Automated Metaphase Chromosome Image Selection Techniques for Karyotyping: Current Status and Future Prospects

Seema A. Bhalegaonkar [a], Mousami V. Munot [b], Alwin D. Anuse [c], Rupali S. Kute [d]

[a] Assistant Professor, Dept. of E&TC Engg., PES's MCOE, Pune, India, seema.bhalgaonkar@gmail.com
[b] Associate Professor, Dept. of E&TC Engg. SCTR's PICT, Pune, India, mvmunot@pict.edu
[c] Associate Professor, School of ECE, Dr. Vishwanant Karad MIT-WPU, Pune, India, alwin.anuse@mitwpu.edu.in
[d] Assistant Professor, School of ECE, Dr. Vishwanant Karad MIT-WPU, Pune, India, rupali.kute@mitwpu.edu.in

Abstract: Identification of good metaphase is a prerequisite and an essential step in human karyotyping used for analysis of genetic abnormalities in human beings. Most of the research work is focused on automation of karyotyping process but despite being crucial step very scant attention is given to metaphase selection. The conventional method still used by cytogeneticists is a manual visual search of good metaphase spread from microscopic slides. This system is highly dependent on individual observations and suffers from majority of drawbacks such as complexity, tediousness, subjective, time consuming and needs a trained expertise. Today, there is an increasing demand in automating the process of metaphase identification to speed up the Automated Karyotyping so as to provide speedy, consistent and accurate diagnostic results and by that effective treatment. This paper is the systematic literature review covering thorough analysis and comparison of various reported selection techniques. It also provides directional pointer to future prospects in the research on automated metaphase selection tools.

Keywords: Chromosome, Karyotyping, Metaphase Selection

1. Introduction

A Cell is a basic building block of all living organisms which contains thread-like structures called chromosomes in its nucleus. Chromosomes contain DNA (deoxyribonucleic acid) which is tightly coiled around protein. They carry valuable hereditary information regarding the individual's health in the form of genes **[Yunis 1974**]. Each human cell normally has 23 pairs of chromosomes - 22 pairs of autosomes and one pair of Gender chromosomes [**Rooney et al., 2001, Tjio 1956**]. DNA has the instructions for the synthesis of proteins which help in movement of molecules, perform maintenance and structure building activities of the human body. The mutation or a change in the gene can change the instructions for making a particular type of protein that causes malfunctioning of the protein or absence of the gene **[Yunis 1974**]. This can result in a medical condition known as genetic disorder. The alteration in either number of chromosomes or the structure of the chromosomes leads to genetic disorder. Even a small deviation in chromosome count (mutation) results in physical abnormalities [**Gajendran et al., 2004**]. One can get the genetic mutation from either one parent or both the parents; moreover, it may also occur during the lifetime. It is important to the doctors to analyse chromosomes for detecting genetic disorders in an individual **[Munot et al., 2018]**. This procedure requires generating a layout of chromosomes organized by decreasing size in pairs for each testing cell; this is called a Karyotyping process and performed at metaphase stage of the cell cycle.

A cell cycle has three different stages: interphase, mitosis and cytokinesis. Among which Mitosis has four stages - prophase, metaphase, anaphase and telophase [**Rooney et al., 2001**]. Chromosome analysis is carried out during the metaphase stage as chromosomes becomes relatively shorter and wider and hence visible under microscope which are otherwise slick, slender and invisible even under a microscope **[Munot et al., 2018]**. These metaphase images are used in karyotyping to prepare Karyogram of an individual which is a standard representation having a layout of chromosomes pairs organized by their decreasing size [**Mousami et al., 2016]**. In karyogram, chromosomes are arranged according to Denvar classification system based on their lengths. Cytogeneticists can then perform chromosomal analysis according to the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN, 2013) on metaphase images [**Polipalli et al., 2016**]. Abnormalities in Chromosomes can be structural like distortions in band patterns, rearrangement, translocations or variations in chromosome count due to absence or duplication of a chromosome [**Lijiya et al., 2012**].

Such abnormalities are the root cause for many genetic diseases like cancers, mental retardation and autism to name a few. By analysing karyotype, cytogeneticists can exactly probe into the chromosomes abnormalities in the cell which immediately reveals the possible genetic disorders and thus assist cytogeneticists in early diagnosis and effective treatments [**Howard et al., 2017**]. Chromosome analysis involves a lot of clinical steps such as collecting and storing blood samples, culturing and harvesting metaphase, preparing glass slides, selecting best metaphase spread and then karyotyping [**Munot et al,. 2013**] as shown in figure1.

1.1. Karyotyping

Earlier karyotyping process was performed manually by cytogeneticists that involved identification metaphase on specimen slides, identification of chromosome in metaphase and then its classification in respective groups. This manual method suffered from various drawbacks such as time consuming, tedious, laborious and lengthy [**Munot et al,. 2019**]. This demanded the need of Automation or use of computers in Karyotyping. Initially Ledley had presented the idea of using computers to which enormous contribution has been made by researchers in development of an automated karyotyping system (AKS) **[Joshi et al., 2013, Ding et al., 2010**]. The extensive efforts of researchers in proposing and implementing various method and methodologies has lead to availability of AKS system providing promising results with advantages like highly efficient, speedy Karyogram completion with less human intervention, facility of interactive and graphical environment, long term storage and aids in ease of understanding and analysis of the image and decreased labour cost. These karyotyping systems are highly dependent on selection of good metaphase spread and accuracy of these systems gets pivoted by metaphase spread. A Metaphase image having good spread, clear banding pattern of chromosomes and having minimum number of touching and overlapping chromosomes results in greater accuracy of classification of chromosomes in their respective groups during karyotyping **[Piper et al., Arora et al. , 2016**] as shown in figure2.

1.2. Metaphase Selection

Although tremendous efforts are taken in automaton of karyotyping, staggeringly metaphase selection step still remained manual. Before karyotyping a technologist prepares at least 8-10 glass slides from patient's samples and uses high end microscope to visually search these slides and identify cells with best metaphase spread having well separated chromosomes with clear band patterns and less overlaps [**Qui etal. 2010**] as shown in figure3. Each slide can have approximately 20 metaphase spreads but not necessarily all will be including analyzable metaphases. This metaphase spread count highly depends upon the investigation or type of disorders and sometimes merely giving low quality or scarce spreads. Analysts prefer to have at least 20-30 best metaphase images with well spread and banded chromosomes for their examination or karyotyping [**Polipalli et al., 2016**]. Technologist thus has to rigorously search large number of cells approximately around 200 metaphases and should select number of best metaphases for AKS **[Uttamatanin *et al.*,2013].**

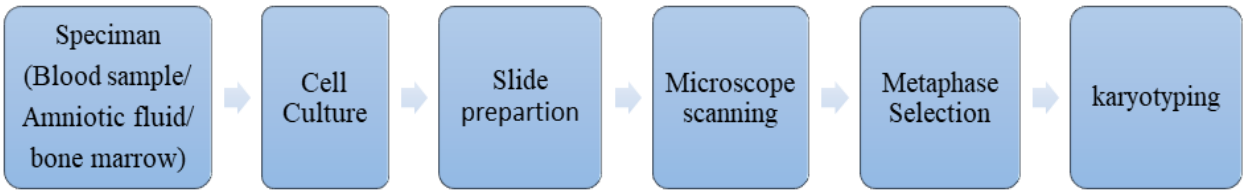

Figure 1. Clinical steps in Karyotyping

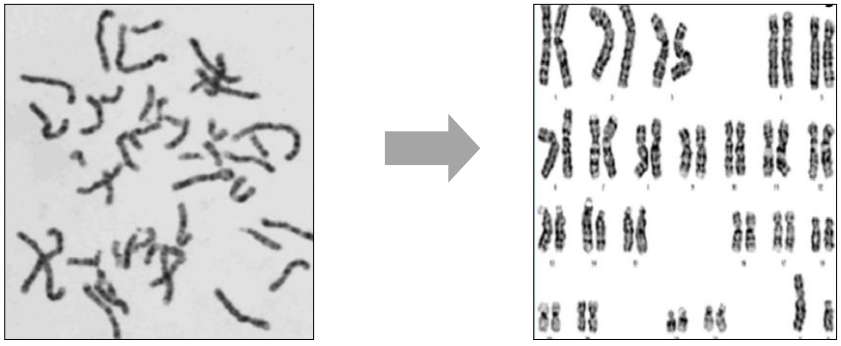

Figure 2 (a). A metaphase spread of a normal human cell; (b). The corresponding karyotype

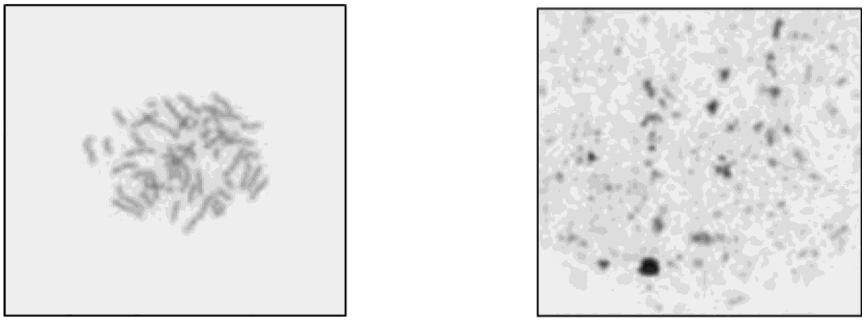

Figure 3 a). Analyzable metaphase; b). Non-analyzable metaphase

1.3. Manual Method of metaphase selection

Manual method of metaphase selections has several major setbacks like time consuming, labour intensive, complex due substantial number of metaphase scan, tiresome, subjective or biased as it is highly human dependent and needs a trained expertise [**Korthof et al., 2008**], consequently is not an efficient solution for selection of the best metaphase images [**Kovác et al., 2009**].

This procedure of metaphase finding apparently needs to be automated using computerised schemes so as to facilitate faster diagnosis and effective treatment, highly efficient due to elimination of intra observer variability and can be more accurate as quality of metaphase spread can significantly improve karyotyping results which in turn improves diagnosis accuracy [**Moazzen et ai, 2019]**.

1.4. Basic Steps in Automated Metaphase Selection:

A typical automated system for metaphase selection generally includes four fundamental steps: pre-processing, segmentation, feature extraction followed by Metaphase classification as shown in Figure4. The microscopic images acquired need to undergo some pre-processing to improve the quality of desired metaphase and remove undesired noise from images. Scanned images may contain small noise objects of low contrast or dried border drops of dye etc. once clean image is acquired the Segmentation techniques can be applied to extract feature set of metaphase. By suitably employing feature extraction techniques and classification techniques, best analyzable metaphases can determined from the scanned slides of specimen.

2. Literature Survey

The earliest preliminary study was presented by **[Castleman, 1992**] on commercially available automated metaphase finder. This metaphase finder used Bayes type classifier trained for different specimen types on binary images. Specific pattern recognition algorithms are used for the selection of metaphases and rejection of non-metaphase material. This suffered from drawbacks like time consuming and performance affected by quality of input samples and variability in spread count per metaphase and wasn't able to provide accuracy more than 80%.

Another system Metafer2 presented by Grell et al. (1991) and Weber et al. (1992) reported to be more time efficient but haven't included the detailed analysis of the same. [**R. Huber et al. 1995]** have assessed the performance and efficiency of this Metafer 2 system on metaphases of rat liver cells. This Metafer2 is based on a binary image analysis technique having programmable threshold and uses three-step algorithm for detection of metaphase on scanned slide frame.

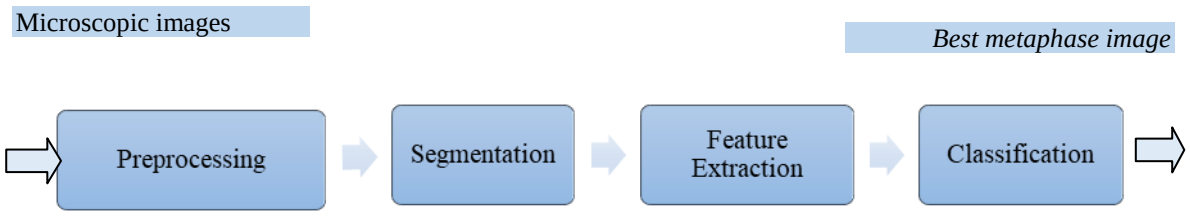

Figure 4 Steps in Automated metaphase Selection

In the first step the system finds candidate metaphase which has higher line values of band pass filtered object. In second step, system looks for higher column values of these candidate metaphases. Last step uses rapid contour following algorithm on binary image within the rectangle set by first two steps for further detail analysis. The system computes its features and classifies it into two classes as 'metaphase' or 'non-metaphase' using multivariate statistical classifiers and provides higher accuracy in simple cases having separated chromosomes.

The classification is highly dependent on binary images generated by comparing input images with a programmable threshold hence threshold selection is crucial and prone to lose small structural details during this step. System's classification accuracy gets affected by the number of additional, overlapping or touching chromosomes present in the binary image. Moreover the system performance is not evaluated on non-aberrant metaphases hence it remains unexplored.

Corkidi et al. 1998 introduced a novel feature Mean Depth-Width Ratio of Extrema (MDWRE) which is a roughness feature of surface-intensity image and used it for the automatically estimating mitotic index in cell proliferation. A mitotic index is the percentage of cells that are in the process of division stage. This helped in identifying variable-shaped metaphases and interphase nuclei on microscopic images even in the presence of artefacts. After acquiring images, segmentation performed by Otsu's method which had two problems, firstly it failed to detect stimulated nuclei brighter than set threshold and secondly it segmented chromosomes of the scattered metaphases as single objects instead of a cluster.

A stereological-inspired approach was implemented wherein density of particular two-dimensional extrema (roughs), is estimated to extract texture roughness of low-resolution images. It analyzes features of roughness such as mean value and the shape of the distribution of the depth-width ratio of selected extrema. Both these features claimed to be distinctive feature of mitotic chromosome clusters and interphase nuclei.

MDWRE feature extraction provided excellent results in detection of artefacts, but did not perform well in detection of metaphases and nuclei. Secondly, as MDWRE uses mean value, it fails to detect fine

Further work to improve the accuracy and speed of detection of a neural network classifier has been devised by [**Cosio et al. 2001**]. Neural network is used to classify each segmented object using its ten distinct morphological features into three classes: metaphases, nuclei and artifacts. A data set of 909 patterns (191 metaphases, 331 nuclei and 387 artifacts) partially used for training and testing of NN which have three-layer feed forward architecture.

Although the neural network classifier out performs previous systems, it faces difficulty in automation of MI because of higher probability of finding artifacts than metaphases.

Machine Learning based methodology was reported by [Qui **2016**] for identifying and classifying metaphases into analyzable and unanalysable groups. Pre-processing and segmentation techniques such as Median filtering, adjustable threshold and Four-connectivity component labelling algorithm are used to detect chromosomes and lessen the effect of background and artifact noise in microscopic images. Five image features such as number of labelled regions, size, circularity, average grey value and radial length of each region to the cell centre are extracted from these labelled regions and are applied to machine learning classifiers. Two different machine learning algorithms which are explored and tested are Decision Tree (DT) and Artificial Neural network (ANN). Both the classifiers are implemented to classify images into two classes analyzable and non analyzable.

The DT with four horizontal layers and five nodes compares computed features of individual chromosomes with a set threshold at each node and classifies the respective class. Metaphases having higher individual and recognizable chromosomes are classified as analyzable by DT.

Another approach using ANN works on features computed from all labelled regions in one acquired image region of interest (ROI). Features considered for classification are six features, which are number of labelled regions, their average size, standard deviation of region size and pixel value, average pixel value of all regions and average radial length of all regions.

Both the classifiers performed well in classifying metaphases with DT accuracy slightly higher in detecting analyzable whereas ANN in non-analyzable metaphases. Performance of the system in terms of accuracy is curbed by use of limited databases and preselected microscopic images with at least one metaphase cell. Moreover there is no substantial verification of system results by different cytogeneticists (merely one) hence cannot be proven against inter observer variability parameters.

Kovács et al. 2009 suggested and implemented a two pass algorithm on digitized microscopic slides for faster detection of metaphases. First step is coarsely localizing possible metaphase structures in image and in second step performs recognition of finer details such as size of metaphase. The diameter of the metaphases is

estimated as two times the diameter of the biggest round cells localized in the first step. The method uses Otsu threshold and component labelling algorithm to detect location of metaphase. To find the size of chromosome metaphases, it uses Hough- transform to find round cells on the scan which helps to calculate coarse resolution of recognizable metaphases.

System performs faster in detection and improved time efficiency. However, for their algorithm the diameter of chromosome metaphases (metaphase size) is measured manually. Furthermore, the system suffers from need of human intervention to note the chromosome metaphases manually on the scans and need to be improvised.

Uttamatanin et al. 2013 proposed applying a rule based classification of chromosomes to address the issue of good metaphase spreads selection. This Methodology worked on classification of chromosomes into four main classes with straight individual chromosomes as first, bended individuals as second, overlapping chromosomes and artifacts as third and fourth class respectively. After performing pre-processing and segmentation on the scanned image, segments are arranged in vertical orientation to facilitate calculation of its geometrical features; width, height, and estimated area ratio. Width and height parameters of chromosome segments have been proved pivotal in classifying the chromosomes into respective classes.

Based on statistical models, threshold value for each parameter is decided i.e. Gaussian model gives a 67.84% threshold value for area ratio classifying chromosomes above this being in straight class. To remove artifacts from this class, threshold check on average width is performed; Width ratio between 0.98 and 1.15 classified in class 1 as straight chromosome and outliers being discarded as artifacts (class 4). This threshold value for object width is determined by empirical and Gaussian probability distribution of width ratio.

Chromosomes having area ratio less than threshold belong to either bended individual or overlapping chromosomes. Such objects are rechecked on its height parameter for elimination of artifacts. For separation of bended individuals and overlapping chromosomes, threshold check is performed on maximum width ratio. The proposed system performs efficiently with satisfying accuracies with straight chromosome class but accuracy declines for of bended and overlapped chromosomes. After classification the images are ranked according to their total number of individual chromosomes present in class 1 and 2, with higher ranking are considered to be of good quality and analyzable.

Although a special software tool called Metasel is developed for quickly ranking metaphases using the above method but major hindrance is the need of cytogeneticists to select metaphase spread image from rankings and provide it to perform karyotyping. So the system doesn't automatically select the best metaphase spread rather it assists cytogeneticists in choosing the most probable best metaphases.

Another similar approach was presented by **Arora et al. 2017** for detecting and ranking of metaphases but lacked in touching upon automatic selection of metaphases. One more study investigated and presented by Hakan et al. [28] which implemented again only the detection of metaphases from microscopic images and did not address selection techniques. The System uses a filter called Meta filter for detection and is claimed to be a low cost system for metaphase finding.

A two-stage automated metaphase-finding scheme proposed by **Moazzen et al**. **2019** has two steps: metaphase detection and selection. For metaphase detection, image processing techniques are employed whereas selection stage has been implemented with a deep convolutional neural network.

The Candidate Metaphase Detection (CMD) finds all candidate metaphase cells by image segmentation techniques on 10x microscopic images. This eliminates stains and undivided cells by observing morphological properties like circularity and pixel information of these objects. A complete metaphase spread cell's regions are saved by dilation methods to connect neighbour chromosomes of metaphase cells. All these detected ROI applied to a pre-trained deep CNN to find the best analyzable metaphases.

Recently, with emerge of deep neural networks(DNN) it has become possible to increase the accuracy in medical diagnosis while avoiding the chromosome segmentation and feature extraction steps **[Cheikh et al., 2020** , **Bar et al., 2015]**. DNN are made of multiple layers of neurons which automatically learns and classifies the images into appropriate classes after suitable training[**Qui et al., 2020**]. Because of its interesting trademark, this profound learning technique has been applied in the clinical field to diagnose various diseases like cancer **[Navab et al., 2015] ,** chest pathology[**Bar et al., 2015**], brain tissue identification [**Zhang et al., 2015**].

One such attempt made by **Qiu et al. 2020** developed a computer aided detection (CAD) scheme using eight layer neural network. First six layers learns the features while last two MLP based layers identifies analyzable metaphases from images. Receiver operation characteristic (ROC) method is used to analyse performance of CAD system which proved the system resulting higher the accuracy with AUC of 0.8. The major drawback of the study is limited dataset which demands for further in-depth study.

Remya R. S. et al. 2020 proposed a sequential Convolutional Neural Network (CNN) model having five layered structure with four convolution-pooling and one completely connected and last layer of output neurons. The output layer contains two neurons for two classes as analyzable/ un-analyzable. Experiment results of model shows a training and validation accuracy of 87 and 88% respectively. Experimentation on the limited dataset, handling of only fixed size images in RGB space due to limited computational resources and ranking of analyzable metaphases needs to be addressed further.

3. Discussion and Conclusion

In Cytogenetic analysis Metaphase selection can be broadly viewed as a two process: Detection of metaphase on scanned microscopic image and selection of the detected metaphases for AKS system.

The traditional manual method suffers from numerous drawbacks such as exhaustive, complex, subjective, time consuming, storage problem, expensive, need of expertise and hence necessities the automation through present day technology. Research carried out in this area has still not given the optimum accuracies in detecting analyzable metaphases and hardly any work is done in selecting best metaphase and passing it automatically for karyotyping which expedites end to end atomization.

The suboptimal accuracies of metaphase detection and finding analyzable metaphases have underlying causes such as type of sample, quality of specimen dependant on slide preparation techniques, magnification power of microscope lens, inherent count variation of number of metaphases due to genetic disorders, staining methods etc.

Analyzable metaphase images should contain a large number of individuals, well-separated straight chromosomes, clear band patterns and fewer artifacts. Most of the efforts were put in to identify metaphases in microscopic slides and group them into analyzable or un analyzable metaphases and very few have worked towards analyzing the quality of the detected metaphases as that's the basis for AKS. Few such attempts were made by researchers which used a ranking system for detected metaphases and metaphases with higher ranking are considered to be of 'best quality'.

Commercial systems are available earlier since not more than the 90's decade. One of such systems **[Castleman 1992]** was developed and deployed in practice but fails in giving accuracy more than 80% and even does not suffice on faster detection. One more such system metafer2 **[Huber 1995]** has highly dependent classification accuracy on the set threshold and presence of overlapping or touching chromosomes. Recently introduced metasel [**Uttamatanin, 2013**] uses rule based classifiers for identification and ranking of metaphases and performs very well in terms of accuracies reaching 99.42% in case of straight, individual chromosomes but experiences decline for bended and overlapped chromosomes. Besides this, a major drawback is human intervention needed to select a metaphase spread image from rankings and pass it for AKS.

Irrespective of these progressing systems available commercially, they lack in gaining acceptance and practical deployment by genetic laboratories because of performance issues and thus still provide scope for further research. Thirst for producing accurate results has kept the researchers zestful towards exploring different image processing techniques in various stages of metaphase selection.

With the aid of simple and effective image pre-processing algorithms such as Median filtering **[Qiu *et al* 2016]** band pass filtering facilitates on improving signal to noise ratio, background suppression and increasing the quality of low contrast metaphase images which indirectly improves chromosome classification accuracy.

For segmentation of metaphases thresholding techniques have been popularly used and are proved effective. Some of the methods employed empirical values while some used statistical models to eliminate the bias in setting it. Performance of automated metaphase selection system significantly hinges upon selection of segmented images and Extraction methods used.

Feature extraction of a metaphase images puts a strenuous challenge on automated selection as it is highly affected by parameters like resolutions and quality of the image, number of overlapping and touching chromosomes, bended and highly curved chromosomes etc.

Geometrical features are the most used feature set for metaphase selection. Researchers have developed systems by working on diverse number of features from as few as three to as many as ten **[Cosío, , 2010 , Yilmaz , 2017].** These geometrical features include width, height, estimated area ratio, labeled region, size, circularity, average gray value and radial length of each region, normalized area, centromeric ratio or arm ratio etc. Few attempts also have been made on working with morphometrical, photo metrical and textural features, while a novel feature MDWRE which is a roughness feature of surface-intensity image is used by **Cosío et al. 2001]**. Another such method devised by **[Uttamatanin et al. 2006]** that works on shape and band features of metaphase images for its classification have provided encouraging results .

The final step in an automated metaphase system is classification of metaphases. Majority of the systems works on classifying the metaphase into two classes: analyzable or un analyzable. While some work goes a step ahead and provides ranking or quality factor for the analyzable metaphases so as to assist cytogeneticists in effective karyotyping.

A wide variety of classification algorithms like rule-based classifiers, decision tree, Bayes classifiers and ANN have been proposed and implemented in literature. Among these, statistical algorithms and artificial intelligence approaches have been proved better performing. Recently introduced DNN based approaches **[Qiu 2020 , Remya 2020]** proved to be improving with detection accuracy but still need further research on use of extensive dataset so as to make the system robust. Table I compares the classification accuracies of methods reported in the literature. Numerous commercial and non-commercial developments presented and implemented by research community have limited success in building intelligent systems with complete automation, consistent and promising accuracies regardless of quality parameters of metaphases and appreciable time efficiency.

Table 1. Performance comparison of the various methods

Reference.	Features used	Approach	No. of classes	Classification method	Accuracy	Time
Castleman 1992	-	Detection, Ranking	2	-	80%	Slow
Huber 1995	Geometric		2	multivariate statistical classifier	*	Slow
Corkidi 1998	Geometric and textural	Detection	2	-	85%	-
Cosio 2001	Morphometrical, photometrical and textural	Detection	3	Neural Networks	91%	
Gajendran 2004	Geometric		0	Not used	*	Slow
Qiu 2010	Geometric and Intensity		5	Decision trees and ANN	-	Slow
Kovac 2009	Geometric	Detection	2	fast component analysis	80%	Fast
Uttamatanin 2013	Geometric and intensity	Detection, Ranking	2	Rule based Classifier	-	Fast
Tanvi 2016	Geometric	Detection, Ranking	5	CVR classifier	96.5%	Fast
Yilmaz 2017	Geometric	Detection	2	-	98.8%	Fast
Maozen 2019	Morphological	Detection, Selection	2	Rule based Classifier and NN	99.33%	Fast
Qui 2016	NA	Detection	2	MLP	AUC of 0.886±0.043	Fast
Remya 2020	NA	Detection	2	CNN	88.34% On Validation	Fast

The developed systems have yet not proved to be fully successful in terms of detection accuracy as compared to an expert cytogeneticist and hence suffer from having error rates which are unacceptable in sensitive field of medical treatments. Research in machine learning is also increasing exponentially leading to many advanced algorithms like GAN, DNN whose utility can be further be explored for development of automated metaphase

selection technique for karyotyping. The designed selection tools can further be customised and tailored for which the karyotyping is addressed. Such a selection tool will have a wide scope of applications in genetic labs and will serve as a boon for cytogeticists.

References

1. Arora and R. Dhir (2016), "A review of metaphase chromosome image selection techniques for automatic karyotype generation," Med. Biol. Eng. Comput., vol. 54, no. 8, pp. 1147–1157, 2016, doi: 10.1007/s11517-015-1419-z.
2. Arora and R. Dhir (2017) , "An automatic human chromosome metaspread image selection technique," Knowl. Inf. Syst., vol. 52, no. 3, pp. 773–790, 2017, doi: 10.1007/s10115-017-1024-6.
3. Bar, I. Diamant, L. Wolf, and H. Greenspan (2015), "Deep learning with non-medical training used for chest pathology identification," Med. Imaging 2015 Comput. Diagnosis, vol. 9414, p. 94140V, 2015, doi: 10.1117/12.2083124.
4. Castleman (1992), "The PSI Automatic Metaphase Finder," J. Radiat. Res., vol. 33, no. SUPPLEMENT, pp. 124–128, 1992, doi: 10.1269/jrr.33.supplement_124.
5. Cheikh, A. B. Mbacke, and S. Ndiaye (2020), "Deep learning in medical imaging survey," CEUR Workshop Proc., vol. 2647, no. 4, pp. 111–127.
6. Corkidi (1998) "Roughness feature of metaphase chromosome spreads and nuclei for automated cell proliferation analysis," no. November, pp. 679–685, 1998.
7. Cosío, L. Vega, A. H. Becerra, R. P. Meléndez, and G. Corkidi (2001), "Automatic identification of metaphase spreads and nuclei using neural networks," Med. Biol. Eng. Comput., vol. 39, no. 3, pp. 391–396, 2001, doi: 10.1007/BF02345296.
8. Gajendran (2004) and J. J. Rodríguez, "Chromosome counting via digital image analysis," Proc. - Int. Conf. Image Process. ICIP, vol. 2, pp. 2929–2932, 2004, doi: 10.1109/icip.2004.1421726.
9. Howard et al. (2017), "MobileNets: Efficient convolutional neural networks for mobile vision applications," arXiv, 2017.
10. Joshi, M. Munot, P. Kulkarni, and M. Joshi (2013), "Efficient karyotyping of metaphase chromosomes using incremental learning," IET Sci. Meas. Technol., vol. 7, no. 5, pp. 287–295, 2013, doi: 10.1049/iet-smt.2012.0160.
11. Huber (1995), U. Kulka, T. Lörch, H. Braselmann, and M. Bauchinger, "Automated metaphase finding: an assessment of the efficiency of the METAFER2 system in a routine mutagenicity assay," Mutat. Res. Mutagen. Relat. Subj., vol. 334, no. 1, pp. 97–102, 1995, doi: 10.1016/0165-1161(95)90035-7.
12. Korthof and A. D. Carothers (1991), "Tests of performance of four semiautomatic metaphase-finding and karyotyping systems," Clin. Genet., vol. 40, no. 6, pp. 441–451, 1991, doi: 10.1111/j.1399-0004.1991.tb03116.x.
13. Kovács, (2009) B. Kajtár, A. Fazekas, and G. Méhes, "Fast detection of chromosome metaphases in digitalized microscopic slides," ISPA 2009 - Proc. 6th Int. Symp. Image Signal Process. Anal., pp. 454–458, 2009, doi: 10.1109/ispa.2009.5297711.
14. Lijiya, M. K. Sangeetha, and V. K. Govindan (2012), "Segmentation and Classification of M-FISH Human Chromosome Images," pp. 7–10, 2012, doi: 10.1109/ICACC.2012.22.
15. Ming and J. Tian (2010), "Automatic pattern extraction and classification for chromosome images," J. Infrared, Millimeter, Terahertz Waves, vol. 31, no. 7, pp. 866–877, 2010, doi: 10.1007/s10762-010-9640-1.
16. Moazzen, A. Çapar, A. Albayrak, N. Çalık, and B. U. Töreyin (2019), "Metaphase finding with deep convolutional neural networks," Biomed. Signal Process. Control, vol. 52, pp. 353–361, 2019, doi: 10.1016/j.bspc.2019.04.017.
17. Mousami VM, J. Prachi, J. Madhuri, and K. Parag (2016) , "An incremental approach for efficient karyotyping systems," J. Med. Imaging Heal. Informatics, vol. 6, no. 1, pp. 221–225, 2016, doi: 10.1166/jmihi.2016.1612.
18. Munot, J. Mukherjee, and M. Joshi (2013), "A novel approach for efficient extrication of overlapping chromosomes in automated karyotyping," Med. Biol. Eng. Comput., vol. 51, no. 12, pp. 1325–1338, 2013, doi: 10.1007/s11517-013-1105-y.
19. Munot and A. Anuse (2019), "Research Journal of Pharmaceutical , Biological and Chemical Sciences Automated Pattern Recognition For Multispectral Chromosome Anlaysis Using Statistical Classifier And Fuzzy Inference Engine .," vol. 10, no. 13, pp. 13–29.
20. Munot M. V et al. (2109), "A Development Of Computerized Systems For Automated Chromosome Analysis : Current Status And Future Prospects," Int. J. Adv. Res. Comput. Sci., vol. 9, no. 1, pp. 782–791, 2018, doi: DOI: http://dx.doi.org/10.26483/ijarcs.v9i1.5436.

21. Navab, J. Hornegger, W. M. Wells, and A. F. Frangi (2015), "Medical Image Computing and Computer-Assisted Intervention - MICCAI 2015: 18th International Conference Munich, Germany, October 5-9, 2015 proceedings, part III," Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics), vol. 9351, no. Cvd, pp. 12–20, 2015, doi: 10.1007/978-3-319-24574-4.
22. Piper, E. Granum, D. Rutovitz, and H. Ruttledge (1980), "Automation of chromosome analysis," Signal Processing, vol. 2, no. 3, pp. 203–221, 1980, doi: 10.1016/0165-1684(80)90019-5.
23. Polipalli et al.(2016), "Cytogenetic analysis for suspected chromosomal abnormalities; A five years experience," J. Clin. Diagnostic Res., vol. 10, no. 9, pp. GC01–GC05, 2016, doi: 10.7860/JCDR/2016/19926.8494.
24. Qiu et al.(2010), "Automated detection of analyzable metaphase chromosome cells depicted on scanned digital microscopic images," Med. Imaging 2010 Image Perception, Obs. Performance, Technol. Assess., vol. 7627, p. 762718, 2010, doi: 10.1117/12.843915.
25. Qiu et al.(2016), "Applying deep learning technology to automatically identify metaphase chromosomes using scanning microscopic images: an initial investigation," Biophotonics Immune Responses XI, vol. 9709, p. 97090K, 2016, doi: 10.1117/12.2217418.
26. Remya, S. (2020) Hariharan, M. Sooraj, V. Keerthi, A. S. Raj, and C. Gopakumar, "Deepnet for Detecting Analyzable Metaphases," Proc. - 2020 Adv. Comput. Commun. Technol. High Perform. Appl. ACCTHPA 2020, pp. 1–7, 2020, doi: 10.1109/ACCTHPA49271.2020.9213212.
27. Rooney and B. Czepulkowski (2001), "Human cytogenetics: constitutional analysis: a practical approach", vol. 1.Oxford University Press, USA, 2001.
28. Van den Berg, H. F. de France, J. D. F. Habbema, and J. W. Raatgever, (1981) "Automated selection of metaphase cells by quality," Cytometry, vol. 1, no. 6, pp. 363–368, 1981, doi: 10.1002/cyto.990010602.
29. Tjio and A. Levan, (1956) "The Chromosome Number of Man," Hereditas, vol. 42, no. 1–2, pp. 1–6, 1956, doi: 10.1111/j.1601-5223.1956.tb03010.x.
30. Uttamatanin et al (2013)., "MetaSel: A metaphase selection tool using a Gaussian-based classification technique," BMC Bioinformatics, vol. 14, no. SUPPL16, 2013, doi: 10.1186/1471-2105-14-S16-S13.
31. Uttamatanin, (2013) P. Yuvapoositanon, A. Intarapanich, S. Kaewkamnerd, and S. Tongsima, "Chromosome classification for metaphase selection," 13th Int. Symp. Commun. Inf. Technol. Commun. Inf. Technol. New Life Style Beyond Cloud, Isc. 2013, pp. 464–468, 2013, doi: 10.1109/ISCIT.2013.6645903.
32. Yilmaz, (2017) "FahamecV1 : A Low Cost Automated Metaphase Detection System," vol. 7, no. 6, pp. 2160–2166, 2017.
33. Yunis, (1974) "1 - Structure and Molecular Organization of Chromosomes," in Human Chromosome Methodology (Second Edition), Second Edi., J. J. Yunis, Ed. Academic Press, 1974, pp. 1–15.
34. Zhang et al.(2015), "Deep convolutional neural networks for multi-modality isointense infant brain image segmentation," Neuroimage, vol. 108, pp. 214–224, 2015, doi: 10.1016/j.neuroimage.2014.12.061.