

Bachelor Thesis

---

# **Hi-C interaction matrix correction using ICE in Rust**

---

Felix Karg

Examiner: Prof. Dr. Backofen

Advisers: Joachim Wolff, Dr. Mehmet Tekman

Albert-Ludwigs-University Freiburg

Faculty of Engineering

Department of Computer Science

Chair for Bioinformatics

July 10<sup>th</sup>, 2019



**Writing Period**

10.04.2019 – 10.07.2019

**Examiner**

Prof. Dr. Backofen

**Advisers**

Joachim Wolff, Dr. Mehmet Tekman



# Erklärung

Hiermit erkläre ich, dass ich diese Abschlussarbeit selbständig verfasst habe, keine anderen als die angegebenen Quellen/Hilfsmittel verwendet habe und alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen wurden, als solche kenntlich gemacht habe. Darüber hinaus erkläre ich, dass diese Abschlussarbeit nicht, auch nicht auszugsweise, bereits für eine andere Prüfung angefertigt wurde.

---

Ort, Datum

---

Unterschrift



# Abstract

foo bar





# Zusammenfassung

German version is only needed for an undergraduate thesis.

**(TODO: Schreiben!)**



# Contents

<b>1</b>	<b>Introduction</b>	<b>1</b>
1.1	Task Definition . . . . .	2
<b>2</b>	<b>Background</b>	<b>5</b>
2.1	Chromosome Conformation Technologies . . . . .	5
2.1.1	Common steps . . . . .	5
2.1.2	3-C . . . . .	7
2.1.3	4-C . . . . .	8
2.1.4	5-C . . . . .	8
2.1.5	Hi-C . . . . .	8
2.1.6	Other methods . . . . .	9
2.2	HiCExplorer . . . . .	9
2.2.1	Analysis . . . . .	10
2.2.2	Visualization . . . . .	10
<b>3</b>	<b>Related Work</b>	<b>15</b>
3.1	Python implementation . . . . .	15
3.1.1	Advantages . . . . .	15
3.1.2	Disadvantages . . . . .	16
3.2	KR-Algorithm . . . . .	16
3.2.1	Implementation . . . . .	17
3.2.2	Advantages . . . . .	17

3.2.3	Disadvantages . . . . .	17
<b>4</b>	<b>Approach</b>	<b>19</b>
4.1	Problem Description . . . . .	19
4.2	Iterative Correction and Eigenvector decomposition (Algorithm) . .	19
4.3	Introducing Rust . . . . .	21
4.3.1	History . . . . .	21
4.3.2	Categorization . . . . .	21
4.3.3	Language Features . . . . .	22
4.3.4	Code Examples . . . . .	24
4.3.5	Advantages of Rust . . . . .	27
4.3.6	Disadvantages of Rust . . . . .	27
4.3.7	Comparing Rust and Python . . . . .	28
4.3.8	Comparing Rust with C/C++ . . . . .	28
4.3.9	Choosing the right API to call Rust from Python . . . . .	29
4.3.10	Integration of Rust in Python . . . . .	32
4.3.11	Using this implementation . . . . .	33
4.4	General Approach . . . . .	33
<b>5</b>	<b>Experiments</b>	<b>35</b>
<b>6</b>	<b>Conclusion</b>	<b>39</b>
	<b>Bibliography</b>	<b>46</b>

# List of Figures

1	Major Chromatin Structures . . . . .	6
2	Comparison between 3C and its derived methods . . . . .	7
3	Summarised Hi-C protocol . . . . .	12
4	Excerpt of HiCExplorer visualizations . . . . .	13
5	Models relating to HiC-Data . . . . .	14
6	Pairplot . . . . .	37



# List of Tables

1	Runtime Comparison Between Rust/C/C++ . . . . .	29
2	Comparison of different Interfaces between Rust and Python . . . .	32





# 1 Introduction

(DRAFT: more formal language)

(DRAFT: rewrite. Make more fluent for readers.) (DRAFT: remove “stuff” and (stuff))

Even though much is known about the one-dimensional structure of our DNA, it also organizes itself in three-dimensional ways. Recently, with the advent of chromosome conformation capture (3C) technologies, it has become possible to gain insight into the three-dimensional structure as well. Gene-regulators have been intensively looked at in the one-dimensional case, but enhancer and promoter also have an influence on their three-dimensional surroundings, making knowledge about the spatial organization relevant.

In this work, data gotten through Hi-C [1] will be used. Hi-C is a method for getting 3D-information of genomes. This is done by ‘strapping’ together parts of the genome that are close by, cutting apart the genome with restriction enzymes, combining the ends of strapped-together fragments and sequencing them. This will be explained in detail in Section ??.

However, such technologies tend to suffer from technical (e.g. sequencing, mapping) [2] and biological factors (e.g. distinct chromatin states) [3], making them inherently inaccurate. Biases are unavoidable, in particular, as some regions are more sensible for biotin labeling enrichments (See Section ?? why this is relevant) they will be

measured more often when compared to others. PCR artifacts may be one of the reasons [?]. Mapping locations may be unclear or not unique, introducing even more sources for possible biases. Sequencing methods have certain biases themselves. Some of the measured interactions are questionable, it is unclear if these are actual, spatially close points, or if it simply is a technical error or a randomly happened interaction.

**(EXTEND: not however so many times!)** However, a basic but strong assumption about the structure of the genome can be made, which is that every location has the same amount of interactions (with other locations) as every other location. The data does not show this due to the several aforementioned inaccuracies. Algorithms such as ICE [4] (Iterative Correction and Eigenvector decomposition, Section 4.2) or KR [5] (Knight-Ruiz, Section 3.2) can be applied to normalize the matrix nonetheless.

## 1.1 Task Definition

**(TODO: Bilder: Image dapted from anstatt Image from ...)**

**(TODO: Prüfungsamt abklären: Digitale Kopie? -> AJ Müller)**

In the three-dimensional space of a cell the DNA forms a structure that is scrambled all around the place. Obviously, there exist many points of contact in the DNA, they even form noticable structures, such as DNA loops. However, many of these contacts are random contacts or measurement errors that need to be corrected. A Python implementation exists but is limited for high resolution data due to high memory usage. This thesis aims to reimplement a more resource efficient method in Rust.

The main goals of this thesis include testing the integration between Rust (a systems programming language recently gaining in popularity, more information in Section 4.3) and Python (Section 4.3.10), how easy it is to let both these languages interact, and

how it compares to the other two current implementations (ICE in Python and KR in C++, more information can be found in Section 3.1 and Section 3.2 respectively). The overall goal however, is to try to implement a more resource efficient version, able to make effective use of parallel computations.



## 2 Background

Chromatin usually describes different levels of how DNA organizes itself. The well-known double-helix is only the lowest of several structural layers (major chromatin structures are shown in Figure 1). Looking at it from the outside (highest structural layer), DNA is scrambled all around the place. With the help of chromosome conformation technologies spatial proximity can be visualized.

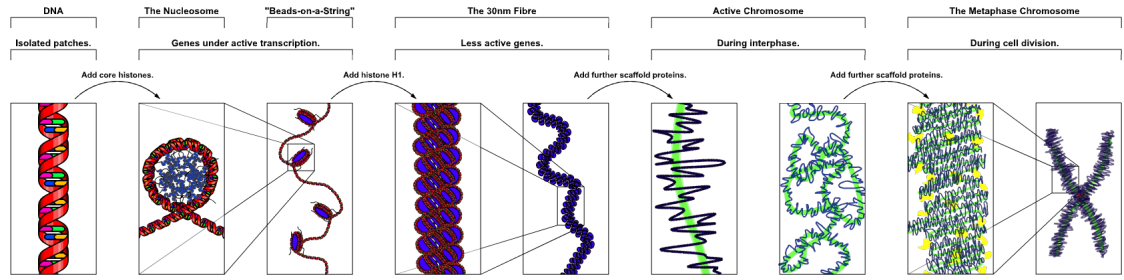
### 2.1 Chromosome Conformation Technologies

#### 2.1.1 Common steps

As can be seen in Figure 2, The first steps, crosslinking, digestion, ligation and the reversal of crosslinking, are the same for all 3-C-based methods.

**Cross-linking DNA** The first step is to cross-link DNA strands that are close to each other spatially (see Figure 2 or Figure 3 for reference). This is done by adding formaldehyde, which connects (links) sufficiently close strands together.

A chromatin cross-link is two entirely different parts of the genome held together by a chemical bond with formaldehyde. This process cannot be specifically controlled, so only ‘regions near each other’ are connected, but not necessarily all regions that are known to be spatially close.



**Figure 1: Major Chromatin Structures.**

Note that the rightmost structures are only found during cell division, the cell is in interphase during most of the time.

Image from [6].

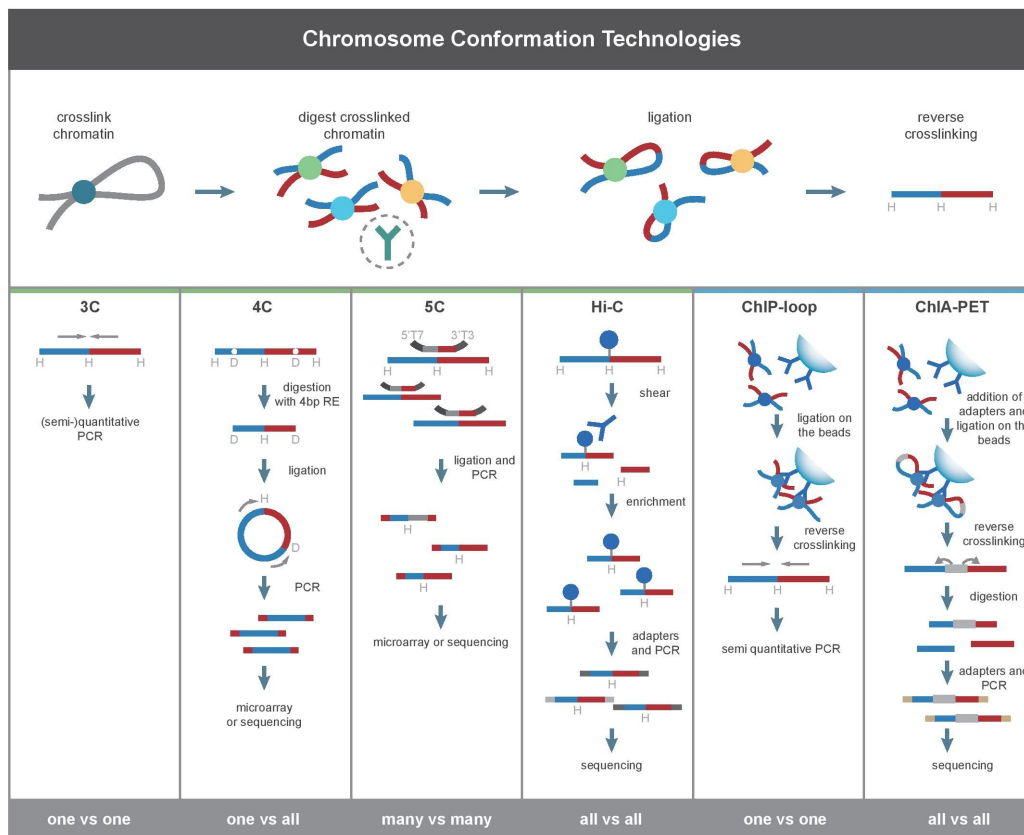
**Digestion** The next step is cutting the DNA apart in intervals. For this, restriction-enzymes are used (specifically restriction endonuclease). Commonly used enzymes for this are DpnII, NcoI or HindIII [1]. This will result in a lot of cross-linked fragments, as well as not-cross-linked ones.

**Ligation** After reducing the concentration of fragments, DNA ligase is added, to ligate (weld together) dangling fragment ends. For this a reduction in concentration is done since mostly fragments close together are ligated, it is intend to ligate fragments linked together by formaldehyde.

In HiC, Biotin is added in this step to mark ligated fragments. This allows filtering out most fragments that have not been ligated in a later step.

**Reverse Cross-links** Adding a high concentration of salt for some time will reverse the cross-linking through formaldehyde, leaving us with our two originally spatially close fragments ligated and with a biotin-marker.

Note that at this point, the fragments are too long to sequence them. They are ligated fragments of around 8000 base-pairs, but most current sequencing methods can only deal with sequence lengths of a few hundred base-pairs at most.



**Figure 2: Comparison between 3C and its derived methods.**

Image taken from [7].

### 2.1.2 3-C

In 2002 Dekker et al. [8] developed a method test for interactions between a single pair of genomic loci. Candidates for promoter-enhancer interactions can be tested using this method. After the reversal of cross-links (Section 2.1.1), the fragments (EXTEND: what is (semi-)quantitive PCR?)

### 2.1.3 4-C

Chromosome conformation capture-on-chip (4C) was developed in 2006 by Simonis, Zhao et al. [9] [10], a method to test interactions between one genomic loci to all others. This is done by adding a second ligation-step (see Section 2.1.1, Figure 2 for reference) creating loops from the DNA fragments and applying inverse-PCR (method to specifically amplify the unknown parts when beginning and ending parts are known, for this the loops are cut within the known section). Afterwards, this may get sequenced. Due to inverse-PCR knowledge about both interacting chromosomal regions is not needed. Results are highly reproducible for close regions (**DRAFT: find source for this**).

**(EXTEND: What is a microarray?)**

### 2.1.4 5-C

Chromosome conformation capture carbon copy (5C) was developed in 2006 by Dostie et al. [11], this method is able to test a region for interactions with itself, such region being no bigger than a megabase. This is done by adding universal primers to all fragments from such a region. 5-C has relatively low coverage, but is useful to analyse complex interactions of specified loci of interest. Genome-wide interaction measuring would require millions of 5C primers, making this method unsuitable.

### 2.1.5 Hi-C

Hi-C (as shown by Figure 3) was developed in 2009 by Liebermann-Aiden et al. [1]. After the common steps noted earlier, unique to Hi-C is the following sequence of sonication, pulldown (filtering based on biotin markers) and sequencing.



**Sonication** Putting the ligated DNA-fragments under the influence of ultrasonic waves is breaking them apart in much shorter fragments (due to long sequences not being able to absorb frequent shocks well), shearing them apart in sequences short enough to enable sequencing.

**Filtering and Removal of Biotin** ‘pulling-down’ fragments marked with biotin leaves only those having been marked, and thus ligated, earlier (see Section 2.1.1). Subsequently the marker is removed, as it would hinder further sequencing.

**Sequencing** Sequencing, short for DNA sequencing, describes processes of measuring a DNA sequence. There are several techniques for doing this, most use PCR (Polymerase Chain Reaction) before or while sequencing, which duplicating fragments several times, allowing them to be sequenced more accurately.

### 2.1.6 Other methods

Other methods, such as ChIP-loop or ChIA-PET exist, however as they are different from the digestion step onwards, and their subsequent steps are fundamentally different, they will not be covered in depth.

(DRAFT: explain them at least somewhat, reference to some other source)

(EXTEND: placeholder)

## 2.2 HiCExplorer

HiCExplorer [13] is a collection of tools that are used to process, analyze and visualize Hi-C data. Part of this collection are tools to convert between formats, correcting the data (which this work is part of), normalizing it, analysing it in various ways, and extensively plotting it. Facilitated is, among others “the creation of

contact matrices, correction of contacts, topologically associating domains (TAD) detection, A/B compartments, merging, reordering or chromosomes, conversion from different formats including cooler and detection of long-range contacts.”<sup>1</sup> Those contact matrices may then be visualized, also showing other types of data, including “genes, compartments, ChIP-seq coverage tracks, long range contacts and the visualization of viewpoints”<sup>1</sup>. An excerpt of possible visualizations can be seen in Figure 4. HiCEXplorer can be installed on Linux and MacOS.

### 2.2.1 Analysis

Corrected Hi-C data can then be further analysed, with `hicFindTADs` one can search for TADs (topologically associated domains) [15], for this a TAD-separation score is computed and local minima indicative of TAD boundaries are searched for. A visualized result of such a computation can be seen in Figure 4I (created with `hicPlotTADs`).

DNA is compartmentalized [1] in different domains, a model for this can be seen in Figure 5. They can be found by computing the Eigenvectors as described in [1] or [4] first by using `hicPCA` and `hicTransform`. With this, a better understanding can be achieved when additionally visualized. Useful metrics include difference, ratio and log2ratio between two matrices. For this, `hicCompareMatrices` can be used. Replications or samples from different conditions can easily be compared when visualized. More information about the analysis capabilities of HiCEXplorer can be found in [13].

### 2.2.2 Visualization

Visualizations are necessary when the goal is to understand complex structures fast or even at all. It is impossible look at rows of numbers and notice that one significantly

---

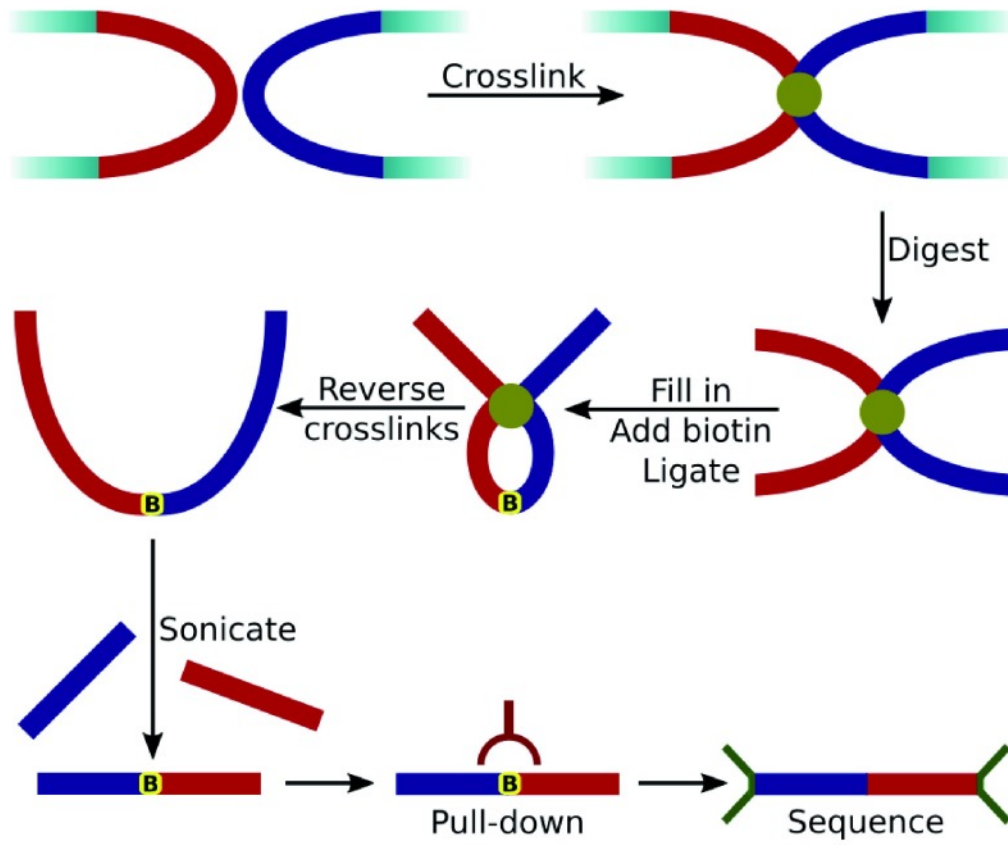
<sup>1</sup><https://github.com/deeptools/HiCEXplorer>, accessed 2019-06-26

higher value, which immediately catches the eye as a heatmap.

Basic plotting of contact matrices (as seen in Figure 4F) can be done using `hicPlotMatrix`. Options include region, color and value ranges, as well as plotting A/B compartments or other additional data when added. `hicPlotViewpoint` can visualize the number of interactions around a specific reference region or point in the genome (see Figure 4G).

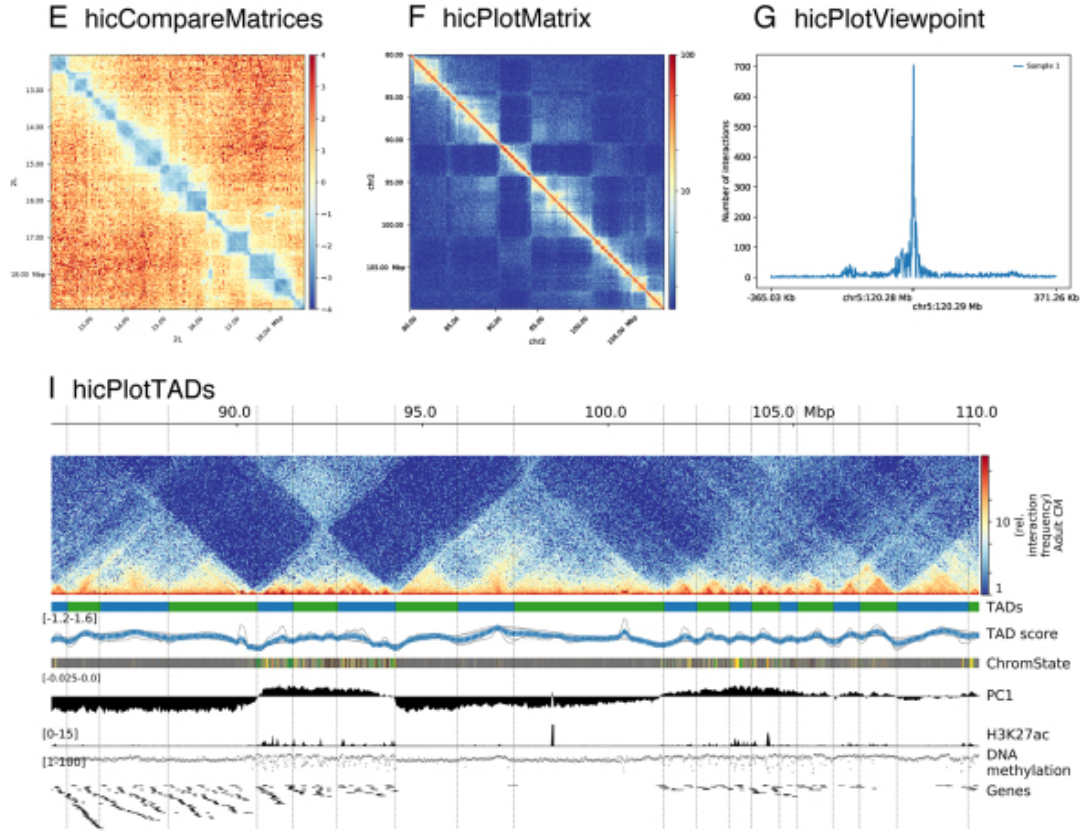
Computed TADs (as described in Section 2.2.1, see Figure 4I) can be plotted using `hicPlotTADs`. This tool can plot multiple matrices and additional data. Contact matrices are rotated by 45°, and TADs are marked with triangles. The colormap, different ways for visualization, and several configurations to plot coverage tracks can be selected.

More information about plotting with HiCExplorer can be found in [13].



**Figure 3: Summarised Hi-C protocol.** Biotin is shown by a yellow marker, while the red and blue parts are different parts of cross-linked fragments. The steps are explained in detail in Section 2.1.1 and Section 2.1.5.

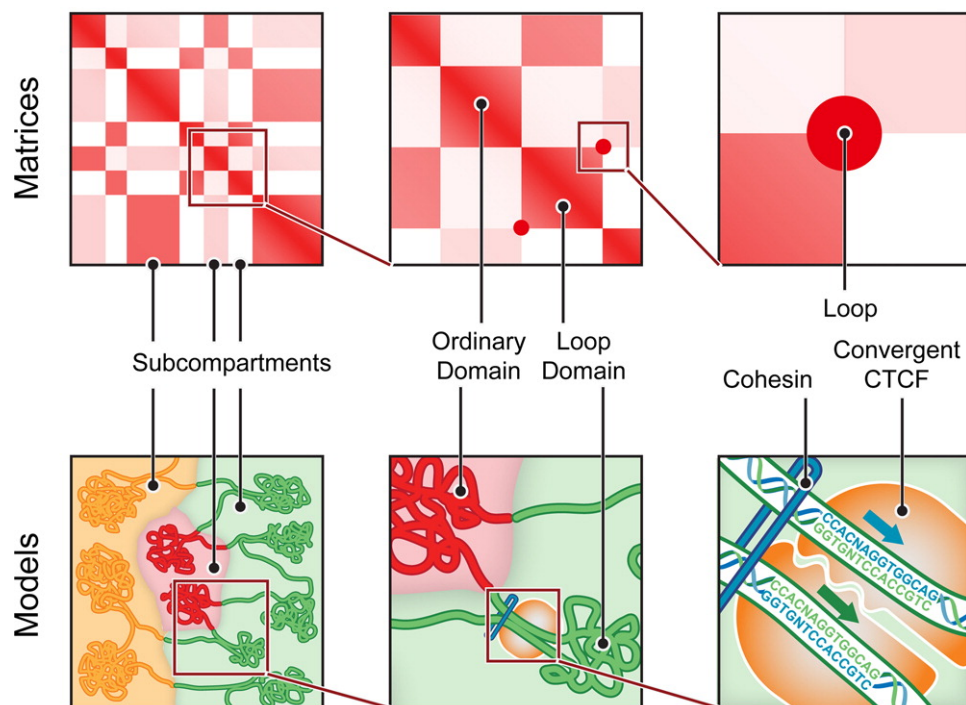
Image taken from [12].



**Figure 4: Excerpt of HiCExplorer visualizations** **E)** Pixel difference computed using `hicCompareMatrices` and visualized using `hicPlotMatrix` of a Hi-C corrected matrix for wild type condition and knock down. **F)** Plot of a 80 to 105 Mb region contact matrix of chromosome 2 in log scale. **G)** Corrected number of Hi-C contacts shown using `hicPlotViewpoint`, for a single bin in chromosome 5 (output similar to 4C-seq). **I)** Human chromosome 2 visualization (region 85-110 Mb) using tracks from different tools found in the HiCExplorer toolbox (primarily TAD-related information).

Image taken from [13].

## Hi-C Matrices and Models



**Figure 5: Models relating to HiC-Data.**  
Image taken from [14].

## 3 Related Work

(EXTEND: some section here to fill another page)

Since the main work is the implementation in Rust as well as the testing of an integrating in Python, related work includes the original implementation in Python as well as the recent implementation of the KR-algorithm in C++. Disadvantages and advantages of the respective implementations will be evaluated in the following.

The description of the implemented algorithm can be found in Section 4.2.

### 3.1 Python implementation

The original implementation was written in Python, since HiCEXplorer is written in Python. This implementation is using common python dependencies extensively, including the Compressed Sparse Row Matrix (CSR Matrix) implementation from `scipy`, as well as the scientific number manipulation library `numpy`.

#### 3.1.1 Advantages

The implementation itself is considerably short, the file having only 86 Lines, including imports and frequent comments. The advantage of using Python here is showing, as most lines are not for functionality itself, but for timing, logging and ease of

debugging. With the iteration itself starting no earlier than Line 40, most are High-Level `numpy` / `scipy` commands, some being themselves implemented in C/C++ to be sufficiently fast.

Another advantage of Python in general are fast implementation times, which are possible through the concise syntax making the spotting of mistakes easier.

### 3.1.2 Disadvantages

(DRAFT: Include: Multithreading is locking the execution of the same part of code, processes allow that but require the same memory for writing in it, reading is fine)

The downsides of this implementation being that datastructures in Python are extensively objectified, meaning they require more working memory, and that even though Python has existing parallelism, a global interpreter lock (python is only interpreted usually, but this still holds for compiling with cpython) prevents multiple threads to use the same parts of memory without duplication. Since Python already has comparatively high memory requirements (DRAFT: link high memory needs), it is not practicable to add the same amount for every further core.

For reference, the Python-implementation can be found here<sup>1</sup>.

## 3.2 KR-Algorithm

What follows is a short description to the Algorithm known as Knight-Ruiz from [5]. Essentially, the algorithm is computing the same things, however it is taking advantage of conjugate gradients to solve the iterative steps faster.

---

<sup>1</sup><https://github.com/deeptools/HiCEXplorer/blob/master/hicexplorer/iterativeCorrection.py>, accessed 2019-06-26



### 3.2.1 Implementation

The KR-algorithm was originally implemented in Matlab, here we compare with a version in C++. Calls from Python to C++ can be done over the C-API with considerable help through Python-header files.

### 3.2.2 Advantages

A commonly mentioned advantage of C++ is the speed of execution, and fine-grained control over Memory available. However, implementations in C++ can be several orders of magnitude faster than their respective implementation in Python. An advantage of the Algorithm itself is that as long as the matrix itself has total support (meaning that at least one diagonal has only positive nonzero values, this can be artificially done setting zeros to some small positive value), it will converge. Thus, it will converge for way more matrices than the ICE-algorithm.

### 3.2.3 Disadvantages

Even though the execution is fast, the development process tends to be slow. This is due to the free memory control, which is hard to get right as this requires upholding of implicit assumptions at several places. As these assumptions are implicit only, it is easy to forget them or 'cut corners' when not possible. Those bugs leading to Segmentation-faults (accessing invalid memory) are notoriously hard to find, as they do not follow determinism. Parallelism is even harder to add, since data races (also nondeterministic) and other sources for hard-to-get right problems are added. Additionally, the syntax is considerably complex, making it rather hard to understand. For reference, the implementation of the KR algorithm can be found here<sup>2</sup>.

---

<sup>2</sup><https://github.com/deeptools/Knight-Ruiz-Matrix-balancing-algorithm>,  
accessed 2019-06-26



## 4 Approach

### 4.1 Problem Description

As noted in Section 1.1, the main goals include testing the integration of Rust within Python, by implementing a counter-version to the original Python implementation of the iterative part of the ICE algorithm, and then comparing it with the original Python-implementation as well as the recent implementation of the KR-algorithm in C++. It will be tested if the memory efficiency can be improved, also how well the parallelization using Rust works really, and how the integration from Python to Rust works. For C/C++ there exist the Python headers, and extensive support from Pythons package manager `pip` and common packaging tools like `setuptools`. For Rust, as it turns out, the support using Python headers is not as easy, and the support for building packages from Pythons side is early at best. Both of this will be covered in Section 4.3.10 and Section 4.3.9 respectively.

### 4.2 Iterative Correction and Eigenvector decomposition (Algorithm)

The Algorithm was proposed by Imakaev et al. 2012 [4], and is described in detail in their supplementary material.

The goal is to obtain the the vector of biases  $B_i$  and the ‘true’ contact map  $T_{ij}$  with their relative contact probabilities. This is done by explicitly solving the system of the following two equations:

$$O_{ij} = B_i B_j T_{ij} \quad (1)$$

$$\sum_{i=1, |i-j|>1}^N T_{ij} = 1 \quad (2)$$

Equation (1) is stating, that when applying  $B$  back again on our corrected matrix  $T_{ij}$ , it will be the same as the original matrix  $O_{ij}$  again. Equation (2) states, that the sum over the corrected matrix, over arbitrary elements in the upper left triangle, but only one from each column, sums up to one.  $T_{ij}$  is doubly stochastic ( $\forall_j \sum_{i=1}^N T_{ij} = 1$  and  $\forall_i \sum_{j=1}^N T_{ij} = 1$ ), **(EXTEND: does it say that it is the same? why is this actually valid?)**

In the algorithm, this is achieved in the following way. First,  $W_{ij}$ , a copy of  $O_{ij}$  is created. This matrix will converge to  $T_{ij}$  during the iterative process. The elements of  $B$  are initialized with 1.

$$S_i = \sum_j W_{ij} \quad (3)$$

$$\Delta B_i = S_i / \text{mean}(S) \quad (4)$$

Each iteration starts by first calculating the coverage by summing up each row (or column, matrix is symmetric so this does not matter) (Equation (3)) and additional biases based on this by dividing them through their own mean (Equation (4)).

$$W_{ij} = W_{ij} / \Delta B_i \Delta B_j \quad (5)$$

$$B_i = B_i \cdot \Delta B_i \quad (6)$$

Then  $W_{ij}$  is iterated by dividing by  $\Delta B_i \cdot \Delta B_j$  (Equation (5)), after which  $B_i$  is iterated by multiplying with the current biases (Equation (6)).  $W_{ij}$  accumulates divisions by  $\Delta B_i$ , just as  $B_i$  accumulates the products of  $\Delta B_i$ . This is repeated until the variance of  $\Delta B$  becomes negligible, at which point  $W_{ij}$  has converged to  $T_{ij}$ .

## 4.3 Introducing Rust

### 4.3.1 History

Rust started out 2006 as a personal project of Graydon Hoare, a Mozilla employee [16]. Mozilla started sponsoring in 2009 [16]. The first compiler was written in OCaml, but 2011 rustc was able to compile itself with the llvm backend [17]. Starting with Rust 1.0, which itself got released on May 15, 2015 [18], there was a new stable point version every six weeks [18]. Early on, Rust had frequent breaking changes [19], recently barely anyone had breakage when updating [20].

### 4.3.2 Categorization

Rust is classified as a high-level language, even though fine low-level control is possible. This is due the high amount of high-level zero-cost abstractions. Rust has a type system with strong guarantees, promising e.g. that all references (pointers) are valid, or thread safety (memory access from other threads does not result in data races / undeterminism). This is possible through concepts such as ownership and lifetimes. Even though one can program in an object oriented way, Rust is primarily not object-oriented. Additionally it is imperative, procedural, generic and functional.

### 4.3.3 Language Features

**Syntax:** The concrete syntax seems similar to C/C++ (curly braces, function signatures), however it is more similar to that of ML or Haskell. A particular example for this case are type classes called “traits” here, similar to C++ templates but inspired from Haskell, supporting polymorphism and generic types. Generic parameters can be constraints, by requiring that generic type to implement a certain Trait.

**Memory safety:** Rust is designed to be memory safe, and does not permit dangling pointers, null pointers, data races in safe code, or usage of uninitialized variables. In case a ‘null’ is needed, the Option-type is provided. Thus, the compiler can guarantee the validity of all references at compile time using its borrow-checker.

**Memory management:** Rust does not have a garbage collector, instead, the resource acquisition is initialization (RAII) convention is used, with optional reference counting. Resource management is deterministic with very little overhead, favoring stack allocation without implicit boxing. References are not run time counted, as their usage is verified at compile time. with this, memory safety can be guaranteed, limiting possible undefined behaviour tremendously.

**Ownership:** In Rust, all values have a unique owner, and the scope of the value is the same as the owners. Immutable references can be passed using `&T`, mutable references by `&mut T`. Pass by value works by passing `T`. Only **one** mutable reference can exist at any point, or any number of immutable ones. This is enforced at compile-time.

**Borrowing:** Borrowing results directly from the concept of ownership. As mentioned, only one mutable borrow (reference) can happen at a time, however that borrowing variable can further borrow it to other variables or functions. The number of immutable borrows is unlimited, meaning there can be multiple references reading

but not modifying part of the memory. This is necessary to guarantee memory safety, as only one mutable reference can write to it at any point in time, wherever that is (in the code).

**Lifetimes:** Lifetimes are the simple concept of keeping track how long each variable and each reference is ‘alive’, this is preventing the simple case of ‘variables going out of scope’ and returning a pointer to it, but can do the same in much more complex environments. Non-Lexical-Lifetimes also work together with borrowing, resulting in variables returning their borrow before the end of the scope, as can be see in the first example of Section 4.3.4.

### **Tooling:**

The reason Rust is loved [21] this much is at least partly due to tooling. This includes the dedicated package manager `cargo`, the linter `rustfmt` or `cargo-fix` (a subcommand that can be added later), that format Rust code after predefined guidelines, or fix most compiler lint warnings automatically and upgrade to newer conventions, respectively.

More information about Rust can be found here<sup>1</sup>.

---

<sup>1</sup><https://www.rust-lang.org/>, accessed 2019-06-26

### 4.3.4 Code Examples

#### Demonstrating Ownership and Borrowing:

By executing Code Example 1 the output from Output 1 will be returned.

Code Example 1

```
1 fn main() {
2     let mut v = vec![];      // ---| v owns the (empty) vector
3     v.push("Hello");         // <--| vector gets first element
4                               //    |
5     let x = &v[0];           // -| | x borrows the first element from v
6     v.push("world");         // <X-| v cannot mutably borrow the vector
7                               //   | | while x has immutably borrowed it
8     println!("{}", x);       // -| | x needed at least until here
9 }                             // ---| x borrowing v ends, x, v go out of scope
```

Output Nr. 1

```
error[E0502]: cannot borrow `v` as mutable, it is also borrowed as immutable
--> src/main.rs:5:5
   |
5 |     let x = &v[0];
   |           - immutable borrow occurs here
6 |     v.push("world");
   |     ~~~~~ mutable borrow occurs here
7 |
8 |     println!("{}", x);
   |           - immutable borrow later used here
```



As the compiler is complaining, `v` needs a *mutable* borrow to modify `v`, however `x` still has an *immutable* borrow! The borrow from `x` cannot be ended yet, because it should be printed later. As the mutable borrow from `v` could modify it in a way such that the reference `x` would be invalid (e.g. delete `v`), this is a potential memory safety problem. However it is fine to print `x` first, and modify `v` afterwards. In Output 2 can be seen what happens when printing the second element of `v` instead of `x` in the last line, and printing `x` before adding the second element of `v` (as shown in Code Example 2).

Code Example 2

```
1 fn main() {
2     let mut v = vec![];      // ---| v owns the (empty) vector
3     v.push("Hello");         // <--| vector gets first element
4                               //      |
5     let x = &v[0];           // -| | x borrows the first element from v
6     println!("{}", x);       // -| | x needed only until here
7                               //      | x returns the borrow here
8     v.push("world");          // <--| v can now modify the vector
9                               //      | (mutable borrow needed)
10    println!("{}", v[1]);     // <--| v can be printed without trouble
11 }
```

Output Nr. 2

```
Hello
world
```

## Demonstrating Ease of Parallelization:

Due to the strong guarantees from the compiler, Memory Safety can be extended to thread safety. In Code Example 3 the function `heavy_operation` is applied to every element in the list. For this, over the list `somelist` is being iterated, and `heavy_operation` mapped over by taking the values from the map-closure - Closures are comparable with lambda-functions from Python, in that they can take arguments and are unnamed functions.

Code Example 3 and Code Example 4 demonstrate how easy it is to turn non-parallel code (Code Example 3) in parallelized code (Code Example 4). The difference here being the imported `rayon::prelude::*` and instead of `iter` now applying `par_iter` to the original list.

Code Example 3

```
1  let otherlist = somelist.iter()
2                                .map(|&v| heavy_operation(v))
3                                .collect();
```

Code Example 4

```
1  use rayon::prelude::*;
2
3  let otherlist = somelist.par_iter()
4                                .map(|&v| heavy_operation(v))
5                                .collect();
```

### 4.3.5 Advantages of Rust

**In General:** As seen in Section 4.3.3 and Section 4.3.4, Rust has several high-level features ready to use, supporting the developer tremendously. Strong compiler guarantees allow easy parallelization and using Rust libraries without worry, since the compiler will complain if it is used wrong. In combination with semantic versioning<sup>2</sup>, this allows adding and using dependencies without worry. The result are many small libraries which depend upon each other, instead of few big ones. Dependencies upon one hundred Rust libraries are not uncommon, and the strong guarantees from the compiler enforce correct usage.

**For this project:** Even though high modularity exists, the Rust ecosystem is comparatively young. meaning even though many libraries exist, they are not as complete as their Python/C/C++ counterparts. Since no implementation of CSR-Matrix having the required features, even though several existed, it was implemented again, adding the one necessary feature, and not implementing any other. This has the advantage of being a very specific solution, possibly faster and smaller than the general ones available.

Also, as seen in (TODO: Link section results), Parallelization can decrease the elapsed computation time.

### 4.3.6 Disadvantages of Rust

**In General:** General disadvantages of Rust include the young ecosystem with slightly less diversity, or too much feature-incomplete diversity. The steep learning curve in the beginning, needs to be mentioned, since Rusts features cannot be selectively activated. Compared to languages like GO, Rust has considerably higher initial compile times. Even though breakage rarely happens [20], a new point-release

---

<sup>2</sup><https://semver.org/>, accessed 2019-06-26

happens every six weeks, frequently introducing new features. The userbase is still growing, and many features frequently used in other languages, such as `async` or specializations, are not available for users of the stable compiler.

**For this project:** In particular, the unavailability of a CSRMatrix implementation with the needed features is concerning. Thus, the current implementation does not have any more features than are needed for the current algorithm, being only a tiny subset of the features provided by the `scipy` implementation.

### 4.3.7 Comparing Rust and Python

Rust and Python are two quite different programming languages, a direct “translation” is not possible. Both implementations are the same semantically, however details differ. Since Rust has a much finer control of memory and the applying of functions to data structures, some operations have been explicitly separated while others have been combined.

Depending on the questions asked, either language may prevail. While Python allows (seemingly) faster development cycles, it is more prone to runtime errors and library misuse. For Rust, the compiler provides strong guarantees, requiring more development time up front but less to fix bugs.

### 4.3.8 Comparing Rust with C/C++

As can be seen in Table 1, the runtime of Rust is very close to that of C and C++ (as is memory, not compared here). Both C and C++ are currently much more widely used, but there are already voices calling to replace C++ with Rust<sup>4</sup>.

---

<sup>3</sup> Rust comparison with C: <https://benchmarksgame-team.pages.debian.net/benchmarksgame/fastest/rust.html>, and with C++: <https://benchmarksgame-team.pages.debian.net/benchmarksgame/fastest/rust-gpp.html>, both accessed 2019-06-26

<sup>4</sup><https://hub.packtpub.com/will-rust-replace-c/>, accessed 2019-06-26

<b>Speed comparison</b>	C	Rust	C++
n-body	7.49	<b>5.72</b>	8.18
binary-trees	3.48	<b>3.15</b>	3.79
pidigits	<b>1.75</b>	<b>1.75</b>	1.89
reverse-complement	1.78	1.61	<b>1.55</b>
spectral-norm	1.98	<b>1.97</b>	1.98
fannkuch-redux	<b>8.61</b>	10.23	10.08
k-nucleotide	5.01	5.25	<b>3.76</b>
fasta	1.36	1.47	<b>1.33</b>
mandelbrot	1.65	1.96	<b>1.5</b>
regex-redux	<b>1.46</b>	2.43	1.82
Fastest in:	3/10	4/10	4/10

**Table 1: Comparing Runtime speeds.** Runtime measured in seconds. Numbers from the benchmarksgame<sup>3</sup>.

Resource needs may be close to the same, but from a developer standpoint Rust has consistently been the ‘most loved Language’ [21] for the last four years, whereas both C and C++ both rank considerably high in the category ‘dreaded’ [21]. Reasons why Rust may be such a loved language are listed in Section 4.3.5. Due to the barely integrated external static analysis done for C/C++-code, both C and C++ are more prone to memory bugs [22], thus having higher developer time requirements.

#### 4.3.9 Choosing the right API to call Rust from Python

There are three main ways to execute Rust code from Python. In the following, the feasibility of them for this thesis is evaluated. And compared in Table ??.

**rust-cpython:** One common way is rust-cpython. This library requires Rust 1.25 or higher, the current stable version at the time of writing is 1.33. Rust-cpython grants access to the Python gil (global interpreter lock) with which Python code can be evaluated and Python objects modified. The resulting library can easily be

## Code Example 5

```
1  #[macro_use]
2  extern crate cpython;
3
4  use cpython::{PyResult, Python};
5  // add bindings to the generated python module
6  // N.B: names: "librust2py" must be the name of
7  // the `.so` or `.pyd` file
8  py_module_initializer!(librust2py,
9      initlibrust2py, PyInit_librust2py, |py, m| {
10     m.add(py, "__doc__",
11         "This module is implemented in Rust.")?;
12     m.add(py, "sum_as_string",
13         py_fn!(py, sum_as_string_py(a: i64, b:i64)))?;
14     Ok(())
15 });
16 // logic implemented as a normal rust function
17 fn sum_as_string(a:i64, b:i64) -> String {
18     format!("{}", a + b).to_string()
19 }
20 // rust-cpython aware function. All of our python
21 // interface could be declared in a separate module.
22 // Note that the py_fn!() macro automatically converts
23 // the arguments from Python objects to Rust values;
24 // and the Rust return value back into a Python object.
25 fn sum_as_string_py(_: Python, a:i64, b:i64)
26     -> PyResult<String>
27 {
28     let out = sum_as_string(a, b);
29     Ok(out)
30 }
```

imported into Python after renaming the compiled library. Native Rust code requires some wrapping first, as can be seen in Code Example 5. The code is taken from the `rust-cpython` example which can be found here<sup>5</sup>. **(TODO: correctly align!!)**

This wrapping, though quite common and based on the Python C-API makes it hard to write purely idiomatic Code in Rust. Since Python is directly affected, the interactions with Python need to be considered while writing Rust-Code, including the affecting of memory Python is managing. This was deemed too much complexity overhead.

**pyO3:** Another common approach is using the `pyO3`-library, which started off as a fork of `rust-cpython`, but has since seen drastic changes. However, as `pyO3` continues to use unstable Rust features, it is only possible to compile this library with the nightly version of the compiler. Even though most unstable features have been stabilized by now, Specialization<sup>6</sup> is still missing several steps, as it is not sound regarding the type system yet, and not all questions are answered. Nightly features are subject to tremendous change, making this option a questionable at best for this implementation, as the goal is to have a stable implementation.

**Generate dylib:** The described way in the official rust docs is to create a `dylib` and import the resulting library in the respective language dynamically<sup>7</sup>. Here is no renaming necessary, but the communication between Rust and Python is more low-level. The main wrapper is on the side of Python, transforming Arguments to pointers and C-Representations. Rust needs to export an interface usable from C, which can be done by a simple `extern`. Rust has the `\#[no_mangle]` and `\#[repr(C)]` (procedural) macros, preventing the compiler to mangle (renaming of functions) and guaranteeing the representation in the memory layout to be as it would be in C, when they need to be exported.

---

<sup>5</sup><https://github.com/dgrunwald/rust-cpython>, accessed 2019-06-26

<sup>6</sup><https://github.com/rust-lang/rust/issues/31844>, accessed 2019-06-26

<sup>7</sup><https://doc.rust-lang.org/1.2.0/book/rust-inside-other-languages.html>, accessed 2019-06-26

<b>API Comparison</b>	rust-cpython	pyO3	dylib
any renaming needed	Yes	Yes	<b>No</b>
stable Rust	<b>Yes</b>	No	<b>Yes</b>
platform-specific compilation flags	Yes	Yes	<b>No</b>
using Memory managed by Python	Yes	Yes	<b>Optional</b>
additional implementation effort	Medium	Medium	<b>Low</b>
difficulty of creating python packages	<b>Easy</b> <sup>8</sup>	<b>Easy</b> <sup>8</sup>	Normal
Good in:	2/6	1/6	<b>5/6</b>

**Table 2: Comparing different API interfaces.**

When comparing the available options as can be seen in Table 2, dylib appears to be the best option. Thus, it got selected.

#### 4.3.10 Integration of Rust in Python

The integration of both C and C++ in Python are considerably straightforward and well-supported. Even though Rust is considerably new, a multitude of options exists. For this application however, None offered what was needed specifically.

**(TODO: interface from python to C but missing to RUST)**

**(TODO: Answer this question later!!)**

One of the main questions for this work was to find out if it is possible to integrate Rust in Python for the HiCE Explorer, and evaluate the advantages versus disadvantages.

**(TODO: rewrite)**

For Rust and Python to interact, there are of course several ways. Those integrating a library written in Rust to allow them to be called from Python will be described in depth in Section 4.3.9.

<sup>8</sup>This was not tested, but pyo3-pack is a zero-configuration package builder, <https://github.com/PyO3/pyo3-pack>, accessed 2019-06-26.



(DRAFT: building process: several options, milksnake was used here)

#### 4.3.11 Using this implementation

### 4.4 General Approach

(TODO: Introduce main approach)

(TODO: Link to github repository)



## 5 Experiments

Experiments were run on a Server with the following specs (Specifics here<sup>1</sup>):

Processor	Intel® Xeon® Processor E5 v4 Family
Number	E5-2630V4

---

### Performance

---

Number of Cores	10
Number of Threads	20
Base frequency	2.2 GHz
Max Turbo frequency	3.1 GHz
Working Memory (RAM)	120 GByte

(TODO: The time-resource-measures done)

(DRAFT: Something is off with my numbers, they show something entirely different from earlier. I'll check that again.)

See Figure 6 (more of that will follow).

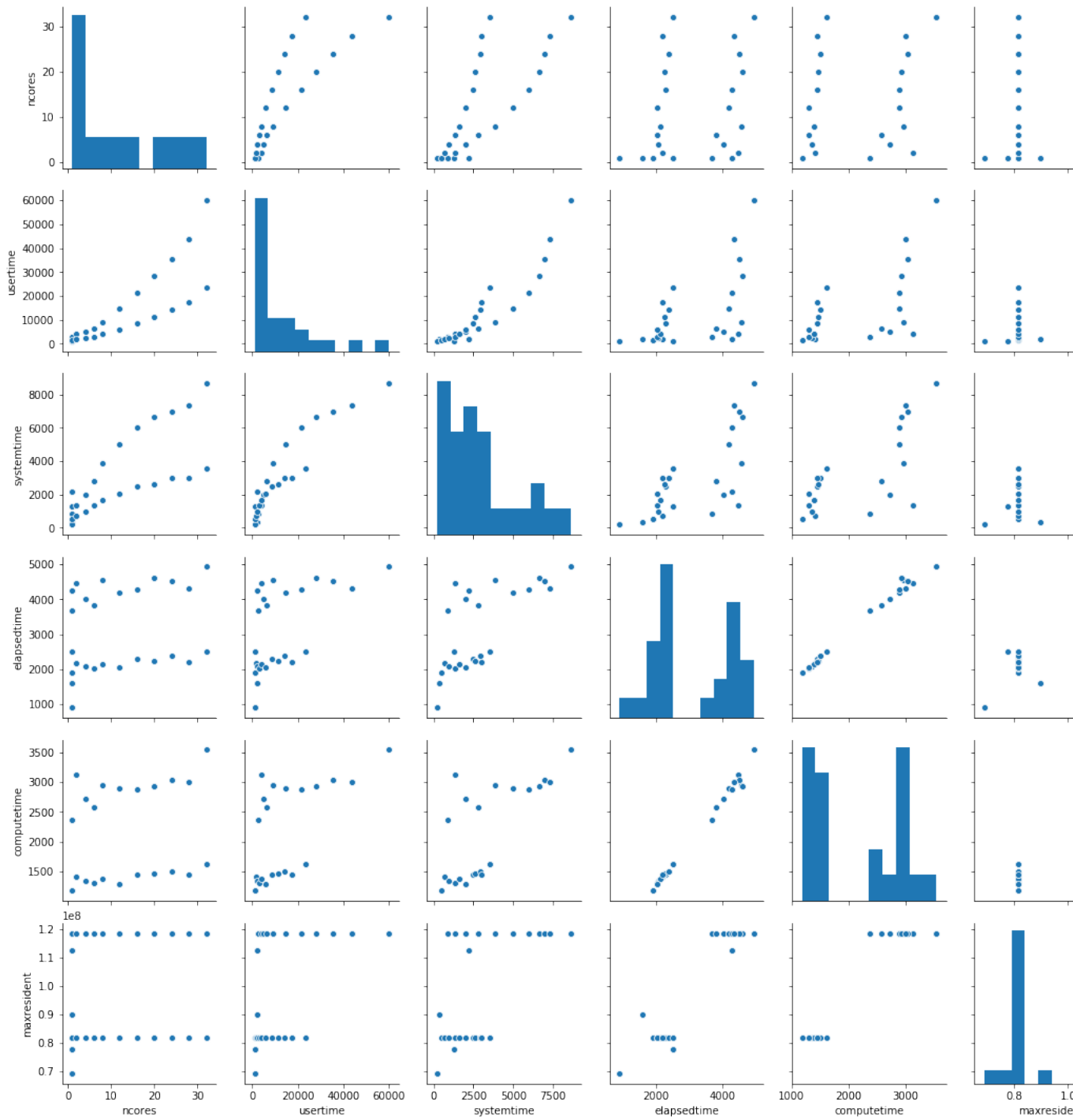
(TODO: add where the matrices / data is from)

(TODO: graphics: runtime vs cores (RUST), RAM vs variants, runtime vs variants)

---

<sup>1</sup><https://ark.intel.com/content/www/us/en/ark/products/92981/intel-xeon-processor-e5-2630-v4-25m-cache-2-20-ghz.html>, accessed 2019-06-26

(TODO: add plot of matrix uncorrected vs corrected ice vs corrected KR  
vs corrected ice\_rust)



(a) Pairplot over some variables

Figure 6: Pairplot over some of the variables ... moar info here



## 6 Conclusion

(TODO: general evaluation; worked better or worse, why, what could be tried / changed, evaluation of rust in this context, ...)

(TODO: clean up repository)

(TODO: remove deepTools entirely)

(TODO: Add Glossary?)

(DRAFT: use more formal language)

(TODO: biggest points: Python to rust and how it worked, give code examples but not too much)

(TODO: clean up github and add documentation)

(TODO: for presentation: 1/3 for everyone, 1/3 for supervisor + experts, 1/3 only I am expert)





## ToDo Counters

To Dos: 21; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21

Parts to extend: 6; 1, 2, 3, 4, 5, 6

Draft parts: 10; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10



# Bibliography

- [1] E. Lieberman-Aiden, N. L. Van Berkum, L. Williams, M. Imakaev, T. Ragozy, A. Telling, I. Amit, B. R. Lajoie, P. J. Sabo, M. O. Dorschner, *et al.*, “Comprehensive mapping of long-range interactions reveals folding principles of the human genome,” *science*, vol. 326, no. 5950, pp. 289–293, 2009.
- [2] M. S. Cheung, T. A. Down, I. Latorre, and J. Ahringer, “Systematic bias in high-throughput sequencing data and its correction by BEADS,” *Nucleic Acids Res.*, vol. 39, p. e103, Aug 2011. [PubMed Central:PMC3159482] [DOI:10.1093/nar/gkr425] [PubMed:20824077].
- [3] L. Teytelman, B. Ozaydin, O. Zill, P. Lefrancois, M. Snyder, J. Rine, and M. B. Eisen, “Impact of chromatin structures on DNA processing for genomic analyses,” *PLoS ONE*, vol. 4, p. e6700, Aug 2009. [PubMed Central:PMC2725323] [DOI:10.1371/journal.pone.0006700] [PubMed:12067662].
- [4] M. Imakaev, G. Fudenberg, R. P. McCord, N. Naumova, A. Goloborodko, B. R. Lajoie, J. Dekker, and L. A. Mirny, “Iterative correction of hi-c data reveals hallmarks of chromosome organization,” *Nature methods*, vol. 9, no. 10, p. 999, 2012.
- [5] P. A. Knight and D. Ruiz, “A fast algorithm for matrix balancing,” *IMA Journal of Numerical Analysis*, vol. 33, no. 3, pp. 1029–1047, 2013.

- [6] R. Wheeler, “Chromatin structures.” [https://commons.wikimedia.org/wiki/File:Chromatin\\_Structures.png](https://commons.wikimedia.org/wiki/File:Chromatin_Structures.png), 2005. Licensed under CC BY-SA 3.0; No changes have been made; accessed 2019-06-26.
- [7] G. Li, L. Cai, H. Chang, P. Hong, Q. Zhou, E. V. Kulakova, N. A. Kolchanov, and Y. Ruan, “Chromatin interaction analysis with paired-end tag (chia-pet) sequencing technology and application,” *BMC Genomics*, vol. 15, p. S11, Dec 2014.
- [8] J. Dekker, K. Rippe, M. Dekker, and N. Kleckner, “Capturing chromosome conformation,” *science*, vol. 295, no. 5558, pp. 1306–1311, 2002.
- [9] M. Simonis, P. Klous, E. Splinter, Y. Moshkin, R. Willemsen, E. De Wit, B. Van Steensel, and W. De Laat, “Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture–on-chip (4c),” *Nature genetics*, vol. 38, no. 11, p. 1348, 2006.
- [10] Z. Zhao, G. Tavoosidana, M. Sjölander, A. Göndör, P. Mariano, S. Wang, C. Kanduri, M. Lezcano, K. S. Sandhu, U. Singh, *et al.*, “Circular chromosome conformation capture (4c) uncovers extensive networks of epigenetically regulated intra-and interchromosomal interactions,” *Nature genetics*, vol. 38, no. 11, p. 1341, 2006.
- [11] J. Dostie, T. A. Richmond, R. A. Arnaout, R. R. Selzer, W. L. Lee, T. A. Honan, E. D. Rubio, A. Krumm, J. Lamb, C. Nusbaum, *et al.*, “Chromosome conformation capture carbon copy (5c): a massively parallel solution for mapping interactions between genomic elements,” *Genome research*, vol. 16, no. 10, pp. 1299–1309, 2006.
- [12] S. Wingett, P. Ewels, M. Furlan-Magaril, T. Nagano, S. Schoenfelder, P. Fraser, and S. Andrews, “Hicup: pipeline for mapping and processing hi-c data,” *F1000Research*, vol. 4, 2015.

- [13] J. Wolff, V. Bhardwaj, S. Nothjunge, G. Richard, G. Renschler, R. Gilsbach, T. Manke, R. Backofen, F. Ramírez, and B. A. Grüning, “Galaxy hicexplorer: a web server for reproducible hi-c data analysis, quality control and visualization,” *Nucleic acids research*, vol. 46, no. W1, pp. W11–W16, 2018.
- [14] S. S. Rao, M. H. Huntley, N. C. Durand, E. K. Stamenova, I. D. Bochkov, J. T. Robinson, A. L. Sanborn, I. Machol, A. D. Omer, E. S. Lander, *et al.*, “A 3d map of the human genome at kilobase resolution reveals principles of chromatin looping,” *Cell*, vol. 159, no. 7, pp. 1665–1680, 2014.
- [15] F. Ramírez, V. Bhardwaj, L. Arrigoni, K. C. Lam, B. A. Grüning, J. Villaveces, B. Habermann, A. Akhtar, and T. Manke, “High-resolution tads reveal dna sequences underlying genome organization in flies,” *Nature communications*, vol. 9, no. 1, p. 189, 2018.
- [16] G. Hoare, “Rust faq.” <https://prev.rust-lang.org/en-US/faq.html>, 2019. accessed 2019-06-26.
- [17] G. Hoare, “stage1/rustc builds.” <https://mail.mozilla.org/pipermail/rust-dev/2011-April/000330.html>, 2011. accessed 2019-06-26.
- [18] “Version history.” <https://github.com/rust-lang/rust/blob/master/RELEASES.md>, 2019. accessed 2019-06-26.
- [19] “The rise and fall of languages in 2013.” <http://www.drdobbs.com/jvm/the-rise-and-fall-of-languages-in-2013/240165192>, 2013. accessed 2019-06-26.
- [20] “Rust survey 2018 results.” <https://blog.rust-lang.org/2018/11/27/Rust-survey-2018.html>, 2018. accessed 2019-06-26.

- [21] “Stack overflow survey.” [https://insights.stackoverflow.com/survey/2019#technology\\_-\\_most-loved-dreaded-and-wanted-languages](https://insights.stackoverflow.com/survey/2019#technology_-_most-loved-dreaded-and-wanted-languages), 2019. accessed 2019-06-26.
- [22] “Memory management bugs: An introduction.” <https://backtrace.io/blog/backtrace/introduction-to-memory-management-errors/>, 2016. accessed 2019-06-26.

