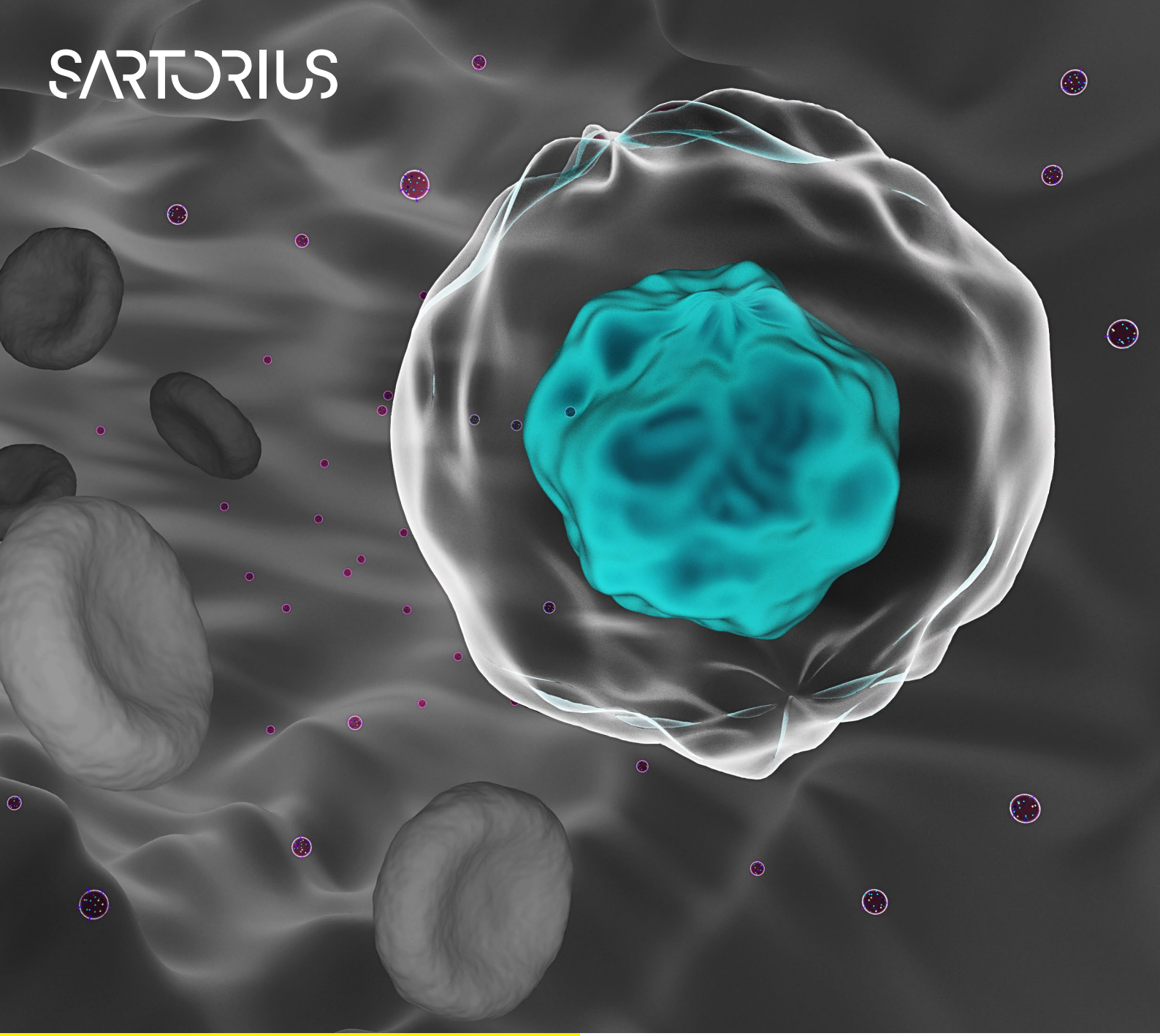


Cell Selection and Retrieval

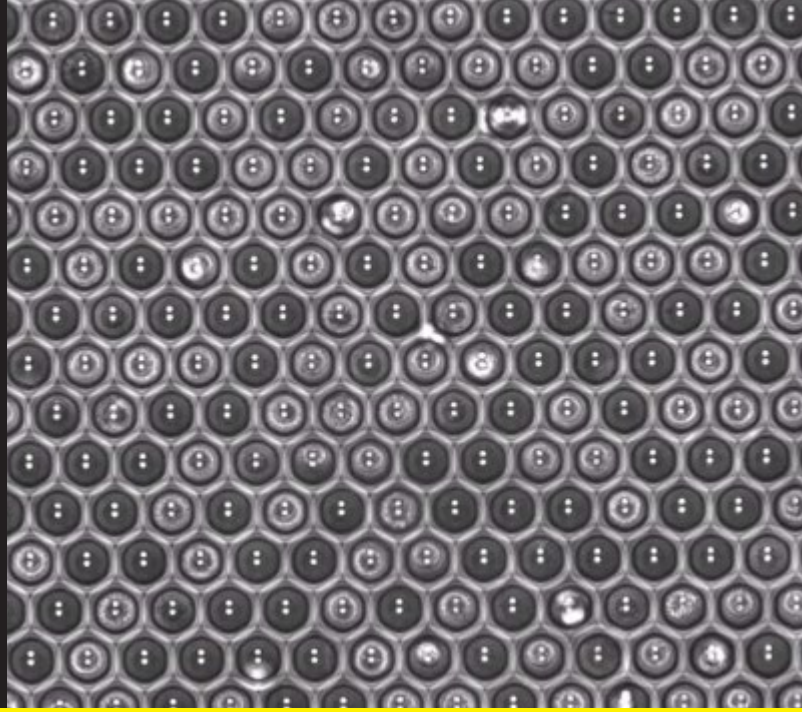
Rare Cell Isolation

Rapid and Accurate Identification of Circulating Tumor Cells

- Custom designed consumables to facilitate the rapid identification of rare target cells
- Advanced software to rapidly classify cells based on morphological and fluorescent parameters
- No cell loss during isolation, classification or transfer
- No cross-contamination within or between assays

Rare Cell Isolation

Understanding the heterogeneity within a cell population can reveal a wealth of insight into cell fate and function, with tumor heterogeneity playing an especially crucial role in both disease progression and resistance to therapies. One of the main obstacles in the treatment of cancer is tumor metastasis, which describes how new tumors originating from a primary site are established at secondary locations. Advances in high-throughput genome sequencing, gene editing, advanced cell models and instrument technology therefore enable scientists to dissect the underlying mechanisms that support metastasis, predominantly comprising of circulating tumor cells (CTCs) and CTC clusters.

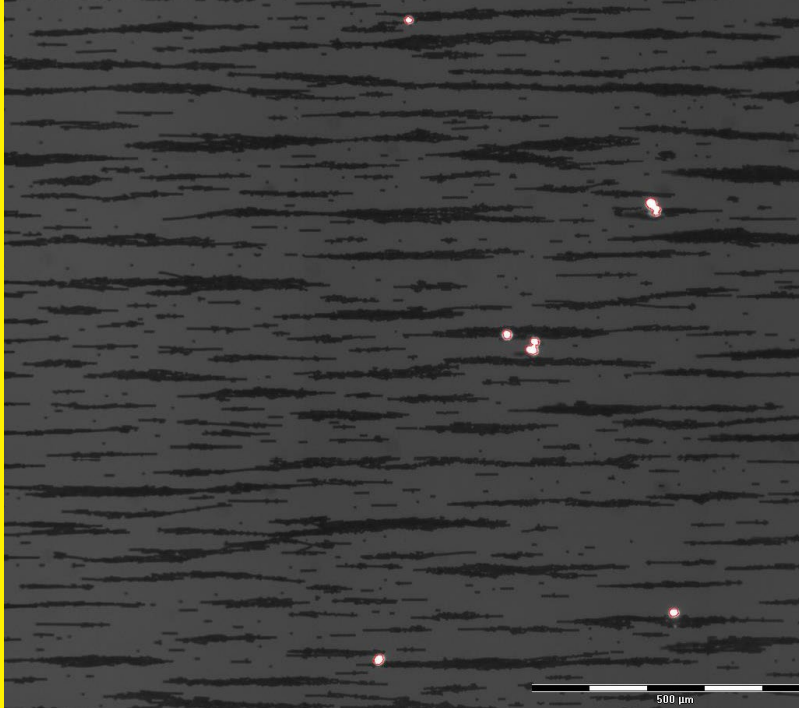


Advantages of the CellCelector Flex in Rare Cell Isolation

- Highly accurate isolation of individual rare cells and clusters using custom designed consumables
- Accurate image-based cell selection using over 140 morphological parameters and gates to accurately identify the rare cell of interest
- Gentle isolation of cells at low pressure and without sheer force
- Image-based single cell capture verification
- Robust cross contamination controls preventing any cellular carryover between wells or assays

Circulating Tumor Cells

Metastasis is responsible for over 90% of cancer-related deaths and is a key area of oncological research. In order to colonize a secondary site, CTCs require a host of advantageous mutations that allow them to escape immune surveillance and exit the circulatory system. Standard CTC isolation and characterization techniques, however, can be challenging due to low target cell numbers and subsequent cell loss, prolonged processing times, and leukocyte contamination.



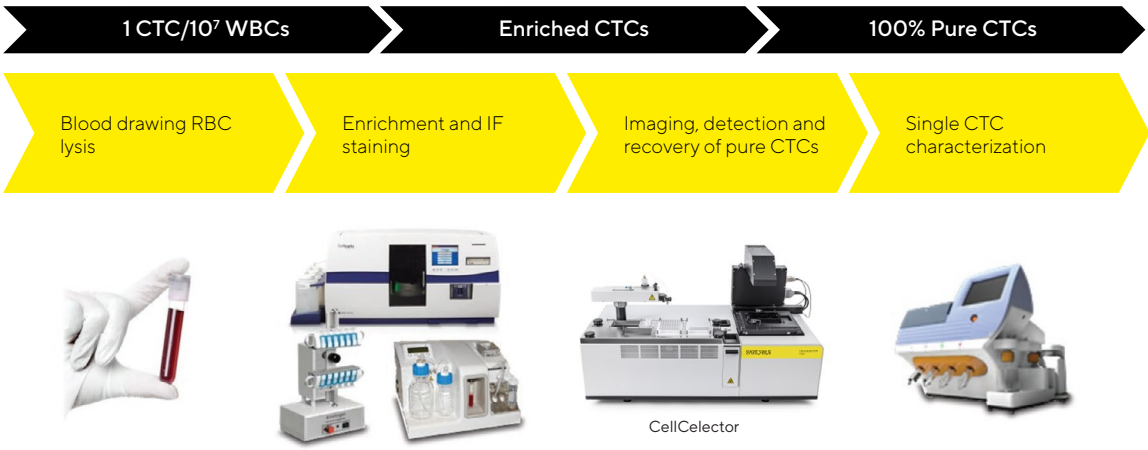
CTC Enrichment and Isolation
A typical CTC isolation and analysis workflow therefore involves the following steps:

1. Blood draw and sample processing
2. CTC enrichment and staining
3. Imaging and isolation of pure CTCs
4. Single-CTC characterization

A wide range of analytical methods have been developed for CTC detection, enrichment and isolation, with CTC-specific properties such as surface marker expression or morphological features used to identify cells of interest. Nevertheless, existing cell culture protocols can influence the health, viability and function of cells, with cell loss common during enrichment protocols, and carry-over with contaminating background cells resulting in interference with downstream studies.

Automated Rare Cell Isolation
Automated systems for the identification and isolation of pure single cells offer many advantages over traditional methods for rare cell isolation and retrieval. Platforms like the CellCelector can reliably deliver 100% pure single CTCs or CTC clusters from samples processed using common enrichment techniques.

The CellCelector system scans cells in brightfield, phase contrast or fluorescence to identify cells of interest, before liquid buffered single-use glass capillaries transfer cells through low pressure, gentle aspiration.
CTCs are then recovered into the destination vessel of choice for downstream analysis or recultivation, with full documentation and traceability of each cell retrieval event, complete with before and after picking images.



Circulating Tumor Cell Research: CellCelector Advantages

Sievewell Nanowell Arrays

Sievewell chips consist of a thin membrane containing 370,000 Nanowells, with outer dimensions corresponding to that of a standard microscope slide. Within each Nanowell there are two 2 µm diameter pores which connect the volume above the chip to a microgap situated below the membrane (Fig. 1). This facilitates a one-directional flow from the top of the liquid chamber, through the pores, into the liquid gap underneath. The separation and isolation of hundreds of thousands of single cells within each Nanowell, coupled with the complete absence of any cell loss, makes Sievewell technology extremely attractive for rare single cell applications. Two Sievewell arrays are available with differing Nanowell sizes. More information can be found in the **"CellCelector Flex – Capillaries, Tips and Consumables"** technical guide.

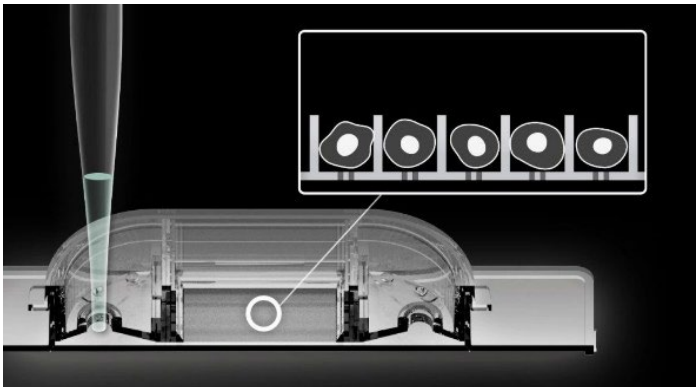


Figure 1: Sievewell Nanowell Arrays.

Nanowell Arrays for Rare Cell Isolation

The use of Nanowell arrays offer researchers a rapid solution for the screening and isolation of single cells. A two macrowell, 25 µm sized Nanowell glass slide has also been specifically developed for rare cell isolation, providing researchers a total of 400,000 Nanowells per slide to screen for their rare cell of interest. The ability to separate single cells in small wells with a defined spacing allows extremely high seeding densities of tens of thousands of cells, without compromising on the ability to screen, analyze or pick individual cells (Fig. 2). More information can be found in the **"CellCelector Flex – Capillaries, Tips and Consumables"** technical guide.

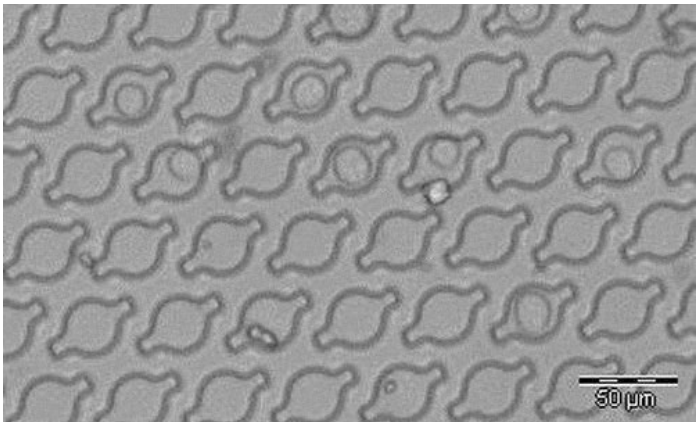


Figure 2: Nanowell Arrays for Rare Cell Isolation.

MagnetPick Rare Cell Isolation Slides

MagnetPick slides have been designed for the specific isolation and picking of rare single cells in suspension that have been processed using positive immunomagnetic enrichment methods. After enrichment, samples are loaded straight onto the MagnetPick glass slides where a magnetic field aligns the beads (Fig. 3), whilst holding the cells in place to keep their position stable for the screening, detection and picking of individual cells or clusters using the CellCelector Flex. More information can be found in the **"CellCelector Flex – Capillaries, Tips and Consumables"** technical guide.

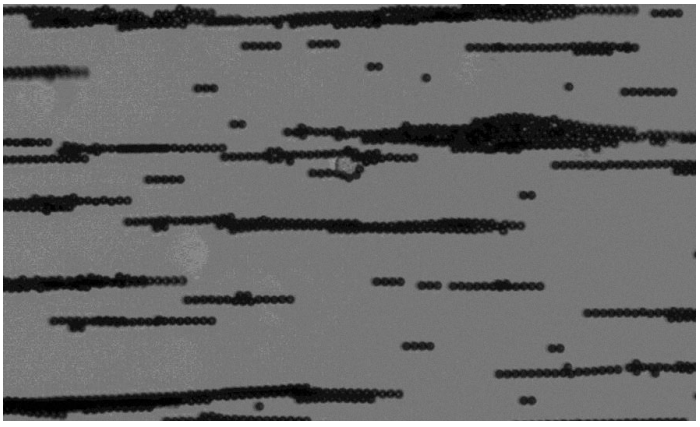


Figure 3: MagnetPick Rare Cell Isolation Slides. The magnetic field generated by the MagnetPick slide adapter immobilizes magnetic beads with attached target cells on the slide surface.

Rapid Cell Identification and Classification

Rare cells can be rapidly identified based on the gating of common morphological parameters during the analysis stage, which include area, diameter, elongation, shape factor and sphericity variables. Over 140 different parameters are available with short descriptions provided for each parameter within the software to ensure that target CTCs are rapidly identified. In addition, up to 5 fluorescent channels can be used to identify specific cell markers, allowing for a total of 14 different fluorochromes to be used in tandem. Common fluorochromes can be selected from a pre-configured library within the CellCelector software, with additional fluorochromes entered manually or imported in bulk. Corresponding excitation and emission filters can be configured, with additional filters easily added for the detection of non-standard fluorophores. More information can be found in the **"CellCelector Imaging and Image Analysis"** technical guide.

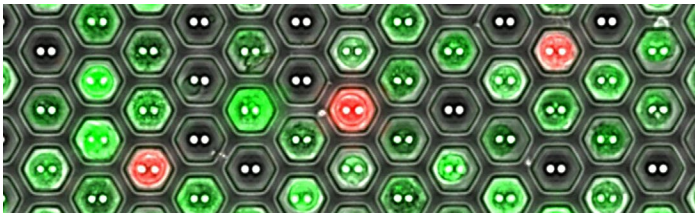


Figure 4: Single Cell Fluorescence. Individual cells within a Sievewell Nanowell Array stained with green and red fluorescent markers.

Robust Cross-Contamination Control

Single cell glass capillaries can be automatically sterilized between picks using 70% Ethanol, or any other sterilization solution of the operator's choice. Pre-defined volumes of sterilization solution are aspirated into the glass capillary and then dispensed back into the sterilization container, with the process being repeated according to the number of desired rinsing loops. Capillaries remain in the sterilization solution for a configurable amount of time before being removed, dried, and then moved to the next object to be transferred. More information can be found in the **"CellCelector Sample Deposition"** technical guide.

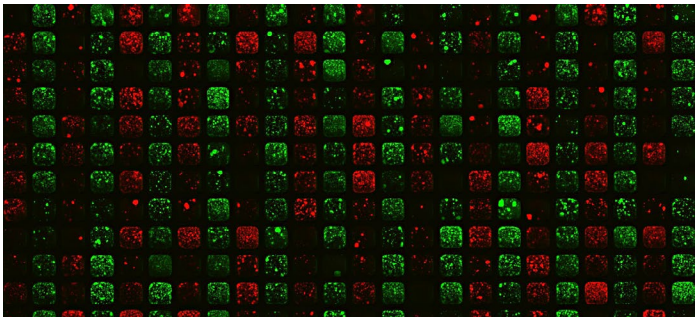


Figure 5: Robust cross-contamination control. GFP and RFP cells were alternatively picked and grown to identify any individual cell cross-over between wells.

Circulating Tumor Cell Publications

CTC Isolation From Various Cancer Types

Breast Cancer

Gkountela, S *et al.,* Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell* 176 (1–2): 98–112 (2019)

Acheampong, E, *et al.* Powering single-cell genomics to unravel circulating tumour cell subpopulations in non-small cell lung cancer patients. *J Cancer Res Clin Oncol* (2023)

Diamantopoulou, Z., *et al.* Implementing microwell slides for detection and isolation of single circulating tumor cells from complex cell suspensions. *Nature* 607, 156–162 (202)

Yang, L., *et al.* Implementing microwell slides for detection and isolation of single circulating tumor cells from complex cell suspensions. *Cytometry* 2022, 1–11. (2022)

Donato, C. *et al.,* Hypoxia Triggers the Intravasation of Clustered Circulating Tumor Cells, *Cell Reports,* 32 (10), 108105 (2020)

Franken, A., *et al.,* Detection of ESR1 Mutations in Single Circulating Tumor Cells on Estrogen Deprivation Therapy but Not in Primary Tumors from Metastatic Luminal Breast Cancer Patients, *The Journal of Molecular Diagnostics,* 22 (1), 111-121 (2020)

Sprouse, M.L. *et al.* PMN-MDSCs Enhance CTC Metastatic Properties through Reciprocal Interactions via ROS/Notch/Nodal Signaling. *Int. J. Mol. Sci.* 20(8), 1916, (2019)

Reinhardt, F. *et al.* Diagnostic Leukapheresis Enables Reliable Transcriptomic Profiling of Single Circulating Tumor Cells to Characterize Inter-Cellular Heterogeneity in Terms of Endocrine Resistance. *Cancers,* 11(7), 903 (2019)

Ao Z. & Liu X. Fiber-Optic Array Scanning Technology (FAST) for Detection and Molecular Characterization of Circulating Tumor Cells. In: *Circulating Tumor Cells,* 235–246 (2017)

Neumann M.H. *et al.* Isolation and characterization of circulating tumor cells using a novel workflow combining CellSearch® and CellCelector™. *Biotechnol Prog.* 33(1):125-132 (2016)

Schneck H. *et al.* EpCAM-Independent Enrichment of Circulating Tumor Cells in Metastatic Breast Cancer. *PLOS One.* 10(12):e0144535 (2015)

Heidary, M. *et al.* The dynamic range of circulating tumor DNA in metastatic breast cancer. *Breast Cancer Res.* 16(4), 421 (2014)

Szczerba, B. *et al.* Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* 566: 553-557 (2019)

Lampignano, R. *et al.* The combination of Parsortix™ and CellCelector™ enables the characterisation of EpCAMneg CTCs in breast cancer. *ISMRC 2016 Hamburg.*

Brain Cancer

Lynch, D., *et al.,* Isolation of Circulating Tumor Cells from Glioblastoma Patients by Direct Immunomagnetic Targeting. *Appl. Sci.,* 10(9), 3338, (2020)

Bang-Christensen, S.R. *et al.* Capture and Detection of Circulating Glioma Cells Using the Recombinant VAR2CSA Malaria Protein. *Cells* 8(9):998, (2019)

Krol I, *et al.* Detection of circulating tumour cell clusters in human glioblastoma. *British Journal of Cancer* 119, 87–491 (2018)

Colorectal Cancer

Adalsteinsson, V.A. *et al.* Single cells from human primary colorectal tumors exhibit polyfunctional heterogeneity in secretions of ELR+ CXC chemokines. *Integr. Biol. (Camb).* 5(10):1272-81 (2013)

Lung Cancer

Ding, P.N. *et al.* The predictive and prognostic significance of liquid biopsy in advanced epidermal growth factor receptor-mutated non-small cell lung cancer: A prospective study. *Lung Cancer* 134:42-45 (2019)

Yao, X. *et al.* Tumor cells are dislodged into the pulmonary vein during lobectomy. *J Thorac Cardiovasc Surg.* 148 (6), 3224-31 (2014)

Ovarian Cancer

Blassl C. *et al.* Gene expression profiling of single circulating tumor cells in ovarian cancer - Establishment of a multi-marker gene panel. *Molecular Oncology* 10(7):1030-42 (2016)

Giannopoulou L. *et al.* Liquid biopsy in ovarian cancer: recent advances on circulating tumor cells and circulating tumor DNA (Review). *Clin. Chem. Lab. Med.* Epub (2017)

Pancreatic Cancer

Kim, D.U. *et al.* Comprehensive characterization of single circulating rare cells through automated picking and high-throughput mRNA profiling in patients with pancreatic cancer. *Meeting abstract Gastrointestinal Cancers Symposium* (2019)

Prostrate Cancer

Nimir, M. *et al.* Detection of AR-V7 in liquid biopsies of castrate resistant prostrate cancer patients: a comparison of AR-V7 analysis in circulating tumor cells, circulating tumor RNA and exosomes. *Cells* 8(7);688 (2019)

Ladurner, M., *et al.* Validation of Cell-Free RNA and Circulating Tumor Cells for Molecular Marker Analysis in Metastatic Prostate Cancer. *Biomedicines.* 9(8):1004 (2021).

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Luk, A., *et al.* CTC-mRNA (AR-V7) Analysis from Blood Samples—Impact of Blood Collection Tube and Storage Time. *Int. J. Mol. Sci.* 18(5), 1047 (2017)

Ma Y. *et al.* Droplet Digital PCR Based Androgen Receptor Variant 7 (AR-V7) Detection from Prostate Cancer Blood Biopsies. *Int. J. Mol. Sci.* 17(8), E1264 (2016).

Lohr, G.J. *et al.* Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer. *Nature Biotechnology* 32, 479–484 (2014)

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