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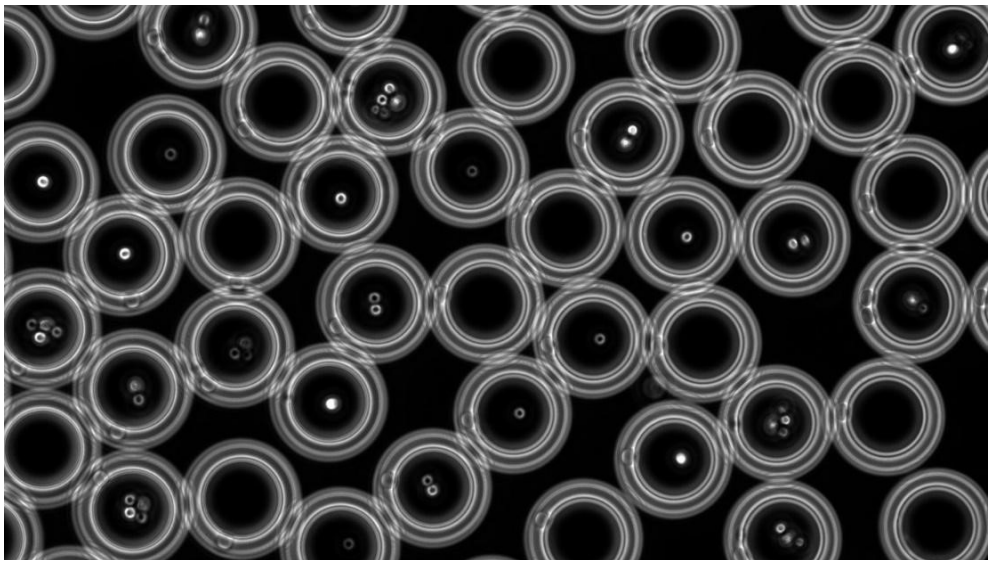
BACTERIA AND YEAST ENCAPSULATION METHOD IN SMALL DOUBLE EMULSIONS

APPLICATION NOTE



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INTRODUCTION

In recent years, bacteria and yeast encapsulation methods have expanded exponentially. In particular, droplet microfluidics has become a powerful technique for bacterial and fungal encapsulation due to its unprecedented performance, efficiency, and precision.

Bacteria and Yeast encapsulation has become a vital method in the research of fungi and bacteria, which are ubiquitous in the ecosystem and play a significant role in many ecosystem functions. Due to high surface area to volume ratio and easy genetic manipulation, fungi and bacteria show an impressive metabolic capacity to produce biopharmaceuticals and recombinant proteins (1, 2). Fungi and bacteria feature simple cellular structures and rapid growth rates, enabling certain microorganisms as powerful model organisms for studying cellular aging and genetic expression.

To carry out the Bacteria and Yeast encapsulation method, emulsions containing the fungi or bacteria of interest must be generated. Unlike single water-in-oil (w/o) emulsions, double w/o/w emulsions (DE) provide an **aqueous carrier fluid**, which makes the **emulsion compatible with most flow cytometry and cell sorting setups.** Typically, double emulsions are produced in batches by a two-stage emulsification process (mixing in bulk), resulting in a highly polydisperse population with low encapsulation efficiency. Compared to currently existing techniques, droplet-based microfluidic techniques offer maximum control over droplet generation, enabling the production of highly stable and monodisperse double emulsions for Bacteria and Yeast encapsulation (3,4).

However, to ensure the compatibility of these droplets in analysis devices, they must be significantly smaller (< 60 µm in diameter) than commercial cell sorter nozzles (typically 70-130 µm in diameter) and, at the same time, large enough to encapsulate variants of interest within the volume of the inner core (5).

Consequently, the method of yeast and bacteria encapsulation within double emulsions small enough for further analysis represents a breakthrough in numerous applications, as it simplifies the process of analysis and screening of bacteria and fungi, and offers distinct advantages in the assessment of cell viability and function.

Therefore, in this application note, we perform a Bacteria and Yeast encapsulation using the Cell Encapsulation Platform, developed by Secoya and consisting of Fluigent's fluid handling system and Secoya's emulsification technology, the RayDrop™.

We demonstrate an easy-to-use and robust workflow for encapsulating the yeast strain S.Cerevisiae CEN.PK 113-7D and the bacterial strain L.cremoris MG1363_GFP within highly monodisperse DE droplets small enough (42µm) for high-throughput cell screening/sorting.

This application note was created in collaboration with Secoya and TU Delft. The images used are courtesy of TU Delft; Marijke Luttik, Sagarika B. Govindaraju and Rinke van Tatenhove-Pel.

HOW TO ENCAPSULATE BACTERIA AND YEAST IN SMALL DOUBLE EMULSIONS

1. Materials

1.1. Materials: Products

To perform Bacteria and Yeast Encapsulation, the Cell Encapsulation platform is used. The platform allows users to keep all components in one place. This helps maintain a good overview, keeps the RayDrop™ standing vertically to drain air to the top, and allows continuous monitoring thanks to its horizontal microscope.
The Cell Encapsulation platform, developed and manufactured by Secoya, combines Secoya's emulsification technology: the RayDrop™ and the pumping technology of Fluigent.

Figure 1. *Cell Encapsulation Platform.*

Emulsification technology:

Droplet generator:

• The RayDrop™ is used to control droplet generation. It allows the generation of the double emulsion in a single step, thus providing a continuous and highly reproducible process.

• The RayDrop™ relies on the alignment of two glass capillaries inside a pressurized chamber. A 3D-printed micro-nozzle is additionally connected at the tip of the injection capillary, enforcing the dripping of small droplets. This non-embedded design presents both the characteristics of a co-flow (axisymmetric geometry) and a flow-focusing (dramatic local accelerations of the continuous phase), and is called non-embedded co-flow-focusing.

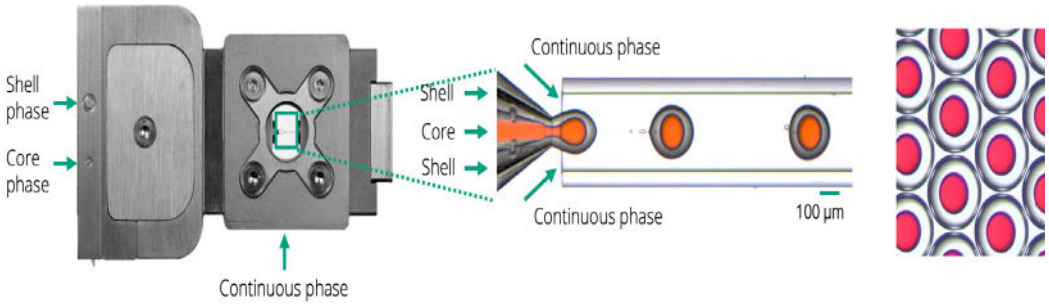


Figure 2. *The RayDrop™ Double Emulsion.*

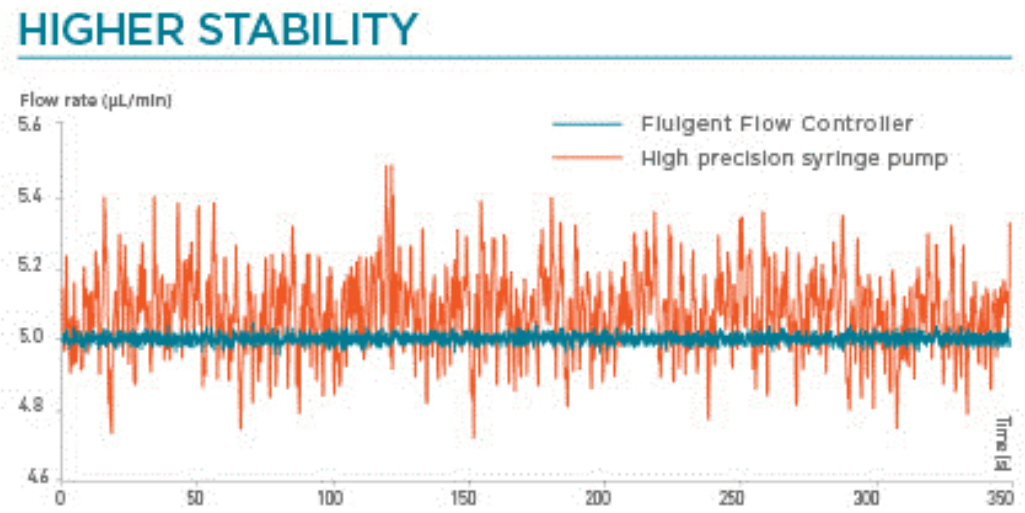
Fluid handling system:

Microfluidic flow controller:

Flow EZ

• Our pressure-based flow controllers are a great alternative to traditional syringe pumps, since, unlike the latter, they offer high stability, pulseless flow, fast response time, the possibility to handle fluid volumes of several liters, and the ability to control fluids in dead-end channels. Their high accuracy and stability allow us to perform a continuous and reproducible droplet generation process, and therefore, a Bacteria and Yeast encapsulation with high monodispersity.

• The Flow EZ is the most advanced flow controller for pressure-based fluid control. It can be combined with a Flow Unit to control pressure or flow rate. **It can be used without a PC.** Three Flow EZs with 7 bars of full-scale pressure are used in the setup presented below.



Flow sensor:

• The Flow Unit is a flow sensor that allows real-time flow rate measurement. By combining a Flow Unit with the Flow EZ, it is possible to switch from pressure control to flow rate control, allowing for the generation of highly monodispersed droplets over a long period of time.

•Two Flow Unit M's (max flow-rate 120 µL/min) are used here to monitor and control the flow rates of the core and shell phases while a Flow Unit L is used to control the flow rates of the continuous phase during the run.

Optical system:

• The Cell Encapsulation Platform for Bacteria and Yeast encapsulation includes an optical system optimized for the observation of the emulsion formation inside the Raydrop™.

• It is composed of a HI-power LED, 10x magnification objective and a ccd camera.

OPTIONAL MODULE: Injection Loop

The Cell Encapsulation platform with an injection loop allows users to work with samples with very small amount of material (e.g. working with cells of limited availability, such as stem cells, primary cells from patients, etc.). It is ideal for the use of rare cells or expensive reagents.

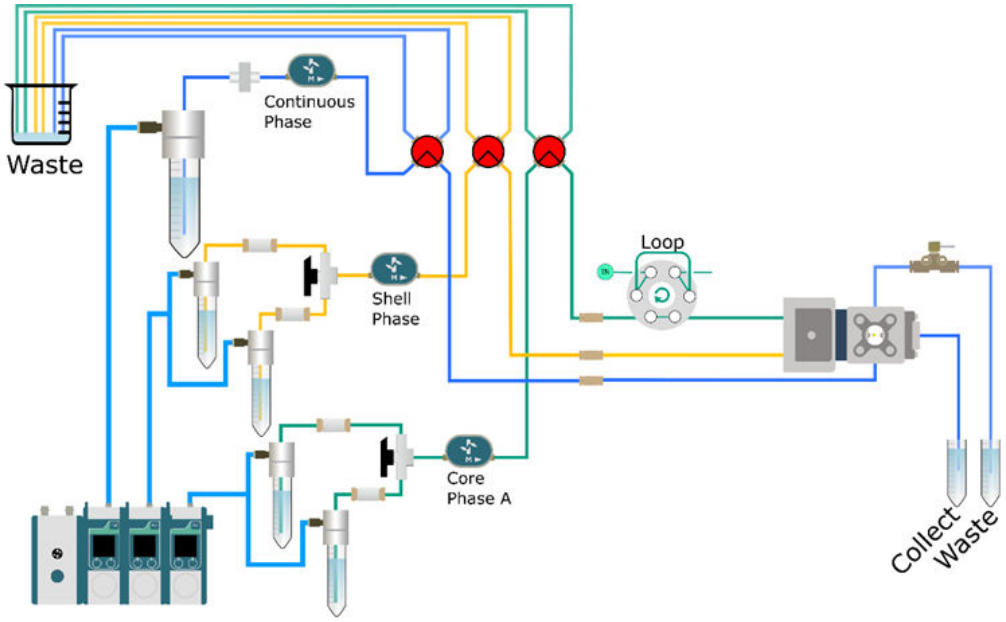


Figure 3. *Cell Encapsulation platform set-up with an injection loop (L-Switch).*

1.2. Materials: Reagents

	Yeast Encapsulation	Bacteria Encapsulation
Continuous phase	Water + 2% Tween20	Water + 2% Tween20
Shell phase	dSurf (HFE7500 + 2% biocompatible surfactant)	dSurf (HFE7500 + 2% biocompatible surfactant)
Core phase	S. Cerevisiae CEN.PK 113-7D was grown on SM, a chemically defined medium for Yeast.	Lactococcus lactis subsp. cremoris MG 1363 was grown on Chemically Defined Medium for prolonged cultivation (CDMPC).

2. Methods: Bacteria and Yeast Encapsulation

The versatility of the RayDrop™ allows a wide range of sizes of monodisperse double emulsions to be achieved by changing the dimensions of the counter-nozzles or double nozzles. There are several RayDrop™ configurations adapted to the encapsulation of very small biological materials, such as bacteria and yeasts.

The following bullet list provides a summary of the main steps involved, but it should be noted that it is not a detailed description of the manipulation. For further information, readers may refer to other manuals. Visit the Cell Encapsulation Platform datasheet and User Manual to learn more.

• Prepare the shell and core solutions by filtering them using filters with pore sizes of 0.2 µm and 40 µm.
• Fill the appropriate reservoirs with the filtered shell and core solutions.
• Create a single emulsion of the shell solution by adjusting the flow rates of the continuous and shell phases.
• Initiate the encapsulation process by introducing the core solution into the single emulsion to form a double emulsion. This is done by adjusting the flow rate and directing the main phase valve in the reservoir towards the device used for emulsification (such as the RayDrop™).
• After a few seconds, collect the encapsulated bacteria or yeast in Falcon tubes for further analysis. The double emulsions will have a protective shell surrounding the core solution, which contains the bacteria or yeast allowing for in vitro analysis.

RESULTS

Once the Bacteria and Yeast encapsulation method is complete, the generated double emulsions are visualized under a microscope to check their monodispersity and stability.

1. Yeast Encapsulation in Small Double Emulsions

Figure 4 shows a successful encapsulation of yeast (S. cerevisiae CEN.PK 113-7D) in DE droplets of 42 µm in size.

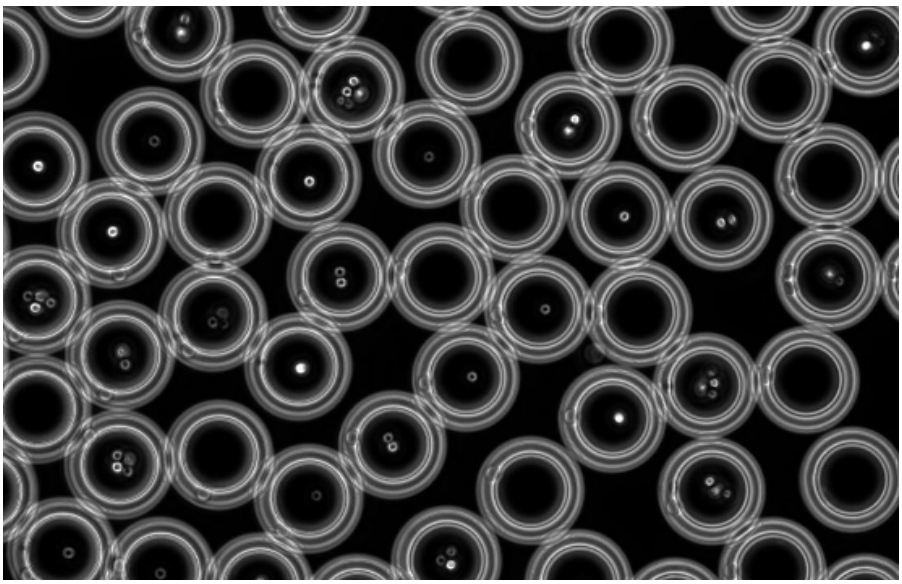


Figure 4. *The yeast strain S. Cerevisiae CEN.PK 113-7D encapsulated within double emulsions of 42 µm.*
Courtesy of TU Delft Marijke Luttik, Sagarika B. Govindaraju and Rinke van Tatenhove-Pel.

2. Bacteria Encapsulation in Small Double Emulsions

Figure 5 shows bacteria successfully encapsulated inside double emulsions with a diameter of 42µm. In both images, a high monodispersity of the droplets can be observed, as well as a homogeneous distribution of the biological material.

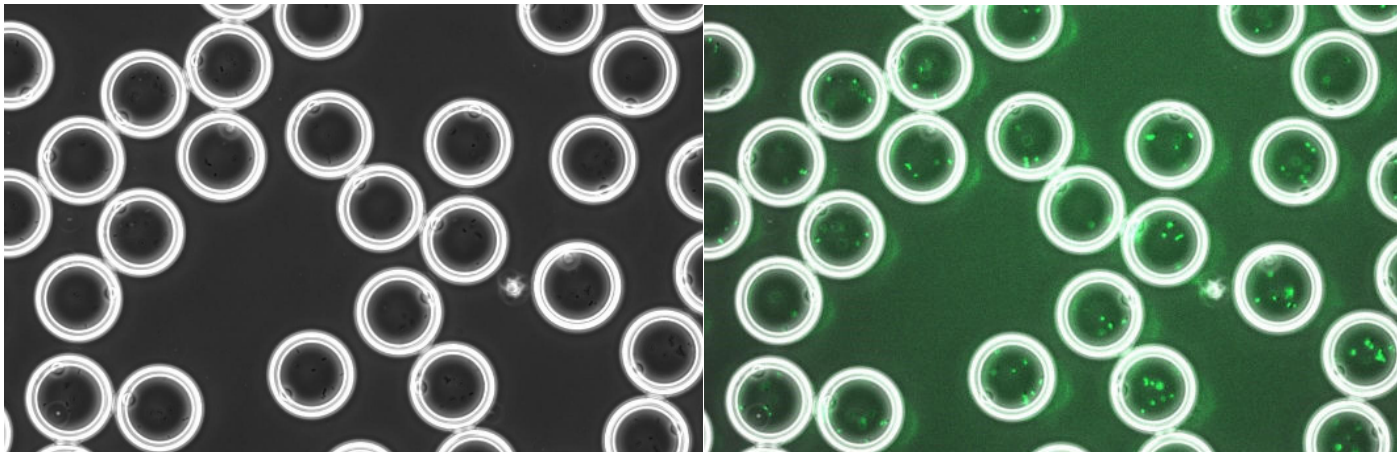


Figure 5. *The bacterial strain L. cremoris MG1363_GFP, encapsulated within double emulsions of 42 µm in diameter. Bright field (up); Overlay of brightfield and fluorescence (down). Courtesy of TU Delft Marijke Luttik, Sagarika B. Govindaraju and Rinke van Tatenhove-Pel.*

CONCLUSION

In this application note, we have demonstrated that the Raydrop™ is able to produce monodisperse double emulsions with an outer diameter below 60 µm, small enough for further analysis. We also demonstrate that the Cell Encapsulation Platform can perform Bacteria and Yeast encapsulation in w/o/w double emulsions with precise droplet size control.

The yeast strain S.Cerevisiae CEN.PK 113-7D and the bacterial strain L.cremoris MG1363_GFP were successfully encapsulated in double emulsions of 42 micrometers in diameter. The high monodispersity and stability achieved are due to Fluigent's stable and pulseless pumping technology and Secoya's emulsification technology: the RayDrop™. Other RayDrop™ configurations and nozzle dimensions are available to target different ranges of droplet sizes and different applications.

References

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