

Delivery to Green Microalgae Cells

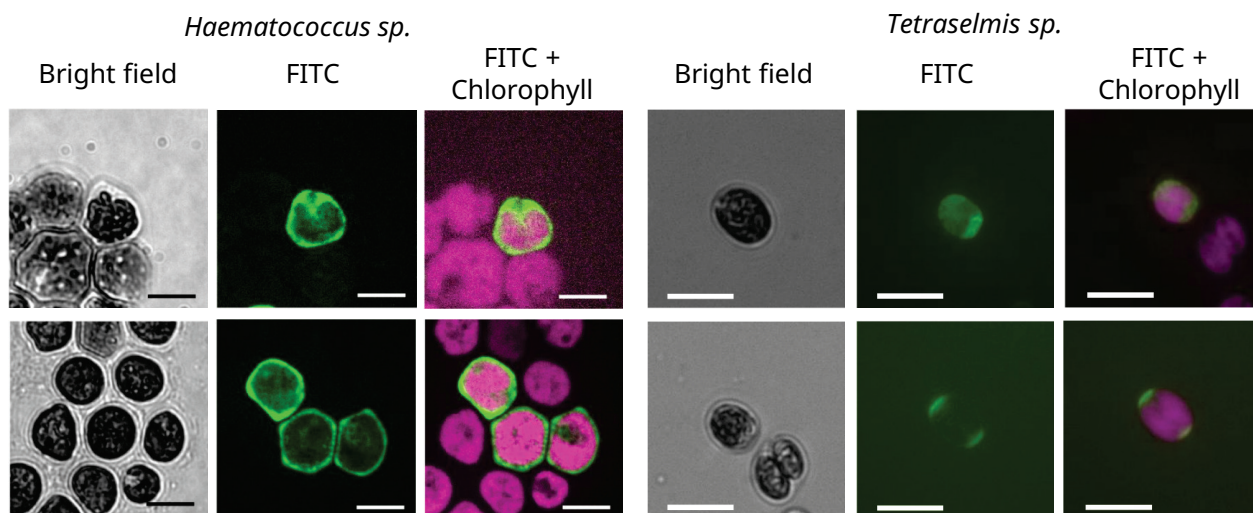
Single Cellome™ Unit SU10



Yokogawa's nano-injection unit, the SU10, provides a technology that can deliver various target substances into single cells by using a "nano"-pipette made from glass capillaries with an outer tip diameter of just tens of nanometers. Injection is possible either directly into the nucleus or into the cytoplasm. Efficient delivery into microalgae has proven to be challenging due to the presence of the cell wall, which significantly hinders the intracellular injection of target substances like nucleic acids or proteins.

This application note presents two successful examples of automated delivery of fluorescent reagents in green microalgae species with different morphologies, demonstrating the potential for efficient genetic transformation, including genome editing and subsequent metabolic engineering.

Delivery to microalgae cells with the SU10



Microalgae species	Total delivered cells	Delivery success rate	Conventional method*
<i>Haematococcus sp.</i>	85	44%	1.3-2.7%
<i>Tetraselmis sp.</i>	40	45%	1.3-2.7%

*Electroporation

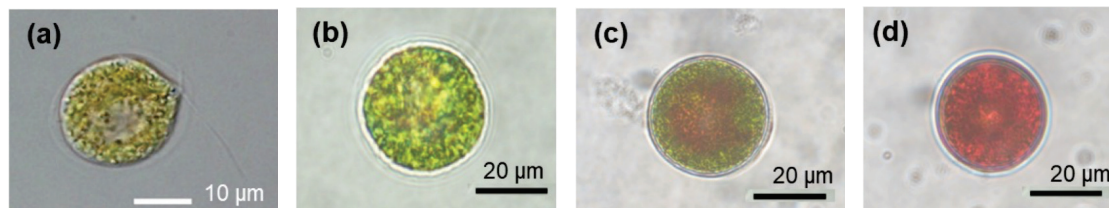
Courtesy of Dr. Tsuyoshi Tanaka, Tokyo University of Agriculture and Technology

Experimental Conditions

Specimen:	<i>Haematococcus sp.</i> (Left) and <i>Tetraselmis sp.</i> (Right). Microalgal cell suspensions were immobilized on agarose gel pads and immersed in 3 mL PBS within a 35 mm Petri dish.
Injection reagent:	FITC-labeled dextran (10 mg/mL, MW: 70,000)
Results:	A fluorescent signal was observed directly after injection, and cell division was confirmed after 4 days post-injection, indicating the viability of the microalgae. FITC-dextran localization was predominantly confined to regions surrounding chloroplasts. This restricted distribution in microalgae can be attributed to their limited cytoplasmic space, as chloroplasts occupy the majority of the cell volume.

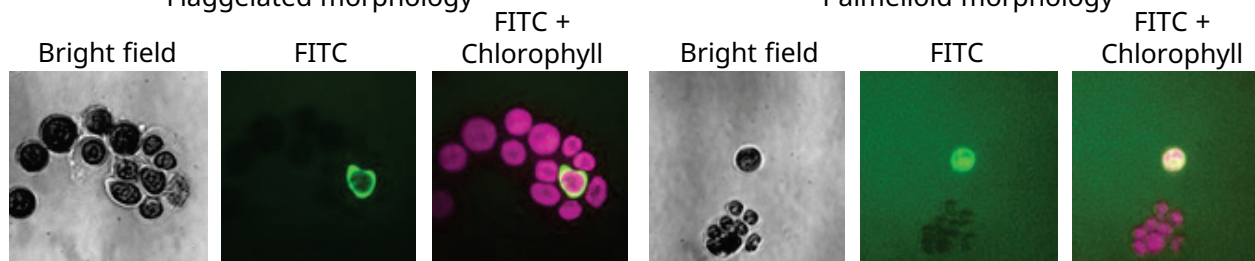
Delivery into microalgae with different cell morphologies

The SU10 can deliver into cells of the same species with different morphologies. The green microalga *Haematococcus* sp. undergoes significant morphological transitions throughout its life cycle, progressing through four distinct stages: flagellated cells (a), palmelloids (b), intermediate cells (c), and cysts (d).



Flagellated morphology

Palmelloid morphology



Experimental Conditions

Specimen: *Haematococcus* sp. (different morphological stages). Microalgal cell suspensions were immobilized on agarose gel pads and immersed in 3 mL PBS within a 35 mm Petri dish.

Injection reagent: FITC-labeled dextran (10 mg/mL, MW: 70,000)

Results: Delivery of fluorescent reagents were successful in two of four morphological stages: Flagellated form and palmelloid form. The SU10 has the potential to deliver substances to different morphological stages of the same species, despite an increasing thickness and rigidity of the cell wall of microalgae.

Conclusion

- ✓ The SU10 can be applied to automatically deliver fluorescent reagents into different species of eukaryotic microalgae.
- ✓ It achieves higher delivery success rates than conventional methods for cells that are difficult to deliver into.
- ✓ The SU10 provides a minimally invasive and precise delivery method at the femtoliter scale.

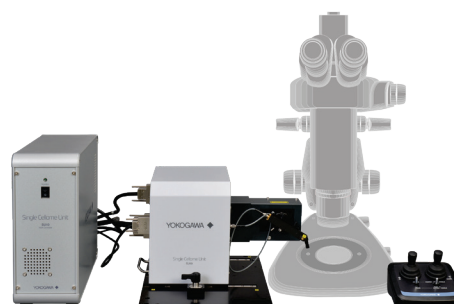
Reference

- 1) Tanaka et al., Electroosmotic Flow-Based Nanoinjection Technique Using a Nanopipette for Green Microalgae. *Mar Biotechnol*, 2025, 27, 105 .Doi: 10.1007/s10126-025-10487-0



Photo on the left:
Inverted microscope and SU10

Photo on the right:
Stereo microscope and SU10
(Note: The SU10 for stereo microscopes is a custom-made model)



Yokogawa Electric Corporation

2-9-32 Nakacho, Musashino-shi, Tokyo, 180-8750 Japan

E-mail: SingleCell@cs.jp.yokogawa.com

URL : <https://www.yokogawa.com/solutions/products-and-services/life-science/single-cellome/>

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