Recovery percentage of remineralization according to severity of early caries

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ABSTRACT: Purpose: To analyze the cutoff severity of early lesions according to recovery rate after fluoride treatment. **Methods:** 100 specimens were demineralized over 3 to 40 days. Specimens were immersed in 2% sodium fluoride solution for 4 minutes, and then in artificial saliva for the rest of the total 24 hours. After 10-time repetition of this cycle, the ΔF recovery rates ($R_{\Delta F}$, %) were calculated from the ΔF values before (ΔF_{base}, %) and after (ΔF_{tx}, %) remineralization using the QLF-D system. For the discrimination of $R_{\Delta F}$ based on ΔF_{base}, the sensitivities versus 1-specificities were analyzed in receiver operating characteristic (ROC) curves, the 95% confidence interval (CI) as well as the significance of differences. The histological features of lesions were observed and lesion depths were digitally measured by polarized light microscopy (PLM). A paired t-test was also performed to assess the differences in ΔF and lesion depth before and after applying fluoride. **Results:** For a threshold recovery percentage of 40%, the suggested ΔF_{base} cutoff value was -19.15%, whereas for a threshold recovery percentage of 50%, the suggested cutoff value was -14.60% ($P< 0.0001$). According to the QLF-D system and PLM analysis, recovery percentage was greater for shallower lesions. Based on fluoride treated recovery percentages, the findings suggested that it is possible for early caries lesions to make more than 50% recovery when the ΔF_{base} value was greater than -14.60%. Visually and numerically, the relative recovery percentages were highest during the earlier stages of caries. (*Am J Dent* 2013;26:132-136).

CLINICAL SIGNIFICANCE: The results indicated that prognosis can be estimated after fluoride treatment using the quantitative light-induced fluorescence digital system. These results could be used to create clinical guidelines for the remineralization of early caries.

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Introduction

Over the past 40 years, most industrialized nations have witnessed a substantial decrease in the prevalence of dental caries among 12 year-olds.[1,2] This change in epidemiology highlights the importance of preventive care in dentistry. The first basic principle of preventive care is the remineralization of early caries lesions rather than the traditional surgical approach, and the most common method of remineralization is topical fluoride application. During the early stage of caries lesions, fluoride can be used to control and treat caries, and thus, prevent lesion progression.[3-6] Although numerous studies have been conducted on the effects of fluoride on shallow (up to 100 µm) lesions, comparatively few studies have addressed deeper lesions.[7,8] However, the severity of early caries lesions can affect remineralization recovery rates.[9] Accordingly, if we could predict recovery rates after fluoride treatment, early caries lesions could be successfully controlled. Nevertheless, quantitative information on recovery rates after fluoride treatment with respect to early caries severity is lacking.

It is essential to detect diseases and accurately diagnose pathological changes for successful recovery of early caries.[1,10-12] In general, white opaque early caries lesions are an enamel defect with a relatively intact surface layer and some subsurface mineral loss.[13] Because of these histological features, early caries is difficult to diagnose. Failure to accurately detect caries could lead to missing an optimal therapeutic opportunity. However, the development of quantitative methods now makes it possible to detect caries lesions at earlier stages. In particular, quantitative light-induced fluorescence (QLF) has emerged for early caries detection.[14] QLF is a non-destructive diagnostic method for the longitudinal assessment of early caries lesions.[15] It has been used for the detection and quantitative analysis of early caries based on the established correlation between mineral loss and fluorescence loss in enamel following demineralization.[14] The QLF digital (QLF-D) system differs slightly from the QLF system, because it employs a modified filter set (D007[a]), a narrow-band blue light (405 nm), and a high-specification digital single-lens reflex (DSLR) camera. The QLF-D system can capture endogenous porphyrin-induced red fluorescence at high resolution,[16] and has been used for the detection and quantitative analysis of early caries lesions in dental clinics. Furthermore, this device can be used to determine the immediate visual and quantitative effects of treatment,[17] and it can sensitively detect early caries lesions because it can detect and analyze even slight mineral changes.[17-20]

Thus, we hypothesized that if we could quantify the cutoff severity of early caries based on recovery percentage after fluoride treatment by the QLF-D system, we would be able to better prevent and manage early caries lesions and to provide patients with more accurate prognostic information. Therefore, this study identified a cutoff point for early caries lesions severity based on fluoride treated recovery percentages.

Materials and Methods

Preparation of enamel specimens - One hundred bovine incisors without cracks or white spots were selected. All specimens were sectioned (6×3×3 mm) from the labial surface of incisors using a low-speed saw equipped with a diamond blade. All specimens were embedded in epoxy resin, and prepared using 600 to 1,200 grit abrasive disc paper (SiC Sand Paper[b]) on a water-cooled polishing unit to form a smooth flat enamel surface.

Artificial caries lesion creation - Before generating artificial early caries lesions, acid-resistant nail varnish was applied on a third of each specimen surface for a reference area. Dried specimens were then immersed in deionized water for 30 minutes. To generate subsurface lesions, specimens were then immersed in 40 ml of demineralizing gel for 3, 5, 10, 15, 20, 30, or 40 days at 37°C and rinsed with deionized water after the entire lesion formation period.[8] Specimens were then divided into seven groups according to ΔF values of the QLF-D system. All specimens were stored in 100% relative humidity at 4°C until needed.

The demineralizing gel (pH 4.8), 0.1 M lactic acid gel containing 1% carbopol (Carbopol ETD 2050[c]) polymer was 50% saturated with hydroxyapatite (calcium phosphate tribasic[d]).

QLF-D system measurements - After de- and remineralization, the QLF-D system (bilumination[a]) was used to measure fluorescence loss (ΔF) values to evaluate mineral loss. Since the lesion window was identical in size for all specimens, only average ΔF values were recorded at the 5% threshold level between sound and demineralized or remineralized enamel using the Subtract[a] program (version 1.1.0.7). All specimens were air-dried for at least 30 minutes at room temperature and then analyzed by the QLF-D system in a controlled, dark environment. The distance between the QLF-D lens and enamel block surfaces were kept constant (10 cm) throughout the experiment.

Analysis of recovery percentage after applying fluoride - After subsurface lesion formation, half of the remaining portion of each specimen (2×3 mm) was treated with acid-resistant varnish for lesion reference area. Dried specimens were treated with 3 ml of fluoride solution [2% sodium fluoride (NaF) solution, 9,050 ppm F, pH 7] for 4 minutes. After completely removing fluoride with paper, specimens were immersed in artificial saliva (0.22% mucin, 30 mM KCl, 10 mM KH_2PO_4, 13 mM NaCl, and 3 mM $CaCl_2$, final pH 6.8; refilled daily), and stirred for the rest of the total 24 hours. This cycle of fluoride and artificial saliva treatment was repeated 10 times. Specimens were measured daily using the QLF-D system, and based on these measurements, the recovery percentage of ΔF ($R_{\Delta F}$, %) value was calculated to evaluate the extent of remineralization after fluoride application using the following equation:

Recovery Percentage of ΔF (%) =
[(ΔF measured post-demineralization – ΔF measured post-fluoride treatment) / ΔF measured post-demineralization] × 100%

$$R_{\Delta F}\ (\%) = [(\Delta F_{base} - \Delta F_{tx}) / \Delta F_{base}] \times 100\%$$

The SigmaPlot 2000[e] was used to create a scatter plot of the distribution of $R_{\Delta F}$ versus ΔF_{base} and the trend was analyzed.

Polarized light microscopy (PLM) - After finishing the fluoride treatment, specimens were cut perpendicular to the treated surfaces. A 300 µm-thick section was cut (TechCut 4[f]) from each specimen, and ground (1,200 grit, SiC paper[b] to a thickness of 100 µm. Sample thickness was measured using a digital micrometer with a precision of 0.001 mm (ID-C125B[g]). Specimens were mounted on glass slides. The slabs were placed in deionized water and their images were captured at a magnification of ×100 using a polarized light microscope (CX31-P[h]). The histological features of lesions were observed in PLM micrographs. An image analysis system (ImagePro-Plus,[i] version 6.0) was used to measure lesion depths.

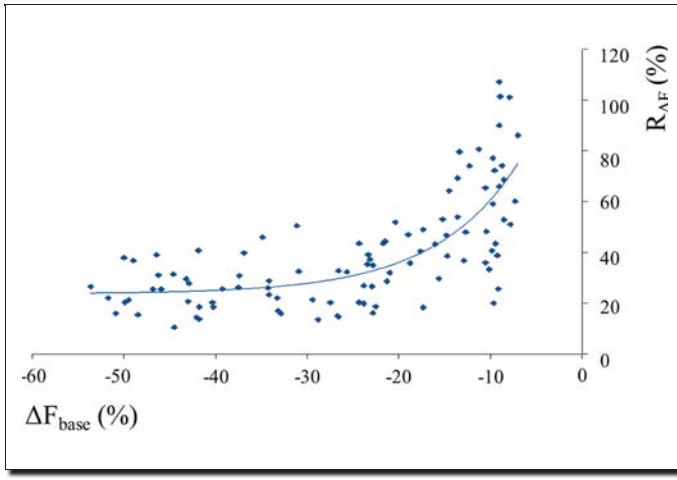

Fig. 1. Scatter plot of the recovery percentages of ΔF ($R_{\Delta F}$) values for the ΔF_{base} values in lesions after fluoride application. The blue line shows an exponential trend.

Statistical analysis - The efficacies of different numbers of fluoride treatments were analyzed using one-way repeated measures ANOVA. The paired t-test was used to assess differences between ΔF values before and after applying fluoride. To discriminate $R_{\Delta F}$ values based on ΔF_{base} values, sensitivities versus 1-specificities were analyzed by receiver operating characteristic (ROC) analysis using areas under the curve (AUC) and 95% confidence intervals (CIs), and the significances of differences were determined. Additionally, the sensitivity and specificity of ΔF_{base} cutoff values for threshold $R_{\Delta F}$ values were analyzed. The paired t-test was performed to assess lesion depth differences before and after applying fluoride. The correlation between ΔF values and histological lesion depths was determined using the Spearman's rank correlation coefficient. Statistical analysis was performed with SPSS[j] version 18.0 for Windows.

Results

ΔF recovery percentages by QLF-D system - The ΔF recovery percentages ($R_{\Delta F}$) of all lesions are presented in Fig. 1. The relationship between ΔF_{base} values and $R_{\Delta F}$ values followed an exponential pattern. For ΔF_{base} values above -20%, recovery percentages drastically increased. $R_{\Delta F}$ value was highest (62.84%) when ΔF_{base} values were between 0 to -10%, and $R_{\Delta F}$ value was 48.01% when ΔF_{base} values were between -10 to -20% (Table 1). However, $R_{\Delta F}$ value was below 30% when the ΔF_{base} values were less than -20%.

The ROC curve was analyzed to determine the ΔF_{base} cutoff values for $R_{\Delta F}$ values (Table 2). When ΔF_{base} cutoff value was -19.15%, early caries lesions made up to a 40% recovery (sensitivity = 0.91, specificity = 0.82), and when the ΔF_{base} cutoff value was -14.60%, early caries lesions made up to a 50% recovery (sensitivity=1.00, specificity = 0.81). With associated AUC values of 0.90 (CI: 0.84–0.96, $P< 0.0001$) and 0.93 (CI: 0.88–0.98, $P< 0.0001$), $R_{\Delta F}$ values of 40% and 50% were very good, respectively (Table 2).

Histological features and lesion depths by PLM - From the initial 100 specimens used in this study, 35 specimens broke during preparation for PLM measurement due to high mineral loss. Observations of histological features in PLM micrographs of lesions revealed less mineral loss and a higher recovery

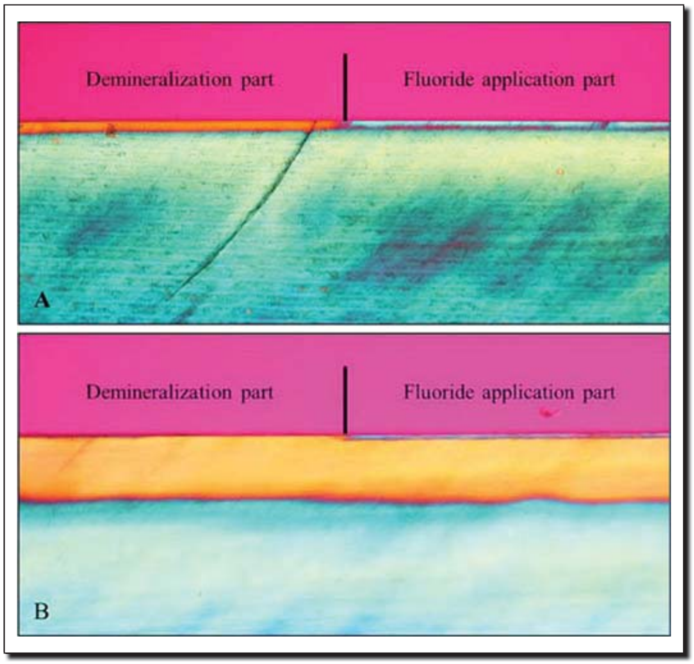

Fig. 2. Representative polarized light microscope (PLM) micrographs (×100) after fluoride application (lesion depth of 35.00 µm (A) and lesion depth of 146.86 µm (B).

percentage for shallow lesion than deep lesion (Fig. 2). Fig. 2A shows that lesion pore sizes and depths reduced after fluoride treatment. This was confirmed by lesion color changes. For deeper lesions, although surface layers were clearly remineralized, the depth of body of the lesion still remained (Fig. 2B).

Histological lesion depths were significantly reduced by fluoride treatment (Table 3, P< 0.0001). Reductions in lesion depth were greatest in Group 1 (approximately 21%) and lowest in Group 5 (approximately 9%). No significant difference in lesion depth reduction was observed between Group 2 (13%), Group 3 (12%), Group 4 (12%), and of Group 6 (11%).

According to correlation analysis, ΔF values were strongly correlated with histological lesion depths before and after fluoride treatment (P= -0.94, P= -0.90, P< 0.0001).

Discussion

Fluoride treatment is an ideal treatment to remineralize early caries lesions. Most of the studies on this subject have primarily evaluated shallow lesions (approximately 100 µm), nevertheless, it is essential that the efficacy of fluoride is determined based on severity of early caries lesions. However, it is difficult to detect pathological changes during caries lesion formation because early caries lesions only cause slight mineral changes. For these reasons, the clinical diagnosis of early caries tends to vary considerably. We considered that some cutoff point is required to determine the severity of early caries lesions based on recovery percentages after fluoride treatment, so as to allow early caries lesions to be classified in terms of recovery or not after fluoride treatment and clinicians to recommend fluoride treatment or other treatment plans, such as resin infiltration, to patients with early caries lesions. Therefore, we hope our results can be used to construct a clinical guideline for the appropriate diagnosis and optimal treatment of early caries.

In this study, specimens were demineralized for 3, 5, 10, 15, 20, 30, or 40 days. In a pilot study (data not shown) that used the same demineralizing protocol as this study, it was confirmed that 10 days of exposure caused approximately 113 µm deep lesions, 20 days 218 µm, 30 days 268 µm, and 40 days 310 µm. Accordingly, we produced artificial early caries lesions of different severities (depths) in order to determine the characteristics of early caries lesions, such as mineral loss, by the QLF-D system.

After fluoride application, ΔF recovery percentages ($R_{\Delta F}$) increased as ΔF_{base} values increased (Table 1), and the relationship between ΔF_{base} and $R_{\Delta F}$ values followed an exponential pattern (Fig. 1). $R_{\Delta F}$ values increased sharply when ΔF_{base} values were more than -20%. In a previous study,[21] shallow lesions (approximately 50 µm) were more remineralized after fluoride treatment than deep lesions (approximately 200 µm). Another study[22] suggested that fluoride treatments had no impact on remineralization of the inner enamel or dentin parts of the lesions. These findings indicated that lesions with larger

Table 1. ΔF values of measurements before and after fluoride treatment and the recovery percentages of ΔF values by fluoride treatment (unit: %). All the values are means (SDs).

Groups	Ranges of ΔF_{base}	N	ΔF_{base}	ΔF_{tx}	$R_{\Delta F}$
1	0 to -10	19	-8.92 (0.82)	-3.37 (2.31)	62.84 (24.28)
2	-10 to -20	22	-14.20 (2.70)	-7.53 (3.08)	48.01 (15.97)
3	-20 to -30	23	-23.97 (2.45)	-17.33 (3.86)	28.28 (10.61)
4	-30 to -40	15	-35.08 (3.04)	-25.53 (4.53)	27.44 (9.89)
5	-40 to -50	18	-45.02 (2.95)	-33.76 (4.17)	24.90 (8.99)
6	-50 to -60	3	-51.57 (1.39)	-40.70 (1.61)	20.98 (5.08)
Total		100	-25.24^{a}(13.83)	-17.41^{b}(12.11)	38.23 (20.83)

[a,b] letters denote statistically significant differences by the paired t-test (P< 0.05). ΔF_{base}, ΔF values after demineralization; N, number of specimens; ΔF_{tx}, ΔF values after fluoride treatment; $R_{\Delta F}$, recovery percentage of ΔF values by fluoride treatment.

Table 2. Results of the statistical analysis of the ability of QLF-D system to determine the ΔF_{base} values of recovery percentages of ΔF values by fluoride treatment.

	ΔF_{base} by QLF-D measurements					
	ROC					Cutoff of ΔF_{base} (%)
RΔF %	AUC	95% CI	P	SE	SP	
40	0.90	0.84-0.96	<0.0001	0.91	0.82	-19.15
50	0.93	0.88-0.98	<0.0001	1.00	0.81	-14.60

Areas under the receiver operating characteristic (ROC) curves (AUCs) with 95% confidence intervals (CI) and the significances of differences (P) are given. In addition, sensitivity (SE) and specificity (SP) for the suggested ΔF_{base} cutoff values are provided.

Table 3. Histological lesion depths before and after fluoride application (unit; µm). All values are means (SDs).

Groups	N	Baseline	After F application
1	10	41.93 (7.35)	33.17 (5.32)
2	15	54.70 (11.63)	47.52 (11.88)
3	14	92.27 (13.01)	81.44 (11.02)
4	10	123.93 (26.89)	109.36 (29.89)
5	13	200.99 (51.47)	181.89 (46.52)
6	3	262.16 (71.21)	230.60 (78.27)
Total	65	115.28 (74.44) [a]	102.15 (68.56) [b]

[a,b] letters denote significant differences by the paired t-test.

ΔF_{base} values, a shallow depth, and lower mineral loss can be easily remineralized. It is reasonable to assume that mineral loss at baseline impacts the remineralization of enamel white spot lesions.

When ΔF_{base} cutoff values for threshold $R_{\Delta F}$ values were analyzed after fluoride application, the ΔF_{base} cutoff value for a $R_{\Delta F}$ of 50% was -14.60%, and the ΔF_{base} cutoff value for a $R_{\Delta F}$ of 40% was -19.15% (Table 2). These results indicate that a lesion can be recovered up to 50% if its ΔF_{base} value is -14.60% when assessed using the QLF-D system. This finding can help decision making in patients with early caries. In other words, it provides a criterion of diagnosis for early caries that can be consistently applied, enables recovery percentage to be predicted using the QLF-D system, and patients to be more accurately informed of their prognosis.

In PLM micrographs, the histological features of lesions were evaluated. The present study confirmed that lower mineral loss increased the recovery percentage. Fig. 2A shows lesion depth to be approximately 30 µm at post-fluoride treatment, while histological features seemed sound and the recovery percentage was 75%. This result concurs with those of previous studies. One study[23] reported almost 80% recovery of original density levels after fluoride application although almost complete recovery was observed in the lesions on the image. Another study[24] reported microhardness measurements with over 85% recovery after a 10-day treatment but micro-radiographic images of the 10-day remineralization indicated complete recovery. On the other hand, as shown in Fig. 2B, only the surface layer was remineralized by fluoride treatment and there were no changes in the lesion body of deeper lesions. The $R_{\Delta F}$ value of this lesion was only 25%. This result indicates that fluoride with high concentration could have precipitated on the surface layer before infiltrating into the body of the lesion. Thus, the lesion body remained demineralized. A previous study[9] found that white spot lesions that progressed through the outer half of enamel were difficult to reverse by fluoride treatment. Accordingly, it was confirmed that the recovery rate of remineralization is dependent on the severity of early caries lesions by PLM analysis. In addition, based on PLM micrographs, histological lesion depth values were significantly reduced by fluoride treatment (Table 3). This result agrees with that of a previous study[26] which analyzed lesion depth using a confocal laser scanning microscope. In this study, PLM analysis produced a similar result pattern as the QLF-D analysis, that is, shallower lesions had a higher lesion depth reduction rate. The lesion depth was reduced the most in Group 1 (21% at ΔF_{base} of -8.92%), and the reduction of lesion depth was similar in all other experimental groups after fluoride treatment.

In this study, ΔF values were found to be strongly and negatively correlated with histological lesion depth. Several previous studies have reported that fluorescence loss is correlated with lesion depth as determined by transverse microradiography (TMR), which is the 'gold standard' for quantifying mineral loss. Furthermore, the relationship between lesion depth and ΔF yielded a correlation coefficient of more than -0.8.[20,27,28] Therefore, the QLF-D system can be utilized to assess the lesion progression which is impossible clinically as there is no method to measure lesion depth. Moreover, the QLF-D system has several major advantages over TMR. The QLF-D system monitors the effect of remineralization after fluoride treatment because it can assess longitudinal changes in mineral status within caries lesions. In addition, the QLF-D system allows immediate visual and quantitative comparison of current images with past images from the same patient. Furthermore, it can be used at chair-side and images can be immediately shown to the patient. The traditional visual-tactile and radiographic methods of detecting dental caries can only detect lesions that are well advanced at least by 300-500 µm into enamel.[9] However, the QLF-D system can detect early caries lesions sooner than traditional diagnostic methods. Furthermore, a consistent treatment plan can be easily suggested if using the fluorescence loss (ΔF) of early caries lesions detected by the QLF-D system rather than lesion depth, which cannot be measured clinically.

In the present study, although fluoride treatment showed a significant effect, net mineral change was not significantly different by repeated fluoride treatments. It is reasonable to assume that the CaF_2 layer, formed on the lesion surface after applying a high concentration of fluoride, functions as a barrier.[29] Thus, although fluoride treatment is repeated, mineral ions, such as fluoride, calcium, and phosphate, do not significantly penetrate the lesion body. Moreover, the recovery rate found during this study is lower than that reported in previous studies.[23,24] There is a possible reason for this discrepancy. The method used to form lesions in this study is different from those used in previous studies. The mineral distribution characteristics of lesions depend on the method used to create the artificial caries lesions.[30] In this study, a polymer method was used and an acidified polymer gel was partially saturated with respect to hydroxyapatite.[8] Previous studies[31,32] reported that lesion type by the polymer method, the so-called 'low-R lesions', has a relatively higher degree of surface zone mineralization although this lesion has a similar degree of mineral loss compared with other types of artificial caries lesions. This is because the surface protective polymer, carbopol, holding calcium and phosphate ions, can protect the surface layer during the formation of early caries lesions.[8] Thus, by using this approach, remineralization can be potentially reduced in lesions because fluoride cannot penetrate deeper lesion regions.[33] In addition, low-R lesions are probably deeper and have narrower diffusion pores than other types of lesions.[32,34] Thus, low-R lesions had a lower response rate because the area of enamel per unit volume of the remineralizing solution is lower. Nevertheless, the artificial caries lesions used in this study were more physiologically relevant because lesions with a higher degree of surface layer mineralization and different degrees of mineral loss at baseline were obtained.

Further studies should be conducted using an in situ model with high and low concentration fluoride treatments. The findings of this study could result in clinical guidelines for the remineralization of early caries. For example, when measuring caries lesions by the QLF-D system, if the ΔF_{base} value is more than approximately -15%, clinicians can suggest fluoride application for their patients. If the ΔF_{base} value is less than approximately -15%, clinicians can suggest an alternative treatment, such as resin infiltration. Furthermore, the remineralization of early caries is more likely to be successful because we would be able to constantly monitor and control early caries lesions using the QLF-D system.

In conclusion, based on fluoride treated recovery percentages, it was possible to estimate that early caries lesions made a 50% recovery when the ΔF_{base} cut off value was -14.60%. In addition, this study also showed visually and numerically that relative recovery percentages were the highest for caries of earlier stages. Furthermore, the QLF-D system was useful for this type of research and for dental clinicians.

a. Inspektor Research Systems BV, Amsterdam, The Netherlands.
b. R&B Inc., Daejeon, South Korea.
c. Noveon Inc., Wickliffe, OH, USA.
d. Sigma, St. Louis, MO, USA.
e. Systat Software, San Diego, CA, USA.
f. Allied High Tech Products, Inc., Rancho Dominguez, CA, USA.
g. Mitutoyo Corporation, Kawasaki, Japan.
h. Olympus, Tokyo, Japan.
i. Media Cybernetics, Inc., Silver Spring, MD, USA.
j. SPSS Inc., Chicago, USA.

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