Supplementary Information

Processing of Pyrex Wafers

**Amorphous silicon**

- Pyrex wafer, coated with 150 nm of amorphous silicon

**Photoresist**

- Front side photolithography (photolithography steps are omitted in subsequent steps)
- Dry etch silicon, remove photoresist mask
- Repeat process on backside and etch Pyrex wafer in hydrofluoric acid (HF)
- Remove silicon in KOH, decontaminate wafer with HCl/H2O2/H2O

Supplementary Figure 1A: Major steps of Pyrex wafer processing.
Supplementary Figure 1B: Major steps of silicon wafer processing.

- Oxidize silicon wafer
- Wet etch openings
- Deposit and dry etch silicon nitride thin film
- Deposit and wet etch PSG
- Deposit and dry etch second silicon nitride layer
- Etch holes and channels through the wafer (STS DRIE)
- Sacrifice oxide in HF to create hollow silicon nitride injector structure
Device Assembly by Anodic Bonding

Supplementary Figure 1C: Device assembly through anodic bonding of processed silicon and Pyrex wafers.

Supplementary Table 1: Operational states of syringe pump and valves during chip operation (O - valve open, C - valve closed, P - three-way valve connects oil reservoir with pressurized air, A - three-way valve connects oil reservoir with ambient air).

<table>
<thead>
<tr>
<th>Task</th>
<th>Syringe Pump</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
<th>V7</th>
<th>V8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo loading into microfluidic system</td>
<td>On</td>
<td>C</td>
<td>C</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>A</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Embryo injection</td>
<td>Off</td>
<td>C</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>C</td>
<td>A</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>Embryo transport to external reservoir</td>
<td>Off</td>
<td>O</td>
<td>C</td>
<td>O</td>
<td>C</td>
<td>C</td>
<td>P</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Repeat cycle (loading of next embryo)</td>
<td></td>
<td></td>
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</table>
Supplementary Figure 2: Detection of embryos with integrated photodiodes and LEDs, integrated on opposing sides of the package. A) Bottom part of the chip package with integrated photodiode chips (Digikey, Thief River Falls, MN); the size of each photodiode chip is approximately 1 mm x 2 mm). B) Top part of the package with integrated LED chip (Digikey). C) Assembled package as viewed from the top side. D) Measurements of photodiode output voltages in response to light from a red laser pointer, moved slowly across all photodiodes, indicate consistent performance of photodiodes. E) A single photodiode/LED pair was used to detect moving objects in the main channel of the microfluidic injector chip. The syringe pump was operated at 140 µL/min. The photodiode signal was measured every 2.3 ms. While air bubbles cause relatively large but short signal fluctuations up to 70 ms, light attenuation through embryos is less pronounced, but lasts for approximately 300 ms. Photodiode/LED pairs could be used to track embryos at multiple locations inside the microfluidic system.
Supplementary Figure 3: Survival (development of mouth hooks after 24 hours) of dechorionated and desiccated embryos which were cycled through a system without microinjector structure. After collection, embryos were stored under a 1 mm thick film of Klearol white mineral oil in a humid environment. Below a flow rate of 140 µl/min embryos were not reliably transported.