Multiresolution registration of whole IHC slide images to evidence and quantify virtual colocalization of tissue-based biomarkers

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Whole slide imaging (WSI) using a NanoZoomer 2.0-HT is an essential step of the integrated solution which is developed since 2010 by DIAPath, the digital pathology platform of the CMMI (Gosselies, Belgium), ensuring the analysis and the validation of tissue-based biomarkers. This solution combines immunohistochemistry (IHC), special staining, WSI, image processing and data analysis. It is based on standardized laboratory procedures and quality controls ensuring reproducibility and traceability.

Extracting relevant information from actors involved in complex biological processes (such as cancers or treatment responses) requires targeting different antigens simultaneously. Multichromogenic (brightfield) IHC is used for multiple antigen labeling on the same slide but suffers from limitations. In addition to antibody cross-reaction problems, this approach also prevents the analysis of proteins expressed in the same cellular compartment (because of color merging or masking). We thus developed an alternative which consists in applying standard IHC on consecutive (also labeled "serial") tissues sections to target different proteins (see Fig. 1, left-hand panel). These slides were then digitalized and superimposed by a registration algorithm. Our approach uses the fact that histological structures (such as glands or epithelium) are often well conserved across a few serial slides because the slide thickness obtained with microtomy is 3 to 5 µm (i.e. smaller than the mean size of human cells).

To superimpose the serial slide images we developed an efficient 2-step registration algorithm [1]. The first, low-resolution, registration step is applied on the 1X equivalent magnification images (showing the whole slides to register in small-sized images such as 4,000 by 3,000 pixels). Briefly, this step enables to roughly superimpose two virtual slides on the basis of the slice outlines, holes and/or large histological structures. After this first step a series of high-resolution registrations were independently performed on the set of high-resolution (i.e. 20X equivalent) fields of view constituting a whole slide image.

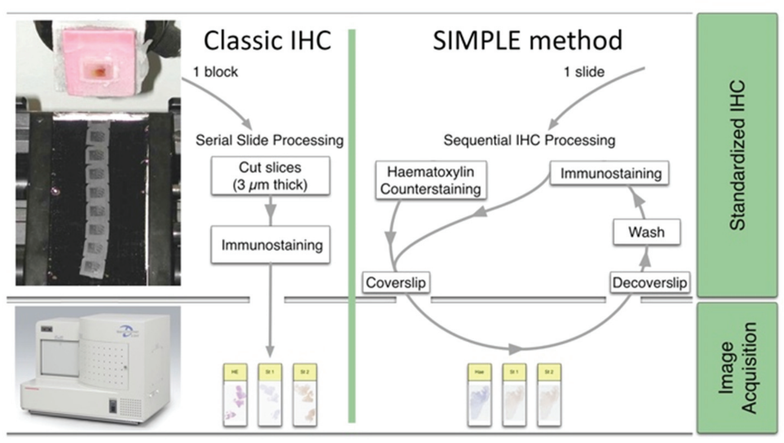


Figure 1: IHC on serial tissue slides (left-hand panel) and SIMPLE technique applied on the same slide (right-hand panel). Both provide slide images to superimpose for colocalizing different antigen expressions.

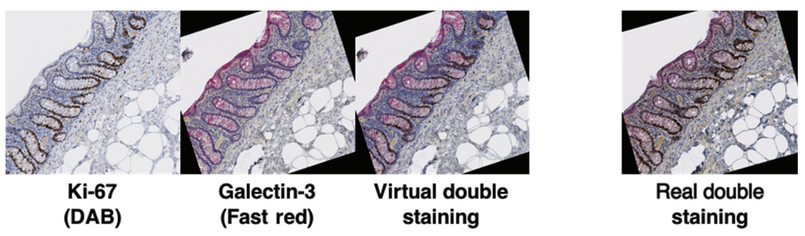


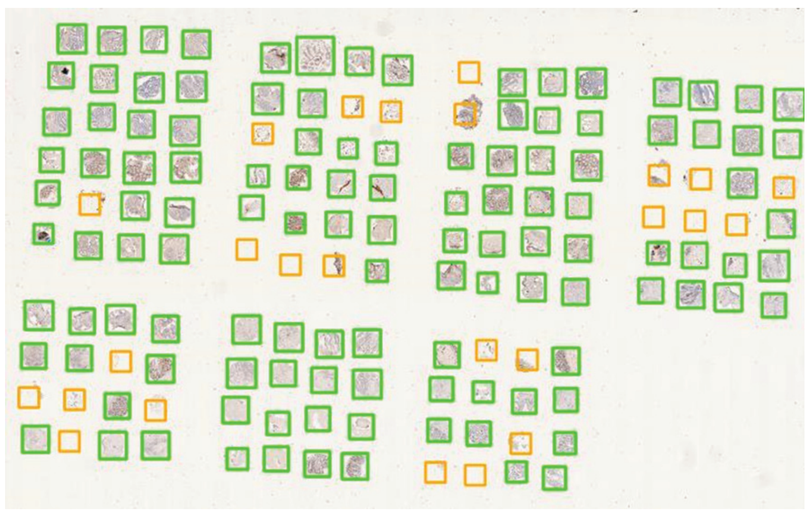
Figure 2: **Virtual double IHC staining**: Result of image registration applied to serial slides independently submitted to IHC and compared to double IHC staining (targeting Ki-67 and galectin-3) on a colonic tissue sample.

We evaluated image registration accuracy by measuring the distances between pairs of control points (located on each image) which should match if the images were perfectly superimposed. Our approach shows accuracy levels compatible with biomarker colocalization characterization. Indeed, on serial slides the distances between control points were evaluated to be at most 20 µm in presence of histological structures in the tissue (e.g. in colonic tissue, see Fig. 2). We then implemented a method to extract biomarker colocalization measurements taking the level of registration accuracy into account and validated our complete procedure by comparison to colocalization information obtained by means of double staining (with different cell locations, Fig. 2).

Because registration accuracy was lower for tissue without histological structures (e.g. the distances between control points being at most 80 µm for serial brain tumor slides), we developed a sequential IHC technique applied on the same slide (Fig. 1, right-hand panel). This "Sequential Immunoperoxidase Labeling and Erasing" (SIMPLE) method is based on cycles of staining/digitization/erasing, where after IHC staining and slide digitization, staining is "washed" through an antibody elution technique. We improved the original SIMPLE method by including a new elution methodology which preserves tissue and antigen epitopes for the next staining step. We successfully used this approach to identify antigens expressed in the same cellular compartment of high-grade glioma samples. We tested our registration method on the virtual slides so obtained and achieved very good results, i.e. about 5 µm of distance between control points [1].

Afterwards we adapted our registration process to tissue microarray (TMA) slides for high-throughput analysis of biomarker colocalization. Registration is easier for these slide images because TMA assembles cores extracted from tissue blocks in array fashion. As expected, we obtained very accurate registration results on serial TMA slides, even for unstructured tissue (i.e. distance between control points less than 10 µm) [2]. Fig. 3 shows the different steps of the process requiring (1) the correct identification of the circular samples (from the cores) on each TMA image, (2) the registration of core images extracted from serial slide images and (3) the computation of a colocalization map on small squared tiles from which a global colocalization measurement (adapted from immunofluorescence) can be computed [1,2]. A first (not illustrated) step consists in computing in each tile the value of a staining feature characterizing each IHC marker, e.g. the labeling index (LI), which is the percentage of positive (i.e. DAB-stained) tissue area [3]. The colocalization map evidences local staining overlap, which increases when both LI increases in a tile (see Fig. 3.3). The value of the global overlap index (r0) is the sum of the tile contributions and takes values between 0 and 1.

1) Core identification on a virtual TMA slide



2) Registration of pairs of core images

3) Colocalization map overlap

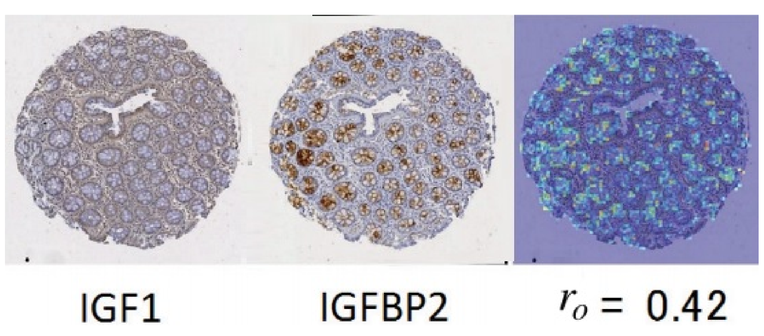


Figure 3: IHC staining colocalization on serial TMA slides showing IGF-I and IGFBP2 IHC expression in colonic tissue. After 1) core identification on each TMA image, 2) core images are extracted and registered to compute 3) staining overlap map and index (r0). In the map blue indicates no overlap whereas yellow to red show increasing overlap levels.

References:

[1] X. Moles Lopez, et al. Registration of whole immunohistochemical slide images: an efficient way to characterize biomarker colocalization. J Am Med Inform Assoc 22(1):86–99, 2015.

[2] Y.-R. Van Eycke, et al. High-throughput analysis of tissue-based biomarkers in digital pathology. In: 2015 37th Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. IEEE, Aug. 2015, pp. 7732–7735.

[3] C. Decaestecker, et al. Requirements for the valid quantification of immunostains on tissue microarray materials using image analysis. Proteomics 9(19):4478–94, 2009.

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