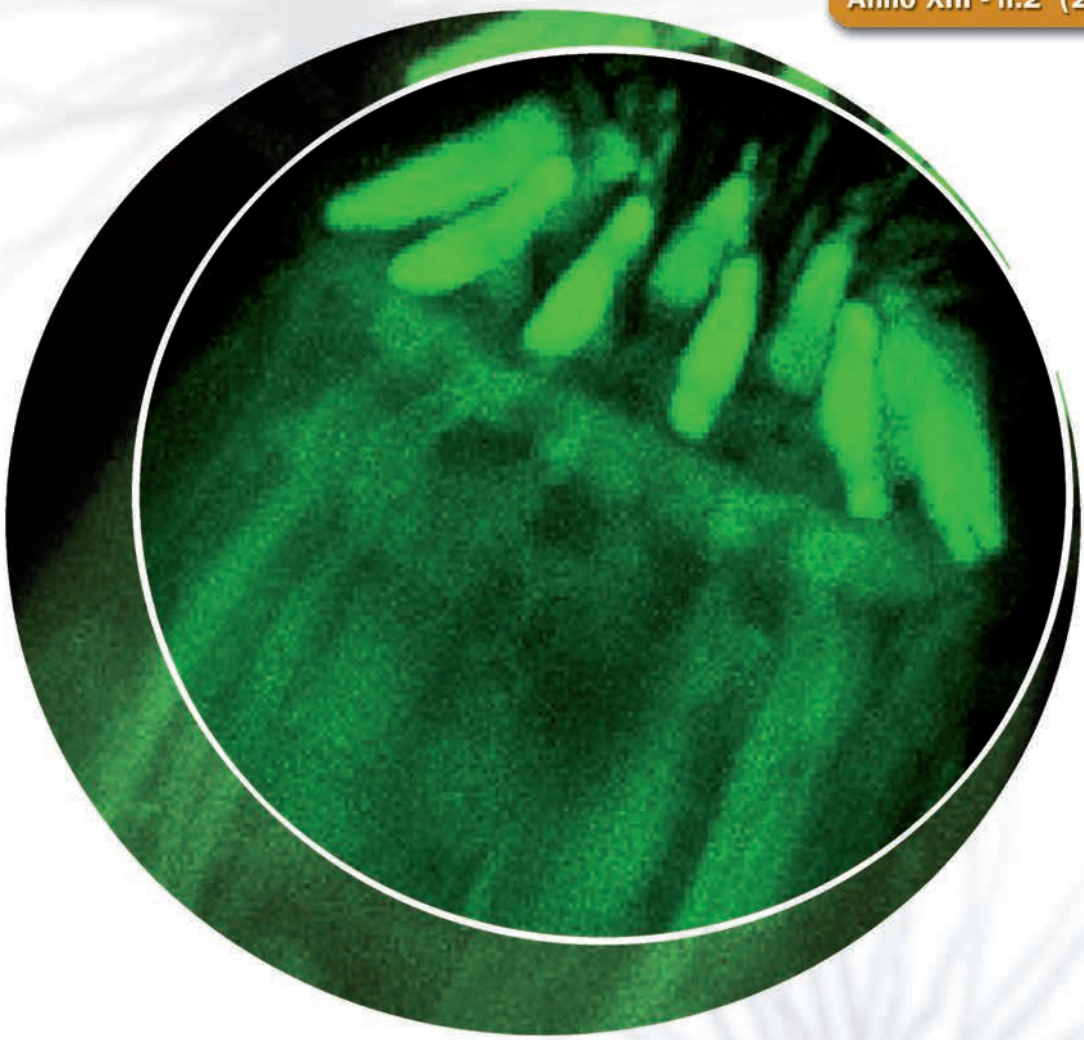
microscopie

Anno XIII - n.2 (26) Settembre 2016



Attività SISM 2016

Vincitori dei Premi SISM per tesi di dottorato

Contributi scientifici

Società Italiana
Scienze Microscopiche

www.sism.it

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Organo Ufficiale della Società Italiana Scienze Microscopiche
http://www.sism.it

Direttore Responsabile
Manuela Malatesta

Comitato di Redazione
Consiglio Direttivo della Società Italiana Scienze Microscopiche

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In copertina: *Dettaglio a microscopia confocale del nematode Craspodema reflectans* (Semprucci e collaboratori).

Indice

ISCRIZIONE

Possono iscriversi alla Società i ricercatori e gli operatori professionali comunque attivi nel campo delle diverse microscopie. Per l'iscrizione alla Società è necessario compilare la richiesta di associazione ed inviarla al Presidente. La scheda di associazione può essere compilata direttamente sul sito web della società all'indirizzo www.sism.it oppure può essere reperita in questo periodico ed inviata via fax. Le richieste verranno valutate dal Consiglio DIrettivo nella prima riunione utile e l'approvazione dei nuovi Soci sarà comunicata personalmente agli interessati. Dopo tale comunicazione il nuovo Socio può procedere al pagamento della quota sociale secondo le modalità riportate sotto.

QUOTA SOCIALE

La quota sociale è di euro 35 per i Soci ordinari e di euro 25 per i non strutturati. I Soci non strutturati, unitamente alla quota sociale, dovranno far pervenire al Presidente della Società una dichiarazione attestante il proprio status.
Modalità di pagamento:

a) mediante carta di credito dal sito www.sism.it
b) mediante invio di un assegno bancario non trasferibile intestato a S.I.S.M.
   l'assegno deve essere spedito alla Prof.ssa Elisabetta Falcieri, Dipartimento di Scienze della Terra, della Vita e dell'Ambiente (DiSTeVA), Università degli Studi di Urbino "Carlo Bo", Campus Scientifico "E. Mattei", via Ca' Le Suore 2, 61029 Urbino (PU)
c) mediante bonifico bancario intestato a S.I.S.M.
   codice IBAN IT43Q0200802455000103039142
   Presso Unicredit, Agenzia 3305 "Bologna Dante"
   Causale: "NOME del SOCIO"

SEDE SOCIALE

Prof.ssa Elisabetta Falcieri
Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino "Carlo Bo", Campus Scientifico "E. Mattei", via Ca' le Suore 2, località Crocicchia, 61029 Urbino
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SOCIETÀ ITALIANA SCIENZE MICROSCOPICHE

Richiesta di associazione

Si ricorda che le richieste di associazione verranno valutate dal Consiglio Direttivo e l'approvazione dei nuovi Soci verrà comunicata personalmente agli interessati.
Il pagamento della quota di associazione deve essere effettuato solo dopo il ricevimento della comunicazione dell'approvazione, da parte del Direttivo, della richiesta di associazione.

Il sottoscritto rischiede l'ammissione alla SISM in qualità di:

❑ Socio ordinario (35 euro)

❑ Socio non strutturato (25 euro)

Titolo, Nome e Cognome

Data di nascita

Titolo di studio e qualifica

Tipo di istituzione

❑ Università ❑ CNR ❑ Industria ❑ Commerciale ❑ Altro ente pubblico di ricerca

Istituto/Ente/Ditta

Dipartimento

Indirizzo

Città CAP

Telefono Fax E-mail

Indirizzo cui inviare la corrispondenza, se diverso dal precedente

Settore di attività

❑ Biomedico ❑ Scienza dei materiali ❑ Commerciale ❑ Altro (specificare) _____

Come deliberato nell'Assemblea Generale del 24/09/2001 ogni Socio SISM è anche Socio EMS.
Questi stessi dati saranno pertanto automaticamente inviati anche all'EMS, di cui la SISM fa parte. I dati dei Soci sono utilizzati dalla Segreteria EMS per distribuire il Notiziario in forma elettronica, per annunciare informazioni importanti come Congressi, Corsi, Scuole e per pubblicare l'Annuario dei Soci EMS.
Se si desidera che i propri dati personali non compaiano nell'annuario EMS, selezionare l'apposita opzione.

❑ Chiedo che il mio indirizzo privato non compaia nell'annuario EMS
❑ Chiedo che il mio numero di telefono/fax non compaia nell'annuario EMS

Data _____ Firma _____

Inviare via fax a:

Prof.ssa Elisabetta Falcieri
Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino "Carlo Bo"
Campus Scientifico "E. Mattei", via Ca' Le Suore 2, 61029 Urbino (PU)
Tel. +39.0722.304284 Fax: +39.0722.304244

Editoriale

Cari Amici e Colleghi,

Nel corso del 2016 la SISM ha organizzato un serie di attività, che hanno visto un'ampia partecipazione di ricercatori.

Presso il Campus Scientifico di Urbino è stato organizzato, il 14, 15 e il 16 marzo, il Workshop su "La microscopia elettronica SEM/ESEM nello studio dell'ambiente". Nei primi due giorni, presso l'Aula Magna ci sono stati interventi di relatori - provenienti da varie università e centri di Roma, Torino, Bolzano, Ancona, Faenza, Pallanza, Parma, Milano, Cosenza ed Urbino - sulla microscopia applicata a fattori abiotici, in ambiente acquatico e in ambiente terrestre. Nel secondo giorno, oltre alle relazioni, hanno avuto luogo una sessione dedicata alle Aziende del settore e una di esercitazioni pratiche in laboratorio. La giornata del 16 marzo, sempre di carattere teorico-pratico si è svolta presso i laboratori ESEM dell'ARPAM di Pesaro, che ospitano un microscopio ESEM di proprietà dell'Ateneo di Urbino. Il workshop ha visto una trentina di iscritti e ha contribuito a rafforzare questo ambito trasversale che impegna, oltre a microscopisti con competenze biomediche, fisici, chimici ed altri studiosi. Gli abstract delle presentazioni sono pubblicati in questo fascicolo di Microscopie.

A Pavia, il 7 e l'8 luglio 2016, è stato organizzato il simposio "Nuclear structure and dynamics, through the microscopes", con il prezioso contributo di M. Malatesta, C. Pellicciari e M. Biggiogera e con il supporto della Società Italiana di Istochimica. L'Aula Magna del Collegio Alessandro Volta ha visto gli interventi di autorevoli ricercatori di Bologna, Roma, Camerino, Milano, Parma, Urbino, Verona e Pavia che hanno presentato lo stato dell'arte sui meccanismi alla base del funzionamento del nucleo cellulare, nelle sue varie fasi, con gli approcci più diversi, in modelli animali più semplici e in patologia. Anche gli Atti di questo simposio sono pubblicati in questo fascicolo.

Con il patrocinio SISM hanno avuto luogo la giornata "Sopravvivenza e morte cellulare: nuove acquisizioni e ricadute applicative", organizzata da S. Meschini presso l'Istituto Superiore di Sanità di Roma il 18 aprile scorso, e il congresso "Nanomedicine Viterbo 2016", presso l'Università della Tuscia a Viterbo, il 21 e il 23 settembre.

Anche quest'anno, dal 22 al 24 settembre, presso il Centro Interfacoltà Grandi Strumenti di Modena è stato nuovamente proposto il "Corso Base integrato di Microscopia Confocale e Microscopia Elettronica TEM/SEM"; M. Tonelli e A. Tombesi sono stati tra gli organizzatori.

Il 29 e il 30 settembre 2016 C. Albonetti ha organizzato a Bologna il workshop "STSPM16" e il 21-22 novembre sarà nuovamente organizzato, ad Urbino, il workshop "La microscopia elettronica applicata allo studio dei Beni Culturali".

Ultimo evento SISM 2016 sarà la Scuola TEM "Pier Giorgio Merli" organizzata, come nelle precedenti edizioni, da R. Balboni presso i laboratori CNR di Bologna CNR di Bologna in novembre e in febbraio.

Ha avuto grande successo il bando di quattro Premi SISM di 500 Euro per Tesi di Dottorato, finalizzati alla partecipazione ad un evento scientifico di ambito microscopico, che ha consentito ai dottori Gabriele Fisichella (Scienze dei Materiali), IMM-CNR, Catania, Maria Chiara Spadaro (Scienze dei Materiali), EPFL, Losanna, Valentina Palmieri (Scienze Biomediche), Università Cattolica di Roma, e Luigi Rosati (Scienze Biomediche), Università degli Studi di Napoli Federico II, di partecipare a recenti eventi congressuali nazionali ed internazionali.

La prossima estate, ci sarà a Rovigno il MCM 2017, per la cui organizzazione da tempo ci stiamo impegnando R. Balboni ed io, in qualità di membri dell'International Board, in collaborazione con tutto il Consiglio Direttivo. Nell'occasione la Società bandirà ancora una volta dei Premi di Partecipazione, per promuovere la ricerca italiana e stimolare la partecipazione dei giovani.

Grazie ancora, quindi, a tutti coloro che ci sostengono, con l'augurio che il nostro impegno porti sempre un contributo per il progresso della microscopia.

Elisabetta Falcieri

Consiglio direttivo della SISM

Verbale della riunione del 4 dicembre 2015

Il giorno 4 dicembre 2015 alle ore 10:30 presso il Dipartimento di Scienze Anatomiche Umane, aula E, in Via Irnerio, 48 a Bologna, si è svolta una riunione del Consiglio Direttivo SISM, per discutere il seguente OdG:

1. Approvazione del verbale della riunione precedente
2. Risultati elezioni rinnovo componenti CD per il biennio 2016-2017
3. Nomine dei Vicepresidenti e del Direttore Responsabile della Rivista
4. Responsabile sito web
5. Situazione economica della Società
6. Attività SISM 2015-2016
7. Rivista Microscopie
8. Gestione della Società
9. Approvazione ammissione nuovi Soci
10 Varie ed eventuali

Sono presenti: *Cristiano Albonetti*, *Roberto Balboni*, *Regina Ciancio*, *Elisabetta Falcieri*, *Stefania Meschini*.
Assenti giustificati: *Manuela Malatesta e Andrea Tombesi*.
Presiede *Elisabetta Falcieri*; svolge le funzioni di segretario verbalizzante *Sara Salucci*.

1. Il verbale della riunione del Consiglio Direttivo del 27 agosto 2015 viene approvato all'unanimità.

2. Il Presidente Elisabetta Falcieri riferisce sulle votazione del CD SISM per il biennio 2016-2017, trasmesse dalla commissione elettorale composta da Paolo Mengucci (Presidente), Gianni Barrucca e Michela Battistelli (scrutatori), che si è riunita il 19 novembre 2015 presso l'Università Politecnica delle Marche (Ancona).
   Elisabetta Falcieri viene riconfermata Presidente per il biennio 2016/2017 con voti 37
   I Consiglieri della Società Italiana Scienze Microscopiche eletti per il biennio 2016-2017 sono:
   Albonetti Cristiano voti 21
   Balboni Roberto voti 31
   Ciancio Regina voti 12
   Malatesta Manuela voti 29
   Meschini Stefania voti 21
   Tonelli Massimo voti 27

3. Viene nominato nuovamente vicepresidente Roberto Balboni. Stefania Meschini, in considerazione dell'alternanza tra i due ambiti, sarà l'altro vicepresidente. Manuela Malatesta sarà ancora, avendone dato la disponibilità, direttore responsabile della rivista per il biennio 2016-2017.

4. Il Consiglio Direttivo, lamentando una certa rigidità del sito web della società, mette in evidenza la necessità di avere un sito più dinamico. Si propone di affidare la cura del sito ad una Società esterna o ad un tecnico esperto in costruzione di siti web, mantenendo Roberto Balboni come figura referente. Il Presidente, Manuela Malatesta, Stefania Meschini e Regina Ciancio provvederanno a fornire a breve delle offerte, in modo da poterne discutere nella riunione del prossimo CD.

5. Il Presidente riferisce sulla situazione economica della società, illustrando dettagliatamente il bilancio 2015 che ad oggi è di circa 24000 euro di attivo. La buona riuscita degli eventi SISM, a fronte dell'investimento della Società in premi per giovani ricercatori pari a 7500 euro, ne garantisce una certa solidità.

6. Il Presidente si complimenta con la dr.ssa Regina Ciancio per il successo dell'iniziativa relativa alla "Scuola di microscopia elettronica a scansione su materiali nanostrutturati e applicazioni innovative" (CNR-IOM, Trieste, 12-14 ottobre).
   Il Presidente relazione sugli eventi che si terranno nel 2016 e per alcuni dei quali sono aperte le iscrizioni:
   "La microscopia elettronica SEM/ESEM nello studio dell'ambiente" (Università di Urbino, 14-16 marzo)
   "Sopravvivenza e morte cellulare: nuove acquisizioni e ricadute applicative" (Roma ISS, 18 aprile, con patrocinio SISM)
   "Nuclear structure and dynamics, through the microscopes" (Pavia, 7-8 luglio 2016, SISM+SII)
   Workshop: "STSPM" (Bologna, fine settembre, 2016)
   "La microscopia elettronica applicata allo studio dei Beni Culturali" (III edizione, Urbino, ottobre 2016)
   Scuola TEM ''Pier Giorgio Merli'' 2016 (Bologna, novembre/febbraio 2016)

7. Il Presidente riferisce che per poter accedere all'impact factor occorre che la rivista sia corredata di numerosi articoli scientifici. Fa nuovamente presente, infatti, che pochi Soci contribuiscono con i loro articoli al buon andamento della rivista. Il Consiglio Direttivo invita fortemente coloro che sono risultati vincitori del premio SISM 2015 a scrivere lavori scientifici da pubblicare sulla rivista nell'anno 2016. A tal proposito, si propone di ufficializzare, nel prossimo bando premi SISM, l'obbligatorietà della pubblicazione scientifica da parte dei vincitore.

8. Il Presidente affronta la questione dei rimborsi per i relatori invitati alle scuole. In particolare, viene affrontato il rimborso chilometrico per chi viaggia in automobile. A tal proposito Roberto Balboni prende l'impegno di studiare la situazione e di inviare delle tabelle ragionevoli che saranno condivise dal CD per il rimborso chilometrico.

9. Approvazione elenco nuovi associati:
   Berti Raffaele
   Conventi Alberto
   Coscia Francesca
   Feltracco Veronica
   Laucello Simone
   Leuzzi Maria Celeste
   Liguori Laura
   Manca Rosarosa
   Papa Valentina
   Pinzari Flavia
   Pontoglio Enrico
   Roncati Carlo
   Saler Marco
   Silvestre Mattia
   Tria Giancarlo

10. Il Consiglio Direttivo ritiene opportuno riconsiderare l'idea del questionario.

Alle ore 13:13, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta.

Elisabetta Falcieri
Roberto Balboni
Cristiano Albonetti
Regina Ciancio
Stefania Meschini

Consiglio direttivo della SISM

Verbale della riunione dell'8 febbraio 2016

Il giorno 8 febbraio 2016 alle ore 14:00 presso il Dipartimento di Scienze Anatomiche Umane, aula E, in Via Irnerio, 48 a Bologna, si è svolta una riunione del Consiglio Direttivo SISM, per discutere il seguente OdG:

1. Comunicazioni del Presidente
2. Approvazione del verbale della riunione precedente
3. Aggiornamento gestione amministrativa della Società
4. Attività SISM 2016
5. Approvazione ammissione nuovi Soci
6. Varie ed eventuali

Sono presenti: *Cristiano Albonetti*, *Roberto Balboni*, *Elisabetta Falcieri*, *Stefania Meschini e Massimo Tonelli*.
Assenti giustificati: *Regina Ciancio e Manuela Malatesta*.
Presiede *Elisabetta Falcieri*; svolge le funzioni di segretario verbalizzante *Sara Salucci*.

1. Il Presidente Elisabetta Falcieri riferisce su preventivi richiesti a diverse Società per rendere più dinamico il sito SISM. Il CD concorda sulla proposta inviata nei giorni precedenti da Manuela Malatesta, che coinvolge una Società esterna, la Seahorse, con sede legale a Lugano. Il CD nomina referente del sito Roberto Balboni che si interfaccerà costantemente con la Società nel lavoro di Design e Sviluppo del sito dedicato alla SISM. Si raccomanda di evitare difficoltà nell'utilizzo del sito in corrispondenza con le iscrizioni alle attività, che ne hanno bisogno.

2. Il verbale della riunione del Consiglio Direttivo del 4 dicembre 2015 viene approvato all'unanimità.

3. Il Presidente riferisce sulla situazione economica della società, illustrando dettagliatamente il bilancio 2015 che ad oggi è di circa 24000 euro di attivo. Viene poi affrontata la questione dei rimborso per i relatori invitati alle scuole. In particolare, viene approvata la formula proposta da Roberto Balboni: rimborso forfettario = (1/7 del costo carburante + indennità di 0.4€) x chilometri effettuati. A tal proposito il Presidente suggerisce di optare, nel limite del possibile, per i mezzi pubblici, nella ottica di contenere le spese in funzione di altri investimenti rivolti alla formazione microscopica e ai giovani.

4. Il Presidente relaziona sugli eventi che si terranno nel 2016, per alcuni dei quali sono aperte le iscrizioni:
   "La microscopia elettronica SEM/ESEM nello studio dell'ambiente" (Università di Urbino, 14-16 marzo)
   "Nuclear structure and dynamics, through the microscopes" (Università di Pavia, 7-8 luglio 2016, SISM+SII)
   Corso teorico-pratico: "Corso Base integrato di microscopia confocale e microscopia elettronica TEM/STEM" (Università di Modena, settembre, 2016)
   Workshop: "STSPM" (CNR, Bologna, 29,30 settembre, 2016)
   "La microscopia elettronica applicata allo studio dei Beni Culturali" (III edizione, Università di Urbino, autunno 2016)
   Scuola TEM "Pier Giorgio Merli" 2016 (CNR, Bologna, novembre/febbraio 2016)
   La SISM patrocinerà, inoltre, i seguenti eventi (verranno messi sul sito e le locandine inviate ai soci):
   "Sopravvivenza e morte cellulare: nuove acquisizioni e ricadute applicative" (Roma ISS, 18 aprile)
   "Nanomedicine" (organizzato dall'ISS, 21-23 settembre Viterbo 2016).

5. Nuovi Soci SISM, nessuno.

6. Il CD decide all'unanimità di non bandire contributi di partecipazione al congresso di microscopia ECM2016 che si terrà a Lione, Francia.
   Il CD decide di attribuire premi SISM a tesi di dottorato (1-2 di area materiali e 1-2 di area biomedica) di taglio microscopico; il Presidente si assume l'onere di formularne il bando.

Alle ore 15:30, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta e incontra le ditte per presentare le iniziative SISM 2016.
Sono presenti Nikon, Gambetti e 2M strumenti a cui vengono presentate le attività SISM 2016.

Elisabetta Falcieri
Roberto Balboni
Cristiano Albonetti
Regina Ciancio
Stefania Meschini

Elenco delle attività promosse dalla SISM nel 2016

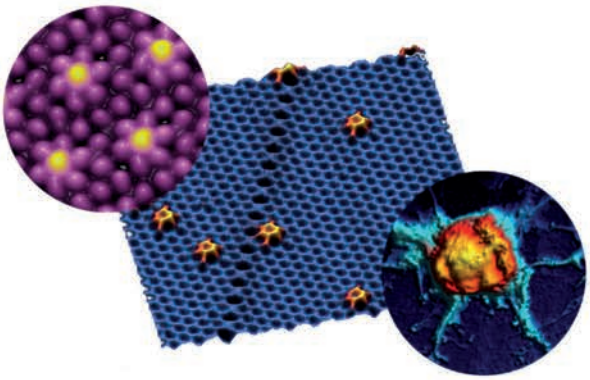
20-21 Ottobre 2016, Bologna: **Workshop Science through Scanning Probe Microscopy 2016 (StSPM'16),** a cura del Dott. Cristiano Albonetti e dei suoi collaboratori.

14-18 Novembre 2016; 6-10 Febbraio 2017, Bologna: **Scuola teorico-pratica di Microscopia Elettronica in Trasmissione in Scienza dei Materiali "Pier Giorgio Merli" 2016**, a cura del Dott. Roberto Balboni e dei suoi collaboratori.

21-22 Novembre 2016, Urbino: **Workshop teorico-pratico La microscopia elettronica applicata allo studio dei beni culturali**, a cura della Prof.ssa Elisabetta Falcieri e dei suoi collaboratori.

Società Italiana Scienze Microscopiche

CNR ISMN



Science through Scanning Probe Microscopy (StSPM'16)

Bologna, 20 – 21 Ottobre 2016

Consiglio Nazionale delle Ricerche

Area della Ricerca di Bologna
Via Gobetti 101 – 40129 Bologna

Direzione scientifica
Cristiano Albonetti, Francesco Valle e Marco Brucale
(CNR-ISMN)

Comitato organizzatore
Denis Gentili, Marianna Barbalinardo, Massimiliano Cavallini, Patrizia A. Fulle, Alessandro Tugnoli

Informazioni Generali

La società **SISM** in collaborazione con l'Istituto per lo Studio dei Materiali Nanostrutturati (**ISMN**) del **CNR** (Area della Ricerca di Bologna), organizza il workshop Science through Scanning Probe Microscopy 2016 (StSPM'16). L'evento si propone come triennale *meeting point* italiano per microscopisti a scansione di sonda, al fine di illustrare gli avanzamenti scientifici ottenuti grazie alle tecniche SPM.
Nel workshop si alterneranno la scienza dei materiali e quella della vita per evidenziare l'apporto della microscopia SPM alle due discipline. Sono previsti interventi di microscopisti di chiara fama e di ricercatori esperti, ma sarà dato spazio anche ai risultati scientifici ottenuti da giovani ricercatori (laureandi, dottorandi e giovani post-doc). È prevista l'assegnazione di 7 *slots* da 10 minuti ciascuno per interventi non ancora programmati; il comitato scientifico/organizzativo li selezionerà tra gli **abstracts** pervenuti entro il **26 Settembre 2016** (per informazioni consultare il sito internet – vedi frontespizio della brochure).
Le presentazioni e le discussioni saranno in lingua Inglese.
Il workshop si rivolge a tutti i professori, ricercatori, tecnici e studenti interessati alla microscopia SPM quale strumento fondamentale per l'indagine scientifica alla scala sub-micrometrica, nanometrica ed atomica. I partecipanti riceveranno un attestato di partecipazione.
I lavori inizieranno alle **14:30** del **20 Ottobre** e termineranno il **21 Ottobre** alle **13:30**. La **scadenza** per le domande di **partecipazione** è fissata al **10 Ottobre 2016**.
Le principali ditte leader nel settore della microscopia SPM presenteranno le ultime novità strumentali del settore.

Il workshop prevede un massimo di **85 partecipanti**
È richiesto un numero **minimo** di **20 partecipanti** per attivare il workshop.

Per ulteriori informazioni rivolgersi a:
Dr. Cristiano Albonetti c.albonetti@bo.ismn.cnr.it
Tel.: 0516398531, Fax: 0516398540
Dr. Francesco Valle f.valle@bo.ismn.cnr.it
Tel.: 0516398512
Dr. Marco Brucale marco.brucale@ismn.cnr.it
Tel.: 0516398519

http://www.sism.it/eventi_sism.php?Lingua=IT
http://www.bo.ismn.cnr.it/StSPM16/

Con il patrocinio di

DSCTM Dipartimento Scienze Chimiche e Tecnologie dei Materiali
CNR ISMN
AREA DELLA RICERCA DI BOLOGNA

Con il supporto di:

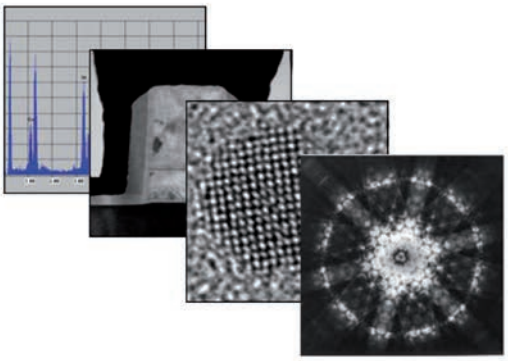
JEOL
ElbaTech srl
FEI
LOT Quantum Design
assing
Gambetti Vacuum Technology and Related Solutions
NT-MDT
Nanosurf
Pra.Ma.

CNR – IMM Bologna

SISM

Electron Microscopy School

"Pier Giorgio Merli"



6th Theoretical - Practical course on

Transmission Electron Microscopy in Materials Science

Bologna, Area della Ricerca del CNR

November 14 - 18 , 2016

February 6 – 10, 2017

temschool.bo.imm.cnr.it

Director: *Roberto Balboni*

The school, jointly organised by SISM and CNR-IMM, consists of two full weeks of theoretical and practical lessons and will provide researchers and microscopists a qualified introduction to transmission and scanning transmission electron microscopy techniques for materials science. After an introduction to the working principles of the instrument, a description of the main techniques for structural and analytical characterization will be provided. The topics covered include: electron emission and electron optics, theory of electron diffraction, mechanism of contrast formation, atomic resolution via coherent (HREM) and incoherent (HAADF/STEM) imaging techniques, electron holography and interferometric methods, convergent beam electron diffraction, analytical methods (EDX and EELS).

The school is divided into a theoretical part (14-18 Nov 2016) and a practical part (6-10 Feb 2017). It is possible to participate to either the whole course or the theoretical part only (it is not possible to attend the practical course only).

The practical course will take place at the Electron Microscopy Laboratory of the CNR-IMM Institute, where the students will directly operate on the FEI Tecnai F20ST, under teachers supervision, practicing with the techniques exposed during the first week. To guarantee enough operating time to all students, the total number of participants to the practical week will be limited. In case of much larger request, an additional practical week may be arranged.

An overview of some of the available simulation and data processing softwares for transmission electron microscopy will be also provided.

Representatives of TEM/STEM manufacturers, sample preparation instruments and accessories for electron microscopy will also give brief presentations of their new products .

As participation to the School is open to people from all countries the official language will be English.

General information

Dr. R. Balboni: balboni@bo.imm.cnr.it

Accomodation

For information on accommodation and how to reach CNR-IMM Bologna, please refer to www.bo.cnr.it or contact:
Mrs. Giorgia Giovannini, CNR – IMM Sezione di Bologna
Tel: +39 051 6399143
giovannini@bo.imm.cnr.it

Teachers: *Aldo Armigliato, Roberto Balboni, Gianluca Calestani (Univ. Parma), Matteo Ferroni (Univ. of Brescia), Giorgio Lulli, Andrea Migliori, Vittorio Morandi, Giuseppe Nicotra, Luca Ortolani, Andrea Parisini.*

Organised by CNR-IMM, Bologna, Italy and the Italian Society for Microscopical Sciences (SISM)

S.I.S.M. Società Italiana Scienze Microscopiche

VINCITORI DEI PREMI SISM TESI DI DOTTORATO

Il Consiglio Direttivo della SISM, nel corso della riunione del 13 maggio 2016, dopo avere esaminato le domande pervenute, le tesi di dottorato ed i curricula dei candidati, ha stilato una graduatoria e proclamato i vincitori.
Come prescritto dal bando, i premi, dell'importo di € 500,00 ciascuno, sono stati utilizzati per la partecipazione ad un evento correlato, nazionale o internazionale:

Il Dott. **Gabriele Fisichella** (Scienze dei Materiali) IMM-CNR di Catania, ha partecipato all'EMRS SPRING MEETING, Lille Grand Palais, Francia, 2-6 Maggio 2016.

La Dott.ssa **Valentina Palmieri** (Scienze Biomediche), Università Cattolica Sacro Cuore di Roma ha partecipato al SEM XIII International Congress and Exposition on Experimental and Applied Mechanics, Lake Buena Vista, Florida, 6-9 Giugno 2016.

Il Dott. **Luigi Rosati** (Scienze Biomediche), Università degli Studi di Napoli "Federico II" ha partecipato al 62° Convegno GEI, Napoli, 20-26 Giugno 2016

La Dott.ssa **Maria Chiara Spadaro** (Scienze dei Materiali), EPFL di Losanna, ha partecipato all'EMC 2016, Lione, Francia, 28 Agosto -2 Settembre 2016.

Il Consiglio Direttivo si congratula vivamente con i vincitori.

Il Presidente SISM

Elisabetta Falqui

Eventi nazionali

2016

70° CONGRESSO NAZIONALE SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI)
Università Cattolica del Sacro Cuore, Roma, 15-17 settembre 2016
http://www.siai.unifi.it/siai2016/Call%20for%20abstract%20SIAI%20Roma%2016.pdf

NANOMEDICINE VITERBO 2016 (Evento patrocinato dalla SISM)
Viterbo, 21-23 settembre 2016
http://www.nanodrug.cnr.it

XXXIV CONFERENZA NAZIONALE DI CITOMETRIA e SCUOLA NAZIONALE DI CITOMETRIA
Corsi Teorico-Pratici Residenziali di Formazione e Aggiornamento
Urbino, 27-30 settembre 2016
http://gic.casaccia.enea.it

CORSO DI MICROSCOPIA NIKON
Bari, 29 settembre
http://www.mariolippolis.it/corso-di-microscopia-nikon/

SCUOLA DI MICROSCOPIA - SUPER RESOLUTION (Evento patrocinato dalla SISM)
Istituto Ortopedico Rizzoli, Bologna, 12-14 Ottobre 2016
http://www.scuoladimicroscopia.it/index.php

ANALISI DELL'AMIANTO. MICROSCOPIA OTTICA IN CONTRASTO DI FASE E IN DISPERSIONE CROMATICA. MICROSCOPIA ELETTRONICA A SCANSIONE
INAIL Centro Ricerche Monte Porzio Catone, Roma, 24-26 ottobre 2016
https://www.inail.it/cs/internet/attivita/prevenzione-e-sicurezza/formazione/calendario-corsi/corso_analisi_amianto_microscopia_ottica_contrasto_fase_dispersi.html

ANALISI DELL'AMIANTO. DIFFRATTOMETRIA A RAGGI X E SPETTROSCOPIA IN TRASFORMATA DI FOURIER
INAIL Centro Ricerche Monte Porzio Catone, Roma, 27-28 ottobre 2016
https://www.inail.it/cs/internet/attivita/prevenzione-e-sicurezza/formazione/calendario-corsi/corso_analisi-dell_amianto_diffrattometria-a-raggi-x.html

CORSO INTRODUTTIVO ALLA MICROANALISI (EDS)
MEDIA System Lab di Macherio (MB), 9-10 novembre 2016
Informazioni: *matteo.mariani@m-s.it*

Eventi internazionali

2016

MSE CONGRESS - SYMPOSIUM D01 - ADVANCED AND IN-SITU MICROSCOPIES AND SPECTROSCOPIES OF FUNCTIONAL NANOSTRUCTURES IN MATERIALS SCIENCE AND ENGINEERING
27 to 29 September 2016
Darmstadt University of Technology – Darmstadt – Germany

ENHANCING YOUR IMAGE – HOW TO COLOUR EM IMAGES USING PHOTOSHOP
28 September 2016
John Innes Centre – Norwich – United Kingdom

MICROSCOPY: ADVANCES, INNOVATION, IMPACT
30 September 2016
The Geological Society – London – United Kingdom

CONFERENCE ON IN-SITU AND CORRELATIVE ELECTRON MICROSCOPY - CISCEM 2016 (EMS sponsored)
11 and 12 October 2016
Saarbrücken – Germany

SSOM INTERDISCIPLINARY SYMPOSIUM ON 3D MICROSCOPY
18 to 21 October 2016
Congress Center – Les Diablerets – Switzerland

6TH "PIER GIORGIO MERLI" TEM SCHOOL IN MATERIALS SCIENCE
14 November 2016 to 10 February 2017
CNR-IMM Bologna, via Gobetti 101, Bologna, Italy – Bologna – Italy

BASICS OF RESIN EMBEDDING AND SECTIONING FOR ELECTRON MICROSCOPY
16 to 18 January 2017
UMC Utrecht – Utrecht – Netherlands

BASICS OF CRYOSECTIONING AND IMMUNOLABELING (TOKUYASU TECHNIQUE)
20 to 23 January 2017
UMC Utrecht – Utrecht – Netherlands

Eventi internazionali

WINTERSCHOOL 2017: PRACTICAL COURSE IN ADVANCED MICROSCOPY
22 to 27 January 2017
University of Zurich and ETH Zurich – Zurich – Switzerland

FOCUS ON MICROSCOPY 2017
9 to 12 April 2017
Bordeaux – France

FOURTH CONFERENCE ON FRONTIERS OF ABERRATION CORRECTED
30 April to 4 May 2017
Kasteel Vaalsbroek – Netherlands
Organisation: Ernst Ruska-Centre in Jülich

QUANTITATIVE ELECTRON MICROSCOPY SCHOOL
22 May to 2 June 2017
St-Aygulf – France

MICROSCOPY CONFERENCE 2017 (EMS extension)
21 to 25 August 2017
SwissTech Convention Center – Lausanne – Switzerland
Microscopy conference jointly organised by: SSOM – Swiss Society for Optics and Microscopy and ASEM – Austrian Society for Electron Microscopy DGE – German Society for Electron Microscopy e.V.

RMS INTERNATIONAL FLOW CYTOMETRY COURSE
12 to 16 September 2017
University of York – United Kingdom

SOURCES, INTERACTION WITH MATTER, DETECTION AND ANALYSIS OF LOW ENERGY ELECTRONS 2017 / SIMDALEE2017
18 to 22 September 2017
Hotel Flamingo – Pula, Sardinia – Italy

13th MULTINATIONAL CONGRESS ON MICROSCOPY (EMS extension)
24 to 29 September 2017
Hotel Lone – Rovinj – Croatia

DREILÄNDERTAGUNG
The MC 2017 is an EMS extension, and jointly organized by
SSOM – Swiss Society for Optics and Microscopy
ASEM – Austrian Society for Electron Microscopy
DGE – German Society for Electron Microscopy e.V.

GENERAL INFORMATION

MC 2017 Lausanne
Microscopy Conference

Date
21–25 August 2017

Venue
SwissTech Convention Center
www.tstcc.ch

Conference Chairs
Dr. Marco Cantoni
EPFL SB CIME-GE, MXC-135
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EMPA, Electron Microscopy Center
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Program & Topics
Plenary Lectures, (Poster-)Sessions, Workshops
- Materials Science
- Life Sciences
- Instrumentation and Methods

Conference Organizer & Industrial Exhibition
Conventus Congressmanagement & Marketing GmbH
Phone +49 3641 31 16-341 • mc@conventus.de

More information available soon at
www.mc2017.ch

Rovinj, Croatia, 2017

Registration to the MCM 2017 conference will open on November 30, 2016.

After 14 years, the Multinational Congress on Microscopy will again be organized in Croatia on September 24-29, 2017. In its 13th issue, the traditional conference series is returning to Istria, this time to the beautiful coastal town of Rovinj.

MCM2017 is jointly organized by 8 societies: Austrian Society for Electron Microscopy (ASEM), Croatian Microscopy Society (CMS), Czechoslovak Microscopy Society (CSMS), Hungarian Society for Microscopy (HSM), Italian Society of Microscopical Sciences (SISM), Serbian Society for Microscopy (SSM), Slovenian Society for Microscopy (SDM) and Turkish Society for Electron Microscopy (TEMD).

MCM2017 will bring together leading experts and young researchers that develop microscopy methods and apply them in the fields of life and material sciences. It will also include a trade exhibition in order to encourage exchange between the producers of microscopy-related equipment and researchers.

MCM conferences have always been an excellent opportunity for microscopists to exchange ideas and experience and to establish new cooperations and joint projects. Our aim is to provide an optimal balance between talks given by world-renowned scientists and a possibility for talented young scientists to introduce themselves to an international audience.

We believe this conference will be a highly rewarding professional and networking experience for all. Additionally, we encourage you to take this opportunity to explore the highlights of coastal town Rovinj with its beautiful surroundings and to experience the unique local blend of nature, culture and gastronomy.

We look forward to meeting you at MCM2017 in Rovinj.

On behalf of the Organizing Committee,

Sincerely
Co-chairs
Igor Weber & Andreja Gajović

Rovinj is situated on the west coast of Istria, the biggest peninsula on the Adriatic coastline, and only within one hour drive from Trieste, 2.5 hours from Zagreb and 6 hours from Munich.

People of Rovinj make their living mostly out of tourism, fishing, agriculture, winemaking and viticulture.

Rovinj is one of the most developed tourist destinations in Croatia, boasting rich natural and cultural heritage with beautiful landmarks such as the old town and the lush Zlatni rt (Punta corrente) forest. Zlatni rt is a protected forest park, while Rovinj's islands and coastal area are protected landscapes/seascapes.

In the last 40 years Rovinj has developed into a popular tourist destination thanks to its lovely nature, indented coastline with a necklace of islands and mild weather.

These features are complemented by diverse activity programs, world-class amenities and historic treasures.

Rovinj's tourist offer is closely related to sports, recreation and entertainment. Rovinj features entertainment for people of all age groups. Guests will enjoy spending time at numerous bars and restaurants along the seaside promenade.

Workshop teorico-pratico

La microscopia elettronica SEM/ESEM nello studio dell'ambiente

14-16 Marzo 2016
Aula Magna Campus Scientifico "Enrico Mattei", Urbino
ARPAM, Dipartimento di Pesaro

AMIANTI E ALTRE FIBRE INORGANICHE, NATURALI E SINTETICHE, PRESENTI NELL'AMBIENTE: INDAGINE TRAMITE SEM-EDS PER LA VALUTAZIONE DEL FONDO AMBIENTALE E DI SITUAZIONI DI INQUINAMENTO

Elena Belluso, Silvana Capella

Dipartimento di Scienze della Terra, Università di Torino e Centro Interdipartimentale per lo Studio degli Amianti e di altri Particolati Nocivi "G. Scansetti", Università di Torino

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Anche se non è sempre evidente, viviamo in un mondo in cui le polveri aerodisperse (intese come particolato fine) sono ubiquitarie e in quantità anche molto elevate. Con un singolo respiro possiamo introdurre nell'apparato respiratorio anche 50 milioni di particelle con dimensioni pari o inferiori a 50 nm. Elevate quantità di particelle presenti nelle vie respiratorie, come è noto, possono provocare malattie anche gravi.

Tra i vari tipi di particelle "respirabili" vi sono anche fibre inorganiche (artificiali e naturali) con specifiche caratteristiche dimensionali: lunghezza > ≥5 μm, diametro (larghezza) ≤3 μm, rapporto lunghezza/diametro 3: 1 (WHO, 2006). Alcune, quali le fibre di amianto o particolari fibre inorganiche naturali (per esempio fluoroedenite asbestiforme) respirate in elevate quantità (come nel caso di esposizione professionale) possono determinare l'insorgenza di malattie cancerose. Secondo studi recenti, analoghi effetti negativi si possono avere anche a dosi basse se continuative. Al fine della prevenzione è perciò particolarmente importante determinare la presenza di possibili sorgenti di aerodispersione di tali fibre.

Per quanto riguarda le 6 specie mineralogiche classificate amianto, in Italia tremolite d'amianto e actinolite d'amianto sono aerodisperse da rocce presenti nel territorio (sorgenti naturali) e non da materiali antropici, non essendo state utilizzate industrialmente. L'aerodispersione di fibre di crocidolite e amosite (grunerite d'amianto) si ha invece solamente da manufatti (sorgenti antropiche) poiché in Italia non sono naturalmente presenti rocce che li contengano. I restanti due amianti (crisotilo e antofillite d'amianto) sono invece presenti sia in sorgenti naturali sia in sorgenti antropiche (la seconda specie in quantità molto basse).

Numerose altre specie minerali con caratteristiche simili agli amianti (e definite asbestiformi) sono naturalmente presenti (localmente anche abbondanti) in rocce del territorio italiano e quindi aerodisperdibili e respirabili (per esempio sepiolite asbestiforme, antigorite asbestiforme). Sulla base di quanto sopra specificato, l'indagine su presenza e concentrazione in aria dei vari tipi di fibre inorganiche con dimensioni respirabili è particolarmente importante perché permette di rilevare situazioni di inquinamento e di identificare, per determinate specie, le specifiche sorgenti di dispersione. La tecnica che offre buona accuratezza e precisione nell'indagine, soprattutto laddove la concentrazione di fibre è modesta, è quella della microscopia elettronica in scansione (SEM) combinata con la spettrometria di dispersione di energia (EDS). L'indagine, a partire dalla prima fase di raccolta dell'aria, è regolamentata dal Decreto Ministeriale 6 settembre 1994, Ministero della Sanità (G.U. n.288, supplemento ordinario del 10 dicembre 1994); solamente i laboratori che hanno superato positivamente i programmi di qualificazione del Ministero della Salute hanno il riconoscimento ufficiale per effettuare analisi sugli amianti.

L'identificazione della natura delle fibre dimensionalmente respirabili è basata sulla composizione chimica per cui è necessario che il laboratorio possegga una banca dati interna (costruita mediante analisi di campioni a composizione chi-

mica nota) e che l'operatore abbia adeguata esperienza. Sulla base delle specie identificate e della loro concentrazione è possibile stabilire se è in atto una situazione di inquinamento e, in casi specifici, anche il tipo di sorgente di dispersione (Figura 1). In caso di identificazioni ambigue (per esempio per distinguere tra crisotilo e antigorite asbestiforme) sono necessarie indagini al microscopio elettronico in trasmissione (TEM) utilizzando osservazioni morfologiche, dati chimici (spettri EDS) e informazioni strutturali (diffrazioni elettroniche di aree selezionate: SAED) raccolti da singole fibre. Queste indagini permettono identificazioni univoche, ma in Italia non sono previste dalla normativa vigente (lo sono invece in altri paesi dell'Unione Europea, per esempio in Francia). Un altro tipo di indagine, particolarmente utile nel caso di sospetta presenza di sorgenti nascoste di aerodispersione di fibre inorganiche, prevede l'utilizzo di animali sentinella e l'impiego della tecnica SEM-EDS (Figura 1), analogamente a quanto previsto per i campioni di aria.

Anche per queste indagini, nel caso di identificazioni dubbie l'uso complementare di TEM-EDS è risolutivo. A Casale Monferrato (AL), dove è stato attivo fino al 1986 uno stabilimento di produzione di cemento-amianto, la presenza di materiale non compatto e respirabile (chiamato "polverino") non è ancora stata del tutto identificata. A tal fine, congiuntamente con ricercatori dell'Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, è stata condotta una ricerca su polmoni di ratti selvatici catturati in zone definite preventivamente in base a dati storici riportati su attività industriali e dei trasporti relativi ad amianto. Frammenti di polmoni prelevati dai ratti sono stati chimicamente digeriti secondo una procedura collaudata (Fornero *et al.*, 2009); la sospensione ottenuta è stata filtrata e i materiali inorganici depositati sul filtro sono stati esaminati tramite SEM-EDS. È stato così possibile identificare vari tipi di fibre inorganiche, attribuibili a differenti sorgenti sulla base delle conoscenze dell'area in esame, tra cui: specie non classificate amianto (biossido di titanio) e fibre di amianto (crocidolite e amosite) derivanti da sorgente antropica; amianto da sorgente naturale (tremolite d'amianto); amianto (crisotilo) da sorgente indefinita poiché attribuibile sia a materiali antropici sia a sorgenti naturali (Ardizzone *et al.*, 2014).

Attraverso il confronto con il residuo inorganico di polmoni di ratti vissuti in aree prive di amianto da sorgenti antropiche, è stato quindi possibile rilevare che in alcune zone dell'area in esame c'è una situazione di inquinamento. Gli stakeholders hanno pertanto la possibilità di organizzare una campagna di indagini per identificare le specifiche sorgenti di inquinamento e programmare opportune bonifiche.

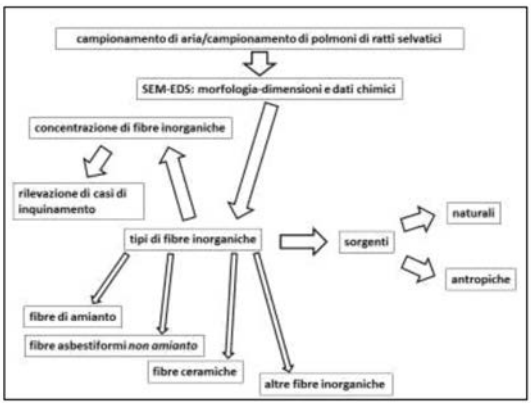

Figura 1. Schematico diagramma di flusso relativo a rilevazione e quantificazione di fibre inorganiche a partire da campioni di aria o campioni biologici di animali sentinella.

Bibliografia

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IL RUOLO DEL SEM NELLA VALUTAZIONE DELLE ALTERAZIONI MORFOLOGICHE INDOTTE DA INQUINANTI NEGLI EPITELI RESPIRATORI DI ORGANISMI ACQUATICI

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Tra le cause più comuni d'inquinamento degli ambienti acquatici rientrano le alterazioni di alcuni parametri abiotici quali: temperatura, pH e salinità e la contaminazione causata dal massiccio uso di sostanze chimiche a fini agricoli, industriali

e cosmetici. Gli effetti dell'inquinamento sugli ecosistemi vengono indagati attraverso analisi ecotossicologiche che possono essere mirate alla valutazione dei danni indotti a diversi livelli dell'organizzazione biologica; la finalità di tali studi, tra le altre, è di individuare i marker più efficaci in grado di segnalare situazioni a rischio nel loro stadio iniziale. In particolare le indagini ecotossicologiche su Vertebrati "non bersaglio", quali gli Anfibi, sono molto importanti alla luce degli attuali sforzi conservazionistici mirati a preservare gli ambienti umidi e limitare la perdita di biodiversità.

Per effettuare una corretta valutazione degli effetti di sostanze inquinanti si possono utilizzare diversi approcci metodologici e negli esperimenti qui riportati abbiamo valutato la risposta di diversi endpoint nelle medesime condizioni sperimentali. Prendendo in considerazione parametri diversi abbiamo avuto l'opportunità di mettere in risalto gli aspetti diagnostici dell'utilizzo della microscopia in generale e del SEM in particolare, negli studi ecotossicologici e di confrontare le capacità predittive di endpoint morfologici rispetto ad altri più comunemente utilizzati marker di stress e inquinamento. Abbiamo utilizzato come organo target l'apparato branchiale degli Anfibi perché altamente permeabile e direttamente esposto alle variazioni dell'ambiente acquatico.

Il declino della popolazioni di Anfibi negli ultimi anni ha richiamato l'attenzione su questo complesso fenomeno. Oggi vi è un accordo generale sul fatto che il declino degli Anfibi sia una conseguenza delle attività umane oltre che alle normali fluttuazioni delle popolazioni animali.

L'acidificazione degli ecosistemi acquatici è ritenuta essere una delle possibili cause del declino degli Anfibi in particolare in Europa e Nordamerica. Abbiamo dimostrato, utilizzando larve di *Lissotriton italicus*, che dopo sole 48 ore di esposizione ad un valore di pH non letale (4.5) le alterazioni dell'apparato branchiale sono cospicue. L'analisi al SEM ha mostrato notevoli alterazioni morfologiche ma il fenomeno più evidente è stato la comparsa di uno strato esterno cheratinizzato che ricopre in maniera piuttosto uniforme la branchia. Tali alterazioni possono interferire con la respirazione e l'osmoregolazione e possono rappresentare un importante fattore di disturbo per le popolazioni naturali, contribuendo così al fenomeno di declino delle popolazioni di Anfibi nelle regioni acidificate. Accanto all'analisi morfologica al SEM abbiamo testato due endpoint di tipo comportamentale, comportamento trofico e attività di nuoto, nelle medesime condizioni sperimentali. Questi test hanno richiesto un numero di individui maggiore rispetto a quelli utilizzati per l'analisi al SEM; inoltre è stato necessario l'utilizzo di un apparato sperimentale complicato e del successivo uso di un software di analisi specifico. Altrettanto complessa è stata la procedura sperimentale che ha richiesto la manipolazione degli animali per isolarli nel corso delle analisi comportamentali, con il rischio potenziale di alterare i risultati dell'osservazione. È interessante notare come questi endpoint, molto utilizzati nei test ecotossicologici, non siano risultati maggiormente sensibili e dopo 24 ore non hanno messo in evidenza alterazioni significative così come non erano evidenti processi patologici a livello della morfologia branchiale.

Un altro fattore di inquinamento da noi valutato è l'aumento della concentrazione salina nelle acque dolci. Il fenomeno della salinizzazione secondaria degli habitat d'acqua dolce viene attribuita prevalentemente all'utilizzo dei sali antigelo, alla desertificazione ed a numerose altre attività antropiche quali disboscamento, eccessivo sfruttamento delle falde, attività industriali, urbane e agricole.

In questo caso abbiamo utilizzato le larve due specie, (*Bufo balearicus* e *Bufo bufo*), la prima delle quali maggiormente eurialina, esponendo i girini a concentrazioni saline crescenti e valutando poi la morfologia branchiale dopo 24, 48 e 96 ore. Abbiamo dimostrato che esiste una relazione negativa fra l'aumento della concentrazione salina e l'integrità della branchia per entrambe le specie considerate. I nostri risultati confermano che l'entità delle alterazioni è fortemente correlata con le preferenze ecologiche delle specie ma evidenziano inoltre che un aumento della salinità ha il potenziale di impattare anche le popolazioni di quelle specie che sono in grado di tollerare salinità moderate.

L'analisi delle branchie di *B. bufo* ha mostrato inoltre che lo stress salino induce alterazioni non solo della struttura ma anche della composizione cellulare ed è particolarmente interessante notare la comparsa della cellula tubulo-vescicolare (TVC). Anche in questo caso abbiamo valutato altri endpoint (mortalità) ed abbiamo dimostrato che concentrazioni saline molto basse, sebbene non siano letali durante una esposizione acuta, inducono precoci e importanti alterazioni a carico

dell'apparato branchiale.

Un'altra categoria da noi analizzata è quella dei pesticidi. In questo caso abbiamo scelto l'endosulfan, un insetticida organoclorurato altamente persistente. Abbiamo valutato gli effetti sull'apparato branchiale di *B. bufo* in risposta a diverse modalità di esposizione (acuta e cronica) ed a diverse concentrazioni. Oltre alle notevoli alterazioni dell'apparato branchiale abbiamo evidenziato, anche in questo caso, la comparsa della cellula tubulo-vescicolare dopo solo 96 ore di esposizione. La presenza delle TVC lascia ipotizzare che tali cellule possano avere un ruolo specie-specifico nella risposta della branchia alle alterazioni del mezzo acquatico. Abbiamo in seguito studiato gli effetti di tale pesticida dopo un'esposizione a lungo termine utilizzando concentrazioni molto basse e rilevabili in natura. I nostri esperimenti hanno messo in evidenza che concentrazioni che risultavano subletali durante l'esposizione acuta, sono divenute letali dopo esposizione a lungo termine o hanno avuto effetti negativi sulla crescita, il comportamento, le caratteristiche metamorfiche, causando inoltre l'insorgenza di gravi malformazioni e ritardi nello sviluppo. Fra le concentrazioni testate solo la più bassa (10 µg/l) sembrava non causare effetti negativi sui girini di *B. bufo*. Tuttavia l'analisi morfologica al SEM ha evidenziato che anche gli individui esposti alla concentrazione più bassa subiscono le stesse alterazioni presenti dopo esposizione alle altre concentrazioni con un andamento oltre che dose anche tempo dipendente. Risulta chiaro che per tutte le concentrazioni testate il primo effetto dell'endosulfan sulla specie studiata è l'alterazione dell'apparato branchiale seguita dall'alterazione di parametri comportamentali.

Un'altra sostanza da noi considerata è il chlorpyrifos (CPF), un pesticida che appartiene agli organofosfati. In questo studio, abbiamo esposto larve di *Rana dalmatina* a 3 concentrazioni di CPF rilevabili in natura per l'intero periodo larvale. Anche in questo caso abbiamo valutato diversi parametri nelle medesime condizioni sperimentali. Dall'analisi dei dati è emerso che il CPF non è in grado di indurre significativi effetti sulla sopravvivenza o su altri parametri di crescita e sviluppo. Tuttavia l'analisi al SEM ha dimostrato che in Rana dalmatina il CPF induce intense e precoci alterazioni a carico dell'apparato branchiale e che il grado delle alterazioni istopatologiche è strettamente legato alla concentrazione dell'inquinante ed alla durata dell'esposizione. Il primo effetto osservabile è l'alterazione branchiale dopo 8 giorni di esposizione che precede qualunque altra alterazione (i.e. deformità, disordini comportamentali).

Dalle evidenze qui riportate si evince con chiarezza che l'analisi morfologica di organi target deve essere considerata un importante strumento per indagare gli effetti subletali dei contaminanti in particolar modo per la sua sensibilità e per la facilità di identificazione e quantificazione.

THE ELECTRON MICROSCOPY APPLIED TO STUDY ADHESION AND SURVIVAL STRATEGIES OF BACTERIA IN RESPONSE TO ENVIRONMENTAL STRESSES

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Unfavorable environmental conditions induce bacteria species to adhere to different surfaces and, subsequently, develop a biofilm or to activate a survival state called viable but nonculturable (VBNC).

Biofilms are defined as oriented aggregations of microorganisms attached to each other or to a surface and enclosed in extracellular polymeric substance produced by themselves (Costerton *et al.*, 1999; Fleming and Wingender, 2010), that protect themselves and, in the case of human pathogens, to express their pathogenicity. The formation and development of biofilms is affected by many factors, including the specific bacteria strain, material surface properties, and environmental parameters such as the pH and nutrient levels and temperature (Abdhallah *et al.*, 2014). Biofilms are prevalently constituted by a single species, but in the oral cavity biofilms are communities with several microbial species. The microbial diversity of oral communities can be studied by scanning electron microscopy (SEM), a useful imaging approach for the visualization of bacterial biofilms in their natural environments, including dental and biomaterial surfaces. Biofilm cells are more resistant to antimicrobial agents than planktonic bacteria, as they have a barrier which prevents or lessens the contact with antimicrobial agents (O'Toole and Kaplan, 2000).

In reference to this, we evaluated by SEM the effect of two antimicrobial substances, Carvacrol (1%) and Chlorhexidine (2%), on dual-species biofilm *in vitro* model grown up to 64.5 h on titanium disc surface and formed by *Streptococcus mutans* ATCC 25175, an usual inhabitant of the oral cavity, in combination with *Porphyromonas gingivalis* ATCC 33277 or *Fusobacterium nucleatum* ATCC 25586, microrganisms implicated in infection diseases. At established time-points (16.5, 40.5 and 64.5h), a visible decrease of cocci (*S. mutans*) and rod cells (*P. gingivalis* or *F. nucleatum*) in both treated biofilms, compared with control untreated samples, was observed. Figure 1 shows, at the representative time-point 40.5h, micrographs of different bacterial combination treated with Carvacrol and Chlorhexidine, respectively. The results indicates that the SEM could be used as preliminary tool for monitoring biofilm development and the effect of antimicrobials agents to delay bacterial colonization ability. Parallel, TEM analysis was performed to directly observe the ultrastructural changes induced by Carvacrol in the same oral bacterial strains. As shown in Figure 2, the exposure of pathogens to Carvacrol revealed that this compound seem to penetrate the cytoplasmic membrane, increasing the permeability and causing the shrinkage of the protoplasm and the loss of cytosolic material.

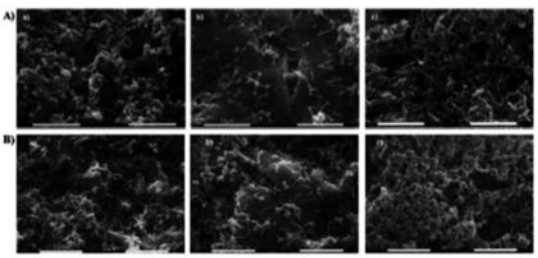

Figure 1. Scanning electron micrographs of multi-specie biofilms formed by S. mutans ATCC 25175 and F. nucleatum ATCC 25586 (A) and S. mutans ATCC 25175 and P. gingivalis ATCC 33277 (B) on titanium discs. The effect of Carvacrol (a) and Chlorhexidine (b) on biofilms development after 40.5h was compared to the untreated disc (c).

In the VBNC state, although still expressing various degrees of metabolic activity and possibly being able to cause infection (Ramamurthy *et al.*, 2014), the bacterial cells cannot be cultured on conventional laboratory media that normally support their growth. During entry into the VBNC state, bacteria acquire a coccal morphology, become smaller in size, and show changes in their protein and fatty acid profiles (Ramamurthy et al, 2014). For this we have applied SEM in association with Flow Cytometry (FCM) to investigate how low temperature affects the morphological and physiological status, respectively, of an environmental *V. parahaemolyticus* strain during incubation in artificial seawater at 4°C. Moreover, since some authors have demonstrated the ability of this microorganism to "resuscitate" from the VBNC state (Oliver, 2005), SEM analyses was used to evaluate morphological features of the *V. parahaemolyticus* vegetative form after temperature upshift as shown in Figure 3. The used microscopical methodologies have provided useful data in relation to the state transitions of *V. parahaemolyticus* regarding the quantification of morphological variations during its entry into the VBNC state and following resuscitation after re-established optimal environmental conditions.

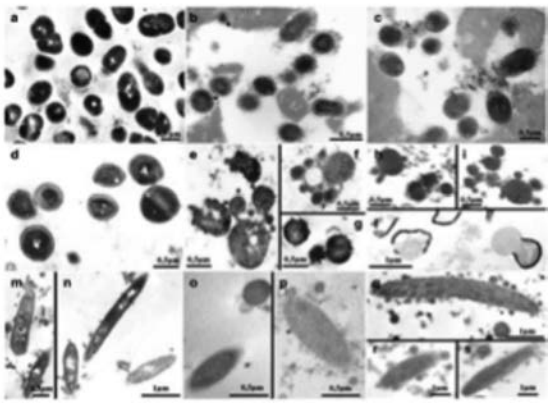

Figure 2. Transmission electronmicrographs (TEM). Effect of Carvacrol on S. mutans ATCC 25175: (a) untreated cells; (b) 5 min exposure; (c) 30 min exposure. Effect of Carvacrol on P. gingivalis ATCC 33287: (d) untreated cells; e, f, g 5 min exposure; c, h, i, l, 30 min exposure. Effect of Carvacrol on F. nucleatum ATCC 25586: m, n, untreated cells; o, p, 5 min exposure; q, r, s, 30 min exposure.

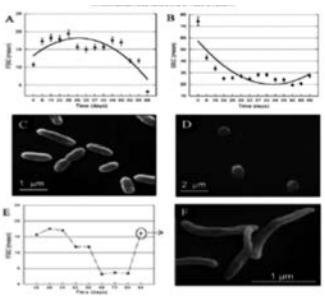

Figura 3. Morphological changes in V. parahaemolyticus, in ASW at 4°C until the VBNC state, examined by FCM and SEM analyses: (A) FCM analyses by scattering signal as Forward Light Scatter (FSC); (B) FCM analyses by scattering signal as Side Light Scatter (SSC); (C) SEM micrograph of V. parahaemolyticus cells maintained in ASW at 4°C at day 0 and day 42 (D) showing bacterial morphological changes from bacillar to coccal shape; (E) FCM analyses of FSC signal and SEM micrograph (F) of V. parahaemolyticus cells after resuscitation by temperature upshift.

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CARATTERIZZAZIONE CHIMICO-FISICA DEL PM2.5 NELL'AREA DI ROMA

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Numerosi studi epidemiologici sono stati condotti dal '90 sugli effetti del particolato urbano aerodisperso (PM) sulla salute della popolazione esposta. Tali studi hanno evidenziato una significativa associazione tra livelli di PM10, in particolare di PM2.5, e aumento di morbidità e mortalità per malattie respiratorie e cardiovascolari. Alcuni studi hanno mostrato inoltre una relazione tra gli effetti sulla salute osservati e alcune componenti del PM, in particolare del PM2.5 (Bell et al, 2014; Gent et al, 2009). Studi tossicologici *in vitro* hanno evidenziato che la tossicità del PM deriva dalla sua capacità di indurre stress ossidativo ed infiammazione nel polmone. Nonostante le evidenze epidemiologiche acquisite ed i numerosi studi tossicologici *in vivo ed in vitro* condotti in questi anni, non sono state ancora individuate le componenti o le caratteristiche fisiche e chimiche del PM in grado di spiegare la tossicità di tale inquinante. Per spiegare i meccanismi mediante i quali le particelle inalate possono produrre specie reattive dell'ossigeno sono state formulate differenti ipotesi, strettamente legate alle caratteristiche fisico-chimiche del particolato: 1) dimensioni delle particelle; 2) area di superficie delle particelle; 3) composizione chimica delle particelle; 4) chimica di superficie delle particelle, in particolare metalli di transizione, chinoni o idrocarburi poliaromatici sulla superficie delle particelle che, in presenza di riducenti biologici e ossigeno, generano radicali liberi attraverso cicli redox.

Appare quindi evidente l'importanza di caratterizzare da un punto di vista fisico-chimico le singole particelle che costituiscono il PM per determinarne forma, granulometria, composizione chimica e studiare la capacità del particolato fine e ultrafine di veicolare sulla superficie sostanze o composti tossici.

La microscopia elettronica a scansione e trasmissione, insieme alle spettrometrie ed esse associate (SEM/EDX, TEM/EDX, TEM/EELS), si è rivelata una tecnica analitica particolarmente adatta per caratterizzare le singole particelle e individuare le componenti del PM e può fornire un utile contributo nello studio della chimica di superficie delle singole particelle, della loro capacità di veicolare composti organici ed inorganici, nell'analisi dei processi di trasformazione che il PM subisce in atmosfera e nell'individuazione delle caratteristiche fisico-chimiche delle particelle in grado di spiegare gli effetti sulla salute della popolazione esposta. La microscopia elettronica a scansione munita di spettrometria a raggi X a dispersione di energia (SEM/EDX) è la tecnica analitica più versatile per la caratterizzazione delle singole particelle del PM. L'introduzione di sistemi di analisi semi-automatici ha fortemente ridotto i tempi di analisi. In tali sistemi le particelle vengono automaticamente rivelate mediante un

aumento del segnale degli elettroni secondari e/o retrodiffusi al di sopra di un livello di soglia fissato manualmente dall'operatore, corrispondente al segnale dovuto al substrato su cui è stato raccolto il PM. Questo sistema di analisi consente di determinare automaticamente una serie di parametri morfologici (diametro minimo, diametro massimo, diametro di Feret, fattore di forma, aspect ratio) e di acquisire, sempre automaticamente una volta terminata l'analisi morfologica, uno spettro EDX su un intervallo di energie (ROI) scelte dall'operatore, compiendo una scansione su ciascuna delle particelle individuate. Il set di dati ottenuto dalla microanalisi a raggi X è poi elaborato mediante tecniche di analisi statistica multivariata per individuare le componenti del PM.

In questo lavoro sono stati campionati, oltre al PM2.5, presso la stazione di monitoraggio dell'Istituto Superiore di Sanità, i seguenti inquinanti gassosi: gli ossidi di azoto (NO_x), i composti organici volatili (COV), l'ozono (O_3) ed il biossido di zolfo (SO_2) ed il monossido di carbonio (CO). In quanto i precursori NO_x, COV, O_3 , SO_2 contribuiscono, attraverso una serie di reazioni chimiche, alla formazione del particolato secondario.

La buona correlazione (0.8 coefficiente di Pearson) riscontrata durante il periodo invernale tra la concentrazione media oraria del CO e del PM2.5 indica una sorgente comune per questi due composti individuata nel traffico autoveicolare. Infatti il CO è considerato un marker del traffico in quanto l'origine antropica di tale inquinante è principalmente legata alla combustione incompleta dei carburanti usati negli autoveicoli. Lo stesso valore di correlazione (0.8 coefficiente di Pearson) è stato riscontrato per il periodo invernale tra la concentrazione media oraria degli NO_x e il PM2.5.

L'analisi delle singole particelle mediante microscopia elettronica a scansione e la successiva elaborazione dei dati di microanalisi a raggi X mediante Cluster Analysis hanno permesso di individuare nel PM2.5 campionato sette tipologie (cluster) di particelle nel periodo invernale: particelle ricche di C (cluster 1), silicati (cluster 2), particelle metalliche (cluster 3), particelle ricche di Fe (cluster 4), carbonati (cluster 5), silice (cluster 6), solfati (cluster 7). Nel periodo estivo sono stati individuate le stesse tipologie tranne i solfati. Le particelle ricche di carbonio rappresentano la tipologia più abbondante in entrambi i periodi. Tali particelle derivano prevalentemente dal traffico autoveicolare e/o da impianti di combustione e all'osservazione al SEM si presentano generalmente come sferule di dimensioni fra i 40 e i 70 nm. La caratterizzazione con la microanalisi a raggi X ha mostrato che gli aggregati "C-rich" agiscono come carrier di diversi elementi adsorbiti sulla loro superficie. Una significativa frazione delle particelle carboniose, trasporta un coating contenente zolfo legato alla presenza di solfati e di acido solforico adsorbito sulla superficie di tali particelle dovuti ai processi di ossidazione fotochimica dell'SO_2 atmosferico. Le particelle metalliche rappresentano il secondo cluster più importante nel periodo invernale ed il terzo nel periodo estivo. In questo gruppo sono state classificate particelle costituite da Al, Cr, Ni, Ti, Zn, Cu. I silicati comprendono feldsfati, argille, inosilicati e silicati vetrosi, derivanti dall'erosione dei suoli e degli edifici. Queste particelle sono il secondo cluster nel periodo estivo ed il terzo nel periodo invernale. L'abbondanza più elevata durante il periodo estivo è dovuta all'effetto dei venti meridionali (scirocco) che trasportano particelle di argilla dall'Africa. Le particelle ricche di ferro sono costituite solo da ferro ed ossigeno. Le particelle appartenenti al gruppo dei carbonati sono carbonati di Ca e Mg e derivano dai processi di erosione del suolo e delle rocce e dal deterioramento degli edifici. Le particelle che costituiscono il cluster dei solfati sono composte principalmente da solfati di Ca e derivano da reazioni tra i materiali composti da carbonato di Ca (marmo, pietre calcaree, ecc.) ed i composti solforosi presenti nell'atmosfera, ma anche dal deterioramento di coperture e vernici che rivestono le pareti degli edifici. Questa tipologia di particolato è risultata presente nel solo periodo invernale con un'abbondanza dello 1%. L'origine antropica del biossido di zolfo deriva dalla combustione domestica degli impianti non metanizzati e in misura molto minore (5%) dalle emissioni dei veicoli diesel. Per questo motivo la concentrazione di questo inquinante presenta una variazione stagionale molto evidente, con i valori massimi nella stagione invernale, in particolare nel sito di campionamento i valori riscontrati in inverno erano quattro volte superiori rispetto a quelli del periodo estivo.

In conclusione nel sito in esame il traffico autoveicolare è la principale sorgente del PM2.5, come dimostrato dall'elevata correlazione riscontrata tra la concentrazione media oraria del CO e del PM2.5. Inoltre, l'utilizzo della microscopia elettronica a scansione munita di spettrometria a raggi X

a dispersione di energia consente di individuare le principali componenti del particolato dovute al traffico veicolare (particelle ricche di C e metalli) e l'identificazione degli elementi presenti su di esse fornisce informazioni utili per lo studio dei processi di trasformazioni che le particelle subiscono in atmosfera.

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OBSERVATION OF ROTIFER JAWS AT SEM IN ECOLOGY AND EVOLUTION

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The phylum Rotifera is a group of microscopic animals, usually much shorter than 1 mm, living in any habitat where water is available: rotifers can be found in permanent water bodies such as lakes, ponds, and rivers, but also in the water layer between soil particles, mosses and lichens, and in the meltwater of glaciers (Fontaneto and De Smet 2015). Rotifers are very diverse, and occupy different dietary niches, with species that are filter-feeders, predators, browsers, piercers, parasites, etc. Such diversity in the feeding strategies is revealed in the wide variety of shape of the hard pieces that form the masticatory apparatus, the trophi (Figure 1). Trophi reflect taxonomic differences (so that detailed differences are used for species identification), evolutionary relationships (trophi are used in the morphological classification of most taxonomic ranks), and ecological adaptations (trophi reflect different feeding adaptations).

The diversity of trophi shape and functions found applications in functional ecology. Given that different species are adapted to different feeding strategies in what are called feeding guilds (Obertegger *et al.* 2011) and that trophi reflect such feeding strategies, observation of the proportion of trophi types in a sample, for example the ratio between the abundance of predator and microphagous trophi, can be used to identify the pollution level of the water body where the sample come from. Such approach in functional ecology started years ago with the saprobic system in rotifers (Sláde ek 1983), and has been already applied and extended in different studies.

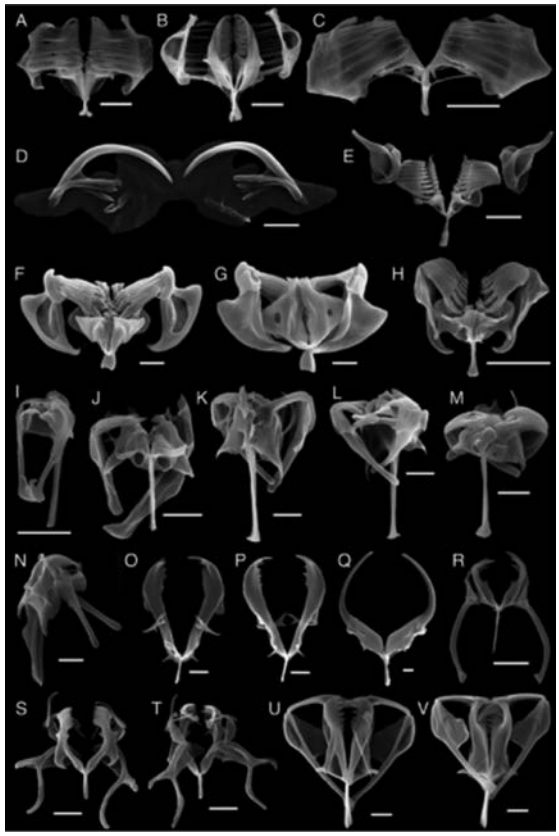

Figure 1. Different types of trophi in rotifers at SEM (modified from Fontaneto and De Smet, 2015). Scale bar = 10 micrometers.

Analyses of more detailed differences in trophi shape have been used also to address evolutionary questions on the role of adaptation in speciation processes, especially comparing different groups of rotifers (Fontaneto and Barraclough 2015), and applying quantitative approaches in the description of shapes, such as geometric morphometrics.

The literature of rotifer trophi and their applications in taxonomy, systematics and ecology is rather vast. For the reader interested in the use of SEM pictures of rotifer trophi, several SEM pictures, together with instructions on how to prepare the samples for SEM observation, can be found on the Rotifer Trophi Web Page (http://www.rotifera.hausdernatur.at/Rotifer_data/trophi/), within

the large framework of the Rotifer World Catalogue (Jersabek and Leitner 2013). An exhaustive explanation on how to prepare rotifer trophi for SEM is reported in De Smet (1998).

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THE APPLICATION OF ELECTRON MICROSCOPIES TO THE ENVIRONMENTAL BIOMONITORING: A BENTHIC FORAMINIFERAL PERSPECTIVE

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Benthic foraminifera, single-celled eukaryotes, have been proven to be suitable and reliable proxies of pollution impacts in marine and transitional marine environments (1). They respond to adverse ecological conditions including pollution by changing assemblage compositions and parameters (diversity and density) as well as cellular ultrastructure. Although major advances have been achieved over 50-60 years, we are still far from fully understanding the benthic foraminiferal response, particularly at ultrastructure level, to pollution. On the basis of mesocosms' experiments with lead and mercury coupled with techniques of electron microscopes (transmission electron microscopy and environmental scanning electron microscopy associated with energy dispersive spectrometer), it was possible to identify changes in the ultrastructure of *Ammonia parkinsoniana* (2,3). An increase of lipid droplets characterized by a more electron-dense core, proliferation of residual bodies, a thickening of the organic lining, mitochondrial degeneration, autophagosome proliferation and the development of inorganic aggregates are among the main cytological alterations of benthic foraminifera in response to pollutant exposure. Interestingly, the presence of Hg within the foraminiferal cell has been, for the first time, identified. Mercury has mainly been localized in the organic linings of the foramen/septa, at the basal part of pores and as cytoplasmic accumulations (Figure 1). All these cytological modifications might be related to the pollutant-induced stress and some of them such as the thickening of organic lining might suggest a potential mechanism of protection adopted by foraminifera.

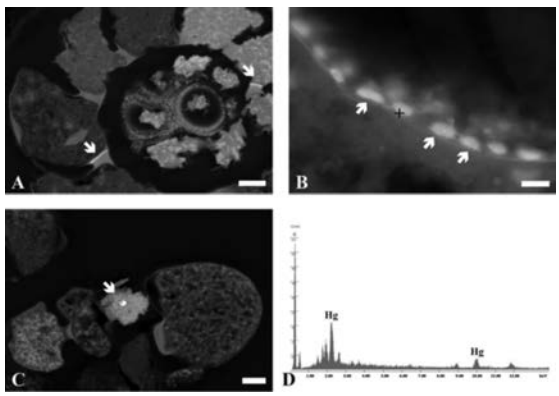

Figure 1. Hg accumulations (➘) in the organic linings of the foramen/septa (A), at the basal part of pores (B) and as cytoplasmic accumulations (C). EDS spectrum of Hg accumulation (+: point analysis at picture B) (D). Bars: A: 20 µm; B: 1.25 µm; C: 15 µm.

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ELECTRON MICROSCOPY IN THE STUDY OF THE ECOLOGICAL DIVERSITY OF SELECTED MEDICINAL SPECIES PRESERVED AT THE GHIRARDI BOTANIC GARDEN (TOSCOLANO MADERNO, BRESCIA)

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The Ghirardi Botanic Garden (GBG, Toscolano Maderno, Brescia) of the Department of Pharmaceutical Sciences of the University of Milan is part of the Network of the Botanic Gardens of Lombardy. It has a long tradition in the cultivation, study and preservation of the genetic resources of medicinal species, in accordance with the priority tasks of the Convention on Biodiversity.

The plant heritage of GBG is the core-topic of a dedicated research project (*IRIS code-2014-PDF-0363*) integrating multidisciplinary studies on target-species. The main objective of the project is the characterization of the morphological and functional complexity of these plants, "observed" as interacting, dynamic components of their own ecosystem.

Indeed, a dual perception drives the whole work program: *phytocentric*, through the study of the biotic mutualistic interactions, mediated by the emission of secondary metabolites, and *anthropocentric* with the aim of assessing their importance for humans in the therapeutic, food and cosmetic sectors.

The work program includes: *(i)* the morphological characterization of the secretory tissues, *(ii)* the analysis of the phytochemical profiles, *(iii)* the analysis of the biological activities of the secondary metabolites; *(iv)* case-studies on their ecological and functional roles. In this perspective, GBG represents a "natural lab" for studying the morphological and phytochemical diversity of the selected plants in an integrated ecological framework.

As preliminary activities of the project, we addressed our interest towards a better understanding of the micro-morphology of the secreting structures of target-species belonging to the Lamiaceae family, well-known to produce terpenoidic and phenolic substances, generally bioactive: *Lavandula dentata* L, *Salvia greggii* Grey, *Salvia blepharophylla* Brandegee, *Salvia verticillata* L, *Salvia uliginosa* Benth, *Scutellaria caucasica* A.Ham, *Scutellaria altissima* L, *Scutellaria brevibracteata* Stapf, *Teucrium chamaedrys* L.

Plant material was collected at anthesis in June 2015 and the samples were treated with the routine procedures for the micromorphological investigation using light microscopy and electron microscopy, allowing to observe: *(i)* the structure and distribution of glandular *indumentun* on both vegetative and reproductive organs, *(ii)* the histochemical nature of the secreted substances, *(iii)* the mode of production and release of the secretory products.

Indeed these structures, sites of the synthesis of bioactive compounds, are key-topics in the scientific research field, in viewing of a possible exploitation by humans.

The glandular *indumentun* displayed both peltate and capitate trichomes; four main morphotypes have been recognized (Figures 1 and 2):

- type A, present on leaves and inflorescences of all of the species, is a typical peltate hairs. It is constituted by a basal epidermal cell, a neck-cell and by a multicellular glandular head surrounded by a large subcuticular space in which the secretion is stored. In *Lavandula dentata*, the secretion is exclusively composed by terpenes, but in the other species its composition is more complex. Indeed, the responses to all the lipophilic stainings are positive as well as to Ruthenium Red and $AlCl_3$, indicating the presence of terpenes and also of major polysaccharidic and flavonoidic fractions. The ultrastructure of the active secreting cells confirms the results of the histochemical survey: the presence of both lipophilic and hydrophilic components appear as osmiophilic droplets immersed in an abundant granular matrix. The secreted material is released after cuticle rupture.
- type B is a short capitate hair widespread on both the vegetative and the reproductive organs of all the examined species. It is constituted by a basal epidermal cell, a neck-stalk cell and by a glandular head of 2-4 cells surrounded by a thin subcuticular space. Generally these trichomes present an exclusive polysaccharide secretion released through the intact cuticle. The ultrastructural observations confirm the histochemical results.
- type C is a long capitate trichomes observed only on *Salvia* species. It is composed by 1-2 epidermal cells, 2 stalk cells, 1 neck cell and by a globose head of 1-2 secretory cells surrounded by a storing chamber. The secretion proved positive to all the lipophilic stainings, particularly to the Nadi reagent, indicating that they are exclusive terpene producers. The cytoplasm of the secreting cells is rich in plastids containing starch granules and a well-developed smooth endoplasmic

reticulum, cellular compartments typical of a terpenoidic secretion.

- type D is a long capitate trichomes observed only on the inflorescences of *Scutellaria* species. It is composed by 2 epidermal cells, 2-4 stalk cells, 1 neck cell and by 8 up to 18-20 secretory cells. Each secretory cells presents a small subcuticular space at the apex. The secretion is characterized by a complex composition, positive to lipophilic and hydrophilic dyes. Therefore, the secretory products are constituted of polysaccharides, terpenes and polyphenols. On the apex of each secreting cell a small portion of the cuticle layer raises, originating a small chamber in which electrondense fibrillar or granular material is stored. Occasionally some prearranged openings for the release of the secretion were observed during SEM investigation, but most part of the secreting material seems to be extruded through the outer periclinal wall.

These highly-specialized secretory structures are characterized by different types of secretions: type B trichomes produce and release exclusively polysaccharides in all of the species; type C hairs in *Salvia* and type A hairs in *Lavandula dentata* are exclusive terpene producers. On the contrary, in all of the other species the secretion of type A, as well as type D trichomes in *Scutellaria*, is characterized by a complex mixture of both hydrophilic and lipophilic substances.

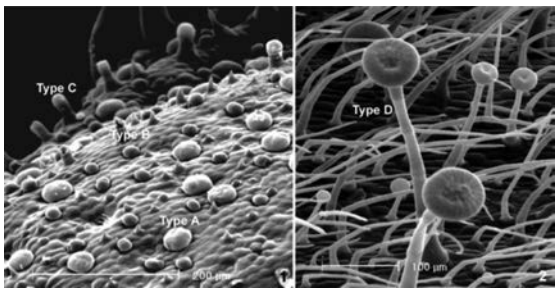

Figure 1. Glandular trichomes on the calyx abaxial side of *Salvia greggii*: peltate type A, type b short capitate, type C long capitate.
Figure 2. Type D long capitate trichomes on the calyx abaxial side of *Scutellaria altissima*.

The micromorphological diversity of the target-species was compared with their phytochemical profiles, through the analysis of the volatile organic compounds (VOCs) emitted from both leaves and flowers. The results indicated that distinctive volatilomes characterize each species, presumably determining heterogeneous spectra of visitors and/or effective pollinators. In addition, each species exhibited divergent vegetative and floral bouquets, probably crucial in acting different functional roles.

With the aim of combining these data with ecological information, we also performed some preliminary observations on the spectrum of insect visitors. These data, in combination with the micromorphological and phytochemical diversity, enabled us to raise some intriguing considerations about the adoption of different pollination strategies across the target-species.

SEM/EDX AND ESEM/EDX ON PLANT SAMPLES FOR METALS AND SEMIMETALS ANALYSIS

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Biological samples are non-compatible with the standard operational requirement of Scanning Electron Microscopy or transmission electron microscopy, that are high vacuum and electrons conductivity. For this reason they undergo several steps of "sample preparation" in order to achieve the best possible state to be inspected using electron beams. The more wide spread preparation techniques are: cryo drying followed by freeze etching and C (or Au) vacuum coating and chemical procedure consisting of fixation, staining, dehydration, resin embedding, sections cutting and coating with C (or Au) [(1)]. However physical and chemical interactions with the sample may lead to artifacts and loss of features. In the case when microanalysis is to be applied in combination with Scanning electron microscopy, most sample preparations strategies are counterproductive because they remove the original biological molecules and substitutes with other that are chemically analogous, but containing different atomic species. The starting elemental content is no longer available for the X-ray acquisition. When both the morphology and the elemental content are to be analyzed a gentler sample treatment is required. In the cases reviewed here, the scopes were: the elemental mapping of plant micro, macro-nutrients and contaminants to highlight organs and tissue distribution, to achieve a semi-quantitative measure of the most important elements, to correlate the presence of specific elements with morphological traits at the level of cell structure. This led us to the application of a simple, but effective sample preparation protocol: 0.5 - 1 mm cross-sections were cut from roots and stems, leaf were left untouched; drying at room temperature under fume hood. Samples with the smoothest surface were chosen after optic microscopy inspection, mounted on SEM slides or stubs using carbon

tape, vacuum coated with colloidal graphite. By means of this procedure the surface topography of the plant sections and of the leaf can be analyzed for both morphology using SEM and elements abundance using EDX. The analyses were performed under high vacuum with a Jeol 6400 Scanning Electron Microscope equipped with an Oxford X-ray Si(Li) detector with a Be window. Excitation voltage was set at 20 kV, ideal for heavy metals and metalloids K , and L , emission lines; the working distance was 12-14 mm, and the effective acquisition time for one scan was 60 s plus a lag time of 20 - 25%. The penetration depth, considering the above parameters and an average sample density of 1.5 - 2 g cm^{-3}, was calculated to be 5 mm for the thickest tissues, rising to 5.5 mm for the less dense ones. The diameter of the electron beam, the most important factor for the quality of the horizontal resolution, was estimated at 1 mm for the 1000x magnification. These parameters were verified to be conserved though the different experiments. With the support of the LINK-ISIS, after 2007 INCA, software to implement the Oxford microprobe, two main categories of X-ray emission-based analyses were available. Point semi-quantitative analyses, using internal standards, and dot maps of elements of interest for some samples with a particularly uniform surface. Background subtraction and spectra deconvolution were automatically performed by the software through its internal standards databank.

Metal-hyperaccumulator plants constitute a cornerstone In the study of plant-metals interaction. According to the original definition, these are "terrestrial higher plants which arc able to accumulate metallic elements in their dry matter to an exceptional degree" without showing symptoms of toxicity [2]. Among these *Alyssum bertolonii*, growing in the Tuscany (Italy), was reported to contain an extraordinarily high Ni content in the shoot dry matter, above 10 000 μg g^{-1}. *A. bertolonii* and its cogener non-hyperaccumular *A. montanum*, growing on the same serpentine site, were analyzed for metals concentration and localization using SEM/EDX to acquire dot-maps of elements distribution within roots, shoots and leaves, and to perform a semi-quantitative analysis on the same organs. In *A. bertolonii*, a specific pattern of Ni distribution was detected, highest concentrations in parenchyma and sclerenchyma for the roots; in the stem epidermis for the shoots, and the leaf trichome base. This distribution was not observed in *A. montanum*. Other mineral nutrients, Mg, Ca, K, Fe, instead, had a similar distribution in the two species [3].

Populus spp. are widely used for the commercial production of paper, pulp, and wood, they constitute a fundamental ecological resource, and physiology, phenology and genetics of a number of species in this genus have been thoroughly investigated. The Western poplar (*P. trichocarpa*) in particular has been adopted as a model tree species and its genome has been fully sequenced [3]. The ability of several poplar clones to take-up Cd, its distribution and interaction with other elements, morphological changes associated with Cd content have been investigated using SEM/EDX. This was instrumental to associate plants physiological response to Cd with their genetic background, evaluated as SNPs level in target genes, and the proteome ensuing from Cd treatments. Cd tolerant clones shared a common increase in stem tissues lignification and suberization, these tissues were used for Cd sequestration [4, 5].

Tomato is a major horticultural crop in both Europe and the US, but its fruit can be compromised by As contamination. Feeding of Si to tomato crops has been used to alleviate drought and salinity stress, but to date no attempt has been made to correlate Si supplementation with As uptake and its translocation to the aerial part of the plant, however Si treatment influenced As uptake in a strongly cultivar-dependent way. SEM/EDX dot maps of As, Si, and other nutrients, distribution within tomato organs cross sections were acquired to correlate the elements tissue distribution with the type of treatment and with the different cultivar types and genetic background [6].

To overcome the drawbacks of high vacuum SEM/EDX, new technical solutions for the scanning microscope set-up have been investigated, so far the most successful is the environmental SEM (ESEM), where a low vacuum is maintained in the sample column, thanks to new-conception detectors scattered and backscattered electrons are acquired for imaging and, at the same time, a standard EDX detector can acquire the elements X-ray spectra. This technique is particularly useful to analyze fragile in vitro grown plants. In this case the delicate model plant *A. thaliana* was mounted on the microscope stub as fresh, morphology aberrations and elemental content were investigated, then associated with particular growth regimes. The experiments were performed using a ESEM FEG2500 FEI, operating in low-vacuum, the LFD (large field detector) allowed optimal SE imaging, while the cone PLA 500μm improved the signal available to the Bruker X-ray detector (manuscript in preparation)

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ADVANTAGES IN SEM USE DURING ENVIRONMENTAL REMEDIATION

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INAIL scope is workers protection and assistance from any kind of damage resulting from work related accidents and occupational diseases freeing employers from any civil liability. INAIL Department of Production Plants and Human Settlements (DIT) in order to protect the health and environmental safety in conjunction with the presence of production facilities and human settlements realizes environmental investigations, evaluates safety and reliability of systems, proceeds to the overall risk assessment, measures and assess the physical and chemical hazards in air, water and soils, gives instructions in adverse events or emergency situations, identify possible remedies and corrective actions for remediation activities in contaminated sites, identifies best available techniques and procedures for the reduction of impacts on the territory, evaluates new and emerging risks related to the use of biotechnology in industry and agriculture, environmental microbiology, bioremediation, biotreatment of wastewater, etc..

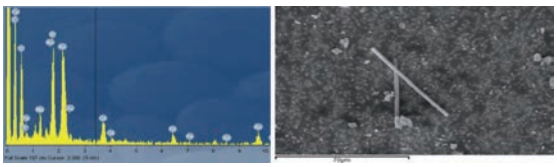
To provide adequate technical and scientific contribution on asbestos problems, DIT created a specific Group focused on Asbestos risk to work, on health and environmental asbestos legislation, prevention and safety plans, training and information on asbestos risk, asbestos mapping, asbestos risk assessment, asbestos monitoring, planning and evaluation of remediation projects, naturally occuring asbestos, asbestos contaminated soils, asbestos waste and contaminated leachate. This Group works on applied researches for the identification and mapping of contaminated sites in Italy and to create specific operational protocols. These, for the safety of operators engaged in environmental remediation work and to protect surrounding living environments. The Group, moreover, manage three laboratories for analysis of environmental matrix (two mobile laboratories) to make sampling and analysis, often on-site. These are equipped with SEM, Gas-mass, PCOM-PLM Microscopes, airborne samplers, and all the equipments for preparation for on-site analysis (vaporization, filtration, sputtering, balances, cupboards, etc.) and a meteo-climatic control unit.


LAMA 1 LAMA 3 out-side LAMA 3 in-side (portable SEM)

The importance of the use of such equipments, and in particular SEM on vehicle, is in:
1. on-site inspections;
2. immediacy of sampling and analytical response;
3. quality of the analytical response (on-site certification by a Public Body);
4. possibility of elaborate on-site immediate and long term risk reduction methodologies;
5. timely technical and scientific assistance during joint inspections with local Environmental and Health Authorities and Environmental Police;
6. action to deter illegal activity or mismanagement of emergency responses, environmental remediation and restoration of contaminated sites.
7. environmental and personal samplings during remediation different steps.
8. restituibility certification for site re-use (compulsory by SEM in Italian law).

Considering that in Italy there are 40 Superfund requiring remediation, for about 3% of the national territory distributed in all Italian regions, with different risk factors for human health and the environment, it is clear the importance of the use of such laboratories and in particular of SEM for analytical detection. A practical example is the Biancavilla case. Biancavilla Etnea (Catania, Sicily, Italy) is located on south-western side of the Etna volcano and in 1999 were noted an anomalous range of asbestos related diseases (~ 17%). Specific monitoring campaign realized by INAIL, ISS, University of Catania, ARPA Sicilia, etc, using SEM have shown that the pollutant is the mineral Fluoro-edenite, with fibres very thin (less than 1 μm in diameter) and relatively long (up to 50-80 μm). The main source of pollution turned out to be two breccia quarrying sites and all municipality soil, analyzed by SEM, was polluted at variable

concentrations. INAIL realized an early environmental investigation in urban areas, detecting very high concentration of Fluoro-edenite fibers (60-80f/l detected - threshold limit =1 f/l) in the quarries and the constant presence of airborne Fluoro-edenite all over the urban area.



Hence safety measures were implemented as: closing down quarrying sites and paving dirt roads, disposal of filling material, landfilling debris deriving from urbanization works and the excavation of metropolitan tunnel, special precautions during street sweeping, replacing the sweeper in use with asbestos specific equipment, all wet cleaning operations also at home, resurfacing the area of the contaminated sports ground with grassy layer, etc. After the adoption of the above-mentioned measures, to assess their effectiveness, INAIL carried out an additional monitoring by SEM to evaluate the airborne concentration in various urban neighborhoods. The analytical results confirmed the presence in all samples of Fluoro-edenite fibres in the urban area, but this time never exceeding the threshold value for urban environments of 1f/l as set by the WHO document "Air Quality Guidelines for Europe". So this paper highlights the important role of SEM use during monitoring campaigns carried out to identify, firstly the contamination source, and secondly, the most adequate remediation actions to adopt in contaminated sites.

NEMATODI MARINI: ADATTAMENTI E STRATEGIE DI SOPRAVVIVENZA ALL'AMBIENTE

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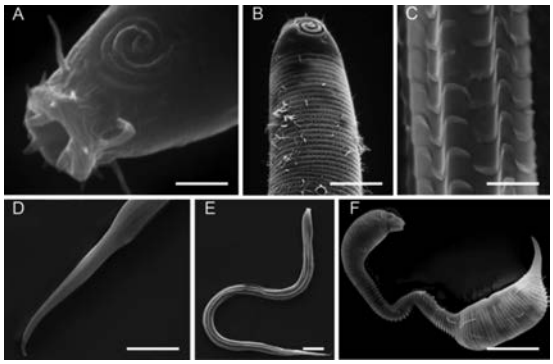
La granulometria del sedimento è un fattore chiave nel determinare la struttura e la biodiversità delle comunità meiobentoniche e dei nematodi [1-2]. Pertanto, un'analisi statistica è stata effettuata su un set di campioni raccolti in due crociere scientifiche (2005 e 2007) nell'arcipelago maldiviano. In quest'occasione abbiamo testato le possibili variazioni di alcuni caratteri morfologici e funzionali dei nematodi (es. cavità boccale, forma dell'anfidio, ornamentazione della cuticola, forma della coda, e strategia di vita) a variazioni nella granulometria dei sedimenti e a diverse condizioni idrodinamiche (A-F). Nella crociera del 2005, sono stati analizzati i possibili cambiamenti di caratteri morfo-funzionali in nematodi di sabbie da fini a molto grossolane: tutti i caratteri sono risultati significativamente differenti. In particolare, le sabbie medio-grossolane sono apparse quelle a più elevata diversità sia tassonomica che morfo-funzionale. L'ornamentazione della cuticola e la strategia di vita erano ben diversificate anche in sabbie molto grossolane, mentre la forma della coda lo era solo in sedimenti fini. Questi dati contribuiscono a sottolineare quanto le sabbie medio-grossolane siano ricche di nicchie ecologiche per gli organismi della meiofauna, che non solo risultano qui più diversificati dal punto di vista tassonomico[3], ma anche per aspetto morfologico e strategia adattativa.



Osservazione al SEM dei tratti morfo-funzionali considerati in questo studio: A, apparato boccale del genere Dorylaimopsis; B, anfidio multispiralato del genere Desmodora; C, cuticola ad anelli orizzontali con creste longitudinali del genere Ceramonema; D, coda del genere Halalaimus; E, habitus del genere Sabatieria a strategia-r; F, habitus del genere Metepsilonema a strategia-k. Barre: A = 2,5 µm; B =28 µm; C = 10 µm; D = 14 µm; E = 60 µm; F = 30 µm.

Quando gli stessi tratti biologici sono stati analizzati per verificare l'eventuale influenza di diversi livelli di idrodinamismo, ad eccezione della forma della coda, tutti hanno mostrato significative differenze. In particolare, il livello intermedio di energia è quello che è risultato correlato alla maggiore diversità: soltanto la forma della coda è apparsa più diversificata nel transetto caratterizzato dal livello di energia più basso. Questo confermerebbe anche la diversità tassonomica rilevata, e sarebbe in linea con la capacità dei nematodi di adattarsi ad un disturbo fisico di limitata intensità che anzi può favorirne la diversità (si veda la

teoria del disturbo intermedio di Connell e Slatyer, [4]). In conclusione, sabbie medio-grossolane e habitat con livello di energia intermedio sembrano favorire non solo un'ampia diversità tassonomica dei nematodi, ma anche una grande diversità di adattamenti morfologici e funzionali che riflette la loro grande eterogeneità in micro-nicchie.

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ULTRASTRUCTURAL APPROACH IN ENVIRONMENTAL MONITORING OF PHYTOPLANKTON, MICROPHYTOBENTHOS AND TOXIC MICROALGAE

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Algae are a heterogeneous group of organisms that accomplished their evolutive history in aquatic environment. These organisms live in a variety of aquatic habitats (e.g, seawater, lakes, rivers, saltmarshes, ice, thermal waters) even if some occur in terrestrial ones (e.g, soil, barks etc.). Some algal species can also establish important symbiotic relationships with other organisms.

The study of marine microalgae (i.e, unicellular microscopic algae) for monitoring purposes is traditionally carried out through light microscopy (LM), as it is a fast and low-cost method. However, the structural details that can be resolved through LM often do not allow their identification neither at the species level nor at higher taxonomical ranks.

Significant progresses have been achieved in the study of algal cells with the coming of electron microscopy era as new ultrastructural details have been observed. Therefore, a number of new species have been described while others have been revised and their taxonomic position emended. Nowadays, modern systematics cannot disregard a deep ultrastructural analysis (associated with molecular analysis of gene sequences as well).

The capability to characterize a microalgal population at the species level is important for a number of ecologically significant reasons: some species could be used as indicators of climate changes, some others may have been introduced by anthropic activities (i.e, alien species), and some are toxin producers, with heavy impact in human and ecosystem health.

The scanning electron microscopy observation can be successfully applied to the study both of microalgae having (e.g, diatoms, thecate dinoflagellates, coccolithophyceans, or lacking (e.g, naked dinoflagellates, cryptophytes) a rigid cell wall.

The Critical Point Drying (CPD) preparation method allows the conservation of the organic structures and can be useful to observe the structure of the microalgal community, as it was originally arranged *in vivo*. This method has been used for studying both the epiphytic and the epizoic communities. Totti *et al.* (1) investigated the epiphytic diatoms on several Icelandic seaweeds, highlighting that highly branched seaweeds with articulated showed a high level of colonization, while flat thalli with smooth surface mainly allowed the growth of erect diatoms. Regarding the epizoic microalgae, Bavestrello *et al.* (2) studied the relationships between benthic diatoms and hydrozoans, highlighting that hydroid colonies represent a mosaic of different microhabitats: in some species, each colony portion hosts different diatom species. This approach can also allow ecological studies, as algae can be identified and counted: the annual cycle of microphytobenthos community on *Eudendrium racemosum* showed that diatoms peaks occur in winter-spring, while cyanobacteria peaks in summer (3). The same approach has been applied to study the relationships between diatoms and Antarctic sponges: the diatom *Porannulus contentus* has been observed only in association with some sponge species where it form monospecific populations (4), while a diversified and complex community dominated by the diatom *Hyalodiscus* sp. live in association with the sponge *Sphaerotylus antarcticus* (5).

In the study of diatom systematics, and/or for a correct diatom identification, cleaning preparation methods are required to remove the organic component from the diatom frustule, allowing the observation of its siliceous nanostructures. New diatom species of both phytoplankton and microphytobenthos are currently described using such morphological ultrastructural approach (e.g, [6]). A number of phytoplankton studies used the SEM

ultrastructural approach to revise some important genera, such as the planktonic diatom *Skeletonema*, for which a number of new species have been described [7], allowing to define the actual biogeographic distribution of one of the most important planktonic genera [8].

The taxonomical resolution through EM appears particularly important whenever it is focused on potentially toxic taxa. For example, Lundholm *et al.* [9] resolved the taxonomy of *Pseudo-nitzschia pseudodelicatissima* complex highlighting that it included four species (among which only two produce domoic acid - Amnesic Shellfish Poisoning) identifiable only by the *striae* details. The toxic *Alexandrium minutum* (involved in Paralytic Shellfish Poisoning) is distinguishable from the non-toxic *A. tamutum* only for the details of the plate tabulation [10]. The EM was applied even to the studies on the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*, to define both morphometric [11] and morphological details [12].

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STUDIO ULTRASTRUTTURALE DEGLI EFFETTI SUGLI APPARATI IPO ED EPIGEI DELLA PIANTA IN RISPOSTA AD APPROCCI AGRONOMICI ECO-SOSTENIBILI

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L'applicazione della microscopia a scansione elettronica per studi ultrastrutturali nel settore agronomico sono di estremo interesse, soprattutto al fine di identificare strategie per limitare l'uso degli input chimici per la fertilizzazione, la protezione dalle fisiopatie e fisiopatologie, nonché il contenimento delle erbe infestanti in agricoltura. Si riportano di seguito alcuni esempi di ricerca che hanno previsto l'applicazione della microscopia elettronica in pressione variabile (30-10 Pa) su campioni vegetali. Lo strumento utilizzato è stato il microscopio EVO-MA 10 (Zeiss), equipaggiato con Beem-Sleeve Technology e lampada LaB_6 ad alta brillanza, in grado di garantire elevate prestazioni analitiche, senza alcun pre-trattamento del campione biologico.

Tecniche di innesto delle ortive per fronteggiare le fisiopatologie - Una delle possibili strategie per fronteggiare le fisiopatologie dei alcuni vegetali si basa sull'uso dell'innesto, ossia sulla selezione di portinnesti resistenti al patogeno, in grado di trasferire tale resistenza anche all'innesto. In tal caso, la microscopia SEM in pressione variabile è stata utilizzata per uno studio sulla compatibilità istologica innesto/portinnesto tra il carciofo [innesto : *Cynara cardunculus* L. var. *scolymus* L.), cv. Romolo, R] ed il cardo (portinnesto: *Cynara cardunculus* subsp. *cardunculus*) coltivato (cv. Belgio, CC) e selvatico (cv. Sardo, WC), con l'obiettivo di ridurre la sensibilità del carciofo alla verticillosi. Tale studio ha permesso di identificare le combinazioni di innesto più promettenti attraverso la valutazione dell'evoluzione della unione

d'innesto all'interfaccia tra innesto/portinnesto, lo sviluppo del callo e la formazione di una completa vascolarizzazione (Trinchera *et al.*, 2013). Inoltre, è stato possibile evidenziare la formazione di desmotubuli contenenti plasmodesmata, quale primordi di connessione tra il portinnesto e l'innesto, mai rilevati nella combinazione cardo-carciofo (Figura 1, A-F), nonché la presenza di materiale pectinico sulle cellule dei tessuti all'interfaccia dell'innesto, in grado di favorire l'adesione fra i due bionti ed evitare la disidratazione dei tessuti durante l'innesto (Figura 1, G-L).

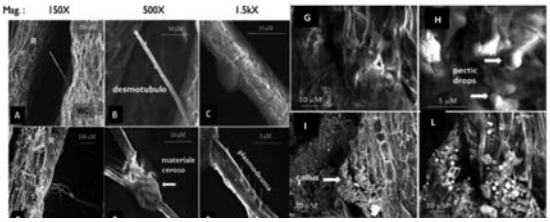

Figura 1. Desmotubulo e plasmodesma all'interfaccia dell'innesto tra carciofo (R) e cardo (WC e CC) (A-F); gocce di materiale pectico sulla superficie di innesto (G-L) (Detectors: VPSE e BSE; Chamber pressure: 20 Pa; Source: LaB6; Mag.: A, D: 150X; B, E: 500X; C, F: 1.5 kX ; I: 1.0 kX; G, L: 2.0 kX; H: 2,5 kX) .

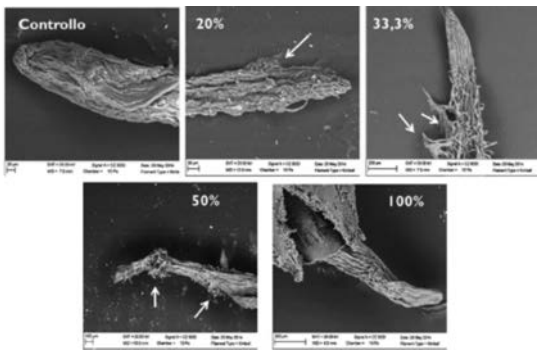

Figura 2. Effetto dell'apporto di estratti di segale a concentrazioni crescenti (0 - Controllo, 20, 33,3, 50 e 100% v/v) sullo sviluppo radicale del romice (Detector: BSE; Chamber pressure: 20 Pa; Source: LaB_6, Mag: 100-250X).

Effetto dell'uso di colture di copertura sullo sviluppo delle infestanti - Un altro esempio interessante di applicazione della microscopia SEM è costituito da studi sugli effetti allelopatici di piante cerealicole, ai fini del contenimento delle infestanti attraverso metodi compatibili con l'agricoltura biologica. Specificatamente, attraverso l'analisi SEM in pressione variabile, è stato valutato nella prima fase di germinazione l'effetto dell'apporto di estratti allelopatici di segale (*Secale cereale* L.) sullo sviluppo radicale di una infestante target tipico dell'area mediterranea, il romice (*Rumex crispus* L.), simulando quanto si verifica in campo. I risultati hanno mostrato come l'apporto di estratti a concentrazione crescente (dil.: 0, 20%, 33,3 %, 50% e 100% v/v estratto in acqua) comporti una progressiva riduzione dello sviluppo della radichetta, fino alla degenerazione tissutale (al 50%) e alla completa inibizione di crescita quando applicati senza alcuna diluizione (al 100%) (Figura 2).

Effetto della gestione agronomica sulla micorrizazione radicale - Un'ultima esperienza riguarda l'applicazione della microscopia elettronica all'uso della pacciamatura verde in agricoltura biologica ed i suoi effetti sulla micorrizazione radicale del carciofo. Nello specifico, in questo caso sono stati confrontati apparati radicali di piante di carciofo [*Cynara cardunculus* L. var. *scolymus* (L.), cv. Jesino, Je] cresciute in presenza di pacciamatura verde (mix di erbe, selezionato, LM) e gli stessi apparati radicali di carciofo, cresciuti in assenza di pacciamatura (in presenza di erbe spontanee, no LM) studiandone lo sviluppo e l'entità della simbiosi micorrizica come positivi effetti della pacciamatura. Lo studio, che ha previsto campionamenti di campo biennali e analisi delle radici fresche in SEM-VP, hanno rivelato come la pacciamatura verde (Je LM) induca un incremento nello sviluppo di peli radicali ed un netto miglioramento nella simbiosi micorrizica rispetto al controllo (Je no LM), con indubbi vantaggi da parte del carciofo in merito alla sua capacità di assimilare il fosforo (Figura 3).

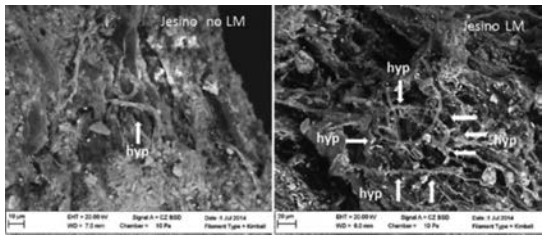

Figura 3. Ife micorriziche (hyp) di radici di carciofo (cv. Jesino), in assenza (no Je LM) e presenza (Je LM) di pacciamatura verde (Detector: BSE; Chamber pressure: 20 Pa; Source: LaB_6; Mag. 750X).

In conclusione, l'uso della microscopia SEM negli studi ultrastrutturali sulle colture fin qui proposti hanno permesso di individuare e teorizzare interessanti meccanismi fisiologici ed ecofisiologici, successivamente verificati anche attraverso analisi di tipo chimico, biochimico e fisiologico. Per questo, la microscopia SEM può essere considerata in taluni casi un «apripista» per lo sviluppo di ricerche innovative, soprattut-

to nel campo della eco-fisiologia vegetale mirata alla tutela ambientale.

IDENTIFICAZIONE E ANALISI DI MARKER AMBIENTALI MEDIANTE MICROSCOPIA ELETTRONICA AMBIENTALE

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Questo lavoro riporta una serie di casi di applicazione della microscopia elettronica a scansione (SEM) in problematiche ambientali, attraverso recenti ricerche condotte da diversi gruppi afferenti all'Università di Urbino, talora in collaborazione con altre università ed enti, pubblici e privati. Lo strumento utilizzato è un SEM di tipo ambientale (FEG-ESEM, Quanta 200, FEI, The Netherlands), provvisto di microsonda a raggi X (Energy Dispersive Spectroscopy EDS, EDAX, Mahwah, NJ, USA), di rilevatore per elettroni secondari (SE) e di rilevatore per elettroni retrodiffusi (BSE). Le analisi sono state condotte talora in condizioni di alto vuoto (pressioni attorno ai 10^{-6} torr), talora in condizioni di basso vuoto (pressioni fra 10^{-1} e 1 torr), oppure in modalità ESEM (pressioni fra 0,1 e 20 torr). I casi di studio riguardano tre grandi ambiti: aria, suolo e acqua.

Settore Aria

A - Un importante capitolo di questo settore riguarda la individuazione e caratterizzazione di materiali fibrosi aero-dispersi. In determinate situazioni a rischio, fra cui le bonifiche di siti, è necessario mettere in atto quelle procedure previste dalla normativa vigente allo scopo di monitorare la presenza di fibre aero-disperse, di classificarle e quantificarne la presenza per unità di volume di aria campionata. Fra le tante tipologie di fibre naturali o artificiali esistenti, è prioritario il riconoscimento di materiali fibrosi classificati come cancerogeni o come pericolosi per la salute umana, quali le fibre di amianto, le fibre ceramiche e le vetrose (Figura 1).

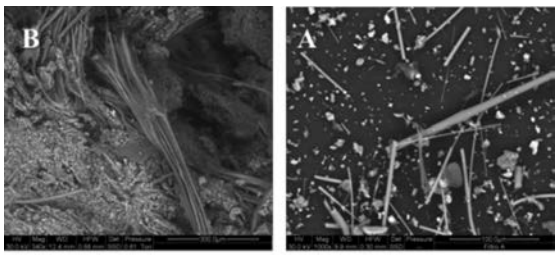

Figura 1. Fasci di fibre di crisotilo (A); fibre ceramiche e vetrose su filtro (B).

In particolare, i numerosi minerali dell'amianto (fra i più diffusi il crisotilo, la crocidolite e l'amosite), utilizzati in passato in vari ambiti (industria, edilizia, agricoltura, etc.), hanno la peculiarità di separarsi in fibre sottilissime, riconoscibili e classificabili in maniera precisa in microscopia elettronica, attraverso la combinazione fra l'analisi morfologica e l'analisi chimica in EDS.

B - I minerali dell'amianto, associati geneticamente a determinate tipologie di rocce (ofioliti e serpentiniti), sono presenti naturalmente nell'ambiente. Una recente ricerca svolta sul "Complesso di Pietra Parcellara", deposito caratterizzato dalla presenza di olistoliti con serpentiniti e brecce ofiolitiche (località La Costa, Parma), ha messo in evidenza la presenza di una fitta rete di vene riempite da minerali carbonatici. Il SEM ha permesso il riconoscimento di cristalli di calcite intimamente associati a crisotilo in fibre sottili, passando dall'aspetto massivo della calcite a quello fibroso del crisotilo (Figura 2).

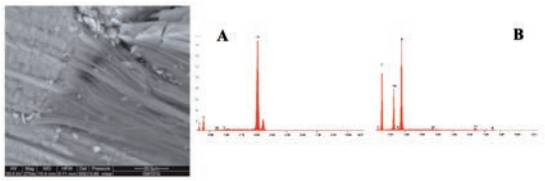

Figura 2. Passaggio dall'aspetto massivo (calcite; spettro A) a quello fibroso (crisotilo; spettro B).

Le fibre di crisotilo appaiono ben formate e come una estensione degli stessi cristalli di calcite. Una serie di analisi in EDS lungo l'interfaccia fra le fasi ha evidenziato una transizione composizionale graduale fra i due termini estremi. E' stata pertanto documentata per la prima volta la crescita di crisotilo entro vene di calcite; poiché tali vene carbonatiche sono estremamente diffuse in questo tipo di rocce, la presenza di crisotilo è un dato che potrebbe costituire un nuovo elemento di rischio ambientale [(1)].

C - Il 14 aprile 2010, in seguito ad una eruzione esplosiva del vulcano islandese Eyjafjallajokull, è stata prodotta una nube di ceneri che si è innalzata nella troposfera fino a circa 9 km e, per effetto dei venti dominanti, si è dispersa progressivamente in direzione SE. Il passaggio della nube, documentato in Norvegia, in Olanda, in Germania ed in Grecia, ha provocato numerosi disagi, fra cui il blocco per giorni del traffico aereo in Europa. Analisi granulometriche, morfologiche e composizionali svolte su campioni di particolato filtrato da acqua piovana (i campioni sono stati raccolti a Rimini nell'arco temporale 19 aprile - 11 agosto

2010), hanno testimoniato il passaggio della nube anche in Italia[2]. Nell'eterogeneo particolato su filtro, infatti, sono comparsi in un momento preciso frequenti frammenti vetrosi dalla tipica morfologia spigolosa (glass-shards) oltre a cristalli sciolti (k-feldspato e pirosseno) riconducibili anche composizionalmente alla eruzione del vulcano (Figura 3).

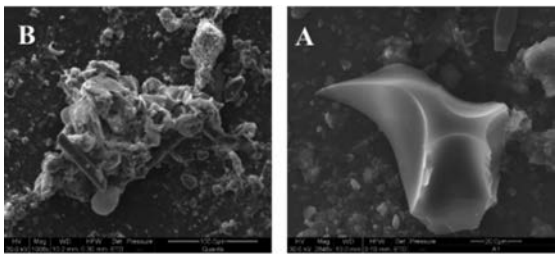

Figura 3. Aggregati particellari con cristalli (A); frammento vetroso (glass-shard; B).

D – Una interessante applicazione legata ai beni culturali riguarda una indagine in corso sulle superfici interne del giardino pensile del Palazzo Ducale di Urbino. Le cornici delle finestre sono realizzate con blocchi di arenaria (Arenaria di Sant'Ippolito) e calcare (Calcare Massiccio) e si presentano in uno stato di conservazione non ottimale essendo interessate da diverse tipologie di degrado (de-coesione, disgregazione, esfoliazione, etc.), oltre a diffusi sbiancamenti su tutta la superficie. Il campionamento di piccoli frammenti di materiale ha permesso di documentare la presenza di particelle estranee, legata a possibili trattamenti di pulitura e/o consolidamento o dovuta ad inquinamento ambientale. Frequente la presenza di particelle di: S e Ca (gesso connesso a processi di degrado da inquinanti gassosi o alla presenza di malte gessose di restauro); Ba e Sr (probabilmente dovuti ad un metodo di consolidamento utilizzato negli anni '60 per la sua azione de-solfatante e per ridurre la porosità); composti di Pb, Fe, Cr, Ti, Sn, Cu, Zn e Ni (probabilmente legati ad emissioni industriali e/o scarico degli autoveicoli; il Pb può essere legato anche a lastre di protezione ancora in parte presenti sulle cornici). Da approfondire la presenza occasionale di W, Au e terre rare.

Settore Suolo

E – È questo il caso di uno studio morfologico e chimico focalizzato sulle ghiandole digestive (hepatopancreas) di isopodi terrestri (*Porcellio scaber*) e rivolto alla valutazione di contaminanti nei suoli (Figura 4). Numerosi individui sono stati raccolti in area agricola non coltivata e presumibilmente non contaminata, di cui è stato analizzato anche il suolo. L'analisi in EDS sulle cellule di hepatopancreas ha indicato la presenza degli stessi elementi chimici rilevati nel suolo (N, P, Cd, Mn, Cu, Ni, S, Zn, Al Y, Fe, Cr e Th). Allo scopo di evidenziare la capacità di bio-accumulo di contaminanti da parte delle cellule digestive, alcuni isopodi sono stati trattati con un percolato di discarica (metalli pesanti, inquinanti inorganici, pesticidi, idrocarburi, solventi). In EDS è stata confermata la presenza di gran parte dei contaminanti, come elementi in traccia (V, Cr, Co, Ni, Cu, Zn, As, Cd, Sb e Pb), accumulati nelle cellule di hepatopancreas, sottolineando la loro capacità di assimilare e accumulare elementi naturali o inquinanti contenuti nei suoli [3].

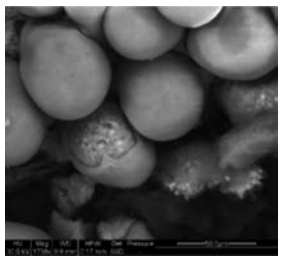

Figura 4. Cellule di hepatopancreas di isopodi terrestri (*Porcellio Scaber*).

Settore Acqua

F – Fondamentale il contributo del SEM nell'evidenziare la capacità di accumulo di metalli pesanti in foraminiferi bentonici. Immagini in BSE, accoppiate ad analisi in EDS, hanno permesso una chiara identificazione di accumuli di Hg sia a livello citoplasmatico che nei pori della superficie esterna di foraminiferi (Figura 5), testimoniando il loro ruolo di bio-indicatori in ambiente marino.

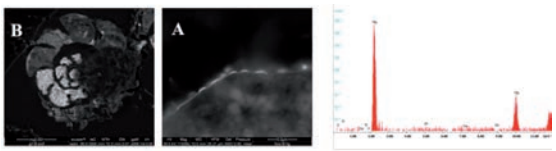

Figura 5 Cellule di hepatopancreas di isopodi terrestri (*Porcellio Scaber*).

G – Infine una ricerca che ha messo in evidenza le potenzialità del SEM anche in campo paleoambientale. Attraverso una serie di campioni di ittioliti è stata esaminata tutta la fase evaporitica (Formazione Gessoso Solfifera) legata alla crisi di salinità del Messiniano, affiorante lungo la falesia del Monte Castellaro (Pesaro). Indagate le differenze morfologiche e composizionali tra individui pachiostosici e non pachiostosici (indice di pachiostosi = diametro/lunghezza dell'apofisi) in

due specie fossili: *Aphanius crassicaudus* (Agassiz, 1839) e *Gobius* sp. (Linnaeus, 1758), entrambi pesci di laguna eurialini. Negli individui non pachiostosici è evidente la presenza di vasi sanguigni, talora interessati da un inizio di precipitazione di idrossiapatite. Tale tessuto osseo è infatti legato ad ambiente evaporitico in presenza di Mg (modulatore della formazione di idrossiapatite), il quale viene assorbito dall'individuo: l'analisi in EDS delle apofisi ne evidenzia la presenza, contrariamente a quanto accade nel sedimento inglobante che ne risulta privo. Le apofisi pichiostosiche, viceversa, sono caratterizzate da un tessuto osseo compatto e non vascolarizzato: con la precipitazione di Mg, l'individuo ne assorbe in minor misura dall'acqua, nelle apofisi non si rileva Mg, mentre compare nei sedimenti corrispondenti [(4)].

Ringraziamenti - Queste ricerche sono state svolte in collaborazione con: M. Letizia Amadori[1], Nicoletta Bedosti[1], Luciano Benini[2], Gaetano Cecchetti[1], Erica Cesarini[1], Rodolfo Coccioni[1], Davide Curzi[1], Giovanni De Falco[3], Federica Fiesoletti[4], Fabrizio Frontalini[1], Matteo Giordani[1], Pietro Gobbi[1], Anita Manti[1], Gabriele Matteucci[4], Michele Mattioli[1], Roberto Mazzeo[1], Emanuela Molinaroli[5], Stefano Papa[1], Elena Pari[4], Claudio Pizzagalli[2], Alberto Renzulli[1], Paolo Rossini[4], Riccardo Santolini[1], Pierpaolo Tentoni[4], Alessio Testoni[4], Malamati Tsiola[1] ([1]Università di Urbino; [2]Arpam, Pesaro; [3]IAMC-CNR, Oristano; [4]Istituto di Ricerca Gruppo C.S.A, Rimini; [5]Università Ca' Foscari, Venezia).

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Simposio

Nuclear structure and dynamics, through the microscopes

Pavia, 7-8 Luglio 2016
Collegio Alessandro Volta

Organizzato congiuntamente da
Società Italiana Scienze Microscopiche e Società Italiana di Istochimica

TRANSCRIPTION TIME-WINDOW APPLIED TO RNA MODIFICATIONS IN HELA CELLS

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We have applied a new method which combines the high spatial resolution of transmission electron microscopy with information on the dynamics of transcription. The incorporation of two different RNA precursors, iodo- and chlorouridine, was used to define a Time Transcription Window on cultured cells treated with hypometabolising peptides which are known to modulate transcription [1,2]. This procedure allows to detect a single fibril of newly synthesized RNA in the time range in which it is transcribed. The same fibril is finally visualized by a selective staining for RNA with terbium citrate[3]. Briefly, D-Ala2-D-Leu5 enkephalin (DADLE) is a synthetic peptide, capable of mimicking the hypometabolising action of the hibernation induction trigger, an opioid δ-receptor agonist occurring in the blood of lethargic hibernators[4] and able to induce lethargy when injected in summer active animals[5]. Similarly to the hibernation induction trigger, DADLE injection can make summer active hibernators enter lethargy[5]; moreover, once injected, this molecule can determine a transient torpor state in rats[6] and, finally, it can induce a hibernation-like state in cultured cells.[2,7] Differently from DADLE, its isomer DALE (D-Ala2-Leu5-enkephalin) is not degradable by enkephalinases; however, data on the hypometabolising effects of DALE are still preliminary[8]. These treatments represent a good model to investigate the possible role of 5mC-containing RNA, whose function is still unknown.

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DOSE-DEPENDENT TYPES OF NUCLEAR ALTERATIONS INDUCED BY ETOPOSIDE ON HUMAN LEUKEMIA CELLS

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Cytotoxic anticancer drugs at low doses (metronomic therapy) perform efficacious anti-angiogenetic activity; moreover, they exert additional direct actions against tumor cells, which are less explored. Here, we investigate the molecular nature of such actions. We performed cytofluorimetric and ultrastructural analyses on the myelocytic tumor cell line U937 treated with low dose (0.5 μM) of the topoisomerase inhibitor etoposide, compared with the cytotoxic (50 μM) dose.

At variance with the high dose, 0.5 μM etoposide promotes deep nuclear invaginations eventually resulting in granulocyte-like differentiation. The differentiation process requires cell cycle arrest via ATM-mediated DNA damage response, indicating that etoposide-induced DNA breaks are the

differentiation mediators.

Caspases are activated also by low dose etoposide; all treated cells eventually dye acquiring all the hallmarks of apoptosis, but surprisingly in a caspase-independent way (unlike 50 μM etoposide). Instead, caspase inhibition reverts differentiation: molecular analyses point to caspase 6-dependent cleavage of cyclin B1 and nuclear lamins as the events that determine granulocytic differentiation by low doses etoposide.

At the morphological level, TEM images of apoptotic cells induced by the two doses show striking differences.

Similar results were obtained in myeloid leukemia HL-60, KG1 and THP-1 cells, indicating general responsiveness. Importantly, 0.5 and 50 μM etoposide possess equal pro-apoptotic efficiency on leukemia cells, whereas it is reported that normal blood precursors are induced to apoptosis only by high doses. This indicates that metronomic doses of etoposide, unlike the cytotoxic ones, display selective killing of tumor cells, promising to successfully eliminate tumor cells with scarce side effects. These results provide for the first time to our knowledge a biological mechanism implying reprogramming (anakoinosis) of leukemia cells towards normal differentiation behavior by low-dose antitumor therapy with etoposide.

In conclusion, we have shown that metronomic doses of etoposide induce granulocytic differentiation via caspase- and DNA-damage response-dependent events, followed by "turnover" (rather that damage-induced) apoptosis, without requiring maturative cell division.

FROM MICROSCOPY TO CYTOMETRY THROUGH FLUORESCENCE: CELLULAR EVENTS AT THE NUCLEAR LEVEL

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Confocal Laser Scanning Microscopy constitutes a powerful tool to analyse the intracellular location of various components through the high-resolution optical imaging and the three-dimensional reconstruction. There are a large number of DNA-specific fluorescent dyes, and each of these has individual characteristics (binding affinity, specificity for DNA or other macromolecules and wavelength of excitation and emission). Some nucleic acid fluorescent probes are able to enter live cells to visualize nucleus and DNA-containing organelles, and can be used as vital stains[1,2]. Other DNA stains diffuse through the cell membrane as a marker to identify the double-stranded DNA breaks in dead cells[3].

Flow cytometry (FC) is a biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering and can be easily used to analyse nucleic acids and chromatin compaction, important elements in cell differentiation and pyknosis of nuclei. Furthermore, one of the most useful cytometric approach is the evaluation of DNA content and cell cycle analyses, by means of several probes[4]. Nuclear fluorochromes can be applied to indirectly measure ROS levels and membrane permeability[5]. Finally, FC offers the possibility of rapid enumeration of parasitemia. It relies on staining the parasite DNA to distinguish between infected and non-infected red blood cell populations. In biological research and medical diagnosis, volume, shape, DNA content, and chromatin pattern of nuclei it can be important for the diagnosis and prognostic impact of many cancers. Some models are here presented to demonstrate the importance of microscopy and flow cytometry in highlighting nuclear domains.

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EFFECT OF ADAPTED PHYSICAL EXERCISE ON THE FINE DISTRIBUTION OF PAX7 AND MYOD IN MYONUCLEI OF OLD MICE

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Satellite cells (SCs) are mitotically quiescent cells located between the basal lamina and the sarcolemma of myofibers[1]. Following injury, muscle tissue may regenerate through the activation of SCs that first proliferate and then differentiate into myocytes which fuse either together or with pre-existing myofibers. Activation, proliferation and differentiation of SCs may undergo alteration during ageing[2,3] with a consequent reduction in

the efficacy of muscle renewal. In fact, during ageing a progressive decline of muscle mass, strength and quality take place, a condition termed sarcopenia[4]. Although no specific therapy is presently available to counteract the onset and progression of sarcopenia, it has been demonstrated that physical exercise may efficiently mitigate the age-related muscle atrophy. In particular, we demonstrated that an adapted aerobic physical exercise (treadmill running) leads to the reactivation of nuclear activity, increasing transcriptional and post-transcriptional processes in both myofibers and SCs of old skeletal muscles[5,6].

The process of SC activation is finely regulated by the expression of specific factors, among which the paired box protein 7 (Pax7) and the myogenic differentiation factor D (MyoD). Although largely investigated in SC nuclei, the distribution and function of Pax7 and MyoD in myonuclei are poorly known. We have therefore focused our attention on the possible effects that age-related atrophy as well as exercise-related nuclear reactivation may induce on the fine distribution and relative amounts of Pax7 and MyoD in myonuclei of old mice. Our results shed light on the possible functional role played by Pax7 and MyoD in the myonuclear response to physical exercise and, more generally, in skeletal myofiber regeneration.

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HISTOCHEMICAL STUDY OF MYOCARDIAL ISCHAEMIC TISSUE IN ADVANCED PUTREFACTION: PRELIMINARY RESULTS

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Histologic diagnosis in forensic context it's often hampered by autolysis and putrefaction. Specimens from autopsies performed on exhumed cadavers or on bodies in advanced state of decomposition render recognition of pathological alterations quite difficult. Moreover tissue decomposition can sometimes simulates non-existent histopathological processes[1]. In forensic pathology practice it is common to encounter heavily putrefied bodies. In these cases to prove the possible presence of an acute myocardial infarction as the cause of death is very important. Moreover, the appearance of myocardial ischemia can be masked or even imitated by autolysis and putrefaction[2].

The use of hematoxylin-eosin (HE) stain is not sufficient to investigate decomposed myocardial tissue[3]. The use of histochemical techniques could help in the histopathological analysis of this type of material to increase the diagnostic specificity[4]. The purpose of this research is the evaluation of the efficacy of histochemical stains to identify ischemic areas in the putrefied myocardial tissue.

Heart tissue specimens was taken from eight cases of macroscopically evident acute myocardial infarction (AMI) during diagnostic autopsies. Specimens was obtained from an area containing ischemic and non-ischemic myocardium. One tissue fragment was immediately fixed in a 10% buffered formalin and used as control. Specimens from AMI were placed in an open case and stored at controlled room temperature, ranging between 16 °C and 20 °C. At time interval of 15 and 30 days of putrefaction the samples of AMI tissues were fixed 42 hours in 10% formalin, processed and embedded in paraffin. Sections of 4µ were cut from paraffin blocks and stained with standard HE and Mallory trichrome stain.

Preliminary results showed in all cases: after 15 days of putrefaction HE stains was no longer able to detect AMI areas. Instead, after 30 days of decomposition, Mallory trichrome showed strongly positive staining of non-ischemic cardiac fibers (red colored), while ischemic myocardium was very less intense.

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HIGHLIGHTING THE APOPTOTIC NUCLEUS: THE PRECIOUS CONTRIBUTION OF THE ELECTRON MICROSCOPE

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Apoptosis is a peculiar form of physiological, genetically controlled cell death, characterized by an intriguing and exclusive cell behavior.(1) Apoptotic chromatin investigations took great advantage by freeze-fracture techniques, which allowed the identification of novel types of chromatin fibers in the complex scenario of apoptotic nucleus.(2) On the other hand, cytochemical approaches revealed the correlation between nuclear domain changes and DNA role. When analysed at TEM, apoptotic chromatin clusters at nuclear periphery, forming cup-shaped dense masses and determining a characteristic nuclear pore translocation.(3) Micronuclei progressively appear throughout the cytoplasm and are finally released into the extracellular space. Secondary necrosis represents a frequent final fate or, when possible, apoptotic cells are engulfed by circulating macrophages. The underlying machinery is not yet completely understood, even if an extrinsic pathway ,or, more frequently, an intrinsic, mitochondria-mediated one , are commonly accepted. DNA cleavage, in oligo-nucleosomic or larger fragments(4) occurs. This has been widely demonstrated *in vitro*, by electrophoretic techniques and *in situ*, by TUNEL reaction, successively more detailed by TUNEL/TEM, associating gold particles to DNA breaks.(5) These patterns have been demonstrated in a variety of cell models, even long considered resistant, in response to different stimuli.(6,7) *In vitro* models of skeletal muscle cells, human chondrocytes and keratinocytes have been studied by a multiple approach(8), and a common project in apoptotic cell death can be identified, which could be then considered also a target for potential anti-apoptotic agents.(9,10)

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DYNAMICS AND REGULATORY MECHANISMS INVOLVED IN IMMATURE OOCYTE CHROMATIN REMODELING

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During meiotic arrest, and particularly during the oocyte growth phase leading to the formation of fully-grown oocyte, the chromatin enclosed within the oocyte nucleus, also known as Germinal Vesicle (GV), is subjected to several levels of regulation controlling both its structure and function. These events include mechanisms acting both locally, on specific loci, and on a large scale to remodel wide portions of the oocyte genome. Morphologically, the chromosomes lose their individuality as well as their characteristic appearance and form a loose chromatin mass, which in turn undergoes profound and dynamic rearrangements within the GV before the meiotic resumption. These 'large-scale chromatin configuration changes' are temporally correlated with the process of transcriptional silencing in the oocyte nucleus as well as with epigenetic modifications such as histone tail modifications and changes in the global level of DNA methylation. Moreover, chromatin configuration rearrangements are tightly associated with the acquisition of meiotic and developmental competence. The molecular mechanisms governing changes in large-scale chromatin configuration still remain largely unknown. Most likely, strategies set in place for the control and coordination of these events are part of a complex physiological process that ultimately confers the oocyte with meiotic and developmental competence. Here, we summarize some studies intended to explain the mechanism(s) regulating this complex process.

BARRIER-TO-AUTOINTEGRATION FACTOR INVOLVEMENT IN PRELAMIN A-RELATED CHROMATIN REMODELING

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Defects in lamin A precursor maturation pathway cause nuclear morphological changes and chromatin remodeling. It has been previously described that the accumulation of lamin A precursors by pharmacological treatments or by transfection with prelamin A uncleavable constructs, induces heterochromatin markers redistribution within the nucleus and nuclear size increase(1) Similar nuclear defects were observed in a group of rare diseases named laminopathies which arise from mutations of the nuclear lamina or the nuclear envelope proteins. Among laminopathies, chromatin structure alterations seem to be more severe in those forms showing prelamin A accumulation. Recently we demonstrated a molecular link between prelamin A and Barrier-to-Autointegration Factor (BAF) a 10 kDa chromatin-interacting protein identified as a component of chromatin dynamics-related molecular complexe.(2,3) Since this finding suggested a possible BAF role in prelamin A chromatin remodeling function, we wondered if prelamin A effects on chromatin could be due to prelamin A-BAF interaction. In particular, we took advantage of the study of the heterochromatin marker H3K9me3 distribution in Néstor Guillermo Progeria Syndrome (NGPS) cells, treated with mevinolin, a prelamin A processing interfering drug. We observed that the BAF mutant (Ala12Thr) identified in this rare disease prevented H3K9 intranuclear localization in response to prelamin A accumulation. In order to confirm this finding we performed additional experiments in HEK-293 cells induced to accumulate lamin A or prelamin A in combination with wild type BAF (WT-BAF) or mutated BAF forms (BAF-A12T, BAF-G47E). Interestingly, we observed that when prelamin A-BAF interaction could not occur properly prelamin A was unable to modify the intranuclear localization of chromatin-related proteins HP1a and LAP2a or to affect chromatin ultrastructural organization. Similar results were obtained in HEK293 cell BAF depleted by siRNA treatment transfected with prelamin A constructs. Finally we confirmed BAF involvement in prelamin A related chromatin organization effects in Hutchinson Gilford Progeria cells. In particular, we were able to rescue the proper intranuclear localization of chromatin-related protein LAP2a affecting progerin-BAF interaction.(4) Our results demonstrate that BAF permits prelamin A chromatin remodeling functions and that the perturbation of this mechanism due to BAF mutations, or its permanent activation by impairment of lamin A precursor processing, could be involved in the pathophysiological mechanism of progeroid syndromes linked with alterations of nuclear lamina proteins.

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MOLECULAR COMPOSITION AND DYNAMICS OF NUCLEAR FOCI IN MYOTONIC DYSTROPHY

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Myotonic dystrophies (DM) are genetically heterogeneous neuromuscular disorders with autosomal dominant inheritance: DM type 1 (DM1) is caused by a CTG repeat expansion in the 3' UTR region of the DMPK gene, while DM type 2 (DM2) is linked to the instability of a CCTG repeat in the CNBP (also known as ZNF9) gene. Both DMs are characterized by multisystemic pathologic features including myotonia, muscular dystrophy, dilated cardiomyopathy, cardiac conduction defects, insulin resistance.

Combining biomolecular and cytochemical techniques, it has been demonstrated that the

basic mechanisms of both DMs reside in the nuclear sequestration of the expanded RNAs: CUG- and CCUG-containing transcripts accumulate in intranuclear foci of DM1 and DM2 cells respectively, and alter the regulation and intranuclear localization of the RNA-binding proteins CUGBP/Elav-like family member 1 and muscleblind like 1[1]. It has been found that DM foci also sequester snRNPs and hnRNPs, splicing factors involved in the early phases of transcript processing[2]. This likely causes a general alteration of the pre-mRNA post-transcriptional pathway in DM-affected cells. Accordingly, splicing and cleavage factors have been found to accumulate in skeletal muscle myonuclei of DM1 and DM2 patients, indicating an impairment of pre-mRNA processing[3]. Finally, by analyzing the dynamics of the DM-specific intranuclear foci, it has been demonstrated that, in cycling cells, they undergo disassembly to be reformed in the nucleus at each mitosis whereas, in non-cycling cells, they progressively increase in size[4], likely leading to the worsening of the pathological traits.

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THE NUCLEAR LAMINA AS A CHROMATIN ORGANIZER

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The nuclear lamina (NL) has been evolutionary expressed in metazoan, and the V-type intermediate filaments, the lamins B and A/C, which undergo polymerization/depolymerization phases, determine the NE assembly/disaggregation along the cell cycle. The role of nuclear lamins in tissue-specific cell differentiation has been highlighted by studies on the pathogenic mechanism leading to a variety of human diseases, collectively referred to as laminopathies[1]. Laminopathic cells are characterized by the expression of immature forms of lamin A (prelamin A), whose accumulation in the nucleus determines loss of peripheral heterochromatin, as revealed by the reduction of typical heterochromatin markes (tri-H3K9) and by electron microscopy[2]. These nuclear alterations and the transcriptional activity can be recovered by drugs that interfere with prelamin A accumulation[3]. The NL and mainly lamin A/C determine stable interactions with particular districts of the genome, characterized by a high histone H3 dimethylation and block of transcriptions. These lamin A/chromatin interactions, in correspondence with a thousands of LADs (Lamin-Associated-Domains) are mediated by epigenetic repressors such as HADCs, and by chromatin-associated proteins including HP1 and BAF[4]. Among laminopathic phenotypes, the most severe is represented by the Hutchinson-Gilford progeric syndrome (HGPS) in which tissues degenerate undergoing premature senescence. Animal models, and cell from laminopathic patients have been treated with drugs that interfere with prelamin A accumulation; the results obtained encouraged the applications of analogous treatments in three international clinical trials. These studies suggested that accumulation of prelamin A could also affect normal cell and organism aging. We obtained further evidence on the crucial role on chromatin organization and gene expression of prelamin A processing and on the particular prelamin A expression that characterizes a special group of aged persons, the centenarians, which present a nuclear arrangement which is closest to young than aged people[5].

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ULTRASTRUCTURAL ANALYSIS OF NUCLEIC ACID METHYLATION

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Transmission electron microscope analysis of 5-methylcitosine (5 mC) distribution represents a different approach to study epigenetic modifications on nucleic acids. Moreover, this allows to specifically identify RNA fibrils carrying these epigenetic markers. We have investigated this distribution on HeLa cells, due to their high transcrip-

tion rate, while rat liver were considered as a control cell model.

We found 5mC labelling in the perichromatin region, where transcription by RNA polymerase II occurs[1]. The colocalization of 5mC and fluoro-uridine, an RNA precursor, together with specific staining methods confirmed the presence of this modified base on RNA fibrils. Since data in the literature indicate that lncRNAs are localized at the periphery of condensed chromatin region to regulate genome activity[2], we have used several markers to elucidate the 5mC presence on mRNA. This mRNA modification is indeed not clearly understood[3]. We identified labelling for 5mC near ribosomes in the cytoplasm, both alone and in combination with labelling for poly(A) tail. Furthermore, our EM data show that RNA methylation occurs cotranscriptionally and is stable during RNA life until its translation. We are setting up a panel of antibodies for discriminating the different types of RNA in the perichromatin region.

Finally, in order to deeper investigate the role of this early and stable epigenetic modification on mRNAs, we propose an analysis on exosomes where the presence of miRNA and mRNA has been described[4]. Terbium positive molecules were found at the periphery of the exosome near the membrane and 5mC was also detected.

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A PUTATIVE 2N=26 ROBERTONIAN MOUSE EMBRYONIC STEM CELL LINE MAINTAINS ITS KARYOTYPE STABILITY DURING CULTURE

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The potential use of mouse embryonic stem cell (mESC) for investigating *in vitro* early embryonic development or for screening the effects of new drugs or xenobiotics depends on capability to maintain their genome integrity during prolonged culture and differentiation. Culture-induced variations of specific chromosomes or genes are almost all unpredictable and, as a whole, differ among independent cell lines. They may arise at different culture passages, suggesting the absence of a safe passage number maintaining genome integrity and rendering the control of genomic stability mandatory since the very early culture passages.

To date, all mESC lines available have been obtained from *Mus musculus* laboratory stains with the 2n=40 all telocentric standard karyotype. These inbreed laboratory strains posses a mixed genome background, a condition that can influence the genome stability.

We have recently derived the first wild-type putative mESC line from mice with a reduced chromosome number (2n=26) for the presence of 8 Robertsonian (Rb) metacentric chromosomes. This cell line expresses pluripotency markers and it is able to form embryoid bodies and the three germ layers.

Putative Rb mESCs were cultured for a period of up three months, monitoring, at regular time intervals, the chromosome complement and the appearance of chromosome abnormalities.

Rb mESCs were cytogenetically analysed between passages 2 and 28 and, on average, 50 metaphase spreads were scored for each passage. This mESC line displayed a stable diploid Robertsonian karyotype during culture (ranging from 84 to 100%), with a very low percentage of numerical chromosomal aberrations (subtetraploid and tetraploid populations). Also, after freezing/thawing procedure, the karyotype stability was maintained.

At each passage, DAPI banding of 5 randomly chosen metaphases showed normal chromosome complement. In just one metaphase at passage 10, although numerically euploid, a chromosome 1.3 trisomy coupled with a chromosome 7 unisomy was found. This aberration was not recovered in the subsequent passages, suggesting that cells with abnormal karyotypes were negatively selected during culture.

This line, characterized by a metacentric phenotype of the chromosomes and reduced chromosome number, represents a precious resource for the study of the centromere function and of the mechanisms involved in the onset of both numerical and structural chromosome abnormalites.

NUCLEAR DIFFUSION ASSAYS FOR THE STUDY OF DNA DAMAGE AT THE SINGLE CELL LEVEL

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The need for express screening of the DNA damaging potential of chemicals has progressively increased over the past twenty years due to the wide number of new synthetic molecules to be evaluated, the adoption of more stringent chemicals regulations such as the EU REACH[1] and risk reduction politics. To this regard, DNA diffusion assays such as the microelectrophoretic comet assay paved the way to a new concept of rapid genotoxicity testing, but a further and more significant simplification and speeding up of the experimental processes was achieved with the fast halo assay[2] (FHA). This assay operates at the single cell level and relies on the radial dispersion of the fragments of damaged DNA from intact nuclear DNA: fragmented DNA is separated by virtue of diffusion in alkaline solvent, stained, visualized at the microscope, and finally quantified using appropriate computer-assisted image analysis programs to assess the extent of DNA breakage caused by different types of DNA lesions. FHA has proven to be sensitive, reliable and flexible. To our best knowledge, FHA is currently the simplest, cheapest and quickest assay for studying DNA damage and repair in living cells. The assay (the preparation of samples ready to be visualized at the microscope) can be performed in about forty min, i.e. one fourth of the mean time required to execute the comet assay and it is very cheap since it requires no electrophoretic apparatus nor expensive reagents. Moreover, recent modifications further implemented FHA allowing a full exploitation of its analytical potential as a technique for large-scale, rapid genotoxicity screening.

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NUCLEAR DUALISM AND GENOME ORGANIZATION IN CILIATES

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In multicellular organisms, the germline and somatic genomes are physically and functionally separated during the development into distinct cell types. In contrast, the ciliates, a group of evolutionarily very successful unicellular eukaryotes, are unique to carry both their germline and somatic genomes enclosed in the same cytoplasm. The germline genome, transcriptionally silent, resides inside spherical micronuclei that divide mitotically and undergo meiosis to generate gamete-nuclei during every sexual event. The somatic genome, transcriptionally active, resides inside larger and variously shaped macronuclei that divide amitotically and are destroyed and built ex-novo starting from mitotic products of the synkaryon formed by fertilization of the gamete-nuclei. These two genomes have remarkably diversified organizations[1]. While the DNA of the micronuclear genome is organized as conventional chromosomes, the DNA of the macronuclear genome forms unconventional nano-chromosomes, or linear 'gene-sized' molecules which range in size from 500 to 20000 bp and usually contain a single coding region[2]. The generation of the gene-sized genome from the chromosomic genome involves impressive DNA rearrangements including chromosome polytenization, fragmentation and elimination of up to 90% of the original DNA sequences[3,4]. The conclusive developmental step is the amplification of the relatively low number of genes that are essential for the cell life into hundreds, or thousands of copies[5].

This contribution will focus on the sophisticated epigenetic mechanisms that, driven by RNA molecules as mediators, are responsible for the complex but precise rearrangements that transform the germinal transcriptionally silent micronuclear genome into the somatic transcriptionally active macronuclear genome.

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TIME-LAPSE IMAGING OF CHROMATIN AND CYTOPLASMIC MOVEMENTS OCCURRING DURING THE GV-TO-MII TRANSITION: IN SEARCH FOR MARKERS OF MOUSE OOCYTES DEVELOPMENTAL COMPETENCE

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The transition from the germinal vesicle to the metaphase II stage (GV-to-MII transition) is crucial to the acquisition of the oocyte developmental competence. Here, using live, time-lapse, imaging we describe the movements occurring during the GV-to-MII transition to the chromatin (CHR-MOV) and to the cytoplasm (CYTO-MOV) of mouse oocytes of known developmental competence or incompetence.

Fully-grown cumulus-oocyte-complexes were punctured from the ovarian surface, the GV oocytes isolated and stained with the supravital Hoechst 33342 fluorochrome (Ho) which allowed the identification of gametes whose nucleolus is surrounded by a ring of Ho-positive chromatin (surrounded nucleolus, SN, oocytes) from those that lack this ring (not surrounded nucleolus, NSN, oocytes). Importantly, when *in vitro* cultured to MII and inseminated with sperm, whilst SN oocytes may develop to term, NSN oocytes arrest development at the 2-cell stage.

The time-lapse observation of CHR-MOV describe distinct chromatin changes in NSN compared to SN oocytes, with a longer GV-to-MII transition in NSN oocytes that reach the M-phase without the gathering of heterochromatin regions around the nucleolus.

Furthermore, by coupling bright-field time-lapse observations with the Particle Image Velocimetry method, we analysed the CYTO-MOV of these two types of oocytes. We showed that SN and NSN oocytes exhibit distinct profiles and, at four main time-frame intervals, their CYTO-MOV velocity is significantly different. In addition, we integrated the information of the CYTO-MOV profile of each single oocyte with an artificial neural network analysis that blindly identified the oocyte as SN or NSN with a robust probability.

The presence of SN and NSN oocytes in all mammals, including humans, extends the interest of these results to the field of assisted reproductive technologies (ART).

Application of confocal laser scanning microscopy in the taxonomy of free-living marine nematodes

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Summary
Confocal laser scanning microscopy (CLSM) can provide high-resolution images of thick nematode specimens and highly detailed three-dimensional reconstructions. Several permanent slides of free-living marine nematode species including a species collected in the 1951 were analyzed with the aid of CLSM. The specimens were excited using an argon laser at 488 nm under the conditions used for Fluorescein Isothiocyanate (FITC) and 543 nm for Tetramethyl Rhodamine Isothiocyanate (TRITC). New details on the morphology of various diagnostic features (the cephalic region and male reproductive system) have been captured for several species allowing a re-description for some of them. Spicules and *gubernaculum*, followed by cuticularised parts of the buccal cavity and precloacal supplements were the most fluorescent parts of the nematode body. The morphological approach here adopted highlights new chances for the study of Museum type material for which CLSM may be decisive in capturing additional, important taxonomical details. Material collected in the 1951 and 1973 still resulted fluorescent making possible the detection of crucial taxonomical data.

Key words: taxonomy, free-living marine nematodes, confocal laser scanning microscopy, autofluorescence.

Introduction

The *phylum* Nematoda includes arguably the most successful free-living metazoans on the Earth (Da Rocha *et al.* 2006; Balsamo *et al.*, 2010). Nematodes have a central role in the energy flows, mineralization rates and nutrients recirculation of the marine ecosystems (Zeppilli *et al.*, 2015). Accordingly, they can give an important and direct contribution in functioning of marine environments (Danovaro *et al.*, 2008; Semprucci and Balsamo, 2012) and are effective tools in the assessment of environmental health and in biomonitoring (Semprucci *et al.*, 2015).

So far ~ 7000 marine species have been described that represent only 19% of the total number of the predicted nematode biodiversity (Appeltans *et al.*, 2012). Biodiversity estimates are crucial for developing conservation programs focused on marine ecosystems. Nematodes are fine and slender worms, mostly one to few millimetres long, with a body diameter 20-40 times less than total length. The body wall is made of a thick, layered cuticle mainly constituted by collagen. The cuticle may appear smooth, transversely annulated, or dotted, and bears a number of sensory structures called *sensilla*, the length and pattern of distribution of which as well as the size, shape and position of the lateral organs (i.e. amphids) are important diagnostic characters for taxonomy. The morphology of the buccal cavity is quite various reflecting the wide range of feeding habits: it may be from absent or minute to armed with mandibles. Cuticularized structures of the male reproductive system such as spicules and *gubernaculum* may considerably vary in shape and size and are normally of great value for the taxonomical identification as are the presence and features of precloacal supplements.

Among the difficulties which hamper the global biodiversity estimate of the *phylum* are the small body size, and the low number of the diagnostic characters that are often minute (Semprucci and Balsamo, 2012). Therefore, new techniques to make their identification easier and capture additional morphological features need to be explored. Furthermore, museum taxonomical collections of nematodes include significant numbers of type materials. These are fundamental not only as reference points for specialists but also as potential sources for obtaining new taxonomical data. However, most types or paratypes of many species have been deposited in the last 10 decades as permanent slides and their conservation status is damaged in some cases. In this regard, advanced microscopic techniques such as Confocal Laser Scanning Microscopy (CLMS) may provide integrative information and even highly detailed tri-dimensional reconstructions of morphological structures thanks to the autofluorescence of specimens.

Autofluorescence may be fixative-induced (e.g. using aldehyde fixatives) or natural and the emission spectra are generally broad compared to the spectra of the dyes or probes (Monici, 2005; Leischner *et al.* 2010). In particular, natural fluorescence has been documented from morphological structures of many animals, plants, fungi and microorganisms when they absorb light (Wu and Warren, 1984; Monici, 2005). The most commonly observed autofluorescing molecules in biological samples are structural molecules such as collagen and elastin as well as molecules involved in metabolic and functional processes (i.e. NADPH and flavins) (Monici, 2005). Forge and MacGuidwin (1989) documented that several nematode genera accumulate fluorescent lipofuscin compounds (lipids) in intestinal cell globules. Instead, Daugschies *et al.* (2001) documented that the eggs of some parasite nematodes emit light and can be easily distinguished from debris. No hypotheses have been advanced about the origin of the eggshell autofluorescence, but nematode eggs consist of vitelline, chitin and lipid-rich layers (Brownell and Nelson, 2006; Altun and Hall, 2009). Zullini and Villa (2006) re-described three species of freshwater nematodes belonging to the Altherr's collection just thanks to their autofluorescence under confocal microscopy. This approach was also used by Semprucci and Burattini (2015) on recent and ancient collection slides of marine nematodes.

Thus, the aid of confocal microscopy may be crucial both to describe new species and possibly also to re-describe some already known species for which morphological and morphometric details are very poor. Accordingly, this study explores possible explanations of nematode autofluorescence and highlights the advantages of CLMS technique in the taxonomical study of free-living marine nematodes.

Materials and Methods

Dorylaimopsis pellucidum (1 specimen), *D. variabilis* (Comesomatidae) (3), *Laxus gerlachi* (Desmodoridae) (1), *Rhinema retrosum* (Monoposthidae) (1), *Craspodema reflectans* (1), *C. octogoniata* (Cyatholaimidae) (2, 1) were the species analyzed in this study. Specimens were fixed with 4% buffered formaldehyde in sea water solution and mounted as permanent slides (Seinhorst, 1959). *C. octogoniata* belongs to the collection of the Zoologisches Institut und Zoologisches Museum of the Hamburg University and its specimens are conserved as permanent slides for which the preparation was not reported in the original description. *C. octogoniata* specimens were collected in French Coasts (St. Honorat and Pierres Noires) in the 1951 and 1973. *D. pellucidum*, *L. gerlachi*, *R. retrosum* and *C. reflectans* were collected in Maldives (Suvadiva atoll, 2009), while *D. variabilis* was collected in Korea (East Sea, 2012).

All specimens were studied with a CLSM, and excited using an argon laser either at 488 nm under the conditions used for Fluorescein Isothiocyanate (FITC, 495 nm excitation and 520 nm emission spectrum peak wavelengths) or at 543 nm for Tetramethyl Rhodamine Isothiocyanate (TRITC, 557 nm excitation and 576 nm emission spectrum peak wavelengths). As reported in Semprucci and Burattini (2015), images were taken with a Leica TCS-SP5 Confocal connected to a DMI 6000 CS Inverted Microscope (Leica Microsystems CMS GmbH), and analysed using the Leica Application Suite Advanced Fluorescence (LAS AF) software. Samples were examined using oil immersion objective lenses (63x N.A. 1.40). CLSM images are presented as single-plane images or Z-stack projections (3D-recostructions) obtained by ImageJ software.

Results

Generally the most fluorescent morphological structures were the buccal cavity (i.e. walls and teeth) and the male spicules and *gubernaculum*. The cuticle, even if visible with both wavelengths, did not present a high fluorescent emission. An exception was represented by some more cuticularized parts such as the helmet (cephalic capsule), the precloacal supplements and the *setae* insertions. Morphological structures were generally visible under both green and red emission with some exceptions reported below.

Dorylaimopsis pellucidum (Cobb, 1920) is a representative species of the family Comesomatidae. As belonging to this genus it is characterized by 3 teeth, cylindrical buccal cavity, 6 + 4 cephalic setae, cuticle with lateral longitudinal rows of dots; spicules elongated and *gubernaculum* apophysis directed caudally. Furthermore, it has a multispiral amphideal fovea with 2.5 turns. Cuticle with 2 lateral longitudinal rows of coarse dots in the middle body region, and 4 in the anterior (i.e. from amphideal fovea to pharyngeal end) and posterior (from spicule to tail) body regions of both sexes. Spicule 110 µm long, with a small distal hook and proximally cephalate. *Gubernaculum* with lateral projections and long apophysis. Pre-cloacal supplements are present, but fine and tubular.

In the male specimen analysed using CLMS, cuticle ornamentation showed a moderate fluorescence that resulted of the same intensity in both green and red emissions (Figure 1A). This was roughly also applicable to the teeth (Figure 1B,C). Instead, spicule and *gubernaculum* showed a relevant emission, especially in green (Figure 1D,E).

Dorylaimopsis variabilis Muthumbi, Soetaert and Vincx 1997 reflects the general features of the genus. In particular, it is characterized by the multispiral amphideal fovea with 2.5–3.0 turns, the cuticle with 2 lateral longitudinal rows of coarse dots in the middle body region, and 3 in the anterior and posterior body regions of both sexes. Spicules 73-127 µm long, arcuate and with a well-developed *capitulum*. *Gubernaculum* with long caudal apophysis. Precloacal supplements with very fine ducts.

The buccal cavity and cuticle ornamentation appeared poorly fluorescent and equivalent in both emissions as in the previous *Dorylaimopsis* species. Spicules and *gubernaculum* showed a higher emission signal and comparable in both the wavelengths (Figure 2A). The 3D-recostructions by CLMS revealed the presence of a greater morphological complexity of the spicule tip than that reported in the original description (Figure 2B,E). These details were also visible to a further analysis by scanning electron (SEM) and differential interference contrast (DIC) microscopies (Figure 2C,D,F).

Laxus gerlachi (Hopper and Cefalu, 1973) belongs to Stilbonematinae (family Desmodoridae). The genus is characterized by the cuticle with fine transverse *striae*. Cephalic cuticle thick with a surface irregularly annulated, reticulated, or sculptured in a fingerprint pattern. Amphideal fovea small, coiled with ~1.5 turns, situated close to the apex. Anterior pharynx slightly swollen and not sharply separated from the narrow middle region. *Gubernaculum* directed dorsally, no dorso-caudal apophysis. Tail short, conical, mostly 1.4 – 2 anal diameters long. Symbiotic bacteria coccoid. In particular, *L. gerlachi* showed cuticle ornamentation starting posteriorly to the amphideal fovea and made of fine transverse *striae* covered by a coat of symbiotic coccoid bacteria. Amphideal fovea multispiral with ~ 3 turns close to the apex. Buccal cavity small without teeth. Spicule 44 µm long and proximally cephalate. *Gubernaculum* present and directed dorsally. Tail short and conical.

The cephalic cuticle emitted a fluorescence in both red and green fluorescence (Figure 3A) and highlighted globular structures that likely were coccoid bacteria. Red fluorescence seemed to better discriminate morphological details than green fluorescence because in the latter the signal emission was too high, so making a clear visualization difficult. The only cuticular parts really fluorescent were the inserting points of the somatic setae that appeared more clearly in green fluorescence (Figure 3A). Single morphological details of the male reproductive system were more fluorescent under FITC than TRITC condition. The tri-dimensional reconstruction of the *gubernaculum* structure was highly informative (Figure 3B). Also the subventral *setae* on the male tail were well-visible (Figure 3C).

Rhinema retrosum Cobb, 1920 belongs to the family Monoposthidae. The head cuticular annules fuse forming a helmet. Buccal cavity is characterized by three teeth (one dorsal and two subventral teeth). Amphideal *fovea* is circular without interruption of its edge. Cuticle exhibits 12 longitudinal rows of *alae* throughout the body. Paired ovaries. Spicules (45 µm long) and *gubernaculum* present.

The specimen analyzed was a female that showed a signal emission in both the wavelengths consid-

ered. In detail, the whole cephalic region (e.g. amphid and helmet) showed a high emission: labial region, buccal cavity and the surrounding pharynx resulted slightly more fluorescent under FITC conditions, while the cuticle of the helmet was comparable with both emissions (Figure 3D,E).

Craspodema reflectans Gerlach, 1964 belongs to the family Cyatholaimidae. Amphideal fovea spiral (3.5 turns), hand-mirror shaped and based on a plaque. The buccal cavity deep with three teeth (one well-developed dorsal tooth and two smaller and equal subventral ones). Lateral differentiation of body cuticle is very prominent as longitudinal rows of enlarged punctations with broad lateral fields between them. Spicules are arcuate (41 µm long) and *gubernaculum* present. Well-developed ventral preanal supplementary organs heavily cuticularized is observed.

Both FITC and TRITC conditions excited the cephalic region of *C. reflectans* (e.g. *rugae* and buccal cavity) (Figure 4A,D). However, *rugae* were slightly more evident using the FITC conditions (Figure 4B), while the buccal cavity walls was slightly more fluorescent under TRITC conditions (Figure 4C). Cuticle and amphideal fovea showed a moderate fluorescence with both wavelengths, while

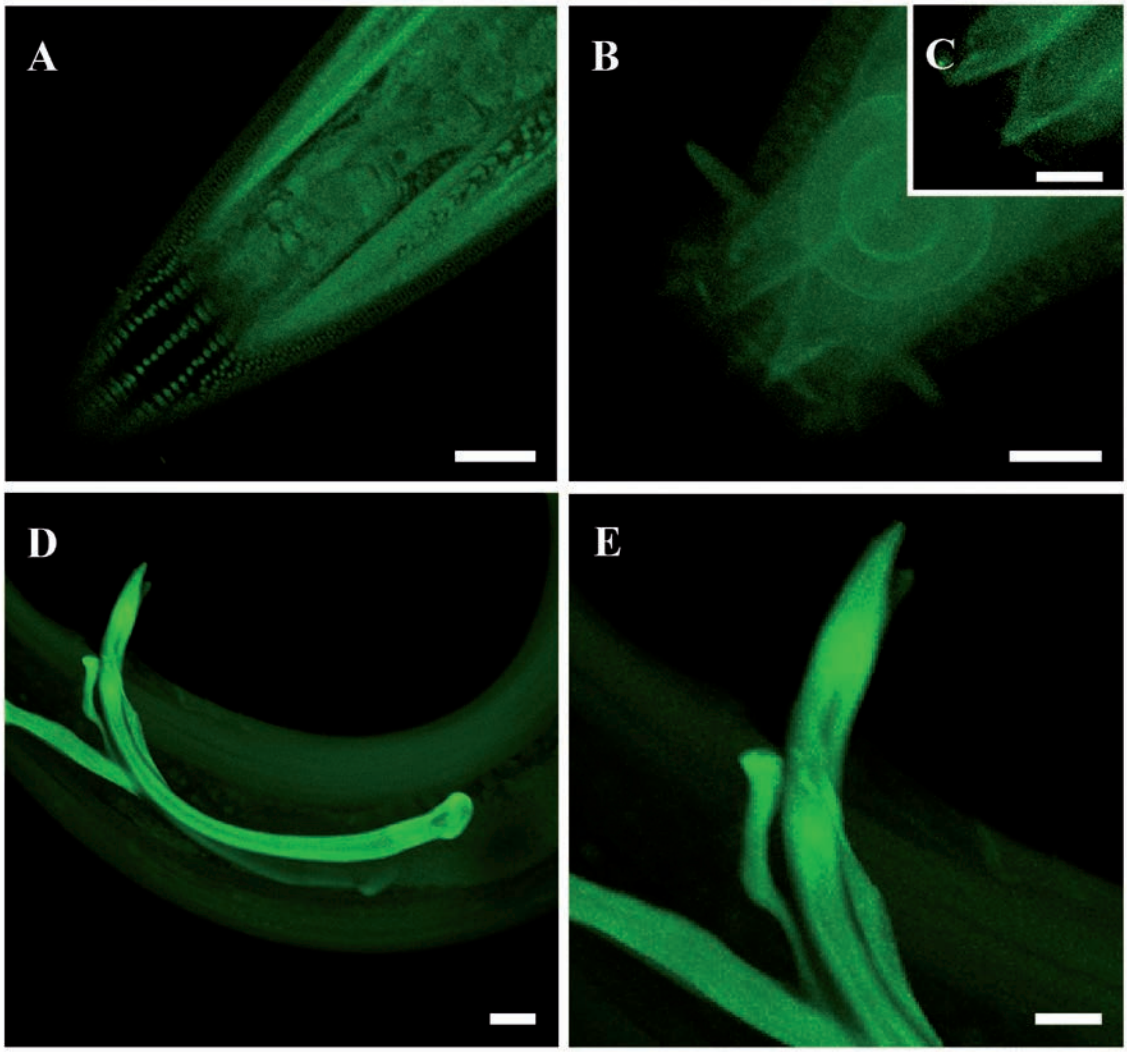

Figure 1. *Dorylaimopsis pellucidum*. A) Detail of cuticular ornamentation by Confocal Laser Scanning Microscopy (CLSM); B) Reconstruction of the buccal cavity by CLSM; inset C. Detail of the teeth; D) Reconstruction of the male copulatory apparatus by CLSM; E) Detail of the spicule tip by CLSM. All images are excited in FITC emission spectra. Scale bars: A, D = 10µm; B,C, E = 5µm.

spicule, *gubernaculum* and precloacal supplements resulted slightly more fluorescent under TRITC conditions. CLMS observations highlighted new details on the *gubernaculum* morphology that have been used to redescribe the species (see below).

Craspodema octogoniata (Gerlach, 1954) shares with *C. reflectans* the cuticle ornamentation typical of the genus, but shows a shallower buccal cavity and a multispiral amphideal *fovea* with ~ 3 turns not based in a plaque. Spicules are arcuate and 42 µm long, the *gubernaculum* is present as well as precloacal supplements. The specimens analyzed belong to the Zoological Museum of the Hamburg University (Germany). In particular, the holotype (collected in the 1951) and the paratype material (1973) showed both fluorescence emissions. However, the conservation state of the holotype is not perfect, especially in the cephalic region. This region, the cuticle as well as the male reproductive structures of *C. octogoniata* appeared fluorescent with both green and red emissions (Figure 5A-D). Also the precloacal supplements were well visible (Figure 5C).

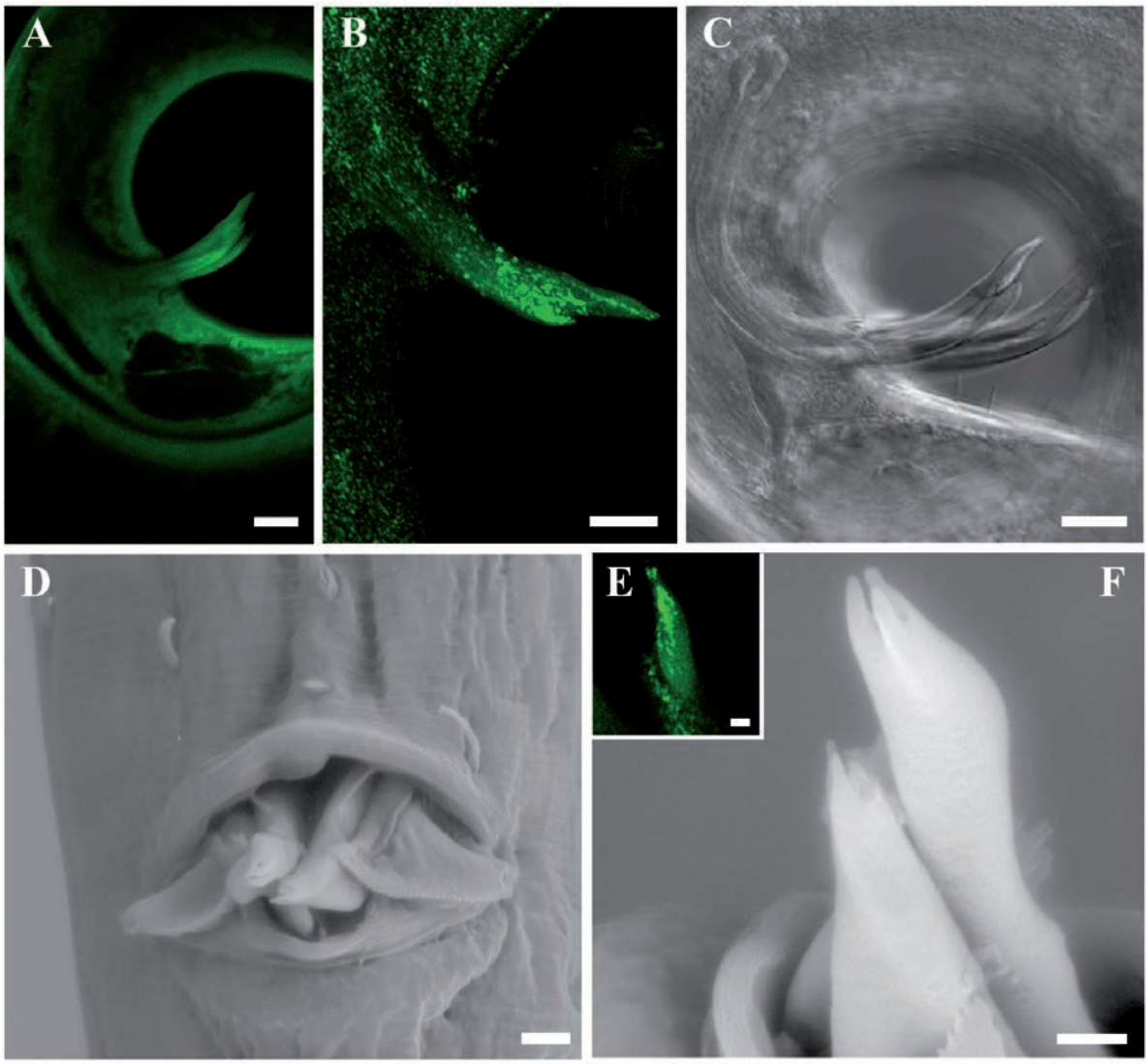

Figure 2. *Dorylaimopsis variabilis*. A) Male spicules by Confocal Laser Scanning Microscopy (CLSM); B) Spicule tip by CLSM; C) Male copulatory apparatus by differential interference contrast microscopy (DIC); D) Male cloaca and distal part of the spicules by scanning electron (SEM); E) Detail of spicule tip region by CLSM; F) Detail of the spicule tip by SEM. Images A,B,E are excited in FITC emission spectra. Scale bars: A, B, C = 10µm; D, E = 2µm; F = 1µm.

Discussion

The autofluorescence of free-living nematodes was documented for the first time by Zullini and Villa (2006), but no possible explanation was given by authors. In all the species observed in the present study, the emission was more marked in spicules and *gubernaculum*, two diagnostic characters that have a fundamental role for the taxonomical identification of nematodes. Depending on the level of the cuticularization of the buccal cavity, the teeth and the buccal cavity walls can be detected, while denticles (very small in size) generally not (see *R. retrosum*). In Cyatholaimidae, Comesomatidae as well as Monoposthidae species, in which the labial region may be rather complex, the 3D-reconstruction may be possible and give spectacular results that may be

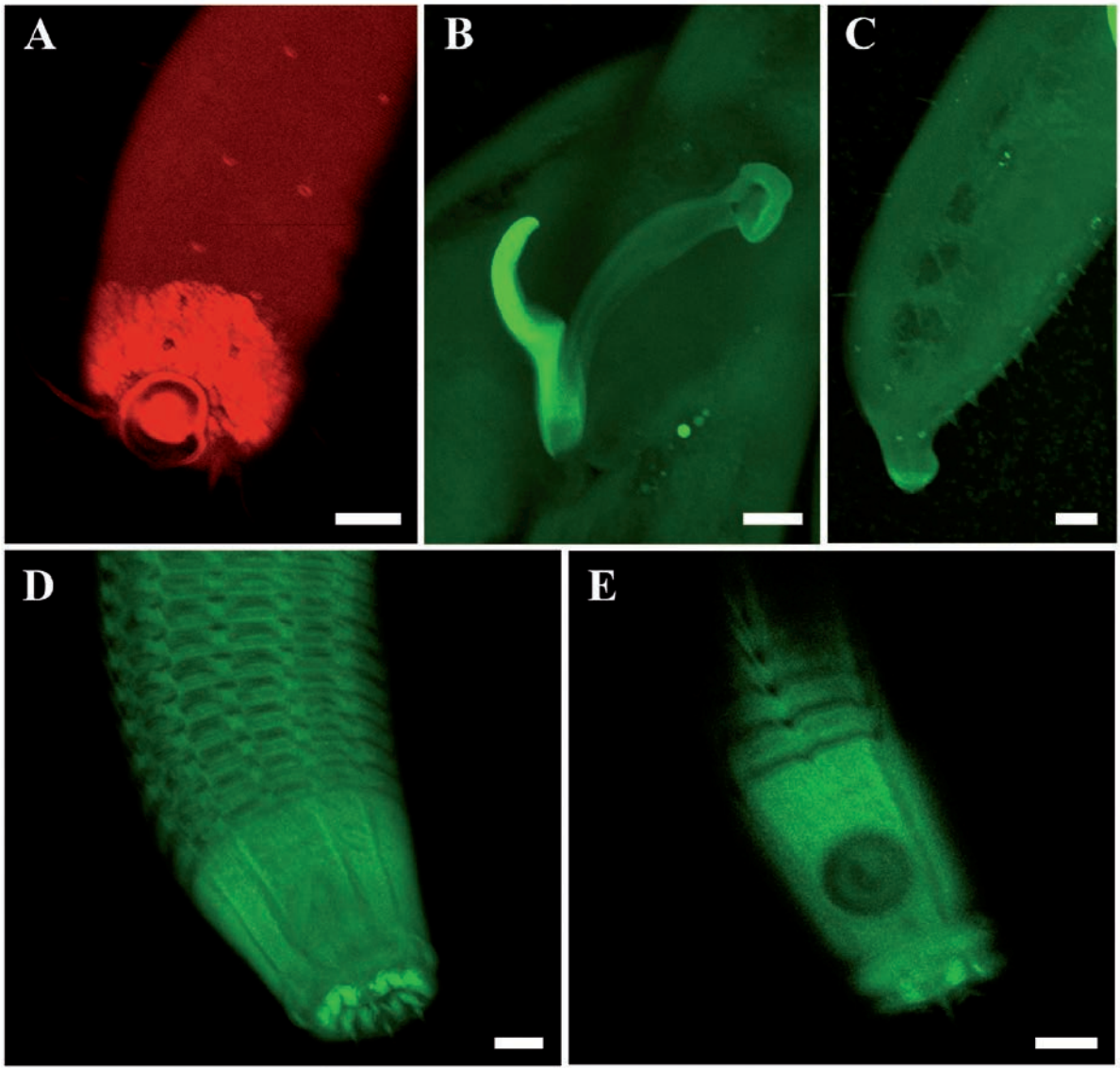

Figure 3. *Laxus gerlachi and Rhinema retrosum* species. A) Detail of helmet region of *L. gerlachi* by Confocal Laser Scanning Microscopy (CLSM); B) Reconstruction of the male spicule and *gubernaculum* of *L. gerlachi* by CLSM; C) Reconstruction of the caudal region of *L. gerlachi* by CLSM; D-E) Detail of helmet region of *R. retrosum* by CLSM. Image A is excited in TRITC emission spectra; images B-E are excited in FITC emission spectra. Scale bars: A, B, C = 10µm; D, E = 5µm.

used for taxonomical purpose (see e.g. Figure 4). The emission from the cuticle ornamentation is often not high, and depends on the cuticle thickness. In general, a greater emission of fluorescence was mainly detected in the cephalic region, the heavy cuticular punctuation or precloacal supplements (e.g. Figures 1A; 3A,D,E; 4A,B,D). The structures of the nematode species that emitted a higher fluorescence were mainly composed by of scleroproteins (e.g. collagen and elastin) (Chitwood and Timm, 1954; Page and Johnstone, 2007) that are known to be autofluorescent because they contain several fluorophores (Georgakoudi *et al.*, 2002; Gerson *et al.*, 2009). In particular, Roshchina (2012) reported their fluorescence spectra over a range of excitation wavelengths: max. 400–430, 465, 495 and 520 nm. This could explain the emission of spicule, *gubernaculum*, cuticularized parts of the buccal cavity, precloacal supplements and cuticle ornamentation both in FITC and (in to a lesser extent) in TRITC conditions. However, it was possible to observe that also internal parts of the nematode bodies, without collagen and elastin, appeared slightly fluorescent. This could be related to the action of formalin that may react with amines and proteins and generate fluorescent products (Leischner *et al.* 2010). Despite this possible formalin influence, the various microscopic techniques that we used (for instance with *D. variabilis*) gave consistent results on the structure of the spicule tip. Thus, formalin seems to amplify the signal emission already released by scleroproteins, and also to induce fluorescence in molecules that are not autofluorescent, as e.g. biogenic amines (Falck *et al.*, 1962; Corrodi and Jonsson, 1967; Rost, 1995). The

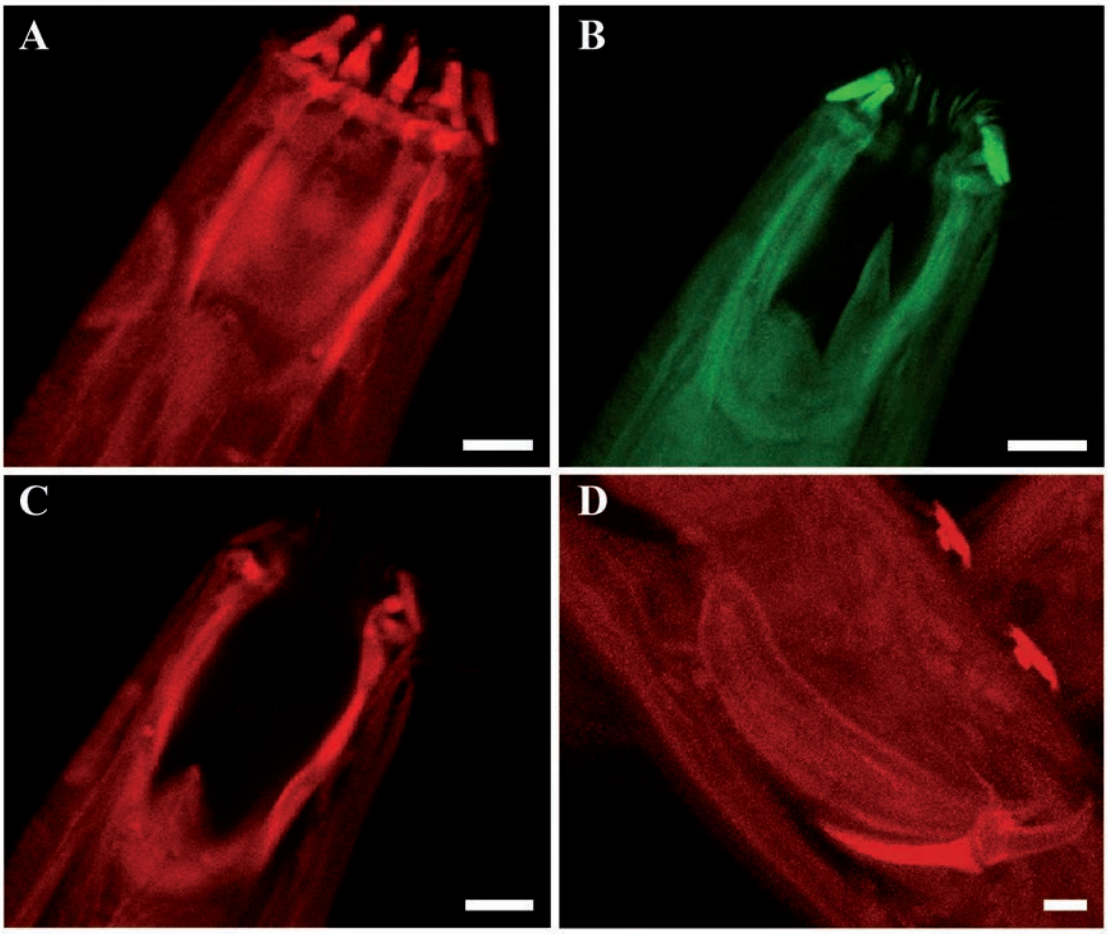

Figure 4. *Craspodema reflectans*. A) Reconstruction of rugae by Confocal Laser Scanning Microscopy (CLSM); B) Detail of teeth by CLSM; C) Buccal cavity walls by CLSM; D) Reconstruction of the male reproductive system. Image B is excited in FITC emission spectra; Images A, C, D are excited in TRITC emission spectra. Scale bars: A, B, C = 5µm; D = 10µm.

details captured by CLMS both in the *gubernaculum* and cephalic region of *C. reflectans* and in the spicule of *D. variabilis* were crucial for the re-description of these species (Figure 2) showing that CLMS is a very powerful tool for nematode study and opens new perspective in the taxonomy of this *phylum*. Indeed, the exploitation of autofluorescence involves low labour costs and make possible excellent 3D-reconstructions of morphological structures without the application of fluorescent tags for target cells or tissues. Furthermore, the approach here adopted highlights new chances for the study of Museum type material. Indeed, old descriptions and drawings of nematodes do not always fit the current taxonomical standards and require further analysis. Type material preserved as permanent slides can

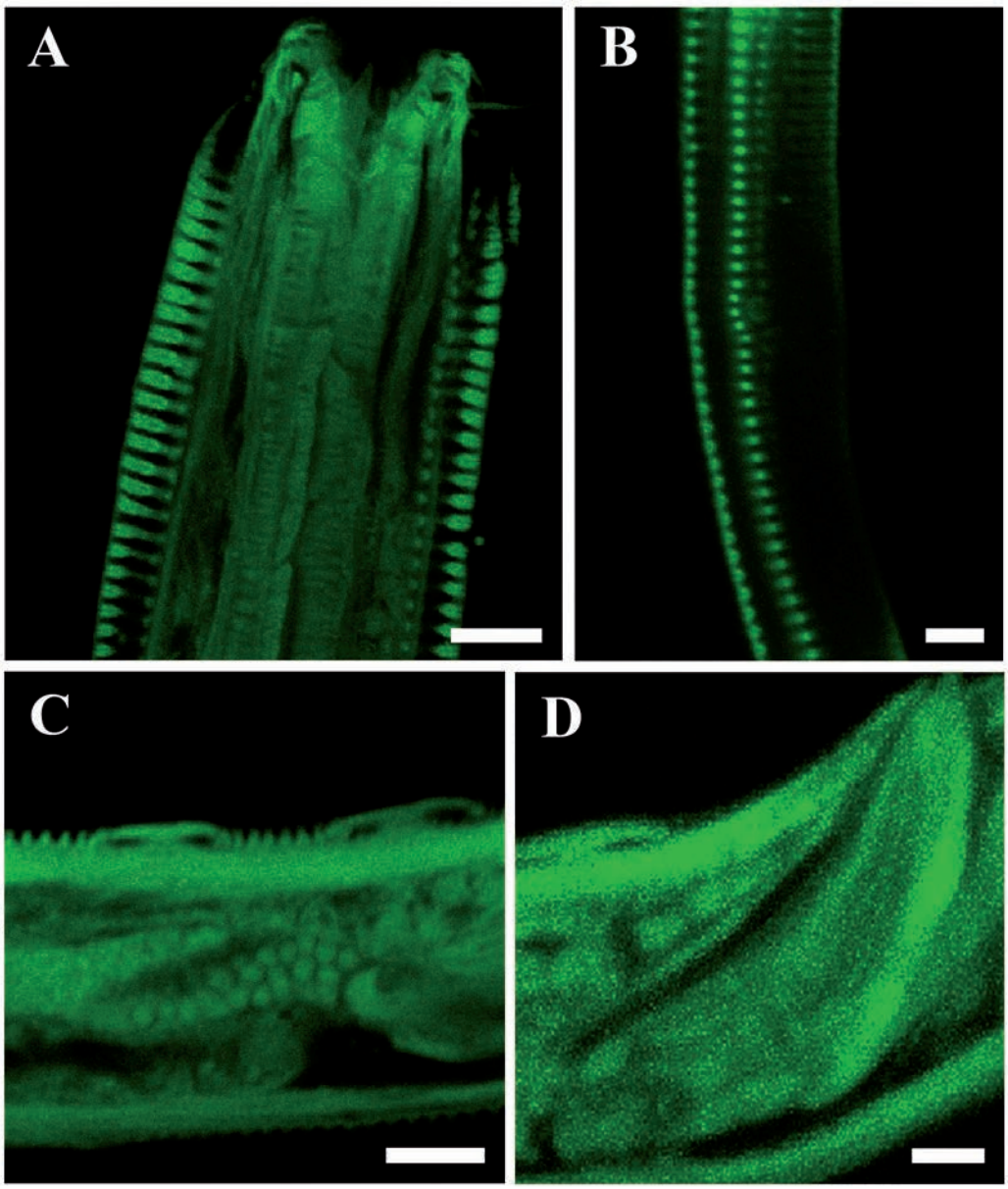

Figure 5. *Craspodema octogoniata*.A) Reconstruction of teeth by Confocal Laser Scanning Microscopy (CLSM); B) cuticle ornamentation in the middle body by CLSM; C) Detail of the precloacal supplements by CLSM; D) Detail of the spicule by CLSM. All images are excited in FITC emission spectra. Scale bars: A, B, C, D = 5µm.

undergo a deterioration or simply cannot be utilized for additional observations such SEM analysis because a part of the necessary diagnostic elements are inside the specimen body and so not directly accessible. In this regard, confocal microscopy can provide high-resolution images of buccal cavity, spicule and *gubernaculum* allowing a 3D-reconstruction of these structures without any damage like the degradation of the cuticle that would be necessary for SEM analysis. The detection of fluorescence in ancient samples of Altherr's collection (deposited in the Museo Cantonale di Storia Naturale of Lugano, Switzerland) (Zullini and Villa, 2006) as well as Gerlach's collections (deposited in the Zoological Museum of the Hamburg University, Germany) (Semprucci and Burattini, 2015) is promising because it really shows that museum specimens may still provide new, precious information. Accordingly, CLSM technique seems to be a significant tool in the effort of making the taxonomical material deposited at the Natural History Museums more accessible and available for scientific purposes.

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Ageing of GeTe nanowires

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Summary
In this paper we report a TEM study on the ageing of GeTe nanowires, for scaled phase memory devices application, when exposed to normal atmospheric conditions. Selective oxidation of Ge occurs, leading to the formation of a Ge oxide amorphous shell around the wire, with $GeTe_4$ nanocrystals embedded within. The oxidation process takes place in a few weeks after the sample preparation, seriously endangering the device integrity and correct functioning.

Key words: TEM; Phase change memory; nanowires; ageing.

Introduction

Phase change memories (PCMs) are an innovative class of memory devices whose working principle relies on peculiar properties of chalcogenide materials. The active region of the memory cell can be written by inducing a phase change between the amorphous and crystalline phases using ns long current pulses. The phase transition is very fast, as it is achieved on the time scale of the tens of ns, and reversible, meaning this that the stored information can be written and erased at will. The stored information is then read measuring the cell resistance: low resistance, i.e. the active region is in its crystalline phase, translate as a binary "1" while the high resistance state, amorphous phase, translate as a binary "0". Therefore, as long as the state of the active region does not undergoes any phase transformation the information is safely stored inside it. PCMs offer the potential for better endurance, data retention, speed and scalability with respect to the current Flash memories. (Raoux et al., 2010; Lacaita and Wouters, 2008)

The most studied chalcogenide phase change alloys are $Ge_2Sb_2Te_5$ and GeTe. The latter, in particular, is the system of choice for fundamental studies owing to its well-understood atomic structure and simple binary chemical composition, which allows for an easier result interpretation in comparison with other Ge–Sb–Te ternary alloys. Self-assembled GeTe nanowires (NWs) received a great interest in the field of PCM applications, because of their potential to allow a defect-free scaling down in the fabrication of high performing, highly integrated, and low-power memory devices (Raoux, 2009; Yu *et al.*, 2008; Lee *et al.*, 2008; Longo, 2014). Reducing the size of the PCM cells to the nano-scale reduces the active material volumes to be programmed, so that shorter and less intense current pulses are required, as already demonstrated in previous studies (Jung *et al.*, 2008; Lee *et al.*, 2008; Jung *et al.*, 2009; Lee *et al.*, 2006; Meister *et al.*, 2006; Jennings *et al.*, 2009).

Besides these positive effects, as the device sizes continuously scale down, insufficiency in the material properties gradually emerged. One of the most important figures of merits that characterize the PCMs quality is the data retention time: namely how long the information can be stored before spontaneous phenomena modify the active region resistivity (Raoux *et al.*, 2010).

The main failure mechanism of PCMs is the recrystallization of the amorphous phase that decreases its resistivity and destroys the stored information. Under normal working condition, the process takes several years to be completed. However, under harsh conditions, such as high temperature/high power (automotive) applications, it speeds up notably (Gleixner *et al.*, 2007). Moreover, highly integrated devices fail more quickly than low integrated ones, indicating a reduced stability of the amorphous

phase at smaller dimensions, as suggested by experiments on the recrystallization time of amorphized nanowires (Lee *et al.*, 2008).

In this paper, we report on the ageing effect that turns the GeTe NWs crystalline phase into an amorphous one, induced by a selective surface oxidation of the Ge, that results in the assembling of Te-rich nanocrystals. The phenomenon happens on a shorter time scale than the recrystallization and has dramatic effects on both the structure and morphology of the NWs under investigation, being potentially able to destroy the memory cell itself in a few weeks. We constantly followed the NWs ageing for several weeks after the NWs synthesis, enabling a deeper understanding and description of the chemical and structural transformations involved.

Methods

The self-assembled growth of GeTe NWs was performed in an MOCVD AIX 200/4 reactor on Si(100)/SiO_2 (50 nm thick, thermally grown) substrates. The substrates were preliminarily loaded into an evaporator, where an Au metal catalyst layer with nominal thickness of 2 nm was deposited by e-beam evaporation at a low deposition rate of 0.1 nm/s. The MOCVD self assembly of the studied NWs was carried out at the temperature of 400°C and pressure of 50 mbar; more details are contained in Longo *et al.*, 2011. The NWs ageing proceeded under normal atmospheric conditions, by keeping them in the laboratory without any particular precaution. In order to assess the chemical and structural transformations undergone by the sample, High Resolution and Analytical TEM investigations were performed on the NWs dispersed on holey-carbon grids, by means of a JEOL 2200FS Field Emission microscope, operating at 200 kV, and equipped with in-column Ω-filter, X-ray energy dispersive (EDX) Spectroscopy and two high angle annular dark field (HAADF) detectors.

Results and discussion

The as-grown NWs (Figure 1) crystallize in the high-temperature stable, rock salt β-GeTe phase (Rabe and Joannopoulos, 1987). They present good crystalline quality and no extended defects are visible, as confirmed by the electron diffraction pattern of Figure 1b): it shows six equivalent reflections having lattice parameters d=0.212 nm consistently with the [111] zone axis. The ageing effects are already visible on the sample after a few days from the synthesis, even if the oxidation process is in its early stage. A close inspection at the High Resolution (HR) TEM image, reported in Figure 1a), reveals that a thin native oxide layer, less than 5 nm thick, is already present on the NW sidewalls. Moreover, the NWs surface itself is not smooth and in some points it appears to be distorted, as within the highlighted white circles.

The amorphous shell is very thin and it is difficult to characterize its nature, the same goes for the surface distortions. However, at this stage, it will not affect the correct device functioning.

TEM observations are repeated on the same sample six weeks after the growth and the results are summarized in Figure 2.

The situation is now completely different: the oxide layer, which has progressively grown on the surface at the expenses of the wire, has doubled its thickness (around 10 nm thick). The distortions observed at the NWs surface have evolved into clear Moirè fringes (circled in red), suggesting the presence of crystal grains at the surface, as a result of a non-complete oxidation of the wire.

The diffraction pattern of the NW is reported in Figure 2b). Here two sets of diffraction spots appear.

The first set, composed by the six outer and brighter spots, corresponds to the (220) reflections family of the β-GeTe. The six less intense and inner spots (red circled in Figure 2b) correspond to a plain spacing d = 0.38 nm, which is not compatible with any crystalline phase of the GeTe. Instead, in the literature, the lattice parameter matches with a Te rich Ge-Te phase, namely the $GeTe_4$ (JCPDS PDF-33-0585).

Close to the outer set of GeTe reflections there are other six spots corresponding to a slightly larger d-spacing than that of the GeTe (d=0.226 nm). These are the (422) diffraction spots of the $GeTe_4$. They appear to have a ring-like displacements due to the presence of satellites spots produced by dynamical scattering (multiple diffraction).

Interestingly, the diffraction spots coming from nanometric crystalline grains randomly located along the NW are sharp, indicating a strong epitaxial relationship with the underlying GeTe NW core.

Further insight on the crystal grains comes from the HAADF-STEM image reported in Figure 3a). They appear as the brighter spots decorating the surface of the wire. The observed contrast is consistent with the chemical composition of the grains, because

the $GeTe_4$ has a higher density than GeTe.

In Figure 3b the EDX map of the same region depicted in Figure 3a is reported. Even if it is not possible to spot the $GeTe_4$ crystal on the map, interesting information on the chemistry of the NWs surface arises: the outermost region of the NWs contains only Ge.

This can be clearly observed in the intensity line profiles of the Ge and Te signals across the wire, reported in Figure 3c). In between the red lines taken as a reference on the images, Ge and Te are uniformly distributed, while only the Ge signal extends outside the red lines. This observation implies that the amorphous shell surrounding the NWs is mainly composed by Ge-oxide.

The effect of the selective Ge oxidation keeps increasing over time, as reported in Figure 4. After twelve weeks from the growth, the thickness of the oxide amorphous layer further increases, reaching an average thickness of 20 nm. The amount of $GeTe_4$ nanocrystals also increased and now they completely cover the whole surface of the NWs. Most of the grains are entirely embedded in the thick amorphous shell which covers the NWs, but they still retain a good epitaxial relationship with the GeTe core, as confirmed by the Fast Fourier Transform (FFT) reported as inset.

A computer algorithm offers the possibility to obtain a distribution map of the intensity exhibited by each spatial frequency present in a HRTEM image. It has been applied to the image in Figure 4a to obtain the map reported in Figure 4b.

The map has been colour-coded, according to the circles on the FFT inset: the intensity of the lattice fringes with the spatial frequency pertinent to the GeTe is shown in green, while for the $GeTe_4$ clusters the red colour has been used.

Interestingly the two signals are well separated, in perfect agreement with the results discussed so far. The GeTe lattice is present only in the core region of the NW, while the $GeTe_4$ nanocrystals are embedded in the amorphous oxide shell.

In the light of the results gathered during the different investigations, we are allowed to suggest the following mechanism for the ageing process. It is graphically summarized in Figure 5.

Due to the air exposure, preferential oxidation of the Ge occurs at the surface of the NWs. The Ge oxide forms the outer amorphous shell, which keeps growing over time. Simultaneously, the excess Te aggregates forming the $GeTe_4$ nanocrystals. Because both the GeTe and $GeTe_4$ belong to the cubic system, the latter can easily form, retaining an epitaxial relationship with the GeTe it originated from.

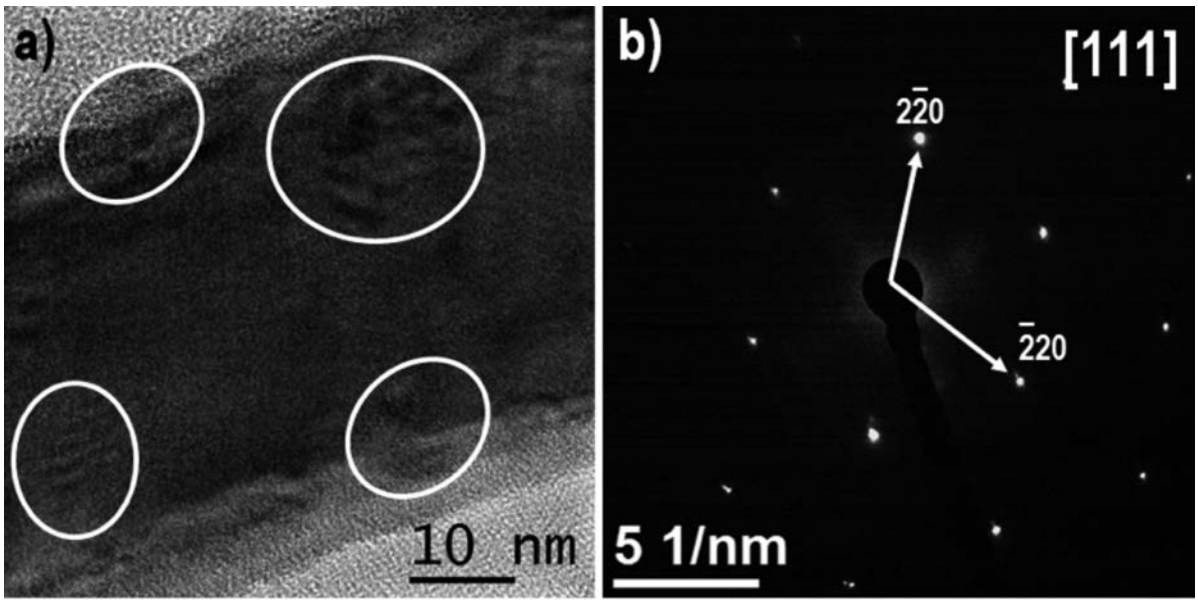

Figure 1. a) TEM image of an as-grown NW exhibiting the rock salt β-GeTe phase and b) its electron diffraction pattern recorded along the [111] zone axis.

As the Ge oxidation precedes and the shell thickness increases, the nanocrystals are eventually embedded into the oxide layer.

We can further argue that the selective oxidation of Ge that we observe is a peculiar behaviour of the GeTe NWs. In fact, similar studies on bulk material indicate a congruent oxidation of both the atomic species and the formation of a GeO_2 and TeO_2 mixture (Yashina *et al.*, 2008).

Conclusion

In this paper we bring compelling evidence of a selective Ge oxidation at the surface of GeTe NWs. The excess Te self-assembles forming $GeTe_4$ nanocrystals. As the oxidation continues, the thickness of the amorphous shell surrounding the NWs embeds those crystals.

The process is very fast: we observed the forma-

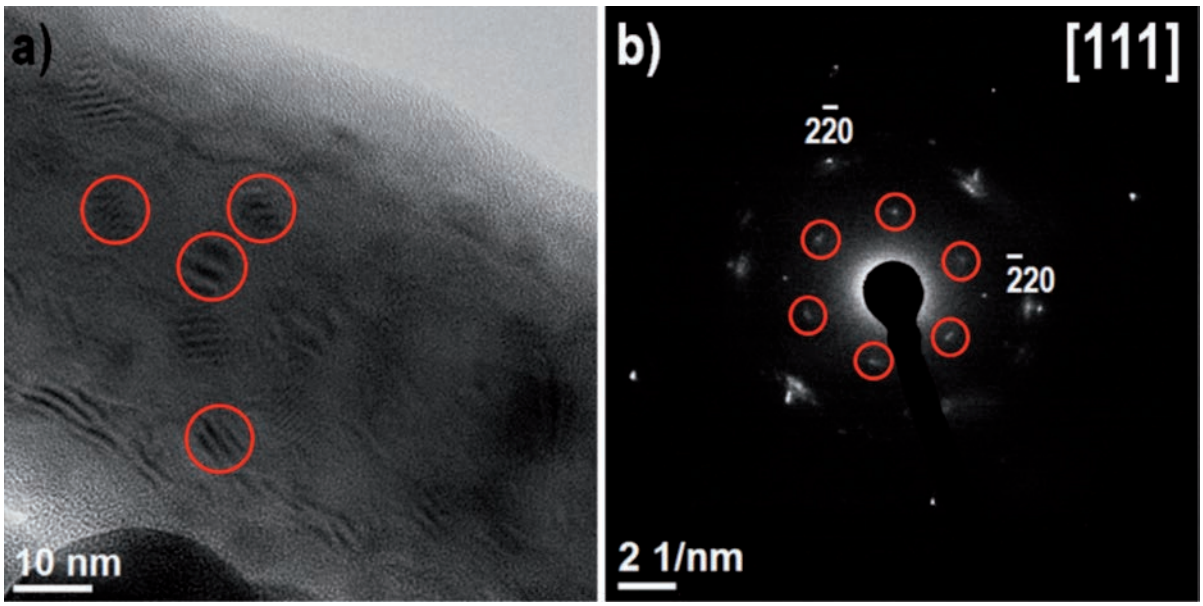

Figure 2: a) TEM image of a GeTe NW after six weeks from the synthesis and b) its electron diffraction pattern recorded along the [111] zone axis. The six inner spots (red circles) come from the nanosized grains circled in a) and correspond to the (220) reflection of the $GeTe_4$.

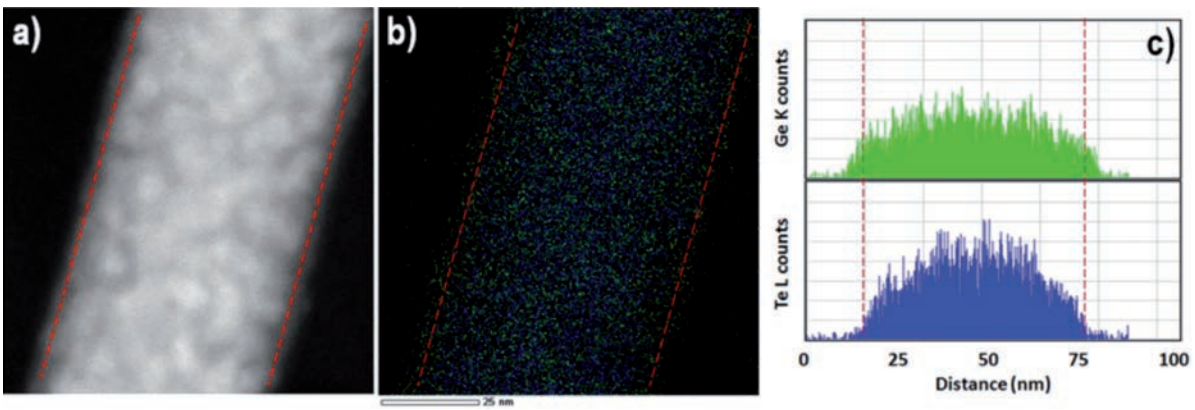

Figure 3: a) STEM-HAADF image of a six weeks aged NW. b) EDX map of the elemental distribution of Ge (green) and Te (blue) and c) intensity line profiles across the NW diameter.

tion of a 20 nm thick amorphous shell, corresponding to a consistent part of the overall NWs diameter, in less than three months, starting from the NWs synthesis.

The presence of an amorphous shell is certainly able to alter the electrical properties of the NWs, and consequently, of the device: the electrically insulating oxide shell prevents any further writing or reading of the memory cell basically destroying it. Considering the velocity of the process, that largely exceeds the commonly studied device failure mechanism, our results will help designing future PCMs devices.

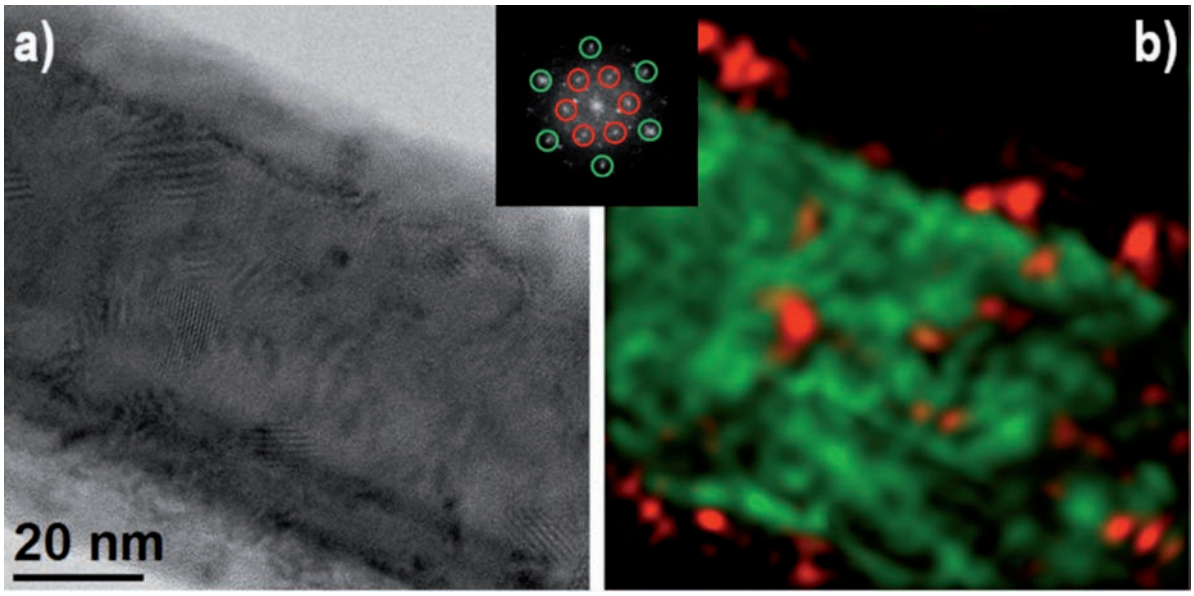

Figure 4: a) TEM image of a twelve weeks aged NW. b) Map representing, in false colors, the region where the GeTe (green) and $GeTe_4$ (red) atomic structures are observed. The map is color-coded according to the FFT reported as inset.

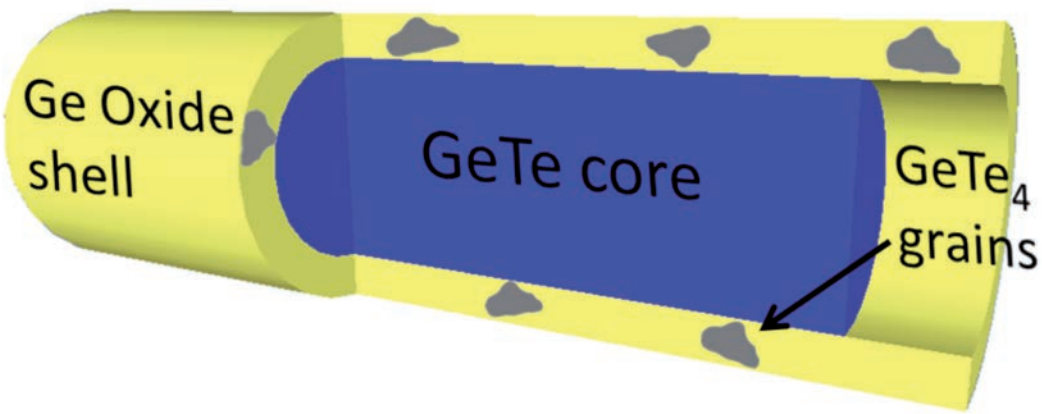

Figure 5. A graphical representation of the ageing process.

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