



**Universitatea POLITEHNICA din București
CENTRUL DE MICROSCOPIE- MICROANALIZA
SI PROCESAREA INFORMATIEI**

DEZVOLTARI PRIVIND NOI TEHNICI DE INVESTIGATIE IN MICROSCOPIA OPTICA CU BALEIAJ SI APLICATII ALE ACESTORA

Cod CNCISIS: 1566

**Director proiect:
Prof. dr. Gheorghe A. Stanciu**



I. Indicatori de performanta realizati

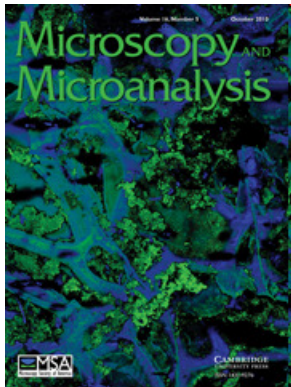


(Factor de impact 1.850)

[Automated Compensation of Light Attenuation in Confocal Microscopy by Exact Histogram Specification](#)

Stanciu, SG; Stanciu, GA and Coltuc, D
MICROSCOPY RESEARCH AND TECHNIQUE

Volume: 73 Issue: 3 Pages: 165-175 Published: 2010 (cu ack.)



(Factor de impact 3.035)

[On the Suitability of SIFT Technique to Deal with Image Modifications Specific to Confocal Scanning Laser Microscopy](#)

Stanciu, SG; Hristu, R; Boriga, R and Stanciu GA
MICROSCOPY AND MICROANALYSIS

Volume: 16 Issue: 5 Pages: 515-530 Published: 2010 (cu ack.)



(Factor de impact 2.067)

[Influence of Confocal Scanning Laser Microscopy specific acquisition parameters to the detection and matching of Speeded-Up Robust Features](#)

Stanciu, SG; Hristu, R and Stanciu GA
ULTRAMICROSCOPY

Lucrare acceptata pentru publicare, (cu ack.)



CdS nanocrystals for laser scanning microscopy

Savu, B; Peretz, S; Stanciu, GA
OPTOELECTRONICS AND ADVANCED MATERIALS-RAPID
COMMUNICATIONS

Volume: 4 Issue: 4 Pages: 453-456 Published: 2010 (cu ack.)

Two photon emission and nonlinear optical imaging of acetonitrile treated quasi-spherical nanoscale PbS systems

Dutta, N; Mohanta, D; Ahmed, GA; Choudhury, A; Hristu, R; Stanciu, SG; Stanciu, GA;

IEEE PHOTONICS JOURNAL

Volume: PP Issue:99 Published: 2010 (cu ack.)

DOI: 10.1109/JPHOT.2010.2093513

Sum-modified-Laplacian Fusion Methods Experimented on Image Stacks of Photonic Quantum Ring Laser Devices Collected by Confocal Scanning Laser Microscopy

Stanciu, SG; Dragulinescu, M and Stanciu, GA
UPB SCIENTIFIC BULLETIN – SERIES A

Lucrare acceptata pentru publicare (cu ack.)





[Optical induced current technique used to investigate the photonic quantum ring laser](#)

Stanciu, G.A. Hristu, R. Stanciu, S.G. O'Dae Kwon Kim, D.K. Proceedings of IEEE International Conference on Transparent Optical Networks 2010 (lucrare indexata ISI)

DOI: 10.1109/ICTON.2010.5549187 Published: 2010 (cu ack.)



[Image Fusion Methods for Confocal Scanning Laser Microscopy experimented on Images of Photonic Quantum Ring Laser Devices](#)

Stanciu, SG

Capitol in cartea intitulata "Image Fusion", Intech Open Access Publisher, ISBN: 978-953-7619-X-X, Va apare in Ianuarie 2011 (cu ack.)

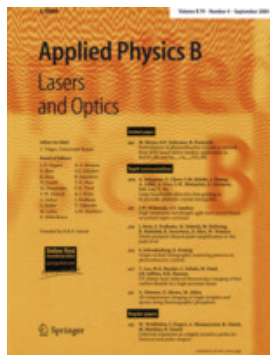


[Two-photon excited photoluminescence of photonic quantum ring laser structures](#)

Hristu, R; Stanciu, SG; Kao, FJ; Kwon, O'D and Stanciu, GA

In review la Optics Express, (cu ack.)

(Factor de impact 3.278)



[Optical beam induced current microscopy of photonic quantum ring lasers](#)

Hristu, R.; Wu, SJ; Kwon, O'D, Stanciu, SG; Kao, FJ and Stanciu, GA

In review la Applied Physics B: Lasers and Optics, (cu ack.)

(Factor de impact 1.992)



[Digital Image Inpainting and Microscopy Imaging](#)

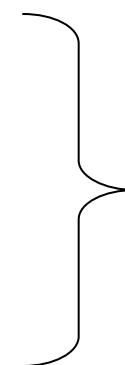
Stanciu, SG; Hristu, R and Stanciu GA

In review la Microscopy Research and Technique, (cu ack.)

(Factor de impact 1.850)

Lucrari din domeniul ‘Microscopie’ care urmeaza a fi premiate in urma participarii la competitia CNCSIS pentru premiarea rezultatelor cercetarii in 2010 :

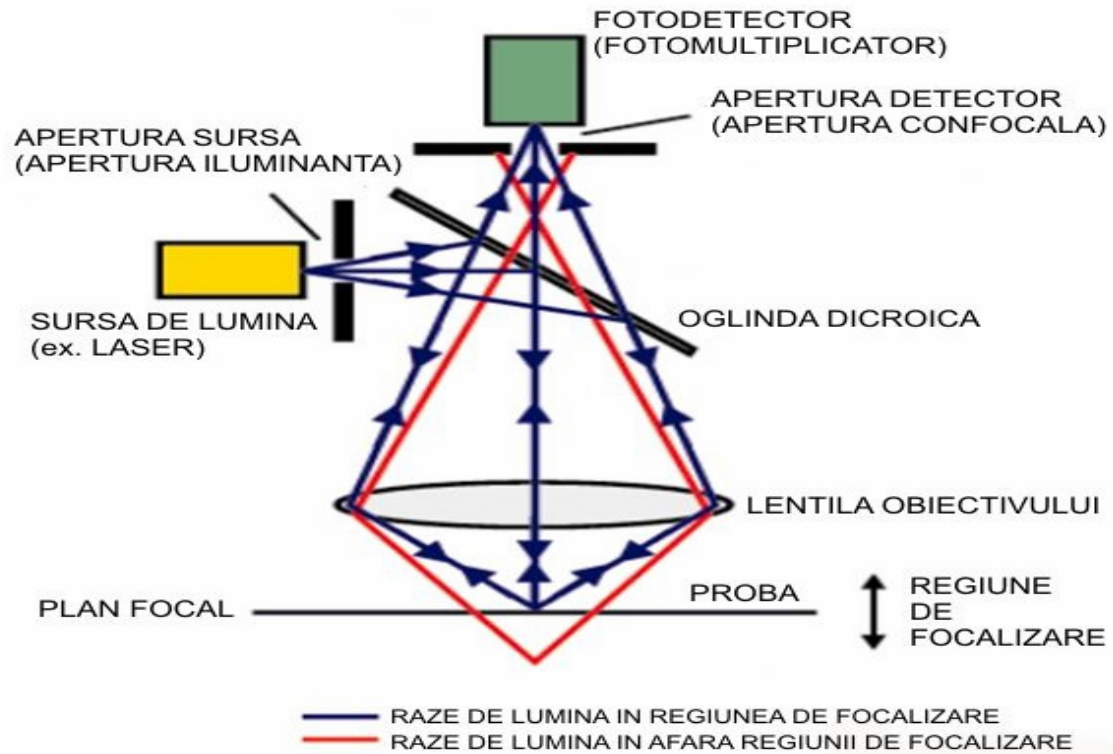
COD CNCSIS	TITLU ARTICOL	DATE_REVISTA	DOMENII_STIINTIFICE
86	COLLAGEN-CHONDROITIN SULPHATE-HYDROXYAPATITE POROUS COMPOSITES: A HISTOCHEMICAL AND ELECTRON MICROSCOPY APPROACH Zarnescu O, Craciunescu O, Moldovan L.	MICROSCOPY AND MICROANALYSIS, VOL. 16, NO. 2, 137-142.	MICROSCOPY
266	INTEGRATED METHODOLOGY FOR THE EVALUATION OF CLEANING EFFECTIVENESS IN TWO RUSSIAN ICONS (16TH-17TH CENTURIES) Sandu IC, Bracci S, Lobefaro M, Sandu I.	MICROSCOPY RESEARCH AND TECHNIQUE, 73, 8: 752-760, August 2010	MICROSCOPY
359	AUTOMATED COMPENSATION OF LIGHT ATTENUATION IN CONFOCAL MICROSCOPY BY EXACT HISTOGRAM SPECIFICATION Stanciu SG, Stanciu GA, Coltuc D.	MICROSCOPY RESEARCH AND TECHNIQUE, 73 (3), 165-175, Martie 2010	MICROSCOPY
360	ON THE SUITABILITY OF SIFT TECHNIQUE TO DEAL WITH IMAGE MODIFICATIONS SPECIFIC TO CONFOCAL SCANNING LASER MICROSCOPY Stanciu SG, Hristu R, Boriga R, Stanciu GA.	MICROSCOPY AND MICROANALYSIS, VOL. 16 NO. 5, 515-530	MICROSCOPY



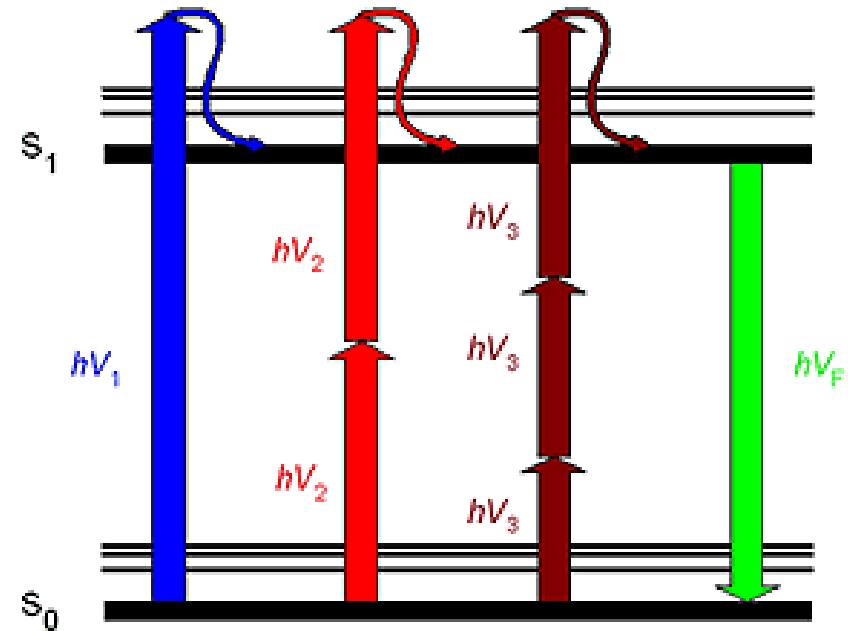
Lucrari rezultate din proiectul curent

II. Principalele rezultate noi, originale,
valoroase din punct de vedere stiintific

Microscopie confocala cu baleiaj laser



Imagistica bazata pe excitatia cu doi fotoni si pe efecte neliniare coerente in microscopia cu baleiaj laser



Excitatiea mediului in microscopia confocala si in microscopia bazata pe excitatiea cu doi fotoni si pe efecte coerente neliniare

Avantajele utilizarii efectelor neliniare fata de microscopia confocala

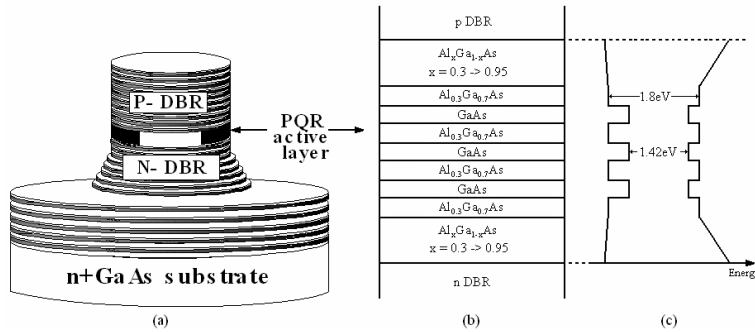
- Patrundere in adancime datorita utilizarii radiatiei laser IR
- Imprastiere Rayleigh scazuta datorita lungimii de unda mari (imprastierea Rayleigh $\sim 1/\lambda^4$)
- Excitatie doar in planul focal ceea ce inlatura efecte ca fototoxicitatea si fotodistrugerea in restul volumului probei
- In cazul SHG si THG nu este nevoie de marcari pentru a obtine imagini ale probei

Microscop confocal spectral Leica



Investigatii pe lasere cu inel fonic cuantic utilizand tehnica LBIC

Colaborare cu Pohang University of Science & Technology (Coreea)



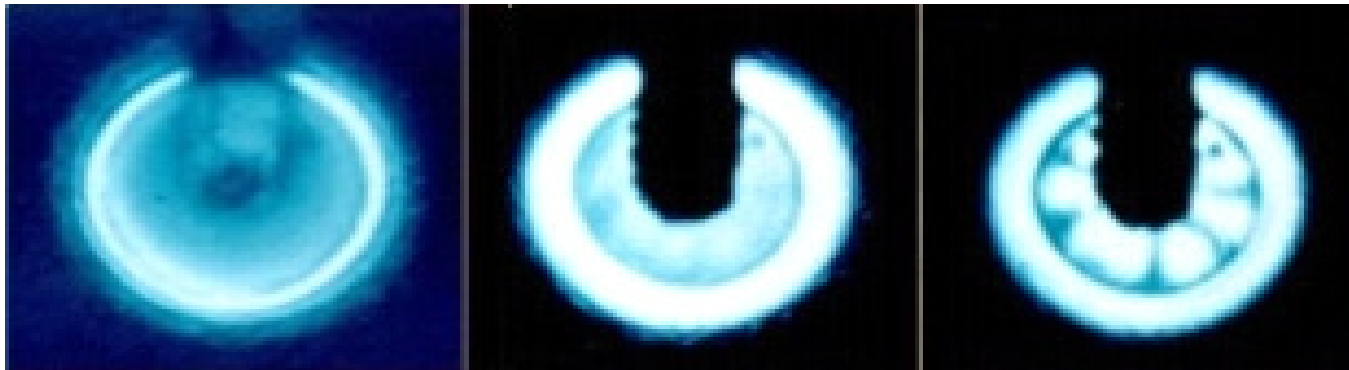
Structura similara cu VCSEL

Confinare fotoni: - verticala realizata de oglinzi Bragg distribuite

- in plan realizata de diferenta de indice de refractie intre mediu activ si aer

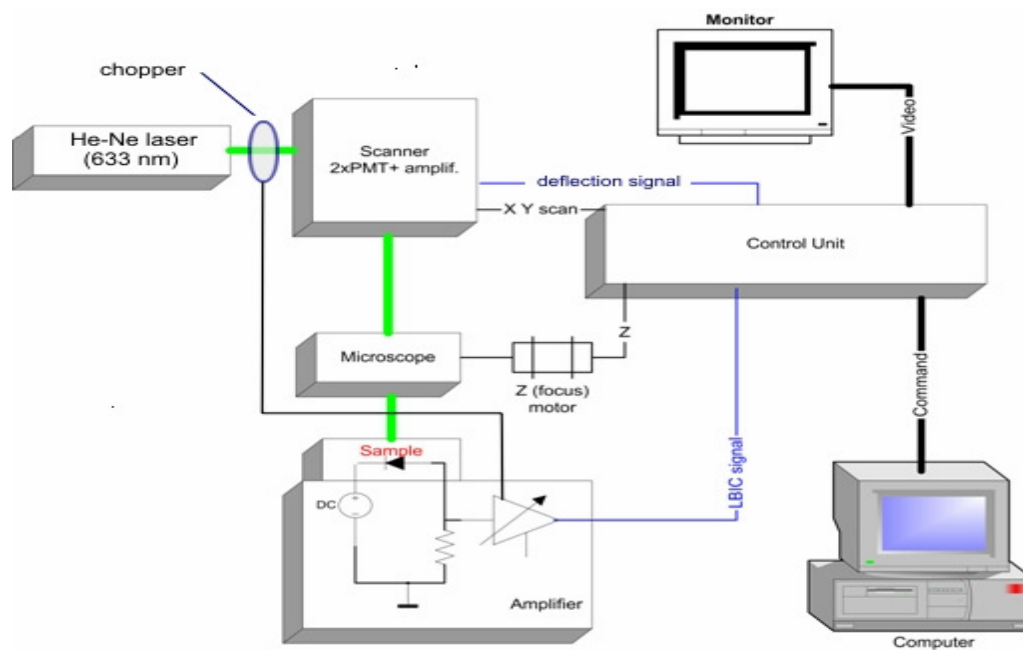
- ⇒ Dubla confinare genereaza un toroid Rayleigh (definit de banda Rayleigh)
- ⇒ Doua regimuri de lasare: - mod PQR, curenti de prag mici, lasare pe inel periferic

- mod VCSEL tipic

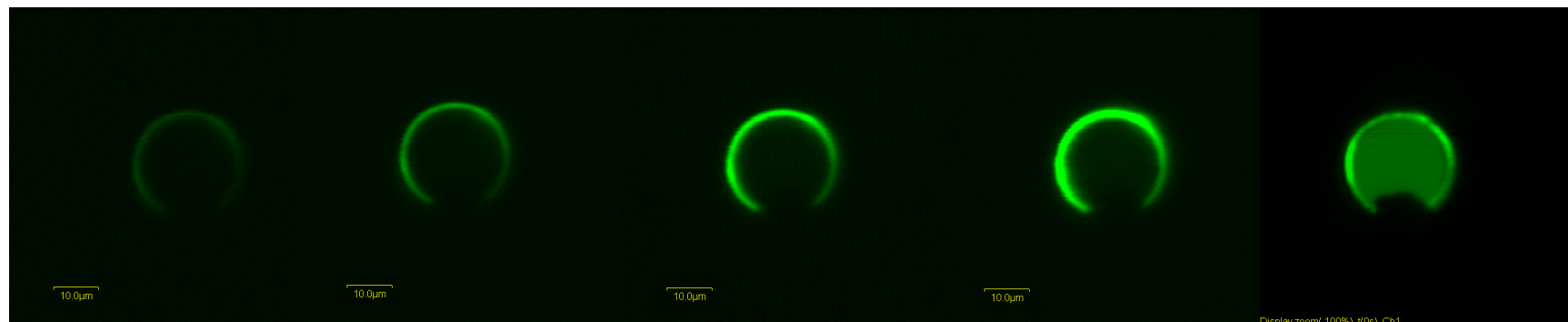


Imagini ale emisiei laser la: 12 μ A (sub prag PQR), la 11.5 mA, sub pragul VCSEL si la 12.2 mA, deasupra pragului VCSEL

Investigatii pe lasere cu inel fonic cuantic utilizand tehnica LBIC



Investigatii pe lasere cu inel fonic cuantic utilizand tehnica LBIC



a

b

c

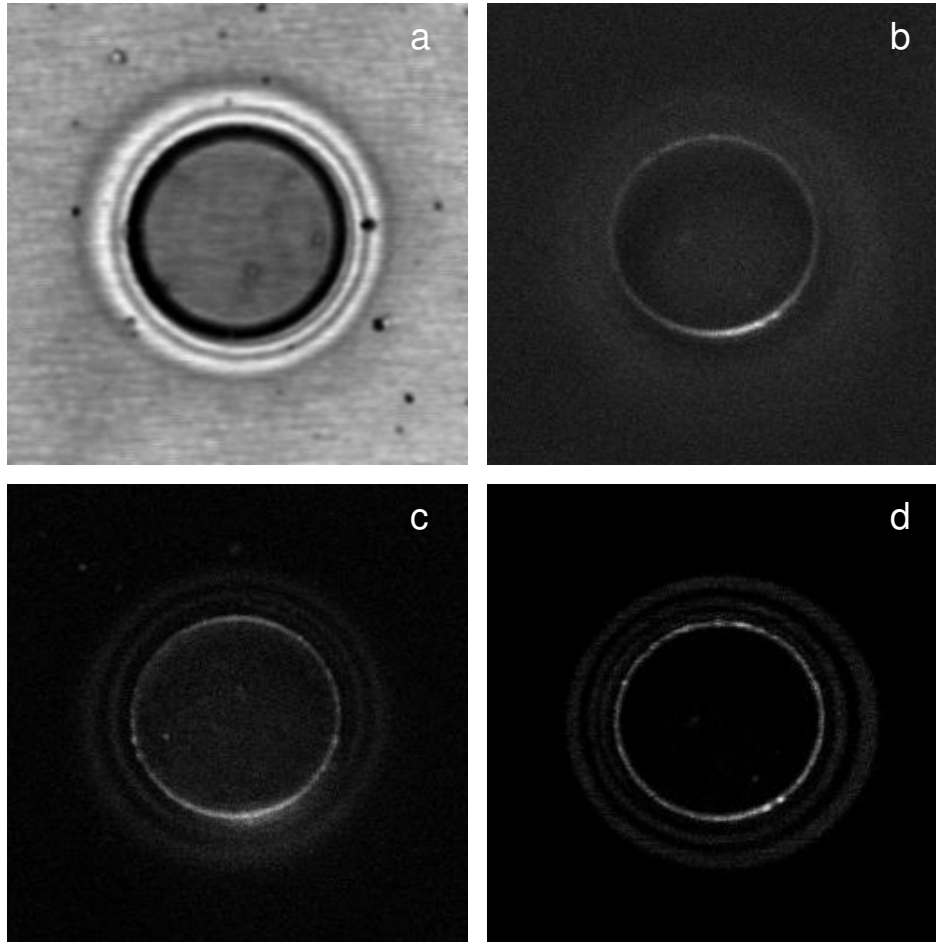
d

e

Imagini ale densitatilor de fotocurenti in functie de puterea laser

a) 5 mW, b) 10 mW; c) 20mW, d) 50 mW, e) 60 mW

Investigatii pe lasere cu inel fonic cuantic prin tehnici neliniare

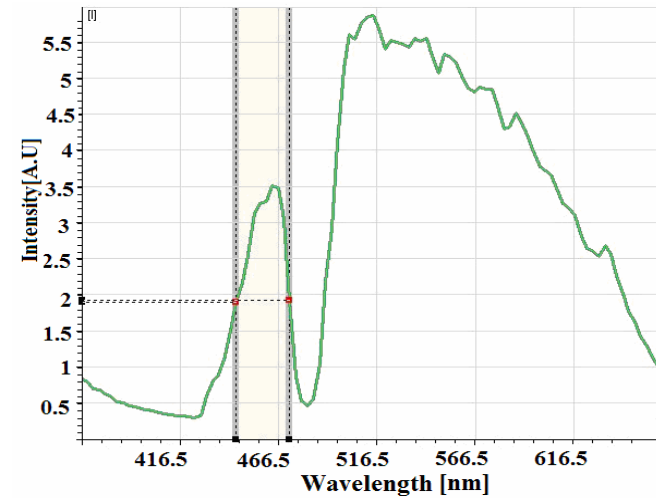
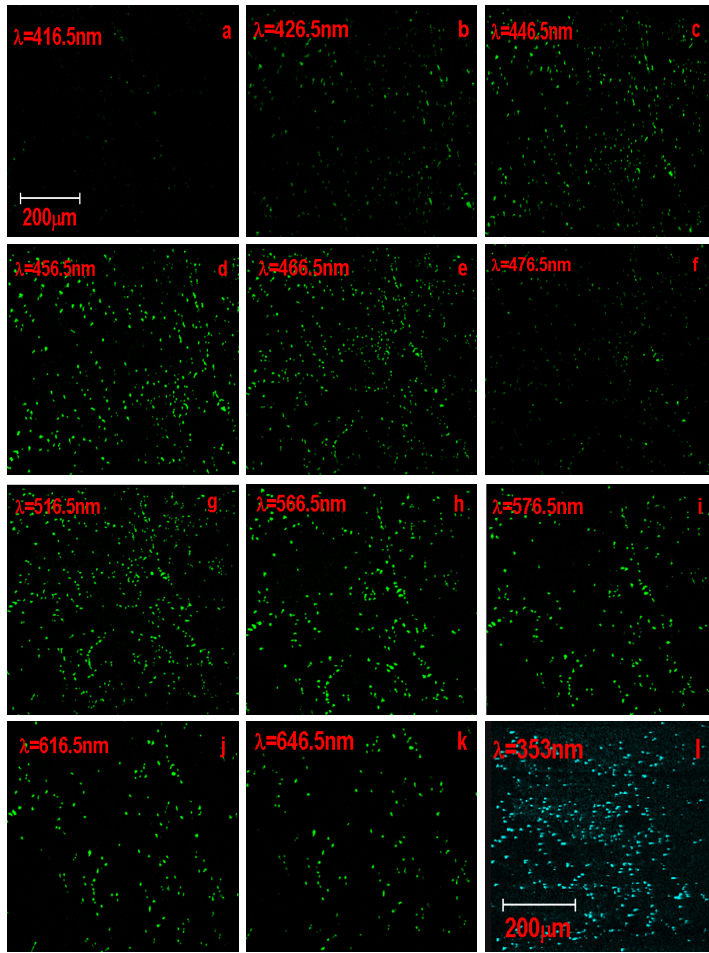


- (a) Imagine in reflexie cu un laser PQR cu diametrul de $27\ \mu\text{m}$
- (b) Imagine de luminescenta la o lungime de unda de excitatie $\lambda = 750\ \text{nm}$
- (c) Imagine de luminescenta la o lungime de unda de excitatie $\lambda = 790\ \text{nm}$
- (d) Imagine de luminescenta la o lungime de unda de excitatie $\lambda = 810\ \text{nm}$

Prin analiza imaginilor de fotoluminescenta excitata cu doi fotoni se pune in evidenta un comportament similar al structurii cu situatia de mod de lasare PQR.

Investigatii pe nanocristale semiconductoare: PbS

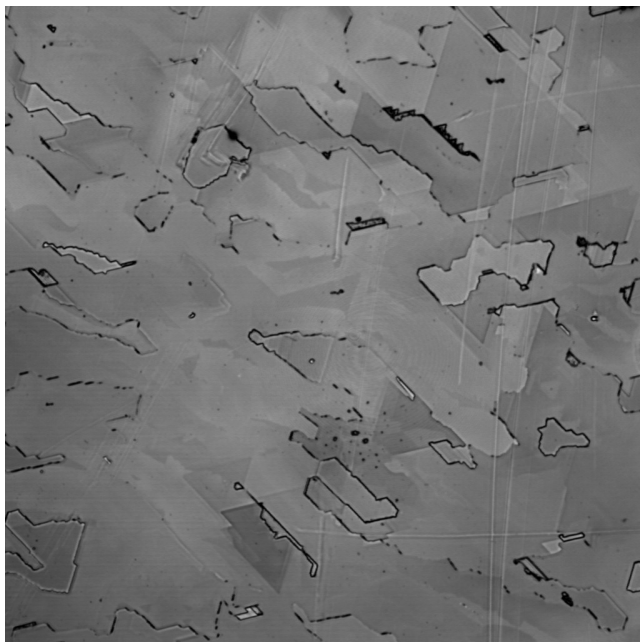
Localizarea si stabilirea maximului emisiei



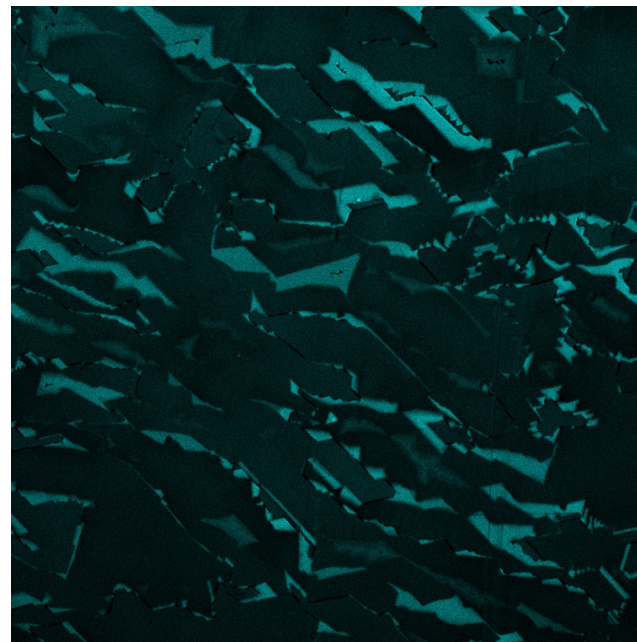
Spectru de luminescenta excitata cu doi fotoni pentru nanocristale de PbS

Investigatii pe SiC

a) Localizarea defectelor in volumul SiC

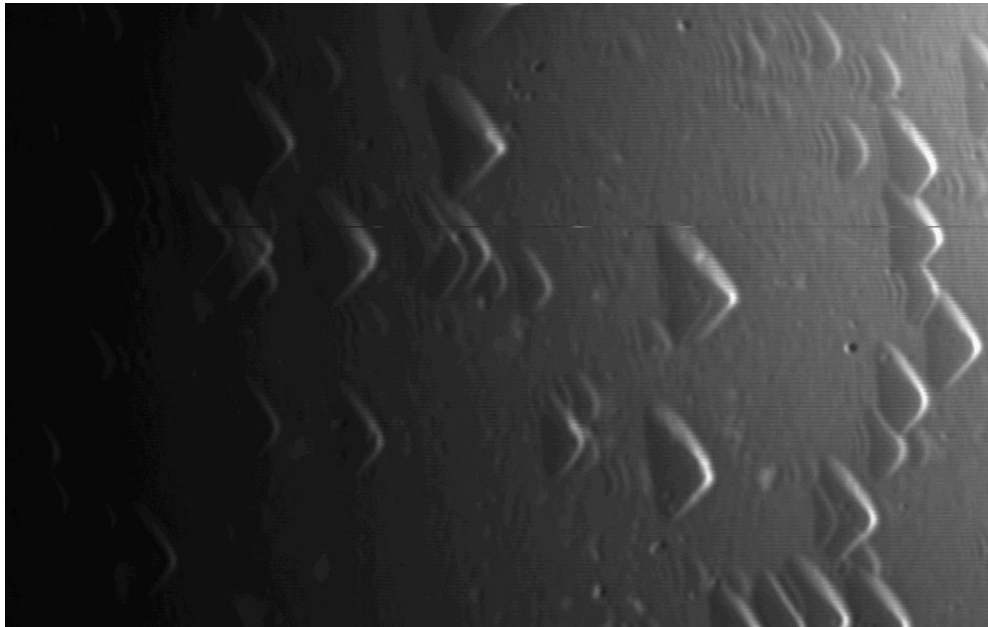


Imagine reflexie



imagine SHG

Defecte la interfata 3C- 6H (SHG)

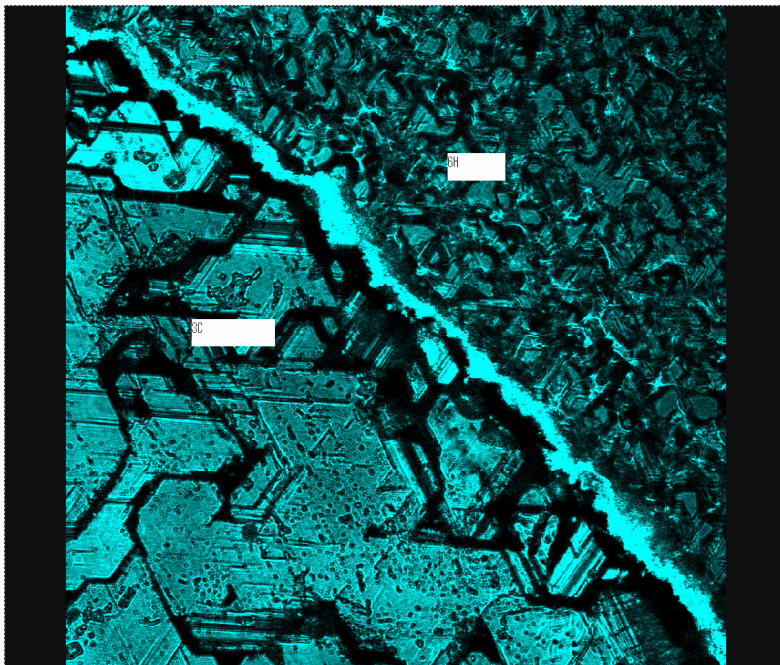


xy

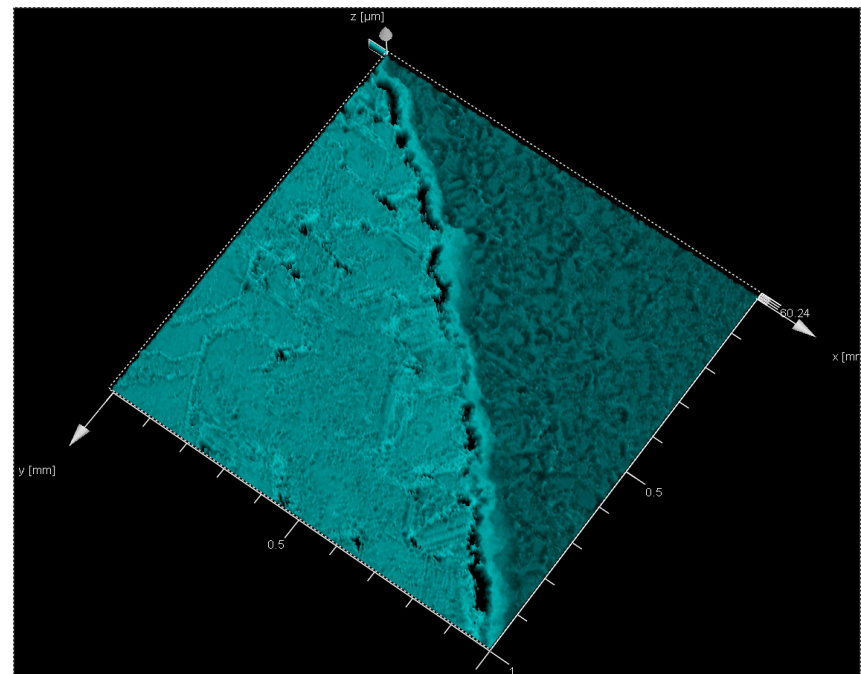


xz

SiC interfata 3C-6H

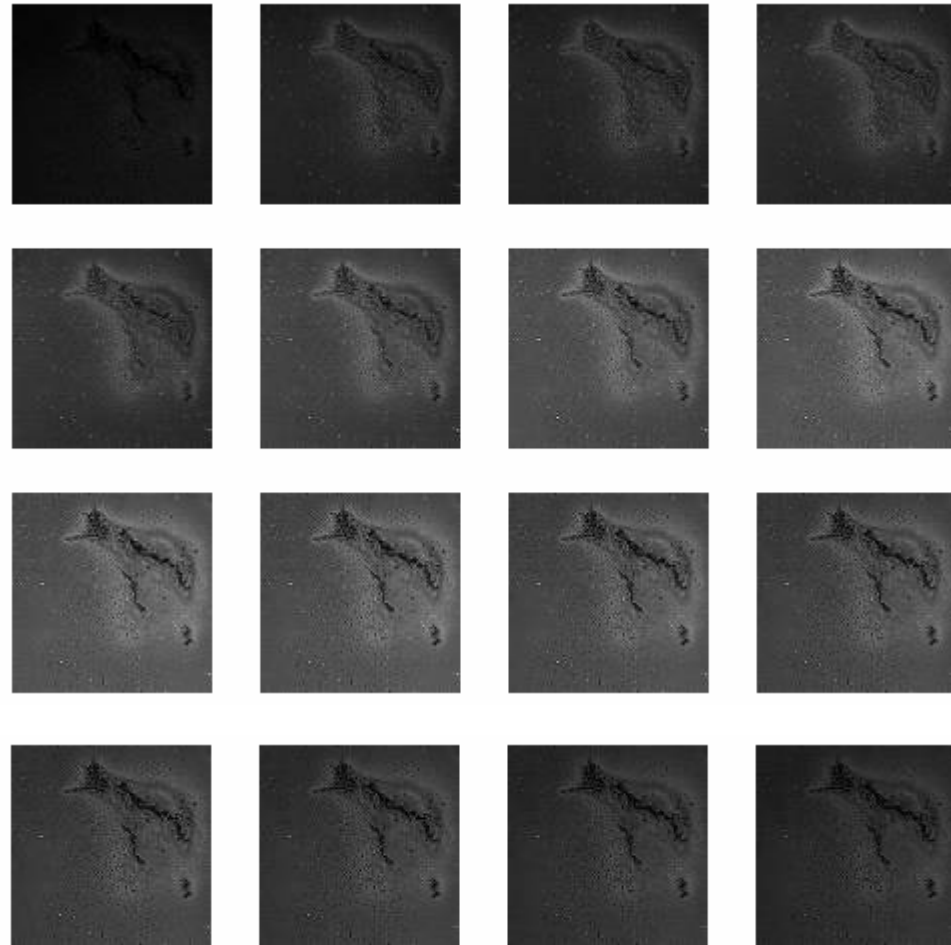
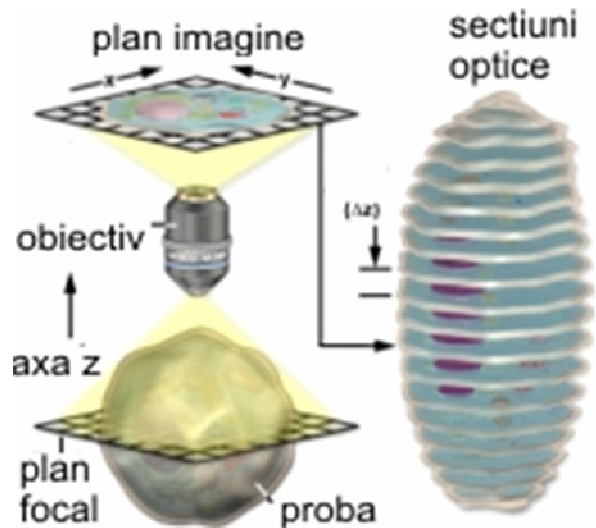


suprafata



Interfata

Metoda pentru compensarea atenuarii luminii in microscopia confocala cu baleiaj laser



[Automated Compensation of Light Attenuation in Confocal Microscopy by Exact Histogram Specification](#)

Stanciu, SG; Stanciu, GA and Coltuc, D

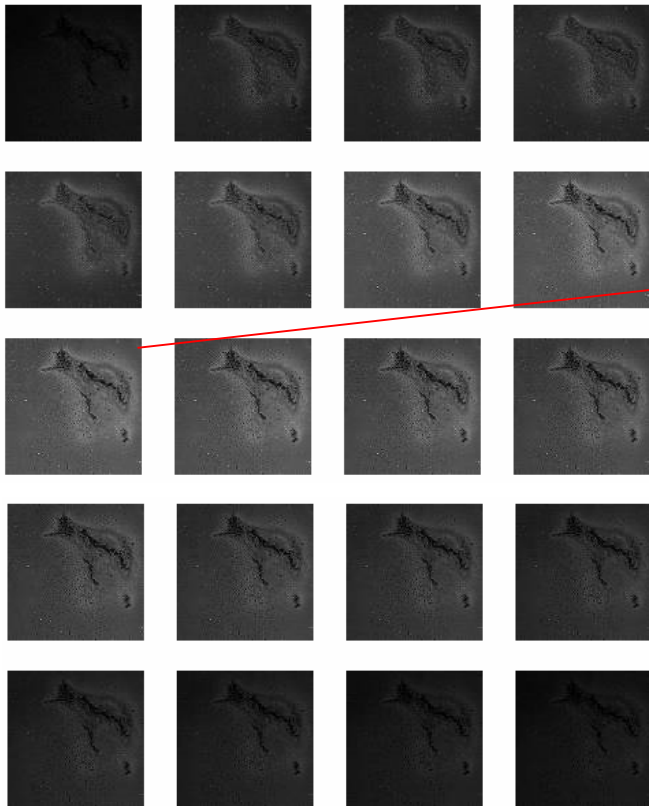
MICROSCOPY RESEARCH AND TECHNIQUE (factor de impact 1.850)

Volum: 73 Fascicula: 3 Paginile: 165-175 Anul publicarii: 2010

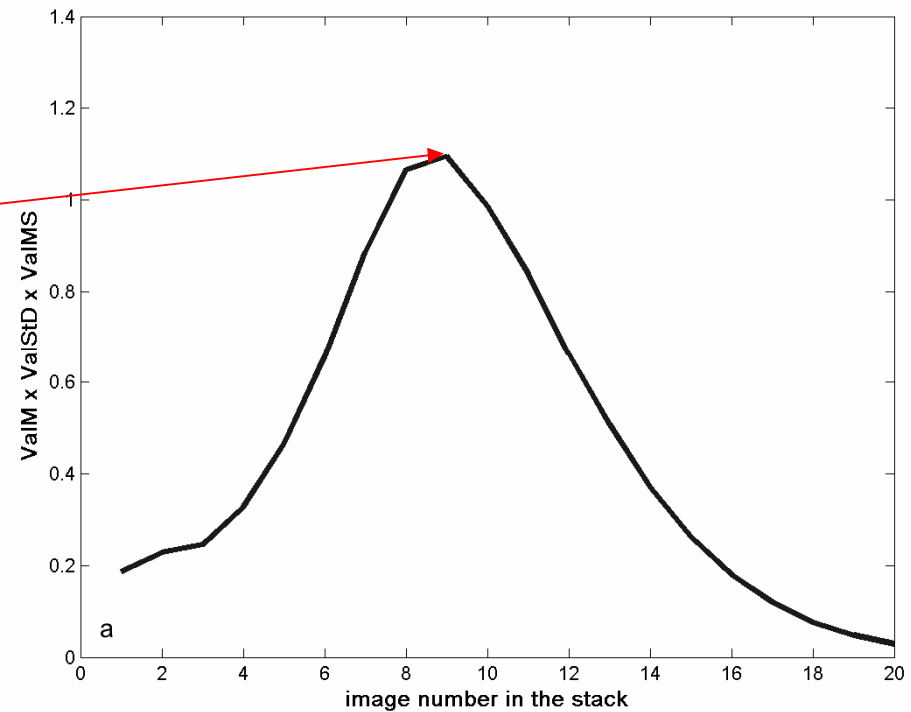
Pasul 1. Detectia automata a imaginii de referinta

Se realizeaza prin intermediul unui operator de evaluare calitativa a imaginilor CSLM obtinute la diferite adancimi de focalizare pe care l-am elaborat tinand cont de caracteristici precum luminozitate, contrast sau claritatea conturilor obiectelor din imagine.

Proba : Matrice Sol-gel



Raspunsul operatorului elaborat indica imaginea de referinta



Pasul 2. Specificarea exacta a histogramei imaginii de referinta

$$\phi_1 = [1] \quad \phi_2 = \frac{1}{5} \begin{bmatrix} 0 & 1 & 0 \\ 1 & 1 & 1 \\ 0 & 1 & 0 \end{bmatrix} \quad \phi_3 = \frac{1}{9} \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix}$$

$$\phi_4 = \frac{1}{13} \begin{bmatrix} 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \end{bmatrix}$$

$$\phi_5 = \frac{1}{21} \begin{bmatrix} 0 & 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 0 \end{bmatrix}$$

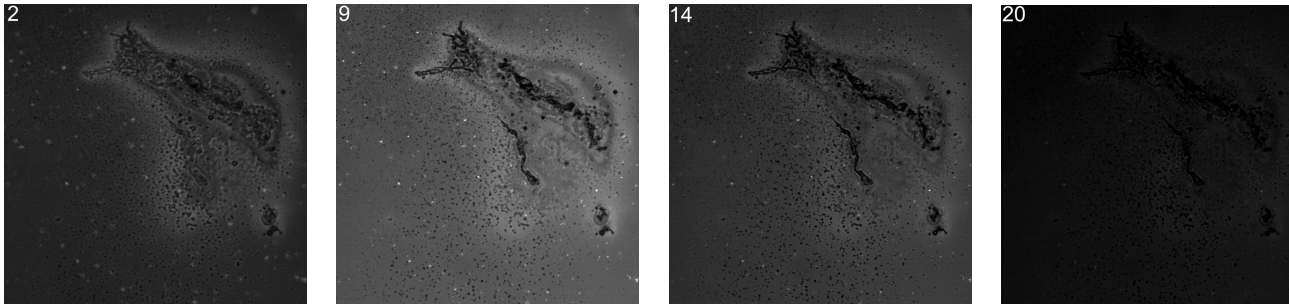
$$\phi_6 = \frac{1}{25} \begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

- Fiecare filtru extrage informatie locala despre nivelele de gri din jurul unui pixel curent $f(x,y)$.

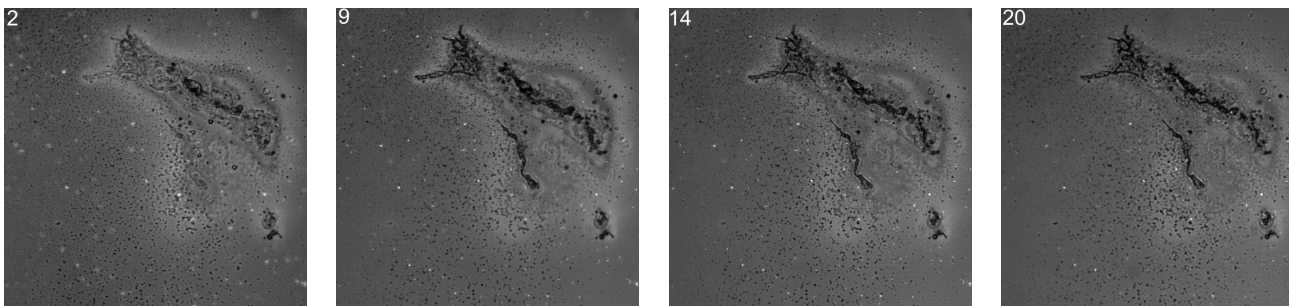
- Fiecarui pixel is se asociaza un vector $\Phi(f)(x,y) = \{\phi_1(f)(x,y), \phi_2(f)(x,y), \dots, \phi_K(f)(x,y)\}$

- Ordonarea strict crescatoare a pixelilor dintr-o imagine se realizeaza prin ordonarea lexicografica a seturilor de k elemente $\Phi(f)(x,y) = \{\phi_1(f)(x,y), \phi_2(f)(x,y), \dots, \phi_K(f)(x,y)\}$

Imagine de referinta identificata la pasul 1



Imagini initiale



Imagini obtinute prin specificare exacta a histogramei imaginii de referinta

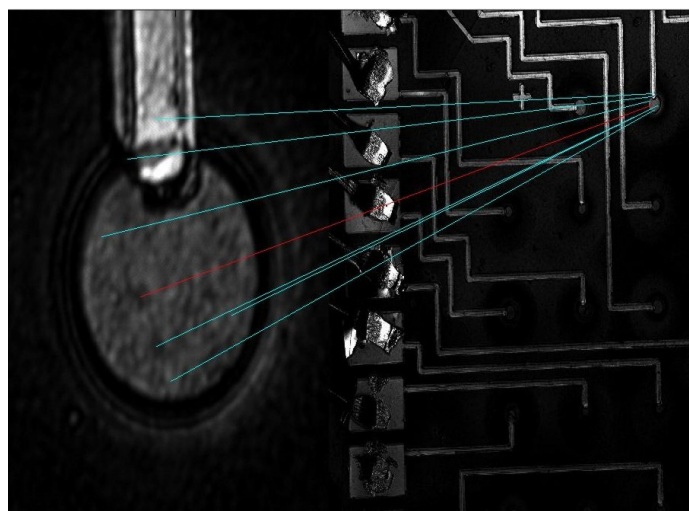
Trasaturi locale invariante

In ultimul deceniu au fost dezvoltate un numar considerabil de tehnici care permit detectia si descrierea trasaturilor invariante in imagini (SIFT, PCA-SIFT, SURF, GLOH, SUSAN, etc.)

Prin intermediul acestor metode este posibila identificarea regiunilor comune din imagini achizitionate in conditii diferite (iluminare, unghi de vizualizare, grad de marire).

Aplicatii

- Alinierea imaginilor (homografie, matrici fundamentale)
- Crearea de imagini panoramice
- Reconstructii 3D
- Urmarirea miscarii (motion tracking)
- Recunoasterea de obiecte
- Indexare si recuperare din baze de date

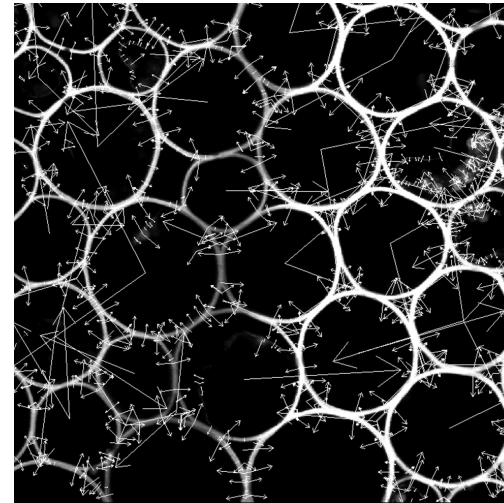


Invarianta trasaturilor locale in microscopia confocala

[On the Suitability of SIFT Technique to Deal with Image Modifications Specific to Confocal Scanning Laser Microscopy](#)

Stanciu, SG; Hristu, R; Boriga, R and Stanciu GA
MICROSCOPY AND MICROANALYSIS
Volum: 16 Fascicula: 5 Pagini: 515-530 Anul publicarii: 2010
Factor de impact ISI 3.035

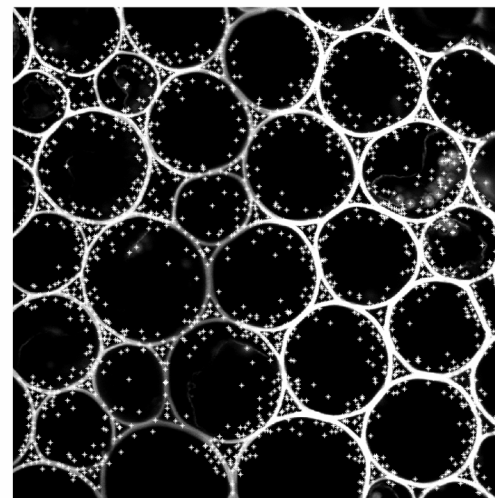
Analiza influentei parametrilor de achizitie specifici CSLM asupra detectiei trasaturilor locale prin tehnica SIFT (Lowe, 2004), tehnica reprezentativa pentru familia metodelor de detectie cu nucleu Laplacian.



[Influence of Confocal Scanning Laser Microscopy specific acquisition parameters to the detection and matching of Speeded-Up Robust Featu](#)

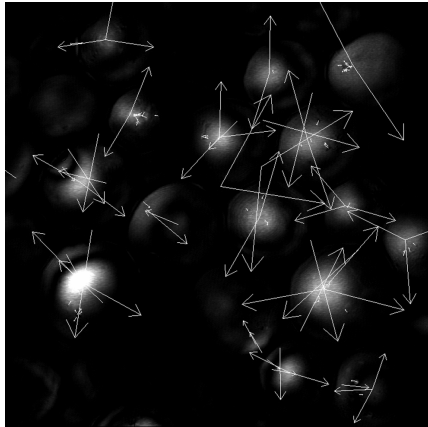
Stanciu, SG; Hristu, R and Stanciu GA
ULTRAMICROSCOPY
Factor de impact ISI 2.067
Lucrare acceptata pentru publicare

Analiza influentei parametrilor de achizitie specifici CSLM asupra detectiei trasaturilor locale prin tehnica SURF (Bay et al.,2008), tehnica reprezentativa pentru familia metodelor de detectie avand ca suport transformari in imagini integrale.

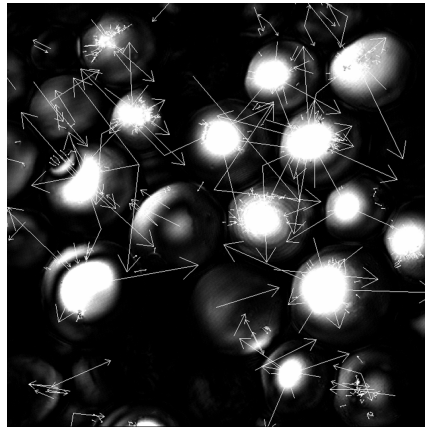


Invarianta la modificarea aperturii detectorului (pinhole)

Reflexie

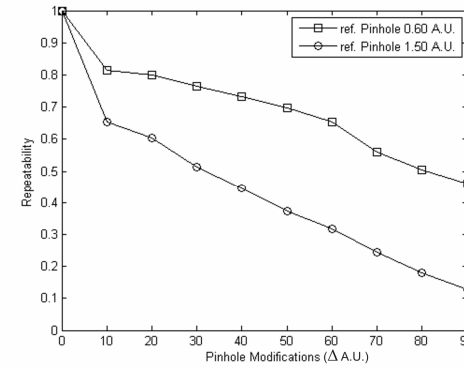


0.5 Airy

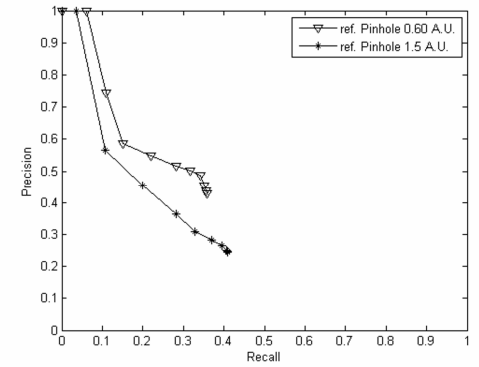


1.5 Airy

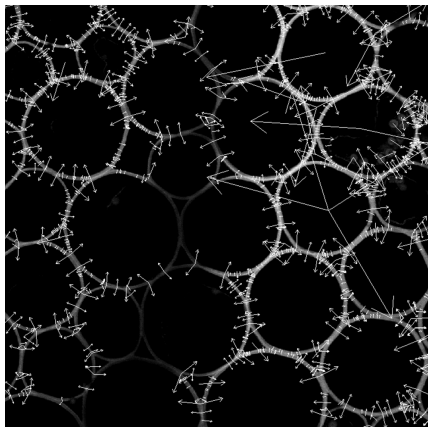
Repetabilitatea trasaturilor



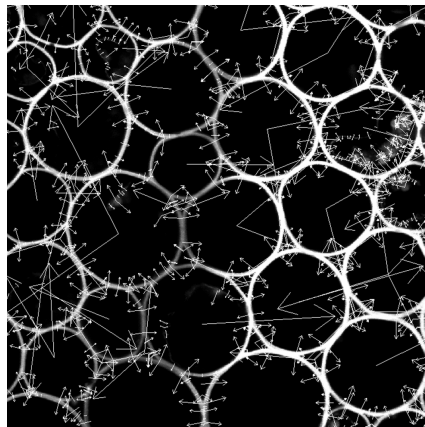
Precizia stabilirii corespondentelor între trasaturile extrase



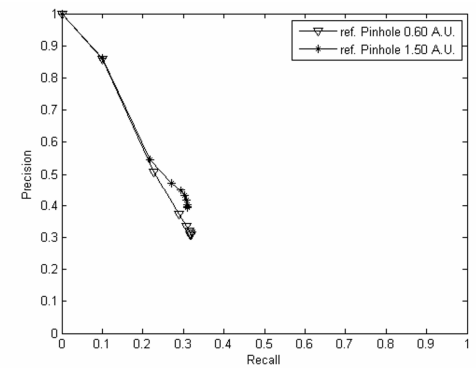
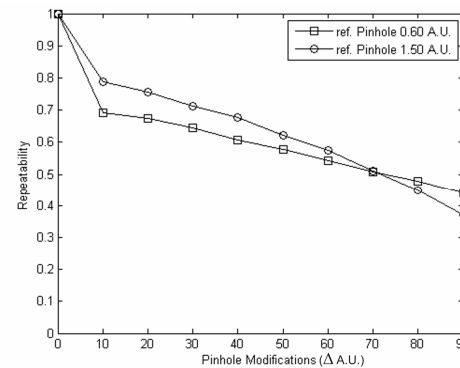
Fluorescenta



0.5 Airy

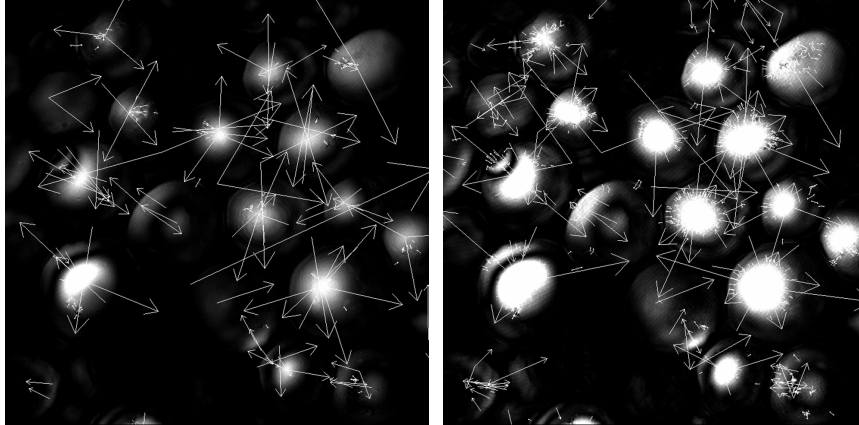


1.5 Airy



Invarianta la amplificarea fotomultiplicatorului (PMT)

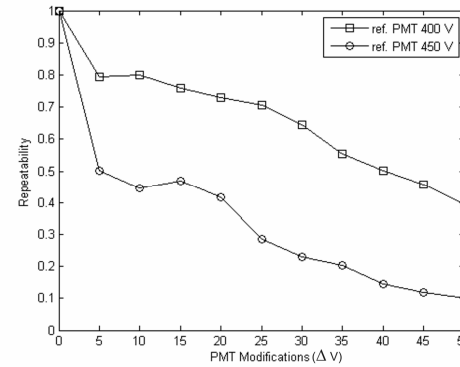
Reflexie



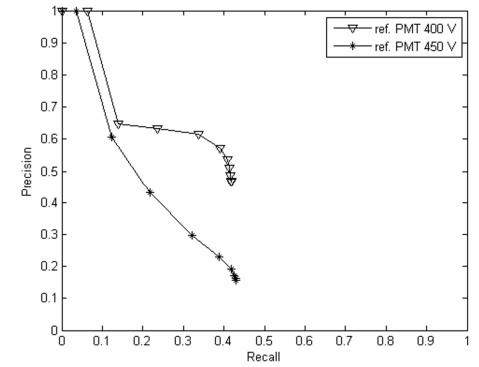
400 V

450 V

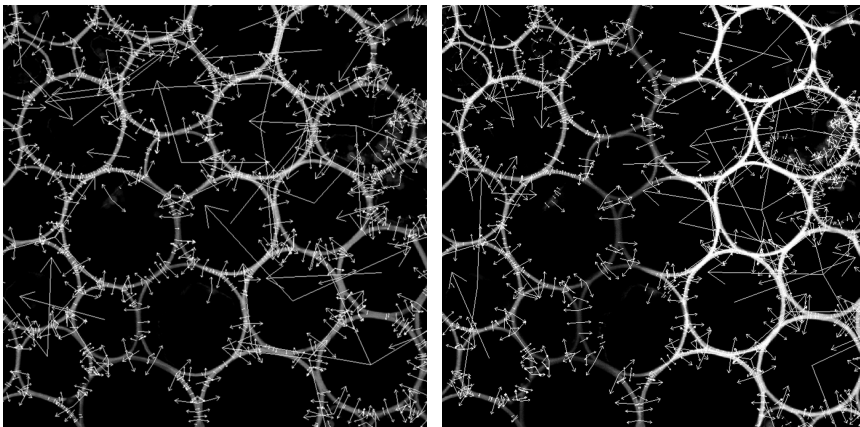
Repetabilitatea trasaturilor



Precizia stabilirii
corespondentelor intre
trasaturile extrase

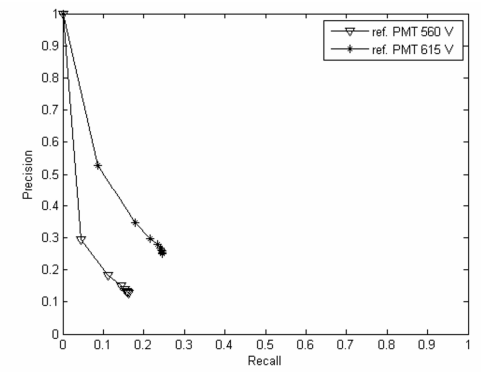
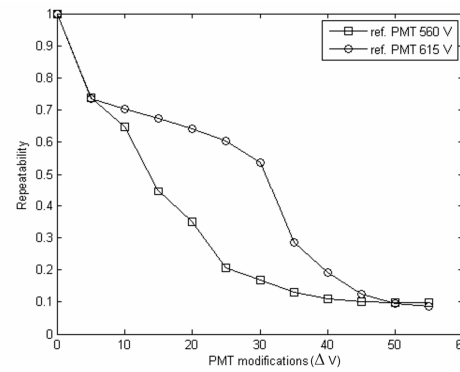


Fluorescenta



560 V

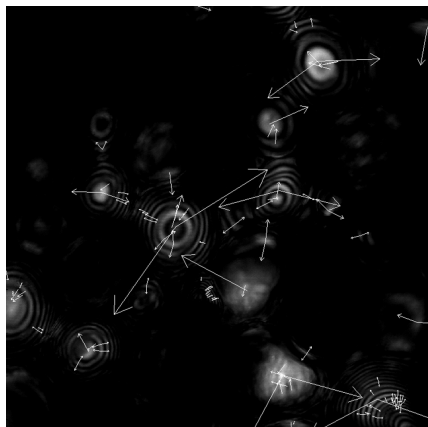
615 V



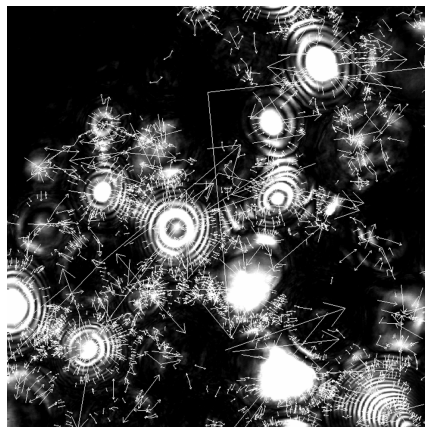
Fascicul laser folosit pentru baleiaj: Ar 488nm, putere la AOTF 100% - 1μW

Invarianta la puterea fascicului laser (reglata prin intermediul AOTF)

Reflexie

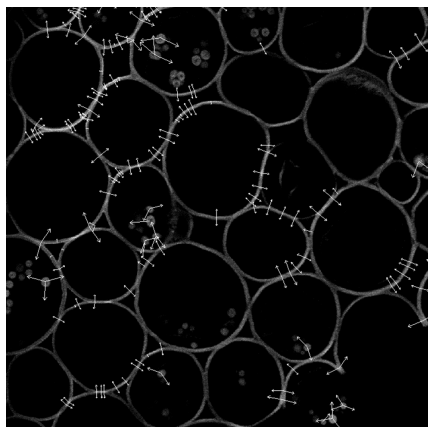


20 % AOTF

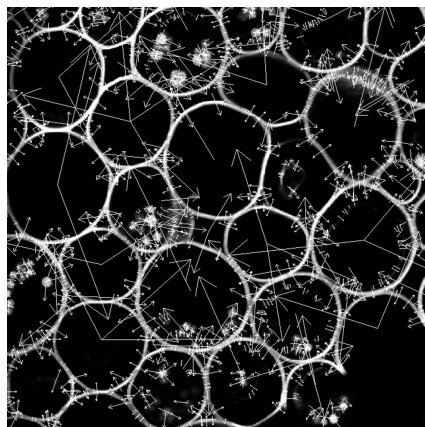


80 % AOTF

Fluorescenta

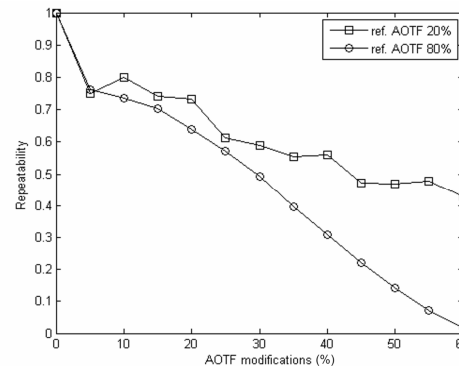


20 % AOTF

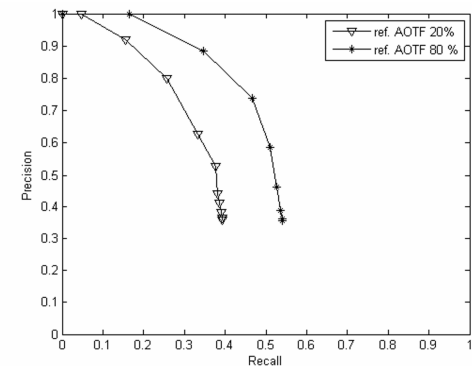
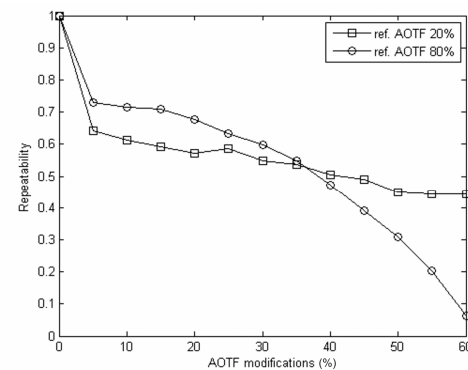
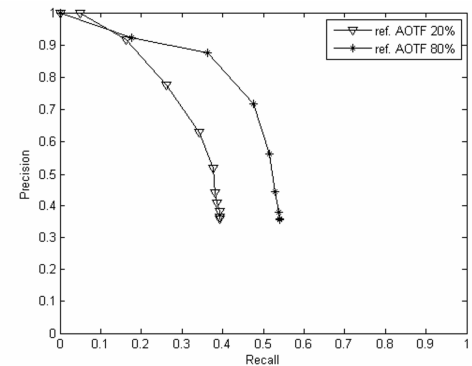


80 % AOTF

Repetabilitatea trasaturilor

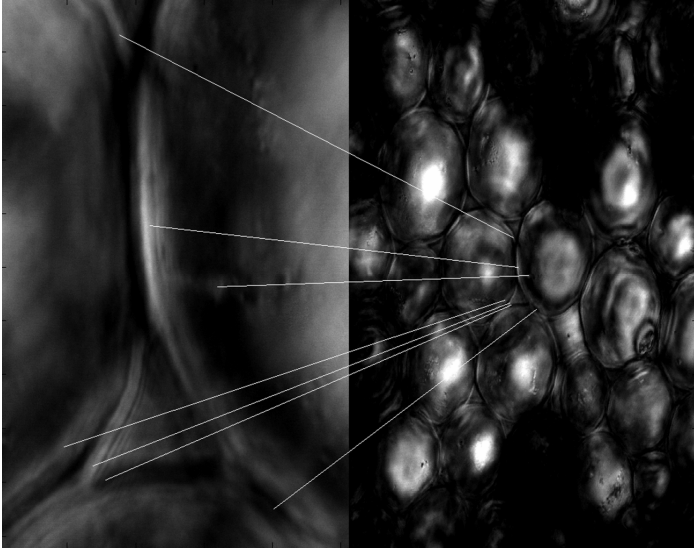


Precizia stabilirii
corespondentelor între
trasaturile extrase

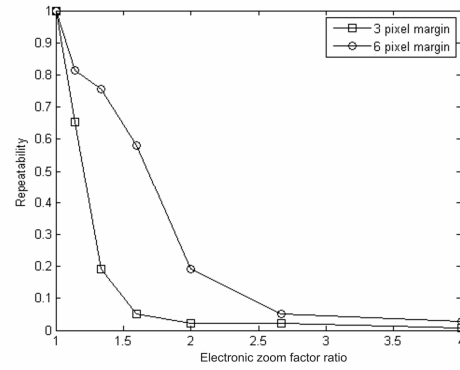


Invarianta la zoom electronic

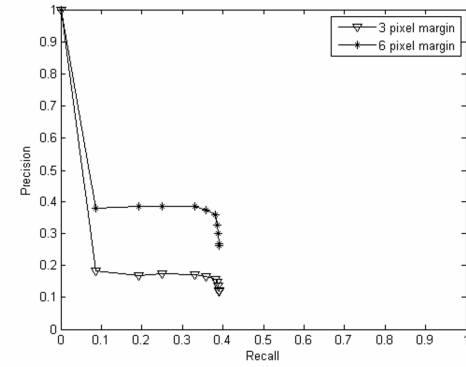
Reflexie



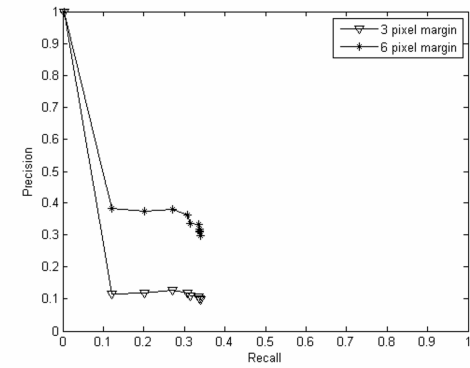
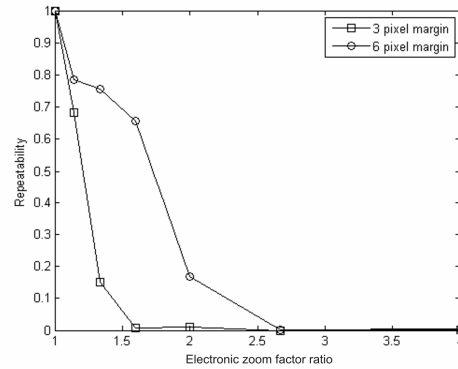
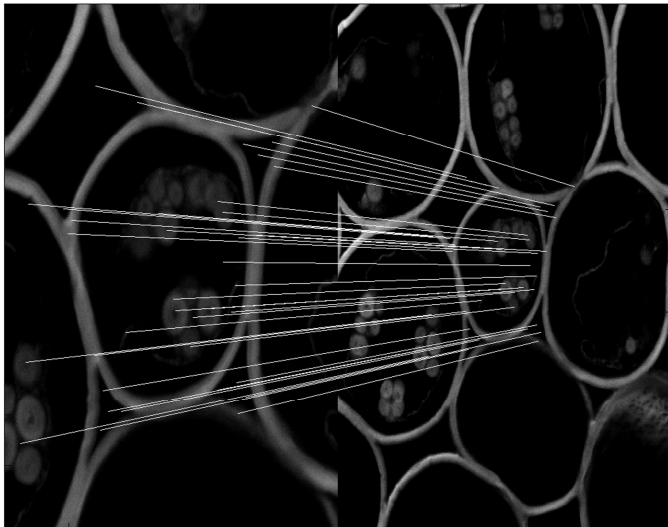
Repetabilitatea trasaturilor



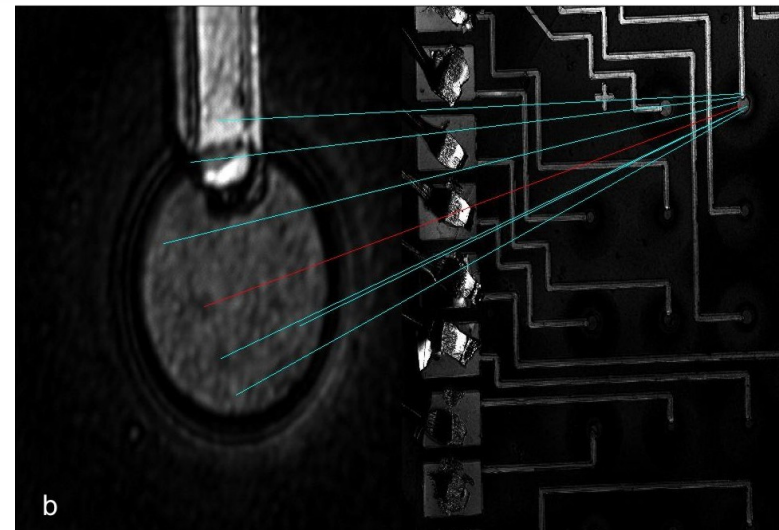
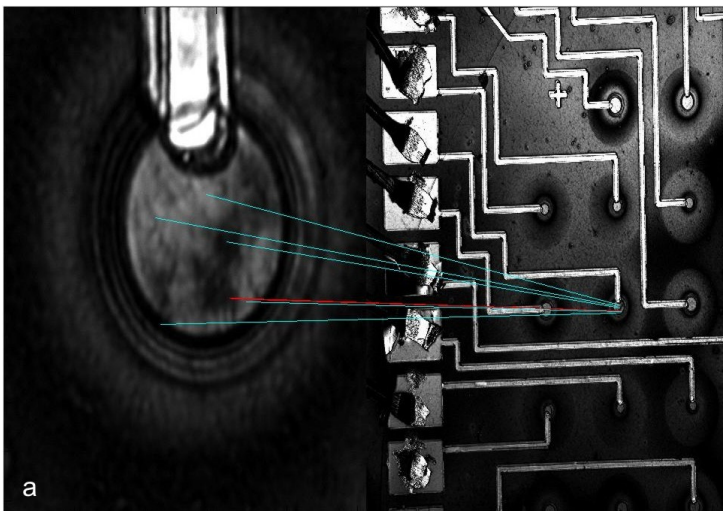
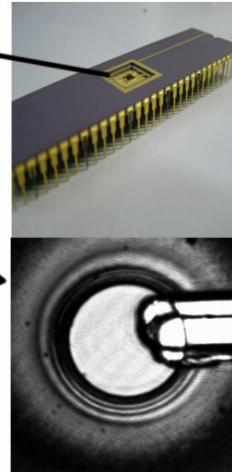
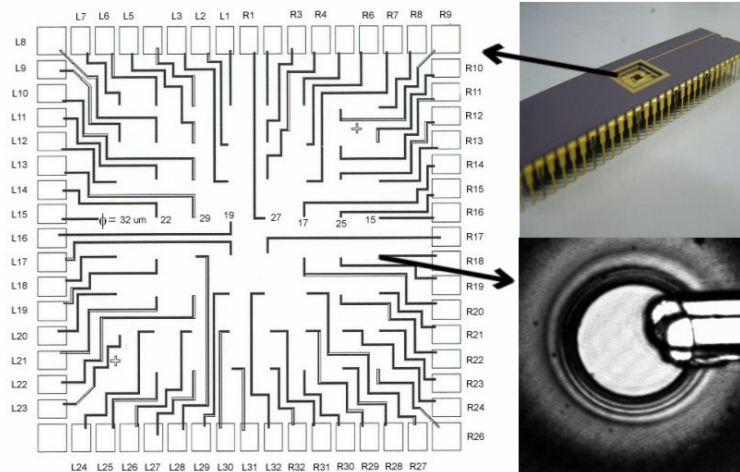
Precizia stabilirii
corespondentelor intre
trasaturile extrase



Fluorescenta

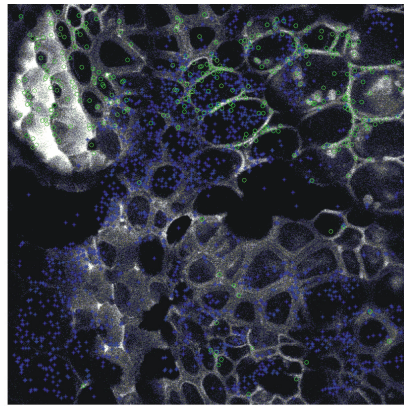
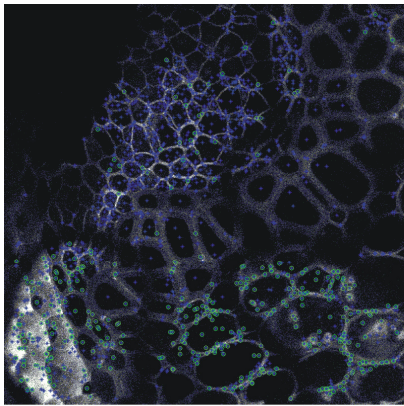


Recunoasterea obiectelor prin intermediul trasaturilor locale invariante

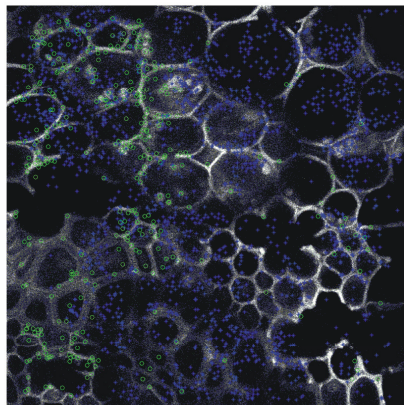
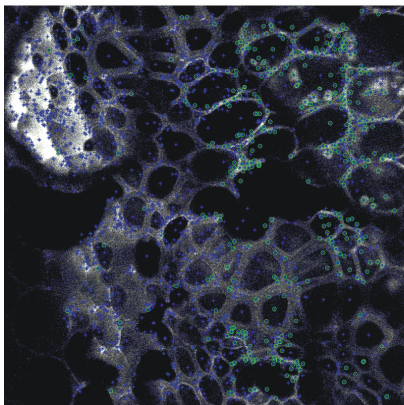


Corepondenta intre trasaturile locale detectate in imagini CSLM achizitionate la factori de marire diferiti pe a) dispozitiv PQR de 29 μm b) dispozitiv PQR de 32 μm

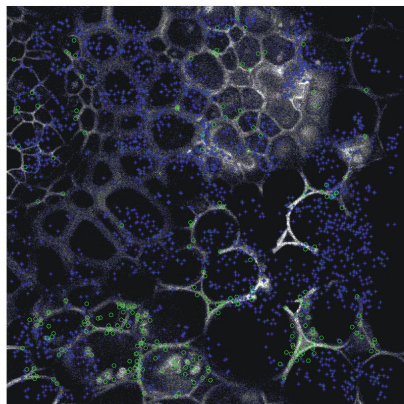
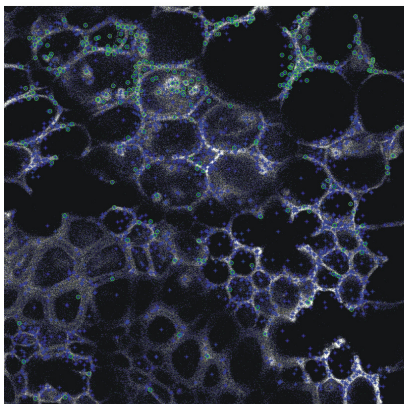
Alinierea automata in vederea obtinerii unor imagini de tip panoramic



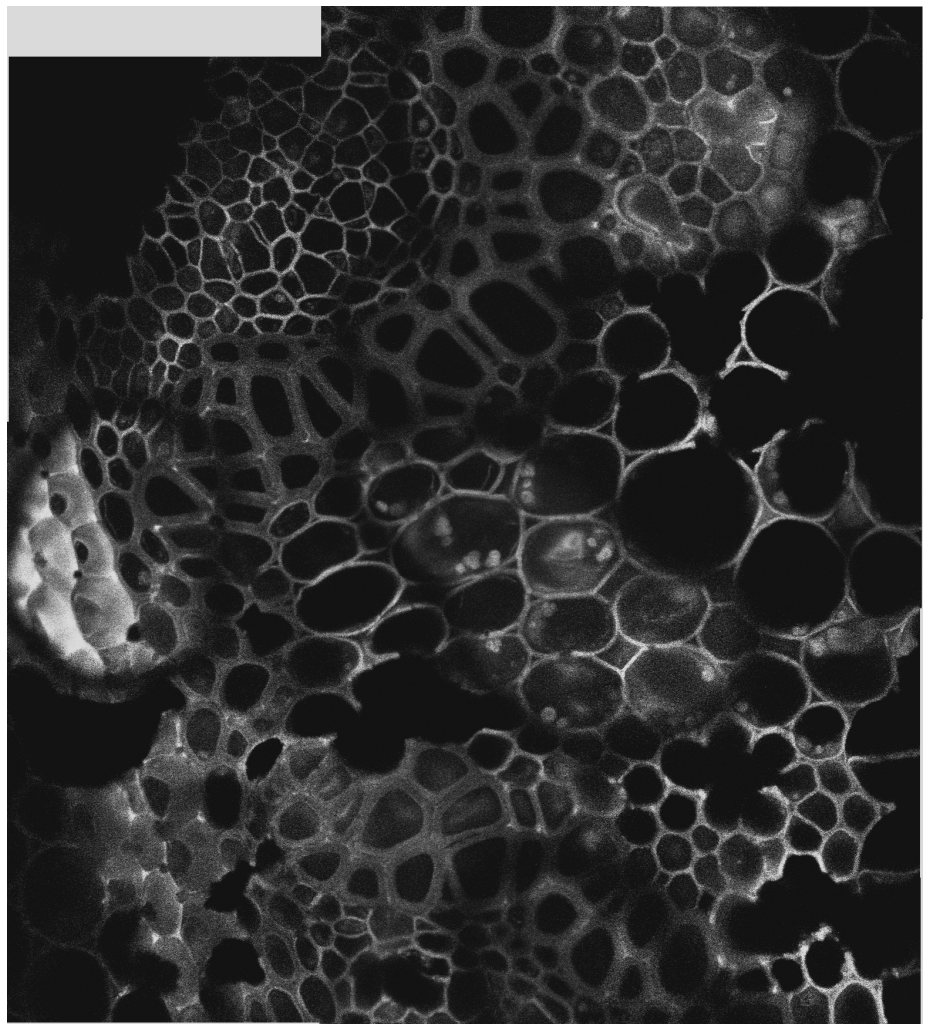
386 Matched keypoints between top left image and bottom left image



428 Matched keypoints between bottom left image and bottom right image



302 Matched keypoints between bottom right image and top right image



III. Implicarea tinerilor cercetatori

- **Savu Bogdan, doctorand (2005)**

Domeniu abordat:

- studii asupra efectelor neliniare (excitatie cu doi fotoni, imagistica bazata pe armonica a doua) in nanocristale semiconductoare.

Teza a fost sustinuta in catedra si va fi sustinuta public in ianuarie 2011

- **Hristu Radu, doctorand (2007)**

Domenii abordate:

- studii asupra efectelor neliniare (excitatie cu doi fotoni, imagistica bazata pe armonica a doua) in nanocristale semiconductoare;
- studii asupra laserelor PQR prin microscopie confocala si excitatie cu doi fotoni;
- studii asupra materialelor semiconductoare cu banda larga prin microscopie bazata pe imagistica armonicii a doua (SHG);
- investigatii pe probe biologice prin microscopie bazata pe imagistica armonicii a doua (SHG).

Teza va fi finalizata in 2011

- **Stanciu Stefan, doctorand (2007)**

Domenii abordate:

- procesari de imagini in microscopia cu baleiaj laser ;
- studii asupra laserelor PQR prin microscopie confocala si excitatie cu doi fotoni;
- studii asupra materialelor semiconductoare cu banda larga prin microscopie bazata pe imagistica armonicii a doua (SHG)

Teza va fi sustinuta in catedra in decembrie 2010 si va fi sustinuta public in ianuarie 2011

- In cadrul CMMPI au fost atrasi 3 studenti de la EITTI, care au obtinut premii la Sesiunea stiintifica cu teme legate de imagistica bazata pe efecte neliniare in microscopia cu baleiaj laser

IV. Dificultati intampinate in derularea proiectului

- imposibilitatea lucrului in laboratoarele partenerului italian, datorita lipsei fondurilor;
- reducerea ariei de investigatie, datorita imposibilitatii achizitionarii de oglinzi laser pe domeniul 750 nm - 1080 nm si a detectorilor sub 350 nm;
- imposibilitatea lucrului cu armonica a treia datorita unei limitari inferioare a laserului cu Ti:Safir ($\lambda_{\max} = 850$ nm);

Motive: Fonduri reduse

V. Obiectivele si rezultatele asteptate
in anul 2011 cu estimarea realista a
bugetului solicitat

Dispozitive semiconductoare

Lasere PQR:

Studii asupra regiunii active (inelului cuantic) prin utilizarea unei radiatii de excitatie (pompaj) in vizibil si IR in vederea stabilirii legaturii dintre fotoluminescenta si emisie laser si a caracterizarii inelului cuantic fonic

Cercetarile vor fi efectuate in colaborare cu Pohang University of Science & Technology si École Polytechnique Fédérale de Lausanne

Materialle semiconductoare

SiC- material semiconductor cu banda larga :

- identificarea defectelor structurale in volumul SiC, utilizand imagistica armonicii a doua;
- stabilirea unei legaturi intre armonica a doua si banda interzisa a diferitelor structuri cristaline dintr-o proba SiC
- metoda bazata pe generarea armonicii a doua pentru identificarea diferitelor structuri cristaline dintr-o proba de SiC

Colaborare cu Aristotle University of Thessaloniki

Nanocristale semiconductoare

Studiul efectelor neliniare in:

- TiO_2 , ZnS si CdS

colaborare cu Tezpur University

Probe biologice

- complementaritate metodelor neliniare TPEF, SHG. SHG metoda care nu necesita marcarea si poate evidenta peretele celular + TPEF care prin folosirea diferitilor markeri pune in evidenta diferite organele celulare;
- utilizarea metodelor de imagistica bazate pe excitatia cu doi fotoni si armonica a doua in investigatii la interfetele bio-nonbio (tesut – biomaterial) in cadrul proiectului FP 7 (Electrically Modified Biomaterials Surface: From Atoms to Applications) la care CMMPI este partener.

Colaborare cu parteneri din proiectul FP 7

Procesarea imaginilor CSLM:

- Studiarea comportamentului tehnicilor de detectie si descriere a trasaturilor locale invariante fata de parametrii specifici tehnicii de excitatie cu fotoni multipli in microscopia confocala (TPE)
- Optimizarea performantelor tehnicilor de detectie si descriere a trasaturilor locale pentru aplicatiile specifice tehnicilor de microscopie studiate
- Dezvoltarea unor aplicatii de specific “computer vision” bazate pe trasaturi locale invariante : identificare si recunoastere de obiecte, forme si texturi, indexarea imaginilor, etc.

Colaborare cu University of Genoa si University of Valencia

Buget solicitat 2011

- Manopera 3 tineri: 140 000 lei
- Manopera un cercetator experimentat: 25 000
- Cheltuieli deplasare: 23 000 lei
- 1. Conferinta "Focus on Microscopy-2011" : 6000 lei
- 2. Conferinta International Conference for Optical Transparent Network ICTON- 2011- 6000lei
- 3. Italia: Universitatea din Genoa (2 tineri - doua saptamani): 9000 lei
- 4. O deplasare a partenerului italian in Romania (o saptamana): 2000 lei
- Cheltuieli materiale:
 - Componente optice si consumabile : 45000 lei
- Echipamente:
 - achizitionarea unui laser cu Cr:fosterite: 75000lei;

REGIE (25 % din cheltuieli directe): 58250 lei

Total: 366 250 lei