# 多光子顯微鏡系統在皮膚的診斷治療之應用

# Multiphoton Microscopy in the Application of Skin Diagnosis and Treatment


## 中文摘要

雷射光療廣泛應用於治療許多種組織包括皮膚的疾病。鉺雅鉻雷射是一種常用的雷射來治療皮膚腫瘤和磨皮；紫外光已知是造成皮膚鬆弛老化的一個重要因子；而電磁波拉皮機器靠著加熱真皮，已被用來做非侵入性的皮膚緊緻治療，治療鬆弛的老化現象。爲了找到適合不同個體差異的治療能量，需要評估組織的即時反應和變化。多光子顯微鏡是一種非侵入性的系統，可提供細胞層次的高解析度，近年來被應用於觀測活體生物的研究。此研究目的主要在活體上利用多光子及二倍頻顯微術，做爲評估雷射和電磁波對組織作用的系統。鼠皮在使用不同能量的鉺雅鉻雷射、紫外光及電磁波作用後，使用多光子顯微鏡，針對皮膚的自發螢光和二倍頻等非線性光學訊號做型態以及定量分析。二倍頻的量已經被證實和膠原蛋白的量有正相關。鉺雅鉻雷射在低能量下可以把角質層震鬆，而沒有傷害到底下的組織，這個效應可用來進一步研究造成雷射輔助藥物經皮穿透的機轉。鉺雅鉻雷射在高能量下所造成的傷灼現象，以及底下表皮、真皮結締組織的破壞可以清楚得由多光子顯微鏡看到。我們能夠更進一步定量分析二倍頻減少的程度，來評估雷射後剩餘熱傷害的程度。我們利用多光子顯微鏡研究經過紫外光照射後的光老化現象，在紫外光照射的組別其膠原蛋白的二倍頻訊號比起對照組有顯著的降低，而光老化形成的彈性纖維也可在多光子顯微鏡下以自發螢光表現。同時，我們評估電磁波拉皮治療皮膚老化的效果，二倍頻減少的程度和電磁波的強度成正比，表示膠原蛋白因爲電磁波加熱的關係而分解。但是在電磁波後的一個月，其二倍頻的強度反而增加到比治療前還要強，表示膠原蛋白在破壞後經過一段時間有增生，這是解釋電磁波能緊緻皮膚的可能機轉。多光子顯微鏡能夠得到細胞層次高解析度的影象，觀察皮膚角質層、表皮層、真皮細胞外間質的變化。由於其非侵入性，可以觀察電波及光線治療後依序的皮膚變化。多光子顯微鏡是一個能應用在活體上評估雷射、紫外光、電磁波對於皮膚組織作用的一個良好工具。此研究將可以使我們更清楚非線性造影術在瞭解電波及光線與組織交互作用的詳細過程的價值，以及進一步應用於臨床研究。

## 英文摘要

Laser and light is frequently used in treating various diseases, including skin disorders. Erbium:YAG laser is a commonly used laser to treat skin tumors and dermabrasion. Ultraviolet (UV) light is known as a major factor in skin aging and laxity. Radiofrequency have been introduced for nonablative tissue tightening by volumetric heating of the deep dermis to treat redundant skin laxity, which is a major feature of aging. Real-time evaluating tissue reaction is necessary in order to adjust the laser or radiofrequency energy to suit individual. Recently, multiphoton microscopy is an emerging non-invasive technology, which can provide cellular level high resolution images in vivo. The aim of this study was to validate the usefulness of MPM as an imaging modality for monitoring tissue reaction after light, laser and radiofrequency in vivo. Nude mouse skin was irradiated with an Erbium:YAG laser and radiofrequency device at various fluence and the skin was imaged using a MPM. The alterations of cutaneous non-linear optical properties including multiphoton autoflurescence and second harmonic generation (SHG) associated with laser and radiofrequency treatment was evaluated morphologically and quantitatively. SHG intensity has been shown to correlate with the amount of collagen. Out study showed that, at low fluence, Erbium:YAG laser selectively loosened the compact stratum corneum without detectable damage to the viable skin. This effect may contribute to laser-assisted transcutaneous drug delievery. At high fluence, the residual epidermal and dermal structures could be visualized after ablation. Furthermore, the degree of collagen damage in the residual thermal zone was evaluated by quantitative analysis of second harmonic generation signals. UV light induced photodamaged skin was studied with multiphoton microscopy. There was significant decrease of collagen SHG signals in UV-irradiated group compared to control. Solar elastosis was found to emit autofluorescence in MPM. Also, we studied antiaging effects of radiofrequency by MPM. The dermal collagen SHG signals decreased in accordance to energy of irradiation, which means collagen disruption after heating effects of radiofrequency. The SHG signals increased over baseline one month after radiofrequency. The effects displayed collagen regeneration after heat-induced disruption, which could be the mechanism of skin tightening with radiofrequency. Multiphoton imaging with cellular resolution clearly visualized the reaction of the stratum corneum, keratinocytes and dermal extracellular matrix caused by laser, radiofrequency and ultraviolet irradiation. Due to its non-invasiveness, we can obtain serial change of skin. In conclusion, MPM is an ideal tool for monitoring tissue reaction to laser, UV light and radiofrequency in vivo. The study support the usefulness of non-linear optics in studying mechanism of tissue remodeling after laser or radiofrequency treatment, which can be applied in further clinical research.