We demonstrate multiplexed single-cell phospho-protein abundance detection in adherent cancer model cells using a fitting combination of Fluorescent Cell Barcoding (FCB) and intracellular staining. Using phosphorylated ERK (pERK) levels in response to EGF as an example, we prove that this novel approach is well suited for short time scale activated protein estimation in clinical diagnostics.

**Introduction**

- Most cancer tissues are adherent in nature
- Short time scale activated protein levels can offer useful insights
- MAPKs strongly implicated in many cancers
- Signal amplifier in tumor microenvironment
- Activated ERK (pERK), a terminal MAPK, abundance at single-cell level a useful biomarker

High-throughput, intracellular, single-cell phospho-protein detection in adherent cells

- Flow cytometry
- rapid, multiparameter detection
- statistically sufficient number of cells
- multiplexing possible – minimizes uncertainty [1]

- Signal preserving cell detachment is key
- Three model cell lines considered (Table 1)
- EGF-stimulated pERK levels as an example

FCB preserves information (signal) flow:

Reliable pERK detection

**Table 1:**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell lineage</th>
<th>Elasticity at 37°C (at RT – 25°C) captured by Young’s modulus (kPa)</th>
<th>Cell-cell detachment force at 37°C (nN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>Cervical adenocarcinoma</td>
<td>4.18 (2.49) [2]</td>
<td>0.04 - 0.06 [3]</td>
</tr>
<tr>
<td>A549</td>
<td>Lung epithelial carcinoma</td>
<td>2.03 (2.12) [2]</td>
<td>0.8 - 1.7 [4]</td>
</tr>
</tbody>
</table>

Table 1: Cell lineage, elasticity at 37°C and RT (25°C – numbers within brackets) and cell-cell detachment force at 37°C for the three different cell lines.

**Detachment methods preserve signaling to ERK**

**References**