# Blast through your problems: ID inorganic and metal particles with LIBS on Hound

## Introduction

The presence of visible and subvisible particulate matter is a risk throughout the development, packaging, and delivery of biologic drugs. There are many sources of potential particulate contamination. Inherent particles, like protein aggregates, come from the formulation itself. Significant contamination risks can come from intrinsic sources such as metal fragments or filter fibers from processing equipment, or glass chips from primary packaging. Extrinsic sources like hair or clothing fibers are also contamination risks. The list of potential contaminant spans protein, organic, inorganic and metal particulates.


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 or 785 nm) and LIBS to identify the composition of particles, helping users to track down the source of particles.

Hound combines automated microscopy, Raman spectroscopy, and Laser Induced Breakdown Spectroscopy (LIBS) in a single instrument to identify visible and subvisible particles across a wide range of chemical compositions (Figure 1). Hound can either fully automate the identification of up to 5,000 particles or be used manually to quickly identify a few particles. Automated microscopy can be used to rapidly count and size all particles in a sample. Raman spectroscopy is used to chemically fingerprint protein, organic, and inorganic particles. LIBS is used to perform elemental analysis to identify inorganics and metals. Particles are identified by matching the chemical and elemental fingerprints with built-in Raman and LIBS reference databases. In addition to the built-in database, custom reference spectra can be added to tailor identification to a specific process.

In this application note, Hound was used to identify the elemental composition of four common inorganic contaminants; a metal vial cap fragment, an unknown metal particulate, copper wire, and quartz shard. The metal cap fragment was compared to a custom reference library of items found in our laboratory to identify the exact source of the particle. The composition of each of the four particles was identified with manual LIBS analysis.

## Methods

### Sample preparation

Visible particles were made by cutting or shattering materials commonly found in a laboratory. Metal particles were collected from three different sources; a Wheaton Industries metal vial cap, copper jumper wire, and an unknown metal fragment from a machine shop. Silicon particles were prepared from a quartz cuvette. Samples were prepared by placing particles on a cellulose nitrate (CN) membrane, which was then glued to an aluminum mesh backing, creating an adhesive round. Adhesive rounds were allowed to dry completely before particle identification with Hound.

### Sample identification

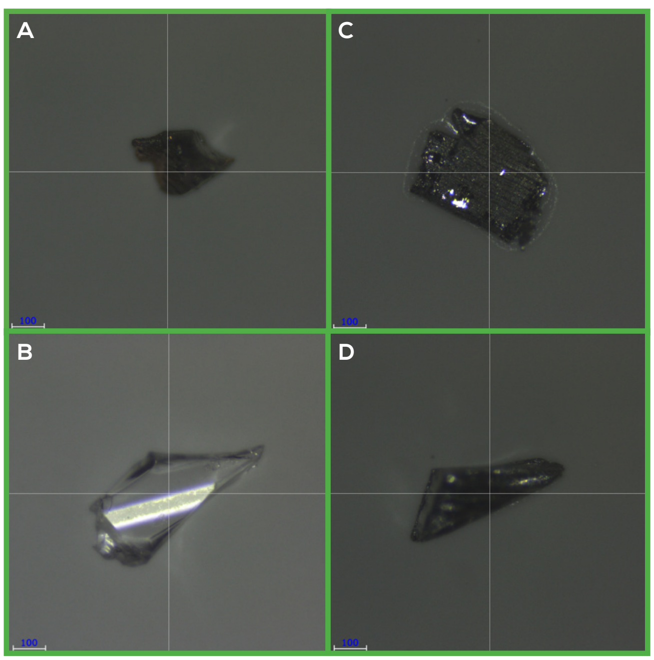
Each of the adhesive rounds contained a single particle that was analyzed manually with LIBS on Hound. Adhesive rounds were placed on Hound and each particle was located using a 5x scanning objective to image the entire particle.

Most particles were identified with a single LIBS measurement. Particles with an outer coating (copper wire in this case) required repeat measurements at a single spot to first burn through the outer coating, then collect a measurement for particle identification.

### LIBS match criteria

Spectra from all particles were compared to the built-in LIBS reference library on Hound for identification. 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. All spectra were matched to the built-in reference library in the Hound Spectral Tools software.

Following manual identification of the metal vial cap particle, the resulting spectrum was compared to a custom reference library that contained a user-created reference spectrum for the exact Wheaton Industries vial cap used. The spectrum from the metal vial cap particle was compared to the custom reference library in the Hound Spectral Tools software.




| Particle | Match name | Particle Source |
|---|---|---|
| A | Copper (Cu) | Copper wire |
| B | Silicon (SiC F100) | Quartz cuvette |
| C | Aluminum | Unknown metal |
| D | Aluminum | Metal vial cap |

Figure 2: Images captured by manually scanning four adhesive rounds at 5x. Each adhesive round contained a single particle. Each particle was manually identified by LIBS based on a match rank of greater than 700. Spectra were compared to Hound's built-in reference library.

## Results

### LIBS analysis

Images were captured of each particle using the 5x objective (Figure 2), followed by identification with LIBS and the built-in Hound reference library. Hound identifies the composition of a particle by comparing the spectrum obtained during analysis to spectra in the reference library to find a match.

Two repeated LIBS measurements were needed to identify the composition of the copper wire. The first measurement returned an unidentified result, but burned through the outer coating on the wire. The second measurement in the same location identified the composition of the particle as copper. The copper wire was identified as copper (Cu) with a rank of 763 (Figure 2A).

The quartz particle was identified as silicon (SiC F100) with a rank of 789 (Figure 2B). Quartz is a mineral made of silicon, so silicon is the closest match in the built-in reference library used. The unknown metal particle was identified as aluminum with a rank of 846 (Figure 2C).

The metal vial cap particle was identified as aluminum with a single measurement when compared to Hound's built-in reference library (Figure 2D). The spectrum obtained from the metal vial cap particle was a high match to the reference library spectrum for aluminum with a rank of 825 (Figure 3).

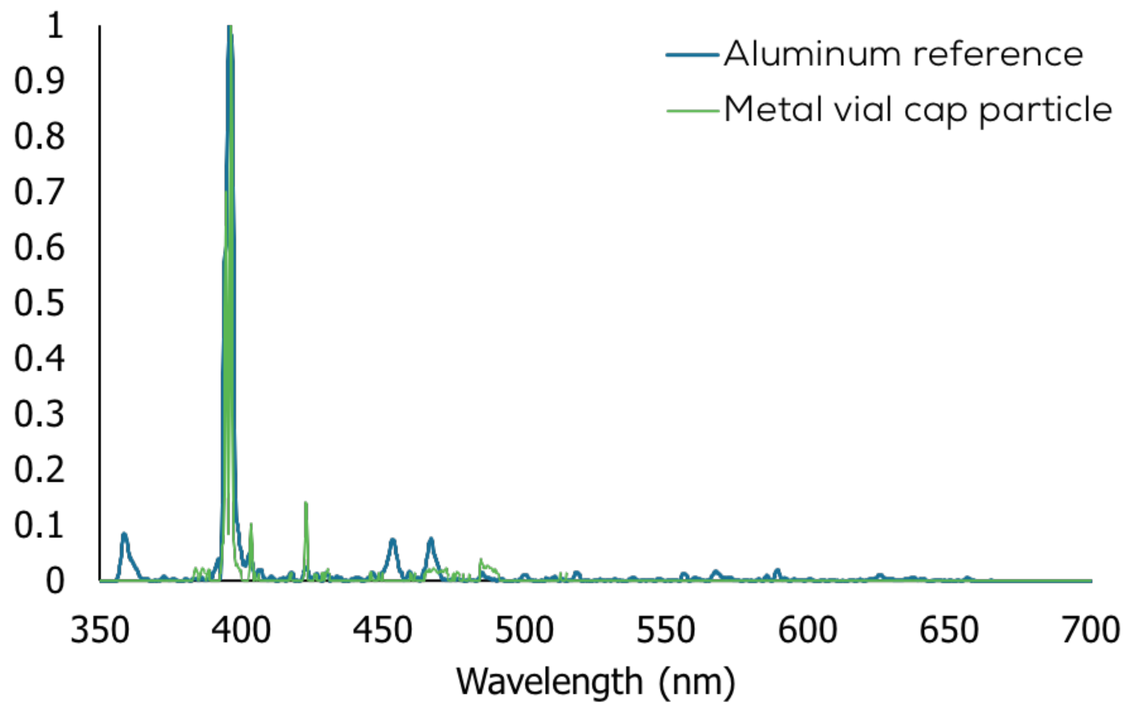



Figure 3: The spectrum captured by Hound of a metal vial cap particle (green) compared to the reference spectrum for aluminum (blue) in the built-in LIBS reference library. The metal vial cap was matched to aluminum with a rank of 825.

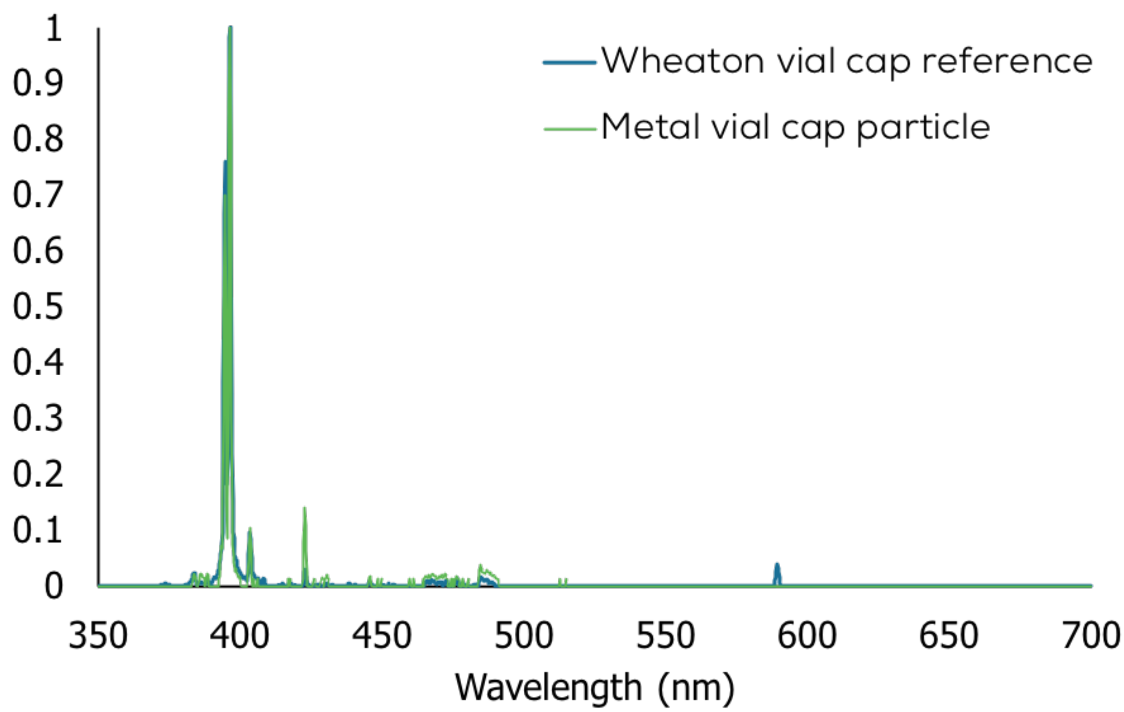



Figure 4: The spectrum captured by Hound of a metal vial cap particle (green) compared to the custom mean reference spectrum for the brand of aluminum vial cap used in this study (blue) in the custom reference library. The metal vial cap was matched to the aluminum vial cap custom reference with a rank of 986.

The spectrum from the metal vial cap was re-analyzed and compared to a custom reference library containing the LIBS spectrum for the Wheaton Industries vial cap used to make the particle in this study. The metal vial cap matched the Wheaton vial cap reference spectrum with a rank of 986 out of 1000 (**Figure 4**).

## Conclusion

Hound with LIBS was able to identify the composition of four elemental particles. Using the built-in reference library for Hound, a user can obtain a particle's elemental identification to narrow down the particle source. A custom reference library makes it possible to narrow down the exact source of a particle. In this application, a particle was identified as aluminum with the built-in reference library and specifically identified as a Wheaton vial cap by adding a custom reference spectrum. With data in hand it is possible identify the source of a particle and perform root cause analysis for particle contamination.

UNCHAINED LABS

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



Rev A