Fat tissue histological study at indocyanine green-mediated photothermal/photodynamic treatment of the skin in vivo

Irina Yu. Yanina
Valery V. Tuchin
Nikita A. Navolokin
Olga V. Matveeva
Alla B. Bucharskaya
Galina N. Maslyakova
Gregory B. Altshuler
Fat tissue histological study at indocyanine green-mediated photothermal/photodynamic treatment of the skin in vivo

Irina Yu. Yanina,1 Valery V. Tuchin,2abc Nikita A. Navolokin,4 Olga V. Matveeva,4 Alla B. Bucharskaya,4 Galina N. Maslyakova,4 and Gregory B. Altshuler5

1Saratov State University, Research-Educational Institute of Optics and Biophotonics, 83 Astrakhanskaya Str., 410012 Saratov, Russia
2Laboratory of Laser Diagnostics of Technical and Living Systems of Precision Mechanics and Control Institute of the Russian Academy of Sciences, 24 Rabochaya, 410028 Saratov, Russia
3University of Oulu, Optoelectronics and Measurement Techniques Laboratory, P.O. Box 4500, FIN-90014 Oulu, Finland
4Saratov State Medical University, Department of Pathological Anatomy, 112 B. Kazachaya Str., 410026 Saratov, Russia
5Palomar Medical Technologies, Inc., Burlington, Massachusetts

Abstract. Histological slices of skin samples with the subcutaneous adipose tissue after photothermal/photodynamic treatment are analyzed. In the case of subcutaneous indocyanine green injection and 808-nm diode laser exposure of the rat skin site in vivo, the greatest changes in tissue condition were observed. Processes were characterized by dystrophy, necrosis, and desquamation of the epithelial cells, swelling and necrosis of the connective tissue, and widespread necrosis of the subcutaneous adipose tissue. The obtained data are useful for safe layer-by-layer dosimetry of laser illumination of ICG-stained adipose tissue for treatment of obesity and cellulite. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.5.058002]

Keywords: adipose tissue; histological analysis; laser photothermal treatment; photodynamic action; indocyanine green; necrosis.

Paper 11596P received Oct. 15, 2011; revised manuscript received Feb. 25, 2012; accepted for publication Mar. 27, 2012; published online May 7, 2012.

1 Introduction

Obesity is characterized by an increase in adipose tissue mass. The consequences of obesity are serious and risk increases progressively as body mass index rises. Obesity is among the most important risk factors for type 2 diabetes, heart disease, stroke, and some forms of cancer. More and more diseases are being linked to obesity, including asthma, osteoarthritis, and sleep apnea.[1–3]

Abdominal obesity characterized by increased fat under the skin of the belly and between the abdominal organs (visceral obesity) is considered especially harmful, as it is particularly powerful in increasing the risk of heart disease and diabetes. It is increasingly clear that disturbed functioning of adipose tissue may be an important factor in obesity-related health risks. As people get more obese and their fat cells expand, the adipose tissue becomes less able to store fat and the body starts storing it in other tissues, such as muscle and liver, which may disturb cell function and lead to disease. Also, it has become clear that adipose tissue is a very active organ, secreting hormonal signals that may affect metabolism in other organs.[4–6]

There are many methods for removing of unwanted body fat. Laser liposuction is new and developing technology for removing fat deposits.[7–9] The application of this method is accompanied by necrosis of adipocytes which are destroyed, but not removed at liposuction.[10–12]

Near-infrared (NIR) laser radiation has a selective thermal action on fat tissue caused by rather strong absorption bands of lipids at 915, 1210, and 1720 nm.[13] Laser liposuction/ lipoplasty or laser lipolysis is one of the prospective ways to remove fat deposits.[14,15] Laser lipolysis is based on a thermal process (selective photothermolysis) allows one to heat up gently and destroy fat cells, leaving them in a liquefied state, which makes it easier to remove them, or for the body to naturally metabolize the fat. Laser lipolysis was first described in 1994.[16] This is an invasive technology; however, less trauma, bleeding and pain are among the main advantages of this technique.[17] After adequate infiltration of an anesthetic solution, a flexible fiber optic delivered through a small caliber cannula is inserted inside the fat tissue. The positioning of the 1 mm cannula is highlighted via trans-illumination from a red guiding beam. The laser energy is transmitted to the adipocytes, which absorb the energy, expand their volume, and rupture.[18] Histological analyses of the effects of the 1060 nm-Nd:YAG laser and the CW 980 nm-diode laser on human fat tissue have shown reversible cellular damage (cell tumefaction) as well as irreversible damage (cell lysis).[19–21]

Lipolysis can be defined as a partial or total destruction of fat cells to release their contents in the interstitial space.[22] The cell death can occur under the action of various physical, chemical, and biological factors. Depending on the inductor, cell apoptosis or necrosis may take place. These two forms of cell death differ by biochemical and morphological signs.[23]

The mechanisms leading to laser lipolysis are temperature dependent. First, for low laser energy and consequently low temperature increase, only tumefaction of the adipocytes is observed. When using a higher laser energy, the histological assessment, carried out in Ref. 11 on tissue samples taken...
immediately after treatment, showed rupture of adipocytes, but also the coagulation of small vessels in the fatty tissue. Because the heat is confined inside the adipocyte, it leads to the rupture of its membrane. The effect is not only thermal but also thermo-mechanical.

More importantly, the degree of tumefaction and lysis varied proportionally to the amount of energy accumulated to the target. Conventional liposuction produces less reversible damage and tumefaction, than laser lipolysis with 1000 J-light energy. For a laser having a wavelength of 1064 nm, and energy applied in the range from 1000 to 12,000 J, it was observed that the higher the energy, the greater the tissue volume reduction. Typically, a 5-cm² reduction of fat volume is observed at 3000 J. A 20-cm²-volume reduction is obtained with 12,000 J. All these studies clearly show that two major parameters must be considered for laser lipolysis: (1) The wavelength, because the interaction between the laser and tissue is achieved by the absorption of laser energy by the receptive chromophores, thus producing sufficient heat in fat tissue to cause the desired thermal damage. (2) The energy applied, because there exists a dose-response relationship. The heat affects fatty cells and the extracellular matrix to produce both reversible and irreversible cellular damage. This facilitates laser liposuction through less traumatic and bleeding, when compared with the conventional liposuction. The major result of energetic laser lipolysis is adipocyte necrosis.

Another optical method of fat tissue destruction, namely photothermal/photodynamic method, may provide reduction of regional or site-specific accumulations of abdominal or subcutaneous adipose tissue least-invasively by inducing cell apoptosis and controlled necrosis in small amounts of fat tissue. In particular, it relates to the employment of localized optical (laser) radiation of the appropriate wavelength and power, which may also be integrated in conjunction with localized specific fat tissue staining and/or lipolytic agent application, to noninvasive and non- or least-destructive downsizing fat tissue volume, and thereby modify contour/shape local target adipose tissue.

In this paper we have undertaken a histological study of subcutaneous fat tissue for indocyanine green (ICG)-mediated photothermal/photodynamic treatment of rat skin in vivo. The motivation for such a study is to find optimal NIR-laser exposures and ICG-staining technology in order to provide a controllable fat-cell lipolysis and gentle reduction of fat tissue with less uploading of waste in the organism.

2 Model and Hypothesis

In the visible and near-infrared spectral region, adipose tissue is weakly absorbing, and selective staining is needed to enhance the efficiency of interaction with light. When using exogenous chromophores efficiency of light interaction with tissue may be substantially higher. For such purposes, ICG is widely used. The ICG has a strong absorption in the NIR and low toxicity. With intravenous administration, ICG effectively binds to albumin in blood plasma, and is rapidly cleared from the plasma of parenchymal liver cells in bile. With topical administration, ICG locally binds to proteins in tissues. It was shown that strong absorption of laser radiation at a wavelength of approximately 810 nm by ICG-stained tumor cells induces local heating and subsequent destruction. The ICG may also induce photodynamic and phototoxic effects. A relatively narrow absorption peak of this dye allows for effective use in selective laser destruction of tissues, but caution should be accounted for because dye molecules interact with organic molecules of a tissue and change their absorption spectrum peaks up to 10 to 20 nm. The intensity and position of the absorption bands depend on the ICG solvents used. By dissolving ICG in saline, the dye tends to aggregate into larger molecular complexes. It also leads to a shift of the absorption peak. ICG solutions based on complex compositions, such as water-glycerol-alcohol, are more stable with significant and stable absorption peaks corresponding to monomers.

The alcohol component in an ICG-solution is also beneficial to make cell membranes more permeable for ICG because of the dissolving lipid component of a biological membrane. The next step of ICG interaction with a cell is controlled by NIR light exposure. The biological response of the photosensitized cell to light exposure can lead to bigger damaging areas in the cell membrane, and their subsequent transformation into actual pores act as gateways for free fat acids (FFAs) leakage outside the cell, because molecular size of FFAs does not exceed a diameter of 1 to 2 nm.

Cell lipolysis can be observed as an optical clearing process of photodynamically/photothermally modified fat tissue samples. Due to light-induced cell membrane porosity, the intercellular content of the cell (FFAs) percolates through the arising temporal pores into the interstitial space. As a consequence, the refractive index of the interstitial fluid (initially equal to \( n_1 = 1.36 \)) becomes closer to the refractive index inside the adipocytes (fat refractive index, \( n_a = 1.44 \)) and because of the refractive index matching effect, the optical medium becomes optically more homogeneous and more transparent to light. The following diagram shows the possible mechanisms of laser action on the photosensitized adipose tissue (Fig. 1).

Histological analysis allows one to characterize the processes occurring in cells and tissues and their structural features. In the histological analysis of the fatty tissue treated with the laser, areas of reversible cellular damage (tumefaction), irreversible cellular damage (lysis), and a reduced intensity of

![Fig. 1](https://ebooks.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics-May2012/images/Fig_1.png)
bleeding were observed. Histological analysis of the effects of photothermal/photodynamic action on adipose tissue should be also of great interest for optimization of controllable fat cell damage.

3 Materials and Methods

A group of ten 2-year old female rats were kept for 14 days in accordance with the European Convention for the Protection of animals used for experimental and other scientific purposes. In addition to the standard diet, the animals received 5 g sugar, 5 g dry milk, 5 g of sunflower oil, and 5 g of egg powder to cause alimentary obesity. Animals were divided into two groups of five individuals. Hairs were removed within the region of ribs on the back, where treatment was carried out. Each sampling zone was about 1 cm in diameter (Fig. 2). The first zone (zone 1) was used as a control and this skin site was not administered any treatment (no stain, no radiation) (Fig. 3). In zone 2, the ICG in concentration 0.5 mg/ml was injected subcutaneously. The first group of rats was injected with ICG dissolved in saline (solution 1), while to the second group was injected with ICG dissolved in a mixture of 25% alcohol, 25% glycerol, and 50% distilled water (solution 2). Zone 3 was irradiated with a diode NIR-laser (VD-VII DPSS LASER DRIVER, 808 nm); power density, 16 to 24 W/cm². The exposure time ranged from 0.5 to 8 min. A wide range of exposures was used in preliminary testing experiments to determine the appropriate dose effects. Exposure ranged from 0.5 to 2 min during the main experiment. An optical fiber tip delivering laser radiation was directed perpendicular to the tissue surface. Within zone 4, ICG (solution 1 or 2 of the similar concentration) was injected first, and then irradiated with a diode NIR-laser (808 nm). Anesthesia of rats was carried out with a zoletil solution. Zoletil was administered intraperitoneally at a dose of 80 mg/kg. The rats were decapitated after 1 h of exposure. Pieces of skin and subcutaneous adipose tissue from zones 1 to 4 were fixed in a 10% formalin solution. Paraffin sections were stained with hematoxylin-eosin (H&E) accordingly to standard technique. Specimens of skin with subcutaneous tissue were cut across all layers. To evaluate the degree of photothermal/photodynamic treatment, morphological examination of the received histological materials were performed.

Statistical data analysis was performed using “Statistics 6.0,” in particular, Cochran’s $Q$ test, a nonparametric test which is applied to the analysis of two-way, randomized block designs where the response variable can take only two possible outcomes (coded as 0 and 1). It provides a quick test of whether $k$ treatments have identical effects simply by counting the number of times each treatment is better than the others.

4 Results and Discussion

4.1 Selection of Parameters of Laser Radiation

At the initial stage, we searched for the radiation dose that resulted in minimal damage in the nonstained tissue. For skin site irradiation using the maximal power density of 24 W/cm² and maximal exposure time of 8 min, histological preparations showed marked tissue destruction with irregular shape, erased boundaries between the epidermal cells, and fragmented cell nuclei. There was marked desquamation of the stratum corneum in these samples. In the dermis, marked signs of destruction of connective tissue were also seen as homogenization and swelling of the fibers and destruction of cell nuclei. For exposure times of 1.2 min and less, and the same laser power density (24 W/cm²), the signs of skin damage were expressed to a much lesser extent in corresponding histological preparations. Major changes were localized in the dermis and presented by swelling of connective tissue fibers without their destruction. The smallest changes were observed with the minimal impact at laser power density of 16 W/cm² with an exposure time of 0.5 min.

4.2 Histological Analysis of Control Samples

The samples were skin slices with a layer of subcutaneous adipose tissue. In the control samples (zone 1) no change in the epidermis and dermis (Fig. 4), or subcutaneous adipose tissue (Fig. 5) were seen. The epidermis is normally thin and cells do not have any degenerative changes. The dermis is represented by collagen fibers, which are distributed in different areas and located close to each other. The contours of the fat cells were distinct. Muscle fibers with distinct cross striation were also seen. Skin appendages were not changed.
4.3 Histological Analysis of Samples after Injection of Dye

The first group of rats were subcutaneously injected with ICG dissolved in saline (solution 1), while the second group was injected with ICG dissolved in a mixture of alcohol, glycerol, and water (0.25:0.25:0.5 ratio) (solution 2). In the samples corresponding to zone 2, i.e., the subcutaneous injection of ICG solution 1, no essential changes in the epidermis and dermis were observed [Fig. 5(a) and 5(b)]. However, in the dermis of the first group there were pockets of dissociated collagen fibers. This is probably due to mechanical impact of water caused by osmotic phenomenon. In most cases, fat is not altered. Fat cells have the form of optically transparent vacuoles with a thin shell [Fig. 6(a)]. In some parts, the cell membrane is destroyed. For animals of the first group, normal epidermis cells are typical. In contrast, animals of the second group, under the influence of alcohol and glycerol (solution 2), a reduction of the thickness of the maintained epidermis and collagen-elastin (protein) matrix was found [Fig. 5(b)]. Injection of ICG solution 2 leads to swelling of adipocytes, and in some cases, to their necrosis [Fig. 6(b)]. In the dermis, there is swelling, comparable in severity to group 1. The samples presented some foci of necrosis, and necrotic fat cells revealed cellular infiltration of white blood cells, plasma cells, and macrophages [Fig. 6(c)].

Fig. 4 Histology (H&E staining) of non-treated skin with subcutaneous adipose and muscle tissue. Control sample. Objective 4x.

Fig. 5 Histology (H&E staining) of skin (epidermis and dermis) when ICG solution 1 (0.5 mg/ml in saline) injected subcutaneously (a), objective 10x and objective 4x (b).

Fig. 6 Histology (H&E staining) of subcutaneous adipose tissue when ICG (0.5 mg/ml) injected subcutaneously: (a) solution 1 (ICG in saline); (b) and (c) solution 2 (alcohol:glycerol:water = 0.25:0.25:0.5) at different sites; (c) one of the site, where necrotic zones (marked by rectangles) with cellular infiltration of white blood cells, plasma cells, and macrophages are seen. Objective 40x.
the cells were located around plethoric blood (full-blooded) vessels. At the site of the dye solution, local separation of collagen fibers in the dermis, and formation of optically transparent (unstained by H&E) areas were seen upon histological examination (Fig. 7).

Described fat cell morphology alterations are associated with the impact of the alcohol/glycerol mixture. This confirms our hypothesis that alcohols may induce reversible/irreversible cell membrane damage with pore formation.45–47

4.4 Histological Analysis of Samples after Laser Irradiation

In the samples corresponding to zone 3, after NIR diode laser irradiation (power density of 16 W/cm² and exposure time 1 min), we observed a slight swelling and changes in the epidermis in the form of cell destruction—acanthosis (Fig. 8).

The epidermis is maintained. In the dermis, edema is not evident. Collagen fibers are compact and somewhat thickened. Fat cells are increased in size, have a more elongated shape than in the controls. Cell matrix is intensively stained with hematoxylin (Fig. 3). In comparison with the control sample [see Fig. 4(b)], changes in subcutaneous fat were significant. Because of transmembrane cell lipolysis, fat cells change their shape. The appearance of macrophages indicates the inflammatory response [Fig. 4(a)].54,55

In this case, changes in fat cells are noticeable but not significant because the adipose tissue does not absorb infrared radiation strongly, which also confirms our hypothesis.

4.5 Histological Analysis of Samples after ICG Subcutaneous Injection and Laser Irradiation

In the samples taken from the skin site in zone 4 after subcutaneous injection of ICG solution 1 (ICG 0.5 mg/ml in saline), the superficial layers of the epidermis were typically damaged with preservation of the basal layer. Necrosis of the upper layers of epidermis can be explained by remained traces of the solution at subcutaneous injection site, which was subjected to laser irradiation. In the dermis, swelling of collagen fibers occurred which is associated with their dissociation. In some cases, the collagen fibers were thickened and had a homogeneous form, which may be an expression of degenerative processes [see Fig. 10(b)]. There are collagen fibers in a state of fibrinoid necrosis.

In the samples taken from the skin site in zone 4 after subcutaneous injection of ICG solution 2 (ICG 0.5 mg/ml; alcohol:glycerol:water = 0.25:0.25:0.5) and subsequent diode laser irradiation (power density of 16 W/cm² and exposure time of 1 min), necrosis is registered in the epidermal cells with papilliform outgrowths, which are then exfoliated [see Figs. 10(b) and 11]. These processes are similar to those found in Ref. 56, where after skin ablation by a high energy single pulse crater appeared, a somewhat lower energy pulse
for extrusion of the skin material existed, and for the lowest energies, the drying and forming the deflection surface occurred. In our experiments, because of selective ICG staining, necrosis was extended to the dermis [Fig. 10(b)] and subcutaneous adipose tissue (see Fig. 12). The ICG-sensitized fat cells absorbed infrared radiation effectively and induced a photothermal/photodynamic effect that led to cell death; this experimental finding also confirms our hypothesis.

In this study we could not separate photodynamic and photothermal reactions, because we were looking only for biological effects, such as cell damage with follow-up lipolysis and necrosis. Basing on our previous in vitro studies, where thin slices of adipose tissue sensitized with ICG water-ethanol solution (1 mg/ml) were irradiated for 1 min by NIR laser at 808 nm with 250 mW/cm² at constant temperature (thermostat, T = 40°C), and where cell lipolysis occurred within 50 min...
Table 1  Statistics for the necrosis appearance in subcutaneous adipose tissue determined from histological studies of rat skin sites at different treatments in vivo.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Zone 1 (control)</th>
<th>Zone 2 (dye)</th>
<th>Zone 3 (laser)</th>
<th>Zone 4 (dye and laser)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solution 1</td>
<td>Solution 2</td>
<td>Solution 1</td>
<td>Solution 2</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>% of cases</td>
<td>0%</td>
<td>20%</td>
<td>40%</td>
<td>20%</td>
</tr>
</tbody>
</table>

after laser irradiation, we can hypothesize that photodynamic action may also have some inclusion in cell damage in our in vivo studies. We understand that both experiments are not easy to compare; however, we have to account for a much higher power density which is applied to the skin surface in the vicinity of a delivering fiber tip in in vivo experiments, and the power density should be much less for tissue depth in animal studies. We must also account for a 35°C body temperature. These two reasons may impact both in vitro and in vivo studies, and the range of parameters which allows for a photodynamic reaction to be possible on the background of the possibly stronger photothermal reaction. Evidently, some synergy of these two reactions is also possible as they are both on the level of primary photophysical/photochemical processes, as well as on the level of biological responses. Because all key processes, such as dye and oxygen diffusivity and cell membrane permeability, are temperature-dependent, cell damage due to photodynamic action may help for thermally-induced damage and vice versa.

The Cochran $Q$ test can be used to evaluate the relationship between two variables that are measured on a nominal scale. One of the variables may consist of only two possible values (dichotomous scale). In our case, six different combinations of treatment were evaluated with 10 laboratory animals. The scale of evaluation was dichotomous; the necrosis in subcutaneous adipose tissue either was present in the histological samples (=1) or did not present (=0).

The value of $Q$ becomes greater if there is statistical association between the variables. If there is no association and only chance is operating, $Q$ reaches exactly the same values as Chi squared ($\chi^2$-distribution). The degree of freedom equals to $k - 1$, where $k$ is the number of cases or treatments, thus for our study we have degree of freedom = 5. On the basis of data presented in Table 1, the $Q$ value of 17.25 was calculated using the “Statistics 6.0” program. It is known that results would be significant when the $Q$ has a higher value than 15.09 for significance level $p = 0.01$. Therefore, the Cochran $Q$ test shows that the empirically found difference between the treatment alternatives is statistically significant, because in our case $Q > 15.09$. So, the combined treatment of the dye and the laser (last column in Table 1) is more effective in comparison with the other treatments. The level of significance of such a conclusion is rather high, $p = 0.01$.

5 Conclusion

Based on the analysis of histological examination of tissue samples taken from the ICG-stained subcutaneous tissue after NIR laser treatment, we may conclude the following:

1. When injected subcutaneously, ICG dissolved in saline produced changes in tissues that were minimal and characterized by signs of swelling.

2. When injected subcutaneously, ICG dissolved in the alcohol mixture produced similar swelling in tissues, and the development of necrosis with an inflammatory reaction was observed around them.

3. The major features of the morphological alteration in tissues caused by laser radiation were associated with an increase in the size of fat cells and destruction of the cell membrane in individual fat cells.

4. The combined action of ICG and NIR laser radiation causes the development of the most pronounced changes; it affects all skin and subcutaneous layers: epidermis, dermis, fat, and muscle tissues. The epidermis shows dystrophy and necrosis, and desquamation of epithelial cells. In the dermis, there is disorganization of the connective tissue in the form of fibrinoid swelling and necrosis. The subcutaneous adipose tissue presents widespread necrosis.
The data obtained are important for safe layer-by-layer dosimetry of laser irradiation used in the photothermal/photon-mediated treatment of obesity and cellulite.

To provide adipose tissue cell lipolysis, apoptosis, or necrosis with no damage to upper skin layers, selective staining of adipose tissue, for example by using a multiple injections directly to target adipose tissue, washing out dye residuals from the skin surface, and its follow up cooling before laser irradiation should be provided. Besides ICG, other absorbers, such as gold nanoparticles with a tuned absorption band to 810 nm and their mixtures with ICG, are prospective.

Acknowledgments
This study was supported in part by RFBR Grant Nos. 10-07-00526-a, 224014 PHOTOSENS4LIFE of FP7-ICT-2007-2, Project No. 1.4.09 of RF Ministry of Education and Science, RF Governmental contracts No. 02.740.11.0879, and 11.519.11.2035; FiDiPro, TEKES Program (40111/11), Finland; and RF President’s grant NSh-1177.2012.2.

References
27. V. V. Tuchin et al., “Fat tissue staining and photodynamic/photothermal effects,” *Proc. SPIE* 7563, 75630V (2010).