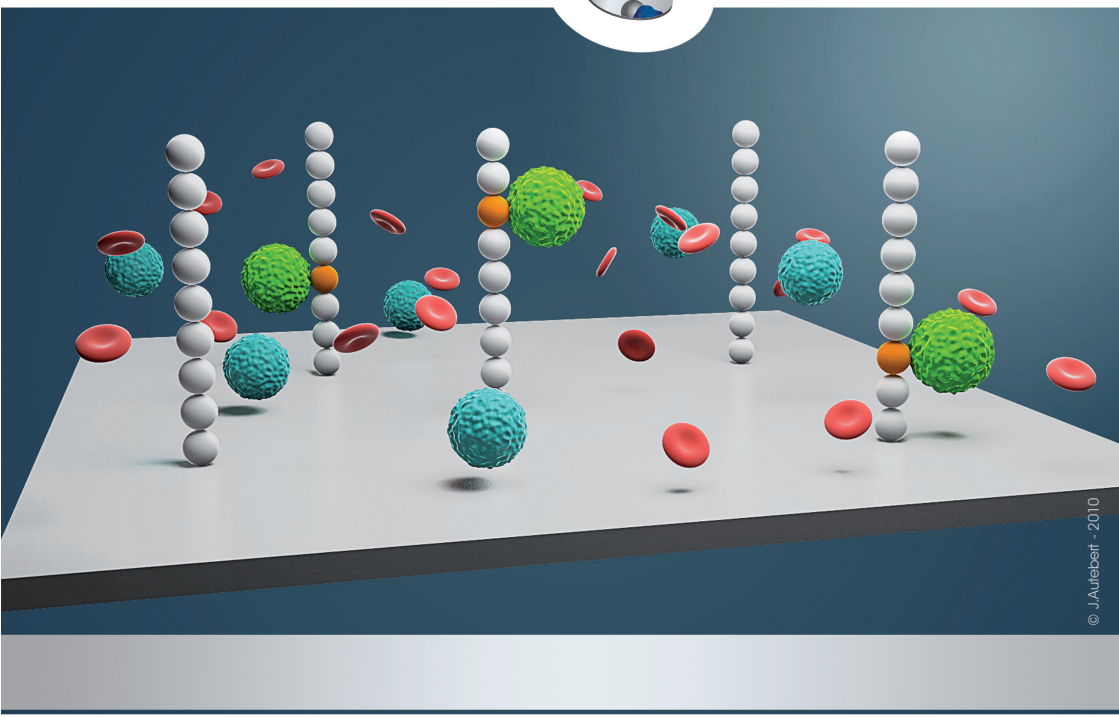
CAMINEMS



Integrated Micro-Nano-Opto Fluidic systems for high-content diagnosis and studies of rare cancer cells

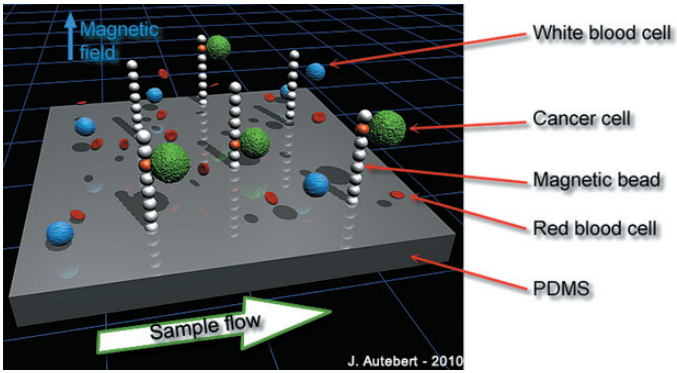
A European Project supported through the Seventh Framework Programme for Research and Technological Development

Outline & objectives

The project aimed at developing new tools based on microfluidics and nanotechnologies, to improve cancer diagnosis and prognosis. Cancer causes about 13% of all deaths in the world and thus represents a huge problem in public health.

In particular, today, about 90% of cancer deaths are due to metastases and therapeutic escape. At the origin of metastases are **Circulating Tumour Cells** (CTC), individual cells or small cellular issued from the primary cancer and transiently circulated in the blood. It would thus be a major breakthrough for treatments to be able to **perform a detailed molecular characterisation on CTC as a «liquid biopsy» providing information on known or unknown metastases.** Besides this major diagnosis and prognosis application, **being able to capture and to study CTC** would also be a highly valuable help in research, for understanding their metabolism and their response to existing or candidate drugs.

For all these applications, current technologies are insufficient both in sensitivity and specificity. They can detect micrometastases only in patients with advanced cancer, and they only allow the identification of a few biomarkers. **Providing a tool for overcoming these limitations, based on innovations in converging sciences, was the main objective of the CAMINEMS project.**



Capture principle: Epithelial circulating tumor cells are captured on the magnetic beads columns while the unwanted blood cells pass freely through the device.

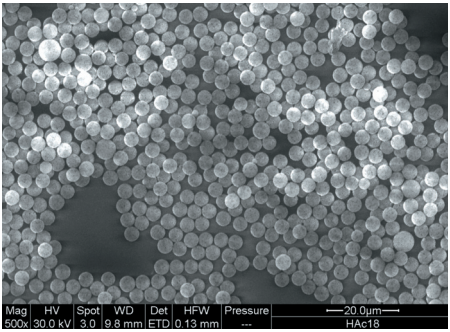
Achievements

The developed EPHESIA technology consists in self-assembling an array of antibody-bearing magnetic particles in a high throughput microfluidic device.

These beads create a self-assembled "micro-posts" array, with an aspect ratio much higher than that of microfabricated post arrays, allowing the use of innovative high resolution imaging with very little optical interference. Blood depleted from red blood cells (RBC) is then flown in the array with a uniform flow velocity, **CTC are captured and complex characterisation protocols** (membrane and cytosol immunophenotyping, detailed morphological analysis, genetic analysis by FISH) **can be performed in situ in a fully automated way**. This reduces the risk of cell loss or damage, as compared to the release and collection of the cells for delayed analysis in a separate device.

New routes for biofunctionalisation of magnetic particles

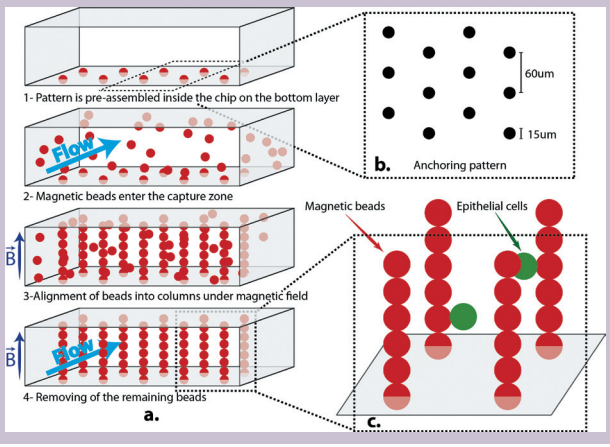
CAMINEMS's work on magnetic micro and nanoparticles has allowed several generations of particles to be synthetised. These new particles expand the range of biofunctionalisation available, as compared to state of the art systems. Notably capture using **HER2 surface antigen** was demonstrated. This protein is a major biomarker regarding personalised medicine of breast cancer, and **the possibility to specifically capture and screen CTC for this antigen opens new routes to CTC characterisation.**



Scanning electron micrograph of P(GMA-MCMEMA-EDMA) microspheres.

Principle of operation of the EPHESIA technology

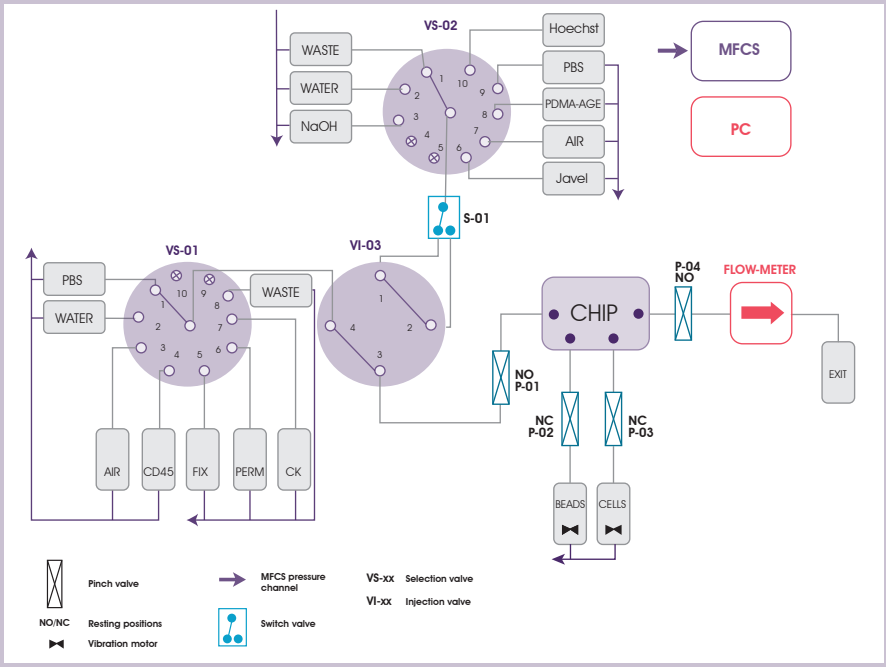
***a.** Columns formation process. A 30mT magnetic field is used to make the columns, and remaining beads are washed away with the flow.*
***b.** Anchoring pattern, composed of 48 rows per 1000 columns. Holes are 5um deep.*
***c.** Capture principle. Epithelial cells pass through the columns pattern and get caught by an antibody/antigen interaction.*



New microfluidic chips for the capture of CTC

New microfluidic chips allowing large blood volumes (7.5 ml) throughput were designed and developed successfully, using the new EPHESIA technology. A new approach for the preparation of magnetic arrays in these chips was also developed. **This technique is based on an original capillary self-assembly method. Column formation, CTC capture and staining were fully automated during the project.** This work has overall led to several **oral and poster presentations** at conferences, **two publications** so far in the Proceedings of the National Academy of Science USA and in Lab on Chips, and a **joint patent**.

Fluidic circuit of the CAMINEMS device



Innovative optics and high imaging performance

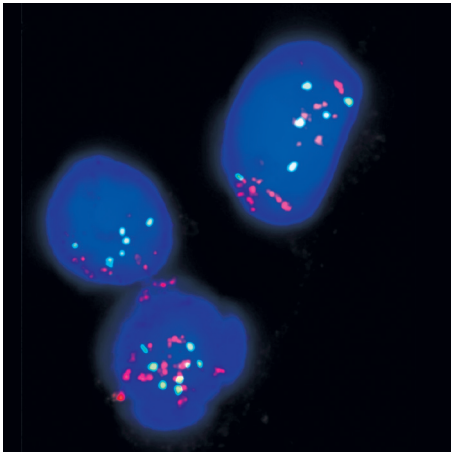
CAMINEMS developed a high sensitivity generation system and high resolution imaging system. Denoising has yielded results beyond expectations: decrease by a factor >10 of acquisition time as compared to conventional analysis.

Samples multicentre and multi-techniques validation

CAMINEMS system was first validated on the characterisation of lymphoma. An agreement of 100% on 20 patients was achieved in a blind comparison with a combined use of flow cytometry and cytological observation[1] (PNAS 2010).

The capture efficiency (90%) and specificity (x2500 enrichment versus white blood cells) was then characterised by spiking cell lines with different expression levels. Using various cell lines (SKBR3, MCF7 for breast cancer, PC3 for prostate cancer, Raji and Jurkat as lymphoid cells), we obtained **a capture efficiency up to 90.6%** for cell quantity as low as 50 cells per sample, the **best result worldwide to our knowledge, and a specificity better than 99.6%**[2].

The in situ quantitative FISH genetic analysis of HER2 amplification was also demonstrated. Finally, the system was tested for CTC capture in patients with breast, prostate and lung cancer, in a multi-centers, blind protocol. Sensitivity comparable to that of CellSearch® was achieved.



On-chip FISH result of SKBR-3 cell lines.

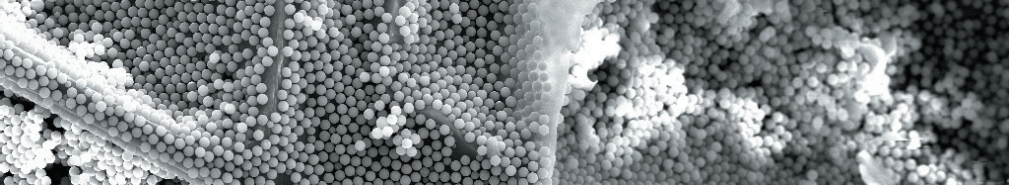
Perspectives

Very encouraging results suggest that the CAMINEMS project will allow routine quantitative genetic testing by direct in situ Fluorescence hybridisation (FISH) of the captured CTC, in a much less labor-intensive and with a higher success rate than currently achieved by state of the art methods.

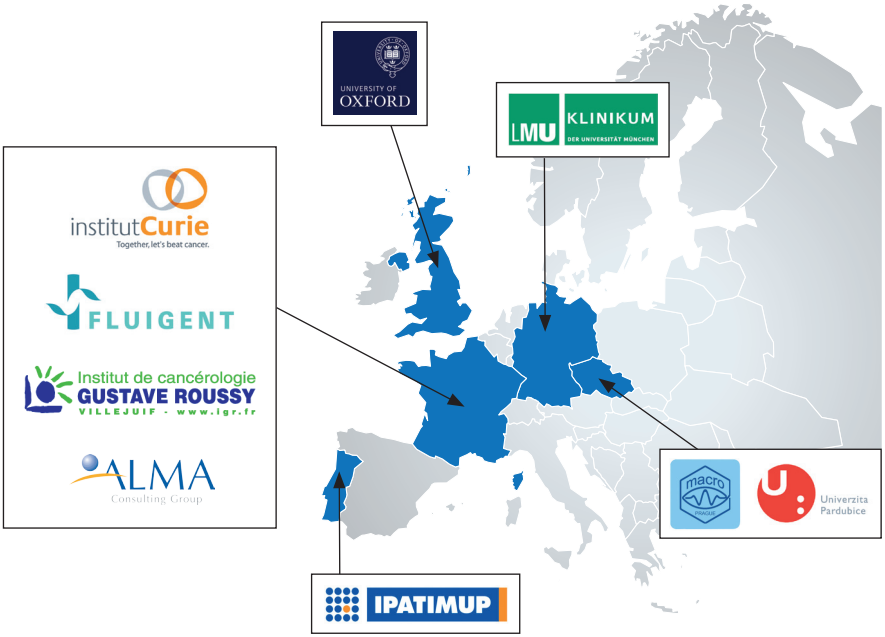
The promises of the project are strong enough, that we consider future industrial exploitation. CAMINEMS is now working on a second generation pre-industrial prototype, and searching partners for industrialisation and commercialisation.

1. *Microfluidic Sorting and High Content Multimodal Typing of Cancer Cells in Self-Assembled Magnetic Arrays, Saliba et al., Proc Natl Acad Sci USA, 107, 14524-529 (2010)*

2. *Microfluidic: an Innovative Tool for Efficient Cell Sorting, Julien Autebert, Benoit Coudert, François-Clément Bidard, Jean-Yves Pierga, Stéphanie Descroix, Laurent Malaquin, and Jean-Louis Viovy, Methods (Elsevier) in press, available online : DOI : 10.1016/j.ymeth.2012.07.002*



Consortium



Acknowledgement

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CAMINEMS

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