Multi-physics Automated Reconfigurable Separation (MARS) System Enables High Throughput Cell Isolation With High Efficiency and High Recovery

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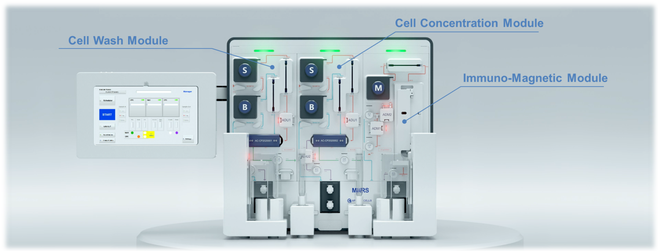
INTRODUCTION

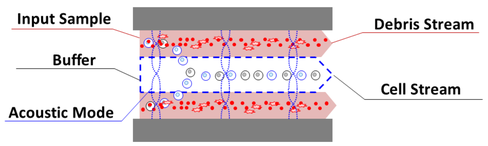
Modular



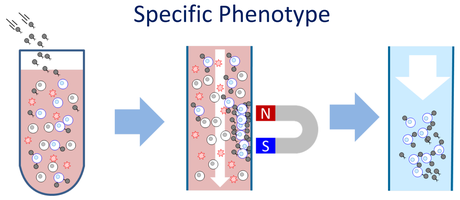
Multi-Physics

Novel **Acoustic** and **Magnetic** device designs to allow high speed cell selection based on

Physical Parameters



Specific Phenotype



Versatile Applications

Rare Cell Enrichment (CTC, DTC, MRD)

Immune Cell Isolation

Sample Prep (leukocyte purification without centrifugation)

impacts

Single Cell Genomics | **Sequencing** | **Genetic Data Analysis**

Immune Cell Therapy Development

Flow cytometry | **Fluorescent Microscopy**

Rare Cell Enrichment (CTC model)

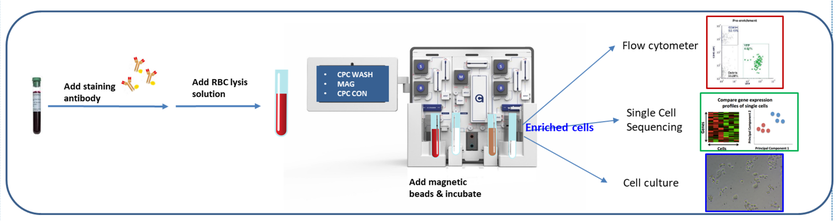


Fig 1 (a), illustration of circulating tumor cell (CTC) enrichment from peripheral whole blood on MARS: staining whole blood with desired antibody conjugates (CD45-biotin), lysing red blood cells, and loading sample on MARS; MARS performs removal of red blood cells debris, concentrate purified white blood cells, mixing white blood cells with magnetic beads and magnetic depletion of CD45+ cells in series automatically through software programs. The enriched tumor cells are ready for downstream analysis. (*Processing speed 10ml whole blood per hour*)

Recovery of Tumor Cells Spiked in Whole Blood By MARS

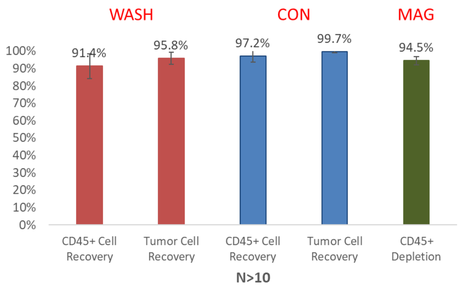


Fig 1 (b), in tumor cell spiking experiments, analyzing individual module's performance by counting CD45+ cells and spiked tumor cells in each collection portion -- "sample" "waste" "positive" and "negative"

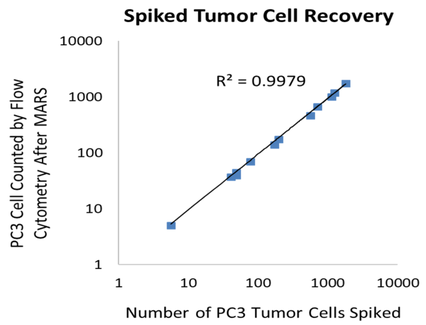
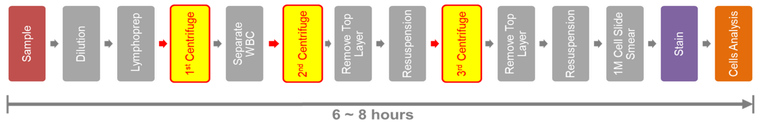


Fig 1 (c), tumor cell recovery linear range: 1-1000 PC3 cell line cells were added into 1ml of whole blood with averaged white blood cell count 6x10e6. Enriched cells were analyzed on flow cytometer (CD45-EpCAM+)

Rare Cell Enrichment (DTC case study)

- **Clinical Problem: Breast Cancer Recurrence**
- **Technical barrier: finding extreme rare disseminated tumor cells from patients bone marrow**

Conventional Method



MARS Process Flow

MRD Incorporating MARS

- Eliminates ~ **60%** steps
- Reduce overall time by **6x - 8x**
- **Full automation**
- **Lower human factor**
- **Higher recovery**

Fig 2 (a), comparing DTC analysis workflow with conventional centrifuge involved manual sample preparation to MARS involved automatic cell enrichment. Benefits gained from MARS are listed.

Tumor Cell Recovery on MARS Across Range of Spiking Frequencies

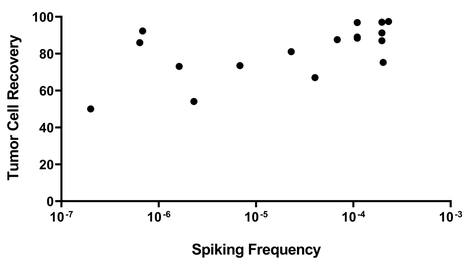


Fig 2 (b), accurate number of breast cancer cell line cells were sorted in human bone marrow samples across range of spiking frequencies, lowest frequency is 1 tumor cell per 5 million bone marrow cells; enriched cells were counted on flow cytometer based on DTC phenotypic panel

CTC model tumor cells recovery on MARS (evaluation results at Penn)

Control	746	548	84	60	16	8
MARS Recover	748 (100%)	532 (97%)	63 (75%)	53 (84%)	8 (50%)	4 (50%)

Table 1, accurate number of YFP+ cancer cells were spiked in 1ml of human whole blood, recovery of spiked YFP+ cells from MARS listed

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Immune Cell Isolation

Sterile Isolation of CD3+ T Cells On MARS

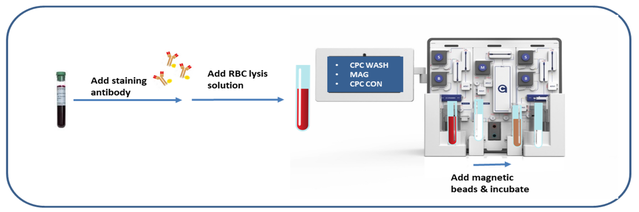
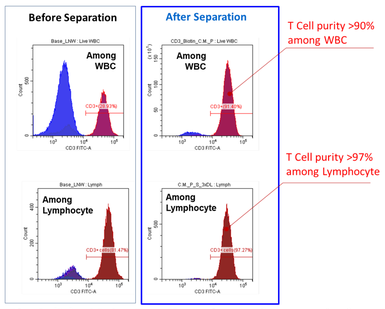


Fig 3 (a), illustration of CD3+ T cell isolation from peripheral whole blood on MARS: staining whole blood with antibody conjugates (CD3-Biotin), lysing red blood cells, and loading sample on MARS. MARS performs removal of red blood cell debris, mixing magnetic beads with purified whole blood and magnetic separation of CD3+ T cells automatically. (MARS fluidic line was sterilized through an easy-to-operate protocol)



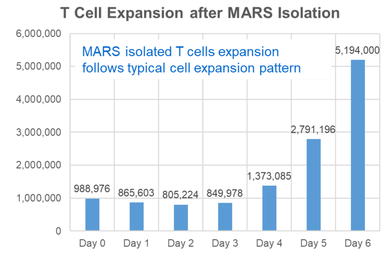


Fig 3 (b), CD3+ T cell purity before and after MARS separation (left); 3(c), isolated T cells were stimulated with CD3/CD28 Dynabeads and cultured for days. Cell expansion progress is shown at right

Cell Sample Preparation

MARS Automates Sample Preparation Workflow for Cell Analysis

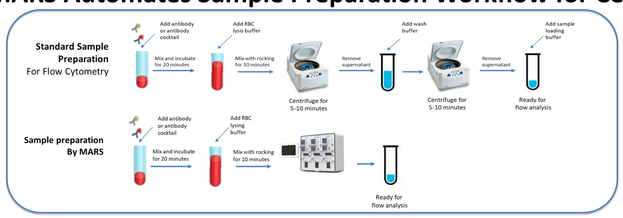


Fig 4 (a), comparing manual sample preparation workflow for flow cytometer analysis with "centrifuge-free" sample preparation workflow using MARS

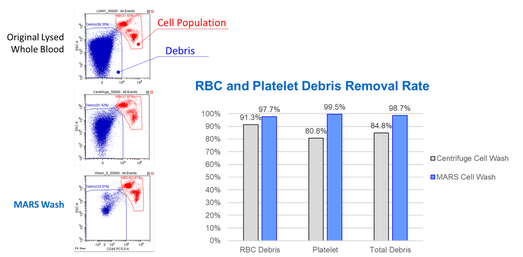


Fig 4 (b), comparing removal of red blood cell debris and platelets by centrifuge process to MARS

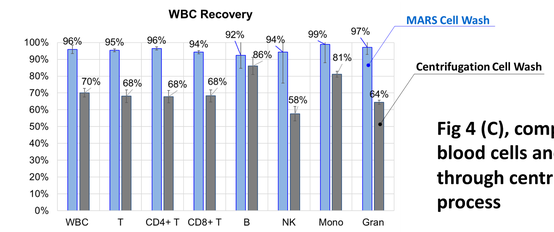


Fig 4 (C), comparing recovery of white blood cells and major subpopulations through centrifuge wash and MARS process

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