

# HER-2 FISH Analysis and H&E Staining of Circulating Tumor Cells Pre-Isolated by CellSieve™ High Porosity Precision Microfilters

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## ABSTRACT

CellSieve™ high porosity microfilters are lithographically fabricated filters with precision pore dimensions, able to rapidly capture Circulating Tumor Cells (CTCs) from whole blood. CTC antibody stains were used to identify breast cancer cell lines, MCF-7 and SK-BR-3, from whole blood, which was followed by molecular subtyping or histological staining. Cells were captured on the filter, immunofluorescently stained and imaged. Filters were then analyzed by Hematoxylin & Eosin stain using standard cytology methods and reagents. Alternatively, samples were subtyped as HER-2 over expressing or normal expressing using a fluorescent *in situ* hybridization (FISH) probe. CellSieve™ microfiltration and immunoidentification of CTCs followed by histological or molecular subtyping has various implications in utility for patient cancer diagnosis as a noninvasive liquid biopsy, as well as research applications.

## INTRODUCTION

CTCs are cancer cells originating from a primary tumor and transiting the circulatory system which can be used to monitor therapy response and predict disease outcome.<sup>1-4</sup> Isolation of CTCs by size exclusion is a technique with the advantage of capturing cells without reliance on surface marker expression.<sup>1,2,4</sup>

We have previously shown that CellSieve™ microfilters can rapidly and efficiently isolate CTCs from whole peripheral blood using fluorescent antibody stain as a detection platform. As initial cancer diagnosis is still largely determined using standard histological stain techniques, we further identify CTCs based on their morphological characteristics based on the standard pathological stains Hematoxylin and Eosin Y.

Cancer subtyping by molecular determinants is used to characterize the mutational status for diagnosis, prognosis and drug therapy<sup>3</sup>. Among the various nucleic acid based assays for cancer mutations, FISH is one of the common methods. In this assay we use a commercially available HER-2 mutational FISH probe, whose mutation status correlates with poor prognosis in 20-30% of breast cancer patients and which is used in treatment determination.

## Material & Methods

MCF-7 and SKBR3 cell lines were used to evaluate the ability to enumerate isolated CTCs using a standard panel of immunofluorescent antibodies, followed by further subtyping using HER-2/chromosome 17 fluorescent *in situ* hybridization probes or staining by Hematoxylin & Eosin.

Cells were counted to obtain precise input CTC numbers, then spiked into 7.5 mL whole human blood. The sample was pre-fixed and passed through the CellSieve™ microfilter by negative pressure (~90 seconds). Microfilters were then post-fixed, permeabilized and stained using the fluorescent antibody panel (FITC-cytokeratins 8, 18 & 19; r-phycoerythrin-EpCAM; Cy5-CD45). Microfilters were mounted, stained with DAPI, and CTCs enumerated under a fluorescent microscope.

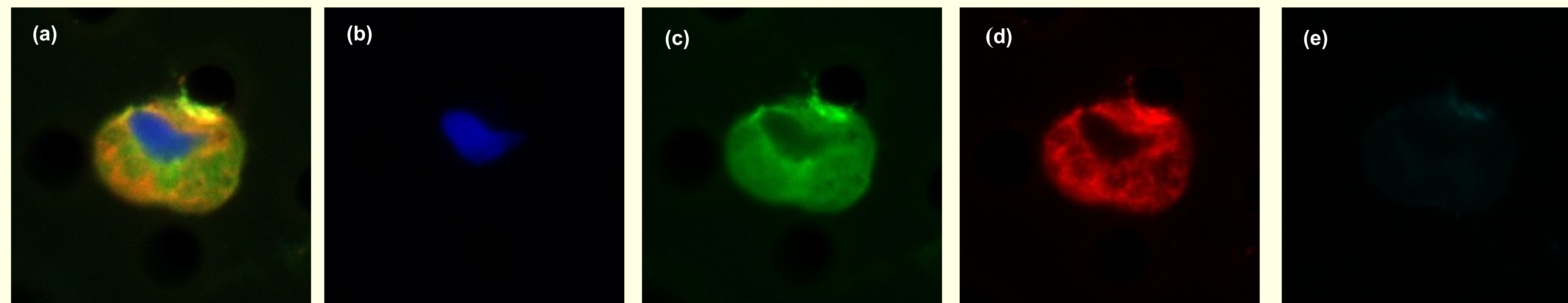
After demounting, filters were reacted with a Hematoxylin stain, rinsed in water and counterstained with Eosin Y. Alternatively, after demounting, filters were attached to a microscope slide and digested in a pepsin solution, washed and probed with HER-2/Chromosome 17 (CytoCell) FISH amplification probes overnight. Filters were then washed, mounted with DAPI, and analyzed using a fluorescence microscope.

## RESULTS

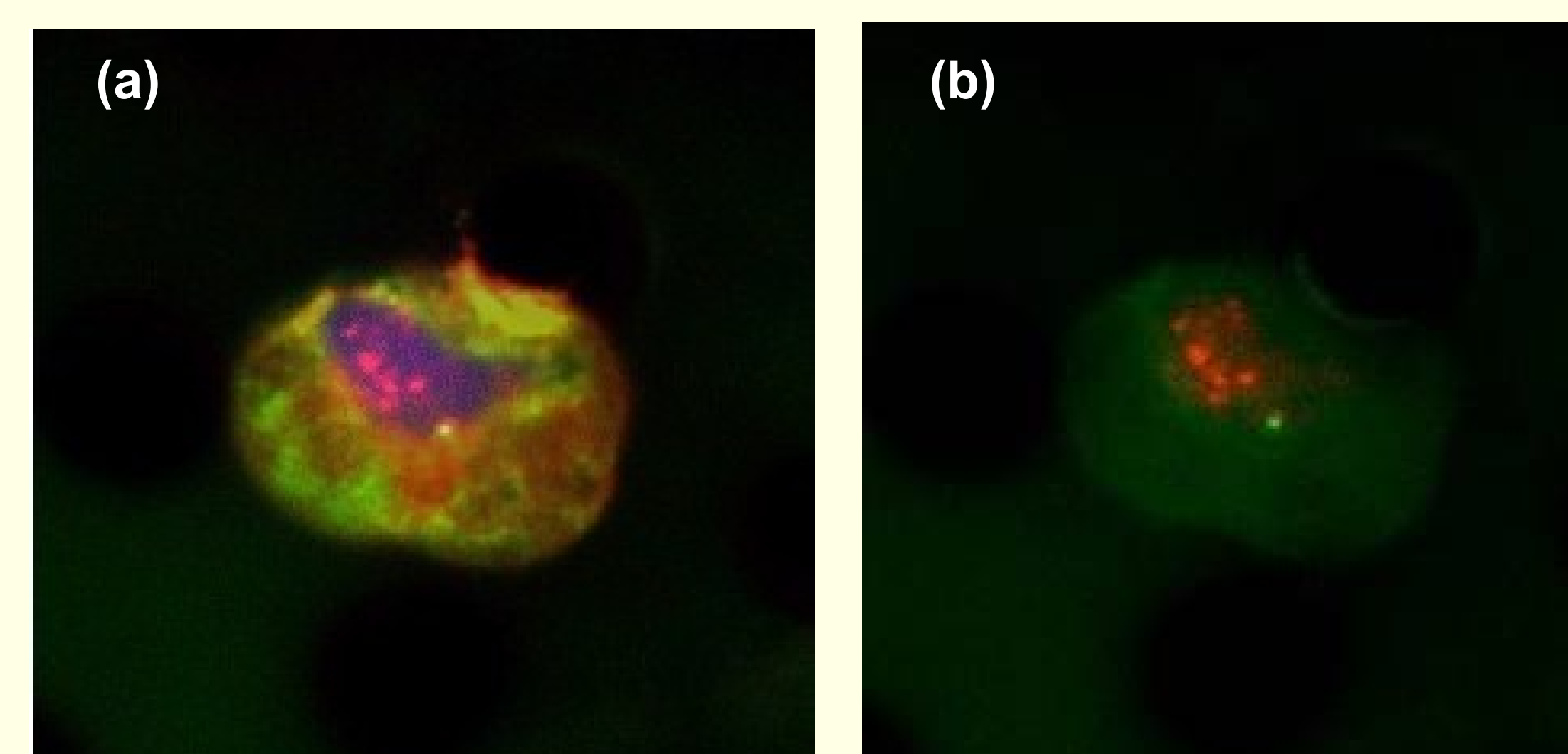
Consistent with previously presented data, the total assay time from capture through staining was less than 2 hours. In addition to high capture efficiency, simple immunostaining of EpCAM cytokeratin 8,18, & 19 and CD45 was performed rapidly on the microfilter. Fluorescence imaging and interpretation were straightforward. Background fluorescence was negligible (Figure 1).

Cells were tracked while probed against HER-2 & Chromosome 17 and reimaged after completion of the FISH assay. Figure 2b shows a normal expression of Chromosome 17 (green) with two dots, while over expressing HER-2 (red) with +12 dots. Figure 2a shows a full overlay of all four stains, as well as the overlay of the two FISH probes.

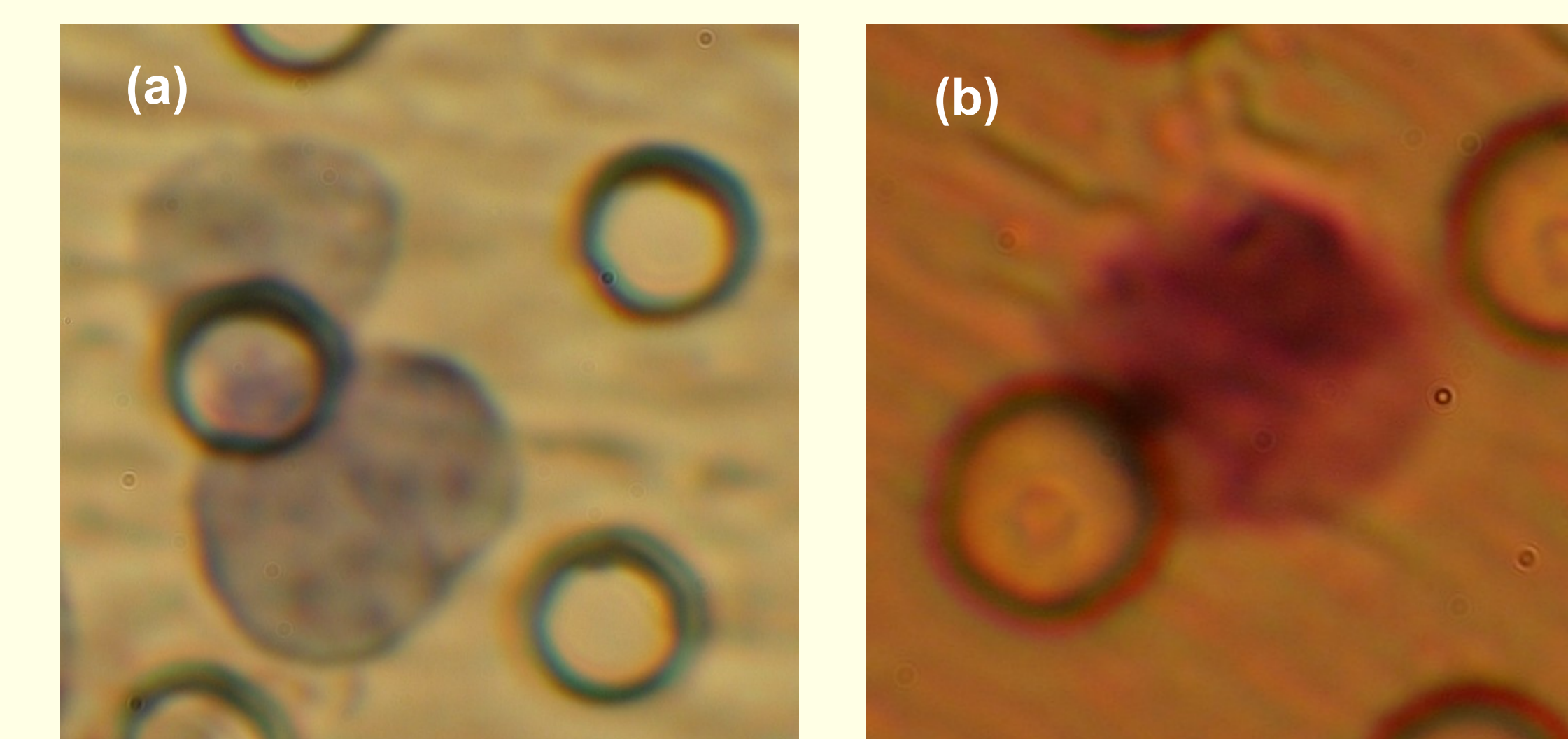
Nuclear profiles and cytoplasmic staining of cells using both Hematoxylin and then Eosin Y stains show that standard histological stains are applicable to the CellSieve™ microfilter (Figure 3).



**Figure 1.** Individual and merged immunofluorescent images of stained SKBR3 cell and on CellSieve™ microfilter: (a) Overlay image, (b) DAPI filter for nucleus, (c) FITC filter for CK 8, 18, 19, (d) TRITC filter for EpCAM, and (e) Cy5 filter for CD45.



**Figure 2.** Merged fluorescent images of stained SKBR3 cell on CellSieve™ microfilter: (a) combined image from Figure 1 with overlay of HER-2 (red) and chromosome 17 (green), (b) HER-2 over expression: Texas Red (red) & chromosome 17 normal expression FITC (green)



**Figure 3.** SKBR3 cells stained by Hematoxylin and Eosin Stain: (a) Hematoxylin Stain (b) Hematoxylin and counterstained with Eosin Y.

## CONCLUSIONS

- Filtration of whole blood using CellSieve™ microfilters takes < 2 min.
- CTCs can be identified by fluorescent immunostaining in < 2 hours
- CTCs captured by CellSieve™ can be further subtyped by standard fluorescent *in situ* probes
- CTCs captured by CellSieve™ can be further characterized by standard histopathological stains

## References

1. Pachmann, K., *et al.* (2008). "Monitoring the response of Circulating Epithelial Tumor Cells to Adjuvant Chemotherapy in Breast Cancer Allows Detection of Patients at Risk of Early Relapse." *J. of Clin. Oncol.* 28 (8):1208-1215
2. Vona, G., *et al.* (2000). "Isolation by Size of Epithelial Tumor Cells A New Method for the Immunomorphological and Molecular Characterization of Circulating Tumor Cells." *American Journal of Pathology* 156(1): 57-63.
3. Lang, J., *et al.* (2009). "HER2 status predicts the presence of circulating tumor cells in patients with operable breast cancer." *Br Can Res Treat.* 113:501-507
4. Farace, F., *et al.* (2011). "A direct comparison of CellSearch and ISET for circulating tumor-cell detection in patients with metastatic carcinomas." *BJC* advance online publication 9 August 2011: 1-7.