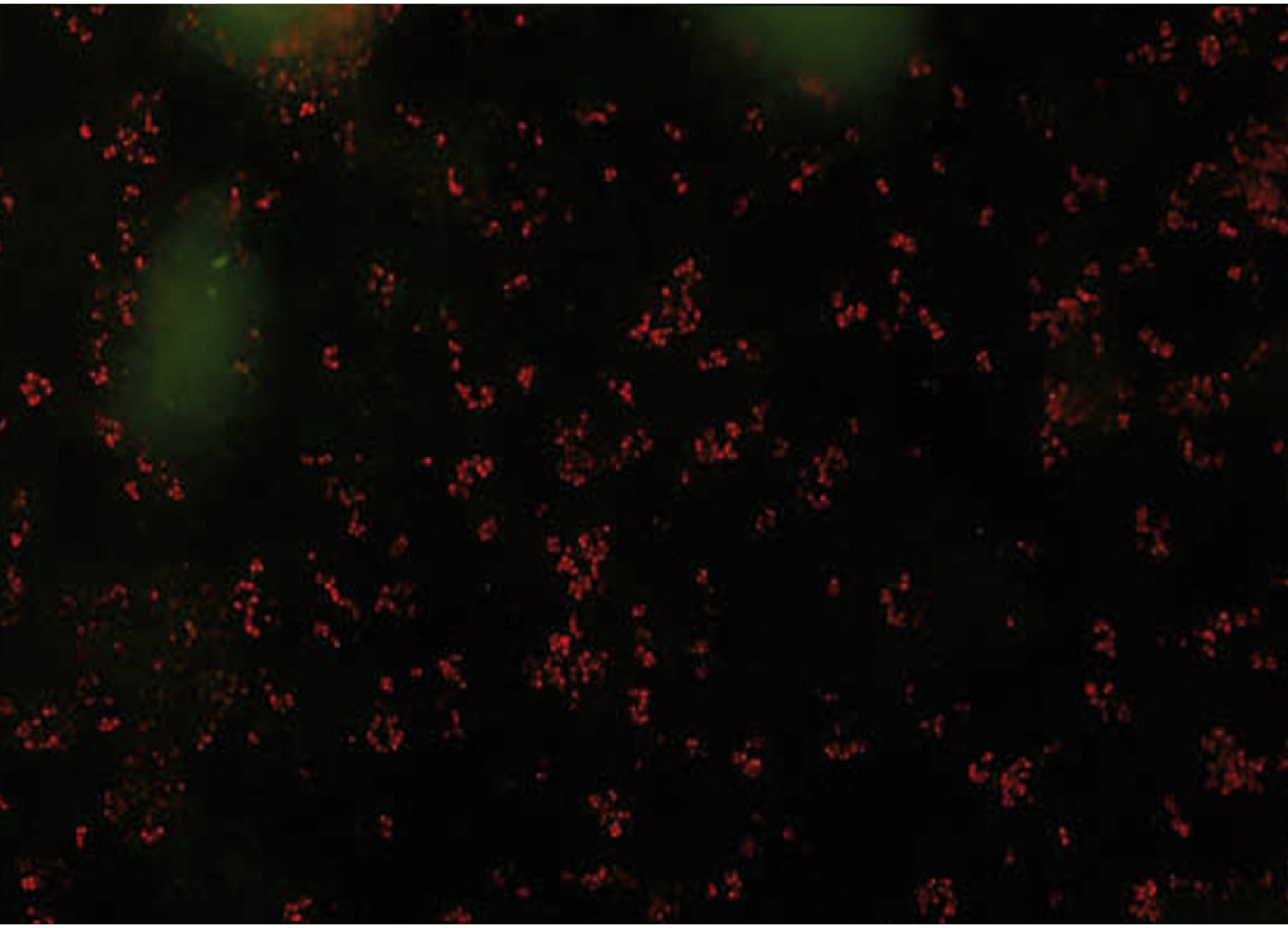

Light Microscopy in Clinical Routine Activities

An Overview of ZEISS Axio Imager and Metafer from MetaSystems

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Introduction

Several cell types, such as white blood cells, can also divide in adult human bodies in locations where tissue regularly needs to be regenerated. The advantage of this for researchers and physicians is that, with suitable preparation, appropriate staining, and a high-quality lens, it is possible to make the chromosomes of human cells visible under a light microscope during metaphase (a stage of cell division).

For many years, this light microscope application has made the interpretation of morphological chromosome changes caused by environmental influences or diseases possible. Finding and interpreting the chromosomes with the help of manual microscopy techniques is an extremely time-consuming and often error-prone task, however. With its metaphase finder – a device that uses a motorized microscope to determine the location of dividing cells in a specimen and that has been well established in the field for three decades – MetaSystems has developed a comprehensive, automated solution for large laboratories. Today, medical facilities and research institutes all over the world use these state-of-the-art image-processing systems. As a result, many other areas of application have been discovered in addition to the applications in clinical cytogenetics, such as in toxicology, pathology, microbiology, and a variety of other fields.

The Metafer Metaphase Finder

In 1986, MetaSystems developed a device named Metafer, which made it possible to automatically detect metaphases in microscopic specimens. In its current form, this metaphase finder is based on the fully motorized ZEISS Axio Imager.Z2 light microscope. It is equipped with a motorized stage, a high-resolution CCD camera, and a state-of-the-art automatic feeder with a capacity of up to 800 slides (SlideFeeder ×80).

The device was originally developed for biological dosimetry. This technique makes it possible to retrospectively determine

Figure 1 *Metafer from MetaSystems is a versatile, automated microscopic image-capture and image-analysis platform. Today's Metafer systems are based on continuous development and diversification that has been carried out over a period of more than 30 years.*

Figure 2 *Dicentric chromosomes (chromosomes with two centromeres; see the green circle) form as a result of DNA breaks caused by exposure to radiation and act as markers for biological dosimetry. The early Metafer system was already equipped with software to automatically detect these chromosome mutations.*

from a blood sample the level of radiation a person has been exposed to, for example as a result of an accident. In this context, chromosome changes are quantified as markers for the level of the dose received. For this method to be effective, it is important to analyze as many cells as possible, which is why a reliable metaphase finder represents a revolutionary leap forward for the user.

It quickly became apparent that laboratories active in the field of clinical cytogenetics could also benefit from such a device. These types of laboratories, which analyze both the chromosomes of unborn children as well as those of cancer patients, create a certain number of karyograms from each patient. The goal is to detect changes to the number of chromosomes – for example, in the case of trisomy 21 (also known as Down Syndrome, in which patients have three copies of chromosome 21) or chromosome mutations. For example, patients with certain types of leukemia exhibit very specific chromosome changes, which can be analyzed using the chromosomes' banding patterns visible in these karyograms. A metaphase finder, in conjunction with the ability to capture high-magnification images and analyze them on the Ikaros karyotyping system's screen, helped many laboratories significantly increase the number and quality of the cases they processed.

Users across the globe benefit from the combination of the high-grade ZEISS Axio Imager microscope with a platform that not only finds metaphases and captures them in high quality but also manages the images and all associated data and transmits it to any number of location-independent evaluation stations. This results in multiuser installations that seamlessly integrate into the workflows of laboratory facilities and hospitals.

Only a few years after the birth of the Metafer metaphase finder, it became apparent that this one-of-a-kind combination of microscopy and automation had the potential to become a platform for numerous applications – a revelation primarily brought on by a technology that was going to bring color to the applications.

Colors: Automated Signal Analysis

The term fluorescence in situ hybridization (FISH) refers to a technique that, through the use of fluorescently labeled segments of DNA, makes it possible to make specifically defined sequences of a cell's genome visible in color under a fluorescence microscope. One way to accomplish this is through *whole chromosome painting*, by which entire chromosomes are stained. In the event of a chromosome mutation such as a translocation, in which entire sections of a chromosome are replaced with those of another, this can be easily analyzed when both of the affected chromosomes have been tagged using FISH. The usefulness of this application relatively quickly led to the development of MetaSystems' fluorescence imaging system Isis.

Metafer also had the ability to automatically capture color FISH images in addition to monochrome images. Nevertheless, the analysis of the metaphase images was initially still left to the user. This changed after MetaSystems took over the analysis and later also the generation of what are called locus-specific probes. Locus-specific FISH probes label one or several specific portions of the genome, whereby these sections are usually those affected by chromosome mutations in the case of well-known syndromes (such as certain types of leukemia, for example). This results in the formation of dot-shaped signals, which are even recognizable in interphase cells – that is, cells that are not currently in the process of dividing.

The result is signal patterns that immediately reveal whether a chromosome mutation has occurred at the location in

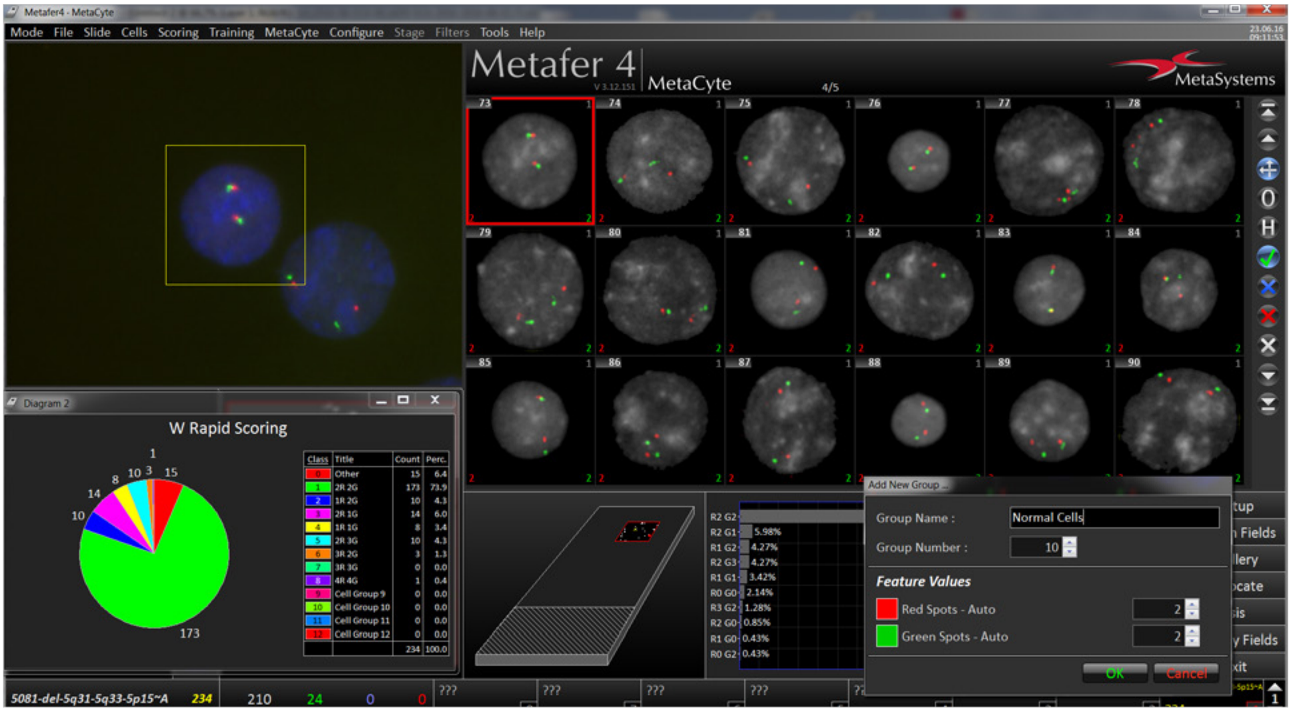

Figure 3 *The modern Metafer program interface in RapidScore mode. Fluorescence signals that represent specific loci on the chromosomes are automatically analyzed. The results provide insight into certain chromosomal changes, such as those that occur in the event of leukemia.*

question. As such, a simple count of the patterns is enough to provide a complete assessment of the cell's condition.
In the year 2000, Metafer was upgraded to include the automatic analysis of these signals in conjunction with the ability to capture images at several focus levels with the help of the microscope's motorized focus adjustment. As a result of this step, the original Metafer metaphase finder evolved into a versatile platform for automating the capture and analysis of microscopic images.

Diversification: From Metaphase Finder to Cross-Application Platform

Since MetaSystems' founding in 1986, the number of Metafer users around the globe has been increasing exponentially. In this context, the company always took great pains to remain in close contact with all of its users – a strategy that led to a constant influx of new ideas and feature requests. The combination of continuous communication with users, an extremely flexible system architecture, and the unconditional will to innovate led to an unprecedented diversification of the system in the following years.

The company recognized relatively early on that users from the field of biological dosimetry also used other protocols, the automation of which could also be automated. This includes applications like the micronucleus test, in which the fragmentation rate of DNA after exposure to radiation is quantified on the basis of micronuclei. The comet assay is a test that measures the fragmentation of DNA at the level of the individual cell. With the γH2AX assay, it's possible to make clusters of repair proteins, and thus double-strand DNA breaks, visible directly under a microscope. It was quickly revealed that the laboratories that also dealt with toxicology used these and other tests, which is why today Metafer is also used in the pharmaceuticals industry and water sector. In this context, radiation biologists such as toxicologists are appreciative of the fact that the variable and error-prone manual analysis can be completely standardized and as result also evaluable, without changing any part of the test itself.

Every search with Metafer creates images and a list of positions on the microscope slide. This is carried out with precision in the micrometer range. From there, it's fairly easy to create composite images. As such, at the beginning of its third decade in use, Metafer was upgraded to include the ability to digitize entire specimens. Here, it proved particularly valuable that a full research microscope was integrated

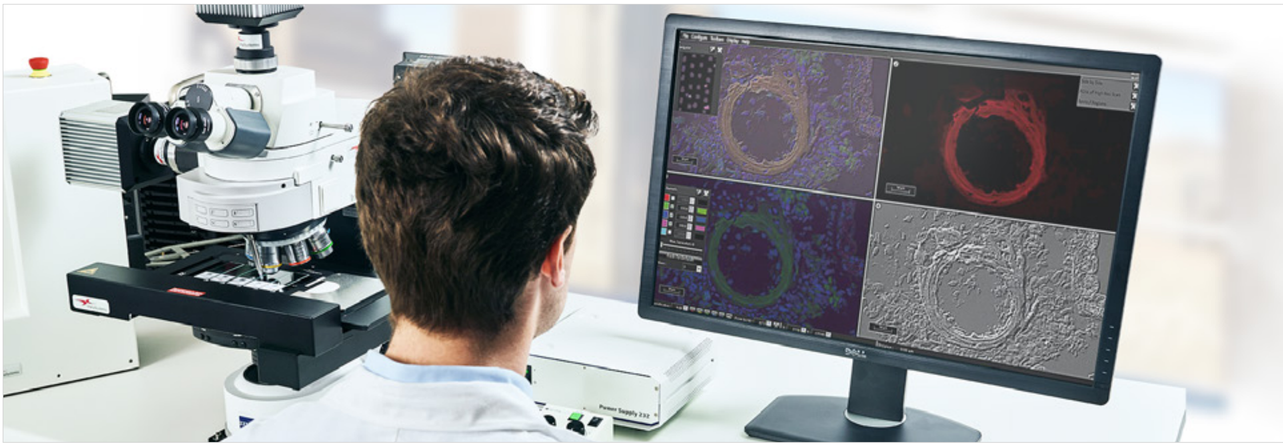

Figure 4 *Metafer uses the different possibilities offered by the microscope (such as phase contrast microscopy and fluorescence) to generate highly complex and versatile image data.*

into the device. In contrast to other devices, there was basically no limit to the magnification levels, contrast enhancement methods, and specimen sizes that could be used when creating large, high-resolution images. As a result, it's easy to combine to and save phase contrast images, fluorescence channels, and color transmitted-light images.

The rapid developments in computer technology, cameras, and also Metafer itself today open up many new areas of application. The most interesting of these is certainly microbiology. MetaSystems got its start in the field of microbiology by configuring Metafer as a digitization system for Gram slides. With the help of Gram staining – a widespread, classic method used in microbiology – bacteria in patient samples are classified in order to make more targeted treatment possible – for example, with antibiotics. With Metafer, these specimens can be completely digitized and evaluated on screen. An additional benefit is the ability to archive the images, which makes it possible to get a second opinion.

In recent years, MetaSystems range of products in the field of microbiology has grown significantly and is marketed under the MetaSystems Indigo brand. Today, Metafer can be used for the automated detection of tuberculosis pathogens and malaria parasites. The most promising new application is the rapid identification of bacteria using a multiplex FISH assay. Depending on the application, this technique can be used to detect and quantify over 90% of the clinically relevant pathogens on the basis of specific FISH signals. Hybridization is a special technique that makes an analysis with Metafer possible after only 30 minutes. As a result, *direct multiplex imaging* (DMI) technology is one of the fastest methods that can be used in day-to-day clinical operations to assist in the treatment of patients, provide targeted antibiotics management, and reduce wait times.

Summary

Beginning with a metaphase finder for biological dosimetry, MetaSystems has now developed a wide range of fully automated image analysis systems for clinical and research-related applications. The history of Metafer, one of the fastest and most versatile microscopic analysis platforms, mirrors both technological progress and the trend toward increasingly automated, location-independent, and connected workstations in research and clinical settings.

Figure 5 *MetaSystems discovered many possible areas of application in the field of microbiology. The automatic identification of pathogens based on a multiplex FISH assay with subsequent analysis by Metafer (using Direct Multiplex Imaging, or DMI for short) is only one of many applications.*

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