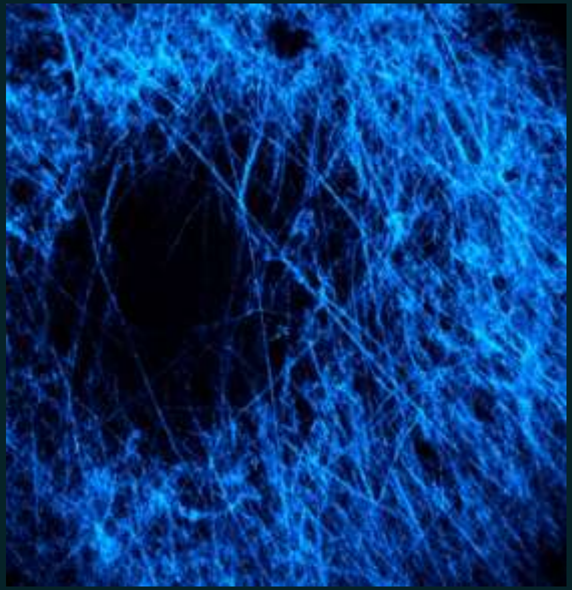

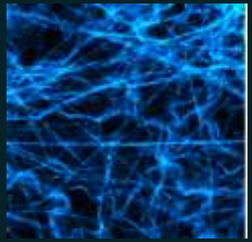

Collagen micro-architecture investigation in tumor sections by means of second harmonic generation signal multiphasor analysis coupled with non-supervised machine learning techniques

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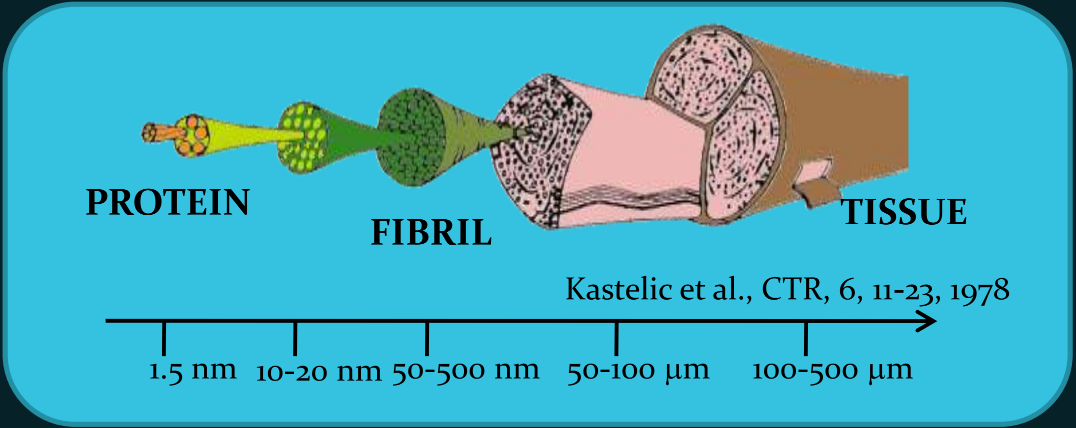
Collagen role in diseased tissues

AIM

Retrieving and exploiting features able to describe the collagen fibrils microstructure to separate tumor from healthy regions into the tissue.



COLLAGEN

- It is characterized by non centrosymmetric microscopic structure.
- The collagen micro-architecture is different depending on tissue and in presence of pathologies (aging, fibrosis, <u>tumor growth</u>)
- Collagen can be exploited as an <u>early diagnostic marker</u>

Polarization Dependent Second Harmonic Generation (P-SHG)

- SHG is a non linear coherent optical process, label-free signal, sensitive to molecular symmetry and polarization.

$$P_i^{(2)}(2\omega) = \sum_j \sum_k \chi_{ijk}^{(2)}(\omega,\omega) E_j(\omega) E_k(\omega)$$

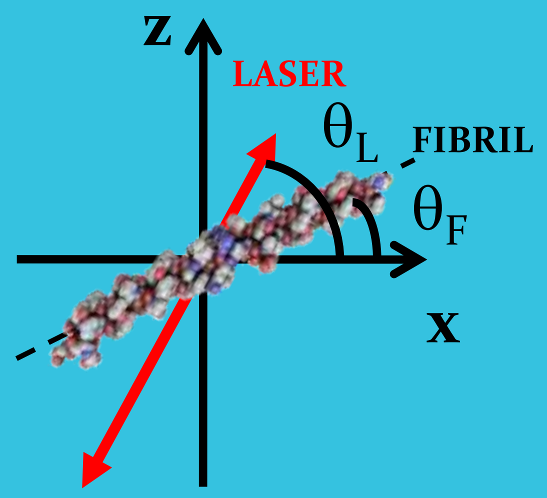
$$\gamma = \chi_{zzz}^{(2)} / \chi_{zxx}^{(2)}$$

Model: Williams, R. et al. Biophys. J. 88, 1377–1386 (2005)

$$I(\theta_L^n) = k\left\{\sin^2\left[2(\theta_L^n - \theta_F)\right] + \left[\sin^2(\theta_L^n - \theta_F) + \gamma\cos^2(\theta_L^n - \theta_F)\right]^2\right\}$$

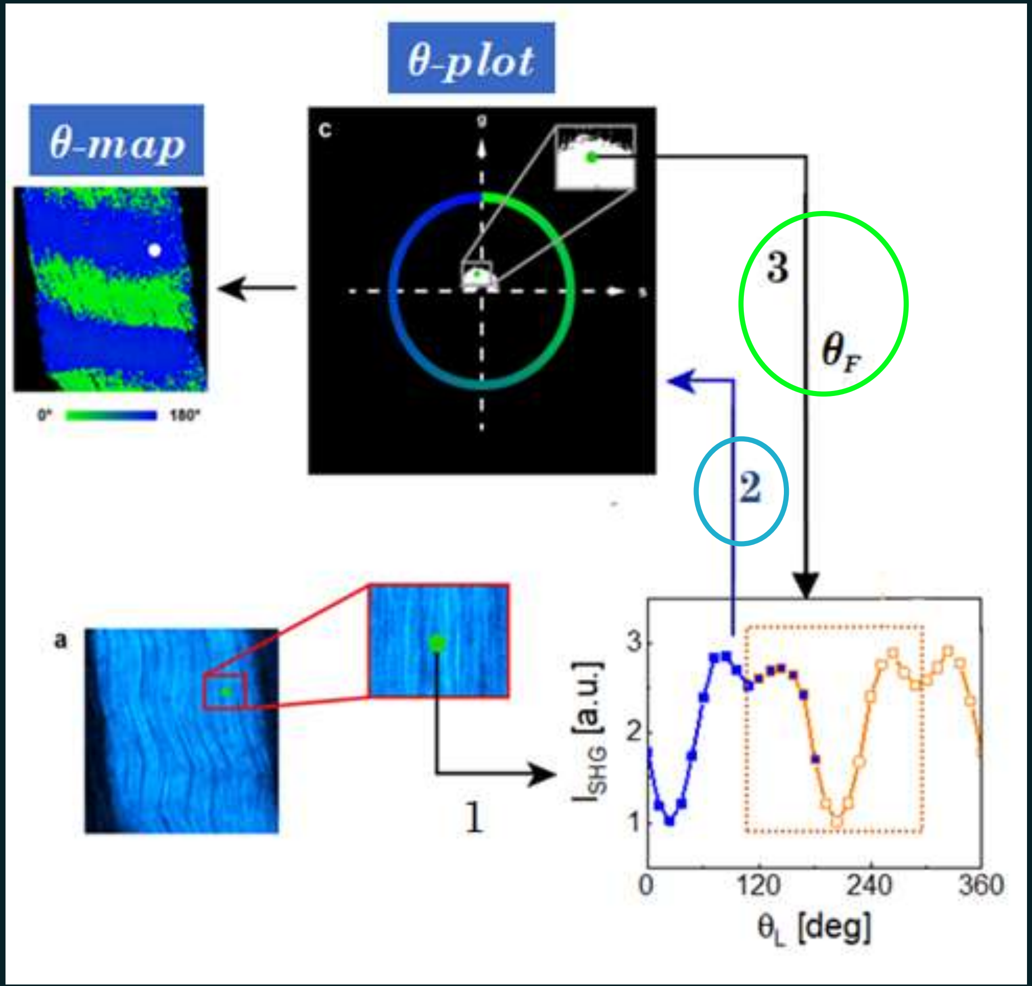
Mean fibril orientation

Mean fibril internal disorder



- Fitting procedures are difficult and slow.

Microscopic Multiparametric Analysis by Phasor projection of Polarization dependent SHG (µMAPPS)



Radaelli et al. Sc.Rep. 7, 17468, 2017

Pixel-by-pixel retrieval of features able to describe the collagen micro-structure, without fitting procedures.

$$g_\theta = \frac{\sum_{n=0}^{N-1} I(\theta_L^n)\cos(\theta_L^n K_\theta)}{\sum_{n=0}^{N-1} I(\theta_L^n)}$$

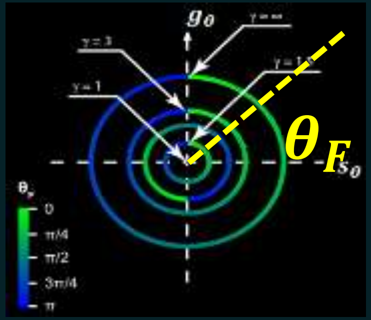
$$s_\theta = \frac{\sum_{n=0}^{N-1} I(\theta_L^n)\sin(\theta_L^n K_\theta)}{\sum_{n=0}^{N-1} I(\theta_L^n)}$$

$$K_\theta = 2\pi(N\Delta\theta)^{-1}$$

$$\theta_L^n = n\Delta\theta$$

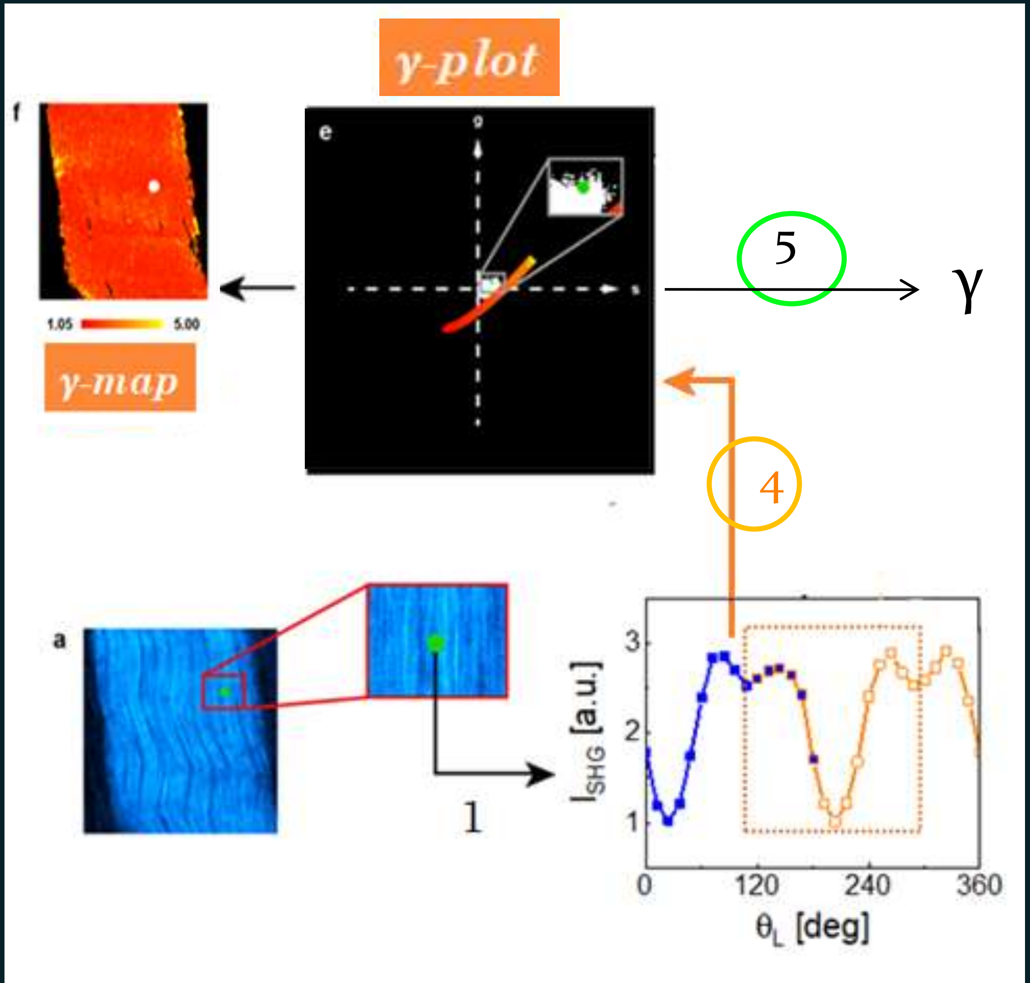
$$N = \frac{\pi}{\Delta\theta}$$

$$0 \le \theta_L \le \frac{3}{2}\pi$$



$$cos(2\theta_F) = \frac{g_\theta}{\sqrt{g_\theta^2 + s_\theta^2}}$$

Microscopic Multiparametric Analysis by Phasor projection of Polarization dependent SHG (µMAPPS)



Radaelli et al. Sc.Rep. 7, 17468, 2017

Pixel-by-pixel retrieval of features able to describe the collagen micro-structure, without fitting procedures.

$$g_\gamma = \frac{\sum_{n=0}^{\left(\frac{N}{2}\right)-1} I(\theta_L^n + \theta_F)\cos((\theta_L^n + \theta_F)K_\theta)}{\sum_{n=0}^{N-1} I(\theta_L^n + \theta_F)}$$

$$s_\gamma = \frac{\sum_{n=0}^{\left(\frac{N}{2}\right)-1} I(\theta_L^n + \theta_F)\sin((\theta_L^n + \theta_F)K_\theta)}{\sum_{n=0}^{N-1} I(\theta_L^n + \theta_F)}$$

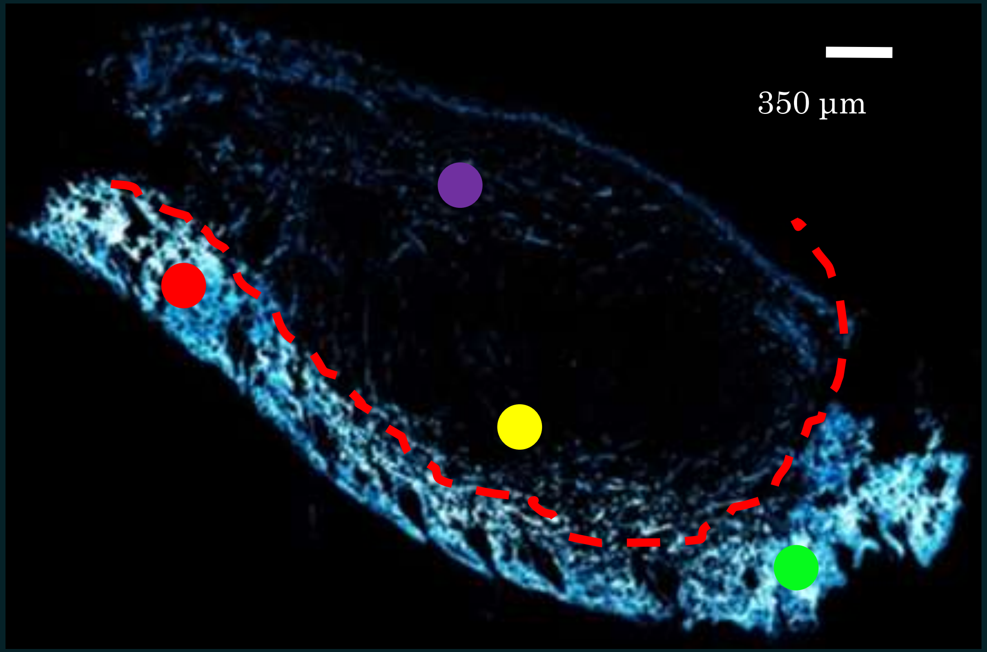
$$\theta_L^n = n\Delta\theta \quad N = \frac{\pi}{\Delta\theta} \quad K_\theta = 2\pi(N(\Delta\theta + \theta_F))^{-1} \quad \theta_F \le \theta_L \le \theta_F + \frac{\pi}{2}$$

$$d_{e-RC} = \sqrt{(g_e - g_{RC})^2 - (s_e - s_{RC})^2}$$

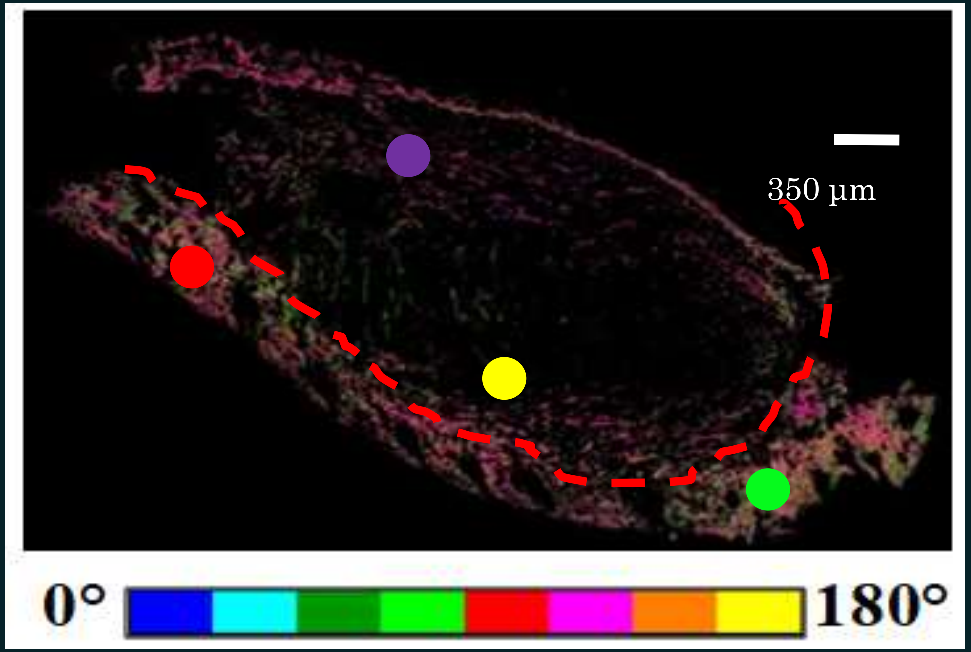
$$\gamma_e = \gamma_{RC|min(d_{e-RC})}$$

Tumor section analysis by µMAPPS

ISHG Map

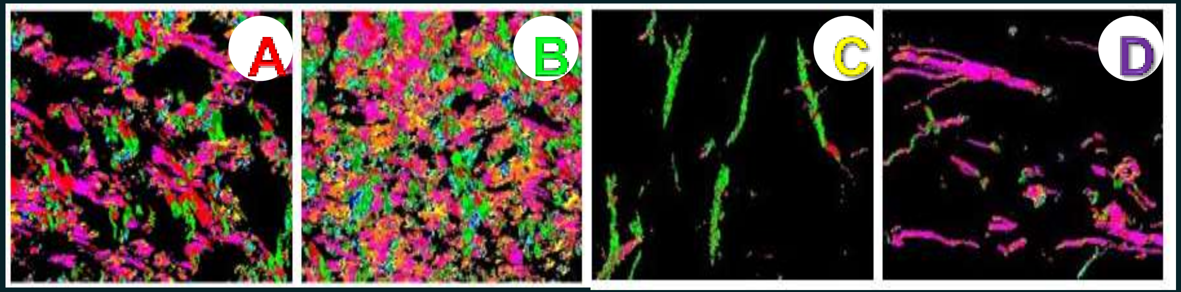


θF-values Map

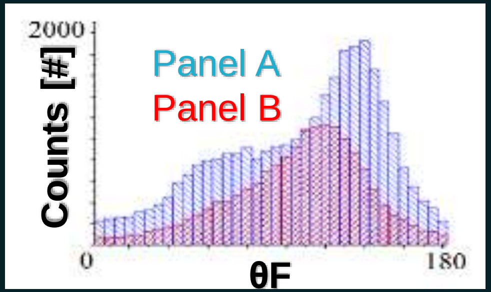


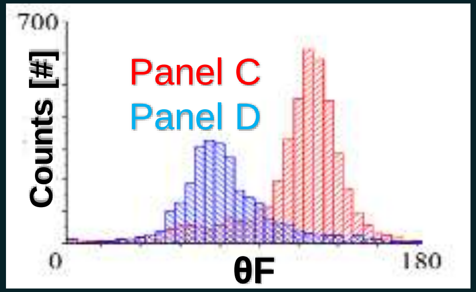
Skin

Tumor



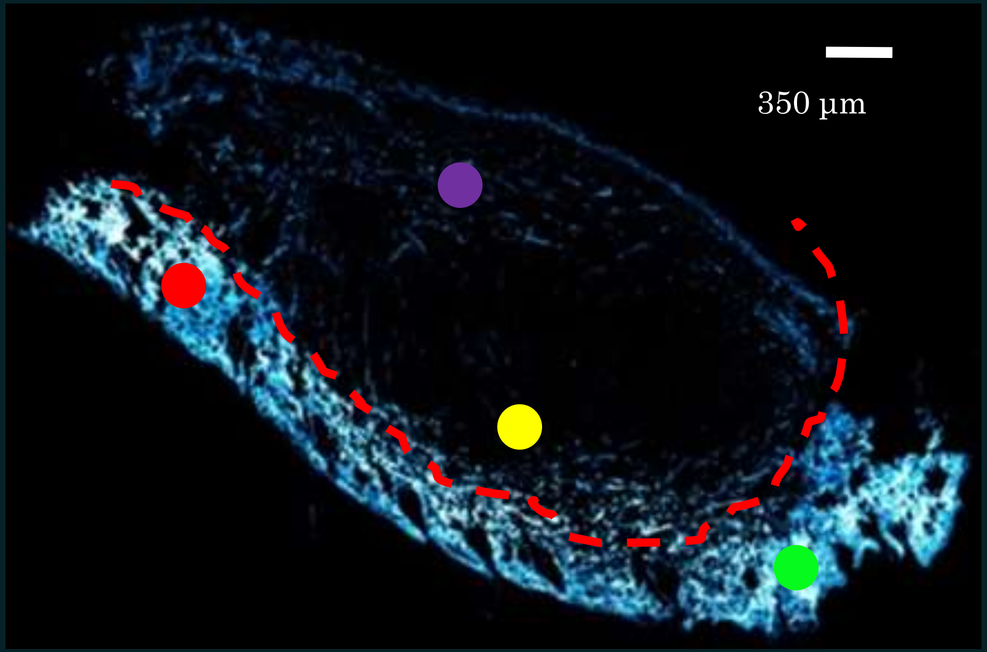
θF-values Histograms



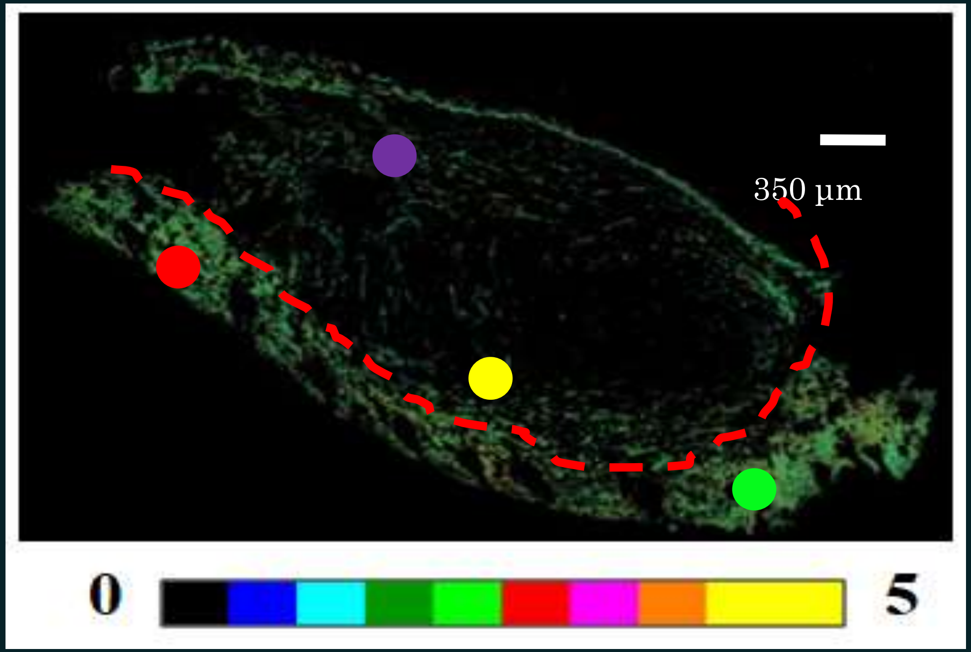


Tumor section analysis by μMAPPS

ISHG Map

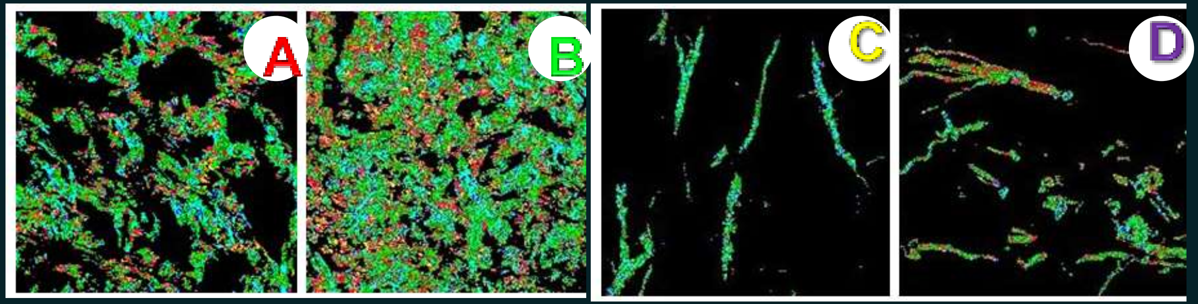


γ-values Map

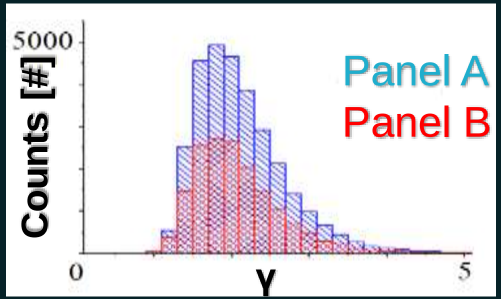


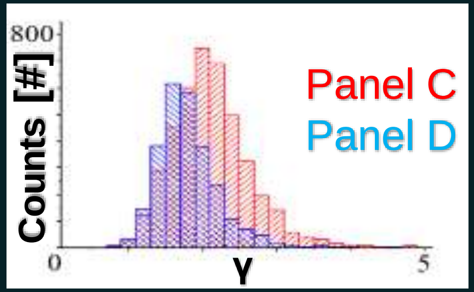
Skin

Tumor



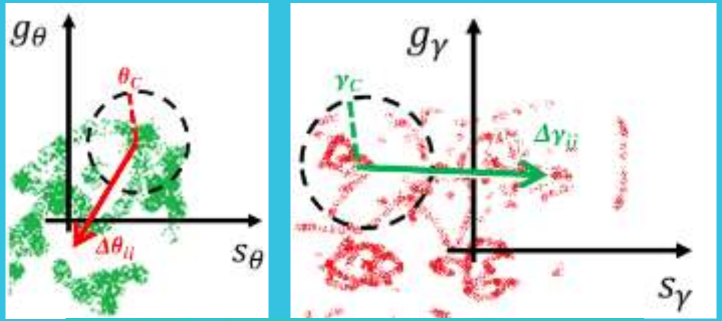
γ-values Histograms





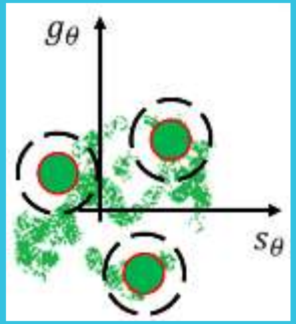
Clustering Procedure

Assignment phase



$$\rho_i = \sum_j \chi(\Delta\theta_{ij} - \theta_C)\chi(\Delta\gamma_{ij} - \gamma_C)$$

Aggregation phase

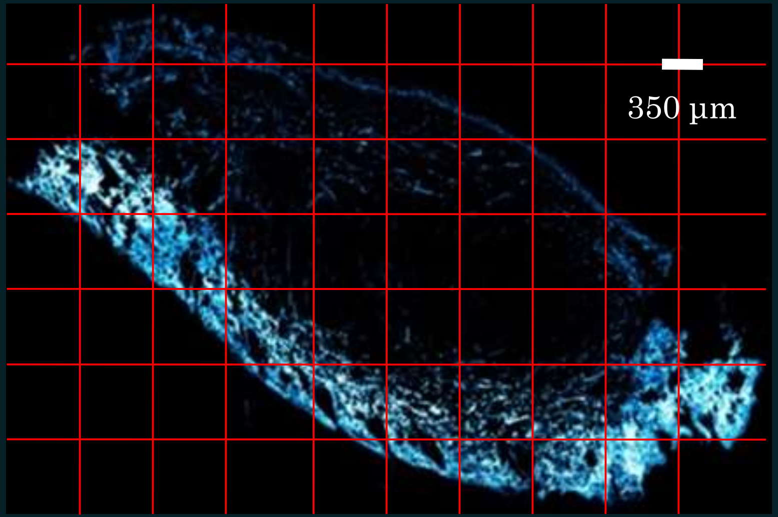


$$d_{ij} = \sqrt{\left|\frac{\Delta\theta_{ij}}{\theta_C}\right|^2 + \left|\frac{\Delta\gamma_{ij}}{\gamma_C}\right|^2}$$

Id Pixel	ρ
180	1501
792	1407
.	.
.	.
47	0

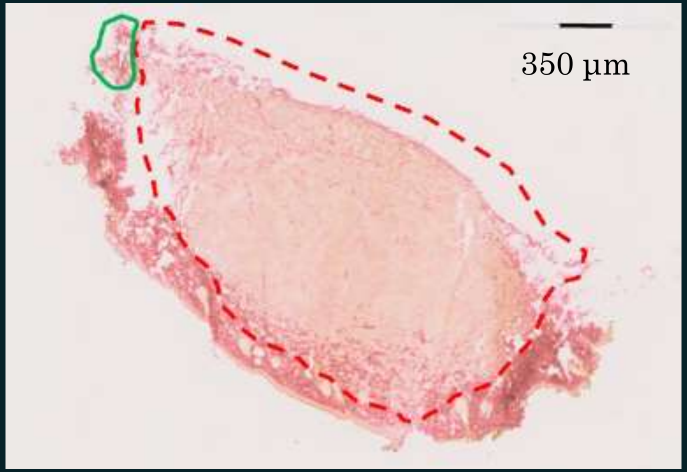
θ_c = Theta radius
γ_c = Gamma radius
$\Delta\theta_{ij}$ = Point-point distance in theta phasor space
$\Delta\gamma_{ij}$ = Point-point distance in gamma phasor space

ROI-based procedure, which exploits both θ_F and γ values.

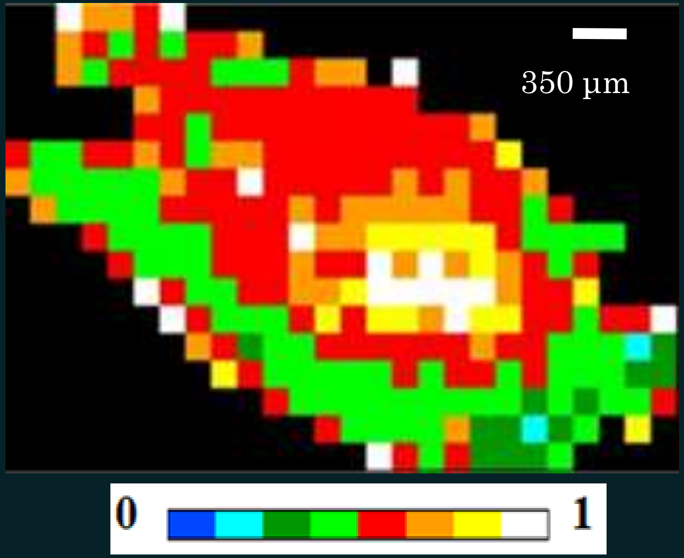


Results

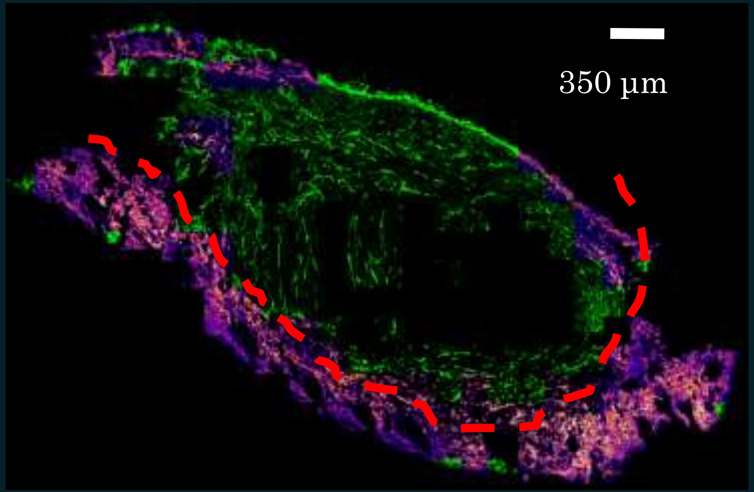
PicroSirius Red Staining



Fibril Entropy (H) Map



Tumor/Skin Map



New feature: FIBRIL ENTROPY (H)

$$H = \frac{-\sum_{i=1}^{N} \frac{x_i}{x_k} log \frac{x_i}{x_k}}{-log \frac{1}{x_k}}$$

X_i = # elements of the i-cluster
X_k = # total clustered elements

Accuracy (%)	True Negative	True Positive
Colon carcinoma	83.0 ± 4.5	91.8 ± 4.4
Breast carcinoma	87.5 ± 3.9	91.0 +- 6.0

Scodellaro et al. Front. Oncol. 9:527, 2019

Perspectives

- Fast microstructural analyses to assist the histo-pathological evaluation.
- In-situ diagnosis of pathologies and diseases.
- Cluster-based machine learning algorithms with diagnostic capability of tumors.
- Application to 3D samples.

Our team

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