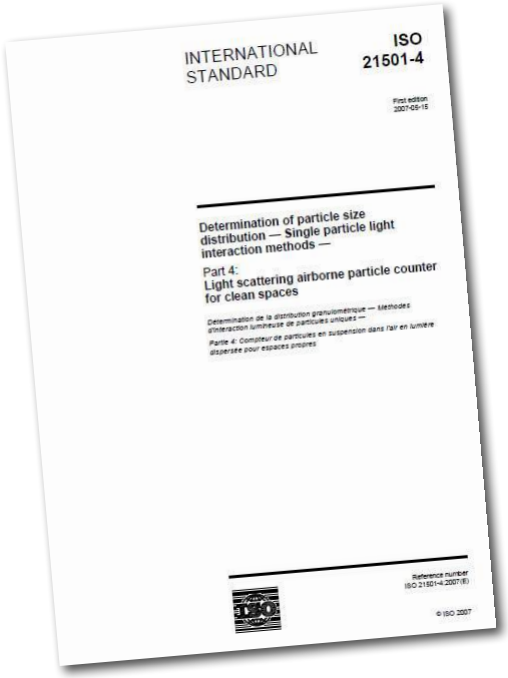
Understanding ISO 21501-4

Introduction

The ISO 21501-4 is the recognized standard for optical particle counter calibration. The International Organization for Standardization (ISO) introduced ISO 21501 in 2007 with the following purpose:

> *"…to provide a calibration procedure and verification method for particle counters, so as to minimize the inaccuracy in the measurement result by a counter, as well as the difference in the results measured by different instruments."*


INTERNATIONAL STANDARD

ISO 21501-4

Determination of particle size distribution — Single particle light interaction methods —

Part 4: Light scattering airborne particle counter for clean spaces

Performance and counting efficiency

The publication includes 4 different sections, with each dedicated to a specific particle counting technology/application:

Part 1: Light Scattering Aerosol Spectrometer
Part 2: Light Scattering Liquid-Borne Particle Counter
Part 3: Light Extinction Liquid-Borne Particle Counter
Part 4: Light Scattering Airborne Particle Counter for Clean Spaces

This document will analyze **Part 4**, which has the title "**Determination of particle size distribution—Single particle light interaction methods**" and specifically refers to the Light Scattering Aerosol Particle Counter (LSAPC).

Before ISO 21501

Prior to the introduction of ISO 21501, there were two standards:

- **JIS B 9921:1997** – "Light scattering automatic particle counter". A Japanese standard that defines Optical Particle Counters performance and counting efficiency
- **ASTM F 328-98** – "Standard Practice for Calibration of an Airborne Particle Counter Using Monodisperse Spherical Particles". The standard was withdrawn in 2007.

The introduction of ISO 21501-4, in 2007, requires the adoption of Pulse Height Analysis (PHA) particle sizing technology and particle standard spheres with international traceability and uncertainty equal to or less than ± 2.5%

Moreover, the standard defines two main goals: *improve the instrument-to-instrument data correlation* and *improve the particle count accuracy*.

The documentation and approval of ISO 21501 replaces ISO 13323-1:2000. The ISO 21501 standard widens the scope of analysis to include methodology for both airborne and liquid particle counting (light scattering and extinction methods).

Relationship with ISO 14644-1:2015

The recent release of ISO 14644-1:2015 establishes a stronger relationship between the two standards and proposes the need for ISO 21501-4 compliant particle counters in cleanroom certification and monitoring applications.

Section B.2.2 of the previous standard, ISO 14644-1:1999, required particle counting to be performed using "calibrated" instruments, and did not require a specific calibration technique.

> **B.2.2 Instrument calibration**
> The instrument shall have a valid calibration certificate; the frequency and method of calibration should be based on current accepted practice.

ISO 14644-1:2015 subsection A.2.2 requires instrument calibrations specified in ISO 21501-4.

> **A.2.2 Instrument calibration**
> The particle counter shall have a valid calibration certificate: the frequency and method of calibration should be based upon current accepted practice as specified in ISO 21501-4.
>
> *Note: Some particle counters cannot be calibrated to all of the required tests in ISO 21501-4. If this is the case, record the decision to use the counter in the test report.*

As the note above states, not all instruments will meet the requirements. Non-compliant instruments will require an additional explanation and instrument approval for the cleanroom's certification audits.

ISO 21501-4 Calibration Requirements

This section will list all the ISO 21501-4 calibration requirements, with a brief description of the test method adopted:

Sampling Flow Rate

The standard uncertainty of volumetric flow rate shall be equal to or less than **5%**.

Note: If the LSAPC does not have a flow rate control system this subclause does not apply, however the manufacturer shall specify the allowable limit of its flow rate of the LSAPC.

The flow rate calibration is performed to ensure a known volume is presented for counting particles. Specifically, the standard provides two different requirements based upon the type of instrument (Optical Particle Counter) under test:

Instrument with a Flow Control System (i.e. pump or blower)

- Compare the Unit Under Test (UUT) flow to certified reference flow meter
- Passing range is ± 5% of nominal

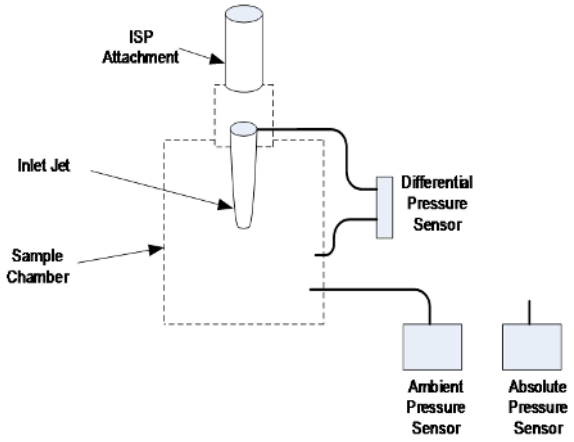
Instrument without a Flow Control System

- UUT samples from a flow set by certified flow system
- Manufacturer specifies the allowable limits of its flow rate

How Particle Measuring Systems Instruments Control the Flow Rate

Particle Measuring Systems (PMS) particle counters measure the mass flow using a differential pressure sensor that regulates the pressure drop in the sampling region.

- Pressure drop across the inlet and outlet jets is measured and provides the most precise measurement of flow
- Mass flow is corrected to volumetric flow by using data from an additional atmospheric sensor, then calculating the volumetric flow using the mass flow measured value and the atmospheric pressure



The Lasair® Pro Aerosol Particle Counter and the IsoAir® Pro Aerosol Particle Sensor use a sophisticated system described by U.S. Patent 6,167,107 that controls the volumetric flow. Competitive particle counters do not provide this type of flow control.

IsoAir® Pro-E

Lasair® Pro

Why Volumetric Flow?

- Particle concentrations are characterized as particles per-unit-volume, so controlling volumetric flow is essential
- Changes in barometric pressure can introduce errors as large as 3% when using a mass flow meter. Particle Measuring Systems' flow control adapts to changes in elevation and atmospheric pressure.

Counting Efficiency

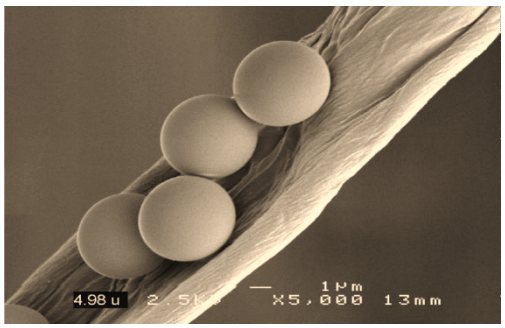
The counting efficiency shall be **50% ± 20%** for calibration particles with a size close to the minimum.

The counting efficiency shall be **100% ± 10%** for calibration particles with a size of 1.5 to 2 times larger than the minimum detectable particle size.

The Counting Efficiency is a ratio of the measured particle data between a UUT and a reference instrument.

The test is performed using calibration particle standards, known as *polystyrene latex spheres* (PSLs), with two sizes: one that is close to the minimum detectable reported size range and another that is 1.5 to 2 times larger than the minimum detectable size. An image of PSLs is shown at right.

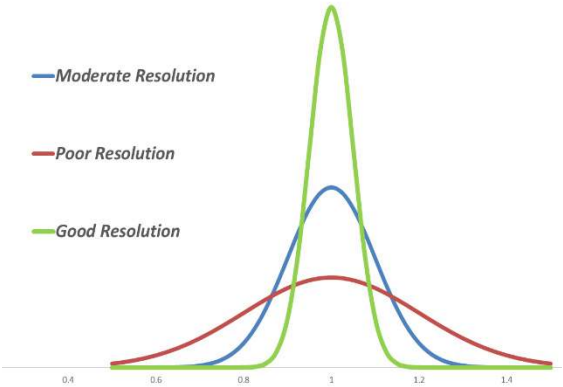
PMS employ Universal Reference Instrument (URI) particle counters that are calibrated against a Scanning Mobility Particle Sizer and Condensation Particle Counter.



Polystyrene latex spheres (PSLs)

Resolution

The size resolution shall be equal to or less than **15%** for calibration particles of a size specified by the manufacturer.



The resolution test verifies the instrument's ability to resolve small differences in particle size.

How is Resolution Calculated?

Simply, the resolution is the standard deviation divided by the mean size of the particle.

$$\text{Resolution (\%)} = \left(\frac{\sqrt{\sigma^2 - \sigma_P^2}}{X_P} \right) \times 100$$

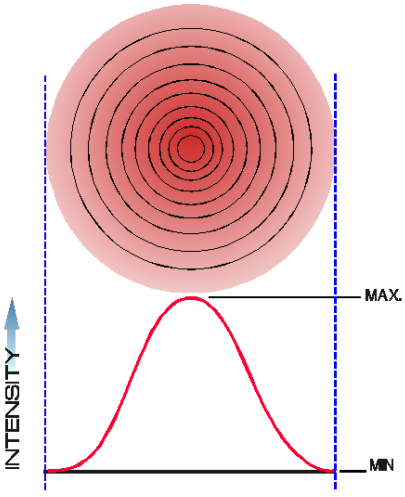
where

σ is the standard deviation reported by the instrument while sampling a particle standard (e.g. PSL)

σ_P is the standard deviation reported by the PSL's published value

X_P is the mean particle size for the particle standard being sampled

The calibrated PHA device analyzes the calibration curves and automatically determines optimal resolution by calculating the absolute value of the differences between the PSL particle size standard and the particle size measured by the instrument. Further details can be found in the ISO 21501-4 document.



Why Isn't Resolution Perfect?

The laser beam's intensity is highest in the center and degrades towards the edges, as shown in the diagram at left. As a result, particles size larger when crossing through the beam's center than similar particles crossing the beam's edge. The Gaussian-shaped curve (shown below the beam pattern) illustrates the resultant light scattering peak as a particle passes through the laser beam.

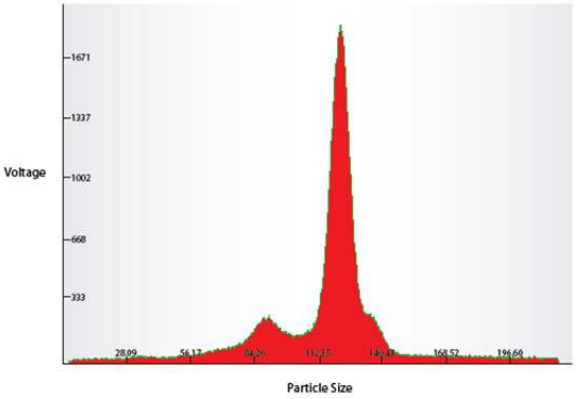
Resolution is improved if the particles are illuminated by the most uniform and highest intensity light. During instrument design and development, PMS optimizes resolution by integrating beam-shaping lenses and masks that can produce more consistent particle sizing, with accurate and repeatable results.

What is the Pulse Height Analyzer (PHA)?

Particle counters employ solid-state photodetectors that convert scattered light energy into electrical signals.

The signals, known as *pulses*, are proportional amplitudes of energy that represent the particles or photons witnessed by the detectors. The pulses are sent to the PHA, and the PHA correlates the relative pulse amplitude to the instrument's particle size thresholds or *bins*.

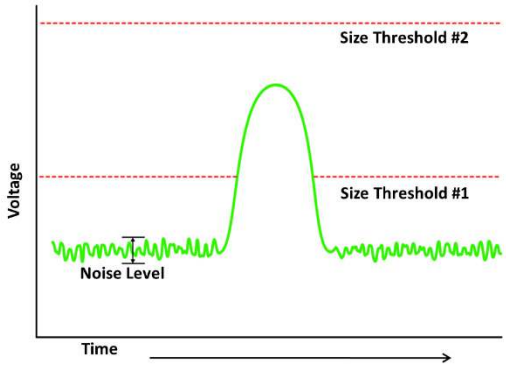
Each particle channel is represented by a counting bin and totaled with other pulses that are of the same amplitude.



False Count Rate

The false count rate is determined y measuring the particle number concentration in the unit of counts-per-cubic-meter at the minimum reported size range when sampling clean air.

No Range Limits



The zero count test is a measurement of "electrical or signal noise".

ISO requires these specifications to be met when performing the zero count test:

> *"...the measured particle number concentration per m^3 when the LSAPC is set to the minimum detectable size and particle free air flows to the LSAPC."*

In addition, the ISO 21501-4 standard requires that only the count rate be reported. Some standards, including the Japanese Industrial Standard (JIS), do not have this requirement.

Going beyond this requirement, PMS particle counter calibrations include a final zero count test, which challenge the instrument to achieve restrictive limits on false counts.

The use of restrictive limits, even when not required by the standard, guarantee higher accuracy in the instrument performance and avoids false counts that appear as particle counts.

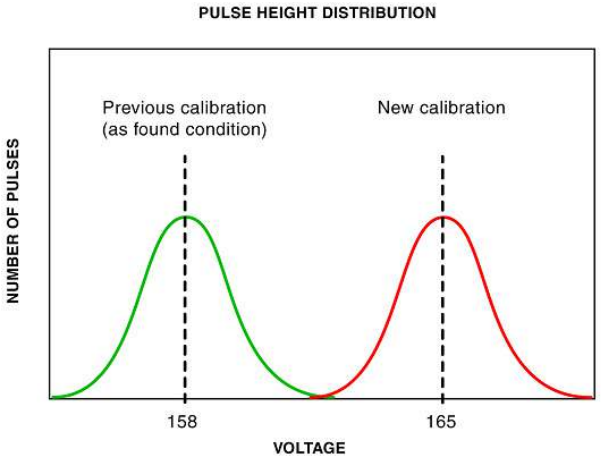
Particle Size Setting

The error in the particle sizes shall be less or equal than **10%**.

The particle sizing error is calculated for the minimum detectable size and additional sizes. The test demonstrates that if an instrument accurately sizes particles, it will also accurately count particles. ISO 21501-4 requires the use of a PHA to determine the particle distribution of three different calibration particles that span the range of particle sizing.

During instrument design, PMS develops a particle sizing curve. The curve defines calibration points and their associated threshold voltages or values. The curve also shows the associated values for other particles, across the entire sampling range of the instrument.

The graph at left shows a threshold/voltage values between the "as found" condition and the calibration's final result. The sizing error is the difference between the instrument's reported particle size range and the calculated particle size.



Additional ISO 21501-4 Calibration Requirements

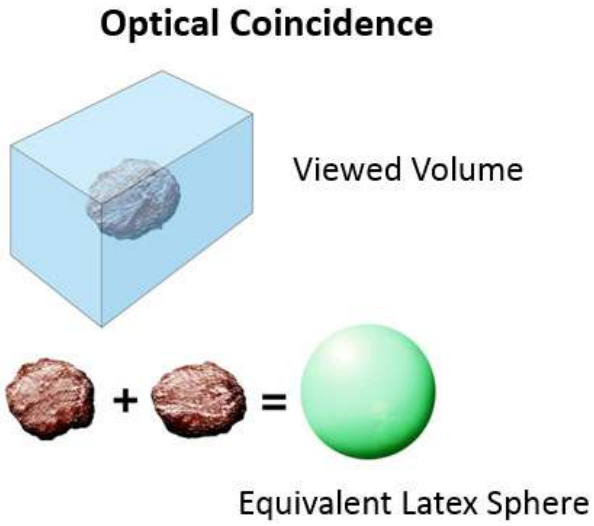
Determined during the design, development and proof of concept

Coincidence Loss at Maximum Particle Concentration

The coincidence loss, at the maximum measurable particle number concentration specified by the manufacturer, shall be **≤ 10%**.

Coincidence loss is the percentage of error when counting large particle concentrations and is typically, accompanied by a perceived shift in size distribution. There are two primary contributors to coincidence loss:

1. Optical coincidence - caused by having more than one particle in the laser "viewed volume" at the same time
2. Electronic coincidence - which limits the number of particle events that can be processed within the system electronics per unit of time
    - In modern particle counter electronic designs this typically exceeds optical limitations by a wide margin.



Except in the most extreme cases, the presence of coincidence loss is visible as a shift in particle distribution.

Maximum Particle Number Concentration

≈ as specified by the manufacturer

The maximum particle number concentration occurs at 5 – 10 % coincidence loss. The value is calculated using the particle transit time through the laser beam and the instrument's flow rate.

Sampling Time

The standard uncertainty in the duration of sampling time shall be **≤ ± 1%**.

Response Rate

The response rate of the LSAPC shall be **≤ 0.5%**.

Conclusion

Cleanrooms that manufacture products adopt procedures to improve yield and increase throughput. Consequently, many manufacturers follow ISO 14644-1 for determining particle counts and classifying cleanrooms (based upon airborne particle data) and ISO 14644-2 for instrumentation guidelines that demonstrate continued compliance.

The newly released ISO 14644-1:2015 introduces the need for particle counters calibrated to ISO 21501-4. The calibration methods assure data accuracy and repeatability.

Pharmaceutical manufacturers and other cleanroom users should then look to ISO 21501 as a method to meet current Good Manufacturing Practice (cGMP), EU Good Manufacturing Practice (EU GMP), ISO 14644-1, and other requirements.

The cGMP and EU GMP guidelines define limits for airborne particle counts, which are based upon specific cleanroom/ clean space classification (e.g. Grade A, Grade B, Grade C, Grade D). However, the guidelines do not provide:

- The methods to determine the particle counts within a clean space
- The instrument to measure particle counts within a clean space
- The calibration methods for particle counters to assure data accuracy

The methods outlined in ISO 14644 and ISO 21501-4 represent an important step in more accurate evaluations of clean room contamination and improved process control.

More information about ISO 14644 and how PMS can provide specific and innovative compliance support and regulatory undersrtanding. See the ISO 14644-1/2 – Cleanroom Classification and Monitoring page on the Particle Measuring Systems website.

For more information about the new ISO 21501-4, contact your Particle Measuring Systems local representative or navigate to our Contact Us webpage.

Authors

Daniele Pandolfi

Global Product Specialist, Aerosol
Life Science Division
Particle Measuring Systems

Steve Kochevar

Senior Global Applications Engineer
Electronics Division
Particle Measuring Systems

© 2023 Particle Measuring Systems. All rights reserved.
Lasair® and IsoAir® are registered trademarks of Particle Measuring Systems.
Reproduction or translation of any part of this work without the permission of the copyright owner is unlawful. Requests for permission or further information should be addressed to Particle Measuring Systems, Inc. at 1-303-443-7100
App Note 214. 10232023