Two Dimensional Laser Scanner for Blood Perfusion Imaging Through Curved Sections of Human Forearm

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Blood flow in microcirculation has found vital role in maintenance of body temperature. Its measurement is vital in the field of skin-vessel reactivity, ageing of skin, micro vascular repair of the ulnar artery and wound healing in association with total elbow replacement. The existing techniques for blood flow measurements like Electromagnetic blood flow measurement, Thermal convection method, Dual radiographic method, Ultrasonic Blood flow measurement and NMR method are not found suitable for measurement of blood flow in microcirculation due to their large size, complexity, invasive nature of measuring element and possibility of radiation exposure to the patient. Hence, a new technology is developed for micro vascular blood flow or velocity measurement using He-Ne laser of wavelength 632.8nm.

Key words: Laser Doppler Effect, Perfusion Values, Occlusion, Rubber phantom, Interpolation.

Blood flow in a micro vessel, which is the integrated displacement of cells/unit time is usually estimated as the product of mean blood velocity and luminal cross-section area.

The existing techniques for blood flow measurements like Electromagnetic blood flow measurement, Thermal convection method, Dual radiographic method, Ultrasonic Blood flow measurement and NMR method are not found suitable for measurement of blood flow in microcirculation due to their large size, complexity, invasive nature of measuring element and possibility of radiation exposure to the patient**.**

Temporal correlation and spatial correlation methods are the other methods which use optical signal and video signal for measurement. These methods are complex and costly, and also the measuring blood vessel should be visible enough to capture the image. Continuous measurement is not possible by these methods[1,2].In order to get continuous, non-invasive measurement signal, some new technology using Lasers is introduced. But the Blood flow by this method has been manual and confined to flat tissue surfaces.

Hence, to get the blood flow in micro circulation of curved surfaces, the measurement is carried out in two stages
1. Development of a 2-D curved surface laser scanner for automatic scanning.
2. To get the perfusion images of human forearm (on a specified area) on the monitor using the laser Doppler flow meter.

Importance of Laser

The laser (abbreviation for light amplification by stimulated emission of radiation)

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is monochromatic source of light and posses coherence (temporal and spatial) properties. Each bundle of photon carry much more energy than the conventional light sources and also depending upon the wavelength the spot of the light could be focused precisely.

When laser interacts with living tissues or skin, at the site multiple reactions take place[3]. These are collimated transmission, immediate absorption, absorption following several scattering, diffuse transmission and backscattering. (Fig.1) For these interactions the dimension of the cells should be large compared to the wavelength of light and their index of refraction differs from that of the embedding liquid. Thus the cells act as a large scatters. Skin consists of Horney layer, epidermis, dermis and other structural components. The thickness and chromophores of epidermis is determined by genetic and environmental a factor, which overlies the thick fibrous and light scattering dermis .In the epidermis, absorption by melanin pigment rapidly increases as wavelength decreases[4]. Absorption and Backscattering of tissues are wavelength dependant properties. At lower end of the visible spectrum absorption dominates, whereas, at higher wavelength the scattering is increased. The penetration of light at the therapeutic window (600 to 1300 nm) is more due to lower scattering and absorption .This allows both substantial penetration of light into the tissue and high remittance of light scattered out of tissue after deep penetration .

Laser Doppler Velocimetry

Depending upon the velocity of the movement of the particles the backscattered or reflected laser light is associated with change in frequency which is known as 'Doppler effect'. Advances in optical system design and development of signal processing enabled this system to measure the absolute value of average velocity or blood flow volume.[5, 6] demonstrated a laser Doppler velocimetry and measured the velocity profile for laminar flow in a circular tube. In this technique, laser beam is used as an incident light source, which is focused on the small scattering region to be measured[7] The light scattered by small particles in this region is then collected by a lens and aperture system which falls on a photo detector. The spectral power density of the photo current thus obtained is called the homodyne spectrum.

Laser speckle Velocimetry

In addition to the laser Doppler velocimetry technique, several researchers have studied laser scattering phenomenon of dynamical laser speckle effects. Speckle pattern is formed by the interference of coherent light scattered from a diffuse object and it moves with a velocity proportional to the object velocity[8] showed the 2-D maps of the blood flow level in a human retina by the use of single exposure speckle photography and optical filtering techniques.

Recently, a method is developed to visualize the microcirculation map of human skin surface through analysis of speckle[16,17]

Laser Doppler Blood Flow Velocimetry

On the basis of laser Doppler effect, several research groups have developed laser Doppler flow meter to measure blood flow in micro vessels[9] .These instruments are found to be useful for their non-invasive, sensitive and repeatable character. The monochromatic laser beam is diffusely scattered and its frequency is shifted by moving red blood cells[10].The reflected light signal is mixed coherently with another portion of the light scattered from static tissue generates a Doppler beat signal in the photo-detector output current.

From the spectral analysis of the beat signal, the blood flow level in the tissue can be estimated. The instrument based on this principle is laser Doppler flow meter which provides the blood perfusion value of single point penetration, in the form of percentage of blood cell flux on its display panel. In order to get the value of perfusion at a specified area the laser probe is moved manually and each time the reading is noted down.
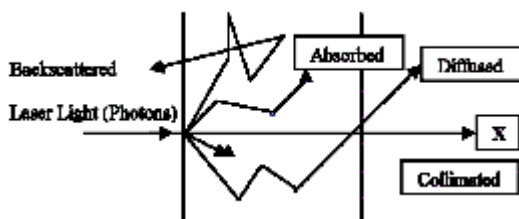

Fig. 1. Laser interaction with Biological system

MATERIALS AND METHODS

Laser Doppler flow meter provides the information on tissue perfusion in the form of blood flow through that location. In order to get the value of perfusion at a specified area the LDF probe is moved manually and each time the reading has to be noted down. In order to make the scanning process automatic, a two dimensional laser blood flow scanning system is designed and to get the perfusion images of a specified area of a human forearm on the monitor, software is developed in Turbo-C. The 2-D laser scanner is designed indigenously in such a way that scanning on curved surface of the human forearm could be achieved. This is in contrast to scanning system which was used for scanning of flat biological surfaces[11], [12].(Fig2)shows the schematic of the experimental set up to obtain perfusion images using 2-D laser scanner and LDF.

Laser Scanner - Design and Development

The system mainly comprises two stepper motors A and B. These motors are connected and programmed in the system, providing orthogonal movement to the probe. Motor 'A' and its shaft is permanently fixed between two stands. The two stands are attached permanently to the base plate.
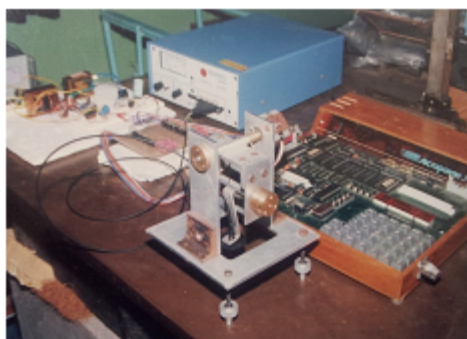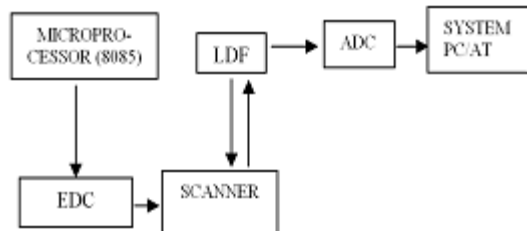
.Motor 'A' moves the whole unit of ' Motor 'B and it provides the curved path movement of the scanning head. The radius of curvature produced by Motor A is 15 cm. The whole unit of Motor B is connected to the shaft of Motor 'A' which provides the vertical or 'y' direction movement of the scanning pattern. The shaft of Motor 'B' has the provision for the probe holder. In the base plate 8x8 cm slot is made for free movement of the probe holder. According to the design of the system both movements along curved surface are possible for probe head. The movement of stepper motors is controlled by programming of microprocessor 8085 and electronic driving circuits. Aluminium and brass materials are used for fabricating the scanning system. The whole unit is put into a small aluminium box. The height of the system can be varied by few centimeters from the base plate by using three supporting adjustable screws. (Fig.3) shows the schematic diagram of the 2-D laser scanner assembly and(Fig 4)shows the actual photograph of indigenously developed 2-D laser scanning assembly.

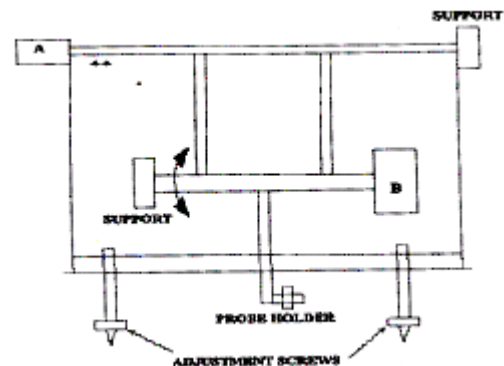**Fig 3.** Schematic View of 2D-Laser Scanner

LDF	→	LASER DOPPLER FLOW METER
EDC	→	ELECTRONIC DRIVING CIRCUIT
ADC	→	ANALOG TO DIGITAL CONVERTER

Fig. 2. Experimental Setup of Perfusion Image

Fig. 4. 2D-Laser Scanner

Stepper Motor Details

Stepper motor is a device which converts digital pulses into precise angular steps. It is basically a motor with two phase salient pole stator and a permanent magnet rotator. The stator is housed in a steel body and rotator is mounted on sealed ball bearings which assures permanent lubrication. These stepping motors are of bifilar type with six leads. Each of the two phases of a motor has a double winding with a center tap. Switching the supply from one phase to another phase causes the reversal of the magnetic polarity without actually reversing the polarity of the supply. The stepper motor can follow either of the two switching logics, (1). 4 step input logic; (2). 8 step input logic. The four step input logic gives 1.8 ° step, and eight step input gives 0.9° step.

The switching sequence from top to bottom (Table.1) rotates the shaft in clockwise, whereas, the sequence operated from bottom to top rotates the shaft in anticlockwise direction. The required sequences are generated with the microprocessor and are supplied to the electronic driving circuit to run the stepper motors.

Laser Scanner - Design and Development

To generate the sequence logic for system operation, a microprocessor Intel 8085 with its standard peripheral devices is employed. The software is designed in such a way that under a single control command the scanning is initiated. The scanning process is automatically stopped by the CPU (central processing unit) when the process is completed. After completing scanning the probe returns to its same starting position. The output of microprocessor is insufficient to drive the stepper motor. To amplify this signal electronic driving circuit is used.

Electronic Driving Circuit

This contains two electronic driving circuits for two stepper motors. Each EDC has four Darlington pairs comprising of BC109 (low power transistor), and 2N5293 (power transistor) to provide higher current.

Periflux PF2-Laser Doppler Flowmeter

The block diagram of laser Doppler flowmeter is shown in Fig.5.

The LDF utilises the Doppler shift, i.e the frequency change that light and other radiations of a wave nature undergo when reflected by objects in motion, such as red blood cells. All wave movements are charactrised by the relation,

$$v = f \times l$$

where   v   propagation velocity

f   wave frequency

l   wavelength

A beam of low power He-Ne laser light is guided by an optical fiber to the probe head. When this is applied to a tissue the light enters into it and is reflected, refracted and gradually absorbed. This multiple scattering produces a volume of almost isotropic illumination inside the tissue in front of the measuring head. All blood cells traversing this volume interact with light beam leading to Doppler frequency signal.

Thus, the volume illumination infront of the probe is a mixture of unshifted and Doppler shifted light, the magnitude and frequency distribution of the latter component being related to the number of blood cells moving through the volume and to their velocity , but virtually

Table 1. Switching logic-stepper motor

Memory location (µp)	Clockwise Rotation				Memory location (µp)	Anticlockwise rotation			
	Ph-1		Ph-2			Ph-1		Ph-2	
	A_1	B_1	A_2	B_2		A_1	B_1	A_2	B_2
5110	0	1	0	1	5117	0	1	0	0
5111	0	0	0	1	5116	0	1	1	0
5112	1	0	0	1	5115	0	0	1	0
5113	1	0	0	0	5114	1	0	1	0
5114	1	0	1	0	5113	1	0	0	0
5115	0	0	1	0	5112	1	0	0	1
5116	0	1	1	0	5111	0	0	0	1
5117	0	1	0	0	5110	0	1	0	1

independant of the movement direction of the individual blood cells.

Parts of the illuminating light is back scattered from the skin and is picked up by two efferent optical fibres arranged in parallel with the afferent fibre carrying the laser light to the point of measurement. The efferent optical fibres having original and Doppler shifted wave frequencies are detected by photo detectors which convert it to electric signals. These electric signals also have the same mixture of frequencies as the laser light which enter into the photo detectors.

Such a mixture produces new signals with frequencies constituting the difference between the component frequencies and their summation. The light frequency and summmed up frequency are so high (1015 Hz), that they cannot be contained by electric circuits. These signals therefore leak away and disappear.

Only the frequency differences, which obviously are identical to the Doppler evoked frequencies changes, and which lie within the frequency range from 0 to about 15KHz appear as a Doppler signal at the photo detector output.

This signal is stochastic in nature, still well defined function of velocities and the number of RBC moving through the part of the illuminated volume.

The capillary blood velocity is only a million millionth of the light velocity. The Doppler shift is the same minute fraction of the light frequency. So, the light should monochromatic and noise free. Even lasers cannot achieve this. This problem is solved by double channel arrangement which produces a clean cell motion correlated doppler signal and also effectively suppresses noise from certain other sources. The signal thus extracted is subsequently processed to compensate for variations in laser intensity and for transmission differences in the fibres. It is finally made subject to an electronic transformation corresponding to the mathematical relation between the unprocessed Doppler signal and the tissue perfusion. The algorithm used for transforming the primary signal into a flux reading can be expressed as,

$$\mathbf{V}_{out} = [\mathbf{K} \int_{\omega l}^{\omega u} \omega P(\omega) d\omega]$$

where

w	: Doppler frequency
Vout	: output signal
P(w)	: total power spectral density of the difference amplifier output signal
wu	: upper cut-off frequency
wl	: lower cut-off frequency
K	: proportionality constant.

Basically this instrument reacts to all movements of definite structures mainly red blood cells, within its measuring volume (radius of 1.5mm). Movement of WBC, thrombocytes doesn't affect the reading because these components form part of the blood flow. Movement of muscle cells, vessel walls and various membranes (which are not related to blood flow) contributions as a rule are insignificant.

Fig 5. Block diagram of Laser Doppler Flow meter

The LDF can perceive signals from a tissue depth of 1 to 1.5 mm. Hence microvessels (e.g) capillaries, microshunts, arterioles and venules contribute to the measurement. Larger vessels in the path do not affect the reading because they are too thick and tight to permit more than a limited amounts of light to pass in and out.

The LDF produces an output signal that is proportional throughout the entire clinical range to a quantity of great physiological significance, the blood cell flux, representing the microvascular blood cell transport through the tissue:

Blood cell Flux = No. of blood cells moving in measuring volume x Mean velocity of these cells.

To achieve linear perfusion measurements throughout the measuring range, the flowmeter should operate with the 12KHz upper frequency limit. 4KHz upper limit is used at less well perfused tissue, such as forearm, back, legs where the cell concentration scarcely reach levels where nonlinearity occurs in the first place.

The laser probe which transmits He-Ne laser light is gently connected to the probe holder of the 2-D laser scanner assembly. Ultimate care is taken to avoid the twisting of the probe, in order to avoid the internal breakage of the probe. Ample probe length is made available near the scanner so that any obstruction for free movement of the probe does not occur.

Data Acquisition

PC/AT System

To collect and process the value of perfusion variation of the tissue throughout its area and to obtain a color coded image personal computer PC/AT is used. It consisting of a real time digitizer (A/D) card. It digitizes the perfusion variations of the tissue (analogue value) to match the requirement of the Personal Computer for processing.

Details Of A/D Converter

PCL-201 card is used for A/D conversion. It is a successive approximation type of A/D converter. It has 16 channel of conversion, 12-bits of resolution and conversion time of 30 micro-sec. Accuracy of this converter is 0.5 % min. at 25°C. Input voltage is 0-5 volts. Hence, the output flux value of LDF is attenuated 2.5 times and is given to the A/D converter. Before acquiring the data for imaging, calibration of the system is carried out.

Calibration

To check the performance of the above system, a rubber phantom of surface curvature equivalent to the mapping curvature of the scanning system is made. The rubber phantom is kept under the probe holder of the scanning system. The movement of the probe holder along and 0.5 mm above the rubber surface is carried out. Data acquisition software is developed in Turbo-C and is installed in the computer. A grid showing outline of rubber phantom is generated and displayed on the screen of the monitor (Fig.6).The LDF is switched on and its 4KHz upper frequency limit is selected. The time constant switch is put to 1.5 seconds position.

Scanning is started by pressing 'execute' button of the microprocessor. The probe is made to follow five horizontal curved paths and four vertical steps alternatively to scan an area of (2x3 cm) by programming the microprocessor. For each movement of the flow meter beam , curser on the grid pattern also moved correspondingly and entered to store the data. After following data processing methods (interpolation and median filtering) data acquired is displayed on the monitor which is shown in (Fig.7). The output of LDF is zero while scanning static surface and hence the image shows the lower grey level. This show that the scanner follows only the curved surface and assigned colors for different values of grey level are correct.

For acquiring data from the curved surface of human forearm, the subject is asked to keep the forearm under the scanning area of the probe and the initial setting is made in the LDF. The grid pattern and organ outline for collecting data is created in the monitor which is shown Fig 8.The free movement of the laser probe is checked for any skin surface obstruction by adjusting the slope of the forearm before starting scanning. By pressing execute button of microprocessor, the scanning is started. For each movement of the laser beam in both directions 'Enter' key is pressed to collect the data. According to the program all the data collected is automatically stored in a specified file. Data collection is done at three locations of human forearm (A, B and C).

Data Interpolation

The observation points are not regular because of the structure of the forearm. To get the

regular pattern (Davis, 1980). of all collected data a software is developed Equally spaced points of 100 x 100 grid pattern is developed and is superposed on the observed data. The estimation is carried out by locating the nearest data points to the grid point k. The distance Dik from observation point i to grid point k is determined by the Pythagorean equation,

$$Dik = Ö(Xk-Xi)2 + (Yk-Yi)2.$$

By using Dik value , the perfusion value at the grid point is calculated by ,Perfusion Pk=S(Pi/Dik)/(1/Dik)i=1 to n.By this procedure irregular points are translated into regular points , and an 100x100 image

Median Filtering

To eliminate the noise introduced during data acquisition and interpolation, median filtering is carried out. It is a non-linear filter which preserves edges and does not introduce any new grey level .Software is developed for median filtering which is implemented on the image matrix. The input pixel is replaced by the median of pixels contained in a 3x3 window. The algorithm for median filtering requires arranging the pixel values in a window in increasing or decreasing order and picking up the middle value.

Display

Software is developed for assigning a suitable grey level to the data. The whole set of data is mapped on to 14 colors (grey level). Scaling is done according to the maximum value. Thus, perfusion image of forearm is displayed on the monitor with their color code

RESULTS AND DISCUSSION

Fig.9 shows the perfusion image of left forearm (ventral view) at places A, B and C of normal male subject I. At place A (i.e) near the wrist region of the forearm (ventral view), perfusion values are moderate and they vary gradually. The microvasculature of the skin is an intricate network of small blood vessels which play a major role in temperature regulation of the body.

The numerical density of capillaries may vary widely from one skin region to another as does the number of arterioles, venules and shunting vessels. Depending upon these and number of moving scatters in these vessels, perfusion value varies.

This fact is supported by [13] as adjacent skin areas may have different values of blood flow. The image at place A is obtained by scanning an area of 2x3 cm with spatial resolution 2.9 mm. From the above figure this can seen that point to point variation of perfusion is not there. It implies that, along the same branch of capillary micro vascular blood flow is constant. At place B, i.e., at mid region of the forearm (ventral view), upper part shows higher value of variation whereas the lower portion shows minimum variation of perfusion. Depending upon the muscle composition and the increased blood circulation the flow pattern of the upper portion shows higher perfusion. If a large artery passes along the laser penetration path, then the laser light is not able to penetrate through artery. This may one of the reasons for lower perfusion values [14]. At place C, i.e., approximately 2 cm above the distal end of forearm, perfusion image show a minimum variation. An increasing value of the capillaries could be a contributing factor for point to point variation in this region [15].

Fig.10 shows the perfusion images of left forearm of subject II at locations A, B and C. Similar to subject I the location A is close to the wrist.
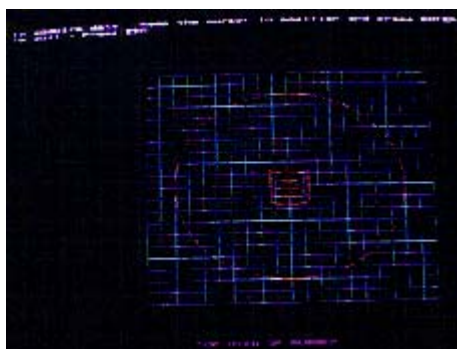

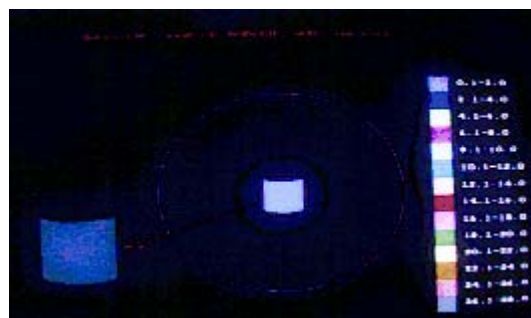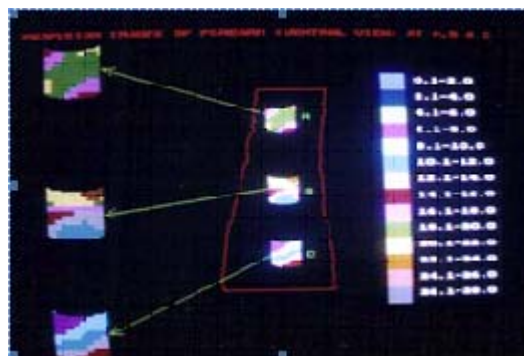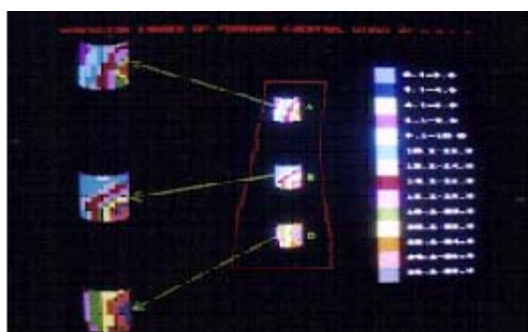**Fig. 6.** Grid pattern with outline of rubber Pattern

Fig.7. Perfusion image of rubber Phantom

Fig. 8. Grid pattern of forearm

Fig. 9. Perfusion image of forearm (Sub I)

Fig. 10. Perfusion image of forearm (Sub II)

Due to closeness of the blood vessels near the skin, an increased flow is measured at these regions. This pattern continues to vary with the location of blood vessels and the tissue composition near and above these vessels. This is also evident from the images as obtained at locations B and C.A large variation is observed at region A compared to that at B and C. The images obtained by this procedure show the dynamic changes in tissue perfusion. Functional muscles can further affect the flow pattern as an increased perfusion is observed in the right handed persons compared to that in their left hand[16]. In contrast to the laser reflectance imaging technique this technique deals with blood perfusion only[17].The tissue composition and pigmentation variation does not interfere in this measurement. Hence this procedure may lead to automation to detect blood flow changes under conditions such as burn-injury, plastic surgery and many others in curved tissue geometrics from the above result, this can be observed that blood flow is not a constant parameter. It is spatial and a time dependant parameter. Also, these images are not same for different persons as a person to person variation

has been observed. A number of studies in this field can help to make this method very success in the immediate use of clinical application[18].

CONCLUSION

With the use of indigenously developed 2-D system incorporated with laser Doppler flow meter, scanning on the curved surface of human forearm without modifying the scanning surface is carried out. It is a non-contact, non-invasive method of measurement. The scanning time is reduced noticeably compared to that of the manual method of probe movement which requires only one operator to operate the system. By attaching this whole unit to a movable stand, the usage of the system is further improved. With the present modification of the system and adequate software, the laser Doppler flow meter is upgraded as a laser Doppler perfusion imager. These images find applications in the fields of skin-vessel reactivity, micro-vascular repair, vasoactive drug development, plastic and reconstructive surgery, ulcer and wound studies, intra-operative organ perfusion imaging and others.

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