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Capturing and displaying microscopic images used in medical diagnostics and forensic science using 4K video resolution – an application in higher education

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Summary

To analyze, interpret and evaluate microscopic images, used in medical diagnostics and forensic science, video images for educational purposes were made with a very high resolution of 4096 × 2160 pixels (4K), which is four times as many pixels as High-Definition Video (1920 × 1080 pixels). The unprecedented high resolution makes it possible to see details that remain invisible to any other video format. The images of the specimens (blood cells, tissue sections, hair, fibre, etc.) are recorded using a 4K video camera which is attached to a light microscope. After processing, this resulted in very sharp and highly detailed images. This material was then used in education for classroom discussion. Spoken explanation by experts in the field of medical diagnostics and forensic science was also added to the high-resolution video images to make it suitable for self-study.

Introduction

Self-produced pictures of microscopic images, for example, blood smears and histological specimens, were used to train students in interpreting these images at Avans University of Applied Sciences in the early 1990s. The pictures served as illustration of this type of specimens to students. With the technical means used at that time, this resulted in a moderate image quality due to the low resolution. Many important details in these images were therefore poorly visible or not visible at all. Learning how to assess these specimens well is an essential part of the training and requires a lot of practice for students. This study examines the usefulness of 4K video technology to capture and display microscopic images.

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4K video is a new video format (Fig. 1), developed in Japan in the early 21st century, in which video can be recorded and displayed with a resolution of 4096 × 2160 pixels (Kanazawa *et al.*, 2003). The name was derived from the number of horizontal pixels, approximately 4000. The benefits of using 4K video shows up best on a very large screen with details needing to be assessed from only a short distance away (Hoorn *et al.*, 2010). The display of these video images remains very sharp, even at short range, where the current resolutions until now fell short.

The degree program at Avans University of Applied Sciences has been extended to the field of forensic trace evidence, where microscopic slides, including hairs, fibres, minerals, etc., are examined and where the perception of details can be crucially important.

Materials and methods

Medical diagnostics

Blood smears (Bauer, 1982) and liver sections were used for the recording of the footage. Fixed blood smears were stained using the May-Grünwald Giemsa staining method (Lee *et al.*, 1998).

Routine liver sections were prepared, fixed and stained with the haematoxylin-eosin staining method (Leong, 1996) and the staining method according to Papanicolaou (Boon & Suurmeijer, 1993).

The specimens were visualized using an upright microscope (Zeiss Axio Scope A1, Carl Zeiss Microscopes & Imaging Solutions, the Netherlands) using a 10×/0.25 (Zeiss N-Achroplan), a 40×/0.65 (Zeiss N-Achroplan) and a 100×/1.3 Oil (Zeiss EC Plan-Neofluar, immersion oil Merck, refractive index n = 1.515 to 1.517) objective.

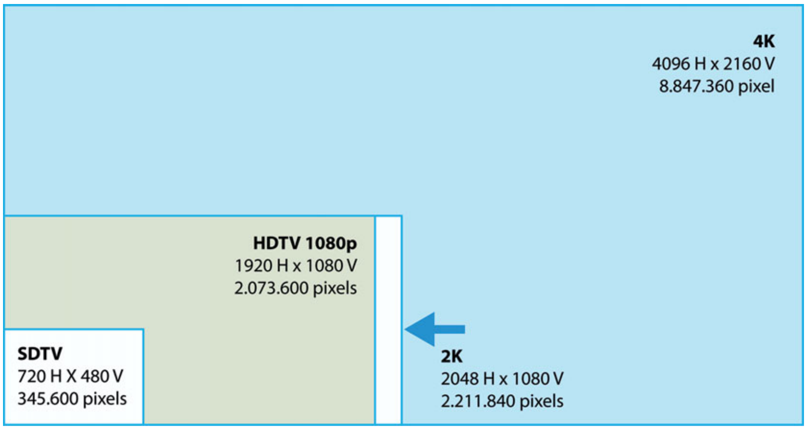

Fig. 1. 4K resolution in comparison to other resolutions.

Forensic trace evidence

Hair specimens of various origins (human, dog, cat, etc.) and fibres and crystals of various kinds were used for the recording of the footage.

These specimens were visualized using an upright microscope (Zeiss Axioskop 40), equipped with a polarization filter. In addition, a 20×/0.1 (Zeiss EC Plan-Neofluar) was used with a 40×/0.75 (Zeiss EC Plan-Neofluar) objective. The specimens were examined using transmitted white light and polarized light.

Before use, the illumination of the microscope needs to be adjusted according to Köhler (1893).

Capturing the microscopic images

Footage was recorded using a RED ONE 4K digital video camera[1] (Red Digital Cinema Camera Company, Irvine, CA, USA) connected to the microscope (Fig. 2). The connection was achieved by means of a T2/T2 camera adapter/optical tube (Zeiss), dimensions: outer diameter × height = 42 × 88 mm. This ensures that the film plane of the RED ONE camera always lies in the image plane of the microscope adapter. The optical tube on one side was provided with a C-mount for connection to the microscope and on the other side it was provided with an F-mount for connection to the camera. For this purpose, the original PL-mount from the camera was replaced by a Nikon F-bayonet (Fig. 3).

The camera was set to a resolution of 4K (4096 × 2304 pixels), a 16:9 aspect ratio and a frame rate of 24 frames per second. The colour temperature was 3200 K and the compression ratio Red code 36. Shutter speed was 1/48 s, with a sensitivity of ISO 800.

Fig. 2. The RED ONE video camera connected to the microscope.

Video postproduction and display

The RED ONE video camera produces video images in RAW (R3D) format. No adaptation took place with these images and therefore they initially appear blurry. The images are subsequently sharpened using an unsharp mask within Adobe Premiere Pro (Adobe Systems Software, Dublin, Ireland). To view these images, a Dell/intoPix 4K playback station (Mont-Saint-Guibert, Belgium) was used. This station applies the file format JPEG2000 (J2K) for playing the video. To achieve this format,

[1] http://www.red.com/products.

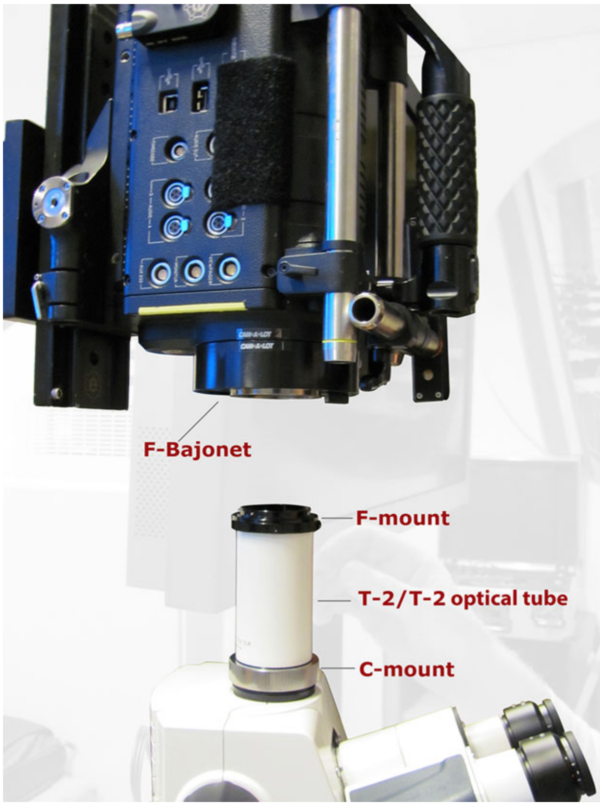

Fig. 3. Optical tube with F-mount and C-mount; the RED ONE video camera with the Nikon F-bayonet.

the R3D files were converted to TIFF format (uncompressed, RGB, colour depth 8 bits per colour) using REDCINE-X software (Red Digital Cinema Camera Company). The TIFF files were converted to J2K format using intoPix Pristine Board software to display the video in full 4K resolution.[2]

The 4K images were displayed using a special 64 inch (diagonal screen) video display EYE-LCD6400-4K (EYEVIS, Reutlingen, Germany), dimensions: 1564 mm × 889 mm × 130 mm, resolution = 4096 × 2160 RGB pixels, active screen area = 1428.5 × 7533 mm, brightness = 500 cd/m2, wide viewing angle = $176^0/176^0$, 1300:1 contrast (Fig. 4). The control of this display was established by Eyevis software for configuring the display. The output of the footage was established via an intoPIX Pristine 4 ultrahigh definition lossless encoder/decoder board (Eyevis, 2009). A corresponding 4 channel 3Gbit/s Serial Digital Interface established an actual link between the 4K display with the intoPIX Pristine Board (Fig. 5).

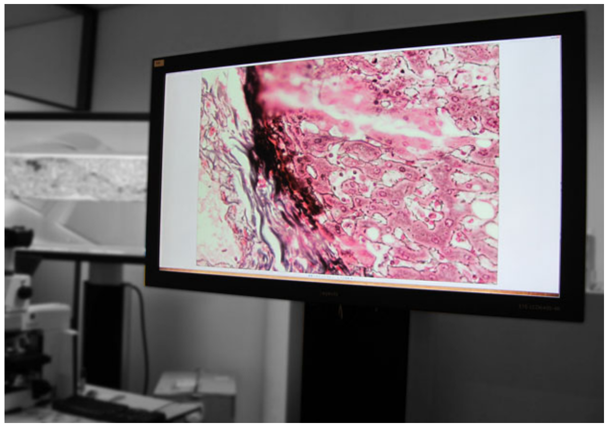

Fig. 4. The 64-inch Eyevis 4K video screen.

Future developments

4K resolution will become the next standard for video and audio. Since August 2012, the official standard is labelled ultrahigh definition which has a minimum resolution of 3840 × 2160 pixels (ITU, 2012). 4K cameras and displays, whether or not in the segment of the more expensive consumer technology, are rapidly cheaper, smaller and easier to use so that they come within reach of a wider audience. In May 2013, the Japanese television network NHK has completed developing an even higher resolution of 7680 × 4320 pixels (8K).

Avans University has buildings that are spread over several locations in three cities. These locations are connected to each other by means of a 10 GB Optical Private Network. Currently, (spring 2014) the possibility is explored to stream 4K video over this network with the JVC GY-HMQ10 4K video camera. This camera delivers a 4K signal, which is presented to the optic network by means of a Modex 4K video interface (True Light, Budapest, Hungary). With this situation a reality, the time-consuming postproduction of 4K images is no longer necessary. Streaming 4K video images via the above connection provides the opportunity for students to follow location-independent education through an expert at a distance, accompanied by ultrahigh-resolution video images.

Results and conclusion

In this project about 4K video applications in higher education, we have investigated the possibility of using this technique for capturing and assessing microscopic slides. The blood smear (Fig. 6)[3] and liver section (Fig. 7)[3] showed very sharp details. Even if the 4K resolution was reduced to High-Definition resolution, it would still be superior to an original high-end HD

[2] Since the 4K technique has become more user-friendly this rather laborious and time-consuming postproduction is not necessary with today's 4K video cameras. Today's transcoding graphics display cards are able to transcode real-time R3D files for real-time 4K playback.

[3] Notice that this image is a print of a 4K video still and gives a poor representation of the 4K resolution. The benefits of 4K resolution can only be seen using a special screen which has a resolution of 4096 × 2160 pixels in combination with a 4K video graphics display card. For the new standard ultrahigh definition, a screen with a minimum of 3840 × 2160 pixels (ITU, 2012) is required.

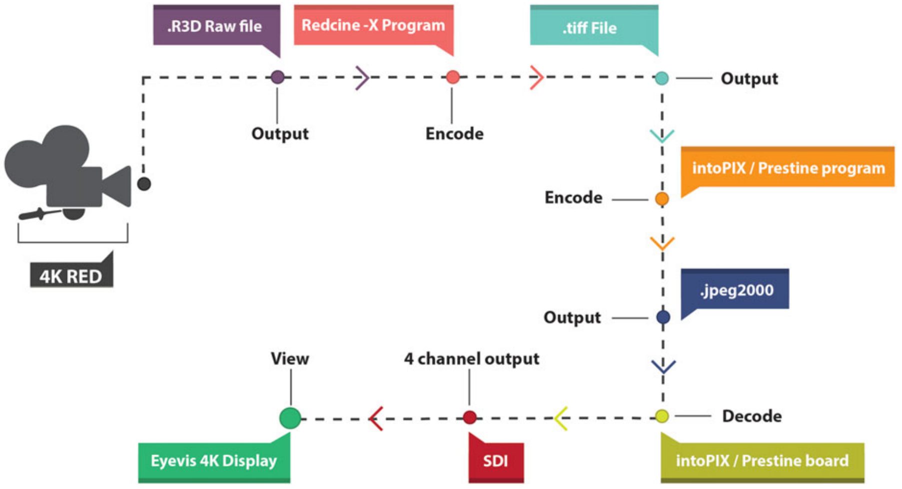

Fig. 5. Flowchart of the video postproduction.

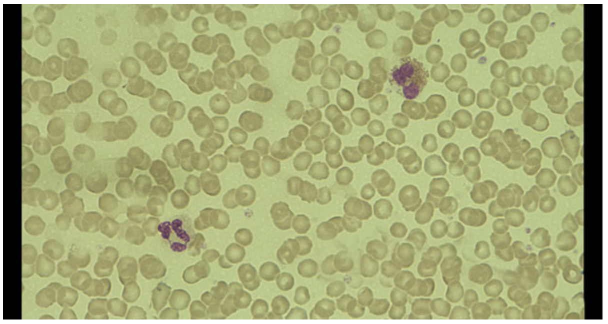

Fig. 6. Blood smear (400×).

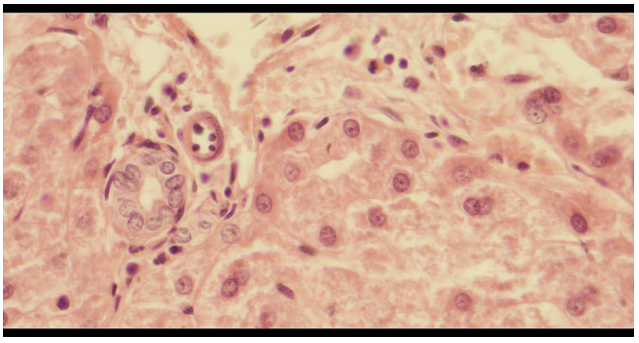

Fig. 7. Liver section (haematoxylin-eosin staining, 100×).

camera recording. This is due to the high quality of the source material, which includes among other things better colour, dynamic range and lower noise threshold in relation to a standard HD recording.[4]

[4] http://www.thedailynote.net/2014/05/why-4k-video-looks-better-on-1080p-monitors.html.

The added value of using *video* to capture these images is apparent from the microscopic examination of blood smears. This usually starts by using a 10× or 20× objective to get an impression of the quantity and quality of the cells. It is then switched to a 100× oil immersion objective to assess the morphology of erythrocytes and for the differentiation of the leukocytes. Here, we look at different parts of the smear, particularly at the edges where the abnormal cells are often located (Heckner *et al.*, 1988). All the 'moving' stages of this procedure are shown with detail and focus by the use of video.

For the forensic trace evidence, recordings of the microscopic images of hair, minerals and fibres were made using both transmitted white light and polarized light and then compared (Kubic & Petraco, 2009). The 4K recordings were, as expected, very sharp and showed a lot of details (Fig. 8),[3] even more than were observed through the microscope.

The added value of using 4K video is mainly in the sharp and detailed image that 4K video provides, even after digital zooming, unlike traditional video recordings. 4K video also provides the ability to go from global to detail without loss of sharpness which is very important in this type of application. 4K video teaching and adjoining learning materials can be used effectively during a class discussion about microscopic slides. After adding expert commentary to the video by the teacher or another expert, it is also useful for students for self-study (Video on Demand).

Due to the very high resolution, this technique provides the ability to capture and display high-quality detailed images of microscopic material. In particular, this 4K video material of microscopic images can be used in an educational context in which analysis, interpretation and evaluation of such images has to be taught as part of professional skills.

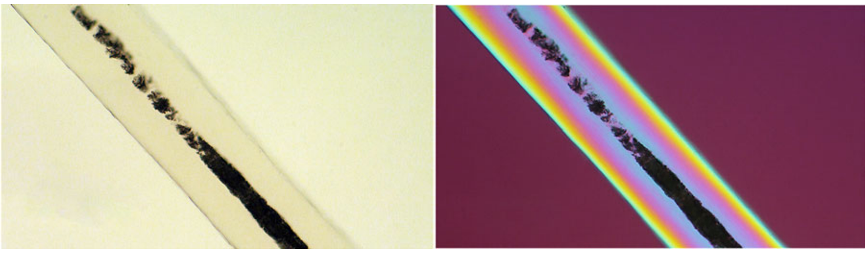

Fig. 8. Female head hair (200×) visualized with transmitted light (left) and polarized light (right).

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