HTS tests for toxicity assessment and their adaptation to nanomaterials

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Introduction

IBCH PAS offers an integrated and well-structured research infrastructure that supports the identification of bioactive chemical compounds, evaluation of their biological effects and „hit–to-lead" optimisation. The high-throughput screening (HTS), super-resolution and chemical biology platforms, located within the Center for Chemical Biology ERIC – a certified **EU-OPENSCREEN ERIC** and **POL-OPENSCREEN node** – enables automated biochemical and biological assays using miniaturised formats, acoustic dispensers, and multimodal readers. It is also equipped with imaging platforms: high content imaging system Opera Phenix and MINFLUX super-resolution microscope. The platform provides access to both the EU-OPENSCREEN compound library and national chemical collections, as well as expert support in assay development and data analysis.

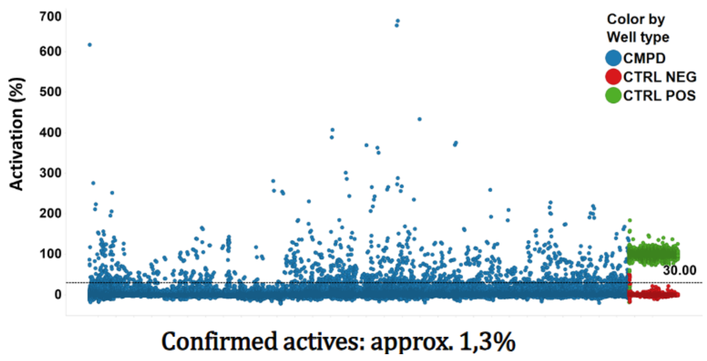
In the iCare project, **IBCH PAS** is involved in development of high throughput screening (HTS) and high content screening (HCS) assays to investigate chemical reactivity and biological effects of pristine and industrial nanomaterials exposed to different environmental and biological conditions. **This unique panel of multiparametric and high throughput assays** on biological models of neuro-nanotoxicity will contribute to linking physico-chemical behaviour of nanomaterials to their biological effects and will demonstrate a unique possibility to high throughput evaluation of the safety of nanomaterials in faster, cheaper and more reliable way than current mono-parametric in vitro methods.

HTS – detection of ROS generation

Implementation of high-throughput assays for identification of generation of **reactive oxygen and nitrogen species** caused by nanomaterials:

- **Resazurin-based assays (DTT/TCEP)** - fluorescence
- **Phenol Red-HRP-DTT/TCEP assays** – for H_2O_2 detection – absorbance
- **FRAP assay -** absorbance

Data from primary screening for resazurin -TCEP based assay



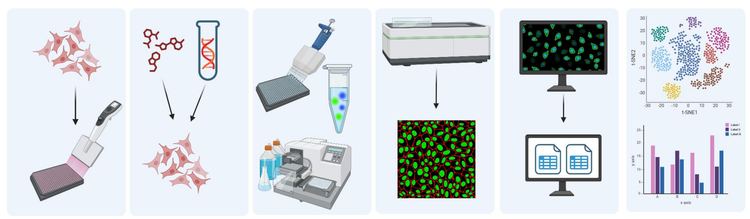
Confirmed actives: approx. 1,3%

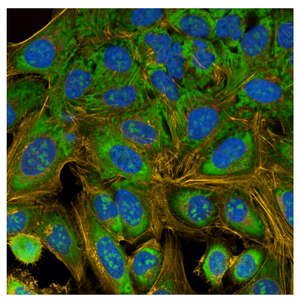
Diversity set (approx. 100k compounds) of European Chemical Biology Library (ECBL)

HCS – toxicity in *in vitro* models

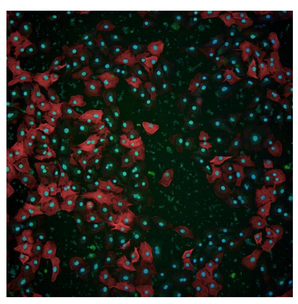
Identification of neurotoxicity in biological models exposed to nanomaterials:

- **Phenotypic Cell Painting Assay**
- **Multiparametric neurotoxicity detection Assays**





U2-OS cell line stained using Cell Painting Assay. Images acquired using Opera Phenix microscope (63x water objective)



A549 cell line stained with Caspase-3/7 detection kit. Images acquired using Opera Phenix microscope (20x water objective)

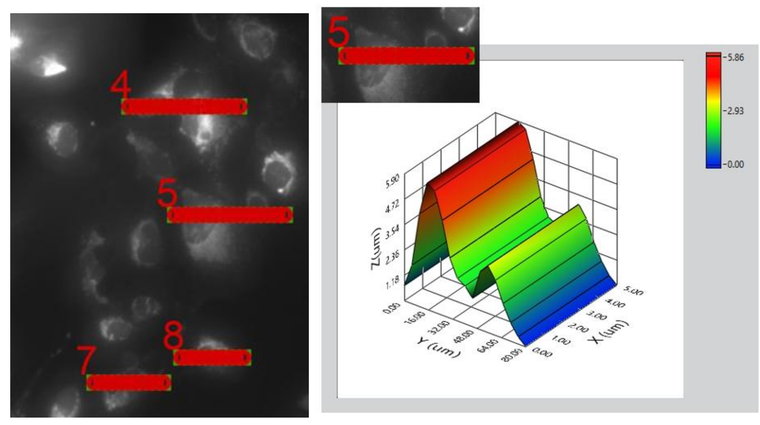
Karczewska N. et. al (2024) Postepy Biochem. 1;70(2):230-245.
Schreiner A. (2025). Phenotype discrimination using the PhenoVue cell painting and multi-organelle staining kits. (Application Note)

PAVONE – Biomechanical studies

PAVONE – first in the world high-throughput mechanical screening platform performing measurements of **fluorescently labelled cells** exposed to nanomaterials:

- Detection of shifts in **viscoelastic parameters**
- Changes in the **3D distribution** of cellular compartments

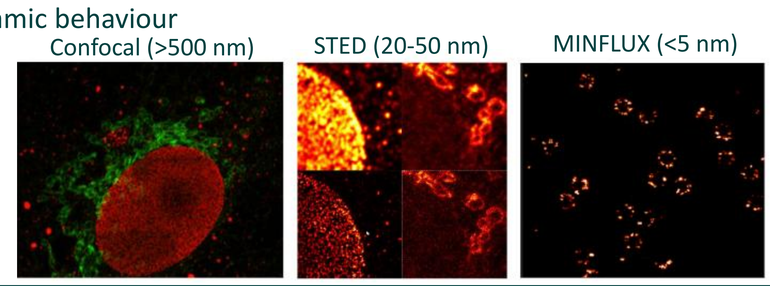
Identation and measurement of topography for U2-OS cell line:



MINFLUX – monitoring of nanomaterial aggregation

Super-resolution fluorescent imaging platform – one of only few systems in the world, performs imaging with a resolution below 2 nm. This work compares benefits of two super-resolution imaging techniques - **STED** (Stimulation Depletion Microscopy), **MINFLUX** (Minimal Photon Flux) to dynamic fluorescent microscopy for

- Monitoring and quantification of **aggregation** of nanomaterials in different matrices;
- Validating auto-fluorescence vs fluorescent tags as mean of visualisation of nanomaterials and their dynamic behaviour



Summary

- Combining all presented techniques allows to establish novel methodologies to resolve questions about the impact of different environments on the stability and reactivity of tested nanomaterials, which might potentially impact nanotoxicity.
- Implementation of three independent imaging platforms results in obtaining complex data on the condition of biological models exposed to tested nanomaterials, which might detect their potential early onset nanotoxicity more reliably and with higher toxicity.
- Unique iCare panel of HTS and HCS assays allows to collect more informative, multiparametric data thereby reducing time and costs of performed experiments.

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Ensuring long-term sustainability of excellence in chemical biology within Europe and beyond' — 'EU-OPENSCREEN-DRIVE project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 823893 (page 43 of the contract, art. 27.3)

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