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# One hour in vivo-like phenotypic screening system for anti-cancer drugs using a high precision surface Plasmon resonance device

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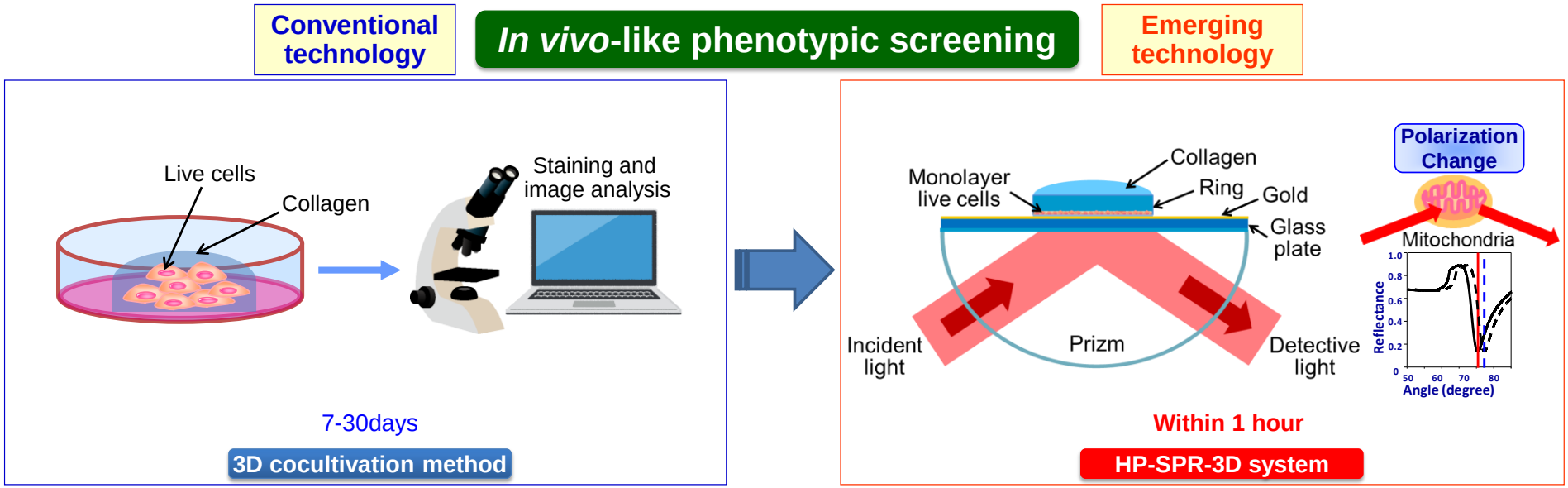
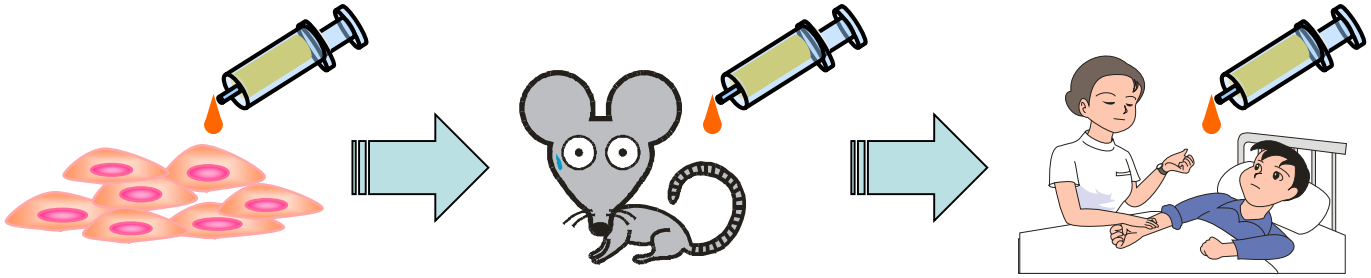
## Graphical Abstract



**Abstract:** In anti-cancer drug (candidate) screening, the demand exists for evaluation at physiological concentrations similar to *in vivo*. This is often performed by 3D cultured cells. It necessitates a long culture period of 2-4 weeks with tedious experimental procedures based on endpoint assay and labeling agents causing low reliability. The previous device methods depend on the pharmaceutical mode of action, little related to the conventional method. Furthermore, a separate set of experiment is required to obtain both efficacy and toxicity. Here, we report on a high precision surface Plasmon resonance-3D system to overcome these all problems sensing dynamic cellular reaction against target compound(s) by laser. We developed the system with average fluctuation of 50 ndeg/s combined with 2D cultured cells attached onto a sensor chip by applying collagen on the top. The 3D cell activity was shortly obtained by this without cell division. New system gave real-time monitoring of mitochondrial membrane potential (MMP) within live cells without both labeling and invasion. It allowed in vivo-like phenotypic screening for anti-cancer drugs within 1h of drug addition. The data were collected as the stable linear signal change parts for at least 5min after 25min following drug addition. The results provided compatibility to clinically related chemosensitivity test ($P<0.001$) using two cell lines of pancreatic cancer and three anti-cancer drugs to represent differences in individual gene expression and drug mode of action. Early MMP change rate is concluded as a key to quantitatively predict the efficacy and toxicity.

**Keywords:** phenotypic; screening; cancer; device; *in vivo*-like

# Steps for efficacy and toxicity prediction of bioactive compounds



*In vitro* cell-based test  Animal test  Clinical test

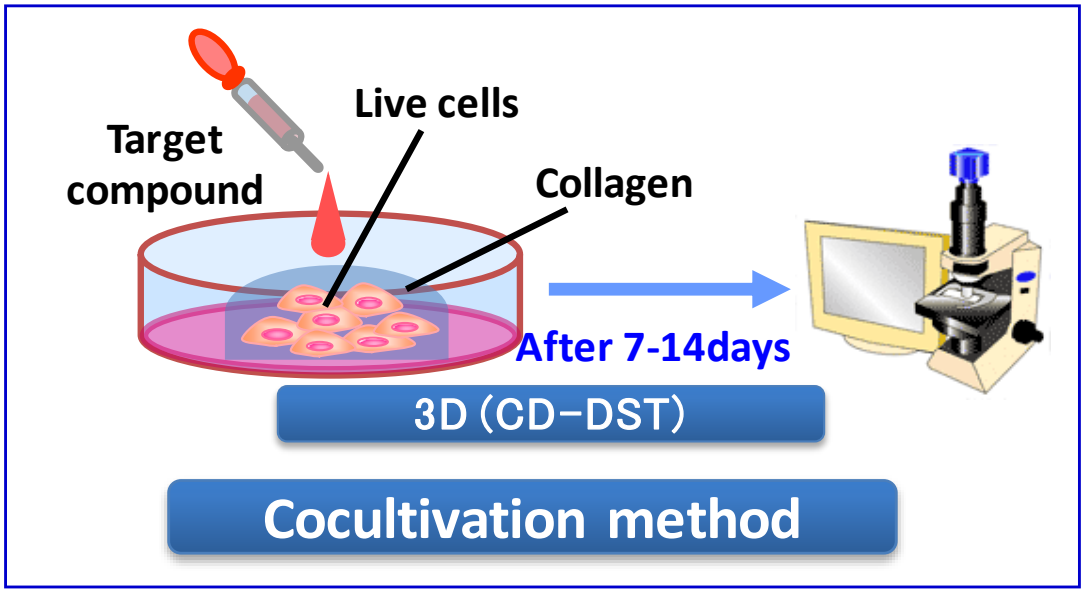
**Animal test should be avoided based on opinions.**

**Rapid and reliable i*n vitro* cell-based test is demanded for discovery of bioactive compounds**

**Key is efficacy and toxicity prediction technology extremely before endpoint of test!**

# Cocultivation method（Cell-based assay）

- Measurement of traits such as cell viability by fluorescence stain and microscope or flow cytometer after 7-30 days of cocultivation of target compounds with live cells embedded in extra cellular matrix such as collagen (3D cell culture)



# Introduction: Advantage & disadvantage of 3D cell culture

## Cocultivation method（Cell-based assay）

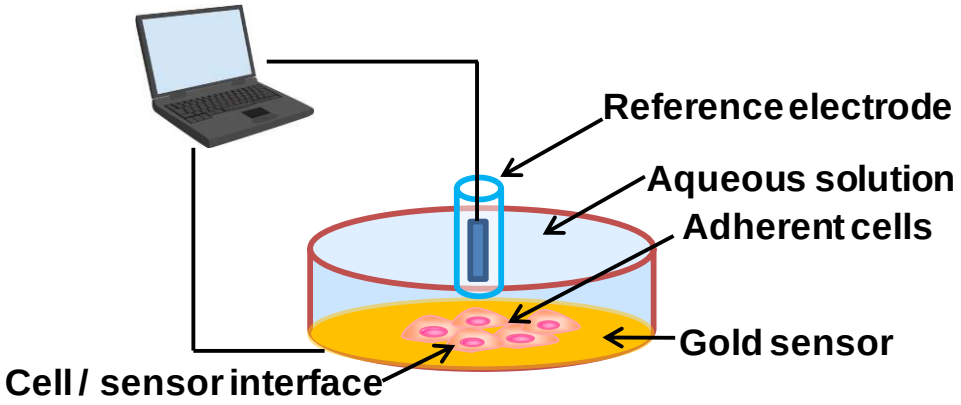
- 3D cell culture with extra cellular matrix
  ---Reproduction of in vivo cell condition
  ---Long decision duration with medium exchange (~30days)--- Slow---
  Large test error--- Low reliability
  ---Standard method---High reliability
  ---Independent to compound mode of action and to personal difference
  --- High reliability
- Label requirement---Large test error---Low reliability
- Difficulty in evaluation of efficacy and toxicity with one experimental set-up--- Low reliability
- Complicated handling---Laboring + Automation shortage---Slow
- Many cells required ($10^6$) ---Difficulty in applying to tumors

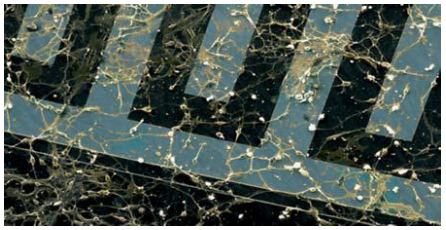
**Rapid, *in vivo*-like label-free cell-based assay is demanded**

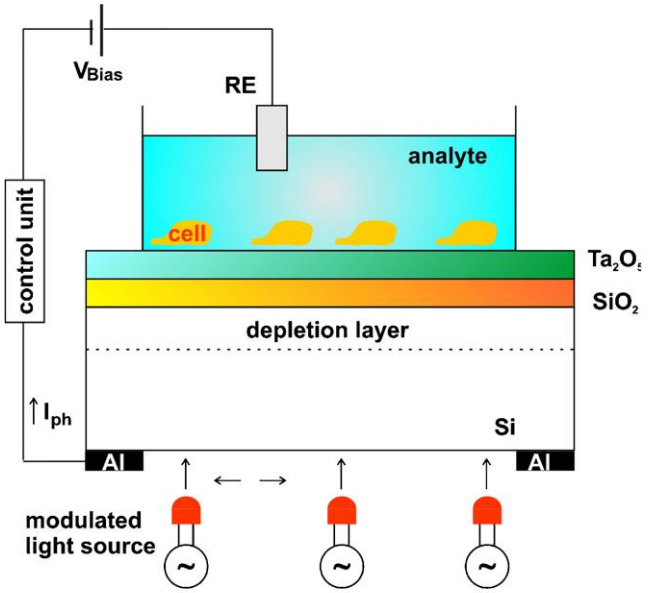
# Introduction: Commercialized devices

## Commercialized devices（Microphysiometer etc）

- Measurement of cellular statuses such as extracellular fluxes of $H^+$, $O_2$, and cell morphology changes using impedance and semiconductor during 1-4 days of cocultivation of target compounds with live cells



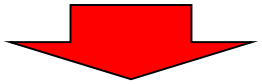


# Introduction: Disadvantege of commercialized devices

## Commercialized devices（Microphysiometer etc）

### Disadvantage

- Medium exchange
- Long decision duration-half of endpoint
- Cause of artifact on live cells by impedance etc
- Large test error
- Low reliability in polypharmacy
- Difference from physiological concentration
- Low compatibility with chemosensitivity test

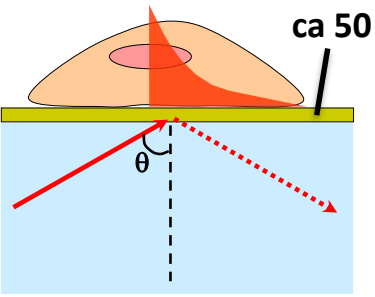


**Rapid, *in vivo*-like label-free and non-invasive cell-based assay is demanded**

# Introduction: Original technology – HP-SPR

## High Precision-Surface Plasmon Resonance (HP-SPR)

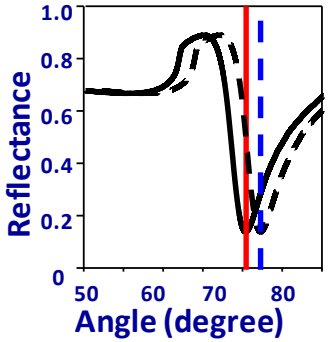
– One of laser spectroscopic methods with non-label and real-time analysis to detect refractive index change derived from interaction between molecules etc. –




**Incident angle (θ) of light for exciting SPR is expressed as:**

$$\sin\theta = \frac{1}{np}\sqrt{\frac{\varepsilon_m \cdot \varepsilon_s}{\varepsilon_m + \varepsilon_s}}$$

**Resonance angle (θ) depends on sample dielectric constant ($\varepsilon_s$)**




**Beam mold** | **Chip design** | **Algorithm** | **Temp. control** | **Low vibration**

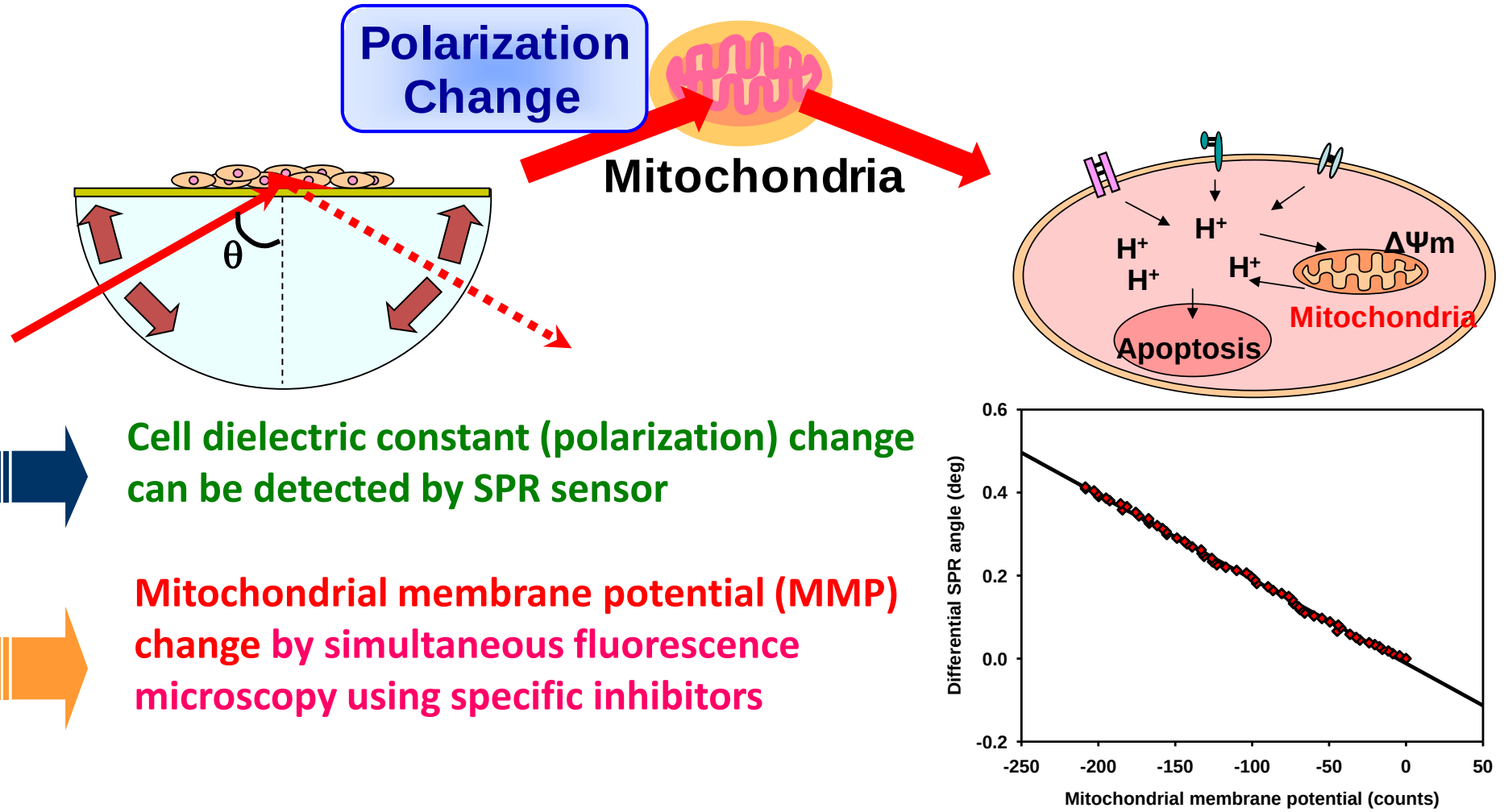
10K to 1M times higher sensitivity compared to commercial instrument !

**Cell reaction = dielectric constant of a few 100 n/s**

Detection of live cell reaction – Apply to efficacy prediction of target compounds –

# Introduction: Original technology – HP-SPR

## HP-SPR

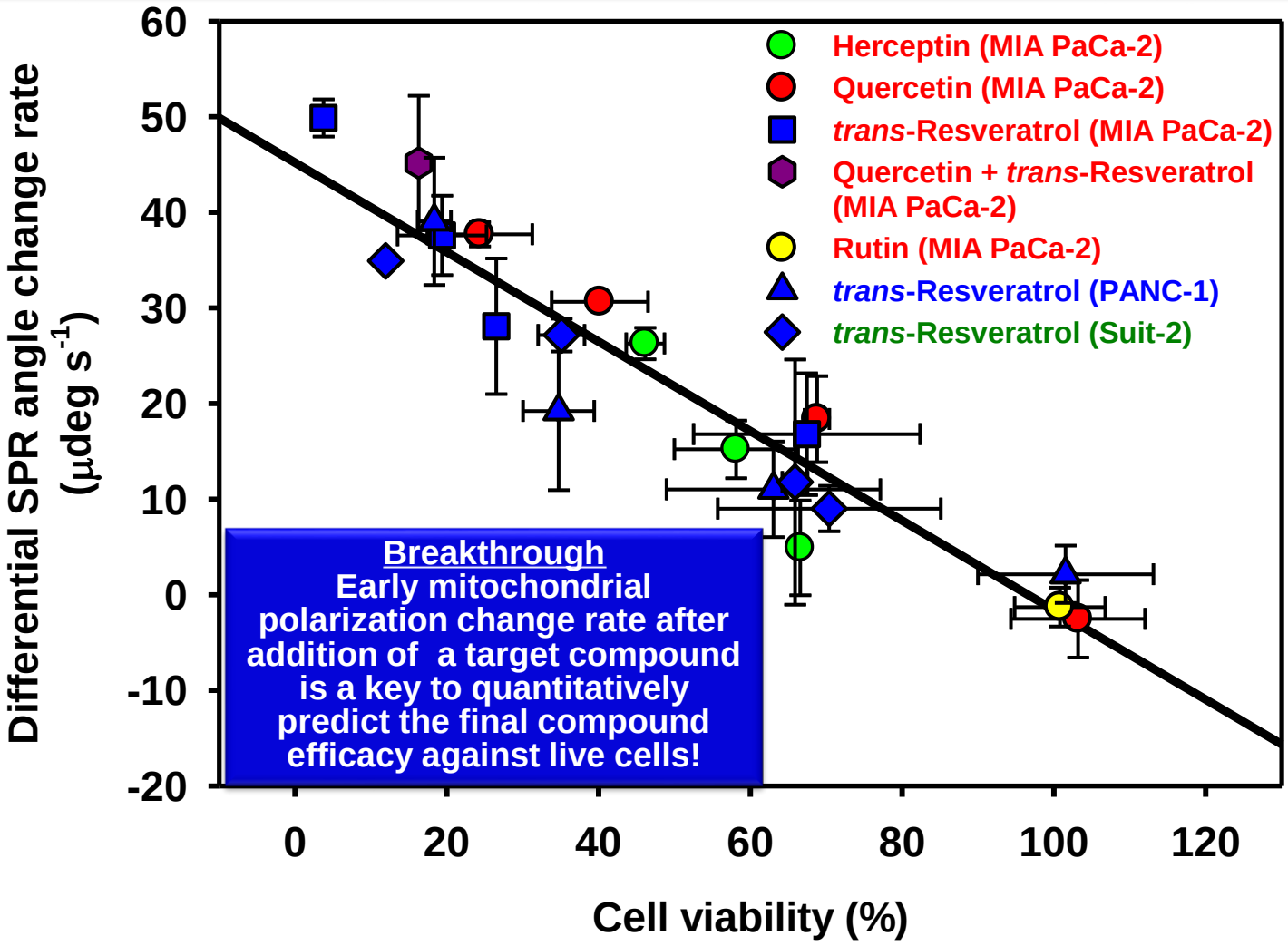



Cell dielectric constant (polarization) change can be detected by SPR sensor

Mitochondrial membrane potential (MMP) change by simultaneous fluorescence microscopy using specific inhibitors

# Introduction: Original technology – HP-SPR-2D

## HP-SPR-2D



## Objectives

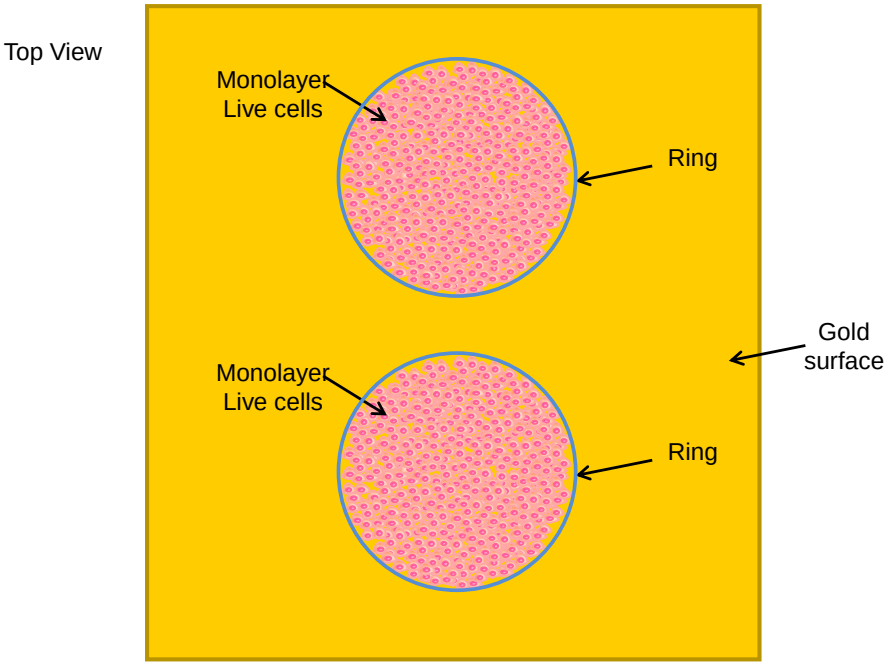
The HP-SPR-2D system uses 2D cells attached onto a sensor chip, which does not allow anti-cancer drug evaluation at physiological concentrations. Theoretically, SPR can detect only materials attached or very close at nearly half of incident light wavelength. Consequently, we cannot use 3D cultured cells for the HP-SPR system.

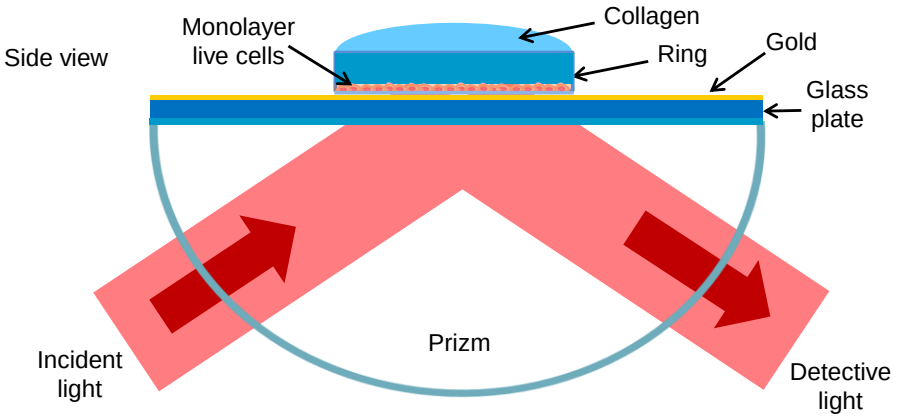
Here, we report on the newly proposed HP-SPR-3D system, which evaluates the efficacy of an anti-pancreatic cancer drug at physiological concentrations. Because pancreatic cancer does not respond to many anti-cancer drugs efficiently contrary to other types of cancer, the number of deaths is increasing in pancreatic cancer patients. Thus, our first target focused on pancreatic cancer.

First, HP-SPR-3D was examined by the activation of 2D cells into *in vivo*-like status on a sensor chip without cell division by applying collagen on the top of the cells.

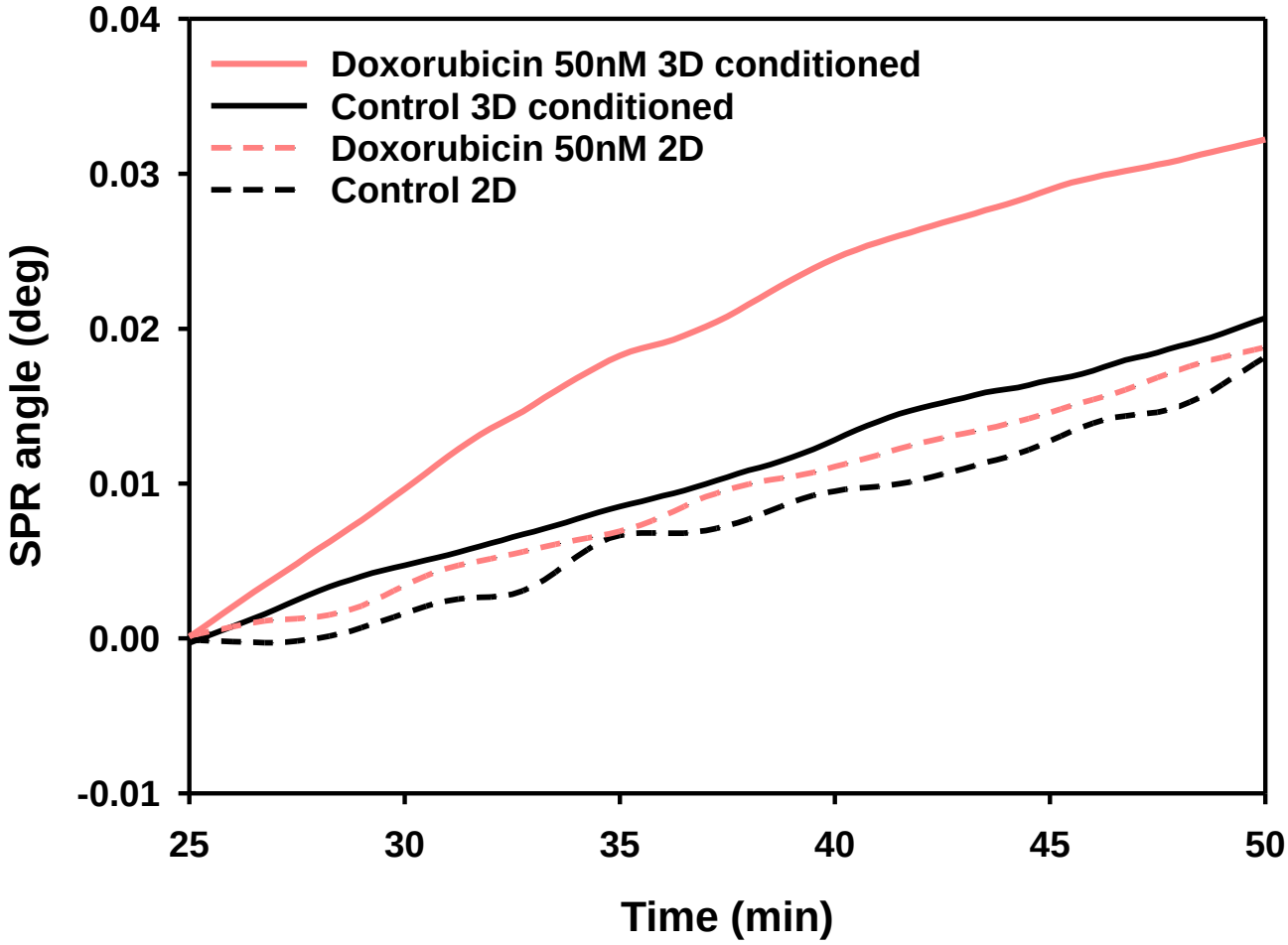
Second, the HP-SPR-3D results obtained were compared to the results of a clinically related chemosensitivity test for pancreatic cancer, collagen droplet embedded culture drug sensitivity test (CD-DST), and the HP-SPR-3D was validated as an *in vivo*-like system.

# Results and discussion: Schematic diagram of HP-SPR-3D

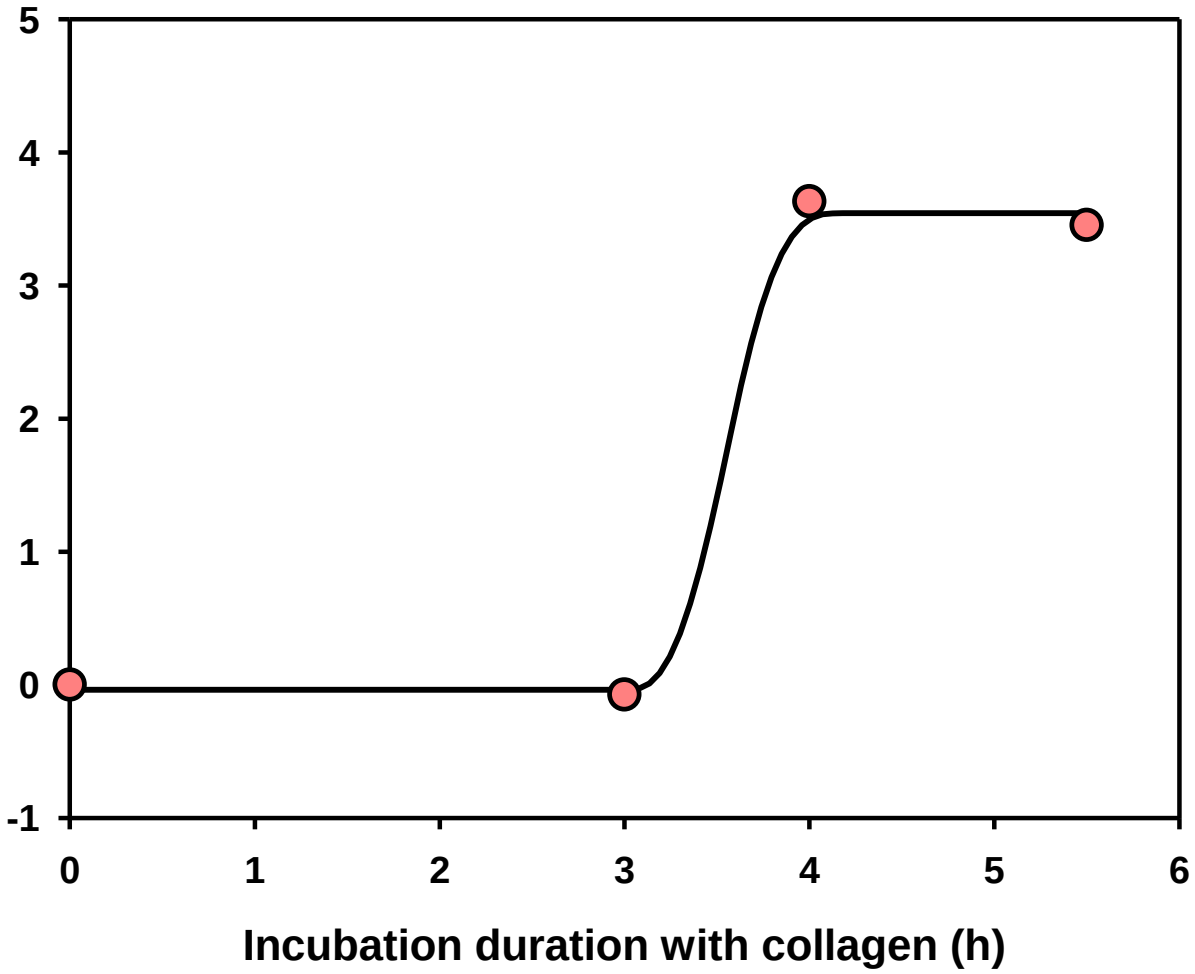


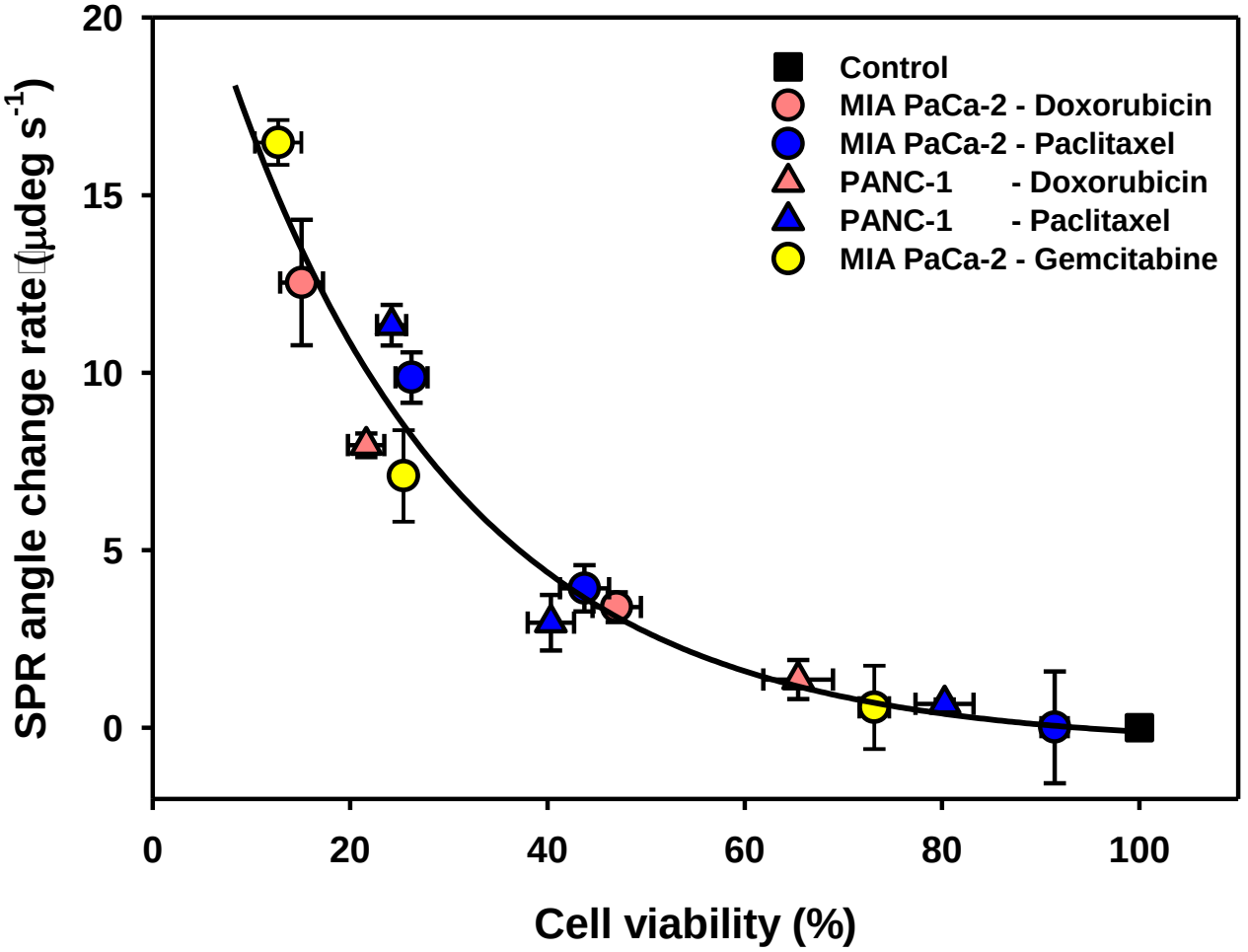
# Results and discussion: SPR angle trend in MIA PaCa-2 cancer cells with 2D and 3D conditioned status exposed to 50nM doxorubicin



# Results and discussion: Change rate in SPR angle in MIA PaCa-2 cancer cells with incubation duration with collagen exposed to 50nM doxorubicin



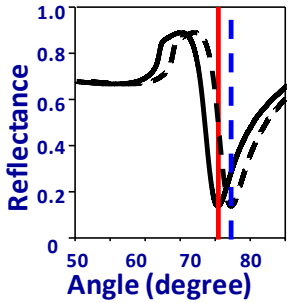
# Results and discussion: Relationship between cell viability by CD-DST and Change rate in SPR angle exposed to various concentrations of drugs (Error bar shows SEM)

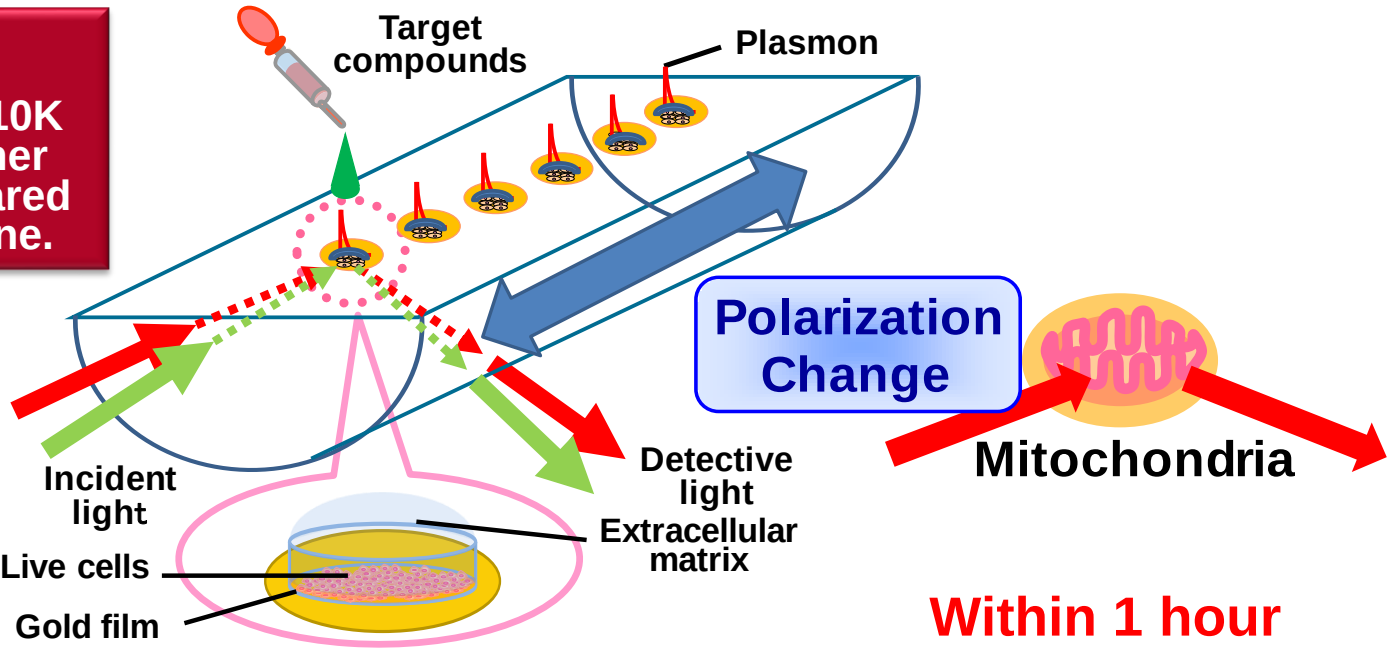


# Conclusions

## HP-SPR-3D

**Breakthrough**
**Custom-made instrument with 10K to 1M times higher sensitivity compared to commercial one.**



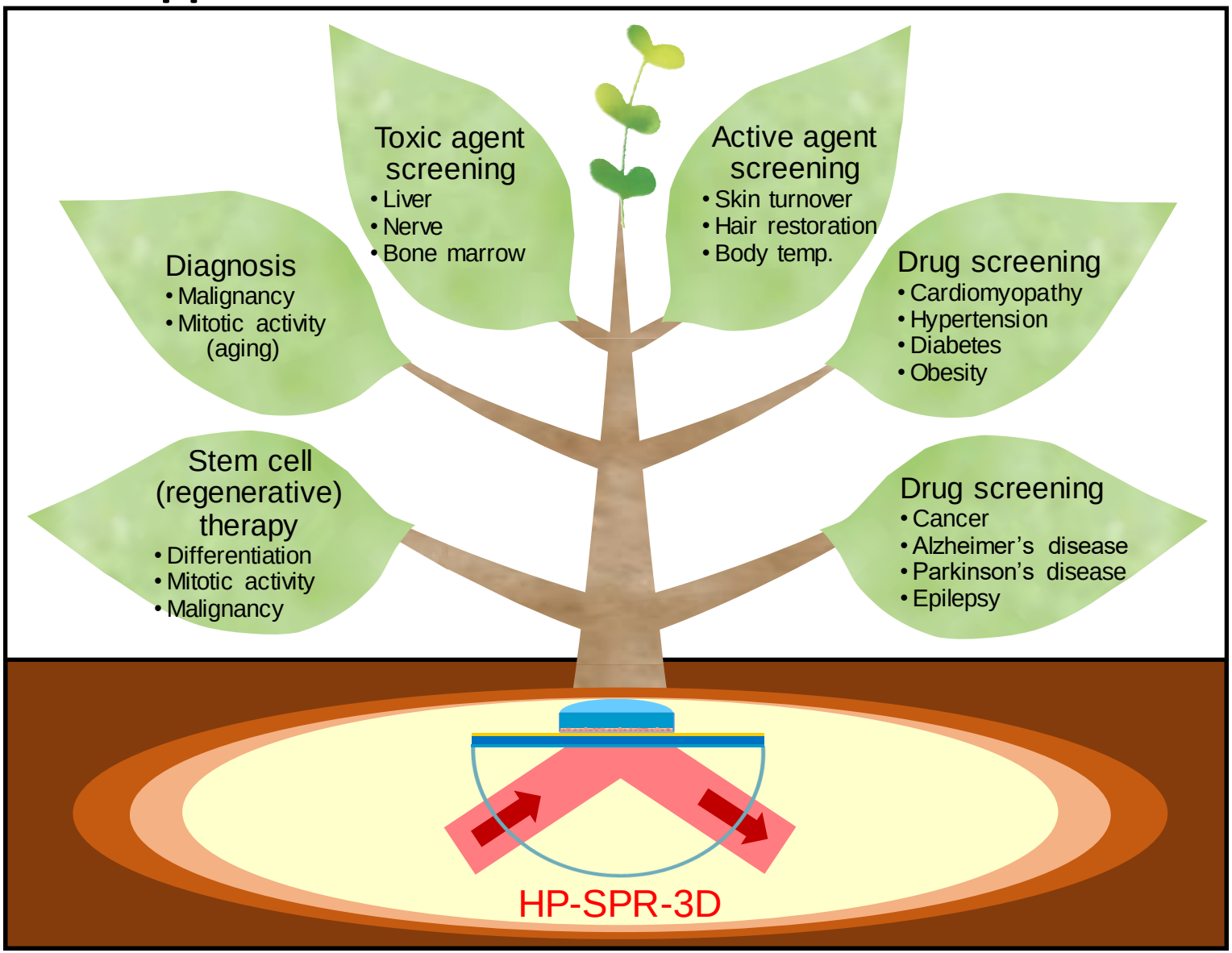




**Breakthrough**
**3D cell activity is obtained by conditioning of 2D attached cells covered by extra cellular matrix for short time with no cell division !**

**Breakthrough**
**Early mitochondrial polarization change rate after compound addition is a key to quantitatively predict the final efficacy and toxicity properties!**

**HP-SPR-3D method**

# Potential application for HP-SPR-3D



# References

Kosaihira, A. and Ona, T. Rapid and quantitative method for evaluating the personal therapeutic potential of cancer drugs. Analytical and Bioanalytical Chemistry, Vol. 391 (5): 1889-1897 (2008).

Ona, T. and Shibata, J. Advanced dynamic monitoring of cellular status using label-free and non-invasive cell-based sensing technology for the prediction of anti-cancer drug efficacy. Analytical and Bioanalytical Chemistry, Vol. 398 (6): 2505-2533 (2010).

Johzuka, J., Ona, T. and Nomura, M. One Hour in vivo-like phenotypic screening system for anti-cancer drugs using a high precision surface plasmon resonance device. Analytical Sciences, Vol. 34 (10): 1189-1194 (2018).

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