

# Efficient Encoding and Compression Techniques for the SLOW5 File Format

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## ABSTRACT

Contemporary data storage of raw nanopore signals in the FAST5 file format doesn't benefit from parallel file access. A more computationally resourceful and space efficient file format could result in significant improvements in the runtime and storage size of nanopore sequencing pipelines.

To address this issue, binary and compressed binary equivalents to the existing SLOW5 format were designed. Parallel access was implemented using SLOW5 index files and multithreading.

Benchmarking experiments to determine the access time and file size of each SLOW5 format and their corresponding FAST5 files were performed using a sequenced human genome dataset on a rack-mounted server. The binary SLOW5 format was found to have the fastest access time with on average roughly 5000 reads accessed per second using 32 threads. Whilst the compressed binary SLOW5 file format was the most space efficient, using 250kB per read on average. In comparison, using the FAST5 format on average 100 reads could be accessed per second, with each read using roughly 300kB on average.

Therefore, in a standard HPC environment the use of SLOW5 format delivered up to a ~50-fold increase in compute time and a ~20% reduction in storage.

By exploiting modern CPU architectures with multithreading whilst employing space efficient compression techniques, the runtime and space requirements of nanopore sequencing pipelines can be significantly improved.

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## 1 BACKGROUND

Genome sequencers read DNA strands in segments and convert each segment into a packet of data known as a *read*. DNA sequencing devices from Oxford Nanopore Technologies (ONT) are no different and record disturbances in ionic current as DNA molecules are passed through a biological nanopore [1]. These measurements can be translated to determine the sequence of each DNA molecule analysed.

The time series current signal data is written in a format called FAST5, which stores the raw data for each nanopore sequencing read. FAST5 is a Hierarchical Data Format 5 (HDF5) file [2] with a specific schema defined by ONT. HDF5 is a complex file format for storing and managing high volume data. It works well with the time series current signal of a nanopore read, since it uses B-trees to index table objects.

However, the official library used to access the HDF5 file format is not scalable with threads [3]. This means that tasks performed with the FAST5 file format suffer from an inefficient utilisation of parallel resources. Therefore, the process of basecalling FAST5 data into DNA sequence reads and other common analyses that utilise

signal-level data (such as DNA methylation calling with Nanopolish [4] or f5c [5]) slow down the typical sequencing pipeline.



2 INTRODUCTION

A new file format is needed to improve the efficiency of these bioinformatics tasks. SLOW5 is a simple file format designed to address this [6]. It is a tab-separated values (TSV) file with a header marked by lines beginning with a '#', followed by the metadata and time series current signal data for one nanopore read per line. An example is shown in table 4.

In order to read a SLOW5 file using multiple threads of execution, a corresponding SLOW5 index file is used [6]. It is also a TSV file and begins with a single header line prepended by a '#' to define the column names and their order. Each following line represents the location of a particular read in the corresponding SLOW5 file by storing its file offset and length in bytes. See table 1 for an example.

Table 1: Example of a SLOW5 index file.

#read_id	file_offset	length
id-0	120	8073
id-1	8193	72705
⋮	⋮	⋮
id-N	397774	56481

In the current readable format, a SLOW5 file containing multiple reads takes up much more memory than its corresponding FAST5 files, each containing one read. Thus, the aim is to efficiently compress the SLOW5 file format to a size smaller than or equal to the total size of its corresponding FAST5 files. Whilst still maintaining multithreaded access to the SLOW5 file format and achieving the best possible level of performance within these constraints.

3 METHODS

3.1 Encodings

Two encodings of the SLOW5 file format were designed and implemented. The first is a binary encoding where each numerical field is stored in binary rather than in the ASCII format. This is analogous to how SAM [7, 8] and VCF [9], two gold standard genomics file formats, have their own binary counterparts BAM and BCF. This encoding is more natural for almost all modern computers since digital memory is designed to store data in binary. It is also significantly more efficient in theory, with one byte of data capable of storing the 16 digits in ASCII versus 256 (= 2<sup>8</sup>) numbers in binary.

The binary encoding also removes the commas, tab characters and the newline characters separating signal data, fields and reads respectively since the size of each numerical field is now static. For all remaining variable length fields such as the read identifier (ID), the encoding prepends them by a static sized numerical field storing their length in bytes. This prepended field is also stored in binary. An example is displayed in figure 3.

The second encoding is a compressed binary encoding in the gzip file format [10]. This is achieved by separately compressing the header and each read of the binary encoded SLOW5 file format using the default level of compression, internal memory allocation

and compression algorithm of the zlib C library [11]. An example is shown in figure 4.

3.2 Multithreading

Reading reads from the SLOW5 file format can be performed by multiple threads in parallel as was mentioned in section 2. The method is the same for all encodings (ASCII, binary and compressed binary) and works as follows.

The algorithm takes as input a SLOW5 file and a list of read IDs. It then outputs the list of reads from the SLOW5 file corresponding to the given read IDs. First of all, it generates a SLOW5 index file (section 2) for the SLOW5 file of interest, if it does not exist already. This index file is then stored in memory as a hash table where the key is the read ID and the values are the location and size in bytes of each read. Then, the list of read IDs are processed one batch at a time to avoid an out of memory state, with the maximum number of read IDs in a batch set arbitrarily to 2<sup>12</sup>. Each thread is then allocated an equal amount of read IDs from the current batch. The threads then begin iterating in parallel through their allocated read IDs.

For each read ID, the offset and length of the corresponding read are found from the hash table. The offset is then used to locate the beginning of the read in the SLOW5 file and the length dictates how many bytes to read from that position into memory for later output.

Work stealing between threads occurs in the case that a thread completes the above task for all their allocated reads earlier than another. Once all read IDs in the current batch have been processed, the stored reads are written as output and freed from the program's memory. The next batch then begins if there are remaining read IDs to process from the initial list.

This algorithm displays the superiority of the simple SLOW5 file format over FAST5 since it very basically overcomes the thread scalability issue outlined in section 1. In fact, it implements parallel access without any synchronisation mechanisms and is inherently thread-safe since the SLOW5 file is read-only throughout the entire algorithm and each stored read is written to a pre-allocated position in memory.



4 EXPERIMENTS

4.1 Design

Experiments to evaluate the performance of each file type and encoding were designed and executed. The dataset of FAST5 files used for these experiments was obtained by sequencing a genome sample from an adult male human on the ONT GridION. It's dimensions are summarised in table 2.

Dimensions of the resulting dataset are summarised in Table 2

A list of read IDs representative of a typical analysis's order of read requests was generated by sorting the dataset's BAM file [7] by chromosome number and then by the base location on each chromosome. The result is a list of 534 000 read IDs sorted by their genomic coordinates with 480 004 being unique. This covers 96.7% of the number of reads in the dataset, making it a robust list of read IDs for performance benchmarking.

The experiments were executed on a rack-mounted server capable of running up to 40 threads of execution in parallel. This amount is beneficial for observing the full relationship between

**Table 2:** The dataset used for the experiments.

<b>Description</b>	Adult male human genome
<b>Sequencer</b>	ONT GridION
<b>Sequencing time (days)</b>	3
<b>No. of bases (Gnt)</b>	10.56
<b>No. of reads</b>	496 368
<b>Avg. read length (nt)</b>	21 278
<b>Max read length (nt)</b>	1 328 419

performance and the number of threads used. It's specifications (table 3) are typical for a small high performance computing (HPC) server which is usually available for genomics research scientists. The server's disk cache was also cleared before executing each experiment in order to prevent inaccurate I/O results.

**Table 3:** Specifications of the server used for the experiments.

<b>Description</b>	Dell PowerEdge C4140 Server Rack
<b>CPUs</b>	2 × Intel Xeon Silver 4114
<b>CPU cores</b>	2 × 10
<b>CPU threads</b>	2 × 20
<b>RAM (GB)</b>	376
<b>Disk System</b>	6.4TB NVMe drive
<b>File System</b>	ext4
<b>OS</b>	Ubuntu 18.04.5 LTS

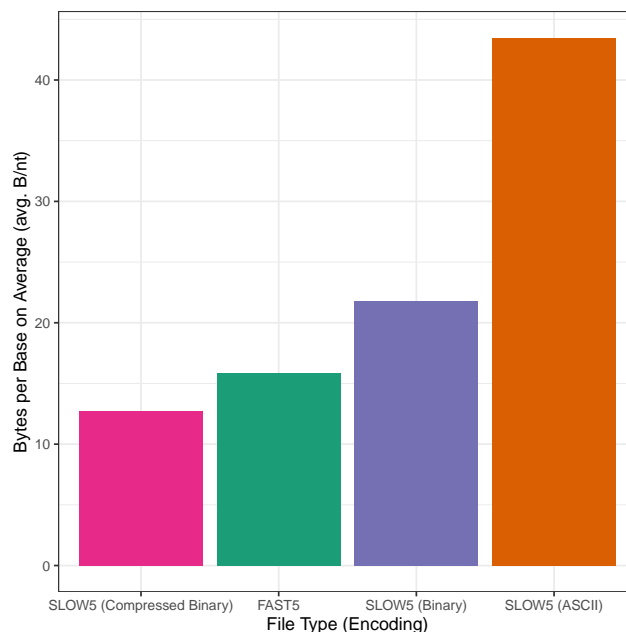
Two experiments were designed to evaluate performance. The first aimed to compare the size of each file type and encoding. Using the dataset's FAST5 files, equivalent SLOW5 files encoded in ASCII, binary and compressed binary were created. Each file's size was then measured in bytes using the Unix utility `wc` [12].

The second experiment aimed to compare the read access time of each file type and encoding using a varying number of threads. The motivation for varying the number of threads was to visualise the thread scalability issue from section 1 and how it was overcome in section 3.2. For each file type and encoding, the total time taken to retrieve and store each read corresponding to the list of read IDs described in section 4.1 was recorded. However, in order to reasonably compare the different encodings, each read was additionally decoded into the ASCII format which would be necessary in most user applications. This decoding time was also recorded, and was combined with the total read access time. For each file type and encoding six such sub-experiments were performed using 1, 2, 4, 8, 16 and 32 threads of execution. These powers of 2 were chosen for pragmatic reasons and because the relationship between performance and the number of threads is typically concave down.

However, the file sizes and total read access times recorded by both experiments are specific for this dataset and list of read IDs. Hence, more normalised metrics were devised based on the number of bases in the dataset and corresponding reads of the list of read IDs. In particular, rather than comparing the raw file sizes, the average number of bytes per base (avg. B/nt) was used. And instead of comparing the total read access times, the average number of kilobases accessed per second (avg. knt/s) was used.

## 4.2 Results

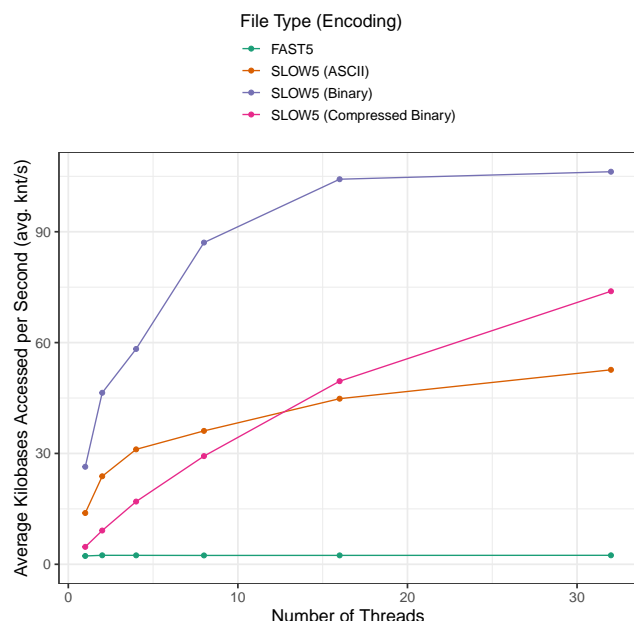
The results from the first experiment are shown in figure 1. The vertical bar plot visualises the bytes stored per base on average for each file type and encoding. It shows that the compressed binary encoding of the SLOW5 file format (in pink) is more space efficient than the FAST5 format (in green) with a 20% reduction in size, or on average 12.73 bytes stored per base versus 15.87 for FAST5. However, the binary encoding (in blue) is less space efficient, taking up 21.75 bytes per base on average, with a 37% increase in size from the FAST5 format.

**Figure 1:** Bar plot of the bytes stored per base on average (avg. B/nt) for each file type and encoding technique.

The second experiment's results are displayed in figure 2. The line chart graphs the average number of kilobases accessed per second using each file type and encoding over a varying number of threads. It clearly visualises the thread scalability issue (section 1) of the FAST5 file format (in green) with its read access speed remaining at roughly 2.42 knt/s on average from 2 to 32 threads. In comparison, the performance of all the SLOW5 file format encodings (in blue, orange and pink) improves with the number of threads used. This relationship appears to be positive, concave-down and non-linear for the server and range of threads used in this experiment.

In particular, the binary encoding of the SLOW5 format (in blue) was found to be the fastest to access for all number of threads, with an access speed of 106.25 knt/s on average using 32 threads (roughly 44 times faster than using FAST5). The compressed binary and ASCII encodings of the SLOW5 format (in pink and orange) performed in between the binary encoding and FAST5 file format (in blue and green). From 1 to 8 threads, the ASCII encoding (in orange) performed better than the compressed binary encoding (in pink), whilst the relationship reversed between 16 to 32 threads. Using 32 threads, 73.90 knt/s could be accessed with the compressed

binary encoding (in pink), in comparison to 52.63 knt/s for the ASCII encoding (in orange). In other words, the compressed binary and ASCII encodings of the SLOW5 file format performed 31 and 22 times faster respectively than the FAST5 file format.



**Figure 2:** Number of kilobases accessed and decoded per second on average (avg. knt/s) for each file format and encoding technique against the number of threads used.

## 5 DISCUSSION

### 5.1 Evaluation

The results displayed in section 4.2 show how storing ONT-sequenced reads in the SLOW5 file format can significantly improve the run-time of nanopore-specific pipelines. In particular, the binary encoded reads could be accessed roughly 44 times faster than FAST5-stored reads. This is the difference between waiting 1.36 minutes for the binary encoding, which is only about 1.4 times longer than it takes for the binary encoding. The regular ASCII encoding would take 2.73 minutes.

The differences in access speed between the SLOW5 encodings can be explained by the size of each read and the process of decoding each read into the ASCII format. The original ASCII-encoded SLOW5 format takes up roughly 2 and 3.4 times more space than the binary and binary compressed encodings respectively. This translates into higher I/O costs for reading the ASCII encoding. In comparison the binary compressed encoding suffers fewer I/O costs but each read must be decompressed and converted from binary into the ASCII format. This memory-decoding trade-off seems to favour decoding as the number of threads increase, as is evident in figure 2 as the binary compressed encoding (in pink) overlaps

the ASCII encoding (in orange) between 8 and 16 threads. This suggests that decoding is an overhead that's overcome by more parallel threads of execution.

For the typical nanopore sequencing pipeline, the results suggest that the compressed binary encoding of the SLOW5 file format is most appropriate, with a 20% reduction in file size and 31 times increase in read access performance. However, for maximum performance the compressed binary encoding could be used for long-term storage and then decompressed.

Might be good to comment on how long this decompression/compression approach would take for a typical dataset?

For adoption into the nanopore sequencing community, the content and structure of the SLOW5 file format should first of all be finalised. Then, a simple toolkit for lossless conversion between FAST5 and the SLOW5 encodings, reading, sorting, indexing and other operations should be built. This should take the form of a command line interface (CLI) and libraries for common bioinformatics programming languages. Afterwards, SLOW5 should be integrated into popular third party software tools (e.g. Bonito [13], Nanopolish [4] and f5c [5]) and released as an open source resource for the bioinformatics community.

### 5.2 Limitations

The design of the experiments could be improved on with more datasets capturing a diverse range of organisms each with multiple lists of read IDs. Furthermore, different servers with various configurations such as a RAID data storage system, should have been used to generate more robust and generalisable conclusions.

## 6 CONCLUSION

In conclusion, the aims of this paper were to efficiently compress the SLOW5 file format to a size smaller than its FAST5 counterparts, maintain parallel access in the process and achieve the best possible level of performance within these constraints. Each was successfully accomplished and detailed hitherto.

Moreover, by exploiting space efficient compression techniques and multithreading, the space requirements of FAST5 files can be reduced by 20% whilst performing read accesses 31 times as fast. The impact of these conclusions are far-reaching for the nanopore sequencing community and the future of bioinformatics.

## 7 FUTURE WORK

In addition to the steps for adoption into the research community detailed in section 5.1, future work includes experimenting with different levels of compression and alternative algorithms used by the zlib library for the compressed binary encoding of the SLOW5 file format.

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## A APPENDIX

**Table 4:** Example of the SLOW5 file format in the ASCII encoding.

##file_format=slow5v0.1						
#read_id	n_samples	digitisation	offset	range	sample_rate	raw_signal
id-0	6028	8192.0	3.0	1467.6	4000.0	1373, 712, 738, 715, 716, ...
id-1	59676	8192.0	23.0	1467.6	4000.0	1039, 588, 588, 593, 586, ...
⋮	⋮	⋮	⋮	⋮	⋮	⋮
id-N	45690	8192.0	5.0	1467.6	4000.0	1255, 773, 617, 574, 568, ...

```
##file_format=blow5v0.1
#read_id n_samples digitisation offset range sample_rate raw_signal
^D^A^@^@^@^@id-0^@^@^@^@^@^@^@^@^@F^@^@^@^@A<85>s.D^@^@zE^@D^L^B^L^BQ^@B^J^@B>^B:^B...
^D^A^@^@^@^@id-11^@^@^@^@^@^@^@^@^@F^@^@^@^@A<90>A<85>s.D^@^@zE^@D^d^Bc^@B=^B^E^@Q^@B<^B...
...
^D^A^@^@^@^@id-N^@^@^@^@^@^@^@^@^@F^@^@^@^@A<85>s.D^@^@zE^@D<92>^B^@^B^V^@B^@B^L...
```

**Figure 3:** Binary encoding of the example SLOW5 file shown in table 4. Note: newline characters between the header and each read have been added for readability and ‘...’ indicates where more information is not displayed.

```
1 ^_<8b>^H^@^@^@^@^@^@^@^@^@C%Ê1
2 <80>0^L^@^@A9yFwÑÑÑ. <84>HÓ^RHSi<82>~_ÄÖ, <9c><(c^]^3S^@S<8e>gzxeKy2^U<94>^B<86>NyRv(Ö$Ä)d^X
<8c>Z<9d>^C&Ycø^GN
3 pãA<97>fœé^E^<99>ro^@^@^@
4 ^_<8b>^H^@^@^@^@^@^@^@^@^@C<<9d>^E, <94>ÄÜÆ<99>^Aé<94>n$E^»^@é<92>^RP@1^P;0±^U^SQTBT^P...
5 ^_<8b>^H^@^@^@^@^@^@^@^@^@C<<9d>^E, TÖÜöÜ<8b><94>NAB^Zi*»^@^@R^J<83> ^B^J^K© <9d>çð<9d><82>...
6 ...
7 ^_<8b>^H^@^@^@^@^@^@^@^@^@C<<9d>^GÜ<8f>öÿÿ<9d>c^
8 i^<9b><94><91>=JF)Ü;{eo¢<8c>^P^J^o{<90>^URQDî 2";3dî-;Üÿçëußzçëñ^Y+ö^1@s<9d>óþöH<96>...
```

**Figure 4:** Compressed binary encoding of the example SLOW5 file shown in table 4. The header found in figure 3 is compressed in lines 1, 2 and 3. Whilst the compressed reads corresponding to read IDs ‘id-0’, ‘id-1’ and ‘id-N’ are found in lines 4, 5 and 7/8 respectively. Note: newline characters between the header and each read have been added for readability and ‘...’ indicates where more information is not displayed.

## NOMENCLATURE

ASCII	American Standard Code for Information Interchange: An encoding for representing text on computers, page 2
Avg.	Average, page 3
B	Bytes, page 3
CLI	Command-line interface, page 4
CPU	Central processing unit, page 1
DNA	Deoxyribonucleic acid, page 1
Gnt	One billion bases (nucleotides), page 3
HDF5	Hierarchical Data Format 5, page 1
HPC	High performance computing, page 3
I/O	Input/output, page 3
ID	Identifier, page 2
knt	Kilobases (kilo nucleotides), page 3
No.	Number, page 3
nt	Nucleotides, page 3
ONT	Oxford Nanopore Technologies, page 1
OS	Operating system, page 3
RAID	Redundant Array of Inexpensive Disks, page 4
RAM	Random-access memory, page 3
Read	Data generated from sequencing a segment of DNA, page 1
TSV	Tab-separated values, page 2