Supporting Information:

Moving perfusion culture and live-cell imaging from lab to disc: Proof of concept toxicity assay with AI-based image analysis

Lina Gruzinskyte[1,2]†, Laura Serioli[2,3]†*, Giulia Zappalá[2,4], En Te Hwu[2], Trygvi Zachariassen Laksafoss[2,5], Peter Lunding Jensen[6], Danilo Demarchi[4], Anette Müllertz[1,2], Anja Boisen[2,3], Kinga Zór[2,3]

[1]*Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*

[2]*The Danish National Research Foundation and Villum Foundation's Center for Intelligent Drug Delivery and Sensing Using Microcontainers and Nanomechanics (IDUN), Department of Health Technology, Technical University of Denmark, Denmark*

[3]*BioInnovation Institute Foundation, Copenhagen N 2800, Denmark*

[4]*Department of Electronics and Telecommunications, Politecnico di Torino, Italy*

[5]*Present address: H. Lundbeck A/S, Valby, Denmark*

[6]*Cellari (Almond IVS), Frederiksberg, Denmark*

† The authors contributed equally to this work

*e-mail: lauser@dtu.dk, phone: +45 45 25 63 37

Figure S1 - Photo of the mammalian Cell culture on Disc (CoD) integrated with the optical detection unit (MoD) from the Cell culture and Microscope on Disc (CMoD)

Figure S2 - Classical microscope vs. Microscope on Disc (MoD)

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Video S1 - Real-time video of HeLa cells growth recorded with the mammalian Cell culture and Microscope on Disc (CMoD)

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Photo of the mammalian Cell culture on Disc (CoD) integrated with the optical detection unit (MoD) from the Cell culture and Microscope on Disc (CMoD) (Figure S1)

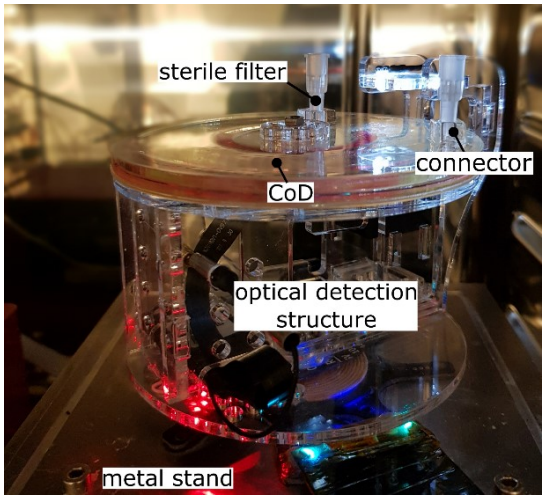

Figure S1. CMoD, CoD integrated with the MoD placed on a spin stand (composed of spindle motor and metal stand) in the incubator.

Classical microscope vs. Microscope on Disc (MoD) (Figure S2)

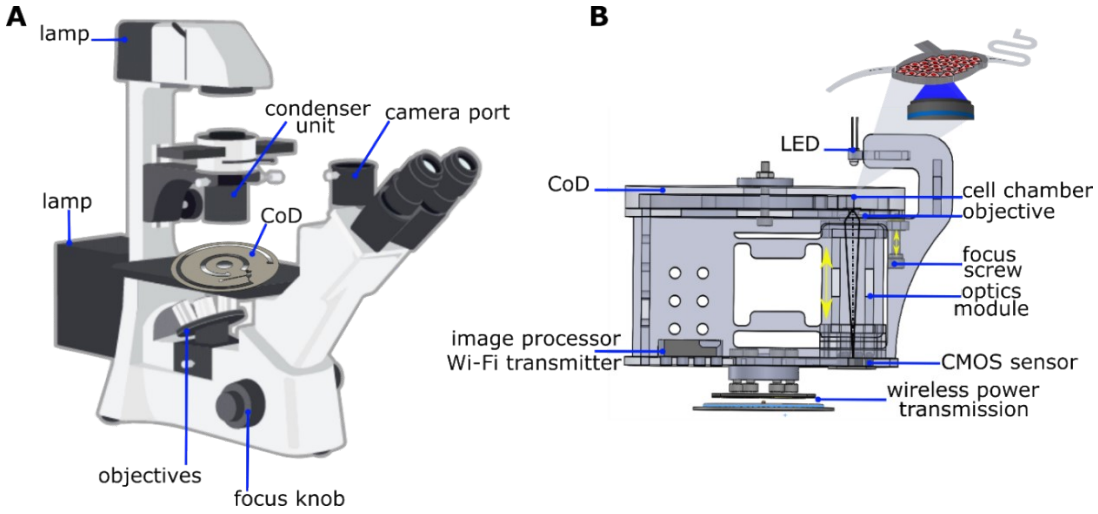

Figure S2. (**A**) A schematic representation of the CoD placed on an inverted microscope and (**B**) CoD integrated with the MoD, showing the location of the miniaturized camera in relation to the culture chamber on the disc.

Differences between the mammalian Cell culture on Disc (CoD) and the Bacterial Culture on Disc (BCoD) (Figure S3)

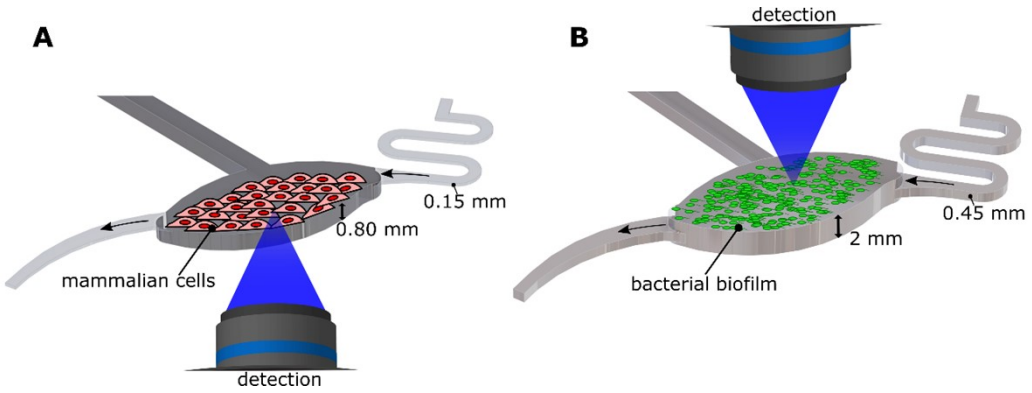

Figure S3. (**A**) CoD vs (**B**) BCoD.

Dependency between the flow rate and rotational frequency, and COMSOL simulation of flow velocity in the cell culture chamber (Figure S4)

The centrifugal platform was developed to accommodate a perfusion cell culture at low flow rates (<2 µL/min) to limit shear stress on the cells. The flow rate calibration curve (Figure S4A) was constructed after the CoD was assembled and prior to the cell seeding, as described in detail by L. Serioli et al.[1]. Briefly, the inlet reservoir was filled with cell culture medium and filters were put in place, sealing the venting holes. Thereafter, the disc was placed on an optical spin stand and a frequency of 3 Hz was used to prime the system and to create the front of the liquid in the waste reservoir. Before calibration, the rotational frequency was set to 0.35 Hz for 2 h in order to dissipate the flow acceleration caused by high frequency at the priming step. Next, the rotational frequencies were gradually increased from 0.70 Hz up to 1 Hz and the volume of the liquid moved from the inlet reservoir to the waste was measured at defined time points. The optical images of the liquid for each frequency were captured at three different times, using the camera software PCO Camware (PCO Camware 64, PCO AG) as previously described 60. The volume of the moved liquid was calculated using MATLAB (MATLAB 9.3, Natick, Massachusetts) code as previously described by T. Rajendran et al.[2]. The flow rate was later calculated in Excel using the formula $\Delta Q = \Delta V/\Delta t$, where ΔQ is the flow rate, ΔV is the volume of the moved liquid and Δt is the time between every measurement. The variation in flow rate between platforms and the stability of the flow rate over time was also evaluated.

The shear stress calculation was carried out with COMSOL (COMSOL Multiphysics 5.3a, Stockholm, Sweden) as previously described[1]. Computational fluid dynamics (CFD) simulations were used to calculate the flow velocity through the culture chamber in three spatial dimensions and the shear stress was evaluated at the bottom of the culture chamber, where the cells adhere and grow (Figure S4B).

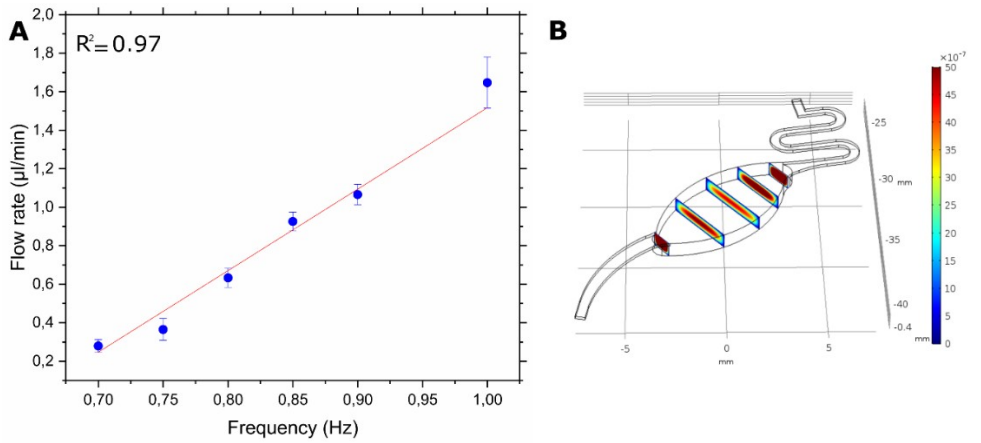

Figure S4. (**A**) Linear dependency between the flow rate and rotational frequency in the CoD. (**B**) Flow velocity profile in the cell chamber at a flow rate of 0.6 µL/min. Error bars represent standard deviation, n=3.

Microscope on Disc (MoD) first generation vs. second generation (Figure S5)

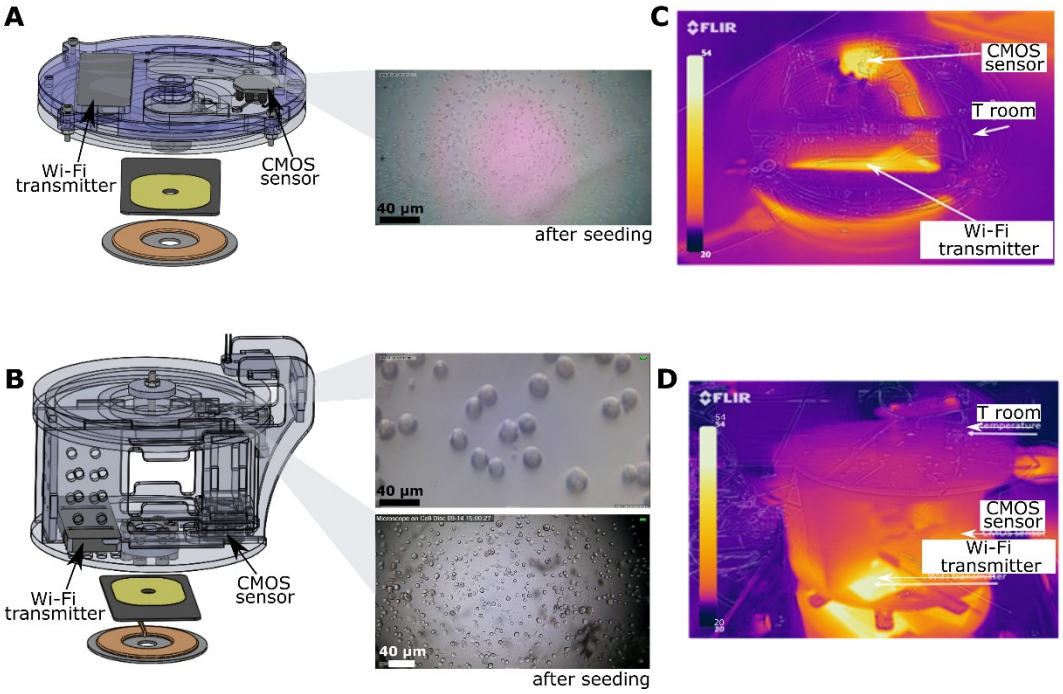

Figure S5. A comparison between (**A**, **C**) the first and (**B**, **D**) second generation optical detection unit developed for the CMoD. Exploded view of (**A**) the first and (**B**) second generation showing the difference in height of the platform, insets are representative optical images collected after seeding of cells. In the second generation unit it is possible to see the cells with the two different magnifications. Thermal imaging analysis of (**C**) the first and (**D**) second generation units, during operation.

Bright-field images of HeLa cells taken with a low magnification Microscope on Disc (MoD) showing attached elongated cells (blue arrows) and round dead cells (red arrows) after 1 h and 24 h of 10 µM doxorubicin (DOX) treatment (Figure S6)

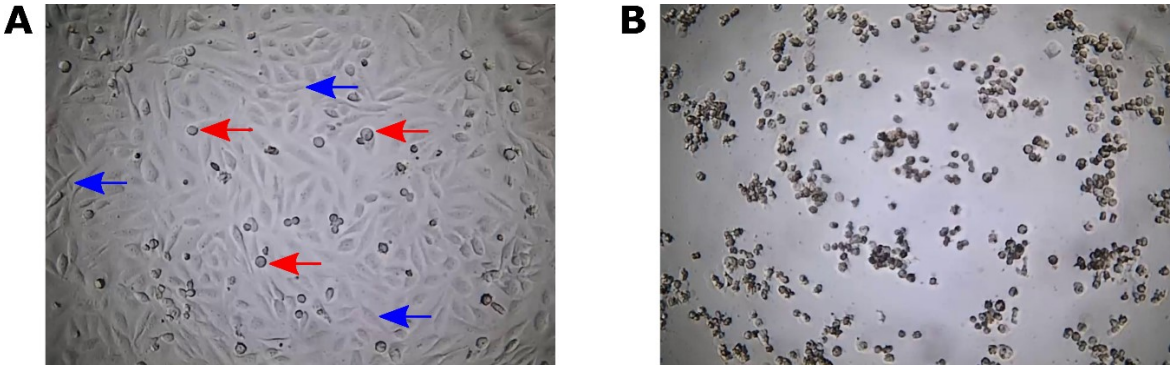

Figure S6. (**A**) Real-time bright-field images of HeLa cells on the CoD taken with the MoD showing attached (blue arrows) and dead (red arrows) cells after 1 h of DOX (10 µM) treatment and (**B**) after 24 h (all cells are dead).

Bright-field images of HeLa cells taken with a high and low magnification Microscope on Disc (MoD) when using different doxorubicin (DOX) concentrations (Figure S7)

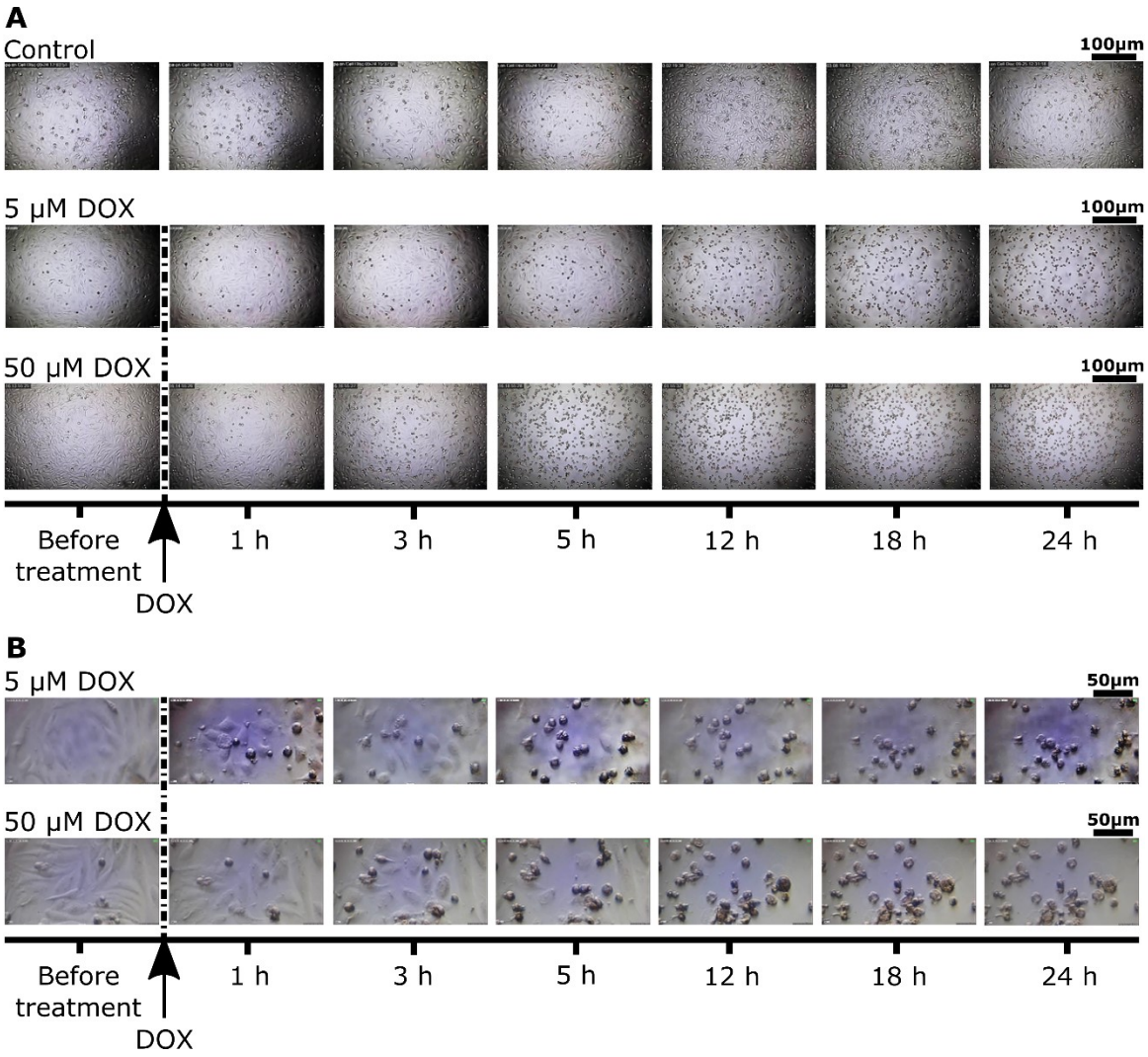

Figure S7. Representative real-time bright-field images of HeLa cells before treatment and at 1 h, 3 h, 5 h, 12 h, 18 h and 24 h after 0 µM, 5 µM and 50 µM DOX treatment on the CoD at 0.6 µL/min flow rate taken with (**A**) low (~10 X) and (**B**) high (~40 X) magnification.

Real-time video of HeLa cells growth recorded with the mammalian Cell culture and Microscope on Disc (CMoD) (Video S1)

Step 1: HeLa cells just after the seeding (seeding concentration 0.75 x 10^5 cells/cm^2).

Step 2: HeLa cells attachment to the cell culture chamber surface.

Step 3: HeLa cells growth until forming a confluent monolayer in the cell culture chamber.

Real-time video of the effect of the drug treatment (10 µM doxorubicin (DOX)) on HeLa cells recorded with the mammalian Cell culture and Microscope on Disc (CMoD) (Video S2)

Step 1: 10 µM DOX is perfused through the cell chamber.

Step 2: HeLa cells start to detach and round up due to the DOX treatment.

Step 3: Dead HeLa cells detached form the surface.

References

1 L. Serioli, T. Z. Laksafoss, J. A. J. Haagensen, C. Sternberg, M. P. Soerensen, S. Molin, K. Zór and A. Boisen, *Analytical Chemistry*, 2020, **92**, 13871–13879.

2 S. T. Rajendran, E. Scarano, M. H. Bergkamp, A. M. Capria, C. H. Cheng, K. Sanger, G. Ferrari, L. H. Nielsen, E. Te Hwu, K. Zór and A. Boisen, *Analytical Chemistry*, 2019, **91**, 11620–11628.