# Optical platform for ion channel drug screening

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## Scientific background

Numerous top-selling drugs are ion-channel modulators. One critical aspect of the market of new drugs in this field is represented by the screening of candidate molecules, which is an expensive and time consuming phase of the discovery process. This primary screening is currently based on automated electro-physiology or optical assays. Unfortunately, neither fits the market requirements regarding the optimal combination of information content, throughput and operative costs.

## Proposal

We propose a novel optical drug screening platform to investigate libraries of molecules that exploits a proprietary approach (WO2011009825) Briefly, a direct measurement of the conductance of channels or transporter is extrapolated by measuring VSD fluorescence variations during membrane charging, both under control conditions and after exposure to specific treatments. This makes it possible to estimate the effect of pharmacological treatments on the ion channel state.

## Conclusions

We provide evidence that our platform has the following distinctive features:

-Time resolution: ion channel activity studied on a sub-second time scale

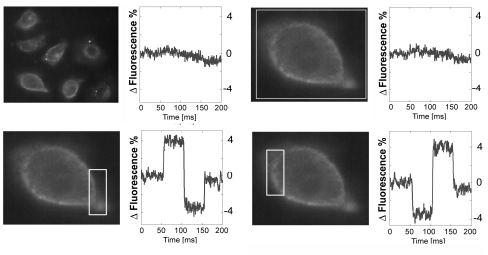
-Spatial resolution: single cell

-Sensitivity: ion channel overexpression not required

-Specificity: no restrictions on either the kind of ion channel or the type of cell

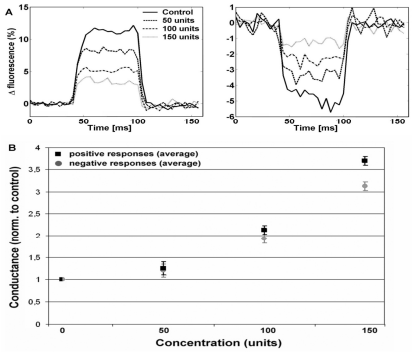
-Scalability: compatible with high throughput requirements

## Imaging membrane potential changes



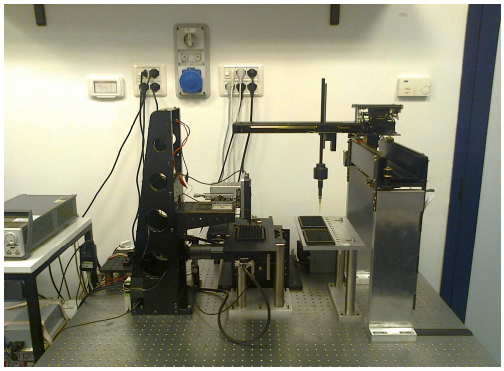

The application of a bipolar electrical pulse induces local changes in the membrane potential that are monitored by fast VSD as variations of the fluorescence. The average signal from a whole field (upper left panels) or even from a single cell (upper right panel) shows no variations upon stimulation, due to the combination of the opposite responses at the two sides (facing electrodes) of the cells. Accordingly, a selective detection of the subcellular responses is necessary to see membrane potential changes.

## Data analysis



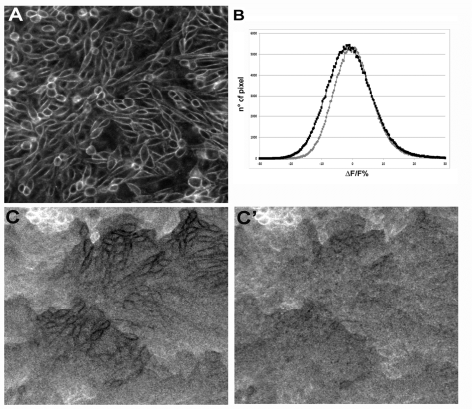

The amplitude of the response to a pulse is a function of membrane conductance (the higher the conductance, the lower the plateau). In this way, quantification of drug effects can be extrapolated from changes in amplitude. A: examples of subcellular fluorescence responses after exposure to different concentration of Streptolysin O, a membrane porator. B the concentration-response relationship.

## Process automation



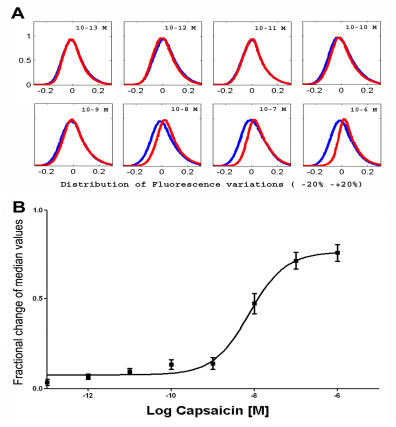
The whole process is fully automated and includes the following steps: auto focus, local electric pulse delivery, drug administration, image acquisition and calculation of the concentration-response curve. Subjective intervention/interpretation of the operator is not required at any step. Typically, a concentration-response curve (9 point dilution series, each point calculated from several thousand cells) can be obtained in 3-4 minutes.

## Data extraction from images




A: VSD-stained h-TRPV1cells. B-C: Examples of the analysis of the fluorescence values, before and after capsaicin (specific agonist) treatment, are shown as distributions (B black and grey traces, respectively) as well as black and white maps of positive and negative variations (C and C')

## Concentration /response curves




A: distributions of fluorescence responses before (blue) and after (red) administration of increasing concentrations of capsaicin. B: concentration-response curve expressed as the fractional change of the median of the responses in A.

## Final considerations

By our HCS/HTS platform it is possible to study any channel, independently of the way it is operated, of its specific permeability and of the extent of the ionic flux sustained by the electrochemical potential.

The methodology has been successfully tested also for the analysis of GABA effects on Staminal differentiated GABAergic neurons.

Noticeably, our platform can be used for screening drugs in complex cellular models such as genetically modified subpopulations, primary models or staminal differentiated neurons.

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