

MERIT Self-Initiated Interdisciplinary Research Report

Identification of Budding Yeast Strains with Different TAG Productivities Using Raman-Activated Cell Sorting

Ryuji Nakao¹, Satoshi Mabe²

¹ Department of Chemistry, Graduate School of Science, Goda Laboratory

² Department of Integrated Biosciences, Division of Signal Transduction, Ohya Laboratory

Student Applicants and Contributions

Ryuji Nakao (Student Applicant 1): Constructed the cell-tracking Raman-activated cell sorting (RACS) system; performed Raman measurement and sorting experiments using BY4741 and AWRI1482; conducted data analysis.

Satoshi Mabe (Student Applicant 2): Provided expertise in evaluating lipid production capacity in budding yeast and cultured BY4741 and AWRI1482.

1. Research Background

For the microbial production of useful compounds, it is important to efficiently select strains with superior productivity for a target metabolite from diverse cell populations generated by genetic engineering or mutagenesis. The budding yeast *Saccharomyces cerevisiae* is widely used to develop strains for useful-compound production because it is readily amenable to genetic manipulation and because extensive knowledge is available regarding its cultivation, metabolism, and cell biology [1], [2]. Student Applicant 2 has been developing budding yeast strains for the production of useful metabolites; however, accelerating this breeding process requires a screening technology that can evaluate target-metabolite productivity in large numbers of cells and recover cells exhibiting the desired phenotype.

Metabolite productivity is commonly evaluated by bulk measurements, in which metabolites are extracted from cell populations and analyzed, or by methods using fluorescent reporters or fluorescent staining. Although these methods provide high sensitivity, bulk measurements cannot readily capture heterogeneity among individual cells within a population and do not allow the measured cells themselves to be recovered. Fluorescence-based sorting also requires the introduction of a reporter corresponding to the target metabolite or an additional staining procedure.

For example, fluorescent dyes such as Nile red are used to evaluate intracellular neutral lipids, and their combination with flow cytometry enables the rapid analysis of large numbers of cells. However, fluorescence intensity may be affected by dye uptake, the states of the cell wall and cell membrane, and staining conditions; therefore, the resulting signal does not necessarily directly represent the amount of the target metabolite itself [3]. A method that can read out intracellular chemical composition and recover target cells without relying on exogenous reporters or fluorescent staining is therefore desirable.

Raman spectroscopy provides label-free information on intracellular components such as lipids, proteins, and nucleic acids from spectra originating from molecular vibrations. Raman-activated cell sorting (RACS), which is being developed by Student Applicant 1 [4], measures the Raman spectrum of each cell moving through a flow channel and physically sorts target cells based on the spectral information. RACS may

therefore serve as a screening platform that reads out intracellular chemical information associated with metabolite productivity at the single-cell level and recovers viable cells that can be used for strain breeding.

In this self-initiated interdisciplinary research project, triacylglycerol (TAG) was selected as a model metabolic phenotype for applying RACS to the breeding and screening of metabolite-producing strains.

2. Research Objective

This self-initiated interdisciplinary research project aimed to determine whether RACS could distinguish specific metabolite productivity at the single-cell level by using two budding yeast strains with different TAG productivities as a model system.

Specifically, the laboratory strain BY4741 [5] and the wine yeast strain AWRI1482, which has higher TAG productivity [6], were used to investigate whether the two strains could be distinguished on the basis of lipid-associated Raman bands.

3. Methods

3.1 Budding Yeast Strains and Culture Conditions

Two budding yeast strains, *Saccharomyces cerevisiae* BY4741 and AWRI1482, were used in this study. Glycerol stocks stored at -80°C were streaked onto yeast extract peptone dextrose (YPD) agar plates and incubated at 30°C for 2–3 days. A single colony was inoculated into 2 mL of YPD liquid medium and precultured at 30°C for 17 h using a rotator. To induce TAG accumulation, 25 μL of the preculture was transferred into 8 mL of nitrogen-limited minimal mineral medium (MM/N-lim1; C/N = 189) prepared according to a standard protocol [7]. For BY4741, 40 mg/L uracil, 100 mg/L leucine, 30 mg/L histidine, and 40 mg/L methionine were additionally supplemented to support auxotrophic growth. Both strains were cultured at 30°C for 96 h using the same rotator to allow TAG accumulation.

3.2 Measurement of BY4741 and AWRI1482 Using RACS

Cell suspensions of TAG-accumulating BY4741 and AWRI1482 were diluted with D-PBS to a final concentration of 1.5×10^5 cells/mL, and their Raman spectra were measured using the RACS system (Figure 1) [4]. During measurement, the cell suspension was introduced into a microfluidic chip using a syringe pump, and an acoustic standing wave generated by a piezoelectric transducer was used to focus the cells into a single stream within the flow channel. The arrival of each cell was detected from the backscattered signal of an 808 nm laser. This signal triggered a two-axis galvanometric scanner to scan a 561 nm excitation laser along the direction of cell flow. Raman spectra were thereby acquired while the excitation beam tracked cells moving through the flow channel. For Raman measurements of yeast cells, a 561 nm laser was used for excitation, with an excitation power of approximately 133 mW after the scan lens and an exposure time of 70 ms. The acquired Raman spectra were baseline-corrected using the Savitzky–Golay coupled advanced rolling circle filter (SCARF) [8].

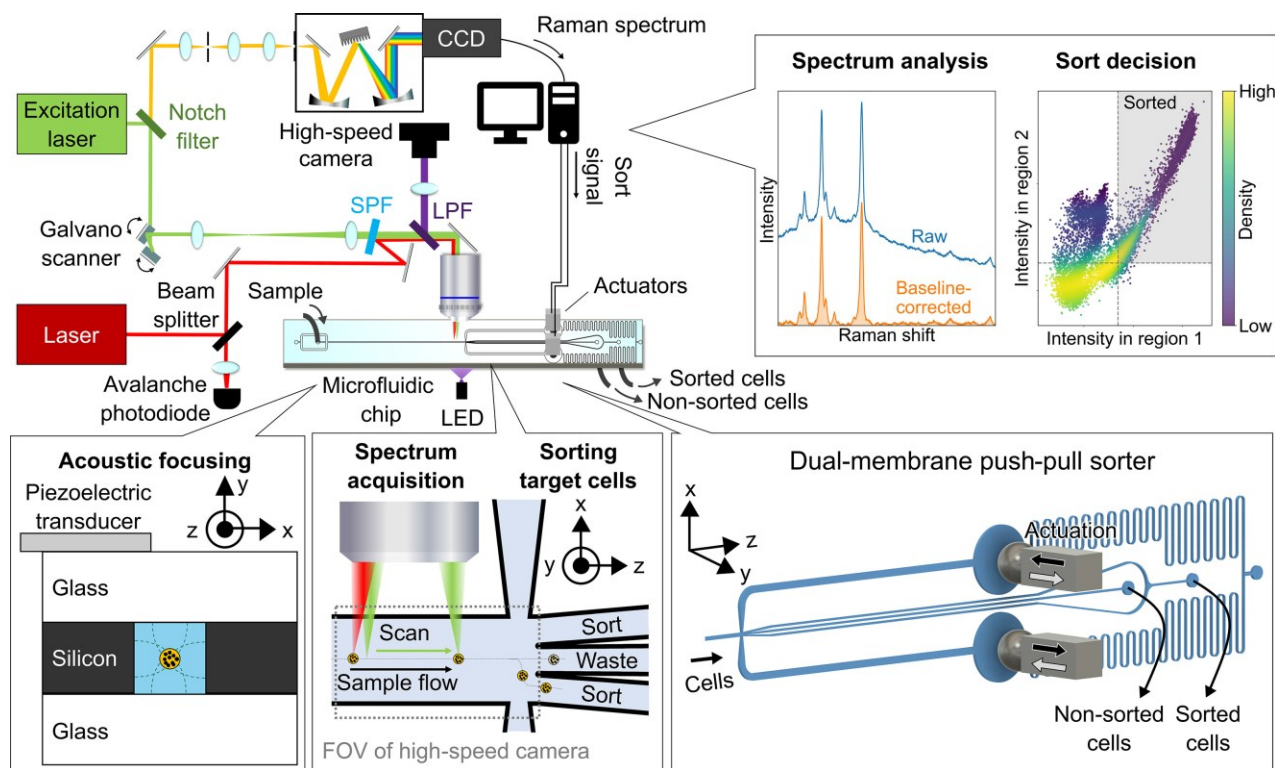


Figure 1. Schematic of the RACS system.

The cell-tracking Raman analysis system consists of a laser-scanning confocal Raman microscope, a spectral analysis and control unit, and a microfluidic device. Cell suspensions are aligned into a single stream by hydrodynamic and acoustic focusing before being directed to the Raman measurement region. During measurement, the excitation laser beam continuously tracks flowing cells while Raman spectra are acquired. The system is also equipped with a cell-sorting function based on the acquired spectral information. CCD, charge-coupled device; SPF, short-pass filter; LPF, long-pass filter; FOV, field of view. Reproduced from Nakao et al., *Matter & Light*, 2026 [4].

4. Results and Discussion

Figure 2 shows Raman spectra acquired using the RACS system from BY4741 and AWRI1482 cultured under nitrogen-limited conditions. The data shown in Figure 2 include measurement results obtained in this self-initiated interdisciplinary research project and were subsequently reported by Nakao et al. [4]. In Figure 2, baseline-corrected Raman spectra acquired from 1,000 cells of each strain are shown as the median and the first-to-third quartile range.

In particular, differences in spectral intensity between AWRI1482 and BY4741 were observed in the region around $2,850\text{ cm}^{-1}$, corresponding to the symmetric stretching vibration of CH_2 , and in the region around $1,650\text{ cm}^{-1}$, associated with $\text{C}=\text{C}$ stretching vibrations. These Raman bands are known to reflect the chemical structures of lipids, particularly neutral lipids including TAG, and are therefore considered to reflect differences in intracellular chemical composition between strains with different TAG productivities.

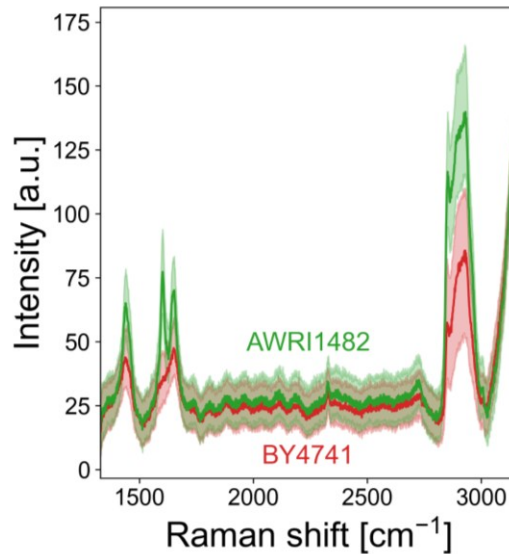


Figure 2. Baseline-corrected Raman spectra acquired from AWRI1482 and BY4741 cells in flow.

The spectral distribution for each strain is shown as the interquartile range; the solid line represents the median, and the shaded region represents the range from the first to the third quartile. $N = 1,000$ for each strain. Reproduced from Nakao et al., *Matter & Light*, 2026 [4].

Because AWRI1482 was selected as a strain with relatively higher TAG productivity than BY4741, the observed differences in lipid-associated Raman bands are considered to reflect differences in intracellular chemical composition related to lipid productivity between the two strains. These results indicate that RACS can potentially read out metabolic phenotypes associated with lipid productivity at the single-cell level without fluorescent staining or exogenous reporters.

5. Summary and Future Perspectives

Toward the breeding of budding yeast strains with high lipid productivity, this self-initiated interdisciplinary research project examined whether RACS could be used to screen two strains, BY4741 and AWRI1482, that differ in TAG production. This proof-of-concept experiment demonstrated that BY4741 and AWRI1482 could be distinguished on the basis of lipid-associated Raman bands. These results confirmed that RACS can read out metabolic phenotypes associated with lipid productivity in budding yeast without labels and can potentially physically sort target cells. In experiments performed after this self-initiated interdisciplinary research project, the difference in auxotrophic requirements between BY4741 and AWRI1482 was used to determine the proportion of target cells (AWRI1482) among the sorted cells, showing that approximately 90% of the sorted cells were target cells [4].

In the future, combining RACS-based screening of budding yeast strains with high lipid productivity, as validated in this study, with yeast breeding and metabolic analysis is expected to contribute to the efficient discovery of budding yeast strains suitable for sustainable lipid production.

Acknowledgments

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