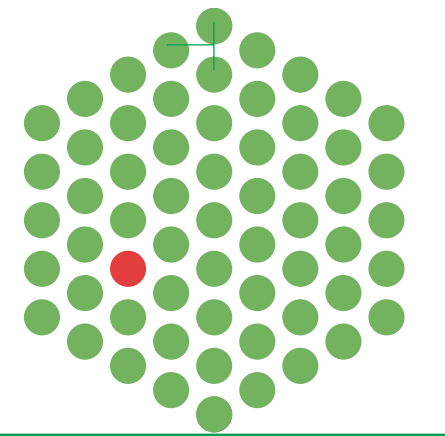


Microscopy-guided subcellular analysis

with femtosecond infrared laser ablation and on-line electrospray post-ionization for high resolution accurate mass spectrometry

ThermoFisher
SCIENTIFIC

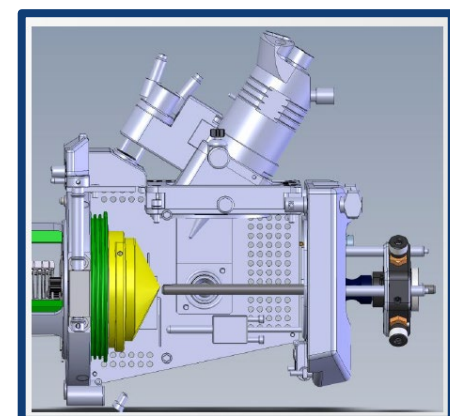
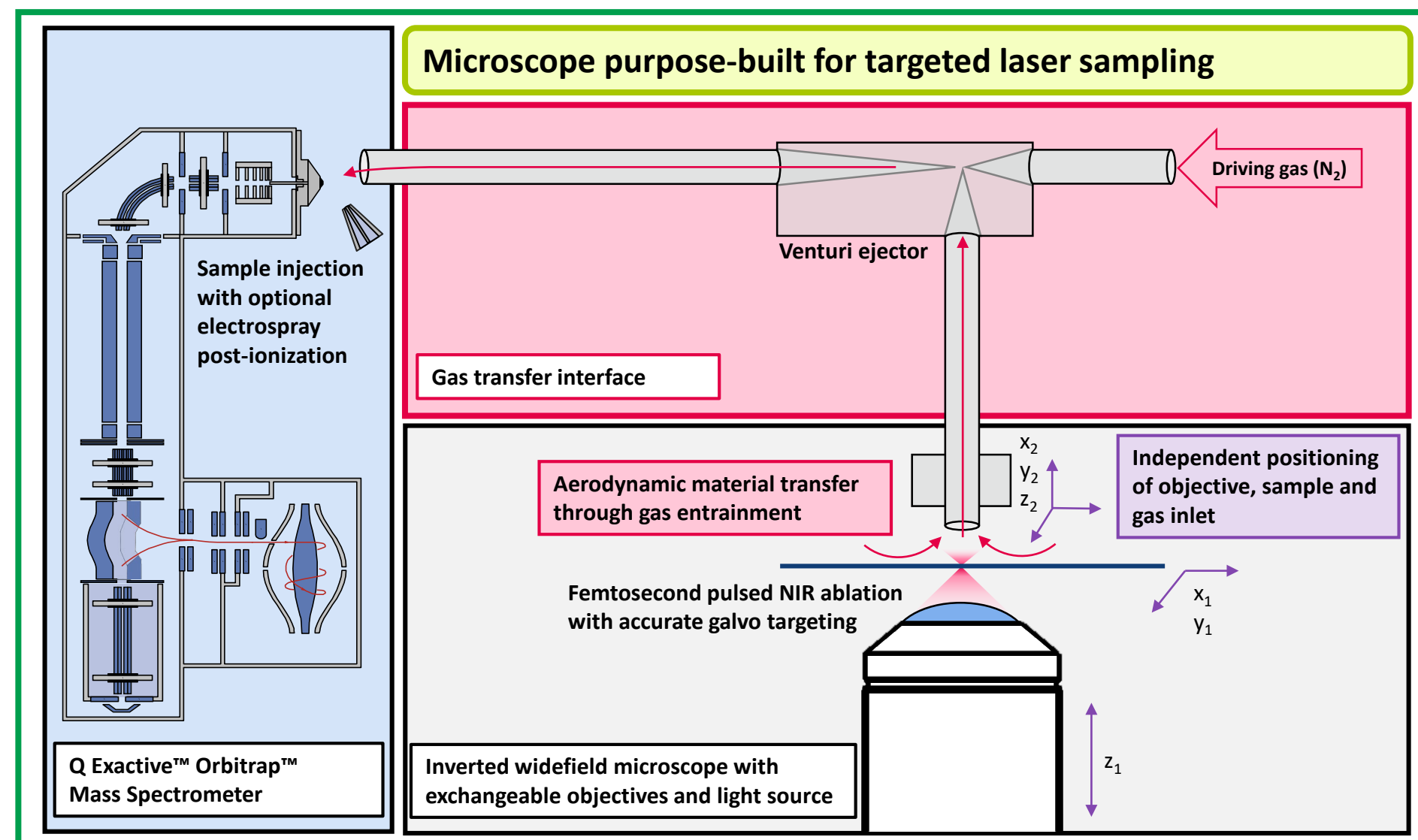
EMBL



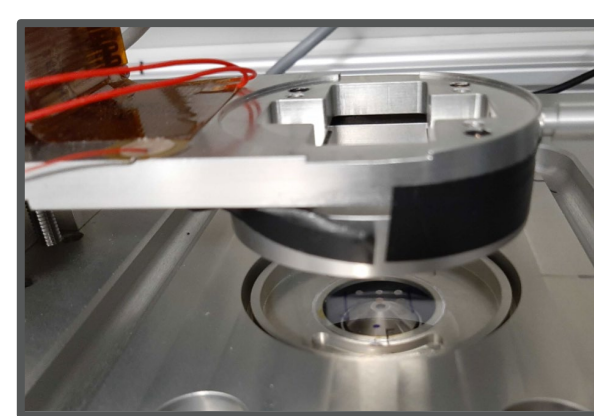
Måns Ekelöf¹, Rainer Daum², Kyle L. Fort³, Alexander Makarov³, Theodore Alexandrov¹

(1) EMBL, Heidelberg, Germany, (2) Thermo Fisher Scientific, Planegg-Martinsried, Germany, (3) Thermo Fisher Scientific, Bremen, Germany

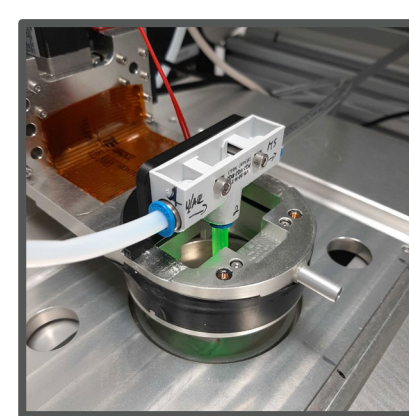
Instrument Design



Gas injector for Ion MAX housing as used in imaging demonstrations

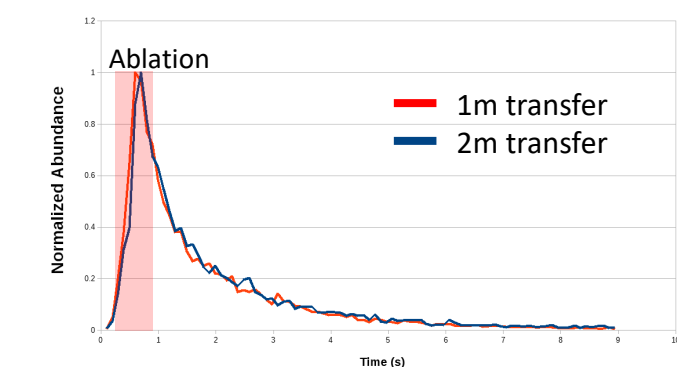


Sample stage for 35mm cover slip mounted samples, with independent positioning of inlet. Here shown with and without mounted venturi gas ejector.

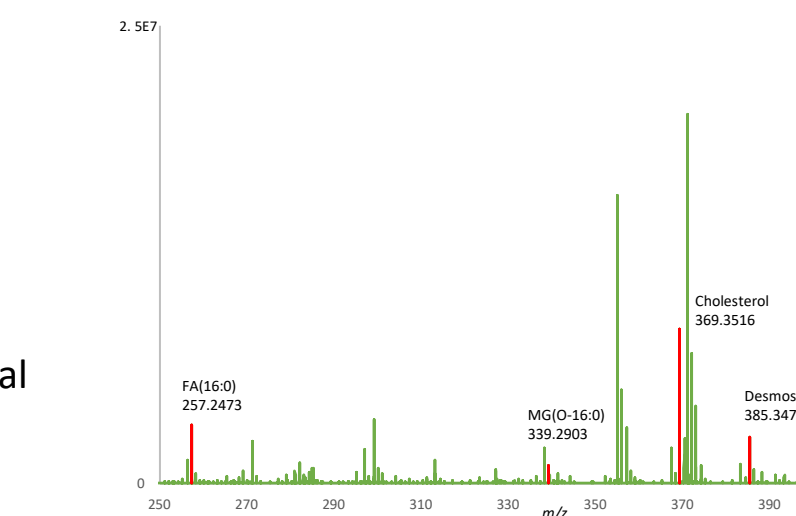


Flexible design gives access to multiple ionization modalities including IR-LDI, IR-MALDI, IR-ELDI and IR-MALDESI

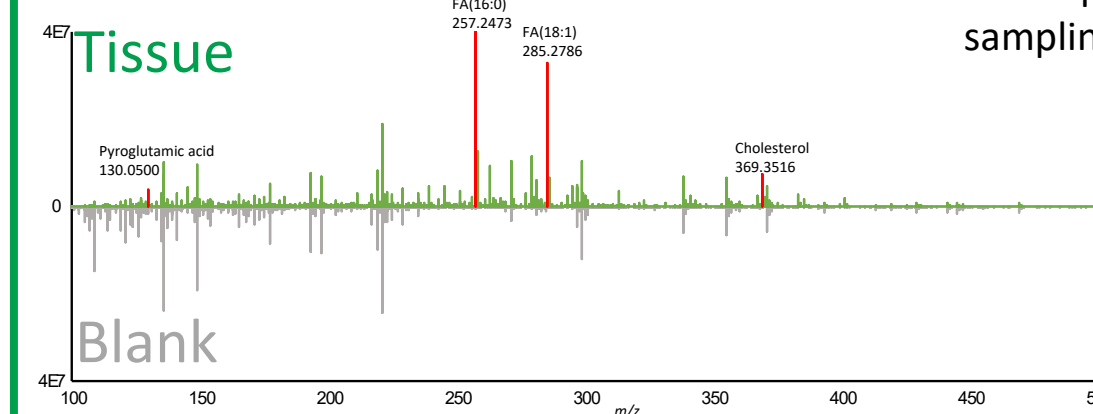
Performance Evaluation



Signal rise and decay in gas system measured by ablation of rhodamine B from red marker pen. Signal magnitude and decay times remain constant with transfer distance

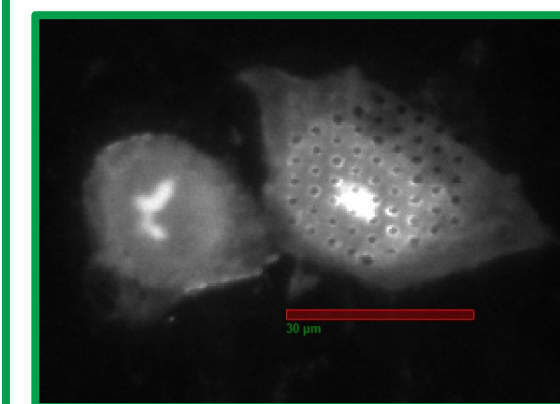


Mass spectrum with lipids and lipid fragments from direct sampling of whole adherent HeLa Kyoto cells

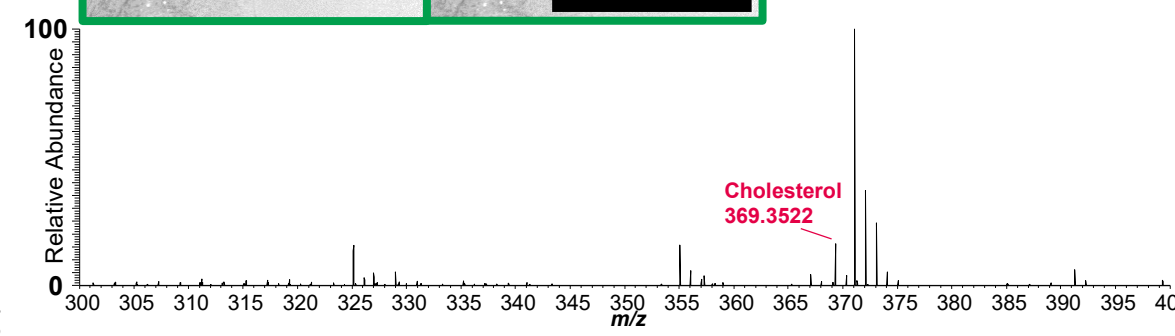
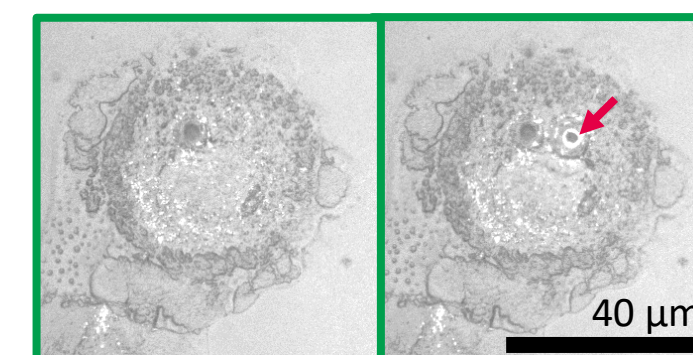


Spectrum from high-power ablation of desiccated mouse liver (approx. 1000 μm³)

Remote femtosecond NIR laser sampling without resonantly absorbing matrix yields LDI-type breakdown products in the high fluence regime (>60 J/cm²)

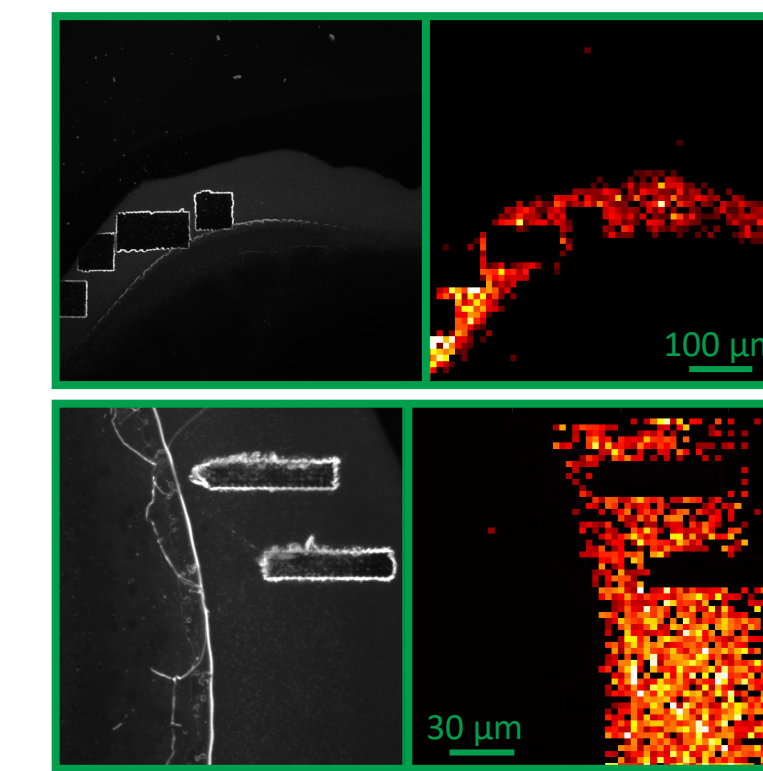


Accurate subcellular ablation with minimal thermal damage. 4',6'-diamidino-2-phenylindole (DAPI) fluorescence (460 nm) unchanged in stained HeLa cells after laser sampling



Targeted sub-cellular ablations of HeLa Kyoto cells

Mass Spectrometry Imaging



Raster scan of Rhodamine B deposit with pre-etched voids

- Irradiation duration 1 ms / pixel
- 150 fs pulse width
- Injection time 100 ms on Q Exactive™ Focus

Feature resolution down to 3 μm³
Ideal LOD <10 pg (<20 fmol)

Conclusions

- A mass spectrometry sampling interface built on a widefield microscopy platform
- Precise subcellular ablation achievable, guided by fluorescence microscopy
- Allows on-line point targeting or full-raster imaging mass spectrometry experiments
- Pico- to femtosecond ablation for efficient material transfer and minimal damage to surrounding areas
- Detection of endogenous small molecules and lipids

Relevant Literature

Infrared laser ionization

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3. Schäfer, KC, et. Al., *Anal. Chem.* 2011, 83, 5, 1632–1640 (IR-LDI)

Ultrashort pulse sampling

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5. Zou J, et. Al., *Anal. Chem.* 2015, 87, 24, 12071–12079 (PIRLAESI)

Aerodynamic devices for mass spectrometry sampling

6. Dixon RB, Bereman MS, Muddiman DC, and Hawkrigde AM, *J Am Soc Mass Spectrom* 2007, 18, 10, 1844–1847 (Venturi ejector)
7. Dolatmoradi M, Fincher JA, Korte AR, Morris NJ and Vertes A, *Analyst*, 2020, 145, 5861–5869 (Ablation chamber)