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Single cell analysis for intrinsic stemness potential of cancer

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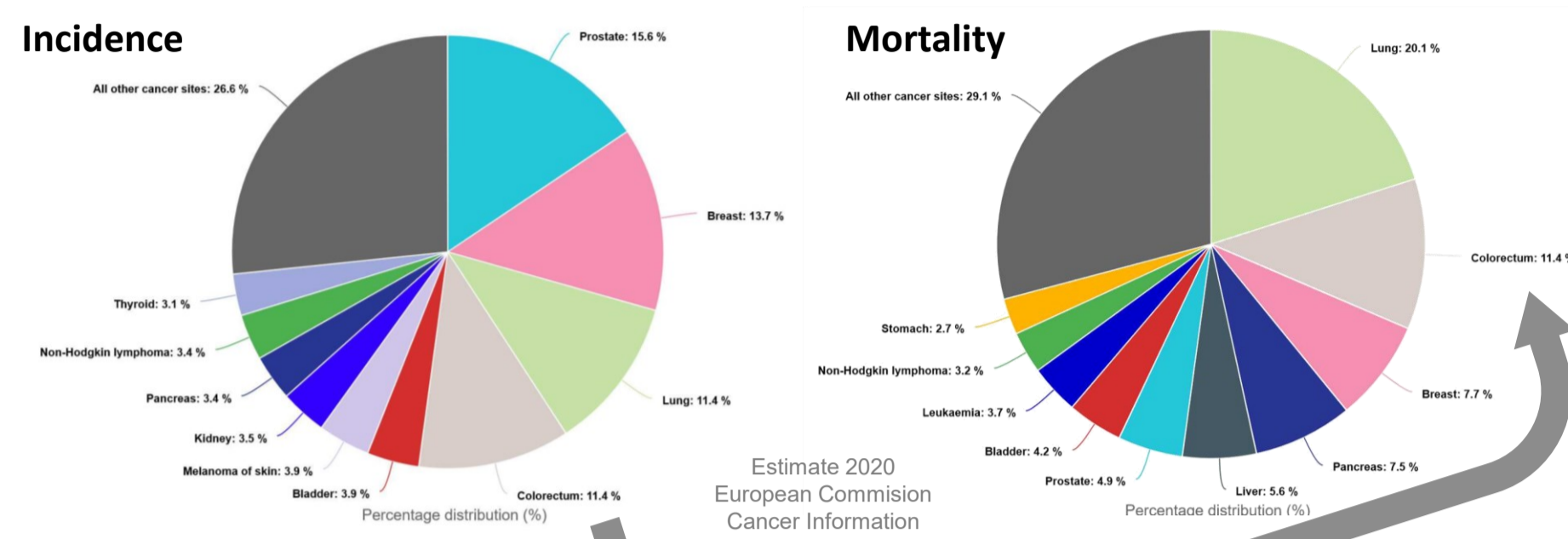
CANTHER – Cancer Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France

°Univ. Lille, CNRS, Centrale Lille Institut Junia, Univ. Polytechnique Hauts-de-France

IEMN UMR CNRS 8520, 59652 Villeneuve d'Ascq, France.



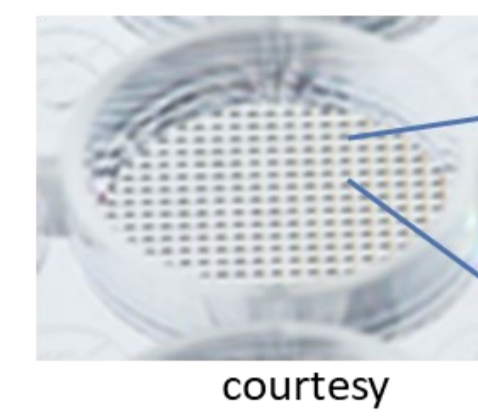
Cancer prevalence in France



Colorectal cancers (CRC)

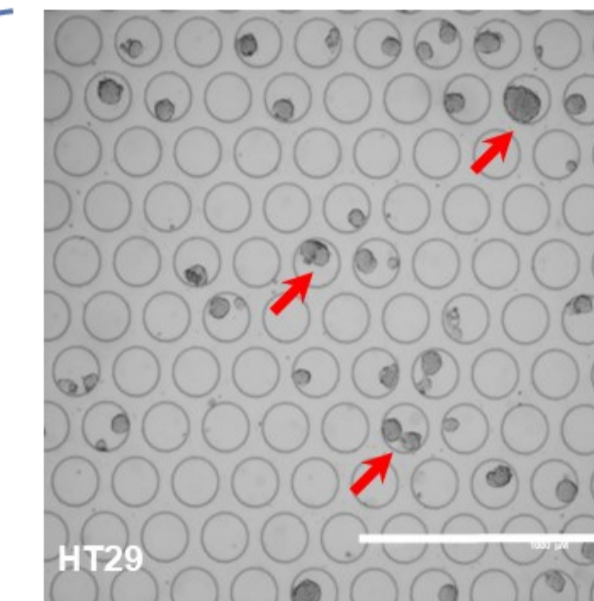
- Poor survival rates are largely due to resistance to chemotherapeutic treatments and the development of metastatic lesions.
- More than 15% of patients have metastatic lesions at diagnosis and another 20% develop them. Metastatic CRC has a poor 5-year survival rate (14%)
- CRC is treated with targeted therapy, immunotherapy and/or surgery combined with chemotherapy (e.g. FOLFIRINOX: 5FU, folic acid, oxaliplatin and irinotecan)

HT29 cells in custom micro-wells plates



Seeding of a (diluted) cell suspension in a 6-well plate with a custom μwell Stencil.

7d culture



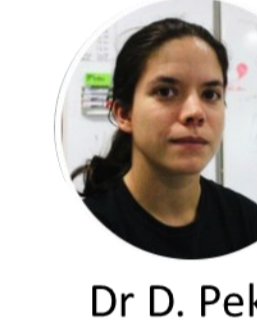
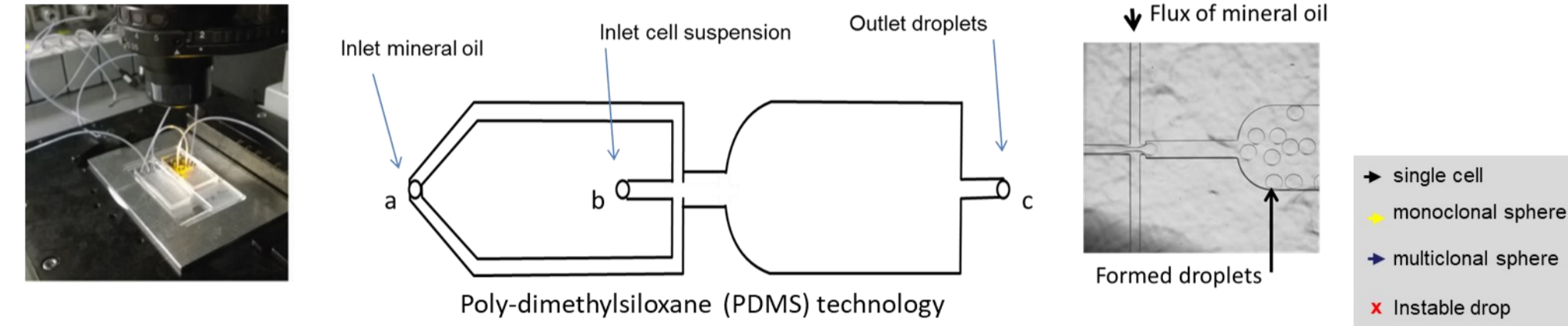
Individual cell ~15 μm
Spheres ~150 μm

μwell = 175 μm

- More than 1 cell / well, no single cell analysis
- Cell distribution sensible to vibrations

HT29 cells in microfluidic droplets

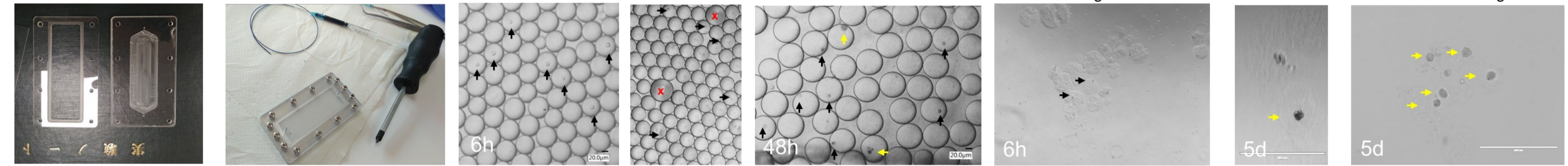
Separation of individual cells in medium-containing droplets followed by tumorsphere culture



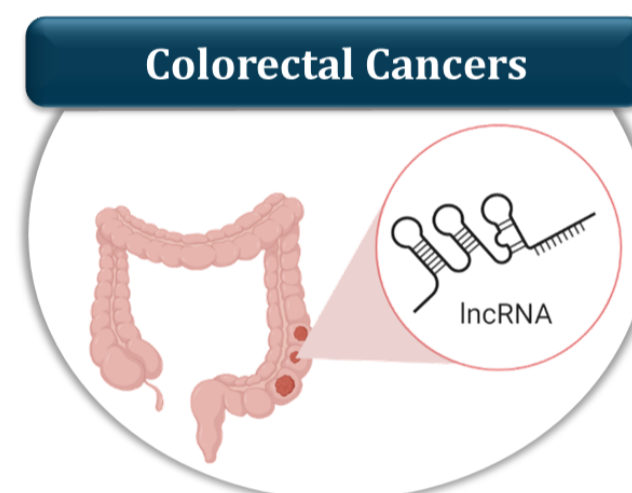
Dr D. Pekin

- Microfluidics setup in culture room
- Matrigel to stabilize drops
- Each experiment needed fine-tuning of flux, difficult to standardize using matrigel

Culture Incubation chamber

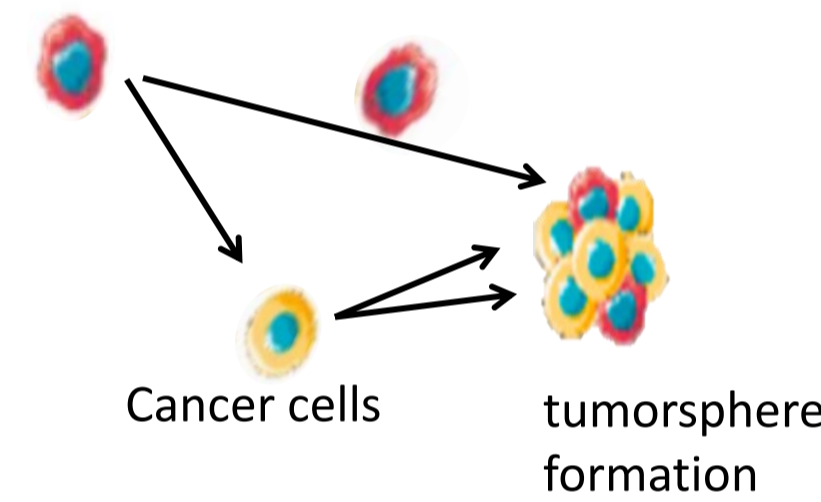


Role of lncRNAs in cancer persistent cells

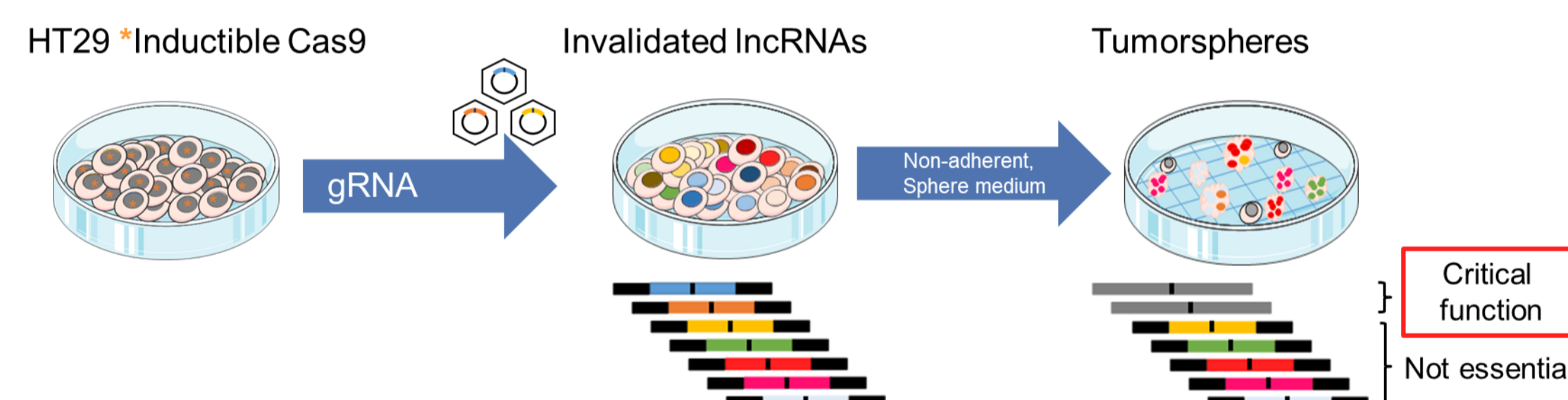


| lncRNA | Fold-Change |
|-----------|-------------|
| GASS | 4.1 |
| LINC01133 | -3.6 |
| Linc-UBC1 | 2.9 |
| CRNDE | 3.6 |
| H19 | 2.7 |
| UCA1 | 2.6 |
| MALAT1 | 2.0 |

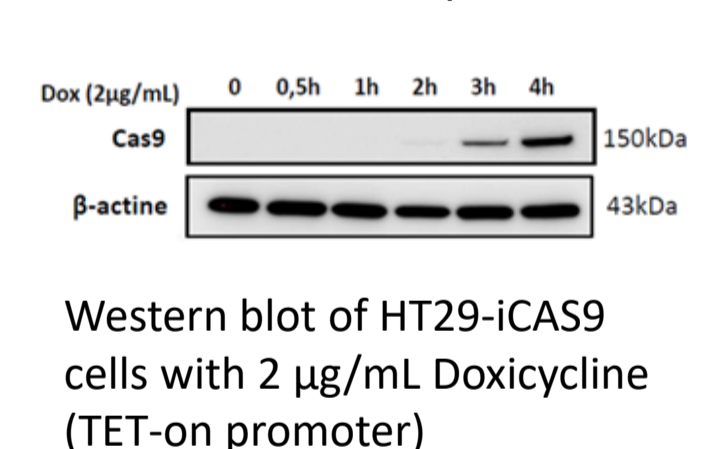
Cancer stem cells



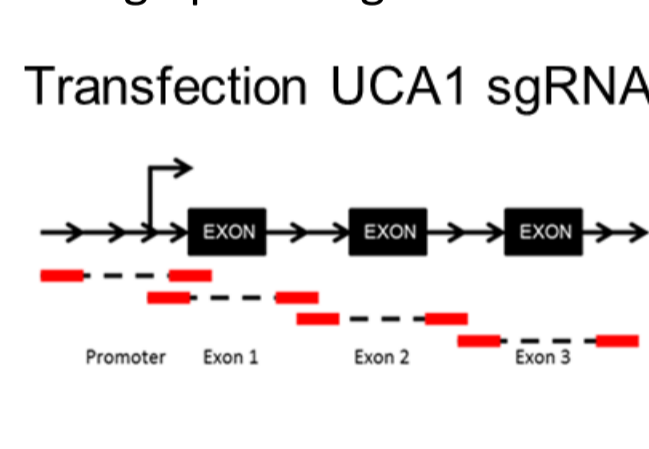
Lentiviral CRISPR-Cas9 library screen



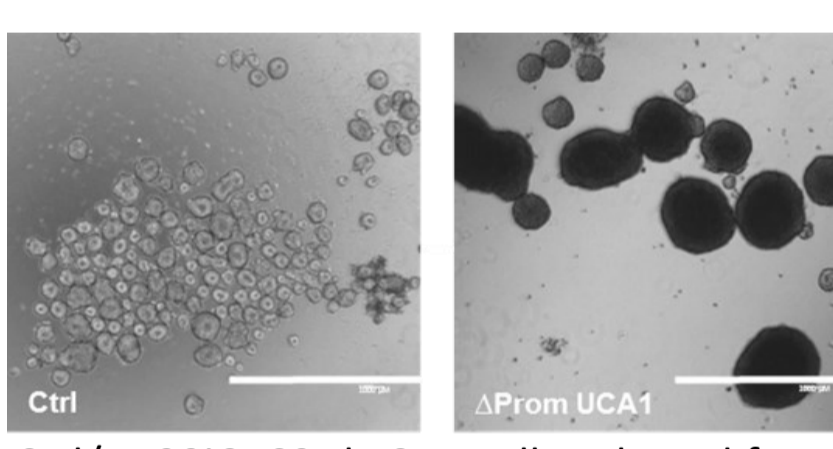
Induction of Cas9 protein



Design paired sgRNAs

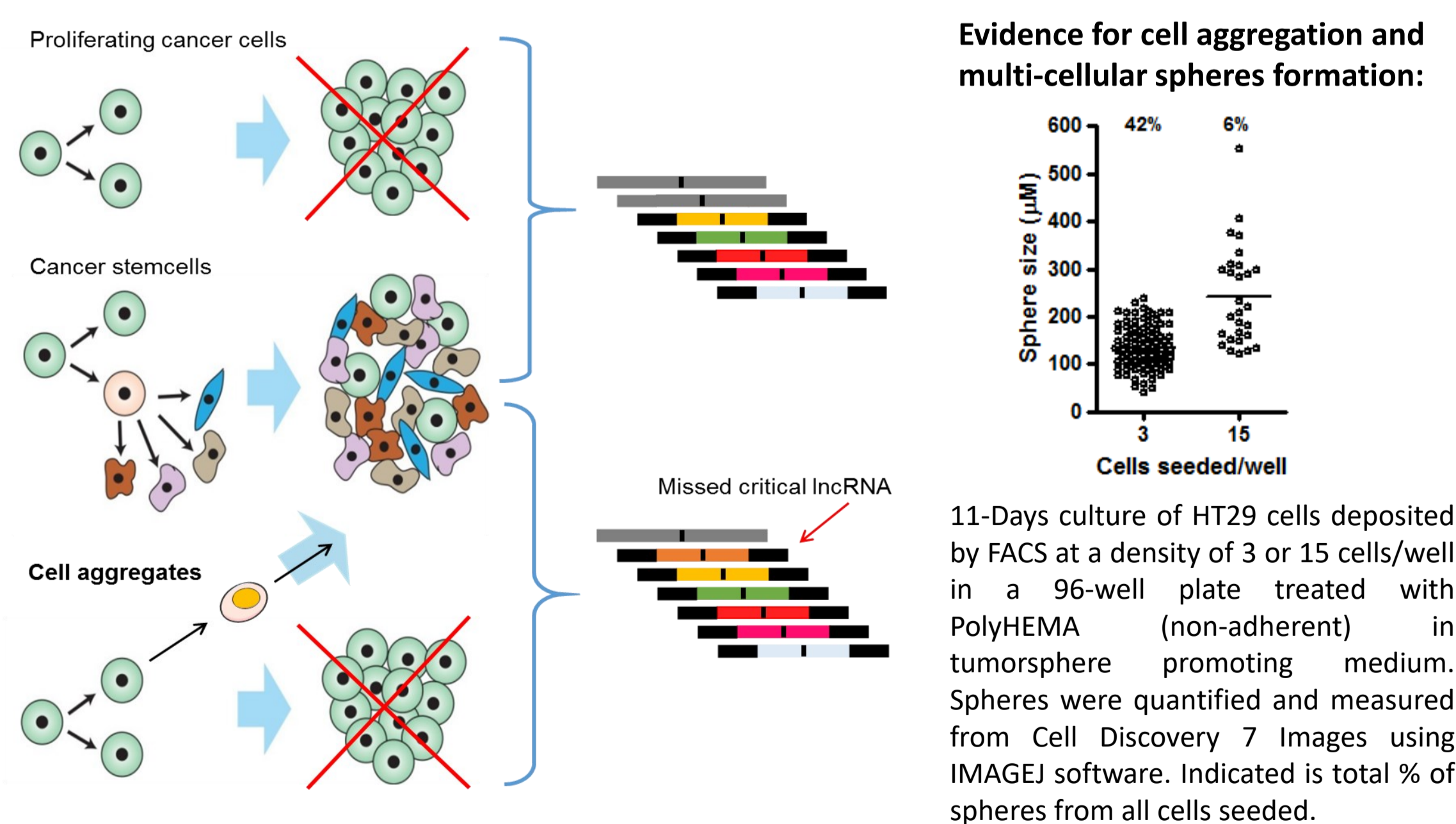


Non-adherent cell culture

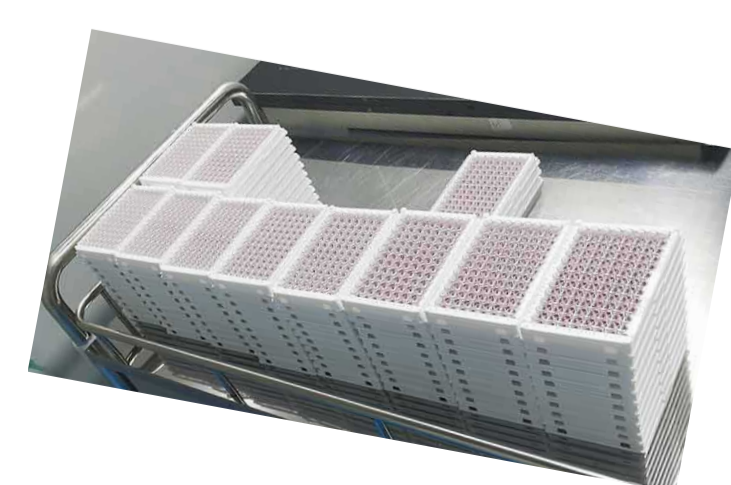


Ctrl/HT29iCAS9-dUCA1 cells cultured for 7 days in tumorsphere promoting conditions

Need for single cell analysis

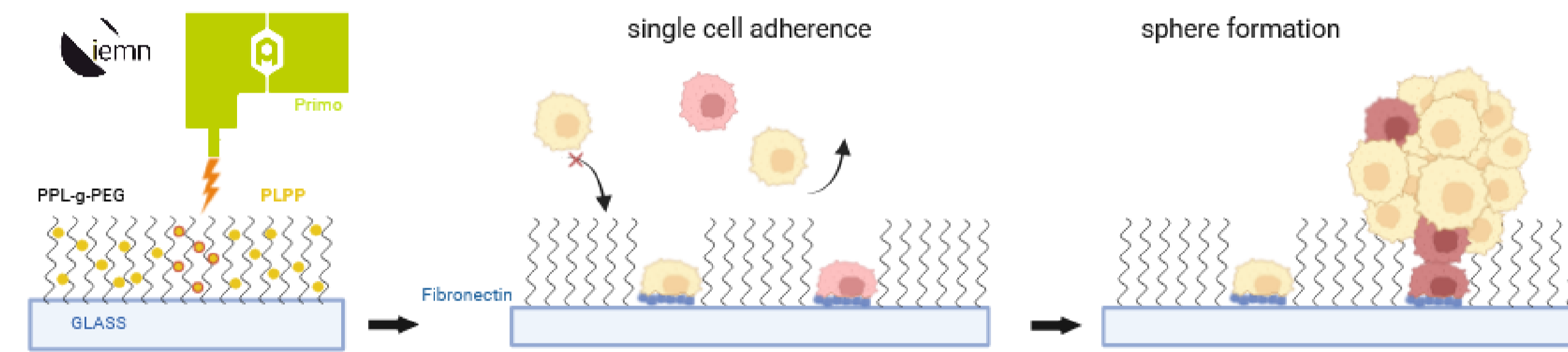


Need for accessible high-throughput readout assay

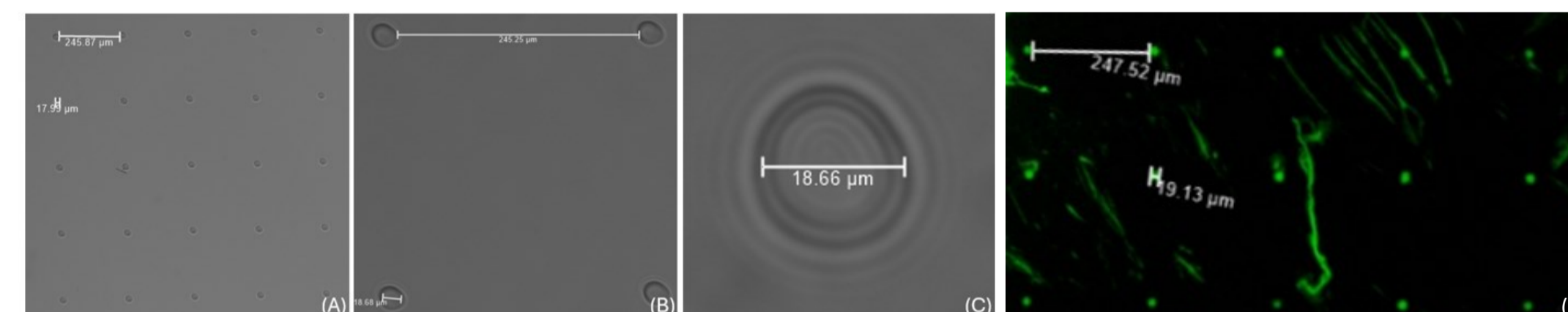


- FACS sorting 1 cell / well
- One sgRNA-pair / cell => low MOI, more cells to infect
- Negative screen: each sgRNA 1000x represented in library
- RNA analysis of all spheres: 96-well format not practical

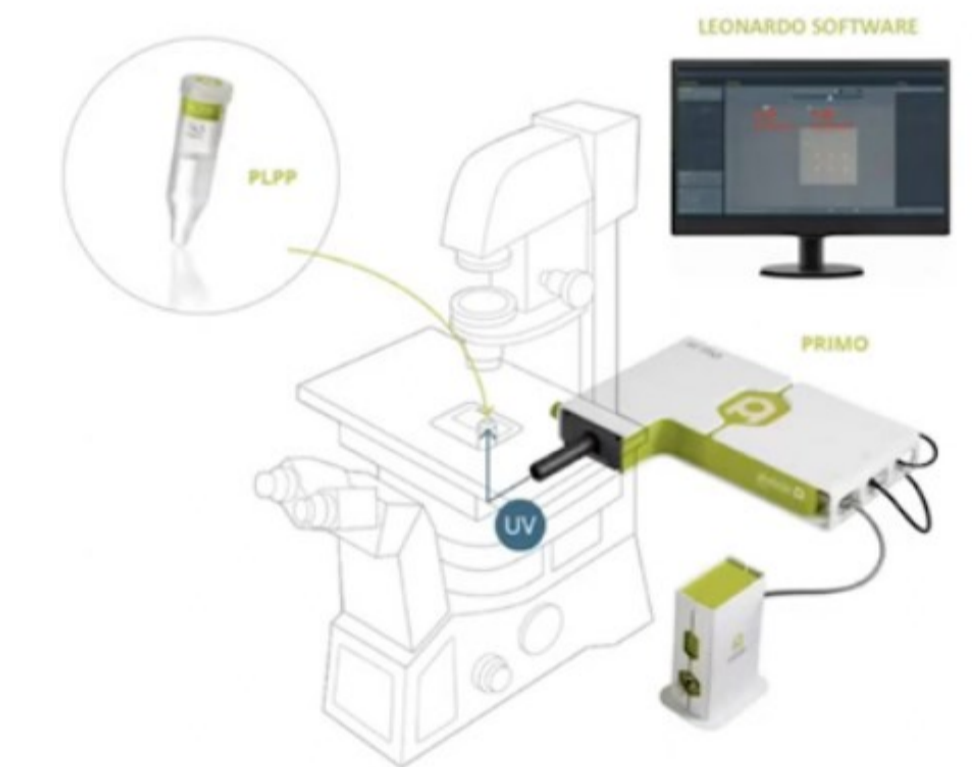
HT29 cells in single cell adherence plates followed by tumorsphere culture



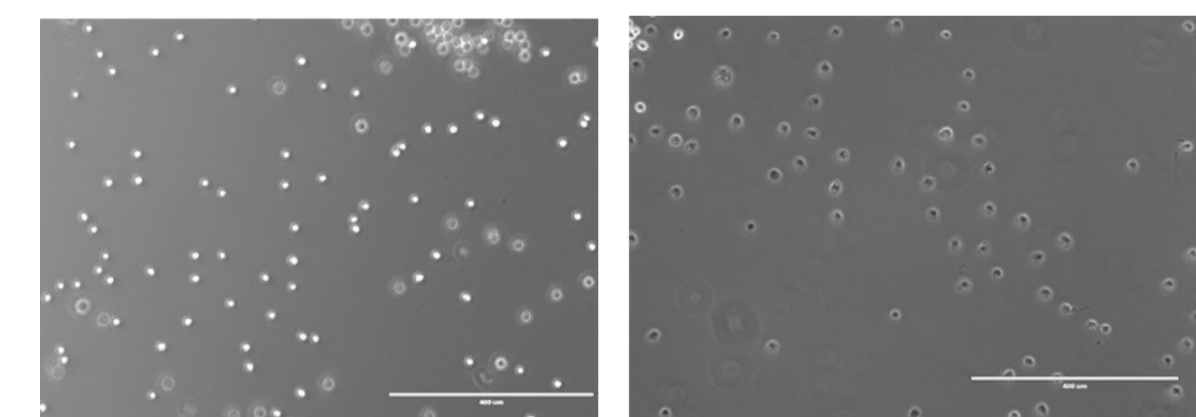
Photopatterning Primo bioengineering technology (Alvéole, France)



Images from Léo Wurthlin (M1): Photopatterning realized with a dose of 30 mJ/mm² to obtain a matrix of 50 x 50 circles with a diameter of 18 μm separated by 250 μm. (A-C) Images with different magnification acquired by a DMI8 inverted microscope, after the photopattern was visualized by DWS LAB Vitra™ 429 ink. (D) DMI8-acquired image of a green-fluorescent Alexa Fluor® 488 fibrinogen conjugate deposited in a 6-well glass bottom plate after photopatterning.

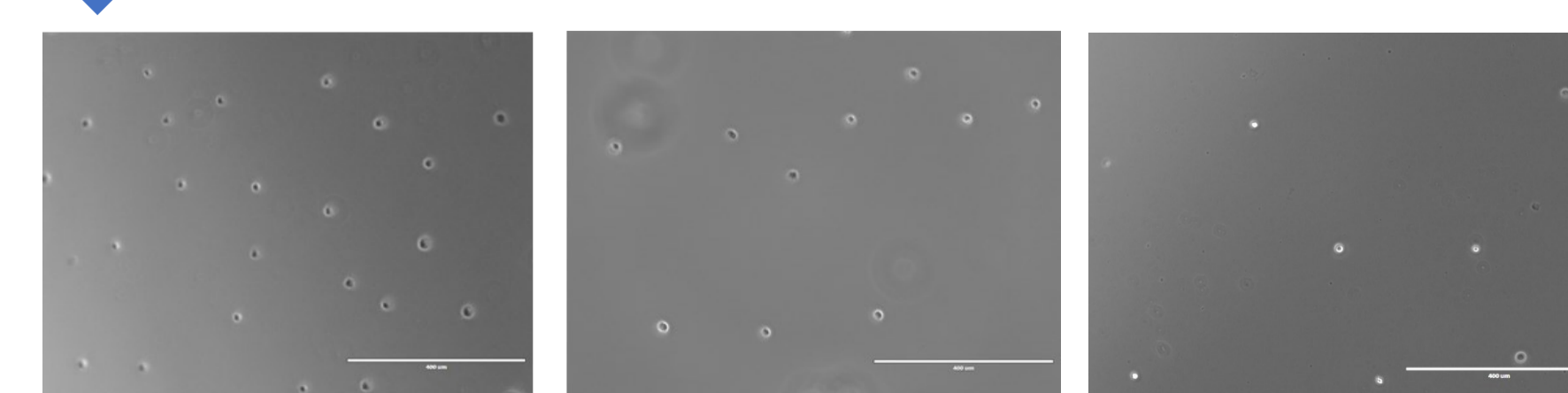


Seeding of HT29 in a Primo-treated well



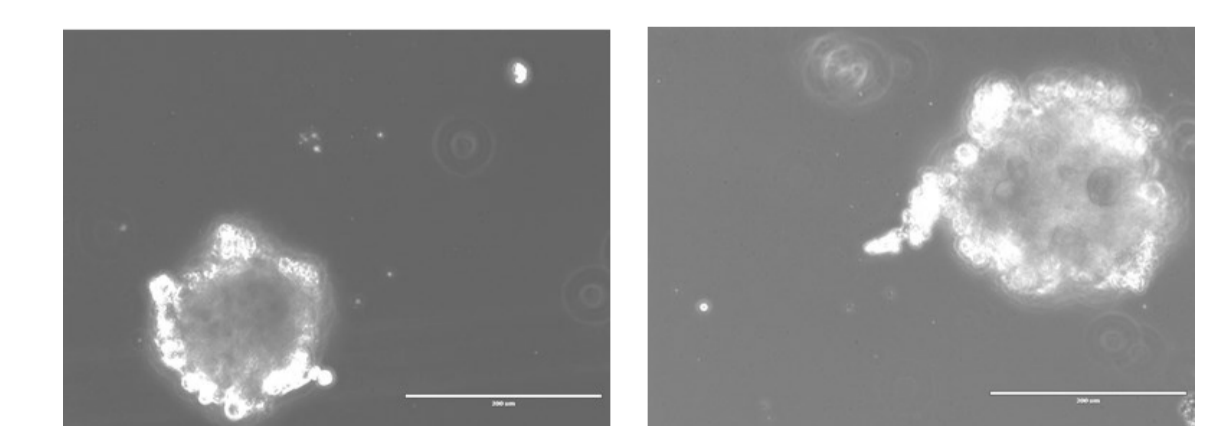
HT29 cells incubated at 37C in standard culture medium for at least 2h. O/N culture results in inert cells

Washes with PBS to remove cells not in adherence spots

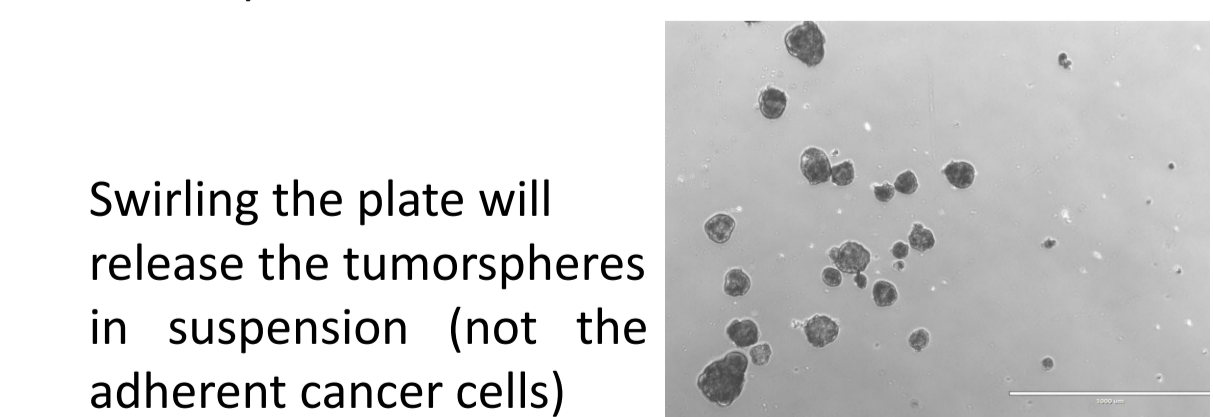


HT29 cells seeded at 200K cells/well adhere in an individual cell pattern. Primo-treated plates are best used within 1-2 days for best results.

- Easy transport of 6-well plate to (lentiviral) culture room
- Seeding of a cell suspension, but individual cell culture



After adherence HT29 cells were cultured in 3D Tumorsphere Medium XF (Promocell). Only stem-like cells will proliferate and develop tumorspheres



Swirling the plate will release the tumorspheres in suspension (not the adherent cancer cells)

- This method is suitable as high-throughput readout assay for library screens
- Single-cell originated tumorspheres can easily be recolted for RNAseq analyses assessing sgRNA expression and transcriptomes

