ELECTRONIC SUPPLEMENTARY INFORMATION

Controlled pharmacokinetic anti-cancer drug concentration profiles lead to growth inhibition of colorectal cancer cells in a microfluidic device

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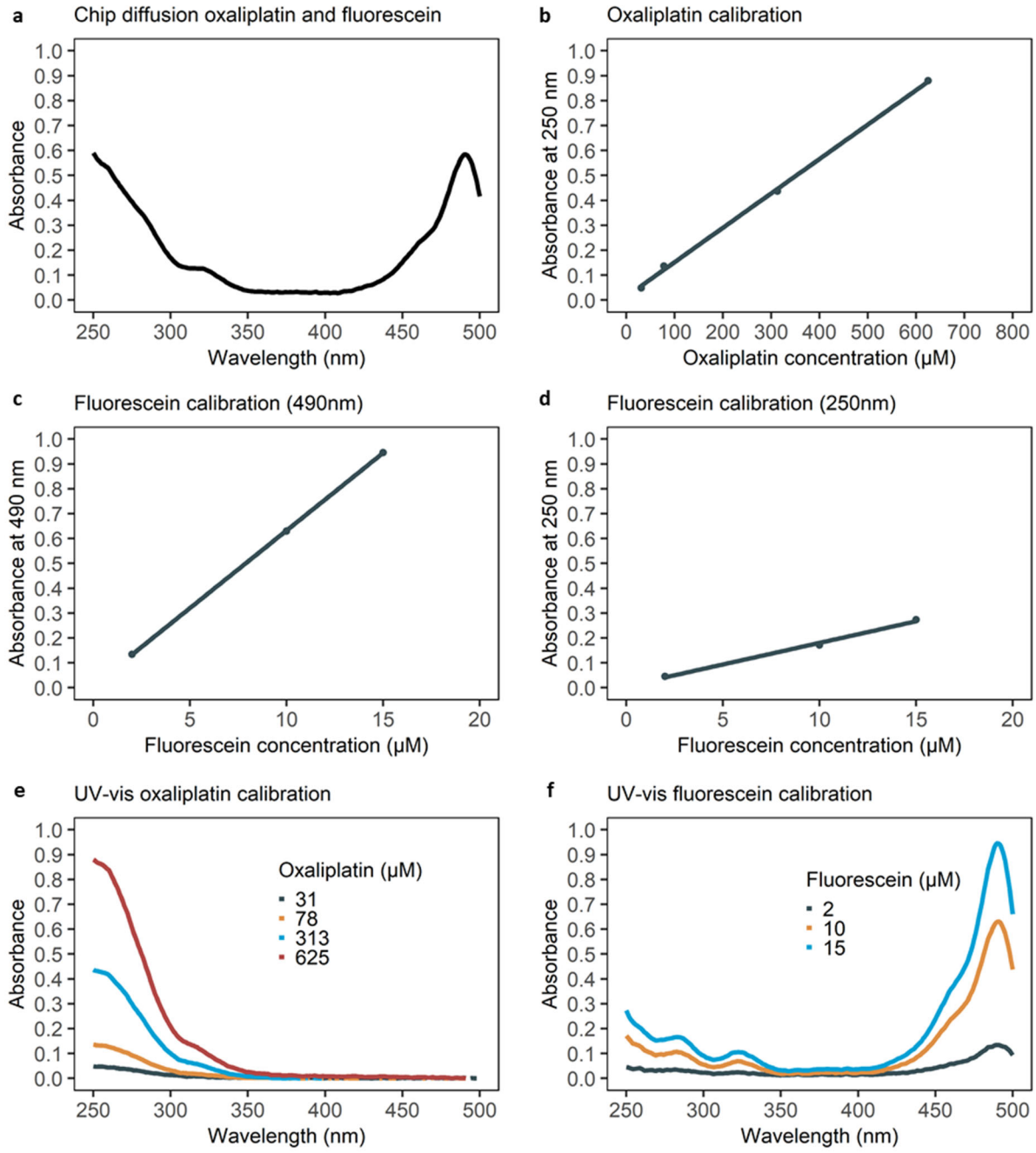
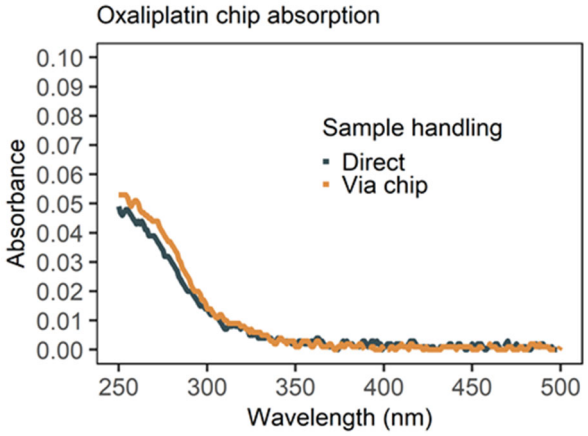
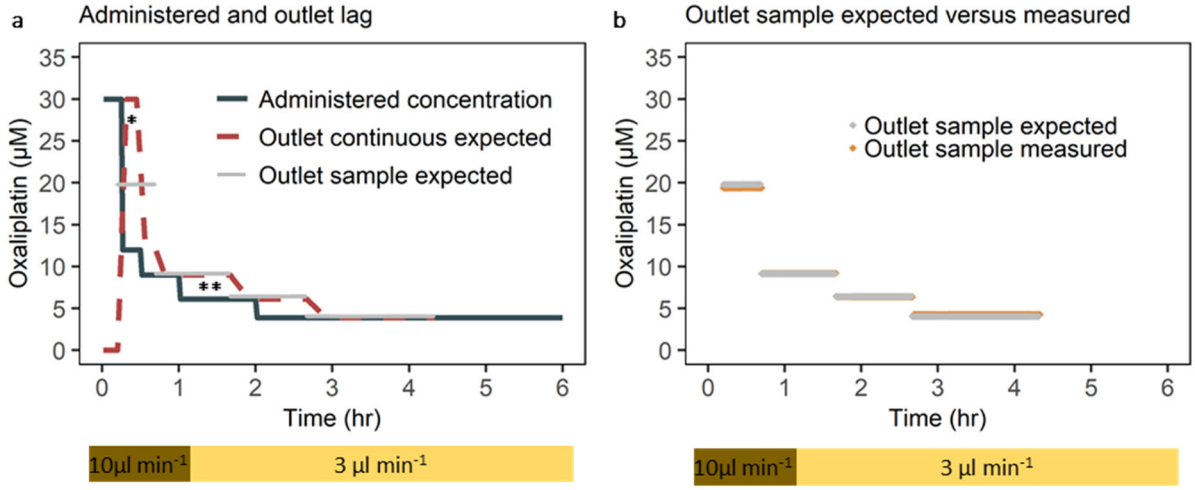


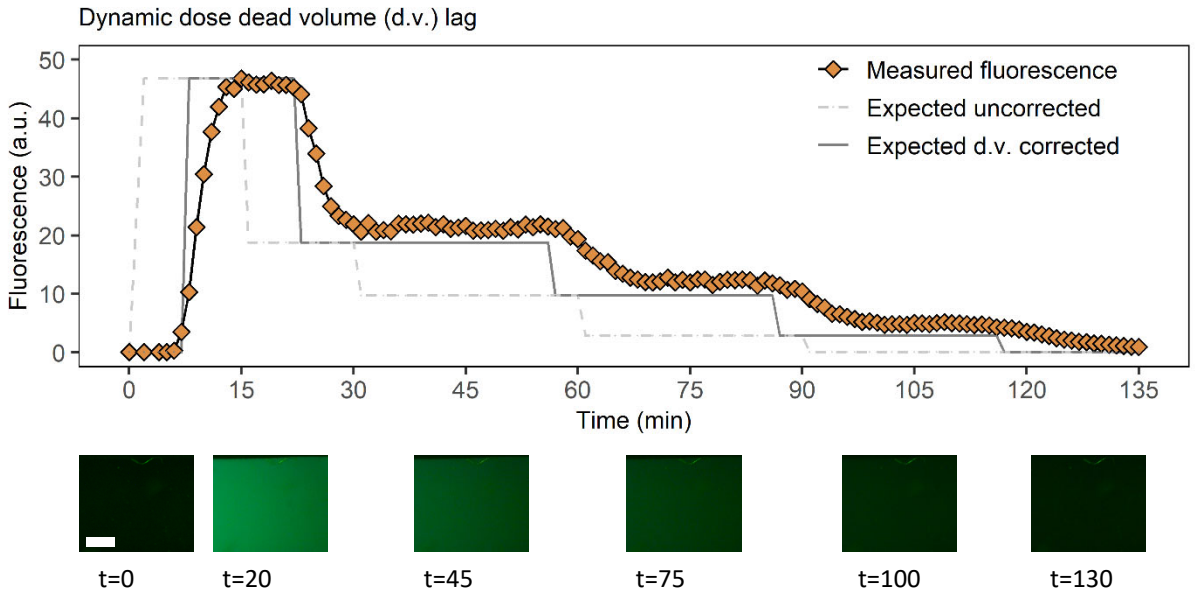
Supplementary Fig. 1 UV-vis spectrophotometry for comparison of oxaliplatin and fluorescein diffusion on chip. (a) UV-vis spectrophotometry of the bottom channel after 16 hours of diffusion from the top channel loaded with 200 µM Fluorescein and 6250 µM Oxaliplatin. A 16 hour time period was chosen as a numerical simulation indicated the average concentration in the bottom channel would be substantial, but not yet in equilibrium (Supplementary figure 11). Bottom channel concentration would be at 48% of the concentration at the start in the top channel, while top channel concentration after 16 hours would still be 80% of the starting concentration, which is due to diffusion time and the difference in volume between the top and bottom channel (Supplementary figure 11). Concentrations in the bottom channel, based on the calibration plots in b-f, are 10 µM fluorescein and 300 µM oxaliplatin, which is a similar ratio as the loaded solutes in the top channel, which implies similar diffusion coefficients, . The 20x lower concentration in the bottom channel compared to the top channel is due to 10x dilution of the bottom channel volume for achieving the minimum volume needed for analysis, and ~2x dilution due to diffusion. (b,c,d) calibration graphs for oxaliplatin at 250 nm (Rsq=0.99), fluorescein at 490nm (Rsq=0.99), and fluorescein at 250nm (Rsq0.99). (e,f) Absorbance profiles for different dosages of oxaliplatin and fluorescein for comparison of the absorbance profile of (a).



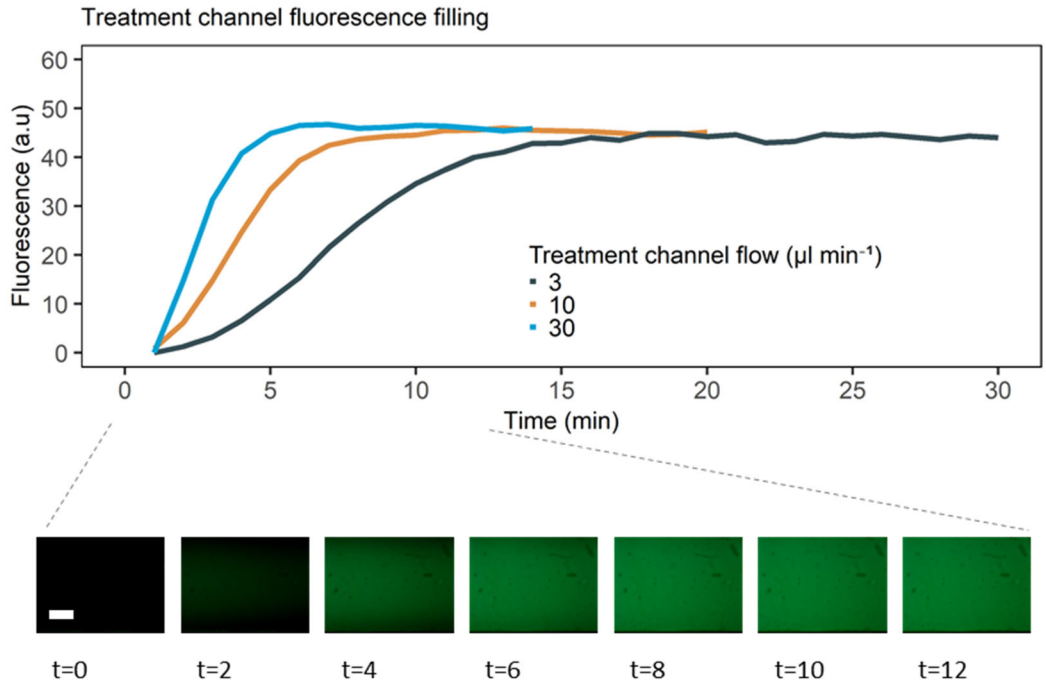
Supplementary Fig. 2 Oxaliplatin absorption on chip. No absorption detectable at 31 µM Oxaliplatin flown through the top channel at 10 µl/min as the absorbance profile is ~equal to 31 µM Oxaliplatin of the calibration sequence that was not flown through a chip.



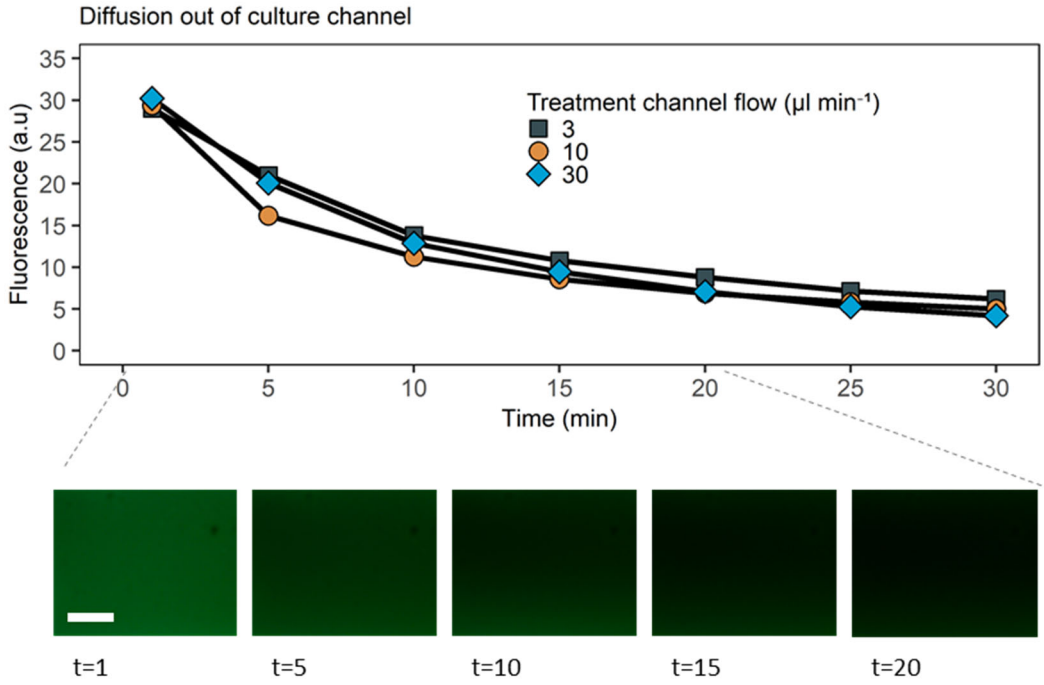
Supplementary Fig. 3 Timing difference in oxaliplatin concentration between administration at the Y splitter and outlet collection due to dead volume lag. (a) Inlet tubing and top channel volume combined is 120 µl, giving rise to an initial (*) dead volume lag of 12 minutes at a flow of 10 µl min^{-1}, which increases to 40 minutes (**) at 3 µl min^{-1}. To match samples with the administered concentration steps, sample timing is adjusted accordingly. (b) Concentration in outlet samples collected matches outlet sample measured well.



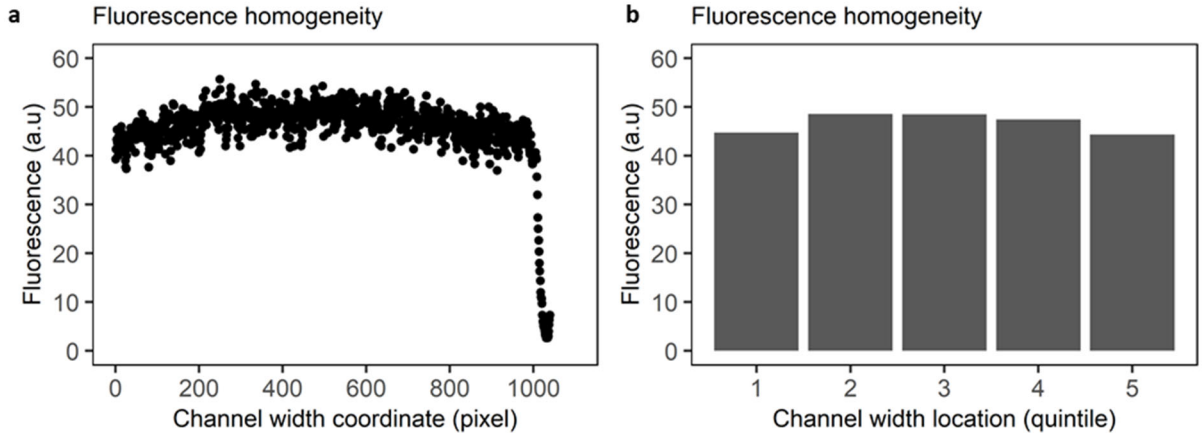
Supplementary Fig. 4 to support main Fig 2b. The expected and measured fluorescence profile of main text figure 2b is shown. Pictures used for fluorescence quantifications are shown below the time axis. On the top of the pictures the top edge of the top channel, on the inlet side can be observed. Scale bar is 0.5 mm.



Supplementary Fig. 5 to support main Fig. 2c. The increase in fluorescence at 10 µl min^{-1} is illustrated by the pictures below the time axis. On the bottom of the picture the bottom edge of the top channel, on the inlet side can be observed. Virtually the entire 2mm width of the channel can be observed. Fluorescence across the channel is homogeneous, which is further quantified in Supplementary Fig. 7. Scale bar is 0.5 mm.



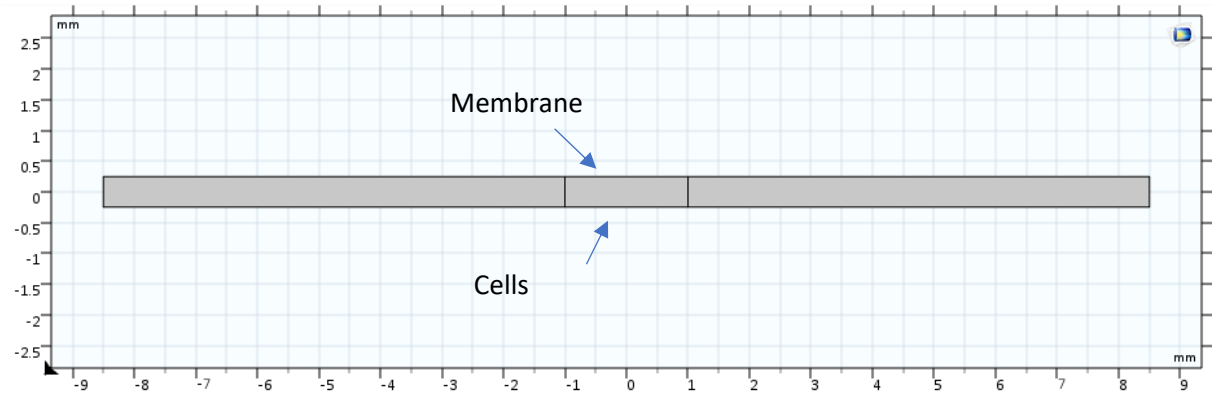
Supplementary Fig. 6 to support main Fig 2d. The decrease in fluorescence due to diffusion across the membrane of main text figure 2d is repeated. The bottom channel is filled with fluorescein. Subsequently the bottom channel is closed and PBS is continuously flown through the top (treatment) channel and fluorescence decline at the membrane is monitored. Scale bar is 0.5 mm.



Supplementary Fig. 7 Fluorescence homogeneity across the width of the top channel after flowing fluorescein 10 µM for 10 minutes at 10 µl min^{-1} through the top channel. (a) The drop beyond 1000 pixels in figure a indicates crossing the boundary of the channel. (b) excluding the pixels beyond the boundary, the difference in fluorescence between the middle quintile and outermost quintiles is ~10%.

Supplementary description of simulations

For the simulation of the diffusion of oxaliplatin, a 2D, time dependent model was made in which the diffusion-equation was solved numerically. The bottom channel was simulated with the following geometry:



Supplementary Fig. 8 Bottom channel geometry used in simulations.

The equation solved was:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D\nabla c) = 0 \tag{1}$$

With c, the oxyplatin concentration, t, the time and D, the diffusion coefficient.
A no-flux condition is set at the walls and at the cells;

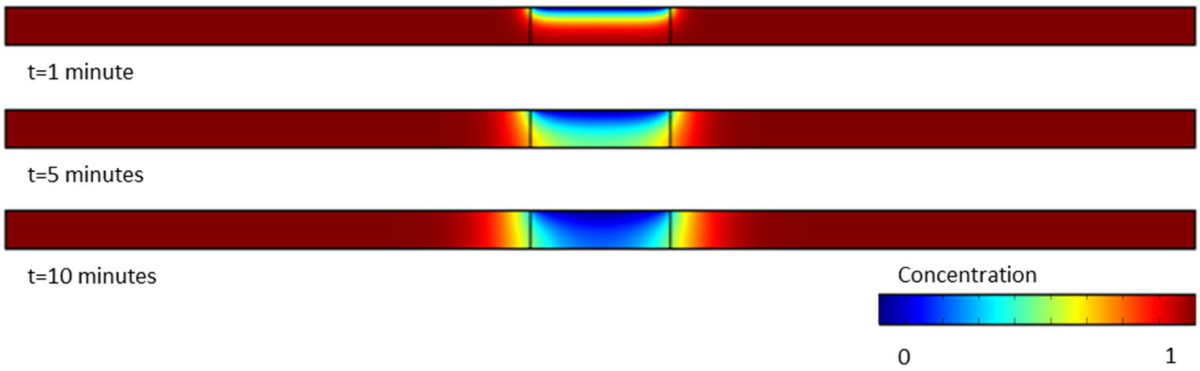
$$J_c \cdot \boldsymbol{n} = 0 \tag{2}$$

Although the cells consume part of the medicine, this is considered to be neglectable compared to the available oxaliplatin within the channel and therefore not taken into account in this simulation.
And a flux condition in the middle to simulate the flux through the membrane:

$$J_c \cdot \boldsymbol{n} = D * k * \frac{C_0 - c}{w} \tag{3}$$

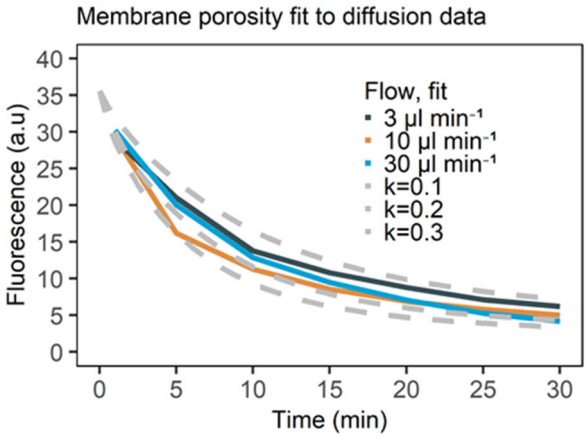
With ,C_0 the concentration in the top channel, k, the permeability constant of the membrane and w the thickness of the membrane. The flow velocity in the top channel was considered to be sufficiently high to neglect the local depletion in the top channel just above the membrane. A triangular mesh was used with the following parameters; Maximum element size: 0.0255 mm, minimum element size: 0.001 mm, maximum element growth rate: 1.15, curvature factor 0.3, resolution of narrow regions: 1.

To determine the permeability factor, the average concentration in the square area between the membrane and the cells was taken and fitted to experimental fluorescence data, with k as the fitting parameter. Where C_0 was set at zero and the concentration in the bottom channel over time was evaluated. This gave a permeability factor of k = 0.2 (Supplementary Fig 3). A graphical depiction of the simulated concentration wash out with a membrane porosity of 0.2 is shown in Supplementary Fig 3.

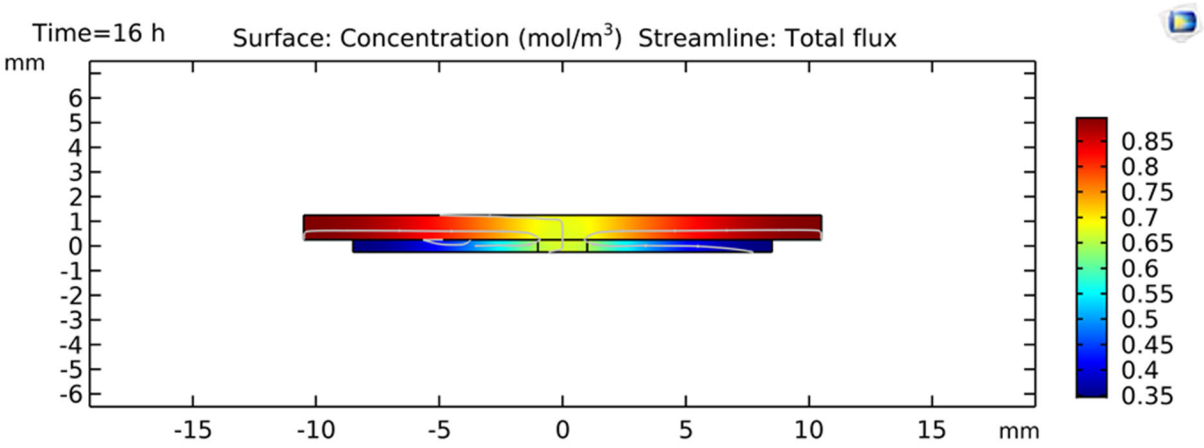


Supplementary Fig. 9 Comsol simulated wash out of fluorescein out of bottom channel through a membrane of porosity 0.2. Starting concentration is set at 1. Diffusion coefficient for fluorescein is 4.2×10^{-6} cm^2 s^{-1}.

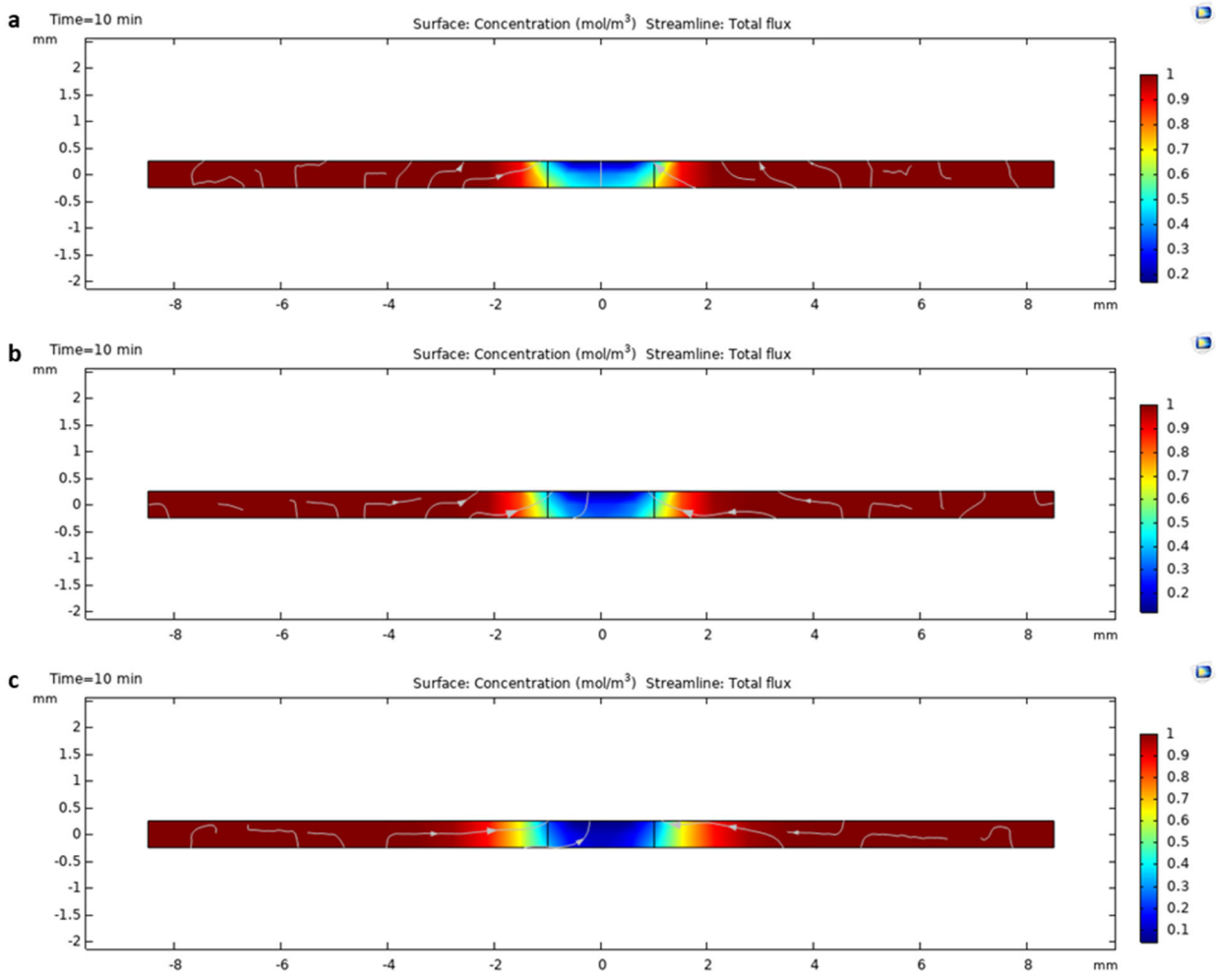
For the simulation of the administering of variable Oxaliplatin doses, a variable $C_0(t)$ was given, and the concentration was determined by using the mean values, at the bottom of the channel over the entire length of the membrane over where the cells are located as a function of time.



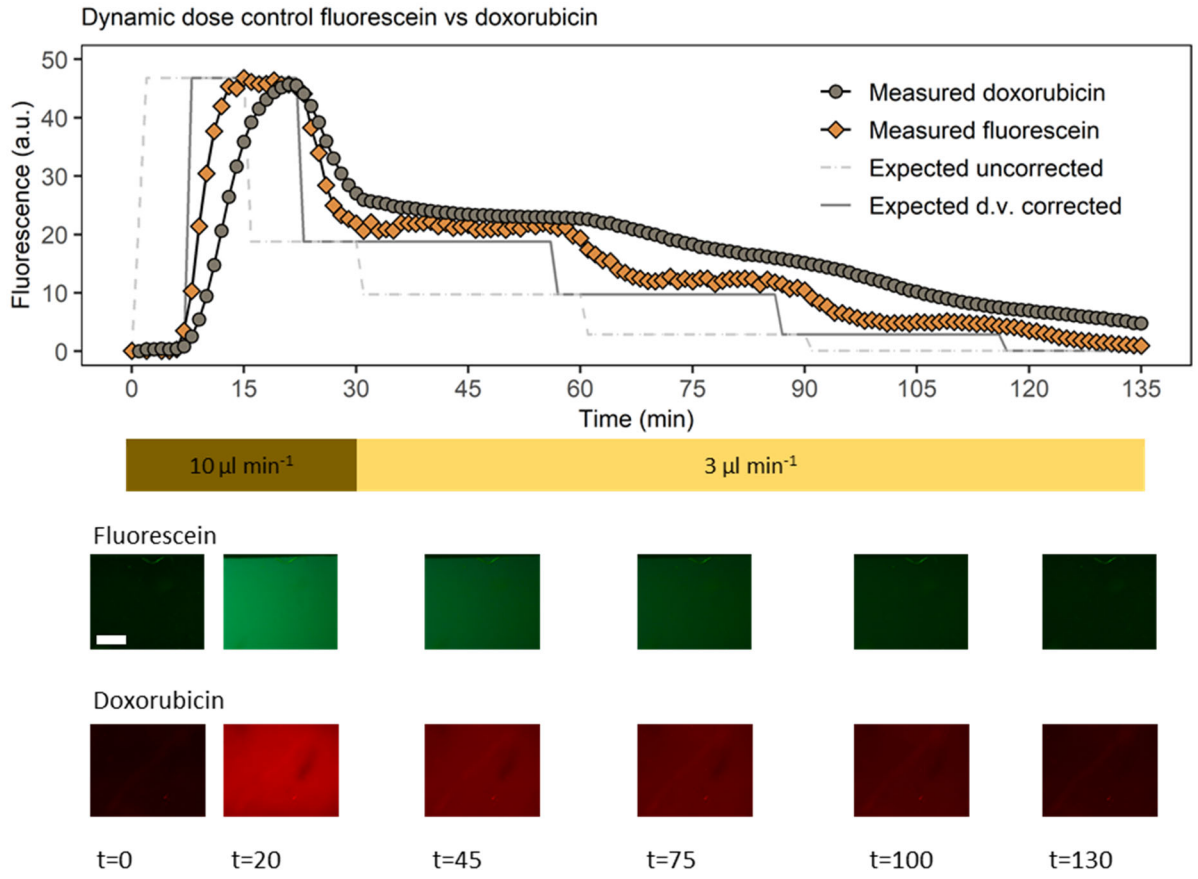
Supplementary Fig. 10 Numerical simulation of diffusion out of bottom channel for different membrane porosities (k) plotted next to diffusion for different flow rates. 0.2 porosity has a good fit with the diffusion experiments.



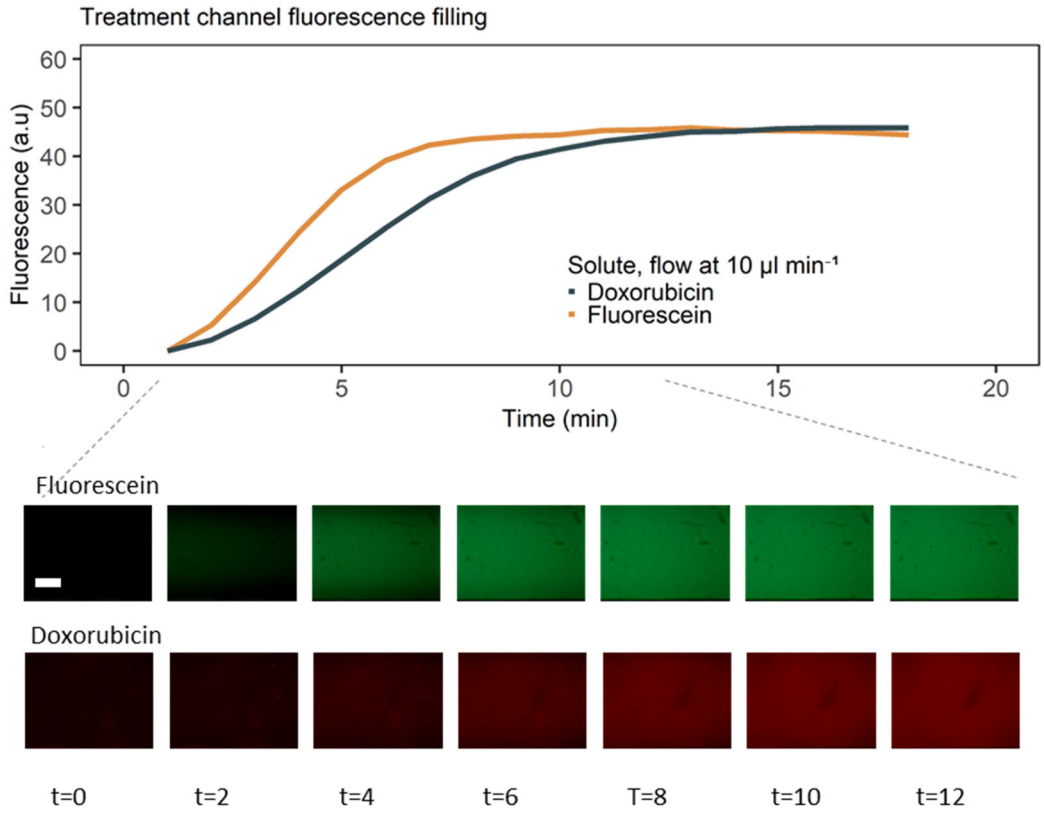
Supplementary Fig. 11 Numerical simulation of loading the top channel with a solute with concentration 1, and diffusion across the membrane into the bottom channel over 16 hours for a diffusion coefficient of 4.2 10^{-6} cm^2 s^{-1}. Average concentration in the bottom channel is 0.48, and 0.8 in the top channel. For the diffusion coefficient range of small molecules of 2.9- 9.0 10^{-6} cm^2 s^{-1} concentration in the bottom is 0.41 to 0.62.



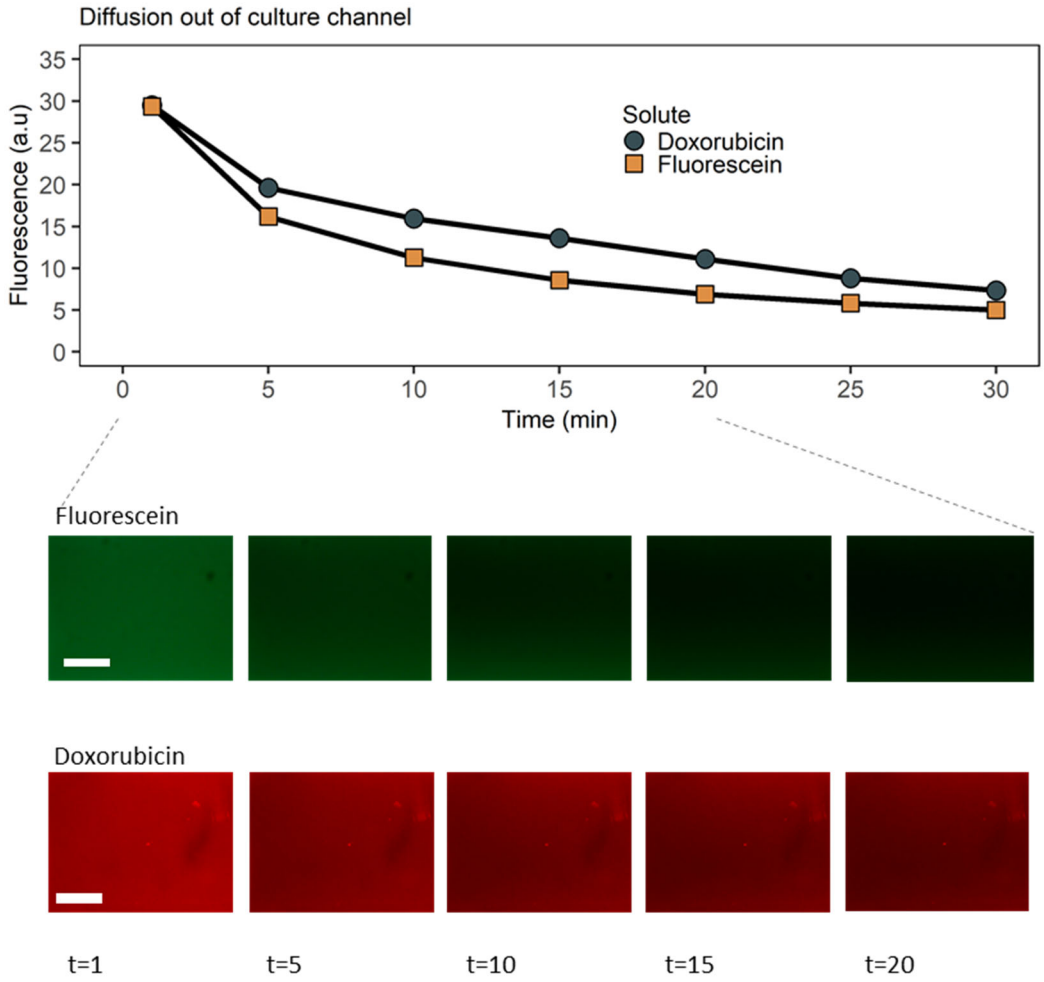
Supplementary Fig 12 Concentration decrease in the bottom channel for diffusion coefficients of (a) 2.9, (b) 4.2, (c) 9.0 10^{-6} cm^2 s^{-1}. Time until 50% of the concentration washes out of the cell culture area is 3, 6, 8 minutes respectively.



Supplementary Fig. 13 Doxorubicin dynamic dosing is compared to fluorescein by adding doxorubicin data and images to Supplementary Fig. 4. Doxorubicin follows fluorescein and expected fluorescence reasonably well but with an increasing delay at lower concentrations and flow rates, which might be due to some absorption. Doxorubicin (10 µM) fluorescence was imaged with a Texas Red filter on a ThermoFisher EVOS microscope at 5×. Doxorubicin fluorescence was quantified by subtracting background fluorescence and subsequently scaling doxorubicin maximum to fluorescence maximum.

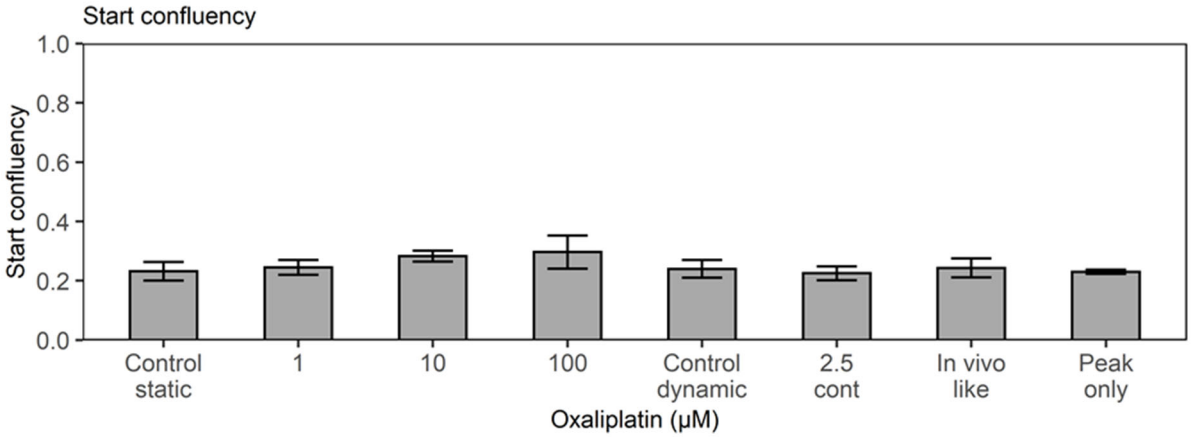


Supplementary Fig. 14 Time until equilibrium fluorescence is achieved for doxorubicin is compared to fluorescein by adding doxorubicin data and images to Supplementary Fig. 5. Equilibrium fluorescence occurs at ~12 minutes for doxorubicin, several minutes later than for fluorescein. Doxorubicin fluorescence was quantified by subtracting background fluorescence and subsequently scaling doxorubicin maximum to fluorescence maximum.

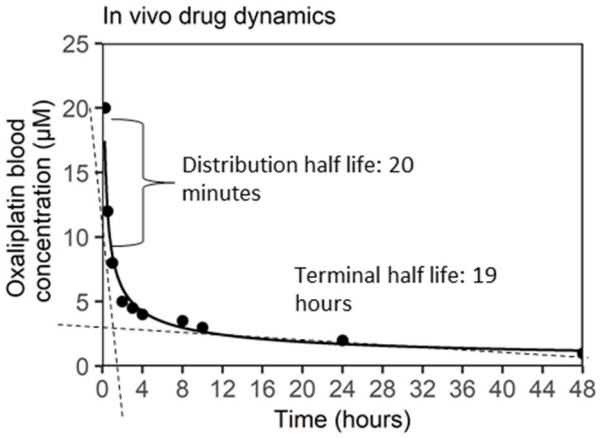


Supplementary Fig 15 Diffusion of doxorubicin out of the culture channel across the membrane is compared to fluorescein by adding doxorubicin data and images to Supplementary Fig. 6. First the bottom channel is filled with doxorubicin. Subsequently the bottom channel is closed, and PBS is flown through the top channel at 10 µl min^{-1}. Decrease of fluorescence of doxorubicin across the membrane is about 5 minutes slower than for fluorescein at the membrane. Doxorubicin fluorescence was quantified by subtracting background fluorescence and subsequently scaling doxorubicin maximum to fluorescence maximum.

These figures show doxorubicin follows the dynamic dosages, channel filling and diffusion found with fluorescein reasonably well, indicating dynamic control for approximation of in vivo like drug concentrations is feasible. One potential point of concern is the increasing lag in concentration decline in Supplementary Fig 13 at lower flow rates and concentrations. Slight, but statistically insignificant sorption of doxorubicin has been reported[2]. To exclude sorption and desorption to have an effect on the concentration in the chip 60 µM doxorubicin at a flow of 10 µl/min for an hour was administered to the PDMS top channel bound to a layer of PDMS. Subsequently PBS at 10 µl/min flow for 30 minutes was applied. Doxorubicin collected from the outlet, and analysed with UVvis at 480 nm, after the first hour was 6% lower at 52 µM, than 55 µM expected. A dilution of 60 µM to 55 µM was expected as the top channel has a volume of 42 µl and was prefilled with PBS. Doxorubicin in the PBS flush was 7.8 µM, slightly lower than 8.4 µM which was expected based on 42 µl of 60 µM doxorubicin in the channel and a subsequent 300 µl PBS flush. Hence we suspect there is slight sorption and negligible de-sorption, which neither have a significant influence on the doxorubicin concentration in the channel. Therefore we conclude dynamic dose control for in vivo approximation on chip would be feasible for doxorubicin.



Supplementary Fig 16 Starting confluency is comparable between 0.2 and 0.3 for different conditions. ANOVA p=0.80.

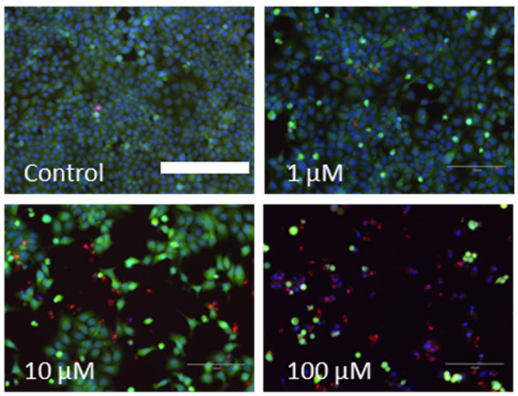


Supplementary Fig. 17 Oxaliplatin concentration in blood over time. Blood concentration values found in mice. Based on graphical depiction by *Li et al. 2011* of measurements at 15, 30 minutes, 1, 2, 3, 4, 8, 10, 24, 48 hours.

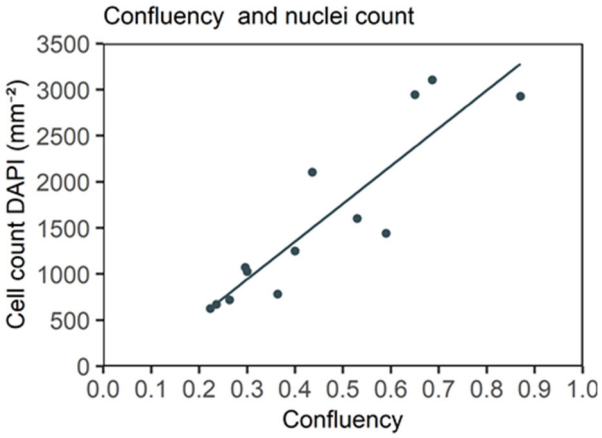
a

	Oxaliplatin concentration (µM)			
	0	**1**	**10**	**100**
Growth vs control				
On chip	1.00	100%	40%	13%
96 well	1.00	48%	28%	5%
External 96 well - NCI60	1.00	36%	31%	14%
Growth (start=1)				
On chip	2.5	2.5	1.6	1.2
96 well	5.0	2.9	2.1	1.2
External 96 well - NCI60*	4.9	2.4	2.2	1.5

b



Supplementary Fig. 18 Comparison of on chip growth versus control for different Oxaliplatin dosages versus 96 well plate and NCI60 data. (a) Growth versus control quantified with confluency for on chip and 96 well, and SRB/protein mass for NCI60. The NCI formula for growth versus control is ((end cell mass treated-cell mass seeded)/(end cell mass control-cell mass seeded). 96 well in triplicate. *no explicit growth data available from NCI60 screen data, however numbers are deduced from doubling time and growth versus control which are available. (B) Staining of internal 96 well plate at 20x magnification; cell nuclei (Nucblue/Hoechst 33342, blue), viability (Calcein AM, green), late stage apoptosis (Ethidium homodimer, red). Scale bar 150 µm.



Supplementary Fig. 19 Relation between cell nuclei per mm^2 and confluence. Typical control growth of 20% confluence to 50% confluence (2.5x) would be ~3.5x growth based on DAPI nucleus count, explaining part of the difference between control growth found on chip and in well plates (R^2=0.81).

Supplementary description of cell seeding movie

About 3000, 5 µl of 0.5 mln ml^{-1} cells, HCT116 cells were spiked with a pipette into the bottom channel of the device. After letting the cells sink to the bottom, flow of 20 µl min^{-1} was applied. The time lapse has 1 frame per 10 seconds.