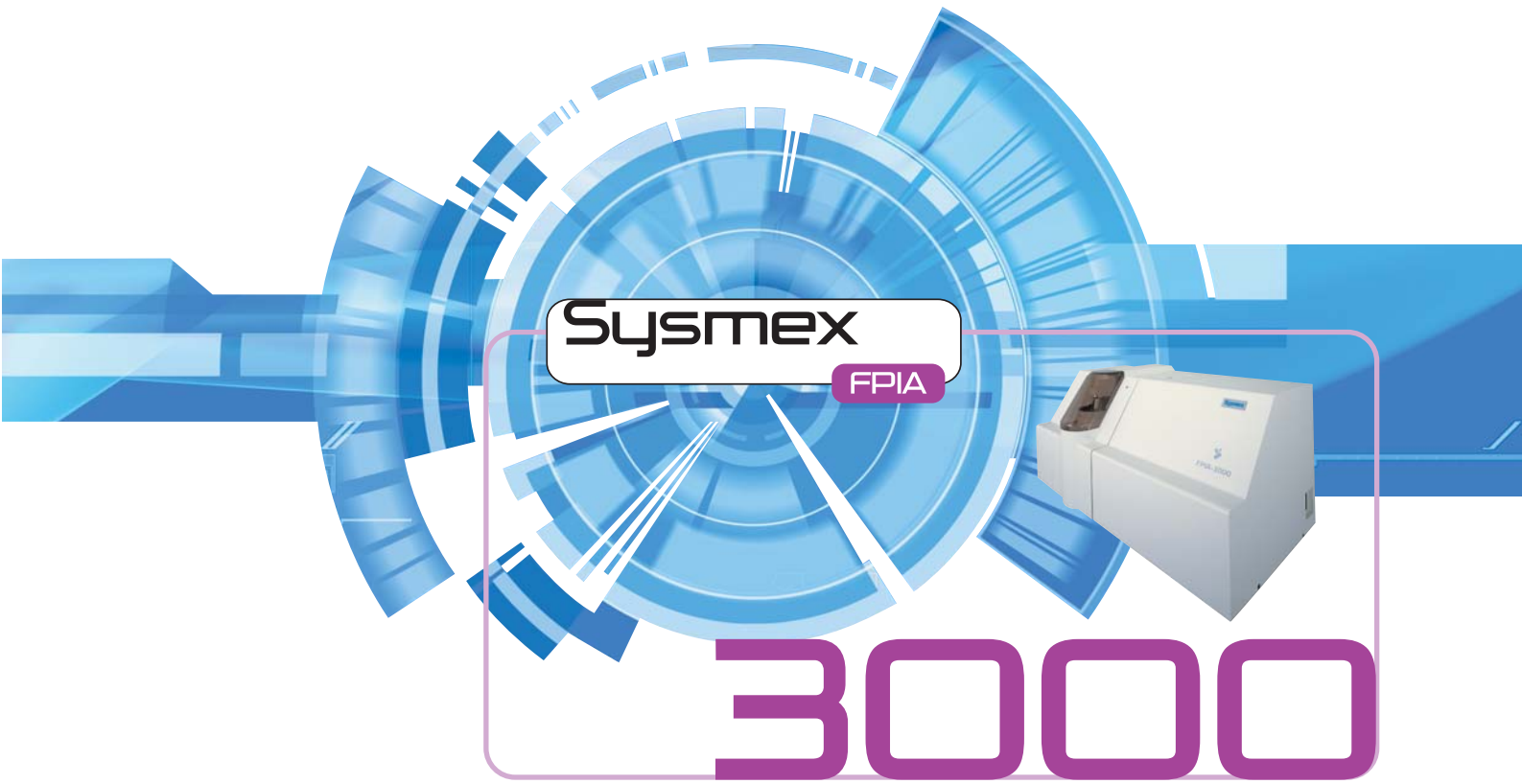
Particle size

Particle shape




# Flow particle image analysis of size and shape

detailed specification sheets from www.malvern.co.uk

Sysmex

MALVERN INSTRUMENTS

# Flow particle image analysis of size and shape

As your understanding of your product and the associated manufacturing process continuously progresses you may increasingly recognize the need for a higher-sensitivity analysis tool. Image analysis is an enabling technology which is both sensitive to subtle variations in particle shape and the presence of small numbers of very small particles. Samples which could not previously be differentiated can now be "fingerprinted" with the high-sensitivity Sysmex FPIA-3000.

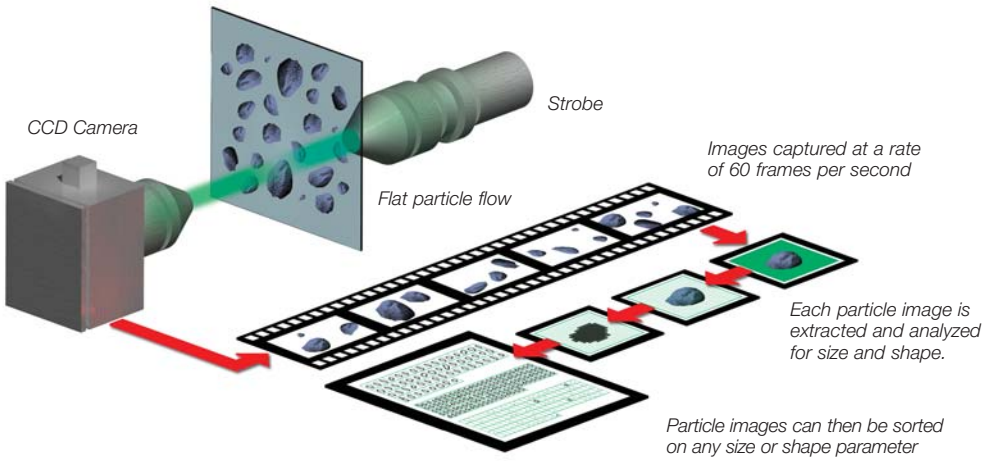
The Sysmex FPIA-3000 Flow Particle Image Analyzer offers high resolution, automated analysis of particle size and shape in a reliable, repeatable and routine manner. Comprehensive particle characterization data is generated in a short time, typically less than 3 minutes and with minimal sample preparation. Extensive information about each individual particle is acquired from the sample and displays of size and shape distributions are supported by images of the particles to provide further visual verification of the measurement data.

# What the FPIA-3000 delivers

| You asked for | We give you |
|---|---|
| *A simple, robust solution* | Ease of use. Prepare your sample and press GO! It really is as simple as that. With no complex configuration or setup procedures the FPIA-3000 has been designed for a QC environment where simplicity and speed are paramount. |
| *Sensitivity to shape* | Particles are fully characterized using a number of morphological parameters such as circle equivalent diameter, circularity and convexity. For each of these parameters a statistical distribution is calculated and combined in a scattergram. The scattergram is a unique fingerprint of the sample – high quality information that can be used to make more informed decisions earlier and distinguish between materials that can appear identical to a microscope or traditional particle sizer. |
| *Statistical significance* | A statistically significant number of particles (up to 300,000) are automatically analysed in a short space of time. |
| *Images I can see* | All particle images are saved for future reference. Each of these images retains its link to the distribution enabling visualization of the actual particles at different areas of the distribution for the purposes of anomaly or agglomeration detection for example. |
| *Compatibility with all the dispersants I use* | Two versions of the FPIA-3000 are available – the standard aqueous version, designed for water and common alcohols and the solvent-compatible version which is designed to work with a range of more aggressive solvents. |
| *Controlled orientation* | Largest area orientation. Random orientation significantly reduces the validity of data. Consistency of orientation is important for statistically significant size and shape measurement. The patented Sheath Flow Mechanism ensures that all particles are in focus and consistently orientated with their largest area facing the camera. |
| *Regulatory compliance* | Provides technical compliance with the requirements of 21 CFR part 11. |
| *A product and company with a secure future* | Malvern Instruments is committed to providing global service and support structures, a high degree of applications knowledge and customer service. |

# How does it all work?

## 1 Sample dispersion and image capture



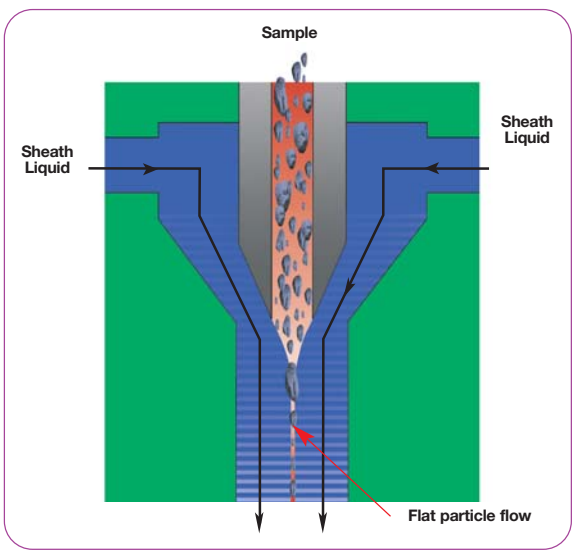

A sample is taken from a dilute suspension of particles. This suspension is then passed through a measurement cell where images of the particles are captured using stroboscopic illumination and a CCD camera.

The system incorporates a patented high-speed image processor. Through a series of sophisticated digital imaging stages each particle is extracted and quantified.

### Flat particle flow

The Sheath-Flow cell is the heart of the FPIA-3000. Its design and technology are pivotal to the unique imaging capabilities of the instrument. Its purpose is to optimize the sample flow to produce an ideal particle presentation for imaging.

The sample is introduced in the Sheath-Flow cell through a jet nozzle. This sample flow is 'sandwiched' by the sheath liquid. This transforms the particle suspension into a flat flow by hydrodynamic effects.




### Successful image analysis every time

Flat particle flow ensures that all particles in a sample lie in the same focusing plane and are orientated with their largest area facing the camera. Consequently, image capture is always successful and every particle is clearly defined.

Up to 300,000 particles can be analyzed and their images saved, producing qualitative data with a high degree of quantitative significance.

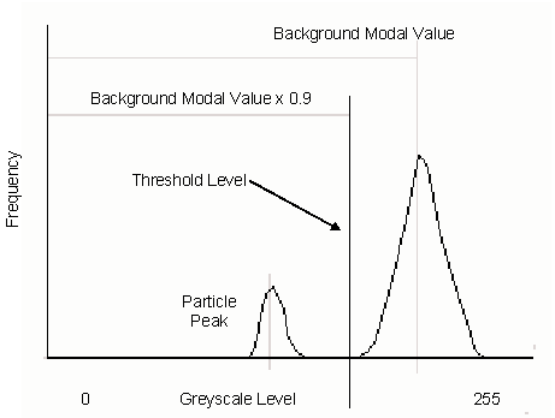
# 2 Image extraction

## Thresholding

Identifying particle pixels from background pixels using differences in greyscale levels

## Edge definition

Tracing the perimeters of individual particles and calculating their areas

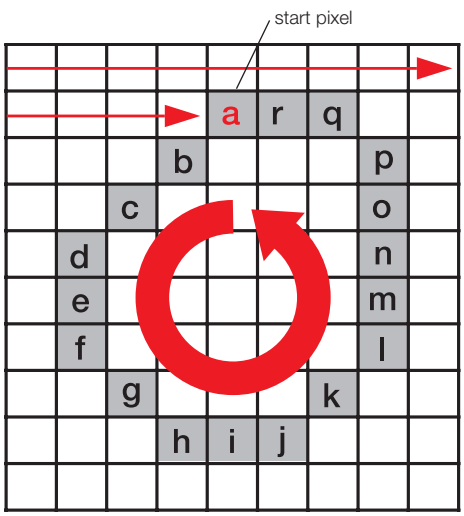



### Thresholding

The raw data is first cleaned to remove erroneous noise and a background correction is applied to remove any nonuniformity of the illumination conditions. A threshold value, which is a % greyscale value is then applied. This value is user definable depending upon the sample but is usually set at around 90% of the background modal value. Every pixel darker than this threshold value is then defined as particle and everything lighter than this is defined as background.

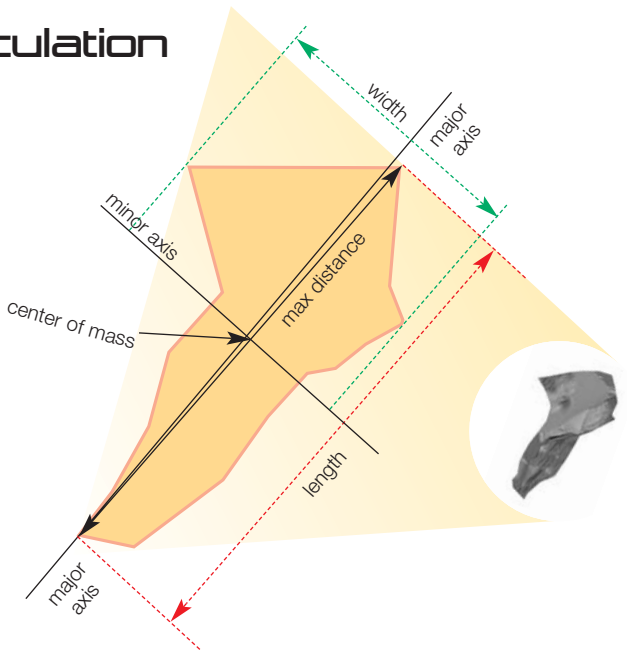
### Edge definition

The particle perimeter is then traced using a technique known as chain code which assigns a value to each pixel reflecting its relationship with its immediate neighbours. For example – a pixel in a straight line with two of its neighbours (pixel 'e' in the diagram) will have a different value than a pixel on a corner (pixel 'd'). All these chain code values are then summed together for the entire particle giving a more accurate perimeter value than if it was calculated by just counting the pixels and giving them all an equal weighting.



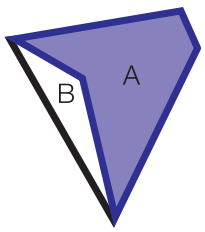
# 3 Morphological parameter calculation

Following image extraction a number of morphological parameters are calculated. These shape parameters enable a much more sensitive characterization process as they capture very subtle variations in particle form that could remain undetected when using manual microscopy or traditional particle sizing techniques.




| Parameter | Definition |
|---|---|
| CE diameter (N, A and Volume based) | The diameter of a circle with the same area as the particle - 3 values are dispayed - (N) weighted by number (all particles weighted equally in the distribution), (A) weighted by area (particles weighted proportional to their area - a particle of twice the area will contribute twice as much to the %density on the distribution), and (V) weighted by volume (particles weighted proportional to their volume) |
| Perimeter | Actual perimeter of particle |
| Area | Actual area of particle |
| Length | All possible lines from one point of the perimeter to another point on the perimeter are projected on the major axis (axis of minimum rotational energy). The maximum length of these projections is the length of the object. |
| Width | All possible lines from one point of the perimeter to another point on the perimeter are projected on the minor axis. The maximum length of these projections is the width of the object. |
| Max. distance | Largest distance between any 2 pixels in particle |
| Min. distance | Projection at 90 degrees to max distance |
| Ferret (horizontal and vertical) diameter | Depending upon how the particle is orientated the horizontal and vertical external caliper dimensions |
| Martin (horizontal and vertical) diameter | Depending upon how the particle is orientated the horizontal and vertical dimensions through the centre of mass |
| Krumbein (horizontal and vertical) diameter | Depending upon how the particle is orientated the maximum internal horizontal and vertical dimensions measured parallel to the horizontal and vertical |
| Equivalent perimeter diameter | Diameter of a circle with equivalent perimeter to particle |

# Morphological parameter calculation continued




$$\text{Convexity} = \frac{A}{A + B}$$

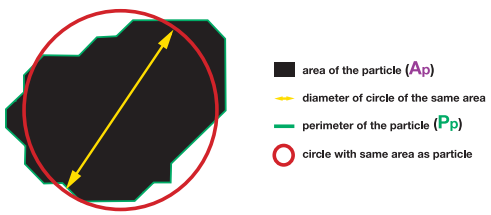
## Convexity

Convexity is the object area divided by the area enclosed by an imaginary "rubber band" wrapped around the object. The convexity has values in the range 0 -1. A convex shape has convexity 1.0, while a concave shape has a lower value, close to 0.

| Parameter | Definition |
|---|---|
| Convex hull perimeter | Perimeter of convex hull (shape defined by analogous elastic band stretched around particle) |
| Convex hull area | Area of convex hull |
| Intensity mean | Average of all the greyscale values of every pixel in the particle |
| Intensity standard deviation | Standard deviation of all the greyscale values of every pixel in the particle |
| Aspect ratio (Feret) | Maximum Feret diameter divided by minimum Feret diameter |
| Aspect ratio (width/length) | Width divided by length |
| Aspect ratio (min/max distance) | Minimum distance divided by maximum distance |
| Convexity (perimeter) | Convex hull perimeter divided by actual particle perimeter |
| Convexity (area) | Actual particle area divided by convex hull area |
| Circularity | Circumference of circle of equivalent area divided by the actual perimeter of the particle. The more spherical the particle the closer the circularity is to 1, the more elongated or rough-edged the particle is, the lower the circularity. |

Shape parameters such as Circularity, Convexity and Aspect Ratio provide the user with a series of highly sensitive tools in order to identify and quantify subtle variations in particle shape and provide a "fingerprint" of each sample. Each parameter is usually normalized between 0 and 1 in order to provide quick and easy comparability. Traditional qualitative human descriptions such as "jagged", "smooth" or "needlelike" can be accurately quantified and hence correlated against important process or end-product variables such as flowability, active area and grinding efficiency.

$$\text{Circularity} = \frac{\text{Circle circumference}}{\text{Perimeter of projected particle image}} = \frac{2\sqrt{\pi A_p}}{P_p}$$

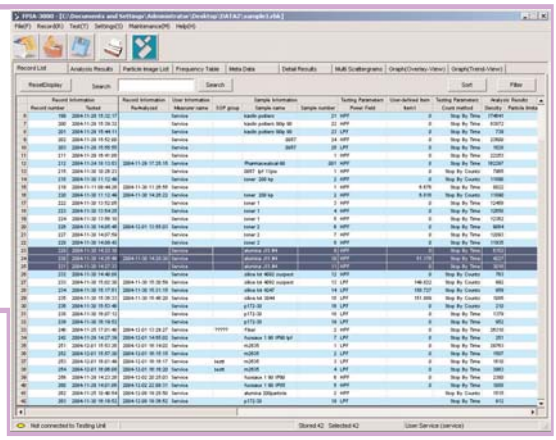



| Shapes | | | | | | |
|---|---|---|---|---|---|---|
| Circularity | 1 | 0.886 | 0.777 | 0.660 | 0.509 | 0.4 |
| Aspect Ratio | | | | 1 in 4 | 1 in 10 | 1 in 20 |

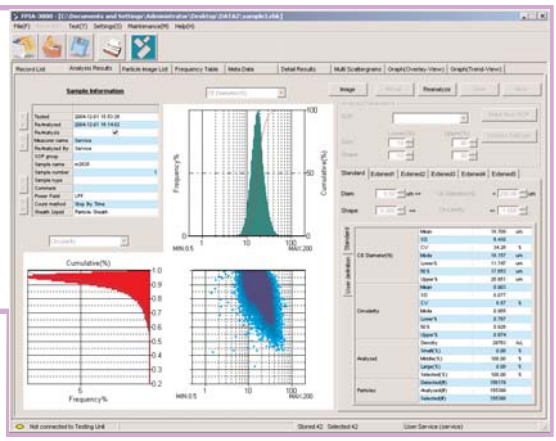
# Software to make it happen

A high level of functionality with a priority placed upon ease-of-use is built into the FPIA-3000 software. Malvern Instruments and Sysmex together have vast experience and expertise in programming and testing such analysis software which means you get the best out of the instrument and the data it generates.

**Record view:** A configurable record view allows you to view a summary of the measurement results and define the information which is displayed for each record. Selecting a record or multiple records allows you to view the details (scattergram graphs, particle images, raw statistical data etc) associated with each record.



**Results view:** The results of a single analysis are represented in a 3 graph format – a particle size distribution (green), a particle shape distribution (red) and a scattergram plot of size against shape (blue). The statistical parameters associated with each distribution (mean, mode, lower, median and upper percentile values etc) are also displayed.

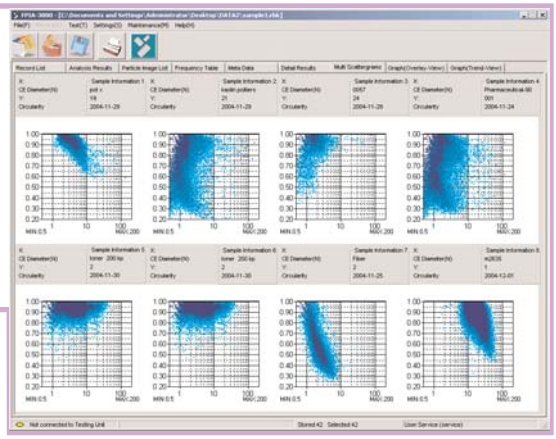


**Particle view:** Images of all particles are saved. These images can be viewed and manipulated through the particle viewer. The images can be magnified and sorted on any size or shape parameter allowing the operator to quickly and easily identify anomalies – perhaps agglomerates or the presence of unexpected foreign particles for example.

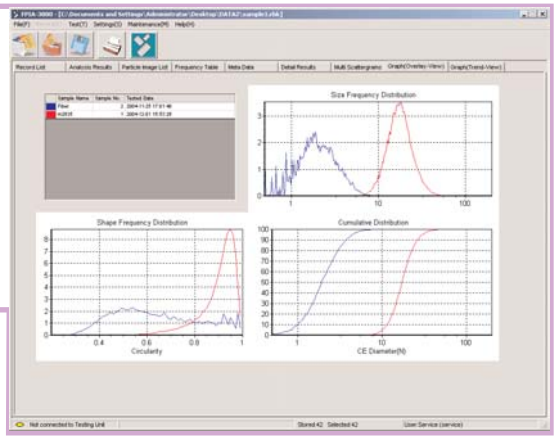
## Software continued

Along with detailed data on individual records the software has the ability to further manipulate the data and compare multiple records to identify subtle differences and trends of key parameters

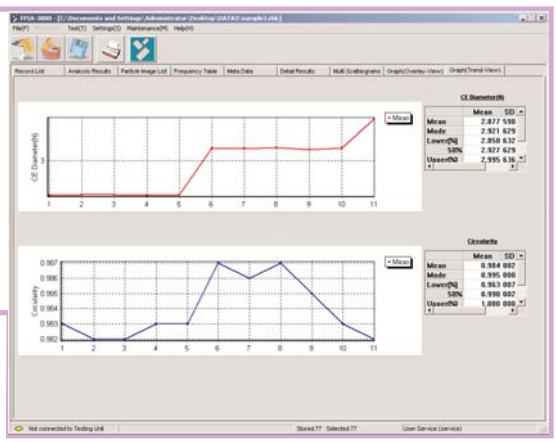
**Multiple scattergram view:** View up to 8 scattergrams side by side for a quick visual comparison of results. This allows the operator to spot gross differences or trends quickly without having to deeply probe the statistics behind each record. Any anomalies or significant differences can be investigated further by drilling down deeper into the raw data.



**Overplot view:** Overplot multiple records for a more detailed comparison. Data is displayed on three graphs – a size frequency distribution, a shape frequency distribution and a size cumulative distribution. The cumulative distribution plot is particularly useful for assessing reproducibility during the method development phase of an analysis for example.



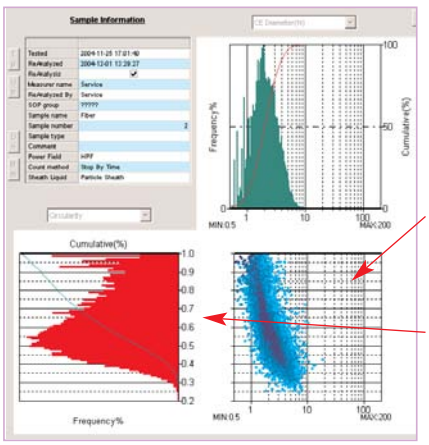

**Trend view:** Plot multiple measurements and compare the trend of parameters. The displayed parameters are user-definable and cross-measurement statistics are calculated such as mean of means, standard deviation of means etc. This ability to plot trends enables data presentation in a form which allows immediate action to be taken by the operator as it avoids the need to export data for any further manipulation.



# Data interpretation

Quick and easy interpretation of analysis data is vital in today's busy and efficiency-conscious R&D and QC labs. In order to facilitate quick decision making the FPIA-3000 software maintains links between the qualitative data, presented in graph format, and the qualitative images. This allows the user to quickly and easily switch between both types of data and retain the relevance at all times. Limits can also be applied to each distribution in order to exclude certain values and the statistics are automatically recalculated without the excluded particles. The two examples below illustrate how both the statistical distribution and the particle images are used together to fully characterize and understand a sample.

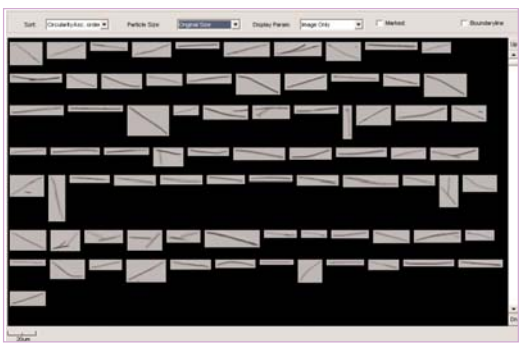
## Example 1: Fiber samples



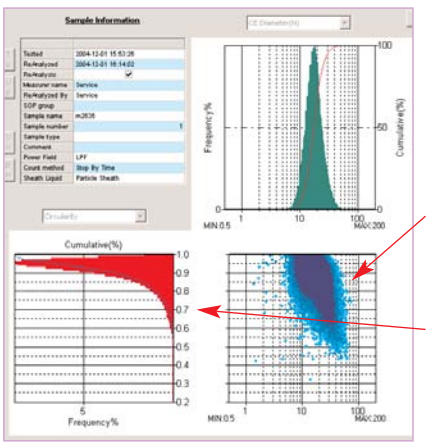

Trend showing decreasing circularity with increasing size

Mean circularity of 0.6

Interpretation of the statistics alone suggests that the sample is fiber or needlelike and a quick look at the images below confirms this



## Example 2: Agglomerated abrasive samples



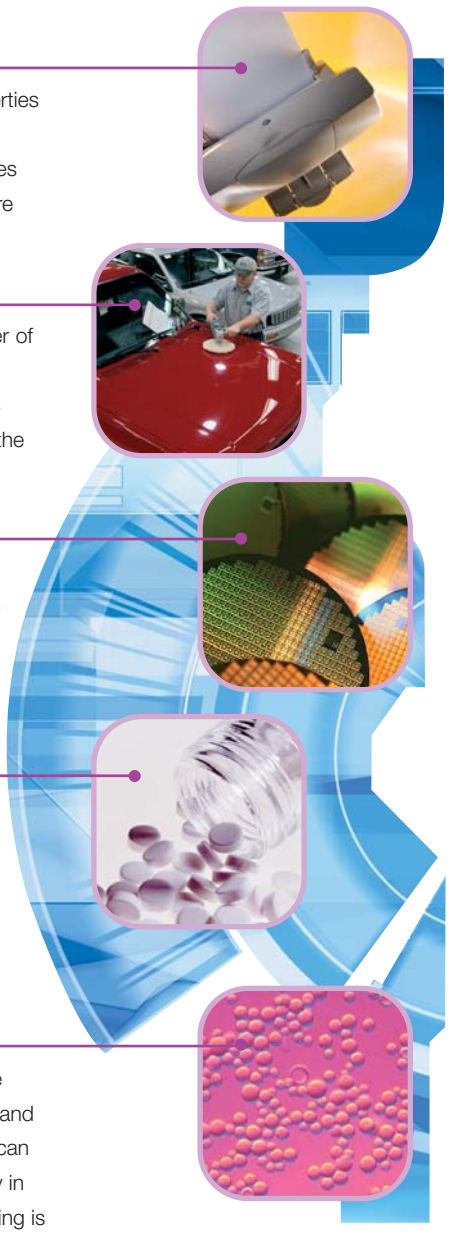

Trend showing decreasing circularity with increasing size

Mean circularity of 0.9

Interpretation of the statistics alone suggests that the sample includes some agglomerates and a quick look at the images below confirms this

# Key applications

The FPIA-3000 has applications across all industry sectors. Wherever and whenever your requirement is for accurate, reliable and repeatable size and shape analysis, the FPIA-3000 is an ideal solution. At any point in your manufacturing process from early research and development, through process-analysis, manufacturing trouble-shooting and root-cause analysis to final product quality control, this instrument gives you an unprecedented level of product and process understanding.



## Toners

Toner manufacturers require defined size and shape characteristics to optimize the flow properties and charging capability. Toner particles that are close to, but not perfect spheres (i.e. with circularity of approx 0.95) are ideal. Too low a circularity (~ 0.8) will result in poor flow properties and increased wear, whereas toner particles produced as perfect spheres (circularity of 1.0) are self-lubricating and do not stick to the print medium sufficiently well.

## Ceramics

The FPIA-3000 is used for precise measurement of size and shape characteristics in a number of different ceramic applications, one of which is the mould design for the 'lost foam casting' of complex metal components in the automotive industry. Unless the permeability of the ceramic mould - a variable closely associated with particle size and shape - is correct, the integrity of the cast item will be compromised.

## Abrasive/cutting powders

Abrasive grains are produced from a variety of different materials, for example alumina ($Al_2 0_3$), silicon carbide (SiC), carbon boron nitride (CBN) or diamond. Along with hardness it is particle shape which gives these materials their abrasive qualities when incorporated into a suitable matrix; for example as sandpaper, grinding wheels, wire sawing, sandblasting etc.
The FPIA-3000 can be used to accurately define and control different grain shapes.

## Pharmaceuticals

Differences in the physical properties of excipient components of pharmaceuticals can cause final formulation variability. Differences can occur in batches from different raw-materials suppliers even though specifications are identical. Size data alone is often not sufficient to predict differences in product performance. Since the FPIA-3000 orientates and disperses particles when wet, it can be used even on delicate needle-shaped crystals to establish the causal links between manufacturing process variables and final product performance.

## Biotechnology

Many processes associated with the Biotech industry increasingly require high-sensitivity shape analysis. Column chromatography is used to separate and purify complex mixtures of proteins and the FPIA is used to control packing density and identify out-of-spec particles. Protein crystals can now be manufactured in sizes and morphologies which are suitable for formulation and delivery in bulk process and the FPIA can be used for the QC of such crystals. Environmental toxicity testing is now required to be applied to all chemical entities manufactured above certain quantities. A simple test based on the growth inhibition of algae is commonly used and the FPIA can be used to quickly and easily measure the level of growth inhibition using a combination of size and shape parameters.

# Sysmex FPIA-3000 technical specifications

## Dynamic size and shape measurement of wet samples

| | | |
|---|---|---|
| **Technical specifications** | | |
| *Measurement technique* | Automated image analysis—flow cytometry | |
| *Light source* | White light stroboscope (60Hz) | |
| *Detector* | CCD (charge-coupled device) | |
| **Measuring mode** | **High power field (2x secondary lens)** | **Low power field (0.5x secondary lens)** |
| *Particle size range (standard unit) Primary lens = 10x* | 1.5µm – 40µm (total mag = 20x) | 6µm – 160µm (total mag = 5x) |
| *Particle size range (high-mag unit) Primary lens = 20x* | 0.8µm – 20µm (total mag = 40x) | 3µm – 80µm (total mag = 10x) |
| *Particle size range (low-mag unit) Primary lens = 5x* | 3µm – 80µm (total mag = 10x) | 12µm – 300µm (total mag = 2.5x) |
| *Standard sample volume* | 5ml | 5ml |
| *Minimum sample volume* | 1ml | 1ml |
| *Measurement time* | 2.5 minutes | 2.5 minutes |
| **Sheath liquid compatibility** | **Standard version** Aqueous 'Particle Sheath Reagent' Methanol, ethanol, isopropyl alcohol and ethylene glycol solution (25%) | **Solvent version** Compatible with most commonly used solvent dispersants such as: Toluene, Acetone, Heptane and Hexane |
| *Reagent consumption* | Approximately 130ml per sample | |
| **Requirements** | | |
| *Minimum PC specification (Supplied with system)* | Windows XP, 3.0GHz Intel Pentium IV processor with 1MB cache, 1GB RAM, 160GB hard drive, DVD +/- RW, 17" flat panel monitor | |
| *Operating environment* | Temperature 15 – 30°C, Humidity 35 – 80% | |
| *Power requirements* | 100V to 240V with Standard IEC inlet socket | |
| **Dimensions** | | |
| *Main unit* | 900(w) x 455(h) x 475(d) weight 59.5kg | |
| *Pneumatic unit* | 280(w) x 400(h) x 355(d) weight 20.7kg | |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FPIA-3000

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