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## **ACCEPTED MANUSCRIPT**

Cyanine5-doped silica nanoparticles as ultra-bright immunospecific labels for model circulating tumour cells in flow cytometry and microscopy

Claire L. O'Connell<sup>1\*</sup>, Robert Nooney<sup>2</sup>, Colette McDonagh<sup>1</sup>.

#### **Abstract:**

In this work, ultra-bright fluorescent silica nanoparticles (NPs) labels have been shown to selectively bind to a model circulating tumour cell (CTC) line, MCF-7, a metastatic breast cancer by targeting epithelial cellular adhesion molecule (EpCAM) present on the MCF-7 cell membrane. Silica NPs approximately 40 nm in diameter were doped with different concentrations of Cyanine5 dye molecules, using the reverse microemulsion method. The NPs were two orders of magnitude brighter than Cyanine5 free dye and the measured fluorescence intensity matched a homo-Förster Resonance Energy Transfer model. NPs were conjugated with anti-EpCAM antibody to the NP surface for immunospecific targeting. In flow cytometry experiments the NPs were twice as bright as two commercial anti-EpCAM red fluorophore conjugates, APC and AlexaFluor®647. This increase is achieved while keeping non-specific binding low as established in control tests with a non-metastatic cancer cell line (HeLa). The NPs were also immunospecific in fluorescence microscopy experiments performed at room temperature on non-fixed cells. Confocal

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