Cell Counting, Death and Proliferation – Cellular Analysis

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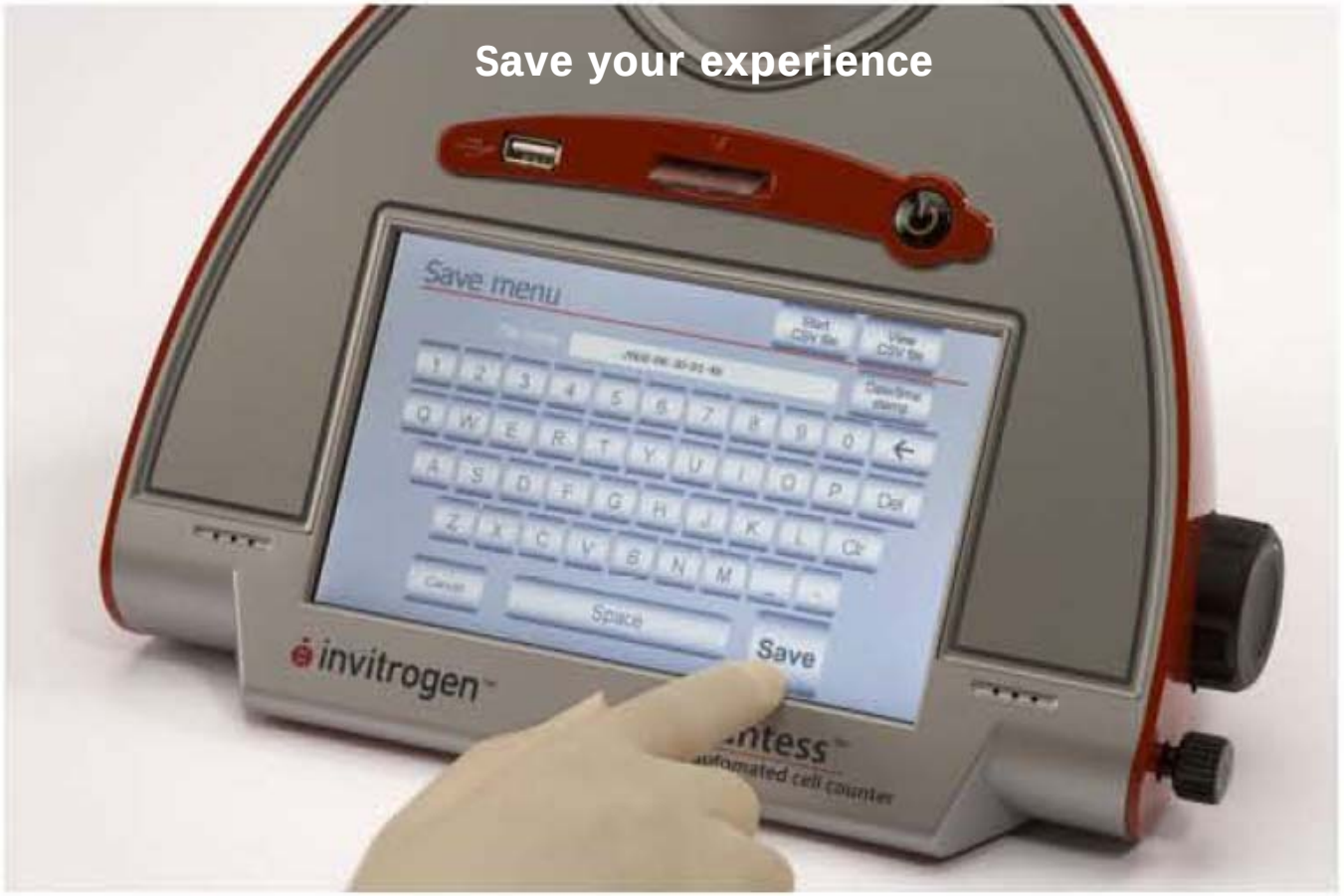
What is the Countess™?

- **Countess™ automated cell counter**
  - Accurately counts cells in 30 seconds
  - Calculates % viability
  - Measures average cell size
  - Includes dilution calculator

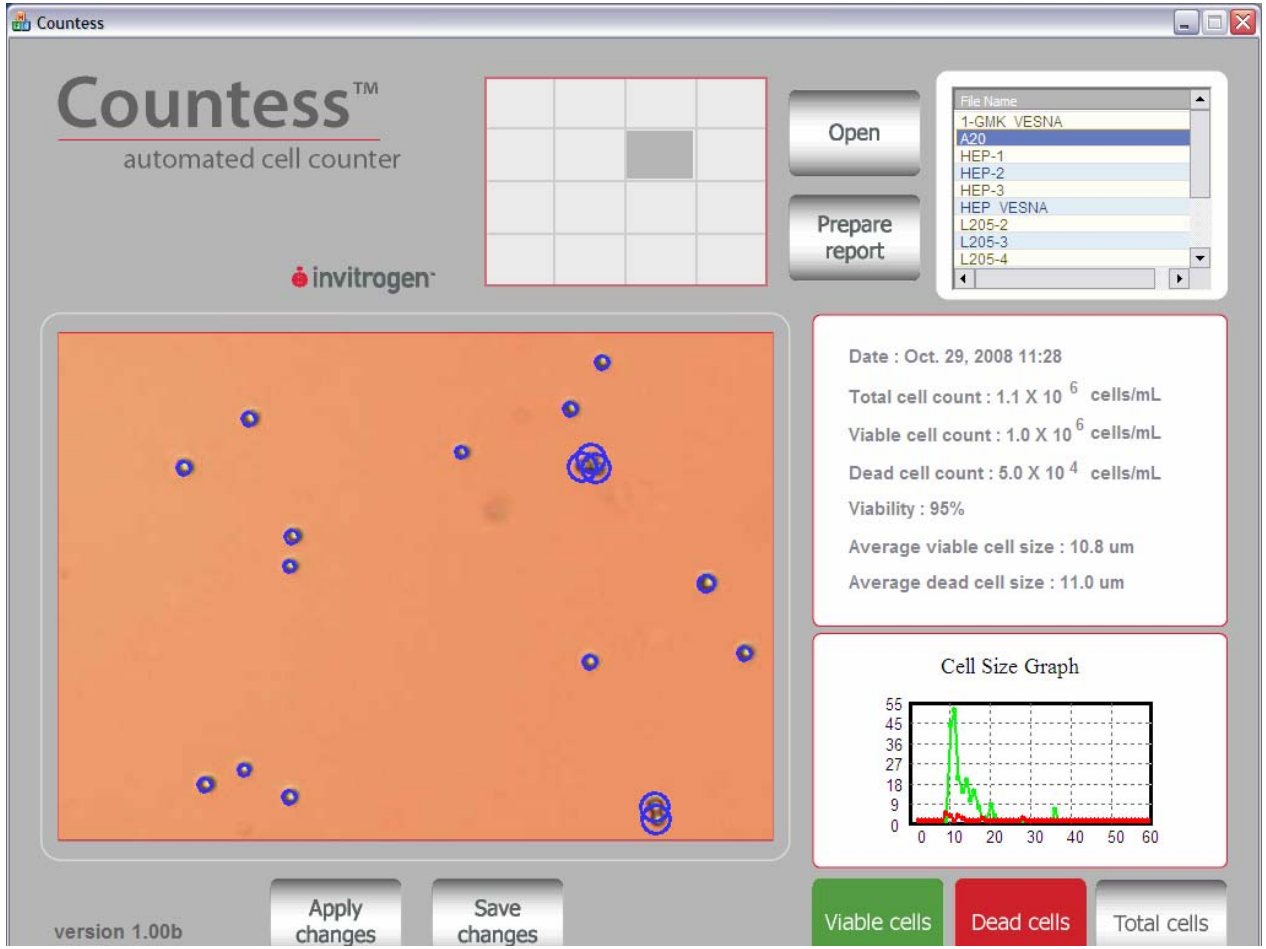
- NEVER USE A HEMOCYTOMETER AGAIN!

Countess™: Appreciate How Easy it can be



How often do you "recount" your cells?

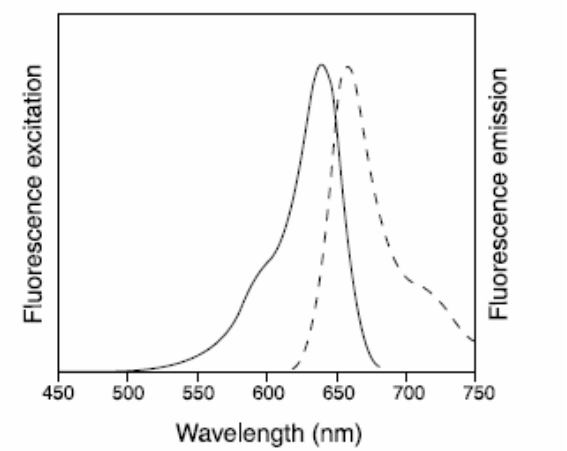
Countess Data

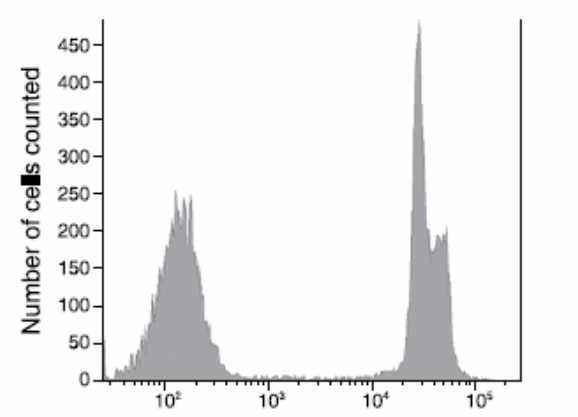


Alternative to Propidium Iodide – SYTOX Red

- High-affinity nucleic acid stain.
- Membrane Impermeant –
  - Can only pass through the membranes of dead cells.
- Allows discrimination between live and dead cells in flow cytometry experiments.
- Single-step procedure with quantitative results.
- Excite with the at 633 or 635nm laser light.
- Frees up the 488 laser for other analyses.
- Discrete emission in the APC channel – sharp, steep curve.
- Perfect for FL4 on the FACScalibur

SYTOX Red

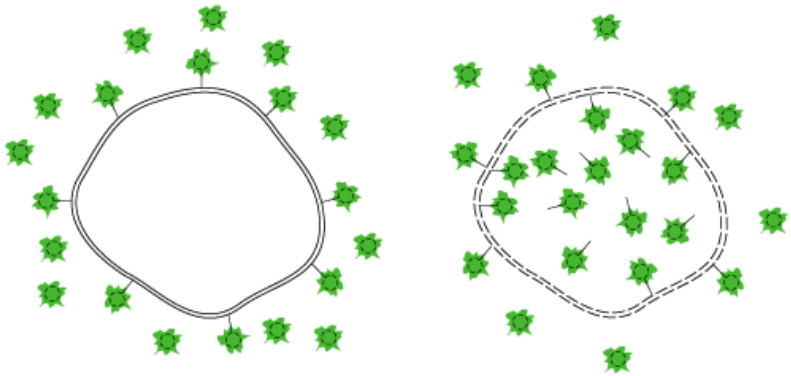




- Fluorescence excitation and emission spectra of the SYTOX Red stain bound to DNA

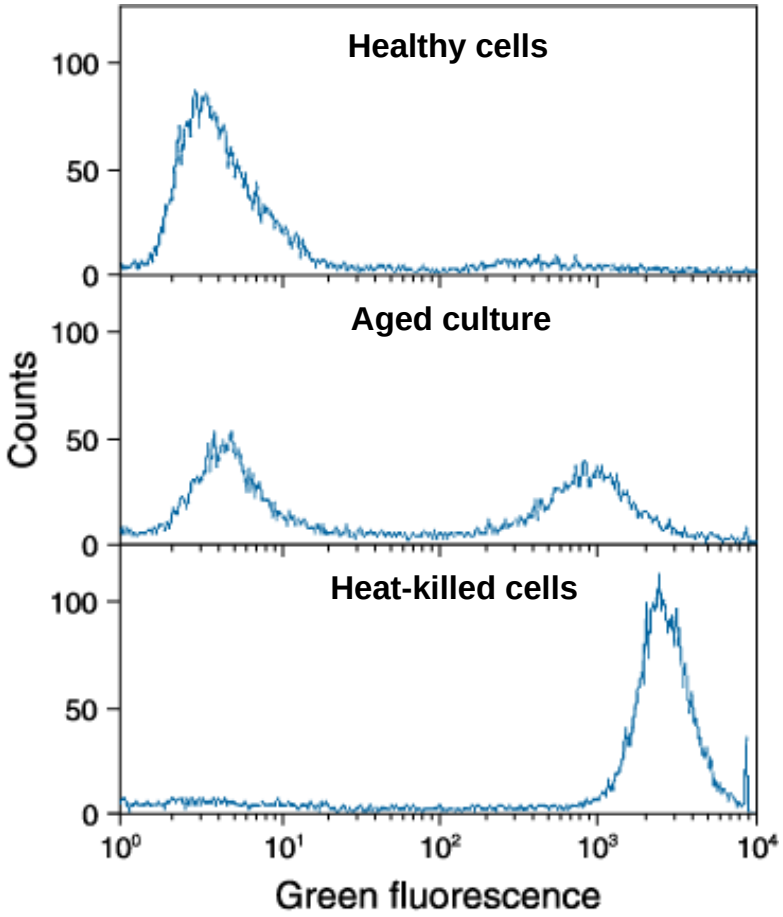
- A mixture of heat-killed and untreated Jurkat cells were stained with 5 nM SYTOX Red stain for 15 minutes.
- Live cells are easily distinguished from the dead cell population

Fixable LIVE/DEAD™ Dead Cell Stains



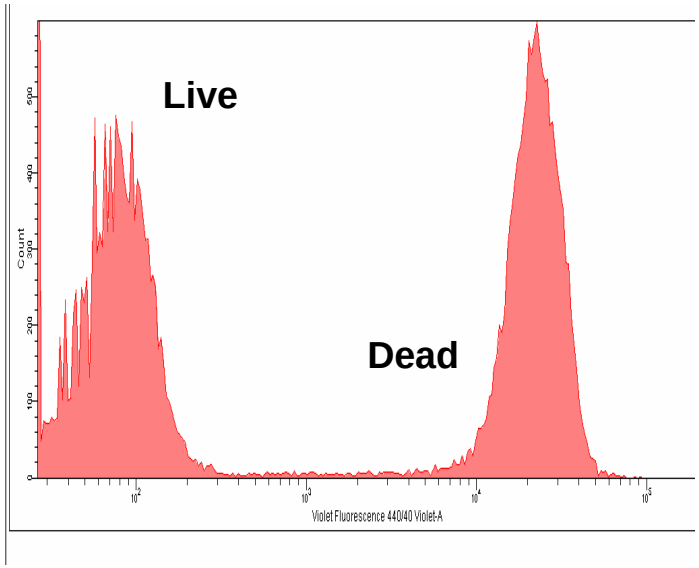
- **Live cells (left)** react with the fluorescent reactive dye only on their surface to yield weakly fluorescent cells.
- **Cells with compromised membranes (right)** react with the dye throughout their volume, yielding brightly stained cells.
- In both cases, the excess reactive dye is subsequently washed away.
- Dye does not redistribute upon fixation as it covalently attaches to proteins (via free amines) within the cell.

LIVE/DEAD® Fixable Green Dead Cell Stain Kit (L23101)

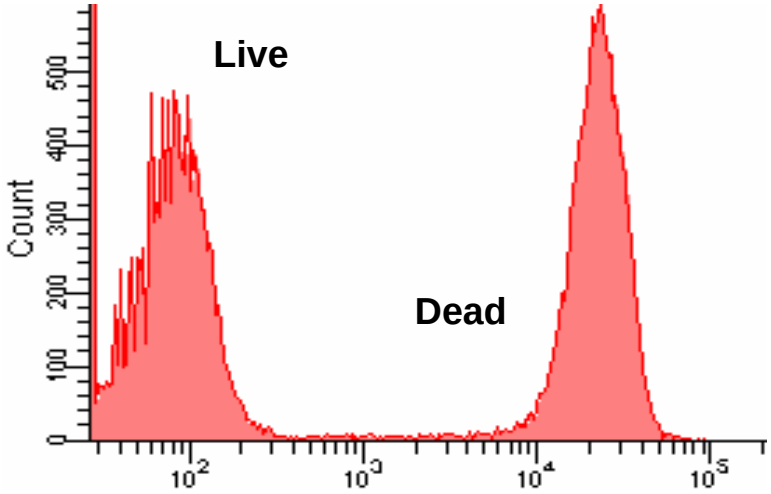


LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit

Before fixation



After fixation



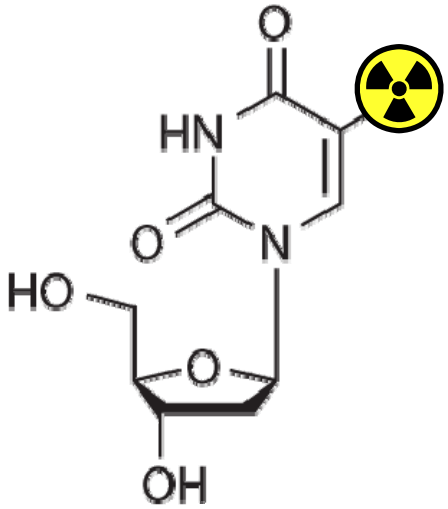
Population Discrimination shows same pattern before and after fixation.

The Family of Fixable Dead Cell Stains

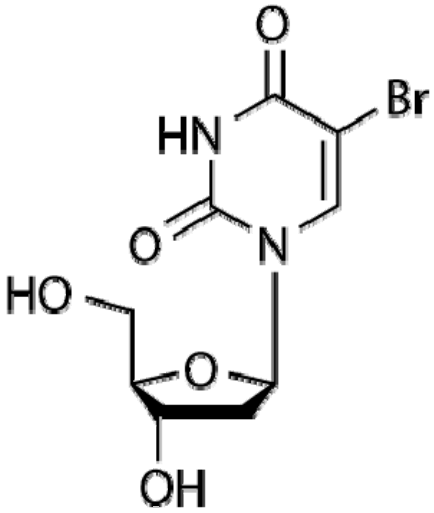
Dye	Laser	Emission
Blue	UV	450 nm
Violet	UV, 405 nm	451 nm
Aqua	405 nm	525 nm
Green	488 nm	520 nm
Red	488 nm	615 nm
Far Red	633 nm	665 nm
Near IR	633 nm	775 nm

Measurement of new DNA Synthesis - Cell Proliferation

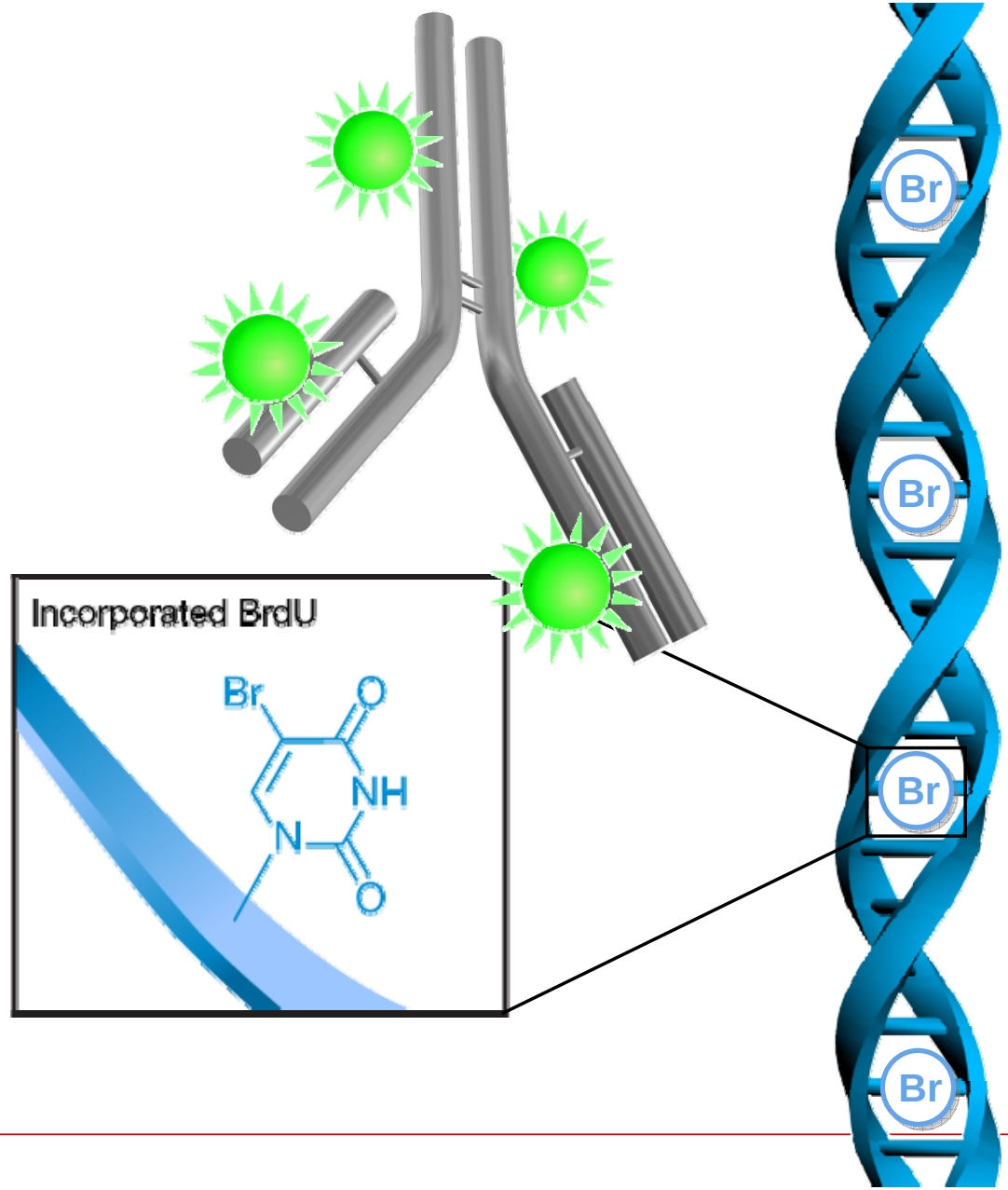
- Based upon Nucleoside incorporation in proliferating cells.
- Measures DNA synthesis at the individual cellular level.
- Current Methods
  - ^{3}H-thymidine
  - BrdU
- Novel Method from Invitrogen
  - Click-iT™ EdU



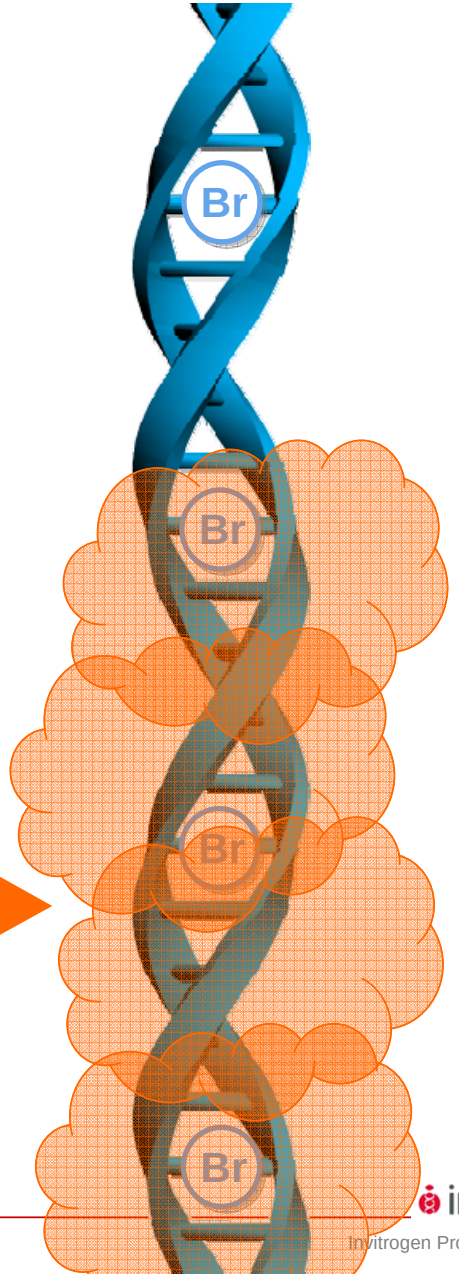
Tritiated (^{3}H) thymidine

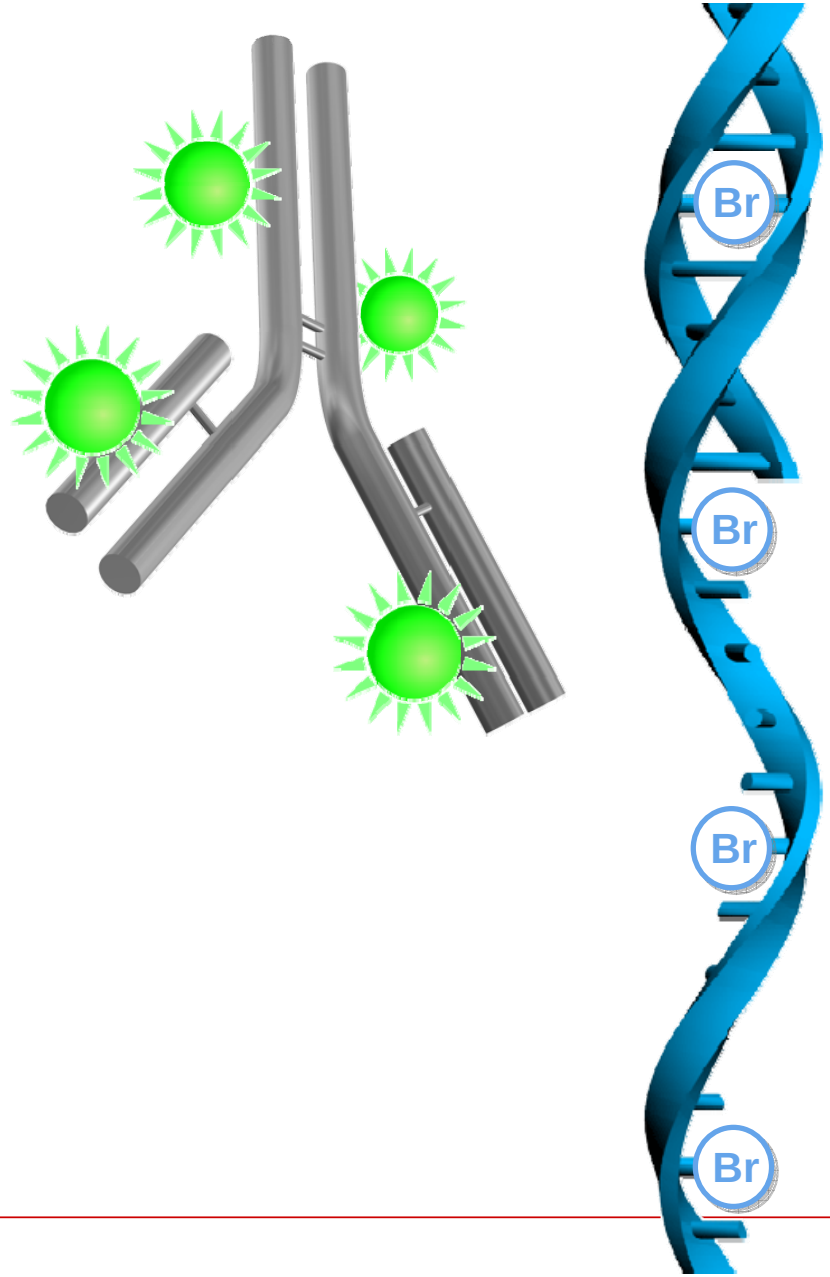


BrdU (5-bromo-2'-deoxyuridine)


Incorporated BrdU
Br
O
NH
N
O
Br
Br
Br
Br

Acid or DNase




Br
Br
Br
Br

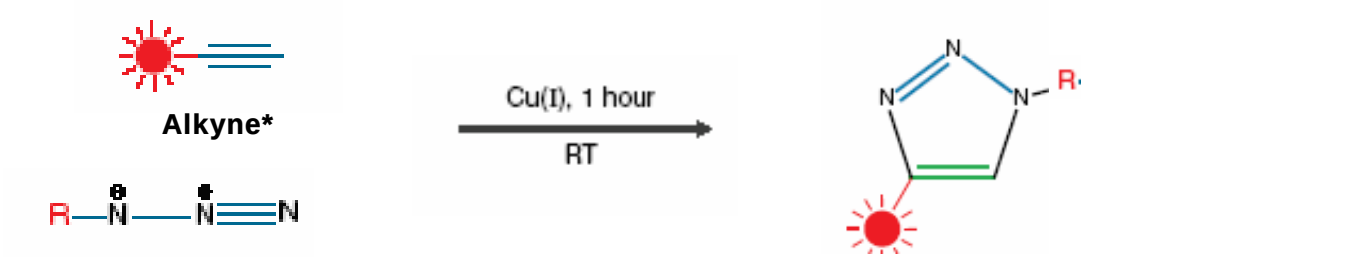
Click-iT™ is a specific Azide/Alkyne Reaction

- Provides a unique “chemical handle”

- Azides and Alkynes absent from nearly all species
- .
- Azides do not react appreciably with water, resistant to oxidation.

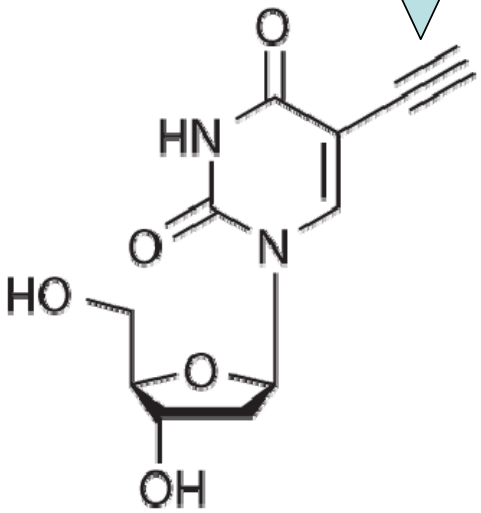
- Compact structure

- For Cell Proliferation
  - EdU is linked to the alkyne.
  - The Azide is linked to the fluorophore.

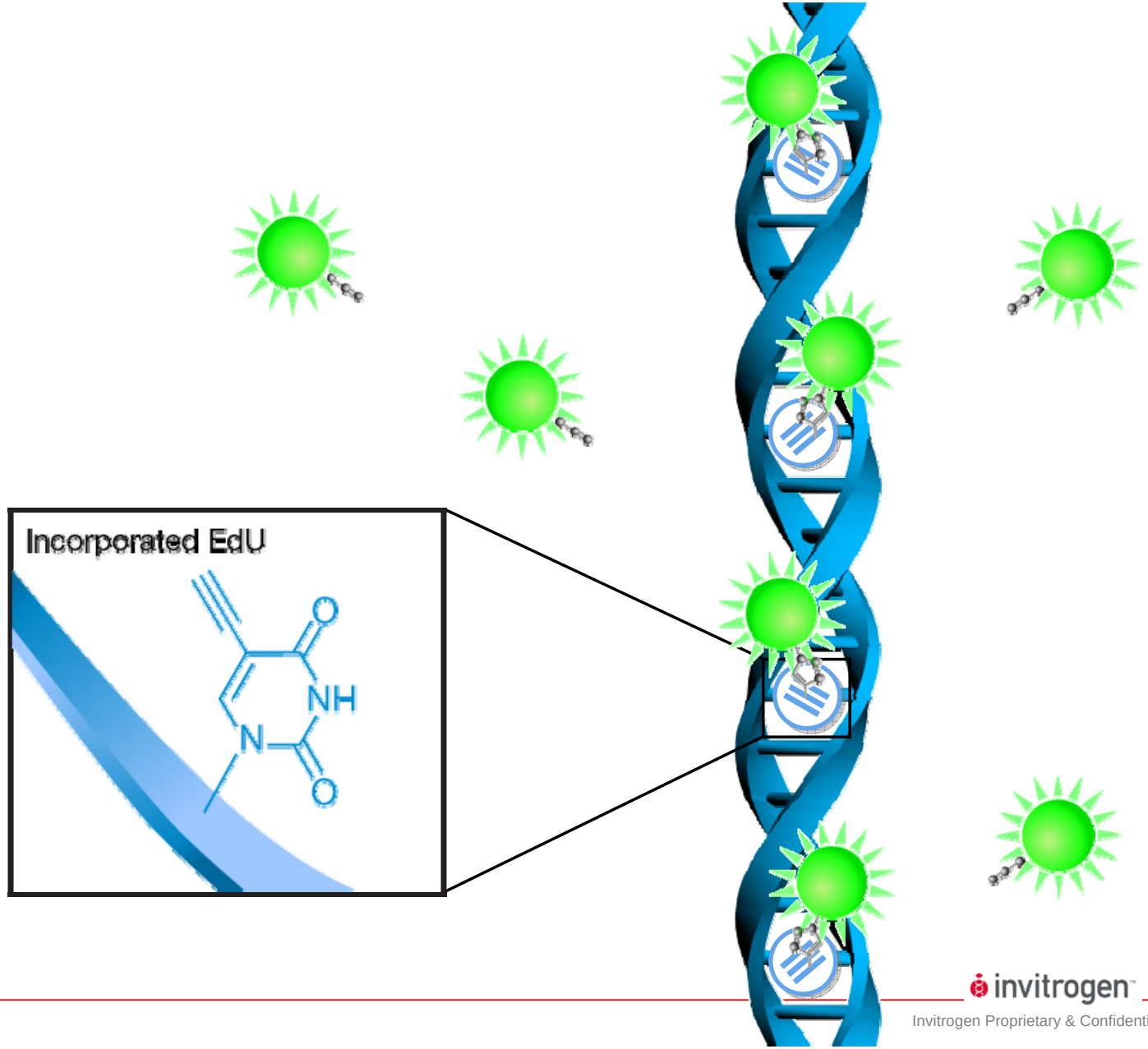


***The azide and alkyne can change in Click-iT™ product – in the Click-iT™ EdU assay, the azide detection reagent and the alkyne is the labeling reagent**

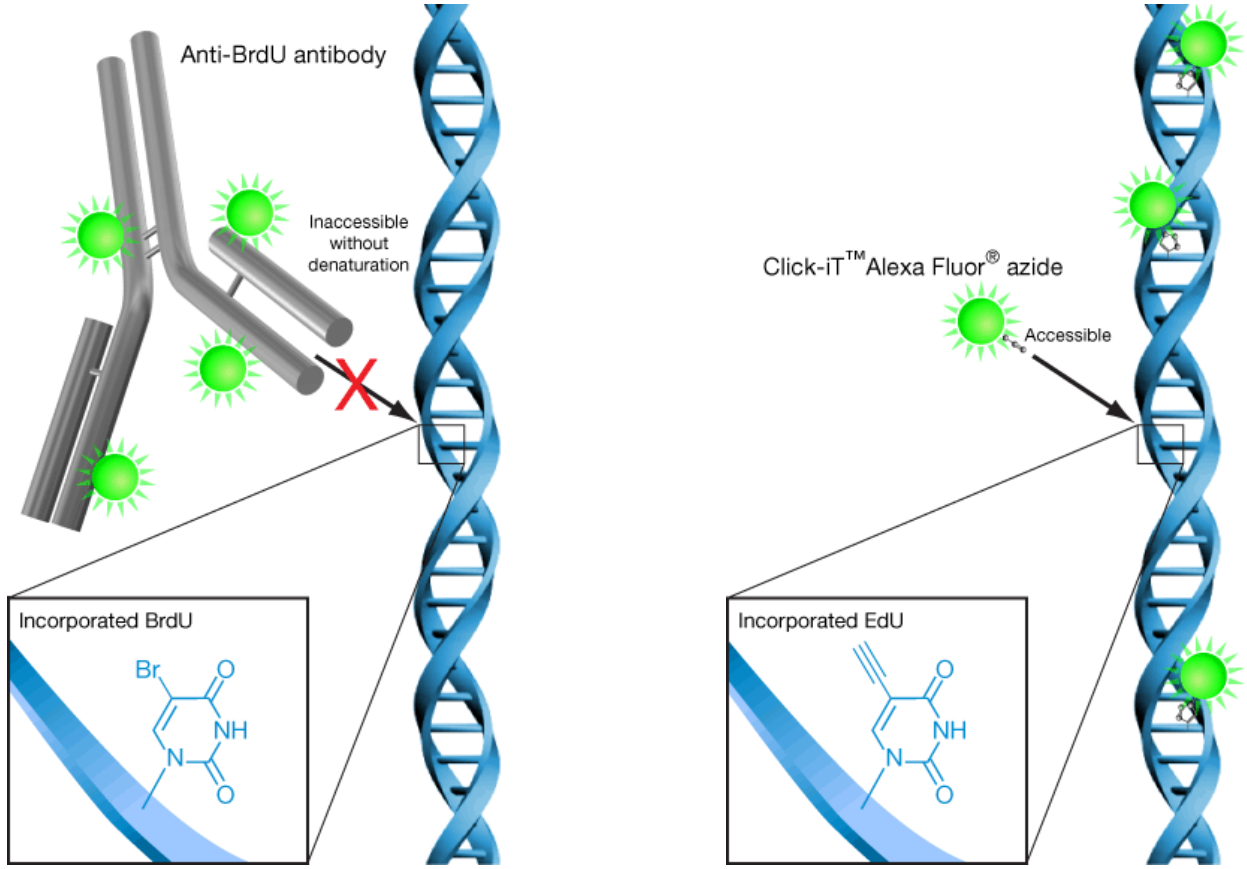
Alkyne reactive group



EdU (5-ethynyl-2'-deoxyuridine)


Incorporated EdU
O
NH
N
O

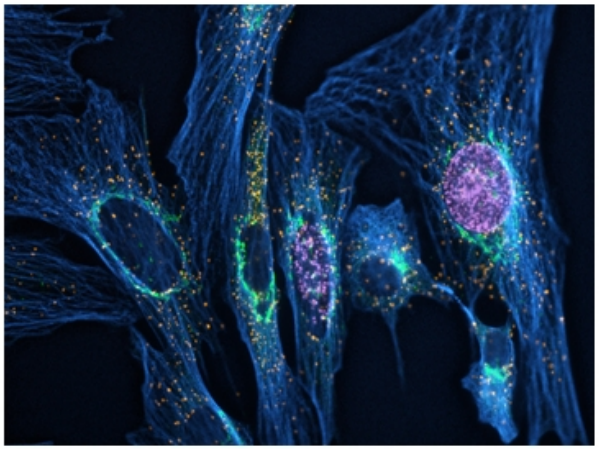
Click-iT™ EdU vs BrdU

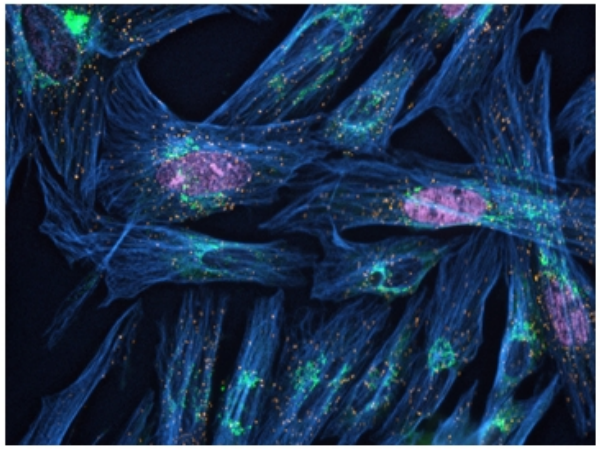


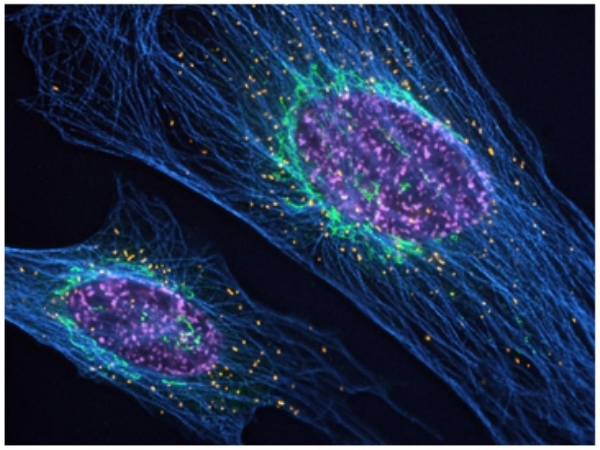
Advantages of Click-iT EdU Cell Proliferation Assays

- **Eliminate Radioactivity**
- **Simplified Procedure**
  - Shorter assay time ~2 hours
  - Only mild fixation / permeabilization required – retains sample morphology and antigen recognition sites
  - No DNA denaturation required
  - Works out of the box – No "magic recipe" or skill set required
- **Content rich results**
  - Measure cell proliferation at the individual cell level
    - With simultaneous cell cycle analysis
    - With simultaneous intracellular and/or extracellular biomarker detection
    - With simultaneous cell cycle analysis and biomarker detection

Multiplexed Imaging is a Snap with Click-iT™ EdU

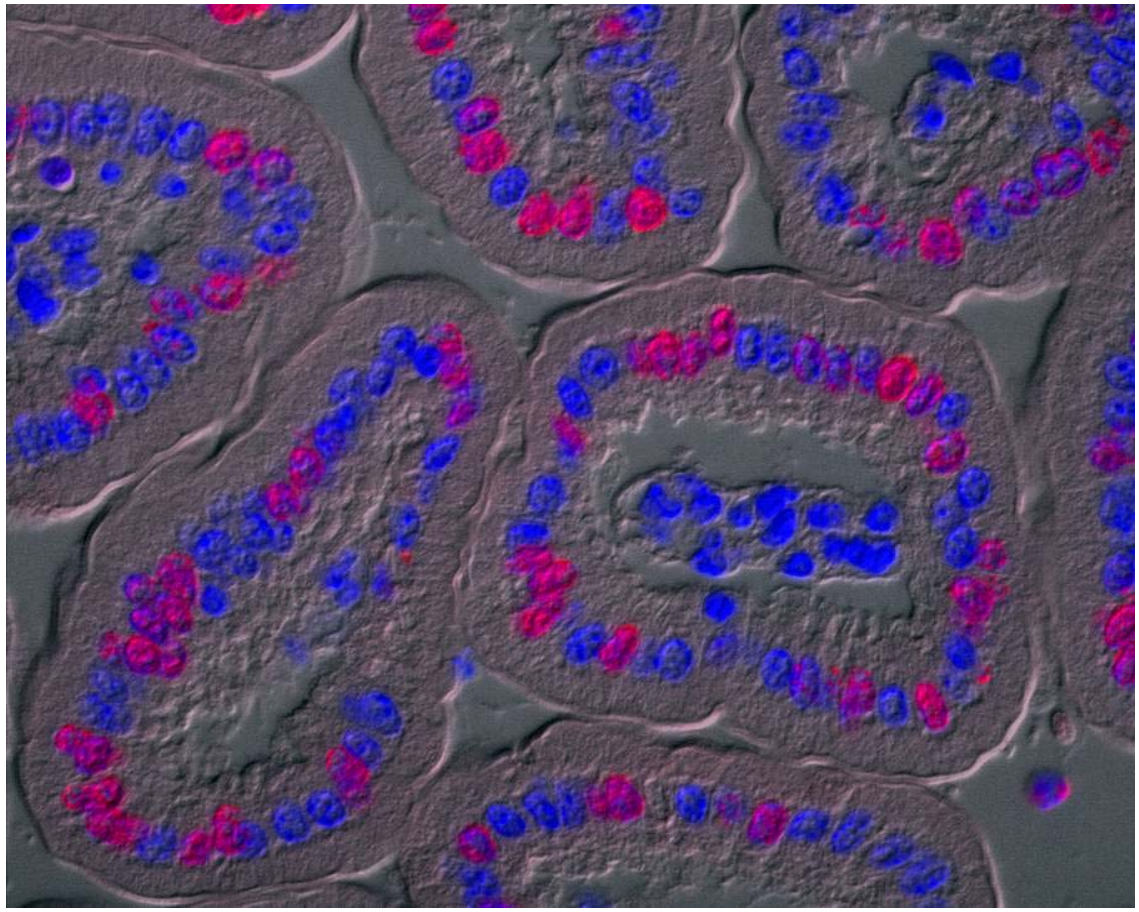






Muntjac cells were treated with 10 μM EdU for 45 minutes. Cells were then fixed, permeabilized and EdU that had been incorporated into **newly synthesized DNA was detected by the far red-fluorescent Click-iT™ EdU Alexa Fluor® 647** High-Throughput Imaging (HCS) assay (A10208) utilizing the technical tip for converting the HCS assay to conventional fluorescence microscopy (X10027). **Tubulin was labeled with an anti-tubulin antibody and visualized with an Alexa Fluor® 350** goat anti-mouse IgG antibody. **Golgi was stained with the green-fluorescent lectin HPA** from *Helix pomatia* (edible snail), **Alexa Fluor® 488 conjugate** and **peroxisomes were labeled with an anti-peroxisome antibody** and visualized with **orange-fluorescent Alexa Fluor® 555** donkey anti-rabbit IgG antibody.

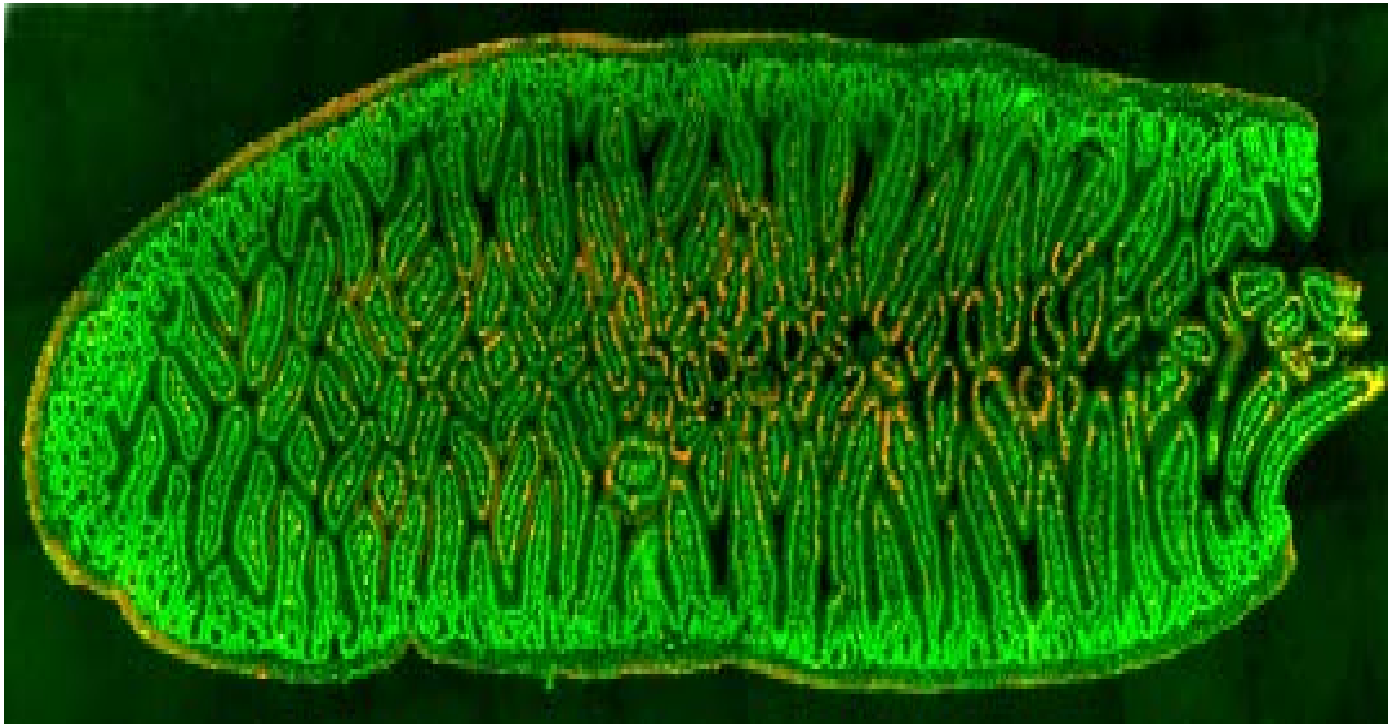
Click-iT™ EdU in vivo: Mouse intestine



Red: Click-iT™ EdU

Blue: DNA

Click-iT™ EdU in vivo: Mouse intestine



an entire oblique section through the intestine - about half a centimeter in length.

Red: Click-iT™ EdU

Green: DNA (Hoechst) ***Image courtesy of Adrian Salic, Harvard University***

Visualise Cell Proliferation in the Colour you want

- **Click-iT™ EdU Flow Cytometry**
  - Alexa Fluor 488
  - Alexa Fluor 647
  - Pacific Blue
- **Click-iT EdU High Throughput (HCS) Assay**
  - Alexa Fluor 594
  - Alexa Fluor 647
- **Click-iT EdU Imaging**
  - Alexa Fluor 488
  - Alexa Fluor 594
  - Alexa Fluor 647
- **Click-iT™ EdU Microplate Assay**
  - Oregon Green

Click-IT™ EdU Flow Cytometry and Imaging Kits are NOT interchangeable

Thank you for your **Attention**

Questions…

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