Using a “Non Uniform Pulse Sequence” can Improve Selective Coagulation With a Nd:YAG Laser (1.06 μm) Thanks to Met-Hemoglobin Absorption: A Clinical Study on Blue Leg Veins

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Background and Objectives: Evaluation of the efficacy, on 1–2 mm blue leg telangiectasia, of a 1,064 nm Nd:YAG laser emitting in a non uniform pulse sequence calculated to consider Met-Hb formation during laser irradiation of a blood vessel.

Materials and Methods: A 1,064 nm Nd:YAG laser (Quantel Medical, Athens, France) was used in a non uniform pulse sequence mode, fluences: 300–360 J/cm² spot: 2 mm, + 5 °C contact cooling. The clinical evaluation was performed on 11 female patients, average age: 43 (25–57) years, phototype I–VI. All subjects were previously examined with Doppler ultrasound. A treatment site (6 × 4 cm) was selected on each patient. The topography of the vessels network was reported on a tracing plastic frame before each session and 6 weeks after the last one. These frames were digitized and the number of vessels was determined using the Digitized Tracing Frames Technique. Side effects were noted before and after every treatment, and 6 weeks after the last one. This study lasted for 10 months.

Results and Discussions: Patients tolerated the procedure without anesthesia. Moderate pain, transient erythema and edema, one hyperpigmentation and one matting were noted. There was no hypopigmentation. 55% (P < 0.002) vessels clearance after one session, 86% after two sessions (P < 0.001), and 98% (P < 0.001) after three sessions were obtained. On two patients, the treatment was completed after two sessions with a full clearance. Data reported in this study were obtained thanks to a computerized calculation of vessels clearance. They are similar or superior to those reported in the literature about 1,064 nm Nd:YAG lasers and leg telangiectasia.

Conclusions: Since, it was developed to consider the modification of blood absorption and the methemoglobin formation which leads to an increase of the 1.06 μm wavelength absorption, the non uniform pulse mode emphasizes the efficacy of this 1,064 nm Nd:YAG laser concerning the treatment of blue leg veins telangiectasia between 1 and 2 mm. This mode gives the possibility to deliver high energy while preserving the surrounding tissue and leads to a rapid vessel clearance with reduced pain and few side effects when compared to previously published clinical studies using a 1.06 μm laser. Lasers Surg. Med. 32:160–170, 2003. © 2003 Wiley-Liss, Inc.
transfer of heat from the target to surrounding tissue. This third possibility can be considered as the worst solution to obtain selective destruction of a blood vessel.

The third and last phase is the tissue denaturation process. This last phase determines the final damage. Several essential phenomena must be taken into consideration here: not all tissues react the same way to a rise in temperature. This is due to the structure of their protein content. For example, egg white cooks more quickly than egg yolk [1]. This stage produces a change in the tissue structure and in its optical properties. Egg white goes from translucent to white on cooking. It is generally accepted that optical absorption increases after this phase by a factor of 3–4 [2,3]. The higher the increase in temperature, the quicker the tissue is denatured, but tissue denaturing takes place mainly after the laser has been fired. Indeed the effect continues until the tissue temperature has returned to normal. This phenomenon was particularly studied by Welch. It is schematically illustrated in Figure 1 [4]. The important observation is more than 50% of the final tissue damage is obtained after the laser has been switched off.

These fundamentals give rise to some important conclusions: (i) tissue change caused by heat induces better light absorption and thus improves target selectivity as compared to surrounding tissue which has not been changed; (ii) the change is only obtained several tens or even several hundreds of milliseconds after the laser pulse has stopped.

**Oxy-Hemoglobin (Hb-O₂) and Desoxy-Hemoglobin (Hb) to Methemoglobin (Met-Hb) Conversion**

When considering a blood vessel, several studies have demonstrated that heating induces hemoglobin modification [5,6]. The change is due to oxidative reactions with formation of met-hemoglobin. When blood is subject to heating, the first observable event is Met-Hb formation, followed by distorted heme protein formation and protein

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**Figure 1a:** Each tissue has a curve showing the thermal damage as a function of temperature.

**Figure 1b:** Rise in tissue temperature. At the end of the laser pulse, the temperature decreases.

**Figure 1c:** Thanks to the curve in figure 1, resulting damage can be linked to temperature. The higher the temperature, the larger the damage.

**Figure 1d:** The final damage is the sum of damage obtained at each different temperature. It can be seen that the greater damage is produced after the laser pulse has been fired.

Fig. 1. Computation of the damage Ω. Data from reference Welch [4].
denaturation. Hb-O2 and Hb are converted to Met-Hb [5,7]. Met-Hb is readily denatured and precipitates via hemochrome formation [5]. Even under mild heating (50–54°C), Met-Hb is formed from Hb-O2 and Hb. This phenomenon is responsible for the change of blood absorption after laser irradiation as clearly demonstrated by Randeberg et al. [8]. They have shown that the change in the blood absorption coefficient due to Met-Hb has a maximum at approximately 72°C.

Absorbance of hemoglobin species including oxy, deoxy, carboxy, and methemoglobin has been studied in the visible and the near infrared regions up to 2,500 nm by Kuenster and Norris [9]. Using their data, Met-Hb has an absorbance about \( \times 4.75 \) higher than that Hb-O2, and Met-Hb has an absorbance about \( \times 20 \) higher than that Hb (Fig. 2). These values are similar to those reported by Barton et al. [7], respectively \( 3 \times \) higher for met-Hb versus Hb-O2 and about \( 13 \times \) higher for Met-Hb versus deoxy-Hb.

This phenomenon can explain why the infrared laser (Diode or Nd:YAG) are efficient in treating leg veins. The optical coefficients for blood (Hb and Hb-O2) usually used for calculation of light extinction inside the target, do not consider the blood absorption modification during the treatment. When considering the Met-Hb, it is evident that the infrared wavelengths can be well adapted for leg vein treatment. After considering the optical absorption of blood and its different hemoglobin species, it becomes interesting to consider a single pulse versus a pulse sequence.

**Role of a Pulse Sequence**

The principle of a pulse sequence is the following: using one light pulse implies that the user must choose an energy (or fluence) which is a compromise between efficiency at the target and safety for the surrounding tissue. This energy can be described as critical (100%). The reinforcement of tissue absorption obtained by the tissue denaturation process is not used.

The aim of a pulse sequence is to improve the selective action of the laser. Instead of firing all the energy in one pulse, with the inherent risk of overdosing and damaging adjacent structures, several pulses of lower energy levels can be used. One can distinguish two different techniques: (i) a pulse sequence of identical height or width, (ii) a pulse sequence of varying height or width also called a “non uniform pulse sequence.”

**Sequence of Identical Pulses**

This technique uses a series of identical pulses with an energy level much lower than that of a single pulse (critical 100% energy). Usually pulses with an energy of only 50% of the critical level are used. The risk of damaging surrounding tissue is minimized. The time between the first and the second pulse is determined so that tissue denaturation process can take place. The second and subsequent pulses then enter a tissue that has an increased absorption compared with surrounding tissue. The total energy delivered is much higher than can be delivered in a single pulse.

With this technique, the progressive build up of heat causes a progressive increase in target tissue temperature (mainly due to a better absorption by the target tissue, blood for example), the level of which must be controlled, either by increasing the time between pulses (which lengthens treatment time), or by reducing the energy of each pulse (in which case the first pulse is not sufficient to heat up the target and change its optical properties). The Figure 3 illustrates this principle.

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![Fig. 2. Absorption spectrum of each of the hemoglobin species: A is carboxyhemoglobin, B is desoyhemoglobin (Hb), C is oxyhemoglobin (Hb-O2), and D is methemoglobin (Met-Hb) (data from reference Kuenstner and Norris [9]).](image-url)

![Fig. 3. Conventional pulse sequence. The increase of absorption due to tissue denaturation also produces an increase in temperature which becomes progressively higher in the target tissue as compared to the surrounding tissue. The global increase in temperature after each pulse can also be observed. (Principle of the vasculight (ESC-Lumenis)).](image-url)
Non Uniform Pulse Sequence

This solution has been proposed first by Mordon in 1986 [10]. This new approach has been particularly studied by Glenn et al. [11]. They have demonstrated that in order to maintain a relatively “constant” temperature inside the vessel, a non uniform pulse sequence would be required. In the study published by Glenn et al., issues of coagulation control were considered by simulating a controlled temperature irradiation using thermal feedback to execute an appropriate temporal profile for laser power. It appeared that ‘constant’ temperature irradiation avoided high temperature effects and achieved relatively slow, steady progression of damage, allowing control of coagulation front position. Furthermore, the difference in damage prediction was much less for a “constant” temperature irradiation than for a free running laser irradiation [12]. For the particular application of leg vein treatment, an x3 increase of blood absorption was considered based on data reported by Barton [7]. Consequently, in the non uniform pulse sequence implemented in a Nd:YAG laser, the second and the third pulse should have an energy three times lower than the first one (Fig. 4). This principle is patented: US 3,330,517.

To summarize, the advantages of a non uniform pulse sequence are: (i) a great increase of the temperature differential between the treatment site and surrounding tissue; (ii) a relatively “constant” temperature inside the target, since blood absorption modification is considered; (iii) a considerable reduction of the treatment time because it is no longer necessary to wait between pulses to avoid a too high temperature increase inside the target. Consequently, more energy can be deposited for a larger or deeper effect while preserving non-targeted structures.

Based on these considerations, this study aimed to evaluate the treatment of 1–2 mm blue leg veins using a Nd:YAG laser modified to emit a non uniform sequence of pulses.

MATERIALS AND METHODS

Laser

In this study, a 1,064 nm Nd:YAG laser (Quantel Medical) was used. This laser has a non uniform emission delivery mode (called Multipulse™ mode). It consists of three 3.5 millisecond pulses, with a 250 milliseconds delay between each pulse. In this sequence of three pulses, the energy of each pulse is different: the energy distribution is explained in Figure 4: 60% of the total energy is delivered in the first pulse, 20% in the second pulse, and 20% in the third one.

The light was delivered with a hand-piece giving a 2 mm diameter beam on the surface of the skin. Spots were juxtaposed by tracing the vessel. A contact cooling device was associated (Fig. 5). This cooling device is composed of a sapphire plate drilled by a hole of 2 mm of diameter and surrounded by a steel duct chilled by a cryogenic gas. The cooling temperature was set at +5°C. The laser beam was fired through the hole. Total fluence (3 pulses) applied on skin was adjusted from 300 to 360 J/cm² as a function of vessel diameter and phototype.

Digitized Tracing Frames Technique (D.T.F. Technique)

The topography of the vessels network was reported on a 6 × 4 cm rectangular transparent plastic tracing frame.
before each session. To further ensure reproducibility at each stage of the procedure, a new tracing frame of same size was used systematically and positioned using anatomical landmarks selected at the first session and noted on the frames. The tracing of the vessels on the plastic frames was performed by the same physician (NF) with the same felt pen (Fig. 6) and were overseen by an independent observer (SM). When performing the tracing, the veins were illuminated with a white light model SNL 319 (Waldmann, Villingen, Germany) placed above. These plastic tracing frames were then digitized using a graphic tablet (Wacom Technology Corporation, Vancouver, WA, USA). This allowed copying the vessels noted on the frame, into a computer giving a digital picture of the original tracing frame. The number of vessels and their lengths (through the number of pixels that each of them represents), were then automatically determined using this imaging software (Sigma Scan Pro 5, SPSS Science, Chicago, IL, USA) allowing calculation of the percentage of disappearance of the vessels after each session.

Photographs
Photographs were taken pre-op, immediately post-op and at 6 weeks for each session using a digital camera (Nikon Coolpix 950). The image size was $8 \times 6$ cm.

Doppler Ultrasound
Every patient had first a doubled clinical examination (NF and DB). All subjects were examined clinically and using a Doppler ultrasound, model Katana (5, Hitachi, Tokyo, Japan) by one author (DB, Angiologist) in order to confirm that saphenous veins and collaterals were competent with no reticular and no perforant veins.

Clinical Protocol
Contra-indications for enrolment were as follows: pregnancy, age under 18, patients with blue deep telangiectasia, patients suffering from dermatosis associated with Koebner’s signs, usual contraindications of vascular lasers. For each patient, age, sex, prototype were recorded. Phototype was evaluated using Fitzpatrick’s classification (I–VI). All of the patients signed a consent form, which was approved first by the local ethical committee. Sun exposure was totally forbidden two months after treatment, but not before.

Procedure and Follow up
Treatments were carried out within a $6 \times 4$ cm rectangular area. Treatments were performed without anesthesia. The treatment consisted in juxtaposing spots along each vessel one spot consisting of three pulses within a Multipulse sequence. The clinical endpoints were an immediate change of the vessel aspect or a complete or partial disappearance of it. A slight overlapping and several passes were accepted till the clinical endpoint was reached. Immediate or late adverse effects observed after and just before each treatment were systematically noted before and after every treatment (1-no effect, 2-erythema, 3-oedema, 4-blisters, 5-hyperpigmentation, 6-hypopigmentation, 7-purpura, 8-skin whitening, 9-immediate vessel disappearance, 10-immediate noticeable change in appearance of vessel, 11-matting). Pain experienced by the patient, during the treatment, was evaluated on a scale going from 1 to 4 and recorded (1-none, 2-minimal, 3-bearable, 4-unbearable). Patient’s satisfaction scale was recorded mainly taking into account the improvement obtained (1-none, 2-improvement in color/texture of the vessel, 3-partial disappearance, 4-complete disappearance). The treatments were performed every 6 weeks and were checked 6 weeks after the last treatment; so, on the whole, the study lasted for 10 months. Using the same parameters for every treatment, re-treatments took place every 6 weeks until vessel disappearance based on patient’s estimation. Maximum number of treatments was limited to three. At each session, digital pictures were taken and a plastic frame was traced.

![Fig. 6. Digitized tracing frame technique: the tracing of the vessels is performed on a plastic frame. This plastic tracing frame is digitized using a graphic tablet (Wacom). The number of vessels and their lengths (through the number of pixels that each of them represents), are then automatically determined using this imaging software (Sigma Scan Pro 5) allowing calculation of the percentage of disappearance of the vessels after each session.](image-url)
Data Analysis

All data were analyzed by an independent statistician. Whenever possible, and depending on the level of data distribution, Student or Wilcoxon tests were performed in order to highlight results.

RESULTS

Eleven female patients, aged from 25 to 57 years (average: 43 years) with phototypes from I to VI (1 phototype I, 4 phototypes II, 5 phototypes III, no phototype IV, and V and 1 phototype VI) were enrolled. All vessels were blue or violin ranged from 1 to 2 mm and had no previous treatment. Reflux of the sapheno-femoral and sapheno-popliteal junctions was ruled out by performing Doppler ultrasound before enrolment. For 11 patients, leg veins were located on thigh (5), on legs (2), on knee (2), and on ankle (2). Overall 28 treatments were performed, 1 patient had 1 treatment, 5 patients had 2 treatments, 6 patients had 3 treatments. The interval between sessions was 6 weeks. The number of treatment was decided according to the results obtained (stop when disappearance) and the satisfaction (stop when 4) of the patient.

Pain experienced was quoted by the patient 2.3 at the first treatment, 2 at the second, 1.4 at the third. Immediate adverse effects: erythema was observed in 18/28, edema in 2/28, and immediate change of aspect of the vessels or disappearance in 17/28. No whitening of the skin was noted. Erythema was staying few hours or days. Edema was transient, localized to the area over the vessel treated and lasted a few hours, only.

There was no correlation between the fact of getting good results and obtaining an immediate disappearance of the vessel or any immediate change of aspect, which where in this study considered as endpoints.

Late adverse effects: hypopigmentation was never observed. One long term hyperpigmentation was observed on phototype VI. Matting was found in 1/28 particularly and it stayed definitely. No blister, no purpura, no scar, no textural change on the surrounding of treated vessels was observed. No thrombosis were seen post treatment.

Patient’s satisfaction was 3.3 before the second treatment, 3.5 before the third treatment, 3.5 before the fourth treatment. This indicates a high level of satisfaction for the majority of the patient.

Results of the tracing frames: after 1 treatment, clearance of the vessels was 55% ($P < 0.002$). It reached 86% ($P < 0.001$) 6 weeks after 2 treatments, 98% ($P < 0.001$) 6 weeks after 3 treatments (Figs. 7 and 8). The vessels cleared completely after 2 sessions for 2 patients. Figures 9 and 10 show clinical results and the corresponding tracing frames.
Six months after the last treatment, no recurrence was noted. There was no correlation between the skin type and the success rate.

**DISCUSSION**

Higher fluences are necessary to get high clearance rates of leg veins telangiectasia from 0.5 to 3 mm with Nd:YAG lasers than with other wavelengths, for instance 200–350 J/cm² in the study of Suthamjariya, and 320–350 J/cm² in Coles’ one [13,14]. However, these high energies with a single pulse emission lead to a considerable pain for the patient even using an effective cooling. But reducing the energy also seems necessary for darker or tanned skin. In our study, we used 300–360 J/cm² with a 2 mm spot. The matting observed on one patient was attributed to a too high energy used on a too small diameter of vessels.

The usual immediate responses considered as a clinical endpoint in most of the published studies are vessel disappearance (vasospasm), or changing aspect and color (thermocoagulation). Sometimes multiple passes in the same session are necessary to get these effects. In our study, only 17/28 sessions showed this kind of clinical effects, despite 98% of vessels disappearance after 3 sessions over all the patients. The lack of immediate clinical visible endpoint is particularly true when the treated vessel is small (1–1.5 mm). This should be related to the non uniform pulse mode which confines heat inside the vessels. This means that when high fluences are delivered using the non uniform pulse mode, multiple passes are not required even if no clinical endpoint is noticeable. The risk of unnecessary passes is to destroy the surrounding tissues of the treated vessel and induce unwanted effects.

Side effects include crusting, purpura, postinflammatory hyperpigmentation. Long term hyperpigmentation and matting are related to the individual response and the type of vessels treated, specially with large vessels. Efficacy and risk of side effects are related to fluence, spot size, and length of pulse. Although other visible wavelengths have shown some efficacy to treat small leg veins up to 1 mm in diameter, they failed on larger vessels [15,16]. Long pulsed Alexandrite lasers for instance provide good results on larger leg veins but bring long term dyschromia, and cannot be used on tanned skin or high phototypes [17]. A comparative study of 1064, 810, and 755 nm lasers was done by Eremina on 0.3–3 mm leg telangiectasia of 22 women, phototype I–V, and a follow up of 3 months [18]. Results were graded as percent resolution on photographs and clinically. 75% improvement was observed at 88% of the Nd:YAG sites compared to 29% with the diode sites and 33% at the Alexandrite sites. Purpura and matting were a significant drawback for the Alexandrite. Pain was sometime sufficient for patient to decline further treatment. He is concluding that the 1,064 nm is very safe and efficient for these vessels diameters despite the high pain induced, but the superlong pulsed diode 810 nm gives unpredictable results and the usefulness of the Alexandrite is limited by the large amount of important unwanted effects.

In the study of Coles et al., pain with laser treatment was the most common reason for subjects to prefer sclerotherapy, but on the other hand, they preferred the Nd:YAG laser for the degree of vessel clearance and the absence of long term hyperpigmentation [13]. This author confirms that with a single pulse shot, pain is related to the highness of the fluence used. In the beginning of her study she applied 150–190 J/cm² and 50–100 milliseconds, 3–5 mm
spot with the coolspot hand-piece and a cold gel on vessels from 1–3 mm. Seventeen of her 20 patients graded the pain from moderate to severe, and had a tendency to rate the pain higher on consecutive treatments. She even tried to use topical anesthesia on 2 patients, but this brought no improvement on pain relief, as it is also mentioned in other studies. She concluded that it was most helpful to use smaller spot sizes to reduce the pain despite using higher...

Fig. 9. Patient 13: Before, after 2 and 3 treatments.
fluences to make the treatment more tolerable. She obtained good results with 1.5 mm spot, 15–30 milliseconds, 320–350 J/cm². These fluences and this spot sizes are very close to the ones we used in our study where the patients graded the pain 2.3 at the first session, 2 at the second one, and 1.4 at the third session. This is in favor of the pain relief given by the cooling device, the special way of delivering the energy due to the non-uniform pulse mode. Another point is also remarkable in our study: pain is decreasing in relation to the increase of the number of sessions. It is mainly due to the number of vessels decreasing, as there is less target to the beam.

The degree of vessels clearance after 3 sessions was very high (98%) in our study compared to the percentages previously published with another 1,064 nm laser. On 24 subjects, phototypes I–IV, 50–95% of clearance was obtained on 0.5–4 mm vessels, at 3 months follow up based on visual ranking of severity [14]. Bowes in her study tried one Nd:YAG laser 25 milliseconds, 6 mm spot, 125 J/cm², compared to another laser with a 5 mm spot, 200 J/cm², 25 milliseconds [19]. Results were 75–72% vessel clearance one month after the last treatment depending on the cooling system of the laser utilized.

Adrian in his study compared the parameters of two Nd:YAG lasers. All vessels from 0.5 to 3 mm showed some response. Large vessels respond better to longer pulse duration at equivalent fluences [20].

Rogachefsky has treated 0.25–4 mm vessels, from all colors, on 10 patients, 2 sites each, phototypes I–IV, two times at 4–6 weeks interval, with fluences 90–187 J/cm² and 10–50 milliseconds pulse duration, with a follow up of 3 months [21]. 71% of the vessels had a significant improvement. A 62% rate of postinflammatory hyperpigmentation was observed at 3 months. Sadick recommended the 1,064 nm to treat leg veins 1–4 mm in diameter in its bimodal approach of dual short and long wavelengths [22]. Kauvar had determined clearance rates using a quartile grading system based on standardized 35 mm photographs [23]. She treated 20 sites, phototypes I–IV, with fluences from 150 to 160 J/cm² and 30–50 milliseconds pulse

Fig. 10. Patient 8, before, and 6 weeks after 3 treatments.
duration. Long pulsed 1,064 nm lasers can bring high clearance rates on vessels 0.5–3 mm. Veins greater than 1.5 mm often clear in one session.

In Omura’s study, 20 patients received a single treatment on 1–3 mm leg veins with 100 J/cm² and 50 milliseconds pulse duration, 10 mm spot size, and a contact cooling device [24]. He had some clearance on all sites. 67% of treated sites had 76–100% clearance 3 months after the treatment. They experienced two weeks of bruising (21%) immediately after and a local inflammatory reaction. Some developed tender thromboses (33%) and required needle aspiration. Larger vessels improved better than smaller. At last, 17% had hyperpigmentation.

Lupton compared sclerotherapy and long pulsed Nd:YAG laser treatment on 20 patients [25]. He obtained a better response on very small vessels by sclerotherapy in fewer sessions. The two groups had minimal and equivocal sequelae with mainly post inflammatory hyperpigmentation.

Weiss in his study with a multiple synchronized pulsed 1,064 nm laser confirms that this wavelength gives better results on blue leg telangiectasia from 0.5 to 3 mm than shorter wavelengths [26]. He had among all his patients 42% of transient hyperpigmentation, very similar to post-sclerotherapy hyperpigmentation, but this resolved in 72% of the cases at 3 months follow up. In our study, only one case of hyperpigmentation was observed on a phototype VI. This skin was treated with the lowest fluence, 300 J/cm², but nevertheless we finally obtained a long term hyperpigmentation due to the phototype. However, no hyperpigmentation was noticed on phototypes I–III.

To summarize, these various clinical studies demonstrate that Nd:YAG laser emitting a high energy single shot (50–100 milliseconds pulse duration) provides a safe and effective treatment for vessels from 1 to 4 mm of diameter while sparing the epidermis but is associated to pain and side effects (hyperpigmentation).

This clinical study demonstrates that the non uniform pulse mode is able to deliver high energy, but with a much better protection of the surrounding tissues and consequently with a very moderate pain and less side effects. Three main reasons can explain the efficacy of the particular non uniform pulse sequence: (i) it was developed to consider the modification of blood absorption and the Met-Hb formation which leads to an increase of the 1.06 µm wavelength absorption by a factor 3, (ii) the duration of this non uniform pulse sequence is 500 milliseconds and consequently matches the Thermal Relaxation Time of a 1–2 mm blood vessel, (iii) much more energy is deposited inside the blood vessel (typically 300–360 J/cm²) when compared to the previous studies using a similar wavelength.

CONCLUSION

Since, it was developed to consider the modification of blood absorption and the Met-Hb formation which leads to an increase of the 1.06 µm wavelength absorption, the non uniform pulse mode emphasizes the efficacy of this 1,064 nm Nd:YAG laser concerning the treatment of blue leg veins telangiectasias between 1 and 2 mm. This mode gives the possibility to deliver high energy while preserving the surrounding tissue and leads to a rapid vessel clearance with reduced pain and few side effects when compared to previously published clinical study using a 1.06 µm laser.

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