Water immersion objectives for automated high-content imaging to improve precision and quality of complex biological assays

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Overview

The purpose of these studies was to determine if water immersion objectives, used to improve image quality in complex biological assays, could be used in a high-throughput environment.

Introduction

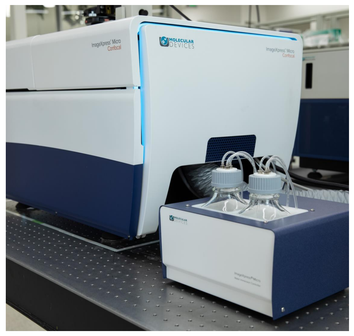
All of the assays were conducted in 96-384 well microplates or a commercially available organ-on-a-chip microplate. The workflow was identical between air and water objectives including laser auto-focus of samples. We saw that significantly shorter exposure times were usually required using the water immersion to still yield the same signal to background ratio, therefore overall plate acquisition times were equal or up to 30% shorter than using standard air objectives, depending on the number of wavelengths collected, fluorescence intensity and whether the sample was acquired in 2D or 3D. Additionally, hundreds of plates can be imaged using water objectives before the water reservoirs require attention.

Water vs. Air objective comparison experiments included:

- A mitochondria fusion and membrane integrity assay using rotenone and chloroquine to generate dose response curves.
- A live dead assay using spheroids grown from HCT116 colon cancer cells after treatment with compounds.
- Acquisition of a complex 3D angiogenesis sample grown in an organ-on-a-chip plate treated with an angiogenesis inhibitor.

In addition, we tested out a workflow that allows an entire microplate to be imaged at low magnification in order to identify rare objects of interest that can be re-imaged automatically using the high magnification water objectives.

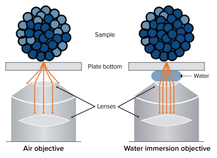
Materials and Methods



Water immersion option for ImageXpress® Micro Confocal High-content Imaging System

Experimental models:

- Labware:
  - Corning® spheroid microplates (384 well format)
  - Mimetas OrganoPlate® 3-Lane
  - InSphero Akura™ 384 well plate
  - Greiner SensoPlate™ - 96 and 384
- Cells:
  - 3D InSight™ Tumor Microtissues (InSphero)
  - HUVEC (human primary endothelial cells)
  - HCT-116 (human colorectal cancer)
  - Caco-2 (human colorectal cancer)
  - PC-12 (rat neuroblastoma)



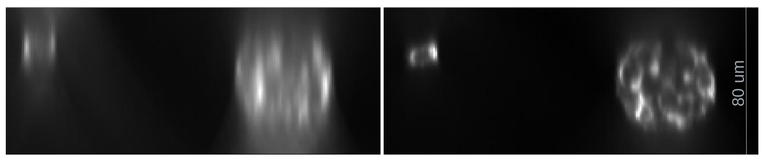
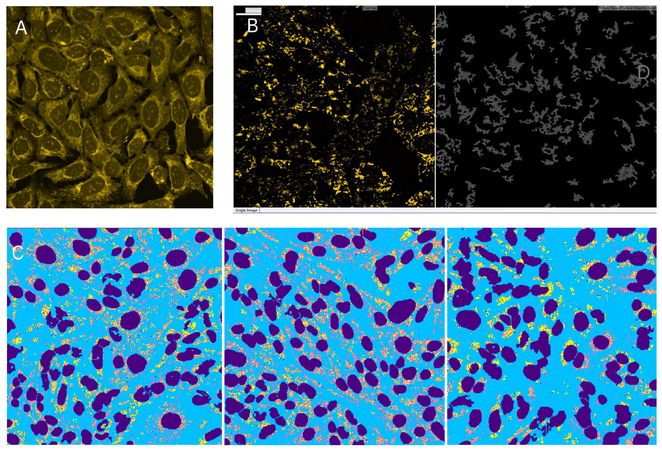


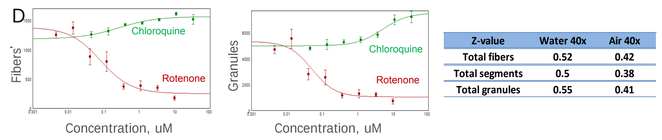
Figure 1. Reconstructed image side view of a spheroid comparing 20X 0.75NA air objective vs. 20X 0.95NA water objective demonstrates the improvement in image quality due to reduced spherical aberrations. Since the index of refraction of water more closely matches that of the medium that the samples are suspended in, images of tumor spheroids located >300 um above the plate bottom surface more accurately reflect the shape of the cells and the overall object without appearing stretched in the z plane. In addition,1/3 exposure time was required to generate the same signal intensity using water immersion (FITC wavelength).

Results

Mitochondria Integrity and Fusion Assay

Characterizing mitochondria shape and integrity is important for understanding mechanisms of diseases and for toxicity assessment. The number, brightness and the length of mitochondria can change during mitochondria recycling, metabolic changes, or the process of apoptosis. The intensity and shape can be determined by using tools in MetaXpress software that allows measurement of “granules”, “fibers” or “segments” by user–defined criteria, that can be used to calculate dose-responses and effective concentrations of various compounds.



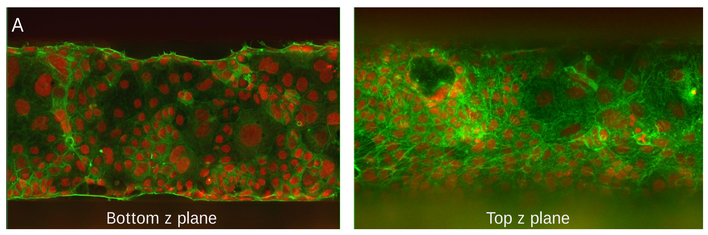


Z-value	Water 40x	Air 40x
Total fibers	0.52	0.42
Total segments	0.5	0.38
Total granules	0.55	0.41

Figure 2. PC12 neuroblastoma cells were treated with chloroquine that inhibits mitochondria recycling, and rotenone, an inhibitor of oxidative phosphorylation, at indicated concentrations for 24 hours. (A) Cells were stained with MitoTracker Orange CMTMRos (in yellow) and Hoechst (not shown). Images were taken at 40x magnification with and without water immersion (WI) and processed using MetaXpress software (B) Data analysis identified “granules” (left) or “fibers” (right) (C) Segmentation masks show how intact granules, fibers or segments were quantitated and used to calculate EC50s (quadruplicate samples) (D) Water Immersion allowed improved quality of images and more accurate calculation of the numbers of particles with resulting higher Z-values.

Organ-on-a-chip

Water immersion objectives improve resolution and sphericity of objects that are not lying flat on a 2D surface near the objective. This confers an advantage when imaging 3D models, especially organ-on-chip samples. We used water immersion for imaging gut-on-a-chip and angiogenesis models in the OrganoPlate 3-Lane format.



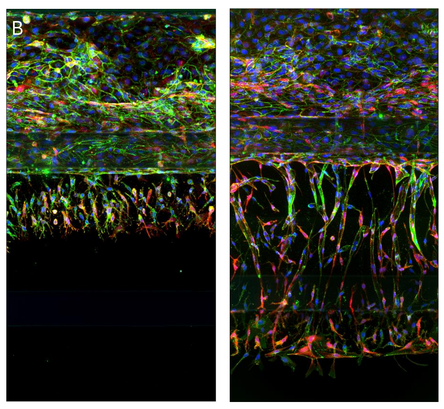


Figure 3. (A) Caco2 cells were grown according to the protocol from Mimetas to form an intact tube. Cells were stained with Hoechst and AlexaFluor-488 conjugated phalloidin and a z stack of confocal images was taken through the entire tube. Bottom (left) and top (right) layers of cells imaged using 40x objective with water immersion. (B) Angiogenesis model of HUVEC cells stained with Phalloidin-AlexaFluor 555, Hoechst, and anti-VE cadherin. 2D projection from 50 z planes acquired with 20X water objective shows untreated cells (right) and treated with anti-angiogenic compound (left).

Screening in 3D (QuickID/Targeted Imaging)

There are several methods for culturing spheroids for screening anti-tumor drugs. In some formats, the spheroids are not always centered in the well, which may make it difficult to acquire the object of interest in a single field-of-view (FOV) at high magnification. QuickID allowed us to automatically image each spheroid in the center of the FOV using a 20X water immersion objective to collect z stacks in multiple wavelengths without acquiring extra sites.

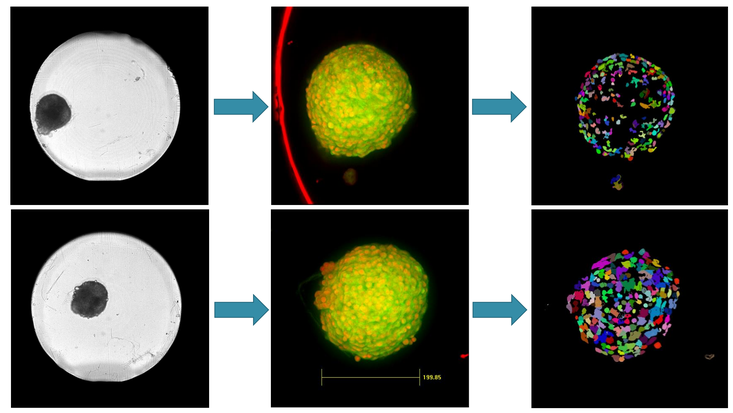
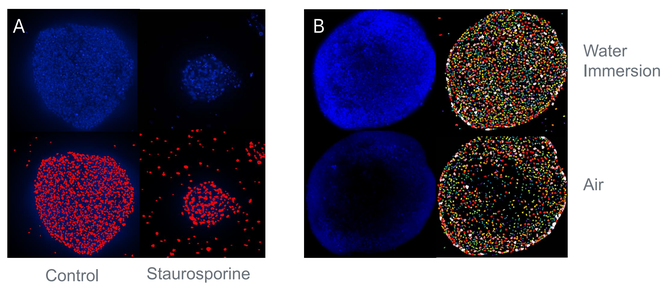


Figure 4. QuickID was performed with a 10X objective to quickly image an entire microplate. Spherical objects were then identified and their X,Y coordinates were used to automatically acquire only sites that contained a tumor microtissue with a 20X water immersion objective in multiple wavelengths and z planes. Image analysis was performed to find and measure objects within the 3D volume.

Quantitative Measurements of Spheroid Health

3D tumor models are increasingly used for cancer research. In this example, we used 3D spheroids formed from HCT116 colon cancer cells to measure cytotoxic effects of drugs on the numbers of live and dead cells in spheroids.



Z-value	20x WI	20x Air
Nuclei Count	0.56	0.46
Live Cells Count	0.65	0.54

Figure 5. Spheroids were formed using 4,000 HCT116 cells in U–shape 384 well plates. Spheroids were treated with anti-cancer compounds for 48h and then stained live with Hoechst and EthD-1 for 3h (A). The plates were then imaged using confocal option with 20X objective, with or without water immersion (B). Z-stacks of 11 images 10um apart were taken then maximum projection images were analyzed using count nuclei or Custom Module Editor. The numbers of nuclei or live cells were used for quantitation of EC50s for studied compounds. Using water immersion resulted in greater Z-values due to more accurate cell counts throughout the spheroid.

Conclusions

- Water immersion objectives were well-suited to automated imaging assays in standard microplates or organ-on-a-chip formats.
- Spherical aberration was reduced in objects 100s of um from the well bottom if water immersion objectives were used during acquisition.
- Acquisition time was reduced by up to 30% in a 2 color assay in a 384 well microplate.
- Image quality, and therefore data quality, was improved in these complex biological assays.