

# Visualizing the Mitotic Stages of Circulating Tumor Cells (CTCs)

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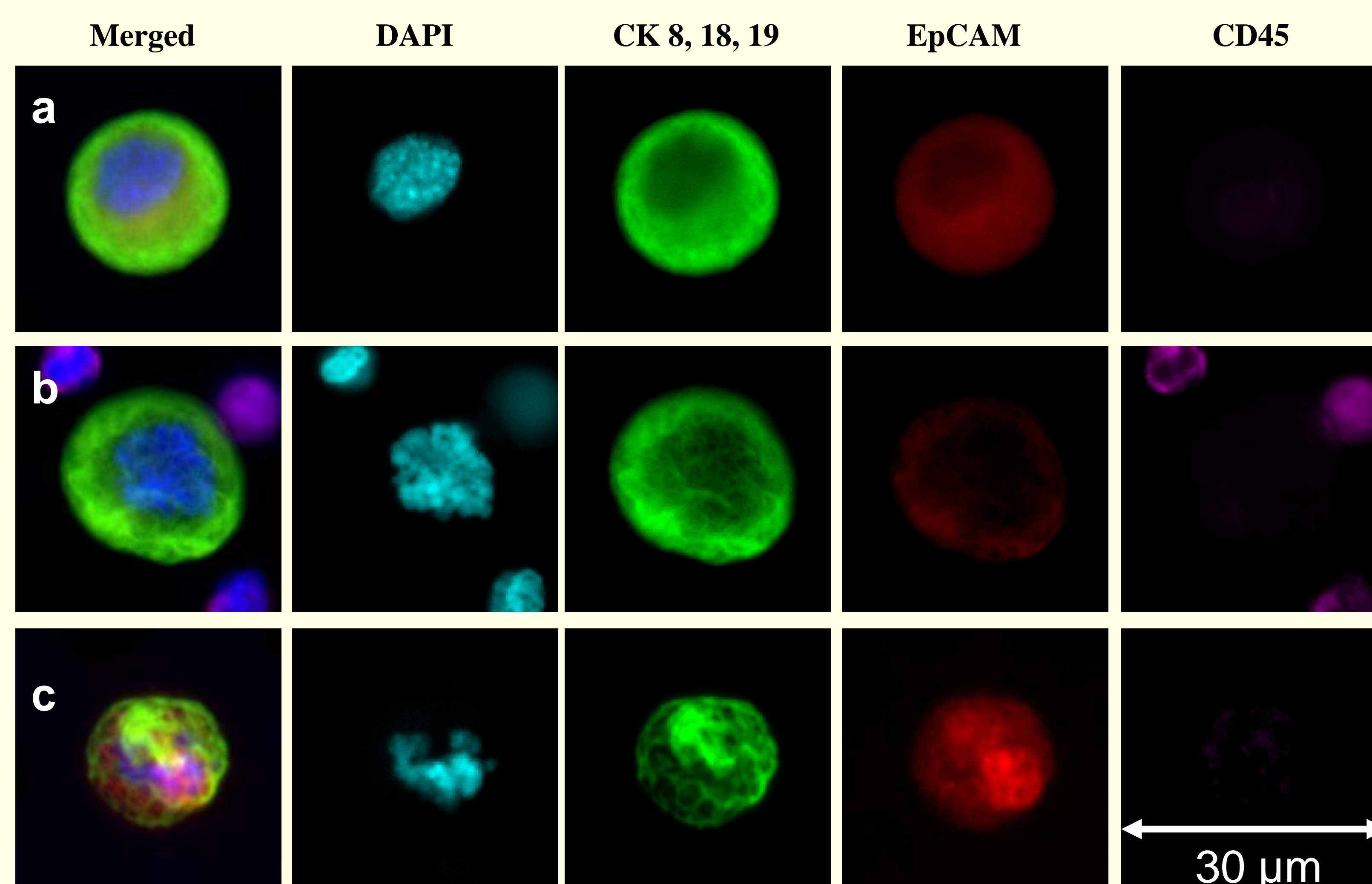
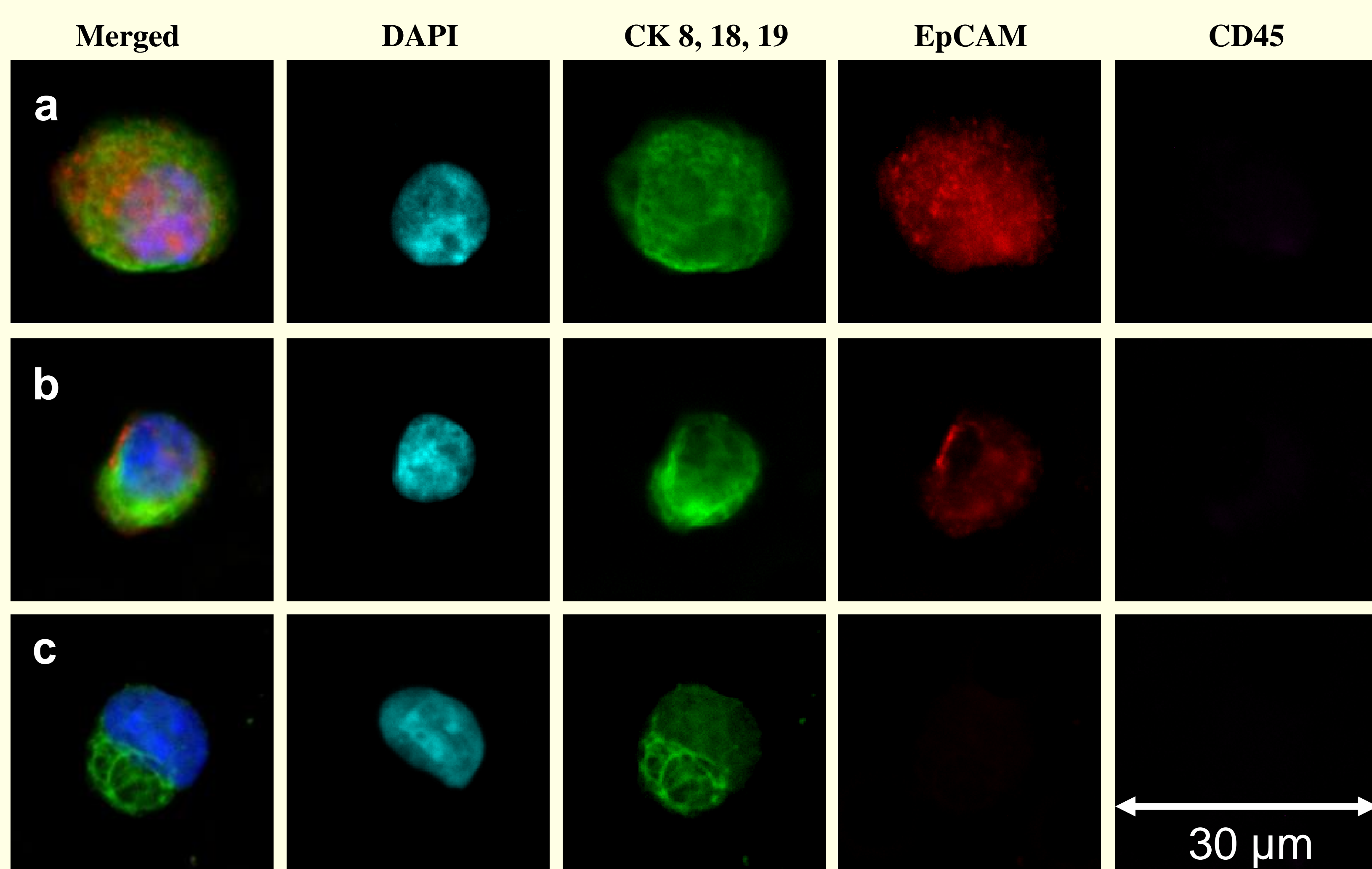
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## ABSTRACT

It has been well documented that microfiltration can be used on peripheral blood as a non-invasive "liquid biopsy" for both circulating tumor cell (CTC) detection and subtyping<sup>1-3</sup>. Typically, CTC identification relies on various immunohistochemical (IHC) stains which are used in an absent/present method (i.e. cytokeratin-positive and CD45-negative). However, in cellular histology, organelle staining is a requisite for proper cell identification. Here we apply high resolution images to the commonly accepted fluorescent CTC stains to categorize cells by the various stages of mitosis, or interphase in preparation for cell division. Using basic histology criteria, we show that actively dividing CTCs are present in the peripheral blood metastatic breast cancer patients and that these cells can be identified, then grouped, into the distinct mitotic phases.

**Figure 1. Typical CTC in interphase**

- (a) CTC with high EpCAM expression and cytokeratin filamentation  
(b) CTC with low EpCAM and cytokeratin filamentation  
(c) CTC with no EpCAM, but with cytokeratin filamentation



**Figure 2. Prophase through metaphase. (a) Prophase-condensing chromatin, (b) prometaphase/metaphase-condensed chromatin with vesicles; (c) metaphase/anaphase-chromosomes starting to split along metaphase plate**

## RESULTS

- CTCs were found in 85% (n=22/26) of patient samples tested, mostly in Interphase (Figure 1a-c).
- High resolution microscopy identified additional CTCs in various stages of mitosis including: prophase (Figure 2a), prometaphase (Figure 2b), metaphase (Figures 2b-c), anaphase (Figures 2c and 3a), and telophase (Figures 3b-c).
- CTCs in active mitosis were found in 23% (n=6/26) of patient samples.

## CONCLUSIONS

- CellSieve™ microfiltration isolates CTCs while retaining the fine morphological features of cytopathological organelles.
- CTCs, from patient samples, can be found in various stages of mitotic division using standard fluorescent microscopy.
- Mitotic subtyping may provide a useful strategy for tracking highly proliferative cancer cells transiting the circulation of cancer patients.

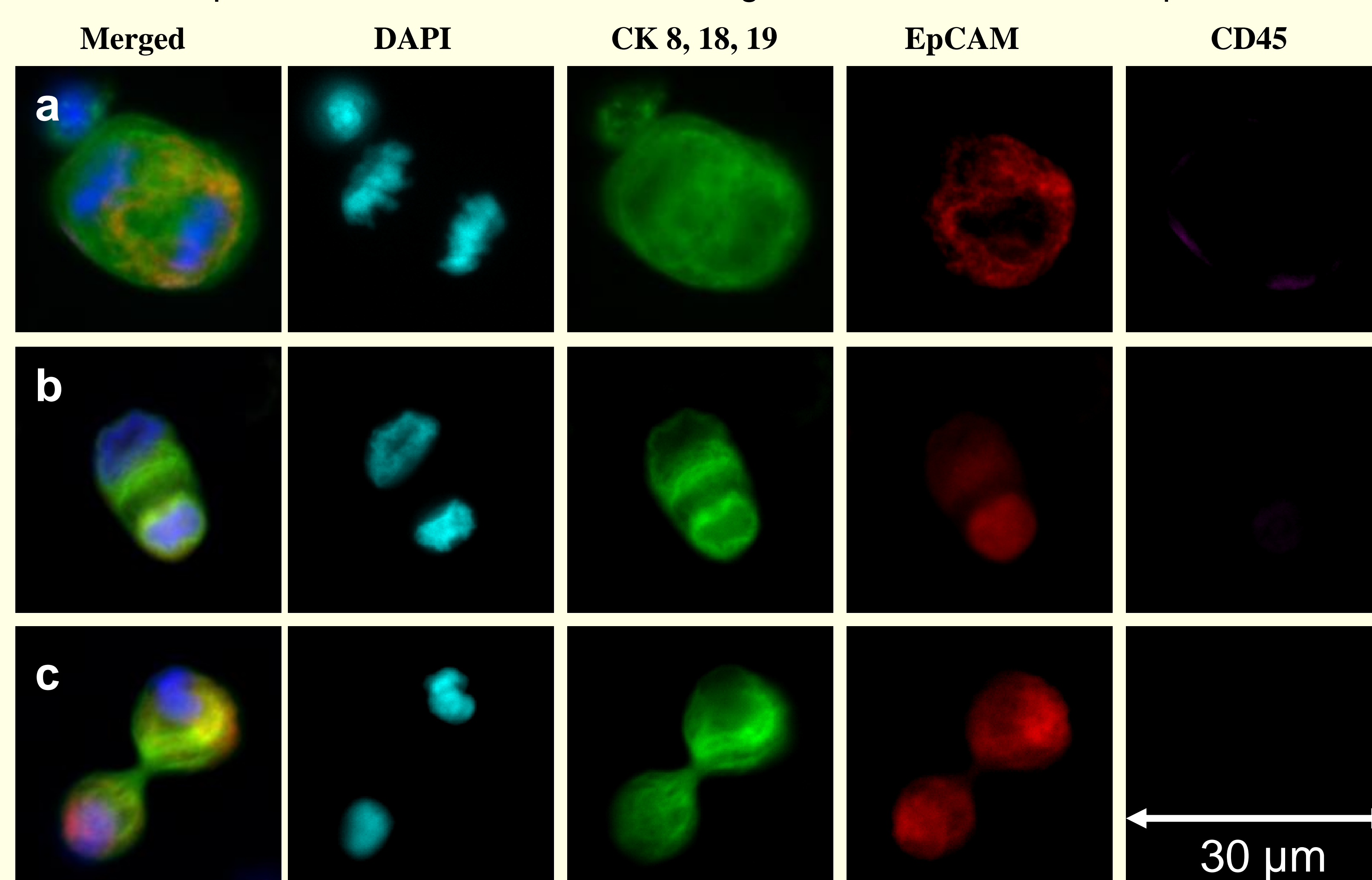
## INTRODUCTION

CTCs are cells that originate from a primary solid tumor and are found transiting the circulatory system. It has been well established that CTC enumeration can be used to monitor therapy response and predict outcome.<sup>1-3</sup> Size exclusion is a technique for isolating CTCs from patient samples, irrespective of their surface marker expression.<sup>1-3</sup>

CellSieve™ microfilters are lithographically fabricated membranes with high porosity, precise pore dimensions, and patterned pore distribution. We previously reported that CellSieve™ rapidly and efficiently isolates CTCs from whole peripheral blood, using fluorescent antibody stain as the detection platform. In addition to enumerating CTCs, subtyping by phenotypic determinates may aid in identifying the CTCs cellular status for diagnosis, prognosis and therapy determination.<sup>1-3</sup>

## MATERIALS & METHODS

Stage III/IV breast patient samples were provided by Fox Chase Cancer Center (FCCC) and University of Maryland at Baltimore (UMB), and analyzed by Creatv MicroTech, Inc. CellSieve™ microfilters, with precision 7 micron diameter pores distributed in uniform arrays were employed. 7.5 mL of whole blood was diluted in pre-fixation solution and filtered (~3 minutes). CTCs were fixed, permeabilized, and stained with DAPI, an antibody cocktail against cytokeratin 8/18/19 (FITC), EpCAM (PE), and CD45 (Cy5). CTCs, defined as cytokeratin-positive and CD45-negative, were then imaged. CTC images were then screened for cells in active stages of mitotic division.



**Figure 3. Anaphase through cytokinesis. (a) Anaphase-chromosomes moving to opposite poles, (b) telophase-chromosomes unwinding, cleavage furrow has not formed, (c) telophase/cytokinesis-contraction ring is cleaving cell**

## References

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