

Структурная биология в прожекторе синхротронного излучения

Вазина А.А.

Институт теоретической и экспериментальной биофизики РАН, Пущино

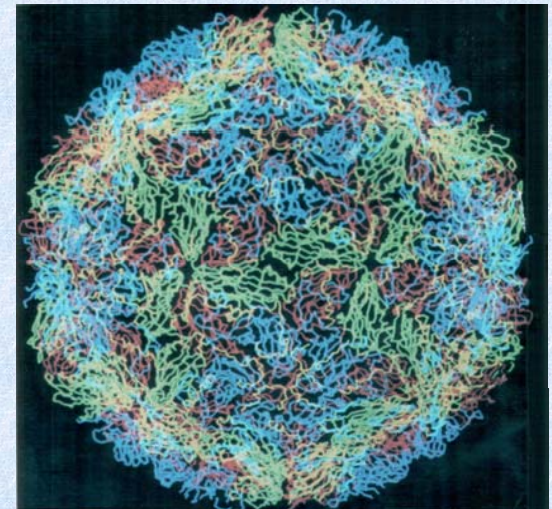
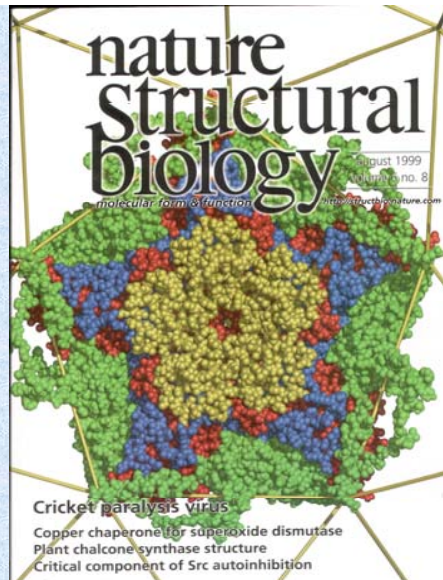
vazina@mail.ru

ESRF, Гренобль



ОСОБЕННОСТИ
синхротронного излучения:

- Высокая интенсивность рентгеновского излучения
- Широкий спектр излучения
- Минимальная расходимость пучка в вертикальном направлении
- Высокая степень когерентности излучения на накопителях третьего поколения





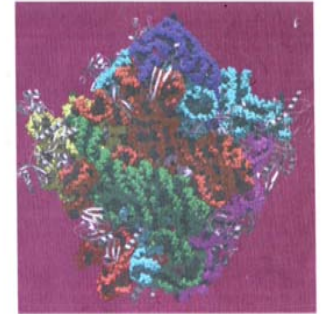
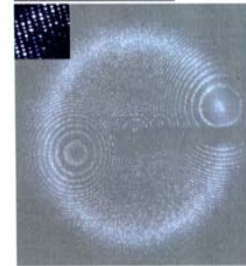
Crystallography-monochromatic

For large structures assemblies

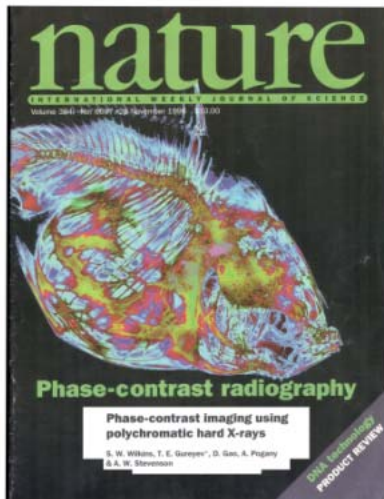
$$I = I_0 \lambda^3 N^2 t V \cdot e^4 / (2\pi m_e^2 c^4) \cdot L_p |F^2|$$



Viruses

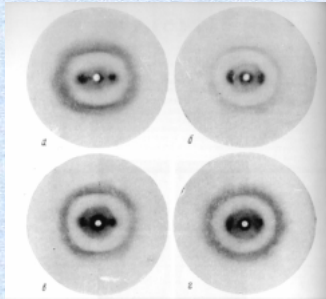


50S ribosomal subunit



**«Дифракционное кино»
 Структурная динамика
 МЫШЦЫ**

Рентгенограммы под большими углами дифракции различных тканей *k-m-f*-группы (кератин, миозин, эпидермис, фибриноген)



В первой половине прошлого века была проведена классификация некоторых типов тканей по дифракционным картинам под большими углами. Однако дифракция в области больших углов не несет информации о функциональном состоянии целостной ткани: рентгенограмма ткани бедна отражениями.

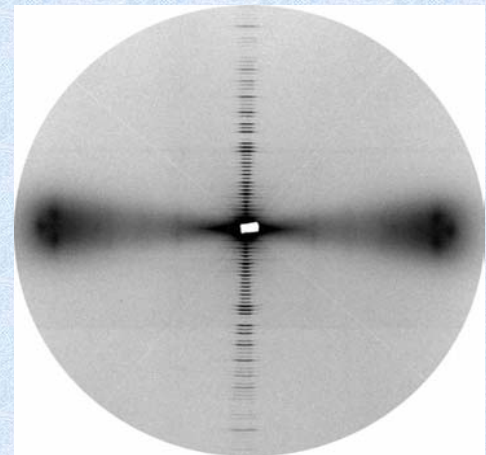


- на лабораторном рентгеновском генераторе GX-20 с вращающимся анодом (Пушкино) с использованием тороидальной фокусирующей камеры Эллипта $U = 40 \text{ kV}$, $I = 40 \text{ mA}$, $\lambda = 0.154 \text{ nm}$; детектор – рентгеновая пленка (Retina, Германия); расстояние образец – детектор 75 – 150 мм; время экспозиции 10-18 часов
- на канале К 1.3 накопителя «Сибирь-2» (Москва) $I = 100-60 \text{ mA}$, $\lambda = 0.16 \text{ nm}$; детектор – рентгеновская пленка (Retina, Германия); расстояние образец – детектор 500 мм; время экспозиции 10-30 мин.

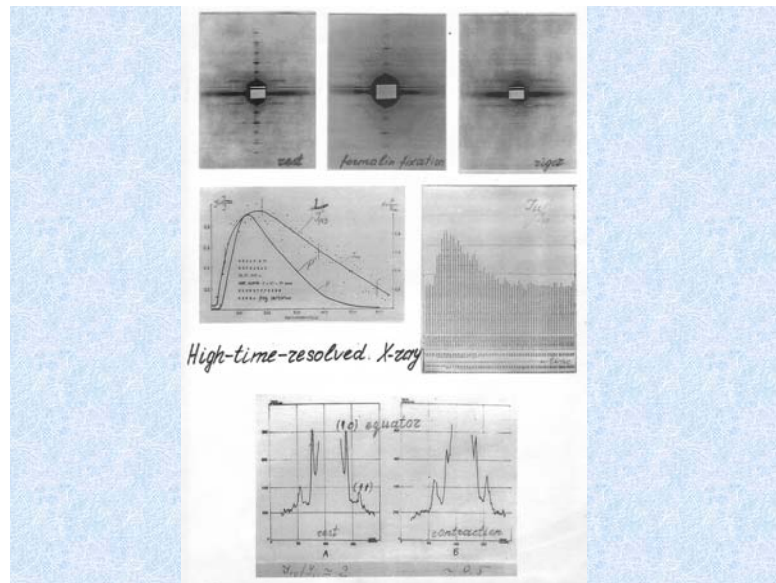
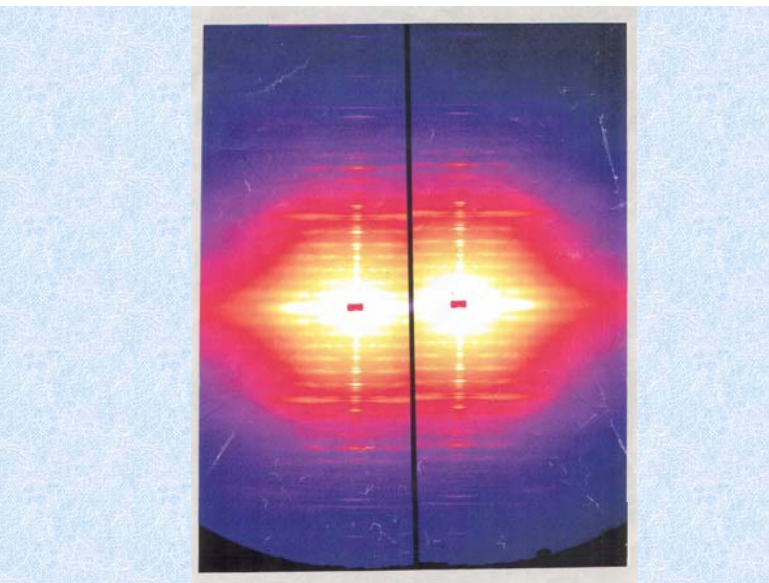
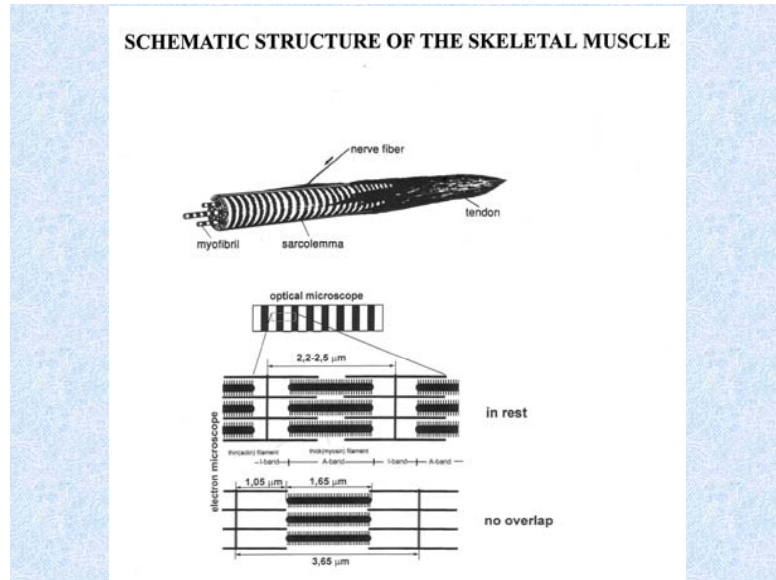
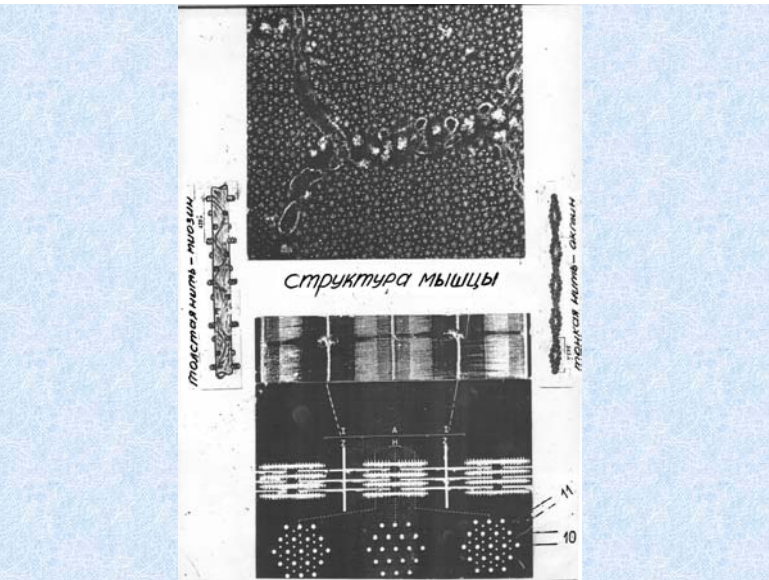


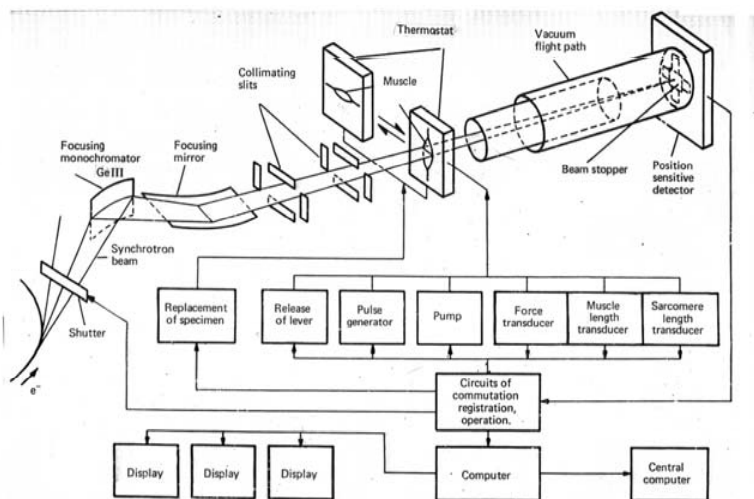
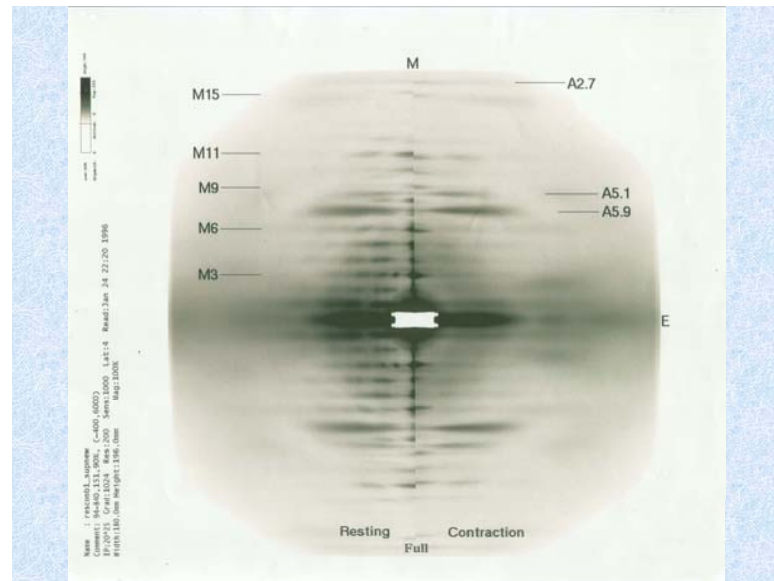
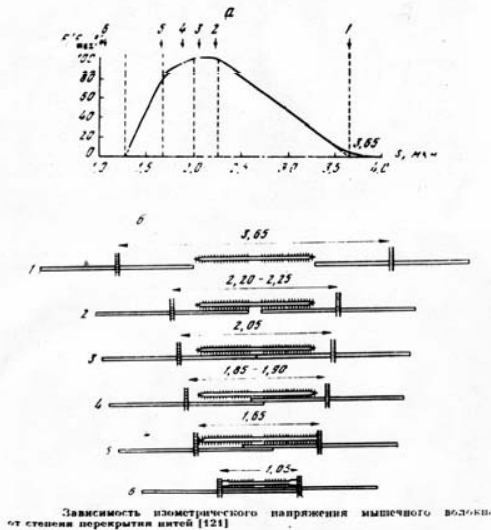
DUBBLE SAXSWAXS BM26, ESRF

- на синхротронном источнике ESRF (Гренобль, Франция) DUBBLE SAXSWAXS BM-26 с энергией 12 keV; $\lambda = 0.124 \text{ nm}$; размер пучка 0.2 мм x 0.2 мм; CCD детектор; расстояние образец - детектор 1.5 - 7.5 м; время экспозиции 1 - 3 мин.

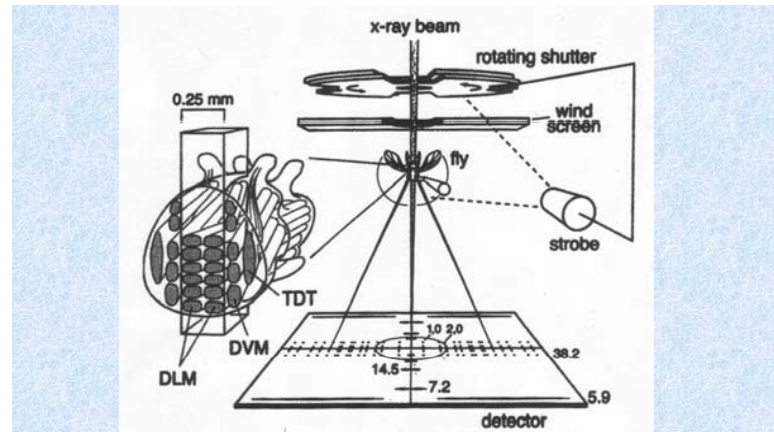


Соединительная ткань хвоста крысы (коллаген)





Schematic diagram of small-angle focusing camera FRAKS on 5th beam-line of storage ring VEPP-3



Experimental set-up and preparation. The incident-beam intensity of the synchrotron x-ray source was modulated by aluminum filters of varying thickness (maximum flux, $\sim 2.0 \times 10^{13}$ photons/s; wavelength, 0.103 nm). The camera length was 1.5 m (fly-to-detector distance); the spot size at the CCD-based detector was $\sim 30 \times 200 \mu\text{m}$ (full width, half maximum); the spot size at the sample was $\sim 250 \times 250 \mu\text{m}$.

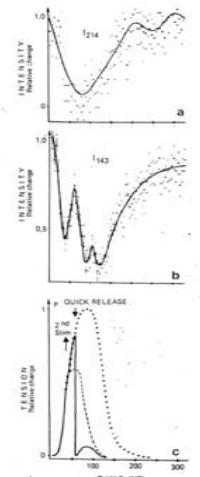
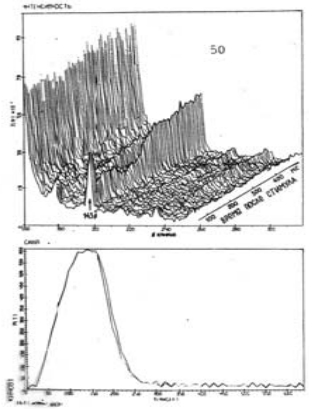
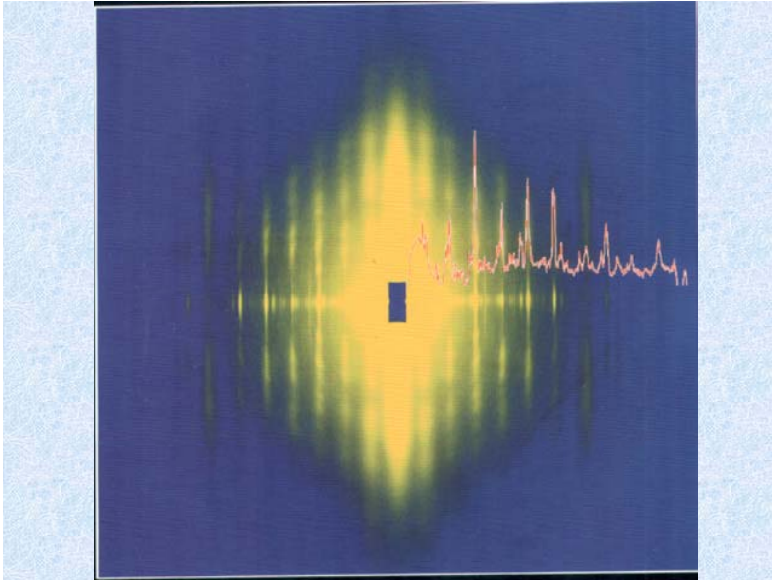
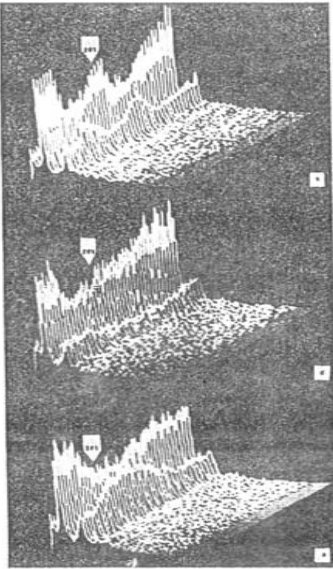
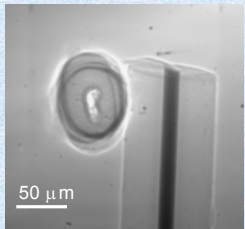


Fig. 3. Time course of changes in the intensity of the meridional reflections in isometric contractions initiated by double stimulation with a quick release applied 25 ms after the stimulus: 214 Å (a), 143 Å (b) and P(1) (c). Tension: (dashed line) during a twitch, (dotted) double stimulation, (full line) a twitch with a quick release.

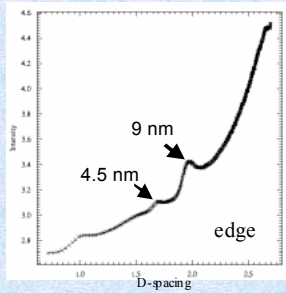
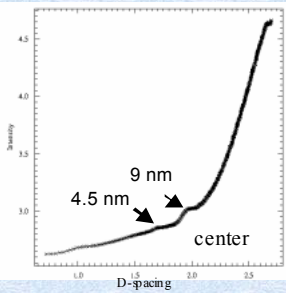
Микродифракция

Scanning SAXS of the hairs breast cancer patient

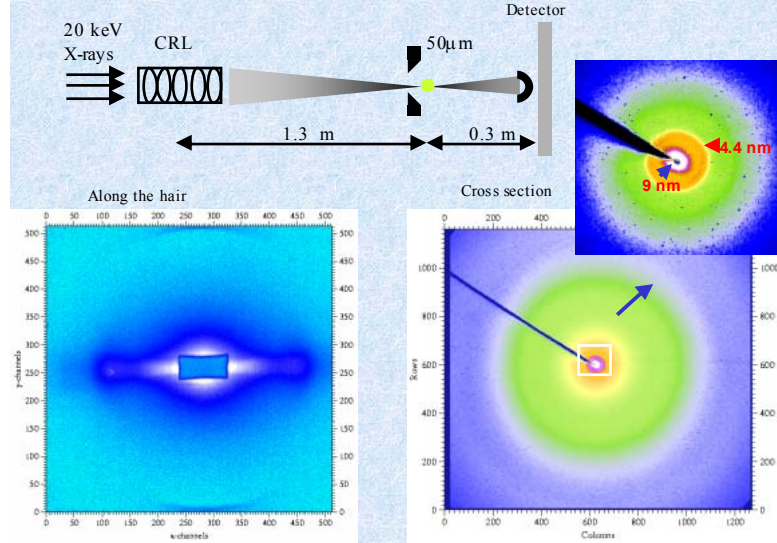


Phase contrast image

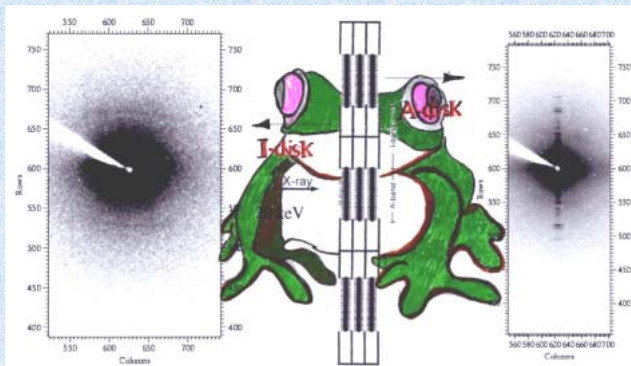
E = 20 keV
 Focusing with CRL
 beam size 1*8 μm²
 Focal distance 1.3 m
 Sample-to-detector distance 0.3 m



SAXS study of the archeological hairs with microbeam



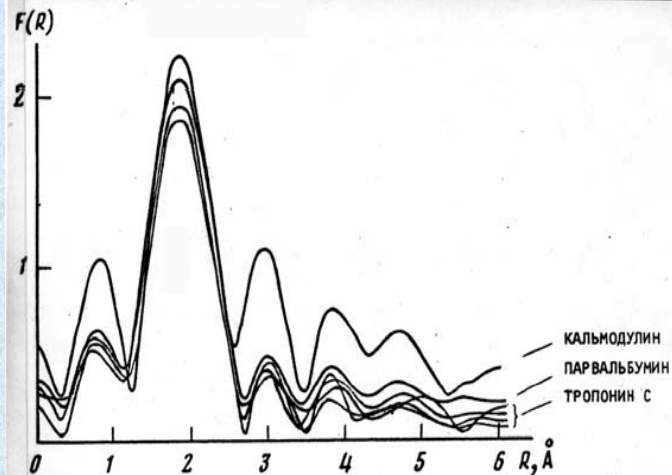
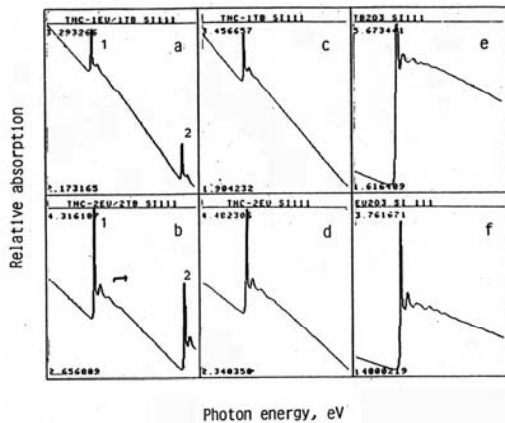
Fibre Diffraction from A- and I- disk of Cross-striated Skeletal Muscle with Microbeam at ESRF



The high resolution of the technique allows one to make visible a fine structure of various biological objects with a period of few microns: the diffraction patterns from A- and I-disks were registered separately.

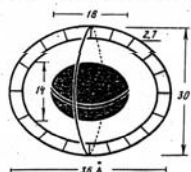
EXAFS- спектроскопия

Experimental EXAFS spectra of TnC (a-d) and of reference compounds Tb₂O₃ (e) and Eu₂O₃ (f)
 a - TnC 1Eu/1Tb; 1-Eu L_{III}, 2 - Tb L_{III};
 b - TnC 2Eu/2Tb; 1-Eu L_{III}, 2 - Tb L_{III};
 c - TnC 1 Tb; d - TnC 2Eu

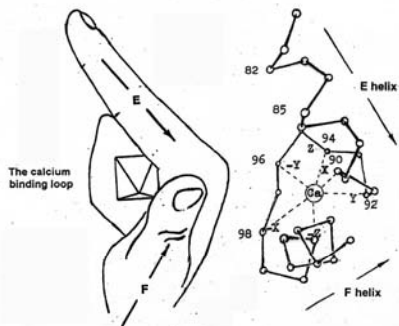


Для всех исследованных белков наибольший максимум $F(R)$ ($R=1,99 \text{ Å}$), характеризующий первую координационную сферу, соответствует расстоянию Tb-O, равному $2,43 \pm 0,02 \text{ Å}$ (с учетом сдвига на $0,44 \text{ Å}$ по эталонному соединению Tb₂O₃).

Schematic representation of a parvalbumin molecule

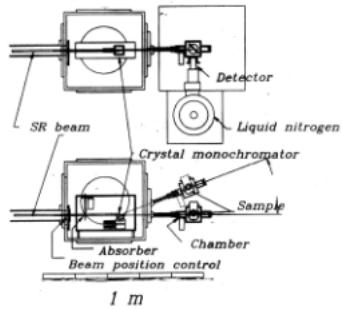


The symbolic EF hand represents (Kretsinger, 1973)



Флуоресцентный многоэлементный анализ

SRXFA station at VEPP-3



Technical characteristics :

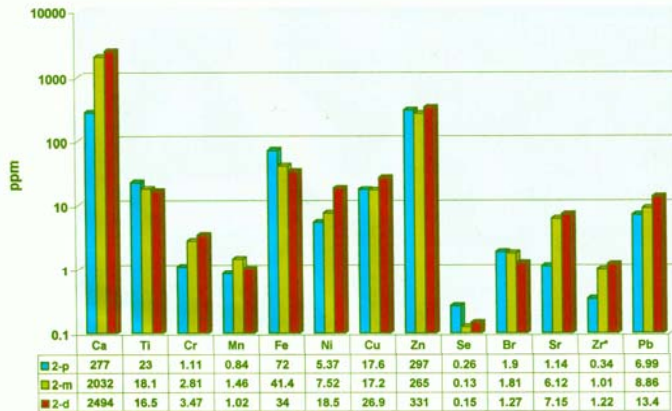
Monochromator :
 energy region : 5 - 46 keV
 energy resolution : 0.1 %
 koef. of reflection : 0.7

Detector Si(Li) :
 energy region : 3 - 50 keV
 energy resolution : 180 eV on 5.9 keV
 counting rate : 2 kHz

SRXFA- station for Element Analysis

Energy excitation : 20 KeV
 Detector : Si(Li), semiconductor (180 eV)
 Exposure : 900 sec
 K, S, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Se, Br, Sr, Hg, Pb

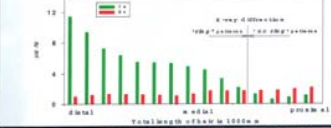
Koca (p,m,d)



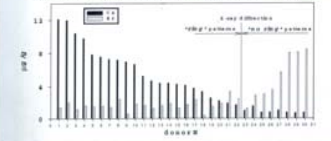
Correlation between contents of Ca-Br & Ca-Sr and typical X-ray hair patterns



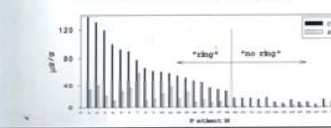
Content of Ca(450) and Br in hairs of individual donor was obtained by scanning along the sample point by point from the root region to the tip at 5-100 nm interval



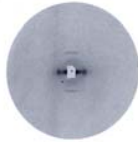
Content of Ca(450) & Br in hairs of 30 donors from Novosibirsk & Penzance



Content of Ca(20) & Sr in hairs of breast cancer patients



The archaeological samples from *Pazyryk mound* of Altai mountains were studied. Undamaged state of hair samples is due to constant extremely low temperature conditions.



The structure of keratin intermediate filaments in hair tissue is invariable, the extracellular matrix structure is varied.

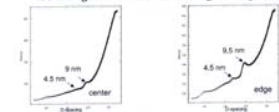


Altai ice maiden

The element content in the hair of Altai ice maiden demonstrates an anthropogenic pressure of civilization of the Bronze Age.

The archaeologically found people's hair have got tenfold excessive accumulation of the dangerous elements such as Cu and Pb that probably results in fivefold deficiency of essential element Zn (zinc-anemia).

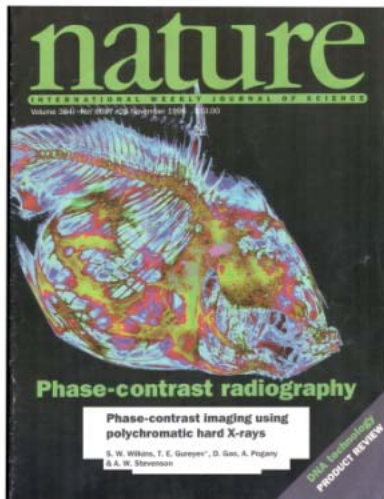
Scanning SAXS of the archaeological single hair



Phase contrast image of single hair (cross section)



Фазово-контрастное изображение биологических объектов



What is phase contrast? *Snigirev, FIRA*

Phase contrast method consist in

- separating the diffracted and non diffracted parts of the light
- treating them differently
- recombining them under conditions such that they produce controlled visible interference effects

light

- Phase contrast light microscopy Zernike, 1935
- In-line holography Gabor, 1948

soft X-rays

- Phase contrast microscope Schmitt et al., 1988
- X-ray holographic microscopy Aoki et al. 1972
- X-ray Fourier holography Howells(ALS), Jacobsen(BNL), Polish (LURE) McNulty & Kirz, 1992

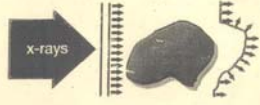
hard X-rays

- interferometer Bonse & Hart, 1965
- phase contrast imaging & tomography Ando & Hoya, 1972; Momose, 1995
- phase contrast based on multicrystal setup
 - "Mikrosim"
 - "infracton"
 - "diposicion"
 - "phase-contrast"

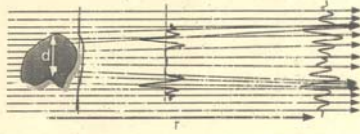
Gozz, Kalozhnikov, Mikhailov, Skolov, Fomenko, Zemanov, 1978; Shilovits & Podurets, 1986; Sagal & Belikovskaya, 1991; Davis, Gao, Guenzl, Stevenson, Wilkins, 1995

Image Formation

- phase-shift inside the object deteriorates wavefront
- direction of propagation is normal to wavefront



- interference of different parts of the wavefront with different direction
- intensity distribution is a function of the object-to-detector distance



ABSORPTION CONTRAST PHASE CONTRAST HOLOGRAPHY

contrast is due to absorption only edges are enhanced (Outline Mode) at $r > d^2/\lambda$

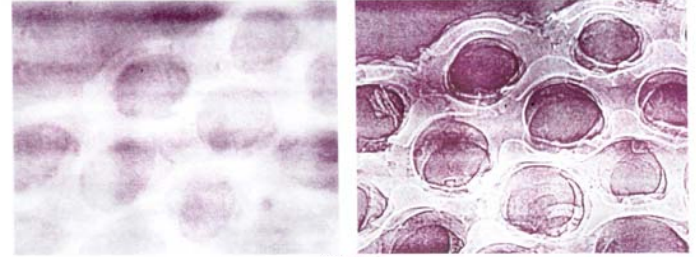
C. Raven: X-ray tomography with micrometer spatial resolution, Buffalo, NY April, 30, 1997 4

X-Ray imaging of seashell *Sorites orbiculus* (Belize) at 18 keV

C. Raven, I. Snigireva, A. Snigirev, ESRF, Grenoble

Amplitude mode
distance sample to detector 0.5 cm

Phase contrast mode
distance sample to detector 20 cm

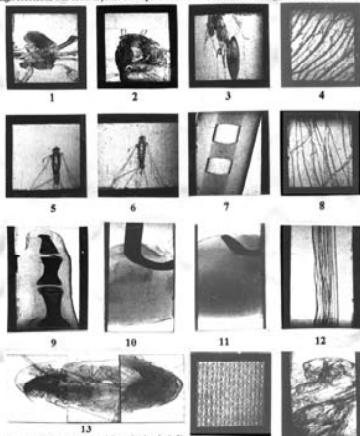


40 μ m

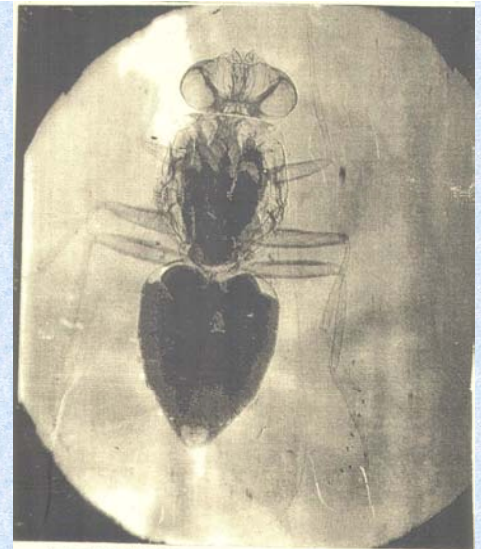
μ -FID₂₂

The refraction images of biological objects obtained using the SR (VEPP-5, INP SB RAS, Novosibirsk)

The shaping of the SR beam was realized in the plane of electron orbit by means of two crystals. The first crystal-monochromator (Si, plane 111, $\lambda=0.15$ nm, asymmetry index is 0.3) was located at the distance of 17 m from SR source. The second crystal (Si, 511) was used as an analyzer. It was cut out from the monocrilline block of silicon and had two parallel geometrical diffraction surfaces separated by a slit of width 15 μ m. The weak-resolution was about 15 μ m. The exposure time was about 1 sec. The magnification is 5.8 \times .

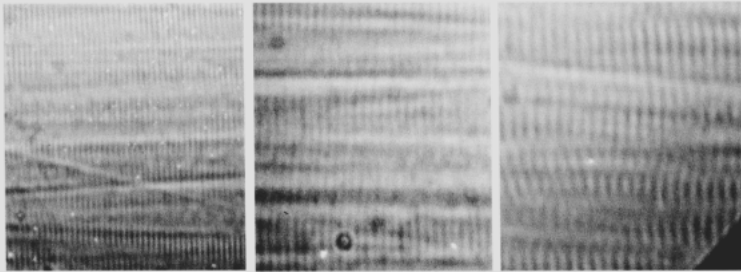


1) Bug (*Anobium portitarsis*); 2) Bug (*Scolytidae*); 3) Ant (*Formicidae*); 4) Wing of seed of maple (*Acer platanoides*); 5, 6) Monopile (*Chalcididae*); 7) Polyethylene capillary with water and bubbles; 8) Threads of polyethylene; 9, 10, 11) Images of Bug organ (*Arctidae exulcens*): Foreleg, Heart and Eye (Pigments); 12) Collagen filaments isolated from the tail of rat



ABSORPTION CONTRAST. 1958.

Phase contrast X-ray images of living cross-striated skeletal muscle *Sartorius* of frog *Rana ridibunda*



In rest
Sarcomere length 2.2 μm

Stretched to 25%
Sarcomere length 3 μm

Stretched to no overlap
Sarcomere length 4 μm

Расчетные параметры контраста и изображений и дозы облучения для рефракционной интроскопии в сравнении с обычной рентгеновской диагностикой

Энергия (кэВ)	15	20	30	40	50
Длина волны (Å)	0.83	0.62	0.41	0.18	0.14
Число фотонов/мм ² ·сек (X 10 ¹¹)	7.8	3.5	1.1	0.3	0.07
Контраст, % (рефракция)	18	15	11	8	7
Контраст, % (поглощение)	1.3	0.5	0.1	0.06	0.04
Доза, рад (рефракция)	19	1 · 10 ⁻²	4 · 10 ⁻⁴	2 · 10 ⁻⁴	2/10 ⁻⁴
Доза, рад (поглощение)	93	0.3	0.1	0.2	0.4

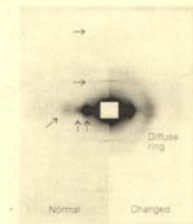
Объект – маммографический фантом: капроновая леска диаметром 1 мм внутри цилиндра воды диаметром 10 см

Структурная биология ткани

Is SAXS a Good Tool to Screen for Breast Cancer?

We have studied hair using fibre X-ray diffraction studies with SR and find that hair from breast-cancer patient has a different intermolecular structure to hair from healthy subjects.

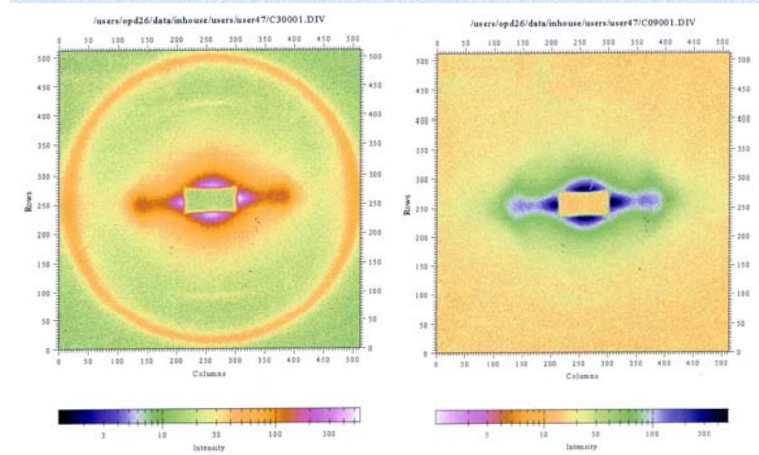
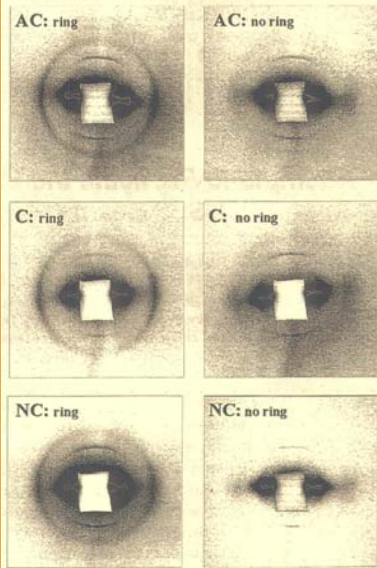
By Veronica James,
John Kearley,
Tom Irving,
Yoshiyuki Amemiya,
David Cookson
Nature 398(1999) 33-34



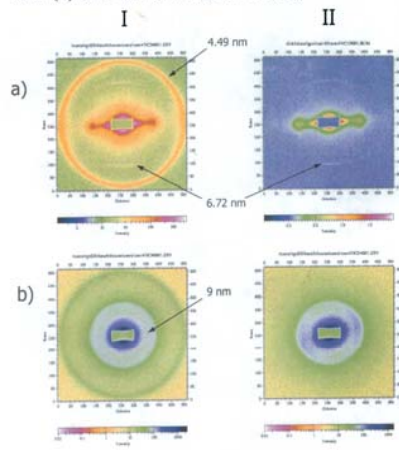
Possibility of Using Synchrotron Fibre Diffraction of Hair to Screen for Pathologic Conditions such as Breast Cancer proposed by Veronica James has not confirmed.

- by
- 1) Fatma Briki et al., LURE, France, *Nature* 400 (1999) 226
 - 2) Michael Hart, Brookhaven NSLS, USA
 - 3) H. Amenitsch et al. Synchrotron at Trieste, Italy
 - 4) Klans Schroer et al., Brookhaven NSLS Beam Line X12B, USA
 - 5) Benjamin Chu et al., Brookhaven NSLS X27C, USA

6) Alvina Vazina et al., *NIM* A470(2001) 380-387



Two types of the hair X-ray diffraction patterns: “ring” (I) and “no ring” (II) along hair (a) and cross-section hair (b)



Корреляция между интегральной интенсивностью рентгенограммы и элементным содержанием волос

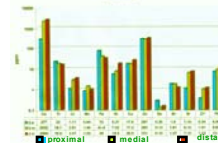
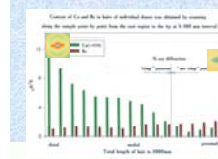
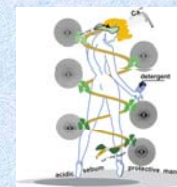
Набор рентгенограмм, полученных в разных точках волоса 18-летней девушки (длина косы более 1000 мм) при сканировании вдоль волоса от корня к кончикам

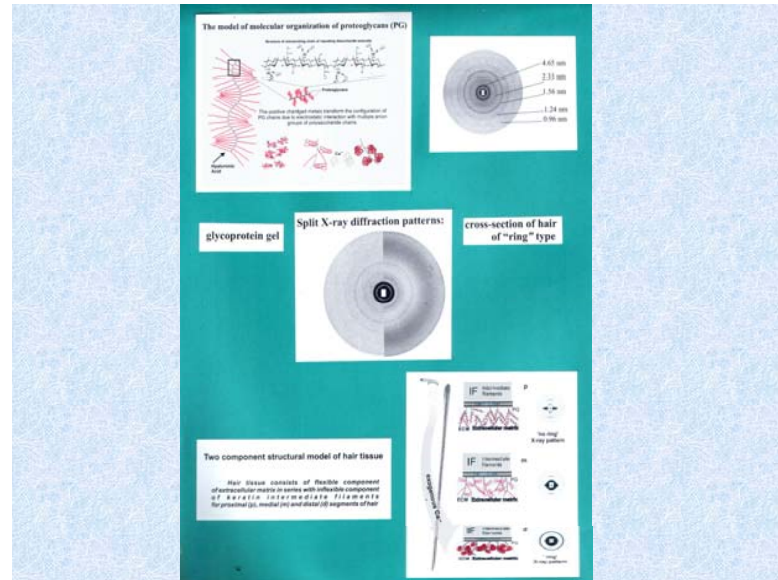
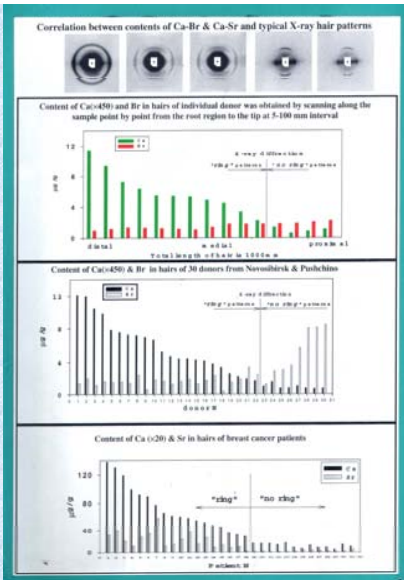
Для этого набора рентгенограмм закон сохранения интенсивности не выполняется – наблюдается значительное увеличение интегральной интенсивности в малоугловой области дифракции:

$$I_1(s)ds < I_0(s)ds$$

Рентгеновские флуоресцентные спектры образцов позволяют определить относительное содержание в них целого ряда элементов: K, Ca, Cu, Zn, Fe, Mn, Cr, Se, Br, Co, Mo, Ni, V, Sr, Rb, Y, Sc, Ti, Ga, Zr, Nb, I, Sn, Sb, Ba, La, Ce, Nd, As, Pr, and Pb.

- Показано, что пул элементов сохраняется в течение всей жизни волоса, а содержание большинства элементов увеличивается от корня к концу волоса.
- Кальций является основным элементом набора волоса; его содержание возрастает вдоль волоса в десятки раз.
- Увеличение интенсивности экваториальной дуги 4.5 нм вызванное, вероятно, окрашиванием кальцием другими элементами, теперь может быть объяснено накоплением атомов металлов в протекликаных структурах. Увеличение электронной плотности приводит к появлению нескольких порядков рефлекса 4.5 нм.



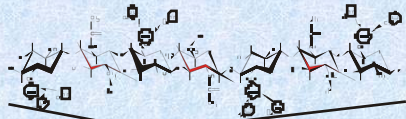


Модель молекулярной организации протеогликанов (PG)

Структура называемая? или построившая дисахариды х-сульфиниц



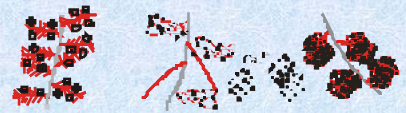
Электрон-микротография и анализированных агрегатов PG



Гликозил-группы, образуются в результате взаимодействия с белком

Влажные свойства протеогликанов с клеточной мембраной

Это обусловлено тем, что полисахаридная часть протеогликанов, являющаяся полифункциональной, может отклоняться конфигурируя

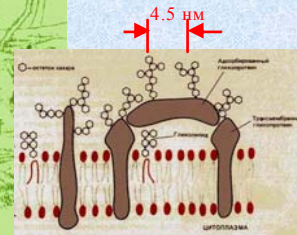
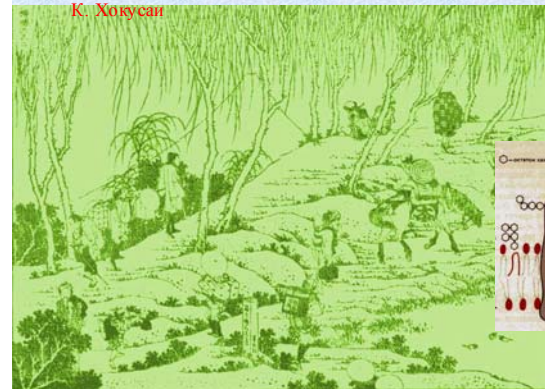


Гиперурсия в листьях

«Вопрос о том, где кончается плазматическая мембрана и где начинается внеклеточный матрикс, можно считать чисто семантическим»

Дж. Уотсон

К. Хаксан



Изменение дифракционных картин нативной ткани волоса под воздействием Ca^{2+}

4.5 nm

4.5 nm

4.5 nm

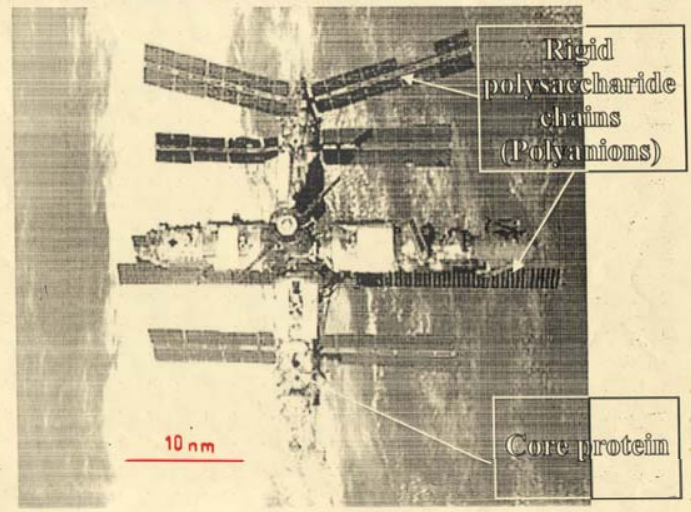
4.5 nm

Модели структуры протеогликанов

Белковые и кор Полисахарида

К. Хокусан

Simulation of proteoglycan structure



Set of X-ray diffraction patterns – "ring free"

There is a class of donors who have hair fibers giving X-ray diffraction patterns without ring throughout the hair length. We call that diffraction patterns as "ring free" patterns.

In this case the law of conservation of intensity is realized always:

$$\int I_1(\theta) d\theta = \int I_2(\theta) d\theta = \int I_3(\theta) d\theta \quad n = 5-10 \text{ in our experiments}$$

Within each experiment the exact exposure time was used to scale the intensity data together.

The ring on X-ray patterns of each hairs can't be obtained under the wide range of various treatments.

Transition of X-ray hair diffraction patterns from "no ring" to "ring"



It was studied the structure changes of ECM due to using of different detergents acting on hairs *in vivo* and *in vitro*

Experiments *in vivo*: about 100 hair samples were collected from two donor's groups of tundrian nenets (Samburg, Tyumen' region):

- (1) adult men and women, who use traditional methods of care for hair, and
- (2) children, living in public school panstation and using modern means for hair washing, especially girls permitting themselves to use cosmetic shampoo.

"No ring" type of X-ray patterns was registered for adult men and women in the majority cases. There are high rate of "ring" type of X-ray hair patterns among the donors of second group, especially for teenagers.

Experiments *in vitro*: two different X-ray patterns were obtained from the same segment of hairs in native state and after:

