

# Meet Hound: three lasers make particle ID a breeze

## Introduction

Parenterals and other drug products are subjected to strict requirements to be essentially free of visible particles. Injectable drug products must be free of visible particles  $>50\ \mu\text{m}$ . Guaranteeing reproducibility and consistency requires significant resources throughout formulation, development, and manufacturing of a drug product. When unidentified particles are found, the root cause must be determined and eliminated before production can continue.

Tablet, inhaler, and topical cream drug products are also subjected to strict requirements for the composition, purity, and distribution of active particles. The distribution of active pharmaceutical ingredients (APIs) and excipients must be analyzed and consistent across lots. Analyzing particle and API distribution can be a time-consuming step in the drug manufacturing process.

Hound combines automated microscopy, dual Raman at 532 nm and 785 nm, and Laser Induced Breakdown Spectroscopy (LIBS) in a single instrument (**Figure 1**). Hound enables scientists to count, size, and shape particles, as well as identify particle composition at a chemical or elemental level to help you find the source. The dual Raman lasers on Hound identify a wide range of protein, organic, and inorganic particles, while LIBS IDs metals, glass, and other elemental particles. Particles can be identified down to the exact source material in just minutes.

Hound contains a digital camera and five microscope objectives – 5x scan, 10x scan, 20x bright field/dark field, 20x LIBS, and 50x Raman – to image and analyze particles. These objectives pair with an automated stage and manual joystick controls for easy use.

Hound can be used to directly identify contaminants. With specialized filter rounds, you can filter and distribute visible and subvisible parti-



**Figure 1:** Hound counts and identifies the composition of visible and sub-visible particles with both automated and manual modes. Hound uses Raman (532 nm and 785 nm) and LIBS to identify the composition of particles, helping users track down the particle source.

cles from a vial or a larger solution volume. Specialized wet rounds are also available to identify particles in suspension. Both materials are gold coated to minimize background noise during Raman spectroscopy (**Figure 2**). Gold coated microscope slides can be used on Hound to spread products, such as topical creams.

Hound software has 21 CFR part 11 compliance tools throughout data collection and analysis. The software has four data collection applications to accommodate a variety of particle analysis needs.

The Image application lets a user quickly capture images and mosaic scans of the sample area. Particle counting provides details on particle number, size, and morphology automatically. Particles larger than a user-defined size can be counted in a custom region of interest with this application.

The Identify application uses Raman and LIBS to identify the chemical and elemental composition of particles based on spectral data. Spectra

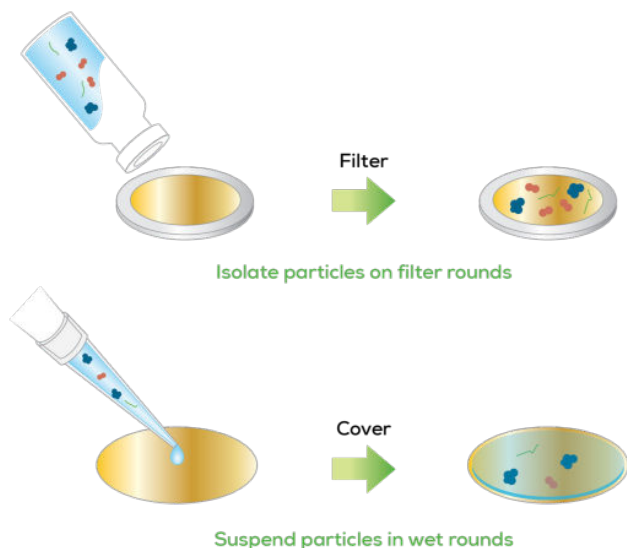


Figure 2: Hound uses gold coated filter rounds and wet rounds to isolate or suspend particles for counting and identification. The gold coating minimizes background interference during Raman spectroscopy.

collected by Hound are automatically compared to a built-in reference database and any custom databases to find the exact composition match of the particle. The Identify application allows users to change the Raman wavelength used, exposure time, and laser intensity to optimize Raman spectra collected. Users can also switch to LIBS to analyze metal and elemental particles in the same experiment.

Automated versions of each application allow users to load up to four samples and walk away while Hound analyzes thousands of particles per sample. The automated Count, Size and Shape application takes user-defined size binning and particle thresholding parameters to count all particles in the sample area while gathering size and morphology information. Count, Size, and Shape can image the entire defined sample area, capture high quality images of particles based on their size and shape, and determine the size distribution of particles in a mixture.

The automated Image-directed ID application provides the count, size and shape of particles and identifies particles with Raman and LIBS. Hound Client allows users to define what particles to identify based on particle size, various

morphology parameters, or a total number of the largest or random particles based on the size distribution of particles. High quality images can be captured of every particle analyzed with Raman or LIBS during automated image-directed ID.

Raman 785 nm and Raman 532 nm can both be used to identify particles in a single experiment. The user defines which Raman laser to use to ID particles. The user also defines if Hound should use the second laser to ID all particles again, or only unidentified particles. Following automated analysis, verification mode allows users to reanalyze any particle, change Raman wavelengths, exposure times, and laser intensity, or switch to LIBS if an elemental particle is suspected.

The automated image-directed ID application also allows for automated LIBS analysis of numerous elemental particles. For automated LIBS, particles are adhered to the surface of a nitrocellulose adhesive round to prevent movement during the experiment. Following automated LIBS, verification mode can again be used to reanalyze particles, or to use either of the Raman lasers to identify any non-metal particles.

In this application note, Hound was used to manually ID a few visible particles found in a drug product, and to automatically count and ID particles found in a sample. In each experiment, Raman 532 nm, Raman 785 nm, and LIBS were used to identify various types of particles.

## Methods

### Match criteria

Spectra from all particles were compared to the built-in Raman and LIBS reference databases on Hound for identification. Particles were also compared to a custom reference database containing common laboratory supplies. A match rank between the sample and the reference spectra was calculated by multiplying the Pearson correlation by 1000. A match rank greater than 700 (out of 1000) was considered a high-quality match.

## Manual particle identification

A protein sample was inoculated with three unknown, visible particles for analysis on Hound. The visible particles were extracted and placed on a filter round. The particles were then analyzed with the Identify application. Raman 785 nm, Raman 532 nm, and LIBS were used to identify the composition of the three particles.

## Automated particle identification

A sample containing numerous particles was prepared by inoculating Kimwipe fibers, plastic vial cap particles, and a metal crimp cap particle into a particle-free vial. The visible particles were analyzed with the automated image-directed ID application on Hound. Visible and subvisible particles were captured on a gold filter round with 0.8  $\mu\text{m}$  pores, and all particles  $>100 \mu\text{m}$  were counted. Spectra from these particles were compared to a custom reference database of common lab supplies.

Raman 785 nm, Raman 532 nm, and LIBS were all used to identify the particles. The automated experiment was set up to analyze particles with Raman 785 nm first, then Raman 532 nm. Verification mode was used to ID metal particles with LIBS. At 785 nm spectra were collected with a laser intensity of 38% for 60 seconds, at 532 nm a laser intensity of 58% was used for one second. Three main particle types were identified across the sample. Verification mode was used to capture any additional spectra from the sample in the same experiment.

## Results

### Manual particle identification

Three unknowns were manually identified from the protein sample using all three lasers and the Identify application. A metallic particle was found in the sample and analyzed with LIBS. It was identified as copper with a match rank of 939 (Figure 3A). A fiber was found in the sample and analyzed with Raman 785 nm. The fiber was identified as cellulose with a match rank of 918 (Figure 3B). A third unknown particle was found in the sample and identified as polystyrene with a

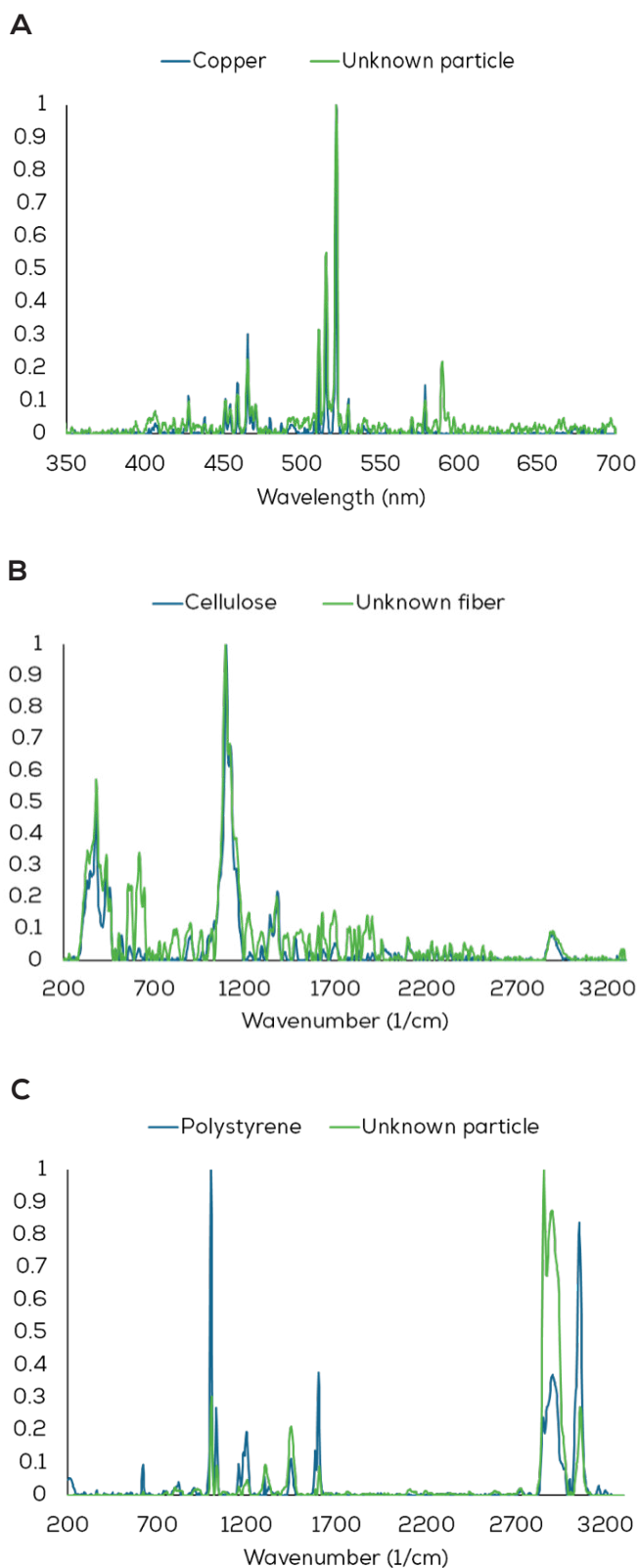


Figure 3: Manual identification of particles inoculated into a protein sample. **A:** LIBS analysis of a particle in the protein sample (green) identified copper with a match rank of 939 to the reference (blue). **B:** Analysis of a fiber with Raman 785 nm identified cellulose (green) with a match rank of 918 to the reference (blue). **C:** Analysis of a particle with Raman 532 nm identified polystyrene (green) with a match rank of 972 to the reference (blue).

match rank of 972 (Figure 3C), using the Raman 532 nm laser.

### Automated particle identification

75 large particles (>100  $\mu\text{m}$ ) were found after filtering the contents of the sample vial (Figure 4). Three types of particles were identified from the sample with the automated image-directed ID application. Raman spectroscopy at 785 nm analyzed particles first, followed by Raman 532 nm analysis. Cellulose fibers from a Kimwipe were identified with Raman 785 nm throughout the sample (Figure 5A). Polypropylene from a plastic vial cap was also found in the sample, with the 532 nm Raman laser being optimal to identify the plastic particles (Figure 5B). A metal particle was found and identified using LIBS. The metal was found to be aluminum from a Wheaton crimp cap (Figure 5C).

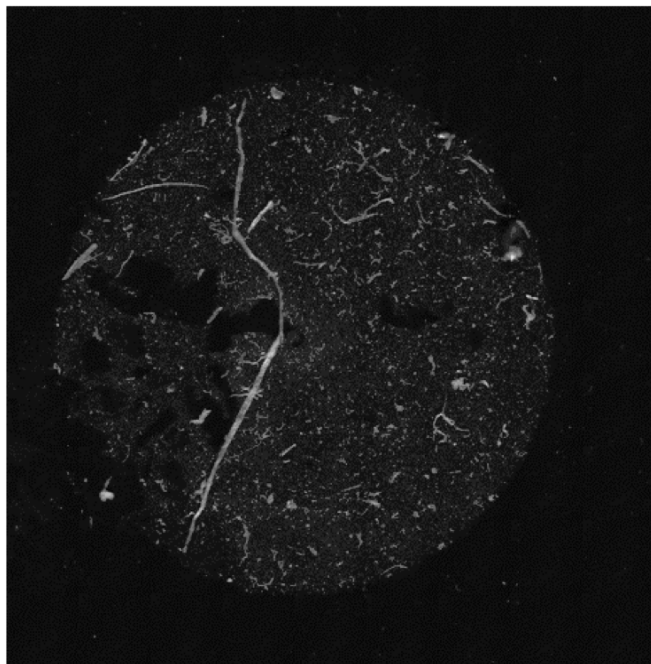


Figure 4: A mosaic scan of the entire filter area showing all particles captured from the sample vial. 75 particles >100  $\mu\text{m}$  were automatically counted and analyzed with Raman 785 nm, Raman 532 nm, and LIBS.

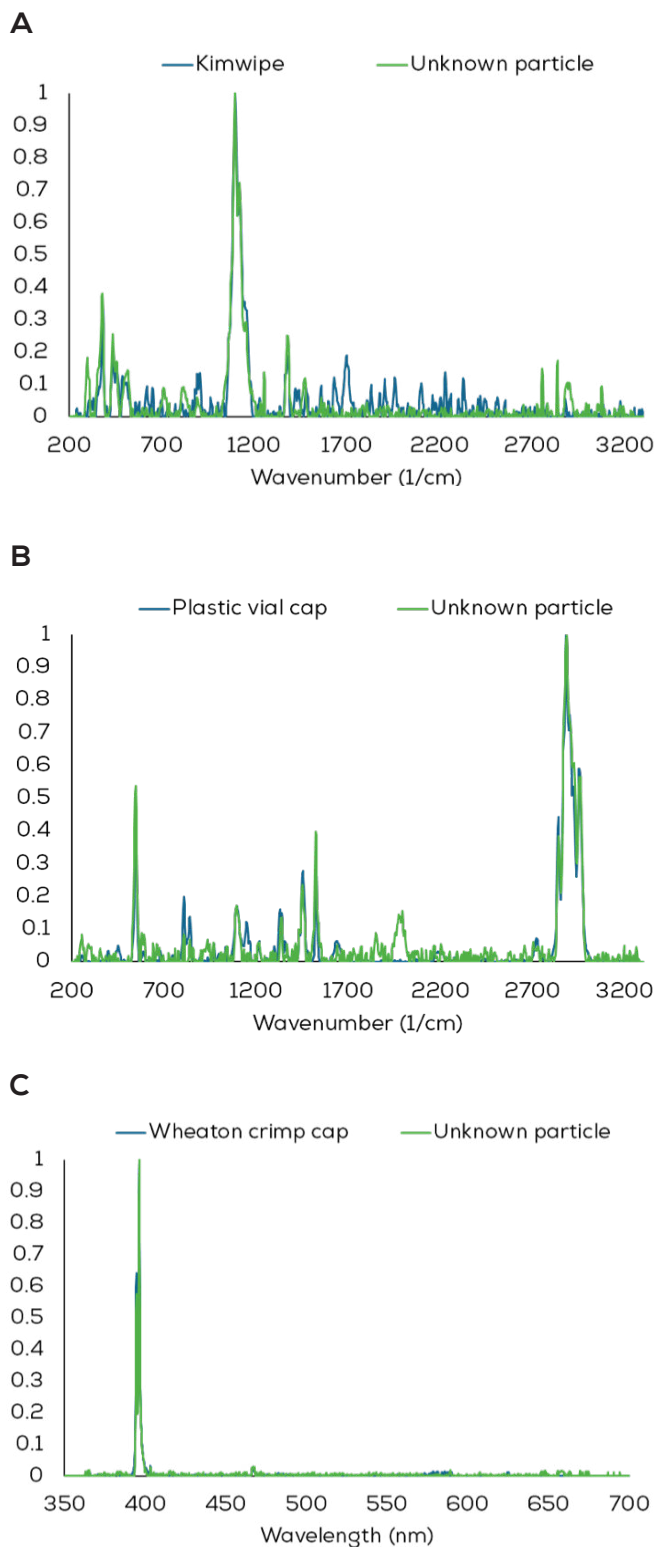


Figure 5: Automated identification of particles known to come from Kimwipe fibers, plastic vial caps, or metal crimp caps. **A:** Raman 785 nm analysis of a fiber identified as cellulose from a Kimwipe (green) with a match rank of 927 to the reference (blue). **B:** Analysis of a particle with Raman 532 nm identified as polypropylene from a plastic vial cap (green) with a match rank of 965 to the reference (blue). **C:** LIBS analysis of a metal particles identified the particle as aluminum from a Wheaton crimp cap (green) with a match rank of 995 to the reference (blue).

## Conclusion

With three lasers, Hound can identify a wide range of contaminants in a single experiment. Hound can identify organic, inorganic, protein, and elemental particles in solution to help identify the root cause of contamination. Manual and automated particle counting and identification modes enable the operator to ID a few particles quickly, or thousands of particles with minimal hands on time. Hound uses three lasers, spanning two spectroscopy types, paired with a fully customizable reference database to identify the composition of unknown particles, leading you to the exact source of particles in your process.



**Unchained Labs**  
6870 Koll Center Parkway  
Pleasanton, CA 94566  
Phone: 1.925.587.9800  
Toll-free: 1.800.815.6384  
Email: [info@unchainedlabs.com](mailto:info@unchainedlabs.com)

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