our knowledge, GKAGE is now the fastest existing method to genotype an individual. Additionally, GKAGE can run on commercial hardware, requiring less than 4GB of GPU memory. KAGE and GKAGE are accessible from the web and can be found at https://github.com/kage-genotyper.

The final KAGE pipeline with GPU acceleration integrated can be seen in figure 31 below.

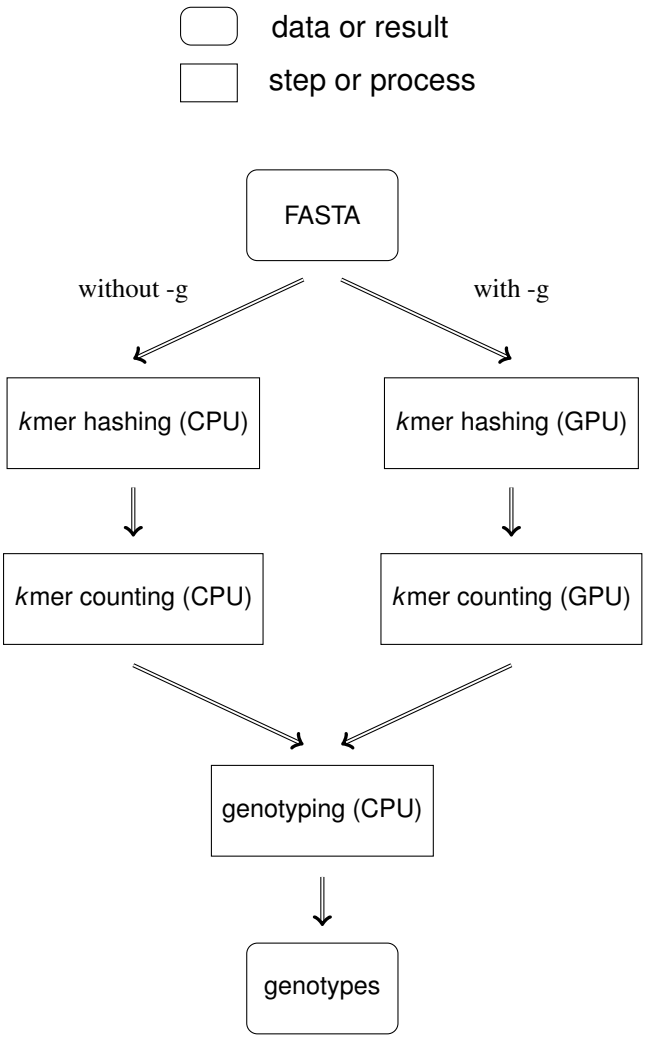


Figure 31: To run GKAGE, you simply run KAGE with the -g flag to indicate that you want to use the GPU to accelerate the processing. Out of the three components that we explored to GPU accelerate, only two made it into GKAGE. Thus, only *k*mer (encoding and) hashing and *k*mer counting is GPU accelerated when running KGAKE. The genotyping step, which constitutes a small portion of the runtime even on the CPU, is identical and runs on the CPU in both KAGE and GKAGE.

4.3 GPU Acceleration Methods

In our expedition to GPU accelerate components of the KAGE genotyping pipeline we found three distinct methods for GPU accelerating existing Python code based on NumPy. Each method was revealed to have certain advantages and

drawbacks. Through practical experimentation, we have uncovered interesting insight into how these methods can be utilized to accelerate a potentially vast field of existing tools by both developers and researchers.

4.4 bioRxiv Manuscript

As a part of this master's project, we, along with the KAGE developers, wrote a manuscript presenting GKAGE [28]. This manuscript has been deposited to bioRxiv - the preprint server for biology [29]. The manuscript, *Ultra-fast genotyping of SNPs and short indels using GPU acceleration*, presents GKAGE as a software tool and briefly details the runtime speedup gained over KAGE and how GKAGE was implemented. This makes GKAGE a contribution to international bioinformatics research, contributing to cutting down the time and cost of genotyping. The full manuscript can be found in appendix A.

5 Discussions

In section 3 we demonstrated three distinct methods of GPU accelerating existing Python code that uses NumPy. We used a combination of these methods to implement GPU acceleration for KAGE, resulting in a new and improved version: GKAGE. In section 4 we showed that GKAGE achieves a 5-11 times speedup compared to KAGE, depending on the type of hardware it is benchmarked on.

In this section we discuss some topics relevant to the project presented in thesis. This will include:

- ***Not using Gerbil for kmer counting***: why did we implement our own GPU accelerated *k*mer counting tool when a previous *k*mer counting tool, Gerbil [16], already had GPU acceleration support?
- ***Advantages and drawbacks of the GPU acceleration methods used***: Why do several possible GPU acceleration libraries and methods exist and why is there no one-size-fits-all solution?
- ***Drawbacks of GPUs***: what challenges do GPUs introduce?
- ***Significance of GKAGE***: what do GKAGE and its results mean for bioinformatics?
- ***Future work***: what are interesting and perhaps obvious improvements that can still be integrated into GKAGE?

5.1 Implementing Our Own *k*mer Counting Tool

In section 4.1 we showed that Gerbil spent more time performing just *k*mer counting, even with GPU support, than GKAGE spent performing the full genotyping pipeline. Our reason for implementing a new tool for *k*mer counting on the GPU, and why our tool seemingly performed so much better than Gerbil, was because the problems solved by Gerbil and GKAGE's *k*mer counting tool are inherently different. Recall from section 2.3, we defined *full k*mer counting as the problem of counting the occurrence of every valid *k*mer in a set of reads, and *partial k*mer counting as the problem of only counting the occurrences of *k*mers present in a predefined set of *k*mers in a set of reads. Unlike the *k*mer counting tool we imple-

mented for GKAGE, Gerbil solves the *full* *k*mer counting problem - a significantly more processing and memory demanding task compared to *partial* *k*mer counting. While Gerbil could have been used in GKAGE for the *k*mer counting portion, we would not only be counting the occurrences of superfluous *k*mers, we would additionally be required to parse Gerbil's output for the wanted *k*mer counts. It was for this reason that we decided to implement a new *k*mer counting tool targeted for our particular purpose.

5.2 Advantages and Drawbacks of Methods

In section 3 we explored three different methods for GPU accelerating existing Python code. Each of the methods we explored has a unique set of advantages and drawbacks. The discipline of designing and implementing GPU programs is quite distinct from the more mainstream discipline of implementing programs meant to run serially on the CPU. Becoming an expert GPU programmer can in many instances take years with all the technologies and tools available today. Thus, an interesting dimension when assessing the advantages and drawbacks of the GPU acceleration methods we have explored is how seamless it is to implement the solution, particularly for someone with little or no experience in GPU programming. We will additionally consider how seamless integration into Python is, and of course, the potential for performance.

5.2.1 Using CuPy as a NumPy Drop-in Replacement

The first GPU acceleration method we explored in section 3.2, was to use CuPy, a GPU accelerated array library with an interface designed to closely follow NumPy, as a drop-in replacement for NumPy.

5.2.1.1 Advantages

This method is ideal in cases where an already existing Python solution based on NumPy exists. Depending on the complexity of the program architecture, replacing the NumPy module with CuPy can yield seamless and immediate GPU acceleration without the need to implement any GPU functionality, understand

the GPU hardware or leave the Python ecosystem. This technique is in such instances, by a large margin, the fastest way of implementing GPU acceleration. In cases where no existing solution exists to transform, CuPy is still an adequate tool for "quick and dirty" testing with seamless GPU acceleration directly in Python. Additionally, this method does not require deep knowledge or understanding of the GPU hardware and architecture, as the routines and functions are implemented by the CuPy developers. That being said, some knowledge about GPUs may still be helpful, as it can guide decision making about what type of NumPy code may best be suited for GPU acceleration and how the data flow may be best optimized.

5.2.1.2 Drawbacks

While an advantage of CuPy is that it allows for seamless "quick and dirty" testing directly in Python, this conversely also introduces a drawback of this method. Many solutions implemented using NumPy are designed for fast processing on the CPU. While such solutions may seem like good candidates for GPU acceleration, this match is not guaranteed. Additionally, because NumPy and CuPy are Python libraries, they suffer from many unnecessary data allocations and copies when nested Python expressions with arrays are computed. An example highlighting this issue is the log of the probability mass function (LOGPMF) described in section 3.5. As the nested LOGPMF expression is computed using CuPy arrays, Python's evaluation rules dictate that the expression must be evaluated one operation at a time. Thus, a simple expression may allocate and produce many temporary arrays, although only a single output array remains when the expression is fully computed. These allocations and copies result in a significant spike in global memory requests and memory usage on the GPU, and can be circumvented when implementing custom kernels using either CuPy's JIT compiled kernel support or CUDA in C++.

5.2.2 Custom C++ Implementations Using CUDA

The second GPU acceleration method we explored in section 3.3, was to implement our own solution directly in C++ using the CUDA framework. We then used pybind11 to create Python bindings for our C++ functionality to gain access

directly inside Python.

5.2.2.1 Advantages

The clearest advantage of this method is the granular control over the hardware achieved when implementing the solution directly in Nvidia's programming platform: CUDA. This provides the possibility of closely tailoring the implementation to the problem and using all of CUDA's technologies to optimize and gain the best performance possible. Additionally, since such an implementation would be created using C++, this allows access to C++ features that are otherwise out of reach in Python, such as thread parallelization.

5.2.2.2 Drawbacks

For this method too, its main advantage also yields its greatest drawback. The CUDA programming platform is vast and provides support that can be extremely useful to solve certain problems effectively, but that also requires deep knowledge and understanding of the GPU hardware- and programming model. Additionally, since CUDA features are used in C++, the programmer would need to be, at the very least, comfortable with writing software in C++. Integration to Python also becomes significantly more difficult, as solution are implemented in C++. Python bindings, using tools such as ctypes or pybind11, are necessary to integrate solutions into the Python ecosystem. This, in addition to the fact that C++ is a significantly more verbose language than Python, results in production time being vastly greater for solutions implemented using this method, even for an experienced CUDA programmer.

5.2.3 Custom JIT-Compiled Kernels in Python

The third and final GPU acceleration method we explored in section 3.3.3, was to implement our solution using CuPy's support for JIT (just-in-time) compiled custom kernels. CuPy, directly in Python through its module interface, provides access to certain CUDA functionality as well as support for creating kernels directly in Python code that are then compiled when the program first encounters them.

5.2.3.1 Advantages

This method has many of the same advantages as the method discussed in 5.2.1, and can be viewed as an extension of the CuPy drop-in for NumPy method. What it brings in addition to being a drop-in replacement for NumPy is its JIT compiled custom kernel support and, although limited, CUDA functionality support. This allows for simple array-programming similar to NumPy where it is suitable, and more detailed usage with custom kernels and some other CUDA functionality in cases where the straight-forward array-interface does not suffice. In section 3.3.3, we showed that we could fully re-implement our CUDA hash table directly in Python using CuPy's custom kernel support. This was all achieved while never leaving Python, meaning a programmer does not need to know C++ in order to implement custom kernels this way. More additional functionality not used in our implementation of GKAGE is also supported through CuPy, such as CUDA streams cooperative groups and more [30].

5.2.3.2 Drawbacks

While it is helpful to be able to implement custom kernels directly in Python code, it is uncertain how much simplicity this in effect introduces. The kernels implemented using CuPy's custom kernel support still need to adhere to the same programming model as CUDA kernels implemented in C++. A programmer with little or no knowledge of how the GPU hardware and programming model works will not with ease be able to implement effective kernels this way. Therefore, we would argue that this advantage does not do much more than alleviate the need to delve into C++ and set up proper compiling and Python bindings.

5.3 Drawbacks of Graphical Processing Units

While GPUs can provide excellent acceleration for many problems in scientific computing, they do come with some notable caveats.

The reason why GPUs are powerful when it comes to accelerating certain parallel programs, is because they are designed for such problems. GPUs are highly specialized compute accelerators that perform poorly when applied to any problem

that does not fit its compute architecture. Additionally, today's GPUs are expensive and less accessible than traditional CPUs. Because of the CPU's flexibility in regards to the problems it can solve, CPUs exist in most - if not all - existing computers today. GPUs, however, are less common, partly because of cost.

Furthermore, GPU programming is its own discipline, as the programming models and paradigms used when developing GPU programs are quite different from the typical sequential programs written for the CPU. This leads to a higher bar for entry when it comes to developing effective GPU programs for difficult problems, as fewer expert programmers have the knowledge and training to implement such solutions.

5.4 Relevance for Bioinformatics

In section 4, we showed that GKAGE can genotype a full human genome at 15x coverage up to an order of magnitude faster than KAGE. KAGE was already an order of magnitude faster than any other known genotyping tool that also provided competitive accuracies [4]. Thus, to the best of our knowledge, GKAGE is now the fastest existing genotyping tool available.

One of GKAGE's primary strengths is how well it performs on consumer grade hardware. While it is commonplace for other genotyping tools to require tens of gigabytes of memory, GKAGE can genotype a full human genome without exceeding 4GB of allocated GPU memory. We believe that the improvements yielded by GKAGE are highly beneficial considering the rate of which whole-genome sequencing is becoming more available and tools for analyzing sequence data is becoming increasingly necessary.

We also showed that since KAGE was implemented using NumPy [7] array-programming for performance in Python, GPU acceleration could be added quite seamlessly using CuPy [30]. In addition, we demonstrated how developers with more knowledge of how GPUs work could use either CuPy's just-in-time compiled custom kernel feature to implement GPU acceleration directly in Python, or write kernels directly in C++ using CUDA [19] and then creating Python bindings

using pybind11 [25]. We see these methods as highly feasible opportunities for unlocking performance enhancements via GPU acceleration. This potential can be leveraged by those developing bioinformatics tools, as well as more broadly in the domain of scientific computing, in Python.

5.5 Further Work

While GKAGE demonstrates that alignment-free genotyping can be sped up through GPU acceleration, we believe that the current GKAGE runtimes can still be significantly improved. We will here identify some possible avenues where the current implementation can be altered or improved in order to better use the hardware and technology available to gain more speedup.

5.5.1 Better GPU Hash Table

While the GPU hash table used in GKAGE for *k*mer counting is plenty adequate to demonstrate the effectiveness of GPU acceleration in alignment-free genotyping, alternative hash tables exist that, with correct integration, should perform better. Since optimizing GPU hash tables can be considered a discipline in and of itself, and beyond the scope of this thesis, our GPU hash table implements a naive solution for collision handling and probing. An interesting avenue would be to integrate a version of a bucketed static cuckoo hash table from Awad et al., (2023) [31] to evaluate how a state-of-the-art static GPU accelerated hash table would perform compared to our implementation.

5.5.2 Parallelization of *k*mer Chunk Preparation

Recall that in the *k*mer counting step in KAGE, the input FASTA file is read in chunks. Each chunk of data is then 2-bit encoded and all valid *k*mers are hashed from the 2-bit encoded data chunk. Finally, the chunk of hashed *k*mers are counted. In GKAGE, the 2-bit encoding, *k*mer hashing and *k*mer counting are performed on the GPU. Thus, the chunk of data read from the FASTA file is copied to the GPU's memory before processing begins. Currently, GKAGE performs all of these processes sequentially.

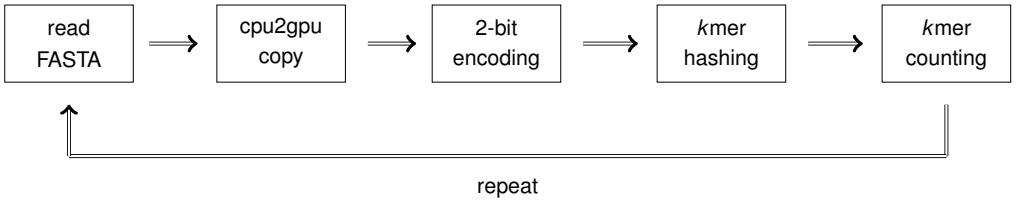


Figure 32: GKAGE's current *k*mer counting pipeline performs several steps in a sequential fashion. While these steps are performed on many *k*mers in parallel at once, each chunk (containing many *k*mers) is processed sequentially.

By utilizing CUDA streams we can parallelize the copying of data to the GPU and the processing of the previous chunk, creating a new and more efficient pipeline.

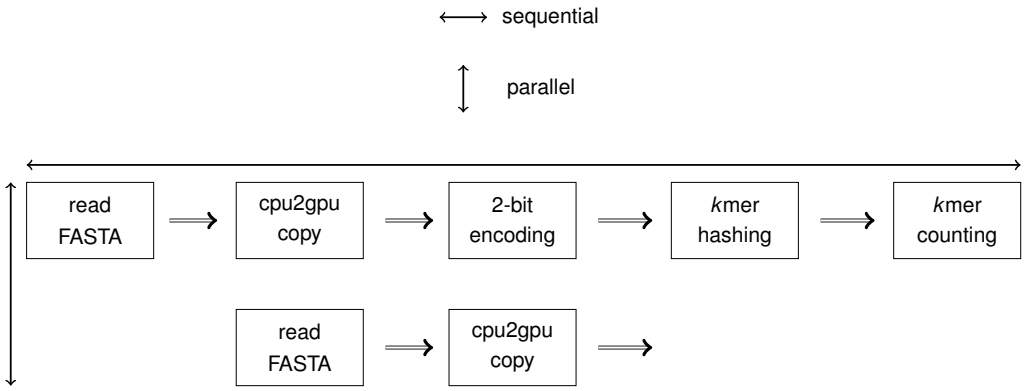


Figure 33: An illustration of a more optimal *k*mer counting pipeline. By utilizing CUDA's streams, we can parallelize copying of data to the GPU and actual GPU processing.

5.5.3 Integrated Graphical Processing Units

While only discrete graphical units (dGPUs) were used in this project, interesting further work may lie in exploring how systems with integrated graphical processing units (iGPUs) can be used for genotyping. Particularly as genotyping may become increasingly common on consumer grade hardware, with fewer processing cores and less memory than larger and more powerful systems, iGPUs may contend with the more powerful dGPUs. The advantage of using iGPUs are that they share the physical memory region with the CPU, meaning that the overhead of copying memory back and forth between the RAM and GPU is alleviated.

6 Conclusion

In section 1.1, we stated that one of the two goals of this thesis was to explore whether alignment-free genotyping could be sped up in any significant way by using the GPU. To investigate this, we attempted to GPU accelerate an existing genotyper, KAGE, which recently showed that it was an order of magnitude faster than any other known genotyper [4]. As a result of GPU accelerating components of KAGE, we presented GKAGE (GPU KAGE), a new GPU accelerated version of KAGE. GKAGE achieves up to 5X speedup compared to KAGE on a high-end compute server, and more than 10X speedup on commercial hardware, all while using very little GPU memory - a scarce resource. We believe that GKAGE is a useful contribution to the world of bioinformatics, considering the rate of which whole-genome sequencing is becoming more accessible and tools to analyze the generated sequence data is becoming increasingly necessary.

The second goal we presented in section 1.1, was to investigate and experiment with different ways of GPU accelerating existing Python code that relies on array-programming libraries such as NumPy [7], and to reveal the advantages and drawbacks of such methods. We achieved this by GPU accelerating different components of KAGE using a suite of different methods. For each method, we discussed its advantages and drawbacks, where dimensions such as ease-of-use, seamless integration, and performance were central. We see the insights revealed by exploring these methods as showing that GPU acceleration is a feasible avenue to unlock performance enhancements in existing tools, particularly tools relying on large array-computations. Large array-computations are commonplace in computational biology. Additionally, Python GPU acceleration libraries such as CuPy [30] are making GPU acceleration more accessible, even to programmers with limited knowledge of the GPU's programming model and hardware. Given these factors, we believe that there could be an avalanche of existing methods and tools that could greatly benefit from integration such acceleration.

References

[1] National Human Genome Research Institute. *The Cost of Sequencing a Human Genome*. https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost. Accessed: 2023-04-24. 2023.

[2] Illumina. *Read length recommendations*. https://www.illumina.com/science/technology/next-generation-sequencing/plan-experiments/read-length.html. Accessed: 2023-04-9. 2023.

[3] Aaron McKenna et al. "The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data". en. In: *Genome Res* 20.9 (July 2010), pp. 1297–1303.

[4] Ivar Grytten, Knut Dagestad Rand, and Geir Kjetil Sandve. "KAGE: Fast alignment-free graph-based genotyping of SNPs and short indels". In: *bioRxiv* (2021). DOI: 10.1101/2021.12.03.471074. eprint: https://www.biorxiv.org/content/early/2021/12/20/2021.12.03.471074.full.pdf. Address <https://www.biorxiv.org/content/early/2021/12/20/2021.12.03.471074>.

[5] Adam Auton et al. "A global reference for human genetic variation". In: *Nature* 526.7571 (Oct. 2015), pp. 68–74.

[6] Luca Denti et al. "MALVA: Genotyping by Mapping-free ALlele Detection of Known VAriants". In: *iScience* 18 (2019). RECOMB-Seq 2019, pp. 20–27. ISSN: 2589-0042. DOI: https://doi.org/10.1016/j.isci.2019.07.011. Address <https://www.sciencedirect.com/science/article/pii/S2589004219302366>.

[7] Charles R. Harris et al. "Array programming with NumPy". In: *Nature* 585.7825 (Sept. 2020), pp. 357–362. DOI: 10.1038/s41586-020-2649-2. Address <https://doi.org/10.1038/s41586-020-2649-2>.

[8] National Human Genome Research Institute. *Deoxyribonucleic Acid (DNA) Fact Sheet*. https://www.genome.gov/about-genomics/fact-sheets/Deoxyribonucleic-Acid-Fact-Sheet. Accessed: 2023-03-30. 2023.

[9] Gautam .B Singh. *Fundamentals of Bioinformatics and Computational Biology. Methods and Exercises in MATLAB*. Springer, 2015.

[10] National Human Genome Research Institute. *Chromosomes Fact Sheet*. https://www.genome.gov/about-genomics/fact-sheets/Chromosomes-Fact-Sheet. Accessed: 2023-03-31. 2023.

[11] Jason A Reuter, Damek V Spacek, and Michael P Snyder. “High-throughput sequencing technologies”. en. In: *Mol Cell* 58.4 (May 2015), pp. 586–597.

[12] M. Mahmoud et al. “Utility of long-read sequencing for All of Us”. In: *bioRxiv* (2023). DOI: 10.1101/2023.01.23.525236. eprint: https://www.biorxiv.org/content/early/2023/01/24/2023.01.23.525236.full.pdf. Address <https://www.biorxiv.org/content/early/2023/01/24/2023.01.23.525236>.

[13] Stepanka Zverinova and Victor Guryev. “Variant calling: Considerations, practices, and developments”. en. In: *Hum Mutat* 43.8 (Dec. 2021), pp. 976–985.

[14] Can Alkan, Bradley P Coe, and Evan E Eichler. “Genome structural variation discovery and genotyping”. en. In: *Nat Rev Genet* 12.5 (Mar. 2011), pp. 363–376.

[15] The International Genome Sample Resource. *Encoding Structural Variants in VCF (Variant Call Format) version 4.0*. https://www.internationalgenome.org/wiki/Analysis/Variant%20Call%20Format/VCF%20(Variant%20Call%20Format)%20version%204.0/encoding-structural-variants. Accessed: 2023-04-7. 2023.

[16] Marius Erbert, Steffen Rechner, and Matthias Müller-Hannemann. “Gerbil: a fast and memory-efficient k-mer counter with GPU-support”. In: *Algorithms for Molecular Biology* 12.1 (Mar. 2017), p. 9.

[17] Swati C Manekar and Shailesh R Sathe. “A benchmark study of k-mer counting methods for high-throughput sequencing”. In: *GigaScience* 7.12 (Oct. 2018). giy125. ISSN: 2047-217X. DOI: 10.1093/gigascience/giy125. eprint: https://academic.oup.com/gigascience/article-pdf/7/12/giy125/27011542/giy125_reviewer_3_report_(original_submission).pdf. Address <https://doi.org/10.1093/gigascience/giy125>.

[18] Thomas Alsop. *PC graphics processing unit (GPU) shipment share worldwide from 2nd quarter 2009 to 4th quarter 2022, by vendor*. https://www.statista.com/statistics/754557/worldwide-gpu-shipments-market-share-by-vendor/. Accessed: 2023-05-7. 2023.

[19] NVIDIA. *CUDA C++ Programming Guide*. https://docs.nvidia.com/cuda/cuda-c-programming-guide/. Accessed: 2023-05-7. 2023.

[20] Dave Kuhlman. *A Python Book: Beginning Python, Advanced Python, and Python Exercises*. https://www.davekuhlman.org/python_book_01.pdf. Accessed: 2023-04-28. 2023.

[21] Stack Overflow. *Most popular technologies*. https://insights.stackoverflow.com/survey/2021#most-popular-technologies-language-prof. Accessed: 2023-04-28. 2023.

[22] Pauli Virtanen et al. "SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python". In: *Nature Methods* 17 (2020), pp. 261–272. DOI: 10.1038/s41592-019-0686-2.

[23] Knut Dagestad Rand and Ivar Grytten. *npstructures*. https://github.com/bionumpy/npstructures. 2022.

[24] Knut Dagestad Rand and Ivar Grytten. *bionumpy*. https://github.com/bionumpy/bionumpy. 2022.

[25] Wenzel Jakob, Jason Rhinelander, and Dean Moldovan. *pybind11 – Seamless operability between C++11 and Python*. https://github.com/pybind/pybind11. 2017.

[26] F Mölder et al. "Sustainable data analysis with Snakemake". In: *F1000Research* 10.33 (2021). DOI: 10.12688/f1000research.29032.1.

[27] Austin Appleby. *MurmurHash3*. https://github.com/aappleby/smhasher/blob/master/src/MurmurHash3.cpp. Accessed: 2023-04-20. 2023.

[28] Jørgen Wictor Henriksen et al. "Ultra-fast genotyping of SNPs and short indels using GPU acceleration". In: *bioRxiv* (2023). DOI: 10.1101/2023.04.14.534526. eprint: https://www.biorxiv.org/content/early/2023/04/14/2023.04.14.534526.full.pdf. Address <https://www.biorxiv.org/content/early/2023/04/14/2023.04.14.534526>.

[29] bioRxiv. *bioRxiv - the preprint server for biology*. https://www.biorxiv.org/. Accessed: 2023-05-11. 2023.

[30] Ryosuke Okuta et al. "CuPy: A NumPy-Compatible Library for NVIDIA GPU Calculations". In: *Proceedings of Workshop on Machine Learning Systems (LearningSys) in The Thirty-first Annual Conference on Neural Information Processing Systems (NIPS)*. 2017. Address <http://learningsys.org/nips17/assets/papers/paper_16.pdf>.

[31] Muhammad A. Awad et al. “Analyzing and Implementing GPU Hash Tables”. In: *SIAM Symposium on Algorithmic Principles of Computer Systems*. APOCS23. Jan. 2023. Address <https://escholarship.org/uc/item/6cb1q6rz>.

Appendices

A bioRxiv Manuscript - *Ultra-fast genotyping of SNPs and short indels using GPU acceleration*

Ultra-fast genotyping of SNPs and short indels using GPU acceleration

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Ultra-fast genotyping of SNPs and short indels using GPU acceleration

Abstract

As decreasing DNA sequencing costs leads to a steadily increasing rate of generated data, the development of efficient algorithms for processing of the sequence data is increasingly important to reduce costs and energy consumption. Recent work have shown that genotyping can be done efficiently and accurately using alignment-free methods that are based on analyzing kmers from sequenced reads. In particular, we have recently presented the KAGE genotyper, which uses an efficient pangenome representation of known individuals in a population to further increase accuracy and efficiency. While existing genotypers like KAGE use the Central Processing Unit (CPU) to count and analyze kmers, the Graphical Processing Unit (GPU) has shown promising for reducing runtime for similar problems.

We here present GKAGE, a new and improved version of KAGE that utilizes the GPU to further increase the computational efficiency. This is done by counting and analyzing large amounts of kmers in the many parallel cores of a GPU. We show that GKAGE is, on hardware of comparable cost, able to genotype an individual up to an order of magnitude faster than KAGE while producing the same output, which makes it by far the fastest genotyper available today. GKAGE can run on consumer-grade GPUs, and enables genotyping of a human sample in only a matter of minutes without the need for expensive high-performance computers. GKAGE is open source and available at https://github.com/kage-genotyper/kage.

Introduction

The cost of sequencing a full human genome has fallen drastically in recent years, and consumers can now get their whole genome sequenced for a few hundred dollars [1], a fraction of what was the price only a few years ago. As millions of genomes are likely to be sequenced in the coming years, there is an ever-increasing need for efficient methods for analyzing this genomic data. At the core of such analysis is variant detection, determining which genetic variants are present in a sample based on the sequenced reads.

Recent methods [2,3,4,5] have shown that detecting variants in a human sample can be performed efficiently and with high accuracy by genotyping the sample against an existing database of known human variation. Such methods use prior knowledge from e.g. the 1000 Genomes Project [6] about where in the genome individuals frequently have variation, and for each such genetic variant use the genomic reads from the sample to infer the most likely genotype. While such methods have traditionally been based on aligning reads to a reference genome, which is slow, recent alignment-free methods have shown that drastic speedup can be achieved by instead analyzing kmers from the sequenced reads. In a recent publication [2], we proposed a highly efficient alignment-free method, KAGE, that uses prior knowledge from a population to achieve high genotyping accuracy while being more computationally efficient than other alignment-free genotypers. Alignment-free genotypers, like KAGE, generally rely on analyzing kmers from reads against kmers associated with genomic variants. Prior to genotyping, kmers that represent alleles of variants of interest are collected and stored in an index (e.g. a hashmap) that enables lookup of kmers from reads to variant alleles. This is typically done once for a set of variants, and this index can then be reused for genotyping any individual against those variants. When genotyping an individual, kmers from reads are collected and looked up in the kmer index, to obtain kmer counts for each variant allele. Generally, genotype probabilities are then found by analyzing the counts of how many reads support the various alleles of the variants of interest. In KAGE, these counts are combined with expected counts sampled from a population to obtain better estimates.

In contrast to other genotypers, especially alignment-based genotypers like GATK [7], the time-consuming steps of KAGE (like processing and counting kmers) can readily benefit from the massive parallelization that the Graphical Processing Unit (GPU) offers [9]. Here, we show that applying GPU acceleration to the compute-heavy parts of KAGE leads to a genotyper GKAGE of unsurpassed computational efficiency. GKAGE uses the GPU to process genomic reads, extract kmers and count the number of kmers that support alleles of genetic variants. We show that this results in a substantial speedup of up to 10X over the original KAGE genotyper (which was already faster than any other genotyper), while producing the exact same output. GKAGE has been implemented so that it is able to run even on standard consumer GPUs, and is able to genotype a whole human sample in a matter of minutes.

Results

GKAGE is a GPU-accelerated version of the recently published KAGE genotyper. GKAGE uses CUDA-enabled GPUs to efficiently parse and encode kmers from reads, to genotype a set of known SNPs and indels based on the kmer counts. GKAGE produces output that is identical to that of KAGE, with reduced runtime on systems that support the CUDA interface for GPU acceleration. The software is open source and available at https://github.com/kage-genotyper/kage. As part of GKAGE, we have also implemented a static GPU hashmap for counting kmers through a Python interface, available at https://github.com/kage-genotyper/cucounter.

We have recently shown that KAGE is an order of magnitude faster than existing genotypers while giving better or comparable accuracy [2]. We thus only benchmark GKAGE against KAGE to show the effect of GPU acceleration. We do this by running GKAGE and KAGE on a human whole genome sample (15x coverage) on two different systems:

1. A high-performance server with an AMD EPYC 7742 64-Core CPU and two NVIDIA Tesla V100 GPUs. KAGE was run using 16 threads and GKAGE was run using one GPU.

2. A regular desktop computer with an 11th Gen Intel(R) Core(TM) i5-11400F @ 2.60GHz CPU with 6 cores and a NVIDIA GTX 1660 super GPU. KAGE was run using 6 threads.

Table 1 shows the runtimes on these two systems. GKAGE is approximately 5x faster on the high-end system and more than 10x faster on the desktop computer. Since GKAGE only needs the kmer counts of a predefined set of kmers (those associated with variants), and no existing GPU-based kmer counter is able to count only a given set of kmers, we have implemented our own kmer counter as part of GKAGE. An alternative solution would be to count all kmers using an existing tool and filter out those kmers that are relevant. Table 1 also shows the time spent by the GPU kmer counter Gerbil [8] to only count kmers.

Table 1: Running times of KAGE, GKAGE and Gerbil (only kmer counting)

	KAGE	**GKAGE**	**Gerbil (only kmer counting)**
Desktop computer	1993 sec	178 sec	464 sec
High-performance computer	510 sec	94 sec	438 sec

Methods

Implementation

Here we describe more in detail how GKAGE has been implemented. While GKAGE is implemented as part of KAGE, and shares large parts of its code with KAGE, compute-heavy parts have been reimplemented so that the GPU is utilized. GKAGE implements GPU support for two bottleneck components of KAGE that were suitable for GPU acceleration:

Reading and encoding kmers from a FASTA/FASTQ file is achieved in KAGE by using BioNumPy [10], a Python library built on top of NumPy [11]. BioNumPy uses NumPy to efficiently read chunks of DNA reads from fasta files, encode the bases to a 2-bit representation, and then encode the valid kmers as 64-bit integer representations in an array. GPU support for this step was achieved by utilizing CuPy [12], a GPU accelerated computing library with an interface that closely follows that of NumPy. This component was implemented in GKAGE by replacing the NumPy module in BioNumPy with CuPy, effectively replacing all NumPy function calls with calls to CuPy's functions providing the same functionality with GPU acceleration. This strategy worked out of the box for most parts of the BioNumPy solution, with only a few custom modifications having to be made due to certain functions in NumPy's interface not being supported by CuPy.

Counting kmers As part of GKAGE, we have implemented a static hashtable for counting a predefined set of kmers on the GPU. The implementation supports parallel and high-throughput hashing and counting of large chunks of kmers simultaneously on the GPU. This static hashtable is implemented as a C++ class in CUDA [13], with two arrays of 64-bit and 32-bit unsigned integers to represent kmers (keys) and counts (values), respectively. CUDA kernels are implemented that handle

insertion (only once during initialization of the hashtable), counting and querying of kmers. The hashtable uses open addressing and a simple linear probing scheme with a murmur hash [14] for the keys.

To find the position of a kmer k in the hashtable, the initial probe position p_0 is found by computing

$$p_0 = hash(k) \bmod c$$

where $hash$ is a murmur hash function and c is the capacity of the hashtable. If p_0 is occupied by a different kmer than k, the next probing position p_i can be computed given the previous probing position p_{i-1} with

$$p_i = (p_{i-1} + 1) \bmod c$$

The probing will continue until either k or an empty slot in the hashtable is observed (See Figure 1 for an illustration of this).

The hashtable supports three main operations: insertion, counting and querying. In each of the cases, the input is an array of 2-bit encoded kmers. When querying, the return value is an array of counts associated with the input kmers. For insertion, counting or querying of n kmers, n CUDA threads are launched. Each thread is then responsible for fulfilling the relevant operation associated with the kmer, i.e. incrementing or fetching the count associated with the kmer in the hashtable, all achieved using the probing scheme previously described. Furthermore, for insertions and count updates, CUDA atomic operations are used to avoid race conditions. To use the hashtable class in Python, C++ bindings are implemented using pybind11 [15].

Since KAGE only needs to count kmers that are preselected to represent alleles of known variants, which typically is only a small subset of the kmers present in genomic reads, the hashmap needed for this requires only a few gigabytes of memory. Thus, when genotyping 28 million variants of a human sample, a GPU with 4GB of memory is sufficient (see details below).

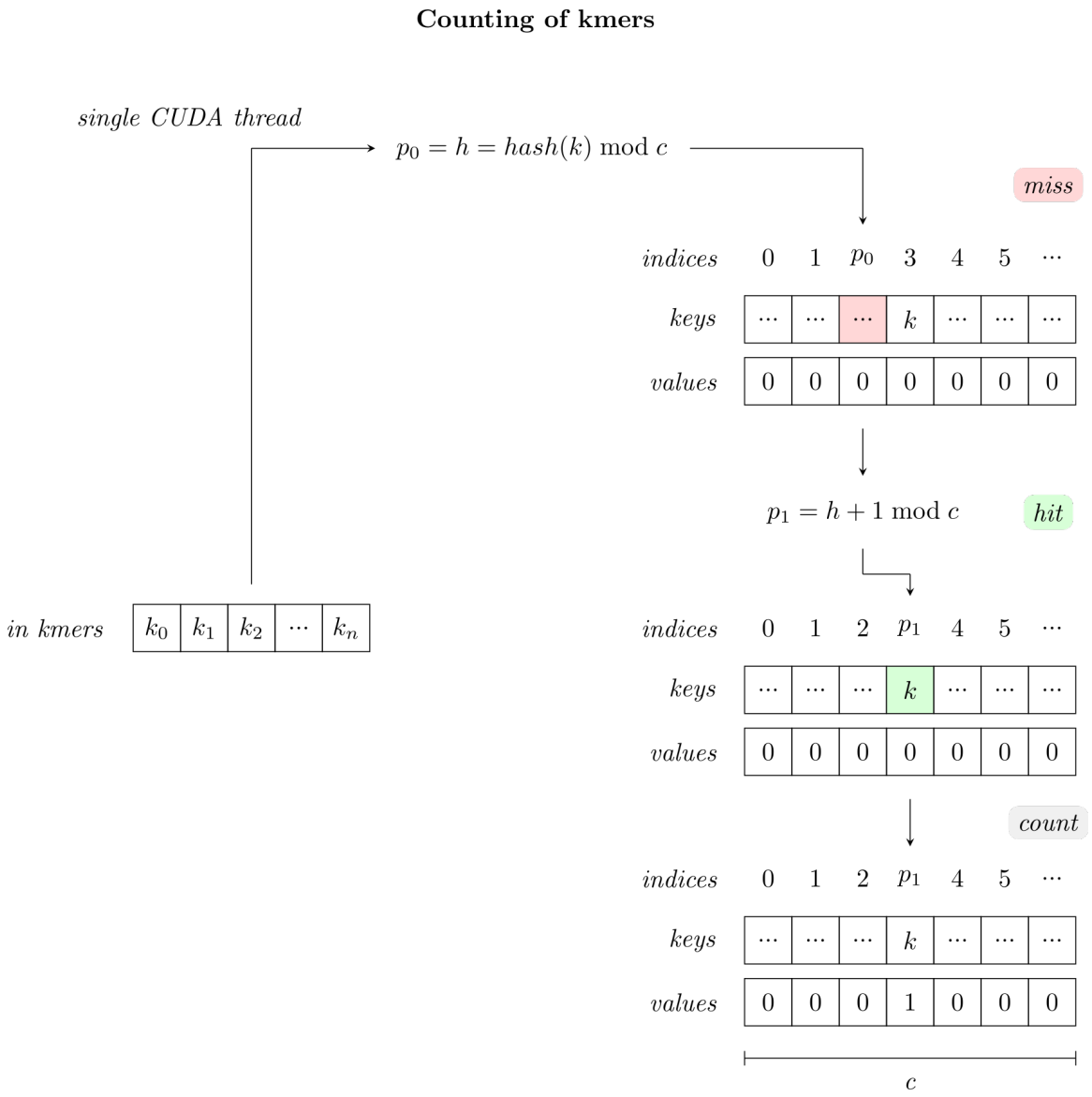

Figure 1: As an array of 64-bit integer encoded kmers are counted by the hash table, each CUDA thread will compute the first probe position p_0 for each individual kmer, and then continue probing by linearly moving up to the next consecutive slot until either an empty slot or the original kmer handled by the thread is observed. If an empty slot is observed, the thread terminates. If the original kmer is observed, the value at the current slot is increased.

Memory Usage

The memory usage for GKAGE is influenced by two factors: The size of the kmer index, and the number of sequences to handle at a time (chunk size). Since the chunk size can be set to a suitable number for a given system (we used 10 MB in our experiments), the main factor will be the size of the kmer-index.

The size of the kmer-index itself is again influenced by three factors: The number of variants to be genotyped (N_v), the average number of kmers per variant (k), and the loading factor of the hash table (L). Given these, the memory will be in the order of $O(N_v * k/L)$. Thus, for a fixed loading factor (default 0.57), the memory consumption of GKAGE is primarily driven by the number of variants to be genotyped. For systems with little memory, the loading factor can be increased to remove the memory consumption, but that can severely impede performance as the loading factor approaches 1. For genotyping the 28 million variants in the experiment in table 1, with an average of 3.4 kmers per variant and a loading factor of 0.57, the memory consumption of the index is $(28 * 10^6 * 3.4/0.57) * (8 + 4)B = 2GB$ (using 8 bytes for keys and 4 bytes for counts).

Benchmarks

Benchmarking was performed on two different computer systems, as described in the Results section, using simulated reads for a whole genome sample with 15x coverage. A Snakemake [16] pipeline for reproducing the benchmarking results can be found at https://github.com/kage-genotyper/GKAGE-benchmarking.

Discussion

We have presented GKAGE, a GPU accelerated, alignment-free genotyper based on a previously published CPU-based genotyper KAGE. Our results show that alignment-free genotyping is an ideal problem for GPU acceleration. While the existing KAGE genotyper is already fast by today's standards, GKAGE is considerably faster, enabling rapid genotyping even on consumer-grade computers. We see these improvements of computational efficiency as highly beneficial considering the continually decreasing cost and expanding capacity at the experimental side of whole-genome sequencing.

Since the original KAGE genotyper was implemented mainly using the array programming libraries NumPy and BioNumPy in Python, GPU support could be added to the existing code base in a clean way by using the CuPy library combined with some custom CUDA kernels with Python wrappers. We thus see our work as a strong example of how the addition of GPU support to existing tools is typically highly feasible and beneficial in cases where many independent operations are performed on an array of data, which is common for problems in computational biology. As GPUs are becoming steadily cheaper and more available, we thus see a huge potential in improving the computational efficiency of existing methods and tools, which in many cases can be achieved quite easily through the Python ecosystem with packages such as CuPy [12], Numba [17] and BioNumPy [10].

References

1. **A New Wave of Genomics for All**
Diana Crow
Cell (2019-03) https://doi.org/gfw9g5
DOI: 10.1016/j.cell.2019.02.041 · PMID: 30901548

2. **KAGE: fast alignment-free graph-based genotyping of SNPs and short indels**
Ivar Grytten, Knut Dagestad Rand, Geir Kjetil Sandve
Genome Biology (2022-10-04) https://doi.org/grpzvf
DOI: 10.1186/s13059-022-02771-2 · PMID: 36195962 · PMCID: PMC9531401

3. **MALVA: Genotyping by Mapping-free ALlele Detection of Known VAriants**
Luca Denti, Marco Previtali, Giulia Bernardini, Alexander Schönhuth, Paola Bonizzoni
iScience (2019-08) https://doi.org/gmhw28
DOI: 10.1016/j.isci.2019.07.011 · PMID: 31352182 · PMCID: PMC6664100

4. **Pangenome-based genome inference allows efficient and accurate genotyping across a wide spectrum of variant classes**
Jana Ebler, Peter Ebert, Wayne E Clarke, Tobias Rausch, Peter A Audano, Torsten Houwaart, Yafei Mao, Jan O Korbel, Evan E Eichler, Michael C Zody, ... Tobias Marschall
Nature Genetics (2022-04) https://doi.org/grp6v6
DOI: 10.1038/s41588-022-01043-w · PMID: 35410384 · PMCID: PMC9005351

5. **Graphtyper enables population-scale genotyping using pangenome graphs**

Hannes P Eggertsson, Hakon Jonsson, Snaedis Kristmundsdottir, Eirikur Hjartarson, Birte Kehr, Gisli Masson, Florian Zink, Kristjan E Hjorleifsson, Aslaug Jonasdottir, Adalbjorg Jonasdottir, … Bjarni V Halldorsson
Nature Genetics (2017-09-25) https://doi.org/gbx7v6
DOI: 10.1038/ng.3964 · PMID: 28945251

6. **A global reference for human genetic variation**
, Adam Auton, Gonçalo R Abecasis, David M Altshuler (Co-Chair), Richard M Durbin (Co-Chair), Gonçalo R Abecasis, David R Bentley, Aravinda Chakravarti, Andrew G Clark, Peter Donnelly, … Nature (2015-09-30) https://doi.org/73d
DOI: 10.1038/nature15393 · PMID: 26432245 · PMCID: PMC4750478

7. **Scaling accurate genetic variant discovery to tens of thousands of samples**
Ryan Poplin, Valentin Ruano-Rubio, Mark A DePristo, Tim J Fennell, Mauricio O Carneiro, Geraldine A Van der Auwera, David E Kling, Laura D Gauthier, Ami Levy-Moonshine, David Roazen, … Eric Banks
Cold Spring Harbor Laboratory (2017-11-14) https://doi.org/ggmrvr
DOI: 10.1101/201178

8. **Gerbil: a fast and memory-efficient k-mer counter with GPU-support**
Marius Erbert, Steffen Rechner, Matthias Müller-Hannemann
Algorithms for Molecular Biology (2017-03-31) https://doi.org/gkzhfr
DOI: 10.1186/s13015-017-0097-9 · PMID: 28373894 · PMCID: PMC5374613

9. **GPU Acceleration of Advanced k-mer Counting for Computational Genomics**
Huiren Li, Anand Ramachandran, Deming Chen
2018 IEEE 29th International Conference on Application-specific Systems, Architectures and Processors (ASAP) (2018-07) https://doi.org/grp3kq
DOI: 10.1109/asap.2018.8445084

10. **BioNumPy: Fast and easy analysis of biological data with Python**
Knut Rand, Ivar Grytten, Milena Pavlovic, Chakravarthi Kanduri, Geir Kjetil Sandve
Cold Spring Harbor Laboratory (2022-12-22) https://doi.org/grp3k6
DOI: 10.1101/2022.12.21.521373

11. **Array programming with NumPy**
Charles R Harris, KJarrod Millman, Stéfan J van der Walt, Ralf Gommers, Pauli Virtanen, David Cournapeau, Eric Wieser, Julian Taylor, Sebastian Berg, Nathaniel J Smith, … Travis E Oliphant
Nature (2020-09-16) https://doi.org/ghbzf2
DOI: 10.1038/s41586-020-2649-2 · PMID: 32939066 · PMCID: PMC7759461

12. **CuPy: NumPy/SciPy-compatible Array Library for GPU-accelerated Computing with Python**
Ryosuke Okuta, Yuya Unno, Daisuke Nishino, Shohei Hido, Crissman Loomis
Thirty-first Annual Conference on Neural Information Processing Systems (NeurIPS)

13. **CUDA C++ Programming Guide**
NVIDIA Corporation & Affiliates
(2023) https://docs.nvidia.com/cuda/cuda-c-programming-guide/#

14. **MurmurHash3**
Austin Appleby
(2023) https://github.com/aappleby/smhasher/blob/master/src/MurmurHash3.cpp

15. **pybind11 -- Seamless operability between C++11 and Python**
Wenzel Jakob
GitHub (2023) https://github.com/pybind/pybind11

16. **Sustainable data analysis with Snakemake**
Felix Mölder, Kim Philipp Jablonski, Brice Letcher, Michael B Hall, Christopher H Tomkins-Tinch, Vanessa Sochat, Jan Forster, Soohyun Lee, Sven O Twardziok, Alexander Kanitz, … Johannes Köster
F1000Research (2021-04-19) https://doi.org/gj76rq
DOI: 10.12688/f1000research.29032.2 · PMID: 34035898 · PMCID: PMC8114187

17. **Numba**
Siu Kwan Lam, Antoine Pitrou, Stanley Seibert
Proceedings of the Second Workshop on the LLVM Compiler Infrastructure in HPC (2015-11-15) https://doi.org/gf3nks
DOI: 10.1145/2833157.2833162