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REVIEW ARTICLE



# Advances in liquid biopsy on-chip for cancer management: Technologies, biomarkers, and clinical analysis

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**ABSTRACT**

Liquid biopsy, as a minimally invasive method of gleaning insight into the dynamics of diseases through a patient fluid sample, has been growing in popularity for cancer diagnosis, prognosis, and monitoring. While many technologies have been developed and validated in research laboratories, there has also been a push to expand these technologies into other clinical settings and as point of care devices. In this article, we discuss and evaluate microchip-based technologies for circulating tumor cell (CTC), exosome, and circulating tumor nucleic acid (ctNA) capture, detection, and analysis. Such integrated systems streamline otherwise multiple-step, manual operations to get a sample-to-answer quantitation. In addition, analysis of disease biomarkers is suited to point of care settings because of ease of use, low consumption of sample and reagents, and high throughput. We also cover the basics of biomarkers and their detection in biological fluid samples suitable for liquid biopsy on-chip. We focus on emerging technologies that process a small patient sample with high spatial-temporal resolution and derive clinically meaningful results through on-chip biomarker sensing and downstream molecular analysis in a simple workflow. This critical review is meant as a resource for those interested in developing technologies for capture, detection, and analysis platforms for liquid biopsy in a variety of settings.

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Liquid biopsy; cancer biomarker; biosensor; lab-on-chip; point of care

## 1. Introduction

Cancer remains the second highest cause of death in the United States, with over 1.5 million new diagnoses and over half a million deaths expected in 2017 [1]. It is expected that almost one quarter of people who live in the developed world will die of cancer, and most of these cases present with symptoms to a healthcare practitioner [2]. It is widely known that survival rates from cancer are higher when the disease is diagnosed earlier because there is a greater chance of successful treatment [1,3–5]. Successful treatment also depends on accurate monitoring of cancer dynamics, which can often entail many molecular studies, expensive imaging, and invasive tissue biopsies [6–8].

There is a trend toward individualized molecular testing of cancer patients to determine genetic alterations, treatment progress, and tumor immunohistochemistry for monitoring [8,9]. For these analyses, a tissue biopsy is typically used and the molecules of interest are extracted and analyzed. Standard molecular analysis of proteins and nucleic acids is performed using robust clinical equipment for sequencing, immune profiling, and cell processing. These analyses are also time-consuming, expensive, and require extensive technical expertise from clinicians [10]. In settings outside of the clinic, complicated instrumentation will not be available, or there may be situations in which a simpler, sample-to-answer technology is required.

Liquid biopsies, or capture of tumor-derived biomarkers in a fluid sample rather than a tissue sample, are growing in popularity because of their minimal invasiveness, ease-of-use, and high throughput for personalized analyses [7,10–12]. Among the biomarkers of interest are cells, proteins, vesicles, and nucleic acids that shed from the tumor site into the bloodstream, saliva, urine, or cerebrospinal fluid (CSF), among others. The presence of these biomarkers allows for less invasive technologies to analyze tumor biomarkers rapidly in single step assays. Thus, there has been a trend toward on-chip technologies that allow for tumor biomarker capture and analysis in a single simple workflow. These technologies hold a lot of promise for patient diagnosis, therapeutics, monitoring, and prognosis in a

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variety of settings without requiring invasive sample acquisition [13].

This article will first review the typical structure of a biosensor for capture of an analyte and readout. This framework will be applied to point of care applications to try to understand the required properties of a sensor for use in a variety of settings. Then, liquid biopsy and the biogenesis of individual biomarkers will be discussed, including typical biomarker concentration in blood samples. Then, body fluids that are used for cancer liquid biopsy will be reviewed, including whole blood and its derivatives, saliva, urine, and CSF. Technologies for capture, detection, and analysis of the biomarkers will be reviewed based on their methodologies, clinical utility of analysis, and technology readiness for point of care of settings. Finally, recommendations will be made for the direction of technology development for single, sample-to-answer platforms for liquid biopsy on chip (Figure 1).

## 2. Biosensors design

A biosensor is a device that takes a biological material and puts it in contact with a recognition element to capture an analyte, and then a transduction element to allow for a measurable signal [6,15–19]. For design of the technology, there are a number of steps that require decision making, including how the sample is handled and how biomarkers are quantitated.

The sample handling is the first step and involves potential preprocessing to get the analyte in the sample to the recognition element. The recognition element is the piece that allows for analyte specificity and can be immune recognition, size, or physical parameter based, or based on electrochemical cues [13,20]. The choice of recognition element is generally based upon the biomarker analyte desired and the required specificity of the system. Then, that binding of the analyte must somehow be converted to a measurable signal, which is accomplished through a transduction element. This transduction can be done through optical, magnetic, chemical, electrical, or a number of other signaling methods with the main goal of producing some sort of quantitative signal [6]. The purpose of this step is to convert the presence and quantity of analyte in the sample into an output that can be analyzed and processed computationally if necessary. In most cases, it is ideal if the measurable signal scales linearly to the quantity of analyte captured, because this will give the sensor a simple working range with easy readout.

Testing at the point of care allows a technology to be made available to a larger group because it can be used in a variety of settings [6]. When applying biosensors to point of care settings, there are a number of important qualities that improve their ease of use, as shown in Figure 3. In order to allow the sensor to be used in a widespread way, it should be generally low cost and easy to use. It is also advantageous for the sensor to use a small sample volume, and be high throughput with a rapid sample-to-answer format. Finally, for storage and transport of point of care devices, a small footprint, and no need for additional equipment improve their applicability.

We will use this framework to evaluate the devices for liquid biopsy that will be reviewed in later sections. Technologies will be reviewed based on their applicability to the point of care based on their technology readiness and the degree to which they satisfy the factors put forth in Figure 2.

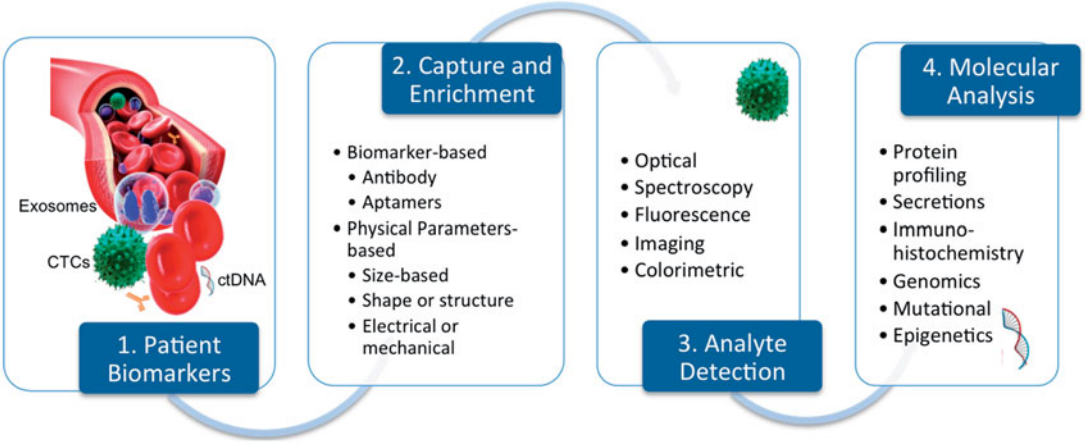


**Figure 1.** Overview of liquid biopsies on-chip. An overview of integrated capture and analysis of biomarkers for point of care cancer diagnosis. Adapted in part from Ref. [14] with permission of The Royal Society of Chemistry.

## 3. Liquid biopsy biomarkers and biogenesis

Liquid biopsy involves gathering information about a disease through a liquid sample rather than a tissue sample [7,12,13,21–23]. Typically, cancer is diagnosed through clinical pathology, with a tissue biopsy or imaging to screen or diagnose various types of cancer. Liquid biopsy provides a promising approach for early diagnosis, therapeutic and prognostic decision making, and monitoring through minimally invasive fluid sample collections rather than a more invasive biopsy [22]. Liquid biopsy also is expected to be lower cost because of the ease of sample collection and its ease of use in the clinic. Before liquid biopsy technologies can be discussed in full detail, it is important to understand the types of biomarkers and their biogenesis in cancer. The main biomarkers that have been studied in liquid biopsy for cancer include circulating tumor cells (CTCs), extracellular vesicles called exosomes, and circulating nucleic acids such as DNA and miRNAs [13,21–23]. These biomarkers are shed off into the bloodstream, urine, saliva, or CSF from the tumor site and can be detected in the fluid and further analyzed to improve clinical understanding of the patient condition, as shown in Figure 3.

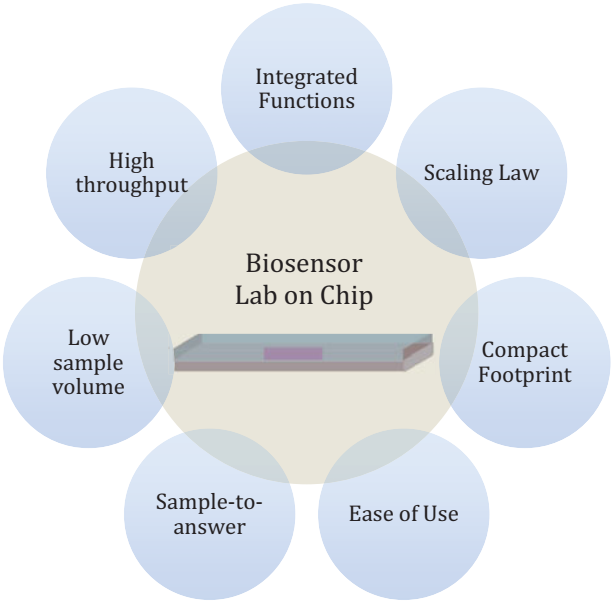


**Figure 2.** Advantages of biosensor and Lab on chip for liquid biopsy, including integration, miniaturization, and clinical advantages.

### 3.1. Circulating tumor cells

CTCs are tumor cells that shed off of the tumor site into the bloodstream, and they are the main mechanism for metastasis [13,24,25]. The major obstacle to easily capturing and analyzing the CTCs lies in their rarity, as CTCs are quite literally one in a million or billion among other cells in the blood [26]. Another challenge has to do with the heterogeneity of CTCs, as they can vary in surface protein expression, size, and physical characteristics, and often travel through the bloodstream in clusters [13]. CTCs are captured in a number of ways, including immune recognition, separation based on size or stiffness, and other chemical recognition methods. Once they are captured, though, CTCs can provide valuable insight into the dynamic fluctuations and characteristics of the disease without requiring access to the tumor site [21].

### 3.2. Circulating vesicles: Exosomes

Exosomes are small, membrane-bound vesicles that are shed from cells for signaling and contain a number of biomolecules such as nucleic acids and proteins

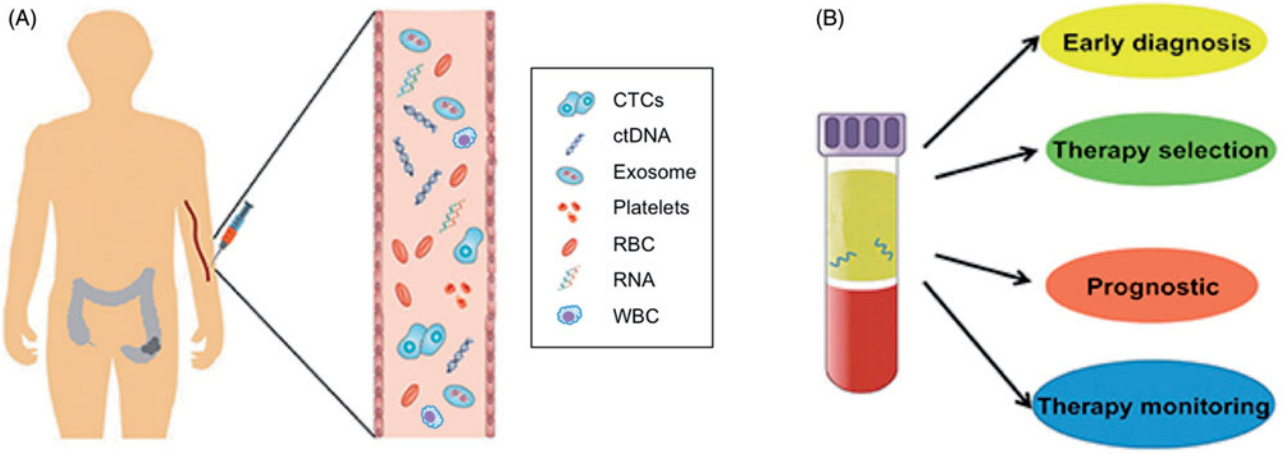


**Figure 3.** Typical circulating biomarkers and applications of their capture and analysis to diagnostics, prognostics, and therapy. Adapted with permission under the Creative Commons Attribution License 3.0 from Ref. [21].

[21,27–29]. They tend to be in the tens to hundreds of nanometers in size and have the advantage that their contents are protected from degradation within a lipid membrane [28]. Because of similarities between the exosome membrane and CTC membranes they can be isolated using the same immune recognition. Other methods of separation include, but are not limited to, size differentiation and ultracentrifugation procedures [21,30–32]. Exosomes circulate in the blood in concentrations above $10^9$ individual exosomes per milliliter of blood and the total quantity scales with the severity of the tumor [21,33].

### 3.3. Circulating nucleic acids: ctDNA and miRNAs

Nucleic acids are shed into the bloodstream through cell apoptosis, necrosis, or secretion [34–37]. Anker et al. showed that while small amounts of free-floating DNA circulate in healthy plasma and serum, cancer patients have increased concentrations due to the presence of ctDNA [36]. These DNA strands exhibit the same point mutations that are found in the tumor and also contain epigenetic alternations such as variable copy number and methylation [38]. In a test of over 150 cancer patients and over 50 healthy controls, Anker et al. found that healthy patients have circulating DNA concentration of about 13 ng/ml, while cancer patients had over 10 times that with an average of 180 ng/ml [36]. More recently, the presence of RNA in plasma and serum have also been found, indicating that both DNAs and RNAs are biomarkers of interest for liquid biopsy [35].

## 4. Sample fluid types for liquid biopsy

Liquid biopsy uses body fluids to detect biomarkers that would otherwise be analyzed from tissue samples, and there are a number of sample types that have been used for liquid biopsy. These include whole blood, blood derivatives, such as plasma and serum, CSF, urine, and saliva, among others [28,39–41]. Given the relative novelty of liquid biopsy, there is still active research in which of these fluids are suitable for a given application, and the proper sample handling procedures for their use. While the bulk of the studies have focused on serum and plasma, saliva has been used mainly for oral and lung cancers [42], CSF has been used for central nervous system cancers [43], and urine has been used for those relating to the urinary tract [44,45]. These fluids and the potential biomarkers that can be derived from them are shown in Figure 4.

### 4.1. Whole blood

Whole blood is the most common body fluid use for analysis of CTCs and is a common starting point for deriving plasma or serum for nucleic acid or vesicle studies [13,47]. For point of care applications, starting with whole blood is convenient because it requires no sample preparation between collection from the patient and analysis. Because CTCs are similar in size to blood cells, whole blood must be used for their analysis because centrifugation will also remove CTCs from the sample [48–50].

### 4.2. Plasma and serum

Both plasma and serum are derivatives of whole blood without the cells and are acquired through centrifugation of blood [16,51,52]. Plasma and serum cannot be used for capture and analysis of CTCs because they are the remnants after removal of blood cells, which also removes CTCs. For the purposes of liquid biopsy, these fluids are most commonly used for capture and analysis of exosomes and circulating nucleic acids. The difference between serum and plasma is that plasma still contains many of the anticoagulants that are in blood, so serum requires more sophisticated equipment to derive from a blood sample [53]. Thus, for point of care applications, plasma might be slightly easier to acquire than serum, although the difference is negligible. Both plasma and serum are used widely in liquid biopsy for a variety of cancers.

### 4.3. Saliva

Saliva is made up of mostly water with cells and other organic and inorganic molecules that can be studied for clinical purposes [54]. It is an attractive sample type because of its ease of collection and storage, and biomarker validation for saliva samples is an active area of research [42]. Compared to blood and the blood derivatives described above, saliva collection is a truly noninvasive procedure. Research has linked salivary biomarker profiles to systemic diseases, oral diseases, and pharmacology for drug response [42]. Saliva has been linked to head and neck cancer, oropharyngeal cancers caused by human papilloma virus (HPV), and lung cancers.

In terms of sample collection, some work has been done to study the collection and storage methods required for successful analysis of biomarkers [42,54]. There is not a wide consensus over whether the saliva sample should be centrifuged prior to processing and how quickly the sample should be analyzed, but some

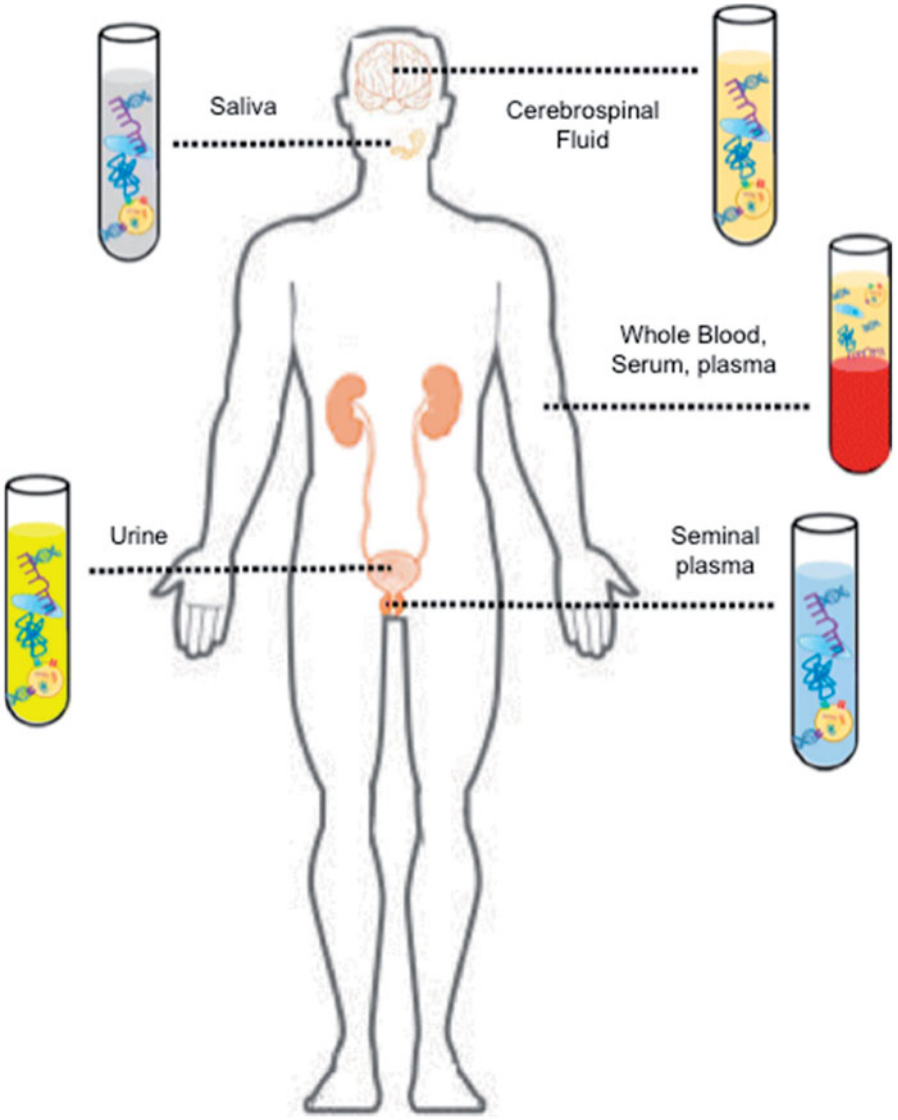


**Figure 4.** Sample fluid types for liquid biopsy, including blood and its derivatives, cerebrospinal fluid, saliva, and urine. This figure is adapted with permission under the Creative Commons License 4.0 from Ref. [46].

research has shown that samples should be used within an hour and that centrifugation can be performed depending on whether it is cells or smaller molecules to be analyzed [42]. In this article, technologies that use saliva for lung cancer-related applications are reviewed.

### 4.4. Urine

Urine is another fully noninvasive sample type that can be used for liquid biopsy and is most commonly associated with use in bladder and prostate cancer diagnosis and analysis [44,46,55]. There have been a number of studies that compare the biomarkers in urine to those in plasma among the same patients, and there is a range of results. It has been shown that the total level of mutant circulating DNA in the urine of non-muscle-invasive bladder cancer patients correlates linearly with the tumor size and stage [44]. There have also been studies comparing the integrity of biomarkers in urine among cancer and healthy patients, which showed that cell-free DNA has elevated integrity compared to healthy controls [46]. There is some variability in whether the sample is centrifuged prior to analysis, and this also depends on the biomarker of interest [44]. There is still more work to be done to validate the use of urine for other cancers and to determine the ideal sample handling procedures.

### 4.5. Cerebrospinal fluid

CSF is the fluid that surrounds the brain and the spinal cord and requires a relatively invasive procedure for collection: a needle stick between the vertebrae [43]. Because of the invasiveness of sample collection, it has

only been investigated in a few studies for central nervous system cancers. One of the most conclusive studies investigating CSF showed that ctDNA that is captured from CSF better represents the genomic changes during brain cancer than ctDNA from plasma. This makes sense given the human anatomy and the characteristics of the blood brain barrier, but this study showed that ctDNA from brain tumors in CSF is more abundant and shows the same actionable mutations as the tumor [43]. These mutations can also be better monitored over time in CSF than in plasma because they better track what is going on in the brain. While CSF is a better fluid for brain cancers and has a lot of utility in a full clinical setting, it may not be the best option for point of care testing due to its invasiveness.

## 5. Technologies for circulating tumor cell capture and analysis

CTCs pose a number of challenges to the capture and analysis from whole blood, namely their rarity and their similarity in size to white blood cells [56,57]. They are among the most widely studied biomarkers in liquid biopsy, and are of particular interest because of their link to metastasis through shedding from the tumor site, moving through the bloodstream, and entering new sites, forming metastases [13,25,58,59]. Among the most common methods of CTC capture from whole blood is immunomagnetic separation, which uses immunorecognition of surface proteins on CTCs and magnetic particles to separate the CTCs from other blood cells. Another commonly used difference exploited for CTC capture is size or geometry-based capture from other cells.

Once the cells are captured from whole blood, there are many protein and nucleic acid-based analyses that can be performed. Most technologies simply leave the viable CTCs for downstream analysis off-chip, but some technologies perform interesting on-chip analyses that are well suited to point of care settings. These include genomic profiling, CTC secretion profiling, and drug resistance measurements.

The capture and analysis technologies will be evaluated in the text of this article based on a number of device performance criteria. These metrics include (1) throughput, which is the amount of sample that can be processed in a given time, (2) recovery rate of CTCs, the percentage of CTCs in the sample that are captured, (3) the removal rate of other blood cells, and (4) the integrity of captured CTCs for downstream analysis. All of these factors will play into evaluation of its quality as a liquid biopsy on chip for capture and analysis of CTCs. A survey of relevant CTC technologies on-chip is shown in Table 1. Some of these will be highlighted in the text, and the relevant performance metrics will be provided.

### 5.1. Biomarker-based capture of CTCs

Immune or biomarker-based capture of CTCs is the use of antibodies that are targeted to CTC capture on the surface of magnetic particles for capture and separation of CTCs from other cells. The most well-known immunomagnetic technology is called CellSearch and is the only FDA approved immunomagnetic liquid biopsy technology [79]. The most common target protein is EpCAM, an epithelial surface protein on CTCs. But there is evidence to show that a cocktail of capture antibodies works better than a single probe because of the heterogeneity of CTCs in patient samples [80–82]. In this section, we will review technologies that use immunomagnetic capture to separate CTCs from whole blood to perform molecular analysis on chip.

Earhart et al. developed an immunomagnetic method of TC separation called a magnetic sifter, and then used the separated CTCs to do mutational analysis of the EGFR gene on chip [60]. This platform works using a miniature microfluidic chip with an array of magnetic pores. The cells are labeled with magnetic nanoparticles, and the magnetic pores allow for high throughput capture of labeled CTCs onto the magnetic sifter, as shown in Figure 5. The tested flow rates for the device were from 5 to 25 ml of blood per hour; the optimal results were at 10 ml/h providing a 95.7% capture efficiency of CTCs. These CTCs are then lysed and characterized using an EGFR antibody, that is, specific to a clinically relevant mutation and is able to detect the mutation levels in captured CTCs. In this case, the capture and analysis were performed on two separate devices, but because they are both performed on microdevices this workflow could be integrated onto a single chip.

Hoshino et al. developed a device for capture and enrichment of CTCs using immunomagnetic detection [83]. By combining the advantages of microfluidics and immunomagnetics, the group was able to optimize the capture rate of CTCs onto glass slides. Recently, a commercial platform was developed by NanoLite Systems based on this innovation, which was used to carry out combined CTCs isolation and cellular metabolism detection [84]. An illustration of the device prototype and the commercial platform are shown in Figure 6. In operation, blood samples are mixed with magnetic particles conjugated with an antibody for CTC capture. The blood is then flowed over a magnet and the CTCs bound to magnetic beads are attached to a coverslip. After capture, the cells can be imaged by

**Table 1.** Technologies for CTC capture, detection, and analysis on chip.

| Sample type | Cancer type | Capture/detection method | Analysis? On/off chip? | Technology readiness | Reference |
|---|---|---|---|---|---|
| Biomarker-based capture | | | | | |
| Whole blood | Lung cancer | Immunomagnetic "Magnetic Sifter" | EGFR mutation, on-chip | ++ | [60] |
| Whole blood | Prostate cancer | Magnetic immunoaffinity with velocity valley chip | CTC and PSA mRNA profiling, on-chip | +++ | [47] |
| Whole blood | NSCLC | Immunomagnetic | CTC single-cell in nanowell PDMS, on-chip | ++ | [61] |
| Whole blood | Colon cancer | Immunomagnetic separation on-chip | Off-chip downstream | ++ | [62] |
| Whole blood | NSCLC | Magsifter immunoisolation | Single cell seeding, multigene expression, on-chip | +++ | [63] |
| Whole blood | Lung | Magnetic nanoparticle labeling | microNMR (off-chip) for detection | + | [64] |
| Whole blood | Colorectal | Immunomagnetic nanocarriers | Cell staining on-chip | ++ | [49] |
| Whole blood | NSCLC | Immune recognition, microfluidic | Off chip | ++ | [65] |
| Physical parameter-based capture | | | | | |
| Whole blood | Prostate | Nanovelcro chip | Laser microdissection, off-chip NGS | ++ | [66] |
| Buffer | Prostate | Geometrically enhanced differential immunocapture | Nuclei extraction, copy number analysis, off-chip | + | [67] |
| Whole blood | Breast, lung | Enrichment using spiral microfluidics | Immunophenotype, FISH, off-chip | + | [68] |
| Whole blood | Prostate | Size difference on-chip | Single cell Drug resistance measurement on-chip | ++ | [69] |
| Whole blood spiked | Colorectal | Size-based enrichment | Droplet digital PCR Off-chip | + | [70] |
| Whole blood | Hepatocellular carcinoma | On-chip micropillar separation | Off-chip enzymatic treatment for release and analysis | + | [71] |
| Whole blood | Breast, Colon, Lung | Size and deformability microfluidic | Cells were viable and harvested for off-chip analysis | ++ | [72] |
| Whole blood | Breast, colorectal, prostate, cervical | Microsieve for size-based capture | Fluorescence in-situ hybridization | + | [50] |
| Whole blood | Breast | Acoustic chip | Immunohistochemistry | ++ | [73] |
| Whole blood | Breast, lung | Vortex inertial focusing | Imaging and deformability studies | ++ | [74] |
| Other methods | | | | | |
| Whole blood | Lung cancer | Magnetic upconversion nanoprobes | Microfluidic silicon nanowire-array, on-chip | + | [75] |
| Whole blood | Pancreatic | Geometrically enhanced mixing for capture | Release on same chip, cells viable, analysis off-chip | + | [76] |
| Whole blood | Lung cancer | Implantable | Cell collector, molecular analysis downstream | + | [77] |
| Whole blood | Prostate | Microfluidic blood cell depletion | Downstream molecular analysis | +++ | [78] |

Technology readiness from + to+++based upon the level of clinical testing, completeness of technology, throughput, and viability of the recovered CTCs. Details about selected technologies will be covered in the text.

immunofluorescence staining for verification and quantitation. Compared to other existing technologies, this microchip-based technology is able to achieve similar capture rates at significantly higher flow rates through the device. The device was tested with both colorectal and breast cancer cells, with capture rates of 90% and 86%, respectively. The flow rate of whole blood through the device can be regulated from 2.5 to 10 ml/h.

Park et al. developed a method for molecular analysis of single CTCs after magnetic separation from blood [63]. Their nanowell array platform is able to compartmentalize CTCs into single cell wells and then perform multigene expression analyses using quantitative real-time PCR. The workflow is shown in Figure 7 and demonstrates how the cells are separated immunomagnetically before individual seeding and PCR for gene mutation and expression analysis. This technology can be evaluated in comparison to traditional PCR, and shows superior performance because 25,600 nanowells can be run with extremely small 20 picoliter volumes. Such a technology is extremely promising as a single-chip method for capture of individual CTCs directly from blood and then subsequent analysis for therapeutic and monitoring purposes.

### 5.2. Physical parameter-based capture of CTCs

As an alternative to immunomagnetic separation of CTCs, some technologies use size-based isolation of CTCs to separate them from blood. These technologies have the advantage of a label-free approach, meaning that they are less likely to harm the cell's viability through binding to surface proteins [48,69,85]. These technologies often use microfluidics to manipulate the individual cells into capture wells for downstream analysis.

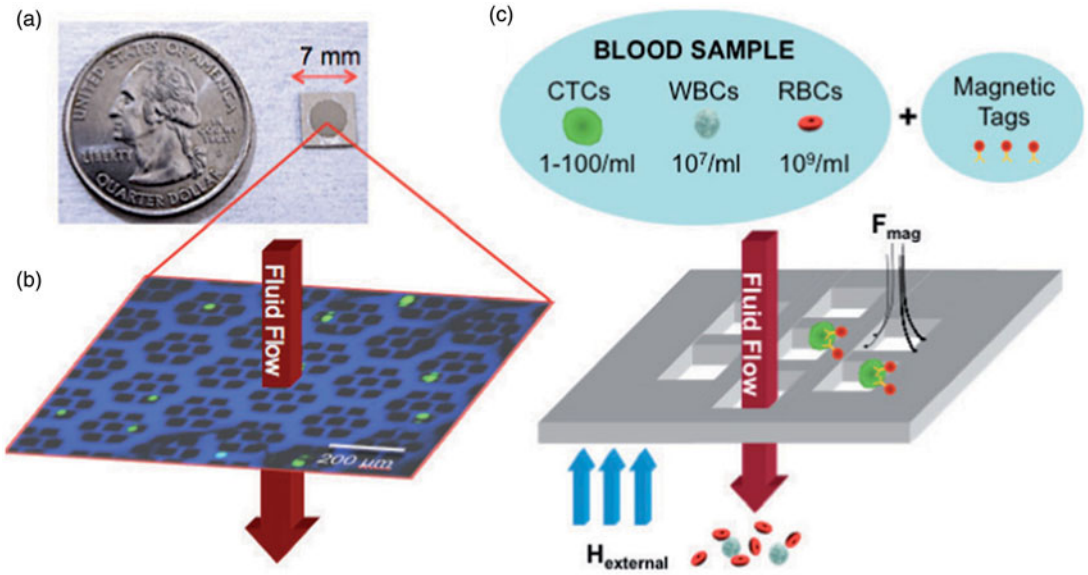


**Figure 5.** Magnetic sifter chip for capture and downstream analysis of CTCs using immunomagnetic separation and mutational analysis. Reproduced from Ref. [60] with permission of the Royal Society of Chemistry.

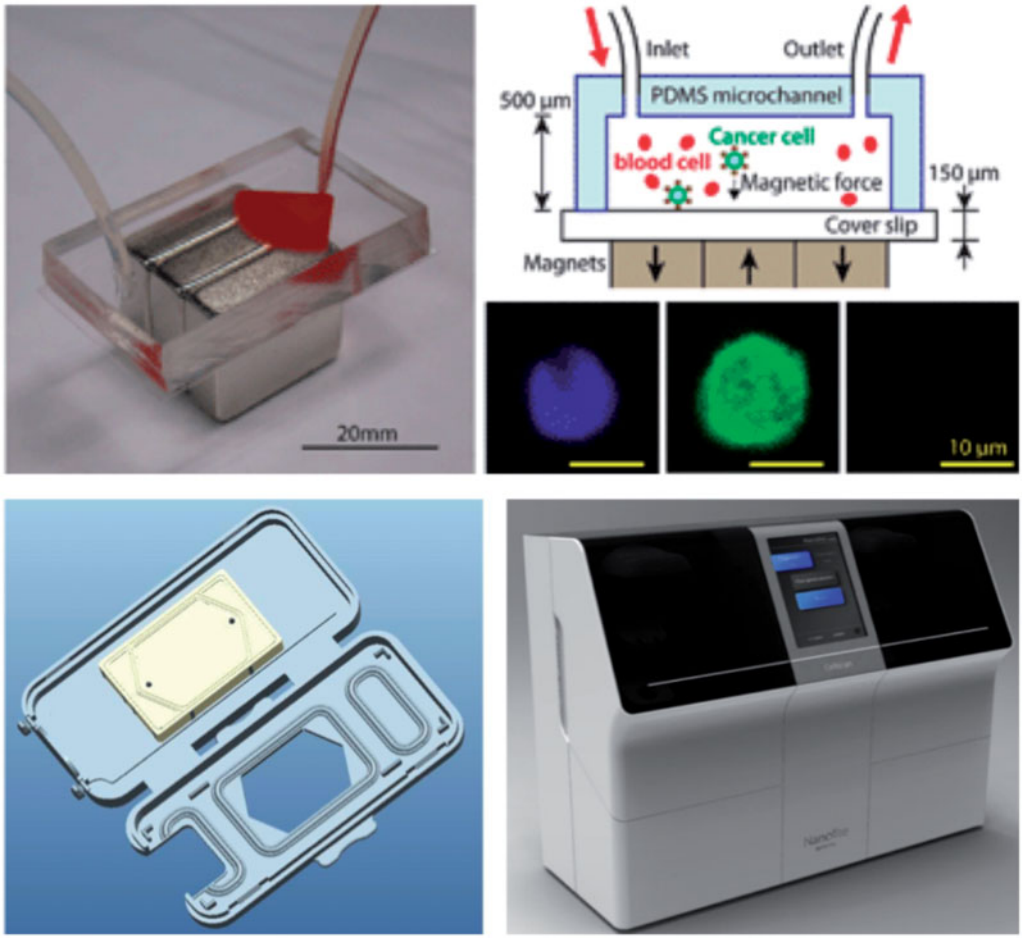


**Figure 6.** Immunomagnetic microchip and platform for CTC separation. Top row: Picture of the device, schematic of operation, and images of captured cells. Reproduced from Ref. [83] with permission of the Royal Society of Chemistry. Bottom row: commercial NanoLite CellRich™ Systems with embedded microfluidic cartridge developed based on the prototype devices.

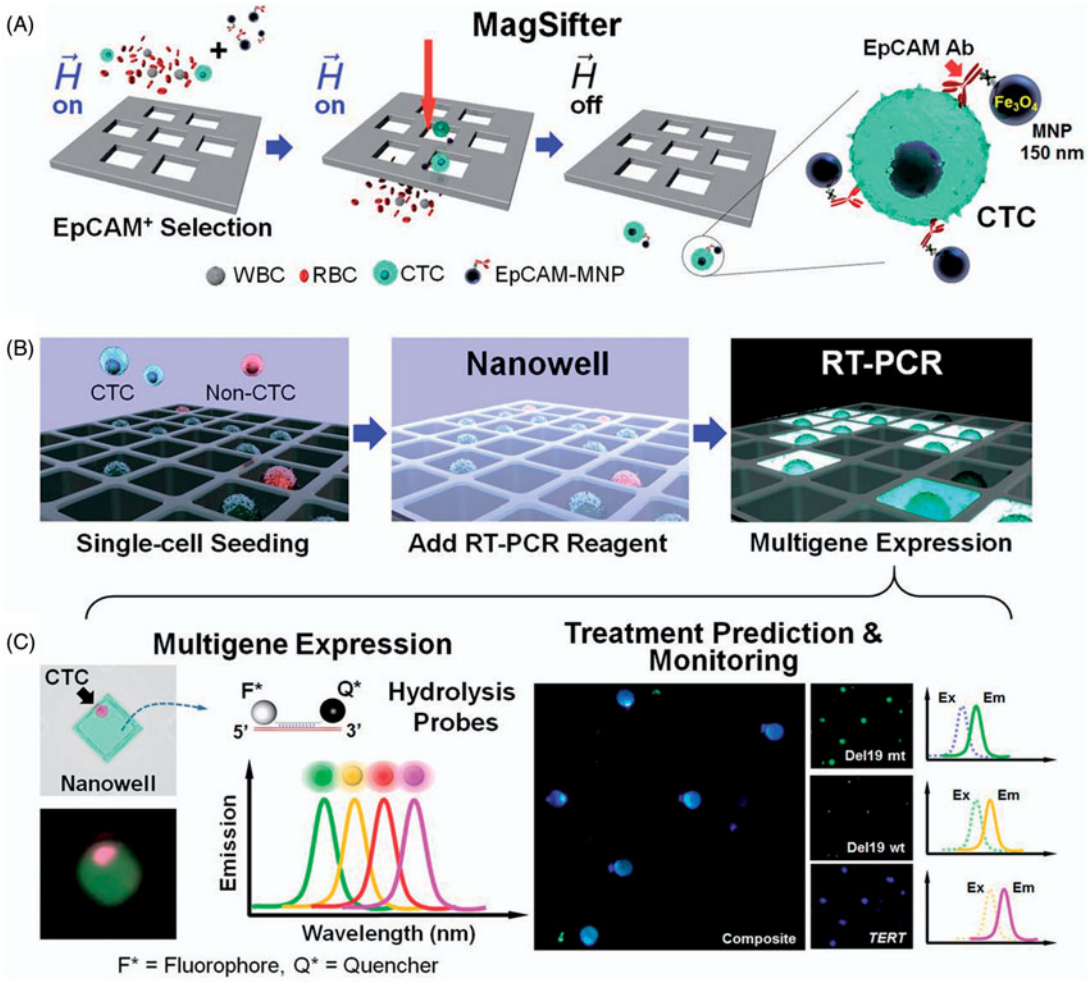


**Figure 7.** Integrated nanoplatform for single CTC seeding and mutational analysis. Reproduced with permission from Ref. [63].

Khamenehfar et al. developed a method to isolate prostate cancer cells from blood cells and measure drug accumulation at the single cell level on a single microfluidic chip [69]. The platform works through size-based separation of CTCs out of other blood cells and then collection into single cell reservoirs for drug loading as shown in Figure 8. For testing purposes, a ratio of one cancer cell in 4000 blood cells was tested with successful cell capture and trapping of single cells into a chamber using dielectrophoresis electrodes. Although this article does not describe patient testing, one performance criterion that can be measured is the time to result, and this technology took about 33 min for the first cancer cell to pass through the channel and another 4 min for the cell to be captured and retained. Then, anti-cancer drugs were introduced into the cell and the accumulation was measured using fluorescence. This platform has the advantage of fast isolation and measurement of drug accumulation in a single cancer cell.

Li et al. developed a method of separating CTCs using an acoustic-based microfluidic device [73]. The device used standing surface acoustic waves to isolate CTCs from breast cancer patient blood samples. In spiked samples in this device, the recovery rate of CTCs was better than 83%, demonstrating the potential of acoustic-based separation as a label-free CTC separation method. Parameters could be modified within the device to tune the recovery rate of CTCs and the removal rate of white blood cells, with a removal rate as high as 99% at best and 90% when balanced with CTC recovery. The recovered CTCs obtained were viable without damage due to the non-contact nature of the acoustic separation.

Sollier et al. developed a method hinging on size-based separation of CTCs using microfluidic vortices and inertial focusing [74]. The basic working principle of the separation has to do with using the effects of fluid flow and inertia to separate particles within a channel by balancing the forces on the particle. The device operated at a fairly high throughput with flow rates around 4 ml/min during buffer testing. Cell viability was quite high with almost all trials demonstrating CTC viability above 80%. The results of the device showed

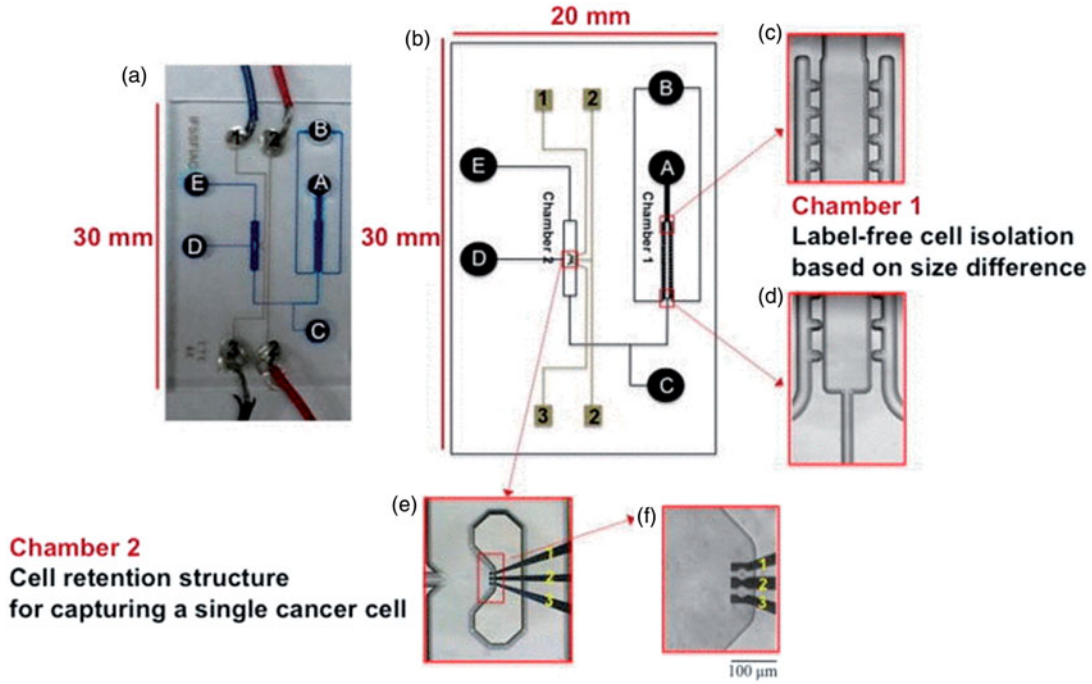


**Figure 8.** Workflow for CTC capture and drug accumulation on an integrated microchip. Reproduced pending permission from Ref. [69].

that the capture efficiency across cell lines was quite low, between 10 and 30% for a range of cell types.

### *5.3. Other or combined methods for capture of CTCs*

An interesting method that uses the combination of the techniques covered in this review is the CTC-iChip from the Toner Lab [78,86]. The technology involves a combination of inertial focusing and magnetic immunoregnition to remove leukocytes. The multistep protocol uses a first section with posts to separate out red blood cells and platelets, then inertial focusing and removal of white blood cells using functionalized magnetic particles. This platform achieves a 97% yield or recovery rate of CTCs from a whole blood sample with significant removal of white blood cell contaminants. A schematic of the CTC-iChip is shown in Figure 9.

## 6. Exosomes

Exosomes are vesicles that act as transport vehicles for biologic molecules including proteins and RNA [87,88]. Exosomes are vesicles released by nearly all cells that act as transport vehicles for biologic molecules including proteins and RNA [27,89]. The capture, detection, and analysis of exosomes on chip as a segment of liquid biopsies are an active field of research. Here, we will describe current methods for both the capture and detection of exosomes and the analysis of these biomarkers as a means for cancer diagnosis on-chip.

On-chip capture of exosomes could aid greatly in the availability of exosomes for analysis. Current procedures, such as ultracentrifugation, require a large amount of time and have poor yields. Many exosome capture methods have been developed on-chip to counter these poor yields and process time. Physical filtration by ciliated micropillars [41], immunomagnetic isolation [29,90,91], immunoisolation on beads [92], and immuno-affinity with nanoshearing [93] are some of the methods used for the capture of exosomes on-chip. As for on-chip analysis of exosomal biomarkers, there are many advantages when compared to standard protocols, including high throughput, sensitivity, and automation. Analysis of surface proteins [29], immunoelectrophoresis [94], colorimetric [93], qPCR [91], on-chip ELISA detection [45], and mass quantitation [95] are some of analytical methods offered on-chip for exosome characterization. We will discuss in-depth some of the more interesting cases.

A similar set of performance criteria can be applied to technologies for exosome separation and analysis. These include (1) throughput, the amount of time required to process a volume of sample (2) recovery rate of exosomes among other sample components and (3) condition of the exosomes for downstream analysis. All of these factors went into the technology readiness scores that are in Table 2, and within the text, these criteria will be presented when available.

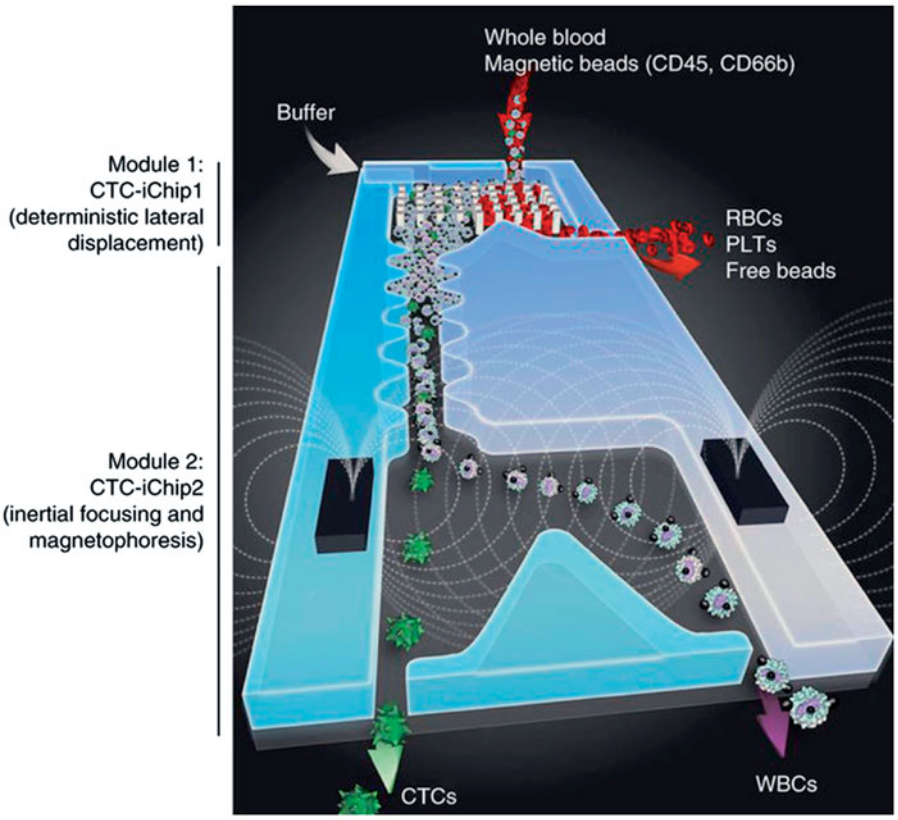


**Figure 9.** Schematic of the CTC-iChip. A schematic of the workflow starting with posts designed for RBC filtration, then inertial focusing of CTCs with removal of WBCs using magnetophoresis. Reprinted by permission from Ref. [78].

He et al. [29] developed a microfluidic approach for both the immunomagnetic capture of various exosome subpopulations and the fluorescence analysis of the exosomal surface proteins. The method was developed around testing of the blood plasma of non-small lung cancer (NSCLC) patients, which are difficult to obtain biopsies from, making them great candidates for the development of a liquid biopsy. The capture can be run with 1/100th typical plasma volumes used in standard procedures. The PDMS chip developed uses a multistep immunomagnetic method and is shown in Figure 10. Antibody-labeled magnetic beads are introduced to the plasma sample, which bind to the target exosomes. The beads are then drawn to the bottom of the chamber using a magnet placed underneath the chip, allowing for the capture and retaining of the bead-exosome conjugate. They showed a >99.9% bead recovery by adjusting flow rates to increase capture efficiency. The time to result was only 100 min for a 30 μl sample of plasma, indicating that the technology is minimally invasive and provides a relatively quick sample-to-answer. Second, a lysis buffer was added to release the desired intravesicular proteins into the microchannel. Again, antibody-labeled magnetic beads were used to capture these proteins and a magnet was again used to retain the protein-bead conjugate in another secondary chamber. In the third step, a sandwich immunoassay is deployed for the detection of the proteins by releasing both detecting antibodies and chemifluorescence reagents into the chamber. The exosome capture showed very high specificity of exosome capture, being able to determine between exosome subpopulations, which methods such as ultracentrifugation cannot do. This was done by the analysis of surface phenotypes of the exosomes. They targeted specific tumor markers, including the overexpression of IGF-1 R, and showed more statistical significance in concentration differences between healthy and diseased samples than ultracentrifugation and ELISA. This method offers high capture rates of the exosomes and streamlined single-chip capture and analysis approach, promising for point of care diagnostics.

Yoshioka et al. [92] developed a chip-based assay that also makes use of immuno-targeting but relies on an excitation of photosensitizer-beads for the detection of the exosomes rather than a magnet, shown in Figure 11. The assay is called ExoScreen, and is similar to the previous immunomagnetic capture method discussed, with a low sample volume (5 μl) and significantly reduced process times when compared to more conventional methods such as ultracentrifugation. This method was used to look at extracellular vesicles, which

**Table 2.** Capture, detection, and analysis technologies for exosomes.

| Sample type | Cancer type | Capture/detection method | Analysis? On/off chip? | Technology readiness | Reference |
|---|---|---|---|---|---|
| Plasma | NSC Lung cancer | Immunoisolation chemi-fluorescence detection | Surface proteins, on-chip | +++ | [29] |
| – | – | Nanoscale DLD | – | + | [96] |
| Serum | Colorectal | Immunoisolation on beads | Detection and quantification on chip | +++ | [92] |
| Plasma | Breast cancer | Gold standard ultracentri-fugation off-chip | On-chip immunoelectrophoresis | ++ | [94] |
| Plasma | – | Gold standard ultracentri-fugation off-chip | Microarray sandwich immu-noassays on-chip | ++ | [95] |
| Cell culture supernatant | Melanoma | Traditional methods | Lateral flow immunoassay (dipstick) on-chip | ++ | [45] |
| Serum | Breast cancer | Nanoshearing and immune affinity | Naked eye detection from colorimetrics | ++ | [93] |
| Cell culture | Glioblastoma | Immunomagnetic exo-some separation, standard isolation | qPCR on-chip, cartridge components from enrich-ment, capture, PCR | + | [91] |
| Plasma | Ovarian | Immunomagnetic capture | Continuous flow mixing, fluorescence labeling | + | [97] |
| Buffer | – | Cilliated micropillar | Fluorescence, visualization with QDs | ++ | [41] |

Technology readiness from + to+++based upon level of clinical testing, completeness of technology, throughput, and recovery rate. Details about selected technologies will be covered in the text.

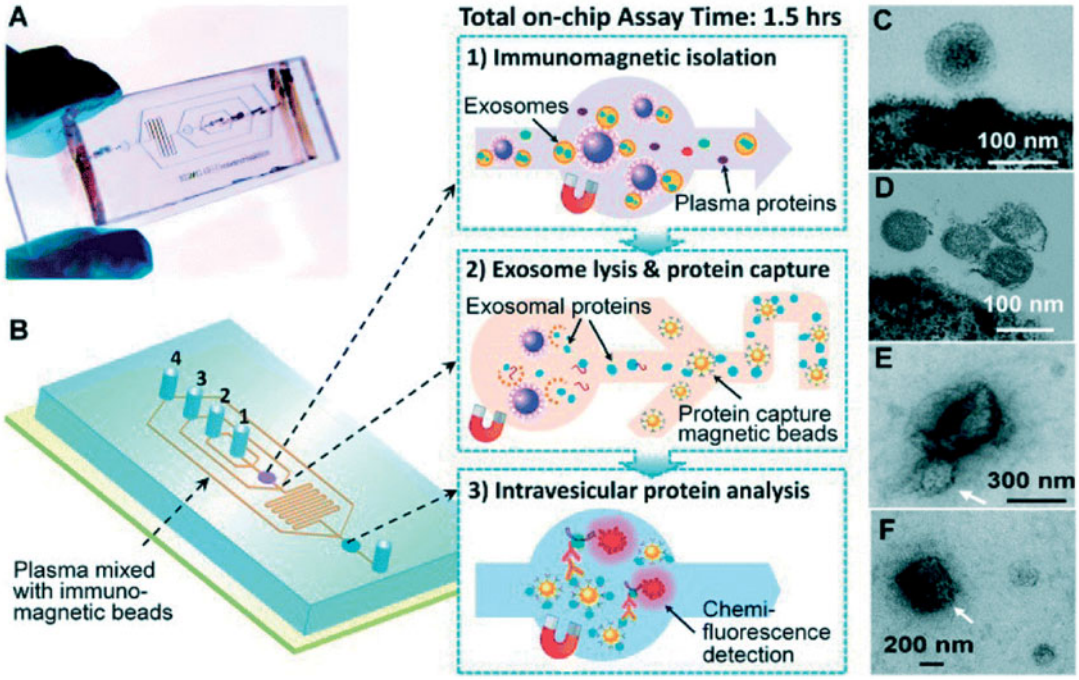


**Figure 10.** The multistep immunomagnetic isolation microfluidic set up for capturing specific sub populations of exosomes, lysing to release their surface proteins, and the subsequent immunomagnetic capture of the surface proteins for analysis. Reproduced by the Royal Society of Chemistry [29].

includes exosomes along with microvesicles released by the cells. The method makes use of a streptavidin-coated "donor beads" and "acceptor beads." The two bead types are synthesized to bind to both ends of the desired analyte. Excitation of the donor bead with a 680 nm laser causes the donor bead to release energy in the form of oxygen to excite a fluorescent signal in the acceptor bead at 615 nm. This system acts as both a capture technique but also an analytical tool for targeting specific biomarkers on the surface of exosomes. The assay only works within a certain size regime of the target analyte, which allows for the exclusion of larger vesicles. Serum samples from patients with colorectal cancer were used to detect various proteins including

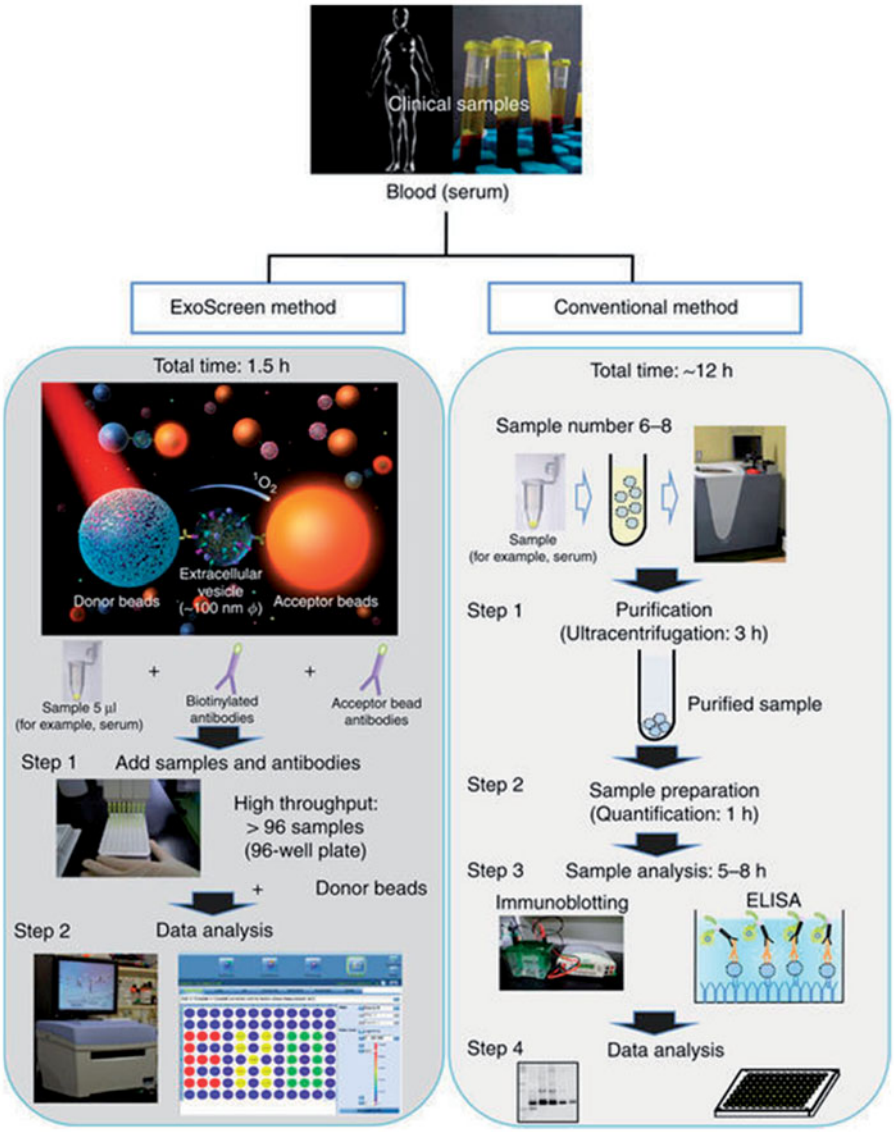


**Figure 11.** The use of photosensitizer-beads acting as a screening assay for immuno-targeted exosomes. Reprinted by permission from Macmillan Publishers Ltd. [92].

the double positive CD9 and CD147 which showed the ability to differentiate between exosome species. This assay has shown potential for the monitoring of patients after surgery or during chemotherapy, avoiding more invasive measures for monitoring disease progress as it is difficult to retrieve multiple colorectal biopsies after the initial surgery. The ExoScreen technology requires as little as 1.5 h for the total sample processing and requires less than 5 µl of sample. Compared to ELISA, it has only two steps and requires no washes with a larger analytic range for output, demonstrating its promise.

Vaidyanathan et al. [93] developed an interesting method for improving immuno-targeted capture of specific exosomes by introducing an electric field, shown in Figure 12. The method utilizes a multiplexed microfluidic device, capable of being tuned for various exosome populations. Through introducing a tunable alternating current to the electrodes that the target antibodies are attached to, an electrohydrodynamic flow is created within a few nanometers of the electrode. This flow will remove weakly bound, nonspecific targets allowing for more opportunities for the capture of the desired, targeted exosome species. This technique showed a 5-fold increase in the exosome capture rate when compared with regular hydrodynamic flow through the microfluidic chip, with a limit of detection of 2760 exosomes per microliter. The total pumping time for operation was around 2 h, with additional antibody incubation for 45 min. The exosome surface proteins were then analyzed using a colorimetric readout with a UV–visible spectroscopy from the commonly

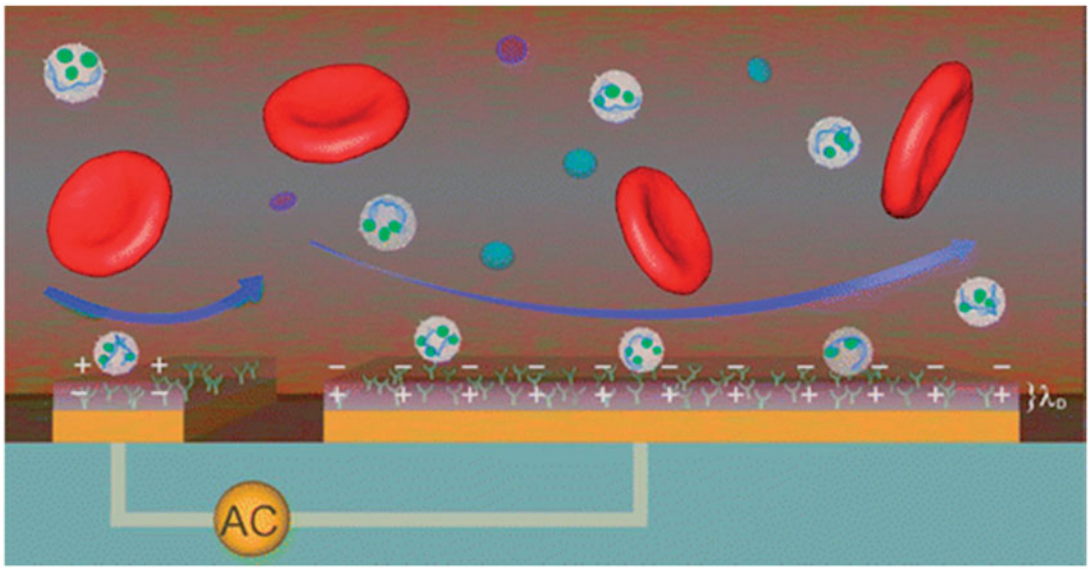


**Figure 12.** A schematic of the nanoshearing aided immunoisolation technique for exosome capture. Reprinted with permission from Ref. [93]. Copyright 2014, American Chemical Society.

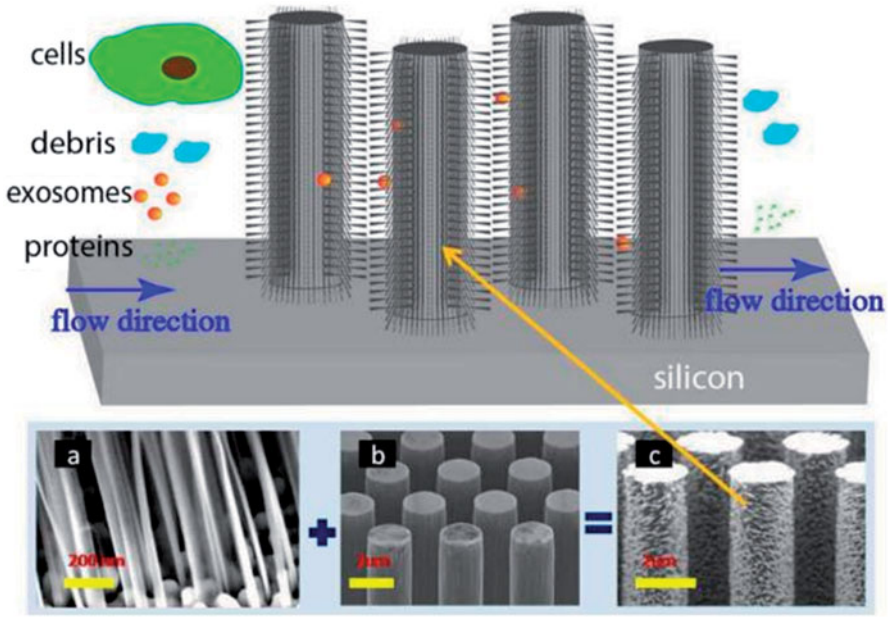


**Figure 13.** Schematic of ciliated micropillars for exosome capture and retention Reproduced from Ref. [41] with permission of the Royal Society of Chemistry.

used horseradish peroxidase and tetramethylbenzidine reaction for color release.

Wang et al. used unique microfluidic device geometry to preferentially trap lipid vesicles similar to exosomes while filtering out other debris [41]. The device contained ciliated micropillars that are made up of porous silicon nanowires, shown in Figure 13. The wires combined with the micropillars were demonstrated to trap exosome-like lipid vesicles while proteins and larger cell debris would pass by. The geometry, placement, and density of the micropillars were optimized to provide ample binding sites for exosomes without providing excessive hydrodynamic resistance within the microfluidic channel. The highest retention rate for tested liposomes was almost 60%, demonstrating the efficacy of the device for capture and enrichment of exosomes, and the typical reaction time is 10 min. This prototype showed the highest specificity for particles of the size of exosomes, demonstrating its utility for size-based separation of exosomes.

Exosome capture and analysis on-chip has been an active area of research for liquid biopsy development as exosomes are excreted by almost all cell types and are abundantly available in cancer patient serum and

plasma samples. The reviewed technologies being developed for exosome capture improve upon conventional methods and allow for a streamlined analysis on the same chip. Although, most developed technologies are for cancer applications, as exosomes become implicated in other diseases these strategies could be adapted.

## 7. Technologies for capture, detection, and analysis of circulating nucleic acids

For the purposes of liquid biopsy, circulating DNAs and RNAs that shed off from the concentration tumor sites can provide valuable insight into tumor progression and metastasis [34,35,37,98]. Both quantitation and genotyping of tumor DNA collected in a biological sample are clinically relevant for diagnosis and monitoring, as cancer patients tend to have higher concentrations and characteristic mutations [99–101]. Because the cell turnover increases at a higher rate as the tumor grows, the level of circulating DNA increases from apoptosis and necrosis [102]. This means that liquid biopsy technologies that target circulating nucleic acids can perform meaningful analyses on both the number and molecular characteristics of nucleic acid fragments found in a sample.

Although whole -blood derivatives, such as plasma and serum are the most commonly used body fluids for circulating nucleic acid (cNA) analysis, recent research has expanded to include a number of other bodily fluids. These include CSF for neurological-related cancers [43], and saliva for lung cancers [103], among others. Given the stability limitations of ctNA, as described earlier, the sample handling procedures for these biological samples vary widely. The types of analysis required also vary widely based on the clinical goals of the platform, with simple capture and detection being most important for diagnosis, but genotyping taking precedence for monitoring and treatment.

One important distinction for liquid biopsy of nucleic acids is that the workflow differs from CTC or exosome analysis. Unlike a cell or vesicle contained within a lipid bilayer, circulating nucleic acids have the advantage that simply their capture and detection can be a meaningful analysis: just verifying their presence and quantity is a meaningful contribution to the clinic. In fact, if we revisit the kinds of analysis done based on CTCs and exosomes, genetic profiling was amongst the most meaningful. Because ctNAs are free-floating nucleic acids, getting an understanding of the overall trend is a powerful analysis on its own. This type of analysis is even more meaningful when you take into account the kinds of information that come from mutational analysis. Simply counting the number of ctDNA molecules that are wild type and the number that have a particular oncogenic mutation can provide a foundation for diagnosis, monitoring, or prognosis of a patient. Another nuance of ctNA technologies is that they often involve on-chip amplification for further downstream analysis, and a number of such technologies are reviewed below.

### *7.1. On-chip amplification methods*

The bulk of circulating nucleic acid measurement technologies requires amplification of the target before detection and analysis. These typically use a modification of polymerase chain reaction (PCR), a procedure that uses temperature cycling to amplify DNA based upon a template sequence [30,104]. This process allows for amplification of the number of DNA molecules, combating the problem of extremely low molecule numbers of circulating nucleic acids in blood.

A number of methods of chip or droplet-based digital PCR has been developed to provide replicable multiplexed quantitative PCR results in conjunction with a PCR machine [105,106–109]. Sefrioui et al. evaluated whether existing methods of chip-based digital PCR could be used to quantify circulating DNA in cancer patients [105]. They tested 34 metastatic colorectal cancer patients looking for a K-ras mutation in Exon 2 for ctDNA detection. The study found that chip-based digital PCR is successfully able to quantify cell-free and ctDNA using patient plasma. The time to result is about 5 h, but the workflow is relatively simple and low cost, indicating that this might be a clinically useful strategy for patient sample analysis. That being said, this sort of analysis requires a full kit and is not done on a microchip, requiring quite a bit of equipment, making it suitable for clinical use, but not use in a field with limited resources.

Li et al. developed a technology for amplification and quantification of nucleic acids at a constant temperature [110]. Although this was not applied for the purposes of capturing ctNA, it could be an interesting approach for point of care applications. The platform works consists of a 27,000 picoliter well chip that performs individual isothermal reactions for DNA quantitation using recombinase polymerase amplification at 39 °C. This method was developed to minimize cross-contamination, and used a novel imaging method to allow wide field imaging over the whole chip area. The whole amplification process required about a half hour and because of its constant temperature, has potential to be well suited to resource-limited environments.

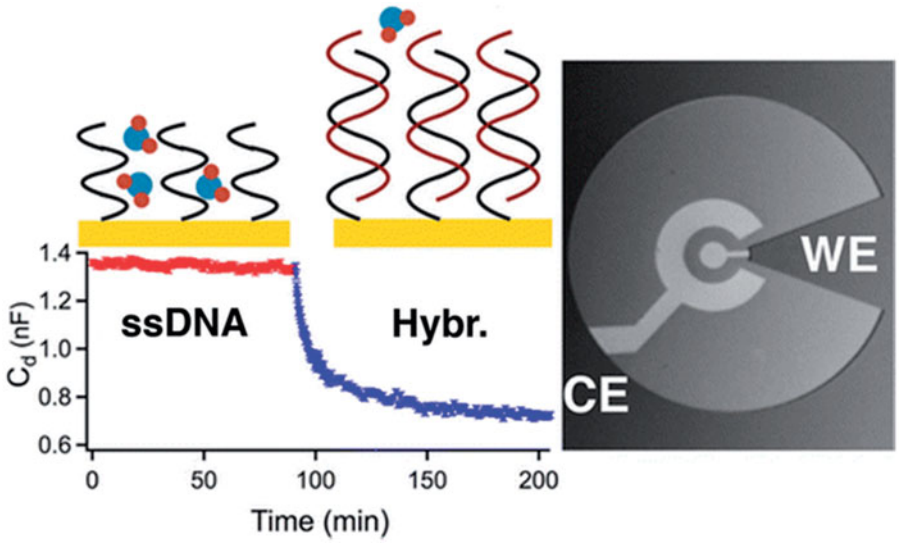


**Figure 14.** Principle of operation and SEM image of microfabricated gold electrodes (working electrode and counter electrode). Reprinted with permission from Ref. [20]. Copyright 2016 American Chemical Society.

### *7.2. Electric or magnetic field-based detection*

An alternative to amplification and fluorescence-based detection that is frequently used in on-chip devices is electrode or magnetic based capture and detection of circulating nucleic acids. A number of devices employ gold electrodes, impedance measurements, or magnetic resistance as transduction elements to read out biomarker binding.

Capaldo et al. developed a method of quantifying microRNAs in human plasma using electrochemical impedance spectroscopy [20]. The transduction method uses a change in capacitance upon binding at an electrode surface [20,111]. This particular technology uses single stranded DNA probes to capture miRNAs by measuring this biorecognition over time. They also demonstrated that this method of sensing is reproducible and reusable while remaining specific to miRNAs and not is affected by nonspecific binding of other markers in the sample. In this particular application miRNAs that were specific to cardiac disease were tested, but it would be equally applicable to cancer-related miRNAs through the same principle. This device was able to detect microRNAs at the picomolar range in real time, and the principle of operation and SEM of the device are shown in Figure 14.

Labib et al. developed an electrochemical method to sense microRNA levels from human serum samples using gold nanoparticle modified carbon electrodes [112]. A series of three electrochemical detection modalities that sense miRNA binding, protein binding, and protein displacement allow for detection of very low target analyte concentration without any amplification steps. The device was tested for sequential analysis of miR-32 and miR-122 on a single electrode, demonstrating the applicability for capture and sensing of multiple miR target molecules. The base transduction element of the sensor is a gold nanoparticle modified screen-printed electrode, and the nanoparticles are functionalized with thiolated capture probes. Biomarker binding is measured by square wave voltammetry, and then there are additional binding steps to improve the limit of detection. This process is shown in Figure 15. The electrochemical measurements for this application are carried out using an electrochemical analyzer connected to a computer, which may need to be miniaturized or modified for point of care measurements.

Another interesting application uses electrochemical sensing of epidermal growth factor receptor (EGFR) mutations in non-small cell lung carcinoma using saliva from patients. This is unusual compared to many of the other technologies reviewed, which use patient whole blood, serum, or plasma, and may be better suited to the point of care because no blood draw is required. The novel technology in this study was an electric field-induced release and measurement that can measure the circulating DNA in a multiplexed fashion. The device was tested *in vitro*, with mouse xenografts, and then in a blinded study with patients. This technology provides very convincing diagnostic capabilities from its patient study, with particular promise as a stress-free method for rapidly identifying oncogenic mutations.

In addition to these electrochemical methods, there are a number of other technologies that employ magnetic fields for detection of bound nucleic acids. Dias et al. used an array of magnetoresistive sensors on a portable biochip for specific target detection of cell-free DNA [109]. They chose fragments ALU115 and ALU247, both of

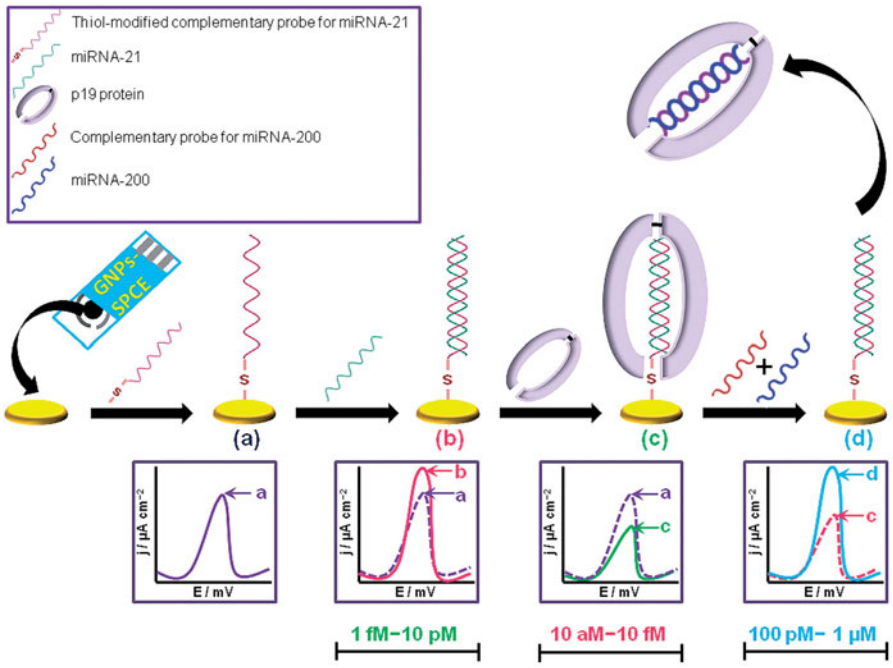


**Figure 15.** Schematic of gold nanoparticle patterned electrode for electrochemical sensing of miRNAs. Each subsequent step shows binding of successive molecules for improvement in detection. Reprinted with permission from Ref. [112]. Copyright 2013 American Chemical Society.

which are promising cancer-related cell-free DNA targets, and used magnetic labeling to capture the sequences followed by detection using the magnetoresistive sensors on a single platform. This technology performed enrichment and detection of the cell-free DNA fragments together on the microfluidic channel in a multistep process. This device was validated in the picomolar range without any prior amplification, and for use in a clinical application, it would be ideal for this to be lowered.

### 7.3. Spectroscopy or optical analysis

There are a number of technologies that use light-based detection methods for detection of circulating nucleic acids. The ones reviewed here use confocal spectroscopy, optical absorbance measurements, and spectral detection. Their detection method is a component that they have in common, as these technologies all measure spectra of light and use them to determine binding and quantity of nucleic acids within the sensor from a blood sample.

Cai et al. developed a microfluidic optical system that is able to extract two cell-free nucleic acid mutations that are melanoma biomarkers from whole blood [113]. The system uses a multistep optofluidic process to extract, enrich, and analyze the biomarkers using microfluidic circuits. This analysis system is unique in that it is a multilayer system that performs filtration of the blood first, then enriches the targets, labels them using fluorescence, and then detects them based upon wavelength spectra on an optical chip. The schematic of the components of this chip is shown in Figure 16. In this system, the researchers look for two mutated circulating nucleic acids: BRAFV600E and NRAS, and can

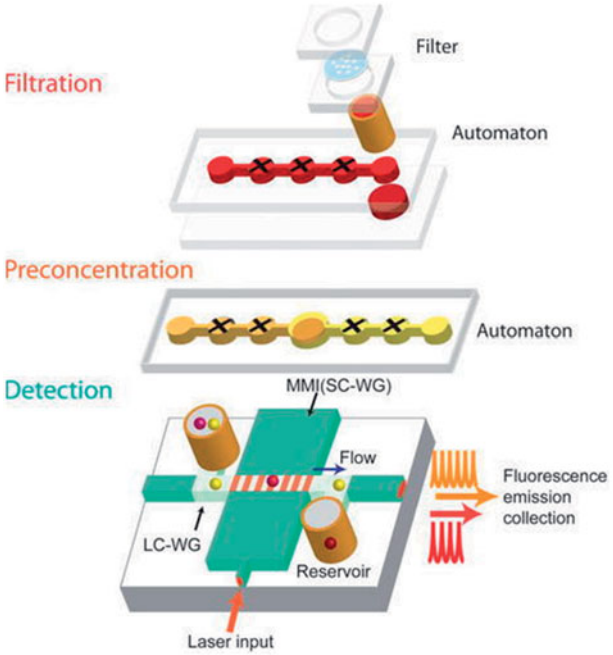


**Figure 16.** Schematic of components of system with all interconnected PDMS chips, gate microvalves, and the multiplex detection chip. Reprinted from Ref. [113] with the permission of AIP Publishing.

**Table 3.** An overview of the reviewed circulating nucleic acid capture, detection, and analysis technologies. They are split based on whether they require amplification and by detection method.

| Sample type | Cancer type | Target biomarker | Capture/detection method | Analysis? On/off chip? | Technology readiness | Reference |
|---|---|---|---|---|---|---|
| Amplification-based methods | | | | | | |
| Plasma | Colorectal | ctDNA | DNA extraction from Qiagen | Chip-based digital PCR | ++ | [105] |
| buffer | – | NAs | Recombinase polymerase amplification | Picowell array chip for quantification | ++ | [110] |
| Electric and magnetic fields | | | | | | |
| Saliva | NSC lung cancer | ctDNA | Electric field induced release and measurement (EFIRM) | EGFR mutation detection, on-chip | +++ | [103] |
| Buffer | – | ctNAs | – | Electrochemical impedance spectroscopy on-chip | + | [20] |
| Serum | – | miRNA | – | Gold nanoparticle-modified electrode | ++ | [112] |
| Plasma | – | cfDNA | Magnetic particle capture | Magnetoresistive sensors, on-chip | ++ | [115] |
| Optical and spectral | | | | | | |
| Whole blood | Melanoma | cfDNA | Chip-based optofluidic system | Wavelength based on-chip spectral detection | +++ | [113] |
| Serum | Lung cancer | cNAs | No enrichment | Microfluidic cylindrical illumination confocal spectroscopy for size, not sequence | ++ | [34] |
| Plasma | Pancreatic cancer | miRNAs | Plasma extraction | LSPR gold nanoprisms | +++ | [114] |

Technology readiness from + to+++based upon testing level, completeness of technology, throughput, and limit of detection.

detect the targets at the same time with a 96% success rate and concentration of 300 nM. Furthermore, the detection method used in this technology only requires a single photodetector and allows it to remain a small chip-based system, making it extremely useful for point of care capture and analysis of circulating nucleic acids.

Another technology that uses a spectrum-based sensing technique was developed by Joshi et al. to detect miRNA 10-b in pancreatic cancer patients [114]. This sensor is able to capture miRNAs that are free floating and miRNAs from within exosomes in buffer and patient plasma samples. The miRNA sensor uses localized surface plasmon resonance (LSPR) to specifically detect miRNA binding to the substrate stably and without fluorescent labeling. The transduction method works such that upon nucleic acid binding to the sensor which is conjugated with a ssDNA probe, the resonant spectra-shifts allowing fully quantitative detection without pre-purification. The sensing method is also sensitive to a single base pair mutation and is able to differentiate miR 10-b and miR 10-a. This sensor was tested with pancreatic cancer patients, those with chronic pancreatitis, and healthy patients, and was able to quantify attomolar concentrations. For point of care applications, this technology is very promising because it requires no pre-concentration of target within the sample, and does not require any fluorescence for quantitation, simply light absorbance through the sample. In order to make it fully suitable to point of care, the light source and spectrometer must be miniaturized and integrated so that additional large equipment is not necessary (Table 3).

A slightly different technology to quantify circulating nucleic acids in serum was developed by Liu et al., and is able to analyze circulating DNA size and concentration [34]. The device is a microfluidic device coupled with cylindrical illumination confocal spectroscope with fluorescence analysis of a DNA intercalating dye. The whole process does not require any DNA isolation or amplification, but is able to size circulating nucleic acids to the femtomolar range using only one picoliter of patient serum. The detection method uses a one-dimensional microfluidic device with excitation and detection channels to analyze DNA fluorescence and measure sizes, as shown in Figure 17. The device, however, requires quite a few optical components and, therefore, will require miniaturization of these optical components for use at the point of care.

### *7.4. ctNA chip design considerations for point of care*

Given these descriptions of existing lab-on-chip technologies for ctNA capture and analysis, there are a number of key takeaways that can be applied to design of new technologies. Some of the capture, detection, and analysis choices made by the technologies are better suited to point of care than others, and these are explored further in this section to determine the best formula for POC development.

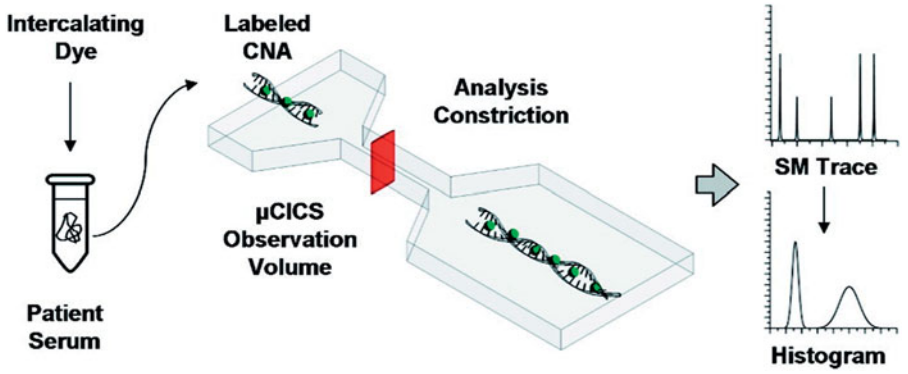


**Figure 17.** Components of microfluidic cylindrical illumination for quantitation and sizing of circulating DNA in serum. Reprinted with permission from Ref. [34]. Copyright 2010 American Chemical Society.

One of the common threads seen in a number of the reviewed ctNA technologies is the need for an off-the-shelf extraction before the nucleic acids can be captured and analyzed. This requirement is due to the low concentration of circulating nucleic acids in a patient sample, but may make the technology less likely to be used in a point of care setting. To keep a technology low-cost and fast, it would ideally be a single step, while the requirement for an extraction step before device use would require additional time and resources such as centrifuges for multiple spin steps. Thus, the technologies that have been reviewed earlier that require that extra extraction might be better suited to clinical setting than the point of care.

Another key takeaway from this section is that in general, fluorescence based detection is less suited to point of care than colorimetric or electronic/optical methods. Colorimetric sensing can be the most robust because it requires no additional equipment to get readout, and technologies that are label-free, such as, electrode or optical based sensors do not have the issue of photobleaching and tend to be easier to read out instantly. The additional development that would be required to move optical and electrical readout based sensors to the point of care is miniaturization of the readout mechanism.

Overall, based upon the review of technologies here, circulating nucleic acids are a promising biomarker for point of care liquid biopsy. The major reason for this is that nucleic acid screening technologies are quite developed, with an increasing understanding of the relationship between individual mutations and cancer prognosis [51,116]. Another reason is that, compared to CTCs or exosomes, circulating nucleic acids require relatively little downstream analysis, as their sequence, mutation status, and copy number can be known based upon capture without further processing. Because, there is no need to break a cell membrane or bilayer and purify internal molecules for downstream analysis, the capture and detection can serve as a meaningful clinical analysis. Thus, the reviewed technologies hold a lot of promise for application to point of care liquid biopsy for cancer, and design choices for capture, analysis, and detection can make devices more suited to a point of care setting.

## 8. Discussion and conclusions

Recent advances in liquid biopsy technologies, combined with molecular profiling of captured biomarkers, have led to many integrated systems for biomarker capture, detection, and analysis. Further understanding of the cancer metastasis and its molecular basis requires tracking the real time dynamics of the tumor development, and liquid biopsy provides a valuable tool for minimally invasive diagnosis and monitoring of tumor fluctuations. For ease of use in and out of a laboratory setting, technologies must be able to effectively capture biomarkers and perform these downstream molecular analyses on an integrated and simple workflow.

In this article, we reviewed the key technologies and provided a framework to evaluate recent progress toward on-chip liquid biopsy for cancer at the point of care. The platforms are able to capture and analyze CTCs, exosomes, and circulating nucleic acids with high throughput, relatively low cost, and minimal need for typical laboratory resources. Integration of these capture and analysis strategies can provide sample-to-answer platforms for diagnosis and monitoring of cancer from biomarker isolation to molecular analysis.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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