ORION Concept | COSM'O Lab | TECHN'O

# La MICROSCOPIE CONFOCALE DE REFLECTANCE de la PEAU

*Training Days – Mars 2012 - Tours*

Together

*To think differently ...*

Cosmetic Valley

ORION Concept | COSM'O Lab | TECHN'O

## Microscopie Confocale IN VIVO
## Les appareils

*VivaScope 1500 mono et trilaser*

*VivaScope 3000*

Cosmetic Valley

# Microscopie Confocale 3000

## VivaScope® 3000 Multilaser - Données techniques

- *Résolution optique : horizontale: <1.25 µm au centre du champ d'image ; verticale : <5.0 µm au centre du champ d'image*
- *Profondeur d'imagerie : jusqu'à 200 µm (selon la nature du tissu)*
- *Section visualisable, image individuelle : 1000 µm x 1000 µm*
- *Résolution d'image : 1000 x 1000 pixels (optimisé Nyquist)*
- *Cadence d'images : 8 images / seconde*
- *Puissance d'exploitation optique : CDRH classe 1, EU classe 1M (max. 16mW)*
- *Longueur d'onde imagerie : 830 nm*
- *Objectif inclus : Lucid StableViewTM F.L. = 4.3 mm, 0.9 NA immersion*
- *Certifications : FCC classe A, marqué CE*



ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale 1500


## VivaScope® 1500 Multilaser - Données techniques

- *Résolution optique : horizontale : <1.25 µm au centre du champ d'image ; verticale : <5.0 µm au centre du champ d'image, l'épaisseur optique de la coupe transversale est réduite avec une longueur d'onde plus courte mais toujours inférieure à 5.0 µm*
- *Profondeur d'image : selon la longueur d'onde sélectionnée*
- *Section visualisable, image individuelle : 500 µm x 500 µm (section écran utilisable)*
- *Champ d'image affiché : +/- 4.0 mm en direction de x et y (8-mm2)*
- *Résolution d'image : 1000 x 1000 pixels (optimisé Nyquist)*
- *Cadence d'images : 9 images / seconde*
- *Puissance d'exploitation optique : laser rouge : CDRH classe 1, EU classe 1M (pour lasers classe 1M, regarder la sortie de laser avec certains instruments optiques dans 100 mm peut représenter un danger pour les yeux) ; laser bleu : à déterminer, par conséquent une protection pour les yeux est recommandée*
- *Longueur d'onde imagerie : 785 nm, 658 nm, 488 nm*
- *Objectif inclus : Lucid StableViewTM F.L. = 4.3 mm, 0.9 NA immersion eau*
- *Certifications : FCC classe A, marqué CE*

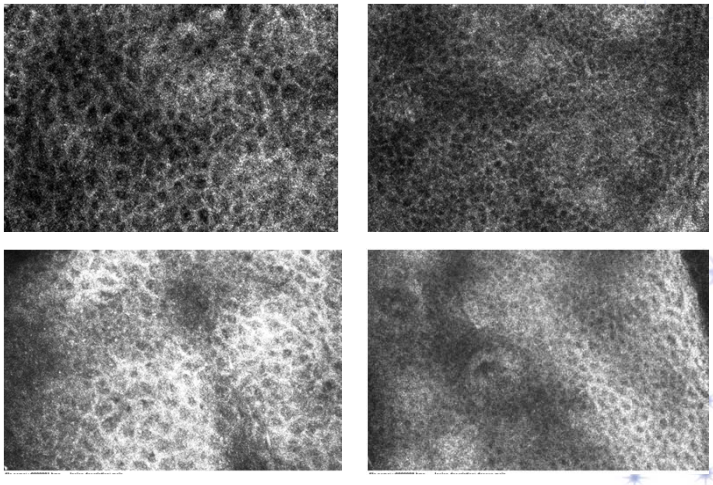
Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

## Microscopie Confocale 1500 multilaser

Infrared Laser 830 nm

Blue Laser 445 nm



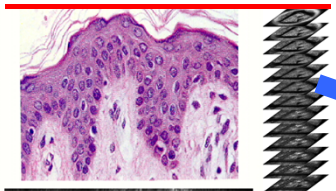
Granular Layer | Spinous Layer

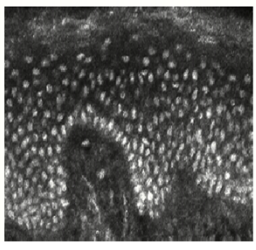
Cosmetic Valley France

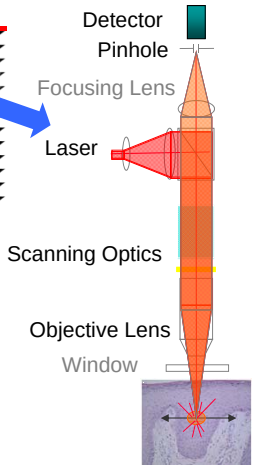
ORION Concept | COSM'O Lab | TECHN'O

## Microscopie Confocale IN VIVO

*VivaScope 1500*








**500 µm**

**Ep coupe : 4-5µm**

**Résol XY: 0.5 - 2µm**

**Pas : 1µm**

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

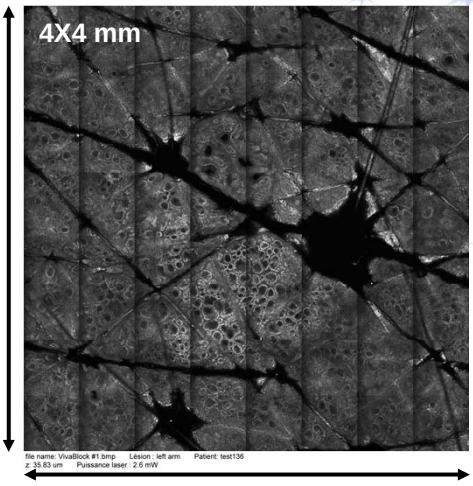
# Microscopie Confocale IN VIVO

- *Acquisitions de « Stacks » (Piles Img. équidistantes)*
- *Acquisitions de « Blocks » (Mosaïques) à différentes profondeurs*
  *(Résolution conservée)*

**Jusqu'à 8 mm de coté**

**1Mo par acquisition élémentaire**

**Meilleure représentativité de la peau**

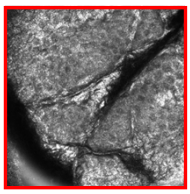



Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

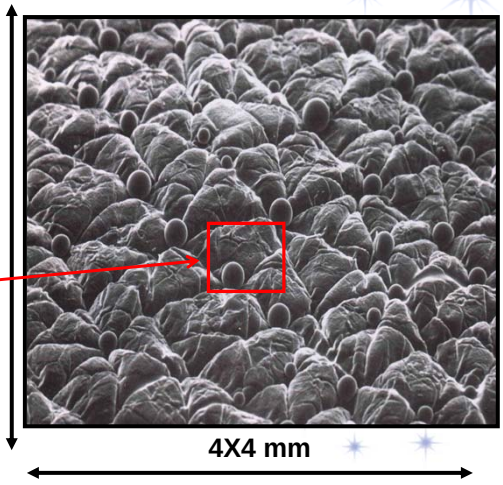
# Microscopie Confocale IN VIVO

- *A cette échelle, la peau n'est pas lisse !!*
  *(Image de MEB)*



**500 µm**

**Acquisition élémentaire VivaScope**

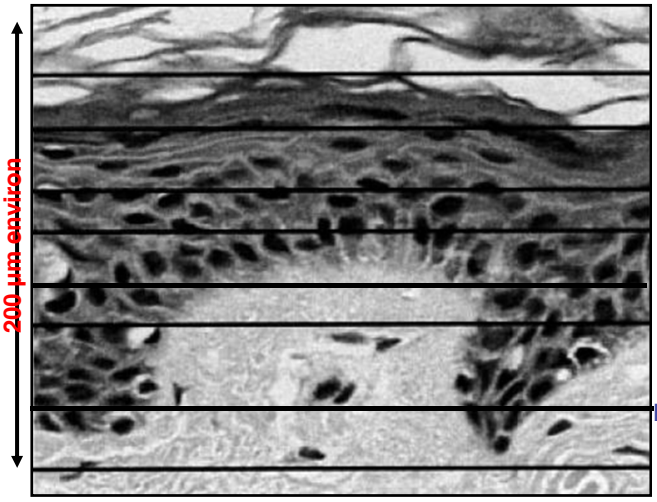


**4X4 mm**

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

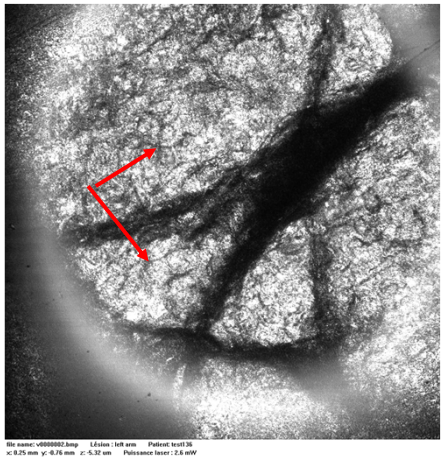



- Vieillissement
- Pigmentation
- Stratum Corneum
- Granular layer
- Spinous layer
- Basal layer
- Upper Papillary dermis
- Papillary dermis
- Lower Papillary dermis
- Superficial reticular dermis

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

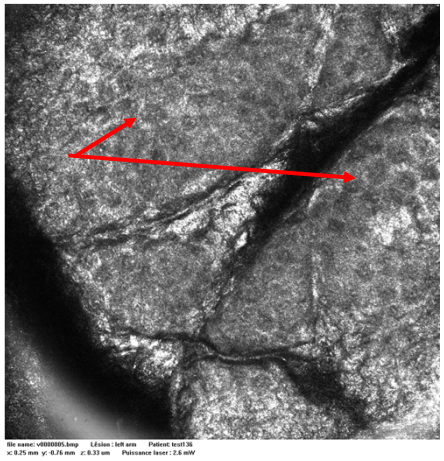


- Vieillissement
- Pigmentation
- Stratum Corneum
- Granular layer
- Spinous layer
- Basal layer
- Upper Papillary dermis
- Papillary dermis
- Lower Papillary dermis
- Superficial reticular dermis

Cosmetic Valley France

ORION CONCEPT | COSM'O LAB | TECHN'O

# Microscopie Confocale IN VIVO



- Vieillissement
- Pigmentation

Stratum Corneum

**Granular layer**

Spinous layer

Basal layer

Upper Papillary dermis

Papillary dermis

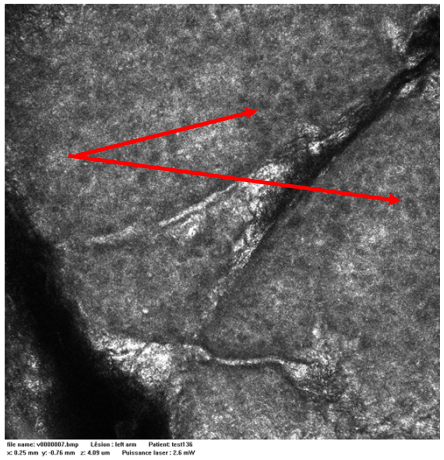
Lower Papillary dermis

Superficial reticular dermis

COSMETIC VALLEY FRANCE

ORION CONCEPT | COSM'O LAB | TECHN'O

# Microscopie Confocale IN VIVO



- Vieillissement
- Pigmentation

Stratum Corneum

Granular layer

**Spinous layer**

Basal layer

Upper Papillary dermis

Mid Papillary dermis

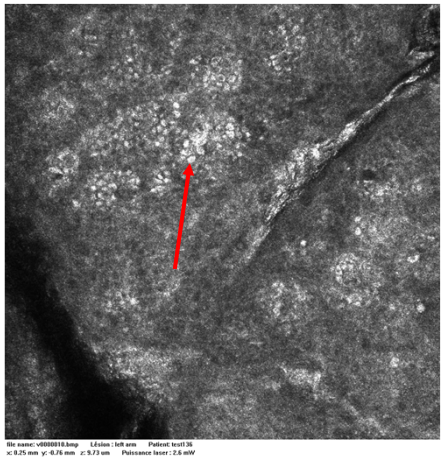
Lower Papillary dermis

Superficial reticular dermis

COSMETIC VALLEY FRANCE

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO



- Vieillissement
- Pigmentation

Stratum Corneum

Granular layer

Spinous layer

Basal layer

Upper Papillary dermis

Papillary dermis

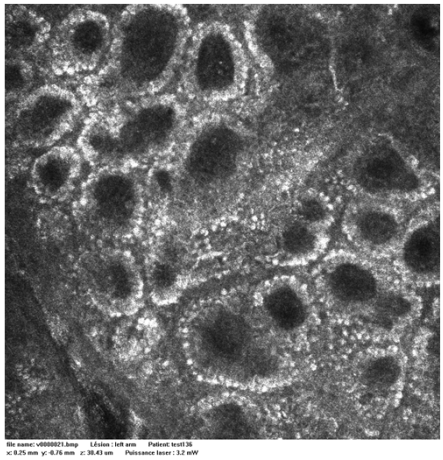
Lower Papillary dermis

Superficial reticular dermis

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO



- Vieillissement
- Pigmentation

Stratum Corneum

Granular layer

Spinous layer

Basal layer

Upper Papillary dermis

Papillary dermis

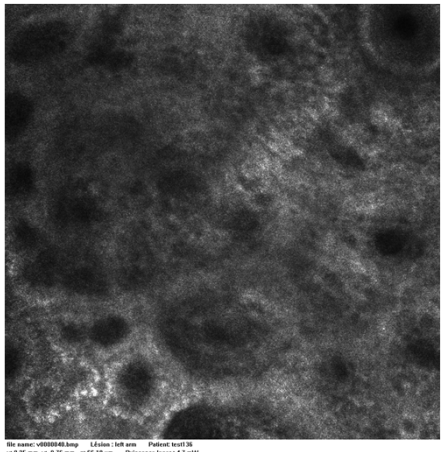
Lower Papillary dermis

Superficial reticular dermis

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO



- Vieillissement
- Pigmentation

Stratum Corneum

Granular layer

Spinous layer

Basal layer

Upper Papillary dermis

Papillary dermis

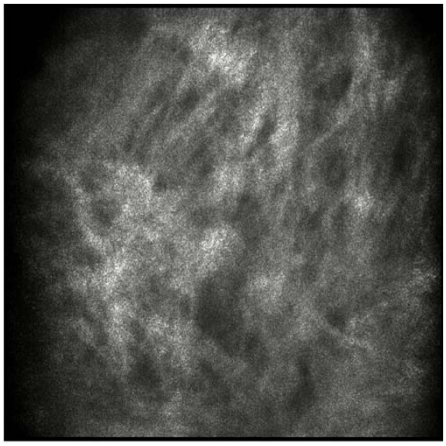
Lower Papillary dermis

Superficial reticular dermis

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO



- Vieillissement
- Pigmentation

Stratum Corneum

Granular layer

Spinous layer

Basal layer

Upper Papillary dermis

Papillary dermis

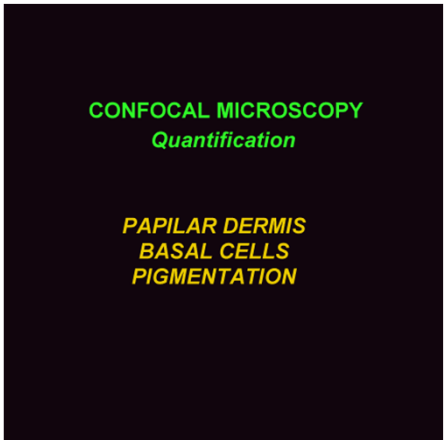
Lower Papillary dermis

Superficial reticular dermis

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO




Vieillissement
Pigmentation
Stratum Corneum
Granular layer
Spinous layer
Basal layer
Upper Papillary dermis
Mid Papillary dermis
Lower Papillary dermis
Superficial reticular dermis

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

***Analyses répétables*** ***ConfoScan® V02.0.7***

***Exigence d'analyses d'images pour « comparer » :***

- ✓ ***Un repérage en profondeur précis***
  - ***Mesurer au même niveau à différents temps d'observation***
- ✓ ***Travailler sur une région représentative***
  - ✓ ***Piles ou Mosaïques***
  - ✓ ***Définition de protocoles d'acquisition selon étude***
- ✓ ***Une gestion d'un échantillon dans le temps :***
  - ***Maîtrise des paramètres d'analyses pour un individu en fonction du temps***
  - ***Un outils dédié utilisateur***

Cosmetic Valley France

# Microscopie Confocale IN VIVO

***Analyses répétables*** ***ConfoScan® V02.0.7***

***Finalités premières : Vieillissement et Pigmentation***

- ✓ ***Pigmentation :***
  - ***Nombre, densité (charge) et répartition cellules basales sur N coupes ou mosaïques (mélanocytes?)***
- ✓ ***Vieillissement : Niveau SC, Papillaire, Réticuliaire***
  - ***Mesures hauteur sur coupes reconstruites en Z :***
    - ***Épaisseur SC, Epiderme***
    - ***Hauteur/largeur des papilles dermiques***
  - ***Mesure dans le plan :***
    - ***Texture du microrelief SC***
    - ***Papilles (hauteur, morphologie, densité de répartition, taille, nombre…)***
    - ***Réseau collagène/élastine (derme réticulaire, structure, densité)***

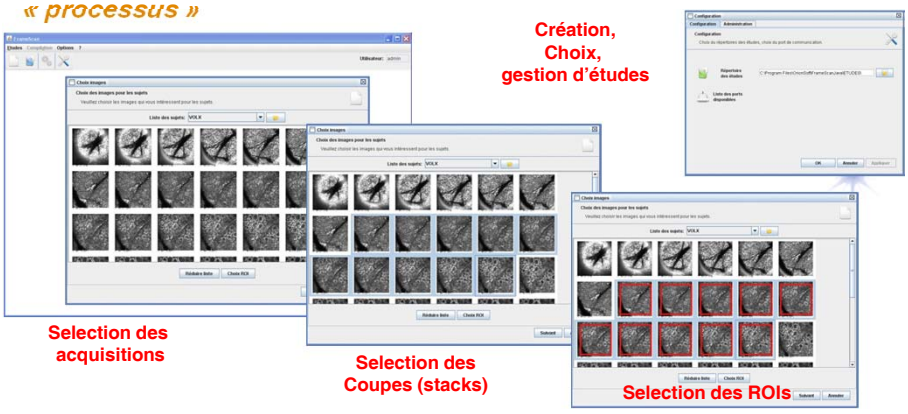


ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

***ConfoScan® V02.0.7***

- ***L'interface logiciel – Gestion d'échantillon et/ou mesure ponctuelle par « processus »***




Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

*Lancement d'un processus d'analyse*

- ✓ *Pigmentation : Derme Papillaire*
- ✓ *Vieillissement : Derme Papillaire, Réticuliare*
- ✓ *Reconstruction coupes Z pour mesures d'épaisseurs*

*Compilation des résultats de l'échantillon en fonction du temps*

- ✓ *Ficher Excel, Texte, PDF*

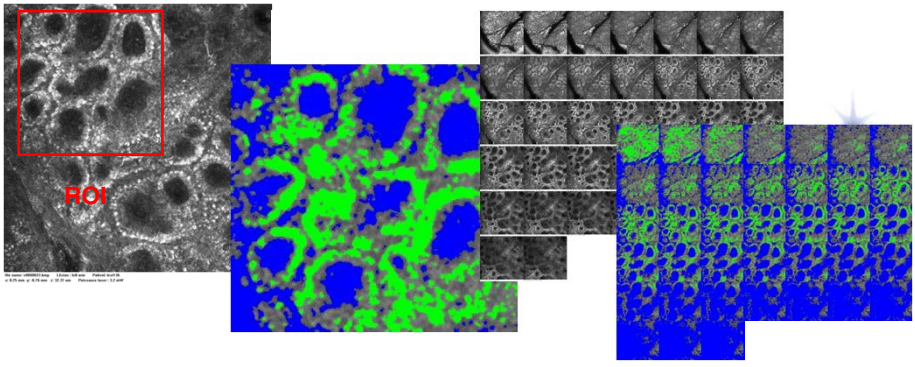
Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

*Analyse de pigmentation*

- ✓ *Nombre et charge en mélanine des cellules basales*




Cosmetic Valley France

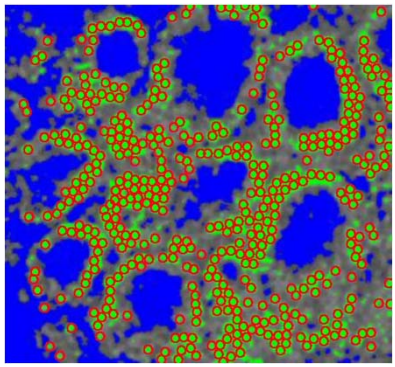
ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

*ConfoScan® V02.0.7*

*Analyse de pigmentation*

✓ *Nombre et charge en mélanine des cellules basales(et forme)*



Analyses par "modèle"

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

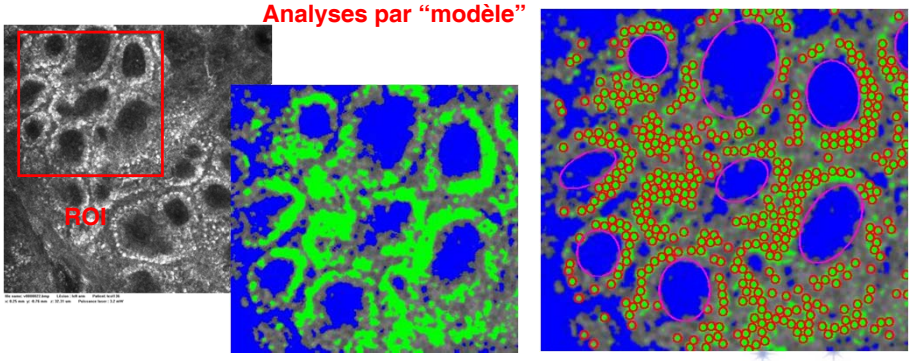
# Microscopie Confocale IN VIVO

*ConfoScan® V02.0.7*

*Analyse du vieillissement - Papilles*

✓ *Nombre, densité, orientation et critère forme des papilles*

Analyses par "modèle"




Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

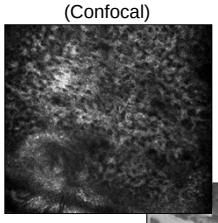
# Microscopie Confocale IN VIVO

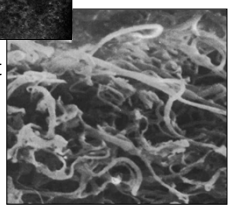
*ConfoScan® V02.0.7*

*Analyse du vieillissement - Derme réticulaire superficiel*

✓ *Texture et densité : un aspect différent*

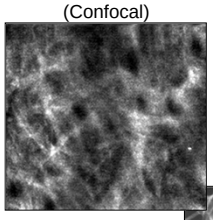
Photodamaged Elastin
(Confocal)

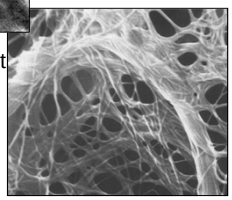




75 year old panelist
In-vivo confocal

Normal Skin
(Confocal)





41 year old panelist
In-vivo confocal

***Exemple : Elastose***
***Photo-Vieillissement***

Cosmetic Valley France

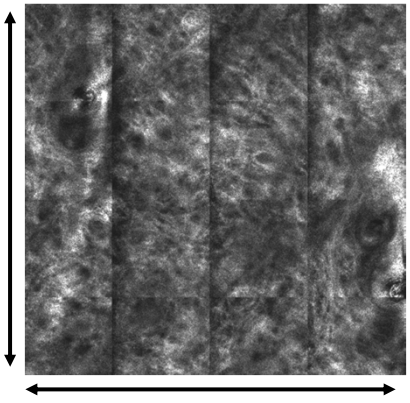
ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

*ConfoScan® V02.0.7*

*Analyse du vieillissement - Derme réticulaire superficiel*

✓ *Texture et densité : une organisation régulière (jeune)*



**2X2 mm**

**Mosaïque réticulaire jeune**

Cosmetic Valley France

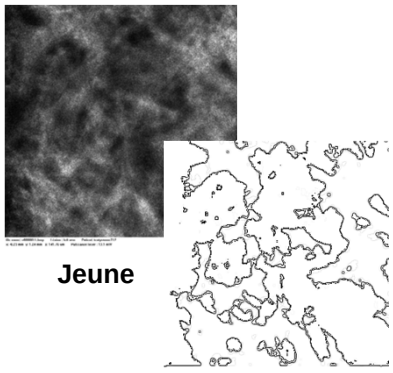
ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

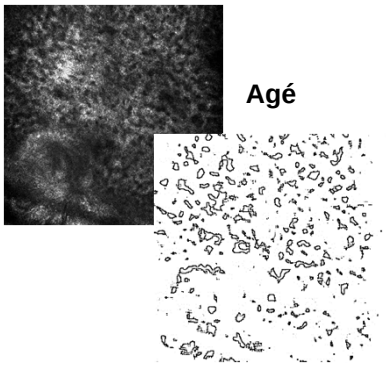
*ConfoScan® V02.0.7*

*Analyse du vieillissement - Derme réticulaire superficiel*

✓ *Texture et densité : différence d'arrangement des fibres*



Jeune



Agé

Cosmetic Valley

ORION Concept | COSM'O Lab | TECHN'O

# Microscopie Confocale IN VIVO

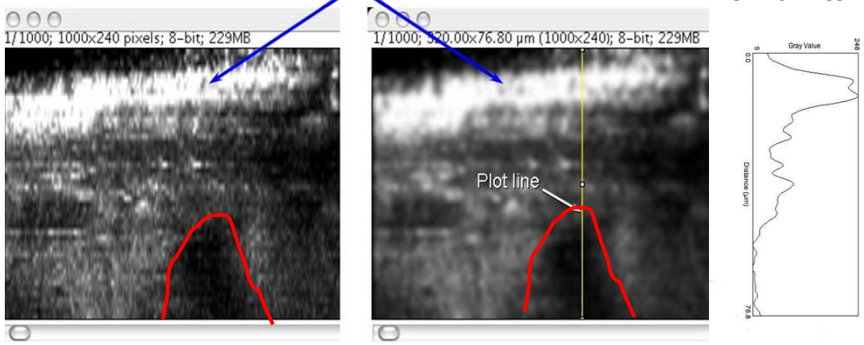
*ConfoScan® V02.0.7*

*Analyse du vieillissement – Mesures hauteur des papilles, du SC…*

✓ *Reconstruction de coupes en Z*

✓ *Mesures : Hauteur, largeur mi-hauteur…*

**Profil d'intensité**




Cosmetic Valley

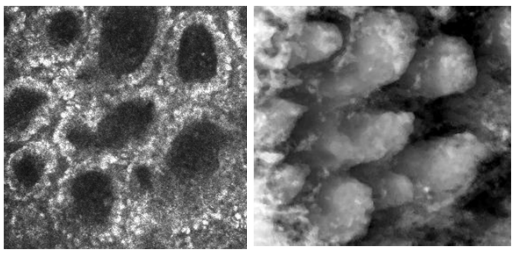
ORION Concept | COSM'O Lab | TECHN'O

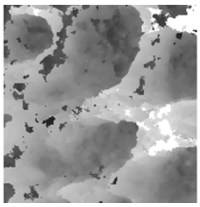
# Microscopie Confocale IN VIVO

## ConfoScan® V01

*Analyse du vieillissement – Mesures dimensionnelles des papilles*

- ✓ *Reconstruction de coupes en Z*
- ✓ *Mesures : Hauteur, largeur*





**Hauteur des papilles**

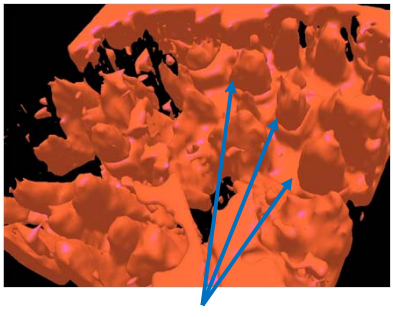
Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

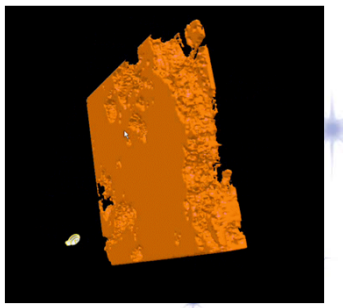
# Microscopie Confocale IN VIVO

## ConfoScan® V01

*…La reconstruction 3D surfacique*
*et aux nouvelles quantifications possibles…*



**Papilles**



*Pour demain … (!)*

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

LVMH RECHERCHE
PARFUMS & COSMETIQUES

*Exemple d'étude :*
*« Cells of the epidermis »*

# EVALUATION OF IN VIVO KERATINOCYTE SIZE WITH CONFOCAL LASER SCANNING MICROSCOPY AT 830 AND 445 NM


***J.C. Pittet**, J. Descoubes*, C. Faucheux*, A. Bernois**, C. Heusèle**, S. Schnebert****
**ORION-Concept, 26 rue Pasteur-37000 Tours, France*
***LVMH Recherche, St Jean de Braye, France*


Together
*To think differently ...*

COSMETIC VALLEY FRANCE

ORION Concept | COSM'O Lab | TECHN'O

## Introduction and Objectives

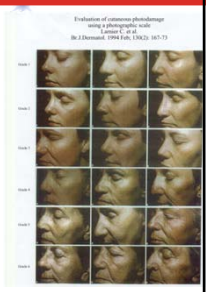
***Confocal Laser Scanning Microscopy (CLSM) allows to obtain "optical biopsies" of the skin and realtime imaging of the keratinocytes of the different layers of the epidermis.***

***The aim of this study was to study, in vivo, on the forehead and the ventral forearm, the size, density and shape of the keratinocytes of the granular and spinous layers with the new VivaScope® 1500 Multilaser and to investigate the age effect these parameters.***

COSMETIC VALLEY FRANCE

ORION Concept | COSM'O Lab | TECHN'O

# Methodology 1/2

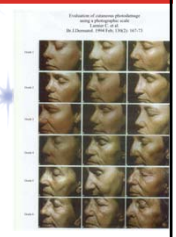


- ✓ **Panel :**
  - *98 healthy Caucasian women aged 18 – 70*
  - *Phototype III*
- ✓ **Localizations:**
  - *On the forehead (photo-exposed area) and the ventral forearm (photo-protected area)*
- ✓ **Measurements :**
  - ✓ *Photoageing scored according to the Larnier scale*
  - ✓ *Biomechanical properties of the skin measured with cutometer SEM 575 (Courage & Khazaka)*
    - *2 mm probe and a 300 mBar suction*
  - ✓ *Image acquisitions obtained with the VivaScope® 1500 Multilaser with 2 wavelengths: 430 nm and 830 nm*
    - *3 stacks separated by 5 mm – 2µm step up to 150 µm depth*
    - *2 mosaïcs (4X4mm) in the granular and spinous layers*

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Methodology 1/2



- ✓ **Panel :**
  - ✓ *98 healthy Caucasian women aged 18 - 70 was recruited*
    - ✓ *Phototype II*
- ✓ **Measurements : on forehaed and the ventral forearm**
  - ✓ *Photoageing was scored according to the Larnier scale*
  - ✓ *Biomechanical properties of the skin were measured with Cutometer SEM 575 (Courage & Khazaka)*

*2 mm probe and a 300 mBar suction on the forehead and the ventral forearm.*

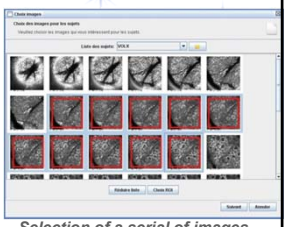
- ✓ *Image acquisitions were obtained with the VivaScope® 1500 Multilaser (Lucid - Mavig GmbH) with 2 wavelengths: 445 nm and 830 nm*
  - *3 stacks separated by 5 mm – 2µm step up to 150 µm depth*
  - *2 mosaïcs (3X3mm) in the granular and spinous layers*
  - *On the Forehead and Forearm*

Cosmetic Valley France

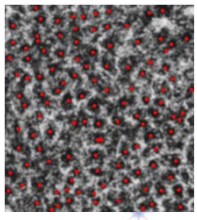
ORION Concept | COSM'O Lab | TECHN'O

# Methodology 2/2

- ✓ **Software:**
  - ✓ ConfoScan V02 (ORION Concept, France)
    - ✓ On mosaïcs of the granular and spinous layers
    - ✓ Between 100 and 500 cells counted per subject (From 5 to 10 images /mosaïc)
- ✓ **Analysis:**
  - ✓ Processing of images for nucleus detection
- ✓ **Quantified parameters for each layer:**
  - ✓ Cell surface/density (per unit of surface)



Selection of a serial of images into a mosaïc



- ✓ **Remarks :**
  - ✓ Verification that parameters were independent of the wavelenght :
    - ✓ No difference between the lasers for cell detection (445 vs 830 nm)
  - ✓ Verification of the size variations of spinous cells with the depth :
    - ✓ No variation

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Results 1/2

- ✓ **Cutometry :**
  - ✓ Both on Forehead and Forearm :
    - ✓ Significant decrease of firmness (↓ Uf and - Ua/Uf – p<0.05)
    - ✓ Significant decrease of the elasticity (↑ Uv/Ue, ↓ Ur/Ue – p<0.05)
    - ✓ Significant decrease of the elastic recovery (↓ Ur – p<0.05)
    - ✓ Skin of the forearm was more firm and elastic than on the forehead
- ✓ **Clinical evaluation of skin ageing : (Scored with the Larnier Scale)**
  - ✓ Significant increase of the ageing signs with the age (p<0.05)
  - ✓ Significant correlation between clinical evaluation and mechanical properties measurements (p<0.05)
- ✓ **Validation of the panel in terms of correlation between « age of subject and age of their skin »**

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Results 2/2

✓ *Confocal microscopy : In average on the whole panel (98 Women)*

| Location Parameters | Granular layer | SD *(interindividual variation)* | Spinous Layer | SD *(interindividual variation)* |
|---|---|---|---|---|
| **Forehead** | | ** homogeneous density for locations | | |
| X diameter (µm) | 21.4 | 0.9 (CV 4%) | 12.0 | 1.0 (CV 8%) |
| Y diameter (µm) | 26 | 1.4 (CV 5%) | 14.8 | 1.3 (CV 8%) |
| Density (nb/mm²) | 1810 | 143 (CV 8%) | 5698 | 820 (CV 14%) |
| **Forearm** | | | | |
| X diameter (µm) | 21.6 | 1.8 (CV 8%) | 11.7 | 1.4 (CV 10%) |
| Y diameter (µm) | 26.4 | 2.1 (CV 8%) | 14.7 | 1.5 (CV 12%) |
| Density (nb/mm²) | 1781 | 256 (CV 14%) | 5991 | 1287 (CV 21%) |

* **

**No statistical differences between locations for all parameters**
**Significant difference between granular and spinous layer**

Cosmetic Valley

ORION Concept | COSM'O Lab | TECHN'O

# Results 2/2

✓ *Confocal microscopy : Relation with the age*

*5 groups : (19-27 years), (28-37 years), (38-47 years), (48-57 years), (58-70 years)*

- ✓ *GRANULAR layer : Significant decrease with the age of the cell density on forearm (-15.7% - p<0.05) and on the forehead (-5.4% - Limit p<0.1) = **cells are larger for old skin .***
- ✓ *SPINOUS layer : No Significant modification of the cell density and size both on forearm and the forehead*
- ✓ *For all locations and layers, no modifications were observed on the shape of cells with the age (diameter X and Y)*
- ✓ *Statistical correlation between the firmness (Uf – Onto the face), clinical evaluation of the ageing signs and the size of the granular cells on the forehead : more the cells are big, more the firmness is bad.*

Cosmetic Valley

ORION Concept | COSM'O Lab | TECHN'O

CHANEL
RECHERCHE ET TECHNOLOGIE
RESEARCH AND TECHNOLOGY

*Exemple d'étude :*
*Reflectance Confocal Microscopy – Basal pigmentation*

Whitening effect of a dermio-cosm »tique product
Comparison of confocal microscopy and skin surface imaging

***Jean-Christophe Pittet1**, Claire Ezanno2, Agnès Lavoix3, Etienne Camel3, Jérôme Jacqueline-Bessiere2, Danielle Mougin2*

*1 ORION Concept, Tours, France*
*2 CHANEL Parfums Beauté, Pantin, France*
*3 Institut d'Expertise Clinique, Lyon, France.*

Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

## Methodologie 1/4

***Preliminary study in Korea** (IEC Korea)*
- ✓ ***Clinical Evaluation Clinique***
- ✓ ***Chromametric measurements***
  - ✓ *Chromamter Minolta CM2600D (L*a*b*, ITA°)*

Cosmetic Valley France

ORION CONCEPT | COSM'O LAB | TECHN'O

# Methodology

***PRELIMINARY IN KOREA*** *(IEC Korea)*

- ✓ ***Clinical Evaluation Clinique***
- ✓ ***Chromametric measurements***
  - ✓ *Chromamter Minolta CM2600D (L*a*b*, ITA°)*

***MAIN STUDY IN FRANCE*** *(IEC France)*

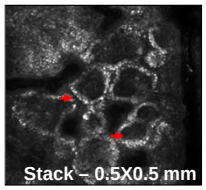
- ✓ ***Panel :***
  - ✓ *23 Asian women (19-70 years old) with lentigines onto the face*
  - ✓ *Whitening product onto the face – twice daily application – 3 months*
- ✓ ***Measuremnts :***
  - ✓ *Digital acquisitions of the skin surface (HR – 6Mpx) of spots (Vivacam, Mavig, Allemagne)*
  - ✓ *Confocal mocroscopy acquisitions (830 Nm) (VivaScope 1500, Mavig, Allemagne)*
    - *2 Stacks in the spot (step 2 µm for the quantification of the melanin)*
    - *1 mosaïc 7X7 mm for iconography*

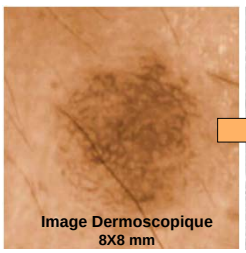
COSMETIC VALLEY FRANCE

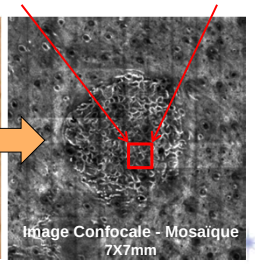
ORION CONCEPT | COSM'O LAB | TECHN'O

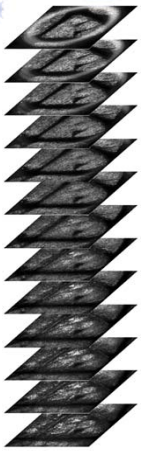
# Methodology

VivaCam



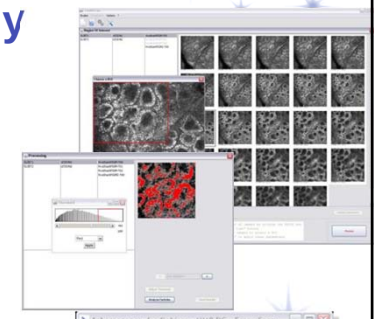



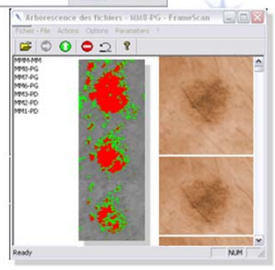



COSMETIC VALLEY FRANCE

ORION Concept | COSM'O Lab | TECHN'O

# Methodology





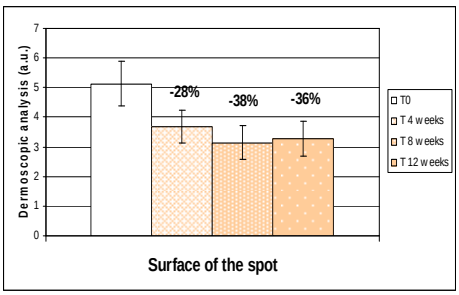
- ✓ **Images analysis**
  - ✓ **Confocal imaging : ConfoScan V1.1.0** *(ORION Concept, France)*
    - ✓ *2 stacks*
    - ✓ *Quantification of the melanin density*
  - ✓ **Dermoscopic imaging : FrameScan V2.95** *(ORION Concept, France)*
    - ✓ *Quantification of color : L*a*b*, ITA°, DeltaE, contrast skin/spot and morphology of spots*

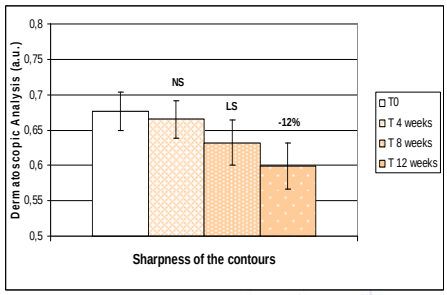
Cosmetic Valley France

ORION Concept | COSM'O Lab | TECHN'O

# Result

- ✓ **Dermoscopic analysis**
  - ✓ **Significant decrease of the spots surface** *(times 1, 2 et 3 months)* **and of the clearness of the edge** *(Time 3 months)*




*LS : Limit significant (0,05≤p<0,10)*
*NS : Not Significant (p≥0,10)*

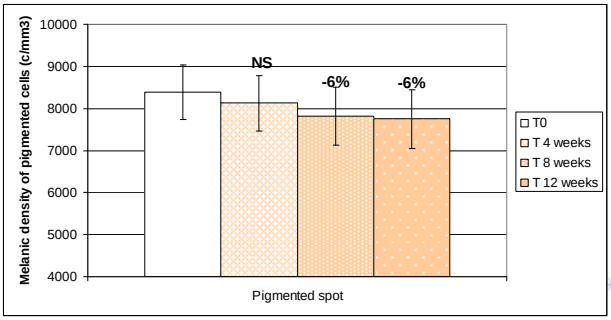
Cosmetic Valley France

ORION CONCEPT | COSM'O LAB | TECHN'O

# Results

✓ *Microscopie Confocale Laser*

✓ *Significant decrease of the melanin density (Times 2 et 3 months) related to the unpigmented skin around.*



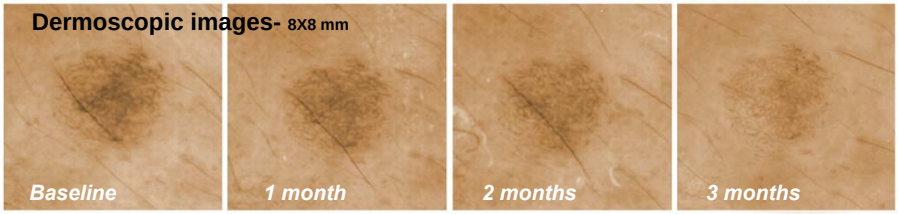

COSMETIC VALLEY FRANCE

ORION CONCEPT | COSM'O LAB | TECHN'O

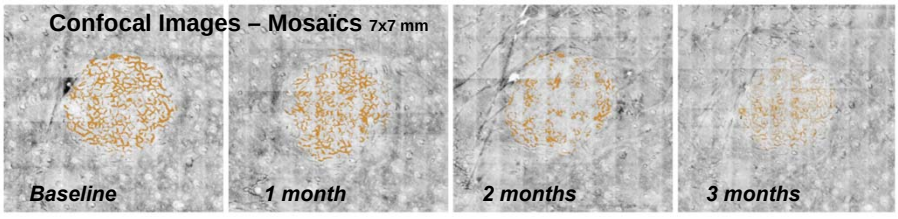
# y

*Sujet 1 : Cas significatif*

**Dermoscopic images-** 8X8 mm




**Confocal Images – Mosaïcs** 7x7 mm

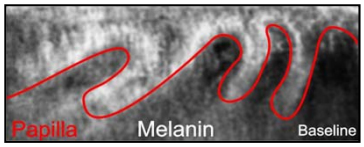


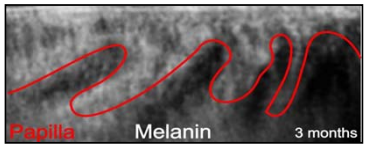

COSMETIC VALLEY FRANCE

ORION CONCEPT | COSM'O LAB | TECHN'O

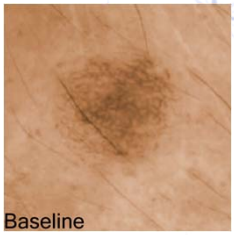
# Iconography

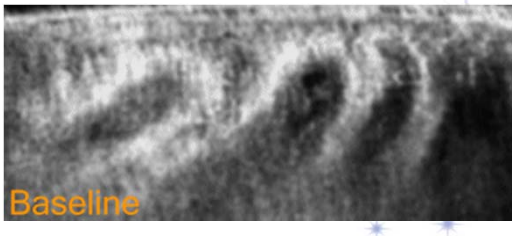
***Subject 1***




**Dermoscopic image**




**Reconstruction in Z direction– Papillar dermis**

COSMETIC VALLEY FRANCE