microscopie

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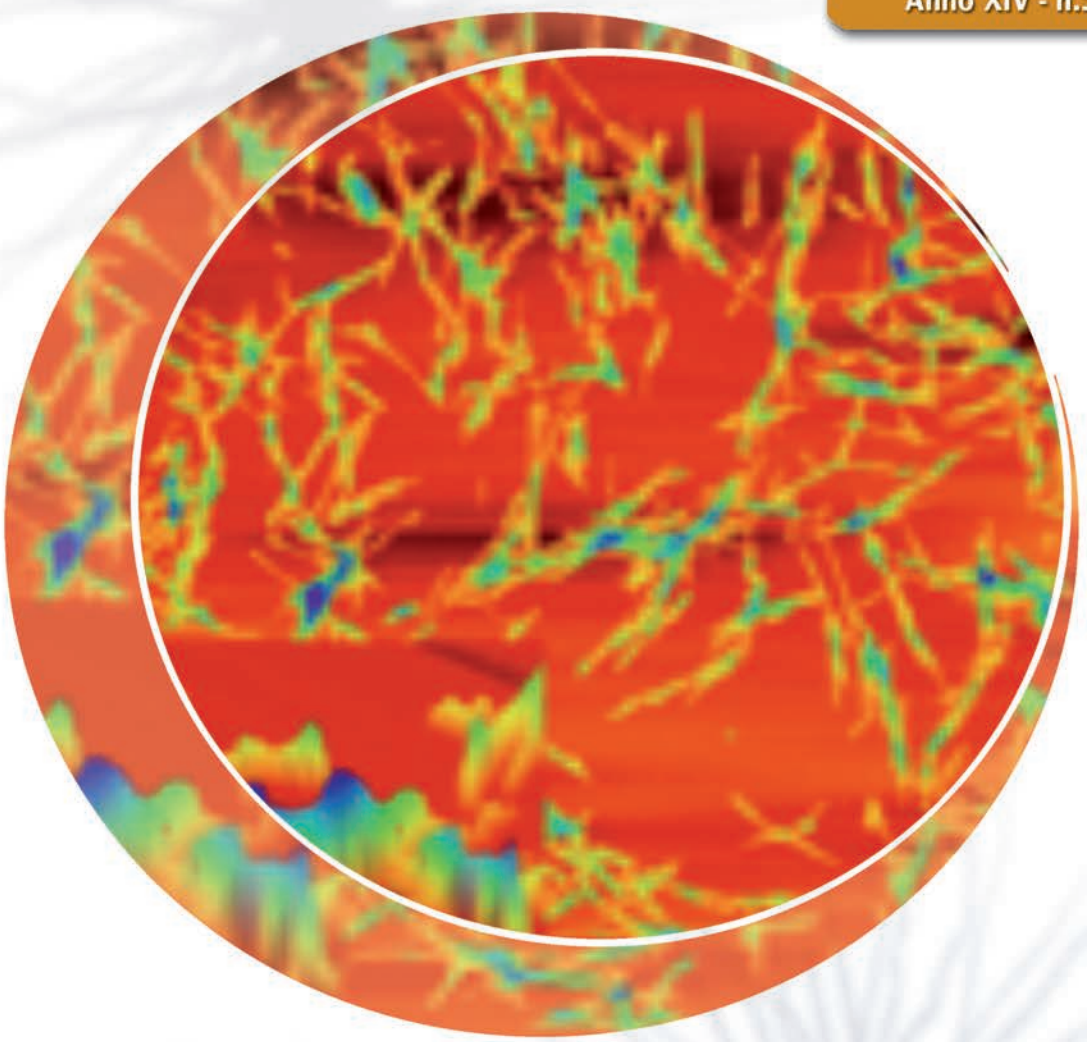

Attività SISM 2017

Bando premi per MCM 2017

Bando premi tesi di dottorato

Auguri al Prof. Ugo Valdrè

Contributi scientifici StSPM'16

Contributi Workshop Beni Culturali

Società Italiana
Scienze Microscopiche

www.sism.it

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http://www.sism.it

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In copertina: *Dettaglio di fibrille amiloidi in AFM* (Bednarikova *et al.*).

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

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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:
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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,

L'attività scientifica della Società si è aperta nel 2016 con il workshop su "La microscopia elettronica SEM/ESEM nello studio dell'ambiente", dal 14 al 16 marzo 2016. Questo evento, il primo in questo ambito, è stato organizzato presso l'Università di Urbino e, in parte, presso l'ARPAM di Pesaro. Il 14 e il 15 marzo ci sono stati, presso l'Aula Magna del Campus Scientifico, interventi di vari relatori, provenienti da diverse sedi italiane, sulla microscopia applicata a fattori abiotici, in ambiente acquatico e in ambiente terrestre. Il secondo giorno, oltre alle relazioni, una sessione è stata dedicata alle Aziende del settore e una ad esercitazioni pratiche in laboratorio. Il 16 marzo il workshop è continuato con una sessione teorico-pratica presso i laboratori ESEM dell'ARPAM di Pesaro. I contributi delle relazioni e dei poster sono stati pubblicati sul fascicolo di settembre di *Microscopie*.

A Pavia, il 7-8 luglio 2016, la SISM è stata parte attiva nel simposio "Nuclear structure and dynamics, through the microscopes". L'evento, organizzato da Manuela Malatesta, Carlo Pellicciari e Marco Biggiogera, con il supporto della Società Italiana di Istochimica, si è svolto presso il Collegio Volta, in cui sono stati ospitati relatori e iscritti. Gli abstract delle presentazioni, che hanno coperto numerosi aspetti delle strutture e delle funzioni del nucleo cellulare, sono stati pubblicati, come nel caso precedente, nel fascicolo di *Microscopie* di settembre 2016.

Come ormai da anni, presso il CIGS dell'Università di Modena, si è tenuto, dal 22 al 24 settembre 2016, il "Corso Base integrato di Microscopia Confocale e Microscopia Elettronica TEM/SEM". L'organizzazione, che ha visto il raggiungimento del massimo numero previsto di iscritti, è stata curata da Massimo Tonelli e da Andrea Tombesi.

Il 20 e il 21 ottobre 2016, Cristiano Albonetti ha organizzato, presso l'Area della Ricerca CNR di Bologna, il workshop "Science through Scanning Probe Microscopy" di cui, in questo stesso fascicolo, vengono dati ulteriori dettagli, oltre che pubblicati i minipapers dei partecipanti.

Con il patrocinio SISM, il 18 aprile 2016 si è svolta, presso l'ISS di Roma, la giornata su "Sopravvivenza e morte cellulare: nuove acquisizioni e ricadute applicative", organizzata da Stefania Meschini. La SISM ha patrocinato anche "Nanomedicine Viterbo 2016", evento organizzato dall'Università della Tuscia il 21-23 settembre 2016.

Ancora una volta, dal 14 al 18 novembre 2016 e dal 6 al 10 febbraio 2017, la Scuola TEM "Pier Giorgio Merli" è stata organizzata da Roberto Balboni presso i laboratori CNR-IIM di Bologna. Anche in questa edizione c'è stata un'ampia partecipazione internazionale.

Nel corso del 2016 sono state concluse le attività connesse con i quattro Premi SISM di 500 euro per Tesi di Dottorato: i vincitori, dottori G. Fisichella (Catania) e M.C. Spadaro (Losanna), dell'ambito Scienza dei Materiali, e V. Palmieri (Roma) e L. Rosati (Napoli), dell'ambito Biomedico, hanno potuto utilizzare il premio per partecipare ad eventi nazionali ed internazionali inerenti l'argomento della tesi di Dottorato. Visto il successo dell'iniziativa, abbiamo riproposto il bando anche nel 2017, con scadenza 30 aprile 2017.

Infine, ricordo a tutti che, dal 24 al 29 settembre 2017, si svolgerà a Rovinj (Croatia) il 13th Multinational Congress on Microscopy, organizzato, come è noto, da otto società (ASEM, CMS, CSMS, HSM, SISM, SSM, SDM, TEMD). La SISM è stata da subito coinvolta dagli organizzatori e quasi tutti i membri del Consiglio direttivo sono chairperson nelle sessioni di riferimento. Proprio in questo mese di marzo sono in corso i contatti con potenziali relatori invitati, con l'intento condiviso di valorizzare il congresso, e, soprattutto, dare spazio a giovani ricercatori.

Editoriale

Una particolarità del MCM 2017 sarà un Meeting Satellite organizzato da Regina Ciancio presso il laboratorio del Sincrotone a Trieste il 23 settembre 2017: la proposta di questo evento, dal titolo "Combining electrons with X-rays for integrated in-operando experiments" è stata accettata con grande interesse dagli organizzatori del congresso, e la realizzazione del Meeting è in progress a Trieste.

Anche per il congresso di Rovinj, la SISM ha bandito 10 premi di partecipazione di 750 euro ciascuno, con l'intento di favorire la partecipazione di ricercatori italiani non strutturati: la scadenza è il 15 maggio 2017…. approfittatene!

Speriamo quindi, con il nostro supporto e con queste iniziative, di contribuire alla riuscita di questo evento e di poter contare su una grande partecipazione di giovani ricercatori italiani.

Non mi resta che chiedere a tutti un aiuto nel diffondere queste nostre proposte in cui, pur nelle difficoltà che le scarse risorse e gli appesantimenti burocratici ci creano, continuiamo a credere.

Ringrazio i nuovi soci, che, in occasione delle nostre articolate attività, si iscrivono, entrando a far parte della nostra vivace comunità. E ringrazio i Soci storici, così come le Aziende che, in vario modo, ci supportano da sempre. Un caro saluto a tutti.

Elisabetta Falcieri

Editoriale

Cari Soci,

Il 2017 sarà un anno importante per la nostra Rivista! A partire da questo numero, infatti, *Microscopie* diventerà, nella sua interezza, una rivista on-line, mentre continuerà ad essere pubblicato in forma cartacea un limitato numero di copie, per il deposito legale dei documenti di interesse culturale destinati all'uso pubblico o riservate alle Aziende che sponsorizzano le attività della SISM.

È una novità importante, decisa dal Consiglio direttivo della Società per rendere *Microscopie* sempre più moderna, ed è solo il primo passo di una progressiva trasformazione della rivista verso una forma editoriale più dinamica e fruibile. Ciò avverrà contemporaneamente all'aggiornamento della pagina web, che permetterà di trasmettere ai Soci, con maggiore completezza e tempestività, tutte le notizie sulle iniziative scientifiche, tecniche e di formazione offerte dalla SISM, dalla EMS e dalle altre Società Italiane e straniere che si occupano di microscopia.

Microscopie manterrà il suo ruolo informativo sui lavori del Consiglio Direttivo e sulle attività nazionali ed internazionali in campo microscopico, accanto a quello più strettamente scientifico. L'accesso alle informazioni riservate (delibere del Consiglio, verbali e bilanci societari) sarà consentito solo ai Soci, attraverso l'uso di una password che verrà loro trasmessa, mentre saranno visibili a tutti gli utenti i resoconti dei Corsi e delle Scuole, e gli articoli scientifici di natura sperimentale e tecnico-strumentale.

È negli auspici del Consiglio Direttivo che, intraprendendo un'operazione di aggiornamento, la nostra Rivista divenga ancor più lo strumento che i Soci utilizzano per divulgare i risultati della propria attività scientifica e per illustrare le proprie iniziative didattiche e formative.

Come è ormai consuetudine, anche questo primo numero dell'anno contiene gli atti di due importanti workshop teorico-pratici SISM, svoltisi negli scorsi mesi: "Science through Scanning Probe Microscopy 2016 (StSPM'16)", svoltosi a Bologna il 20-21 Ottobre 2016, in collaborazione con l'Istituto per lo Studio dei Materiali Nanostrutturati (ISMN) del CNR (Area della Ricerca di Bologna), sotto la direzione scientifica di Cristiano Albonetti, Francesco Valle e Marco Brucale, e "La Microscopia Elettronica applicata allo studio dei Beni Culturali", tenutosi a Urbino il 6-7 febbraio 2017, per l'organizzazione di Elisabetta Falcieri e Laura Baratin. Per entrambi i workshop, i testi degli interventi sono riassunti come *mini-lavori*, spesso corredati da immagini fotografiche e grafici, in forma dunque ampia ed esauriente. La parte scientifica è completata da una minireview sul ruolo delle tecniche microscopiche in nanomedicina, un campo in grande sviluppo e di estrema attualità.

In questo fascicolo festeggiamo anche i novant'anni del Professor Ugo Valdrè, eminente figura di scienziato che tanto ha dato anche alla nostra Società, sin dai lontani anni nei quali ancora ci chiamavamo Società Italiana di Microscopia Elettronica.

Editoriale

Nel breve articolo di Marco Vittori Antisari e Giulio Pozzi viene affettuosamente ricordata la carismatica figura del Professore che, negli anni cinquanta del secolo scorso, fondò il Laboratorio di Microscopia Elettronica presso l'Istituto di Fisica "A. Righi" di Bologna, contribuendo grandemente allo sviluppo della microscopia elettronica in Italia, specialmente nel campo dell'ottica elettronica e della fisica dei materiali. Anche da parte della redazione di *Microscopie*, i più sinceri auguri, Professore!

E a tutti Voi Soci, l'augurio di buon lavoro in questo 2017 e di buona lettura virtuale!!!

Manuela Malatesta

Consiglio direttivo della SISM

Verbale della riunione del 13 maggio 2016

Il giorno 13 maggio 2016 alle ore 10:45 presso il Dipartimento di Scienze Anatomiche Umane, in Via Irnerio, 48 a Bologna, si è svolta in aula E una riunione del Consiglio Direttivo SISM, per discutere il seguente OdG:

1. Approvazione del verbale della riunione precedente (in allegato)
2. Valutazione candidatura Catania per EMC 2020
3. Aggiornamento gestione amministrativa della Società
4. Aggiornamento sito web della Società
5. Valutazione domande e graduatoria Premi dottorato SISM
6. Approvazione ammissione nuovi Soci.
7. Varie ed eventuali.

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

1. Il verbale della riunione del Consiglio Direttivo del 8 febbraio 2016 viene approvato all'unanimità.

2. Il Direttivo è concorde con la candidatura di Giuseppe Nicotra (CNR Catania) per l'organizzazione del EMC 2020. Alle 14:20 dello stesso giorno, il dr. Nicotra, in presenza dei membri del Direttivo e di qualche responsabile aziendale, ha illustrato la sua candidatura che presenterà all'ECM 2016 a Lione. I membri del CD hanno ampiamente commentato, e in particolare, è emerso il suggerimento di organizzare due meeting satellite sulla microscopia applicata alle nanotecnologie e sulla quella avanzata in 3D, con il coinvolgimento scientifico e organizzativo di tutti i membri del CD. Il dr. Nicotra ha anche chiesto di poter investire un piccolo budget (circa 1000 euro) per la promozione della proposta al EMC 2020.
   Il CD approva all'unanimità.

3. Il Presidente riferisce sulla situazione economica della società, illustrando un bilancio positivo di circa 25000 euro.

4. Balboni riferisce sulla situazione del nuovo sito web della SISM (a cura della società Seahorse) che è in fase di definizione.

5. Il CD valuta le domande pervenute per il premio bandito dalla SISM sulle quattro migliori tesi di dottorato. Le domande sono 8 divise in Scienze Biomediche e Scienze dei Materiali. Vengono valutati i curricula di ogni singolo partecipante. I contributi di 500 euro verranno destinati in seguito a rilascio da parte dei vincitori dell'attestato di partecipazione all'evento prescelto.

6. Nuovi Soci: Valentina Bonanni, Fabio Brazzatti, Elvira Brunelli, Simone Dal Zilio, Elena Bianca Donetti, Silvio Mario Luciano Greco, Antonello Guardia, Rachele Macirella, Alberto Mariani, Marta Marmiroli, Alessia Matruglio, Manuela Nazzari, Daniela Parrinello, Luca Piantanida.

7. Il CD valuta l'opportunità di pubblicare Microscopie solo nella versione online in quanto le copie stampate sono eccessive in rapporto al numero di soci in regola. Il presidente contatterà la responsabile del giornale, per chiederle di valutare con la casa editrice le seguenti soluzioni:
   - mettere tutto on-line
   - fare le copie cartacee solo per i soci realmente paganti (circa una cinquantina....su 350, purtroppo)
   - prevedere una copia cartacea solo in occasione di particolarità a cui è bene dare un certo valore (ricorrenze, celebrazioni, attività della Società).

   È stato, inoltre, discusso il rapporto con l'EMS e la convenienza della SISM di pagare annualmente una quota pari a 1770 euro. L'Italia, infatti viene sempre meno considerata nei congressi ECM (Lione, Manchester…). Si propone di pagare la quota e concordare una strategia al riguardo.

Alle ore 15:30, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta.

Roberto Balboni
Regina Ciancio
Elisabetta Falcieri
Stefania Meschini
Massimo Tonelli

Consiglio direttivo della SISM

Verbale della riunione del 24 ottobre 2016

Il giorno 24 ottobre 2016 alle ore 10.30 presso il Dipartimento di Scienze Anatomiche Umane, in Via Irnerio, 48 a Bologna, si è svolta in aula E, una riunione del Consiglio Direttivo SISM, per discutere il seguente OdG:

1. Approvazione del verbale della riunione precedente (in allegato)
2. Candidatura Catania per EMC 2020
3. Situazione finanziaria della Società
4. Aggiornamento sito web della Società
5. Microscopie
6. Borse di partecipazione al MCM2017 a Rovigno
7. Approvazione ammissione nuovi Soci.
8. Varie ed eventuali.

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

1. Il verbale della riunione del Consiglio Direttivo del 13 maggio 2016 viene approvato all'unanimità.

2. Il Direttivo discute sull'esito sfavorevole della candidatura del dr. Giuseppe Nicotra (CNR Catania) per l'organizzazione del EMC 2020. I vicepresidenti, presenti a Lione, riferiscono al CD sulla presentazione di Nicotra, non consona alle aspettative, fuori dai tempi tecnici, non competitiva con le altre candidature e, per questo, non sostenuta dagli stessi. S. Meschini ringrazia R. Balboni di aver gestito in maniera diplomatica la situazione con una lettera di spiegazione inviata allo stesso G. Nicotra e a tutto il CD. Il presidente riferisce di aver avuto una lunga telefonata con G. Nicotra, al quale piacerebbe in qualche modo collaborare con la Società. R. Ciancio ritiene che Nicotra abbia visto nei vicepresidenti i veri ostacoli alla sua candidatura. M. Tonelli, pur apprezzando l'entusiasmo, la ritiene poco matura rispetto a quella dei competitor. M. Malatesta suggerisce di considerare questa esperienza come spunto di autocritica. Il CD ritiene conclusa la vicenda lasciando aperta a Nicotra la possibilità di collaborare e proporre attività legate all'ambito della microscopia.

3. Il Presidente riferisce sulla situazione economica della società, illustrando un bilancio positivo di circa 26800 euro, che, oltre che dai soci, provengono dagli sponsor e sono frutto degli eventi organizzati dalla SISM. M. Tonelli riferisce sul "Corso Base integrato di microscopia confocale e microscopia elettronica TEM/STEM", svoltosi a Modena nei giorni 21-23 settembre, che ha raggiunto il numero di iscritti previsto. C. Albonetti riporta i dettagli della scuola "Science through Scanning Probe Microscopy 2016 (StSPM'16)" che si è svolta a Bologna nei giorni 20-21 ottobre e che ha visto la partecipazione di circa 50 ricercatori. Infine, R. Balboni riferisce di aver raggiunto il numero di iscritti per la Scuola TEM "Pier Giorgio Merli" che si terrà a Bologna nei giorni 14-18 novembre.

4. R. Balboni riferisce sulla situazione del nuovo sito web della SISM (a cura della società Seahorse). In particolare, le difficoltà di comunicazione non hanno permesso ancora alcuna modifica. Si decide all'unanimità di ricontattare il responsabile della Seahorse e, in caso di non collaborazione, di rivolgersi ad un altro operatore. La presenza di un sito web moderno ed efficace è, ora più che mai, indispensabile e urgente.

5. M. Malatesta riferisce che la PagePress farà un preventivo per mettere Microscopie solo online, che dovrebbe aggirarsi sui 1460 euro comprensivi di IVA, permettendo di far risparmiare alla società circa 1000 euro l'anno. La quota sarà comprensiva di 25 copie stampate che potranno essere utili quale pubblicità nel corso dei vari eventi. Nell'occasione, R. Ciancio propone di pubblicare online con una certa periodicità (bimensile o trimestrale, da concordare) una Newsletter - per valorizzare la vivacità della Società - da inviare sia ai soci sia ai partecipanti alle singole scuole. Di questi ultimi suggerisce di creare una mailing list separata dai Soci.
   Il CD approva all'unanimità.

6. Il CD decide di bandire, per il prossimo MCM, che si terrà a Rovigno nel settembre 2017, 10 borse di partecipazione dell'importo di 750 euro divise equamente tra Scienze della Vita e quelle dei Materiali. Inoltre, propone, visto il successo della prima edizione, di bandire di nuovo 4 premi di 500 euro alle migliori tesi dottorato per favorire la partecipazione di giovani ricercatori a scuole, congressi e stage. I dottori di ricerca dovranno aver conseguito il titolo al massimo 3 anni prima dalla data del bando. Inoltre, il CD concorda sulla possibilità che si possono vincere i due premi dalla stessa persona. Nell'occasione del congresso di Rovigno e vista la vicinanza con Trieste (circa 40 km), R. Ciancio proporrebbe di organizzare un workshop satellite. Il CD approva e lascia alla dr.ssa Ciancio il compito di pensare all'organizzazione.

7. Nuovi Soci: Alzari Valeria, Carton Flavia, Catara Stefania, De Stefanis Cristiano, Ferlenghi Ilaria, Marotta Roberto, Pascucci Luisa, Zanotti Fregonara Carlo

8. Il presidente riferisce sugli eventi che saranno organizzati nel 2017.
   Ricorda al CD lo spostamento del workshop " Microscopia Elettronica applicata allo studio dei Beni Culturali" che sarà organizzato ad Urbino il 6-7 febbraio 2017.
   La seconda settimana della scuola TEM "Pier Giorgio Merli" organizzata a Bologna, come previsto verrà organizzata il 6-10 febbraio da Balboni.
   M. Tonelli, dietro suggerimento di alcuni partecipanti al corso di Modena dello scorso settembre, propone un evento sulla microscopia applicata alla botanica: i dettagli verranno riportati alla prossima riunione del CD. Inoltre, verrà di nuovo organizzato il Corso Base integrato di microscopia confocale e microscopia elettronica TEM/STEM che si terrà a Modena nel settembre 2017.
   S. Meschini riferisce sull'evento che organizzerà a fine 2017 presso l'ISS di Roma, che potrebbe avere carattere trasversale tra la microscopia correlativa e la criomicroscopia. R. Ciancio le suggerisce di focalizzarsi su un solo topic, coinvolgendo personalità di spicco, e di mettere un numero massimo di partecipanti provenienti dal ISS. Il CD è concorde nel concentrarsi sulla microscopia correlativa e appoggia l'iniziativa proposta da S. Meschini quale evento SISM. A gennaio la dr.ssa Meschini riferirà gli aggiornamenti.
   C. Albonetti propone di organizzare il prossimo Multinational Congress (MCM 2019) a Bologna, e presenterà la sua candidatura a Rovigno. Il CD approva all'unanimità e ne sostiene la candidatura. Il presidente suggerisce però ad Albonetti di coinvolgere i microscopisti di Bologna di ambito biomedico per organizzare il settore Life Sciences, di notevole rilevanza in questi congressi.
   Inoltre, Albonetti propone di organizzare a Bologna una scuola SEM nel 2017.
   Nel 2017 a Rovigno verrà convocata l'assemblea generale SISM per l'elezione del nuovo CD.
   R. Balboni riferisce che sta maturando la possibilità di organizzare le prossime elezione online.
   Balboni riferisce, inoltre, che ZEISS è diventata ZEISS Italia, per cui ASSING ha trasferito materiale e strumentazioni di microscopia elettronica a questa nuova azienda.
   Infine, E. Falcieri riferisce di essere in una COST action, che finanzia la mobilità di giovani ricercatori e sponsorizza eventi, ritenendola una buona opportunità anche per la Società.

   Alle ore 14:00, null'altro essendovi da deliberare, il Presidente dichiara chiusa la seduta.

Cristiano Albonetti
Roberto Balboni
Regina Ciancio
Elisabetta Falcieri
Manuela Malatesta
Stefania Meschini
Massimo Tonelli

Elenco delle attività promosse dalla SISM nel 2017

Workshop: "La microscopia elettronica applicata allo studio dei Beni Culturali", Università degli Studi di Urbino (Urbino, 6-7 febbraio 2017)
Con il supporto del Dipartimento di Scienze Biomolecolari dell'Università degli Studi di Urbino "Carlo Bo", la SISM organizzerà la terza edizione di questo evento, presso il Campus Scientifico di Urbino. Esercitazioni pratiche saranno dedicate alla preparazione e osservazione dei campioni. Verrà inoltre organizzata una sessione di poster e i contributi scientifici saranno pubblicati su Microscopie.
Per informazioni: Prof.ssa Elisabetta Falcieri (*elisabetta.falcieri@uniurb.it*)

"Microscopia applicata alla botanica" organizzata dal Dott. M. Tonelli, presso l'Università degli Studi di Modena e Reggio Emilia (organizzazione in progress, fine anno 2017)
Per informazioni: Dott. Massimo Tonelli (*massimo.tonelli@unimore.it*)

Corso Base integrato di microscopia confocale e microscopia elettronica a trasmissione, Modena, C.I.G.S. (Università degli Studi di Modena e Reggio Emilia) Settembre 2017
La SISM, con il supporto della Società Italiana di Immunobiologia Comparata e dello Sviluppo (SIICS), del Dipartimento di Scienze della Vita e del Centro Interdipartimentale Grandi Strumenti (C.I.G.S.) dell'Università degli Studi di Modena e Reggio Emilia, organizza due corsi integrati che sono indirizzati a tutti coloro che sono interessati ad acquisire gli elementi di base di entrambe le tecniche di indagine, con particolare riferimento all'ambito biomedico. I corsi prevedono sia sessioni teoriche che pratiche, più una sessione, comune ad entrambi i corsi, dedicata all'utilizzo e alla gestione delle immagini digitali.
Per informazioni: Dott. Davide Malagoli (*davide.malagoli@unimore.it*)

"Correlative microscopy in life and material sciences", evento organizzato dalla Dott.ssa S. Meschini presso l'Istituto Superiore di Sanità, Roma (organizzazione in progress, prima metà di novembre)
Per informazioni: Dott.ssa Stefania Meschini (*stefania.meschini@iss.it*)

"Scuola SEM in Scienze dei Materiali", evento organizzato dal Dott. R. Balboni, Dott. V. Morandi e Dott.ssa R. Ciancio presso CNR-IMM, Bologna (organizzazione in progress, seconda metà di novembre)
Per informazioni: Dott. Roberto Balboni (*balboni@bo.imm.cnr.it*)

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

PREMI DI PARTECIPAZIONE AL 13th MCM 2017, ROVINJ (Croatia)

Si comunica che la Società Italiana Scienze Microscopiche in collaborazione con le Aziende del settore della Microscopia, bandisce

n. 10 PREMI

dell'importo di **€ 750,00** ciascuno, per favorire la partecipazione di ricercatori italiani al MCM2017 (www.mcm2017.irb.hr) che si terrà in Croazia a Rovinj, dal 24 al 29 settembre 2017.

I Premi sono riservati a **ricercatori non strutturati**.

I partecipanti devono inviare **entro il 15 maggio p.v.** (scadenza anche del congresso):

1) **Domanda**
2) Copia dell'**Abstract** (completo di tutto) inviato al Congresso
3) Un **Curriculum Vitae** di massimo due pagine con autocertificazione della propria posizione lavorativa.

L' iscrizione alla SISM, alla data della domanda, a parità di giudizio, costituirà titolo preferenziale.

Per partecipare alla selezione del bando, che verrà effettuata a giudizio insindacabile del Consiglio Direttivo, la documentazione richiesta va inviata per e-mail al Presidente della SISM, prof.ssa Elisabetta Falcieri (elisabetta.falcieri@uniurb.it)
Al ricevimento della documentazione verrà inviata una e-mail di conferma dell' avvenuta ricezione.
E' fatto obbligo, per i vincitori, di essere presenti per tutta la durata del Congresso, pena esclusione dalla graduatoria. Inoltre, i vincitori saranno tenuti ad inviare i propri contributi scientifici che saranno pubblicati sulla rivista Microscopie.
I risultati della selezione verranno comunicati per e-mail ai candidati e pubblicizzati sulla pagina web della SISM, all' indirizzo www.sism.it

Urbino, 16 gennaio 2017

Il presidente SISM
Elisabetta Falcieri

PRESIDENTE
Prof.ssa Elisabetta Falcieri
Dipartimento di Scienze delle Terra, della Vita e dell' Ambiente (DiSTeVA) - Università degli Studi di Urbino Carlo Bo, Campus Scientifico "E.Mattei"
Via Ca' le Suore 2, località Crocicchia, 61029 Urbino
Tel. 0722-304284 Email: elisabetta.falcieri@uniurb.it
Sede Sociale S.I.S.M:
Dipartimento di Scienze delle Terra, della Vita e dell' Ambiente (DiSTeVA) - Università degli Studi di Urbino Carlo Bo, Campus Scientifico "E.Mattei", Via Ca' le Suore 2, località Crocicchia, 61029 Urbino
SISM P.IVA 05089821002 C.F. 80181630155

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

PREMI SISM TESI DI DOTTORATO

La Società Italiana Scienze Microscopiche bandisce

4 PREMI dell'importo di **€ 500,00** ciascuno

a 4 tesi di dottorato (2 nell'ambito delle Scienze Biomediche e 2 nell'ambito delle Scienze dei Materiali, discusse negli anni 2014, 2015 e 2016) nelle quali la microscopia, in tutte le sue forme e applicazioni, sia parte integrante e rilevante.

Il premio sarà finalizzato **alla partecipazione**, entro un anno dalla data di scadenza del bando, **ad un evento correlato nazionale o internazionale.**

I partecipanti devono inviare **entro il 30 aprile 2017**:

1) **domanda**
2) **copia della tesi di dottorato** (in formato pdf)
3) **curriculum vitae** di massimo due pagine, con autocertificazione della propria posizione.
4) indicazione dell'**evento** a cui si intende partecipare

L' iscrizione alla SISM, alla data della domanda, a parità di giudizio, costituirà titolo preferenziale.

Per partecipare alla selezione del bando, che verrà effettuata a giudizio insindacabile del Consiglio Direttivo, la documentazione richiesta va inviata per e-mail al presidente della SISM, prof.ssa Elisabetta Falcieri (elisabetta.falcieri@uniurb.it)

Al ricevimento della documentazione verrà inviata una e-mail di conferma dell'avvenuta ricezione.

I risultati della selezione verranno comunicati per e-mail ai candidati e pubblicizzati sulla pagina web della SISM, all' indirizzo www.sism.it

Elisabetta Falcieri

Urbino, 16 gennaio 2017

Eventi nazionali

2017

Corso di perfezionamento in Citologia Diagnostica
Università Cattolica del Sacro Cuore
Roma, 23 Febbraio - 14 Luglio 2017
http://roma.unicatt.it/2016-2017-citologia-diagnostica
Informazioni: *segreteria.corsiperfezionamento-rm@unicatt.it*

Advanced fluorescence application in biotechnology & biology
Consiglio Nazionale delle Ricerche
Roma, 3-4 Maggio 2017
https://www.cnr.it/en/event/14988/advanced-fluorescence-application-in-biotechnology-biology

FOTONICA 2017 - 19° Convegno Italiano di Tecnologie Fotoniche
Associazione Italiana di Elettrotecnica, Elettronica, Automazione, Informatica e Telecomunicazioni
Padova, 3-5 Maggio 2017
http://convegni.aeit.it/fotonica2017/

Seconda Riunione Nazionale ISCCA
Società Italiana per l'Analisi Citometrica Cellulare (ISCCA)
Bologna, 8-10 Maggio 2017
http://www.iscca.eu/Meeting.aspx

Tirocinio di citologia cervico-vaginale avanzato
Istituto per lo Studio e la Prevenzione Oncologica (ISPO)
Firenze, 24-26 maggio e 13-15 novembre 2017
http://www.citologia.org/index.php/altri-eventi/53-seminario-ispo-2

44th Meeting of the SCUR
Society for Cutaneous Ultrastructure Research - The Skin Imaging Society
Milano, 8-10 giugno 2017
www.scur.org

Corso Avanzato di Citologia
Unione Italiana Società Veterinarie (UNISVET)
Milano, 17-19 Giugno e 1-3 Luglio 2017
http://www.unisvet.it/corso.php?id=406

Corso "toolbox" La condotta e la gestione dei citometri a flusso convenzionali e del relativo reagentario
Società Italiana per l'Analisi Citometrica Cellulare (ISCCA)
Urbino, 19 - 23 Giugno 2017
http://www.iscca.eu/Didattica.aspx#

Terza edizione della Scuola permanente di Citologia
Ordine Nazionale dei Biologi
Roma, Settembre 2017 - Febbraio 2018
http://www.onb.it/2017/03/14/terza-edizione-della-scuola-permanente-di-citologia/

71° Congresso Nazionale della Società Italiana di Anatomia e Istologia (SIAI)
Taormina, 20-22 settembre 2017
www.siai.unifi.it

37° Congresso Nazionale della Società Italiana di Istochimica (SII)
Taormina, 22-23 settembre 2017
www.siai.unifi.it

La Microscopia FT-IR (Fourier transform infrared microscopy)
Bruker Corporation
Milano, 28 settembre 2017
https://www.bruker.com/about-us/offices/local-offices-web-pages/italy/corsi-di-formazione/la-microscopia-ft-ir.html

XXXV Conferenza Nazionale di Citometria - Scuola Nazionale di Citometria
Gruppo Italiano di Citometria - GIC
Paestum (SA), 3-6 ottobre 2017
http://gic.casaccia.enea.it/giconf/GIConf-2017/Annuncio%20Conferenza%20GIC%20Paestum%202017.pdf

Society for Cutaneous Ultrastructure Research - The Skin Imaging Society

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE BIOMEDICHE PER LA SALUTE

SIAI Società Italiana di Anatomia e Istologia

Società italiana di istochimica

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

INSIDE OUT
OUTSIDE IN
THE SKIN

44th meeting of the SCUR
Milan, 8-10th june 2017

Meeting President: E.B. Donetti

SCUR Committee: V. Huck, R. Abdayem, M. Bedoni, A. Ishiko, W. Muss, F. Quondamatteo

Scientific Committee: E. Berti, E. Donetti, F. Prignano, S. Veraldi

Organizing Committee: L. Cornaghi, F. Arnaboldi

Thursday, 8th june (Aula Napoleonica)

14.00 - 15.00: Registration

15.00: Welcome adddress

15.30 - 16.30: Opening Lecture: Electron microscopy in chronic hystiocytoses (E. Berti, University of Milan)

16.30 - 17.30: Laser Microdissection: A bridge from morphology to molecular biology and functional studies (M. Baron, Leica Microsystems)

18 - 21: Walk around downtown Milano and welcome cocktail in Piazza Duomo

Friday, 9th june (Città Studi)

8.30 - 9.30: Registration (Città Studi)

9.30 - 10.00: Reflectance Confocal Microscopy in monitoring medical treatment modalities for actinic keratoses and field of cancerization (L. Bianchi, University of Rome "Tor Vergata")

10.00 - 11.00: Oral presentations

11.00 - 11.30: Coffee break

11.30 - 12.00: Ultrastructure in genodermatoses (G. Tadini, University of Parma)

12.15 - 12.45: Oral presentations

12.45 - 13.45: Lunch

13.45 - 14.30: SCUR Assembly

14.30 - 15.30: Poster Presentation

15.30 - 16.00: Coffee break

16.00 - 16.30: Dendritic cells and psoriasis: a never end story (F. Prignano, University of Florence)

16.30 - 17.30: Oral presentations

19.00 - 20.00: Happy hour on the boat on Navigli

20.30: Gala Dinner at "El Brellin"

Saturday, 10th june (Città Studi)

9.30 - 10.00: Elasticity in skin physiopathology (D. Quaglino, University of Modena – Reggio Emilia)

10.00 - 10.45: Oral presentations

10.45 - 11.15: Coffee break

11.15 - 11.45: Epithelial-to-mesenchymal transition: the good and the bad in skin biology (N. Gagliano, University of Milan)

11.45 - 12.30: Oral presentations

12.30 - 13.00: Awards and Closing remarks

GENERAL INFORMATION (www.scur.org)

Key dates

- Early registration until 31 march 2017
- Late registration until 15 may 2017
- Last day to submit abstract: 15 may 2017 (**scurmilano@scur.org**)

Registration Fees (euros)	Early registration 31 march 2017	Late registration until 15 may 2017
SCUR member	380	450
NON SCUR member	450	500
Student member	250	280
Student non member	300	340
Accompanying person	180	180

The registration fees **include**:

- Admission to all scientific sessions
- Congress materials (congress bag, final program booklet, access badge)
- Welcome Cocktail (Thursday 8 june, registration compulsory)
- Coffee breaks and lunch on Friday 9th june and coffee break on Saturday 10th june
- Happy hour on Navigli and Gala Dinner at "El Brellin" (Friday 9th june, registration compulsory)

The registration fees **does not include** lodging (and its booking); as a participant you will be responsible for booking your own accommodation.

Fee payment

Only bank Transfer (SEPA) can be accepted. Please clearly indicate the reference: SCUR MILANO 2017
BANK: INTESA SANPAOLO S.p.A RETE CA.RI.P.LO.
AGENCY: MILANO, SERVIZIO TESORERIA ENTI, Via Giuseppe Verdi 8, 20121 Milan
CURRENT ACCOUNT: 000000463971
ABI CODE: 03069
CIN CODE: G
IBAN CODE: IT97G0306909400000000463971
BBAN CODE: G0306909400000000463971
SWIFT CODE: BCITITMMXXX
After the fee payment, please send the copy of your bank transfer to **scurmilano@scur.org** with your personal data for the invoice (VAT registration number, fiscal code, residence, date and birthplace).

Official Language

All the congress will be proceeding in **English.** No simultaneous translation will be provided. Papers and materials are published in English.

Abstracts: Abstracts have to be submitted to **scurmilano@scur.org**. Please use the prepared RTF form.

Oral Presentation: 10 minutes + 2 minutes of discussion

Poster: Dimension: 50 cm x 70 cm

Awards: One 'best oral presentation award'; One 'best poster presentation award'; One 'best young scientist presentation award' will be offered at the end of the Meeting.

Travel grants: travel grants can be requested to the Secretary by young Scientists (up to 35 years)

Eventi internazionali

2017

Focus on Microscopy 2017
9 to 12 April 2017
Bordeaux – France

Microscopy of Semiconducting Materials - MSM XX
9 to 13 April 2017
Lady Margaret Hall – Oxford – United Kingdom

Porto AFM Training Workshop 2017
10 to 13 April 2017
Porto – Portugal

Fourth Conference on Frontiers of Aberration Corrected
30 April to 4 May 2017
Kasteel Vaalsbroek – The Netherlands

Advanced Course on Cryo-Electron Tomography
6 to 12 May 2017
Vienna – Austria

HSM 2017 - Annual meeting of the Hungarian Society for Microscopy
11 to 13 May 2017
Siófok, Lake Balaton – Hungary

2nd Slovene Microscopy Symposium
11 and 12 May 2017
PIRAN – Slovenia

EDGE 2017: Enhanced Data Generated by Electrons
14 to 19 May 2017
JAL Okuma Resort – Okinawa – Japan

15th International Congress of Histochemistry and Cytochemistry
18 to 21 May 2017
Antalya – Turkey

23th National Congress of Electron Microscopy
19 to 21 May 2017
Antalya – Turkey

Quantitative Electron Microscopy school
22 May to 2 June 2017
St-Aygulf – France

ISM 2017 - 51st Annual Meeting of the Israel Society for Microscopy
22 and 23 May 2017
Weizmann institute of science – Rehovot – Israel

4th edition of Quantitative Electron Microscopy School QEM2017
22 May to 2 June 2017
Balaruc-les-Bains, Etang de Thau, France

DPC Workshop at Regensburg University
22 and 23 May 2017
Regensburg – Germany

17th International European Light Microscopy Initiative (ELMI) Meeting
23 to 26 May 2017
Dubrovnik – Croatia

Electron Microscopy with High Temporal Resolution EMHTR 2017
29 to 31 May 2017
Strasbourg – France

2nd France-BioImaging CLEM course - Correlative microscopies: theory and applications
29 May to 2 June 2017
Paris – France

CCEM Summer School on Electron Microscopy
5 to 9 June 2017
McMaster University – Canada

SCANDEM 2017 Annual Conference of the Nordic Microscopy Society
5 to 9 June 2017
Reykjavik – Iceland

FEBS Advanced Course "Functional imaging of cellular signals"
11 to 16 June 2017
Amsterdam – The Netherlands

EMAT Workshop on Transmission Electron Microscopy
12 to 23 June 2017
University of Antwerp – Antwerp – Belgium

Summer School in Advanced Light Microscopy 2017
12 to 16 June 2017
Ghent – Zwijnaarde – Belgium

3rd International TEM Spectroscopy Workshop in Materials Science
19 to 22 June 2017
Uppsala – Sweden

Advanced course Correlative Light Electron Microscopy
22 to 27 June 2017
Utrecht – The Netherlands

Microscience Microscopy Congress 2017
3 to 6 July 2017
Manchester – United Kingdom

1st EUFN workshop (Former DACH Workshop): general FIB and FIB applications
4 and 5 July 2017
Graz – Austria

RMS Light Microscopy Summer School
16 to 19 July 2017
York – United Kingdom

RMS Getting the Most from your Confocal Course
20 and 21 July 2017
York – United Kingdom

ESRIC Super-Resolution Summer School 2017
31 July to 4 August 2017
United Kingdom

Microscopy & Microanalysis 2017 Meeting
6 to 10 August 2017
St. Louis – Missouri – USA

CenErgy II: International PhD Summer School on Advanced transmission and scanning electron microscopy in Materials Science
14 to 25 August 2017
Denmark

Microscopy Conference 2017
21 to 25 August 2017
SwissTech Convention Center – Lausanne – Switzerland

Advanced Methods in Biomedical Image Analysis (AMBIA)
3 to 9 September 2017
Brno – Czech Republic

17th Congress of the European Society for Photobiology
4 to 8 September 2017
Pisa – Italy

"Microscopy at the Frontiers of Science 2017" (5th Joint Congress of the Spanish and Portuguese Microscopy Societies)
6 to 8 September 2017
Zaragoza – Spain

RMS Flow Cytometry Course
10 to 15 September 2017
York – United Kingdom

15th Methods and Applications in Fluorescence (MAF15)
10 to 13 September 2017
Bruges – Belgium

12th European Congress for Stereology and Image Analysis (ECSIA)
11 to 14 September 2017
Kaiserslautern – Germany

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

21st EFUG meeting during ESREF : semiconductor and device applications of FIB
25 to 29 September 2017
Bordeaux – France

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.

The MCM 2017 sessions:

Session: Life sciences

L1. Live Cell Imaging and Intracellular Dynamics

L2. High-Resolution Microscopy in Biological Sciences

L3. Structure and Imaging of Biomolecules

L4. Nanobiology and Nanomedicine

L5. Microscopy in Microbiology, Plant and Environmental Sciences

L6. Neuroscience and Histopathology

L7. Multidisciplinary Approaches in Natural and Biomedical Sciences

Session: Instrumentation

I1. Tomography, 3D Imaging and Image Processing, Phase-Related Techniques (including holography, vortex beams, Bessel beams etc.)

I2. In-situ and Environmental Microscopy (including cryo-microscopy, heating, low-vacuum etc.)

I3. Correlative Microscopy and other Imaging Modalities (including AFM, STM etc.)

I4. Light and Electron Optics, Super-Resolution Microscopy

I5. Specimen Preparation Techniques

I6. Advances in Instrumentation and Techniques (including aberration correction, low voltage SEM & TEM etc.)

I7. Electron Spectroscopy, Diffraction and Analytical Microscopy

Session: Materials

M1. Thin Films, Coatings, Surfaces and Interfaces

M2. Polymers, Organic and Soft Materials

M3. Materials in Geology, Mineralogy and Archaeology, Ceramics and Composites

M4. Metals, Alloys and Intermetallics

M5. Nanostructures and Materials for Nanotechnology and other Applications

M6. Semiconductor Materials and Devices

M7. Biomaterials and Biosensors

Important Dates

- Abstract submission site open: January 17, 2017
- Registration site open: December 30, 2016
- Abstract submission deadline: May 15, 2017
- Notification of accepted abstracts: June 15, 2017
- Early registration payment deadline: June 30, 2017
- Preliminary program available online
- Late poster abstract submission: August 20, 2017
- Regular registration and payment deadline: August 20, 2017
- MCM 2017: September 24-29, 2017

Buon Compleanno, Professore!

Il Professore Ugo Valdrè ha compiuto novanta anni proprio lo scorso anno (2016).
Vogliamo qui fare gli auguri al Professore, ancora attivo e che segue da vicino le vicende della nostra Società. A questo scopo, due suoi allievi, Marco Vittori Antisari e Giulio Pozzi, entrambi soci onorari SISM, ricordano in queste righe l'atmosfera del Laboratorio di Microscopia Elettronica dell'Istituto di Fisica di Bologna, diretto dal Professor Valdrè, che è stato riferimento fondamentale per studenti di Scienze dei Materiali e microscopisti elettronici italiani.

La nascita e lo sviluppo iniziale della Microscopia Elettronica in Italia è stata in gran parte favorita ed assistita dalla presenza di due poli di eccellenza, l'Istituto Superiore di Sanità di Roma, principalmente impegnato in campo biomedico, ed il Laboratorio di Microscopia Elettronica presso l'Istituto di Fisica "A. Righi" di Bologna, per il campo dell'ottica elettronica e della fisica dei materiali. Quest'ultimo si è identificato per molti anni anni nella persona del Professor Ugo Valdrè, che lo ha fondato negli anni cinquanta e diretto per quasi mezzo secolo, fino al suo ritiro dalla vita accademica attiva.

Non vogliamo qui ripercorrere la storia dettagliata di queste Istituzioni ed il loro ruolo nello sviluppo della microscopia elettronica nel nostro Paese, aspetti peraltro minuziosamente descritti in un articolo dello stesso Professor Valdrè dal titolo "Electron Microscopy in Italy", pubblicato nel 1996 sulla Rivista Advances in Imaging and Electron Physics, ed. P.W. Hankes, vol. 96, pag. 193-215.

In questo contesto, vorremmo brevemente ripercorrere le sensazioni ed il modo di fare ricerca che si viveva in questo Laboratorio, quando, grazie alla personalità carismatica del Professore, divenne un centro di attrazione e formazione per molti studenti che vedevano nella scienza dei materiali e nella microscopia elettronica un campo affascinante in cui mettersi alla prova. Si trattava, in quegli anni, di ricerche che facevano riferimento a settori nati da relativamente poco tempo, quali l'ottica elettronica e la scienza dei materiali, in fase di rapida evoluzione e di grande interesse a livello internazionale, per cui il Laboratorio di Microscopia Elettronica ben presto si conquistò un suo ruolo ben definito e di grande prestigio all' interno di un Istituto di antiche e ben consolidate tradizioni.

Nel Laboratorio il Professore era la figura di riferimento, circondato da un'aura di rispetto e ammirazione per i suoi successi scientifici, ottenuti per lo più in collaborazione con le prestigiose Università di Cambridge e Oxford. A questi sentimenti si aggiungeva poi un profondo affetto che si veniva portati naturalmente a provare per lui, probabilmente grazie al suo carattere schivo ma sempre paterno e vicino ai collaboratori più giovani.

Il Professore aveva raccolto attorno a sè professionisti di altissimo livello che costituivano uno staff di supporto di assoluto valore ed in grado di completare, con una guidata e competente pratica di laboratorio, la formazione nelle attività sperimentali dei giovani ricercatori. Le ecclettiche competenze di Antonio Grilli, la precisione di Libero Morini, la vulcanica creatività di Teo Bartolucci ed il supporto di progettazione meccanica di Ricciotti, hanno contribuito in maniera sostanziale alla formazione dei giovani che hanno iniziato la loro vita di ricercatori nel Laboratorio del Professor Valdrè.

La stretta connessione, poi, con i laboratori Inglesi e principalmente con il Cavendish di Cambridge assicurava che le tematiche di ricerca fossero di stretta attualità e di grande impatto a livello internazionale.

Come giustamente sottolineato da Pier Giorgio Merli in occasione della nomina del Professor Ugo Valdrè a Socio Onorario della Società Italiana di Microscopia Elettronica avvenuta durante il Congresso della SIME tenutosi a Bologna nel 1985, il Professore ha rappresentato per tutti noi il Maestro Silenzioso e il metro ideale a cui rapportarsi e tendere.

È un dato di fatto che, almeno a nostra memoria, nessuno lo ha mai chiamato per nome, come invece facevano familiarmente i colleghi inglesi e americani che venivano in Italia per partecipare alle Scuole di Microscopia Elettronica di Erice o per tenere seminari a Bologna.

Tuttavia fra di noi (e pensiamo che ne fosse al corrente anche se non lasciava trapelare nulla) lo chiamavamo familiarmente il “babbo”, e chissà quante volte gli saranno fischiate le orecchie quando, a margine di congressi e scuole, ci si trovava attorno ad un tavolo con una bottiglia a discutere di microscopia elettronica, prospettive e sogni.

Per tutti noi il periodo trascorso presso il Laboratorio, che coincideva con quello magico della nostra giovinezza, rappresenta un ricordo indimenticabile, dominato dalla figura del Professore a cui, in questa occasione, con immutato affetto e rispetto diciamo, finalmente:

“Grazie babbo per quanto silenziosamente ci hai saputo dare!”

Marco Vittori Antisari e Giulio Pozzi

Science through Scanning Probe Microscopy 2016 (StSPM'16)

20 – 21 ottobre 2016, Bologna, CNR – Area della Ricerca

Il workshop Science through Scanning Probe Microscopy 2016 (StSPM'16), organizzato dall'Istituto per lo Studio dei Materiali Nanostrutturati (ISMN) e dalla Società Italiana di Scienze Microscopiche (SISM), in collaborazione con l'Area della Ricerca di Bologna, ha replicato il successo dell'edizione 2013 raccogliendo circa 60 ricercatori provenienti da molte regioni italiane, dal sud fino al nord, afferenti ad università, enti di ricerca e laboratori industriali, ma soprattutto ha visto la partecipazione di molti giovani ricercatori.

StSPM'16 ha mostrato una comunità SPM viva, in linea con i più recenti avanzamenti scientifici nelle due macro-aree principali della scienza dei materiali e della vita. Una menzione speciale va a Bruno Samorì, il quale, con eleganza e sobrietà, ha aperto i lavori del workshop raccontando un frammento della storia della microscopia attraverso la sua carriera. Sono stati presentati 29 contributi orali, di cui 9 ad invito per professori e ricercatori di chiara fama. I contributi delle due macro-aree sono stati alternati per mantenere costante l'attenzione dei partecipanti e condensati nel pomeriggio del 20 e nella mattina del 21 Ottobre. L'ambiente collaborativo e spontaneo che si è instaurato ha permesso a tutti i partecipanti, studenti inclusi, di sentirsi liberi di fare domande, proporre idee e discutere.

Di fondamentale importanza alla riuscita dell'evento è stata la partecipazione e il contributo economico delle ditte SPM, sponsor dell'evento e della SISM. In tutto hanno partecipato 11 rappresentanti di 10 ditte connesse alla microscopia a scansione di sonda. La loro partecipazione è stata valorizzata da una sessione speciale dove sono state presentate le ultime novità commerciali in quanto a microscopi, sonde ed accessori. La sessione è stata organizzata in stile "Elevator pitch", ovvero l'oratore doveva descrivere la propria ditta sinteticamente, chiaramente ed efficacemente nei limiti di tempo imposti dalla corsa dell'ascensore (specificatamente 10 minuti). Il risultato è stata una sessione frizzante e snella da seguire.

Infine, seguendo lo spirito educativo della SISM, abbiamo colto l'occasione del workshop per realizzare questi proceeding su "Microscopie", la rivista della società. Al momento, la rivista è indicizzata DOI, ma speriamo di assegnarle un Impact Factor internazionale nel prossimo futuro.

Ringraziamo pubblicamente tutti coloro che hanno partecipato all'organizzazione e hanno supportato l'iniziativa in qualsiasi forma e, per concludere, vi aspettiamo a StSPM'19 dove, siamo convinti, parteciperete con entusiamo e nuovi risutalti scientifici.

Il comitato scientifico

Cristiano Albonetti
Francesco Valle
Marco Brucale

Magnetic force microscopy with controlled magnetization of the tip: Toward truly quantitative nanomagnetometry

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Key words: nanoparticles, Magnetic Force Microscopy, Atomic Force microscopy

Introduction

Magnetic nanoparticles exhibit very particular magnetic properties (superparamagnetic character), which can be exploited in several diagnostic and therapeutic applications.[1] The development and optimization of these systems require a deep understanding of the magnetic behavior of the used nanomaterials, and, therefore, a detailed characterization of their main magnetic properties. NPs systems are conventionally characterized by statistical techniques which allow the measurement of the overall magnetic parameters of numerous ensembles of NPs in the form of patterns or ferrofluids, which, however, do not allow the complete comprehension of all the mechanisms regulating the magnetic NPs system behavior, such as the dependence with other chemical and physical properties (e.g. the composition, the structure, the size, the shape) and the effects of the mutual inter-particles dipolar interactions. For this reason several efforts are focused on the development of high resolution and nano-element sensitive techniques able to measure the magnetic parameters of single nanoparticles and, thus, deepen the understanding of all the factors influencing the magnetic behavior of single elements and affecting the efficiency of the overall system. Among other high resolution techniques, Magnetic Force Microscopy, thanks to its nanometric lateral resolution, high sensitivity, applicability to all kind of magnetic nanomaterials without particular sample preparation and not expensive instrumental apparatus, is emerging as a potential tool for the characterization of single magnetic nanomaterials.

Nevertheless, some open issues have been identified as the main drawbacks limiting the application of the technique to the quantitative magnetic characterization of single magnetic nanoparticles, which can be summarize as follows: i) the presence of non-magnetic tip-sample interactions, which produce an additional signal in MFM measurements, making difficult the extrapolation of the "pure" magnetic contribution and, therefore, the quantitative interpretation of the measured data; ii) the lack of a theoretical model describing the magnetic tip-NPs interactions consistently with experimental data and the consequent difficulty in "converting" the measured data in the values of real physical parameters such as the NP magnetization.[2]

The evidence of the necessity of a methodology to evaluate and eliminate the electrostatic effects in MFM images encouraged us to conceive a new MFM approach, we called Controlled Magnetization MFM (CM-MFM), with the aim of depurating MFM images from electrostatic contributions and detect the pure magnetic signal.

In this work we present a synthesis of the results obtained with CM-MFM technique and its possible applications.

Materials and Methods

In CM-MFM, two subsequent MFM images of the same area are collected. Each MFM image is acquired using the so called "lift height mode". The first MFM image, an example of which is reported in Figure 1 a), is acquired using the probe magnetized along a certain direction, allowing the measurement of the signal resulting by the superimposition of the electrostatic and the magnetic tip-sample interactions. Then, a second image is detected using the probe "demagnetized", allowing the detection of the contribution due to the sole electrostatic effects, as shown in the example reported in Figure 1 b). The image representative of the "pure" magnetic signal is retrieved by subtracting the second image to the first one. An example is reported in Figure 1 c). The in situ demagnetization of the probe (i.e. without moving the probe from the scan area) is obtained through the application of its remanent coercitive magnetic field, determined by a calibration procedure performed using a reference sample with periodically patterned magnetic domains and measuring

the phase contrast in two adjacent domains after applying and switching off magnetic fields with different intensity.[3]

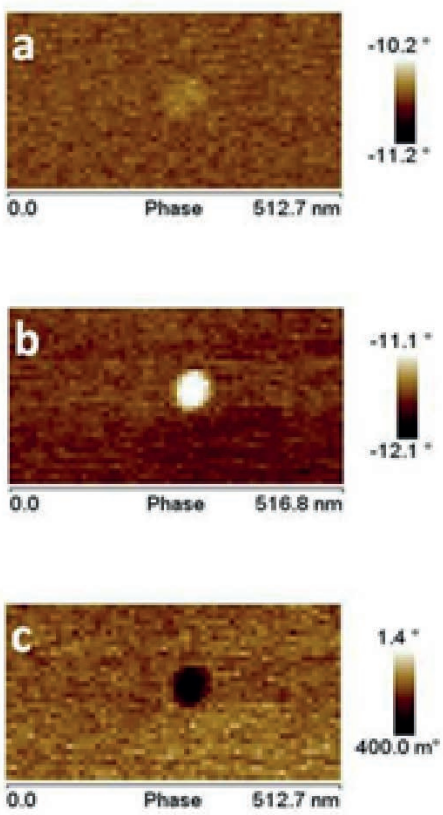

Figure 1. Standard MFM image (a), electrostatic image (b) and CM-MFM image (c) obtained by the subtraction of image (b) to image (a) of a Fe_3O_4 NP of 18nm (diameter).

Results and Conclusions

The effectiveness of CM-MFM technique has been demonstrated through a challenging case study, i.e., the characterization of superparamagnetic NPs in absence of any applied external magnetic field.[3] Once the electrostatic artifacts are removed, the tip-NP interaction has been demonstrated to be well described by that of two single-point magnetic dipoles, indicating the effectiveness of our technique in the removal of electrostatic artifacts in MFM maps and the possibility of retrieving quantitative information about single NPs properties.

As an example, a possible application of CM-MFM consists in the measurement of the thickness of the non-magnetic coating of core-shell magnetic NPs. As a verification of the effectiveness of the technique we carried out a preliminary analysis on two Fe_3O_4 and two Cu-coated Fe_3O_4 NPs. The "dipole model" has been used to calculate the thickness of the coating of the core-shell NPs. The coating thickness values obtained presented good agreement with the average values obtained by the statistical analysis carried out by AFM on the two kinds of NPs. Nevertheless, further more statistically significant analysis needs to be performed in order to assess the accuracy and the reproducibility of the technique.[4]

Another possible application consists in the measurement of the magnetization curve of single magnetic NPs, by performing in-field CM-MFM measurements.

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AFM study of amyloid self-assembly

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Key words: AFM, amyloid, morphology.

Introduction

Formation of non-native conformers of poly/peptides and their subsequent self-assembly into amyloid aggregates is one of the hallmarks of more than 30 amyloid diseases such as Alzheimer's disease or lysozyme systemic amyloidosis. Interestingly, although the amyloid fibrils have similar features, they can differ in morphology, stability and cytotoxicity depending on the conditions of fibrils formation.[1] This polymorphism could be important for understanding the molecular basis and the natural variability of amyloid diseases.

The mechanism of amyloid aggregation is still

poorly understood. Peptide-membrane and peptide-lipid interactions are thought to be crucial in this process[2]. Therefore, we have studied the effect of phospholipid on amyloid aggregation of lysozyme at two concentrations.

Materials and Methods

Atomic Force Microscopy (AFM)

Samples of protein were placed on a freshly cleaved mica surface, let adsorb for 5 min, washed with ultrapure water and left to air dry. Unfiltered AFM images were taken in tapping mode using a Scanning Probe Microscope (Veeco di Innova, Bruker AXS Inc., USA) with an uncoated NCHV cantilever at a scan rate of 0.5 kHz. No smoothing or noise reduction was applied. The image analysis was performed using Gwyddion software.

In vitro amyloid fibrillization of lysozyme

Hen egg white lysozyme amyloid fibrils (LAF) were prepared through the incubation of lysozyme for 2 h at 65°C with constant stirring (1200 rpm) (i) in acidic (pH 2.7 - LAF2) and neutral (pH6.0 - LAF6) conditions or (ii) in the presence of phospholipid DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine). The formation of fibrillar aggregates was confirmed using Thioflavin T fluorescence assay.

Cell culture

LLC-PK1 (porcine epithelial kidney) cells were grown according to their specifications. $5x10^3$ cells/ well in 24 well-plates were incubated in the presence of 0.1, 10 and 100 μg/ml LAF2 or LAF6 for 5 days. The cells were counted using a Bürker-Türk hemacytometer. All experiments were performed in triplicates.

Results and Conclusions

Atomic force microscopy was used to visualize the morphology of the obtained lysozyme fibrils. LAF2 and LAF6 formed fibrils with features typical for amyloid species; however, the morphology was significantly different (Figure 1A). LAF2 represent long fibrillar structures, whereas LAF6 were thicker and shorter and showed a strong tendency to lateral association. The morphology of lysozyme assemblies was determined in more detail by extracting further information about the height, diameter and length of fibrils from the AFM images and respective distribution functions are presented in Figure 1B. The image analysis has shown that the LAF2 fibrils are fiber-like objects with average heights of 6 nm, average length of 955 nm and the average diameter of 7 nm. In contrast, the LAF6 fibrils self-assembled into large bundles of fibrils with average heights of 53 nm. It was not possible to obtain the average length and diameter of LAF6 fibrils due to their lateral association and interfibrillar interactions.

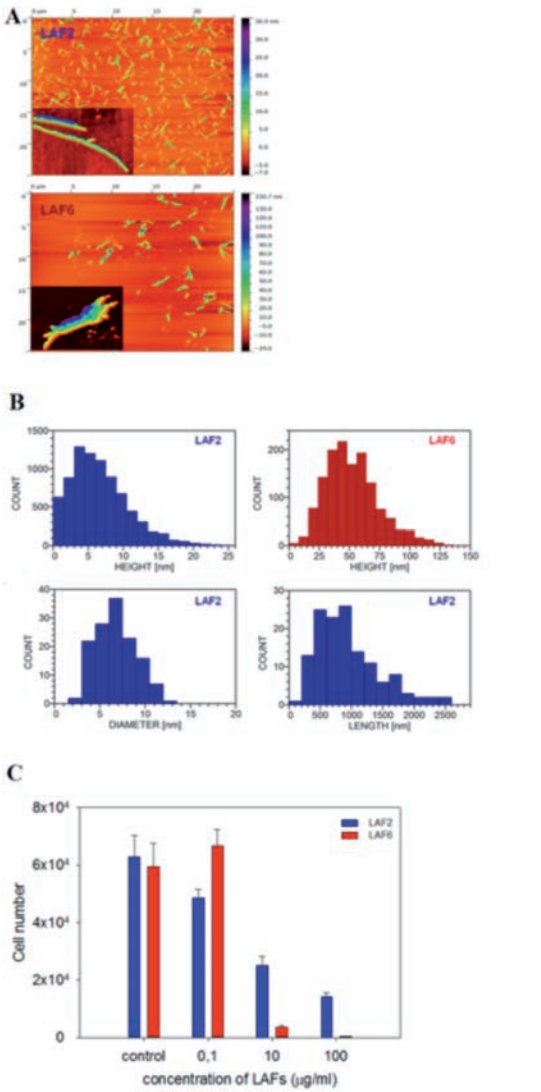
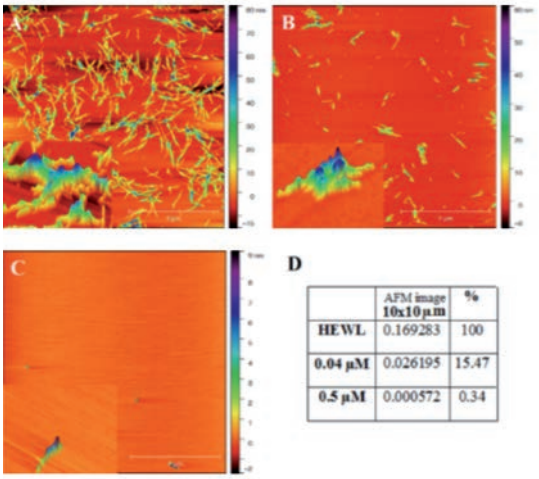


Figure 1. (A) AFM images of LAF 2 and LAF6 in the spectral palette prepared with Gwyddion software. Their 3D details are shown as insets; (B) The distribution function of height, diameter and length of LAF2 (blue) and LAF6 (red); (C) cytotoxic effect of LAF2 (blue) and LAF6 (red) on LLC-PK1 cell line after 5 days.

The morphologically different LAFs also inhibited growth of renal LLC-PK1 cells. Figure 1C illustrates that LAF6 were more toxic than LAF2 at higher concentrations (10 and 100 μg/mL) and at longer incubation time. Our findings indicate that protein aggregation can give rise to fibrillar

species with different degrees of cytotoxicity due to intrinsic differences in pathways of fibrils formation resulting from a formation of various partially unfolded species at the beginning of the process. The influence of DMPC phospholipid on the lysozyme fibrillization was also investigated by AFM to directly visualize their inhibitory abilities. Representative AFM images are presented in Figure 2. The incubation of DMPC with lysozyme led to extensive reduction of the overall amount of the fibrillar structures compare to untreated lysozyme. The fibril/background ratios calculated from AFM images confirmed concentration-dependent inhibitory effect of DMPC - low concentration (40 µM) decreases amount of fibrils to 15.5% whereas 500 µM leads to reduction of fibrils to 0.34% of image area.



	AFM image 10x10 µm	%
HEWL	0.169283	100
0.04 µM	0.026195	15.47
0.5 µM	0.000572	0.34

Figure 2. AFM images obtained for lysozyme amyloid fibrils formed alone (A) or in presence of DMPC at 40 µM (B) and (C) 500 µM concentrations. Bars represent 4 µm. (D) fibril/background ratios were calculated using Otsu method in Gwyddion software.

These results have shown that AFM is a very useful tool for a direct observation of amyloid aggregates, their morphological properties and the process of amyloid aggregation in general.

Acknowledgments

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A new scanning probe microscopy standard based on diblock copolymers and holey silicon

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Key words: Length Standard, SPM, AFM, Self Assembly, Diblock Copolymers

Introduction

The direct self-assembly (DSA) of diblock copolymers (DBCs) is widely used as patterning and nanofabrication technique, combining the top-down and bottom-up approaches to the DBC capability of phase separate into the small size and high-density features of different shape. These characteristics suggest the possibility of using the DBCs in order to address the gap in nano-structured lateral standards for nanometrology, consequently supporting the miniaturization processes involved in the semiconductor industry and in nanostructured device fabrication. In this frame, we systematically studied the orientation and ordering process of cylinder forming PS-*b*-PMMA DBC patterns confined within periodic trenches and the variation of its characteristic dimensions (i.e. center-to-center distance L_0 and diameter d) after their propagation into the Si, obtained by means of reactive ion etching (RIE).

Materials and Methods

The self-assembly (SA) of DiBlock Copolymers (DBC) based on the phase separation into different morphologies of small size and high-density features is widely investigated as patterning and nanofabrication technique.[1-3] The integration of the conventional top-down approaches with the bottom-up SA of DBC discloses the possibility to address the lacking of lateral length standards for nanometrology, consequently supporting the miniaturization processes in device fabrication. In

particular, these characteristics suggest the possibility to use DBCs films as organic template to promote the realization of a silicon lateral standard for Atomic Force Microscopy (AFM) calibration in the length scale ranging from 10 nm to 70 nm. In this frame, we studied the DBC behavior when confined within periodic SiO_2 trenches of different width (W, ranging between 75 and 600 nm) and fixed length (L, 5 µm) through a Rapid Thermal Processing (RTP) machine.[4] On one hand, we systematically studied the variation of the characteristic dimension (*i.e.* center-to-center distance L_0) of cylinder forming PS-*b*-PMMA (54 kg mol^{-1}, Styrene fraction 70%) DBC patterns, and on the other hand, we evaluated incidence of confined film thickness on the BCP domain orientation and morphology by varying the process parameters (annealing time and temperature, trench features).

Experimental Results and Conclusion

DBC disposition inside periodic trenches

The layout of the lateral standard consists in periodic gratings of ten trenches defined by conventional top down approaches and subsequently neutralized using a P(S-r-MMA) random copolymer (RCP). When the ordering process is accomplished on a flat surface, in a temperature range between 180 and 250°C, cylindrical microdomains perpendicularly oriented with respect to the substrate are observed irrespective of annealing temperature. In contrast, when the ordering process occurs on topographically patterned substrates, different phenomena have to be considered.[5]

The study of the DBC disposition inside the periodic trenches is a fundamental step in the realization of the holey silicon stripes, since it strongly affects the pattern transfer into the Si substrate. Indeed, the simultaneous effect of the flow around the gratings and the DBC flux from the zone located between adjacent trenches (mesa) into the inner part of the trenches results in significant thickness variations of the confined DBC film. Therefore, the amount of DBC inside the trenches depends on the width of the mesa region, which acts as a DBC reservoir. Moreover within each trench group, the DBC thickness progressively decreases from the external to the central trenches composing the periodic grating. The thickness variation of the DBC film within the trenches influences the ordering process, ultimately leading to different orientation of the microdomains in the periodic grating.[6]

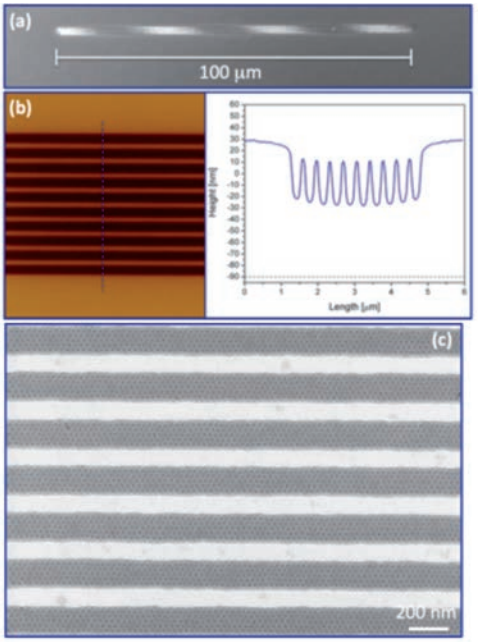

Figure 1. (a) SEM image of the entire lateral standard. (b) AFM analysis of the DBC disposition inside the periodic grating. (c) SEM micrograph representing the directed self-assembly of DBC inside the periodic gratings.

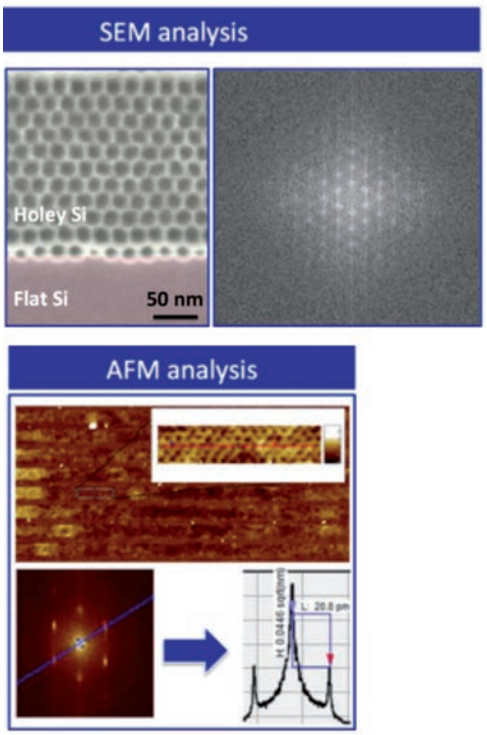

Figure 2. SEM and AFM analysis performed on both DBC and holey silicon stripes for the determination of the characteristic dimensions of the nanometric cylinders.

In particular only in a small range of temperatures a precise confinement of the DBC within the trenches featuring a perpendicular cylinder morphology is observed. At higher temperatures mixed or parallel orientations of the microdomains are obtained depending on the width of the trenches composing the periodic grating

Definition of the holey silicon stripes

After the optimization of the DBC ordering inside the periodic trenches, the nanopatterned stripes were transferred into the Silicon layer by means of Reactive Ion Etching (RIE) with two different methods. In the first approach, pattern transfer has been done directly on Silicon by means of Plasmalab 100 and using cryogenic mixing mode of SF_6/O_2 gases. The second approach is using hard mask of SiO_2, in which pattern transfer into the SiO_2 has been performed in Plasmalab 80 Plus by using CHF_3/Ar gases. For Silicon etch step, Pseudo Bosch process (SF_6/C_4F_8) has been adopted and performed again by Plasmalab 100.

Finally, in order to determine the exact values of the geometrical parameters of the confined nanodomains, SEM and AFM analysis have been systematically performed on the holey silicon stripes and compared with that obtained on the flat surface.

The object of this was to develop the first prototype of lateral standard for SPM traceable calibration based on holey silicon. The standard have been developed using the self-assembly properties of DBCs with tunable characteristic dimensions between 13 nm and 50 nm.

Acknowledgments

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Measurement of magnetic vortex chirality by field-dependent local hysteresis loops with MFM

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Key words: MFM, magnetic vortices, chirality.

Introduction

Magnetic patterned media are considered interesting materials for a variety of applications, including magnetic storage and microwave oscillators.[1] In the form of dots with suitable thickness to diameter ratio, magnetic vortices can be formed which can be exploited to permanently store information, coded in the vortex chirality. Many techniques for imposing a chirality in magnetic dots are available, which include rotating field or field impulses, suitable tailoring of the dots shape to break their symmetry, and many-body interactions. For reading the vortex chirality, X-ray magnetic circular dichroism, SEM with polarization analysis, planar Hall effect, magneto-resistance, Lorentz Effect TEM are very powerful yet extremely complex experimental techniques.

Magnetic force microscopy (MFM) is relatively common and extremely effective for this purpose. In this paper, we will briefly show how MFM can be used to investigate the formation of the vortex chirality in magnetic dots.[2] Besides the usual approach of collecting MFM images at different applied magnetic field values, field-dependent MFM techniques have been recently developed that allow the measurement of local hysteresis loops in individual patterned structures.[3,4] Common measurement and modelling tools do not offer sensitivity on the vortex chirality, because magnetic hysteresis loops are normally degenerate with respect to it. Conversely, in this paper we will show that local hysteresis loops measured by MFM are able to distinguish between chirality states and offer a means of studying more effectively the magnetization reversal processes in magnetic dots.

Materials and Methods

$Ni_{80}Fe_{20}$ dots with a thickness of 30 nm have been prepared by sputtering on SiO_2 substrates using electron beam lithography. The lateral size of the dots is of approximately 800 nm. The dots

are arranged in a square array where individual elements are spaced by more than 2 μm in order to minimize magnetostatic interactions.

Local hysteresis loops have been measured by means of Magnetic Force Microscopy (MFM, Bruker Multimode V Nanoscope 8 equipped with a fully non magnetic head and scanner) using a recently developed technique,[2,3] consisting in disabling the slow scan axis of the microscope while synchronizing the magnetic field variations with the end of line signal. As a result, an image consisting of phase, pass 2 lines of the same profile acquired each at a different applied field value is obtained. Arbitrary magnetic field histories can be generated. Each image contains therefore the information on the evolution of the magnetization under the application of a magnetic field which can assume several hundreds or even thousands different values. A suitable image analysis can reveal the local hysteresis loops irreversible features and provide details on the magnetization reversal of micrometric and sub-micrometric structures. The equilibrium configuration of the magnetization has also been calculated using micromagnetic simulations; then, the corresponding MFM images have been reconstructed by assuming a tip uniformly magnetized along the vertical axis and not affected by the stray field of the sample or by the applied magnetic field.[2]

Results and Conclusions

Figure 1 reports the four possible evolutions of the magnetization of a dot submitted to a magnetic field that cycles from positive to negative saturation and back. On the left of the figure, the MFM images reconstructed from the micromagnetic simulations are reported, together with the corresponding local hysteresis loops.[2] Even though the images look quite similar, their detailed analysis reveals four different cases, leading to significantly different local hysteresis loops. The four cases correspond to the relative position of the MFM scan line and of the edge along which vortex nucleation and expulsion occurs. In particular, type I and II loops correspond to vortices nucleating along the same edge of the dot both in the first and second loop branch (which means that the chirality is inverted in the two branches), with the tip scanning respectively close to the opposite and same edge. Type III and IV loops correspond to vortices nucleating along opposite edges of the dot in the first and second loop branch (which means that the chirality is preserved in the two branches), again for the two different positions of the tip scan line.

The comparison with the experimental data (right side of Figure 1) reveals a striking agreement with the simulations, both in terms of appearance of the MFM images, and especially concerning the local hysteresis loops shape, where the branches relative disposition, cross points and main features perfectly match. Type I loops are notably missing from the collected experimental data, whereas type II loops turn out to be observed most of the time. A careful investigation of the tip-sample interaction has been performed,[2] revealing the influence of the tip in inducing the nucleation side of the vortex and therefore the probability of appearance of the different loop types.

In conclusion, the proposed field-dependent MFM technique can be exploited to both measure and control magnetic vortex chirality in patterned dots. The validity of the technique has been tested experimentally and by comparison with numerical results.

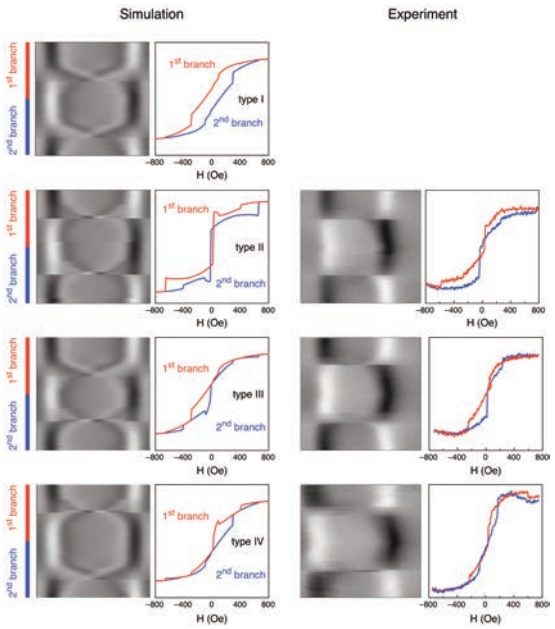

Figure 1. Comparison between simulated (left) and experimental (right) MFM images, and corresponding local hysteresis loops, of a $Ni_{80}Fe_{20}$ square dot submitted to an in-plane magnetic field.

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A combined STM and optical investigation of the solid-liquid interface

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Key words: Scanning probe microscopy, reflectance anisotropy spectroscopy, solid-liquid interface.

Introduction

The use of scanning probe microscopy (SPM) to investigate low dimensional systems represents the most common and standard characterization of surfaces, interfaces or more generally nanostructures. Furthermore, the possibility of coupling SPM with different spectroscopies often opens larger perspectives, to solve long-standing scientific issues. In this article we present two significant examples of how SPM can be combined with optical spectroscopy, to investigate: i) a complex case of reconstructed surface in Ultra High Vacuum (UHV) and ii) a liquid/solid interface.

Materials and Methods

In both examples we exploit the versatility of a particular surface-sensitive optical technique, reflectance anisotropy spectroscopy (RAS), and the unsurpassed spatial resolution of STM.

Reflectance anisotropy spectroscopy (RAS) mimics ellipsometry at normal incidence, measuring the anisotropy of light linearly polarized along two orthogonal directions of the sample surface.[1] It is a surface sensitive optical technique, non-destructive (using low energy photons, in the range 300-800 nm), and can be applied in UHV, liquid and air. The signal is averaged on the light spot size (2-3 mm^2), and can also provide information from the buried interface (within the penetration length of light). The STM experiments were performed in UHV by a commercial VT-STM Omicron, and in liquid by a home-made instrument able to investigate the solid/liquid interface of a sample immersed in an electrochemical cell.[2] Cyclic voltammetry data can be acquired in situ on the same sample.

Results and Conclusions

Ge/Si (105) surfaces exhibit a complex strained reconstruction known as rebounded-step (RS),[3] where the subsurface layer, hidden from probe microscopy, has a key role to determine electronics and optical properties of the entire reconstruction. At different values of the Ge coverage, STM images (left panel of Figure 1) show that the zig-zag motif typical of RS is always clearly present, while defects density changes. On the contrary, RAS spectra (central panel) have a well-marked dependence upon the surface preparation stage. Density Functional Theory (DFT) results (right panel) confirm the experiments and explain how the spectral line-shape depends upon the stoichiometry below the topmost layer of the surface, producing true surface states inside the bulk band gap.[4]

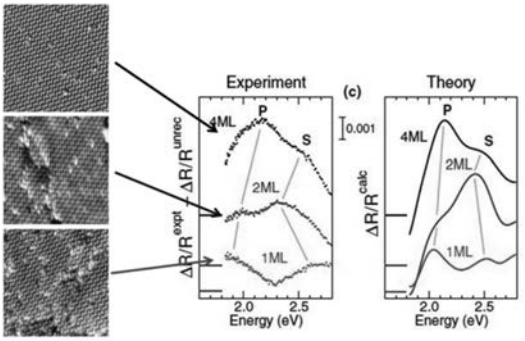

Figure 1. STM images (50 x 50 Å²) of the (105) surface for increasing coverage θ of Ge and corresponding measured RAS spectra (central panel) for the same coverage values. Computed RAS spectra are also compared (right panel). Lines are a guide for the eye.

When it is used to study surfaces immersed in liquid, RAS allows to investigate the surface–liquid interface in situ and in real time during the evolution of reactions at particular surfaces or in specific reagents.[2,5] Electrochemical Scanning Tunnelling Microscope (EC-STM) is also well suited for liquids. In Figure 2, STM images of a Cu(110) surface in hydrochloric acid solution are presented at selected values of the electric potential applied to the metal sample in an electrochemical cell.[2] The evident unidirectional stripes are due to adsorption of chlorine on the copper surface. The RAS signal recorded at fixed wavelength (corresponding to the peak of the chlorine stripes-related anisotropy, at 2.5 eV) is then measured during the cyclic variation of the electric potential. Once correlated with the STM images acquired during the same cycle, it provides a fast response to monitor in real time the surface modification. The obtained curve (Figure 2), signifying the modification of the RAS signal (RAS) with respect to the clean copper surface, represents the time evolution of the adsorption/desorption of chlorine.

We believe this approach will provide meaningful developments in particular for the solid/liquid interface, where the incompatibility of normally used surface probes (often restricted to UHV) with liquid still represents a significant limit for investigation.

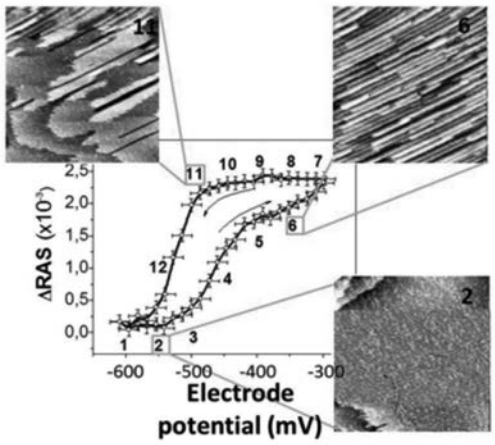

Figure 2. Evolution (at fixed photon energy, 2.5 eV) of the optical anisotropy signal ΔRAS as a function of the sample potential for the Cu(110) surface in HCl solution. STM images (81 nm2) acquired at selected potential values are reported. The arrows inside the cycle indicate the scan direction of the potential (positive and negative directions). The numbers represent the potential value at which STM pictures have been acquired in liquid. Some images are reported: (2) clean copper surface, (6) stripes of adsorbed chloride running along the [0 0 1] direction of the surface, and (11) copper terraces reappearing after partial desorption of chloride (channels due to chlorine are clearly evident).

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Characterization of electrical nanoscale properties of Si-based thin films for photovoltaic applications

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Key words: conductive AFM, photovoltaic, silicon oxynitride.

Introduction

Actual research on solar cells is focused on efficiency improving, as well as cost reduction and optimization of production processes feasible at commercial and industrial scale. Within this perspective, silicon heterojunction (SHJ) solar cells with thin films were produced, following a multijunction concept, with a record efficiency of 25.6% in 2014.[1,2]

In SHJ solar cells, a doped amorphous silicon layer (a-Si:H) is deposited on top of the crystalline silicon (c-Si) active material, to create the electric field of the pn junction, with the addition of passivation layers to avoid surface recombination.[1] Notwithstanding the good passivation qualities of a-Si:H, carriers with short lifetime are generated in this material and a large fraction of them recombine, causing a high parasitic light absorption.[3] Within thin films solar cells technology, silicon oxy-nitride (SiO_xN_y) turns out to be a promising material as a substitute for a-Si:H, for its tunable large bandgap and high conductivity (up to 2.5 eV and 44 S/cm), as well as its low contact resistance with the transparent conductive oxide.[3] Our previous studies have shown that the physical properties of the SiO_xN_y layers are affected by both nitrous oxide dilution and thermal treatment, due to different O inclusion and relocation.[4,5] These parameters cause a variation in crystalline fraction of the layers, their macroscopic electrical conductivity and morphological properties at the nanoscale[4,5]

Conductive Atomic Force Microscopy (c-AFM) is a useful non-destructive and cheap technique, to study nanometric electrical properties of complex samples with several phases. It consists of a conductive tip put in contact with the sample surface, while the current flow between them is measured at constant bias.

In the present contribution, c-AFM technique

has been used to study the electrical properties at the nanoscale of B doped silicon oxy-nitride deposited with different parameters, to extract a model of electrical conduction in these materials.

Materials and Methods

P-type SiO_xN_y layers (nc-SiO_xN_y) are deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD) on FZ-Si substrate. Silane (SiH_4), hydrogen (H_2) and nitrous oxide (N_2O) are used as precursor gases, with diborane (B_2H_6) diluted in hydrogen (0.5%) to achieve p-type doping. The deposition temperature is 300°C and the radio frequency (RF) is set at 13.56 MHz. The flow of N_2O and the ratio of B_2H_6 are both referred to their dilution in silane. The samples are deposited with a fixed diborane dilution (2.34%) and then annealed at 800°C in a nitrogen atmosphere to promote nanocrystals formation.

The investigated samples are selected by changing only one deposition parameter each time: N_2O dilution (R) and annealing (TT), respectively. This choice is made to show how the change in the parameter affects the nanometric electrical properties of the samples. The analyzed samples are the following: A (R=9.09%, TT=0 h), B (R=9.09%, TT=3 h), C (R=47.4%, TT=3 h), with thickness in the range of 200 nm. It has to be noted that higher values of R correspond to a higher O content within the layers, causing a lower crystalline fraction and a macroscopic electrical conductivity decrease.[3,4]

C-AFM acquisitions are performed using a Park NX10 system in contact mode with Pt probe, with nominal tip radius smaller than 20 nm. A fixed bias is applied to the sample through the silver paste contact on the top side of the sample, while the current is extracted from the tip.

Results and Conclusions

Current maps of 1x1 μm^2 have been recorded in several fresh areas of the nc-SiO_xN_y layers. As an example, a map on sample A is reported in Figure 1a. The dark areas in the image correspond to conductive regions of the sample. Grain-like structures in a low conductive matrix are visible on the surface of all the samples, however the grain conductivity is affected by annealing time and oxygen content. In sample B, the high conductive grains agglomerate forming clusters; this effect is in accordance with the observed coalescence and clustering of nanocrystals and the O relocation.[4] Moreover, sample B shows the highest conductance at the nanoscale, since both annealing and low O content promote electrical transport. In addition, it is the only sample that show enhanced conductance at positive biases, due to holes' conduction. This means that low O content promotes B-dopant activation in p-type layers.

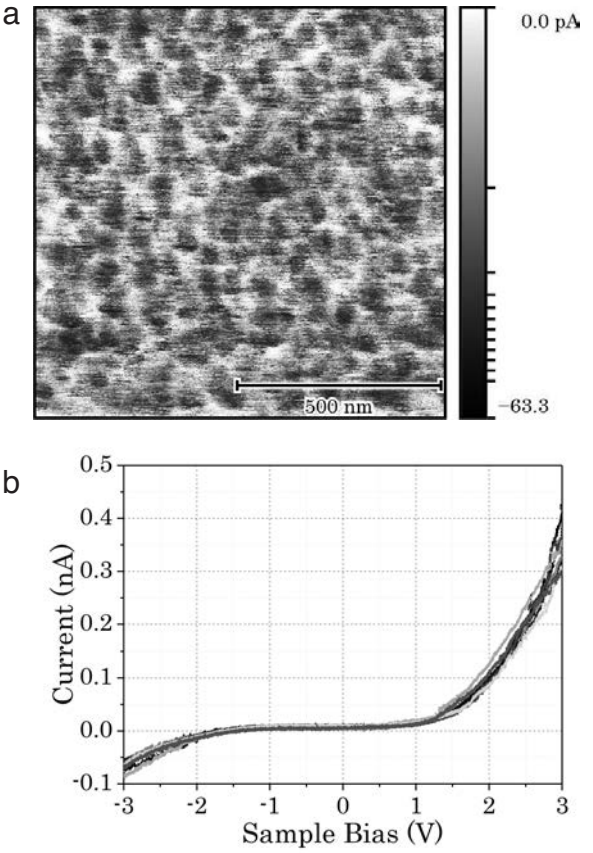

Figure 1. (a) Current AFM map on sample A at negative bias (-1.5 V); a non-linear scale is used. (b) Example of several I-V characteristics on sample A with a linear change in bias ([-3, 3] V).

Current-voltage (IV) characteristics have been measured locally on high conductive points (grains) of sample A (Figure 1 b). The tip-sample junction can be described as a Schottky contact, so the IV curves are well fitted by a thermionic emission theory, in accordance with the fact that the barrier of the highest conductive sample is the lowest one.

C-AFM analyses of microscopic transport properties of nc-SiO_xN_y thin films for photovoltaic applications have demonstrated that different deposition parameters (annealing, O concentration) strongly affect nanoscale electrical properties; in particular, low O content promotes B activation in p-type samples. Conductive AFM has demonstrated to be a very useful tool for the study of these multi-phase and non-stoichiometric thin films.

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Surface roughness in Al-implanted 4H-SiC substrates for different Al concentrations and after 1950°C post implantation annealing

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Key words: 4H-SiC, Al doping, ion implantation

Introduction

Silicon carbide (SiC) is a wide bandgap semiconductor suitable to fabricate low-loss, high-power and high-frequency devices for harsh environments. Selective area doping by ion implantation is often used in the fabrication of SiC electronic devices. To obtain p-type regions the preferred dopant species is aluminum (Al), that can be implanted on a wide concentrations range (from 10^{17} to 2×10^{20} cm^{-3}) with an accurate spatial distribution. After the implantation, a high-temperature (up to 1950°C) post implantation annealing is required to recover the lattice disorder produced by ion bombarding and electrically activate the implanted impurities. However, during such treatments a surface degradation of SiC is usually observed and the mirror-like surface is completely lost, due to Si desorption and atom migration on the surface.[1] This is a problem both for device fabrication and for material properties investigation, as the conditions required for a proper optoelectronic characterization are not fulfilled if the SiC surface is too rough. Proper SiC surface capping during the post-implantation annealing effectively suppresses the roughening phenomenon and, among the several capping materials proposed in the last years, a thin carbon layer (C-cap) gives the most successful results.[2]

In this study the surface roughness of Al-implanted on-axis 4H-SiC substrate is investigated by Atomic Force Microscopy (AFM) for implanted Al concentrations in the range 5×10^{18} – 1.6×10^{20} cm^{-3} after a 1950°C/12 min annealing with C-cap.

Materials and Methods

A High Purity Semi-Insulating (HPSI) on-axis <0001> 4H-SiC wafer was Al^+ implanted by using a Tandentron 1.7 MV accelerator (High Voltage Engineering Europa B.V.). Different ion energies and ion doses were used so to obtain implanted Al depth profiles of almost box shape next to the surface with fixed 400 nm thickness but different plateau heights in the range 5×10^{18} – 1.6×10^{20} cm^{-3} on different pieces of the same SiC wafer. After the implantation, a resist film was spun on the implanted surface of each piece and transformed in a carbon layer (C-cap) by a pyrolysis at 900°C for 2 min in forming gas.[3]

Post implantation annealing processes were performed in a conventional inductively heated furnace in high purity Ar atmosphere (for a comprehensive description of the annealing system, see ref. [4]) at 1950°C for 12 min. The same heating and cooling transients were used for all the samples.

After annealing, the C-cap was removed from the samples surface by a 850°C/15 min dry oxidation process. The surface morphology of the as-implanted and of the annealed samples after C-cap removal was characterized by atomic force microscopy (AFM) in contact and non-contact mode, respectively. The surface morphology of the samples was evaluated by measuring the root-mean-square (RMS) roughness over an area of 10 μm × 10 μm and 5 μm × 5 μm for the annealed and virgin samples, respectively.

Results and Conclusions

Figure 1 shows the surface morphology of a 4H-SiC sample implanted by an Al concentration of 1.6×10^{20} cm^{-3} before and after the post implantation annealing. The RMS roughness of the virgin and annealed samples are 0.39±0.04 and 0.56±0.08 nm, respectively. The AFM image of the annealed sample shows the formation on the surface of few nanometer deep circular pits with a diameter of ≈ 1 μm (Figure 1b). A similar morphology is observed in the annealed samples implanted by the other Al concentration values of this study, not shown in Figure 1. No significant dependence of the surface roughness on the implanted Al ion dose was observed, as shown in Figure 2. Similar trend was previously reported for P-implanted 4H-SiC.[5] With the exclusion of the 5×10^{19} cm^{-3} sample (presently considered a scattered point), the ave-

rage RMS roughness for the implanted concentrations is (0.57±0.2) nm, that is comparable with the RMS roughness before annealing and highly acceptable for microelectronic device fabrication. These results show the high quality of the C-cap used in this study, which can withstand annealing temperatures up to 1950°C.

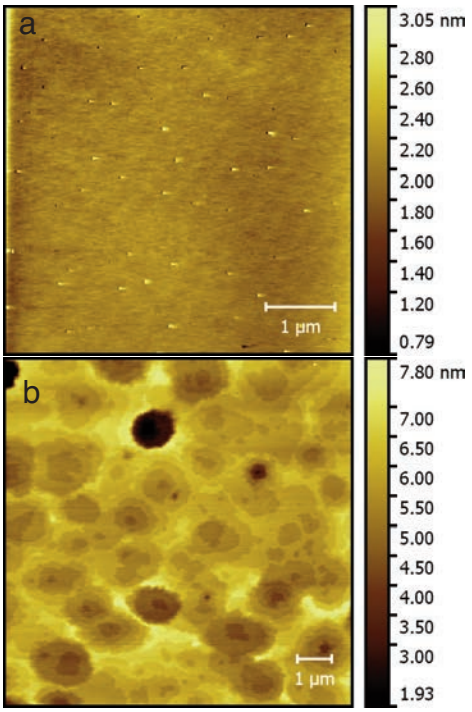

Figure 1. AFM images of the same 1.6×10^{20} cm^{-3} Al+ implanted 4H-SiC sample before (a) and after (b) the 1950 °C/12 min post implantation annealing with C-cap (removed before AFM measurements).

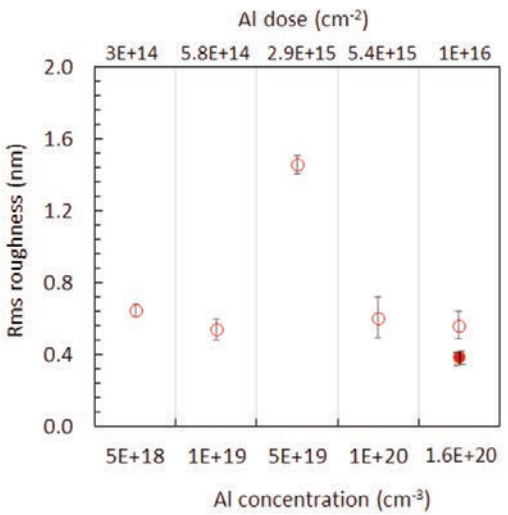

Figure 2. RMS of 4H-SiC samples versus implanted Al concentration (Al dose): (●) as-implanted, (○) after post implantation annealing.

Acknowledgments

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Effect of an antimicrobial peptide on model membranes studied by Atomic Force Microscopy and Fluorescence Microscopy

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Key words: antimicrobial peptides, Atomic Force Microscopy, Fluorescence Microscopy.

Introduction

Antimicrobial peptides (AMPs) are small amphipathic molecules produced in plants and animals to fight bacterial infections. Magainin, a 23-residue peptide secreted by the skin of an African frog, is a classical and widely studied α-helical AMP.

The proposed mechanism of action of AMPs suggests, at low peptide-to-lipid ratios, a lateral expansion of the lipid membrane due to peptide surface binding.[1] The lateral expansion induces a corresponding membrane thinning due to the almost constant volume of lipid bilayers.

Studies have shown that magainin binding causes membrane thinning[2] and softening.[3] In particular, Atomic Force Microscopy (AFM) studies showed membrane thinning due to a magainin analog, MSI-78.[4] However, the study of the effect of exogenous molecules on Supported Lipid

Bilayers (SLBs), the model system usually studied by AFM and considered 'at constant area', could provide different results with respect to freely suspended liposomes, a model considered 'at constant lateral tension'. Here, we used AFM-based force spectroscopy to measure the force required to punch through the membrane, and Fluorescence Microscopy to observe the effects of mag-H2 binding on SLBs composed of PalmitoylOleoylPhosphatidylCholine (POPC). The measurements aimed at understanding if the presence of a nearby solid substrate could induce some artifacts on the obtained results for SLBs with respect to liposomes.

Materials and Methods

POPC, solubilized in chloroform, was mixed with 1% molar DHPE-Texas Red and used to prepare Giant Unilamellar Vesicles (GUVs) by the electroformation method in 100 mM sucrose solution. In a homemade chamber with mica as the bottom surface, GUVs were deposited in 105 mM glucose, and then ruptured by adding 1-3 drops of a concentrated $MgCl_2$ solution to obtain lipid bilayer patches on the surface. The glucose solution was then replaced with the imaging buffer (150 mM KCl, 8 mM Hepes, 3 mM $CaCl_2$, pH 7). Fluorescent images were acquired with an inverted optical microscope (Olympus IX70). AFM force curves (tip speed: 1 µm/s; nominal spring constant: 0.24 N/m) were acquired with a Bioscope I microscope equipped with a Nanoscope IIIA controller. The peptide mag-H2 was hydrated in Millipore water and diluted to the desired concentration. The reported mag-H2 concentrations indicate the final concentrations in solution.

Results and Conclusions

For force spectroscopy analysis, lipid bilayer patches with no lipids on top were considered. The bilayer was exposed to concentrations of 0, 1.5 and 3 µM mag-H2. The area of the patch, obtained from fluorescence images, exhibited a significant increase at 3 µM. When taking force curves on this patch, we observed the clear presence of a jump-through event only at 0 and 1.5 µM (Figure 1). Apparently, at 3 µM, where a large lateral expansion had occurred, the structure of the membrane was already compromised so that there were not the conditions to obtain a clear jump through event. The most probable jumpthrough force increased from 2.3 nN at 0 µM to 4.5 nN at 1.5 µM, suggesting a stiffening of the membrane. When considering liposomes, it has been found that magainin typically induces a softening of the lipid bilayer.[3]

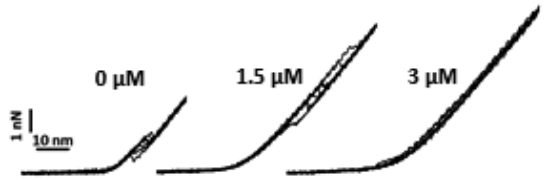

Figure 1. Representative force curves for a POPC bilayer exposed to three different mag-H2 concentrations.

We also studied a patch, 200 µm in diameter, with some small patches on top, using only Fluorescence Microscopy (Figure 2). This sample was exposed to a 20 µM mag-H2 concentration. Just after adding the peptide, lipid tubes began to form from the surface of the patch. After 60 seconds of peptide exposition, the area of the patch began to increase reaching a final 5% total area variation (Figure 2c). In contrast, one of the small patches on top, ~2 µm in diameter (see inset to Figures 2a and 2b), began to laterally grow immediately after adding the peptide, reaching a final 300% total area variation. These results suggest a strong interaction between the SLB and the mica surface which restricts the lateral expansion of the membrane. The accumulated tension due to peptide binding is released through the formation of lipid tubes, while for the patch over the SLB, the lateral expansion has no restrictions.

We conclude that the surface under the SLB affects significantly the effects of mag-H2 binding. This could lead to different results when comparing with studies using other model membranes.

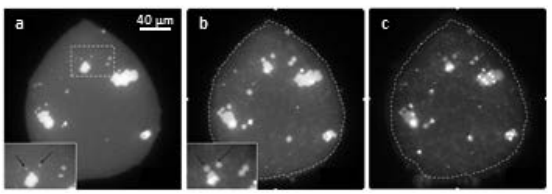

Figure 2. a) a POPC patch on mica before the injection of mag-H2 peptide. The inset is a magnification of the rectangular area shown by a dashed white border; b) The same patch after been exposed to a 20 µM mag-H2 concentration for 60 s. The inset is the same as in a). The dashed white line represents the border of the patch in a); c) The same patch after 200 s. The dashed line is again the border of the patch in a).

Acknowledgments

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Hydroxy-silicate substrates for biomolecules characterization

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Key words: substrates, nanolithography, single biomolecules.

Introduction

The ability to confine the deposition of single biomolecules onto specific nanosized areas is a requirement of paramount importance for their characterization. The substrate should be reasonably flat, homogeneous and chemically stable. Common ways of patterning surfaces make use of self-assembly of molecules combined with nanolithography. AFM nanolithography can be exploited to create nanosized areas with a specific chemical affinity onto an inert and uniform surface for bionanopatterning; then the molecules of interest will be deposited on these surfaces and will bind specifically on the patterned areas. Most of the substrates are not atomically flat, are chemically uniform across the surface and requires time consuming preparation procedures.

In this work we show that, by taking into account the crystal chemistry and surface potential properties of a magnesium–aluminum hydroxide–silicate substrate it is possible to perform well-controlled removal of the sub-nanometer thick structural layers of the substrate at the nanometer level. Furthermore, the resulting atomically flat and charged pattern is used for effective characterization of biomolecules at the nanometer scale.

Magnesium–aluminum hydroxide–silicate is an electrostatic crystal made up of regularly stacked negatively charged TOT (tetrahedra–octahedra–tetrahedra) mica-like layers, 1 nm thick, sandwiching a positively charged Mg–Al-hydroxide octahedral layer, 0.4 nm thick. The material contains several per cent of Al both in the tetrahedral sites of the TOT substituting silicon and in the hydroxide layer substituting Mg. The double Al substitution confers a positive charge to the hydroxide layer and a negative to the TOT surface. The two layers are bonded to each other by a weak electrostatic force. Once cleaved the material can expose simultaneously regions of the TOT (negative and hydrophilic), and of hydroxyl groups belonging to the hydroxide layer (positive and hydrophobic). The surface potential difference drives the deposition of charged biomolecules.[1-3]

Materials and Methods

The Mg–Al-hydroxide–silicate was prepared and characterized at the Laboratory of Biomaterials and Applied Crystallography of the University of Bologna (Italy). Its composition was previously determined by means of electron microprobe analysis in the wavelength-dispersive mode and the crystal structure by a Bruker X8-Apex fully automated four-circle diffractometer.[1]

A Nanonis SPM control system (Nanonis—SPECS Zurich GmbH, Zurich, Switzerland) equipped with two oscillation controller modules (with digitally integrated PLL/lock-in) and a software lock-in detector module was used for topography measurements, Kelvin probe analysis and nanolithography. The precise control of the relative tip–sample position was achieved by an nPoint closed-loop MultiMode scanner (nPoint, Inc., Madison, WI, USA). Single-pass amplitude-modulation Kelvin probe was used for surface potential measurements.

Results, Discussion and Conclusions

Figure 1 B shows as an example an AFM topographic image of a just prepared surface of the magnesium–aluminum hydroxide–silicate. Bright areas represent the Mg–Al-hydroxide layer, 0.4 nm thick in good agreement with single crystal x-ray diffraction results, extending over the mica-like layer (darker background). Terraces were observed to be atomically flat and extended in size from about 700 nm down to few tens of nm. In general, for this material the surface potential difference between the TOT basal plane (dark areas in Figure 1, B) and the hydroxide layer upper sur-

face (bright areas in Figure 1 B) gave figures, as measured by Kelvin probe force microscopy, ranging from 50 to 500 mV (at room temperature, atmospheric pressure and relative humidity of 30–70%), because of the variable surface crystal chemistry and environmental conditions. Here, these surface potential differences were applied to drive the deposition of single glycine molecules. To this aim AFM nanolithography was used to produce a custom nanopattern. Mechano-voltage nanolithography was performed in contact mode to expose at the surface a wide TOT area (Figure 1 A). Figure 1 A shows the central part of the surface in Figure 1 B after controlled removal of a portion of the 0.4 nm thick hydroxide layer and after deposition of glycine molecules. A specific procedure was developed to investigate the same nanosized area before and after ex situ biomolecules deposition. Single biomolecules (bright dot-like structures) were observed to be preferentially adsorbed in a stable manner onto the hydroxide surface, whereas no stable adsorption was observed onto the mica-like surface.

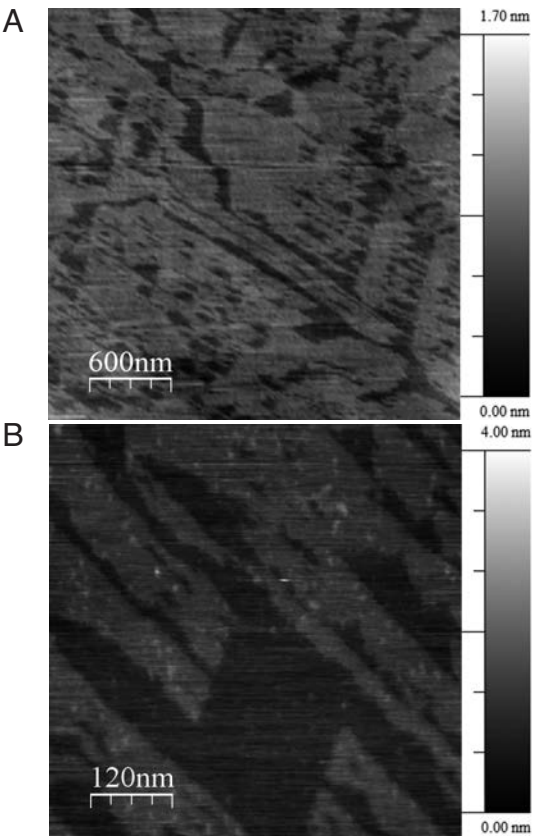

Figure 1. (A) Just prepared surface. (B) After nanolithography and glycine deposition.

This material allows the construction of atomically flat and charged patterns, designed to guide biomolecules deposition and stable adsorption without the need of any chemical functionalization of the surface. It is a promising substrate that could offer unique applications in several areas of bio-nanopatterning and particularly for surface driven deposition of biomolecules. This substrate was also found to be effective in the manipulation of single DNA molecules, single RNA molecules and nucleotides.[1-3] Similar procedures can be readily extended to other charged organic molecules for life science and polymer research, where the exploitation of self-assembled mechanisms on a large scale is in high demand.

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Exosomes distribution in cells via X-ray Fluorescence Microscopy

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Key words: Extracellular Vesicles, X-Ray-Fluorescence Microscopy, Atomic Force Microscopy.

Introduction

Exosomes are small vesicles (50-200 nm diameter) released in the extracellular space by most of the mammalian cells and represent one of the major routes of intercellular communication.[1] The content of such vesicles (miRNAs, mRNAs, proteins, etc.) can indeed alter and modulate the response of recipient cells. The ability of exosomes to travel in all body fluids (blood, saliva, urine) allows them to get in contact with cells localized very far from the originating cell, making them one of the main suspects of the elusive mechanisms of metastatic spreading (pre-metastatic niche). In fact, a strong effect has been attributed to exosomes derived from tumour cells in modulating the evolution of tumour microenvi-

ronment itself. This effect has been observed in different classes of tumours (glioblastoma, ovarian cancer, gastric cancer, breast cancer)[2-5] and we have recently shown a prominent role of glioma-derived exosomes in enhancing the aggressiveness of the tumour.[6] Yet, the complexity and heterogeneity of exosome origin and composition and their small size, make structural/functional studies on exosome uptake challenging to many available microscopy techniques. X-Ray Fluorescence Microscopy could have the potential of unravel mechanisms of exosome/cell interactions due to its high chemical sensitivity that can reveal subtle metabolic changes affecting elemental composition inside cells.[7] These details can be resolved within micrometer resolution by exploiting the XRSM, making possible a biochemical profiling of individual cells. Here we present our preliminary results of X-Ray Fluorescence Microscopy to evaluate the distribution of exosomes functionalized with Fe_2CoO_4 nanoparticles (NPs) in glioma cells, with a 500-800 nm lateral resolution.

Materials and Methods

Glioma Associated Stem Cells have been cultured on silicon nitride membranes in the presence or not of exosomes purified from Glioma Cells as reported in ref. 8. In order to monitor the internalization of exosomes by GASC cells, purified vesicles have been labelled with antiCD9 functionalized $CoFe_2O_4$ NPs.[9] After 72 h in culture, unconditioned and conditioned cells have been fixed and dehydrated. We measured 3 types of samples: fixed GASC cells not conditioned (Control); fixed GASC cells conditioned with NPs (NP Control); fixed GASC cells conditioned with NP decorated exosomes (EXONP). Fixed cells have been then imaged by means of XRM and XRF measurements allowing for the mapping of the distribution of exosomes inside the cells.[10] Atomic Force Microscopy images of GASC fixed cells have been acquired with XE-100 instrument (Park Instruments) in contact mode in air. 40x40 μm^2 images at 256/512 pixel per line have been acquired and analysed using XEI (Park Instruments) and Gwyddion softwares.

Results and Conclusions

In Figure 1 we report the Atomic Force Microscopy image of a GASC cell after 72 h exposure to EXONPs, and the corresponding low energy x-ray fluorescence (XRF) maps relative to different elements O, Mg, Co and Fe. O and Mg maps reveal the main structure of the cell (been rather homogeneously distributed in the cells), whereas the Fe and Co maps show the colocalization, sign of presence of NPs, and therefore of the occurred interaction of the Exosomes. The EXO-NPs are present mostly in the cytoplasmatic region, in good agreement with optical fluorescence measurements performed on similar cell cultures.[6] In all the other samples the Co and Fe fluorescence signal was completely absent (Control, not shown here) or sporadically present (NP, not shown here), demonstrating the specific interaction of exosomes with the cells. These preliminary results demonstrated the feasibility of the study of uptake of extracellular vesicles by means of XRF microscopy. Moreover the combination of AFM (real 3D topography) and XRFM could also allow to map the elemental distribution inside the cells and to extract atomic concentrations[7]: this label-free characterization of cells of different origin upon exposure to exosomes could bring to the discovery of new specific fingerprints of exosome interaction.

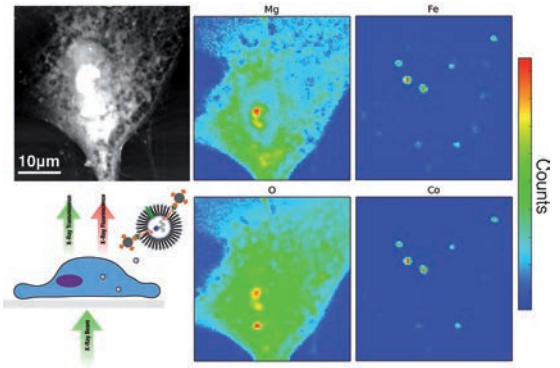

Figure 1. Atomic Force Microscopy topographic image and corresponding elemental maps from low energy X-Ray Fluorescence measurements of a Glioma Associated Stem Cell. In the low left corner we report a scheme of the experiment and the sketch of the EXO-NPs. XRF maps were collected at 1 keV at the TwinMic beamline of Elettra synchrotron.[11]

Acknowledgments

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Nanomechanical characterizations with contact resonance atomic force microscopy

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Key words: contact resonance AFM, elastic modulus, viscoelasticity, nanomechanical map.

Introduction

Accurate mechanical characterization of nanomaterials and nanosystems is fundamental to achieve advancements in a broad range of applications, e.g., in nano-electromechanical systems, nanocomposites, coatings, as well as in nano-biotechnology. Materials to be characterized vary from hard protective coatings to soft polymers or biological samples. Standard methods such as nanoindentation, tensile tests, or ultrasound-based techniques, can be limited in the analysis of nanomaterials due to their poor spatial resolution and the difficulty in selecting specific locations on the sample surface and/or requiring macroscopic specimens. Also, depending on sample dimensions and mechanical properties, standard tests like nanoindentation can be destructive. Finally, results obtained with conventional methods on thin films on substrates can be dramatically affected by the mechanical properties of the substrate itself. Therefore, innovative techniques enabling truly nondestructive, accurate and reliable mechanical characterizations of nanosized volumes of materials, with nanometer scale positioning, must be developed. Atomic force microscopy (AFM) has been demonstrated to be a powerful platform for the development of methods for mechanical characterizations at the nanoscale. Among them, contact resonance AFM (CR-AFM) allows one to measure the local elastic modulus of materials by analyzing the resonances of the cantilever when the tip is in contact with the sample surface.[1] Here, we describe CR-AFM technique and give a synthetic overview of the current capabilities of this technique.

Results and Conclusions

CR-AFM is a contact mode technique in which out-of-plane oscillations at ultrasonic frequencies of the system constituted by the cantilever, the tip, and the sample are excited through a piezoelectric transducer coupled with the cantilever holder or with the sample back surface. By analyzing the resonances of the system, the contact resonance frequencies (CRFs) of the cantilever are measured, which can be used to evaluate the local indentation modulus of the sample.[2] Taking advantage of the 'stiffening' of the cantilever at ultrasonic frequencies, CR-AFM allowed the nanomechanical characterization and the indentation modulus mapping of relatively stiff samples, which cannot be studied with AFM based indentation.[3] As an example, Figure 1 shows the topography (left) of a diamond-like carbon (DLC) coating deposited with laser ablation from a glassy carbon target to a molybdenum substrate. The map of the second CRF (center) shows dark agglomerates which indicate less stiff regions. The corresponding indentation modulus map (right) allows us to evaluate the indentation modulus of the stiffer region (higher content of sp^3 carbon) in the range 160-230 GPa. The softer agglomerates, with indentation modulus of 40-50 GPa, indicate sp^2 carbon deposited on the target without bond rearrangement.[3]

Since its invention, CR-AFM has been constantly

improved to enable more accurate characterization of different mechanical parameters of broader classes of materials. For instance, by exciting shear waves in the sample and analyzing both flexural and torsional CRFs of the cantilever, Hurley and Turner used CR-AFM to independently evaluate sample Young's modulus and Poisson ratio.[4]

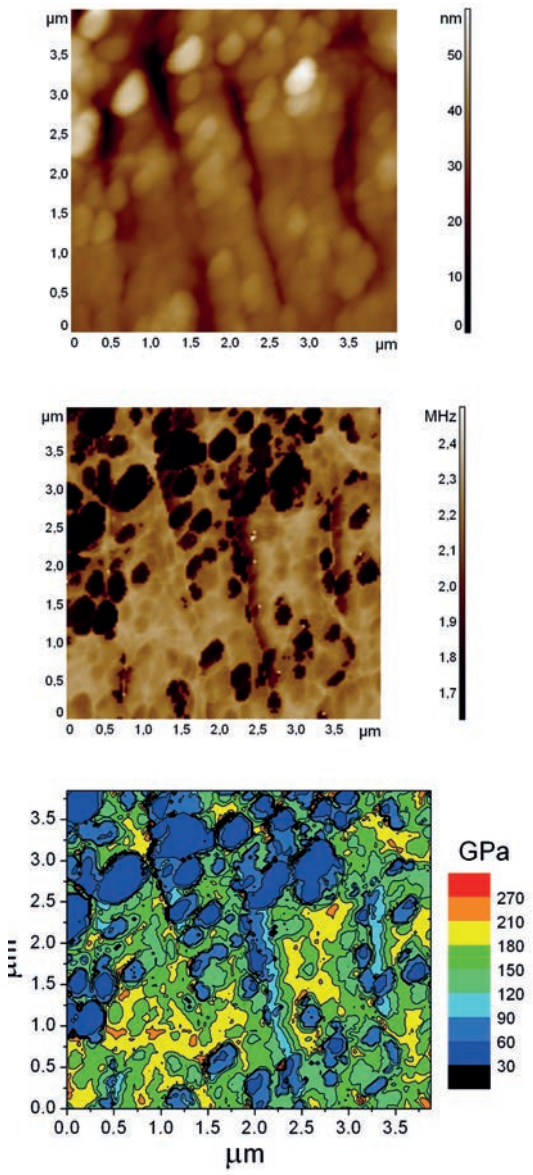

Figure 1. Atomic Force Microscopy topographic image and corresponding elemental maps from low energy X-Ray Fluorescence measurements of a Glioma Associated Stem Cell. In the low left corner we report a scheme of the experiment and the sketch of the EXO-NPs. XRF maps were collected at 1 keV at the TwinMic beamline of Elettra synchrotron.[11]

A major improvement of CR-AFM is represented by the possibility of characterizing viscoelastic materials, the mechanical response of which can be described by a complex elastic modulus, the real and imaginary part of which are the storage and loss modulus modulus, respectively. By acquiring the cantilever CRFs and the corresponding quality factors, indeed, storage and loss moduli as well as their ratio, i.e., the loss tangent, can be evaluated.[5] These viscoelastic parameters can be mapped with nanometer lateral resolution and, thus, CR-AFM is a powerful tool in the study of polymer blends.[6] Moreover, the sample can be mounted on a temperature controlled heating stage in order to measure and map the elastic and viscoelastic moduli of the sample at variable temperature.[7,8] Finally, the use of CR-AFM for the characterization of viscoelastic properties of soft materials at the solid-liquid interface has been reported.[9]

In conclusion, CR-AFM is a powerful technique for nanomechanical characterizations, greatly improved since its invention in terms of accuracy, range of measurable elastic moduli, accessible mechanical properties, in liquid or at variable temperature. CR-AFM is expected to be further improved, e.g., to allow the investigation of softer samples like biological materials.

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Ultrasonic Force Microscopy and subsurface imaging of two-dimensional materials

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Key words: scanning probe microscopy, graphene, MoS2, 2D-materials, ultrasonic force microscopy, elastic properties, subsurface imaging.

Introduction

Scanning probe Microscopy (SPM) represents a powerful tool that, in the past thirty years, has allowed one to investigate material surfaces in unprecedented ways at the nanoscale level. However, SPM's have shown very little power of depth penetration, whereas several nanotechnology applications would require it. Subsurface imaging has been achieved only in a few cases, when subsurface features influence the physical properties of the surface, such as the electronic states or the heat transfer. Ultrasonic Force Microscopy (UFM), an adaption of the contact mode Atomic Force Microscopy (AFM) contact mode, can dynamically measure the stiffness of the elastic contact between the probing tip and the sample surface. In particular, UFM has proven highly sensitive to the modulation of the near-surface elastic field due to the presence of non-homogeneous structures in the subsurface.

In this paper, we present an investigation of two-dimensional (2D) materials, namely flakes of graphite and molybdenum disulphide placed on structured polymeric substrates. We show that UFM can non-destructively distinguish suspended and supported areas. Specifically, UFM can probe small variations in the local indentation induced by the mechanical interaction between the tip and the sample. Therefore, any local change in the elastic modulus within the volume perturbed by the applied load or the flexural bending of the suspended areas can be detected and imaged.

Materials and Methods

Ultrasonic Force Microscopy (UFM) is a technique invented by Kolosov and Yamanaka,[1] resulting from an adaption of Atomic Force Microscopy (AFM) working in Contact Mode (CM-AFM). UFM has specifically proven a valid tool to localize subsurface defects in materials.[2] This can be achieved working at very low load values and eliminating the shear stress at contact thanks to a superlubricity phenomenon ultrasonically induced.[3]

We have carried out a study of samples made of very stiff two dimensional (2D) materials,[4] targeting the exploration of subsurface details and buried interfaces by means of UFM. In particular, we have deposited thin flakes of graphite and molybdenum disulphide (MoS_2) on structured polymeric substrates in order to obtain suspended and supported areas (see a schematic of the experimental setup in Figure 1A). UFM data are also compared to topographic data obtained with tapping mode AFM (TM-AFM).

Graphite or MoS_2 thin flakes can be transferred to a given substrate exploiting a PDMS stamp based technique.[5] In our case the substrate chosen is a film of Cyclic Olefin Copolymer (COC) polymer patterned via hot embossing in order to produce periodic flat mesas with randomly distributed voids in the regular array of grooves on a macroscopic scale (around 5x5 mm^2). The height and the periodicity of the grooves are approximately 250 nm and 1 µm, respectively. Given the typical lateral size of the flakes in the range from 5 to 20 µm, they present adjacent regions alternatively supported by the COC mesas and suspended over the voids (see Figure 1 B).

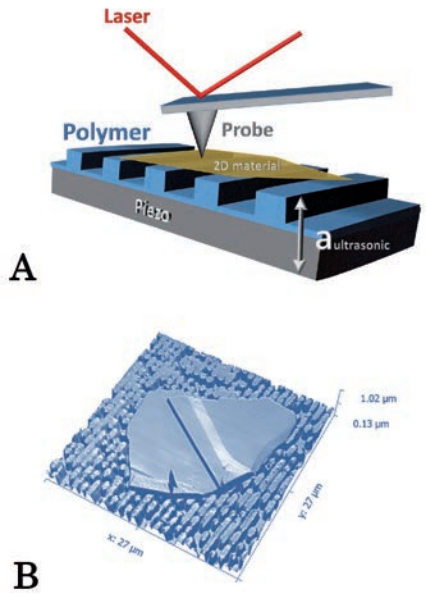

Figure 1. (A) Schematic of the experimental setup. (B) Topographic image of a graphitic flake on top of a patterned COC substrate.

The UFM setup is based on a standard CM-AFM including a vibrating sample stage, capable of producing out-of-plane ultrasonic vibrations with amplitude $a_{ultrasonic}$. These vibrations are transferred to the specimen, reversibly bonded to the stage, while the AFM tip and the sample surface are in contact under a fixed average load F_N. Our experimental setups are hybrid systems made of commercial and custom components, that can perform both UFM and TM-AFM measurements. One is based on a *Multimode*-type head with a *Nanoscope III* controller (Bruker), the other on a *SMENA*-type head (NT-MDT) with home-built electronic controller. Both these systems are equipped with a custom sample holder made of a piezo disc with having a thickness typical resonance around 2 or 4 MHz (Physik Instrumente).

Results and Conclusions

In Figure 2 A-B, we show some data obtained for a graphite flake (around 50 nm in thickness, excluding folded areas) deposited on a patterned COC film, as described above. Figure 2 A shows the topography obtained with standard TM-AFM: the topography image is featureless and smooth, and it is not possible to identify suspended or supported regions within the flake.

Figures 2 B shows the same flake imaged with UFM: the nanomechanical UFM contrast clearly discriminates the supported from the suspended portions of the flake, the latter appearing darker. Finally, in Figure 2 C the superposition of a UFM image with a topography one (also obtained with standard TM-AFM) of a MoS_2 flake is presented: again the presence of voids underneath the flake is well visible (darker contrast).

In summary, we have shown how UFM can detect and image subsurface features on the nanoscale in the case of two-dimensional materials, namely graphite and molybdenum disulphide.[6] In particular, we have investigated flakes of a few tens of nanometres in thickness placed on structured polymeric substrates with suspended and supported areas. We have demonstrated that UFM can identify the different regions in a non-destructive way, as is it highly sensitive to the flexural bending induced by the elastic field applied by the tip on the sample.

For all these reasons, we believe that this particular SPM technique is a very promising candidate for the mechanical characterization and testing of nano-devices based on 2D-materials where high spatial resolution may be requested.

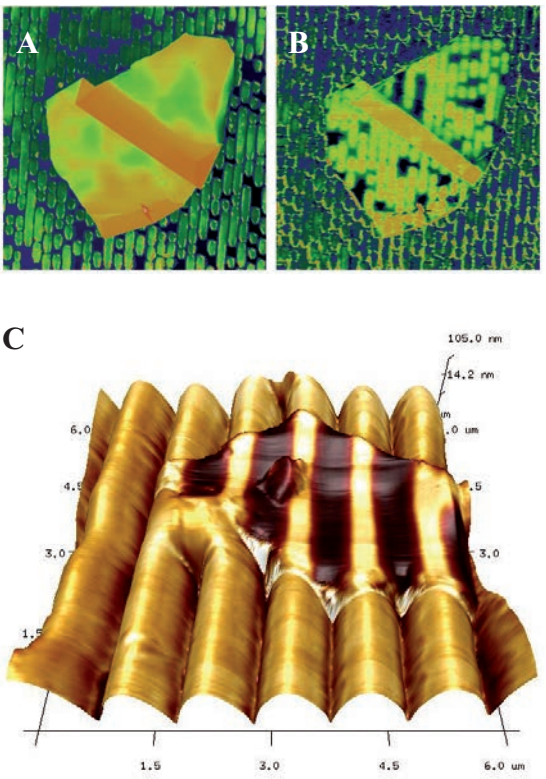

Figure 2. (A) Topography image of a graphitic flake on deposited on top of the patterned COC substrate. (B) UFM image of the same graphene flake showing a contrast due to its subsurface imaging capability. (C) UFM image superimposed to a topographic one of a MoS_2 flake: also in this case UFM demonstrates its subsurface sensitivity showing the presence of grooves under the 2D-material flake. The topography images were both obtained with TM-AFM.

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Workshop

La microscopia elettronica applicata allo studio dei Beni Culturali

Urbino, 6-7 febbraio 2017

Petroarchaeometric characterization of ancient materials by SEM-EDS

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The characterization of lithic artefacts, mainly obsidians and other kind of rocks used as tool stones (millstones, pestles, axes, pigments and so on) can provide important information on cultural, social and economic relationships between peoples in Mediterranean history and prehistory. For this reason, the study of the distribution of these rocks has provided a well-defined outline of their circulation during the past, in southern Italy and in the Mediterranean basin.

These archaeomaterials may be characterized using partial destructive investigations or absolutely non-destructive techniques. For this purpose the Scanning Electron Microscopy coupled with microanalytical spectrometers is a powerful tool, since 1960s, to characterize simultaneously the morphology and the chemistry of rocks and of minerals, even if very small.

At the Earth and Geoenvironmental Sciences Department of Bari University, over the past three decades, numerous researches about the characterization of obsidian provenance were performed: the provenance of over 390 obsidian artefacts present in 35 Neolithic sites in central and southern Italy was determined (Acquafredda *et al.* 2016). The procedure involves the analysis of the obsidian glass (Acquafredda *et al.* 1999) or of the very small microphenocrysts present in the rocks (Acquafredda and Paglionico, 2004) using a SEM equipped with ED spectrometers (Si(Li), Ge or Silicon drift). To perform a quantitative chemical analysis of the samples, their surfaces must be sputtered with a thin carbon film. The research revealed that obsidian provenances of 35 Neolithic sites in central and southern Italy (Acquafredda *et al.* 2016) were attributed chiefly to Lipari island (91,3%) and secondarily to Palmarola island (4,9%) and to Monte Arci (3,8%) in Sardinia.

The SEM can also be used with the chamber set in variable pressure: this procedure is particularly useful in the case of archaeomaterials whose surface cannot be sputtered with carbon; in fact, frequently the conductive film can't be easily removed from the surface of the specimen after SEM investigations as in the case of the characterization of precious Neolithic artefacts (Figure 1), whose surface is not as smooth as that of obsidians.

Nowadays many SEM are equipped with the last generation of solid state Silicon Drift Detectors (SSD), that normally have a very thin polymeric SuperAtmosphere Thin Window©; these ED spectrometers give an output signal with much higher count rates, up to about 100,000 cts on the whole spectrum, that guarantee a better sensitivity of light elements, even at a very low probe current of the beam, and very accurate X-ray maps still at high resolution, which proves very useful when studying the inclusions distribution in crystals (Figure 2). Moreover, this peculiarity of the SSD detector allows to study minerals used as pigments also in glassware enamels and pottery of different epochs and provenances, as in the case of lazurite or other sodalite group blue minerals like haüyne (Figure 2), that easily vaporize under the electron beam. The discovery of enamelled glass objects in Frederick II Melfi castle (South of Italy) and the presence, in the same area, of two rocks that contain haüyne, Phonolite of Toppo San Paolo and Haüynophire of Melfi, led to question

the real nature of the blue enamels raw materials. Therefore, a research on haüyne crystals behaviour with heating started: it was found that the blue chromophore S^{3-} presence in the haüyne crystals appears increased and that its distribution is different after heating. This phenomenon is probably controlled by sulphur-containing inclusions, such as pyrite, depending on temperature and oxygen-fugacity (Caggiani *et al.* 2014).

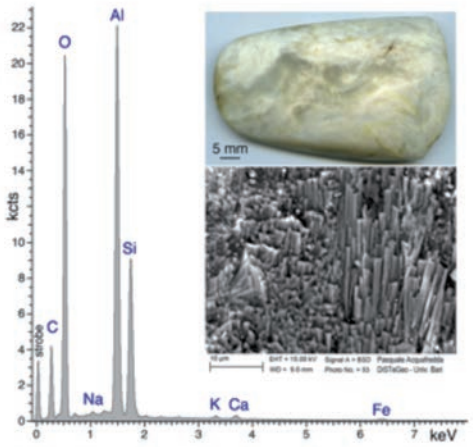

Figure 1. SEM-EDS spectrum of a little Neolithic axe with Al_2SiO_5 composition; the SEM was set in variable pressure (chamber at 10 Pa) to avoid the spattering of the surface of the specimen that would compromise the perfect cleaning of the sample after SEM investigations. In the upper right part of the figure, the macrophotograph of a Neolithic axe (sample SAdE 2) from Sant'Agata di Esaro (CS); in the bottom right part of the figure the SEM BSD image of the axe surface where the elongated prismatic sillimanite crystals whose shape corresponds to only one of the three polymorphs of Al_2SiO_5 are clearly recognizable.

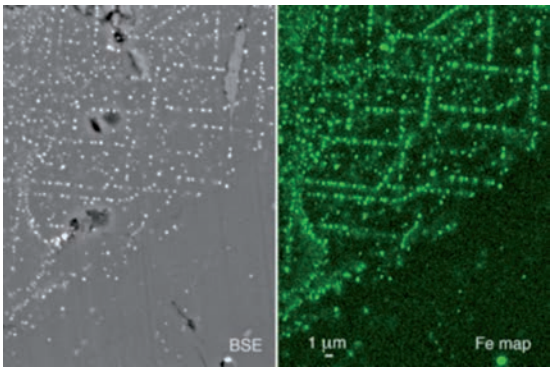

Figure 2. left: back scattered electron SEM image of a haüyne crystal (lazurite and haüyne are members of the sodalite group) from Monte Vulture (Melfi, PZ) with very small inclusions (<1 µm) of pyrite and magnetite aligned along the cleavage planes; right: the relative Fe distribution.

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Analisi delle tecniche esecutive della "Madonna col Bambino e Santi" del Pio Sodalizio dei Piceni in Roma

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Il manufatto raffigurante la *Madonna col Bambino, i Santi Geronzio e Maria Maddalena e donatori* è attribuito alla bottega del celebre maestro urbinate, Federico Barocci (Figura 1). L'opera, proveniente dalla chiesa di San Francesco di Cagli (PU) e attualmente conservata presso il Pio Sodalizio dei Piceni in Roma, è databile alla fine XVI secolo. La tela versava in un discreto stato di conservazione. Tuttavia, la pellicola pittorica era offuscata da un vistoso ingiallimento della vernice applicata in un precedente intervento di restauro (non documentato). L'intervento di restauro[1] è consistito prevalentemente nella rimozione della vernice non originale alterata e dei numerosi ritocchi alterati e debordanti sulla pittura originale. Il restauro del manufatto ha dato avvio a una nutrita campagna di indagini di natura invasiva e non invasiva, ai fini di caratterizzare la

tecnica esecutiva mediante l'identificazione dei materiali costitutivi e la descrizione del disegno soggiacente e dei processi compositivi. Il dipinto, eseguito su supporto tessile in fibra vegetale ad armatura a spina di pesce, è composto da due teli uniti in verticale con unione a sopraggitto ed è ancorato a una struttura di sostegno non originale costituita da un telaio ligneo rettangolare. La tela è inchiodata al telaio in maniera puntuale, tramite chiodi industriali, posti lungo lo spessore del telaio.

Le analisi scientifiche hanno permesso di studiare il *modus operandi* dell'artista, intimo collaboratore del Barocci, indagando i pigmenti impiegati e le ridipinture presenti sulla pellicola pittorica.

Le indagini non invasive sono state fondamentali per la scelta dei punti di campionamento, effettuati su diverse campiture. Lo studio delle stratigrafie è stato realizzato mediante microscopia ottica in luce riflessa nel campo del visibile e mediante microscopia elettronica a scansione con microanalisi EDS (Field Emission Gun Environmental Electron Scanning Microscope, FEG-ESEM, Quanta 200, FEI, The Netherlands, microsonda a raggi X a dispersione di energia (EDS, EDAX, Mahwah, NJ, USA), voltaggio: 30 kV, *lifetime*: 60 s, pressione della camera: 0,8 Torr).[2]

In generale è stata rilevata una preparazione di spessore medio e di colore bruno, composta da due o tre strati sovrapposti, internamente più scura che schiarisce verso gli strati pittorici, a base di terra d'ombra, bianco di piombo e carbonato di calcio (Figura 2).

Le campiture sono state realizzate per stesure successive di colore, progressivamente più intenso.

Nelle campiture blu è stata individuata la presenza di due o tre strati pittorici a base di oltremare e bianco di piombo, con tracce di terra rossa, dolomite, lacca e silicati riferibili a vetro, principalmente a smaltino, che caratterizza lo strato steso sulla preparazione in corrispondenza del manto dell'angelo in alto a sinistra (Figura 2). Anche gli incarnati si caratterizzano per la presenza di due o tre strati pittorici; tutti i campioni studiati presentano un sottile strato a base di silicati, riferibili a vetro, steso sulla preparazione. I pigmenti utilizzati sono bianco di piombo, terra rossa, cinabro e lacca. Le campiture rosse sono realizzate con pigmenti a base di piombo (principalmente minio), lacca, cinabro e terra rossa. Le campiture gialle sono molto diverse, una si presenta caratterizzata da bianco di piombo e terra rossa, mentre un'altra da giallolino, litargirio e terra rossa. In questo secondo campione, è presente uno strato di smaltino tra gli strati pittorici appena descritti e la preparazione (Figura 3). Le campiture verdi sono state realizzate utilizzando un'ampia gamma di pigmenti: pigmenti a base di piombo, pigmenti a base di rame, giallo di Napoli, giallolino, terra rossa, ematite, dolomite e silicati, riferibili anche a smaltino.

Figura 1. Bottega del Barocci (attr.), Madonna col Bambino, i Santi Geronzio e Maria Maddalena e donatori; cm 214 x 324,5, olio su tela, fine XVI sec. Pio Sodalizio dei Piceni, Roma.

Confrontando i risultati ottenuti con l'opera di Federico Barocci, *Il deposto di Croce*, conservato nella cattedrale di Perugia,[3] emerge una maggior complessità stratigrafica degli strati pittorici, più numerosi nell'opera conservata a Roma. La tavolozza pittorica risulta sovrapponibile anche se sono presenti delle differenze nella realizzazione delle diverse campiture. Nell'opera oggetto di questo studio non è stata rilevata azzurrite nelle campiture blu; nei verdi sono stati individuati

[1]*Il restauro è stato eseguito da Daphne De Luca (Responsabile Tecnico), Silvia Fioravanti e Giulia Cappelletti (febbraio 2016-marzo 2017). Direzione dei lavori dott.ssa Lucia Calzona, Soprintendenza Speciale per il Patrimonio Storico Artistico ed Etnoantropologico e per il Polo Museale della città di Roma.*

[2]*Per l'utilizzo del microscopio elettronico si ringrazia la dott.ssa Laura Valentini e il prof. Pietro Gobbi dell'Università di Urbino e il Direttore dott.ssa Patrizia Ammazzalorso dell'ARPAM Pesaro.*

[3]*F. Talarico, G. Sidoti, Caratterizzazione chimica dei materiali pittorici, in F. Abbozzo, M. T. Castellano (a cura di), Federico Barocci, "Il deposto di Croce" alla Cappella di San Bernardino nella Cattedrale di Perugia. Il restauro. Studio e conservazione, Il lavoro editoriale 2010, pp. 79-99*

[4]*Si veda in proposito D. De Luca, Indagini sull'underdrawing nella bottega del Barocci, in B. Cleri (a cura di), Barocci in bottega, giornata di studi, 26 Urbino ottobre 2012, Editoriale Umbra 2013, pp. 261-280 e D. De Luca, Restauri a San Girolamo, in B. Cleri (a cura di), Raccolte d'Arte, 1, Casa Editrice Guerrino Leardini, Macerata Feltria 2014, pp. 155-159.*

anche giallo di Napoli, giallolino e smaltino; nei rossi è stato individuato anche minio e negli incarnati anche lacca; mentre nei gialli non si registra l'uso di orpimento.

Così come le opere autografe del Maestro, anche questa tela realizzata da uno dei suoi collaboratori più dotati sembra caratterizzata dalle stesse ricerche coloristiche: si riscontra infatti una complessità delle stesure pittoriche, una raffinatezza delle campiture e una ricerca di effetti di cangiantismo per le vesti delle figure che contraddistingue di norma le opere di Federico, a riprova della volontà di normalizzare la produzione artistica che usciva dalla sua celebre bottega-impresa.[4]

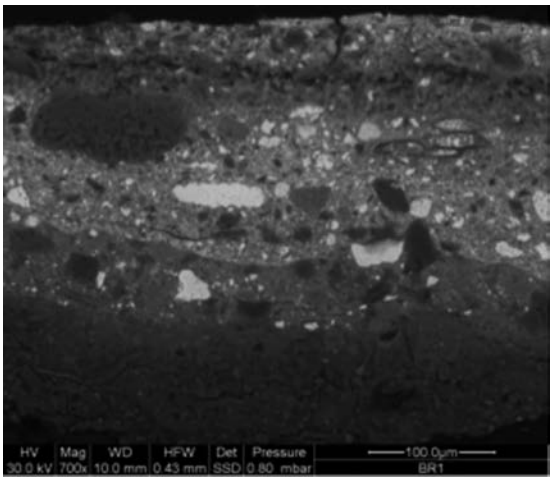

Figura 2. Immagine BSE, manto blu dell'angelo.

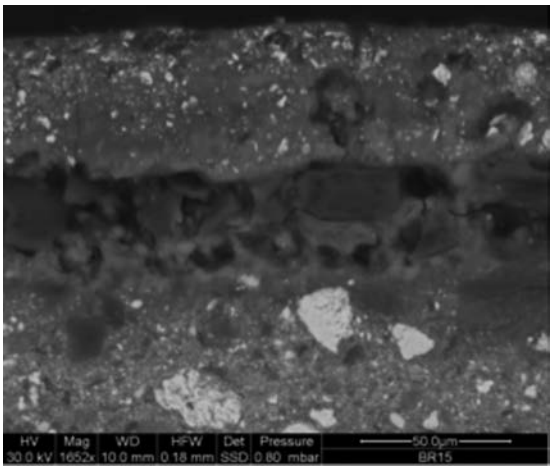

Figura 3. Immagine BSE, veste gialla della fanciulla.

Electron Microscopy and Numismatics

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Coins are widely studied in archaeometry because they provide a lot of information on social, economic and technological history of people and territories which they are related to. In several studies the chemical analysis of number of numismatic materials have been performed with different methods (XRF, ICP, PIXE, NAA). On the contrary only a few of metallographic data are known, so lack of information exists on the technological process (casting, mechanical working, heat treatment) used to produce coins of different composition.

The combination of chemical analysis with metallurgical investigation with OM and SEM on cross sections results in a complete description of the coin and its production steps, from melt alloy to final coin shape. This paper reports a summary of the results on the Cu and Ag base emission of different periods with the aim to develop a protocol of investigation based on microstructural and chemical analysis in order to reinforce the numismatic classification.

The composition of all samples has been determined, at first, by an XRF. The microstructure was investigated on cross-sections by optical microscopy (Leica DM 100) and SEM (Leica Cambridge Stereoscan 440) and the micro-analysis EDS was used for elemental analysis. The cross sections were degreased, polished and etched with $FeCl_3$ according to the standard metallographic techniques. The EDS line profiles were obtained on cross-sections. The estimation of the limits of the surface non-destructive investigation techniques (XRF, EDS) used in numismatic was evaluated.

Roman coins of the imperial ages

On the basis of the chemical compositions determined on cross sections, the coins can be divided in three main groups: pure Cu coins (with traces of Pb, Sn, Fe), named as group 1, brass or orichalcum coins (with Zn 17-24%, with traces of Pb and Sn), and Cu-Pb-Sn coins (with Sn 0-5%, Pb 8-30%),

All the coins of pure Cu and Cu-Zn have a recrystallized structure; the grains are not oriented and have the size of 10 - 80 μm. These features confirm the cold rolling and annealing processes. In the

Cu-Pb-Sn the Sn content is less than 5%, while Pb is in the range of 8 - 30%.

All the examined coins have dendritic or partially dendritic microstructure, with porosities and elements segregation (typical of cast and not homogenized alpha-bronze alloys), a few delta phase inclusions and Pb globules or elongated islands with orientation on the longitudinal direction are present. In some coins a moderate amount of recrystallized grains and slip lines have been detected. These features suggest that the alloy was solidified without any homogenization treatment and the flans were slightly mechanically worked before the striking process.

12th century Venetian denari

The results demonstrate that under Enrico Dandolo the mint produced debased coins, if compared to those pertaining to his predecessor Orio Malipiero. In addition, it is possible to assert that the silver content of the Venetian coinage at the end of the 12th century was around 25%. Such silver content made the Venetian coins probably the cheapest pieces available in the economic panorama of Italy and it can justify their great success.

The blanks were produced by casting, subsequent mechanical working and a final process of chemical blanching testified by a modified surface that has a thickness in the range 10-80 µm. The SEM-EDS investigation on the cross sections had confirmed that both the types of coins were subjected to an enrichment surface process. The destructive approach, in this research, is resulted indispensable to study the morphology and the composition of the coins and to interpret correctly the EDXRF results.

Diffusione degli elettroni nella materia

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Utilizzando una simulazione tipo Montecarlo si mostrano le diverse profondità di penetrazione degli elettroni su alcuni materiali in funzione dell'energia del fascio incidente.

La simulazione, sviluppata presso il laboratorio LAMA dell'Università IUAV di Venezia, ripercorre quanto già fatto da altri studiosi e in particolare segue le indicazioni di D. C. Joy in "Monte Carlo Modeling for Electron Microscopy and Microanalysis", 1995.

Una caratteristica del software sviluppato è di mostrare la traiettoria degli elettroni in una proiezione tridimensionale con colori diversi a seconda dell'energia posseduta dalla particella.

In Figura 1 si riportano i colori e le corrispondenti energie in percentuale rispetto all'energia del fascio incidente.

In ogni immagine di Figura 2 sono riportate l'energia del fascio incidente, la tipologia di materiale, la densità e la scala di riferimento.

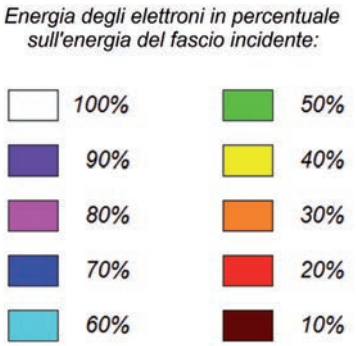

Figura 1.

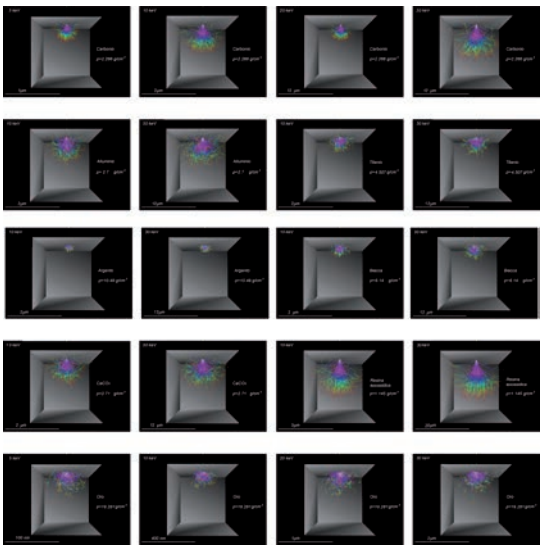

Figura 2.

La diffusione degli elettroni nella materia e l'interpretazione dei dati sperimentali

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Con riferimento ad un software di simulazione del comportamento degli elettroni nella materia sviluppato presso il Laboratorio LAMA di Venezia e presentato ad Urbino durante il precedente workshop sulla microscopia elettronica applicata ai beni culturali (cfr *Microscopie*, marzo 2016), il presente lavoro illustra alcune caratteristiche e modifiche apportate al software al fine di renderlo utilizzabile anche su piattaforma windows.

Alcuni semplici casi di studio dimostrano l'utilità della simulazione nel contribuire alla corretta interpretazione dei dati sperimentali.

Descrizione delle modifiche apportate al software

Il software è stato importato dall'ambiente Linux, in cui era stato sviluppato originalmente, all'ambiente windows. Sono state implementati alcuni menù che permettono all'utilizzatore di cambiare l'energia del fascio, le dimensioni del campione simulato e la sua composizione. È stata ulteriormente sviluppata l'idea, già abbozzata nella precedente versione, di rappresentare il cammino degli elettroni all'interno della materia con colori diversi a seconda dell'energia posseduta dalle particelle (Figura 1).

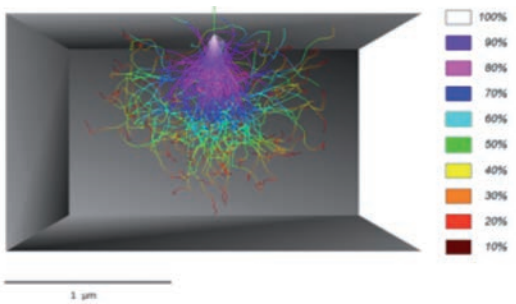

Figura 1. Simulazione di un fascio di elettroni da 10 keV incidente su un campione di quarzo. A destra, l'energia degli elettroni in percentuale rispetto all'energia del fascio incidente.

Applicazione del software di simulazione ai beni culturali

Nel caso dell'analisi di monete molto ossidate in superficie la simulazione conferma che l'analisi SEM fornisce informazioni limitatamente alla composizione della superficie alterata. In particolare nel caso della antica moneta romana sezionata e mostrata in Figura 2 appaiono ben visibili due strati di alterazione (B e C in Figura 3), spessi rispettivamente qualche centinaio di micrometri (B) e qualche centinaio di nanometri (C).

Figura 2. La freccia indica una moneta di epoca romana in bronzo, sezionata per l'analisi.

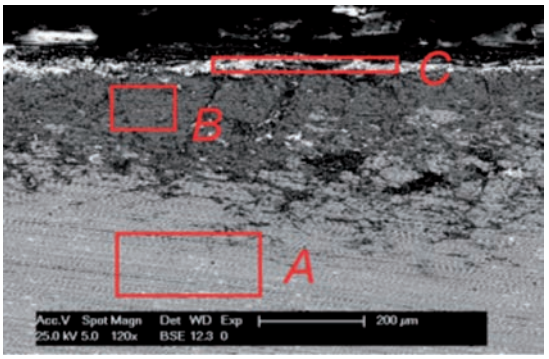

Figura 3. Immagine al SEM in sezione della moneta di Figura 2.

La zona C contiene una alta percentuale di Pb (dal 60% all'90% in peso, a seconda delle zone).

L'applicazione del software al caso di studio (Figura 4) ha dimostrato che l'impiego di un fascio incidente con energia pari a 10 keV sulla superficie della moneta non permette l'analisi composizionale della stessa. Infatti l'energia del fascio non risulta sufficiente per oltrepassare il primo strato di alterazione (C) pari a 200 nm.

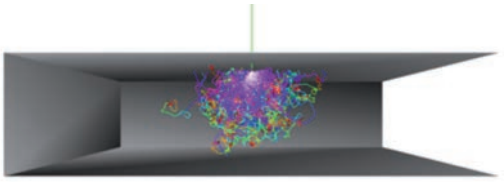

Figura 4. – 10 keV su lamina di piombo dello spessore di 200 nm.

Un altro caso in cui la simulazione ha dimostrato la propria utilità è la caratterizzazione di un vecchio chiodo in rame trovato nella Laguna di Venezia.

Il chiodo, trovato da un restauratore delle procuratorie di San Marco di Venezia, Enrico Pinzan, presenta sulla testa una interessante effigie (Figura 5) e sullo stelo si sono trovate delle striature di una lega di piombo e antimonio (frequente in epoca medioevale) (Figura 6).

Figura 5. Testa di chiodo in rame. Ritrovamento: Laguna di Venezia, Isola di Sant' Erasmo.

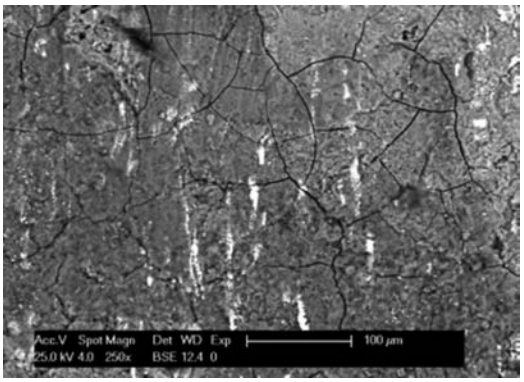

Figura 6. Superficie del gambo del chiodo, le striature chiare risultano essere di piombo e antimonio - 25 keV elettroni retrodiffusi

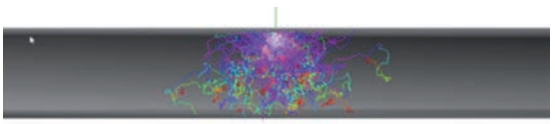

Figura 7. Simulazione di una lamina sottile di 50 nm di piombo, fascio incidente 5 keV.

Facendo incidere il fascio su una di queste striature si è notato che abbassando l'energia da 10 keV a 5 keV il picco L del rame viene minimizzato mentre il picco M del piombo resta ben visibile.

In questo caso, l'utilizzo della simulazione ha reso possibile la stima dello spessore massimo della "strisciata".

Si vede che un fascio di elettroni da 5 keV su una lamina di piombo da 50 nm esce dalla lamina in bassissima percentuale (Figura 7). Questo ci permette di attribuire alla striatura uno spessore non superiore ai 50 nm. L'eventuale presenza di fluorescenza secondaria o indotta da bremsstrahlung contribuirebbe ad abbassare ulteriormente lo spessore stimato.

Probabilmente il chiodo era stato infisso su di una lastra di piombo e i residui trovati in superficie indicano il modo in cui il chiodo era stato utilizzato.

Una versione di test del software descritto è scaricabile dal sito *www.semsage.it*

I materiali e la tecnica pittorica di Giuseppe Alberti alla luce delle indagini scientifiche

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Obiettivo della ricerca è l'integrazione degli studi di carattere storico, documentario e iconografico della produzione dell'artista con quelli più strettamente scientifici focalizzati sulla materia pittorica. Ci si riferisce all'identificazione mediante tecniche non invasive o micro-invasive dei materiali originali impiegati dall'artista: vale a dire i pigmenti negli strati pittorici, le cariche minerali nella preparazione del supporto e la natura dei leganti del colore. Tali informazioni permettono di avere informazioni volte a comprendere le scelte tecnologiche e le modalità operative del pittore nell'eseguire il dipinto, la possibilità di intuire la storia dell'opera rivelando la presenza di materiali non originali nonché elementi utili alla valutazione dello stato di conservazione del manufatto artistico in oggetto. Si presentano qui i risultati delle analisi effettuate al microscopio ottico ed elettronico di micro-frammenti di materiale pittorico (di dimensioni dell'ordine di 1 mm^2), mirate alla caratterizzazione della stratificazione dei vari film sovrapposti.

Il progetto diagnostico

La metodologia scelta ha previsto dapprima

l'esecuzione di indagini elementali mediante la fluorescenza ai raggi X (XRF) con l'ausilio di uno strumento portatile (Thermo Fisher Scientific, Niton XL6t GolD+) che non necessita di prelievi di materiale pittorico. Sulla base delle informazioni acquisite con l'analisi XRF è stato possibile guidare il campionamento riducendo al minimo il numero di prelievi necessari per le successive indagini chimiche e stratigrafiche.

Lo studio delle sezioni stratigrafiche di colore è stato realizzato seguendo una metodologia analitica consolidata[1] e in accordo con la normativa corrente riguardo la diagnostica per i beni culturali.[2] Le sezioni trasversali lucide così ricavate sono state osservate al microscopio ottico, con sorgenti di luce visibile ed ultravioletta; i componenti sono stati identificati mediante test microchimici, prove istochimiche per l'identificazione dei leganti direttamente sulla sezione. L'analisi elementare è stata effettuata al microscopio elettronico (JEOL JSM 7401) con energia di impatto 25 KeV e microsonda (Bruker XFlash 5030) secondo quanto riportato nel Documento UNI-NORMAL 8/81.

I risultati delle indagini

In Figura 1 si mostra un particolare dell'opera Caino e Abele in cui è indicato il punto sottoposto ad analisi chimico-stratigrafica. In Figura 2 si riporta l'immagine ottenuta al microscopio ottico della sezione stratigrafica del campione prelevato. L'immagine ottenuta al microscopio elettronico della stessa sezione è mostrata in Figura 3. In Figura 4 si riporta una mappa chimica ottenuta tramite spettroscopia X a dispersione di energia.

Conclusioni

In tutte le opere studiate lo strato di preparazione del supporto tessile è composto da una miscela di terre naturali argillose ricche di ossidi di ferro impastate con olio siccativo come legante. In alcune di queste sono state ritrovate piccole quantità di pigmenti a base di piombo utilizzati come essicanti del medio lipidico. Gli strati pittorici sono ad olio; i pigmenti identificati sono: bianco di piombo, nero carbone vegetale e animale, ocre rosse e gialle, vermiglione, lacche rosse, giallo di Napoli, terre naturali verdi e brune. Di grande interesse è, infine, la natura dei pigmenti blu, quattro diversi nelle opere studiate: l'azzurrite, l'oltremare naturale, lo smaltino e il blu di Prussia.

Figura 1. Particolare del Caino e Abele in cui è segnato il punto sottoposto ad analisi chimico-stratigrafica.

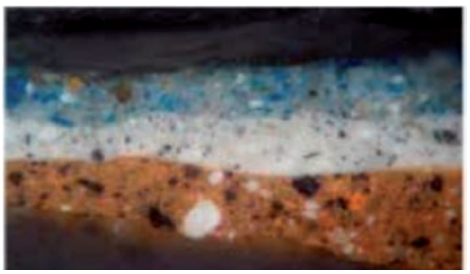

Figura 2. Sezione stratigrafica del campione studiato in cui si vede la spessa preparazione bruna (in basso) coperta da un film pittorico grigio chiaro e dal colore del cielo composto da azzurrite naturale macinata.

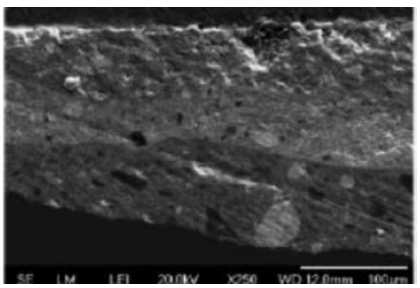

Figura 3. La sezione stratigrafica osservata al microscopio elettronico a scansione (JEOL JSM 7401F).

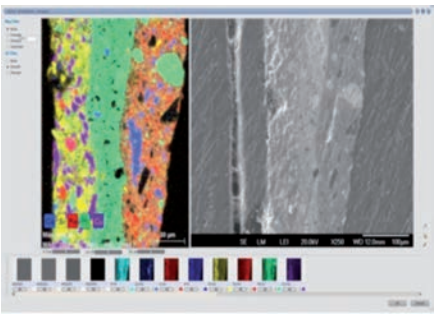

Figura 4. Mappa chimico-elementale della sezione stratigrafica): Ca (blu), Si (giallo), Fe (rosso), Pb (verde), Cu (viola).

[1]*Plester J., Cross-sections and chemical analysis of paint samples, in "Studies in Conservation", 2 (1956), pp.110-157*
Van Asperen De Boer J.R.J., An introduction to the scientific examination of paintings", in "Nederlands Kunsthistorisch Jaarboek", 26 (1975).
Martin E., Sonoda N., Duval A.R., Contribution a l'etude des preparations blanches des tableaux Italiens sur bois, in "Studies in Conservation", 3 (1992), pp.82-92
[2]*Documento UNI-NORMAL 14/83 "Sezioni sottili e lucide di materiali lapidei: tecnica di allestimento", Ed. CNR-ICR, Roma 1983.*

Degrado biologico delle mura di Urbino

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Scopo del presente studio è stato quello di analizzare l'entità della colonizzazione biologica in relazione allo stato di degrado e alle caratteristiche composizionali dei materiali (mattoni e malte) utilizzati nella realizzazione della cinta muraria di epoca rinascimentale della città di Urbino. Sono stati selezionati tre siti in relazione alla diversa esposizione e differente stato di conservazione: 1) Bastione San Polo, situato nella parte sud della città, che si trova in uno stato di conservazione apparente buono; 2) Pian del Monte, esposto a nord, caratterizzato da un notevole dissesto della superficie muraria; 3) Fortezza Albornoz, esposta nord-ovest, che presenta una situazione eterogena con parti ben conservate e altre con evidente degrado.

Studio mineralogico-petrografico

Le indagini sono state condotto con metodologie mineralogico-petrografiche (Diffrattometria a raggi X, petrografia ottica in sezione sottile, microscopia elettronica a scansione con apparato analitico EDAX. Per quanto concerne i laterizi, le analisi mineralogiche condotte in DX hanno evidenziato un'importante variabilità dei tenori dei minerali presenti imputabile alla disomogeneità dei sedimenti dai quali proveniva la materia prima.

La composizione mineralogica è risultata costituita, in ordine di abbondanza relativa, da quarzo, calcite, plagioclasi, feldspati potassici, fillosilicati micacei (muscoviti), e minerali di neoformazione quali: pirosseni diopsidici, gehelenite e sporadicamente ematite; questi ultimi formatisi durante le fasi di cottura. Nelle zone più superficiali, sottostanti i licheni, talvolta è risultato presente un ossalato di calcio, identificato come whewellite (Figura 1).

Studio della colonizzazione biologica

È stata indagata la presenza di licheni, il cui studio è stato approfondito mediante Miscroscopia Elettronica a Scansione (SEM), di muschi e piante vascolari. Per i muschi e le piante è stato valutato il grado di copertura seguendo il metodo di Braun-Blanquet (1964) e l'Indice di Pericolosità (I.P.) secondo Signorini (1996). Sono considerate specie molto pericolose quelle con un I.P. tra 7 e 10. La crescita lichenica interessava quasi tutta la superficie muraria. Sono state identificate tre specie di licheni crostosi e calcicoli: *Caloplaca aurantia* (Figura 2), *Lecanora albescens e Verrucaria nigrescens.* Tali specie non sono considerate particolarmente pericolose; cionondimeno la loro presenza diffusa può essere causa di un progressivo degrado in corrispondenza dell'interfaccia mattone-lichene, come evidenziato dalla microscopia elettronica (Figura 3).

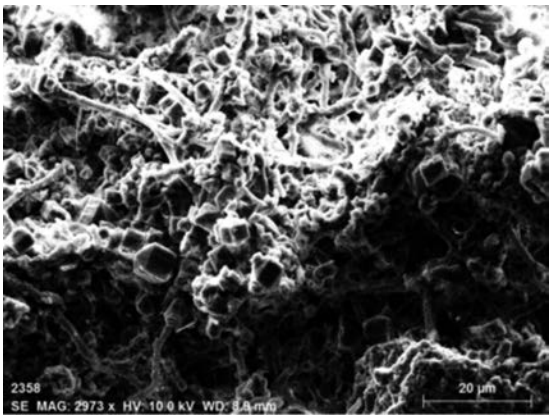

Figura 1. SEM. Cristalli di Whewellite.

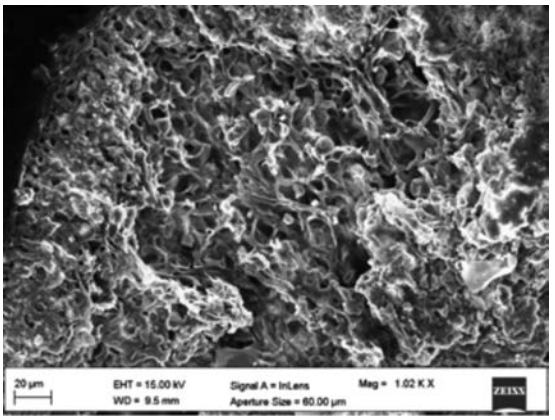

Figura 2. SEM. Sezione di *Caloplaca aurantia*.

La colonizzazione delle superfici murarie da parte delle specie vegetali si presentava con un grado di copertura disomogeneo. Le opere di parziale ristrutturazione, con conseguente diserbo e

di stilatura con malta cementizia, potrebbero essere alla base della ridotta colonizzazione riscontrata nella maggior parte dei siti relativi al Bastione San Polo e in alcune parti della Fortezza Albornoz. In generale si è evidenziata una prevalenza di piante erbacee annuali, bienni o perenni con I.P. medio basso. Importante era la presenza nella parte alta della mura in alcuni tratti della Fortezza Albornoz di specie arboree *(Quercus pubescens*, *Taxus baccata*, *Pinus nigra)* con un I.P. di 9. Le loro radici, insediandosi tra i mattoni, hanno causato, in alcuni punti, un vero dissesto della struttura. Tuttavia, anche le specie poco o mediamente pericolose possono risultare dannose se presenti in popolamenti. Infatti, la presenza di numerosi apparati radicali con la loro azione chimica e meccanica sulle malte contribuisce nel tempo al deterioramento della struttura.

In conclusione, il rilievo floristico delle superfici murarie costituisce un valido strumento utilizzabile per stabilire l'opportunità o meno di interventi al fine di eliminare la crescita biologica e di scegliere le procedure più adatte per impedirne o ritardarne la ricrescita.

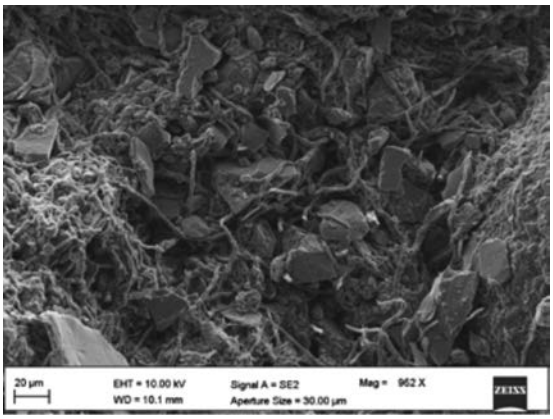

Figura 3. Effetto di disgregazione causato dalle ife di *Lecanora albescens*.

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Caratterizzazione metallurgica di manufatti artistici in ghisa per arredo urbano tra ottocento e novecento

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L'arredo urbano in ghisa costituisce un bene culturale spesso trascurato, a causa del prevalere di aspetti puramente funzionali rispetto alla necessità di salvaguardia dei beni storici.[1] A tale riguardo, spesso non si coglie la preziosità della testimonianza offerta dai getti in ghisa artistici che popolano le vie e le piazze cittadine. Tali oggetti testimoniano i progressi siderurgici, che hanno portato la ghisa a prevaricare sul ferro battuto tradizionalmente utilizzato in ambito decorativo, grazie alla possibilità di ottenere dei getti in serie che riproducessero minuziosi dettagli ornamentali. I complementi per arredo urbano sono, inoltre, una testimonianza artistica del gusto che muta con le epoche e della manualità dei modellatori che, come abili scultori, realizzavano il modello che avrebbe poi dato origine al getto.[2,3]

Nel presente studio, particolare attenzione è stata rivolta agli aspetti storico-artistici legati alla produzione della ghisa, che vedono quest'ultimo materiale da un lato considerato di scarso pregio e dall'altro esaltato per la sua democratizzazione dell'arte.[4]

L'attività sperimentale, condotta in collaborazione con la Fondazione Neri – Museo Italiano della Ghisa, ha permesso di identificare le principali caratteristiche metallurgiche di elementi di arredo urbano in ghisa e di individuare specifiche correlazioni temporali e/o geografiche. In particolare, sono stati indagati ventiquattro campioni, risalenti al periodo che va dalla seconda metà dell'Ottocento fino ai tempi recenti e provenienti da fonderie site principalmente in Italia, Francia ed Inghilterra (Figura 1A). I parametri microstrutturali, valutati mediante microscopia ottica ed elettronica a scansione (SEM+EDS) (Figura 1 B-D) sono stati i seguenti: frazione volumetrica di steadite ($Vf_{steadite}$), frazione volumetrica dei solfuri ($Vf_{solfuri}$), frazione volumetrica, dimensione media dell'asse maggiore e fattore di forma della grafite

($Vf_{grafite}$, MA_{max} e SF, rispettivamente).

I risultati ottenuti hanno evidenziato come la $Vf_{steadite}$, la $Vf_{solfuri}$ e la MA_{max} variano maggiormente nel periodo preso in esame; al contrario, la $Vf_{grafite}$ e SF non sembrano aver risentito delle evoluzioni tecnologiche introdotte in campo siderurgico negli ultimi centocinquant'anni.

Con riferimento alla fonderia di provenienza, nella seconda metà dell'Ottocento e nei primi anni del Novecento si osserva una tendenza alla ispirazione dell'architettura italiana della ghisa al modello inglese e francese; tale tendenza sembra venire meno con l'affermarsi di una produzione italiana originale.

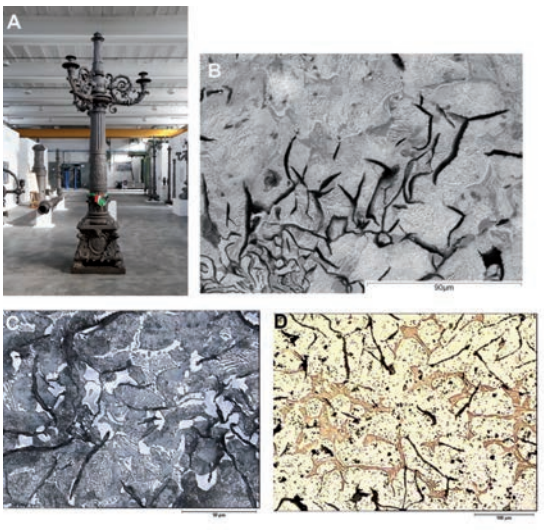

Figura 1. Lampione per illuminazione proveniente dalla città di Bologna (1896 ca.): (A) immagine fotografica dell'elemento di arredo attualmente collocato presso il Museo Italiano della Ghisa, Longiano (FC), Italia; (B) microstruttura osservata attraverso microscopio elettronico a scansione (SEM+EDS); (C) microstruttura osservata mediante microscopio ottico (MO) dopo attacco metallografico con reattivo Nital 4; (D) microstruttura osservata mediante microscopio ottico (MO) dopo attacco metallografico con reagente Murakami.

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SEM on metal archaeological findings: case studies

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Ancient metallurgy is fascinating and offering a very interesting insight on the material culture of our ancestors. Metal objects represent a quite unique opportunity to gather information not only about materials selection and usage but also on technical skills and practical advances in the field of metals working. This because the microstructural features are directly related to the metal composition and the thermomechanical history (i.e. the manufacturing process and the usage) while the corrosion (i.e. alteration patina) is also linked to the environment (e.g. soil, water) of permanence during the abandoning period. In other words, the metal artifact behaves as a solid state memory where the suffered events are recorded. The tool to recall and read such information is the metallography, a discipline started at the end of the XIX century. with the introduction of both metallographic microscope and suitable polishing procedures. A well prepared surface (i.e. mirror like polished with the support of calibrated diamond pastes) showing the cross section or the surface of micro-specimens (e.g. a few square millimeters fragment sampled from the original object) allows the investigation through optical and scanning electron microscopes which are the main tools of the metallographer. Moreover, SEM are also suitable for not prepared surface observations and chemical analysis. The latter is possible when such devices are equipped with Energy or Wave Dispersive X-ray spectroscopes (i.e. EDS and WDS) and offers the opportunity to identify the composition of the alloy, its phases and inclusions, and of the corroded layers.

The questions that might be faced by using metallography are: nature of the alloy and its relationship with the manufacturing procedure and the usage; nature of the minor elements and the inclu-

sions offering information on the ores; technical solution (e.g. casting and crafting processes); finishing processes (e.g. gilding, silvering, whitening procedures); environment-object interaction (e.g. corrosion process) and patina formation. To maximize the information one can gather from the metallography it is important to face some issues related to the representativeness of the sample toward the whole object and to the possibility to cut a fragment for further preparation. The correlation of microstructural and compositional details with corrosion features and macroscopic aspects leads to the formulation of consistent hypothesis which can be verified throughout experimental archaeology (e.g. objects manufacturing following the same process suggested by the results) and can be used by the restaurateurs during the conservation process.

Three case studies are hereafter presented as an example of the huge potential offered by the application of metallography to the characterization of archaeological metal findings.

Alteration patina

Figure 1 reports a quite classical aspect of the corrosion process suffered by a bronze thin plate belonging to an ancient shield form the III century B.C. where the bottom of the picture corresponds to the external edge where the patina is more tough and homogeneous while the top of the picture shows the intergranular penetration of the corrosion into the metal matrix. This picture taken by SEM using backscattered electrons highlight the changes in composition in the dense patina (i.e. a changing Cu/Sn ratio mainly with lighter zones richer in Sn) well distinguished from the metal matrix which is more homogeneous (i.e. well annealed solid solution). The corroded compounds are darker than the metal matrix due to the presence of light elements like oxygen and carbon. Whenever the Cu/Sn ratio is more favorable to the Sn the grey shade is getting lighter. The intergranular corrosion also highlights the microstructural features showing grain and mechanical twins boundaries. Figure 2 is taken for a bronze sheet belonging to a Celtic trumpet dated approximately in the same period showing a completely different microscopic aspect. The metal matrix is still visible and homogeneous but the corrosion penetration is not following the grain boundaries. This typology of corrosion was recently defined as “tentacle like” and correlated to a microbiological induced alteration typical of objects abandoned in anaerobic environment (e.g. river bed, pit bottom).

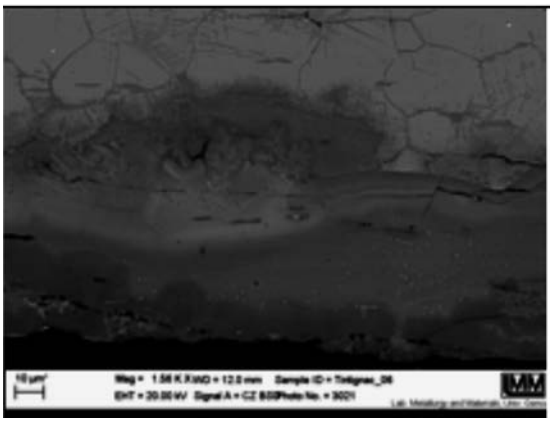

Figure 1.

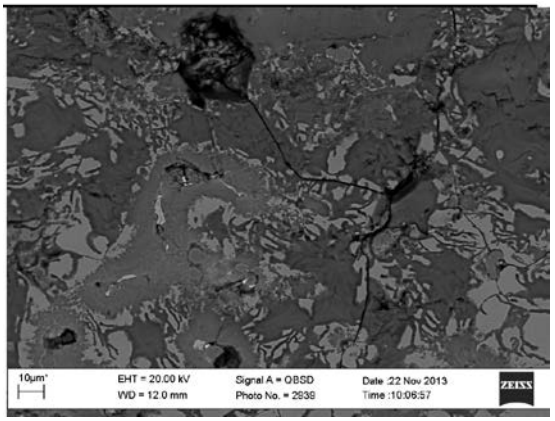

Figure 2.

Ghost microstructures in carbon steels

As previously observed in Figure 1, the corrosion process is usually etching the grain boundaries and then the bulk of the metal matrix. The alteration is highly sensitive to the chemical nuances that might be found in the alloy and to the presence of phases. Figure 3 reports a classical example a carbon steel composed by a ferritic-perlitic matrix. The sample comes from a Celtic blade and the grains shape suggests a thermal treatment at high temperature (i.e. at a T where the austenite is stable like 850°C) followed by a cooling process in air (i.e. normalizing thermal treatment). To have sound metal from iron and steel made objects is by the way a rare opportunity due to the quite high reactivity of iron base alloys to the oxygen reach environments. However, a chance to read the microstructural features is still possible in the corroded samples due to the higher chemical resistance of the cementite (i.e. iron carbide) composing together with alpha ferrite the perlitic eutectoid.

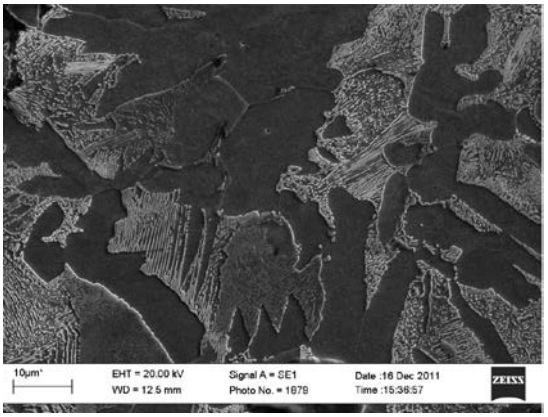

Figure 3.

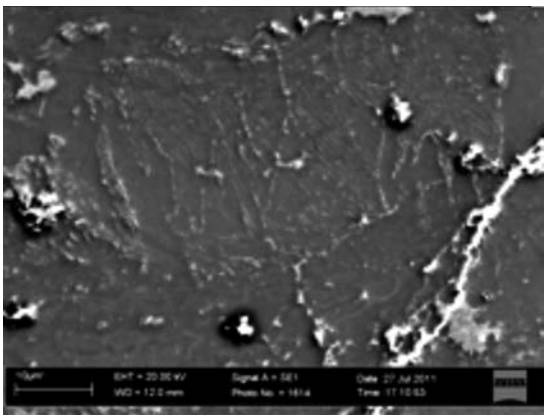

Figure 4.

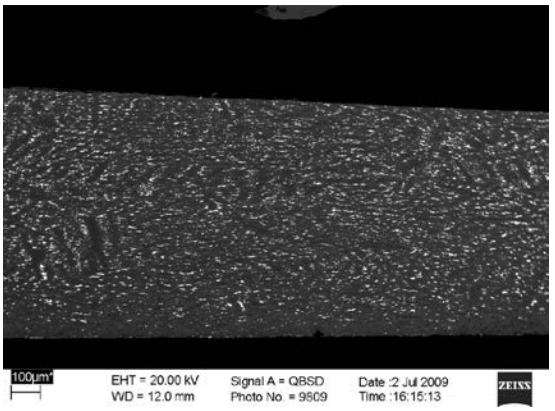

Figure 5.

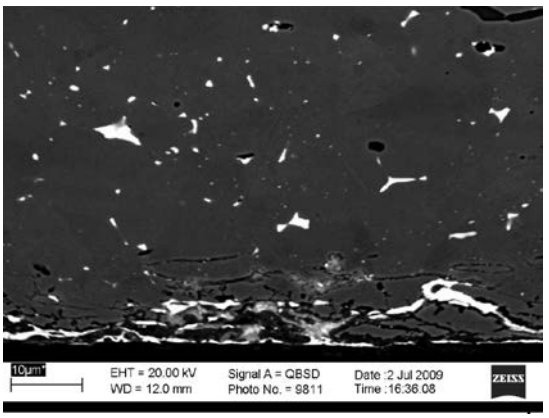

Figure 6.

Figure 4 shows an example of it where, using a simple trick as inverting from positive to negative the SEM-BSE image, the tiny white lines correspond to the cementite in a ferritic matrix. The image thus represents what remains of the original microstructure which is then defined as "ghost microstructure".

Copper-Silver alloys with surface whitening

A fragment of roman coin dated I century AD nominally identified as made of a silver-copper alloy is presented in Figure 5. The chemical composition have confirmed the presence of silver but as a minor alloying element of a quaternary copper base alloy with Sn, Pb as other elements. Figure 6 shows the reason of the silvery aspect of the coin: the inverse segregation occurring to Pb rich phases during the first solidification. Lead is a natural "solvent" for silver and tin and the visible lack of lead rich phases (white in the figures) from the external edge clearly suggests that during the solidification lead moves to the surface together with the other alloying elements.

The coin is then mechanically worked, annealed (as indicated by the recrystallized grains visible in Figure 6) and chemically whitened by selective Pb and Sn dissolution (e.g. with vinegar) in order to leave the silver on the surface. The final minting process consolidate the silver rich layer.

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Analisi archeometriche mediante SEM e microanalisi su manufatti in pietra ollare

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Il termine pietra ollare indica diversi tipi di rocce Alpine di colore dal grigio al verde (11 litologie riconosciute da Mannoni et al., 1987) formatesi per metamorfismo regionale (facies degli Scisti Verdi) di rocce basiche e ultrabasiche. Al fine di semplificare possiamo distinguere: un litotipo particolarmente "tenero" di colore grigio (i.e. "soapstones"), contenente talco ed un litotipo relativamente più "duro" di colore verde (e.g. cloritoscisti, serpentinoscisti, scisti ad anfibolo). Dal punto di vista archeologico e della cultura materiale, il termine pietra ollare deriva dal suo impiego nella produzione di olle, recipienti utilizzati per la cottura del cibo, ma anche per stampi, crogioli, stufe, ecc. Le aree alpine in cui la pietra ollare affiora più estesamente sono Valle d'Aosta, Val di Lanzo, Valli del Ticino e del Toce, Valtellina, Val Chiavenna e Val Malenco. L'utilizzo della pietra ollare inizia più di 2000 anni fa, ma è a partire dalla tarda età romana e Alto Medioevo, con l'introduzione della lavorazione al tornio idraulico (IV secolo) che la produzione e standardizzazione dei manufatti raggiunge una vasta espansione anche verso le regioni a sud del fiume Po. Le principali caratteristiche che hanno determinato il vasto uso di questa litologia sono l'elevata refrattarietà e buona stabilità termica accompagnate da lento accumulo e lenta restituzione dell'energia calorica; una bassa porosità anche in presenza di una tessitura scistosa e soprattutto una buona lavorabilità (rocce molto tenere per la presenza di talco). Questo contributo è focalizzato su frammenti di contenitori provenienti da siti archeologici dell'Emilia Romagna (Sant'Agata Bolognese, Nonantola e Comacchio) che presentano caratteristiche macroscopiche e microscopiche molto diverse rispetto a frammenti di pietra ollare "classici" utilizzati sul fuoco di tipo domestico. Solo attraverso un attento studio al microscopio ottico a luce polarizzata accoppiato alla microscopia elettronica SEM+BSE e analisi EDS è stato possibile stabilire che dal punto di vista mineralogico si tratta di rocce sottoposte a decomposizione termica con evidenti forme di "breakdown" di strutture cristalline originarie (Mini, 2014). Come confronto possiamo considerare che i manufatti in pietra ollare normalmente usati per la cottura del cibo, potevano subire un riscaldamento fino a temperature di circa 540°C non sufficienti ad innescare reazioni di dissociazione nei minerali se non a 520°C con la decomposizione della magnesite in periclasio. È stato comunque possibile individuare almeno due tipologie di materiali (i) rocce a talco e clorite riconducibili a talcoscisti a magnesite e cloritoscisti e (ii) rocce a pirosseno e olivina (non identificabili negli schemi classificativi di Mannoni et al., 1987). Grazie al confronto con dati relativi al comportamento termico (TGA+TDA) eseguiti su campioni di pietra ollare da affioramenti alpini (Antonelli et al., 2006) è stato possibile stabilire che le rocce talcose non dovrebbero avere superato la temperatura di 800°C poiché presentano forme deidrate della clorite (T compresa tra 590-630°C) e talco non trasformato (T<800°C); mentre le rocce a pirosseno e olivina potrebbero avere superato la soglia di 800°C dal momento che sono caratterizzate dalla deidratazione del talco (T>800°C) e dalla presenza di protoenstatite (trasformazione del talco tra 700-1000°C). Si tratterebbe molto verosimilmente di frammenti provenienti da crogioli in pietra ollare utilizzati per la fusione del vetro, la composizione chimica di roccia totale dei manufatti evidenzia delle anomalie come la presenza di Pb, Sb, Cu, Sr e Rb che potrebbero proprio testimoniare probabili processi di contaminazione tra la roccia/pareti del contenitore e il materiale all'interno del crogiolo portato alla fusione. Focalizzando l'attenzione su frammenti con le stesse caratteristiche macroscopiche ma con tracce (mm) di vetro nella parte interna del crogiolo, è stato possibile distinguere uno strato di interfaccia tra la roccia alterata (parete del crogiolo) e lo strato di vetro residuo. In questo sottile spessore (fino a 1 µm) si evidenzia una struttura a "palisade" formata da microliti di clinopirosseno e di olivina che in alcuni casi mostrano una forma a "coda di rondine" riconducibile a processi di rapida cristallizzazione. Nell'interfaccia i microliti presenti sono circondati da una fase amorfa arricchita in MgO e FeO che renderebbero conto della fusione incipiente della parete del crogiolo e di una sua successiva ricristallizzazione. Infine, sono stati evidenziati fenomeni di scambio chimico tra crogiolo e vetro attraverso l'acquisizione di spettri EDS ogni 300 µm lungo un transetto di 8.5 mm.

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Accuracy improvement by means of porosity assessment and standards optimization in SEM-EDS elemental analyses on archaeological and historical pottery and porcelain

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SEM-EDS analyses of pellets produced with powdery clay and ceramic standards and fired at increasing temperatures showed a systematic overestimation of the abundance of heavier detected elements (K, Ca, Ti and Fe) using the conventional procedure of calibration with massive mineral certified materials followed by normalization of the detected values. Errors were particularly noticeable for samples fired in the typical range of temperatures of archaeological and historical pottery (600-900 °C) and for unfired samples, and were attributed to material porosity. An extremely simple method based on the SEM-BSE image analysis is proposed for the semi-quantitative evaluation of porosity. A remarkable increase of accuracy was evidenced when the calibration is performed using a standard with porosity comparable to the samples, with regard to the pottery temperature range.

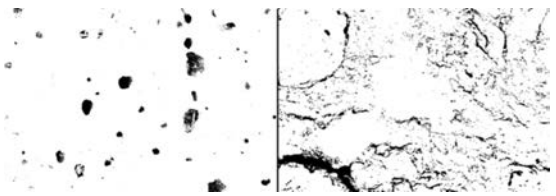
Sample name	Raw material	Preparation procedure	Firing temperature
CL1	Clay standard	Hydraulic press	-
CL2	Clay standard	Hydraulic press	600 C°
CL3	Clay standard	Hydraulic press	900 C°
CL4	Clay standard	Hydraulic press	1200 C°
CL1k	Clay standard	Kneading moistened clay	400 C°
CL2k	Clay standard	Kneading moistened clay	600 C°
CL3k	Clay standard	Kneading moistened clay	900 C°
CL4k	Clay standard	Kneading moistened clay	1200 C°
CE1	Ceramic standard	Hydraulic press	-
CE2	Ceramic standard	Hydraulic press	600 C°
CE3	Ceramic standard	Hydraulic press	900 C°
CE4	Ceramic standard	Hydraulic press	1200 C°

Two commercial Standard/Certified Reference Materials, a clay (CL) and a ceramic (CE) powder, were subjected to different preparation procedures and to firing at diverse temperatures to obtain series of simulated samples of historical pottery (600 and 900 °C) and porcelain (1200 °C).

EDS analyses on these samples gave detected compositions quite different with respect to the certified composition, showing an apparent composition change vs. firing temperature.

Analyses were performed by using two different instruments, an EVO-50 Zeiss and a Cambridge S-360 SEM equipped with an Oxford Instruments INCA Energy 200 EDS spectrometer and a X-Act3 SDD-EDS detector, respectively. All measurements were performed on polished section. All these variations are quite comparable for the two instruments. The good reproducibility indicates that the observed difference should be a matter of accuracy.

Errors are ascribed to the different path in the sample with respect to a non-porous material; a semi-quantitative evaluation of the porosity of these materials was hence carried out to correlate these results with the compositional data obtained by SEM-EDS. BSE images was employed to obtain a binary image, the porosity calculation was carried out by simply dividing the number of black pixels (voids on surface) of this image by the total number of pixels. The results show a general increasing trend up in porosity to 900 °C for all CL, CLk and CE series and then an abrupt decrease at higher temperatures. The porosity was also estimated on historical (a fragment of porcelain, picture below, left) and on archaeological (a ceramic sherd, picture below, right) respectively, demonstrating that the obtained standards are representative for real materials, as far as the porosity is concerned.



Therefore, the ceramic standard samples were used as calibration standards in the analyses of the clay standard samples, in order to check for an accuracy improvement when the calibration is performed using a standard with comparable porosity with respect to the analysed sample. RMS (Root Mean Square) differences between the certified compositions and the values obtained ana-

lyzing samples CL1, CL2, CL3, CL4 and CL4k using massive mineral standards and CE1, CE2, CE3, and CE4 as calibration standards showed an evident tendency of the RMS differences to decrease the more the estimated porosity of the sample and of the calibration standard are similar.

The results clearly showed that the use of a standard characterized by a porosity similar to the porosity of the samples is advisable for the determination of chemical composition of ceramic materials, considerably increasing data accuracy. This observation is particularly essential in the temperature interval (600-900 °C) of the typical firing step of ancient pottery, which usually shows a porosity of even few tens percent in volume. The influence of the porosity is definitely less important in the case of high temperature fired samples, such as porcelain, since porosity is low and its effect is negligible. The proposed method for a semi-quantitative evaluation of ceramic and porcelain materials porosity is extremely simple and rapid and it is suitable for calibration optimization.

The proposed calibration method seems particularly suitable for studies on production technologies or in the SEM-EDS examination of multilayer ceramics, where trends in the composition of each layer (i.e. body, slip, glaze) need to be evaluated. Moreover, it seems seminal in the case of characterization studies.

The full discussion, including analogous considerations about XRF technique, is available in Turco et al. *Journal of Archaeological Science: Reports*, 2017, 12, 54-65.

Topography and nanomechanical properties of cellulose fibers: application to ancient paper

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Cellulose is the main component of paper since the beginning of its production in the Far East in the first century A.D. and in the Western world in the Middle Ages. The remarkable properties of this material are due to the specific hierarchical structure that this polysaccaride assume within each fiber. The micro-fibrils that are stacked in the different layers are in fact composed by distinct regions where cellulose polymers are arranged either in crystalline or in amorphous domains. The different and opposite mechanical properties of these regions impart most of the mechanical properties to the fibers and thus to the paper sheets. Paper plays also an important role in cultural heritage due to the large number of precious artefacts, however the many degradation processes that take place within its structure might affect its preservation.[1-3]

In this frame studying in the less invasive way the morphology and the mechanical properties of cellulose fibers open the way to novel diagnostic tools to monitor the degradation processes of these artefacts.

We have applied two different techniques, atomic force microscopy (AFM) and optical profilometry, to measure over different lengthscales the three dimensional (3D) morphology of paper from the whole sheet to the sub-fiber constituents. Moreover, we have explored[3] the possibility to measure the mechanical properties of a single fiber down to the nanometer scale to map it as a function of the aging of the sample.

Profilometric measurements were performed using a Smart-WLI 3D Prime Optical Non-contact Profilometer (GBS-Ilmenau GmbH); images were collected at five different position in each sheet, near each corner and in the center.

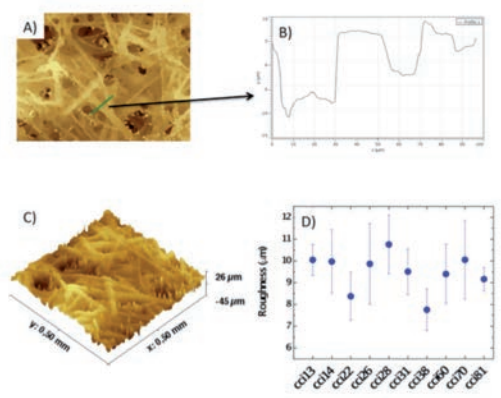

Figure 1. Profilometric image of an ancient paper sheet. A) The top view with the cross section (green line). B) The profile corresponding to the cross section of A. C) A 3D rendering of the image where the z-scale can be appreciated. D) The roughness measured onto different sheets of the *Corpus Cartarum Italicarum*.

AFM experiments were performed as described in ref. 3; briefly a single cellulose fiber was extracted from each sheet and positioned on a OH enriched glass slide that was then placed under

the AFM probe. The microscope used was a Multimode VIII (Bruker, Santa Barbara, CA, US) equipped with a Nanoscope V controller and operated in the Quantitative nanomechanic mode.

Optical profilometry is a powerful technique able to map the morphology of a sample based on the interference of the light passing through an interferometric objective thus providing a 3D topography with a lateral resolution limited by the optical diffraction limit and with a few nanometer resolution in the vertical direction. It is non invasive and can map large areas; in Figure 1 a profilometer image is reported both as a top view and as a 3D rendering. One can appreciate the kind of information available from these data ranging from the single fiber features to the statistical characterization of the whole area such as the roughness. These informations can be related both to the fabrication procedure of the sheets, such as the size and spacing of the strands (*filoni*) and the chainlines (*vergelle*).

AFM was employed to scale down with the lengthscale towards the nanometer range. As already described in a previous work,[3] quantitative nanomechanics was performed on single fibers extracted from each sheet in order to measure the Young modulus and the deformation at each point of the AFM image. The measured values were then correlated with the degradation induced by hydrothermal aging on reference paper sheets.

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Recovering the casting process of ancient bronzes with SEM analysis: Metallographic investigations of Bronze Age as-cast bronzes and experimental casting ingots

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In the Cultural Heritage field, the electron microscopic analysis is a fundamental tool for the investigation of metallic artefacts. In the particular case of as-cast materials, it allows the observation and the elemental characterization of each phases constituting the alloy, permitting also the estimation of its compositional heterogeneity (microsegregation), and the determination of the chemical composition of the inclusions.

The present research aims to explore through practical examples coming from Bronze Age artefacts up to casting experiments how SEM-EDS analysis could provide precious information regarding the casting process of ancient bronzes.

In first instance, a complete metallographic study of several archaeological corpuses of objects from Bronze Age hoards in France and Switzerland has been performed in order to highlight some microstructural features as for instance the composition of the alloy and the different phases (solid solution and second phases). A particular attention has been paid on the inclusions observed in the bronze matrix (copper sulphides and tin oxides) as they constitute an evidence of native mineral used for the alloy. In a second part, experimental castings of tin bronzes have been carried out in order to correlate experimental parameters to final microstructure. Therefore, the impact of mould material on the different phases composition has been investigated through casting of tin bronzes and leaded tin bronzes in different moulds (clay, metal, sand). The influence of recycling on the features of inclusions has also been studied in the particular case of the addition of tin oxides in the copper matrix.

In all experiments, SEM-EDS analysis plays a preponderant role compared to light-optical microscopic (LOM) observation as it gives access to the chemical composition of the different phases, which directly depends on casting experimental conditions. As information regarding the casting process of archaeological artefacts may be sometimes barely accessible, SEM investigations might provide a new reading key of ancient bronze microstructure and ancient manufacturing practices.

Microscopy techniques in nanomedical research

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Summary
In recent years, the application of nanotechnology to biomedicine has been exponentially increasing. The physical and chemical properties, quality and safety of nanomaterials designed for biomedical application need to be accurately evaluated by means of reliable and robust techniques. Among the methods used, microscopy techniques play a primary role. This paper presents a brief overview of the contribution of different microscopy techniques to the study of the structural and functional aspects of nanoconstructs and their relationships with the biological milieu, demonstrating the great impact that microscopy sciences have in nanomedical research and applications.

Key words: electron microscopy, fluorescence microscopy, nanomedicine, nanoparticles.

Introduction

In recent years, the application of nanotechnology to biomedicine for the development of e.g. new drug delivery systems, diagnostic tools, sorting systems, scaffold components (Lim *et al.*, 2010; Bobo *et al.*, 2016; Fernandes *et al.*, 2016; Soica *et al.*, 2016) has been exponentially increasing. Understanding the structure of nanocomposites is crucial to elucidate their physical and chemical properties, quality and safety, as well as their distribution and behavior *in vivo*. All these features, in fact, strongly affect the efficiency of nanoconstructs in the living organism, from the molecular to the systemic level. It is therefore essential to perform accurate studies by means of reliable and robust techniques. Among the methods used for evaluating the structural and functional aspects of nanoconstructs and their relationships with the biological milieu, microscopy techniques play a primary role. This paper presents a brief overview of the contribution of different microscopy techniques to the development of nanomedicine.

Microscopy to characterize nanoconstructs for biomedical application

A wide variety of analytical methods have been used for evaluating the physico-chemical characteristics of manufactured nanomaterials (for a review, see Lin *et al.*, 2014): these include chromatography, electrophoresis, magnetic resonance, X-ray scattering and spectroscopy, mass spectrometry, circular dichroism spectroscopy, zeta-potential measurements, as well as techniques of microscopy on which the present article will especially be focused.

In fact, transmission electron microscopy (TEM) is one of the most efficient tools for the characterization of nanomaterials. TEM provides high resolution of minute structural details, which is essential, for instance, to obtain information about the crystalline structure and granularity of the nanoparticles (Williams and Carter, 2009). Through TEM it is also possible to detect alterations in nanoparticle morphology due to the incorporation of drugs at different concentrations, thus representing an indispensable technique for the development of drug delivery systems (Govender *et al.*, 2000). To be suitable for observation at TEM, nanomaterials usually need to be dehydrated, but it is also possible to freeze them (cryo-TEM), thus better preserving their original morphology (Williams and Carter, 2009). Although TEM provide 2D images, the technique of electron tomography can be used to create 3D images using a sequence of micrographs taken at different tilts (Williams and Carter, 2009).

Scanning electron microscopy (SEM) uses electrons for high resolution imaging of the sample surface (Reimer, 2000), and represents a valid tool to investigate some nanomaterials (Bogner *et al.*, 2005). The topography of the nanostructured samples can be preserved using special techniques that avoid any manipulation (environmental or wet SEM) or pre-

serve their morphology by rapid freezing (cryo-SEM). The environmental SEM, allowing analyses on hydrated materials without fixing, drying, freezing or coating the specimen (Bogner *et al.*, 2005), is especially suitable to characterize microspheres and microcapsules (Xiong *et al.* 2012). Cryo-SEM method has been applied for the characterization of microspheres (Allan-Wojtas *et al.*, 2008) and nanoemulsions (Hoesli *et al.*, 2012).

Polarized light microscopy (PLM) may be used for the preliminary identification of many liquid-crystalline structures (Gaisin *et al.*, 2010). The anisotropic systems cause a deviation in the plane of polarized light and show typical black and white or colored textures. Based on this texture, liquid-crystalline structures can be classified in: (i) lamellar liquid crystalline phase which reveals oily streaks with inserted "maltese crosses" in the micrograph; (ii) hexagonal liquid-crystalline structure which is indicated by a fanlike texture (Müller-Goymann, 2004; Carvalho *et al.*, 2010; Rissi *et al.*, 2014). However, PLM can be applied to particles whose size approaches the wavelength of visible light (400 to 700 nm); for liquid crystal particles presenting smaller dimensions, TEM is necessary to resolve them (Müller-Goymann, 2004).

Atomic force microscopy (AFM) is one of the most popular scanning probe microscopy methods (Binning *et al.*, 1986) and the interaction of nanoparticles with the AFM probe has been extensively studied from different experimental points of view (AFM tip modification, nanoparticle manipulation, substrate influence) (Theil Hansen *et al.* 1998; Lee *et al.*, 1998; Klapetek *et al.*, 2011; Henry, 2005). AFM allows detection and imaging of nanoparticles from 0.5 nm in diameter and, although it has been mostly applied to inorganic nanoconstructs, it is also suitable to characterize hydrated nanomaterials.

Microscopy for visualizing nanoconstructs in living organisms

To be used in nanobiology and nanomedicine, nanoconstructs need to be tested in living organisms. Cells cultured *in vitro*, which ensures simple and controlled conditions, represent the experimental model of choice. The preliminary utilization of *in vitro* systems also allows short experimental times and reduction of the number of laboratory animals for the following *in vivo* studies, thus implying a significant decrease of the research costs.

Light microscopy has been largely applied for the safety assessment of nanomaterials and for designing efficient administration strategies for biomedical use. Microscopy techniques proved to be useful to study the interaction of nanoparticles with the cells and to visualize their intracellular fate, while allowing to simultaneously evaluate signs of cell damage or death (for a review, see Ostrowski *et al.*, 2015).

By definition, nanoparticle are less than 100 nm in size and cannot be resolved as single entities even at the highest magnification in conventional light microscopy. Thus, only nanoparticulates that form clusters or aggregates of more than 200 nm in size can directly be visualized in single cells or tissues. Depending on their chemical composition, some nanoconstructs (e.g., carbon nanotubes, iron oxide or titanium dioxide nanoparticles) can be directly observed as naturally colored deposits (Porter *et al.*, 2010; van Landeghem *et al.*, 2009; Adachi *et al.*, 2010). Enhanced darkfield microscopy has also been used to detect metal oxide nanoparticles in histological samples (Roth *et al.*, 2015). In addition, some histochemical techniques are suitable to stain either inorganic or organic nanoparticles; Prussian blue may be used to stain iron containing nanoconstructs (Bumb *et al.*, 2011 and Figure 1a) while nanoparticles con-

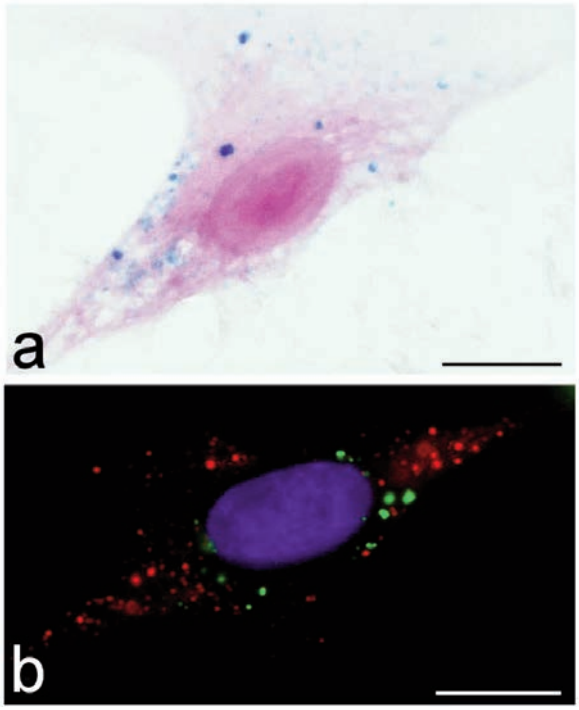

Figure 1. a) Brightfield microscopy. Iron oxide nanoparticles (Prussian blue staining) inside a murine fibroblast (hematoxylin countestaining). b) Fluorescence microscopy. Chitosan nanoparticles (green) inside a human epithelial cell; lysosomes (red) are visualized by specific immunostaining; DNA (blue) is stained with Hoechst 33258. Bars: 20 µm.

taining polysaccharide with negatively charged sulfate groups have been successfully visualized by Alcian blue staining (Holzhausen *et al.*, 2013).

No doubt, fluorescence microscopy is the most widely used approach to investigate the biodistribution and the intracellular localization of nanoconstructs at light microscopy. To this purpose, nanoparticles are usually labelled with fluorochromes (Figure 1b), which must be selected for their structure, molecular weight and charge not to alter the physicochemical characteristics of nanoconstructs.

The interaction of fluorochrome-conjugated nanoparticles with specific cells or intracellular organelles may be visualized by the simultaneous immunofluorescence labelling of marker proteins (Cho *et al.*, 2009; Malatesta *et al.*, 2015 and Figure 1b). A more precise spatial localization of nanoparticles in their interactions with cells may be obtained by confocal laser scanning microscopy: by this technique, serial optical sectioning of the sample are obtained, which allows 3D reconstructions of single cells or tissues sections. However, confocal microscopy is diffraction-limited as much as conventional fluorescence microscopy, so that the X-Y resolution is restricted to about 200 nm, substantially larger than the <100 nm size of nanoparticles. Techniques of super-resolution light microscopy may overcome this limitation, allowing to significantly increase X-Y resolution up to about 30 nm (Willig *et al.*, 2006; Sonnefraud *et al.*, 2014; Guggenheim *et al.*, 2016).

TEM, thanks to its higher resolution, is however the most appropriate approach to obtain detailed and unequivocal information on each step of nanoconstruct interactions with the cell components, from their uptake at the cell surface to their intracellular degradation (Figure 2). A clear analysis of nanoparticle internalization mechanism(s) can be

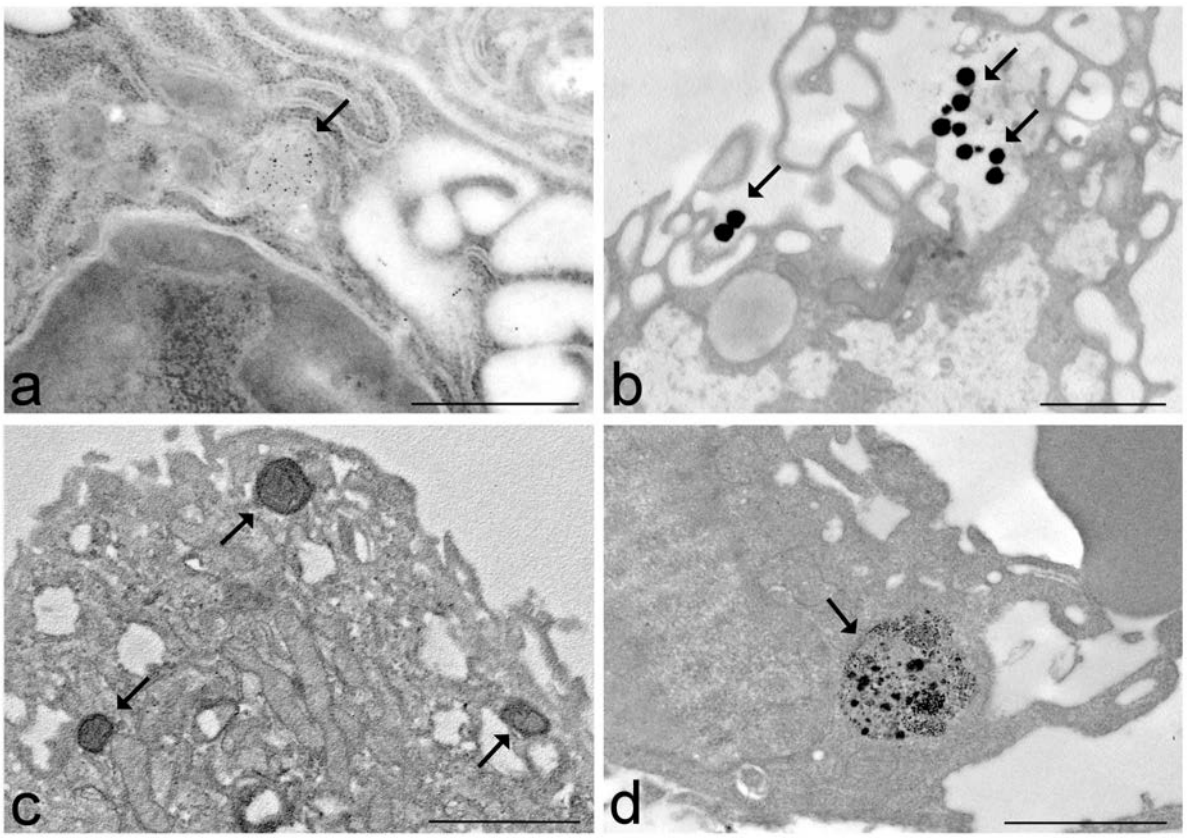

Figure 2. Transmission electron microscopy. a) Gold nanoparticles (arrow) internalized in a human macrophage. b) Lipid nanoparticles (arrows) entering a human epithelial cell. c) Polymeric nanoparticles (arrows) in a human myoblast. d) Quantum dots (arrow) inside a murine macrophage. Bars: a,c 200 nm; b,d 1000 nm.

obtained, visualizing the contact with the plasma membrane and the passage into the cell by endocytosis, phagocytosis or membrane fusion (as in the case of nanoconstructs of lipid nature) (e.g., Zhang *et al.*, 2011; Malatesta *et al.*, 2012; Costanzo *et al.*, 2016a,b; Boyles *et al.*, 2015; Poussard *et al.*, 2015; Lopes *et al.*, 2016; Messerschmidt *et al.*, 2016; Zielinska *et al.*, 2016). The distribution of the nanoparticulates in the cellular compartments provides information on their fate: the entrapment into endosomes or phagosomes prefigures their rapid degradation in the lysosomal compartment, while their free (organelle-unbound) occurrence in the cytosol indicates their ability to escape endosomes and, consequently, the enzymatic lysis (Panyam *et al.*, 2002; Varkouhi *et al.* 2011). However, TEM observations revealed that these free nanoparticles may re-enter the lytic pathway by autophagosomal processes (Costanzo *et al.*, 2016a,b). Importantly, TEM allows to distinguish the presence of intact nanoparticles or their remnants after enzymatic lysis, thus providing unequivocal information on their biodegradability. TEM can also provide clear evidence for the distribution of nanoparticulates inside the cell nucleus: some nanoparticles may, in fact, enter the nucleus by passing through the nuclear pores or being entrapped therein at the end of mitosis (Nabiev *et al.*, 2007; Colonna *et al.*, 2011; Guan *et al.*, 2012; Malatesta *et al.*, 2013, 2015; Zhang *et al.*, 2015). This is a crucial information for evaluating the safety of nanoconstructs, since the persistence of exogenous materials in close proximity of nucleic acids may have unpredictable consequences on whole cell activity.

An important contribution to nanomedical research has been given also by correlative microscopy. Light (especially fluorescence) microscopy was combined with advanced TEM methods (conventional, immuno and energy-filtered electron microscopy, and electron tomography) to analyze the biodistribution of different types of nanoparticles (Mühlfeld *et al.*, 2007). Quantum dots were identified in *in vitro* and *ex vivo* samples by combining fluorescence microscopy, TEM and scanning transmission electron microscopy (STEM) (Dukes *et al.*, 2010; Killingsworth and Bobryshev, 2016), and the combination of TEM and Serial Block Face SEM allowed to quantify their intracellular uptake (Hondow *et al.*, 2016). The intracellular distribution of gold nanoparticles was investigated by using interferometric photo-activated localization microscopy and electron microscopy (Shtengel *et al.*, 2014), while their identification inside tumor masses was performed by combining optical microscopy and SEM (Kempen *et al.*, 2015). The uptake and intracellular fate of ZnO-based nanoparticles were analyzed combining dynamic confocal imaging, low resolution bright field TEM and dark field STEM (Othman *et al.*, 2016). Cryo-soft X-ray tomography was used to obtain three-dimensional information on the interaction of super-paramagnetic iron oxide nanoparticles with cancer cells (Chiappi *et al.*, 2016). Fluorescence microscopy and SEM were combined to investigate macrophage uptake of cylindrical nanoparticles (Tscheka *et al.*, 2015).

Concluding remarks

In the last 15 years, more than 190,000 articles have been published in qualified journals on nanoparticles, (source: Scopus database, https://www.scopus.com), and in about 57,000 papers of these, microscopy techniques were used among the experimental methods. This clearly indicates the great impact that microscopy sciences have in nanomedical research and applications. It is easy to foresee that this will even increase in the years to come, thanks to the continuous progress in microscopy technology and instrumentation. TEM still is the most informative approach for investigating the interaction of nanoconstructs with cells and intracellular organelles, but super-resolution light microscopy may be envisaged as the future in the field: multicolor histochemical techniques will allow to simultaneously detect the interactions of nanoparticles with several subcellular components at the nanodimension of super-resolved fluorescence microscopy.

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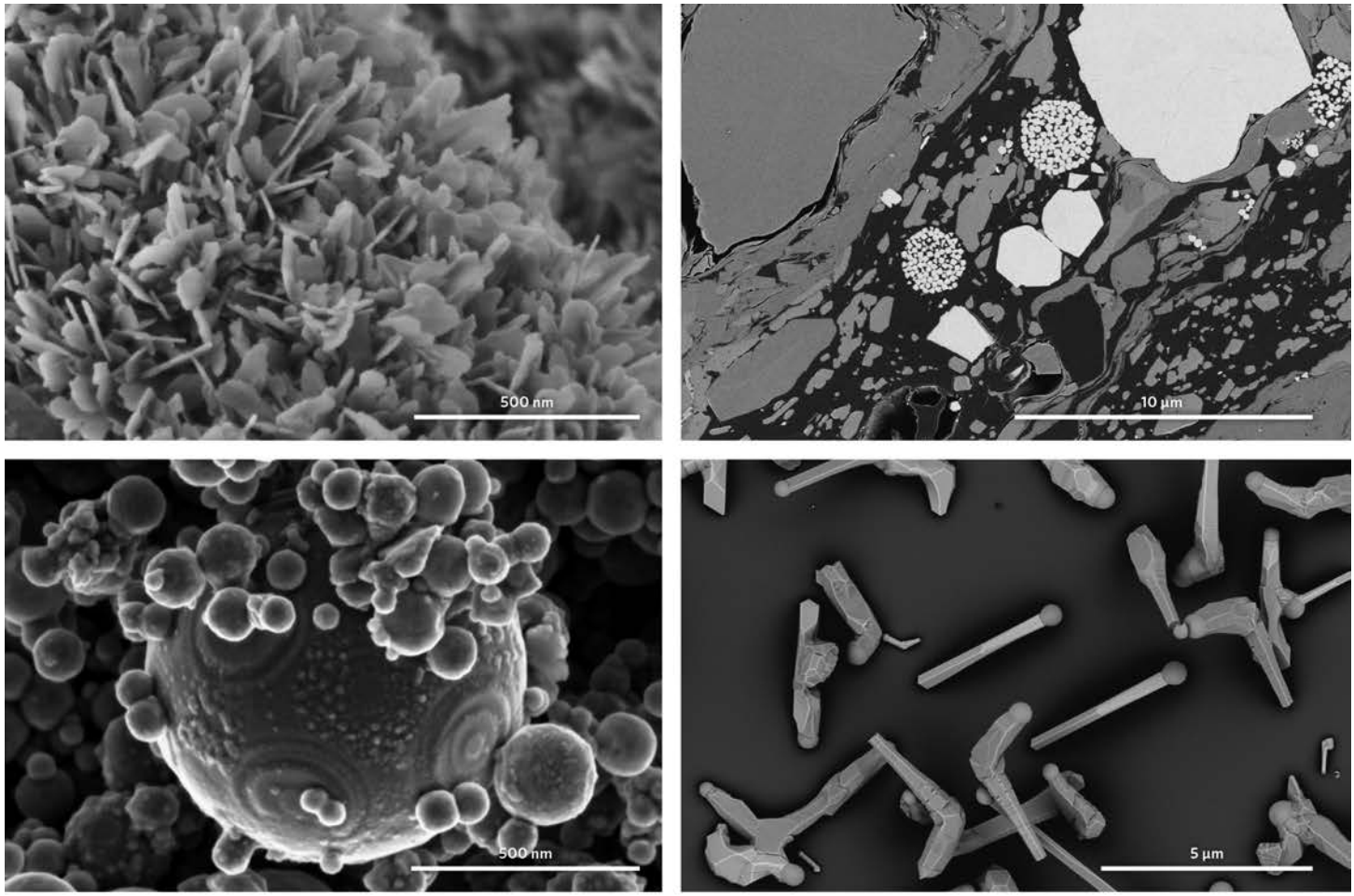

Top left, *Hydroxyapatite crystals*. Sample courtesy of Devin Wu, FEI China and Shanghai Institute of Ceramics. **Top right, *Shale*.**
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