

1536-well FLIPR: A Possibility or Probability? Or, "How I Spent Last Week!!!"

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"High throughput screening (HTS) does not have a pot of gold at the end of the Leads rainbow". The continued growth of compound collections at Pharmaceuticals has brought the costs of HTS under serious scrutiny, particularly in the chemokine screening arena in which FLIPR assays are extremely important but becoming increasingly expensive. In screening in excess of 1 million wells, the quantities of synthetic chemokines required can cost upwards of \$50,000. This represents the most expensive reagent in a FLIPR screen. Reducing these costs while maintaining throughput for increasing compound collections and/or improving data quality through replicate data generation is extremely important. It is against this backdrop that we have generated very preliminary data in low volume 384 and 1536 well plates and contrasted it against 'regular' 384 well screening. This data has been generated using a chemokine receptor stably transfected in CHO cells and current in automated HTS; (see the presentation of David Harding, RTS Thurnall, for more details of our approach to FLIPR automation).

CHO cells were dispensed using either a 1536 well Aquamax (MDC) or a Flexispense (Asys) at 3×10^5 cells/ml, 5ul per well. After 24 or 48 hours of incubation, media was removed using a 1536 well washer (Skatron) and 5ul of Fluo-4 containing brilliant black was added to each well again using each of the dispensers. Plates were incubated for at least 60 minutes at 37°C then 1ul of pre-diluted DMSO (final concentration 0.5%) was added to each well using either a Platemateplus (Agilent Technologies) or a Cybiwell (Cybio), to model the compound addition step. This was followed by a 15 minutes incubation at 37°C. Within FLIPR, a 384 well agonist plate containing the chemokine at either the EC80 concentration or in a concentration-response range was used to stimulate chemokine-induced calcium responses by dispensing varying volumes from 1 to 5ul within FLIPR. Across plate and within column controls for Z factors were calculated and compared between 'regular' 384, low volume 384 and 1536 well plates. Comparable data was obtained between these three formats and suggests that both low volume 384 and 1536 well FLIPR assays are possible. Further enhancements in equipment are required to make this format viable for 'real' HTS, but these will be discussed during the presentation for feedback.