1 **Fine Sieving of Collected Atmospheric Particles using** 2 **Oil Electrophoresis (iSCAPE)**

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TOC

Abstract

It is rather challenging to separate atmospheric particles from nano- to micro-metre mixed in a sample. Here, a system named iSCAPE was invented to efficiently sieve particles out from a mixture by employing an electrostatic field and a non-conductive mineral oil. Tests with atmospheric particles of different cities as well as soil and road dust samples demonstrated that the iSCAPed particles under different operating conditions moved rapidly with different velocities and both directions. Particles of different sources such as ambient air, soil or road were shown to have different polarity-charged particle fractions, and exhibited clearly different particle electrical mobility graphs after the iSCAPE sieving from seconds to minutes. Data also revealed that after the sieving some particles were enriched at specific mobility ranges. Bacterial ATP measurements implied that the iSCAPE can be also used to efficiently separate bacteria of different sizes and charge polarity. Experimental data here suggest that the iSCAPE sieving strongly relies on the electrostatic field strength, mineral oil viscosity and the run time. In theory, the iSCAPE system can be used to extract any desired targets from a complex sample, thus opening up many outstanding opportunities for environmental, biomedical and life science fields.

Keywords: Atmospheric Particles, Sieving, Electrical Mobility, iSCAPE, Size Distribution

51

52 **Introduction**

53 Air pollution, especially the particulate matter (PM), has become one of the most
54 important environmental problems in the world. Exposure to PM has resulted in
55 millions of deaths globally (1). The components of atmospheric particles are very
56 complex, including biological such as bacteria, fungi, viruses, pollen and chemical
57 components - sulfate, nitrate, ammonium and other non-biological particles (2). There
58 are some commercially available instruments for studying size distributions of
59 atmospheric particles (3-4), but they do not automatically provide samples for post
60 analysis. In addition, collection of nanoscale particles requires expensive equipment
61 and high power source (5). Differential mobility analyzer (DMA) with up to 192 size
62 channels is otherwise used to study size distributions of nanoscale particles (1
63 nm-1µm) (6-7). In addition to its limited size ranges, it is also difficult to collect
64 enough nano-sized particles for post analysis due to its small size and low flow rate.
65 For studying PM health effects, it is also challenging to differentiate the toxicity
66 between different particles since they are often mixed together in a sample. Separation
67 and classification of atmospheric particles using currently available methods are often
68 restricted in terms with their sizes and species, especially for post-analysis.

69

70 On the other hand, biological detection of certain microbial species is often
71 prohibitive due to complex environmental matrix of the samples, e.g., PCR inhibition
72 problems encountered in many studies (8-10). In microbiology field, the method of
73 gel electrophoresis has been extensively used in separating the DNAs since its earlier
74 invention (11-12). On another front, it was revealed that particles in the atmosphere
75 likewise carry different polarity charges and levels (13-15). For example, it was
76 shown that bacterial particles in indoor and outdoor air carried about 21-92 elemental
77 unit charges (15). Here, we invented a novel particle mixture sieving system named
78 iSCAPE by employing an electrostatic field together with a non-conductive mineral
79 oil medium. Under the same operating conditions, particles in a sample with different

electrical mobility would move at varying velocities in the mineral oil, thus ending up at different locations on the particle moving line within a given time. Depending on objectives, targeted particles or molecules can be thus efficiently extracted from a complex particle of environmental or medical origin using the iSCAPE developed.

Materials and Methods

Experimental setup

In this work, we pioneered a novel system named iSCAPE (fine Sieving of Collected Atmospheric Particles using Oil Electrophoresis) by using an electrical field together with a non-conductive liquid (mineral oil) as shown in Fig. 1. The iSCAPE sieves particles of different sizes from collected atmospheric particle mixture based on their electrical mobility difference. The system consists four major components: high voltage supply (BertanTM, Model 205B-20R, Hicksville, New York), two copper electrodes, mineral oil (M5904, Sigma- Aldrich, USA), and the electrophoresis container (electrical insulation support). In addition, the iSCAPE system is also provided with a ruler that is used to measure the distance from the PM feed point as illustrated in Fig 1. The dimensions of the container are 60×20×4 mm (length × width × height) . The power supply can provide a voltage of up to 20 kV. The mineral oil has a viscosity of 14.2-17.2 cSt (11.9-14.5 mPa*s) and density of 0.84 g/mL at 25 °C.

Fig 1 Experimental setup for fine sieving of atmospheric particles using mineral oil electrophoresis (iSCAPE) powered by a high voltage supply together with a mineral oil. The dimensions of the container are 60×20×4 mm(length × width × height).

iSCAPE of atmospheric particles of various sizes

To test the iSCAPE system, we used atmospheric samples previously collected using automobile air conditioning filters for Beijing, Zurich, and San Francisco (16). Here, we also collected Beijing's soil and road dust samples for testing the system. When operating the iSCAPE, approximately 1 mL mineral oil was first added into the electrophoresis container. Secondly, the power supply with desired voltage was turned on until being stable without air breakdown between the two electrodes. Lastly, approximately 20 μ L mineral oil suspension with the tested samples dissolved (atmospheric particulate matter, soil sample or road dusts) was pipetted into the oil container from the sample feeding point as illustrated in Fig 1. Depending on the experimental objectives, the tests could last from seconds to minutes to sieve particles or extract desired size particles from the sample mixture. Under the used experimental

118 conditions, particles with different electrical mobility would travel a different velocity
119 in the mineral oil, thus ending up in different locations away from the sample feeding
120 point. For particles with positive charges, they moved toward the negative electrode,
121 while particles with negative charges moved toward the opposite.

122

123 **Analysis of iSCAPEd samples**

124 In this work, for different samples, we took samples from various points away
125 from the sample feed points, e.g., 0.5, 1, 1.5, 2 and 3 cm. The samples were further
126 subjected to microscopic analysis using a microscope (BX 63, Olympus Co., Tokyo,
127 Japan). In addition, using a slightly modified iSCAPE system, the particle
128 electrophoresis was also directly conducted on a microscopic slide (S2112,
129 Matsunami Co., Osaka, Japan) such that particles at different points between the
130 electrodes can be continuously imaged using the microscope (corresponding videos
131 are provided as Supporting Information; use of the microscopic slide however could
132 impact the original particle charge distribution in the sample). For particles retrieved
133 from different points between two electrodes, various analyses were conducted. Here,
134 as an example analysis we have calculated their electrical mobility, performed
135 microscopic imaging, and bacterial ATP measurements. The particle electrical
136 mobility was calculated using the following equation (17):

$$137 \quad \mu_d = V_d / E = K \times Q / (d \times \eta) \quad (1),$$

138 where μ_d is the particle electrical mobility ($\text{m}^2/(\text{V}\cdot\text{s})$), V_d (m/s) is the particle velocity,
139 E (V/m) is the uniform electrostatic field, K is a constant, Q is the particle charge, d is

140 the particle diameter, and η is the medium viscosity. Bacterial ATP measurements, as
141 an example analysis for bacterial separation, were performed using a device
142 (SystemSure Plus, Hygiena, Camarillo, CA). For measuring their ATP levels, 5 μ L of
143 mineral oil sample retrieved from different locations was taken and analyzed.

144

145 **Statistical analysis**

146 We have tested the iSCAPE system using different samples (atmospheric PM, soil
147 and road dust samples) under different experimental conditions (different electrostatic
148 field strength (3.17 kV and 6.33 kV/cm), different run time (20 s to 6 min). For each
149 sample retrieved, at least five images were taken from different microscopic views. In
150 addition, we have provided videos of imaged particles along the particle moving lines
151 of the mineral oil. Here, mineral oil (microbiology grade) was also imaged to
152 eliminate the possible particle contamination before any experiments as shown in Fig
153 S1.

154

155 **Results and Discussion**

156 *Atmospheric particle sieving by iSCAPE under different electrostatic field strength*
157 *and run time*

158

Fig. 2 Fine sieving of atmospheric particles collected from three global cities (Beijing, Zurich, and San Francisco(SF)) using the iSCAPE at 3.17 kV/cm for 2 min. Bacterial ATP results for different sampling points are shown in Fig S2. For each sampling point, at least five images (200 μm scale bar) taken from different microscopic views of 10 μL sample.

As shown in Fig. 2, for particles from different cities iSCAPE has demonstrated different sieving capabilities. For atmospheric samples from Beijing, observed particles seemed to have higher electrical mobility (3.95) compared to those (1.32) of the particles from San Francisco and Zurich. In contrast with the control without the iSCAPE, a large amount of particles travelled to 1.5 cm location from the particle feeding point at a speed of 125 $\mu\text{m/s}$ under the experimental conditions tested. As observed in Fig 2, particles with smaller sizes generally moved faster than those larger particles, nonetheless the mobility was proportional to the ratio of particle charge over

174 diameter. Particles from different sources had different particle mobility graphs as
 175 shown in the figure under the same iSCAPE operating conditons (3.17 kv/ cm for 2
 176 min). The differences observed for different cities via the iSCAPE were likely due to
 177 the different components such as bacteria and metals, and the size distributions of their
 178 PMs (5, 16,18). For example, air samples from Zurich were shown to have higher
 179 fraction of nanoscale particles than those from Beijing (5). For the sampling points
 180 listed above, Fig S2 showed the ATP measurements for Beijing's samples that were
 181 iSCAPed. As seen in the figure, most of bacteria moved to the postive electrode
 182 (56%), concentrating within 1.5 cm range from the particle feed point (B-0). For the
 183 negative electrode, about 21% was located within 1 cm range from the feed point
 184 (B-0). These data suggest that the iSCAPE system can be also used to separate
 185 bacterial particles, and a higher fraction of them were shown carrying negative
 186 charges. Apparently, a stronger electrostatic field or longer run time are needed to
 187 sieve large particles from Zurich and San Francisco.

188

189 **Fig.3.** Fine sieving of atmospheric particles from Beijing using the iSCAPE at 3.17
 190 kV/cm for 6 min. Beijing's PMs iSCAPEd videos for 3 min with particles along the
 191 particle moving line are further provided in File S1 (negative charges) and S2
 192 (positive charges) (Supporting Information). For each sampling point, at least five
 193 images (200 μm scale bar) taken from different microscopic views of 10 μL
 194 sample.

195

196 To further test the iSCAPE capability, we have repeated the test with Beijing's PM
 197 samples but with longer run time, i.e., 6 min, at the same electrostatic field strength
 198 (3.17 kV/ cm). Compared to shorter time shown in Fig 2, more particles travelled
 199 away from the PM feed point. It can be again seen that particles with smaller sizes
 200 generally travelled much faster (55.6 μm/s) than those with larger sizes (13.9 μm/s).
 201 In addition to these sampling points, we have provided particle separation information

(iSCAPEd for 3 min) along the particle moving line in videos (File S1 and S2, Supporting Information) from which imaged particles can be seen at any locations between the electrodes. Also, as observed from the figure there were more particles carrying negative charges than those carrying positive ones. Data in these videos also demonstrated that the iSCAPE system can efficiently sieve out the particles. Depending on the targets to be obtained, the run time and electrostatic field strength can be fine adjusted.

209

210 *Soil and road dust sample sieving by the iSCAPE under different electrostatic field*
211 *strength and run time*

212

213 **Fig. 4** Fine sieving of Beijing's soil samples using the iSCAPE at 6.33 kV/cm for 20
214 seconds. Beijing's soil sample iSCAPEd videos are provided in File S7 (negative
215 charges) and File S8 (positive charges) (Supporting Information). For each sampling
216 point, at least five images (200 μm scale bar) taken from different microscopic views
217 of 10 μL sample.

218

219 To further validate the iSCAPE system, we have also performed the same tests
 220 with Beijing soil and road dust samples (Fig S3, S4, and Fig 4) at 3.17 kV/ cm for 2
 221 min. As observed in Fig S3, and S4 along with videos (File S3 S4, S5, and S6), the
 222 iSCAPE system was shown to efficiently sieve soil and road dust particles using their
 223 electrical mobility. Again, particles of different sources have demonstrated different
 224 mobility graphs under the same operating conditions (the electrostatic field strength
 225 and the same run time) given the same viscosity mineral oil. To test high electrostatic
 226 field strength, a modified iSCAPE was used, e.g., shorter electrode distance (3 cm)
 227 but with the same voltage (19 kV), and the results with Beijing soil sample are shown
 228 in Fig 4. As observed from the figure, under higher electrostatic field strength (6.33
 229 kV/ cm), all particles travelled much faster up to 500 $\mu\text{m/s}$ for the location of 1 cm
 230 than the lower electrostatic field (3.17 kV/cm), and even within 20 seconds the
 231 particles can be well sieved as seen in the figure. There was a clear contrast between
 232 samples before and after the iSCAPE test. Images of particles at other particle moving
 233 points on the line can be seen in File S7 and S8 (Supporting Information). In addition
 234 to air, these data showed that the iSCAPE system can be also applied to many other
 235 samples, and the particle sieving can be fine controlled by adjusting the electrostatic
 236 field strength and the run time.

237

238 In this work, we report an invention (the iSCAPE) that can be used to fine sieve,
 239 enrich and extract desired particles including bacteria, fungi, pollen and viruses, out

240 of a particle mixture based on their electrical mobility. For particle health or haze
241 formation mechanism study, the iSCAPE can be used to extract selected particles
242 from an air sample using pre-determined operating parameters. The iSCAPE system
243 also holds an immense potential in separation and purification of protein and chemical
244 molecules from a biological sample. In principle, the iSCAPE system can be used to
245 extract any desired targets from a sample of environmental or medical origin, e.g., for
246 an improved PCR detection, by modifying electrical field, mineral oil viscosity, run
247 time and particle electrical mobility. The particle electrical mobility per equation (1) is
248 a function of particle charge, electrophoresis medium viscosity and particle diameter
249 (17). The bacterial particle charge can be attributed to two factors: ionizable groups
250 ((NH₂) and carboxyl (COOH)) or others present on the cell surface and the external
251 particle frictions (19). To some extent, the latter can be modified by a manual
252 charging process. Therefore, biological and non-biological particles with similar
253 particle sizes could move differently under the same iSCAPE operating condition.
254 The iSCAPE system could be negatively impacted by the moistures in the sample and
255 possible ions in the mineral oil. Certainly, a large amount of work needs to be rapidly
256 explored for the innovative applications of the invented iSCAPE in many different
257 fields such as air pollution, clinical microbiology, and sample purification.

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259

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263 developed here prior to this manuscript submission. M. Yao conceived the research

idea, and S. Xu and X. Li performed experiments with equal contributions.

References

1. Forouzanfar, M.H.; Alexander, L.; Anderson, H.R.; Bachman, V.F.; Biryukov, S.; Brauer, M.; Burnett, R.; Casey, D.; Coates, M. M.; Cohen, A., et al., Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: A systematic analysis for the global burden of disease study. *Lancet*. **2013**, 386, 2287–2323, DOI: 10.1016/S0140-6736(15)00128-2
2. Zhang, T.; Li, X.; Wang, M.; Chen, H.; Yao, M., Microbial aerosol chemistry characteristics in highly polluted air. *Sci China Chem*. **2019**, 62(8), 1051-1063, DOI: 10.1007/s11426-019-9488-3
3. Yao, M., & Mainelis, G., Effect of physical and biological parameters on enumeration of bioaerosols by portable microbial impactors. *J Aerosol Sci*. **2006**, 37 (11), 1467-1483, DOI: 10.1016/j.jaerosci.2006.06.005
4. Agranovski, V.; Ristovski, Z.; Hargreaves, M.; Blackall, P.J.; Morawska, L., Real-time measurement of bacterial aerosols with the UVAPS: performance evaluation. *J Aerosol Sci*. **2003**, 34 (3), 301-317, DOI: 10.1016/S0021-8502(02)00181-7
5. Yue, Y.; Chen, H.; Setyan, A.; Elser, M.; Dietrich, M.; Li, J.; Zhang, T.; Zhang, X.; Zheng, Y.; Wang, J., et al., Size-resolved endotoxin and oxidative potential of ambient particles in Beijing and Zürich. *Environ Sci and Tech*. **2018**, 52 (12), 6816–6824,

287 DOI:

288 10.1021/acs.est.8b01167

289 6. Hewitt, G.W., The charging of small particles for electrostatic precipitation. *Trans*

290 *Am Inst elect Engrs.* **1957**, 76, 300-306 , DOI: 10.1109/TCE.1957.6372672

291 7. Knutson, E.O.; Whitby, K.T., Aerosol classification by electric mobility: apparatus,

292 theory, and applications. *J Aerosol Sci.* **1975a**, 6, 443, DOI:

293 10.1016/0021-8502(75)90060-9

294 8. Alvarez, A.J.; Buttner, M.P.; Stetzenbach, L.D., PCR for bioaerosol monitoring:

295 sensitivity and environmental interference. *Appl Environ Microbiol.* **1995**, 61 (10),

296 3639–3644, DOI: 10.1007/BF00871823

297 9. Hospodsky, D.; Yamamoto, N.; & Peccia, J., Accuracy, precision, and method

298 detection limits of quantitative PCR for airborne bacteria and fungi. *Appl. Environ.*

299 *Microbiol.* **2010**, 76(21), 7004-7012, DOI: 10.1128/AEM.01240-10

300 10. Xu, S., & Yao, M., NanoPCR detection of bacterial aerosols. *J Aerosol Sci.* **2013**,

301 65, 1-9, DOI: 10.1016/j.jaerosci.2013.06.005

302 11. Ünlü, M.; Morgan, M.E.; Minden, J.S., Difference gel electrophoresis. A single

303 gel method for detecting changes in protein extracts. *Electrophoresis.* **1997**, 18(11),

304 2071-2077, DOI: 10.1002/elps.1150181133

305 12. Panyim, S., & Roger, C., High resolution acrylamide gel electrophoresis of

306 histones. *Arch Biochem Biophys.* **1969**, 130 (1), 337-346, DOI:

307 10.1016/0003-9861(69)90042-3

308 13. Wei, K.; Zou, Z; Yao, M., Charge levels and Gram ($\pm$) fractions of environmental

309 bacterial aerosols. *J Aerosol Sci.* **2014**, 74, 52-62, DOI:
310 10.1016/j.jaerosci.2014.04.002

311 14. Yao, M., & Mainelis, G., Utilization of natural electrical charges on airborne
312 microorganisms for their collection by electrostatic means. *J Aerosol Sci.* **2006**, 37(4),
313 513-527, DOI: 10.1016/j.jaerosci.2005.05.006

314 15. Xie, C.; Shen, F.; Yao, M., A novel method for measuring the charge distribution
315 of airborne microbes. *Aerobiologia.* **2011**, 27(2), 135-145, DOI:
316 10.1007/s10453-010-9183-x

317 16. Li, J., Cao, J.; Zhu, Y.; Chen, Q. ; Shen, F. ; Wu, Y. ; Xu, S. ; Fan, H. ; Da, G.;
318 Huang, R.J., et al, Global survey of antibiotic resistance genes in air. *Environ Sci and*
319 *Tech.* **2018**, 52(19), 10975-10984, DOI: 10.1021/acs.est.8b02204

320 17. Hinds, W. C., Aerosol technology: properties, behavior, and measurement of
321 airborne particles. John Wiley & Sons, 1999.

322 18. Li, J.; Chen, H.; Li, X.; Wang, M.; Zhang, X.; Cao, J.; Shen, F.; Wu, Y.; Xu, S.;
323 Fan, H., et al, Differing toxicity of ambient particulate matter (PM) in global cities.
324 *Atmos Environ.* **2019**, 212, 305-315, DOI: 10.1016/j.atmosenv.2019.05.048

325 19. Mainelis, G.; Willeke, K.; Baron, P.; Reponen, T.; Grinshpun, S.A.; Gorny, R.L.;
326 Trakumas, S., Electrical charges on airborne microorganisms, *J Aerosol Sci.* **2001**, 32
327 (9), 1087-1110, DOI: 10.1016/S0021-8502(01)00039-8
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Supporting Information

Figures:

Fig. S1 Microscopic images (200 μm scale bar) of mineral oil (microbiology grade) (Sigma-Aldrich) control used for by iSCAPE system: no particle contamination for the mineral oil was detected during the experiments.

Fig. S2 Bacterial ATP detection results for the Beijing's PM iSCAPed as shown in Fig 2. Numbers in the figure represent the distances (cm) away from the sample feed

344 point (B-0); and minus and plus signs represent locations toward negative and positive
345 electrodes, respectively. Due to limited sample volume, 5 μ L was used for ATP
346 measurement for each sampling point.

347

348 **Fig.S3.** Fine sieving of Beijing's soil samples using the iSCAPE at 3.17 kV/cm for 2
349 min. Beijing's soil sample iSCAPEd videos are provided in File S3 (negative charges)
350 and File S4 (positive charges). For each sampling point, at least five images (200
351 μ m scale bar) taken from different microscopic views of 10 μ L sample.

352

353 **Fig. S4** Fine sieving of Beijing's road dust samples using the iSCAPE at 3.17 kV/cm

for 2 min. Beijing's road dust samples iSCAPed videos are provided in File S5 (negative charges) and File S6 (positive charges). For each sampling point, at least five images (200 μ m scale bar) taken from different microscopic views of 10 μ L sample.

Supporting Files

File S1 Beijing's PMs with negative charges iSCAPed videos at 3.17 kV/cm for 3 min with imaged particles (200 μ m scale bar) along the particle moving line.

File S2 Beijing's PMs with positive charges iSCAPed videos at 3.17 kV/cm for 3 min with imaged particles (200 μ m scale bar) along the particle moving line.

File S3 Beijing's soil samples with negative charges iSCAPed videos at 3.17 kV/cm for 2 min with imaged particles (200 μ m scale bar) along the particle moving line.

File S4 Beijing's soil samples with positive charges iSCAPed videos at 3.17 kV/cm for 2 min with imaged particles (200 μ m scale bar) along the particle moving line.

File S5 Beijing's road dust samples with positive charges iSCAPed videos at 3.17 kV/cm for 2 min with imaged particles (200 μ m scale bar) along the particle moving line.

File S6 Beijing's road dust samples with negative charges iSCAPed videos at 3.17 kV/cm for 2 min with imaged particles (200 μ m scale bar) along the particle moving line.

File S7 Beijing's soil samples with negative charges iSCAPed videos at 6.33 kV/cm for 20 seconds with imaged particles (200 μ m scale bar) along the particle moving

381 line.

382

383 **File S8** Beijing's soil samples with positive charges iSCAPed videos at 6.33 kV/cm

384 for 20 seconds with imaged particles (200 μ m scale bar) along the particle moving

385 line.