

MigraSee®

Migrasome and submicron-to-micron
particle observation chip

SE001

Instruction manual

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Catalog number	Pack size
SE001	42 tests

This product is designed for scientific research and is not intended for clinical diagnosis. Read the detailed risk and liability statement in this manual carefully before using the product.

Product overview

This chip is used for high-magnification microscopy and counting of vesicles and particles from 0.3 to 10 μm in size. Fluorescence labeling allows the morphology and concentration of specific targets to be assessed in mixed samples without first isolating and purifying the target particles.

Product contents

Component	SE001
MigraSee® chip	42 tests
Plasma sample stabilizer	100 µL
Instruction manual	1 copy
MigraSee® product file package	1 package

Materials and equipment required but not supplied

- Reagents and consumables for immunofluorescence staining.
- Confocal fluorescence microscope.
- ImageJ or equivalent analysis software.

Storage and shipping

Transport and store the MigraSee® chip at ambient temperature.

Principle

The MigraSee® chip precisely defines the volume sampled during confocal z-stack imaging. Particle concentration is calculated from the number of particles within this volume. In a conventional confocal imaging dish, surface tension gives the sample droplet a curved profile. The imaging volume cannot therefore be controlled, preventing accurate, consistent particle counting (Figure 1).

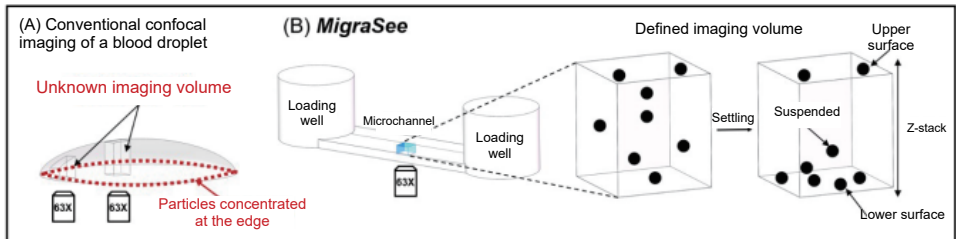
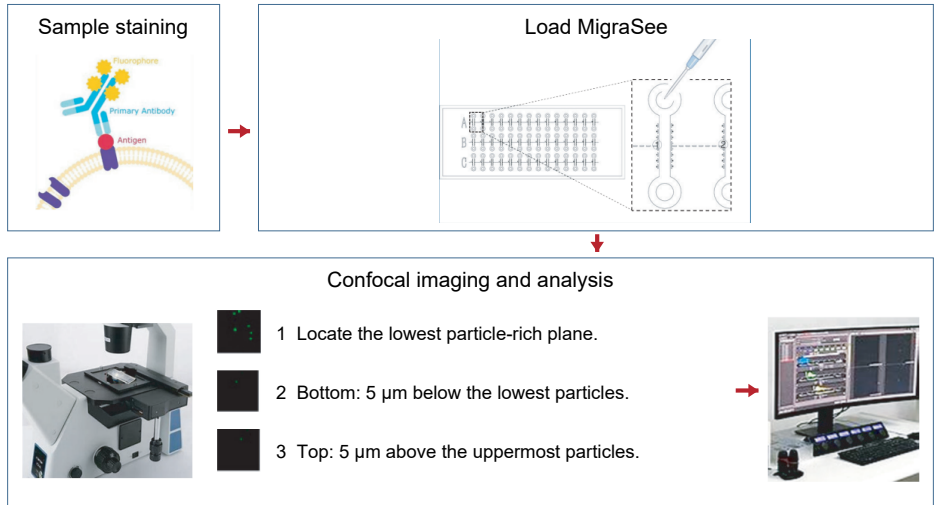


Figure 1. Principle of MigraSee®.

A: A sample droplet in a conventional confocal dish has an unknown imaging volume, with particles concentrated at the droplet edge. B: MigraSee® has loading wells connected by a microchannel that defines the imaging volume. The settling diagram distinguishes suspended particles from particles attached to the upper and lower surfaces; the z-stack spans the full channel height. Objective magnification: 63×.

Concentration = particle count / imaging volume = (number of suspended particles + number of particles attached to the upper and lower surfaces) / (channel height × field-of-view area).

Workflow



Sample staining, loading onto MigrasSee®, confocal imaging, and analysis.

Staining labels: fluorophore, primary antibody, and antigen. The chip has rows A, B, and C.

Stain the sample with a fluorescent antibody.

Load the stained sample into a MigrasSee® channel.

Locate the lowest layer with the most fluorescent particles. Set the bottom of the z-stack 5 μm below the lowest particles and the top of the z-stack 5 μm above the uppermost particles.

Acquire the z-stack and analyze the images.

Procedure

1. Sample preparation

- 1-1. Stain the sample with a suitable fluorescent antibody. Antibody background can substantially affect counting, so establish suitable staining conditions.
- 1-2. Place the chip on a dark background, peel back the sealing film, and load 1.5 to 2 μL stained sample into the MigraSee® sample channel.
- 1-3. Replace the sealing film after loading. Label the film or the side of the MigraSee® chip.

Allow the sample to settle for at least 10 min, according to its sedimentation properties. A stabilizer is recommended for biological samples that drift.

2. Imaging

- 2-1. Once the sample has settled, switch on the confocal microscope and select an objective with a magnification of 60 $\times$ or higher.
- 2-2. Place the chip upright on the microscope stage. In bright field, align the objective with the sample channel and adjust the focus until the channel edges are clearly visible through the eyepiece. Move the objective to the center of the channel.
- 2-3. Select the fluorescence channel appropriate for the antibody. Adjust the laser intensity and contrast, and set the pinhole, Nyquist sampling, z-step, and other parameters for optimal imaging.

Fluorescence intensity, background, and related factors strongly affect particle counting and size analysis. These parameters vary between samples and must be optimized by the user.

2-4. Select the channel region indicated by the arrows. Set the z-stack positions and scan height, then acquire images. The channel is 20 μm high. Generally, include an additional 5 μm above and below the channel, for a total scan height of 30 μm .

Acquire images within the region bounded by the four position markers, I to IV, above and below the channel center. Select at least three imaging positions. Use more positions for dilute samples to support reliable results. Confirm that the z-stack includes all particles within the imaging volume, including those near the upper and lower surfaces, to avoid undercounting.

3. Analysis

The product file package includes a counting guide, an Excel counting and calculation worksheet, and an analysis program.

3-1. Use ImageJ for small image sets; Imaris is recommended for batch analysis. See the operating guide for detailed analysis procedures.

Inspect the detected particles to determine whether they are the intended targets, and set the threshold according to the image.

3-2. Analyze the images using the analysis program workflow to obtain particle counts. Use the Excel calculation worksheet to calculate particle number and concentration.

If required, particle-size distributions can be obtained from the accompanying raw data. Fluorescence halos introduce approximately 20% error into size analysis.

Frequently asked questions

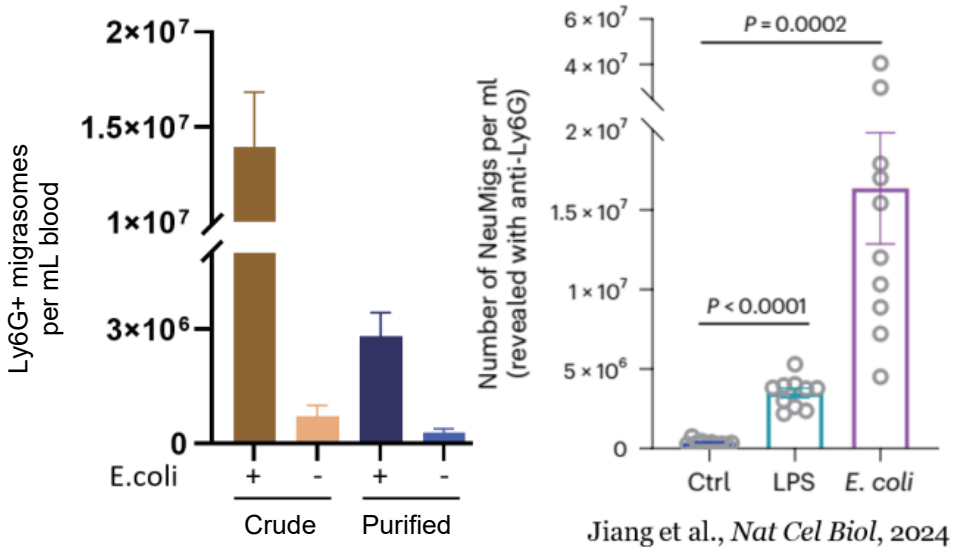
How can I locate the sample in a channel and focus on its lowest plane using a 60× or higher oil-immersion objective?

Begin in bright field with the objective directly beneath the channel. Adjust the z position while looking through the eyepiece until the channel edges are sharp. Use the channel-center markers to adjust the x and y positions and locate the channel center. Switch to the fluorescence channel to find the target sample.

How can I reduce the time required for confocal z-stack imaging?

Use the largest suitable z-step and an appropriate scan height. A microscope with an automated stage is recommended. Multiposition z-stack acquisition allows images to be collected at several positions in multiple channels in one batch.

Representative results



Counts of neutrophil-derived Ly6G+ migrasomes in blood from bacteria-injected and uninjected mice obtained with MigaSee® (left) were broadly consistent with the imaging flow cytometry results reported by Jiang et al., *Nat Cell Biol*, 2024 (right).

Left: Ly6G+ migrasomes per mL blood in crude and purified preparations; + and - indicate the presence or absence of *E. coli* injection. Right: NeuMigs per mL detected with anti-Ly6G in the Ctrl, LPS, and *E. coli* groups. The comparisons shown have $P = 0.0002$ and $P < 0.0001$.

Reference: Jiang et al., *Nat Cell Biol*, 2024.

Precautions

1. The chip base is a 0.17 mm thick coverslip and is very fragile. Handle it carefully.
2. Each sample channel is for single use. Mark used channels clearly on the sealing tape to prevent repeated loading.
3. Do not immerse the chip in liquid. Immersion can damage the chip, and such damage is not covered by the warranty.
4. This counting method requires particles to remain stationary during z-stack imaging. Before imaging, determine a suitable settling time and sample concentration. A stabilizer is recommended where possible for samples that drift, such as plasma or concentrated samples.
5. Higher objective magnification, a smaller pinhole, and suitable laser intensity and contrast facilitate particle counting and size analysis. Select the z-step according to the target particle size. For example, use a 0.5 μm z-step for particles of 0.5 μm or larger.
6. The recommended protein concentration for loading is 100 to 200 $\mu\text{g/mL}$, as determined by BCA assay. Protein concentration varies between samples. On first use, examine an undiluted sample to assess settling and the time required. If the sample is too concentrated, test a series of dilutions. At a suitable concentration, particles settle and remain stationary.
7. Stabilizer use: Mix the stabilizer thoroughly with the stained sample at 1:1. Use a low-volume pipette to load 1.5 to 2 μL into MigraSee® and allow at least 10 min for settling. Check settling under the microscope. If particles still drift, allow additional settling time.

Important risk and liability statement

This product is for research use only. It has been designed and manufactured in accordance with the standards and regulatory requirements applicable to research reagents. Read this manual carefully before use and comply with all applicable biosafety, legal, ethical, and privacy requirements, including, but not limited to, the following:

- **Compliance:** Do not use this product for clinical diagnosis, treatment, or any other medical purpose.
- **Biosafety:** Take appropriate biosafety measures to prevent infection and strictly follow the procedures for handling and storing biohazardous materials.
- **Ethics and privacy:** Sample collection must comply with all relevant ethical review requirements, protect personal privacy, and be supported by the appropriate informed consent.
- **Cross-contamination:** Follow this manual to prevent sample cross-contamination and ensure accurate experimental results.
- **Storage and stability:** Store the product correctly to maintain its stability and effectiveness. Storage outside the specified conditions may reduce performance.
- **Waste disposal:** Dispose of used reagents and samples safely in accordance with local regulations.
- **Performance limitations:** Product performance and limitations are described in this manual. Migrasome Therapeutics accepts no responsibility for use outside these instructions.

Migrasome Therapeutics accepts no legal liability for any direct or indirect consequences of improper use of this product or failure to comply with applicable regulations. Contact customer service if you have questions or require further information.

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