In-vitro and in-vivo flow cytometry of circulating tumor cells – development of sorting, detection, characterization and possible treatment strategies.

Major collaborators from both sides:
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Background and major aims:

Dissemination of cells from the primary tumour in the form of metastasis in distant organs is responsible for 90% of cancer-related deaths. A cellular link between the primary malignant tumour and the peripheral metastases has been established in the form of circulating tumour cells (CTCs) in peripheral blood. It is of major interest to decipher the biology of this very unique cellular population, and ultimately, improving our level of understanding of how cancer “spreads and kills”. In the proposed project, we want to address this question in a concerted effort, and by developing and using a combination of in-vivo and micro-fluidic, in-vitro flow cytometry.

A major, still largely open question is in which ways and to what extent cancer cells entering the blood can circumvent the body’s defence system and settle at other locations. A major issue is that in patients with cancer, the amount of CTCs may be as low as 1 out of $10^5$ cells in blood (one cell per ml of blood). It is a tremendous challenge to specifically highlight these cells for selection and isolation, to separate them physically, and when separated, to perform analyses onto the sorted CTCs and extract a maximum amount of information out of this very minor amount of sample material. Aman Russom and colleagues have developed an inertial cell sorter which shows great promise for use in this context [2]. The research group of Jerker Widengren has developed and is in the very forefront of fluorescence-based readouts offering high specificity, single-molecule sensitivity [3] and spatial resolution far beyond the optical diffraction limit. This combination of sorting and fluorescence-based characterization has a considerable potential for CTC diagnostics. However, additional improvement of detection, sorting and characterization may also be obtained by use of nanoparticles. These particles can be coated with CTC-specific affinity molecules, harnessed with supermagnetic and fluorescence properties for sorting and/or detection purposes, or eventually even be designed for drug or gene delivery to the CTCs. Expertise in this area will be provided by the research group of Hongchen Gu. In addition, this project will explore the possibilities to implement the developed in-vitro flow cytometry on the flow cytometry technology developed by the Xunbin Wei group that has the capability to detect and quantify continuously the number and the flow characteristics of fluorescently labelled cells in vivo [1], and was recently demonstrated to be able to quantify fluorescently labelled circulating tumor cells introduced in live mice in real-time and in a reproducible manner.

Brief description of proposed research and research plan:
With the overall aim to improve our understanding of how CTCs spread and kill, and our abilities to monitor and eventually interfere with these events, we plan several activities. These activities can be run in parallel, and will benefit from the joint, complementary expertise of our research groups.
Based on ultrasensitive fluorescence spectroscopy/imaging concepts (JW), procedures will be tested for how the sensitivity and specificity can be increased further in in-vivo flow cytometry (XW).

In breast and prostate cancer diagnostics there is a choice between using core-needle biopsy or fine-needle aspiration (FNA) sampling of suspect cancer cells. A decisive, but very difficult parameter to measure is the risk of cancer cell dissemination at the time of sampling. In-vivo flow cytometry (XW) may provide the tool necessary to assess the risks of cancer cell dissemination, for FNA versus core needle sampling, and will be applied in collaboration with the JW group and clinicians in Stockholm.

The development of microfluidic in-vitro flow cytometry for CTC detection, sorting and characterization (AR and JW) will proceed in Stockholm, and will be combined with nanoparticle-based detection and sorting (HG). For the latter, both a fluorescence signal as well as magnetic properties of the nanoparticles can be used. In this context, these properties will be analysed in detail and new strategies will be developed to increase the sensitivity and specificity of both the detection and the sorting.

The ability of nanoparticles (HG) to adhere to cells of interest will be investigated by Fluorescence Correlation Spectroscopy (FCS) and high-resolution STED microscopy, combined with FCS and related methods can also be used to monitor the efficacy of molecular delivery from nanoparticles to cells. Based on the outcome the properties of the nanoparticles can be modified and further improved.

The subprojects described above define a starting point for the interaction between the groups. As a starting point, a PhD student will be engaged at KTH, in the group of Jerker Widengren, supported by Aman Russom and his research group. Given the complementary nature of the groups, and the overall common aim, these sub-activities can be extended and additional joint research activities are likely to follow. The described activities will be run in parallel, will be coordinated via regular contacts between the four PI s. The research can be expected to improve our fundamental understanding on cancer biology and will have important diagnostic and therapeutic implications.

REFERENCES