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Superficial blood perfusion evaluated by quantitative laser speckle flowmetry as a biomarker of neurogenic inflammation and burn severity

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Abstract
The following short review summarizes how superficial blood perfusion monitored by laser speckle contrast imaging can be used as a multipurpose experimental and clinical biomarker of various conditions. These include, but are not limited to immunogenic and neurogenic inflammation (clinical as well as experimental occurrences), burn severity assessment and real-time intra-operative blood-flow monitoring during neurosurgery [1–5]. Here, focus will be on how to monitor and quantify superficial blood perfusion assessed by laser speckle flowmetry as a “proxy outcome measure” or biomarker for experimentally induced aseptic neurogenic inflammation and as a clinically applicable biomarker of burn severity. Note that “flare” is used to denote a localized increase in superficial skin perfusion. Test.

The imaging technique
The laser speckle imaging technique works by illuminating an area with a preset laser light pattern in the wavelength of the 700-800 nm [3, 6, 7]. This is just above the spectrum for visible light at the threshold of infrared (IR) light (see figure 1B). The reflection of the laser light from the investigated surface produces a high contrast laser pattern known as a “speckle pattern” (see figure 1A) that can be evaluated in close to real-time [7, 8]. Any blood cells flowing through the investigated will cause the speckle pattern to change and appear blurred, subsequently leading to a reduction in image contrast locally in a specific area of the image. This means that areas of high blood flow show up as areas of decreased contrast and that low flow rate regions maintain high image contrast. In other words, image analysis software instantly transforms the speckle pattern to velocity distributions. For convenience, this information is typically converted into an intensity map (often a red to blue heat map), which is a better visualization to provide an overview for an investigator and/or clinician [3, 6–8].
Using laser speckle imaging as a biomarker of underlying processes in the dermis and epidermis

Superficial blood flow can be increased by a number of different mechanisms and has a variety of physiological purposes.

Examples that can increase superficial blood flow include[4]:

Figure 1: A) Depicts a typical laser speckle pattern reflected from a non-perfusion and non-moving surface. B) The light wavelength spectrum. Notice that laser speckle is typically conducted with wavelengths just above the visibility threshold at 710 nm, which means that the rays are invisible to the human eye. C) A temporal profile of the increased perfusion induced by the topical vasodilatory compound methyl nicotinate. Notice the shift from blue (i.e. low perfusion) to bright red (i.e. high perfusion) measurable on the heat-map scale in arbitrary units. Image from [31]
1. Immunogenic flare (typically an inflammatory reaction to the presence of a pathogen)
2. Increased activity in the sympathetic nervous system (e.g. in response to intense exercise or stress)
3. Neurogenic inflammation or “aseptic inflammation” (induced by release of vasoactive substances from activated peptidergic nerve fibers)

In many cases these reactions can overlap. For instance, if a pathogenic antigen is recognized by a mast-cell (an innate immune cell), it will cause a release of histamine leading to activity in histamine-receptor-positive mechano-insensitive C-fibers (CMi-fibers) giving rise to a sensation of itch, but also releasing CGRP into the surrounding skin thus leading to widespread neurogenic inflammation also known as an axon-reflex flare. I.e., this process would be a combination between immunogenic flare (initiating factor) and neurogenic flare (maintenance and extension of flare) [4]. Interestingly, different nociceptive and pruriceptive fibers have different capabilities when it comes to generating neurogenic flare [1, 5, 9–20]. This is due mainly to the extent of their terminal branching, allowing certain fiber subtypes to create more spatially extensive inflammation than that of others. The following fibers are typically considered capable of producing neurogenic inflammation to some extent:

- **Aδ-fibers** are responsive for the epicritic aspects of pain and only capable of producing a very modest neurogenic flare [21–23].
- **Polymodal C-fibers** (PmC-fibers) are capable of producing intense local neurogenic inflammation but a relatively small axon-reflex flare typically extending only a few millimeters to a few centimeters beyond the site of the physical or chemical insult/trauma [1, 23].
- **CMi-fibers** are capable of producing an extensive axon-reflex-flare extending far beyond the area of the insult/trauma [1, 24–26].

This distinct pattern of neurogenic flare induced by different fibers makes it possible to determine which fibers that are involved in e.g. a response to an irritant substance (see figure 2 for an example [27]). In other words one can say that in relation to neurogenic inflammation laser speckle contrast imaging works as a **mechanistic** and/or **severity biomarker**.
Figure 2: The images (left) represent typical images of the superficial skin perfusion in an area after insertion of a few cowhage spicules either coated with histamine/capsaicin or native spicules containing the pruritogen mucunain. Notice how histamine, a principal CMI-fiber-activator, induces a pronounced and extensive flare, while capsaicin, a prototypical PmC-activator, induces intermediary flare and mucunain (cowhage), an inducer of itch through non-peptidergic C-fibers and Aδ-fibers, only produces a very small local increase in blood flow. Right side of the image shows the quantified averaged areas. Image and graph from [27].

Laser speckle perfusion assessment for neurogenic flare can in the way described above be useful to observe activity in cutaneous innervation within the field of e.g. experimental neuroscience and dermatology [1]. However, it could also be useful for more commercial applications e.g. in the cosmetics business for testing the degree and duration of neurogenic pro-inflammatory activity of an active ingredient or additive (which could be both desired and undesired depending the situation).

Using laser speckle imaging as a biomarker of burn severity

Laser speckle imaging has been proposed as a valuable and objective biomarker in the assessment of burns to the skin [3]. Sufficiently severe burns (i.e. deep 2nd degree and 3rd degree burns) require grafting of healthy skin from elsewhere on the body to heal optimally (see figure 3) [28–30]. The problem with the current manual assessment is that it relies on a subjective visual assessment of the affected area, which is often highly damaged, flaring, exudative and/or exfoliating at the time of inspection.
Because deep 2nd or 3rd degree burns damage dermal and subcutaneous vasculature it decrease perfusion while superficial 2nd or 1st degree burns will lead to local aseptic inflammation (i.e. result in highly increased superficial perfusion) the measure is useful in a clinical setting for **prognostic and choice-of-intervention purposes**[3, 29]. See figure 4 as an example of how laser speckle contrast imaging can be used in clinical practice.

**Figure 3**: A simple overview of burn severity classification. 2nd degree burns are often sub-categorized into superficial 2nd degree burns and deep 2nd degree burns. Notice that as the depth of the burns increase the damage to vasculature increase as well, which if sufficiently severe will result in very little or no perfusion in the area.

**Figure 4**: Shows a partial thickness (2nd degree burn) that has a deeper burn area and a more superficial burn area. Notice that the elongated area of very low perfusion (i.e. deep burn) is very clearly identifiable on the laser speckle contrast image (B & C), but less so on the regular picture (A). In this case the area size was deemed to small and not located in an area where slight scaring is an aesthetic concern and hence no skin grafting was conducted.
References


