

Année de publication : 2017

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M Serra, I Pereiro, A Yamada, J-L Viovy, S Descroix, D Ferraro (2017 Jan 24)

**A simple and low-cost chip bonding solution for high pressure, high temperature and biological applications.**

Lab on a chip : 629-634 : [DOI : 10.1039/c6lc01319h](https://doi.org/10.1039/c6lc01319h)

**Résumé**

The sealing of microfluidic devices remains a complex and time-consuming process requiring specific equipment and protocols: a universal method is thus highly desirable. We propose here the use of a commercially available sealing tape as a robust, versatile, reversible solution, compatible with cell and molecular biology protocols, and requiring only the application of manually achievable pressures. The performance of the seal was tested with regards to the most commonly used chip materials. For most materials, the bonding resisted 5 bars at room temperature and 1 bar at 95 °C. This method should find numerous uses, ranging from fast prototyping in the laboratory to implementation in low technology environments or industrial production.

Bruno Teste, Jerome Champ, Arturo Londono-Vallejo, Stéphanie Descroix, Laurent Malaquin, Jean-Louis Viovy, Irena Draskovic, Guillaume Mottet (2017 Jan 17)

**Chromatin immunoprecipitation in microfluidic droplets: towards fast and cheap analyses.**

Lab on a chip : 530-537 : [DOI : 10.1039/c6lc01535b](https://doi.org/10.1039/c6lc01535b)

**Résumé**

Genetic organization is governed by the interaction of DNA with histone proteins, and differential modifications of these proteins is a fundamental mechanism of gene regulation. Histone modifications are primarily studied through chromatin immunoprecipitation (ChIP) assays, however conventional ChIP procedures are time consuming, laborious and require a large number of cells. Here we report for the first time the development of ChIP in droplets based on a microfluidic platform combining nanoliter droplets, magnetic beads (MB) and magnetic tweezers (MT). The droplet approach enabled compartmentalization and improved mixing, while reducing the consumption of samples and reagents in an integrated workflow. Anti-histone antibodies grafted to MB were used as a solid support to capture and transfer the target chromatin from droplets to droplets in order to perform chromatin immunoprecipitation, washing, elution and purification of DNA. We designed a new ChIP protocol to investigate four different types of modified histones with known roles in gene activation or repression. We evaluated the performances of this new ChIP in droplet assay in comparison with conventional methods. The proposed technology dramatically reduces analytical time from a few days to 7 hours, simplifies the ChIP protocol and decreases the number of cells required by 100 fold while maintaining a high degree of sensitivity and specificity. Therefore this droplet-based ChIP assay represents a new, highly advantageous and convenient approach to epigenetic analyses.

Karla Perez-Toralla, Guillaume Mottet, Ezgi Tulukcuoglu-Guneri, Jérôme Champ, François-Clément Bidard, Jean-Yves Pierga, Jerzy Klijanienko, Irena Draskovic, Laurent Malaquin, Jean-Louis Viovy, Stéphanie Descroix (2017 Jan 4)

**FISH-in-CHIPS: A Microfluidic Platform for Molecular Typing of Cancer Cells.**

*Methods in molecular biology (Clifton, N.J.)* : 211-220 : [DOI : 10.1007/978-1-4939-6734-6\\_16](https://doi.org/10.1007/978-1-4939-6734-6_16)

**Résumé**

Microfluidics offer powerful tools for the control, manipulation, and analysis of cells, in particular for the assessment of cell malignancy or the study of cell subpopulations. However, implementing complex biological protocols on chip remains a challenge. Sample preparation is often performed off chip using multiple manually performed steps, and protocols usually include different dehydration and drying steps that are not always compatible with a microfluidic format. Here, we report the implementation of a Fluorescence in situ Hybridization (FISH) protocol for the molecular typing of cancer cells in a simple and low-cost device. The geometry of the chip allows integrating the sample preparation steps to efficiently assess the genomic content of individual cells using a minute amount of sample. The FISH protocol can be fully automated, thus enabling its use in routine clinical practice.

Davide Ferraro, Jérôme Champ, Bruno Teste, M Serra, Laurent Malaquin, Stéphanie Descroix, Patricia de Cremoux, Jean-Louis Viovy (2017 Jan 4)

**Droplet Microfluidic and Magnetic Particles Platform for Cancer Typing.**

*Methods in molecular biology (Clifton, N.J.)* : 113-121 : [DOI : 10.1007/978-1-4939-6734-6\\_9](https://doi.org/10.1007/978-1-4939-6734-6_9)

**Résumé**

Analyses of nucleic acids are routinely performed in hospital laboratories to detect gene alterations for cancer diagnosis and treatment decision. Among the different possible investigations, mRNA analysis provides information on abnormal levels of genes expression. Standard laboratory methods are still not adapted to the isolation and quantitation of low mRNA amounts and new techniques need to be developed in particular for rare subsets analysis. By reducing the volume involved, time process, and the contamination risks, droplet microfluidics provide numerous advantages to perform analysis down to the single cell level. We report on a droplet microfluidic platform based on the manipulation of magnetic particles that allows the clinical analysis of tumor tissues. In particular, it allows the extraction of mRNA from the total-RNA sample, Reverse Transcription, and cDNA amplification, all in droplets.

Ayako Yamada, Renaud Renault, Aleksandra Chikina, Bastien Venzac, Iago Pereiro, Sylvie Coscoy, Marine Verhulsel, Maria Carla Parrini, Catherine Villard, Jean-Louis Viovy, Stéphanie Descroix (2016 Nov 1)

**Transient microfluidic compartmentalization using actionable microfilaments for**

**biochemical assays, cell culture and organs-on-chip.**

Lab on a chip : [DOI : 10.1039/C6LC01143H](https://doi.org/10.1039/C6LC01143H)

**Résumé**

We report here a simple yet robust transient compartmentalization system for microfluidic platforms. Cylindrical microfilaments made of commercially available fishing lines are embedded in a microfluidic chamber and employed as removable walls, dividing the chamber into several compartments. These partitions allow tight sealing for hours, and can be removed at any time by longitudinal sliding with minimal hydrodynamic perturbation. This allows the easy implementation of various functions, previously impossible or requiring more complex instrumentation. In this study, we demonstrate the applications of our strategy, firstly to trigger chemical diffusion, then to make surface co-coating or cell co-culture on a two-dimensional substrate, and finally to form multiple cell-laden hydrogel compartments for three-dimensional cell co-culture in a microfluidic device. This technology provides easy and low-cost solutions, without the use of pneumatic valves or external equipment, for constructing well-controlled microenvironments for biochemical and cellular assays.

Année de publication : 2016

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Renaud Renault, Jean-Baptiste Durand, Jean-Louis Viovy, Catherine Villard (2016 May 27)

**Asymmetric axonal edge guidance: a new paradigm for building oriented neuronal networks.**

Lab on a chip : 2188-91 : [DOI : 10.1039/c6lc00479b](https://doi.org/10.1039/c6lc00479b)

**Résumé**

We present a novel kind of directional axon guides for brain-on-a-chip applications. Contrarily to previous works, the directionality in our design is created by rerouting axons growing in the unwanted direction back to their original compartment while leaving the other growth direction unaffected. This design yields state-of-the-art levels of directionality without the disadvantages of previously reported technologies.

Evangelos Gogolides, Angeliki Tserepi, Gerhard Jobst, Jean-Michel Friedt, David Rabus, Bruno Dupuy, Zuzana Bilkova, Stephanie Descroix, Jean-Louis Viovy, George Papadakis, Electra Gizeli (2016 May 27)

**Micro-Nano-Bio Diagnostic System for Food Pathogen Detection Revolutionizes Food Safety Management & Protects Consumers Health.**

*Studies in health technology and informatics* : 67-72

**Résumé**

The development of integrated, fast and affordable platforms for pathogen detection is an emerging area where a multidisciplinary approach is necessary for designing microsystems

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employing miniaturized devices; these new technologies promise a significant advancement of the current state of analytical testing leading to improved healthcare. In this work, the development of a lab-on-chip microsystem platform for the genetic analysis of Salmonella in milk samples is presented. The heart of the platform is an acoustic detection biochip, integrated with a microfluidic module. This detection platform is combined with a micro-processor, which, alongside with magnetic beads technology and a DNA micro-amplification module, are responsible for performing sample pre-treatment, bacteria lysis, nucleic acid purification and amplification. Automated, multiscale manipulation of fluids in complex microchannel networks is combined with novel sensing principles developed by some of the partners. This system is expected to have a significant impact in food-pathogen detection by providing for the first time an integrated detection test for Salmonella screening in a very short time. Finally, thanks to the low cost and compact technologies involved, the proposed set-up is expected to provide a competitive analytical platform for direct application in field settings.

Ayako Yamada, Maéva Vignes, Cécile Bureau, Alexandre Mamane, Bastien Venzac, Stéphanie Descroix, Jean-Louis Viovy, Catherine Villard, Jean-Michel Peyrin, Laurent Malaquin (2016 May 13)

**In-mold patterning and actionable axo-somatic compartmentalization for on-chip neuron culture.**

*Lab on a chip* : 2059-68 : [DOI : 10.1039/c6lc00414h](https://doi.org/10.1039/c6lc00414h)

**Résumé**

Oriented neuronal networks with controlled connectivity are required for many applications ranging from studies of neurodegeneration to neuronal computation. To build such networks *in vitro*, an efficient, directed and long lasting guidance of axons toward their target is a prerequisite. The best guidance achieved so far, however, relies on confining axons in enclosed microchannels, making them poorly accessible for further investigation. Here we describe a method providing accessible and highly regular arrays of axons, emanating from somas positioned in distinct compartments. This method combines the use of a novel removable partition, allowing soma positioning outside of the axon guidance patterns, and in-mold patterning (iMP), a hybrid method combining chemical and mechanical cell positioning clues applied here for the first time to neurons. The axon guidance efficiency of iMP is compared to that of conventional patterning methods, e.g. micro-contact printing (chemical constraints by a poly-l-lysine motif) and micro-grooves (physical constraints by homogeneously coated microstructures), using guiding tracks of different widths and spacing. We show that iMP provides a gain of 10 to 100 in axon confinement efficiency on the tracks, yielding mm-long, highly regular, and fully accessible on-chip axon arrays. iMP also allows well-defined axon guidance from small populations of several neurons confined at predefined positions in  $\mu\text{m}$ -sized wells. iMP will thus open new routes for the construction of complex and accurately controlled neuronal networks.

Davide Ferraro, Jérôme Champ, Bruno Teste, Marco Serra, Laurent Malaquin, Jean-Louis Viovy,

Patricia de Cremoux, Stephanie Descroix (2016 May 10)

**Microfluidic platform combining droplets and magnetic tweezers: application to HER2 expression in cancer diagnosis.**

*Scientific reports* : 25540 : [DOI : 10.1038/srep25540](https://doi.org/10.1038/srep25540)

### Résumé

The development of precision medicine, together with the multiplication of targeted therapies and associated molecular biomarkers, call for major progress in genetic analysis methods, allowing increased multiplexing and the implementation of more complex decision trees, without cost increase or loss of robustness. We present a platform combining droplet microfluidics and magnetic tweezers, performing RNA purification, reverse transcription and amplification in a fully automated and programmable way, in droplets of 250nL directly sampled from a microtiter-plate. This platform decreases sample consumption about 100 fold as compared to current robotized platforms and it reduces human manipulations and contamination risk. The platform's performance was first evaluated on cell lines, showing robust operation on RNA quantities corresponding to less than one cell, and then clinically validated with a cohort of 21 breast cancer samples, for the determination of their HER2 expression status, in a blind comparison with an established routine clinical analysis.

**Année de publication : 2015**

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Reza M Mohamadi, Zuzana Svobodova, Zuzana Bilkova, Markus Otto, Myriam Taverna, Stephanie Descroix, Jean-Louis Viovy (2015 Oct 22)

**An integrated microfluidic chip for immunocapture, preconcentration and separation of  $\beta$ -amyloid peptides.**

*Biomicrofluidics* : 054117 : [DOI : 10.1063/1.4931394](https://doi.org/10.1063/1.4931394)

### Résumé

We present an integrated microfluidic chip for detection of  $\beta$ -amyloid ( $A\beta$ ) peptides.  $A\beta$  peptides are major biomarkers for the diagnosis of Alzheimer's disease (AD) in its early stages. This microfluidic device consists of three main parts: (1) An immunocapture microcolumn based on self-assembled magnetic beads coated with antibodies specific to  $A\beta$  peptides, (2) a nano-porous membrane made of photopolymerized hydrogel for preconcentration, and (3) a microchip electrophoresis (MCE) channel with fluorescent detection. Sub-milliliter sample volume is either mixed off-chip with antibody coated magnetic beads and injected into the device or is injected into an already self-assembled column of magnetic beads in the microchannel. The captured peptides on the beads are then electrokinetically eluted and re-concentrated onto the nano-membrane in a few nano-liters. By integrating the nano-membrane, total assay time was reduced and also off-chip re-concentration or buffer exchange steps were not needed. Finally, the concentrated peptides in the chip are separated by electrophoresis in a polymer-based matrix. The device was applied to the capture and MCE analysis of differently truncated peptides  $A\beta$  (1-37, 1-39, 1-40, and 1-42) and was able to detect as low as 25 ng of synthetic  $A\beta$  peptides spiked in

undiluted cerebrospinal fluid (CSF). The device was also tested with CSF samples from healthy donors. CSF samples were fluorescently labelled and pre-mixed with the magnetic beads and injected into the device. The results indicated that A $\beta$ 1-40, an important biomarker for distinguishing patients with frontotemporal lobe dementia from controls and AD patients, was detectable. Although the sensitivity of this device is not yet enough to detect all A $\beta$  subtypes in CSF, this is the first report on an integrated or semi-integrated device for capturing and analyzing of differently truncated A $\beta$  peptides. The method is less demanding and faster than the conventional Western blotting method currently used for research.

D Ferraro, Y Lin, B Teste, D Talbot, L Malaquin, S Descroix, A Abou-Hassan (2015 Oct 6)

**Continuous chemical operations and modifications on magnetic  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> nanoparticles confined in nanoliter droplets for the assembly of fluorescent and magnetic SiO<sub>2</sub>@ $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>.**

*Chemical communications (Cambridge, England)* : 16904-7 : [DOI : 10.1039/c5cc07044a](https://doi.org/10.1039/c5cc07044a)

### Résumé

We present a microfluidic platform that allows undergoing different chemical operations in a nanoliter droplet starting from the colloidal suspension of magnetic iron oxide ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles « NPs » (ferrofluid). These operations include: mixing, flocculation, magnetic decantation, colloidal redispersion, washing, surface functionalization, heating and colloidal assembly. To prove the platform capabilities, we produced fluorescent and magnetic nanoassemblies composed of fluorescent silica and magnetic NPs.

Thanh Duc Mai, Iago Pereiro, Mohamed Hiraoui, Jean-Louis Viovy, Stéphanie Descroix, Myriam Taverna, Claire Smadja (2015 Jul 25)

**Magneto-immunocapture with on-bead fluorescent labeling of amyloid- $\beta$  peptides: towards a microfluidized-bed-based operation.**

*The Analyst* : 5891-900 : [DOI : 10.1039/c5an01179e](https://doi.org/10.1039/c5an01179e)

### Résumé

A new sample treatment approach for sensitive determination of three amyloid- $\beta$  peptides (A $\beta$  1-42, A $\beta$  1-40 and A $\beta$  1-38) with capillary electrophoresis coupled with laser induced fluorescent detection is reported herein. These A $\beta$  peptides are considered an important family of biomarkers in the cerebrospinal fluid (CSF) for early diagnosis of Alzheimer's disease (AD). Due to their extremely low abundance in CSF (down to sub nM ranges), batch-wise preconcentration via magneto-immunocapture with enrichment factors up to 100 was implemented. The A $\beta$  peptides were first captured onto magnetic micro-beads. Then, on-beads fluorescent labeling of the captured A $\beta$  peptides were carried out to avoid the unwanted presence of extra fluorescent dye in the eluent as in the case of in-solution labeling. Finally thermal elution was performed and eluted labeled peptides were analyzed off line with CE-LIF. The A $\beta$ -capturing efficiencies of different commercially available

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antibodies grafted onto magnetic beads were tested. A $\beta$  peptides in CSF samples collected from AD's patients and healthy persons (used as controls) were measured and evaluated. As a proof of concept, the developed strategy was adapted into a miniaturized fluidized bed configuration that has the potential for coupling with a microchip separation system.

Renaud Renault, Nirit Sukenik, Stéphanie Descroix, Laurent Malaquin, Jean-Louis Viovy, Jean-Michel Peyrin, Samuel Bottani, Pascal Monceau, Elisha Moses, Maéva Vignes (2015 Apr 23)

**Combining microfluidics, optogenetics and calcium imaging to study neuronal communication in vitro.**

*PloS one* : e0120680 : [DOI : 10.1371/journal.pone.0120680](https://doi.org/10.1371/journal.pone.0120680)

**Résumé**

In this paper we report the combination of microfluidics, optogenetics and calcium imaging as a cheap and convenient platform to study synaptic communication between neuronal populations in vitro. We first show that Calcium Orange indicator is compatible in vitro with a commonly used Channelrhodopsin-2 (ChR2) variant, as standard calcium imaging conditions did not alter significantly the activity of transduced cultures of rodent primary neurons. A fast, robust and scalable process for micro-chip fabrication was developed in parallel to build micro-compartmented cultures. Coupling optical fibers to each micro-compartment allowed for the independent control of ChR2 activation in the different populations without crosstalk. By analyzing the post-stimuli activity across the different populations, we finally show how this platform can be used to evaluate quantitatively the effective connectivity between connected neuronal populations.

Julien Autebert, Benoit Coudert, Jérôme Champ, Laure Saias, Ezgi Tulukcuoglu Guneri, Ronald Lebofsky, François-Clément Bidard, Jean-Yves Pierga, Françoise Farace, Stéphanie Descroix, Laurent Malaquin, Jean-Louis Viovy (2015 Mar 28)

**High purity microfluidic sorting and analysis of circulating tumor cells: towards routine mutation detection.**

*Lab on a chip* : 2090-101 : [DOI : 10.1039/c5lc00104h](https://doi.org/10.1039/c5lc00104h)

**Résumé**

A new generation of the Ephesia cell capture technology optimized for CTC capture and genetic analysis is presented, characterized in depth and compared with the CellSearch system as a reference. This technology uses magnetic particles bearing tumour-cell specific EpCAM antibodies, self-assembled in a regular array in a microfluidic flow cell. 48,000 high aspect-ratio columns are generated using a magnetic field in a high throughput (>3 ml h<sup>-1</sup>) device and act as sieves to specifically capture the cells of interest through antibody-antigen interactions. Using this device optimized for CTC capture and analysis, we demonstrated the capture of epithelial cells with capture efficiency above 90% for concentrations as low as a few cells per ml. We showed the high specificity of capture with only 0.26% of non-epithelial cells captured for concentrations above 10 million cells per ml. We investigated the capture

behavior of cells in the device, and correlated the cell attachment rate with the EpCAM expression on the cell membranes for six different cell lines. We developed and characterized a two-step blood processing method to allow for rapid processing of 10 ml blood tubes in less than 4 hours, and showed a capture rate of 70% for as low as 25 cells spiked in 10 ml blood tubes, with less than 100 contaminating hematopoietic cells. Using this device and procedure, we validated our system on patient samples using an automated cell immunostaining procedure and a semi-automated cell counting method. Our device captured CTCs in 75% of metastatic prostate cancer patients and 80% of metastatic breast cancer patients, and showed similar or better results than the CellSearch device in 10 out of 13 samples. Finally, we demonstrated the possibility of detecting cancer-related PIK3CA gene mutation in 20 cells captured in the chip with a good correlation between the cell count and the quantitation value Cq of the post-capture qPCR.

**Année de publication : 2014**

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Karla Perez-Toralla, Guillaume Mottet, Ezgi Tulukcuoglu Guneri, Jérôme Champ, François-Clément Bidard, Jean-Yves Pierga, Jerzy Klijanienko, Irena Draskovic, Laurent Malaquin, Jean-Louis Viovy, Stéphanie Descroix (2014 Dec 5)

**FISH in chips: turning microfluidic fluorescence in situ hybridization into a quantitative and clinically reliable molecular diagnosis tool.**

Lab on a chip : 811-22 : [DOI : 10.1039/c4lc01059k](https://doi.org/10.1039/c4lc01059k)

**Résumé**

Microfluidic systems bear promise to provide new powerful tools for the molecular characterization of cancer cells, in particular for the routine detection of multiple cancer biomarkers using a minute amount of the sample. However, taking miniaturized cell-based assays into the clinics requires the implementation and validation of complex biological protocols on chip, as well as the development of disposable microdevices produced at a low cost. Based on a recently developed microfluidic chip made of Cyclic Olefin Copolymer for cell immobilization with minimal dead volume and controlled shear stress, we developed a protocol performed entirely in the liquid phase, allowing the immobilization and fixation of cells and their quantitative characterization by fluorescence in situ hybridization. We demonstrated first in cell lines and then in two clinical case studies the potential of this method to perform quantitative copy number measurement and clinical scoring of the amplification of the ERBB2 gene, a decisive biomarker for the prescription of HER2+ related targeted therapies. This validation was performed in a blind protocol in two clinical case studies, in reference to the gold standard and clinically used method based on glass slides. We obtained a comparable reproducibility and a minor difference in apparent amplification, which can be corrected by internal calibration. The method thus reaches the standard of robustness needed for clinical use. The protocol can be fully automated, and its consumption of samples and DNA probes is reduced as compared to glass slide protocols by a factor of at least 10. The total duration of the assay is divided by two.



Publications de l'équipe  
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