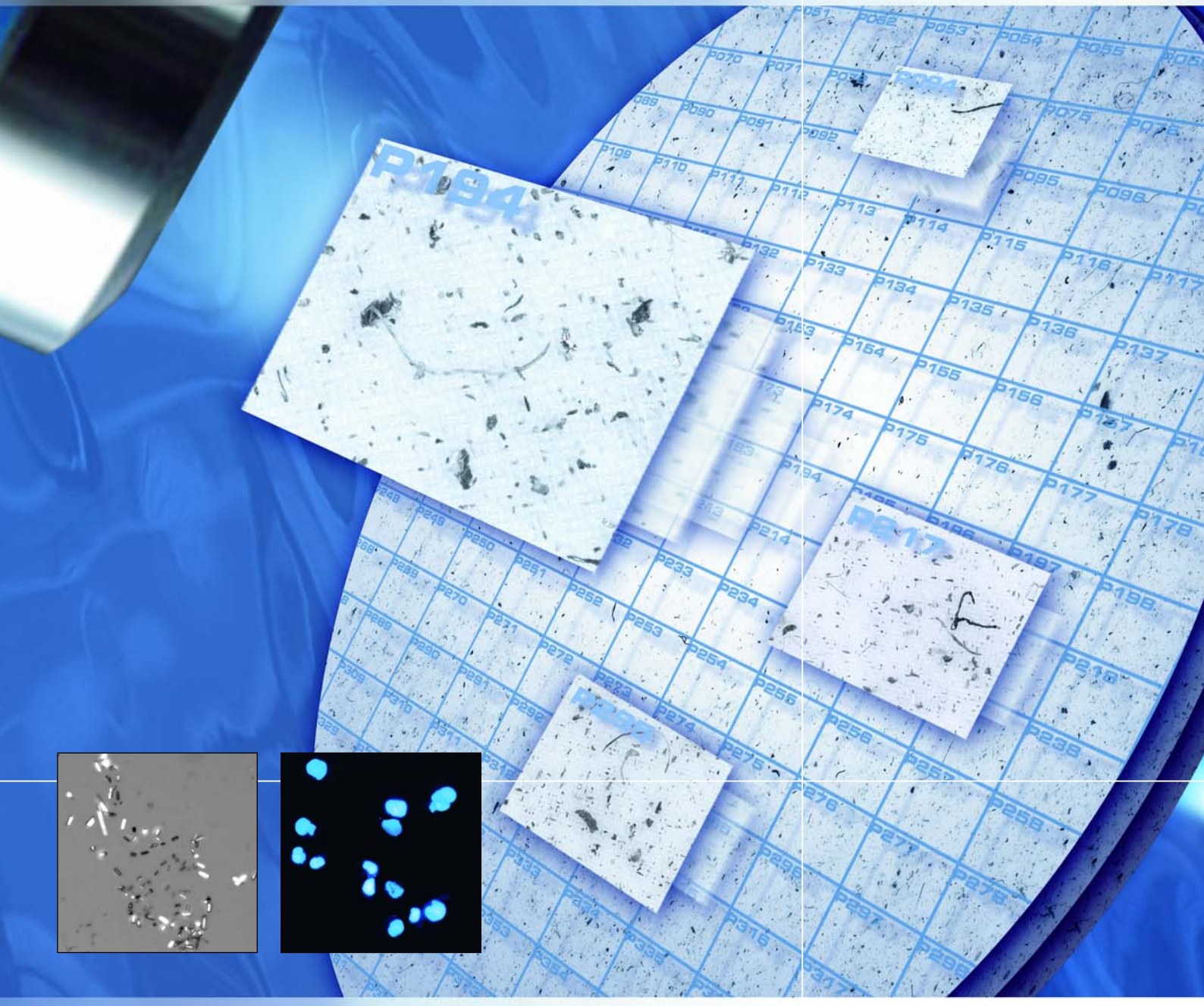
Particle Analyzer

First Class for all Particle Size Categories



The Fully Automated Complete System for Measuring and Documenting Particles of All Size Categories in Accordance with Standards

ZEISS

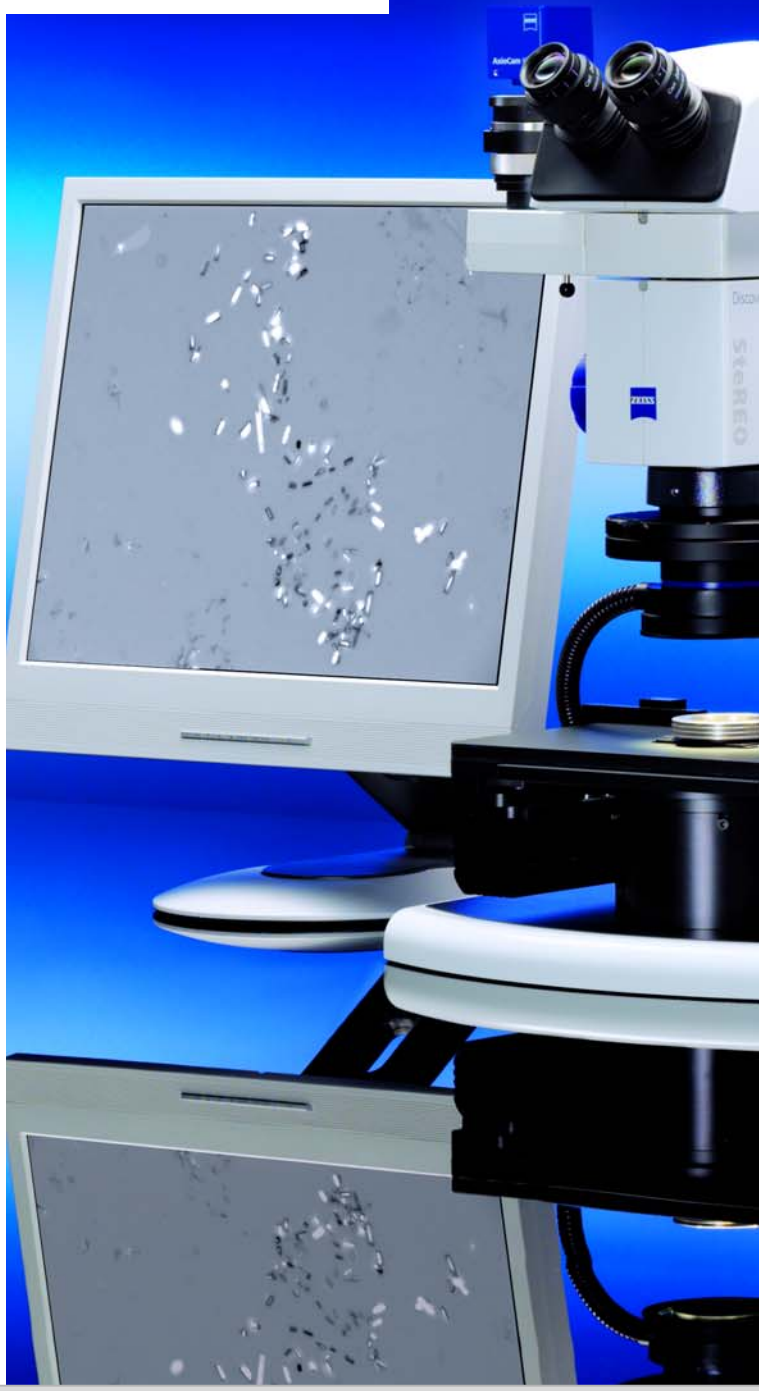
We make it visible.

Every Technology has Its Limits – in Particle Analysis, We have Redefined Them Once Again

The trend in industry is for continuing miniaturization and ever greater packing densities. This, combined with increasingly high quality requirements, makes it inevitable that the problems faced will also increase – for example, extremely small particles that reduce the quality of pharmaceutical products or impair the functionality of technical components. Many sectors, from the pharmaceuticals industry through to the automotive supply sector, are today obliged to safeguard and document the fact that their products are clean on the basis of guidelines such as the FDA 21 CFR part 11 regulation for GxP*-compatible validation, or guidelines and standards such as ISO 16232. They must also be able to provide evidence of this. The requirements range from the evaluation of complete round filters for particle analysis through to the key data for electronic data management. Requirements that, until now, could only be partially met in practice.

A solution is needed that goes beyond the limits of established systems – in terms of the analysis of even very large samples, the resolution of images and the operating concept. A solution that is simple, self-explanatory and easily configurable for a variety of applications – as well as being flexible enough to adjust to future requirements without difficulty. A solution that will bring a new level of quality to industrial testing practice - Particle Analyzer from Carl Zeiss.

*Option not available in all countries



The system variants of the Particle Analyzer: based on SteREO Discovery and Axio Imager

Particle Analyzer

A System for Particle Testing in Industrial Environments Must Be One Thing Above All Else: Practical

Particle Analyzer is designed for use in practice and is oriented towards routine work in industry. Above all, it offers the analysis of complete mosaic images, can be adapted easily to comply with company-specific test specifications and can also be integrated into GxP applications. Other extremely practical features include the versatility and flexibility of the system and its full integration into the Carl Zeiss family of microscopes, plus simple handling... Features which are unique. And, quite simply, well thought through.

No more limits: analysis of large mosaic images

Until now, on account of the fact that the high image resolution demanded for the acquisition of even the smallest particles in accordance with standards leads to data volumes that exceed the capacities of user memories, it has been necessary to measure small and large particles separately. It is now possible, however, to measure specimens in one step. The technical basis for this is an innovative analysis technique from Carl Zeiss – with convincing results. Particle Analyzer makes it possible to analyze and document even a complete mosaic image in its overall context without difficulty. This means that a system is available to industry which allows precise measurements of particles of all size categories to be performed in a reliable and reproducible way – in order to actually meet the requirements of industry standards.

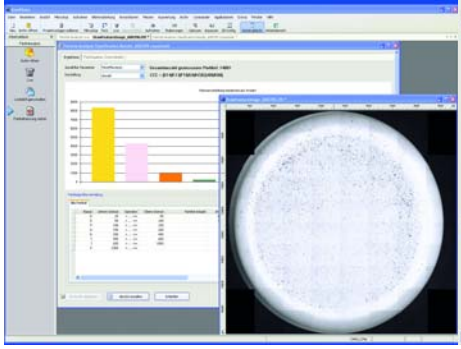
Automatic and decisively faster: particle classification

The reliable classification of particle types is a central issue during the analysis of residual dirt in particular. Metallic particles are especially critical and classification into metallic and non-metallic particles is, therefore, one of the most important problems faced. Before now, two different scanning procedures were required for this – with polarized light being used in the first step to identify the particles and bright field being used in the second step to determine the degree of reflection. All in all, an extremely time-consuming process. With Particle Analyzer, classification can be performed with just one image acquisition procedure using a specially integrated segmentation function. A procedure that is automatic, secure and reliable. This means that, using Particle Analyzer, the definition of the classification can be checked by the operator at any time and adjusted flexibly to specific requirements. All available measurement parameters can be used to describe a type of particle and combined with one another as desired.

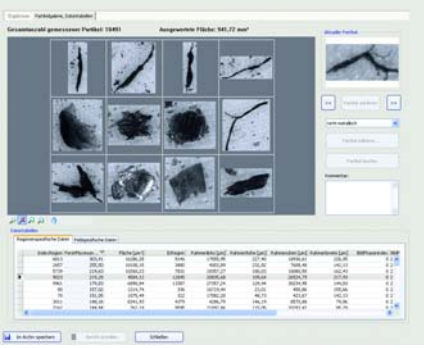
Simple, reliable and automatic: operation and configuration

Operating reliability is an essential requirement for industry, and one that is met 100% by Particle Analyzer. The fully motorized microscope platform ensures that all settings are selected correctly – from the camera's exposure time to the objectives, contrast procedure and illumination settings – and in a reliable and reproducible way. The work steps, which are integrated into the intelligent architecture of AxioVision, can be executed with just a few clicks of the mouse: from entering project data through to generating reports and archiving. The system is also extraordinarily easy to configure – an important advantage if the system needs to be adjusted, quickly and without any external assistance or additional programming, by the authorized system administrator to comply with new test specifications or standards.

1. *User interface with overview image of a filter and classification results*
2. *Gallery of the largest particles for individual assessment*



1.



2.

Protection of your investment ensured: the flexibility of the system

Analyzing inclusions in steel, measuring lengths of components, simply acquiring an image in order to document events of damage – the complete system from Carl Zeiss offers you the freedom for flexible use far beyond the analysis of particles, even in the standard version. This applies, for example, to the integration of additional methods of analysis, as well as to the retrofit of polarization and other contrast techniques to the microscope. It also applies to new developments in the future – whether these are new AxioVision modules or microscopy techniques. You can therefore be confident that, with Particle Analyzer, you will have a technologically current analysis tool even for the long term.

Standard-compliant, certified: the integrated guidelines

Particle Analyzer supports all important national and international norms and standards, including ISO 4406, ISO 16232, VDA Band 19 and others. The corresponding classes and class boundaries are set automatically and the appropriate measurement parameters are selected. Within GxP projects Carl Zeiss provides the required software and documentation. One crucial advantage lies in the possibility of editing the Carl Zeiss analysis system software. New standards can be added easily to the existing system – without having to wait for new versions of software. Furthermore, on request, the system can be delivered with a calibration certificate. Optional certifiable particle normals are also available for system inspection – these can be used for transmitted light and reflected light.

Complete for particles of all size categories: the system variants

Particle Analyzer is the complete solution for reliably quantifying and assessing particles. The microscopes:

1. Axio Imager for particle sizes upwards of 2.5 µm
2. SteREO Discovery for particles upwards of 25 µm

Fully equipped with a motorized stage, bright field, dark field and polarization*, PC, TFT monitor as well as all the relevant AxioVision components.

Whether you are working in traditional or stereo microscopy, since they were introduced, the two youngest generations of microscopes from Carl Zeiss have been setting standards in the areas of optical quality as well as motorized precision and, therefore, with their high reproduction accuracy. With the SteREO Discovery.V12, motorized magnification setting can be performed with a reproduction accuracy of more than 99%. Furthermore, Particle Analyzer is naturally also available to you as a retrofit for your existing Carl Zeiss work environment.

*Dark field and polarization optional with SteREO Discovery

Versatility is the Key

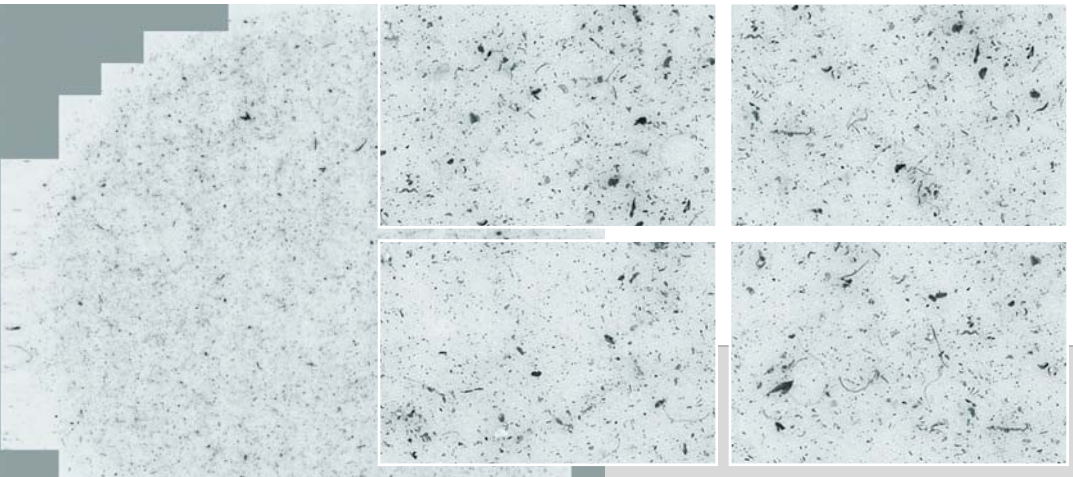
There is more to Particle Analyzer than you could previously expect from conventional particle analysis technologies. The system can be used by various users for a wide variety of tasks relating to materials testing and quality assurance. We have given just four examples here, but the range of possible uses for the system is virtually unlimited.

Residual dirt determination on filter membranes

Analyzing particles on round filters is one of the standard procedures in quality assurance. Following the production process, random samples of components are rinsed with a defined amount of fluid, which is then pressed or sucked through a filter membrane. Any particles of dirt that may be present are caught by the filter and can then by analyzed under the microscope. This procedure is also used at wind energy plants, for example, for carrying out checks during regular service intervals. Here, lubricants are analyzed for dirt particles. In all areas of use, large particles up to the mm range are particularly relevant. These can only be reliably captured through the acquisition of mosaic images. Particle Analyzer has made it possible, for the first time, to acquire an image of an entire round filter completely and carry out the associated evaluation without the need for multiple measurements. The result is a reliable analysis of all the defined size categories.

Defects in diecast components

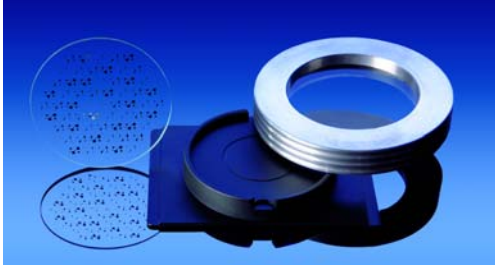
The mechanical properties of a material are negatively influenced (increased internal stress concentration) by manufacturing-related casting defects such as pores, blowholes or hollows. With aluminum or magnesium casting materials in particular, which are becoming increasingly relevant, contraction-related blowholes and gas pockets are virtually impossible to avoid. Today, industry is particularly interested in automatically determining the proportion of the area made up of casting defects that present a risk of fracture (especially around the material surface) as well as in classifying the sizes of the defects. The main focus is on large defects which could previously only be analyzed inadequately using single image measurements. The functionality of Particle Analyzer for analyzing mosaic images means that reliable results can be obtained in this area for the first time.



Residual dirt determination

Residual dirt particles on filter membrane
Mosaic image consisting of over 200 single images
Microscope: Axio Imager
Objective: EC Epiplan Neofluar 5x/0.13
Camera: AxioCam MRc

Particle normal and filter mount



Active substances in crystalline form in ointments

Ointments such as fungicidal preparations for the skin and mucous membrane are tested for the distribution of the active substance, which is present in crystalline form. The samples come either from new products that are being developed or from finished tubes of ointment, the quality of which is being checked during production. Under the transmitted light microscope, the ointment is spread onto the slide and analyzed in polarization contrast (10x and 20x objective). The crystals of the active substance are clearly visible as dark structures and can be assessed using image analysis. In order to validate the system, an area normal (circle with area of 100 µm²) is measured each month and the measured value compared with the reference value. With Particle Analyzer it is possible, for the first time, to detect and measure both large crystal agglomerates and extremely small particles in just one step and compare the particle distribution with the distribution stipulated in the Standard Operating Process (SOP).

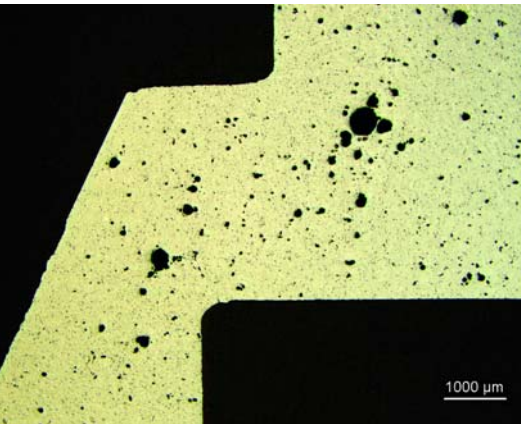
Plaques in microtiter plates

The manufacture of vaccines and serums requires extensive testing to determine efficacy and dosage. For this purpose, the active substance is brought into contact with the pathogen – in various concentrations – in 24-well microtiter plates as a dilution series. Cells are then added to the virus/antibody mixture and the plates are incubated. Once all the viral particles have been neutralized, a confluent cell lawn is formed. Viruses that have not been neutralized multiply in the cells that are added, and this leads to the formation of holes (plaques) in the cell lawn. Following immortalization of the cells/viruses using UV, the entire microtiter plate is scanned, quickly and with no time wasted, and immediately saved using Particle Analyzer.

After scanning, the images are loaded from the hard disk and the plaques are automatically detected, separated and measured. Artifacts which are below the minimum size entered are automatically excluded from the measurement. Measured values from each microtiter plate, as well as the input and output image, are saved for subsequent controls. As an option, it is also possible to ensure that the entire process can be validated in accordance with GxP using Particle Analyzer.

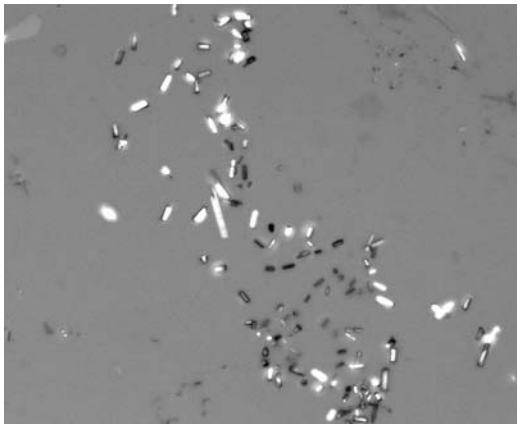
Diecast components

Structural defects in diecast components
Mosaic image of a total area of approx. 38 mm²
Microscope: Axio Imager
Objective: EC Epiplan Neofluar 5x/0.13
Camera: AxioCam MRc



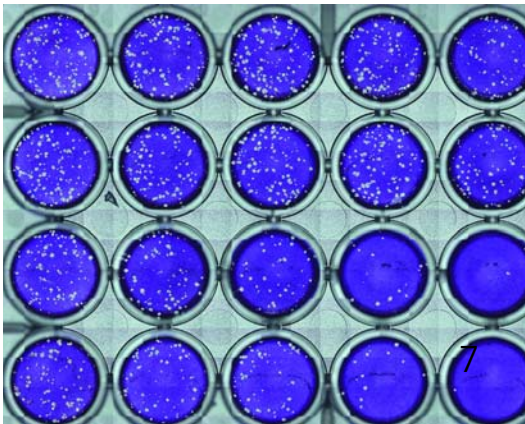
Ointment preparation

Active substance in crystalline form in an ointment sample
Microscope: Axio Imager
Objective: A-Plan 10x
Camera: AxioCam MRm



Microtiter plates

Plaques in microtiter plates
Microscope: SteREO Discovery.V12
Objective: Plan S 1.0x
Camera: AxioCam MRc

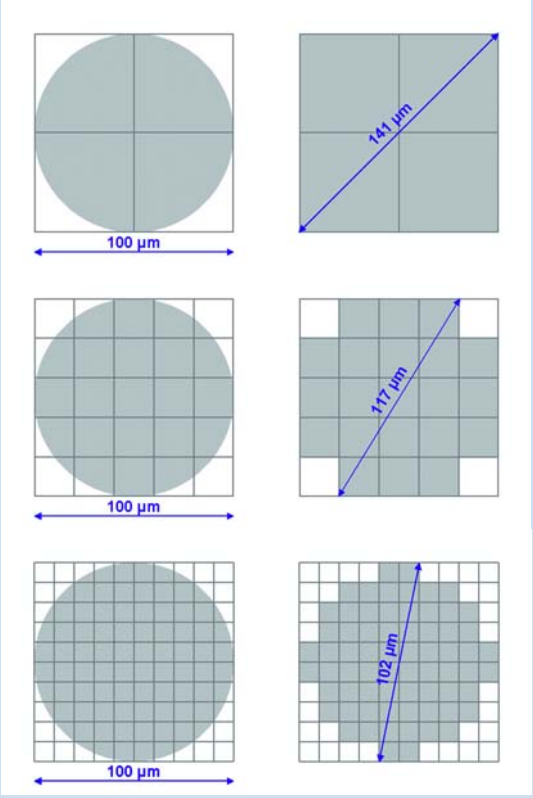


Particle Analyzer: the Technical Principle

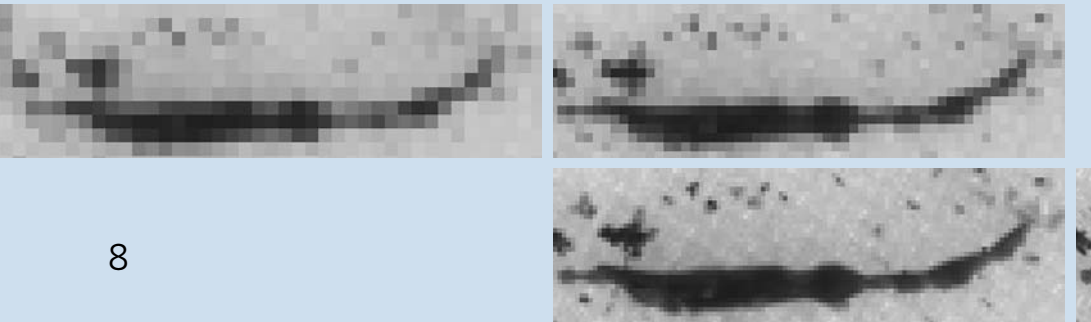
In order to evaluate samples using computers, the samples must first be digitized through a camera and loaded onto a PC. Two partly conflicting points should be taken into consideration here. The resolution is crucial for displaying the image details. The higher the resolution, the more precise the image will be. However, the limitations of the PC hardware stand in the way of this. Doubling the resolution quadruples the memory required, with correspondingly longer evaluation times. A sensible middle ground is therefore being sought between resolution of detail on the one hand and the available PC technology on the other.

Resolution: the theoretical basis

The theoretical basis for the minimum necessary resolution is provided by the Nyquist-Shannon sampling theorem. This theorem states, with regard to the analysis of images, that the smallest detail to be resolved must be sampled using at least two pixels. For the measurement of a circle this would mean that the circle is sampled with four pixels. The result in the image would therefore be a square! If the circle has a diameter of 100 µm, the measurement of the longest diameter would produce a value of 141 µm. This means that it would be measured with an error of more than 40%. Even by increasing the sampling to 5 pixels, at 117 µm, the result would be 17% above the correct value. Sampling using 5 pixels can be used perfectly well for estimating the number in less important size categories with many small particles. However, this should not be referred to as a measurement in this case. In some guidelines it is therefore requested, quite justifiably, that in order to produce a correct measurement, the smallest particle to be measured should not be sampled with 2 or 5 pixels but with 10. This results in a circle measurement of 102 µm or an acceptable error of 2%.



Influence of the resolution selected on the accuracy of the measurement
Left: Circle with diameter of 100 µm sampled with two, five and ten pixels
Right: Results when measuring the longest diameter



Improving the display of a particle by changing the resolution

Edge errors in the measurement of single images: particles at the edge of the image are cut off and cannot be measured. It is primarily large particles that are not acquired

The problem with single images

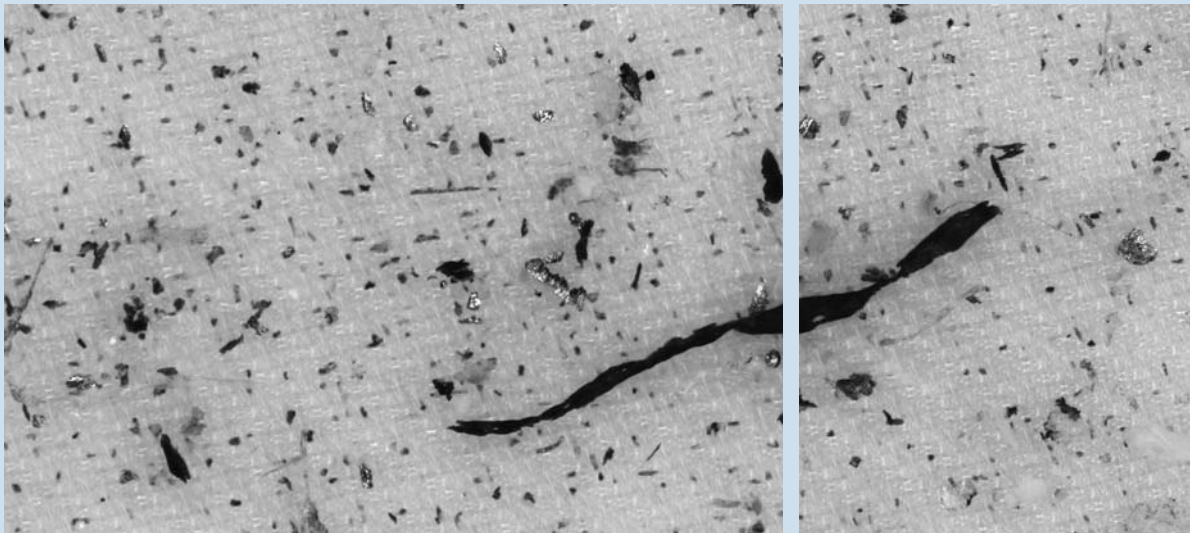
When acquiring images of small particles, with the appropriate resolution, it is inevitable that only a very small frame of the sample is captured by a camera image. In the past, in order to acquire images of large particles which did not fit into an image field, several single images were often taken. In this case it is difficult to handle the edges, as particles at the edge of the image are cut off. To avoid this problem, particles that crossed over fields were previously either not measured at all or the particles were located later and measured interactively. The third option was to measure the sample several times: once with low magnification so that the large particles could be captured, then with a high magnification for the small particles. All in all these are time-consuming and error-prone procedures.

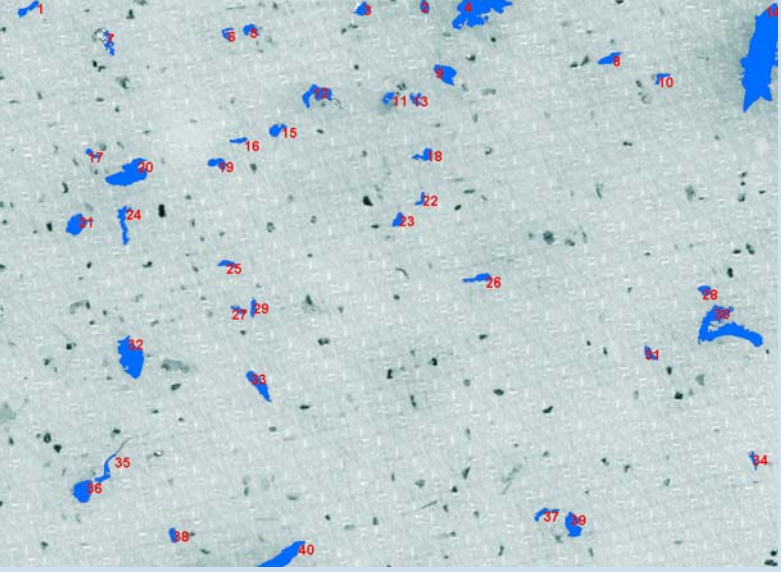
The problem with mosaic images

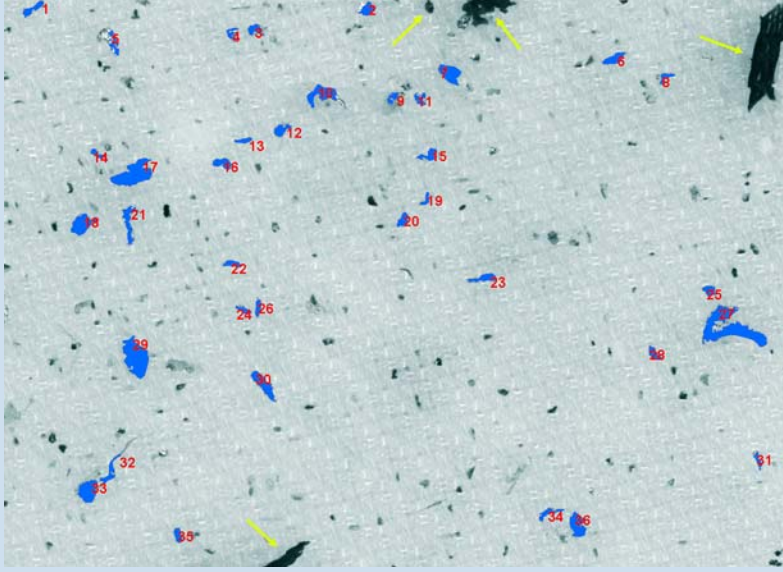
Today, motorized stages are used for large samples and single images are acquired which are then put together to form a large overall image (Mosaic). This generates huge data volumes, up to the GB range. Such volumes can still be loaded in a stable way for display provided that sufficient memory is available.

It is during measurement that problems occur. The processing and measurement functions can still only process one of the single images. This means that the security of genuinely having acquired images of and classified all parts of the particles correctly is lost – not only for particles which cross over fields, but also for small particles at the edges of the images. Handling the overall image as a single entity becomes impossible due to the limitations of the memory.

When mosaic images are acquired, large particles, which are cut off during the acquisition of single images, are captured in their entirety

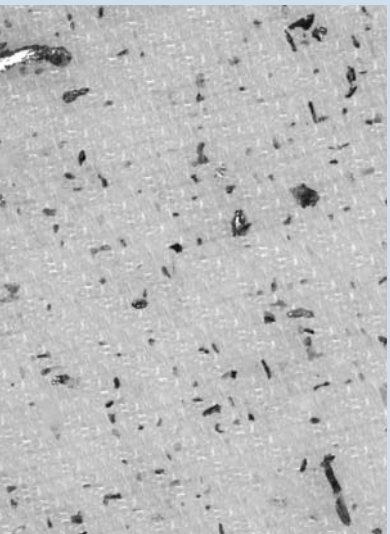






The solution

In principle, the solution for displaying large images irrespective of memory is simple. The overall image is not stored in the memory in its entirety, but in a resolution-reduced form. Only the relevant sub-regions of the image are loaded for display on the monitor, meaning that the memory is only ever burdened with the current frame. This is a well-known technique that has already proven itself for some time, e.g. on the Internet. What is new, however, is the expansion of this technique to the processing and measurement functions of Particle Analyzer. Using the innovative analysis system from Carl Zeiss, it is now possible to evaluate even large mosaic images in one step with full resolution. This means that you no longer have problems with varying particle sizes: dirt particles which cross over fields can be measured in their entirety – the particles are no longer cut off at the edge of the image. Consequently, for the first time, you now have the security of really being able to capture, display and measure all particles, totally regardless of their size – in just a single measurement process and irrespective of memory. For industry this means a completely new level of analysis quality and a considerable increase in efficiency for routine tasks..



Function-relevant particle size	Pixel scaling required	Image size in MB* Grayscale	Color	Evaluation time in min.
10 µm	1.0 µm/Pixel	1500	4500	approx. 20 min
25 µm	2.5 µm/Pixel	200	600	approx. 4 min
50 µm	5.0 µm/Pixel	50	150	approx. 2 min
100 µm	10.0 µm/Pixel	15	45	approx. 1 min

*Filter with diameter of 44 mm (area approx. 1,500 mm²)

The Camera Resolution

Resolution plays a central role in the acquisition and measurement of microscopic images. A distinction is made here between two meanings. The optical resolution describes the distinguishability of fine structures, that is to say, the smallest distance between two points that is still perceptible. In contrast, camera resolution – also known as image resolution – denotes the number of pixels from which a digital image is composed. The image resolution is of great significance when measuring digital images: the higher the image resolution, the more detailed the display and the more precise the measurement results.

The limitations of visibility

The problem is well-known: like the observer when he or she is looking through the eyepiece, a camera also only ever sees a part of the sample. In an ideal situation, however, the whole of the microscope's intermediate image should be imaged directly onto the chip of the camera. In the case of a light microscope, this intermediate image has a diameter of 18, 20, 23 or 25 mm. As a general rule, however, the sensors are a great deal smaller. Consequently, only a part of the image that is visible through the eyepiece is captured by the camera and displayed on the monitor. This means, for example, that with the 8 mm diagonal of a 1/2" CCD chip, approximately 1/8 of a visual field of 18 mm is acquired.

Loss-free acquisition

The resolution required to ensure loss-free acquisition, depends on the resolution of the objective and camera adapter used. Two pixels are required on the camera sensor to display each line pair imaged by the objective. If a camera adapter with image-narrowing optics is used, this must be included in the calculation. The calculation for the necessary resolution is based on the following formula:

$$\text{Resolution}_{\text{mikroscope}} = \frac{3000 \times \text{numerical aperture of objectives}}{\text{magnification}_{\text{objective}} \times \text{magnification}_{\text{camera adapter}}}$$

To ensure loss-free sampling of the image, the result is doubled and multiplied by the dimensions of the surface area of the sensor:

$$\text{Horizontal resolution}_{\text{camera}} = 2 \times \text{resolution}_{\text{microscope}} \times \text{sensor lenght [mm]}$$

$$\text{Vertical resolution}_{\text{camera}} = 2 \times \text{resolution}_{\text{microscope}} \times \text{sensor width [mm]}$$

SteREO Discovery

Zoom optics magnification factor	Numerical aperture	Resolution at object plane [µm]	Resolution in sensor plane [lp/mm]	Number of pixels horizontal (H)	Number of pixels vertical (V)
1.00	0.022	15.2	66	832	634
2.00	0.039	8.6	59	737	562
4.00	0.067	5.0	50	633	482
5.00	0.079	4.3	47	597	455
8.00	0.116	2.9	44	548	418
10.00	0.144	2.3	43	544	415

Objective: Plan S 1.0x

The tables show selected values for the Axio Imager light microscope and SteREO Discovery stereo microscope. The resolution at the object plane represents the smallest object structure that can be resolved by the objective in question.

Camera: AxioCam ICc1
Camera adapter: 1.0x

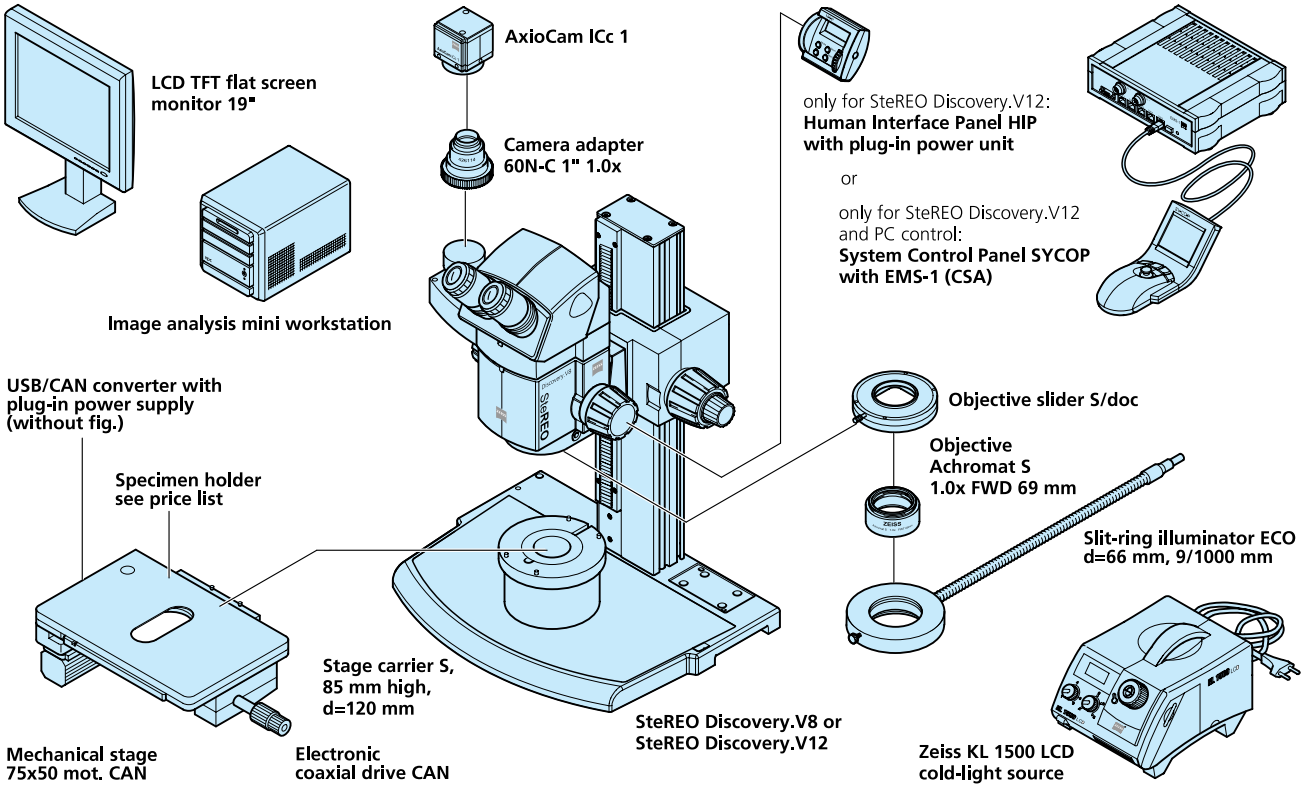
Axio Imager

Magnification of the objective	Numerical aperture	Resolution at object plane [µm]	Resolution in sensor plane [lp/mm]	Number of pixels horizontal (H)	Number of pixels vertical (V)
1.25	0.03	9.17	72	907	691
2.50	0.06	4.58	72	907	691
5.00	0.13	2.12	78	983	749
10.00	0.25	1.10	75	945	720

In the number of pixels (H) and number of pixels (V) columns, the number of camera pixels (horizontal and vertical) is stated that is required for correct imaging.

Resolution: 1392 (H) x 1040 (V) = 1.4 megapixels
Sensor size: 1/2" corresponds to 6.3 mm (H) x 4.8 mm (V)

Particle Analyzer: Configuration With SteREO Discovery



Microscope
SteREO Discovery V.12 with
"SyCoP" system control panel or
SteREO Discovery.V8

Illumination
ECO annular slit illuminator d = 66 mm,
9/1000 mm
Zeiss KL 1500 LCD cold light source

Objektiv
Achromat S 1.0x

Equipment
Motorized mechanical stage, range 75 x 50 mm
CAN electronic coaxial drive
S/doc objective slide

Camera
AxioCam ICc1 digital microscope camera

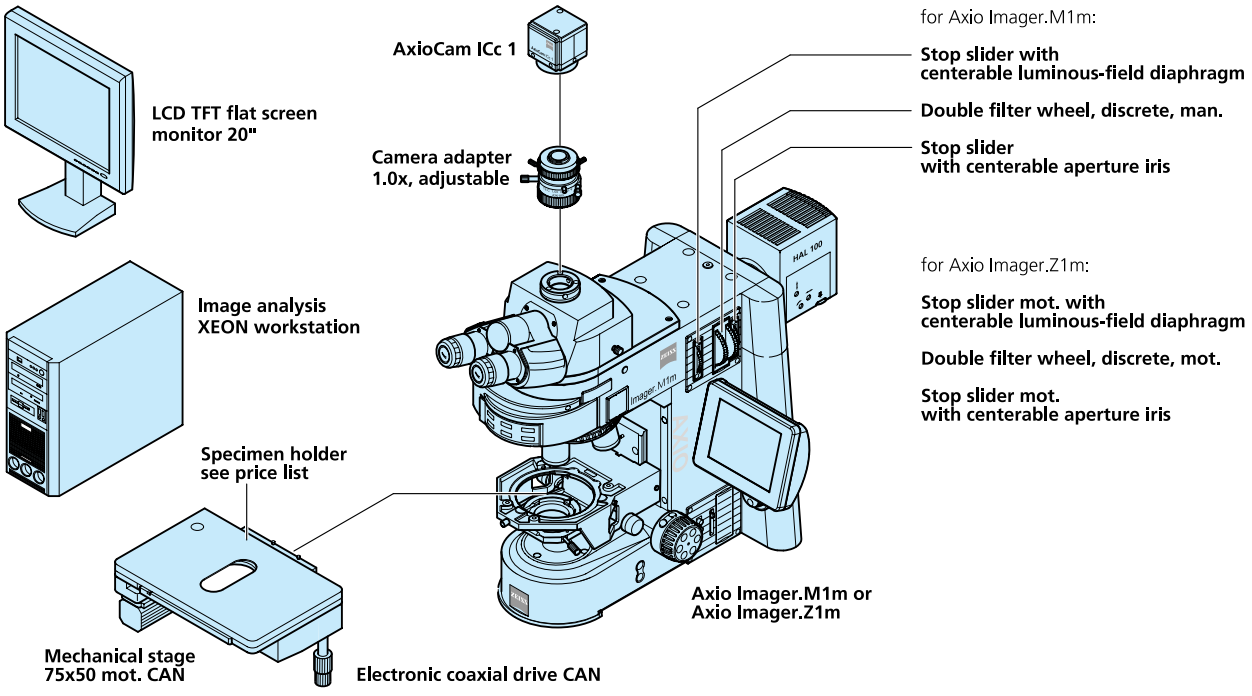
PC hardware
Mini image analysis workstation
2048 MB user memory
80 GB hard disk capacity
19" LCD TFT monitor

Software
Microsoft® Windows XP Professional SP2
AxioVision basic software
AxioVision MosaiX, Asset Archive and Particle Analyzer Projects modules

Additional modules
AxioVision AutoMeasure Plus, Commander and GxP modules

Please note: the example shows the typical configurations. They are expandable depending on applications. Individual components can be exchanged for special applications.

Particle Analyzer: Configuration With Axio Imager



Microscope
Axio Imager.M1m with TFT monitor or
Axio Imager.Z1m with TFT monitor

Contrast procedures
Reflected light: bright field, dark field,
DIC/polarization

Objektive
Epiplan 5x/0.13 HD und 10x/0.20 HD

Equipment
Motorized mechanical stage, range 75 x 50 mm
CAN electronic coaxial drive

Camera
AxioCam ICc1 digital microscope camera

PC hardware
XEON image analysis workstation with RAID 0 link
3072 MB user memory
480 GB hard disk capacity
20.1″ LCD TFT monitor

Software
Microsoft® Windows XP Professional SP2
AxioVision basic software
AxioVision MosaiX, Asset Archive and
Particle Analyzer Projects modules

Additional modules
AxioVision Module Autofokus, AutMess Plus,
Commander, GxP

Please note: the example shows the typical configurations. They are expandable depending on applications. Individual components can be exchanged for special applications.

Particle Analyzer has Many Strengths – Here are the Most Persuasive Ones

The system	Fully automatic complete system for particle analysis
The microscopes	SteREO Discovery • Optical design for brilliant images rich in contrast • Automatic scaling • Uniquely high level of reproduction accuracy • All acquisition parameters can be read out Axio Imager • Objective for outstanding contrast and resolution • Full motorization for 100% reproducible system settings • Intuitive operation with touch screen • Highly robust stand design
The innovation	The system for evaluating even large mosaic images
The advantage in practice	• Simple operation and simple configurations, without having to spend time on programming • Particle classification without having to acquire several images of the sample • Digital documentation of complete samples
The range of uses	Can be used in a variety of applications from documenting cases of damage through to materials analysis and biomedical applications
The software	AxioVision • Universal Imaging System • Modular concept • Simple operating philosophy
Precision reliability	Available with optional calibration certificate, supplied with particle normal and special mount for filters up to 50 mm
Results according to standards	Evaluation according to ISO 4406, ISO 16232, VDA 19 etc., or make the selection of your choice. Optional: software and documentation for GxP*-validated applications according to FDA 21 CFR part 11
Protecting your investment	Fully integrated into the Carl Zeiss system world, and can therefore be expanded to meet future requirements

*Option not available in all countries

Carl Zeiss MicroImaging GmbH

P.O.B. 4041, 37030 Göttingen, Germany
Phone: +49 551 5060 660
Fax: +49 551 5060 464
E-mail: micro@zeiss.de

www.zeiss.de/particle

Printed on environmentally-friendly paper, bleached without the use of chlorine.
Subject to change.

46-0123 e 02.2007