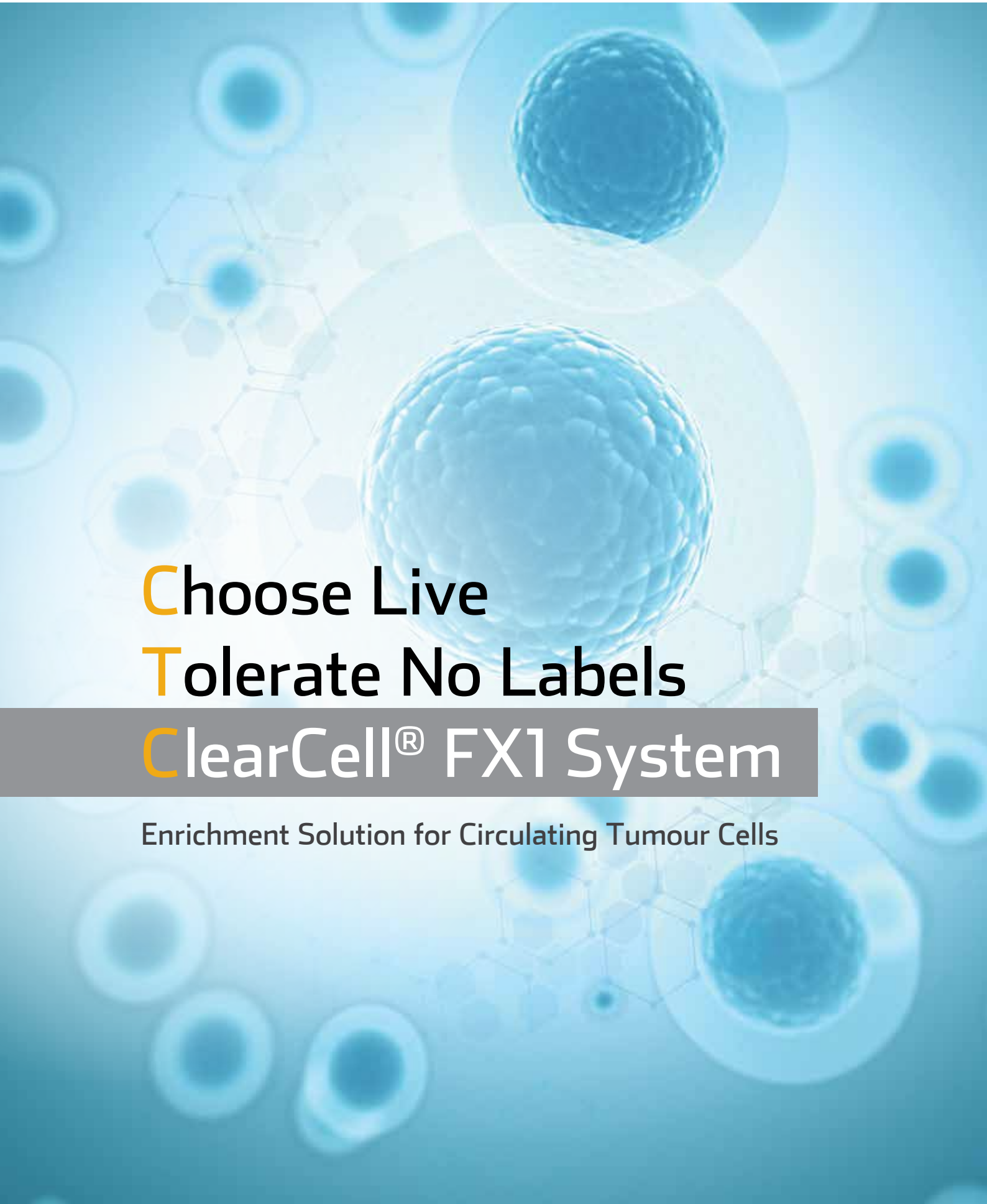
PROTEIGENE

Biolidics



Choose Live
Tolerate No Labels
ClearCell® FX1 System

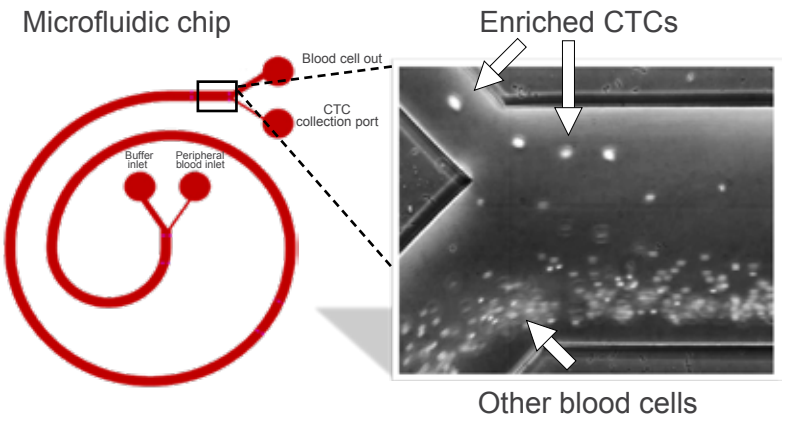
Enrichment Solution for Circulating Tumour Cells

Biolidics

Product Introduction

ClearCell® FX is driven by the proprietary microfluidic CTChip® FR. It is one of the world's first automated system that can rapidly and efficiently enrich circulating tumour cells (CTCs) from the patients' blood. By leveraging on the process of Dean Flow Fractionation (DFF), CTCs can be isolated based on size, deformability and inertia relative to other blood components.

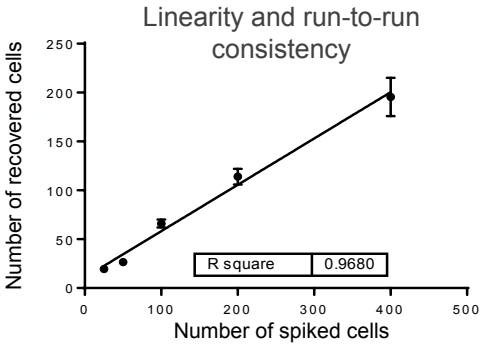
Through this process of DFF, blood cells are distributed by themselves within the channels, with the larger cells along the inner wall and the smaller cells away from the inner wall. This allows for effective and fast separation without compromising on the quality of the retrieved cells.

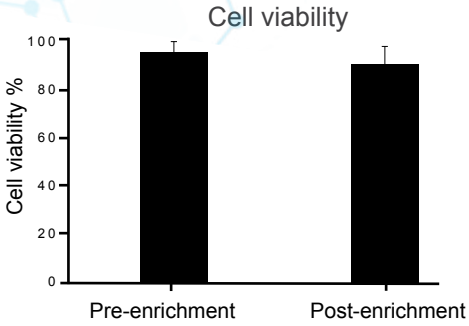


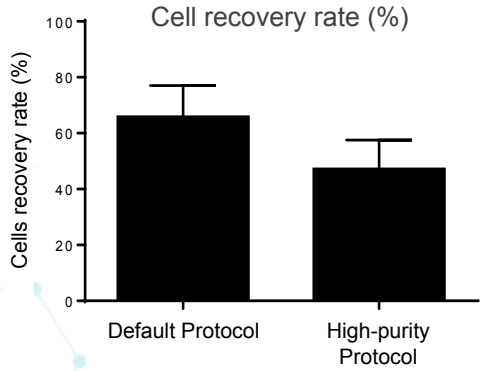
Unique Selling Proposition

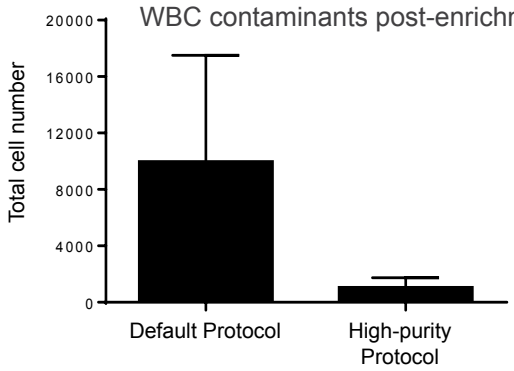
1. Fully automated CTC enrichment completed within 1 hour.
2. High CTC recovery rate - Maximum sensitivity in integrated diagnostic assay.
3. High-purity of CTCs - >99.99% removal of white blood cell.
4. High cell viability - Cell viability is about 90% after ClearCell FX® enrichment.
5. "Label-free" approach - Unbiased enrichment of heterogeneous cancer cells, from different cancer types.
6. Both cfDNA and CTC can be analyzed from a single 10 mL tube of blood.

System Performance Parameters



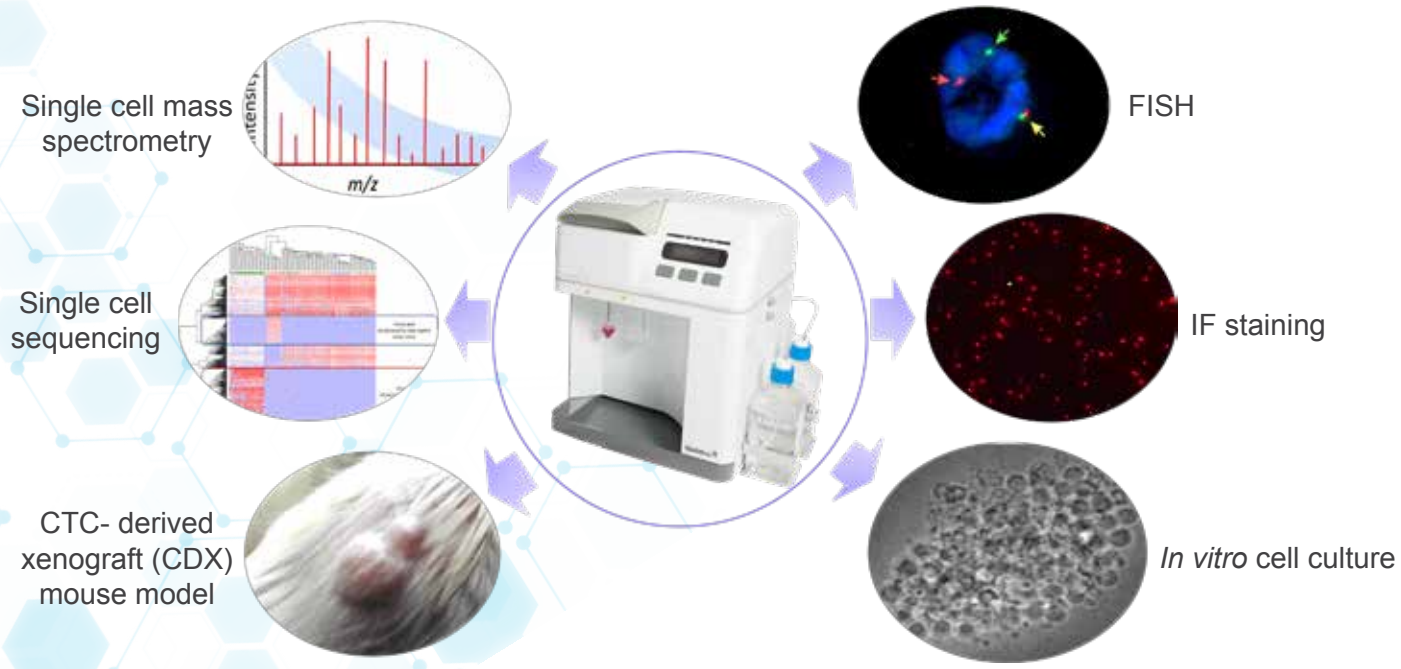






Wide Range of Applications

CTCs isolated by ClearCell® FX system are label-free, intact and viable, which enables cancer discovery as well as better patient management.



Product Specifications

Unit Dimensions (Unboxed)

Height	51 cm
Width (Including reagent bottle)	51 cm
Depth	40 cm
Weight	35 kg

Power Specifications

	50/60Hz
Required Voltage	100-240 VAC, 1A
Power Rating	96 W

Environmental Specifications

Operating Temperature	18-32°C
Storage Temperature	5-40°C
Operating Environment	For indoor use only
Humidity	20-60%

About Biolidics Limited

Incorporated in 2009, Biolidics (formerly known as Clearbridge Biomedics Pte Ltd) is a Singapore- based medical technology company focusing on the development of cell enrichment systems which, when combined with other analytical tests, have a wide range of applications for cancer diagnosis, prognosis, treatment selection and treatment monitoring.

Biolidics has developed the ClearCell® FX1 System, a fully automated IVD medical device which relies on a novel patented technology to separate and enrich cancer cells from blood. Biolidics' ClearCell® FX1 System allows users of the system to perform liquid biopsies to test for the presence of cancer cells (specifically circulating tumour cells, or CTCs) in blood samples or perform further analysis on cancer cells.

Biolidics' quality assurance capabilities have been recognized through its ISO certification. Europe: CE-marked. North-America: The ClearCell® FX1 System is registered with the US FDA Class 1 medical device.

Ordering Information

Biolidics Limited (formerly known as Clearbridge Biomedics Pte Ltd)

PROTEIGENE
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Biolidics is an ISO 13485 certified company

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E&OE
Oct 2019

Advancing Cancer Discovery

Biolidics

Introducing the ClearCell® Systems

ClearCell® FX

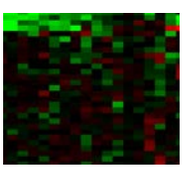
ClearCell® FX

- Simple and standardized workflow for high reproducibility
- Robust workflow for co-harvesting of cell free DNA (cfDNA) and circulating tumor cells (CTC)
- Minimal hands-on time and off-line processing
- No biomarkers used in CTC isolation — Capture heterogeneous population of tumour cells to fit your research and discovery needs
- Wholly intact, live CTCs

Clearcell® LX

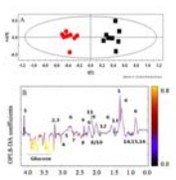
ClearCell® LX

- Automated image capture of CTC in less than 10 min per sample
- Scans up to 4 samples
- Easy to use software for CTC identification and analysis



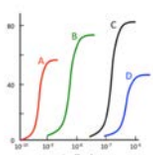
1. **Single cell gene expression profiling**

2. **Next generation sequencing**

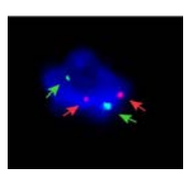


3. **Single cell metabolic profiling**

4. **Patient-derived xenograft**



5. **Drug sensitivity screening**



6. **CTC genotyping (FISH)**

Selected Publications

1. Single-cell gene expression profiling of patient-derived CTCs for recurrence prediction

Addressing cellular heterogeneity in tumor and circulation for refined prognostication. PNAS (2019)

- Specific gene signatures with distinct gene expression profiles in CTCs from patients with differing metastatic potential were identified by single cell CTC analysis (Fig. 1A)
- The use of CTC-derived gene expression signature further refines a prognostic risk model in predicting recurrence in non-small cell lung cancer that takes into account intratumor heterogeneity (ITH) (Fig. 1B)

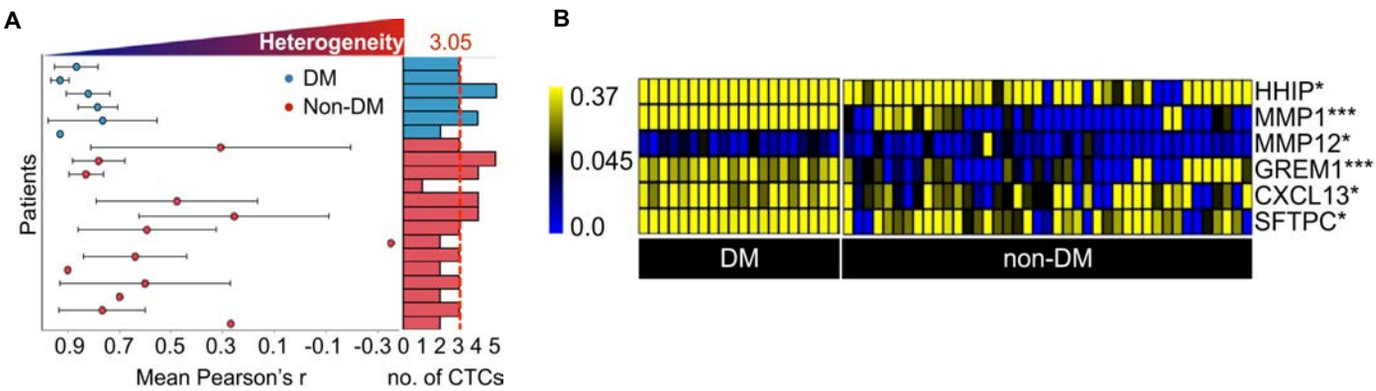


Fig. 1: A. Heterogeneity in 15-gene matrisome expression across all CTCs detected within the same patient with (blue) or without (red) distant metastases (DM) **B.** Distinct signature gene expression profiles in patients with (DM) and without (non-DM) distant metastases

2. Next Generation sequencing (NGS)

Succinct workflows for circulating tumor cells after enrichment: From systematic counting to mutational profiling. PLOS ONE (2017)

- Immunofluorescence-based CTC enumeration workflow established with 80.4% sensitivity and 85.7% specificity in 56 cancer patients and 21 healthy donors (Fig. 2)
- Next-generation sequencing (NGS) workflow integrated to detect somatic mutations in CTCs (Table 1)

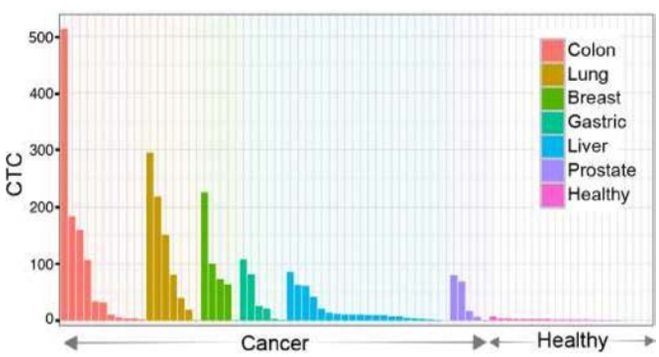


Fig. 2: No. of CTCs per 8mL of blood detected in individual cancer patient and healthy donor

Sample	CTC number	Genomic coordinate	Mutation	Somatic *p* value in technical repeat (coverage)
Patient-12	10	Chr17: 7573937	A364S	***Run 1***
				$p = 1.8 \times 10^{-7}$ (448952)
				Run 2
				$p = 1.9 \times 10^{-3}$ (335959)
Patient-19	45	Chr6: 152419988	S559A	***Run 1***
				$p = 7.9 \times 10^{-4}$ (57352)
				Run 2
				$p = 0.023$ (62598)

Table 1. Sequencing results of CTCs from patients

Selected Publications

3. Single cell metabolic profiling for biomarkers discovery

Live single cell mass spectrometry reveals cancer- specific metabolic profiles of circulating tumor cells. Cancer Science (2019)

- Metabolomic profile of single CTCs obtained from gastric cancer (GC) and colorectal cancer (CRC) patients were analysed using live single cell mass spectrometry
- Cancer origin-specific biomarkers and metabolic profiles were elucidated in gastric cancer and colorectal cancer CTCs (Fig. 3A & 3B)

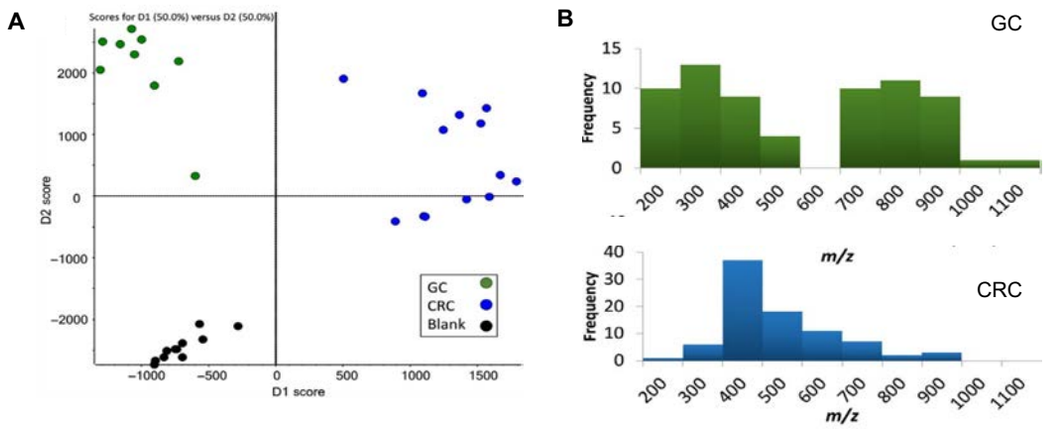


Fig. 3: A. Principle component analysis (PCA) clustering of single cell profiles of CTCs from gastric cancer (GC) and colorectal cancer (CRC) **B.** Histogram of the frequency of peak distribution across m/z scale in GC and CRC

4. Patient-derived xenograft mouse model for drug resistance analysis

Xenograft tumors derived from malignant pleural effusion of the patients with non-small-cell lung cancer as models to explore drug resistance. Cancer Communications (2018)

- Malignant tumor cells were isolated from the pleural fluid of two non-small cell lung cancer (NSCLC) patients using the ClearCell® FX system and subcutaneously inoculated into female CB17-SCID mice to generate xenograft tumor model (Fig. 4)
- Drug-resistant (crizotinib or osimertinib) xenografts were generated by prolonged treatment with the drugs
- Whole exome sequencing (WES) showed that while the genotypes of xenograft and patient tumor are similar, acquired somatic mutations can be identified in drug-resistant xenografts (Table 2)

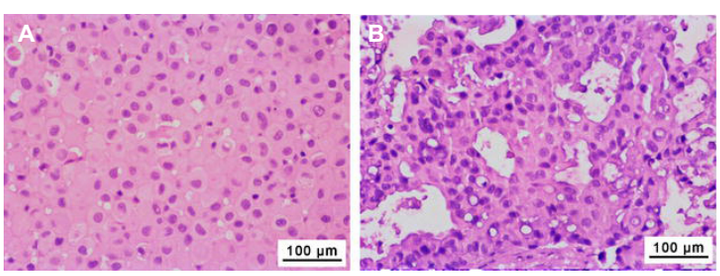


Fig. 4: H&E staining of the tumor biopsy from **A.** patient and **B.** xenograft tumor with matched histology

Sample	Key mutations
Biopsy from patient CTC15035$_{EML-4-ALK}$	EML4–ALK fusion (EML4 exon18–ALK exon 20)
Crizotinib-6 xenograft	EML4–ALK fusion (EML4 exon18–ALK exon 20) ALK: E1210K (9%)
Biopsy from patient CTC15063$_{EGFR, L858R,T790M}$	EGFR: L858R (85.7%) T790M (71.5%)
Osimertinib-3 xenograft	EGFR: L858R (53.6%) , T790M (41.7%); PIK3C2A: R86fs (11%); BRAF: G7V (11.5%)

Table 2. Sequenced mutations in patient tumor and drug-resistant xenografts. Drug-resistance associated mutations are shown in red

Selected Publications

5. *In vitro* drug sensitivity testing

Detection of CTCs in portal vein was associated with intrahepatic metastases and prognosis in patients with advanced pancreatic cancer. Journal of Cancer (2018)

- The portal vein or peripheral blood samples from 29 patients with advanced pancreatic cancer were processed using ClearCell® FX system. CTCs counts in the portal vein were significantly higher as compared to peripheral blood
- The overall survival was significantly shorter in patients with portal vein CTCs over 150 per 7.5 mL of blood than those portal vein CTCs less than 150 per 7.5 mL of blood (Fig. 5A)
- *In vitro* drug sensitivity testing showed that CTCs derived from portal vein blood were highly resistant to several chemotherapy regimens (Fig. 5B)

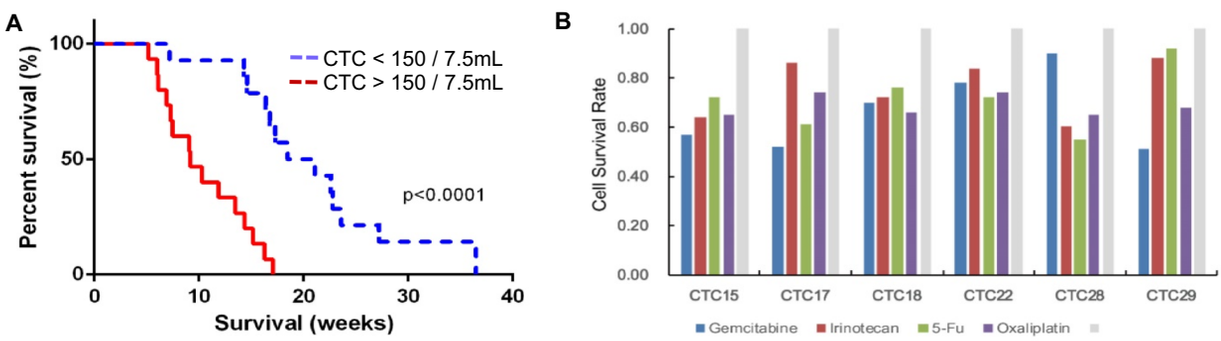


Fig. 5: A. Overall survival of patients with portal vein CTCs above or below 150 CTCs/7.5mL **B.** Drug sensitivity of portal vein CTCs

6. Fluorescent *in-situ* hybridization (FISH) for personlised treatment and monitoring

Concordance of Anaplastic Lymphoma Kinase (ALK) Gene Rearrangements Between Circulating Tumor Cells and Tumor In Non-small Cell Lung Cancer. Oncotarget (2016)

- Anaplastic lymphoma kinase (ALK) gene rearrangement in CTCs from non-small cell lung cancer (NSCLC) was evaluated by fluorescent *in-situ* hybridization (FISH) hybridization
- Over 90% concordance rate in ALK rearrangement pattern was observed between CTCs and primary tumor tissues
- An index case suggests that ALK-positive rearranged CTCs can dynamically monitor efficacy of crizotinib treatment and disease progression

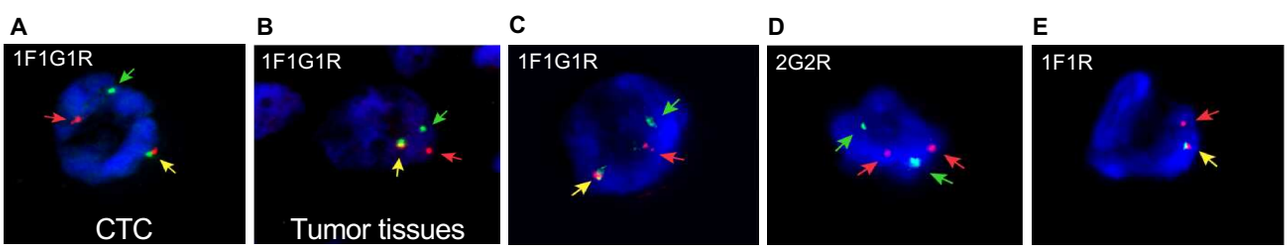


Fig. 6: Representative FISH images showing concordant ALK F1G1R rearrangement in **A.** CTC and **B.** tumor tissue. **C.**, **D.** & **E.** show representative FISH images of multiple ALK rearrangement patterns in a patient with progression after crizotinib treatment.

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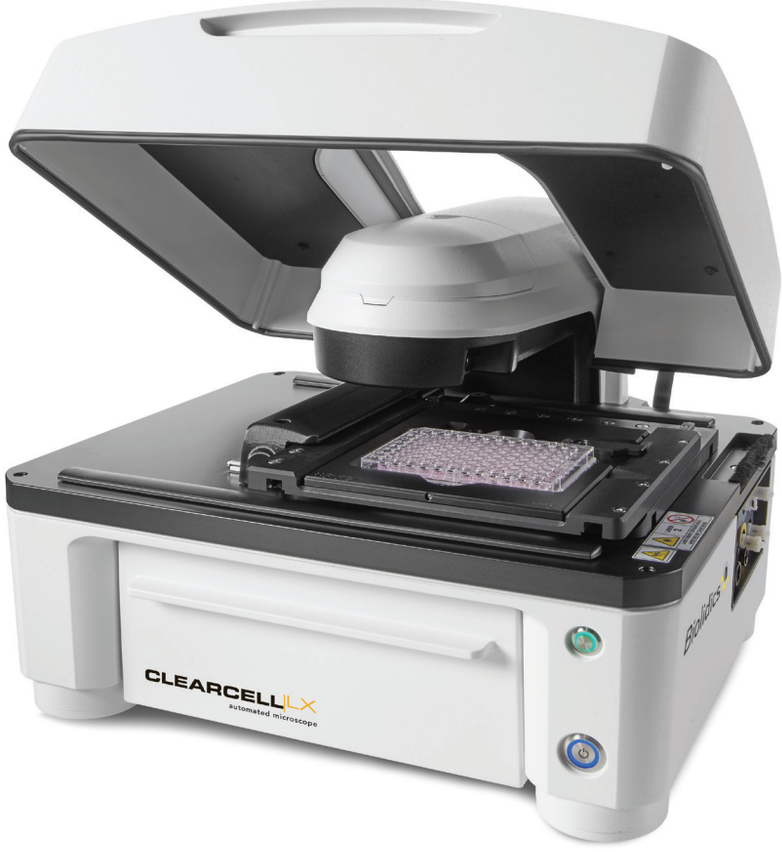
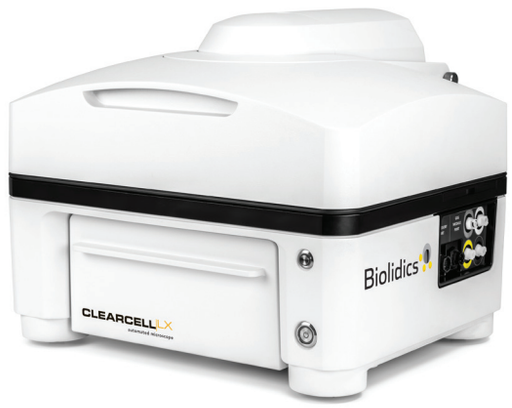
ClearCell® LX

Fully Integrated Imager for Automated Imaging & Analysis of Circulating Tumour Cells

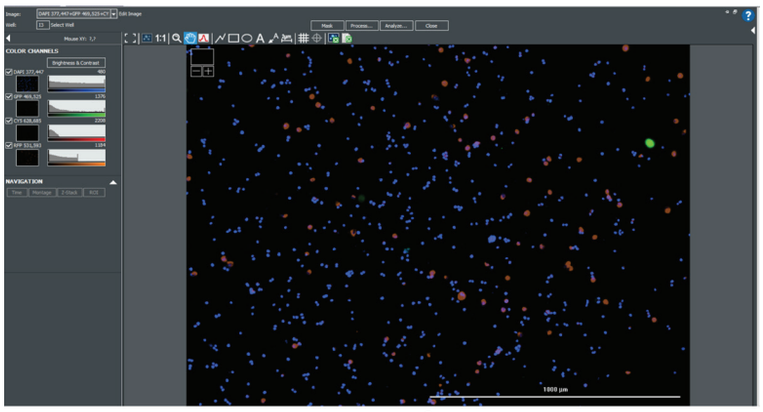


Key Features



ClearCell® LX is designed specifically for the detection and analysis of your circulating tumour cell specimens. The system comes pre-configured with the objectives, filters, and software configurations necessary for automated scanning and analysis of CTC samples on glass slide.

Together with ClearCell® FX's CTC enrichment solution, ClearCell® LX offers an integrated workflow from whole blood to cancer insights.



User Interface

Pre-Configuration
Software comes pre-configured for CTC characterization and enumeration

Easy-to-Use
User-friendly software for the review and rapid characterization of CTC

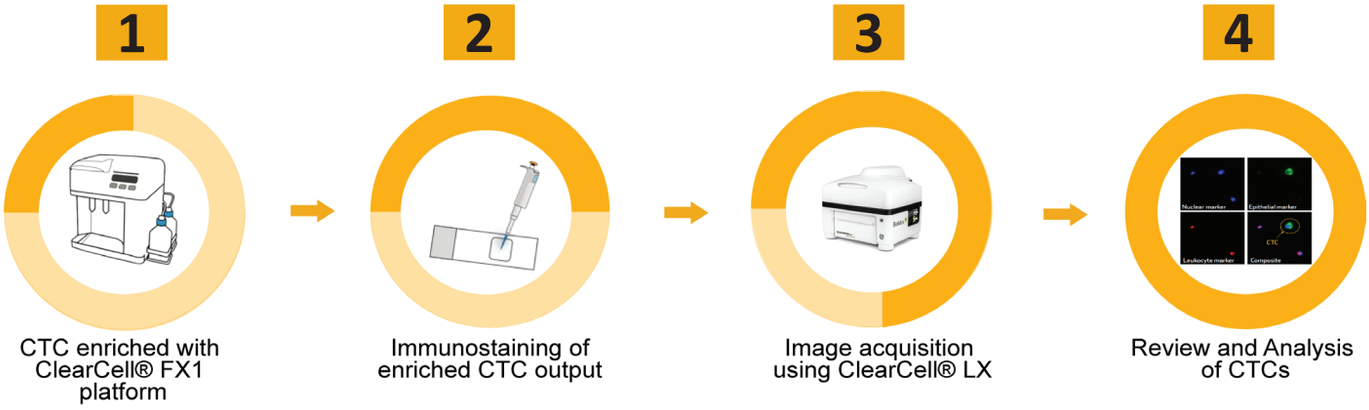
High Throughput
Rapid and easy scanning of the entire sample. User can scan up to 4 samples in a single load

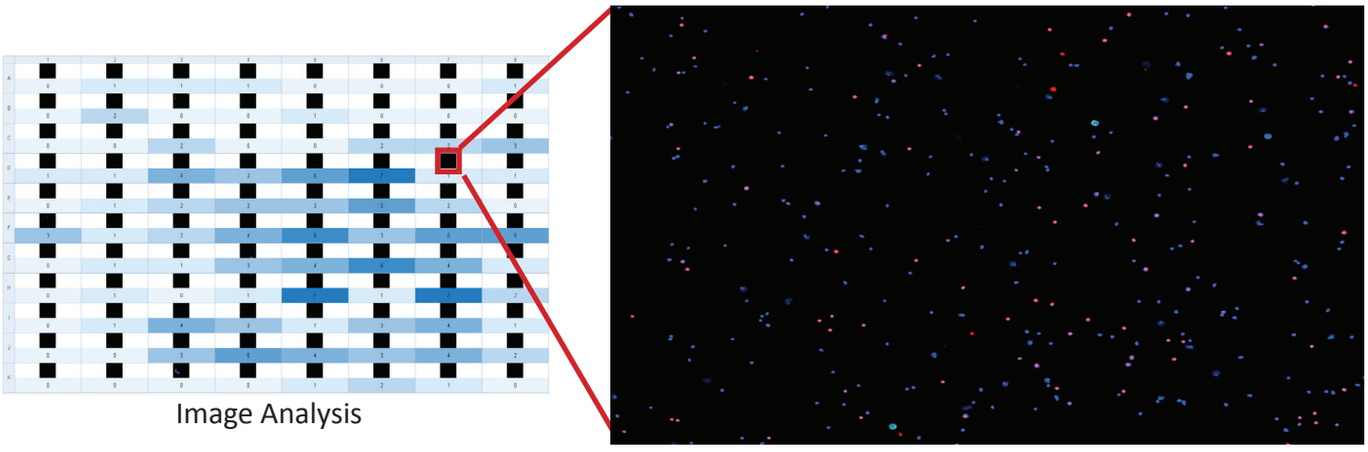
Automation
Automated digital microscopy with motorized XY stage, auto focus, auto image capture

Offline Review & Analysis
Software allows for the offline review and analysis of CTC with the biometric data of interest (E.g cell area, signal intensity)

Customization
Customizable protein marker detection made possible with an additional fourth fluorescence channel available (e.g., PD-L1, AR-V7, etc.)

Workflow Overview



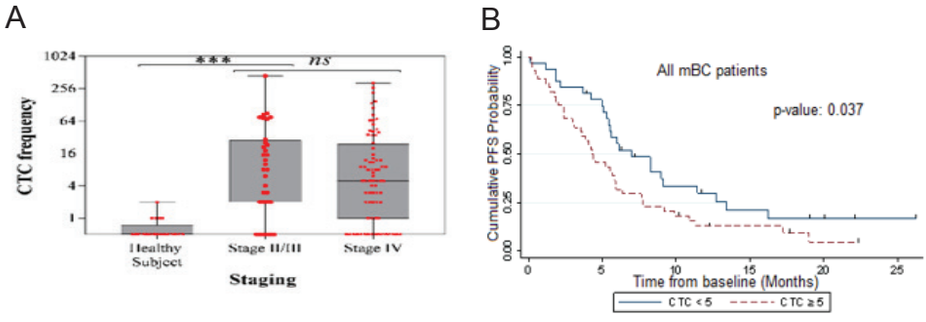


Selected Publications on CTC Enumeration

CTC frequency is associated with poor survival outcome in breast cancer patients

A. CTCs are detected in breast cancer patients from Stage II-IV

B. Metastatic breast cancer (mBC) patients with 5 or more CTCs/7.5mL of blood are associated with poor progression-free survival (PFS)

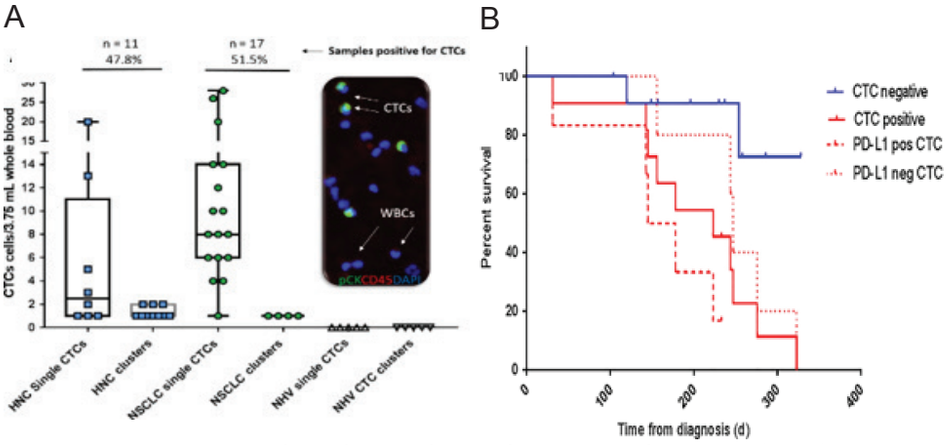


Yap, Yoon-Sim, et al. PloS one 14.9 (2019): e0221305

CTCs are predictive of poorer outcomes in head and neck cancer patients

A. CTCs are detected in patients with head and neck cancer (HNC) and non-small cell lung cancer (NSCLC), but not in normal healthy volunteers (NHV)

B. HNC patients with CTC-positive counts are associated with poor progression-free survival (PFS)



Kulasinghe, Arutha, et al. Cancer medicine 7.12 (2018): 5910-5919

Product Specifications

GENERAL	
Top cover	Light-tight, imaging, dust cover. Keeps the optics dust-free and blocks excessive light
X/Y stage resolution	Lead screw driven stage with 0.1 micron resolution
Software	CTC identification software included. Generates image and list of potential CTCs for user review and confirmation. Software optimized to support CTC counting
Unit dimensions	18.3” D, 17.9” W, 14.1” H (46.5 cm x 45.5 cm x 35.8 cm)
Weight	51 lbs (23.1 kg)
Power	60 Watts maximum consumption
IMAGING	
Modes	Fluorescence, high contrast brightfield and color brightfield imaging capability with 4-position slide holder
Light source	High power LEDs in 365nm, 465nm, 523nm, 623nm wavelengths
Camera	16-bit gray scale, Sony CCD, 1.25 megapixel
Objectives	4X (NA: 0.13)
Filter cubes	Blue(DAPI), Green(GFP), RFP and CY5
Collection rate	Angle well fastest frame rate capture (integration time dependent): Full resolution: up to 10 frames per second for single color images 2x2 Binning: up to 20 frames per second for single color images
OTHER ACCESSORIES	
Imaging system controller	Pre-configured computer optimized for the imaging systems. Includes monitor, key-board and mouse

Ordering Information

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