

XRI³ *CTP* 2006

**International Workshop on X-ray
spectromicroscopy and imaging**

in conjunction with

**1st Meeting of the International
Consortium for Coherent X-ray Diffractive
Imaging (ICCDXI)**

and

Round Table for Diffractive X-ray Optics

May 20th – 22nd, 2006

GENERAL INFORMATION

Location of the conference

The conference XRI3CTP in conjunction with the 1st Meeting of the ICCDXI consortium and the Round Table on Diffractive X-ray Optics will take place in the Adriatico Guesthouse of the Abdus Salam International Center for Theoretical Physics in Grignano, Trieste, Italy. The meeting room is the so-called Kastler room on the ground floor.

International Center for Theoretical Physics (ICTP)
Adriatic Guesthouse (AGH)
Via Grignano, 9
34014 Trieste, Italy
Tel: (+39) 040 2240 112
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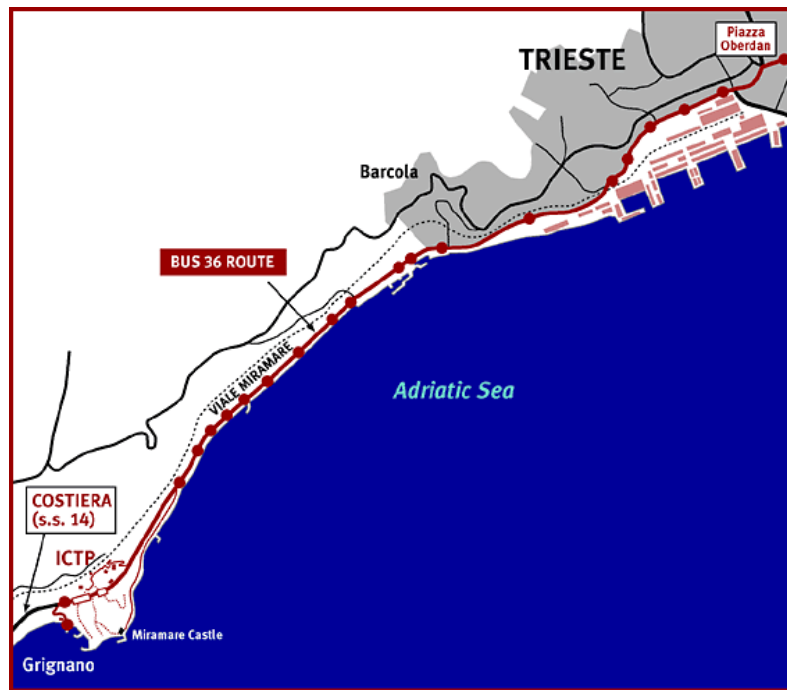


Bus, taxi, train and boat services link the Trieste Rail Station, located 7 km east of [ICTP campus](#), to the Centre. If you arrive in Trieste by train, you will need to reach ICTP from downtown Trieste.



Bus No. 36 to ICTP

The map shows where the bus stop is near to the train station (which is called Trieste Centrale). Bus No. 36 will take you to ICTP as shown next.

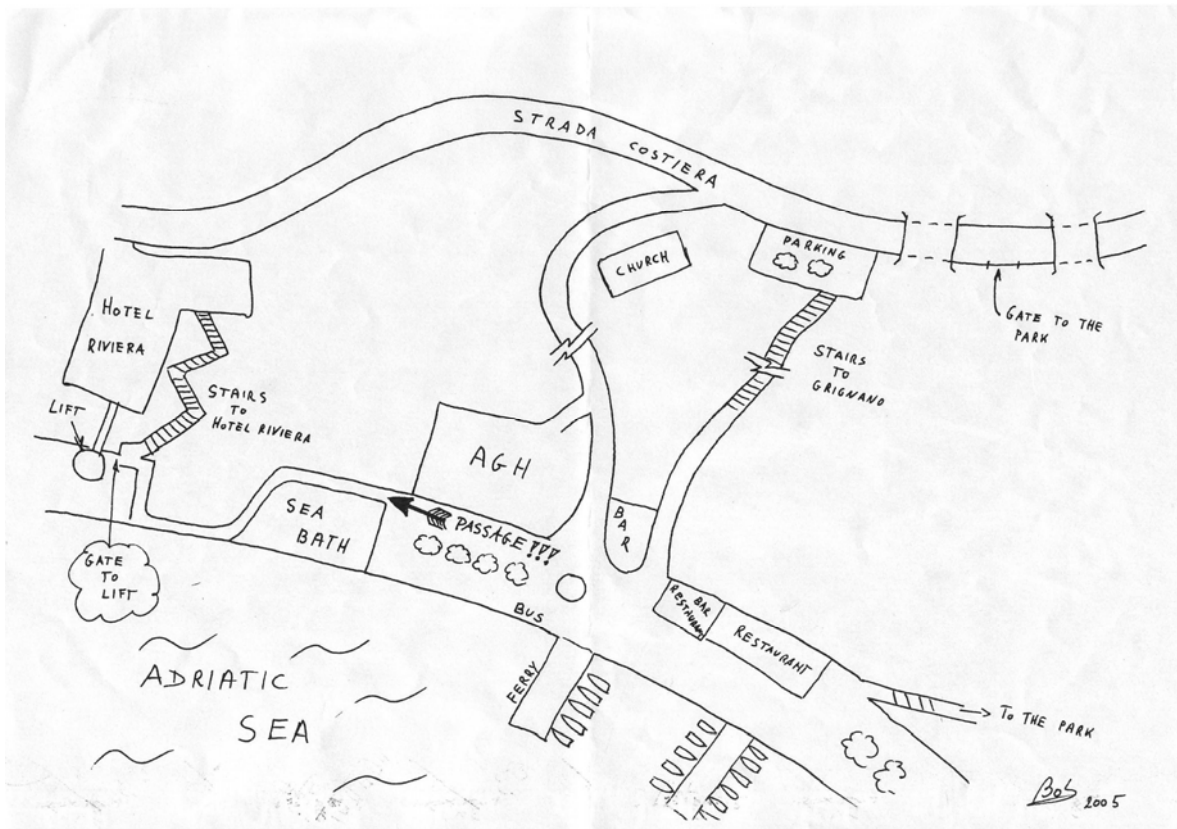


Bus No. 36 runs from Piazza Oberdan in downtown Trieste to Grignano just a short distance from the [Adriatico Guesthouse](#). The stop before Grignano (when coming from Trieste) is closest to the Main Building. Bus 36 runs during the day every 20 minutes. In summer (between June and September), the bus runs more often. From 21:00 until midnight, Bus C runs between Piazza Goldoni and Barcola. Up-to-date bus timetables are available on the Web ([Trieste Trasporti](#)) or the information desk during the conference. You must purchase your tickets beforehand and they must be validated using the machines provided on the buses. Bus tickets are available at news stands or tobacconists in Trieste and the surrounding area or at the Reception desks of the Adriatico and Galileo Guesthouses. When you are in the tunnel, ring the bell to get off the bus. In the Grignano area, next to the road going down to the Adriatico Guesthouse, you will find the signposts indicating where the buildings of the ICTP campus are.

A boat service has been established recently. Detail information will be provided at the information desk.

How to get from the Hotel Riviera to the Conference venue

It is not necessary that you use the main street “Strada Costiera” to reach the AGH. From Hotel Riviera, take the lift which takes you down to the seaside, walk along the seaside till you pass the sea bath and the lower parking of the AGH.



Lecture hall

The “Kastler” room is located on the ground floor of the Adriatico Guesthouse.

Contact to Organizing Committee during the Workshop

An information desk will be located in front of the Kastler conference room.

Lunch and Coffee breaks

Lunch and coffee will be served in the restaurant of the Adriatico Guesthouse (1st floor). Lunches and coffee breaks are free of charge for registered participants.

























Social dinner

The conference dinner will take place on May 21st at 19:00 in the Hotel Riviera. Non-invited participants can purchase a dinner voucher during registration (35 €).

Computer and internet access

Computer terminals will be available at and close by the information desk. Those registered participants who wish to use the ICTP wireless network, have to make an account request to the Organizing Committee not later than May 8th.

WORKSHOP PROGRAMME - OVERVIEW





Saturday, May 20th 2006		Sunday, May 21st 2006		Monday, May 22nd 2006	
Session 1		Session 4		Session 2/2	
9:00	Welcome address	9:00	E. Di Fabrizio - Univ. Catanzaro, I 	9:00	H. Hertz - KTH Stockholm, SE 
9:20	J. Susini - ESRF, F 	9:40	S. Rehbein - BESSY, D 	Session 6	
10:00	Q. Shen - APS, US 	10:20	K. Jefimovs - SLS, CH 	9:40	J. Kirz – LBL/ Stony Brook, US 
10:40	C. Knoechel - ISA, DK 	10:50	Coffee break	10:20	I. Robinson - DIAMOND, UK 
11:10	F. Polack - SOLEIL, F 	Session 5		10:50	Coffee break
11:40	Coffee break	11:10	C. Jacobsen - NSLS/ Stony Brook, US 	11:20	P. Thibault - Cornell Univ., US 
Session 2/1		11:50	H. Stoll - MPI Stuttgart, D 	11:50	F. Parmigiani - Univ. Trieste/ ELETTRA, I 
12:10	S. Wilkins - CSIRO, AU 	12:20	M. Wieland - Univ. Hamburg, D 	12:20	Lunch break/ Poster Session
12:50	C. David - SLS, CH 	12:50	A. Barty - LLNL, US 	Round Table - ICCDXI	
13:30	Lunch break	13:20	Lunch break/ Poster Session	14:00	Introduction (S. Wilkins)
Session 3		Round table		14:15 - o.e.	ICCDXI Round Table
14:30	G.R. Morrison – KCL, UK 	15:00	Introduction (B. Kaulich)		
15:10	T. Wilhein - FH Koblenz, D 	15:15 - o.e.	Round table - Diffractive X-ray Optics		
15:50	Coffee break				
16:20	A. Bravin - ESRF, F 				
16:50	E. Tasciotti - ICGEB, I 				
17:20	L. Rigon - ICTP, I 				
17:50 - 18:20	A. Bronnikov - Bronnikov-Algorithms, NL 	19:00	Social dinner in Hotel Riviera		

PROGRAMME

SATURDAY, MAY 20th



Session 1 – X-ray spectromicroscopy and imaging with synchrotron light sources

(Chair: **M. Kiskinova**, ELETTRA, Italy)

9:00	Welcome address by C. Tuniz & B. Kaulich
9:20	J. Susini , European Synchrotron Radiation Facility (ESRF), France <i>X-ray Fluorescence microscopy and micro-spectroscopy: Multidisciplinary tools</i> 
10:00	Q. Shen , Advanced Photon Source (APS), Argonne National Lab, USA <i>X-ray microscopy and imaging at the Advanced Photon Source</i> 
10:40	C. Knoechel , Institute for Storage Ring Facilities, Denmark <i>Status of the Cryo X-ray Microscopy at Aarhus</i> 
11:10	F. Polack , SOLEIL, France <i>Microscopy beamlines at SOLEIL</i> 
11:40	<i>Coffee break</i>





Session 2/1 Imaging and microscopy with lab sources I

(Chair: **T. Wilhein**, RheinAhrCampus Remagen, D)

12:10	S. Wilkins , Australian Commonwealth Scientific and Research Organization (CSIRO), Australia <i>X-ray phase-contrast microscopy and microtomography using lab-based system</i> 
12:50	C. David , Paul-Scherrer Institute, Swiss Light Source (SLS), Switzerland <i>Interferometric phase contrast imaging and tomography using incoherent radiation sources</i> 
13:30	<i>Lunch break</i>

Session 3 – Microtomography, medical imaging and phase sensitive imaging techniques

(Chair: **G. Tromba**, ELETTRA, Italy)

14:30	G. R. Morrison , King's College London, United Kingdom <i>Simultaneous absorption and phase contrast imaging using a scanning transmission x-ray microscope</i> 
15:10	T. Wilhein , IXO, RheinAhrCampus Remagen, Germany <i>Differential interference contrast x-ray microscopy with diffractive x-ray optics</i> 
15:50	<i>Coffee break</i>
16:20	A. Bravin , European Synchrotron Radiation Facility (ESRF), France <i>Phase contrast x-ray imaging in biomedicine: the ESRF experience</i> 
16:50	A. Tasciotti , International Centre for Genetic Engineering and Biotechnology (ICGEB), Italy <i>Molecular imaging in cells and live animals for biomedical research</i> 

Session 3 (cont.) – Microtomography, medical imaging and phase sensitive imaging techniques

(Chair: **G. Tromba**, ELETTRA, Italy)

17:20	L. Rigon , International Center for Theoretical Physics (ICTP), Italy <i>Recent developments in diffraction enhanced imaging</i>
17:50-18:20	A. Bronnikov , Bronnikov-Algorithms, Netherlands <i>Reconstruction algorithms for phase-contrast tomography</i>

SUNDAY, MAY 21st

Session 4 – Focusing optics and its application for imaging

(Chair: **C. David**, Paul-Scherrer-Institute, Swiss Light Source (SLS), Switzerland)

9:00	E. Di Fabrizio , University of Catanzaro, Italy <i>Diffraction nano-optics and their use for X-ray synchrotron radiation</i>
9:40	S. Rehbein , Berliner Elektronenspeicherring für Synchrotronstrahlung (BESSY), Germany <i>Zone plate fabrication at BESSY</i>
10:20	K. Jefimovs , Paul-Scherrer-Institute, Swiss Light Source (SLS), Switzerland <i>Fresnel zone plates for hard x-ray nanofocusing</i>
10:50	<i>Coffee break</i>

Session 5 – Spectromicroscopy and imaging with FEL's, ps- and fs-time resolved imaging

(Chair: G.R. Morrison, King's College London, UK)

11:10	C. Jacobsen , Stony Brook University, NSLS, USA <i>Spectromicroscopy analysis: clustering, error finding, and interpolation</i>
11:50	H. Stoll , Max-Planck Institute for Metal Research, Germany <i>Time resolved transmission x-ray microscopy</i>
12:20	M. Wieland , University of Hamburg, Germany <i>A VUV/ VIS cross-correlator for the temporal characterization of VUV-FEL pulses</i>
12:50	A. Barty , Lawrence Livermore National Lab (LLNL), USA <i>Ultrafast coherent diffraction imaging with a soft x-ray free electron laser</i>
13:20	<i>Lunch break/ Poster session</i>


Round Table on Diffractive X-ray Optics

15:00	B. Kaulich , ELETTRA, Italy <i>Introduction</i>
15:15 to open end	<i>Discussions</i>
16:30	<i>Coffee break</i>

MONDAY, MAY 22nd





Session 2/2 – Imaging and microscopy with lab sources

(Chair: **C. Jacobsen**, NSLS & Stony Brook Univ., US)

9:00	H. Hertz , Institute for Biomedical and X-ray Physics (BIOX), Royal Institute for Technology, Sweden <i>X-ray microscopy with laboratory sources</i> 
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Session 6– Coherent imaging and approaches for data analysis and image processing

(Chair: C. Jacobsen, NSLS & Stony Brook Univ., US)

9:40	J. Kirz , Lawrence Berkeley Lab (LBL)/ National Synchrotron Light Source (NSLS), USA <i>Diffraction microscopy – past, present and future</i> 
10:20	I. Robinson , DIAMOND, United Kingdom <i>Retrieval and Interpretation of Complex Images of Nanocrystals</i> 
10:50	<i>Coffee break</i>
11:20	P. Thibault , Cornell University, USA <i>Reconstruction in the real world</i> 
11:50	F. Parmigiani , ELETTRA/ University of Trieste, Italy <i>Science with UV/ X-ray free electron lasers</i> 
12:20	<i>Lunch break</i>

Round Table on Diffractive X-ray Optics

14:00	S. Wilkins , Australian Commonwealth Scientific and Research Organization (CSIRO), Australia <i>Introduction</i>
14:15 to open end	<i>Discussions</i>
16:00	<i>Coffee break</i>

ABSTRACTS
ORAL PRESENTATIONS

X-ray Fluorescence microscopy and micro-spectroscopy: Multidisciplinary tools

Jean Susini

*European Synchrotron Radiation Facility,
BP220, F-38043 Grenoble Cedex , France
susini@esrf.fr*

The X-ray microanalysis techniques follow today the evident trend in the development of nano-technologies by pushing further spatial resolution. Hence, considering the concomitant developments of laboratory instruments and dedicated synchrotron beam lines worldwide, a very competitive context can be anticipated for the coming years. Towards this perspective, synchrotron based analytical techniques (diffraction, imaging and spectro-microscopies) will play an important role by offering unique capabilities in the study of complex systems. Ultimately, this complexity can be envisioned in three dimensions: compositional, temporal and spatial. Typical experiments can be broadly divided into two categories. On one hand, morphological studies, which require high spatial resolution and are, therefore, well adapted to 2D or 3D full-field imaging microscopy. On the other hand, studies dealing with co-localization and/or speciation of trace elements in heterogeneous systems. Scanning X-ray microprobes using various detection modes – transmission and fluorescence - are better suited for the latter cases, which often require both low detection limits and spectroscopic analysis capabilities for chemical composition and chemical state, respectively.

Compared to other techniques, Synchrotron X-Ray Fluorescence (SXRF) microprobes display a unique combination of features. Today, SXRF microprobes using undulator sources provide micron spatial resolution and sub-ppm detection limits for $Z > 20$. When associated with a high collection detection system, the radiation damage is minimal and accurate quantification is possible. Furthermore, the possibility of in-situ experiments remains a unique attribute of synchrotron based analytical methods. Physical penetration of hard X-rays enables specific sample environments to be developed to study realistic systems in their near-native environment rather than model systems. Ability to analyze in-situ in environmental chambers such as high or low temperature, high pressure, or wet cells explains the increasing interest from communities such as Planetary and Earth, environmental science and microbiology. More recently, fluorescence tomography has been developed, where 2-D slices are obtained through a 3-D object without physical sectioning.

Among the 40 beamlines in operation at the European Synchrotron Radiation Facility (Grenoble, France), three beamlines are fully dedicated to X-ray microscopy and micro-spectroscopy techniques in the multi-keV energy range. The main fields of applications are driven by the unique attributes of X-ray microscopy in this spectral range: i) access to K-absorption edges and fluorescence emission lines of medium-light elements and L,M - edges of heavy materials for micro-spectroscopy, chemical or trace element mapping; ii) higher penetration depths compared to soft X-rays allowing imaging of thicker samples; iii) favorable wavelengths for diffraction studies and iv) generally large focal lengths and depths of focus which are advantageous for the use of specific sample environments (in-situ, high pressure, controlled temperatures....).

This presentation will be biased towards sub-micron microscopy developed on the X-ray microscopy beamlines at the European Synchrotron Radiation Facility (Grenoble). Following a brief account on the characteristics of these instruments, strengths and weaknesses of X-ray microscopy and spectro-microscopy techniques in the 1-20keV range will be discussed and illustrated by examples of applications.

X-ray Microscopy and Imaging at the Advanced Photon Source

Qun Shen

*X-ray Microscopy and Imaging Group
X-ray Science Division / Advanced Photon Source
Argonne National Laboratory
Argonne, IL 60439, USA*

X-ray microscopy and imaging is widely used in a variety of synchrotron applications to investigate structures from nanometer to millimeter scales in materials science and biology. This talk will provide an overview of current research activities in the X-ray Microscopy and Imaging Group at the Advanced Photon Source, including both full-field and scanning probe applications. Applications cover a wide range of scientific areas such as: functional materials research at nanometer scale in thin-films and self-organized and self-assembled structures; biological and biomedical research on trace-element distributions in subcellular organelles, single cells, and tissues; environmental research on natural and externally introduced nutrients and toxicities in soil and marine systems; materials microstructure studies on internal strain, grain boundaries, deformation and sintering; and small animal and soft tissue research on vascular networks and pulmonary ventilation. In addition, significant R&D efforts are directed to advance to state-of-the-art x-ray microscopy and imaging facilities such as phase-contrast and coherent diffraction imaging capabilities, as well as nano-focusing optics developments.

This work at the Advanced Photon Source is supported by the U.S. Department of Energy under contract number W-31-109-ENG-38.

Status of the Cryo X-ray Microscope in Aarhus

C. Knöchel

ISA

University of Aarhus

Ny Munkegade, Building 1520

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The Institute for Storage Ring Facilities at the University of Aarhus operates an X-ray microscope in the soft X-ray region at a bending magnet source on the ASTRID storage ring. In the course of an upgrading process an additional vacuum object chamber for investigating cryogenically fixed samples was implemented. For mounting an object under cryogenic conditions in the microscope a modified Gatan sample holder is used. A light microscope for prealigning the sample in the vacuum chamber is under construction at the moment. A crucial aspect for the operation of the X-ray microscope is the supply of optics. This is especially true for the condenser optics which are practically no longer available. In cooperation with other groups we are trying to establish a fabrication process for these large area optics. Some first test experiments will be presented.

Microscopy beamlines at SOLEIL

François Polack and Mourad Idir

Synchrotron SOLEIL, L'Orme des Merisiers, BP 48, 91192 Gif-sur-Yvette, France

Micro-focused beams and micro-imaging instruments are more and more requested from synchrotron radiation facilities. SOLEIL expects to be able to fulfill this demand in almost all the spectral range it covers from IR to hard X-rays. In the long wavelength range, two beamlines, SMIS and DISCO, will be equipped with spectromicroscopes respectively in the 1 - 10 μm and 200 – 900 nm ranges. In the short wavelength domain, an X-PEEM will be installed on a side branch of the μfocus beamline. The X-ray microscopy needs should be covered by three beamlines.

One of these, LUCIA, is already in operation at the SLS since 2004. It covers the 0.8 – 8 keV range with a DC monochromator, and delivers on the sample, by means of a bendable KB mirror pair, a medium focus probe whose minimum size is $\sim 2.5 \mu\text{m}$. The transfer of the beamline back to SOLEIL is scheduled for 2008.

Soft X-ray and hard X-ray beamlines are still in preliminary project state. The hard X-ray beamline will cover the 4 – 20 keV energy range. It will be a multi technique beamline, equipped for $\mu\text{-XFS}$, $\mu\text{-XAS}$, $\mu\text{-XRD}$ and micro tomography. Probe focusing will be primarily achieved by KB mirrors and produce μm sized spots. Producing smaller spots is still an issue due to the horizontal electron beam size and the available source to experiment distance. It will almost certainly require the use of hard x-ray zone plates.

In establishing a proposal of soft X-ray beamline, equal number of demands has been received from users for an Imaging Transmission Microscope TXM as for a scanning microscope STXM. The accepted project considers one microscope of each kind on two branch lines of the same beamline. The two microscopes will share the same APPLE II type undulator and the same grating monochromator. The spectral range will extend from 200 to 2000 eV with a moderate resolution of several thousands, by use of one standard lamellar grating and one multilayer grating. For the adequate illumination of the TXM from the small emittance beam of the undulator, a dynamic synthesis of the input aperture with flipping mirrors is foreseen. The performances of these microscopes will mainly depend on the quality of the zone plate that will be available. The procurement of these elements is still considered as a critical issue. French microfabrication facilities have been contacted, and have declared interested. Other collaboration offers will be welcomed.

X-ray Phase-Contrast Microscopy and Microtomography using a Lab-Based System

S. W. Wilkins¹, S.C Mayo¹, P.R. Miller¹, D. Gao¹, T.E. Gureyev, J. Sheffield-Parker²

¹*CSIRO Division of Manufacturing and Infrastructure Technology, Private Bag 33,
Clayton South, VIC 3169*

²*XRT Limited, A3.0, 63 Turner Street, Port Melbourne, VIC 3207*

We have developed an X-ray microscope, the XuM, based on a scanning electron microscope in which the fine focus of the electron beam is used to produce a submicron x-ray source down to 100nm in size [1,2]. This is used in a point projection imaging regime in which the sample is much closer to the x-ray source than to the detector. This geometry results in natural magnification, and, due to the small source-size and large gap between sample and detector, it also produces in-line phase-contrast.

Whereas conventional x-ray imaging relies solely on x-ray absorption, inline phase-contrast exploits the refraction of x-rays by a sample to enable imaging of non- or weakly absorbing specimens and to enhance the visibility fine features, edges and boundaries. Extending this form of imaging to microscopy and microtomography enables us to look inside a weakly-absorbing microscopic samples without cutting them physically at resolutions down to <100nm (imaging) or 1 or 2 microns (tomography).

We will show examples of microscopy and microtomography of a wide variety of specimens using this instrument. The importance of phase-retrieval in tomographic reconstruction, and methods of improving tomographic resolution will be discussed. These techniques are equally relevant to synchrotron-based applications.

Some References

1. S.C Mayo, P.R. Miller, S.W. Wilkins, T.J. Davis, D. Gao, T.E. Gureyev, D. Paganin, D., D.J. Parry, A. Pogany, and A.W. Stevenson, *J. Microsc.*, **207**, (2002) pp. 79-96.
2. S.C. Mayo, T.J. Davis, T.E. Gureyev, P.R. Miller, D. Paganin, A. Pogany, A.W. Stevenson, S.W. Wilkins, *Optics Express*, **11**, (2003), pp. 2289-2302.

Interferometric phase contrast imaging and tomography using incoherent radiation sources

C. David*, C. Kottler, F. Pfeiffer, O. Bunk, M. Stampanoni, C. Grünzweig, G. Frei,
E. Lehmann

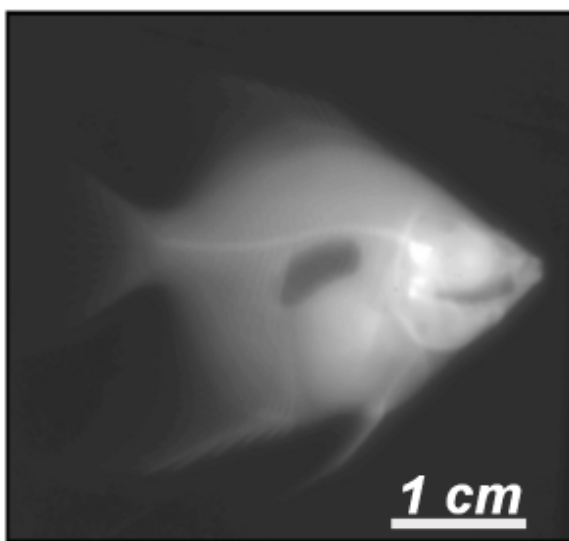
Paul Scherrer Institut, Switzerland

We report how an interferometric method can produce quantitative x-ray and neutron phase contrast images. The interferometer is based on diffraction gratings fabricated using microlithography techniques. Separate phase and absorption images are recorded simultaneously [1]. By taking data sets under many viewing angles, a tomographic reconstruction of both the real part and the imaginary part of the objects complex refractive index distribution can be obtained.

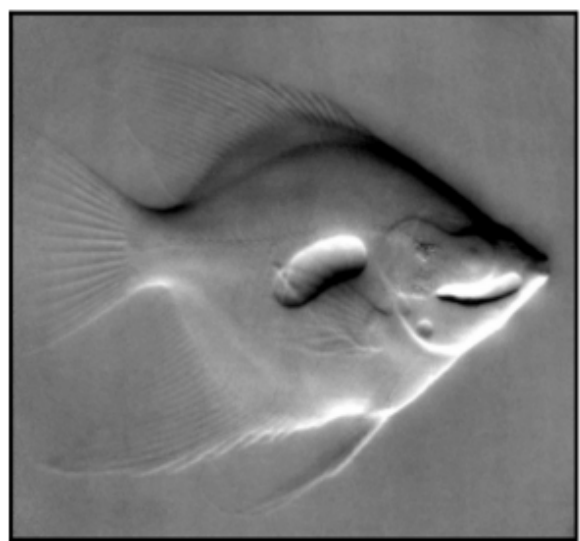
In the x-ray case, the method can be used to enhance the contrast in medical radiography and it has the potential to reduce the applied radiation dose. As opposed to existing techniques, the method requires only little coherence and can be scaled up to fields of view of many centimetres (see Fig. 1). Its application is therefore not limited to be used at synchrotron light sources, but it can be used with standard x-ray tube sources [2]. This opens up a wide range of applications in medical imaging and non destructive testing.

In addition, very recent experiments with cold neutron radiation are presented [3]. Again phase contrast images and tomographic data sets were recorded. Our technique opens up the way for combining an imaging approach with information obtained through the quantum mechanical interactions of neutrons with matter.

- [1] T. Weitkamp, A. Diaz, C. David, F. Pfeiffer, M. Stampanoni, P. Cloetens, E. Ziegler, *Optics Express* **13** (2005) 6296-6304
- [2] F. Pfeiffer, T. Weitkamp, O. Bunk, and C. David, *Nature Physics* **2** (2006) 258 – 261
- [3] F. Pfeiffer, C. Grünzweig, O. Bunk, G. Frei, E. Lehmann, C. David, in preparation



X-Rays - Absorption



X-Rays – Phase-Contrast

Fig. 1: X-ray images of a fish in absorption and phase contrast taken with an incoherent Mo x-ray tube

Simultaneous Absorption and Phase Contrast Imaging using a Scanning Transmission X-ray Microscope

G R Morrison¹, A Gianoncelli², B Kaulich³

1. *Dept of Physics, King's College London, Strand, London WC2R 2LS*
2. *C2RMF - UMR CNRS 171, AGLAE, Palais du Louvre, Paris, France*
3. *ELETTRA - Sincrotrone Trieste, I-34012 Trieste-Basovizza, Italy*

The use of a segmented x-ray detector with a software-configurable response function makes it possible for the scanning transmission x-ray microscope to form images in a number of different contrast modes from a single raster scan of the specimen. In particular the use of symmetric and anti-symmetric detector responses allows both absorption and differential phase contrast images to be derived from a single scan of the specimen.

This paper describes measurements made using the Twinmic end-station at the Elettra synchrotron. This uses a Peltier-cooled, fast-readout CCD (FRCCD) detector having 128 by 128 pixels, with visible light coupling to a phosphor screen located downstream of the sample. The FRCCD records a full frame of data for every pixel in the STXM raster scan, potentially generating large volumes of data; simple real-time processing of these data yields absorption and differential phase contrast image signals, while the full volume of image data is retained to allow more sophisticated off-line analysis when required.

As the configurable FRCCD is a straightforward substitute for the more conventional single detector used in STXM, the spatial resolution of the STXM is not compromised, and is determined by the characteristics of the focusing optics, so at present, the complex specimen transmittance can be studied at a resolution of just over 0.1 μm . Measurements made at energies on either side of an x-ray absorption edge show there are considerable practical benefits to having both absorption and phase contrast information simultaneously available to the user, while through-focal series of images allow the relative importance of the real and imaginary parts of the complex refractive index to be investigated.

Differential Interference Contrast X-Ray Microscopy with Diffractive X-Ray Optics

¹**Thomas Wilhein**, ²**Uli Vogt**, ³**Burkhard Kaulich**, ⁴**Enzo Di Fabrizio**,
⁵**Pambos Charalambous**

¹*IXO, RheinAhrCampus Remagen*; ²*BIOX, KTH Stockholm*, ³*ELETTRA Trieste*;
⁴*Cantanzaro University*; ⁵*zoneplates.com*

In the X-ray wavelength region, the real part of the atomic scattering factor, describing the phase shift, exceeds the imaginary part, responsible for absorption, by far. As a result of this fact, several methods for converting the phase signal of the specimen into image contrast in X-ray imaging have been developed in the last years, especially for X-ray microscopy. One of these methods utilizes zone plates not only as imaging optics but also for beam splitting and combining. An appropriate combination of two zone plates result in an optical device that forms a high resolution image in X-ray differential interference contrast (X-DIC). The strong Nomarski-like contrast enhancement for low absorbing specimen could be demonstrated at 4 keV photon energy at ESRF beamline ID21. The X-DIC optics works in full field mode as well as with the STXM.

In order to reduce difficulties in the demanding manufacturing process of the first X-DIC zone plates, so called zone plated doublets, single diffractive optical elements (DOEs) showing the same DIC imaging properties were designed, manufactured and tested. These DOEs can be treated as computer generated holograms that virtually superimpose two spherical waves originating from two points shifted with respect to each other either in lateral or axial position with a plane wave. Imaging and interference properties – soon including partially coherent illumination – of the DOEs can be simulated by especially created wave propagation software tools.

An important aspect of full field X-ray microscopy – not only but in particular for X-DIC – is the illumination arrangement in the microscope setup, as it deals with the intensity distribution in the object plane as well as spatial coherence properties. Especially at undulator beamlines, the high degree of spatial coherence in the illumination path often creates trouble when applied to full field imaging. To somehow control the spatial coherence and at the same time provide an evenly illuminated object field for X-ray microscopy, a special DOE acting as condenser has been developed, tested and successfully implemented at the TWINMIC microscope installed at ELETTRA, resulting in better imaging quality of the X-ray micrographs and opening up the possibility to employ X-DIC for the full field mode of the TWINMIC.

Phase contrast X-ray imaging in biomedicine: the ESRF experience

Alberto Bravin

European Synchrotron Radiation Facility, Grenoble, France

It has been widely shown in the literature that phase contrast techniques are the most suitable imaging modalities to reveal tissue structures without the aid of a contrast agent. Strong development in this field is carried out at several synchrotron radiation facilities, covering a wide range of applications, from basic studies up to clinical trials.

At the ESRF, the free propagation (FP) and the analyzer-based imaging (AI) techniques are applied in in-vitro and in in-vivo preclinical bio-medical studies using single projection and 3D micro-tomography modalities.

FP and AI have found successful application in various medical-related fields. In-vitro human samples are examined in the frame of mammography and breast cancer characterization programs, and of osteoporosis and bone ingrowth studies; in-vivo techniques are applied to in-vivo animal models to follow up the development of diseases like osteoarthritis.

X-ray beams suitable for clinical application of phase contrast techniques (collimated, highly intense and quasi monochromatic) are presently available at synchrotron radiation facilities only. It is expected that the exciting preclinical and clinical results at these facilities stimulate the engineering of new intense table top sources to be introduced in the clinical diagnosis routine.

Molecular imaging in cells and live animals for biomedical research

Ennio Tasciotti

International Centre for Genetic Engineering and Biotechnology & Centro Biomedicina Molecolare, Trieste, Italy

Most of the biological events taking place inside a eukaryotic cell depend on the coordinated assembly of multi-component biological machineries. In particular, all DNA transactions in the cell's nucleus (replication, recombination, repair and transcription) are carried out by multi-molecular protein and nucleic acid complexes that assemble in a timely, regulated manner in specific subnuclear locations. Recent advances in live imaging microscopy and the possibility of tagging biological molecules with fluorescent probes or quantum dots now permit the visualization of these events inside the living cells and in real time. Among several available techniques, fluorescence resonance energy transfer (FRET) permits the visualization of direct protein-protein interactions; fluorescence recovery after photobleaching (FRAP) monitors protein trafficking inside different subcellular compartments; fluorescence correlation spectroscopy (FCS) studies the diffusion of molecules inside biological microenvironments.

The in vivo application of imaging techniques would allow the visualization of molecular and cellular processes in living organisms, thus representing a powerful tool to understand the mechanisms underlying biological and pathological events. The exploitation of visible light imaging in whole organisms, however, is rather limited by the adsorption properties of several body constituents. To overcome these limitations, imaging is performed in the near-infrared range (NIR), using radioactive tracers (PET, SPECT) or by exploiting the magnetic resonance properties of the tissues (MRI). Some of these techniques are already extensively used in clinics (PET, CT-SPECT, MRI); NIR imaging is currently limited to animal research, although its prompt transposition to humans is highly desirable and expected over the next few years.

In this presentation, I will review some of our applications of the above mentioned imaging techniques in the field of HIV-1 research and gene therapy for cardiovascular disorders.

Recent developments in Diffraction Enhanced Imaging

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Although Diffraction Enhanced X-ray Imaging (DEI) was pioneered 25 years ago, only in the last decade several research groups (most of them belonging to synchrotron radiation community) have deeply investigated this phase sensitive x-ray imaging technique.

DEI is based on the utilization of an analyzer crystal, placed between the sample and the imaging detector. The rocking curve of the analyzer system acts as an angular band-pass filter, whose width is comparable with the tiny deviations suffered by X-ray photons traversing the sample (typically in the order of 1-100 μrad). Therefore, an intensity modulation is recorded on the detector. In particular, refraction and ultra-small angle scattering are exploited and provide extra contrast in addition to X-ray absorption.

More precisely, the name DEI is used to indicate an algorithm capable to produce separate images of refraction effects and of absorption effects (including extinction, i.e. the rejection of ultra-small angle scattering). Despite the spectacular images produced by DEI, the method has significant imperfections. In particular, as highlighted in recent studies, the DEI algorithm gives too coarse a treatment of ultra-small angle scattering. Several researches have investigated this issue, suggesting possible alternatives. In this presentation, the limits of the original approach will be discussed and an overview of the new generation methods will be presented.

Theory and algorithms for phase-contrast CT

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Applications of x-ray computed tomography (CT) vary from medical and biological imaging to material research and industrial inspection. We briefly discuss the state of the art in conventional absorption-based CT and make a link to a new imaging technique: phase-contrast CT. This technique can be implemented at third generation synchrotron radiation sources or by using a microfocus x-ray tube. Unlike conventional absorption-based imaging, phase-contrast imaging uses the phase shifts in the x-ray beam rather than the differences in attenuation. Promising experimental results have been obtained by several research groups. At the same time, the lack of a mathematical theory comparable to that of conventional absorption-based CT limits the progress in this field. We suggest such a theory and prove a fundamental theorem that plays the same role for phase-contrast CT as the Fourier slice theorem does for absorption-based CT. The theory holds for both purely phase objects and the mixed phase and amplitude objects with weak absorption. In contrast to other methods, the suggested approach requires no intermediate steps of phase retrieval and provides exact quantitative reconstruction of the refractive index from intensity measurements. The fundamental theorem plays an important role in the derivation of the algorithms. In particular, the theorem establishes a straightforward relation between the 3D object function and its phase-contrast projections and allows us to derive reconstruction algorithms in the form of filtered backprojection (FBP). FBP is a relatively simple and fast image reconstruction algorithm. The latter makes it a suitable candidate for routine processing of huge volumes of high-resolution phase-contrast data. The filter function of the FBP algorithm can be derived in the Fourier and spatial domains. Depending on the type of phase-contrast measurements, different filter functions are suggested. For the measurements based on the scheme of in-line holography, a low-pass filter function has been derived (Ref. 1-2). The measurements based on using a grating interferometer require the filter function of the Hilbert transform. The reconstruction algorithms have been implemented in the commercially available software (Ref. 3). The results of application of the algorithms are discussed; a comparison with the conventional FBP algorithm is given.

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Diffractive nano-optics and their use for X-ray synchrotron radiation

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The current intense interest in extreme ultraviolet and x-ray microscopy is mainly due to the availability of a nearly ideal optical source for nano-optics based on diffraction, that is, a source with low divergence whose wavelength can be tuned over a range of several keV and whose spectrum can be monochromatised with a band pass $\Delta\lambda/\lambda$ of less than 10^{-4} . Synchrotrons of the latest generation and free electron lasers (in the near future) are devices that produce x-rays with these characteristics. Zone plate, that can be now considered a well established focusing element for x-rays, was invented more than hundred years ago but due to technological difficulties, they have been implemented only in the last three or two decades.

In this presentation we show that it is possible to design, fabricate and easily use new optical elements that, beyond focusing, can perform new optical functions. In particular, the intensity of light in the space beyond the optical elements can be redistributed with almost complete freedom. In other words, already available extreme ultraviolet and x-ray sources are suitable as ideal sources for diffractive optical elements designed to perform new optical functions useful in scanning and transmission X-ray microscopes. Finally, results on the possibility of combining X-ray beams with other optical tools in different wavelength regime such as optical tweezers will be presented.

Zone Plate Fabrication at BESSY

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The full-field x-ray microscope installed at the electron storage ring BESSY II is dedicated for applications in life, environmental and material sciences. It covers the photon energy range between 250-750 eV. Currently, the spatial resolution of the zone plate based x-ray microscope is about 20 nm.

At BESSY II we started an in-house fabrication of Fresnel zone plates to supply the x-ray microscope with tailored optics and to improve their spatial resolution in the future. For a future improvement of the spatial resolution new fabrication processes are required as discussed in the following:

State-of-the-art Fresnel zone plates with an outermost zone width of 20 nm can be described by scalar diffraction theory neglecting the three-dimensional shape of the zone structures. According to this theory their diffraction efficiency scales as $1/m^2$ where m is the diffraction order. While keeping the zone height constant, the aspect ratio of the zones increases inversely with decreasing outermost zone width. For photon energies below one keV, it is shown by applying electrodynamic theory that scalar theory is no longer suited to describe zone plates with outermost zone width below 20 nm and aspect ratios of about 10:1 [1,2].

Full electrodynamic theory predicts that the diffraction efficiency decreases continuously if the lateral dimensions of the zone width approach the wavelength used for imaging. This result is obtained for zone structures parallel to the optical axis.

Unlike the diffraction properties of parallel zone structures, rigorous coupled wave theory (RCWT) predicts for zone structures tilted to the optical axis according to the local Bragg condition that the diffraction efficiency can be up to 50 % [3]. In addition, RCTW calculations show that similar diffraction efficiency values can be obtained in any high order of diffraction $m > 1$.

The resolving power of zone plates scales with the order of diffraction m . By applying high orders of diffraction, it is possible to increase the resolution without the need for manufacturing increasingly smaller outermost zone width far below 20 nm.

Applying high orders for imaging requires manufacturing tilted zone structures with aspect ratios of about 20:1 [2]. To overcome the extremely difficult problem of manufacturing tilted zones with high aspect ratios of 20:1, we will work on manufacturing zone plates on top of each other with slightly decreasing zone radii [3]. In good approximation – depending only on the number of layers – the zones can be tilted according to the local Bragg condition and each single layer requires only moderate aspect ratio structures.

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Fresnel Zone Plates for Hard X-ray Nanofocusing

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The focusing of hard X-rays ($h\nu > 8$ keV) is important prerequisite for many techniques such as micro-fluorescence, microimaging, and microdiffraction. At present, the best resolution for x-ray focusing is obtained by using Fresnel Zone Plates (FZPs). In the soft x-ray range FZPs can reach a resolution down to below 20 nm [1], and sub-100 nm values are routinely achieved. The efficient focusing of hard X-rays is more difficult, since in order to obtain acceptable diffraction efficiencies, the zone plate structures should be made from heavy materials, but even then the required height of the structures needs to be a micron or more. This means that the aspect ratios of the structures of FZPs have to be very high, when both high resolution and efficiency are needed [2]. This is the reason why the high resolution potential of FZPs could not be exploited in the hard X-ray regime.

We developed a method which allows us to produce gold structures with aspect ratios up to 10. FZPs with an outermost zone width of 100 nm and diameters ranging from 20 μm to 200 μm were fabricated and tested in MicroXAS beam line of Swiss Light Source. FZP of 30 μm diameter (see Fig. 1) was used in nanofocusing experiments. In order to match transverse coherence length of the incident illumination we used 20 μm aperture in front of the FZP, which means that only zones down to 150 nm were illuminated. A spot size below 250 nm and diffraction efficiency of 8.4 % were measured at X-ray energy of 8 keV and focal distance of 19.4 mm.

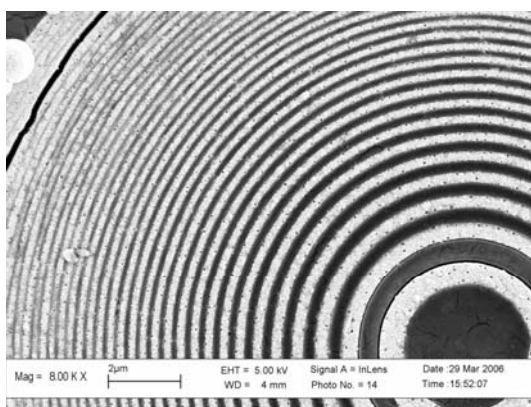


Fig.1. SEM-image of 30 μm diameter FZP with outermost linewidth of 100 nm.

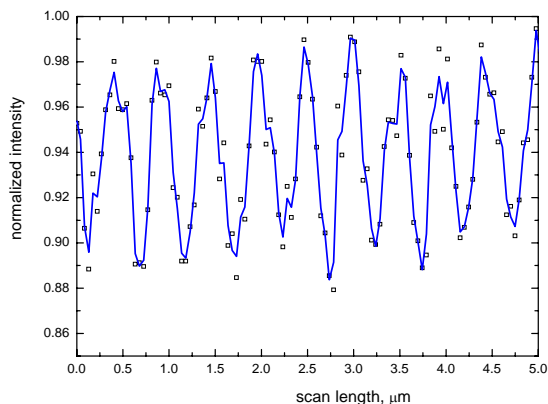


Fig.2. Scan across a grating with 250nm lines and spaces in the focal plane of FZP.

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Spectromicroscopy analysis: clustering, error-finding, and interpreting

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Soft x-ray spectromicroscopy provides the means for studying chemical speciation at the 30-50 nm resolution scale, and it is finding wide use in studies in biology, environmental science, astrobiology, polymer research, and other fields. For a specimen that can be characterized in terms of a set of known spectra, a variety of approaches^{1,2} can be used for compositional mapping. However, this is rarely the case in biology or environmental science where the complexity of the specimen and reactivity of components precludes advance knowledge of all signature spectra.

Cluster analysis provides a way to find the signature spectra that exist in a specimen, and form compositional maps based on these “discovered” spectra. Following preliminary work³, we have carried out a systematic development⁴ of this approach to soft x-ray spectromicroscopy analysis, and have extended it with methodologies aimed at classifying only on compositional variations rather than specimen thickness⁵. New developments include the recovery of the “true” incident flux spectrum from a dataset, and new methods for the analysis of mixtures such as non-negative matrix factorization. These and other developments will be reviewed and illustrated with examples from studies in biology and environmental science.

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Time-Resolved Transmission X-Ray Microscopy

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Fast magnetization dynamics of ferromagnetic elements on short length scales is currently attracting substantial scientific interests for both technological and fundamental reasons. Measurements with a time resolution of 70-100 ps combined with a lateral resolution of 20-40 nm were performed using two different sample geometries (magnetic ‘in-plane’ excitation by a microcoil and ‘out-of-plane’ excitation by a stripline) at two different microscopes: a full-field soft X-ray microscope (XM-1, ALS beamline 6.1.2) and a scanning transmission X-ray microscope (STXM, ALS beamline 11.0.2). The scanning microscope equipped with a fast avalanche photo diode (APD) detector allowed us to speed up time-dependent measurements by about a factor of 10.

Complementary to the time-domain ‘pump-and-probe’ measurements [1] a frequency-domain ‘sine excitation’ technique [2] was implemented into X-ray microscopy. Spin precession [1] and gyrotropic vortex motion [2] in micron-sized ferromagnetic patterns have been studied. Magnetic vortices show low frequency modes, appearing as translational gyrotropic motions of the vortex when the system is excited with an in-plane sine magnetic field. This gyrotropic motion was imaged not only in Permalloy (Py), but also in Py Cu Co trilayers [3]. By tuning the photon energy to the absorption edges of Ni or Co, we could study separately the vortex dynamics in the Permalloy and in the Co layer as well as the magnetic interaction between them. The vortex structures in the Permalloy and the Co layer show the same sense of in plane flux closure and the same polarisation of the out-of-plane vortex core and thus the same handedness. But remarkable is a 180 degrees phase shift between the vortex motions of the Permalloy and the Co layers caused by the magnetic coupling between them.

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A VUV/VIS-cross-correlator for the temporal characterization of VUV-FEL pulses

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Soft x-ray- and VUV-free electron lasers (FEL) as fourth generation synchrotron sources deliver radiation with unequaled brilliance, in particular photon flux and pulse duration promise to enable new classes of experimental studies including the possibility to investigate dynamic processes with temporal resolutions in the order of a few 10fs.

FEL radiation originates from statistically fluctuating self amplified spontaneous emission (SASE). As a result, the time of emission slightly changes from pulse to pulse with respect to a synchronized visible laser by a certain amount, the so-called ‘jitter’ δt . Pump-Probe experiments rely on proper timing of pump and probe pulse, i.e. a fixed delay Δt is necessary. Since at present there is no possibility to reduce or even extinguish the FEL jitter, the only way to overcome this limitation is the measurement of the jitter on a shot-to-shot basis.

The presented project aims at the development and implementation of a novel cross-correlation technique for the temporal characterization of VUV-FEL pulses, i.e. both, pulse duration and jitter for each FEL pulse. The measurement principle relies on the simultaneous interaction of the FEL- and a visible laser pulse in a target gas and the subsequent spatially resolved detection of the generated energy-shifted photo electrons with an imaging electron spectrometer.

In this contribution, we present first results from recent measurements at the VUV-FEL facility at HASYLAB/DESY at a photon energy of 38eV. The feasibility of the developed cross-correlator setup consisting of (i) interaction chamber including multiple diagnostic tools for spatial and temporal overlap of the two pulses, (ii) electron optical system, (iii) adjustable gas inlet, (iv) optical setup for visible laser steering and focussing and (v) mounting platform for alignment of the vacuum chamber with respect to the FEL beam is demonstrated. So far, the obtained data is integrated over several FEL pulses and thus limited regarding the temporal resolution. Further improvements to obtain single-shot data are discussed.

Ultrafast coherent diffraction imaging with a soft X-ray free-electron laser

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Diffraction microscopy is an imaging mode that relies on the numerical inversion of a diffraction volume to recover the object function, and offers the potential for high-resolution aberration-free diffraction-limited 3D imaging. Theoretical studies and simulations predict that with a very short and very intense coherent X-ray pulse a single diffraction pattern may be recorded from a large macromolecule, a virus, or a cell without the need for crystalline periodicity. Measurement of over-sampled X-ray diffraction patterns permit phase retrieval and hence structure determination. Although individual samples will be destroyed by the X-ray pulse, a three-dimensional data set could be assembled when copies of a reproducible sample are exposed to the beam one by one. Here we report the first experimental verification of the principle of flash diffraction imaging using a soft X-ray free-electron laser. The results show that an interpretable diffraction pattern can be obtained before the sample turns into a plasma when exposed to an intense 25 fs long photon pulse at 32 nm wavelength (focused to a peak intensity of up to 10^{14} W/cm²). Significantly, the image we obtain by phase retrieval and inversion of the diffraction pattern shows no discernible sign of damage, and the object can be reconstructed to the resolution limit. Damage occurs only after the pulse traverses the sample. A second exposure shows scattering from the hole that was created by the first pulse. These results provide experimental evidence for the basic principle of flash imaging, and have implication for studying non-periodic molecular structures in biology, and in any other area of science and technology where structural information with very high spatial and temporal resolution is valuable.

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X-Ray Microscopy with Laboratory Sources

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X-ray microscopy in the water-window region ($\lambda = 2.3\text{-}4.4$ nm) is an attractive technique for high-resolution imaging. In this wavelength region state-of-the-art optics has demonstrated sub-20 nm resolution and the sample preparation techniques are maturing. Unfortunately present operational x-ray microscopes are based on synchrotron radiation sources, which limit their accessibility. Many biological investigators would benefit from having the x-ray microscope as a tool among other tools in their own laboratory. For this purpose we recently demonstrated the first compact x-ray microscope with sub-visible resolution.¹

We have recently developed a flexible, compact x-ray microscope operating at $\lambda = 2.48$ nm. This wavelength should provide improved imaging of thicker structures compared to the $\lambda = 3.37$ nm microscope in Ref. 1. The microscope is based on a 100 Hz liquid-nitrogen-jet-target laser-plasma x-ray source², in-house fabricated diffractive condenser optics³, in-house fabricated 25 nm Ni zone plates⁴, and CCD detection. The sample holder is positioned in a helium atmosphere with silicon nitride membranes separating it from the vacuum in the condenser and imaging module. Initial images of test objects show structures down to 30 nm lines and spaces.

This presentation will discuss the source, the diffractive optics, the imaging properties and systems' issues. Some emphasis will be placed on the fabrication and testing of our uniform high-aspect-ratio diffractive optics with small other zone widths due to their importance for microscope performance. Furthermore, we will discuss the possibilities of DIC microscopy with compact sources by tailored diffractive optical elements (DOEs)⁵. If time allows we will touch upon operation of the microscope at $\lambda = 3.37$ nm with a methanol-liquid-jet laser plasma⁶ and novel normal-incidence Cr/Sc multilayer condenser optics showing 2.5-3% average reflectivity.

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Diffraction microscopy - past, present and future

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The idea that the image of an object could be reconstructed from the recorded intensities of its diffraction pattern has been developed by David Sayre [1]. He has led the effort over the years to bring the idea to fruition. [2]. The Stony Brook group constructed an apparatus designed to collect the necessary data [3]. This apparatus is currently installed on an undulator beamline at the ALS. [4]. The Stony Brook group, in collaboration with the Cornell group, succeeded in the reconstruction of the diffraction pattern of a freeze-dried yeast cell [5], and is well on the way to extend this effort to three dimensional imaging, and to the reconstruction of frozen hydrated specimens. The same apparatus is being used by the Arizona State/Livermore/LBL group to image non-biological specimens [6].

A very interesting extension of this technique has been proposed by Spence et al., to collect the diffraction pattern from a stream of laser-aligned macromolecules, dubbed “serial crystallography” [7]. Beyond that, there are plans to mount an experimental program at the LCLS to reconstruct macromolecules from the diffraction patterns obtained using the XFEL beam. [8]

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Retrieval and Interpretation of Complex Images of Nanocrystals

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Inversion of Coherent X-ray Diffraction is especially interesting when Bragg diffraction from the sample object is considered. First of all, this allows individual objects to be cleanly separated from their neighbours in a typical sample. In this sense it is a "dark field" method. Secondly, the problems associated with eliminating the "direct beam" from the measurements are neatly avoided. However the large momentum transfer involved makes high demands on the quality of the sample: the diffraction becomes highly sensitive to displacements of atoms even at the level of fractions of Angstroms within the object. The nominal "resolution" is much less than this, of course. Being sensitive to tiny displacements can also be an advantage if they can be understood as arising from deformation fields within the sample object.

Here we demonstrate a new formalism that maps the displacement field onto the imaginary part of the object density. In this presentation, we demonstrate the recovery of a 3D image of strain within a lead nanoparticle and show that this is consistent with the theory of elasticity. Phasing the diffraction from the complex object turns out to be no more difficult than for real objects. The method is expected to be general because deformation is common in nanocrystals due to the presence of mechanical strain.

Reconstructions in the real world

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The last decade has seen many breakthroughs in the development of diffraction microscopy. This new imaging method involves the accurate measurement of a specimen's diffraction pattern and the use of an algorithm for the reconstruction of the image. The benefits of this method are numerous, but there are also many outstanding challenges. An essential requirement of any diffraction microscopy experiment is the reproducibility of the reconstructions.

Uniqueness of the reconstruction can be compromised in very many ways. It is now well known that missing data in the center of the diffraction pattern (due to the beam stop) can lead cross a threshold where information is irretrievably lost. Noise is important as well, as it replaces, from the algorithm's perspective, the unique solution with an ensemble of near solutions. Here averaging procedures are indispensable for restoring reproducibility.

Another casualty of noise is the elimination of vital interference effects in the diffraction pattern when these are suppressed due to pathological distributions of intensity. This situation arises in some electron diffraction experiments with specimens containing crystalline domains, such as carbon nanotubes or atom clusters. Information about the large-scale shapes of the domains is encoded in small regions around the diffuse Bragg peaks in the diffraction pattern. Weak interference between the diffuse signal in distinct peaks encodes the relative positions of domains and is easily lost to noise.

Science with UV/X-ray free electron lasers

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While the most advanced synchrotron light sources are reaching the limit of their fundamental performances the fast growing field of sub-picosecond and subfemtosecond time resolved experiments is rising a strong demand for intense, coherent and ultrashort IR-hard x-ray radiation sources. Depending on the wavelength, ultrashort pulses can be produced, for example, by plasma sources, high harmonic generation by femtosecond coherent laser pulses or by free electron laser (FEL).

Development of FEL started when in 1970 J.M. Madey recognized that coherent electromagnetic radiation is generated and amplified by stimulated emission of bremsstrahlung, when relativistic electrons propagate co-linearly with the radiation field in a periodic magnet array or undulator. While other ultrashort radiation sources are suitable for specific applications the FEL would combine most of the behavior of both a laser and a synchrotron light source.

In this lecture will focus some of the most interesting application of very brilliant ultrashort and coherent radiation pulses. The emphasis will be given to those experiments where the main parameters to be considered are the magnitude of the e.m field magnitude, time structure of the radiation pulse, transversal and longitudinal coherence and state of polarization.

ABSTRACTS
POSTERS

Utilization of X-ray radiography and femtosecond Laser Induced Breakdown Spectroscopy (fs-LIBS) for monitoring of the heavy-metal hyperaccumulation in vegetal tissues

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Very promising and natural method for detection and subsequent removal of the (toxic) metals accumulated in the environment seems to be the phytoremediation [1]. This technique is based on the removal of the contaminants by means of their absorption in the above-ground part of the plants, specially cultivated for this purpose and then harvested and liquidated properly [2]. In order to select appropriate species for environmental-cleaning and to minimize the possibility to transfer the toxic elements into the human body through the food-chain, detailed study of the exact mechanism of phytoremediation is needed.

The study of these problems presents various aspects, and consists in the detection of contaminants, in the comparison of accumulation properties of the various plants and in the mapping of possible biological structures, which can specifically accumulate metals within a given tissue.

Among the different diagnostic techniques for metal-detection in vegetal tissues here we are focusing on two particular one – X-ray radiography (using synchrotron radiation) and Laser Induced Breakdown Spectroscopy (LIBS).

A monochromatic synchrotron radiation beam allows to select a very thin spectral range and, by a specific optical set-up, to localize in the observed sample the absorption of a given chemical element. This possibility has greatly enhanced the interest of microscopic elemental analysis. X-rays from a synchrotron are in an energy range much higher than the energy of chemical bonds (typically of the order of few eV), so that the absorbing electrons are the K or L, M... electrons. Since this absorption is independent from any chemical bond, an absorption measurement can give directly the number of contaminant atoms/cm² which are present in the sample [3]. Here we report on the results of dual-energy X-ray micro-radiography and micro-tomography experiments at ELETTRA Synchrotron, Trieste (Italy).

The capability of the LIBS technique for direct determination of Pb, Al, Ca, Cu, Mn, Zn, Mg and Fe in plant materials was shown already in 1999 for powdered leaves of different plants [4]. More recently the possibility of high-spatial resolution analysis on *Helianthus annuus* leaf was presented [5]. Presently this technique is capable of in situ heavy metal detection with sub-micron spatial-resolution. Here we report on a possible utilization of LIBS for preliminary qualification of a big number of 2D (leaf) samples.

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Images of small brain phantom with contrast agents and rat kidney, using 20, 30 and 40 keV synchrotron X-rays: Utilization of DEI and CT.

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X-ray imaging techniques are extensively used for clear visualization of the internal micro-architecture and the associated hard and soft-tissue with better contrast in the field of medicine and biology. It is difficult to obtain high contrast X-ray images of soft-tissues with conventional methods, because X-ray absorption coefficients of light elements (H, C, N, and O, and many of these constitute the soft-tissue) are extremely small and therefore comparatively transparent to X-rays and generate poor contrast. Soft-tissues weakly absorb the X-rays, so the contrast in conventional X-ray images cannot reveal the internal micro-architecture to a more visible level.

An ideal monoenergetic radiation for this purpose would come from a synchrotron source, for example, high X-ray flux and considerable monochromacy. Synchrotron radiation provides much higher fluxes on a wide range of energies, with the subsequent possibility of monochromatizing the beam while preserving the sufficient flux.

There are several ways to carry out a 3D tomography using possible reconstruction algorithms. The sample used in the study is composed of inner and outer parts and our motivation is to examine the inner and outer parts by visualization. This technique requires the use of a narrow beam in which the radiation is approximated by parallel rays. The scattered intersections between different planes can be considered negligible. In this way the 3D visualization is a repetition of 2D (single plane) reconstruction, i.e., any slice of the sample is separately reconstructed.

The experimental work at multiple energies will allow us (1) to know the internal features with acceptable visibility (2) identification of optimum energy (3) use of optimum energy for further analysis.

Images of small brain phantom with contrast agents and rat kidney are acquired using 20, 30 and 40 keV synchrotron X-rays utilizing X-ray CT and diffraction-enhanced imaging technique. The choice of energy is chosen based on the quality of the image. Visualized the embedded features at different regions within the rat kidney. The choice of optimum energy allowed us clear visibility of the internal structural features. The sensitivity of X-ray imaging to soft-tissues must be improved with monochromacy, for better contrast, in particular, utilizing the new X-ray modalities, such as, diffraction-enhanced imaging. Recently, refraction properties of X-rays turn to be more attractive advantages for

imaging over the absorption properties. Refraction is orders of magnitude more sensitive, particularly for biological or low materials. Diffraction-enhanced imaging (DEI) technique exploits these refraction for differentiating the biological soft-tissue with high collimated synchrotron X-rays. Images of the brain phantom, with contrast agents, for example, water, Iodine and physiological saline provided us some new source of information, related to the contrast. Estimated the dose contribution at each of the energy, with contrast agents and with and without inclusion of the sample. Further images acquisition is in progress with medical samples.

The TWINMIC full-field transmission x-ray microscope at ELETTRA

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In this contribution we present the current status of the TWINMIC full-field transmission x-ray microscope. The TWINMIC x-ray microscope, which combines for the first time a possible operation in scanning or full-field transmission mode in a single instrument, went in operation last year. It is currently installed at the BACH beamline at the ELETTRA synchrotron in Trieste, Italy, but will be moved to its own dedicated beamline next year. At both beamlines x-ray radiation from an undulator source is used, which imposes special demands on the condenser design because of the high degree of spatial coherence in the undulator beam.

We have developed a single-element diffractive optic consisting only of a large number of small diffraction gratings [1]. The condenser produces a Koehler-like homogeneous intensity distribution in the sample plane and eliminates the object illumination problems connected to the spatial coherence of the undulator beam. The use of the condenser is as simple as the use of a normal condenser zone plate.

We will present results obtained with the TWINMIC microscope using this condenser setup, including resolution tests with standard test structures. An outlook on the final setup of the microscope at the new TWINMIC beamline will be given.

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Coherent x-ray production by x-ray waveguides and application to imaging

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X-ray waveguides have proved to be optical elements allowing imaging in phase contrast with nanoscale spatial resolution [1]. In ten years, the efficiency of x-ray waveguide (WG) optics has been improved by three orders of magnitude [2] and different schemes of coupling between radiation and optics have been tested. WG optics at present can produce nanometer size beams [3,4] with unique coherence properties [5].

In addition to the optical properties concerning the beam compression and the spatial coherence filtering, it is interesting to study the dispersion properties of a WG [6], in view of ultrafast x-ray sources. In particular, 4th generation Synchrotron Radiation sources, such as FEL, will need outstanding dispersion-free x-ray optics able to preserve their exceptional time and coherence properties. In the EUV and soft x-ray region only front coupling vacuum gap WG can operate. In this report we present theoretical evaluation of the dispersion properties of such waveguides, demonstrating that WG are dispersion-free optical elements far from absorption edges of the cladding materials, and that have strong dispersion effects close to those absorption edges. These effects can in principle be usefully exploited to manipulate the time properties of chirped pulses. Moreover we present advances in fabrication methods of vacuum-gap waveguides, with some preliminary experimental results. The potentialities of waveguides in time-resolved imaging at nanoscale resolution will be presented, in particular using phase contrast methods which allow investigating biological samples.

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