



Introduction

Image analysis technology has been applied to various investigational purposes in the cosmetic and dermatological industries and has been a useful tool for determining the safety and efficacy of topically applied cosmetic and consumer products as well as cosmeceuticals. Applications of this methodology include the investigation of skin topography, such as roughness, wrinkling and wound characterization, skin coloration, including pigmentation or inflammation, light reflectance or shine of the hair and analysis of follicular biopsy slides. Because image analysis is well suited for measuring small-scale changes in dimension, it provides an ideal system for the evaluation of the efficacy of mascara products to increase the length, diameter and/or volume of eyelashes. The data collected by image analysis of treated and untreated lashes determines dimensional changes resulting from treatment, with accuracy and precision that far exceeds that of more subjective mascara efficacy evaluation methods. The capability to assess the magnitude of changes in lash size is very appropriate to the current trends in mascara packaging and marketing, for which volumizing, lengthening and thickening claims are frequently reported in units of proportional increases. For example, currently marketed products list statements such as "65% longer lashes," "gives you lashes that appear up to 30% longer," "making even tiny lashes 100% fuller" and "three times fuller lashes." Such percentage increases in dimension are easily measured by our method described herein.

Purpose

The purpose of this investigation was to introduce a method of determining the effects of mascara treatment on eyelash dimensions, including length, diameter and volume.

Methods

A white card, measuring approximately 2.5 cm x 14 cm, was marked at ten positions spaced at 1 cm intervals. Double-sided tape was placed over the marks, aligned with the edge of the card. Ten individual hairs were removed from a false eyelash made of human hair (Andrea® International Industries, Los Angeles). The base of each lash hair was placed onto the double-sided tape at 1 of the 10 marked positions, such that most of the length of the lash extended from the edge of the card. The bases of the lashes, affixed to the double-sided tape, were covered with white tape to hold the lashes in position. Another strip of a white index card was affixed on top of the card with the mounted lashes. By lifting this strip like the cover of a book, a constant edge was provided at the base of the lashes for images taken before and after treatment. The strip was lifted during the application procedure, and any mascara inadvertently applied to the mounting card was covered to avoid mis-measurement of lash dimension. The untreated, mounted eyelashes were placed on top of a white background and covered with a glass slide. One image was taken of each of the 10 eyelash hairs. Graph paper with 1 millimeter units was used for calibration of image size.

After the images of untreated lashes were captured, the glass slide was removed, and the attached white strip was lifted. The mascara was applied to the lashes, affixed to the index card strip. Each mascara was applied in a consistent manner for all evaluations. The eyelashes were allowed to dry for a 10 minute period, the lashes were covered with a glass slide, and images were captured. To determine the effects of an additional coat of test material, the application procedure was repeated at approximately 30 minutes from the initial application. The treated lashes were again covered with a glass slide, and images were taken of the individual treated hair shafts.

Methods (Continued)

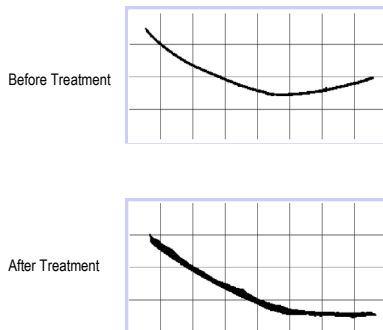


Figure 1. Images captured from a single lash before and after treatment with a mascara product (after bilevel thresholding).

Use of individual lashes separated from neighboring lashes by a 1 cm distance allows for easy manipulation of the lash during image analysis and prevents erroneous measurements caused by clumping of multiple lashes during application.

Image Analysis Procedure

Calculation of the Average Diameter of a Single Lash



High-resolution digital images of eyelash fibers, before and after treatment, are analyzed using calibrated image processing software. Before taking any measurements, the image is processed to remove any background noise due to lighting, optics, and equipment. The image is then converted into a bi-level image through thresholding. By averaging the length of a series of radial lines traversing the width of the lash image in a perpendicular orientation, the average diameter of the untreated or treated lash can be determined. The length of each lash is measured from a skeletonized image. The data is captured and stored in a data file.

Statistical Methods

Measurements of diameter and length were taken from each of the individual hair shafts. Volume was calculated for each lash ($V = \pi r^2 L$). Standard paired t-tests were used to determine the statistical significance of changes in lash dimensions after application. Statistical significance was declared for all two-tailed p-values less than or equal to 0.05.

Results

Lash Number	Diameter (mm)			Length (mm)			Volume (mm ³)		
	Baseline	Post-Treatment	% Change	Baseline	Post-Treatment	% Change	Baseline	Post-Treatment	% Change
1	0.09102	0.37693	314%	5.84392	6.39225	9%	0.83553	3.78473	353%
2	0.09032	0.37117	311%	7.89284	10.65480	35%	1.11979	6.21209	455%
3	0.10782	0.35140	226%	5.96750	6.31679	6%	1.01068	3.48673	245%
4	0.10456	0.23808	128%	6.08856	6.12436	1%	1.00000	2.29036	129%
5	0.10641	0.37383	251%	3.96881	4.08964	3%	0.66338	2.40148	262%
6	0.11305	0.42046	272%	6.25086	6.98766	12%	1.11002	4.61505	316%
7	0.10858	0.35837	230%	4.83901	5.00703	3%	0.82533	2.81859	242%
8	0.11887	0.35622	200%	4.33842	5.22280	20%	0.81007	2.92241	261%
9	0.07564	0.31675	319%	6.75829	8.76805	30%	0.80299	4.36254	443%
10	0.08339	0.31538	278%	5.96460	7.15298	20%	0.78130	3.54357	354%
Mean	0.09997	0.34786	253%	5.79128	6.67164	14%	0.89591	3.64376	306%
St. Dev.	0.01401	0.04899	60%	1.15730	1.90416	12%	0.15341	1.18730	99%

Table I. Length, Diameter and Volume of Untreated and Treated Lashes.

The table includes the dimensions measured for the evaluation of a marketed mascara with volumizing claims, which was used in our investigation as a reference product for the demonstration of parity. Ten individual lashes were analyzed for each test mascara, which provides a sufficient sample size to measure changes with statistical significance. The small sample size allows for generation of results in a timely fashion. The average diameter and length measured from sections of an individual lash are used to calculate volume. The measured dimensions are accurate to 0.00001 mm.

Figure II. Average Changes in lash dimensions following treatment with mascara products.

The percentage change in lash size for an individual treated lash is calculated relative to the untreated baseline lash measurements. The average changes in diameter, length and volume were calculated for the 10 lashes treated with each test material. We have studied the effects of over 19 mascara products. The effects observed for these 4 mascara products are representative of the range of typical results obtained, which can be expressed as the percentage increase that was measured.

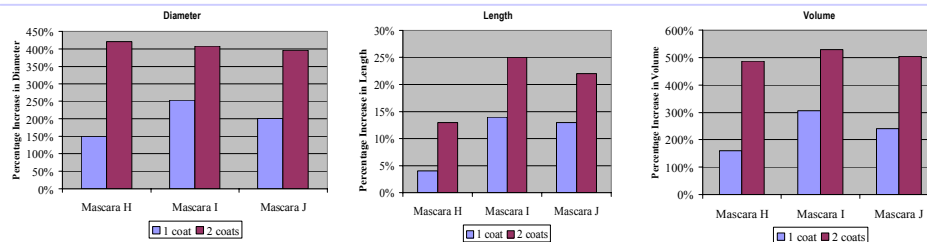
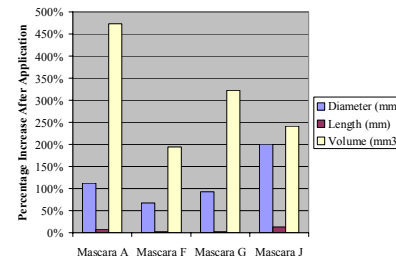


Figure III. Evaluation of the Effects of Two Coats of Mascara.

To assess the effect of a 2-coat application, the mascara is applied in the described manner twice, with a 30 minute drying time between applications. The data collected is useful for determining the expected effect of multiple applications, generating instructions for consumer packaging or evaluating the feasibility of marketing the test material as a single-coat mascara.

Conclusion

Use of image analysis to determine the efficacy of mascara products is a reproducible, accurate, non-invasive method that provides data that may be used to support one-coat, thickening, lengthening and volumizing claims as well as to demonstrate parity between test materials, including marketed products for which volumizing, lengthening and/or thickening claims are reported. This method is a quick and efficient, non-invasive manner with which to quantitatively assess the increases in dimensions of individual eyelashes resulting from mascara application.