Biomarker Colocalization Analysis of a Virtual 12-plex using Discovery Chromogenic Dyes and Tissuealign™ Co-registration Software

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Introduction

The tumor micro-environment plays an important role in the diagnosis, prognosis and treatment regimens that patients receive. As such, understanding the specific changes in the immune-oncology (I/O) milieu presentation for tumors is key to developing novel therapeutics, new treatment regimens and ultimately increasing the survival and long-term prognosis for cancer patients.

Imaging the tumor milieu has inherent problems as there are many different types of immune cells to identify. In order to increase the number of biomarkers that can be analyzed concurrently, we propose a combination of multi-chromogen dyes and software for co-registration of sequential serial sections. These co-registered images produce highly multiplexed virtual images (8-, 10-, 15-plex or more) in which the tumor microenvironment can then be interrogated.

Here we assess the accuracy of our hypothesis utilizing 3 sequential serial sections in which the purple dye represents the same biomarker in each section. This continuity allows us to identify the same cell occurring in 1,2 or all 3 serial sections and to provide a visual and statistical analysis of our hypothesis.

Methods

Three tonsil tissue sections cut at 4-micron intervals were stained with Discovery Purple CD8 plus combinations of Discovery Teal, Discovery Yellow Ki67, and hematoxylin counterstain, then digitized using an Aperio scanner and co-registered using Visiopharm's Tissuealign™ module..

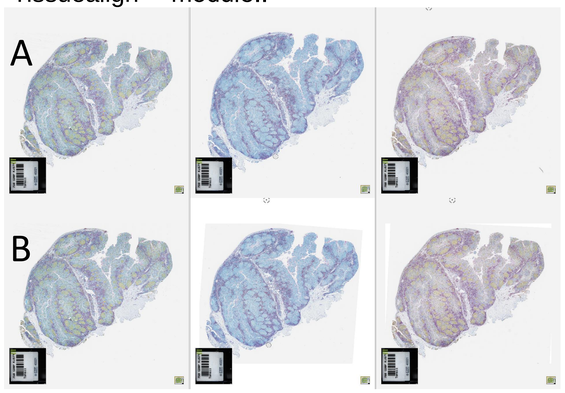

Figure 1:Co-registration of sequential serial sections

Three sequential sections labeled with CD8 (Discovery Purple), Discovery Teal, and Ki-67 (Discovery Yellow). (A) Raw Images, (B) Images after processing with Tissuealign™.

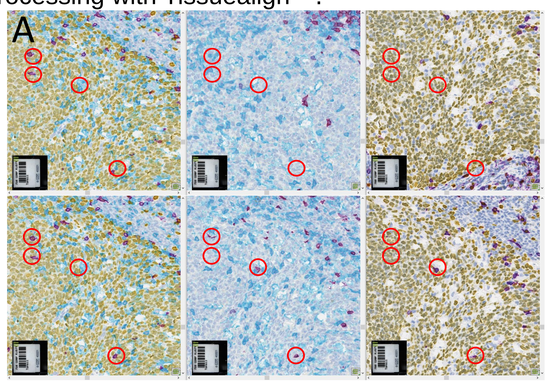

Figure 2. CD8+ Cell Identification in Sequential Serial Sections

Identification of CD8+ cells (Purple) in (A) unaligned and (B) aligned serial sections. Red circles indicate cell locations.

Results

The results of the study are described in the charts below. We observe no relationship between cells in sections that are not aligned (data not shown). However, data that are aligned with Tissuealign™ show high degrees of cellular correlation throughout the virtual stack of 3 serial sections. The occurrence of CD8+ cells in all three images in the virtual stack was very high (77%) if the cell was present in the middle section. As expected, cells that were identified at the top or bottom plane were identified to much less of a degree (36% top section and 44% bottom section throughout all three sections. Sampling error, measured by the presence of cells in the top section and bottom section was minimal (14%) and is most likely explained by the presence of a second cell appearing in the stack.

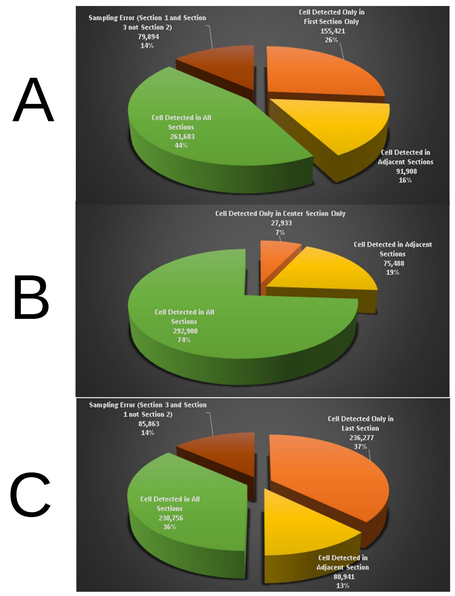

Chart 1. The Likelihood of CD8+ Cell Identification in Sequential Serial Sections

The likelihood of finding a CD8+ cell in adjacent serial sections if cell appears in the (A) top section, (B) middle section, and (C) bottom section of the virtual stack.

Conclusions

- We have demonstrated that it is practically feasible to work with high-plex study designs, using a combination of physical and virtual multiplexing.
- This approach requires the ability to achieve highly accurate/precise alignment of several serial sections, close to cell-to-cell alignment, even in the presence of non-linear tissue deformations across serial sections.
- The virtual multiplexing software developed by Visiopharm provided both a high level of precision and speed, which made it well suited as a tool for this type of study designs.
- The image analysis software, makes it efficient / feasible to analyze high (even hyper)-plexed datasets regardless of whether it is physical, virtual or hybrid multiplex designs.

Acknowledgements

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