25 May 2021 - on-line event

Vibrational imaging approaches for cancer diagnosis: status, needs and perspectives

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CNR IFN Istituto di Fotonica e Nanotecnologie

Laserlab Europe

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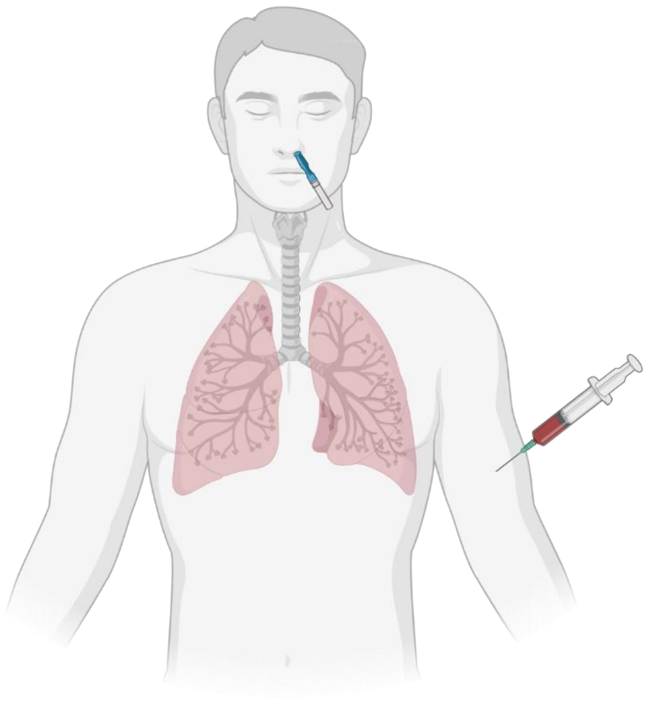
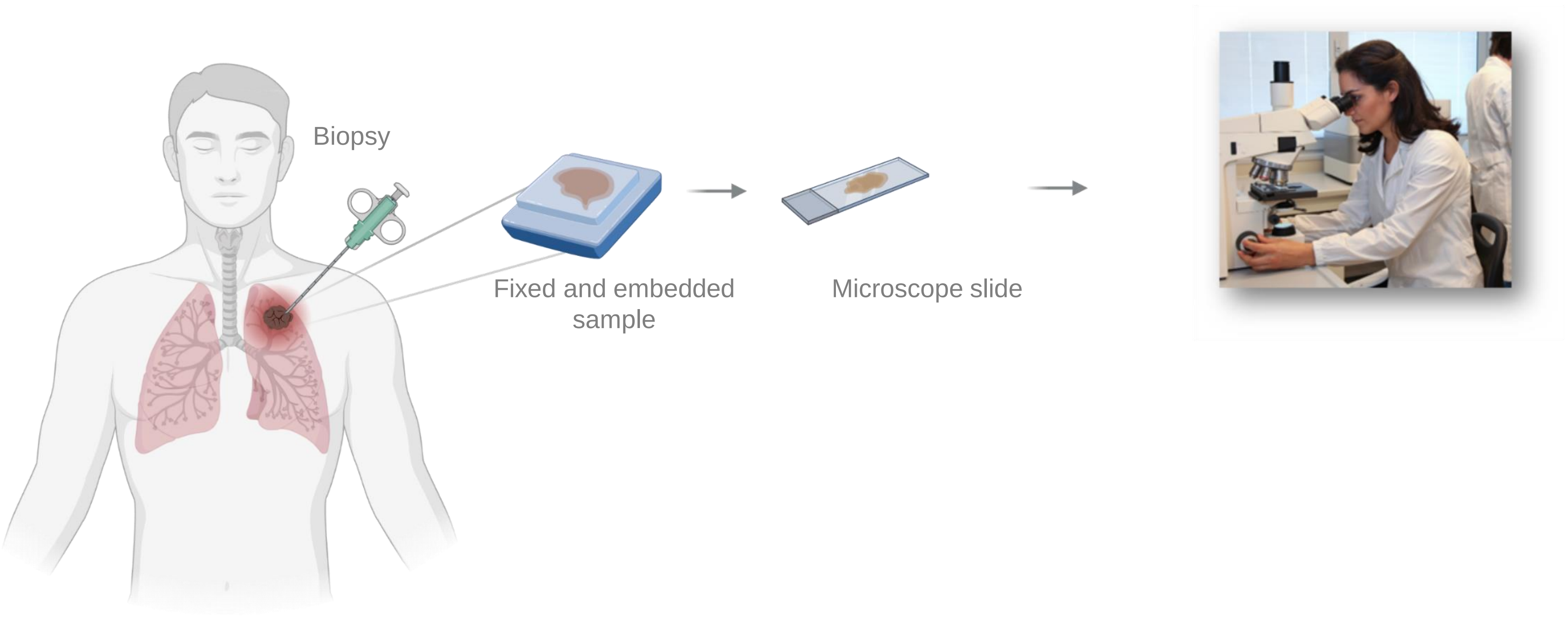
For some diseases we have clear biomarkers



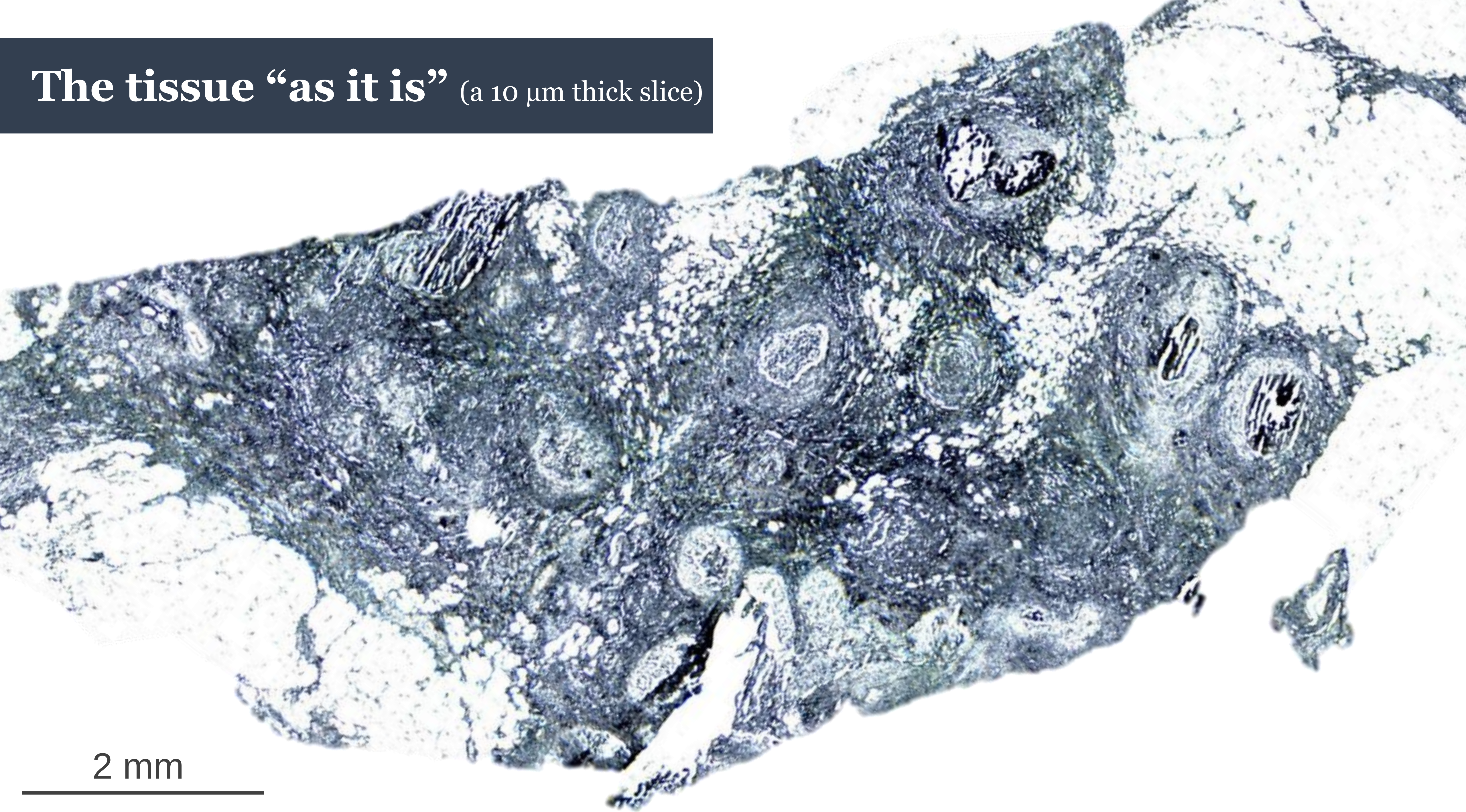
- Genetic diseases (e.g. huntington disease)
- Metabolic diseases (e.g. diabetes)
- Infective diseases (e.g. COVID-19)

… **blood**, **nasal swab** or even **saliva** can be enough for diagnosis.

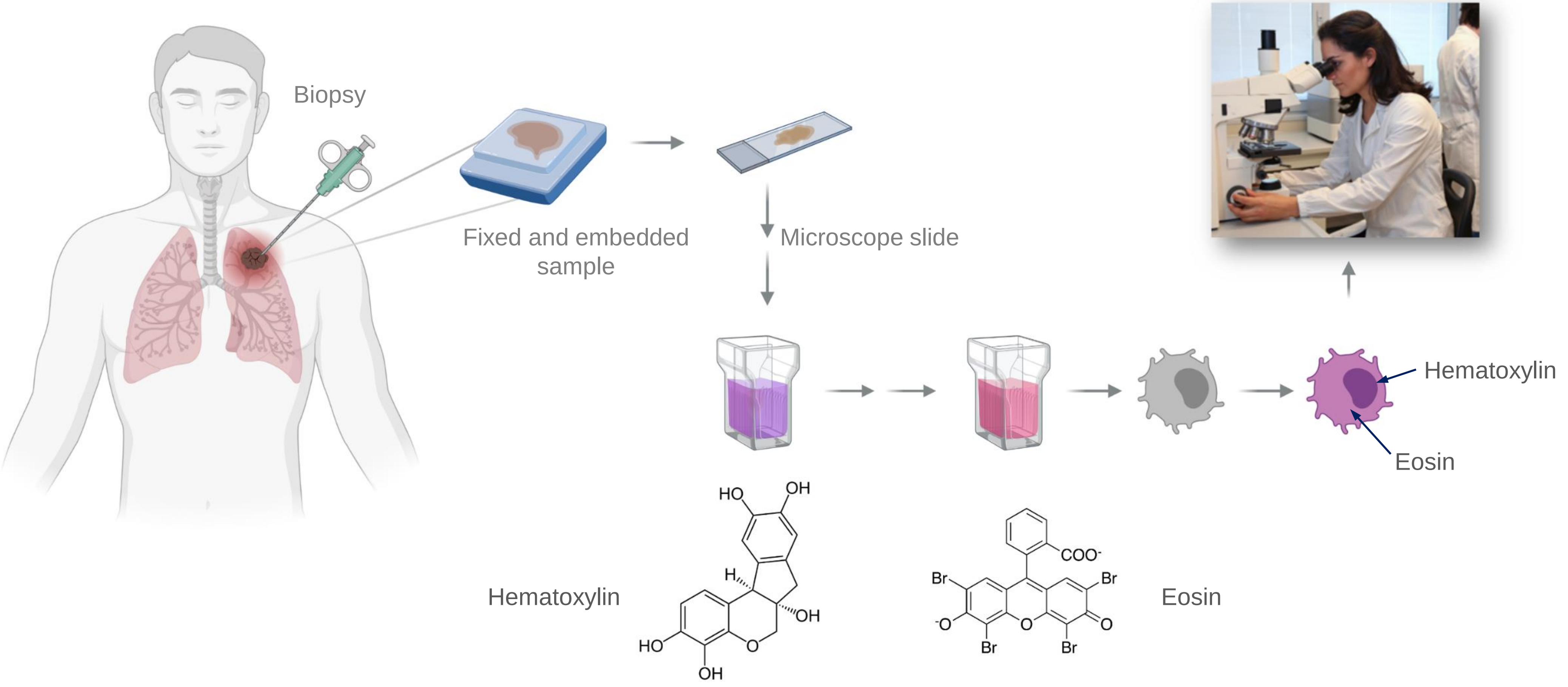
For cancer diagnosis we need to "observe it" (most of the time)



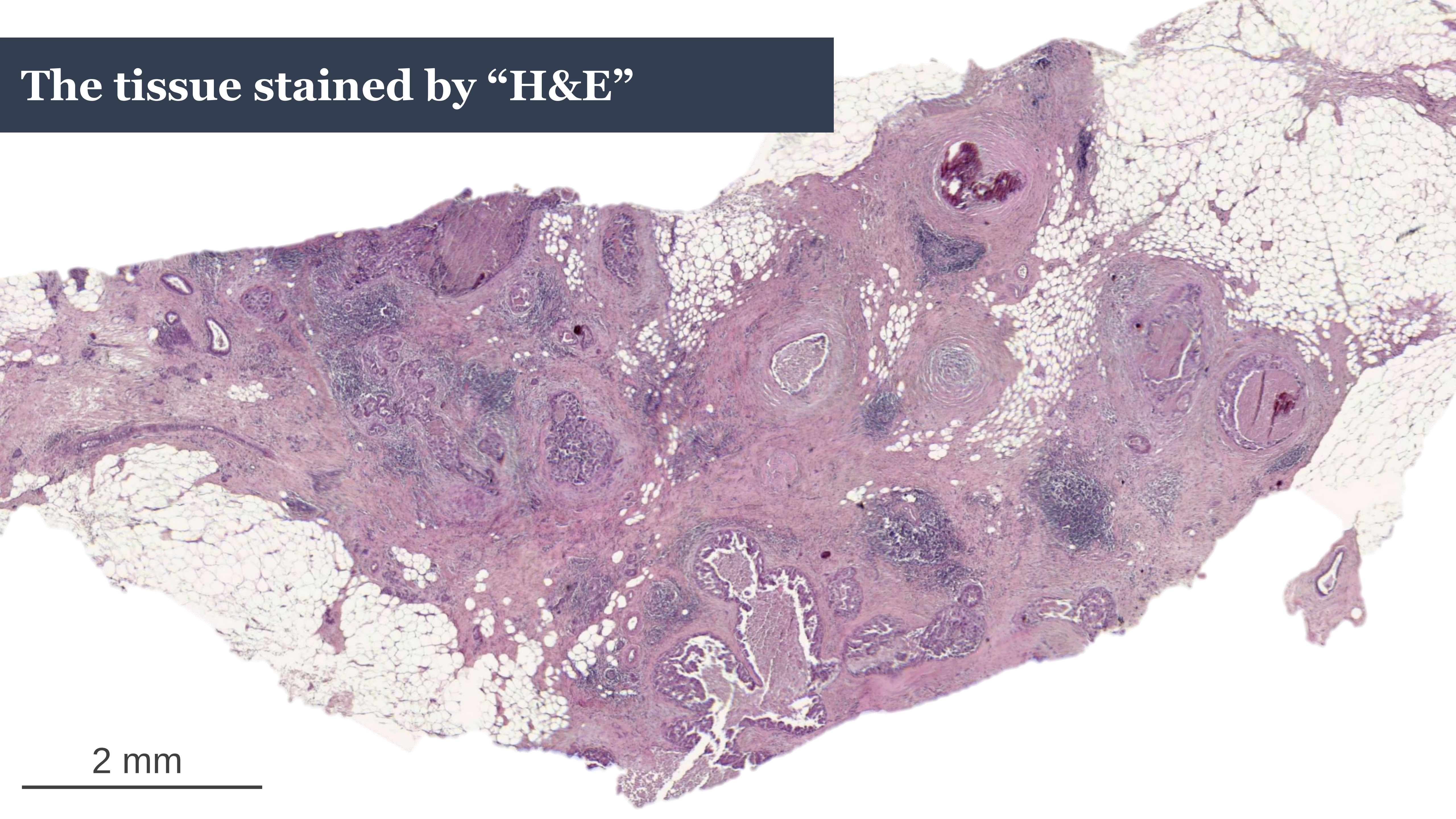
The tissue “as it is” (a 10 µm thick slice)

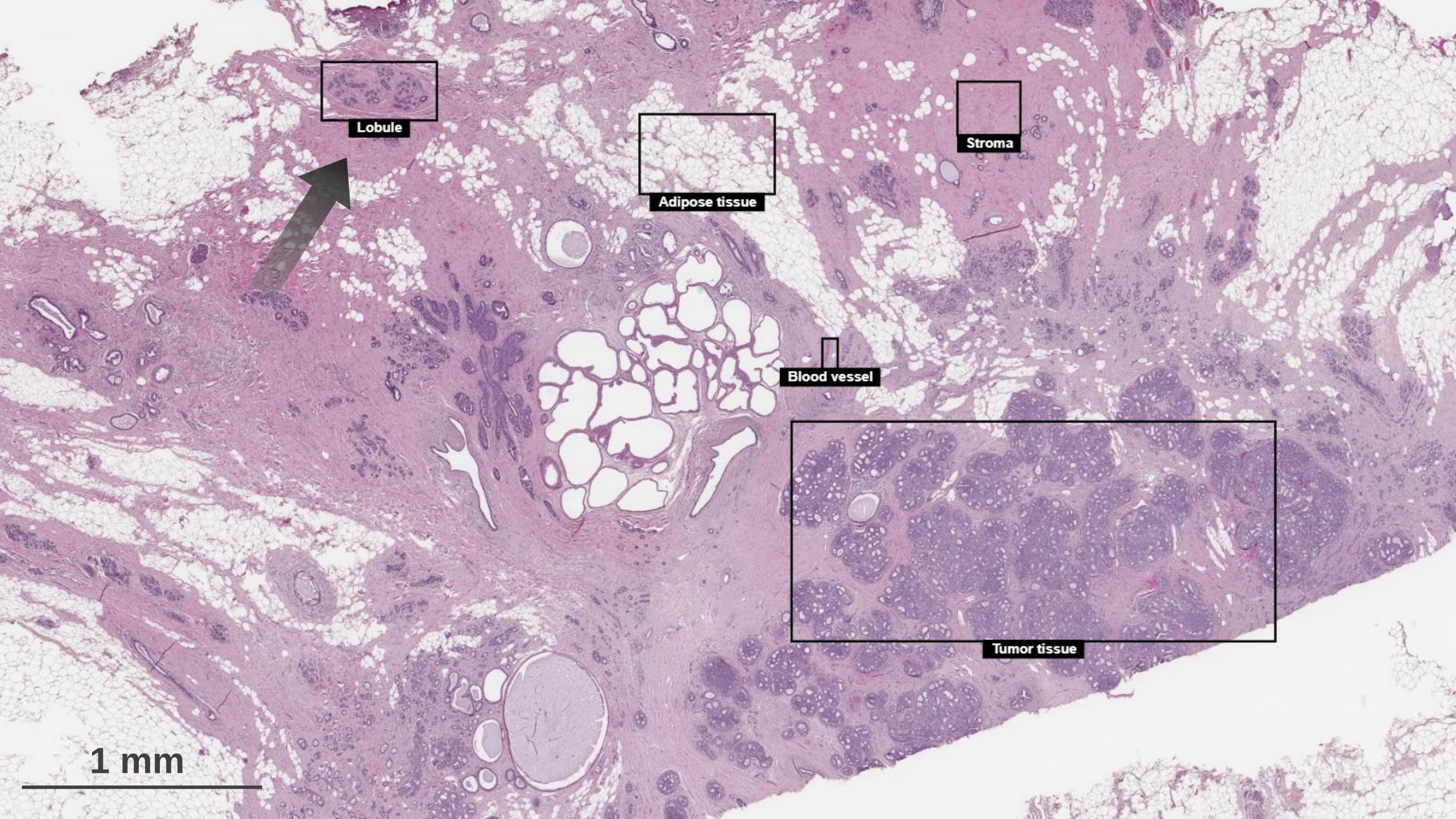


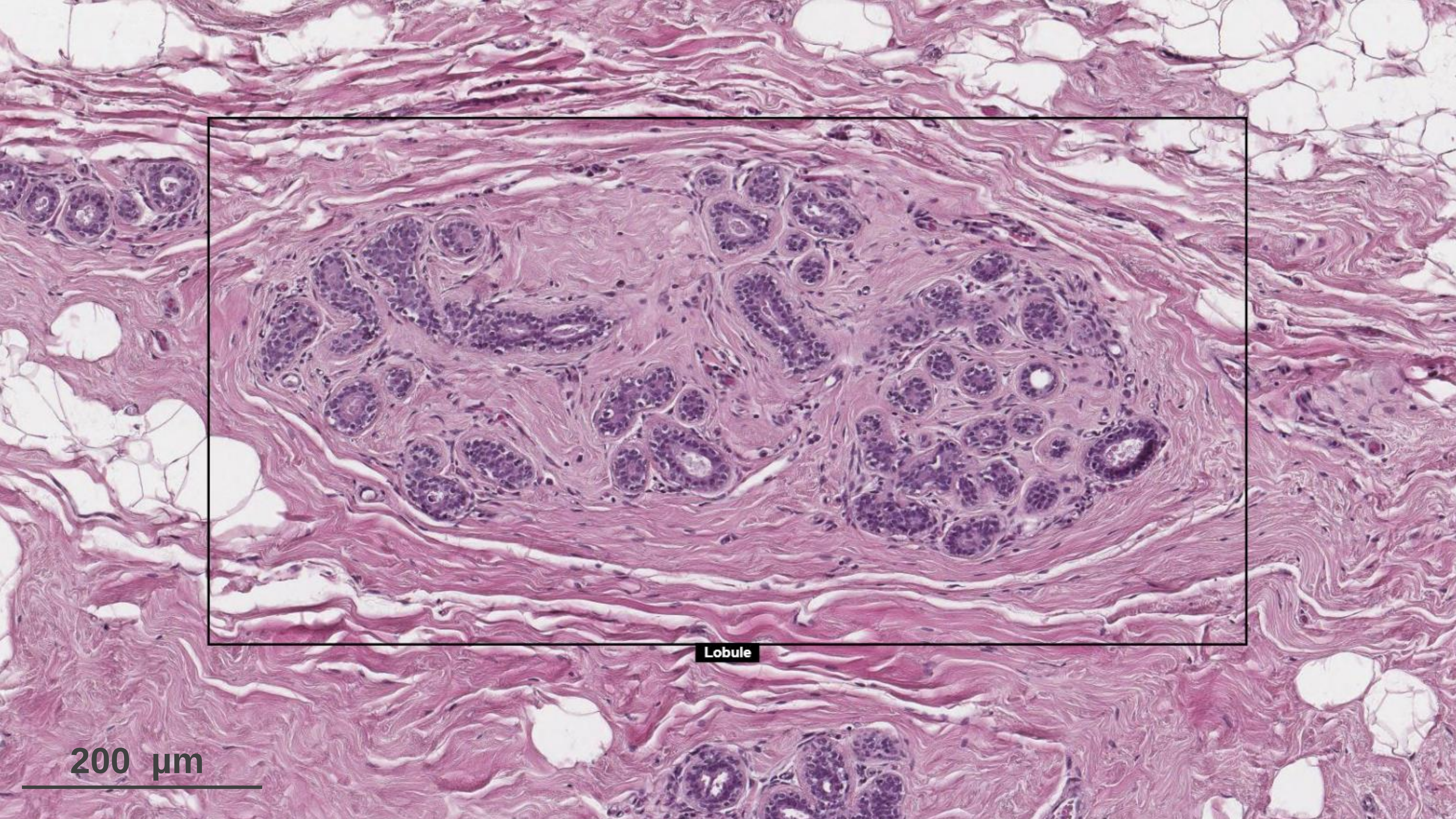
We need to define a contrast. Today, by chemical staining

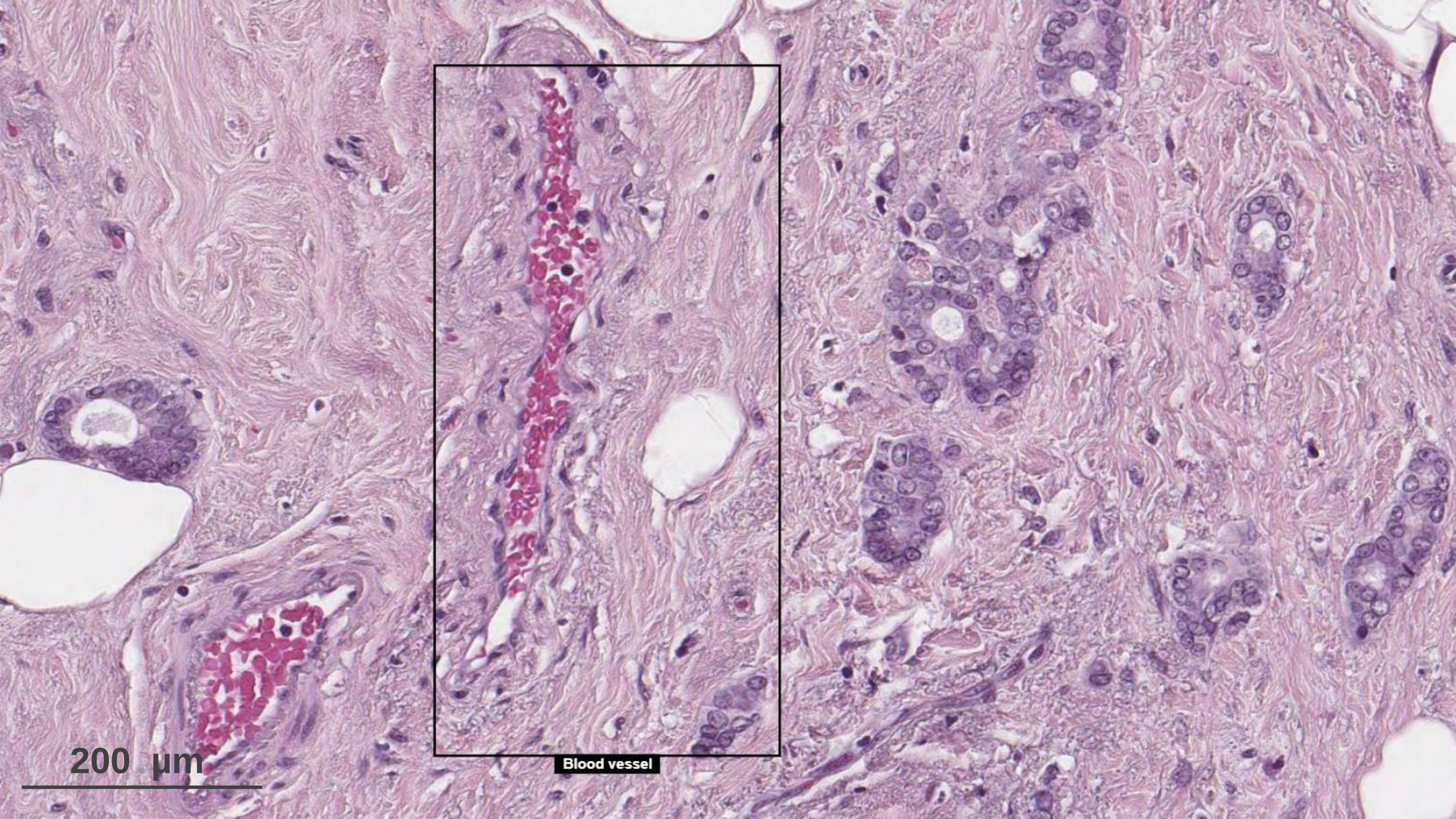


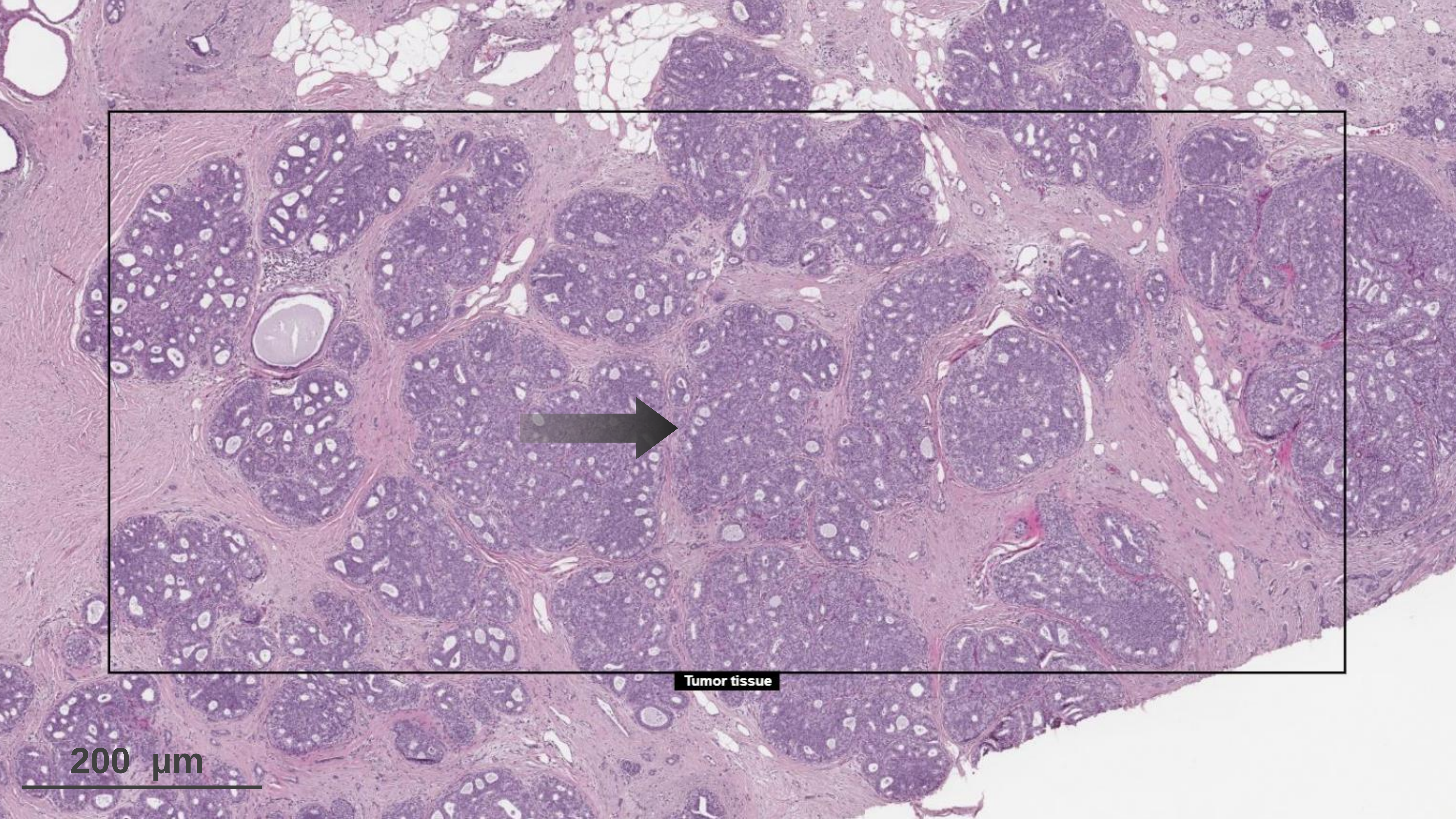
The tissue stained by “H&E”

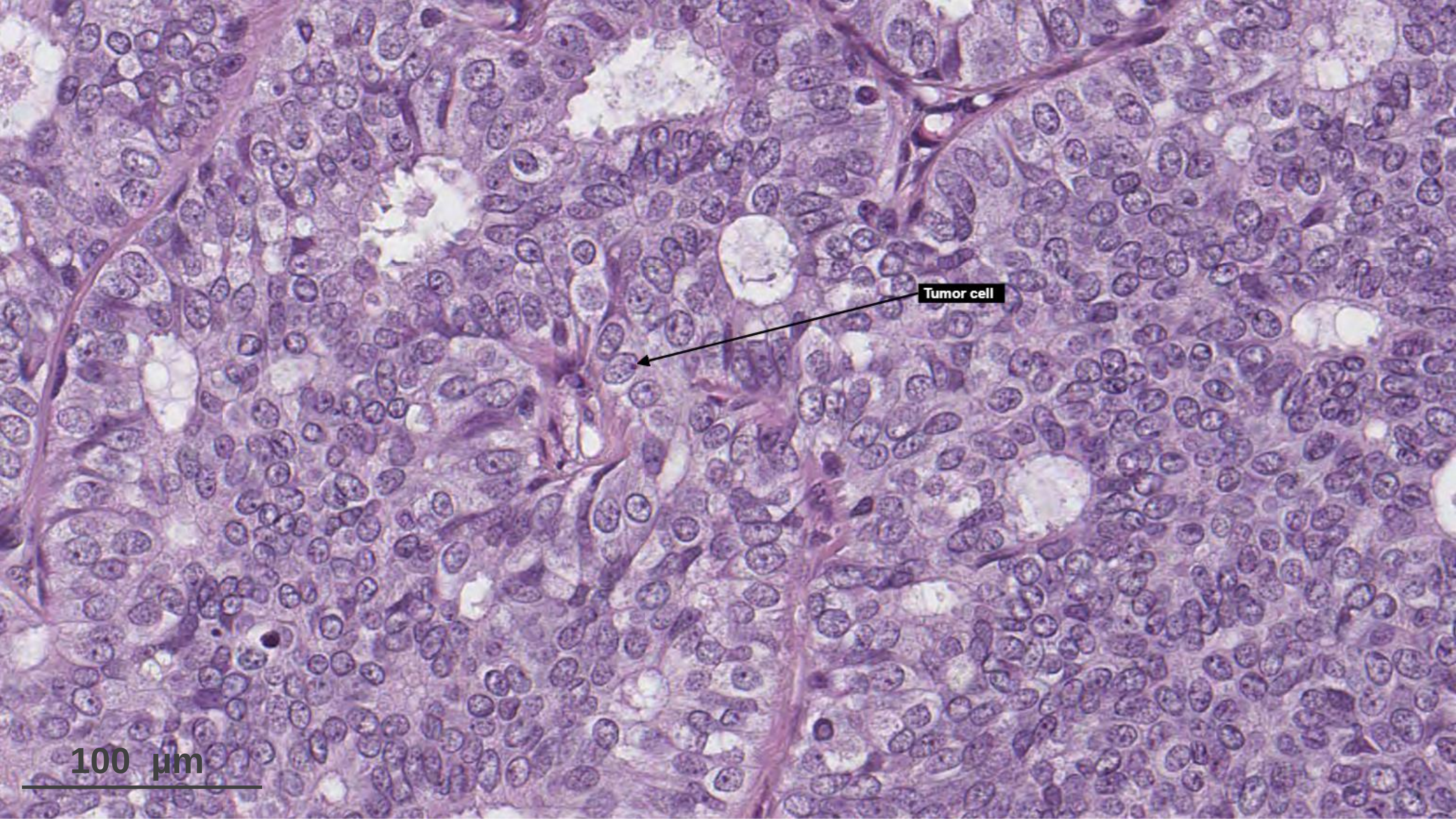



Lobule
Adipose tissue
Stroma
Blood vessel
Tumor tissue
1 mm


Lobule
200 µm


Blood vessel
200 µm


Tumor tissue
200 μm


Tumor cell
100 µm

Main Limitations

- High Subjectivity and Error prone
- Time-consuming (sample preparation and staining)
- Scarce molecular characterization (only morphological)
- Other biomolecular characterization is normally required

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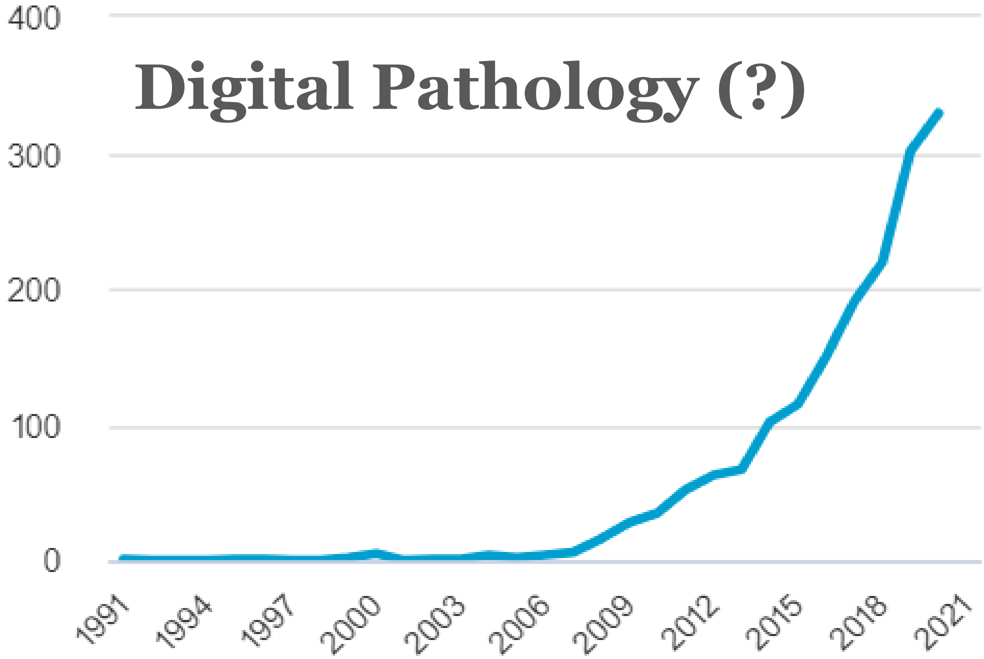
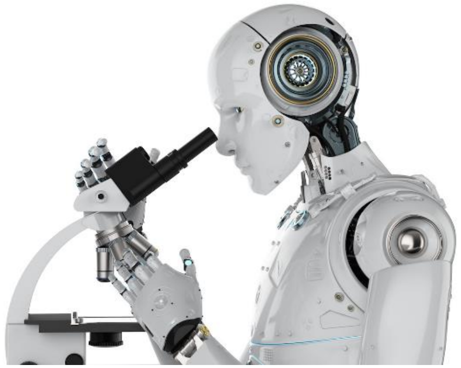
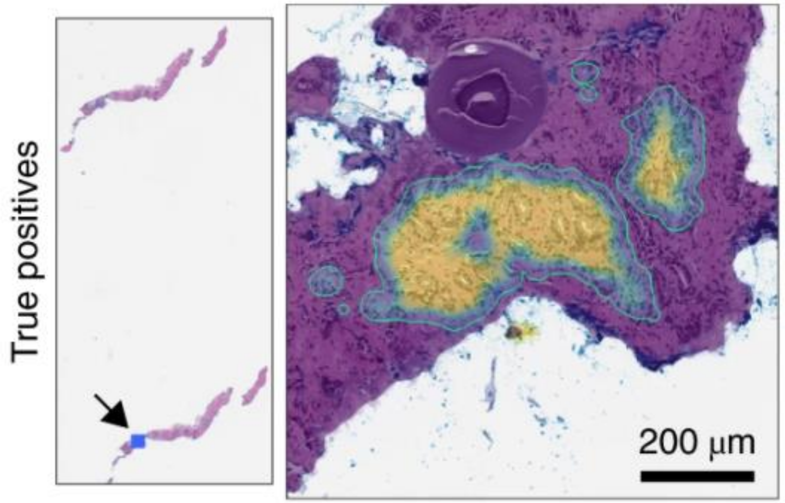


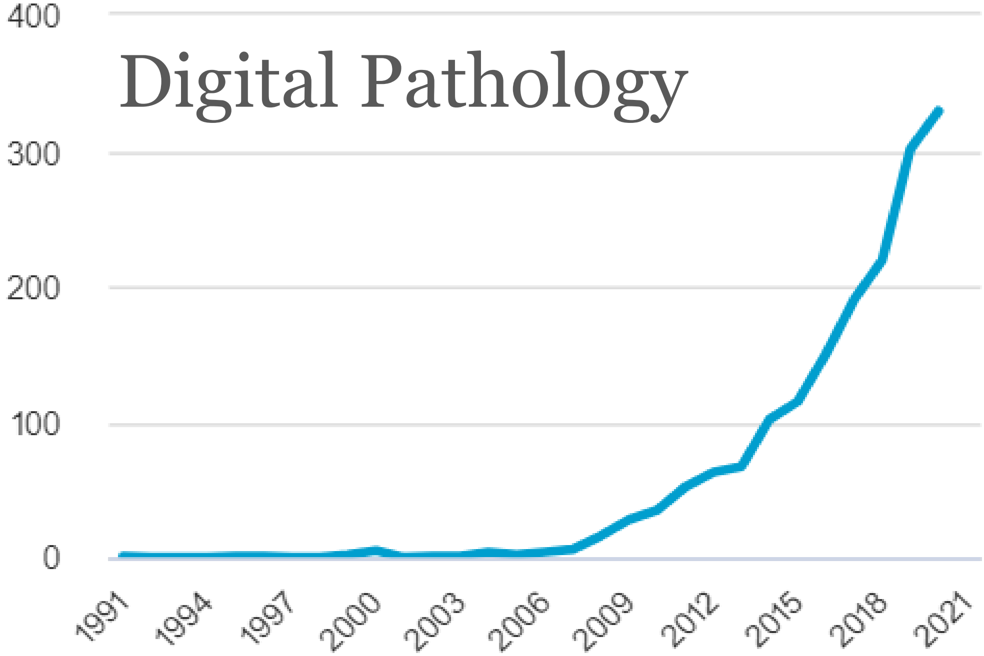


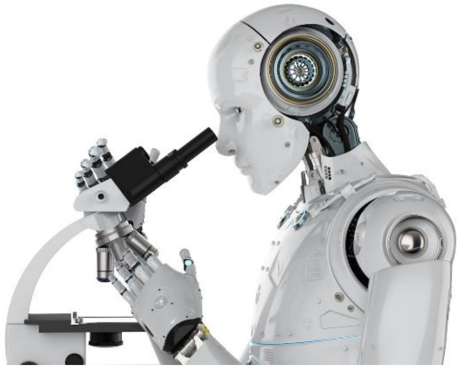
Bera, Kaustav, et al. *Nature reviews Clinical oncology* 16.11 (2019): 703-715.

Main Limitations



- **More objective**
- **Fast (visualization)** – same time for staining
- Scarce molecular characterization (only morphological)





Campanella, Gabriele, et al. *Nature medicine* 25.8 (2019): 1301-1309.

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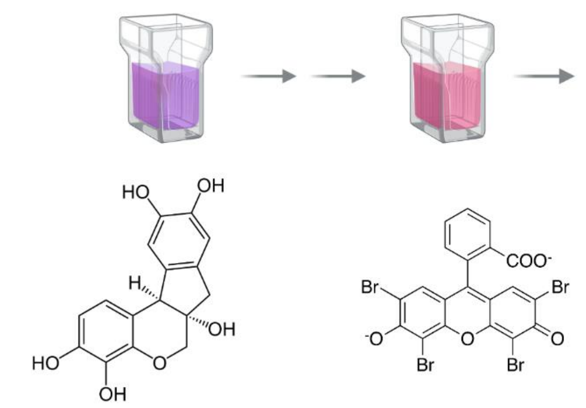

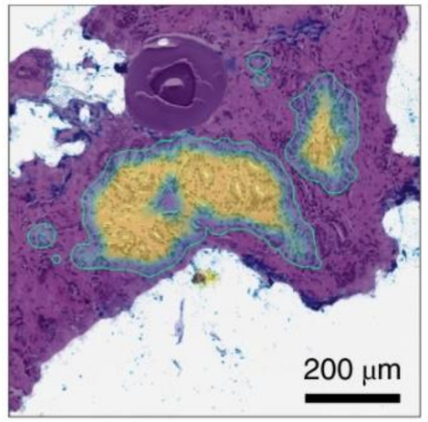

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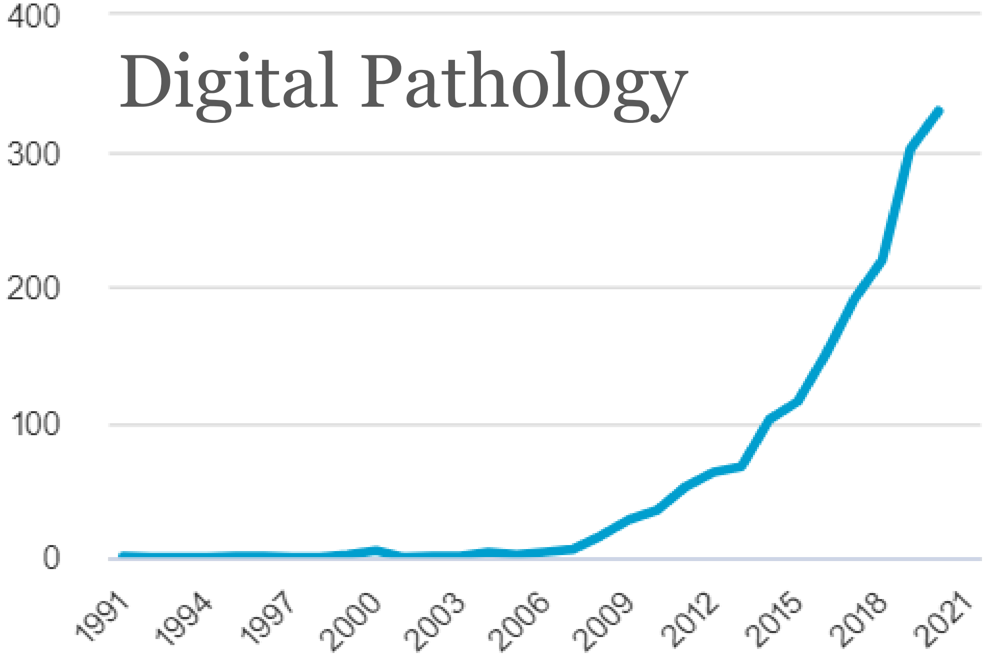
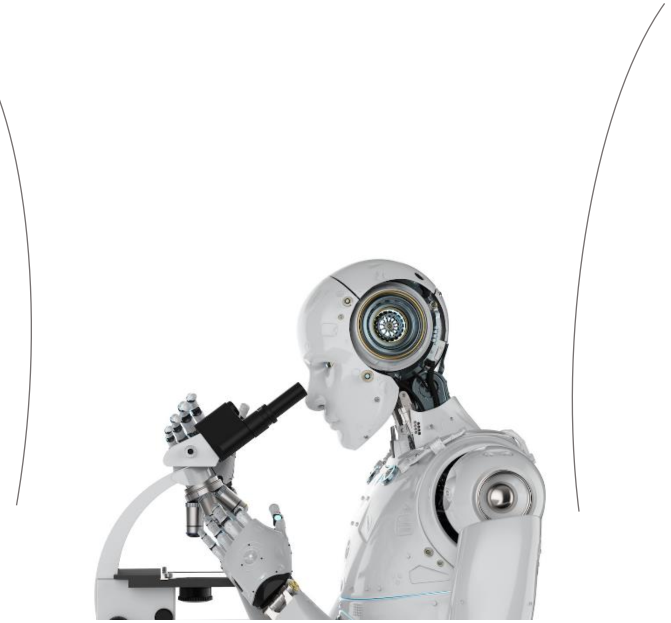
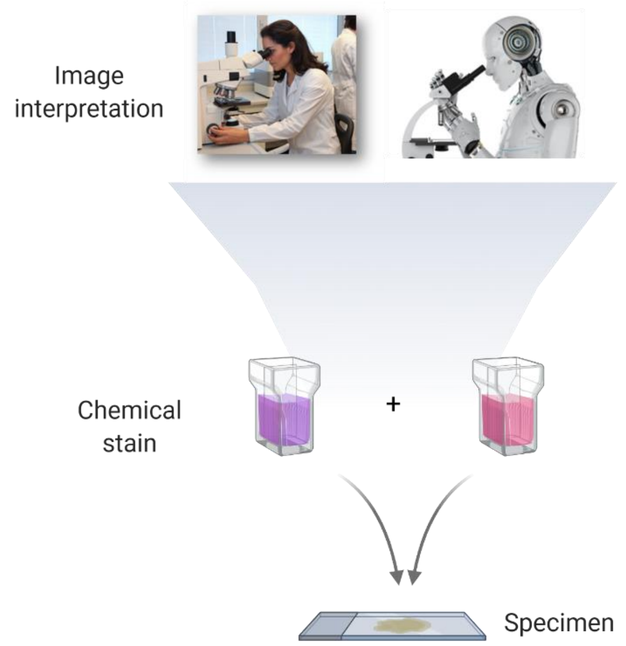


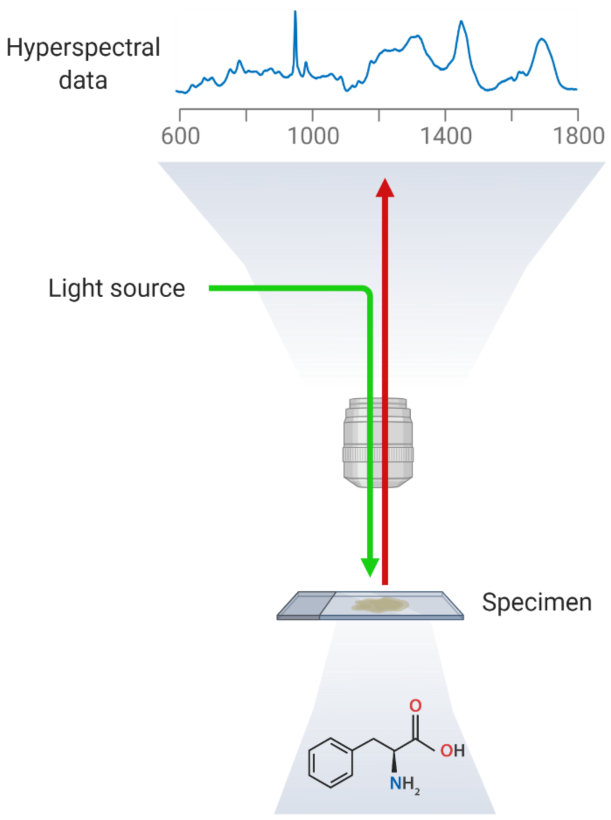


Campanella, Gabriele, et al. *Nature medicine* 25.8 (2019): 1301-1309.

Vibrational imaging for cancer diagnosis

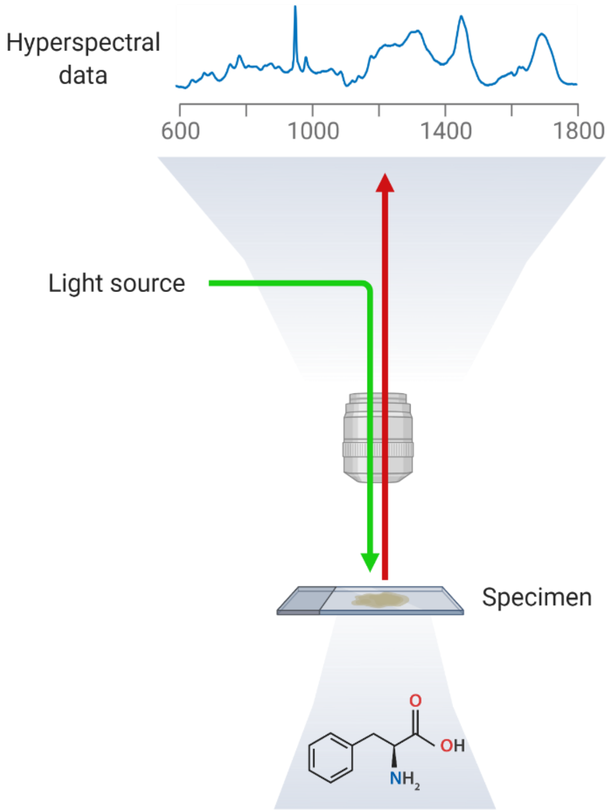
Chemical staining *vs* **Vibrational imaging**

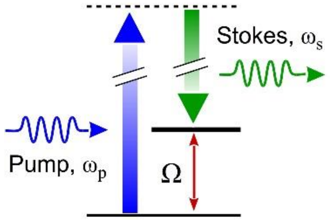




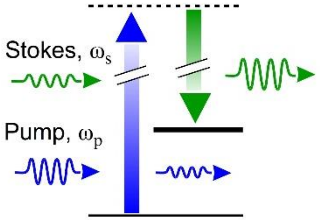
- Highly informative
- Objective
- Label-free
- Compatible with automatic procedures

Vibrational imaging for cancer diagnosis

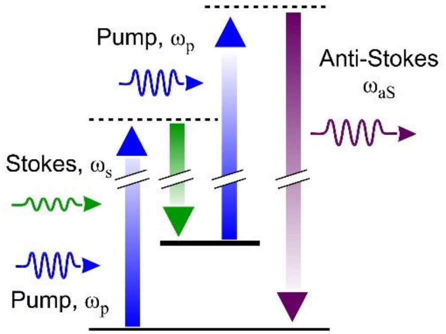




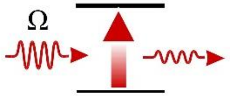
Raman scattering



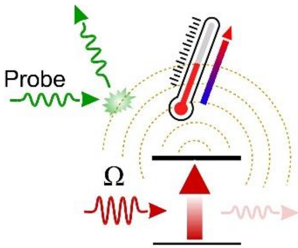
Stimulated Raman Scattering (SRS)



Coherent Anti-Stokes Raman Scattering (CARS)



Mid Infrared Adsorption
(FT-IR spectroscopy)
(QCL IR spectroscopy)



Photothermal effect

Vanna, Renzo, et al. *La Rivista del Nuovo Cimento (in preparation),* (2021)

Renzo Vanna, PhD

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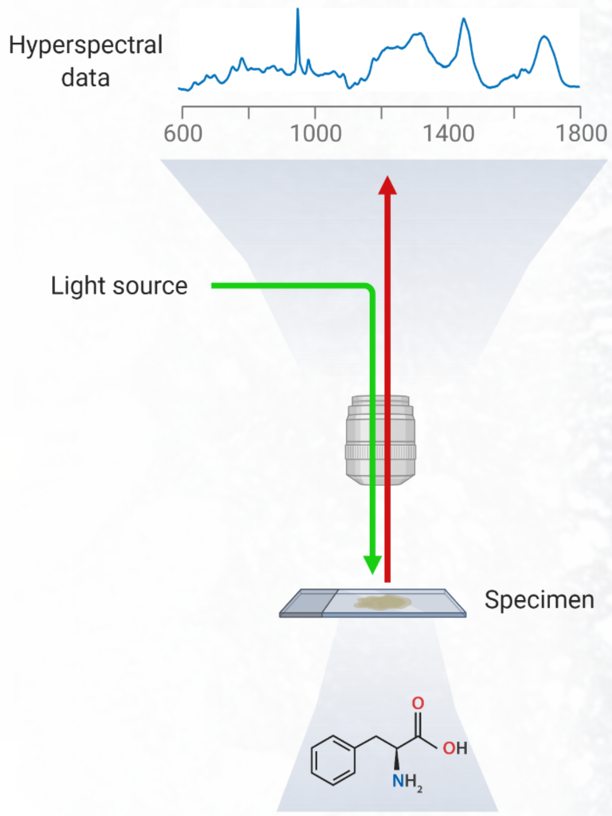
Vibrational imaging for cancer diagnosis: how it works



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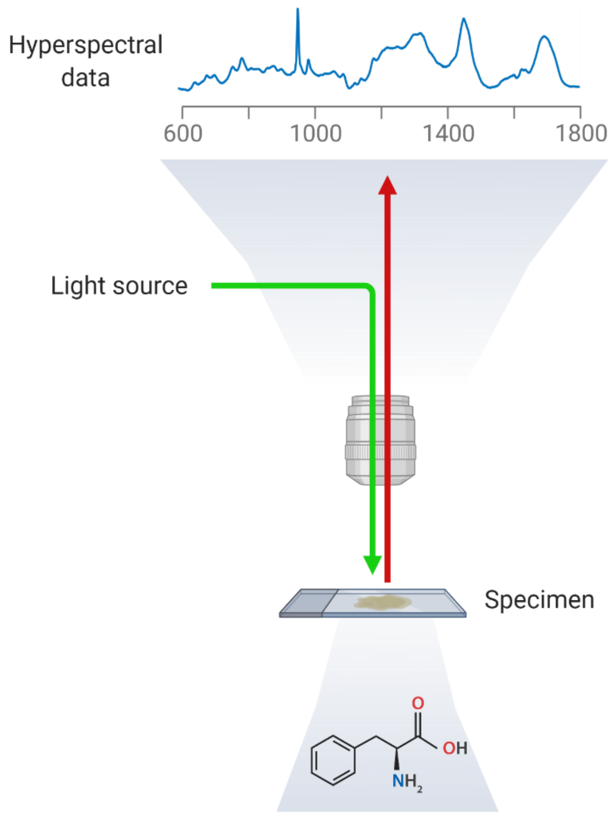

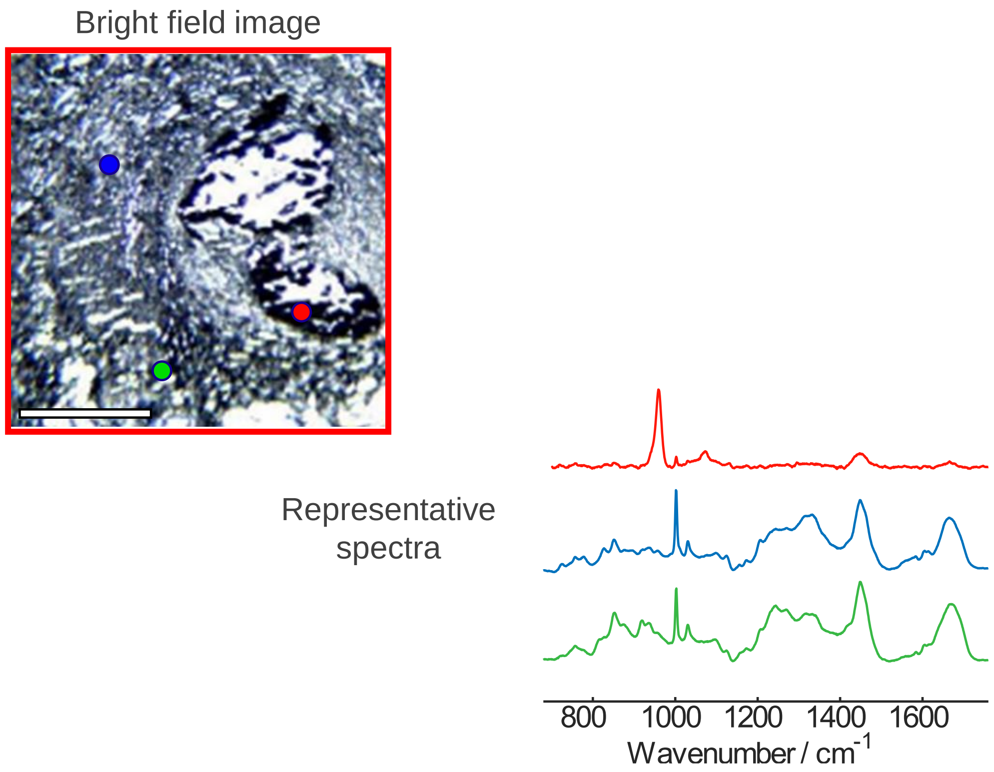

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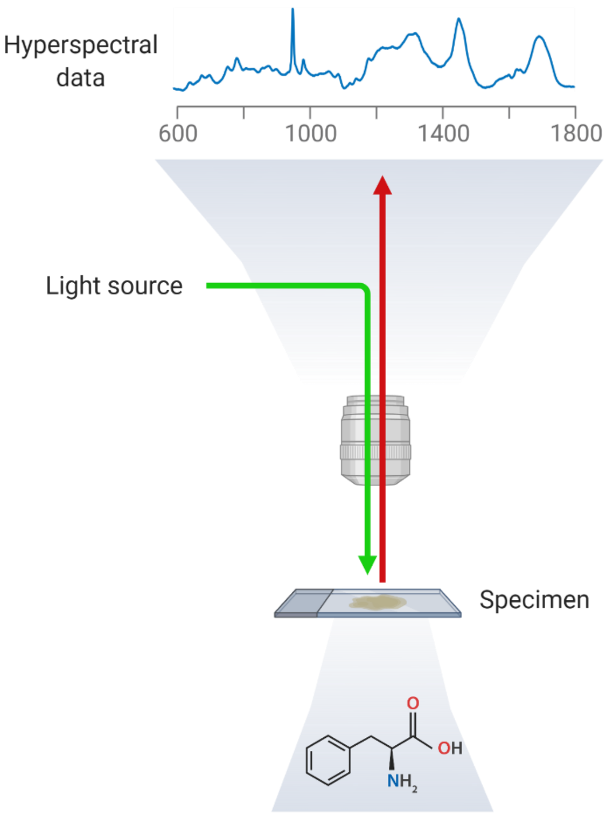

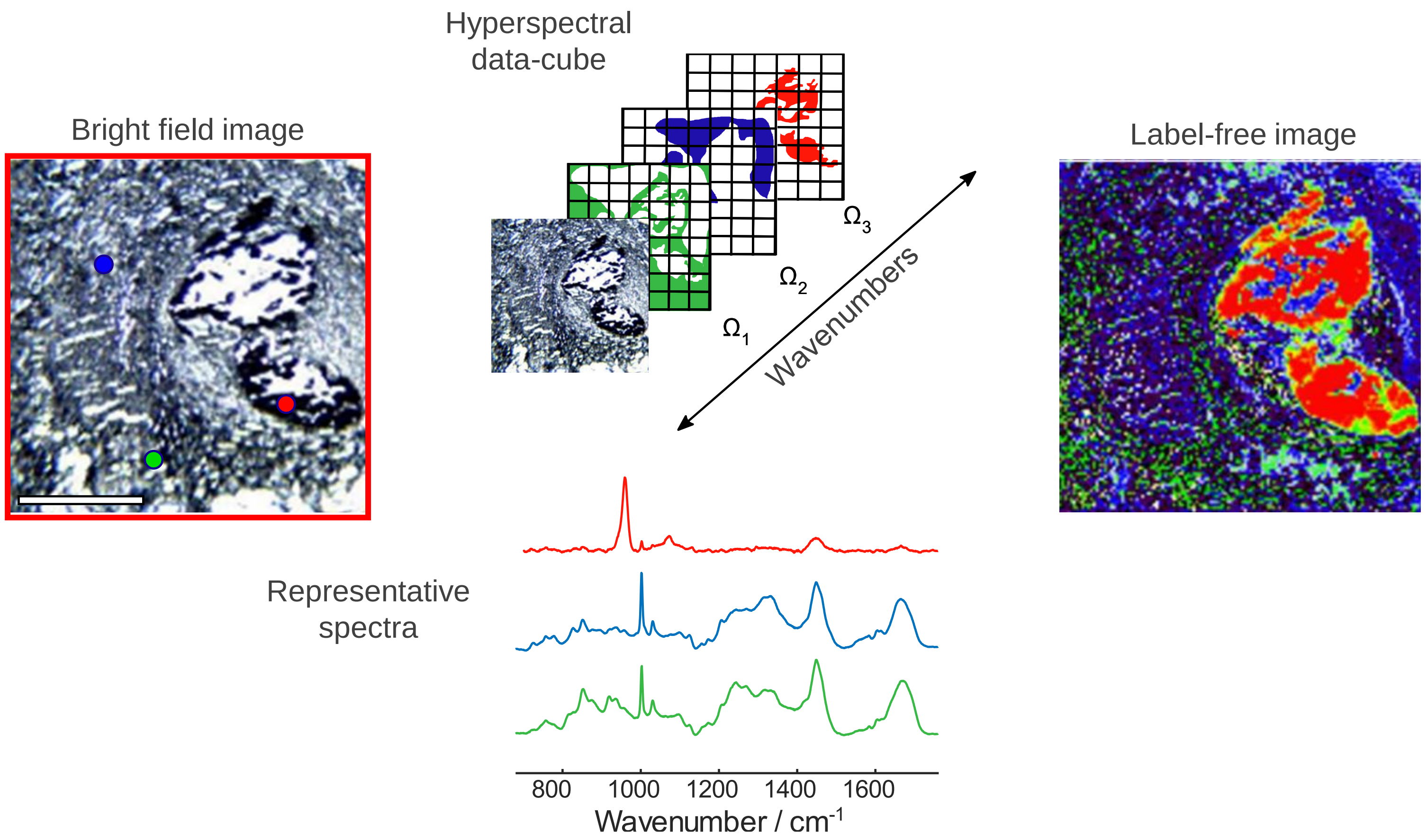

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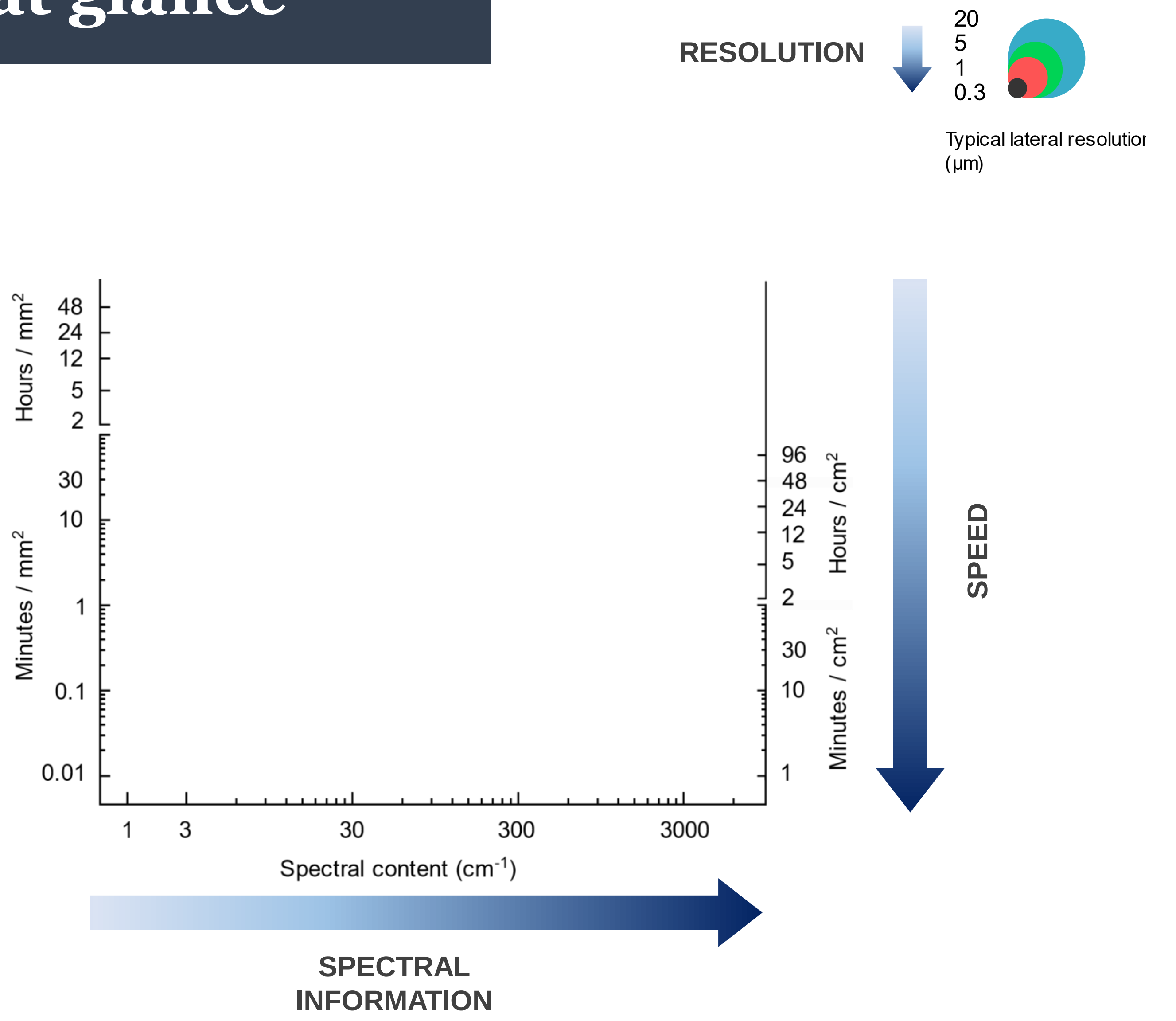
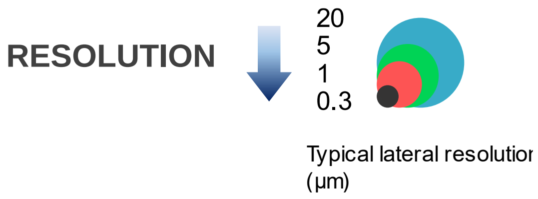
Vibrational spectroscopies at glance



Vanna, Renzo, et al. *La Rivista del Nuovo Cimento (in preparation),* (2021)

Raman microscopy



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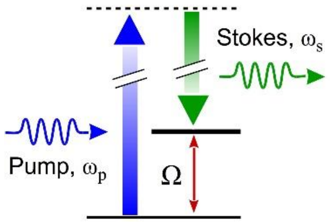
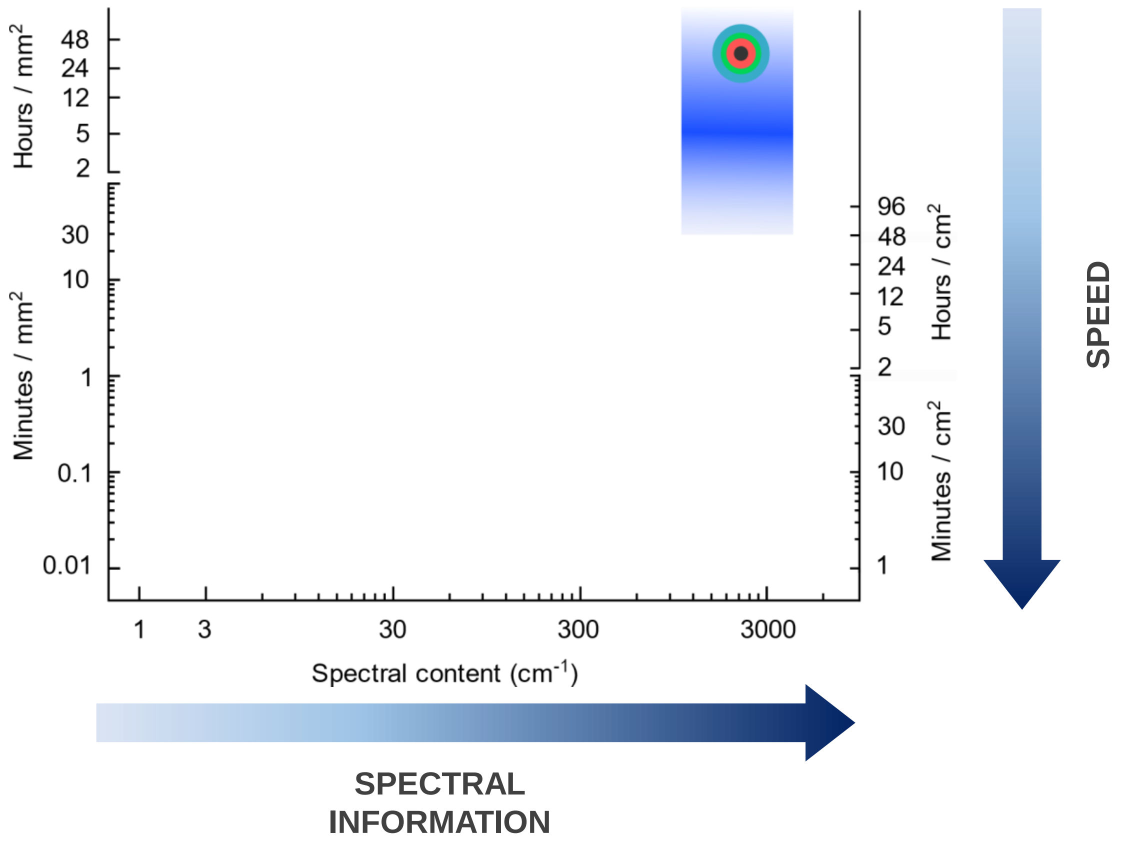


Raman scattering
(Raman microscopy)



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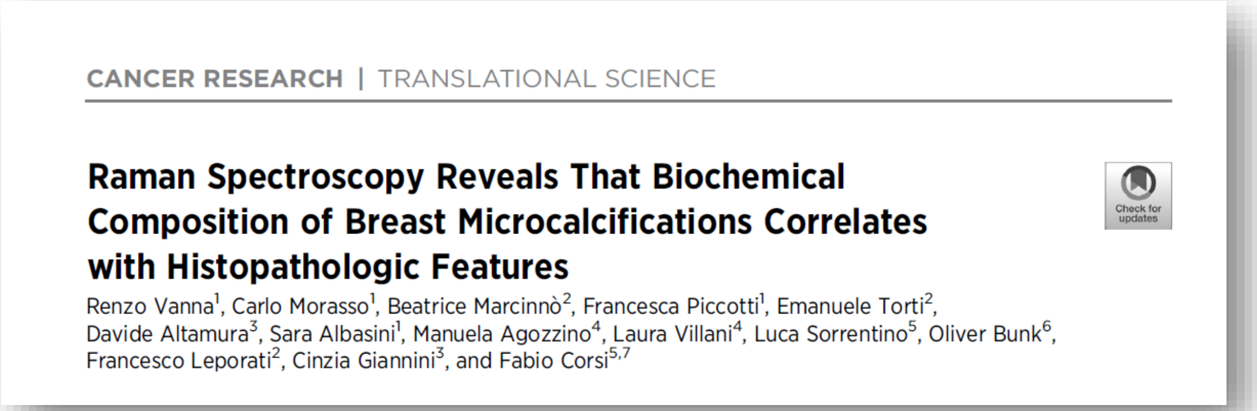
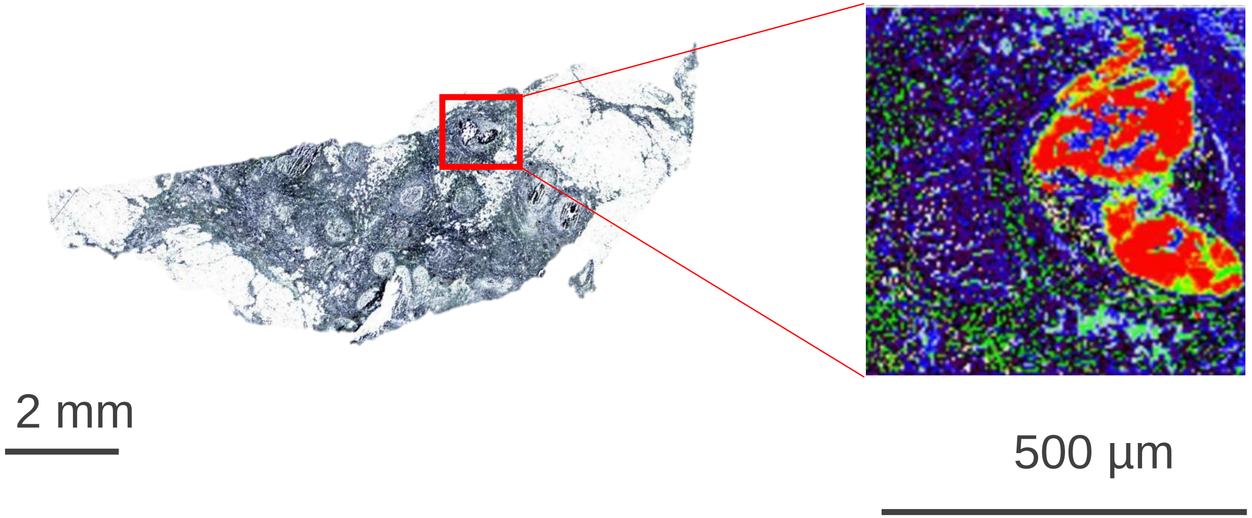
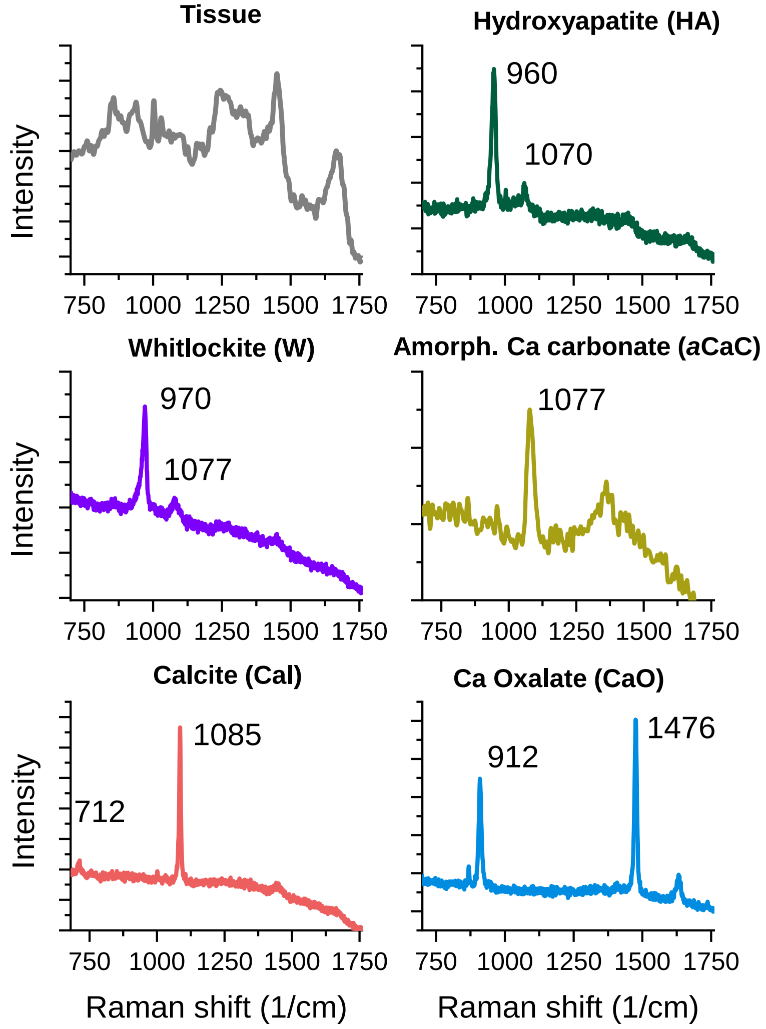
Raman imaging: example 1


CANCER RESEARCH | TRANSLATIONAL SCIENCE

Raman Spectroscopy Reveals That Biochemical Composition of Breast Microcalcifications Correlates with Histopathologic Features

Renzo Vanna[1], Carlo Morasso[1], Beatrice Marcinnò[2], Francesca Piccotti[1], Emanuele Torti[2], Davide Altamura[3], Sara Albasini[1], Manuela Agozzino[4], Laura Villani[4], Luca Sorrentino[5], Oliver Bunk[6], Francesco Leporati[2], Cinzia Giannini[3], and Fabio Corsi[5,7]





Vanna, Renzo, et al. *Cancer research* 80.8 (2020): 1762-1772.

Raman imaging: example 1

CANCER RESEARC

Raman Spectr
Composition o
with Histopath

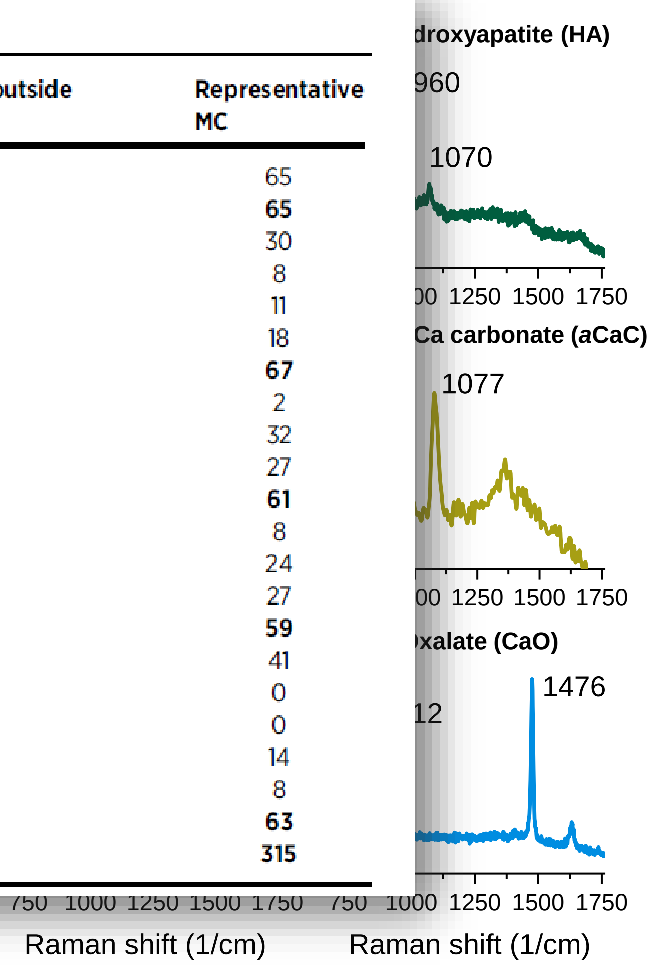
Renzo Vanna[1], Carlo Mo
Davide Altamura[3], Sara
Francesco Leporati[2], Cir

Table 1. Number of microcalcifications detected from different subjects.

B category	Histological classification	Patients	Total MC	MC found outside the lesion	Representative MC
B1 "normal tissue"	NOR	6	65	—	65
Total		**6**	**65**	**—**	**65**
B2 "benign"	FAD	4	30	—	30
	FIB	2	8	—	8
	FNE	1	11	—	11
	UDH	2	18	-	18
Total		**9**	**67**	**—**	**67**
B3 "of uncertain malignancy"	PAP	2	2	—	2
	FEA (DIN1A)	2	32	—	32
	ADH (DIN1B)	4	27	—	27
Total		**8**	**61**	**—**	**61**
B5a "carcinoma *in situ*"	DCIS DIN1C	3	19	*11*	8
	DCIS DIN2	8	32	*8*	24
	DCIS DIN3	6	46	*19*	27
Total		**17**	**97**	***38***	**59**
B5b "invasive carcinoma"	IDC G2	12	133	*92*	41
	IDC G3	1	2	*2*	0
	ILC G2	1	8	*8*	0
	ILC G3	1	33	*19*	14
	IMC	1	8	*0*	8
Total		**16**	**184**	***121***	**63**
Grand Total		**56**	**474**	***159***	**315**

2 mm

500 µm



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Raman imaging: example 1

CANCER RESEARCH | TRANSLATIONAL SCIENCE

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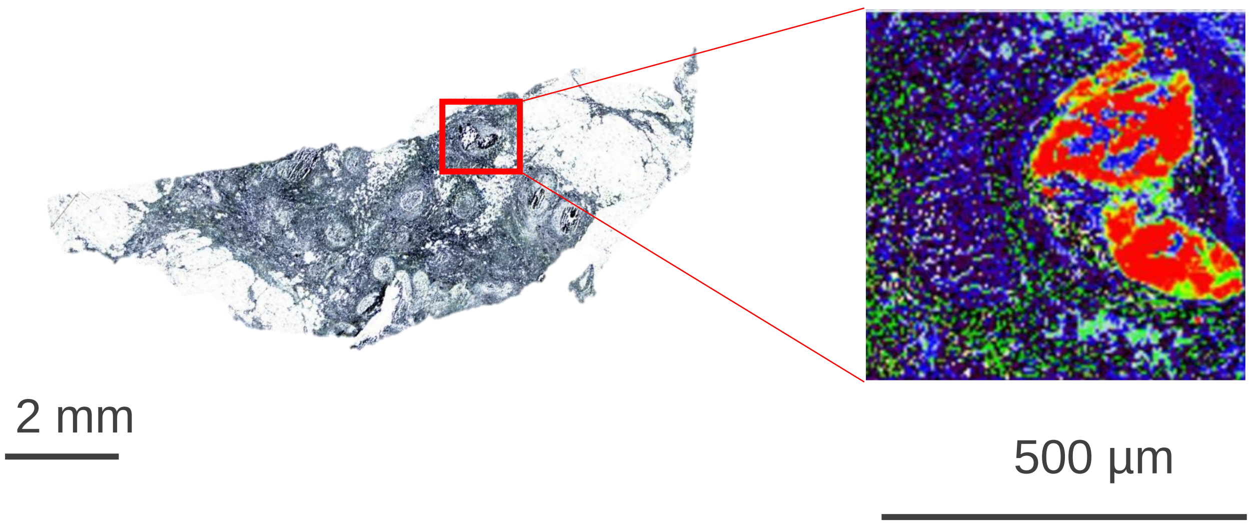

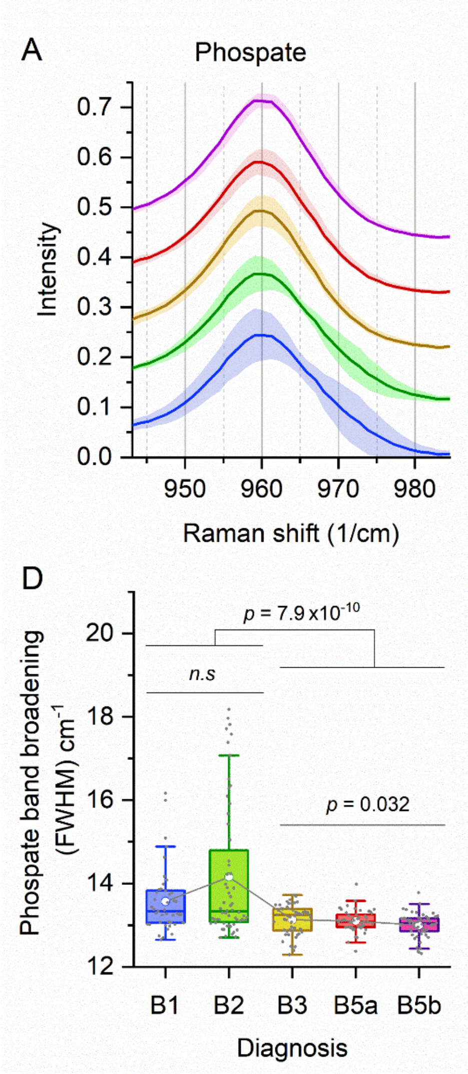

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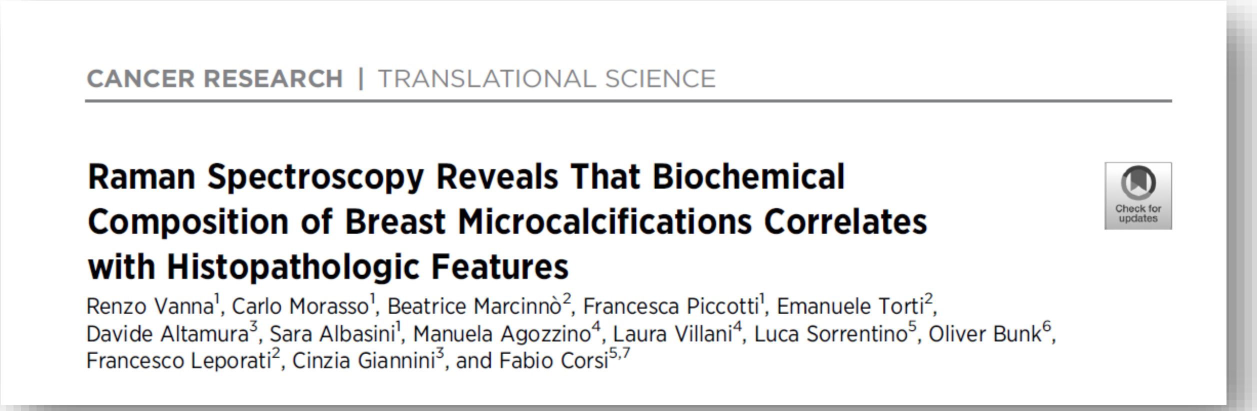
Raman imaging: example 1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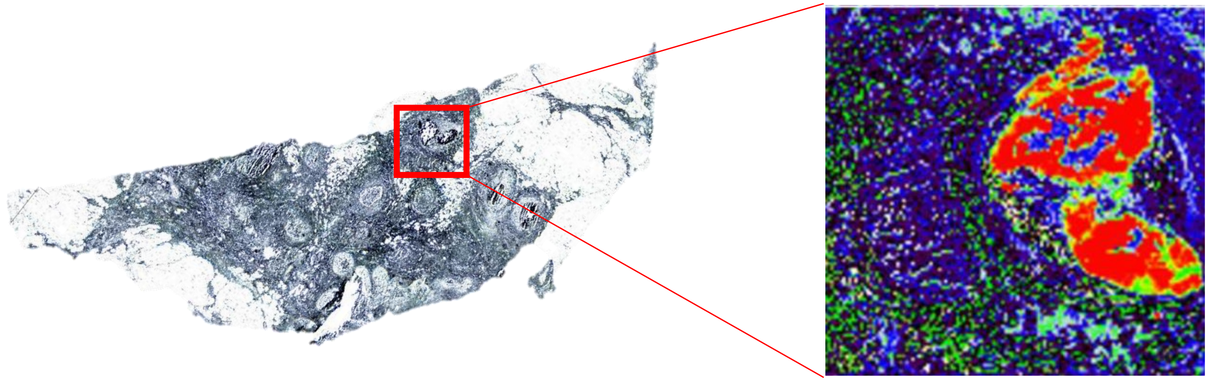
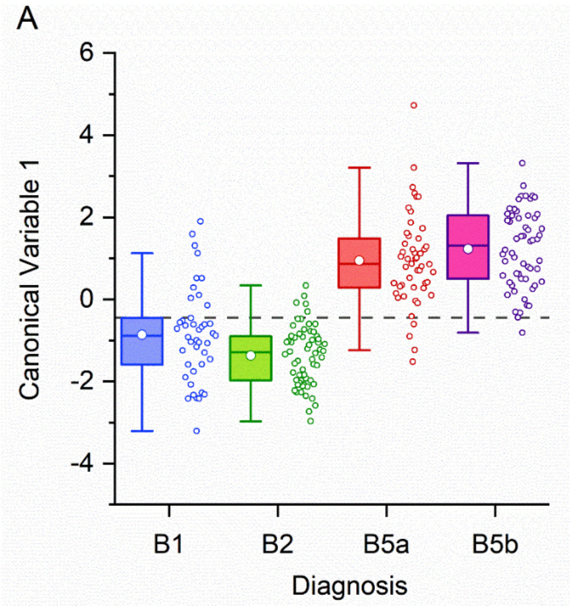
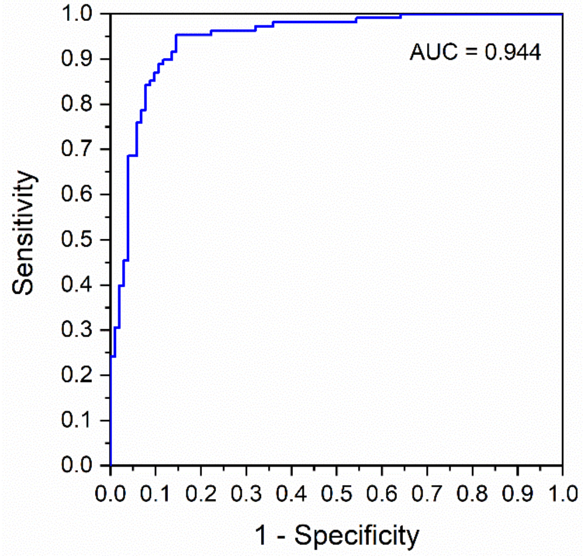


2 mm

500 µm (a few hours)





Vanna, Renzo, et al. *Cancer research* 80.8 (2020): 1762-1772.

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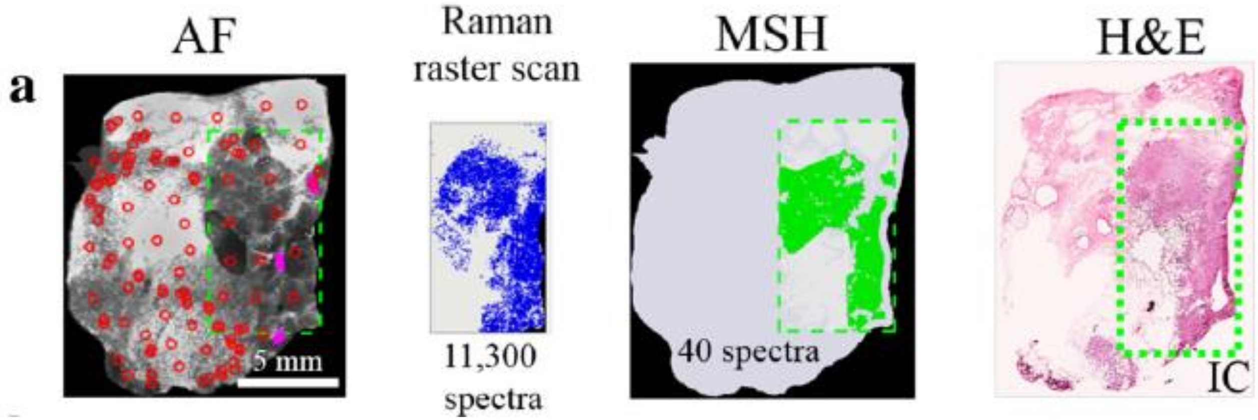
Raman imaging: example 2


Shipp *et al. Breast Cancer Research* (2018) 20:69
https://doi.org/10.1186/s13058-018-1002-2
Breast Cancer Research

RESEARCH ARTICLE Open Access

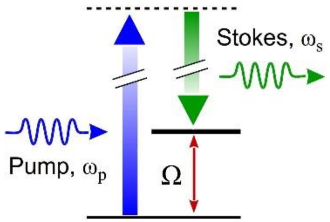
Intra-operative spectroscopic assessment of surgical margins during breast conserving surgery

Dustin W. Shipp[1], Emad A. Rakha[2], Alexey A. Koloydenko[3], R. Douglas Macmillan[4], Ian O. Ellis[2] and Ioan Notingher[1*]

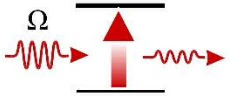


Shipp, Dustin W., et al. *Breast Cancer Research* 20.1 (2018): 1-14.

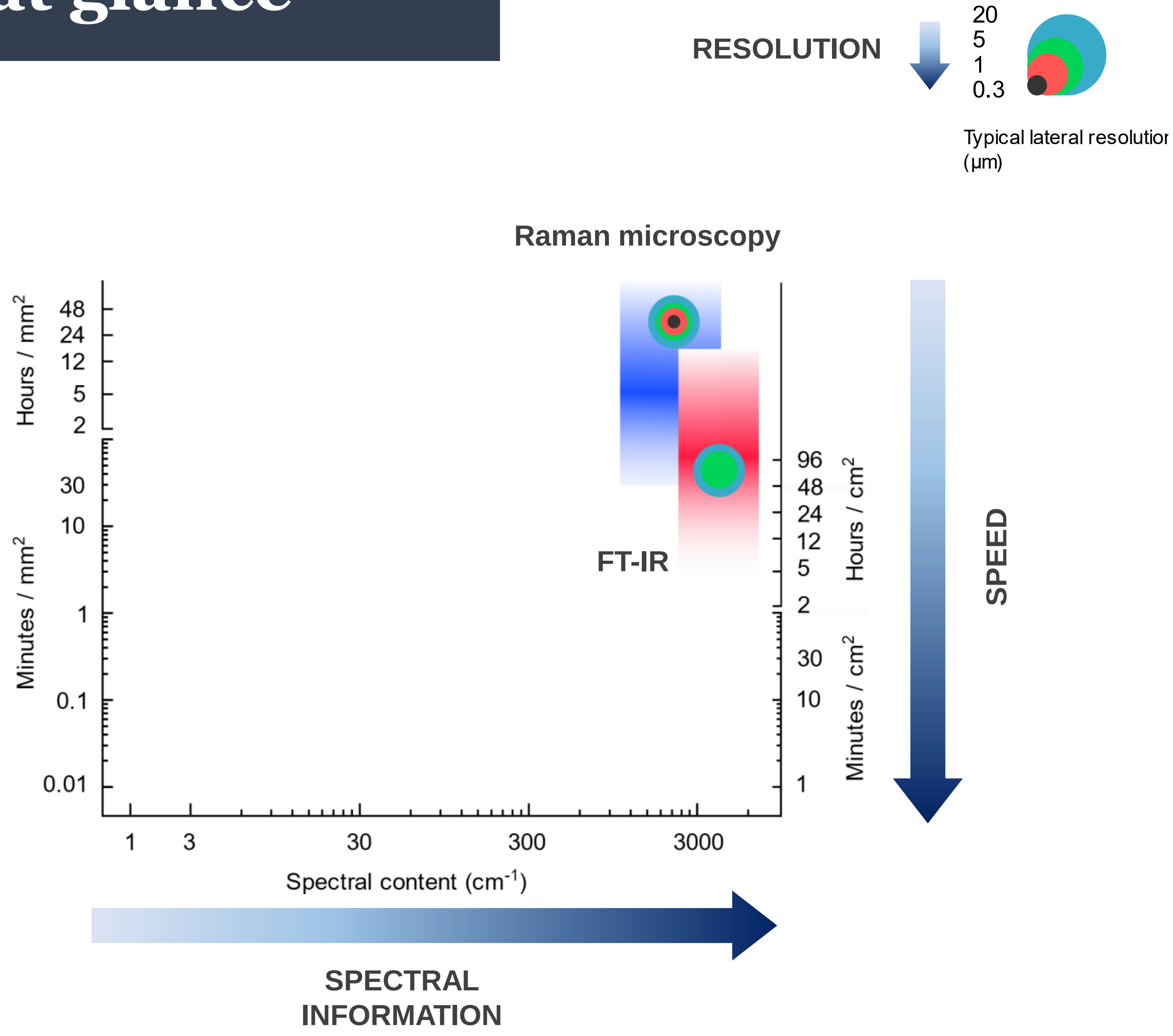
Vibrational spectroscopies at glance



Raman scattering
(Raman microscopy)



Mid Infrared Adsorption
(FT-IR spectroscopy)



Vanna, Renzo, et al. *La Rivista del Nuovo Cimento (in preparation),* (2021)

FT-IR imaging: example


nature biotechnology

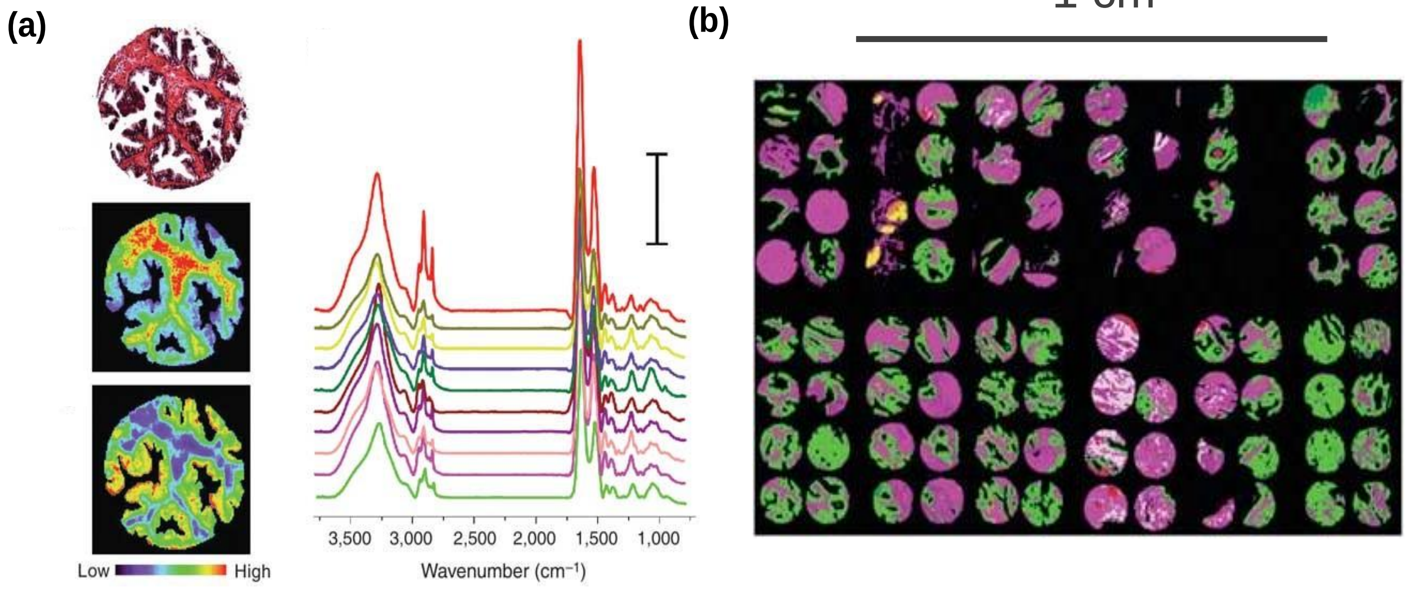
Infrared spectroscopic imaging for histopathologic recognition

Daniel C Fernandez[1,3,4], Rohit Bhargava[1,4], Stephen M Hewitt[2] & Ira W Levin[1]

- 262 samples from 40 patients
- 3 million spectra
- 6.25 um spatial resolution

700 µm

(40 min)

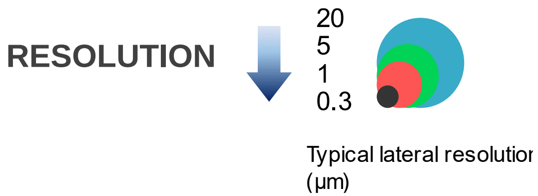


1 cm

(days)

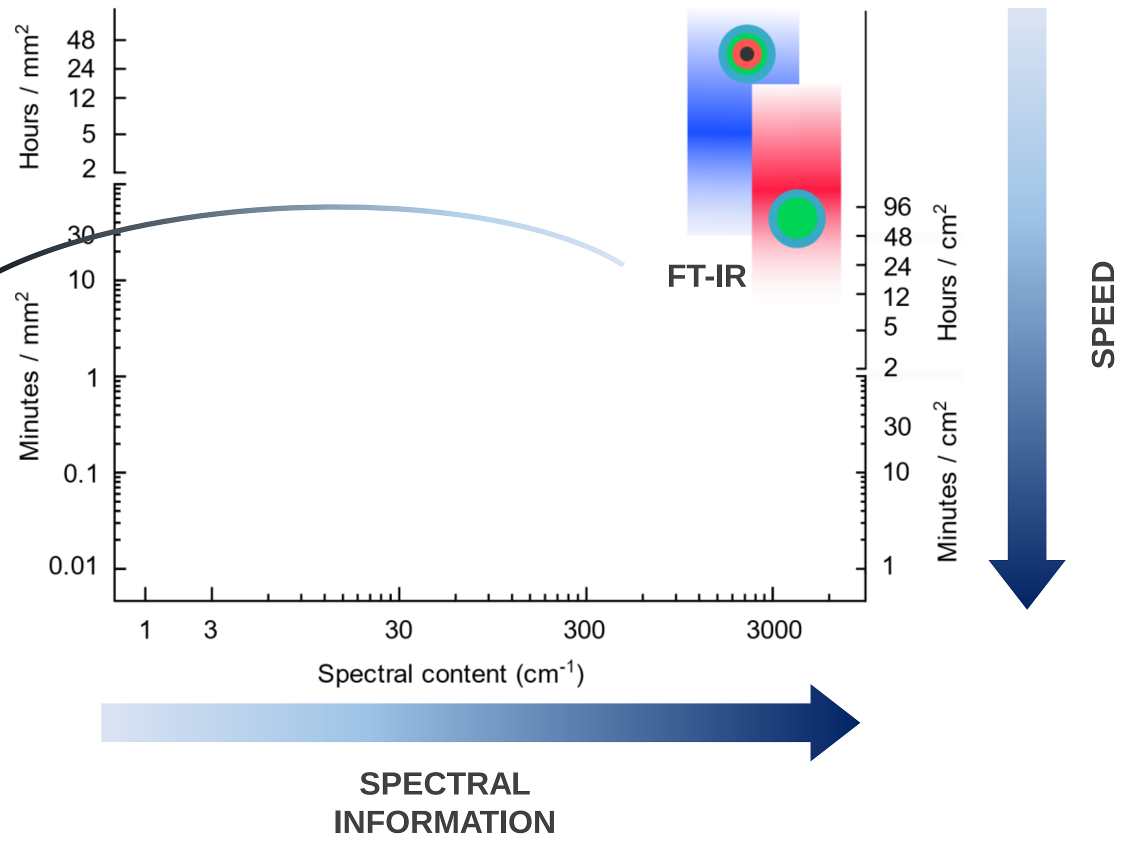
Fernandez, Daniel C., et al. *Nature biotechnology* 23.4 (2005): 469-474.

Vibrational spectroscopies at glance



- Super-detailed information
- High resolution
- Simple technology
- Compatible with water
- Low imaging speed

- Super-detailed information
- Medium resolution
- Simple technology
- Medium/Low imaging speed
- Not compatible with water



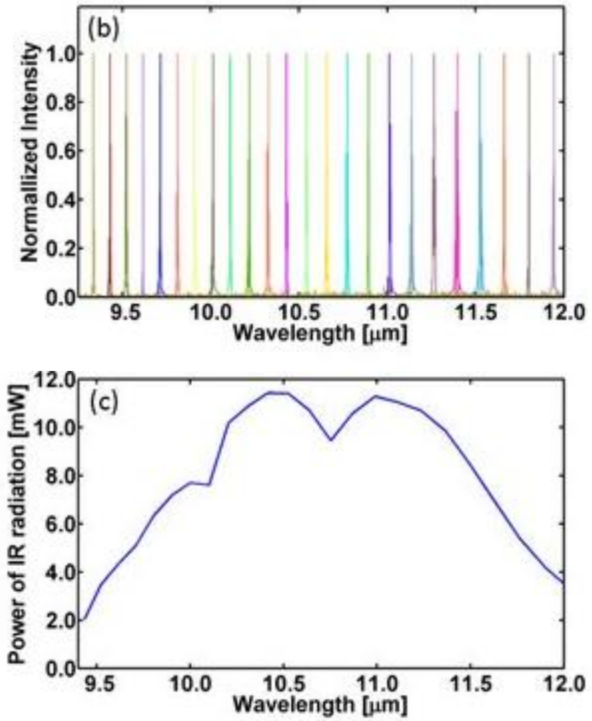
Vanna, Renzo, et al. *La Rivista del Nuovo Cimento (in preparation),* (2021)

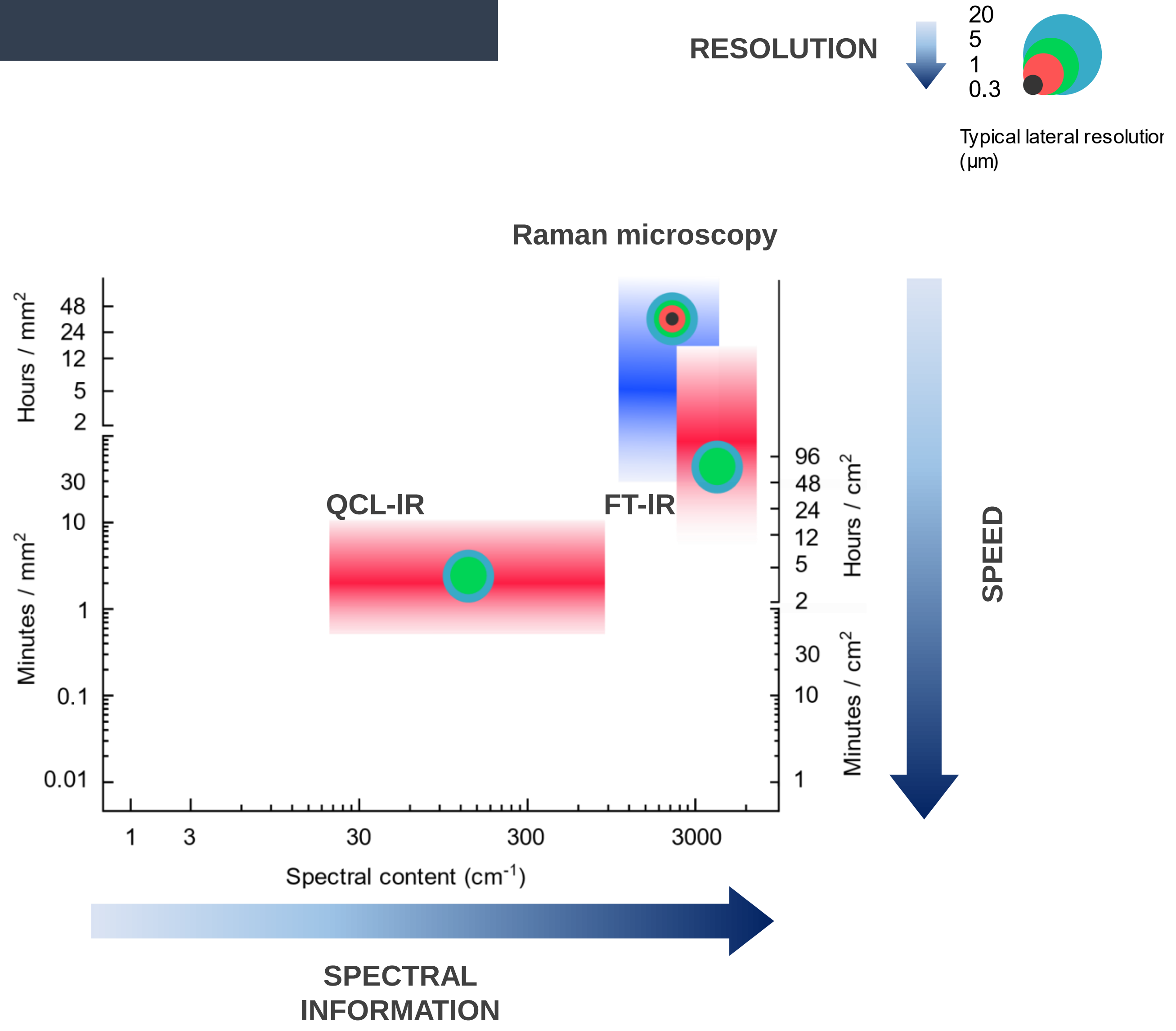
QCL IR-Imaging

Tunable Quantum Cascade Laser (QLC) Source

QCL source

single-element mercury cadmium telluride (MCT)





Mittal, Shachi, et al. *PNAS* 115.25 (2018): E5651-E5660.

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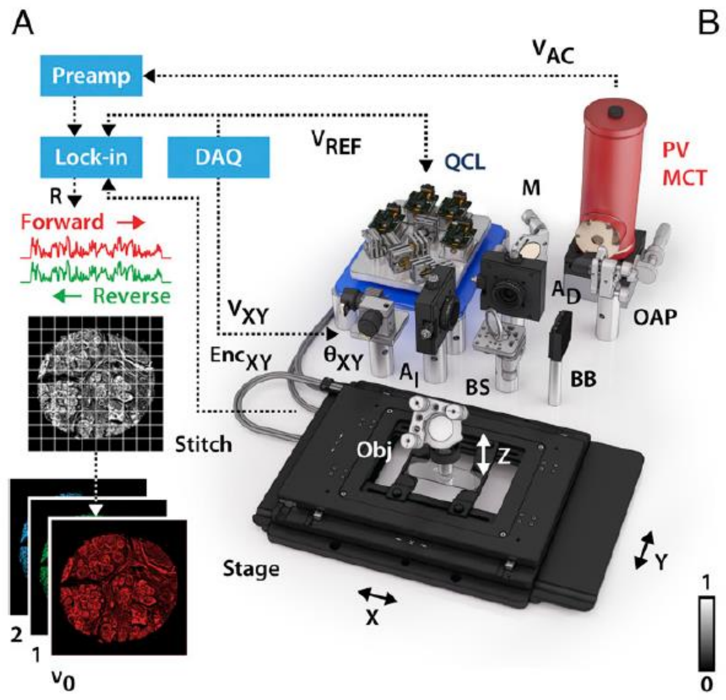
POLITECNICO MILANO 1863

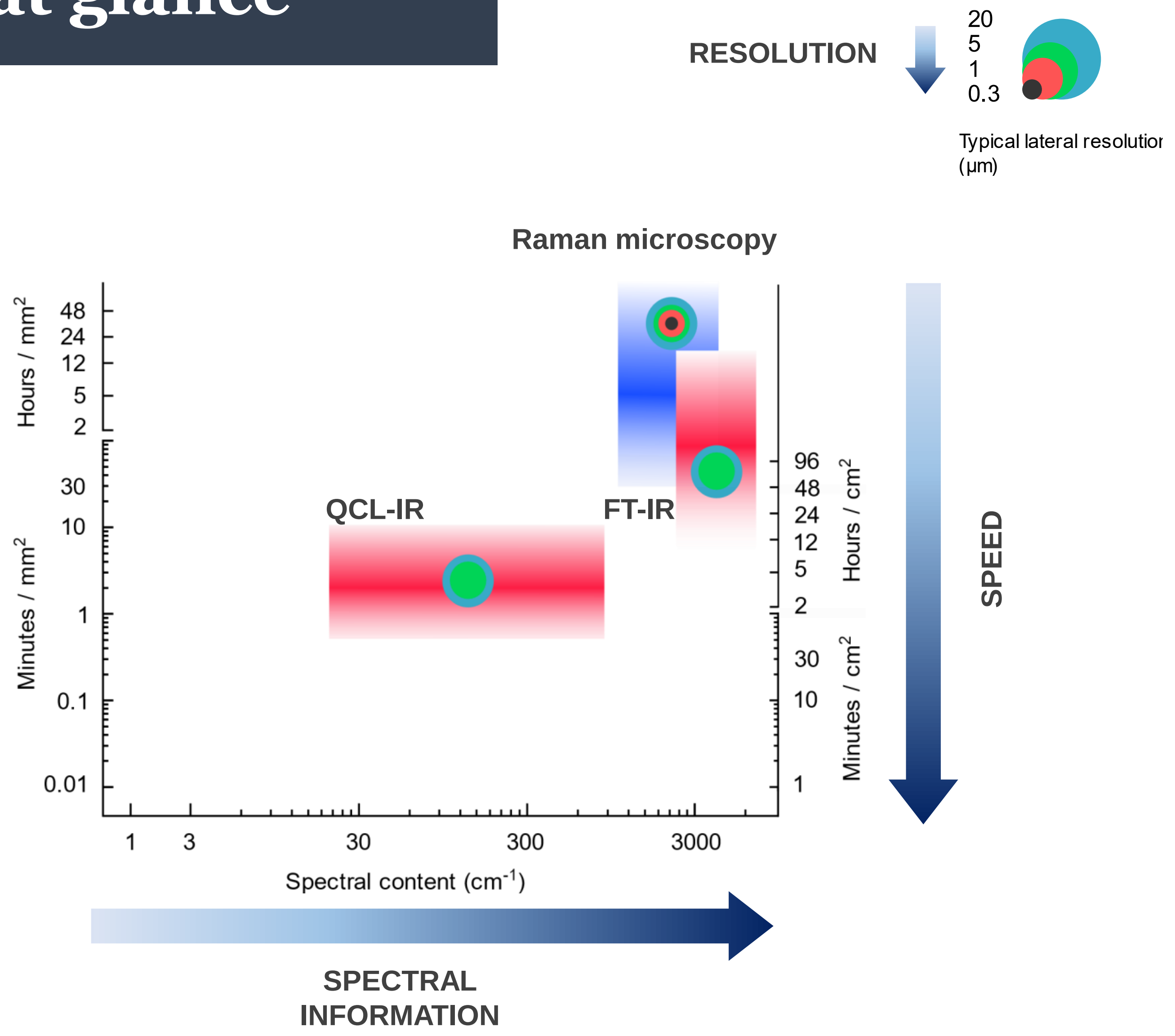
Vibrational spectroscopies at glance

Tunable Quantum Cascade Laser (QLC) Source

QCL source

single-element mercury cadmium telluride (MCT)





Mittal, Shachi, et al. *PNAS* 115.25 (2018): E5651-E5660.

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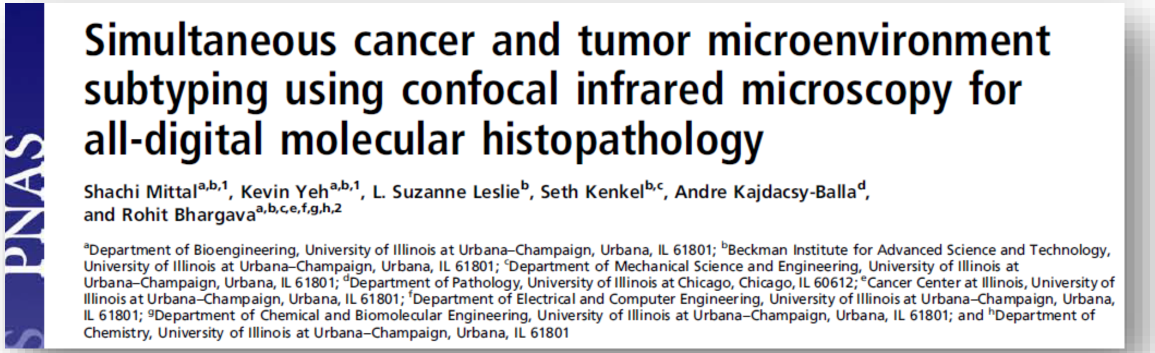
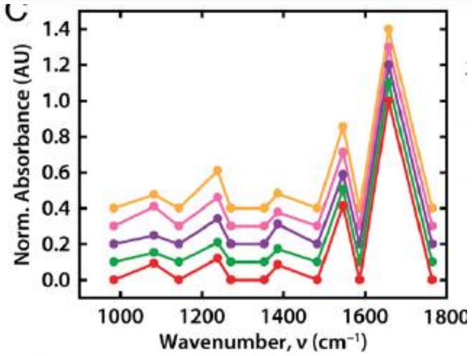
QCL-IR examples

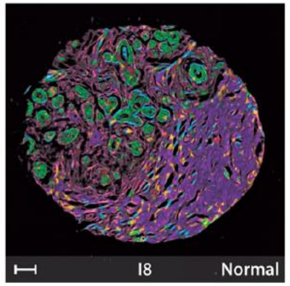

PNAS

Simultaneous cancer and tumor microenvironment subtyping using confocal infrared microscopy for all-digital molecular histopathology

Shachi Mittal[a,b,1], Kevin Yeh[a,b,1], L. Suzanne Leslie[b], Seth Kenkel[b,c], Andre Kajdacsy-Balla[d], and Rohit Bhargava[a,b,c,e,f,g,h,2]

[a]Department of Bioengineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [b]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [c]Department of Mechanical Science and Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [d]Department of Pathology, University of Illinois at Chicago, Chicago, IL 60612; [e]Cancer Center at Illinois, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [f]Department of Electrical and Computer Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [g]Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; and [h]Department of Chemistry, University of Illinois at Urbana–Champaign, Urbana, IL 61801





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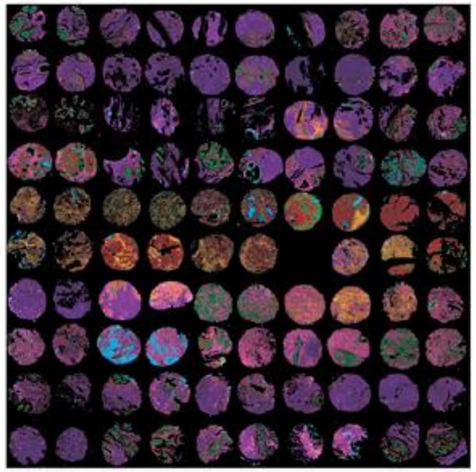

1 cm

(8h)

Mittal, Shachi, et al. *PNAS* 115.25 (2018): E5651-E5660.

QCL-IR examples

PNAS

Simultaneous cancer and tumor microenvironment subtyping using confocal infrared microscopy for all-digital molecular histopathology

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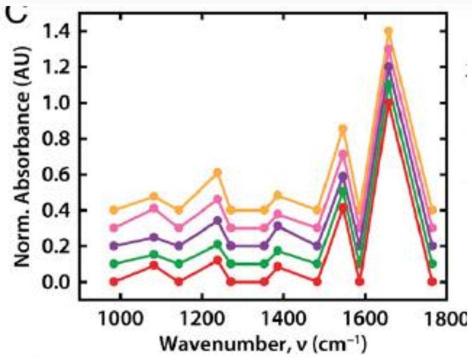
[a]Department of Bioengineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [b]Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [c]Department of Mechanical Science and Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [d]Department of Pathology, University of Illinois at Chicago, Chicago, IL 60612; [e]Cancer Center at Illinois, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [f]Department of Electrical and Computer Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; [g]Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana–Champaign, Urbana, IL 61801; and [h]Department of Chemistry, University of Illinois at Urbana–Champaign, Urbana, IL 61801

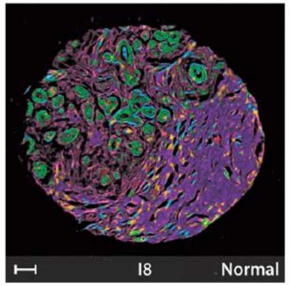
OPEN

Quantum Cascade Laser-Based Infrared Microscopy for Label-Free and Automated Cancer Classification in Tissue Sections

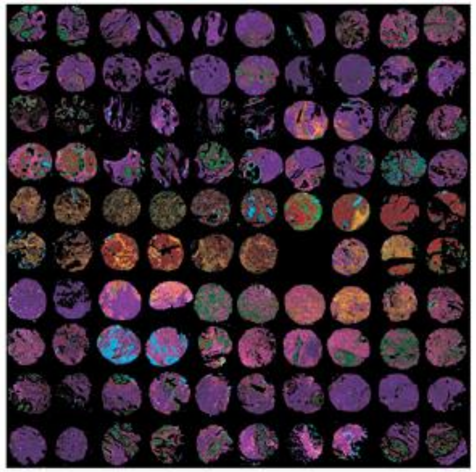
Received: 7 December 2017
Accepted: 2 May 2018
Published online: 16 May 2018

Claus Kuepper[1], Angela Kallenbach-Thieltges[1], Hendrik Juette[2], Andrea Tannapfel[2], Frederik Großerueschkamp[1] & Klaus Gerwert[1]



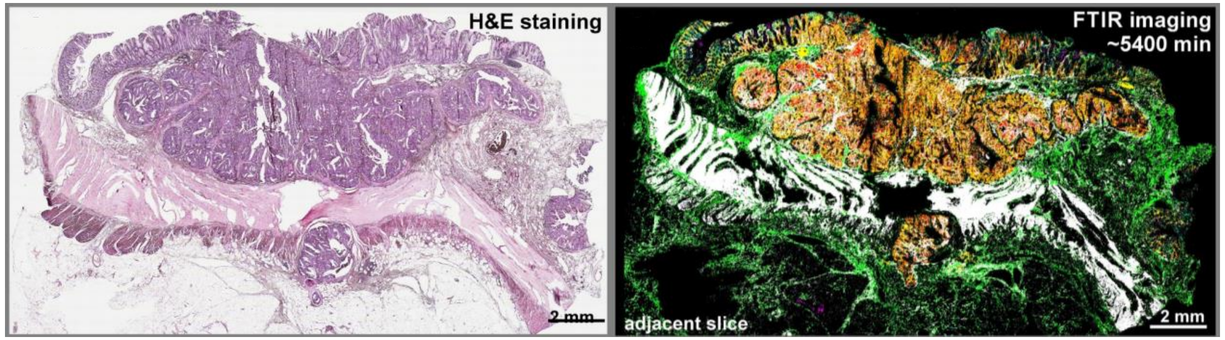


1 mm



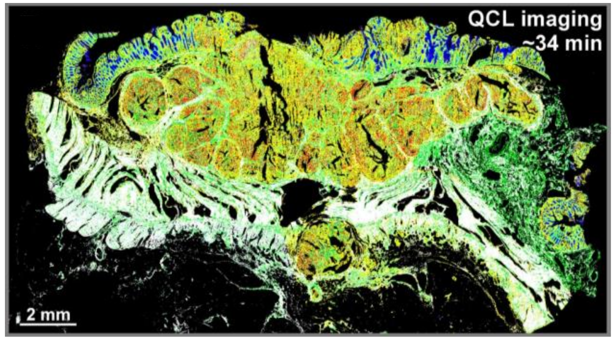
1 cm

(8h)



(4 days)

(34 min)

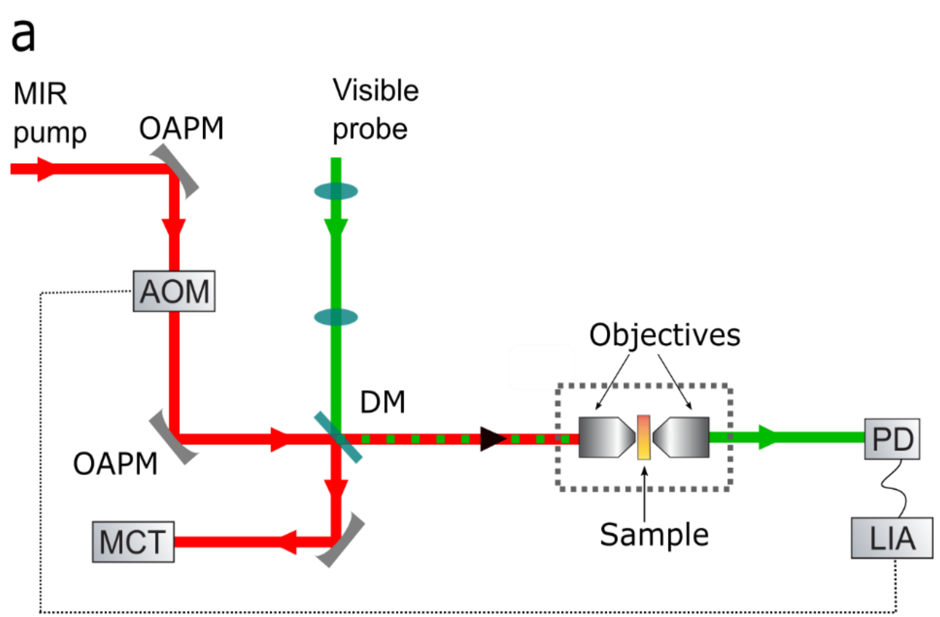


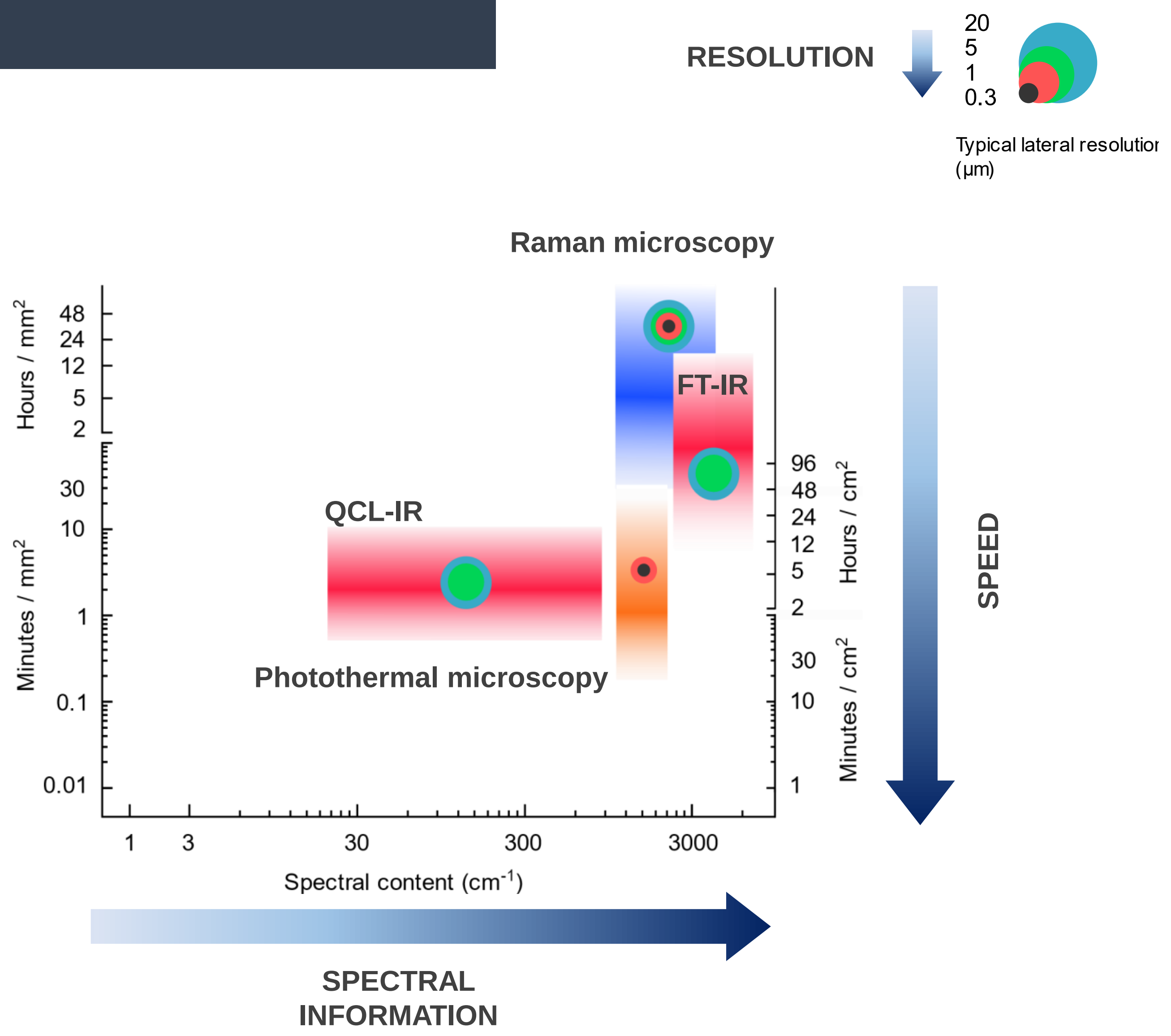
2 cm

Mittal, Shachi, et al. *PNAS* 115.25 (2018): E5651-E5660.

Kuepper, Claus, et al. *Scientific reports* 8.1 (2018): 1-10.

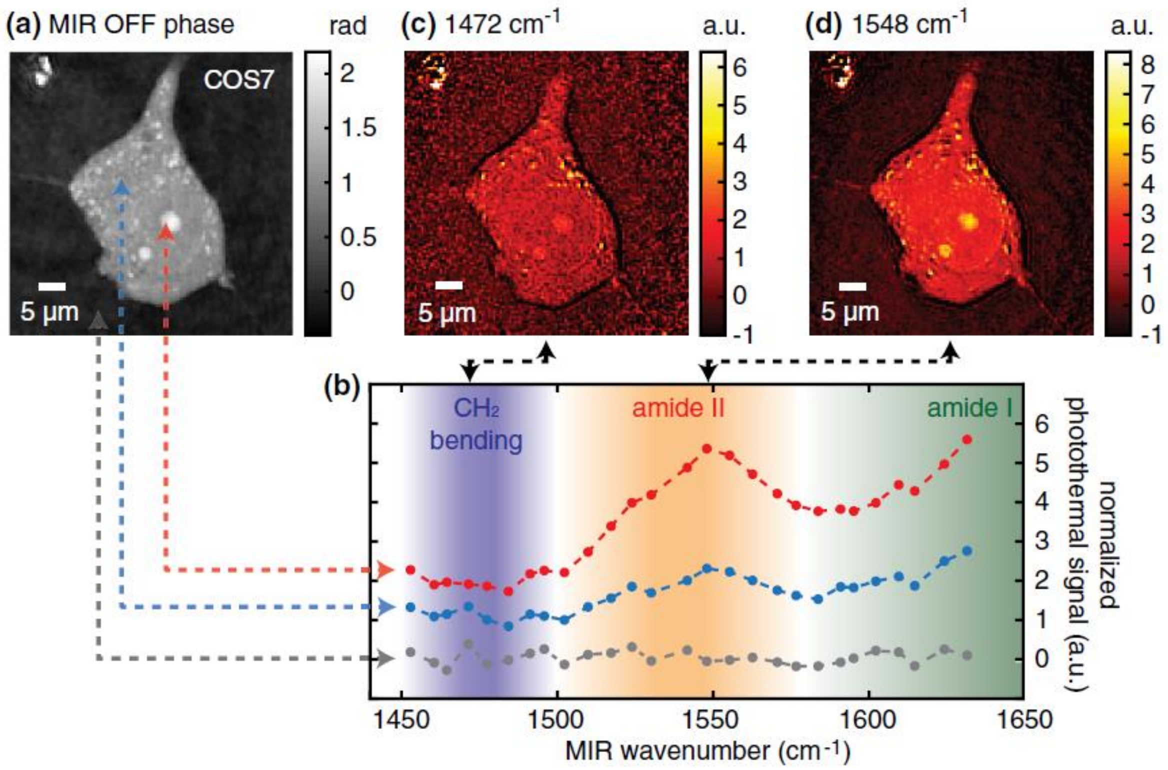
Photothermal microscopy

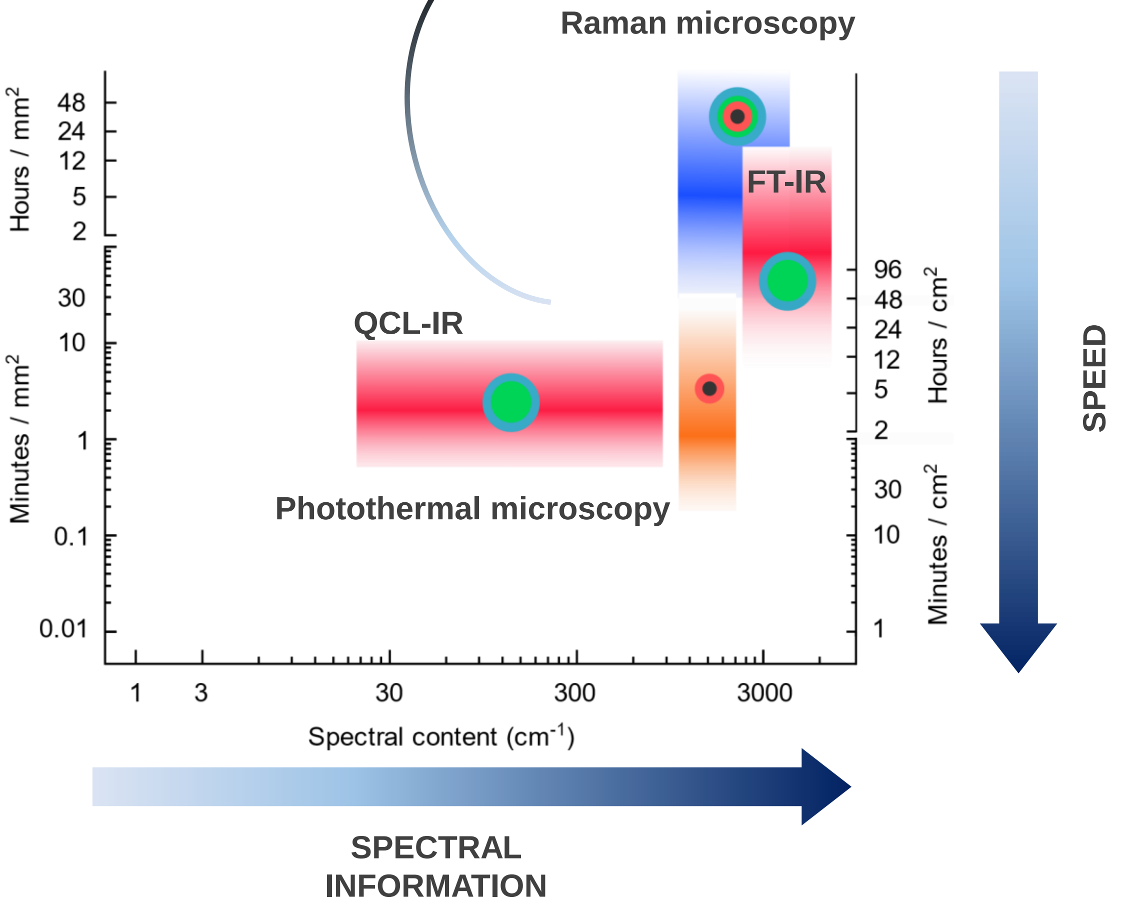




Photothermal microscopy

- spectral informative
- Fast
- High resolution
- Compatible with water
- At the early stage





Tamamitsu et al. *Optica* 2020

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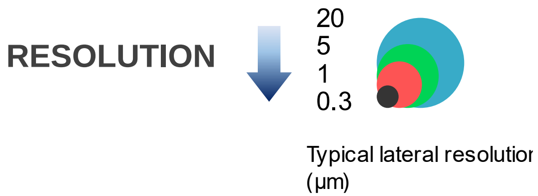
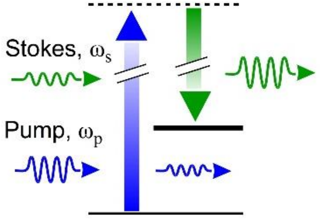
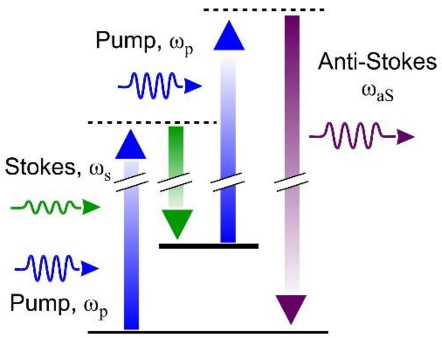
Coherent Raman Spectroscopies



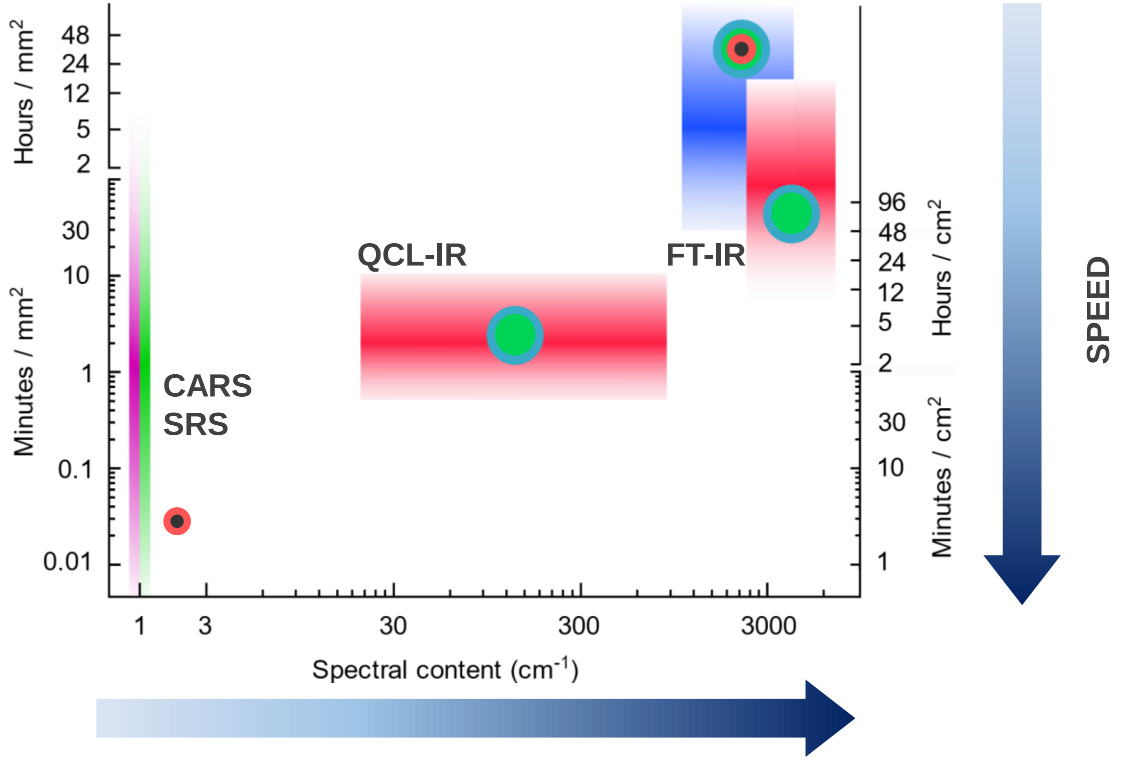


Stimulated Raman Scattering (SRS)



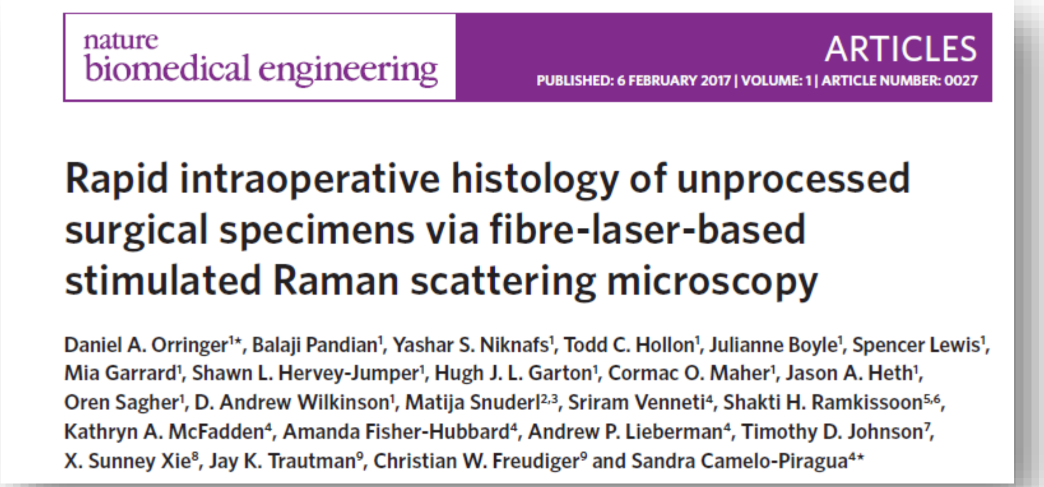
Coherent Anti-Stokes Raman Scattering (CARS)

Raman microscopy



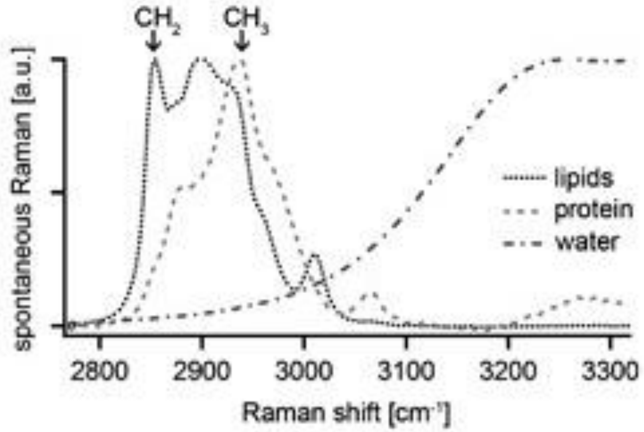
SPECTRAL INFORMATION

Stimulated Raman Spectroscopy: example

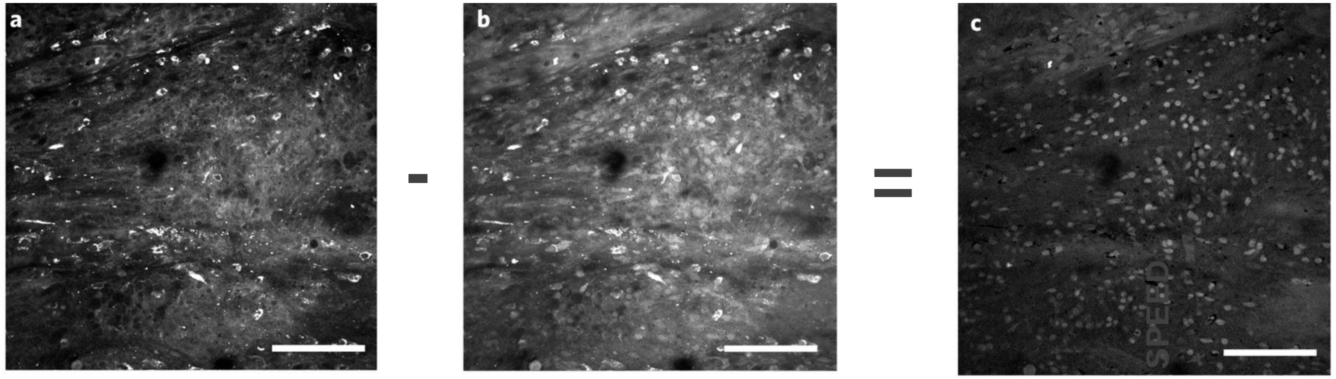

nature biomedical engineering
ARTICLES
PUBLISHED: 6 FEBRUARY 2017 | VOLUME: 1 | ARTICLE NUMBER: 0027

Rapid intraoperative histology of unprocessed surgical specimens via fibre-laser-based stimulated Raman scattering microscopy

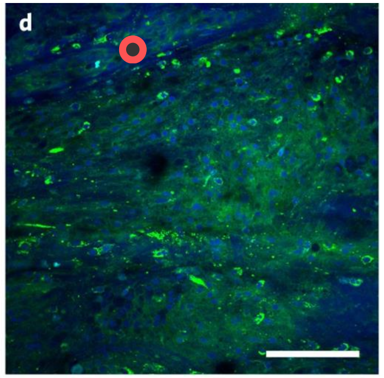
Daniel A. Orringer[1*], Balaji Pandian[1], Yashar S. Niknafs[1], Todd C. Hollon[1], Julianne Boyle[1], Spencer Lewis[1], Mia Garrard[1], Shawn L. Hervey-Jumper[1], Hugh J. L. Garton[1], Cormac O. Maher[1], Jason A. Heth[1], Oren Sagher[1], D. Andrew Wilkinson[1], Matija Snuderl[2,3], Sriram Venneti[4], Shakti H. Ramkissoon[5,6], Kathryn A. McFadden[4], Amanda Fisher-Hubbard[4], Andrew P. Lieberman[4], Timothy D. Johnson[7], X. Sunney Xie[8], Jay K. Trautman[9], Christian W. Freudiger[9] and Sandra Camelo-Piragua[4*]



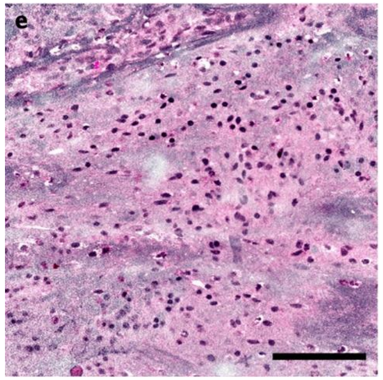
CH_2 (2845 cm^{-1}) CH_3 (2390 cm^{-1}) $CH_3 – CH_2$



CH_2 (proteins) / $CH_3 – CH_2$ (nuclei)

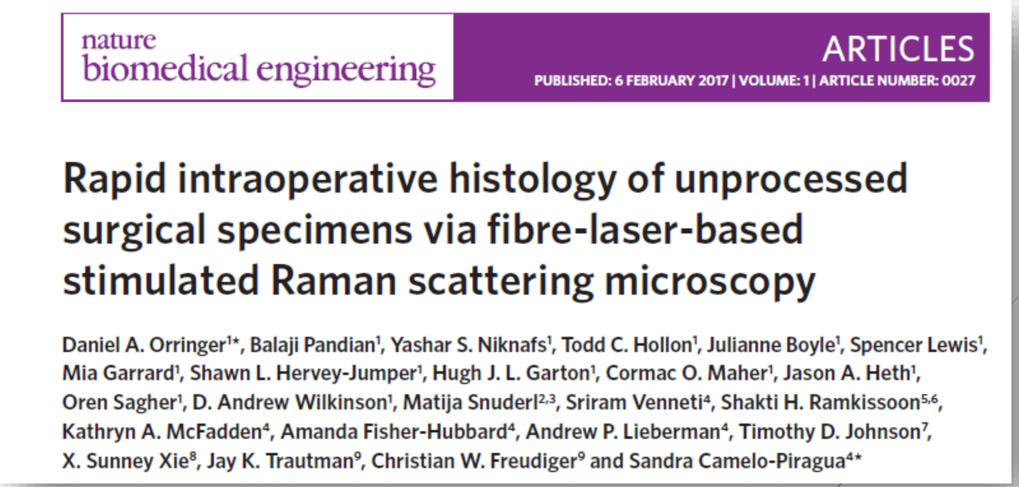


Virtual stain (pseudo-H&E)



Mittal, Shachi, et al. *PNAS* 115.25 (2018): E5651-E5660.

Stimulated Raman Spectroscopy: example


nature biomedical engineering

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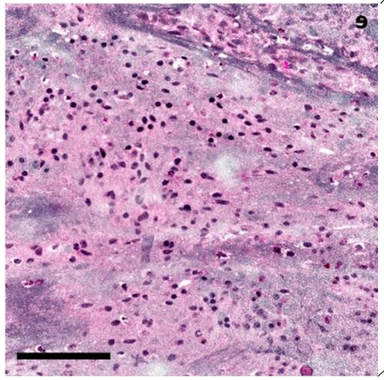
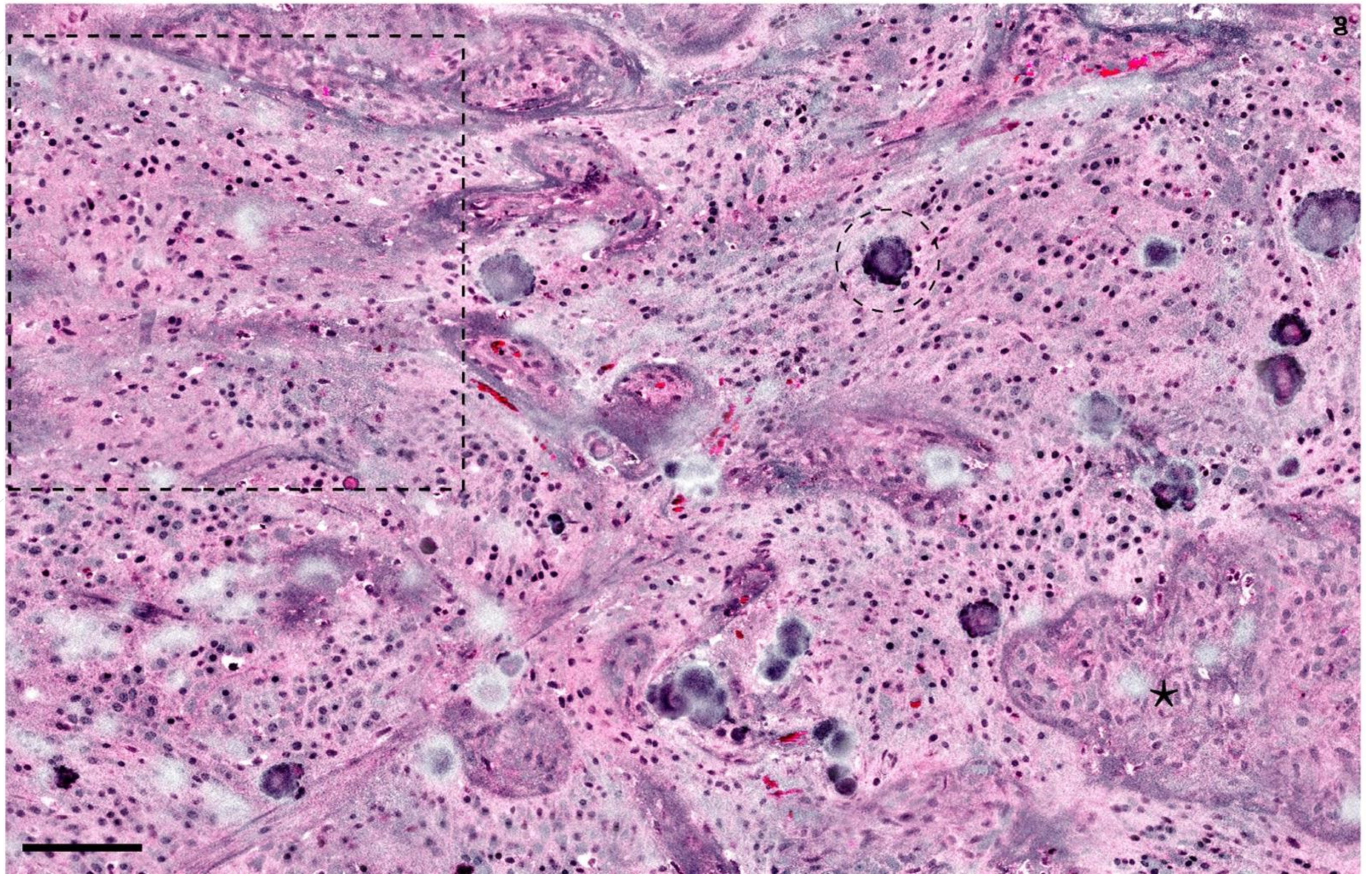


Virtual stain (pseudo-H&E)

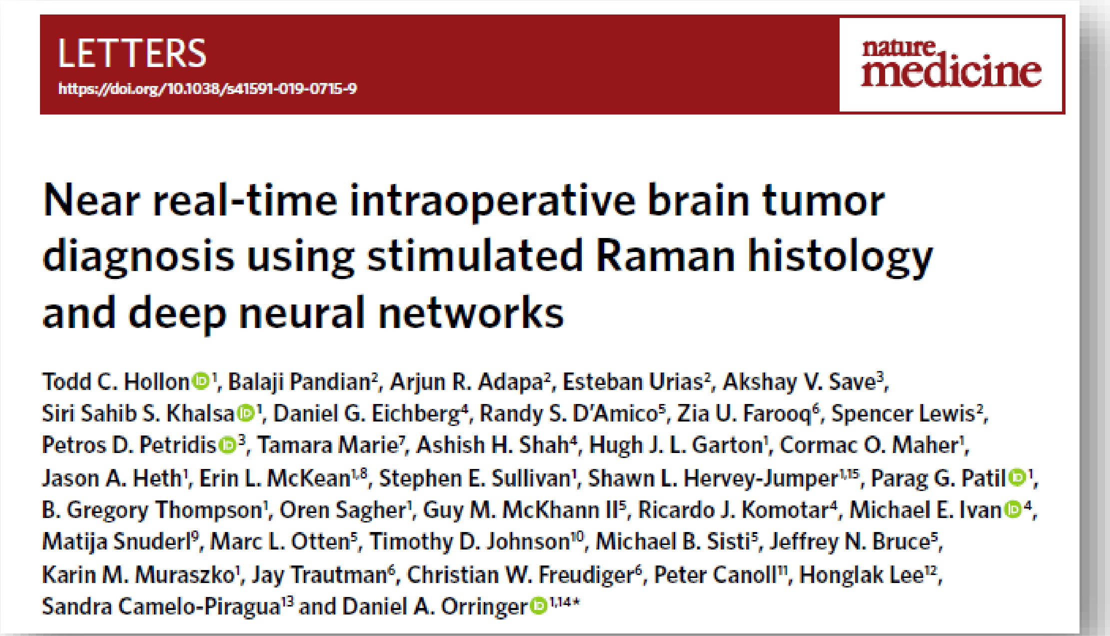


1 mm

(120 sec)

Orringer, Daniel A., et al. *Nature biomedical engineering* 1.2 (2017): 1-13.

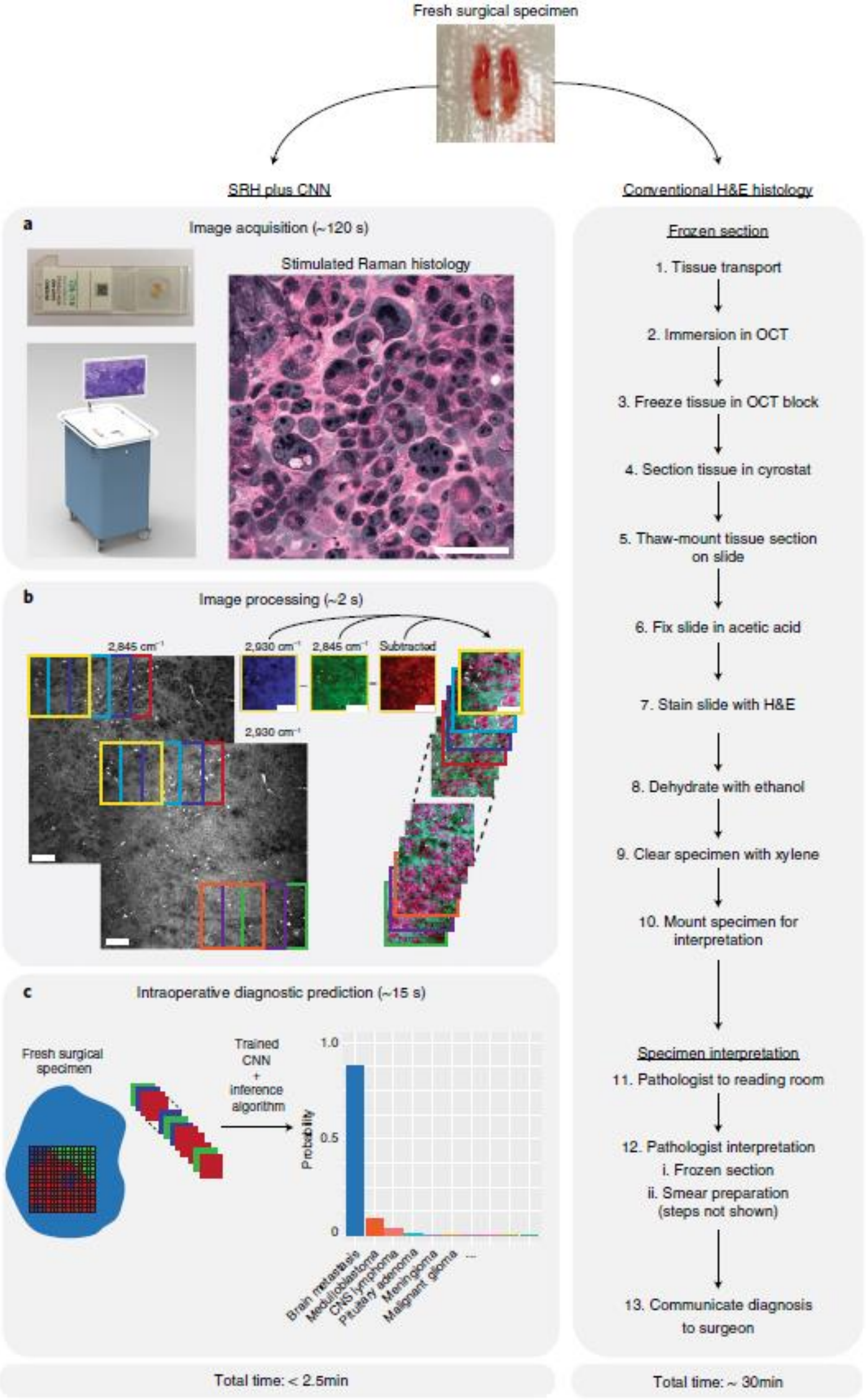
Stimulated Raman Spectroscopy


LETTERS
https://doi.org/10.1038/s41591-019-0715-9
nature medicine

Near real-time intraoperative brain tumor diagnosis using stimulated Raman histology and deep neural networks

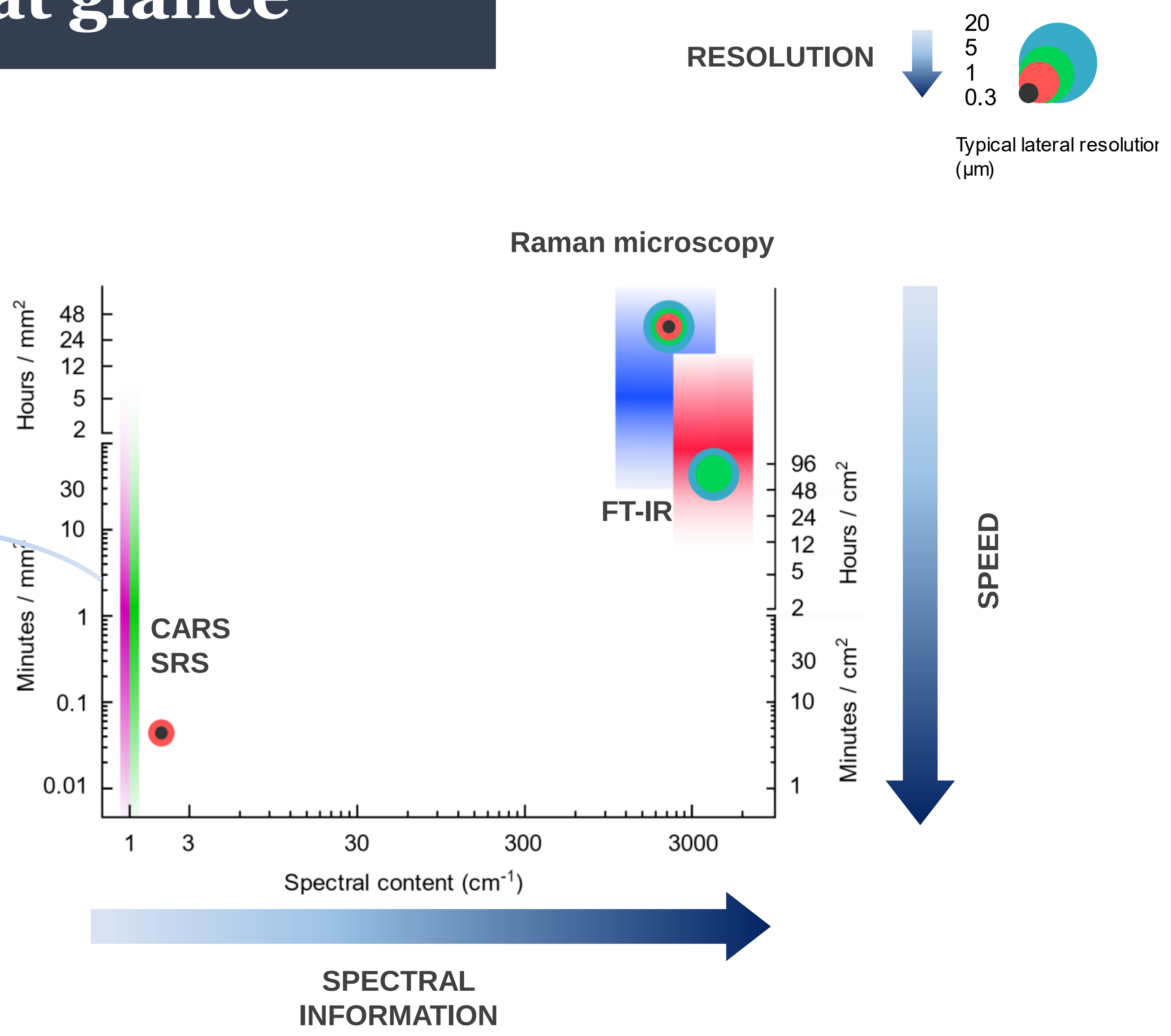
Todd C. Hollon[1], Balaji Pandian[2], Arjun R. Adapa[2], Esteban Urias[2], Akshay V. Save[3], Siri Sahib S. Khalsa[1], Daniel G. Eichberg[4], Randy S. D'Amico[5], Zia U. Farooq[6], Spencer Lewis[2], Petros D. Petridis[3], Tamara Marie[7], Ashish H. Shah[4], Hugh J. L. Garton[1], Cormac O. Maher[1], Jason A. Heth[1], Erin L. McKean[1,8], Stephen E. Sullivan[1], Shawn L. Hervey-Jumper[1,15], Parag G. Patil[1], B. Gregory Thompson[1], Oren Sagher[1], Guy M. McKhann II[5], Ricardo J. Komotar[4], Michael E. Ivan[4], Matija Snuderl[9], Marc L. Otten[5], Timothy D. Johnson[10], Michael B. Sisti[5], Jeffrey N. Bruce[5], Karin M. Muraszko[1], Jay Trautman[6], Christian W. Freudiger[6], Peter Canoll[11], Honglak Lee[12], Sandra Camelo-Piragua[13] and Daniel A. Orringer[1,14*]

- **2.5 min** (**vs 30 min H&E**) [smaller tissue regions]
- 278 patients
- 4 systems in 4 hospitals
- > 2.5 million images
- 94.6% accuracy (vs 93.9% H&E)



Hollon, Todd C., et al. *Nature medicine* 26.1 (2020): 52-58.

Vibrational spectroscopies at glance



- Very fast !
- High resolution
- Scarce spectral information

Vibrational spectroscopies at glance

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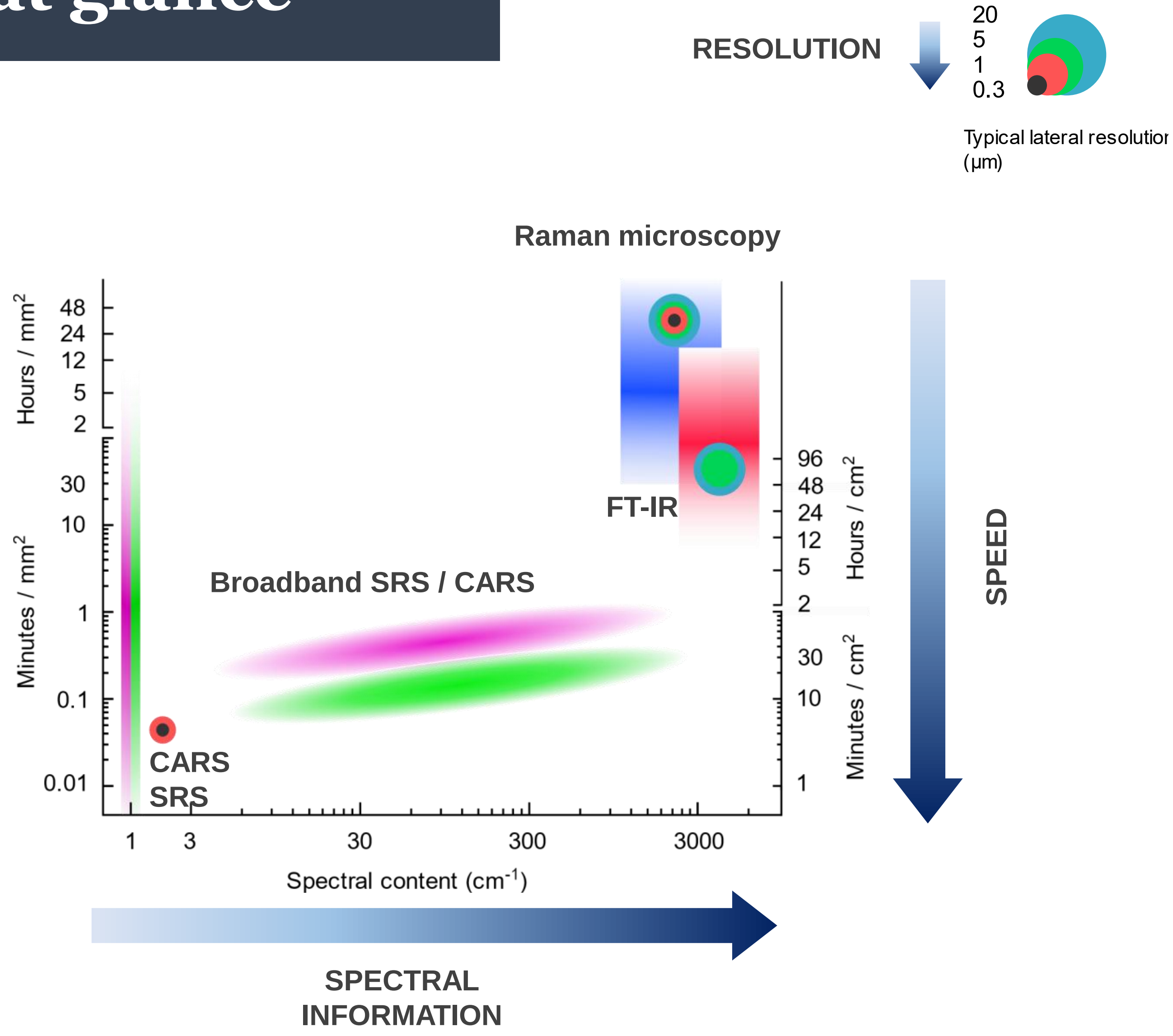
Inserm

activefiber systems

3rdPlace user & customer AI

LIGHTCORE technologies

CRI chemometric imaging



Polli, Dario, et al. Laser & Photonics Reviews 12.9 (2018): 1800020..

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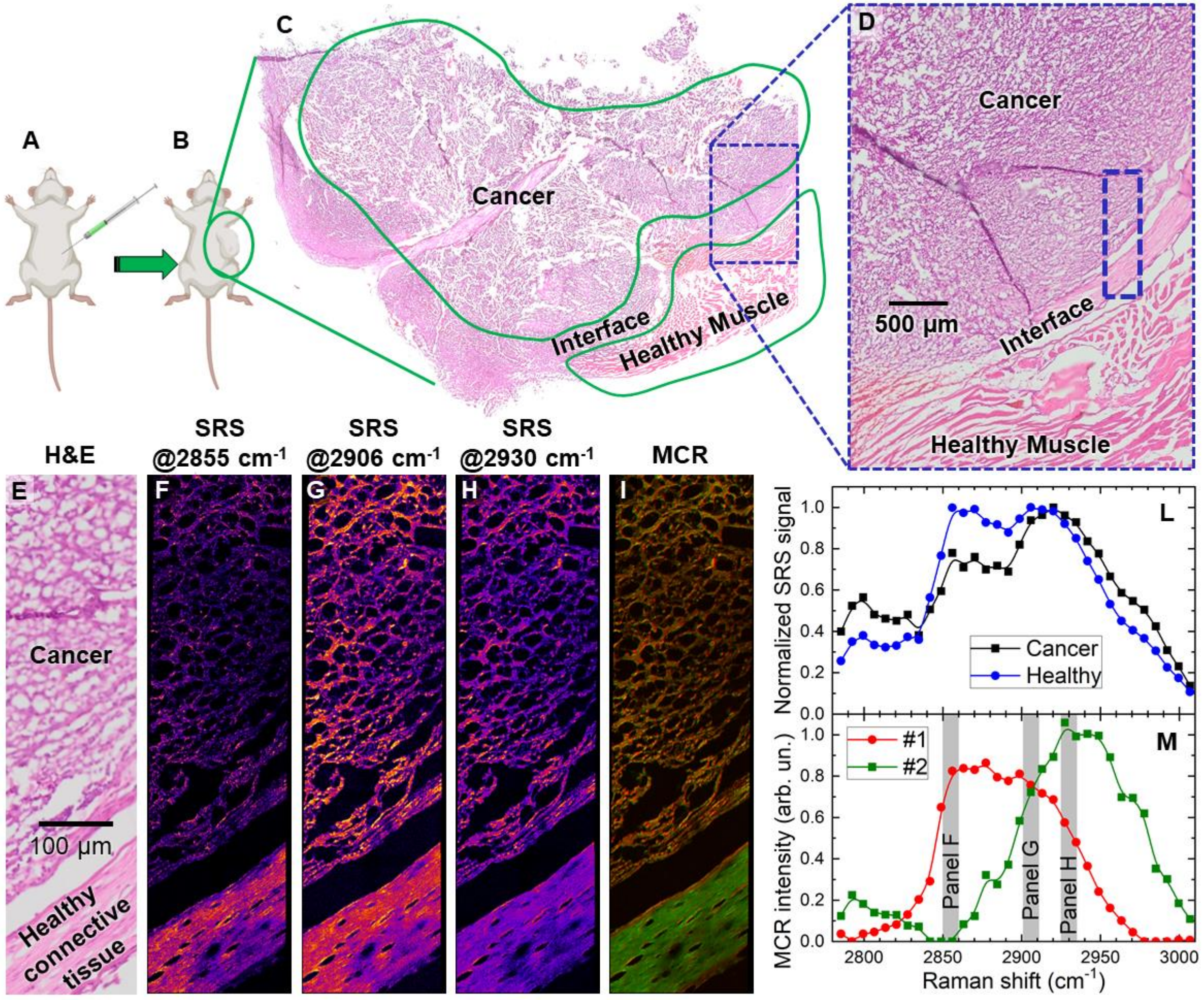
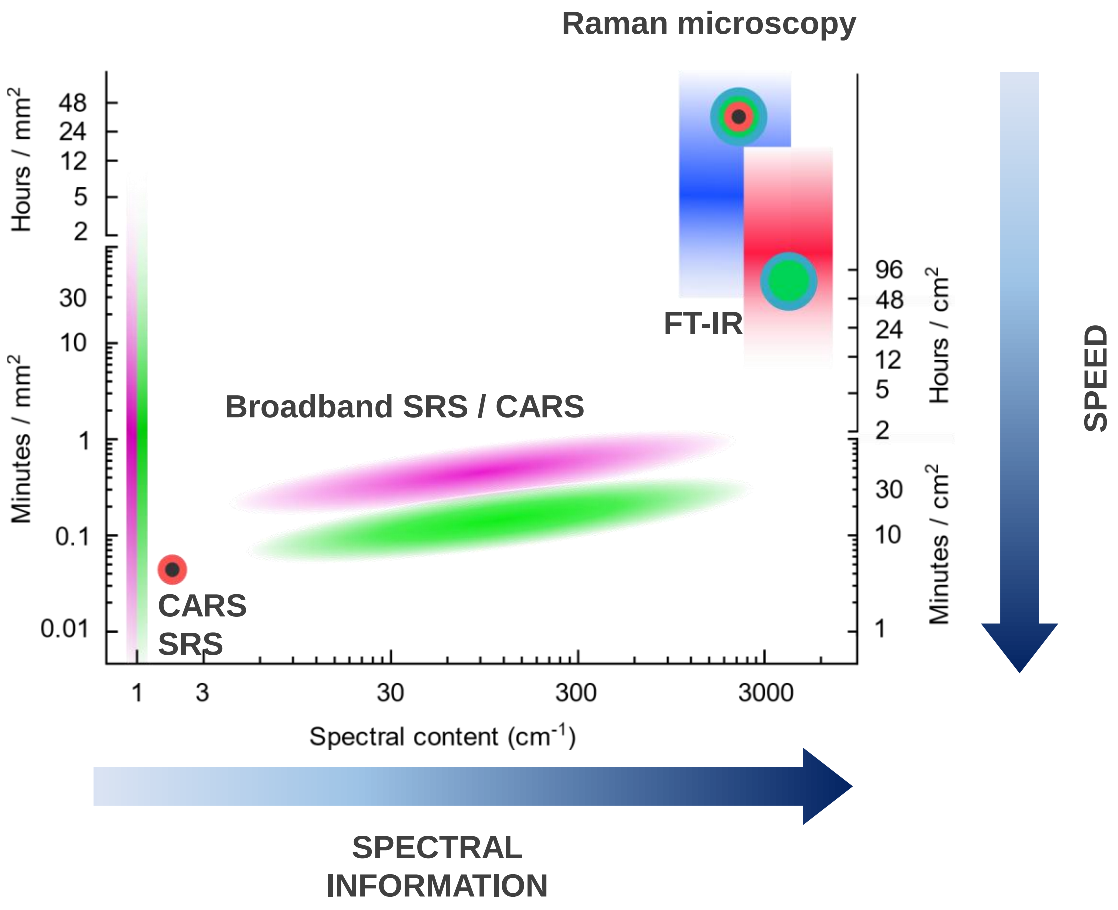
Broadband SRS example





De la Cadena, Alejandro et al. (2021) ***in preparation***
Polli, Dario, et al. *Laser & Photonics Reviews* 12.9 (2018): 1800020..

What’s next ?

Powerful technologies

Promising data

Space for improvements
..to move technologies to clinical use

Next steps: How to meet clinical needs ?

0) Technology improvement (…)

Next steps: How to meet clinical needs ?

0) Technology improvement

1) **Validation, networking, validation**

CLIRSPEC

Raman4Clinics

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

analytical chemistry

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Cite This: *Anal. Chem.* 2020, 92, 15745−15756 Read Online

analytical chemistry

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Surface Enhanced Raman Spectroscopy for Quantitative Analysis: Results of a Large-Scale European Multi-Instrument Interlaboratory Study

Stefano Fornasaro, Fatima Alsamad, Monica Baia, Luís A. E. Batista de Carvalho, Claudia Beleites, Hugh J. Byrne, Alessandro Chiadò, Mihaela Chis, Malama Chisanga, Amuthachelvi Daniel, Jakub Dybas, Gauthier Eppe, Guillaume Falgayrac, Karen Faulds, Hrvoje Gebavi, Fabrizio Giorgis, Royston Goodacre, Duncan Graham, Pietro La Manna, Stacey Laing, Lucio Litti, Fiona M. Lyng, Kamilla Malek, Cedric Malherbe, Maria P. M. Marques, Moreno Meneghetti, Elisa Mitri, Vlasta Mohaček-Grošev, Carlo Morasso, Howbeer Muhamadali, Pellegrino Musto, Chiara Novara, Marianna Pannico, Guillaume Penel, Olivier Piot, Tomas Rindzevicius, Elena A. Rusu, Michael S. Schmidt, Valter Sergo, Ganesh D. Sockalingum, Valérie Untereiner, Renzo Vanna, Ewelina Wiercigroch, and Alois Bonifacio*

Cite This: *Anal. Chem.* 2020, 92, 4053−4064 Read Online

Guo, Shuxia, et al. *Analytical Chemistry* 92.24 (2020): 15745-15756.
Fornasaro, Stefano, et al. *Analytical chemistry* 92.5 (2020): 4053-4064.

Next steps

0) *(not only)* **Technology improvement**

1) **Validation, networking, validation**

2) **- Early definition of clinical and final user need**

- Strong interdisciplinarity

- Deep involvement of clinicians

Orringer, Daniel A., et al. *Nature biomedical engineering* 1.2 (2017): 1-13.
Hollon, Todd C., et al. *Nature medicine* 26.1 (2020): 52-58.

Next steps

0) *(not only)* Technology improvement

1) Validation, networking, validation

2) - Early definition of clinical and fina

- **Strong interdisciplinarity**
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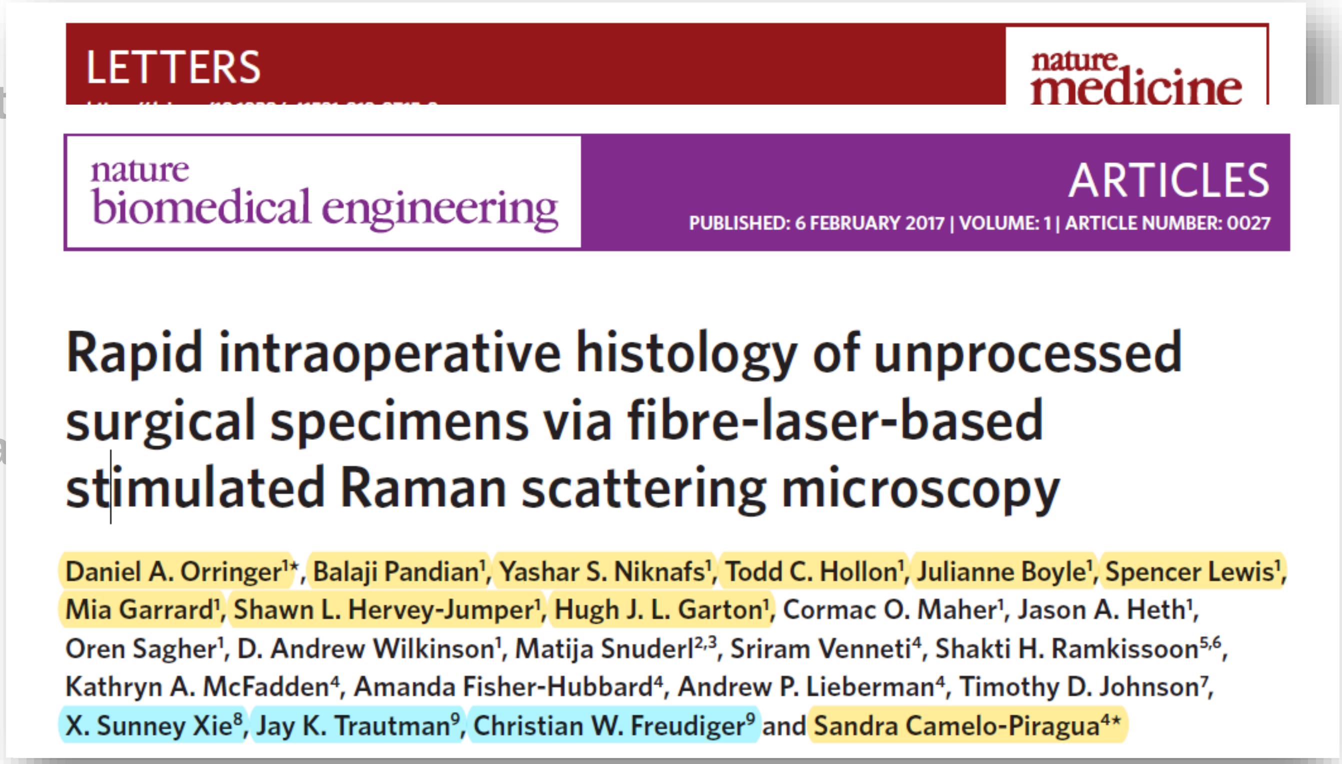
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Hollon, Todd C., et al. *Nature medicine* 26.1 (2020): 52-58.

Acknowledgments

Team at IFN-CNR – Politecnico di Milano

Dario Polli
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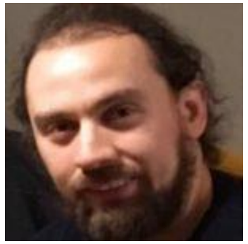
Marco Marangoni
Cristian Manzoni

Alejandro de la Cadena
Bendetta Talone
Arianna Bresci
Federico Vernuccio
Chiara Ceconello



Collaborations

Carlo Morasso (ICS Maugeri Hospital)
Nick Stone (Exeter University)
Cinzia Giannini (IC-CNR)

Michael Schmitt (IPHT), Tomas Mayer (IPHT), Hervé Rigneault (Fresnel), Orlando Guntinas (UniMed Jena), Cambridge Raman Imaging (CRI srl), Italia Bongarzone (INT), Cristina Sobacchi (Humanitas)

Fundings

H2020 No 101016923 (CRIMSON)
Regione Lombardia POR FESR 2014-2020 (NEWMED)

CRIMSON NewMed

PHOTONICS21
PHOTONICS PUBLIC PRIVATE PARTNERSHIP

Thank you!

Team at IFN-CNR – Politecnico di Milano

Dario Polli
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Cristian Manzoni


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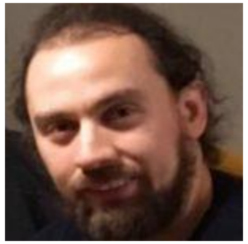
Bendetta Talone
Arianna Bresci
Federico Vernuccio
Chiara Ceconello







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Fundings

H2020 No 101016923 (CRIMSON)
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