Microscopia SHG e THG na detecção do câncer

X WORKSHOP TEÓRICO-PRÁTICO DO INFABIC
17-21 e 24-27 de outubro de 2022

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I B B

ORGANIZAÇÃO

- ✓ Microscopias não lineares
- ✓ Propriedades das microscopias não lineares
- ✓ Contraste nas microscopia SHG e THG
- ✓ Implementação
- ✓ Aplicações na detecção do câncer

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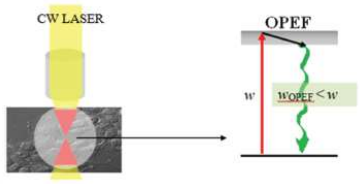
MICROSCOPIAS NÃO LINEARES

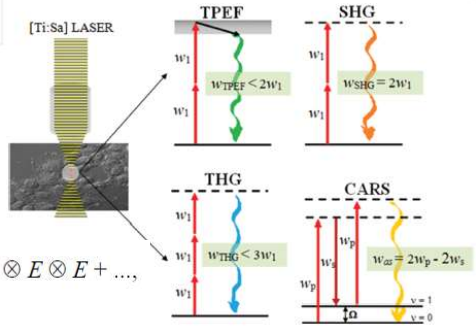
LINEAR

$$P = \chi^{(1)} \otimes E$$



NÃO LINEAR

$$P = \chi^{(1)} \otimes E + \chi^{(2)} \otimes E \otimes E + \chi^{(3)} \otimes E \otimes E \otimes E + \ldots,$$



J. Adur et al. (2016) Chapter 6, Nonlinear microscopy techniques…. Intech Open Science

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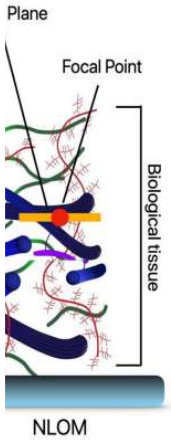
MICROSCOPIAS NÃO LINEARES

As sinais não lineares dependem da probabilidade de encontrar mais de um fóton no espaço e no tempo, o que aumenta dramaticamente no foco de um laser pulsado.



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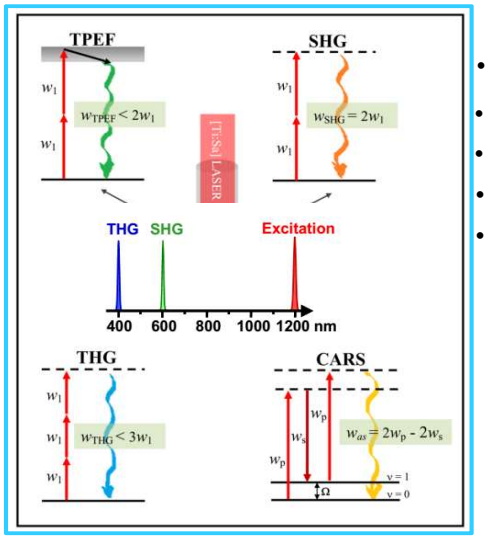
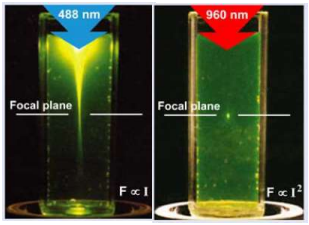
PROPRIEDADES



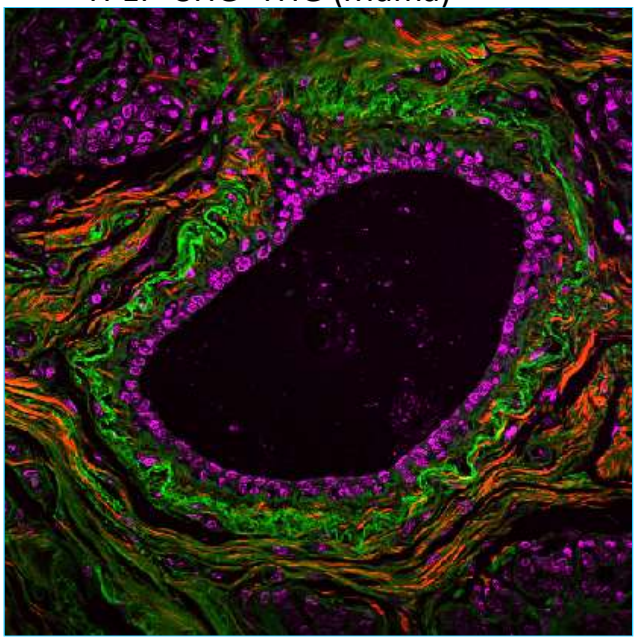
- São processos intrinsecamente confocais
- São processos elásticos e coerentes
- Não precisa de marcadores
- Maior penetração
- Fácil de separar com filtros



J. Adur et al. (2014) Chapter 3, Nonlinear Optics: Fundamentals, Applications…. Nova Science Publishers

5

TPEF+SHG+THG (Mama)



J. Adur et al. (2012) Journal of Biomedical Optics 17(8), 081407

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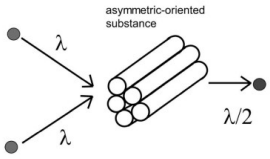
MECANISMOS DE CONTRASTE

La microscopía de segundo y tercer armónico se basan en los efectos ópticos que son inducidos por las propiedades físicas inherentes de la muestra.



SHG

-Permitido solo en moléculas sin simetría de inversión
-Favorecido en estructuras con alto grado de orientación y organización.

THG

-Permitido en cualquier medio
-Proviene de las interfaces ópticas y las heterogeneidades.

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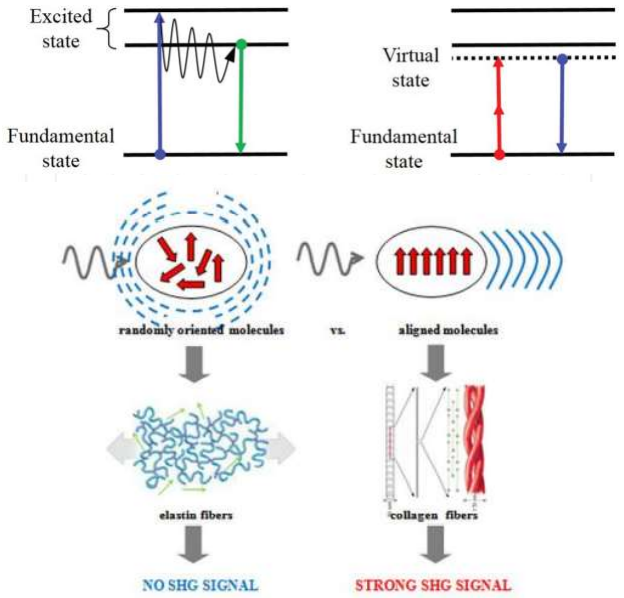
SEGUNDO HARMÔNICO



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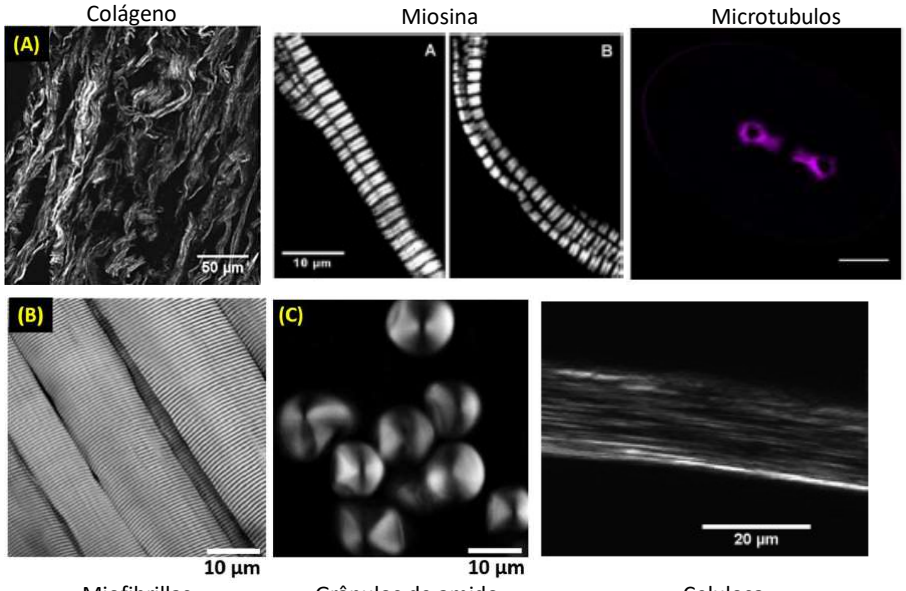
SEGUNDO HARMÔNICO

Colágeno | Miosina | Microtubulos



Miofibrillas | Grânulos de amido | Celulosa

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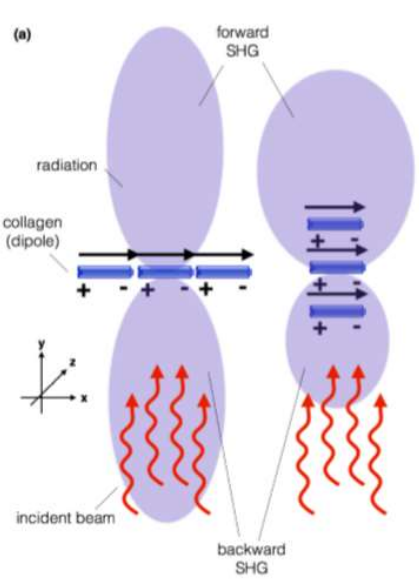

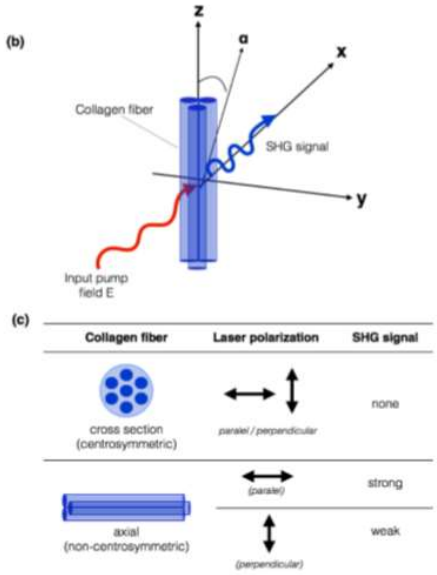

F / B ratio

P - SHG

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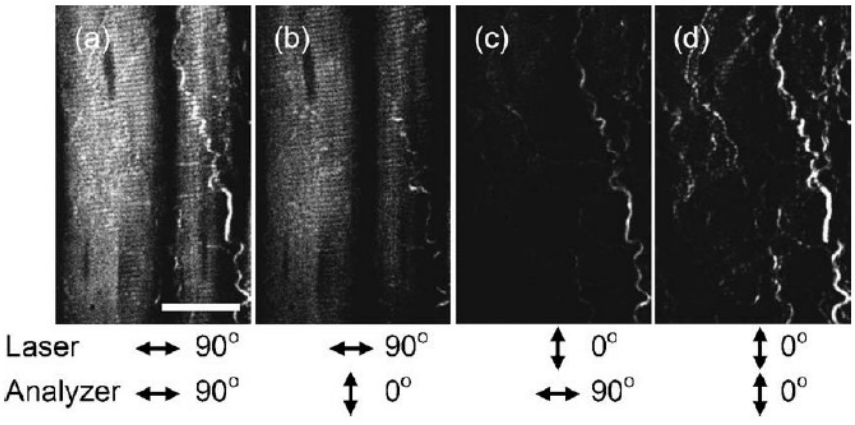

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CONTRASTE THG

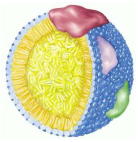
- Permitido em qualquer médio
- Derivado de interfaces e heterogeneidades óticas

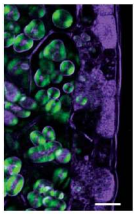
TABLE 4 Optical properties of lipids and glycerol at 1180 nm and comparison with water

	$\alpha = \chi^{(3)}/n_{3\omega}(n_{3\omega}-n_{\omega})$ $(10^{-22}\ m^2\ V^{-2})$	$\chi^{(3)}$ $(10^{-22}\ m^2\ V^{-2})$	$\lvert\alpha-\alpha\text{water}\rvert^2$	$\lvert\chi^{(3)}-\chi^{(3)}\ \text{water}\rvert^2$
Water	61.8 ± 3.6	1.68 ± 0.08	0	0
NaCl 1mol/L	63.9 ± 4.2	1.79 ± 0.09	0.012	4.41
Triglycerides	83.7 ± 5.0	2.58 ± 0.5	0.8	480
Triglycerides + cholesterol 1%	83.7 ± 5.0	2.58 ± 0.5	0.8	480
Vegetable oil	90.0 ± 5.0	2.71 ± 0.5	1.1	795
Glycerol	85.2 ± 5.0	2.63 ± 0.5	0.9	550

D. Débarre et al 2006. Nature Methods 30(1): 47-53

$$I_{\mathrm{THG}} = \left(\frac{3\omega}{2n_{\omega}c}\right)^2 \chi^{(3)} I_{\omega}^3 \int_{z1}^{z2} \frac{e^{i\Delta kz}}{(1+2iz/b)^2} dz,$$





O contraste de uma determinada estrutura depende do tamanho da mesma relativo ao volume focal.

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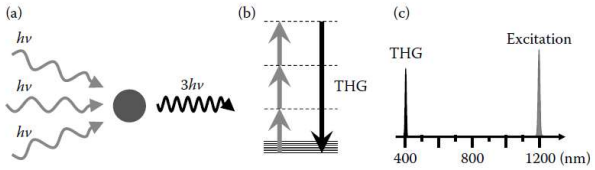
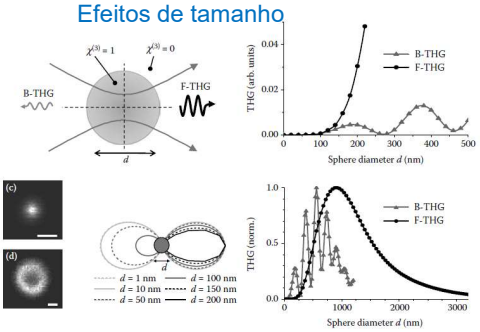
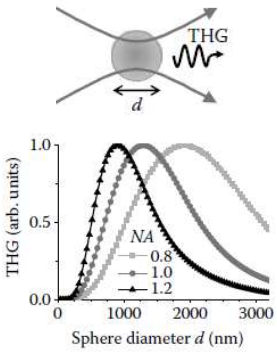
Processo THG



Efeitos de tamanho



Influência da Excitação NA



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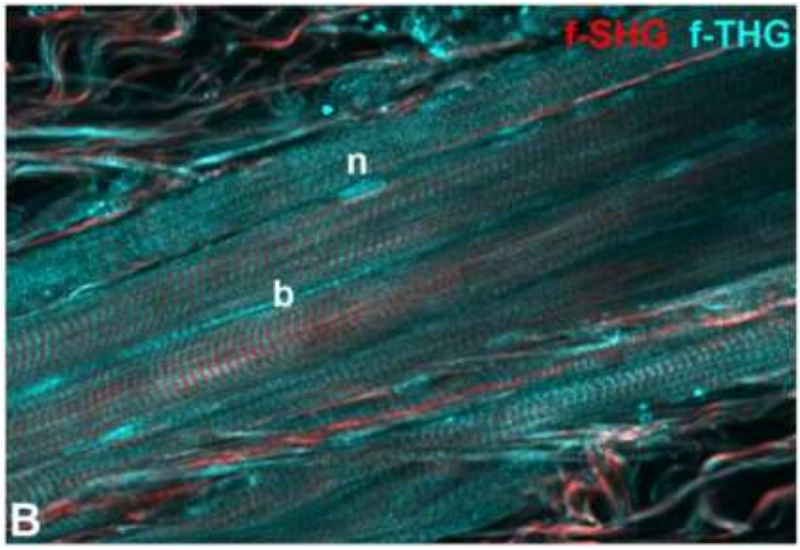

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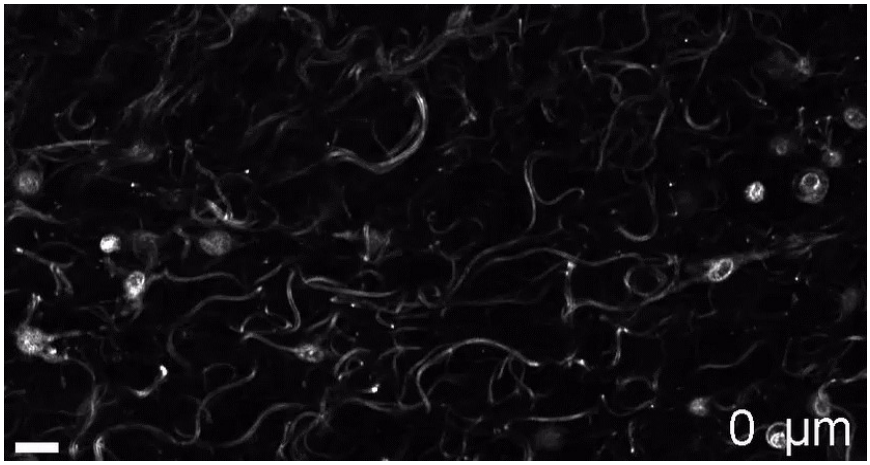

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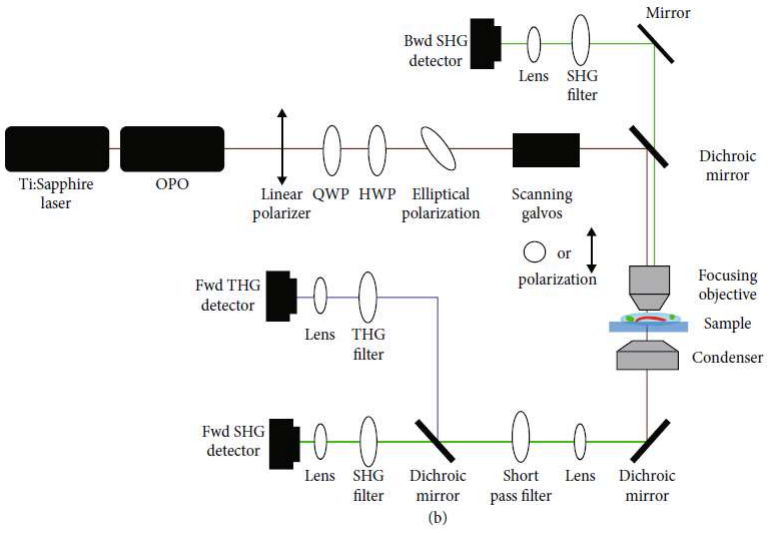
IMPLEMENTAÇÃO



(b)

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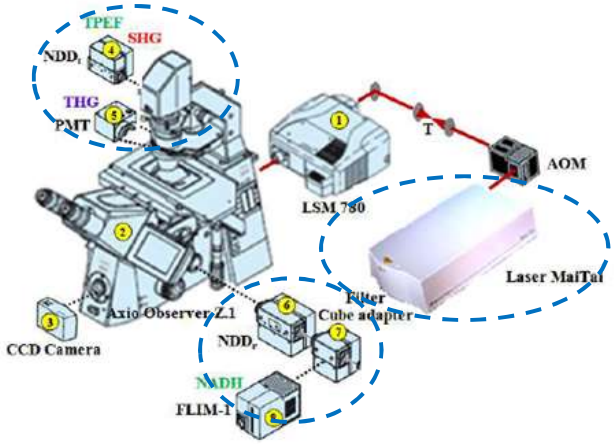
IMPLEMENTAÇÃO



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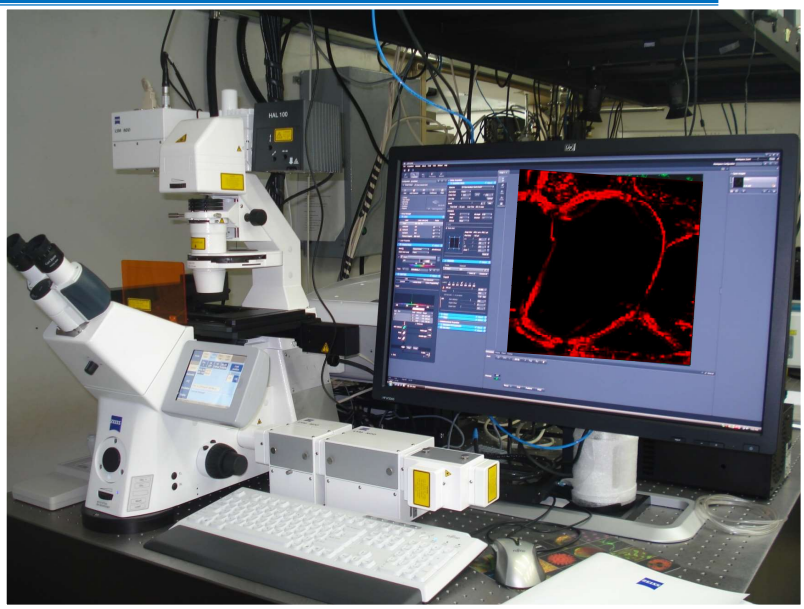

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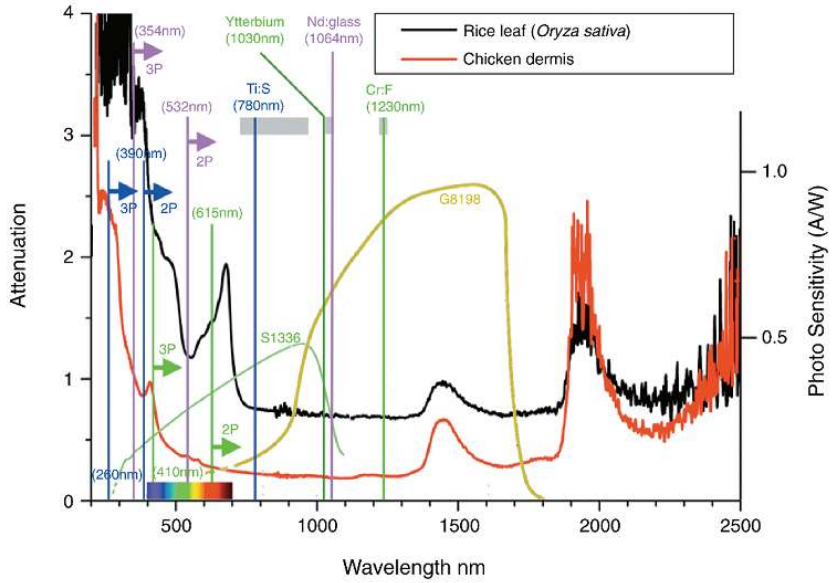

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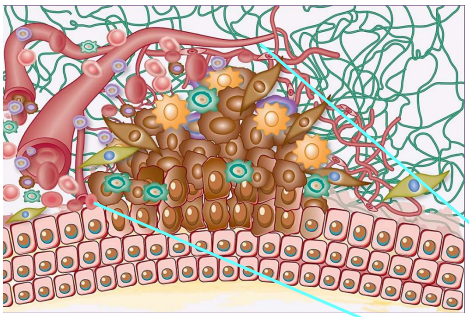
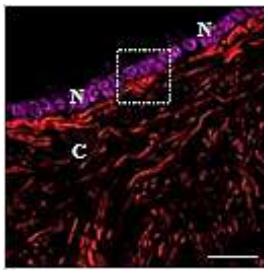
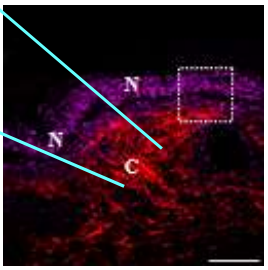
APLICACOES NA DETECAO DO CÂNCER



Ioana Berindan-Neagoe and George A. Calin, Clin Canc. Res. 2014





Adur 2014

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PLOS ONE

Optical Biomarkers of Serous and Mucinous Human Ovarian Tumor Assessed with Nonlinear Optics Microscopies

Javier Adur[1,2*], Vitor B. Pelegati[1], Andre A. de Thomaz[1], Mariana O. Baratti[6], Diogo B. Almeida[1], L. A. L. A. Andrade[3], Fátima Bottcher-Luiz[4], Hernandes F. Carvalho[5,6], Carlos L. Cesar[1,6]

1 Biophotonic Group, Optics and Photonics Research Cen
Brazil, 2 Microscopy Laboratory Applied to Molecular an
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and Gynecology, Faculty of Medical Sciences, State Univ
and Biophysics, Institute of Biology, State University of C
Photonics Applied to Cell Biology, Campinas, São Paulo

J. Biophotonics 1–13 (2012) / DOI 10.1002/jbio.201200108

Journal of BIOPHOTONICS

FULL ARTICLE

Second harmonic generation microscopy as a powerful diagnostic imaging modality for human ovarian cancer

Javier Adur[*,1,2], *Vitor B. Pelegati*[1], *Andre A. de Thomaz*[1], *Mariana O. Baratti*[3], *Liliana A. L. A. Andrade*[4], *Hernandes F. Carvalho*[3,5], *Fátima Bottcher-Luiz*[3,6], *and Carlos Lenz Cesar*[1,3]

Epithelial Ovarian Cancer Diagnosis of Second-Harmonic Generation Images: A Semiautomatic Collagen Fibers Quantification Protocol

Angel A Zeitoune[1,2], Johana SJ Luna[3], Kynthia Sanchez Salas[3], Luciana Erbes[1,2], Carlos L Cesar[4,5], Liliana ALA Andrade[6], Hernades F Carvahlo[4,7], Fátima Bottcher-Luiz[4,8], Victor H Casco[2] and Javier Adur[1,2,3]

Cancer Informatics
1–12

DOI: 10.1177/1176935117690162
SAGE

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Nonlinear Optical Microscopy Signal Processing Strategies in Cancer

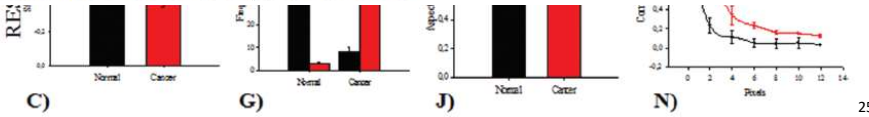
Javier Adur[1,2], Hernandes F. Carvalho[2], Carlos L. Cesar[2] and Víctor H. Casco[1]

CANCER INFORMATICS 2014:13 67

Table 2. Summary of the different methods for the analysis of nonlinear images.

METHODS	INDEX/VARIABLE	VALUES	MEANING	CALCULATION
SAAID	Collagen/elastin ratio	Positive (more collagen) Negative (more elastin)	Cancer tissues have high collagen concentration	Integrated density option in measure menu of ImageJ
TACS	Fiber angle (relative to epithelium)	TACS-2 ($\theta \equiv 0°$) TACS-3 ($\theta \equiv 90°$)	Normal tissues present TACS-2 while adenocarcinomas show TACS-3, which may be indicative of the invasive and metastatic growth potential	Angle tool of ImageJ
AR	Fiber direction	AR = 0 (anisotropic) AR = 1 (isotropic)	Normal tissues fibers show isotropic behavior. While in adenocarcinomas the fibers are more anisotropic	FFT process of ImageJ
GLCM	CORRELATION (Fiber separation) ENERGY	C ≡ 1 (displaced image = non displaced one, non-periodic fibers) C θ ≡ 0 (displaced image ≠ non displaced one, periodic fibers) [0, +1]	Normal tissues show more defined and periodic fibers While adenocarcinomas tissues show more random and non-periodic fibers. Energy is highest in images with uniform gray level or uniform gray level differences at the specified separation and lower for those with more variation in gray levels	GLCM plugin of ImageJ

Abbreviations: SAAID, second harmonic to autofluorescence ageing index of dermis; TACS, tumor-associated collagen signatures; AR, aspect ratio; θ, angle; C, correlation; FFT, fast Fourier transform; GLCM, gray level co-occurrence matrix.



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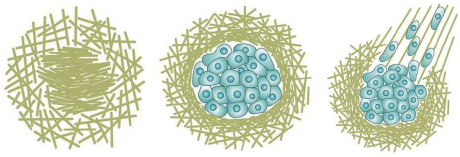
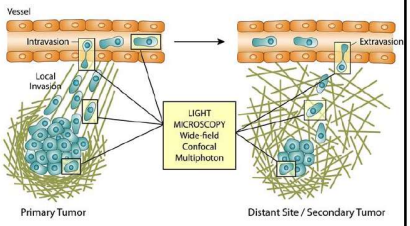
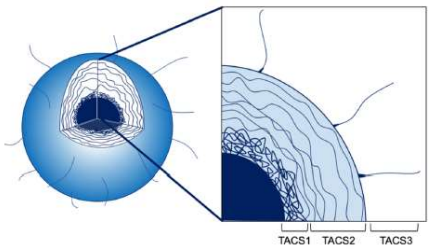
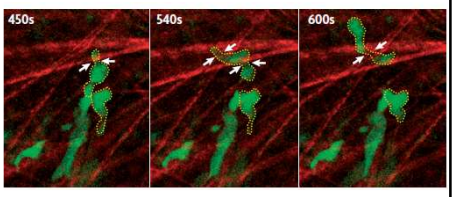
Tumor-associated collagen signatures (TACS)









E. Sahai 2007. Nature Reviews 7: 737-749

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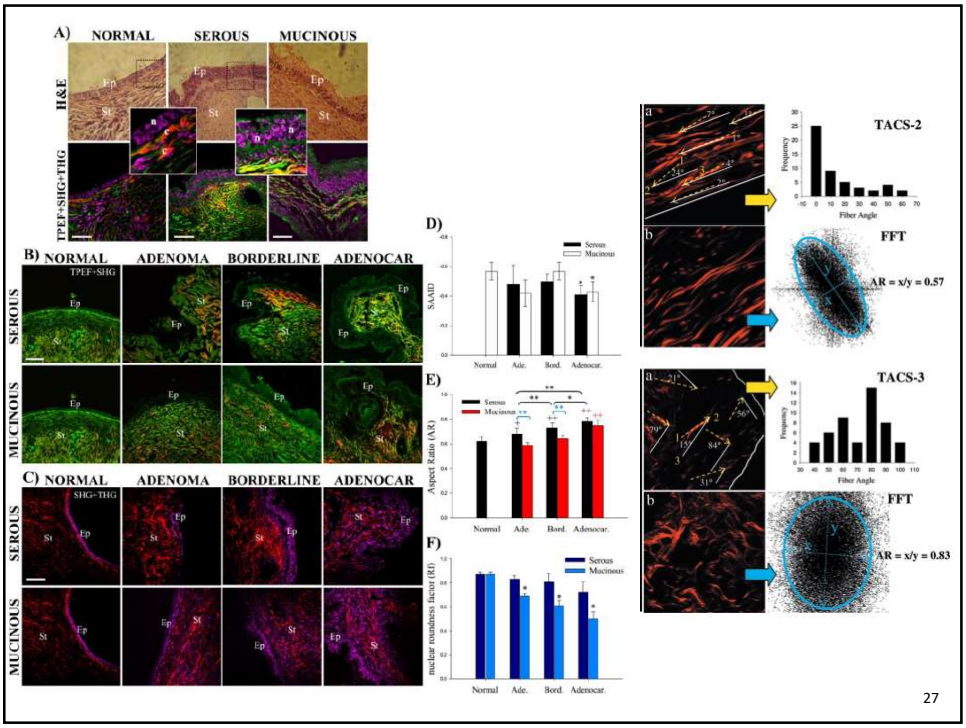

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Table 2. Summary of TPEF and SHG imaging studies and techniques to probe collagen remodeling of the ECM in ovarian cancer.

TECHNIQUE	RESULTS	CONCLUSIONS	AUTHORS
GLCM on SHG images, spatial frequencies + TPEF redox ratios	GLCM $Corr_{50}$ Normal and cancer statistically different	Variable redox ratios of high-risk women combined with collagen morphology may lead to improved detection	Kirkpatrick et al.[11]
	Spatial frequency: Cancer tissues had increase in low ($\leq$10 μm^{-1}) and decrease in high (72–92 μm^{-1}) spatial frequencies		
	Redox ratio: Low risk > high risk > cancer		
Support vector machine (SVM) using GLCM, FFT	Classified cancer vs normal: Sensitivity = 81.5% Specificity = 81.1%	SVM of GLCM and FFT is moderately successful in classifying collagen alterations of cancerous tissues.	Watson et al.[34]
TPEF, SHG, and THG combined with FFT, TACS, and cellular signatures	**FFT:** Normal: 0.65 ± 0.12 Benign: 0.74 ± 0.11 Boderline: 0.80 ± 0.10 Serous: 0.79 ± 0.11	Multimodal imaging approaches are useful in classifying normal, benign, borderline, and serous ovarian tissues	Adur et al.[31]
	TACS: Normal and benign tumors demonstrate TACS-2 Serous demonstrate TACS-3		
Texton classification	Classified normal and high-grade serous with 97% accuracy	Highly specific and versatile approach to classify ovarian tissues	Wen et al.[35]
SHG creation and anisotropy (β)	Type / F_{SHG} / β Normal / 93% / 0.76 Cancer / 77% / 0.88	Cancer is denser and more organized and has better packed fibrils than normal tissues	Nadiarnykh et al.[29]
Intravital TPEF and SHG imaging using STICK objective	Intrinsic tumor fluorescence is red-shifted relative to normal; Collagen is thicker in neoplasia lesions than normal tissues	Intravital TPEF and SHG imaging provides a means to detect small neoplastic regions using both collagen and cellular features	Williams et al.[13]

K Tilbury & P. J. Campagnola. Perspectives in Medicinal Chemistry 2015:7 21–32 doi: 10.4137/PMC.S13214.

Theranostics 2021, Vol. 11, Issue 7 3229

IVYSPRING INTERNATIONAL PUBLISHER

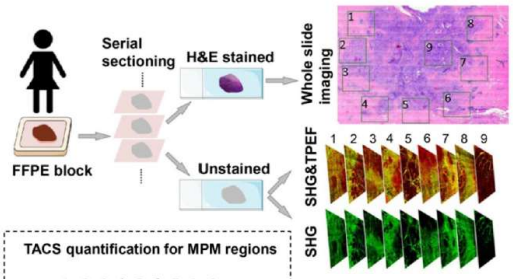
Theranostics

2021; 11(7): 3229-3243. doi: 10.7150/thno.55921

Research Paper

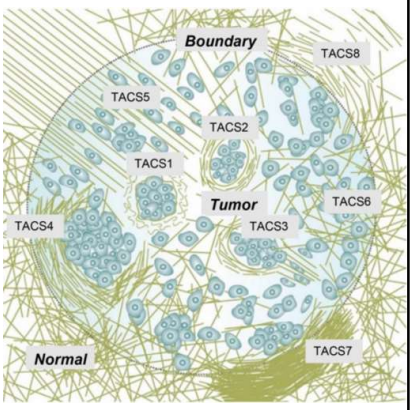
Large-scale tumor-associated collagen signatures identify high-risk breast cancer patients

Gangqin Xi[1*], Wenhui Guo[2*], Deyong Kang[3*], Jianli Ma[4*], Fangmeng Fu[2], Lida Qiu[1,5], Liqin Zheng[1], Jiajia He[1], Na Fang[1,6], Jianhua Chen[1,7], Jingtong Li[8], Shuangmu Zhuo[1], Xiaoxia Liao[9], Haohua Tu[10], Lianhuang Li[11✉], Qingyuan Zhang[4✉], Chuan Wang[2✉], Stephen A. Boppart[10✉], and Jianxin Chen[1✉]



TACS quantification for MPM regions

	1	2	3	4	5	6	7	8	9	percent
C_{TACS1}	✓				✓					2/9
C_{TACS2}	✓								✓	2/9
C_{TACS3}							✓			1/9
C_{TACS4}				✓	✓	✓		✓		4/9
C_{TACS5}			✓							1/9
C_{TACS6}	✓									1/9
C_{TACS7}				✓				✓		2/9
C_{TACS8}						✓				1/9



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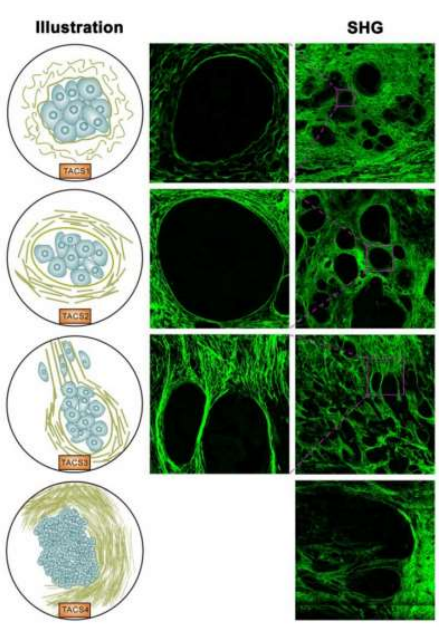

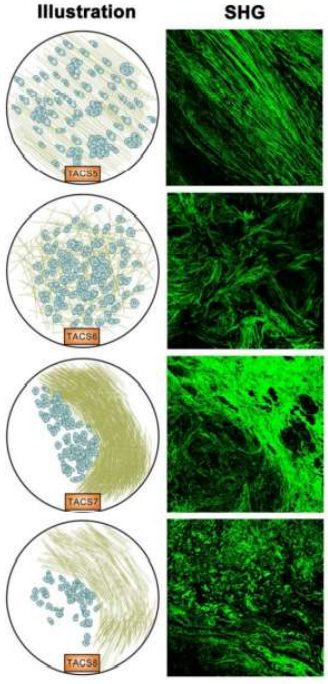

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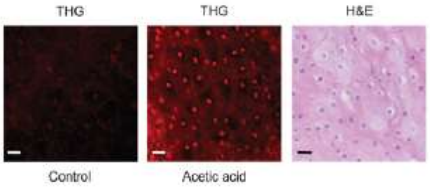
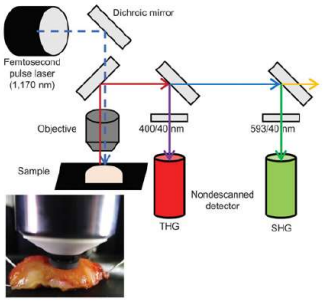
CANCER RESEARCH | CONVERGENCE AND TECHNOLOGIES

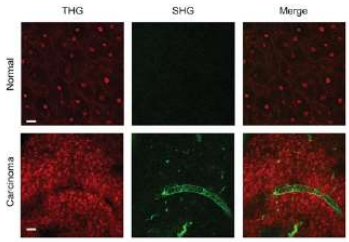
July 23, 2020; DOI: 10.1158/0008-5472.CAN-20-0348

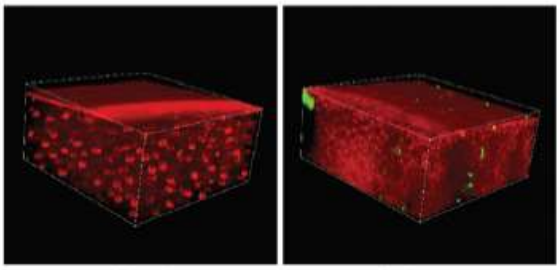
Nonlinear Optics with Near-Infrared Excitation Enable Real-Time Quantitative Diagnosis of Human Cervical Cancers

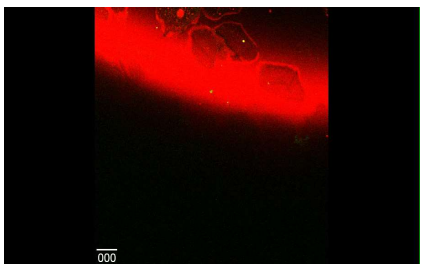
Takahiro Matsui[1], Ryo Tamoto[2], Akio Iwasa[2], Masafumi Mimura[2], Seiji Taniguchi[1], Tetsuo Hasegawa[1],











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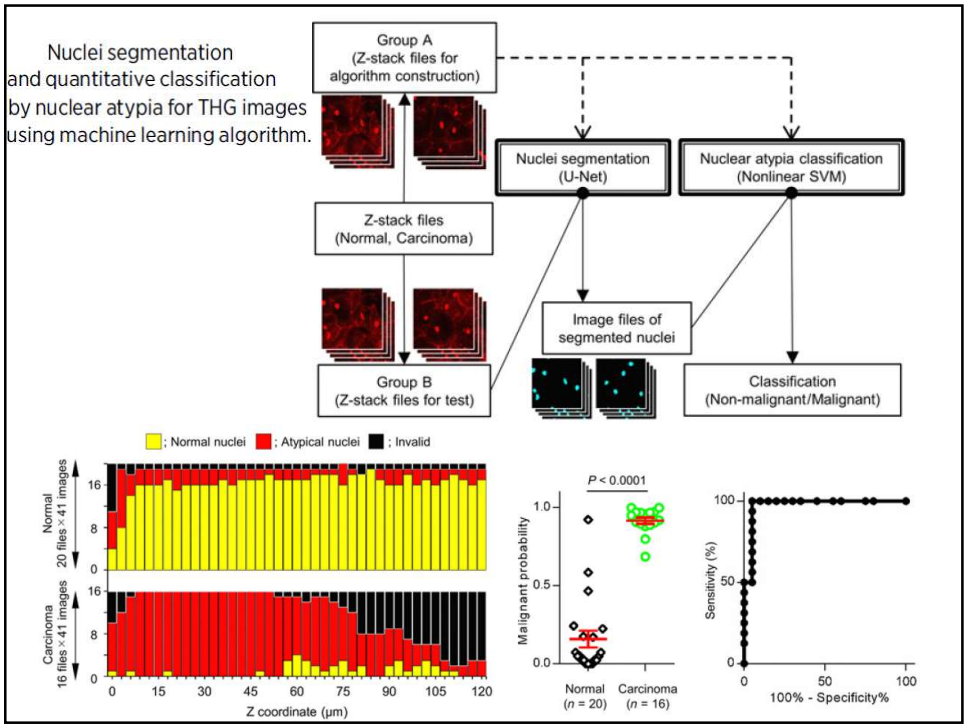
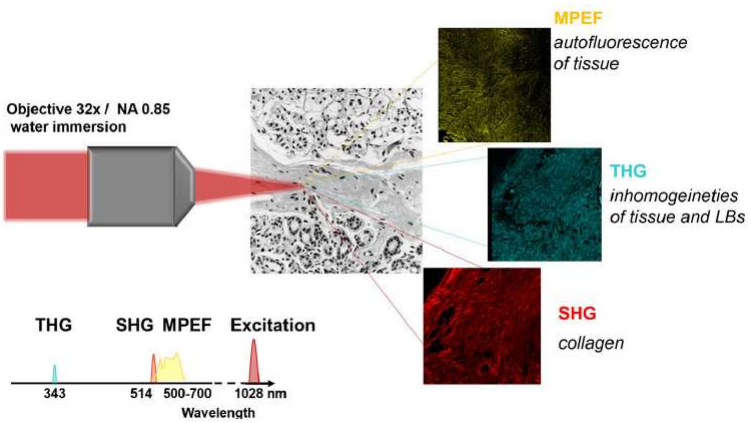
Nuclei segmentation and quantitative classification by nuclear atypia for THG images using machine learning algorithm.



Third Harmonic Generation microscopy distinguishes malignant cell grade in human breast tissue biopsies

SCIENTIFIC REPORTS
nature research

Evangelia Gavgiotaki[1,2], George Filippidis[1✉], Vassilis Tsafas[1,3], Savvas Bovasianos[1,3], George Kenanakis[1], Vasilios Georgoulias[2], Maria Tzardi[2], Sofia Agelaki[2] & Irene Athanassakis[4✉]



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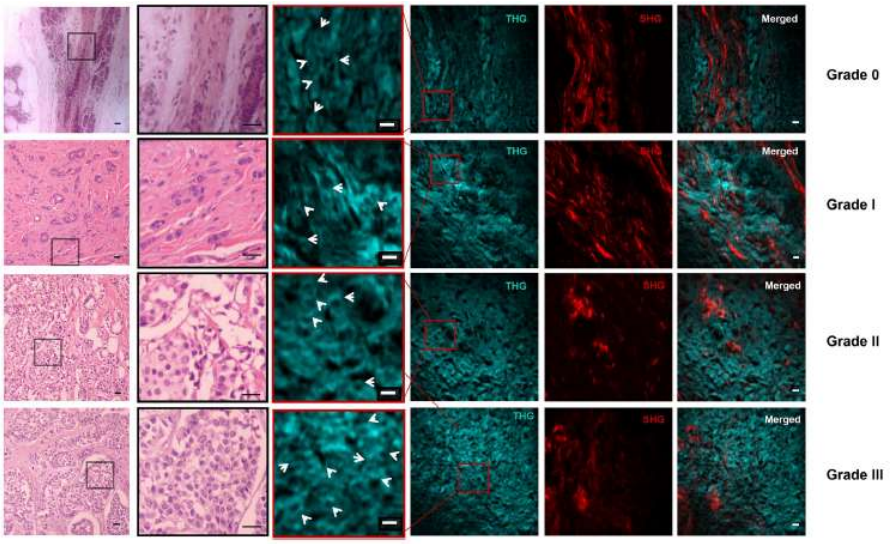
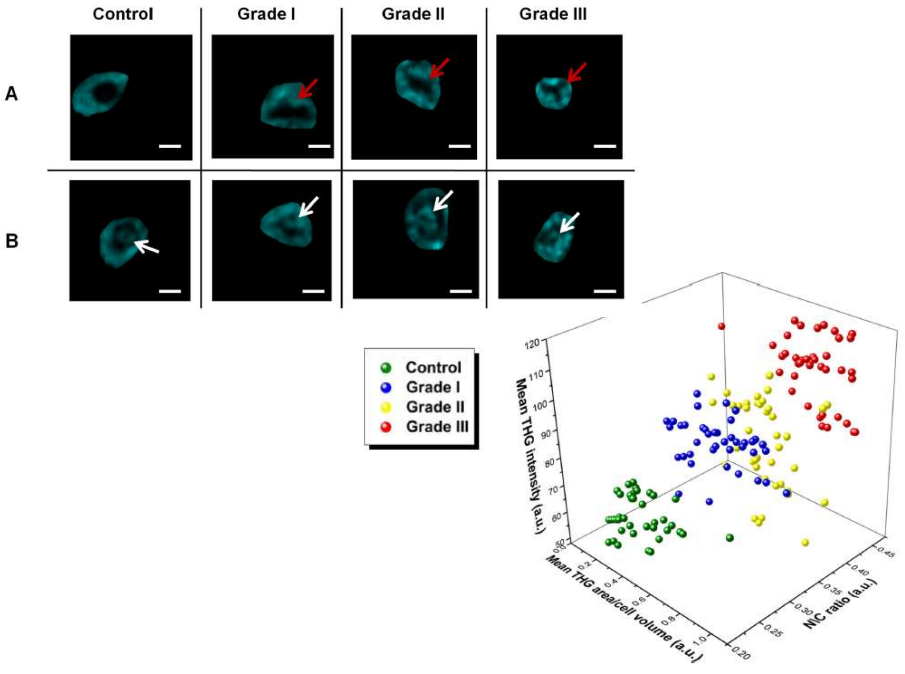

THG
SHG
Merged
Grade 0
Grade I
Grade II
Grade III

35


Control
Grade I
Grade II
Grade III
A
B
Control
Grade I
Grade II
Grade III
Mean THG intensity (a.u.)
Mean THG area/cell volume (a.u.)
N/C ratio (a.u.)

FULL ARTICLE

TRANSLATIONAL BIOPHOTONICS

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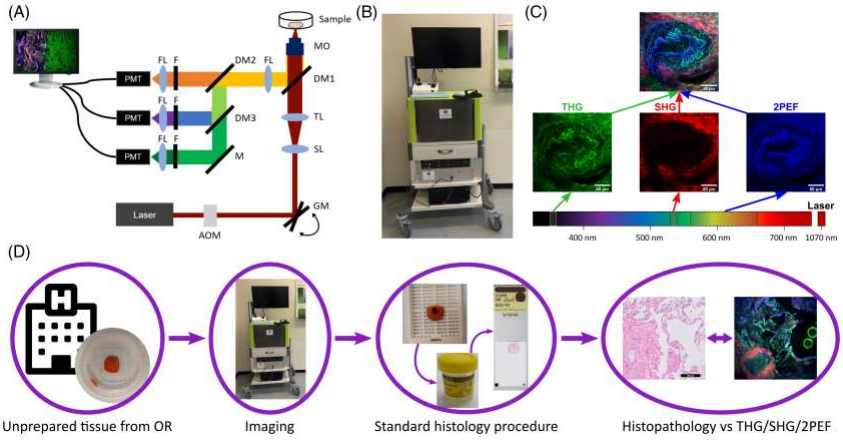
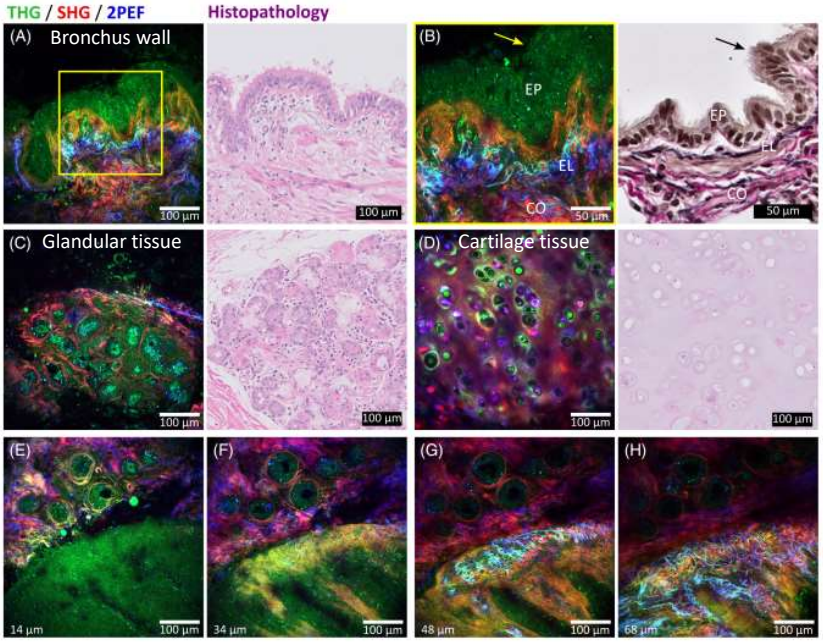
Compact portable multiphoton microscopy reveals histopathological hallmarks of unprocessed lung tumor tissue in real time



Imagens de tecido não tumoral da parede do brônquio



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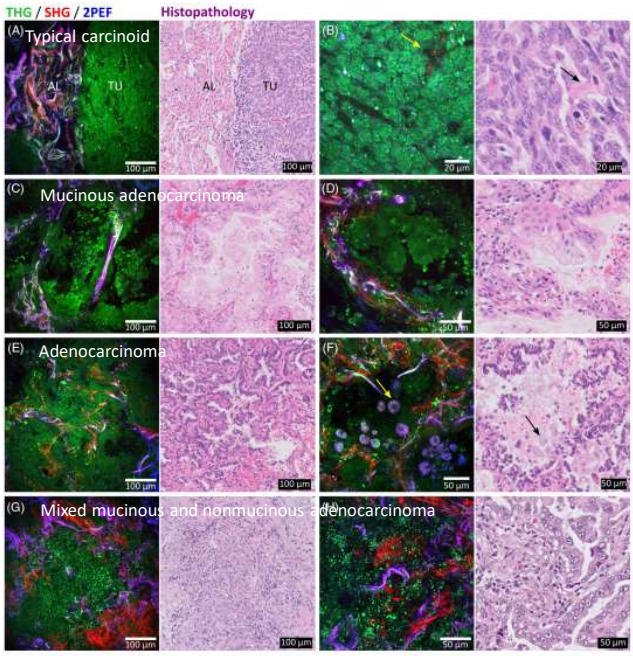
Imagens de vários tipos de tecidos tumorais



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Cytometry
Cytometry Part A • 95A: 47–55, 2019

In Vivo Metabolic and SHG Imaging for Monitoring of Tumor Response to Chemotherapy

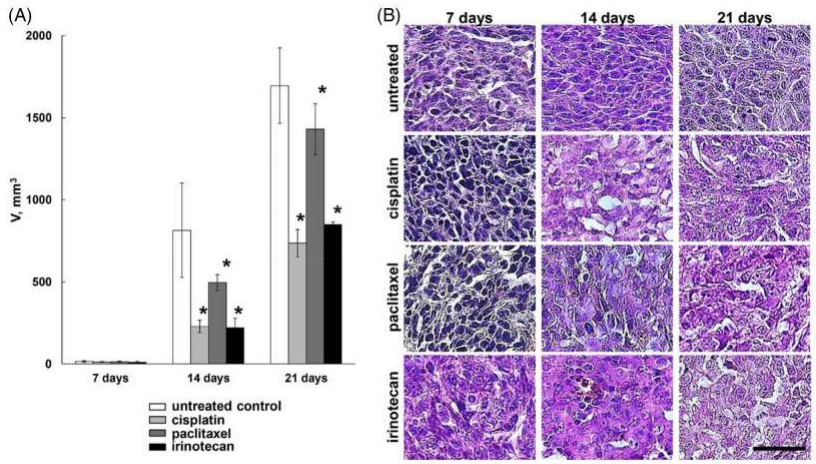

Figure 1. Effects of chemotherapy on ☒T26 tumors in mice. (**A**) Tumor volume dynamics in response to cisplatin, paclitaxel, and irinotecan. Mean ±SEM, $n = 3$. *Statistically significant difference between treated and untreated tumors, $P \leq 0.05$. (**B**) Posttreatment histopathology of tumors. Representative tissue sections stained with H&E (original magnification × 40). Bar is 25 μm. [Color figure can be viewed at wileyonlinelibrary.com]

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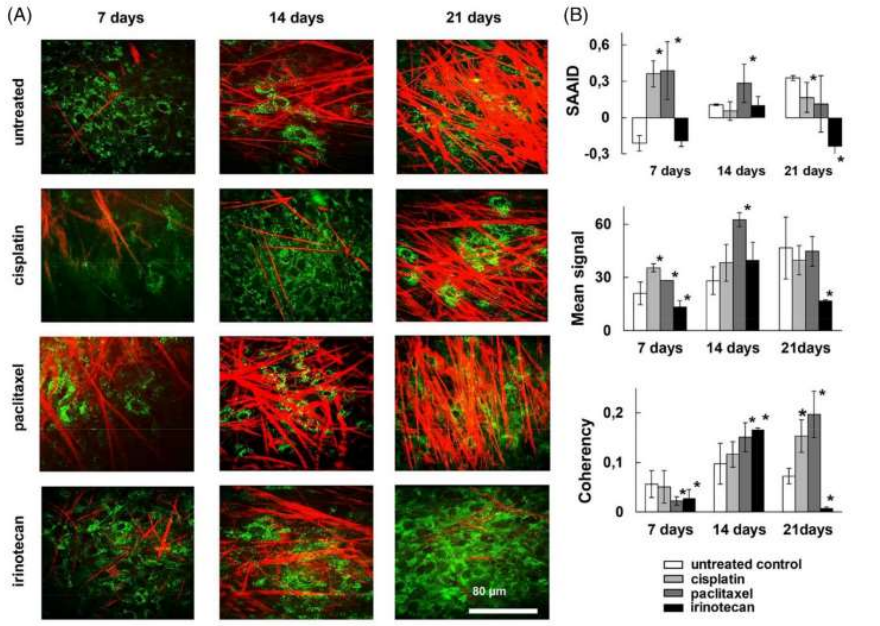
(A)
7 days
14 days
21 days
untreated
cisplatin
paclitaxel
irinotecan
80 µm
(B)
SAAID
0,6
0,3
0
-0,3
7 days
14 days
21 days
Mean signal
60
30
0
7 days
14 days
21days
Coherency
0,2
0,1
0
7 days
14 days
21days
untreated control
cisplatin
paclitaxel
irinotecan

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Obrigado por sua atenção...

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