436b Analysis of Cell Population Distributions Using Fluorescence Microscopy

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Cell populations are heterogeneous systems in the sense that intracellular content is distributed amongst the cells of an isogenic cell population. Moreover, the phenotype of each individual cell is tightly controlled by the function of gene regulatory networks. Therefore, in order to rigorously design biological systems for biotechnological applications it is important to first elucidate the relationship between the architecture of gene regulatory networks and cell population heterogeneity.

As shown by Collins and Richmond [1], the reaction and division rate distributions of a cell population can be obtained through inverse population balance modeling if the average specific growth rate and the number density functions of the entire population, the dividing cell and newborn cell subpopulations are known. Therefore, the decomposition of the cell population distribution into growing, newborn and dividing subpopulation distributions in conjunction with the ability to numerically simulate cell population balance models [2] can offer valuable quantitative insight into the dynamics of cell populations.

A new assay was developed to quantify these subpopulations using E.coli cells carrying an artificial gene-switching network, known as the genetic toggle. This network is composed of two coupled inducible promoter-repressor pairs and uses a green fluorescent protein (GFP) gene to report the expression levels of one of the two repressor proteins [3]. Based on fluorescence microscopy and digital image processing, this assay is easier to implement than standard flow cytometry techniques and can provide information about spatial variations of GFP intensity. E. coli cells were immobilized and visualized with a 100x oil-immersion objective and a green bandpass filter set (Omega XF100-2, 455-490 nm excitation range, 515-555 nm emission range). Digital images were acquired using a cooled CCD camera (Photometrics Coolsnap) and a frame grabber controlled by an image processing software package (Metamorph, Universal Imaging Inc.).

Automated algorithms were developed for identifying the dividing cell subpopulation using two cell properties. First, dividing cells are longer than newborn or growing cell. Also, dividing cells develop a characteristic constriction that eventually leads to division or septation. Fluorescence microscopy images revealed that all cells satisfying these two criteria exhibited a distinct minimum of GFP intensity in the constriction area. The GFP intensity in the constriction zone dropped by almost 1/3 below the maximum, allowing for easy identification and separation of the two cell halves. In general, however, the fluorescence peaks computed for the two halves were unequal, indicating that the two daughter cells had unequal partitioning of the GFP.

Using this assay, the distributions of GFP in the growing, dividing and newborn populations were obtained. These results indicate that application of fluorescence microscopy techniques can significantly contribute towards obtaining an in depth understanding of both the sources and implications of cell population heterogeneity.

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[3] Gardner, T.S., C.R. Cantor, and J.J. Collins, Construction of a genetic toggle switch in Escherichia coli. Nature, 2000. 403: p. 339-342.