The RareCyte™ system for enumeration of circulating tumor cells that retains all nucleated cells for analyses and does not rely on capture of proteins expressed on cells

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Abstract

• Detection and molecular characterization of circulating tumor cells (CTCs) are useful for diagnosis, prognosis and measuring therapeutic response of malignant tumors.

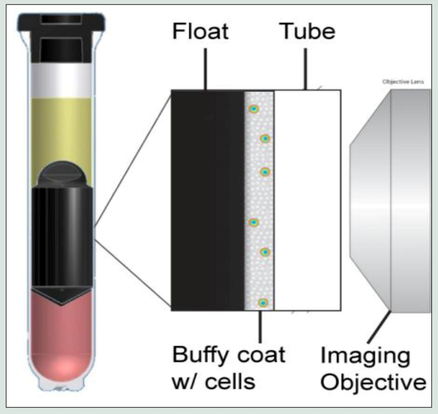
• Many CTC detection methods are based on capture of cells that express EpCAM, limiting the method to cells with expression of this or other markers.

• The RareCyte™ system is based on density separation and the spread of nucleated cells onto an imaging surface.

• Our technology is not limited to a specific set of cancer biomarkers and requires no pre-enrichment step.

Technology Overview

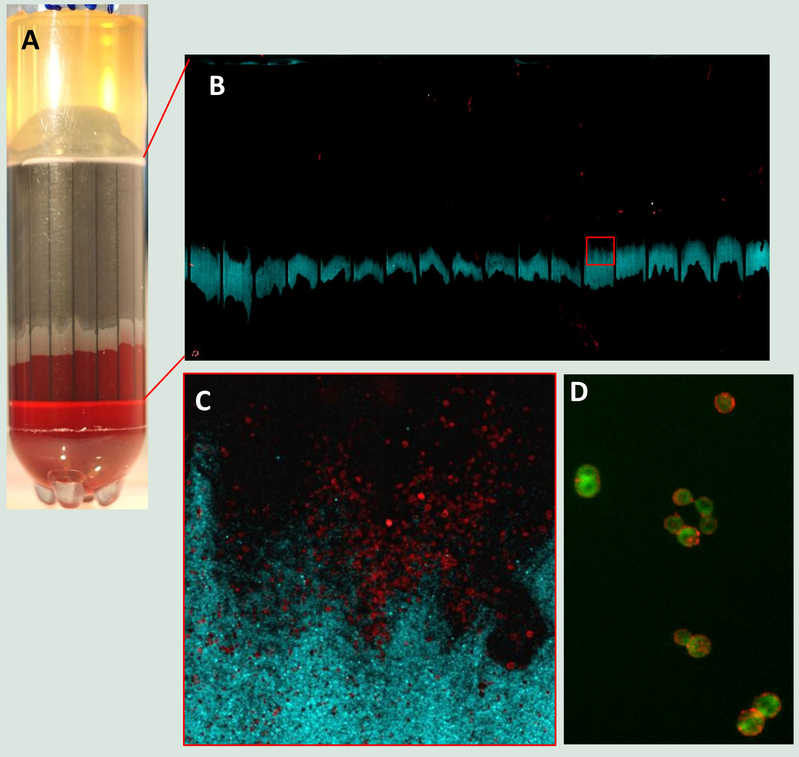
The system uses a proprietary tube and float combination to separate the Buffy coat, which contains white blood cells and CTCs, from other blood components, and create a 30 µm layer for imaging. The entire Buffy coat is imaged in our automated fluorescence microscope.



Experimental Protocol:
1. Add antibodies to tube containing blood sample
2. Incubate for 1 – 2 hours
3. Insert float and then centrifuge for 30 minutes
4. Image on RareCyte™ scanner
5. Run detection algorithm to find CTCs
6. Review images to confirm identity of CTCs

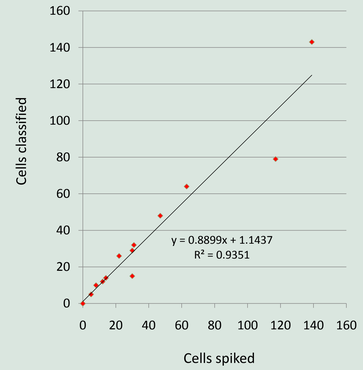
Imaging Cancer Cells in Blood

Prototype Scanner



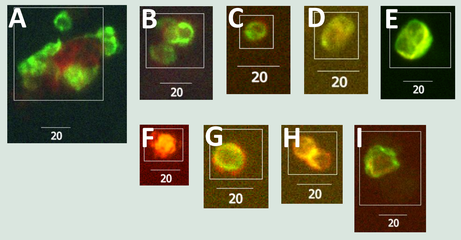
Separation of blood components and imaging.
Panel A shows a tube and float that have been centrifuged with 3 ml of blood, separating the plasma, Buffy coat, and red blood cells and creating the imaging layer around the float. Panel B is a stitched 10X image of an entire float (3040 images) with CD45 antibody in cyan showing the Buffy coat and SkBr3 cells labeled with EpCAM in red. Panel C is a zoomed-in image of the float scan showing SkBr3 cells just above the white blood cell layer. Panel D is a close-up of cells showing CK in green and EpCAM in red.

Spike-in Recovery of Cells



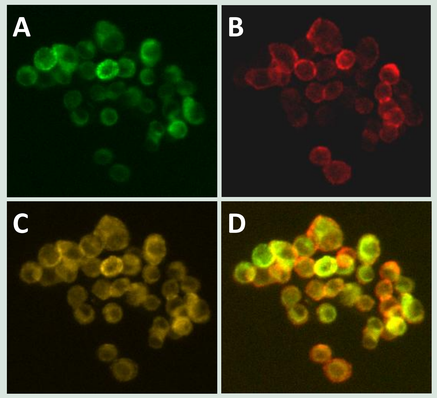
Spike-in results.
• MDA-MB-453 cells were spiked into 3 ml of blood from 14 healthy individuals and counted on the RareCyte™ system.
• Average recovery was 90%
• Sensitivity is one CTC within one billion blood cells.
• System could detect CTCs even 7 days after spiking in cells.

Clinical Samples



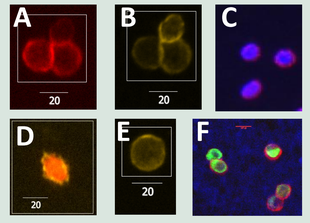
Images of CTCs obtained with the RareCyte™ system.
CTCs have been identified in over 50 clinical samples from patients with prostate, breast, colorectal and skin cancer using the RareCyte™ technology. Panels A and B are clusters of CTCs from a breast cancer patient stained for CK in green and EpCAM in Red. Panels C-I are CTCs from prostate cancer patients stained with CK in green, PSMA in yellow and EpCAM in red.

Imaging and Characterization



Cell characterization.
SkBr3 cells imaged in 3 wavelengths to detect expression of Cytokeratin (Panel A), EpCAM (Panel B) and Her2 (Panel C). Composite image shown in panel D.

Open System



Multiple markers can be detected.
The RareCyte™ technology is an open platform that allows users to chose markers relevant to their interests. Nine markers have been used in our system to characterize CTCs: EpCAM (Panel A), PSMA (Panel B), Her2 (Panel C, in red), CD146 (Panel D, yellow), CEA (Panel E), CK (Panel F, in green), CD24, CD56 and EGFR.

Conclusions

• The RareCyte™ technology is a new platform for CTC detection and characterization that does not rely on capture of cells through expression of a biomarker.
• Our system can image CTC clusters in the circulation. It is believed that these clusters have a higher metastatic potential than individual CTCs.
• We obtain a high recovery of spiked-in cells with virtually no false positives.
• Molecular characterization of CTCs can be customized and automated for deployment in clinical laboratories.

RARECYTE