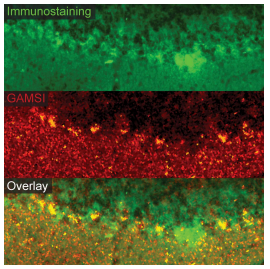
Dec 10, 2024

Gel-Assisted Mass Spectrometry Imaging (GAMSI) Protocol



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We use this protocol and it's working

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Abstract

Gel-Assisted Mass Spectrometry Imaging (GAMSI) is an accessible and robust sample preparation and imaging workflow to enhance the spatial resolution of MALDI-MSI to the single-cell scale and beyond. Using mouse brain sections as an example, we describe a step-by-step GAMSI protocol that increases the spatial resolution of MALDI-MSI ~3-6-fold to the sub-micrometer level without changing the existing mass spectrometry hardware or analysis pipeline. The protocol is expected to be applicable to a diverse array of tissue types.

Attachments

XLSX

Materials_for_GAMSI....

10KB

Image Attribution

Single-cell lipidomic profiling in mouse cerebellum with Gel-Assisted Mass Spectrometry Imaging (GAMSI) (image credit: Gao Lab, University of Illinois Chicago)

Materials

Tissue preparation

	Materials	Shape/size	Vendor	Product Number
	Isopentane (2-methylbutane)	1 L	Sigma	320404-1L
	Glass microscope slides	25 × 75 mm	Midsci	MID7100-45

Day 1: Sample fixation

	Materials	Shape/size	Vendor	Product number
	Paraformaldehyde, 20%	10 mL	Thermo	047340.9M
	10x PBS	500 mL	Thermo	70-011-069

Day 1: Sample anchoring

	Materials	Shape/size	Vendor	Product number
	Acryloyl-X	5 mg	Thermo	A20770
	Anhydrous DMSO	3 mL	Thermo	D12345

Day 2: Sample gelation

	Materials	Shape/size	Vendor	Product number
	Sodium acrylate	25 g	Thermo	50-750-9773
	Acrylamide	100 g	Sigma	A9099
	N,N-Methylenebisacrylamide	5 g	Sigma	294381
	Sodium chloride	500 g	Sigma	S9625
	4-Hydroxy-TEMPO (4HT)	1 g	Sigma	176141-1G
	NNN′N′-Tetramethylethylenediamine (TEMED)	25 mL	Sigma	T7024-25ML
	Ammonium persulfate (APS)	25 g	Thermo	PI17874
	0.5-mil Polyimide (Kapton) tape	7.94 mm W x 33 m L	Caplinq	PIT0.5S-UT/7.94

Day 2: Sample homogenization

	Materials	Shape/size	Vendor	Product number
	Trypsin	50 mg	Thermo	20233
	Proteinase K	2 mL	NEB	P8107S

Day 4: Sample expansion and immobilization

	Materials	Shape/size	Vendor	Product number
	No. 1.5 cover glass	36 × 60 mm	TedPella	260461-100
	ITO-coated glass slides	70 - 100 Ω/sq	Delta Technologies	CB-90IN-S111,

(Optional) Matrix sublimation and recrystallization

	Materials	Shape/size	Vendor	Product number
	1,5-diaminonaphthalene (DAN)	36 × 60 mm	TedPella	260461-100
	Acetone	1 L	Thermo	A929-1
	Isopropyl alcohol	1 L	Sigma	1027811000
	PYREX petri dishes	100 mm diameter	Thermo	08-747C

Recipes and Reagents:

4% PFA

	Materials	Amount
	Paraformaldehyde, 20%	10 mL
	10x PBS	5 mL
	Water	35 mL

Note

4% PFA solution can be stored at 4 °C up to 2 months.

3% PFA/0.1% GA

	Materials	Amount
	Paraformaldehyde, 20%	7.5 mL
	Glutaraldehyde, 8%	625 µL
	10x PBS	5 mL
	Water	36.875 mL

Note

3% PFA/0.1% GA solution can be stored at 4 °C up to 1 month. Store in 1 mL aliquot at -20 °C for long term storage.

AcX stock ([M] 10 mg/mL)

- Thaw Acryloyl-X (5 mg tube) to room temperature.
- Dissolve Acryloyl-X (5 mg tube) in 500 µL anhydrous DMSO.
- Aliquot in 20 µL batches.
- Store in a desiccated environment at -20 °C .
- **DO NOT** reuse AcX after thawing.

4HT stock ([M] 5 mg/mL)

- Dissolve 0.5 g of 4HT in 100 mL of water.

TEMED stock ([M] 100 mg/mL)

- Dissolve 10 g of TEMED in 100 mL of water.

APS stock ([M] 100 mg/mL)

- Dissolve 10 g of APS in 100 mL of water.

Note

Store 4HT, TEMED and APS stock solutions in 100 µL aliquots at -20 °C

Stock-X solution (for 4-fold expansion) (Excluding 4HT, TEMED, APS):

	A	B	C	D
	Sodium acrylate	38	2.25	8.55
	Acrylamide	50	0.5	2.5
	N,N-Methylenebisacrylamide	2	0.75	0.15
	Sodium chloride	29.2	4	11.7
	10x PBS	10x	1	1x
	Water		0.9	
	Total		9.4	

A: Materials; B: Stock solution concentration (g/100 mL); C: Amount of stock solution (mL) to be mixed; D: Final concentration (g/100 mL).

Note

- The final concentration is calculated by accounting for the volumes of 4HT, TEMED, and APS to be added.
- Follow columns A-C of the table to prepare the Stock-X solution.
- Store Stock-X in 1 mL aliquots at -20 °C .

Stock-S solution (for 6-fold expansion) (Excluding 4HT, TEMED, APS):

	A	B	C	D
	Sodium acrylate	38	2.721	10.34
	Acrylamide	50	2.840	14.2
	N,N-Methylenebisacrylamide	2	0.025	0.005
	Sodium chloride	29.2	3	8.76

	A	B	C	D
	10x PBS	10x	1	1x
	Water		0.084	
	Total		9.67	

A: Materials; B: Stock solution concentration (g/100 mL); C: Amount of stock solution (mL) to be mixed; D: Final concentration (g/100 mL).

Note

- The final concentration is calculated by accounting for the volumes of 4HT, TEMED, and APS to be added.
- Follow columns A-C of the table to prepare the Stock-S solution.
- Store Stock-S in 1 mL aliquots at -20 °C .

Trypsin stock ([M] 2.5 Mass / % volume or [M] 25 mg/mL)

- Dissolve 50 mg of Trypsin in 2 mL of 1x PBS.
- Store trypsin stock in 100 µL at -20 °C .

Tissue preparation

1 Euthanize mouse via CO_2 asphyxiation followed by decapitation.

2 Dissect mouse brains and immediately freeze on prechilled isopentane (-80 °C).

Note

The dissected mouse brains can be stored at -80 °C up to 3 months until sectioning.

3 Make a 25 µm cryosection of the dissected mouse brain using a cryostat of choice (e.g., CryoStar NX50 Cryostat, Epredia).

4 Multiple sections can be mounted on a single slide, but they must be gelled together. Thaw-mount the 25 µm brain sections on positively charged glass microscope slides (e.g., MID7100-45, Midsci).

Note

1. Multiple sections can be mounted on a single slide, but they may need to be gelled together in the subsequent steps.
2. The mouse brain sections can be stored at -80 °C up to 3 months until further analysis and procedure.

Day 1: Sample fixation

30m

5 Thaw the 25 µm mouse brain sections to Room temperature . 5m

6 Fix the 25 µm mouse brain sections in 4% PFA or a combination of 3% PFA and 0.1% glutaraldehyde at Room temperature for 00:10:00 . 10m

Note

Each mouse brains section on the glass slide requires at least 1 mL of fixatives.

7 Remove the fixatives and wash sample 3 × 5 mins in 1 mL with 1 mL 1 mL of 1x PBS.

7.1 Remove the fixatives and wash sample 00:05:00 in 1 mL of 1x PBS. (1/3) 5m

7.2 Remove the fixatives and wash sample 00:05:00 in 1 mL of 1x PBS. (2/3) 5m

7.3 Remove the fixatives and wash sample 00:05:00 in 1 mL of 1x PBS. (3/3) 5m

Day 1: Anchoring 6h 10m

8 Thaw Acryloyl-X (AcX) stock solution ([M] 10 mg/mL). 5m

9 Dilute the AcX stock solution 1:100 in 1x PBS to a concentration of [M] 0.1 mg/mL . Vortex well. 5m

10 Incubate the brain section(s) 06:00:00 with the AcX solution at room temperature. 6h

Day 2: Sample gelation (4-fold expansion) 3h 21m

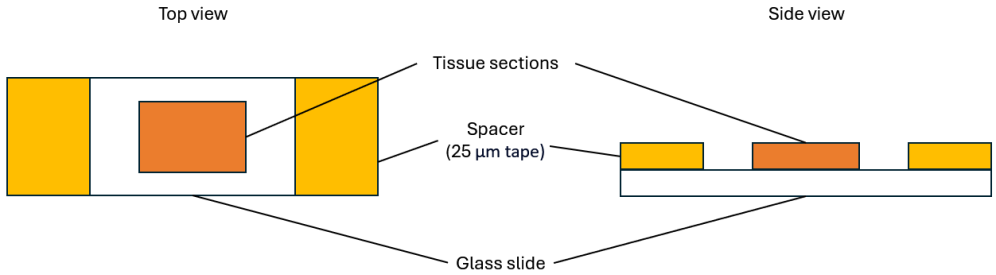
11 Wash sample 2 × 15 mins with 1 mL of 1x PBS.

11.1 Wash sample 00:15:00 with 1 mL of 1x PBS. (1/2) 15m

11.2 Wash sample 00:15:00 with 1 mL of 1x PBS. (2/2) 15m

12 Thaw Stock-X solution as well as 4HT, TEMED and APS stock solutions. Vortex well and keep the solutions On ice . 5m

13 Prepare the gelation chamber for gelation. Wipe off excess 1x PBS surrounding sample with disposable wipes (e.g., Kimwipes, Kimberly-Clark). Put the 25 µm tape on both sides of the tissue section(s). 5m



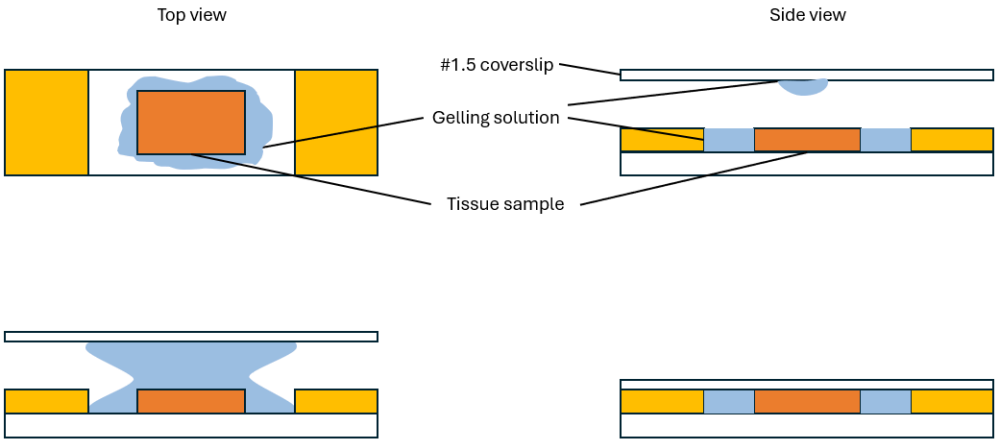
14 Pipette 50 µL of the Stock-X solution on top of each tissue section. Incubate the slide at room temperature for 00:10:00 . 10m

15 Mix the Stock-X solution and the 4HT, TEMED, and APS stock solutions at a ratio of 94:2:2:2. Vortex to obtain the gelling solution. 1m

Note

1. Follow the exact order when mixing the gelling solution, i.e., adding the Stock-X solution first and the APS stock solution last.
2. Move to the next step immediately.

16 Remove the Stock-X solution. Add 50 µL of the gelling solution and gently place a coverslip over the gelation chamber. Gently press to seal the coverslip and incubate at 4 °C for 00:30:00 . 30m



17 Polymerize the gel at 37 °C for 01:30:00 to 02:00:00 . 2h

(Alternative) Day 2: Sample gelation (6-fold expansion) 4h 41m

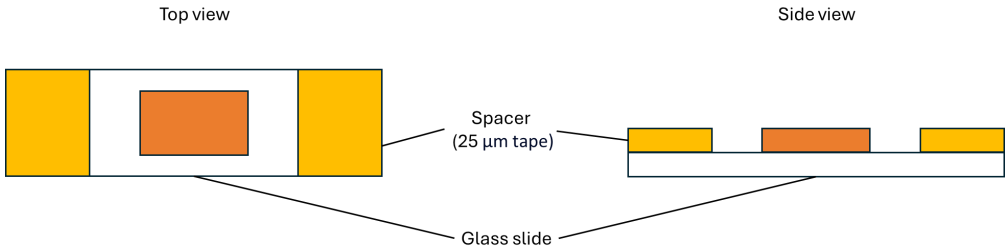
18 Wash sample 2 × 15 mins in 1 mL of 1x PBS.

19 Wash sample 00:15:00 in 1 mL of 1x PBS. (1/2) 15m

20 Wash sample 00:15:00 with 1 mL of 1x PBS. (2/2) 15m

21 Thaw Stock-S solution as well as 4HT, TEMED and APS stock solutions. Vortex well and keep the solutions On ice .

22 Prepare the gelation chamber for gelation. Wipe off excess 1x PBS surrounding sample with Kimwipe. Put the 25 µm tape on both sides of the tissue section(s).



23 Pipette 50 µL of Stock-S solution on top of each tissue section. Incubate the slide at room temperature for 00:10:00 .

10m

24 Mix the Stock-S solution and the 4HT, TEMED, and APS stock solutions at a ratio of 96.7 : 0.3 : 1.5 : 1.5. Vortex to obtain the gelling solution.

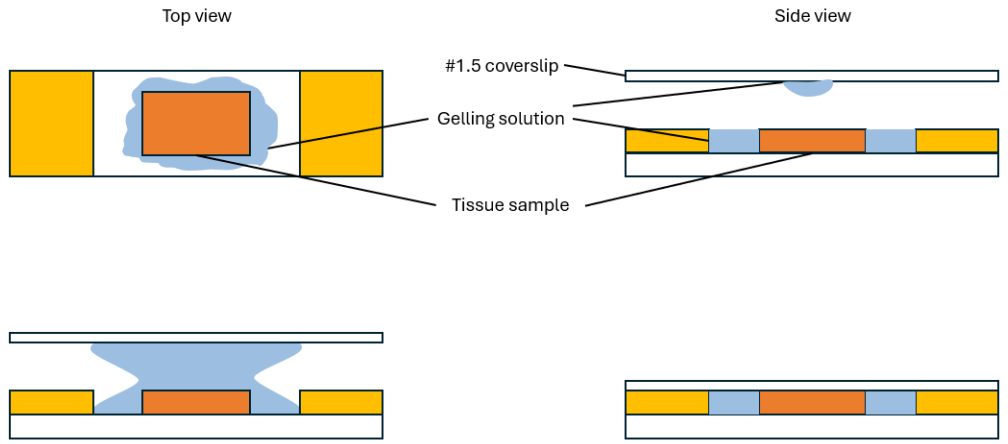
1m

Note

1. Follow the exact order when mixing the gelling solution, i.e., adding the Stock-S solution first and the APS stock solution last.
2. Move to the next step immediately.

25 Remove the Stock-S solution. Add 50 µL of the gelling solution and gently place a coverslip over the gelation chamber. Gently press to seal the coverslip and incubate at 4 °C for 00:30:00 .

30m

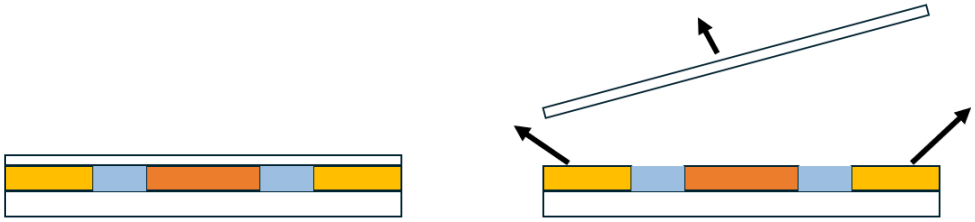


26 Polymerize the gel at 37 °C for 01:30:00 to 02:00:00 . 3h 30m

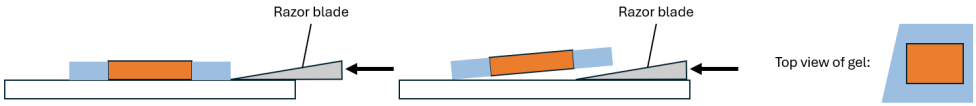
Day 2: Sample homogenization 2d 0h 15m

27 Thaw trypsin stock solution and dilute it 1:10 - 1:100 in 1x PBS to prepare trypsin digestion buffer [0.025-0.25% (w/v) in 1x PBS] at 37 °C for 2-4 days with the trypsin digestion buffer freshly prepared each day. 5m

28 Remove the chamber lid with a razor blade. Trim the gels into a right trapezoid to track the orientation of the sample. 5m



29 Remove the sample from the slide with a paintbrush that has been wetted with a small amount of 1x PBS and transfer it to a 2 mL Eppendorf containing the trypsin digestion buffer. 5m



30 Incubate each sample with 1 mL of the trypsin digestion buffer at 37 °C (or Room temperature) 48:00:00 to 96:00:00 . 2d

Note

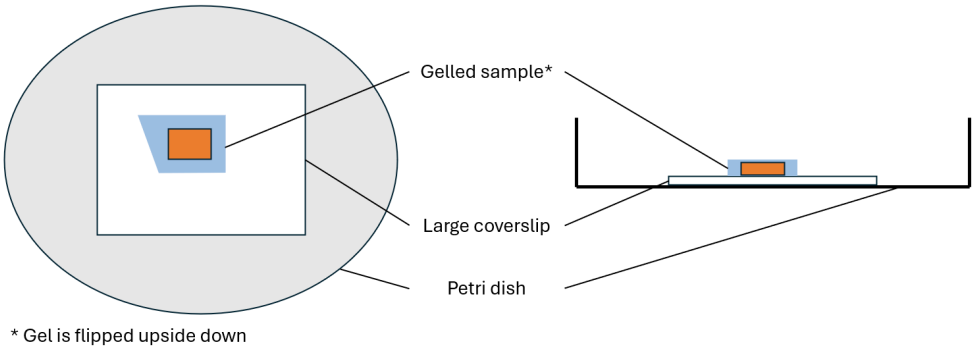
If tears or wrinkles appear after the trypsin digestion, Proteinase K (ProK) digestion (8 U/mL of ProK in 1x PBS) is an alternative for stronger homogenization.

Day 4: Sample expansion and immobilization

13h 24m

31 Place a large coverslip (e.g., 260461-100, Ted Pella) on a petri dish to prepare the expansion chamber. 1m

32 Transfer the sample onto the coverslip in the expansion chamber. 1m



Note

It is crucial to flip the sample (so that the tissue section side of the gel is facing up) in this step to maximize the MSI signal.

33 Wash the sample in an excess volume of 0.5x PBS for 00:20:00 . 20m

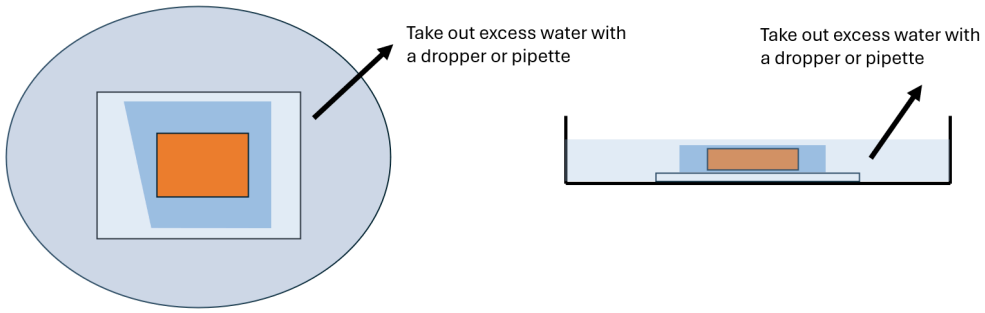
34 Wash the sample 3 × 20 mins in an excess volume of purified water until gels are fully expanded.

34.1 Wash the sample 00:20:00 in an excess volume of purified water until gels are fully expanded. (1/3) 20m

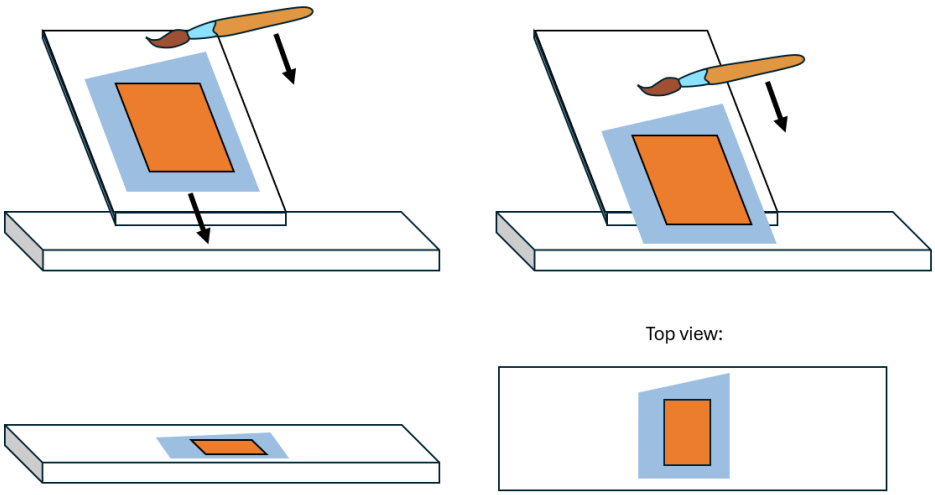
34.2 Wash the sample 00:20:00 in an excess volume of purified water until gels are fully expanded. (2/3) 20m

34.3 Wash the sample 00:20:00 in an excess volume of purified water until gels are fully expanded. (3/3) 20m

35 Keep the sample on the large coverslip and remove excess water with a dropper or pipette.



36 Transfer the expanded sample onto a MALDI substrate (e.g., stainless steel MALDI plate, Applied Biosystems SCIEX or ITO-coated glass slide, PL-IF-000010-P25, Hudson Surface Technology, Inc. or MALDI IntelliSlide, 1868957, Bruker) with the help of the large coverslip.



37 Dry-mount the sample under vacuum in a vacuum desiccator filled with Drierite (Sigma) at Room temperature for 04:00:00 to 12:00:00 .

12h

38 (Optional) Dry the mounted samples under high vacuum (e.g., in a CentriVap Benchtop Vacuum Concentrator, Labconco) at 37 °C for 00:02:00 to ensure complete removal of moisture.

2m

Day 5: Matrix application (1,5-diaminonaphthalene) 30m

39 Dissolve 50 mg of 1,5-diaminonaphthalene (DAN) in 2 mL of acetone. 5m

40 Aspire the DAN acetone solution onto the bottom of the sublimation flask, and blow-dry it using nitrogen to form a thin layer of white solid. 2m

41 Heat up the hotplate to 105 °C while placing digital thermometer in contact with the bottom of the flask to monitor the temperature. 15m

42 Add ice slush to the cold finger of the apparatus, to which the MALDI plate or the glass slide with the sample is adhered on the underside with copper tape. 5m

43 Place the sublimation system under a vacuum of 80 mTorr using a rough pump. 1m

44 Sublime DAN for 00:02:00 . 2m

Day 5: (Optional) Matrix recrystallization 3m

45 Add 2 mL of 5% isopropyl alcohol to a glass petri dish to prepare the recrystallization chamber. 1m

46 Attach the sample coated with the matrix to the cover of the recrystallization chamber and place the chamber at 55 °C for 00:02:00 . 2m

Day 5: Mass spectrometry imaging

47 To enhance signal intensity, it is recommended to increase laser power and/or the number of shots per pixel. Transfer the MALDI plate or the glass slide with the sample to a MALDI mass spectrometer of choice and start data acquisition.

Note

1. To enhance the signal, it is recommended to increase the laser power and/or the number of shots per pixel.
2. For example, a rapifleX MALDI Tissuetyper (Bruker) can be operated in negative ion reflection mode with a mass range (*m/z*) of 500-2000, 10 µm or 50 µm raster distance, and 200 laser shots per pixel to to acquire image data.
3. A timsTOF fleX MALDI-2 (TTF) equipped with a Smartbeam 3D 10 kHz Nd:YAG (255 nm) laser and microGRID (Bruker) can be operated in negative ion transmission mode (MALDI-1 mode) with a mass range (*m/z*) of 300-2500, 5 µm, 10 µm, or 20 µm raster distances, and 45, 150, and 200 laser shots per pixel, respectively, to acquire image data.
4. In general, the total image time increases for GAMSI sample because the imaging area increases proportionally to the square of the expansion factor and more time per pixel may be needed for better signal.

Protocol references

Chan, Y.H., Pathmasiri, K.C., Pierre-Jacques, D. *et al.* Gel-assisted mass spectrometry imaging enables sub-micrometer spatial lipidomics. *Nat. Commun.* **15**, 5036 (2024). https://doi.org/10.1038/s41467-024-49384-w

Acknowledgements

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