OP-31

Speed improvement of automated fluorescent digital slide scanning

Viktor Sebestyén Varga[1], Levente Ficsor[1], Barnabás Galamb[2], Viktor Kamarás[2], Béla Molnár[1], Zsolt Tulassay[1]

1 2nd Dept. of Int.Med., Semmelweis University, Budapest, Hungary.
2 3DHISTECH Ltd., Budapest, Hungary.

viktor.varga@3dhistech.com

Abstract

Introduction: Fluorescent whole slide imaging became commercially available in the last years. Our goal was to enhance the scanning speed of the MIRAX system by new hardware components and software algorithms. Materials: We used MIRAX Midi and Scan slide scanners with Plan-Apochromat 20x / 0.8NA objective, AxioCam MRm camera with a resolution of 0.32 µm / pixel and a filter changer with fluorescent filters for DAPI, FITC and Rhodamine. The light source was an HXP-120 triggered metal halide lamp.

Methods: The AxioCam MRm can provide 12 bit images but the system stores 8 bit images only. The additional bits are utilized for digital gain. If the digital image is shifted by 1 bit then the exposure time is halved. This way the exposure time can be decreased by a factor of 16 if the image is shifted by 4 bits. As the exposure time is decreased the noise of the image is increased. The user can select what digital gain setting will be used for each channel.
Scanning fish spots and thick samples requires grabbing every field of view in several different focal planes. By an extended focus method all layers can be acquired but not all layers are necessary in every digitized channel. We made individual extended focus setting available for each channel.

Results: The monochrome camera halved scanning time. Four times digital gain provided 33% speed increase. Selectable extended focus settings provided a speed increase from 20% to 50% in three channels depending on the different settings.

Conclusions: The speed of fluorescent scanning could be increased without compromising image quality. Speed could be further increased using binning and a camera with more pixels and higher frame rate. The sample is bleached less because it is exposed for a shorter period.