Correlating longitudinal single-cell analyses with populational biochemical assays using microfluidics

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INTRODUCTION

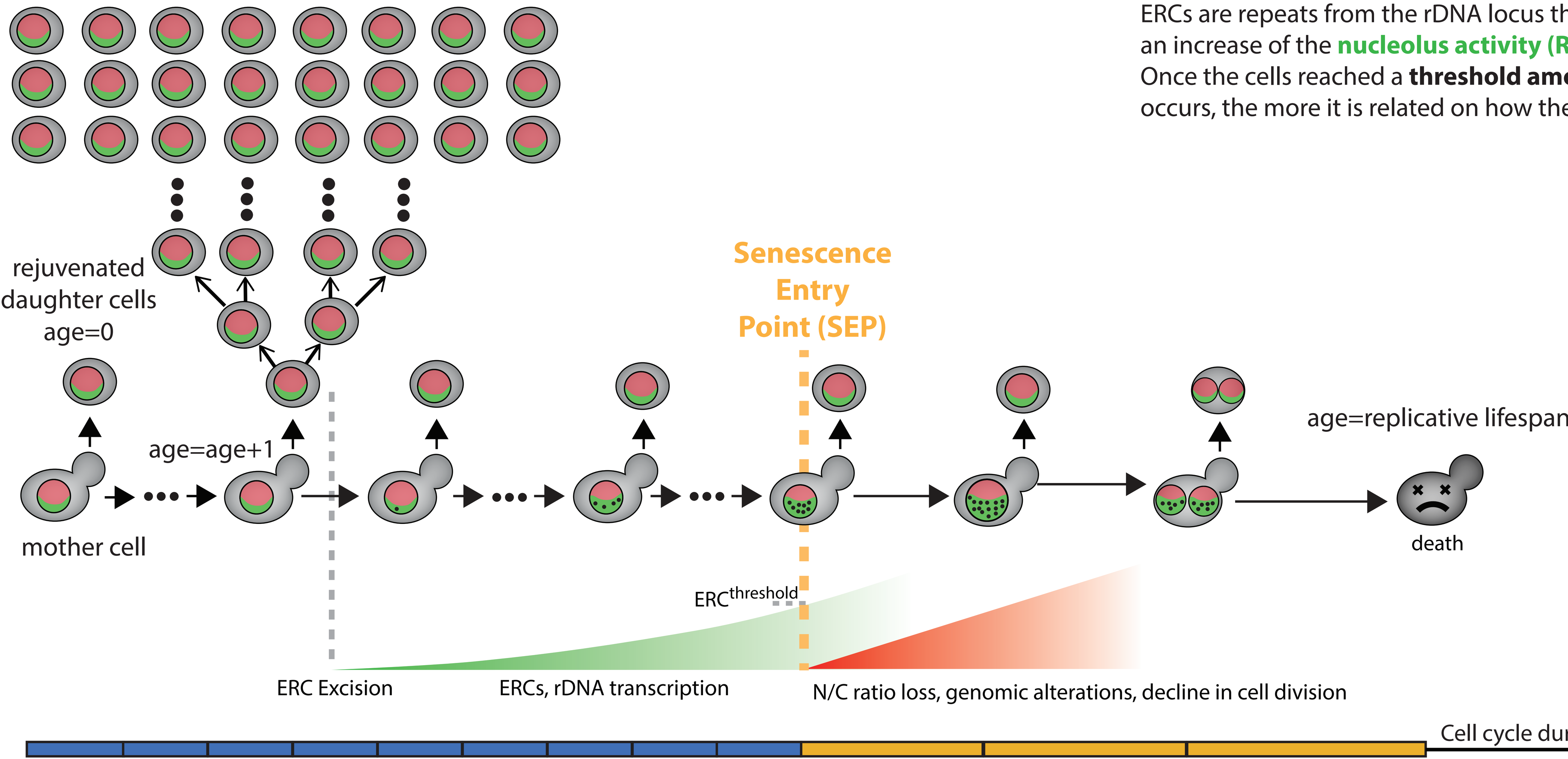
Budding yeast has an asymmetrical division pattern and a **limited replicative lifespan**: mother cells undergo about 20 - 30 rounds of division before dying.
However, daughters of ageing mothers recover a full replicative lifespan (**rejuvenation**). Therefore, mother cells are thought to accumulate and retain 'aging factors', which become toxic and cause senescence.

1) Old cells are rare in a growing population

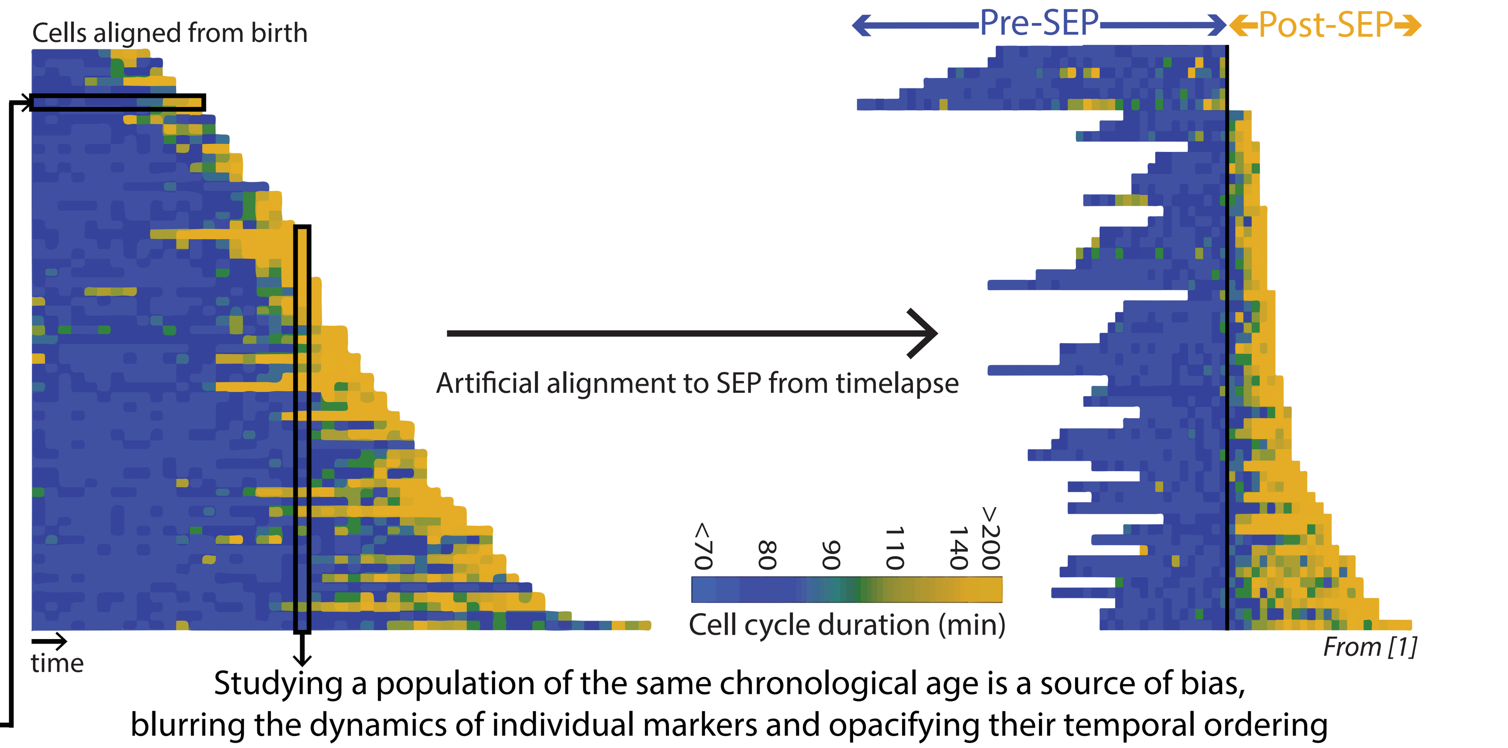
At each round of division, the number of cells doubles with rejuvenated individuals, diluting the old cells. After N divisions, 2^N cells and only one of age N.
eg: In 1mL (10^8 cells), one cell of age 25, two cells of age 24,... and 5.10^7 cells of age 0

2) Most cells follow a common pathway to senescence...

Using timelapse microscopy, our group showed that **budding yeast sharply slows down its division time** after a variable number of divisions (**Senescence Entry Point=SEP**) [1]. This correlates with an **increase of the nucleus volume** and is followed by death.
More recent work [2] showed that this abrupt change of physiology was caused by **Extrachromosomal rDNA Circle (ERC) excision**.
ERCs are repeats from the rDNA locus that can replicate, leading to an amplification at each division and their **accumulation** is concommitant with an increase of the **nucleolus activity (RNA Pol I transcription)**.
Once the cells reached a **threshold amount of ERCs**, the SEP is triggered and and number of various phenotypes appears. The later an event occurs, the more it is related on how the cell survives and dies rather than how and why it aged.



3) ...but the senescence dynamics are highly variable among an isogenic population



Studying a population of the same chronological age is a source of bias, blurring the dynamics of individual markers and opacifying their temporal ordering

HOW TO STUDY REPLICATIVE SENESCENCE FROM A HETEROGENEOUSLY SENESCENT POPULATION?

How is the upregulation of nucleolar activity causal for the loss of nuclear homeostasis and entry into senescence? Are the commonly described aging pheotypes causes or byproducts of SEP? Refining our model of senescence requires approaches complementary to timelapse microscopy, which demands populations of cells from each step of the senescence pathway. To this end, we propose a **new framework** based on **microfluidics** and **FACS**, to **culture and harvest old cells**, before **sorting them according to their senescence stage**.

PRODUCE, SORT AND ASSESS OLD CELLS

SUMMARY OF THE METHOD

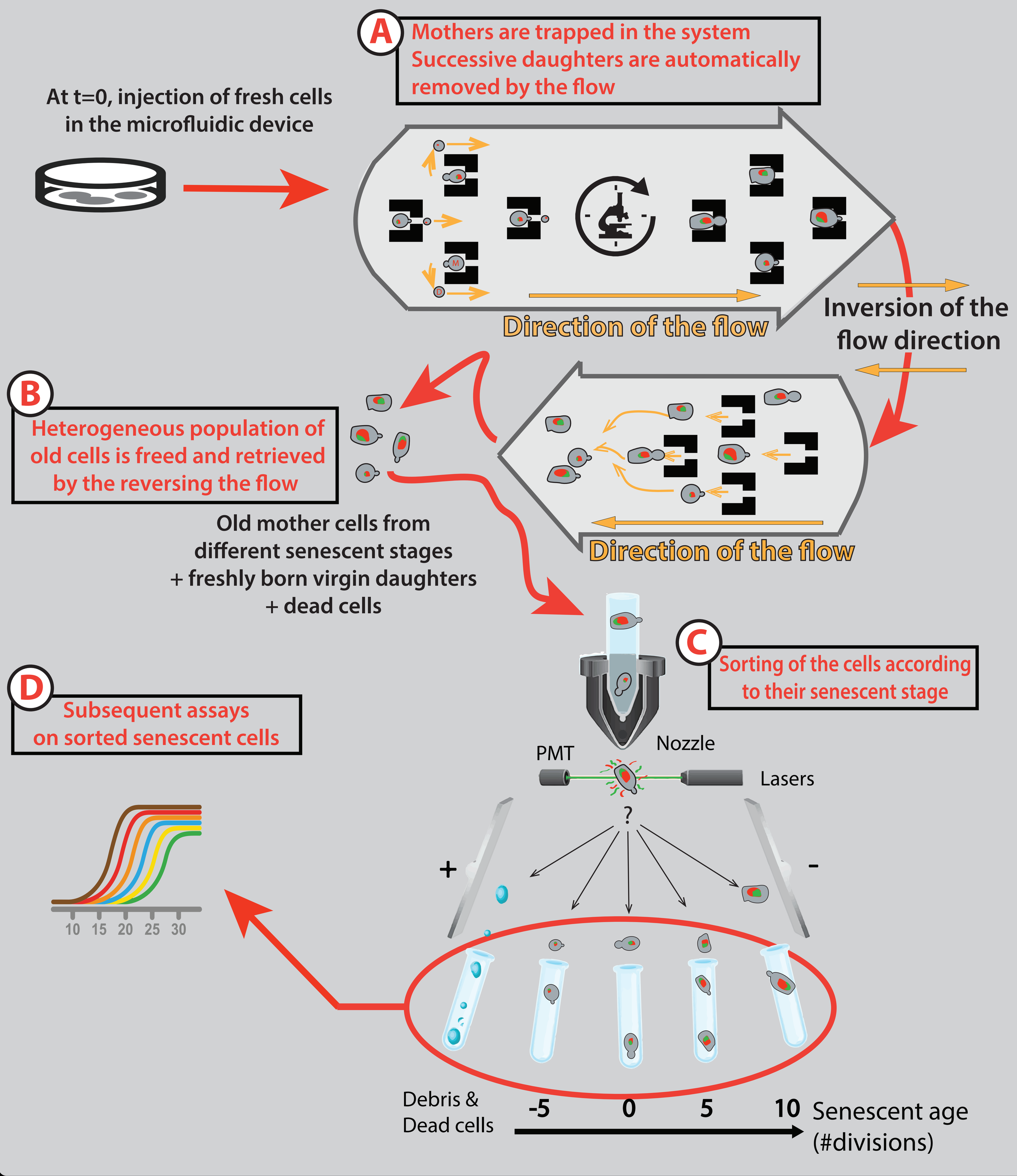
GOAL: Assay synchronized populations regarding senescence, at different stages

Use of a microfluidic system to (A) **trap and age mother cells** inside specific structures that automatically remove the progeny.

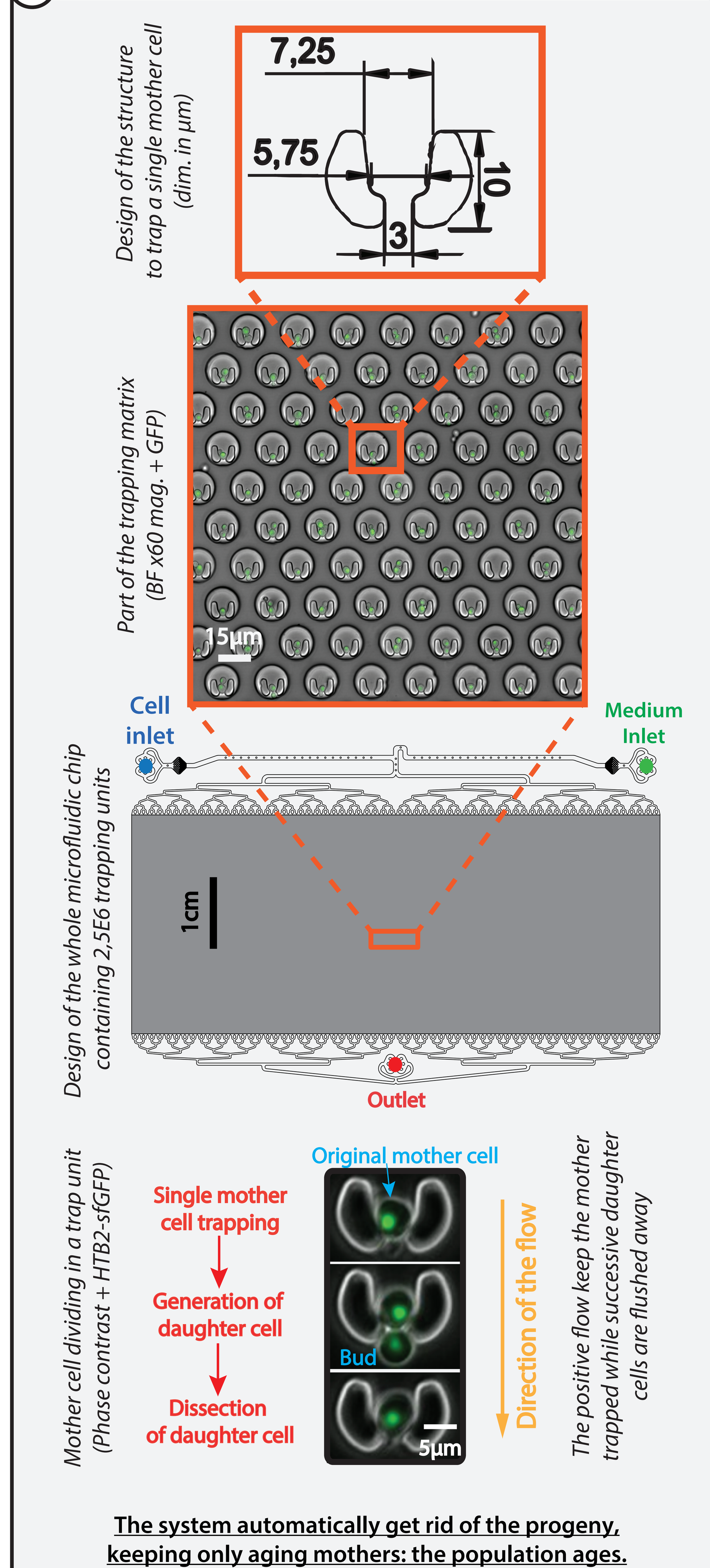
(B) **Harvesting of the old cells** from the traps with a negative flow .

(C) FACSorting to **synchronize cells in population of the same senescent age** (using fluorescent markers).

(D) Finally, standard methods (transcriptomic, tomography...) are applied on the categories to characterize each stage and temporally order events.

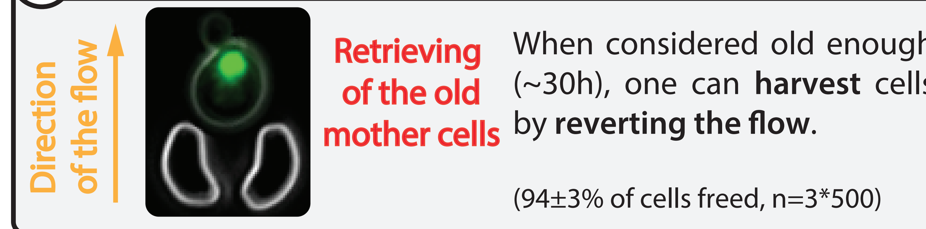


A AGEING A POPULATION OF CELLS



The system automatically get rid of the progeny, keeping only aging mothers: the population ages.

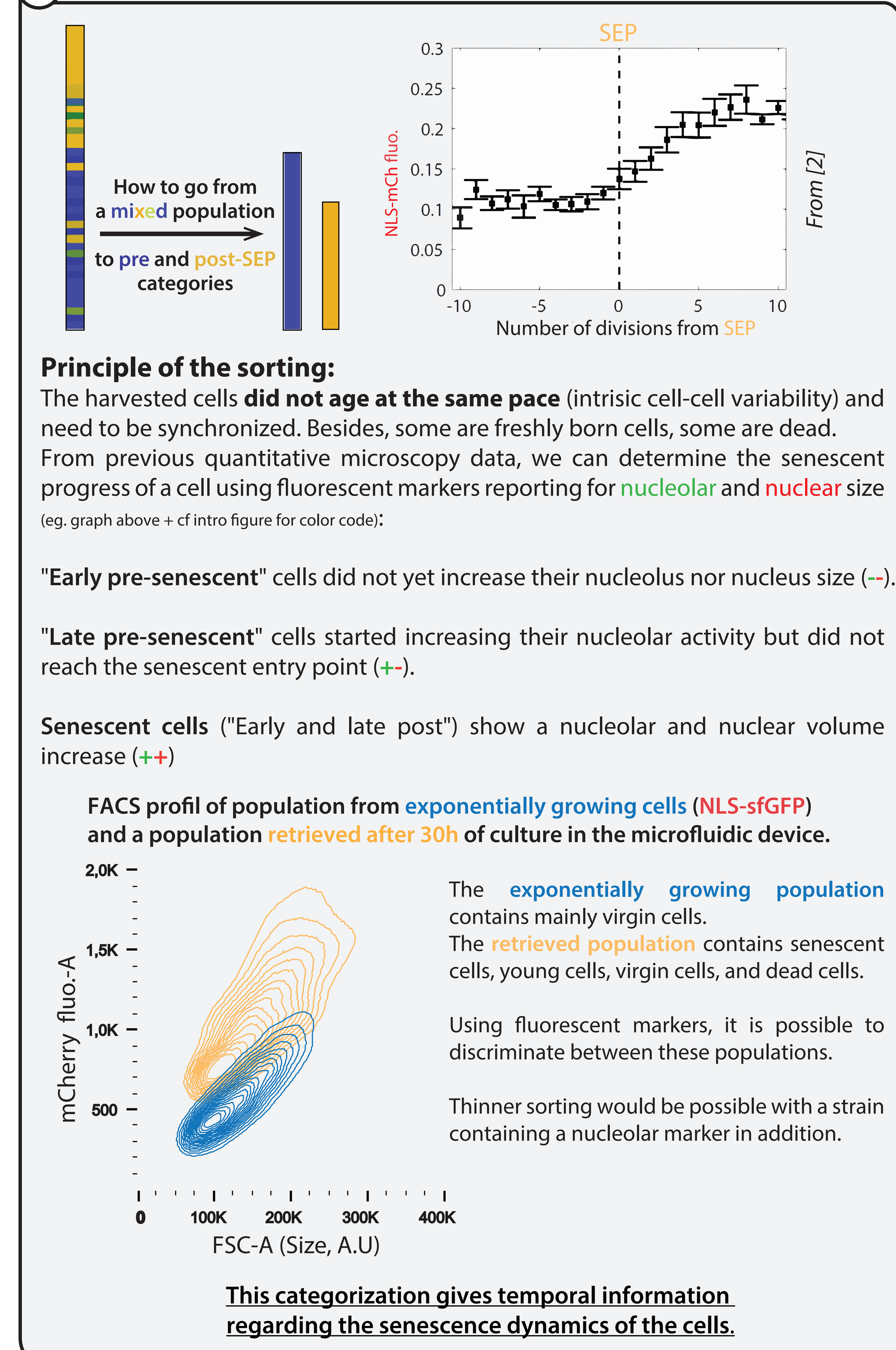
B HARVESTING THE AGED POPULATION



When considered old enough (~30h), one can **harvest** cells by **reverting the flow.**

(94±3% of cells freed, n=3*500)

C SORTING INTO SENESCENT STAGES



Principle of the sorting:
The harvested cells **did not age at the same pace** (intrisic cell-cell variability) and need to be synchronized. Besides, some are freshly born cells, some are dead.
From previous quantitative microscopy data, we can determine the senescent progress of a cell using fluorescent markers reporting for nucleolar and nuclear size (eg. graph above + cf intro figure for color code):

"Early pre-senescent" cells did not yet increase their nucleolus nor nucleus size (--).

"Late pre-senescent" cells started increasing their nucleolar activity but did not reach the senescent entry point (+-).

Senescent cells ("Early and late post") show a nucleolar and nuclear volume increase (++)

This categorization gives temporal information regarding the senescence dynamics of the cells.

D AND NOW, WHAT TO ASSESS?

Once the categories representing different senescence stages are isolated, we can assess different readouts on them, to **gain insight into the actors of the senescence pathway and their dynamics.**

What triggers nucleolar overactivity?
ERCs deregulate the RNA pol1 activity?
>Expression of transcription machinery VS ERCs levels with qPCR.

How is it relayed to the nucleus, inducing a global loss of homeostasis and eventual death ?
Why does nuclear content globally increase? Are the NPCs clogged or malfunctional?
> FIB-SEM.

Cause or byproduct of SEP: where stand the commonly described events?
Loss of nucleosomes and chromatin silencing > ATAC-seq.
Genomic instabilities, sterility, ribosomal processing.

Validated assays:
- ✔ Transcript levels by qRT-PCR
- ✔ ERCs levels by qPCR

SUMMARY

→ Senescence occurs **sharply** in budding yeast, mechanisms are unknown.
→ A **common pathway of senescence** seems to be shared by most of the cells implying **Extrachromosomal rDNA Circle excision and amplification**...
→ ...but **high cell-cell variability** regarding the dynamics of entry into senescence.
→ Quantitative microscopy allows to observe senescence longitudinally but complementary techniques are required to better characterize each step of the process.
→ **Old cells are rare and from heterogeneous senescence stages** in a bulk population.

→ DEVELOPMENT OF A METHOD TO PRODUCE SYNCHRONIZED SENESCENT CELLS BASED ON MICROFLUIDICS AND FACS ←

REFERENCES
[1] Aging yeast cells undergo a sharp entry into senescence unrelated to the loss of mitochondrial membrane potential. Fehrmann S, Paoletti C, Goulev Y, Ungureanu A, Aguilaniu H, Charvin G. Cell Rep. 5(6):1589-99, 2013
[2] Excessive rDNA transcription drives the disruption in nuclear homeostasis during entry into senescence in budding yeast Morlot S, Jia S, Léger I, Matifas A, Gadal O, Charvin G. Cell Rep, (28)2:408-422, 2019