

To count the number of pixels belonging to DAB-stained nuclei, N_D , we choose the following (r, g, b) intervals:

$$(<40, 115>, <6, 80>, <10, 75>) \quad (6)$$

To calculate N_H and N_D the whole slide may be subdivided into any number of disjoint parts or even read into RAM pixel by pixel. It is important since it makes possible to analyze specimens even on a PC with 16 GB RAM. N_H and N_D are then used to calculate proliferation index PI_C (2). For one specimen time necessary for calculations varied from about 50 seconds to about 7 minutes depending on the size of the whole specimen.

3 Results

We have calculated proliferation index PI_C for 9 whole slides of DLBCLs and we juxtapose the results with proliferation index PI_P evaluated for the same slides by a trained pathologist (Fig. 2).

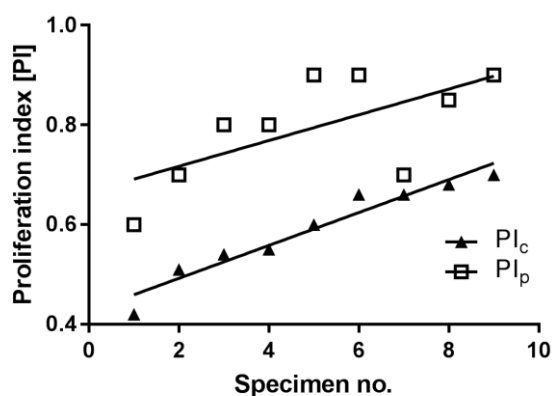


Fig. 2. Proliferation indices PI_C and PI_P for 9 specimens.

The ranges of PI_C for whole slides and of PI_P evaluated for these slides by pathologist are comparable - 0.28 vs. 0.30 - but absolute values of PI_C are about 0.20 lower than corresponding values of PI_P . This difference is obvious since pathologist while visually evaluating a specimen tries to find hot-spots and PI_P takes its highest values locally just for the hot-spots. The calculated PI_C and the PI_P evaluated by pathologist change concurrently i.e. if for a given case PI_P is equal or greater than for another case then also PI_C computed for the given slide is equal or greater than that for the other case. Statistics shows that correlation coefficient of PI_C vs PI_P is equal 0.71. Only one specimens behaves not in agreement with this rule. The most important is that calculated PI_C still shows that these are high grade DLBCLs.

4 Conclusions

CFPP method of calculating proliferation index PI_C for grading DLBCL neoplasms from stained histological slices is quick and simple (cf. [7]). It gives the results that well correlate with proliferation index PI_P estimated by a trained pathologist. The method is much simpler and much less time consuming than the methods that try to emulate pathologist's way of thinking when estimating PI_P . Of course, medical decision still remains to be made by a pathologist, but such a quick computer-assisted grading of DLBCLs may be quite helpful in diagnosis. By appropriate changing of color filtration thresholds CFPP method may be adapted to analysis of other problems in digital pathology.

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References:

- [1] R.K.Kumar et al. Virtual microscopy for learning and assessment in pathology, *The Journal of Pathology*, 304:613-618, 2004.
- [2] W. Klonowski, Applications of Chaos Theory Methods in Clinical Digital Pathology, in: *Handbook of Applications of Chaos Theory*, Ch.H.Skiadas & C.Skiadas, Eds. CRC Press, Boca Raton, New York, 2016, pp.681-690.
- [3] W. Klonowski et al. Application of Higuchi's Fractal Dimension in Analysis of Images of Anal Intraepithelial Neoplasia, *Chaos, Solitons and Fractals*, 48:54-60, 2013.
- [4] S.H.Swerdlow et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 127:2361-2364,
- [5] T.Markiewicz, A.Korzynska et al. MIAP - web-based platform for the computer analysis of microscopic images to support the pathological diagnosis. *Biocybernetics & Biomedical Engineering* 36(4):597-609, 2016,
- [6] Z.Swidarska, A.Korzynska et al. Comparison of the manual, semiautomatic, and automatic selection and leveling of hot spots in whole slide images for Ki-67 quantification in meningiomas. *Anal Cell Pathol* 2015:1-15.
- [7] E.M.Brey, Automated selection of DAB-labeled tissue for immunohistochemical quantification, *The Journal of Histochemistry & Cytochemistry*, 51:575-584, 2003.