HORIBA
Scientific

PSA300

State of the art, static image analysis solution

Featuring an automated microscope with high-resolution camera and seamless integration of powerful particle characterization software.

PSA300

Automated Image Analysis to Measure Particle Size and Shape

The HORIBA PSA300 is a state-of-the-art, turnkey image analysis system for particle size and shape characterization. The seamless integration of Clemex-designed image analysis software with automated microscopy creates a powerful, but easy to use workstation for use in a wide range of applications from pharmaceutical research to materials science. Automated image analysis is rapidly replacing manual microscopy in laboratories wanting unparalleled accuracy and resolution in size and shape coupled with the statistical confidence from inspecting thousands more particles than otherwise possible.

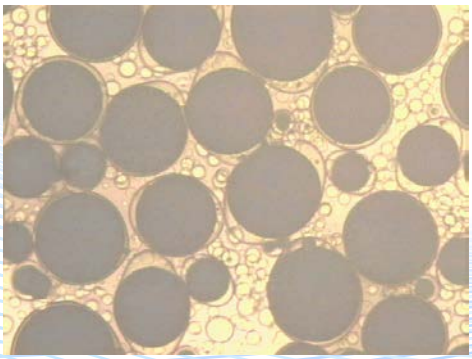
Why Image Analysis?

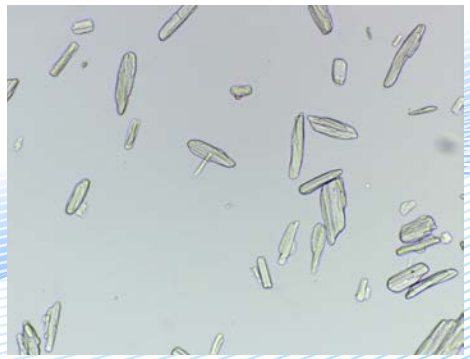
Accuracy

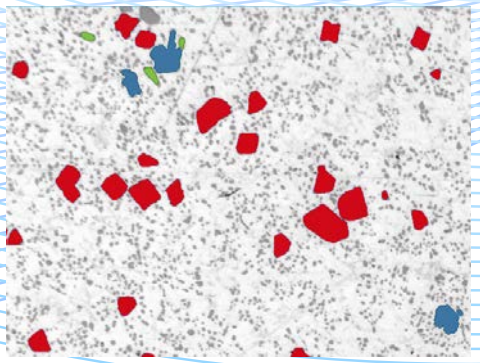
Microscopy is the preferred technique for particle size analysis since it is the most direct measurement possible. When searching for the “real particle size distribution,” only one technique provides the most accurate results – image analysis.

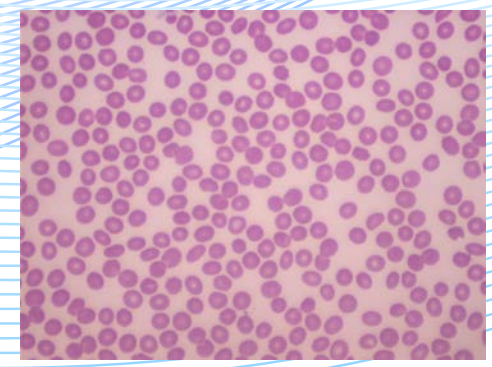
Statistical Certainty

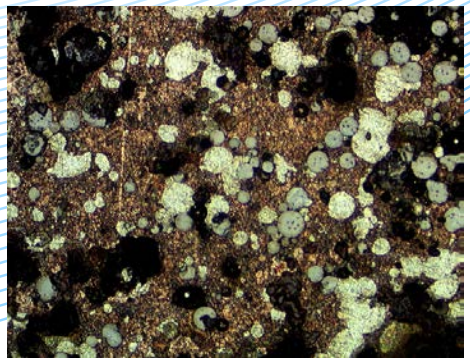
The challenge with manual microscopy is inspecting enough particles to assure statistical validity to reported distribution values. Automated image analysis quickly inspects thousands (or hundreds of thousands) of particles, ensuring confidence not only at the mean, but also at the tails of the distribution (d10 and d90).











Particle Shape Information

Particle shape is extremely valuable information since morphology affects many critical performance characteristics including powder flow and compaction. Image analysis is the only technique that directly reports particle shape distribution using a multitude of parameters such as roundness, aspect ratio, convexity, and many others.

Images

A single picture of a particle next to a size scale has settled many an argument over the presence of large particles in a sample. The PSA300 can store an image of every particle inspected, providing intuitive confirmation of the kind of particles in a sample.

Finding Outlier Populations

All techniques that inspect particles one at a time are inherently higher resolution than ensemble methods. Since image analysis is a high resolution counting technique, it can effectively detect outlier populations (both larger and smaller than the main population). For active pharmaceutical ingredients, this is a critical advantage for finding small amounts of large particles that could negatively impact dose uniformity.

Size Analysis of Non-spherical Particles

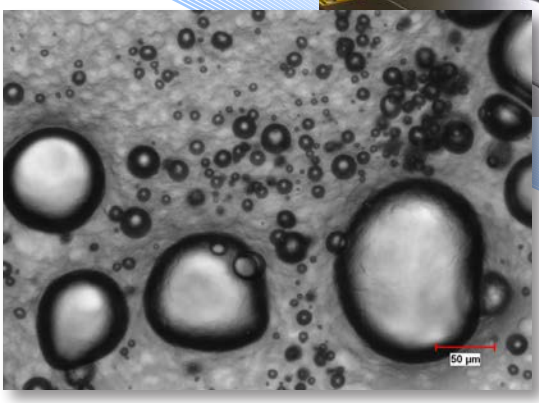
All light scattering techniques, optical counters, and electric sensing zone counters are based on equivalent spherical diameter models. Microscopy/image analysis is the only technique that provides accurate size and shape distribution information for particles of any shape. Several industry-leading pharmaceutical companies now regularly switch to image analysis for particles beyond a defined aspect ratio.

Support Method Development and Validation

Even when laser diffraction will be the release test for an API, image analysis can play a critical role in both method development and validation. Since microscopy/image analysis is the referee technique in particle size analysis, image analysis could be used as an accuracy test during method validation. The FDA guidance document for method validation states, “Several methods of determining accuracy are available....Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined.”

Principle of Operation

Due to advances in both hardware and software, image analysis is now one of the easier techniques for particle characterization. It’s as simple as preparing the sample, loading the sample onto a slide, putting the slide in the PSA300, clicking the run button and then viewing the measurement result.



Unique Software Features

The image analysis software created by Clemex comes from a group of scientists working in the field of image analysis for many years. The software has now been customized for particle size and shape applications for use with the PSA300. This is a radically different approach than the software designed by particle sizing companies who recently learned to apply image analysis to this application. Because of the difference in development path, the PSA300 software contains many powerful software features that are standard image analysis tools, but are unique in the field of particle characterization. The new Dashboard Routine mode provides an easy to use GUI that facilitates optimizing advanced software features into application specific analysis routines.

Clemex microscope shown running with image analysis software.

Thresholding

All image analysis programs offer some kind of threshold function to separate the particles from the background. The PSA300 software provides the flexibility to achieve this goal regardless of sample difficulty. Features unique to the PSA300 software include Auto Gray Threshold and Contrast Thresholding.

Auto Gray Threshold automatically detects phases in a grayscale image. With Auto-thresholding, you no longer need to manually set intensity intervals because the software chooses the optimum conditions.

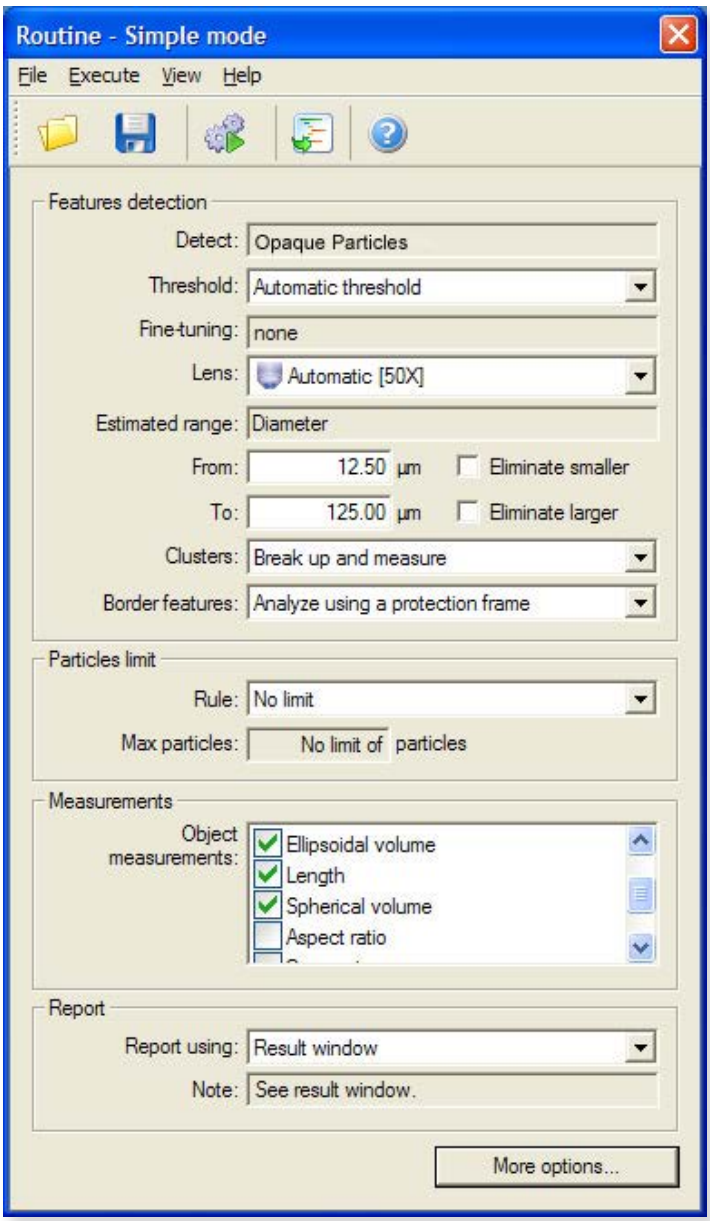
The Contrast Threshold function is extremely useful when measuring particles where the particle/background contrast is weak.

Multilayer Grab

This is a unique function that is useful for samples with a wide range of particle sizes. Depth of field limitations make it difficult to have both small and large particles in focus at the same time. By adjusting the height (z-axis), focusing on different portions of the image, and combining these views, a sharp image can be generated despite the fact that various portions of the image are within different focal planes. The past alternative of using multiple objectives and then combining the data not only takes more time, but introduces error in the combination. It's easy to see why the Multilayer Grab is innovating image analysis.

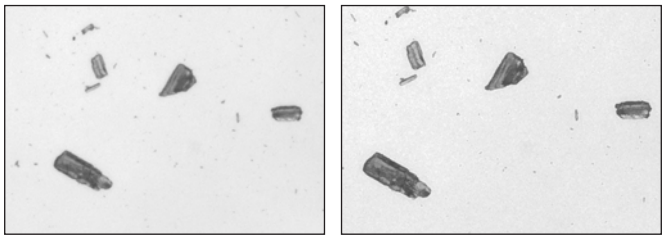
Simple Automation

The PSA300 Dashboard organizes all of these advanced software features into an easy to use single screen. The operator simply chooses the desired operations and calculated results using the single window shown above.



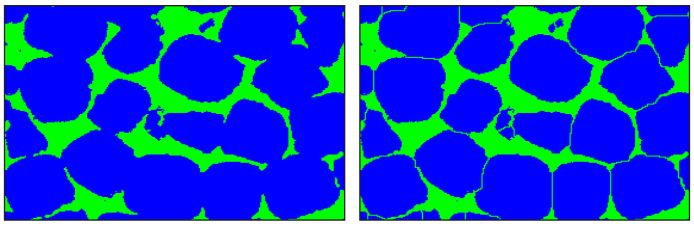
Delineation

The camera detects intermediate gray levels wherever there is a transition from light to dark or dark to light at the edge of the particle. The Delineation function removes intermediate gray levels to increase contrast by changing the gray value of pixels in the transition zone by assigning them either the maximum or minimum value in the kernel depending on which is closest to their original value. The images below show the original image (left) and the resultant image (right) after the delineation function has been applied.

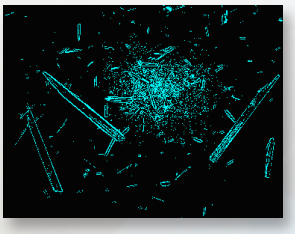


Bridge Removal

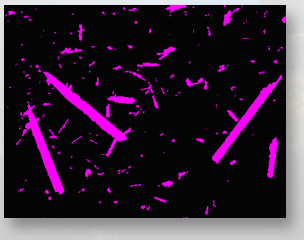
This feature separates juxtaposed or slightly superimposed particles. Two particles touching each other may be measured as one large particle without bridge removal. With Bridge Removal, those particles are accurately separated and measured. The images below show an image before (left) and after (right) applying the bridge removal function. The Bridge Removal function can be adjusted to optimize particle separation depending on surface roughness and the number of pixels touching each other.



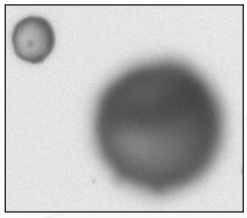
Example of Thresholding

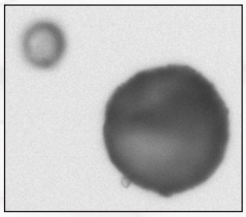


The top image is before thresholding. The center image shows the effect of conventional thresholding and the bottom image shows the effect of using contrast threshold.

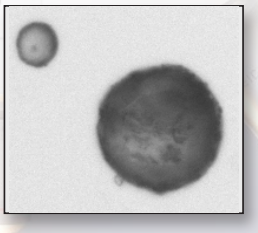


Example of Multilayer Grab

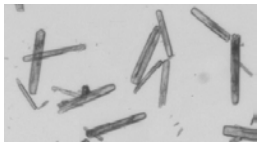


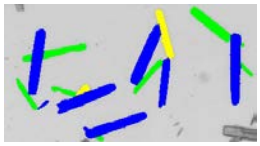


The images to the left show the same field with the small particle in focus, the large particle in focus and the combined view with all particles in focus using the unique Multilayer Grab.



Separate Long Objects

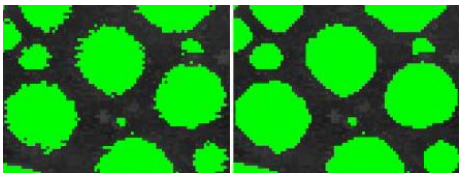




This unique PSA300 feature is used to separate fibers or acicular crystals for accurate chord length distribution. The other Bridge Removal function would break crossed fibers into four individual segments, none of them equal to the actual fiber length. Using Separate Long Objects, the PSA300 separates crossing fibers as individual particles and assigns each one accurate size and shape parameters. The images to the right show fibers before and after applying the Separate Long Objects function.

Convex Hull

At high magnifications, the particle edges can become too rough (pixelated), leading to erratic perimeter measurements. Applying the Convex Hull function is similar to what would happen if a rubber band were placed around an object. This filter selectively dilates concave portions of a particle's contours until they become convex. This improves the accuracy of perimeter measurements which are used in several shape calculations. The images below show the original (left) and improved (right) images after applying the Convex Hull function.



Accurate Volume Distributions

Image analysis results are collected as a number distribution which is often later converted to a volume distribution. Transforming the two-dimensional projected area into a volume distribution requires an assumption on the shape of the particles. Other software packages always assume spherical shaped particles. The PSA300 software can build the volume distribution based on spherical, ellipsoidal, cylindrical, and trapezoidal shapes. Using the length and width measurements of each particle generates a more accurate volume distribution.

Powder Disperser

An automated sample disperser subjects powder samples to a controlled quantity of force by subjecting the sample to a burst of air as a vacuum is released. The sample disperser breaks agglomerates and assures an even spread of sample across the slide. Unit includes disperser column, two slides base, high vacuum dry pump assembly, and software.

Applications

APIs

The most common application for static image analysis systems is currently the characterization of active pharmaceutical ingredients (APIs). Pharmaceutical laboratories around the world are investing in image analysis systems as a valuable tool for complete particle size and shape characterization of solid oral, aerosol and transdermal dosage forms.

Excipients

Another common application of image analysis is the physical characterization of excipients. The particle size and shape of excipient powders is well understood to play a crucial role in powder flow, compaction, and tablet quality. In addition, image analysis has proven to be an important tool for screening excipients when a new, less expensive source claims to be the same size and shape as the approved supplier.

Aerosols

Pharmaceutical aerosols including metered-dose inhalers (MDIs) and dry powder inhalers (DPIs) are devices that deliver a specific quantity of drug to the lungs. The particle size distribution and shape of the delivered dose is more critical for inhalation aerosols than for most other conventional drug products because these factors greatly influence the deposition profile in the lungs of the patient. Although the compendial tests for pharmaceutical aerosols are based on cascade impactors, microscopic analysis can provide valuable information on particle size and shape distribution of the drug particles such as:

- The presence of large particles
- Changes in morphology of the drug substance particle
- Extent of agglomeration
- Crystal growth
- Presence of foreign particulate matter

Abrasives

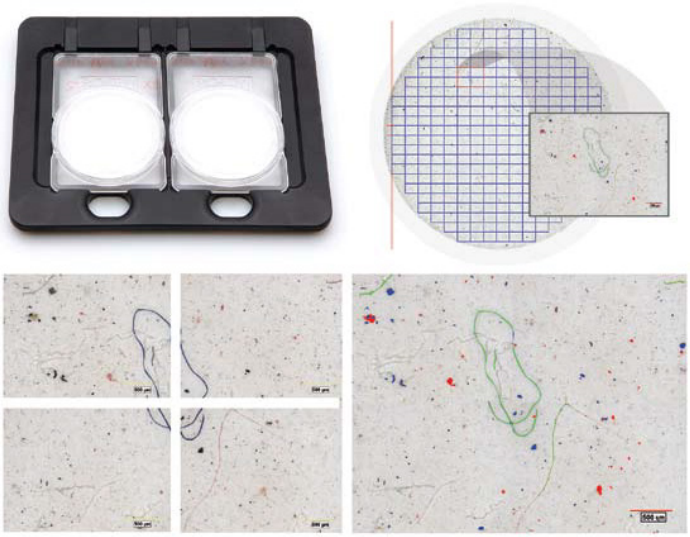
The shape of abrasive particles has a direct impact on abrasion rate. Abrasive particles typically have rough edges in order to decrease the surface area in contact with the work piece and increase the localized contact pressure. Several of the parameters reported by the PSA300 can be used to quantify particle shape allowing it to be used as a critical performance indicator, including a custom calculation involving counting sharp tips on the particles.

Filter Testing

One way to count and size particulate contamination is to pass the sample through a filter, trapping the particles on the filter. The filter can then be inspected using a microscope. Automated image analysis is replacing manual microscopy to count and size particles trapped on a filter surface. This approach can be applied to parenteral drugs or ophthalmic solutions following the procedure in USP <788> and <789>.

The PSA300 Particulate Filter System accepts standard size circular paper filters in a special sample holder without operator involvement. Large fibers, fibers that overlap, and tiny dirt particles are all identified in the same run. Outsized fibers larger than the field of view are stitched together using the mosaic function to deliver a single, high integrity measurement. An image of the entire filter may be reconstructed this way.

The PSA300 Particulate Filter System is easy to use and delivers accurate, reproducible, and guaranteed results.



The PSA300 segments the filter paper into multiple fields for measurement. Outsized fibers covering multiple fields are combined to give a single, coherent particle image.

Cleanliness Evaluation as per IEST-STD-CC1246D

CLEMEX intelligent microscopy

Sample name:	ABC123	**Calibration:**	1.4784 µm/pixel
Analyst:	John Smith	**Magnification:**	50x
Sample Size:	4 inch wafer	**# Fields:**	489
Number of Samples:	1	**Target Level:**	100
ID of replicate samples:			

Date procured: 2009-Jun-11 **Date tested:** 2009-Jun-11

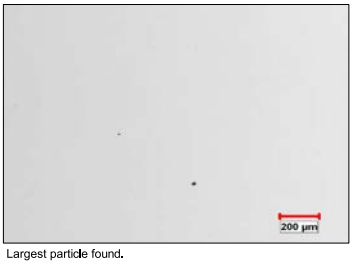
Aditionnal information:
Note: Complete surface analyzed
Operating condition of item during test procurement.
Environment in which sample was taken
Equipment used to take sample
Method used to obtain sample.
Method used to perform test

Results

		Normalized to 0.1 m2
Total area analyzed:	1386692127 mic2	0.1 m2
Total particle count:	23	1658.6
Total particle area:	985.735611 mic2	71085.4 mic2

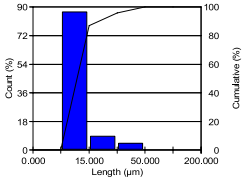
Percent Area Coverage: 0.000071 %
UCL 34.5
LCL 15.3

Particle Size (mic)	Max. concentration for 0.1 m2	Count (normalized)	Over
5	1784.8	1658.6	no
15	265.0	216.3	no
25	78.4	72.1	no
50	10.7	0.0	no
100	1.0	0.0	no



Largest particle found.

Particle Size Distribution



Particulate Contamination as per ISO 4406 - 4407 (2002)

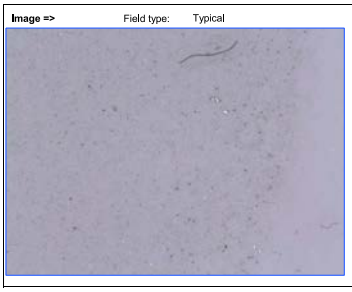
Date:	2009-Oct-28, 3:51:24 PM -04'00'		**Covered area:**	2106.38	mm²	**Magnification:**	16x		
Analyst:	John Smith		**Covered ratio:**	1.21		**Calibration:**	5.0441 µm/pixel		
Sample ID:	Filter 1		**Effective filtration diameter:**	47	mm	**Units:**	microns		
Pore size:	1.0	mic.	**Effective filtration area:**	1734.94	mm²	**# Fields:**	97		
Sample volume:	100	ml	**Illumination type:**	reflected					

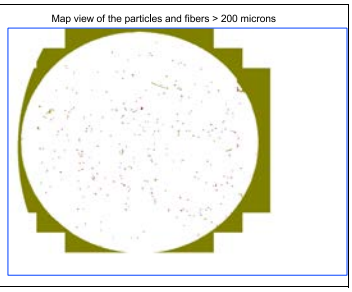
Particle count data and Scale number											**Fibers[d] Count**
Size range (mic)	[a]7.5 - 15	15 - 25	25 - 50[b]	50 - 100	100 - 150	150 - 200	200 - 400	400 - 600	600 - 1000	> 1000[c]	0
Blank count (can be filled in manually or linked to an exported Vision file)											**Total**
Particle counts	-	796	450	222	96	611	341	102	61	0	2679
Estimated particles for the whole filter	-	655.6	370.6	182.9	79.1	503.3	280.9	84.0	50.2	0.0	2206.6
Particle count per 100 ml	-	655.6	370.6	182.9	79.1	503.3	280.9	84.0	50.2	0.0	2206.6
Scale number	--	17	16	15	13	16	15	14	13	0	18
Final classification	-- / -- /17/16/15/13/16/15/14/13/0										

Largest particle => **Maximum dimension:** 1620.43 microns **Nature:** Fiber

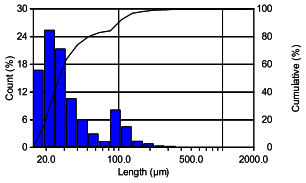
Image => Field type: Typical

Map view of the particles and fibers > 200 microns





Distribution of particles per category

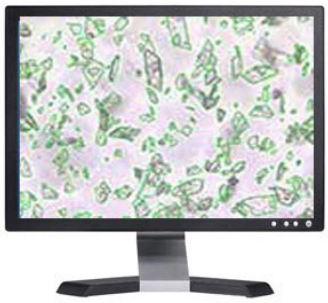


Remarks / Comments:

a) At 16x, features smaller than 15 microns cannot be discriminated from artefacts and are not reported here.
b) At 16x, the precision decreases for particles under 50 microns. If a better precision is needed on smallest particles, use the routine for 55x instead.
c) At 16x, measurements over 1000 microns are based on Tiled features, so the precision decreases past this limit
d) ISO 4406 identifies fibers as features longer than 100mic. with a Length / Width ratio greater than 10. The Vision routine is based on String Length / String Width ratio. This can be modified if desired.

Specifications

Measuring range	Recommended range is 0.5 to 1000 microns in size (2000 microns size with 1.25X optional objective)
Size Parameters	Main length (longest particle dimension), circular diameter (equivalent diameter based on particle area), feret average (caliper measurements circumscribing particle), string length (curved fiber type particle length) and any user defined parameter.
Shape Parameters	Aspect ratio (particle length/particle width), particle Sphericity (area/perimeter ratio), particle Roundness (area/circle area ratio), particle Roughness (edge smoothness), string aspect ratio (fiber type particle aspect ratio), and any user defined shape parameters.
Volume Parameters	Spherical volume (volume based on spherical particle, closest to laser diffraction results), cylindrical volume (volume of rod type particles), tetragonal volume (volume of crystal shaped particles) and any user defined volume parameters.
Hardware Package	• Enclosed automated microscope 2.5X, 5X, 10X, 20X and 50X objectives. • Computer-controlled motorized nosepiece, condenser and focus @ 0.05 mm/step • CLEMEX controller for motorized stage with 215 x 75 mm travel @ 0.5 mm/step • High-resolution 2.1 Megapixel, USB 2.0 CCD mono camera (color camera upon request) • Three 4-slide holders • NIST-traceable calibration slide • Ultra-high performance computer with state of the art Intel processor • Two 20” LCD UXGA (1600 x 1200 pixels) color monitors and dual monitor stand
Software Package	• Report generator and measurement templates • Available user and data management module (21 CFR PART 11 compliant) • R’KIVE Explorer for file archiving • Microscope automation software
Physical	• The instrument operates and meets all specifications on a power source of 100-115 Volts/60 Hz. • The instrument operates under conditions of 5° C to 40° C. • The instrument weighs approximately 70 lbs. (excluding the computer) and has dimensions of 27” x 20” x 12” (without powder disperser).



Please read the operation manual before using any of these products to ensure safe and proper operation.

www.horiba.com/scientific • email: labinfo@horiba.com

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