



COMPUTER ASSISTED MICROSCOPY.

The era of Small Size slides and 4M microscopes



- ★ Prof.Dr.O.Ferrer-Roca et al. 2010
at the BIOSTEC 2010.
Valencia.Spain

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The situation SSVS & 4M

★ SSVS technique

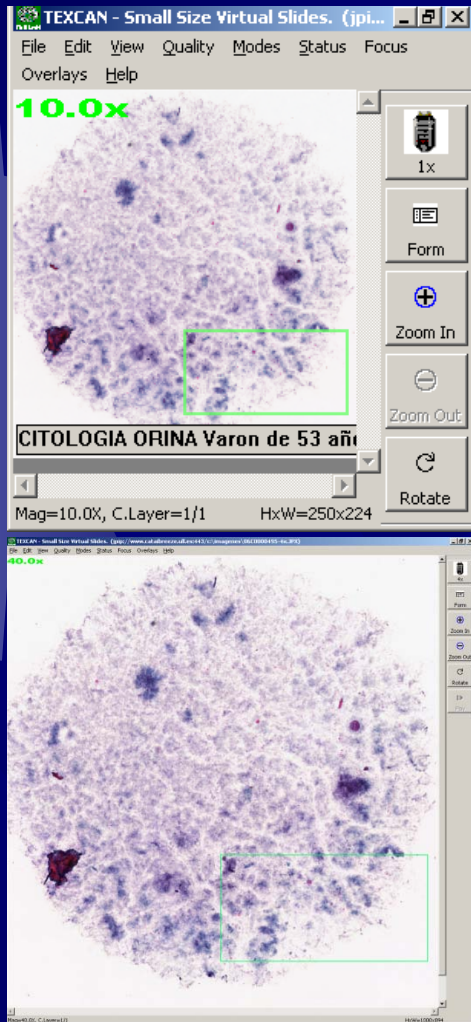
Slide digitized at low microscopic power (4x).

Digitization carried out with a high resolution camera at the super-optical resolution

Stored in JPEG2000 format maintaining diagnostic quality of original slides.

In SSVS the zoom-in and the zoom-out is handled by software. ZF-Zoom Focus (©-Ferrer-Roca) technique.

★ Due to chip miniaturization microscopes can be built on hand-held devices. This is the case of the 4M or Multi-Modal Miniature Microscopes of less than two centimetres.





Material & Methods



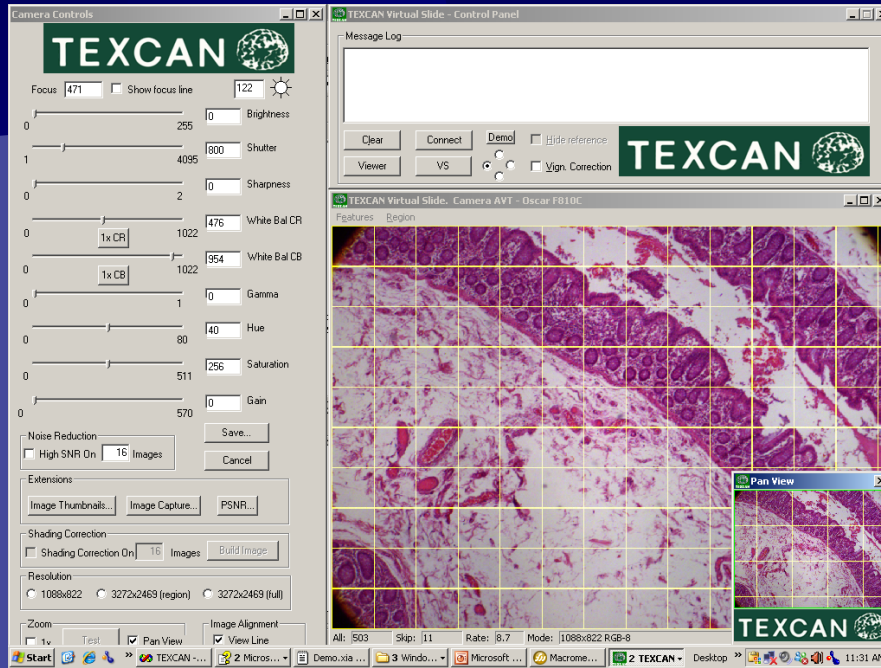
- ★ Olympus BH-2 microscope
- ★ High resolution CCD camera.

It was an 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 monochip was a color mosaic (R-G x G-B) with 2x2 pixel sensitivity. Signal to noise ratio (SNR) was 36,19dB.



PARAMETERS-I



★ **Projection magnification onChip (PMoC)**

★ **Field of view (FOV)** → 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

★ → Projection magnification onChip - **PMoC of 3x** ($4 \times 2.5 \times 0.3$).



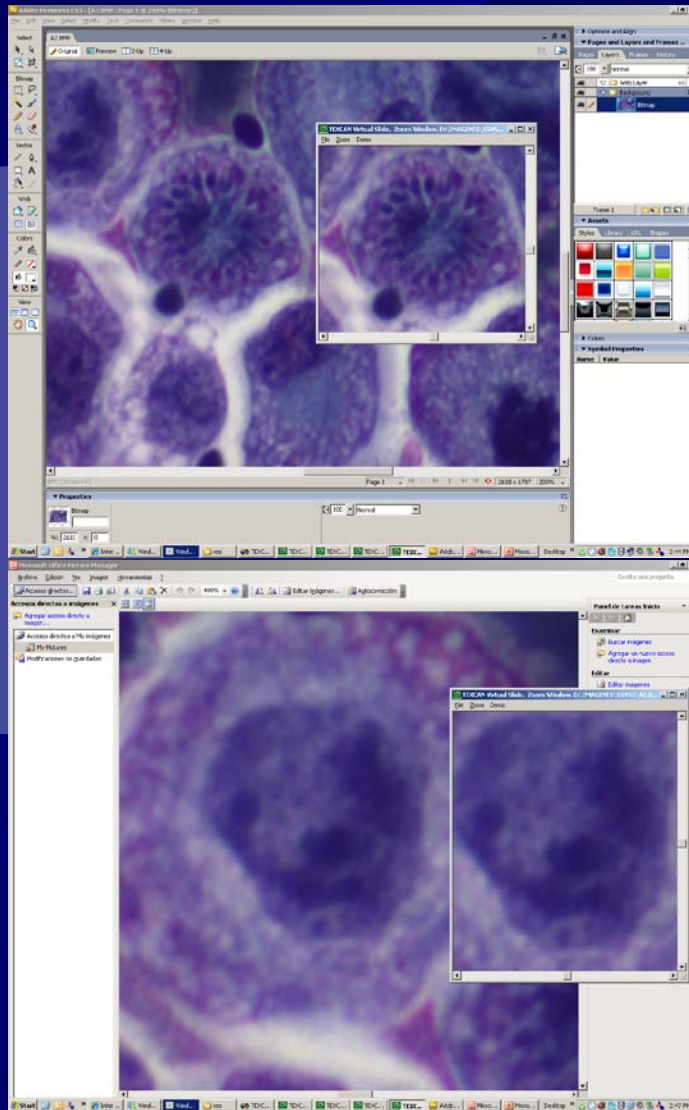
★ **Region of interest (ROI)** was scanned with a 0.46 NA SPlan 20x objective

★ → at a **PMoC of 15x** ($20 \times 2.5 \times 0.3$).





PARAMETERS-II



- **Overall Magnification (OM)** lenses ($Obj * Oc$) and the distance (D) over which the image is projected.

Digital images

- **The Digital resolution (DR)** effective pixel size (Epx) of the sensor divided by the total projection magnification onChip. $Epx \mu m / PMoC$. =sampling density.

- **Visual magnification (VM)** through a 10x wide field ocular (Oc) in a standardized projection of 250 mm minimum distance for 20/20 eyes, considering the $\frac{1}{4}$ mm eye resolution.

FOV scan objective = 40x , ROIs objective= 200x.

- **Total Screen magnification (SM).**
Relationship between the size of Screen pixel and the CCD pixel= $Spx/CCDpx$
1:1 zoom = $(264\mu m/2.7\mu m = 97.77)$, near 100x.
The digital zoom-in and zoom-out magnification factors depend of the JPEG2000 tile format.

- **Useful magnification (UM)** ranged from $500*NA$ up to $1000*NA$.



RESULTS-1- DR

OBJ.	PMoC	OR μm	ST $\mu\text{m}/\text{px}$	RGB-demosaic $\mu\text{m}/\text{px}$	RAW $\mu\text{m}/\text{px}$	RAW-demosaic $\mu\text{m}/\text{px}$
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
60x*	45x	0.29	1/8	1/5	1/15	1/9
100x*	75x	0.20	1/12	1/10	1/30	1/15

DIGITAL RESOLUTION-DR.

- ☀ Compared : (1) original demosaicing RGB 8:1 images, with (2) black and white original RAW images and (3) demosaicing 4:1color displayed RAW images.
- ☀ **PMoC**= Projection Magnification *onChip*. **OR**= Microscopic Optical Resolution.
- ☀ **ST**= Sampling density according to **Shannon Theory**.

*60x and 100x are not tested in the paper. 40x is tested but not used in SSVS.



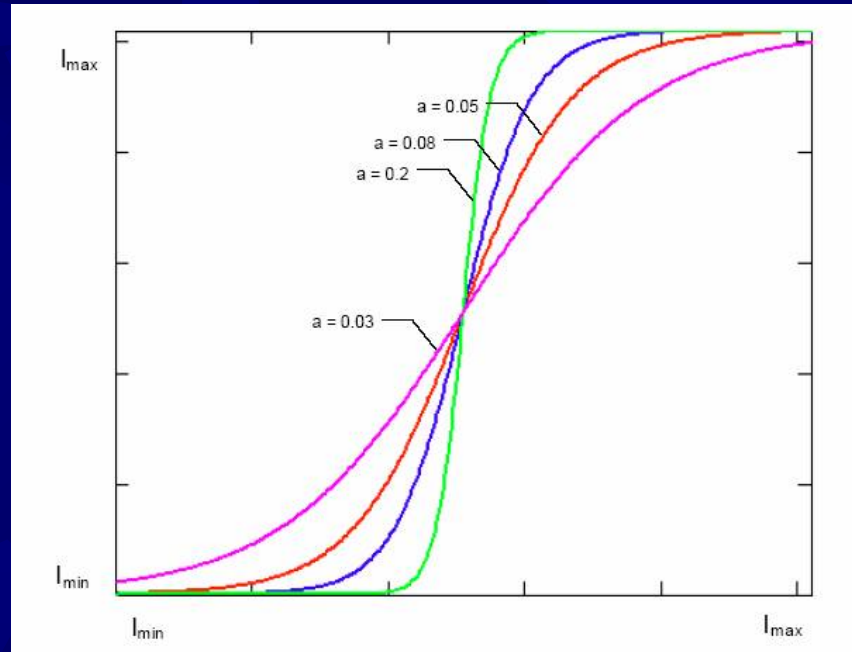
RESULTS-2

Obj(NA)	VM	Low UM 500xNA	High UM 1000xNA	CCD PMoC	Zoom-out (fingerprint)	1:1	Zoom- in
4 x(0.13).	40x	65x	130x	3x	9x	294x	587x
20x(0.46).	200x	250x	460x	15x	46x	1467x	2933x
40x(0.95)*	400x	475x	950x	30x	92x	2933x	5800x
60x(0.95)*	600x	475x	950x	45x	nt	nt	nt
100x(1.4)*	1000x	700x	1400x	75x	nt	nt	nt

- **Analog Visual & Useful magnification (in yellow) versus digital onChip & onScreen magnification (in white) RAW demosaicing images.**
- VM=Visual magnification; UM= Useful magnification range.
- (*)40x included for comparison purposes not used in SSVS. (nt)= not tested.



RESULTS-3 Superresolution algorithm

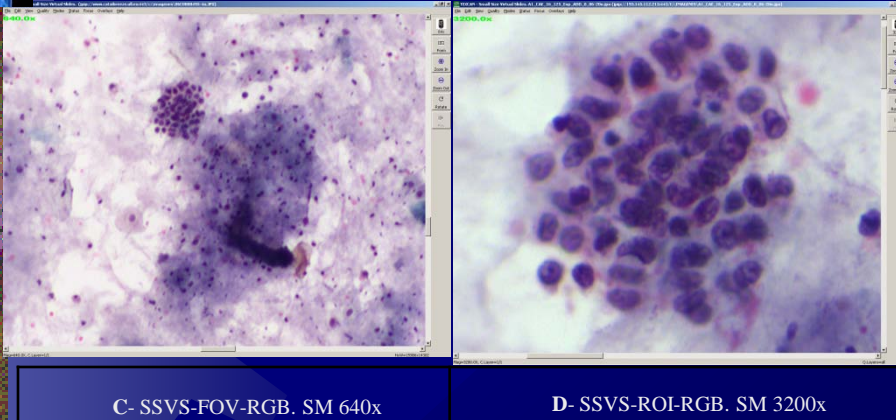


- ★ **Non linear ACE** or adaptive contrast enhancement curve. Acting as an inverse normalized optical modulation transfer function (MTF, see <http://www.microscopyu.com/articles/optics/mtfintro.html>) correcting optical coherence factor (OCF) or relationship between NA of detector (CCD) and the objective $\gamma = NA_{ccd}/NA_{obj}$.
- ★ Light gathering: Comparable L-RGB on **L-RAW** images.
- ★ 16 image averaging.



RESULTS-3

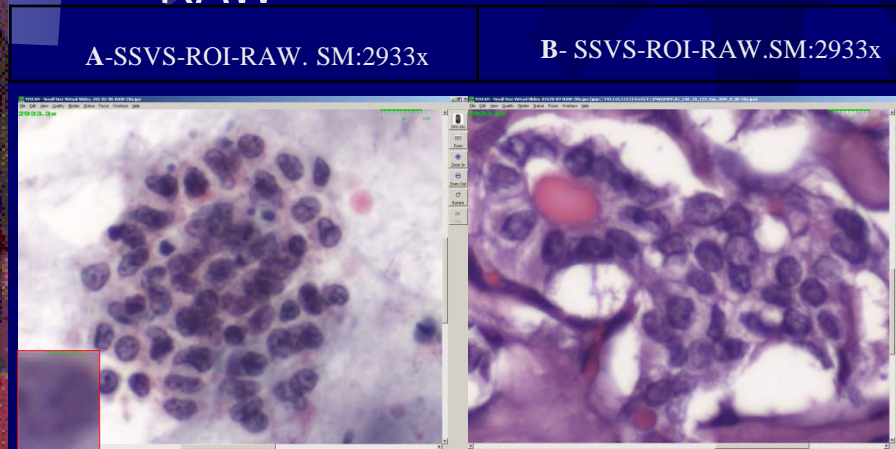
RGB



C- SSVS-FOV-RGB. SM 640x

D- SSVS-ROI-RGB. SM 3200x

RAW



A-SSVS-ROI-RAW. SM:2933x

B- SSVS-ROI-RAW.SM:2933x

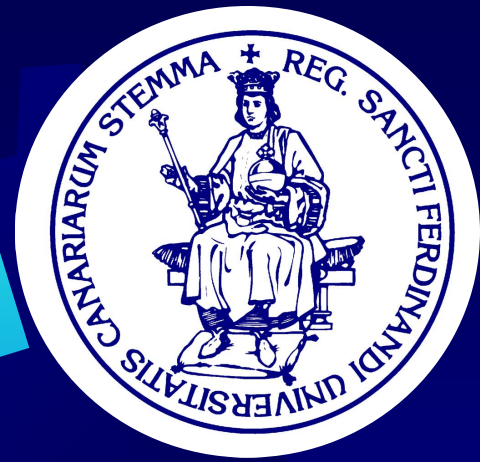
- **Figure 1. C. FOV onScreen** Zoom-in SM 640x. System: OR= $2.12 \mu\text{m}$ and DR= $3 \mu\text{m} / \text{px}$ (one third of the Shannon theory).
- **D. ROI onScreen** . Zoom-in SM 3200x showing a tridimensional papillary structure. System: OR= $0.55 \mu\text{m}$ OR and DR= $1 \mu\text{m} / 2\text{px}$ (half of the Shannon theory).
- **Figure 2. ROI onScreen** SSVS-RAW images. System: OR= $0.55 \mu\text{m}$ and DR= $1 \mu\text{m} / 3\text{px}$ (near to Shannon sampling theory). The rule on the top right is $10 \mu\text{m}$.
- **A- ROI onScreen** . Zoom-in of the cytology of figure 1. SM 2933x. On the left corner SM 8799x showing the pixelated nuclear details of the empty magnification.
- **B. ROI onScreen** . Zoom-in of the surgical specimen of figure 2. SM 2933x.



CONCLUSIONS



1. Projection magnification *onChip (PMoC)* is essential to evaluate the system sampling capabilities, the so-called DR or digital resolution that influenced visibility and digital image quality with or without computer assisted techniques.
2. Differences *OnScreen of RAW /RGB* images were related to **higher DR** and the contrast enhancement & light gathering provided by superresolution algorithm on the RAW images.
3. That *SSVS* are **ideal for hand-held microscopes, 4M** or even **mobile phones** with ad-on capture systems



**THANK YOU
FOR YOUR ATTENTION**

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