Most ovarian cancers are diagnosed today at a late, incurable stage. This leads to a continued emphasis for earlier detection and the development of effective screening strategies. To be considered effective, early detection technologies must have sufficient sensitivity and specificity to impact patient mortality, while minimizing false positives. Magnetic relaxometry (MRX) can differentiate between superparamagnetic iron oxide nanoparticles (SPIONs) bound to cancer cells and those freely floating in the vascular space with high sensitivity and specificity, promising to improve early detection.

**Background on Magnetic Relaxometry**
MRX uses an array of ultra-sensitive superconducting quantum interference detectors (SQUIDs) to measure the relaxation properties of SPIONs in response to a brief magnetizing pulse [1–3]. SPIONs are targeted to selectively bind to cancer cells using antibodies. Characteristic temporal differences in relaxation behavior are used to distinguish free SPIONs, which relax relatively quickly (< 35 ms) by Brownian motion, from bound (or sufficiently immobilized) SPIONs, which relax at a much slower rate (typically > 100 ms). The differences in relaxation behavior are used to selectively bind to cancer cells using antibodies. Characteristic temporal differences in relaxation behavior are used to distinguish free SPIONs, which relax relatively quickly (< 35 ms) by Brownian motion, from bound (or sufficiently immobilized) SPIONs, which relax at a much slower rate (typically > 100 ms). The differences in relaxation behavior are used to distinguish free SPIONs, which relax relatively quickly (< 35 ms) by Brownian motion, from bound (or sufficiently immobilized) SPIONs, which relax at a much slower rate (typically > 100 ms).

**ELISA on SPION pellets**
We verified the reported concentration of anti-HER2 antibody in the SPION solutions using an ELISA assay. Additionally, we verified successful antibody-SPION conjugation through an ELISA performed on the SPION pellets after high-speed centrifugation, confirming the presence of anti-HER2 antibody in the pellets compared with the supernatant, which had a much lower concentration of antibody.

**Flow Cytometry**
Flow cytometry was performed to confirm a high level of specific binding between SKOV3 cells and anti-HER2 antibody, as well as to confirm similar levels of binding between SKOV3 cells and anti-HER2 conjugated SPIONs and SKOV3 cells and free anti-HER2 antibody. Afterward, for our initial study using fixed cells (Fig. 1), we repeated the study with live cells (Fig. 2) to establish relevance to our MRX studies, which used live cells.

**Results**
Our in vitro MRX data (Fig. 3) revealed strong linearity between cell number and MRX signal for both SKOV3 and HEY cells, with cell number vs. MRX signal curves showing a highly correlated linear relationship (r = 0.99 and 1, respectively). Furthermore, there was little to no MRX signal for all cell numbers when SKOV3 cells were incubated with unconjugated, PEG SPIONs, which confirmed the antibody’s selectivity. MRX signal was observed when 10⁷ SKOV3 cells were incubated with anti-HER2 SPIONs, which were 2.1 ± 0.3 and 15 ± 1.4 times higher than the MRX signal for 10³ HEY cells (p < 0.05; one-way ANOVA). There was highly correlated (r = 0.99) with the MRX signal from the corresponding cell sample. When the in vitro portion of the study was repeated several months later, similar MRX signal versus cell number in the injected mice was highly correlated (r = 0.99) with the MRX signal from the corresponding cell sample.

**Conclusion**
MRX appears sufficiently sensitive to detect clusters of 1 million cells in culture or 10 million cells in vivo. Sensitivity may be improved using nanoparticles conjugated with antibodies against antigens that are overexpressed by a larger fraction of ovarian cancers and will be the focus of future work. Efforts towards such evaluation have already begun with the conjugation of anti-CA125 antibodies to SPIONs and verification of binding to ovarian cancer cell lines through flow cytometry. Our next step will be to perform MRX studies evaluating sensitivity and specificity of the anti-CA125 SPIONs in cell culture and in mice.

**References**

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