Supplementary information

High-throughput “read-on-ski” automated imaging and label-free detection system for toxicity screening of compounds using personalised human kidney organoids

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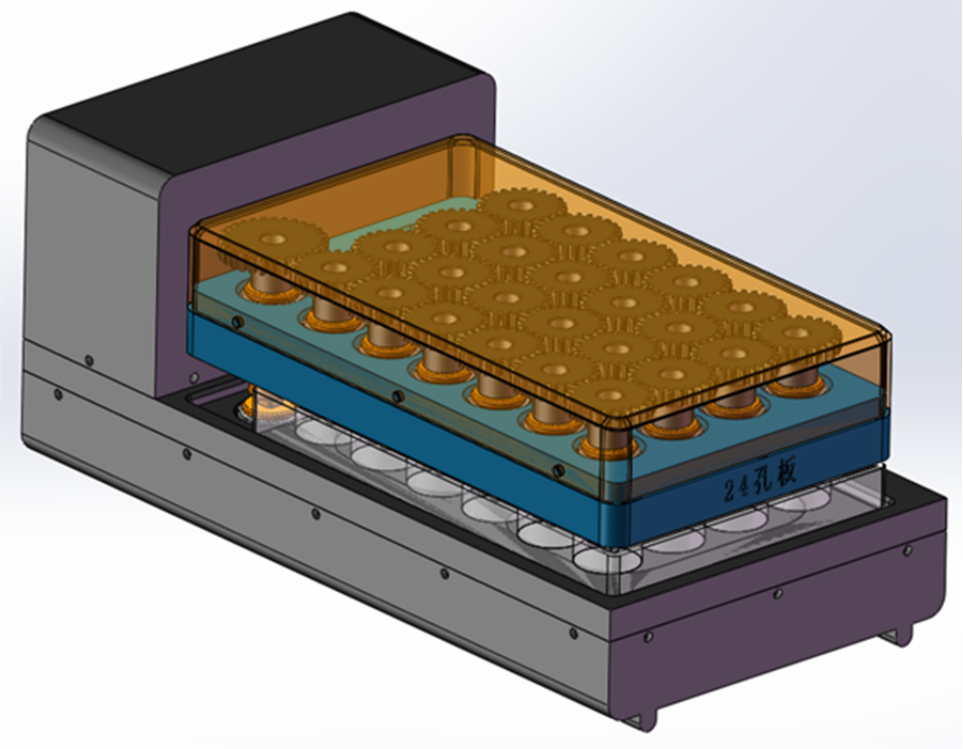


Fig. S1 Multi-well spin device implemented for generating kidney organoids in a batch format. The spin device can operate with 24/48-well plates with the master spin motor and interlinked with multiple stirring impellers.

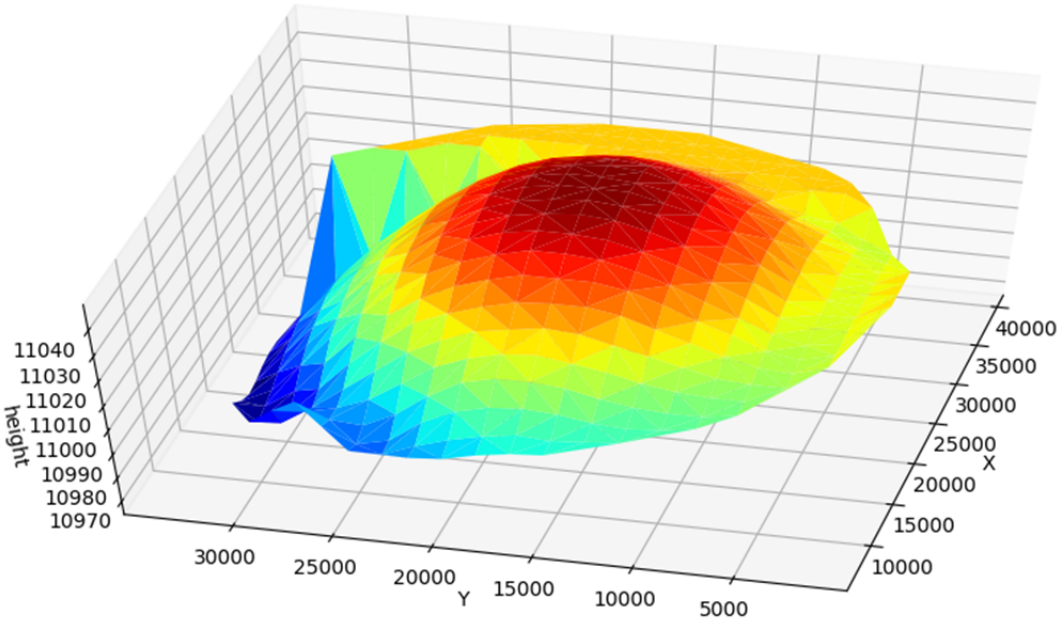


Fig. S2 Example of laser-based measurement for multi-well bottom surface (unit: μm). The scanning map for the height heat map shows that the uneven shape of the bottom has different focal planes for different areas. Based on this heat map, the pre-arranged *Z*-axis lens movement will implement non-stop movement in the autofocus system called "read-on-ski".

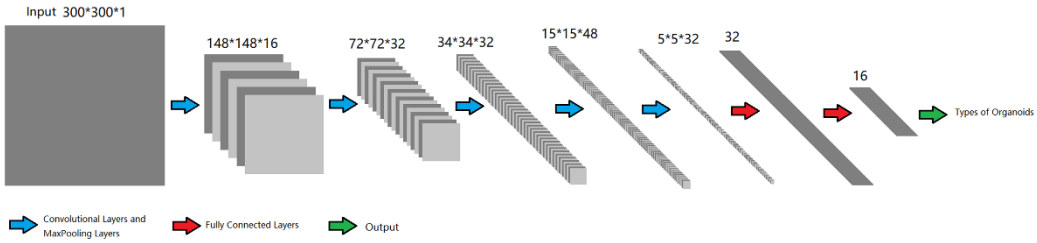
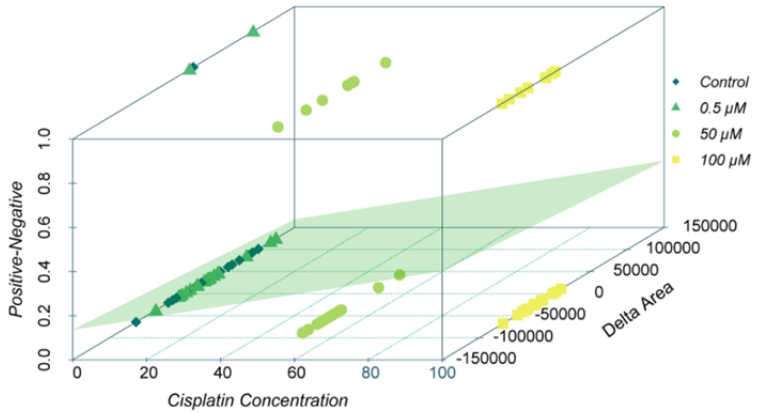


Fig. S3 Neural network architecture for the classification of organoids.



Logistics Analysis Summary

	Estimate	Std. Error	Z value	Pr(>|z|)	Significant level
(Intercept)	-2.31E+00	4.79e-01	-4.827	1.390e-06	***
Cisplatin Concentration	1.66E-02	7.54e-03	2.200	2.780e-02	*
Delta Area	-4.32E-06	7.13e-06	-0.606	5.442e-01	NA

Positive-Negtive ~ Cisplatin Concentration + Delta Area

Fig. S4 Multiple logistic regression analysis. Prediction of treatment outcomes based on cisplatin concentrations and delta area using data from 96-well plate-based experiments. * *P*<0.05, * *P*<0.001.**

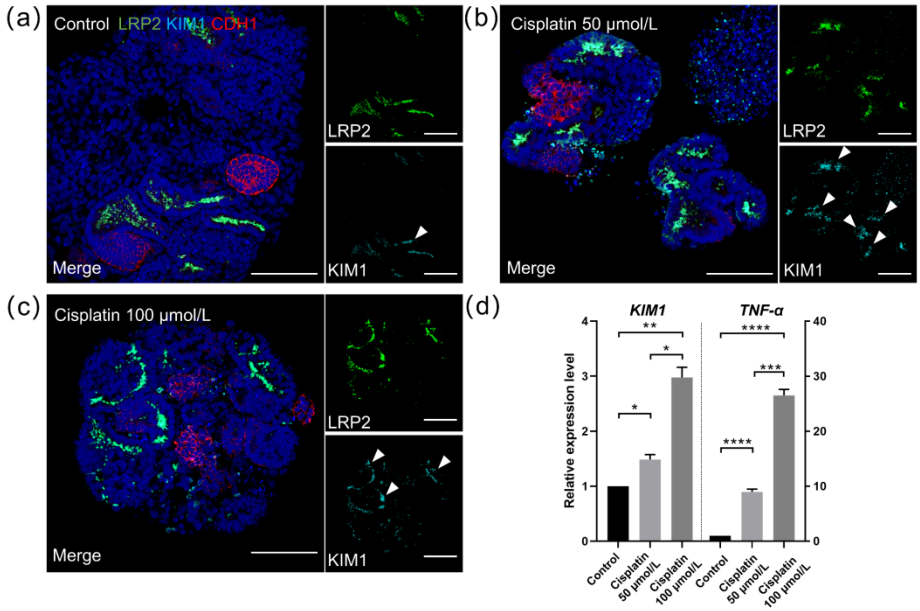
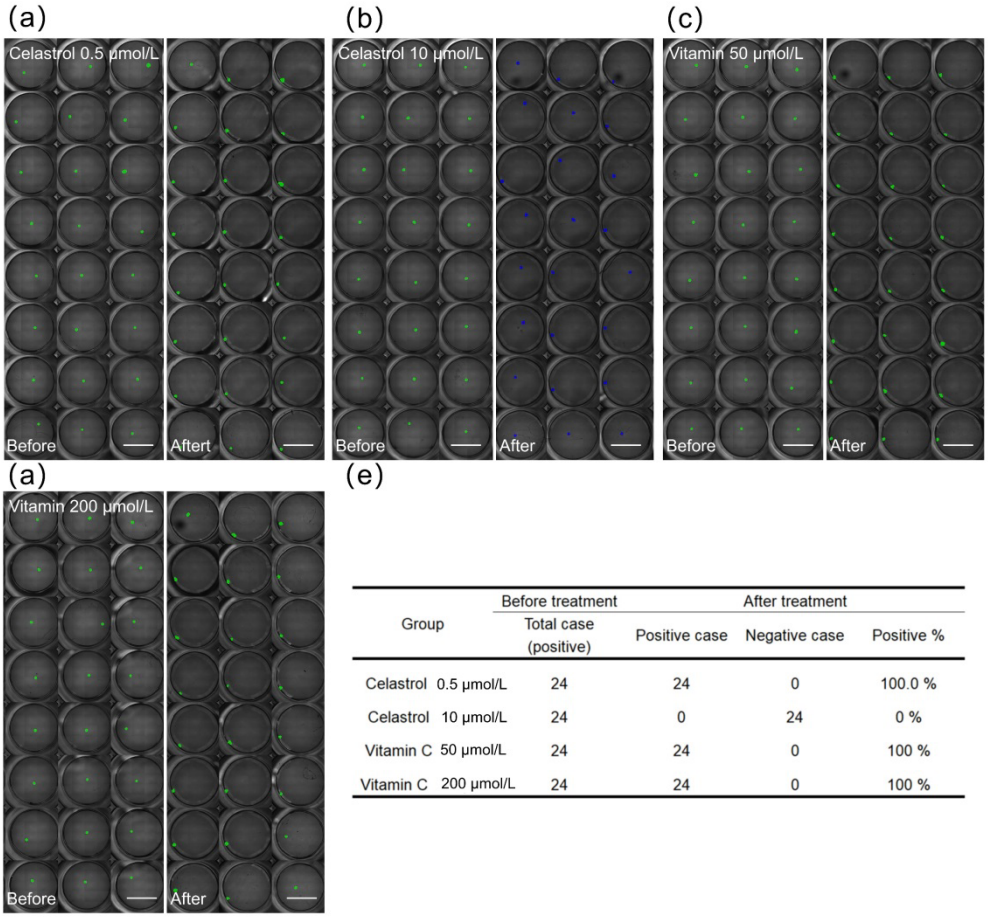


Fig. S5 Immunocytochemical and RT-qPCR analysis. (a–c) Immunofluorescence staining of organoids after cisplatin treatment using proximal tubule marker LRP2 (green), tubular injury marker KIM1 (cyan), and distal tubule marker CDH1 (red), then co-stained with DAPI (blue) in the merged channels. The white arrowheads denote the KIM1+ cells. (d) RT-qPCR analysis for the expression of *KIM1* and proinflammatory cytokine gene *TNF-α* in kidney organoids treated with cisplatin at indicated doses. The left and right y-axis represent the relative expression level of *KIM1* and *TNF-α*, respectively. Data are expressed as mean±SEM. (n=3). * *P*<0.05, ** *P*<0.01, * *P*<0.001, **** *P*<0.0001. LRP2: lipoprotein receptor-related protein-2; KIM1: kidney injury molecule-1; CDH1: cadherin-1; DAPI: 4',6-diamidino-2-phenylindole. Scale bars, 100 μm (a–c).**



Group	Before treatment	After treatment		
	Total case (positive)	Positive case	Negative case	Positive %
Celastrol 0.5 μmol/L	24	24	0	100.0 %
Celastrol 10 μmol/L	24	0	24	0 %
Vitamin C 50 μmol/L	24	24	0	100 %
Vitamin C 200 μmol/L	24	24	0	100 %

Fig. S6 "Read-on-ski" system performance of organoid-based drug screening in 96-well plates. (a–d) The detection of organoids before and after treatment with celastrol and vitamin C at indicated doses. Positive and negative case kidney organoids are marked as green and blue, respectively. (e) Detection of kidney organoids before and after treatment with different compounds. Scale bars, 5 mm (a–d).

Table S1 Number of kidney organoids before and after treatment with cisplatin at indicated doses in each category after detection by the "read-on-ski" system

Group	Positive cases		Negative cases		Adhesion cases		Total Cases	
	Before	After	Before	After	Before	After	Before	After
Control	218	91	28	25	41	86	287	202
Cisplatin 50 μmol/L	181	101	15	58	15	15	211	174
Cisplatin 100 μmol/L	215	8	19	123	15	35	249	166