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Application Note

Cell Cycle Analysis Using Cell Cycle Indicator: Fucci

Introduction

Fluorescent ubiquitination-based cell cycle indicator (Fucci) is a set of fluorescent probes which enables the visualization of cell cycle progression in living cells. Cdt1, which accumulates only in the G1 phase of the cell cycle, is fused with an orange fluorescent protein (monomeric Kusabira-Orange2:mKO2), and Geminin, which accumulates in the S/G2/M phases, is fused with a green fluorescent protein(monomeric Azami-Green1 :mAG1). The fluorescent intensity of each color is dependent on the level of expression of the Cdt1 and Geminin proteins. This application note introduces experimental examples of long-term time lapse analysis of Fucci-expressed HeLa cells observed by CellVoyager CV8000 and analyzed by CellPathfinder, clearly showing dose-dependent effect of Etoposide, an G2 arresting anti-cancer drug, on cell cycle population over time.

Experimental procedure

- HeLa cells (that stably express Fucci) were seeded on a 96-well plate (Greiner #655896) at 2500 cells/well and incubated for 24 hours.
- Replace the medium with fresh medium containing Etoposide. (Figure 1B, Etoposide concentration)
- Images were acquired using CellVoyager CV8000.
  (Excitation wavelength: 488 nm, 561 nm, bright field, objective lens: 10x, 48-hour time lapse observation over 1 hour intervals)
- Nuclear regions were recognized (Figure 1C) and average intensity within the orange and green fluorescent nuclear regions calculated with analysis software CellPathfinder. Each value was plotted and the gate is set to classify cells into four phases : early G1 phase, G1 phase, early S phase, and S/G2/M phase (Figure 1D). The number of cells and percentage of each were calculated.

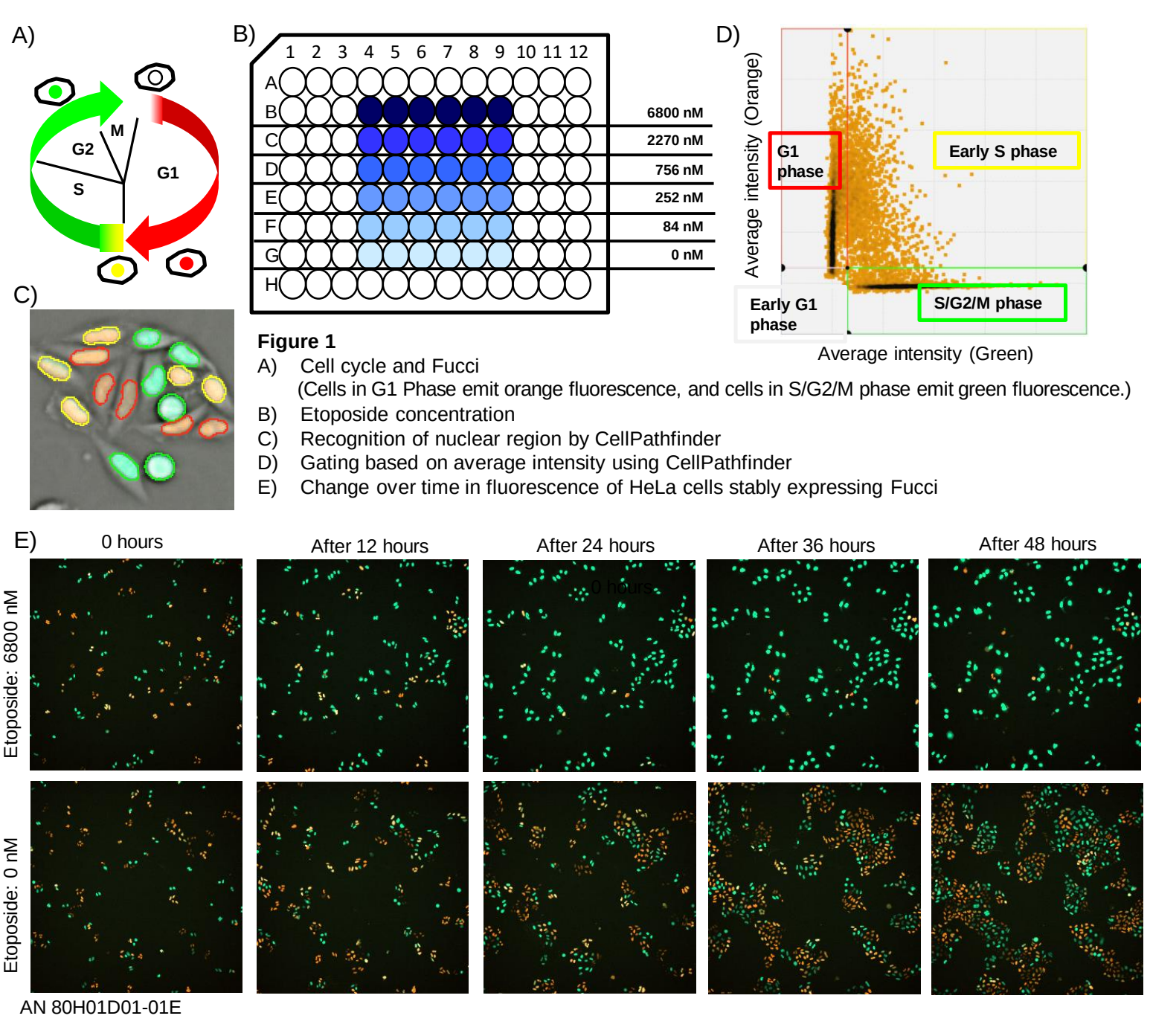
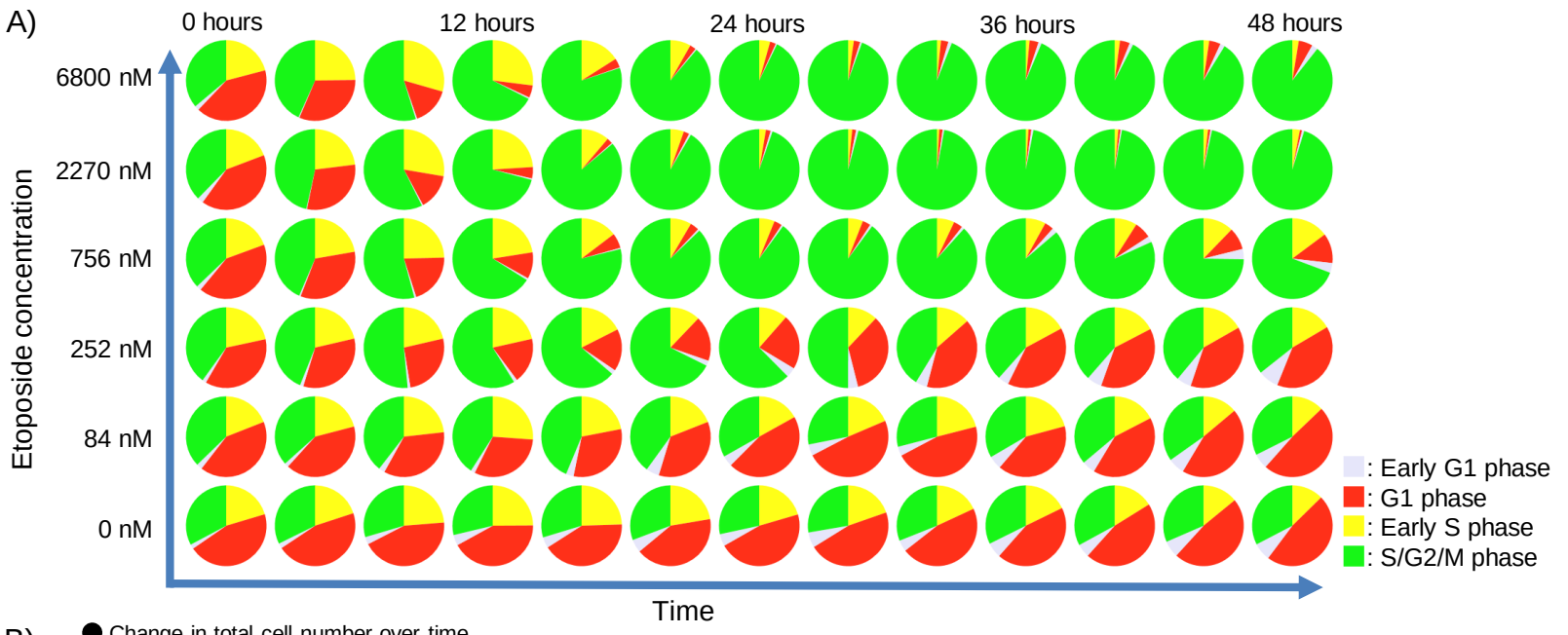


Figure 1

A) Cell cycle and Fucci
(Cells in G1 Phase emit orange fluorescence, and cells in S/G2/M phase emit green fluorescence.)
B) Etoposide concentration
C) Recognition of nuclear region by CellPathfinder
D) Gating based on average intensity using CellPathfinder
E) Change over time in fluorescence of HeLa cells stably expressing Fucci



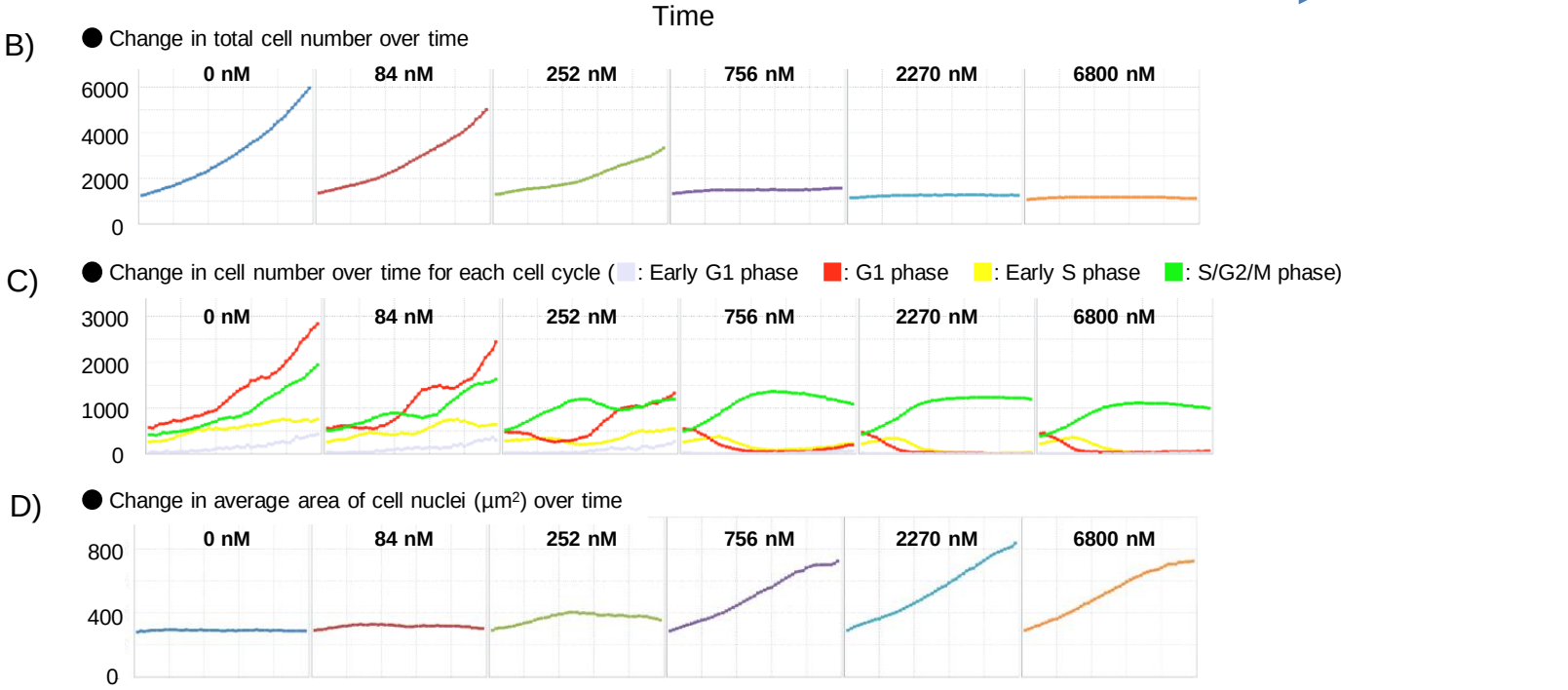


Figure 2
Etoposode dose-dependent effect on cell cycle population over time
A) Change over time in the percentage of cells classified into each cell cycle by gating
B) Change over time in total number of cells at each concentration
C) Change over time in total number of cells at each concentration and cell cycle
D) Change over time in area of cell nuclei at each concentration

Results and Discussion

The progression of cell cycles were observed by time lapse imaging over 48 hours using cells that emit fluorescence of the cell cycle indicator Fucci and CV8000 equipped with a high-performance incubator.
Using the analysis software CellPathfinder, Etoposide was shown to inhibit cell growth during the S/G2/M phase, and the increase in average nuclear area indicated that cells in the G2 phase had accumulated (the nuclear area is larger because there is double the amount of DNA per nucleus). These results show the efficacy of Etoposide (arrests the cell cycle at G2) in multiple ways. (Figure 2)
Thus, CV8000 and CellPathfinder enable the efficient analysis of drug effects.

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