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Microtechnologies Enable Cytogenetics

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1. Introduction

In this Chapter the standard cytogenetic methods are shortly introduced. Furthermore, the existing microtechnologies that improve the cytogenetic analysis are thoroughly described and discussed.

1.1 Traditional and molecular cytogenetics

Cytogenetic analysis is an important tool in pre- and postnatal diagnosis as well as cancer detection. In a traditional cytogenetic technique known as karyotyping the metaphase chromosome spreads are prepared on a glass slide and stained with a Giemsa stain. The stain reveals a specific banding pattern for each chromosome – a chromosome bar code. Karyotyping is often supplemented by the molecular cytogenetic technique Fluorescent *In Situ* hybridization (FISH), which requires the use of fluorescently labeled DNA probes to target a specific chromosome region. In FISH the chromosome preparations (metaphase spreads or interphase nuclei) are heat denatured, followed by application of the probe and hybridization at 37 °C. FISH can be performed on interphase nuclei on non-cultured cells in less than 24 hrs, but the chromosome structure cannot be visualized. On the other hand, metaphase FISH has the advantage of visualizing the entire karyotype at once and can detect potential abnormalities at a high resolution. But, the long analysis time and culturing required for metaphase FISH are important disadvantages.

Recently, a common DNA analysis, such as PCR amplification of a specific DNA region gained more popularity. Such analysis is beneficial as it can be performed on non-cultured cells, providing the results within a few days. Even though DNA techniques hinder the evolution of FISH, it can still provide valuable information on abnormalities, enabling detection of complex chromosome rearrangements. Nevertheless, FISH is now rarely used as the first step in cytogenetic analysis, due to the high cost of the probes, need for skilled technicians and lengthy analysis protocol. However, the use of microdevices for FISH could reestablish the status of this technique as an important tool for high resolution detection of chromosome abnormalities.

The major drawback of the FISH is the long analysis protocol. To perform a complete FISH analysis, even well trained technicians spend several hours in sample preparation as well as the waiting time in between each pre- and post- hybridization washes. There are at least 12-

15 different washes in a standard routine test that in total takes about 45 minutes. Apart from the cell culture work, the hybridization process is very time consuming. At minimum, performing a FISH analysis with centromeric probes (repetitive sequences), will take 2 to 3 hours. Furthermore, in some FISH experiments the hybridization of a probe requires overnight incubation. Another bottleneck of FISH analysis is the cost of the reagents used for the assays, mainly the fluorescent probes. In standard lab protocols 10-15 μl of probe are used per slide containing metaphase spreads or interphase nuclei (Jiang & Katz, 2002). Such analysis is normally performed on a single patient sample, thus the cost of a single analysis is extremely high, as 10 μl of probe cost 100 $. The development of a high throughput device for metaphase or interphase FISH analysis benefits from reduced probe volume per single sample, at the same time reducing the cost per diagnosis. Also, addressing the need for reduction in probe volume for single analysis can greatly increase the application of FISH in routine clinical diagnostics. Moreover, other standard cytogenetic analysis methods, such as karyotyping, also lack the automation. The introduction of automated microfluidic assays for cytogenetic analysis can offer more thorough and routine diagnosis that can be performed in the doctor's office at a lower cost and shorter time.

1.2 Microtechnologies in the cytogenetic field

Traditional cytogenetic analysis has evolved from karyotyping, through FISH techniques, Comparative Genomic Hybridisation (CGH), towards DNA microarrays. A few years ago, a routine chromosome test was carried out by culturing of the patients' blood sample followed by karyotyping, thorough banding analysis and validation by FISH. Nowadays, the first step performed in cytogenetics labs is often a CGH array, which is a genome wide DNA microarray that enables detection of deletions and duplications. It allows for assessment of the chromosome disorders by targeting multiple chromosome regions at once. At first the cytogenetic society was skeptical about their use; however their popularity has gradually increased over the years. Owing to that, microtechnologies gained trust in the cytogenetic scientific community and are now widely accepted.

Unlike microarrays, FISH can only detect few DNA regions in a single experiment, but introducing new microfabricated assays for interphase and metaphase FISH can greatly increase the use of such analysis. However, it should be noted that before these devices will reach cytogenetic laboratories all over the world they need to be tested for high quality and reproducibility of results.

In recent years the integration and automation of cytogenetic techniques has gained more attention. Most reports in this field focus on the development of an integrated microfluidic chip for interphase and metaphase FISH analysis. There are also some reports on the cell culture systems for suspension cells required for cytogenetic analysis. The recent developments in the field of microcytogenetics that address the need for automation, time and cost reduction in chromosome analysis are described here. Moreover, the commercially available machines and assays are also presented.

1.3 Cytogenetic analysis

A typical procedure for cytogenetic analysis is shown in **Figure 1**. The blood sample is collected from the patient and cultured for 3 days. The culturing step normally performed in

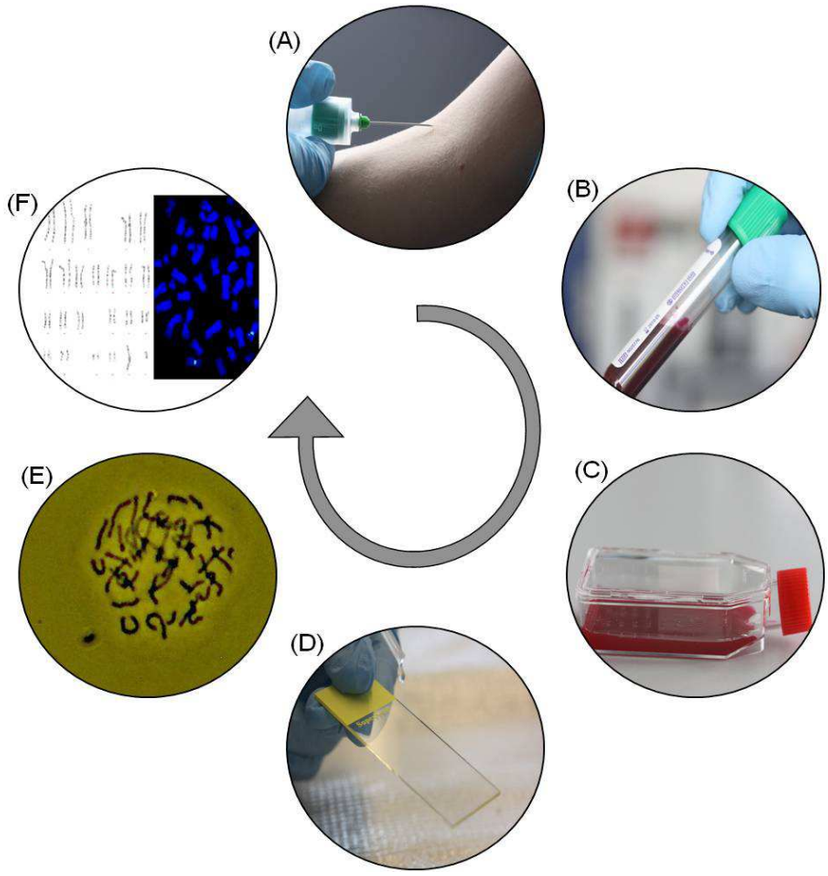


Fig. 1. The cytogenetic analysis starts with culturing of a blood sample, further fixation and splashing, followed by metaphase FISH or karyotyping.

culture flasks is prone to miniaturization and automation. The microdevices for culturing of suspension cells are presented in the following section. The next step requires harvesting of the chromosomes and chromosome spreads preparation on a glass slide. It is traditionally performed manually by dropping a cell suspension on a glass slide. Some machines and microdevices exist, which enable automation of the process. Finally, the analysis of chromosome glass slides is performed by either karyotyping or FISH. The conventional analysis requires the use of coplin jars for washing and incubation steps and usually uses high volumes of expensive reagents such as fluorescent probes. This traditional analysis protocol is far from automated but some examples of the microcytogenetics devices are available and described in this chapter.

2. Microchips for cytogenetic analysis

In this section we describe all available microdevices that enable performing a cytogenetic analysis. Firstly, the microfluidic bioreactors for culturing of suspension cells required for some protocols such as metaphase FISH or karyotyping. The main advantage of the presented cell culture microdevices is the ease of exchanging the liquids, i.e. from cell culture medium to hypotonic solution and finally fixative. All the laborious centrifugation steps can be omitted, which results in a higher yield of cells for the analysis. Further, the

existing machines for metaphase chromosome spreads preparations are described. Lastly, the existing chips for performing miniaturized FISH are presented; with examples of FISH results obtained using these devices.

2.1 Sample preparation: Expansion, arrest and fixation of chromosomes

Cell culture is the primary step performed on patient samples containing lymphocytes, before they undergo metaphase FISH and karyotyping. The primary purpose of this cell culture is to ensure sample expansion and to perform sample preprocessing steps like hypotonic state inducement and arrest of the lymphocytes. Some of the cytogenetic laboratories perform the culture without separation of lymphocytes from red blood cells and plasma, but others prefer to culture lymphocytes purified by centrifugation with Ficoll-Paque®. Normally, the cells are cultured in culture flasks or tubes for 72 hrs in RPMI medium in a humidified incubator at 37 °C and 5 % CO_2. The disadvantage of such a culture method is the large volume of medium used and the fact that handling of suspension cells is tedious. Moreover, the traditional cell culture increases the risk of contamination due to manual sample handling.

To automate this process and reduce the contamination risk and volume of the medium a microfluidic device needs to be considered. Microfluidics-based cell culture devices due to small spatial dimensions have the promising prospect of providing cells with an *in vivo* like microenvironment in microchips. Moreover, for cells which are typically perfused actively via the vascular network, a perfusion based culture system provides a much better alternative to the standard static Petri dish-based cultures. By actively controlling the microenvironments surrounding the cells, we can exert greater control on the cell-cell interaction, the supply of nutrients to the cells and actively remove the biological waste (Kim et al., 2007).

Typically, shear stress applied to the cells is a major issue in case of microfluidic cell cultures and in case of suspension cell cultures the major challenge is to retain the cells in the system. A simple cell culture chip suitable for suspension cells was presented by Liu et al., (2008). They have fabricated a microfluidic device with minimum shear stress. The device was fabricated in two layers of PDMS bonded to a clean glass slide. The device consists of a main channel and side chambers for cell culturing. The medium supply and waste removal is achieved by convective and diffusive mass transport. The culturing of T-lymphocytes was demonstrated without cell losses due to shear stress. The main advantage of this design is that the cell medium perfusion can be started immediately after injection of cells, as the shear stress is too low to remove the cells. The main drawback of this device was the difficulty in extracting the cells for subsequent analysis post culture.

Recently, membrane based microfluidic bioreactors addressing all these issues and providing a simpler protocol for preparation of chromosome spreads were developed (Shah et al., 2011a; Svendsen et al., 2011). The proposed diffusion based microreactor (**Figure 2**) facilitates culturing of lymphocytes but also expansion, hypotonic treatment, and fixation of cells with the possibility to avoid several tedious centrifugation steps (Svendsen et al., 2011). Svendsen et al. developed a membrane based bioreactor for culturing a suspension of cells above the membrane with a microfluidic channel for media perfusion from the bottom. The cell culture in the bioreactor is performed for 72 hrs on lymphocytes purified from the

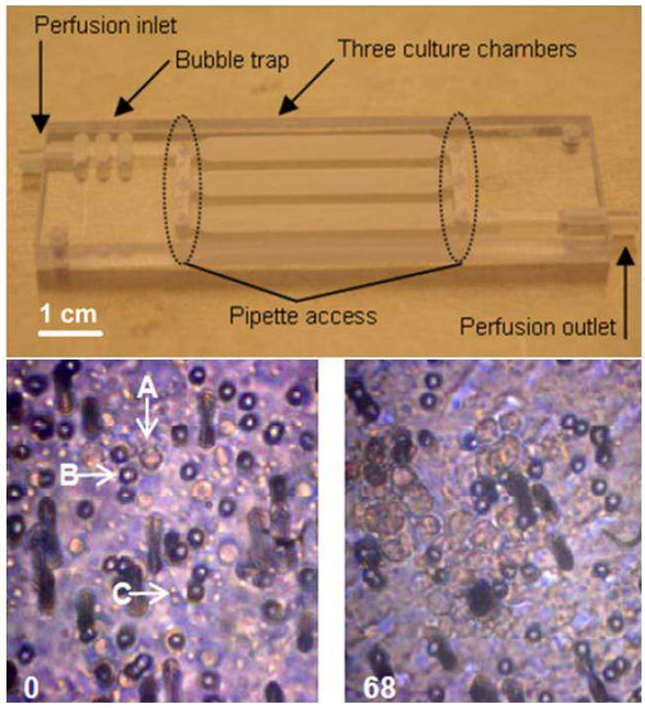


Fig. 2. The membrane based cell culture bioreactor for suspension cell culture. The two pictures below show a bright field image of the cells on the porous membrane at time 0 and after 68h of culturing. At the beginning the cells (A) are just slightly bigger than the pores (B). Platelets (C) can also be observed on the membrane (Reprinted with permission from Svendsen et al., 2011).

blood. The bioreactor was designed to provide pipette-based seeding and unloading of the cells. This ensures easy adaptability for the technicians who are familiar with pipette based traditional techniques. The inlet/outlet ports were sealed using a PCR tape during the cell culture. This is the only step, which requires manual operation of the device; all further steps are performed on a closed chip, which reduces contamination risk. The continuous perfusion of the medium ensures that all the necessary nutrients are delivered to the cells via diffusion from the membrane. It enables fast solution changes for expansion and cell fixation to obtain high quality metaphase spreads. Separation of the culture chamber by a membrane from the perfusion channel is also helpful to protect the cells from air bubbles formed in the flowing medium and allows the perfusion to be started even before the cells settle on the membrane. Svendsen et al. concluded that the cell growth inside the bioreactor was comparable to the control sample with the cells grown in a well-plate. However, the authors have not tested whether the culturing time can be reduced by means of this microfluidic bioreactor.

Shah et al. modified the media perfusion channel to ensure more thorough transport of nutrients across the membrane to the resting cells (Shah et al., 2011a). The pipette accesses were changed into microfluidic inlet and outlet ports connected to 3-port valves which allowed for easy removal of bubbles and change of medium (**Figure 3**). Shah et al. for the first time demonstrated that cell proliferation on the chip is better than in the control experiment on a Petri dish culture (**Figure 3B**).

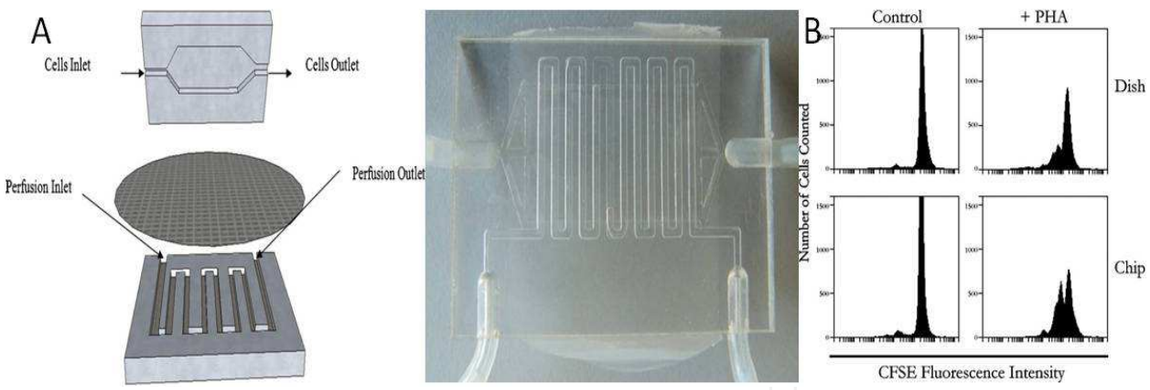


Fig. 3. A-The modified membrane based cell culture device with a perfusion channel that ensures better nutrients delivery to the cells. B-A graph showing proliferation of the CFSE stained cells in the device and on a Petri dish, control experiments without PHA stimulation (Reprinted with permission from Shah et al., 2011a).

One of the further steps required for cytogenetic analysis is harvesting of metaphase cells by hypotonic treatment followed by fixative addition. All these steps are traditionally performed by a series of centrifugation steps, which enables exchange of the solutions. The cultured cells are first centrifuged to remove the cell culture medium and to change the solution to hypotonic buffer. Such a treatment ensures cell swelling necessary for breaking of the membrane during preparation of chromosome spreads on slides. In order to make the membrane permeable the fixative, which is a mixture of methanol:acetic acid in a 3:1 ratio, is added to the pellet after centrifugation; this procedure is repeated up to 3 times. Harvesting of the metaphase chromosomes requires several laborious centrifugation steps, which have to be performed manually by a technician. A company called Transgenomic addressed this issue by introducing Hanabi-PII Metaphase Chromosome Harvester macromachine, which enables the steps to be performed automatically with more consistent results. Also, the presented microfluidic cell culture devices enable easy swelling and fixation of the cells. By simple change in the solution that is perfused below the membrane the hypotonic treatment and further fixation of the cells is performed on chip, in steps of 25 min. This is a simple and a very effective way of reducing the need for trained technicians and opens a possibility of performing a point-of-care analysis.

2.2 Chromosome spreads preparation

For reliable results cytogenetic analysis needs to be performed on a high quality sample. One of the very important steps in the cytogenetic analysis is the preparation of the high quality chromosome spreads on the glass slide. The technique, which is often used, is traditionally called 'splashing'. It is performed manually by skilled technicians and is greatly dependent on the environmental conditions such as temperature or humidity. Many cytogenetic laboratories have designated conditioned rooms to ensure non varying conditions for spreads preparation.

A traditional way of slides preparation varies from lab to lab. However, the splashing is commonly performed on glass slides that are kept, prior to the experiment, in a water container in a fridge to ensure proper wetting of the surface. A single use plastic Pasteur

pipette is used to collect the cells in the fixative, which are further dropped on a tilted glass slide. The excessive liquid is drained on a tissue paper by placing the slide on the side. Afterwards, the slides are dried on a hot plate to facilitate evaporation of the fixative, which in turn enables spread formation.

There are a lot of factors that are presumably affecting the chromosome spreading process. In recent years, there has been considerable interest in fathoming the underlying process of chromosome spreading (Chattopadhyay et al., 1992; Gibas et al., 1987; Sasai et al., 1996). These explorations have also resulted in a number of devices aimed at automating the chromosome spreading protocol (Henegariu et al., 2001; Qu et al. 2008; Yamada et al., 2008). The most important environmental conditions for obtaining high quality spreads are temperature and humidity. These are often controlled by special humidity chambers with a temperature control. Another factor is a proper technique for slides ageing. The most common method is a simple air drying for 2 days, which helps the visualization of chromosome structures. Another technique involves dry-heat and chemical ageing, the latter being beneficial for FISH analysis, as it results in better signals and preserves chromosome architecture (Claussen et al., 2002, Henegariu et al., 2001; Rønne, 1989). Another factor suspected of affecting chromosome spreads quality is the dropping height, but Claussen et al. claim it is not essential. Last but not least, the presence of a thin water layer on the slide before dropping the cell suspension induces cell swelling resulting in better spreads.

Air drying is difficult to achieve in a completely closed microsystem, as the rate of fixative evaporation is crucial for good chromosome spreading. Based on this Vedarethinam et al. fabricated a splashing device, which contains two channels, one for ice cold water and one for cell suspension dropping (**Figure 4**). The injection of these solutions can be done manually, but is amenable for automation by use of syringe pumps. The system is designed to have a fixed dropping height and enables introduction of a thin water layer to the slide.

A ZenTech company produces the ZENDROPPER® macromachine, which is used for preparation of chromosome spreads with high reproducibility. Moreover, it enables high

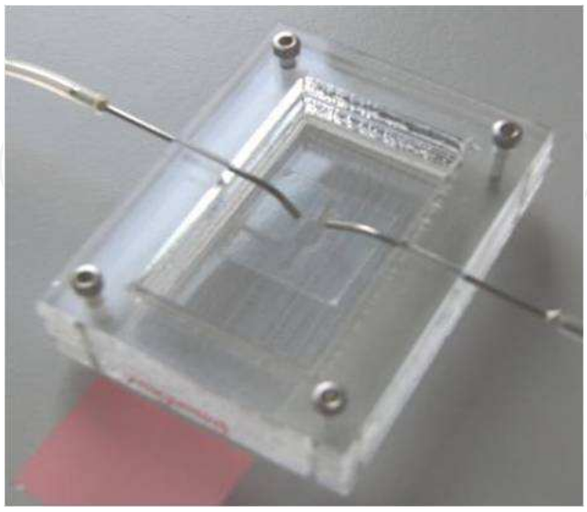


Fig. 4. A splashing microdevice for chromosome spreads preparation (Reprinted with permission from Vedarethinam et al., 2010)

throughput of 40 slides in 40 minutes preparation, which is essential for routine diagnostics laboratories. It allows for variations in the splashing height, but assures proper environmental conditions. However, the price and the size of this equipment prevent its common use in the laboratory, but anyone can benefit from the small sizes of the microdevices.

2.3 Integrated culturing, harvesting and splashing device

Recently, Shah et al. described a novel microfluidic FISHprep device for metaphase FISH slides preparation (**Figure 5**). The device combines the bioreactor for cell culturing (Shah et al., 2011a) with the splashing device for preparation of the chromosome spreads (Vedarethinam et al., 2010). This device consists of a diffusion based cell culture reactor separated from the splashing device by a clip valve. In this device, a 72 hr culture of T-lymphocytes with a CFSE staining was performed to determine the proliferation rate. Further, by means of addition of a clip valve which can be opened after the culture, the cultured and fixed lymphocytes can be splashed on the glass slide. The quality of spreads obtained from the FISHprep was comparable to traditionally obtained spreads. The integration of all the steps required for successful metaphase FISH slide preparation in one FISHprep offers a possibility for an automation of the molecular cytogenetics in future (Shah et al., 2011b).

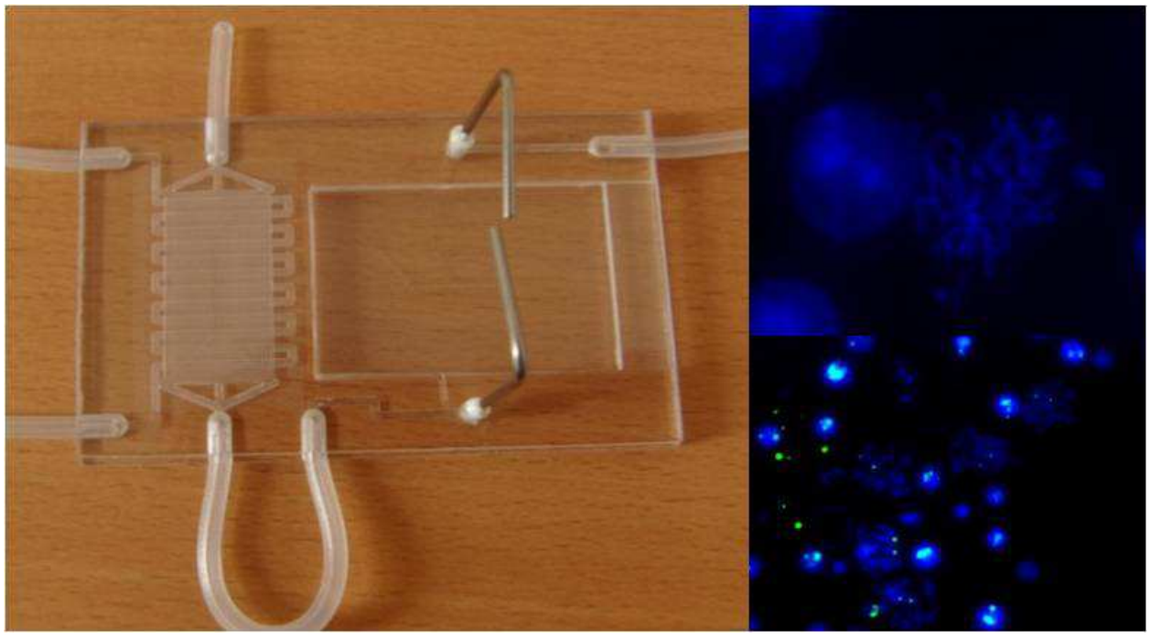


Fig. 5. Integrated cell culture chamber with a splashing device for preparation of the chromosome spreads for FISH analysis (Reprinted with permission from Shah et al., 2011b)

2.4 FISH on chip

In this subsection all commercially available microchips for FISH are introduced. Moreover, examples of the developed devices from the literature are also presented here. These microdevices address the need for probe volume reduction. Some of them miniaturized the

interphase FISH protocol, but a few allow for metaphase chromosomes analysis. One of the interphase chip examples, tried to reduce the hybridization time, thus addressing the issue of long analysis time.

2.4.1 Cytocell multiprobe haematology

One of the very first miniaturized assays available on the market was provided by CytoCell. The devices called Chromoprobe Multiprobe Haematology are designed for FISH analysis of interphase and metaphase chromosomes from cultured peripheral blood cells or bone marrow samples. The Chromoprobe technology uses DNA FISH probes that are reversibly bound to the glass slide. During the experiment the probes dissolve in the solution of hybridization buffer and are readily hybridized to the chromosome preparations. The required denaturation of both chromosome preparations on the slide and probe in the solution occurs in the device itself during heating. This system allows multiple FISH probes to be hybridized to the same chromosome preparation, which enables rapid screening for multiple abnormalities in a single experiment. Such a solution makes FISH analysis easier and quicker, however as the slide is divided into small squares with different immobilized probes it requires troublesome analysis and counting of the results.

2.4.2 BioCellChip

In 2007, Lee et al., presented one of the first examples of miniaturized devices for performing FISH analysis (Lee et al. 2007). This device in **Figure 6** is suitable for interphase FISH, allowing high throughput analysis on a cell array. The authors have shown the possibility to array cells on the glass slide by spotting small amounts of samples onto a supporting PDMS matrix. The chip consists of a patterned glass slide with bonded 1 mm perforated PDMS layer. The glass slide and PDMS lid form an array of 96 cavities of 1.5 mm in diameter for cell spotting. The patterning of a glass slide with 96 squares and numbers for indexing of wells was achieved by a photolithography and thin-film metal deposition process. The PDMS layer was only used for a controlled cell sampling on the glass slide, mainly to stop the cell samples from spreading all over the glass slide. After air drying, the conventional FISH protocol was performed on a glass slide, without the supporting PDMS layer. The main advantage of this device is that for 96 different specimens only 10 μl of the probe can be used. Such a microfabricated bio-cell chip with a PDMS layer is useful in mass screening of microdeletions or aneuploidy with the same probe used for detection of chromosome abnormalities. The authors have mentioned the probe volume reduction used to analyze 96 specimens in one experiment, but other major issues such as manual intervention and time consumption were not stated. Moreover, it is discussed that PDMS could be replaced by cement or paper stickers, to create the spotting chambers and physically separate them.

2.4.3 Interphase FISH on chip

The first demonstration of FISH on chip was showed by Sieben, et al. (2007). They have adapted a conventional interphase FISH protocol to a miniaturized version (**Figure 7**). One of the few differences was immobilization of cells, which was achieved by temperature treatment. Moreover, the authors explored the methods of hybridization time reduction by recirculation of the probe by means of on-chip peristaltic pumps and electro-kinetic transport by external electrodes inserted in the end wells. It was shown that electro-kinetic

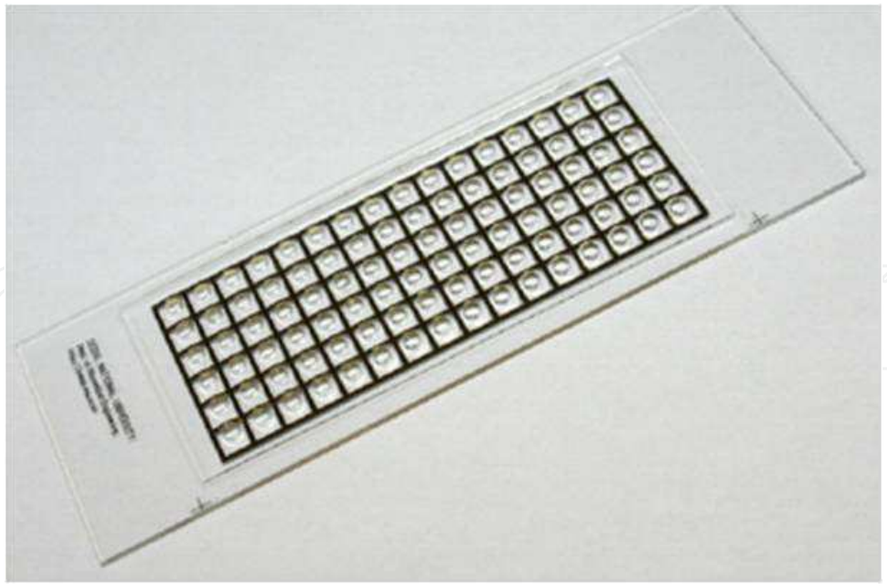


Fig. 6. BioCellChip enabling high throughput analysis of several cell samples (Reprinted with permission from Lee et al., 2007)

probe circulation, which is easy to integrate into the design, performed better than the recirculation. The recirculation method involved a use of on chip valves and pumps, which require complex microfabrication method. Nonetheless, by performing FISH on-chip at microfluidic volumes, they were able to achieve a tenfold reduction in DNA probe consumption per test, with corresponding reduction of a single experiment cost. In 2008,

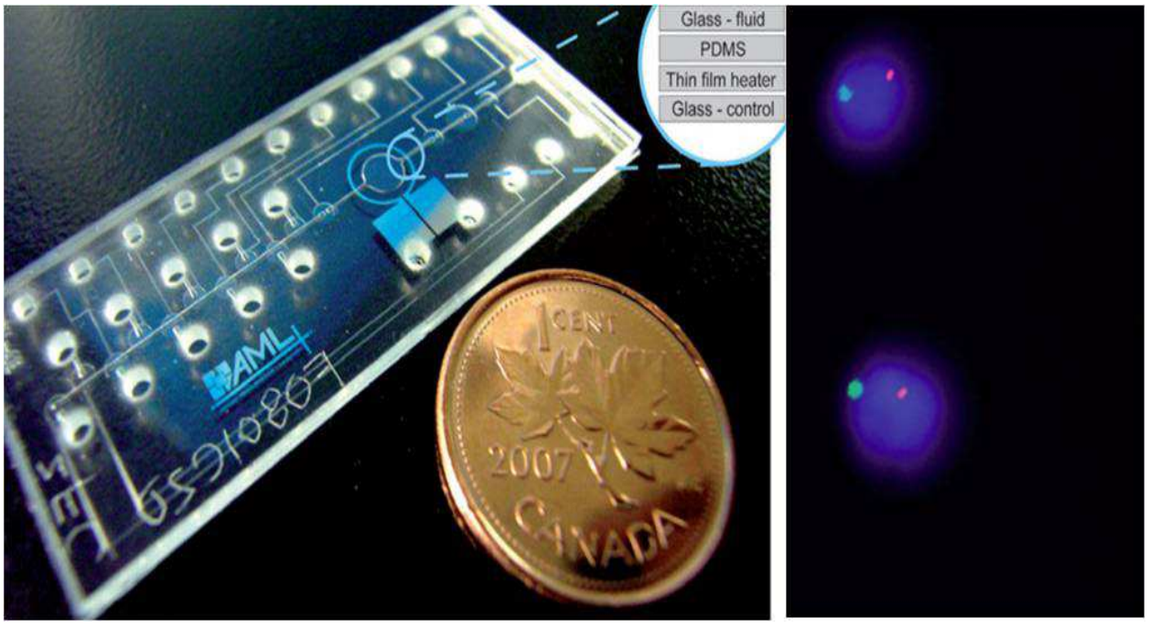


Fig. 7. Interphase FISH on CHIP with integrated mixing elements to enhance the hybridization. The FISH results showing a male sample with one X and Y chromosomes (Reprinted from Sieben et al., 2008 with permission of The Royal Society of Chemistry)

Sieben, et al., published another paper in which all previously performed steps with microfluidic FISH were integrated onto one chip and automated using valves and a computer controlled peristaltic pump (Sieben et al., 2008). Whereas conventional FISH takes nearly an hour of on-and-off attention from a skilled technician, using their time inefficiently, automated microfluidic FISH reduced human intervention to only involve reagent preparation, and results analysis with fluorescence microscope. However, the chip design is very complex and technicians would need additional training to be able to use the device.

2.4.4 Miniaturized interphase FISH

The most recent example of an interphase FISH on chip was presented by Zanardi et al., (2010). They presented a miniaturization of the interphase FISH protocol through microfluidic methods with minimized shear stress exerted on cells (living or fixed) during sample loading. The authors focused on the use of biomaterials and coating to promote cell adhesion, mainly nanostructured TiO_2 coating that triggers a rapid and efficient cell immobilization.

The microfluidic device consists of a PDMS pad with a straight 0.15 µl microchannel with an inlet and outlet that are accessible with an automatic pipette tip. The PDMS pad was manually bonded to a glass slide previously coated with 50 nm of TiO_2 and treated with oxygen plasma to increase hydrophilicity. The coating was achieved by means of cleanroom cluster beam technology, which greatly increases the device fabrication costs. Sample loading into the device was achieved by simply pipetting 1.5 µl of cell sample into the inlet hole and left to enter the channel by capillary force. The entire FISH protocol was performed in the microfluidic device, by passing all the necessary solutions through the channel. After injection of the probe, the inlet and outlet holes were sealed with a drop of mineral oil for overnight probe hybridization to prevent the evaporation. Post hybridization washing steps were performed in a conventional ways on the TiO_2 coated slide without a PDMS pad. The device was fully validated using various clinical samples to analyze chromosome abnormalities. The device performance was compared to that of the conventional method. The miniaturized version of FISH provided accurate, high quality, reproducible results as compared to standard FISH protocol. The main advantage of this microchip is its ease of use and reduction in the probe volume used (10- to 30-fold less probe compared to the standard protocols). Moreover, due to the confinement of cell sample in a relatively small area, the image analysis can be performed rapidly in less than 8 minutes, which is 5 times faster than image analysis after conventional FISH. The device gained attention in the cytogenetic field and is now available commercially as the product microFIND research® from the TETHIS S.p.A. company. It enhances cell retention and a uniform distribution of the cells inside the channel, reducing the reagents volume up to 20 times.

2.4.5 Metaphase FISH on chip

The first implementation of a chip for metaphase FISH analysis was presented by Vedarethinam et al., (2010). Metaphase spreads formation is based on the hydrophilicity of the substrate, a proper rate of evaporation, temperature gradient, humidity and controlled splashing angle, which is difficult to achieve in a closed chip (Deng et al., 2003; Henegariu et al., 2001). The authors have presented a novel splashing device with an open chamber, which is used for dropping the fixed cell sample and ice cold water on the glass slide. In this way the

evaporation is easily controlled and the chromosome spreads achieved by the splashing device are comparable to those achieved by the conventional method. By placing a double adhesive tape stencil on the glass slide the confinement of the metaphase spreads was achieved. The adhesive tape serves as a bonding support for mounting a PDMS lid with two microchannels and a reaction chamber (**Figure 8**). Such a rapid and easy protocol for the microFISH device allows for a quick transformation of a simple glass slide into a metaphase FISH on chip assay. All steps in a conventional metaphase FISH protocol, such as washing, dehydration, probe injection, were performed on the chip. The authors focused mainly on the miniaturization of a conventional protocol, without introducing any innovation to the existing method. The advantage of the microFISH device over traditional method is the 2-fold reduction in the probe volume used, which reduces the cost of the analysis. The presented device is a good solution for integration into existing work routines in cytogenetic labs. Moreover, it is amenable for automation by preloading of the reagents into tubings. The reagents preloading into tubings was tested for a further reduction of a probe volume showing some promising results (unpublished data), which can allow for a widespread use of the microFISH device.

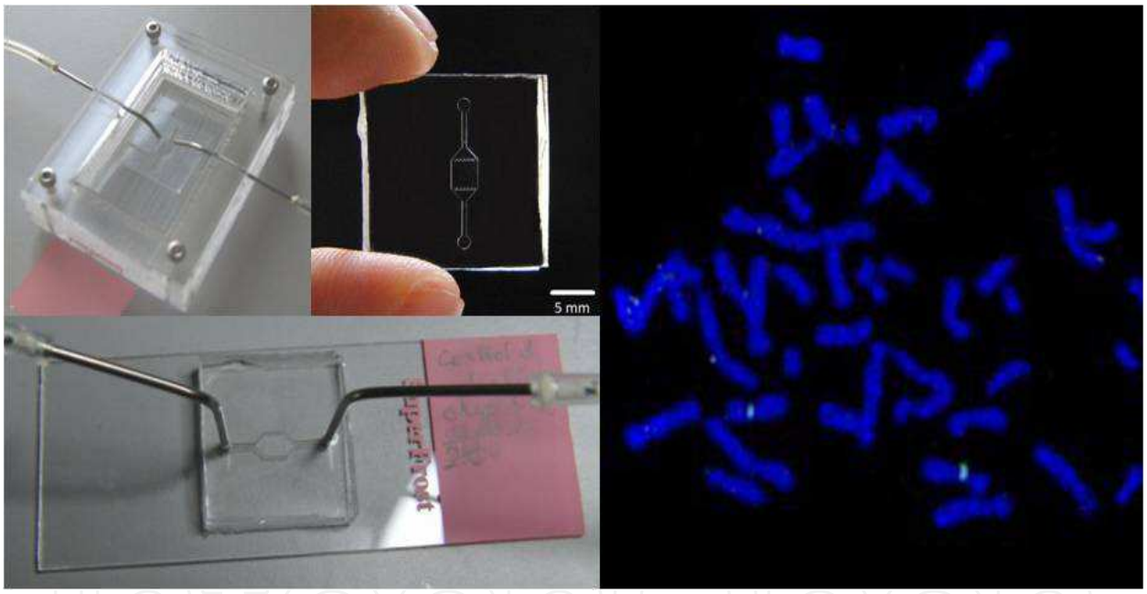


Fig. 8. Metaphase FISH on chip with an open splashing device to enable spread formation. The PDMS lid consists of two channels with a chamber for performing FISH. On the right FISH results with X centromeric probe inside the chip are shown (Reprinted with permission from Vedarethinam et al., 2010).

2.5 Chromosome sorting on chip

The cytogenetic analysis often reveals the chromosome changes present in the sample. Very often the resolution of the techniques is not sufficient to determine the small changes. Cytogenetists often need to obtain the derivative chromosomes carrying the abnormalities for further testing by e.g. next generation sequencing or for recombinant DNA libraries construction. One of the most well known techniques is the chromosome sorting by flow cytometer. However, many disadvantages such as need for high sample volume, lack of

precision and poor reproducibility, have hindered its common use. Moreover, the flow sorters are expensive and require skilled technicians to operate the machines.

Recently, an attempt to replace the traditional chromosomes flow sorter with a microdevice has been reported by Inoue et al., (2008). The authors presented two methods suitable for manipulating the chromosomes – by electric and magnetic fields. The voltage modulation was suggested to replace the conventional gel electrophoresis for chromosomes sorting. The mobility of the chromosomes depends on their size when placed in a time-dependent electric field, with the smallest chromosomes moving faster. As the chromosomes size distribution is very large, the authors classified them into three size based groups. They proved that during 30 minutes experiment in the device the chromosomes have been sorted into these 3 groups, however, high sample density resulted in clogging.

The other presented method requires use of a magnetic field. The authors fabricated a device for continuous flow sorting of chromosomes, with high throughput and high operation speed. The chromosomes labelled with superparamagnetic beads are dragged by a magnetic field against the laminar flow stream and are collected at separate outlets. The force is proportional to the amount of magnetic beads attached to the chromosomes, which in turn depends on the chromosome size. The deflection of larger chromosomes is bigger than that for small chromosomes. Further studies are conducted to improve the chromosome sorting accuracy and throughput by means of the microfluidic device.

3. Future prospects

While cytogenetic analysis continues to be cumbersome, manual and expensive, the recent advances in the microtechnologies enabling cytogenetics offer a new direction and hope for optimizing the protocols and making them simpler, cheaper and available on a wider scale. The successful demonstrations of devices for the various steps involved in the cytogenetic procedures need to now be tested and validated clinically before they can be implemented on a routine basis. The microfluidic bioreactors have proved superior for application in suspension cell cultures, which opens up possibilities for utilizing them for culture of amniocytes or chronic villi, which will speed up the prenatal diagnosis protocols. The device for metaphase FISH offers good prospects for automating the sequential FISH sample injection protocol leading to automated FISH. In the distant future, greater benefits of using microtechnologies for cytogenetics will be reaped when these assays will be automated and multiplexed; and be able to process multiple samples simultaneously.

4. Conclusion

Cytogenetic analysis performed nowadays is a laborious, technically demanding and long process, starting from the culturing of lymphocytes from patient sample through preparation of chromosome spreads to FISH or karyotyping analysis. All these steps are performed manually by skilled technicians; however, to perform the test routinely in the doctor's office there is a need for automation and simplification of the protocol. One of the main drawbacks of FISH is its complexity and price of the probes, which hinders the usage of this technique. Owing to that, DNA based techniques are gaining more popularity, and are nowadays the first analysis step performed routinely in cytogenetic laboratories. FISH is now just a supplementary technique used for validation of the results. Although, some

Microtechnology in Cytogenetic Analysis		
Sample preparation	Liu et al., 2008	*Minimal shear stress cell culture device* Cell culture chambers on a side of the main channel Convective and diffusive nutrient supply Shear stress minimized Difficult cell extraction
	Svendsen et al., 2010	*Membrane cell culturing bioreactor* Adherent and Non-Adherent cells Pipette access holes for cell loading/unloading Cell culture separated from the medium perfusion channel Culture time – 72 hrs Continuous diffusion based perfusion Easy solution changes
	Shah et al., 2011a&b	*Microfluidic Bioreactor for cell culture* *In vivo* like environment Cell culture separated from the medium perfusion channel Culture time – 72 hrs Continuous diffusion based perfusion Better cell growth Easy operation Easy cell retrival Easy solution changes
Glass Slide Preparation	Vedarethinam et al., 2010, Shah et al., 2011b	*Splashing device* Cold water and cell inlets Reliable metaphase spreads Suitable for automation Easy operation
FISH	CytoCell	*Multiprobe Haematology* Interphase/metaphase FISH Sample spotting on a glass slide Probes reversibly bound to the glass slide Conventional washing steps
	Lee et al., 2008	*BioCellChip* Interphase/metaphase FISH 96 cell samples spotted using PDMS stencil Conventional washing steps Conventional hybridization protocol Probe volume - 10 µl Probe volume reduction 10 ul for 96 samples High throughput
	Sieben et al., 2007&2008	*Interphase FISH on chip* Interphase FISH Probe volume reduction 10- to 20-fold Automated protocol Complicated design Expensive fabrication

Microtechnology in Cytogenetic Analysis		
	Zanardi et al., 2010	*Miniaturized interphase FISH* Interphase FISH Cell immobilization on TiO_2 coated glass slide PDMS channel lid Probe volume – 0.3 µl Probe volume reduction – 10- to 30- fold Reduced analysis time Suitable for automation and high throughput
	Vedarethinam et al., 2010	*Metaphase FISH on chip* Interphase/metaphase FISH Glass slide preparation using splashing device PDMS channel lid Probe volume – 5 µl Probe volume reduction – 2 fold Suitable for automation and high-throughput Inexpensieve

Table 1. A summary of available microdevices for cytogenetic analysis

machines for FISH protocol automation exist, their cost and size prevent their usage in non-laboratory settings. Table 1 summarizes the advantages and disadvantages of the microdevices available for cytogenetic analysis described in this chapter.

These microdevices and assays can be easily incorporated in the routine cytogenetic lab environment, but can also be available for point of care diagnostics. The cost of such assays is greatly reduced, due to smaller volumes of probe used. Finally, each step of the protocol can be transformed to a microdevice format allowing a total analysis of chromosomes to be done on a microplatform. Such a miniaturization of a protocol enables high-throughput analysis of several samples at the same time and could drastically speed up the process to provide fast results. Moreover, the manipulation of the probe across the chromosome spreads inside microfluidic devices could reduce the hybridization time down to minutes.

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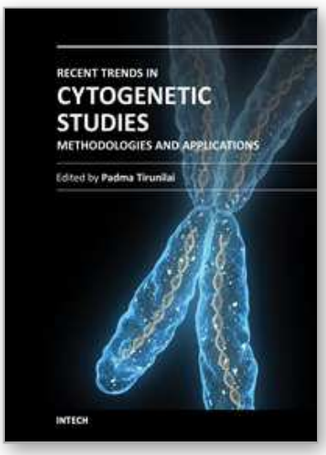
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Recent Trends in Cytogenetic Studies - Methodologies and Applications

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Recent Trends in Cytogenetic Studies - Methodologies and Applications deals with recent trends in cytogenetics with minute details of methodologies that can be adopted in clinical laboratories. The chapters deal with basic methods of primary cultures, cell lines and their applications; microtechnologies and automations; array CGH for the diagnosis of fetal conditions; approaches to acute lymphoblastic and myeloblastic leukemias in patients and survivors of atomic bomb exposure; use of digital image technology and using chromosomes as tools to discover biodiversity. While concentrating on the advanced methodologies in cytogenetic studies and their applications, authors have pointed out the need to develop cytogenetic labs with modern tools to facilitate precise and effective diagnosis to benefit the patient population.

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