Review of: "VALIS: Virtual Alignment of pathoLogy Image Series"

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Potential competing interests: I used to be affiliated to Moffitt Cancer Center which is the affiliation institution for most authors in the paper.

The authors present a tool to register pathology image series. The authors describe well the problem where images from stained tissues using multiple markers are not aligned. Authors illustrated well the problem in Figure 1. Authors also describe a good list of previous work, however from the text it is not fully clear how author's tool compare to previous methods. They also provide a list of tool's functions, however not all functions are illustrated or demonstrated in the text.

I agree with author's pipeline of the method. First reading the images using a memory saving format. Then pre-processing to make the color images gray-scale and similar between markers. Hierarchical feature extraction, however features are not described apart of calling them "tissue features". Then authors sort the images to perform a sequential registration, however I wonder how results would be if instead of sorting the images each image is register to the best quality image used as registration template. The method is described in Figure 3, however the figure should be improved, for instance by no stacking the images so they can be fully visualized, also parts of the figure are blurry or chaotic.

To validate the tool, authors use 613 samples, however it is unclear if several samples are from the same tumor and from how many patients samples were obtained. For validation they use feature distance between images, however as it is unclear which features are used then it is difficult to assess the results. For instance, if those features describe landmarks in the images I would agree with the validation, I think this should be clarified in the text. Then authors describe several applications but I don't think this adds much to the paper. Finally, I would have liked authors to discuss how their results compare quantitatively to other previous work.