# Detecting Glass Particles: Making the Invisible Visible to Avoid the Leading Cause of Drug Recalls

## Introduction

Particles account for 38% of all drug recalls.[1] Out of all inherent, intrinsic, or extrinsic particle types, delaminated glass particles present the gravest threat, accounting for >110 recalls between 2009 and 2017 alone.[2] Glass particles from defective vials are dangerous subvisible particles due to their ability to puncture capillaries and tissue.[3] But why have they been so elusive to analytical detection? Glass is optically transparent and has an exceptionally low refractive index ($n_{glass}$ ~ 1.5), which is close to the refractive index of water ($n_{water}$ ~1.33). This low refractive index of glass in solution makes these dangerous particles virtually invisible to flow based subvisible particle analyzers including flow imaging and light obscuration.[4]

## Methods

Glass particles were obtained by polishing ultrapure glass vials and then sieved via a 200 µm filter. Commercially available mAb solutions varying from 1–200 mg/mL were provided by a leading pharmaceutical company. Mixed samples containing varying amounts of glass (0–100 mg/mL) and a fixed concentration of mAbs (2 mg/mL) were measured in BMI and SIMI using three replicates of 40 µL per well for every glass dilution condition.

Aura's primary particle detection is based on Backgrounded Membrane Imaging (BMI), which is a high optical contrast method for imaging, counting, and sizing subvisible particles. In BMI, the liquid matrix is removed from the measurement and the particles are measured in air ($n_{air}$ =1), producing large optical contrasts Δn≥0.4. In addition, Aura has the ability to assess samples from two light sources including Side Illumination Membrane Imaging (SIMI) and Fluorescence Membrane Microscopy (FMM) which use different incident angles and fluorescence properties respectively to quickly and accurately identify different particle types. In this technical note, we show how BMI and SIMI specifically detect glass particles in commercial antibody formulations.

## Results and Discussion

*Figure 1* shows subvisible particles imaged in BMI (*Figure 1a*) and in SIMI (*Figure 1b*). These low refractive index particles are easily detectable in both imaging modes, due to the inherent sharp contrast imaging and the strong side scattering from grounded glass which protrudes out of plane. The strong positive SIMI signal seen in *Figure 1b* is caused by side scattering from particles protruding out of the plane, indicating that these are glass.

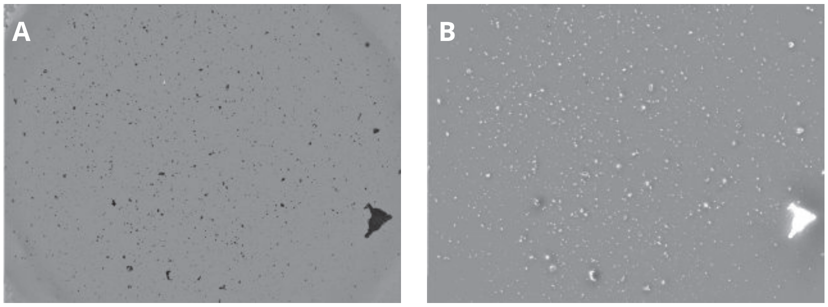


**Figure 1:** Glass particles imaged in (a) BMI and (b) SIMI.

A more quantitative look at the ground glass samples is presented in *Figure 2* which shows the SIMI intensity vs. particle size (Equivalent Circular Diameter, ECD in µm). We observe that SIMI scattering increases proportionally with glass particle size starting as small as 2 µm. Most particles were small but there were also several particles ≥25 µm in size.

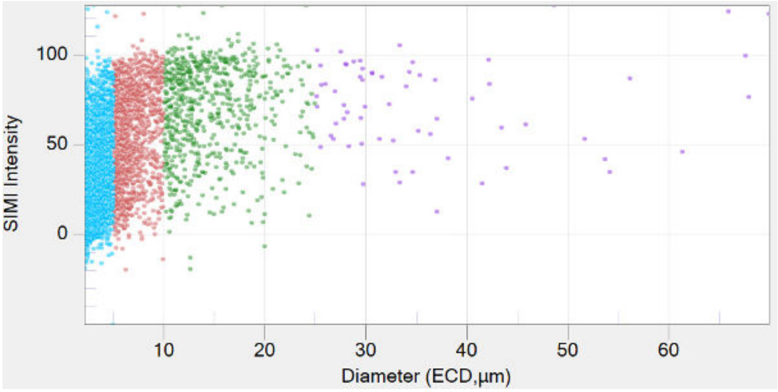


**Figure 2:** Scatter plot of SIMI intensity vs. Equivalent Circular Diameter (ECD) for glass particulates. Note that protein particles were identified using FMM and are excluded from this figure.

In *Figure 3,* it is seen how BMI can detect these nearly invisible glass particles in a commercially available monoclonal antibody formulation. In this experiment, the antibody concentration is kept constant at its commercially specified 2 mg/mL, and the concentration of glass powder varies from 0.01–100 mg/mL. Glass particles were detectable even in the lowest of concentrations. For concentrations below 10 mg/mL of glass powder the linearity was $r^2$>0.99. Membrane coverage is a simple metric that quickly diagnoses when a well is heavily covered with a given particle type and useful as a general screening parameter.

## Conclusions

Aura can detect glass particles in highly concentrated antibody formulations. BMIs high sensitivity/RI contrast measurements, supported by additional illumination modes such as SIMI, make it easy to detect these particles. In addition, the high refractive index contrast of BMI allows accurate counting and sizing of the glass particles, without the limitation of flow imaging where differentiating glass in a medium of similar RI leads to inaccuracy and imprecision. (Learn more in TechNote 5). Aura's SIMI and BMI can help researchers avoid dangerous and expensive recalls from the peskiest of particles.

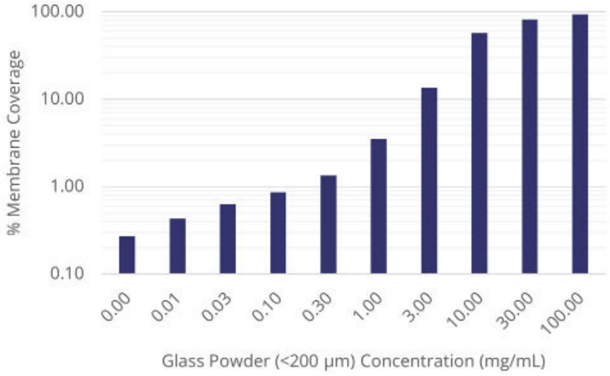


**Figure 3:** Membrane coverage from glass particles with increasing concentrations of glass powder.

## References

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