COMPUTER ASSISTED MICROSCOPY
The Era Small Size Slides & 4m Microscopes

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Abstract: The present paper described the technique to evaluate *digital resolution* (DR), *Visual Magnification* (VM), *onScreen* Magnification (SM) and *Useful magnification* (US) in order to compare image quality and resolution for diagnostic purposes on computer assisted microscopes including Multi-Modal Miniature Microscopes-4M.The study was done on surgical pathology and cytological specimens comparing analog microscopic images versus digital Small Size Virtual Slides (SSVS) images. The SSVS were obtained with an 8 megapixel camera, in JPEG2000 format using a super-resolution algorithm of capture. The field of view-FOV images showed four times higher discrimination power, in spite of the low sampling density. The region of interest-ROI images, with a sampling density close to Shannon theory showed six times higher discrimination power. OnScreen magnification FOV achieved 640x and ROI 3200x augments that could never been reached using analog microscopy. The paper demonstrates that SSVS are ideal for hand-held microscopes or even mobile phones with ad-on capture systems.

1 INTRODUCTION

Several approaches have been proposed for an efficient storage of complete digital slides (DS=digital slide) with distant access: Dynamic Robotic Telepathology (DRT) (Ferrer-Roca, 1998), Virtual Slides (VS) and the present Small-Size Virtual Slides (SSVS) (Ferrer-Roca, 2005, 2007) technique.

SSVS take the advange of the modern digital cameras using digital zoom. It is a JPEG-2000 image (JPEG, 2001) 100 times smaller than VS, easy to transmit and store (Marcano, 2007). In-focus low power images are solved with the ZF-Zoom Focus (Ferrer-Roca, 2005) technique.

Fully digital surgical pathology is progressively being accepted (Ho et al., 2006) but cytology is complex since it requires high power for diagnosis.

Also chip miniaturization allows using hald-held devices. This is the case of the 4M or Multi-Modal Miniature Microscopes of less than two centimetres of diameter using CMOS as well as mobile phones cameras up to 12 megapixels. Being out of the strict optical control of the microscopic vendors it become essential to established the parameters to compare image quality, which are provided in the present paper.

2 MATERIAL & METHODS

Images were obtained on an average quality Olympus BH-2 microscope with a high resolution CCD camera. A AVT-Oscar F-810C fireware IEEE1394 camera, with a CCD 2/3" Sony sensor of 8 Megapixels-Mpx (3288x2470) producing images of 3272x2469, 12 bits/pixel. The chip was a colour mosaic (R-G x G-B) with 2x2 pixel sensitivity. Signal to noise ratio (SNR) was 36,19dB (Noise floor of CCD cameras of 12 bits dynamic range is $2.4x10^{-4}$; SNR= - 10 $\log_{10}$ Fnoise= 36,19dB.)

2.1 Digital Camera-image Processing

The camera control was integrated into the TEXCAN-II®-suite using AVT-Allied Vision Technologies (www.intek-darmstadt.de), CVB (www.commonvisionblox.com) and LeadTools libraries (www. Leadtools .com / SDK / Medical / Medical-Products-n.htm).

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The suite controls white balance, image focus at 1:1 digital zoom (ZF technique) (Ferrer, 2005), image noise reduction, hardware shading correction and FOV images acquisition. Virtual image were obtained stitching all FOV in a JPX-JPEG2000 format building a final 1/10 wavelets compressed JPEG2000 image.

Image noise reduction and contrast enhancement was tested with several algorithms and the better one is presented. Further improvement of the SNR (signal to noise ratio) avoiding aliasing was the colour demosaicing display: Images taken in RAW format were displayed demosaicing while RGB images were stored demosaiced.

2.2 Optical Acquisition System

2.2.1 PMoC

Each field of view (FOV) was taken through a SPlan 4x objective (Obj), 0.13 NA (Numeric Aperture), using a relay tube lens NFK 2.5x LD of 125 on the MTV-3 tube with a 0.3x lens that produce a total *Projection Magnification onChip* - PMoC of 3x (4*2.5*0.3). Exact magnification was checked with a calibration slide of 1 mm in 10μm marks from Graticules LTD, England.

Each region of interest (ROI) was scanned with a 0.46 NA SPlan 20x objective at a PMoC of 15x (20*2.5*0.3).

2.2.2 Diagnostic Image Quality

The *Overall Magnification* (OM) was the product of the lenses (Obj *Oc) and the distance (D) over which the image is projected. Human eye is only capable to discriminate ¼ mm (M= D* Obj*Oc/250 mm). According to the Abbe rules the magnification capable to enlarge an object from ¼ μm to ¼ mm to be seen by human vision is 1000x. Above 1000* NA no further detail are shown and therefore is called an empty magnification (http://www.microscopyu.com/ articles/formulas/formulasmagrange.html).

The following parameters were evaluated on digital images:

- *Digital Resolution* (DR) or sensor effective pixel size (Epx) divided by the total PMoC. Epx μm / PMoC. Equivalent to sampling density.
- *Visual Magnification* (VM) through a 10x wide-field ocular (Oc) in a standardized projection of 250 mm distance for 20/20 eyes. VM was 40 times for FOV scan objective and 200 times for the ROI objective.
- Total *Screen Magnification* (SM) or relationship between screen and CCD pixel size Spx/CCDpx. In 1:1 zoom images SM was almost 100 times (264μm/2.7μm = 97.77). Digital zoom-in and zoom-out magnification factors depend of the JPEG2000 tile format. In these cases SM was previously calculated using a micrometric standardized slide (see diagnostic assessment below) and overlaid on the screen.
- *Useful Magnification* (UM) ranged from 500*NA up to 1000*NA.

2.3 Diagnostic Assessment

On screen observation for diagnosis was standardized in an FTP monitor of 17" with 1280 * 1024 px, 32 bpp at 60 Hz. For comparison purposes a micrometric rule was built using the calibration slide mention above.

Visual diagnostic assessment was carried out with 15 cases taken at random: 3 surgical pathology slides and 12 cytology.

In TEXCAN-II®-suite, the ROI appeared as color overlay on the FOV: red square whether selected at random and green square whether selected by technicians whose name is annotated. The clinical information can also be annotated (see **Figure 2A**).

The SSVS were accessed at distance though the TEXCAN-II®-server using a JPEG2000 transmission protocol (JPIP) (Taubman, 2003; Krishnan, 2006) based on Kakadu 5.2 library (http://www.kakadusoftware.com). Browsing was done with the TEXCAN-II®-viewer also based on Kakadu (see the viewer on **Figures 2-3**).

3 RESULTS

3.1 DR of the System

Optical Resolution (OR) according numeric aperture and objective correction based on the Rayleigh criterion of the diffraction limit ($\Delta x = 0.61 \lambda / n^* \sin \theta$) as taken from http://www.microscopyu.com/articles/formulas/formulasresolution.html was 2.12 μm for FOV and 0.55 μm for ROI images.

Scanning density or pixel density was 3272*2469 in a of 8.8 * 6.6 mm^2 chip area that contain 2.7 * 2.7 μm^2 pixels (px).

Effective pixel (Epx) size

- In RGB-24 bit images colour demosaicing was carried out with three linear bilinear interpolations of 8:1 Bayer color pattern 4G-2R-2B taking 3x3 surrounding pixels, before storage. The result was that although pixel density is maintained, the information was integrated in an 8.1 * 8.1 μm^2 area and the Epx was 8.1 µm.
- In RAW-16 bit format (black and white-BW), images were displayed on screen in colour previous demosaicing. For that purpose the colour integration algorithm used was a B, R, G1+G2/2 taking the 2x2 surrounding colour pixels in a 4:1 Bayer color pattern 2G-R-B. The result was that for visual perception pixel density was maintained but information was integrated in a 5.4 * 5.4 μm^2 area maintaining original data for processing purposes. The resulting Epx size of the RAW colour image was 5.4µm.

3.1.1 FOV Digital Resolution (4x)

RGB-images after demosaicing had a DR= 2.7 (Epx/PMoC= 8.1/3) or around 3µm/px. Since optical resolution was 2.12µm, sampling frequency was one third of the optimal (2.12µm /2.4px ≈ 1µm /px) in Shannon theory.

RAW images had DR=0.9 (Epx/PMoC= 2.7/3) or around 1µm/px, that fulfilled the Shannon theory. After demosaicing to be displayed on the screen in colour, RAW-images had a DR=1.8 (Epx/ PMoC = 5.4/3) or around 2µm/px, which is half of the optimal. (see **Table I**).

3.1.2 ROI Digital Resolution (20X)

RGB-images had a DR=0.54 (Epx/PMoC= 8.1/15) or around 1µm/2px. Since optical resolution at this magnification is 0.55 µm, the sampling frequency was half of the optimal (0.55 µm/ 2.4px ≈ 1µm /4px), in Shannon theory (see **Table 1**).

RAW images had a DR=0.18 (Epx/PMoC= 2.7/15) or around 1µm/5px. The specimen is therefore oversampled according the Shannon theory. After demosaicing, to be displayed on screen in colour, RAW-images had a DR=0.36 (Epx/PMoC = 5.4/15) or around 1µm/3px, close to Shannon theory. (**Table 1**)

3.1.3 Super-resolution Algorithm

The best cost-computation algorithm to reduce image noise was applied to all images. This was a 16 times image averaging that improved signal-to-noise ratio (S/N or SNR) by N/sqrt(N) a factor of 4, which is 12.04 dB. Furthermore, as mention in material and methods, demosaicing for *onScreen* colour display further improve SNR avoiding aliasing.

Table 1: Digital Resolution. Comparison of demosaiced-RGB 8:1 images, with RAW demosaiced images displayed in 4:1color pattern.

ST= Sampling density according to Shannon theory.
40x is tested but not used in the SSVS.

OBJ.	PMoC	OR µm	ST µm/px	RGB-demosaic µm/px	RAW µm/px	RAW-demosaic µm/px
4x	3x	2.12	1	3	1	2
20x	15x	0.55	1/4	1/2	1/5	1/3
40x*	30x	0.29	1/8	1/4	1/10	1/5

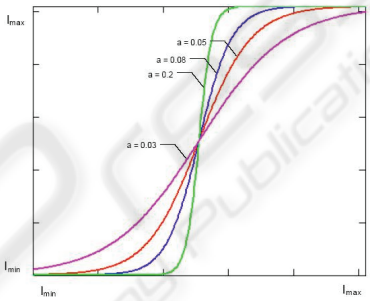

Figure 1: Non linear ACE or adaptive contrast enhancement curve. Acting as an inverse normalized optical modulation transfer function (MTF, see http://www.microscopyu.com/articles/optics/mtfintro.html) correcting optical coherence factor (OCF) or relationship between NA of detector (CCD) and the objective γ= NAccd/NAobj.

In RAW images, after averaging, we further improved adding 6 % in the three channels (RGB) to compensate contrast reduction to build roughly a LRGB image (Luminance RGB image). This was followed by an adaptive contrast enhancement (ACE) 16x16 mask filter to correct low contrast, exponentially adjusted by a factor of 125 in a sigmoid curve. (**Figure 1**).

3.1.4 SM for Diagnostic Purposes

SSVSs size depended on the number of FOV contained. Software zoom-in and zoom-out was limited by JPEG-2000 compression structure.

Screen Magnification-SM was related to image zoom ranging from 9 to 2933 times. Lower implemented SM was 3 times CCD vision (see **table 2**), although JPEG2000 algorithm supports smaller fingerprints. Similarly, higher SM could be displayed, but the result will be an empty magnification.

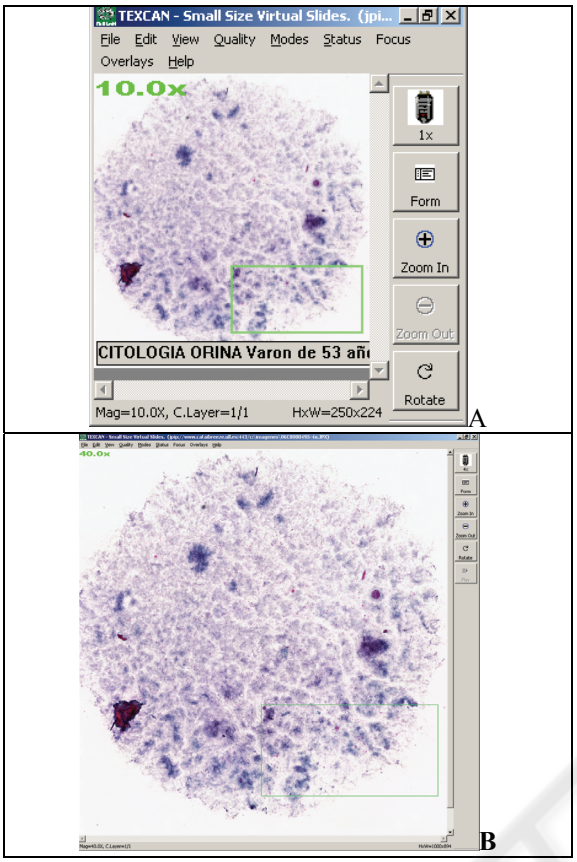

Figure 2: Cytology specimen. SSVS-RGB.**A**. FOV *onScreen*. Zoom-out 10x. The green square indicates a ROI selected by a cytotechnologist. Screen magnification – SM is seen on the upper left corner.**B** FOV *onScreen*. Zoom-out SM 40x.

System: OR= 0.55µm OR and DR= 1 µm /2px. Table 2 compared analogue and digital magnification. Two analogue magnifications were analyzed: Visual and Useful magnification-UM by analogue projection. Two digital magnifications were analyzed: *onChip* and *onScreen* depending on image zooming. Maximum zoom-in was one step before pixel-block (often referred to as pixelation) appeared on the image.

3.1.5 FOV onScreen Magnification 4x

The SSVS can be seen almost from the original magnification (9x), to the maximum image display according to FOV sampling (294x) up to 587x by software zoom-in. Further zoom showed pixelation. See images in **Fig. 2 & 3** AB.

The onScreen magnification-SM when compared with maximum Useful Magnification-UM of analog images (1000*NA= 1000*0.13) was 2 times higher on the original digital 1:1 images (294/130=2.3) and

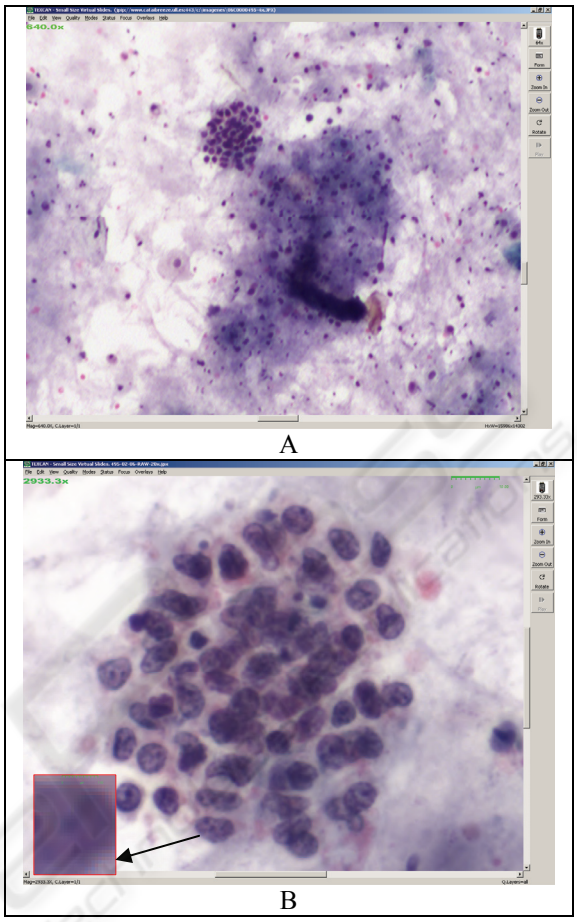

Figure 3: Cytology specimen.SSVS**A**. RGB-FOV *onScreen* Zoom-in SM 640x. System: OR=2.12 µm and DR=3 µm /px.**B**. RAW-ROI *onScreen*. SM 2933x. On the left corner SM 8799x showing the pixelated nuclear details of the empty magnification. System: OR=0.55µm and DR=1 µm /3px. The rule on the top right is 10 µm.

Table 2: Analog Visual & Useful magnification (in grey) versus digital *onChip* & *onScreen* magnification (in white) RAW demosaicing images. VM=Visual magnification; UM= Useful magnification range.

Obj	**VM**	**Low UM** 500xNA	**High UM** 1000xNA
4 x	40x	65x	130x
20x	200x	250x	460x
CCD PMoC	**Zoom-out** (fingerprint)	**1:1**	**Zoom-in**
3x	9x	294x	587x
15x	46x	1467x	2933x

4.5 times higher at the maximum digital zoom-in (587/130= 4.5).

The onScreen RGB images built with half sampling density then required improved by 4.5 times the UM to enter in the so called empty magnification.

In **Figure 2 & 3** A-B we can analyze several zoom-in and zoom-out SM.

4 DISCUSSION

The present paper demonstrates how digital pathology behave as a computer assisted microscopy, because helps to detect details that escape to human eye in the so-called type I low aperture (NA<0.5) widefield incoherent light systems. In other words hybrid systems (in which optical and digital modules are part of the same system) could improve resolution and specifically the CCD systems improve system resolution by 2 (Torok, 2007).

In the presented system, all improvements were low cost computation algorithms: (1) noise reduction increasing depth of field by 16 image averaging, (2) attenuated frequencies were amplified with LRGB images that correct the limited light gathering (proportional to NA^2) of low-NA lenses, and (3) phase recovery improving modulated transfer function with an adaptive contrast enhancement. The displayed RAW-color demosaicing images reached the superresolution level (Nugent, 2003) (Lipson, 2003) even without an optimal digital resolution.

Projection magnification onChip (PMoC) is essential to evaluate the system sampling capabilities and Digital Resolution-DR influence visibility and digital image quality with or without computer assisted techniques.

OnScreen differences for RAW and RGB images were due to higher DR and contrast enhancement with light gathering provided by superresolution algorithms on the RAW images.

Capture is furthermore influenced by Chip quality. Most photographic cameras have 5-9 µm pixel size and big size chips. Microscopy requires smaller chips to avoid aliasing (Koren, 2000-2009) and therefore smaller pixel size; this provides more noise and less sensitivity increasing the cost. This is the reason why high resolution cameras with high SNR are require in microscopic imaging.

Nowadays the public consume CCDs and CMOS chips for imaging are improving. Being the CMOS more noisy but cheaper solutions. There are ultimate regeneration of mobile phones that contain a 12 Megapixel cameras and therefore provide high digital resolution due to the high sampling. Those hand-held solutions including the 4M microscopes only require to be considered in pathology appropriate objective lenses and illumination system preferable base on leads (Ferrer-Roca, 2005).

One of the main drawbacks for distant diagnosis in pathology (telepathology) is sampling error because the essential part of the specimen is not seen because it was not completely digitized. The solution to this is to digitized the whole specimen building a Virtual Slide. The usual VS technique captures images using 40x objectives because optical resolution is adequate (0.29 µm); the result is a huge image (around 10 GB) difficult to handle, that require time consuming compression techniques on which we cannot control lost information and that is difficult to store in the hospital information systems based on DICOM, because the limit image size is 2 GB (Dicom, 2007).

The technique presented here not only provide small images but zoom-in and zoom-out capabilities never explore by pathologist (Ferrer-Roca, 2005)(Marcano, 2007; 2006). As shown in the paper the image showed a super-resolution level to which the oversampling and the super-resolution algorithm applied played a role.

The paper demonstrated the methods to evaluate image quality on computer assisted microscopes displaying digital images. The analysis was focused on resolution and visual magnification in order to be able to apply it to various capture systems for distant diagnosis (4M, mobile phones…).

In summary: The SSVS technique implemented in the TEXCAN-II™ demonstrated that image diagnostic capabilities are higher than analogical image seen in the microscope because they are capable to produce intermediate and high power microscopic magnification entering in the empty magnification showing super-resolution details. The technique of specimen navigation and ROI detection simplify and facilitate diagnosis at distance and prepare the era of the hand-held microscopes based on 4M or integrated into the mobile phones.

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