Title
NONINVASIVE BLOOD FLOW IMAGING FOR REAL-TIME FEEDBACK DURING LASER THERAPY OF PORT WINE STAIN BIRTHMARKS

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Background and Objectives:
Port wine stain (PWS) birthmarks are progressive vascular malformations that occur in ~12,000 live births per year in the United States. The majority (~90%) of PWS birthmarks occur on the head and neck regions, and thus are difficult to conceal. The psychosocial development of individuals with PWS birthmarks is adversely affected. Facial PWS lesions have been associated with increased incidence of glaucoma and seizures. The progressive nature of PWS skin may be due to lack of neuronal regulation of blood vessel size. Progressive development of the PWS results in a darker appearance, soft tissue hypertrophy, nodularity, and overall further disfigurement. Current treatment options have significant limitations in terms of efficacy and risk. With laser therapy, a reduction in size and degree of redness of PWS skin occurs in ~60% of treated patients. After ten treatment sessions, complete disappearance of the PWS occurs in only ~10% of treated patients. To reduce the financial burden and potential risks of repeated treatments under general anesthesia, there is a need for innovative, personalized methods to maximize the reduction in PWS redness per treatment session. Without addressing this need, the overall efficacy of PWS laser therapy will remain variable, because treatment protocols will remain based primarily on the subjective impression and overall experience of the treating surgeon. To address this need, we propose use of laser speckle imaging (LSI) to provide real-time, quantitative feedback during laser surgery. Herein, we present in vitro and in vivo data collected with a clinic-friendly LSI instrument. Collectively, the data suggest the potential of LSI to serve as an intraoperative imaging tool to furnish clinicians with real-time images of blood perfusion. During laser therapy of port wine stain (PWS) birthmarks, regions of persistent perfusion may exist. Immediate retreatment of such regions may improve the blanching efficacy of laser therapy. We developed a laser speckle imaging instrument to be used in the clinic which permits noninvasive, real-time, quantitative feedback during laser therapy and provide aid in identification of regions of persistent perfusion that could be retreated immediately.

Study Design/Materials and methods:
The clinical-friendly LSI instrument (Fig. 1)
consists of a 30-mW, 633-nm helium-neon laser, optical fiber and digital charger-coupled device (CCD) camera equipped with a macro lens. The recorded image sequence was converted to speckle contrast images by applying $7 \times 7$ sliding window to each $1600 \times 1200$ image. Relative flow image were obtained by calculating $1/\tau_c$ at each image pixel; a higher pixel value was assumed to be analogous to higher blood flow. Analysis of blood flow images obtained from subjects undergoing laser therapy of PWS birthmarks at Beckman Laser Institute. We use our clinical LSI instrument to acquire a sequence of raw speckle images prior to and immediately after pulsed dye laser therapy.

**Results:**
We found that the relative difference between blood perfusion in PWS and adjacent normal skin is highly variable. The perfusion of PWS skin sometimes is indistinguishable from that of adjacent normal skin. With laser therapy, we observed a global decrease in blood perfusion, and we frequently observed distinct regions of persistent perfusion (Fig. 2).

The current treatment relies on physicians to observe directly the purpura formation as the endpoint of PDL therapy. However, our clinical data demonstrate that persistent perfusion regions exist even after surgery by a highly skilled physician specializing in PWS laser therapy.

**Conclusion:**
The advantages of LSI over laser Doppler imaging and Doppler optical coherence tomography include speed, cost, simplicity, and potential ease of integration into current laser systems. Our results demonstrate the potential role of image-guided laser therapy of PWS birthmarks. LSI is a promising tool for noninvasive blood flow characterization during laser therapy due to its relative simplicity and low cost.

Figure 2. (A) 24-bit RGB image of a patient subject (B) prior to and (C) 50 min after laser therapy by a highly experienced physician. The images in (B) and (C) were acquired from the region of interest corresponding to (A). Instead of the expected homogeneous blood flow shutdown after laser therapy, we instead observed a heterogeneous flow distribution with regions of persistent perfusion. The mean SFI value decreased to 49% of its pretreatment value.