Resolution Considerations for Photomacrography and Photomicroscopy

Theodore M. Clarke, Case Corporation

My Inter/Micro-84 presentation and subsequent article in *The Microscope* dealt with optimizing diffraction limited depth of field in photomacrography and is referenced by Brian Bracegirdle in his "Scientific Photomacrography.[1,2] My analysis was based upon the classical solution for the diffraction pattern image of a point light source, using the Rayleigh criterion of resolution. H. Lou Gibson's method of treating combined diffraction and geometric blurring away from the object focal plane was used.[3] The calculations and the experimental results indicated that a final print resolution, for the object focal plane, of 7 lines/mm (.29 mm Airy disk) gives an optimum balance of depth of field and resolution. The 7 lines/mm criterion is equivalent to an Abbe criterion of total magnification equal to 440 times the numerical aperture. The 500 times numerical aperture Abbe criterion recommended for microscopists with very acute vision corresponds to a maximum resolution of 6 lines/mm in the final image. This resolution is not as good as our Océ 3045 office copier used to duplicate our metallurgical reports with 4x5 Polaroid images. The Océ 3045, when properly adjusted, resolves 8 lines/mm on standard copier paper in photo mode.

BASIC EQUATIONS

1) The relationship between the maximum print resolution, lens f/number setting, and magnification is as follows (assuming $\lambda = 5.5 \times 10^{-4}$ mm):

Max. print resolution = f/number $(M_{camera}M_{enlarging}+M_{enlarging})\ 6.7 \times 10^{-4}$ (in mm). Where the maximum print resolution is equal to one-half the final Airy disk diameter after enlarging.

2) The relationship between the enlarged circle of confusion diameter in the final print, C, and the geometric depth of field is given by the following equation:

$$\text{Depth of field} = \frac{\text{f/number}\,(1+M_{CAMERA})C}{M_{ENLARGING}M_{CAMERA}^{2}}$$

3) The relationship between the numerical aperture (N.A.), f/number, and camera magnification (M_{camera}) is given by the following equation:[4]

$$\text{N.A.} = \frac{(M_{CAMERA})}{2\ \text{F/no.}\ (M_{CAMERA}+1)}$$

4) Lou Gibson's method is used to calculate the final image resolution at the depth of field/limits:

$(\text{Print Resolution})^2 = (\text{Max. Print Resolution})^2 + C^2$ at depth of field limits. Using equations 1-4, the following Table 1 of depth of field and resolution is derived for full f/stop increments:

TABLE 1

Max Resolution Final Image	Abbe Criterion	f/No.	Depth of Field mm for 8 lines/mm	Depth of Field mm for 6 lines/mm	Depth of Field mm for 3 lines/mm
9 lines/mm 0.11 mm (0.22 mm Airy Disk)	320 N.A.	$160/(M_{Tot}+M_{enl})$	$38/M_{Tot}^{2}$	$85/M_{Tot}^{2}$	$200/M_{Tot}^{2}$
7 lines/mm 0.15 mm (0.29 mm Airy Disk)	440 N.A.	$220/(M_{Tot}+M_{enl})$	--	$70/M_{Tot}^{2}$	$260/M_{Tot}^{2}$
4.5 lines/mm (0.44 mm Airy Disk)	660 N.A.	$330/(M_{Tot}+M_{enl})$	--	--	$330/M_{Tot}^{2}$

Discussion of Optimum Aperture Concept

H. Lou Gibson, at first, strongly objected to the results given in Table 1. His previous analysis included all sources of image blur, including recording and enlarging losses. His main objection was my finding that, for a given maximum final image resolution for the object focal plane, the depth of field was inversely proportional to the square of the final magnification. He had concluded that the greatest depth of field could only be obtained with a large format camera and no subsequent enlarging. Our results were in agreement when an enlarging magnification of 1X was used with my f/number and depth of field equations for a maximum final image resolution of 7 lines/mm. Gibson's original conclusion that the depth of field for 6 lines/mm resolution decreased when a significant part of the final magnification was obtained by enlarging was the result of a mathematical reasoning error in Gibson's treatment of ray optics based depth of field, which was correct only for a 1X enlarging magnification.[3] Subsequent correspondence, facilitated by Dr. Walter C. McCrone, led Gibson to acknowledge my work was valid. Gibson's error was subsequently corrected in his 1986 BPA article, as noted in my letter to the editor published in "The Microscope."[6] I have copies of the Gibson correspondence if someone is interested in doing a historical study of photomacrography. Gibson was the pioneer and great contributor to this field. Gibson agreed with me that the image did not "fall apart" until the resolution was less than 3 lines/mm.

A more stringent criterion than 6 lines/mm is used for depth of field in conventional photography, where the circle of confusion should not exceed 0.25 mm (8 lines/mm) within the depth of field.[7] A one f/stop decrease in f/number from that giving 7 lines/mm resolution in Table 1 gives a maximum resolution of 9 lines/mm (0.22 mm Airy disk diameter) and near a maximum depth of field for 8 lines/mm resolution, but at the expense of a significant loss of depth of field for 3 lines/mm print resolution. Comparison of test photomacrographs indicated that this loss of depth of field reaching 3 lines/mm resolution was not justified by the increased maximum resolution from 7 lines/mm to 9 lines/mm. John Delly preferred a 4.5 lines/mm resolution requirement, which gives the greatest depth of field reaching 3 lines/mm. The test images did not appear to be blurred until their resolution was less than 3 lines/mm. These results are consistent with Abbe's criterion that useful magnification ranges between 500 N.A. to 1000 N.A., corresponding to a print resolution between 6 lines/mm and 3 lines/mm. These values of image resolution can be readily calculated from the common definition of light microscope resolution:

$$\text{Object Resolution} = \frac{.61\lambda}{\text{N.A.}}$$

Electronic Imaging

The standard for recording photomicrographs in industrial laboratories is 4x5 Polaroid film. Its maximum resolving power in prints has previously been claimed to be 20 lines/mm, which is ample to faithfully record an optical image achieving 6 lines or line pairs/mm. (A quick test with Polaroid Type 55 P/N and a 1963 NBS Microcopy Resolution chart imaged at 1X yielded a print resolution of 12.5 lines/mm.) The imaging area on a Polaroid 4x5 print is 89 mm by 114 mm, which is the same aspect ratio as a 1024 by 1280 row and column pixel array. Alternating the 1024 rows bright and dark would yield the finest line spacing and greatest number of horizontal lines recordable with this array. If these 512 lines could then be accurately displayed and recorded on 4x5 Polaroid, their spacing would be 5.7 lines (or line pairs)/mm.[8] Our preliminary experiments with a line test pattern and a Kodak 1.6i Megapixel camera indicate a resolution of 5.6 lines/mm can be recorded with the lines at 45° to the X-Y directions. Alias lines are apparent at about 4 lines/mm when the lines are oriented in the X-Y directions. The experiments with the Kodak camera are done using a Nikon 60 mm f2.8 Micro Nikor lens on a copy stand and imaging a Canon office copier resolution test chart, which is a version of the NBS Microcopy Test Chart, in a 3.5 by 4.5 inch field size. I wish a similar pattern covering a range of 200 to 1000 lines/mm was available for the light microscope. A star pattern or modulation transfer function sine bar pattern might be more suitable than a line pattern to avoid image artifacts.

Obviously the light microscopist and photomacrographer would see further improvement in image quality with a 2000x2000 or 2000x3000 pixel CCD camera which would shift the alias line problem to higher frequencies than resolved by the microscope objective, but it would be difficult to justify the added cost with current technology. A best quality 1024x1280 pixel image could be computed for display from the higher resolution camera output. I suspect this is already done with some of the digital SEM's.

I hope that this information proves useful and leads to a greater emphasis on image display resolution. We have learned that it is necessary to include a resolution test pattern with image files to assure that the digital image files are faithfully reproduced on computer monitors and in hard copy output. We have found that the 4x5 Polaroid camera on our JEOL JSM-5800LV makes the best hard copy of TIFF files from the Kodak 1.6i system. ■

1. Clarke, T.M., "Method for Calculating Relative Apertures for Optimizing Diffraction - Limited Depth of Field in Photomacrography:, Microscope 32, 219-258 (1984).
2. Brian Bracegirdle, "Scientific Photomacrography", RMS Microscopy Handbook, No. 31, BIOS Scientific Publishers Ltd., Oxford, U.K.
3. Gibson, H. Lou, "Photomacrography: Mathematical Analses of Magnification and Depth of Detail", Kodak Publication No. N-15.
4. Shillaber, C.P., "Photomicrography in Theory and Practice", John Wiley & Sons, Inc. (1944).
5. Gibson, H. Lou, "Depth and Enlarging Factors in Ultra-Close-up and Photomacrographic Prints and Slides", BPA 54, 127-142 (1986).
6. Clarke, T.M., Letter to the Editor, Microscope 35, 332-336 (1987).
7. Neblette, C.B. & Murray, A.E., "Photographic Lenses", Morgan & Morgan, Inc. (1973).
8. Clarke, T.M., "Light Microscopy Criteria for Electronic Imaging", Microscope 43:3, 121-124 (1995).