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Flow imaging microscopy-based method for rapid, high-throughput measurement of fiber count and length distributions in air

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Assessing airborne fiber length and number in air samples is crucial for evaluating workplace exposure to asbestos and elongate mineral particles (EMPs). Growing concerns about noncommercial EMPs highlight the need for efficient monitoring methods. Phase Contrast Microscopy (PCM), used in the National Institute for Occupational Safety and Health (NIOSH) Method 7400, is a standard technique but is labor-intensive and time-consuming, examining only about 0.2% of the filter area and yielding 100–200 fiber counts. This study evaluates flow imaging microscopy (FIM) as a rapid, high-throughput alternative for measuring fiber number and length distribution. To validate its accuracy, monodisperse polystyrene latex standards (5–50 μm) were analyzed using 4X and 10X objective lenses. Test glass fibers were prepared as (i) suspensions in deionized water and (ii) aerosols collected on cascade mesh micro-screens to produce fibers of varying lengths. FIM demonstrated accurate sizing for spherical particles (5–50 μm), with biases under 13% for 4X and 3% for 10X. Counting accuracy biases were below 22% for 4X and 10% for 10X, with relative standard deviations (RSDs) of 4.7% and 9.0%, respectively. Fiber length distributions at 10X showed geometric mean lengths of 8.0–26 μm , closely agreeing with PCM (average bias $\sim$ 16.6%). Comparisons of fiber density showed that discrepancies between the two methods decreased as fiber counts increased, highlighting the significance of high-throughput measurement with FIM. The results indicate that FIM's high-throughput ability shows potential for analyzing workplace air samples more quickly and cost-effectively, while still providing superior counting statistics.

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Environmental significance

Reliable assessment of inhalation exposure to airborne fibers, including asbestos and elongate mineral particles (EMPs), is essential for evaluating exposure risks in both ambient and occupational environments. Conventional Phase Contrast Microscopy (PCM) techniques often demonstrate limited precision and poor sampling statistics, particularly at trace concentrations approaching $\sim 0.005 \text{ f cm}^{-3}$. This study examines Flow Imaging Microscopy (FIM) as a promising alternative, offering high-throughput and low-uncertainty measurements. The study presents a robust calibration and measurement methodology to accurately determine fiber-length and diameter distributions in aerosolized fibrous samples. The results demonstrate that this method can potentially provide enhanced sampling statistics and precision, previously unattainable, thereby enabling accurate measurements at ultra-trace concentrations necessary for improved regulatory compliance.

1. Introduction

Fiber counting and length distributions of airborne fibers are important to assess exposures to asbestos and elongate mineral particles (EMPs).^{1–3} It is well known that inhaled airborne asbestos fibers are linked to various respiratory diseases, such as mesothelioma and lung cancer,^{1,4} and that fiber length is considered an important factor in determining

toxicological responses to asbestos and other bio-persistent fibers.^{1,4–17} Exposure to noncommercial EMPs with the potential for asbestos-like health effects is an emerging issue that may affect construction workers in the workplace.^{1,18} These materials can be encountered by disturbing natural deposits during construction activities or using materials such as crushed stone products contaminated with EMPs.¹ These minerals are likely to fracture, creating EMPs of varying lengths, known as cleavage fragments, and potentially causing high exposure levels.¹⁸ Recently, the Environmental Protection Agency (EPA) has proposed an interim existing chemical exposure limit (ECEL) to enhance risk management for chrysotile asbestos in the chlor-alkali industry at 0.005 f cm^{-3} in workplace air,^{19,20} which is significantly lower than NIOSH's

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recommended exposure limit (REL) of 0.1 f cm^{-3} for chrysotile asbestos.

Airborne fibers are typically defined as particles elongated in one direction.⁶ To be classified as a fiber, the length should be at least three times the width (*i.e.*, aspect ratio (length to width) $\geq 3:1$), and regulatory standards for asbestos fibers require fibers to meet a minimum length, which is generally over $5 \mu\text{m}$ in most jurisdictions. Phase contrast microscopy (PCM; National Institute for Occupational Safety and Health [NIOSH] Method 7400)² is typically used to measure inhalation exposure to airborne asbestos, other fibers or EMPs by counting fibers whose length is longer than $5 \mu\text{m}$ with aspect ratio (ratio of fiber length to diameter) larger than 3, whose definition is provided in the NIOSH Method 7400 “A” rules. Although the PCM method is straightforward and user-friendly, this routine exposure assessment relies on manually counting fibers, which is labor-intensive and time-consuming, especially when the air concentration is below or at the proposed ECEL or REL. Additionally, the method may introduce background noise, particularly in samples with significant debris or non-fibrous compact particles, interfering with the clear visualization of fibers. In the PCM method, the sample analyzed is a miniscule fraction of the total number of fibers or particles collected in a typical 8-hour shift. Typically, fiber counting of a sample is stopped after 100 fields of view are analyzed, which amounts to roughly 0.2% of the entire effective filter area (25 mm mixed cellulose ester [MCE] filter; according to the NIOSH 7400 Method²). This leads to very poor Poisson statistics and high counting uncertainty of fiber number concentration measurement, particularly for trace concentrations.

A study conducted by Lorenz *et al.* (2017)²¹ developed an optimized methodology for Flow Imaging Microscopy (FIM) to analyze microplastic fibers, validating an enzymatic digestion protocol through a comparison of particle counts, sizes, and appearances before and after enzyme or detergent treatment. Similarly, Kannan and Banat (2020)²² employed FIM to characterize the morphological parameters of mixed natural and synthetic fibers present in dryer lint, specifically quantifying attributes such as fiber length and straightness to ensure reliable particle differentiation. Furthermore, Kim *et al.* (2023)²³ utilized FlowCam for automated microplastic morphology analysis, quantifying fiber shapes and investigating the impact of natural organic matter on the reliability of shape measurements. Another investigation by Chorán and Örmeci (2023)²⁴ demonstrated that Micro-Flow Imaging (MFI) can automatically measure size, color, intensity, and shape descriptors of microplastics, including fibers, thereby reducing the subjectivity associated with manual microscopy. While these studies demonstrate the capability of FIM and MFI to characterize and identify fibers or microplastic particles in water or liquid samples through morphological analysis, none have examined how the FIM method influences the accuracy of counting and sizing fibers within size ranges relevant to environmental or occupational air monitoring.

To address these drawbacks of the PCM method, in this study, we propose using Flow Imaging Microscopy (FIM) as an alternative method that can provide high precision and high-

throughput measurement for fiber count and length distribution measurements. Fig. 1 shows a schematic diagram of an overall new approach using fiber-in-liquid samples to measure airborne fiber concentrations in the workplace: airborne fibers in the workplace can be collected directly into a liquid (typically water) using a wet cyclone or liquid collector.²⁵ Images of fibers in liquid samples are captured using Flow Imaging Microscopy (FIM) as the liquid samples flow through a microfluidic channel. The FIM has an automated capability to provide length and number distribution of the fibers analyzed in near real time. Then, fiber air concentration is obtained based on measured distributions by FIM. This study is focused on probing the analytical figures of merit of length and count distribution measurement of FIM.

2. Experimental methods

2.1 Flow imaging microscopy method and its counting and sizing accuracy measurement

In our study, we utilized a FlowCam instrument (Model 8100, Yokogawa Fluid Imaging Technologies, Inc, Scarborough, Maine) that employs flow imaging microscopy (FIM) to capture images of both subvisible and visible particles in a liquid as they flow through a microfluidic channel. The FlowCam features several essential components: a flow cell, an optical system (which includes a microscope and camera), and a fluidics system (a syringe pump). A schematic diagram of the instrument is shown in Fig. 1(b). The FlowCam has three key functions: (i) it examines a liquid sample under a microscope, (ii) captures images of magnified particles within the liquid, and (iii) analyzes the particles through various measurements, including particle size and morphology.

Overall procedure of the instrument is as follows: liquid sample is loaded into the injection port of the instrument, and then, the fluidics system draws the sample into a flow cell, and a fluidics sensor initiates data acquisition. As particles pass through the flow cell, the optical system captures high-resolution images of the full width and depth of the flow cell as the sample flows through the optical field of view. Particle images are segmented from the camera images and captured in real-time as they flow through the flow cell. Captured particle images are processed by a software (VisualSpreadsheet version 6, Yokogawa Fluid Imaging Technologies, Inc., Scarborough, Maine), which identifies and classifies particles based on pre-defined criteria such as particle size and aspect ratio.

We first investigated counting and sizing accuracies of the FIM method for spherical particles, with objective lens magnifications of 4X and 10X, using monodisperse polystyrene latex (PSL) count and size standards (Count-CAL Microsphere Size Standards, Thermo Scientific™) with microspheres in the diameter range of $2\text{--}50 \mu\text{m}$. The properties of the PSL count and size standards used in this study are summarized in Table 1. Monodisperse PSL count and size standards with microspheres (2, 5, 10, 20, 50 and/or mixtures of these) in the diameter range of $2\text{--}50 \mu\text{m}$ was used. The FIM counting and sizing accuracies were also compared to those from a reference instrument (AccuSizer A7000AD, Entegris), a liquid particle counter capable

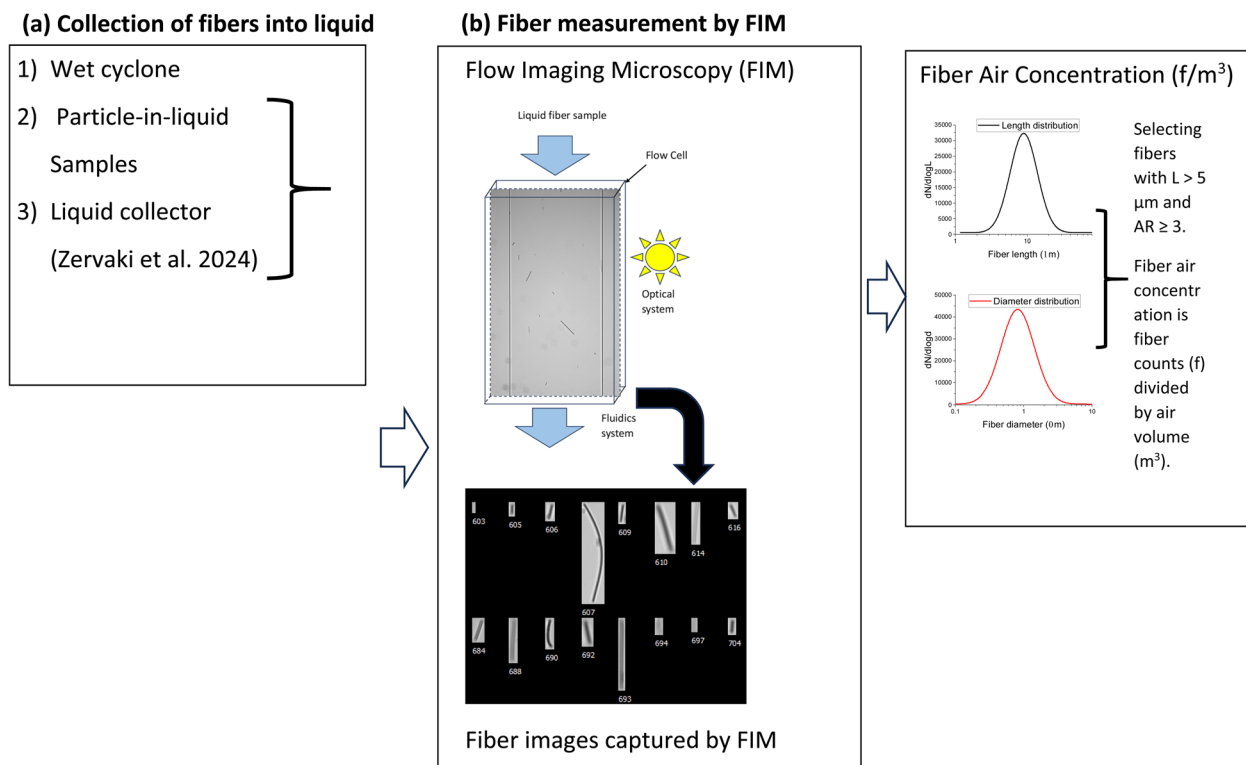


Fig. 1 Schematic diagram of a new approach using fiber-in-liquid samples to measure airborne fiber concentrations in the workplace: flow imaging microscopy (FIM) method was investigated, using a flow cell of a rectangular microfluidics channel where liquid samples pass through. L is fiber length and AR is aspect ratio of fibers.

of measuring both particle size and concentration of suspensions. The AccuSizer utilizes single particle optical sensing (SPOS) technology to provide high resolution, wide dynamic range, and high accuracy. This measurement by the reference instrument was to ensure that FIM measurements are accurate and reliable in the size range studied.

2.2 Fiber sample preparation

Test glass fibers were either (i) aerosolized by vortex shaking of glass fiber powder and collected on cascade mesh micro-screens with different pore sizes or (ii) prepared as a suspension in deionized water from bulk fiber powder to obtain fiber samples with different lengths.

A microscreen system was developed in this study. The system using mesh screens for classifying fibers by length consists of (i) fiber generation vortex shaker, (ii) entrance length unit, and (iii) a series of screens with different pore sizes for fiber penetration and collection. We designed and constructed

a laminar flow entrance length unit upstream a series of screens with different pore sizes (5, 10, 20, 30, 41 & 60 μm). The entrance length unit was made with a conducting tube with 25 mm inner diameter (ID) and 250 mm length to remove the entrance effect of circular tubing and make aerosol flow fully developed, minimizing fiber random orientation (or fluctuation) in the aerosol flow.²⁶

The schematic diagram for the experimental setup is shown in Fig. 2. Glass fibers supplied by the Japan Fibrous Material Research Association (JFMRA)²⁷ were used as a surrogate for asbestos. Glass fibers were aerosolized by vortex shaking from the bulk fiber material at relative humidity (RH) ~ 30 –50% to promote fiber release from the generation system.^{28–30} The aerosol stream of fibers was filtered through screens of different screen pore sizes (5, 10, 20, 30, 41 & 60 μm) and the length-classified fiber samples on the screens (three groups: long, medium, and short fibers, geometric mean length $\sim 46 \mu m$, 19 μm , and 11 μm) were suspended into deionized water by the

Table 1 Properties for spherical particle count and size standards

Nominal particle diameter (μm)	Lower particle limit for counting (μm)	Expected particle count (mL^{-1})	Certified mean diameter (μm)	Coefficient of variation (%)
2	1.3	2700–3300	2.02 $\pm$ 0.015	1.0
5	3.0	2700–3300	5.010 $\pm$ 0.035	1.0
10	7.5	2700–3300	10.13 $\pm$ 0.06	0.9
20	10	2700–3300	20.00 $\pm$ 0.20	1.0
50	30	2700–3300	51.2 $\pm$ 0.5	1.2

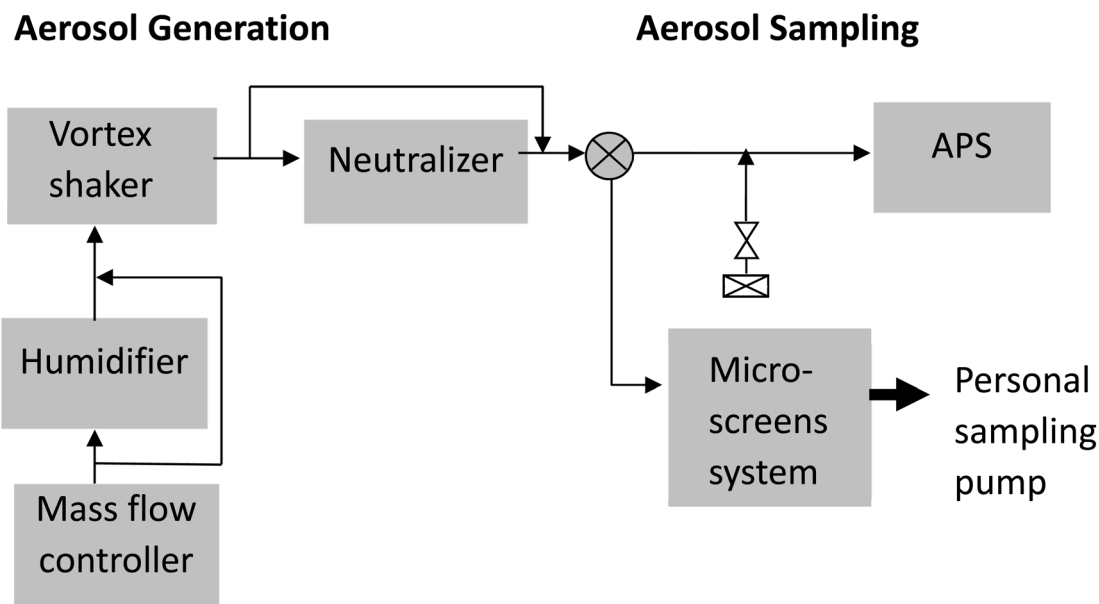


Fig. 2 Schematic diagram of an experimental setup for generating airborne fibers to evaluate the FIM method.

screen washing procedure.²⁷ Six fiber samples from different suspensions were prepared and the same volume of the suspension (*i.e.*, 1 mL to 5 mL) for both FIM and PCM methods was used for different fiber samples; each fiber suspension was analyzed using FIM with the magnifications of 4X and/or 10X, compared to the PCM method.

As a second approach to obtain fiber samples, we used bulk fiber powders to first weigh gravimetrically the mass of each powder and prepared a suspension of each powder in deionized water. If a suspension concentration is too high, we diluted the suspension with deionized water to obtain a suspension of a different fiber concentration. We prepared five fiber samples (samples A to E) from different suspensions to compare PCM measurements conducted by both us and an external laboratory with FIM measurements. Since all suspensions employed in this study were prepared using ultrafiltered or deionized water, there were no interfering fibrous particles within the specified diameter and length ranges. Consequently, a blank analysis was deemed unnecessary. However, when analyzing field samples, which may potentially introduce contamination and interference, it would be necessary to subtract the blank measurement.

For PCM measurement, the fiber suspension was vacuum filtered on a 0.8 μm pore size mixed cellulose ester (MCE, SKC Inc.) filter using a filtration apparatus (Millipore), followed by drying the MCE on a warm plate.³¹ After filter sample preparation, fiber count was performed together with length measurement according to the NIOSH Method 7400. For Flow Imaging Microscopy (FIM), measuring a 1 mL sample volume at a flow rate of 0.15 mL min⁻¹ requires approximately 7 minutes to count between 1000 and 10 000 fibers, depending on the sample concentration. Conversely, the time needed to count fibers on a filter using Phase Contrast Microscopy (PCM) is determined by a standard procedure outlined in NIOSH Method 7400, which mandates counting a minimum of 100 fibers. Furthermore, multiple factors, including sample preparation and the

manual scanning process of the filter, influence the overall time required. It is estimated that either counting a minimum of 100 fibers in at least 20 fields or scanning a maximum of 100 fields per filter if 100 fibers are not counted *via* PCM requires approximately 30 to 90 minutes on the microscope alone, excluding the time needed for sample preparation to transfer the fiber sample from the filter to the microscopy slide.

2.3 Fiber count comparison by FIM and PCM

To investigate how comparable two methods (FIM and PCM) are when they are used to count fibers, one experimental testing was conducted with one fiber sample: instead of using a limited number of fields of view from a test fiber sample filter, resulting in a limited number of fibers (about 200 fibers) to be counted, which is typically done by PCM based on NIOSH Method 7400, many fields of view for fiber counting were scanned and obtained from the test fiber sample filter, as shown in Fig. S1. The test fiber sample filter was scanned from the top of the filter. Basically, scanning was done from left to right along each row of the MCE filter to obtain fields of view by moving PCM camera every 1 mm in the horizontal direction. This process continued from row 1 to row 8. For each row, fiber count was added up as more fields of view were obtained and an averaged fiber density (fiber count per scanned field-of-view area, f mm^{-2}) as a function of fiber count was calculated based on fiber counting. The criteria for fiber measurements were that fiber is longer than 5 μm with aspect ratio equal to or larger than 3.

To compare fiber density measured by PCM to FIM measurements, the images captured by FIM camera were used in a way that more images could provide the increasing number of fibers counted to obtain the averaged fiber density as a function of fiber count. FIM software (VisualSpreadsheet version 6, Yokogawa Fluid Imaging Technologies, Inc., Scarborough, Maine) provided 'Fluid Volume Imaged' and fiber

number counted with the criteria, and then the averaged fiber density was calculated based on fiber count and a known fiber deposited filter area.

2.4 Fiber length measurement by FIM

Fiber suspension samples were first tested by FIM with 4X objective lens and a flow cell with a dimension of 300 μm depth $\times$ 1500 μm width to cover unknown long fibers. Each sample in a tube was dispersed by vortex shaking for one minute, and then the tube was inverted ten times before taking the sample from the tube. Immediately, the sample was manually loaded to the pipette attached to the inlet port of the FIM instrument using a calibrated pipette. The sample volume was typically 1.0 mL, and the flow rate drawn by a syringe pump in the instrument was 0.9 mL min^{-1} . Before measurement, the FIM instrument was checked for a standard calibration with bead standards (ThermoFisher ScientificTM). When changing the objective lens between 4X and 10X, the flow cell of the FIM instrument was auto-focused using autofocus beads (ThermoFisher ScientificTM), using 15 μm and 100 μm size standards for 10X and 4X lenses, respectively, to ensure that particle images taken by the optical system are in focus. The FIM measurement was conducted with three replicates for each fiber sample. Data such as fiber length and aspect ratio (AR) were further analyzed and filtered post-acquisition. For fiber length measurement, the fibers with AR larger than 3 and length longer than 5 μm were measured based on NIOSH Method 7400 (A rules) and Feret length was used, which is defined as the maximum perpendicular distance between parallel tangents touching opposite sides of a particle (VisualSpreadsheet 5 Version 5.9 User Guide, Yokogawa Fluid Imaging Technologies, Inc).

2.5 PCM and TEM measurements by an external laboratory

Five fiber samples were prepared by filtering sample suspensions to compare fiber length distributions using three methods: PCM, TEM, and FIM. These samples included both relatively short and long fibers. Before filtration, each sample was thoroughly mixed using a vortex shaker. Immediately after mixing, a 1 mL aliquot was removed and placed into an Advantec 150 mL glass funnel with an effective filtration area of 210 mm^2 . The samples were deposited onto a 0.8 μm MCE filter (SKC 225-1930, lot MO024276), placed on top of a 5.0 μm backing MCE filter. After adding the sample aliquot, the funnel was thoroughly rinsed with pure water. Once filtered, the 0.8 μm MCE filter was removed and allowed to dry before analysis. This process was repeated twice, yielding three individual filters per liquid sample. One filter was analyzed *via* TEM, while the remaining two were analyzed *via* PCM using NIOSH 7400 Method "A" counting rules.

2.6. Phase contrast microscopy: NIOSH 7400 method, "A" counting rules

Samples were analyzed for fibers using the microscopic techniques specified in the National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods (Fifth Edition), Method 7400, Issue 3, 2019.²⁸ A wedge-shaped section

of each filter was carefully excised and mounted on a standard microscope slide using the acetone vapor/triacetin method to render the filter transparent and adhere the coverslip.

Fibers were defined as particles observable on the filter surface with length-to-width aspect ratios of $\geq 3:1$ and lengths exceeding 5 microns. Fibers were counted using a binocular microscope with positive phase contrast illumination. A calibrated ocular insert projected a circle of known and calibrated area onto the filter image, forming discrete "fields." Fields were randomly selected and examined until one of the following conditions was met:

- (i) A minimum of 100 fibers were counted in at least 20 fields.
- (ii) A maximum of 100 fields was examined.

The raw result was the filter fiber concentration, expressed in fibers per mm^2 . This value, along with the effective filtration area of 210 mm^2 , was used to calculate fiber concentrations for each sample. Each sample calculation assumed a suspension solution volume, with 1 mL of suspension filtered. The final reported units were fibers per mm^2 .

3. Results and discussion

3.1 FIM sizing and counting accuracies for standard spherical particles

Fig. 3 shows particle size accuracy and bias measured by FIM using 4X and 10X lens, and SPOS for monodisperse standard spherical particles in the size range from 2 μm to 50 μm . The particle mean diameter for the size standard was obtained from three runs based on the number of particles detected by the FIM and is represented as the mean $\pm$ standard deviation of the three runs. In Fig. 3(a), the y-axis is the mean diameter, x-axis is a certified standard particle size as shown in Table 1 and in Fig. 3(b) the y-axis is a bias corresponding to each datum in Fig. 3(a). The bias was defined as the deviation (%) of particle size measured by each method (FIM or SPOS) from PSL size standard. It was found that with 4X lens, the FIM method can measure particle sizes larger than 5 μm with a bias less than 13%. For all sizes, relative standard deviations (RSD, defined as a ratio of standard deviation to mean; coefficient of variation) were less than 2%. However, as particle size decreased from 5 μm down to 2 μm , the particle size deviated from the size standard by about 90%. With 10X lens, the FIM method was able to measure particle sizes with a bias of less than 3% in the range of 5–50 μm . Also, the deviation from the size standard at 2 μm was about 35–45%. It is worth noting that the RSD for FIM 4X and 10X was less than 5% in the range of 2–50 μm . All biases and RSDs for FIM 4X and 10X measurements of particle size are summarized in Table 2.

The size measurement of the same standard particles using a particle counter based on SPOS as reference values was found to be better than the FIM measurement. The bias of the size measurement by SPOS was less than 1.5% in the size range of 5–50 μm and less than 3% for the size 2 μm . To further investigate the particle size measurement accuracy by FIM, the FIM measured size distribution for each size standard particle was compared to a theoretical size distribution generated using an AEROCALC Excel program developed by the late Dr Baron. The

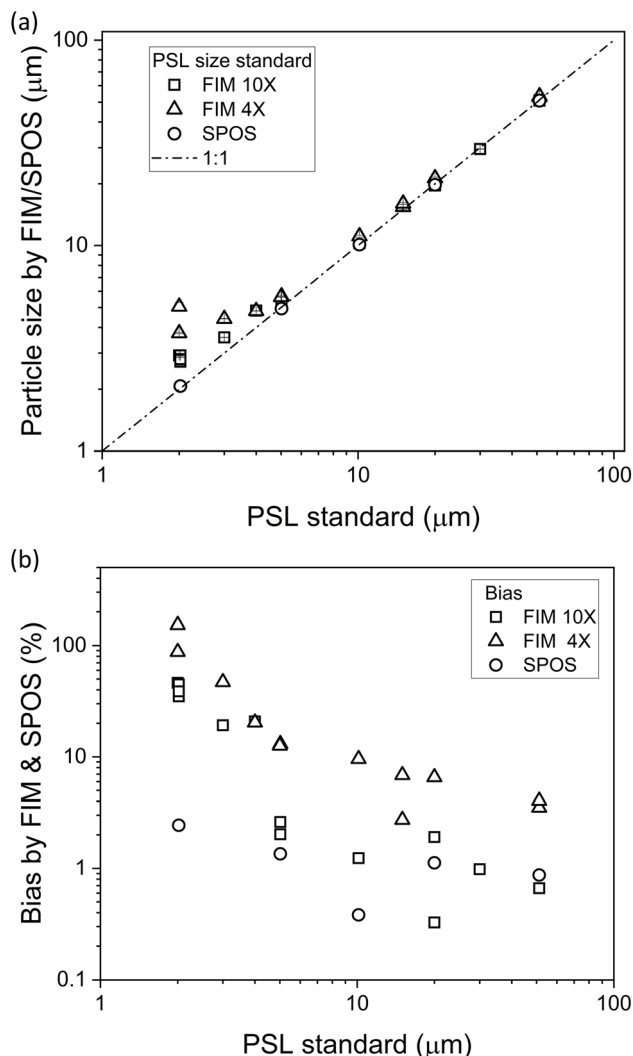


Fig. 3 Particle size accuracy (a) and bias (b) for monodisperse spherical particles by FIM 4X and 10X compared to SPOS. Bias was defined as deviation (%) of particle size measured by each method from PSL size standard.

measured size distributions by FIM were fitted with theoretical size distributions with assumed total particle number, mean, and standard deviation. Fig. S2 and S3 show particle size distributions of PSL size standard microspheres for each 2 to 50 μm in diameter by FIM with 10X and 4X, respectively, compared

to theoretical size distributions. For FIM 10X, the measured size distributions were in good agreement with the theoretical size distributions, with geometric standard deviations (GSD, σ) ranging from 1.05 to 1.25, indicating that the size distributions are monodisperse and that FIM with 10X lens can measure particle size down to 2 μm accurately. In practical cases, the GSD for monodisperse particles usually ranges between 1.0 and 1.2. For FIM 4X, the measured size distributions were also in good agreement with the theoretical size distributions, with geometric standard deviations (GSD, σ) ranging from 1.10 to 1.30, except for 2 μm size measurement, for which the size was overestimated and the GSD was larger than that supposed to be because the resolution of 4X lens is too low for this size to be measured accurately.

Fig. S4 shows size distributions measured by SPOS and fitted by theoretical size distributions for each size standard. Surprisingly, the GSD was about 1.03 for all size standards, confirming that particle size standards are monodisperse as claimed by the company. Fig. S5 (a) and (b) show size distributions of mixed size standards (*i.e.*, 5, 10, 20 & 50 μm) measured by FIM 10X & 4X compared to SPOS. FIM 10X shows a comparable modal diameter at each size, while FIM 4X delivers a little broader size distribution at each size standard compared to SPOS.

Fig. S6 and S7 show typical particle images captured by FIM 10X and 4X for monodisperse size particles, respectively, and Fig. S8 for mixed particles of size standards. The particles passing through the flow cell were captured automatically by the FIM camera and the images were sorted by area-based diameter (ABD). The images taken by FIM with 10X lens have more contrast than those taken by FIM with 4X lens.

Fig. 4 shows particle counting accuracy per standard sample volume (mL) measured by FIM with 4X and 10X and SPOS for different particle sizes. Particle count standards with different sizes (2, 5, 10, 20, and 50 μm) have about 3000 particles per mL with the uncertainty of ± 300 particles per mL and each count standard has a lower size limit for counting, as shown in Table 1. Particle counting was performed down to the lower size limit. According to the vendor, the certified mean diameters of these standards were transferred by optical microscopy from a stage micrometer, a glass slide with a scale with line spacing calibrated by the National Institute of Standards and Technology (NIST, SRM 2800 SN411) in micrometers. The data for FIM 4X

Table 2 Particle mean diameter, standard deviation and bias measured by FIM using 4X and 10X lens for particle size standards

Nominal particle diameter (μm)	FIM 4X		FIM10X		Bias ^a (%)		RSD ^b (%)	
	Particle mean diameter (μm)	Standard deviation (μm)	Particle mean diameter (μm)	Standard deviation (μm)	4X	10X	4X	10X
2	3.76	0.02	2.72	0.12	87.8	34.9	0.6	4.5
5	5.68	0.03	5.11	0.07	13.1	2.0	0.5	1.3
10	11.10	0.12	10.26	0.04	9.6	1.2	1.0	0.4
20	21.31	0.22	20.07	0.07	6.6	0.3	1.0	0.3
50	53.00	0.62	50.86	0.03	3.5	0.7	1.2	0.1

^a Bias was defined as deviation (%) of particle size measured by each method from PSL size standard. ^b RSD stands for relative standard deviation (%) calculated by dividing standard deviation by mean.

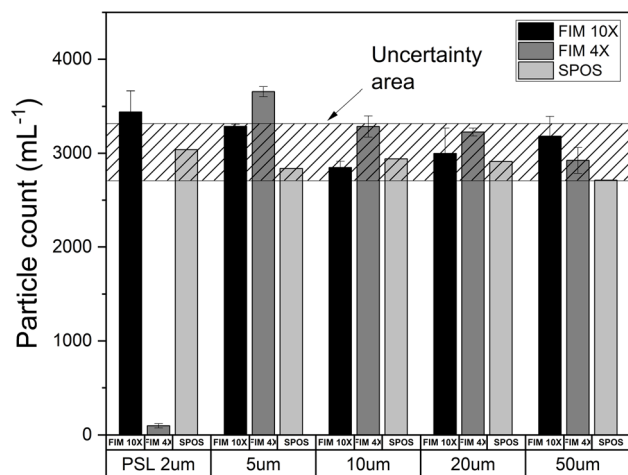


Fig. 4 Particle counting accuracy per standard sample volume (mL) measured by FIM with 4X and 10X and SPOS for different particle sizes.

and 10X in Fig. 4 were obtained with three replicates and are represented as the particle count mean with standard deviation. It was found that both FIM 4X & 10X can count particles in the size range of 5 to 50 μm within the uncertainty provided by the vendor, except for FIM 4X at 5 μm , which shows a little higher particle count than the expected value. Specifically, FIM 4X has a counting accuracy with less than 22% bias, while FIM 10X has a counting accuracy with less than 10% bias in the size range of 5 to 50 μm . It was found that the counting measurement RSD is 4.7% and 9.0%, respectively. For the 2 μm size standard, counting accuracy drastically decreased for FIM 4X, resulting in a bias of approximately 96.7%, while for FIM 10X, the bias increased to 14.6%. The possible reason that the counting accuracy significantly decreased for FIM 4X may be due to the fact that the resolution of the 4X lens is too low for this size to be detected. For a mixture of size standards (5, 10, 20, and 50 μm), particle counting was measured 3367 and 3053 for FIM 4X and FIM 10X, respectively, resulting in 12.2% and 1.8% bias whose corresponding particle images of the mixture are shown in Fig. S8. All bias and RSDs for FIM 4X and 10X measurements of particle counts are summarized in Table 3.

Fig. 5 shows particle count concentration measured by FIM method with 10X lens for the PSL count standard with a size 20

μm for different concentrations. Each datum was obtained from three replicates under the same conditions and represented as a mean with an error bar (one standard deviation of three measurements). It was found that the FIM method was in reasonable agreement with particle concentration in the range from 50 count mL^{-1} to 3000 count mL^{-1} . It is worth noting that as particle count concentration decreased down to 50 count mL^{-1} , the variation of particle count represented as relative standard deviation (RSD) increased from 9% to 36%.

This may be due to the fact that a small number of particles have higher uncertainty confidence limits, accounting for Poisson components according to Table 1 in the NIOSH method 7400 (NIOSH, 2019). Fig. S9 shows geometric mean diameter (GMD) and geometric standard deviation (GSD) measured by FIM 10X for PSL size standard (6 μm , coefficient of variation (CV) < 0.25) for different PSL numbers over three orders of magnitude range from 100 up to 315 000. Fig. S9(a) shows log-normal distributions for different particle numbers and Fig. S9(b) shows GMD and GSD corresponding to Fig. S9(a). GMD increased from 5.52 μm to 5.69 μm as particle number increased from 100 to 315 000. The corresponding GSD decreased from 1.28 to 1.22 with increasing particle number, showing that the measured GSD is within the CV range (<0.25) claimed by the vendor. It is also noticed that regression coefficients (R^2) for the log-normal fittings in Fig. S9(a) are about 0.998–0.999, regardless of particle number, indicating that particle size measured by FIM 10X is very reliable and reproducible with different particle number. The result shows that FIM 10X can measure particle sizes with a high number up to 315 000 with a bias of 5.2–8.0% for the PSL size standard. All bias and RSDs for FIM 4X and 10X measurements of particle counts are summarized in Table 3.

3.2 Length distributions for fiber samples by FIM

Fig. 6 shows fiber mean lengths measured by FIM compared to the PCM method and the corresponding bias in the measurement. The fiber mean lengths were obtained from three runs based on the number of fibers measured by FIM for each run using log-normal fitting of fiber lengths and are represented as the mean $\pm$ standard deviation of the three runs in Fig. 6(a).

For FIM 4X, fiber mean lengths measured by FIM were overestimated compared to those measured by PCM in the fiber

Table 3 Particle mean count, standard deviation and bias measured by FIM using 4X and 10X lens for particle count standards

Nominal particle diameter (μm)	FIM 4X		FIM 10X		Bias ^a (%)		RSD ^b (%)	
	Particle mean count (mL^{-1})	Standard deviation (mL^{-1})	Particle mean count (mL^{-1})	Standard deviation (mL^{-1})	4X	10X	4X	10X
2	98	23	3440	224	96.7	14.7	23.5	6.5
5	3656	52	3286	23	21.9	9.5	1.4	0.7
10	3285	113	2847	67	9.5	5.1	3.5	2.3
20	3226	42	2997	271	7.5	0.1	1.3	9.0
50	2923	138	3181	211	2.6	6.0	4.7	6.6

^a Bias was defined as deviation (%) of particle size measured by each method from PSL size standard. ^b RSD stands for relative standard deviation (%) calculated by dividing standard deviation by mean.

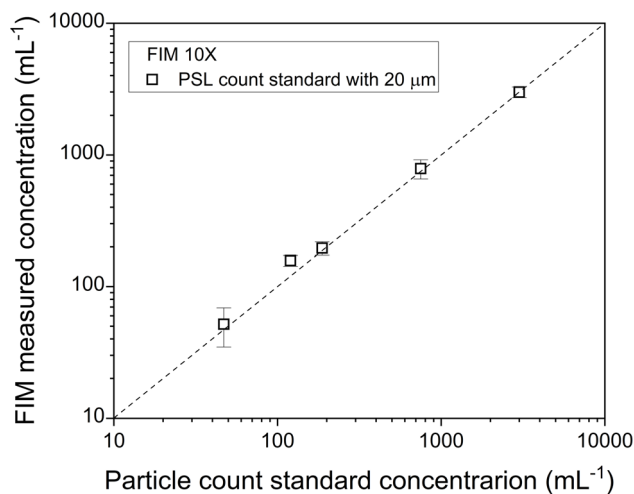


Fig. 5 Particle count concentration measured by the FIM method for PSL count standard (with 20 μm) with different concentrations. The data for FIM 10X in this figure were obtained with three replicates and are represented as the particle count mean per mL with standard deviation.

length range of 14 μm to 48 μm , and the bias increased with decreasing fiber length. It was assumed that the PCM measurement is a reference value, although the PCM method has an inherent measurement uncertainty. The bias increased from 3.4% to 121% as the fiber mean length decreased from 47.8 μm to 14.3 μm , as shown in Fig. 6(b).

For FIM 10X, the fiber mean length was comparable to that measured by PCM in the fiber length range of 10.9 μm to 23.5 μm . The bias increased from 4.3% to 39.1% as the fiber mean length decreased from 23.5 μm to 10.9 μm .

For the sample with a fiber mean length of 20.2 μm , Fig. S10 shows fiber length distributions measured by FIM 4X and 10X compared to PCM. The length distributions were obtained by log-normal fitting of fiber number as a function of fiber length and then normalized by the peak value. The error in the distribution was expressed as the standard deviation of three replicate measurements for FIM 4X and 10X. Based on Fig. 6 and S10, the results show that FIM with 10X can measure fiber length with a bias of less than 40% for the samples compared to the PCM method. All bias values and RSDs for FIM 4X and 10X measurements of fiber lengths are summarized in Table 4.

RSDs for FIM 4X measurements in the fiber length range of 14.3 to 20.2 μm were found to be about 4.3–12.4%, while the corresponding biases were 75–121%. On the other hand, RSDs for FIM 10X measurements in the fiber length range of 10.9 to 23.5 μm were 0.4–5.6%, while the corresponding biases were 9.5–39%, indicating that the RSDs for FIM 4X are relatively small, even though the bias for FIM 4X is very high (up to 121%). In other words, the accuracy of FIM 4X measurements is low, while their precision is high in the fiber length range.

It is worth noting that the fiber sample in Fig. S10 has a wide length distribution, spanning from about 4–5 μm to over 100 μm , and both FIM 10X and PCM measurements of fiber length are in good agreement with each other.

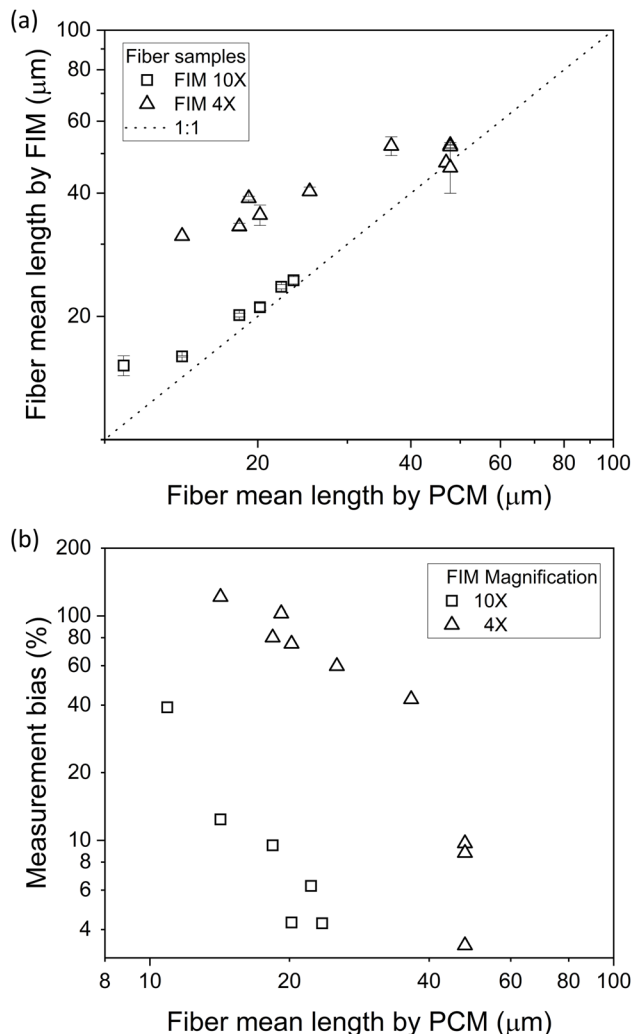


Fig. 6 Effect of magnification of FIM measurement on bias. (a) Fiber mean lengths measured by FIM compared to PCM method. The fiber mean lengths in (a) were obtained from three runs based on the number of fibers measured by the FIM and are represented as the mean $\pm$ standard deviation of the three runs. (b) Measurement bias for different FIM magnifications.

3.3 Fiber counting for fiber samples by FIM

Fig. 7 shows fiber density as a function of fiber count based on PCM and FIM methods. The fiber density measured by FIM is represented by shaded area of two independent measurements, indicating the shaded area is the uncertainty of FIM measurements. The fiber density measured by PCM for each row (from row one to row eight) is compared with FIM data in Fig. 7. From this measurement, several important findings are clear: (i) PCM method for each row shows a lot of variation in fiber density as a function of fiber count; the fiber density increases with increasing fiber count for some rows (rows 1 & row 3) while it increases initially and then decreases with increasing fiber count for other rows (rows 6 & 7). (ii) The averaged fiber density varies considerably, ranging from approximately 500 to 1000 mm^{-2} for several hundred fibers counted, depending on the row. (iii) The averaged fiber density based on the total fiber count

Table 4 Fiber mean length, standard deviation and bias measured by FIM using 4X and 10X lens for different fiber samples

Fiber mean length by PCM (μm)	FIM 4X		FIM 10X		Bias ^a (%)		RSD ^b (%)	
	Fiber mean length (μm)	Standard deviation (μm)	Fiber mean length (μm)	Standard deviation (μm)	4X	10X	4X	10X
10.9	—	—	15.2	0.85	—	39.1	—	5.6
14.2	31.4	—	16.0	0.06	121.1	12.4	—	0.4
18.4	33.1	0.58	20.2	0.21	80.1	9.5	1.7	1.1
19.2	38.9	0.40	—	—	102.4	—	1.0	—
20.2	35.4	2.03	21.1	0.45	75.1	4.3	5.7	2.1
22.3	—	—	23.6	0.29	—	6.3	—	1.2
23.5	—	—	24.5	0.51	—	4.3	—	2.1
25.3	40.4	0.96	—	—	59.7	—	2.4	—
46.9	47.5	—	—	—	1.3	—	—	—
47.8	46.2	6.23	—	—	3.4	—	13.5	—
47.8	52.4	0.75	—	—	9.7	—	1.4	—
47.8	52.0	0.60	—	—	8.8	—	1.2	—

^a Bias was defined as deviation (%) of particle size measured by each method from PSL size standard. ^b RSD stands for relative standard deviation (%) calculated by dividing standard deviation by mean.

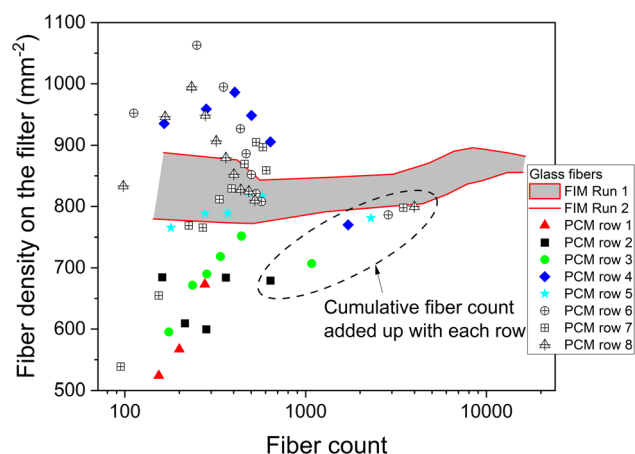


Fig. 7 Effect of fiber number concentration on measurement uncertainty in PCM & FIM. Fiber density as a function of fiber count based on PCM and FIM methods. The average fiber density for each row was obtained as the fibers in the scanned field of view were counted. The shaded area between two FIM runs represents measurement uncertainty of the FIM. The data points in the dashed ellipse show average fiber density on the filter calculated based on the fiber count added up with each row.

of each row is in the range from about 700 to 900 mm^{-2} for fiber counts between 350 and 600. This shows somewhat improved fiber density compared to that by FIM, as shown in Fig. 7 (iii) the averaged fiber density based on fiber count added up with total count from each row, as shown in dashed ellipse in Fig. 7, approaches to the value measured by FIM, indicating that as more fibers are counted, the density difference between the two methods decreases. In contrast to PCM measurements, the fiber density measured by FIM as a function of fiber count remains relatively stable, regardless of fiber count. However, there is more variation in fiber counts of 200–300 compared to counts exceeding 1000–10 000. It is worth noting that the number of fibers counted by FIM is up to about 15 000, while the total fiber counted by PCM for each row is about 500–600. Considering

that the typical number of fibers counted by PCM in the NIOSH method 7400 is 100–200 fibers, the FIM method can count fibers at a scale two orders of magnitude higher than PCM. The high-throughput fiber counting capability by FIM clearly demonstrates a better accuracy in the averaged fiber density, as shown in Fig. 7 and also suggests that the accuracy of the PCM method will be improved with a large number of fibers counted.

3.4 Fiber length and count measurements by PCM, TEM and FIM for different fiber samples

Having demonstrated that the FIM 10X can provide accurate measurements in the size range of 1–2 μm to 50 μm and in the count range of up to 3000 mL^{-1} and 300 000 at 6 μm , fiber length and count measurement accuracies were further investigated using FIM 10X for different fiber samples. To compare our measurement with those from an external laboratory, five fiber samples were prepared and sent to an external laboratory for fiber length and count measurements, while the same measurements were conducted in our laboratory. Typical images taken by PCM by us and PCM by external laboratory for each sample are shown in Figs. S11 and S12 in the SI. Fig. 8 shows that fiber length distributions measured by the PCM, FIM and TEM based on NIOSH method 7400 (A counting rule) for each fiber sample (Samples A to E), and Fig. 9 presents geometric mean lengths (GMLs), bias, and geometric standard deviations (GSDs) for the fiber length distributions shown in Fig. 8 and Table 5 summarizes these values.

Fiber length distributions in Fig. 8 were obtained from two to three runs based on the number of fibers measured by PCM or FIM for each run using log-normal fitting of fiber lengths and are represented as the mean $\pm$ standard deviation of the replicate runs. Compared to the PCM method, the FIM method was found to have a bias of 3.1% to 16.6% for fiber mean lengths while it has a bias of 1.8% to 17.8% compared to the TEM method, as shown in Fig. 9(b). The GSDs for the length distributions ranged from 1.50–2.05 for PCM, 1.59–2.22 for FIM, and

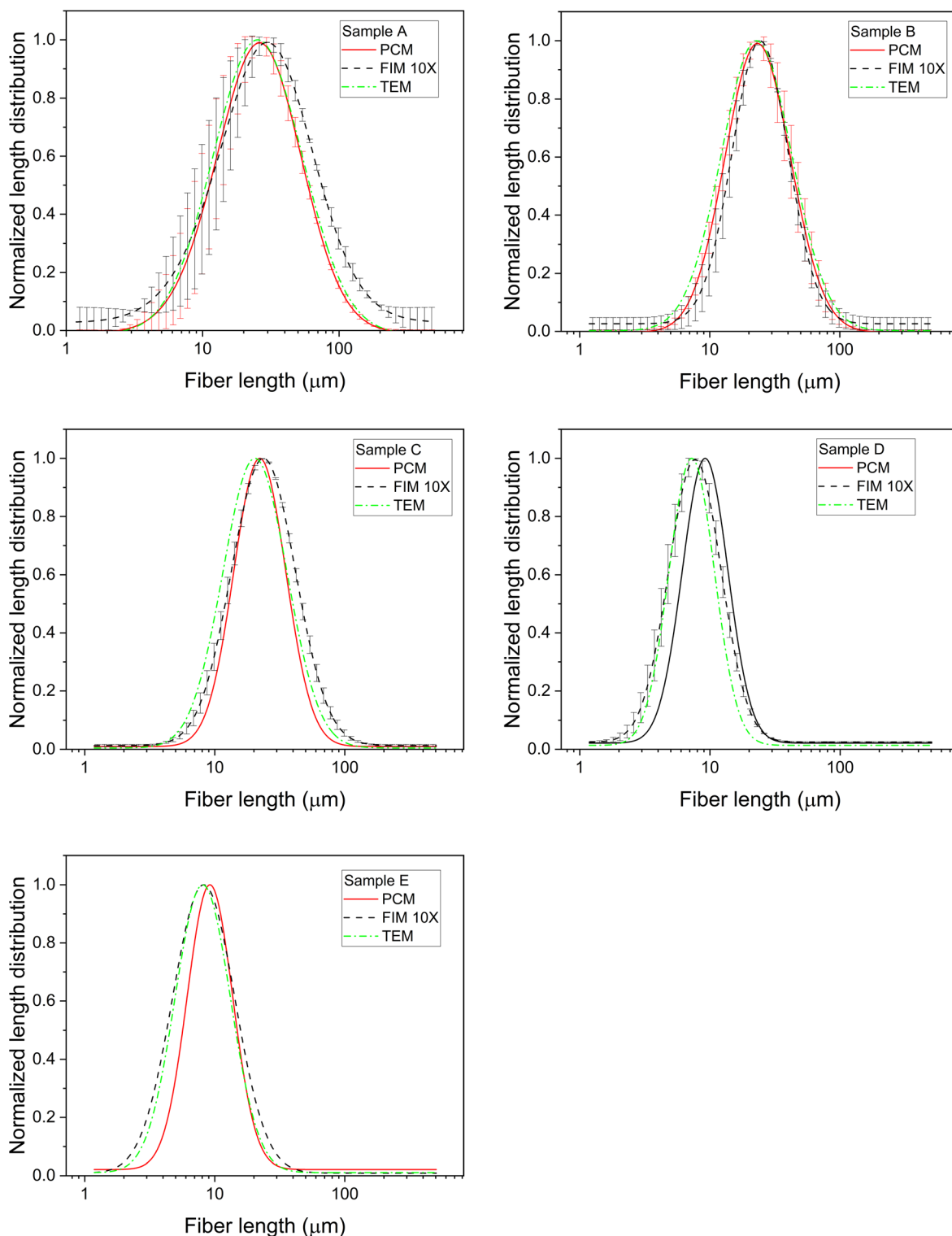


Fig. 8 Fiber length distributions measured by PCM, FIM and TEM for different fiber samples from fiber suspensions.

1.50–2.12 for TEM, indicating that FIM measurements are in good agreement with PCM and TEM methods. It is worth noting that the number of fibers measured by FIM for the length distributions was approximately 2000–20 000, while the number of fibers measured by PCM was about 100–400, and by TEM was about 200.

Table 6 summarizes fiber count comparison based on PCM, TEM and FIM 10X measurements for different fiber samples. Fibers counted by each method was expressed as fiber density, which is defined as the fiber count per unit filter area (fibers per mm^2). The numbers in the parentheses in the table represent the fiber counts used to calculate fiber density.

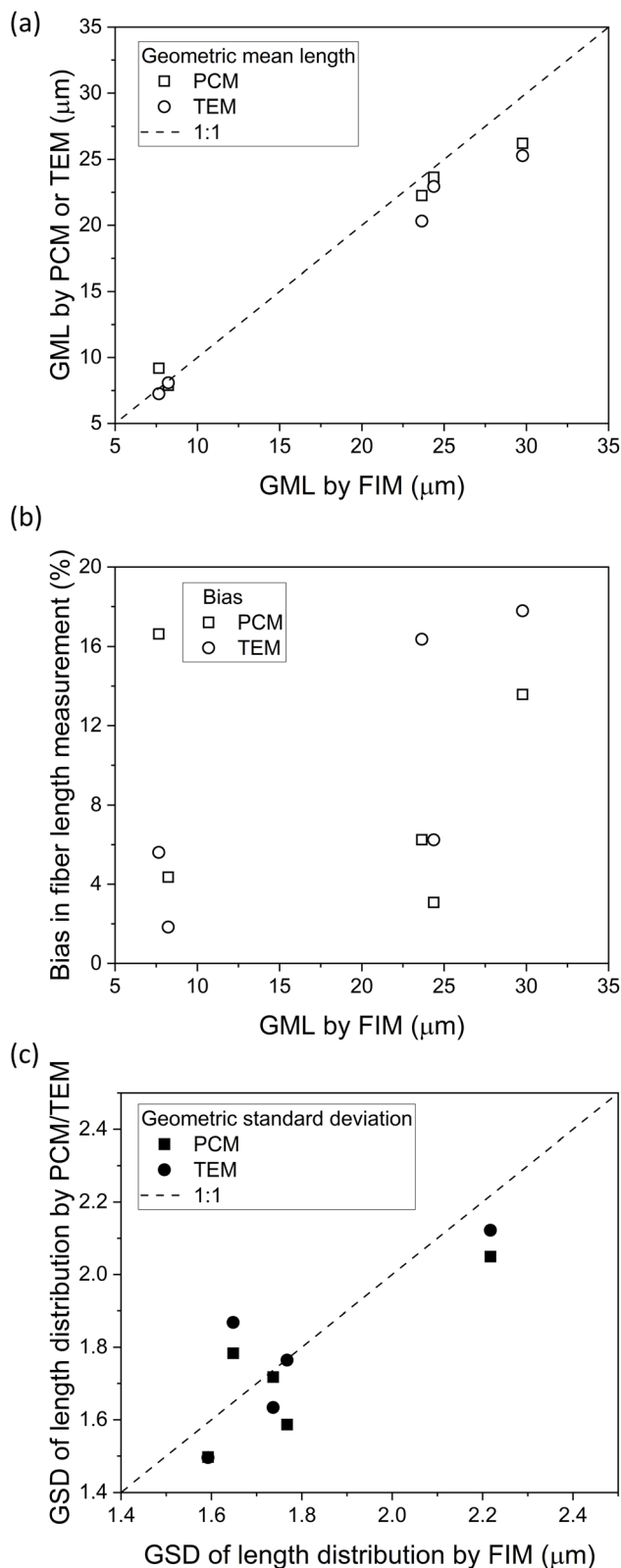


Fig. 9 Comparison of FIM measurements (at 10X) with PCM and TEM measurements. (a) Geometric mean lengths (GMLs), (b) bias, and (c) geometric standard deviations (GSDs) for fiber length distributions measured by PCM, TEM and FIM for fiber samples from fiber suspensions.

Table 5 GML and GSD comparison for PCM, TEM and FIM 10X for different fiber samples

Sample	PCM		TEM by external lab		FIM	
	GML ^a	GSD ^b	GML ^a	GSD ^b	GML ^a	GSD ^b
	(μm)	(μm)	(μm)	(μm)	(μm)	(μm)
Sample A	26.2	2.05	25.3	2.1	29.8	2.22
Sample B	23.6	1.78	22.9	1.9	24.4	1.65
Sample C	22.3	1.59	20.3	1.8	23.6	1.77
Sample D	9.2	1.50	7.2	1.5	7.7	1.59
Sample E	7.9	1.72	8.1	1.6	8.2	1.74

^a GML stands for geometric mean length. ^b GSD stands for geometric standard deviation.

Fig. 10 shows the bias between PCM (or PCM external) and FIM methods and also presents 95% confidence intervals for various fiber counts in a single measurement. Compared to PCM measurements in this study, the FIM-counting bias was in the range of -26% to 90% , with most values falling within the 95% confidence intervals expressed as expanded uncertainty,³² assuming that a subjective interlaboratory relative standard deviation (S_r) is 0.2, as shown in Fig. 10. The expanded uncertainty for 95% confidence intervals was -38% to 63% for 100 fiber counts. However, compared to PCM measurements conducted by an external laboratory, the FIM-counting bias was in the range of about 60% to 378% , with only half of the values falling within the 95% confidence intervals, assuming $RSD = 0.4$, indicating a large discrepancy. According to the NIOSH 7400 method,³² a NIOSH study conducted using field samples of asbestos reported an intra-laboratory S_r ranging from 0.17 to 0.25 and an inter-laboratory S_r of 0.45.³³ Surprisingly, the expanded uncertainty for 95% confidence intervals with $S_r = 0.4$ was -53% to 265% for 100 fiber counts, highlighting the inherent variability of the PCM method for fiber counting, which is strongly influenced by subjective interlaboratory S_r . As mentioned in Fig. 7 in the previous section, the PCM method exhibited significant variation in fiber density for fiber counts below 200, where the fiber density ranged widely from 500 to 1000 mm^{-2} . Fig. 7 clearly demonstrates that counting as many fibers as possible helps reduce the subjective variability in fiber counting. The equivalent area of the total fields of view on the filter scanned by PCM in the experiment shown in Fig. 7 was about 5.0 mm^2 (about 3.25% of the filter area), corresponding to about 637 fields of view and resulting in approximately 4000 fiber counts. According to the NIOSH Manual of Analytical Methods (NMAM, 5th Edition), a major difference between Method 7400 and other analytical methods in the NMAM is that there is no reference method for Method 7400. Therefore, the consensus mean is the “true” value and the interlaboratory results effectively define the method’s accuracy. In this context, our rationale was that the fiber density obtained by the FIM method, based on two orders of magnitude higher fiber counts than the PCM method, would be more accurate than PCM-driven values. As shown in Fig. 10, the PCM counting method

Table 6 Fiber count comparison for PCM, TEM and FIM 10X for different fiber samples

Sample	PCM (f mm ⁻²)	PCM by external lab ^a (f mm ⁻²)	TEM by external lab ^a (f mm ⁻²)	FIM (f mm ⁻²)	FIM bias compared to PCM (%)	FIM bias compared to PCM external (%)
Sample A	124.1 (219) ^b	21.8 (31)	7.2 (38)	92.1(4251)	−25.8	322.5
	119.8 (113)	26.7 (38)		90.1 (4695)	−24.8	237.5
Sample B	276.8 (407)	159.0 (102)	188.1 (203)	353.3 (20 324)	27.6	122.2
	341.7 (201)	145.0 (103)		327.7 (7758)	−4.1	126.0
Sample C	54.4 (96)	27.7(39.5)	22.5 (183)	48.2 (2221)	−11.4	74.0
	—	29.5 (42)		47.1 (2196)	—	59.7
Sample D	341.1 (301)	105.0 (100.5)	179.7 (200)	435.2 (19 906)	27.6	314.5
	249.5 (98)	131.0 (100.5)		472.9 (18 169)	89.5	261.0
Sample E	347.9 (174)	71.9 (100.5)	136.3 (200)	336.7 (15 401)	−3.2	368.3
	342.4 (137)	55.4 (79)		352.6 (16 426)	3.0	536.5

^a These data were provided by external laboratories for the same samples used in this study. Fibers counted by each method was expressed as fiber density, which is defined as count per unit filter area (fibers per mm²). ^b In the format A (B), A means fiber density and B means fiber counts used to calculate fiber density.

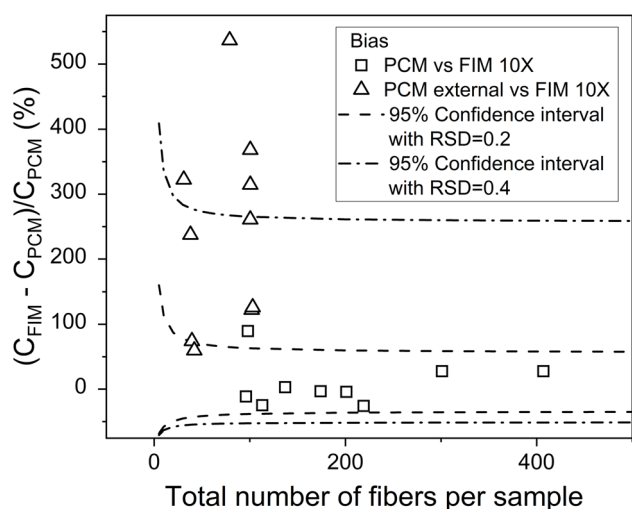


Fig. 10 Bias between PCM or PCM external and FIM method. 95% Confidence intervals with RSD = 0.2 and 0.4 are included for comparison. C_{PCM} and C_{FIM} are fiber counts by PCM and FIM, respectively.

indicates an inherently high uncertainty due to limited fiber count and the subjective variability in fiber counting.

3.5 Limitations of the FIM method

This study demonstrated the capability of Flow Imaging Microscopy (FIM) to quantify fibers and measure their length distribution, highlighting its potential for rapid, high-throughput assessments that may enhance the statistical reliability of fiber enumeration compared to Phase Contrast Microscopy (PCM). Nevertheless, several limitations must be acknowledged.

Given that FIM is based on optical microscopy, it shares the inherent constraint of reduced sensitivity in detecting thin and short fibers—a limitation characteristic of all optical microscopy techniques. This limitation is also shared by the PCM method, which employs optical microscopy similarly.

In FIM, fibers are suspended in a liquid medium and can exhibit rotation during imaging, often resulting in random

orientations with respect to the imaging detector. These orientation effects may contribute to resolution degradation or the broadening of measured length or diameter distributions. The extent of this error can be mitigated by utilizing narrower flow channels.

FIM measurements do not provide specificity to the chemical components of the fiber. The presence of a large number of interfering particles, which possess similar aspect ratios or appearances to the target fibers, could potentially introduce significant bias. The automated classification of particle shape and size utilized by the FIM software may be insufficient for accurately detecting trace amounts of target fibers with high specificity among a multitude of interfering particles. The development of more advanced machine learning algorithms, specifically trained for applications such as asbestos detection, could potentially improve specificity and reduce errors misclassification.

Further studies are needed to explore possible interference from air samples collected in real-world settings. It will be essential to assess the overall uncertainty of the method, including the air sampling process, to demonstrate specificity, and to evaluate the counting statistics of the entire method, taking into account the collection efficiency of the air-to-liquid sampler and the volume of sampled air.

4. Conclusions

We developed and evaluated flow imaging microscopy (FIM) as a rapid, high-throughput method for measuring fiber number and length distribution in fiber samples. To validate the accuracy of length measurement, monodisperse polystyrene latex standards with particle diameters ranging from 5 μm to 50 μm were analyzed using 4X and 10X objective lenses for spherical particles. FIM demonstrated accurate sizing for spherical particles within the 5–50 μm range, with biases of less than 13% for the 4X lens and 3% for the 10X lens. Across all sizes, relative standard deviations (RSD, defined as the ratio of standard deviation to the mean, or coefficient of variation) were less than 2%. FIM at 4X exhibited counting accuracy with a bias of less than 22%, while FIM at 10X achieved counting accuracy with

a bias of less than 10% in the 5 to 50 μm size range. The counting measurement RSD was 4.7% for the 4X lens and 9.0% for the 10X lens.

When measuring fiber length distributions at 10X, geometric mean lengths ranged from 8.0 to 26 μm , closely aligning with PCM results, with an average bias of approximately 16.6%. Comparing fiber density (fiber count per unit filter area) as a function of fiber count revealed that the discrepancy between the two methods decreased as fiber counts increased, highlighting the advantages of the FIM method for measuring trace concentration samples with low count uncertainty. The study suggests that the FIM-based fiber method could be a promising approach for analyzing workplace air samples, significantly reducing analysis time, cost, and counting uncertainty in the overall measurement.

Author contributions

Bon Ki Ku (corresponding author): laboratory experiments and investigation, data curation and interpretation, formal analysis, funding acquisition, methodology, project administration, validation, visualization, writing – original draft, reviewing and editing. Pramod Kulkarni: conceptualization, experimental design and data interpretation, methodology, writing – review and editing.

Conflicts of interest

No potential conflict of interest was reported by the author(s).

Data availability

The data generated or analyzed during this study can be found within the published article and its Supplementary information (SI) files. Supplementary information is available. See DOI: <https://doi.org/10.1039/d5em00411j>.

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