Electronic Supplementary Material (ESI) for Lab on a Chip.

Angry pathogens, how to get rid of them: introducing microfluidics for waterborne pathogen separation to children

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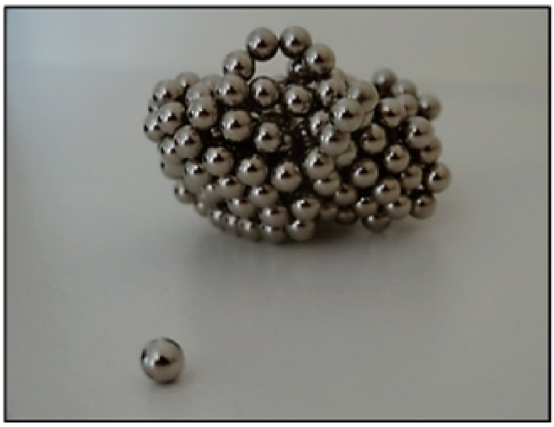
SUPPLEMENTARY INFORMATION

STEP-BY-STEP PROCEDURE FOR MODULE 2-IMS

1. Particles in FIMO® polymer clay.

Different blocks of FIMO® have been used to create the good and bad particles. The FIMO® is a soft polymer clay that can easily be moulded by hands (S1-Left). The simplest option is to create spherical particles by rolling the FIMO®. The size of bad pathogens is here roughly 1.6 cm in diameter and the yellow ones 1.1 cm. Non spherical particles can also be shaped although depending on their size, they can be blocked above a post in the DLD device or have some difficulty to roll down in the microfluidic-based IMS. Several tutorials are proposed on the Internet to create Angry Birds® with FIMO®. One of them has been used here for the eyes of pathogens (red and brow particles). Eyes can also be painted for easing the process although it needs to be waterproof if experiments have to be carried with water. For experiments dealing with IMS, two sets of red particles are modelled: one set with a small magnet inside (Figure S1-Right) and one set without. Small white particles are also shaped with a magnet inside to mimic the antibody-coated magnets (Figure 4 bottom in the paper). Magnets are placed before baking the FIMO®.



S1. Left: FIMO® polymer clay. Reproduced from http://en.wikipedia.org/wiki/Fimo. Right: Magnetic beads incorporated in soft polymer clay before baking.

At the end, a set of red bad particles with and without magnet, white small particles with magnet and yellow, brown and green particles without magnet is baked during 30 minutes at 110°C. A transparent polish can be applied on the beads after baking to avoid colour deterioration with time.

2. **Without Antibodies…**

For the first experiment, red particles without magnet, brown, yellow, brown and green ones are placed with water in a cup. A strong magnet is used to try to remove the particles…unsuccessfully.

3. **With Antibodies…**

Then a new cup is used with red particles containing magnets. Children can then add white antibodies to the water and see that they are attracted to the red particles. Then using a strong magnet above the cup, all the red particles can easily be removed in one shot. Evidently, the red particles are now magnetic and would be removed without the "antibodies" so it is advisable to control the use of the magnet carefully.

STEP-BY-STEP PROCEDURE FOR MODULE 3-Microfluidics

1. Base in FIMO® polymer clay.

FIMO® is used again to build the microchannel. A FIMO® block is flattened using a rolling pin until the thickness of the device is satisfactory (few millimetres here). Edges are then cut using a mould for cakes (roughly 5 cm in length here). Then a mould is required to create the channel. Here, we used wooden letters available in craft shops. The mould in pressed in the FIMO® block until obtaining a satisfactory thickness to allow the liquid to flow (1 mm here) (S3-left). Inlets and outlets are then created using a toothpick (S3-right). The channel is baked during 30 minutes; a weight can be used during the baking to ensure the top surface is flat.

S2. Microfluidic channel with FIMO®.

S3. Y-shape channel (left) without inlet/outlet and with inlet/outlets (right) made with a toothpick.

2. Plexiglas and Silicon

Plexiglas is used to close the channel. Plexiglas can be bought in a DIY shop and cut there. Here the Plexiglas is approximately 4 mm thick. A piece of Plexiglas is placed above the channel and bonded with transparent silicon for bathroom. Silicon is placed near the channel to avoid any leakage of the liquid outside the channel (Y shape here). Specific attention is required near the inlet/outlet to avoid clogging.

3. Liquid injection

After 24 hours (when the silicon is dry), liquid can be injected via the holes. Here a needle-tip plastic bottle (found in a craft shop) is filled with red squash in the device (S4). After use, the system needs to be cleaned with water to avoid any decolouration of the FIMO®. Note that food dye can also be used for colouring the water.

S4. Injection of squash in the device.

STEP-BY-STEP PROCEDURE FOR MODULE 4-Microfluidic-based IMS

1. Y-channel

Using Plexiglas, a Y-channel is created (dimensions in the paper) by cutting different rectangular pieces then fixed with silicon. Angle brackets are used at each straight angle to support the system (in white in Figure 6). A small support is also glued on the side of the channel to support the magnet while being easily removable by children. A simplest version of this system can be realised using a flat plastic bottle for instance (S5).

S5. Microfluidic IMS with a plastic bottle

2.Piezo-electric sensor

The piezoelectric sensor used for this experiment is presented in Figure S6.

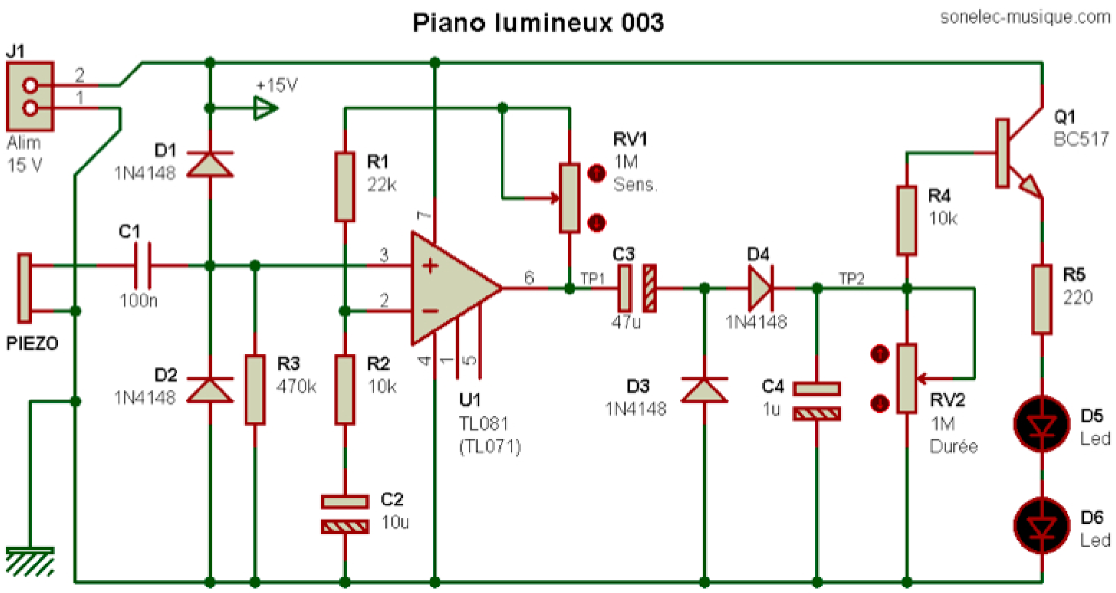


Figure S6. Piezoelectric sensor. Extracted from http://www.sonelec-musique.com/electronique_realisations_piano_lumineux_003.html

C means capacitance, D diode, R resistance, U operational amplifier, RV is a potentiometer and Q a transistor.

Several tutorials are available on the web for "in-house" piezoelectric sensor-based vibration detector. This one was originally used for detecting percussions of certain musical instruments. Although the circuit can seem complex, each electrical compound, whose characteristics are presented in Figure S6, has been found easily in specialised shops. A solderless breadboard is used to avoid the use of solder and have a reusable system. The ceramic piezo (PIEZO in Figure S6) is fixed on the wall of the Y-channel with transparent bluetack. When the sensor detects a vibration, a current is generated that will here turn on a red LED (Led in Figure S6). The vibration is created here by the shocks of magnetic particles bumping onto the magnet. A piece of foam (sheet of blue foam bought in a craft shop here) is used to absorb the vibrations created by particles entering in the channel (*cf.* Figure 6).

3. Experiment

The microfluidic-based experiment is similar to the standard IMS (Module 2). First beads without magnet are passed through the channel. They will roll down by gravity since the device is inclined. If the left outlet is blocked (here with a piece of flexible plastic, missing part of the bottle in Figure S5), all the particles will finish in the right outlet. Note that by simply using a flexible piece of plastic, the water will flow in both outlets but not the particles. Then a new set of beads is used. This time with red magnetic beads preliminary mixed with white antibodies. Note that red pathogens with more than one white bead tend to stagnate in the device; they will thus not roll smoothly in the channel. Once red pathogens are trapped by the magnet, the red light will turn on. The right outlet needs to be closed and the left one opened (by displacing the piece of flexible plastic). By removing the magnet, red pathogens will roll in the left outlet and thus will be separated form the other particles.

STEP-BY-STEP PROCEDURE FOR MODULE 5-DLD

1. LEGO® board and posts

LEGO® posts are placed on the LEGO® board with the geometry presented in Figure S7. This geometry allows particles above 1.5 cm to follow the blue path while smaller ones have an ultimate straight path. For each post, two LEGO® posts are superimposed to create one taller post to avoid particles to quit the DLD device. The LEGO® board is placed in a vase (28 cm in length, 18 cm in height and 8 cm in depth). It can be noted however that a higher vase would allow a better separation effect to be visualised by children.

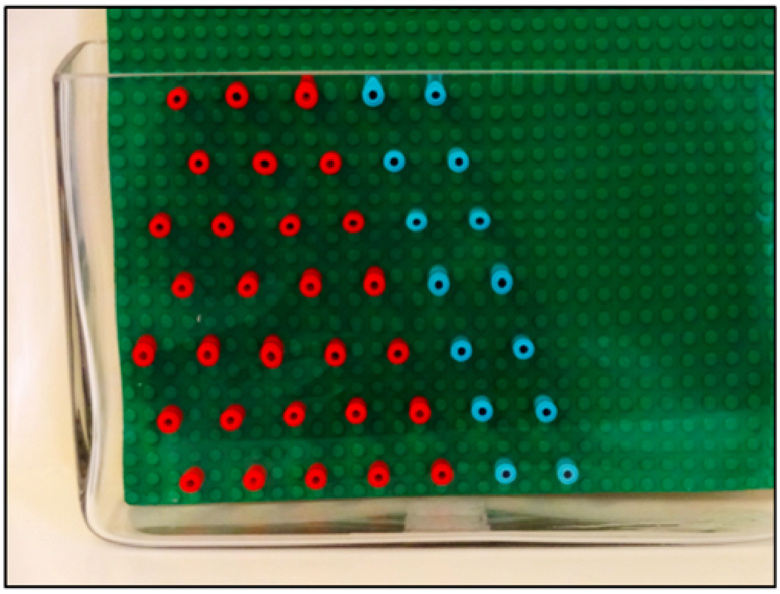


Figure S7. DLD using LEGO®. Particles are introduced at the top of the vase, between the two blue posts. Small particles follow an ultimate straight path and thus finish between red posts while large particles follow the blue path.

2. Shower gel

To reproduce the slow motion characteristic of microfluidics, a viscous liquid is needed. Here transparent shower gel is used. Depending on its viscosity, dilution may be needed. However, mixing shower gel with water will create a lot of bubbles and foam and a certain time is required before being able to use the solution. Here, when the red pathogens were injected between the two blue posts at the top of the board, they needed roughly 7 seconds to reach the bottom of the vase.

3. Experiments

Particles must be injected near the board always between the two blue posts. Red and brown particles will follow the blue path; yellow and green ones a red path. It can be noted that if particles are too small, they tend to stagnate above posts. In order to ease the injection, tweezers were used by children (chopsticks for learners). Removing the particles at the bottom of the vase can be quite tricky, tweezers were used here. It can be noted that posts can be glued to the posts to avoid damage during the experiments. Magnetic beads can also be incorporated in all the particles to be used for the LEGO DLD and then be removed using magnets. However, the deviation should be large enough to avoid attraction of particles in the vase.

STEP-BY-STEP PROCEDURE FOR MODULE 6-Fluorescent microscopy

1. Fluorescent microscope

The fluorescent microscope consists of an insect magnifier placed in a tissue box in cardboard (available in craft shops) (Figure S8). The box is painted inside in black. The magnifier is then placed in the box with the top of the magnifier passing through the holes for tissues (Figure S9). Black paper is used to block light in the top part of the magnifier that is not in the box (Figure S8-Right).

Figure S8. Left: Insect magnifier used for reproducing the microscope. Right: Black cardboard box with the magnifier to visualize by fluorescence the magnetic beads trapped onto the bad red particles.

Figure S9. Insect magnifier in the cardboard box without (left) and (right) with beads before blocking the light with paper on the top of the magnifier. The final microscope is presented in Figure S8-Right.

2. Experiment

The FIMO® used for antibodies is fluorescent. When placed under a lamp for few minutes and then in the dark, the FIMO® glows. By placing the red particles with magnets and the white beads with magnets, detection by fluorescence can be performed (S9). It can be noted however that the fluorescence signal of the FIMO® can be quite low. Placing the beads near a lamp right before the experiment for few seconds will ease the visualization.