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X-Ray Fluorescence Microscopy based Cell Discrimination using Inorganic Nanoparticle Tag

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Cell cultures are a crucial tool in biological science. To accurately mimic complex human tissues, heterogeneous co-cultures consisting of different cell types are essential. However, traditional methods for identifying individual cells in these mixes lack spatial resolution, are destructive, or require thin slicing. Here, we show the use of inorganic nanoparticle tags for X-ray fluorescence imaging (XFI) as a powerful, non-destructive, and high-resolution approach to analysing cell distribution.
We screened 11 different nanoparticle syntheses to identify the 4 best candidates, based on gold (Au), iron (Fe), Nickel (Ni), and Bismuth (Bi) for high-contrast, spectrally unique XFI signals. Using these selected tags, we successfully labelled four different cancerous cell lines (HeLa, MCF7, A549, and 4T1) in a mixed cell culture. Using the hard X-ray microprobe at beamline P06 (PETRA III, DESY), we performed high-sensitivity XFI to achieve simultaneous identification and spatial mapping of all four cell types based on their individual NP signatures, confirming the method's precision and lack of spectral interference.
This work establishes a robust XFI framework for non-destructive, multiplexed cellular imaging, distinguished by its high sensitivity and spatial resolution, enabling precise, non-destructive, and multiplexed cellular mapping in 2D co-cultures. Furthermore, the innate deep-penetration capability of hard X-rays provides a direct and seamless pathway to apply this same methodology to complex 3D tissue models. This versatility unlocks new possibilities for studying cellular interactions in realistic tumour microenvironments and evaluating drug response.

Authors: Dr SKIBA, Marvin (Universität Hamburg); PARAK, Wolfgang (Uni Hamburg)

Co-authors: Dr PENG, Bo (Universität Hamburg); Dr YANG, Chenxi (Universität Hamburg); HAN, Jili (Universität Hamburg); LI, Jinrui (Universität Hamburg); Dr BARRIO CAPUCHINO, Juan Jesus (Universität Hamburg); SOSNIOK, Martin (Universität Hamburg); LUONGO, Maya (Universität Hamburg); Dr FENG, Miao (Universität Hamburg); FELIU TORRES, Neus (UniversitÃ¤t Hamburg); RESZEGI, Rebeka (Universität Hamburg); SHÜTT, Robbert (Universität Hamburg); Dr WANG, Ruixia (Universität Hamburg); ZHOU, Shihao (Universität Hamburg); WANG, Wenbo (Universität Hamburg); HAN, Yuxuan (Universität Hamburg)

Presenter: LUONGO, Maya (Universität Hamburg)

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