The rules of acquiring digital images of the IHC stained tissue samples in terms of colour temperature and tints

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This study concerns the problem of image unification in a sense of colour and contrast as a part of a projected internet platform software for the automatic image analysis. These investigations are mainly focused on tissue samples immunohistochemically stained with DAB&H to perform the quantification of nuclei.

The images received with the developed internet platform are very different, what makes the development of effective image analysis software difficult. Thus, the image unification should be performed as a part of Image preprocessing. In this investigation we focus on the dependence of colour variation in images acquired with the same light conditions and mismatches that appear in WB adjustment. The WB adjustment in WB correction can be easily handled on the stage of the image acquisition. However, if analysis is done with full automatic software on images or virtual slides collected and sent by various users, who have used various acquisition tools, images should be unified in pre-processing stage. Our aim is to establish rules of acquiring digital images to avoid loss of important information, taking in amount segmentation criteria, which is responsible for unreliable results of analysis.

**Materials:**
- disease: meningioma
- immunohistochemistry staining: DAB&H against Ki67
- field of view: no 6 images with empty background
- field of view acquired: no 255
- options of lamp filament: 15
- options of camera WB settings: 15

Each field of view has been acquired 225 times with various microscope light temperature (from 5 up to 12 V) and camera WB settings (from 5 up to 12). Inadequate WB setting causes colour diversity; images acquired with lower WB setting than light temperature are blue tinted, while acquired with higher WB setting are yellow tinted.

**Methods:**
- WB correction was compared with the reference image and among themselves to find if the image acquisition process or image manipulations lead to loss of information. Two types of reference images have been used:
  1. Image with statistical descriptors closest to the averaged for all images acquired with adequate WB settings;
  2. Artificial image developed as the average of images; all images acquired with adequate WB settings.

**RESULTS**

1. Acquiring under mismatched settings causes the images to be bluish or yellowish and that intensity of discoloration is bigger if the difference in camera settings and microscope’s light temperature is bigger.
2. The discoloration is reduced by manual or automatic procedures of the WB correction, the contrast in luminance decreases in such a degree, as this difference increases.
3. The best light colour temperature appears when light filaments are powered by the voltage of 7.5–9.0 V, based on images acquired with matched settings of camera’s WB to the microscope’s light temperature.
4. Powering light with less or more voltage increases MSR but decreases SNR and SSIM.

**CONCLUSIONS**