High-precision extraction and concentration detection of airborne disease microorganisms based on microfluidic chip

Cite as: Biomicrofluidics 13, 024110 (2019); doi: 10.1063/1.5086087

Submitted: 18 December 2018 · Accepted: 13 April 2019 ·

Published Online: 25 April 2019

Peifeng Xu,^{1,2} Rongbiao Zhang,^{1,a)} Ning Yang,^{1,a)} Paul Kwabena Oppong,³ Jian Sun,¹ and Pan Wang¹

AFFILIATIONS

¹School of Electrical and Information Engineering, Jiangsu University, Zhenjiang 212013, China

²Jiangsu Vocational College of Agriculture and Forestry, Jurong 212400, China

³Agriculture Equipment Engineering Institute, Jiangsu University, Zhenjiang 212013, China

^{a)}Authors to whom correspondence should be addressed. Electronic addresses: 675917471@qq.com and yangning7410@163.com

ABSTRACT

Accurate monitoring of the content of specific disease micro-organisms in the air is one of the key technologies for early warning of airborne diseases. Based on the principle of aerosol particle motion in the microenvironment, this paper proposes a microfluidic chip method for accurately extracting diseased micro-organisms directly from the gas stream. The chip consists of a two-stage coupling of parallel double-sheath flow focusing and radial sheath flow acceleration. Considering the case of extracting mold spores (near spherical shape, average particle size 6 μm) and strawberry gray mold spores (near spherical shape, average particle size 10 μm) from the mixture (concentration of the mixture is about 3.4×10^8 /ml), the performance of the chip was evaluated using two indicators: extraction rate and purity. The results showed that the extraction rates of mold spores and gray mildew spores were 89% and 76% and the purges were 98% and 87%, respectively, achieving high-purity and accurate extraction of fungal spores and greatly improving the detection accuracy. It could be used as the development basis of microbial sensor for the early rapid detection of crop fungal diseases.

Published under license by AIP Publishing. <https://doi.org/10.1063/1.5086087>

I. INTRODUCTION

Certain harmful micro-organisms, including bacteria, fungi, viruses, etc., rapidly spread by air to form airborne diseases, which cause great harm to the natural environment, health care, agricultural production, etc., and may even be exploited by bioterrorism.^{1–5} Studies have shown that the number of harmful micro-organisms in the air is closely related to the degree of disease occurrence. Therefore, understanding the number of infecting micro-organisms during the occurrence of diseases has a very important role in understanding the epidemic law of diseases and improving the predicted level of diseases. At the same time, it also helps the functional departments to grasp the best prevention and control opportunities, effectively control and manage diseases, reduce the irrational use of chemical drugs, reduce environmental pollution problems, and delay the occurrence of pathogen resistance.^{6–8}

In the agricultural field, traditional methods of capturing pathogens in the air, such as slide sedimentation, rotating glue

sticks, and fixed spore traps, can only be used for sampling of pathogens and continuous monitoring of the number of pathogens is not possible, and pathogen capture efficiency and effects are susceptible to climate change, especially rainfall and wind speed.^{9–11} At present, these have been replaced by the fixed-capacity spore trap method, but the latter related equipment is large in size, complicated in structure, and expensive, and it is impossible to promote it in a large area. In addition, due to the extremely complex microbial composition in the air, the sample collected by the trap is mixed with a large number of interfering substances, which seriously affects the detection results when using images, optics, etc.^{12,13}

With the development of MEMS and information technology, microbial sensors are gradually being applied in the fields of medicine and environment. Compared with the traditional spore trap method, microbial sensors have high integration, small size, and low price so that they can be deployed on a large scale. It is the future development trend of airborne

microbial detection.¹⁴ In related research, microfluidic chip-based pathogenic microbial sensing detection system is one of the current research hotspots due to the better integration, low consumables, high throughput, concurrency, and microscopic dynamics of microfluidic chips. It exhibits special work performance and is well adapted to the miniaturized microbial sensor functional requirements.^{15–18}

A typical microfluidic chip sensing system includes functional modules for sample transport, pretreatment, separation and extraction, enrichment, detection, and analysis. The separation and extraction module is used for separating and purifying the target microbial object, which is the premise of enrichment counting, accurate identification, and rapid detection, and it plays an important role in the whole detection system. At present, microfluidic separation and extraction can be divided into two types: passive and active. Passive method refers to the effect of not introducing external field force but only using microchannel geometry or internal force of fluid to achieve separation. Related research such as the one by Clime and Hu Guoqing studied the theory of microfluidic inertial aggregation separation.^{19–21} Active method refers to the method of controlling, separating, and extracting the target biological objects by applying external field forces such as electric fields, sound field, and magnetic field to the flow field of microchannel.^{22,23} According to different control objects, microfluidic separation are divided into particle separation in liquid and gas. At present, the theory and practice of separation of particles in liquid flow field are widely studied.^{24–26} However, the separation of particles in the gas flow field has long been considered an extremely difficult task due to the low levels of pathogens in the air, impurity interference, sensor material and structure, etc. There are still significant limitations in terms of detection accuracy and sensitivity, and there are no practical microbial sensors available in the market.¹⁴ Related studies such as the one by Hong constructed a curved channel inertial microfluidic control to achieve the separation of bacteria and viruses of different particle sizes in aerosols,²⁷ but in general, there are few related studies and lack of data.

The above research on particle separation in the gaseous state is mainly based on the inertial principle of the movement of the microenvironment particles, that is, the particles of different masses and particle sizes moving in the microchannel with a certain distance after the airflow, and the motion position is fixed at a certain position, the required particles are then extracted from these locations, which require a sufficiently compact chip structure, but is apt to complicate the structural design.²⁸

This study proposes a simple method of active airflow driven microbial separation. Although the airflow is still used to drive the particles, the active method of focusing and then separating is adopted, that is, the separation process is changed from the passive mode to the active mode, thereby better controlling the movement speed and trajectory of the particles in the airflow. Despite the features of active separation, there is no additional equipment and cost added, because even with passive separation, airflow drive is still required. The method achieves the extraction of high-purity target micro-organisms, thereby laying a foundation for the development of high-detection and high-sensitivity airborne microbial sensors.

II. MATERIALS AND METHODS

A. Principle of airborne spore extraction

The flow of uniformly dispersed microparticles flows in a laminar flow in the microchannel, which is generally considered to be a low Re number of Newtonian fluids. According to Newton's second law, the state of the combined force of the particles in the gas flow is a linear motion that changes with time. Its equation of motion is

$$\frac{d}{dt}(m_p v_p) = F_D + F_{ext}. \quad (1)$$

Here, m_p is the mass of the particles (kg), v_p is the speed of movement of the particles (m/s), and F_D is the drag force. The air is used as the flow medium under normal pressure. In addition to the drag force F_D , the force exerted on particles in the air flow, such as gravity, buoyancy force, pressure gradient force, additional mass force, Magnus force, Basset force, Saffman force, etc., is expressed by F_{ext} here. At the microscale, the flow characteristics of the airflow are shown as rarefied gas effect, under the condition of higher aspect ratio of microchannel, the volume scale of single spore was smaller than 10% of the volume of flow field.²⁹ Therefore, under a certain flow velocity, the influence of airflow's drag force plays a leading role in spore movement, and other forces can be ignored, namely, F_{ext} can be ignored, as shown in Fig. 1(ii).

Drag force F_D is the most important force for particle motion in airflow. It is solved according to the literature²⁹ using the Cunningham modified Stokes resistance equation,

$$F_D = \frac{3\pi\mu d_p |u_f - u_p|}{C_c}. \quad (2)$$

Here, u_f is the speed of the airflow (m/s), u_p is the speed of the particles (m/s), μ is the viscosity coefficient of the airflow (N s/m²), and C_c is the correct coefficient for Cunningham,

$$C_c = 1 + 0.5Kn_p [2.34 + 1.05 \exp(-0.195Kn_p)]. \quad (3)$$

Here, Kn_p is the Knudsen number of the particles (Knudsen) and is defined as $Kn_p = 2\lambda/d_p$, among them λ is the mean free path (m) of the gas molecules.

Figure 1(i) shows the structure of the microfluidic channel with two-stage sheath flow coupling. The airflow containing microparticles enter the microfluidic chip from the left side and is focused by the double-sheath flow into the separation zone on the right side. The radial sheath flow under the zone causes different degrees of migration of the microparticles of different masses and sizes to achieve the purpose of separation. Considering the separation zone as a plane, from the perspective of particle mechanics analysis, before entering the separation zone, it is subjected to the drag force F_{Dx} in the X direction and after entering the separation zone, the radial sheath flow is added and a new drag force F_{Dy} in

FIG. 1. Principle of microparticle extraction. (i) Microfluidic chip channel structure and particle extraction principle; (ii) force of particles in microchannels; (iii) laminar flow in microchannels; (iv) and (v) particle trajectory in microfluidic chip; and (vi) and (vii) particle motion trajectory in curved channels.

the Y direction is generated. The two drag forces are perpendicular to each other. Therefore, it can be considered that the offset of the particles is the result of the combined effect of the Stokes drag force in the X–Y plane, and the particle offset distance can be expressed as

$$S_y = S_x \cdot \tan \theta \approx S_x \cdot \left(\frac{F_{Dx}}{F_{Dy}} \right). \quad (4)$$

When the airflow flows in the microchannel, the flow velocity of the microchannel centerline to the channel wall decreases in a

gradient due to the laminar flow effect, as shown in Fig. 1(iii). When the microparticles are in different laminar flow positions, different speeds are obtained because of the follow-up so that when the radial sheath flow is accelerated when entering the separation zone, the offset distance of the particles is different, and the overlap at the outlet cannot be achieved because of overlapping interference. The purpose of the separation is shown in Fig. 1(iv). The solution to avoid this situation is to allow the particles to concentrate on the centerline of the microchannel as they enter the separation zone, thereby ensuring that the initial positions of the particles are the same and a relatively uniform initial velocity is obtained so that the offset distance of the same type of particles is roughly equal, but the offset distance of the different types of particles are different, and then the separation targets are better achieved, as shown in Fig. 1(v).

Figures 1(vi) and 1(vii) show the separation of particles in a curved channel and relate to the theory of Dean flow, refer to the paper for inertial aggregation.^{30,31} The principle is to use a multi-stage curved channel or a spiral channel with a sufficient number of turns to make the particle moving position stable and then extract. However, if the distance is not long enough, the offset of the particles in the microchannel is also affected by the initial position and the initial velocity. If the initial position is the same, the separation operation is significantly promoted, so the method is equally applicable to particle separation in the curved channel.

According to the above ideas, this study proposes an active pneumatic separation of airborne spore extraction method, which consists of two functional sections: the focus zone and the

separation zone. As shown in Fig. 1(i), two axial sheath flows were added on both sides of the microchannel, and the airflow containing particles in the middle microchannel is squeezed by this double-sheathed airflow, to achieve the airflow containing particles focusing to the center line of the microchannel. After entering the separation zone, different particles are subjected to different degrees of displacement by the action of the radial sheath flow, thereby achieving the purpose of separation.

B. Simulation analysis and optimization

A finite element multiphysical field simulation software COMSOL was used to build the microfluidic chip 3D model, as shown in Fig. 2(i)(a). Using laminar flow module and particle tracking module, we applied drag force to affect the particles in the flow field and to simulate the trajectory of particles moving with airflow in the microchannel. In addition, we collected the statistics data of airflow velocity, channel size, particle size, quality, etc., then the method of comprehensive fuzzy evaluation is adopted to obtain the optimal parameter matching. By the simulation analysis method, for particles with a particle size ranging from $0.5\mu\text{m}$ to $10\mu\text{m}$ and particle mass ranging from 10^{-12}kg to 10^{-11}kg , we set the length of focusing area and separation area to be $4500\mu\text{m}$, the width of the aerosol inlet channel to be $500\mu\text{m}$, and the height of the microchannel to be $70\mu\text{m}$ and found the flow rate of the aerosol inlet to be $1300\mu\text{m/s}$, the focusing sheath flow rate to be $3500\mu\text{m/s}$, and the radial sheath flow rate to be $5000\mu\text{m/s}$. Figure 2(i)(b) shows the flow field distribution of airflow velocity of

FIG. 2. Microfluidic extraction chip simulation, physical and experimental device. (i) Simulation analysis, (ii) the air spore extraction chip physical map, and (iii) the air spore extraction experimental platform.

the chip. The separation effect of 4 model particles is shown in Fig. 2(i)(c). According to the simulation analysis, if the particles have the same particle size, the quality difference is more than 25%, or if the particles have the same mass, the difference in particle size is more than 18%, and these particles fall into the category of efficient separation.

C. Spore mixture sample preparation

The samples of mold spores and strawberry gray mold spores that have sprouted are provided by the Modern Horticultural Engineering Technology Center of Jiangsu Agriculture and Forestry Vocational and Technical College. Three medium blocks of 5 mm diameter were taken with a puncher, and the spore germination was first observed with a microscope. The concentration of mold spores is about $6.79 \times 10^8/\text{ml}$, and the concentration of strawberry gray mold spores is about $7.82 \times 10^5/\text{ml}$. The two types of spores were then mixed into a test tube, and the above procedure was repeated three times, and the entire experiment was repeated five times.

D. Airborne spores microfluidic extraction experimental device

The microfluidic airborne spores separation chip produced is shown in Fig. 2(ii), where (a) is based on a complete chip including focusing and radial sheath flow; (b) is a simplified chip in which only the separation region is retained, and it is used to contrast the extraction efficiency of particles under nonfocusing sheath flow conditions. According to the simulation analysis and optimization results, the microchannel of the extraction chip was designed to have a width of $500 \mu\text{m}$ and a height of $70 \mu\text{m}$. The chip material was made of polydimethylsiloxane (PDMS), and the microchannel was etched on the bottom surface of the PDMS and bonded to the glass substrate.

The experimental platform for aerosol spore separation is shown in Fig. 2(iii). The platform is mainly composed of (1) the microfluidic separation chip, (2) the test tube, (3) the multichannel air pump, (4) collection cups, (5–9) soft catheter, inverted fluorescence microscope (not shown list), and other components. The multichannel air pump provides a multipass airflow outlet that can be set to a constant flow rate per unit time. The gas flow inlet of the microfluidic separation chip was connected to a test tube using a soft conduit and a test spore mixture. The air outlet of the multichannel air pump is connected to the test tube by a soft conduit, wherein conduit 6 is arranged as a pulsed air flow, mainly for agitating the spore mixture in the test tube, conduit 7 is at a constant flow rate, the inlet flow rate is provided for the microfluidic chip, and conduit 8 is connected to the chip. The dual focus sheath flow inlet, conduit 9 is connected to the radial sheath flow inlet of the chip separation zone. The microfluidic chip separation zone has three outlets connected to three collection cups.

In the experiment, the spore mixture in the test tube was subjected to air flow disturbance, injection, focusing, and separation processing procedures and finally placed into the collection cup. The spore extraction rate and purity of the experimental system were then evaluated using a microscope to observe and count the amount of spores in the collection cup and chip. For statistical

purposes, the loss of spores adhering to the walls of the soft conduit during the experiment was ignored.

E. Spore extraction rate and purity evaluation

Defining spore extraction rate $\eta = N / \sum M_i$, where N is the number of spores that pass through the chip and enter the collection cup, $\sum M_i$ is the sum of the number of spores in the wall of the microchannel in the chip, at each outlet position, and in other collection cups, five outlet positions were taken in this experiment. The spore purity was defined as $\zeta = N_1 / M_1$, where N_1 is the number of target spores in the collection cup and M_1 is the number of all spores in the collection cup.

III. RESULTS AND DISCUSSION

A. Capture area division

Particles enter a large separation area. Due to the inertial effect, there is a diffusion process. Particles of different masses and particle sizes are shifted to different extents. Ideally, the target particles are distinguished and reach the set capture area. The capture area needs to be determined according to the trajectory and dispersion range of the target particle motion, and the effect of overlapping with other particles should be minimized. Figure 3(i) shows the dispersion distance of particles of different diameters at the outlet under different radial airflow velocities under two conditions of focusing and nonfocusing. It can be seen that the particles are scattered far away under focusing conditions. Less than no focus, this facilitates the division and setting of the capture area in the chip design. In addition, Fig. 3(ii) is the variation trend of the dispersion distance with the radial flow velocity, it is easy to see that in the case of no focus, as the radial flow velocity increases, the dispersion distance decreases, in the case of focusing with the increase of radial acceleration, the dispersion distance increases, and the spacing between different particles increases, which is also beneficial to the division of separation regions.

B. Influence of focus preprocessing on test results

The extraction efficiency of the chip is evaluated from the extraction rate and extraction purity of the target particles. First, it is necessary to count the effective amount of the extracted target particles. Figures 3(iii) and 3(iv) show no focus and focus under the control conditions, and the number of particles of four different particle sizes captured can be seen. In the case of no focusing, the scattering range of the particles at the outlet is large, different particles overlap each other, and the target extraction effect is not ideal. On the contrary, in the case of focusing, the objects are separated from each other, the mixing phenomenon is slight, and the precise separation and high-purity extraction of the target particles are achieved.

The results of the analysis of the curved microchannels are similar, and the separation test of three kinds of microparticles (particles with a particle size of $10 \mu\text{m}$ added in the paper) is referred to in the literature.²⁷ Figures 3(v) and 3(vi) show the experimental result data. This grade is mainly used to extract A particles with a particle size of $0.5 \mu\text{m}$. It can be seen that in the absence of focusing, the A particles are in regions 1 and 2

FIG. 3. Experimental data analysis. (i) Dispersion distance of particles at the exit of the chip affected by focusing and radial sheath flow; (ii) variation of the distance of the particles at the exit of the chip with radial flow rate; (iii) nonfocusing treatment of particles. The distribution of each capture zone of the chip has (iv) the distribution of the focused processing particles in each capture zone of the chip, (v) the distribution of the nonfocused particles in the capture zone of the curved channel, and (vi) the distribution of the focused particles in the capture zone of the curved channel.

are interspersed, but in region 2 the A particles are mixed with B particles, having a size of $2\mu\text{m}$. Under the condition of focusing, although the region 2 still has some particles B, the particles A are all concentrated in the region 1, and the precision and the purity are improved. The separation and extraction of other particles will continue in the next stage, but after the focus treatment, the particles have shown strong convergence, objectively creating favorable conditions for the next stage of separation operation.

C. Experimental verification

Based on the above simulation results, we built the airborne spore extraction experiment platform shown in Fig. 2(iii), A mixture containing mold spores (near sphere shape, mean particle

size $6\mu\text{m}$), strawberry gray mold spores (near sphere shape, mean particle size $10\mu\text{m}$), and other impurities were filled in the test tube, the spore-containing airflow is driven into the microfluidic chip by air flow disturbance for separation and extraction, and the final extraction rate and purity of spores were calculated to verify the effectiveness of the experimental method.

The chip (a) shown in Fig. 2(ii) was connected to the experimental platform for verifying the spore extraction performance under the condition of focusing control, setting the flow rate of the gas inlet to $1300\mu\text{m/s}$ and the flow rate of the sheath flow in the focus zone to $3500\mu\text{m/s}$. The flow rate of the sheath flow in the separation zone was $5000\mu\text{m/s}$, the spores were blown into the chip for about 1 min, and then the number of spores in the statistical microchannel and the collection cup was observed under a microscope.

TABLE I. Statistics and analysis of spores were extracted.

Spores	Count type	With focusing						Without focusing					
		G1	G2	G3	G4	G5 ^a	AVE ^b	G1	G2	G3	G4	G5	AVE
Spores of mold	P1	35	22	29	33	31	30	21	27	36	28	31	29
	P2	11	15	14	7	8	11	15	12	10	18	22	15
	P3	4	3	6	2	4	4	8	5	5	11	9	8
	P4 ^c	0	0	2	1	0	1	1	0	0	2	0	1
	CC1	520	482	598	519	684	561	353	479	405	433	219	378
	CC2 ^d	25	30	31	18	14	24	167	305	148	281	137	208
	SUM ^e	595	552	680	580	741	630	565	828	604	773	418	638
	EXR ^f				89%						59%		
	PUR ^g				98%						88%		
Spores of gray mold	P1	5	8	2	3	5	5	8	5	8	6	6	7
	P2	2	3	0	2	4	2	8	8	7	4	5	6
	P3	9	11	7	9	6	8	11	10	10	15	10	11
	P4	15	31	18	22	26	22	15	32	38	18	35	28
	CC1	6	8	15	16	7	10	51	45	45	58	53	50
	CC2	92	269	188	98	127	155	73	63	58	77	79	70
	SUM	129	330	230	150	175	203	166	163	166	178	188	172
	EXR				76%						41%		
	PUR				87%						25%		

^aG1–G5 are the experimental groups.

^bAverage.

^cP1–P4 are the capture areas in the chip.

^dCC1 and CC2 are the number of spore collection cup.

^eSubtotal number of captured spores.

^fExtraction ratio.

^gPurity.

The chip (b) shown in Fig. 2(ii) was connected to the experimental platform to verify the spore extraction performance under the condition of no focus manipulation, setting the flow inlet flow rate to 4000 $\mu\text{m/s}$ and the radial sheath flow velocity in the separation zone to 5000 $\mu\text{m/s}$. The mixed spores were blown into the chip for about 1 min, and then the chip was taken out and the number of spores in the microchannel and collection cup was observed and counted by a microscope.

The above experiments were repeated 5 times, and the collected data were analyzed and summarized. The results are shown in Table I. It can be seen that under the condition of focusing manipulation, the extraction rates of the two spores were 89% and 76%, and the purity were 98% and 87%, respectively, which was much higher than the condition of no focus control, indicating that the focus manipulation can greatly improve the detection accuracy of spores.

IV. SUMMARY

The paper introduces a method for accurate extraction of fungal spores based on a microfluidic chip. The chip is driven by focusing and radial two-stage sheath flow. Through experimental analysis, the focused double sheath flow forces the particles to concentrate on the centerline of the microchannel. With consistent initial velocity, according to the difference of the radial offset

distance of particles with different mass and particle size in the separation zone, the separation of different particles can be realized, and the radial sheath flow can effectively adjust the distribution of particles in the capture zone, which can accurately determine the capture area. The effects of focusing sheath flow on the number and purity of captured spores were compared with mold spores and gray mold spores. It was shown that the focusing sheath flow control significantly improved the extraction rate and purity of spores, which lays a foundation for the research and development of biosensors for the rapid detection of fungal disease in early stage.

ACKNOWLEDGMENTS

This work was supported by the Chinese National Natural Science Foundation (Grant No. 61673195), Chinese National Natural Science Foundation (Grant No. 31701324), and the University “Blue_Cyanine project” training plan of Jiangsu, Jiangsu Agricultural Science and Technology Innovation Fund [Grant No. CX(18)3043].

REFERENCES

- W. W. Thompson, D. K. Shay, E. Weintraub, L. Brammer, N. Cox, L. J. Anderson, and K. Fukuda, “Mortality associated with influenza and respiratory syncytial virus in the united states,” *J. Am. Med. Assoc.* **2**, 179–186 (2003).

- ²H. L. Wang and C. Y. Jiang, "Avian influenza h5n1: An update on molecular pathogenesis," *Sci. China* **5**, 459–463 (2009).
- ³X. D. Duan, X. L. He, S. Xu, Z. C. Liu, and X. D. Yang, "Epidemic trend of pseudoperonospora cubensis in cucumber I: Symptom of disease, leaf position disease resistance capacity and spatial distribution of scattered zoosporangia," *J. Northeast For. Univ.* **4**, 107–109 (2010).
- ⁴K. L. Bowen, K. L. Everts, and S. Leath, "Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust," *Phytopathology* **81**, 503–511 (1991).
- ⁵M. Schoch-Spana, "Implications of pandemic influenza for bioterrorism response," *Clin. Infect. Dis.* **6**, 1409–1413 (2000).
- ⁶L. L. Ji, H. Q. Li, Y. Z. Ren, L. M. Qi, B. L. Zhao, G. Y. Li, and C. Li, "Dynamics of sporangium diffusion of plasmopara viticola and its correlation with disease incidence in vineyard," *J. Fruit Sci.* **1**, 94–98 (2012).
- ⁷C. Blanco, B. D. L. Santos, and F. Romero, "Relationship between concentrations of Botrytis cinerea conidia in air, environmental conditions, and the incidence of grey mould in strawberry flowers and fruits," *Eur. J. Plant Pathol.* **4**, 415–425 (2006).
- ⁸S. Y. Yu, C. H. Liang, L. Liu, C. Y. Liu, and J. F. Fu, "Correlation among grape downy mildew epidemic rate airborne sporangium density of plasmopara viticola and environmental factors in shenyang," *J. Plant Protect.* **3**, 434–441 (2016).
- ⁹Y. L. Zhou, X. Y. Duan, and D. F. Cheng, "Estimation of disease severity of wheat powdery mildew by using data of pathogen spore trap," *Acta Phytopathol. Sin.* **3**, 307–309 (2007).
- ¹⁰L. Xia, X. H. Zhang, J. X. Li, and X. Y. Gao, "Design of a continuous aerial fungal spore catcher," *Plant Protect.* **204**, 76–79 (2005).
- ¹¹X. R. Cao and Y. L. Zhou, "Progress in monitoring and forecasting of plant diseases," *Plant Protect.* **42**, 1–7 (2016).
- ¹²Y. Y. Jiang, J. Y. Luo, D. P. Luo, and J. Zeng, "Monitoring effect of remote-controlled spore trap on wheat aero-borne diseases," *Plant Protect.* **41**, 163–168 (2015).
- ¹³X. R. Cao, Y. L. Zhou, and X. Y. Duan, "The application of volumetric spore trap in plant disease epidemiology," in *Proceedings of the 2008 Academic Conference of the Chinese Society of Plant Pathology* (Chinese Society of Plant Pathology, 2008).
- ¹⁴F. F. Christopher and J. Y. Yoon, "Biosensors for monitoring airborne pathogens," *J. Lab. Automat.* **20**, 390–410 (2015).
- ¹⁵S. Julich, M. Riedel, M. Kieplinski *et al.*, "Development of a lab-on-a-chip device for diagnosis of plant pathogens," *Biosens. Bioelectron.* **26**, 4070–4075 (2011).
- ¹⁶L. L. Yang, Z. G. Wang, M. T. Wang, J. Mu, M. Q. Zhao, J. F. Li, N. Wang, and A. D. Qian, "A novel immunoassay of detecting newcastle disease virus by cytometry with flowing microspheres protein carrier," *Chin. J. Anal. Chem.* **36**, 29–33 (2008).
- ¹⁷S. Lee, J. Choi, L. Chen, B. Park, J. B. Kyong, G. H. Seong, and S. W. Joo, "Fast and sensitive trace analysis of malachite green using a surface-enhanced raman microfluidic sensor," *Anal. Chim. Acta* **590**, 139–144 (2007).
- ¹⁸A. Ebrahimi, W. Withayachumnankul, S. Al-Sarawi, and D. Abbott, "High-sensitivity metamaterial-inspired sensor for microfluidic dielectric characterization," *IEEE Sens. J.* **14**, 1345–1351 (2014).
- ¹⁹L. Clime, X. D. Hoa, N. Corneau, K. J. Morton, C. Luebbert, M. Mounier, D. Brassard, M. Geissler, S. Bidawid, J. Farber, and T. Veres, "Microfluidic filtration and extraction of pathogens from food samples by hydrodynamic focusing and inertial lateral migration," *Biomed. Microdevices* **17**, 17 (2015).
- ²⁰C. Liu, G. Hu, X. Jiang, and J. Sun, "Inertial focusing of spherical particles in rectangular microchannels over a wide range of Reynolds numbers," *Lab Chip* **15**, 1168–1177 (2015).
- ²¹Q. K. Wang and R. Sun, "Advances in the research on 'inertial focus of particles' and its application in microfluidics," *Adv. Mech.* **42**, 692–703 (2012).
- ²²J. Guo, R. B. Zhang, and N. Yang, "Magnetic isolating method for aquaculture pathogens detection system," *Chin. J. Sens. Actuators* **30**, 373–377 (2017).
- ²³Z. H. Ni and S. C. Zhu, "Research on bio-particle separation chip based on dielectrophoresis," *J. Southeast Univ. (Nat. Sci. Ed.)* **35**, 70–74 (2005).
- ²⁴Y. C. Tan and A. P. Lee, "Microfluidic separation of satellite droplets as the basis of a monodispersed micron and submicron emulsification system," *Lab Chip* **5**, 1178–1183 (2005).
- ²⁵W. D. Huang, H. Zhang, T. Xu, C. W. Li, L. J. Zhou, and M. S. Yang, "Separation of blood plasma by inertial focusing using microfluidic chips," *Chin. Sci. Bull.* **56**, 1711–1719 (2011).
- ²⁶A. A. S. Bhagat, "Inertial microfluidics for particle separation and filtration," Ph.D. dissertation, University of Cincinnati, 2009.
- ²⁷S. C. Hong, J. S. Kang, J. E. Lee, S. S. Kim, and J. H. Jung, "Continuous aerosol size separator using inertial microfluidics and its application to airborne bacteria and viruses," *Lab Chip* **15**, 1889–1897 (2015).
- ²⁸P. Sajeesh and K. S. Ashis, "Particle separation and sorting in microfluidic devices: A review," *Microfluid. Nanofluid.* **17**, 1–52 (2014).
- ²⁹W. C. Hinds, *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles* (John Wiley & Sons, 2012), Vol. 1.
- ³⁰R. I. Crane and R. L. Evans, "Inertial deposition of particles in a bent pipe," *J. Aerosol Sci.* **8**, 161–170 (1977).
- ³¹Y. R. Zhou and Z. H. Xie, "Numerical simulation of gas-solid two-phase flow in a 90° curved duct," *J. Xi'an Univ. Sci. Technol.* **28**, 46–50 (2008).