Click Chemistry-Based Detection of S-Phase Adherent Cells Using Automated Microscopy and Image Analysis

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Abstract

Labeling, detection, and quantification of cells in the S-phase of cell cycle progression are not only important in characterizing the basic biology but also in defining the cellular responses to drug treatments. Bromodeoxyuridine (BrdU) labeling of cells followed by antibody staining is the standard method for detecting cells in the S-phase that is amendable to high throughput screening. Antibody detection of BrdU involves harsh treatments or nuclease digestion to facilitate epitope access, which could interfere with multiplexing with other probes. We show a new method of labeling cells in the S-phase using ethynyl-deoxyuridine (EdU) and its subsequent fluorescent detection using a Cu(I) catalyzed [3+2] azide-alkyne cycloaddition reaction performed in an aqueous buffer under mild conditions. We demonstrate multiplexing of EdU fluorescence detection with other chemical- and antibody-based fluorescent probes, and image acquisition/analysis using automated fluorescence microscopy.

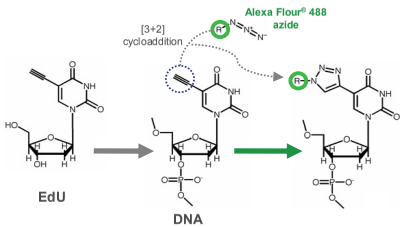
Introduction

Click Chemistry [1] –

a set of chemical building blocks/reactions that works reliably in both small- and large-scale applications:

- modular and wide in scope in applications; high chemical yield; generates inoffensive byproducts; stereospecific; high atom economy;
- has readily available starting materials and reagents; easy product isolation; simple reaction conditions
- no solvent involved or a benign solvent (preferably water)
- physiologically stable
- large thermodynamic driving force
- a fast reaction with a highly selective single reaction product.

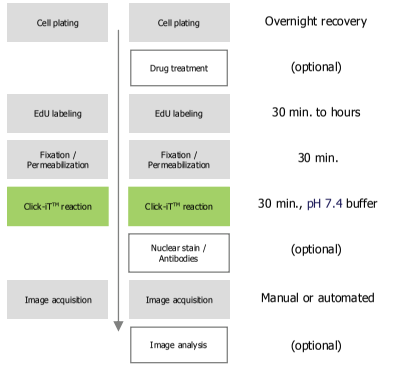
Azide-alkyne cycloaddition reaction is one example of click chemistry[2, 3].



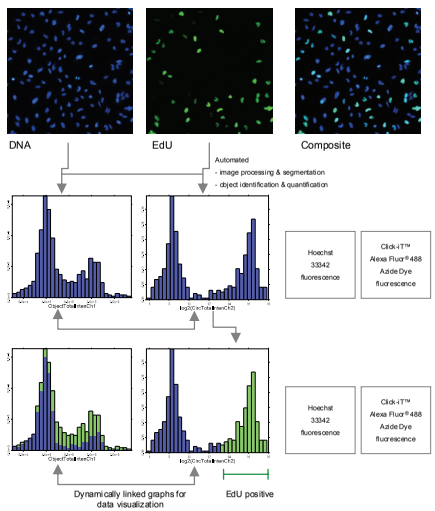
Azide-alkyne cycloaddition-based detection of DNA synthesis -

- **Ethynyl-deoxyuridine (EdU)** as the thymidine analog for incorporation into newly synthesized DNA by cells
- Ligation of fluorescent probe for detection: **Click-iT™ Alexa Fluor® 488 azide dye**

Click-iT™ EdU S-phase detection by imaging - workflow

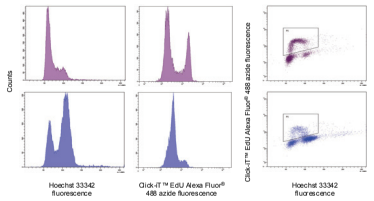


DNA profile and Click-iT™ S-phase detection by imaging

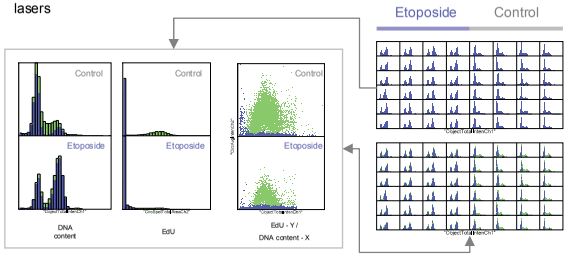


- Images: HeLa cells, EdU labeling for 120 min.; formaldehyde fixed and processed for Click-iT™ EdU reaction; DNA - Hoechst 33342; EdU – Click-iT™ Alexa Fluor® 488 Azide Dye.
- **Automated image acquisition & analysis** – Arrayscan VTI (Thermo Fisher Cellomics, Pittsburgh, PA)
- **Dynamic graphics-based data exploration & plotting** – Panmo (The Chi-Square Works, Inc., Seabeck, WA)
- Histograms show relative frequencies; "EdU positive" is painted green (bottom right); dynamically linked graph of DNA profile (bottom left) shows the distribution of "EdU positive" in DNA profile.

DNA profile and Click-iT™ S-phase detection – flow cytometry and imaging cytometry

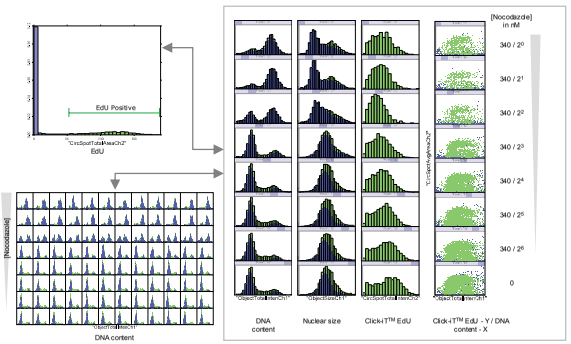


- A549 cells; etoposide 1 μM treatment for 20 hours; EdU labeling for 30 min; processed for Click-iT™ detection reaction
- LSRII flow cytometer (BD Biosciences, San Jose, CA) with 355 nm & 488 nm excitation lasers



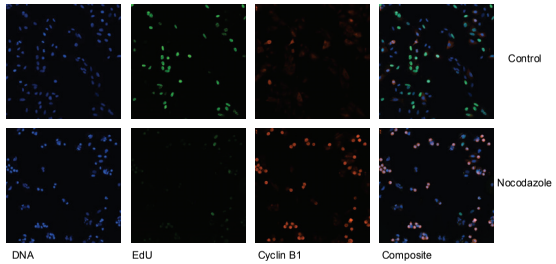
- A549 cells in a 96-well plate were treated as above (etoposide 1 μM - 20 hours, EdU labeling - 30 min.). EdU detection & automated image acquisition/analysis were performed as illustrated in the previous image analysis figure. Histograms show relative frequencies.
- Results comparable to flow cytometry data (above), with consistency among wells in a microplate.

DNA profile and Click-iT™ S-phase detection – Drug effects

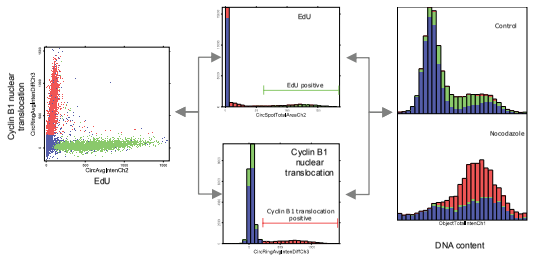


- A549 cells in a 96-well plate; nocodazole treatment (concentration step gradient as illustrated) for 20 hours; labeled with EdU for 30 min., processed with Click-iT™ detection reaction. Image acquisition & analysis were performed as illustrated in the previous panels.
- Drug effects: visualization of changes of DNA content, nuclear size, and EdU signal intensity. Histograms show relative frequencies.

Click-iT™ S-phase detection by imaging – multiplexing



- A549 cells, nocodazole 170 μM treatment for 20 hours, EdU labeling for 30 min.; formaldehyde fixed and processed for Click-iT™ EdU reaction; stained with anti-cyclin B1 (Invitrogen) and Hoechst 33342. DNA – Hoechst 33342; EdU – Click-iT™ Alexa Fluor® 488 Azide Dye; Cyclin B1 – Alexa Fluor® 555 conjugated secondary antibody.



- A sub-set of graphs from visual data exploration of the data set as illustrated by micrographs above. Histograms show relative frequencies. All plots are dynamically linked.
- Scatter plot (left): distinct distribution of EdU incorporation (green) and cyclin B1 nuclear translocation (red).
- Nocodazole effects (histograms, right): shift of DNA profile, increase in the number of cells with cyclin B1 nuclear translocation (marked red).

Summary

- Click-iT™ EdU reaction for S-phase detection:
  - performed in a mild buffer
  - simple, fast, and no harsh treatment
- Click-iT™ EdU reaction with automated imaging & image analysis:
  - comparable with flow cytometry data, readily applicable to adherent cells
  - multiplexing with other probes results in content-rich assays

References

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