



Review

Droplet based microfluidics integrated with machine learning

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ABSTRACT

Droplet based microfluidics (DBMF) has gained huge recognition in the recent years for performing micro-reactions in droplets with high throughput, sensitivity, specificity and minimum cross-contaminations. This technology enables the researchers to realize highly reliable and rapid detection and screening applications in various fields. The high-throughput nature of droplet microfluidics generates large amounts of valuable but complex droplet dataset. Deeper analysis of this intricate droplet data is very essential for detection, classification, characterization and quantification of reactions/content inside the droplets. This can be carried out by Machine Learning (ML), which has proven itself in processing and providing deeper insights and precise predictions of relatively large amounts of complex data with shorter analysis times and exceptional accuracy. The analytical tools of ML enable to imbibe automation and control of many such diagnostic platforms, including DBMF, with minimum human intervention. In recent times, the potential of ML has been explored in microfluidic technology as well to tackle challenges in biomedical and biotechnological applications. The synergy of both the fields, DBMF and ML, helps in development of optimized and automated tools with higher accuracy for numerous applications. Specifically, this enables complete comprehension of the field to eventually realize a truly microfluidic total analysis system (μ TAS). This work comprehends a general review emphasizing the implementation of different ML models with DBMF to automate various activities such as fluid control, droplet size prediction, recognition of flow pattern and identification, classification and sorting of droplets in a microfluidic device.

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1. Introduction

Since its inception, microfluidics has been a boon in many domains, such as biomedical, biochemical, pharmaceutical, cosmetic and microelectronics etc., enabling researchers to employ its complete potential to perform analysis in an efficient, rapid and automated manner. The emerging growth of microfluidic technology has paved way for new technologies that carry out analysis with high specificity and sensitivity, while providing reliable and accurate results using very low samples with an aim to realize micro total analysis systems (μ TAS) [1,2]. Few such microfluidic platforms include open microfluidics [3], miniaturized paper based analytical devices (μ PADS) [4,5] and droplet based microfluidics [6,78]. Even though each microfluidic platform has its own unique capability and functionalities, in recent times, droplet based microfluidics has earned huge importance because of its numerous advantages such as high throughput, individual analysis in picoliter droplets, minimal cross contaminations and multiplexed reactions in droplets with high sensitivity and reliability [9,10]. One of the main advantages of DBMF is that it provides miniaturization, compartmentalization and parallelization in a single microfluidic device enabling to realize several lab-on-chip applications [11].

In a droplet based microfluidic device, droplets are generated when the dispersed phase is sheared off due to the excess energy applied by the continuous phase [12]. Controlled manipulation of these droplets in the microfluidic channel results in various processes such as droplet merging [13], droplet splitting, mixing inside the droplets and also categorization of droplets [14]. These processes with the integration of analysis tools enables to accomplish reactions in individual droplets. In addition, at microscale, owing to very high surface area to volume ratio, the heat transfer rate and the mixing efficiency are enhanced at shorter diffusion distances resulting in lesser reaction times [6,15]. Furthermore, the sample in picoliter volume droplets are less prone to dilution, evaporation and sample absorption to the channel walls during transportation of droplets in a microfluidic device [10,16].

Evidently, the monodisperse nature of generated droplets has paved way for many detection and high throughput screening applications [17,18]. Droplet microfluidics has been leveraged in many applications such as single cell analysis, drug discovery and drug delivery, biomolecule synthesis, protein crystallization, nanoparticle synthesis, droplet-based (Polymerase Chain Reaction) PCR, DNA sequencing etc [19–21]. Droplet microfluidics also plays a major role in determining and comprehending the rheological parameters of fluids at microscale. Owing to its high throughput capability, droplet microfluidic platform is amenable to generate droplets starting from a few hertz to several kHz [22,23]. This signifies that millions of individual reactions can be performed enabling each droplet to behave as a micro-reaction chamber where by large amounts of quality droplet data can be generated from DBMF platform every second. A process run for 10 min with a throughput of 1 kHz can result in half a million droplets with individual reactions in them. Though performing reactions in individual droplets is advantageous, analysis of all the numerous reactions is equally important to comprehend the results. Fig. 1 shows the experimental setup: syringe pumps for infusion of fluids, optical microscope integrated with high speed camera for droplet visualization and a computer for recording the droplet data.

There are several prominent ways of detection and quantification of droplets and its content [24]. Few of them include Optical Imaging techniques such as bright field imaging, fluorescence based detection [25], Absorption spectrometry [26], Laser induced fluorescence detection [27] Fourier transform based infrared spectroscopy (FTIR) [28] and Raman Spectroscopy [29]. Other detection techniques include electrochemical detection [30–32], impedance measurements

[33], mass spectrometry [34] and magnetic resonance spectrometry [35]. Post detection, rapid quantification of the droplets is essential to analyze the reaction in the droplets, whereby the aforementioned methods can also be used for the quantification of droplets. However, as they are not prone to rapid quantification due to limitations such as high generation frequency of the droplets, optical resolution, and requirement of high performance computing for rapid analysis, hence off chip quantification need to be performed. In addition, the utilization of these time invasive processes needs a constant supervision by an expert, minimizing the possibilities for automation. The unification of detection, and rapid and reliable quantification of droplets in a microfluidic device plays a vital role in biomedical field exploring its possibilities even in the detection of SARS-CoV-2 RNA. The key requirements for detection of COVID-19 are sensitivity and high throughput which are inherent features offered by droplet microfluidics [36–38]. In addition, the advantages offered by droplet microfluidics has shown an increased sensitivity in detection of SARS-CoV-2 by droplet digital PCR compared to RT-qPCR [39]. Also, the characterization and classification of droplets is important in applications such as cell sorting and droplet sorting based on either by size, shape or nature, and its content [40]. However, considering the high droplet generation frequency, the sorting of droplets becomes a chaotic process and can lead to oversights during quantification process.

Though there is an incremental growth in the experimental field of DBMF, there is a need for support of computational tools that are capable of identifying, processing and quantifying large amount of intricate quality droplet data. One of the best available options to realize an automated DBMF platform that process and quantifies large amounts of data is by integrating the droplet microfluidics with Machine Learning (ML) and Artificial Intelligence (AI) tools [41]. ML and AI tools have successfully proven their ability in automating most complex systems [42–44]. With proper training and implementation of optimal ML models, automation of the entire microfluidic system, starting from flow control to classification of droplets is possible. Fig. 2 shows different activities of droplet microfluidics that can be automated using Machine learning. ML and AI tools can be successfully implemented for identification and characterization of droplets in a microfluidic channel. In fact, ML tools have been employed in DBMF for understanding i) design automation of device geometry ii) dynamic flow control iii) prediction of droplet size iv) size-controlled droplet generation v) droplet sorting vi) mixing characteristics vii) recognition of flow patterns. In this regard, this review emphasizes an overview of implementation of various ML tools in DBMF platforms through which automation of system is being realized. The structure of the article will be a brief introduction of dynamics of droplets and droplet generation methods followed by overview of ML and AI and their implementation in DBMF for realizing various applications and concludes with future scope and conclusions.

2. Droplet dynamics

Droplet generation in a microfluidic device is primarily because of interaction between two immiscible fluids which are a continuous phase and a dispersed phase [45]. The fluids are usually forced into a microchannel through different inlets either by using passive pumps with a stable, regulated and a measurable force. In addition, hydrostatic pressure/ gravity driven flow is also a known approach for passing fluids into the microchannel [46]. When two different immiscible fluids, with distinct properties and driving forces, are pumped into a specifically designed confined environment, droplets will be generated as a response between both the phases. This interaction between the two phases introduces two important forces: interfacial tension and viscous forces, which are primarily

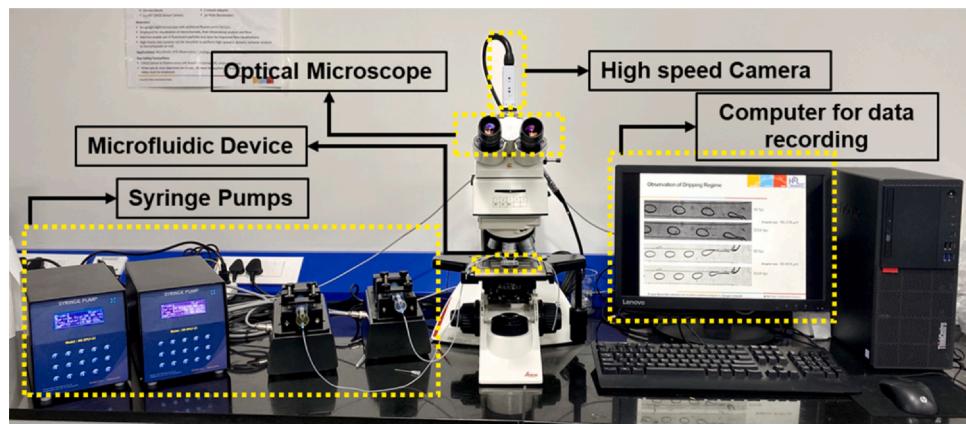


Fig. 1. Experimental setup required for droplet generation, droplet visualization and collection of droplet data (image represents the experimental setup used in our research group).

dependent for droplet generation [47]. Interfacial tension force is responsible for minimization of surface area of the fluid to take the shape of sphere, while the viscous forces are due resistance between the adjacent layers of the fluid [48–51].

To clearly understand the role of these forces, a clear idea of various dimensionless numbers, like Reynolds number, Weber number, Pecllet number and Bond number, giving an insightful comparison of different physical forces, is crucial. Reynolds number, providing a relationship between the inertial forces and viscous forces, is very small in the microfluidic environment [52,53]. Weber number, the ratio between inertial forces to the interfacial tension forces, is also small considering low inertial forces in microfluidic

domain [54]. Peclet number, a ratio of advection to diffusion, represents the diffusion/mixing process in a microchannel and does not depend on any force that lead to droplet generation [55,56]. Finally, Bond number, providing the association between the gravitational forces and interfacial tension forces, can also be considered to be smaller as the flow in the microchannel is independent gravity [57]. It is evident that these dimensionless numbers do not associate any of the two forces which play a vital role in droplet generation. This results in another dimensionless number called Capillary number (Eq. 1) which gives the relationship between interfacial tension force and viscous force [6,58].

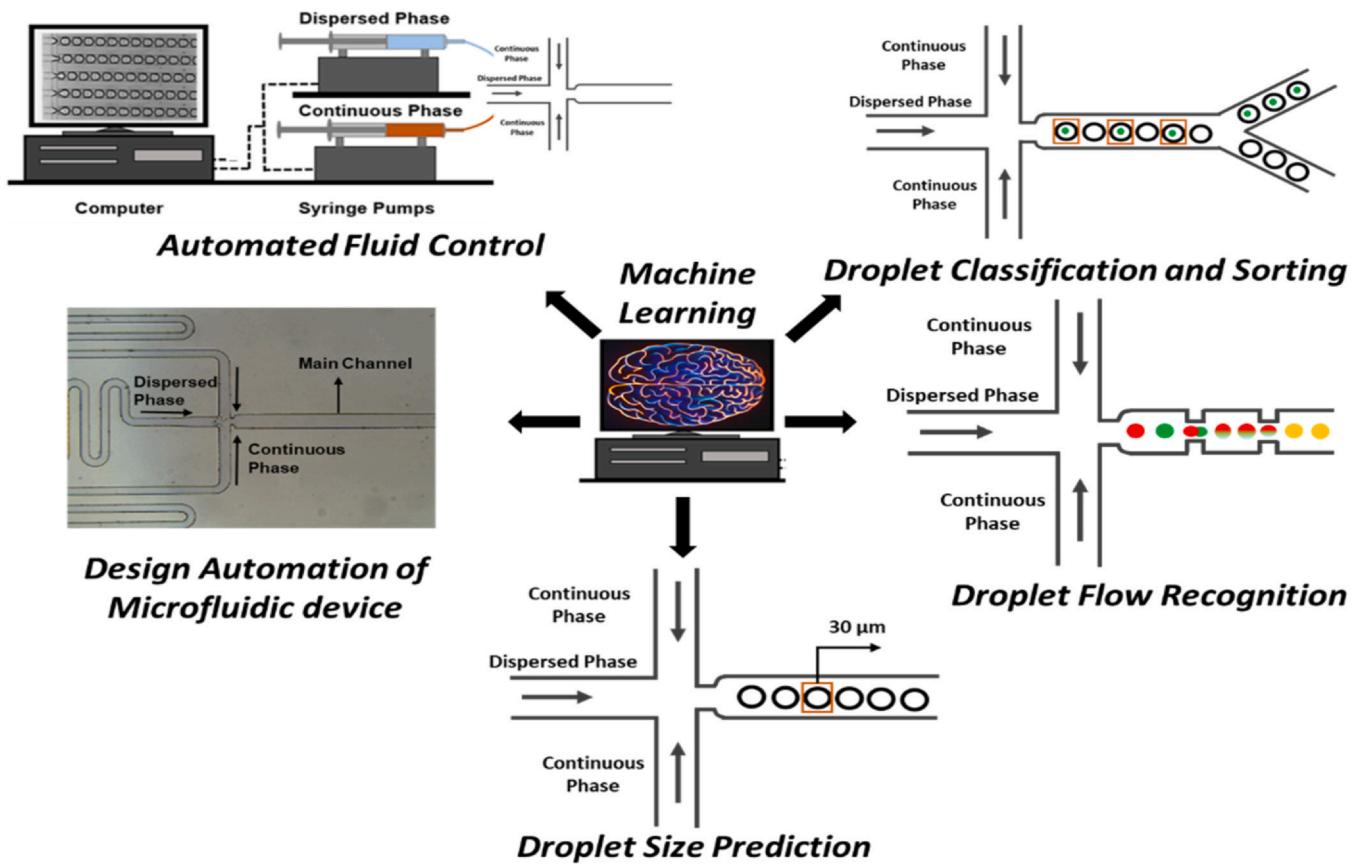


Fig. 2. Applications of machine learning in droplet based microfluidics.

$$Ca = (\mu_c \times U_c)/\sigma \quad (1)$$

where, μ_c is viscosity of the continuous (oil) phase, U_c is velocity of the continuous phase and σ is interfacial tension between the fluids. Lower capillary numbers indicate that the droplet formation in the microchannel is due to the domination of interfacial tension forces over viscous forces which leads to the formation of monodisperse and symmetrical droplets. On the other hand, higher Ca indicates that the droplet generation is because of strong viscous forces leading to deformation in droplet shape and therefore formation of asymmetric droplets [47,59,60]. This suggests that capillary number plays a vital role in the dynamics of the generated droplet.

$$\lambda = \frac{\mu_d}{\mu_c} \quad (2)$$

μ_c = viscosity of the continuous phase: μ_d = viscosity of aqueous (dispersed) phase.

Apart from these dimensionless numbers, viscosity ratio (Eq. 2) and flow rate ratio (Eq. 3) of the fluids also effect the droplet generation in the microfluidic channel [51,61,62]. In addition, parameters such as surface wettability, channel geometry [63,64], nature of the fluids [65] and also surfactants play [66] key role in observation of different droplets.

$$\phi = \frac{Q_c}{Q_d} \quad (3)$$

where, Q_d and Q_c : are Flow rate of aqueous and continuous phase, respectively.

3. Droplet generation in a microfluidic channel

One of the primary requirements of droplet microfluidic technology is to achieve stable, homogeneous and monodisperse droplet generation in a microfluidic device. In applications involving analysis and synthesis, it is very crucial to know the droplet size and the period of generation [67]. In addition, the size of the droplet plays a pivotal role in the level of sensitivity obtained in droplet based diagnostic applications [68]. In order to generate droplets with desired size and shape, a specifically designed geometry is required [69]. In general, three different geometries (Fig. 2) are predominantly used for producing droplets: i) cross flow junction ii) flow focusing junction iii) co-flow geometry.

3.1. Cross flow junction or T junction

Cross flow geometry was first reported by Thorsen et al., wherein the dispersed phase meets the continuous phase at a junction where two microchannels are perpendicular to each other [70–72] as shown in Fig. 3(a). Droplets of different size and shape can be easily achieved in a microfluidic T junction even by altering the widths of the inlet channels [73]. However, apart from orthogonal microchannels, droplets were also generated in microchannels with varying angles [74]. One drawback of the T junction in a 2D plane is that it forms plug-like droplets rather than spherical shaped which leads to consumption of more of the dispersed phase [59]. In order to obtain spherical droplets, the microchannel geometry of the dispersed phase must be scaled down which might raise difficulties such as fluid resistance and low droplet generation frequency [7]. However, this issue can be addressed by achieving 3D crossflow junctions that reduce the surface wettability and fluid resistance. Besides droplet generation, T junctions can be employed in designing micro-valves [75] and micro-actuators [76].

3.2. Flow focusing junction

In a flow focusing geometry, the dispersed phase is squeezed by two counter flowing streams of the continuous phase [77,78]. This kind of geometry (Fig. 3(b)) was first reported by Anna et al. [79]. In case of flow focusing devices, the breakup of the droplets solely depends on the action of shear force and the flow rates of the fluids [80]. Also, at a high droplet generation frequency, monodisperse and spherical droplets can be achieved using flow focusing devices [64,81]. Furthermore, a flow focusing junction, when compared to other geometries allows to achieve droplets with lower coefficient of variation [82]. For applications such as encapsulation of micro particles, a flow focusing junction is usually preferred because of high encapsulation efficiency [83]. In addition, mixing efficiency of droplets can be increased using asymmetric flow focusing junction without the need of serpentine channel which is usually employed for mixing of content in the droplets [84].

3.3. Co-flow junction

Droplet generation in a co-flow geometric devices is realized by integrating a capillary tube in a square or a rectangular micro-channel with two streams flowing in parallel direction [85,86]. The co flow junction consists of an outer channel and an inner channel (usually capillary tube) as shown in Fig. 3(c). In general, the continuous phase flows in the outer microchannel with the dispersed phase flowing inside the capillary [87]. In such geometry, droplet generation frequency and droplet size are highly dependent on the velocity of the continuous phase [69]. In general, generation of monodisperse droplets where non-Newtonian fluids are involved, co-flow junctions are more effective compared to the other methods [88].

4. Introduction to artificial intelligence and machine learning

ML and AI are multidisciplinary tools involving subjects like mathematics, statistics, communication, signal processing and computer sciences. This interdisciplinary tool has been of great help in emerging areas focusing towards automation [89–93]. Though closely related, there is a nuance between ML and AI and is indeed important to differentiate them. According to A V Joshi, AI can be considered as a machine capable of providing very quick responses by processing large sets of data with a sensitive touch of human like behavior [94]. Alternatively, ML is a subset of AI which is essential in developing AI systems. Machine Learning, as the name suggests, the machine learns through user guidance and predicts the results that the user is unaware of. ML works with training a system or a model with sets of desired input and output data to predict the output for a new input. Machine learning helps in providing deep insights in the prediction of output and decisions in intensive data fields of science, commerce and public sector [95–99].

As per the style of learning, ML can be categorized to three main types: i) Supervised learning ii) Unsupervised learning iii) Reinforcement learning [100]. In supervised learning, the model will be trained using a labeled dataset of input and output (x and y) to predict the output y' and for a given input x' . This learning model needs large amounts of dataset to train the model to achieve high accuracy (Fig. 4(a)), which is more related to applications dealing with classifications and regression [101]. Unlike the supervised learning, unsupervised learning tries to predict output from unlabeled dataset of input and output. It tries to interpret regularities and structures of clustered input data to predict the output (Fig. 4(b)). Clustering and segmentation are examples of unsupervised learning [102].

On the other hand, Reinforcement learning uses neither of the labelled and unlabeled data. Instead, it learns itself by interacting

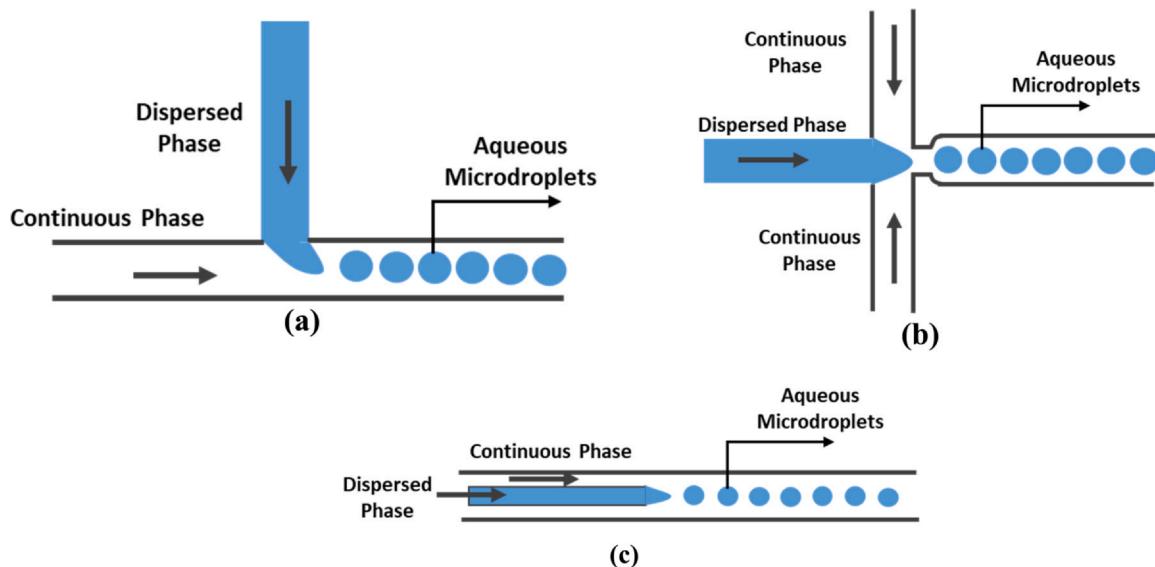


Fig. 3. Different geometries used for generation of droplets (a) Cross flow junction (b) Flow focusing junction (c) Co flow junction.

with the environment in predicting the desired outputs (Fig. 4(c)). Reinforcement learning is mostly used in training robots to realize AI systems [94].

One subset of ML is Deep Learning (DL), which can either be supervised or unsupervised learning technique. DL, recently has gained a huge importance because of its capability of handling large sets of structured data [103]. Deep learning can be considered as an extension of machine learning that implements complex models and transform the data using numerous functions for representation of data in a hierarchical manner [104]. One of the strongest features merits of deep learning is feature extraction i.e., it does the extraction of features from the raw data automatically. This suggests that DL that higher level hierarchies shall be formed from lower level concepts/features [105]. Another advantage of DL is that it is not task specific and is often considered to be universal learning methodology which is capable of solving complex problems in various domains [106]. DL has gained prominence for precision in prediction of various datatypes in applications such as translation of language, recognition of speech, face recognition and object detection and also dataset from computational biology and biotechnology [107]. Deep learning methods are often referred to as representation learning. Representation learning are a set of methods that allow a system to be fed with raw input data and to automatically detect the features needed for classification [103]. While DL uses multiple levels of representation which allows the system to build complex concepts out of simpler ones [108]. Many times, representation learning needs a great deal of human effort and time for extracting features in case of a complex task, while in DL, the feature extraction is not designed by a human instead they are learned from data using a specific learning architecture [109]. Few common architectures involved with DL are Generative Adversarial Networks (GAN), Multilayer perception (MLP), Recurrent Neural Network (RNN), Artificial Neural Network (ANN) and Convolutional Neural Networks (CNN) [110,111]. Each of these architectures have their unique features and are used for specific applications. For instance, RNN, usually referred to as Deep Neural Network (DNN), is capable of providing better predictions when sequential datasets are available [112]. GAN are capable of predicting and generating realistic images and videos from unlabeled dataset [113], while, ANN tries to resemble the human brain by using a web of layers (neurons) and connections to realize functional mapping of the data. ANNs are best suitable for applications involving complex applications such as voice recognition [114]. The ability of CNN is to learn automatically to imitate human vision

and it plays a crucial role in the field of image classification of applications such as facial recognition [115].

In addition, with availability of large data storages and ultrafast Graphical processing units (GPUs) with exceptional computation speed, the field of DL has reached to ultimate heights assisting with applications in several fields [116]. Besides, DL has pioneered in medical industry with precise predictions using large sets of clinical data. DL techniques are successfully implemented in the medical industry in applications for cancer prediction [98], drug discovery [117], genetics and genomics [118], human sperm selection [119], DNA sequencing [120] and even for classification of COVID [121].

In addition, DL techniques have proven to be helpful in realizing AI on microfluidic devices paving way for integration of ML techniques with microfluidic technology in applications such as cytometry [122], Drug susceptibility [123], cell classification [124] and cell sorting [125,126], complete blood count and also several biochemical and biomedical applications [127]. Owing to these applications, the research in integrating DL techniques with microfluidics is also being rapidly increasing [112,128,129]. As said, quantification of large sets of high quality droplet data can be effectively comprehended only with the implementation of ML models. The following sections emphasize the employment of various ML models in droplet microfluidics to realization of an automated DBMF technology.

4.1. Machine learning for design automation of microfluidic device and automated flow control

The emergence of droplet based microfluidic device paved way for achieving many Lab-on-chip (LOC) applications. One primary requirement to meet a specific application is the microfluidic device itself. A DBMF platform must be very precise and more importantly easy to use. Generation of micro-droplets predominantly depends on the microfluidic geometry. Out of the required droplet microfluidic device geometries (Section 3), flow focusing has its unique features in providing monodisperse droplets at a very high frequency. However, with minute changes in the microchannel geometry, droplet characteristics, such as droplet shape, size and the generation frequency, drastically changes. Different applications demand specific droplet size and frequency to reduce the sample size, which are highly dependent on the microchannel geometry. This signifies that different microchannel geometries are required for attaining droplets of different size. In order to achieve this, design experiments with multiple iterations and optimizations have to be performed

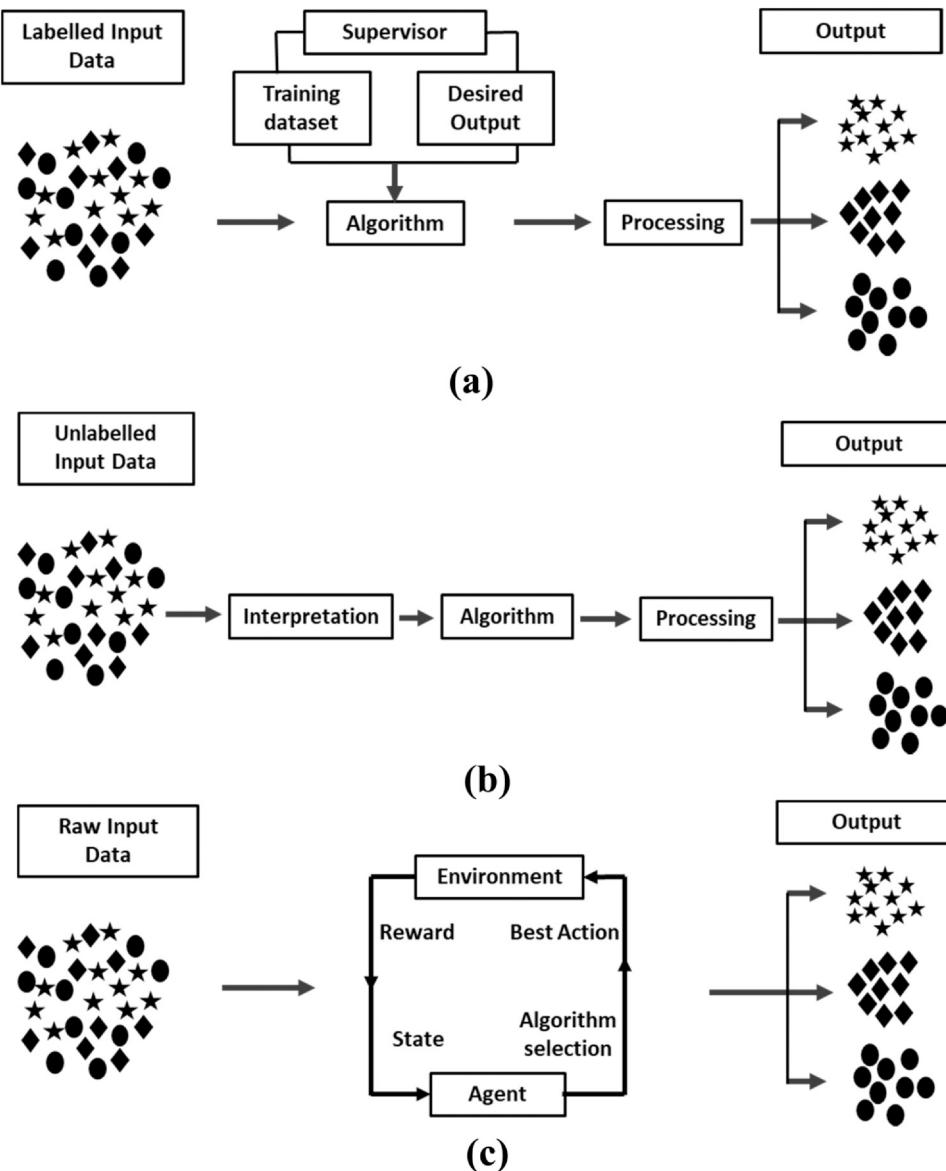


Fig. 4. Three categories of ML (a) Supervised Learning (b) Unsupervised Learning (c) Reinforcement Learning.

until the desired droplet size is achieved [82]. This leads to a laborious process with large consumption of time and money.

One of the best ways to minimize this effort was identified by Ali lashkaripour et al. in order to achieve automation of microchannel geometry using ML techniques [41]. They have developed an automation tool, Design Automation based on Fluid Dynamics (DAFD), that works on machine learning techniques to achieve optimized channel geometry and to predict the performance of the device. Adaptive network based fuzzy inference system (ANFIS) was employed for realize design automation [130]. Large sets of experimental droplet data, obtained from flow focusing junction of different geometries and flow condition, was collected and trained the model to achieve DAFD. For a detailed analysis, reverse predictive models, such as nearest data point, M5P trees and radial basis function, were also explored to predict the output (geometry and flow condition) for a given input (droplet size and generation frequency) [131]. It was identified that nearest data point model was helpful among the three in predicting the microchannel geometry with a minimal error. The automated tool was also tested with active learning algorithms with quality metrics, such as random choice, greedy sampling and query by committee, to understand their role in

predicting the performance of the device [132]. It was observed that group sampling outran the other two metrics suggesting that large amount of quality experimental data is essential for clear-cut predictions. DAFD is an open source tool with reverse and forward predictive models that enables the user to pick the accurate model to predict the device automation and device performance to obtain droplet generators. This tool that works on the basis of ML models is undoubtedly the first automated device automation tool which eliminates design iterations and minimize labor intensive and time invasive process and also reduces the cost of experimentation (Fig. 5(a)) [41].

While optimal geometry is one important parameter, automated and intelligent flow control system is also equally important to obtain droplets of required size and frequency. In a direction towards achieving automated flow control system, Tuam M Tran et al. employed Robot Automated Droplet Microfluidics (RADM) by utilizing commercial fluid handling units. The system comprises of i) Robot for fluid handling ii) microfluidic system iii) interface for changing reagents. All the three components were controlled by master computer. The communication between the master computer and the system components were controlled through LabVIEW. Even

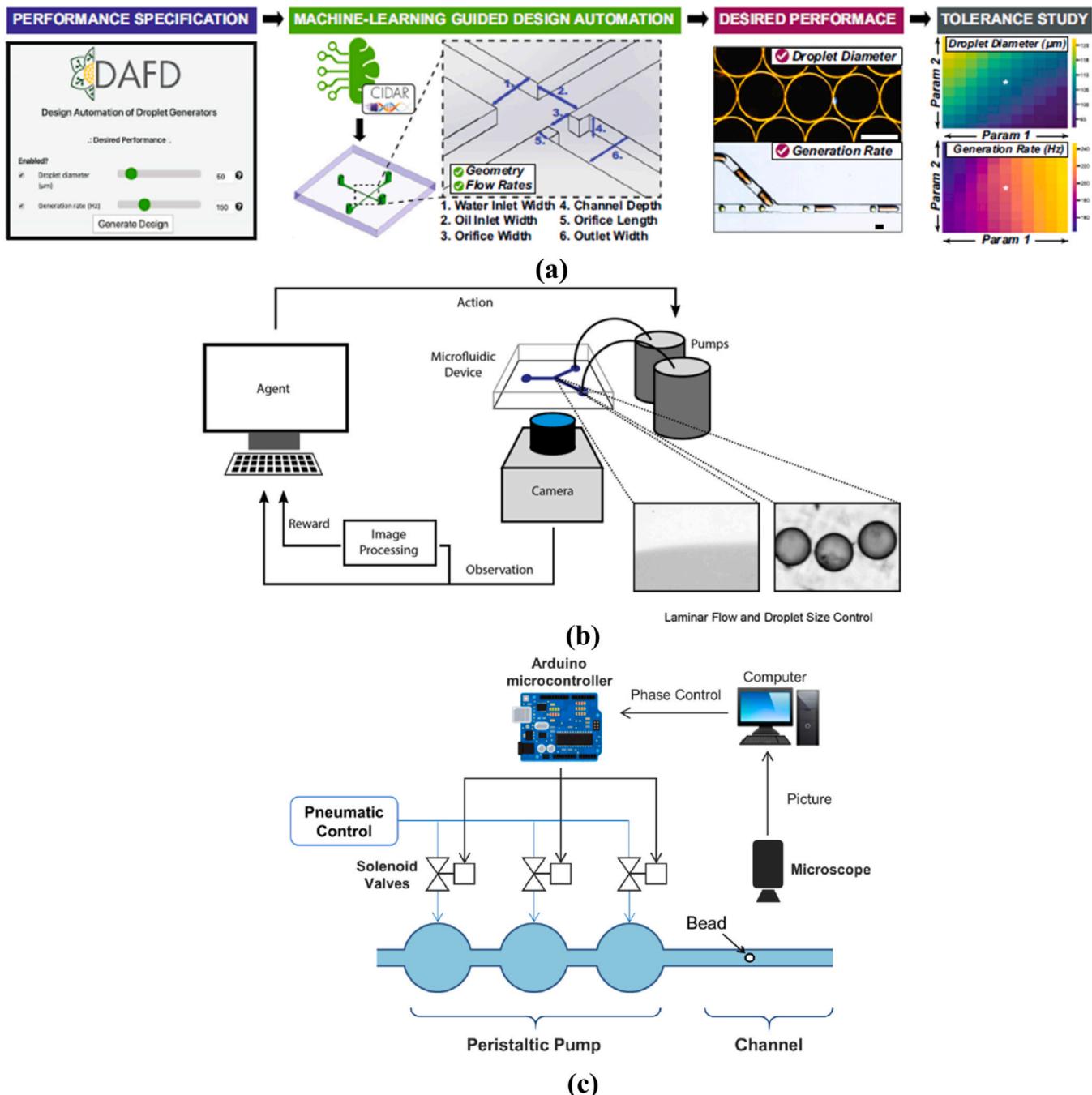


Fig. 5. (a) The open source DAFD tool that operates on ML algorithms takes desired performance parameters which are desired droplet size and generation and provide the required channel geometry and flow rates [41] (b) Reinforcement learning implemented to achieve automated flow control for obtaining co-laminar flow and size controlled droplets in continuous and droplet microfluidics (c) implementation of Reinforcement Learning models to realize an intelligent peristaltic pump that can learn from the environment that regulates the micro-valves to obtain different flow conditions such as micro-mixing and micro-pumping in a microfluidic channel. (b) (with permission from [133]). (c) (with permission from [134])

though, in this work ML techniques was not implemented, it was one of the prominent works directed towards automation of DBMF [135]. In addition, flow control in microfluidic device was also automated through image based analysis [136]. However, it is believed that ML models provide better comprehension for flow control in microfluidic devices.

In a recently reported work, Reinforcement Learning algorithms were employed to regulate the fluidic control in both continuous and droplet based microfluidic systems (Fig. 5(b)) [133]. Learning models, such as Deep Q networks (DQN) and model free episodic controllers (MFEC), were utilized to achieve dynamic flow control in

a microfluidic system. These self-sufficient learning models were trained and were devoted to achieve i) efficient interface between two immiscible fluids in a microfluidic flow at low Reynolds numbers and ii) control of droplet size in two phase flow. It was identified that for laminar flow control, DQN was best suited for long term and complex experiments. While MFEC was suitable for short and simple experiments, as it is easy to train a model with short times in case of MFEC, which was not a possible case using DQN. While for droplet size control, MFEC outran DQN by exhibiting effective learning from the environment and by providing dynamic flow control generation of droplets in very short times. Also, the

Table 1 Summary of different ML models used for design optimization and automated flow control.

S No.	Learning Model	Purpose	Performance / Accuracy	Ref.
1	ANFIS	To predict the device geometry and flow condition for required input of droplet size and generation frequency	–	[130]
2	Reverse Predictive models (Nearest data point, M5P trees and Radial basis function)	Regulation of fluidic control to achieve the desired flow condition	Greater than 90% accuracy	[131]
3	Neural networks	Regulation of fluidic control to achieve the desired flow condition	DQN performed well for laminar flow control while MFEC was more suited for controlled droplet generation	[41]
4	Reinforcement Learning models (DQN and MFEC)	To achieve smart peristaltic pump to realize automated flow conditions such as flow switching an micro-mixing	The trained model led to achieve intelligent peristaltic pump for complex microfluidic systems	[133]
5	Reinforcement Learning			[134]

advantages of Reinforcement learning were made use by Takaki Abe et al., to realize intelligent peristaltic pumps for flow control [134]. The authors implemented reinforced learning algorithms to control the micro-valves of the peristaltic pump to achieve different flow conditions such as flow switching and micro-mixing of fluids inside the microchannel. It suggests that automated microfluidic systems, that can adapt to the changes in environment (experimentations) in real time situations, can be achieved by implementation of ML models for flow control in a microchannel (Fig. 5(c)). Various models used for automated device design and flow control are shown in Table 1.

4.2. Machine learning for prediction of droplet size

The droplet size and shape depends on several parameters such as dimensionless numbers, flow rate ratio, viscosity ratio and channel geometry as discussed in Section 2. However, prediction of droplet is very essential to understand the effect of these parameters on droplet size and shape for applications such microencapsulation, synthesis of nanoparticles and hydrogel beads in a microfluidic platform. In order to determine the size of the droplet image analysis software such as imageJ would be helpful. Other methods to identify droplet size include electrochemical methods [30] and optical imaging methods [137]. These methods can just be utilized to measure the generated droplet and they cannot predict the droplet size beforehand. One best alternative in predicting the droplet features, especially when numerous parameters are involved is implementation of ML approaches. This section showcases few important works that employed ML models for prediction of droplet size.

The effect of four different conditions like Reynolds number and Capillary number of the continuous and dispersed phases (Re_d , Re_c , Ca_d , Ca_c) over the droplet size were successfully predicted by employing ANN model [138]. Droplets were generated in a microfluidic T junction for a wide range of Ca and Re with a total of 742 experiments out of which 520 examples were used for training the model and 111 examples for testing and the rest for validation. A static neural network with four input parameters (Re_d , Re_c , Ca_d , Ca_c) and several hidden layers, and an output layer (size of droplet) was considered to be the structure of NN (Fig. 6(a)). It was identified that NN with ten layers was found to be optimal for effective prediction of droplet size with $R^2 \approx 1$ and with a least square error of 1.4×10^{-6} .

In another work, Ali Lashkaripour et al., implemented ANFIS approach to predict the droplet size in a microfluidic flow-focusing junction for different input parameters such as geometry of the microchannel, fluid properties and different flow conditions [139]. Six major input parameters, orifice width, viscosity of continuous phase, surface tension, velocity of continuous phase and dispersed phase and density of the continuous phase, were considered to comprehend their effect on droplet size. Thoroughly performed experimental data was used to train the ANFIS model to predict the droplet size. The trained ANFIS model was able to predict the droplet size with an accuracy of 96% for a given input (Fig. 6(b)). A similar work was carried out by Sina Mottaghi et al., to predict the droplet size using ANFIS model [141]. In this work, the authors trained the model with other input parameters such as Ca , Re , flow rate ratio and viscosity ratio to predict their effect on the droplet size. The trained model performed efficiently by predicting the droplet size with an accuracy of 92%. Through these works, the authors proposed that ANFIS is an efficient model in exploring large datasets in a very effective manner to make accurate predictions.

In a different work, ANN models were applied to predict the size of PLGA microparticles produced in a flow focusing microfluidic channel [140]. It was very essential to have a desired size of PLGA microparticles which influences the stability of obtained beads which are vital for many biomedical applications [142]. Experimental dataset was obtained in a microchannel for different input

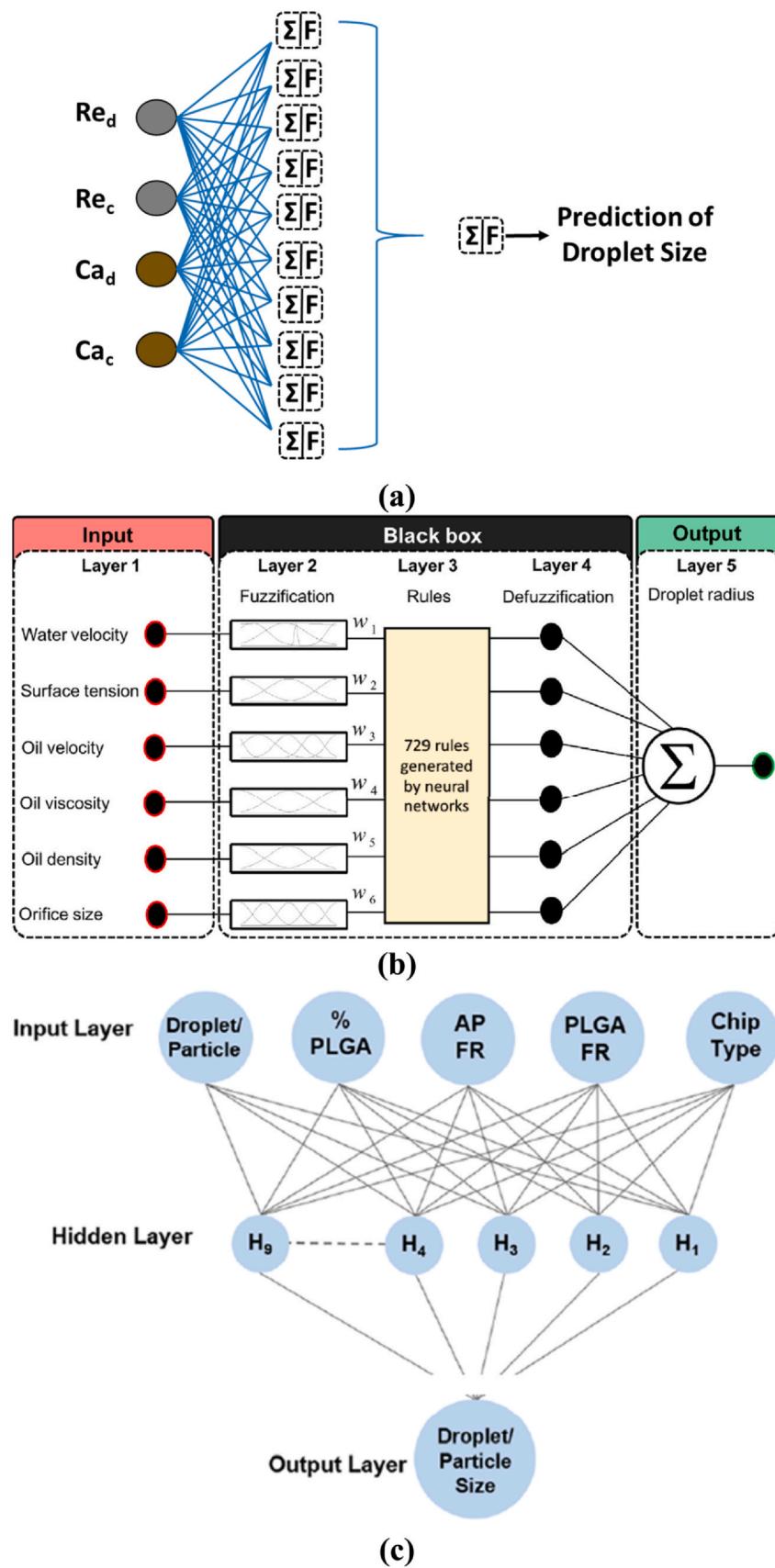


Fig. 6. (a) ANN algorithm applied to train the model with four input parameters. The optimal model was found to have 10 neurons to precisely predict the droplet size for a given.

Table 2
Summary of different ML models used for prediction of droplet size.

S No.	Learning Model	Purpose	Performance / Accuracy	Ref.
1	Artificial Neural Networks	Prediction of droplet size for user inputs such as Reynolds number and capillary number of dispersed and continuous phases	$R^2 \approx 1$ with a least square error of 1.4×10^{-6}	[138]
2	ANFIS	Prediction of droplet size for input parameters such as orifice width, viscosity of continuous phase, surface tension, velocity of continuous phase and dispersed phase and density of the continuous phase	Trained model exhibits accuracy of 96%	[139]
3	ANFIS	Prediction of droplet size based on Reynolds number, capillary number, flow rate ratio and viscosity ratio	Prediction accuracy of 92% was achieved	[141]
4	Artificial Neural Networks	Prediction of PLGA microparticles size for inputs such as concentration and flow rate of PLGA, emulsion type and device geometry	Trained model with nine neurons showed precise prediction with $R^2 \approx 0.97$	[140]

parameters such as PLGA concentration and flow conditions to predict the output parameter (size of the PLGA microparticles) (Fig. 6(c)). Three different conditions were considered for experimentation: i) production of PLGA particles in single junction microfluidic device with single emulsion ii) PLGA microparticles in 7-junction device and iii) generation PLGA microparticles using multiple emulsion. With the intention of obtaining an effective model, three ANN models were individually trained for the above mentioned conditions named ANN-A, ANN-C and ANN-C. It was observed that all the three models performed exceptionally well with an r^2 value of 0.99 for validation dataset. In addition, two more ANN models (ANN-AB and ANN-AC) were developed utilizing the data obtained from the initially trained three models. It was observed that the optimal ANN-ABC consisted of 8 inputs with 9 neurons for predicting one output with an r^2 value of 0.97 for validation dataset. This resulted in obtaining a simple and a generalized for predicting the size of PLGA microparticle effectively for any input parameter. Table 2 shows learning models employed for predicting the size of droplets in a microfluidic device.

4.3. Machine learning for classification of droplet content

Machine learning extended its way even for classification of droplets and its content. In this section delves upon different works that implemented unique ML models, for characterization of droplets and identification of different flow patterns in a droplet based microchannel. Classification of droplets based on its content is essential for applications involving detailed single cell analysis. Gabriele Soldati et al. employed CNN to realize automated droplet classification based on different droplet images [143]. Among all the ML models, CNN has proven to show excellent performance in applications where image classification is involved. Therefore, the authors collected huge experimental data in the form of quality images that include empty droplets, droplets with debris and droplets with cells (Fig. 7(a)). This dataset was used to train the CNN model to automate i) classification of droplet content ii) measurement of pH and volume and iii) segmentation of cellular images. The training was performed on three different CNN models namely ResNeXt, ConvNet and Inception v3 for different epochs. It was identified that after training of 150 epochs, ResNeXt has shown good performance in classification of droplets with an accuracy of 90%. In another study, CNN was employed to identify the concentration of mixture inside the droplet and also the flow rate of the dispersed phase [144]. Large set of droplet data was obtained for different concentrations of water/IPA mixture (4%-7%) and different flow rates of the dispersed phase (0.1 ML/hr. to 1.5 ML/hr.) with a constant flow rate of the continuous phase in a microfluidic flow focusing junction. A CNN model with two convolutional layers was employed and trained with the obtained experimental dataset to predict either the concentration of the mixture or the flow rate (Fig. 7(b)). In addition, the authors also suggested that the proposed model can also be employed for predicting fluidic properties such as surface tension and viscosity.

CNNs were also implemented to recognize different oil-water patterns. CNN models such as LeNet 5, Alex Net and VGG-16 net were trained to identify the flow pattern [146]. All the three models were trained with a total of 29000 images which include 2000 images of slug flow, 13000 oil bubble images and 8000 images of finely dispersed oil with bubbles. Among all the models, VGG-16 showed promising results in identifying the flow pattern. A similar kind of work was reported by Chong Shen et al., wherein CNN model was trained and validated with experimental data of different flow conditions to recognize flow pattern in a microfluidic channel [147]. In order to achieve automated experimentation, the trained recognition model was integrated to automatic flow control system and an online capture system to regulate the flow so that the desired flow pattern can be obtained with a continuous feedback. The model

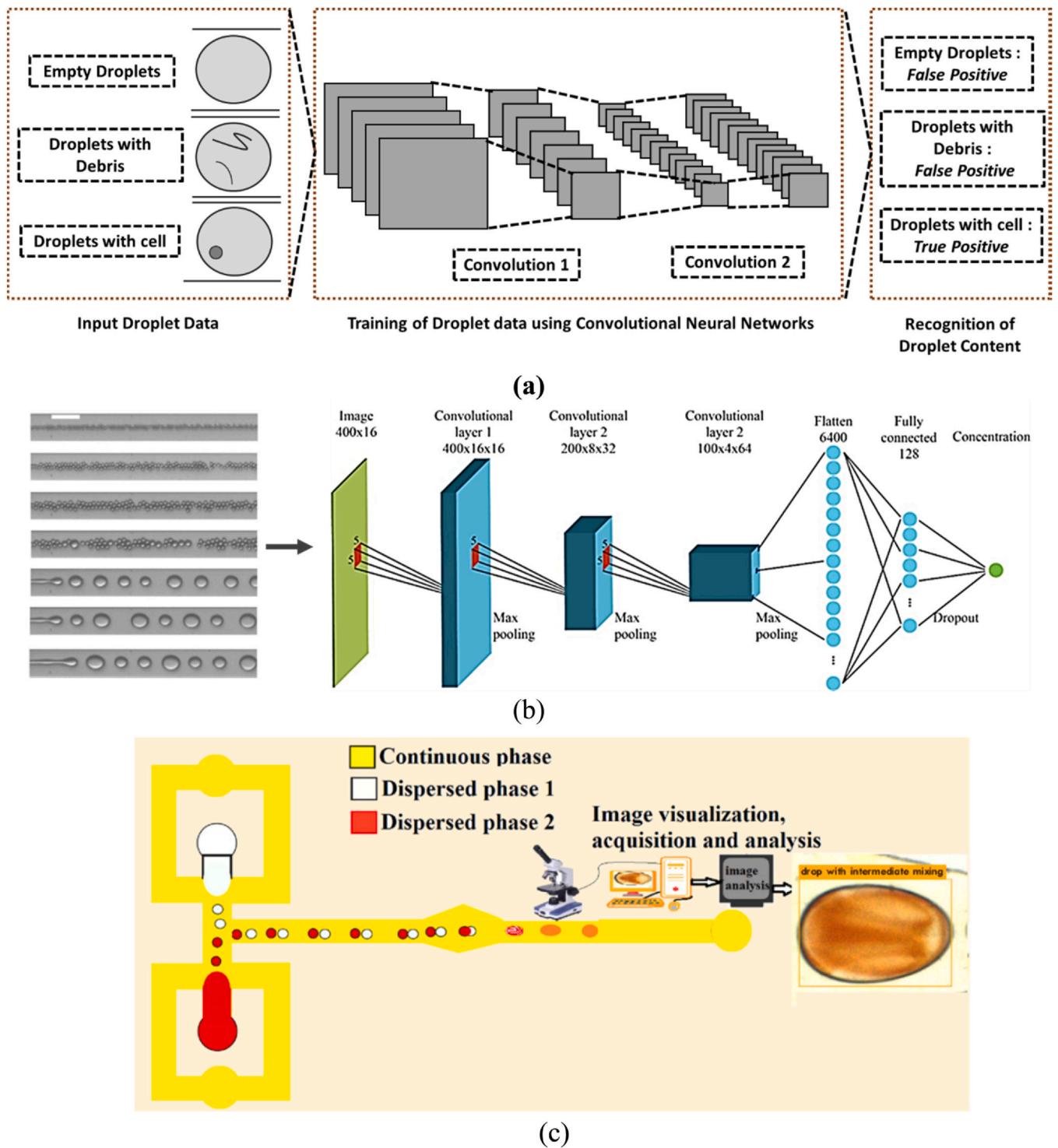


Fig. 7. (a) The CNN model was trained with different droplet set such as empty droplets, droplets with debris and droplets with cells. The model, post training was capable of detecting droplet and cell inside a droplet individually with a 90.2% accuracy [143] (b) high quality droplet data in the form of images were used to train the CNN model with three convolutions and a connected layer for prediction of the concentrations of the inlet fluids [144] (c) Droplets were generated in a microfluidic channel with two different dispersed phases in a specially designed flow focusing junction which enabled the mixing of two individual droplets. This droplet data was acquired through optical imaging system and was fed to CNN models to identify the grade of mixing.

The trained model successfully predicted the mixing grades (lower, intermediate and high) with a very high accuracy (with permission from [145]).

proved to be successful in clear identification of different flow patterns such as annular flow, droplet flow, slug flow and dispersed flow with an accuracy of 98%. Furthermore, CNN tools, such as You Only Look Once (YOLO) and Single Shot Multibox Detector (SSD), which are effective in real-time object detection and classification were implemented to understand the mixing grade inside a droplet

generated in a microfluidic channel [145]. Droplet data obtained from flow focusing junction and cross junction were used to train the two models to predict three degrees of mixing which are low mixing, intermediate mixing and high mixing (Fig. 7(c)). Post training both the models, SSD showed higher accuracy in identifying the mixing quality. Additionally, DeepSORT and YOLO, other CNN

Table 3
Summary of ML models used for classification of droplet content and flow pattern.

S No.	Learning Model	Purpose	Performance / Accuracy	Ref.
1	Convolutional Neural Network (ResNeXt, ConvNet and Inception v3)	To automate droplet classification based on its content and also for measurement of pH and volume of droplet.	An accuracy of 90% was achieved through ResNeXt	[143]
2	Convolutional Neural Network	To predict the flow rate and concentration of mixture (water/IPA) inside a droplet	Trained model predicted concentration with an accuracy of 0.5% change of the mixture and flow rate with a resolution of 0.05 mL/h	[144]
3	Convolutional Neural network (YOLO and SSD)	To predict the degree of mixing inside a droplet	SSD model showed higher accuracy than YOLO	[145]
4	Convolutional neural Network (LeNet 5, Alex Net and VGG-16)	To recognize flow patterns such as droplet flow, annular flow and slug flow	VGG-16 showed better performance	[146]
5	Convolutional Neural Network	To predict droplet shape and track its motion in a microfluidic channel	Trained model exhibited an accuracy of 98% in recognizing different flow patterns	[147]
6	Convolutional Neural Network (DeepSORT)	To predict droplet content and flow pattern in a microfluidic channel involving complex flow condition	The trained models successfully predicted droplet trajectories in a microfluidic channel involving complex flow condition	[148]

models were also used in order to track the droplet movement in a microfluidic channel [148]. The authors suggest that the proposed model helps in understanding the dynamic nature of cells and microorganisms in complex flow conditions (such as emulsion) in a microfluidic channel. Various models utilized to realize content inside the droplet and flow pattern are shown in Table 3.

4.4. Machine learning for droplet sorting

In applications involving encapsulation of microparticles, such as cells, segregation and proper classification of droplets with and without cells gets trickier, consumes time and might lead to lower accuracy when done manually. In single cell analysis, classification of cells is very essential to understand the mechanical behavior of cells and also to identify desired cells from the heterogeneous population [149]. Thus, detection, sorting and quantification of encapsulated droplets is very essential to employ them in many biomedical applications [20]. Few established sorting techniques, such as image based automated sorting of droplets [150] and Fluorescence activate cell sorting (FACS) [151,152], are extensively used. While image based sorting techniques uses image processing algorithms for sorting, FACS needs droplets to be coded with fluorescence tags before experimentation. In addition to these, ML algorithms have also been implemented for accurate and precise sorting, detection and quantification droplets by learning from the experimental datasets. ML models were employed successfully in clinical approach for detection, stratification and even classification of cells [153]. Supervised multi class classification and CNN models were implemented earlier for off-chip analysis of stem cells, blood cell counting and many other applications as discussed in Section 4. ML models were also integrated with microfluidic platform to realize screening, cell counting and sorting applications. Xiwei Huang et al. reported an image based microfluidic cytometer integrated with ML techniques [154]. The authors proposed Extreme Learning Machine (ELM) algorithms were used to perform identification of cells and their count in a continuous flow microfluidic device. Also, recently DL approaches were employed for screening of lung cancer cells in a microfluidic device [155]. Five CNN models were trained with experimental dataset that helped in building a computer aided diagnosis system for effective and highly efficient screening of cells through elimination of user intervention. Supervised learning models such as support vector machines (SVM) was used for detection of leucocytes in a microfluidic device based on pillars [156].

In addition to these models, image based deep learning techniques were proven to be very helpful in automated sorting of cells or droplets in a microfluidic device [157]. One of the best characteristics of image based DL models is their capability of extracting hidden features from images that enables to distinguish any two different images [158]. One such work which implemented DL for image base cell sorting was developed by Keondo Lee and group [159]. In this work, high quality images were captured during experimentation and image processing algorithms were implemented for acquisition, processing and classification of images. The processed image dataset was utilized to train, test and validate the CNN model. The output of the trained CNN model was utilized for sorting of particles that gives signals to a piezoelectric actuator. The final model integrated to sorting mechanism enabled to take decisions on sorting of beads within 3 ms with a sorting efficiency of 98%. In another work, labelled images were used to train CNN model to automatically detect and sort microencapsulated droplets in a DBMF platform [160]. The images were classified as per their generation characteristics and were fed into a CNN model that predicted and distinguished encapsulated droplet and empty droplets. This predictive model was integrated with controllers that utilized the output data from the model to sort droplets using solenoid valves (Fig. 8(a)). Furthermore, the authors suggested that implementation

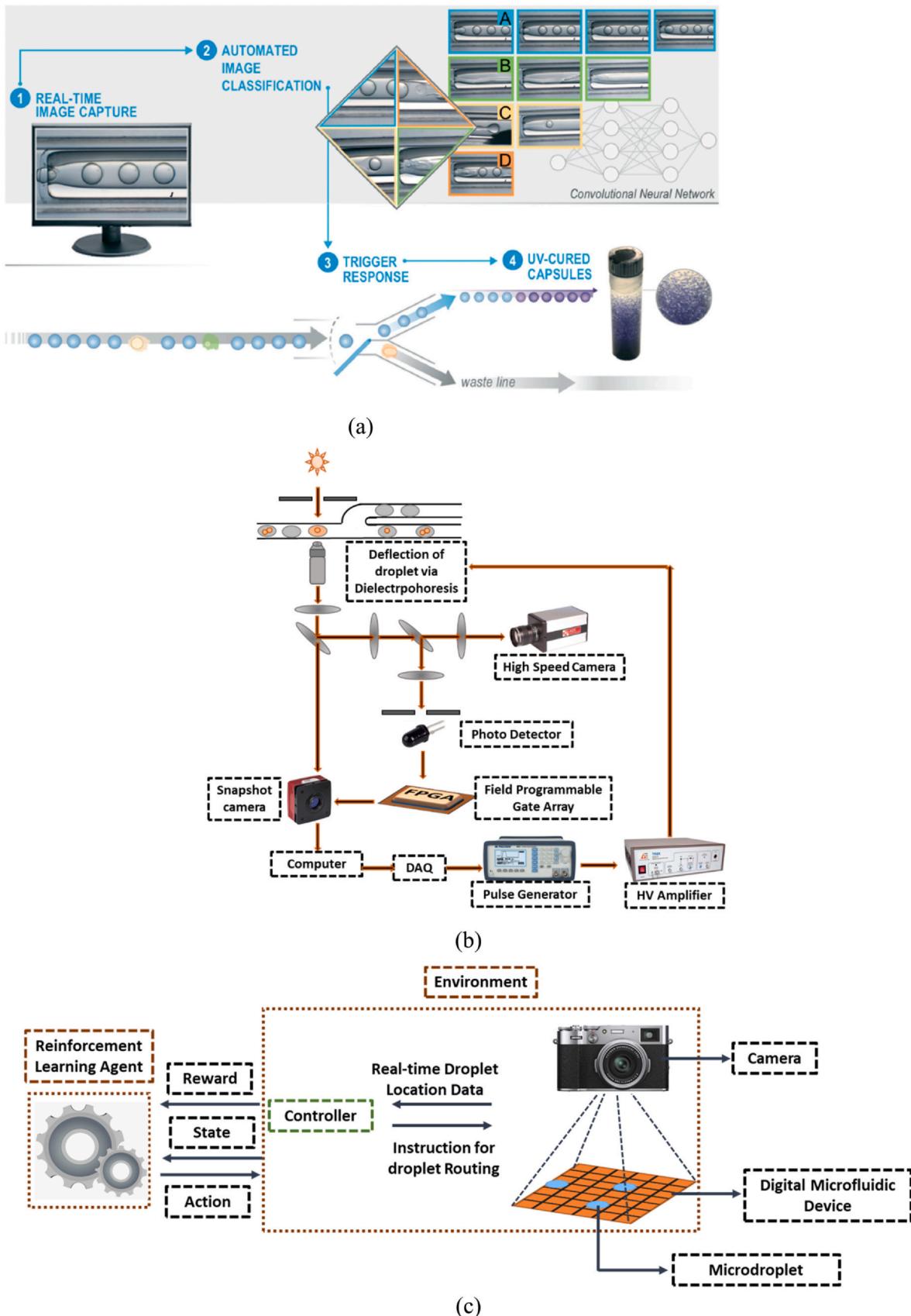


Fig. 8. (a) Real time images were captured during experimentation and post classification of the data, the dataset was fed to the CNN model. The trained model successfully identifies the encapsulated and empty droplet and gives feedback to the controllers which in turn sort the droplets with the help of solenoid valves [160] (b) A CNN model was trained with droplets of three classes namely droplet with no cell, single cell and more than one cell. The CNN model predicts the droplet with single cell and triggers the sorting mechanism to sort the droplet with the help of dielectrophoresis [161] (c) implementation of Reinforcement Learning in digital microfluidic platform. Images of the digital microfluidic device were captured using a CCD camera and were sent to a processor that detects the location of droplets on the chip. This processed information was fed to RL model which routes the droplets through the controller by identifying the degradation of electrode [162].

of more complex algorithms such as RNN helped in improving the accuracy in prediction that helps the controllers for modulating the operating conditions.

A similar approach was adopted by Vasileos and group by implementing DL techniques with image based droplet sorting for analysis of single cells and cell culture [161]. Multiple datasets of droplets, such as droplets with polystyrene beads, polyacrylamide beads, droplets with MCF 7 cells and droplets with combination of beads and cells, were used for training the model to achieve real-time classification of droplets and sort them. The final model was able to identify and sort the droplets with single MCF-7 cells with an accuracy of 90% (Fig. 8(b)).

Carl Magnus Svensson et al. implemented Random Forest Classifier (RFC), a supervised ML technique that used various decision trees for given dataset to provide better predictions for classification of micro-droplets [163]. In their work, RFC was implemented to classify droplets based on colored beads encapsulated in individual droplets. In addition, the RFC model was integrated with Bayesian interface (a statistical paradigm) to identify the experimental conditions as well. Here, droplets were generated in a flow focusing device and polystyrene beads of 8 different colors were encapsulated in the droplets. The experimental dataset was fed to the model for classifying the encoded droplets with different colors. The trained model showed exceptional performance with an accuracy of 99.6% for a droplet population of nearly 10^5 droplets per each experiment of 20 different experimental conditions. This models helped in realizing automated DBMF platform for biological applications involving classification of droplets with biological particles in them.

In addition to droplet microfluidics, ML techniques have also proved to be effective in digital microfluidic platforms for drug discovery and drug development [164]. Development of ML algorithms for digital microfluidics helps in easier classification of multi concentrated samples that helps in drug development. Research in implementing ML algorithms is still in the nascent stage. Recently, RL methods were implemented for routing droplets in a digital microfluidic device [165]. The problems associated with degradation of electrodes over time in a digital microfluidic device were tried to address using RL algorithms. The RL model was trained in such a way that it routes the droplet to a healthy electrode by comprehending the behavior of a degrading electrode and thus increasing the life span of a digital microfluidic chip (Fig. 8(c)) [162]. Table 4 shows various ML models implemented to achieve sorting of droplets.

4.5. Challenges and future opportunities

Droplet based microfluidics and Machine learning are two different emerging areas that have excelled in their individual domains. Both the fields demonstrate unique features that are advantageous for several applications that benefit the scientific community. However, both the fields have their drawback as well. In case of droplet microfluidics, it is evident that, a specifically designed microfluidic device will be required for certain application. It suggests that the device is more of application specific and a single device cannot be applicable for a wide range of applications. Similarly, in the case of ML, precisely tailored algorithms are.

essential to achieve automation. It is indeed very essential to identify the correct model to succeed in precise predictions of the outputs. So, in order to have a better comprehension of both the fields, a SWOT (Strength, Weakness, Opportunity and Threat) analysis was performed as shown in Fig. 9.

It can be seen from Fig. 9 that both the fields have outstanding strengths and unlimited opportunities in several fields. It can also be seen that there are areas of improvements in respective fields that can be perfected with time and with the expansion of technology. As discussed in Section 4, integration of ML with droplet microfluidics

Table 4
Summary of different ML models used for sorting of droplets in a microfluidic channel.

S No.	Learning Model	Purpose	Performance / Accuracy	Ref.
1	Conventional Neural Network	To sort droplets based on their content such as empty droplet or encapsulated droplet	Trained models with the help of sorting mechanism achieved sorting efficiency of around 98%	[159,160]
2	Convolutional Neural Network	To detect and sort droplets with cells, polystyrene beads, polyacrylamide beads and a combination of cells and beads.	The final model with 128 neurons was capable of identify and sort droplets with 90% accuracy	[161]
3	Random Forest Classifier	To classify droplets i.e decoding of droplets with beads and cells and also quantification of content	The model was found to be successful with 99.6% accuracy in decoding the droplets	[163]
4	Reinforcement learning	To route droplets on a digital microfluidic device by learning the degradation of electrode	The final model effectively predicted the electrode degradation and routes the droplet accordingly to healthy electrode	[162,165]

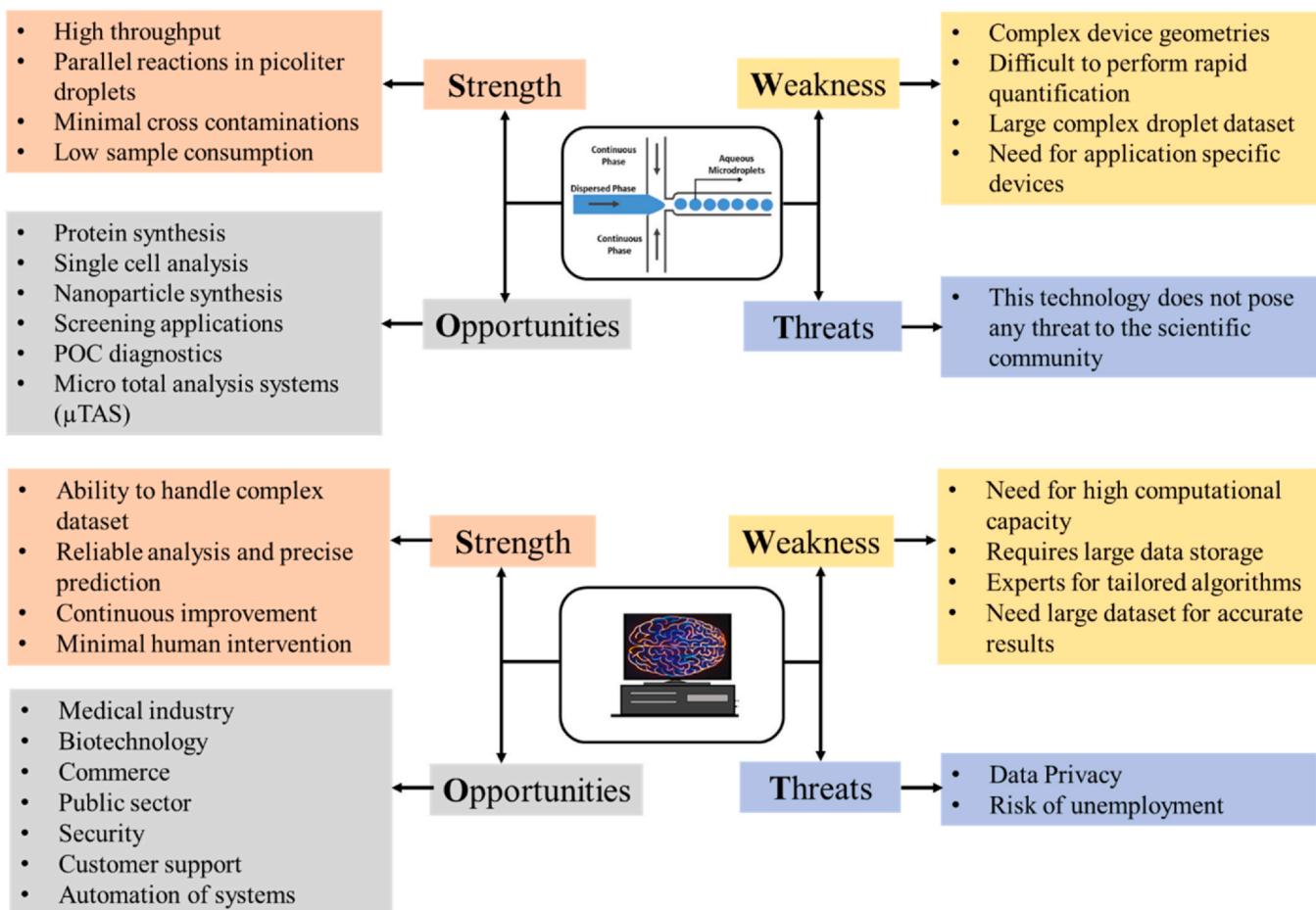


Fig. 9. Detailed SWOT analysis of droplet based microfluidics and machine learning.

facilitates in achieving numerous functions precisely with minimized human involvement. Considering the advantages of amalgamation of these two fields, we strongly believe that these weaknesses can be overlooked. In addition, we trust that the collaboration of DBMF and ML, in near future, will pave way for achieving smart microfluidic systems such as automated organ on chip devices and cloud based microfluidic devices.

5. Conclusions

The synergy of high-throughput nature of droplet microfluidics (DM) and analytical tools of Machine learning (ML) boosts the strength of droplet based microfluidic technology. This integration helps in achieving optimized and automated microfluidic systems with higher accuracy, shorter times and more importantly with minimal intervention of personnel. Each field, as an individual pose several challenges such as fabrication of complex geometries, high throughput droplet generation, large droplet data and quantification of droplets are a few difficult tasks involved in DBMF. Similarly, Machine learning has few critical requirements such as high computational capacity, large data storage, selection of tailored ML models for specific applications and skilled personnel. However, on a large scale, in order to realize a cost-effective and an automated micro total analysis systems (μ TAS) technology with high-throughput and accurate results, the marriage of Microfluidic technology with advanced Machine learning tools is inevitable. In addition, with the development of technology, the above mentioned challenges of individual fields can be easily taken care of to realize intelligent microfluidic technology. We strongly believe that there is

a lot of potential for implementing Machine learning models in achieving complete automation of droplet based microfluidic platforms right from flow control to a detailed quantification of droplet data with highly accurate results without any human intervention.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] G.M. Whitesides, The origins and the future of microfluidics, *Nature* 442 (2006) 368–373, <https://doi.org/10.1038/nature05058>
- [2] N. Convery, N. Gadegaard, 30 years of microfluidics, *Micro Nano Eng.* 2 (2019) 76–91, <https://doi.org/10.1016/j.mne.2019.01.003>
- [3] D. Juncker, H. Schmid, E. Delamarche, Multipurpose microfluidic probe, *Nat. Mater.* 4 (2005) 622–628, <https://doi.org/10.1038/nmat1435>
- [4] X. Li, D.R. Ballerini, W. Shen, A perspective on paper-based microfluidics: current status and future trends, *Biomicrofluidics* 6 (2012) 011301, <https://doi.org/10.1063/1.3687398>
- [5] W. Dungchai, O. Chailapakul, C.S. Henry, Electrochemical detection for paper-based microfluidics, *Anal. Chem.* 81 (2009) 5821–5826, <https://doi.org/10.1021/ac9007573>
- [6] S.-Y. Teh, R. Lin, L.-H. Hung, A.P. Lee, Droplet microfluidics, *Lab Chip.* 8 (2008) 198–220, <https://doi.org/10.1039/b715524g>
- [7] R. Seemann, M. Brinkmann, T. Pfohl, S. Herminghaus, Droplet based microfluidics, *Rep. Prog. Phys.* 75 (2012) 016601, <https://doi.org/10.1088/0034-4885/75/1/016601>
- [8] L. Shang, Y. Cheng, Y. Zhao, Emerging droplet microfluidics, *Chem. Rev.* 117 (2017) 7964–8040, <https://doi.org/10.1021/acs.chemrev.6b00848>
- [9] A. Sua-Ngam, P.D. Howes, M. Sriva-Art, A.J. deMello, Droplet microfluidics: from proof-of-concept to real-world utility? *Chem. Commun.* 55 (2019) 9895–9903, <https://doi.org/10.1039/C9CC04750F>

- [10] S. Mashaghi, A. Abbaspourrad, D.A. Weitz, A.M. van Oijen, Droplet microfluidics: a tool for biology, chemistry and nanotechnology, *TrAC Trends Anal. Chem.* 82 (2016) 118–125, <https://doi.org/10.1016/j.trac.2016.05.019>
- [11] Y. Ding, P.D. Howes, A.J. deMello, Recent advances in droplet microfluidics, *Anal. Chem.* 92 (2020) 132–149, <https://doi.org/10.1021/acs.analchem.9b05047>
- [12] K. Matula, F. Rivello, W.T.S. Huck, Single-cell analysis using droplet microfluidics, *Adv. Biosyst.* 4 (2020) 1900188, <https://doi.org/10.1002/adbi.201900188>
- [13] G.F. Christopher, J. Bergstein, N.B. End, M. Poon, C. Nguyen, S.L. Anna, Coalescence and splitting of confined droplets at microfluidic junctions, *Lab Chip.* 9 (2009) 1102–1109, <https://doi.org/10.1039/b813062k>
- [14] C.-G. Yang, Z.-R. Xu, J.-H. Wang, Manipulation of droplets in microfluidic systems, *TrAC Trends Anal. Chem.* 29 (2010) 141–157, <https://doi.org/10.1016/j.trac.2009.11.002>
- [15] A.B. Theberge, F. Courtois, Y. Schaefer, M. Fischlechner, C. Abell, F. Hollfelder, W.T.S. Huck, Microdroplets in microfluidics: an evolving platform for discoveries in chemistry and biology, *Angew. Chem. Int. Ed.* 49 (2010) 5846–5868, <https://doi.org/10.1002/anie.200906653>
- [16] W.-L. Chou, P.-Y. Lee, C.-L. Yang, W.-Y. Huang, Y.-S. Lin, Recent advances in applications of droplet microfluidics, *Micromachines* 6 (2015) 1249–1271, <https://doi.org/10.3390/mi6091249>
- [17] Z.-M. Liu, Y. Yang, Y. Du, Y. Pang, Advances in droplet-based microfluidic technology and its applications, *Chin. J. Anal. Chem.* 45 (2017) 282–296, [https://doi.org/10.1016/S1872-2040\(17\)60994-0](https://doi.org/10.1016/S1872-2040(17)60994-0)
- [18] H. Song, D.L. Chen, R.F. Ismagilov, Reactions in droplets in microfluidic channels, *Angew. Chem. Int. Ed.* 45 (2006) 7336–7356, <https://doi.org/10.1002/anie.200601554>
- [19] Y. Wang, Z. Chen, F. Bian, L. Shang, K. Zhu, Y. Zhao, Advances of droplet-based microfluidics in drug discovery, *Expert Opin. Drug Discov.* 15 (2020) 969–979, <https://doi.org/10.1080/17460441.2020.1758663>
- [20] S. Sohrabi, N. kassir, M. Keshavarz Moraveji, Droplet microfluidics: fundamentals and its advanced applications, *RSC Adv.* 10 (2020) 27560–27574, <https://doi.org/10.1039/DORA04566G>
- [21] S. Srikanth, S. Dudala, U.S. Jayapiriy, J.M. Mohan, S. Raut, S.K. Dubey, I. Ishii, A. Javed, S. Goel, Droplet-based lab-on-chip platform integrated with laser ablated graphene heaters to synthesize gold nanoparticles for electrochemical sensing and fuel cell applications, *Sci. Rep.* 11 (2021) 9750, <https://doi.org/10.1038/s41598-021-88068-z>
- [22] S.H. Tan, F. Maes, B. Semin, J. Vrignon, J.-C. Baret, The microfluidic jukebox, *Sci. Rep.* 4 (2015) 4787, <https://doi.org/10.1038/srep04787>
- [23] A. Sciambi, A.R. Abate, Accurate microfluidic sorting of droplets at 30 kHz, *Lab Chip.* 15 (2015) 47–51, <https://doi.org/10.1039/C4LC01194E>
- [24] Y. Zhu, Q. Fang, Analytical detection techniques for droplet microfluidics—A review, *Anal. Chim. Acta* 787 (2013) 24–35, <https://doi.org/10.1016/j.aca.2013.04.064>
- [25] T. Fukuda, N. Funaki, T. Kurabayashi, M. Suzuki, D.H. Yoon, A. Nakahara, T. Sekiguchi, S. Shoji, Real-time monitoring of chemical reaction in microdroplet using fluorescence spectroscopy, *Sens. Actuators B: Chem.* 203 (2014) 536–542, <https://doi.org/10.1016/j.snb.2014.06.045>
- [26] Z. Mao, F. Guo, Y. Xie, Y. Zhao, M.I. Lapsley, L. Wang, J.D. Mai, F. Costanzo, T.J. Huang, Label-free measurements of reaction kinetics using a droplet-based optofluidic device, *J. Lab. Autom.* 20 (2015) 17–24, <https://doi.org/10.1177/2211068214549625>
- [27] E.D. Guetschow, D.J. Steyer, R.T. Kennedy, Subsecond electrophoretic separations from droplet samples for screening of enzyme modulators, *Anal. Chem.* 86 (2014) 10373–10379, <https://doi.org/10.1021/ac502758h>
- [28] K.L.A. Chan, S.G. Kazarian, FT-IR spectroscopic imaging of reactions in multi-phase flow in microfluidic channels, *Anal. Chem.* 84 (2012) 4052–4056, <https://doi.org/10.1021/ac300019m>
- [29] G.L. Nelson, S.E. Asmussen, A.M. Lines, A.J. Casella, D.R. Bottenus, S.B. Clark, S.A. Bryan, Micro-raman technology to interrogate two-phase extraction on a microfluidic device, *Anal. Chem.* 90 (2018) 8345–8353, <https://doi.org/10.1021/acs.analchem.7b04330>
- [30] X. Hu, X. Lin, Q. He, H. Chen, Electrochemical detection of droplet contents in polystyrene microfluidic chip with integrated micro film electrodes, *J. Electroanal. Chem.* 726 (2014) 7–14, <https://doi.org/10.1016/j.jelechem.2014.05.005>
- [31] S. Srikanth, J.M. Mohan, S. Raut, S.K. Dubey, I. Ishii, A. Javed, S. Goel, Droplet based microfluidic device integrated with ink jet printed three electrode system for electrochemical detection of ascorbic acid, *Sens. Actuators A: Phys.* 325 (2021) 112685, <https://doi.org/10.1016/j.sna.2021.112685>
- [32] S. Sangam, M.M. Jaligam, A. Javed, S.K. Dubey, S. Goel, Droplet based microfluidic electrochemical detection of uric acid, ascorbic acid and dopamine, *Meet. Abstr. MA2020–02* (2020), <https://doi.org/10.1149/MA2020-02573888mtgabs> 3888–3888.
- [33] Z. Xu, J.-Q. Lu, S.-L. Hu, W.-D. Yang, J.-S. Liu, Z.-X. Zhang, W.-F. Tian, Impedance Monitoring of Droplets in a Microfluidic Chip, (n.d.) 4.
- [34] S.K. Küster, S.R. Fagerer, P.E. Verboeket, K. Eyer, K. Jefimovs, R. Zenobi, P.S. Dittrich, Interfacing droplet microfluidics with matrix-assisted laser desorption/ionization mass spectrometry: label-free content analysis of single droplets, *Anal. Chem.* 85 (2013) 1285–1289, <https://doi.org/10.1021/ac3033189>
- [35] W. Hale, G. Rossetto, R. Greenhalgh, G. Finch, M. Utz, High-resolution nuclear magnetic resonance spectroscopy in microfluidic droplets, *Lab Chip* 18 (2018) 3018–3024, <https://doi.org/10.1039/C8LC00712H>
- [36] N. Gupta, S. Augustine, T. Narayan, A. O'Riordan, A. Das, D. Kumar, J.H.T. Luong, B.D. Malhotra, Point-of-care PCR assays for COVID-19 detection, *Biosensors* 11 (2021) 141, <https://doi.org/10.3390/bios11050141>
- [37] M. Deiana, A. Mori, C. Piubelli, S. Scarso, M. Favarato, E. Pomari, Assessment of the direct quantitation of SARS-CoV-2 by droplet digital PCR, *Sci. Rep.* 10 (2020) 18764, <https://doi.org/10.1038/s41598-020-75958-x>
- [38] M. Rezaei, S. Razavi Bazaz, S. Zhand, N. Sayyadi, D. Jin, M.P. Stewart, M. Ebrahimi Warkiani, Point of care diagnostics in the age of COVID-19, *Diagnostics* 11 (2020) 9, <https://doi.org/10.3390/diagnostics11010009>
- [39] L. Dong, J. Zhou, C. Niu, Q. Wang, Y. Pan, S. Sheng, X. Wang, Y. Zhang, J. Yang, M. Liu, Y. Zhao, X. Zhang, T. Zhu, T. Peng, J. Xie, Y. Gao, D. Wang, Y. Zhao, X. Dai, X. Fang, Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR, *Public Glob. Health* (2020), <https://doi.org/10.1101/2020.03.14.20036129>
- [40] H.-D. Xi, H. Zheng, W. Guo, A.M. Gañán-Calvo, Y. Ai, C.-W. Tsao, J. Zhou, W. Li, Y. Huang, N.-T. Nguyen, S.H. Tan, Active droplet sorting in microfluidics: a review, *Lab Chip* 17 (2017) 751–771, <https://doi.org/10.1039/C6LC01435F>
- [41] A. Lashkaripour, C. Rodriguez, N. Mehdiipour, R. Mardian, D. McIntyre, L. Ortiz, J. Campbell, D. Densmore, Machine learning enables design automation of microfluidic flow-focusing droplet generation, *Nat. Commun.* 12 (2021) 25, <https://doi.org/10.1038/s41467-020-20284-z>
- [42] S. Das, A. Dey, A. Pal, N. Roy, Applications of artificial intelligence in machine learning: review and prospect, *IJCA* 115 (2015) 31–41, <https://doi.org/10.5120/20182-2402>
- [43] V. Dunjko, H.J. Briegel, Machine learning & artificial intelligence in the quantum domain: a review of recent progress, *Rep. Prog. Phys.* 81 (2018) 074001, <https://doi.org/10.1088/1361-6633/aab406>
- [44] I.S. Stafford, M. Kellermann, E. Mossotto, R.M. Beattie, B.D. MacArthur, S. Ennis, A systematic review of the applications of artificial intelligence and machine learning in autoimmune diseases, *Npj Digit. Med.* 3 (2020) 30, <https://doi.org/10.1038/s41746-020-0229-3>
- [45] T.S. Kaminski, O. Scheler, P. Garstecki, Droplet microfluidics for microbiology: techniques, applications and challenges, *Lab Chip.* 16 (2016) 2168–2187, <https://doi.org/10.1039/C6LC00367B>
- [46] N. Shi, M. Mohibullah, C.J. Easley, Active flow control and dynamic analysis in droplet microfluidics, *annurev-anchem-122120-042627*, *Annu. Rev. Anal. Chem.* 14 (2021) 133–153, <https://doi.org/10.1146/annurev-anchem-122120-042627>
- [47] C.N. Baroud, F. Gallaire, R. Dangla, Dynamics of microfluidic droplets, *Lab Chip* 10 (2010) 2032–2045, <https://doi.org/10.1039/c001191f>
- [48] X. Chen, H. Wu, J. Wu, Surface-tension-confined droplet microfluidics, *Chin. Phys. B* 27 (2018) 029202, <https://doi.org/10.1088/1674-1056/27/2/029202>
- [49] L. Peng, M. Yang, S. Guo, W. Liu, X. Zhao, The effect of interfacial tension on droplet formation in flow-focusing microfluidic device, *Biomed. Micro* 13 (2011) 559–564, <https://doi.org/10.1007/s10544-011-9526-6>
- [50] D. Ferraro, M. Serra, D. Filippi, L. Zago, E. Guglielmin, M. Pierro, S. Descroix, J.-L. Viovy, G. Mistura, Controlling the distance of highly confined droplets in a capillary by interfacial tension for merging on-demand, *Lab Chip.* 19 (2019) 136–146, <https://doi.org/10.1039/C8LC01182F>
- [51] J.D. Wehking, M. Gabany, L. Chew, R. Kumar, Effects of viscosity, interfacial tension, and flow geometry on droplet formation in a microfluidic T-junction, *Microfluid. Nanofluid.* 16 (2014) 441–453, <https://doi.org/10.1007/s10404-013-1239-0>
- [52] J.D. Tice, H. Song, A.D. Lyon, R.F. Ismagilov, Formation of droplets and mixing in multiphase microfluidics at low values of the reynolds and the capillary numbers, *Langmuir* 19 (2003) 9127–9133, <https://doi.org/10.1021/la03090w>
- [53] Y. Wang, P. Dimitrakopoulos, Low-Reynolds-number droplet motion in a square microfluidic channel, *Theor. Comput. Fluid Dyn.* 26 (2012) 361–379, <https://doi.org/10.1007/s00162-011-0238-6>
- [54] P. Tirandazi, C.H. Hidrovo, Liquid-in-gas droplet microfluidics: experimental characterization of droplet morphology, generation frequency, and monodispersity in a flow-focusing microfluidic device, *J. Micromech. Microeng.* 27 (2017) 075020, <https://doi.org/10.1088/1361-6439/aa7595>
- [55] P. Mary, V. Studer, P. Tabelin, Microfluidic droplet-based liquid–liquid extraction, *Anal. Chem.* 80 (2008) 2680–2687, <https://doi.org/10.1021/ac800088s>
- [56] H. Song, M.R. Bringer, J.D. Tice, C.J. Gerdt, R.F. Ismagilov, Experimental test of scaling of mixing by chaotic advection in droplets moving through microfluidic channels, *Appl. Phys. Lett.* 83 (2003) 4664–4666, <https://doi.org/10.1063/1.1630378>
- [57] H. Gu, M.H.G. Duits, F. Mugele, Droplets formation and merging in two-phase flow microfluidics, *IJMS* 12 (2011) 2572–2597, <https://doi.org/10.3390/ijms12042572>
- [58] D. Li (Ed.), *Encyclopedia of Microfluidics and Nanofluidics*, Springer, US, Boston, MA, 2013, , <https://doi.org/10.1007/978-3-642-27758-0>
- [59] A. Gupta, R. Kumar, Flow regime transition at high capillary numbers in a microfluidic T-junction: viscosity contrast and geometry effect, *Phys. Fluids* 22 (2010) 122001, <https://doi.org/10.1063/1.3523483>
- [60] M.-C. Julien, M.-J. Tsang Mui Ching, C. Cohen, L. Menetrier, P. Tabelin, Droplet breakup in microfluidic T-junctions at small capillary numbers, *Phys. Fluids* 21 (2009) 072001, <https://doi.org/10.1063/1.3170983>
- [61] I.-L. Ngo, S. Woo Joo, C. Byon, Effects of junction angle and viscosity ratio on droplet formation in microfluidic cross-junction, *J. Fluids Eng.* 138 (2016) 051202, <https://doi.org/10.1115/1.4031881>
- [62] K. Loizou, V.-L. Wong, B. Hewakandamby, Examining the effect of flow rate ratio on droplet generation and regime transition in a microfluidic T-junction at

- constant capillary numbers, *Inventions* 3 (2018) 54, <https://doi.org/10.3390/inventions3030054>
- [63] O. Sartipzadeh, S.M. Naghib, A. Seyfoori, M. Rahmanian, F.S. Fatemiania, Controllable size and form of droplets in microfluidic-assisted devices: effects of channel geometry and fluid velocity on droplet size, *Mater. Sci. Eng. C, Mater. Biol. Appl.* 109 (2020) 110606, <https://doi.org/10.1016/j.msec.2019.110606>
- [64] M. Rahimi, A. Shams Khorrami, P. Rezai, Effect of device geometry on droplet size in co-axial flow-focusing microfluidic droplet generation devices, *Colloids Surf. A: Physicochem. Eng. Asp.* 570 (2019) 510–517, <https://doi.org/10.1016/j.colsurfa.2019.03.067>
- [65] J. Yao, F. Lin, H. Kim, J. Park, The effect of oil viscosity on droplet generation rate and droplet size in a t-junction microfluidic droplet generator, *Micromachines* 10 (2019) 808, <https://doi.org/10.3390/mi10120808>
- [66] J.-C. Baret, Surfactants in droplet-based microfluidics, *Lab Chip.* 12 (2012) 422–433, <https://doi.org/10.1039/C1LC20582J>
- [67] M. Saqib, O.B. Şahinoğlu, E.Y. Erdem, Alternating droplet formation by using tapered channel geometry, *Sci. Rep.* 8 (2018) 1606, <https://doi.org/10.1038/s41598-018-19966-y>
- [68] L. Rosenfeld, T. Lin, R. Derda, S.K.Y. Tang, Review and analysis of performance metrics of droplet microfluidics systems, *Microfluid Nanofluid* 16 (2014) 921–939, <https://doi.org/10.1007/s10404-013-1310-x>
- [69] P. Zhu, L. Wang, Passive and active droplet generation with microfluidics: a review, *Lab Chip.* 17 (2017) 34–75, <https://doi.org/10.1039/C6LC01018K>
- [70] H. Liu, Y. Zhang, Droplet formation in microfluidic cross-junctions, *Phys. Fluids* 23 (2011) 082101, <https://doi.org/10.1063/1.3615643>
- [71] A.M. Leshansky, L.M. Pismen, Breakup of drops in a microfluidic T junction, *Phys. Fluids* 21 (2009) 023303, <https://doi.org/10.1063/1.3078515>
- [72] S. Srikanth, S. Dudala, S. Raut, S.K. Dubey, I. Ishii, A. Javed, S. Goel, Optimization and characterization of direct UV laser writing system for microscale applications, *J. Micromech. Microeng.* 30 (2020) 095003, <https://doi.org/10.1088/1361-6439/ab92ea>
- [73] A. Gupta, R. Kumar, Effect of geometry on droplet formation in the squeezing regime in a microfluidic T-junction, *Microfluid Nanofluid* 8 (2010) 799–812, <https://doi.org/10.1007/s10404-009-0513-7>
- [74] M.Y.A. Jamalabadi, M. DaqiqShirazi, A. Kosar, M.S. Shadloo, Effect of injection angle, density ratio, and viscosity on droplet formation in a microfluidic T-junction, *Theor. Appl. Mech. Lett.* 7 (2017) 243–251, <https://doi.org/10.1016/j.taml.2017.06.002>
- [75] L. Wang, M. Quintard, Nanofluids of the future, in: L. Wang (Ed.), *Advances in Transport Phenomena*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2009, pp. 179–243, https://doi.org/10.1007/978-3-642-02690-4_4
- [76] K. Khoshmanesh, A. Almansouri, H. Albloushi, P. Yi, R. Sofie, K. Kalantar-zadeh, A multi-functional bubble-based microfluidic system, *Sci. Rep.* 5 (2015) 9942, <https://doi.org/10.1038/srep09942>
- [77] S. Takeuchi, P. Garstecki, D.B. Weibel, G.M. Whitesides, An axisymmetric flow-focusing microfluidic device, *Adv. Mater.* 17 (2005) 1067–1072, <https://doi.org/10.1002/adma.200401738>
- [78] L. Yobas, S. Martens, W.-L. Ong, N. Ranganathan, High-performance flow-focusing geometry for spontaneous generation of monodispersed droplets, *Lab Chip.* 6 (2006) 1073–1079, <https://doi.org/10.1039/b602240e>
- [79] S.L. Anna, N. Bontoux, H.A. Stone, Formation of dispersions using “flow focusing” in microchannels, *Appl. Phys. Lett.* 82 (2003) 364–366, <https://doi.org/10.1063/1.1537519>
- [80] T. Fu, Y. Ma, D. Funfschilling, H.Z. Li, Bubble formation and breakup mechanism in a microfluidic flow-focusing device, *Chem. Eng. Sci.* 64 (2009) 2392–2400, <https://doi.org/10.1016/j.ces.2009.02.022>
- [81] P. Garstecki, I. Gitlin, W. DiLuzio, G.M. Whitesides, E. Kumacheva, H.A. Stone, Formation of monodisperse bubbles in a microfluidic flow-focusing device, *Appl. Phys. Lett.* 85 (2004) 2649–2651, <https://doi.org/10.1063/1.1796526>
- [82] A. Lashkaripour, C. Rodriguez, L. Ortiz, D. Denmore, Performance tuning of microfluidic flow-focusing droplet generators, *Lab Chip.* 19 (2019) 1041–1053, <https://doi.org/10.1039/C8LC01253A>
- [83] M. Nooranidoost, R. Kumar, Geometry effects of axisymmetric flow-focusing microchannels for single cell encapsulation, *Materials* 12 (2019) 2811, <https://doi.org/10.3390/ma12172811>
- [84] K.I. Belousov, N.A. Filatov, I.V. Kukhtevich, V. Kantsler, A.A. Evstratov, A.S. Bukatin, An asymmetric flow-focusing droplet generator promotes rapid mixing of reagents, *Sci. Rep.* 11 (2021) 8797, <https://doi.org/10.1038/s41598-021-88174-y>
- [85] P.B. Umbanhower, V. Prasad, D.A. Weitz, Monodisperse emulsion generation via drop break off in a coflowing stream, *Langmuir* 16 (2000) 347–351, <https://doi.org/10.1021/la990101e>
- [86] P. Zhu, X. Tang, L. Wang, Droplet generation in co-flow microfluidic channels with vibration, *Microfluid Nanofluid* 20 (2016) 47, <https://doi.org/10.1007/s10404-016-1717-2>
- [87] S.L. Anna, Droplets and bubbles in microfluidic devices, *Annu. Rev. Fluid Mech.* 48 (2016) 285–309, <https://doi.org/10.1146/annurev-fluid-122414-034425>
- [88] A. Taassob, M.K.D. Manshadi, A. Bordbar, R. Kamali, Monodisperse non-Newtonian micro-droplet generation in a co-flow device, *J. Braz. Soc. Mech. Sci. Eng.* 39 (2017) 2013–2021, <https://doi.org/10.1007/s40430-016-0699-z>
- [89] D. Rafique, L. Velasco, Machine learning for network automation: overview, architecture, and applications [invited tutorial], *J. Opt. Commun. Netw.* 10 (2018) D126, <https://doi.org/10.1364/JOCN.10.00D126>
- [90] THE POWER OF HUMAN-MACHINE COLLABORATION: ARTIFICIAL INTELLIGENCE, BUSINESS AUTOMATION, AND THE SMART ECONOMY *Econ. Manag. Financ. Mark.* 13 (2018) 51 doi: 10.22381/EMFM13420184.
- [91] K.C. Morris, C. Schlenoff, V. Srinivasan, Guest editorial a remarkable resurgence of artificial intelligence and its impact on automation and autonomy, *IEEE Trans. Autom. Sci. Eng.* 14 (2017) 407–409, <https://doi.org/10.1109/TASE.2016.2640778>
- [92] Application of Artificial Intelligence in Automation of Supply Chain Management *JJSIS* 14 (2019) doi: 10.33423/jjsis.v14i3.2105.
- [93] D.S. Nau, Artificial intelligence and automation, in: S.Y. Nof (Ed.), *Springer Handb. Autom.*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2009) 249–268, https://doi.org/10.1007/978-3-540-78831-7_14
- [94] A.V. Joshi, Machine learning and artificial intelligence, *Springer Int. Publ.*, Cham (2020), <https://doi.org/10.1007/978-3-030-26622-6>
- [95] M.I. Jordan, T.M. Mitchell, Machine learning: trends, perspectives, and prospects, *Science* 349 (2015) 255–260, <https://doi.org/10.1126/science.aaa8415>
- [96] C. Park, C.C. Took, J.-K. Seong, Machine learning in biomedical engineering, *Biomed. Eng. Lett.* 8 (2018) 1–3, <https://doi.org/10.1007/s13534-018-0058-3>
- [97] J. Wei, X. Chu, X. Sun, K. Xu, H. Deng, J. Chen, Z. Wei, M. Lei, Machine learning in materials science, *InfoMat* 1 (2019) 338–358, <https://doi.org/10.1002/int.2.12028>
- [98] J.A. Cruz, D.S. Wishart, Applications of machine learning in cancer prediction and prognosis, *Cancer Inform.* 2 (2006) 117693510600200, <https://doi.org/10.1177/117693510600200030> 117693510600200.
- [99] M. Žekić-Sušac, S. Mitrović, A. Has, Machine learning based system for managing energy efficiency of public sector as an approach towards smart cities, *Int. J. Inf. Manag.* 58 (2021) 102074, <https://doi.org/10.1016/j.ijinfomgt.2020.102074>
- [100] T. Oladipupo, Types of machine learning algorithms, in: Y. Zhang (Ed.), *New Advances in Machine Learning*, InTech, 2010, , <https://doi.org/10.5772/9385>
- [101] T. Hastie, R. Tibshirani, J. Friedman, Overview of supervised learning, *The Elements of Statistical Learning*, Springer, New York, New York, NY, 2009, pp. 9–41, https://doi.org/10.1007/978-0-387-84858-7_2
- [102] Unsupervised learning algorithms, in: M.E. Celebi, K. Aydin (Eds.), *Springer International Publishing*, Cham, 2016, <https://doi.org/10.1007/978-3-319-24211-8>
- [103] Y. LeCun, Y. Bengio, G. Hinton, Deep learning, *Nature* 521 (2015) 436–444, <https://doi.org/10.1038/nature14539>
- [104] A. Kamilaris, F.X. Prenafeta-Boldú, Deep learning in agriculture: a survey, *Comput. Electron. Agric.* 147 (2018) 70–90, <https://doi.org/10.1016/j.compag.2018.02.016>
- [105] M.Z. Alom, T.M. Taha, C. Yakopcic, S. Westberg, P. Sidiqe, M.S. Nasrin, M. Hasan, B.C. Van Essen, A.A.S. Awwal, V.K. Asari, A state-of-the-art survey on deep learning theory and architectures, *Electronics* 8 (2019) 292, <https://doi.org/10.3390/electronics8030292>
- [106] Y. Bengio, Learning deep architectures for AI, *FNT Mach. Learn.* 2 (2009) 1–127, <https://doi.org/10.1561/2200000006>
- [107] L. Deng, Deep learning: methods and applications, *FNT Signal Process.* 7 (2014) 197–387, <https://doi.org/10.1561/2000000039>
- [108] I. Goodfellow, Y. Bengio, A. Courville, *Deep learning*, The MIT Press, Cambridge, Massachusetts, 2016.
- [109] L. Deng, Deep learning: methods and applications, *FNT Signal Process.* 7 (2014) 197–387, <https://doi.org/10.1561/2000000039>
- [110] N. Rusk, Deep learning, *Nat. Methods* 13 (2016) 35, <https://doi.org/10.1038/nmeth.3707> 35–35.
- [111] A. Shrestha, A. Mahmood, Review of Deep Learning Algorithms and Architectures 7 *IEEE Access*, 2019, pp. 53040–53065, <https://doi.org/10.1109/ACCESS.2019.2912200>
- [112] J. Riordon, D. Sovilj, S. Sanner, D. Sinton, E.W.K. Young, Deep learning with microfluidics for biotechnology, *Trends Biotechnol.* 37 (2019) 310–324, <https://doi.org/10.1016/j.tibtech.2018.08.005>
- [113] A. Creswell T. White V. Dumoulin K. Arulkumaran B. Sengupta A.A. Bharath Generative adversarial networks: an overview 2018 *IEEE Signal Process Mag* 53 65 doi: 10.1109/MSP.2017.2765202.
- [114] E.S. Wahyuni, Arabic speech recognition using MFCC feature extraction and ANN classification, 2017 2nd International Conferences on Information Technology, Information Systems and Electrical Engineering (ICITISEE), IEEE, Yogyakarta, 2017, pp. 22–25, <https://doi.org/10.1109/ICITISEE.2017.8285499>
- [115] S. Albawi, T.A. Mohammed, S. Al-Zawi, Understanding of a convolutional neural network, 2017 International Conference on Engineering and Technology (ICET), IEEE, Antalya, 2017, pp. 1–6, <https://doi.org/10.1109/ICEngTechnol.2017.8308186>
- [116] P.P. Shinde, S. Shah, A review of machine learning and deep learning applications, 2018 Fourth International Conference on Computing Communication Control and Automation (ICCUBEA), IEEE, Pune, India, 2018, pp. 1–6, <https://doi.org/10.1109/ICCUBEA.2018.8697857>
- [117] A. Lavecchia, Machine-learning approaches in drug discovery: methods and applications, *Drug Discov. Today* 20 (2015) 318–331, <https://doi.org/10.1016/j.drudis.2014.10.012>
- [118] M.W. Libbrecht, W.S. Noble, Machine learning applications in genetics and genomics, *Nat. Rev. Genet.* 16 (2015) 321–332, <https://doi.org/10.1038/nrg3920>
- [119] C. McCallum, J. Riordon, Y. Wang, T. Kong, J.B. You, S. Sanner, A. Lagunov, T.G. Hannam, K. Jarvi, D. Sinton, Deep learning-based selection of human sperm with high DNA integrity, *Commun. Biol.* 2 (2019) 250, <https://doi.org/10.1038/s42003-019-0491-6>

- [120] N.E.M. Khalifa, M.H.N. Taha, D. Ezzat Ali, A. Slowik, A.E. Hassani, Artificial intelligence technique for gene expression by Tumor RNA-Seq data: a novel optimized deep learning approach, *IEEE Access* 8 (2020) 22874–22883, <https://doi.org/10.1109/ACCESS.2020.2970210>
- [121] F. Waselallah Alsaade, T.H.H. Aldhyani, M. Hmoud Al-Adhaileh, Developing a recognition system for classifying COVID-19 using a convolutional neural network algorithm, *Comput. Mater. Contin.* 68 (2021) 805–819, <https://doi.org/10.32604/cmc.2021.016264>
- [122] Y. Li, A. Mahjoubfar, C.L. Chen, K.R. Niazi, L. Pei, B. Jalali, Deep cytometry: deep learning with real-time inference in cell sorting and flow cytometry, *Sci. Rep.* 9 (2019) 11088, <https://doi.org/10.1038/s41598-019-47193-6>
- [123] H. Kobayashi, C. Lei, Y. Wu, A. Mao, Y. Jiang, B. Guo, Y. Ozeki, K. Goda, Label-free detection of cellular drug responses by high-throughput bright-field imaging and machine learning, *Sci. Rep.* 7 (2017) 12454, <https://doi.org/10.1038/s41598-017-12378-4>
- [124] C.L. Chen, A. Mahjoubfar, L.-C. Tai, I.K. Blaby, A. Huang, K.R. Niazi, B. Jalali, Deep learning in label-free cell classification, *Sci. Rep.* 6 (2016) 21471, <https://doi.org/10.1038/srep21471>
- [125] A. Isozaki, H. Mikami, H. Tezuka, H. Matsumura, K. Huang, M. Akamine, K. Hiramatsu, T. Iino, T. Ito, H. Karakawa, Y. Kasai, Y. Li, Y. Nakagawa, S. Ohnuki, T. Ota, Y. Qian, S. Sakuma, T. Sekiya, Y. Shirasaki, N. Suzuki, E. Tayyabi, T. Wakamiya, M. Xu, M. Yamagishi, H. Yan, Q. Yu, S. Yan, D. Yuan, W. Zhang, Y. Zhao, F. Arai, R.E. Campbell, C. Danielon, D. Di Carlo, K. Hiraki, Y. Hoshino, Y. Hosokawa, M. Inaba, A. Nakagawa, Y. Ohya, M. Okawa, S. Uemura, Y. Ozeki, T. Sugimura, N. Nitta, K. Goda, Intelligent image-activated cell sorting 2.0, *Lab Chip* 20 (2020) 2263–2273, <https://doi.org/10.1039/DOLC00080A>
- [126] A.A. Nawaz, M. Urbanska, M. Herbig, M. Nötzel, M. Kräter, P. Rosendahl, C. Herold, N. Toepfner, M. Kubánková, R. Goswami, S. Abuhattum, F. Reichel, P. Müller, A. Taubenberger, S. Girardo, A. Jacobi, J. Guck, Intelligent image-based deformation-assisted cell sorting with molecular specificity, *Nat. Methods* 17 (2020) 595–599, <https://doi.org/10.1038/s41592-020-0831-y>
- [127] A. Isozaki, J. Harmon, Y. Zhou, S. Li, Y. Nakagawa, M. Hayashi, H. Mikami, C. Lei, K. Goda, AI on a chip, *Lab Chip* 20 (2020) 3074–3090, <https://doi.org/10.1039/DOLC00521E>
- [128] E.A. Galan, H. Zhao, X. Wang, Q. Dai, W.T.S. Huck, S. Ma, Intelligent microfluidics: the convergence of machine learning and microfluidics in materials science and biomedicine, *Matter* 3 (2020) 1893–1922, <https://doi.org/10.1016/j.matt.2020.08.034>
- [129] S.R. Dabbagh, F. Rabbi, Z. Doğan, A.K. Yetisen, S. Tasoglu, Machine learning-enabled multiplexed microfluidic sensors, *Biomicrofluidics* 14 (2020) 061506, <https://doi.org/10.1063/5.0025462>
- [130] A. Lashkaripour, R. Sanka, J. Lippai, D. Densmore, Design automation based on fluid dynamics, (n.d.) 3. The Proceedings of the 9th International Workshop on Bio-Design Automation, (2017).
- [131] A. Lashkaripour, C. Rodriguez, D. Douglas, A reverse predictive model towards design automation of microfluidic droplet generators, (n.d.) 3 Proc. 10th Int. Workshop Bio-Des. Autom. 2018.
- [132] D. McIntyre, A. Lashkaripour, D. Densmore, Active learning for efficient microfluidic design automation, (n.d.) 3, Proc. 12th Int. Workshop Bio-Des. Autom. (2020).
- [133] O.J. Dressler, P.D. Howes, J. Choo, A.J. deMello, Reinforcement learning for dynamic microfluidic control, *ACS Omega* 3 (2018) 10084–10091, <https://doi.org/10.1021/acsomega.8b01485>
- [134] T. Abe, S. Oh-hara, Y. Ukiwa, Adoption of reinforcement learning for the intelligent control of a microfluidic peristaltic pump, *Biomicrofluidics* 15 (2021) 034101, <https://doi.org/10.1063/5.0032377>
- [135] T.M. Tran, S.C. Kim, A.R. Abate, Robotic automation of droplet microfluidics, *Bioengineering* (2018), <https://doi.org/10.1101/278556>
- [136] T. Aoyama, A. De Zoysa, Q. Gu, T. Takaki, I. Ishii, Real-time flow-rate control system for cell analysis, Proceedings of the 2015 Conference on Advances In Robotics - AIR '15, ACM Press, Goa, India, 2015, pp. 1–4, <https://doi.org/10.1145/2783449.2783499>
- [137] J. Wu, G. Zheng, L.M. Lee, Optical imaging techniques in microfluidics and their applications, *Lab Chip.* 12 (2012) 3566–3575, <https://doi.org/10.1039/c2lc40517b>
- [138] Y. Mahdi, K. Daoud, Microdroplet size prediction in microfluidic systems via artificial neural network modeling for water-in-oil emulsion formulation, *J. Dispers. Sci. Technol.* 38 (2017) 1501–1508, <https://doi.org/10.1080/01932691.2016.1257391>
- [139] A. Lashkaripour, M. Goharimanesh, A. Abouei Mehrizi, D. Densmore, An adaptive neural-fuzzy approach for microfluidic droplet size prediction, *Microelectron. J.* 78 (2018) 73–80, <https://doi.org/10.1016/j.mejo.2018.05.018>
- [140] S.A. Damiani, D. Rossi, H.N. Joensson, S. Damiani, Artificial intelligence application for rapid fabrication of size-tunable PLGA microparticles in microfluidics, *Sci. Rep.* 10 (2020) 19517, <https://doi.org/10.1038/s41598-020-76477-5>
- [141] S. Mottaghi, M. Nazari, S.M. Fattahi, M. Nazari, S. Babamohammadi, Droplet size prediction in a microfluidic flow focusing device using an adaptive network based fuzzy inference system, *Biomed. Micro* 22 (2020) 61, <https://doi.org/10.1007/s10544-020-00513-4>
- [142] A. Agrawal, S. Rellegadla, S. Jain, Biomedical applications of PLGA particles, *Materials for Biomedical Engineering*, Elsevier, 2019, pp. 87–129, <https://doi.org/10.1016/B978-0-12-816913-1.00004-0>
- [143] G. Soldati, F.D. Ben, G. Brisotto, E. Biscontini, M. Bulfoni, A. Piruska, A. Steffan, M. Turetta, Microfluidic droplets content classification and analysis through convolutional neural networks in a liquid biopsy workflow, (n.d.) 13.
- [144] P. Hadikhani, N. Borhani, S.M.H. Hashemi, D. Psaltis, Learning from droplet flows in microfluidic channels using deep neural networks, *Sci. Rep.* 9 (2019) 8114, <https://doi.org/10.1038/s41598-019-44556-x>
- [145] A. Arjun, R.R. Ajith, S. Kumar Ranjith, Mixing characterization of binary-coalesced droplets in microchannels using deep neural network, *Biomicrofluidics* 14 (2020) 034111, <https://doi.org/10.1063/5.0008461>
- [146] M. Du, H. Yin, X. Chen, X. Wang, Oil-in-water two-phase flow pattern identification from experimental snapshots using convolutional neural network, *IEEE Access.* 7 (2019) 6219–6225, <https://doi.org/10.1109/ACCESS.2018.288733>
- [147] C. Shen, Q. Zheng, M. Shang, L. Zha, Y. Su, Using deep learning to recognize liquid–liquid flow patterns in microchannels, *AIChE J.* 66 (2020), <https://doi.org/10.1002/aic.16260>
- [148] M. Durve, F. Bonaccorso, A. Montessori, M. Lauricella, A. Tiribocchi, S. Succi, A fast and efficient deep learning procedure for tracking droplet motion in dense microfluidic emulsions ArXiv:2103.01572 [Cond.-Mat.] 2021.accessed May 31, 2021 (<http://arxiv.org/abs/2103.01572>).
- [149] K. Matula, F. Rivello, W.T.S. Huck, Single-cell analysis using droplet microfluidics, *Adv. Biosyst.* 4 (2020) 1900188, <https://doi.org/10.1002/adbi.201900188>
- [150] M. Vaithiyathan, N. Safa, A.T. Melvin, FluoroCellITrack: An algorithm for automated analysis of high-throughput droplet microfluidic data, *PLoS One* 14 (2019) 0215337, <https://doi.org/10.1371/journal.pone.0215337>
- [151] J.-C. Baret, V. Taly, M. Ryckelynck, C.A. Merten, A.D. Griffiths, Gouttes et émulsions: criblage à très haut débit en biologie, *Med Sci.* 25 (2009) 627–632, <https://doi.org/10.1051/medsci.2009256-7627>
- [152] S.S. Terekhov, I.V. Smirnov, A.V. Stepanova, T.V. Bobik, Y.A. Mokrushina, N.A. Ponomarenko, A.A. Belogurov, M.P. Rubtsova, O.V. Kartseva, M.O. Gomzikova, A.A. Moskvtsev, A.S. Bukatin, M.V. Dubina, E.S. Kostryukova, V.V. Babenko, M.T. Vakhitova, A.I. Manolov, M.V. Malakhova, M.A. Kornienko, A.V. Tyakht, A.A. Van'yushkina, E.N. Ilina, P. Masson, A.G. Gabibov, S. Altman, Microfluidic droplet platform for ultrahigh-throughput single-cell screening of biodiversity, *Proc. Natl. Acad. Sci. USA* 114 (2017) 2550–2555, <https://doi.org/10.1073/pnas.1621226114>
- [153] H. Raji, M. Tayyab, J. Sui, S.R. Mahmoodi, Biosensors and Machine Learning for Enhanced Detection, Stratification, and Classification of Cells: A Review, (n.d.) 25.
- [154] X. Huang, J. Guo, X. Wang, M. Yan, Y. Kang, H. Yu, A contact-imaging based microfluidic cytometer with machine-learning for single-frame super-resolution processing, *PLoS One* 9 (2014) 104539, <https://doi.org/10.1371/journal.pone.0104539>
- [155] H. Hashemzadeh, S. ShojaeiLangari, A. Allahverdi, M. Rothbauer, P. Ertl, H. Naderi-Manesh, A combined microfluidic deep learning approach for lung cancer cell high throughput screening toward automatic cancer screening applications, *Sci. Rep.* 11 (2021) 9804, <https://doi.org/10.1038/s41598-021-89352-8>
- [156] B. Turan, T. Masuda, W. Lei, A.M. Noor, K. Horio, T.I. Saito, Y. Miyata, F. Arai, A pillar-based microfluidic chip for detection of T-cells and B-cells using machine learning, 2018 International Symposium on Micro-NanoMechtronics and Human Science (MHS), IEEE, Nagoya, Japan, 2018, pp. 1–3, <https://doi.org/10.1109/MHS.2018.8886964>
- [157] M. Senen, G. Whyte, Image-based single cell sorting automation in droplet microfluidics, *Sci. Rep.* 10 (2020) 8736, <https://doi.org/10.1038/s41598-020-65483-2>
- [158] C.A. LaBelle, A. Massaro, B. Cortés-Llanos, C.E. Sims, N.L. Allbritton, Image-based live cell sorting, *Trends Biotechnol.* 39 (2021) 613–623, <https://doi.org/10.1016/j.tibtech.2020.10.006>
- [159] K. Lee, S.-E. Kim, J. Doh, K. Kim, W.K. Chung, User-friendly image-activated microfluidic cell sorting technique using an optimized, fast deep learning algorithm, *Lab Chip.* 21 (2021) 1798–1810, <https://doi.org/10.1039/DOLC00747A>
- [160] A. Chu, D. Nguyen, S.S. Talathi, A.C. Wilson, C. Ye, W.L. Smith, A.D. Kaplan, E.B. Duoss, J.K. Stolaroff, B. Giera, Automated detection and sorting of micro-encapsulation via machine learning, *Lab Chip.* 19 (2019) 1808–1817, <https://doi.org/10.1039/C8LC01394B>
- [161] V. Anagnostidis, B. Sherlock, J. Metz, P. Mair, F. Hollfelder, F. Gielen, Deep learning guided image-based droplet sorting for on-demand selection and analysis of single cells and 3D cell cultures, *Lab Chip.* 20 (2020) 889–900, <https://doi.org/10.1039/DOLC00055H>
- [162] T.-C. Liang, Z. Zhong, Y. Bigdeli, T.-Y. Ho, K. Chakrabarty, R. Fair, Adaptive Droplet Routing in Digital Microfluidic Biochips Using Deep Reinforcement Learning, (n.d.) 11.
- [163] C. Svensson, O. Shvydkiv, S. Dietrich, L. Mahler, T. Weber, M. Choudhary, M. Tovar, M.T. Figge, M. Roth, Coding of experimental conditions in microfluidic droplet assays using colored beads and machine learning supported image analysis, *Small* (2018) 1802384, <https://doi.org/10.1002/smll.201802384>
- [164] S. Momtahan, F. Al-Obaidy, Farah Mohammadi, Machine learning with digital microfluidics for drug discovery and development, 2019 IEEE Canadian Conference of Electrical and Computer Engineering (CCECE), IEEE, Edmonton, AB, Canada, 2019, pp. 1–6, <https://doi.org/10.1109/CCECE.2019.8861842>
- [165] K. Rajesh, A. Tirkey, A. Sarkar, S. Pyne, Reinforcement learning based droplet routing algorithm for digital microfluidic biochips, 2020 24th International Symposium on VLSI Design and Test (VDATE), IEEE, Bhubaneswar, India, 2020, pp. 1–6, <https://doi.org/10.1109/VDATE50263.2020.9190306>

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