











shape and the shape being aligned to it) or the Cohen's kappa coefficient.<sup>9</sup>

Figure 5 shows 3 serial slices, and the segmented markers, before and after the alignment; the reader may note that the alignment allows a trustworthy comparison of the markers' respective localization. After having observed the result of applying our method to several sets of stained serial slices, we believe that our multiscale-hierarchical alignment procedure is a necessary preliminary step allowing any reliable comparative evaluation of densities and respective markers' localization of serial slice sets.

MIAQuant software and its user manual are freely available as supplementary material of this article, for clinical studies, pathological research, and diagnosis.

## Discussion

MIAQuant is a simple mean for the estimation of clinically interesting parameters. Being not affected by subjective variability, it might be a powerful tool to increase sensitivity, objectivity and efficiency in parameter estimation.

It can be adapted to staining methods used in pathology routine practice such as histochemistry and immunohistochemistry, and it is able to mitigate biological inconsistencies and/or technical errors in sample processing, including differential or incomplete slides or different intensity of staining.

MIAQuant is reliable, easy to handle and usable even in small laboratories, since image acquisition can be performed by cameras mounted on standard microscopes, which are commonly used in histopathological routine also in small hospitals, for their cheap cost. Moreover, MIAQuant is flexible since it effectively analyses images characterized by different image formats, pixel size and resolution, thus encouraging image exchange between clinical centers. In conclusion, MIAQuant has the potential to provide valuable assistance to pathologists in their daily practice, substantially enhancing the efficiency and accuracy of diagnostic processes, with benefit for the patient.

We are presently testing MIAQuant in various clinical oncological studies, including the definition of myeloid and immune cell tissue scores in metastatic melanoma and hepatocellular carcinoma. Another important application of MIAQuant in the research field concerns the study of the immunological infiltrate in human arterial plaques and its role in lympho-angiogenesis. Results of these studies, confirming the applicability of MIAQuant in patients setting, will be published elsewhere in the near future. All the aforementioned applications show that MIAQuant is a promising novel image analysis tool that might be successfully adapted to several medical research and clinical studies.

MIAQuant software, its user manual, and further developments, are available online at [www.consorzioomia.org](http://www.consorzioomia.org).

## References

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