How to Reduce the Volume of Data in 3D High-Content Screening Assays

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1 Introduction

To better predict the effects of drug candidates during preclinical screening, more physiologically relevant 3D model systems are being deployed in high-content screening assays. 3D image acquisition is time-consuming and generates huge amounts of data which must be stored and analyzed. Especially if objects are randomly distributed in 3D, a considerable number of images will be empty or out-of-focus. To avoid the unnecessary generation and storage of images, the upcoming release of Harmony 4.9 software includes an update to our intelligent image acquisition solution, PreciScan, which avoids the acquisition of empty images or partially imaged 3D objects and reduces the amount of data that needs to be acquired and analyzed. Here we demonstrate the time saving and data reduction that can be achieved in two example assays, and we also show how PreciScan can identify and automatically acquire high resolution image stacks of tumor cells inside a complex 3D model organism (zebrafish).

2 Problem: 3D imaging generates huge amounts of data

3D cellular objects such as spheroids or organoids are often randomly distributed in x, y and z dimensions when grown in hydrogels. To acquire 3D data for detailed analysis, currently a huge z-stack has to be defined to cover all sample objects.

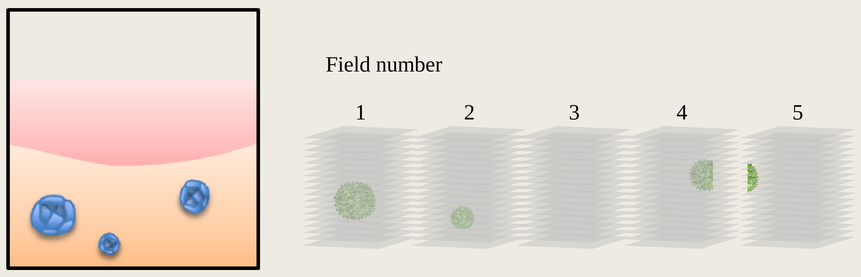


Figure 1: To cover all spheroids an extensive z-stack must be acquired at each field. Some fields might not contain any 3D object at all (field 3) or contain only a partial 3D object (fields 4 and 5)

To reduce the amount of data and accelerate the 3D workflow, we introduce the object-based image acquisition routine PreciScan. PreciScan determines the x, y and z positions of objects of interest from a single color low magnification pre-scan with a low z sampling rate. This positional information is then used to acquire high magnification/resolution z-stacks of centered objects in a re-scan experiment. The re-scan experiment can be set up with the minimal number of planes needed to capture the objects of interest. This reduces the number of planes, prevents the acquisition of empty images or partially imaged 3D objects and reduces the amount of data that needs to be acquired and analyzed. Furthermore, the identified objects of interest will be centered in the re-scan field of view.

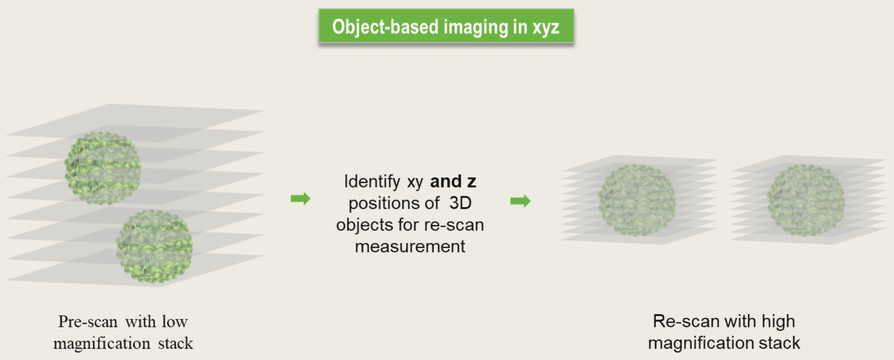


Figure 2: PreciScan allows the determination of x, y and z coordinates of 3D object in a pre-scan experiment and uses them for object-based imaging in a re-scan experiment.

3 Spheroid image acquisition data reduced 35-fold

HeLa cell spheroids were grown in Geltrex and imaged using PreciScan. A 10x air objective was used for the Pre-scan and a 40x water immersion objective was used for the Re-scan.

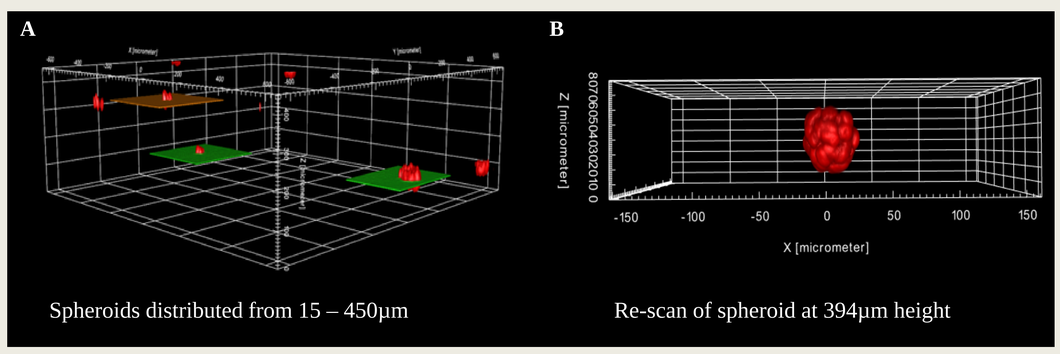


Figure 3: PresciScan allows object-based imaging of spheroids grown in hydrogels. (A) For the pre-scan a 10x air objective was used to acquire a z-stack covering 450µm at 15µm step size. The spheroids are distributed randomly between 15µm - 450µm throughout the gel. (B) For the re-scan a 40x water immersion objective was used to acquire a z-stack covering 80µm with 1µm step size.

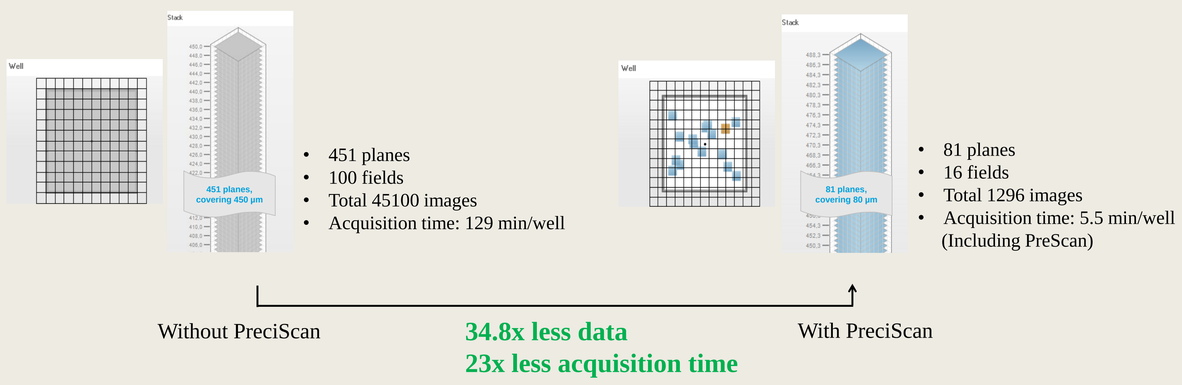
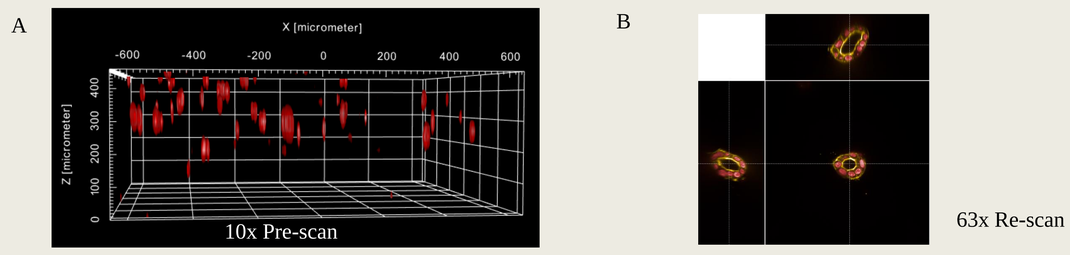


Figure 4: PreciScan reduces the amount of data acquired in the re-scan experiment by a factor of 34.8. The acquisition of 16 spheroids using PreciScan generated ~1.93Gb of data per well. Without PreciScan the whole well needs to be imaged with a z-stack covering the whole 450µm. This would have generated ~67.3Gb of data. Hence, PreciScan reduces the amount of data by a factor of 34.8 and the acquisition time by a factor of 23 (including PreScan).

4 MDCK cyst image acquisition data volume reduced 54-fold

MDCK cells were cultured in *3-D Life* Hydrogels (Cellendes, Germany) and imaged using PreciScan. Imaging of 25 cysts using PreciScan acquired 54x less data than conventional imaging without PreciScan.



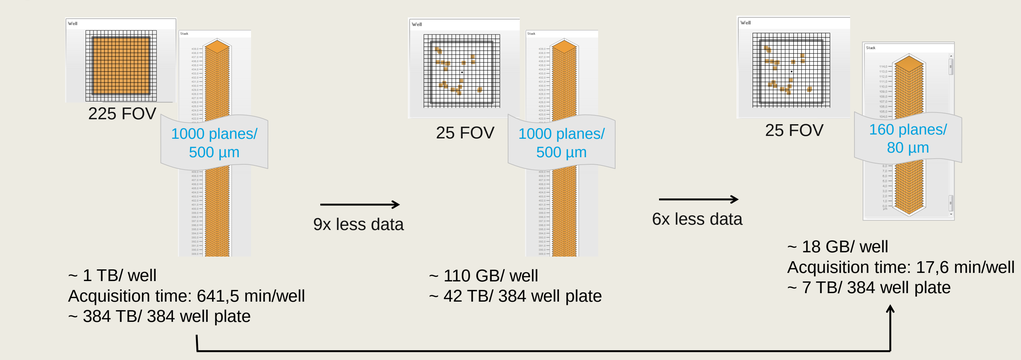


Figure 5: PreciScan allows object-based imaging in xyz and reduces the amount of re-scan data by a factor of 54. MDCK cells were grown in *3-D Life* Hydrogels (Cellendes, Germany). The gels have a height of 500µm and the MDCK cells form 3D aggregates throughout the gel. The cysts should be imaged with a 63x water immersion objective. (A) 3D visualization of pre-scan acquired with a 10x objective (24 planes, 15µm step size). (B) XYZ view of a MDCK cyst grown in the presence of RGD peptide (which promotes cyst formation), stack imaged with the 63x water immersion objective (160 planes, 0.5µm step size). (C) To image, for example, 25 cysts, without PreciScan the whole well needs a z-stack covering the whole 500µm, which generates ~1TB data per well. With PreciScan identification of the x and y positions of objects, only 25 fields need to be acquired, generating ~110Gb/well. Using PreciScan in x, y and z, the acquired data can be reduced to 18Gb/well and the acquisition time by a factor of 36 (including PreScan).

Following acquisition, MDCK cyst morphology was analysed using the Harmony 4.9 3D analysis tools. The lumen of the MDCK cysts was segmented and the volume calculated.

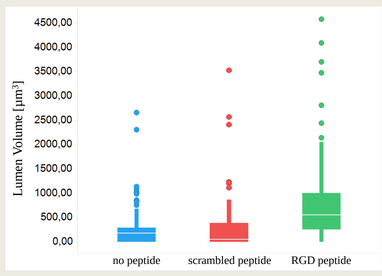


Figure 6: MDCK cyst lumen quantification using Harmony 4.9 3D analysis tools. MDCK cells were grown in 3-D Life Hydrogels (Cellendes) to form cysts. The hydrogels were supplemented with either an integrin binding mortif (RGD peptide) or a scrambled control peptide (scrambled peptide). As an additional control, gels with no added peptide (no peptide) were prepared. For each condition 3 wells and 25 cysts per well were analysed. (A) Shown are box plots of single cell results (n=3 wells).

5 PreciScan driven acquisition of tumor cells in zebrafish

PreciScan can also be used to identify cells inside a larger structure, e.g. zebrafish. RFP positive tumor cells were segmented in a pre-scan using a 5x objective and targeted for re-scan using a 20x water immersion objective. The identified tumor cells varied in z-height between 150µm and 550µm.

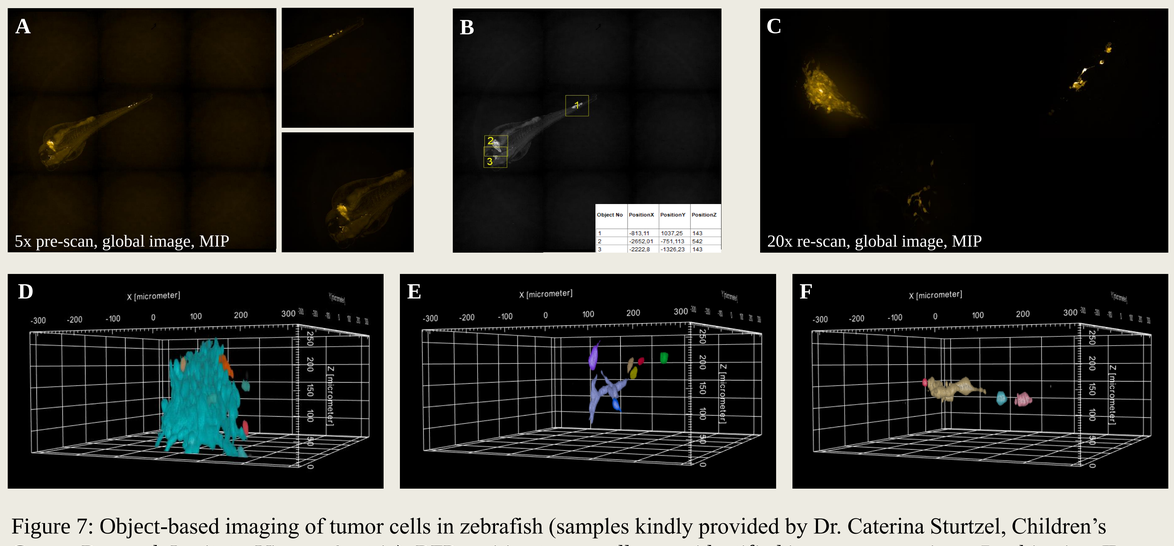


Figure 7: Object-based imaging of tumor cells in zebrafish (samples kindly provided by Dr. Caterina Sturtzel, Children's Cancer Research Institute, Vienna, Austria). RFP positive tumor cells were identified in a pre-scan using a 5x objective. To cover the whole well of a 96well CellCarrier Ultra plate, 9 fields were acquired with a z-stack of 25 planes at 30µm step size. (A) 5x pre-scan, maximum intensity projection of global image and individual images. (B) Segmented tumor cells on global image with coordinates for re-scan. (C) Maximum intensity projection of global image of re-scan acquired with a 20x water immersion objective (280 planes, 1µm step size) (D-F) 3D view of re-scan with segmented tumor cells.

6 Summary

Using epithelial cysts and spheroids grown in hydrogel as examples, we show how PreciScan, which employs a low magnification pre-scan followed by a high-magnification re-scan, decreases the data volume of the re-scan by a factor of 34-54, depending on the assay. We also show how PreciScan can be used to identify and automatically acquire high resolution image stacks of tumor cells inside a complex 3D model organism (zebrafish). With this new version of PreciScan, in Harmony 4.9 software, we are addressing the challenges of the long imaging times and large data volumes associated with high-content analysis of 3D cell models and complex model organisms. Harmony is a single software package for acquisition, visualization and analysis of high-content images, and can directly analyze the re-scan images without the need for data transfer to another software.

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