Improved multiparametric scrape loading-dye transfer assay for a high-throughput analysis of intercellular communication

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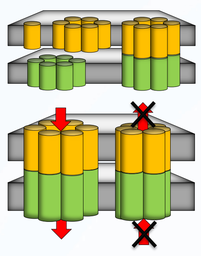
GAP JUNCTIONS

❖ ***Gap Junctions*** [1]
- Closable transmembrane channels
- Connection of adjacent cells

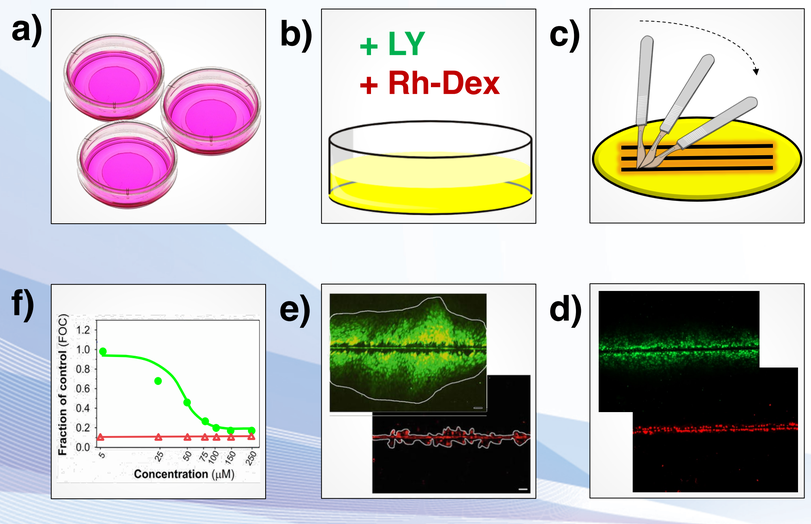
❖ ***Gap Junctional Intercellular Communication*** [2]
- Central homeostatic process
- Exchange of information, signal molecules etc.
- Signal transduction, phosphorylation by kinases



❖ ***Inhibition of GJIC*** [3]
- Tumour-promoting process
- Dysregulation of apoptosis, contact inhibition, proliferation and various cell functions

TRADITIONAL ASSAY

❖ ***Scrape loading-dye transfer (SL-DT) assay*** [4]
- Permeable fluorescent dye Lucifer Yellow (**LY**) as a tracer of GJIC
- Impermeable fluorescent dye Rhodamine-Dextran (**Rh-Dex**) as a "loading control"



Cells are seeded into individual Petri dishes **(a)**, let grow into a full confluency and exposed. The growth (exposure) medium is poured and cells are washed with a buffer, the fluorescent dye mix (Lucifer Yellow and Rhodamine-Dextran) is added **(b)**. After 5-min incubation, three parallel cuts with surgical blade are created **(c)**. Cells are subsequently incubated for a few minutes to allow fluorescent dyes to diffuse through gap junctions. Representative images of dyes' diffusion are taken using fluorescent microscope **(d)**. The area of each of the fluorescent dye is then evaluated using freeware ImageJ software **(e)**. Data are evaluated, compared to negative/solvent control(s) and plotted into graph **(f)**.

❖ ***Disadvantages***
- High material and time consumption
- Costly
  - Fluorescent dyes mix ~ 650 CZK/mL
- Low reusability of materials an chemicals
  - Rh-Dex usable for approx. 1-2 weeks
- Manual performance, not easy to handle
- Only one endpoint
- Manual (subjective) evaluation
  - The evaluator tends to manipulate with the results according to expected values

OBJECTIVES

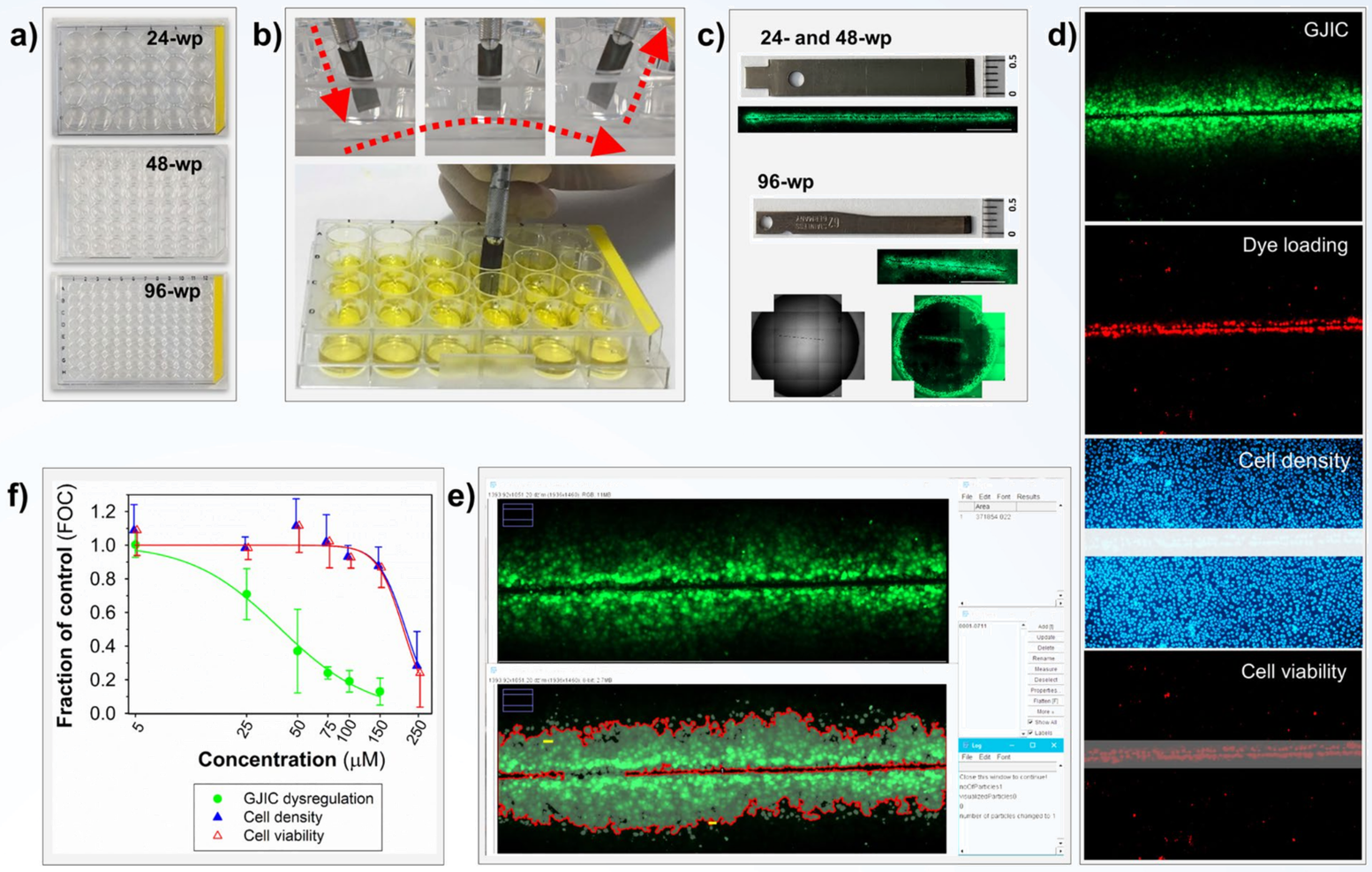
❖ ***Miniaturisation***
- Improved design, high-throughput compatibility

❖ ***Better cost-effectivity***
- Lower material consumption
- Alternative fluorophores

❖ ***(Semi)-automatic performance and evaluation***
- Automatic acquisition program
- Semi-automatic evaluation in ImageJ

❖ ***Multiple endpoints***

NOVEL MULTIPARAMETRIC METHOD



The novel multiparametric *in vitro* assay is suitable for multiple high-throughput-compatible formats, such as 24-well plate (24-wp), 48-well plate (48-wp) and 96-well plate (96-wp; **a**). The evaluation of GJIC dysregulation is based on SL-DT **(b,c)** using Lucifer Yellow as a marker of communicating cells **(d-GJIC)** and propidium iodide as a marker of dye-loaded cells along the cut **(d-Dye loading)**. The number of total cells is assessed by staining cell nuclei with Hoechst 33342 **(d-Cell density)** and the number of dead cells out of the cut by labelling cells with propidium iodide **(d-Cell viability)**. The image analysis and evaluation for all parameters is (semi)automatic using macros in ImageJ software **(e)**. Data are expressed as fraction of control (FOC; **f**).

❖ ***Multiple formats***
- 35mm Petri dish, 24-, 48- and 96-well plate … High-throughput compatible

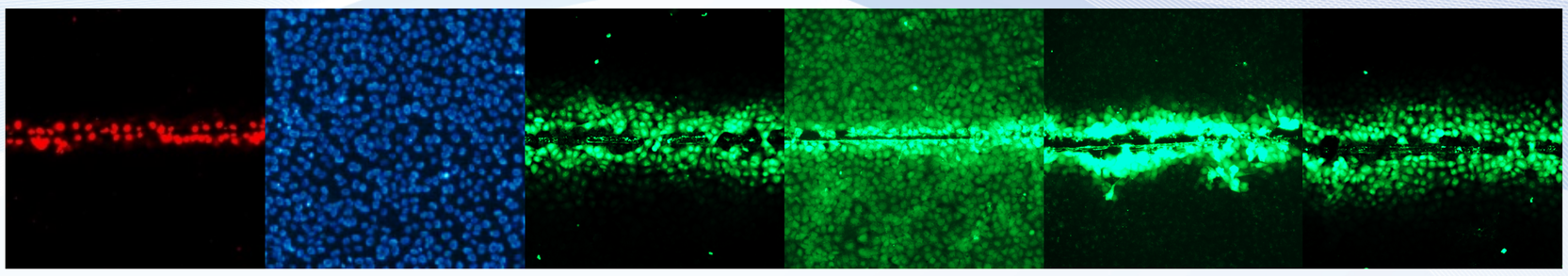
❖ ***Multiple endpoints and fluorophores***
- Loading control, cell viability … Propidium iodide
- Gap junctional intercellular communication … Lucifer Yellow
  … Alternatively Fluorescein 5(6)-isothiocyanate, Calcein-AM, CF™ 488A hydrazide
- Cell density … Hoechst 33342

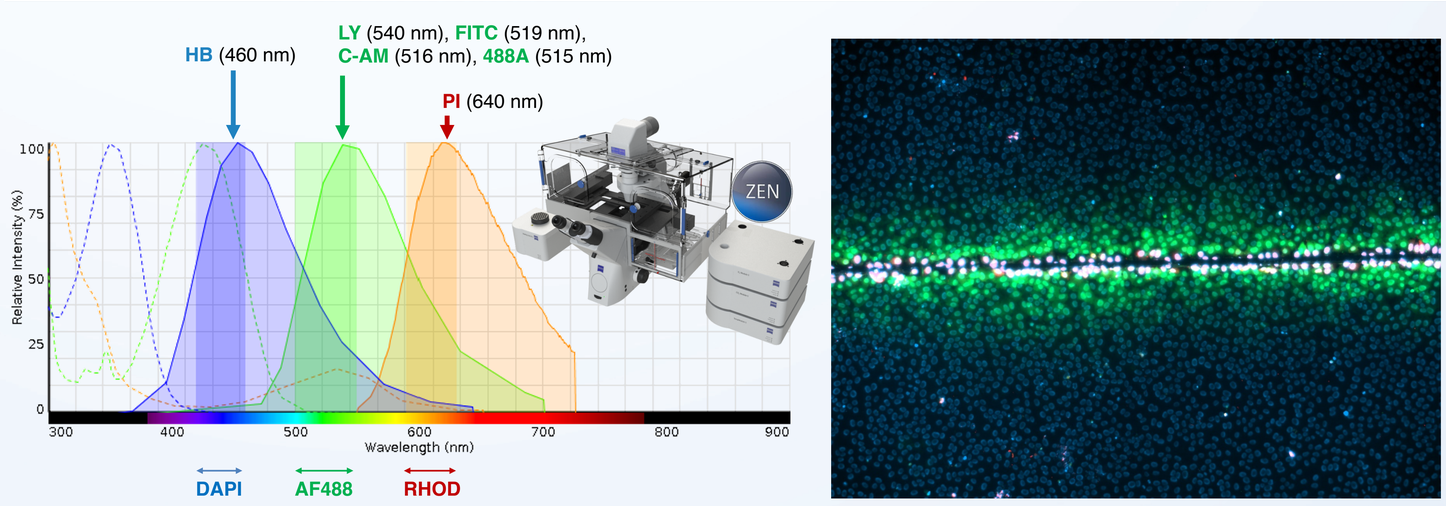
❖ ***(Semi-)Automatic acquisition and evaluation***

❖ ***Cost-effectiveness***
- High reusability and stability … Up to 50-times recyclable fluorescent dyes
- Lower material consumption … Up to 95% less total media consumption
  … Up to 99% less cells needed for an experiment
- Easy to handle and perform

❖ ***Available at*** https://go.nature.com/2yplfGT [5]

Dye loading & Cell viability	Total cell count	Gap junctional intercellular communication			
PROPIDIUM IODIDE (PI)	**HOECHST 33342** (HB)	**LUCIFER YELLOW** (LY)	**Fluorescein 5(6)-isothiocyanate**	**Calcein-AM** (C-AM)	**CF™ 488A hydrazide** (488A)
10 μg/mL, 1500 ms	50 μg/mL, 250 ms	1 mg/mL, 250 ms	(FITC), 1 mg/mL, 500 ms	1 mg/mL, 1500 ms	1 mg/mL, 100 ms
(ex/em 500/640 nm)	(ex/em 350/460 nm)	(ex/em 430/540 nm)	(ex/em 492/518 nm)	(ex/em 496/516 nm)	ex/em 490/515 nm
Filt. RHODAMINE (RHOD)	Filt. DAPI	Filt. ALEXAfluor488 (AF488)	Filt. ALEXAfluor488 (AF488)	Filt. ALEXAfluor488 (AF488)	Filt. ALEXAfluor488 (AF488)





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