Supporting Information

D-CryptO: Deep learning-based analysis of colon organoid morphology from brightfield images

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**Dose-dependent changes in organoid morphology**

To further validate D-CryptO, we used it to assess the dose-dependent response of doxorubicin on opacity and budding following treatment at various concentrations. Doxorubicin is a chemotherapeutic that inhibits DNA and RNA synthesis and induces apoptosis.\(^1\) We applied doxorubicin at concentrations of 50 µM, 5 µM, 0.5 µM, 0.05 µM, and 0.005 µM (Figure S1a). For opacity, the concentration at which 50% of organoids became opaque was 3.6 µM (Figure S1b). For budding, the concentration at which 50% of the organoids still had budding structures was 39.8 µM (Figure S1c). It is important to note that budding did not increase with higher dosages of doxorubicin. Instead, the percentage of non-viable organoids increased which was classified under the budding category. For diameter, the concentration at which 50% of the organoids had a reduction in diameter was 0.5 µM (Figure S1d). Each parameter was impacted at different concentrations, indicating the importance of monitoring these features to assess drug toxicity.
Supplementary Figure 1. Dose-dependent changes in organoid morphology. 

a, Brightfield images of organoids taken on Day 0 and Day 10 of drug treatment with doxorubicin at 5 different concentrations. Scale bar, 500 μm 

b, The percentage of opaque organoids following 10 days of treatment with increasing concentrations of doxorubicin. 

c, The percentage of budding organoids following 10 days of treatment with increasing concentrations of doxorubicin. 

d, The change in diameter following 10 days of treatment with increasing concentrations of doxorubicin.
Supplementary Figure 2. D-CryptO performance on overlapping organoids. a, Examples of overlapping organoids. b, Correct and incorrect classifications by D-CryptO. c, D-CryptO accuracy when classifying overlapping organoids. d, Percentage of overlapping organoids in both 384 and 24-well plates.
References