PATIENT-DERIVED TUMOR ORGANOID DRUG SCREENING

Retire the lysis step. Label-free organoid viability that outperforms CellTiter-Glo 3D.

We screened four patient-derived tumor organoid (PDTO) lines against two compounds and read out the same wells twice: once by brightfield imaging analysed with Orbits 2.0, and once with CellTiter-Glo 3D (CTG). Orbits 2.0 reproduced CTG potency and delivered markedly tighter replicates and robust assay quality – without dyes and without lysis. Because Orbits 2.0 segments viable and dead organoids label-free, its growth-rate metric (NOGR) also distinguishes cytostatic from cytotoxic drug effects: information an ATP endpoint cannot provide.

5×
lower replicate scatter than CTG at doses where organoids are alive

0.73
median Z′-factor, Orbits 2.0 vs -0.57 for CTG

r = 0.98
EC50 correlation with CTG – same potency, median ratio 1.12×

0
dyes, reagents or lysis steps

Why move beyond an ATP endpoint?

Luminescent ATP assays such as CellTiter-Glo 3D are the default viability readout in organoid screening. They are simple, but they are **destructive**, give **one timepoint per well**, and depend on complete lysis and ATP extraction from 3D structures – a step that is difficult to control in dense, large or heterogeneous organoid cultures.

Imaging-based analysis keeps every well alive and measurable over time. The first generation of Orbits (1.0) already quantified organoids label-free in brightfield, but still relied on a fluorescent dead-cell dye to separate viable from dead organoid area.

Orbits 2.0 removes that dependency. A new segmentation and cell-death classification model identifies viable and dead organoid area directly from brightfield images (Figure 1), so a screen needs nothing more than the organoids, the compounds and an imager.

What this note shows

- Orbits 2.0 recovers the same compound potency as CTG from a simple brightfield image.
- Replicates and controls are far tighter than CTG, giving good-to-excellent assay quality in every line.
- Fully label-free Orbits 2.0 matches or exceeds dye-assisted Orbits 1.0.

Study design

Models 4 patient-derived tumor organoid lines (PDAC001, CRC001, LU001N, CCA001)

Compounds 2, 16-point dilution, 5–5000 nM, 5 replicate wells per dose

Controls 16 untreated wells and 5 positive-control wells per line **Format** 384-well plates, Tecan Spark Cyto, T0 and 120 h

Reference CellTiter-Glo 3D measured in the same wells after the final image

Readouts viable organoid area (Orbits 1.0 and 2.0), NOGR growth rate, ATP luminescence

PDAC: pancreatic ductal adenocarcinoma; CRC: colorectal cancer; LU: normal lung; CCA: cholangiocarcinoma.

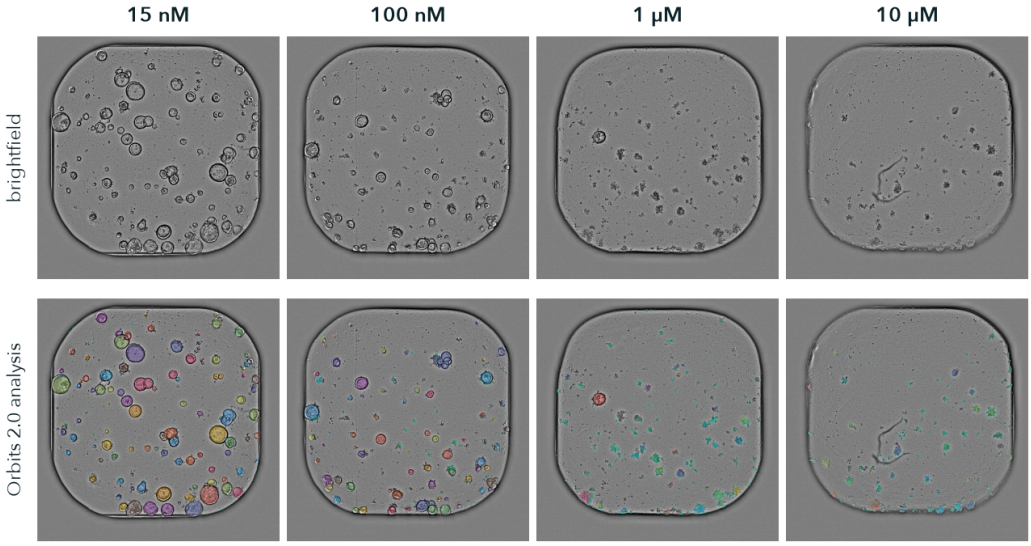


Figure 1. Orbits 2.0 reads viability directly from brightfield. Representative wells across a dose titration (example dataset): raw brightfield (top) and the Orbits 2.0 analysis overlay (bottom), with individual organoids segmented and dead material classified (green) without any fluorescent label.

01 Same potency, without lysis

Normalised to the untreated and positive-control wells of each line, Orbits 2.0 viable organoid area and CTG luminescence produced overlapping dose-response curves (**Figure 2**). For Compound 1, where all readouts gave an EC50 within the tested range, Orbits 2.0 potencies were within 1.05–1.32-fold of CTG (r = 0.98, median ratio 1.12×, Figure 3d; e.g. CRC001 181 vs 190 nM, LU001N 91 vs 98 nM). Compound 2 was more potent than the lowest tested dose in all lines by imaging. By CTG, scattered low-dose wells reading up to twice the untreated signal distorted two of the four fits.

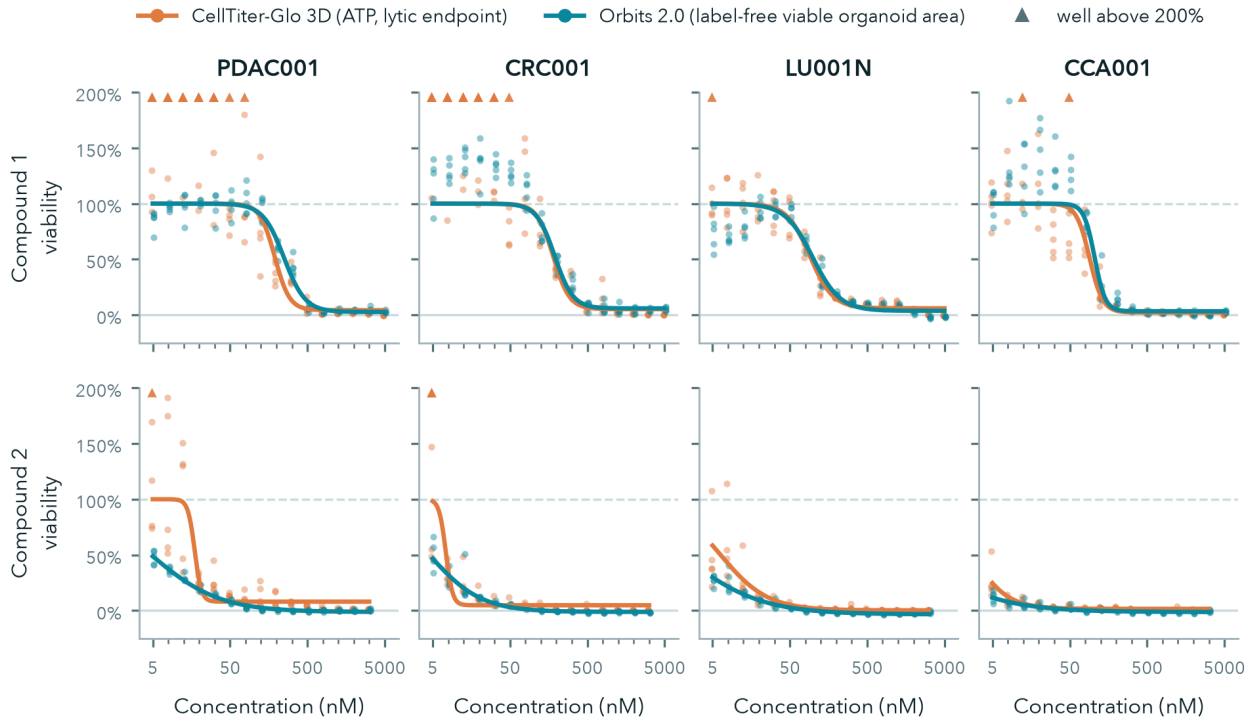


Figure 2. Orbits 2.0 tracks CellTiter-Glo 3D potency. Viability of four PDTO lines (columns) treated with Compound 1 and Compound 2 (rows). Dots are individual wells (n = 5 per dose; no wells removed); lines are three-parameter log-logistic fits. Viability = (well − median positive control) / (median untreated − median positive control). Triangles mark wells above 200%.

02 Tighter replicates, better assay quality

Every screen stands or falls with its controls, so we start there. **Figure 3a** shows each untreated well relative to the median of its line: if a readout is reliable, all points sit close to 1×. The Orbits 2.0 wells do (CV 13–18%), and even more tightly after each well is normalised to its own T0 image (CV 7–10%). The CTG wells do not: CVs of 31–76%, and 11 of the 64 untreated wells read two to four times their line median (shaded area). No Orbits 2.0 control well came close.

These control wells set the **assay quality**, summarised by the Z′-factor (**Figure 3b**): it compares the gap between untreated and positive-control wells with their spread. Above 0.4 is good and above 0.7 excellent; below 0 the two control groups overlap and hits cannot be called reliably. Because of its erratic controls, CTG fell below 0 in 3 of 4 lines (median -0.57). Orbits 2.0 reached a median of 0.47 from the endpoint image alone and **0.73** with T0 normalisation, excellent in 3 of 4 lines. That second step is only possible because imaging leaves every well intact.

The same difference appears in the treated wells (**Figure 3c**). At doses where organoids were still alive – the part of the curve that defines potency – replicate CTG wells typically differed by **68%** of the assay window, against **10%** for Orbits 2.0. At doses that killed the organoids, both readouts were equally tight (1%). The extra CTG noise therefore sits exactly where the biology is, which explains the scattered CTG wells in Figure 2. Despite this, both readouts rank compound potency the same way (**Figure 3d**).

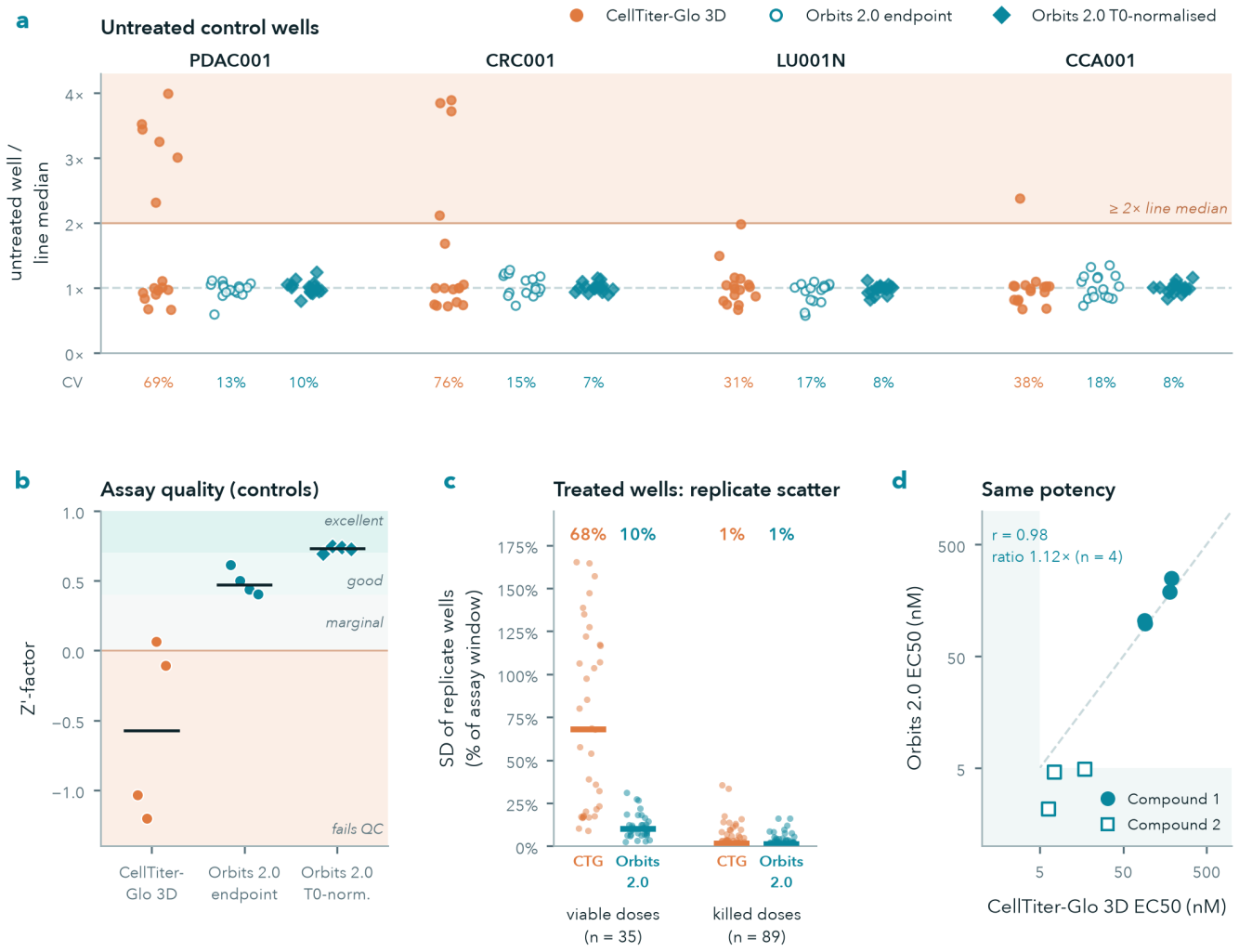


Figure 3. Orbits 2.0 gives tighter controls, better assay quality and tighter replicates than CellTiter-Glo 3D. (a) Each untreated well (16 per line) relative to the median of its line; numbers below are coefficients of variation (CV); shaded area marks wells at 2× the line median or more. (b) Z′-factor per organoid line, calculated from n = 16 vehicle-control (untreated) and n = 5 positive-control wells per line; black bars are medians; background bands mark quality ranges. (c) SD of the five replicate wells at each dose for CellTiter-Glo 3D (orange) and Orbits 2.0 endpoint viable organoid area (teal), as % of the assay window (untreated minus positive control), for doses where organoids were viable (mean viability ≥ 50%) or killed; bars and numbers are medians. (d) EC50 of Orbits 2.0 vs CTG; circles, Compound 1; open squares, Compound 2 (EC50 at or below the lowest tested dose, shaded).

03 Cytostatic or cytotoxic? One label-free metric tells them apart

A CTG signal below the untreated control shows that a drug worked, but not how: organoids may have grown less, or died. Orbits separates the two because it segments viable and dead organoids label-free in every image, measuring viable organoid area directly rather than a proxy such as ATP.

The Normalised Organoid Growth Rate (NOGR) expresses the change in each well's viable area from T0 to the endpoint, scaled to the untreated and positive-control wells. Comparing each well with its own starting point removes differences in seeding, which improves reproducibility. The same metric also shows how a drug acts: **values from 1 to 0 are cytostatic** (organoids still grow, but less than untreated; 0 means complete growth arrest), and **values from 0 to −1 are cytotoxic** (viable organoid area is lost; −1 matches the positive control).

Orbits 1.0 already reported NOGR, but needed Cytotox Green and a second imaging channel to identify dead organoid area. Orbits 2.0 does this from brightfield alone (Figure 1). We compared both versions on the same wells.

The NOGR curves of Orbits 1.0 and 2.0 overlap in every line and resolve both the cytostatic and the cytotoxic range (**Figure 4a**). Replicate wells were tighter with Orbits 2.0 in 7 of 8 dose-response curves (**Figure 4b**), and the T0-normalised assay quality rose from a median Z′ of 0.56 to **0.73**, with every line in the good-to-excellent range (**Figure 4c**). Potency was unchanged: the concentration that halves organoid growth (NOGR50) agreed between versions with r = 0.99 (**Figure 4d**). Dropping the dye therefore costs nothing – it improves the readout.

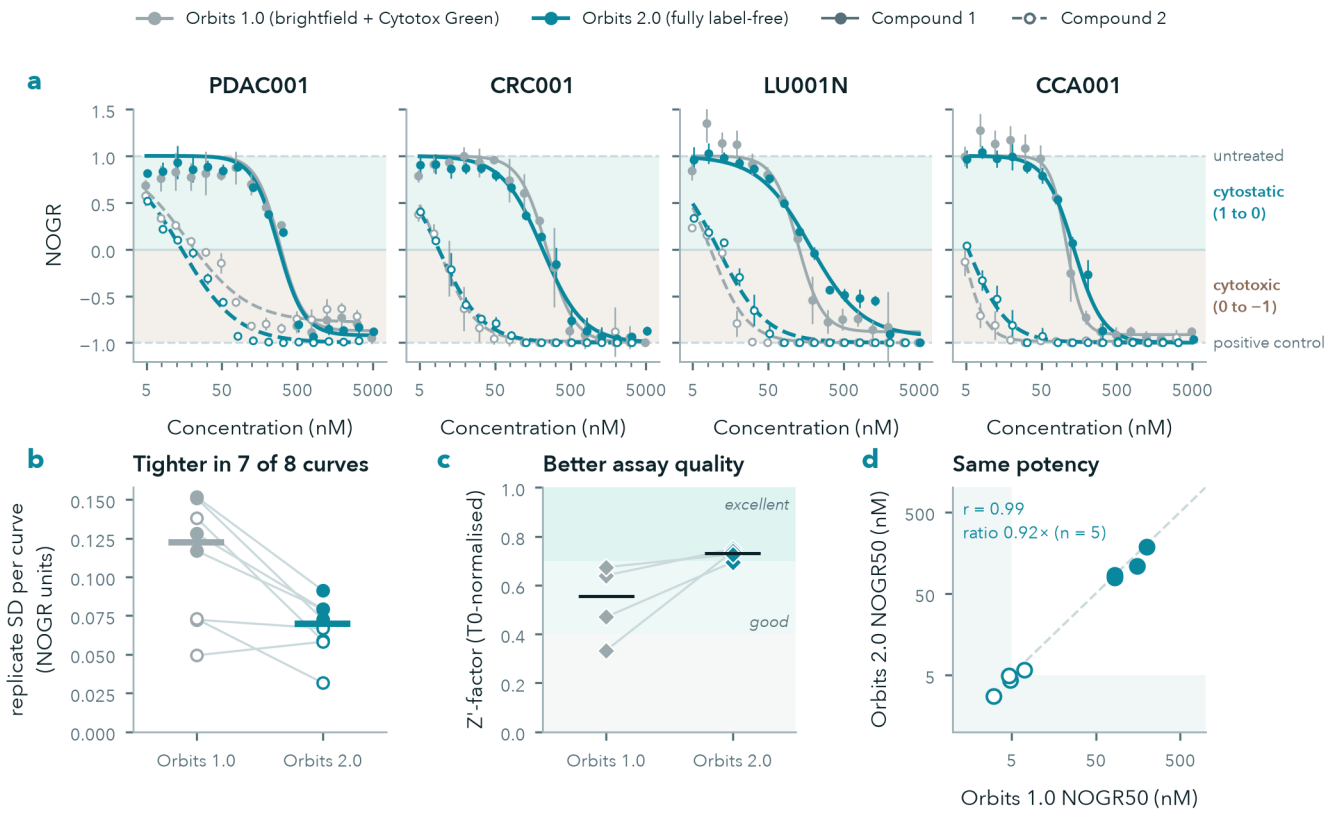


Figure 4. Removing Cytotox Green improves, rather than compromises, the readout. (a) NOGR dose-response for Orbits 1.0 (brightfield + Cytotox Green) and Orbits 2.0 (brightfield only); mean ± SD of 5 wells, fitted curves. Shaded ranges: NOGR 1 to 0, cytostatic (reduced growth; 0 = complete growth arrest); 0 to −1, cytotoxic (loss of viable organoid area; −1 = positive control). (b) Replicate scatter per dose-response curve (SD of replicate wells, pooled over doses); lines connect the same curve. (c) Z′-factor after T0 normalisation per organoid line. (d) NOGR50, the concentration at which growth is inhibited by 50%; filled symbols, Compound 1; open symbols, Compound 2.

At a glance

	CellTiter-Glo 3D	Orbits 1.0	Orbits 2.0
Reagents added to the well	ATP detection reagent (lytic)	Cytotox Green dye	**None**
Wells remain intact	✕ endpoint only	✓	✓
Timepoints per well	1	Multiple (kinetic)	**Multiple (kinetic)**
Cytostatic vs cytotoxic (NOGR)	✕	✓	✓
Replicate SD at viable doses (median)	68%	14%	**10%**
Median Z′ (endpoint / T0-norm.)	-0.57 / not possible	0.46 / 0.56	**0.47 / 0.73**
Imaging channels required	–	Brightfield + green fluorescence	**Brightfield**
Per-organoid size, count, morphology	✕	✓	✓

Methods in brief

PDTOs were seeded in 384-well plates and imaged in brightfield and green fluorescence at T0 and at the endpoint after 120 h on a Tecan Spark Cyto, following the protocol described in ref. 3; CellTiter-Glo 3D was added to the same wells after the final image. Viable organoid area per well was quantified with Orbits 1.0 (brightfield organoid area minus Cytotox Green-positive area) and Orbits 2.0 (brightfield-only segmentation and label-free death classification; the green channel was not used). Endpoint readouts were normalised per line to the median untreated (100%) and positive-control (0%; Compound 2 at 5000 nM) wells. NOGR was calculated from the T0-to-endpoint change in viable area and scaled to the untreated (1) and positive-control (−1) medians (ref. 2). Dose-responses were fitted with a three-parameter log-logistic model with the top fixed at 100% (or NOGR = 1). Replicate variability is the SD of the five wells per dose. $Z' = 1 - 3(SD_{pos} + SD_{neg})/|mean_{pos} - mean_{neg}|$. No wells were excluded. Data are from a single experiment with four PDTO lines and two compounds; CTG performance depends on assay optimisation and may differ in other settings.

References

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2. Deben C, et al. Development and validation of the Normalized Organoid Growth Rate (NOGR) metric in brightfield imaging-based assays. *Communications Biology* 7:1612 (2024). doi:10.1038/s42003-024-07329-5
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About Orbits Oncology

Orbits started in 2019 at the University of Antwerp, where cancer biologists and biomedical engineers set out to make 3D in vitro models reproducible and scalable. The Orbits platform turns live-cell imaging of organoids, spheroids and other complex in vitro models into quantitative readouts: segmentation, cell-death classification, growth rate and drug-response metrics, across imaging systems and plate formats. Orbits Oncology was incorporated in the United States in 2023.

Put your next screen on Orbits.

Send us a plate you have already run with CellTiter-Glo and brightfield images, and we will analyse it side by side with Orbits 2.0 – so you can judge the difference on your own models, compounds and imager.

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