**Abstract**

In the design of FilooT, some of the generated views are manipulatable. In addition to the most common techniques for manipulating the views, which are scrolling and zooming, there are two other techniques that I discuss in this section: the Overview+Detail, and the Focus+Context [24].

**1. Introduction**

Health investigators are interested in finding the relation of the gene substitutions to disease characteristics in a virus gene family of sequences. Aanalysts also need to understand how characteristics of the disease related to virus strain mutations.

Applying the principles of Information Visualization to design biology-specific systems is highly acknowledged in the literature [18, 25, 26, 30, 32]. Advanced hand-drawn pictures in scientific publications prior to existing computers shows that utilizing the human vision system was grounded in biology many years ago [32]. As the biological data-sets scales are increasing rapidly, custom software combined with manual intervention is replacing manual data analysis in biological sciences [18].

These computer-based visualization tools have enhanced our ability to communicate with the large amount of scientific data. Usually these tools are designed for a specific data-set/task-set in the domain. Advantages of these custom tools are twofold. First, they solve target analysts’ problems, which are part of the domain problems. Second, by analyzing the successful tools, researchers can eventually extract the target domain’s design guidelines and patterns. A special issue of Nature Methods gives biologists an overview of current computational methods and tools used for visualizing biological data [32]. Although classical visualization techniques are used in the field of biology, researchers define new and creative ways to meet the target domain visualization needs [15]. One such example is the work of Nielsen et al. in creating a novel graph representation for visualizing genome sequence assembly structures [30].

**2. Literature review**

Once a virus infects a host, it makes copies of itself and spreads to other people. During this replication process, typically some substitutions appear in genetic sequence [31]. One way for characterizing DNA is to compare their sequences with each other [19]. In bioinformatics, Multiple Sequence Alignment helps to compare more than a pair of sequences and to find the similar regions between them [19].

For representing Multiple Sequence Alignment, there are two types of visualizations: the Sequence Logo [36] and Multiple Sequence Alignment viewers [4, 5] and we focus on the latter.

Multiple Sequence Alignment view is a table in which each row corresponds to a sequence and each column is a position in all the sequences. Each cell represents a DNA letter in each sequence. The goal of this view is to show the variations in the sequences to the analyst.

IMAS [39] is a visual analysis tool for rapid analyses of DNA sequences. This tool visualizes the output of common bioinformatics tools such as BLAST and Clustal-W in a unified framework with semantic zooming navigation [39]

Jalview is one of the most commonly installed tools. Its Multiple Alignment view is capable of hiding and grouping multiple sequences (rows) [48]. It also allows the user to sort the sequences with different criteria.

Historically, the table view provides interactive features to allow the users to gain insight about the data [39, 48]. Sequnce-Juxtaposer [42] is an example of applying Focus+Context in bioinformatic sequences alignment explorations.

The multiple alignment views usually accompanied by another metadata matrix view which is a kind of Table Lens where each column contains information about one metadata and each row represents the value of that metadata for each strain [33, 45, 11, 43, 23]. Freire et al. [11] used horizontal bars for encoding each cells’ data, as well as vertical bars on top of each column to show overall column distributions. Others, such as Sopan et al. [43], used colour saturations in different cells to encode their values. The colour saturation usually is a better choice for visualizing the information in a cell as it has higher accuracy for encoding ordered data [23].

For representing a hierarchical structure in data tree is a common visualization technique [5, 15].

The Noblis Team [4] used sunburst layout to represent the evolutionary tree of the current outbreak sequences and utilized colour to represent the degree of the overall danger level of the sequences. Freire et al. [11] used the basic Node-Link layout for the evolutionary tree information. And we adopted the same representation in FilooT.

For other types of relationships between data items, the Network representation is used [15].

ManyNets [11] is a network visualization tool with tabular interface in which its tabular view was a kind of Table Lens that the disease characteristics were shown in columns. This tool enables users to create a new column with the existing characteristics and sort all the rows according to the values of the particular characteristics associated to a column.

GeneTracer [22] provided three views: Gene Sequence view, Disease Characteristic view, and Graph view. Disease Characteristic view used a Table Lens where each column had a different colour and each cell had different saturations of that colour. The Graph View visualized the relations among the sequences via a Minimum Spanning Tee representation where the weight of an edge is the Hamming distance between the two sequences [19].

Wood et al. [50] utilized a heat map for Table Lens representation. It had two levels of sorting of the rows according to one characteristic, and sorting them again within each category of the first sort, according to the second characteristic.

**3. Design**  
FilooT visualization system. (a) An interactive visualization table to represent  
the genetic sequence information. (b) A matrix visualization for interacting with the  
disease characteristics data. (c) The P-Value bars to show a metric (reverse of P-value in Mann- Whitney U test) about each column. (d) The Group View containing the user created groups along with an overview of each group. (e) A graph visualization for representing row (or column) relationships depending on the system mode (Row based or  
Column based). (f) Two buttons enable the user to choose between the Column and Row mode. (g) The Statusbar is being updated after each action that the user makes.  
**3.1. Interactive Tabular view**

The tabular view is an interactive visualization for exploring genetic sequences. The first row represents the genetic information about the original sequence. The second row shows position numbers and numbers start from one and end with the length of the sequences.

Each of the subsequent rows indicates one sequence. Each cell contains the result of the comparison of each sequence with the original sequence appeared in the first row. The purple color is used to represent those cells that did not change in comparison with the original sequence and the yellow color highlights cells with a change in a particular row and column. The letter indicates a change in the information of the specific cell in comparison to the corresponding column in the original sequence.

This view supports the following user interactions.

**Navigation**: The horizontal and vertical scroll bars at the bottom and on the right are so that the user can explore more of the sequences and the positions’ data

**Zoom:** The “+” and “-” buttons allow user to zoom in and out.

**Reset:** To compare different columns with each other, placing the columns close to each other frees up the cognitive load of the users to focus on their desired task [40]. One way of putting columns close to each other is to allow the user to drag and drop the columns next to each other. However, the natural order of nucleotides in a sequence is meaningful Therefore the “reset” button returns the columns to their original sequence from one to the length.

**Filter:**

Tabular view provides two filtering capabilities;

a) Basic Filtering:  the user can separate out a group of columns (or one column). The transition between hidden/ unhidden state is animated so that the view does not jump to a new state.

b)Augmented Filtering: While having basic filtering seems useful for exploring the data, finding relevant columns still requires manual work (exploring all the columns to find relevant ones). Moreover, a small number of substitutions in a column may occur randomly and do not reveal any valuable information to the analyzers. Therefore, an augmented filtering excludes the columns that have fewer yellow cells than the filter number.

These interactions affect the other views linked to Main View.

**3.** **2. Matrix View**

The matrix view enables the user to sort the rows according to the values of different characteristics (for example a disease characteristic such as severity). Design of this view is inspired by the Table Lens [11]. In table lens, the levels are shown by the length of horizontal bars or colour saturation per cell [43]. However, we utilize position and redundantly colour saturations to encode the same property of the data. Each column is divided by the number of its characteristics levels. The coloured label on top of each column shows the different levels in that particular column. The darker the colour is, the higher the level of the characteristics. The coloured labels are placed from right to left respective to color saturation level.

We exploit position channel for representing discrete ordered data-type because it is the most powerful visual property for encoding all kinds of data [23]. In addition, the colour saturation is a better alternative for the length channel for encoding this ordered information [23].

We also used hue to separate different characteristics that are nominal data and the hue channel is appropriate for separating different categories [23].

The user can perform the following list of interactions in Matrix View:

**Sort:** the rows ccan be sorted ascendingly or descendingly according to the values of a selected column header

**Aggregation:** The “add” button enables the user to make a new column by combining the existing ones with a simple mathematic function in between them

**Zoom:** The user can zoom in and out to the view using “+” and “-” buttons from Main View

**Overview:** At the bottom of each column in Matrix View, an overview of that specific column is provided so that the user can see the pattern of the change for all the row values for that specific disease characteristics column, without the need to zoom. When the Row mode is activated, and a sequence header is highlighted to show the mouse position, it also highlights a row in the overview of Matrix View.

Matrix view and tabular view are linked together by shared row labels. Consequently, when the rows’ positions are changed in one view, for example if the user sorts the rows, their vertical positions will be changed in the other view accordingly.

**3.3. The P-Value View**

# 

There is a pattern [4] within some of the columns that makes them interesting candidates to form new hypothesis. This pattern suggests a relationship between substitutions in a particular column and one of the characteristic of the rows.

As humans do not complete pattern-detection tasks very well [28], we cannot rely on them to find this pattern in columns.

Commonly biologists use metrics detect interesting patterns. Mann-Whitney U test’s p-value is one of the metrics used for finding relevant positions [4].

Using the Mann-Whitney U test, the severe rows can be separated from others by splitting all the rows into two groups based on the existence substitutions in them.

The negative of the logarithm of the P-Value suggests likeliness of the significant difference between the two groups.

This value is shown by the bar lengths in P-value View to help users find relevant columns.

The length channel is the second most powerful channel for encoding the ordinal values [reference]. Therefore we used length to represent the p-value metric.

The P-value view also provides the filtering feature.

The filtering feature enables the user to filter out any column where the length of the bar is smaller than the filter number. This view also lets the user sort the positions based on the bar length. The columns will be sorted from high to low and placed from right to left.

In general, sorting all the rows according to one of the characteristics from top to bottom, a significantly larger proportion of substitutions appear at the top rather than the bottom. As the user might want to focus on those columns with the higher bar length, merely hide/unhide all the other columns is not efficient. Instead, it would be more productive to sort the columns based on the reverse of the p-value (length of bars. keep the bars on top of the columns in Main View, so that the user could go over the bars while observing the columns’ pattern.

The Tabular view and the P–Value view are linked so that if the user re-orders the positions in one view, the corresponding column’s order will be changed in the other. Also they can use the reset button to go back to the original domain ordering.

**3.5. Graph View**

Graph View is a node-link representation that visualizes the relationship between the columns (or rows) and helps Users to find related columns (or rows) and group them together to focus on fewer rows (less data dimensions) for future analysis.

Grouping feature is defined in both row and column mode. The user can click on rows (or columns) and add them to a newly created group. The user also can separately load each group into the views for further investigations.

**Column relation (**Column Grouping)

It is more likely that users will make a group from the relevant columns. The idea is to let the user make different groups from a combination of different columns. The user can see an overview of the group and its general pattern.

Between any pairs of columns, two kinds of relationship are supported: Complementary patterns and Correlation

Row Relation:

The relations between rows are hierarchical. The existed Graph Views used to make a Tree for the representation of this relationship.

Some of the submissions [22, 12] used the Minimum Spanning Tree for constructing the evolutionary tree. The weight of the edges was the Hamming distance between the two nodes [19]. The Hamming distance was the number of positions that differed in any two rows that implied the number of changes is needed to transform one sequence to the other. The Minimum Spanning Tree is a tree in a graph that connects all nodes (rows) and its total edge weight is the minimum of total edge weights of all the possible trees

Interactions:

This view provides an overview and filtering mechanism as well as two categories of interactions with groups: basic grouping and augmented Grouping.

In the Basic Grouping, groups are created by user based on their prior knowledge or observations.

In contrast to the Basic Grouping, in the Augmented Grouping, the users can more effectively detect the columns (or rows) of the same group because the system highlights the relationships between columns(or rows) to guide group creation.

The augmented grouping motivates the graph view.

An overview of the distributions should allow analysts to see the big picture, and identify clusters, trends and outliers that may be candidates for detailed inspection [43]. Therefore, an overview of a group consists of a larger window than Main View information (prior to zooming).

If the user clicks on the overview the content of that specific group is loaded in all other Views. There is a predefined group that contains the entire data-set for the user to be able to go back to the original data (with latest changes).

The filter mechanism built into the view removes the different levels of correlation links. Two filters are placed to enable the user to sort out the columns based on the strengths of their connection.

There is linkage between Main View and the selected group. When the user selects a group among the previously created groups from Group View, the chosen group’s data will be uploaded into the system. Therefore, the data in all Views matches the data in the selected group. when the user’s mouse hovers a column, the corresponding column is highlighted in the overview of the currently selected group. The user’s mouse position highlights the corresponding row label in Main View as well as the equivalent node label in Graph View. To show the matching node in graph view.

If the user deletes a row from Main View, the matching node in Graph View will be deleted.

Alternative representation:

One alternative representation for relationship between a pair of columns is the matrix visualization [15]. One benefit of using this matrix is, by re-arranging the rows and columns, some interesting patterns would be revealed. However, it requires a large screen space. One drawback is that we cannot eliminate the cells with 0 correlation from the space.

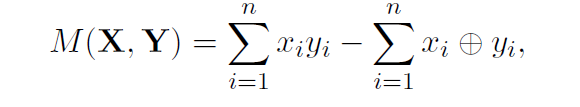
The second option is using a node-link graph, where there is a link between a pair of columns only if their correlation is non-zero. The link is coloured blue for correlation (numbers greater than 0) and red for complementary (numbers less than 0). Colour saturations and line weights are also redundantly used to encode the same information.

As there are a considerable number of columns with zero correlations, this option conserves the space better than the table representation.

Given that the substitutions in this data-set are represented by values 0 and 1, one may define the similarity between two columns with measures such as Pearson’s correlation calculation for any pair of column. This is however not optimal because many zeros (no substitution)in the columns result in a correlation close to 1 indicating they are highly correlated however it is the case.

To alleviate this problem, we propose to use a new measure which ignores the common zeros between columns.

Assuming that two columns, X and Y, each have n members, X = {x1, . . . ,xn} and Y = {y1, . . . , yn}. The measure is defined as follows:



Where ⊕ is the logical XOR operation. This measure ignores entries with no substitution in columns, increases when entries with substitutions occur together and decreases when substitution complements each other. Given that, both positive and negative values are expected.

**4. Evaluation**

To evaluate our tool we followed Nested Model Process (NMP) which was also used for Visualization Design of the tool. Our work falls into data/operation abstraction, and encoding/interaction technique design layers of this framework [27]. For evaluation at the visual encoding/interaction layer, NMP provides two recommendations:

a) The design needs to follow perceptual and cognitive principles [27]. We used heuristics for information visualization to meet this requirement [51].

b) The design should be able to communicate with the analyzer and be useful towards solving their problem [27]. Therefore our general research question was set to: “How the design of FilooT could help the domain users solve the tasks problems”. Considering the formative nature of our research question, we took User Experience scenario (UE) to understand “what do our target users think of the visualization?” [21]. However our evaluation methodology is user-based in which problems are found through the observation of and interaction with users while they use or comment on the interfaces [21, 41].

**4.1. Method**

We conducted a qualitative, User Experience study for system evaluations [21] where users played with the system to answer some tasks. The participants were asked to talk freely about anything comes to their mind about the tool during and after the process. The study follows by an informal interview with open-ended questions to understand user’s opinion about the tool.

The followings are the interview guide questions.

* How each view/features helped you to find the answers? Any of them were more or less useful?
* Did you have any difficulties understanding any of the presented information in any of the views?
* Please explain your process and steps of finding the answers.
* Please give me your feedbacks to make each of the views better. Your suggestions may include your ideas of new interactions or refinement of some parts of the design.
* Are there limitations of the current system that would hinder its adoption?

**4.3. Participants**

//number of participants

Participants are over 19 year old undergraduate/graduate students or postdoctoral researchers in the lower mainland. They are required to be familiar with DNA multiple sequence alignment concepts and they should be interested in this study.

## 4.4. Procedures

The experiment took less than 1.5 hours. The studies took place in the lab environment on a laptop computer.Participants read and signed the consent form and filled out a pre-study about their familiarity with the domain and their experience with similar tools.

Then they were asked to read the Task/Data Description and they were trained for 10 minutes to learn to use the basic features of the software. After that, they used the system for 30 minutes and they were encouraged to write down their findings, think aloud and express their thoughts, concerns and their questions at any time.

The experimenter used pen and paper to take notes from observations of participants’ use of the tool.

After the 30 minutes, in a semi-structured interview participants were asked about their experience with the tool. At the end, they were thanked for the participation and they received the compensation by signing the compensation form.

**4.6. Data**

We use a synthetic data-set for the VAST Challenge 2010, Mini Challenge 3 [20]. It is about an arms dealing scenario in which one of the dealers, Nicolai, died in a hospital with symptoms consistent with Drafa Fever. To help Public health organizations to develop pandemic response plans, analysts seek for more information about the disease. The data-set consists of 56 strains of a particular original virus spread over time to different infected people. Each strain has a gene sequence of 1400 nucleotides with one or more nucleotide changes from the original virus’s sequence. There is also information about some characteristics for each of the evolved viral strains.

**4.7. Tasks**

We used a benchmark data-set/task-set so we assume that the tasks are already validated and they reflect the target domain users’ work.

**Task 1**: Identify mutations that lead to an increase in symptom severity (a disease characteristic). For example, “C → G, 456” shows C changed to G at position 456.

When a combination of the mutations explains a characteristic’s severity, these mutations should be reported together.

**Task 2**: Identify mutations that lead to the most dangerous viral strains.

**Task 3**: Nicolai has a strain identified by sequence 583. One patient has a strain identified by sequence 123 and the other has a strain identified by sequence 51. Which patient contracted the illness from Nicolai and why?

**4.5 limitations**

The given time can interfere with participants’ solving task. However we explained the users that the problem solving process is more important than reaching to answers and their accuracy.

**Results and Discussion**

The results of the user study led me to create a list of requirements as well as develop a guideline for future work. These results informed the initial design of the tool [6].

During the study session, the participants used the tool to find the answers for three tasks (see Section 1.3.1). I used paper and pen to write their comments about the system features. They were also asked to share their findings about the system. There was an interview with open-ended questions to capture their comments at the completion.

Main View Visualization Comments

• The colour choice for mouse hovers (changing from gray to pink) is not discernible for individuals with colour-blindeness.

• One user suggested we choose different colours for each nucleotide.

• The same user suggested that we choose similar colours for T and A, and similar colours for C and G, but that the two categories should be differentiated.

• The labels must be readable, regardless of the zoom level.

Interaction Comments

• According to users’ suggestions, the sliderbar was the most usable feature.

• Another user asked if it would be possible to sort the rows on their ID, and to search for rows by typing their labels directly into a textbox.

• If the fonts are small the labels could pop up in response to a mouseover.

• One user wanted to be able to type a motif and have the system highlight it with its local alignment score. This would be useful in order to know if a column is in a conserved region or not (conserved sequences are similar sequences that occur within DNA sequences). Another user mentioned that the system could be linked to the UCSC Genome Browser 1 to coordinate data so as to be able to highlight information about adjacent columns.  
Main View Observations

• The sliderbar was the first and most common interactions with the tool.

5.1.1.3 Main View Discussion

• Regarding different substations in a column, the user pointed out that in a real dataset different kind of substitutions usually happen in a column, yet there is only one kind of substitution in a column in this study’s data-set. For example, if an “A” changes to “C” in one row in one column, then all other substitutions in that particular column, will also be a “C”. By contrast, in real data-sets, there are the four kinds of substitutions in a column. To make the tool adaptable to a new data-set, as some of the users already suggested, I could use different hues to show different nucleotides (Figure 5.1).

One potential drawback for this suggestion is that it increases the number of hues in the tool. Depending on the number of columns in Matrix View, this could interfere with the hue uses in that view, because using more than eight hue colours on screen is not recommended (see Section 3.1.1). Instead, I suggest using only one hue per column for Matrix View and distinguishing between columns by adding extra space between them. Figure 5.2 shows this idea. More specifically I suggest using Proximity to show the organization of inter and intra columns (see Section 3.1.1). Also the designer must prevent hue overloading. For instance if they want to use red for showing one of the nucleotides, they should use a different hue for edge colours in Graph View.

• The data-set carries no information about adjacent columns. According to the participants’ comments, authentic data-sets contain this information. Figure 5.3 shows an example of neighbouring columns which do have some relationship with each other.

To solve the tasks, researchers need to know if a column is related to its neighbours and what the nature of that relationship is. Examples of these relationships include codon and motif information in data-sets. However, because the VAST Challenge stated that the DNA is non-coding, codon analysis and AA sequence analysis cannot be considered.

• All of the users were familiar with at least one Visual Analytics tool similar to Main View. Although some of the interactions that they used to see in similar tools were not relevant to the study tasks, the users desired to see all of the familiar features in Main View.  
Matrix View Comments

Below are users’ opinions about Matrix View. Matrix View (see Section 3.3.1.4) has an overview to represent the overall trend on each column. A row of this overview will be highlighted when the user is in Row mode and the user’s mouse hovers over that particular row.

Visualization Comments

• A user thought that the as yet unimplemented “add” button could be extremely helpful.

Interaction Comments

• A suggestion was to have the system show the row label in response to a mouseover.

• When the user clicks on the coloured label at the top of each column, all the rows will be sorted according to the values of that column’s characteristics 3.3.1.4. However, when the user clicks again, the rows order might be changed because the sorting algorithm allows for sequences that are different but still correct. Therefore, although the rows will be sorted each time a user clicks on a coloured label, the rows’ order within a level might be changed. The user did not like this and preferred consistency and predictability in the column sorting behaviour.

• Another user liked that the coloured labels at the top of each column were clickable and the clicking on them would sort the rows (see Section 3.24). (He said, “It’s pretty cool”. Moreover, he thought that these labels could be made more useful if the different levels were clickable separately so that the system would jump to a state in which Main View and Matrix View contained the rows with the selected level.

• One user suggested that Matrix View could have a built-in option to keep track, and sort the next column based on previous selections. He mentioned that could be

particularly useful if the analyzer had certain priorities of columns and want to see a 5.1.2.2 Matrix View Observations

• One user sorted the rows based on different characteristics and used the Overview and

P-value bars to see which characteristics contributed more to overall danger.

5.1.2.3 Matrix View Discussion

The compilation of the user’s comments on Matrix View shows that this is one of the most frequently used views. The users’ suggestions could be used as a guideline to make this view more accessible and useful for completing the study tasks. Below is my extension of how one of the suggestions should be considered for further implementation:

• For the user comment about clicking the coloured label in order to update the view with new information, I suggest that instead of Main View suddenly switching to new information, the system automatically move the vertical scrollbar which shifts Main View until the new, selected column is reached. Through this process, the user sees that the change in Main View is fluid, while keeping track of how the information changes.

• The above strategy could be used to link Graph View and Main View. When the user clicks on a node in Graph View, Main View needs to contain the matching row. In the case of a row needing to appear in Main View window, vertical scroll bars could be used. In the case of a column, horizontal scroll bars could be used to move Main View columns.

• It seems implementing the “add” button will be useful. Levels need to be associated with a particular number, and this number should be editable by the users.

5.1.3 P-value View

5.1.3.1 P-value View Comments

P-value View elicited a few comments. This view has a section that filters out the columns based on the reverse of the p-value of the Mann- Whitney U statistical test that suggest a pattern in the columns (see Section 3.2.4.3).