

## **SDF imaging for assessing mucosal microcirculation and its correlation with OSMF**

### **A review**

**Language:** English

### **Authors:**

Senior lecturer Prashant Gupta, Prof Dr Ashok L, HOD, Senior Lecturer Dr Shantala R Naik, MJR Rohilkhand University, Institute of Dental Sciences, Dept of Oral Medicine and Radiology, Bareilly, Uttar Pradesh, India

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### **Introduction**

Tissue oxygenation is important for organ function. An important approach in understanding tissue dysoxia and the proper choice of treatment is to measure the oxygenation status of the microcirculation. Oral and maxillofacial compartments are highly vascularized areas and offer a very approachable site for noninvasively monitoring and assessing the microcirculation. Recently intravital microscopy has been miniaturized and developed for clinical conditions by the implementation of orthogonal polarization spectral (OPS) imaging in a hand held microscope type device.<sup>1</sup> Since it has few drawbacks microvision medical introduced another novel approach as side stream dark field (SDF) imaging.<sup>2</sup>

### **Principle of SDF imaging**

SDF is a stroboscopic LED ring based imaging modality. In SDF imaging illumination is provided by surrounding a central light guide by concentrically placed light emitting diodes (LEDs) to provide sidestream dark field illumination. The lens system in the core of the light guide is optically isolated from the illuminating outer ring thus preventing the microcirculatory image from contamination by tissue surface reflections. Light from the illuminating outer core of the SDF probe, which penetrates the tissue embedded microcirculation by scattering. The LEDs emit a central wavelength of 530nm, chosen to correspond to an isobestic point in the absorption spectra of deoxy and oxyhemoglobin (i.e at 530nm) to ensure optimal optical absorption by the hemoglobin in the RBCs, independent of its oxygenation state. This leads to an image where RBCs are imaged as dark moving globules against a white / grayish background. To improve the imaging of moving structures such as flowing RBCs, the LEDs provide pulsed illumination in synchrony with the CCD frame rate to perform intravital stroboscopy. This stroboscopic imaging partially prevents smearing of moving features, such as flowing RBCs and motion induced blurring of capillaries due to the short illumination intervals.<sup>1</sup>

### **Advantages:**<sup>3,4</sup>

- Non invasive and hence makes SDF a attractive and patient friendly instrument
- Provides stroboscopic illumination
- Superior image quality when compared with OPS imaging
- Low power LED illumination allows computer operation and thereby improved clinical applicability

### **Shortcomings:**<sup>3,4</sup>

- Limited penetration depth limits its use to superficial epithelial layers (upto 500 µm)
- > 1 area is required
- Detectable RBC velocity is physically limited
- Pressure induced microcirculatory alterations occur due to pressure from probe. This leads to false interpretation of actual microcirculatory perfusion.

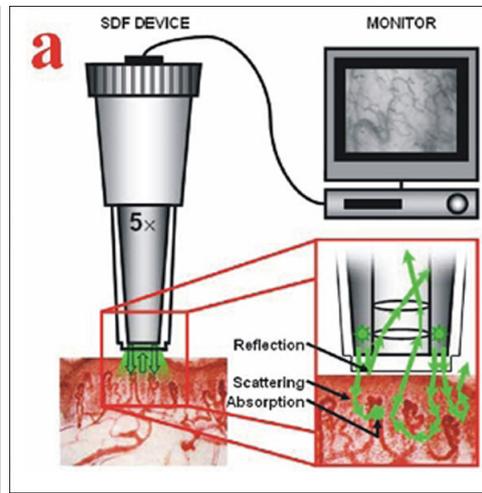
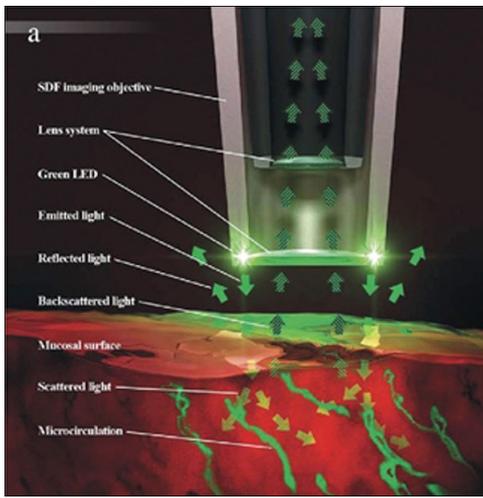


Fig. 1: illustration of SDF imaging probe

Fig. 2: SDF imaging device

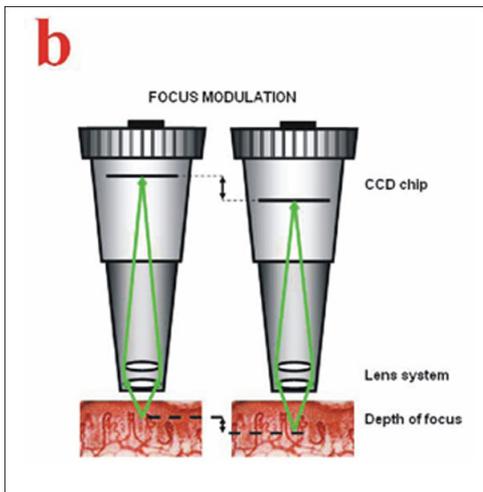


Fig. 3: SDF imaging device

## Material and Methods

The SDF imaging technology is incorporated into a portable hand held video microscopy instrument for performing noninvasive real time intravital microcirculatory observations on mucosal surfaces.<sup>4</sup> SDF imaging is a microscan video microscope (Microvision Medical, Amsterdam, The Netherlands). It is fitted with a 5x objective lens system. The CCD chip is axially translated with respect to the fixed lens system in the tip of SDF probe. It is covered by a sterile disposable cap, the probe can be placed on organ and tissue surfaces to investigate microcirculatory morphology and perfusion under different clinical conditions. To prevent microcirculatory perfusion alterations by applying pressure on imaged area, the probe is placed onto tissue and gently pulled back until contact is lost. Then probe is advanced again slowly to point at which contact was regained and microcirculation is in focus of lens system contained in probe.<sup>4</sup>

Investigation is done bedside with backrest inclined upright. Instrument is placed on buccal mucosa inferior to the 1st and 2nd molar without applying pressure and gently pivoting the SDF imaging probe wedged between molars. A 2 minute video recording of different adjacent sites is obtained.<sup>4</sup> Microcirculation studies requires multiple measurements per time point. Current microcirculatory guidelines dictate that measurements has to be done at 3-5 sites / time point to allow adequate interpretation of results. Length of each image sequence should be > 20 sec.<sup>5</sup> All imaging is captured with a 720 × 576 pixel resolution video camera resulting in a 1.0 × 0.75mm imaged mucosal segment. All measurements are recorded for 2 minutes on a video camera and then transferred on monitor.<sup>4</sup> Video output is visualized on a monitor and connected to a computer via signal converter to directly and digitally record images onto a hard drive to enable offline analysis of images. The device is mounted in a specifically engineered universal holder.<sup>3</sup>

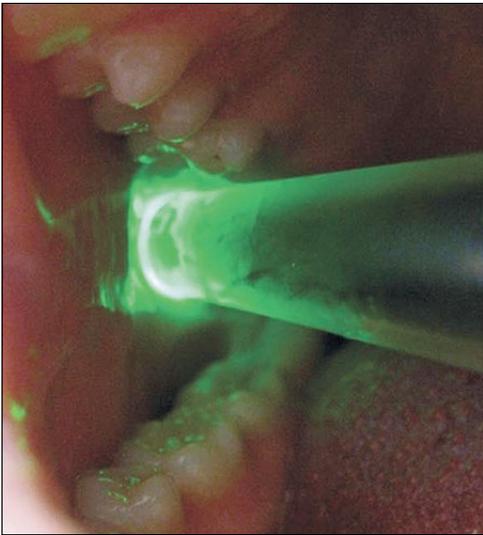


Fig. 4: placement of probe on oral mucosa

Fig. 5: SDF imaging probe

### Interpretation of images

SDF evaluates functional state of circulation (perfusion), morphological characteristics of microcirculation such as capillary density and microvessel morphology. Homogeneous perfusion of capillaries is a prerequisite for normal function. Offline image analysis is performed by counting the number of perfused capillaries in each frame. Baseline capillary density is  $18.9 \pm 2.4$  cpl/mm<sup>2</sup>.<sup>3,4</sup>

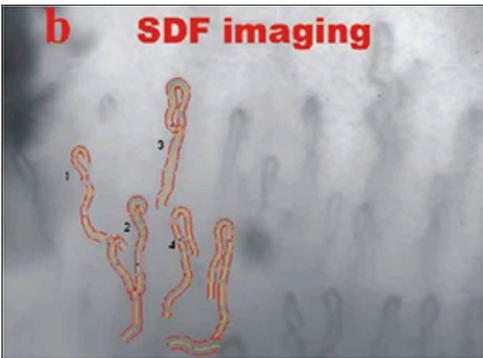


Fig. 6: offline images of capillary diameter



Fig. 7: capillary loop showing individual RBCs and plasma gaps



Fig. 8: pulsatile flow observed during during SDF probing to calculate the RBC velocity

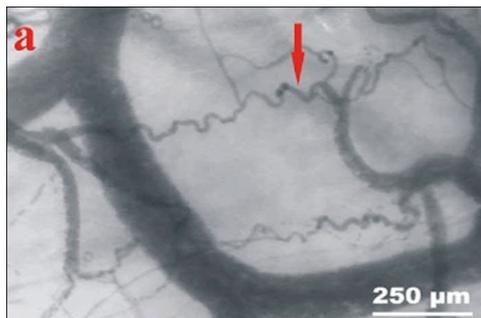


Fig. 9: leucocyte visualization using SDF

### Conclusions

Correlation with the therapy of OSMF: Oral submucous fibrosis still remains enigmatic. The degree of vascularity of diseased mucosa has always been a matter of dispute. Atrophy in OSMF is based on assumption of ischemic epithelium resulting in poorly vascularized stroma. Vasodilators such as Pentoxifylline has also been used in the therapy and found to be an effective adjunct in the treatment of OSMF.<sup>6</sup>

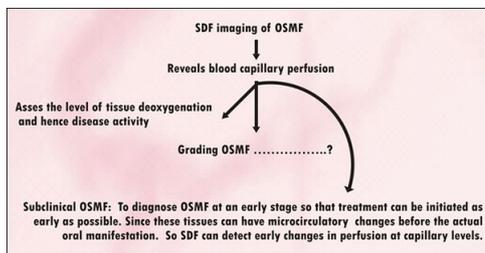


Fig. 10: flow chart correlating SDF imaging with OSMF

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1. D M Milstein, J A H Lindeboom and C Ince: Sidestream dark field imaging and image analysis of oral microcirculation under clinical conditions. AIPCE proceedings of 20th post graduation course in critical care, 2005, Nov 18-21.
2. C Ince: Sidestream dark field imaging: an important technique to observe sublingual microcirculation. Critical care 2005, 9 (suppl 1), p72.
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5. G M Balestra, R Bezemer, E C Boerma, Ze - Yie Yong, Krishan D Sjauw, A E Engstrom, M Koopmans and C Ince: Improvement of SDF imaging with image acquisition stabilizer. BMC Medical Imaging 2010, 10, 15.
6. R Rajendran, Vidya Rani and Saleem Shaikh: Pentoxifylline therapy: A new adjunct in the treatment of oral submucous fibrosis. Indian J Dent Res 2006, 17, 190-8.

## Abbreviations

SDF: side stream dark field  
OSMF: oral submucous fibrosis

*This Poster was submitted by Prashant Gupta.*

## Correspondence address:

Senior lecturer Prashant Gupta  
MJP Rohilkhand University  
Institute of Dental Sciences, Dept of Oral Medicine and Radiology  
Suresh sharma Nagar, Pilibhit bypass road  
Bareilly, Uttar Pradesh  
India

# SDF imaging for assessing mucosal microcirculation & its correlation with OSMF

Gupta Prashant<sup>1</sup>, Ashok L<sup>2</sup>, Naik Shantala R<sup>3</sup>, Dept of Oral Medicine and Radiology.  
 2. Prof and Head, Bopuji Dental College and Hospital, Davangere (INDIA),  
 3. Sr Lecturer, Institute of Dental Sciences, Bannily, (INDIA).

**Introduction :** Tissue oxygenation is important for organ function. An important approach in understanding tissue dysaxia and the proper choice of treatment is to measure the oxygenation status of the microcirculation. Oral and maxillofacial compartments are highly vascularised areas and offer a very approachable site for noninvasively monitoring and assessing the microcirculation. Recently intravital microscopy has been miniaturized and developed for clinical conditions by the implementation of orthogonal polarisation spectral (OPS) imaging in a hand held microscope type device. Since it has few drawbacks microvision medical introduced another novel approach termed as side stream dark field (SDF) imaging.

**Principle of SDF imaging:**

SDF is a stroboscopic LED ring based imaging modality. In SDF imaging illumination is provided by surrounding a central light guide by concentrically placed light emitting diodes (LEDs) to provide sidestream dark field illumination. The lens system in the core of the light guide is optically isolated from the illuminating outer ring thus preventing the microcirculatory image from contamination by tissue surface reflections. Light from the illuminating outer core of the SDF probe, which penetrates the tissue illuminates the tissue embedded microcirculation by scattering. The LEDs emit a central wavelength of 530nm, chosen to correspond to an isosbestic point in the absorption spectra of deoxy and oxyhemoglobin (i.e at 530nm) to ensure optimal optical absorption by the hemoglobin in the RBCs, independent of its oxygenation state. This leads to an image where RBCs are imaged as dark moving globules against a white / grayish background. To improve the imaging of moving structures such as flowing RBCs, the LEDs provide pulsed illumination in synchrony with the CCD frame rate to perform intravital stroboscopy. This stroboscopic imaging partially prevents smearing of moving features, such as flowing RBCs and motion induced blurring of capillaries due to the short illumination intervals.<sup>1</sup>

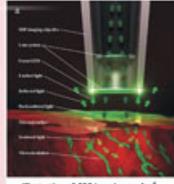
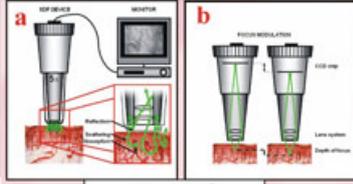


Illustration of SDF imaging probe<sup>1</sup>



SDF imaging device<sup>1</sup>

**Advantages:<sup>1,4</sup>**

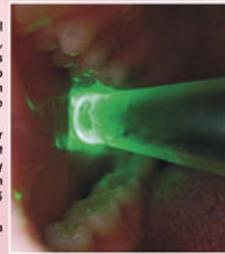
- Noninvasive and hence makes SDF a attractive and patient friendly instrument
- Provides stroboscopic illumination
- Superior image quality when compared with OPS imaging
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**Shortcomings:<sup>1,4</sup>**

- Limited penetration depth limits its use to superficial epithelial layers (upto 500µm)
- Images have smaller surface area and reproducibility is difficult. Hence imaging of > 1 area is required.
- Detectable RBC velocity is physically limited
- Pressure induced microcirculatory alterations occur due to pressure from probe. This leads to false interpretation of actual microcirculatory perfusion

**Materials and methods:**

The SDF imaging technology is incorporated into a portable hand held video microscopy instrument for performing noninvasive real time intravital microcirculatory observations on mucosal surfaces.<sup>1</sup> SDF imaging is a microscan video microscope (Microvision Medical, Amsterdam, The Netherlands). It is fitted with a 5x objective lens system. The CCD chip is axially translated with respect to the fixed lens system in the tip of SDF probe. It is covered by a sterile disposable cap, the probe can be placed on organ and tissue surfaces to investigate microcirculatory morphology and perfusion under different clinical conditions. To prevent microcirculatory perfusion alterations by applying pressure on imaged area, the probe is placed onto tissue and gently pulled back until contact is lost. Then probe is advanced again slowly to point at which contact was regained and microcirculation is in focus of lens system contained in probe.<sup>1</sup> Investigation is done bedside with backrest inclined upright. Instrument is placed on buccal mucosa inferior to the 1<sup>st</sup> and 2<sup>nd</sup> molar without applying pressure and gently pivoting the SDF imaging probe wedged between molars. A 2 minute video recording of different adjacent sites is obtained.<sup>1</sup> Microcirculation studies requires multiple measurements per time point. Current microcirculatory guidelines dictate that measurements has to be done at 3-5 sites / time point to allow adequate interpretation of results. Length of each image sequence should be > 20 sec.<sup>1</sup> All imaging is captured with a 720 × 576 pixel resolution video camera resulting in a 1.0 × 0.75 mm imaged mucosal segment. All measurements are recorded for 2 minutes on a video camera and then transferred on monitor.<sup>1</sup> Video output is visualized on a monitor and connected to a computer via signal converter to directly and digitally record images onto a hard drive to enable offline analysis of images. The device is mounted in a specifically engineered universal holder.<sup>1</sup>



Picture depicting probe and its placement on oral mucosa

**Interpretation of images:**

SDF evaluates functional state of circulation (perfusion), morphological characteristics of microcirculation such as capillary density and microvessel morphology. Homogenous perfusion of capillaries is a prerequisite for normal function. Offline image analysis is performed by counting the number of perfused capillaries in each frame. Baseline capillary density is 18.9 ± 2.4 cpl/mm<sup>2</sup>.<sup>1</sup>



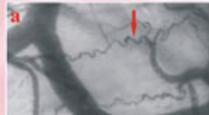
Offline images of capillary diameter



Image of a capillary loop showing individual RBCs and plasma gaps



Pulsatile flow observed during SDF imaging to calculate the RBC velocity



Leucocyte visualization using SDF

**Conclusion:**

Normalizing microcirculatory density and perfusion has become focus of new clinical studies and circulatory images are gaining a more prominent role in clinical monitoring. It is anticipated that SDF imaging will serve as a novel and improved imaging modality to contribute to clinical assessment of microcirculation in various clinical scenarios. It further allows the computer aided image processing and analysis for quantification of microcirculatory alterations associated with disease and therapy.

**Correlation with the therapy of OSMF:**

Oral submucous fibrosis still remains enigmatic. The degree of vascularity of diseased mucosa has always been a matter of dispute. Atrophy in OSMF is based on assumption of ischemic epithelium resulting in poorly vascularized stroma. Vasodilators such as Pentoxifylline has also been used in the therapy and found to be an effective adjunct in the treatment of OSMF.<sup>1</sup>



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