

# Modelling gastrointestinal cancer cell interaction with tumor stroma in a 3D microwell array

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## Background and Purpose

Tumor response to radiotherapy strongly depends on local microenvironment, which is highly influenced by extracellular matrix molecules and nonmalignant cells from tumor stroma. Tumor cells not only receive survival signals from tissue-specific stromal cells, but also recruit circulating mesenchymal cells from blood.

- Recruited stromal cells produce anti-apoptotic cytokines, which enhances survival of cancer cells during therapy.
- Mesenchymal stromal cells have immunomodulatory properties, which may promote cancer cells escape.
- The niche, in which dormant cancer stem cells may survive for years, is formed by stromal cells.

However, knowledge on interaction between tumor cells and tumor stroma is rather limited, and therapeutic implications are still awaited. Aim of the present work was to establish a standardized *in vitro* assay for studying effects of different stromal elements on tumor cells, and to deliver a proof of principle with human gastrointestinal cancer cell lines.

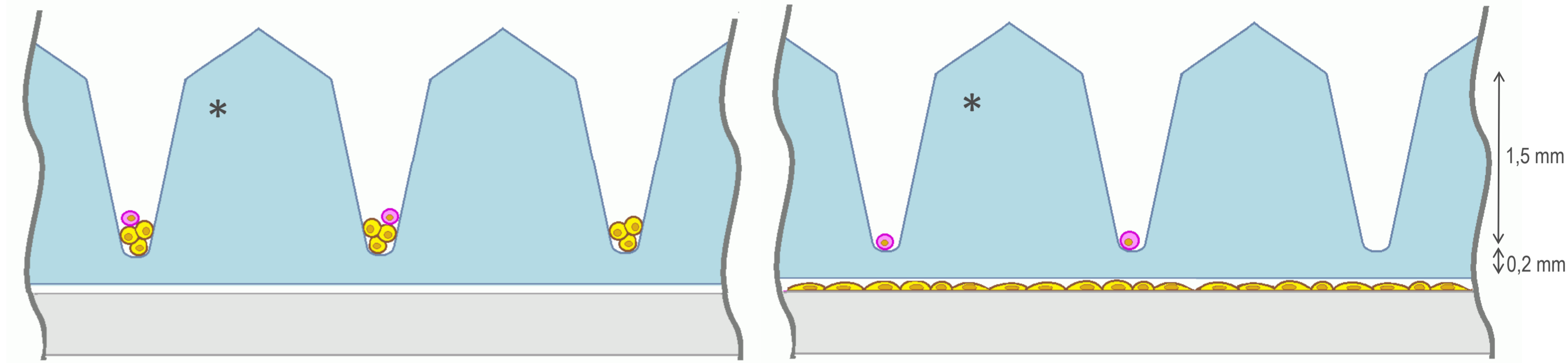
## Materials and Methods

Human pancreatic cancer cell line MiaPaCa-2 was seeded into hydrogel arrays with conical microwells. After loading with cells, microwell arrays were placed on monolayers of primary human bone marrow stromal cells. This setup allows exchange of cytokines and small molecules through the agarose hydrogel by diffusion, but prevents direct contact between the two cell types. In a modified co-culture setup allowing cell-cell contacts, MiaPaCa-2 and HT-29 human colon carcinoma cells were irradiated with 0, 2, 4, 6 or 8 Gy and seeded into microwells preloaded with bone marrow stromal cells. Readout was done on day 10 - 14 by selective staining of viable colonies and by image analysis of high resolution scans.

## Results

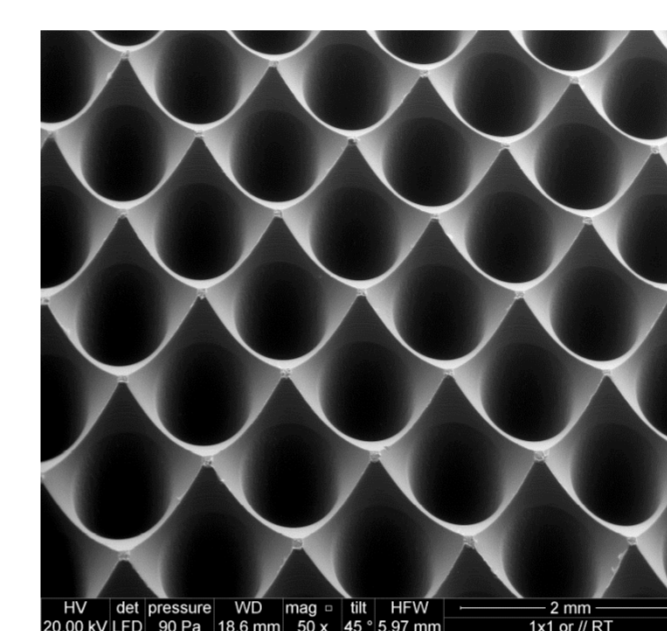
In both cancer cell lines, colony formation was significantly enhanced by co-culture with bone marrow stromal cells (MiaPaCa-2: up to 3.8-fold increase). Interestingly, presence of hMSC resulted in larger, but also more compact pancreatic cancer colonies, as shown by histology.

Results of the microwell array correlated well with soft agar overlay cultures, which were performed in parallel as reference, but experimental setup of the microwell array showed to be less time consuming and allowed a more consistent readout.

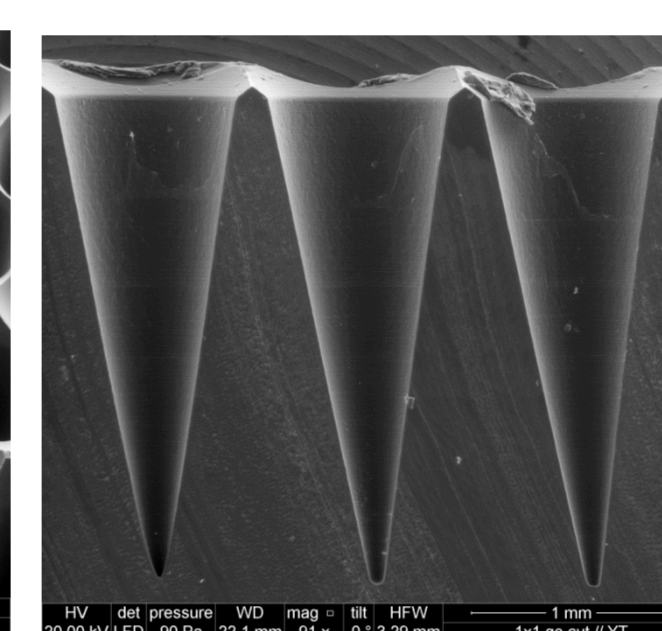


**Fig. 1 Basic principle of Contact Coculture:** Cancer cells (●) and stromal cells (●) are seeded together into conical agarose microwell array (\*).

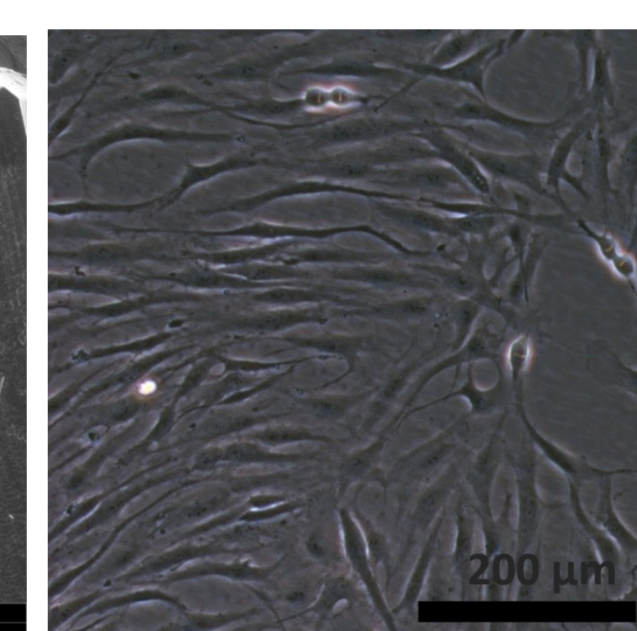
**Fig. 2 Basic principle of Distant Coculture:** Cancer cells (●) are seeded together into conical agarose microwell array (\*), which is then placed on top of a monolayer of stromal cells (●).



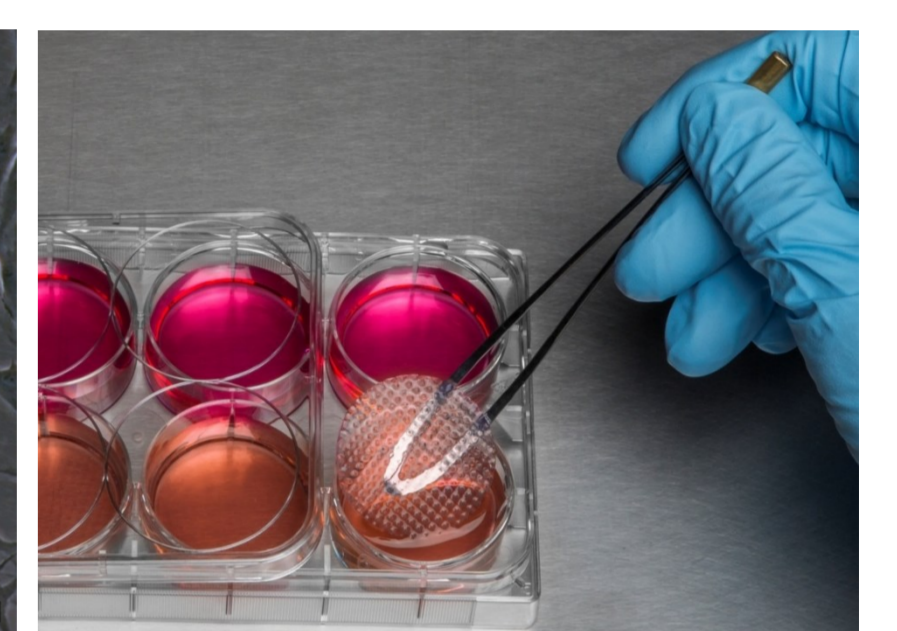
**Fig. 3 SEM image of microwell array geometry, top view**



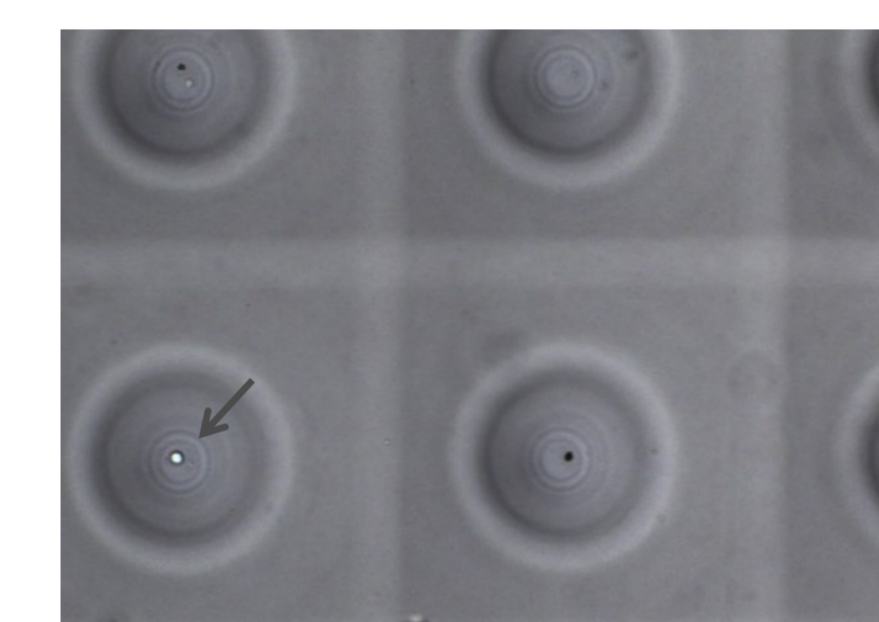
**Fig. 4 SEM image of microwell array geometry, lateral view**



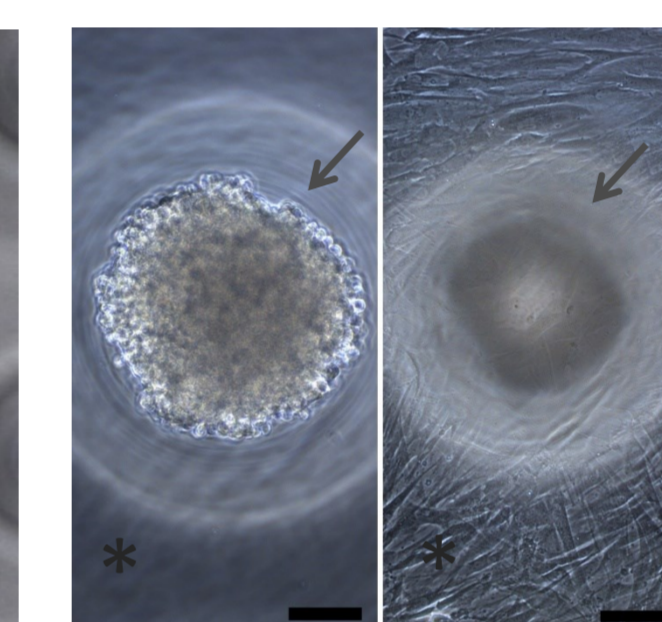
**Fig. 5 Human mesenchymal stromal cells (hMSC) as monolayer**



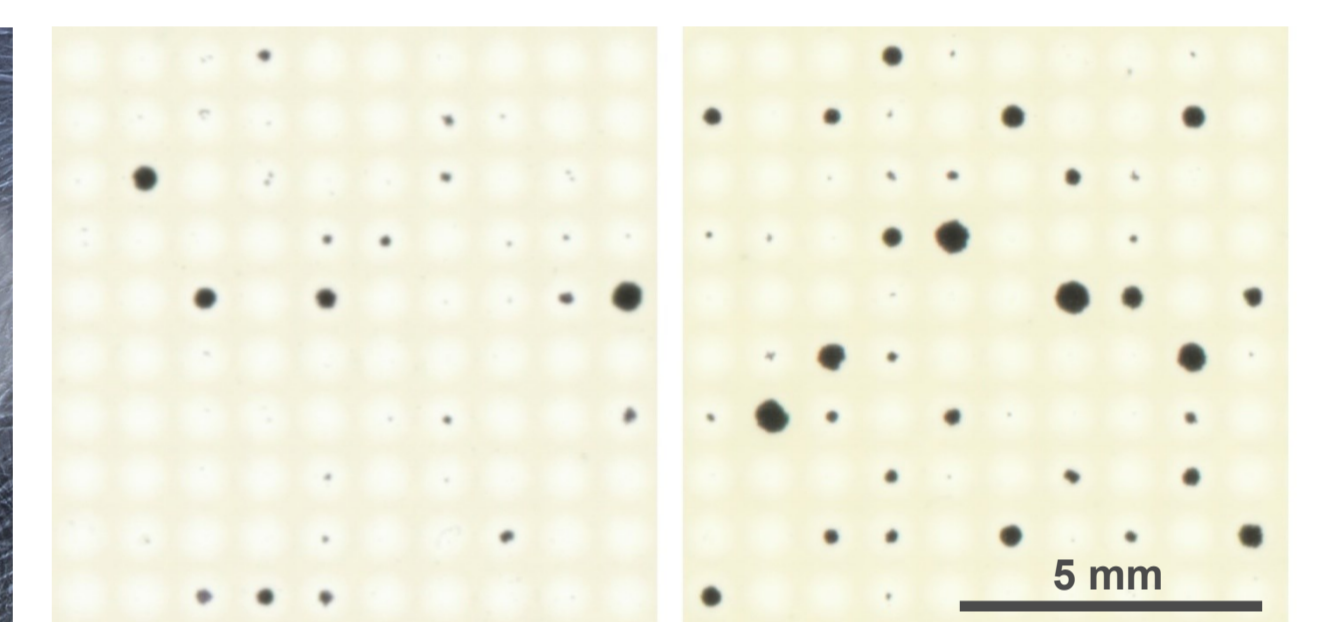
**Fig. 6 Assembly of Distant Cocultures:** An agarose array loaded with cancer cells is placed on top of adherent stromal cells.



**Fig. 7 Individual cancer cell (←) in agarose microwell, day 0.**



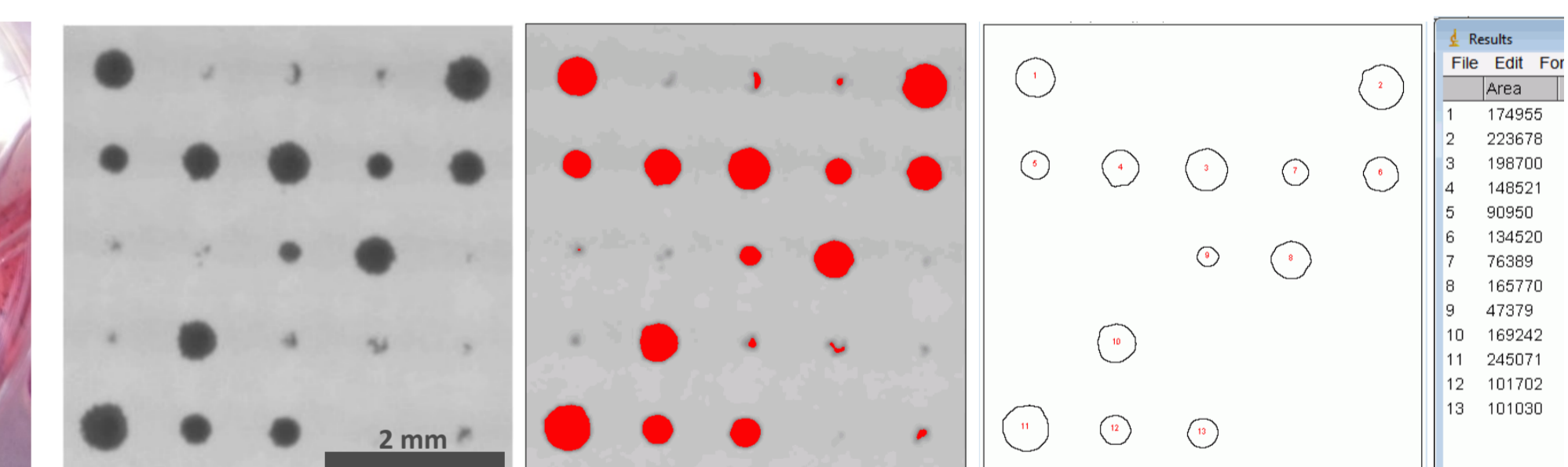
**Fig. 8 MiaPaCa-2 distant coculture on day 14 at two different focal planes: cancer cell colony (←) and hMSC (\*).**



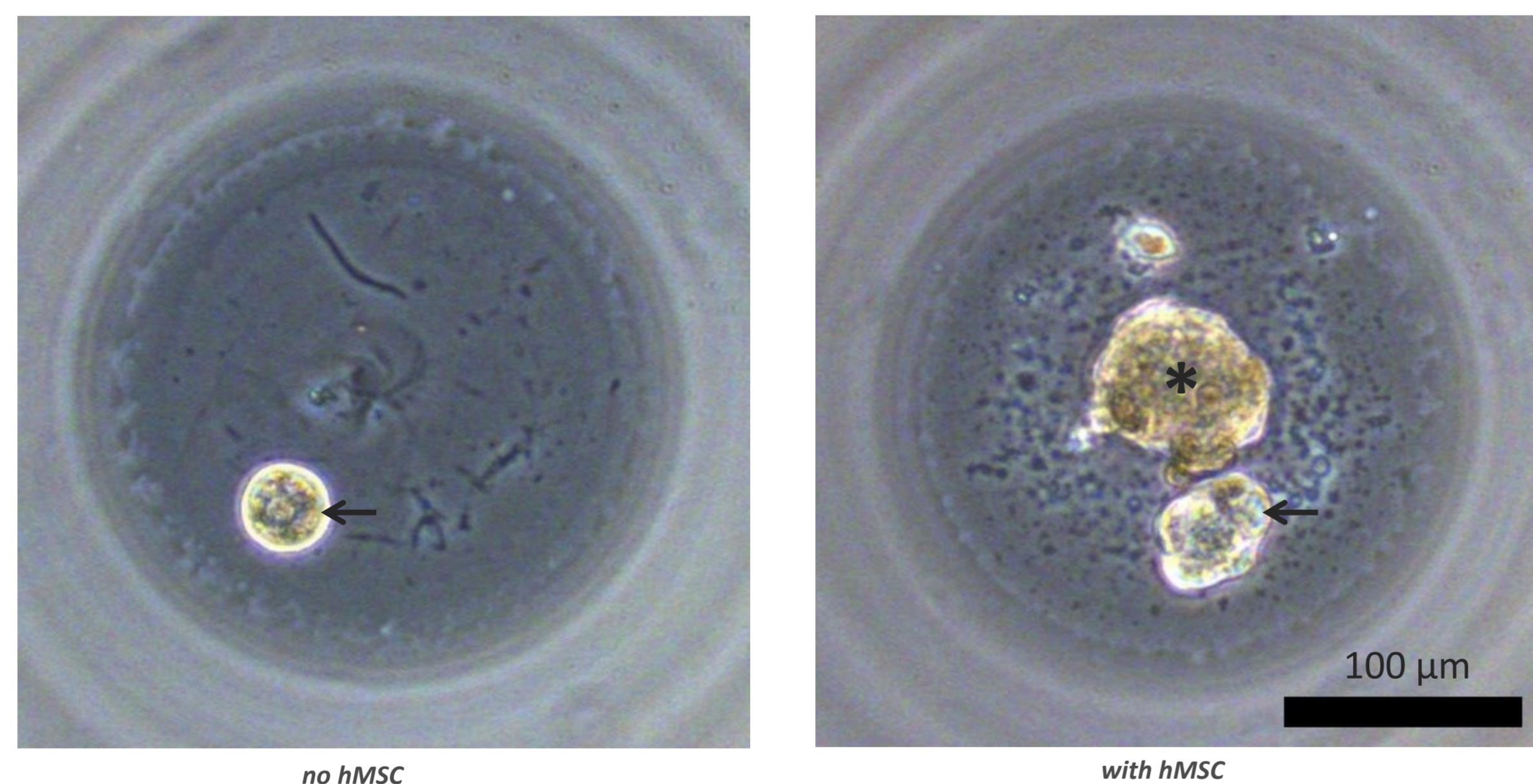
**Fig. 9 MTT-stained MiaPaCa-2 colonies in distant coculture, day 14.**



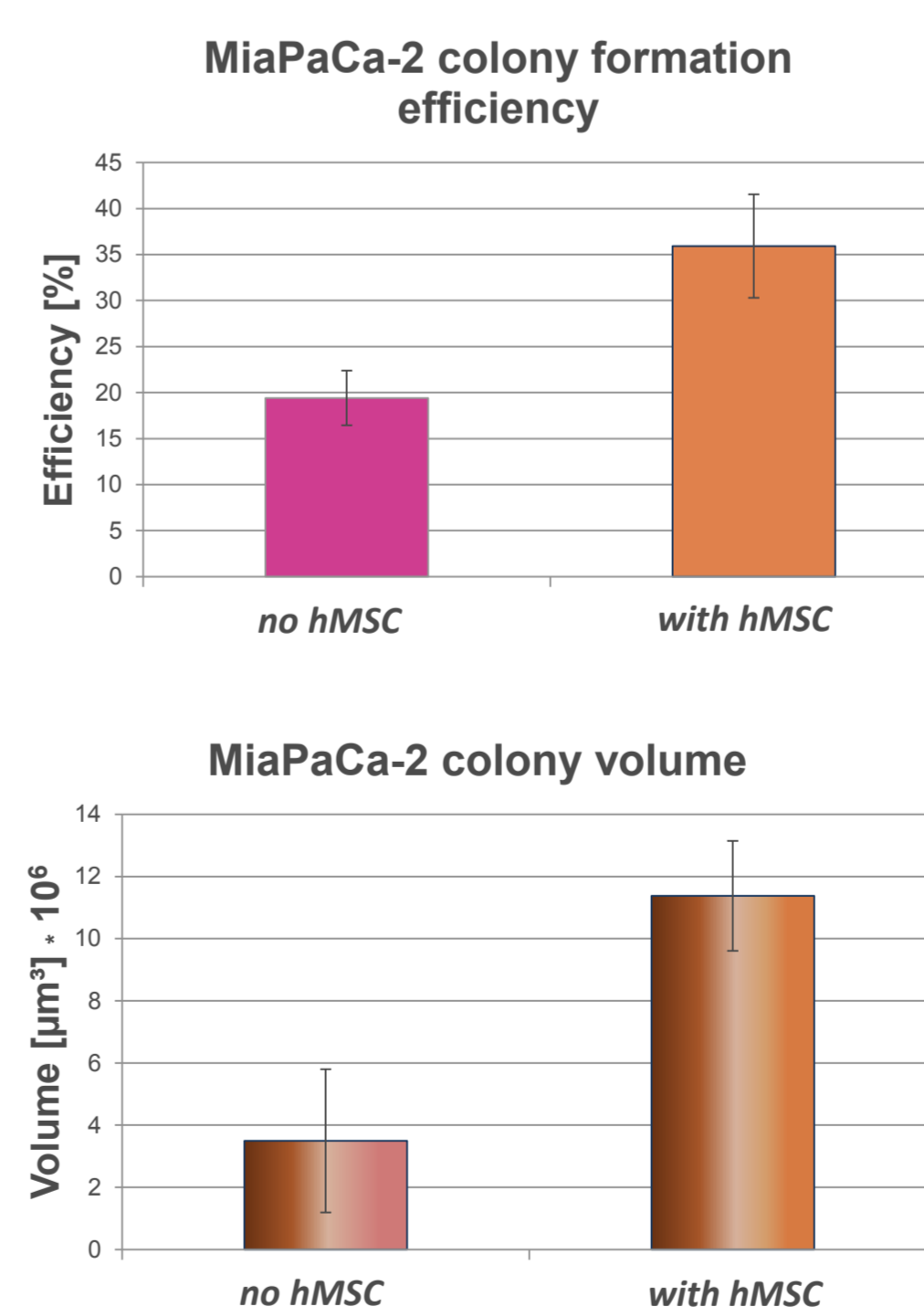
**Fig. 10 For readout, agarose arrays were scanned on a transmitted light office scanner.**



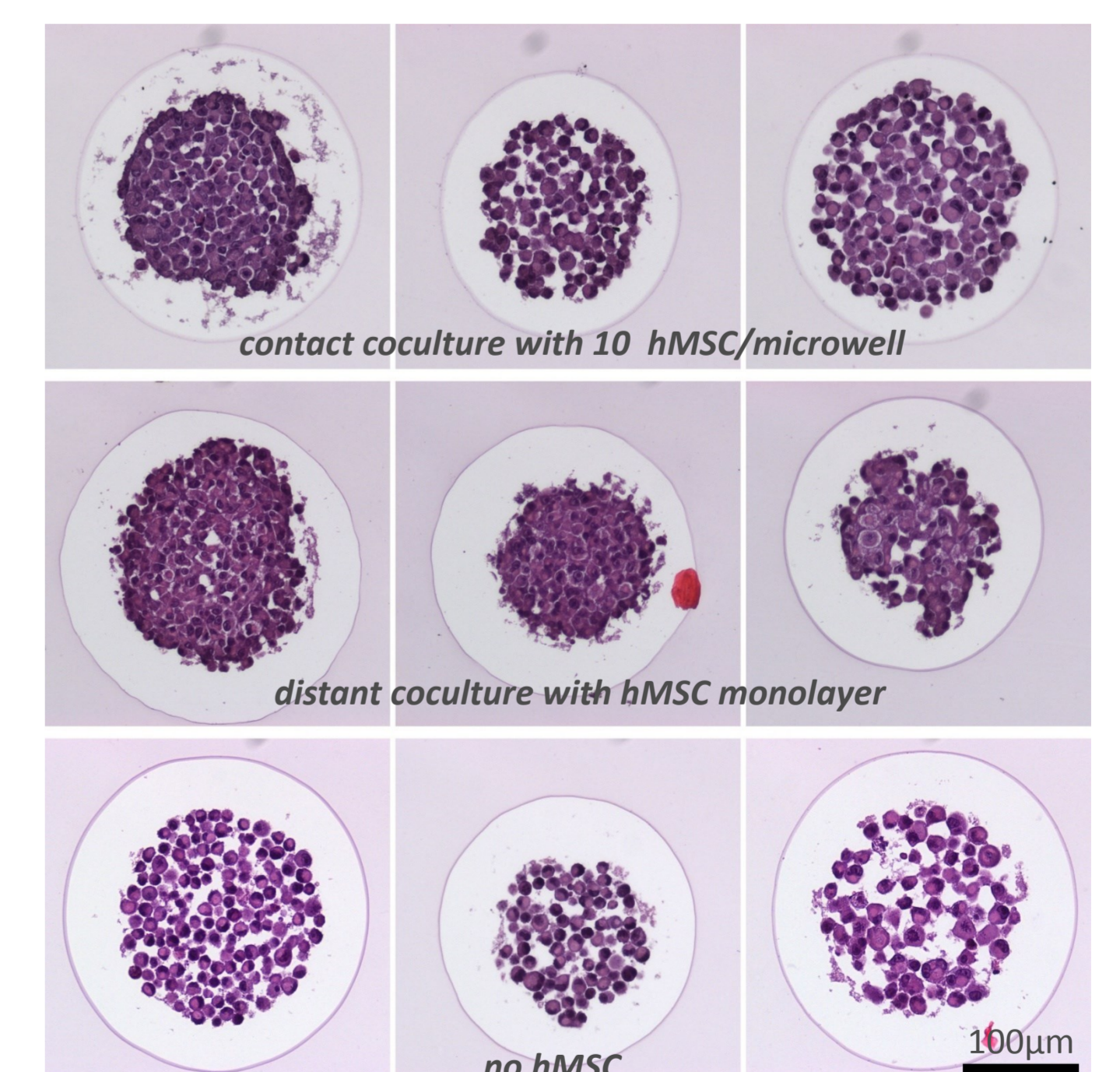
**Fig. 11 Arrangement of colonies allows precise quantification of both number and volumes of colonies by image analysis (ImageJ). In each agarose array, 4 cm² with 400 microwells were evaluated.**



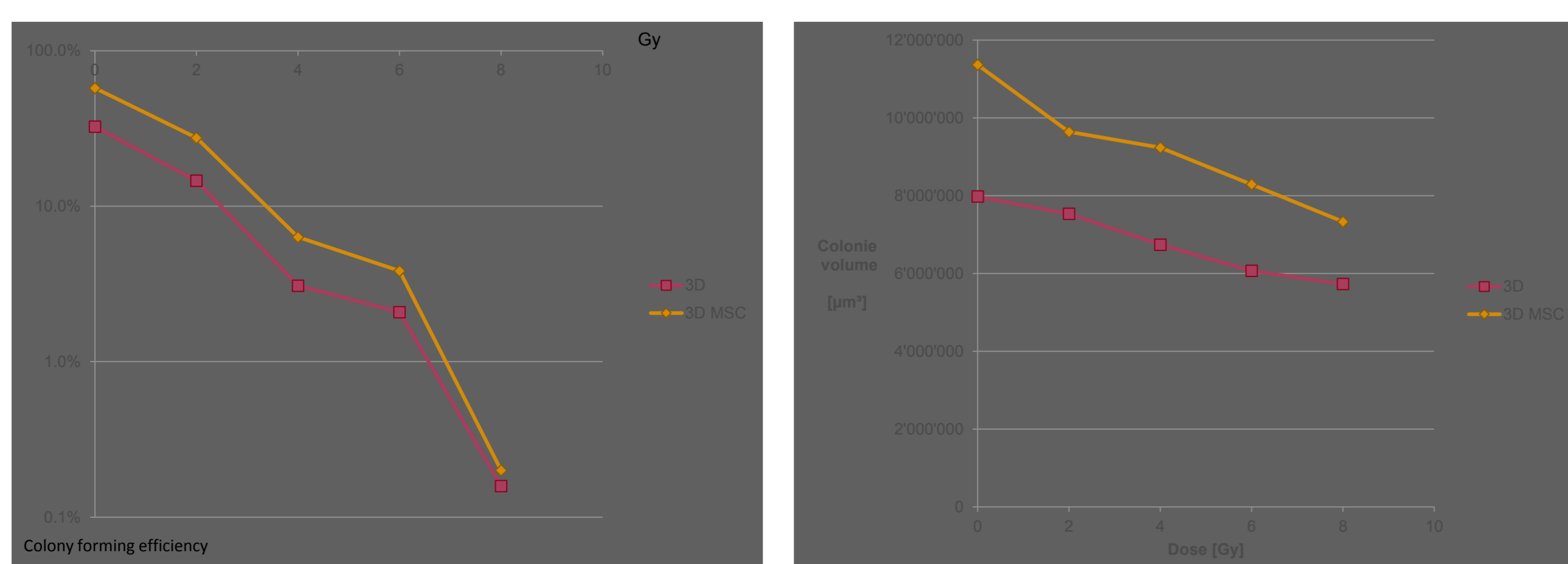
**Fig. 15 HT-29 colonies (←) on day 5 after seeding irradiated single cell suspension into microwells (1x 4 Gy). As contact coculture with hMSC (\*), cancer cell colonies grow faster. Both cell types are immobilized by walls of agarose microwells, but remain distinguishable at this early stage.**



**Fig. 12 + 13 Colony formation of MiaPaCa-2 cells as distant cocultures +/- hMSC. Both colony forming efficiency and mean volume of cancer cell colonies is significantly enhanced by presence of hMSC.**



**Fig. 14 Paraffin histology shows 3D MiaPaCa-2 colonies show a more compact growth in pres of hMSC. H&E staining.**



**Fig. 16 + 17 As would be expected, frequency of clonogenic HT-29 cells decreases with higher irradiation doses. In addition, mean volume of colonies is negatively affected by higher doses. Presence of hMSC increases both clonal survival of cancer cells and volume of colonies.**

## Conclusions

We present a standardized and robust method for testing the effect of stromal cells on 3D colony formation and radiosensitivity of cancer cells. Our data suggest that stromal cells may cause both supportive and phenotype-modulating effects on pancreatic and colorectal cancer cells. As circulating bone marrow stromal cells are known to be recruited into tumors, this cell type could have impact in clinical radiation biology and will be further investigated in our department.