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Multilayer Concentric Filter Device to Diminish Clogging for Separation of Particles and Microalgae Based on Size

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Abstract

Microalgae species have great economic importance; they are a source of medicines, health foods, animal feeds, industrial pigments, cosmetic additives and biodiesel. Specific microalgae species collected from the environment must be isolated for examination and further application, but their varied size and culture conditions make their isolation with conventional methods, such as filtration, streaking plate and flow cytometric sorting, intensive of labour and costly. A separation device based on size is one of the most rapid, simple and inexpensive methods to separate microalgae, but this approach encounters major disadvantages of clogging and multiple filtration steps when the size of microalgae varies over a wide range. In this work, we propose a multilayer concentric filter device that has a varied pore size and is driven with a centrifugation force. The device, which includes multiple filter layers, was employed to separate a heterogeneous population of micro-particles into several subpopulations with filtration in one step. A cross-flow to attenuate prospective clogs is generated by altering the rate of rotation instantly through the relative motion between the fluid and the filter according to the structural design of the device. Mixed micro-particles of varied size were tested to demonstrate that clogging was significantly suppressed, for a highly efficient separation. Microalgae in a heterogeneous population collected from an environmental soil collection were separated and enriched into four subpopulations according to size in one step filtration process. A microalgae sample contaminated with bacteria and insect eggs was also tested to prove the decontamination capability of the device.

Key words: Cross-flow, concentric filter, clog reduction, microalgae separation and decontamination, particle separation, centrifugal microfluidics

Introduction

The biodiversity of microalgae is enormous: 200,000-800,000 species are estimated to exist, of which only about 50,000 species are reported. Over 15,000 novel compounds originating from algal biomass have been chemically determined.[1] The chemical components extracted from microalgae are valuable, and are much applied in the field of pharmaceuticals, cosmetics, functional foods and biofuels.[2, 3] For example, microalgae can be cultivated to produce polyunsaturated fatty-acid oils, which are nutritional supplements of infant formulae,[4, 5] microalgal carbohydrates serve as a source of carbon in the fermentation industry,[6, 7] lipids extracted from microalgal biomass could provide a feedstock for biodiesel,[2, 8] and proteins and pigments extracted from microalgae have been applied in the pharmaceutical industry.[2, 9] The production of these microalgal chemicals and bioactive compounds typically requires monocultures of specific microalgae species, for which the isolation of a single microalgae species is critically important. Traditional methods of microalgae separation such as serial dilution and streaking plate method, both were inefficient and intensive of labour. Sorting microalgae with a flow cytometry to isolate a specific microalgae population is not only an expensive method but also unsuitable for the isolation of microalgae samples of small number. Bacterial contamination is another important issue to be considered in isolating microalgae.[10] Bacteria commonly appear in a microalgae living environment; some bacteria can attach to the microalgae and be co-cultured with the same culture medium. This unwanted co-culture causes difficulty on isolation and serious pollution arises in the industrial manufacture of microalgae products. The isolation of microalgae from a bacterial contamination is hence important for an industrial production of microalgae.

Several microfluidic devices to separate micro-particles and bio-samples have been reported. Various techniques involved in developing these separation devices include hydrodynamically based,[11-16] charge-based,[17-20] wave-based,[21] magnet-based[22, 23] and filter-based[24-26] devices to separate particles and bio-samples. Although these approaches showed promising results, the challenges of stable flow control, a requirement of complicated and expensive equipment and a limitation of a dynamic

sorting range are still applicable in most devices for separation of particles and biological samples, except a device based on a filter. Separation based on a filter is among the simplest, most efficient and least expensive method to separate particles and bio-samples, but clogging is its main disadvantage, such that the efficacy in a separation system gradually decreases. Filtration of a cross-flow type was applied to decrease clogging in many investigations and applications.[27-29] The major advantage of a cross-flow is that clogs at the filter pores can be substantially washed away during the filtration, so that the filter unit can be operated durably.

Lab-on-a-CD systems integrated with miniaturization technology and centrifugal pumping system were widely applied with in vitro diagnostics (IVD), sample treatment and separation. Sample processing steps such as reagents mixing and metering of sample fluids can be easily automated by adjusting different spinning parameter. Compared with the driving force of other chip-based microfluidic systems, centrifugal microfluidic platforms offer many superior properties.[30] For instance, the centrifugal pump requires only a simple centrifuge to provide the drag forces for fluid manipulation. The external, complicated and expensive equipment such as delicate syringe pumps and other electronic devices are unnecessary in a centrifugal microfluidic separation system. Moreover, samples separated in this system are unaffected by electricity, magnetism, heat or waves, which arise in other separation systems. Depending on the species of microalgae, their sizes can range from a few micrometers (μm) to a few hundreds of micrometers. They are hence appropriately separated on a micro-scale of a microfluidic system. In this research, we developed a centrifugally driven, multilayer, concentric filter device, shown in Fig. 1. The separation of micro-particles and microalgae with this device was driven with centrifugal force. A small bench-top centrifuge, which is commonly available in biochemical laboratories, was employed as part of our centrifugal microfluidic separation system. Such a laboratory centrifuge is readily accessible, affordable and portable. The cross-flow in our separation system is generated on altering the rate of rotation, as revolutions per minute (RPM), instantly through the relative motion of the device and the fluid, as shown in Fig. 1. This strong cross-flow can wash away the particle and bio-sample clogs at the filter pores, so increasing the filtration efficiency

without complicated control and expensive equipment. The use of our device enabled variously sized micro-particles and environmentally collected microalgae samples to be readily separated in a few minutes. Similarly sized micro-particles or microalgae were retained and enriched at the same filter layer with minimal clogs due to a cross-flow washing effect. This device was used also to isolate microalgae from a contaminated mixture of bacteria and insect eggs based on their size difference. This concept makes this device extremely effective for the application of separating micro-particles and bio-samples on a small scale and with a minimal volume.

Material and Methods

Fabrication

The microfluidic device was fabricated using photolithographic and soft-lithographic techniques. All device designs were created in AutoCAD 2010 (Autodesk, San Rafael, CA, USA); a transparency mask was created from the CAD file and created with a laser pattern generator (DWL2.0). The silicon wafer was first spin-coated with a photoresist (SU-8 3050) at speed 3000 rpm. A transparent mask created with the negative pattern of the device was placed over the wafer and exposed to ultraviolet light, which cross-linked the photoresist in the exposed areas. The uncross-linked photoresist was then washed away, resulting in a silicon wafer layered with the positive relief of the device. Microfluidic devices were made on replica molding of polydimethylsiloxane (PDMS; Dow Corning, USA) and curing the degassed elastomer mix (10: 1, base: curing agent) against the silicon master in an oven (80 ^{o}C, 2.5 h). Polymerized PDMS devices were peeled from the silicon master; individual devices were cut; inlets and outlets were created with standard punch tools (diameter 2 to 4 mm). PDMS devices were dusted with air, washed multiple times with deionized water and ethanol and dried on a hot plate. The glass cover slips were dusted with air and washed multiple times in deionized water and isopropanol. PDMS surface and cover slips were oxidized with an oxygen plasma for 45 s, and bonded on a hotplate (90 ^{o}C, 30 min).

Filter Size Variant of Concentric Separation Device

Pores of varied size in a concentric filter layer structure were designed and fabricated for the separation of micro-particles and microalgae. Pore sizes 20 and 10 μm of the bilayer concentric filter device were fabricated and tested for the effect of attenuating particle clogging with a cross-flow; pore sizes 25 and 15 μm of the bilayer concentric filter device were tested with particles of heterogeneous diameter to evaluate the efficiency of separation with and without the cross-flow. A concentric filter device with three layers and filter pore diameters 15, 10 and 5 μm was used to separate a microalgae sample from an environmental collection. The channel height of the three devices was 50 μm. As PDMS is an elastic material, circular micro posts of size from 0.8 to 2.4 mm were created in each circle channel to support the thin and wide channels from collapsing, as shown in Fig. 2a. The device was anchored with an adapter, shown in Fig. 2b and c. The anchored device was then mounted on the adaptor in a small laboratory centrifuge (E-Centrifuge, Wealtec Corporation; 4000 rpm maximum), as shown in Fig. 2d. A film covered the top of the inlet and outlet chambers to prevent splash during centrifugation. The device was spun at 4000 rpm for 10 s and then manually stopped abruptly to generate a cross-flow among each concentric filter layer; the procedure of spinning and stopping was undertaken 5 to 15 times (50 to 150 s). For the last spin, no stop was required; the centrifugal force would keep the small particles passing through the filter and prevent them from flowing back to the inner channel. A general microscope was applied to observe the separation results.

Tangential Velocity of the Cross-flow in Each Concentric Layer and Diminution of Particle Clogging

According to the radius of each concentric filter layer, the tangential velocity of the cross-flow in each concentric layer was calculated with a formula, $V = R\ 2\pi/T$, shown in Fig. 3a. The radius is 4.5 mm for the filter layer with pore size 25 μm, and 9.5 mm for the layer with pore size 15 μm; the dimensions of the 20- and 10-μm bilayer

device are the same as for the 25- and 15-μm devices. The radii of the 15-, 10- and 5-μm tri-layer device are 4.5, 7.5 and 10.5 mm respectively. To examine the cross-flow effect on diminishing particle clogging with an experimental operation, particles (20 μm, Polyscience, Inc., Warrington, PA USA) at concentration 6×10^5 particles/mL were tested using the bilayer concentric device with filter pore sizes 20 and 10 μm. A procedure involving spinning without stopping that generated no cross-flow was compared with the procedure involving spinning and stopping that generated a cross-flow. The results of diminution of clogging were observed with a microscope.

Separation of Micro-particles of Varied Size

The efficiencies of separating micro-particles of varied size were measured with the bilayer filter devices (25 and 15 μm) in the condition with and without the cross-flow. Particles of two types were mixed and tested with the device; particles of average size 49 μm (range 25-85 μm, GenScript USA Inc., Piscataway, NJ) at concentration 4×10^5 particles/mL were mixed with particles of average size 20 μm (range 14-22 μm, Polyscience, Inc., Warrington, PA) at concentration 4×10^4 particles/mL to generate a pool of particles of diameter from 14 to 85 μm. The devices were prefilled with water to minimize introduction of bubbles before use. The mixed micro-particle solution (volume 100 μL) was loaded into the central inlet chamber of the device; a film covered the top of the inlet and outlet chambers to prevent splash during centrifugation. After the device was anchored and mounted in the small laboratory centrifuge, it was spun at 4000 rpm for 10 s; the rotation was then manually stopped abruptly to generate a cross-flow. The procedure involving spinning and stopping was repeated 5 to 15 times sequentially. The control was spun for 50 to 150 s without stopping. A general microscope was applied to observe the separation results.

Separation of a Sample of Environmental Microalgae

The microalgal samples from an environmental soil collection were first cultured in the sterilized tubes for 1 to 3 weeks with light incubation. A mass of microalgae was

taken from the tubes and the microalgae were mixed into sterilized water. The original concentration obtained from the collection was 1.12×10^6 cells/mL. The pre-cultured microalgal sample was loaded into the inlet chamber of the tri-layer concentric filter device with water prefilled; the device was operated with spinning and stopping 15 times to separate the microalgae. To investigate the ability of the device to separate microalgae from bacterial contamination, a microalgae sample contaminated with bacteria and unknown insect eggs was tested with the tri-layer filter device. Before separation, trypan blue was added to stain the bacteria for improved observation.

Microscope and Particle Counting

After microparticles and microalgae were separated with the multilayer concentric filter devices, each concentric channel was imaged on a microscope (Leica DM IL LED). Images were recorded at magnifications 10× and 20× with a digital camera (Nikon D5000), and the number of separated micro-particles of a particular size at each concentric space was counted and analysed with a microscope to measure the efficiency of separation. For each data point, the averages of three images were compiled to ensure an adequate sample size. Because of error of the diameter measurement and device fabrication, particles of diameter greater than 27 μm, 27 to 17 μm and smaller than 17 um were counted for the separation efficiency in a 25-μm filter, 15-μm filter and outer layer respectively.

Results

Concentric Separation Device with Varied Filter Size

Concentric multilayer filter devices of varied pore size were designed and fabricated to examine the efficacy of separation and enrichment of micro-particles and microalgae. Devices of three types were fabricated. The first device, with 20-and 10-μm bilayer filter pores, was used to examine the ability of diminution of clogging shown in Fig. 2a. The second bilayer filter device, with 25- and 15-μm pore sizes, was fabricated and used to separate micro-particles of varied size with and without a cross-flow. The third, tri-layer filter device, with 15-, 10- and 5-μm pore sizes, was made to separate and enrich microalgal samples, shown in Fig. 5 and 6. All multilayer

filter devices were made of PDMS; as this material is elastomeric, it would collapse on covering a wide channel. The circles were thus designed around the channel function as a support structure to prevent the PDMS from collapsing, shown in Fig. 2a. The centre inlet was designed to load a sample for separation, and the multilayer filters were designed to separate particles and microalgae into many subpopulations based on the size difference. The outlet in each layer was used to collect a separated sample.

Tangential Velocity of the Cross-flow in Each Concentric Filter Layer and Diminution of Particle Clogging

The tangential velocity of the cross-flow generated along each concentric filter layer of the devices depends on the radius of each filter layer, calculated with a formula for the tangential velocity, $V = R\ 2\pi/T$. The theoretical velocity of the 20-μm pore filter layer is 1884 mm/s and of the 10-μm filter layer is 3799 mm/s. The theoretical velocity of the 25-μm pore filter layer is 1884 mm/s and of the 15-μm pore filter layer is 3799 mm/s; the theoretical velocities of the 15-, 10- and 5-μm pore filter layers are 1884, 3140 and 4396 mm/s respectively, shown in Fig. 3a. All velocities of the cross-flow were calculated based on rotation at 4000 rpm. To demonstrate the effect of the cross-flow on the diminution of particle clogging, particles of diameter 20 μm were loaded into the bilayer filter device (pore sizes 20 and 10 μm). A comparison of devices operated without stopping and stopping was designed to evaluate the washing effect of the cross-flow, as shown in Fig 3b. In the continuous spinning condition, no cross-flow was generated; a serious clog was observed, which also blocked the fluid flow, shown in Fig. 3b1 and b3. In contrast, with the spinning and stopping, particle clogs were diminished significantly through the cross-flow washing effect at the 20-μm concentric filter of the device, shown in Fig. 3b2 and b4.

Separation Efficiency for Particles of Varied Size

The efficiency of the device to separate particles of diameter over a wide range was analysed in the condition with and without the cross-flow. Micro-particles of diameter from 14 to 85 μm were loaded into the device to examine the separation efficiency.

The device was span at 4000 rpm for 10 s and then manually stopped abruptly to generate the cross-flow to diminish the clogging. The procedure with spinning and stopping was repeated 5 to 15 times (50 to 150 s) to evaluate the particle distribution. The spinning and stopping procedure at 50, 100 and 150 s was calculated sequentially to evaluate the particle distribution in each filter layer. The particle distribution for each filter layer was calculated according to the number of particles of each type trapped at each channel divided by the total number of particles trapped at each channel.

In the 25-μm filter layer with the cross-flow, the fraction of particles of diameter greater than 27 μm increased from 49 % to 80.8 % when spinning and stopping was performed from 5 to 15 times (50 s to 150 s). Without the cross-flow, it increased from 49 % to 67.1 %. The fraction of particles of diameter 17 to 27 μm was decreased from 45.8 % to 17.6 % with the cross-flow, it decreased from 39.1 % to 28.5 % without the cross-flow. The fraction of particles of diameter less than 17 μm was decreased from 5.2 % to 1.6 % with the cross-flow; it decreased 8.2 % to 4.4 % without the cross-flow.

In the 15-μm filter layer with the cross-flow, the fraction of particles of diameter 17 to 27 μm increased from 87.9 % to 93 % at 50 s to 100 s and slightly decreased to 90 % at 150 s. Without the cross-flow, it decreased from 72.2 % to 64.6 % at 50 s to 100 s and increased to 75.4 % at 150 s. The fraction of particles of diameter greater than 27 μm was 0 during all processes with or without the cross-flow. The fraction of particles of diameter less than 17 μm decreased from 12.2 % to 10.2 % at 50 s to 150 s with the cross-flow. Without the cross-flow, it increased from 21.9 % to 35.5 % at 50 s to 100 s and decreased to 24.6 % at 150 s.

In the outer channel, the fraction of particles of diameter less than 17 μm increased from 0 to 88.4 % at 50 s to 150 s with the cross-flow. It increased from 0 to 82.9 % at 50 s to 150 s without the cross-flow. The fraction of particles of diameter greater than 27 μm was 0 during the entire process with or without the cross-flow. The fraction of particles of diameter from 17 to 27 μm was increased from 0 to 11.6 % at 50 s to 150 s with the cross-flow. It increased from 0 to 17.1 % at 50 s to 150 s without the cross-flow.

Separation of Heterogeneous and Bacterially Contaminated Microalgal Samples

The tri-layer filter device was used to test the separation and enrichment ability of a mixed variable microalgal sample from an environmental soil collection. The heterogeneous microalgae collection loaded in the centre inlet of the device is shown in Fig. 5a. The heterogeneous microalgae were composed of various species of varied size. After separation with the tri-layer concentric filter device, the microalgae were divided into four groups. Microalgae in a group with size greater than 15 μm were retained and enriched in the inner 15-μm filter layer as shown in Fig. 5c. The distribution of microalgae with size greater than 15 μm was increased from 0.1 % to 12.5 %; it enriched 125 times the original concentration as shown in table 1. Microalgae in a group with diameter ranging from 15 to 10 μm were retained and enriched in the 10-μm layer as shown in Fig. 5d; the distribution of microalgae was increased from 0.5 % to 28.6 %, so enriched 57.2 times the original shown in table 1. Microalgae of diameter from 10 to 5 μm were retained and enriched in the 5-μm filter layer, as shown in Fig.5e; the distribution of microalgae was increased from 3.6 % to 37.7 %, so enriched 10.5 times the original shown in table 1. Other microalgae of diameter less than 5 μm were separated to the outer channel of the device, as shown in Fig. 5f. There were few microalgae larger than 5 μm found in the outer channel. The distribution of microalgae less than 5 μm was increased from 95.8 % to 99.2 %. The microalgal sample contaminated with bacteria and insect eggs was tested with the tri-layer filter device to test its capability of decreasing contamination. The microalgae sample contaminated with bacteria and insect eggs is shown in Fig. 6a. After separation with the tri-layer device, most microalgae were retained in the 10-μm filter layer, shown in Fig. 6b, and few bacteria were found in this layer. The distribution of microalgae was increased from 0.9 % to 51 % and the contamination of bacteria was decreased from 97.9 % to 0.8 % as shown in table 2. The insect eggs were separated in the 5-μm filter layer and the outer channel, shown in Fig. 6c and d; bacteria stained blue were separated in the outer channel, shown in Fig.6d.

Discussion

The multilayer, concentric, filter microfluidic devices tested in this work provide a simple, economical, rapid and highly efficient means of separation based on size discrepancy, micro-particles and microalgae in a heterogeneous population. With the use of an inexpensive, convenient and portable bench-top centrifuge combined with the device design of a concentric filter layer, a centrifugal force and a cross-flow were generated concurrently. The separation required only 3 min for operation. This device can be designed to separate particles in a heterogeneous population according to the particle diameter in a particular range. A conventional filter separates a sample into only two subpopulations, so that further filtration steps are required to separate a sample into subgroups. With the multilayer filter design in this device, particles and bio-samples in heterogeneous populations were separated into several subpopulations in a single filtration. The groups of subpopulation can be freely controlled by the numbers of concentric filter layers. The size of particles to be separated can readily be determined from the pore size of the concentric filter. The device fabrication uses a standard PDMS molding process that can produce large quantities at low cost.

The cross-flow velocity in each concentric filter layer of the device was calculated based on the radius of the filter layer and the top speed of the portable laboratory centrifuge, 4000 rpm. The velocity of the cross-flow attained 1884 mm/s in the 20-μm layer and 3977 mm/s in the 10-μm layer, which are very fast flow velocities relative to those of other microfluidic systems.

The velocity was calculated based on the relative motion on stopping the rotation of the device instantaneously. If the stopping is not implemented abruptly, the velocities of the cross-flow decrease. To minimize this effect, the rotation is stopped abruptly to create a cross-flow at a maximum velocity. To be more consistent, an improved design to add a notch to outside portion of the adaptor plate combined with mechanically spring-mounted rod to stop the rotation automatically shall be performed in the further study. Another condition that might decrease the flow velocity is the viscous dissipation that occurs at the interface of the fluidic and PDMS

structure. The only solvent used in this work is pure water, which has a small viscosity. Moreover, PDMS is an inherently hydrophobic material; the contact angle of water on PDMS is 109^{o}. We thus regard that the viscous dissipation and interface interaction does not perceptibly decrease the velocity of the cross-flow. However, viscosity may have positive effect on clog washing effect due to the larger fluid shear force to particles in fluid dynamics. In conclusion, we regard that both the flow velocity and viscosity should be useful for clog reducing. Therefore, a high viscosity solvent may be applicable in this device by the high shear force instead of fast velocity.

To ensure the diminution of clogging with the cross-flow generated in the device, we undertook a comparison of tests with and without cross-flow. According to the results shown in Fig. 3b, the cross-flow, as expected, was demonstrated to attenuate the clogging significantly. Without the cross-flow, an accumulation of particles in the filter layer is evident, and the clogs even blocked the fluid flow. With the effect of the cross-flow, the clogs were washed away from the filter pores and the fluid passed the filter pore again. The main disadvantage of a device based on a filter was diminished significantly. To quantify further the functionality of the multilayer concentric filter device with cross-flow, we introduced heterogeneous micro-particles of diameter 14 to 85 μm into the bilayer concentric filter device with filter pore sizes 25 and 15 μm. The devices were operated with and without cross-flow for comparison. With the cross-flow in the 25-μm filter layer, the final particle distribution reached 80.8 % for particles of diameter greater than 27 μm at 150 s. Without the cross-flow, it reached only 67.1 %. The particle distribution is only 17.6 % for undesired particles of diameter greater 17 to 27 μm with cross-flow in this layer; it reached 28.5% without the cross-flow. In the 15-μm filter layer with the cross-flow, the distribution of targeted particles of diameter ranging from 17 to 27 μm reached 90 % at 150 s; without the cross-flow, it reached only 75.4 %. Those results provide the quantity analysis to demonstrate the cross-flow significantly increased the efficiency and purity for separation. In addition, according to the separation analysis shown in Fig. 4; we found that, with the cross-flow, the separation efficiency kept increasing during the separation process from 50 to 150 s. In contrast, without the cross-flow, the

separation efficiency did not increase after separation for 100 s, reflecting that clogging occurred with blockage of the filter. The analyses show that the cross-flow could make the filter operated durably and prolong its lifespan.

We found that some particles of diameter 17-19 μm passed the 15-μm filter layer after the spinning and stopping 15 times (150 s). We speculate that, through deformation of the PDMS, the particles of diameter 17-19 μm passed the 15-μm filter. In contrast, no particle of diameter greater than 27 μm passed the 25-μm filter after the spinning and stopping 15 times; we speculate that, because the radius of the 25-μm layer is smaller than that of the 15-μm layer, the centrifugal force in the 25-μm layer is weaker than in the 15-μm layer, such that no particle of diameter greater than 27 μm passed the 25-μm filter after spinning 15 times.

In the outer channel, the fraction of particles of diameter less than 17 μm remained 0 for the spinning and stopping procedure from 50 to 100 s, but increased to 88.4 % for this procedure from 100 to 150 s with cross-flow. We speculate that the centrifugal force is related to the particle mass, such that small particles require greater force to pass the multilayer filter to the outer channel. In contrast, without the cross-flow, the fraction of particles of diameter less than 17 μm reached 86 % at 100 s; we regard this result to be due to the continuous centrifugation force from 50 s to 100 s without stopping procedure.

In this work, we achieved the separation of microalgae from environmental soil collection with a 15-, 10- and 5-μm tri-layer filter device according to the size distribution of the microalgae. The pre-cultured mixed microalgae collection in a heterogeneous population was separated into four subpopulations classified by size related to the number of the concentric filter layer shown in Fig 5. The device enriched each subpopulation of microalgae from 28.6 to 125 times. Compared with traditional filtration, this device could generate many subgroups of particular size of microalgae by one step and prevents potential sample loss with multiple filtrations. This process could be a first step to separate microalgae into many subpopulations for

further isolation. As we can enrich significantly the distribution of each subpopulation of algae, further isolation is facilitated. We found that the aggregation of microalgae is an important issue for the separation efficiency; the aggregated microalgae were retained in the inner filter layer as its size was enlarged. This phenomenon decreases the separation efficiency of microalgae. To disperse the microalgae through a violent vortex or a chemical treatment to diminish the aggregation or a further dilution before the separation would be potential solutions for this aggregation issue. However, the aggregation is expected not to affect much the further isolation of microalgae, as it would be easy to be isolated from other single microalgae by its comparatively large size. An application for decontamination of microalgae was demonstrated with the tri-layer device, in which a microalgae sample contaminated with bacteria and insect eggs was tested. The results of bacterial decontamination show that almost all microalgae are retained in the 10-μm filter layer; most bacteria with a blue stain were not found in the same filter layer, as shown in Fig. 6b. Most bacteria and insect eggs were separated in the 5-μm filter layer and the outer channel, shown in Fig. 6c and 6d. Although some part of the insect eggs were retained in the 10-μm filter layer, it would not be difficult to separate them from microalgae due to the distinct culturing condition of algae and insect eggs. As we mentioned previous, due to the co-cultured condition and bacteria attached with surface of microalgae; it causes a major difficulty to isolate free microalgae from contamination. We speculate that the strong cross-flow and centrifugation force generated in our device during separation might have a large effect on washing and pulling the attached bacteria away from the microalgae. Compared with the traditional serial dilution and streaking plate method, this device could offer a better solution to overcome the current difficulty on decontamination of microalgae. In conclusion, the device could accelerate the progress and decrease the cost of microalgal isolation. The device could potentially enhance many industrial applications on isolating specific and purified microalgal species.

As the total sample volume applied in this device is 200 to 300 μL, it is suitable for a small volume of sample separation. The sample loss is limited compared with other filter methods. For separation of a sample of large volume, the device could be designed to create a large inlet chamber to increase the loading volume. A higher and

wider channel in the outer layer of the device could be also applied to increase the working volume. The device can be further applied as a rapid and cheap diagnostic tool. For example, to isolate white blood cells from total blood based on a size difference, or to separate rare cells such as circulating tumour cells based on their size being larger than other cells, the large cell is retained in the inner filter layer, which is readily observable because of the small inner filter channel area and the enrichment effect. Varied dimensions such as the numbers of concentric filter layers, the radius of the filter layer and the filter pore size and number could be designed to suit a targeted sample for optimal separation parameters.

Conclusion

An efficient and convenient centrifugal concentric filter microfluidic chip and system were fabricated and demonstrated. The microfluidic device is fabricated with a regular PDMS modelling process and is easily adaptable to a portable, inexpensive laboratory centrifuge. With the concentric structural design and operating steps, it can diminish clogging that is the main disadvantage encountered with other methods based on filters. The separation can be accomplished in a few minutes. The device could be applied also to separate particles and bio-samples of varied size on designing an appropriate filter pore size and layer. Future application to blood separation and biochemical diagnosis is expected.

Acknowledgement

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Table. 1 Separation efficiency and enrichment of algae subpopulation in each filter layer

	Before separation %	After separation %	Enrichment
Algae >15μm in 15-μm layer	0.1± 0.05%	12.5± 3.1 %	125
Algae 15-10μm in 10-μm layer	0.5± 0.2%	28.6± 5.3%	57.2
Algae 10-5μm in 5-μm layer	0.9± 0.3 %	25.7± 4.8%	28.6
Algae <5μm in the outer layer	98.52± 1.1%	99.2 ± 0.8%	1

Table. 2 Separation efficiency and enrichment of algae contaminated with bacteria

	Before separation %	After separation %	Enrichment
Algae	0.9±0.1%	51±8.6%	57
Bacteria	97.9±1.9%	0.8±0.5%	-122
Worm eggs	1.2±0.3%	48.2±9.5%	40

Caption of figures:

Fig. 1 Schematic illustration and working principle of a size-based multilayer concentric filter device. The device contains a central inlet for sample loading and a multilayer concentric filter; the pores of the inner filter are larger than those of the outer filter. Using centrifugal force, large particles are retained in the inner filter layer; medium and small ones are separated by each concentric filter layer and retained in the appropriate filter layer according to the size of particles in a serial order. A cross-flow in this system is generated on altering the rate of rotation instantaneously through the relative motion between the fluid and the filter layer. The cross-flow washes away the prospective clogs at the filter pores.

Fig. 2 (a). Appearance of the bilayer concentric filter device; the centre inlet was designed to load a sample and the inner and outer filters were designed to separate particles based on size difference. The circles in the channel function as a support structure prevent the PDMS collapse. (b), (c) and (d) show that the device is anchored by an adapter and mounted in the laboratory centrifuge

Figure. 3 (a)The tangential velocity of the cross-flow in each concentric layer of a device of three types was calculated according to its radius and the rate of rotation, at 4000 revolutions min^{-1}. (b) Comparison of the clogs diminished in the condition with and without cross-flow by microscopic observation. (b1,3) Without the cross-flow, an accumulation of particles in the filter layer is evident; the clogs block the fluid flow as the arrow indicates. (b2, 4) With the washing effect of the cross-flow, the clogs were washed away from the filter pore; the fluid passed the filter pore as the arrow indicates.

Figure .4 (a) Particle distribution of varied microparticles of diameter ranging from 14 to 85 μm in the 25- and 15-μm filter layer with the cross-flow on repeating the spinning and stopping procedure 5 to 15 times (50 to 150 s) sequentially. (b) Particle

distribution of varied micro-particles of diameter ranging from 14 to 85 μm in the 25- and 15-μm filter layer without the cross-flow from 50 to 150 s.

Figure. 5 Microscopic views of separated microalgae obtained from an environmental collection in the tri-layer concentric filter device (a). Mixed microalgae of varied size loaded in the inlet of the device before separation. (b). Appearance of the tri-layer filter device; the pore size of each filter layer from inner to outer was 15, 10 and 5 μm. Microalgae in a heterogeneous population were consecutively separated by size at each filter layer. (c). Microalgae of diameter greater than 15 μm were retained and enriched at the 15-μm filter layer. (d). Microalgae of diameter 15-10 μm were retained and enriched at the 10-μm filter layer. (e). Microalgae of diameter 10-5 μm were retained and enriched at the 5-μm filter layer. (f). Microalgae of diameter less than 5 μm were separated to the outer channel of the device.

Figure. 6 Microscopic views of a microalgae sample contaminated with bacteria and insect eggs separated with the tri-layer filter device (a). The microalgae sample contaminated with insect eggs and bacteria was loaded in the inlet of the device before separation. (b). Most microalgae were retained at the 10-μm filter layer after separation. (c). Insect eggs were retained at the 5-μm filter layer. (d). Insect eggs and bacteria stained in blue were separated to the outer layer of the device.

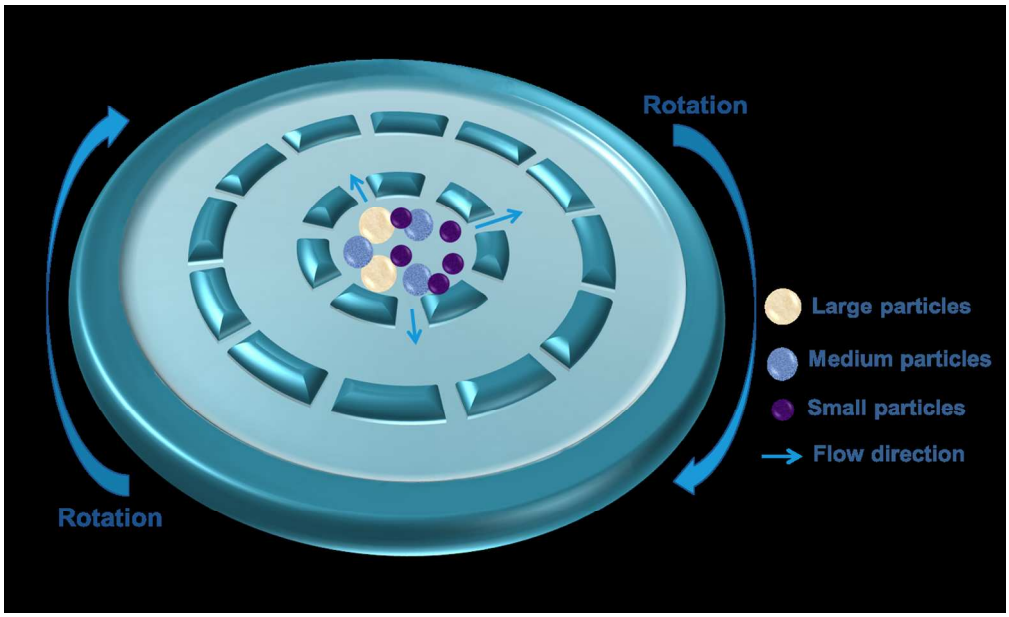

Fig. 1 Schematic illustration and working principle of a size-based multilayer concentric filter device. The device contains a central inlet for sample loading and a multilayer concentric filter; the pores of the inner filter are larger than those of the outer filter. Using centrifugal force, large particles are retained in the inner filter layer; medium and small ones are separated by each concentric filter layer and retained in the appropriate filter layer according to the size of particles in a serial order. A cross-flow in this system is generated on altering the rate of rotation instantaneously through the relative motion between the fluid and the filter layer. The cross-flow washes away the prospective clogs at the filter pores.
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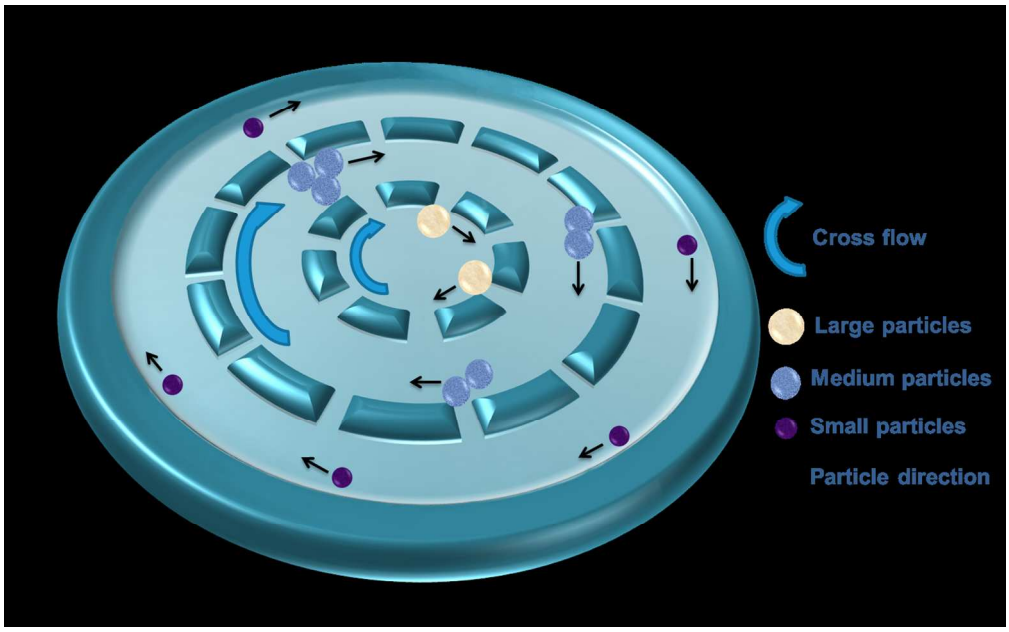

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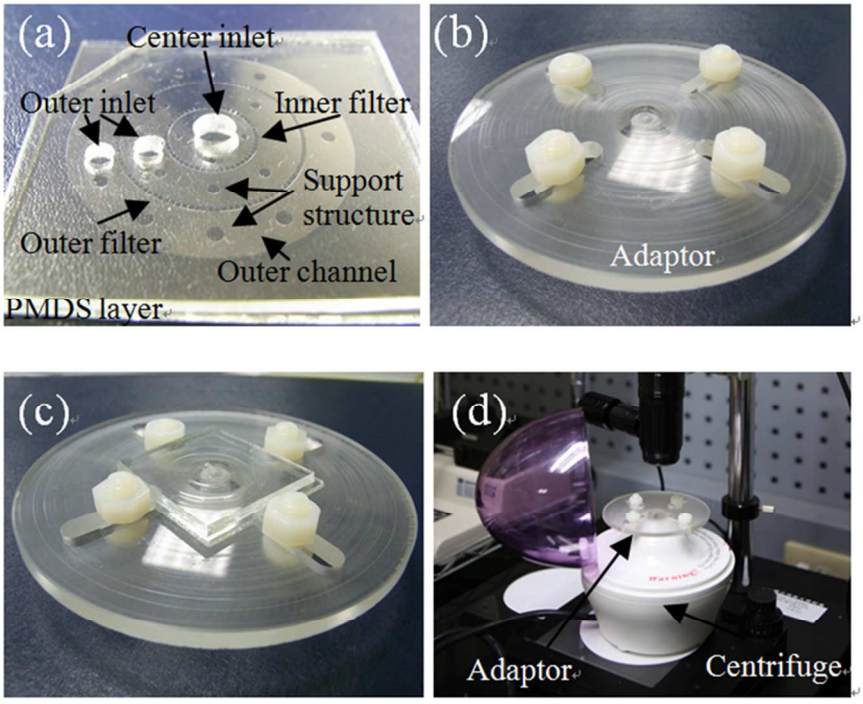
252x156mm (150 x 150 DPI)



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254x190mm (96 x 96 DPI)

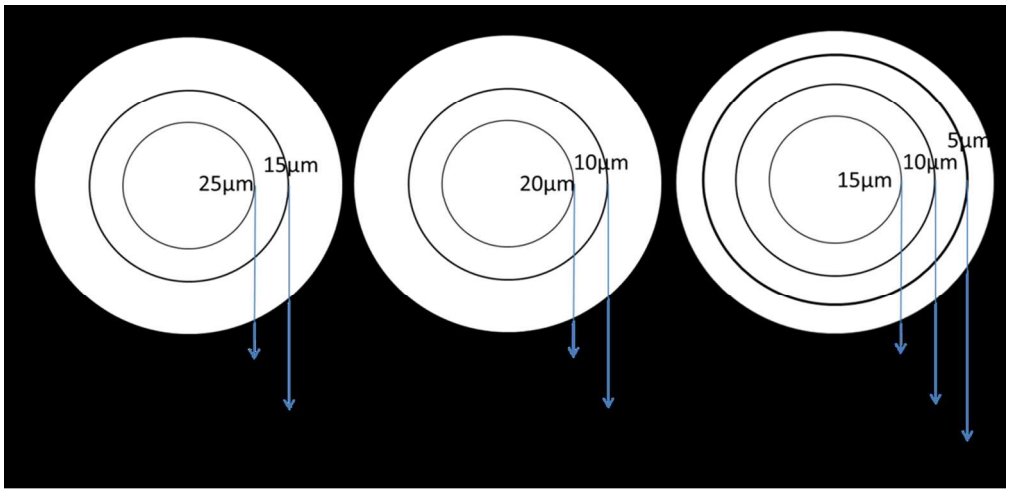

Figure 3(a)The tangential velocity of the cross-flow in each concentric layer of a device of three types was calculated according to its radius and the rate of rotation, at 4000 revolutions min^{-1}.
230x111mm (150 x 150 DPI)

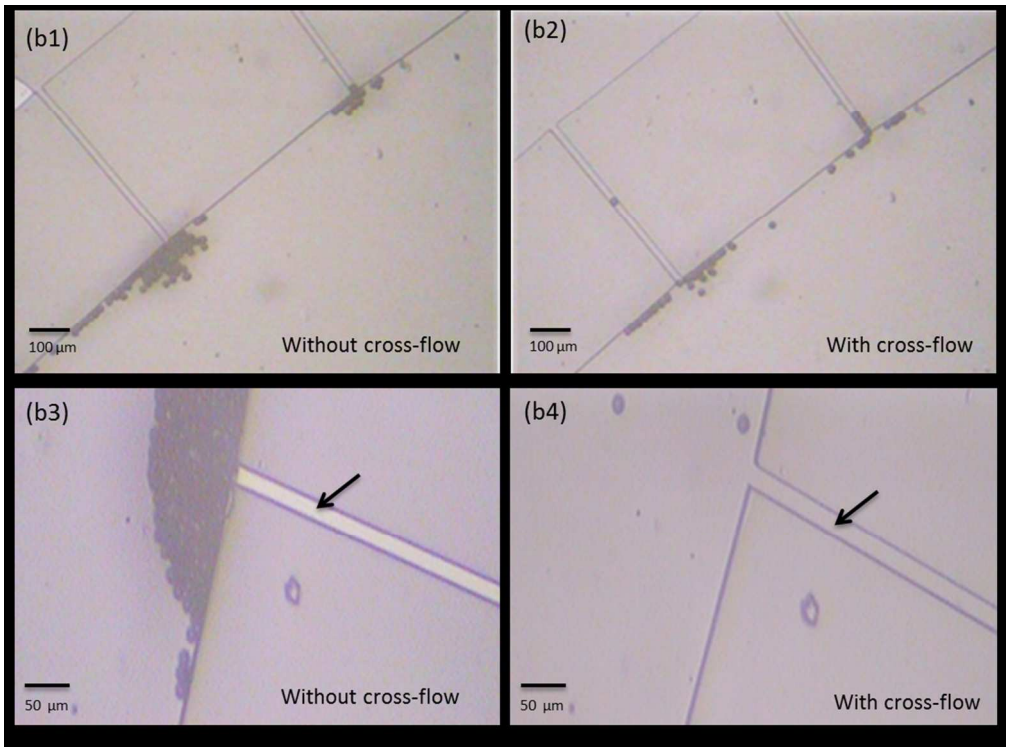

Figure3(b) Comparison of the clogs diminished in the condition with and without cross-flow by microscopic observation. (b1, b3) Without the cross-flow, an accumulation of particles in the filter layer is evident; the clogs block the fluid flow as the arrow indicates. (b2, b4) With the washing effect of the cross-flow, the clogs were washed away from the filter pore; the fluid passed the filter pore as the arrow indicates.
185x137mm (150 x 150 DPI)

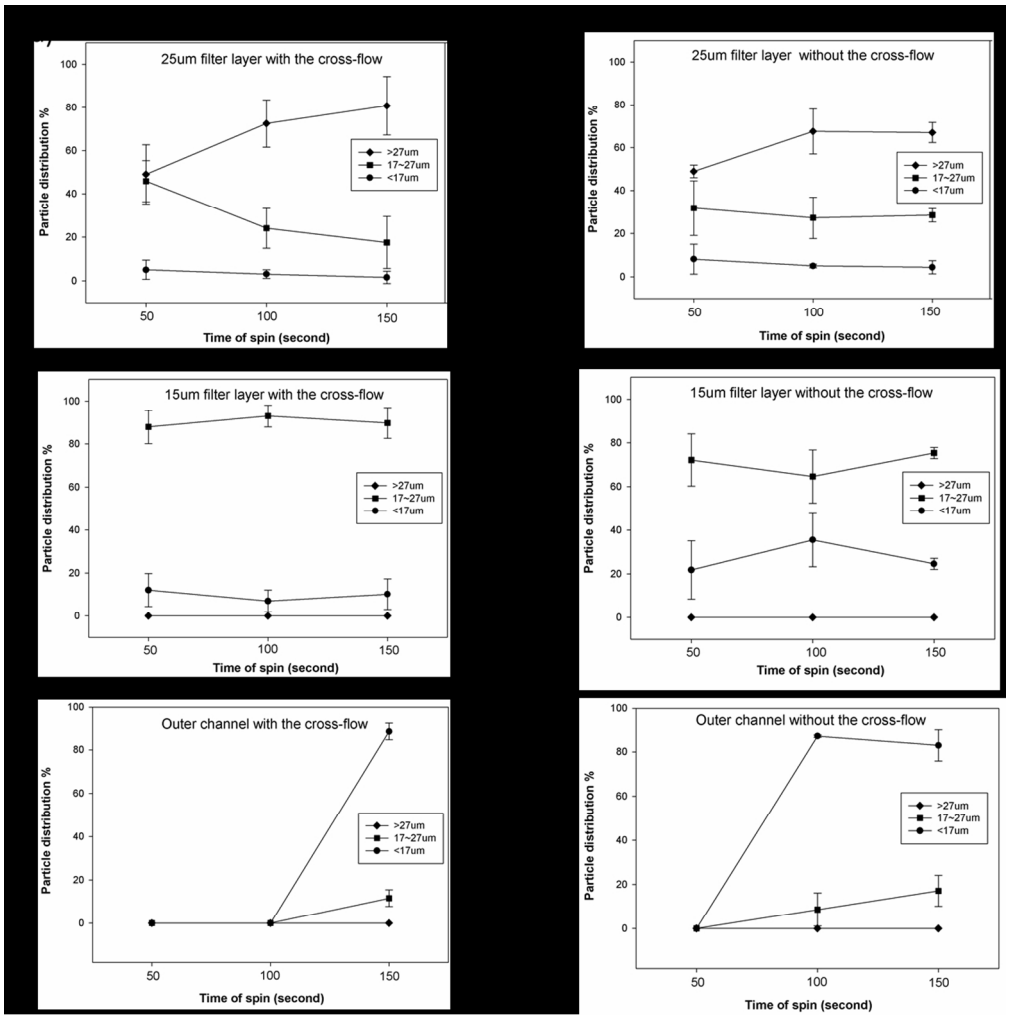

Figure 4: (a) Particle distribution of varied microparticles of diameter ranging from 14 to 85 μm in the 25- and 15-μm filter layer with the cross-flow on repeating the spinning and stopping procedure 5 to 15 times (50 to 150 s) sequentially. (b) Particle distribution of varied micro-particles of diameter ranging from 14 to 85 μm in the 25- and 15-μm filter layer without the cross-flow from 50 to 150 s.
218x219mm (150 x 150 DPI)

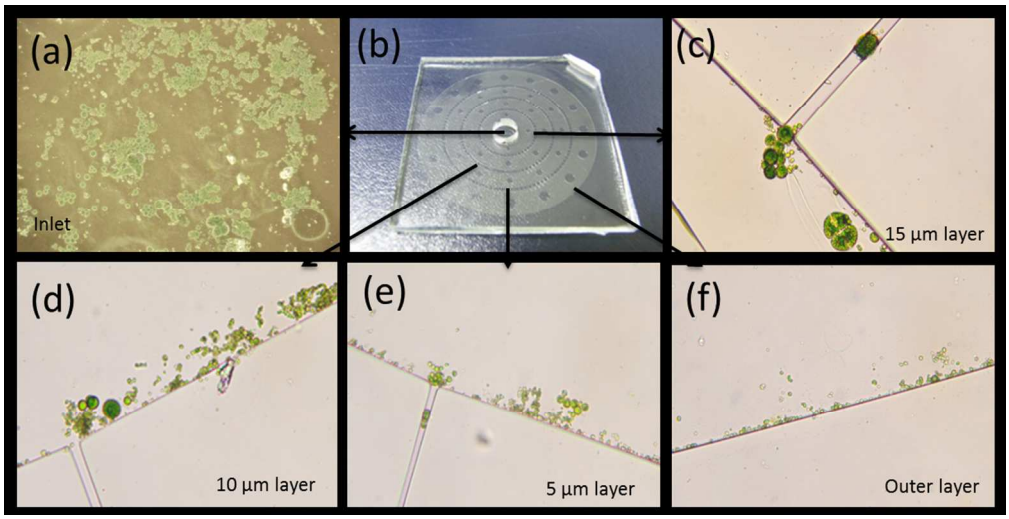

Figure 5: Microscopic views of separated microalgae obtained from an environmental collection in the tri-layer concentric filter device (a). Mixed microalgae of varied size loaded in the inlet of the device before separation. (b). Appearance of the tri-layer filter device; the pore size of each filter layer from inner to outer was 15, 10 and 5 µm. Microalgae in a heterogeneous population were consecutively separated by size at each filter layer. (c). Microalgae of diameter greater than 15 µm were retained and enriched at the 15-µm filter layer. (d). Microalgae of diameter 15-10 µm were retained and enriched at the 10-µm filter layer. (e). Microalgae of diameter 10-5 µm were retained and enriched at the 5-µm filter layer. (f). Microalgae of diameter less than 5 µm were separated to the outer channel of the device.
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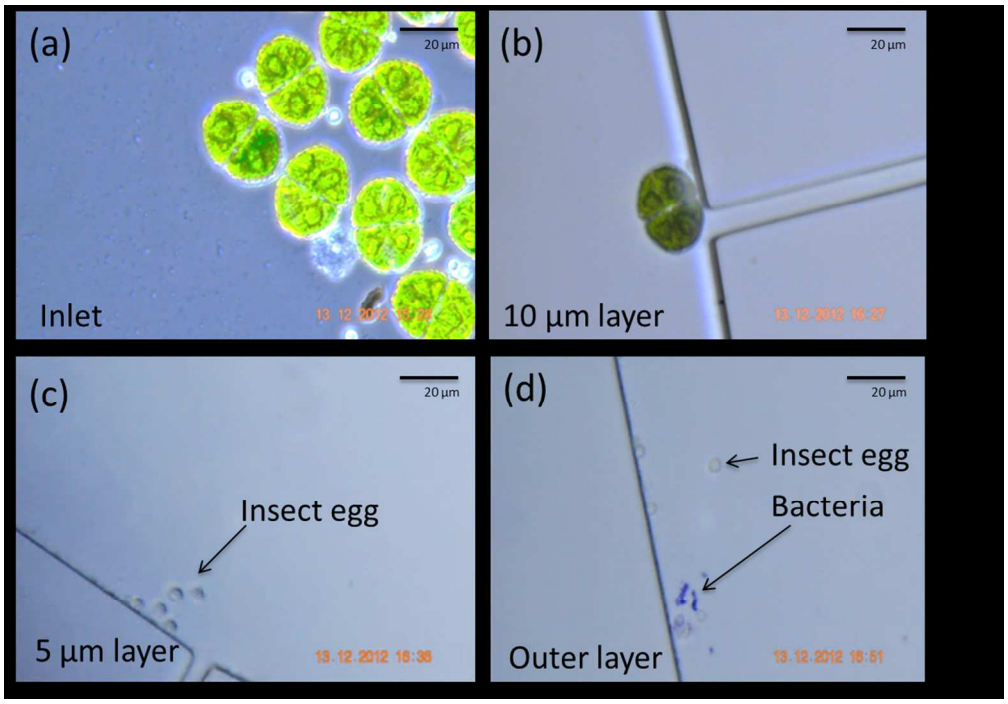

Figure 6: Microscopic views of a microalgae sample contaminated with bacteria and insect eggs separated with the tri-layer filter device (a). The microalgae sample contaminated with insect eggs and bacteria was loaded in the inlet of the device before separation. (b). Most microalgae were retained at the 10-µm filter layer after separation. (c). Insect eggs were retained at the 5-µm filter layer. (d). Insect eggs and bacteria stained in blue were separated to the outer layer of the device.
208x144mm (150 x 150 DPI)