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Balancing image quality and compression factor for special stains whole slide images

Anurag Sharma[a,*], Pinky Bautista[b] and Yukako Yagi[b]
[a]*NEC Laboratories America, Inc., Princeton, NJ, USA*
[b]*Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

Abstract. The objective is to find a practical balance between quality and performance for daily high volume whole slide imaging. We evaluated whole slide images created by various scanners at different compression factors to determine the best suitable quality factor (QF) needed for pathological images of special stains.

Method: We scanned two sets of eight special stains slides each at 0.50 μm/pixel resolution in Hamamatsu scanner at six and five QF levels respectively to generate 72 images which were observed at a calibrated monitor by imaging specialists, a histo-technician, and a pathologist to find the most suitable QF level for special stains in digital slides.

Results: Most special stains images were acceptable at QF 30 except for the stain Reticulin where the lowest acceptable QF was 50. The compression of images from QF 90 to QF 50 reduced the size of the images by 62.73%.

Conclusion: 0.50 μm/pixel images at QF 50 or above were found suitable 12 special stain.

keywords: Special stains, image, quality factor, compression, whole slide, image quality, digital pathology

1. Introduction

The quality of the scanned images is an important factor in Whole Slide Imaging (WSI). Poor quality may result in inappropriate diagnosis, inaccurate analysis or can make an image less useful. The large size of these images is one of the major issues in digital pathology. It is difficult to deal with high quality digital images due to their large size. It makes storage space, network bandwidth, analysis time or Internet access expensive. There are some key technologies in WSI to use images and the system effectively with optimized methods. Image compression is one such method.

It is important to use the compression technologies to balance the quality with overhead costs especially in high volume scanning which we define as over 100 slides per day for a variety of purposes. The key is to find a balance between the quality and the compression for several common special stains by human observation and by computing the difference in file size. A previous study [1] found quality factor (QF) of 80 suitable for H&E stain. This study investigates the lowest acceptable QF for special stains.

1.1. Compression

The image compression is measured in QF. An uncompressed image may be said to have a QF of 100. Though there is no universally accepted definition for QF. The QF varies from manufacturer to manufacturer and is generally the only factor user can control while scanning. Compression can reduce the file size of the images. As a side effect, it can reduce some information or alternatively, may add some artifacts. Higher QF provides better quality but adversely affects the resources. Higher QF has higher infrastructure requirements while an image with lower QF may be smaller in size and faster to access but may be of little use.

*Corresponding author: Anurag Sharma, PICT Lab, Massachusetts General Hospital, 101 Merrimac Street, Ste 820, Boston, MA 02114, USA. Tel.: +1 617 643 7913; E-mail: asharma@nec-labs.com.

There are various image formats used by the vendors employing different compression algorithms. JPEG and JPEG 2000 are two common compressions. While JPEG 2000 compression is a little more efficient at higher quality, the quality drops sharply at lower QFs. On the contrary, JPEG compression maintains a more consistent relationship between quality and compression. JPEG 2000 image size may be smaller but it requires more processing power. It is a more flexible solution compared to JPEG but the later one provides less complexity [2]. The Nanozoomer 2.0 HT (Hamamatsu Photonics K.K., Hamamatsu, Japan) scanner utilized in this study uses JPEG compression which compression can produce irregularities such as pixelation, compression artifacts and noise in the image.

1.2. Special stains

Special stains are used to selectively stain cells and cellular components. These stains are used to answer additional questions that arise after interpreting H&E-stained tissue morphology [3]. These stains are economical in comparison to immunohistochemistry. The special stained slides are prepared as needed after the evaluation of the common H&E slides. The basis of interpretation with special stains is color in addition to morphology.

1.3. The objective

We investigated the lowest appropriate compression (value of QF) at which the image quality is good enough for evaluation and analysis in special stained WSI. We also investigated the reduction in image size due to compression.

2. Method

We started with 16 slides in 12 stains: Trichrome, Periodic Acid Schiff, Reticulin, Gomori methenamine silver, Giemsa, Brown-Hopps-Gram, Steiner, Warthin-Starry, Mucicarmine, Elastic, Periodic Acid Schiff-Diastase, and Congo-Red. There were eight special stained slides each with human tissue and mouse embryo. The slides were scanned by a Whole slide scanner Nanozoomer 2.0 HT. These slides were scanned with 0.50 μm/pixel resolution. The first set (human tissue) was scanned at five QF levels: 30, 50, 70, 80 and 90 while the second set (mouse embryo) was scanned at 30, 50, 80 and 90. This experiment used 72 images, 40 for first set and 32 for the second set.

The images were analyzed with a calibrated monitor MDCC 6130 DL (Barco, Kortrij, Belgium) by four people: two imaging specialists, a histo-technician, and a pathologist for various quality factors with an objective to find the most suitable QF level for special stains in digital slides. Since many special stains deal more with color difference, we tried to benchmark the same. Table 1 explains the stains and other information about the slides and equipment used in the study. These images were observed by a pathologist and a histo-technician using a grading method with two values; "acceptable" and "unacceptable".

3. Results

The Reticulin stain images were acceptable at QF value of 50 and above while other special stains were acceptable at 30 and above. The comparison can be seen in Figs. 1–5.

The compression reduced the size of the images. The average reduction in size between QF 90 and QF 30 was found to be 62.73% with a standard deviation of 3.45. Table 2 provides the relationship between the QF

Table 1
(Special stains used in the study)

Tissue	Human	Mouse embryo
Scanner	Nanozoomer	Nanozoomer
Slides	8	8
Special stains	Mucicarmine, Elastic, Periodic Acid Schiff, Periodic Acid Schiff-Diastase, Trichrome, Elastic, Congo Red	Brown-Hopps-Gram, Gomori methenamine silver, Geimsa, Periodic Acid Schiff, Steiner, Trichrome, Worthin-Starry, Reticulin
Resolution	0.50 μm/pixel	0.50 μm/pixel
Quality factor	30, 50, 70, 80, 90	30, 50, 80, 90
Number of images	40	32

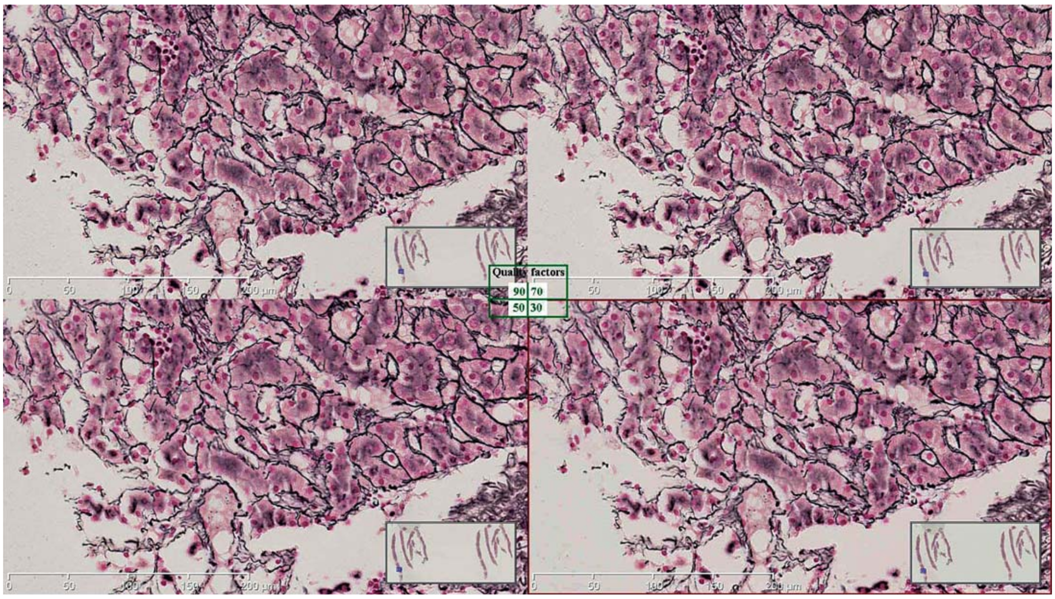


Fig. 1. Four images of the same area on a Reticulin slide at quality factors as 70, 30, 50, 90 (clockwise from top right) look similar.

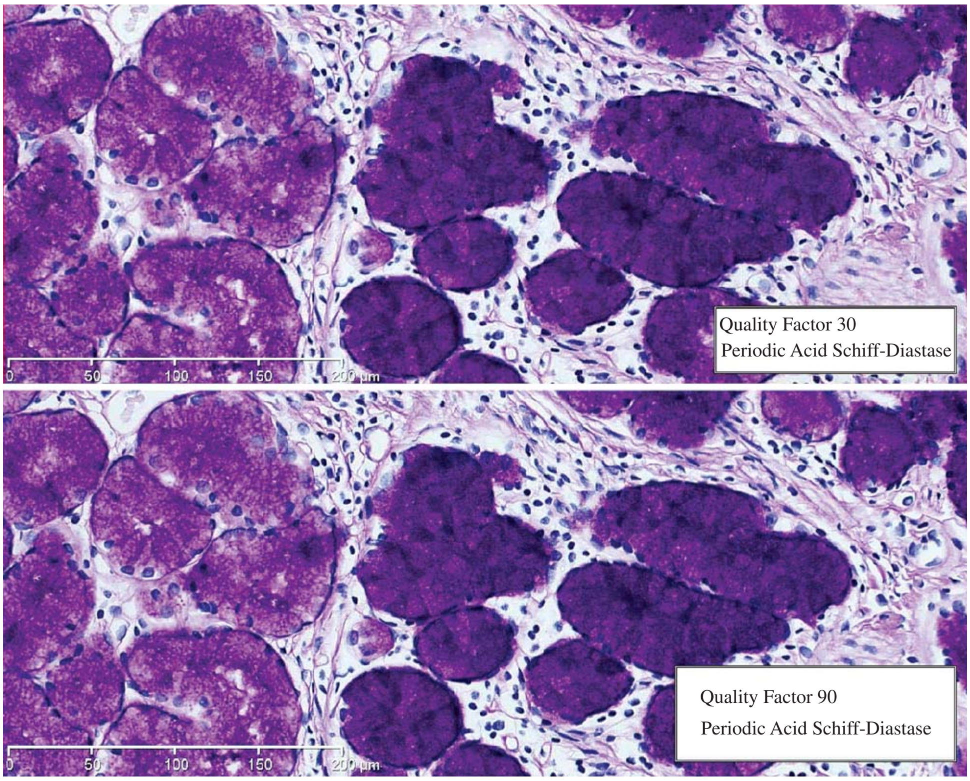


Fig. 2. Periodic Acid Schiff-Diastase slide scanned at 30 and 90 has not lost visual information.

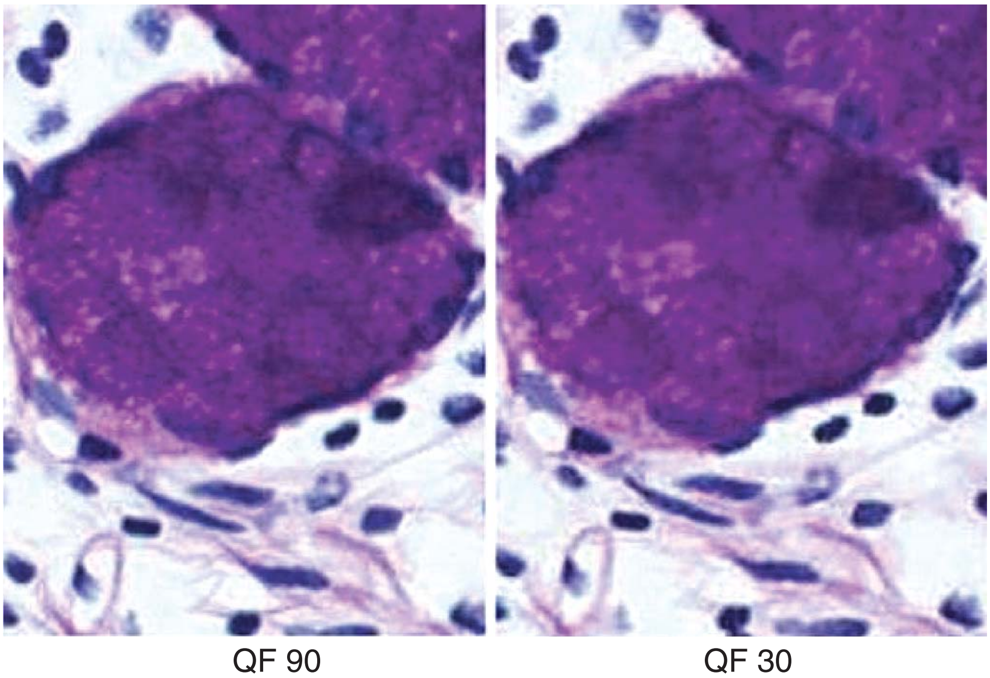


Fig. 3. There is degradation of quality at lower quality but it does not affect diagnosis (periodic Acid Schiff-Diastase stain).

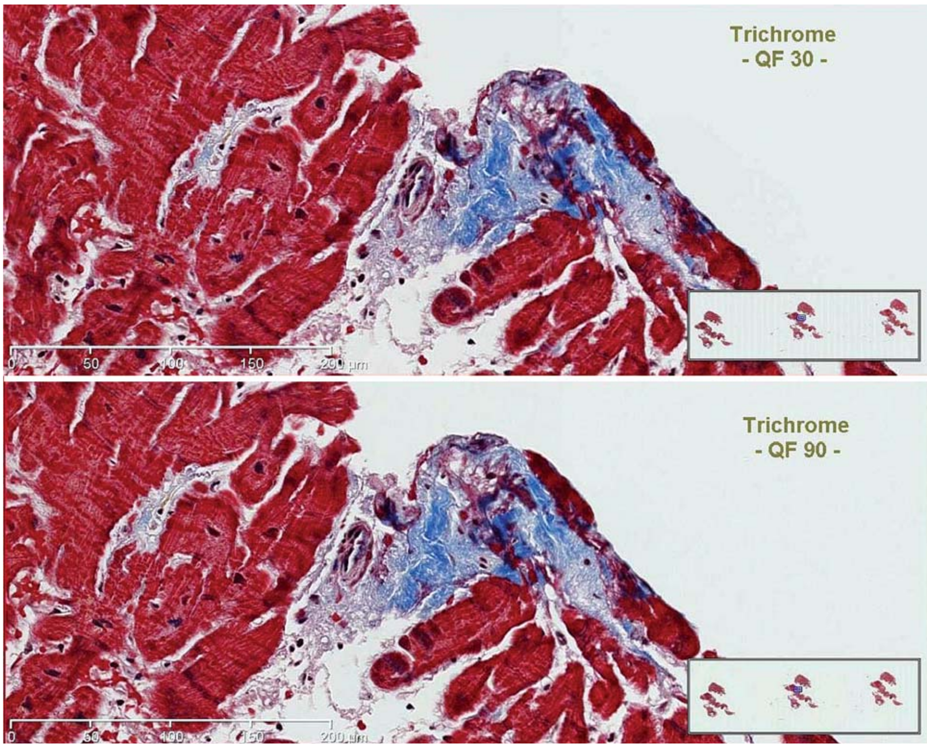
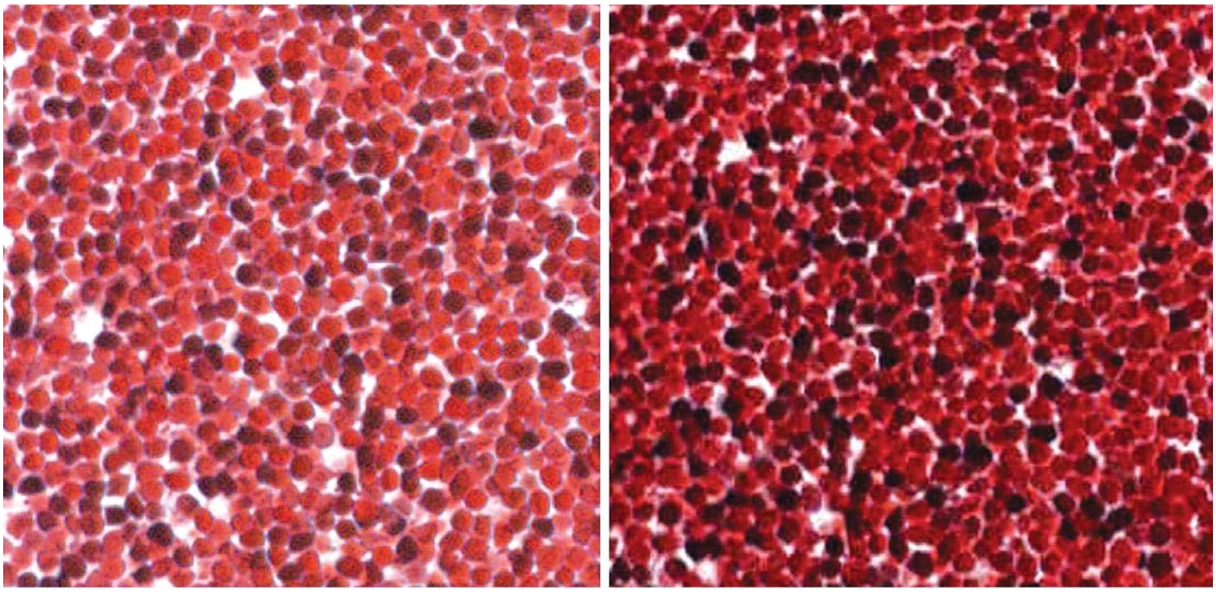


Fig. 4. (Trichrome screen shots at QF 30 and QF 90 display no loss of visual information).



QF 90 compared to QF 30 - mouse embryo (Trichrome)

Fig. 5. (The difference in colors at QF 90 is retained at QF 30).

Table 2
(The relationship between QF and file size) (The file sizes are in megabytes)

Slide	**QF 90**	QF 80	QF 70	**QF 50**	QF 30	Difference in size between QF 90 and QF 50	% reduction in image size
Set 1							
1	**357.00**	163.00	133.40	**97.00**	72.80	223.60	62.63
2	**233.70**	107.00	84.30	**76.50**	51.70	149.40	63.93
3	**226.20**	110.90	91.00	**72.40**	64.80	135.20	59.77
4	**671.80**	370.10	299.00	**219.50**	158.50	372.80	55.49
5	**185.00**	88.00	66.00	**64.20**	39.80	119.00	64.32
6	**97.00**	54.00	40.10	**39.10**	21.70	56.90	58.66
7	**389.00**	139.00	135.80	**106.60**	19.30	253.20	65.09
8	**108.50**	52.00	46.70	**37.80**	21.30	61.80	56.96
Set 2							
1	**672.19**	383.38		**227.77**	177.43	444.42	66.12
2	**610.46**	393.45		**223.51**	170.28	386.95	63.39
3	**558.18**	337.73		**192.33**	163.41	365.85	65.54
4	**676.48**	419.91		**252.15**	274.73	424.33	62.73
5	**779.06**	514.02		**268.28**	204.02	510.78	65.56
6	**533.64**	316.3		**203.84**	156.14	329.80	61.80
7	**709.37**	401.23		**226.25**	194.16	483.12	68.11
8	**661.93**	397.79		**240.89**	174.82	421.04	63.61
Average percentage reduction in image size between QF 90 and QF 50							**62.73**
Standard deviation in % reduction in image size between QF 90 and QF 50							3.45

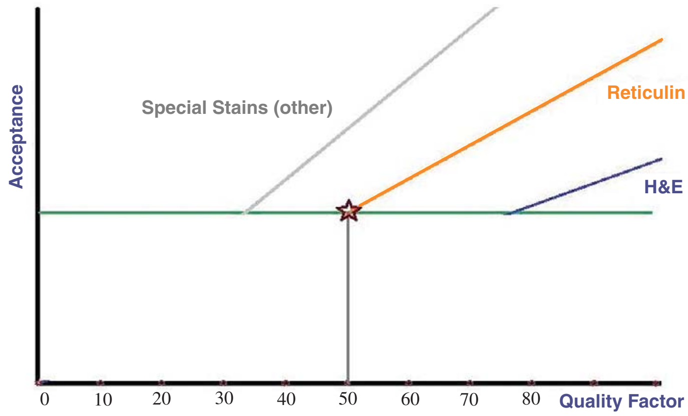


Fig. 6. (Acceptable QF Values for HE, Reticulin and other special stains).

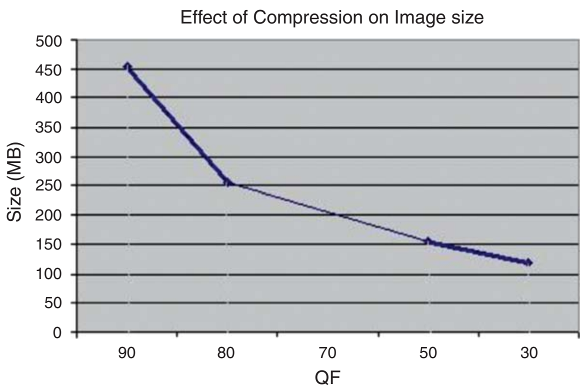


Fig. 7. The image size reduce significantly with compression.

and the size. The result of the qualitative analysis is shown in Fig. 6.

4. Discussion

Special stains are more economic than immunohistochemistry, flow cytometry and other diagnostic and research technologies. The special stained WSI will get even more economical at lower QFs. In special stains, it is more important to be able to see the chromatic variation than features such as architecture or granularity. We are less likely to lose diagnostic information with compression in cases of most special stains than in H&E stains. Compression artifacts were more visible at lower QFs. However we focused to see if the image quality was good enough to evaluate the results of special stains, most special stained images were acceptable at QF 30, except for Reticulin stain where the lowest acceptable QF was 50. We focused to see if the image quality was good enough to show the color difference between region of interest and the background. Though the difference was dependent on the stains it was still above the distinguishable value for average human eye. Most special stained images were still acceptable at QF 30 except for the Reticulin stain.

5. Conclusion

The experiment was done with images of various QF to determine the lowest possible QF for a usable pathological image with special stain. The 0.50 μm/pixel images at QF 50 or above were found to be suitable for high volume special stained WSI. The average reduction in storage space (Fig. 7) by moving from QF 90 to QF 50 is 62.73%. However the experiment can be advanced further by applying a quantitative benchmarking tool for judging the image quality to avoid the limitations and variations of human observation.

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