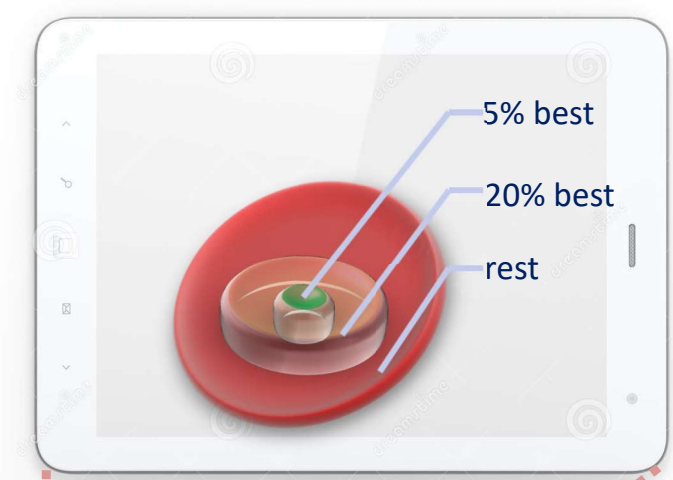
Miniaturized Planar Chromatography as Citizen Science

G. Morlock, JLU Giessen, Germany

A system for Citizen Science



The very first start a decade ago

G. Morlock *J. Chromatogr. A* 1382 (2015) 87-96

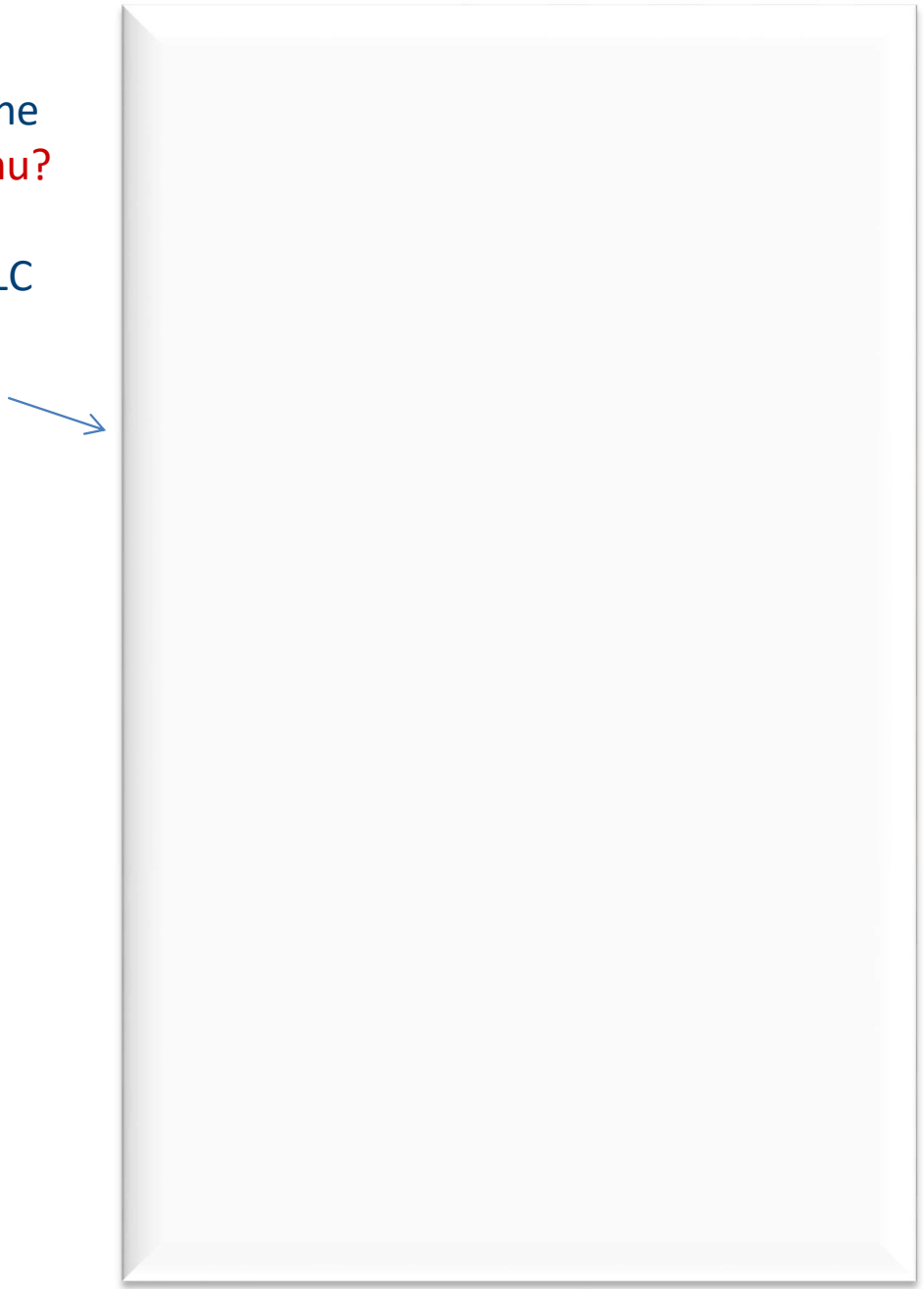
… printed on the HPTLC layer

G. Morlock *J. Chromatogr. A* 1382 (2015) 87-96

The magic dinner card for HPTLC 2006

The most frequently asked question at the symposium dinner was: What is the menu?

It was always pointed to the empty HPTLC foil in the menue cover on each dinner table…



The magic dinner card for HPTLC 2006

The most frequently asked question at the symposium dinner was: What is the menu?

It was always pointed to the empty HPTLC foil in the menue cover on each dinner table…

It took a while until a scientist started to heat the HPTLC plate as a heating plate was noticed on the buffet.

The menue was printed on an impregnated plate not using inks, but the sweetener sucralose. Upon heating a chemical derivatization made the writing visible.


International Symposium for HPTLC
Dinnercruise on the MS Kreuz
10th October 2006

Buffet

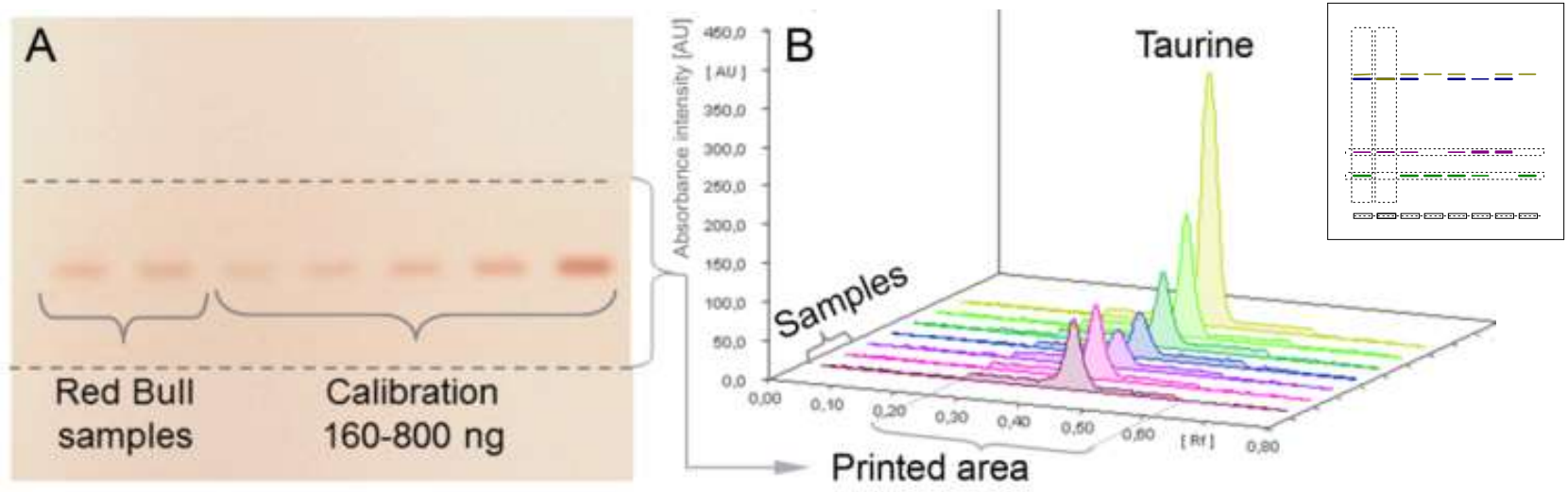
Cold starters
Vitello Tontao
Sweet & soure shallots cooked in red wine & garlic
Country style ham with honeydew
Russian styled herring salad
Variety of fish, lobster and crabs in jelly
Marinated mushrooms & parsley tomato dressing
Mixed green salad with crispy Panchetta, herb-flavored vinaigrette and Caesardressing
Minced egg with chives

Hot dishes
Carott-Orange soup
Traditional Coq au vin with rosemary potatos
Baked Pollock filet on a vegetable creme sauce and Herb-flavored rice
Ragout a la "Stroganoff"

Desserts
Apple Pie
Creme Caramel
Mousse au chocolate with Portwine plums
Variety of Cheese and bread with butter

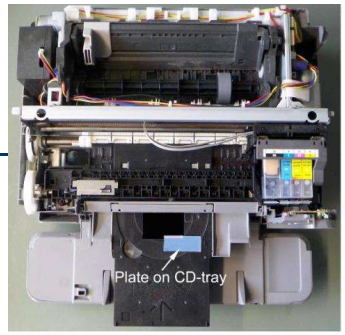
Inkjet printing of derivatization reagent

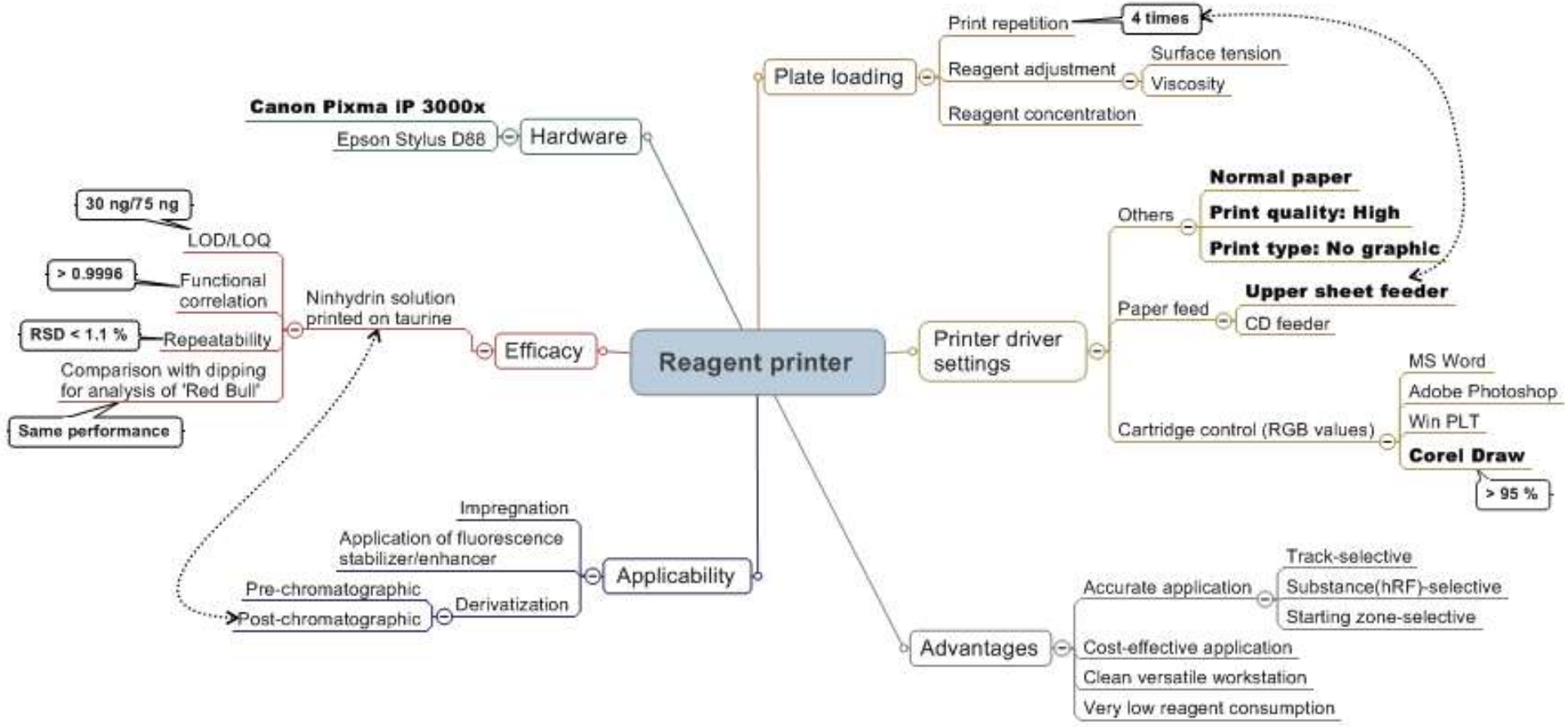
	Dipping[9]	Printing
Functional correlation		
RSD	±0.9%	±1.0%
Correlation coefficient r	0.9998	0.9996
LOD	41 ng	30 ng
LOQ	82 ng	75 ng
Repeatability (RSD, $n = 5$)	±0.9%	±1.0%
Taurine found	0.37% ($n = 4$)	0.35% ($n = 2$)
Recovery rate ($n = 3$)	103% ± 3.0%	98% ± 2.8%



G. Morlock *et al. J. Liq. Chromatogr. Relat. Technol*. 30 (2007) 2171-2184

Reagent printer

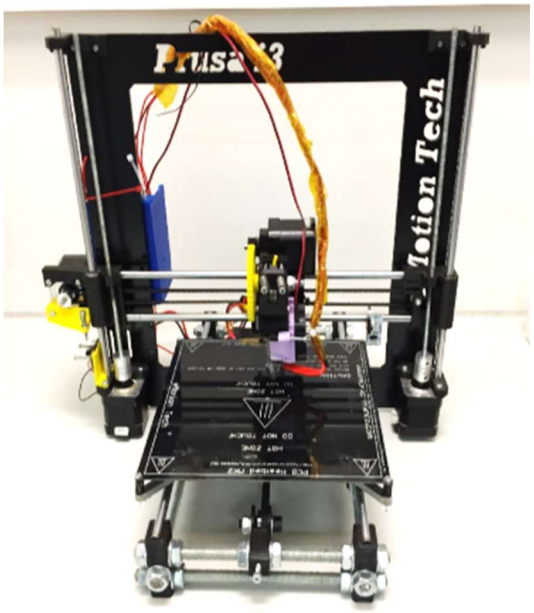


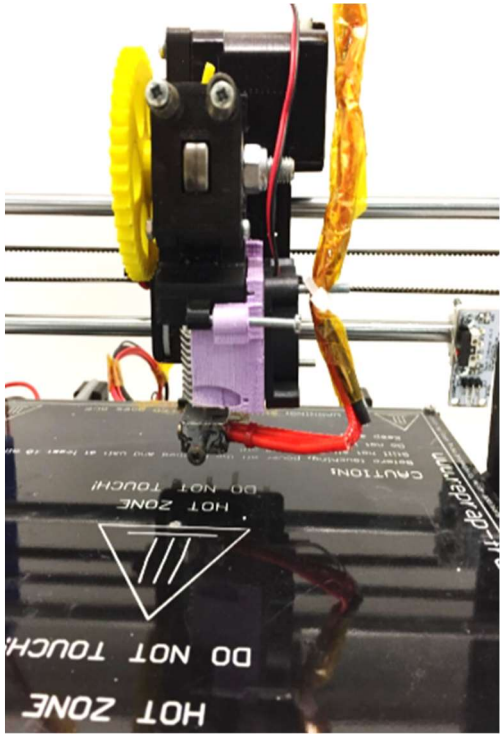


G. Morlock *et al. J. Liq. Chromatogr. Relat. Technol*. 30 (2007) 2171-2184

Open source tools

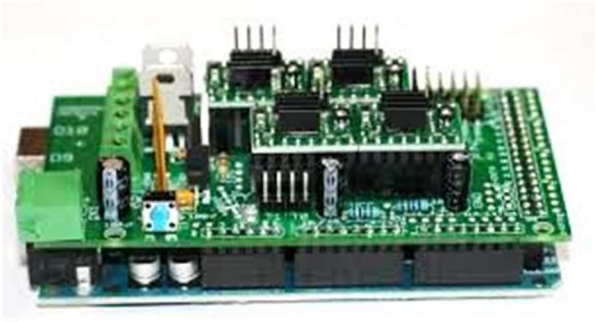
Programming tools





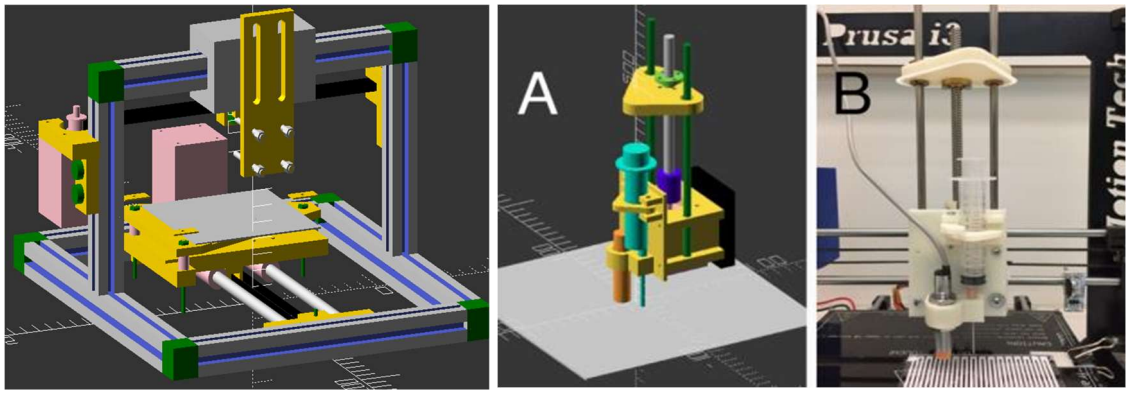
Prusa i3 printer

Internet of things with Raspberry pi

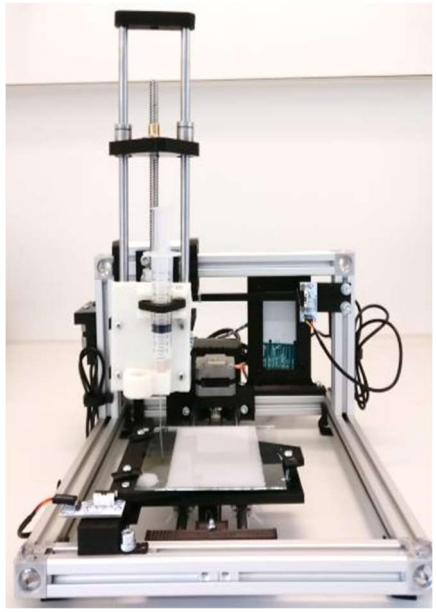


Arduino mega 2560 and Ramps 1.4

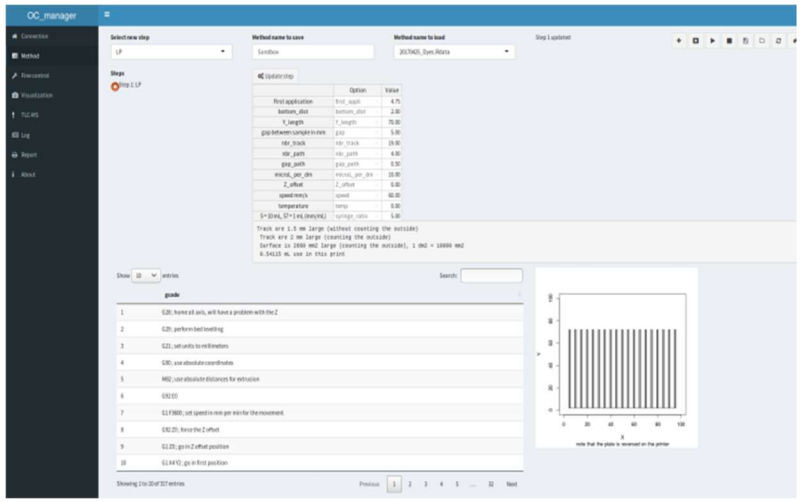
Layer print



CAD of prototype



Open source apparatus

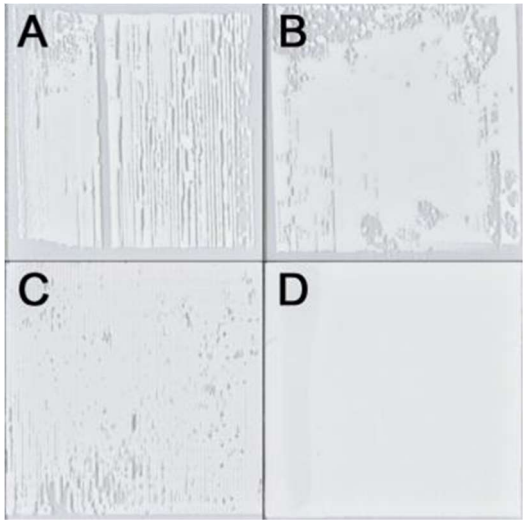


OC_manager software

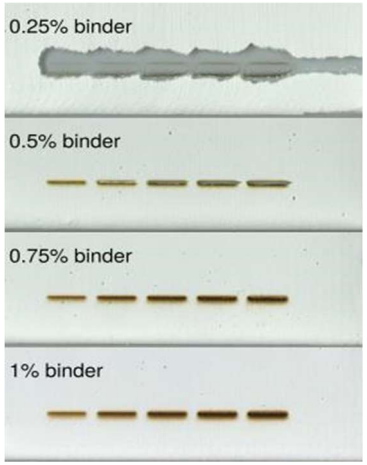
	Option	Value
First application	first_appli	4.75
bottom_dist	bottom_dist	2.00
Y_length	Y_length	70.00
gap between sample in mm	gap	5.00
nbr_track	nbr_track	19.00
nbr_path	nbr_path	4.00
gap_path	gap_path	0.50
microL_per_dm	microL_per_dm	10.00
Z_offset	Z_offset	0.00
speed mm/s	speed	60.00
temperature	temp	0.00
5 = 10 mL, 57 = 1 mL (mm/mL)	syringe_ratio	5.00

D. Fichou, G. Morlock *Anal. Chem.* 89 (2017) 2116–2122

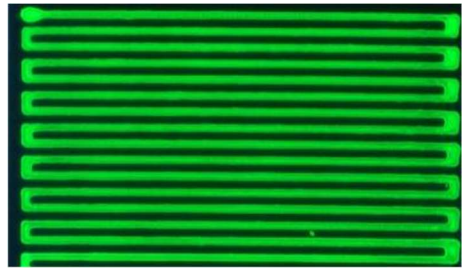
Layer print



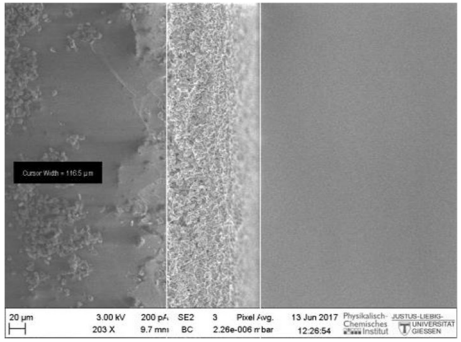
Problems encountered



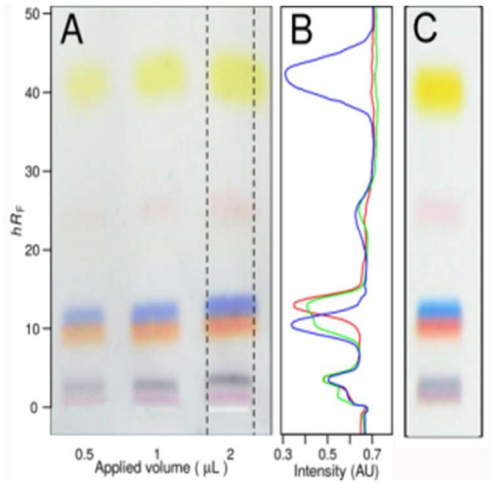
Organic binder



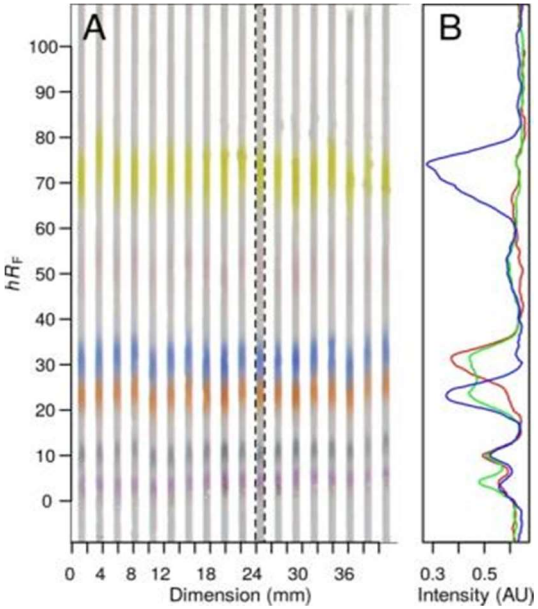
Fluorescence indicator



Layer thickness



Paricle size



Channelled pattern
40 tracks/10 cm

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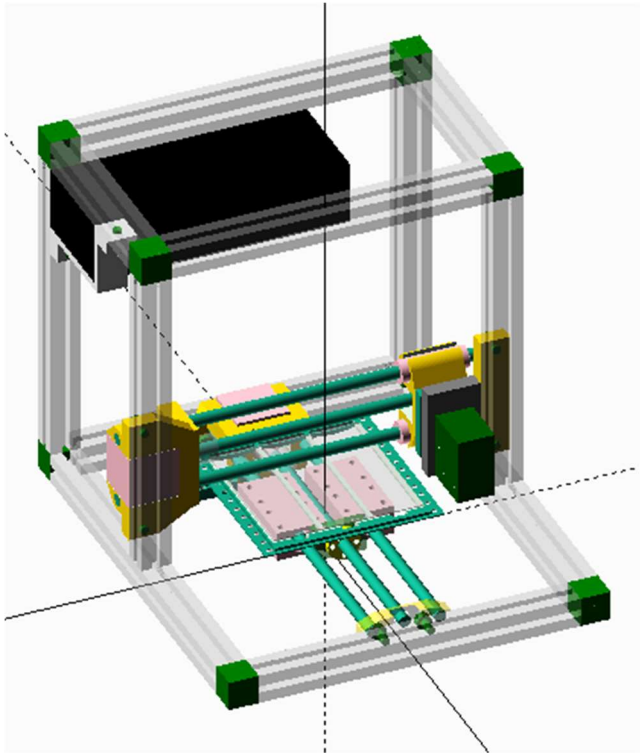
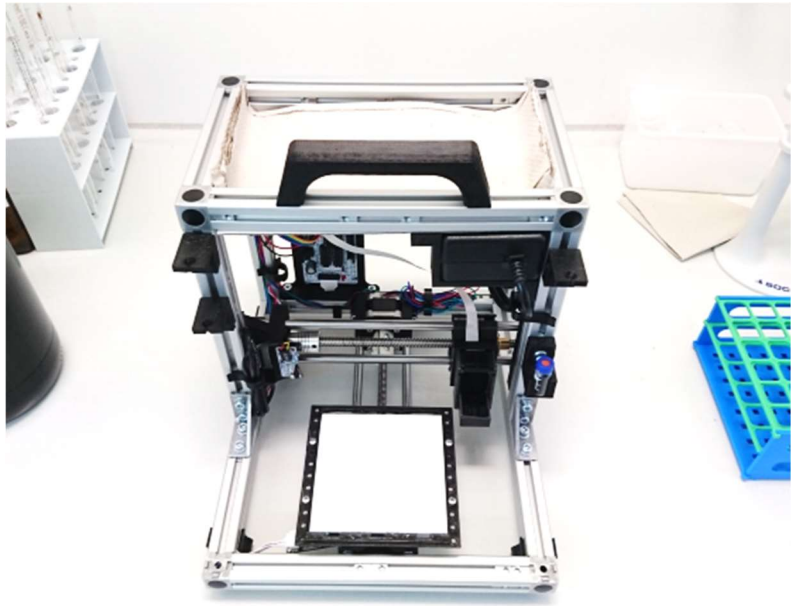
All-in-one open source system



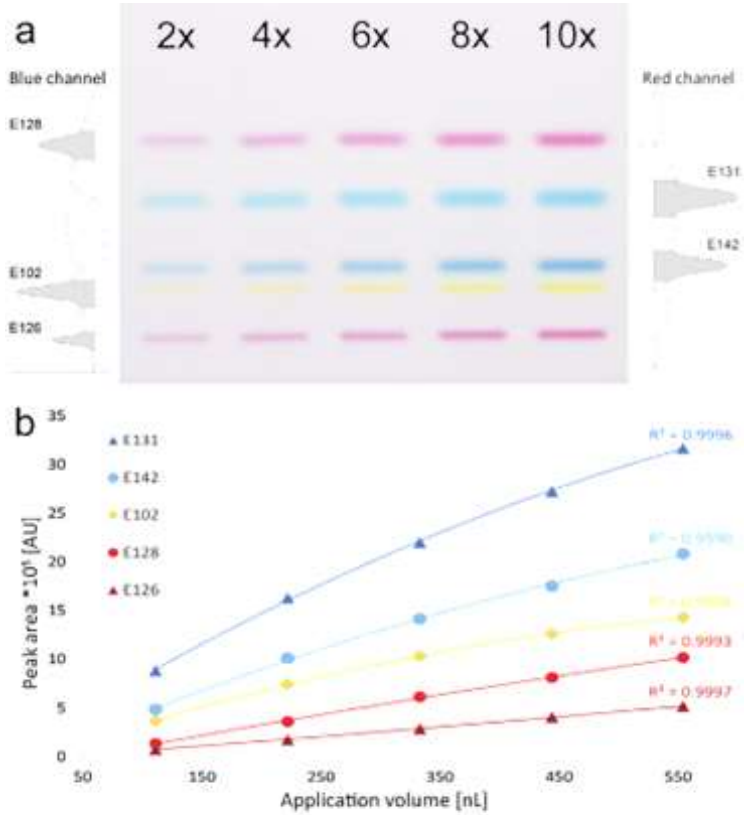
CAD of prototype



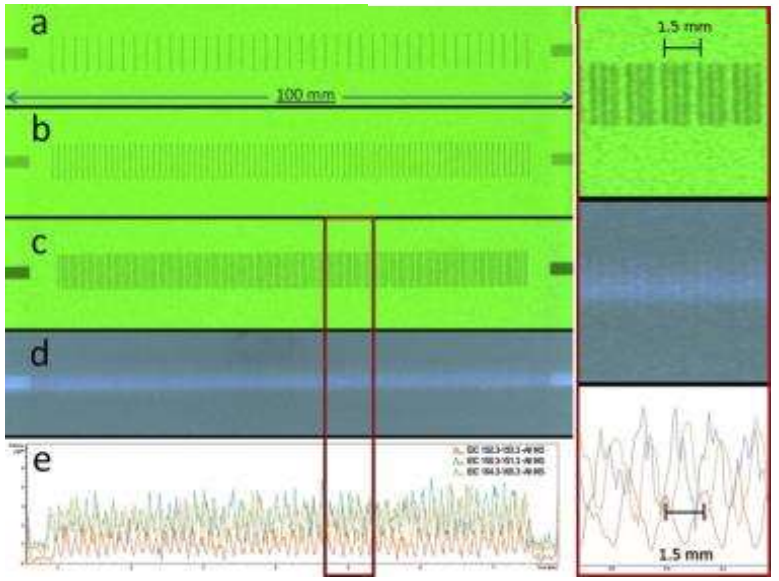
First OC prototype

OC_manager software

Precision of printing

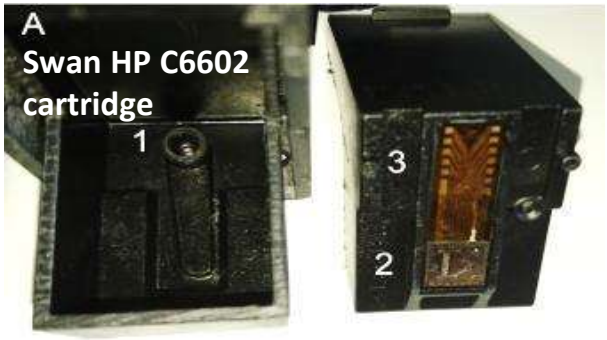


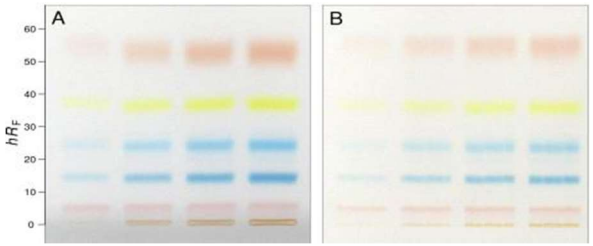
C	hR_F	amount [ng]	mean *RR* [%]	*%RSD* [%]	mean R^2
E126	7	67 - 332	103	4.0	0.9977 ± 0.0033
E102	16	44 - 222	101	3.4	0.9995 ± 0.0008
E142	20	22 - 111	105	6.5	0.9991 ± 0.0007
E131	32	44 - 222	101	2.9	0.9990 ± 0.0007
E128	42	44 - 222	103	7.2	0.9995 ± 0.0005



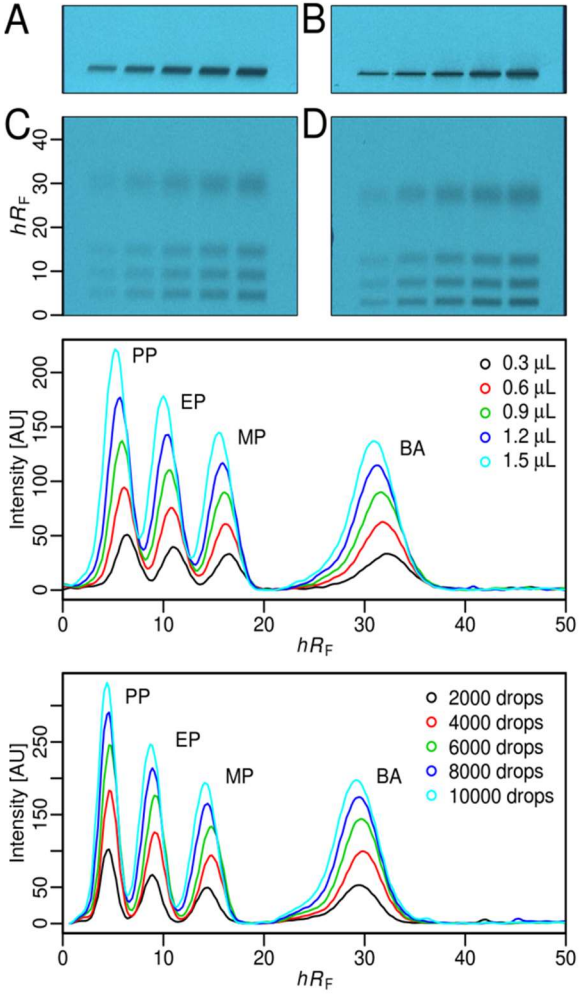
T. Häbe, G. Morlock *J. Chromatogr. A* 1413 (2015) 127–134

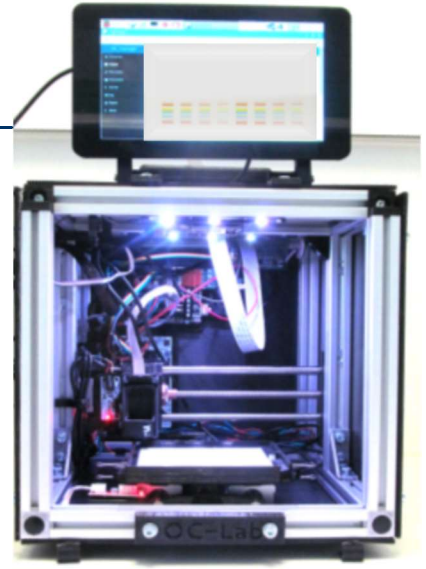
Inkjet print of sample solutions

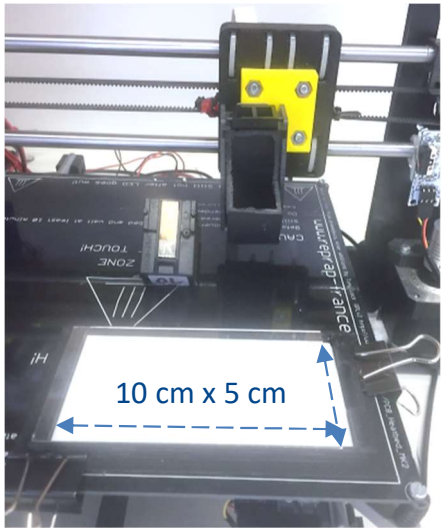




Spray-on (A) *versus* inkjet (B) application



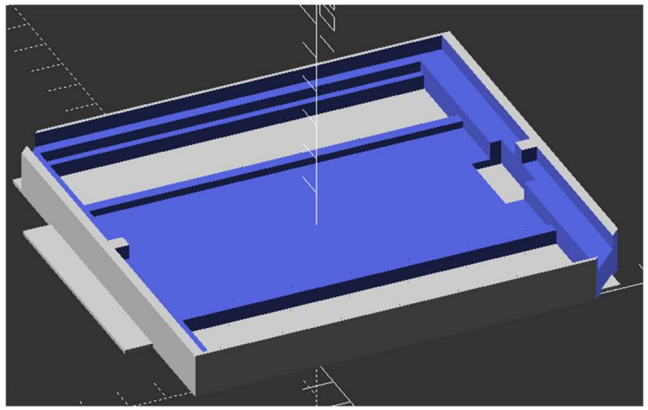




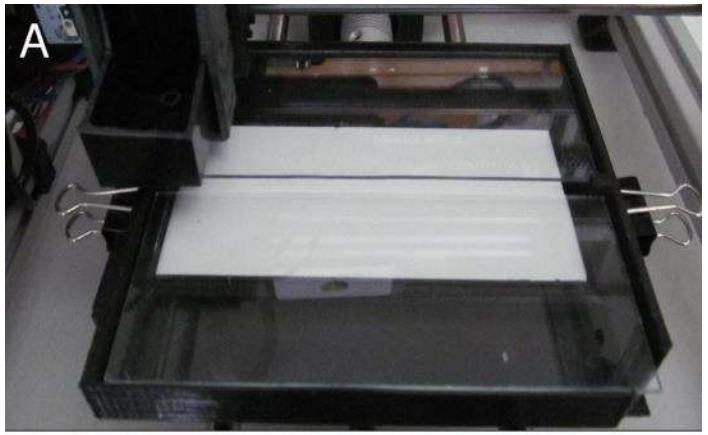
Miniaturized chamber

D. Fichou, G. Morlock *Anal. Chem.* 90 (2018) 12647–12654

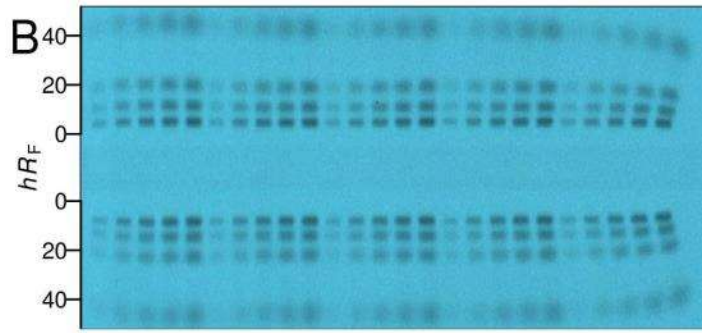
Inkjet print of mobile phase



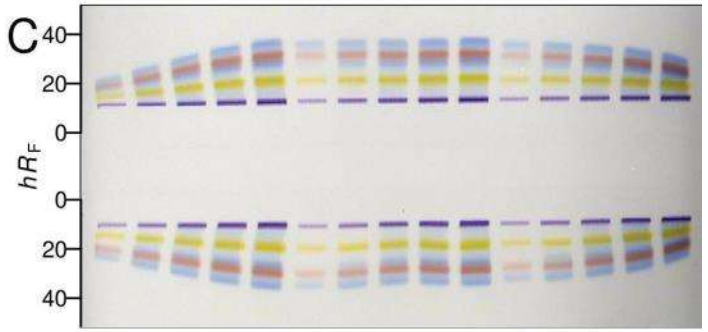
CAD of developing chamber



Parabens in MeOH/H_2O 1:3
EtOH/H_2O/AcOH, 14:40:0.1
CN F_{254} s



Food dye mixture
MeOH/H_2O 5% Na_2SO_4 3:4
RP 18 W



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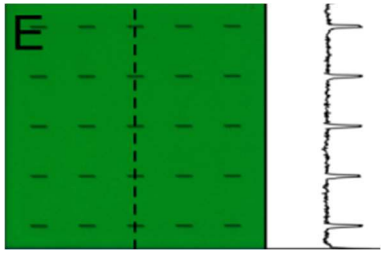
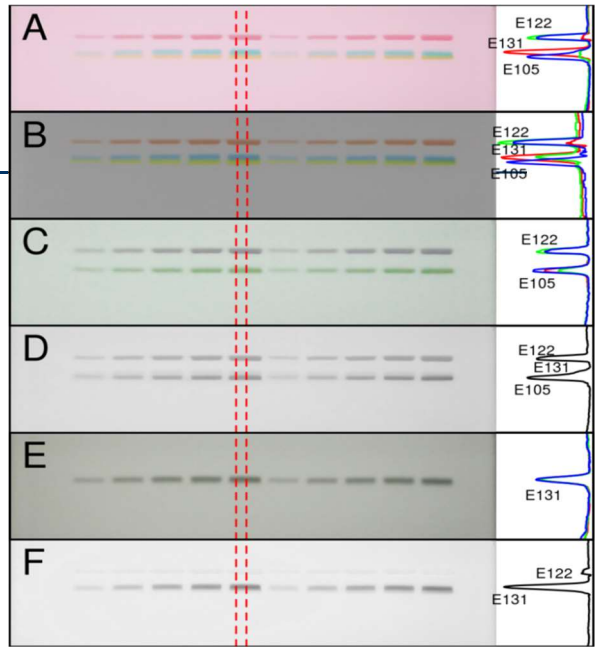
Detection by LEDs

Visualization chamber: Raspberry Pi camera and UV-LED

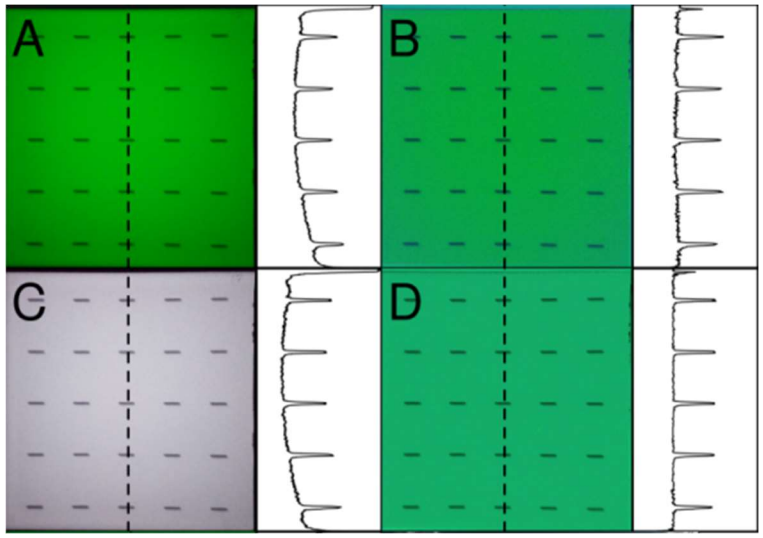
Reference:
50 ms
%RSD = 3.7%





no correction
800 ms, ISO 200
%RSD = 6.4%

corrected
%RSD = 6.0%



200 ms, ISO 800
%RSD = 4.6%

%RSD = 3.4%

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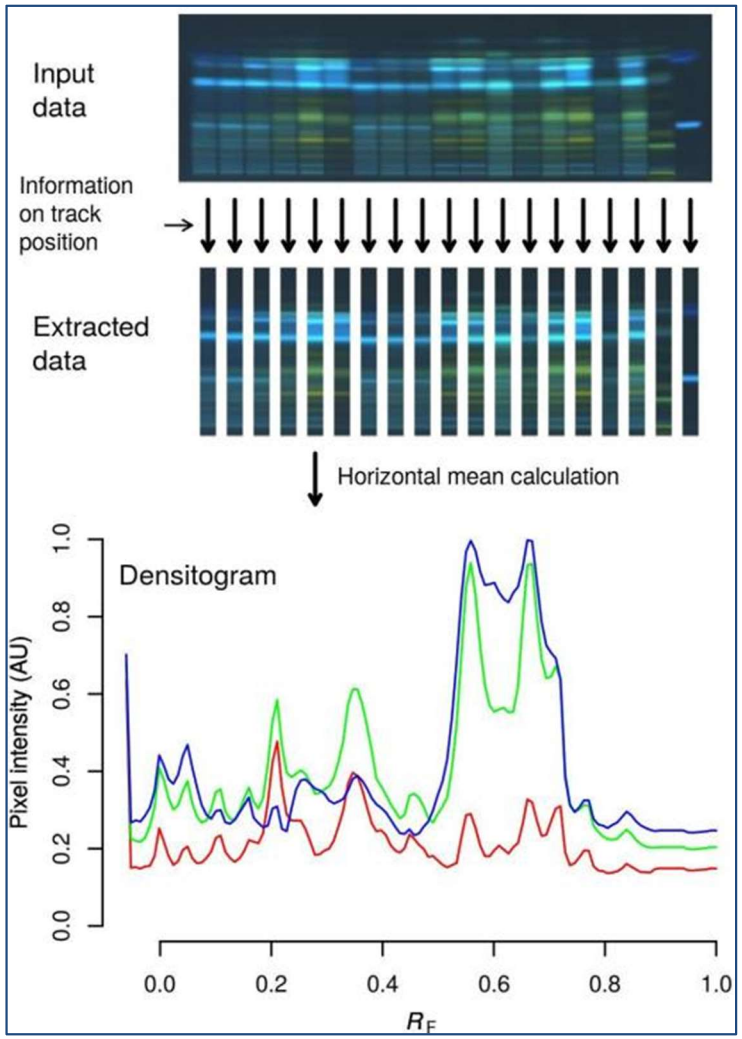
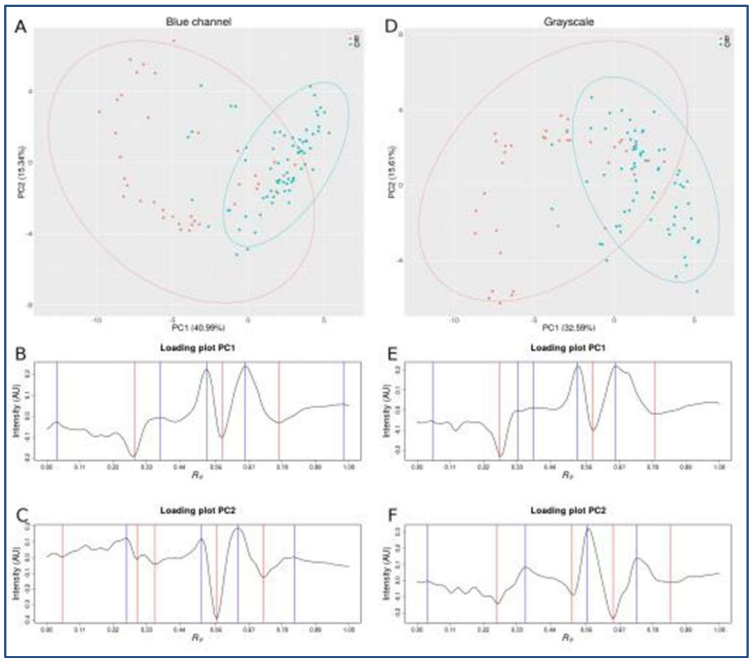
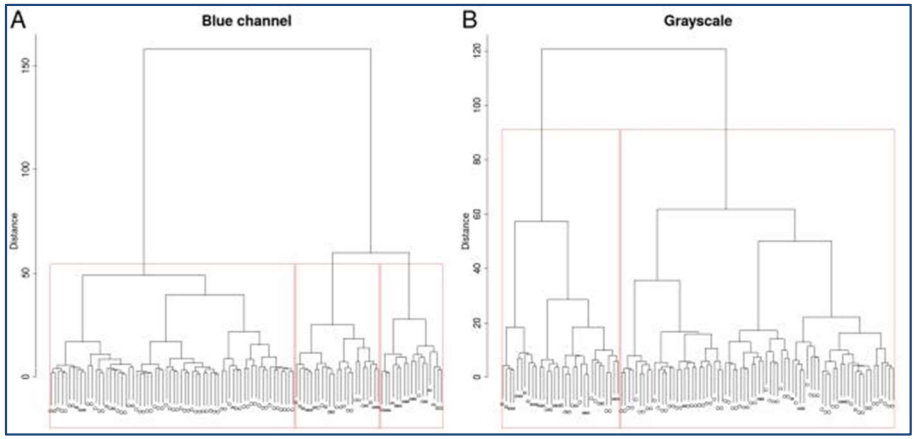
Data analysis: open-source rTLC software



Automatic video densitogram extraction



PCA of German propolis samples

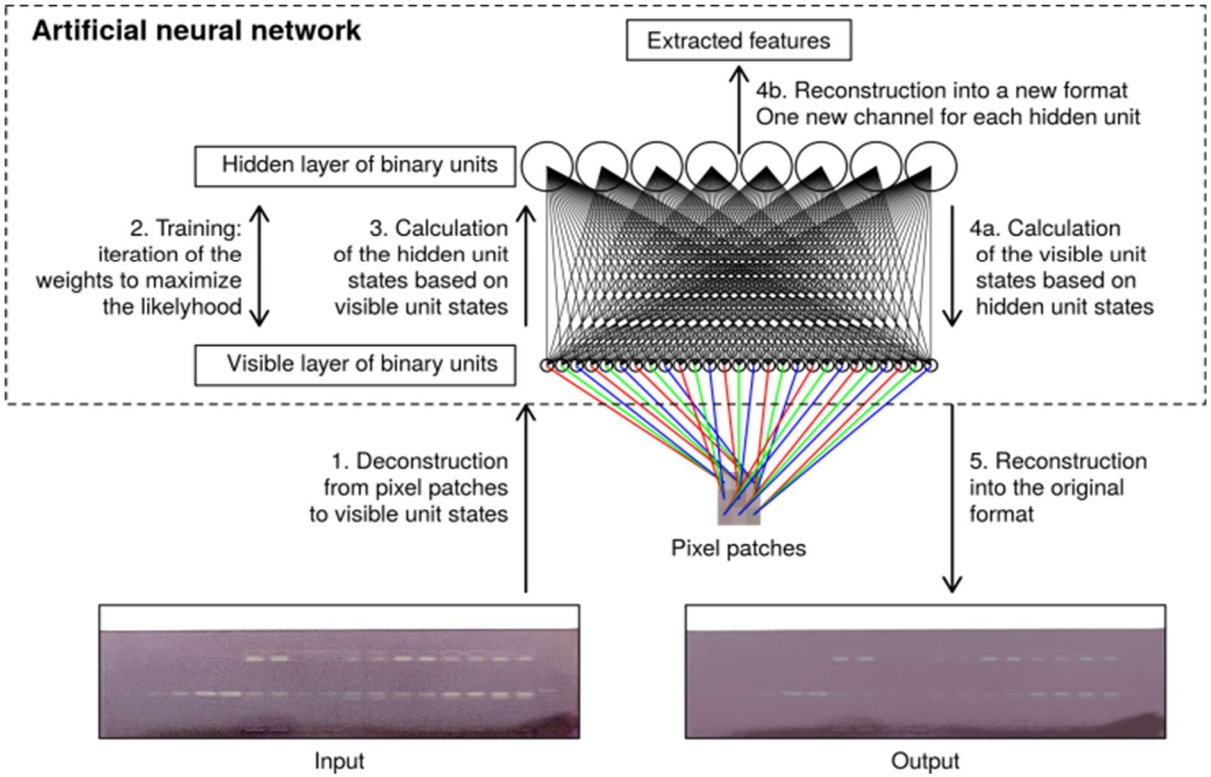


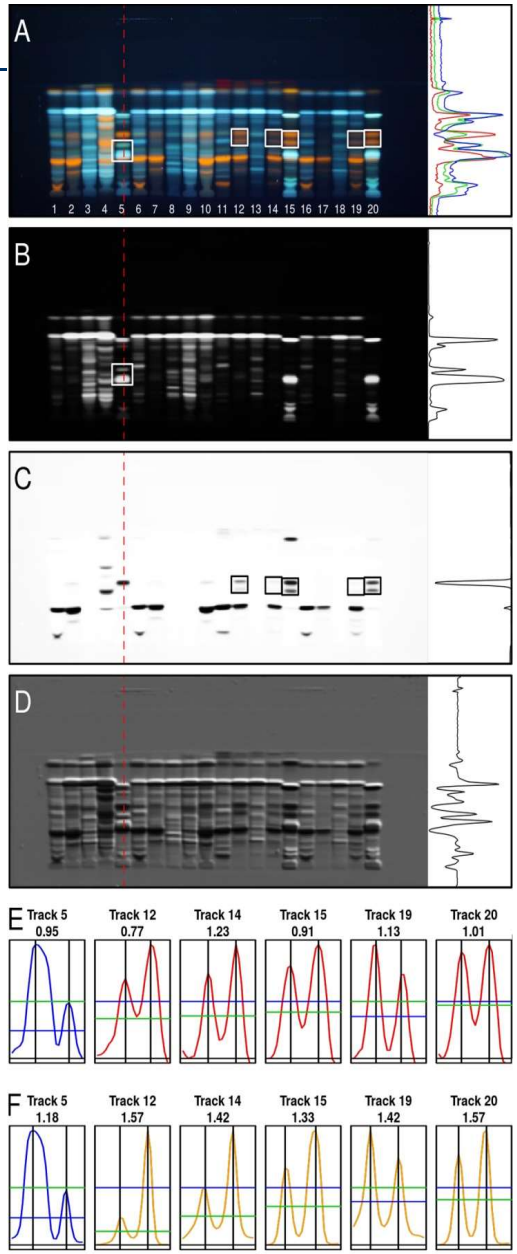
HCA of German propolis samples

D. Fichou *et al. Anal. Chem.* 88 (2016) 12494-12501

JLU

Data analysis: ANN





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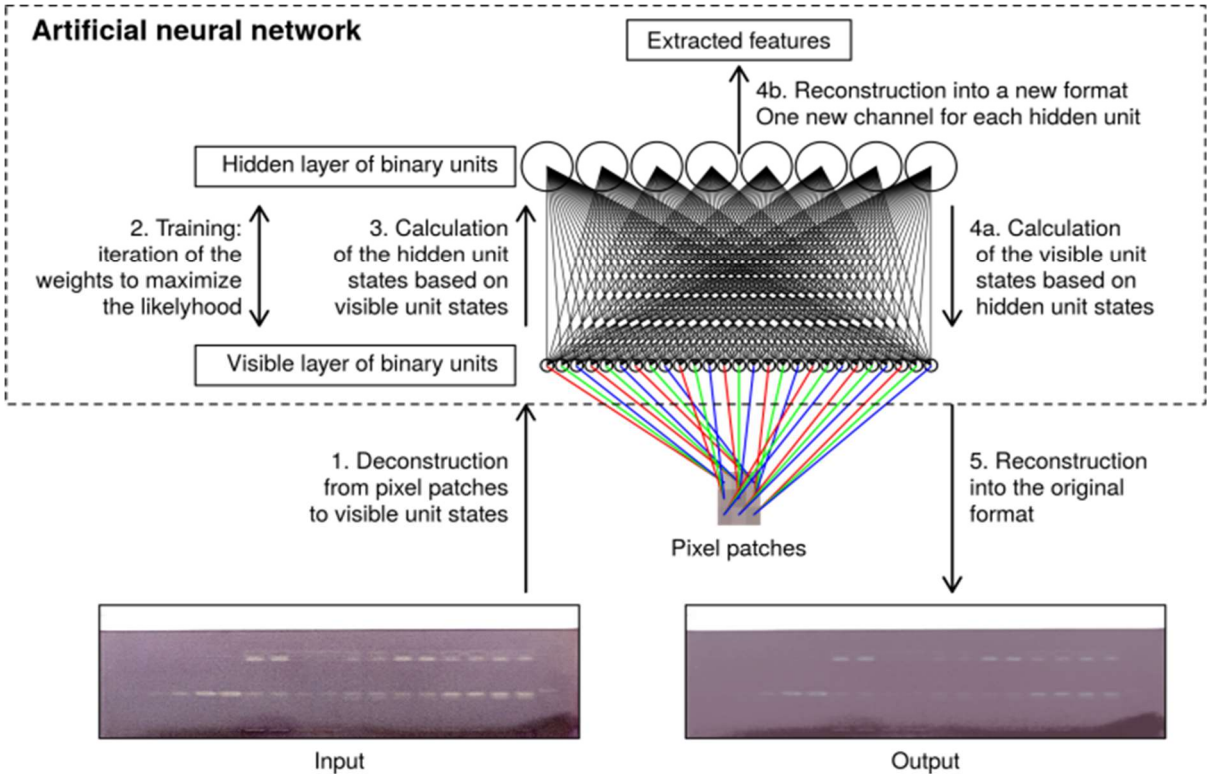
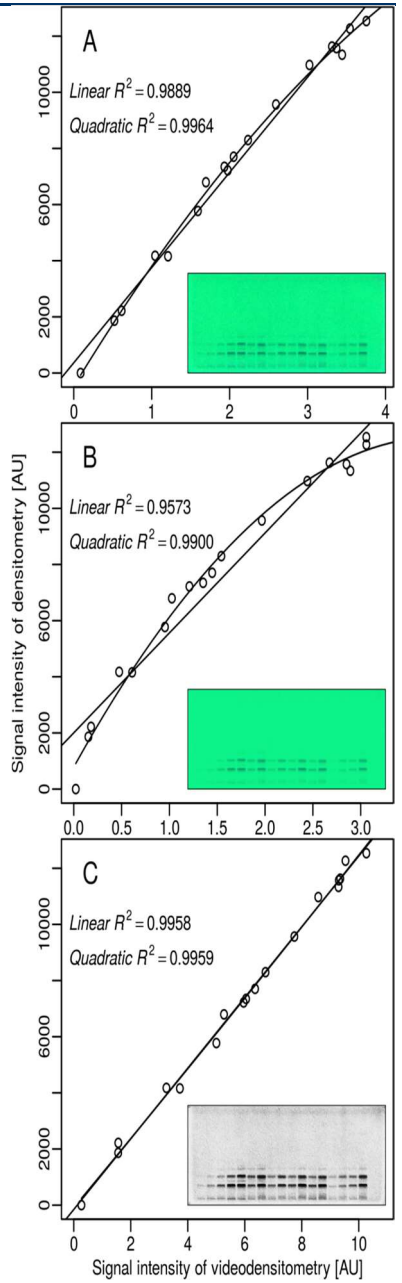
Data analysis: ANN





D. Fichou, G. Morlock *Anal. Chem.* 90 (2018) 6984-6991

All-in-one open-source system

Steps targeted

- Layer/sample/mobile phase print
- Plate drying
- Derivatization/bioassay print
- Detection by LEDs (UV, Vis, FLD)
- Luminescence detection
- Data analysis by ANN
- Cloud computing

Specifications

- ✓ 96 dpi inkjet resolution (250 µm)
- ✓ 50 µm mechanical resolution
- ✓ 100 pL per drop
- ✓ 26 x 31 x 26 cm^3 footprint
- ✓ µL-volumes
- ✓ <10-min separations

Printing cells on the plate

Aliivibrio fischeri bacteria passing inkjet nozzles

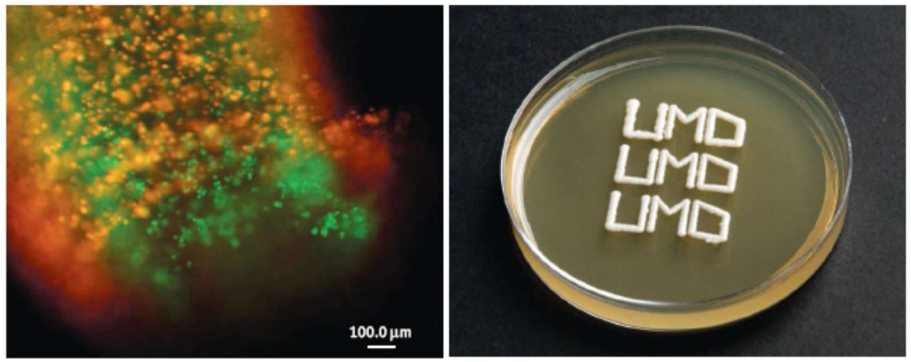
Printed in March 2008, G. Morlock *CBS* 102 (2009) 9

MATERIALS SCIENCE

Printing Cells

Paul Calvert

Inkjet printing technology offers a way to create studying cell interactions and artificial organs.



Cells on demand. (Left) Three-dimensional tube structure made from bioprinted cells. This composite image shows an inner layer of human umbilical endotheial cells (green) and an outer layer of human aortic smooth muscle cells (red). **(Right)** Printed and cultured yeast patterns after 3 days of culture. The patterns were printed at 75, 150, and 300 drops per second, from top to bottom.

www.sciencemag.org **SCIENCE** VOL 318 12 OCTOBER 2007

Effect-directed analysis by HPTLC

A bioanalytical tool!

"…we know there are **known knowns**.

These are things we know that we know.

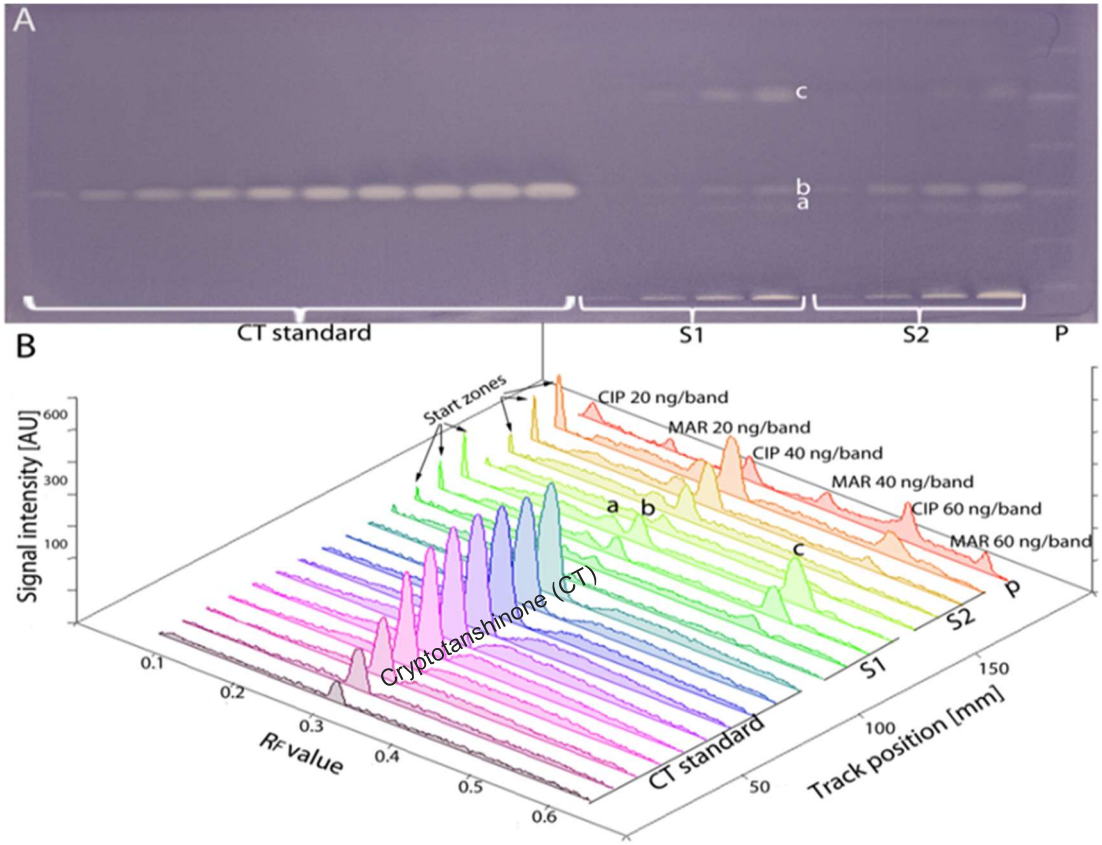
We also know that there are **known unknowns**.

That is to say, there are things that we know we do not know.

But there are also **unknown unknowns –**

the ones we do not know we do not know…" *D. Rumsfeld, 2002*

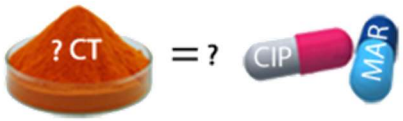
Quantification or equivalency calculation



S. m. roots		S1	S2
		Biodensitometry/densitometry	
CT content (mg/g)		1.8/1.6	3.2/3.9
Repeatability (%RSD, n=2)		8.7/0.4	7.0/0.9
Intermediate precision (%RSD, n=3)		16.3/1.4	15.2/1.6
Mean CT bioequival. content ± SD (mg/g, n=2)	a	1.1 ± 0.1	2.2 ± 0.0
	c	2.7 ± 0.0	< LOD

→ IC_{50} of cryptotanshinone (CT): 65 ng/band

→ equivalent to 365 µg S1 and 203 µg S2 root powders

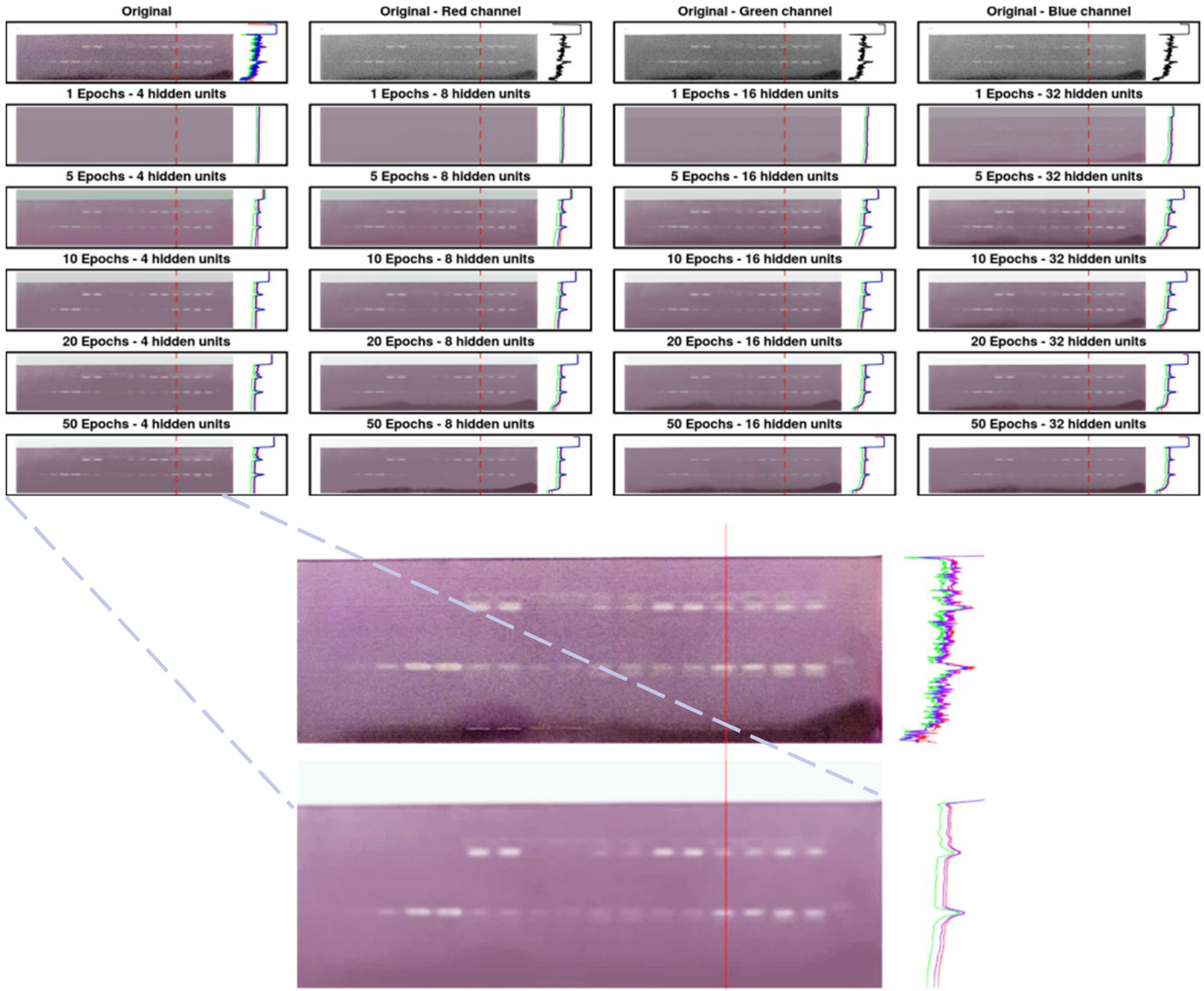


→ Equivalency calculation to synthetic antibiotics

→ 1 ng/band CT ~ 0.6 ng/band ciprofloxacin ~ 2 ng/band marbofloxacin

M. Jamshidi-Aidji, G. Morlock *Anal. Chem.* 88 (2016) 10979−10986

Data analysis: open-source ANN algorithm



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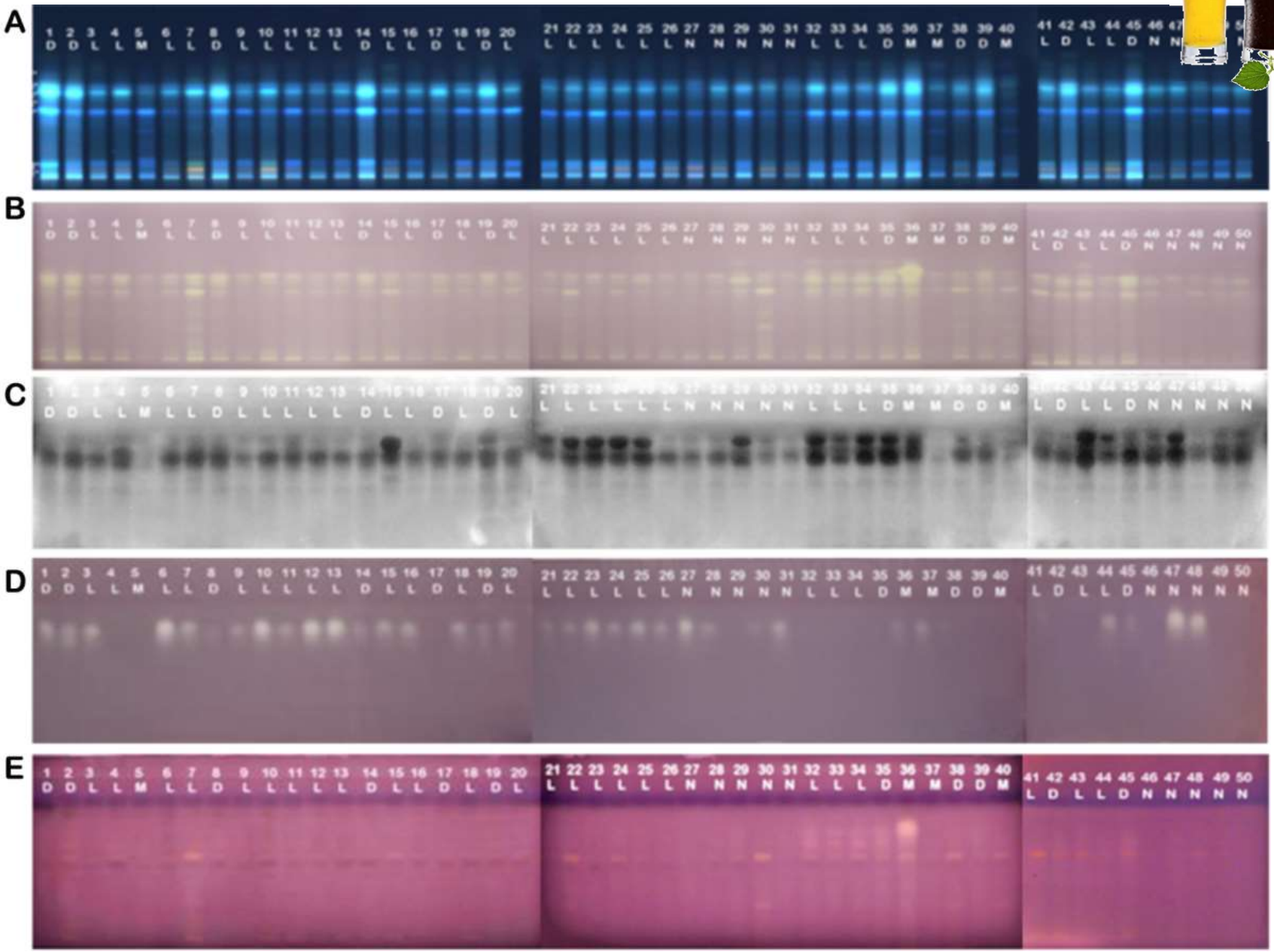
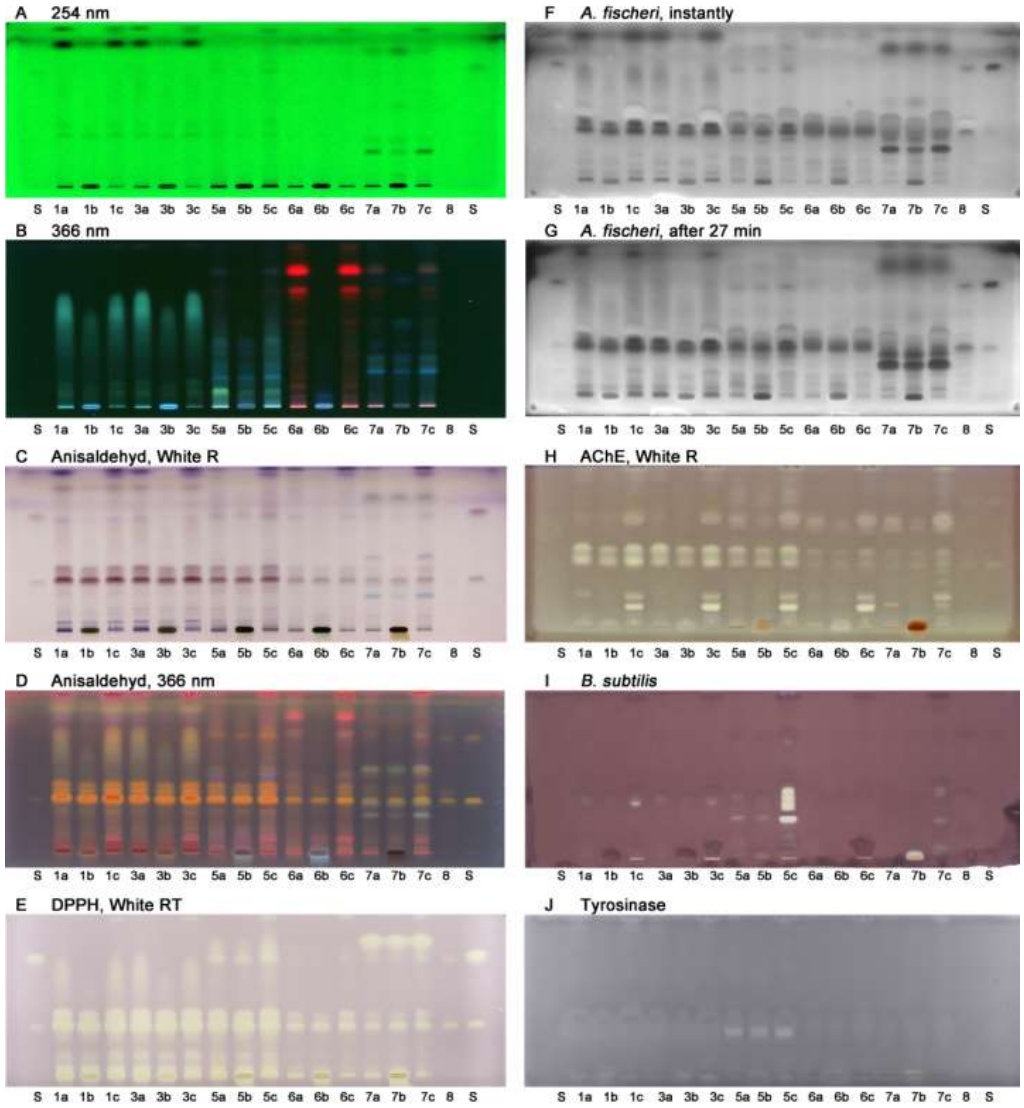
G. Morlock Food Science JLU Giessen

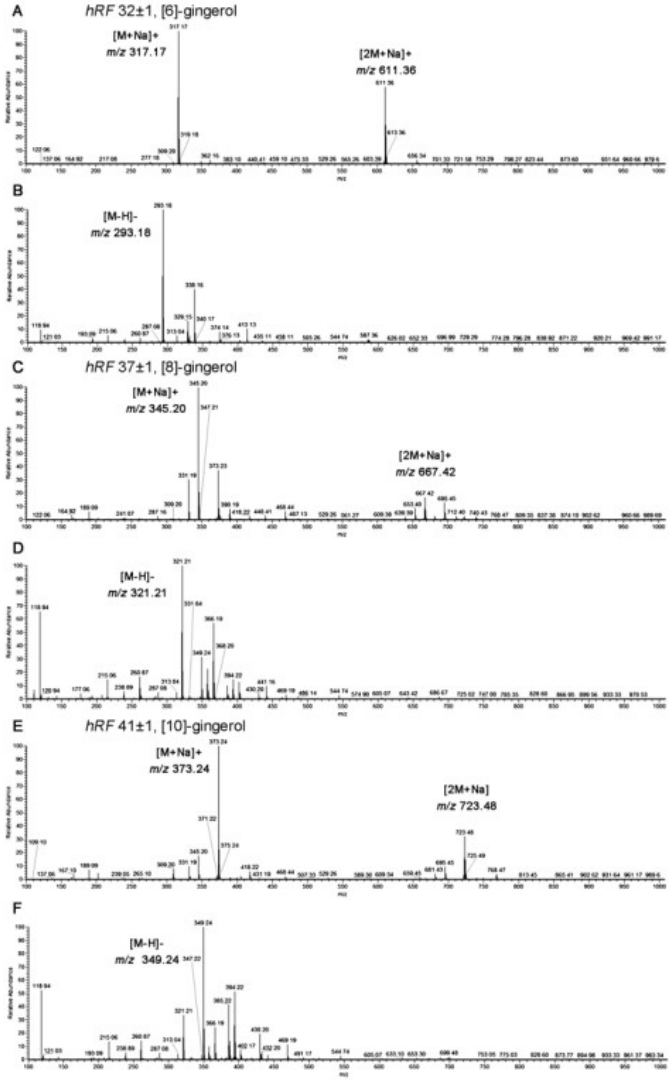
Screening → fingerprints/profiles of 50 beers



Still active after processing?

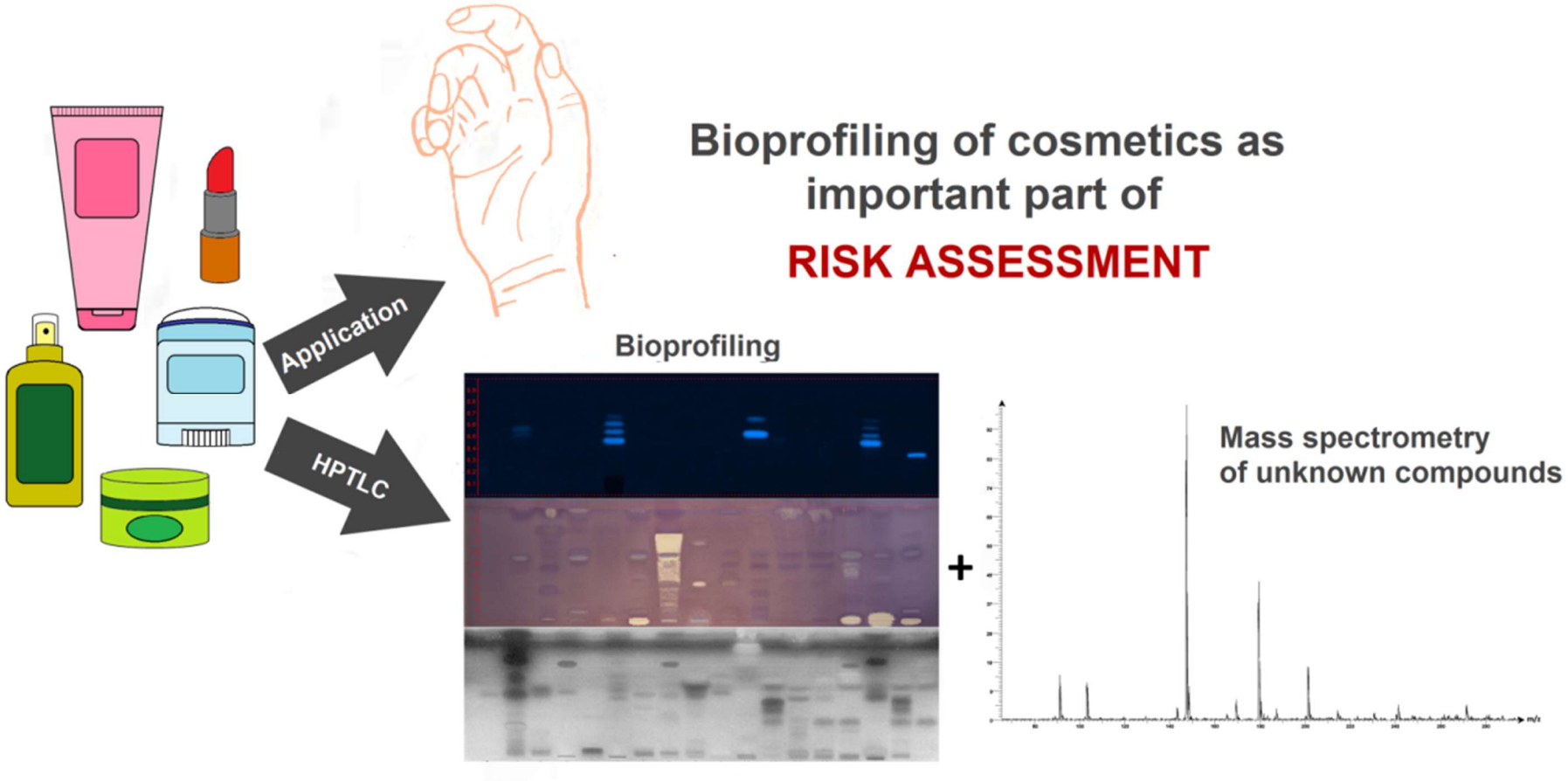
Ginger (*Zingiber officinale*) and its food products





S. Krüger, A. Bergin, G. Morlock, *Food Chem* 243 (2018) 258-268

Safe product?



C. Stiefel *et al*. *ACS Omega* 2 (2017) 5242−5250

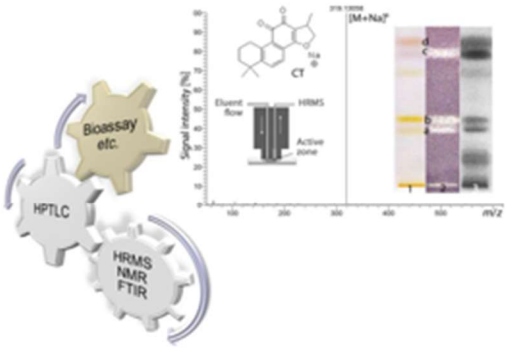
Chromatography + bioassay > 2

TransMIT *Der Partner, der neues Wissen schafft*

Modul
Effect-directed analysis by HPTLC-bioassay-HRMS

Prof. Dr. Gertrud Morlock

- Chromatography combined with assays
- Fast link to single bioactive compounds in complex samples
- Streamlined bioprofiling via biological and biochemical assays in the adsorbent bed
- High-performance thin-layer chromatography combined with effect-directed analysis and high resolution mass spectrometry (HPTLC-UV/Vis/FLD-EDA-HRMS)



FOOD SAFETY AUTHENTICITY RISK ASSESSMENT

PROGRAM 26.02. – 01.03.2020

09.00 Start
10.30 Coffee
12.30 Lunch
15.00 Coffee
17.00 End

Lectures: Prof. Dr. Gertrud Morlock

The 5-day practical course focuses on different assays. The full workflow HPTLC-UV/Vis/FLD-assay-ESI-HRMS or DART-MS is shown on each day.

WEDNESDAY

Gram-negative antimicrobials via *Aliivibrio fischeri* bioassay

THURSDAY

Gram-positive antimicrobials via *Bacillus subtilis* bioassay

FRIDAY

Hormone-effective compounds via planar yeast estrogen/androgen screen (pYES/pYAS)

Genotoxic compounds via SOS/umuC assay

SATURDAY

Enzym inhibitors via cholinesterase/tyrosinase assay

SUNDAY

Enzym inhibitors via α/ß-glucosidase/amylase assay

REGISTRATION

1. Email to gertrud.morlock@uni-giessen.de
2. Payment on receipt of invoice

RESPONSIBLE FOR MODULE

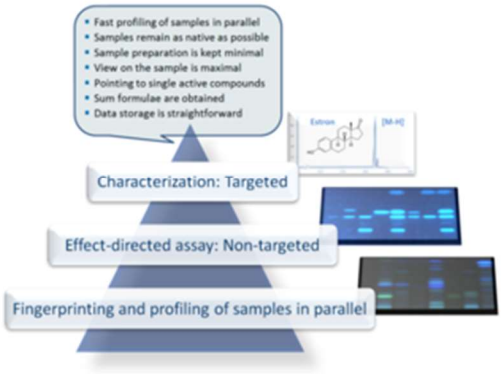
Justus Liebig University Giessen
Prof. Dr. Gertrud Morlock
Full Professor
Chair of Food Science

MODULE AIMS

The participants

- Understand the meaning of effect-directed analysis as well as advantages and disadvantages of the different techniques
- Survey the variety of *in situ* assays (in the adsorbent bed)
- Experience fast effect-directed profilings (3-20 min/sample for 20 samples in parallel)
- Recognize the highly efficient combination of planar chromatography with biological and biochemical or other effect-directed assays
- Realize the power of hyphenated HPTLC
- Know the streamlined workflow on one plate, *i. e.* parallel separation of compounds in complex samples, discovery of active compounds and their characterization by chromatographic, spectroscopic and spectrometric information

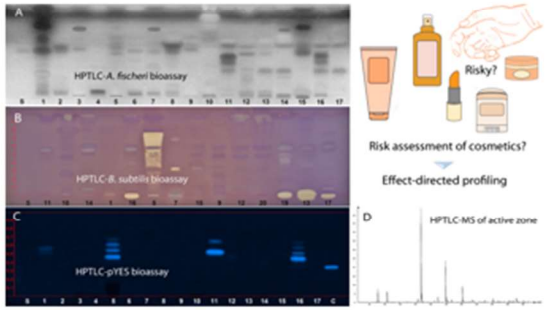
G. Morlock Food Science JLU Giessen



"...we know there are **known knowns**.
These are things we know that we know.
We also know that there are **known unknowns**.
That is to say, there are things
that we know we do not know.
But there are also **unknown unknowns** –
the ones we do not know we do not know."

Donald Rumsfeld, 2002

Let us find it out!



Source: Morlock, G.: Bioassays and further effect-directed detections in chromatography, in Worsfold P.J., Poole, C., Townshend, A., Miro, M. (Eds.): Reference Module in Encyclopedia of Analytical Science, 3rd edn. With permission from Elsevier Science, Amsterdam, 2019

FEE

500 € per day or 1500 € for 5 days

Included in fee:
- Course material on USB stick
- Lunch and coffee breaks
- Certificate on request

LOCATION

Justus Liebig University Giessen
Interdisziplinary Research Center (IFZ)
Department of Food Science
Heinrich-Buff-Ring 26-32
35392 Giessen
Germany
Tel. +49 641 99 391 41
www.uni-giessen.de/food

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN
Food Science

ROUTE TO US

At IFZ, take the red entrance door at Area A.
Go to Area D, 1. floor, Room B 117 (next to elevator)

HOTELS NEAR BY

The participant is responsible for self-accomodation.

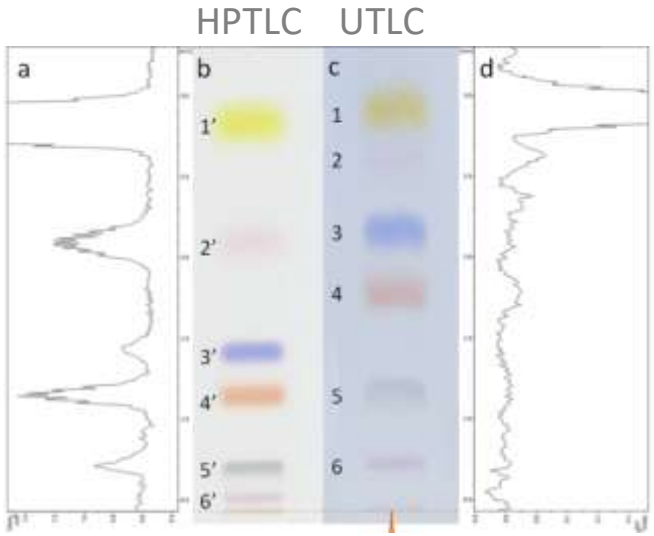
- Hotel Heyligenstaedt, Aulweg 41, 35392 Giessen
  Tel. +49 641 4609650
  info@hotel-heyligenstaedt.de
  www.restaurant-heyligenstaedt.de
- Giessener Bett, Westanlage 5, 35390 Gießen
- Adapt Apartments Giessen, Henriette-Hezel-Straße, 20, 35398 Gießen
- Appartement am Schloss, Landgraf-Philipp-Platz 9, 35390 Gießen
- Gästehaus Wilhelma, Wilhelmstr. 3, 35392 Giessen, Tel. +49 641 79 26 65
  info@gaestehaus-wilhelma.de
  www.gaestehaus-wilhelma.de
- Tourist Information Giessen
  Tel. +49 641 306 18 90, tourist@giessen.de
  www.giessen-tourismus.de

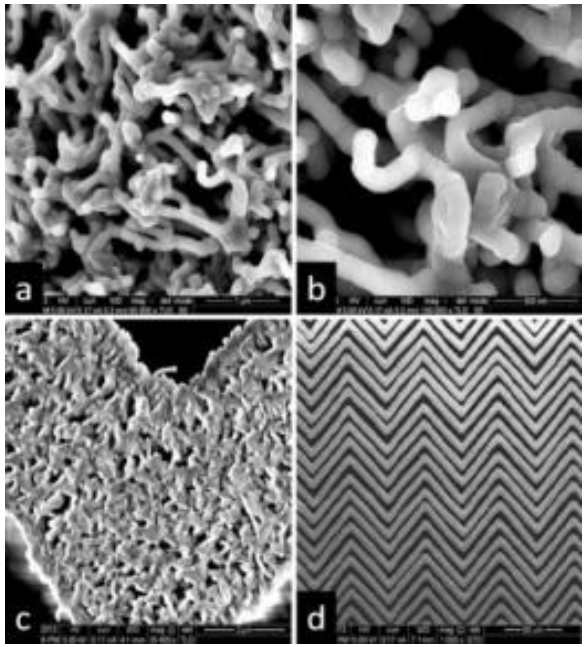
Visit the working place of Justus Liebig:

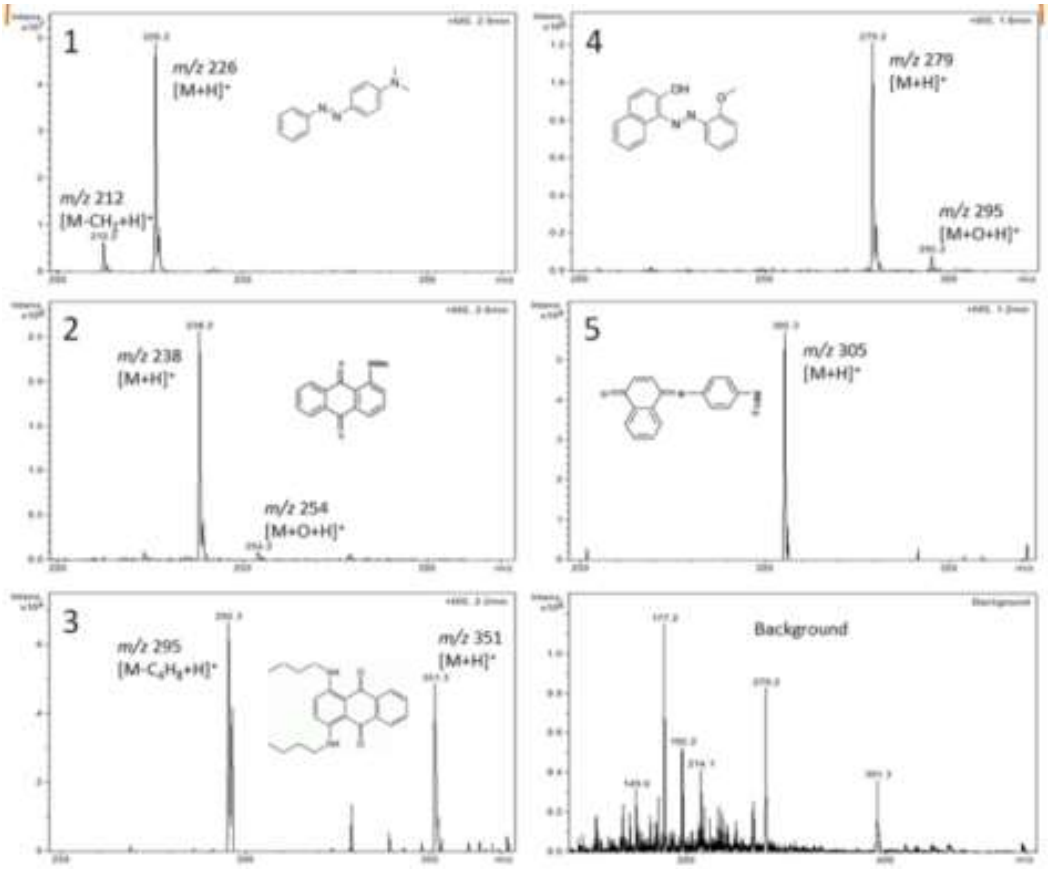
Source: wikipedia.org

JLU

Detection by ESI-MS

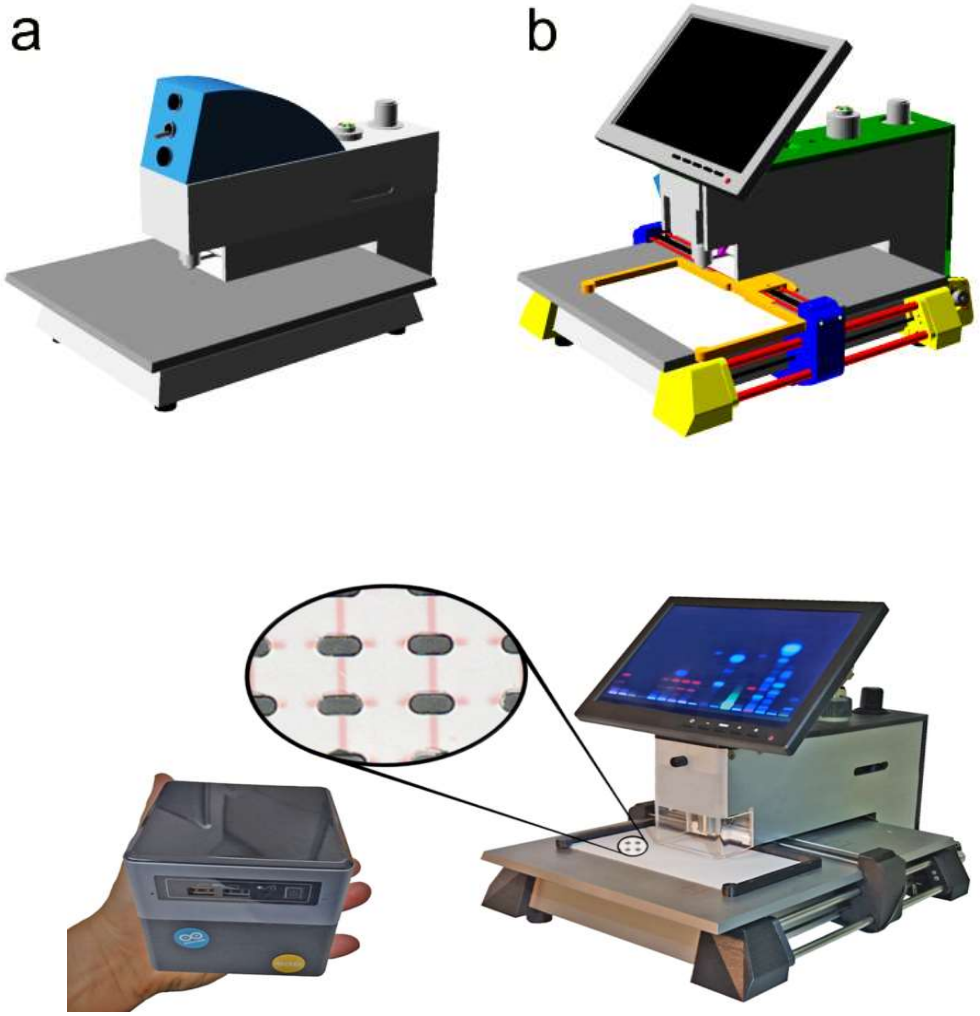






S. Kanyal *et al. J. Chromatogr. A* 1404 (2015) 115-123

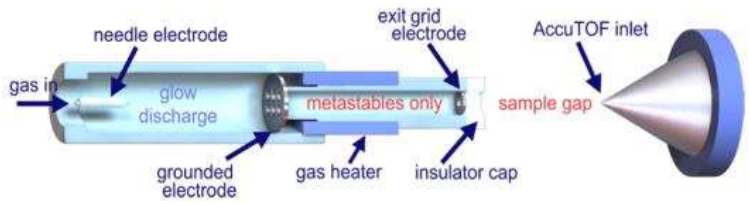
Quantitative automated elution-based HPTLC-MS



T. Häbe, G.E. Morlock, in submission

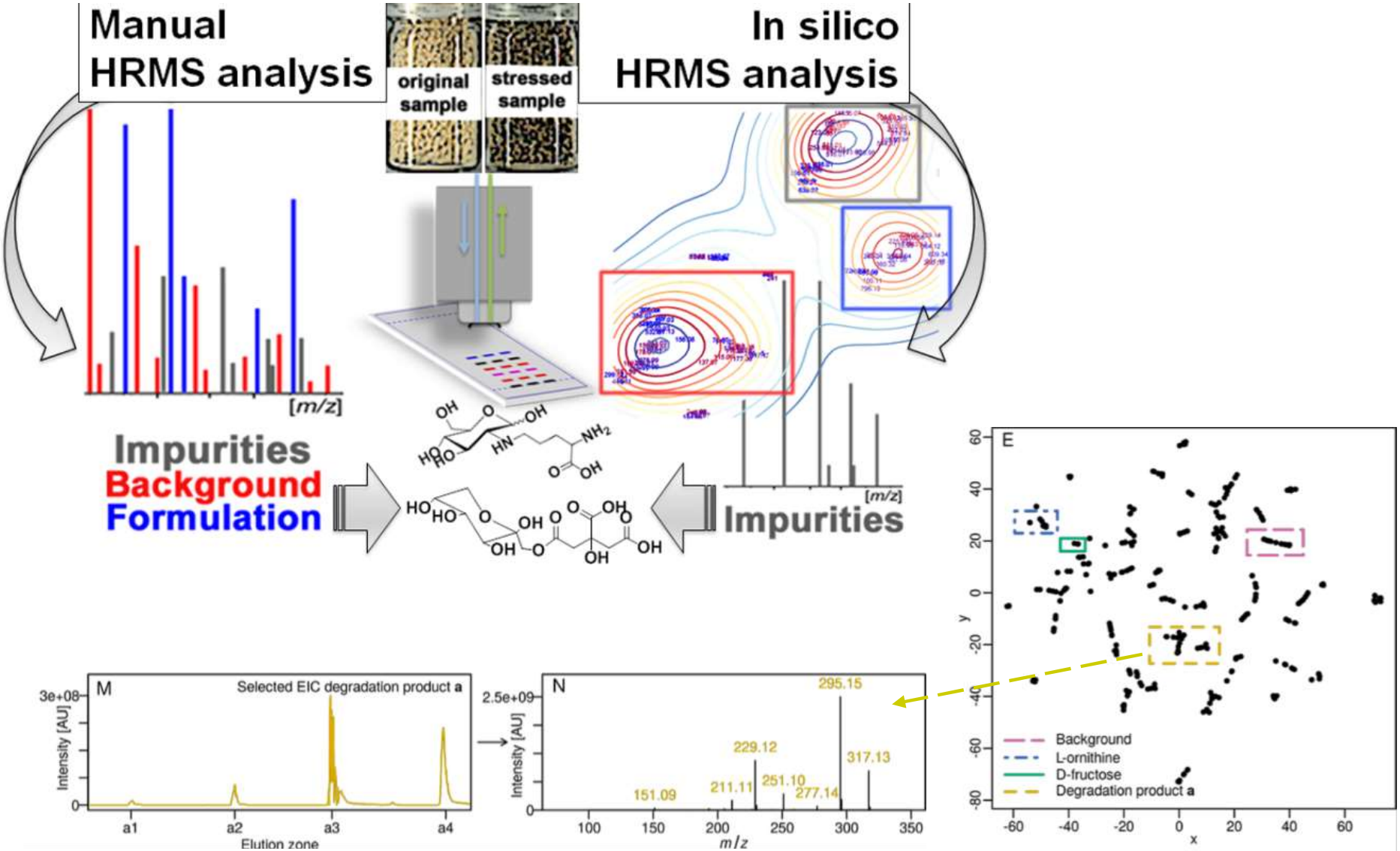
…desorption-based HPTLC-MS

DART ion source



T. Häbe, G.E. Morlock in preparation

Data analysis: open-source eicCluster software

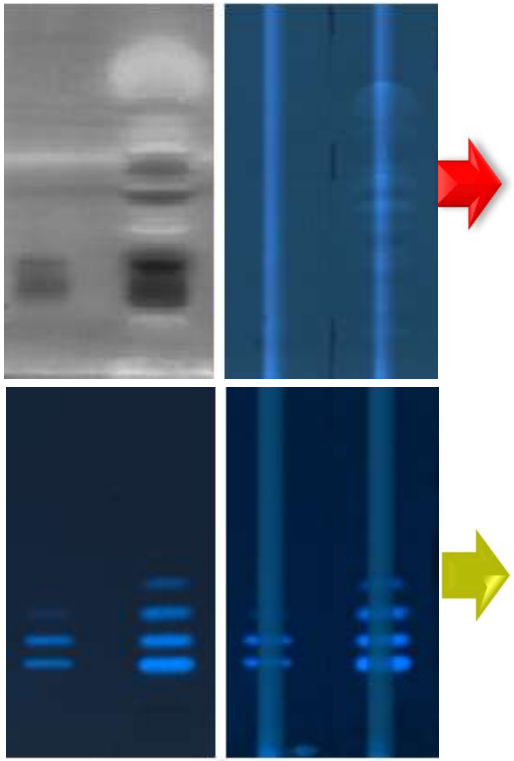


D. Fichou *et al*. *J. Chromatogr. A* 1577 (2018) 101–108

Quantitative HPTLC-EDA-DART-MS

Parabens?

Bioactives?



Reliable quantification

→ Mean r^2 = 0.9992

→ Mean deviation: 4.6% (*A. fischeri vs.* pYES)

→ Method comparison

		Amount in sample [mg/100g]						
		Sample 1			Sample 2			
		ME	EE	PE	ME	EE	PE	BE
without BioAssay	NP	103	56	30	165	75	37	65
	RP	97	59	34	147	69	30	67
A. fischeri	NP	96	51	27	173	69	24	53
	RP	101	51	27	157	59	27	59
pYES	RP	111	53	31	170	60	26	62

T. Häbe, M. Jamshidi-Aidji, J. Macho, G. Morlock, *J. Chromatogr. A* 1568 (2018) 188–196

HPTLC in the age of ultra-rapid separations

Rapid HPLC is like…

http://ams.bg/images

HPTLC is like…

http://images.google.de

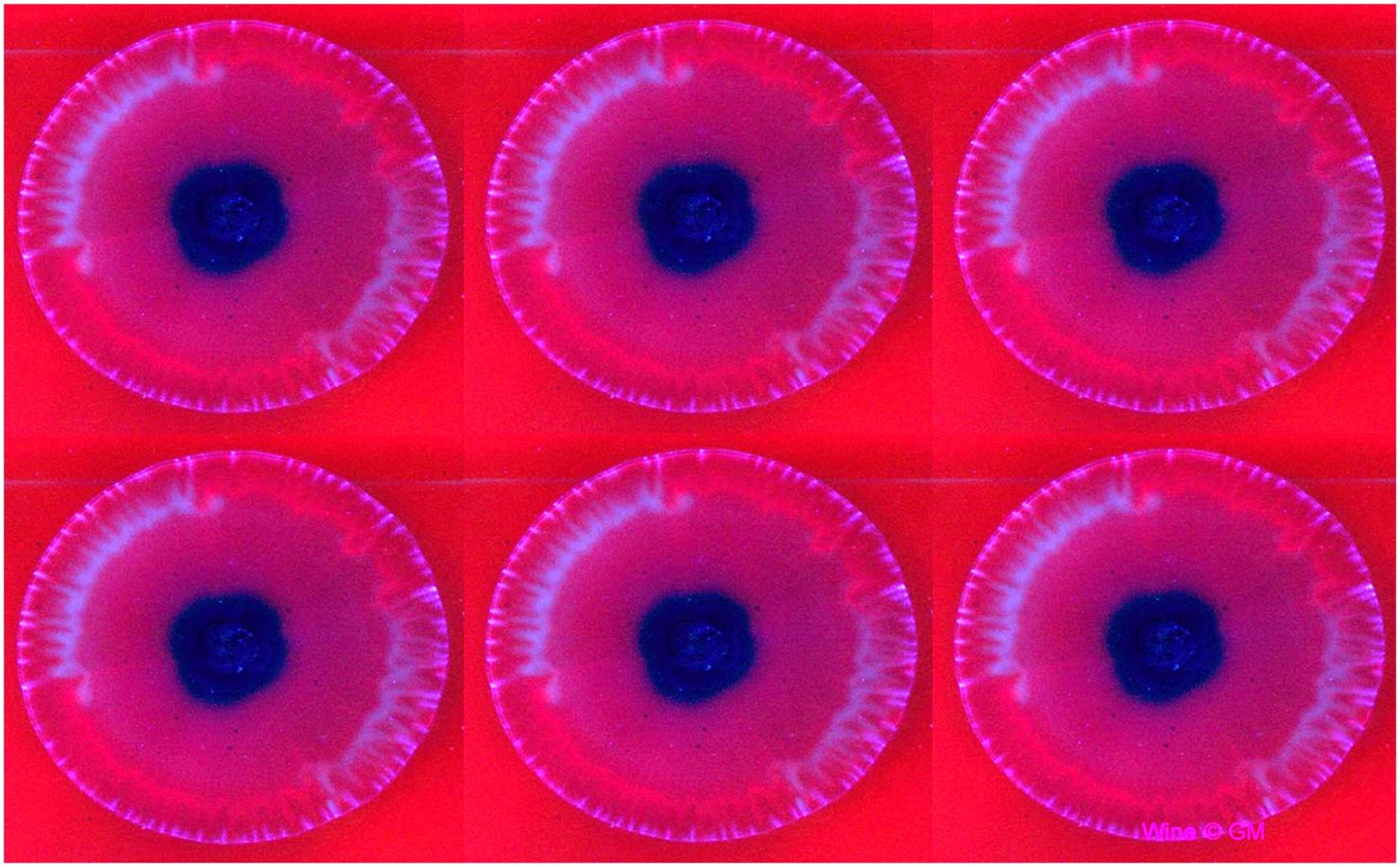
- **Nice feeling?** - But only for 1 or 2 - Convenient feeling for many.
- **Fast?** - What about in the city? - You are faster in the city.
- **High costs?** - Who pays for the high budget? - Saving costs makes sense.
- **High image?** - Unclear for what purpose… - Definitely not - but rational!

What is the ideal transportation system?

⇒ It makes sense not to drive always a Porsche.

⇒ Taking the metro might be a good idea!

If science does not work out, make art!



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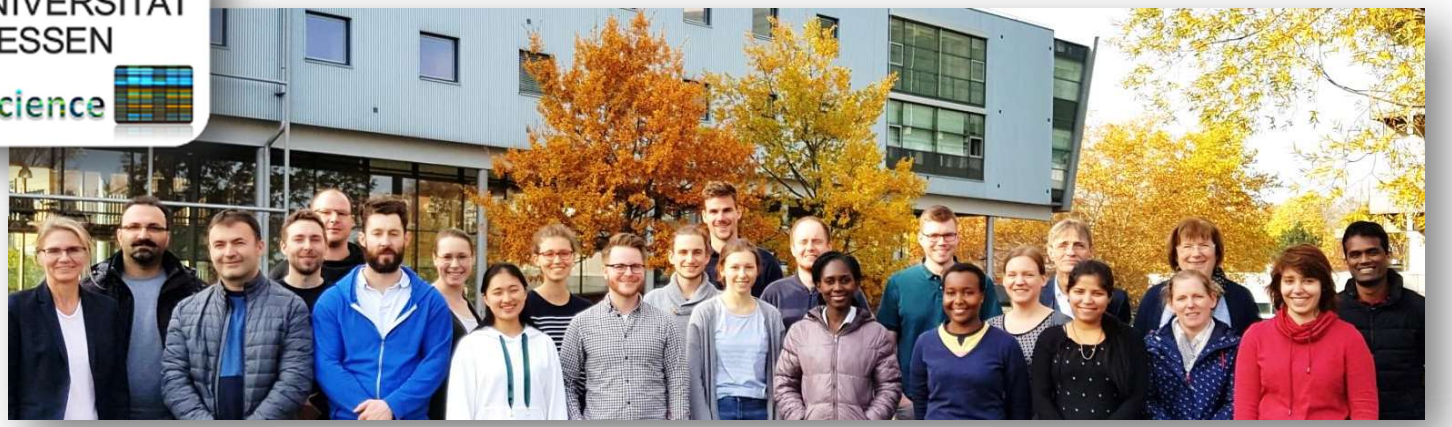

TransMIT

Thank you!

CAMAG

MilliporeSigma

WALA

DFG Deutsche Forschungsgemeinschaft

Nestlé

MERCK

Sanitätsdienst

DAAD

ADISSEO

PiLeJe

Lenzing Innovative by nature

STADA