Personalised treatment of cancer depends on a deep understanding of the unique genetic characteristics of a tumour. We spoke to **Gianni Medoro**, **Maximilian Sergio** and **Elena Bevilacqua** about their work in developing the DEPArray™ technology, a new approach to cell isolation that could open up new possibilities in precision medicine

A single cell technology for personalised medicine

The genetic makeup of a tumour varies significantly among individual cases, and treatment tailored to the precise characteristics of the tumour offers the best chance of leading to a better response. This relies on an accurate molecular diagnosis of the individual case and an understanding of the precise nature of the tumour, a topic that has attracted a lot of attention in clinical research. "There is a clear need to deepen our understanding of the underlying molecular mechanisms, which can be achieved by analysing the genome of tumour cells. This is a new approach that is emerging in the field of personalised medicine," says Gianni Medoro, PhD. As Chief Technology Officer at Menarini Silicon Biosystems, Dr Medoro led the development of DEPArray™, a proprietary technology that could open up new possibilities in precision medicine, particularly with respect to cancer. "What we have developed with the DEPArray™ technology is a new approach to cell isolation, which enables accurate genetic analysis of tumours at the single cell level," he explains.

This is part of a wider paradigm shift in the treatment of disease, away from a generic approach, towards more personalised treatment. The DEPArray™ technology holds clear potential in these terms, enabling scientists to gain deeper insights into a tumour. "The DEPArray™ technology is aimed at enabling more precise molecular diagnosis of a tumour. In future this will allow clinicians to match each tumour with the correct combination of drugs, that will then work more effectively," says Elena Bevilacqua, PhD, Product Manager at Menarini Silicon Biosystems. This kind of personalised approach to therapy may offer several advantages. "For example to avoid the use of therapies that cannot work for some specific patients. This is important to prevent adverse side-effects and to offer alternatives to the patient that might work more effectively," points out Dr Medoro.

The DEPArray™ NxT System, the latest development of the technology, is composed of three elements: a benchtop instrument, a disposable microfluidic cartridge and a proprietary software - the CellBrowser™. The core of the technology is the microsystem cartridge, which integrates a silicon chip, microfluidic chambers and valves. Microelectronics and microfluidics are combined synergically in the cartridge to provide unique single-cell sorting capabilities in a highly-automated platform, providing a simple and reliable system for isolating pure, single, viable rare cells from a heterogeneous sample, for culture or molecular analysis.

The working principle of the DEPArray™ technology is based on the ability of a non-uniform electric field to exert forces on neutral, polarizable particles, such as cells, that are suspended in a liquid. This electrokinetic principle, called dielectrophoresis (DEP), is exploited to create - in the micro-chamber at the core of the cartridge - a patterned field force composed of tens of thousands of microscopic attraction regions (called DEP 'cages') capable of 'trapping' cells in stable levitation and controlled position.

A selection of mages of the core of the technology: the microsystem cartridge, which integrates a silicon chip, microfluidic chambers and valves.

"An array of about 300k micro electrodes integrated in a semiconductor chip allows the generation of up to 30.000 'DEP cages', each able to capture a single cell in stable levitation through the application of gentle dielectrophoretic forces," explains Maximilian Sergio, PhD, integrated circuit design (ICD) manager at Menarini Silicon Biosystems.

The fluorescent microscope integrated in the DEPArray™ instrument allows the acquisition of high-resolution images for each individual cell in the sample, thus enabling accurate cell analysis and selection based on fluorescence and morphology. High-resolution imaging is key to minimizing errors and adding precision and robustness to the system. The proprietary CellBrowser™ software then elaborates the images and enables automatic or operator-assisted selection of the desired cell. "Once identified each target cell can be isolated from the other cell types automatically. DEP cages are moved by a change in the electric field pattern concurrently and independently, step by step, along trajectories calculated by the software, dragging each selected cell from the original location into a dedicated Parking chamber," explains Maximilian Sergio.

From the Parking chamber, target cells are moved into the Recovery chamber and from there are ejected directly into a recovery support through an accurate microfluidic control, without any risk of cross-contamination between different samples. The recovery procedure can be repeated to obtain from the same sample multiple recoveries of individual target cell or group of cells separately.

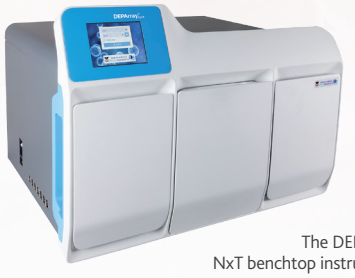
Applying DEPArray™ in liquid biopsy

Liquid biopsies are essentially non-invasive blood tests to detect circulating biomarkers like circulating tumour cells (CTCs) that are shed from tumours into the bloodstream. Genetic analysis of these cells provides all the information necessary to understand the genetic mutations of the tumour and to identify molecular targets for personalized therapies.

DEPArray™ technology sets a new standard of excellence in this setting, as it allows the precise identification and isolation of these rare CTCs from enriched blood, right down to the single cell. "CTCs are very important, as they are a very good representation of patients' tumour genomic variability, so there is a strong motive in isolating and analysing those cells, so as to understand the genetic characteristics of a tumour," continues Dr Medoro. "The problem with circulating tumour cells is that they are extremely rare – they are present in blood at a ratio of around one tumour cell to every billion normal cells. Our technology provides a solution to this problem by offering the capability to identify and isolate those cells with 100 percent precision down to single cells, with a high level of automation and reliability."

Gianni Medoro (right), CTO and Nicolò Manaresi (left), CSO of Menarini Silicon Biosystems presenting the DEPArray NxT at AACR Annual Meeting in New Orleans.

The **DEPArray™ technology** is aimed at supporting precision medicine in oncology by enabling **precise molecular characterization** of a tumour. This will help clinicians to develop and implement protocols to match each patient with the correct combination of drugs, that will then work more effectively and **improve patient outcomes**


The DEPArray NxT benchtop instrument

CTCs play an important role in the advanced stages of cancer, as they are effectively the seeds of metastasis. "Once these cells have entered the bloodstream, they may then reach and colonize a secondary site in the body, which can lead to metastasis," outlines Bevilacqua. The ability to isolate these cells and analyse them, on the single cell level, gives an indication of the state and genetic makeup of the disease.

This will give clinicians the foundations on which they can identify the correct treatment, and then follow the patient's progress as the disease evolves. A tumour by nature evolves and changes over time, also in response to therapy, so it therefore may be necessary to adapt and modify treatment; applying the DEPArray™ technology in liquid biopsy offers a non-invasive means of monitoring the progression of a tumour over time, which can then help to inform therapeutic decisions.

"The great advantage of this type of analysis is that it will allow clinicians, in the near future, to follow the evolution of the disease over time with a simple, non-invasive blood drawing," explains Dr Medoro. "The goal is to develop a blood test that can be done at regular intervals to see if a therapy is working, helping doctors not only to identify what is the best treatment for individual patients but also to follow how the disease is responding to that treatment."

The benefits of the DEPArray™ technology in liquid biopsy are well illustrated by the example of a recent study at the Manchester Cancer Research Centre in the UK (https://www.nature.com/articles/nm.4239). Researchers there used DEPArray™ to isolate circulating tumour cells from the blood of patients affected by small-cell lung cancer, then analysed the genetic profile of those

cells one by one. "By analysing the copy number profiles of single circulating tumour cells, they were able to identify a genetic signature correlated to the response of these patients to chemotherapy. This is an important result, as it represents an example of how useful the DEPArray™ is to isolate those cells individually, to support the study of whether a particular therapy might be an effective treatment for an individual patient," explains Dr Medoro.

This is a clear example of the wider importance of this research. "It is widely understood now that not all patients respond in the same way to therapy, so a generic therapy for a cancer patient may not prove effective," says Bevilacqua. "Therefore you need to go into the genetics of the tumour; you need to understand what is driving it."

Dissecting tumour heterogeneity in tissue biopsies

A further important characteristic of a tumour is its heterogeneity; every tumour is different and even within a single tumour specimen there are different populations of cancer cells and normal cells that have distinct characteristics. DEPArray™ technology holds clear importance to personalized medicine because of its ability to 'dissect' the heterogeneity of tumour biopsies. It is necessary to break the tumour biopsies by breaking the tumor down into its components to enable to analyse them separetely.

Starting from a minute, low-cellularity tumour biopsy, DEPArray™ can isolate and recover groups of tumour cells or individual cells, allowing a complete and precise understanding of tumour biology, which can then guide a decision on the most effective molecular, target treatment.

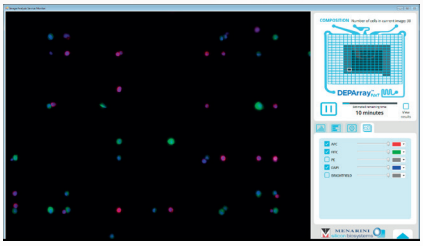
"The technology can be used to achieve a level of purity which enables the genetic analysis of tumour cells, even from small biopsies" outlines Dr Medoro. "Furthermore, each tumour cell may have different genetic characteristics, so analysing just the bulk of the cells in many cases is not sufficient to gain a deeper understanding of the tumour overall. The DEPArray™ technology is able to isolate tumour cells one by one or in pool, with high levels of purity, to reduce the background noise that might make it difficult to interpret the genetic data."

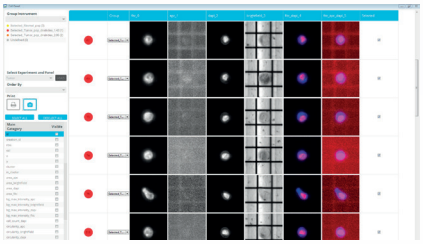
This underlines the wider potential of the DEPArray™ technology, now the team at Menarini Silicon Biosystems are looking to introduce it more widely in clinical cancer research and from there to diagnostic settings. Bevilacqua says there are cultural and technical barriers to overcome before this can be achieved. "There are certain standard, well-established practices in the clinic, and we need to ensure that the value of the technology is well understood. The other major challenge is technological, which is one of the major reasons why Menarini Silicon Biosystems is part of the ADMONT project, an EU-backed initiative developing a pilot line for certain technologies.

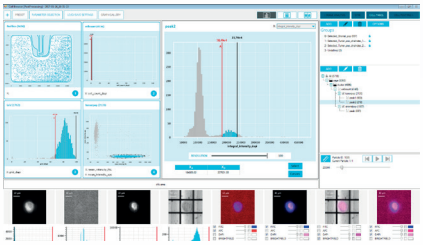
ADMONT project

The DEPArray™ platform is currently installed in several research centres worldwide as a 'Research Use Only' instrument to support clinical research activities in life science, but its potential is not limited to the life-science market. "With our involvement in the ADMONT project, we could accelerate the optimization of the technology to bring it from a research instrument to an instrument that has all the characteristics to be widely adopted in clinical settings," says Dr Medoro. "We are working to introduce into the pilot line all the requirements to ensure that this technology is compatible with the diagnostic market, not only in terms of cost and quality, but also ease of use and automation."

"At the end of the project, we will have a technology which perfectly matches the needs of the users," stresses Sergio.







Representative images of the CellBrowser™ software which enables image-based selection and recovery of specific cells and cell populations in a sample.

ADMONT

Advanced Distributed Pilot Line for More-than-Moore Technologies

Project Objectives

The DEPArray™ systems is an innovative technology, developed by Menarini Silicon Biosystems, to sort, manipulate, and collect individual rare cells or groups of cells from heterogeneous samples. Using an electronic chip-based microfluidic cartridge and fluorescent image-based analysis, the DEPArray isolates 100% pure tumor cells from tissue biopsies or single circulating tumor cells form blood. By participating in the ADMONT project, Menarini Silicon Biosystems is optimizing the DEPArray™ system (CMOS/MEMS, Microfluidics, Automatic machine and Software) to accelerate its penetration in the cancer diagnostic markets.

Project Funding

The ADMONT project has received funding from:

- the ECSEL Joint Undertaking under grant agreement No 661796. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and Germany, Finland, Sweden, Italy, Austria, Hungary.

- the Austrian Ministry for Transport, Innovation and Technology (BMVIT) under the program ICT for future (IKT der Zukunft).

- the Swedish Governmental Agency for Innovation Systems.

Project Partners

https://admont-project.eu/index.php/partners

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Gianni Medoro, Ph.D.

Gianni Medoro, Ph.D. Chief Technology Officer and co-founder of Silicon Biosystems (now Menarini Silicon Biosystems). He is the inventor of the core technology patent of DEPArray™, and co-inventor behind more than 30 European and US patent families. He holds a PhD in Electrical Engineering and Computer Sciences. He is co-author of more than 70 scientific publications in the field of Lab-on-a-Chip.

Admont

DEPArray™ NxT