Lab on a chip: Frontier science in the classroom

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Supplementary information

In this document, practical information is given regarding the implementation of Lab-on-a-chip lessons in the classroom. Part A gives a description of the equipment and alternatives for the individual experiments described in the main article. Production details for low-cost Lab-on-a-chip experiments is given in part B. Part C describes the procedures for cutting and assembling lamination-foil chips, intended as a guideline for learners. In Part D, some examples of assessment during the Lab-on-a-chip lessons are presented.

A: Components in the practical kit

The practical kit, with all components required for the Lab-on-a-chip experiments described, is available from the outreach program at the University of Twente. Teachers at high schools all over The Netherlands can borrow such a kit for several weeks, for as long as the lesson series on Lab on a chip at their school takes. The eight kits available cannot serve a large numbers of schools, so we are working on affordable equipment for the future.

To assist with implementing the lessons elsewhere, all components of the kit, and their suppliers, are listed in table A1. Most items are now commercially available, including those custom made for this project.

The total cost per kit used in the project described are around 4500 Euro, ex VAT. The TCB chip and electronics are not available commercially.

Figure A1: *One of the practical kits containing the equipment for Lab-on-a-chip experiments in the classroom.*

Glass chips and the clamping chip holder are custom-made by Micronit Microfluidics to be included in the practical kit for use in the lesson series. Those glass chips can be used in the simple acrylic (PMMA) chipholder, serving as a low-cost alternative to the metal clamping chip holder. Cheap laser-cut foil chips may serve as a low-cost alternative to glass chips. Foil chips and the acrylic chip clamp are not intended for experiments requiring high volume throughput.

Chip fabrication background information

Wet etching is performed using Hydrogen Fluoride (HF) on rinsed glass substrate, masked with positive resist resin. This technique is used for the production of the channel layers in the FFDG droplet generator and the TCB chip from borosilicate glass. Structures are positioned with 1 µm accuracy.

Powder blasting (Abrasive Jet Machining) is used on glass substrate, covered with negative resist film. After UV mask exposure and rinsing, the exposed glass is removed with fine abrasive powder, with an accuracy of within 2 μm and a feature size accuracy of around 25 μm. Roughness of the structures is between 0.8 and 2.5 μm, depending also on the chosen process. Typically, walls will have an angle of 70 degrees. Channel depth may vary from 25 μm to several mm.
All chips we describe are produced from borosilicate glass. The H-reactor, TD26 mixer chips and the top layers of the droplet generator and the TCB chip are produced by powder blasting. The FFDG droplet generator and TCB chip (channel layers) are HF etched. All glass layers of the chips are bonded by controlled heating. Additional information is available at the Micronit website: http://www.micronit.com.

Maintenance and practical issues

The practical kits have been borrowed on a regular basis starting in 2012, and inspected, refilled and cleaned after each use. Thorough inspection and cleaning of the fluidic chips and other components is done once a year. A number of pumps had to be repaired (broken pump housing), but the original microfluidic components, such as the chip holder, tubings and ferrules, are in excellent condition. Contrary to our expectations, tubings, connectors and syringes have rarely had to be replaced.

Issues, remedies and trouble-shooting

- Students place chips (containing oil or dye) back in the kit. Inspection by school technician after each practical session is required.
- Use of ordinary paper tissue (as commonly used at school) resulted frequently in fibers clotting the channels in the LoC. Thorough rinsing, using 1 M NaOH solution, is needed to remove all organic matter from chips. A supply of dust-free cleanroom tissues in the practical kit, and instructions in the practical description, reduced this problem. Unfortunately, fiber pollution remains difficult to avoid. Once-a-year inspection and cleaning of the kits is necessary.
- Broken chips are rarely seen. In case of incorrect placement of the chip and rough closure of the holder, breakdown is reported.
- Finding suitable dyes. Currently, highly soluble dyes (Brilliant Blue and Conchenille Red) are used. Fountain-pen inks raise problems since they contain traces of oil; a mixture of colors may result in precipitation of debris.
- Pump defects may occur, mostly resulting from extreme pressure build-up. A fully clotted chip, or wrong placement of the ferrule, may prevent fluid from entering the chip. In this case, fluid is unable to leave the syringe, resulting in cracks in the pump housing.
- Experiments with the droplet generator, using oil, water and detergent, are delicate. Finding proper adjustments is difficult. Oil may fill the channels of the chip before water w/ detergent is present, and coat the channel wall. Formation of oil droplets in water is prevented, and oil droplets adhere to the wall, forming large oil droplets. Starting the experiment using water w/ ca 5% detergent (Tween 20) to fill tubing and chip, and subsequently changing to the water w/ detergent and oil is most likely to offer usable results.
- Observation of the phenomena on the chip requires a microscope or camera. Difficulties with adjusting the microscope result in poor observations. The use of the condenser and proper diaphragm width, in particular, have a large influence on contrast and visibility of color. Small diaphragms result in a very dark view of the channels, especially when they are produced using powder blasting (like the H-reactor and the TD26 mixer chips).
- Connection of fingertight nuts with ferrules to the tubing are intended to be mounted once. Users frequently remove the connectors, so the ferrules have to be replaced.

- Foil chip production may result in various errors, which can easily be corrected by repeating the procedure correctly. The most common error reported is cutting channels too wide (resulting in closure of the channel). Sometimes, the entrance of the channels (at the punched hole) is clotted by some glue. Using the tip of a needle or scissors to remove this will help.
- Spontaneous filling of capillaries happens in most cases. Sometimes, a trapped air bubble prevents fluid passage. In most cases, application of under-pressure (suction, using a pipette balloon) will start the fluid stream, which can be continued using pieces of tissue at the exit.
- Before lamination, the foil must be covered in paper. If not, the foil will stick to the hot roll and disrupt the lamination machine. If the machine is not hot enough (e.g., set to thin foil thickness) this will result in opaque-appearing chips that are not well glued. If a machine is used with very soft rolls, this may result in fully closed channels. Testing of foil and lamination machine is required.

Table A1: components of the Lab-on–a-chip practical kit.

Component	Amount	Brand (if relevant)	Identification	Purpose	Remarks	Microscopy	H-reactor	Micromixer	Droplets	TCB chip
Tubing 1/16 inch OD, 0,030 inch ID (Tefzel)	4 x 30 cm, 4 x 50 cm	IDEX	1528 (optional 1528L => 50ft)				4x	3x	3x	2x
Fingertight nuts (PEEK) with ferrules	6 x	IDEX	F-300X				2x	2x	2x	2x
Replacement ferrules (ETFE)	2x	IDEX	F-142NX							
Plug 10-32 threaded	10x	IDEX	U-467W							
Luer connector, PEEK	5x	IDEX	P-659				2x	2x	2x	2x
Ferrule 1/16 inch Perlast and stainless steel ring	10x	Micronit / IDEX	Micronit ferrule (art.no. 00199) SS ring from IDEX P-259X (10-pack)				4x	3x	3x	4x
Syringes 10 ml w. Luer connector	5x	BD (e.g. via VWR)	6133931 vvd:613-0973 (Terumo)				2x	2x	2x	1x
LockIn amplifier with PC and software	1x (not in standard kit)	BIOS group University of Twente	Custom							1x
Shielded wire with connector (see fig 1)	4x	Micronit	Custom		Not in the standard experimental kit					2x
CE chip samples ('lithium chip')	20x	Medimate (supplied by Micronit, or CE-mate if they are willing)				1x				
T junction for fingertight ferrules	2x	IDEX								
H chip (custom made)	2x	Micronit	H150.015.2 will be replaced by art.no. 00755 (H300.015.2) => channel width of 150um is replaced by 300um.		Custom design, can be ordered	1x	1x			
Teardrop mixer	2x	Micronit	TD26		Standard product	1x		1x		
Fast flow droplet generator (adapted version)	2x	Micronit	FFDG.2.50		Custom made, can be ordered	1x			1x	
Trapping and Counting of Beads (custom made)	2x	Micronit, BIOS group University of Twente	TCB		Not standard in the experimental kit.					
EL-DEMO chip	1x	Micronit	Chip with several electrodes for viewing		Not used for experiment					

(Table A1 continued next page)

Tubing cutter 1/16, 1/8 inch	1x	IDEX	A-327							
Polypropylene Waste container 200 ml	1x	VWR (Nalgene PP bottle)	50367				1x	1x	1x	1x
Chip holder (custom made)	2x	Micronit	100705		Can be replaced by home-made PMMA holder		1x	1x	1x	1x
Laser cut lamination foil for self assembly of chips 15 x 45 mm	50 pieces	University of Twente and FabLab	Custom				Foil chip H reactor, as alternative	Other types of serpentine mixers or self produced	Can be cut from Y reactor, difficult to make	
H reactor (2 in, 2 out), Y reactor (3 in, 1 out),	nn	80 or 125 micron hot lamination foil, A4 size, GBC or Leitz (Acco brands) give good results	Custom		To be made from lamination foil using laser cutter					
Serpentine mixer (2 in, 1 out); Serpentine mixer 2 + 1, 2 out)	nn	80 or 125 micron hot lamination foil, A4 size, GBC or Leitz (Acco brands) give good results	Custom		To be made from lamination foil using laser cutter					
45 x 45 mm foil just holes	nn	80 or 125 micron hot lamination foil, A4 size, GBC or Leitz (Acco brands) give good results	Custom		To be made from lamination foil using laser cutter					
Serpentine mixer (2 in, 1 out; channel widts 100, 300 and 1000 micron)	nn	80 or 125 micron hot lamination foil, A4 size, GBC or Leitz (Acco brands) give good results	Custom		To be made from lamination foil using laser cutter					
Silicon ferrules (7 mm OD, 2 mm ID and 3 mm thickness)	4x	Nn	Self made from silicon tubing 40 mm dia, 3 mm wall thickness							
Plastic holder with 1 mm acrylic inlay 15 x 45 mm	2x	Micronit			For mounting lasercut chips in chip holder		(1x)	(1x)		
Syringe pump w. AC adapters	2x	New Era (via ProSense)	NE-300				2x	2x		
Centrifuge tubes for required fluids	4x	Greiner	50 ml				2x	2x		

(Table A1 continued next page)

Demineralized water		Nn					1x	1x	1x	
Dyes, diluted in demineralized water: Conchenille red (ponceau 4R) 3g/L; Brilliant blue 0,2 g/L)	50 ml	Nn					1x	1x	1x	
2-propanol	50 ml	nn								
Organic oil (e.g. olive, sunflower, almond)	50 ml	Nn							1x	
Detergent	10 ml	Tween 20								
USB microscope	1x	SuperEyes	Type B5				1x (or optical microscope)	1x (or optical microscope)	1x (or optical microscope)	
Clean room tissue	30 sheets	Contec, SC USA	Amplitude Sigma, Cell/Poly Non woven				1x	1x	1x	
Sorting box for small components	1x	RS-online	435-0208 (storage box 10 compartments)							
Carrying case	1x	Nn								
Practical instruction manual NLT module 'Lab on a chip'.	1x	University of Twente	To be downloaded from http://www.labochip.org		Creative Commons (share alike)		1x	1x	1x	

(Table A1 end)

B: Design of polymer Lab-on-a-chip devices and chip-holder

Foil chips

The production and practical use of Lab-on-a-chip devices made by laser cutting is described in this section. The designs made for the laser cutter were drawn in 2D vector software (Adobe Illustrator, CorelDraw), and cut using the Trotec Speedy 300 laser cutter. The chips are cut from lamination foil pouches. Best results were obtained using 125 micron sheets (brand: Leitz). We also used 80 micron sheets (brand: GBC). Other brands will also provide good results. In all cases a good combination of foil-characteristics and settings for the specific laser-cutter need to be arranged. See *figure B1* for the

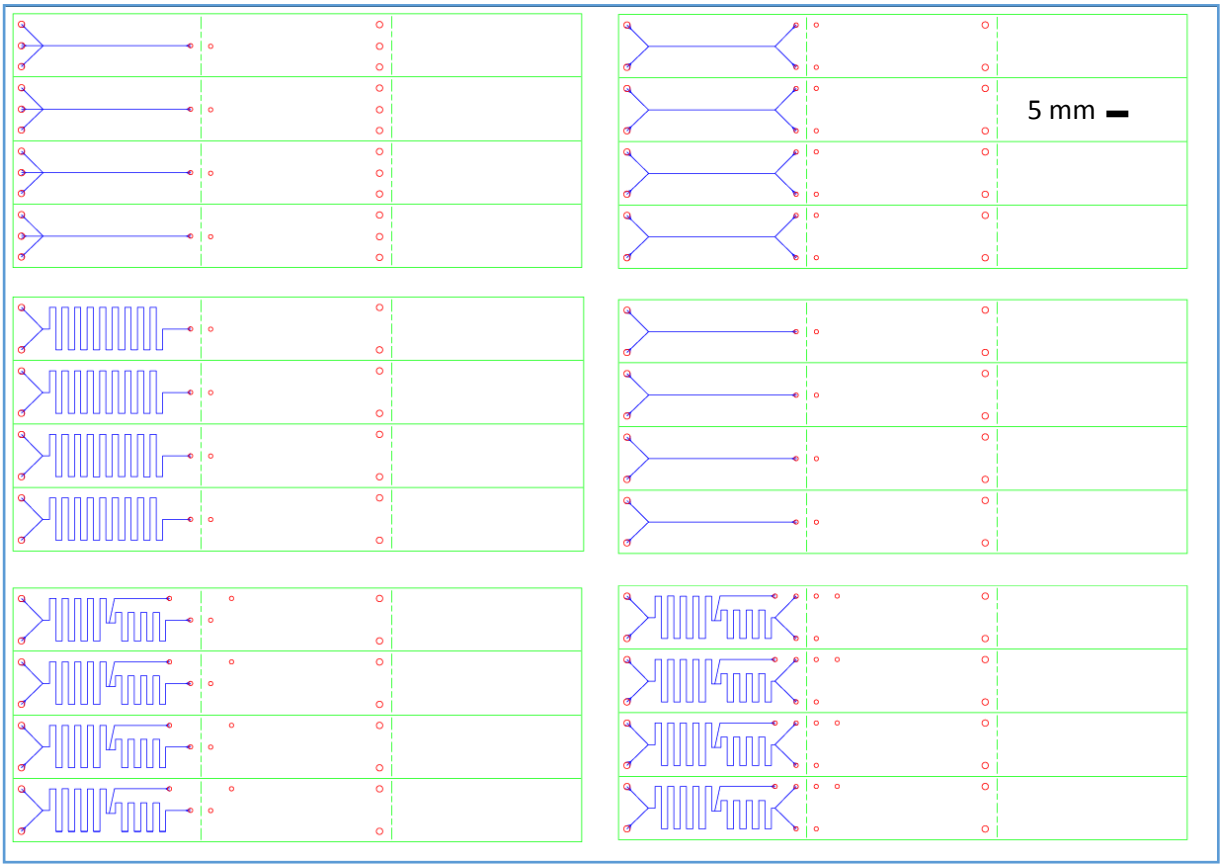

***Figure B1**. Design of the cutting pattern used to produce laser-cut chips compatible with the holder in the practical kit. Bar indicates 5 mm, which is the distance between two holes in the connection grid. For the Trotec 300 laser cutter we used: resolution 500 dpi, pulse frequency 10,000 Hz; for the chip channels: power 40, speed 30, for holes power 40 speed 15, and for folding edges: power 35, speed 45. In the drawings, line colors correspond to laser settings for speed and power, and vary according to thickness of the foil to be cut. Red lines, 80 μm foil: power 40, speed 15; 125 μm foil: power 45, speed 8. Green lines, 80 μm foil: power 35, speed 45; 125 μm foil: power 45, speed 10. Blue lines, 80 μm foil: power 40, speed 30; 125 μm foil: power 45, speed 8. The minimum diameter of the channels is determined by the beam-width of the laser (which is around 100 μm in case of the Trotec Speedy 300 we used). At the connection between a hole and a channel, a small triangle is cut, to avoid clotting the channel during hot lamination. Optimal settings for cutting speed and beam intensity depend on the characteristics of the lasercutter and lamination foil being used.*

design and settings we used.

Low-cost chip holder

A low-cost chip holder was designed and produced from acrylic (PMMA); see *figure B2* for the design. The chip holder plates were cut from 6 mm cast PMMA. Before use, the chip holder is assembled using nuts and bolts (4 mm diameter stainless steel). A rubber ring (3 mm diameter) is placed around each bolt to keep it in place in the bottom plate. Fluidic chips are mounted on the bottom plate, and fixed with a small piece of adhesive tape. Engraved dots, matching the holes in the top plate, are present to align the fluidic chip properly (*see figure B4*). The PMMA chipholder is designed to fit two small 15 x 45 mm chips or larger chips (*figures B3, B4 and B9*). If the foil-chips have holes in the right place, various dimensions can be chosen.

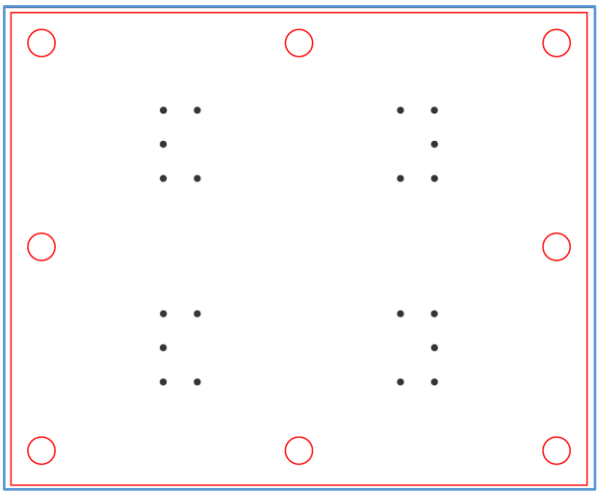

***Figure B2A**: Design of PMMA chipholder bottom plate, with engraved spots for alignment of the fluidic connections. The holder is produced from cast PMMA of 6 mm thickness using the Trotec Speedy 300 laser cutter. Cutting is done at 500 dpi, 10,000 Hz, power 100, speed 0.3. Engraving of dots: 500 dpi, 10,000 Hz, power 100, speed 3.*

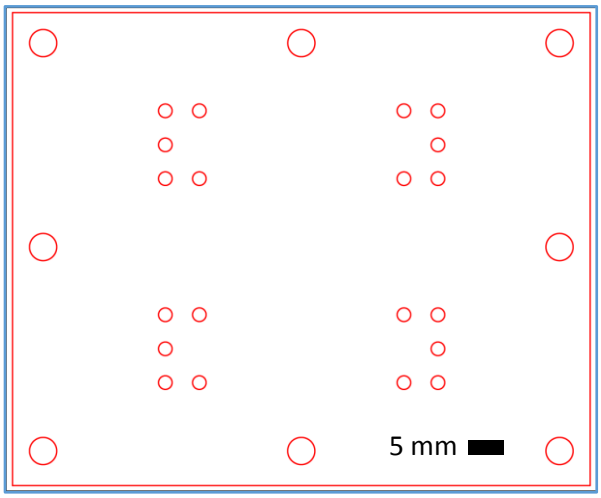

***Figure B2B**: Design of PMMA chipholder top plate, with 2 mm holes (for tubing) and 4 mm (for screws). Bar indicates 5 mm. (A similar plate, with 3.4 mm holes, made from 2 mm PMMA is added between the chip and the top plate to fit perlast ferrules with stainless steel ring). A smaller variety of the chip holder holds only one 15 x 45 mm chip. Bar indicates 5 mm.*

For low pressure fluidic connections, a silicon ferrule was developed, which can be easily made from silicon sheet or tubing. Practicals on microfluidics can be performed with relatively low cost materials and in a short time. Students can manage to produce the foil chips from the prefab lasercut elements and build a working setup using syringe pumps and can perform an experiment on laminar flow or mixing within one hour. Since the foil chips are cheap and disposable, the time needed for the cleaning and disassembly procedure is reduced to a minimum. Inspection of the experiments, especially leaks or clotting, and use of microscopes is easy with the PMMA holder. In this way, we have overcome the need to rent the fluidic kit to perform the *basic experiments* such as (low pressure) laminar flow and mixing. For advanced (higher pressure) experiments, proper mixing, droplet formation and bead trapping and counting, the fluidic chips and chip holder described in the main article will be needed.

Fluidic connections

Tefzel tubing (1/16 inch, inner diameter 0.03 inch, IDEX) is used for connections to the fluidic chip. The diameter of the openings in the chip must not be larger than 2 mm. The chip can be connected to the tubing using Perlast ferrules (including stainless steel ring, 3.4 mm diameter, as described in the main article). If Perlast ferrules (3.4 mm stainless steel ring) are used, an additional PMMA plate with holes of 3.4 mm and 2 mm thickness is placed between top plate and ferrule. Silicon ferrules (7 mm outer diameter, 2 mm inner diameter, punched from 3 mm silicon rubber sheet) can be self-made using hole punches (7 mm and 2 mm). These silicon ferrules are used as an alternative for the Perlast ferrule with SS ring (see figure 1 in the main article) to reduce costs. The silicon ferrule is less pressure-resistant, but performs well for experiments with aqueous solutions in foil-chips and (low pressure) glass chips for the educational purposes we present. *Figures B3 – B6* show various experiments performed in this way using glass and polymer 15 x 45 mm chips.

Figure B3: Detail of the PMMA holder holding the FFDG droplet generator. *For this experiment, Perlast ferrules and an adapter plate (2 mm thickness, with 3.4 mm holes to fit the SS ferrule rings placed directly below the top plate) were used.*

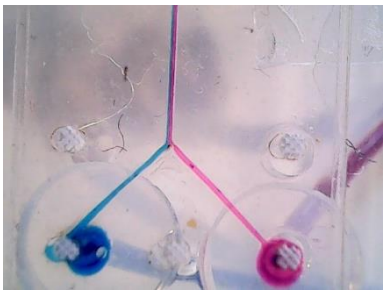

Figure B4: Silicon ferrules connect the H-reactor in the PMMA holder. *Note the dashed alignment indicators in the bottom plate.*

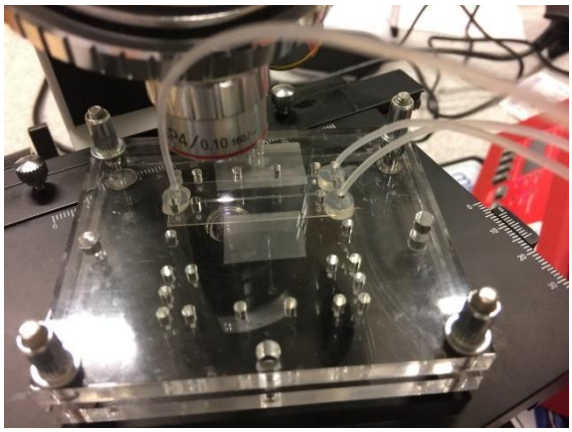

Figure B5*:* ***Assembled chip PMMA holder*** *holding an 15 x 45 mm Y reactor foil chip (the channel of which was enlarged to 0.3 mm by scissors before assembly) and connected to the tubing using silicon ferrules. With this experiment a Calcite precipitate is formed from 0.025 M CaCl2 and 0.1 M NaHCO3 (for details see figure 7 of the main article). The reaction is observed using the 40x magnification of a standard microscope.*

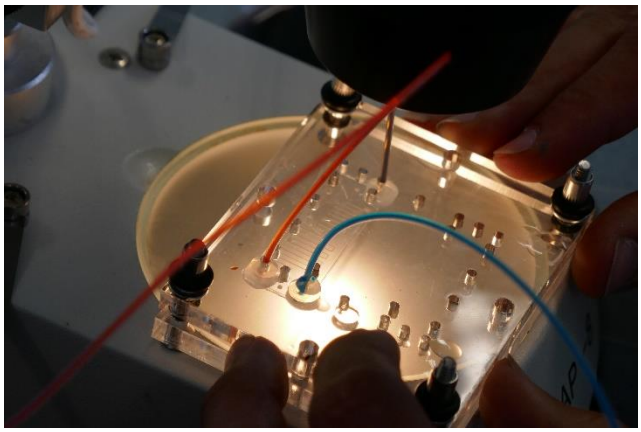

Figure B6: PMMA holder *in an experiment with a 3-inlet serpentine mixer (of which 2 inlets were closed with adhesive tape), used with dyed water to study the mixing properties of the chip. In this case a binocular microscope is used to study the reaction.*

Scissor cutting

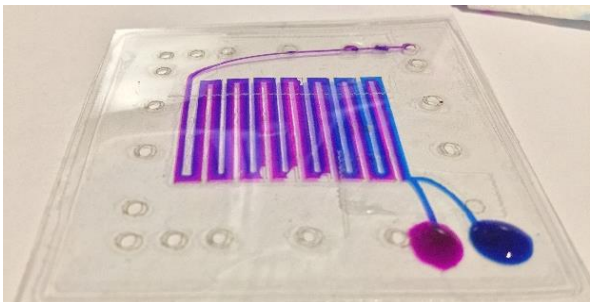

Figure B7*: Example of large 50 x 50 mm foil chip design (laser-cut serpentine mixer, 300 μm channel width) filled with dyed water. The additional holes are in the (universal) top layer allow modification of the channel pattern using scissor or knife before assembly.*

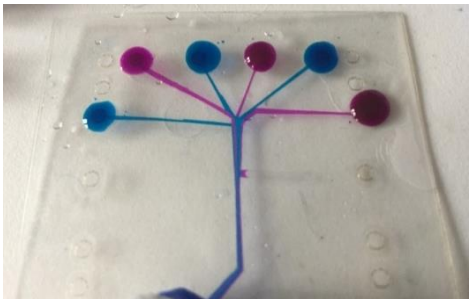

Figure B8*: A 50 x 50 mm chip, with laser-cut entrance holes and scissor-cut channels, filled with dyed water after assembly. See figure 6 in the main article for details. The chip will fit the PMMA holder.*

Pre-fabricated laser-cut foil can be produced with only the main holes, or a few channels, present. Users can cut their own channels in the chip. This can be done in small (15 x 45 mm) foil chips. We also present a larger design, resulting in 50 x 50 mm chips, fitting the PMMA holder we present. *Figure B8* shows scissor-cut channels, made in lamination foil containing a grid of holes of 2 mm diameter around the rim. This corresponds to the holes in the top plate, *figure B2B*. The chip is filled with dye-colored water solely by capillary force. *Figure B9* shows a 50 x 50 mm serpentine mixer (pre-fabricated laser-cut channel, with a width of 300 μm) mounted in the PMMA holder, showing its versatility. This chip is an enlarged version of the serpentine mixer in the 15 x 45 mm chip presented in *figure B2*.

3D printing and PDMS

Some samples of 3D printed molds are included in the practical, of the 15 x 45 mm chip size, to be used for PDMS casting and intended for demonstration purposes (*figure B10*). Experiments with

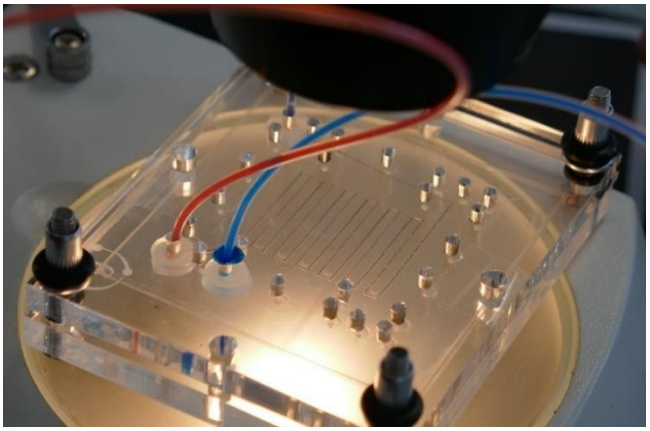

Figure B9: The PMMA holder *in an experiment with the 50 x 50 mm serpentine mixer, mounted using silicon ferrules.*

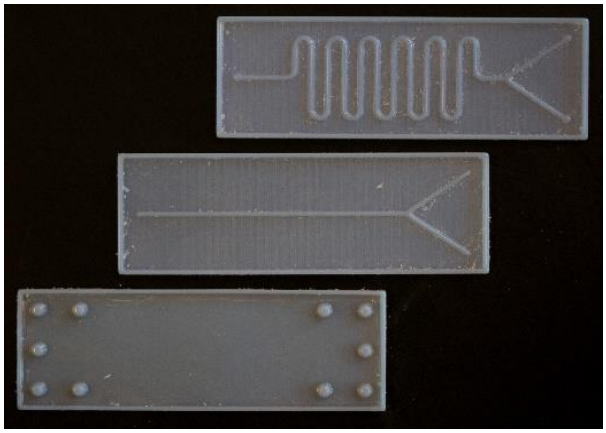

Figure B10*:* ***3D printed molds for PDMS casting****: bottom part (serpentine channel or Y channel) and top part (below).*

PDMS casting are not a standard part of the lessons for high school students, but students perform them in the outreach laboratory at the university. Since PDMS casting takes quite a lot of time and many safety precautions, it is not the preferred procedure in the classroom.

The molds (*figure B10*) are for the bottom (serpentine mixer, Y channel) and the top (entrance holes) of the PDMS chip. The molds were printed on the Objet Scholar 30 3D printer (Stratasys), with Vero Blue 240 plastic. Some STL files for printing the depicted 3D molds can be downloaded from http://www.labochip.org. Molds are cleaned in 70% ethanol prior to use.

The PDMS silicone resin (Sylgard 184, Dow Corning) is mixed with curing agent (10:1) and kept refrigerated until use at -18 ^{o} Celcius to degass. The resin is poured in the mold and remaining air bubbles are removed using a fine needle, and cured on a 65^{o} Celcius heating plate for 1.5 hours. The cured PDMS is gently removed from the mold using forceps, and instantly used for the assembly of chips. The top plate (with holes) and bottom plate (with channels) are gently pressed together, preventing encapsulation of air bubbles. The PDMS top layer may be replaced by a plasma-cleaned glass cover (prepared in the cleanroom) containing holes at the same locations.
The assembled chip may be fitted in a black plastic chip holder to fit in the metal chip holder. The development of the PMMA chip holder makes it easier to use PDMS chips, since chip thickness from 0.1 to several millimeters will fit.

C. The production of lamination foil chips

Here we describe the details of how to produce lamination foil chips using either (1) scissors or (2) a laser-cutter.

1. Large scissor-cut chip from lamination foil.

Required Materials:

- Hot-lamination foil, 80 micron, standard office quality, gloss (a thickness of 125 micron or more can be used to produce wider channels, but cutting is harder)
- Fine, sharp pair of (embroidery) scissors
- Standard office hole punch (make sure it is sharp)
- Hot-lamination machine (an iron with low temperature setting can be used instead)
- Office paper
- Marker (about 0.5 mm line width)
- Tissue paper
- Transparent tape
- Dyed water (use food coloring, printer ink or fountain pen ink), two colors
- Water
- Pipettes or cotton sticks
- A small rubber pipette balloon is helpful in some cases

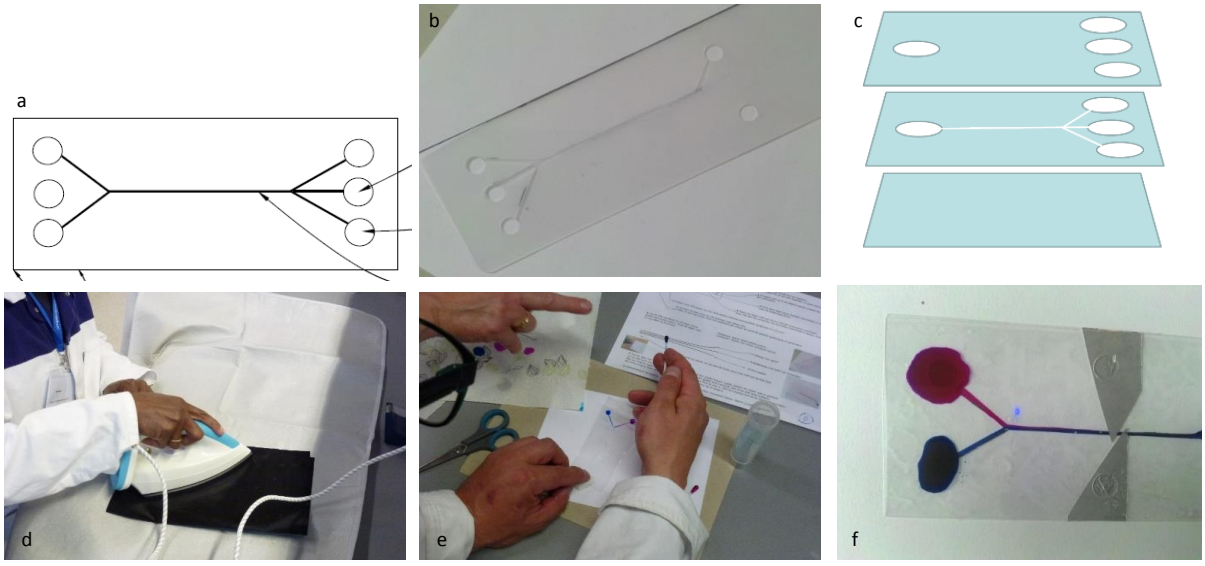

Figure C1: Assembly of large scissor-cut chip from lamination foil. *(a) Design of a chip; circles represent punch holes, black lines channels. (b) Holes punched and channels cut out in the middle layer of foil (dimension ca 4 x 10 cm). (c). Schematic of the layers in a foil chip. (d) Hot lamination with an iron. (e) Application of dyed fluid on the foil chip. (f) Laminar flow in a chip, also showing aluminum foil electrodes; additional holes were punched and aluminum foil was placed between the top and middle foil-layers before hot lamination.*

Procedure:

Cut 3 pieces of lamination foil, dimensions approximately 5 x 5 cm (may be up to 10 x 20 cm). Please note: lamination pouches have two layers of foil; the inner matte side has hot melt glue.

Take 2 pieces of foil, put the glued faces towards each other.
Put a 5 x 5 cm piece of office paper on top and bottom, and punch holes at the desired places (a punch hole is typically 6 mm in diameter; a smaller punch can also be used).

Take one of the pieces of lamination foil, and use the marker to draw lines where fluidic channels should appear (*figure C1a*).
Cut the channels with the scissors, by cutting precisely to the left and right of the marked lines. The cut-out channel will have a width between 0.5 and 1.5 mm. If it is made wider, there is a good chance that it will get blocked during lamination (*figure C1b)*
If desired, small pieces of aluminum or copper foil can be placed between the top and the middle layer, before hot lamination, to serve as electrodes. Punch additional holes, if required (*figure C1f)*.

Alternatively, it is possible to use a razorblade or scalpel knife to cut out channels, but this is not a safe procedure in classrooms.

After finishing the channel pattern, the pieces are stacked up (*see figure C1c*)
The bottom layer (as is) is put glue-face up.
The middle layer (holes and channels) is aligned with the top layer (position of glue-face does not matter)
The top layer (holes only) is put glue-face down.

Make sure that the top and bottom are the gloss faces of the chip.

Line up the holes, and keep the parts together between fingertips or fix with piece of tape.
Take a sheet of (A4, legal) office paper and fold it (a folded piece of aluminum foil can be used instead).
Put the chip into the folded paper, fix with small pieces of tape. Make sure the chip layers stay aligned.
Put the folded paper with chip (fold-first) in the hot lamination machine (if it has a switch: at 125 micron position).

Alternatively, a hot iron can be used (low temperature setting), but this is not a very safe procedure in classrooms (*see figure C1d*).

Make sure the chip is clearly translucent. If it has an opaque appearance, repeat the lamination step. The channels should have still a matte appearance; if there are translucent zones this means the top and bottom layer have glued together and the channels are partly blocked.

Remove paper and tape. Cut the edges of the chip if desired; remove remains of paper or tape.

Testing:
Put the chip, holes upwards, on a surface.
Add a droplet of (colored) water to one of the holes, and wait for the fluid to fill the channels by capillary force. Do not add drops at other holes before the channels are filled properly, to prevent locking-in of air.
After the channels are filled, droplets can be added to the other holes (*see figure C1e,f)*.
One hole is used as the exit. Firmly press a piece of tissue on that hole and observe the behavior of fluids in the chip.

If channels do not fill spontaneously, some suction or pressure can be performed with a small pipette balloon. After channels are completely filled, suction can be done with tissue.

After use, the chip can be cleaned with running water and dried for repeated use.

2. Laser-cut chip.

Preparation:
Tape sheets of lamination foil (one half of a lamination pouch) glue-face up on paper (*see part B, figure B1, for procedures and designs for laser-cutting*).
Put taped sheets glue-face up in the laser cutter.
Cut the chip pattern.
The exact parameters of cutting speed, intensity and cutting frequency must be determined experimentally. Each combination of laser cutter and lamination foil gives slightly different results affecting the final product. The settings given in the caption of figure B1, give good results using the combination of materials reported in this article.

Ready-made sheets of laser-cut foil are brought to the classroom. Students pick out a piece of foil with the chip design they prefer to assemble.

Requirements:

- Laser-cut chips, produced from hot-lamination foil, 80 micron, standard office quality, gloss.
- Hot lamination machine (an iron on low temperature setting can be used instead, but this is not a very safe procedure in classrooms)
- Office paper
- Tissue paper
- Transparent tape
- Dyed water (use food coloring, printer ink or fountain pen ink), two colors
- Water
- Pipettes or cotton sticks
- A small rubber pipette balloon is helpful in some cases

Assembly of the laser-cut LoC:
The foil chip is removed from the backing paper.
Identify the (matte) glue face.
Fold a piece of paper.
Carefully fold the chip, glue-face in and with holes aligned.
Place the folded chip inside of the folded paper and fix with small pieces of tape.
Make sure that the holes remain carefully lined-up.
Put the folded paper with chip (fold-first) in the hot-lamination machine (if it has a switch: at 125 micron position).
After lamination, make sure the chip is clearly translucent. If it has an opaque appearance, repeat the lamination step.
Instead of a lamination machine, also an iron at low temperature setting can be used.

Remove tape and paper from the chip.
Put the chip hole-face up on a surface.
The quality of the chip can be inspected using a magnifying glass or microscope to see if the holes and channels are open.
The chip can be tested before use by adding a droplet of (dyed) water to one of the holes and waiting for the channels to fill. This can take some time.

Testing:
The chip can now be used in an adapted chip holder (containing a 1 x 15 x 45 mm piece of acrylic for support) the same way glass chips are used.

The bonding strength of the lamination foil does not allow high fluidic pressures for longer times. Flow speeds should not exceed *ca* 50 µL/min to prevent delamination. Some testing is required. The bottom layer can be replaced by waterproof clear tape to improve the stability of the chip. The chip can be cleaned with running water and dried for repeated use.

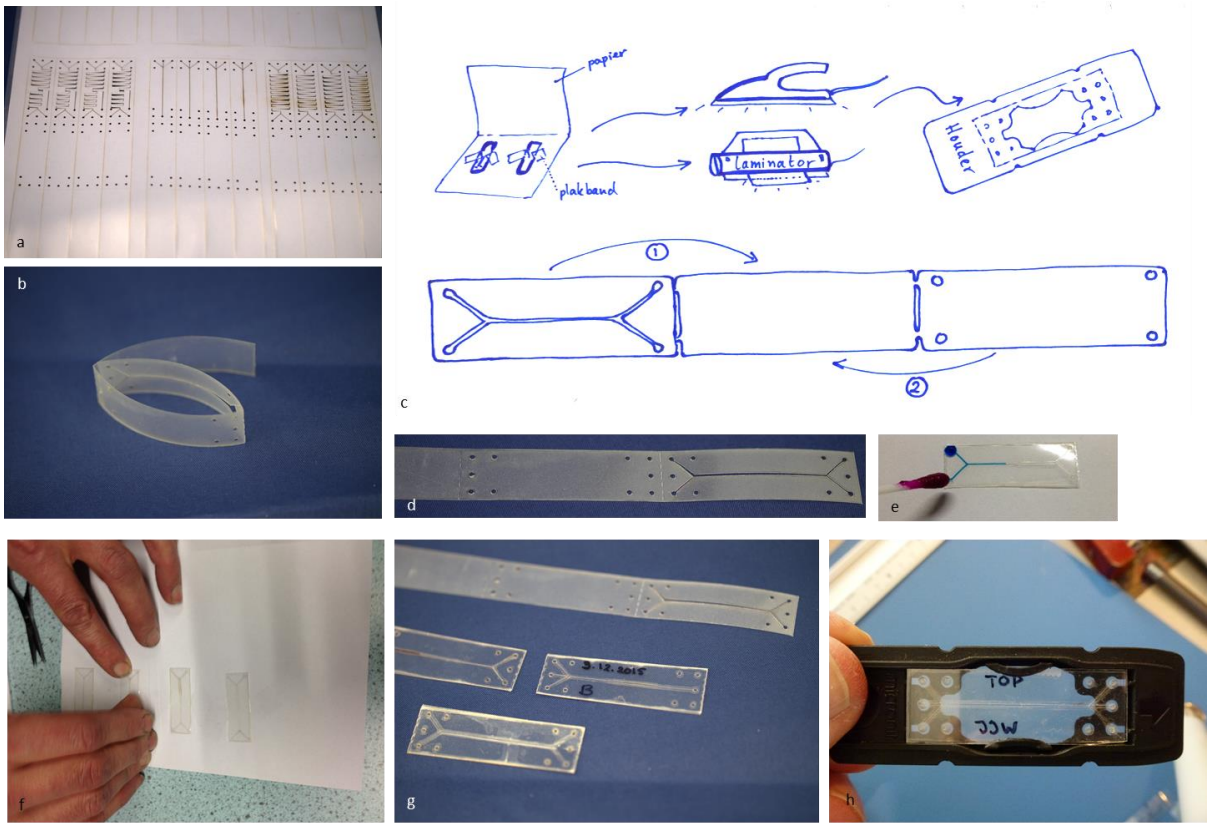

Figure C2 Assembly of chips from laser-cut foil. *(a) Laser-cut sheet of lamination foil, backed with office paper. (b) Folded chip, prior to lamination. (c) The assembly procedure and folding order (1,2). (d) Lasercut foil, just removed from backing paper. (e) Filling the chip (note: add second color when whole channel is filled). (f) Removing chips from cover paper after lamination. (g) Ready to use chips. (h) Foil chip mounted in chip-sleeve, on top of a supportive acrylic layer, ready to place in the chip holder.*

Videos:
Video demonstrating a scissor-cut foil chip (tri-laminar flow):
https://www.youtube.com/watch?v=XwR7ft-TWFc

Video demonstrating a laser-cut foil chip, mounted in a prototype of the acrylic chip holder:
https://youtu.be/j3Km667AebQ

D. Assessment for the Lab-on-a-chip lesson series

Exercise questions

For all chapters of the lesson series, exercise questions are given on the various concepts discussed in the text and practicals. A sample of these exercises is presented below.

Q 1.3. About the Semen-chip

a. What are some disadvantages of testing semen quality, as done with standard methods?
b. In what respects does the semen-chip improve this procedure?
c. Design an advertisement or write a journal-article to promote the development of a lab-on-a-chip device for semen testing. Produce a poster, billboard or a written article.

Q 1.5 Give three arguments why a device should be developed for farmers to measure the calcium level in bovine (cows') blood.

Q 2.3 Chemical experiment

Ask your teacher or use e a chemistry textbook to get a description of a chemical experiment, such as an acid-base reaction.

a. Describe precisely all of the steps required for the experiment.
b. Describe all glassware, manipulations and how you determine if the desired reaction has taken place.
c. Make a precise stepwise description of how to carry out this experiment using the equipment in the Lab-on-a-chip practical kit.
d. If the experiment cannot be carried out using the available equipment, what modifications do you suggest to make the experiment work?

Q 2.9 (Use the internet, or consult your biology teacher).

a. DNA can be copied using Polymerase Chain Reaction. This can be done using Lab on a chip. Find out how the method works, and how it can be performed in a Lab-on-a-chip setup.
b. Find some other applications of Lab on a chip that are available for DNA analysis.

Q 3.3 A tubular channel in a chip is 0.30 mm diameter and 120 mm long. Calculate the inner volume in cubic meters and Liters. Also give the volume in nanoLiters.

Q 3.10 The surface of a circle is πr^2. The surface of a sphere is $4\pi r^2$. The volume of a sphere is $^4/_3\pi r^3$. Calculate A/V for a sphere of 1.0 cm radius. Repeat for a raindrop with 2.0 mm radius.

Q 3.16

a. The channels in glass LoC devices can be made in different ways. Give two.

b. Take glass chips from the practical kit, and study the channels using a microscope. Compare the H-reactor and the focussed flow droplet generator (FFDG). Make a drawing of their channels. What is your observation? Can you conclude which technique was used for each of the chips? Give an explanation for the differences in structure.

c. What consequences will the difference in channel structure have for the experiments using each of these chips?

Q 4.5 For this task use the detailed drawing you made of the lithium chip (practical 7.1) or ask your teacher for a photograph of it. Note for each of the visible parts what function it has. Give each part a

number, and compare this with the schematic in figure 4.4. Next, describe stepwise the order in which the parts are used during a measurement in the chip.

Q 5.1 Explain why capillary force has a greater role when the inner diameter of a tubing gets smaller. (Hint: total adhesion force is directly proportional to the contact surface of fluid and tubing wall; total gravity force is directly proportional to the volume of the fluid column).

Q 5.9 Find in BINAS [student book with scientific lookup tables] the values of ρ and η [density and viscosity] for water. [The Reynolds number equation is given in the module textbook]. The radius of a round channel in a chip is 0.030 mm. Make a calculated estimation of the maximum speed of passing water in this channel without getting turbulent.

Q 5.12 Describe the 'trick' applied in the TD26 chip to get proper mixing in small amounts of fluid, despite narrow channels. See the website at URL 17.

Q 5.23 (see paragraph 5.6). The micro-reactor for production of gold nanoparticles uses a very *long* capillary tubing. What phenomena occur when two fluid streams are joined in one capillary? What should be changed before you can use a *short* capillary tubing?

Paragraph 6.6

A choice of design tasks for the student project, performed in teams of 2-4 students. As an introduction, a general description is given of design and development projects, and how results need to be presented. A poster is created to describe the design and function of the Lab-on-a-chip system, including a prototype, if technically feasible.

Task E. Design a chip for low-cost testing of Ebola or HIV. Developing countries need reliable tests that are cheap and easy to use. They need to be as reliable as classical lab testing. Recently, such an Ebola test has been developed. Find out how such a test works, and how this can be done using Lab-on-a-chip technology.

Task F 1. Design a chip that can sort a mixture of large and small beads.

Task H. Your own design. If you have another idea for a Lab on a chip, discuss with your teacher. To get inspired, look at URL 16.

Final assessment

The final assessment for the Lab-on-a-chip (LoC) lesson series includes a written test and a project poster presentation. Results for two test questions were analyzed to give an indication of the final level students can reach at the end of the lesson series (see also the *Educational Scenario* paragraph in the main article). The first question tests LoC design skills, whereas the second question tests understanding of the layout and workings of a given chip design that is somewhat similar to one of the LoC designs described in the lesson materials and discussed during the lesson series.
These sample questions were used in the final test at one high school involving 70 students.

> **Test question 1: Design a Lab on a chip for the Food Safety Authority (translated from Dutch).**
> The Food Safety Authority (FSA) needs a compact device to check that food that is for sale does not contain illegal amounts of certain components. These components can be proteins, additives, poisons and other compounds. FSA officers often find these illegal (amounts of) components in food. The Lab-on-a-chip system that you must design for the FSA should be able

to test products that are ready to sell, at that location. Five different components are tested in one run.

The samples are currently processed in the laboratory, using indicators showing the components present. A small amount of the sample is mixed 1:1 (vol:vol) with ethanol, the indicator is added and a readout of the color is done. For each compound another indicator is used.

Design a Lab-on-a-chip system to achieve this task. Make a sketch showing the design of the chip, the pumps and electronics attached for measurement.

Take into account the behavior of fluids in a Lab-on-a-chip system, with regard to addition, mixing and analysis of the samples. Also find a way to introduce samples and other compounds into the chip.

Analysis of the results on test question 1 (n = 70)
Average score = 82%, standard deviation = 27%. Fully correct implementation of the requirements in the chip design was achieved by 24 students. Other students made one or more mistakes with mixing (24), logical layout and design issues (24) or omission of a detection method (5).

Test question 2: Lithium chip (translated from Dutch)
The figure below shows the design of the lithium chip. The green circles are contact points, for connection to electrodes.

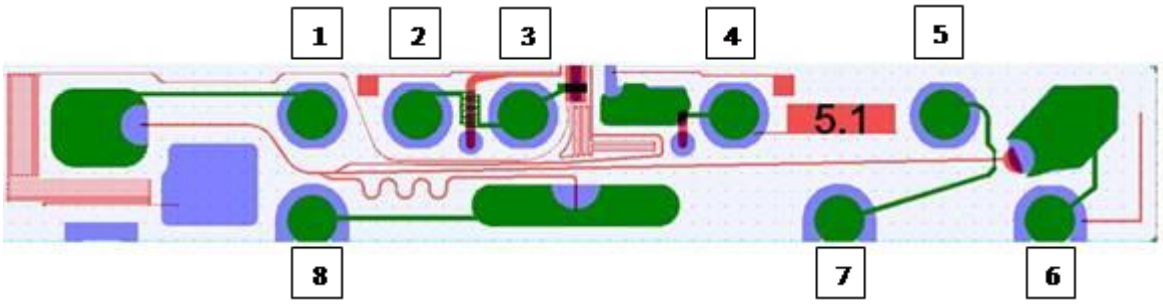

Figure D1: *Schematic of the lithium chip (courtesy of Medimate / CE-Mate, Enschede, The Netherlands)*

(Question 2 continued)

a. Describe briefly what this chip is intended to measure.
b. What do the thin lines on the chip represent?
c. Make a drawing of typical measurement results and indicate the conclusions drawn.
d. The electrodes in the chip operate in pairs. Which pairs? In wat order do they operate during the measurement process?
e. What changes are required to make the chip measure particles with opposite charge?
f. What materials are used to build this chip?
g. The chip is produced on wafers. What does that mean?

Analysis of the results for test question 2 (n = 70)
Average score = 77%, standard deviation = 18%. Errors were made in all questions, but most frequently on questions d and e.

Note: the lithium chip, described by Floris et al., 2010 (ref 18), is discussed in detail in the lesson series, in both the theoretical part as well as the introductory practical: microscopy of a Lithium chip.

Student project

During the final lessons of the series, groups of 3-4 students work on a project to produce a design for a Lab-on-a-chip system. They produce a poster and present their ideas to their peers, and in some cases, a visiting university staff member. One of the posters is presented in the main article. An additional example of student design posters is depicted below (in Dutch). The posters were produced by student teams at two different schools.

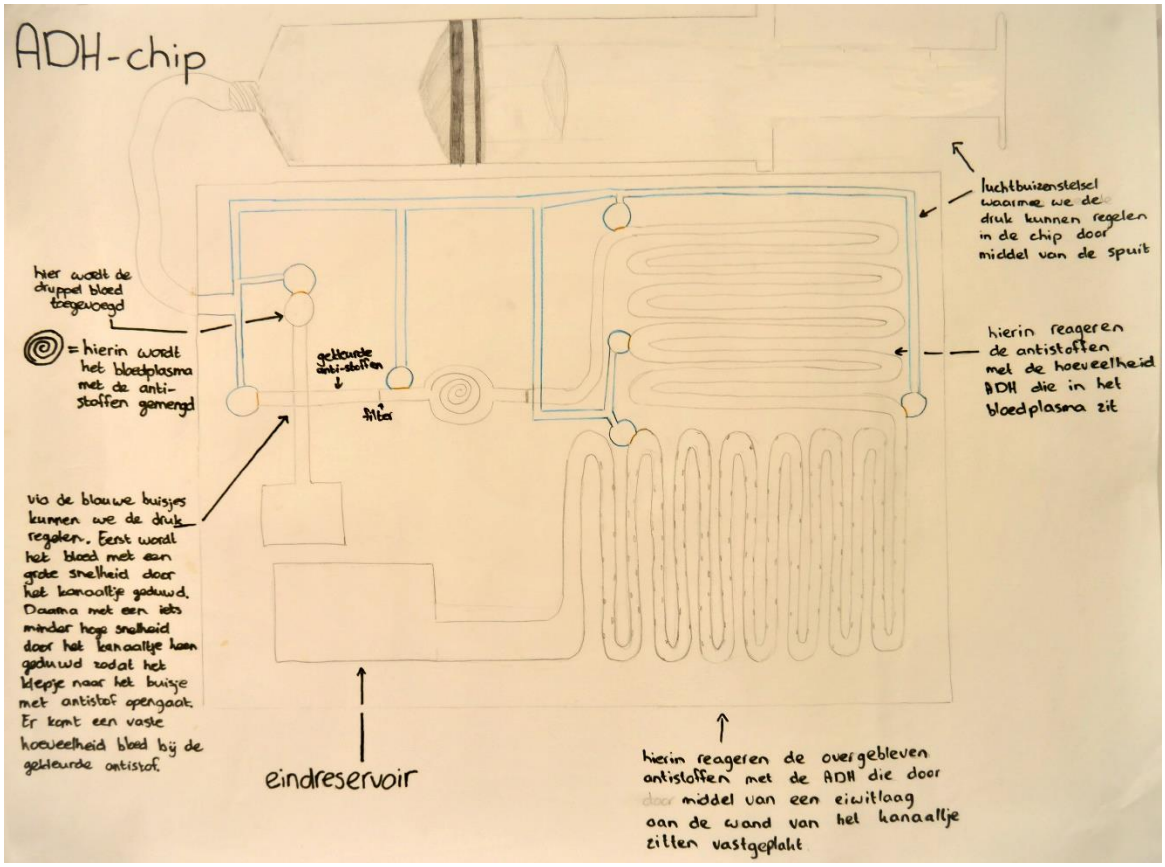

Figure D2: *student poster presenting the idea for an ADH (anti diuretic hormone) detection chip (reproduced with permission).*