# A high-throughput three-dimensional magnetically printed cellular assay (BiO Assay) for toxicity screening for breast cancer applications

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## Background

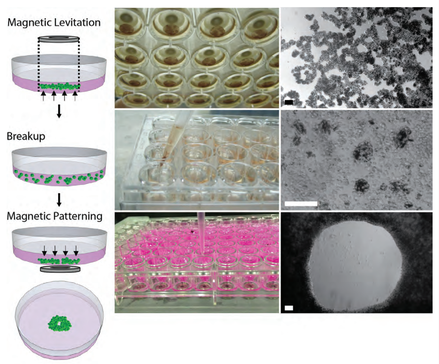
In drug development, the efficient screening of candidate compounds for toxicity and efficacy is an important step. The most common models for such screening are animal models, which are representative of the native tissue but are expensive and low-throughput. Alternatively, *in vitro* models are much cheaper and high-throughput, but are poor representatives of the native tissue, as they are typically two-dimensional (2D) models on rigid surfaces. Instead of choosing between the two extremes, there is a demand for *in vitro* models that represent the native tissue structure, and can be built and assayed quickly and efficiently.

To that end, we introduce a novel three-dimensional (3D) assay, the BiO Assay, for high throughput compound screening. The basis of the assay is 3D cell printing using magnetic nanoparticles; cells bind to the magnetic nanoparticle to render them magnetic, and able to be printed into 3D rings using magnetic forces. These rings will close over time as a result of migration, proliferation, and general cell health, and at a rate that varies with drug concentration. This assay also uses an iPod to image whole plates, yielding a complete assay with representative models and high-throughput and efficiency. In this study, we demonstrated the utility of the BiO Assay for breast cancer applications by investigating the response of MDA-231 breast cancer cells to doxorubicin in the assay.

**Hypothesis: This assay, the BiO Assay, can be used to rapidly print cells into rings that close/shrink over time in a fashion dependent on compound concentration**

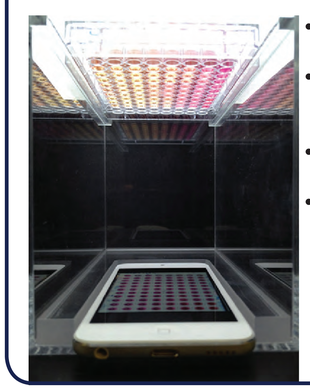
## 3D Magnetic Printing

- Cells are incubated with NanoShuttle (Nano3D Biosciences) overnight
- The next day, cells are levitated to synthesize ECM for a few hours to overnight
- Cultures are broken apart and printed into rings for 15 minutes (150K cells/ring) →
- Magnets are removed and cells are allowed to close for hours to days




**Magnetically levitated cultures can rapidly print 3D cell cultures**

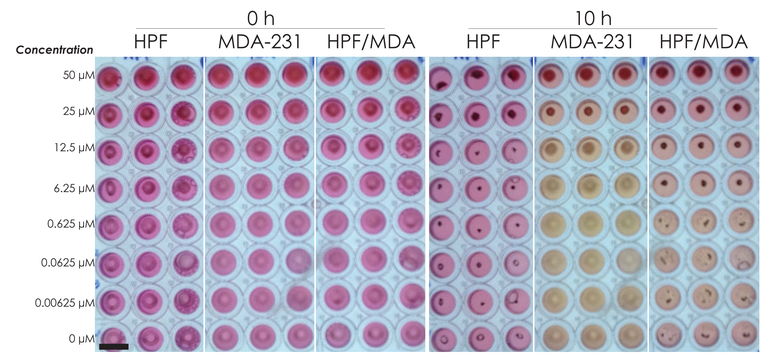
## iPod-Based Imaging System



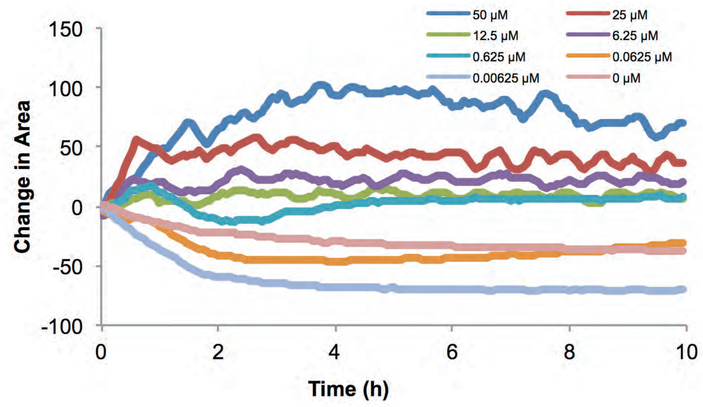
- Images of rings closing are taken with an iPod (Apple Computer)
- iPod is programmed using a freely available app (Experimental Assistant, Nano3D Biosciences) to image ring closure in real-time
- ←Imaging setup fits within a standard incubator
- iPod imaging forgoes imaging with a microscope

**Imaging with an iPod eliminates well-by-well imaging and improves throughput and efficiency**

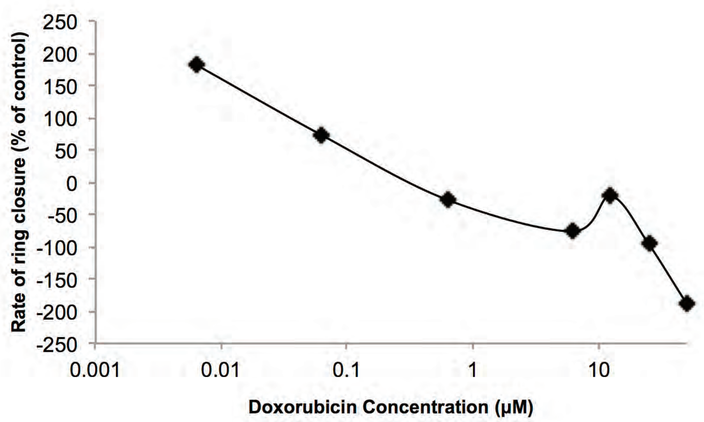
## BiO Assay




*Rings of MDA-231, primary human pulmonary fibroblasts (HPF),and a co-culture of HPF/MDA-231 in a 1:1 ratio after 0 (L) and 10 (R) hours, exposed to varying concentrations of doxorubicin. Note with increased concentrations of doxorubicin, the printed rings are unable to close. Scale bar = 5 mm.*

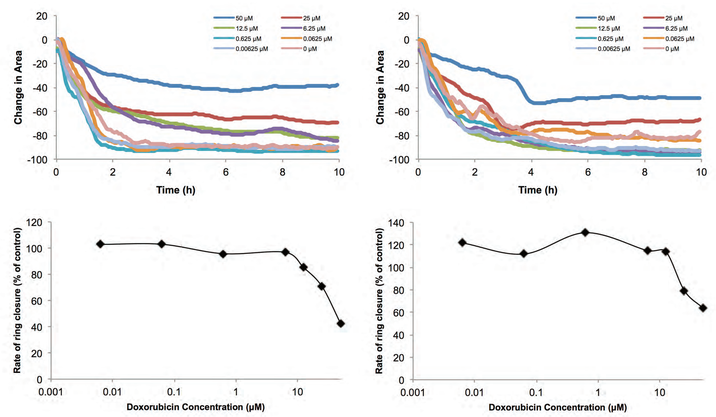



*Change in area of printed rings of MDA-231s (n=3) over 10 hours with varying concentration with doxorubicin. Note as doxorubicin increases, the rate of ring closure decreases.*




*The rate of ring closure for MDA-231s over 10 hours as a function of doxorubicin concentration. As expected, with increasing concentration of doxorubicin, the rings of MDA-231s were less able to close the ring, as a*

## BiO Assay




*Time-dependent ring closure (top) and dose-response curves (bottom) for the co-culture of HPFs and MDA-231 (L) and HPFs alone (R). Note that in adding a fibroblast line in co-culture with MDA-231s, the rings respond differently than just MDA-231 alone. In particular, the co-culture is more sensitive to doxorubicin than MDA-231s, almost similarly to HPFs alone. Additionally, the rings of the co-culture and HPFs close much more than MDA-231s*

## Conclusions

- Magnetic nanoparticles were used to rapidly print 3D rings or dots of cells in 96-well formats
- Ring closure is a label-free metric that leaves closed rings available for further analysis, such as genomics, proteomics, transcriptomics
- iPod imaging system facilitates real-time imaging without imaging each well under a microscope, improving throughput and efficiency
- MDA-231 ring closure was significantly affected with increasing doxorubicin concentration
- Co-culturing with fibroblasts increased the sensitivity of MDA-231s to doxorubicin
- Future directions include expanding into 384-well formats

**The BiO Assay is a complete assay for the printing, imaging, and analysis of 3D cultures for high-throughput drug screening**

## References

1. Haisler, W. L. et al. Three-dimensional cell culturing by magnetic levitation. Nat. Protoc. 8, 1940-9 (2013).
2. ***Timm, D. M. et al. A high-throughput three-dimensional cell migration assay for toxicity screening with mobile device-based macroscopic image analysis. Sci. Rep. 3, 3000 (2013).***

**Scan the QR-code to see the YouTube video of the image analysis of the rings!**

For more information, go to www.n3dbio.com, or email us at info@n3dbio.com

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