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Novel Insights into skin biology and permeation of actives using ToF-SIMS and 3D OrbiSIMS.

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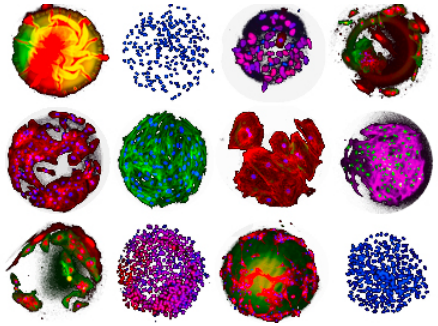
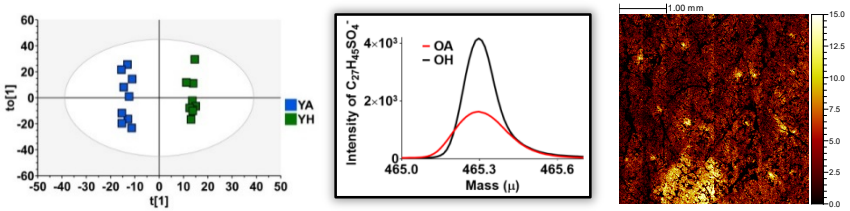
Materials for Healthcare



Cell polymer microarray

Discovery, design and development of novel materials and devices.

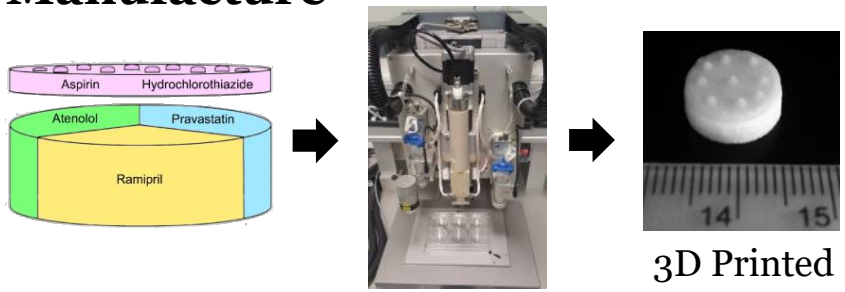
Advanced Analysis



Lipid variations in skin aging

Nano and micro- scale imaging, spectroscopy, mass spectrometry analysis.

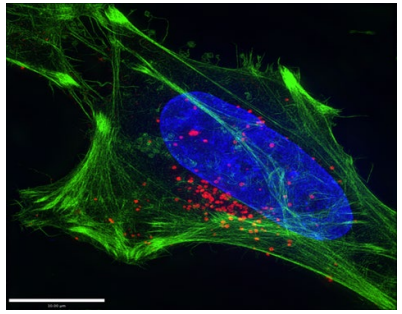
New Methods for Medicines Manufacture



3D Printed 'Polypill'

Accelerating scale-up of medicines for the most pressing needs of global healthcare.

Diagnostic Technologies



NanoSensors in cells

Tools to discover new devices and biomarkers for diagnostic applications in healthcare.

ToF-SIMS & 3D OrbiSIMS (HybridSIMS)

Time of flight (ToF) secondary ion mass spectrometry (SIMS)

- Highly surface sensitive (1 -3 nm) label free imaging Spectrometry.
- Developed for semi-conductor industry with applications now spanning most disciplines.

ToF-SIMS

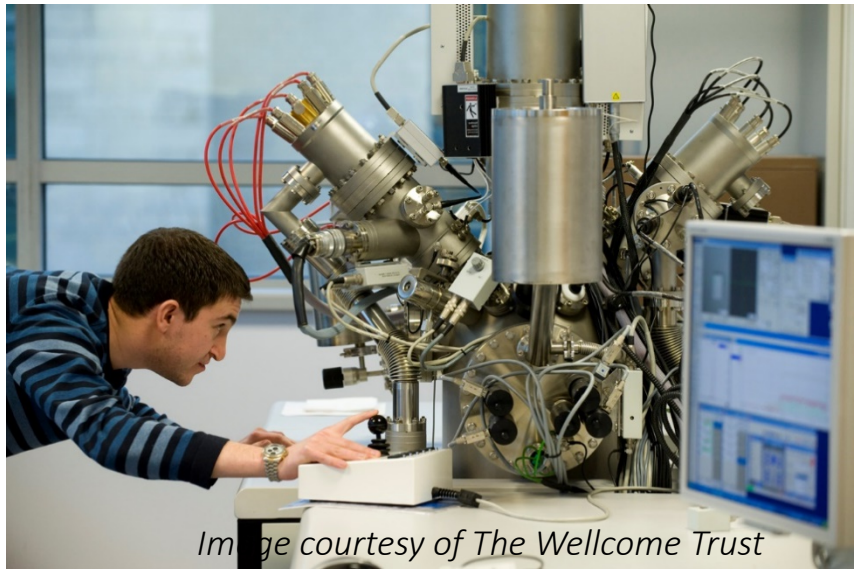
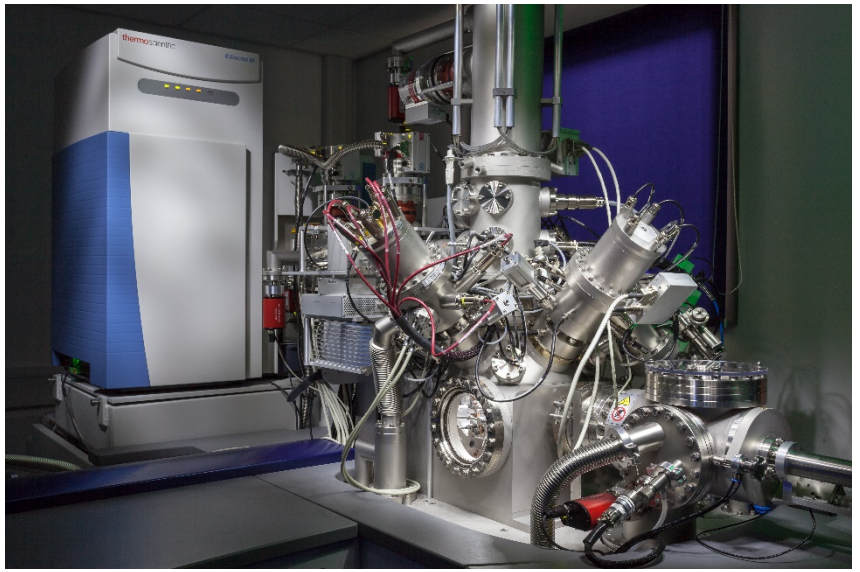


Image courtesy of The Wellcome Trust

3D OrbiSIMS (HybridSIMS)

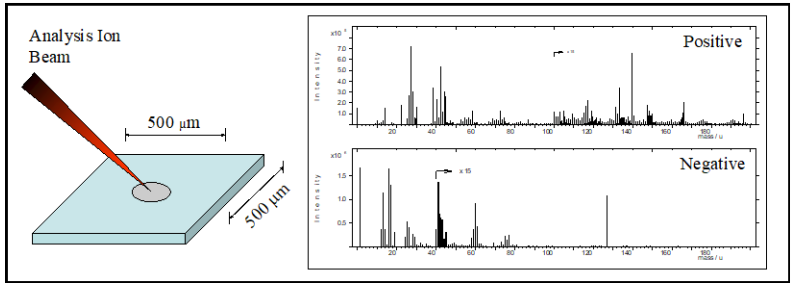


ToF-SIMS – Modes of Operation

1. Surface Spectrometry (static SIMS)

Application of very low primary ion dose densities

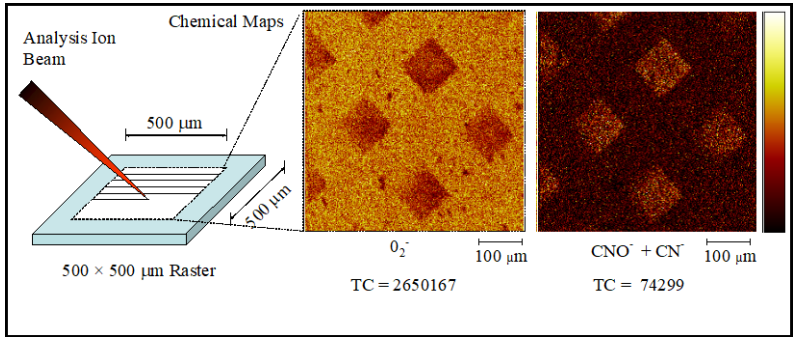
- quasi non-destructive surface analysis



2. Surface Imaging (static SIMS)

Rastering of a finely focussed ion beam over the surface

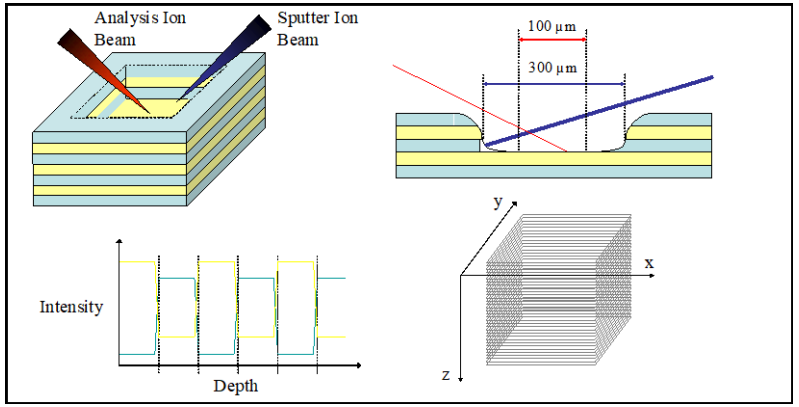
- mass resolved secondary ion images (chemical maps)



3. Depth Profiling (dynamic SIMS) & 3D Rendering

Application of high primary ion dose densities

- successive removal of top surface layers
- elemental in-depth distribution



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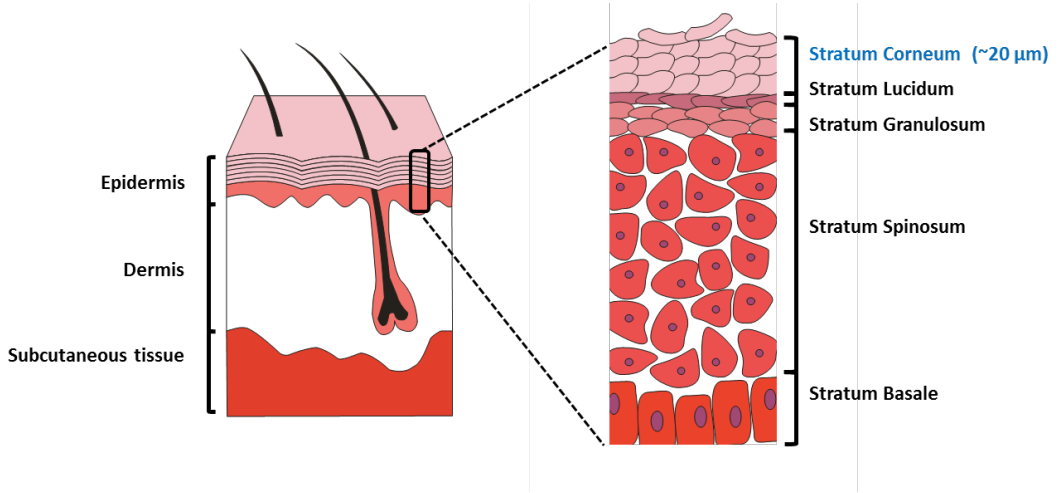
Permeation of actives using ToF-SIMS

Introduction

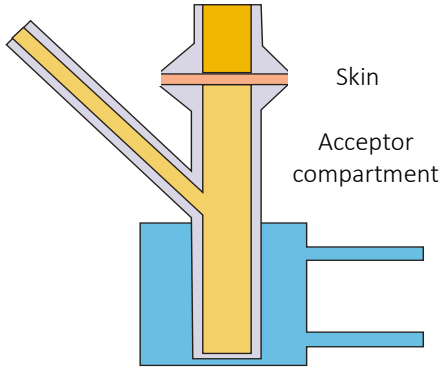
Topical applications include:

1. Antibacterial
2. Pharmaceutical
3. Cosmetic

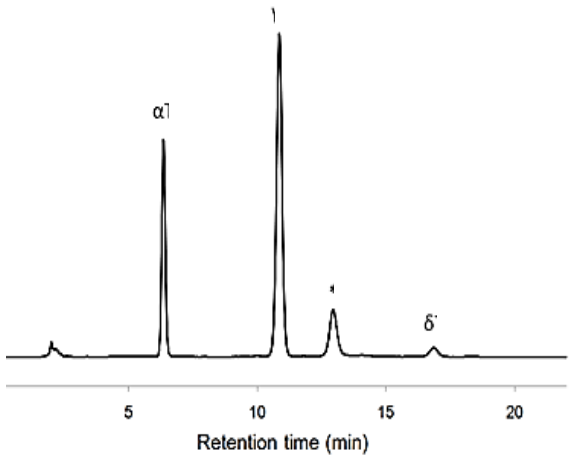


HPLC currently the 'gold standard' skin permeation analysis . . . . . .

Franz Cell



HPLC analysis



- No permeation analysis in the upper layers.
- Relies on extraction of actives from the tape strips/remaining skin tissue.
- No information on spatial distribution.

Imaging:

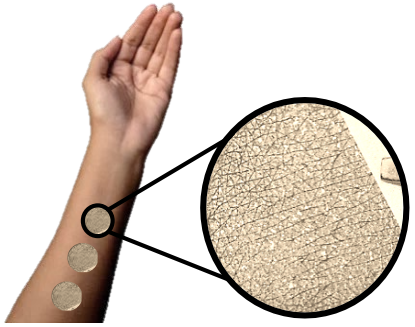
- Raman
- CARS / SRS
- MALDI
- ToF-SIMS

Introduction

Skin sampling

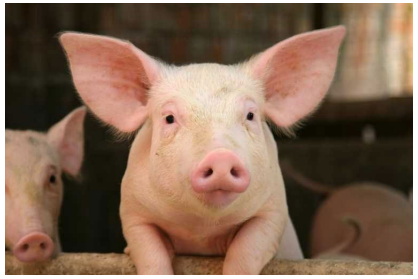
In vivo sampling

- ✓ Tape stripping
- ✓ Simple
- ✓ Minimally invasive
- ✓ Safe to use on human volunteers



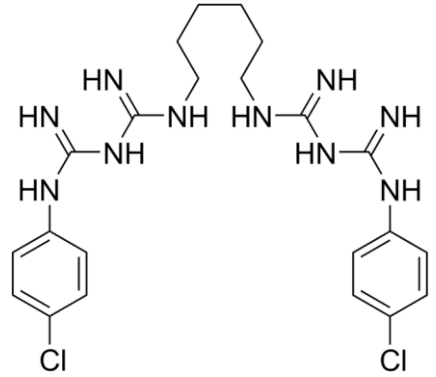
In vitro sampling (by-product of food)

- ✓ Porcine skin
- ✓ Ear tissue
- ✓ Easily removed from cartilage
- ✓ Used in Franz cell testing



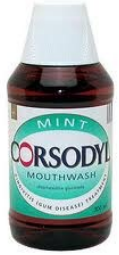
Antibacterial

Chlorhexidine is a chemical antiseptic effective on both Gram-positive and Gram-negative bacteria.



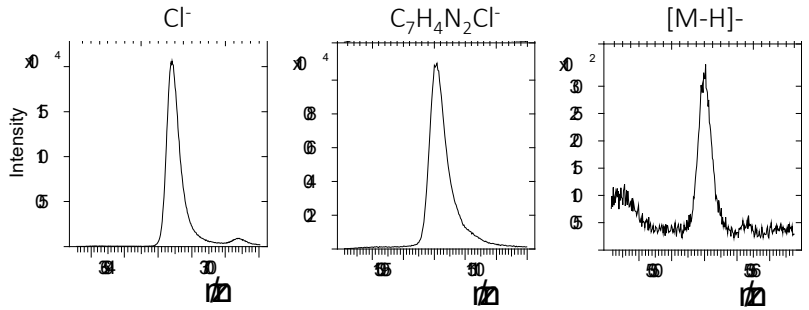
Found used safely in low concentrations in many products, such as:

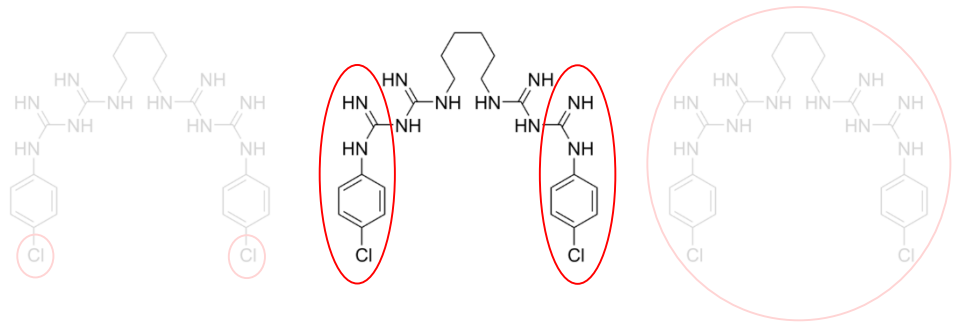
- Mouthwash
- Contact lens solutions
- Pre-surgery skin cleansers



Sufficient tissue Permeation vital, insufficient = bacterial re-colonisation

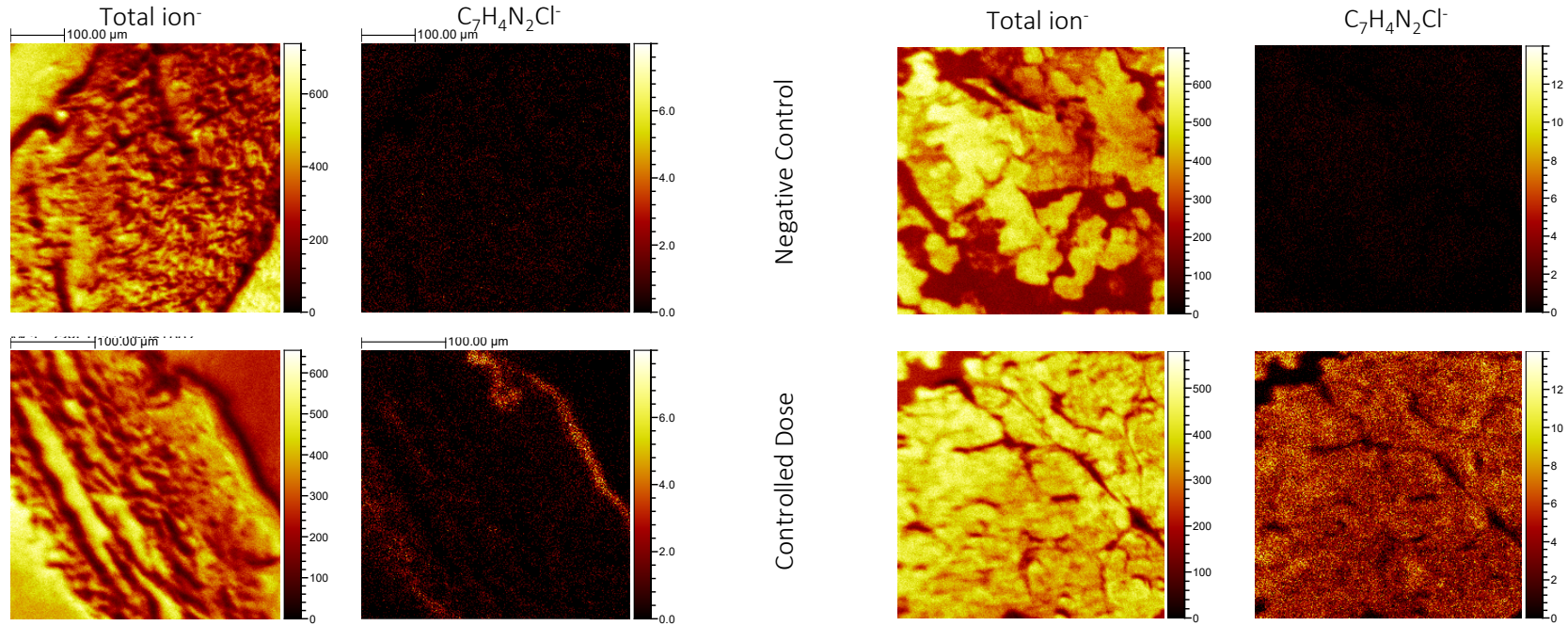
Antibacterial





Tissue Cross-sections

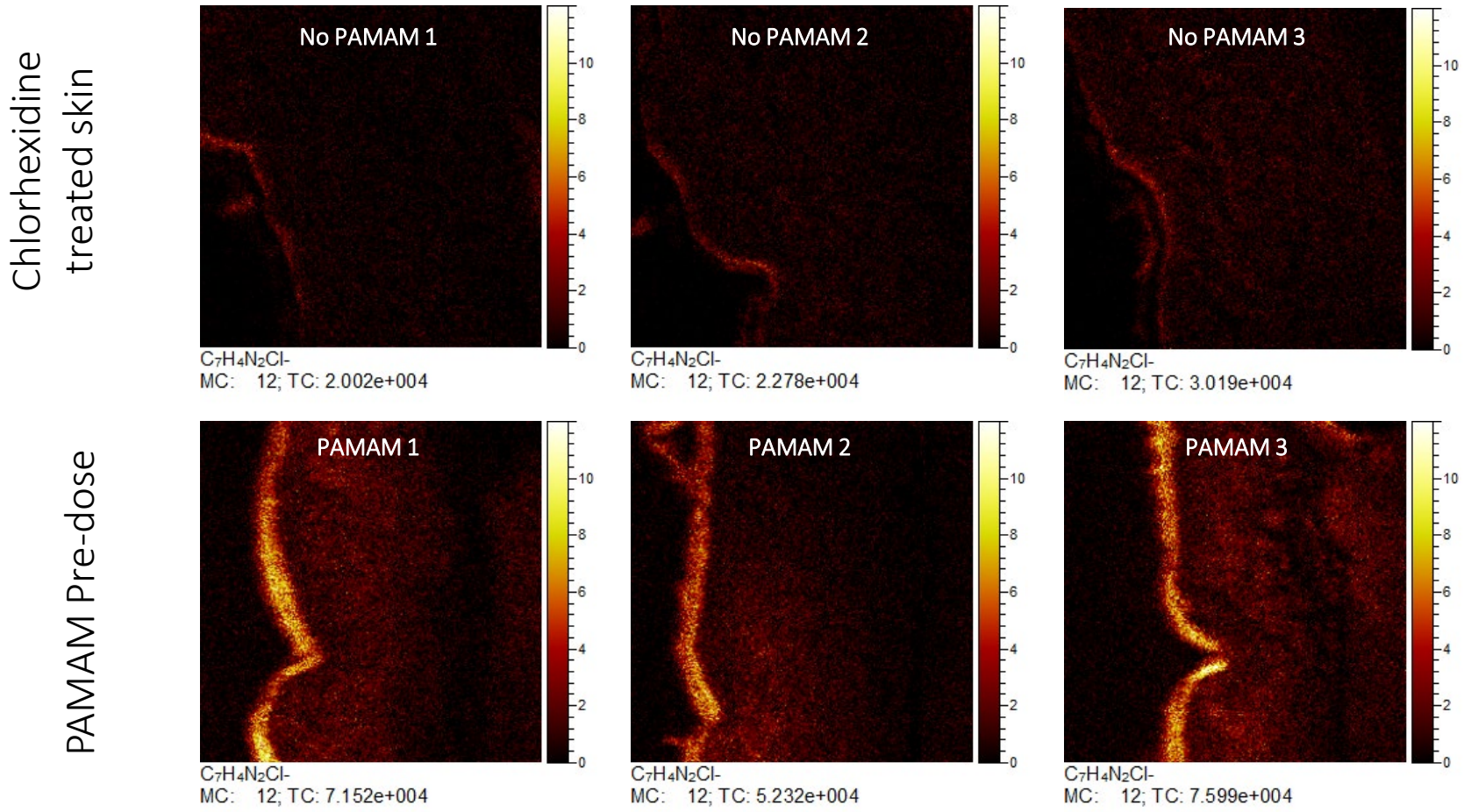
Tape Stripped Tissue



Judd, Scurr, Heylings, Wan & Moss, *J. Pharm. Res.*, **2013**, 30(7)

Antibacterial

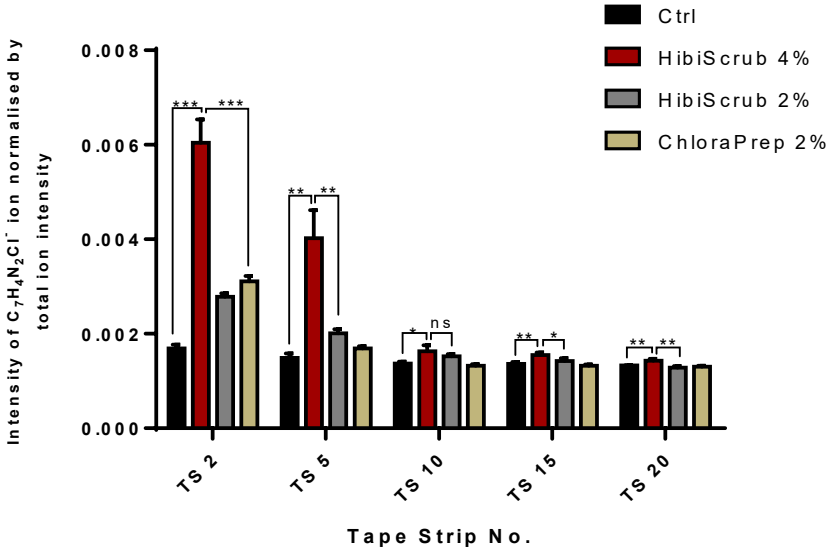
Use of polyamidoamine (PAMAM) dendrimers to enhance topical delivery of chlorhexidine to improve antimicrobial efficacy

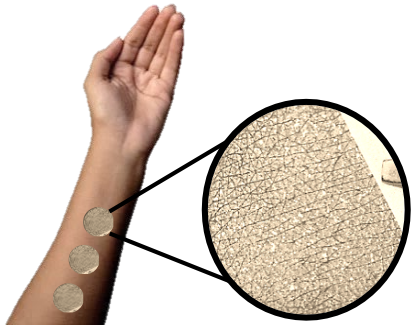


Holmes, Scurr, Heylings, Wan & Moss., *J. Pharm. Sci.*, **2017**, 104

Antibacterial

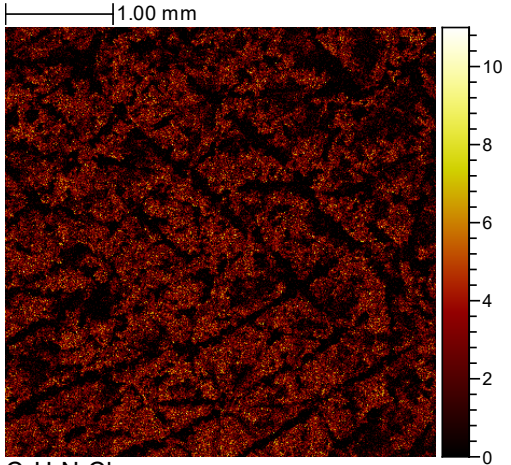
In Vivo (Conc. & vehicle)



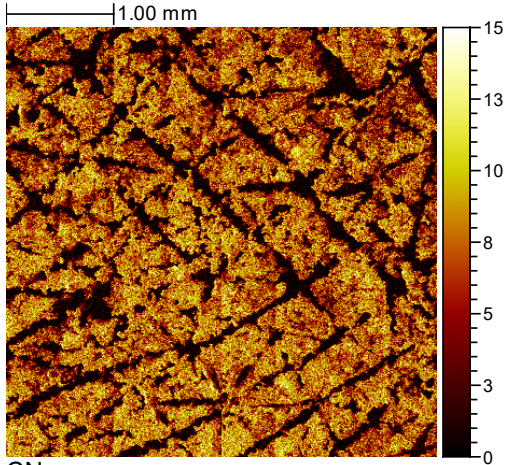


water

isopropyl alcohol



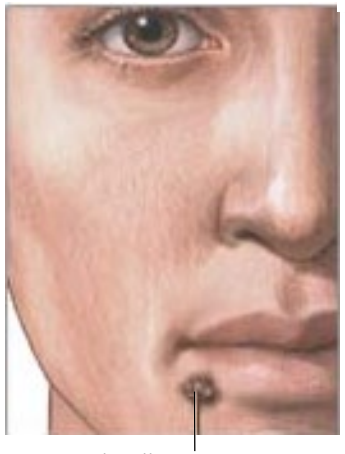
$C_7H_4N_2Cl$-
MC: 11; TC: 2.411e+005



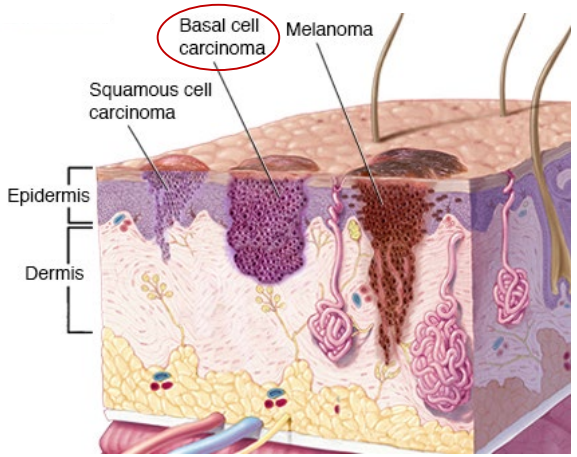
CN-
MC: 15; TC: 9.101e+005

Drug Delivery

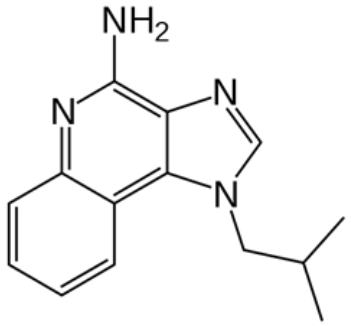
Basal cell carcinoma (BCC) is the most common type of skin cancer which develops from the lowest epidermal layer.



Basal Cell Carcinoma



Source: [Mayo Foundation for Medical Education & Research].



$C_{14}H_{16}N_4$

Molecular weight = 240

Partition coefficient = 2.74

Practically insoluble in water

Soluble in DMSO & oleic acid

Types of BCC:

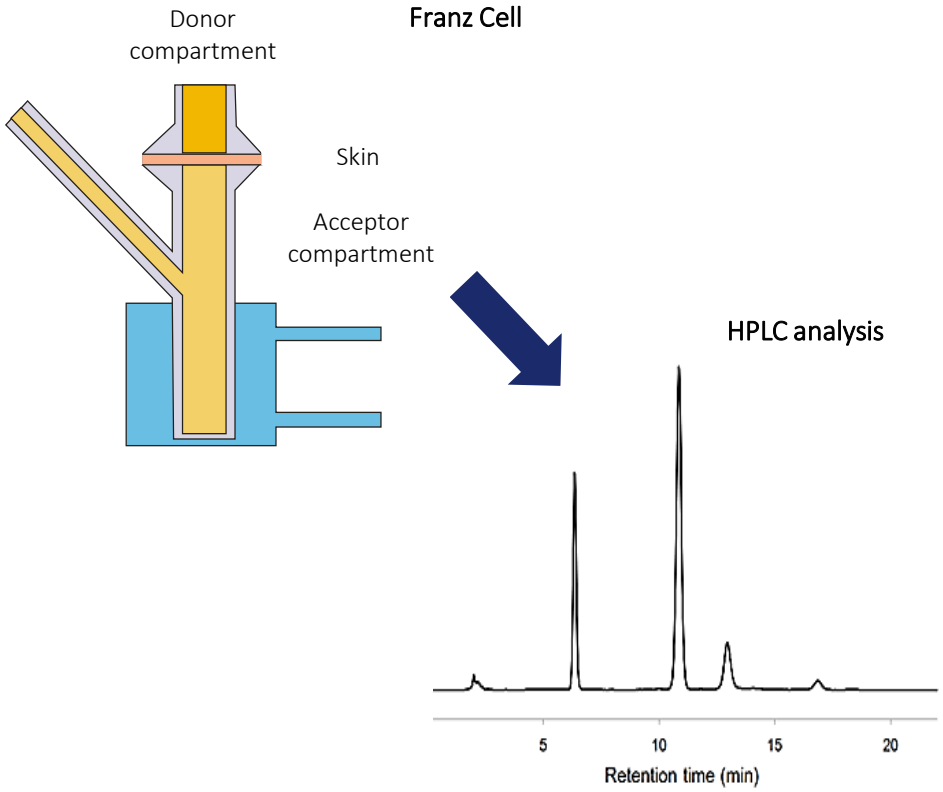
- Superficial - without penetration into the dermis.
- Nodular - with deeper penetration.

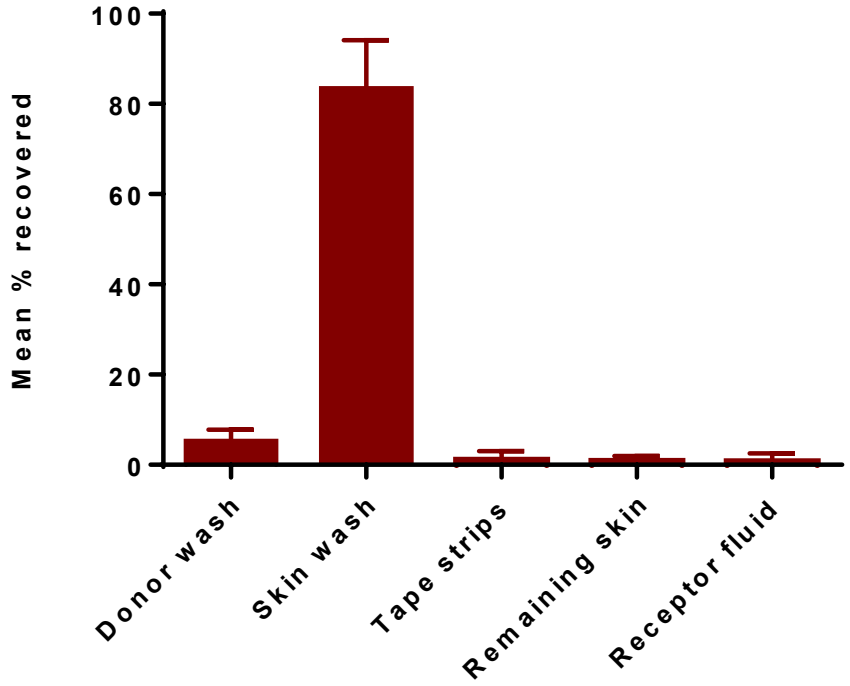
Treatment options:

- Surgery for nodular legions
- Topical with Aldara™ (imiquimod) for the treatment of superficial lesions.

Permeation study of Aldara™ cream

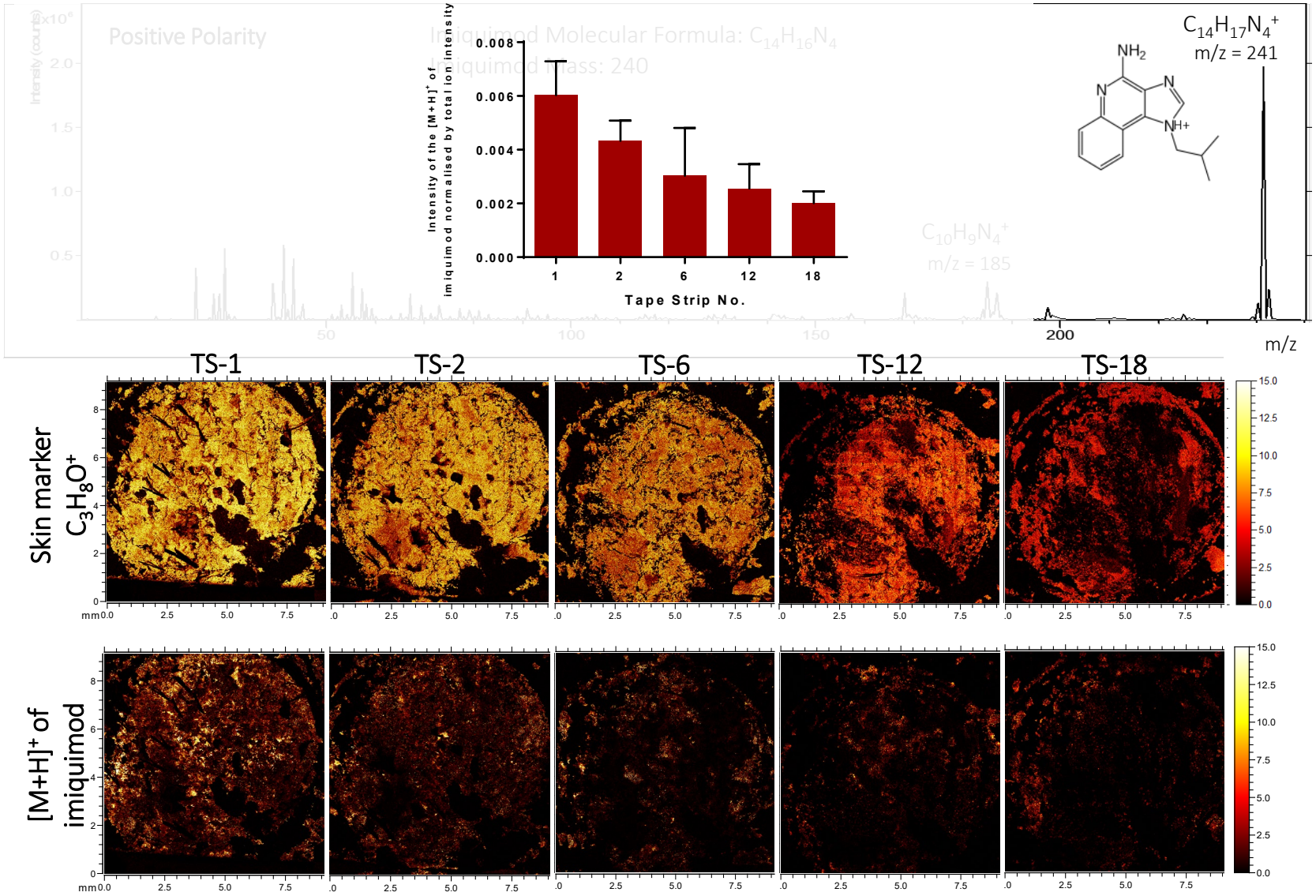
Determination of imiquimod amount recovered from different samples by HPLC





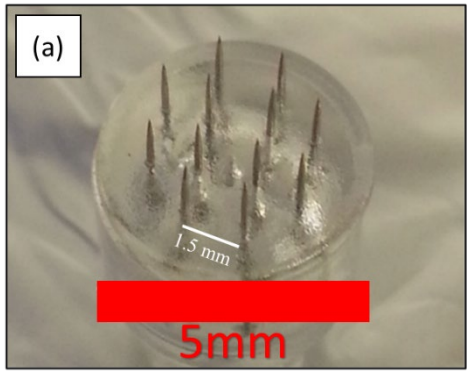
- Most of imiquimod was recovered from the skin wash with a little amount recovered from the remaining skin.

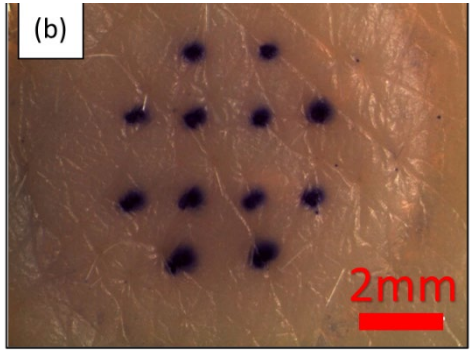
Drug Delivery

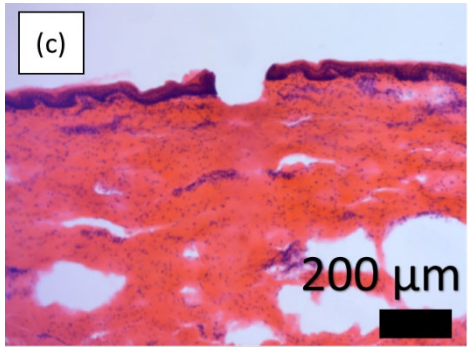


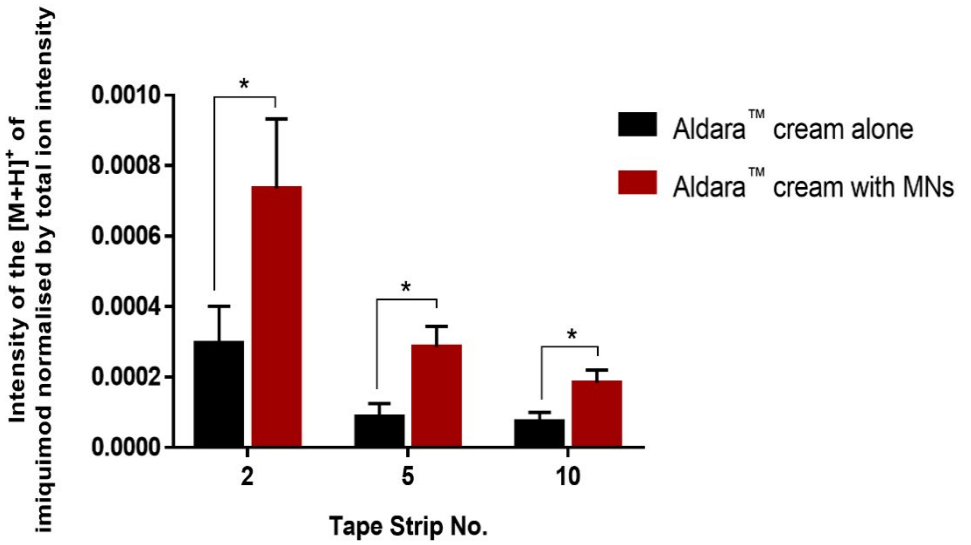
Al-Mayahy, Sabri, Rutland, Holmes, Marlow & Scurr **(2019)** *Eur. J. Pharm. Biopharm,* 139

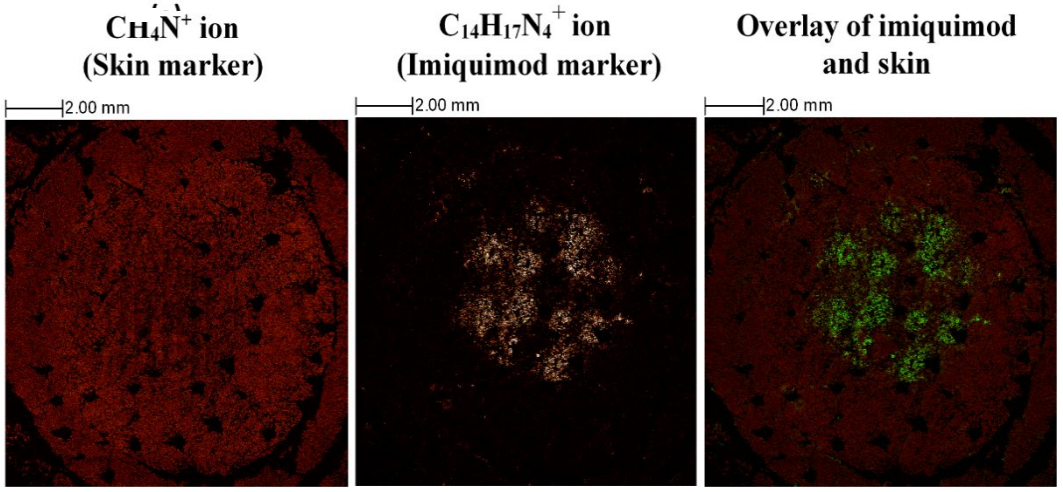
Microneedle Enhanced Drug delivery











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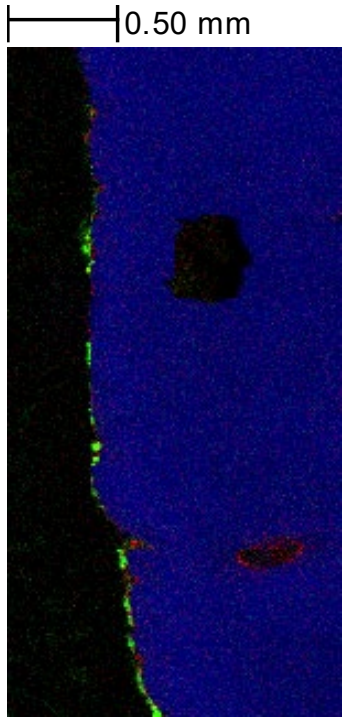
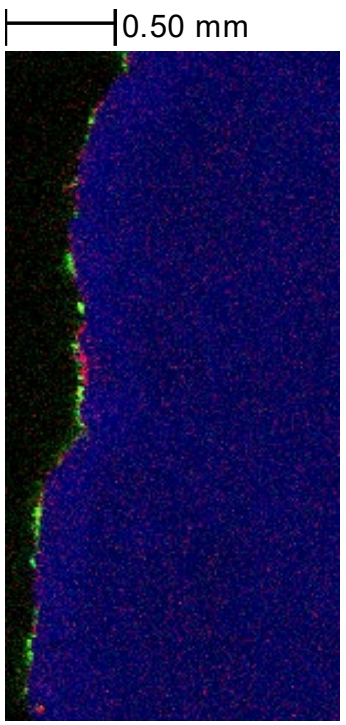
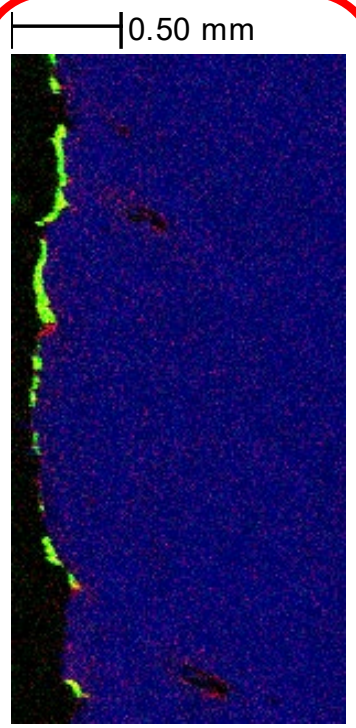


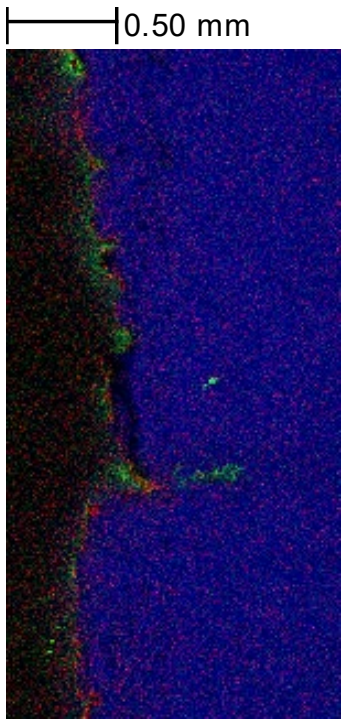
Aldara™ cream only



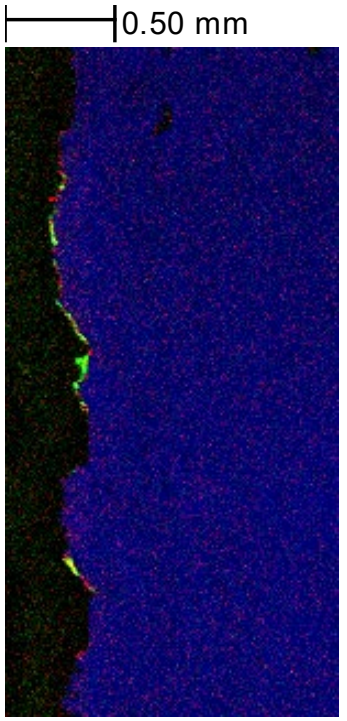
250 µm MN + Aldara™ cream



500 µm MN + Aldara™ cream



750 µm MN + Aldara™ cream



1000 µm MN + Aldara™ cream

0 $C_{17}H_{32}N^+$ 4
(Ceramide fragment ion)

0 $C_5H_{15}NPO_4^+$ 80
(Phosphatidylcholine fragment ion)

0 $C_{14}H_{17}N_4^+$ 12
(Imiquimod molecular ion)

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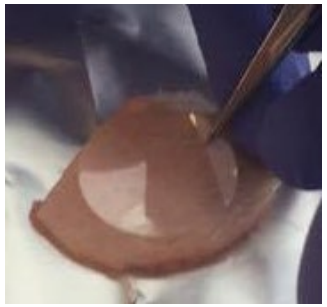
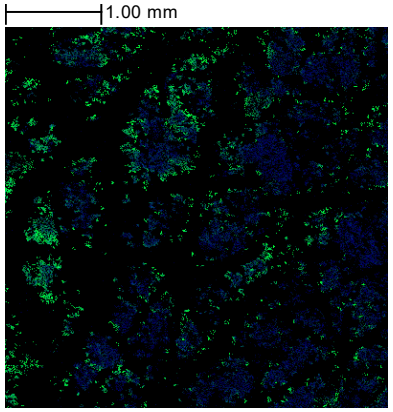
Microneedle Enhanced Drug delivery

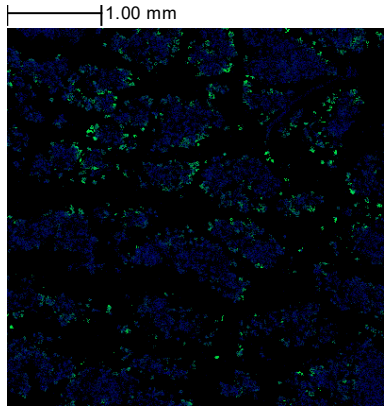


Tape strips

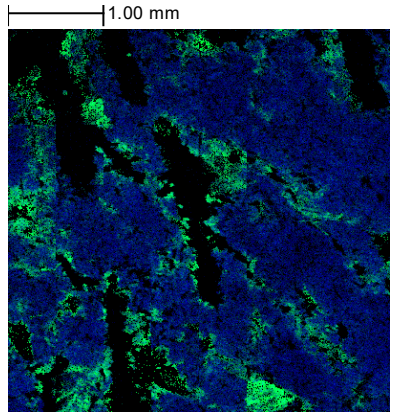
Cream only

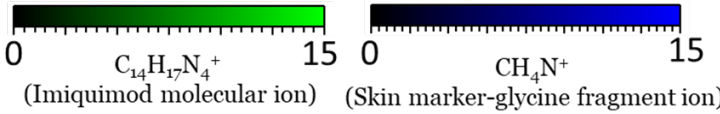


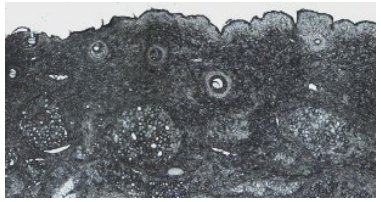
“Poke and patch”



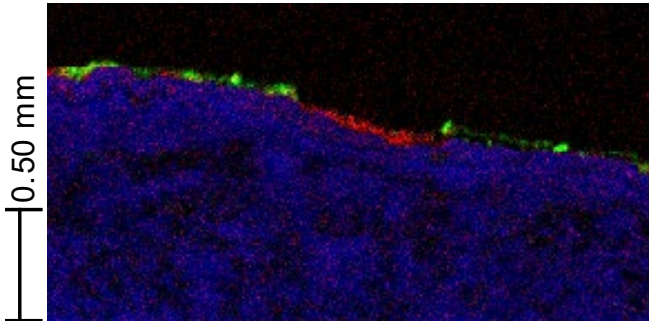
“Patch and poke”

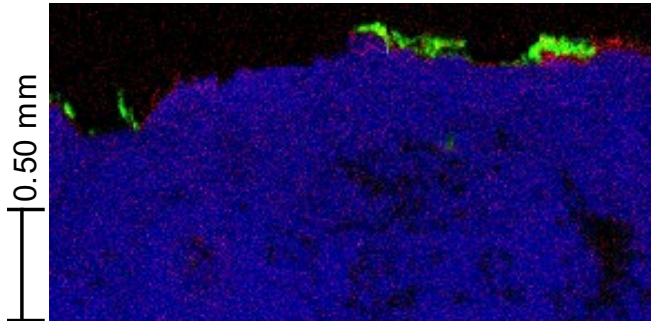


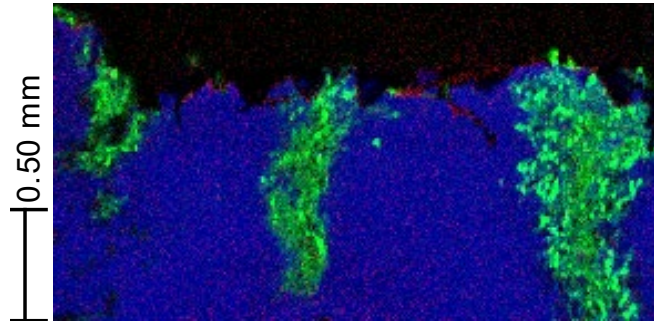


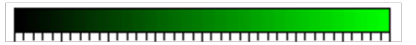


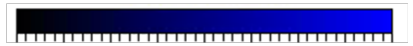
Cross sections







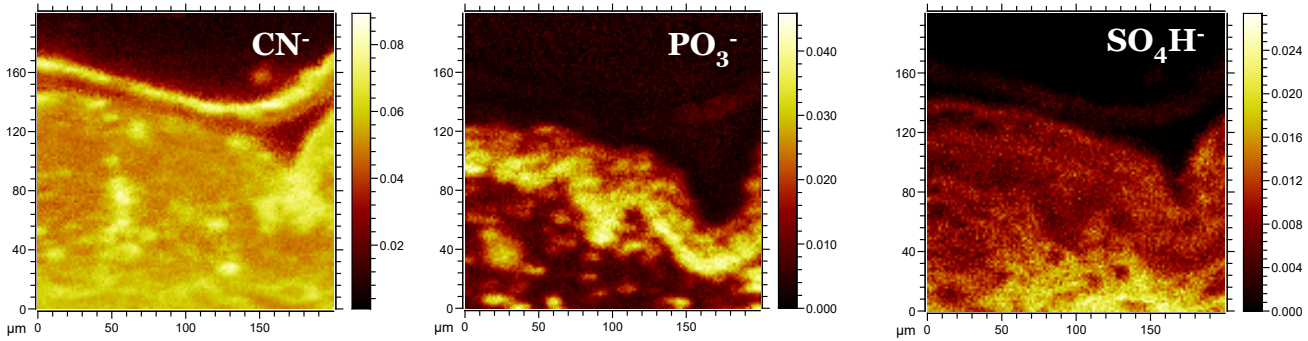




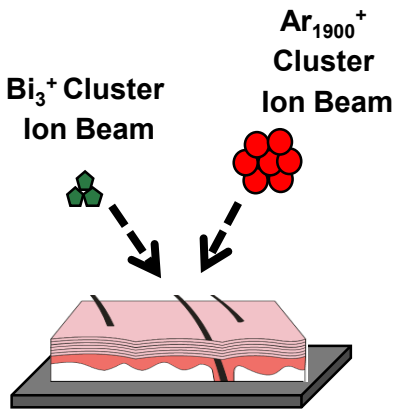
Akmal Bin Sabri
Poster Presentation
(PW34)

Cosmetic

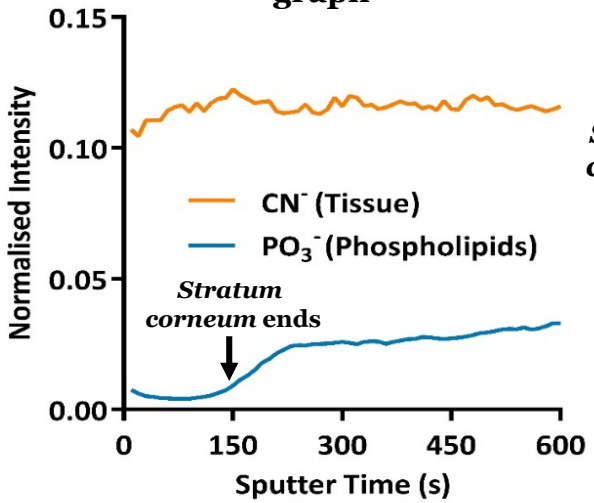
Native tissue cross-section



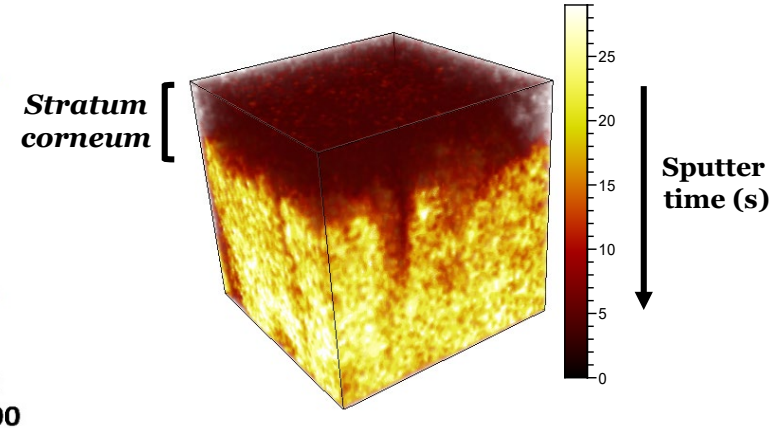
Depth profile - native tissue



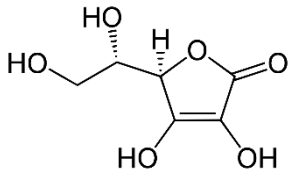
Depth profile graph



3D distribution map (PO_3^-)

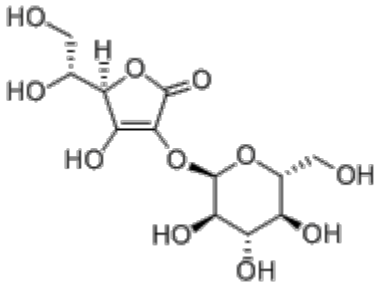


Cosmetic



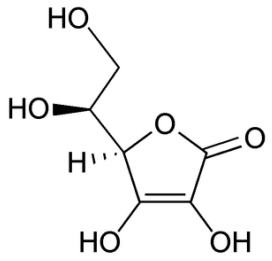
Ascorbic Acid

$C_6H_7O_6^-$



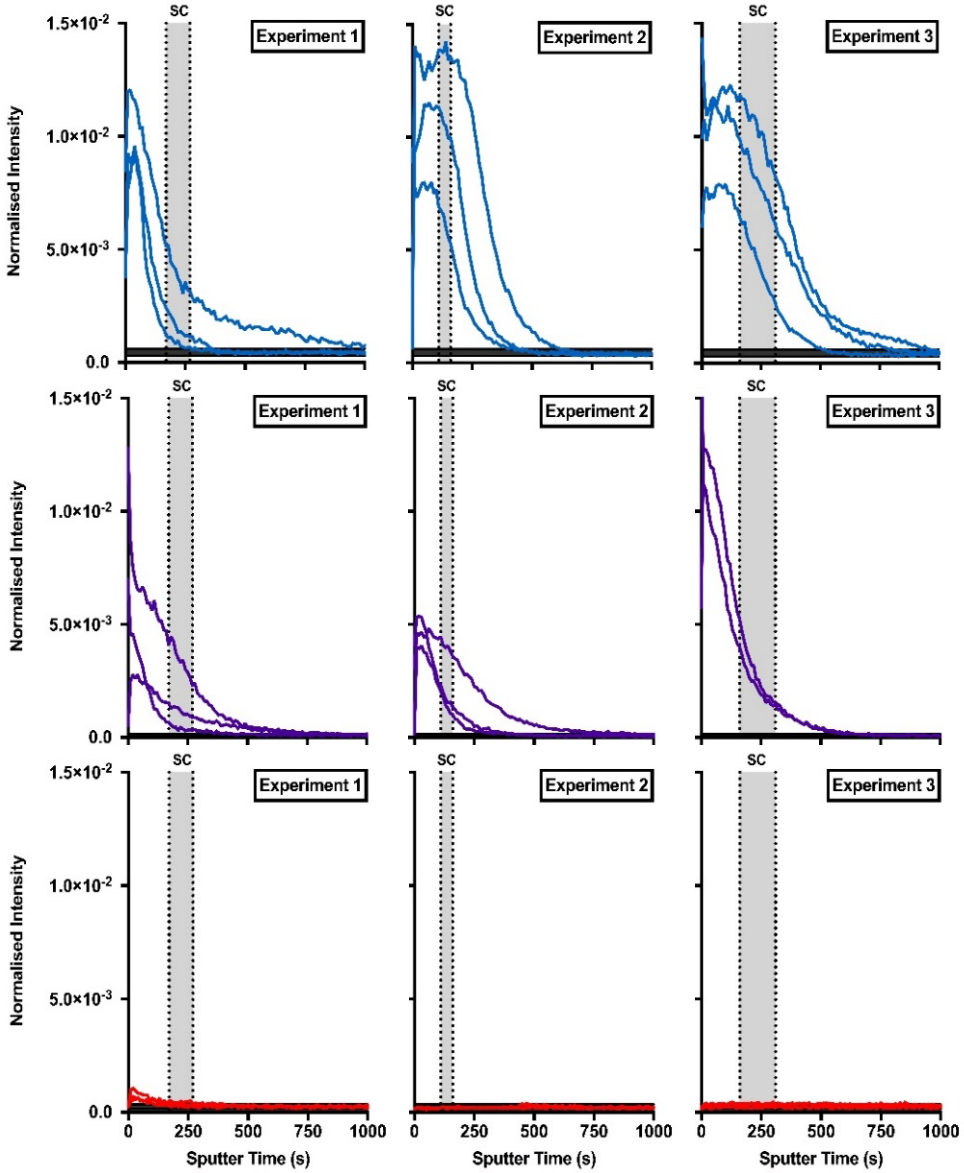
Ascorbyl Glucoside

$C_{12}H_{17}O_{11}^-$



Ethyl Ascorbic Acid

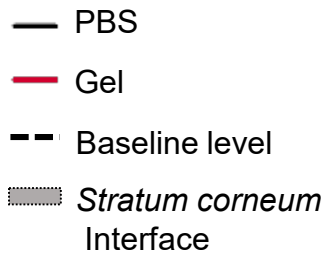
$C_8H_{11}O_6^-$

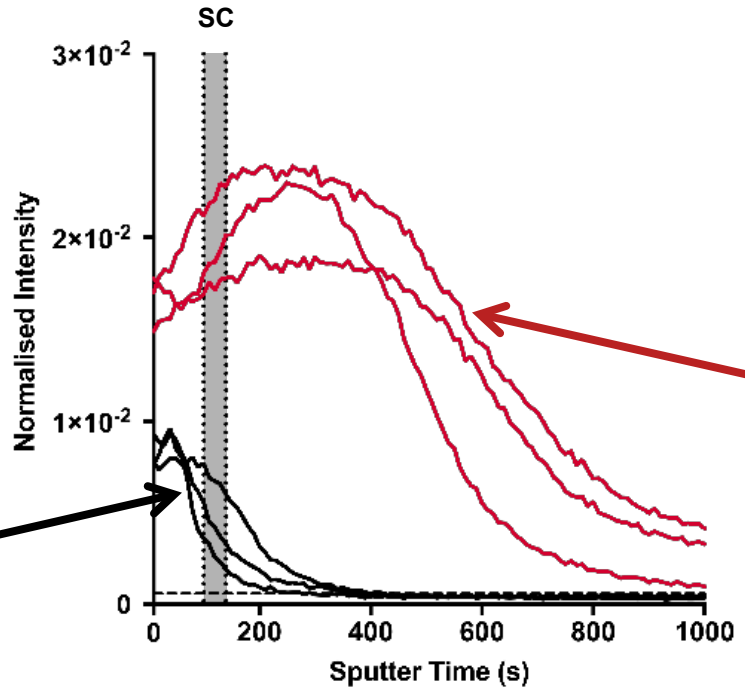


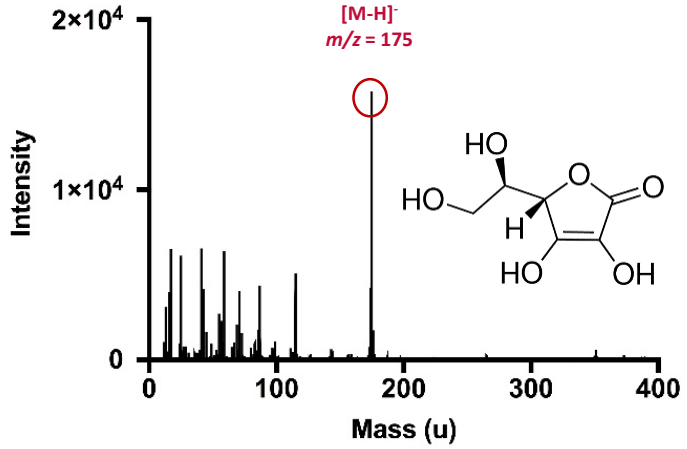
Starr, Hamid, Wibawa, Marlow, Bell, Perez Garcia, Barrett & Scurr **(2019)** *Int. J. Pharm.*, 563

Cosmetic

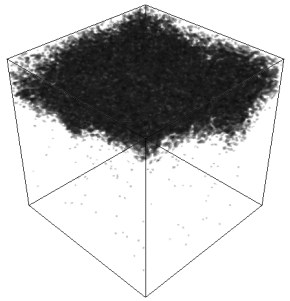
Ascorbic acid (AA) permeation





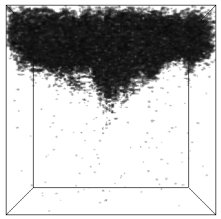


3D ion image

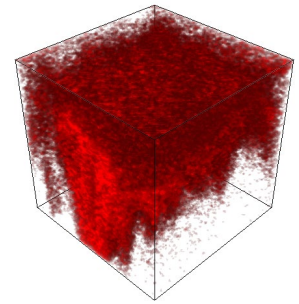


AA in PBS

XZ View

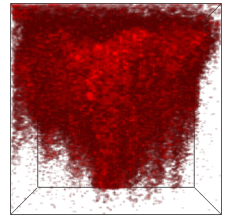


3D ion image



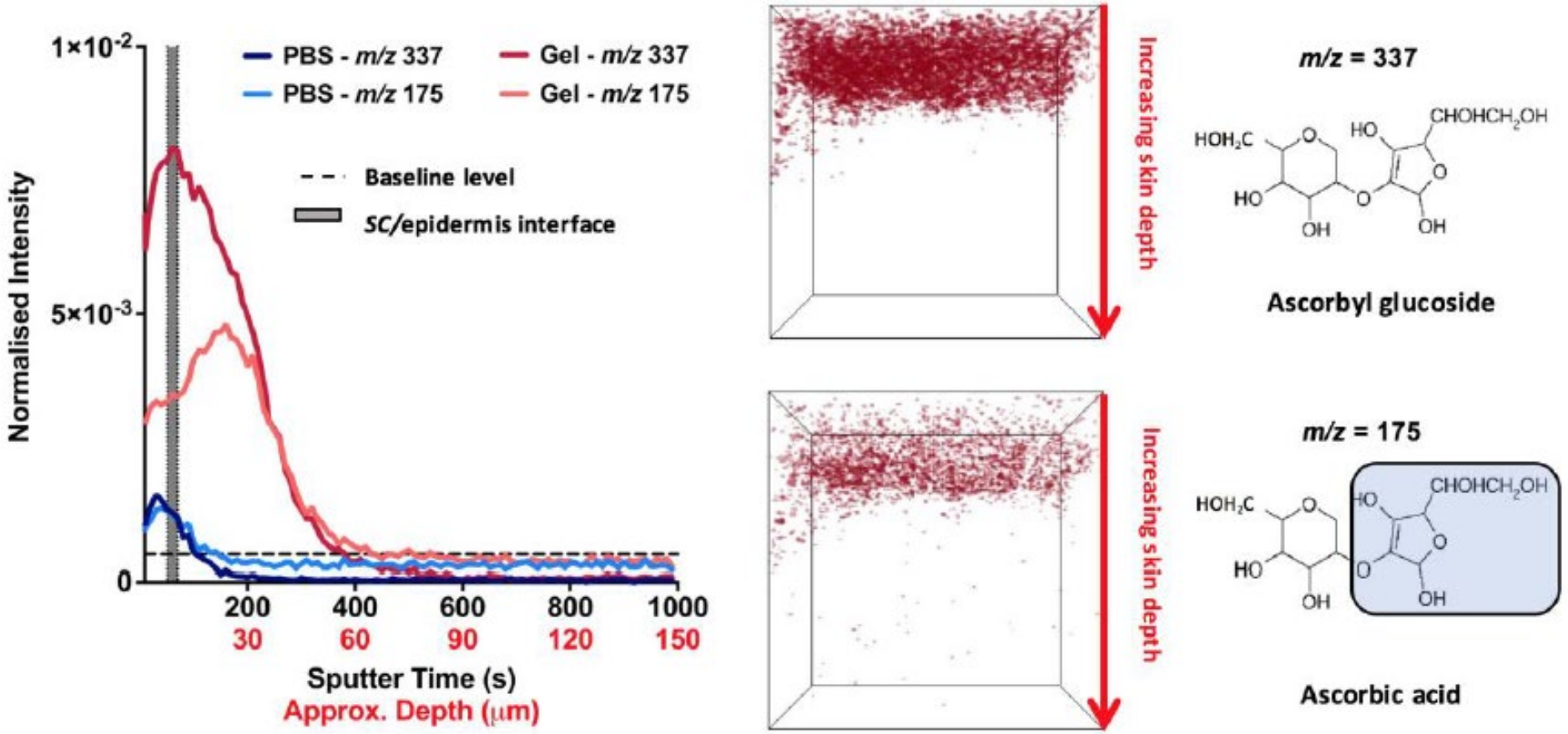
AA in Gel

XZ View



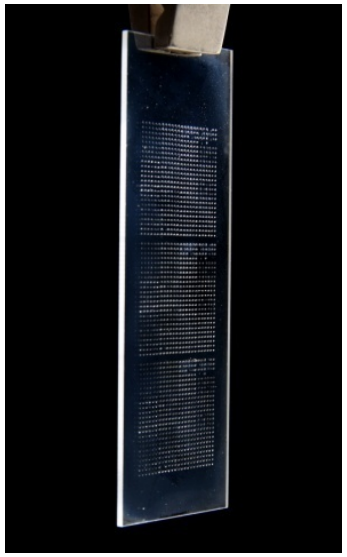
Starr, Hamid, Wibawa, Marlow, Bell, Perez Garcia, Barrett & Scurr **(2019)** *Int. J. Pharm.*, 563

Cosmetic



Starr, Hamid, Wibawa, Marlow, Bell, Perez Garcia, Barrett & Scurr **(2019)** *Int. J. Pharm.*, 563

Microarray Printing



Anderson *et al.* **2004**
Nature Biotech

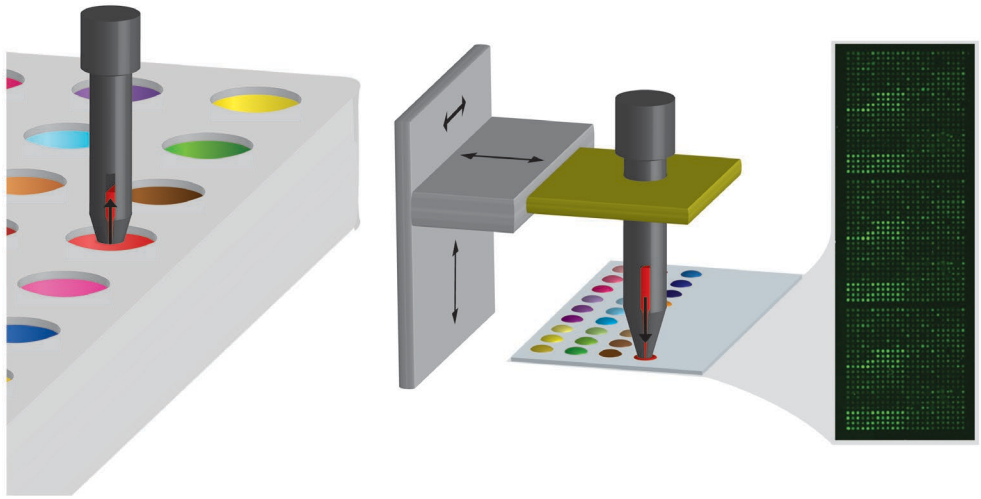
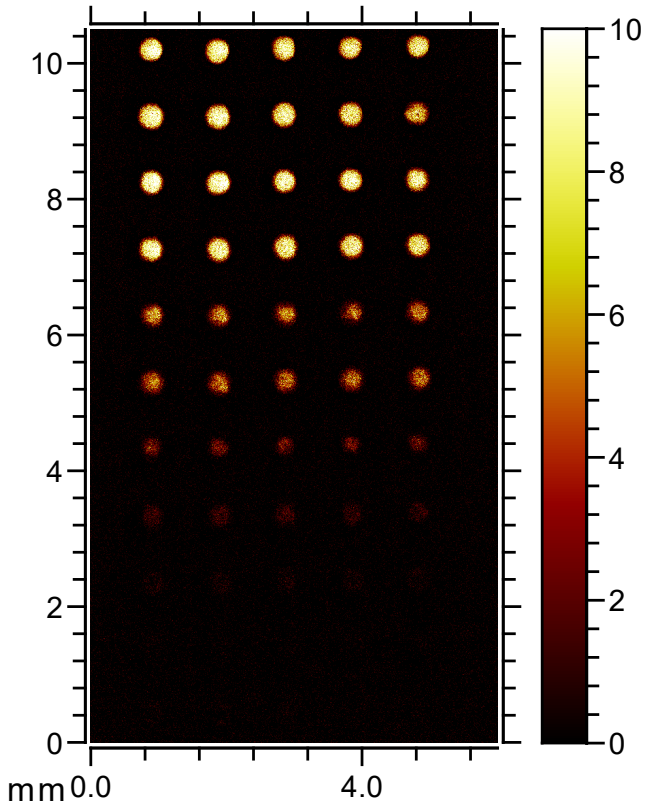


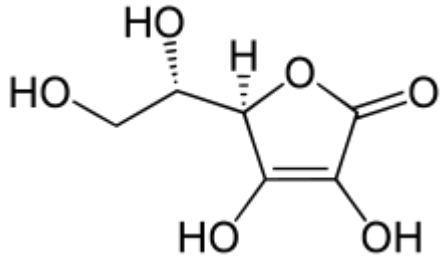
Image courtesy of Andrew Hook

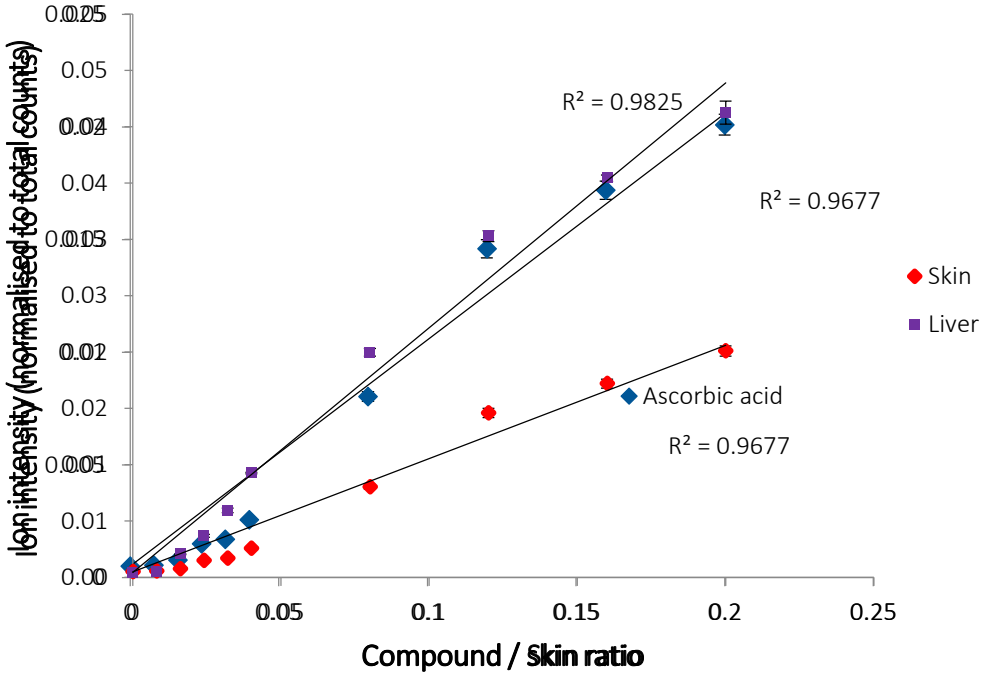
- Requirement to better understand multicomponent systems e.g biological systems
- Key enabling technology for high throughput (HT) materials screening
- Created by contact or inkjet printing = thousands of unique materials on a glass slide

Microarray Printing

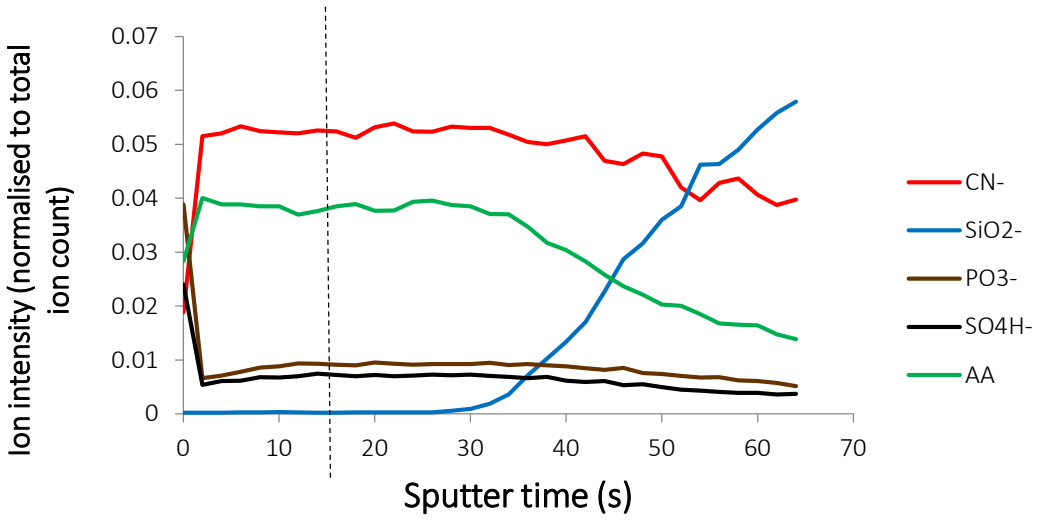


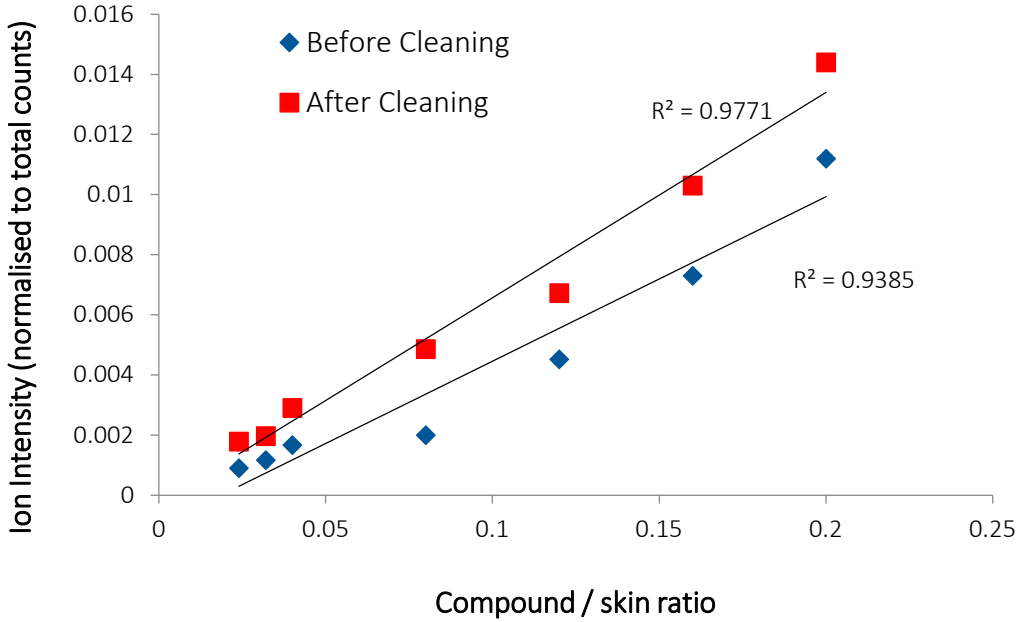
$C_6H_7O_6^-$





Microarray Printing





University of Nottingham
UK | CHINA | MALAYSIA

Novel insights into skin biology using ToF-SIMS and 3D OrbiSIMS

Native Skin Analysis

Chemical composition of the skin

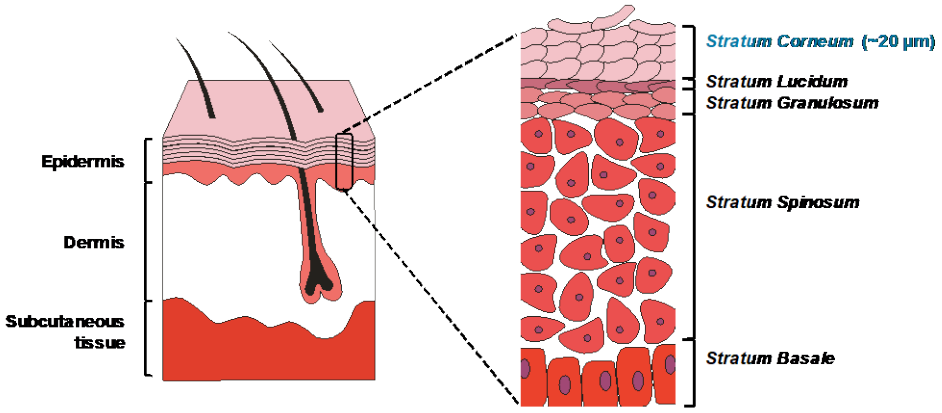


TABLE 1. Distribution of polar and nonpolar lipids in pig, human, and rat epidermal cells

	Pig	Human	Rat
	% (by weight) of total lipids		
Nonpolar (neutral) lipids[a]	30.2 ± 7.8 (5)[b]	35.5 ± 4.0 (4)	64.2 ± 8.5 (6)
Polar lipids			
Phospholipids[c]	62.3 ± 9.7 (5)	53.0 ± 4.0 (4)	34.7 ± 8.5 (6)
Glycosphingolipids (glucosylceramide)[d]	7.3 ± 1.5 (3)	9.5 ± 0.5 (2)	ND[e]
Cholesteryl sulfate	0.3 ± 0.06(2)	1.0 ± 0.14(2)	1.12 ± 0.04(3)

TABLE 2. Composition of total phospholipids in epidermal cells of pig, human, and rat

	Pig (5)[a]	Human (4)	Rat (6)
	% of total phospholipids		
Diphosphatidylglycerol (cardiolipin)	3.4 ± 0.6	3.6 ± 0.7	4.7 ± 1.7
Phosphatidic acid	1.3 ± 0.5	2.4 ± 0.8	4.2 ± 1.6
Phosphatidylethanolamine	17.7 ± 1.5	[illegible]	[illegible] ± 3.2[b]
Ethanolamine plasmalogen	2.4 ± 1.1	[illegible]	[illegible][c]
Phosphatidylserine	[10.0 ± 4.7][b]	[illegible]	7.9 ± 2.9[b]
Serine plasmalogen	[illegible]	[illegible]	[0.4][c]
Phosphatidylinositol	[illegible]	[illegible] ± 2.3	6.2 ± 2.9
Phosphatidylcholine	[illegible]	38.5 ± 2.7	[33.4 ± 7.4][b]
Choline plasmalogen	[illegible]	[illegible]	[1.5][c]
Sphingomyelin	[illegible] ± 2.0	20.8 ± 2.7	19.6 ± 4.0
Phospholipid PL-X	1.3 ± 0.5	2.4 ± 0.8	4.2 ± 1.6
Phospholipid PL-Y	tr[d]	1.9 ± 0.7	tr

phospholipids

TABLE 3. Fatty acid composition of phospholipids and glycosphingolipids in pig and human epidermal cells

Fatty Acid	Pig									Human	
	PE	PI	PS	CL	PA	PL-X	PC	SP	GSL	SP	GSL
14:0	tr	2.7	2.4	4.4	3.6	0.8	14.7	1.7	tr	2.6	5.1
15:0	tr	2.0	2.7	2.3	1.8	1.0	1.2	1.0	tr	1.1	3.4
16:0	11.5	18.5	11.3	15.4	11.9	11.0	26.4	22.5	9.4	14.6	8.2
16:1	5.5	3.6	3.9	6.8	4.5	5.6	6.4	tr			
17:0	tr	tr	2.5	0.5	1.2		tr	tr	tr	2.0	2.4
18:0	16.3	25.2	16.5	7.4	13.5	12.9	7.5	[illegible]	2.7	6.4	4.3
18:1	24.6	13.3	17.4	21.6	19.7	31.5	19.[illegible]		24.7	2.8	17.9
18:2	34.4	14.9	20.7	34.2	22.9	21.[illegible]					
18:3	7.7	0.8	2.1	3.5	3.5						
20:0	tr							24.6	15.8	11.6	7.7
20:1		3.3	5.9								
20:2	tr	1.7									
20:3	tr	1.5				3.3					
20:4		7.2				4.1					
21:0								0.8	tr	1.3	1.7
22:0								8.1	9.8	8.9	4.3
23:0								1.0	1.6	1.6	1.7
24:0						3.1		14.4	14.5	18.8	10.0
24:1								10.5	2.6	9.5	2.0
25:0								0.8	tr	2.0	5.2
26:0								2.8	9.0	5.8	5.4
28:0									7.8	0.7	5.9
24:0:OH									tr		2.6
26:0:OH									tr		5.6
NI		1.8	2.9	1.6	12.5	1.9	1.8	4.2	2.1	10.3	8.6

glycosphingolipids

TABLE 4. Sterol content of pig, human, and rat epidermal cells

	Pig (4)[a]	Human (2)	R[illegible]
	mmoles of sterol[illegible] cell		
Cholesterol	270 ± 2[illegible]	[illegible]	[illegible]50 ± 200
Cholesteryl esters	2[illegible]	[illegible]	330 ± 190
Cholest-7-ene-3β-ol	[illegible]	[illegible] ± 14	74 ± 37
Cholest-7-ene-3β-ol esters	[illegible]	6 ± 2	126 ± 22

sterols

TABLE 5. Composition of nonpolar (neutral) lipids in epidermal cells of pig, human, and rat

	Pig	Human	Rat
		% (by weight) of total nonpolar lipids	
Hydrocarbons	8	6	
Squalene			
Cholesteryl esters	3		11[a]
Wax esters			26
Triglycerides		25	19
Free fatty acids		25	
Cholesterol	[illegible]	25[a]	18[a]
Ceramide	3	3	ND
Unidentified	11	13	22

neutral lipids

Lipid compositions of cells isolated from pig, human, and rat epidermis
G. M. Gray and H. J. Yardley , *Journal of Lipid Research,* 1975, 16, 434-440

Current research focuses

Skin Diseases Associated with the Depletion of Stratum Corneum Lipids and Stratum Corneum Lipid Substitution Therapy

Sahle F.F.[a, d] · Gebre-Mariam T.[d] · Dobner B.[b] · Wohlrab J.[c] · Neubert R.H.H.[a]

Skin Pharmacol. Physiol., 2015, 28, 42-55

Table 1.2 A summary of the detected changes to stratum corneum lipids associated with skin disorders. Adapted from van Smeden et. al, J. Invest. Dermatol. (2014) [56].

Disease	Change in lipid composition
Lamellar ichthyosis	CER [NP] [EOS] ↓
Psoriasis	CER [NP] [EOS] [AP] ↓ CER [AS] [NS] ↑
Netherton	CER [EOS] [EOP] [EOH] [EOdS] [NP] ↓ Short chain lipids ↑ Unsaturated lipids ↑
Atopic dermatitis	CER [EOS] [EOP] [EOH] [EOdS] ↓ CER [AS] [AH] [AP] [AdS] ↑
Chanarin - Dorfman	Acyl-CERs ↓ TAG ↑
X-linked ichthyosis	↑ cholesterol sulfate

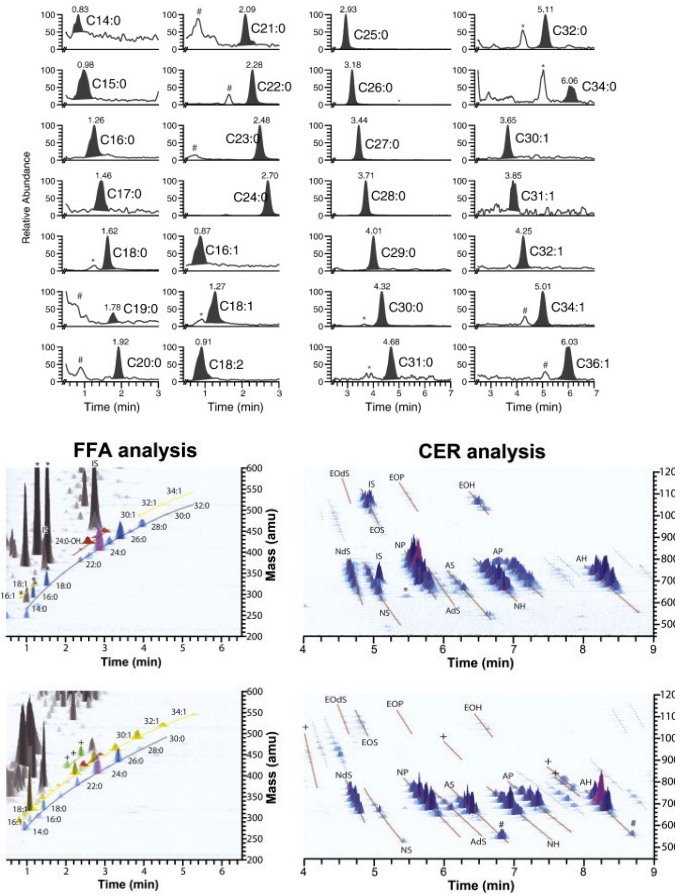
Age and skin structure and function, a quantitative approach (II): protein, glycosaminoglycan, water, and lipid content and structure

Jeanette M. Waller, Howard I. Maibach

Skin Research and Technology, 2006, 12:3, 145-154

Combined LC/MS-platform for analysis of all major stratum corneum lipids, and the profiling of skin substitutes

Jeroen van Smeden [a], Walter A. Boiten [a], Thomas Hankemeier [b, c], Robert Rissmann [d], Joke A. Bouwstra [a], Rob J. Vreeken [b, c]



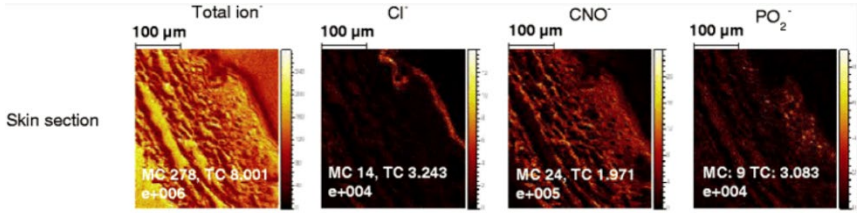
Biochim Biophys Acta., 2014, 1841:1, 70-79

ToF-SIMS Native Skin Analysis

RESEARCH PAPER

Distribution and Visualisation of Chlorhexidine Within the Skin Using ToF-SIMS: A Potential Platform for the Design of More Efficacious Skin Antiseptic Formulations

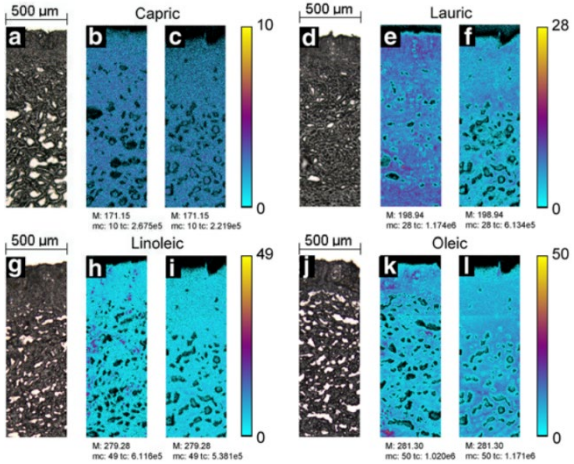
Amy M. Judd • David J. Scurr • Jon R. Heylings • Ka-Wai Wan • Gary P. Moss



Pharm. Res., 2013, 30, 1896-1905

Studying the penetration of fatty acids into human skin by ex vivo TOF-SIMS imaging

Toma Kezutyte[1†], Nicolas Desbenoit[2†], Alain Brunelle[2*] and Vitalis Briedis[1]

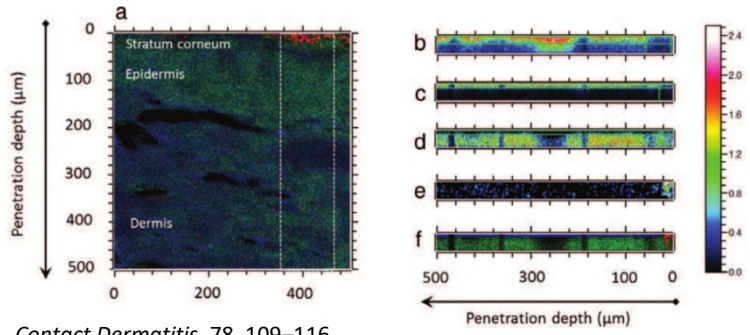


Biointerphases, 2013, 8:3

Imaging mass spectrometry for novel insights into contact allergy – a proof-of-concept study on nickel

Per Malmberg[1], Thomas Guttenberg[1,2], Marica B. Ericson[2] and Lina Hagvall[3]

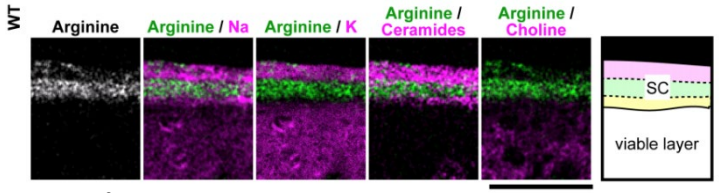
[1]*Department of Chemistry and Chemical Engineering, Centre for Imaging Mass Spectrometry, Chalmers University of Technology, 412 96 Gothenburg, Sweden,* [2]*Biomedical Photonics Group, Department of Molecular Biology and Chemistry, University of Gothenburg, 412 96 Gothenburg, Sweden and* [3]*Occupational Dermatology, Department of Clinical Sciences, Sahlgrenska Academy at the University of Gothenburg, 413 45 Gothenburg, Sweden*



Contact Dermatitis, 78, 109–116

The stratum corneum comprises three layers with distinct metal-ion barrier properties

Akiharu Kubo[1,2], Itsuko Ishizaki[3], Akiko Kubo[4], Hiroshi Kawasaki[1], Keisuke Nagao[1], Yoshiharu Ohashi[3] & Masayuki Amagai[1]



Scientific reports, 2013, 3:1731

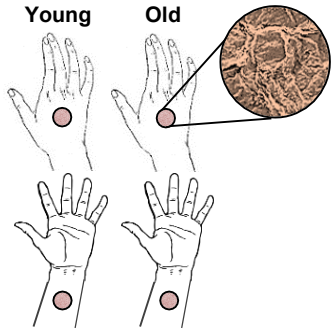
ToF-SIMS Native Skin Analysis

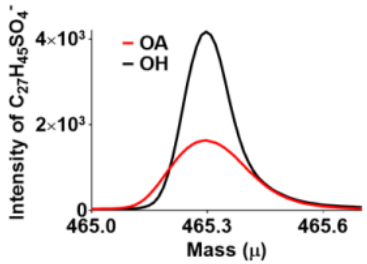
Native skin analysis at University of Nottingham

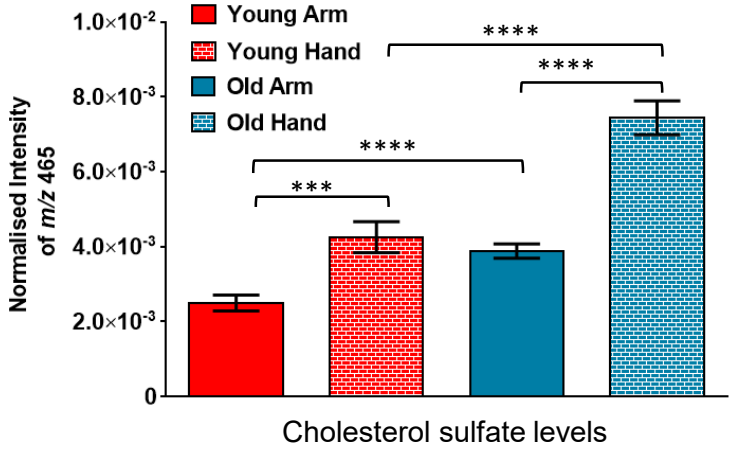
analytical chemistry
Article
pubs.acs.org/ac

Age-Related Changes to Human Stratum Corneum Lipids Detected Using Time-of-Flight Secondary Ion Mass Spectrometry Following in Vivo Sampling

Nichola J. Starr,[†] Daniel J. Johnson,[‡] Judata Wibawa,[§] Ian Marlow,[§] Mike Bell,[§] David A. Barrett,[†] and David J. Scurr*[,†]





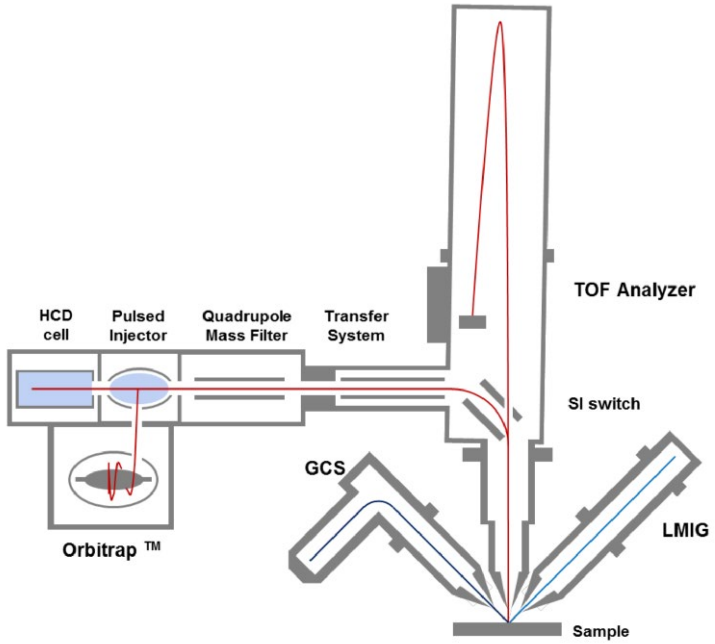


Cholesterol sulfate levels

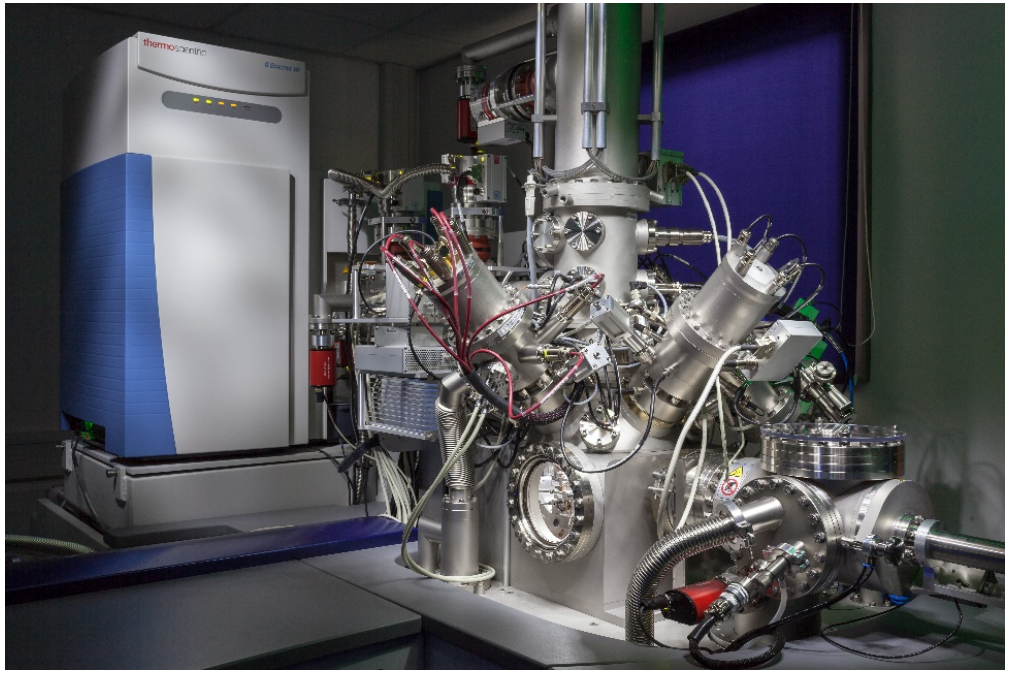
3D OrbiSIMS Native Skin Analysis

3D OrbiSIMS (HybridSIMS)

"Surface analysis meets organic mass spectrometry“
IONTOF



Passarelli *et al., Nature Methods*, **2017**, 14, 1175–1183



- ❖ Mass resolution > 240,000
- ❖ Mass accuracy < 1 ppm
- ❖ High resolution cluster SIMS imaging

- ✓ Unambiguous peak identification
- ✓ MS/MS capabilities

3D OrbiSIMS Native Skin Analysis

In vivo and *ex vivo* analysis methods

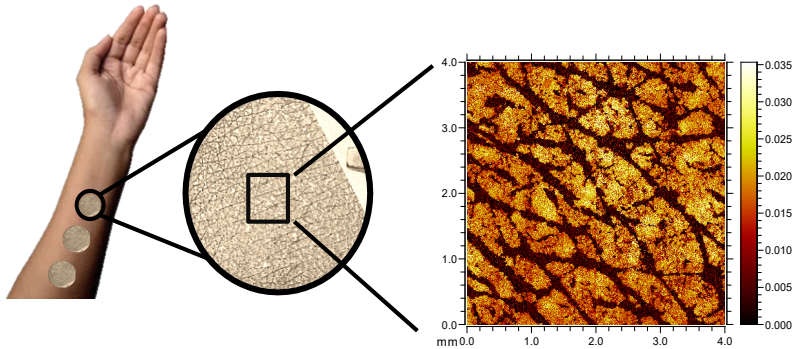
Number of peaks with intensity value > 10^3 for SIMS IV = 621

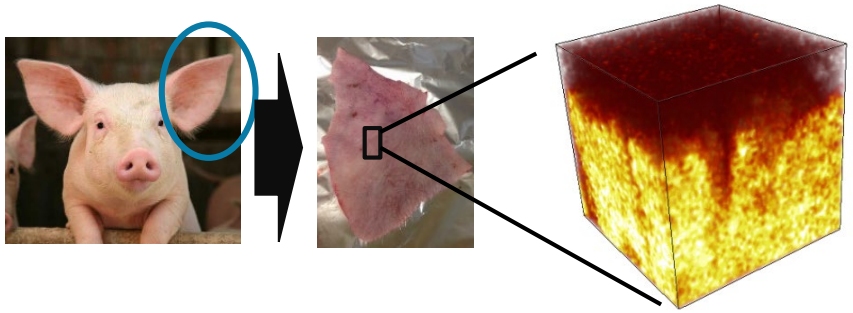
Number of peaks with intensity value > 10^3 for Hybrid SIMS = 9791

Number of peaks with intensity value > 10^5 for Hybrid SIMS = 874

In vivo sampling → surface analysis

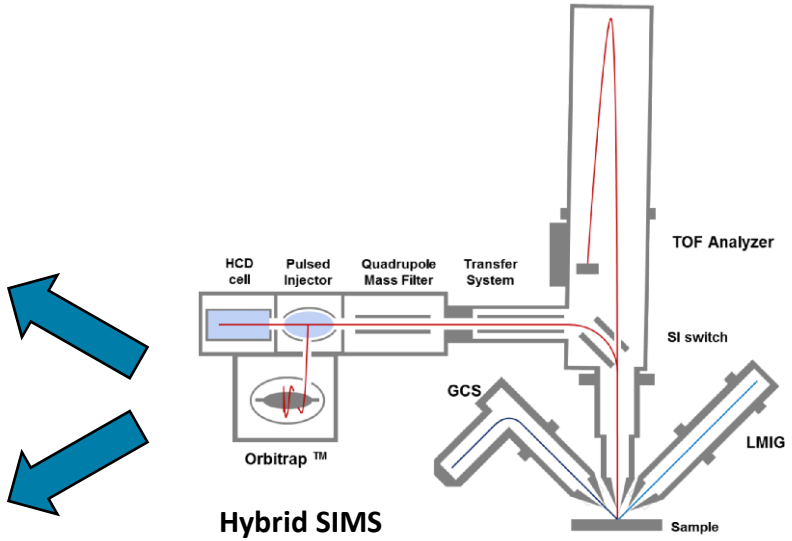
- ✓ Human volunteers
- ✓ Individual layers of SC





Ex vivo sampling → profiling

- ✓ Porcine or human (less used)
- ✓ 3D analysis

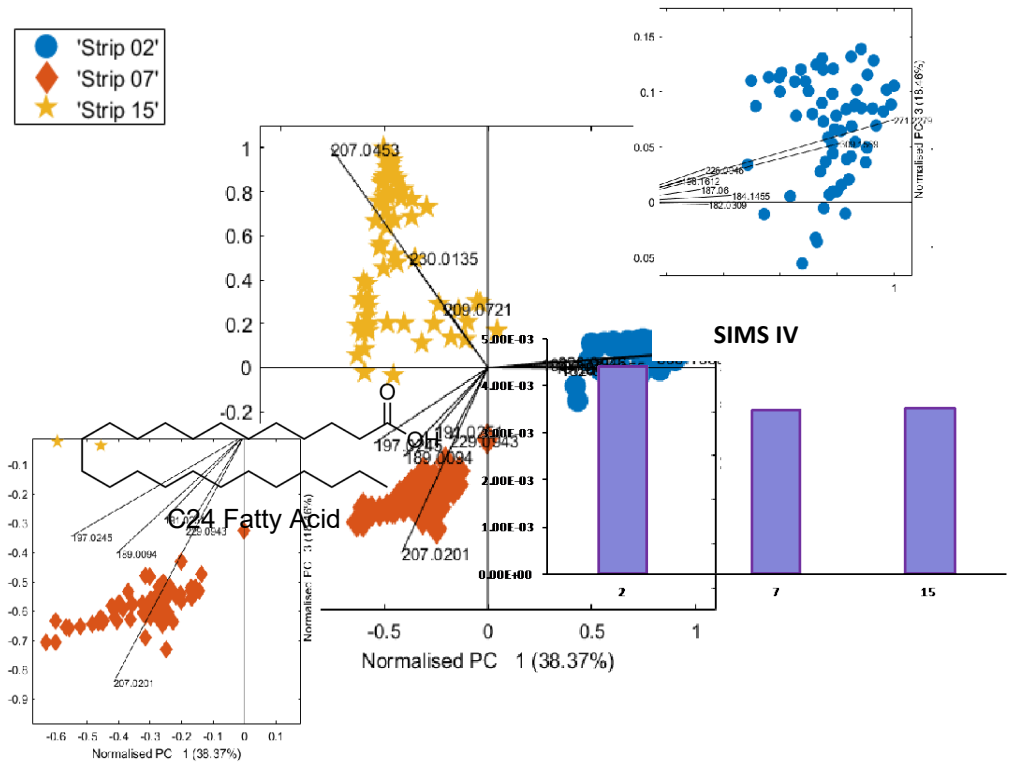


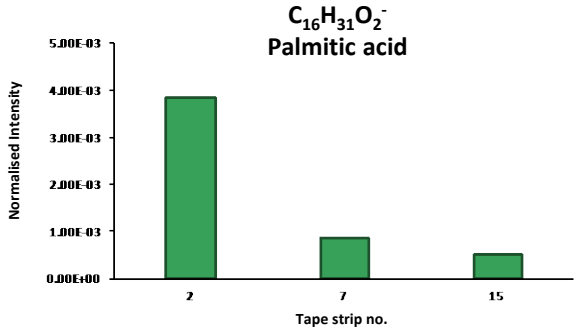
Hybrid SIMS

Orbitrap SIMS single beam –
Single quasi - DC beam (Ar_n , n > 1000)

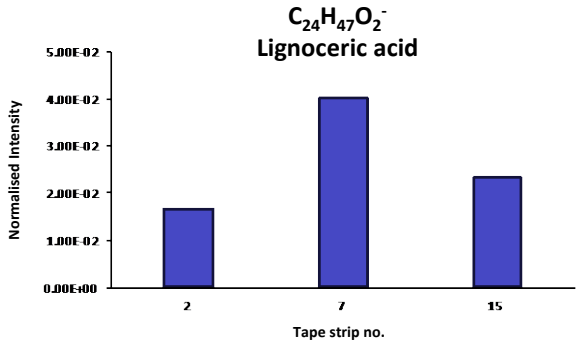
3D OrbiSIMS Native Skin Analysis

Stratum corneum gradients (Tape strips)

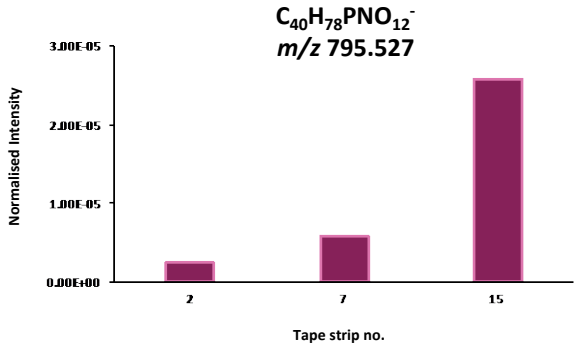




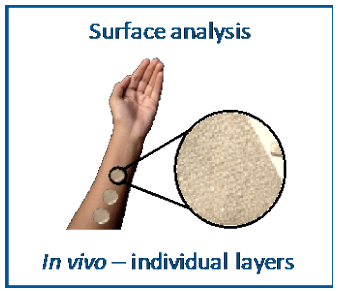
High at the surface



Localised within *SC*

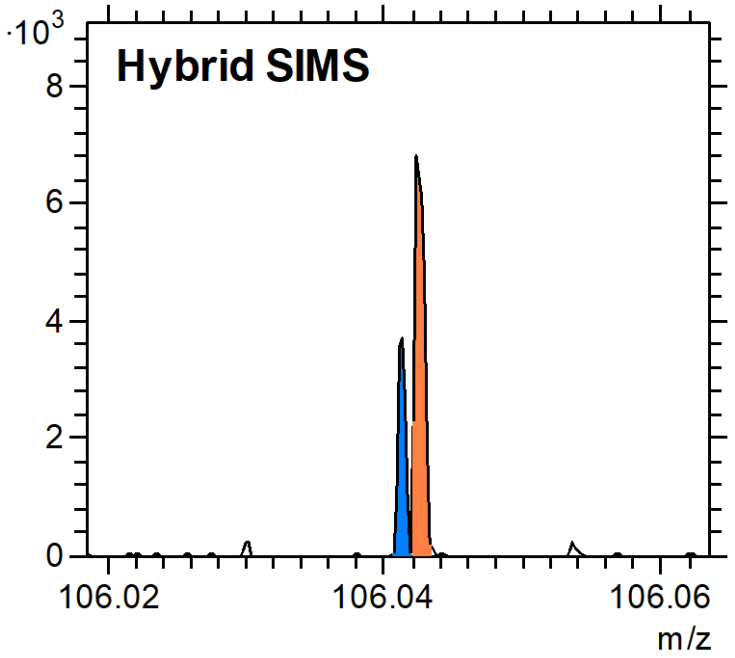


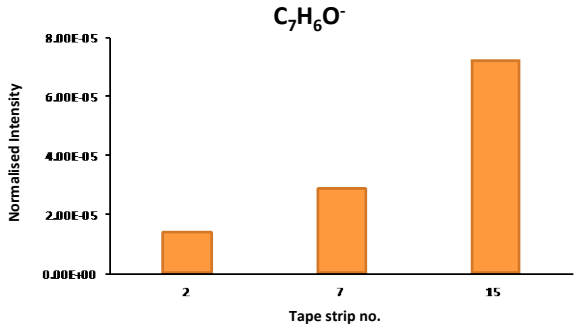
High in underlying epidermis

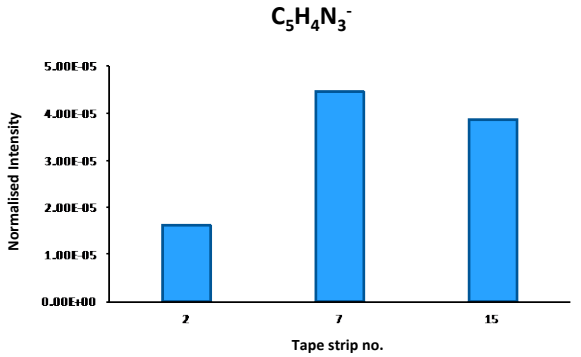


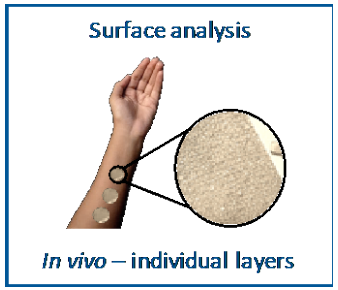
3D OrbiSIMS Native Skin Analysis

Stratum corneum gradients (Tape strips)



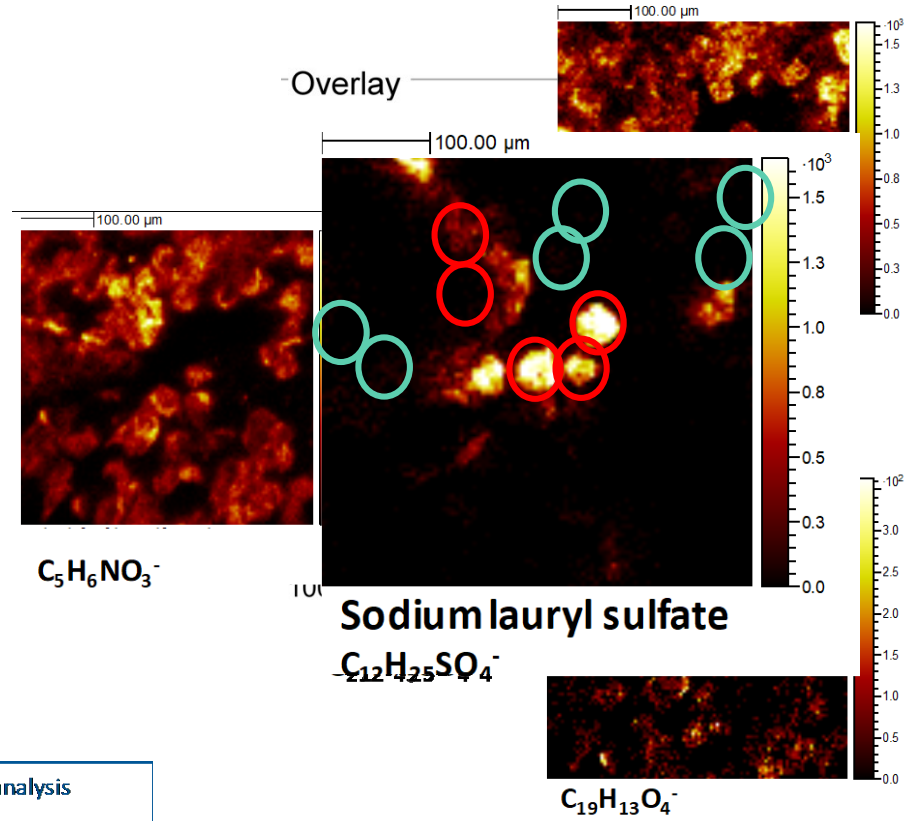


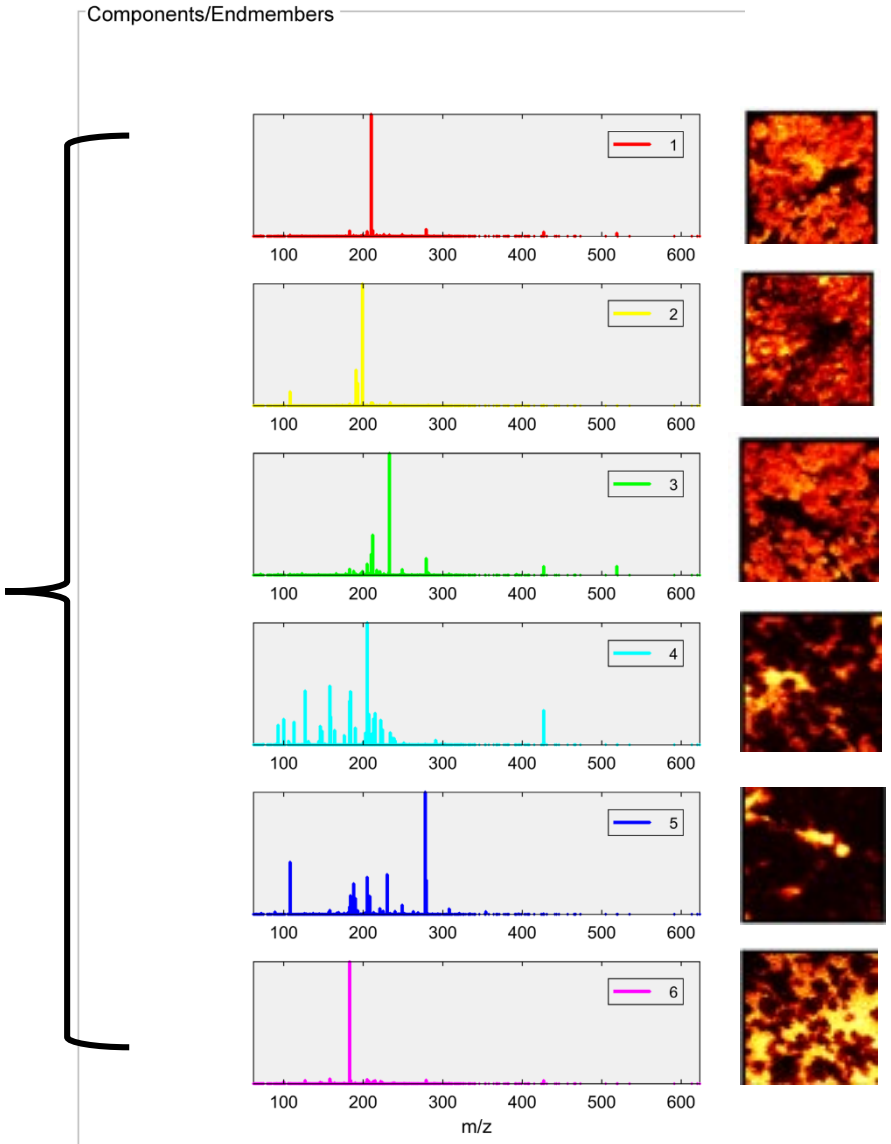


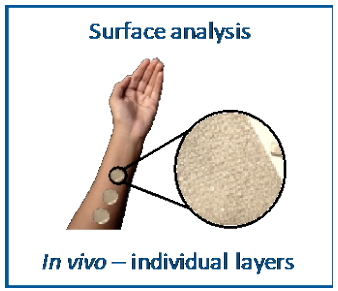


3D OrbiSIMS Native Skin Analysis

Chemical distributions (Tape strips)



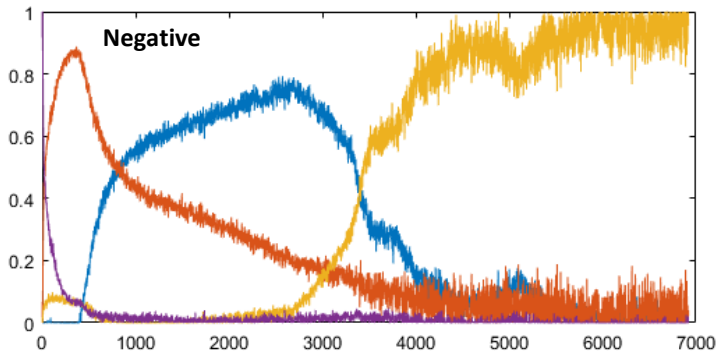


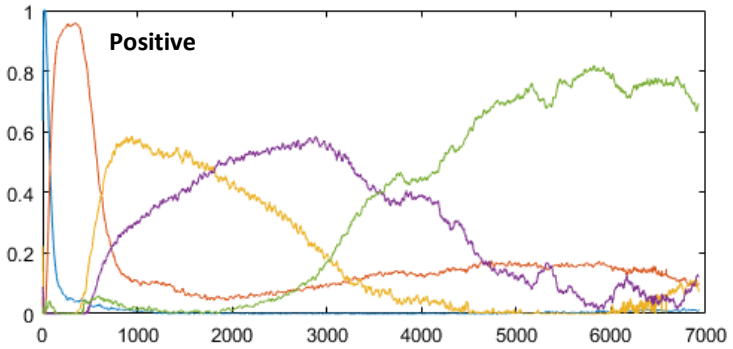


3D OrbiSIMS Native Skin Analysis

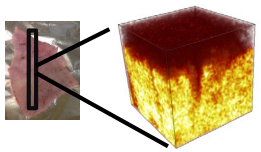
Depth profile analysis

Non-negative matrix factorisation analysis



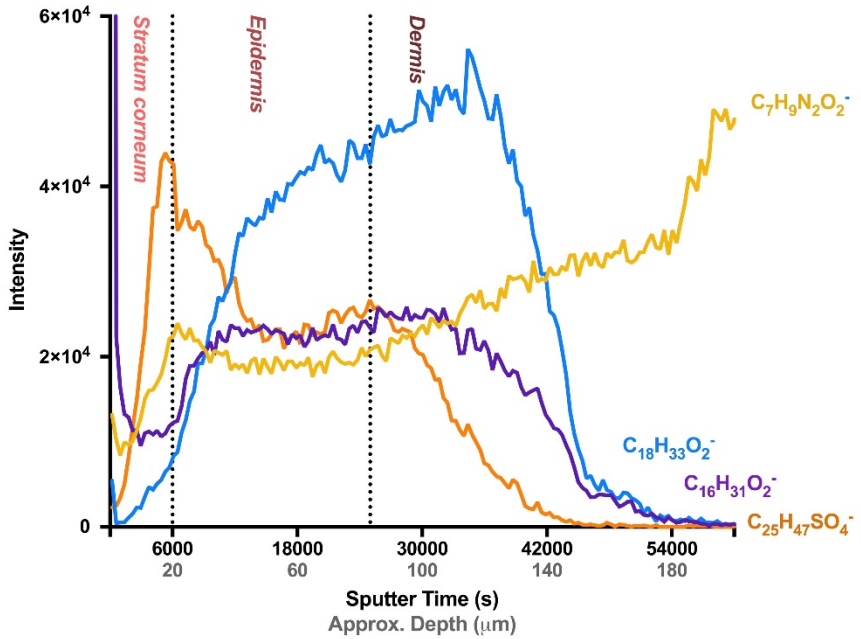


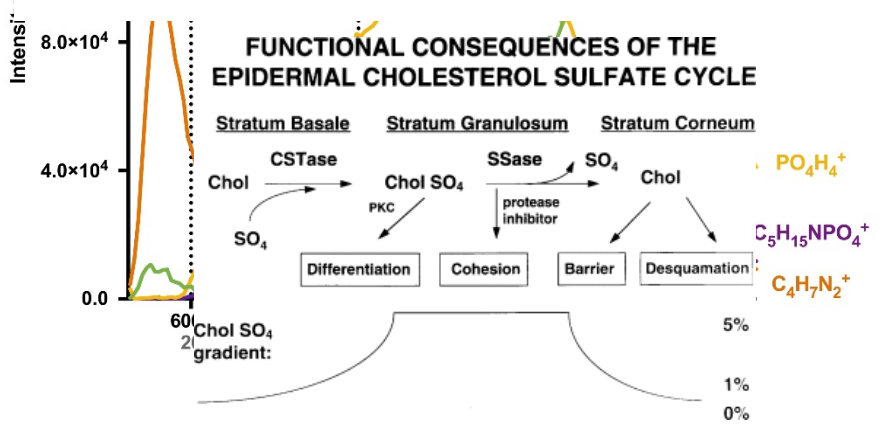
Depth analysis



Ex vivo porcine skin

Hybrid SIMS depth profile





Zetteson *et. al.,* Journal of Investigative Dermatology , 1998, 111, 5, 784-790

Conclusions

- Exogenous compounds can be traced permeating through *in vitro* and *in vivo* skin using ToF-SIMS as demonstrated for:
    - Antibacterial
    - Pharmaceutical
    - Cosmetic
- The hybrid SIMS instrument was able to elucidate differences in lipid composition as a function of depth, previously unattainable using the SIMS IV instrument.
- Differences in the lateral distribution of lipid species could also be obtained, indicating that lipids with different chemistries, such as ceramides and fatty acid species are distributed differently within a single SC layer.

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- Ministry of Higher Education in Iraq

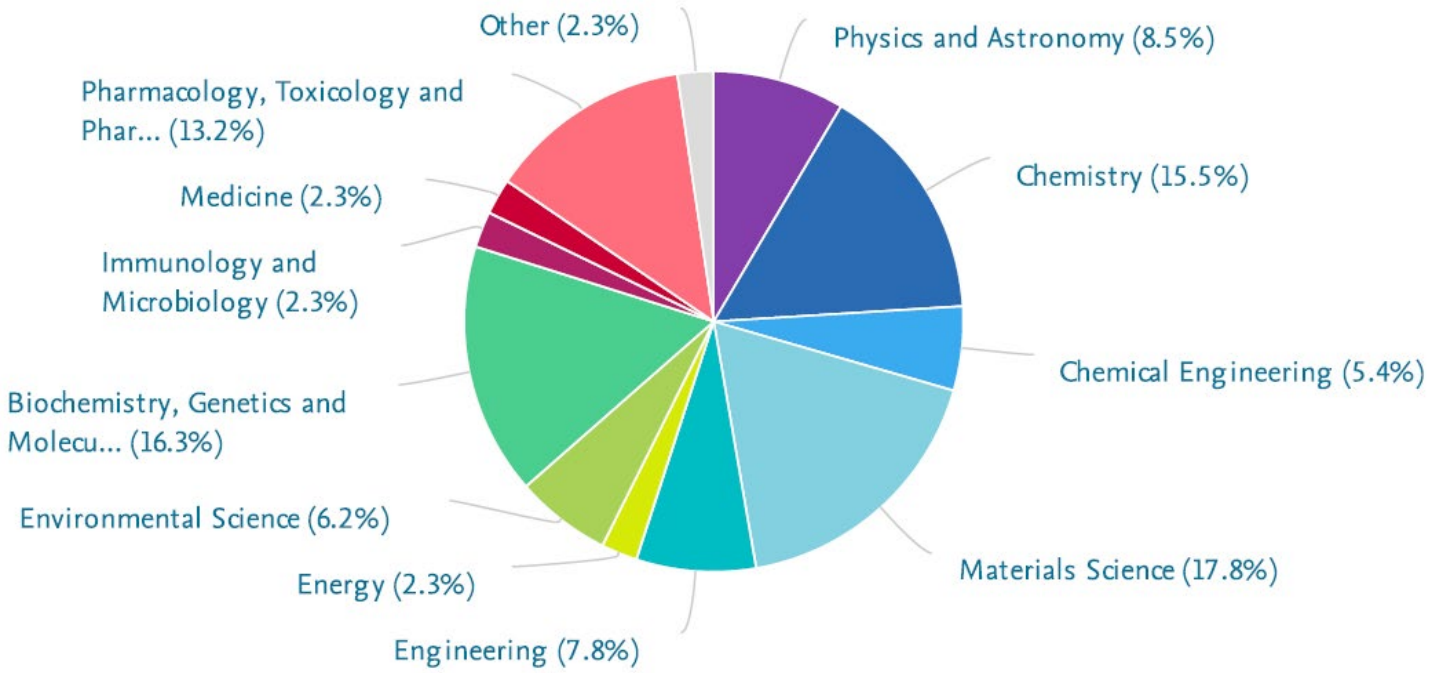
Multivariate Analysis

www.mvatools.com

EPSRC Innovate UK Walgreens Boots Alliance وزارة التعليم العالي والبحث العلمي

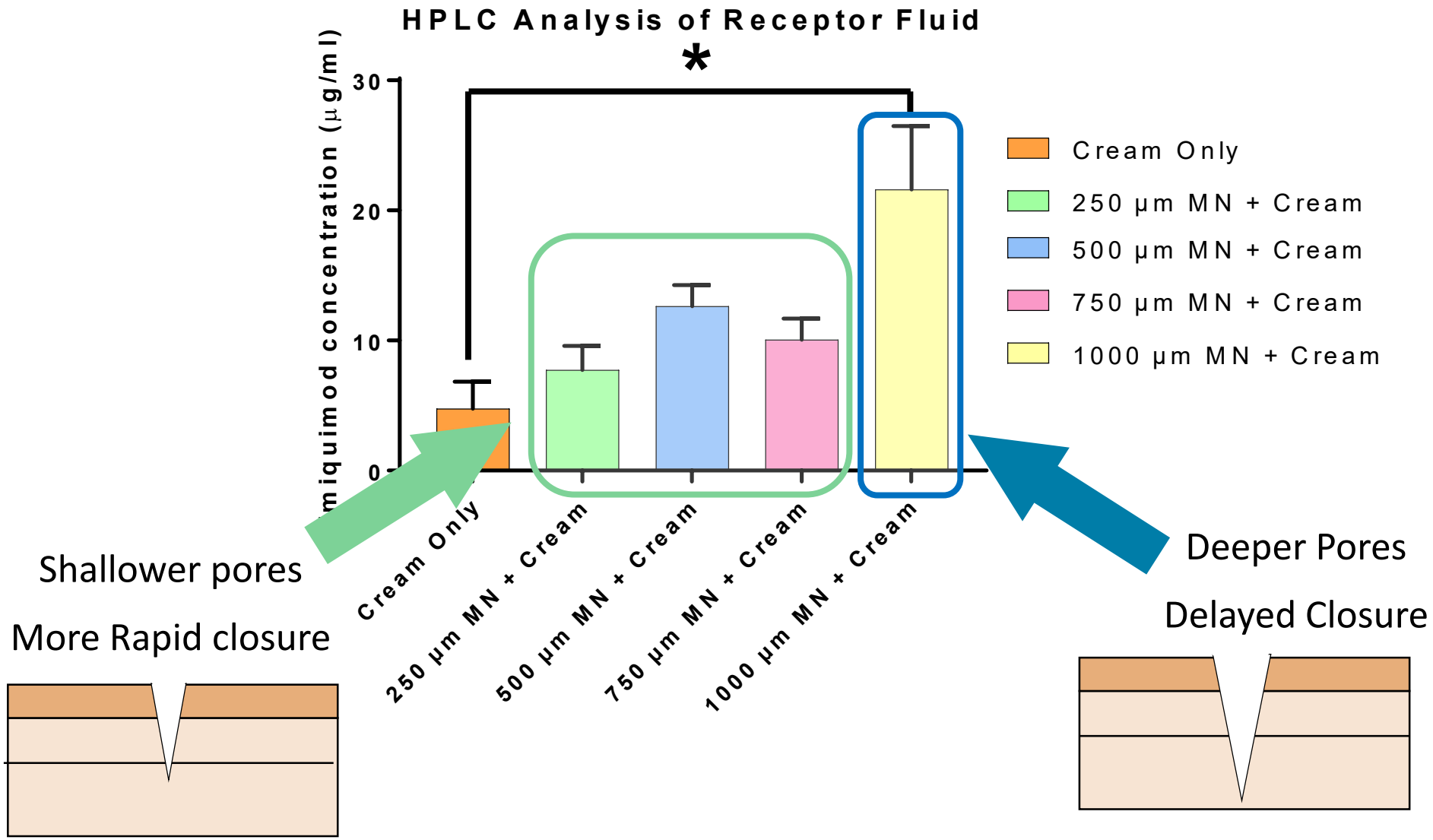
Research Interests

- ***Skin permeation & chemistry***
- *Pharmaceutical device characterization*
- *Multivariate Data Analysis*
- *Drug delivery micro and nanoparticles*
- *Polymer / skin microarrays*
- *Diesel Injector Deposits*



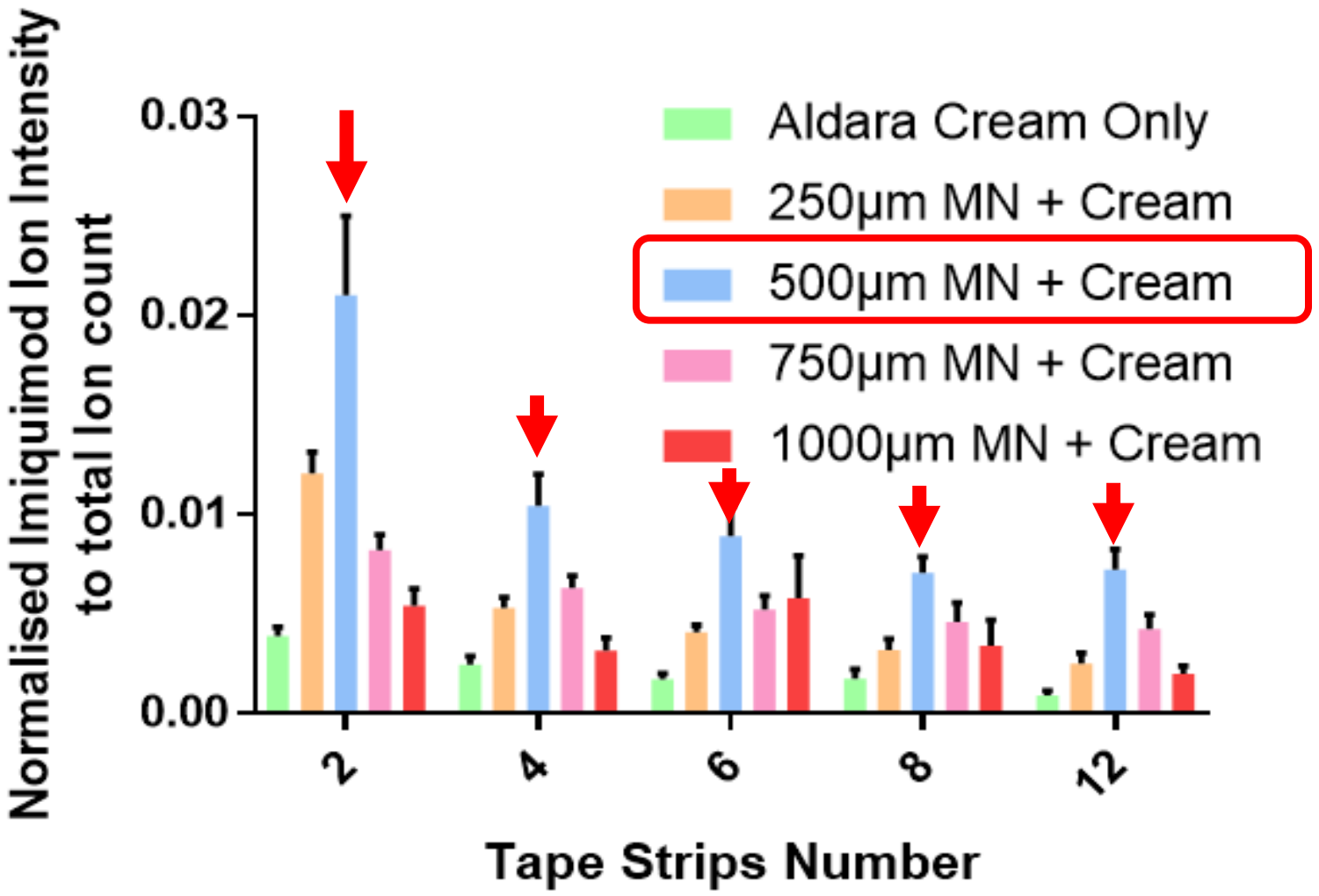
Source: SciVal (55 articles: 2012 - 2017)

Microneedle Enhanced Drug delivery



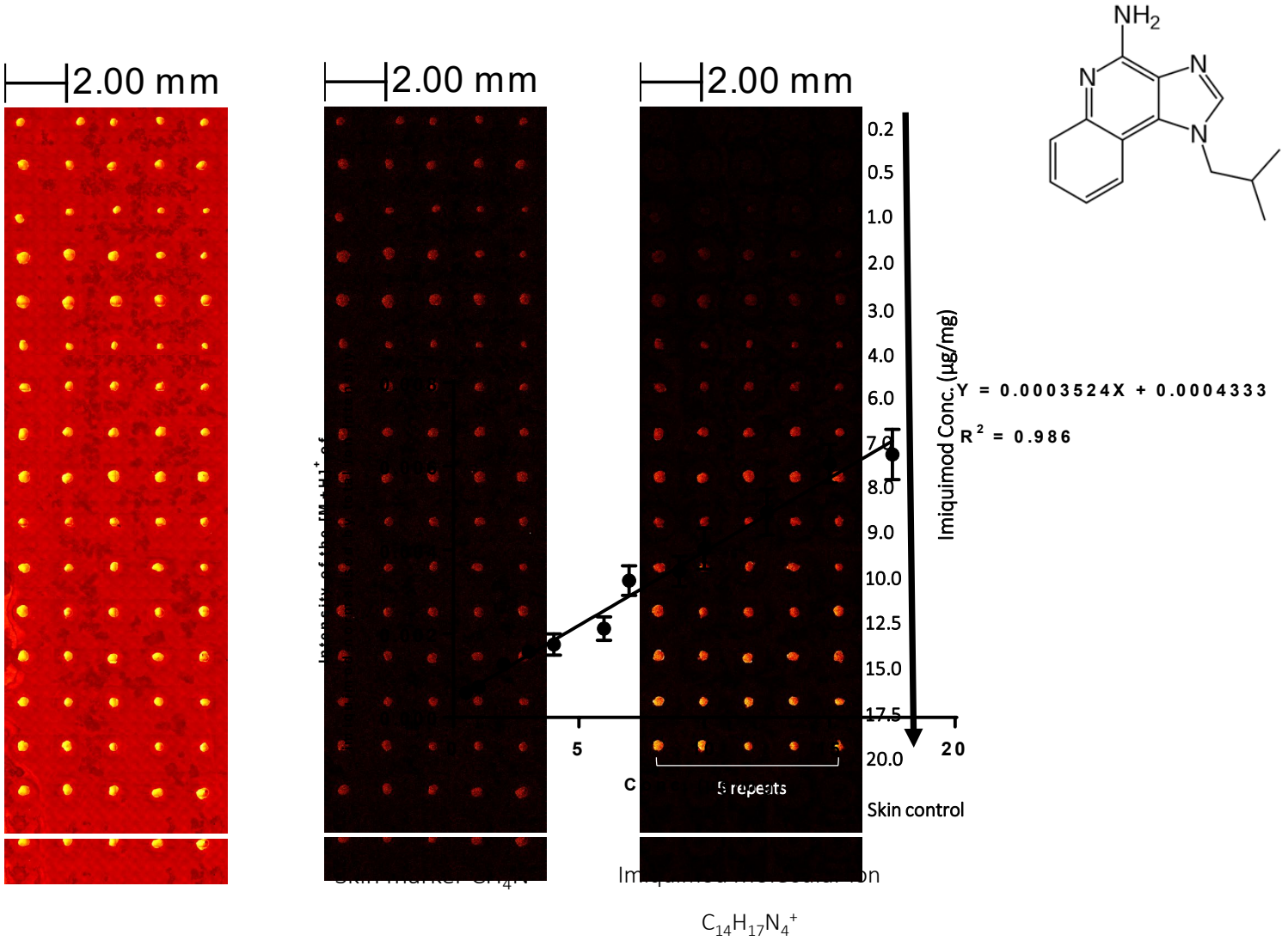
n=4. Mean ± SEM. One- way ANOVA with post hoc Tukey's test. *p < 0.05

Microneedle Enhanced Drug delivery

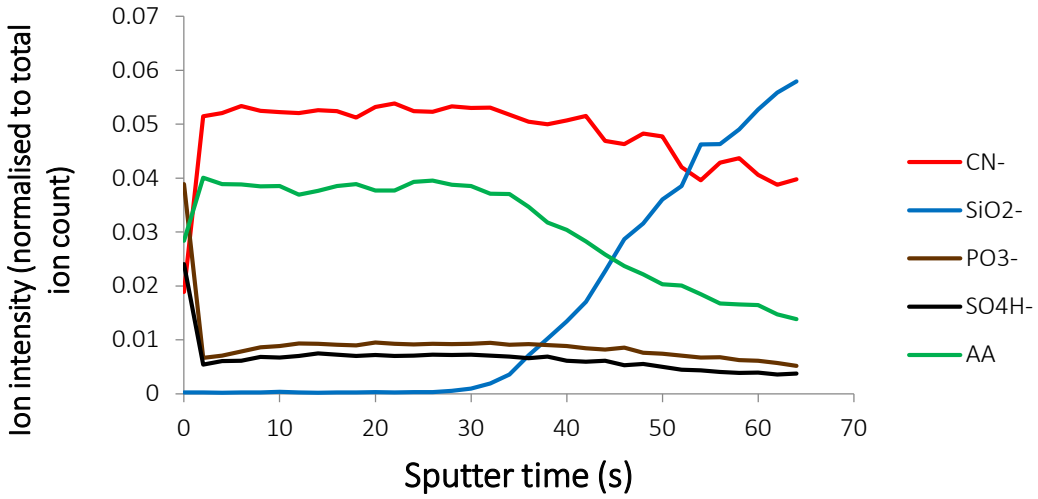


Imiquimod molecular ion $C_{14}H_{17}N_4^+$ intensities for sequential tape strips from porcine skin with different treatment modalities. *n=12* analytical repeats. Mean ± SEM

Microarray Printing



Microarray Printing



CN-
Skin marker

SiO_2-
Substrate

$C_6H_7O_6^-$
Ascorbic acid