





The chemosensitivities of 4 tumor organoids used for validation of the high-throughput organoid imaging assay. Organoid size changes after being treated by 10μ M 5-FU or 10μ M CPT11 in 4 organoid lines. 5-FU-s, 5-FU-r: Organoids defined as chemo sensitive or resistant to 5-Fluorouracil; CPT11-s, CPT11-r: Organoids defined as chemo sensitive or resistant to CPT11. Organoid size data shown are mean \pm SEM, n=3.



Single-layer

Z-stacked

В

Comparison of the single-layer image and Z-stacked image

Single layer		Z-stacked		
Count	Total Area (pixel)	Count	Total Area (pixel)	
8	8787	40	22521	

Additional file 1: Fig. S2

The comparison of the single-layer and Z-stacked image.

A. Representative single-layer image and Z-stacked image under the same field; **B.** Comparison of the single-layer and Z-stacked image (organoids that were blurred in the image were not measured).



Additional file 1: Fig. S3

The comparison of three staining methods under different concentrations. A, Calcein-AM, Hoechst/PI, and CFDA SE of different concentrations were used to stain mice small intestinal organoids. For Calcein-AM dye, "1X" concentration means 2μ M, and for Hoechst/PI and CFDA SE staining, "1X" concentration means the recommended working concentration according to the manufacturers.



Additional file 1: Fig. S4

Comparing CTG method and fluorescence labeling method to test the chemosensitivity of tumor organoids. Cell viability changes and organoid maximum cross-section changes of organoids treated by 5-FU or CPT11. Untreated organoids were used as the starting point for both methods. Data are presented as the mean \pm SD, n=3.