LETTER TO EDITOR

Interactive Karyotyping Training

*Ashwin Kotwaliwale**
Genomic Software, Inc., 10315 Alpine Drive, #2 Cupertino, CA 95014, USA

Abstract:

Despite the wide use of newer techniques in genetic diagnostics, there remains a need for technologists to learn human chromosome morphology, identify abnormal metaphases and report clinical abnormalities. Global shortage of cytogenetic trainers and a time consuming training process makes Karyotyping training difficult. We have developed a web based interactive Karyotyping training tool, KaryoTutor ©, that allows technologists to learn karyotyping in an interactive environment and aids the trainer in the training process. KaryoTutor© provides visual clues for identifying abnormal chromosomes, provides instant test scores and includes a reference library of ideograms, sample chromosome images and reference materials. Trainees are able to recursively work on a case till a satisfactory result is achieved, with KaryoTutor providing interactive inputs. Additionally, trainers can assign cases and monitor trainee progress using audit trail management and other administrative features.

Keywords: Cytogenetic Education, Chromosome Analysis, Interactive Karyotyping, Training, Genetics Training

Interactive Karyotyping Training Salient Features

- Karyotyping is an important step of diagnosing genetic disorders and often very difficult to learn, Karyotutor makes the learning process simple and efficient.

- Karyotutor provides a simple way for trainers to create training sets and for students to work on cases from anywhere at any convenient time.

- Karyotutor provides an easy, intuitive interface for students to work with and allows for interactive feedback defining the correctness of each chromosome image as well as the entire case, providing a score.

- Karyotutor enhances the training process by providing easy access to a reference library as well as sample chromosome images.

- Karyotutor allows the trainer to audit each trainee's progress, thereby allowing an efficient grading system.

In recent years there has been rapid growth in the number of diagnostic laboratories around the world offering genetic testing. In the USA alone there are a few hundred cytogenetic laboratories. Despite the widespread use of newer diagnostic techniques such as Fluorescence-In-Situ-Hybridization [1] and diagnostic microarrays [2], a thorough understanding of human chromosome morphology forms the basis of genetic education at varying levels, ranging from graduate courses to vocational training. Additionally, routine G banded chromosome analysis forms the mainstay for prenatal diagnosis of several aneuploidies such as Trisomy 21 (Down's syndrome), large chromosomal deletions and duplications and importantly cancer causing translocations such as the classic BCR-ABL (Leukemia) gene fusions [3].

The aim of this communication is to define the salient features of KaryoTutor and its applicability in cytogenetic training. The definition of trainer side functionality is beyond the scope of this communication.

Cytogenetic laboratories process cell culture based metaphase spreads and prepare karyotypes upon which genetic reports are published. Image analysis software is used to rapidly cut out chromosome images from metaphase spread images and arrange them on a karyotype template. The segmentation and arrangement algorithm employ pattern recognition techniques of banded chromosomes, comparing them to a standard library and thus determining the chromosome number [4]. It may be convenient to believe that such software packages can solve all issues related to chromosome identification, however, this is usually not the case for complex chromosomal rearrangement such as deletions, translocations, partial trisomies and ring chromosomes, thus requiring human intervention. Shortcomings of such algorithm processes create a continuous requirement for trained staff which form the basis of genetics training in the age of automation and software powered technologies.

Efficient manpower and training tools are required to continue genetic training. Global shortage of trainers and the difficulty of training aspects such as funding and core resources make it a necessity to have smart software tools to aid in the process of training.

The authors are of the opinion that software technologies can be used to address a part of the training process. To address these training needs we have developed KaryoTutor [5] which is 'first of a kind', innovative, unique and interactive cytogenetic training software, specifically designed to teach genetic technologists to learn human chromosome morphology and perform Karyotyping as a part of their vocational training.

KaryoTutor is a web based application developed using Microsoft Silver light 3 [6], Microsoft. NET 3.5 [7] and SQL Server 2008 R2 [8]. Silver light is a modern browser plug in that installs on all operating systems. It allows for silver light applications to execute on the client computer, thereby utilizing the CPU power of the client computer. Additionally, silver light being a plug in system, works within the browsers sandbox and has access to a subset of the operating systems API such as those required for graphics rendering. These were important design considerations as smooth dragging-dropping, image rotation, and zooming of chromosome images, are required for Karyotyping. The user interface was designed to address these requirements and silver light met the requirements 'out-of-the-box'.

As in any modern client-server application, saving the state of a user's work was absolutely required. We used Entity Framework [9] and Windows Communication Foundation (WCF) Services [10] to load and store a case that a trainee was working on. Entity Framework makes it possible to quickly generate lists of objects to work with such as a case or a chromosome and assign attributes to entity objects such as chromosome number and degree of rotation. Entity Framework has built in methods to track changes on an entity thereby allowing high level coding during development. WCF is the preferred route for communication between silver light clients and the databases

as it allows for simple serialization and deserialization of entity objects.

Before commencing a training session in KaryoTutor, minimal preparatory work is required; this starts with the account administrator creating accounts for trainers and students. For trainees to use KaryoTutor, training sets have to be made available. We provide over one hundred cases which have been selected by cy-togeneticists and deemed suitable for training. However, trainers also have the option to create their own custom library of cases from metaphase images using a pre-defined image naming nomenclature. For e.g. Case1-1-1.png would represent chromosome 1, first one of the pair (when referencing diploid cells) and which belongs to case named 'Case1'. Additionally, each chromosome image must have its 'p'
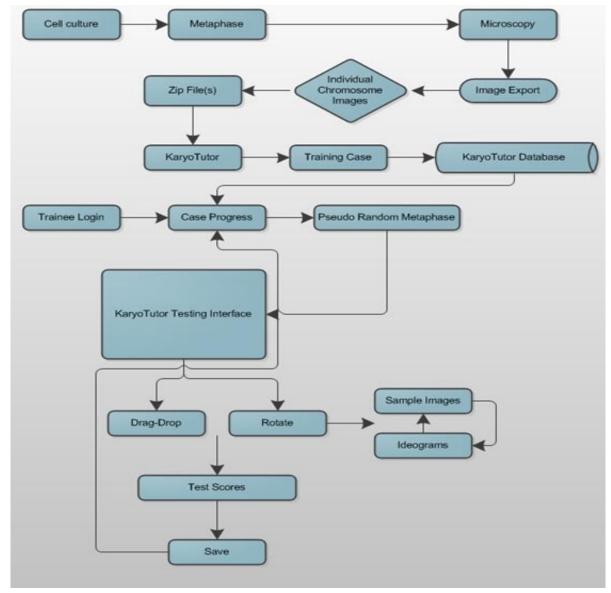
Fig. 1: KaryoTutor Workflow

arm upward and it's 'q' arm in the downward orientation as they would normally appear on a karyotype. It's is image naming nomenclature that KaryoTutor employs to validate student responses as it's able to infer the actual chromosome number and the corresponding slot in which the image was placed by the trainee. The overall workflow that addresses trainer and trainee functionality is outlined in Fig. 1.

A training session in KaryoTutor starts with the trainee using an internet connected computer to login into her account and select an assigned case to work on. Initially, all chromosome images are randomly placed onto a metaphase window. Since we do not store exact locations of each chromosome image as it is found in the original metaphase, KaryoTutor creates a pseudo-random metaphase that simulates a metaphase as seen under a microscope. The

trainees then assign each chromosome image numbers that they thinks correctly identifies the chromosome. The trainee has the functionality to rotate the chromosome image in the metaphase window. Each of the chromosome images can then be transferred to the karyotype template using the export feature, such that each chromosome is placed onto its own slot on the karyotype template as shown in Fig. 2.

The chromosome images may be rotated again if desired. At this stage the trainee may also check to evaluate the correctness of their identification (score). Wrongly identified chromosome images may be moved to other slots. The trainee may also use visual clues such as reference chromosome images and ideograms to develop chromosome identification skills. A zoom in/out feature also allows the user to view a magnified image of each chromosome image.
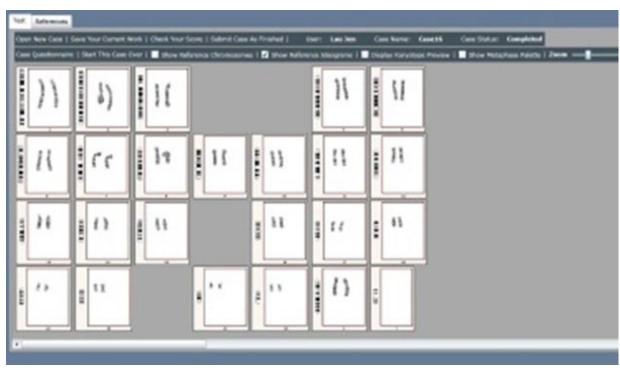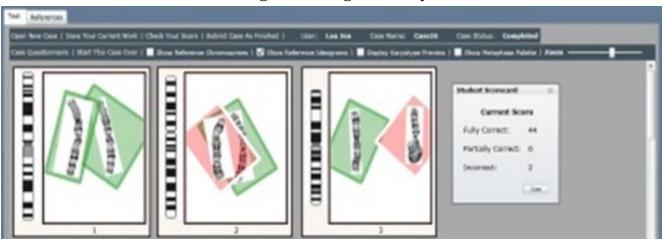
Fig. 2: KaryoTutor Testing Interface

Fig. 3: Scoring Summary

The process of moving chromosomes until a desired result is obtained can be continued indefinitely. At any stage in the training process the users may save their work to finish later.

The core functionality of KaryoTutor is to provide instant feedback to the trainee. According to the International Standard Chromosome Nomenclature (ISCN) rules, a chromosome is considered fully correct when it is in the correct location and orientation on the karyotype template. Fully correct chromosomes are highlighted using the color 'green' whereas those chromosomes which are in the incorrect location are highlighted red. Partially correct chromosomes are marked yellow such as when the chromosomes are in the correct location but

Fig. 4: Completed Case

have incorrect degree of rotation. We allow for 20 degrees of rotational freedom before a chromosome is considered incorrect. A sample scoring summary is provided in Fig. 3.

The results are displayed as a scorecard in a popup window. (Fig. 5)

Several other visual clues required for identifying chromosomes such as reference images and ideogram references are also provided as shown in Fig. 4.

Reference images and ideogram references are also placed adjacent to each chromosome image box that the trainee can turn on or off as desired. Other features included the ability to move chromosome images from one location to another, save the state of the case, reload the case, and access a reference image library and textual description of each chromosome image. A sample of a complete case is shown in Fig. 5.

Discussion:

KaryoTutor is in use at several institutions and is used by trainees undergoing vocational training as well as trainees undertaking undergraduate courses. In both instances trainers are able to successfully assign cases and trainees are able to perform interactive karyotyping, generating test scores and completing assigned course work as shown in Fig. 6. In the following section we discuss some of the shortcomings of KaryoTutor when used in a multiuser scenario and we make design recommendations based on identified technical drawbacks and user feedback.

Since the starting material for KaryoTutor cases is a set of predefined images, there is a limitation for chromosome identification when chromosome crossovers are encountered. In the real world, when a trainee encounters a chromosome crossover situation, which typically involves a pair of chromosomes and appears as 'X' under a microscope, they may think of several possibilities such as the left arms on an

Fig. 5: Reference Library

'X' could be one chromosome or the top 'V' of an 'X' could be another possibility. In KaryoTutor, such an experience is not encountered because each chromosome is a predefined imaged, thereby allowing the trainee to simply mouse point each image and identify.

Modern HTML technologies such as HTML 5 [11] have limited support for graphics, whereas other plugin systems such as Adobe Flash [12], meant primarily for graphics applications lack convenient database connectivity modules, in consideration, silver light was chosen as it runs on a subset of the NET Framework and is tightly integrated with NET for easy data access. We decided to use an n-tier development pattern for architecting KaryoTutor. The database was developed using a RDBMS pattern in Microsoft SQL Server 2008 as it offered scalability, security and reliability. The middle tier which encompasses the business logic was coded in NET 3.5 using both C# and VB.NET.Silverlight was used to develop the entire user interface(s) as it also allows execution of native operating system functions such as smooth drag-drop. Since KaryoTutor' score functional requirement was to allow image manipulation, silverlight was able to meet our design requirements.

Trainees are required to place chromosome images in close proximity as defined by ISCN. Images are rendered as rectangles wherein each pixel is given a color. G banded chromosome images are always black-white images with the chromosomes placed centrally and in black-white bands surrounded by a white area in each chromosome image. The presence of this white area would not allow for close image proximity, thereby preventing overlapping of chromosome images. This is due to one of the limitations of KaryoTutor (and Silverlight) that .png

image format transparency is not fully addressed. The solution to this problem is to make each image's background transparent using an image editing tool. Additionally, Silverlight does not natively support image rotation. To accomplish this, we used template list box controls and added custom behaviour to allow for rotation. The effect was that the image rotation was handled with limited success, although trainees were able to accomplish adequate image rotation with continual practice.

KaryoTutor allows storage of all the information related to every case which leads to requests for several database transactions. We noticed that sporadically, state data was not being saved, and considered that WCF time-out was the reason. Since we used entity framework for database modeling, each SQL query is converted from a class definition to native SQL syntax; such generated SQL query by design is complex and parsing it might also contribute to failed database transaction. One way to solve this issue would be to increase WCF timeout.

KaryoTutor was designed to work with a range of chromosome images which are used as educational material. However, when very long images were loaded (prometaphase chromosome images), it was noticed that the edges of the chromosomes were often cropped when placed in their image slot. This is because chromosome slot dimensions were determined at design time and thus did not take into account images whose dimensions would exceed the slot dimensions. This limitation is illustrated in Fig. 7. To overcome this issue we plan to dynamically adjust the size of the image slot in which the image is dropped.

In KaryoTutor, the trainer has the ability to view all student activity, such as the number of cases attempted as well as the audit associated with

each case such as the score and the number of times the trainee tested their identifications. At times, the audit trail interface would become unresponsive due to the number of records being returned from the database. In future releases of NET, asynchronous operations are better supported and we plan to integrate code that would load data in chunks as opposed to a single dataset.

We developed KaryoTutor to aid the cytogenetics training community in their efforts. While there are several lacunae that we have to fully optimize, KaryoTutor is being successfully used in its third year having trained several hundred trainees to date.

Readers can access a demo of KaryoTutor at http://www.karyotutor.us/demo/karyotutor.aspx

Acknowledgements

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Author for Correspondence: *Ashwin Kotwaliwale, Genomic Software, Inc.10315 Alpine Drive, #2 Cupertino, CA 95014, Email: ak@genomicsoftware.com*