ORIGINAL ARTICLE

THE ROLE OF AI ASSISTANCE IN CONVENTIONAL KARYOTYPING: ENHANCEMENTS, LIMITATIONS, AND THE CONTINUING NEED FOR HUMAN EXPERTISE

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ABSTRACT

Objectives: This study aimed to assess the performance, efficiency, and limitations of AI-assisted karyotyping using Applied Spectral Imaging (ASI) software and to highlight the importance of expert cytogeneticist involvement to ensure that the diagnosis is accurate, reliable, and properly interpreted in light of clinical history and laboratory findings.

Material & Methods: A comparative observational study was conducted at Islamabad Diagnostic Center from October 2025 to December 2025. A total of 2,305 chromosomes were analyzed using ASI AI-based karyotyping software. Each AI-generated karyogram was reviewed by two cytogeneticists. Parameters evaluated in this study included chromosome count accuracy, overlap detection, segmentation errors requiring joining or separation, and the correctness of chromosome placement. The time required for AI-assisted analysis was compared with that of conventional manual karyotyping.

Results: The AI software detected a mean of 49.34 ± 2.63 chromosomes per metaphase, indicating a tendency toward over-segmentation. Chromosomal overlap was observed in 2.30% of cases. Manual joining and separation of chromosome segments were required in 7.50% and 4.59% of chromosomes, respectively. Correct placement without human intervention was achieved in 82.93% of chromosomes. AI-assisted analysis, including manual verification, required approximately 2 minutes per metaphase, compared with 10–15 minutes for manual karyotyping.

Conclusion: AI-assisted karyotyping improves efficiency and reduces turnaround time. Expert review remains essential for resolving errors and interpreting complex patterns. A combined AI–human approach provides the most reliable framework for accurate cytogenetic interpretation.

Keywords (MeSH): Artificial Intelligence, Karyotyping, Cytogenetics, Chromosomal abnormalities

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INTRODUCTION

Examination of metaphase chromosomes under a light microscope using Giemsa banding (G-banding) is a time-tested cytogenetic technique. This method detects microscopic chromosomal anomalies, including aneuploidies, translocations, inversions, deletions, and duplications. Despite the introduction of numerous molecular techniques and advanced methodologies, conventional karyotyping remains indispensable. This is due to its ability to assess abnormalities at the whole-chromosome level by an expert. [1, 2] However, this technique is time-consuming, mainly performed manually, and highly dependent on the capabilities and experience of cytogeneticists, leading to subjective differences in interpretation and prolonged turnaround time. [3, 4] In recent years, software-assisted image analysis and digital microscopy have been developed to reduce these limitations. These approaches improve chromosome segmentation, capture metaphases, and generate ideograms. [5, 6] Artificial Intelligence is emerging as a rapidly evolving component that aids in multiple steps, ultimately leading to markedly reduced analysis time and increased consistency. [7, 8]

Applied Spectral Imaging (ASI) software provides an AI-augmented karyotyping tool. It helps analyze metaphase spreads and align chromosomes in under 2 minutes, compared with the 10–15 minutes typically required for manual analysis. [9] Additionally, data is saved as comprehensive, high-resolution images, which are incorporated into the final report and can be retrieved later if required.

Despite these qualities, AI-driven platforms face several limitations. Expert examination of metaphases and manual correction are required to confirm low-quality metaphase images, metaphases with overlapping or

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closely spaced chromosomes that the software fails to separate, complex structural rearrangements and abnormalities, and marker chromosomes. Also, the software lacks interpretive results and integration with clinical context, which are provided and verified by cytogeneticists. [10]

This emerging situation spotlights the requirement for a hybrid diagnostic model in conventional cytogenetics. The introduction of AI-assisted software in this field markedly affects work speed, but the role of human expertise is irreplaceable. This article examines the role of AI in conventional karyotyping, its benefits and limitations, and emphasizes the need for expert cytogeneticist interpretation to achieve accurate results and diagnoses.

The objective of this study was to assess the strength of software in the correct placement of chromosomes according to ICSN guidelines and the level of human mediation required for correct diagnosis and ultimately authentic reporting.

MATERIAL AND METHODS

This observational study was conducted at the Department of Cytogenetics, Islamabad Diagnostic Center (IDC), Islamabad, from October to December 2025. The study aimed to evaluate the efficiency and accuracy of AI-assisted karyotyping using ASI software (Applied Spectral Imaging, CA, USA) compared with traditional manual karyotyping performed by experienced cytogeneticists.

Following approval from the institutional ethical review board, cytogenetic samples received for evaluation were included based on clinical indications, including suspicion of Down syndrome, Edwards syndrome, Patau syndrome, and other aneuploidies; developmental delay; disorders of sex development (DSD) (including suspected Klinefelter syndrome, Swyer syndrome, MRKH syndrome, gender dysphoria, congenital adrenal hyperplasia, and Turner syndrome); recurrent fetal loss (RFL); preimplantation genetic testing for in vitro fertilization (IVF); acute leukemias; myeloid neoplasms such as chronic myeloid leukemia; and other suspected chromosomal abnormalities. All samples were processed in accordance with standardized cytogenetic laboratory protocols to ensure consistency and reliability.

Conventional karyotyping was performed on peripheral blood lymphocyte cultures, followed by cell harvesting, hypotonic treatment, fixation, and slide preparation. G-banding was used to obtain well-defined chromosomal banding patterns. Slides were pre-screened for quality assurance to ensure optimal metaphase spreads, minimal overlap, and sufficient band resolution before detailed analysis. Only high-quality metaphases meeting established criteria were included in the study to minimize analytical bias.

A total of 2,305 chromosomes from 50 metaphases were selected for analysis. The GenASIs BandView module of the ASI software, an AI-based image analysis platform, was used to automate chromosome detection, segmentation, and preliminary classification for each metaphase. The software's results were then reviewed and validated by expert cytogeneticists to assess concordance with the actual findings, identify any discrepancies, and ensure diagnostic accuracy.

Two experienced cytogeneticists independently reviewed the chromosomal assessments generated by the software. For each metaphase, they carefully examined several parameters, including the number of chromosomes correctly aligned in the initial automated run, the presence of overlapping chromosomes, and instances in which chromosome segments required joining due to improper splitting. They also assessed instances in which chromosomes needed to be separated, as well as the software's overall accuracy in classifying and placing chromosomes.

All observations were recorded using a standardized checklist, and mean ± standard deviation (SD) values were calculated for each metaphase. To minimize interobserver variation, both cytogeneticists discussed discrepancies in their evaluations and reached consensus. This step helped ensure consistency and strengthened the reliability of the findings. In addition, occasional cross-checks of previously reviewed slides were conducted to maintain uniformity throughout the analysis.

For statistical evaluation, descriptive statistics were used to summarize the data. Means, standard deviations, and percentages were calculated for each parameter across all metaphases. Data were entered and analyzed using SPSS version 26.0. Continuous variables are presented as mean ± SD with 95% confidence intervals (CIs), and categorical data are reported as frequencies and percentages, with 95% CIs.

RESULTS

In our study, a total of 2,305 chromosomes from 50 metaphase spreads were evaluated. To assess the performance of the ASI (Applied Spectral Imaging) AI-based karyotyping software, the software was evaluated (Figure 1). The key performance indicators included accurate assessment of chromosome counts, detection of overlapping chromosomes, correction of segmentation needed in metaphases, appropriate placement of chromosomes, and time-saving effectiveness for the expert. Hence, its role is to reduce turnaround time and enable early report generation.

In the study, the software automatically identified, on average, 49.34 ± 2.63 chromosomes per metaphase. This indicates a trend toward over-segmentation of chromosomes, particularly in metaphases with suboptimal band resolution, overlapping chromosomes, and closely spaced chromosomes, ultimately resulting in the splitting of chromosomes into two or more segments. This required manual intervention for correct placement (Figure 2).

Chromosome overlap was observed in 2.30% of chromosomes across the metaphases considered. Manual intervention by expert cytogeneticists was required to resolve overlaps and ensure accurate interpretation and correct placement. Chromosomes requiring manual joining of fragmented segments numbered 3.45 ± 1.84 per metaphase. On the other hand, almost 4.59% of chromosomes (mean: 2.11 ± 1.54) needed manual separation.

AI-based technology mistakenly joined the neighboring chromosomes into a single structure and ultimately placed them in the wrong position. (Figure 3)

On average, in the AI analysis, the number of chromosomes that were incorrectly placed and ultimately required intervention and human correction was 7.85 ± 2.37 per metaphase, corresponding to 17.07%. This shows that 82.93% of all the chromosomes in the considered metaphases were placed appropriately and required no adjustments by the relevant expert.

One of the most effective benefits of ASI software was the marked reduction in required analysis time. In contrast to the 10–15 minutes required for manual karyotyping, AI-assisted karyotyping required only an average of 2 minutes per metaphase. This time included the necessary manual intervention by an expert cytogeneticist. More than an 80% improvement in time savings was observed

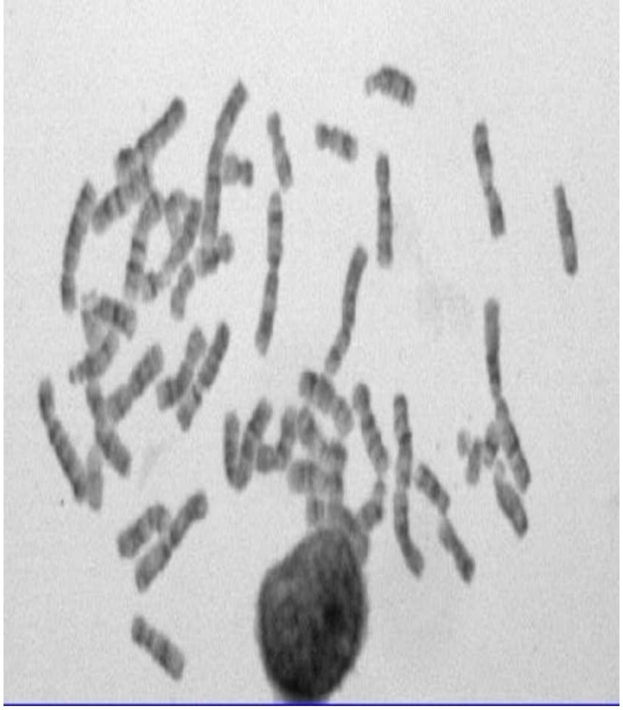

Fig 1: Representative G-banded metaphase spread, which is obtained from cultured peripheral blood lymphocytes (×1000, oil immersion)

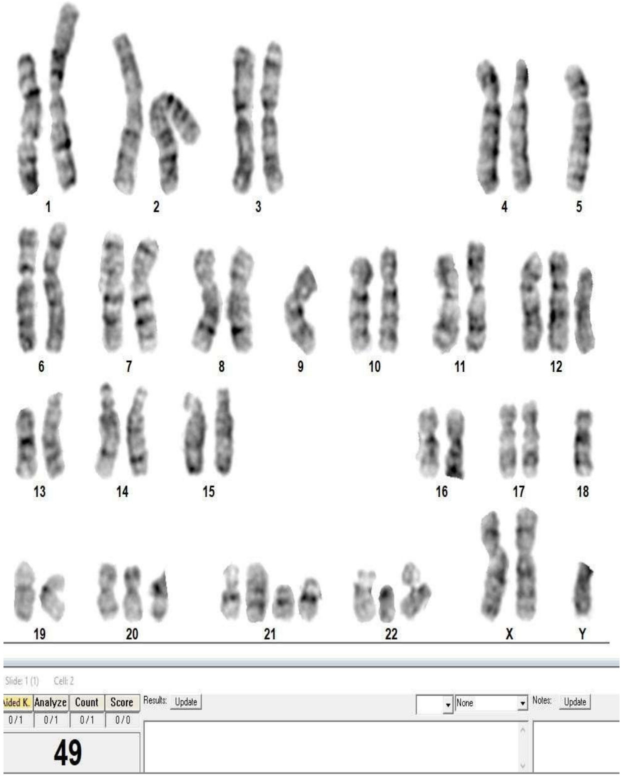

Fig 2: An initial automated karyotype output generated from a metaphase, analyzed by AI-based software, and showing an extra number. This is the unrefined chromosome detection prior to manual rearrangement performed by an expert.

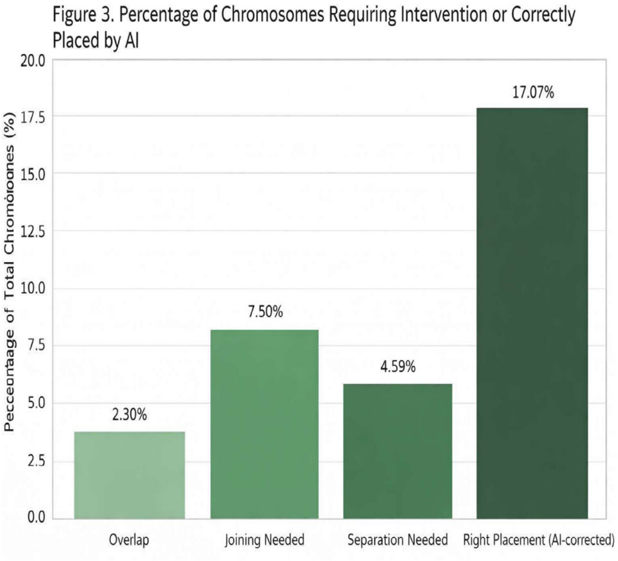

Fig 3: Percentage of Chromosomes requiring intervention or correctly placed by AI

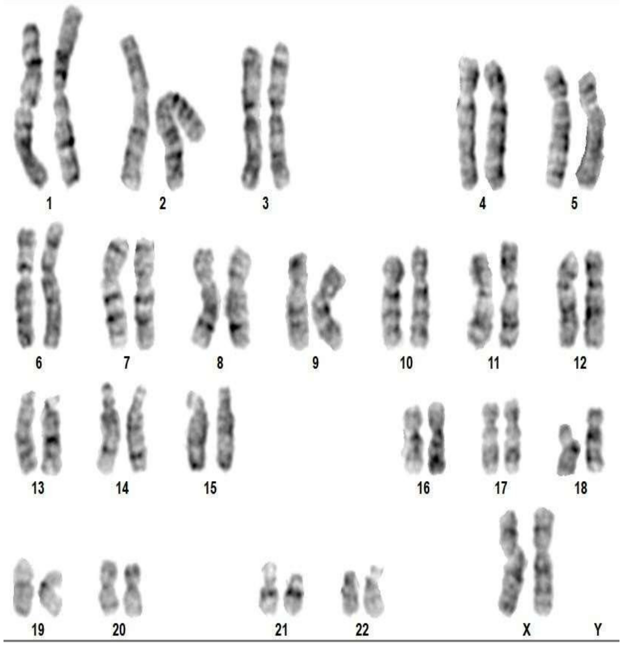

Fig 4: Karyogram showing karyotype of 46, XX. Manual correction of chromosome placement done according to ISCN guidelines

in our study, which significantly affects workflow optimization in high-volume cytogenetics laboratories. In addition, digital data storage, its incorporation into final report generation, and retrieval are strengths of the software. All these factors make the analysis robust and up to the mark. Hence, it positively affects diagnosis and the desired treatment strategy for patients (Figure 4).

DISCUSSION

The use of Artificial Intelligence (AI) in conventional karyotyping has shown very favorable outcomes, particularly in optimizing processes, improving operational efficiency, and reducing manual workload compared with previous cytogenetic techniques. In this study, experts evaluated ASI (Applied Spectral Imaging) software and analyzed a total of 2,305 chromosomes across 50 metaphase spreads.

It was observed that ASI software tends to over-segment, with an average chromosome count of 49.34 ± 2.63 per metaphase. This is above the standard chromosome number, indicating that chromosomes were occasionally split into two or more fragments. This kind of issue has been reported in a few studies that evaluated AI-based chromosome segmentation. It was noted that suboptimal banding quality, artifacts, closely spaced centromeres on chromosomes, and chromosomal crossing over may mislead the software's detection capabilities. [5, 6, 8] However, 2.30% of the chromosomes required manual correction and placement by an expert due to overlap. This finding concurs with studies by Zhou et al. and Chen et al., which showed that even advanced segmentation models still require expert input when dealing with tightly clustered metaphase spreads or chromosomal overlap. [6, 9]

Consistent with earlier studies, our results also confirm that AI-based karyotyping systems still require expert manual correction, particularly for fragmented, overlapped, and fused chromosomes. [10, 11] The observed similarity points to a shared constraint in automated systems, underscoring their limited capacity to distinguish chromosomal continuity from close physical alignment.

A key strength of the ASI software platform was its ability to automatically position 82.93% of the chromosomes in their appropriate locations. This indicates that when image acquisition is high quality, AI-based systems can organize the majority of chromosomal elements. Comparable performance trends have been reported in evaluations of GenASIs and MetaSystems software. These automated karyotyping methods demonstrated high accuracy. [12, 13]

Nevertheless, 17.07% of the chromosomes still required correction by trained professionals. This finding emphasizes that automated analysis can't be a substitute; it's supportive. Expert assessment remains essential, particularly when interpreting complex structural rearrangements or cases of numerical mosaicism, where algorithmic detection may struggle to identify subtle variations.

Importantly, AI-assisted analysis substantially reduced processing time. The average evaluation time per metaphase was approximately two minutes with an automated support system, whereas conventional manual assessment typically required 10 to 15 minutes. This represents a five- to sevenfold improvement in turnaround time. These benefits align with previous studies that identify time reduction as a primary advantage of AI integration in cytogenetics. [11, 12, 14] Faster processing is especially beneficial when prompt diagnostic reporting directly influences patient management decisions. [4, 10, 16]

Additionally, AI systems offer advantages in digital infrastructure. Their platform-based capabilities for remote case review, long-term storage of metaphases and finalized karyotype images, and systematic data archiving are worth noting. These capabilities enhance traceability and enable retrospective analysis. [14, 19, 20]

Despite these benefits, current AI technologies are not without constraints. Automated algorithms may encounter difficulties when analyzing abnormal karyotypes, suboptimal banding patterns, or complex structural abnormalities, where image variability and chromosomal distortion can compromise accuracy. [17] Moreover, while software may aid in chromosomal identification and image arrangement, it lacks the capability to correlate these findings with clinical context and to provide interpretive judgment related to patients' diagnosis and ultimately treatment strategy. This is done by human experts using their knowledge and clinical expertise. [18]

Therefore, the integration of AI in cytogenetics should be viewed as a complementary approach, with the role of cytogeneticists indispensable for validating results, resolving ambiguous findings, and ensuring that interpretations are clinically meaningful. This collaborative model will not only enhance diagnostic accuracy but also support the effective and safe implementation of AI technologies in routine cytogenetic practice. In addition, recent advancements highlight that although AI-assisted karyotyping significantly improves efficiency and reproducibility and reduces turnaround time, its performance remains highly dependent on the diversity of training datasets and the quality of the advancements. [24]

Importantly, our findings emphasize a hybrid approach that combines the speed and standardization of AI with expert human review as the gold standard for clinically relevant diagnoses and final case interpretation.

As molecular genetics and sequencing techniques continue to evolve, AI-assisted karyotyping will likely serve as a bridging technology, improving conventional cytogenetic workflows while integrating them with other modern genomic tools. Ultimately, this will improve patient healthcare in a robust manner. [21] Also, future improvements include enhanced machine learning algorithms through training and the introduction of learning models based on user feedback. [22, 23]

CONCLUSION

This study concludes that the ASI Artificial Intelligence-assisted karyotyping system significantly streamlines conventional chromosome analysis by reducing the time required for metaphase interpretation. With most chromosomes correctly placed without manual correction, the software proves highly effective for the cytogenetic workflow. However, shortcomings such as over-segmentation, inability to resolve complex overlaps, and the need for manual correction highlight the indispensable role of expert cytogeneticists. Despite being an advanced technique, AI-assisted karyotyping still lacks the understanding required to interpret structurally complex or clinically subtle abnormalities.

Thus, the most suitable model combines the computational strength of AI platforms with the interpretive acumen of trained human professionals. With continued development, AI has the potential to enhance the efficiency and accuracy of cytogenetic diagnostics, thereby improving patient management.

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Authors Contribution:

Following authors have made substantial contributions to the manuscript as under

Authors	Conceived & designed the analysis	Collected the data	Contributed data or analysis tools	Performed the analysis	Wrote the paper	Other contribution
Rehan GE	✓	✓	×	×	✓	×
Khan AA	✓	×	✓	✓	✓	×
Uppal R	✓	✓	×	×	×	✓
Malik HS	✓	×	✓	✓	✓	×

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval:

This Manuscript was approved by the Ethical Review Board of Islamabad Diagnostic Center (IDC), Islamabad, Pakistan.

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