

ORION CONCEPT

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La MICROSCOPIE MULTIPHOTONIQUE
« Imagerie moléculaire de fluorescence »

*Training Days – Mars 2012 -
Tours*

Together
To think differently ...

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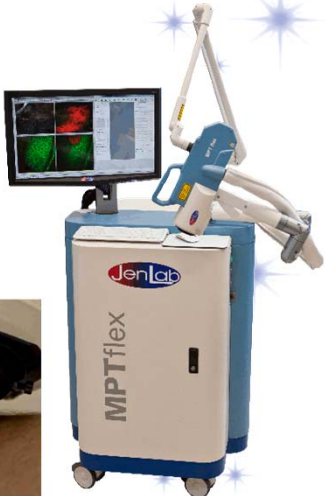
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Appareils

Dermalinspect / MPT Flex

- ✓ Acquisitions de stacks de 300x300 μm jusqu'à 150 μm de profondeur (Reticulum superficiel Elastine et Collagène)
- ✓ Acquisitions simultanée de l'Elastine et du Collagène (seconde harmonique SHG)
- ✓ Acquisition du NADPH contenu dans les cellules de l'épiderme (processus oxydatif lié au vieillissement cutané)

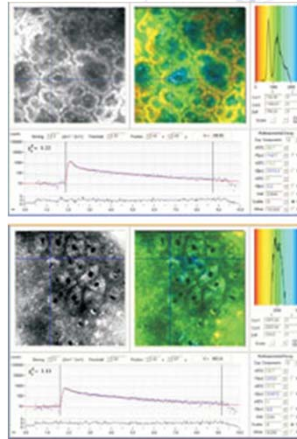
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Principe

Fluorescence des composés cutanés

- ✓ *Laser fento seconde (Haute énergi délivré sur un temps très court)*
- ✓ *Longueurs d'onde variables (proche infrarouge)*
- ✓ *Excitation des molécules telles que : le NADPH, la mélanine, les porphyrines, elastine...*
- ✓ *FLIM : Mesure du temps de fluorescence des composés (in vitro à ce jour...) fonction de leur environnement moléculaire*

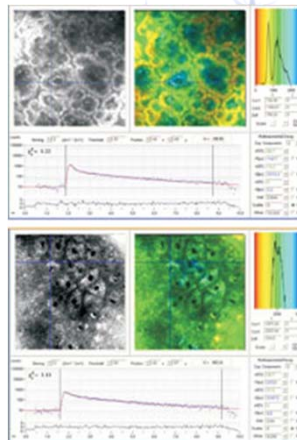



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Caractéristiques

- ✓ *Acquisition de stack de 300X300 jusqu'à 150 µm de profondeur – Pas variable de minimum 1 µm*
- ✓ *Résolution : < 0.5 µm dans le plan des images*
- ✓ *Epaisseur de coupe : 5 µm*
- ✓ *Temps d'acquisition variable selon rapport signal sur bruit (4.2 à 15 secondes par coupe – optimal à 7.3 seconde)*
- ✓ *Coupe dans le plan de la peau*
- ✓ *Longueur d'onde : 710 à 920 nm (Proche IR)*



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Imagerie




Image des cellules de l'épiderme
(Couche granuleuse)

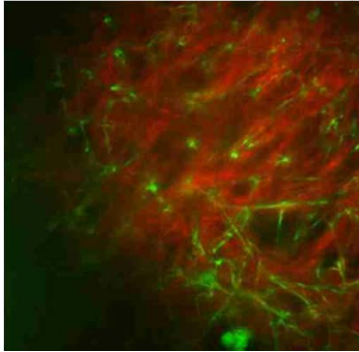



Image simultanée de l'Elastine (vert)
et du collagène (rouge) du derme
superficiel

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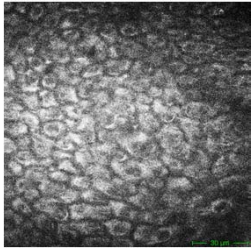

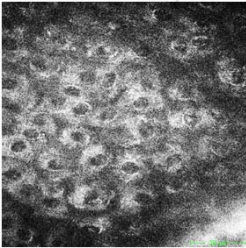
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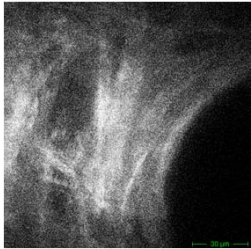
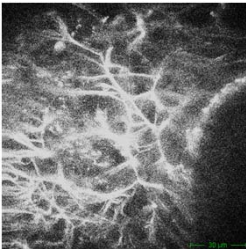
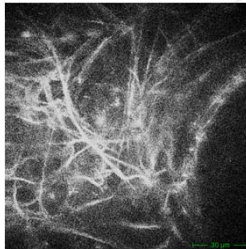
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Exemple d'étude :
Multiphoton Confocal Laser Microscopy

Preliminary studies for the skin ageing process

Studies in progress ...

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Methodology

DermalInspect

- ✓ Acquisitions stacks of 300x300 μm up to 150 μm (superficial reticular networks of Elastic/Collagen)
- ✓ Simultaneous acquisitions of the SHG of collagen
- ✓ Acquisition of the NADPH contained in epidermis cells (oxidative process of ageing)

Faisability

- ✓ To determine functionality of the system *in vivo*
- ✓ Repeatability/reproducibility
- ✓ Definition of a protocol for measurement
 - ✓ Location/number of repetitions/re-positionning of the measurements...

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Methodology

Research Studies

- ✓ Quantification of the NADPH in epidermis cells and relationship with the ageing
- ✓ Quantification of the SAID (ratio between collagen and elastin)
- ✓ Quantification of the evolution of this ratio with the depth into the reticular dermis and the age
- ✓ Relationship with the hormonal status
- ✓ Correlation with Cutometry (validation of the panel)
- ✓ Correlation with the High Frequency echography of the skin (SENEB)
- ✓ Correlation with the clinical signs of ageing
- ✓ ...etc

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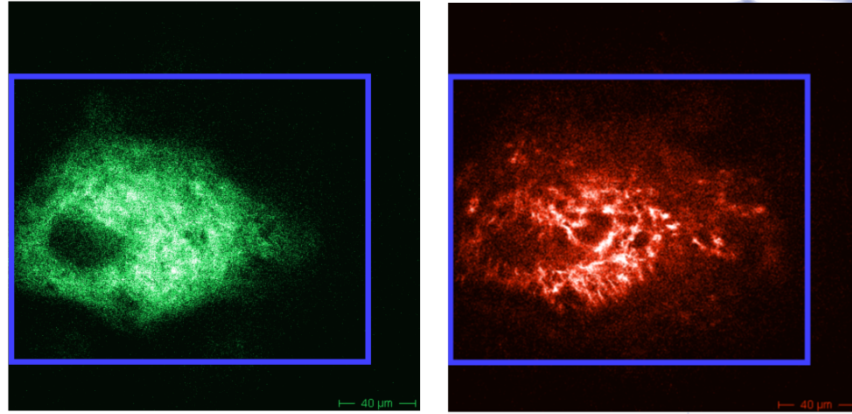
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Example of the variability of the technique for such a purposes Elastin/Collagen ratio

	REPRODUCIBILITE (n=2 days)			
Collagene /Elastin Superficial	Collagène Sup.	Elastine Sup	Ratio Coll/Elast	SAIID
Mean	90,66666667	78,83333333	1,158290836	0,0702
SD	0,73711148	2,375570107	0,034016076	0,014207419
Variation	0,8%	3,0%	2,9%	20,2%

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First results for the validation Elastin/Collagen ratio



Collagen image (left) and simultaneous acquisition of the elastin (right) for the same location into the skin

