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## **Cellular Heterogeneity Analysis: An Innovative Approach to Single-Cell Metabolomics**

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HAL Authorization

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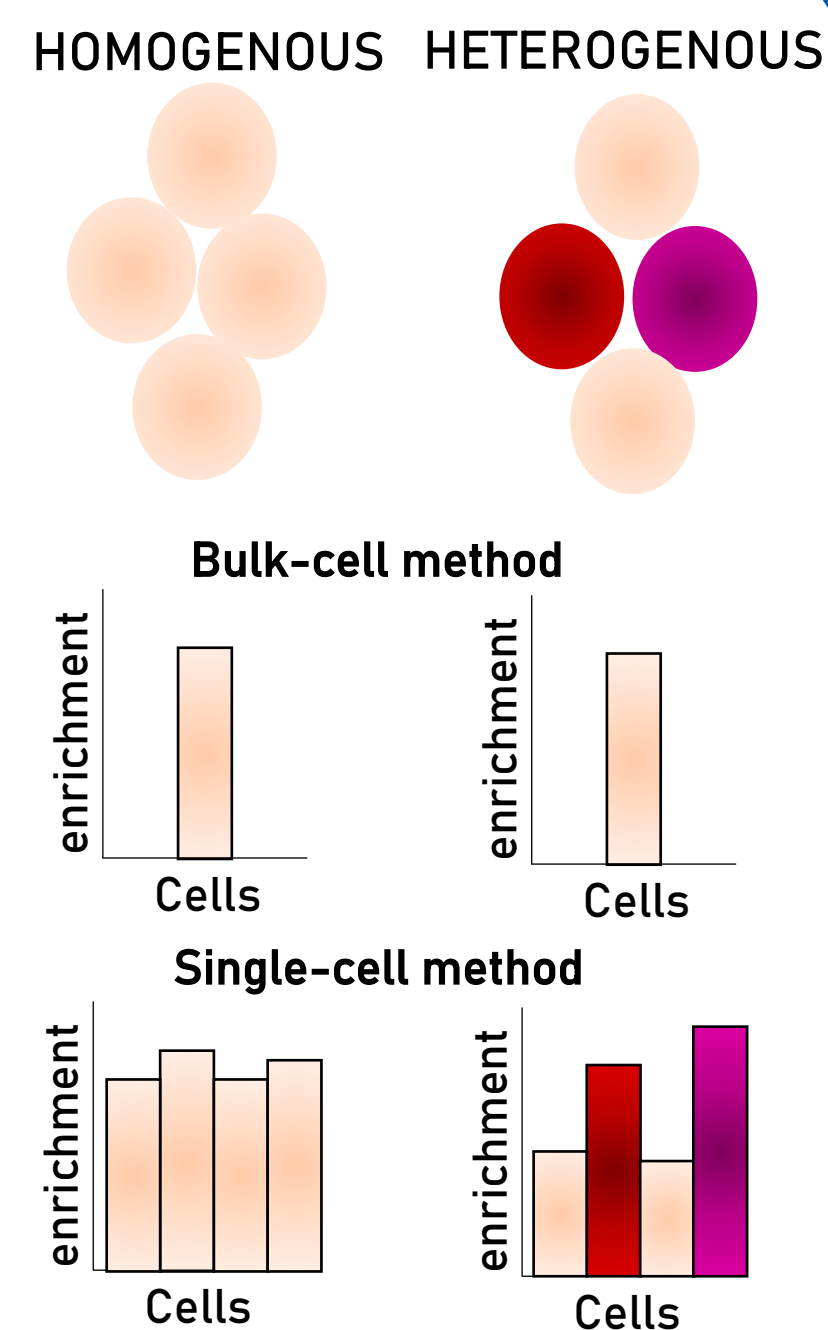
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## Introduction

Cellular heterogeneity significantly influences the phenotype of cultured cells. A better understanding of this heterogeneity is essential in all fields, from biotechnology to healthcare. Single-cell analysis provides a wealth of information for studying cellular physiology and pathology. Among single-cell "omics" approaches, metabolomics stands out by offering rapid and dynamic insights into cellular functions due to the metabolome's immediate response to environmental changes. Single-cell metabolomics is very challenging: low metabolite levels, endogenous and exogenous presence, and the inability to amplify metabolites.

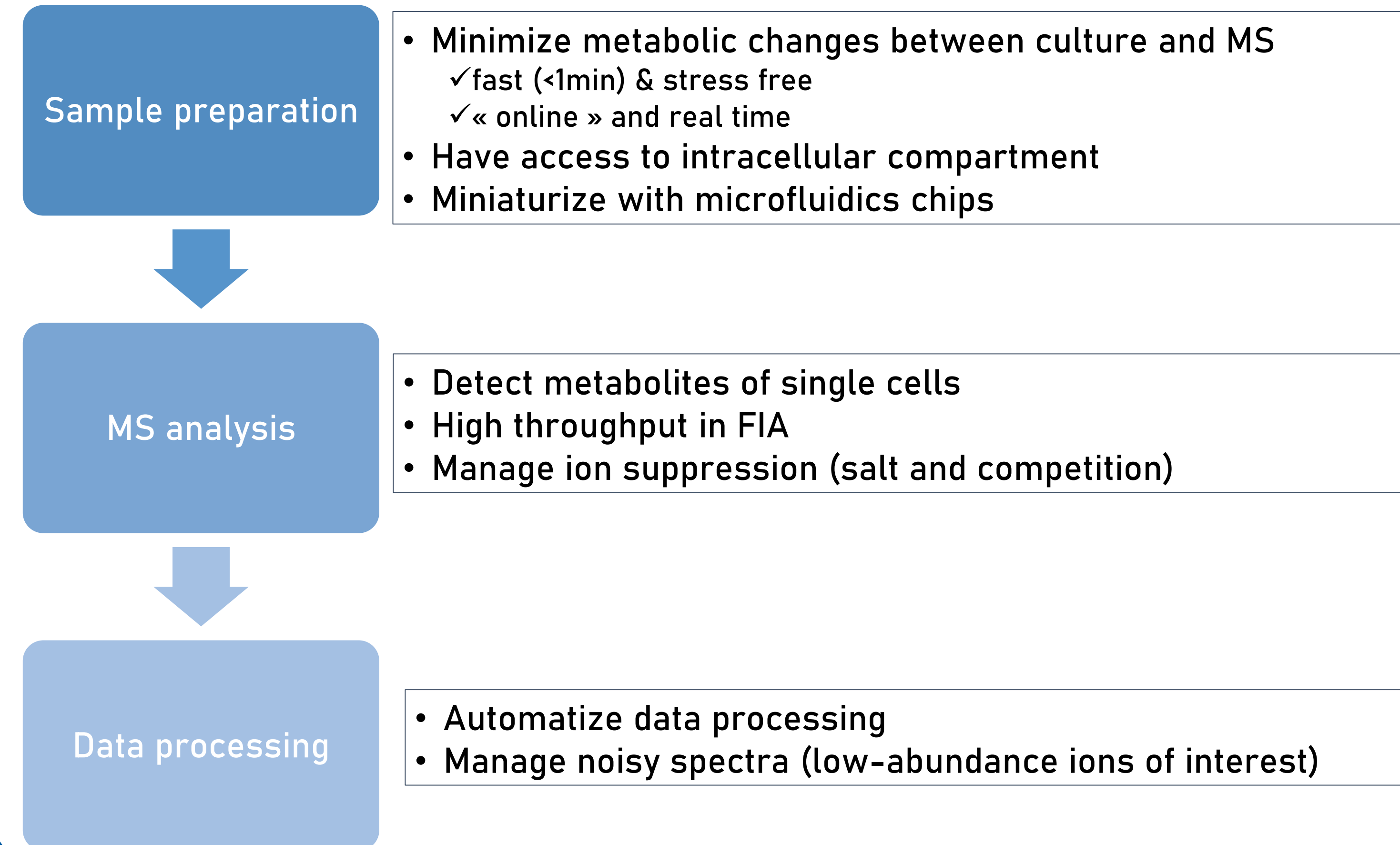
Recent advancements now enable single cell analysis mainly by miniaturizing cell handling, incubation, and enhancing microfluidics chip-coupling to mass spectrometers.

Here we propose an innovative miniaturized system that combines microfluidics with nanoESI-MS for high-throughput on-line detection of cellular metabolites.



## Requirements for functional analysis of metabolism

Challenges at every steps



## Workflow optimisation for single-cell analysis

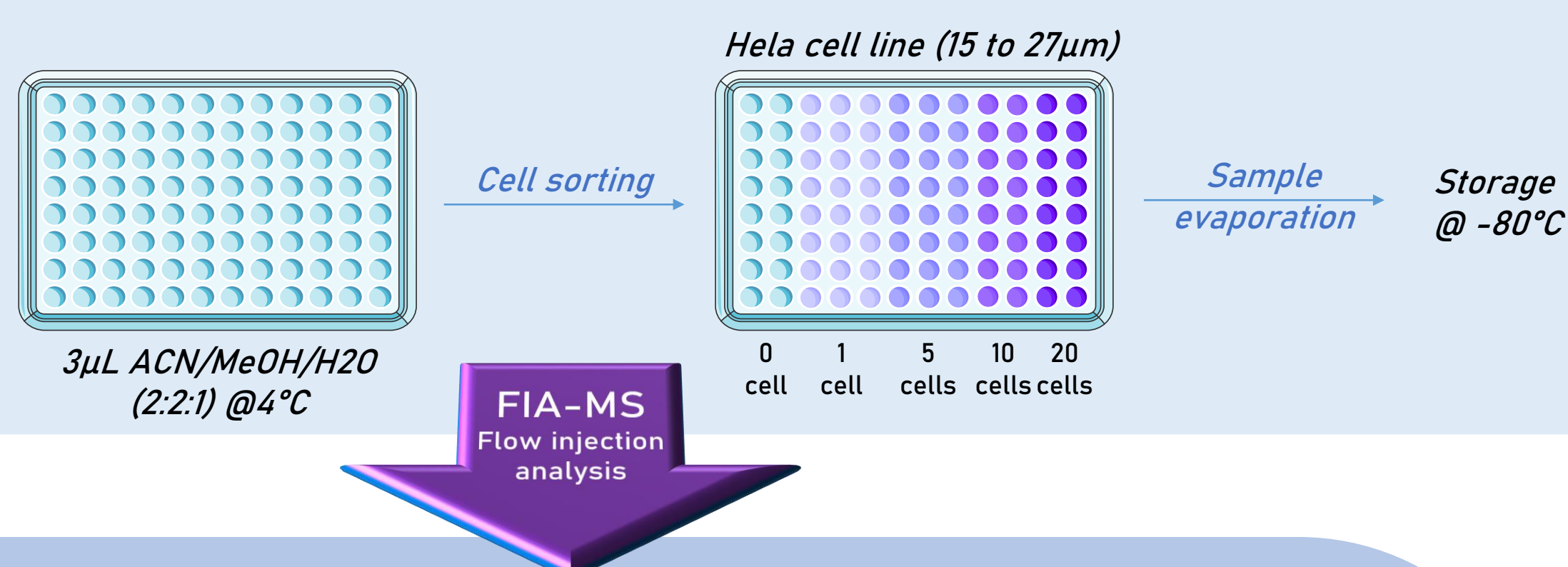
Sample preparation:

### OFFLINE APPROACH

Single cell sorting, isolation & nanoliter dispensing for omics applications



- Image-based technology
- Piezo-acoustic droplet generation for ultra gentle cell isolation → No stress
- Accurate single cell sorting and isolation (~95% accuracy)
- Miniaturized single-cell omics workflows: Metabolomics workflow developed



### MS analysis:

Improve MS detection and interfacing with microfluidics



	Mammalian cell (HeLa)	Yeast (S.cerevisia)	Microorganism (E.coli)
Size	~30µm	5µm	~2µm
Volume	3 000 fL	65 fL	1 fL
Mass	2ng	70pg	1pg
Metabolite Estim. quantity in 1cell	Fmol	Fmol-Amol	Amol



INTERFACE Microfluidics-MS In progress

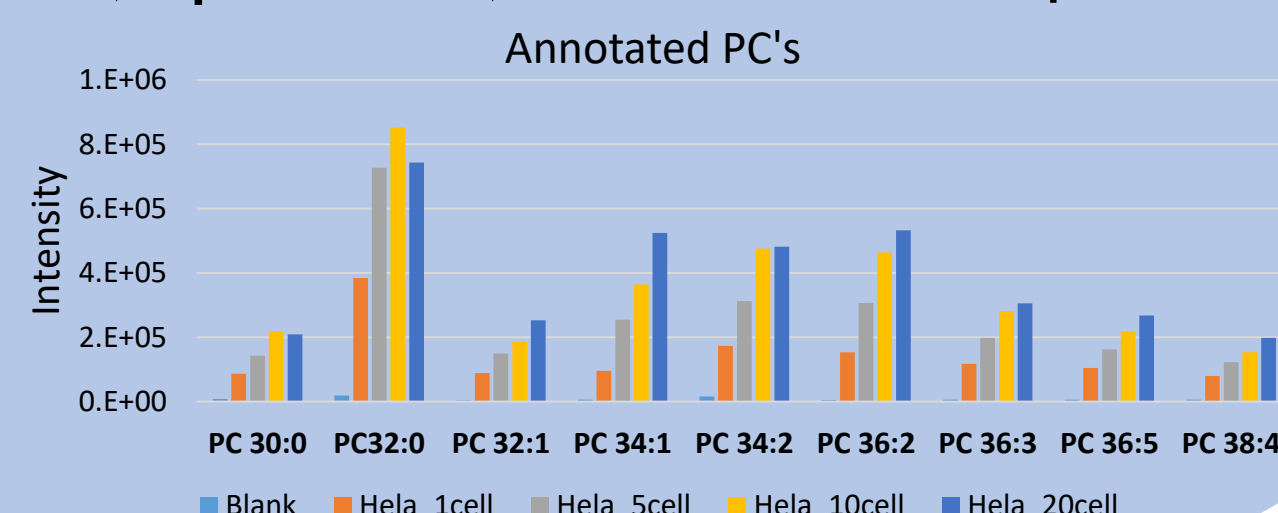
### Sensitivity on metabolites standards (LOD in fmole injected, Orbitrap™ Astral™, neg mode)

Molecule	Precursor	LOD	Molecule	Precursor	LOD	Molecule	Precursor	LOD	Molecule	Precursor	LOD
Ala	88.0404	50 fmol	HexaseP	259.0224	5 fmol	Fumarate-Maleate	115.0037	5 fmol	Uridine	243.0623	5 fmol
Val/Norv	116.0717	5 fmol	GlcNacP	300.0490	5 fmol	Succ-mMalonate	117.0193	50 fmol	dAMP	330.0609	5 fmol
Leu/Ile	130.08774	5 fmol	Pent5P	229.0119	5 fmol	Malate	133.0142	5 fmol	AMP-dGMP	346.0558	5 fmol
Asp	132.0302	5 fmol	Rib5P	308.9782	50 fmol	aKG	145.0142	5 fmol	UDP	402.9949	0.5 fmol
Glu	146.0459	5 fmol	Fru6P	338.9888	50 fmol	Adipate	145.0506	5 fmol	dATP	489.9936	5 fmol
Met	148.0438	5 fmol	GalINIP/GlcN6P	258.0384	5 fmol	Tartrate	149.0092	5 fmol	ADP-dGDP	426.0221	5 fmol
Phe	164.0717	5 fmol	Fru6P	166.9751	5 nM	Orotate	155.0098	5 fmol	UTP	482.9613	50 fmol
Tyr	180.0666	50 fmol	Glycerol3P	171.0064	5 nM	CisAco/TransAco	173.0092	5 fmol	ATP-dGTP	505.9885	5 fmol
Cystine	239.0166	5 fmol	Pser	184.0016	5 nM	Shikimate	173.0455	50 fmol	UDP-Hex	565.0477	5 fmol
Gln	145.06187	50 fmol	Sed7P	289.033	50 nM	Cil/Isocit	191.0197	5 fmol	GDPMan	604.0699	5 fmol

### Sensitivity on biological samples (HeLa cell line, lipidomics, timsTOF Ultra 2®, pos mode)

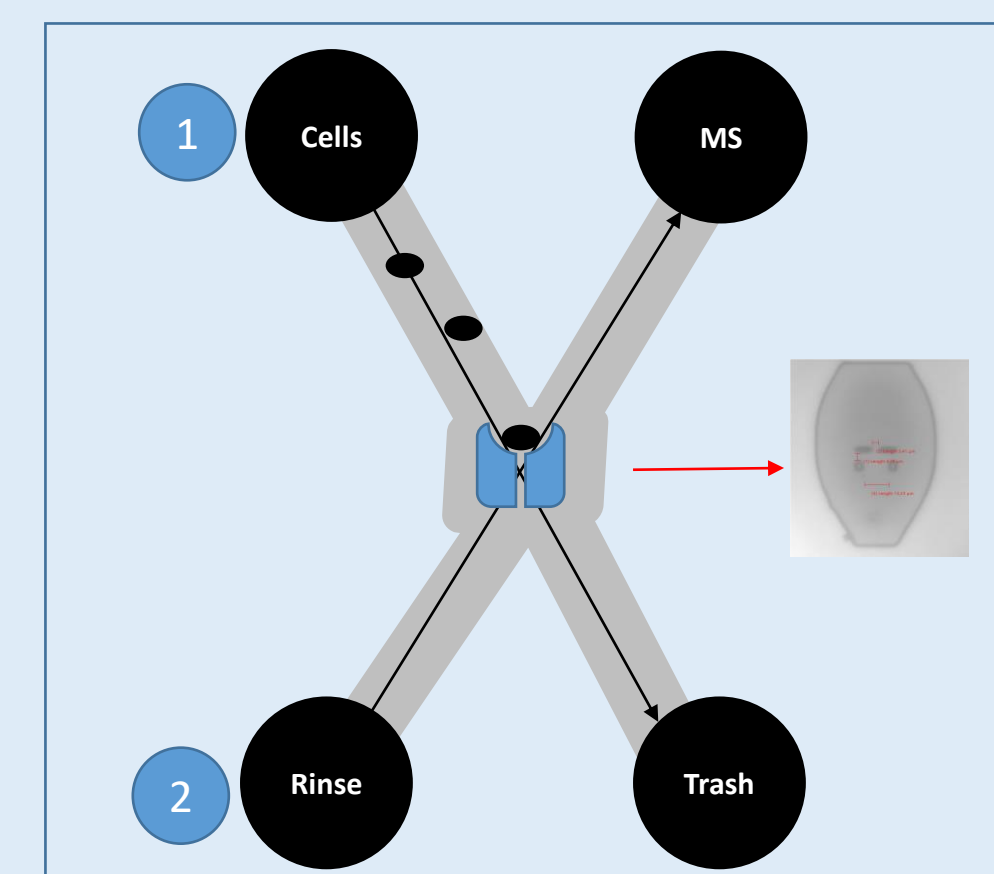
PCA score plot: clustering of samples in function of cell number

- 75 features annotated and 14 detected in 1 cell:
- 11 PC
- 1 TG (54:3)



### ONLINE APPROACH

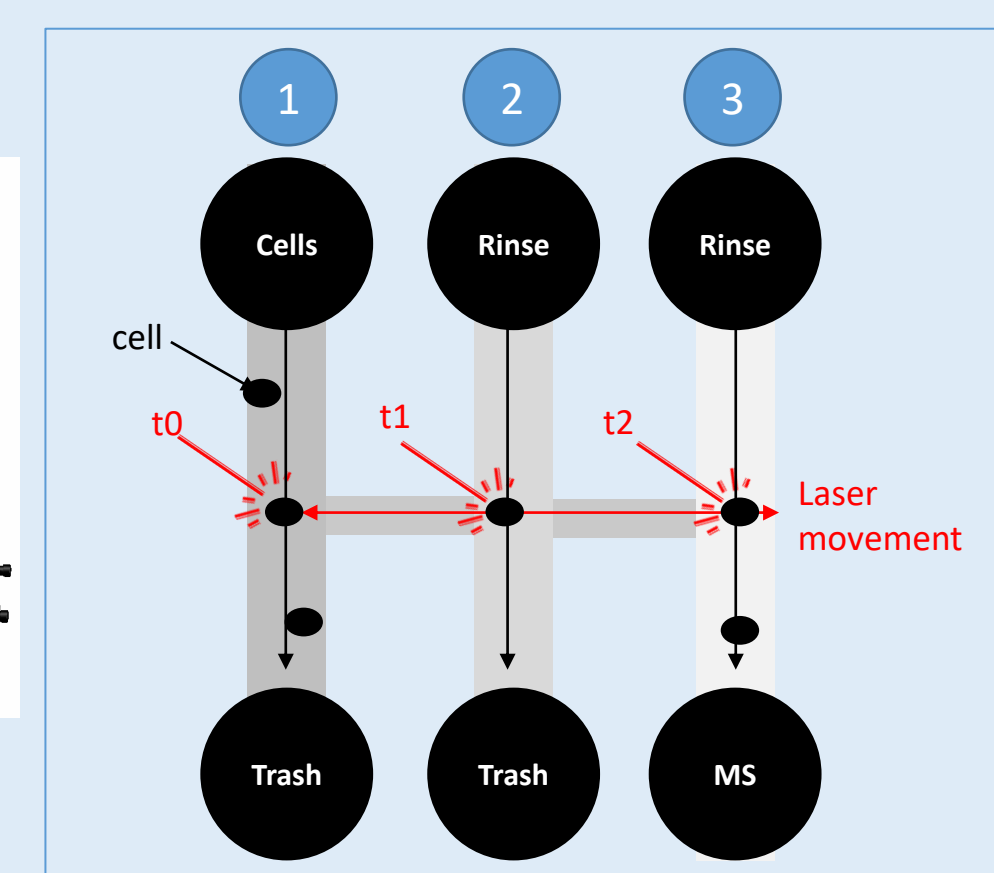
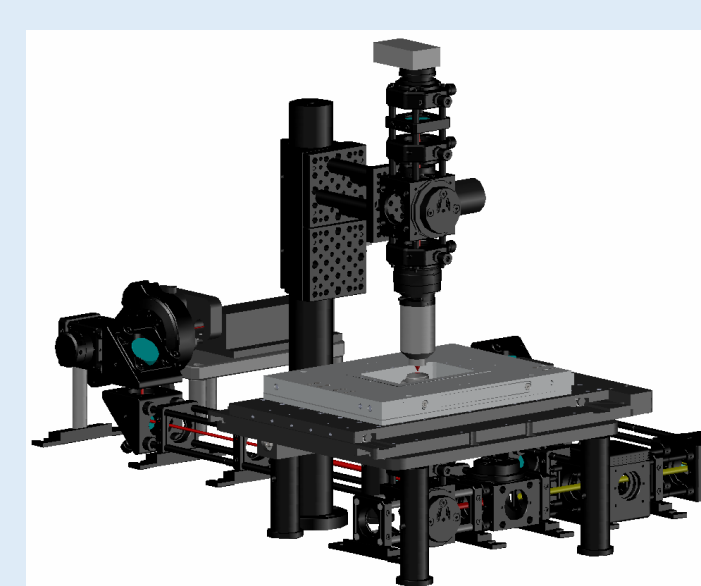
Development of microfluidics devices to manipulate and prepare single cells



- Automation
- High throughput
- Plug and play
- Organism specific (size)
- Physical contact with cell
- Distance between chip and MS

- Cells circulation from culture to trash and 1 cell caught by the trap
- Cell rinsed with 1 stream of rinsing solution and sent to MS

Design 1: Cell Trapping and preparation with PDMS trapping array



- No contact with cell
- Optimal rinsing
- Simple and universal chip
- Complex sample preparation possible
- Complex automation
- Laser (safety)
- space-saving: need optimization

- Cells circulation from culture to trash and 1 cell caught by the laser
- Cell rinsed with 1 stream of rinsing solution
- cell sent to MS with clean rinse solution and laser position reset

Design 2: Cell Trapping and preparation with optical tweezers

## Conclusions

Initial results obtained with the offline FIA-MS approach are encouraging and have enabled us to optimize MS detection. As the sample dilution caused by FIA (leading to a decrease in sensitivity) will be eliminated by direct coupling of microfluidic chips to MS, our energy is now focused on these innovative developments in collaboration with leading laboratories in the field such as LAAS (Laboratory specialising in Systems Analysis and Architecture).

This system is currently being optimized and validated using synthetic cells (with a specific lipid composition of PC, PG and PE) and yeast. Once validated, it will be applied to more complex cell cultures to study cellular heterogeneity. We expect this technology to provide the basis for more sophisticated designs, with the medium-term aim of coupling metabolomic and genomic analyses of single cells to gain a comprehensive understanding of cellular heterogeneity.

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