

Supplementary Material

Exploring Possible Concentration Bases

The possible ways of choosing a subset of 4 valves from a set of 8 valves is given by the binomial coefficient. There are

$$\binom{8}{4} = 70$$

possible concentrations that can be achieved by selecting 4 out of 8 input concentrations, given that there is no redundancy. A simple Mathematica code allows to test for redundancy of different bases and their smoothness which ultimately determines the dynamic range and resolution of the signal generator. Notice that each basis vector c can be multiplied by an arbitrary *tuning factor* without changing the redundancy. This will increase the dynamic range and decrease the resolution. In practice the optimal tuning factor will be determined by the dynamic range that needs to be achieved. To compare several bases we define the performance of each basis as the ratio of the dynamic range and the maximal concentration difference between neighbors (resolution). The performance P is independent of the tuning factor.

$$P = \frac{\max(C_{out}) - \min(C_{out})}{\max(C_{out,i+1} - C_{out,i})}$$

| Basis | Performance |
|-----------|-------------|
| Linear | 16.00 |
| Power | 16.14 |
| Geometric | 20.15 |
| Fibonacci | 21.67 |

Linear Basis

For the linear basis $c = 0\ 1\ 2\ 3\ 4\ 5\ 6\ 7$ the redundancy $R = 53$ and only $N = 17$ distinct output concentrations can be reached. The advantage of this concentration basis is that the mapping from valve state to output concentration is smooth with equidistant resolution between achievable output concentrations (see Supplementary Fig. S1a).

Power Basis

The power basis $c_n = 2^n$ with concentrations $c = 0\ 2\ 4\ 8\ 16\ 32\ 64\ 128$ covers a wide dynamic range but its performance is rather low due to larger gaps in the mapping and hence low resolution (see Supplementary Fig. S1b).

Geometric Basis Variant

An obvious candidate for the concentration basis is the geometric basis $c_n = 1/2^n$. However, a variant of the geometric series $c_n = (3/2)^n$ resulted in smoother results and we here discuss the concentration basis $c = 0\ 3/2\ 9/4\ 27/8\ 81/16\ 243/32\ 729/64\ 2187/128$. The redundancy of this basis is zero $R = 0$ and it has a rather smooth almost linear mapping for intermediate concentrations before it nonlinearly changes towards the boundary (see Supplementary Fig. S1c).

Fibonacci Basis

The Fibonacci sequence $c = 1\ 2\ 3\ 5\ 8\ 13\ 21\ 34$ can often explain geometries that occur in nature and follow the golden ratio. We tried to use the Fibonacci numbers as concentration basis and found a remarkable property. Although the basis has a redundancy of $R = 12$ the mapping is almost perfectly smooth (see Supplementary Fig. S1d).

Optimal Performance

The optimal performance of 27.00 was obtained with the basis $c_n = (1.93992448789999993863375)^n$ which was obtained by iterative numerical optimisation. In practice such accurate concentrations cannot be pipetted.

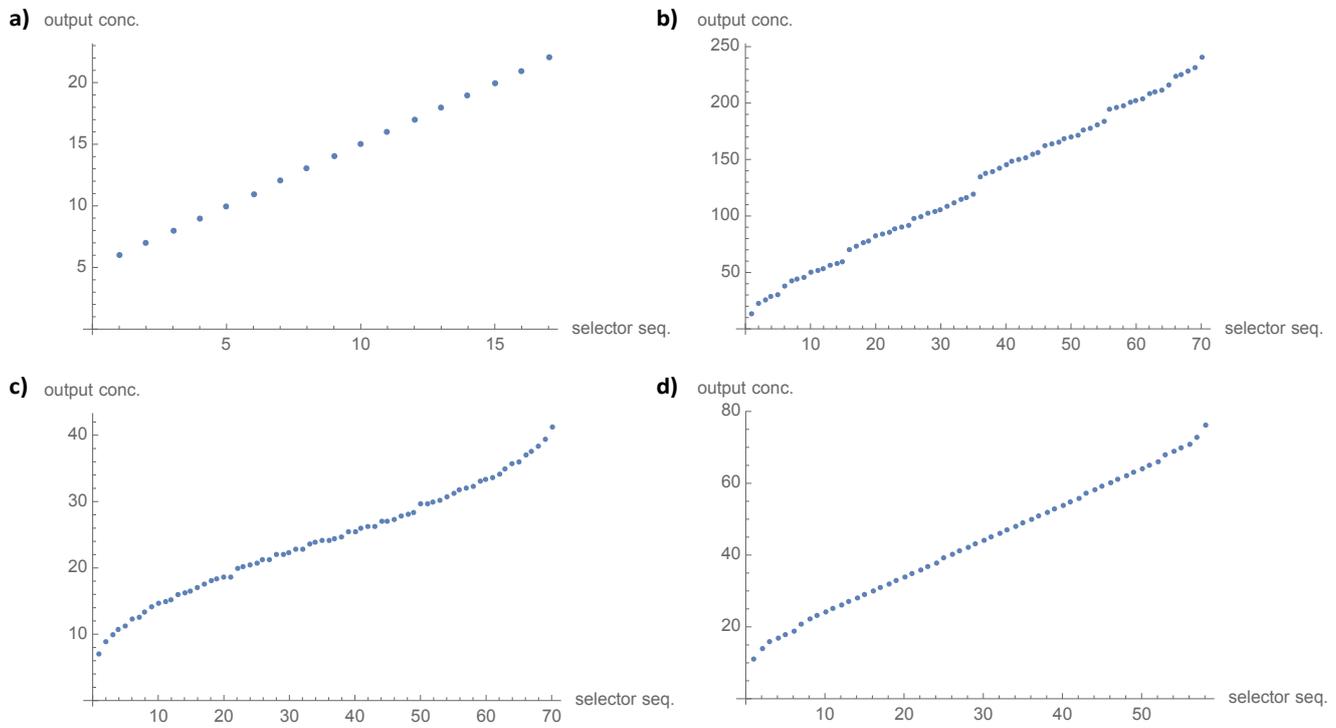


Fig. S1 a) Achievable concentration using a linear concentration basis **b)** Achievable concentration using a power concentration input basis has zero redundancy $R = 0$ and has a unique concentration for each selector sequence. However, the selector-output mapping is not very smooth. **c)** Achievable concentration using a geometric concentration input basis. **d)** Achievable concentration using a Fibonacci concentration input basis.

Data shown in the Bode plot (Fig. 3e) was obtained from Fluorescence Microscopy Images of FITC stimulated cell culture chambers. For each recorded movie the fluorescence time course data was extracted using ImageJ and the amplitude measured for each frequency. The decrease in amplitude was then plotted in units of dB as a function of the logarithmic frequency (Fig. 3e).

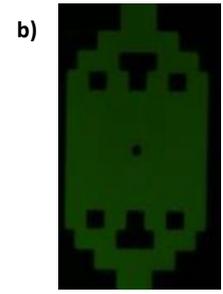
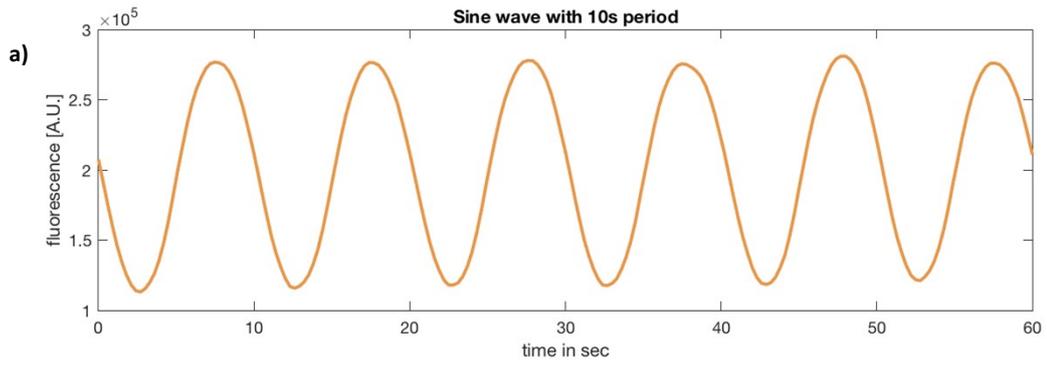


Fig. S2 a) Extracted data from the FITC time course of the cell culture chamber shown in S2b). Horizontal axis shows time, the vertical axis fluorescence intensity in arbitrary units. **b)** Recording of a FITC sine wave with period of 10sec (freq. 360) (See ESI).

Dynamic Range Tuning

A practical and important feature of the presented device is its ability to easily tune the dynamic range without calibration by means of the tuning factor (see Supplementary, Exploring Possible Concentration Bases), to allow the adjustment of the dynamic range of the device to the one of the receptor. While this tuning provides the experimenter with great flexibility, the presented 8 bit bases are not optimized to reach zero concentrations as this introduces further constraints on the basis. In most cases reaching zero is not desired as receptors show a response threshold or ultrasensitivity and do not respond to low concentrations.⁴⁸ In special cases, where reaching smaller concentrations or even zero concentration is necessary, we recommend to optimize the concentration basis and the number of inputs to this objective, i.e. choosing 2-4 zero basis concentrations or to simply increase the number of inputs to reach the desired dynamic range.

Simultaneous representation of stimulation and response

Below the NFκB nuclear localization time course of 7 single cells (grey) during periodic TNF sine wave stimulation is shown. The period of the sine-wave in red is 100 min, the minimum concentration 200pg/ml and the maximum concentration 3000pg/ml.

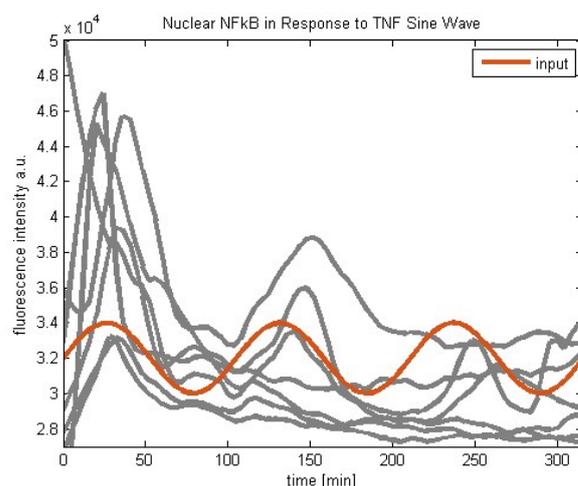


Fig. S3 Time course of nuclear NFκB intensity. Horizontal axis shows time in minutes, the vertical axis represents fluorescence intensity in arbitrary units. The red line represents the TNF input, grey lines NFκB nuclear intensity of single cells.

Signal Generator Cell Culture Chip Design

Below we show the AUTOCAD drawing of the integrated signal generator module and the cell culture chip. Yellow are control channels, and red and blue are flow channels.

