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**Research** article

## Imaging flow cytometry methods for quantitative analysis of label-free crystalline silica particle interactions with immune cells

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Appendix



**Supplementary Figure 1.** Particle size distribution (as volume percent) of crystalline silica (Min-u-sil® 5) in ultra-high purity (UHP) water as measured using static light scatter (Malvern Mastersizer 2000). Measurements correspond to the average of 3 replicate measurements. The volume median diameters (DV) in which 10% (DV0.1), 50% (DV0.5) and 90% (DV0.9) of the particles are greater in size were 0.62  $\mu$ m, 1.74  $\mu$ m and 5.7  $\mu$ m, respectively.



**Supplementary Figure 2.** Correlation between crystalline silica titrated dose and darkfield signals in association with CD14<sup>+</sup> phagocytes within PBMC. A. Correlation between darkfield bright detail intensity (BDI) high gated CD14 monocytes and crystalline silica titrated dose from 0.025 to 25  $\mu$ g/mL, Pearson correlation, r = 0.795, P =  $\leq 0.01$ . **B.** Correlation between darkfield CD14 gated monocytes with internalised darkfield signals and crystalline silica titrated dose from 0.025 to 25  $\mu$ g/mL, Pearson correlation, r = 0.669, P =  $\leq 0.01$ . Experimental set up and acquisition was carried out as described within the main text and accompanying data is shown in figure 4 of the main manuscript.

## Supplementary Figure 3. Calculations of the daily exposure level to crystalline silica.

 $EF = (F \times ED) / AT$ 

Where EF = exposure factor (unitless), F frequency of exposure (days/year), ED = exposure duration and AT = average time (ED x 365 days/year)

D = (C x IR x EF) / BW

Where D = exposure dose (mg/kg/day), C = contaminant concentration (mg/m<sup>3</sup>), IR = intake rate  $(m^{3}/day)$ , and BW = body weight (kg)

Assuming a 8 h shift, 5 days/week, 50 weeks/year

F = 8 h/day x 5 days/week x 50 weeks/year

= 200 h/year

= 83.3 days/year

To calculated EF, we need to assume an exposure duration, here we will assume a 1 year period  $EF = (F \times ED) / AT$ 

= (F x ED) / (ED x 365) = (83.3 x 1)/(1 x 365) = 0.228

If we use the WEL as the contaminate concentration, and assume an average male (70 kg) with a default air intake rate (15.2  $m^3$ /day) (https://www.atsdr.cdc.gov/hac/phamanual/appg.html)

D = (C x IR x EF) / BW

 $= (0.1 \text{ mg/m}^3 \text{ x } 15.2 \text{ m}^3/\text{day x } 0.228) / 70 \text{ kg}$ 

= 0.005 mg/kg/day

Therefore, an average male could be exposed to 0.005 mg/kg/day x 70 kg (BW) = 0.35 mg crystalline silica per day.



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