SMART CATCH

A new step forward in circulating tumor cell detection

At SmartCatch, we believe the future of oncology lies in fully integrated, end-to-end workflows—from sample to actionable insight.

Today, we're excited to share a new application note demonstrating the seamless integration of:

- SmartCatch X-Tracker technology for tumor cell enrichment
- @Thermo Fisher Scientific CellInsight™ CX7 high-content imaging platform
- @NeoVirTech's automated analysis and classification pipeline

Together, these technologies enable a complete, standardized workflow delivering:

- Efficient enrichment of tumor-related cells
- High-resolution, multiparametric imaging
- Fully automated, operator-independent enumeration and classification

The outcome?

A robust, scalable, 96-well–compatible workflow capable of multiplexing up to 7 markers—unlocking advanced phenotyping of circulating tumor cells (CTCs) in melanoma and beyond.

This proof of concept illustrates how integrated solutions can accelerate the adoption of minimally invasive, real-time tumor monitoring, bringing us closer to routine clinical use.

#CTC #LiquidBiopsy #Oncology #CellImaging #Automation #PrecisionMedicine #SmartCatch

THE FULL APPLICATION NOTE IS NOW AVAILABLE ON OUR WEBSITE

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A Standardized Workflow for Melanoma Cell Detection in Blood: X-Tracker® Coupled with High-Content Imaging and Automatized Enumeration

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Melanoma is one of the most aggressive skin cancers, marked by a rising global incidence and pronounced tumor heterogeneity, which contributes to metastatic progression and recurrence in approximately 5% of patients despite early clinical management [Huang 2022]. To overcome the limitations of conventional serum markers such as LDH, circulating tumor cells (CTCs) offer a dynamic and noninvasive tool for real-time assessment of tumor progression, therapeutic response, and monitoring of residual disease [Shoji Y 2022].

The X-Tracker® system has been developed by SmartCatch to isolate circulating tumor cells and other cancer related cells by leveraging their physical traits, particularly size and deformability. This label-free technology uses micro-engineered filters adapted from microelectronics manufacturing, to gently retain target cells while preserving their integrity and enabling downstream applications such as immunostaining or molecular profiling.

High-content imaging platforms such as the CellInsight™ CX7 from Thermo Fisher Scientific™ enable detailed cell characterization through fully automated acquisition and advanced multiparametric, single-cell image quantification across large imaging areas. By discriminating cell types based on fluorescent staining and morphology, this technology converts qualitative observations into robust quantitative datasets with high reproducibility and minimal operator dependency, key requirements for standardized clinical workflows.

To illustrate these capabilities to melanoma applications, this note presents the integration of X-Tracker® with the CellInsight™ CX7, supported by a custom high-level analysis workflow developed by NeoVirTech. This combined approach unites cancer cell enrichment, high-resolution imaging, and automated enumeration into a scalable, clinically adaptable workflow, demonstrated here through a two-color proof of concept with potential expansion to up to seven markers for advanced phenotyping.

Materials & Methods

Samples
Citrate Phosphate Dextrose (CPD) blood bags from healthy donors are acquired through the EFS (Etablissement Français du Sang). To mimic patient blood, spiking experiments are done by adding WM-266-4 cells (metastatic human melanoma cell line) into 10mL healthy CPD blood. These cells are stained before spiking using CellTracker Green CMFDA (Invitrogen™, C7025) at 1µM for 30min at 37°C and with Hoechst at 5µg/mL for 15min at 37°C.

X-Tracker® processing
The instrument protocol applied is “Isolation and fixation” with CTC-InSITU consumable. During this protocol, the blood sample is passed through the filters and captured cells are fixed directly in the machine using Formalin 10% for 20 min. After X-Tracker® processing, the filters are recovered and placed in a 96 well plate containing Hoechst at 5µg/mL and incubated 15 min at room temperature. Samples are then flipped upside down and deposited in a glass bottom 96 well plate (Corning, 3340) containing 200µL of PBS 1X.

Acquisition
96 well plates were imaged with CellInsight™ imaging platform CX7. 25 fields per well were acquired with 10X objective, full resolution (bining 1x1). Individual fields were finally assembled in FIJI (version 1.54p) to obtained complete filtered samples overview.

Data analysis
To quantify cells objects, individual fields containing three channels - brightfield as gray channel, Hoechst signal as blue channel, green fluorescence as green channel - is processed by IA segmentation algorithm. Segmentation mask obtained is used to define regions of interest and measure for each object area [μm^2], mean pixel value in blue and green channels. These values are then processed through Pandas workflow (Python 3) and visualized with Matplotlib.
Thresholding applied: area [155 – 280 μm^2], mean pixel value in blue channel [250:max] and green channel [2200:max]. Total cells are defined as Hoechst+, cells of interest (WM-266-4) are defined as Green+ / Hoechst+.

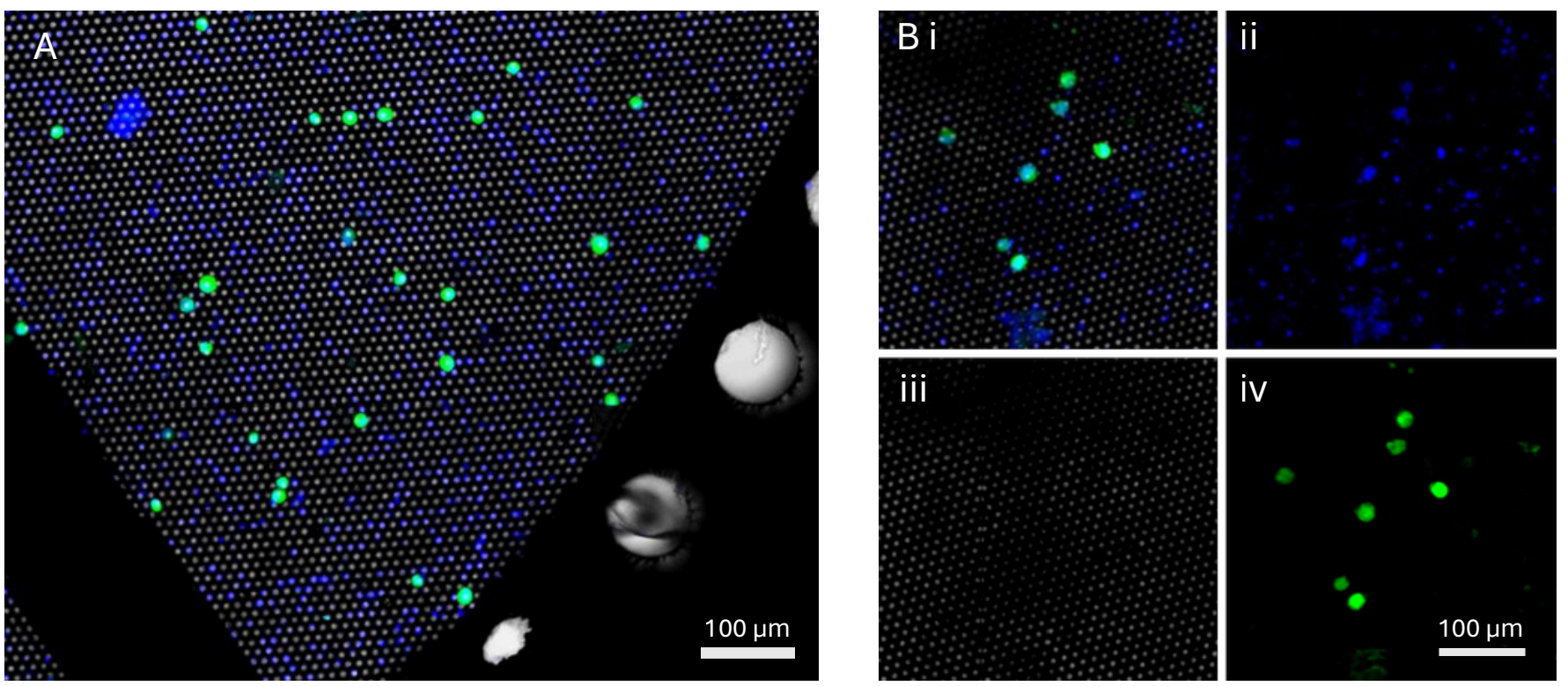

Figure 1 : Image examples of WM-266-4 cells and PBMC captured on the filters; A/ Merge channels; Bi/ Merge channels; ii/ Blue channel; iii/ Bright field; iv/ Green channel

Results

Rapid acquisition at 10× magnification, performed as 25 images per filter, provides full coverage of the filter while maintaining excellent spatial resolution. This resolution allows to clearly visualize individual cells and reliably distinguish nuclear from cytoplasmic staining. In this experiment, nuclei are labeled with Hoechst (blue), whereas the green cytoplasmic marker identifies the cells of interest (Cell Tracker Green CMFDA).

The images shown in figure 1 clearly show that several green-stained cells of interest are retained on the X-Tracker® filters, confirming the device's ability to capture the targeted cells of interest. Overall, the combination of rapid, low magnification acquisition and high-content imaging delivers a robust and interpretable visualization of enriched cells.

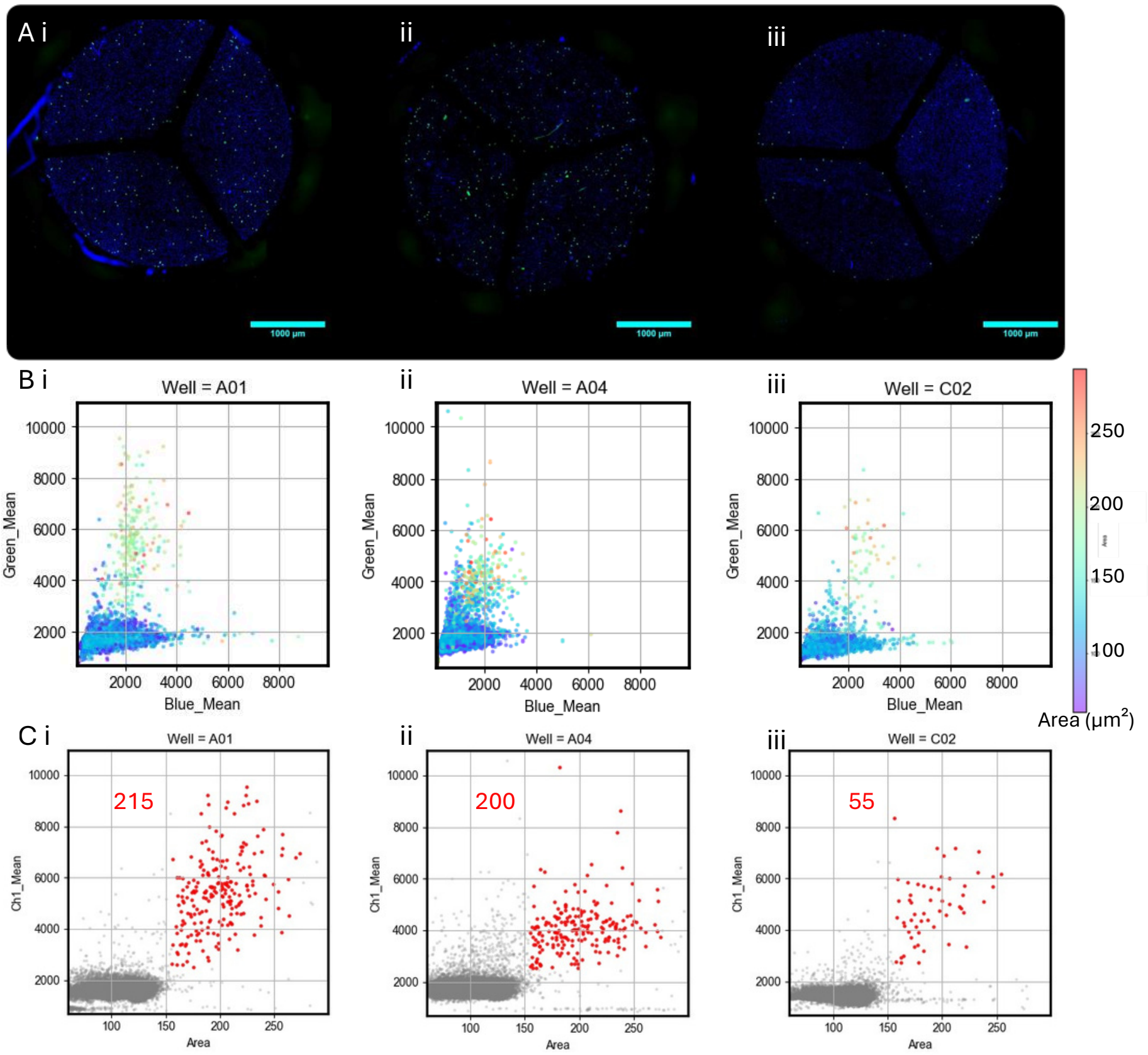

Figure 2 : A/ Full filter reconstruction images; B/ Dotplot of segmented area. Each dot corresponds to a segmented area and is colored depending on its size; C/ Dotplot of each segmented object. A threshold is applied to differentiate cells of interest in red from other objects in gray (other cells, debris, empty pores, etc.). Each column i, ii and iii refers to the same analyzed filter.

In figure 2B, each point in the plot represents a single detected object, with the color encoding its estimated size. The population of green-stained cells of interest shows a consistent and relatively homogeneous size, centered around ~200 μm^2, appearing predominantly in green/yellow tones. In contrast, the remaining detected objects such as nontarget cells and empty filter pores display smaller sizes, represented mainly by blue colored points.

This size-based separation, combined with nuclear (blue) and cytoplasmic (green) fluorescence intensities on the axes, confirms that the cells of interest cluster within a distinct and coherent size range, while background objects form a separate, lower size distribution, in accordance with the expected size of each population. This supports the accurate identification of target cells captured by the X-Tracker® and classified through high content imaging.

Applying size and green intensity thresholds allows the automated selection of the cells of interest (fig 2C). This approach isolates objects that fall within the expected cytoplasmic signal range and the characteristic size window of the target cells, enabling fully automated and operator independent quantification.

Consistent with what is observed in the first two images where over 200 cells of interest are detected, the thresholding procedure successfully highlights the relevant population (shown in red on the scatter plot). In contrast, the last image contains significantly fewer positive events, with 55 cells of interest identified. This reduction is visually apparent in the corresponding filter image, where fewer green stained cells are distributed across the membrane.

Conclusion

SmartCatch technology enables the efficient isolation of target cells directly from whole blood, providing a robust foundation for downstream analytical workflows. The imaging process can be performed in a fully automated and standardized manner thanks to NeoVirTech expertise, ensuring reproducibility and minimizing operator dependent variability. Subsequent data analysis allows the selection, classification, and quantification of specific cellular subpopulations while extracting rich quantitative parameters.
In this proof-of-concept example, only two fluorescence channels were used; however, these technologies can multiplex up to seven markers, which could be applied to CTC identification, generally detected with four markers (Hoechst+, CD45-, EpCam+, PanCK+) and allowing more comprehensive phenotypic characterization. Altogether, the workflow from whole blood processing to the enumeration of cells of interest is fully standardized and compatible with a 96 well format, facilitating routine implementation and method standardization.
This integrated approach demonstrates strong potential for detection of CTC and cancer associated cells, where reliable isolation and detailed characterization of circulating tumor cells can support both research and future clinical workflows.

N. Huang, et al, ‘Current Trends in Circulating Biomarkers for Melanoma Detection’, Apr. 05, 2022, Frontiers Media S.A.
Y. Shoji, et al, ‘Recent Developments of Circulating Tumor Cell Analysis for Monitoring Cutaneous Melanoma Patients’, Feb. 01, 2022, MDPI.