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Comment

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Comment on "Label-Free Single Exosome Detection Using Frequency Locked Microtoroid Optical Resonators"

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ABSTRACT: In a recent paper by Su [*ACS Photonics* **2015** *2*, 1241−1245], a microtoroid resonator was demonstrated that is capable of detecting binding events of single exosomes, as well as providing other physical parameters such as size and mass. While we feel the method presented by Su is likely to find significant application in the basic study of exosomes, the clinical significance of this result is overstated in the article. As discussed below, the proof-of-concept results presented depend critically on a xenograft model that is not readily translated into a clinical setting.

We read with great interest the recent article "Label-Free Single Exosome Detection Using Frequency-Locked Microtoroid Optical Resonators" by Judith Su, published in the September 16th, 2015 issue of *ACS Photonics*.[1] The field of exosome detection and characterization is a new and rapidly evolving one, and many authors (including ourselves) have highlighted the heterogeneity of exosomes.[2−4] Thus, single-exosome detection and characterization technologies such as Su's are needed advancements in the field. In particular, we were impressed by the range of information afforded by her technology, including raw count number, as well as other physical parameters such as size and mass. However, we feel there were some oversights in the biological discussion in this paper that should be corrected, as they may give a false impression to an uninitiated reader. The author suggests that a microtoroid resonator could be used as a "liquid biopsy" to detect exosomes released from cancer cells. This assumption relies on the fact that some antibodies or other capturing agents could be devised that are specific to cancerous exosomes. However, to date, no such biomarker has been discovered. This is widely considered a "holy grail" of exosome research. In fact, even separating exosomes from other nanometer-sized vesicles released by cells is a challenge, as they have no unique surface proteins. Thus, purification of exosomes remains a significant and persistent challenge in the field.[5,6] Furthermore, even if researchers in the field could separate "exosomes", "micro-vesicles", and other nanometer-sized vesicles, without the "holy grail" of a cancer-specific exosome marker, another challenge still remains: to reliably and repeatedly separate tumor-originating exosomes from, for example, exosomes secreted by healthy cells. In her article, Su uses CD81 as a biomarker to capture exosomes in plasma. Since CD81 is a surface protein found in most human cells and also expressed in most human exosomes,[7] it cannot be considered as "exosome-specific" or "cancer-specific." Therefore, the reason Su's experiments in mice showed an undetectable signal in cancer-free mice (Figure 2 of the original paper[1]) is because the anti-CD81 used in this experiment is against human CD81. As Su mentions in the introduction, normal mouse blood (just as human blood) has a high concentration of exosomes, including those expressing *murine* CD81. Therefore, in this paper, human CD81 is a cancer-specific biomarker because no murine exosomes would express human CD81, while the exosomes from the human xenograft cancer cells do. In principle, any human membrane protein expressed by exosomes could have been used with the same results. Nevertheless, to demonstrate the technical (but not clinical) feasibility of the frequency locked microtoroid optical resonator, the xenograft model was a good choice, because it eliminates an extra uncontrolled variable. Therefore, to conclude, we find Su's microresonator concept to be highly innovative, and its ability to sense individual exosomes (as well as to provide some other physical characterization such as size and mass) to be an important contribution to the field of single-exosome analysis. These features suggest that the microtoroid resonator may be an intriguing tool for basic studies of exosome biology. However, given that this paper focuses on the clinical applications of this device, we feel readers should be aware that the experiments shown rely on a xenograft model that cannot be clearly translated to the clinical setting.

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Notes

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