Advanced microtechnologies for cytogenetic analysis

Cytogenetic and molecular cytogenetic analyses, which aim to detect chromosome abnormalities, are routinely performed in cytogenetic laboratories all over the world. Traditional cytogenetic studies are performed by analyzing the banding pattern of chromosomes, and are complemented by molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH). To improve FISH application in cytogenetic analysis the issues with long experimental time, high volumes of expensive reagents and requirement for trained technicians need to be addressed. The protocol has recently evolved towards on chip detection of chromosome abnormalities with the development of microsystems for FISH analysis. The challenges addressed by the developed microsystems are mainly the automation of the assay performance, reduction in probe volume, as well as reduction of assay time. We present here our efforts to introduce automation in the cytogenetic laboratories at a microscale. We have developed membrane based micro perfusion systems capable of expansion of lymphocytes in a shorter time and at a smaller scale. The simulated and experimental results show very efficient exchange of the growth medium to the hypotonic solution and fixative. These are commonly used solutions required for proper preparation of metaphase chromosomes analysis. Further we developed a microfluidic chip for preparation of metaphase chromosome spreads and their analysis by metaphase FISH on chip. All developed devices are capable of performing the entire metaphase FISH protocol in a shorter time and at the same quality as standard methods.

General information

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Event:
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Research output: Contribution to conference › Conference abstract for conference – Annual report year: 2012 › Research › peer-review

Advanced microtechnologies for detection of chromosome abnormalities by fluorescent in situ hybridization.

Cytogenetic and molecular cytogenetic analyses, which aim to detect chromosome abnormalities, are routinely performed in cytogenetic laboratories all over the world. Traditional cytogenetic studies are performed by analyzing the banding pattern of chromosomes, and are complemented by molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH). To improve FISH application in cytogenetic analysis the issues with long experimental time, high volumes of expensive reagents and requirement for trained technicians need to be addressed. The protocol has recently evolved towards on chip detection of chromosome abnormalities with the development of microsystems for FISH analysis. The challenges addressed by the developed microsystems are mainly the automation of the assay performance, reduction in probe volume, as well as reduction of assay time. The recent focus on the development of automated systems for performing FISH on chip is summarized in this review.

General information
Chip connector

General information
Publication status: Published
Organisations: Department of Micro- and Nanotechnology
Contributors: Shah, P. J., Svendsen, W. E.
Publication date: 2011

Publication information
Patent number: WO 20111474421
Filing date: 01/12/2011
Original language: English
Source: orbit
Source-ID: 316973
Research output: Patent

FISHprep: A Novel Integrated Device for Metaphase FISH Sample Preparation

We present a novel integrated device for preparing metaphase chromosomes spread slides (FISHprep). The quality of cytogenetic analysis from patient samples greatly relies on the efficiency of sample pre-treatment and/or slide preparation. In cytogenetic slide preparation, cell cultures are routinely used to process samples (for culture, arrest and fixation of cells) and/or to expand limited amount of samples (in case of prenatal diagnostics). Arguably, this expansion and other sample pretreatments form the longest part of the entire diagnostic protocols spanning over 3–4 days. We present here a novel device with an integrated expansion chamber to culture, arrest and fix metaphase cells followed by a subsequent splashing protocol leading to ample metaphase chromosome spreads on a glass slide for metaphase FISH analysis. The device provides an easy, disposable, low cost, integrated solution with minimal handling for metaphase FISH slide preparation.

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Publication information
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Scopus rating (2011): SJR 0.222 SNIP 0.882
ISI indexed (2011): ISI indexed no
Original language: English
Keywords: Lab on chip, Chromosome spreading, Genetic analysis, Chromosomal translocations, Metaphase FISH, Fluorescence In Situ Hybridization (FISH)
Electronic versions:
micromachines-02-00116[1].pdf
DOIs:
10.3390/mi2020116
Source: orbit
Source-ID: 276952
Research output: Contribution to journal

Micro and nano-platforms for biological cell analysis

In this paper some technological platforms developed for biological cell analysis will be presented and compared to existing systems. In brief, we present a novel micro cell culture chamber based on diffusion feeding of cells, into which cells can be introduced and extracted after culturing using normal pipettes, thus making it readily usable for clinical laboratories. To enhance the functionality of such a chamber we have been investigating the use of active or passive 3D
surface modifications. Active modifications involve miniature electrodes able to record electrical or electrochemical signals from the cells, while passive modifications involve the presence of a peptide nanotube based scaffold for the cell culturing that mimics the in vivo environment. Two applications involving fluorescent in situ hybridization (FISH) analysis and cancer cell sorting are presented, as examples of further analysis that can be done after cell culturing. A platform able to automate the entire process from cell culturing to cell analysis by means of simple plug and play of various self-contained, individually fabricated modules is finally described.

Microfluidic bioreactors for culture of non-adherent cells
Microfluidic bioreactors (μBR) are becoming increasingly popular for cell culture, sample preparation and analysis in case of routine genetic and clinical diagnostics. We present a novel μBR for non-adherent cells designed to mimic in vivo perfusion of cells based on diffusion of media through a sandwiched membrane. The culture chamber and perfusion chamber are separated by a sandwiched membrane and each chamber has separate inlet/outlets for easy loading/unloading of cells and perfusion of the media. The perfusion of media and exchange of nutrients occur through the sandwiched membrane, which was also verified with simulations. Finally, we present the application of this device for cytogenetic sample preparation, whereby we culture and arrest peripheral T-lymphocytes in metaphase and later fix them in the μBR. The expansion of T-lymphocytes from an unknown patient sample was quantified by means of CFSE staining and subsequent counting in a flow cytometer. To conclude on the applicability of μBR for genetic diagnostics, we prepare chromosome spreads on glass slides from the cultured samples, which is the primary step for metaphase FISH analysis.
Metaphase FISH on a Chip: Miniaturized Microfluidic Device for Fluorescence in situ Hybridization

Fluorescence in situ Hybridization (FISH) is a major cytogenetic technique for clinical genetic diagnosis of both inherited and acquired chromosomal abnormalities. Although FISH techniques have evolved and are often used together with other cytogenetic methods like CGH, PRINS and PNA-FISH, the process continues to be a manual, labour intensive, expensive and time consuming technique, often taking over 3-5 days, even in dedicated labs. We have developed a novel microFISH device to perform metaphase FISH on a chip which overcomes many shortcomings of the current laboratory protocols. This work also introduces a novel splashing device for preparing metaphase spreads on a microscope glass slide, followed by a rapid adhesive tape-based bonding protocol leading to rapid fabrication of the microFISH device. The microFISH device allows for an optimized metaphase FISH protocol on a chip with over a 20-fold reduction in the reagent volume. This is the first demonstration of metaphase FISH on a microfluidic device and offers a possibility of automation and significant cost reduction of many routine diagnostic tests of genetic anomalies.
An integrated microfluidic system to isolate leukocytes from whole blood

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Contributors: Shah, P. J., Dimaki, M., Svendsen, W. E.
Publication date: 2009
Peer-reviewed: Yes
Event: Poster session presented at MMB2009, Quebec City, Canada.

A novel passive microfluidic device for preprocessing whole blood for point of care diagnostics
A novel strategy to sort the cells of interest (White Blood Cells (leukocytes)) by selectively lysing the Red Blood Cells (erythrocytes) in a miniaturized microfluidic device is presented. Various methods to lyse cells on a chip exist i.e. electrical, mechanical, chemical and thermal but they need integration of electrodes, traps, reservoirs, heaters, etc which is often difficult at microscale [1 – 4]. On the other hand, FACSlyse protocol uses only osmotic pressure to lyse erythrocytes allowing further isolation of leukocytes. This motivated us to develop a novel herringbone based lyser which works on the principle of mixing whole blood with pure water in time controlled manner to lyse erythrocytes osmotically on a chip.

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Electronic versions:
Shah.pdf
DOIs:
10.1109/SENSOR.2009.5285475

Novel Integrated Lab-on-Chip System for Chromosome Translocations Analysis
This presentation will focus on the development of a chromosome total analysis system (C-TAS) starting from the design strategy and simulations to the integration into a final monolithic plug and play device. Individual modules which perform the sample preprocessing and analysis tasks like - cell isolation, cell culture, cell lysing, chromosome extraction and
Fluorescence In-Situ Hybridization will be presented. How we solved connecting the individual chips and adjusting the microfluidic flows, by using simulations will be discussed.

**General information**
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Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2009

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Source-ID: 249895
Research output: Chapter in Book/Report/Conference proceeding – Annual report year: 2009
Research: peer-review

**Novel integrated Lab-on-Chip System for Chromosome Translocations Analysis**

**General information**
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2009

**Host publication information**
Title of host publication: Proceeding at: Lab On Chip 2009
Source: orbit
Source-ID: 249917
Research output: Chapter in Book/Report/Conference proceeding – Annual report year: 2009
Research: peer-review

**A Microfabricated Platform for Chromosome Separation and Analysis**

**General information**
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Pages: 052047
Publication date: 2008
Peer-reviewed: No

**Publication information**
Journal: Journal of Physics - Conference Series
Volume: 100
Issue number: 5
ISSN (Print): 1742-6588
Ratings:
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.264 SNIP 0.313
Web of Science (2008): Indexed yes
Original language: English
DOIs:
10.1088/1742-6596/100/5/052047
Source: orbit
Source-ID: 231158
Research output: Contribution to journal › Conference article – Annual report year: 2008
Research

**A Microfluidic Lyser to Extract Blood Cells**

**General information**
Comsol Multiphysics Simulations of Microfluidic Systems for Biomedical Applications

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology, Silicon Microtechnology Group, MicroElectroMechanical Systems Section, Theoretical Microfluidics Group, Theory Section
Publication date: 2008

Host publication information
Title of host publication: Proceeding of The Comsol User Conference
Source: orbit
Source-ID: 231198

FISH (Flurescent in Situ Hybridisation) on Chip

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2008

Host publication information
Title of host publication: Proceeding of The 12th Annual European Conference on Micro & Nanoscale Technologies for the Biosciences
Place of publication: Switzerland
Source: orbit
Source-ID: 231237

FISH (Flurescent in Situ Hybridisation) on Chip

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2008
Peer-reviewed: Yes
Event: Poster session presented at The 12th Annual European Conference on Micro & Nanoscale Technologies for the Biosciences, Montreaux, Switzerland.
Source: orbit
Source-ID: 231294
Research output: Contribution to conference – Poster – Annual report year: 2008 – Research – peer-review
FISH on Polymer

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2008

Host publication information
Title of host publication: Proceeding of NanoBioEurope
Place of publication: Barcelona, Spain
Source: orbit
Source-ID: 231235
Research output: Chapter in Book/Report/Conference proceeding – Article in proceedings – Annual report year: 2008 – peer-review

FISH on Polymer

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2008
Peer-reviewed: Yes
Source: orbit
Source-ID: 231292
Research output: Contribution to conference – Poster – Annual report year: 2008 – Research – peer-review

FISH on Polymer

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Publication date: 2008
Peer-reviewed: Yes
Source: orbit
Source-ID: 231293
Research output: Contribution to conference – Poster – Annual report year: 2008 – Research – peer-review

Scanning conductance microscopy investigations on fixed human chromosomes
Scanning conductance microscopy investigations were carried out in air on human chromosomes fixed on pre-fabricated SiO2 surfaces with a backgate. The point of the investigation was to estimate the dielectric constant of fixed human chromosomes in order to use it for microfluidic device optimization. The phase shift caused by the electrostatic forces, together with geometrical measurements of the atomic force microscopy (AFM) cantilever and the chromosomes were used to estimate a value, for the dielectric constant of different human chromosomes.

General information
Publication status: Published
Organisations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology
Pages: 225-228
Publication date: 2008
Peer-reviewed: Yes
Spatially Optimized Mixers to Enhance Mixing of Whole Blood with Water to Lyse Red Blood Cells

General information
Publication status: Published
Organizations: Nano-Bio Integrated Systems Group, Biomedical Micro Systems Section, Department of Micro- and Nanotechnology, Silicon Microtechnology Group, MicroElectroMechanical Systems Section, Theoretical Microfluidics Group, Theory Section
Publication date: 2008

Host publication information
Title of host publication: Proceedings of NanoBioEurope
Place of publication: Barcelona, Spain
Source: orbit
Source-ID: 231228


A Microfluidic Chip for Sorting of Chromosomes

General information
Publication status: Published
Organizations: Department of Micro- and Nanotechnology
Publication date: 2007
Peer-reviewed: Yes
Source: orbit
Source-ID: 206518

Research output: Contribution to conference – Conference abstract for conference – Annual report year: 2007 – Research – peer-review
Fabrication of polymeric microstructures to capture chromosomes on monolayer of antibodies

General information
Publication status: Published
Organisations: Department of Micro- and Nanotechnology, Bioprobes, POEM
Publication date: 2007
Peer-reviewed: Yes
Source: orbit
Source-ID: 206525
Research output: Contribution to conference › Poster – Annual report year: 2007 › Research › peer-review

Scanning Conductance Microscopy of Chromosomes

General information
Publication status: Published
Organisations: Department of Micro- and Nanotechnology
Publication date: 2007

Host publication information
Title of host publication: Proceedings of the AFM BioMed Conference 2007
Source: orbit
Source-ID: 199136
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 2007 › Research › peer-review

Separation of white blood cells from a whole blood sample using pinched flow

General information
Publication status: Published
Organisations: Department of Micro- and Nanotechnology
Publication date: 2007
Peer-reviewed: Yes
Source: orbit
Source-ID: 206529
Research output: Contribution to conference › Poster – Annual report year: 2007 › Research › peer-review

Separation of white blood cells from a whole blood sample using pinched flow

General information
Publication status: Published
Organisations: Department of Micro- and Nanotechnology
Publication date: 2007
Peer-reviewed: Yes
Event: Poster session presented at 33rd International Conference on Micro- and Nano-Engineering, Copenhagen, Denmark.
Projects:

**Microfluidic system for chromosome analysis system**
Shah, P. J., PhD Student, Department of Micro- and Nanotechnology
Svendsen, W. E., Main Supervisor
Dimaki, M., Supervisor
Okkels, F., Supervisor
Almdal, K., Examiner
Becker, H., Examiner
Geschke, O., Examiner
Forskningsrådssfinansiering
15/01/2007 → 24/08/2011
Award relations: Microfluidic system for chromosome analysis system
Project: PhD

**Evolvable Smartphone-based Point-of-Care Systems for In-Vitro Diagnostics**
Patou, F., PhD Student, Department of Micro- and Nanotechnology
Svendsen, W. E., Main Supervisor
Dimaki, M., Supervisor
Madsen, J., Supervisor
Pop, P., Examiner
Romano-Rodriguez, A., Examiner
Shah, P. J., Examiner
Marie Curie (EU-stipendium)
01/02/2013 → 02/11/2016
Award relations: Evolvable Smartphone-based Point-of-Care Systems for In-Vitro Diagnostics
Project: PhD

Activities:

**An integrated microfluidic system to isolate leukocytes from whole blood**
Period: 1 Apr 2009 → 3 Apr 2009
Pranjul Jaykumar Shah (Speaker)
Department of Micro- and Nanotechnology
Biomedical Micro Systems Section
Nano-Bio Integrated Systems Group

**Description**
Place: Presented at MMB2009, Quebec, Canada

**Related external organisation**

**Unknown Organization**
Activity: Talks and presentations › Conference presentations

**talk about “On-Chip Leukocytes Isolation” Presented at The International Conference on Biomedical and Genomic Research(ICBGR)**
Period: 30 Jan 2009
Pranjul Jaykumar Shah (Speaker)
Department of Micro- and Nanotechnology
Biomedical Micro Systems Section
Nano-Bio Integrated Systems Group
Description
Place: Ahmedabad, India

Related external organisation
Unknown Organization
Activity: Talks and presentations › Conference presentations

Comsol Conference 2008
Period: 4 Nov 2008 → 6 Nov 2008
Pranjul Jaykumar Shah (Participant)
Biomedical Micro Systems Section
Nano-Bio Integrated Systems Group
Department of Micro- and Nanotechnology

Description
Talk about "Comsol Multiphysics Simulation of Microfluidic Systems for Biomedical Applications" presented at Comsol User Conference

Place: Hannover, Germany
Degree of recognition: International

Related event
Comsol Conference 2008
04/11/2008 → 06/11/2008
Hannover, Germany
Activity: Attending an event › Participating in or organising a conference