MilliDrop
every droplet counts

HIGH-THROUGHPUT MICROBIAL ANALYSIS

THE REVOLUTION MILLIDROP

PRESS KIT
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SUMMARY

Glossary

High-throughput screening: Identification of the activity or element being sought in thousands of samples.

Culture medium: Liquid or solid medium promoting the multiplication of cells, bacteria, yeast or molds.

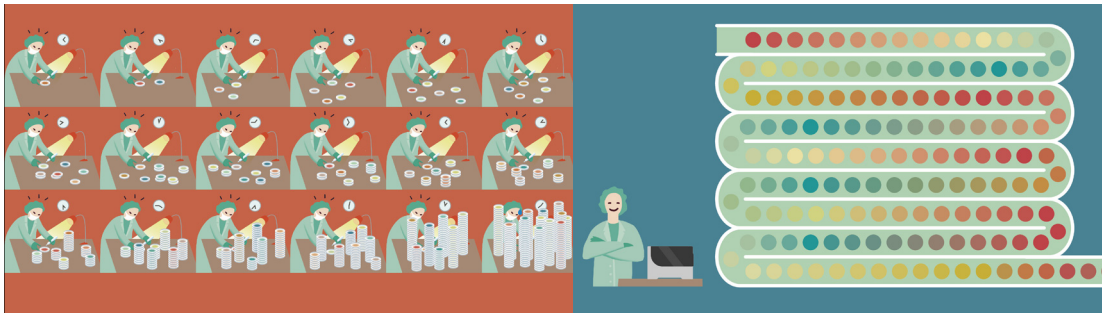
Microorganism: Microscopic living organisms such as bacteria, viruses, single-cell fungi (yeasts) and protists.

Phenotype: An individual's set of observable characteristics.

A DROPLET-BASED CULTURE VESSEL

The days of Petri dishes and flasks are over! With MilliDrop's millifluidic technique, the same growth performances of microorganisms—bacteria, fungi, algae or yeast—can be achieved in a single droplet.

MilliDrop's breakthrough technology will put high-throughput microbiology systems, the likes of which have never been seen before, within reach of public- and private-sector research laboratories. It meets a growing need for analysis of microorganisms—from single cells to entire colonies—with instruments that are easy to use and highly reliable.



Illustrations : Laura Pigeon

Phenotyping: before/after MilliDrop

The instrument developed by MilliDrop is a compact system that interfaces seamlessly with other laboratory instruments, including automated screening platforms. Its miniaturization to the millimeter scale delivers a 1,000-fold leap in productivity.

Benefits of millifluidic technology

Miniaturized, high-throughput, automated → time-saving

1,000 times less reagent → lower-cost

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A revolution in microbiology

The growth of microorganisms and their phenotype can now be studied at high speed.

The first instrument - MilliDrop Analyzer - currently commercialized by MilliDrop, analyzes the phenotypic diversity of micro-organisms—this means the various characteristics observable among thousands of populations cultivated in parallel. Phenotypes of interest can be isolated and their recovery automated.

Dr. Maxime Ardré, research scientist at ESPCI's LGE laboratory led by Paul Rainey

"For comparative microbiological growth studies, a large number of glass vessels and culture media have traditionally been used. What's more, this type of experiment requires a very large number of pipetting operations by a careful technician. Thanks to the MilliDrop Analyzer, I can produce a thousand incubated microcosms in parallel with a single multi-well plate and only 20ml of culture medium, and it can all be done with a few clicks!"

A second generation of machines intended for diagnostics is currently in the pipeline. Health professionals, who are not laboratory analysis experts, will be able to use it unassisted. The MilliDrop IVD under development will be a fully automated in vitro diagnostics instrument able to identify both infectious agents and the doses of antibiotic required to eradicate them. The ultimate aim is to avoid selection of the wrong antibiotic or administration of an excessive dose.

Several MilliDrop Analyzer systems have been installed at our partner academic laboratories, including the ESPCI in Paris, and the Wageningen University in the Netherlands.

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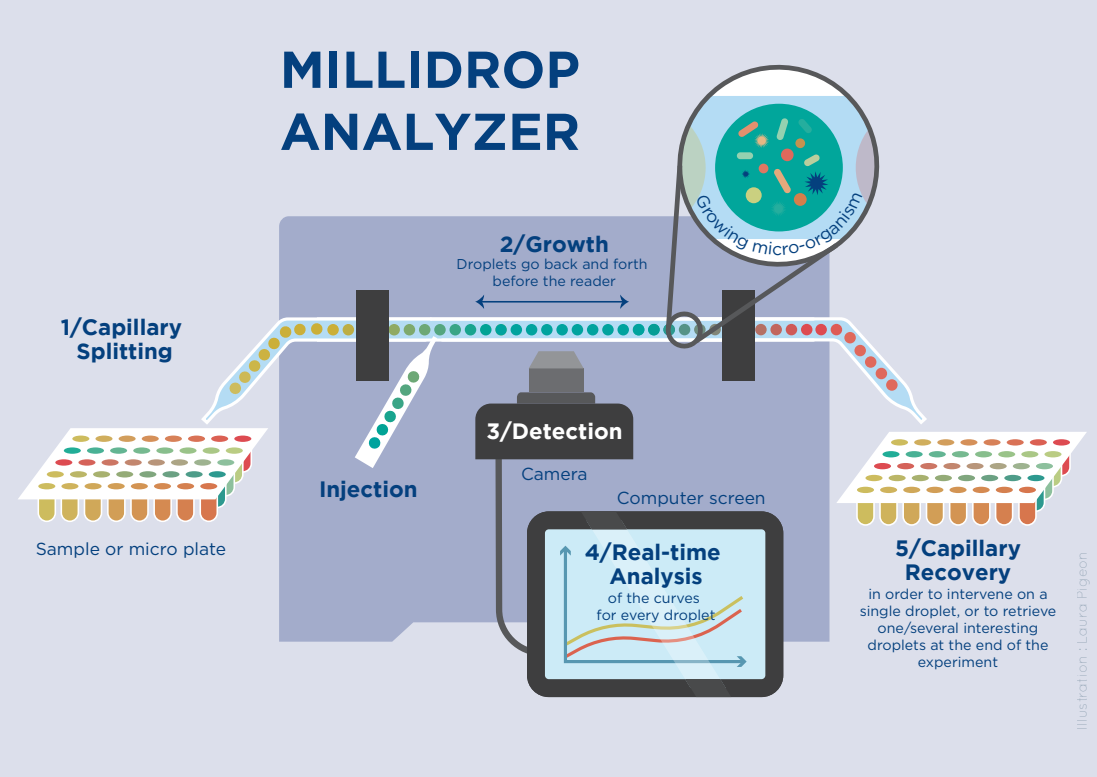
HOW DOES IT WORK?

Microorganisms are placed in suspension in a culture medium. From each sample contained in a simple tube or in a well of microplates, millimeter-scale droplets are formed through aspiration and stored in a capillary containing up to several thousands. During the formation process, the droplets are isolated from each other by air bubbles, which keeps them totally independent and prevents any possibility of cross-contamination and evaporation. The capillary is placed in a temperature-controlled enclosure to incubate microorganisms in the droplets. While maintaining them in their respective order, they are kept circulating at all times and fed in front of an optical system that tracks each of them individually.

The optical system is equipped with fluorescence and light diffusion analysis modules to detect both the growth in the microorganisms and the molecules they express. Compounds can also be injected into the drops during incubation via a small opening on the side of the capillary. At the end of the experiment, droplets of interest may be recovered onto a plate for further analysis.



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Droplet-based microbiology — fast, reproducible results, high-quality analysis and sensitivity

The droplet produces the same growth performances as standard techniques. The culture and incubation conditions are robust and homogeneous. The format of the droplets and the millifluidic techniques employed can achieve a very high level of analytical sensitivity and easily handle thousands of samples at the same time. Handling errors are minimized, reproducibility is excellent, and the quality of analysis is first-rate. Furthermore, users save time compared with the standard techniques.

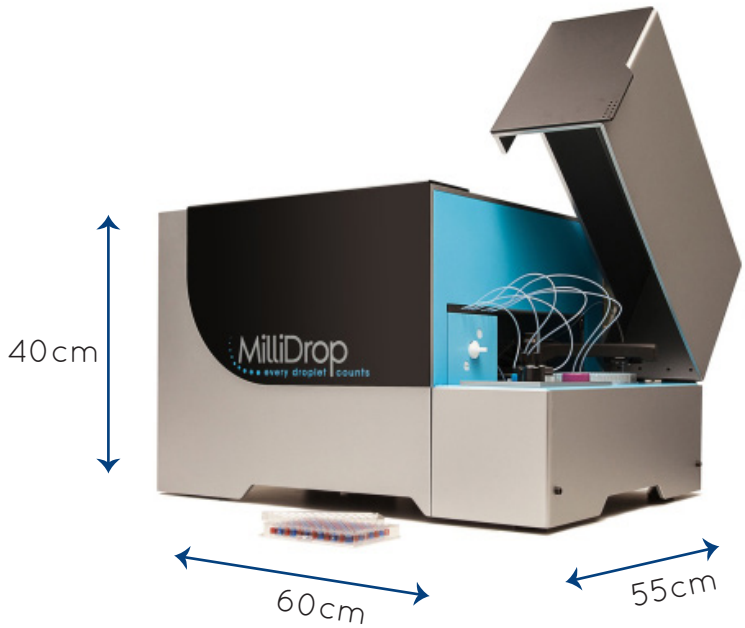
A MODULAR SYSTEM

MilliDrop's offering consists of a basic module that automatically prepares, incubates and analyzes the samples. Other modules may be added:

- **The injection module,** which provides the option of adding compounds into a particular drop at any time during culture.
- **The sorting module,** which collects the droplets targeted by the user for further analysis.

These modules open up a whole new range of possibilities for users to define protocols perfectly geared to the needs of each experiment or analysis. Other modules are under development and will be delivered with forthcoming MilliDrop instruments, such as multiplexing to test the various culture conditions or the ability to add samples as and when they arrive on the analysis platform.

The droplets of interest are cultivated, analyzed and recovered automatically in the MilliDrop Analyzer.



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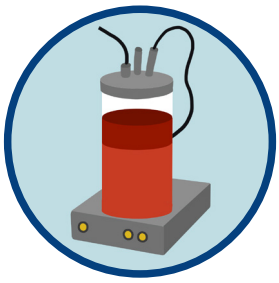
THREE MARKETS SERVED BY MILLIDROP TECHNOLOGY

Given the Analyzer's compact nature, the effectiveness of droplet-based cultivation, and the option of integrating this technology into an automated process, MilliDrop can meet the needs in three segments of the global biological instrument and reagent markets, totalling around €7 billion.

RESEARCH

Academic research laboratories will be able to access high-throughput screening systems that were previously available only to large organizations. This will benefit all areas of microbiological research—healthcare, agriculture, agrifood and biotechnology. This market segment is estimated to be worth around €200 million (MilliDrop data). MilliDrop technology meets the growing demand for high throughput analysis at lower cost by offering dedicated, compact and easy-to-use instruments.



INDUSTRIAL MICROBIOLOGY

Using millimeter-scale droplets reduces the quantities of samples and reagents required by a factor of 1,000, while delivering highly effective microbial cultivation.

The results are obtained rapidly from a very large number of droplets. During cultivation and analysis of the samples by the system, the industrial platform's robots are freed up for other tasks. This also helps to increase the platform's productivity.

Large agrifood and healthcare companies along with biotechnology companies are among the industry players that could benefit from it. Industrial microbiology represents a €4 billion market growing at a rate of 6 % p.a. (Future Markets Insights, source: 2016).

All manufacturing facilities face performance, productivity and cost constraints. MilliDrop's technology can help them to deal with these challenges.

DIAGNOSTICS

A second range of instruments developed by MilliDrop will be added to the existing line of microbiological analysis platforms. Its high-throughput capability delivers a genuine leap forward that will improve treatment of patients suffering from infectious diseases by speeding up the analyses required for a suitable treatment to be prescribed. The clinical microbiology market is worth around €2.8 billion and is growing at a rate of 11 % per year according to Markets&Markets (2016 estimate). Owing to its speed and its high throughput, MilliDrop technology helps to curb the phenomena of antibiotic resistance.

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TECHNOLOGICAL IMPACT ALREADY DEMONSTRATED

Several scientific publications have already demonstrated the benefits of MilliDrop technology for various applications.

1/ Determining the concentration of antibiotics needed to inhibit the growth of a bacteria[1, 2]

The growth dynamics of a microbial population over a number of generations were analyzed in over 1,000 drops in parallel. Researchers measured the growth rate of a species of bacteria as a function of an antibiotic's concentration gradient and thus determined the Minimum Inhibitory Concentration (MIC). Cellular activity is detected by means of automatic detection of an epifluorescent signal, which can be used to track growth in microbial populations of up to one million cells*.

***Therapeutic application** : identify infectious agents and the antibiotic doses needed to eradicate them, and reduce the therapeutic failures associated with the wrong choice or overdose of antibiotics.*

* MilliDrop technology was compared in reference strains with three different antibiotics and demonstrated a high level of accuracy.

2/ Analyzing the heterogeneity in algae cell growth[3]

Single-cell algae carrying the same genes were placed in droplets providing the same culture conditions.

The existence of two sub-populations was demonstrated—one growing very rapidly and the other slowly. This heterogeneity in terms of the growth dynamics may derive from mutations or from variations in metabolites or macromolecules.

***Industrial application** : identify the heterogeneity of strains in fermenters to track their quality and increase productivity.*

1 Millifluidic droplet analyzer for microbiology, L. Baraban, F. Bertholle, M. L. M. Salverda, N. Bremond, P. Panizza, J. Baudry, J. A. G. M. de Visser Lab Chip, 2011,11, 4057-4062.

2 Digital antimicrobial susceptibility testing using the MilliDrop technology ", L. Jiang, L. Boitard, P. Broyer,A.-C. Chareire, P. Bourne-Branchu, P. Mahé, M. Tournoud, C. Franceschi, G. Zambardi, J. Baudry, J. Bibette Eur J Clin Microbiol Infect Dis (2016) 35: 415.

3 A millifluidic study of cell-to-cell heterogeneity in growth-rate and cell-division capability in populations of isogenic cells of chlamydomonas reinhardtii, P. Damodaran, S. Eberhard, L. Boitard, J. Garnica Rodriguez, Y. Wang, N. Bremond, J. Baudry, J. Bibette, F. A. Wollman. PLoS ONE, 2015, 10, 0118987

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3/ Understanding microbial diversity[4]

The accuracy obtained from the millifluidic format helped to detect several competing resistance strategies that co-exist in the sample when subjecct to an antibiotic concentration below the minimum inhibiting concentration. This technology could be used as a means of addressing new challenges such as developing growth systems for several microbial species within a community.

Therapeutic application *: analyze bacterial communities to develop new antibiotics, probiotics, or new therapies targeting the microbiota.*

4/ Determining the phenotype and genotype of an isolated microorganism[5]

Like the DNA sequencing technologies used to detect genetic mutations, MilliDrop technology can detect changes in heritable phenotypes in growing bacterial populations. Cells are collected at regular intervals and inserted individually into a 100 nanoliter droplet. Their growth is tracked over 15 generations by means of a fluorescent protein. The growth trend in an E.coli population was tracked for 30 days without any nutritional injection to reveal a rapidly emerging diversity of heritable phenotypes. The mutations corresponding to each phenotype were then identified using sequencing.

Applications *: facilitate the directed growth of microorganisms to help create proteins used in numerous domains, such as therapeutic proteins, enzymes for molecular biology, industry or biocatalysis.*

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4 Growing microbes in millifluidic droplets, L. Boitard, D. Cottinet, N. Bremond, J. Baudry, J. Bibette. Eng. Life Sci., 2015S.

5 Lineage tracking for probing heritable phenotypes at single-cell resolution, D. Cottinet, F. Condamine, N. Bremond, A. D. Griffiths, P. B. Rainey, J. A. G. M. de Visser, J. Baudry, J. Bibette, PLoS ONE, 2016, 11, 0152395

PARTNERSHIPS AND FUTURE DEVELOPMENTS

Partnerships

MilliDrop is pursuing a partnership strategy with academic and private research laboratories to explore potential new applications. The ESPCI has a special place within MilliDrop's network of partners.

ESPCI (École Supérieure de Physique et de Chimie Industrielle), Paris

ESPCI (major institute of higher education in science and engineering, which recruits students via the most selective competitive examination in France) in Paris

MilliDrop, born at the ESPCI, continues to work closely with the existing laboratories to gain access to the latest technological innovations. Early-stage research takes place at the ESPCI, while development is a core part of MilliDrop's activities.

To enhance its *In Vitro* Diagnostics offering, MilliDrop also works closely with clinical laboratories. This affords it access to patient samples and enables it to compare the results obtained with the MilliDrop Analyzer with those produced using standard methods.

Future development plans

- Project to observe the behavior of several bacteria within a single drop and the relationships between them.
- Project to reproduce anaerobic conditions to cultivate bacteria previously impossible to cultivate.

A very wide range of research areas—biofilms, intestinal or cutaneous microbiota—could benefit from MilliDrop's millifluidic technology.

MIT's Innovators Under 35 prize
The MIT Technology Review ran the rule over the leading talent in France for the fourth year in a row. After considering 200 candidates, it bestowed awards on 10 young French innovators in May 2016, including Laurent Boitard, MilliDrop's Founder and Chairman.

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COMPANY PROFILE

Winner in the French national innovative technology business creation awards, MilliDrop was founded in 2015 and received a grant from the SATT IDF Innov — French equivalent of the *Office of Technology Transfer* — to fund the development of its prototype.

MilliDrop is the product of ten years of research in Colloids and Dispersed Materials and is part of the ESPCI's incubator in the Institut Pierre-Gilles de Gennes, a one-of-a-kind microfluidic research facility. It has 15 research teams made up of around 165 researchers working on numerous international academic and entrepreneurial collaborative projects.

Harnessing its millifluidic technologies, MilliDrop develops and markets systems for microbiology applications that incubate, analyze and handle thousands of hundred-nanoliter-scale samples in parallel. Its proprietary technology is protected by seven patents.

In February 2016, MilliDrop raised €1 million from Quadrivium 1, Seventure Partners' seed fund, for the market introduction of its first instrument.

Laurent Boitard, Cofounder and CEO of MilliDrop Instrument SAS

"This change in scale has opened up a realm of new possible applications across different sectors of industry–from food and health to the environment. Wherever there is a need to identify or understand microbial flora. We design simple systems that integrate with existing automated platforms. Affordable for smaller organizations, these systems bring high-throughput phenotypic screening into the mainstream."

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MANAGEMENT TEAM

Laurent Boitard

CEO & cofounder

Laurent Boitard graduated from the ESCPI ParisTech school of engineering. After a PhD at Université Denis Diderot, he became interested in millifluidic technologies while a research assistant at US-based Raindance Technologies. There realized how these technologies could be applied in microbiology. He then created a model automating micro-organism culture and analysis in the laboratory of Colloids and Divided Materials at the ESPCI in Paris. He developed the technology in the Pierre-Gilles de Gennes (IPGG) Institute's incubator. After securing the intellectual property, he focused on industrializing the production of his automated devices.

Denis Cottinet

CSO & cofounder

Denis Cottinet graduated from the ESPCI ParisTech school of engineering. He then completed a PhD at Université Pierre et Marie Curie. In the laboratory of Colloids and Divided Materials, he worked on demonstrating the potential for millifluidics in analyzing diversity within bacterial populations. He joined MilliDrop's senior management in December 2015.

Jairo Garnica

CTO & cofounder

Jairo Garnica completed a PhD at the University of South Australia. He was responsible for the development of micro-organism screening platforms in the Colloids and Divided Materials Lab for five years. He is the co-inventor of three of the patents licensed by MilliDrop.

Patrick Martin

COO

Patrick has an Electronics, Optronics & Systems PhD and has a 20-year international experience in the High Technologies Industry. He was Production Director at Excico's before coming to MilliDrop.

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STRATEGIC COMMITTEE EXPERTS

A. Permanent members

Jérôme Bibette

Cofounder

Jérôme Bibette is the director of the laboratory of Colloids and Divided Materials Lab, which he founded at the ESPCI in 2001. He has an international reputation for his research in emulsions, magnetic particles and other colloids. He is a member of the prestigious Institut Universitaire de France, has been awarded the CNRS' silver medal and has written over 150 peer-reviewed articles for international journals and holds 45 patents. He is the co-founder of five companies : Ademtech (2000), which synthesizes magnetic particles for biotechnology applications (diagnostics, genetics and immunology); Raindance Technologies (2004), the world leader in digital microfluidics; Capsum (2008), which offers new materials and carriers of active ingredients for the cosmetics market, and won the 2012 prize for innovation; HiFiBiO (2013), a start-up that uses its microfluidic platforms to discover therapeutic antibodies and Biomillenia (2014), a start-up that uses microfluidic platforms to discover industrial enzymes.

Alain Rousseau

IDN Engineer from Lille Central School, holder of a Fluids Mechanics University Degree, Alain Rousseau created, developed the instruments and organized the production of the international success "Diagnostica Stago" range. In 2003, he became Director of Biocode Hycel, which he sold in 2007 to IDS Plc, who launched the conceived system, first called 3X3 then IDS-Isys, applied to specialized endocrinology. In parallel with his activities at Biocode Hycel's, Alain Rousseau conceived, as a consultant, from 2004 to 2008, a high-speed machine in haemostasis for Trinity Biotech, a company bought in 2010 by Stago. He founded Arteion, a Counsel Company for pharmaceutical industry and IVD. Alain Rousseau is the origin of more than 30 patents: sensors, car industry, connections and in-vitro diagnostics...

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Philippe Tramoy

Philippe Tramoy has a biochemistry and an immunology & genetics Degree of Bourgogne University, he graduated from Lyon's École Normale Supérieure, from the Company Administration Institute of Paris Panthéon-Sorbonne (Finances Master's Degree) and from HEC Paris (Intelligence Marketing Master's Degee). He was project manager and Business Development Director in Europe and Israel, in the biotechnologies sector within Rhodia Corporate, Rhodia Chirex and Rhodia Pharma Solutions. In 2004, he founded CBDMT, a market & business intelligence company. Philippe Tramoy has developed a wide network of marketing, strategy, finances and business development experts. Since 2013, he's worked as Investment Director for Seventure Partners' Quadrivium 1 Seed Fund.

B. Guest members

Philippe Nérin

Cofounder

Philippe Nérin, an independent expert in medical instruments and the management of industrial innovation. For ten years, he headed up the Research and Development department of Horiba Medical, a subsidiary of the Japanese group Horiba. He led several research and development teams at both SMEs and large groups where he oversaw clinical trials in conjunction with clinical teams at hospitals in Bordeaux and Montpellier. He has directed the optics and photonics research and innovation department for eight years at Fogale Nanotech as a senior manager and shareholder. He has authored over 15 peer-reviewed articles for international journals and holds 15 patents. During his industrial career, he has been involved in developing and marketing over 10 high-tech products. In 2012, he won the Shinkichi Horiba Award for the most impressive innovations by the Japanese group's employees. He was also involved in filing a patent that is currently used by MilliDrop Instruments.

Paul Rainey

Cofounder

Paul Rainey, research director and professor at the University of Massey (New Zealand), Member of the Max Plank Institute, Blaise Pascal chair holder at ENS Ulm, he possesses over 30 years' experience in microbiology.

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