Microscope Magnification

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Note: This article is intended to be read and a part of two USB Microscope review articles also found on this website titled <u>Examining the Able Eye EHEV3-USBUV USB Uv Microscope</u> and <u>Examining the Microlinks Technology Co., Ltd. UM-CAM.</u>

Most microscope users understand how magnification is optically defined and how it applies when comparing various instruments. Higher magnifications permit seeing smaller specimens and usually the higher the better is the mantra among microscope users.

If you study the optics of microscopes you will soon discover that one of the most important optical "numbers" is the numerical aperture, NA, of the system. The higher the NA value (unitless) the better. If the NA value is not specified you are probably not looking at a high quality instrument. You will also discover that the upper NA limit for "normal" high grade microscopes approaches 0.95 (perhaps as exceptionally high as 1.30) for its NA value.

When you magnify without resolution you have what is often called empty magnification. This is very common in the consumer type USB microscope market. You may make a solid square as large as you wish and it will still be a square. It looks larger but it may not be resolved with the same detail as a smaller (less magnified) square using high quality (high NA) optics. To see this you look at the edges of the square to see how "not smooth" they really are. As the NA value goes up the price goes up exponentially. High quality optics is expensive⁽¹⁾.

If you need more than 500 to 1000x you will need to use oil immersion techniques in order to get the resolution you need to actually see the detail at 1000x+. A very nice comparative illustration may be found in the Optical Microscopy Primer NA entry reproduced below⁽²⁾.

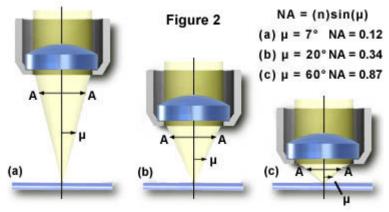


Fig 1 – Examples of NA values found in microscopes.

- (1) Cost often has a labor intensive factor. Grinding lenses and optical testing is a time consuming operation directly related to labor costs. Expertise is another factor. The Internet has brought expertise to everyone so the cost of quality optics keeps coming down, especially for products made in Asia (mostly China).
- (2) The related links to the Optical Microscopy Primer are provided below. Numerical Aperture Light Cones: <u>http://www.micro.magnet.fsu.edu/primer/java/nuaperture/index.html</u> Digital Imaging: <u>http://micro.magnet.fsu.edu/primer/digitalimaging/index.html</u>

Obviously the two low end USB microscopes being reviewed are not to be confused with "high quality." What they do offer, however, is a price that is more affordable so every microscope user may add ease and convenience to their low magnification applications.

Getting used to a low end USB microscope will also serve as an introduction to digital imaging which is nicely described in the Introduction of the second link of footnote A2 above.

"Digitization of a video or electronic image captured through an optical microscope results in a dramatic increase in the ability to enhance features, extract information, or modify the image. When compared to the traditional mechanism of image capture, photomicrography on film, digital imaging and postacquisition processing enables a reversible, essentially noise-free modification of the image as an ordered matrix of integers rather than a series of analog variations in color and intensity. This section addresses a variety of current topics in image acquisition and processing for optical microscopy."

Related to digital image capture is the post image processing. Photo Shop is great, but it is expensive and it has a very steep learning curve. Paint Shop Pro is far less expensive and it is much easier to use. You will also find 50 to 100 free image "processing" programs on the Internet.

In terms of the technology USB microscopes are in the 8 bit 2 - 5 megapixel range. Magnification is given in terms of the screen size divided by the subject size. Most people won't see the empty magnification when they see demonstrations on large LCD screens. In terms of the state of the art 16 bit 54 megapixel USB cameras are available as attachments to standard high quality microscopes.

The excitement of seeing the image on a screen, post processing of the image, and storing it greatly increases the fun of microscopy. If you don't take a photo of what you are looking at, have you really seen it? Setting up a microscope, preparing the specimen, and getting everything just right to see what you want will mean so much more when you may share that "perfect" image with anyone else in the world. Getting started with a low cost USB microscope will spoil you forever in terms of image capture. Start saving your money to "go digital."

An important reality to remember is that magnification IS NOT a factor in determining resolution because only numerical aperture and the wavelength of the illuminating light determine specimen resolution. The images shown in one of the two articles also illustrates that uv lighting improves the image sharpness.

The quality of USB microscopes improves each year. This video camera application is even invading the business world with USB document cameras. These will eventually become common in every office so that the video business conference will soon be supplemented with documents and small subject images included with a second video camera. Some USB microscope cameras also double (usually poorly) as webcams.

Uv Microscope Magnification – "High"⁽³⁾

Using 0.7 in margins for the page, the image in Fig. 2 is 1/2 a column and 3.55 inches wide. Measur-ing the printed document determined that there are 20 squares over 25 mm. One square is 1.25 mm as printed. The actual dimension is 0.10 mm. This makes the magnification of Fig. 2 is 12.5x.

Fig. 3 is the full column version which is printed 7.1 inches wide. Measuring the printed document determines that there are 20 squares over 50 mm. One square is 2.5 mm. The actual dimension is 0.10 mm. This makes the magnification of Fig. 2 equal to 25x. If this image is printed in landscape the magnification would be 33.9x. If I view the image

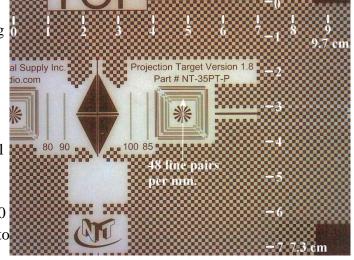


Fig. 2 - Magnification = 12.5x. A square = 0.10 mm.

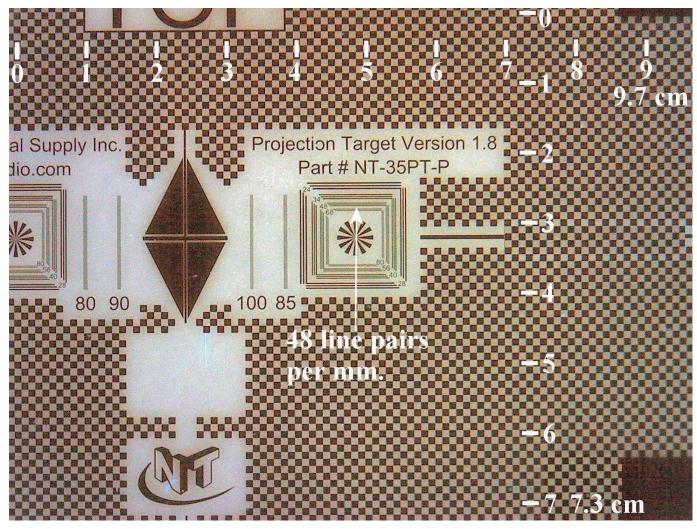


Fig. 3 – The effective magnification as shown is 25x. Each square = 0.10 mm.

⁽³⁾ Holding the microscope on the subject as intended and defined in the article.

on my 19 inch LCD Screen with Word at set at 175% and I measure the screen there are 90 squares in 321.7 mm. One square is 3.57 mm on the screen which is 35.7x - AS VIEWED ON THE LCD SCREEN.

Look at the resolution portion of the test image in Fig. 3. I added a notation pointing out the three lines marked 48 line pairs per millimeter. These are fuzzy. Note that the lines marked 24, 28, 34, & 40 are fairly sharp. We may conclude that the image resolving ability of both USB microscopes reviewed is between 40 and 48 line pairs per millimeter. Also note that there are additional resolution lines to the left (vertical, on each side of the black solid diamond) marked 80, 90, 100, & 95.

The resolution pattern was cut and placed on the 3/8" stage (for use with the uv microscope) and photographed, see Fig. 4, at the highest magnification using uv lighting. Did the resolution change? Can you discern the "48" lines? Fig. 4 is 59% larger than the same area shown in Fig. 3. Even a slight edge of using 375 nm blue light didn't help.

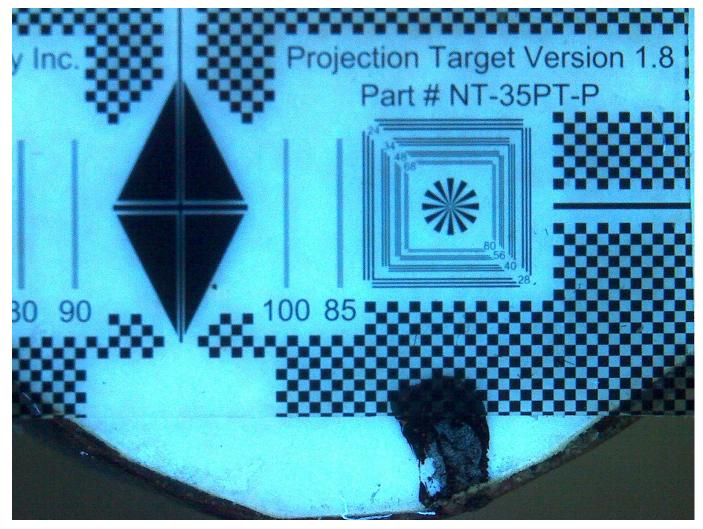


Fig. 4 – Uv image capture at extra high magnification. The image is even larger than that of Fig. 3.

If I look at the same film with my Nikon Stereo Microscope at 20x the three "48" lines are clearly seen and very sharp. All lines (up to the 100 line pairs per mm) are clearly discernable at 40x. The USB

microscope magnification images for Fig. 2 - Fig. 4 are obviously of low quality. Is the Fig. 2 to Fig. 4 increased size (increased magnification?) an example of empty magnification - YES!

Below is a table – provided for perspective only - of video resolution comparisons based on NTSC video standards. A USB microscope uses a video camera.

Correlating the USB digital image with the scan lines (not the same as line pairs) is difficult. The practical way to compare systems is to use an appropriate test chart/standard slide/target (as shown in Figs 2 - 4), and is the easiest way to quantify/compare visual quality (resolution). The golden rule for optics is that quality (resolution) is better than quantity (magnification).

Table A1 – NTSC Video Quality of Commonly Used Equipment

- 1. 350×240 (250 lines): Video CD, good security cameras.
- 2. 330×480 (250 lines): Umatic, Betamax, VHS, Video8
- 3. 400×480 (300 lines): Super Betamax, Betacam (professional)
- 4. 440×480 (330 lines): analog broadcast
- 5. 560×480 (420 lines): LaserDisc, Super VHS, Hi8
- 6. 670×480 (500 lines): Enhanced Definition Betamax
- 7. 720×480 (500 lines): DVD, miniDV, Digital8, Digital Betacam (professional)
- 8. 720×480 (400 lines): Widescreen DVD (anamorphic)
- 9. 1280×720 (720 lines): D-VHS, HD DVD, Blu-ray, HDV (miniDV)
- 10. 1920×1080 (1080 lines): D-VHS, HD DVD, Blu-ray, HDCAM SR (professional)

If you would like two frames of the 35 mm test film send me a self-addressed stamped envelope and I will send them to you.

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