SUPPORTING INFORMATION

Supporting Figure 1: Details of the fabrication process.

Fabrication of thin silicon membranes

The realization of the suspended silicon membrane relied on wet etching of SOI wafer. The 'device' side of the wafer was converted into a silicon membrane by etching away the 'handle' side, forming the reservoir during the process. The middle oxide layer served to stop the selective etch process.

(a) Virgin <100> SOI wafer having a 10μm thick 'device' side, 500μm thick 'handle' side, and a 3μm thick middle oxide layer.

(b) The wafer was thermally oxidized (5000Å thick layer) and nitrified (1800Å thick layer).

(c) Optical lithography was used to define the reservoir on the handle side of the dies simultaneously with the dies outline. The nitride layer was removed by a short CHF_3 plasma etch in a Reactive Ion Etching (RIE) machine. The remains of the exposed oxide layer were then removed by 6 minutes dip in 1:10 hydrofluoric acid (HF) solution at room temperature.

(d) The silicon was etched at a rate of 1μm/minute in 33% KOH solution at 80˚C. The etch process followed the <111> crystallographic planes, forming truncated pyramids having 54.7˚ angles relative to the <100> surface. Etching stopped at the buried oxide layer. Following this stage, the wafer was easily diced into $13mm \times 13mm$ squares along the previously defined outline. The rest of the fabrication was performed on single dies.

(e) The remains of the buried oxide were removed by dipping the dies for 30 seconds in 1:10 hydrofluoric acid (HF) solution, at room temperature.

(f) The nitride layer was removed by a 30 minute dip in hot phosphoric acid (175˚C).

Membrane perforation

(g) Needles were defined by e-beam lithography. The device side of the silicon die was spin coated (5000 rpm for 1 minute, followed by 60 minutes soft bake at 180ºC in an oven) with a positive electron-beam resist (495A5 PMMA by MicroChem, inc.). A $2mm \times 2mm$ array of 500nm diameter circles, 5μm apart, was defined by electron beam lithography (Raith E-Write machine, 300μC/cm^2 dosage) on the membrane and its neighborhood. The resist was developed for 30 seconds in 3:1 IPA:MBIK solution. The pattern was transferred from the PMMA to the oxide layer, using dry oxide RIE at 0˚C and the latter layer was perforated using CHF_3 plasma.

(h) The membrane was perforated in an ICP machine using a modified Bosch process.

(i) Remains of the oxide were removed by 10 seconds dip in HF at room temperature.

Oxidation of the perforated membranes

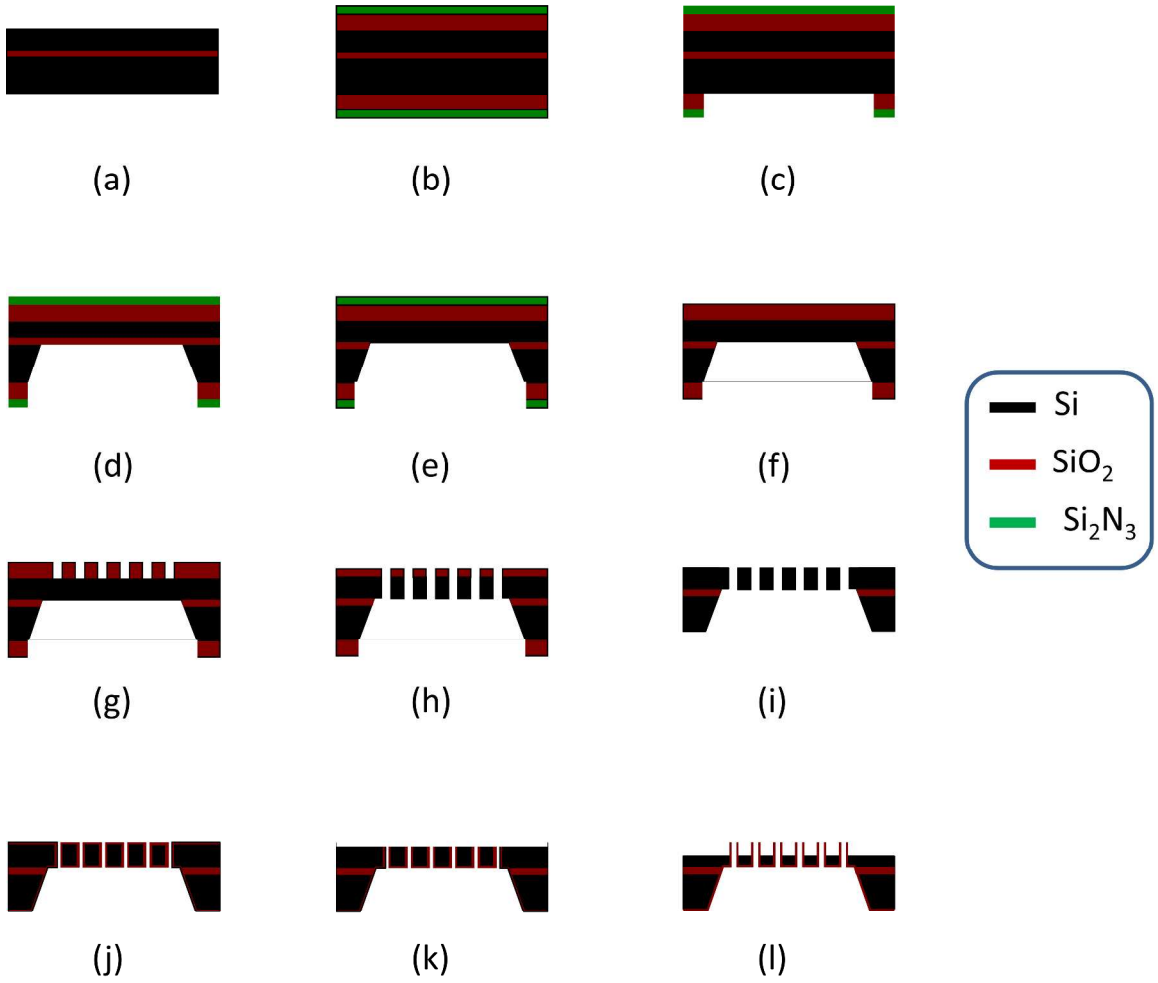
(j) The die was cleaned using a standard RCA cleaning and the entire substrate was thermally oxidized at 1000ºC for 190 minutes to create a 100nm thick oxide layer.

Removal of the front oxide layer

(k) The oxide layer was directionally removed from the device side of the die by CHF_3 RIE.

Selective thinning of the silicon membrane

(l) The exposed silicon layer was thinned by DRIE in ICP. The etching process, being 60 times faster with silicon compared with silicon dioxide, revealed the buried SiO_2 nanoneedles.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
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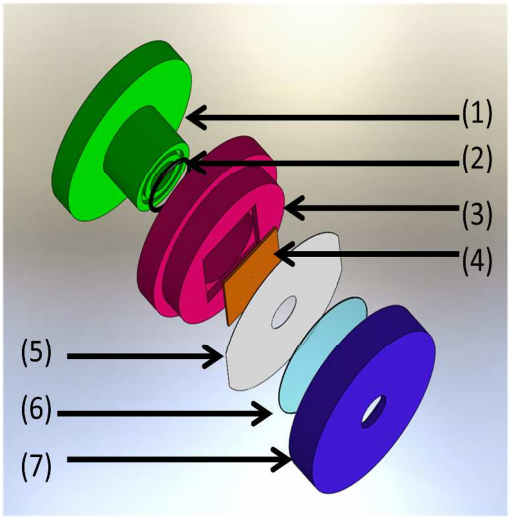
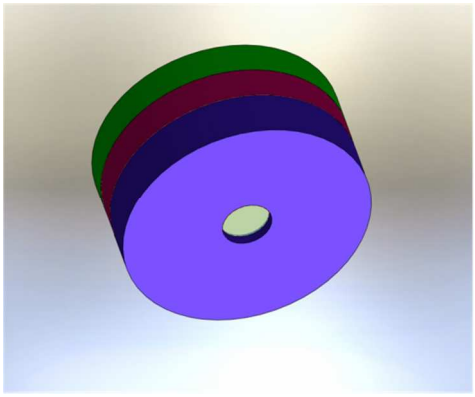
(l)
Si
SiO_2
Si_2N_3

Supporting Figure S2: Sample holder for NNA dies

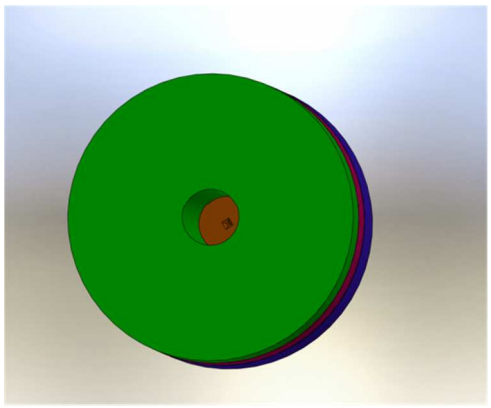
(a) Sample holder assembly. The sample holder was designed to hold the NNA die during the experiment and prevent leaks from the backside reservoir to the front side of the die. The NNA die (marked '4', the brown rectangle), with its front side facing down, is pressed between an "O"-ring seal (marked '2') and a 0.1mm thick Teflon disc (marked '5'). A hole in the Teflon disc creates a volume for the solution at the front side of the device, and enables observation through a glass cover slip (marked '6'). Teflon (marked '1', '2') and alumina (marked '7') parts construct the body of the sample holder and provide mechanical support.



(b) An assembled sample holder; the front side of the NNA die is imaged through a window.



(c) An assembled sample holder; The reservoir is accessible through the hole at the back of the sample holder.



(d) An assembled sample holder containing the NNA die placed on the stage of an inverted ZEISS AxioVert200 microscope. Solution is applied to the reservoir by the pipette (yellow, right). The NNA die and cells face down to the microscope objective.

Supporting information S3: Theoretical estimate of the molecular diffusion time from the back reservoir through the nanoneedles

The molecules current, j, through a needle is governed by diffusion and the corresponding Fick's law

$$j = -AD\frac{\partial C}{\partial z}.$$

Here, A is the needle's cross section area, D is the molecules diffusion constant, and C is their concentration. During the initial stage of diffusion, which is relevant to the appearance of a signal, the concentration gradient between the reservoir side of the needle (where it equals C) and the other side of the needle (where it vanishes) can be assumed constant. For a typical molecule concentration in the reservoir, $dC = 10\mu M$, needle length of $dz = 10\mu m$, needle cross section $A = 6\times10^{-14}\,m^2$, and $D = 10^{-11}\,m^2/s$

$$j = 360\,molecules/s\,.$$

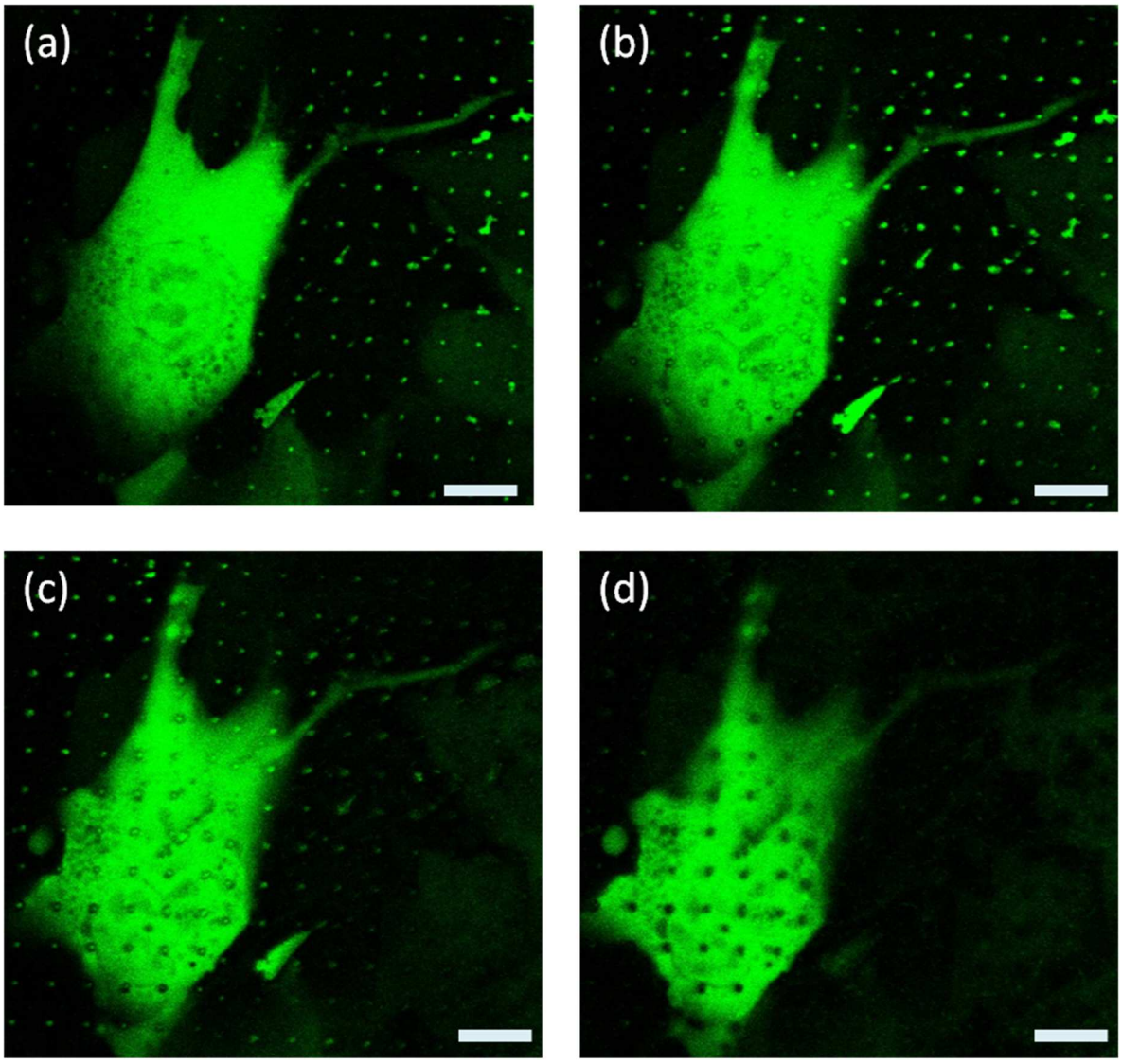
Fluorophore concentration of $100nM = 6\times10^{19}\,molecules/m^3$ is easily detectable by fluorescence microscopy. A typical pixel measures $10^{-18}\,m^3$ and therefore requires 60 molecules for observation. The buildup of that concentration with the calculated molecule current sould thus takes as little as $0.25s$.

This estimate is supported by experiment, as evident from the supporting movie S4.

Supporting Movie S4: Fluorescence signal of Fluorescin passing through the NNA die.

Supporting Figure S5: Confocal images depicting NIH3T3 mouse fibroblast cell growing on a NNA die

NIH3T3 mouse fibroblasts expressing cytosolic GFP were cultured on the NNA dies and imaged by confocal microscopy. The cells spread on the nanoneedles, reaching all the way to the bottom substrate. (a)-(d) Different z-sections of the same cell, taken at 1μm spacing from each other. In all images, the cell body overlaps with the nanoneedles. The 0.5μm-diameter nanoneedles show as excluded points in the images, surrounded by the cytosol. (a) Top plane of NNA. (b) Plane of the nanoneedles, 1μm below their top. (c) Plane of the nanoneedles, 2μm below their top. (d) Base of the nanoneedles. Scale bars: 10μm



Supporting Movie S6: Time-lapse movie of HEK293 cells expressing nucleic GFP cultured on an NNA die. 250,000 cells were seeded on the die 24 hours before the movie onset.