Some challenges in high-throughput high-content (HTHC) phenotypic screening

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« From phenotypes to pathways: inferring genetic architecture from perturbation maps » ESF exploratory workshop, Cambridge, UK

The project

- **Goal**: detect kinases and miRNA whose inhibition affect proliferation and differentiation in prostate cancer
- **Approach**: HTHC phenotypic screening at the single cell level. Monitor differentiation and proliferation on living cells after systematic inhibition of kinases and miRNA
- **Applications**: drug and drug target discovery, patient profiling, pathway elucidation, general HTHC screening pipeline

HTHC Screening pipeline overview

HTHC screening

Pathfinder™ imaging workstation

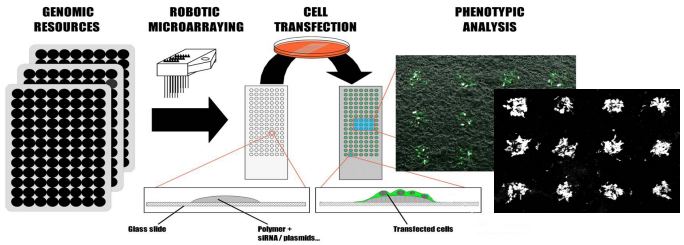
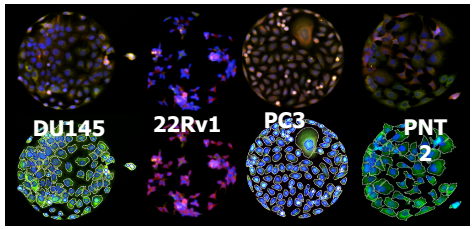
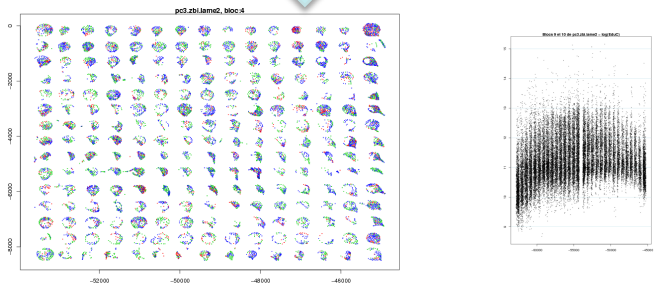
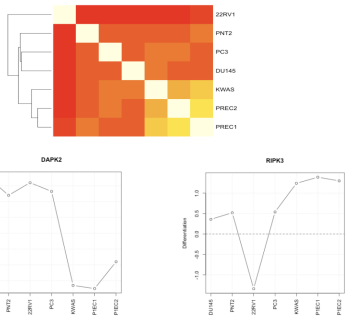


Image processing





Data processing

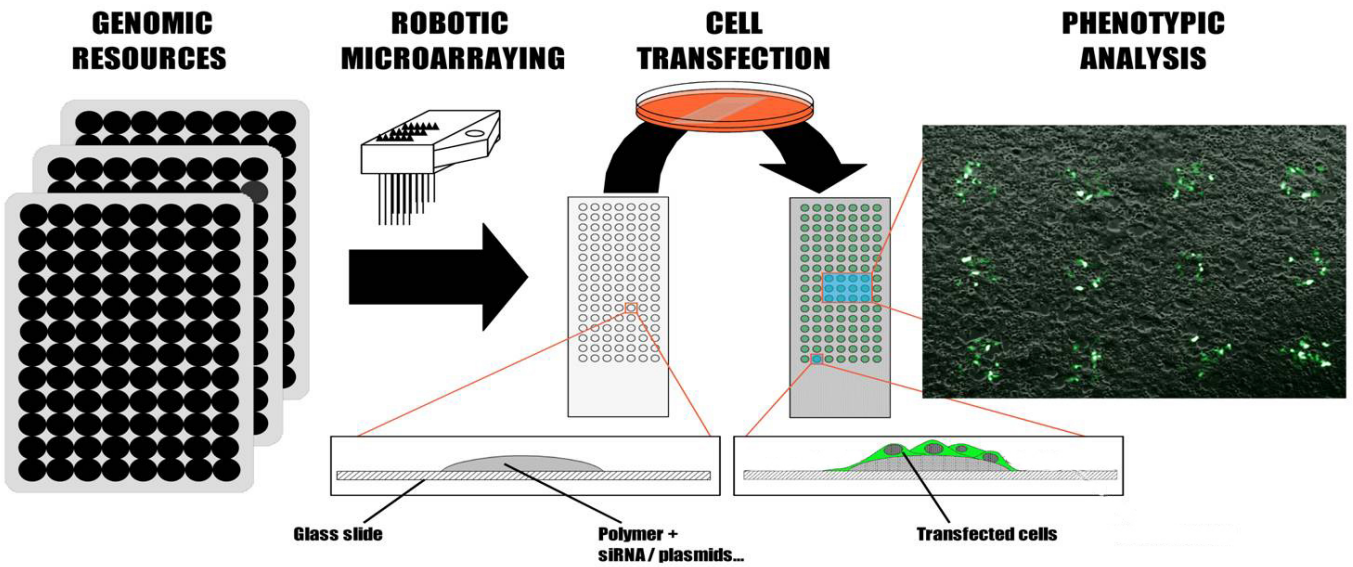


Results analysis

Sample preparation

- Focus on prostate cancer
  - 4 cell lines (PNT2, 22Rv1, DU145, PC3)
  - 3 primary cultures (2 normal prostate, 1 tumour)
- 1284 siRNA targetting 648 kinases
- 902 LNA miRNA inhibitors (Exiqon)
- 15 replicates for each
- Fluorescent markers for:
  - Differentiation: K18/K19
  - Proliferation: EdU
  - DNA: DAPI

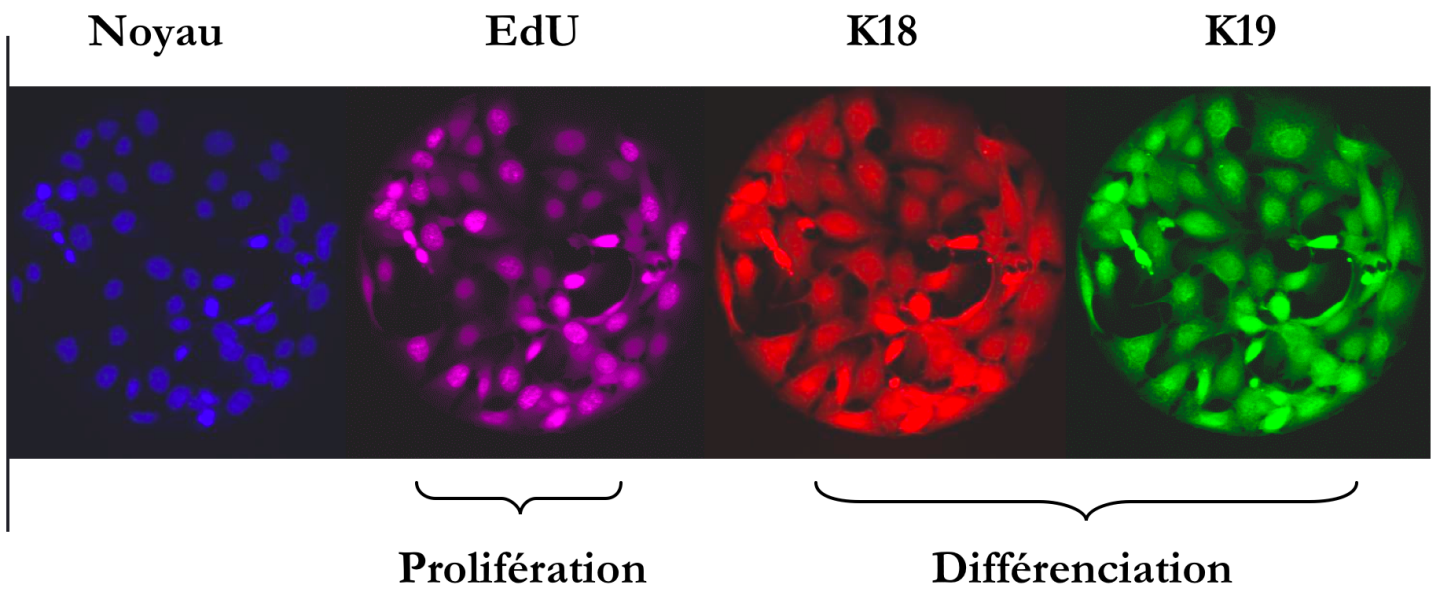
Cell microarrays

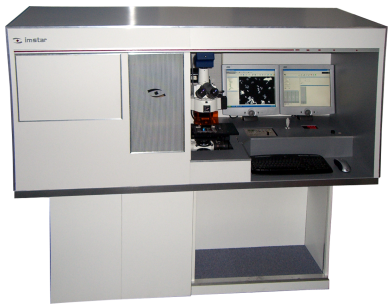


(Gidrol lab)

- **2700 micropatterns/array on cell repulsive glass slides**
- **Extra cellular matrix proteins and transfection agents**
- **100~400 cells per micropattern**

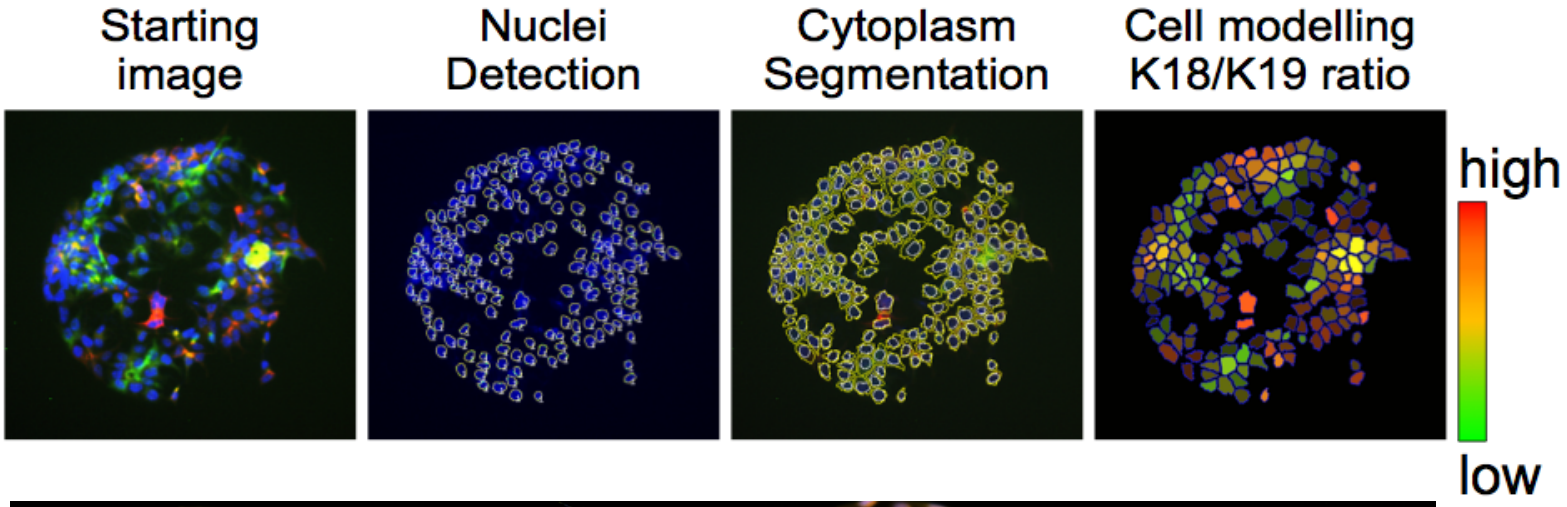
Image aquisition

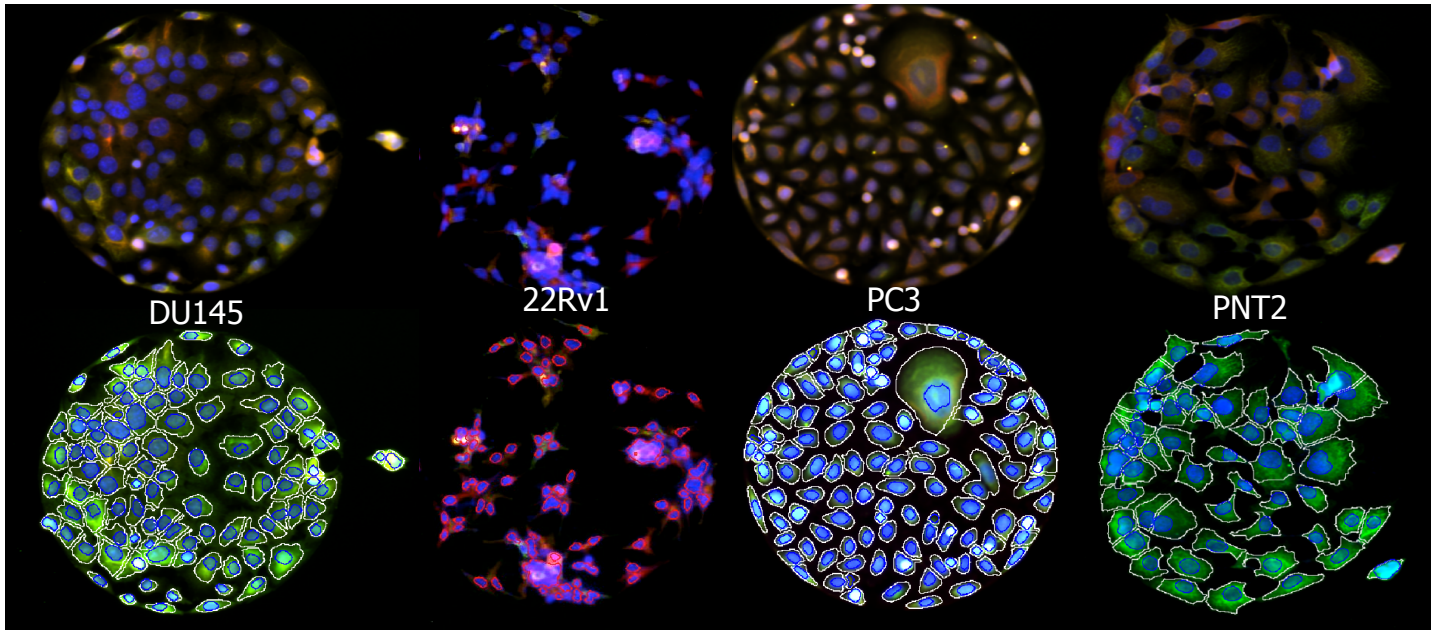




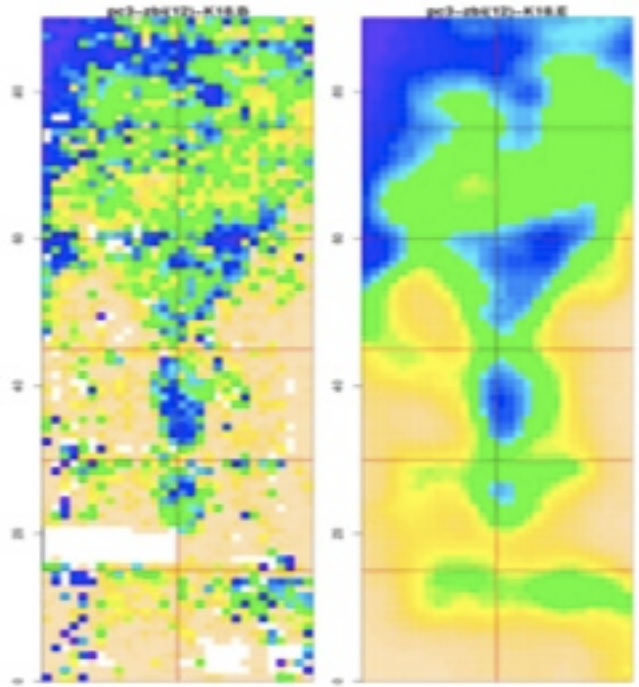
IMSTAR - PathFinder

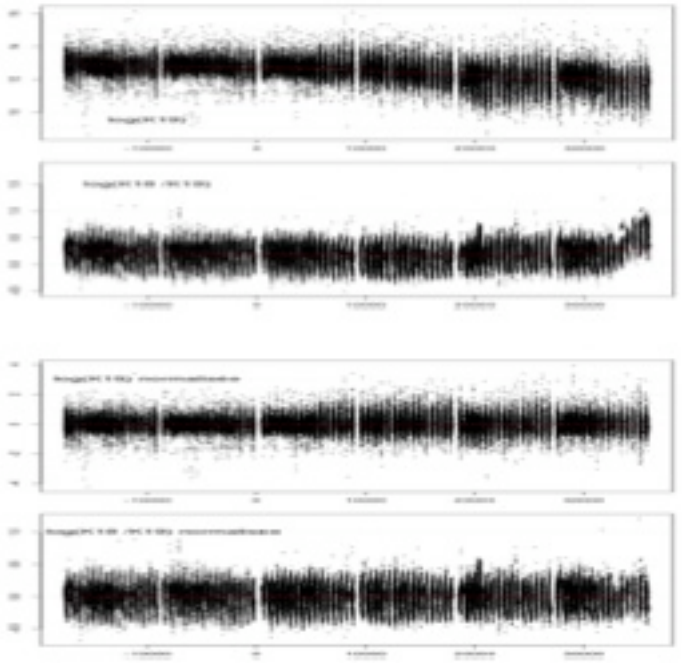
Cell detection and quantification



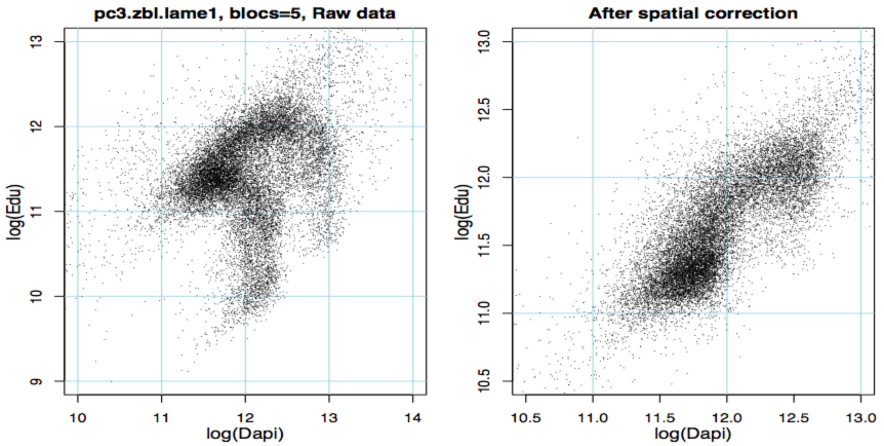


Fighting spatial artefacts…

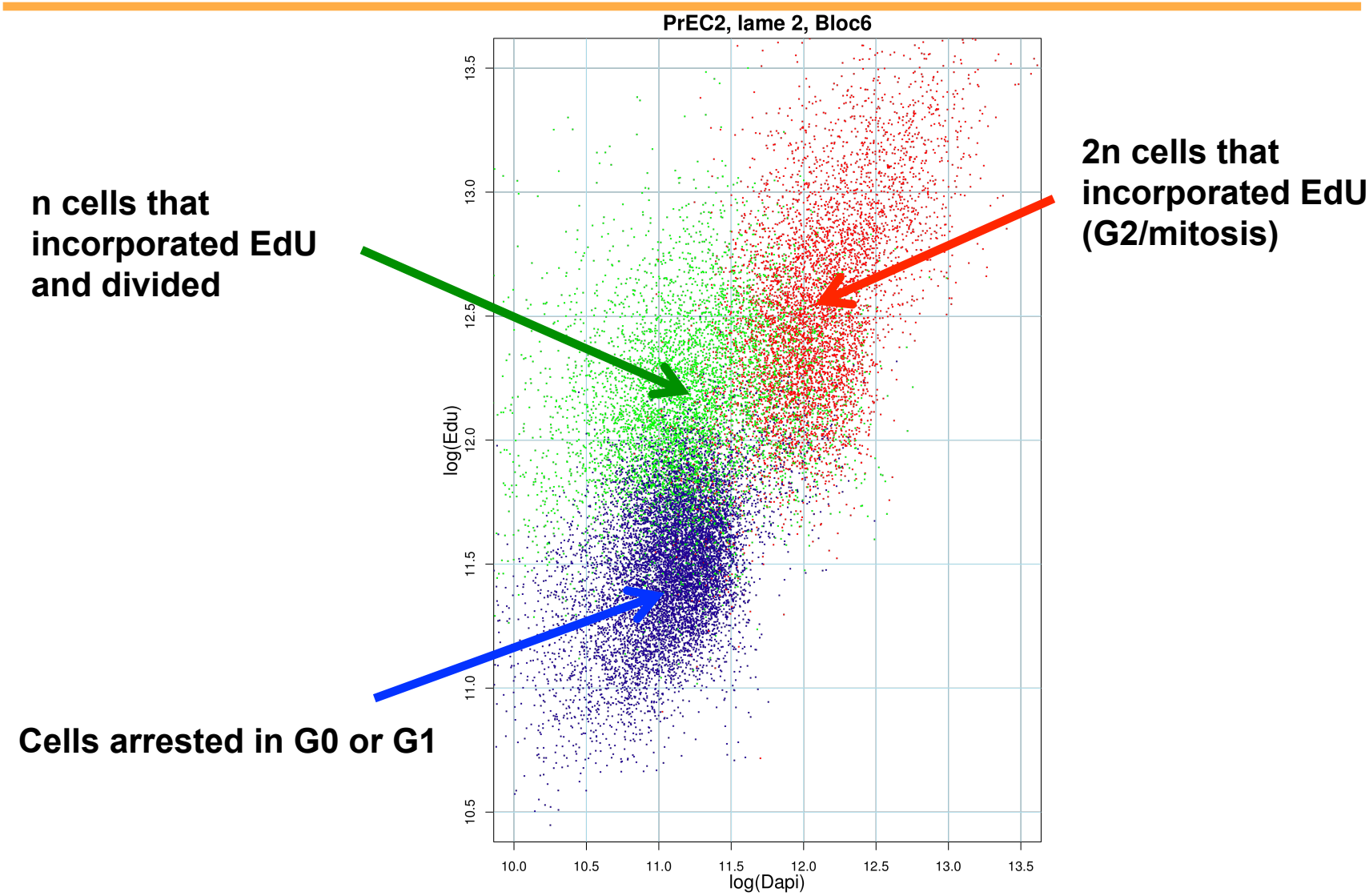




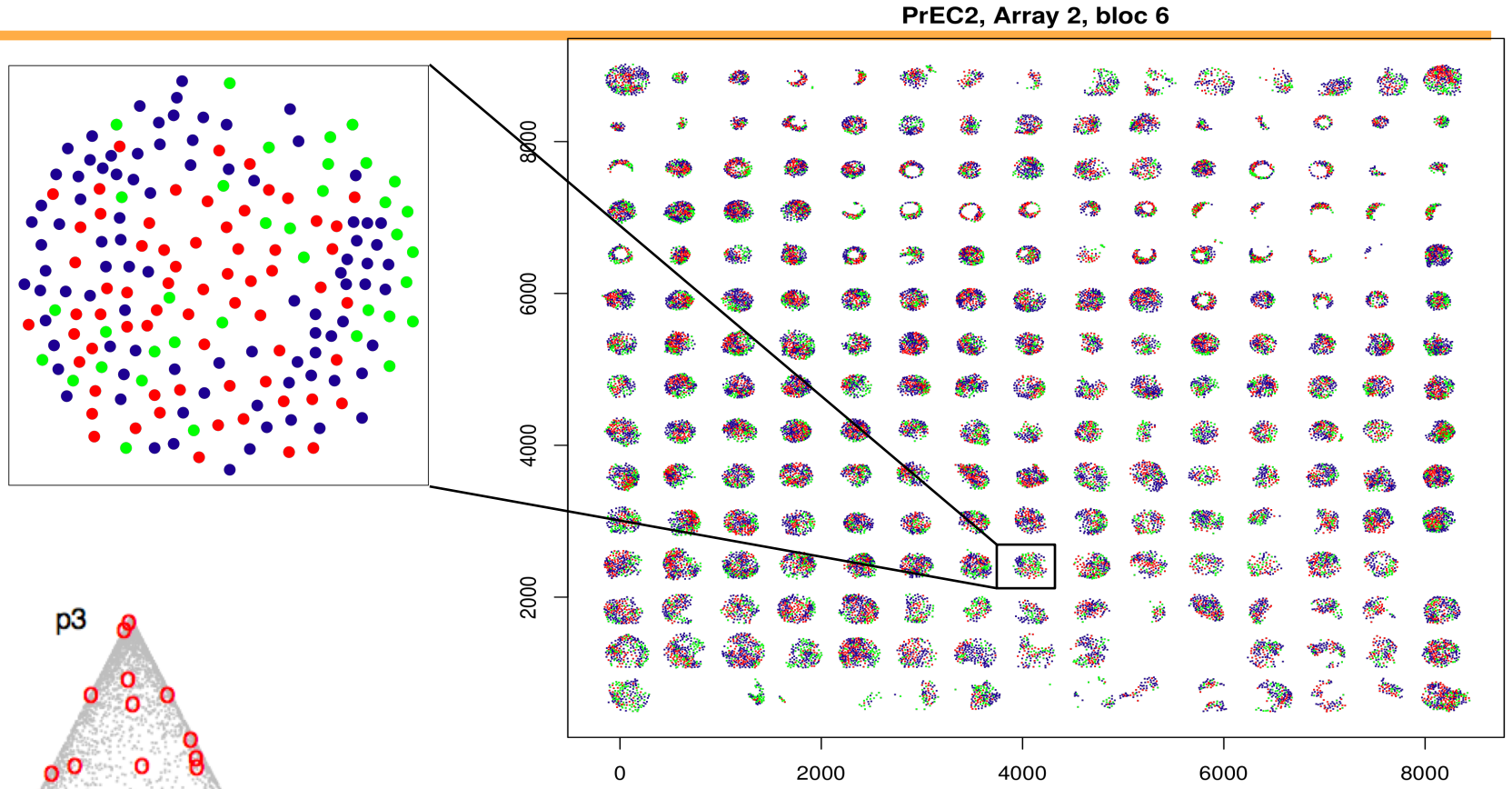
Kriging, quantile SVM…

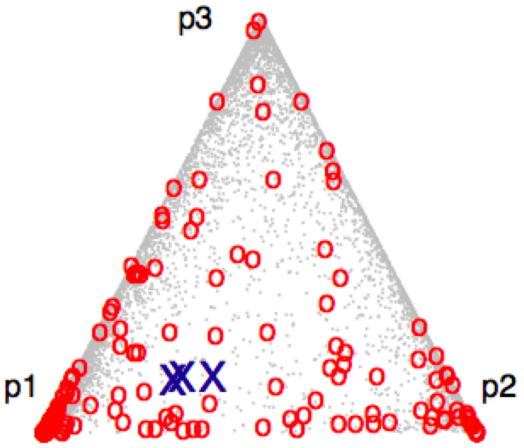


Detecting proliferation



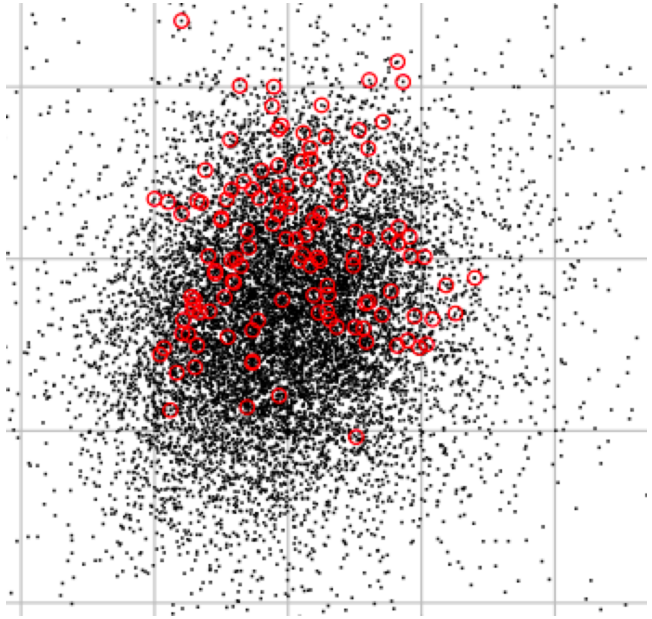
Scoring proliferation of a spot



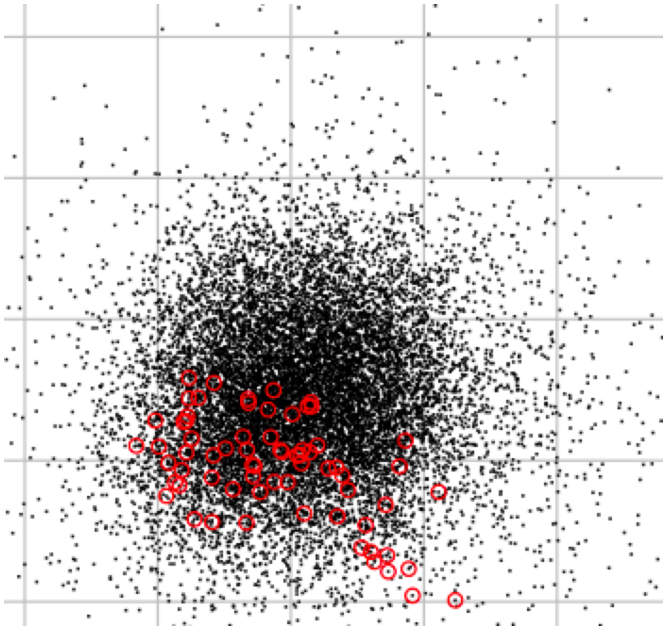


$$Z_s = \frac{1}{n_S} \sum_{i \in S} \frac{P[i \in \text{ population } 3 \cup \text{population } 2]}{P[i \in \text{population } 1]}$$

Scoring differentiation of a spot



Highly differentiated



Poorly differentiated

-MA plots log K19 +/- log K18
-Differentiation score of a spot is the average M of the cells in the spot

Scoring a treatment

- 3 spots x 5 arrays = 15 replicates
- Statistical tests to combine each cell's score and assign proliferation and differentiation score to each treatment

$$Y_i = V_{\tau(i)} + \epsilon_i$$

$$Y_T = \frac{1}{N_T} \sum_{\tau(i)=T} Y_i$$

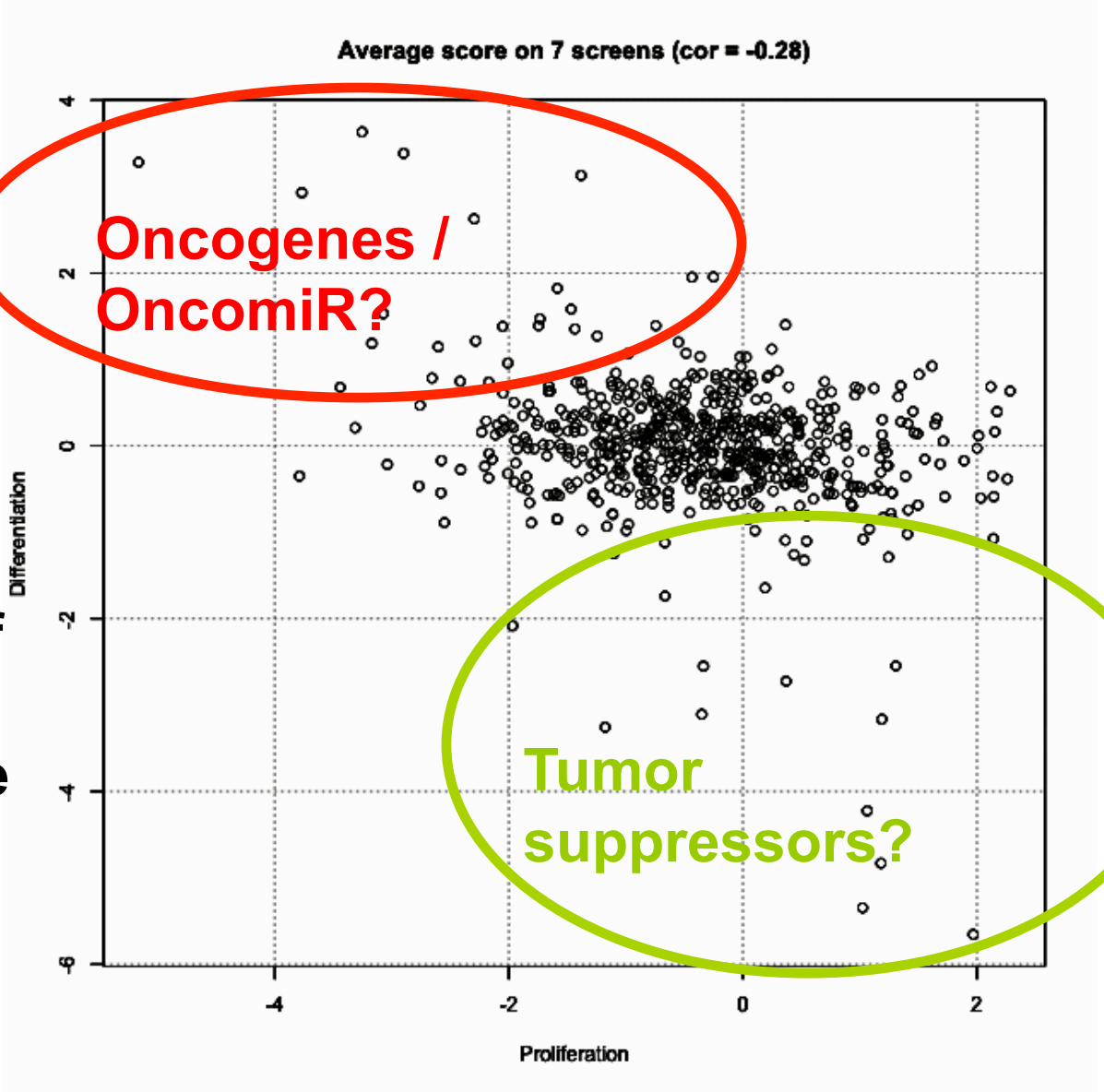
$$\mathrm{var}(Y_T) = \left(\frac{1}{n_T}\right)^2 \sum_S n_S^2 \sigma_S^2 + \frac{1}{n_T} \sigma_\epsilon^2$$

$$U_T = G\left(\frac{Y_T}{\sigma_T}\right)$$

Global phenotypic response

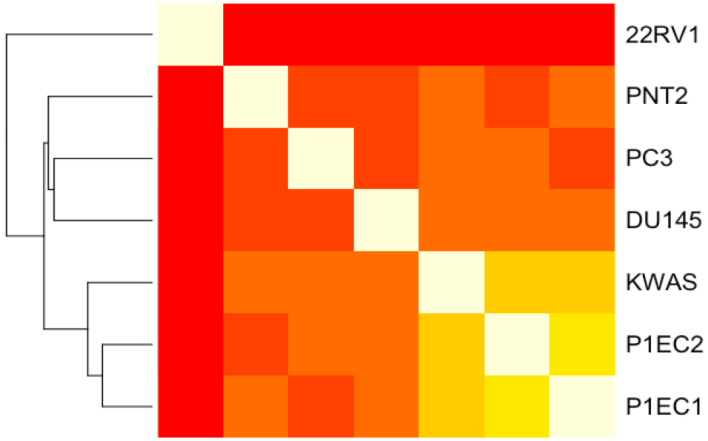
- **Negative correlation between differentation and proliferation**

- **Bi-modal / long tail distribution of the scores -> modular structure of the regulatory pathways?**

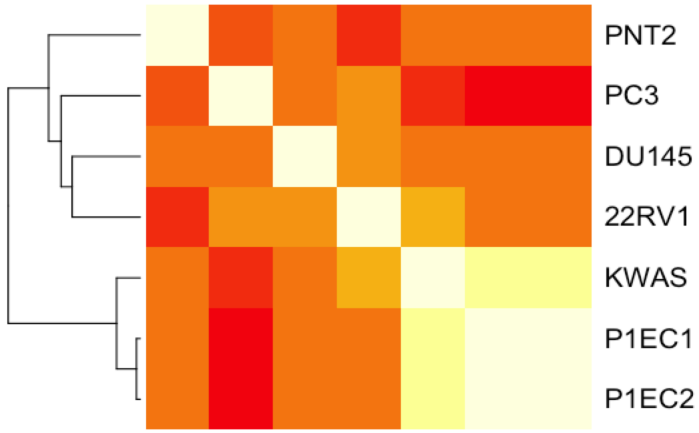


The limits of cell lines

1. Tumour samples are more similar to normal prostate than to cell lines



Differentiation profile



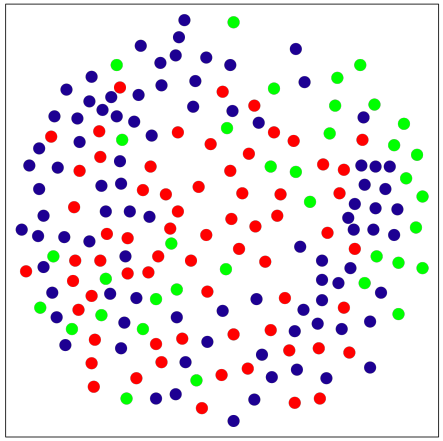
CGH

2. Cell lines are more robust to perturbations than primary cultures, tumour more robust than normal prostate tissue

Sample	DU145	PC3	PNT2	PrEC1	PrEC2	KWAS
siRNA inducing differentiation	0	0	0	6	16	5
siRNA decreasing differentiation	0	0	0	11	17	3

Ongoing/future work…

- Validation of interesting kinases/miRNA inhibitors
- Crossing kinase/miRNA results, modeling pathways…
- Looking at more general « multidimensional » phenotypes
- Investigating spatial structures



What is the next big thing?

- **DATA**: Producing and sharing more HC phenotypic screen results at the single cell level (standardization,repositories…)
- **METHODS**: Modelling / predicting complex phenotypes (multidimensional, spatial dependencie, QSAR…)
- **TECHNOLOGY**: cheap high-quality screening of primary culture from patient's tumours for personalized medicine

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