Walton, S. et al. 92013). Accurate automated quantitative imaging of tortoise erythrocytes using the NIS image analysis system.
Biotechnic & Histochemistry, 88(5): 242 – 249
http://dx.doi.org/10.3109/10520295.2013.765594

Accurate automated quantitative imaging of tortoise erythrocytes using the NIS image analysis system

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Abstract

The standard method for assessing blood cell characteristics using an ocular micrometer is time-consuming and limited. We used the Nikon NIS Elements imaging software and May-Grünwald-Giemsa staining to determine whether automated image analysis is suitable for rapid and accurate quantitative morphometry of erythrocytes. Blood was collected during four seasons from 126 geometric tortoises and the blood smears were evaluated for cell (C) and nuclear (N) characteristics of the erythrocytes. We measured area, length (L), width (W), perimeter, elongation and pixelation intensity, and calculated L/W and N/C areas. Erythrocyte size differed among cohorts; females, the larger sex, had smaller erythrocytes than either males or juveniles. Males had more elongated erythrocytes than females and erythrocytes of adults were more elongated than those of juveniles. Erythrocyte size and shape influence the efficiency of gas exchange owing to surface area to volume ratios, which are greater for small, elongated cells than for large, round cells. The high N/C ratio and low pixelation intensities of males and juveniles indicate that they may have had more immature erythrocytes in their circulation than females. The use of pixelation intensity to indicate the presence of immature erythrocytes was validated by seasonal differences that corresponded to the biology of the tortoises. Pixelation intensity was lowest in winter. We found that automated image analysis is a rapid and reliable method for determining cell size and shape, and it offers the potential for distinguishing among developmental stages that differ in staining intensity. The method should be useful for rapid health assessments, particularly of threatened species, and for comparative studies among different vertebrates.

A thorough hematological evaluation includes red and white blood cell counts and assessment of morphology (Campbell 2004). Collecting blood for blood smears is minimally invasive, easily performed in wild populations, and has been used with success to assess an animal's health and physiological state (Arikan and Cicek 2010). Knowledge of vertebrate blood cell morphology and function is based mainly on mammals (Claver and Quaglia 2009); there are few studies of lower vertebrates, particularly rep- tiles (Campbell 2004, Strik et al. 2007). Leukocyte types correspond broadly among all vertebrate groups, but non-mammalian vertebrates have nucleated thrombocytes and erythrocytes in contrast to the anucleated blood platelets and red blood cells of mammals (Campbell 2004, Claver

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