

**Cytological Profiling of Drug Action****Feng, Yan<sup>1</sup>, Slack, Mike<sup>2</sup>, Perlman, Zach<sup>1</sup>, Wu, Lani<sup>2</sup>, Mitchison, Tim<sup>1</sup>, Altschuler, Steve<sup>2</sup>****<sup>1</sup>Harvard Institute of Chemistry and Cell Biology, Harvard Medical School, Boston, MA, USA;****<sup>2</sup>Bauer Center for Genomics Research, Cambridge, MA, USA**

Drug treatment can induce changes in cellular organelles and protein localization that are readily detected by microscopy. Whether these changes simply reflect cytotoxicity or true mechanism of action, or both, has not been well studied. Here we conducted a systematic study in this area using known drugs. We selected 25 groups of drugs, such as actin and microtubule cytoskeleton modifiers, kinase inhibitors, and DNA or protein synthesis inhibitors. Each group of drugs has multiple compounds with similar activity. A serial dilution of each compound was applied onto HeLa cells for a single 20hr time point. Morphological changes of cellular organelles, including nuclei, nuclear speckles, Golgi apparatus, actin and microtubule cytoskeletons, and protein translocation of several phosphorylated signaling proteins, were recorded using a high throughput automated fluorescence microscope as “cytological markers”. Quantitative image analysis algorithms were developed to score the changes in size, shape, intensity and textures of each marker in every single cell under each drug treatment condition, which generated about >100 “cytological drug descriptors”. Each descriptor under drug treatment conditions was compared with control using a non-parametric Kolmogorov-Smirnov statistics and the difference was scored as a P-value. “Heat plot” of each drug was generated using the P-values and served as a drug activity fingerprint. Drugs were further clustered using a dilution-insensitive method that ranks order the inflection points of each descriptor. Several groups of compound with similar action generated similar “heat plot” and clustered together. For example, tubulin depolymerizers, tubulin stabilizers, actin depolymerizers, protein synthesis inhibitors and CDK inhibitors all showed up in tight clusters. Interestingly, several groups of drugs, such as COX inhibitors, were separated into two distinct clusters, suggesting that they might have different action mechanisms on cells. There are groups of drugs that are not clustered well by the descriptors we used so far. We are now expanding the list of markers and descriptors to see if we can effectively score these drugs, too. In summary, we developed high throughput image acquisition, image analysis, databasing and statistical tools to show that cytology changes could be useful in identifying drug action on cells. It would be interesting to see how this information intensive “high content” method can be used on more broadly drug discovery process.