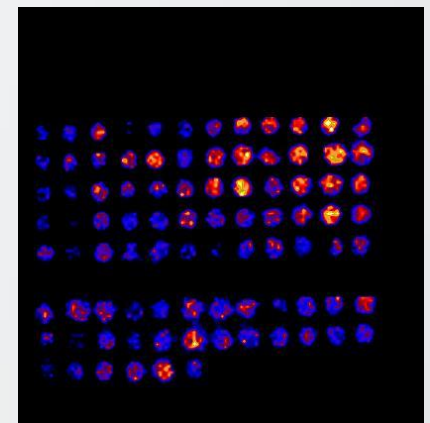
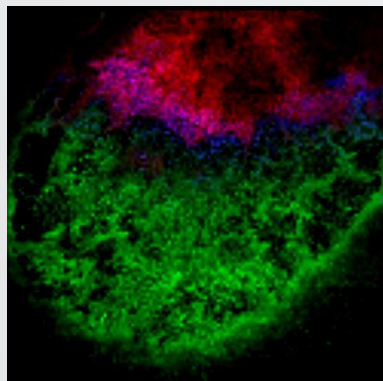


MALDI-Mass Spectrometry Imaging of Hypoxia and Metastasis-associated proteins Combined with quantitative MS

Marie-Claude DJIDJA, PhD

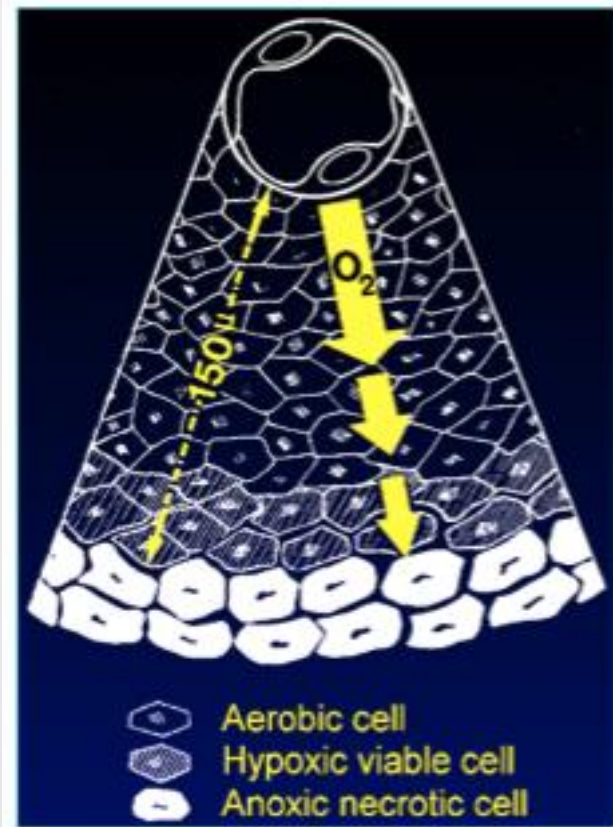
The Institute of Cancer Research, London, UK
Hypoxia and Metastasis Team
Integrative Network Biology Initiative



Early Stage Career Investigator

Email: marie-claude.djidja@icr.ac.uk

Hypoxia and Metastasis targeting with MALDI-MSI



- hypoxia = low oxygen
- Tumour hypoxia is clinically associated with metastasis
- Metastases are responsible for 90% of cancer patient deaths
- Lysyl oxidase (LOX): **A critical mediator of hypoxia-driven metastasis**
- LOX expression is clinically correlated with hypoxia, metastasis and poor survival
- LOX inhibition prevents invasion and metastasis

Erler et al, 2006, Nature 440

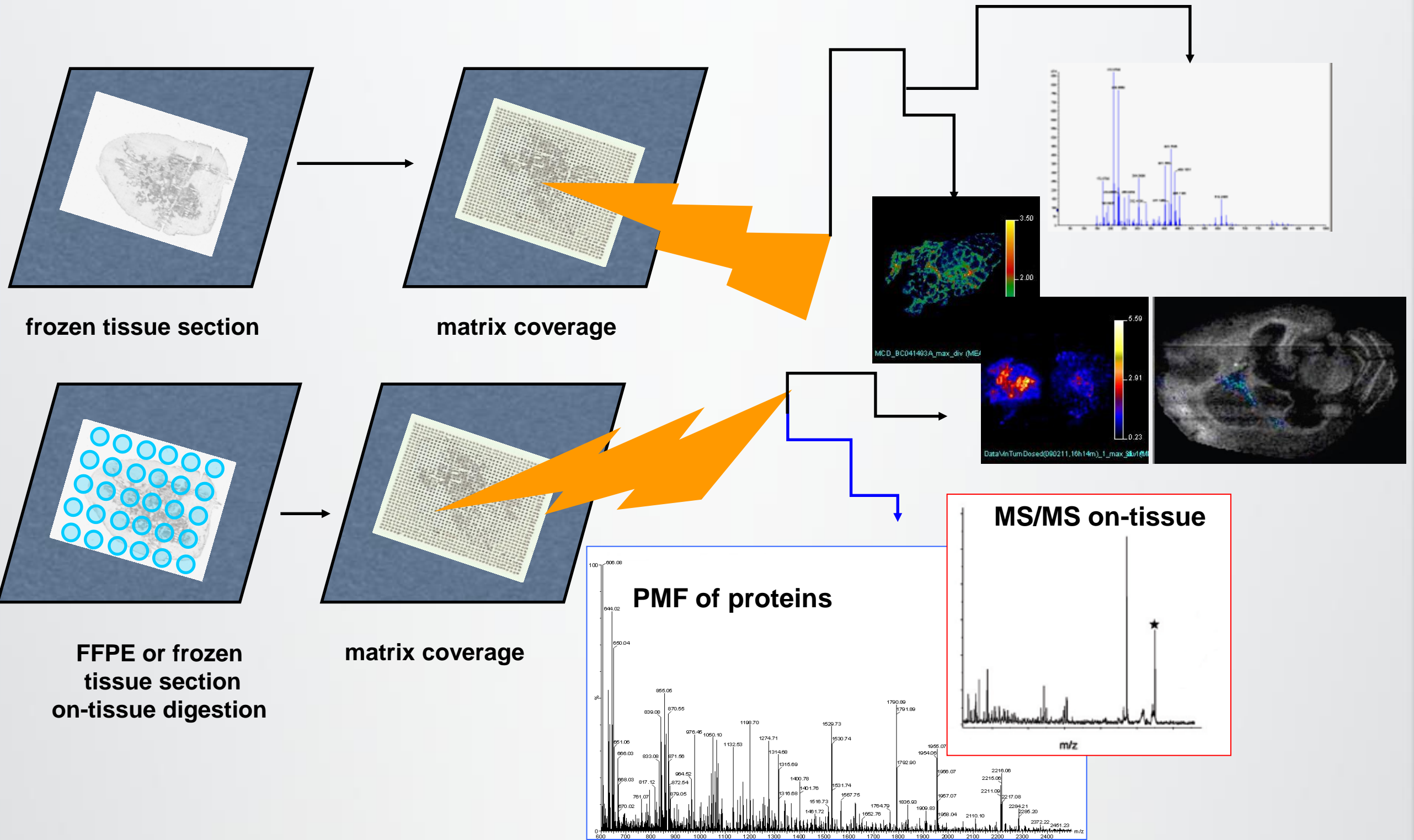
Levental et al, 2009, Cell 139

Le et al, 2009, JCO 27

Hypoxia and Metastasis targeting with MALDI-MSI

- Goal: Understand hypoxia-driven metastasis and uncover novel therapeutic targets and treatment strategies
- Aims: Use of MALDI-MSI to localise and identify proteins present within the tumour-microenvironment and associated with metastatic progression

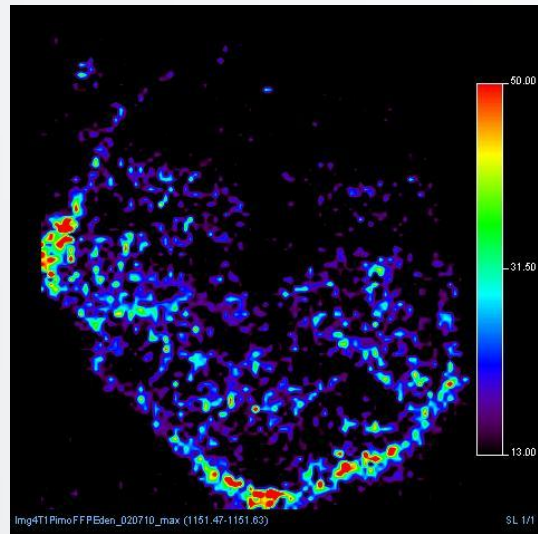
MALDI-Mass Spectrometry Imaging



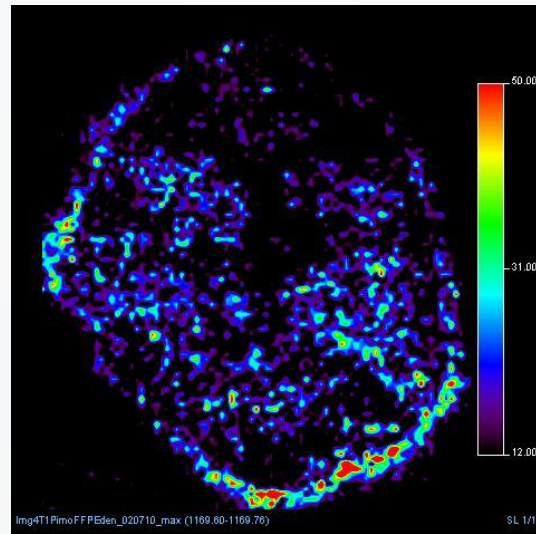
Presentation overview

- 1. Investigation of hypoxia at the primary tumour site**
- 2. Identification of metastasis-associated proteins**
- 3. Investigation of proteins involved at the pre-metastatic niche at secondary sites**

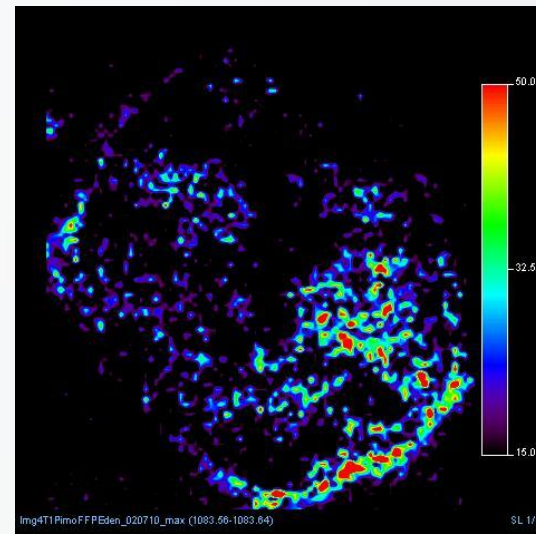
Hypoxia targeting with MALDI-MSI



m/z 1151 fibronectin



m/z 1169 fibronectin

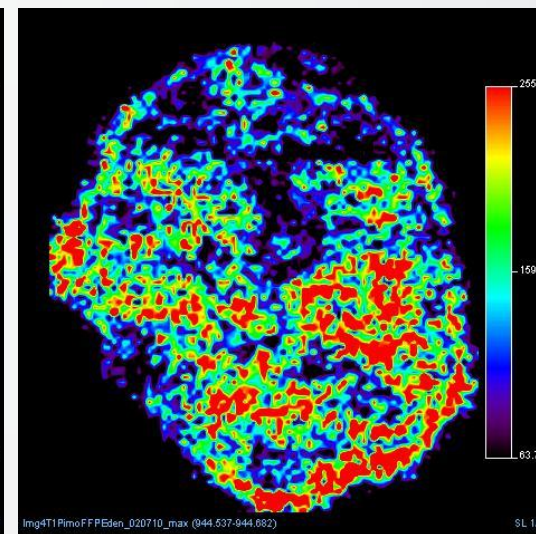
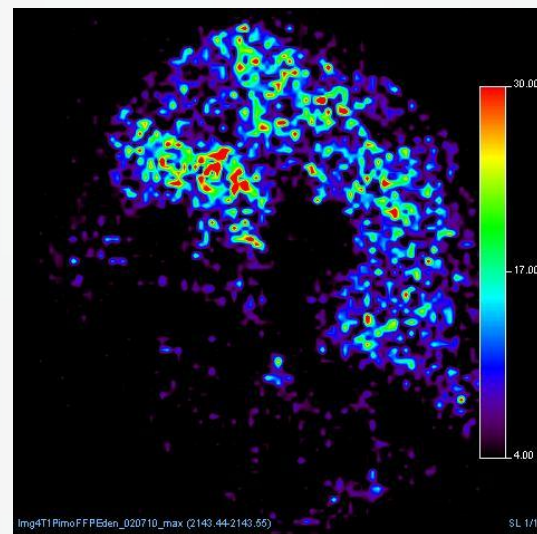
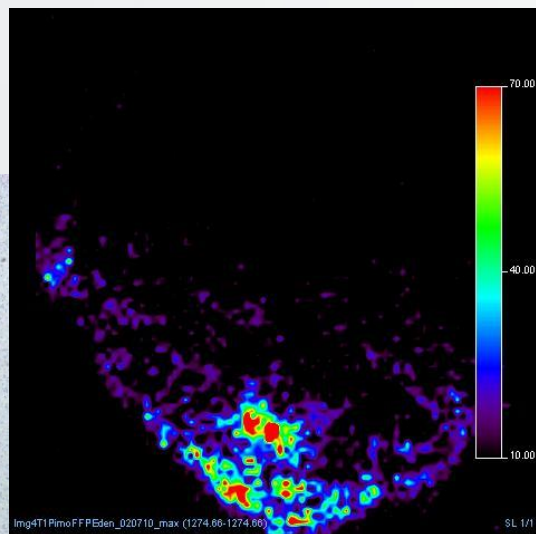
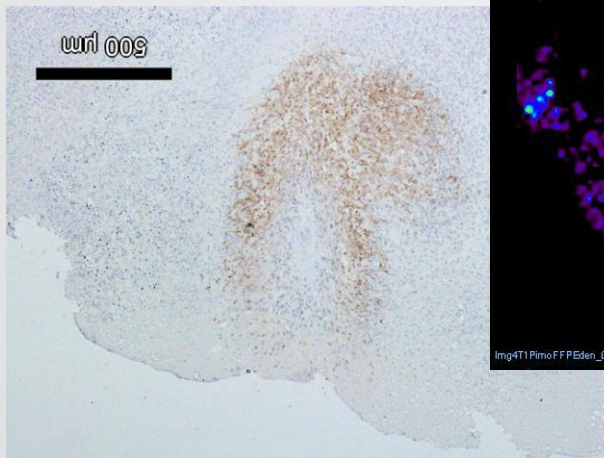


**m/z 1083
phosphoglycerate kinase1**

m/z 1274 haemoglobin β

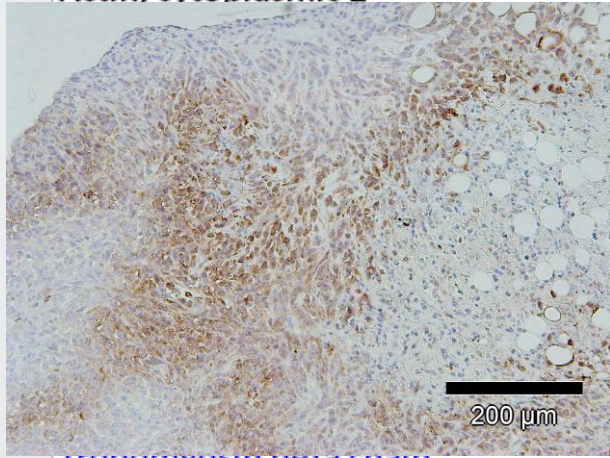
m/z 1490

m/z 944 histone H2A

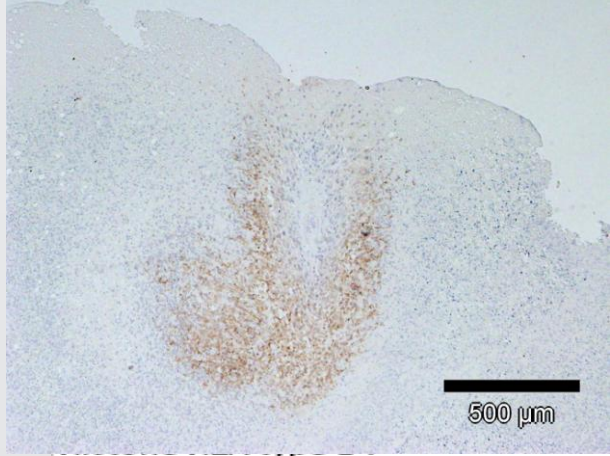


Hypoxia targeting with LCM and ESI-MS

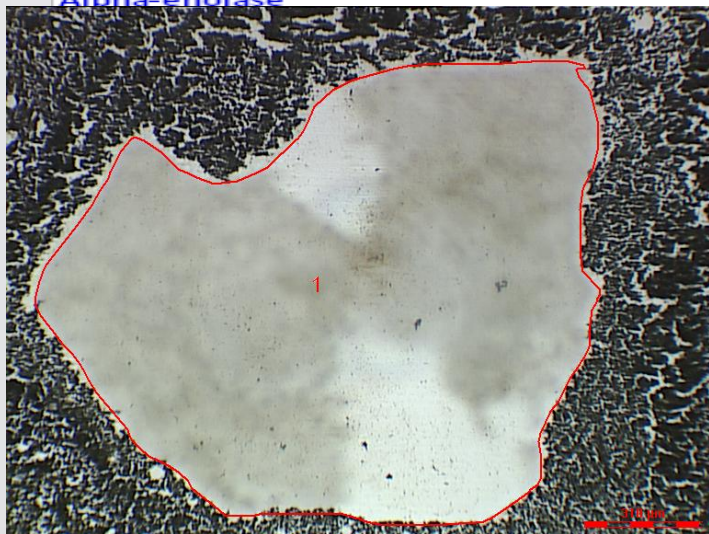
MS M...
 Ser...
 Tropomyosin beta chain
Vimentin
 Keratin, type II cytoskeletal 6A
 Collagen alpha-2(I) chain
 Actin, cytoplasmic 2



Tropomyosin beta chain

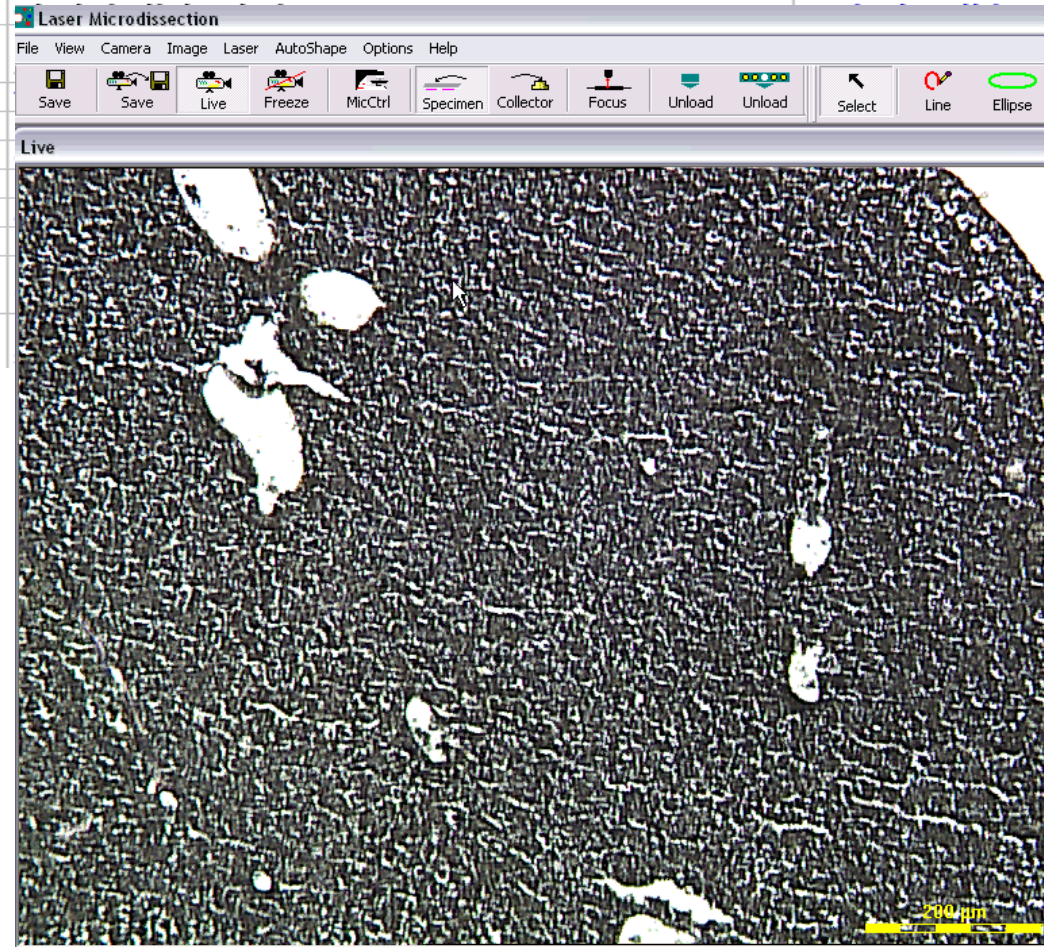


Alpha-enolase



60S ribosomal protein L18
 SAP domain-containing ribonucleoprotein
 Tenascin
 Drebrin-like protein

Heterogeneous nuclear ribonucleoprotein K
 ATP synthase-coupling factor 6, mitochondrial
 Nuclease-sensitive element-binding protein 1
 Creatine kinase M-type



Reticulocalbin-1
 Keratin, type I cytoskeletal 10
 Heterogeneous nuclear ribonucleoprotein A3
 Myristoylated alanine-rich C-kinase substrate
 Cofilin-1
 Annexin A1
 Histone H1.3
 Superoxide dismutase [Cu-Zn]
 Nucleophosmin
 Plasminogen activator inhibitor 1 RNA-binding protein
 Myosin-9
 Myosin-4
 Serine protease inhibitor A3K

Peroxisome biogenesis factor 1
 Peroxiredoxin-2
 Reticulon-4
 Keratin, type II cytoskeletal 73
 Splicing factor, arginine/serine-rich 1

actin
 ain
 isomerase
 unit delta, mitochondrial
 enase, mitochondrial
 ta/alpha
 polymerase II transcriptional
 otein S28
 phosphate aldolase A
 egiion
 neage cell-specific protein
 nuclear ribonucleoprotein D0
 toskeletal 5
 r protein RAD23 homolog B
 ain
 oponent 1 Q subcomponent-
 mitochondrial
 protein
 otein L15
 oport inner membrane
 nit Tim13
 erase N
 otein L5
 ase-activating protein
 lin-specific chaperone A
 opontin
 GDP-dissociation inhibitor 1
 nin-1A

nucleobindin-2
 Hepatoma-derived growth factor
 Troponin T, fast skeletal muscle
 Beta-2-microglobulin
 Macrophage-capping protein
 Charged multivesicular body protein 4b
 Cytochrome c oxidase subunit 5A, mitochondrial
 Beta-enolase
 Far upstream element-binding protein 1
 28 kDa heat- and acid-stable phosphoprotein
 Heterogeneous nuclear ribonucleoprotein M

KEY:									
Standard and robust hypoxia-regulated protein									
Reported hypoxic regulation									
Unreported hypoxic regulation but function associated with hypoxia responses									

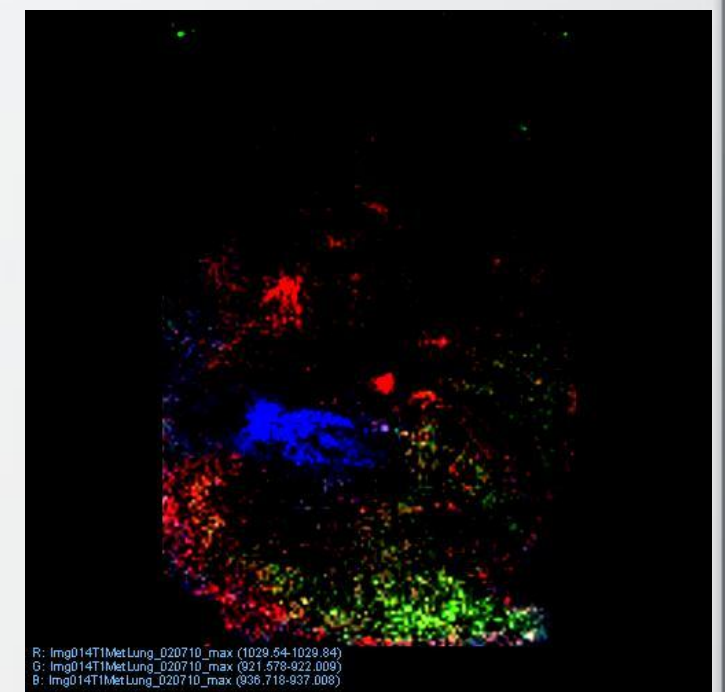
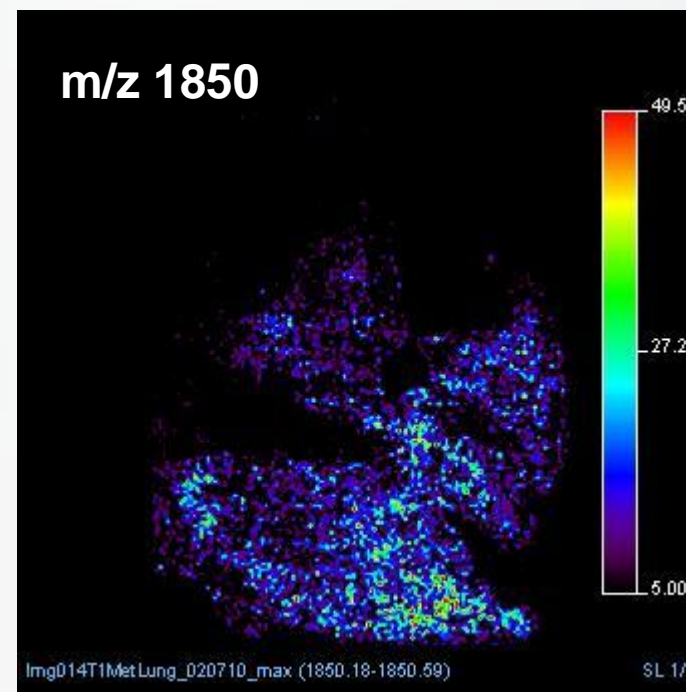
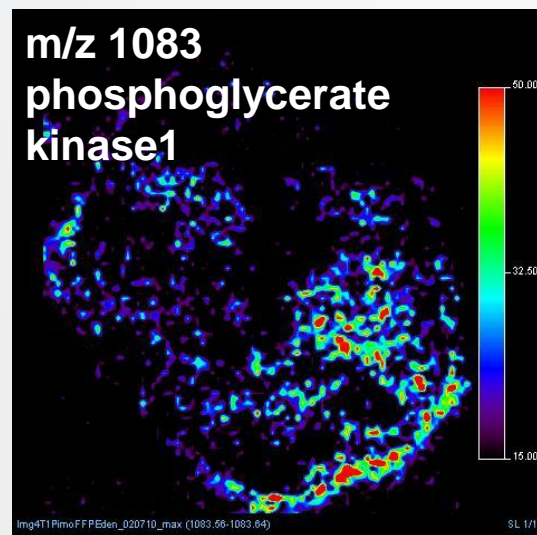
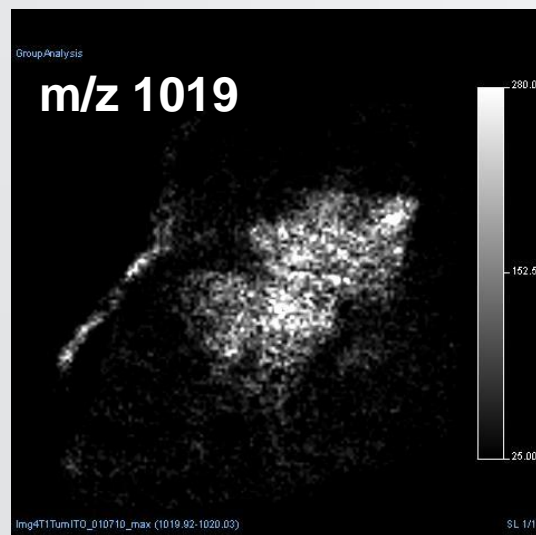
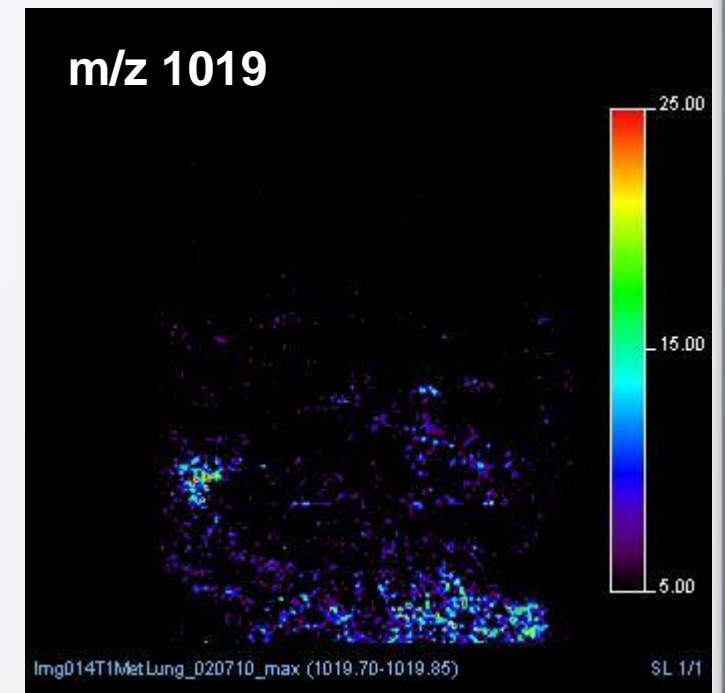
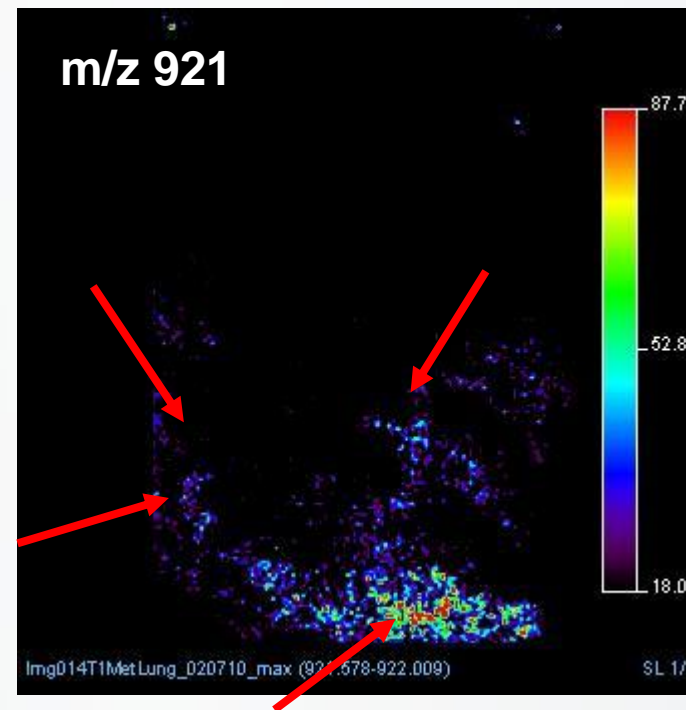
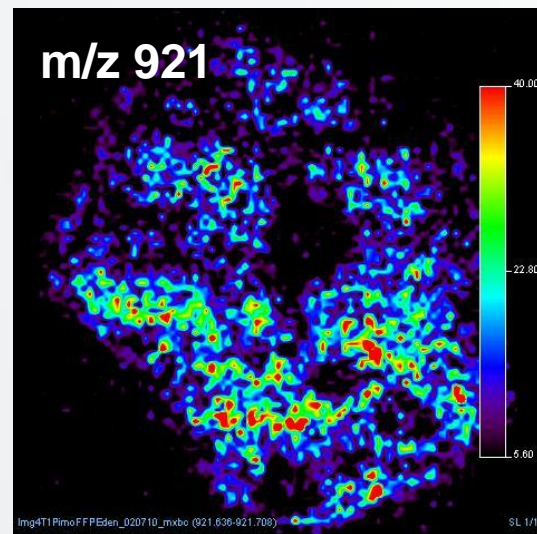
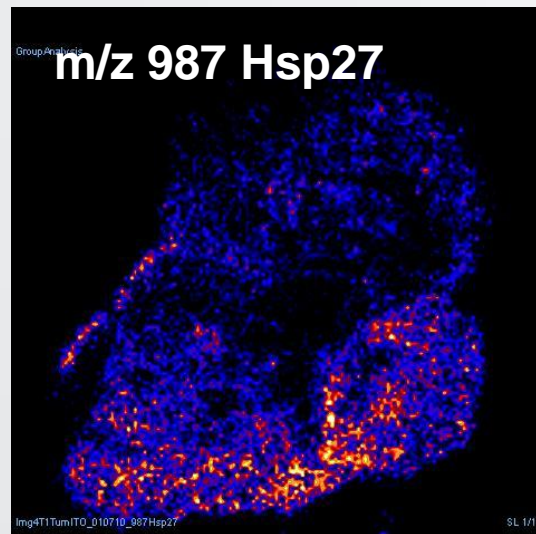
Presentation overview

- 1. Investigation of hypoxia at the primary tumour site**
- 2. Identification of metastasis-associated proteins**
- 3. Investigation of proteins involved at the pre-metastatic niche at secondary sites**

Biological Process	Protein name/ Accession number	Mass (Da)	Observed peptide m/z	Sequence	MOW-SE Score	Molecular function/ Pathways	
Cell cycle control	Tumour protein 63/ Q9H3D4	76736	1127.6	RCPN <u>H</u> ELSR, Oxidation (HW)	15,	Other transcription factor Select regulatory molecule	
	Rola-associated inhibitor/ Q8WUF5	80036	1067.52		de none		
Cell proliferation and differentiation	Tumour protein 63/ Q9H3D4	76736	1127.6				
	Rola-associated inhibitor/ Q8WUF5	80036	1067.52				
Chaperone Protein folding	Heat-shock protein beta-1/ P04732	97768	987.61	WPFSLLR	36	Chaperone, p38 MAPK pathway, VEGF signaling pathway, Angiogenesis	
			1163.61	LFDQAFGLPR	31		
Chromatin packaging and remodeling	Histone H2A.Z/ P0C055	13545	944.53	AGLQFPVGR	41	Gene regulation, histone	
			15204	1032.6	YRPGIVALR		30
			11360	1325.74	DNIQCITKPAIR		30

Biological Process	Protein name/ Accession number	Mass (Da)	Observed peptide m/z	Sequence	MOW-SE Score	Molecular function/ Pathways	
	Histone-lysine N-methyltransferase/ O14686	563831	1095.55	CGAH <u>G</u> RCRCR, Acetyl (N-term); Oxidation (HW)	14	Acts as a coactivator for estrogen receptor, methyltransferase	
			1286.64	NLITM <u>S</u> ELIHKR, Acetyl (N-term); Oxidation (HW); Oxidation (M); Oxidation (P)	23		
Dehydrogenase	2-oxoglutarate dehydrogenase E1 component-like/ Q9ULD0	114409	962.5	FM <u>T</u> ILRR, Oxidation (M)	21	Dehydrogenase, Tricarboxylic acid pathway	
Hydrolase	Na ⁺ /K ⁺ -ATPase alpha 3 subunit variant/ Q53ES0	111779	1002.5	RDLDMKK			
Muscle development	Obscurin-like protein 1/ O75147	152786	1111.50	NGAVVTPG <u>P</u> QR, Oxidation (P)	33	Guanyl-nucleotide exchange factor	
Protein biosynthesis, other metabolism	28S ribosomal protein S18b/ Q9Y676	29377	1105.6	NHKGCVPP <u>Q</u> R, Oxidation (HW)	20	Ribosomal protein	
Transport	Albumin/ P02768	69321	1311.73	HPDYSVLLLLR	57	Other transfer/carrier protein	
			15085	1087.6			
			15806.8	1274.7			
			111779	1002.5			

Identification of metastasis-associated proteins



primary tumour tissue sections

Lung metastases

Presentation overview

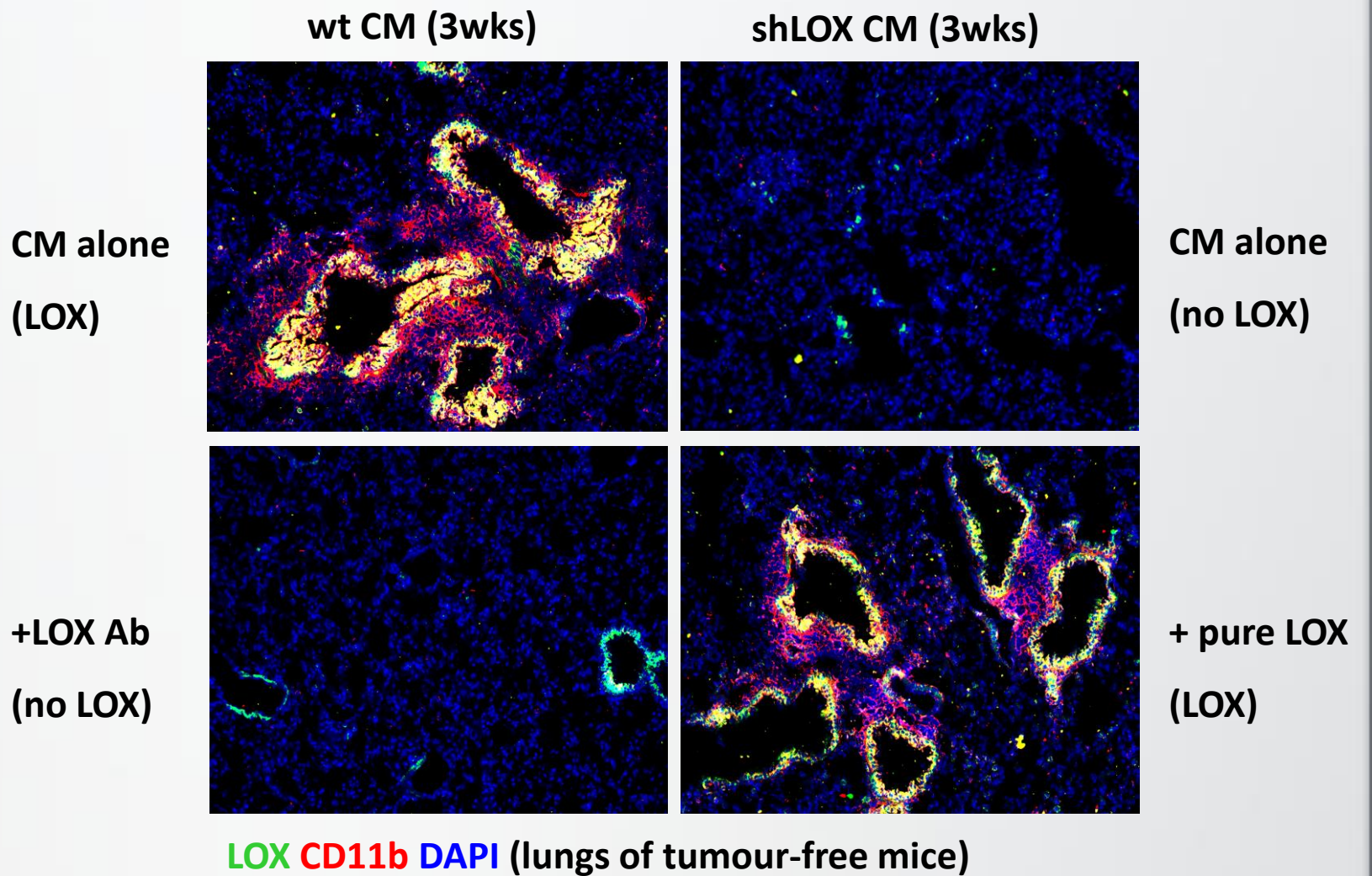
- 1. Investigation of hypoxia at the primary tumour site**
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MALDI-MSI of proteins involved in pre-metastatic niche formation and metastatic progression

▪ Tumour cells secrete factors that elevate fibronectin expression at distant sites of future metastasis (*Kaplan et al, 2005, Nature 438: "The pre-metastatic niche"*)

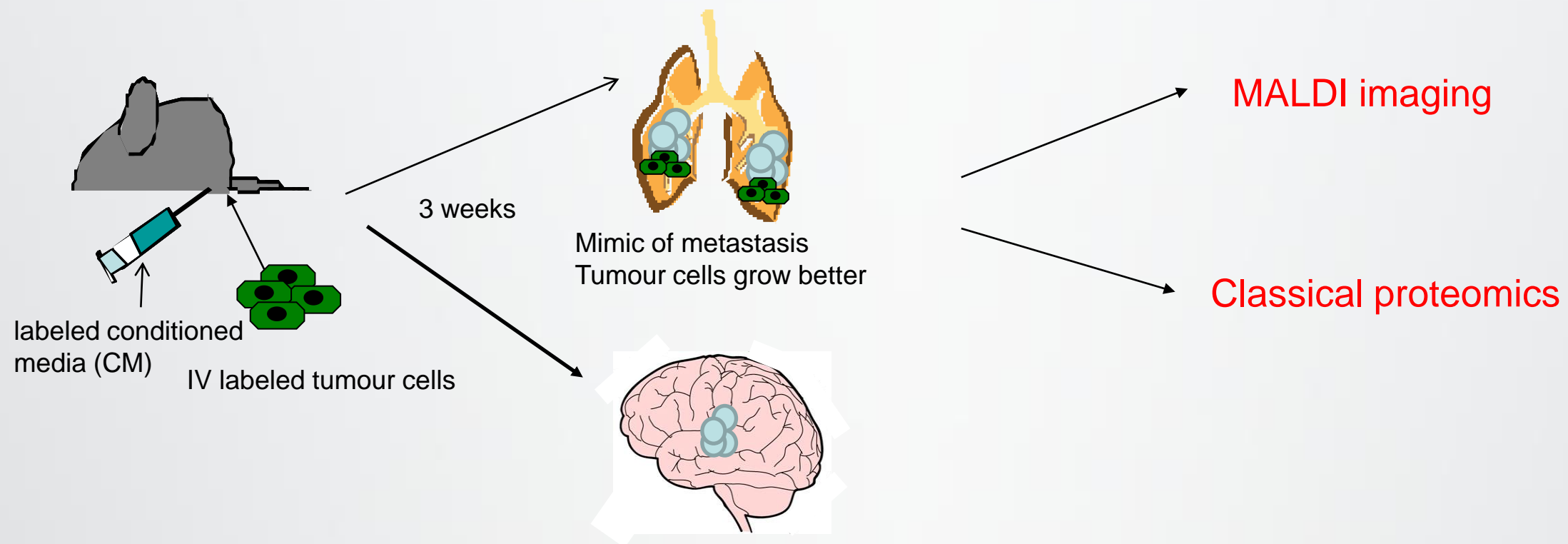
⇒ Bone marrow-derived cells (BMDCs) are recruited to these sites and create a pre-metastatic niche:

- Pre-metastatic niches determine where metastases form
- We noted BMDC accumulation is associated with LOX expression (*Erlor et al., 2009, Cancer Cell*)



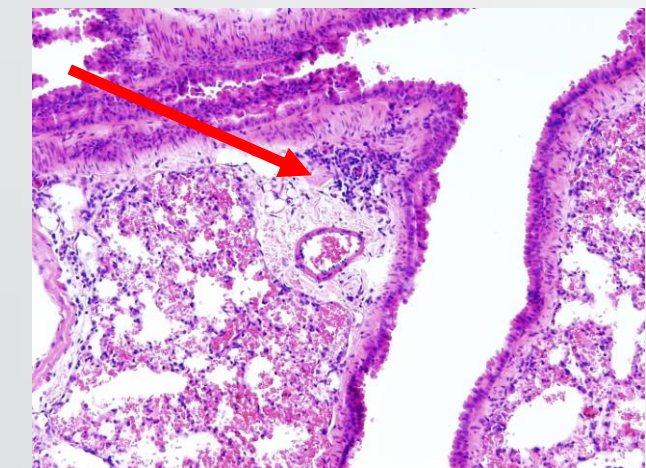
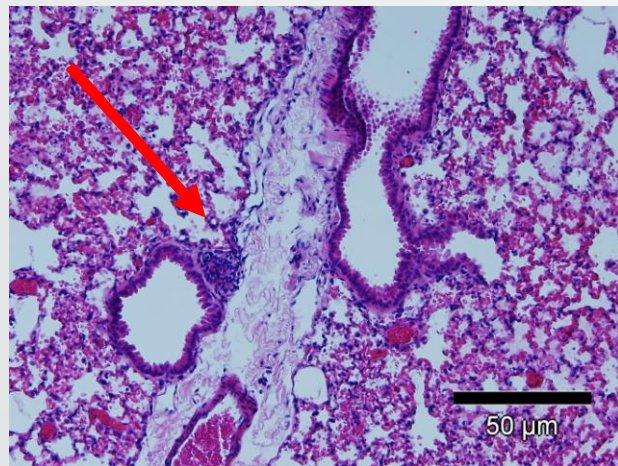
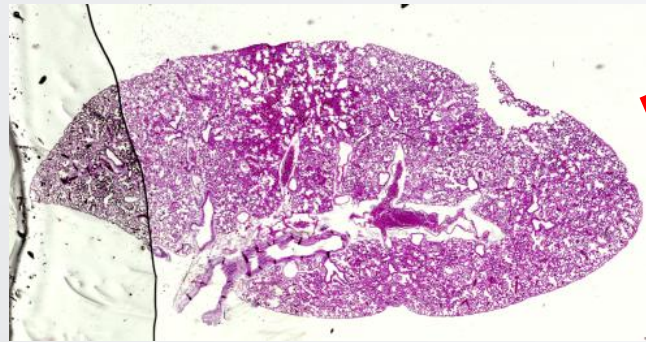
MALDI-MSI of proteins involved in pre-metastatic niche formation and metastatic progression

LOX in CM recruits BMDCs to pre-metastatic sites

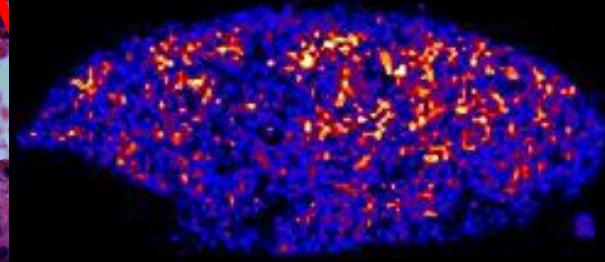


- Use of SILAC (stable isotope amino acid) to monitor the CM and/or the tumour cells
- Use of MALDI-MSI to localise biomolecules involved in these processes

MALDI-MSI of proteins involved in pre-metastatic niche formation and metastatic progression

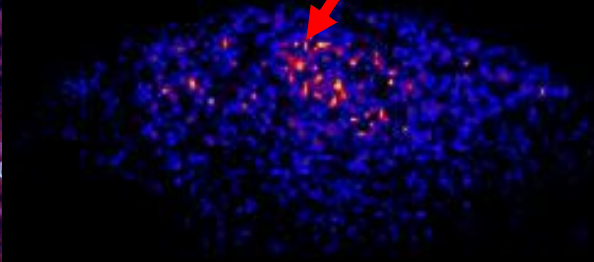


m/z 944 histone H2A



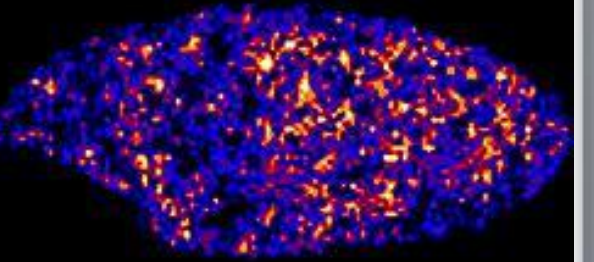
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m/z 1374



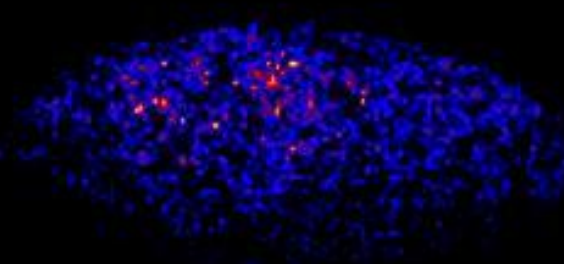
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m/z 1198 actin



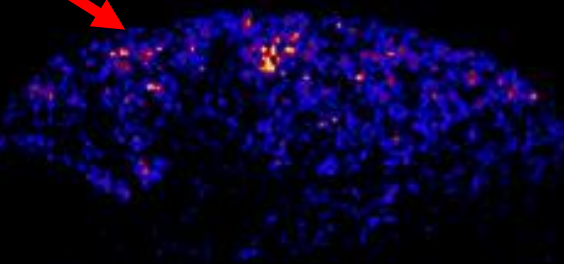
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m/z 1529 HBA



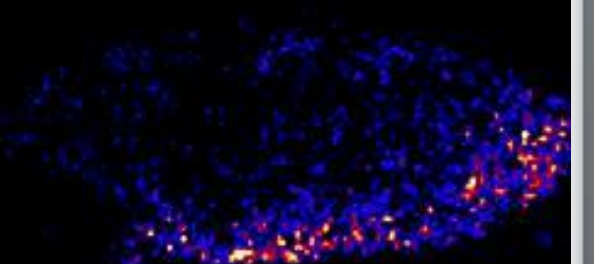
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m/z 1490



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m/z 1029

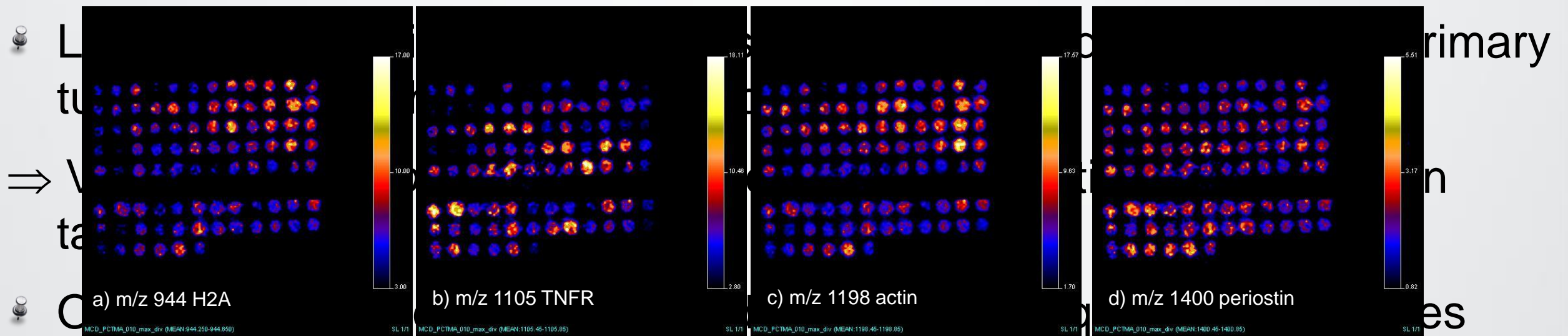


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To summarise: 'Every picture tells a story'

- Possibility to map and localise hypoxia at the primary tumour site. Identification of several hypoxia regulated proteins using LCM combined with ESI-MS.

⇒ Use of quantitative methodologies such as iTRAQ



using SILAC methodologies for the investigation of proteins involved in the pre-metastatic niche formation.

- Application on clinical samples from patients and TMA

Acknowledgments

Hypoxia and Metastasis team

Janine Erler
Holly Barker
Tom Cox
Georgina Lang
Annie Baker
Joan Chang
Demelza Bird
Martina Mrsnik
Andreas Hadjiprocopis



Industrial Collaborators

Applied Biosystems
Waters Corporation
Bruker
Thermo
Denator

Thank you for your attention