

Novel analytical technologies for metabolomics: more for less

X-omics festival, 28th of September 2020

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Analytical Biosciences and Metabolomics group

Mission: We accelerate the application of metabolomics in Biomedical research, Clinical application for personalized health, and Precision medicine.

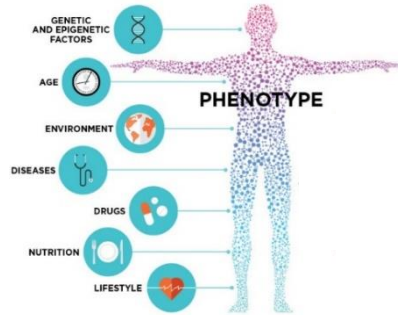
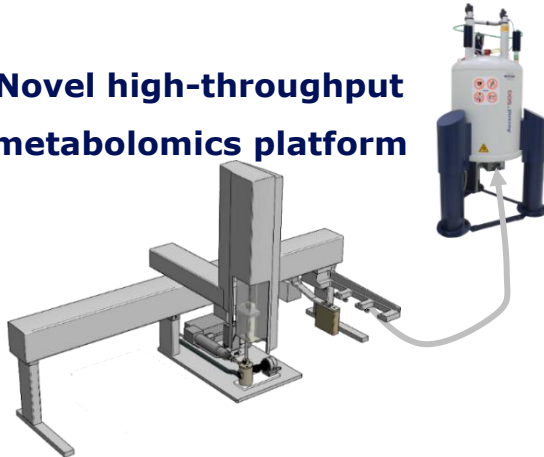


PI: Prof. Thomas Hankemeier

Large-scale studies



Novel high-throughput
metabolomics platform



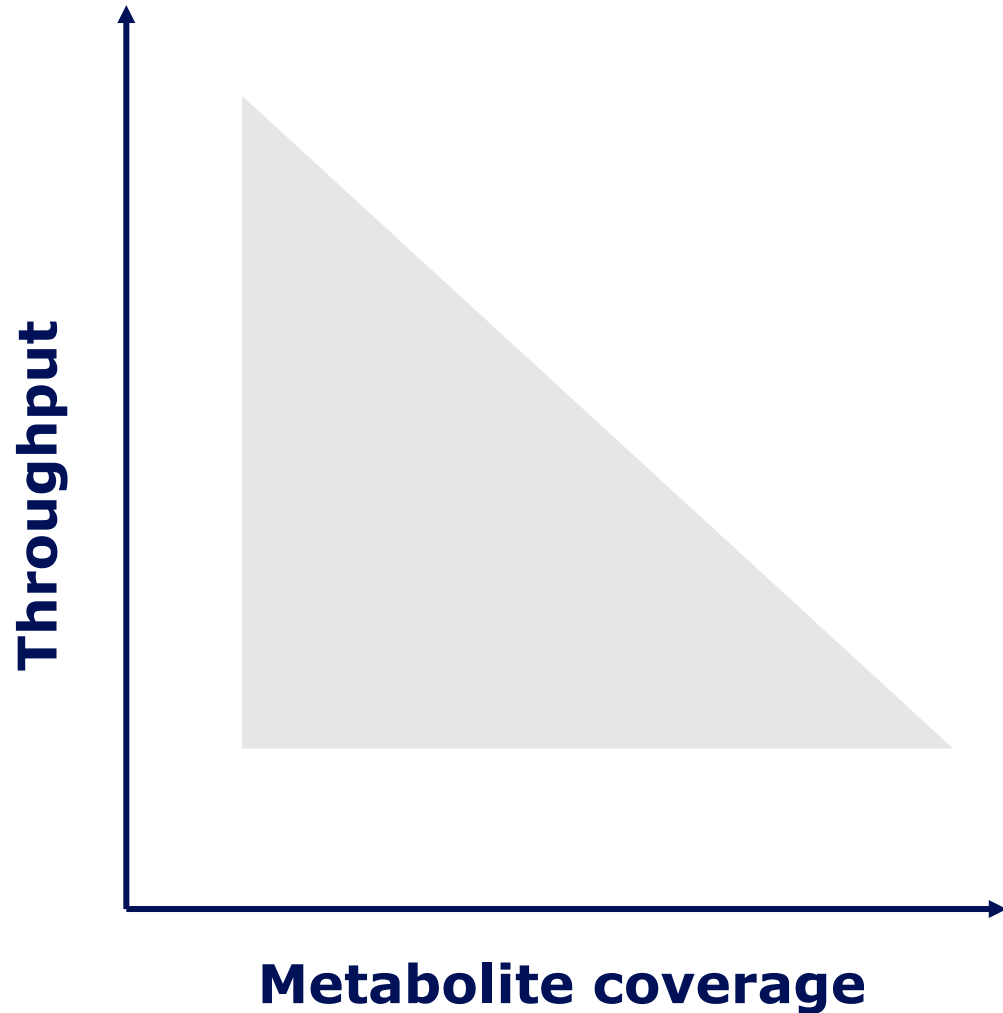
Metabolic profiling



Building automated **X-omics** Infrastructure for 2021

*High-throughput analytical technology enable breakthroughs in **life sciences and beyond***

Sample-handling platform for high-throughput flow-NMR



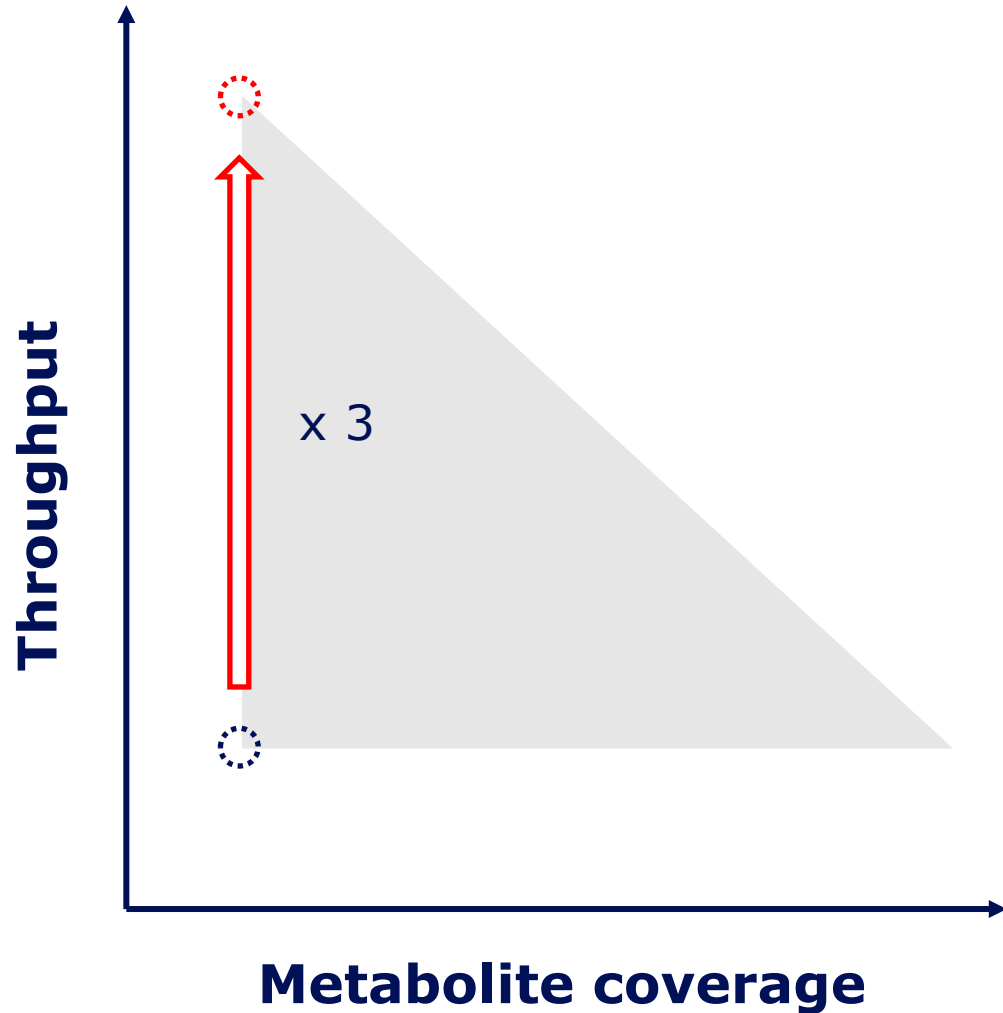
Advantages of NMR:

- Quantitative analysis
- Structural elucidation
- Robust, mature technology

Bottlenecks of NMR:

- Larger sample volume required (*e.g.* 0.5 mL)
- Limited metabolite coverage
- Lower sensitivity (μM concentration)

Sample-handling platform for high-throughput flow-NMR



Advantages of NMR:

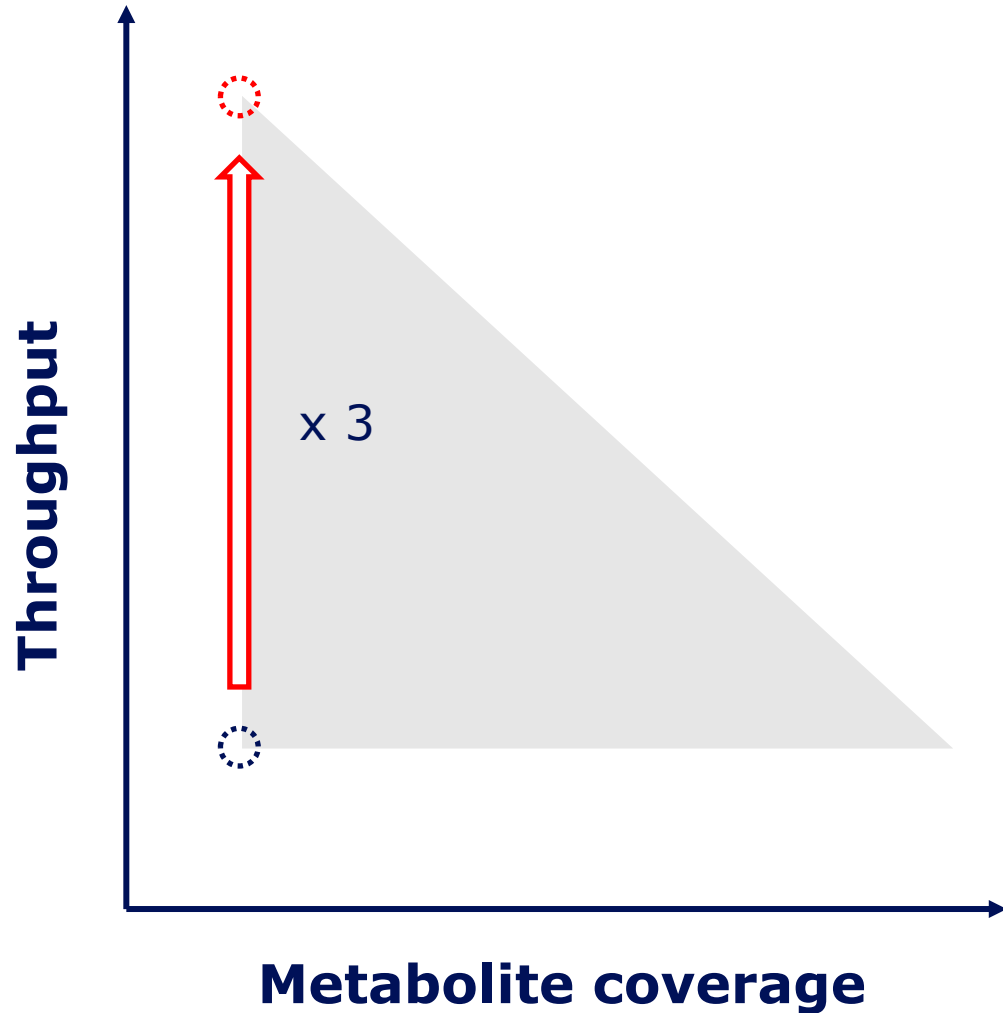
- Quantitative analysis
- Structural elucidation
- Robust, mature technology

Bottlenecks of NMR:

- 200-500 samples/day

Goal of our platform: 3-fold increased throughput

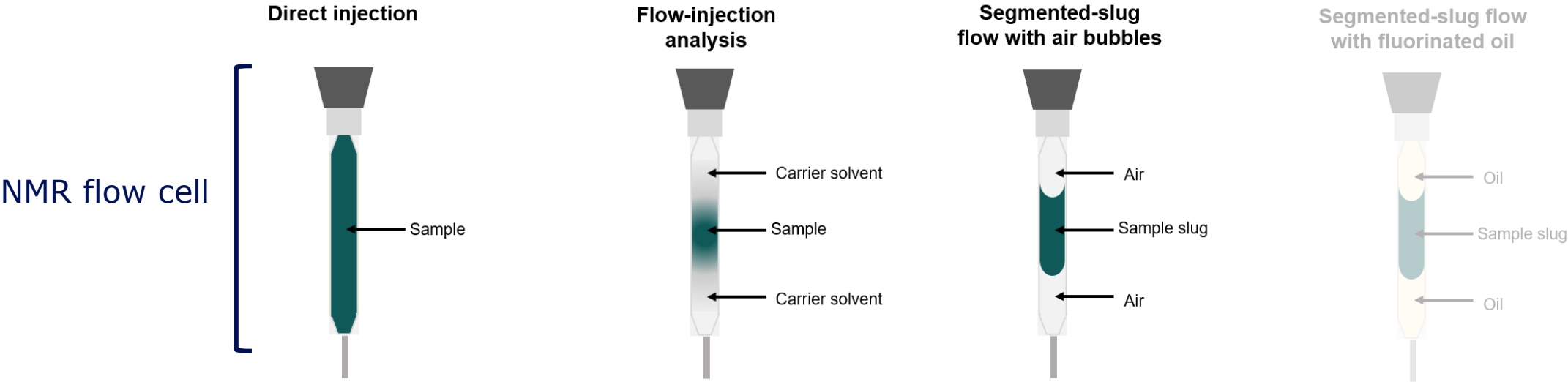
Sample-handling platform for high-throughput flow-NMR



High-throughput screening ^1H -NMR:

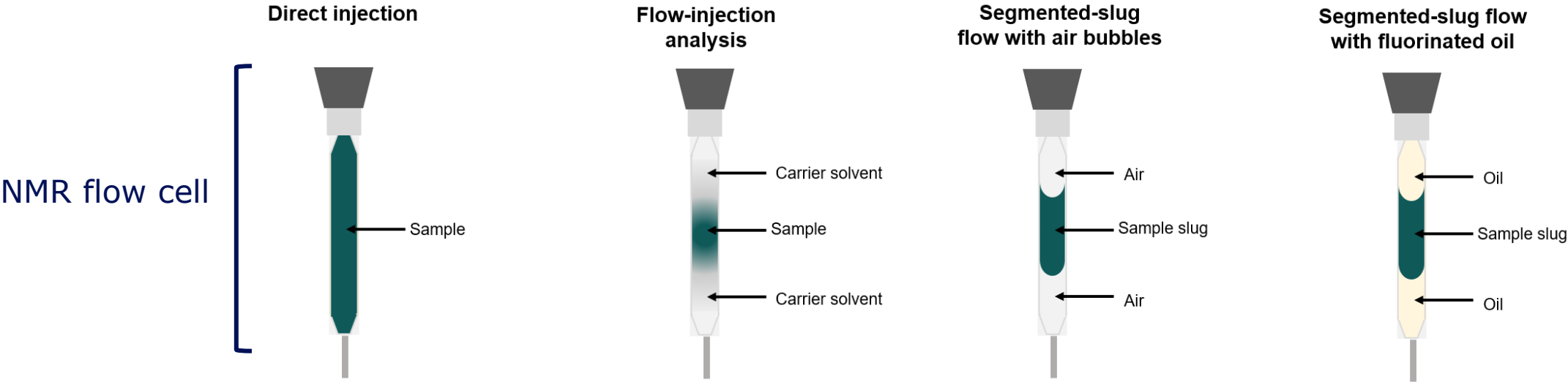
- Typically glass sample tubes, **short analysis time** (seconds to minutes).
- **Time-consuming steps** include sample exchange, temperature equilibration, and shimming.
- Alternative to glass tubes: **flow-NMR** in combination with liquid-handling robot.

Comparison of different flow-NMR techniques



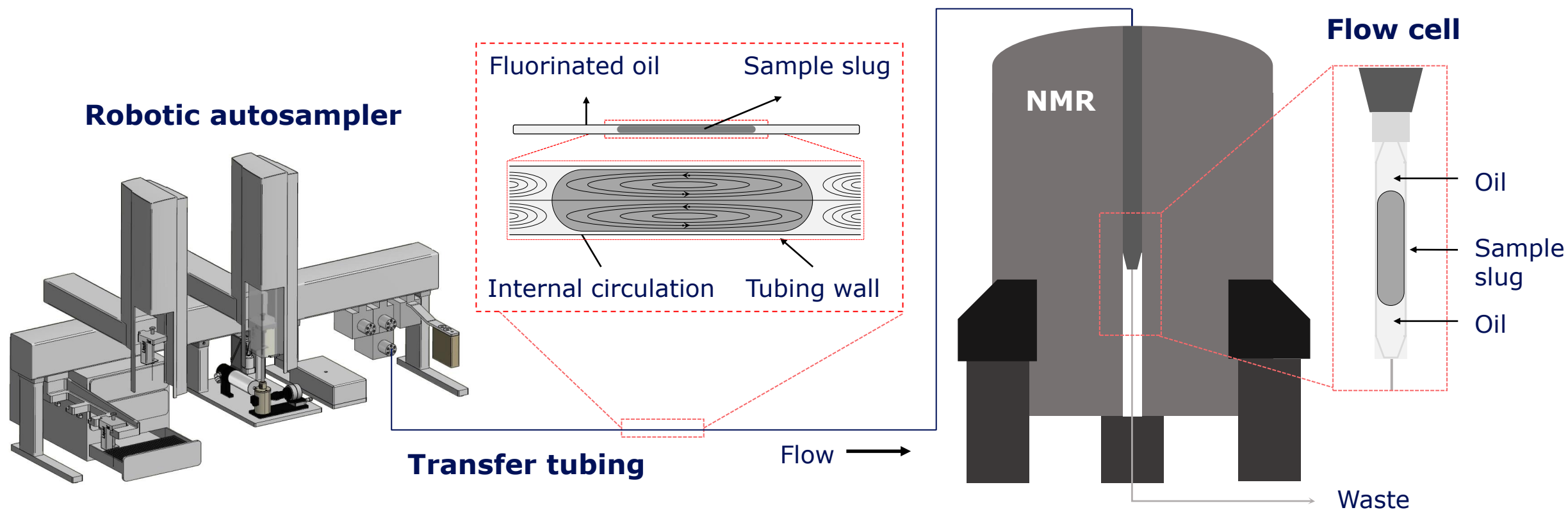
Compared to mechanical tube-based sample changers	Direct injection	Flow-injection analysis	Segmented flow with air
Sample consumption	-	+	+
Throughput	-	+	+
Carry-over	-	-	-
Sensitivity	0	--	-

Comparison of different flow-NMR techniques

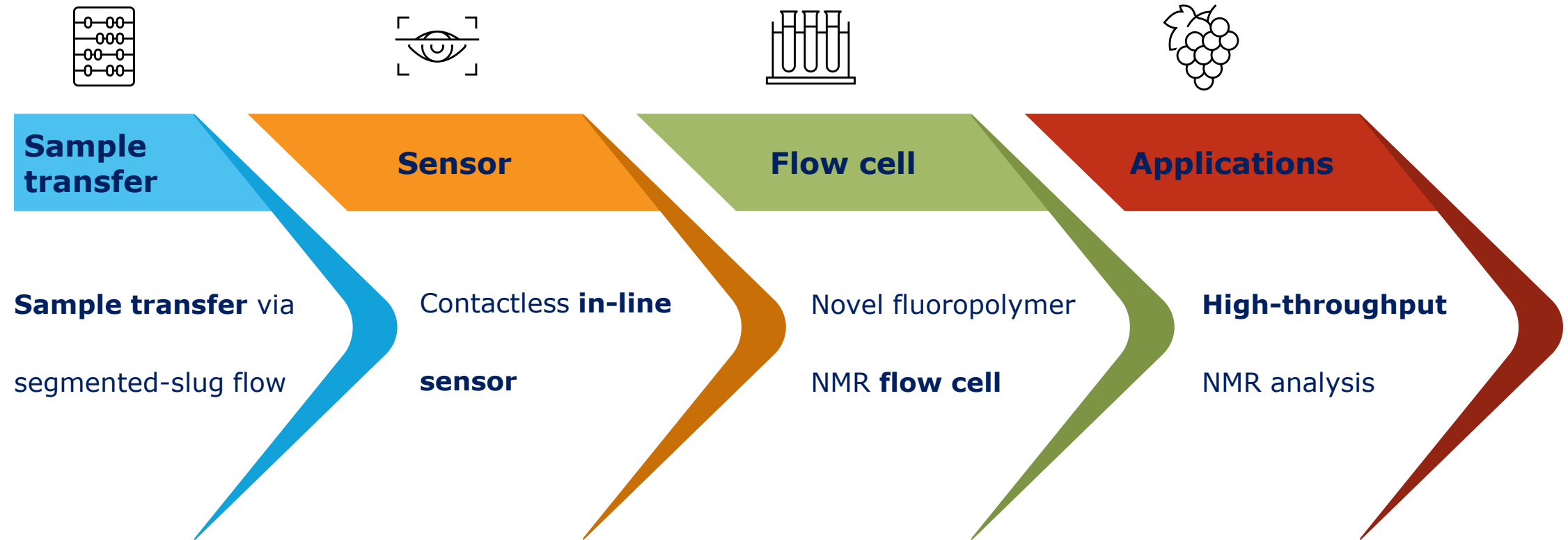


Compared to mechanical tube-based sample changers	Direct injection	Flow-injection analysis	Segmented flow with air	Segmented flow with oil
Sample consumption	-	+	+	+
Throughput	-	+	+	+
Carry-over	-	-	-	0
Sensitivity	0	--	-	0

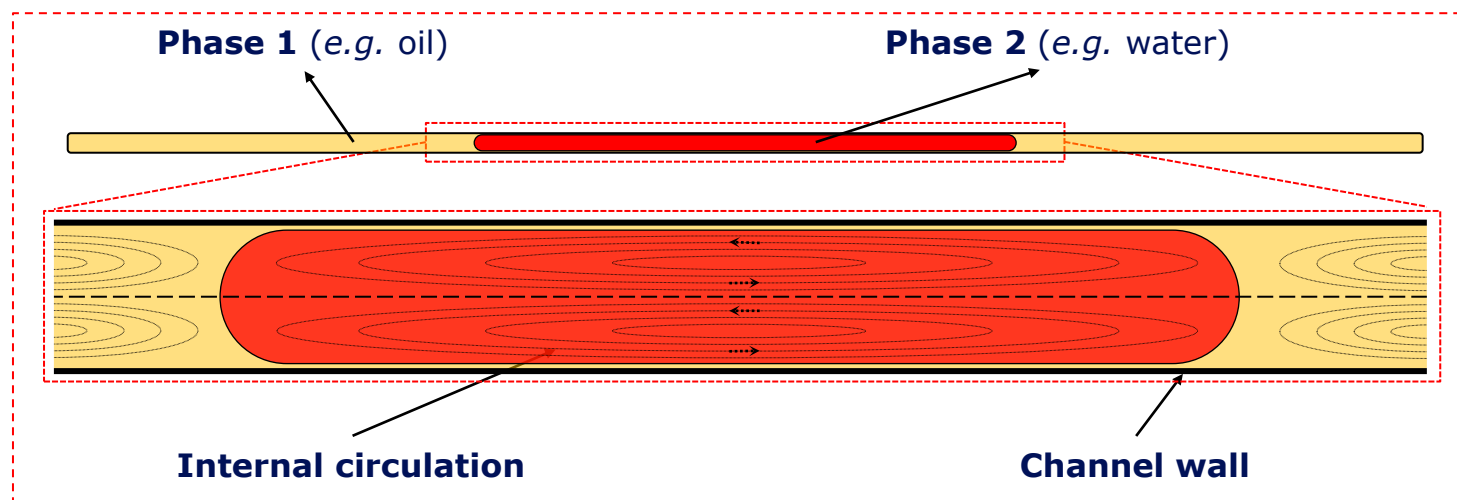
Sample-handling platform for high-throughput flow-NMR



Sample-handling platform for high-throughput flow-NMR



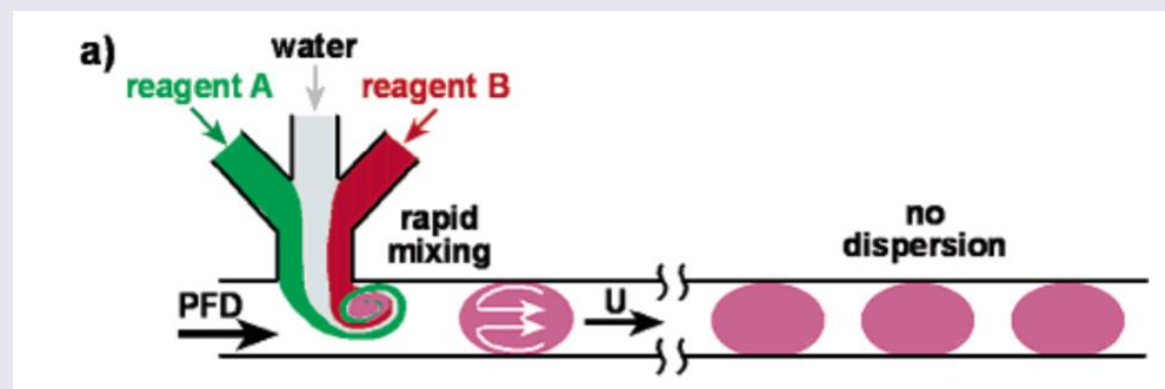
Fluid mechanics of segmented-slug flow



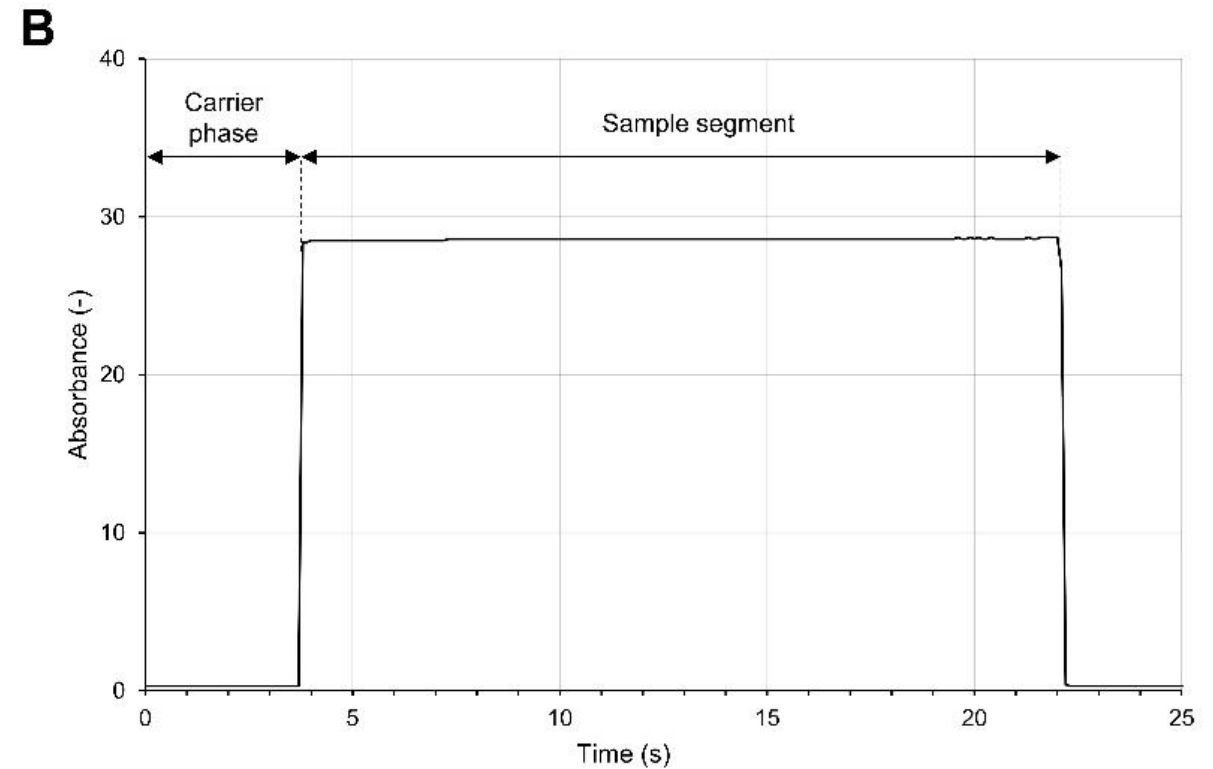
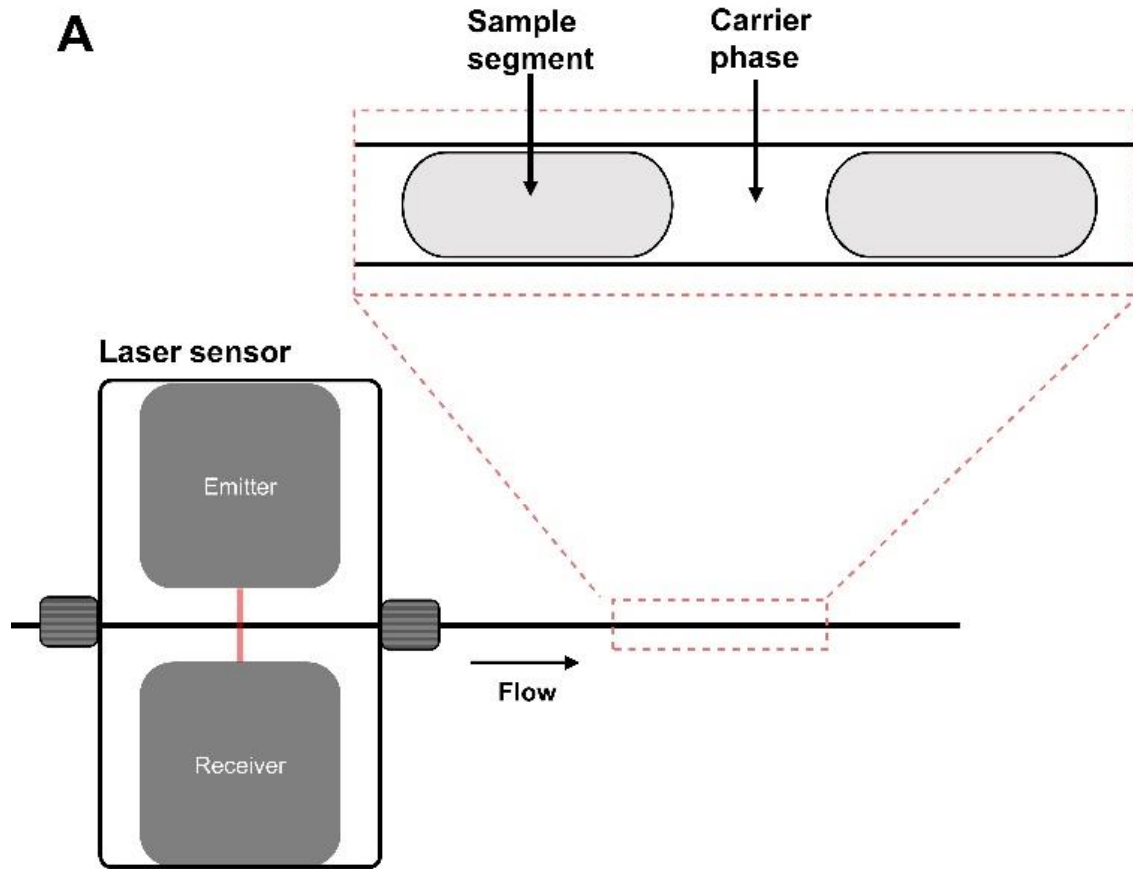
4 forces:

- Viscous forces
- Gravitational forces
- Inertial forces
- Interfacial forces

- Immiscible phases
- Slug or droplet = discrete “micro” reactor
- Can be used for sample transfer, sample storage, mixing, derivatization, high-throughput analysis.

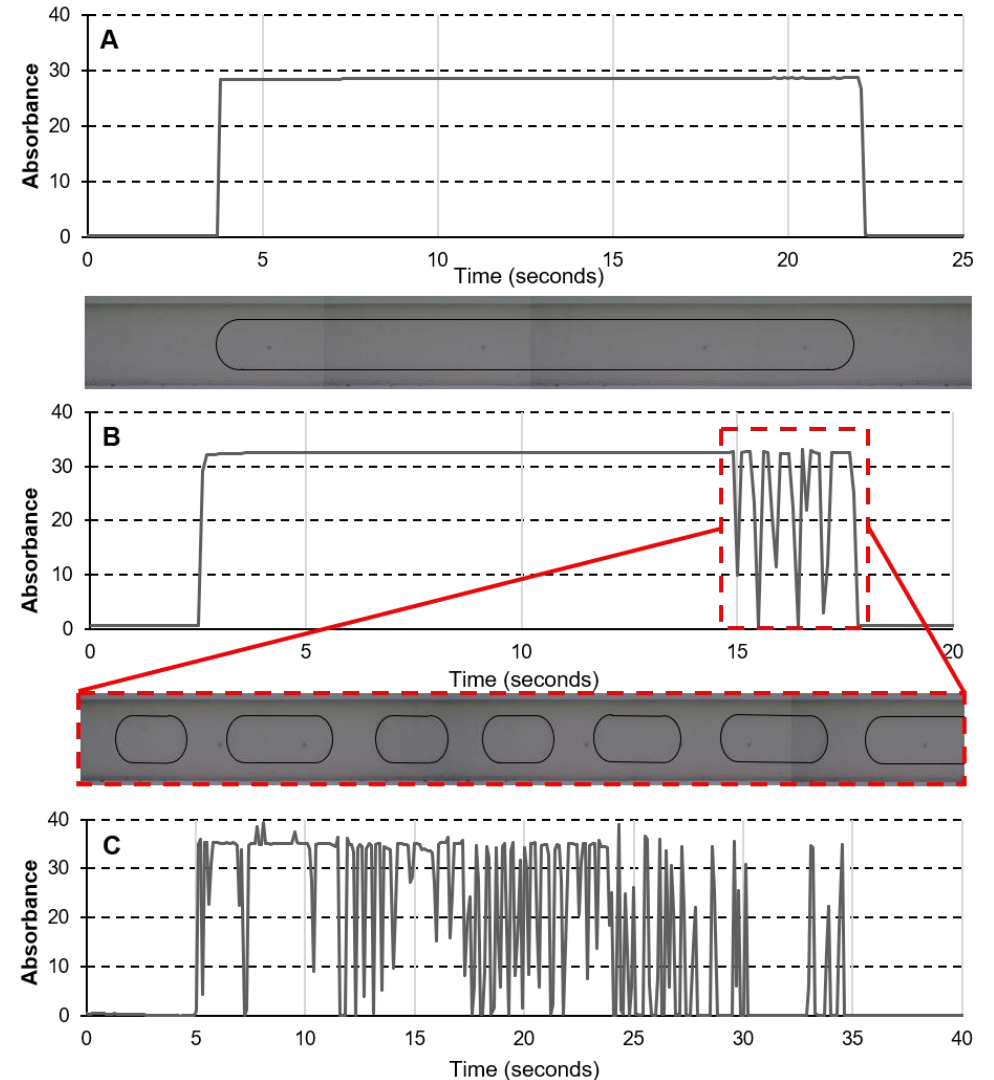


Non-intrusive laser sensor for sample characterization

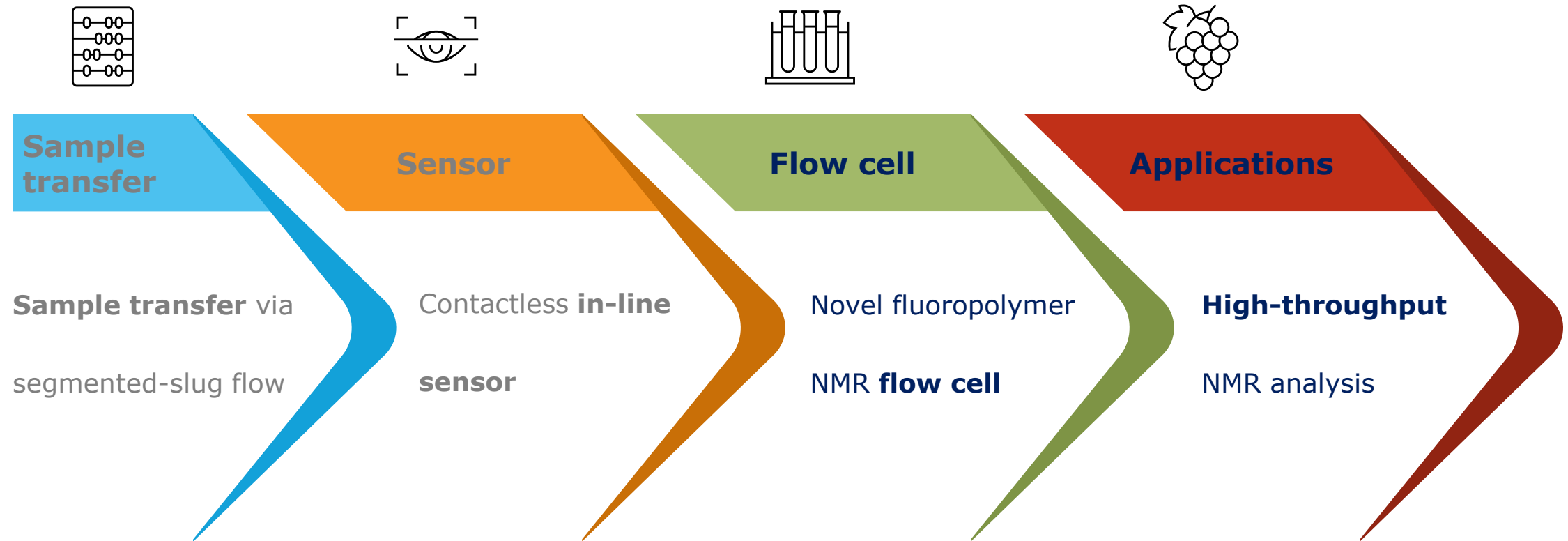


Application: Intact, frothing, or fragmenting slugs

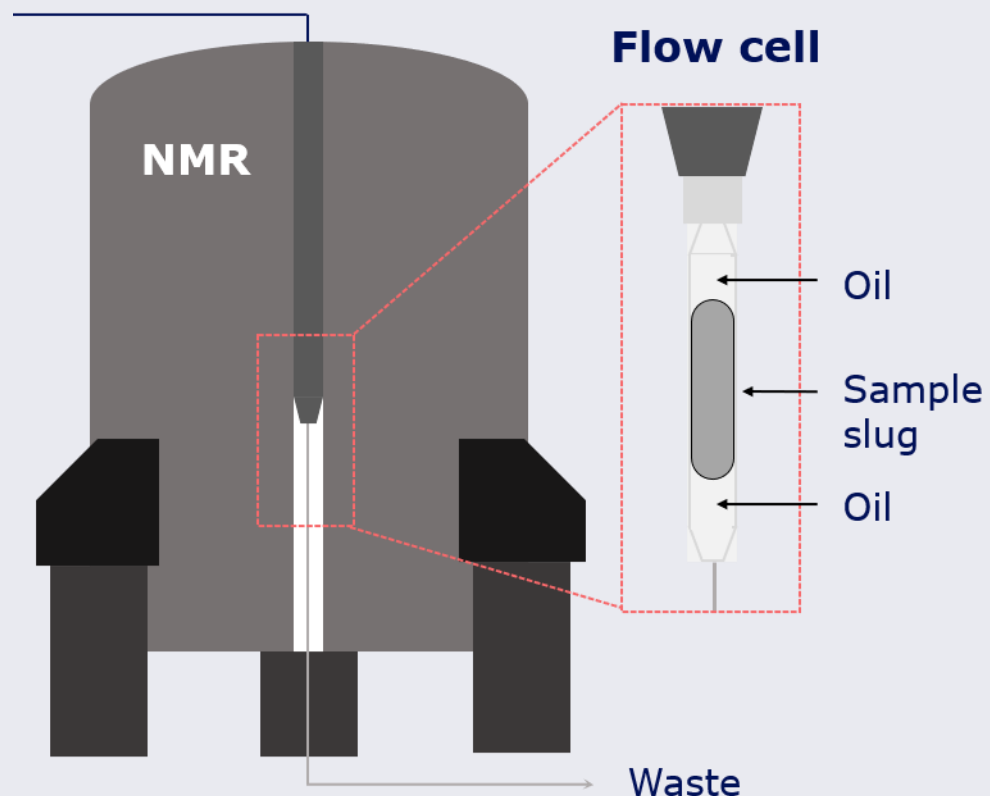
- **Intact slug (A)**
 - Best scenario with maintained slug shape and minimal sample-to-sample carry-over
- **Frothing slug (B)**
 - Fragmentation at the end of a slug and higher risk of sample-to-sample carry-over
- **Fragmenting slug (C)**
 - Whole slug is fragmented, not suitable for NMR measurements



Sample-handling platform for high-throughput flow-NMR



Re-designing the NMR flow cell



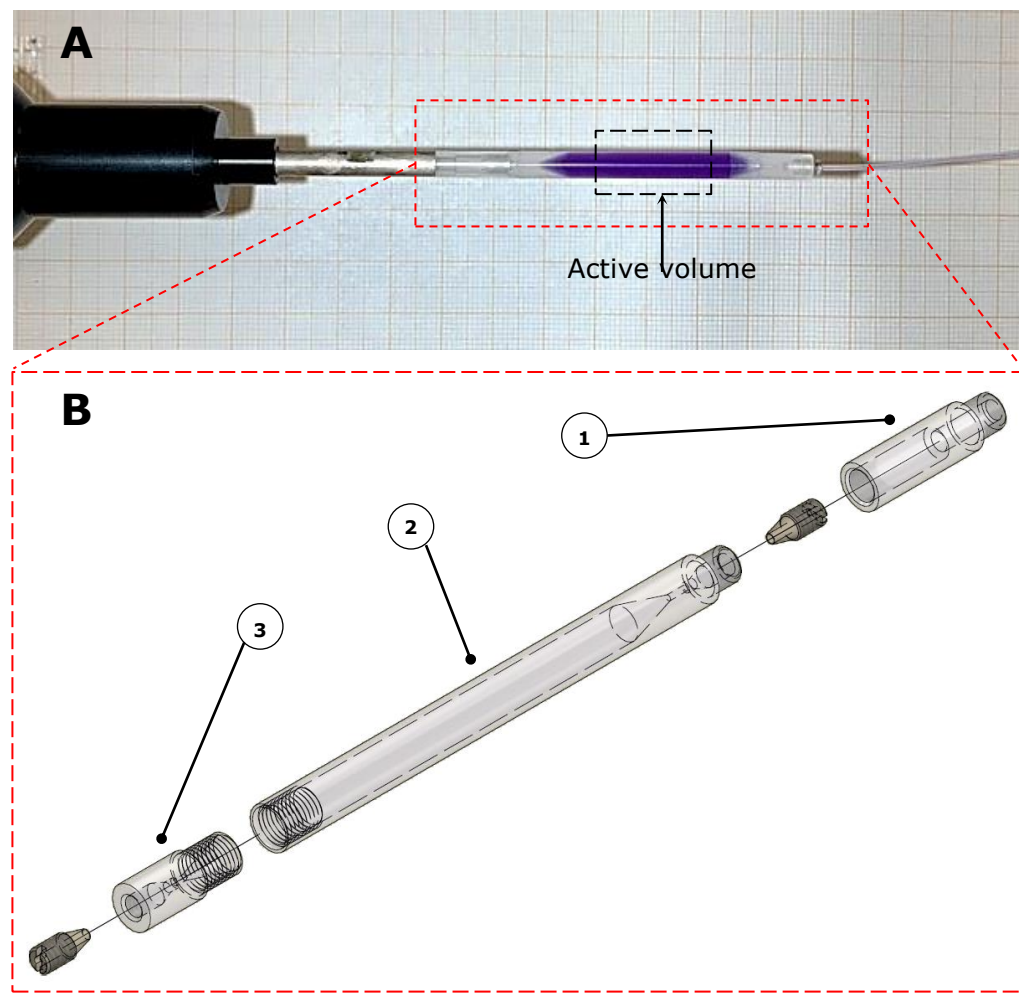
1. Fragile and expensive glass flow cells

2. Limitations of glass material:

- No preferential wetting to the sample.
Temporary fluorosilane coating could wear off, wash step between samples still necessary;

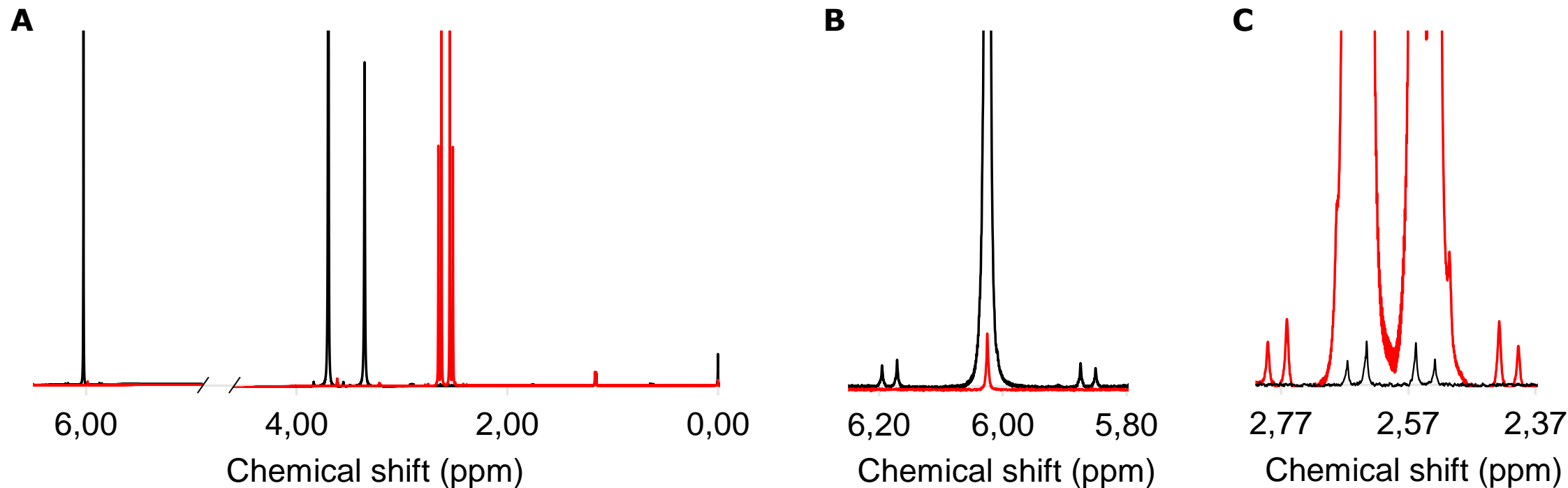
3. Poor compression-style connections risk leak inside the NMR

Novel fluoropolymer NMR flow cells



- Polychlorotrifluoroethylene (PCTFE) material:
 - Fluorinated oil forms a film around the sample;
 - **Reduced carry-over**, no need for intermediate wash step
- Plug-and-play with Bruker CryoFit accessory, **no adjustments.**
- Segmented-slug flow enables **stop-flow analysis**
- High-pressure resistant connections enable fast **sub-30 s** transfer time.
- Fixed flow cell reduces **shimming** time.

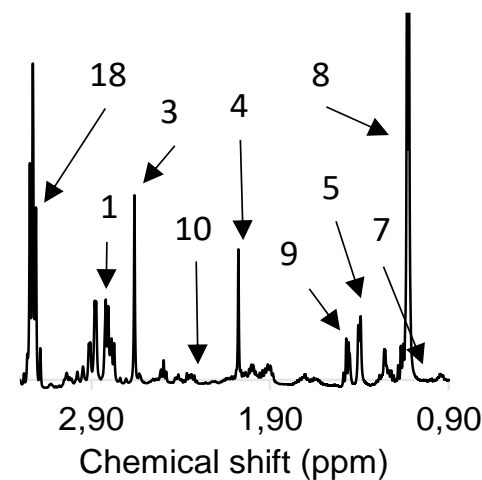
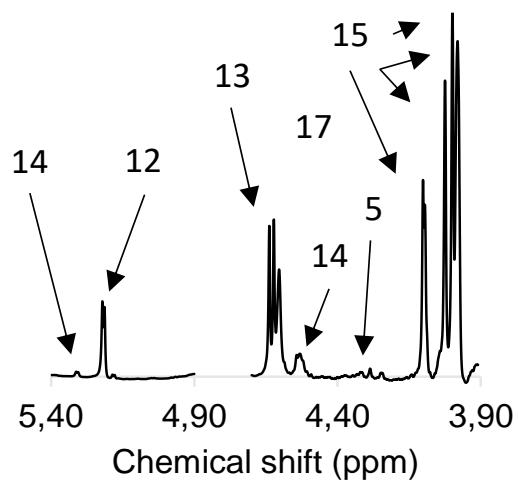
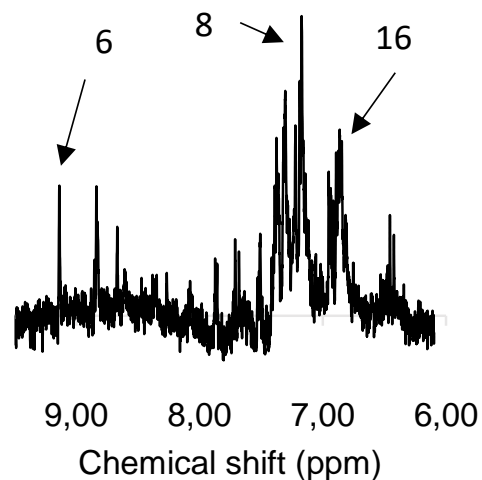
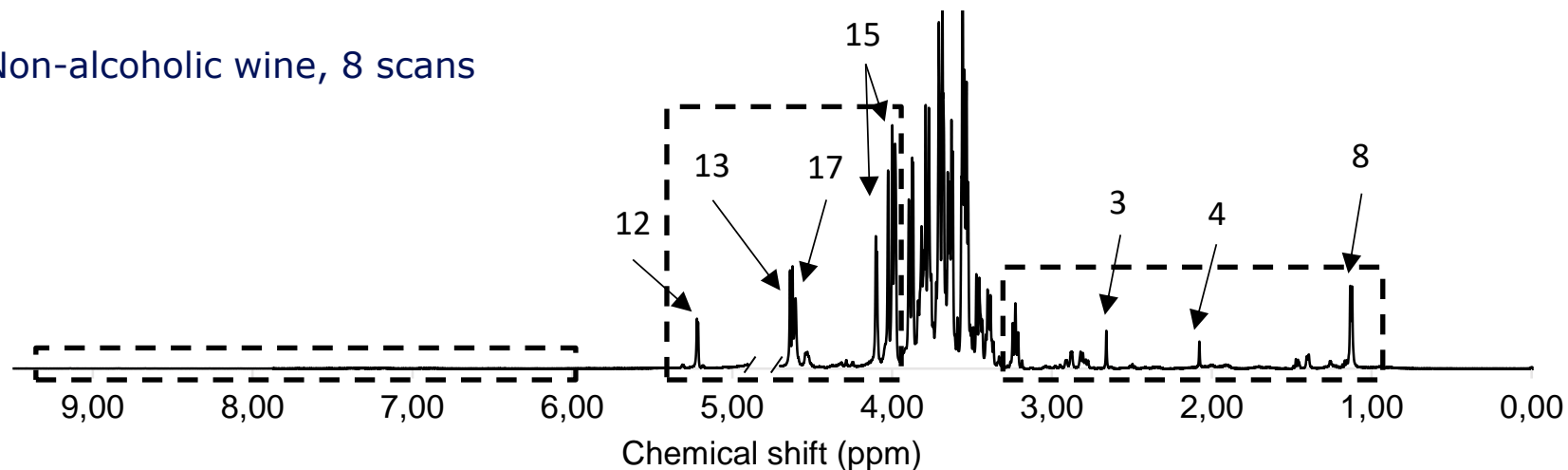
Flow cell comparison: carryover and peak width



Sample type	Chemical Shift signal (ppm)	Flow cell type	Peak width (Hz)	Carryover (%) (n=10)
Maleic acid	6.03	PCTFE	1.6±0.1 (50 %), 25.7±2.3 (0.55%)	0.6
		Glass	1.0±0.04 (50%), 16.7±1.2 (0.55%)	1.5

Application of automated flow-NMR platform

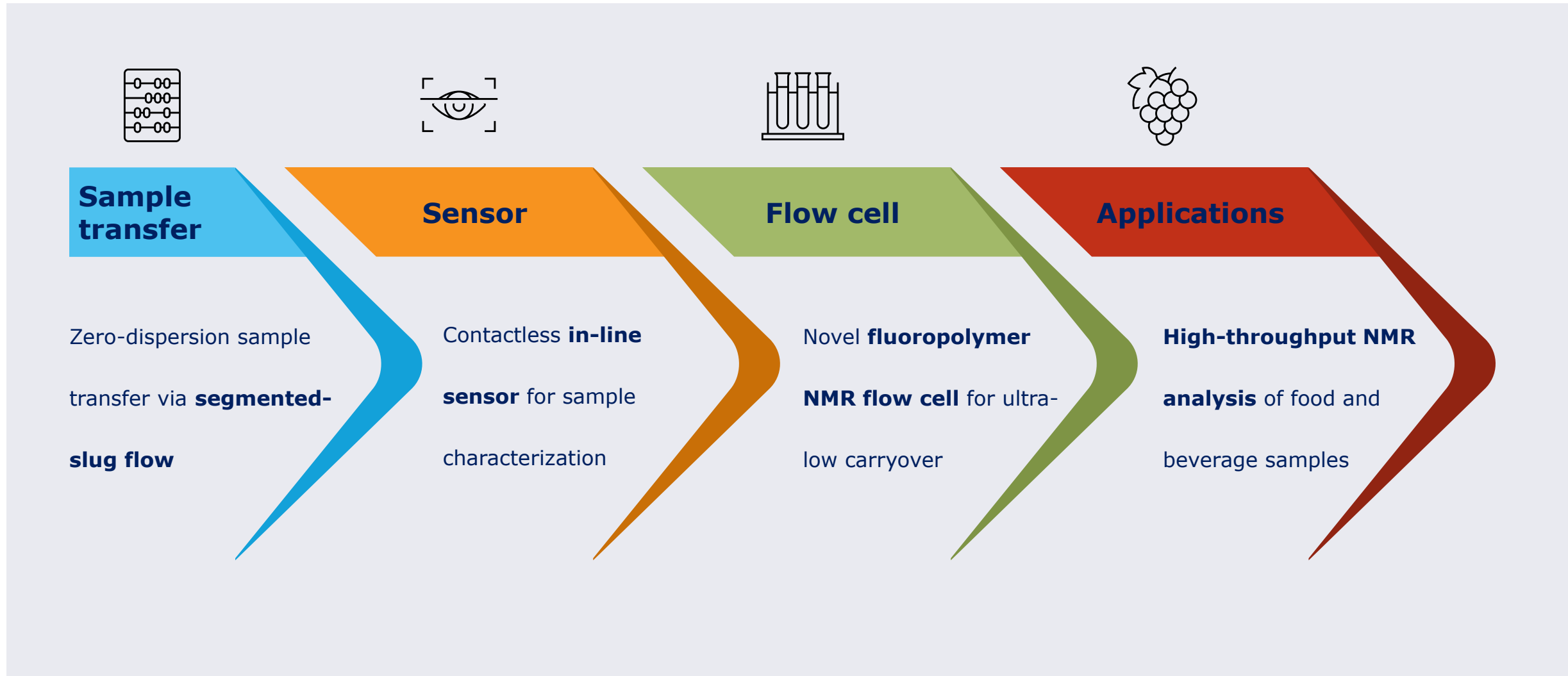
Non-alcoholic wine, 8 scans



Annotations:

- | | |
|--------------------|-----------------------|
| 1. Malic acid | 10. Proline |
| 2. Citric acid | 11. Valine |
| 3. Succinic acid | 12. α -glucose |
| 4. Acetic acid | 13. β -glucose |
| 5. Lactic acid | 14. Galacturonic acid |
| 6. Formic aldehyde | 15. Fructose |
| 7. 2,3-butanediol | 16. Catechin |
| 8. Tyrosine | 17. Tartaric acid |
| 9. Alanine | 18. Choline |

Concluding remarks



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