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

SUPPLEMENTARY INFORMATION

FIGURE 1: Schematic diagram of the experimental setup highlighting the components of the cooling system. The table shows lengths, diameters and material for all tubing used to make fluidic connections.

FIGURE 2: Average temperature of the immobilization microchamber in the chip versus time. After turning on the Peltier units, chilled coolant flow (3.2 ml/min) through the chip is started at 0 seconds with temperature of the coolant in the external reservoir being at 23 ^{0}C (room temperature) and -10 ^{0}C respectively (pre-cooled).

FIGURE 3: Box plots showing all distances moved in between frames by (a) mitochondria at NMJs, (b) cell bodies along VNCs and (c) mitochondria along axons in between video frames collected over 2 minutes for ten animals loaded onto the same chip. The videos were collected with larvae loaded on the microfluidic chip with chilled coolant (5M salt water solution) as well as with coolant at room temperature flowing (3.2 ml/min) through the chip respectively. Each box extends from the 25th to 75th percentiles, the line in the middle is plotted at the median and the whiskers span between the ends of the interquartile ranges to the furthest observations within the whisker length (1.5 times the interquartile range). Observations lying beyond the whiskers are displayed with a red + sign. Pixel resolution is 0.18 μm.

FIGURE 4: Average temperature of the immobilization microchamber in the chip versus time. After turning on the Peltier units, chilled coolant flow (at 3.2 ml/min) through the chip is started at 0 seconds with: (a) Temperature of 5M salt water solution in the external coolant reservoir being at 23 ^{0}C (room temperature), temperature of 5M salt water solution in the external coolant reservoir being at -10 ^{0}C (pre-cooled) and temperature of 40% ethanol solution in the external coolant reservoir being at 23 ^{0}C (room temperature) respectively.

FIGURE 5: (a) Known (accurately controlled) applied static air pressure versus experimentally measured deflection of flexible PDMS membrane. The solid line represents a fit by a third-order polynomial. (b) Fluid (5M salt water solution) pressure exerted on the PDMS membrane versus known (accurately-controlled) fluid flow rate. The solid line represents best fit by linear regression. Error bars indicate standard deviation of three measurements performed on the same device and is given by the data point.

FIGURE 6: Survival rate versus time after six consecutive cycles. Error bars indicate standard deviation of ten animals.

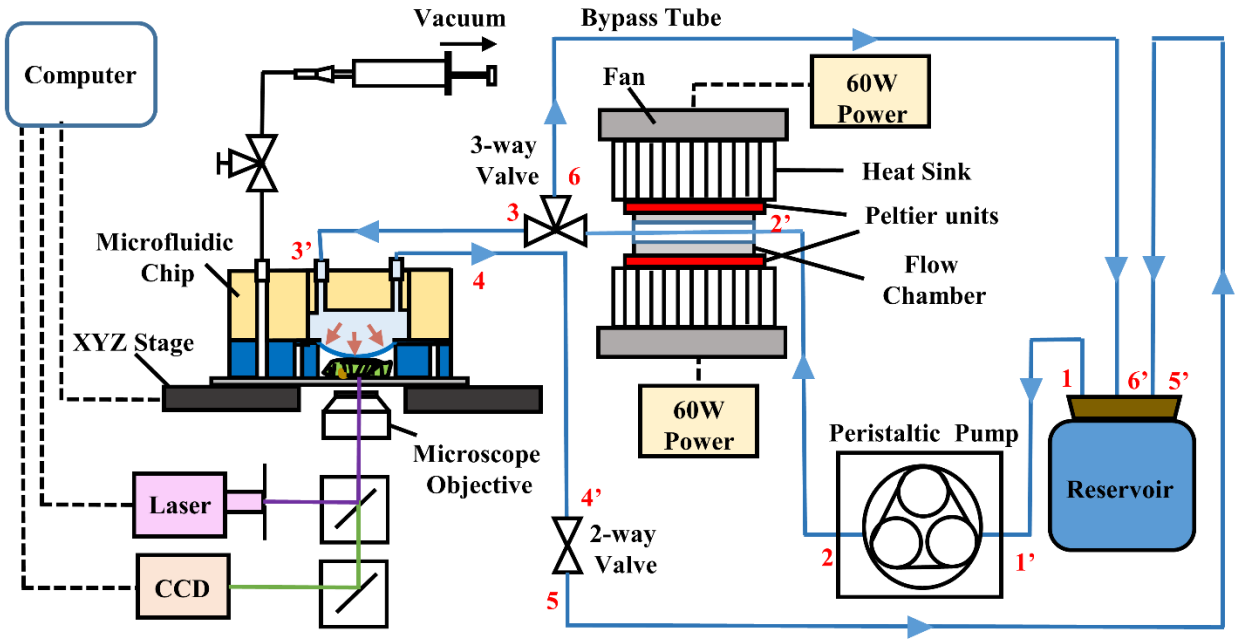
FIGURE 7: Conversion efficiency of seven individual VNCs as measured pre and post conversion in their red:green ratio of the average fluorescent intensities at the cell bodies. Fold change was compared to pre-conversion ratio.

MOVIE 1: Illustration of immobilizing effect on larval movement once chilled coolant was passed under pressure through the chip (time point 0:00:03 in video). A clear and rapid immobilization effect (time point 0:00:10 in video) was observed with movement decreasing and then stabilizing. As soon as flow through the chip was turned off, the larvae started regaining mobility (time point 0:00:33 in video). The movement of the larva was recorded continuously for a period of 540 seconds in real time (acquisition rate: 1 frame/sec). The video has been accelerated ten times (frame rate: 10 frames/sec) to make the file size shorter.

MOVIE 2: Movement at the Neuro-Muscular Junction (NMJ) showing fluorescently labeled mitochondria during immobilization with the larva in the immobilization microchamber and chilled coolant (5M salt water solution) flowing (3.2 ml/min) above it through the cooling microchamber. The movement of the larva was recorded continuously for a period of 120 seconds in real time (acquisition rate: 1 frame/sec). The video has been accelerated three times (frame rate: 3 frames/sec) to make the file size shorter.

MOVIE 3: Movement at the Ventral Nerve Chord (VNC) showing fluorescently labeled neuronal cell bodies during immobilization with the larva in the immobilization microchamber and chilled coolant (5M salt water solution) flowing (3.2 ml/min) above it through the cooling microchamber. The movement of the larva was recorded continuously for a period of 120 seconds in real time (acquisition rate: 1 frame/sec). The video has been accelerated three times (frame rate: 3 frames/sec) to make the file size shorter.

MOVIE 4: Movement at the axons showing fluorescently labeled mitochondria running along the length of the axons during immobilization with the larva in the immobilization microchamber and chilled coolant (5M salt water solution) flowing (3.2 ml/min) above it through the coolant microchamber. The movement of the larva was recorded continuously for a period of 120 seconds in real time (acquisition rate: 1 frame/sec). The video has been accelerated three times (frame rate: 3 frames/sec) to make the file size shorter.



Tubing	Length	Material	Diameter
1-1'	57.4 cm	Polyvinyl	1.60 mm I.D. 3.18 mm O.D.
2-2'	73.9 cm	Polyvinyl	1.60 mm I.D. 3.18 mm O.D.
3-3'	14.0 cm	Polyvinyl	0.58 mm I.D. 0.97 mm O.D.
4-4'	14.0 cm	Polyvinyl	0.58 mm I.D. 0.97 mm O.D.
5-5'	105.2 cm	Polyvinyl	1.60 mm I.D. 3.18 mm O.D.
6-6'	81.0 cm	Polyvinyl	1.60 mm I.D. 3.18 mm O.D.
Pump Tubing	14.0 cm	Silicone rubber	2.03 mm I.D. 3.56 mm O.D.

FIGURE 1

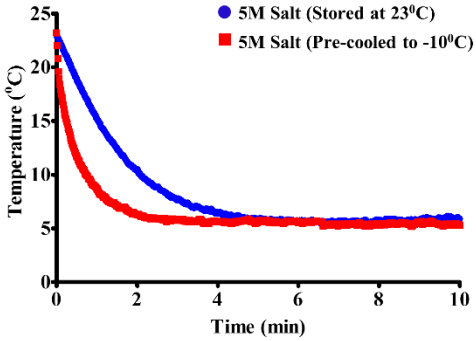

5M Salt (Stored at 23⁰C)
5M Salt (Pre-cooled to -10⁰C)
Temperature (⁰C)
25
20
15
10
5
0
0
2
4
6
8
10
Time (min)

FIGURE 2

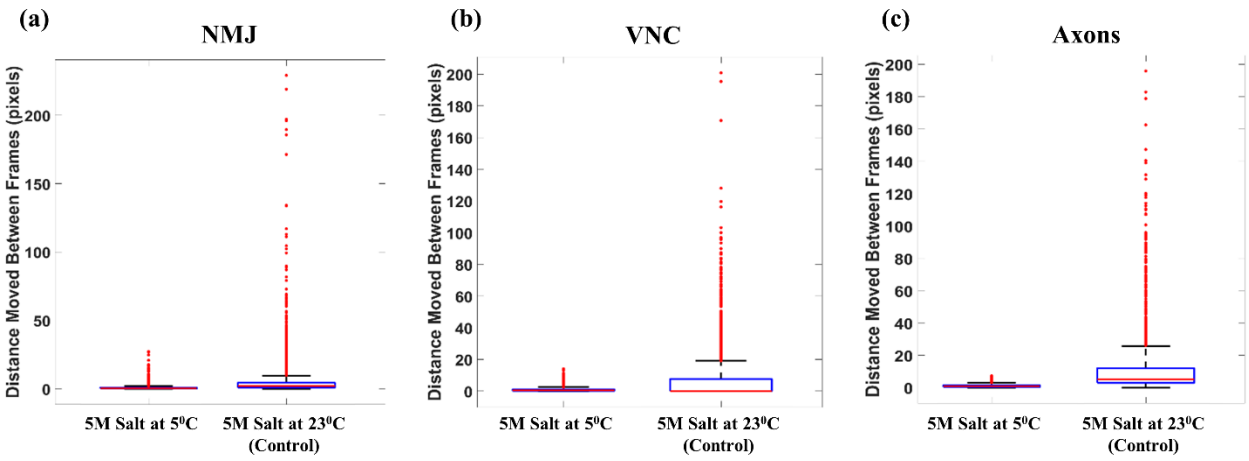

(a)
NMJ
Distance Moved Between Frames (pixels)
0
50
100
150
200
5M Salt at 5⁰C
5M Salt at 23⁰C
(Control)
(b)
VNC
Distance Moved Between Frames (pixels)
0
20
40
60
80
100
120
140
160
180
200
5M Salt at 5⁰C
5M Salt at 23⁰C
(Control)
(c)
Axons
Distance Moved Between Frames (pixels)
0
20
40
60
80
100
120
140
160
180
200
5M Salt at 5⁰C
5M Salt at 23⁰C
(Control)

FIGURE 3

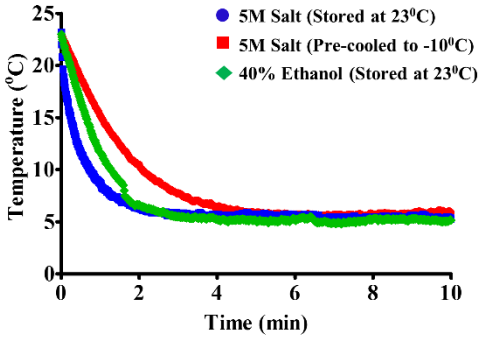

5M Salt (Stored at 23⁰C)
5M Salt (Pre-cooled to -10⁰C)
40% Ethanol (Stored at 23⁰C)
Temperature (⁰C)
25
20
15
10
5
0
0
2
4
6
8
10
Time (min)

FIGURE 4

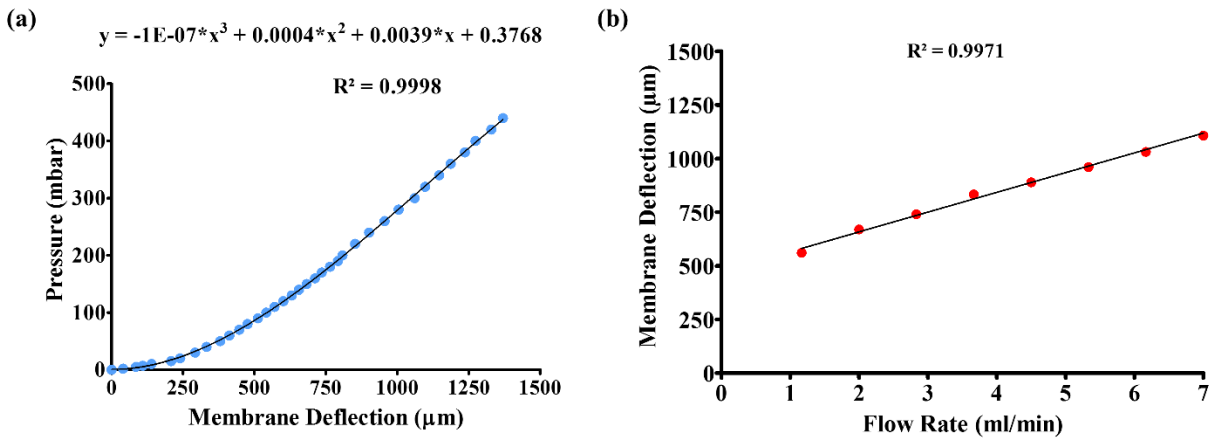

(a)
y = -1E-07*x³ + 0.0004*x² + 0.0039*x + 0.3768
R² = 0.9998
Pressure (mbar)
Membrane Deflection (µm)
0
100
200
300
400
500
0
250
500
750
1000
1250
1500
(b)
R² = 0.9971
Membrane Deflection (µm)
Flow Rate (ml/min)
0
250
500
750
1000
1250
1500
0
1
2
3
4
5
6
7

FIGURE 5

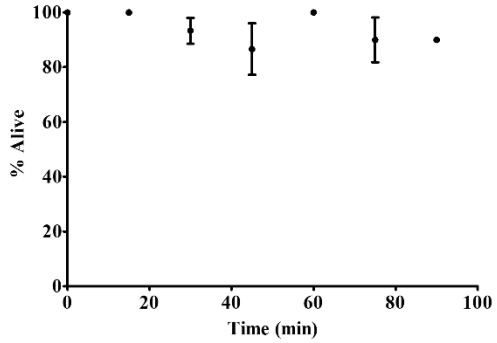


FIGURE 6

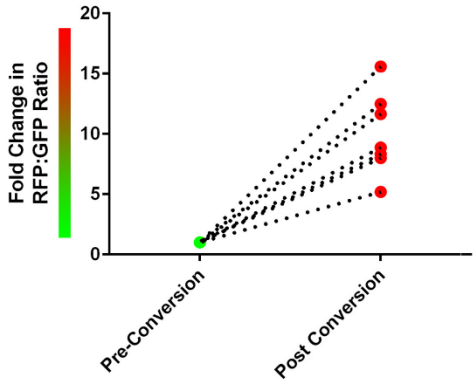

Fold Change in RFP:GFP Ratio
20
15
10
5
0
Pre-Conversion
Post Conversion

FIGURE 7