ANGLE

Size-Based Isolation of Circulating Tumour Cells and Its Application in Advanced Prostate Cancer

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ORCHID
FIGHTING MALE CANCER

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Background

Circulating tumour cells (CTCs), as a liquid biopsy, are promising in molecular diagnosis, therapy selection and treatment monitoring for cancer patients. CTCs are a heterogeneous population of cancer cells and the fate of CTCs varies (**Figure 1**). Immunomagnetic separation using epithelial cell surface markers is the most widely adopted CTC isolation methodology. However, it may lose cells under epithelial-mesenchymal transition (EMT), which are crucial in the metastatic process. In addition, magnetic beads surrounding isolated cells also affect downstream analysis. Size-based CTC isolation systems, which are independent of epithelial cell specific antigen expression, may retain cells with EMT and facilitate downstream molecular analysis, so providing a good alternative to immunomagnetic systems.

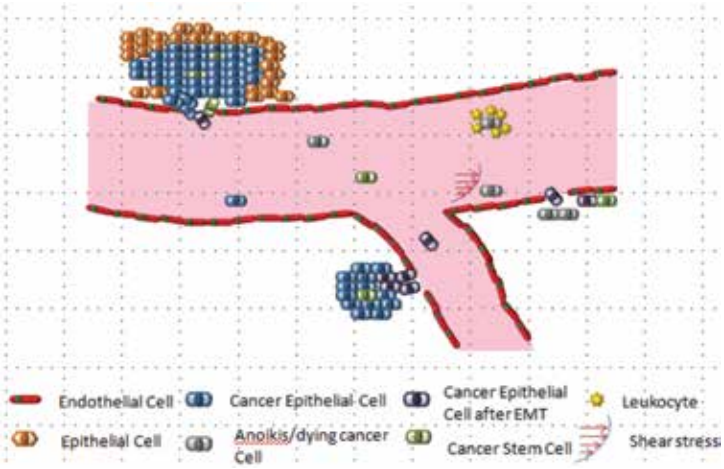
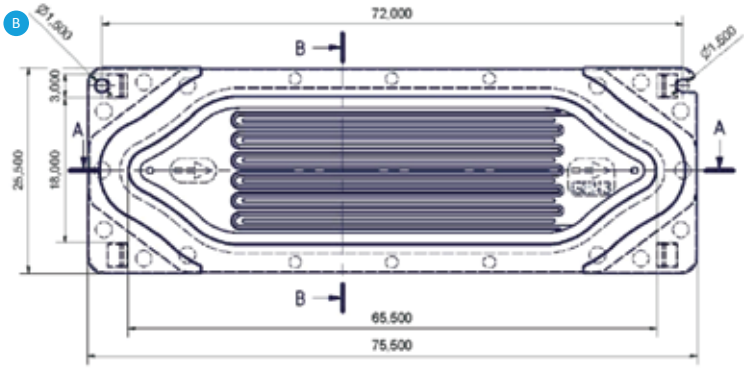


Figure 1. Various fates for CTCs

Aims

To optimise and evaluate CTC capture and harvest by the size-based Parsortix system (**Figure 2**), and assess suitability of harvested cells for downstream analysis. The IsoFlux immunomagnetic microfluidic system (**Figure 3**) was used for comparison.

Figure 2. Overview and isolation principle of Parsortix.
A. Overview of Parsortix system.
B. A diagram of the disposable isolation cassette. Blood is forced along a series of channels and to flow through a 10µm gap which separates particles on the basis of size and compressibility.



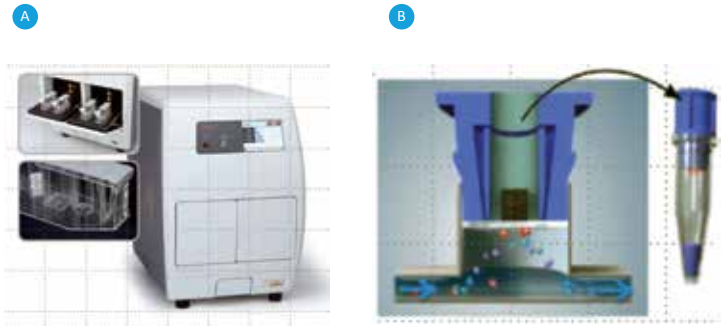


Figure 3. Overview and isolation principle of IsoFlux.
A. Overview of IsoFlux system.
B. The sample passes through an isolation zone where magnetically labelled cells are captured on the disc at the top of the channel in the presence of a magnet.

Materials & Methods

Cell lines of prostate cancer (PC3, DU145 and 22RV1), breast cancer (MCF7) and pancreatic cancer (FA6) were used for spiked experiments. Cells were pre-labelled by CellTracker and spiked in certain amount of blood. Blood samples 5-15mL each from 15 healthy donors were used for spiked experiments and those from 13 castration resistant prostate cancer patients were used for clinical assessment. All were collected from St. Batholomew's Hospital with ethical approval and informed consent.

Immunofluorescence (IF) was used to identify CTCs in clinical samples. A CTC is defined as a CK+, CD45-, nucleated and morphologically intact cell.

FISH was tested as a potential downstream analysis.

Three different ways for sample preparation prior to automated isolation in Parsortix:

1. No treatment was required and whole blood was directly loaded onto the machine;
2. 1:1 dilution of blood in PBS;
3. Ficoll treatment (**Figure 4**) to recover PBMC fraction, followed by resuspending in dilution buffer.

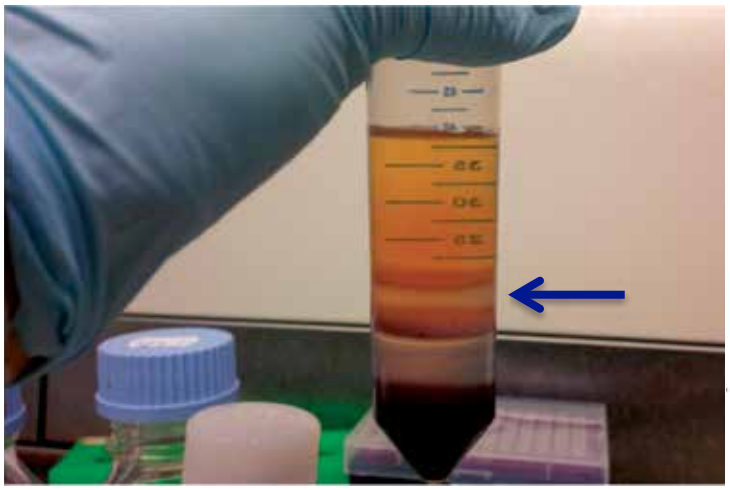


Figure 4. Samples after ficoll treatment. The white layer indicated by the arrow is peripheral blood mononuclear cell (PBMC) fraction. This fraction was collected and centrifuged, followed by resuspension.

Results

An optimal protocol was developed allowing a larger volume of whole blood, reducing separation time and improving harvest rates (**Table 1**).

Table 1. Comparison of three different sample pre-treatments

	Blood volume (mL)	Separation time (minute) Median (range)	Harvest Rate (%) Mean±STD*
Whole blood running	3	102.3	
1:1 dilution	3	79.2	
Ficoll treatment	3	35.4	
1:1 dilution	7.5	196.73	35.94±16.94
Ficoll treatment	7.5	62.3	35.94±16.94

*Not available due to limited volume of blood and number of trials by this method

Spiking experiments: Percentages of tumour cells captured in Parsortix cassettes varies from 38.5% to 73.3% for different cell lines (**Figure 5A**). After harvesting PC3 cells onto slide, a mean harvest rate of 38.21% (21-56%) and a mean purity of 8.11% (3.01-11.27%) of cancer cells were obtained by Parsortix. Using IsoFlux, there is a higher and more stable harvest rate for PC3, 91.5% (90-93%). However, the cancer cell purity is lower, 1.76 (0.60-2.91%) (**Figure 5B**). Representative images of captured cancer cells and leukocytes are shown in **Figure 6**.

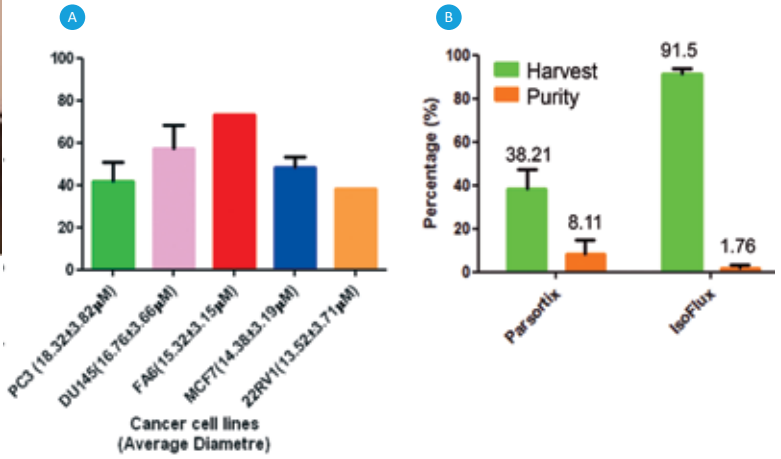


Figure 5. Capture/ harvest rates and purity of harvested cells.
A. Mean capture rates of various cancer cell lines in cassette by Parsortix.
B. After harvest, comparison of harvest rate and purity of cells on slides between two systems using PC3.

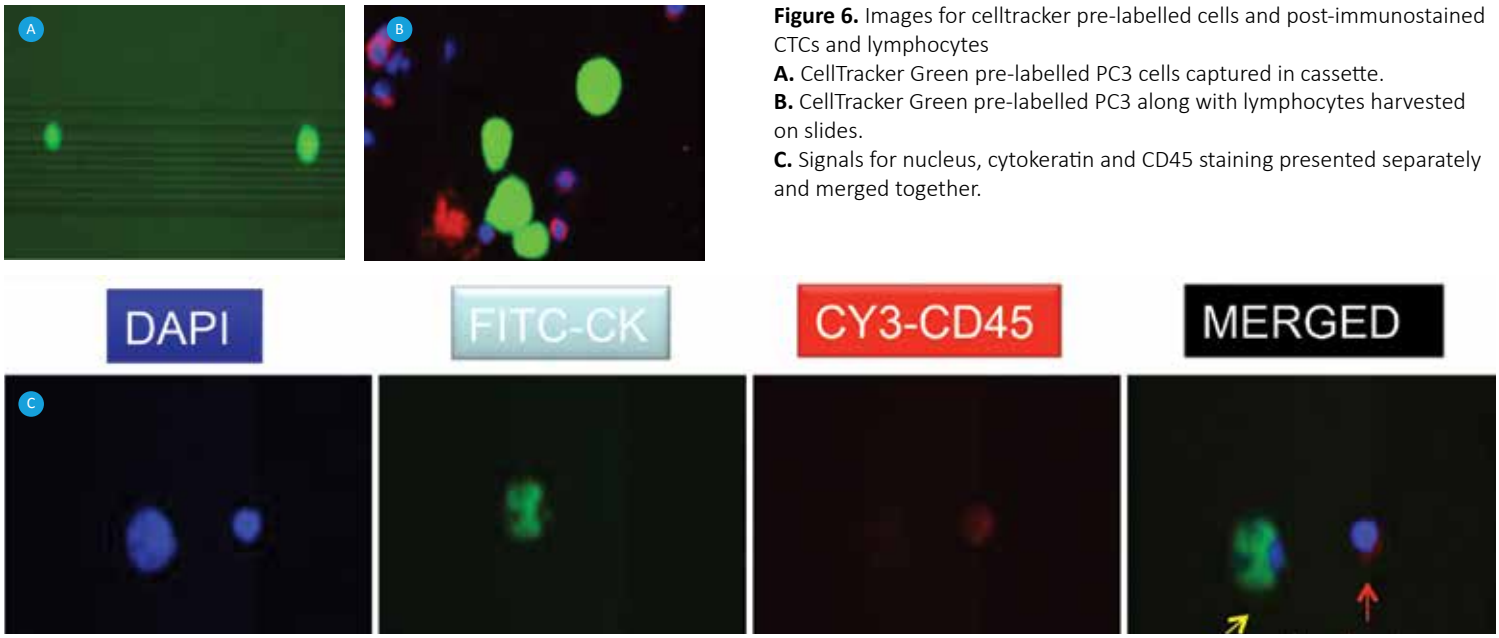


Figure 6. Images for celltracker pre-labelled cells and post-immunostained CTCs and lymphocytes
A. CellTracker Green pre-labelled PC3 cells captured in cassette.
B. CellTracker Green pre-labelled PC3 along with lymphocytes harvested on slides.
C. Signals for nucleus, cytokeratin and CD45 staining presented separately and merged together.

Clinical Samples: Enumeration of CTCs by Parsortix in advanced prostate cancer patients is presented in **Table 2** and a parallel study using IsoFlux is shown in **Figure 7**. All patients had >5 CTCs, and the purity ranged from 1.41% to 45%. The parallel study showed a similar number of harvested CTCs between Parsortix and IsoFlux, but a higher purity from Parsortix. From CTCs isolated by Parsortix, different levels of CK expression were seen; and CTCs from IsoFlux were typically surrounded by beads (**Figure 8**).

Table 2. CTC isolation efficiency in prostate cancer patients by Parsortix

Patient Number	Age	Number of CTCs	Number of other cell	Purity(%)	Blood Volume (mL)
1*	67	9	11	45	5
2*	80	26	380	6.4	5
3	55	23	74	23.71	7.5
4	85	55	522	9.53	7.5
5*	61	63	330	16.03	8
6	80	10	698	1.41	8
7	85	17	467	3.51	9
8	55	10	271	3.56	7.5
9	55	14	135	9.4	7
10	69	11	119	8.46	7.5
11	80	23	161	12.5	5
12*	91	19	743	2.49	7.5
13*	82	54	746	6.75	6

*Paralleled to IsoFlux system

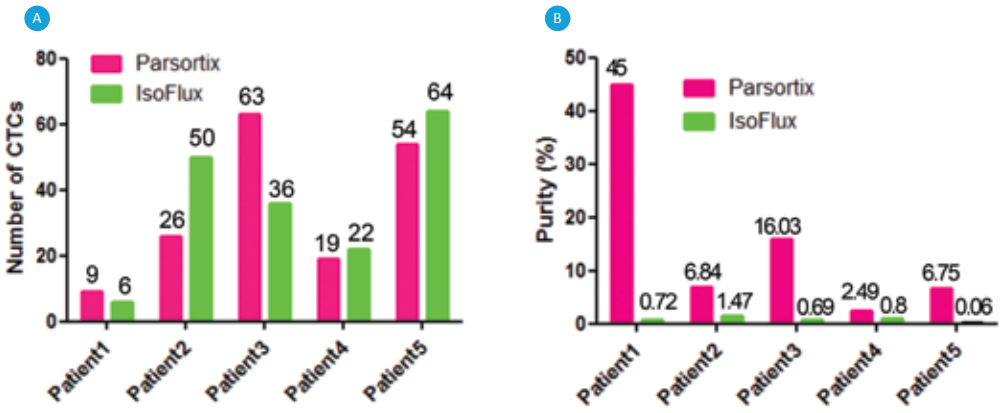


Figure 7. Parallel study between Parsortix and IsoFlux in 5 prostate cancer patients.
A. Comparison of number of captured CTCs.
B. Comparison of purity.

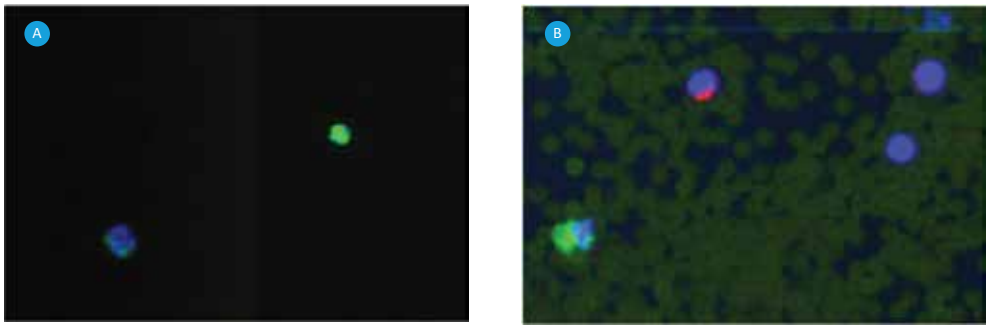
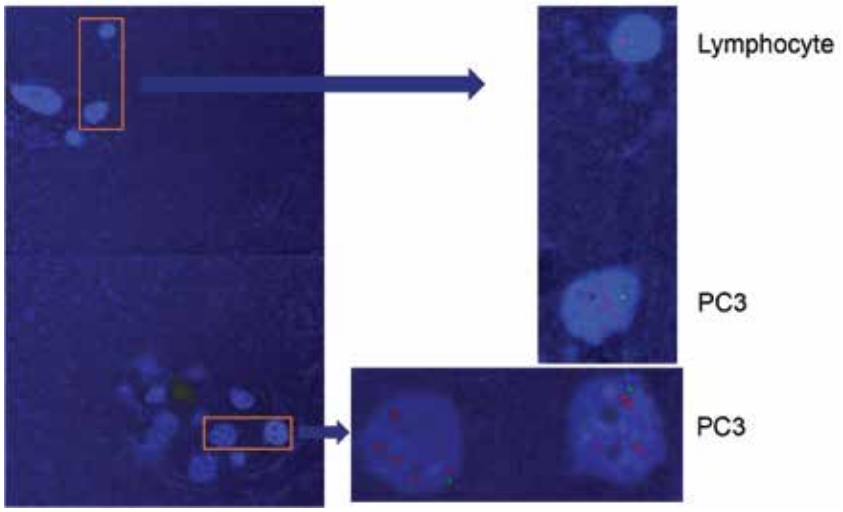


Figure 8. Comparison of image view of CTCs harvested on the slide by Parsortix and IsoFlux.
A. Isolated cells by Parsortix, showing different levels of CK expression. The green signal is stronger for the right upper CTC than the left lower one.
B. Isolated cells by IsoFlux, showing that a CK positive CTC was isolated with some CK negative cells and a large number of beads (small green round dots).



FISH analysis was successfully applied on spiked cells after removing IF signals (Figure 9).

Figure 9. FISH signals on the spiked PC3 cells after IF, using AR (red) and 8p (green) as detective probes. PC3 cells can be distinguished from surrounding lymphocytes by the appearance of aneuploidy.

Conclusions

When tested using cell lines with epithelial cell features (EpCAM positive), the immunomagnetic bead approach was more effective than a size based approach at capture and harvest of these cells. However, when tested using patient samples, this difference disappeared and similar numbers of CTCs were harvested by both systems. The Parsortix size based approach consistently harvested CTCs at a higher purity (lower WBC contamination) than the Isoflux system and does not have magnetic beads in the harvest. This makes the Parsortix harvest particularly suitable for certain downstream molecular analysis, such as FISH.

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