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The PSI Automatic Metaphase Finder

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Metaphase finding/Pattern recognition/Image analysis/Automated microscope/Aberration scoring

The Genetiscanner automatic metaphase finder (AMF) instrument manufactured by Perceptive Scientific Instruments, Inc. is capable of searching a microscope slide and generating a list of the stage coordinates of the metaphases within the 20 mm×40 mm search area in ten minutes. It can process a cassette load of sixty slides in approximately ten hours, ranking the metaphases according to six categories of quality. It uses a robot arm to load and unload slides from a 60-slide cassette onto an automated microscope stage. It searches at approximately 1.5 sq. mm per second and uses automatic focus to keep the image sharp during the search. On good quality blood slides it detects greater than 80% of the metaphases with greater than 80% ranking accuracy and less than 20% false positives. It can also search amniotic fluid, bone marrow and mammalian cell culture metaphase preparations. The pattern recognition algorithms use classifiers specifically trained for each specimen type, and for the rejection of non-metaphase material. Such units have been in continuous operation for up to two years with good performance and reliability.

INTRODUCTION

For two decades automatic metaphase finding has been a topic of research and development in cytogenetics automation[1–7] for use in routine cytogenetics, cancer cytogenetics and aberration scoring.

Perceptive Scientific Instruments, Inc. has developed and is marketing an automated microscope programmed for automatic metaphase finding (Fig. 1). The Genetiscanner automatic metaphase finder (AMF) instrument is capable of searching a microscope slide and generating a list of the stage coordinates of the metaphase spreads found, ranking them into six categories of quality. Searching at approximately 1.5 sq. mm per second, it can process a cassette load of sixty slides in approximately ten hours of unattended operation.

The automated microscope uses a robot arm for automatic loading and unloading of slides from a 60-slide cassette onto an automated microscope stage, and uses automatic focus to keep the image sharp during the search. On good quality blood slides it detects more than 80% of the metaphases with greater than 80% ranking accuracy and less than 20% false positives. It can also search amniotic fluid, bone marrow and CHL metaphase preparations.

The pattern recognition algorithm uses a series of classifiers that are specifically trained for each specimen type, and for the rejection of non-metaphase material. Such instruments have been in continuous operation for up to two years with good performance and reliability.

This paper gives a preliminary description of the instrument. The discussion is necessarily

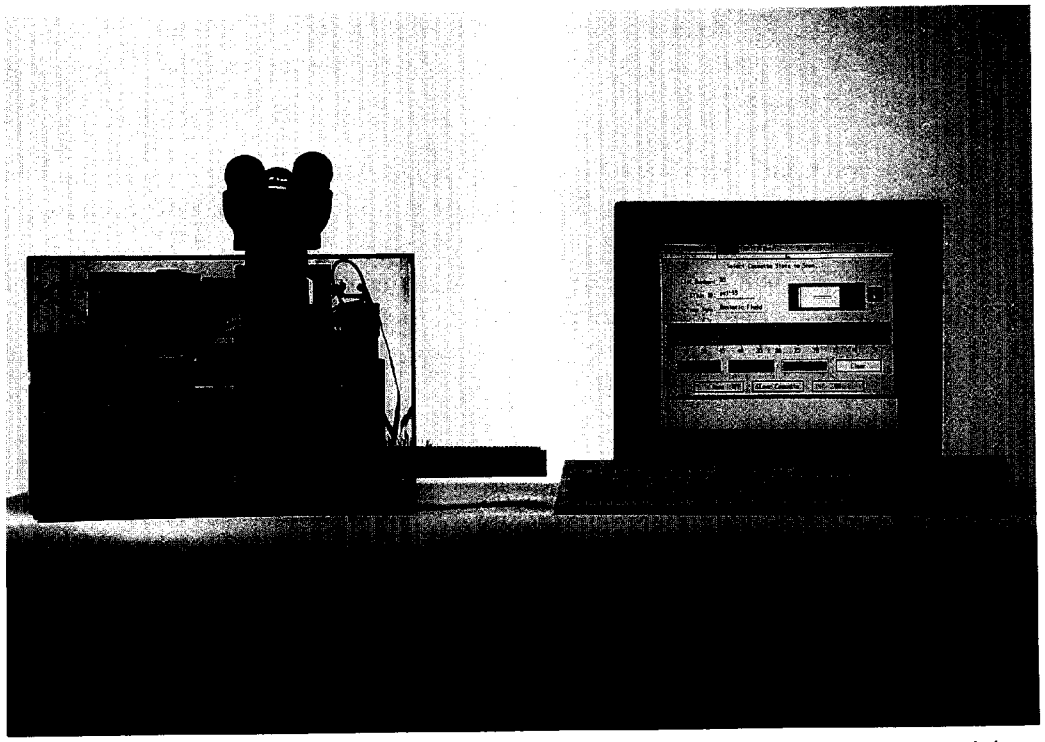

Fig. 1. The PSI Genetiscanner Automatic Metaphase Finder. The automated microscope unit is on the left, and the display console is on the right.

incomplete in certain areas due to proprietary design details that cannot be disclosed at this time.

MATERIALS AND METHODS

General Layout. The instrument consists of a computer, an automated microscope assembly, a motor control interface, and an image processor. The lists of ranked spread coordinates can be printed or transferred to a PSI Genetiscan Relocation Station for automatic retrieval of the metaphases.

The computer controls all the functions of the instrument and provides the interface with the operator. The microscope uses a robot arm with vertical and rotary motion for automatic loading and unloading of slides from a 60-slide cassette onto its automated stage. Vacuum chucks on the arm and stage hold the slide in place. A line array camera feeds a video image to the image processor which feeds digital image data to the computer's memory while a one millimeter wide strip of the slide is being scanned. At the end of each strip scan, the computer processes the image data and adds the metaphases that were found to the spread list.

Computer and Interfaces. The instrument is controlled by a 25 MHz 386 type IBM PC/AT

compatible computer. The computer has a VGA color graphics display and a mouse pointer device. It controls the motor driver interface, the image processor and the vacuum system through parallel interface ports.

Microscope Mechanical Hardware. The X- and Y-direction stage motion and vertical arm motion are effected by stepping motor driven leadscrews, while Z-axis motion comes from a stepping motor driven micrometer that moves the objective vertically. The cassette is belt-driven by a stepping motor. The arm is rotated through 90 degrees by a DC motor. Electromagnetic valves control two vacuum chucks. One holds a slide on the arm and one holds a slide on the stage. Solid state pressure sensors monitor the presence or absence of a slide.

Microscope Optics. The microscope uses a 100 watt tungsten-halogen lamp and a 16X, 0.45 NA objective to form an image of the specimen. Beamsplitters divide the light between the eyepieces, the CCD television camera that is used for remote viewing, and the CCD line array camera. The magnification from specimen to line array is 13. Thus the 13 micron sensor element spacing of the line array camera scales down to 1 micron pixel spacing at the specimen. The 1024 element line array sweeps out a 1 mm wide path across the specimen slide as the stage moves in 1.0 micron steps in the Y-direction.

Electronics. Each of the stepper motors that control the X-, Y- and Z-axis motion, the robot arm vertical motion and the cassette motion is driven by an indexer board and a power driver board in the motor control chassis. The indexer boards operate the motors at 40 microsteps per step and are controlled by the 386 computer through a parallel port.

The line array camera is controlled by an image processing board in the image processor chassis. This component also provides shading correction and digitizing of image data. The vacuum valves and sensors, as well as the mechanical limit switches, are controlled and sensed by the 386 computer through a parallel interface port.

User Interface Software. Specification of specimen type (for each slot position) and the area to be scanned is done with the mouse by menu selection from a graphic representation of the cassette and the microscope slide (Fig. 1). Once all slides have been specified, the analysis proceeds automatically, without the need for operator attendance.

Microscope Control Software. Calibration and setup consists of initialization of the image processor board, stepping motor position counters and shading correction lookup tables. A specially designed calibration slide is automatically loaded and used for this part of the process.

Slide loading is done by moving the cassette to the proper slot position, lowering the (now vertical) arm into the cassette, activating the vacuum chuck and lifting the slide out of the cassette. Then the arm is rotated to the horizontal plane, the stage is moved to the load/unload position, and the arm lowered until the slide is transferred to the stage chuck.

Slide unloading involves moving the stage to the load/unload position and moving the (now

horizontal) arm upward, with vacuum chuck activated, to lift the slide off the stage chuck. Then the stage is retracted, the arm rotated to the vertical plane and lowered into the cassette, and the vacuum released.

Automatic focus[8] keeps the image sharp during the search of the slide. It is done at appropriately spaced points on the slide using the video signal from the line array camera.

Image Analysis Software. The specimen is scanned in 1 mm wide strips at approximately 2.5 mm/sec. The resulting digital image data is transferred from the image processor to computer memory in real time. At the end of each strip scan, the stage movement pauses while the image data is analyzed for the detection of metaphase spreads. The time required for the analysis is appoximately equal to that for scanning the strip.

During the image analysis phase, objects are found and non-chromosomal material is discarded. Then groups of nearby objects are associated into "clusters" and non-metaphase material is discarded. Metaphases are then classified into six categories of quality by a Bayes-type classifier specifically trained for the specimen type (blood, amniotic fluid, bone marrow, CHL, etc.).

RESULTS

Reliability. In system performance testing, slide handling errors were found to be well below 1%. Most load/unload errors are recoverable and do not terminate the unattended operation of the instrument, but may leave one slide unprocessed. Slide breakage is very rare. In operational experience mechanical problems are rare. For most problems, future occurrences have been prevented by slight chances in the software. No recurring problem areas have been identified.

Accuracy. Metaphase finding accuracy is extremely dependent upon specimen quality. On good quality blood slides the instrument detects greater than 80% of the metaphases with greater than 80% ranking accuracy and less than 20% false positives. One unit has been in continuous operation for two years (>50 slides per day) with good performance and reliability.

Performance on CHL specimens is about the same as for blood, and for amniotic fluid it is almost as good. Performance on bone marrow specimens is more difficult to specify in a meaningful way due to the wide variability in the number of spreads per slide, spread quality and artifact content. Nevertheless, it exhibits the potential for significant time savings in the laboratory, since the cases that are difficult for the instrument are also the difficult ones for humans.

Timing. The slide loading and unloading procedure requires an average of slightly over one minute. The average search speed is approximately 1.5 mm^2 per second. This means that the 20 mm by 40 mm active area of a slide can be searched in about 8 1/2 minutes. Including slide loading and unloading, typical times are very close to ten minutes per slide. Setting up the

instrument for the scan of 60 slides takes approximately 30 minutes.

DISCUSSION

Automatic metaphase finding is a complex technology, but considerable development work stands behind currently available systems. Operational experience with the instrument described in this paper is quite encouraging. It suggests that this technology may soon come into widespread usage and provide considerable assistance in the laboratory.

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