

# Clinical Screening Study: Use of the Pharmanex® BioPhotonic Scanner to Assess Skin Carotenoids as a Marker of Antioxidant Status

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A new, non-invasive BioPhotonic Raman spectroscopy method was used to establish relationships between skin (palm) carotenoid levels as a biomarker of the strength of the antioxidant defense system and demographic, dietary and lifestyle parameters. There were 1,375 subjects entered into this population study. Results confirmed and helped validate all expectations for this health assessment instrument. Specifically, measurements appear not to be confounded by general demographic variables, such as age, sex and race/ethnicity, and they show the expected relationships with body composition, oxidative stress (urinary MDA, smoking) and dietary habits (fruit and vegetable consumption and LifePak® usage). Subjects habitually using LifePak® had a 61% higher BioPhotonic skin response than non-users, and similar or higher responses than subjects who reported eating more than five servings of fruits and vegetables daily.

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## Introduction

The ability to measure is the basis of all sciences, and health practitioners rely heavily on biological measurements such as cholesterol tests and blood glucose and insulin concentrations for the diagnosis and management of hyperlipidemia and diabetes. However, most of these measures require blood sampling, which is not only an unpleasant experience but is a safety concern in a virus-conscious society.

Recent advancements in laser technology have offered new opportunities for the health care industry. The application of this BioPhotonic technology increasingly enables non-invasive biological measurements.

Raman spectroscopy is a powerful laser spectroscopy that detects the characteristic vibrational/rotational energy levels of a molecule. Inelastically scattered light ("Raman" scattering) originates when energy is exchanged between incident light photons and the scattering molecules, resulting in a characteristic red shift when comparing the incoming photon with the scattered photon. Raman spectroscopy generates a spectral fingerprint, which depends on a molecule's unique vibrational energy scheme. Since Raman scattering is linear, the intensity of a Raman spectroscopy measurement is directly proportional to the amount of molecules.

Carotenoids are a family of antioxidant nutrients responsible for most of the red, orange, and yellow colors found in nature. Carotenoids play an important role in human health (*Gerster, Int J Vitam Nutr Res* 63:93, 1993). Recently, the protective effects of carotenoids against free radical damage have stimulated intensive research on several specific carotenoids. Beta-carotene, alpha-carotene, lycopene, lutein, and zeaxanthin are of particular importance in human nutrition. Alpha- and beta-carotene are vitamin A provitamins and act as antioxidants (Mortensen et al., *Arch Biochem Biophys* 385:13, 2001; Paiva and Russell, *J Am Coll Nutr* 118:426, 1999). Lutein and zeaxanthin are important for eye health (Mares-Perlman et al., *J Nutr* 132:518S, 2002), while lycopene, the most potent antioxidant carotenoid, may have far-reaching cell-protective benefits (Rao and Agarwal, *J Am Coll Nutr* 19:563, 2000; Heber et al., *Adv Exp Med Biol* 492:29, 2001).

Carotenoids are present in the epidermal and stratum corneum layers of human skin and are believed to confer antioxidant and photo-protective benefits to the skin (Alaluf et al., *J Nutr* 132:399, 2002; Stahl et al., *J Nutr* 131:1449, 2001). In this sense, measurement of skin carotenoids would provide a site-of-action-advantage over conventional HPLC measurements of blood serum or plasma carotenoids.

Carotenoid molecules have characteristic long chains of conjugated double-bonds, which generate strong and unique Raman signals. The dietary carotenoids alpha-carotene, beta-carotene, lycopene, lutein and zeaxanthin can all produce strong Raman spectroscopy signals at 511 nm when excited with 473 nm laser light. The BioPhotonic properties of carotenoids are highly specific and with little or no interference from any other bio-molecules present in human skin, such as melanin, vitamin E or skin lipids. As a result, Raman spectroscopy allows for a non-invasive, rapid, accurate, and safe assessment of carotenoid levels in the skin. Research suggests that skin carotenoid levels correlate with levels of carotenoids in the diet and blood (Hata et. Al., *J Invest Dermatology* 115:441, 2000). Reviews of the application of Raman spectroscopy to measure skin carotenoids were published by Hata et al. (*J Invest Dermatology* 115:441, 2000) and Ermakov et al. (*Optics Letters* 26:1179, 2001).

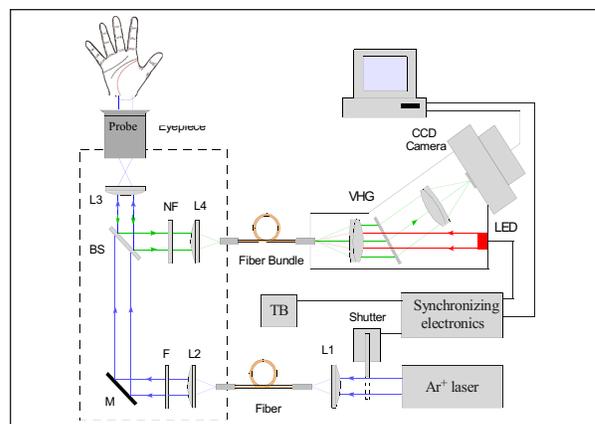
The present study used questionnaires in the large number of subjects to examine whether or not there are relationships between age, gender, race/ethnicity, body mass index (BMI), smoking, usage of dietary supplements (i.e., LifePak®), and consumption of fruits and vegetables, with skin carotenoid readings. This information is important to help validate the Pharmanex® BioPhotonic Scanner as a novel, non-invasive optical tool for *in vivo* dietary assessment.

## Materials and Methods

A total of 1,375 employees of Nu Skin Enterprises® and their family and friends were recruited. No groups were excluded from the study. Subjects participating in this study were instructed to complete a computer-administered questionnaire to assess demographical, dietary and lifestyle variables. The questionnaire contained a food frequency query asking subjects to record their consumption of foods containing more than 1 mg of total carotenoids per serving according to the USDA carotenoids database. Subjects then underwent the measurement of carotenoid levels in the skin on the palm of the hand using the Pharmanex® BioPhotonic Scanner at the Pharmanex Research Institute in Provo, Utah.

Figure 1 shows a schematic drawing of the Pharmanex® BioPhotonic Scanner describing the components and its operation.

**Figure 1: Pharmanex® BioPhotonic Scanner**

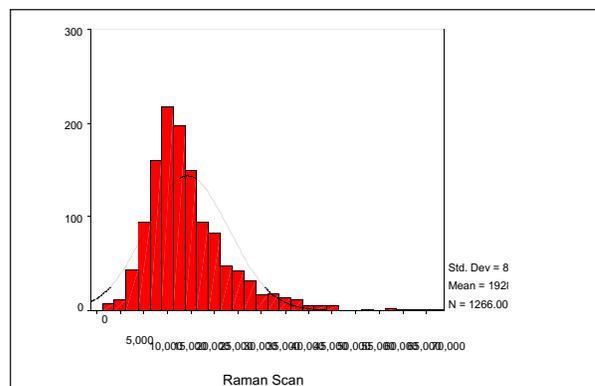


Body fat percentage was measured with a near-infrared device (Futrex-5000/XL, Futrex Inc., Galthersburg, MD). Subjects were asked for voluntary participation in a home urine malondialdehyde (MDA) test (Free Radical Test™, Vespro Life Sciences, Lenexa, KS) using the first morning void, and to report the test results (one of four color-matched outcomes) to the study coordinator. BioPhotonic responses were correlated with the information gathered in the questionnaire and the urinary MDA test to accomplish the study objectives. Information obtained from the subject questionnaire and the carotenoid measurements was tabulated. Correlations (positive or negative) between the individual items on the questionnaire and the carotenoid levels were examined and presented graphically. Statistical significance was examined using appropriate tests (t-test, correlation analysis).

## Results and Discussion

All 1,375 of the recruited subjects participated in the study, which was completed within eight weeks. The Pharmanex® BioPhotonic Scanner was able to accommodate more than 300 subjects per day,

**Figure 2: Histogram of Study Subjects**



owing to the short time required to obtain the measurement (less than one minute). The overall histogram of all study subjects is presented in Figure 2. The overall mean BioPhotonic response was 19,072 with a standard deviation of 8,828 units. The lowest measurement was 1,556 units and the highest measurement was 73,416 units, while the majority of subjects (68%) fell between 10,244 and 27,900 units.

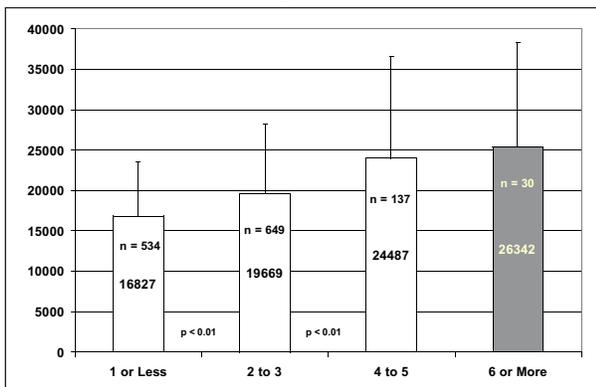
**General Demographics**

There were small, non-significant differences between women (19,244, n=666) and men (18,937, n=704), which can be explained by a slightly higher reported consumption of fruits and vegetables in women (2.34 servings/day) compared to men (2.03 servings/day). There were no significant differences in the BioPhotonic responses between the different age groups. Among race and ethnic groups, Asian subjects measured significantly higher than white-Caucasian, Hispanic and African-American subjects. Again, this can probably be explained with the higher reported consumption of fruits and vegetables of Asians (2.59 servings/day) compared to white-Caucasians (2.15 servings/day). Overall, this study showed that demographic variables do not influence the BioPhotonic measurements, and that any observed differences can be explained by different dietary habits.

**Fruit and Vegetable Consumption**

As expected, there was a pronounced, positive relationship between self-reported fruit and vegetable intake (a dietary source of antioxidants and carotenoids) and the BioPhotonic measurements as follows (see Figure 3): 1 or less serving/day: 16,827

**Figure 3: Scanner Readings vs. Fruit & Vegetable Intake**



± 6,725; 2-3 servings/day: 19,669 ± 8,557; 4-5 servings/day 23,997 ± 12,648; and 6 or more servings/day: 25,377 ± 12,953 units.

These data will help validate the BioPhotonic skin carotenoid measurements as a more convenient marker of fruit and vegetable intake than currently used blood plasma carotenoid measurements (El-Sohemy A et al., *Am J Clin Nutr* 76:172-9, 2002).

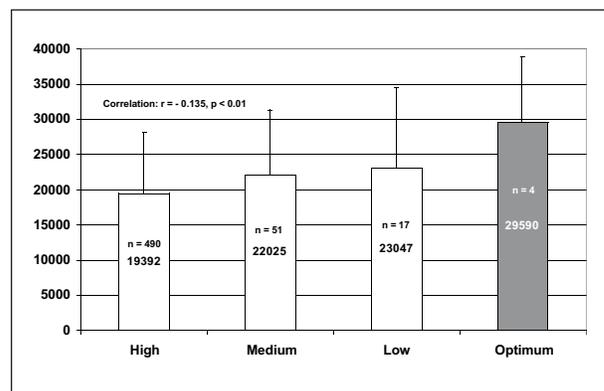
**Carotenoid Consumption**

Analysis of the self-reported consumption of carotenoid-rich foods resulted in a similar relationship as observed with fruit and vegetable intake, although overall carotenoid consumption was probably overestimated. Subjects consuming 15 or less mg carotenoids daily scored lower (16,440 ± 6,876 units, n=541) than those with 15-30 mg/day (20,097 ± 8,879 units, n=516, p<0.05), who in turn scored lower than subjects reporting more than 30 mg/day (21,889 ± 10,376 units, n=318, p<0.05).

**Urinary Free Radical Activity**

Of the 1,375 subjects, 562 completed and reported the results for the urinary MDA test, and the majority (490 subjects) reported high MDA levels (test scores were: “optimum,” “low,” “medium,” and “high” free radical activity). Nevertheless, there appears to be a consistent and inverse relationship between free radical activity (urinary MDA) and the BioPhotonic measurement of skin carotenoids as shown in Figure 4.

**Figure 4: Scanner Readings vs. Urinary MDA**



This result was expected, because carotenoids are singlet oxygen quenchers and therefore an important part of the body’s antioxidant defense system. Further studies using more sophisticated tests of free radical activity and antioxidant status are ongoing to further validate this relationship.

### Smoking

Tobacco smoke is a potent cause of free radical damage and smoking is clearly associated with increased oxidative stress and decreased markers of antioxidant defense (Lesgards JF et al., *Environ Health Perspect* 110:479-86, 2002).

**Figure 5: Smoking**

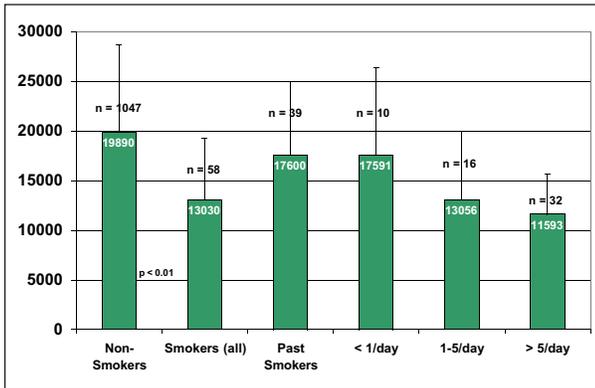


Figure 5 shows the BioPhotonic responses related to smoking status. Smokers scored 34.5% lower than non-smokers, and BioPhotonic skin responses were lowest in those who smoked the most, i.e., more than five cigarettes daily. Fruit and vegetable consumption was similar across smoking categories, except that those who smoked more than five cigarettes daily did report significantly lower fruit and vegetable consumption than those who smoked less than one cigarette daily. These results help validate the BioPhotonic skin carotenoids measurements as a marker of the antioxidant defense system, as similar relationships have previously been shown for blood plasma carotenoids (Walmsley CM et al., *Public Health Nutr* 2:199-208, 1999).

### Body Composition

Previous studies have shown inverse relationships between body mass index (BMI) or body fat content and serum or plasma carotenoid concentrations (Reitman A et al., *Isr Med Assoc J* 4:590-3, 2002; Neuhaus ML et al., *J Nutr* 131:2184-91, 2001). This is believed to be due to a dilution effect of adipose tissue serving as a storage site for carotenoids. The same relationship was observed in the present study. BioPhotonic skin carotenoid responses in the palm declined with increasing BMI as follows: BMI < 25 = 21,347 ± 9,661 (n=564); BMI 25-29.9 = 18,549 ± 7,319 (n=378, p<0.05), and BMI > 30 = 15,432 ± 6,621 (n=184, p<0.05). The same relationship was observed when body fat

was estimated using a near-infrared device: Body fat < 15% = 21,320 ± 9,394 (n=165); body fat 15-24.9% = 19,985 ± 8,665 (n=422, p=0.08); body fat 25-34.9% = 18,998 ± 8,762 (n=460, p=0.09); and body fat >35% = 15,925 ± 7,496 (n=90, p<0.01). Self-reported fruit and vegetable consumption was similar across all BMI and % body fat groups. These findings compare well with conventional traditional blood analysis and help substantiate the BioPhotonic skin carotenoid measurements as an assessment tool of carotenoid status.

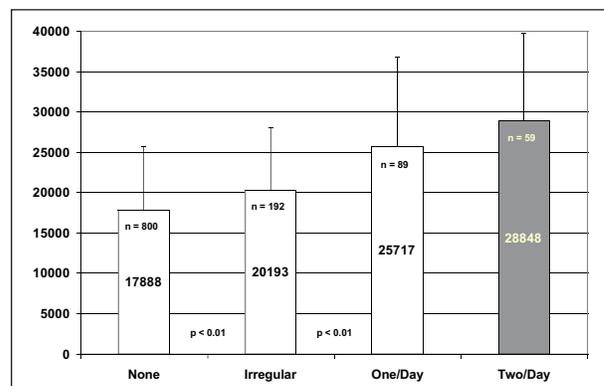
### Sunlight Exposure

Reported sunlight exposure was inversely related to the BioPhotonic skin measurements as follows: Low exposure = 20,085 ± 9,776 (n=550); moderate exposure = 18,660 ± 8,069 (n=716, p<0.01); and high exposure = 16,446 ± 7,508 (n=96, p<0.05). This was observed despite a significantly higher consumption of fruits and vegetables by subjects with high sunlight exposure (2.46 ± 1.33 servings/day, p<0.05) compared to those with low and moderate exposure (2.14 ± 1.22 and 2.17 ± 1.18 servings/day, respectively). This supports the theory that carotenoid antioxidants are protecting the skin from UV-light induced free radical damage and are consumed in the process.

### LifePak Supplement Usage

Antioxidant supplements can improve antioxidant status and this has been shown as well for LifePak® (Pharmanex LLC, Provo, UT) in earlier clinical studies showing increases in serum antioxidant concentrations and improved resistance to *ex vivo* LDL oxidizability (Smidt et al., *FASEB J* 13:A546, 1999). The present study shows a pronounced and positive relationship between LifePak® supplementation and BioPhotonic skin carotenoid measurements as shown in Figure 6.

**Figure 6: Scanner Readings vs. LifePak® Usage**



Subjects habitually consuming LifePak® at the recommended dosage (two packets daily) measured 61% higher than those not using LifePak® ( $p < 0.001$ ). LifePak® users had about the same BioPhotonic skin measurements as people who reported eating more than five servings of fruits and vegetables daily. Fruit and vegetable consumption was similar across LifePak® usage categories, except for irregular LifePak® users who reported less intake ( $2.04 \pm 1.09$  servings/day) than people using 1 packet of LifePak® daily ( $2.39 \pm 1.24$  servings/day,  $p < 0.05$ ). However, this difference is small and not considered a confounding variable based on the magnitude of the effect of fruit and vegetable consumption observed in this study. These results suggest that LifePak® antioxidants and carotenoids are bioavailable and lead to significant strengthening of the body's antioxidant defense system. LifePak® contains over 40 antioxidant nutrients, including 15 mg/day of mixed carotenoids (6 mg beta-carotene, 5 mg lycopene, 2 mg alpha-carotene and 2 mg lutein) (per current U.S. version; formulas vary by market.)

## Conclusions

This is the first large screening study to help validate and gain field experience with the Pharmanex® BioPhotonic Scanner. The study confirmed and helped validate all expectations for this health assessment instrument. Specifically, measurements appear not to be confounded by general demographic variables, such as age, sex and race/ethnicity, and they show the expected relationships with body composition, oxidative stress (urinary MDA, smoking) and dietary habits (fruit and vegetable consumption and LifePak® usage). Further studies are being conducted at Pharmanex and in academic institutions to confirm these and other conclusions.

For more information, please contact Pharmanex:

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