APPLICATION NOTE

ECLIPSE Si upright microscope
***NIS.ai* AI module for microscopes (Segment.ai)**

Mayonnaise observation and label-free analysis of oil droplet particle size distribution using AI

Emulsification technology is used in a wide range of industrial fields, including the manufacture of mayonnaise, margarine, and cosmetic creams. Mayonnaise is an oil-in-water (O/W) type emulsion in which lecithin and lipoprotein contained in egg yolk act as emulsifiers, and oil is dispersed in the aqueous phase. Since droplet size affects the taste of foods and the long-term stability of emulsions, particle size distribution analysis of droplets and observation of membranes are performed during emulsifier development and stability testing. Although droplets can be observed with transmitted light, in bright-field images the contrast between droplets and their background is low, making it difficult to identify droplet regions by conventional binarization methods based on intensity values. This application note introduces a label-free observation method for droplets, and an examples of particle size distribution analysis of mayonnaise by identifying droplets using the deep learning-based Segment.ai module.

Emulsion stability

An emulsion is a system in which one of two liquid phases that do not dissolve in each other is dispersed as fine particles in the other liquid phase. Droplets in the emulsion suppress aggregation due to electrostatic repulsion and maintain a dispersed state (Fig. 1 (C)). Emulsions are unstable, and contact between the emulsions breaks and fuses (unifies) the interface membrane, forming large droplets. For droplets with weak membrane strength, the emulsified state deteriorates due to fusion of the droplets.

Droplet membrane observation using dark-field microscopy

In dark-field observation, the membranes of droplets can be observed in high contrast, since membranes between the oil phase and the aqueous phase look bright against the dark background. It is also suitable for observation of W/O/W type emulsions with an inner aqueous phase (Fig. 2 (B)).

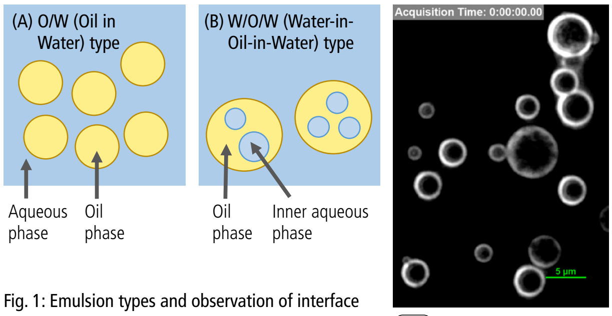


Fig. 1: Emulsion types and observation of interface membranes
(A) O/W Type (B) W/O/W Type
(C) Dark-field time-lapse image of mayonnaise oil droplets dispersed by electrostatic repulsion, Duration: 1 minute, Interval: 500 ms, Scale bar: 5 µm

Click to view the video

Sample video

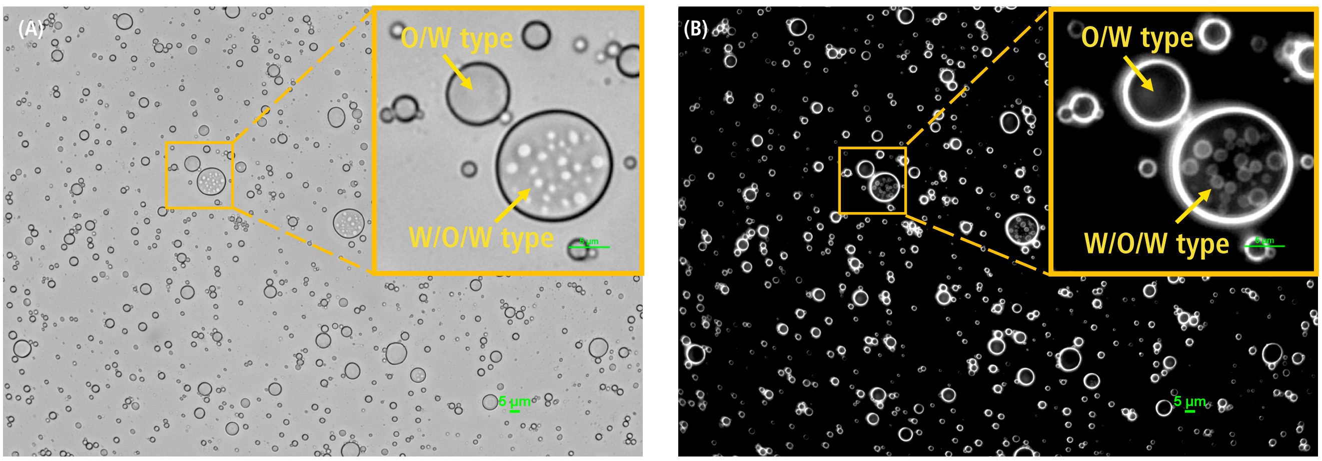


Fig. 2: Images and enlarged images of dispersed mayonnaise oil droplets

Objective: 40X, Scale bar: 5 µm

(A) Bright-field image (B) Dark-field image
Both O/W type and W/O/W type emulsions were observed. As can be seen, oil droplets with a diameter of 0.5 µm to 10 µm or more are dispersed.

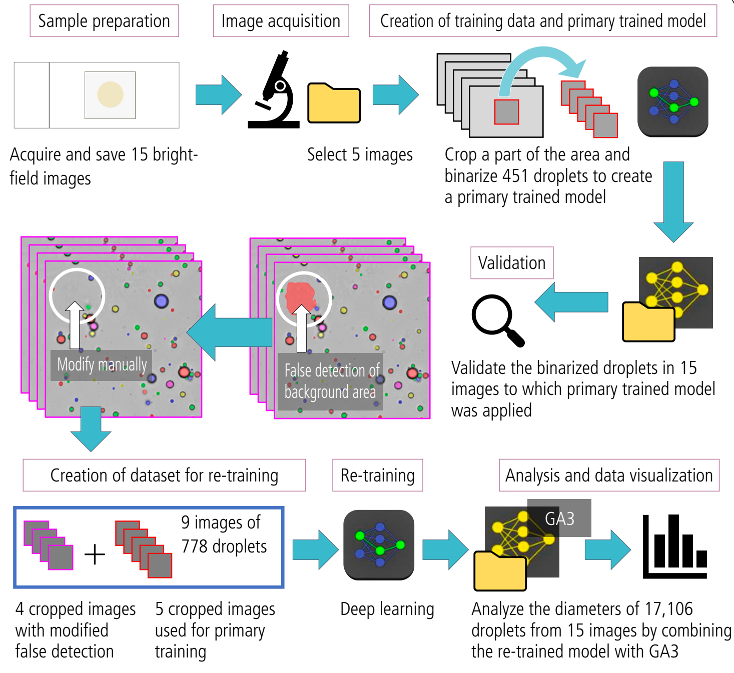
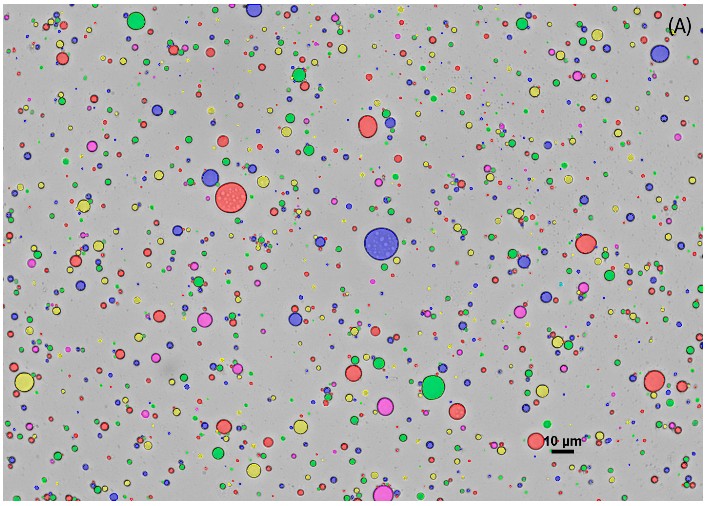


Fig. 3: Experiment overview, and droplet image analysis flow using Segment.ai



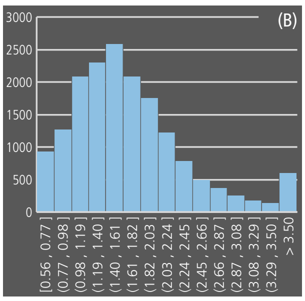


Fig. 4: Oil droplet analysis results of mayonnaise

(A) A bright-field image overlaid with droplet masks (random color) that were obtained by binarization of droplets with a diameter of 0.5 µm or more using Segment.ai. Objective: 40X, Scale bar: 10 µm

(B) Oil droplet diameter histogram. The diameters of a total of 17,106 oil droplets were analyzed from 15 images. It was found that there were many oil droplets with a diameter of about 1 to 2 µm.

Experiment Overview

(1) Put mayonnaise and water into a 35 mm dish and perform pipetting of the mixture to create an emulsion. Drop 5 µL of emulsion onto the glass slide and cover with the cover glass. Acquire 15 bright-field images with the ECLIPSE Si upright microscope equipped with a 40X objective and the DS-Fi3 digital camera.
(2) Select 5 representative images from 15 images.
(3) Crop an area of 0.07 x 0.07 mm from each bright-field image of 0.31 x 0.22 mm.
(4) Manually paint 451 droplets from 5 cropped images using the Auto Detect feature of the Binary Toolbar, and binarize them to create a dataset for primary training.
(5) Create a primary trained model by training with 5 images 1,000 times using "Train Segment.ai."
(6) Using the created primary trained model, binarize the droplets of 15 bright-field images with Segment.ai and validate the accuracy by visual observation.
(7) Manually modify the representative images with false detection of the background area and crop the 0.07 x 0.07 mm area to create 4 images for re-training.
(8) Use a total of 9 images as the re-training dataset, including 5 images used in the primary training and the 4 modified images.
(9) Create a re-trained model by training with a total of 9 images 1,000 times using "Train Segment.ai."
(10) Create a GA3 recipe for measuring droplet diameter by combining the re-trained model with "General Analysis 3 (GA3)."
(11) Analyze droplet diameter from 15 images using "BatchGA3."
(12) Export the droplet diameter measurement results to Microsoft® Excel to create a histogram.

Summary

- The dark-field observation method allows high-contrast observation of oil droplet membranes and the dispersed state of emulsions.
- Label-free particle size distribution measurement of droplets from a bright-field image is possible using Segment.ai.
- Segment.ai is useful for developing emulsions for foods and cosmetics.
- Under the conditions of this experiment, it took about 30 minutes to manually paint 451 droplets to create a dataset for primary training. It therefore takes about 19 hours to manually paint all 17,106 droplets. It took a total of 9 hours, including about an hour required to create the dataset for primary training and re-training, and 4 hours x 2 required for training via deep learning. Since it is possible to leave the desk during training with deep learning, Segment.ai was able to reduce the actual work time to about 1/19 compared to manually binarizing all droplets for this experiment.
  * Using NVIDIA Quadro RTX 4000 8GB
- Image acquisition, training data creation, trained model creation, binarization, and measurement can be performed seamlessly using NIS-Elements AR imaging software alone.

Product information

ECLIPSE Si upright microscope

By inserting a slider for dark-field microscopy* into the condenser slot and using oblique illumination, light scattered by specimens can be visualized. This method is effective for label-free examination of emulsions, colloidal particles, etc. Attaching and detaching the slider allows the user to easily switch between dark-field and bright-field observations.

*Objectives for Si compatible with darkfield sliders have a NA of 0.65 or less. Objectives of 4x or less are not compatible with darkfield observation.

NIS.ai AI module for microscopes (Segment.ai)

The NIS.ai module of NIS-Elements AR imaging software can improve image processing and analysis workflows using deep learning. Segment.ai of NIS.ai can distinguish low-contrast droplets from the background, enabling quantitative analysis from bright-field images, a process which is difficult with conventional binarization.