
Metafer – a Novel Ultra High Throughput Scanning System for Rare Cell Detection and Automatic Interphase FISH Scoring

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INTRODUCTION

Automation of microscopic analysis is highly desirable in numerous diagnostic applications. The two main situations that take particular advantage of automatic slide scanning are rare event detection and statistical scoring of a large number of cells. The detection of fetal nucleated red blood cells (fNRBCs) in maternal blood presents an example of extremely rare events as commonly expected cell rates are in the order of 1 fNRBC in 10^7 maternal nucleated blood cells [1, 2]. Approaches of selective enrichment of the fetal cell population by methods like density gradient centrifugation, selective cell lysis, magnetic cell separation or a combination of these techniques did not succeed in satisfactory detection of fNRBC; cell loss during these procedures is believed to be the limiting factor. Minimizing the treatment and enrichment steps performed on the cells and compensating the low rate of fNRBCs by using a powerful automatic slide scanning system seems to be the most promising strategy for solving the problem of fNRBC assessment. A recently described solution [3] achieved cell rates of 862 cells/s in monolayer preparations and is faster than other image cytometers that have been reported before [4]. However, this development is not commercially available and still requires total scanning times of about 64 hours per patient to scan the 2×10^8 cells that can be expected to include the 10 to 20 fNRBCs that are necessary for subsequent genetic analysis. For routine diagnostic applications an improvement in speed by at least a factor of 10 would be prerequisite to reduce scanning times to a few hours per patient.

Interphase FISH scoring is an example of the second situation that calls for automation. Genetic testing by FISH is a technique closely related to reproductive medicine and fetal diagnostics and has been suggested as a strategy to detect fetal cells in maternal blood by anomalous FISH signals [2]. In a tedious and error-

prone procedure fluorescent spot-like signals have to be scored in hundreds or thousands of cells while constantly changing the plane of focus in order not to miss signals from out-of-focus planes. Manual screening gets particularly awkward if more than one or two different labels need to be detected. Although attempts have been made to automate this process [5, 6] no automatic interphase FISH scoring system has become commercially available so far that provides the speed and three-dimensional resolution to reliably count locus specific probes and to measure their three-dimensional distances in order to detect signal fusions.

The system presented in the following was designed to overcome the described limitations and to provide a fast, flexible and adaptive solution to numerous applications. The system is based on the second generation of the proven Metafer scanning platform that has been available for many years (the first generation was introduced in 1987) in its MSearch operating mode for automatic metaphase search. Two recently developed operation modes of Metafer are RCDetect which provides the fast rare cell detection functionality, and MetaCyte for fully automated high throughput interphase FISH scoring.

METHODS

General set-up

Automated slide scanning is generally performed by moving the slide with reference to the fixed objective lens in a regular meander-like pattern without gaps between the image fields. Image acquisition is done at the lowest possible optical magnification that still allows to resolve the features of interest. Each field of view is captured and analyzed. Depending on the result of the analysis individual objects (cells) within a field may be identified as objects of interest and will be further analyzed and stored in an image gallery along with their feature measurement data. Once the scan has been completed, the on-screen image gallery can be used to review the detected cells and to reject unsuitable cells or to do corrections if necessary. If the gallery image is not sufficiently informative, any cell can automatically be relocated under the microscope for direct visual inspection.

Fig. 1 depicts the main components of Metafer. A fluorescence microscope (*Carl Zeiss Axioplan2 Imaging Mot*) with motorized focus, motorized filter cube revolver with 8 positions, and equipped with a motorized scanning stage with 8 slide positions (*Maerzhaeuser*) is controlled by a microcomputer system (Pentium III, 1 GHz, Windows NT/2000 operating system). The focus motor of the microscope provides a focus resolution of 25 nm. A proprietary 2-axes stepper motor control board in the PC drives the scanning stage in micro-step operation to achieve a positioning accuracy of 1 micron in x and y at a maximum speed of 70 mm/s. All motorized microscope controls can be operated manually in the usual way; for manual stage movement a trackball is provided. A high resolution CCD camera with long time integration capability and fast digital read-out (JAI, Denmark, 1280 x 1024 pixels, 12 images/s at full resolution) captures the images that are analyzed in the computer. Automatic slide scanning always requires capturing large numbers of images, often at multiple wavelengths and multiple focus planes. Reasonable fluorescence intensities are prerequisite to achieve exposure times not exceeding a few seconds per capture. Cooled CCD cameras that provide lower electronic background at long exposure times are not required. However, the fast read-out rate of 12 images per second is essential for obtaining high scanning speed.

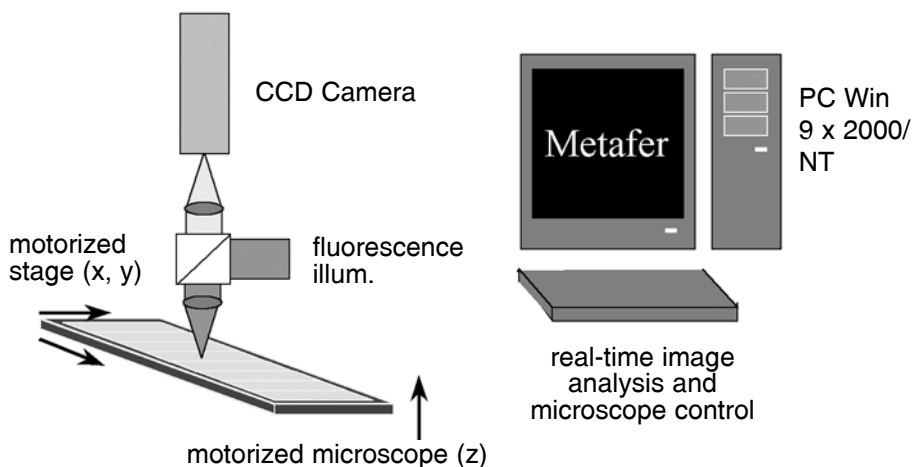


Figure 1. Set-up of slide scanning platform Metafer

Automatic focussing

Precisely maintaining the plane of focus during the scan is of utmost importance. We use a strategy that provides very high speed without sacrificing accuracy. At a number of grid positions that are regularly distributed across the scan area the plane of best focus is determined by automatically moving the stage in the z-direction, capturing images in different focus planes, and analyzing the focus quality based on a local contrast criterion. Typically 11 z-positions are being analyzed within approximately 2 seconds. The number of grid positions used for focus analysis is typically 60 for a complete slide (scan area 36 mm x 20 mm). Based on the grid focus measurements the software reconstructs the focus surface of the scan area by performing a bilinear interpolation and displays it as a pseudo-3D wire plot. During the subsequent scan, the slide is automatically kept within the plane of best focus.

Image acquisition

In fluorescence imaging signal intensity varies significantly between counterstain and fluorescent labels and even between different positions on the same slide. Automatic exposure control is a must to assure correct image quality and high dynamic range. The software estimates the correct exposure time based on the histogram (the intensity distribution) of an image captured without integration. Depending on the histogram shape a reduced exposure time (using the built-in electronic shutter) or long time integration is used. This strategy allows for exposure times from 1/10,000 s to approximately 30 seconds. As exposure times longer than a few seconds indicate an image field without any signal, the maximum exposure time is normally limited by the software to typically 1 to 2 seconds.

Rare cell detection

In rare cell detection a large number of counterstained cells and a small number of specifically labeled cells of interest are present on the slide. The label can consist of a cytoplasmic or a surface marker with a fluorescence tag attached to it and will cover a significant part of the labeled cell. This allows the use of a low optical magnification. For high light efficiency we use a high aperture Fluar 10x/0.5 objective lens. After the initial focus analysis based on the counterstain (usually DAPI) image the system selects the appropriate

filter cube to capture the fluorescence signal of the specific label. The contrast of the automatically captured image is analyzed, and images with insufficient contrast are rejected. Next, in case of a sufficient image contrast a locally adaptive detection algorithm identifies objects that are brighter than their surroundings and at the same time meet predefined size criteria. In contrast to simple thresholding techniques the employed algorithm assures that labeled cells are reliably found despite variable labeling efficiency across the slide, without increasing the likelihood that elevated background is misinterpreted as a labeled cell. If labeled objects have been detected a DAPI (counterstain) image is captured and the software determines if a nucleus of appropriate size is present. If this is not the case the object is considered a staining artifact and is rejected. If the labeled object passes this test, its position is stored together with a gallery image of the cell and its surroundings. This procedure is repeated until the desired scan area has been completely covered. During the scan, the average counterstain intensity of isolated nuclei is automatically determined which allows the software to estimate the total cell count by dividing the total counterstain intensity by the average nucleus intensity. The system can thus provide a quantitative relative occurrence rate of labeled cells.

A simultaneous second cell label tagged with a distinguishable second fluorochrome can be detected during the scan. Logical conditions can be used to increase the specificity of the result. For example, coincidence of two labels or anti-coincidence may be used to select cells of interest.

In many – if not most – applications the rare cell detection is only the first step that will be followed by a second assay. To this aim, the slide may be removed from the scanning stage after detecting the rare cells of interest. A sequential assay (e.g. a fluorescence *in-situ* hybridization) may be performed. After re-inserting the slide in the scanning system, any rare cell can be relocated with a mouse click, and the FISH result can be checked. The sequential correlation of different assays provides a maximum of information in characterizing rare cells.

Speed considerations in rare cell detection

It is obvious that speed is essential in rare cell detection. For optimizing speed the main time-consuming steps in the scanning process need to be identified. Counterstain image as well as cell label are usually pretty bright, so that exposure times will be fractions of a second per image. Stage displacement time (including the settling time necessary to wait for vibrations to fade out before capturing the next image) is typically 0.1 seconds. Image analysis does also not take longer than a fraction of a second and can be performed while the camera is already capturing the next field of view. Changing the filter block position takes approximately 0.4 seconds. Obviously, the key to speed improvement lies in accelerating the filter change. This was achieved by using a dual band filter in combination with individual excitation filters mounted in a modified excitation filter wheel (*Carl Zeiss*). The original stepper motor of the filter wheel was replaced with a more powerful model which is driven by a proprietary controller board. This solution improved the switching time between DAPI and FITC excitation from about 0.4 seconds to 60 milliseconds, resulting in an overall scanning speed of two images per second (for DAPI plus an additional cell marker). The employed high resolution camera (1280 x 1024) with a pixel size of 6.7 micron results in a threefold increase of information per field of view as compared to a regular video type CCD with typically 752 x 572 pixels. Finally, it turned out that an optical magnification of 6.3 x is sufficient for rare cell detection. This was achieved by using a 0.63 x camera adapter instead of the 1 x adapter in combination with the 10 x objective lens. The resulting scanning time per slide is 6 to 8 minutes for a complete slide. In monolayer Cytospin preparations with approximately 3 million cells per slide (scanned area 36 mm by 20 mm) this corresponds to approximately 7,000 scanned cells per second – one order of magnitude above scanning rates of previously reported image cytometers and comparable to flow cytometry [3].

Interphase FISH scoring

In automatic interphase FISH analysis the thickness of the nuclei and the small size of the FISH signals pose additional problems. As a consequence, optical magnification has to be raised to 20x (for relatively large signals, e.g. centromeric probes) or even 40x. Image capture at different focal planes is necessary to avoid losing individual spots. And, if spot counts per cell are required, isolated cells need to be identified while cell clusters that cannot be automatically segmented need to be skipped.

As described above the focus surface is detected using the counterstain information. Next, the counterstain information of each field of view is analyzed. Objects are automatically segmented and suitable cells are identified using appropriate shape criteria. Cell clusters are automatically rejected by detecting the concavity depth of the object contour. If suitable cells have been identified the system selects the filter combination for the FISH signals, otherwise it proceeds with the next field of view. FISH signals are captured in several focal planes. When using the 40x lens typically 5 focus planes separated by approx. 0.7 micron are captured. The individual images are combined to a projection image that includes only in-focus information of the individual focus planes. This projection (or extended focus-) image is used for detecting the FISH spots. This is repeated for additional color channels if more than one FISH label is present. Currently, up to 6 simultaneous color channels (including the counterstain), simultaneously displayable in pseudo colors, are supported. Analyzed nuclei are displayed in an image gallery with their spot counts for on-screen review and interactive correction.

In addition to spot counts the system can measure numerous selectable features per color channel during the scan. These include shape and texture-related features for cell classification as well as intensity features which yield e.g. ploidy information. Three-dimensional spot distances are also measurable, e.g. for automatic detection of signal fusions in translocation analysis. After the scan, any of the features can be displayed as a histogram. Two dimensional scatter plots are equally selectable. Selecting sub-populations based on any one or two feature values (known as “gating” in flow cytometry) allows detailed analysis of the scan data.

While the described single cell analysis approach is essential if counts per cell are required, there are situations where sufficient individual cells are not available or where the cells of interest appear in cell clusters. Smear preparations and tissue sections are specimen types where the analysis has to rely on cell clusters as well. In such situations ratio approaches can be helpful. A special sampling mode of MetaCyte allows measuring spot count ratios within automatically positioned sampling windows within the field of view. For example, gene amplifications in tissue sections can be automatically assessed by measuring the signal area of the probe for the amplified gene, measuring the signal area of a reference signal and calculating the ratio (areas rather than counts are used if high level amplifications yields clustering of FISH spots which makes the identification of individual spots impossible). As problems like cut or incompletely sampled nuclei will affect both signals similarly, they will average out statistically if a sufficiently large area is analyzed.

Speed optimization in spot counting

The limiting factor in interphase spot counting is the image acquisition time. The relatively small signals normally require integration times of up to one second for each image of the focus series. This time has to be multiplied by the number of FISH labels. To keep acquisition times as short as possible, single band filter cubes are preferred, despite the longer filter changing time of approximately one second (compared to the 60 milliseconds of the custom-modified excitation filter wheel. The essential time factor in spot counting is the cell density on one hand, and the necessary optical magnification on the other hand. With optimized cell spreads scanning speeds of up to 1,000 nuclei per minute (large, eg centromeric FISH signals)

are possible at 20x, while scanning with a 40x yields up to approx. 200 analyzed nuclei per minute. This scanning rate corresponds to that of the laser scanning cytometer [6] which provides about 10 times lower spatial resolution and no z-information and is about two orders of magnitude above the previously reported FISH dot counting cytometer [5].

Training

Easy adaptation and parameter optimization to different cell and label types is of crucial importance for making an automated system useful for routine analysis. To facilitate this task the system provides sophisticated training and optimization routines. The principle of optimization by training relies on a set of training images which have been preclassified by the operator. In a first step, the training images are automatically captured using slides that are representative for the type of specimens to be analyzed. Next, the operator can display image by image and can mark positive cells by using the mouse. Based on this *a-priori* information the training routine then performs the optimization by systematically varying within predefinable limits the parameters that are used for cell classification. Each set of parameters is applied to the training images and will detect cells. The number of "hits" is compared to the preclassified training data set until the parameter combination giving the best match between automatic and human classification has been found. The optimization can be applied to the cell selection criteria as well as to the spot counting parameters. Here, the operator will not only preclassify suitable cells to train the cell selection but will also interactively score for each hybridization channel the number of spots. Again, by automatically varying the spot count parameters like minimum/maximum spot size, minimum spot intensity, minimum distance required between two adjacent spots of the same color to interpret them as separate signals, the parameter set resulting in the best match between human and machine interpretation is determined.

RESULTS AND DISCUSSION

Detection of fetal cells in maternal blood

The system is currently being used for the analysis of blood samples drawn from patients between 10 and 13 weeks of gestation after undergoing invasive procedure (chorionic villus sampling). The red blood cells were removed by centrifugation, and the nucleated blood cells were smeared onto poly-lysine coated glass slides with a cell density of $5-10 \times 10^5$ cells on each slide. For the selective staining of fetal NRBCs a FITC-labeled PNA probe for the gamma-globin mRNA of fetal hemoglobin was applied [7]. Cells were counterstained with DAPI. In order to increase the specificity of the detection and to reject granulocytes and other nuclei showing unspecific FITC fluorescence two additional criteria to those described above were applied: 1. the cell label has to have a crescent-type or ring shape that is characteristic for the cytoplasmic RNA marker, and 2. the nuclei have to be round whereas the lobed nuclei of granulocytes are rejected. The screen shot (*Fig. 2*) shows the gallery of the detected fetal cells.

Between 0.5 and 6.0 ml of blood (mean sample volume of 1.9 ml) from 20 pregnant women have been analyzed. This corresponds to having scanned and analyzed between 3-23 slides per patient. Gamma-PNA stained fNRBCs have been identified in 65% of the blood samples with a mean number of 24,4 cells per sample [8].

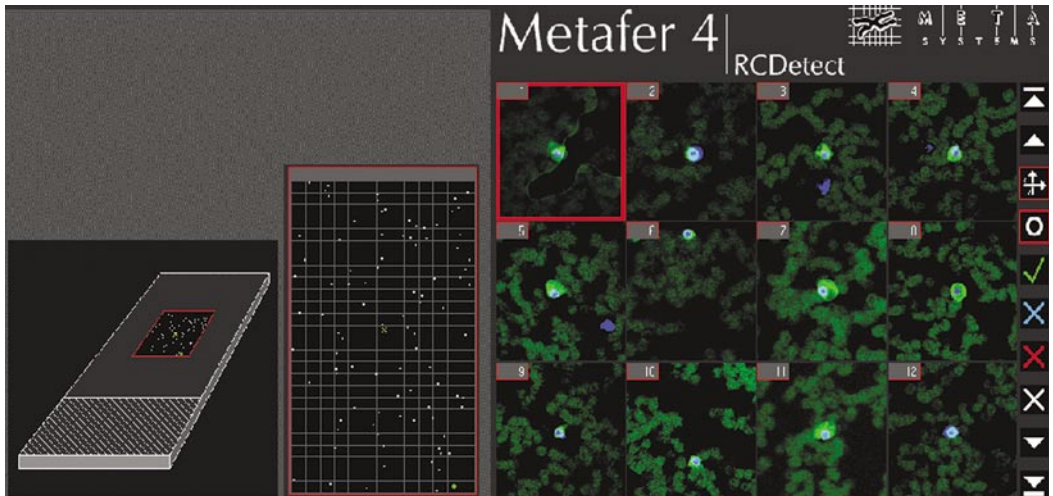


Figure 2. Image gallery of fetal cells detected in peripheral blood of pregnant woman after chorion villus sampling (refer to text for details)

Left-hand side shows slide with scanned area which was 25% of total slide area in this example. Each white dot corresponds to the position of a fetal cell. Center shows enlarged view of scanned area.

In the next step blood from pregnant women that did not undergo an invasive procedure will be analyzed.

Detection of isolated tumor cells

The assessment of occurrence rates of isolated tumor cells in bone marrow and peripheral blood is important in tumor staging and therapy monitoring. *Fig. 3* shows neuroblastoma cells detected in a bone marrow cytospin preparation, identified by using a fluorescence-labeled GD2 antibody. For this application spiking experiments showed that virtually all present cells could be detected, the sensitivity of the system was found to be only limited by the absolute number of cells present [9]. As it is known that false positive reactions do occur, particularly in enzymatic detection systems [10] but also in fluorescence, the correlation of the phenotype with a genetic neuroblastoma marker (deletion of 1p36 or amplification of N-myc detected with a FISH probe) hybridized in a sequential procedure provided the high specificity that is essential in the assessment of minimal residual disease.

The possibility of sequential assays may also provide more precise information on the proliferative potential of isolated tumor cells. Recent studies showed that a significant percentage of isolated tumor cells in patients with advanced breast cancer that were detected using antibodies against cytokeratin and epithelial-type mucin were apoptotic [11]. This should have implications in the diagnostic interpretation of the occurrence of these cells.

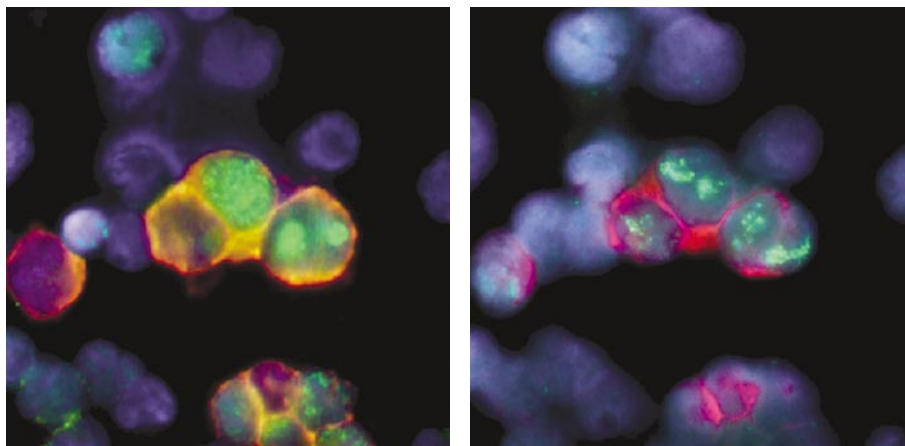


Figure 3. Disseminated tumor cells in bone marrow. Left image shows detected neuroblastoma cells with GD2 antibody label (red) and simultaneous Ki67 antibody label (green). Right image shows result of a sequential FISH assay of the same cells with NMyC amplification (green signal, some residual GD2 antibody label is still visible).

Interphase FISH spot counting

Fig. 4 illustrates a result of an automatic spot counting analysis of sperm cells. Sperm cells hybridized with centromeric probes (*AneuploYsion kit* from *Ysion*) for the X- (SpectrumGreen) and Y-chromosomes (SpectrumOrange) as well as for chromosome 18 (SpectrumAqua) were scanned using a 20x objective lens. DAPI was used as nuclear counterstain. During the scan cells without a chromosome 18 signal were considered hybridization failures and were consequently rejected. The time to score 2006 hybridized sperm cells was approx. 45 minutes (scanning of 1347 cells on the same slide took only 15 minutes, which was due to the fact that the density of hybridized cells varied significantly across the slide so that 2006 cells were found in 123 fields while the 1347 were detected in only 34 fields). Of these 2006 cells, 99.2 % showed one chromosome 18 signal. The 0.8 % with two chromosome 18 signals were due to superimposed cells and were rejected after verification in the gallery. There were no cells detected having more than two chromosome 18 signals. The remaining 1990 sperm cells with monosomy 18 showed the following initial results: 947 cells (47.6 %) had one X and no Y signal, 914 cells (45.9 %) showed no X and one Y signal, 110 cells (5.5 %) had neither an X nor a Y signal (due to poor hybridization efficiency), 15 cells (0.8 %) exhibited one X and one Y signal, and 4 cells (0.2 %) had two Y signals. There was no cell with two X signals.

In a second interactive step, cells without X and Y signals were checked in the gallery, and their spot counts were corrected. This was facilitated by thresholding functions allowing to increase the display contrast to enhance even faint signals. After this procedure which took approximately 15 minutes and left only

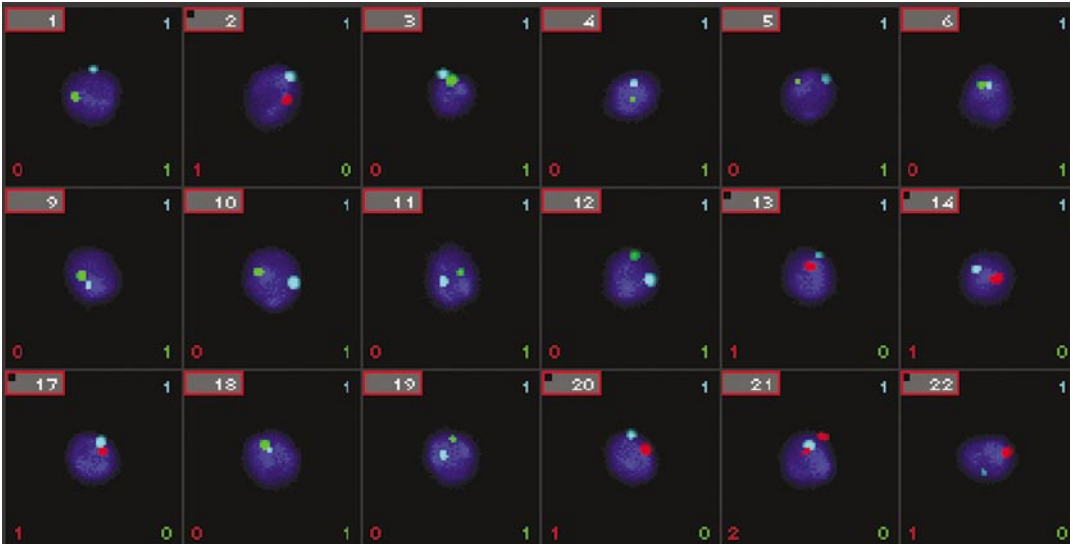


Figure 4. Spot counting in sperm cells hybridized with AneuVysion kit (Vysis)
 Each gallery image shows the cell number (upper left corner), and the automatic spot count for chromosomes Y (red, lower left corner), X (green, lower right corner), and 18 (cyan, upper right corner).

15 cells without any sex chromosome at all, the results were as follows: 987 cells (49.6 %) had one X and no Y signal, 966 cells (48.5 %) showed no X and one Y signal, 15 cells (0.8 %) had neither an X nor a Y signal, 15 cells (0.8 %) exhibited one X and one Y signal, and 6 cells (0.3 %) had no X but two Y signals. There was one cell (0.05 %) with two X signals and no Y.

Interphase translocation analysis

In *Fig. 5* the result of a *bcr/abl* translocation scanning analysis is shown. In this example the majority of cells exhibits the translocation as can be seen from the histogram showing the distribution of minimum distances. In the gallery images the green spot count (lower left), the red spot count (lower right corner) and the minimum 3-dimensional distance between a green and red signal is displayed for each gallery image. Normal cells (no. 10 and no. 12) show 2 green and 2 red signals with a relatively large minimum distance between any green and red signal. Cells with the Philadelphia chromosome are characterized by a small minimum distance between a green and a red signal. In addition, the probe kit used (*Vysis*) shows an extra green signal if the *bcr/abl* translocation is present. This is due to the minor breakpoint involved in this particular case which is located within the sequence spanned by the green signal. The additional information increases the specificity as it allows to discriminate between a signal fusion and an accidental co-localisation of two chromosomes 9 and 22.

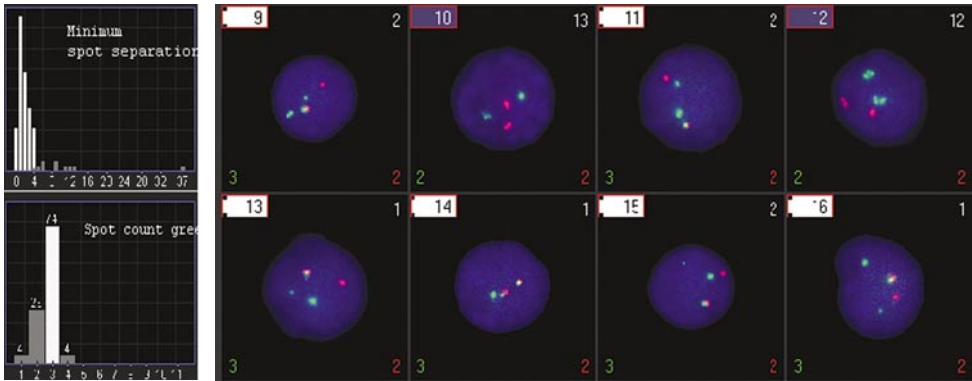


Figure 5. Example of translocation analysis by detecting co-localization of *bcr* and *abl* probes (Vysis)

Gallery images show cell number (upper left corner), green signal count (lower left corner), red signal count (lower right corner), and the smallest distance between a green and a red signal (upper right corner). Cells with a minimum distance between a green and a red signal of 4 pixels and less correspond to aberrant cells having the *bcr/abl* translocation and are highlighted. Cells no 10 and 12 having large minimum distances between a green and a red signal are normal cells. Normal cells have a green count of two whereas aberrant cells show three green signals. Left-hand side shows histograms of minimum spot distance red-green (top) and histogram of spot count of green signal (bottom).

CONCLUSION

In conclusion, it has been shown that the presented slide scanning system Metafer in its RCDetect mode is capable of fast detection of specifically labeled rare cells, such as fetal cells in maternal blood or isolated tumor cells in bone marrow. The scanning speed of up to several thousand cells per second for counterstained cells having an additional fluorescent label is comparable to flow cytometry techniques and is high enough to make enrichment methods unnecessary thus avoiding their inherent risk of losing cells of interest.

The MetaCyte operation mode, on the other hand, provides high throughput interphase FISH scoring, currently in up to 6 color channels. However, due to the higher optical magnification required, the much lower cell concentration necessary to avoid too many cell clusters, and to the necessity of capturing and analyzing several images at different focal planes in each color channel, the maximum scanning speed is at least 1000 times lower than in RCDetect. Spot counting as the initial screening method for the detection of very rare cells by their genetic properties is therefore not practical given the huge number of cells that need to be evaluated.

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REFERENCES

1. Bianchi DW, Williams JM, Sullivan LM, Hanson FW, Klinger KW, Shuber AP: PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. *Am J Hum Genet* 1997; 61:822–829
2. Holzgreve W, Di Naro E, Garvin AM, Troeger C, Hahn S: Prenatal diagnosis using fetal cells enriched from maternal blood. *Croatian Med J* 1998; 39:115–120
3. Bajaj S, Welsh JB, Leif RC, Price JH: Ultra-rare-event detection performance of a custom scanning cytometer on a model preparation of fetal nRBCs. *Cytometry* 2000; 39:285–294
4. Oosterwijk JC, Knepple CFHM, Mesker WE, Vrolijk H, Sloos WCR, Pattenier H, Ravkin I, van Ommen G-JB, Kanhai HHH, Tanke JH: Strategies for rare-event detection: An approach for automated fetal cell detection in maternal blood. *Am J Hum Genet* 1998; 63: 1783–1792
5. Kozubek M, Kozubek S, Lukasová E, Marecková A, Bartová E, Skalníková M, Jergová A: High-resolution cytometry of FISH dots in interphase cell nuclei. *Cytometry* 1999; 36:279–293
6. Kamensky LA, Kamensky LD, Fletcher JA, Kurose A, Sasaki K: Methods for automatic multiparameter analysis of fluorescence *in situ* hybridized specimens with a laser scanning cytometer. *Cytometry* 1997; 27:117–125
7. Larsen et al.: in preparation
8. Christensen B, Philip J: unpublished data
9. Mehes G, Luegmayr A, Hattinger C, Loerch T, Ambros I, Gadner H, Ambros PF: Automatic detection and genetic profiling of disseminated neuroblastoma cells. *Med Pediatr Oncol* 2001; 36:205–209
10. Borgen E, Beiske K, Trachsel S, et al: Immunocytochemical detection of isolated epithelial cells in bone marrow: non-specific staining and contribution by plasma cells directly reactive to alkaline phosphatase. *J Pathol* 1998; 185:427–434
11. Mehes G, Witt A, Kubista E, Ambros PF: Circulating breast cancer cells are frequently apoptotic. *Am J Pathol* 2001; 159:17–20

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