

# Multi-Color Fluorescence Microscopy Application Using Novel Brilliant Violet™ and Alexa Fluor® 594 Conjugated Monoclonal Antibodies

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#### **Abstract**

BioLegend has generated a large library of high affinity antibody clones against different mouse and human antigens that are validated in flow cytometry applications. They are offered as conjugates with the violet-excited Brilliant Violet<sup>™</sup> (BV<sup>™</sup>) fluorophores. Use of the BV™ fluorophores has greatly increased phenotypic multiplexing capabilities in flow cytometry by increasing the utility of the violet laser. Violet-excited BV421™ antibody conjugates are at least as bright as yellow/green-excited phycoerythrin (PE) and red-excited Alexa Fluor® 647 conjugates. The brightness of violet-excited BV510™ is more on par with the green-excited Alexa Fluor® 488. BV421™ and BV510™ are far brighter and more photostable than the violet-excited Alexa Fluor® 405 and Pacific Blue™. Here, using a variety of discrete immunologic cell populations bearing different antigens, BV421™, BV510<sup>™</sup>, Alexa Fluor<sup>®</sup> 488, Alexa Fluor<sup>®</sup> 594 and Alexa Fluor<sup>®</sup> 647 are shown to also increase multiplexing capabilities and increase resolution in fluorescence microscopy applications. The results show that BV421™, Alexa Fluor® 488, Alexa Fluor® 594 and Alexa Fluor® 647 conjugated monoclonal antibodies are valuable new tools for the immunologist wanting to resolve multiple antigens simultaneously with fluorescence microscopy.

#### Introduction

Fluorescence microscopes and fluorophore-conjugated antibodies are powerful tools to visualize the distribution of proteins in/on cells, structures within tissues, or subcellular compartments. Directly conjugated antibodies can shorten sample staining time and simplify dual and triple labeling procedures. Here, we show that violet-excited BV421™ and BV510™, Alexa Fluor® 488, Alexa Fluor® 594 and Alexa Fluor® 647 conjugated antibodies exhibit high fluorescence intensity and photostability. They can be used for cell surface staining and intracellular staining with low background in suspension cells, adherent cells and tissues by using a variety of methods. BV421™, Alexa Fluor® 488, Alexa Fluor® 594, and Alexa Fluor® 647 conjugated antibodies can be combined in cells or tissue sections, thus providing valuable tools for visualizing up to 4 antigens simultaneously.

#### MATERIALS AND METHODS

#### Immunofluorescence cell surface staining:

Human peripheral blood mononuclear cells were fixed with 1% paraformaldehyde (PFA) followed by 5% FBS blocking. Then the cells were stained with indicated antibodies for 30 minutes at room temperature. Nuclei were counterstained with either DAPI or DRAQ5 $^{\text{\tiny TM}}$ .

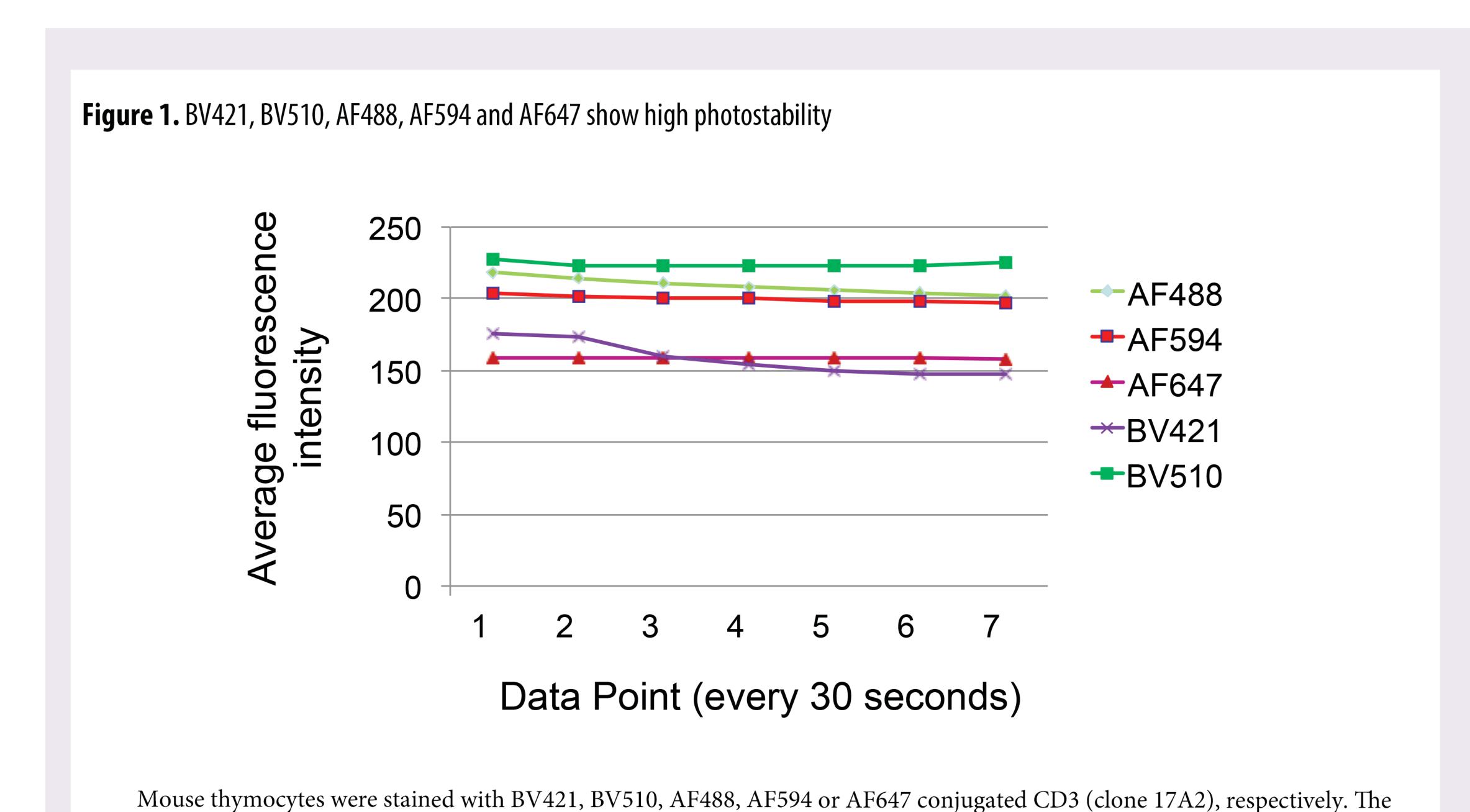
## Immunofluorescence intracellullar staining:

The cultured HeLa cells were fixed with 1% PFA for 10 minutes, permeabilized with 0.5% Triton X-100 for 10 minutes, and blocked with 5% FBS for 30 minutes. Then the cells were intracellularly stained with 5 µg/ml of different formats of human Ki-67 (clone Ki-67) in blocking buffer for 3 hours at room temperature. Nuclei were counterstained with DAPI or DRAQ5™. Immunofluorescence staining with tissue:

C57BL/6 mouse frozen lymph node section was fixed with 4% PFA for 10 minutes at room temperature and blocked with 5% FBS plus 5% rat serum for 1 hour at room temperature. Then the section was stained with indicated antibodies overnight at 4°C. The image was captured by 10X objective.

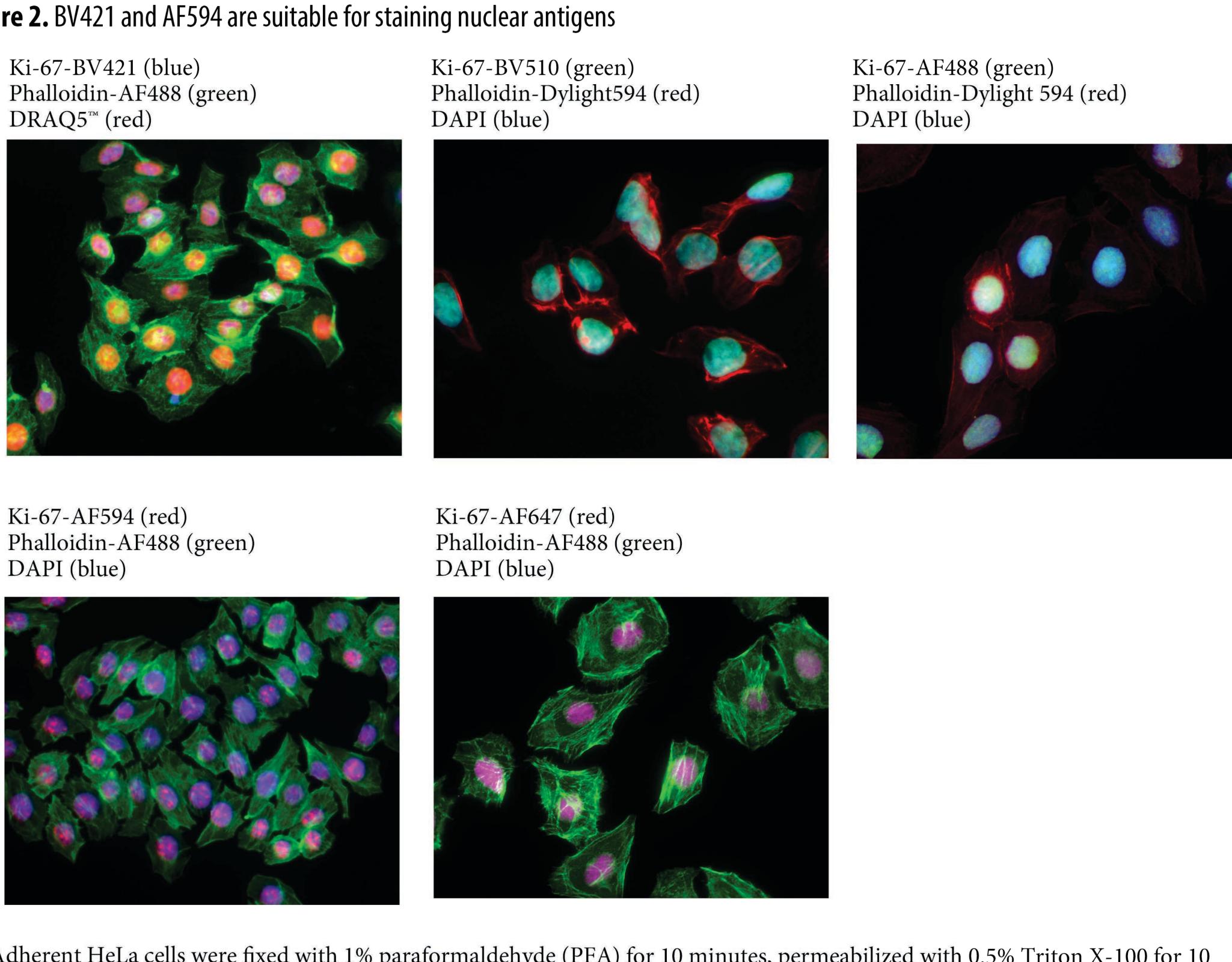
#### Immunofluorescence photostability testing:

C57BL/6 mouse thymocytes were fixed and stained with different formats of 5 mg/ml CD3 (clone 17A2) at room temperature for 30 minutes. Then the cells were spun on a slide with cytospin and mounted with Prolong Antifade mounting solution. The image were captured by using a 40X objective. The plot shows fluorescence intensity from one cell collected every 30 seconds for 500 ms over a span of 3 minutes.



images were captured by 40X objective (data point 1 = 0 second).

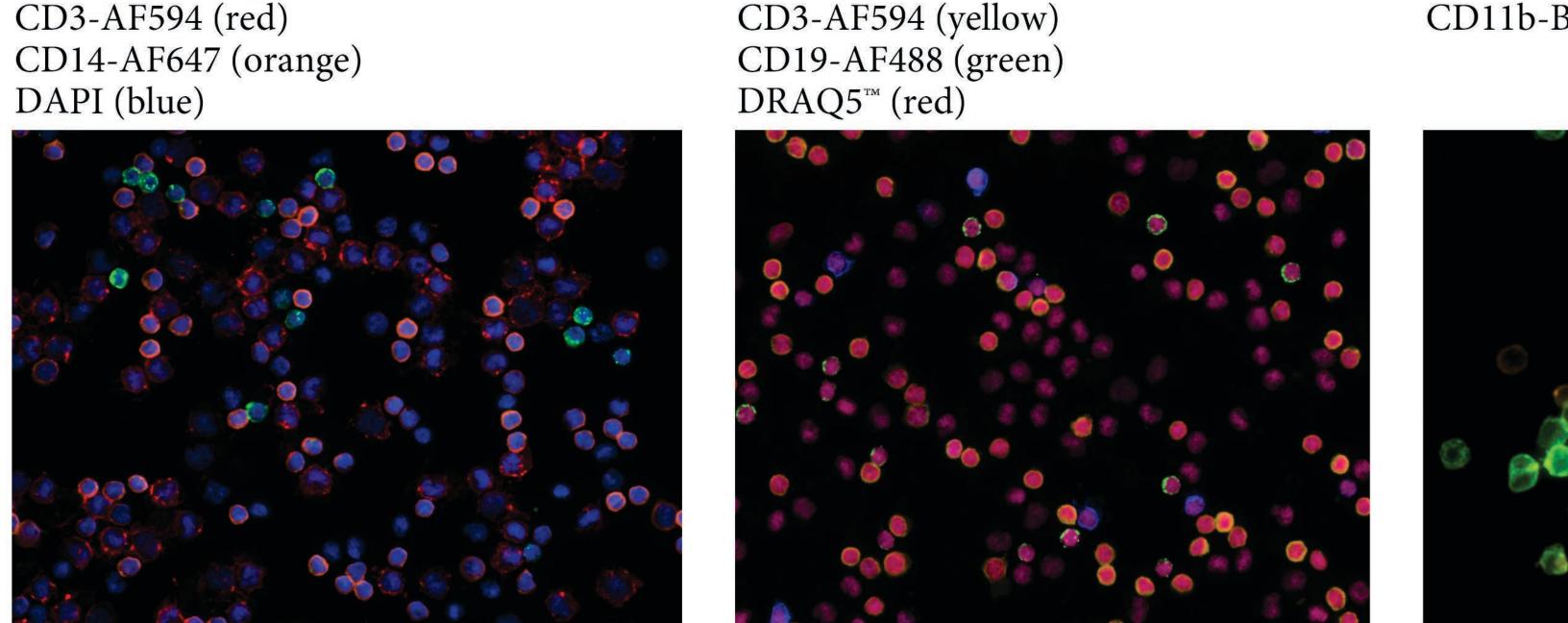
**Figure 2.** BV421 and AF594 are suitable for staining nuclear antigens



Adherent HeLa cells were fixed with 1% paraformaldehyde (PFA) for 10 minutes, permeabilized with 0.5% Triton X-100 for 10 minutes, and blocked with 5% FBS for 30 minutes. Then the cells were intracellularly stained with 5 µg/ml of different formats of human Ki-67 (clone Ki-67) in blocking buffer for 4 hours at room temperature and followed by AF488-Phalloidin or Dylight-594 Phalloidin for 20 minutes at 4°C. Nuclei were counterstained with DAPI or DRAQ5™.

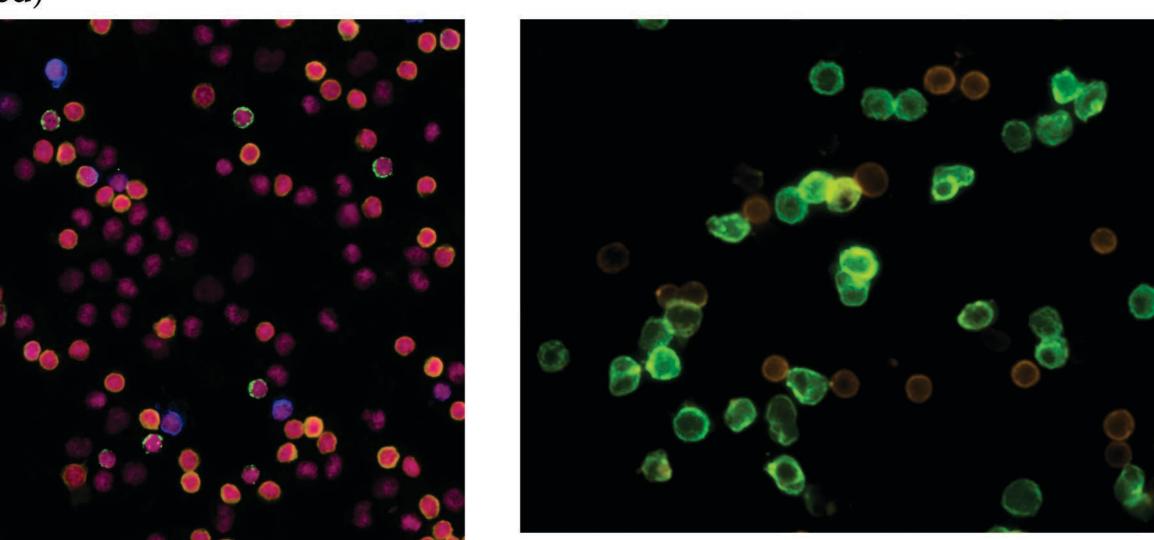
Figure 3. BV421 and AF594 fluorophores are suitable for staining surface antigens

CD19-AF488 (green)



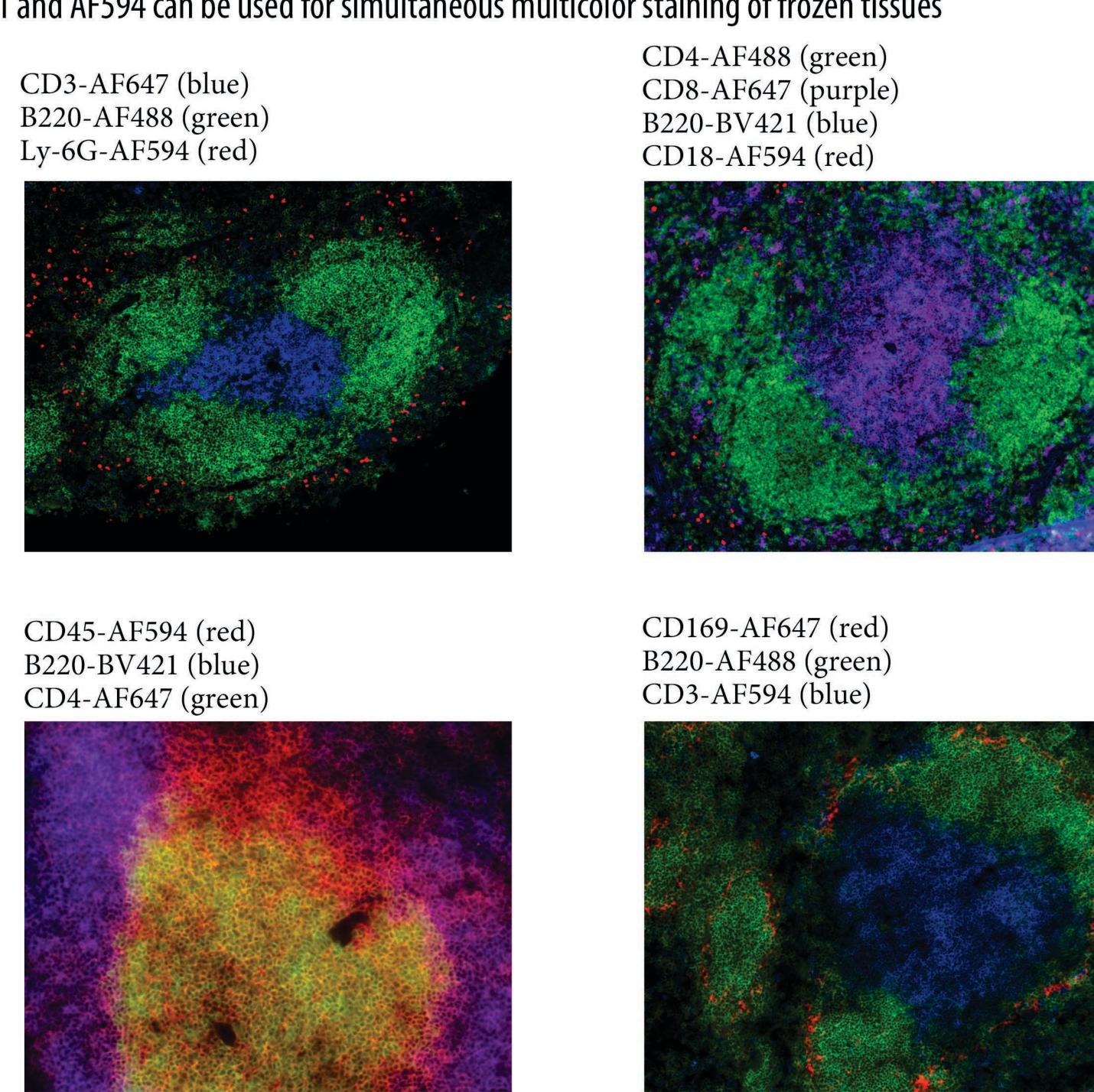
CD56-BV421 (blue)

CD45-AF647 (green) CD11b-BV510 (yellow)



Human PBMCs were fixed with 2% PFA and blocked with 5% FBS. Then the cells were stained with indicated antibodies. Nuclei were counterstained with DAPI or DRAQ5™

Figure 4. BV421 and AF594 can be used for simultaneous multicolor staining of frozen tissues



C57BL/6 mouse frozen lymph node section was fixed with 4% paraformaldehyde (PFA) for 10 minutes at room temperature and blocked with 5% FBS plus 5% rat/5% mouse serum for 1 hour at room temperature. Then the section was stained with indicated antibodies overnight at 4°C. The images were captured by 10X objective.

### CONCLUSIONS

- BV421<sup>™</sup>, BV510<sup>™</sup>, Alexa Fluor<sup>®</sup> 488, Alexa Fluor<sup>®</sup> 594, and Alexa Fluor<sup>®</sup> 647 exhibit high fluorescence intensity
- BV421<sup>™</sup>, BV510<sup>™</sup>, Alexa Fluor<sup>®</sup> 488, Alexa Fluor<sup>®</sup> 594, and Alexa Fluor<sup>®</sup> 647 conjugated antibodies can be used for surface and intracellular staining.
- BV421<sup>™</sup>, Alexa Fluor<sup>®</sup> 488, Alexa Fluor<sup>®</sup> 594 and Alexa Fluor<sup>®</sup> 647 conjugated antibodies can be used to stain up to 4 antigens simultaneously.

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