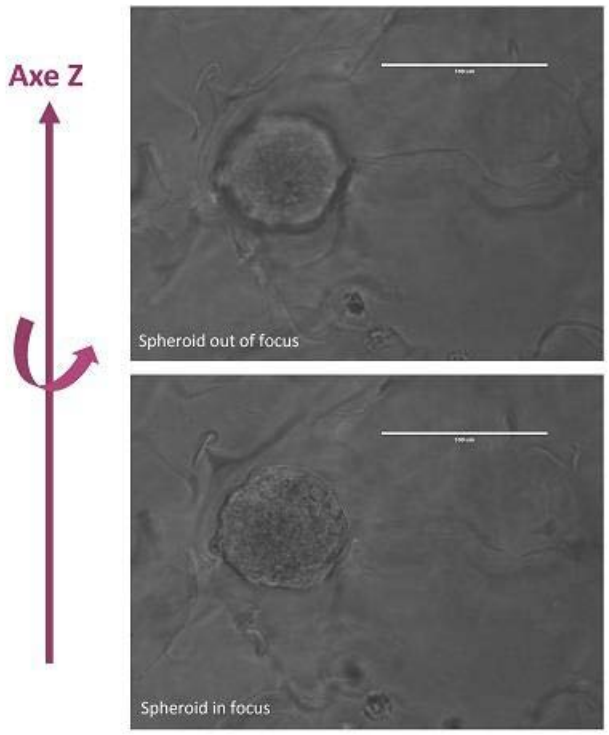
MICROSCOPY

BRIGHTFIELD MICROSCOPY

INTRODUCTION

A microscope is the piece of equipment most regularly used in the laboratory to verify and follow-up cell-culture experiments.
In 3D, the adoption of focal plane merging or Z-stacking, becomes a necessity, this is because 3D brings a new dimension compared with 2D; the field depth.
The use of microscopic and macroscopic screws allows the focal plane to be altered; visualizing not just the cells but also the Hyaluronic Acid and Collagen chains that form the BIOMIMESYS® hydroscaffold.



The translucent properties of the BIOMIMESYS® hydroscaffold means that there is no restriction to clear visualization of the cells.

METHOD

Observation with a conventional microscope is easy; all that is necessary is to place the BIOMIMESYS® plate seeded with cells on the viewing platform.

RESULTS

Microscopes tested: inverted microscope, upright microscope.

FLUORESCENCE MICROSCOPY

INTRODUCTION

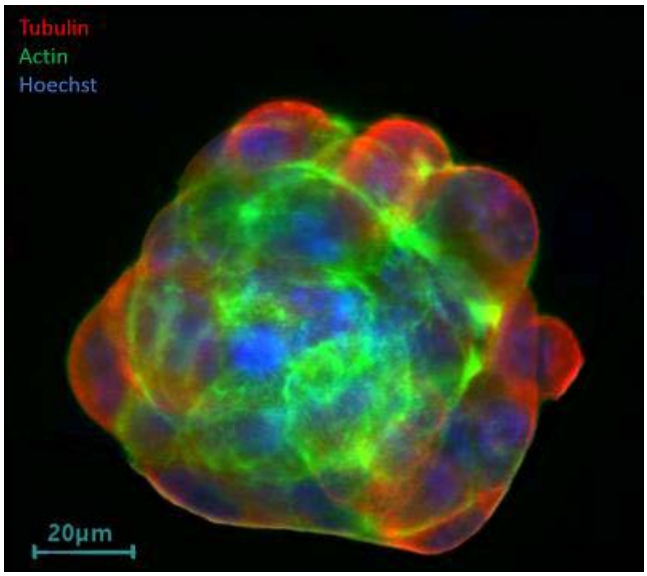
Direct live-cell visualization of cell culture. Confocal or inverted fluorescence microscopy is a useful tool to obtain high-resolution images of cells within the BIOMIMESYS® hydroscaffold and to investigate the localization of the target protein.

METHOD

Cell-labelling is carried out as usual, with the relevant fluorescent marker(s). As above, the visualization is carried out by directly placing the matrix on the viewing platform.

DYES AND ANTIBODIES TESTED

A large number of dyes and antibodies have been successfully used with the BIOMIMESYS® hydroscaffold to label a range of targets. The table shows a non-exhaustive list of such dyes and antibodies:



PANC1 spheroid at day 12 labelled with tubulin, actin and Hoescht immunofluorescence.

Dye/Antibody	Target	Supplier
α-tubulin	cytoskeleton	Sigma-Aldrich
GAPDH	cytoplasm	Sigma-Aldrich
Hoechst	nucleus	Sigma-Aldrich
Phalloidin	cytoskeleton	Life technologies
Perilipin A	Adipocyte identification	Abcam
Fibronectin	ECM secretion	Abcam
Collagen I	ECM secretion	Abcam
BrDU	Cell cycle	Life technologies or BD Biosciences
MRP2	Biliary canaliculus	Abcam
Ki67	Cell cycle	Abcam
Vimentin	Fibroblast identification	Abcam
Beta Actin	cytoskeleton	Fisher Scientific

SCANNING ELECTRON MICROSCOPY

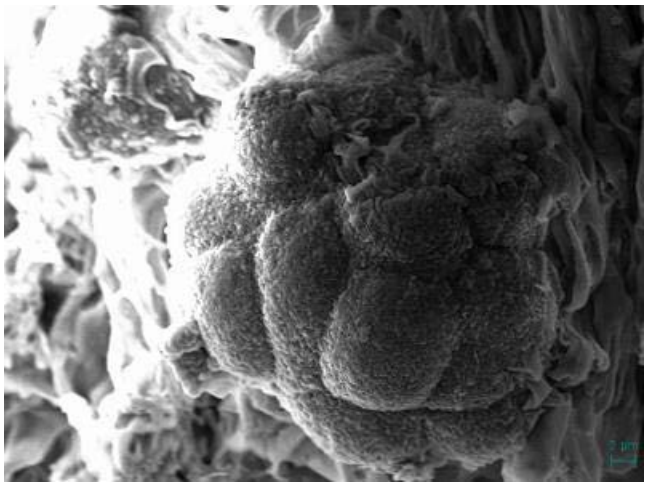
INTRODUCTION

To examine the surface morphology of cells grown in BIOMIMESYS®. Scanning electron microscopy of cultured cell lines enables study of the surface architecture, varying numbers of prominent blebs of different sizes may be identified and also microvilli. The presence of surface blebs appears to be a characteristic feature for secreting and non-secreting myeloma cells.

METHOD

Sample preparation: fix the sample with glutaraldehyde, then follow the protocol for dehydration with alcohol.

RESULTS



HT29 cell at day 14

These figure shows that the SEM technique used preserves the form of the spheroids.

Contact Information

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