# Nanotechnology Research using ImageStream Cytometry


The ImageStream system combines high-speed image capture with image quantification to create a statistically powerful microscopy platform, enabling robust discrimination of cells based on their appearance. This document highlights applications of ImageStream cytometry to the field of nanotechnology as described in publications. For more information, check out the website: (https://www.amnis.com/nanotechnology.html)

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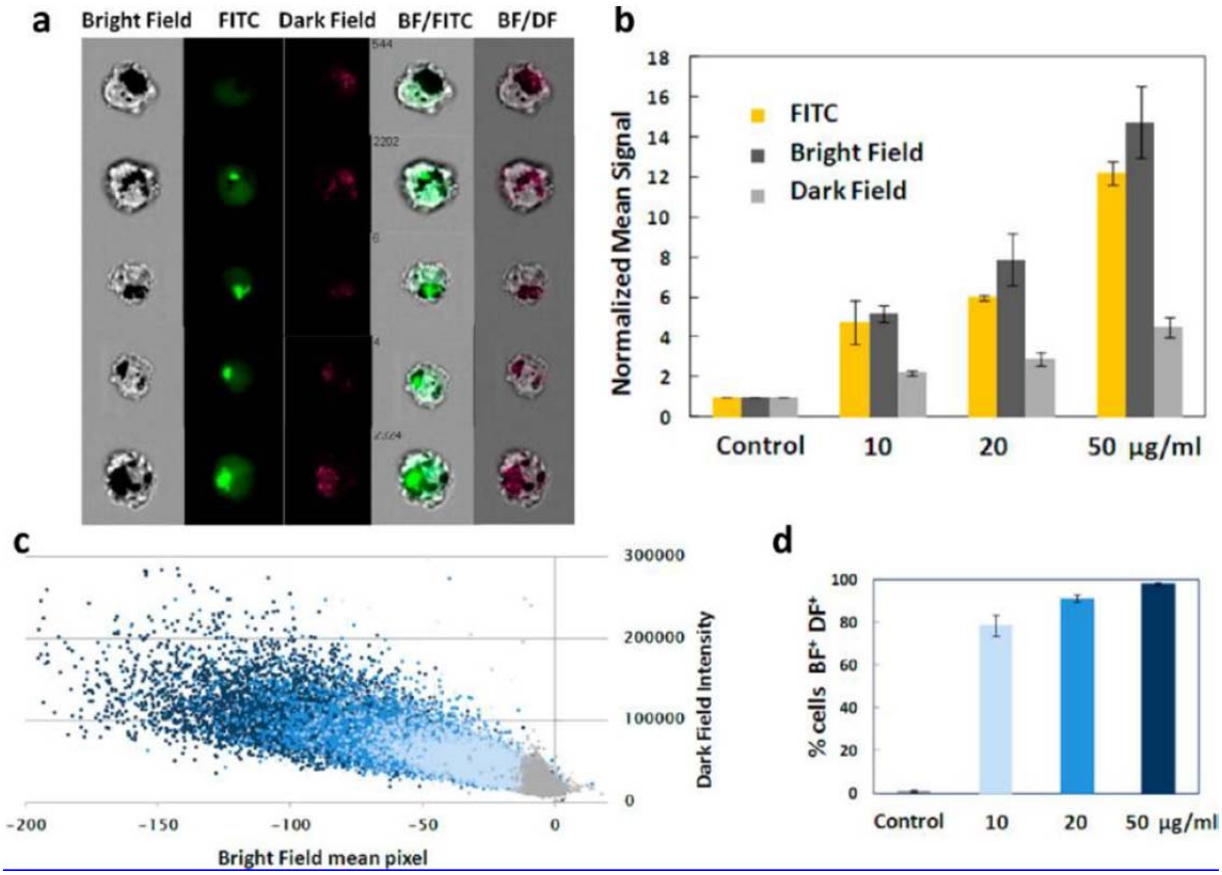
# Label-free detection and quantification of intracellular carbon nanotubes

**Summary: This ImageStream assay quantifies the amount of carbon nanotubes (CNTs) within endothelial cells (ECs). The ImageStream's high speed image capacity enabled analysis of 7,000-10,000 events per sample for robust statistics. Mean signal intensity from FITC, Bright Field, and Dark Field images increase with CNT concentrations in a dose-dependent manner (b) and double Bright Field/Dark Field images act as a label-free classifier for CNT labeled cells (d).**

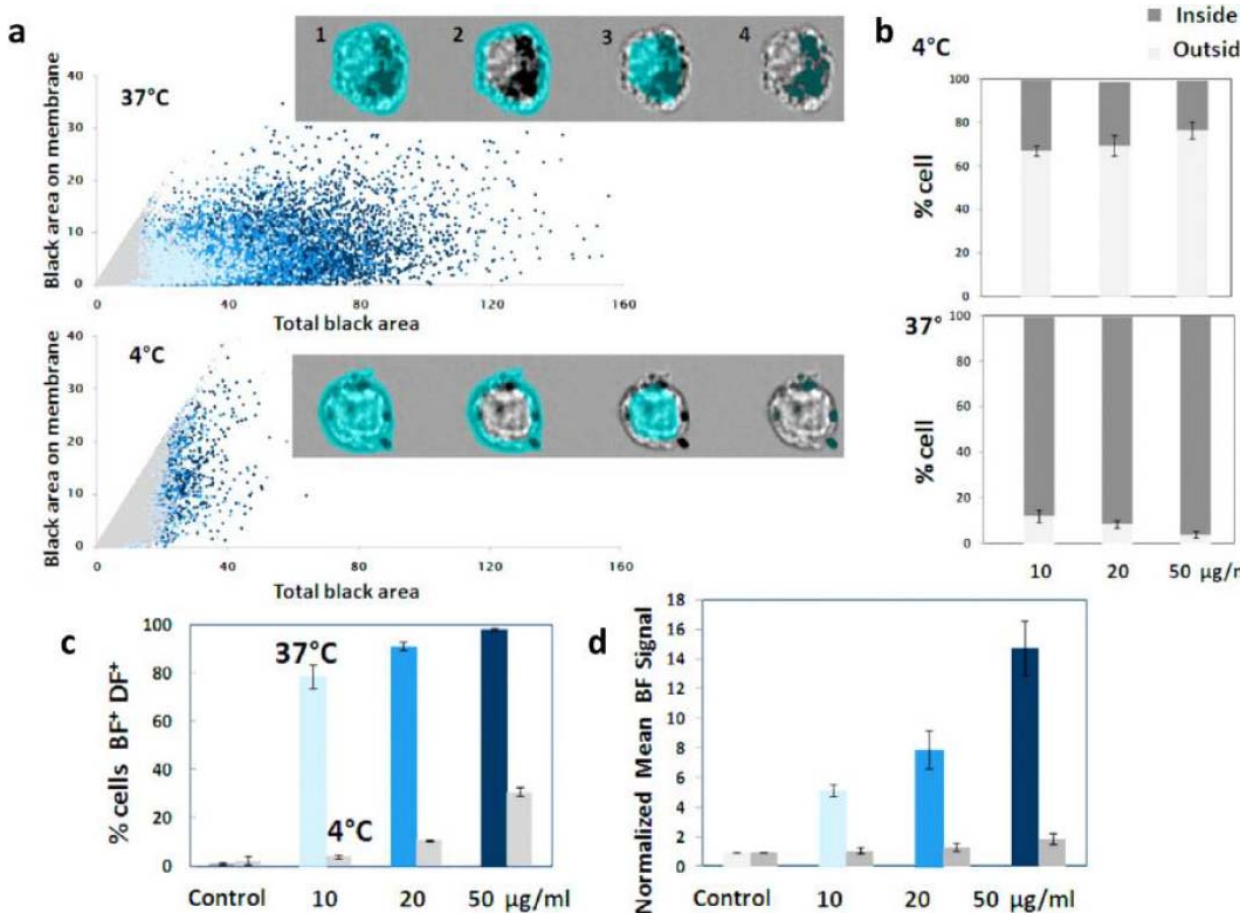



"In summary, we have presented a novel label-free high throughput flow cytometry imaging method, which enables concomitant localization and quantification of carbon nanotubes on a statistically relevant number of cells. This method, based on bright- and dark-field image analysis, could be generalized to study cell interactions with any nanomaterials that induce strong absorbance and scattering of light."

**Reference**: Marangon I, Boggetto N, Ménard-Moyon C, Venturelli E, Béoutis ML, Péchoux C, Luciani N, Wilhelm C, Bianco A, Gazeau F*., Intercellular carbon nanotube translocation assessed by flow cytometry imaging.* Nano Lett. 2012 Sep 12;12(9):4830-7. doi: 10.1021/nl302273p. Epub 2012 Sep 4.

## Energy-dependent internalization of carbon nanotubes

**This ImageStream assay compares the amount of membrane-bound verses internalized carbon nanotubes (CNTs). The ImageStream's high speed image capacity enabled analysis of 7,000-10,000 events per sample for robust statistics. Endothelial cells (ECs) were incubated with CNTs at 4C and 37C, and the IDEAS image analysis masking feature (a) was applied to demonstrate that CNTs are internalized via energy dependent mechanisms.**

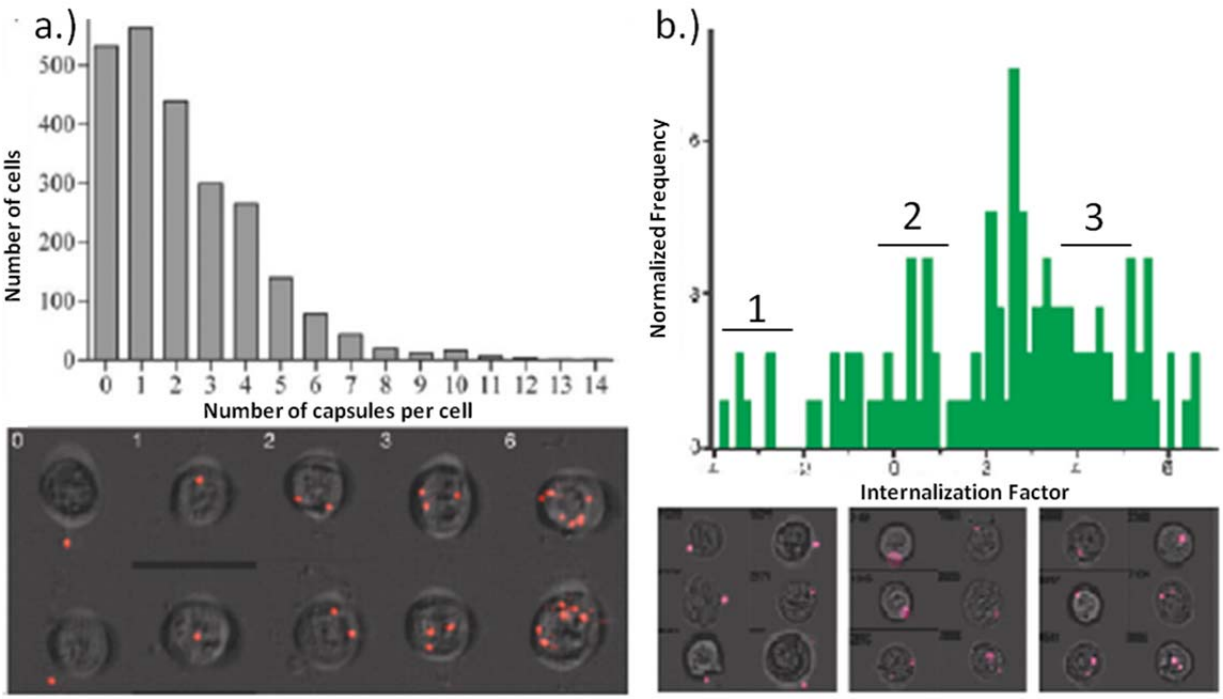



"Moreover, thanks to the high sensitivity and specificity of our ImageStreamX quantification protocol, we could quantify the dose-dependent intercellular transfer of CNTs from homotypic cells (EC to EC or HMM to HMM) and heterotypic cells (cross transfer from EC to HMM and vice versa). The implication of microvesicles in CNT transport and delivery was qualitatively evidenced by confocal and electron microscopy, while ImageStreamX analysis confirmed this transfer pathway on a statistically significant number of cells."

**Reference**: Marangon I, Boggetto N, Ménard-Moyon C, Venturelli E, Béoutis ML, Péchoux C, Luciani N, Wilhelm C, Bianco A, Gazeau F*., Intercellular carbon nanotube translocation assessed by flow cytometry imaging.* Nano Lett. 2012 Sep 12;12(9):4830-7. doi: 10.1021/nl302273p. Epub 2012 Sep 4.

# Uptake and intracellular fate of nanoparticle-based drug delivery vehicles

**Summary: This ImageStream assay quantifies the number of Doxorubicin-loaded nanocapsules within colon cancer cells (a) and their degree of internalization (b). The ImageStream's high speed image capacity enabled analysis of 20,000 events per sample for robust statistics. (a) Images were acquired using extended depth of focus (EDF) mode to focus 15 um deep images onto a single plane, which facilitated automated spot counting. (b) Normal depth of focus was used to calculate the internalization factor, which characterizes nanocapsule localization as (1) capsules bound to the cell membrane, (2) capsules in close proximity to cell membrane, (3) internalized capsules.**

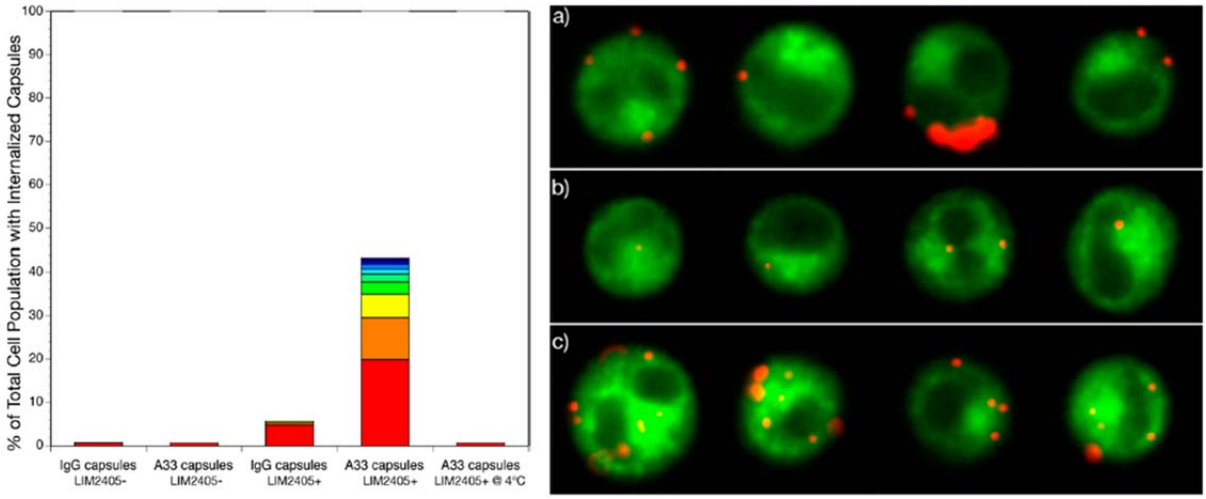



"In agreement with the flow cytometry analysis, IFC revealed a similar time-dependent interaction profile of PMA-SH capsules per cell when analyzed using ImageStream flow cytometry. IFC also revealed that, among the cells that are with capsules, on average only 2-3 endocytic compartments containing capsules are present within each cell after 24 h incubation."

**Reference**: Yan Y, Johnston AP, Dodds SJ, Kamphuis MM, Ferguson C, Parton RG, Nice EC, Heath JK, Caruso F., *Uptake and intracellular fate of disulfide-bonded polymer hydrogel capsules for Doxorubicin delivery to colorectal cancer cells*. ACS Nano. 2010 May 25;4(5):2928-36. doi: 10.1021/nn100173h.

## Binding and internalization of antibody functionalized capsules

**Summary: This ImageStream assay quantifies the specificity of huA33 monoclonal antibody-functionalized nanocarriers in targeting huA33 antigen positive cell lines. The ImageStream's high speed image capacity enabled aquisition of 10,000 events per sample for robust statistics. *Left Panel*: The IDEAS spot count feature was applied to determine the number of antibody-functionalized nanocapsules within individual cells. *Right Panel*: Nanocapsule localization was also investigated, and representative images are shown for (a) surface bound capsules, (b) cells with 1-2 internalized capsules, (c) cells with multiple internalized capsules.**




"Conventional flow cytometry does not allow the direct quantification of the number of capsules bound per cell; however, this can be achieved using imaging flow cytometry."

**Reference**: Johnston AP, Kamphuis MM, Such GK, Scott AM, Nice EC, Heath JK, Caruso F., *Targeting cancer cells: controlling the binding and internalization of antibody-functionalized capsules.* ACS Nano. 2012 Aug 28;6(8):6667-74. doi: 10.1021/nn3010476. Epub 2012 Aug 7.

## Reference list – Nanotechnology applications for the ImageStream

Johnston AP, Kamphuis MM, Such GK, Scott AM, Nice EC, Heath JK, Caruso F., *Targeting cancer cells: controlling the binding and internalization of antibody-functionalized capsules.* ACS Nano. 2012 Aug 28;**6**(8):6667-74. doi: 10.1021/nn3010476. Epub 2012 Aug 7.

Yan Y, Johnston AP, Dodds SJ, Kamphuis MM, Ferguson C, Parton RG, Nice EC, Heath JK, Caruso F., *Uptake and intracellular fate of disulfide-bonded polymer hydrogel capsules for Doxorubicin delivery to colorectal cancer cells*. ACS Nano. 2010 May 25;**4**(5):2928-36. doi: 10.1021/nn100173h.

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