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# TARGET ACHIEVED: SMOOTH AND YOUNG SKIN FOR LONGER

Target

eyes  lips  forehead

leuphasyl<sup>®</sup> effect

Ca<sup>2+</sup> entrance inhibition  
muscle relaxation

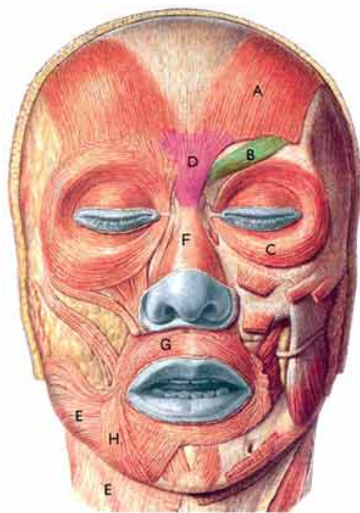
action mechanism

Enkephalin receptor  
leuphasyl  
G1 proteins  
K<sup>+</sup>  
Ca<sup>2+</sup>

# WHAT ARE EXPRESSION WRINKLES?



- ❖ Expression wrinkles (or dynamic wrinkles) are a particular type linked to repeated muscular contraction
- ❖ They are the first wrinkles to appear (as soon as the age of 30)
- ❖ Typical expression wrinkles appear especially in the top half of the face: crow's feet, frown wrinkles, etc.



## PRIMARY MUSCLES OF FACIAL EXPRESSION

- (A) Frontalis
- (B) Corrugator and Depressor supercilli complex
- (C) Orbicularis oculi
- (D) Procerus
- (E) Platysma
- (F) Nasalis
- (G) Orbicularis oris
- (H) Depressor anguli oris

# ...AND THE WRINKLES THEY CAN GENERATE



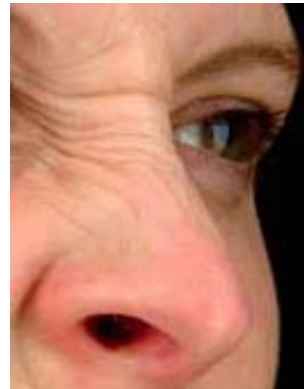
Frontal muscle -  
Horizontal frown lines



Corrugator / Depressor muscle -  
Glabellar lines - vertical rhytides



Orbicularis oculi  
muscle - Crow's feet



Nasalis muscle -  
Horizontal rhytides



Orbicularis oris and mentalis muscles -  
Excessive lip pursing

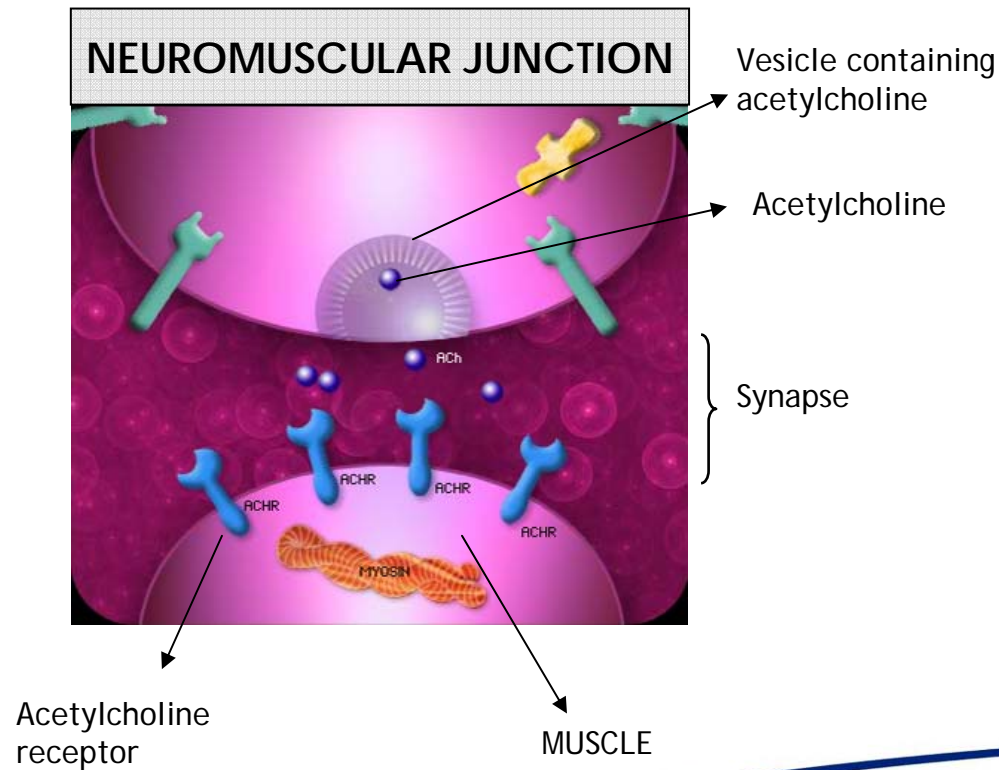
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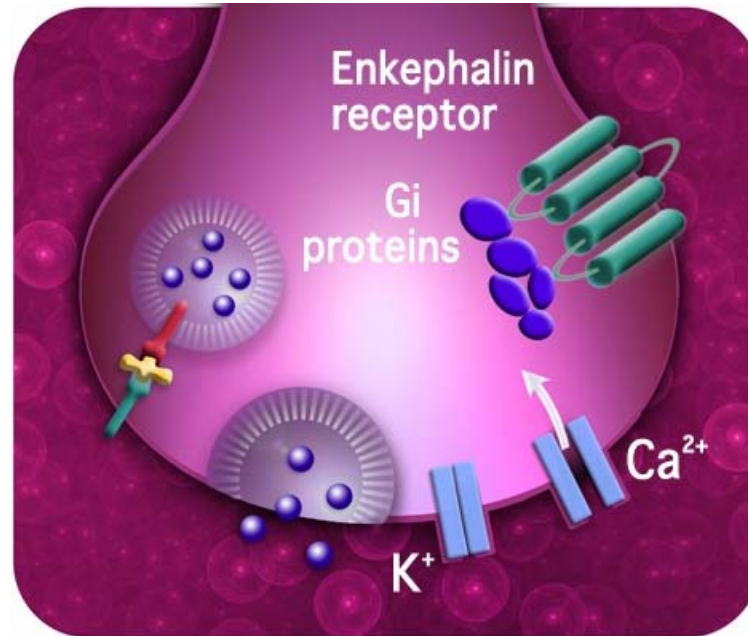
# HOW DOES MUSCLE CONTRACTION WORK?



Muscles are contracted when they receive acetylcholine released from inside a vesicle in a process known as neuronal exocytosis



# NEURONAL EXOCYTOSIS



Two events are required for neuronal exocytosis:

- Vesicles must be loaded with Acetylcholine
- Calcium ions are needed to trigger vesicle fusion



# ANTI-EXPRESSION WRINKLES ACTIVES



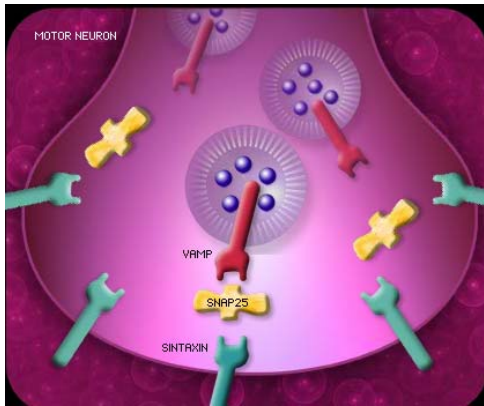
Lipotec has developed two different peptides which prevent the formation of expression wrinkles by attenuating muscular contraction through different mechanisms:

LEUPHASYL<sup>®</sup> and ARGIRELINE<sup>®</sup>

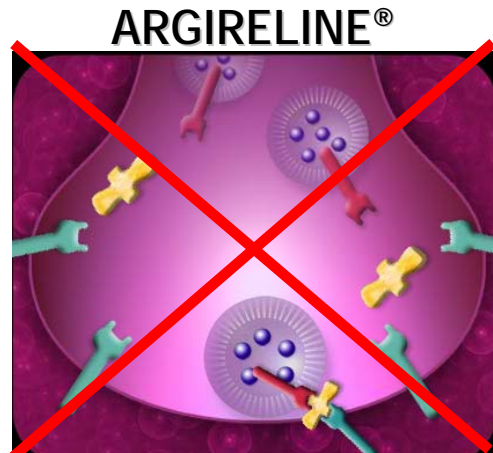
leuphasyl<sup>®</sup> 

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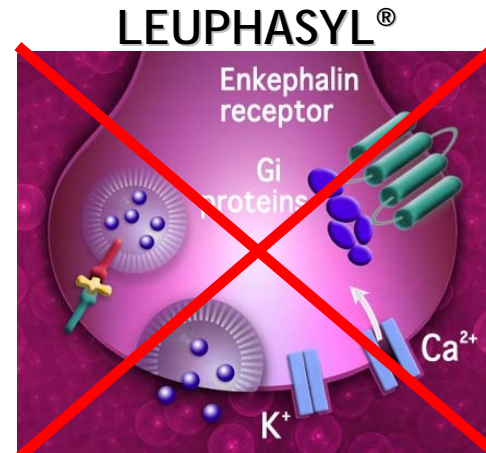
# INTEGRAL TREATMENT AGAINST EXPRESSION WRINKLES



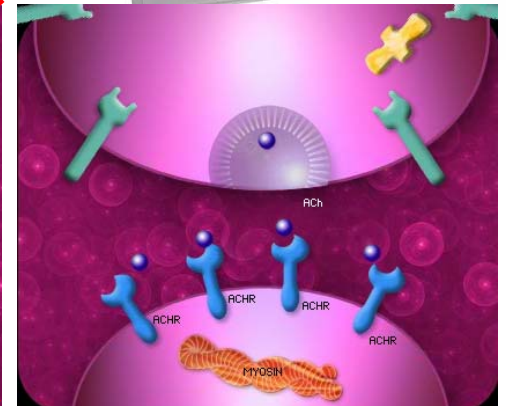
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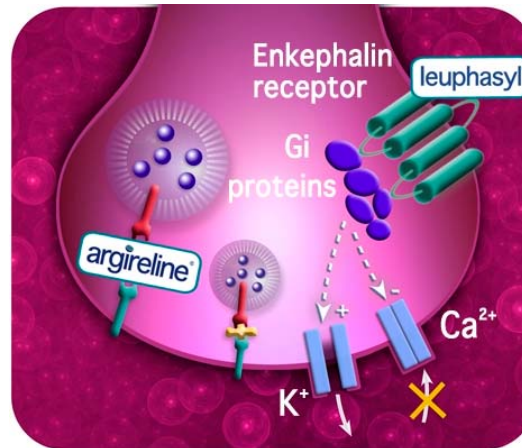
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3



4



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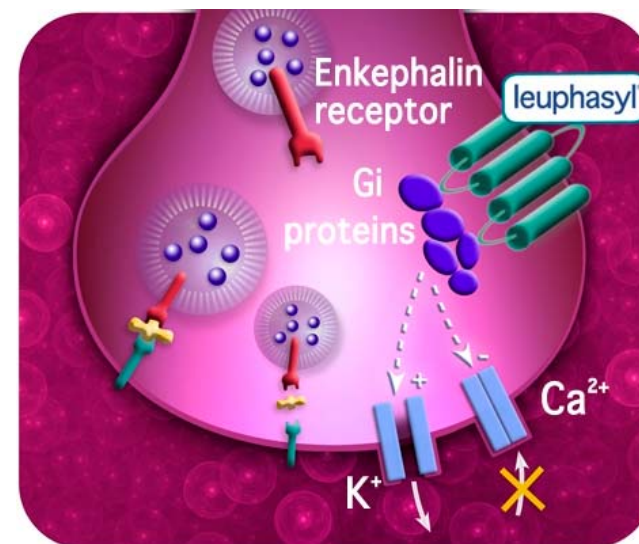


# LEUPHASYL®



❖ LEUPHASYL® is new pentapeptide that mimics in vitro the mechanism of Enkephalins

❖ Coupling of LEUPHASYL® to the enkephalin receptor results in the reduction of the neuron's excitability



This process attenuates muscle contraction, preventing the formation of lines and wrinkles

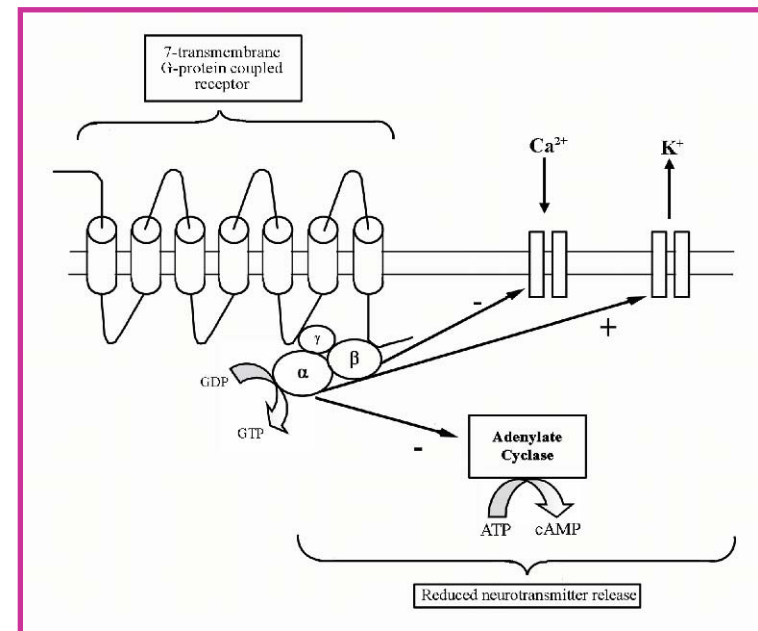
# LEUPHASYL®: MECHANISM



- Enkephalins are endogenous opioids: they inhibit neuronal activity
- Enkephalin receptors are coupled to Gi (inhibitory) proteins
- LEUPHASYL® docking releases a G protein which closes  $\text{Ca}^{2+}$  channels and opens  $\text{K}^{+}$  channels



Neuron excitability is reduced



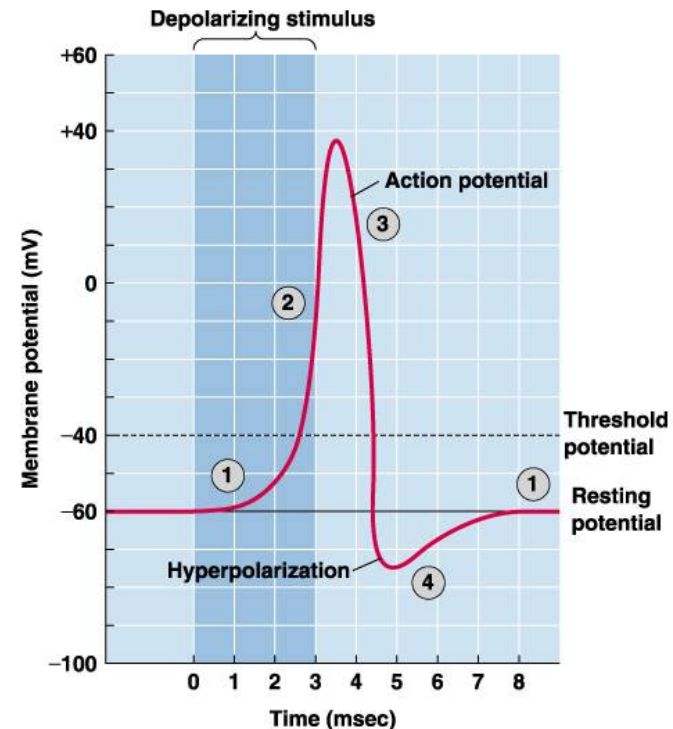
# LEUPHASYL<sup>®</sup>: MECHANISM



- (2) Excitation: Na<sup>+</sup> enters the neuron (making the inside more +, and the potential rise)
- Na<sup>+</sup> open more if the potential rises
- After maintained depolarisation, Na<sup>+</sup> close
- (3) Relaxation: K<sup>+</sup> channels open, + leaves inside and potential decreases



For neuron relaxation we need K<sup>+</sup> channels open and Na<sup>+</sup> channels closed



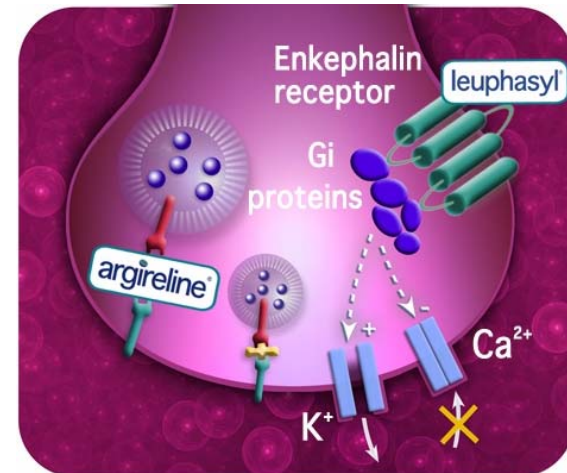
# PROPERTIES



The synthetic pentapeptide offers 2 new properties:

- ✓ A new mechanism to fight expression wrinkles
- ✓ An alternative path means compatibility with other anti-wrinkle peptides

LEUPHASYL® offers an additive/synergistic effect to complement the action of ARGIRELINE®



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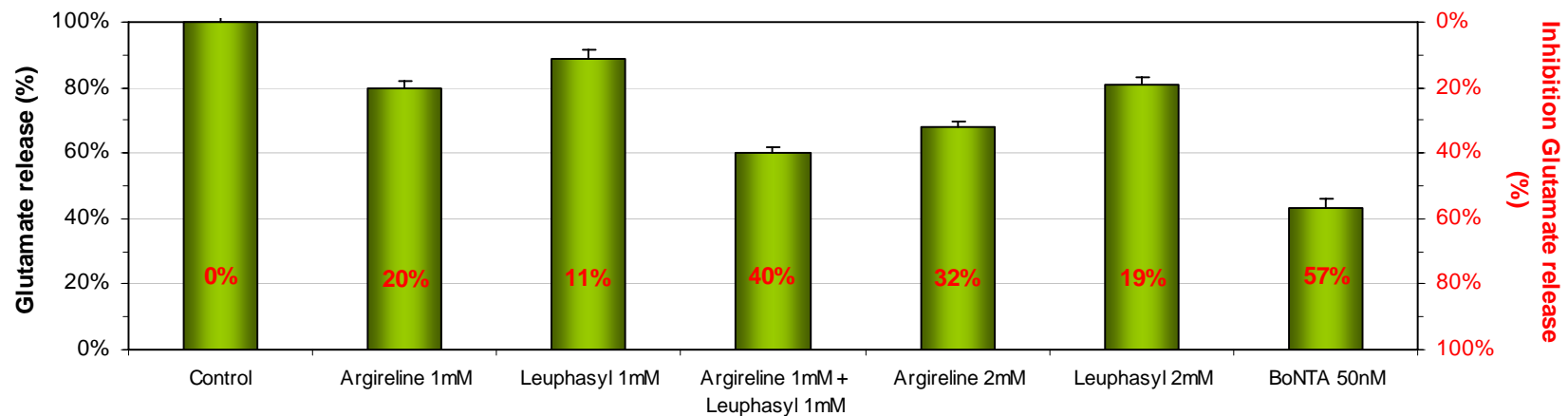
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# IN VITRO TEST

## MODULATION OF GLUTAMATE RELEASE



- ❑ The release of glutamate from the neurons is measured in order to compare the *in vitro* activities of all the peptides
- ❑ Glutamate is the most common neurotransmitter in the brain and its release is used as an estimate for acetylcholine release
- ❑ Values obtained are the average of 6 determinations



**ARGIRELINE®** and **LEUPHASYL®** present an *in vitro* SYNERGISTIC effect, showing that their combination provides better efficacy than using one single peptide even at double concentration

# *IN VIVO* TEST ANTI-WRINKLE EFFICACY



- ✓ Efficacy of peptides for anti-expression wrinkles was evaluated by taking silicon imprints of the wrinkles around the eyes of healthy volunteers, after 28 days of cream application
- ✓ A cream containing 5% LEUPHASYL<sup>®</sup> SOLUTION (0.05%) was applied twice daily around the eyes of 14 volunteers, aged 39 to 64, for 28 days
- ✓ A cream containing 5% ARGIRELINE<sup>®</sup> SOLUTION (0.05%) was applied twice daily around the eyes of 14 volunteers, aged 39 to 63, for 28 days
- ✓ A third test was performed using a combination of both actives: a cream containing 5% ARGIRELINE<sup>®</sup> SOLUTION (0.05%) + 5% LEUPHASYL<sup>®</sup> SOLUTION (0.05%) was applied twice daily around the eyes of 15 volunteers, aged 39 to 63, for 28 days

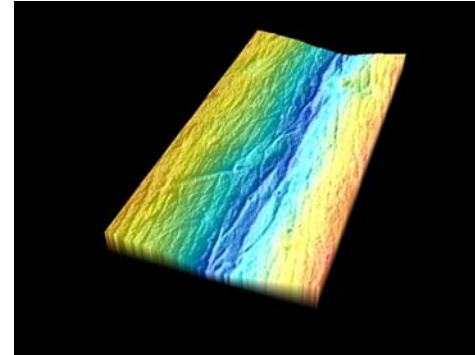
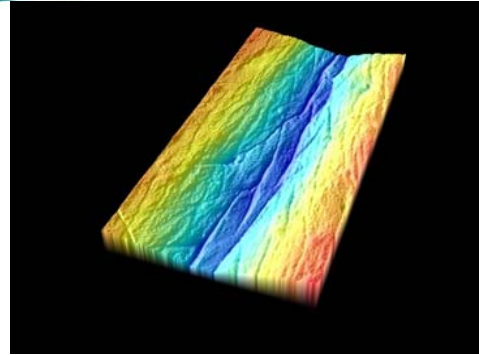
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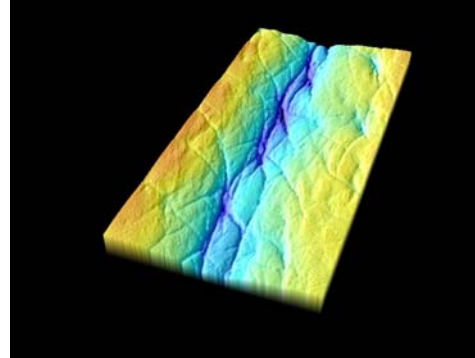
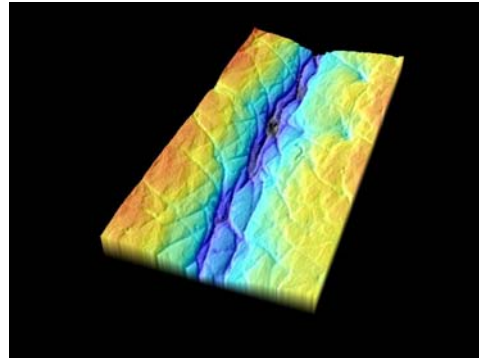
# 5% LEUPHASYL® SOLUTION (0.05%)



Sample 1:



Sample 2:



Day 0

Day 28

n=14

Mean value: -11.64%

Maximum reduction: -23.55%

Dark blue colour indicates maximum depth  
Bright red colour indicates max height

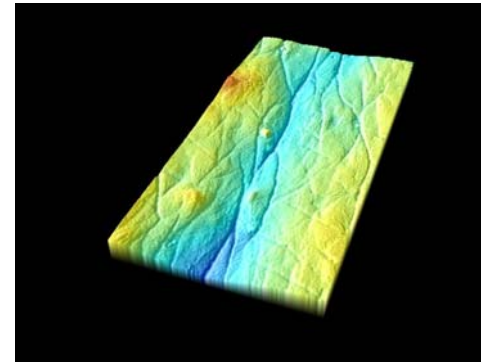
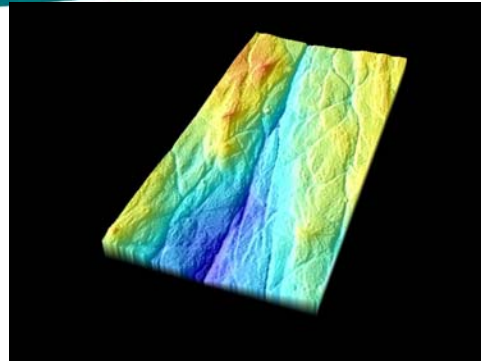
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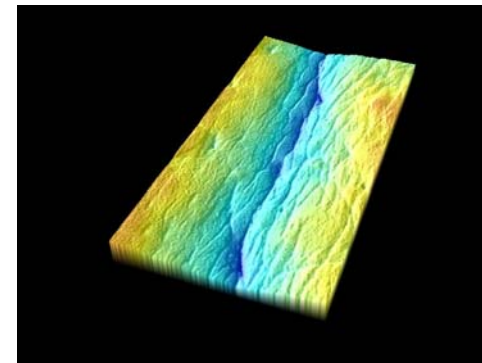
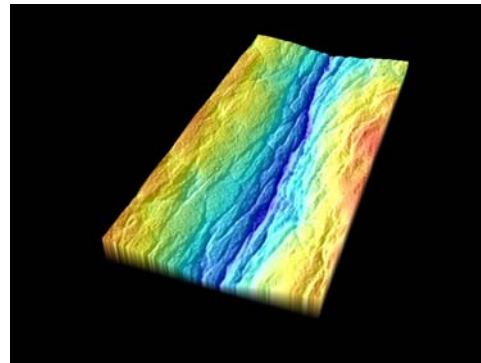
# 5% ARGIRELINE® SOLUTION (0.05%)



Sample 1:



Sample 2:



Day 0

Day 28

n=14

Mean value: -16.26%

Maximum reduction: -31.80%

Dark blue colour indicates maximum depth  
Bright red colour indicates max height

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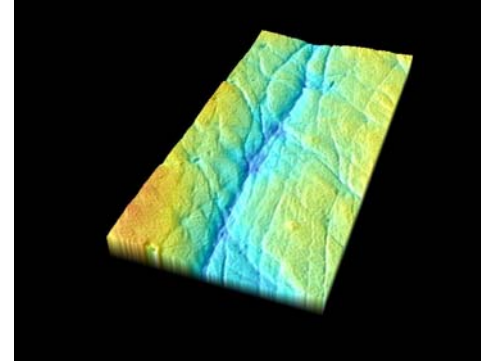
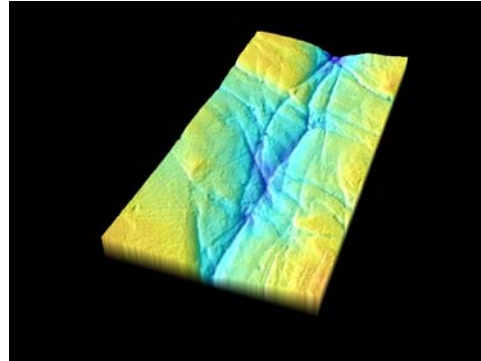
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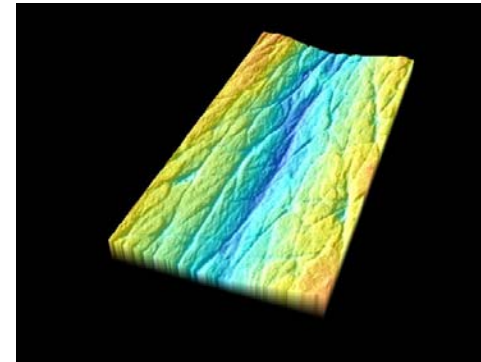
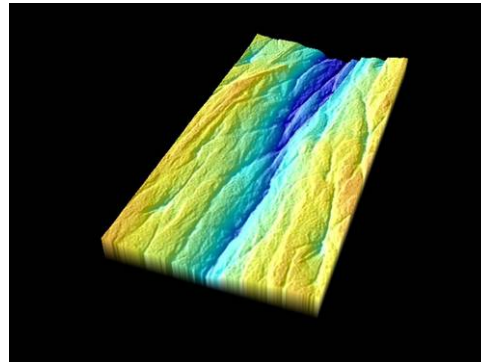
# 5% LEUPHASYL® SOLUTION (0.05%) + 5% ARGIRELINE® SOLUTION (0.05%)



Sample 1:



Sample 2:



Day 0

Day 28

n=15

Mean value: -24.62%

Maximum reduction: -46.53%

Dark blue colour indicates maximum depth  
Bright red colour indicates max height

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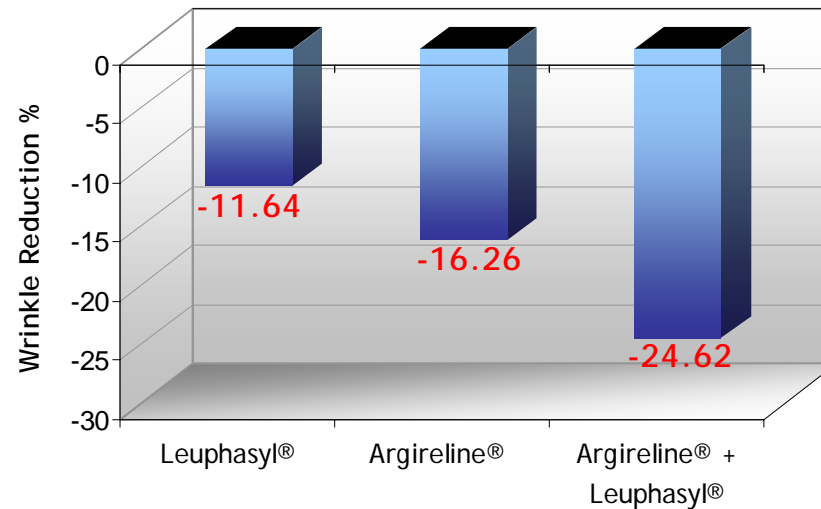
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# IN VIVO TEST SUMMARY



✓ *In vivo*, we do not see the synergistic effect detected *in vitro*, but we can see an additive effect

Anti Expression Wrinkle Peptides  
5% Peptide Solutions (0.05%)



The combination of both peptides shows a reduction in the depth of wrinkles of 25%, with maximum values up to 47%

# CONCLUSIONS



**leuphasyl**<sup>®</sup> offers a new, parallel, complementary pathway to reduce expression wrinkles

This results in a SYNERGISTIC effect *in vitro* and an ADDITIVE effect *in vivo*, enabling the combination of LEUPHASYL<sup>®</sup> with other antiwrinkle peptides such as ARGIRELINE<sup>®</sup>

**leuphasyl**<sup>®</sup> 

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