Confocal laser microscope

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INTRODUCTION

In vivo imaging of human epidermis and superficial dermis is a matter of interest for the dermatologist. The pursuit started with a magnifying glass, continues with a dermoscope and aims towards reaching new heights with the confocal laser microscope.

The confocal laser microscope is a novel and interesting noninvasive tool for imaging skin lesions and subsurface skin lesions that are not visible to the naked eye or even by dermoscopy. Skin can be imaged *in vivo* or freshly biopsied (*in vitro*) skin specimens can be visualized immediately without the processing that is required for routine histopathology.[1] Dynamic events (real time imaging) in the epidermis, papillary dermis and superficial reticular dermis to a maximum depth of 350 μm below the stratum corneum can also be visualized.[2] It has potential for diagnosing skin lesions with precision and could also become a tool for monitoring treatments in some cases.

The confocal microscope was invented by Marvin Minsky in 1955.[3] Since the advent of lasers there has been considerable improvement in the resolution, contrast, depth of imaging and field of view [Figure 1]. Over the years a small, portable confocal microscope similar to a dermoscope has been developed.

MECHANISM OF CONFOCAL MICROSCOPY

It is based on the principle[4] that when a diode laser beam is passed through the skin, reflected light is used to construct detailed images of optical sections through the tissue [Figure 2].

A laser provides excitation light of high intensity. The laser light is reflected from a dichroic mirror. From there, it hits two mirrors which scan the laser across the sample. Emitted light from the sample gets descanned by the dichroic mirror and is focused onto the pinhole after which it gets measured by a detector, i.e., a photomultiplier tube.

At any moment, only one point of the sample is observed, a complete image of the sample is never formed. The detector attached to a computer helps in building up the image, one pixel at a time. The detection of backscattered light along with differences in tissue refractive index help in high

Figure 1: Confocal microscope without skin contact device

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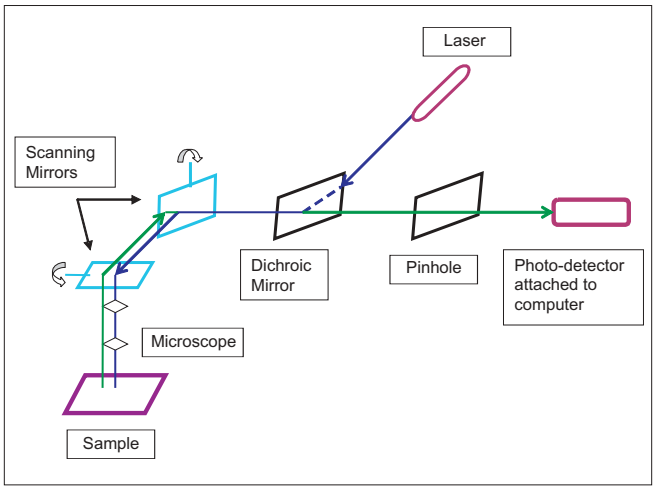

Figure 2: Diagrammatic representation of principle of confocal microscopy

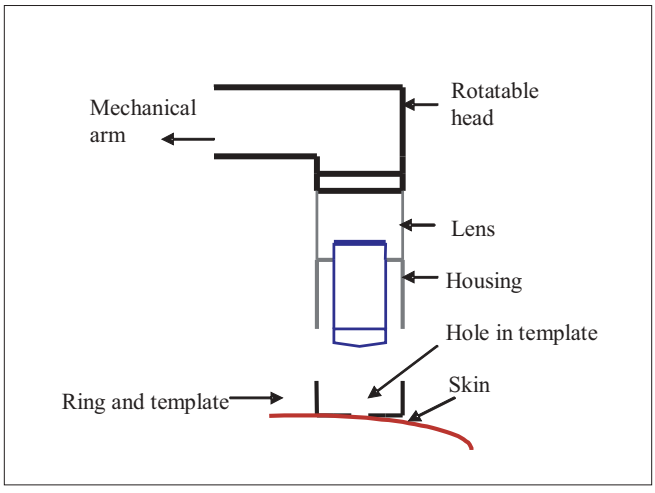

Figure 3: Line diagram showing the structure of skin contact device of confocal laser microscope

resolution of cellular detail and contrast when examining thin sections thus making staining unnecessary.

CONFOCAL MICROSCOPY AND HIGH RESOLUTION

Ordinarily, in fluorescence microscopy, the entire sample on being completely illuminated by the excitation light is fluorescing. The highest intensity of the excitation light is at the focal point of the lens; however, other parts of the sample do get some of this light and fluoresce. This leads to a background haze in the resulting image. This problem is solved by adding a pinhole/screen. The pinhole being conjugate to the focal point of the lens is a confocal pinhole and hence this is known as confocal microscopy. The image is formed from a thin section of the sample. Thus by scanning many thin sections of the sample, a very clear three-dimensional image of the sample is formed. It has better resolution horizontally, as well as vertically.

The imaging depth is directly proportional to the wavelength, the epidermis is imaged with visible 400-700 nm wavelengths; the superficial papillary dermis and blood cells (erythrocytes and leukocytes) in the deeper capillaries are imaged with the near infrared 800-900 nm wavelengths.[5]

Types of lasers that are used in confocal microscopy are Argon ion laser, Helium-Neon laser and Xenon laser.

INSTRUMENT AND METHOD OF USE

Continued research has led to the development of a user-friendly confocal microscope with flexible operating systems unlike earlier models that were large, immobile and unstable and hence only limited areas of the body could be imaged. For stable imaging at different sites on the body, a skin-to-confocal microscope contact device was developed. The microscope is supported on a stand that can be raised or lowered according to the skin site to be imaged.[1] An extended arm (attached to the microscope) with a rotatable head allows easier access to different sites on the arms, legs, back, trunk, face, neck and head [Figure 3].

The skin-contact device encloses the objective lens, a ring and template in housing. The ring and template is attached to the skin with double-sided tape or liquid. The ring forms a well on the skin which holds the immersion medium. The template has a hole which is centered over the site. The subject is placed either directly below or next to the objective lens and the stand is lowered or raised as necessary. The arm is oriented in such a way as to cause the ring and template to get engaged and thus lock into the housing. The skin within the template hole then remains laterally stable relative to the objective lens even though the subject may be moving.

Recent advances have led to the development of a handheld instrument. Skin is swabbed with a

fluorescent dye.[6] After pressing the microscope's handheld probe against the skin one can study the resulting images displayed on a computer monitor.

USES IN DERMATOLOGY

1. Microscopic analysis of skin structures (including hairs and nails) and components at different anatomic sites and in different conditions both physiological and pathological.[7-9]
2. *In vivo* imaging of skin lesions and their margins minimizing the need for skin biopsy.
3. To detect malignant changes in actinic keratoses and other premalignant conditions[10] and to study morphological differences between benign and malignant pigmented skin lesions leading to diagnosis of melanoma *in situ*.[11,12]
4. For diagnosis of dermatophyte infections, to identify fungal hyphae within the stratum corneum after potassium hydroxide application.[13]
5. For *in vivo* noninvasive visualization of mite, *Sarcoptes scabiei*.[14]
6. To monitor treatment for skin disorders e.g., in psoriasis to assess reduction in activity of T-cells after steroid thrapy.[6]
7. Confocal laser microscope has been used to visualize dynamic events at the cellular level in conditions like allergic contact dermatitis, folliculitis etc.[15,16]
8. *In vivo* imaging of intradermal tattoos for accurate laser treatment.[17]
9. To study the hair abnormalities in trichothiodystrophy.[18]
10. To characterize Merkel cells on vellus hair follicles of the facial region and study Merkel cell carcinoma.[19]
11. To study the influence of liphophilicity and vehicle composition on permeation of a drug in a hair follicle.[20]
12. To quantify the number of Langerhans cells and other epidermal cell nuclei per volume unit in skin biopsies. A study has found a single Langerhans cell to be present per 53 epidermal cells.[21]

Many other uses are being explored for dermatological indications.

PROS AND CONS OF CONFOCAL LASER MICROSCOPE

Advantages of confocal microscopy include rapid, noninvasive technique allowing early diagnosis and management and high resolution images[2] as compared to CT scan, MRI and USG for dermatological use. Disadvantages of confocal microscopy include its high cost and relatively smaller field of vision.

Confocal microscopy is currently in a stage of development. Newer modifications in the technique are taking place by leaps and bounds. At present, though its use is limited for the purpose of research, the confocal laser microscope may have enormous clinical implications for dermatology in future.

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