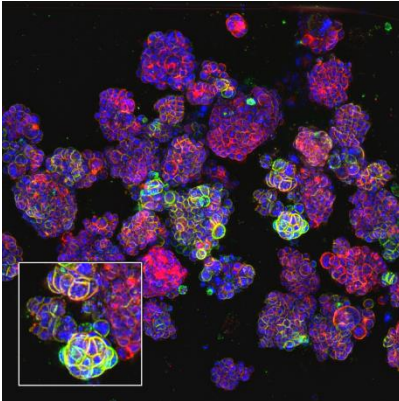


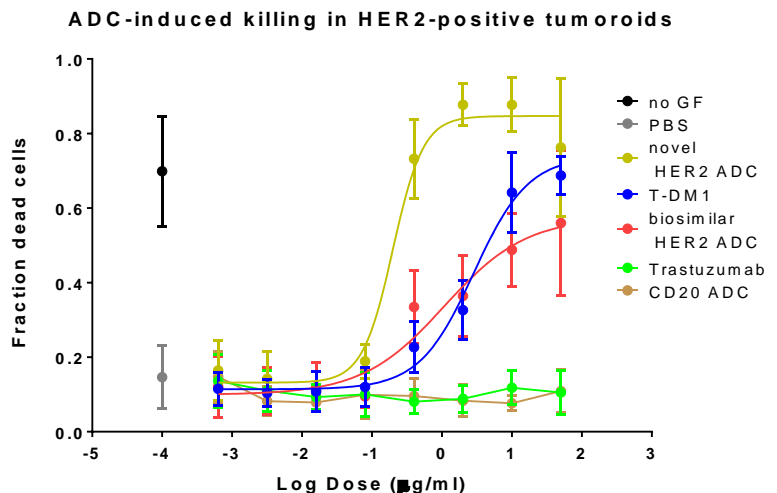
# ADC profiling in cultured human tissues



Actin Nuclei ADC-FITC (T-DM1)

- 3D cultured human tumor tissues
- High content analysis, high throughput testing
- Quantify ADC efficacy, binding and penetration
- Broad range of well characterized tumor models

**ADC binding and tumor killing** in HER2-positive Gastric cancer tumoroids derived from PDX (Charles River Laboratories). T-DM1 and T-DM1 biosimilars are compared alongside Trastuzumab and non-binding CD20-ADC.

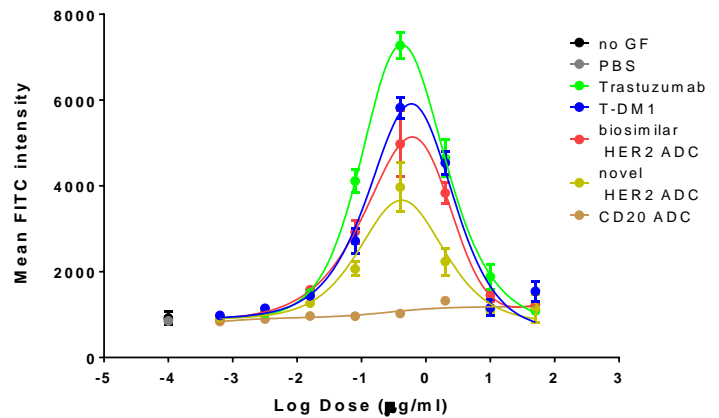


OcellO's high content 3D screening platform enables unique characterization of compound effects including antibody-drug conjugates (ADCs). Using 3D *in vitro* tumor models, compound **efficacy** (killing), antibody **binding and penetration** are evaluated. In addition to cell line derived tumor spheroids, and normal and matching tumor organoids for testing, tumor cultures derived from a broad range of tumor models are available from the Charles Rivers' PDX collection for potential *in vivo* follow up studies.

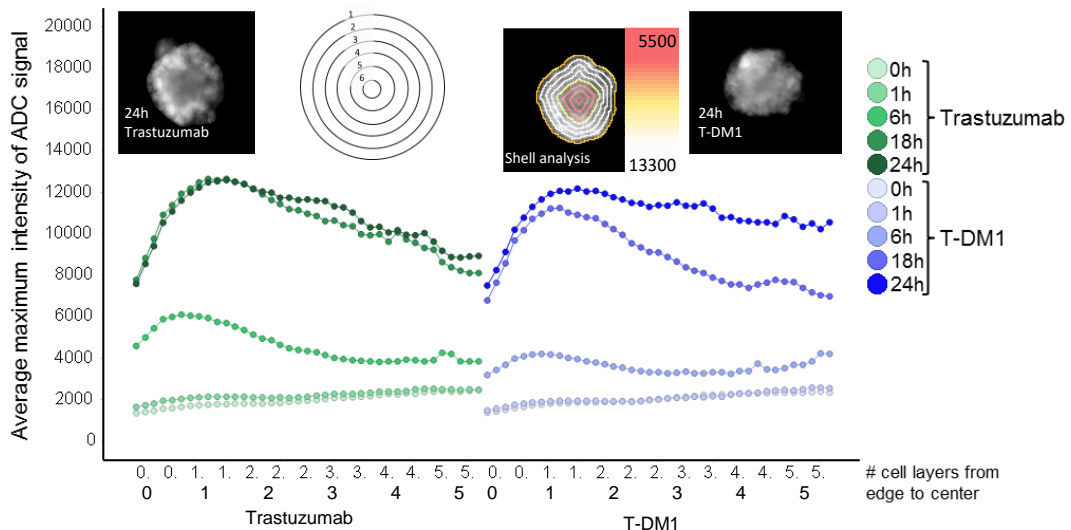
## Quantification of ADC binding to tumor tissue

HER2 targeting Trastuzumab and T-DM1 bind efficiently to HER2-positive GXA-3067 tumoroids, whereas CD20-targeting ADCs do not bind. At high doses, internalization masks the antibody from detection with a secondary anti-human-FITC antibody. The dose at which the ADCs show maximum binding coincides with the onset of killing.

ADC targeting to HER2-positive tumoroids



ADC penetration into HER2-positive tumoroids



## Quantification of ADC penetration into tumor tissue

Tumoroids are exposed to antibody for different time periods, followed by fixation, permeabilization, staining with secondary antibody and 3D imaging. By performing OcellO's unique shell-based analysis on each tumor spheroid optical section, the localization and penetration of the antibody or ADCs is measured from the spheroid perimeter to the center. In this example, Trastuzumab initially binds the outer cell layer more efficiently, whereas T-DM1 eventually penetrates deeper into the tumoroids.

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