APPLICATION NOTE

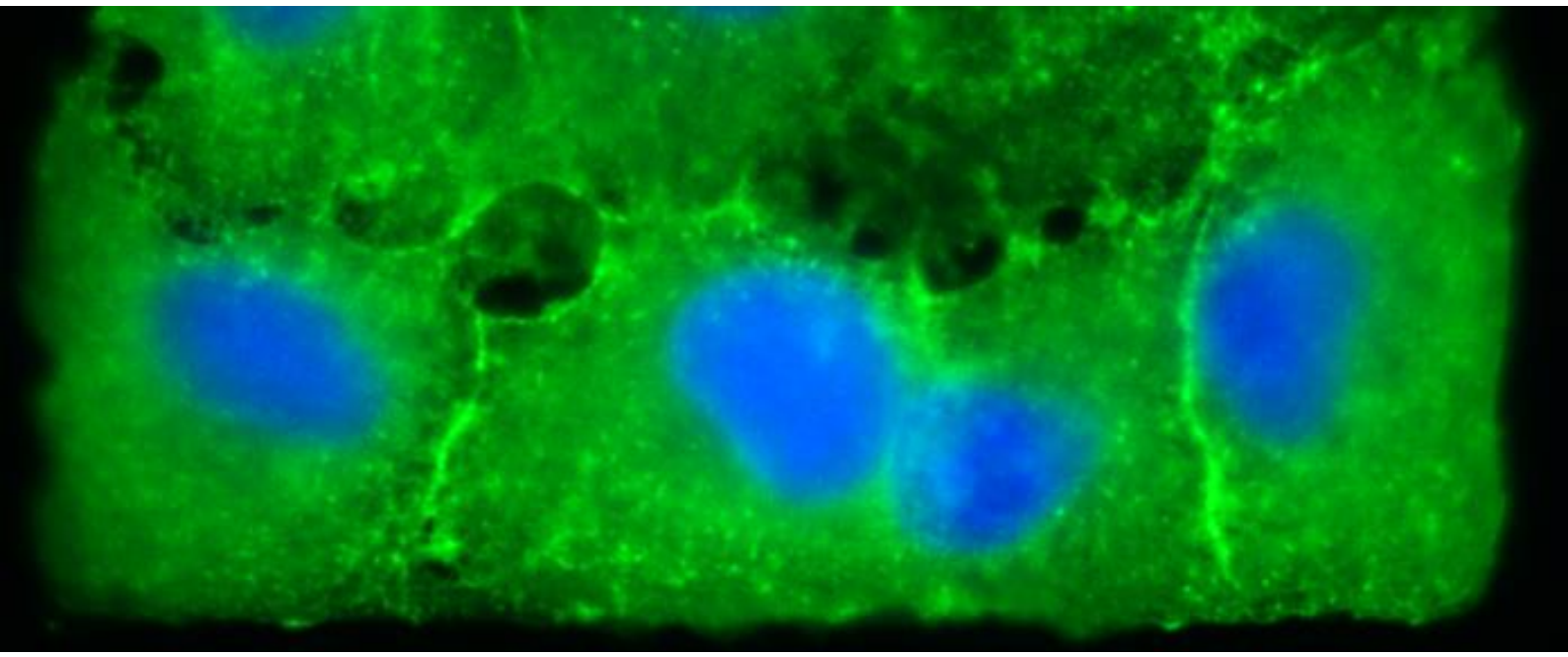
SemaCyte® microcarriers are compatible with common *in-vitro* imaging assays and allow for flexible storage and staining workflows

Daniel Weekes[1], Serena Belluschi[1], Tom Mitchell[1], Jeroen Verheyen[1], and Tarun Vemulkar[1]

[1] Semarion Ltd, UK

Abstract

Assays using cell imaging as endpoints are important in all steps of the drug discovery pipeline. Here we show that SemaCyte® microcarriers are compatible with both standard live cell dyes and common immunofluorescent staining protocols. Currently, flexibility of these imaging workflows are limited by the need to maintain adherent morphology. Here we highlight how the unique properties of SemaCyte® microcarriers can allow for flexible storage and staining workflows for fixed adherent cells.



Introduction

Imaging and staining of adherent cells with fluorescent dyes or antibodies forms part of multiple workflows within the drug discovery pipeline. Live cell dyes allow for cells or subcellular organelles to be rapidly stained and imaged. Whereas antibody staining protocols allow for analysis of the expression and subcellular localisation of specific proteins in fixed cells. Here, we successfully perform staining with live cell dyes and antibody staining (immunofluorescence) of A549 cells on SemaCyte® microcarriers. We demonstrate that SemaCyte® microcarriers are compatible with both methanol and paraformaldehyde fixation.

Currently there are two main options for immunofluorescent staining - grow, fix, and stain in imaging quality plates and perform staining in plates, or to grow, fix, and stain cells on coverslips which can be delicate and tricky to handle. In both cases storage of fixed and stained samples can be cumbersome. Furthermore, a whole well or coverslip worth of cells is needed for each set of staining even if only a small number of cells are needed for downstream analysis. Here we demonstrate that due to their unique properties that allow for the movement and re-dispensing of adherent cell colonies, SemaCyte® microcarriers enable flexible storage and staining workflows for fixed cells and can enable sub-sampling of fixed cells populations.

Materials and Methods

A549 cells were seeded onto a 2-inch SemaCyte® Seeding Dish at a density of 40,000 cells/cm^2. Cells were incubated for 24 h in RPMI-1640 (Gibco)culture medium supplemented with foetal calf serum, glutamine, pyruvate, and pen-strep. SemaCyte® Seeding Dishes were agitated to release cell-loaded SemaCytes. After magnetic separation, the microcarriers were resuspended in cryopreservation medium and frozen at a rate of 1°C/min and stored at -80°C or plated directly for subsequent assaying.

For analysis of viable cell count, SemaCyte® microcarriers were labelled with AM-Calcein (ThermoFisher Scientific), HCS Nuclear Mask Blue (ThermoFisher Scientific) and ethidium homodimer (ThermoFisher Scientific). Cells were imaged using an EVOS microscope (10x objective).

For immunofluorescence, frozen A549 loaded SemaCyte® microcarriers were thawed and allowed to recover for at least 60 minutes before drug treatment and/or fixation. For methanol fixation SemaCyte® microcarriers were washed once with PBS and fixed with -20 °C methanol (100%) for 10 minutes. For paraformaldehyde (PFA) fixation SemaCytes were washed once with PBS and fixed with 4% PFA in PBs for 10 minutes. After fixation SemaCyte® microcarriers were washed once with PBS and either stored in PBS at 4°C or directly used for antibody staining. Prior to staining, cells were permeabilised with 0.5% triton X100 in PBS for 10 minutes. SemaCyte® microcarriers were then incubated with the required primary antibodies for 2 hours at room temperature. After washing three times with PBS, SemaCyte® microcarriers were then incubated with the required secondary antibody for 2 hours at room temperature, washed, and then stained with HCS Nuclear Mask Blue (ThermoFisher Scientific, 1:1000). Antibodies used are summarized in Table 1. Cells were imaged using an EVOS microscope (40x objective).

Target	Antibody	Dilution
p53	D0-1	1:200
Beta actin	ab8226	1:200
Mouse IgG	A28175	1:1000

Table 1. Antibodies used in this application note

SemaCyte® microcarriers are compatible with live/dead cell dyes.

The ability to stain cells with live cell dyes allows for the rapid assessment of the biological state of individual cells in a population and can be used to assess a variety of cellular phenotypes. Two common phenotypes assessed are cell viability and cell death. To demonstrate the compatibility of SemaCyte® microcarriers with a commonly used live/dead cell assay) we treated A549 loaded SemaCytes with two different concentrations of the cytotoxic compound ionomycin for 16 hours. Cells were then stained with AM-Calcein (live cells, green), HCS Nuclear Mask Blue (nuclear stain, blue) and ethidium homodimer (dead cells, red). We saw a dose dependent decrease in viable cell count and related increase in dead cell count demonstrating that SemaCyte® microcarriers are compatible with these dyes (Figure 1).

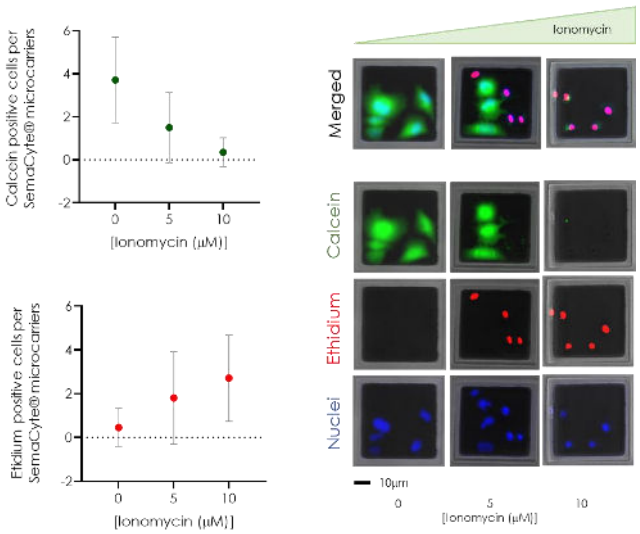


Figure 1. SemaCyte® microcarriers are compatible with live cell dyes. Frozen A549 loaded *SemaCyte® microcarriers* were thawed and treated with the indicated concentration of Ionomycin. 16 hours later, wells were labelled with calcein-AM, ethidium homodimer and HCS nuclear mask blue. SemaCytes were imaged and the number of calcein/ethidium positive cells per SemaCyte counted. Plots show mean and standard deviation from 30 SemaCytes for each condition. Example images of an individual SemaCyte are shown. Esterases in live cells generate fluorescent calcein leading to green cells. Ethidium homodimer is a membrane non-permeable DNA dye that stains the nuclei of dead cells red. HCS nuclear mask stains the nuclei of all cells blue.

SemaCyte® microcarriers allow for flexible Immunofluorescence staining and storage workflows.

Immunofluorescence uses antibodies to stain proteins of interest and allows for the assessment of protein concentration and localisation at the subcellular level. Methanol and paraformaldehyde are two common fixatives used in immunofluorescence workflows. The p53 antibody D0-1 is compatible with both methanol and PFA fixation workflows. To demonstrate the compatibility of SemaCyte® microcarriers with these two fixatives we used the MDM2 inhibitor Nutlin3a to stabilise p53 and then performed p53 immunofluorescence on cells in plates and on SemaCytes. A Nutlin3a dose dependent stabilisation of p53 was observed for cells on SemaCyte® microcarriers fixed with PFA or methanol and this stabilisation was comparable to that seen for cells grown in tissue culture plates (Figure 2).

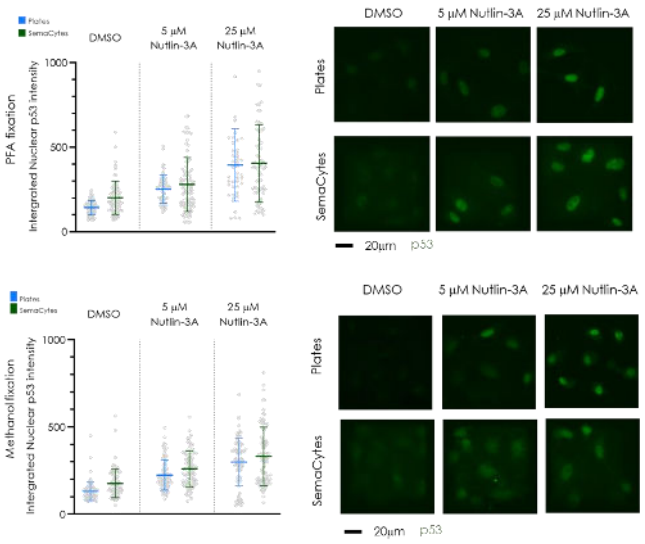


***Figure 2. SemaCyte® microcarriers are compatible with both PFA and methanol fixation**. p53 immunofluorescence was performed in the presence or absence of the p53 stabilising drug Nutlin3a. Semacytes or cells on plates were either paraformaldehyde (PFA, top) or methanol (bottom) fixed as indicated. p53 fluorescent intensity per nucleus was calculated for each condition and plots represent the mean and standard deviation. Each point represents an individual nucleus.*

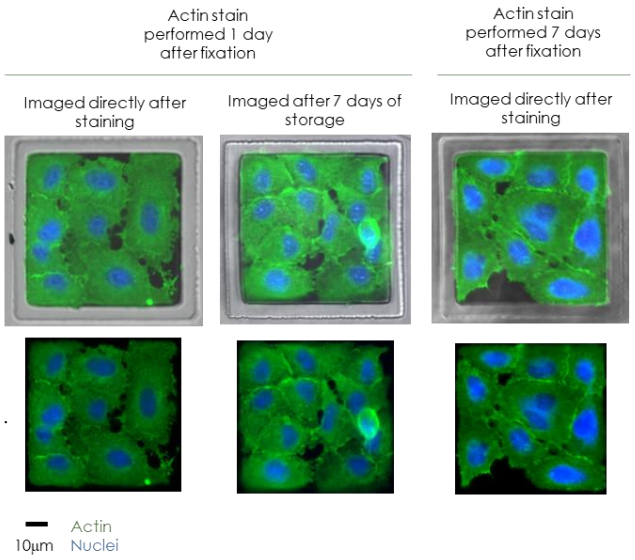


*Figure 4. **SemaCyte® microcarriers can be stored without qualitative reduction in immunofluorescent imaging quality**. Examples of an actin stain on freshly fixed and stored A549 cells on individual SemaCyte® microcarriers. Top image shows the SemaCyte® microcarriers bright field image as well as fluorescent channels, whereas the bottom image shows the fluorescent channels only.*

Currently immunofluorescent workflow flexibility is limited by the need for cells to maintain their adherent morphology. The unique features of SemaCyte® microcarriers allow for the maintenance of adherent morphology whilst increasing workflow flexibility (Figure 3). For example, the use of SemaCytes can allow subsampling of a fixed cell population and flexible staining, imaging and storage workflows (Figure 3). To demonstrate this flexibility, we performed actin staining on methanol fixed A549 loaded SemaCytes. SemaCytes that were stored in tubes for 7 days at 4 °C after fixation produced actin staining of comparable quality to those stained directly after fixation (Figure 4). Similarly, no clear loss of staining quality was observed after storing actin stained SemaCytes for 7 days at 4 °C prior to imaging (Figure 4).

Conclusion

In this application note we have shown SemaCyte® microcarriers are compatible with commonly used adherent cell imaging workflows including live cell dyes and immunofluorescence. Importantly, the unique properties of SemaCyte® microcarriers allow for increased workflow flexibility. In particular, SemaCyte® microcarriers allow efficient storage solutions. They also enable switching between imaging format dependent on the throughput/imaging resolution required for different applications or parts of the workflow. Finally, SemaCytes allow for subsampling of fixed cells populations, which is not possible with cells on plates or coverslips and might be important when using rare cells or for optimising staining protocols.

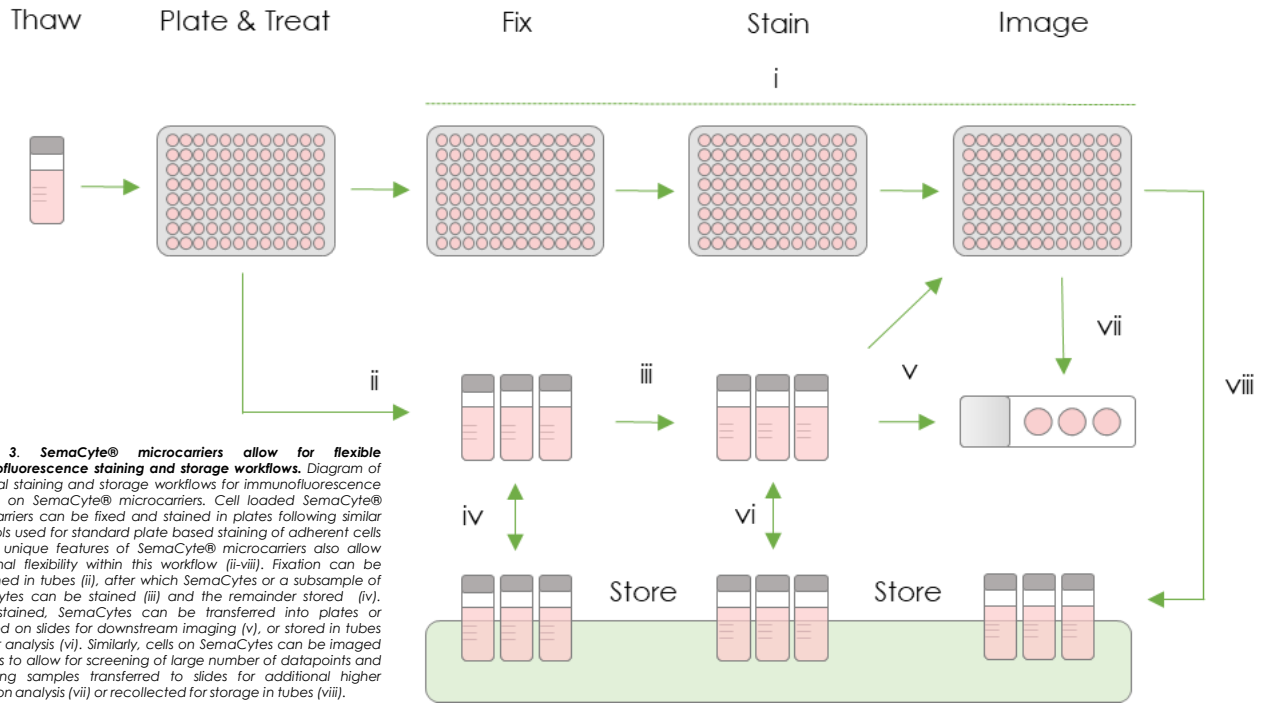


***Figure 3. SemaCyte® microcarriers allow for flexible Immunofluorescence staining and storage workflows.** Diagram of potential staining and storage workflows for immunofluorescence of cells on SemaCyte® microcarriers. Cell loaded SemaCyte® microcarriers can be fixed and stained in plates following similar protocols used for standard plate based staining of adherent cells (i). The unique features of SemaCyte® microcarriers also allow additional flexibility within this workflow (ii-viii). Fixation can be performed in tubes (ii), after which SemaCytes or a subsample of SemaCytes can be stained (iii) and the remainder stored (iv). Once stained, SemaCytes can be transferred into plates or mounted on slides for downstream imaging (v), or stored in tubes for later analysis (vi). Similarly, cells on SemaCytes can be imaged in plates to allow for screening of large number of datapoints and interesting samples transferred to slides for additional higher resolution analysis (vii) or recollected for storage in tubes (viii).*