The History and Future of X-ray Microscopy

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Abstract. We take a somewhat whimsical look at the history of X-ray microscopy, and extrapolate some trends into the future.

1. History

This is a story of spies, heroes, villains, false starts, and a brush with real fame. We divide the history into seven epochs: 1) Ancient history 1895–1945; 2) The Classical Period 1946–1960; 3) The Dark Ages 1961–1971; 4) The Renaissance 1972–1982; 5) Romanticism 1983–1993; 6) The Age of Reason 1994–2002; and 7) The Industrial Revolution 2003–2008.

1.1. Ancient History 1895–1945

This was the time of the pioneers who, following Röntgen's 1895 discovery of X-rays [1], established point-projection microscopy with a resolution of a few microns [2], understood the nature of X rays, and developed absorption and emission spectroscopy. It was, coincidentally, during this period that R.W. Wood demonstrated the use of phase zone plates as focusing elements with visible light [3]. The theoretical starting point of X-ray optics can be traced to the brief paper by Einstein [4] in which he suggested that the index of refraction for X rays in most materials should be just slightly less than 1.

1.2. The Classical Period 1946–1960

Right after World War II several groups became interested in X-ray microscopy. Arne Engstrom in Sweden developed the technique of quantitative elemental imaging [5]. Paul Kirkpatrick at Stanford worked on grazing incidence optics. With Albert Baez he developed the crossed-mirror system that bears their names [6] (see Fig. 1). He convinced himself that this optical element will lead to a very high resolution imaging microscope [7], having neglected to consider off-axis aberrations; additional examples were constructed with Howard Pattee. In Cambridge, Cosslett and Nixon first carried out work in point projection x-ray microscopy (Fig. 2), and then went on to work primarily on x-ray analysis in the electron microscope [8]. These three groups took turns organizing international conferences on X-ray microscopy: Cosslett hosted the first one in 1956 [9], Engstrom the second in 1959 [10], with Kirkpatrick taking responsibility for the third in 1962 [11]. This series of conferences shifted focus from microscopy to microanalysis around the 1962 meeting.

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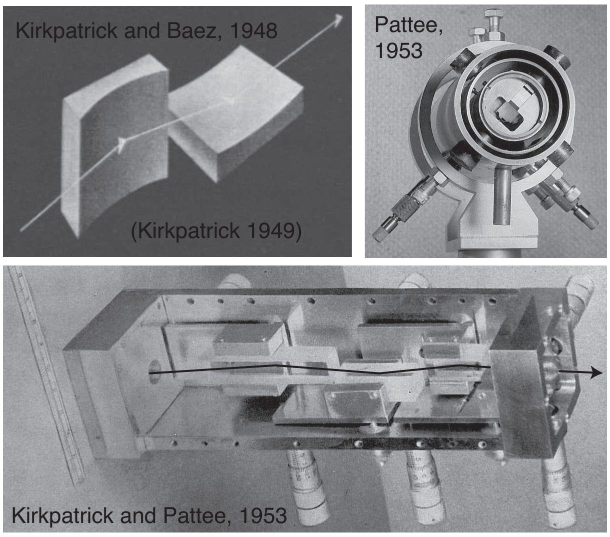

Figure 1. The crossed-1D-lenses focusing scheme developed by Kirkpatrick and Baez using elliptical profile mirrors [6]. At top left is an illustration from Kirkpatrick's 1949 contribution to *Scientific American* [7], where the editor's blurb in the table of contents stated "The X-ray Microscope: It would be a big improvement on microscopes using light or electrons, for X-rays combine short wavelengths, giving fine resolution, and penetration. The main problems standing in the way have now been solved." At bottom is a three-mirror system constructed by Kirkpatrick and Pattee [12], and at top right is an improved, concentric-cylinder mounting scheme developed by Pattee (1953; picture from a 1983 letter by Pattee to M. Howells). More recent focusing schemes involving multilayer Laue lenses [13] return to the crossed-1D-lenses focusing geometry.

It was during this period that Baez published his suggestion that zone plates should be used as X-ray lenses [14, 15], and the book by Cosslett and Nixon [16] summarized everything that was known about X-ray microscopy.

1.3. The Dark Ages 1961–1971

The sixties were a relatively quiet period in X-ray microscopy. The Cambridge group continued to be active, especially Theodore Hall and his collaborators [18]. It was revealed only decades later that Hall was the second Soviet spy in Los Alamos [19], and that Cosslett was also involved in clandestine activities.

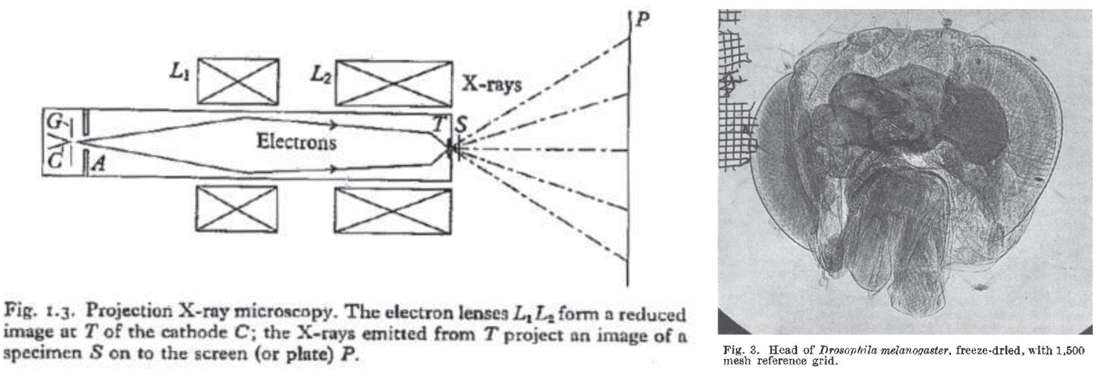

Figure 2. Point projection microscopy scheme developed by Cosslett and Nixon. The scheme is shown at left (from [16]), while at right is shown a point projection image of an insect head (from [17]).

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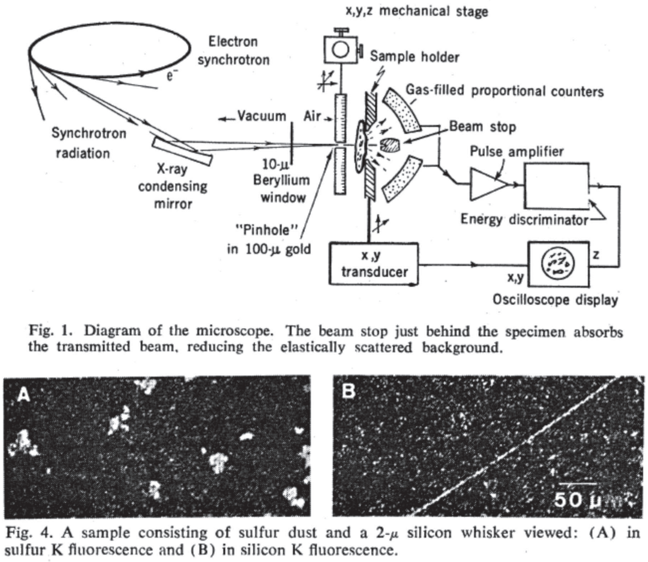

Figure 3. The first synchrotron-based x-ray microprobe was developed by Horowitz and Howell at the Cambridge Electron Accelerator in 1972 [21]. The microprobe used a deep pinhole fabricated by plating around a silicon whisker. Unfortunately this promising instrument was short-lived, as the accelerator was shut down in 1973 due to the high energy physics community moving on to bigger machines.

Of course the seeds for the renaissance were planted during the dark ages: it was toward the end of the decade that Schmahl and Rudolph turned their attention to holographic fabrication of zone plates [20].

1.4. The Renaissance 1972–1982

The decade of the seventies saw the first lightsource-based X-ray microscopes: Horowitz and Howell demonstrated both transmission and fluorescence microscopy at the Cambridge Electron Accelerator [21] (Fig. 3), Aoki and Kikuta did the first holographic experiments in Japan [22], and Schmahl *et al.* built the first TXMs that used zone plates, first using a laboratory source [23] and soon after using synchrotron radiation [24, 25] (Fig. 4). Stony Brook activities in developing STXM started at SSRL [26], and moved to the NSLS [27] (Fig. 5).

1.5. Romanticism 1983–1993

Following the first uses of synchrotron light sources, a lot of new avenues were tried, some more successful than others. The King's College group built a STXM at Daresbury [28] (using

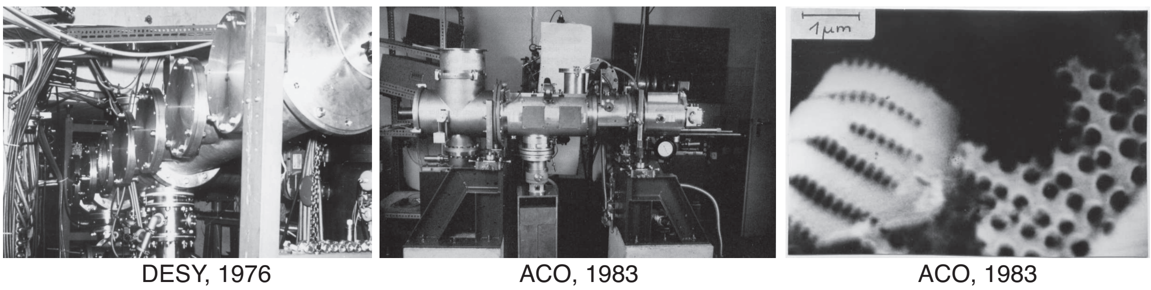

Figure 4. The first zone plate TXMs (transmission x-ray microscopes) developed by Schmahl, Rudolph, and Niemann. At left is shown an instrument operated at DESY in Hamburg in 1976 [24], while an instrument operated at ACO in Orsay is shown in the middle image. At right is shown an image of a diatom obtained at Orsay [25]. Images courtesy G. Schmahl.

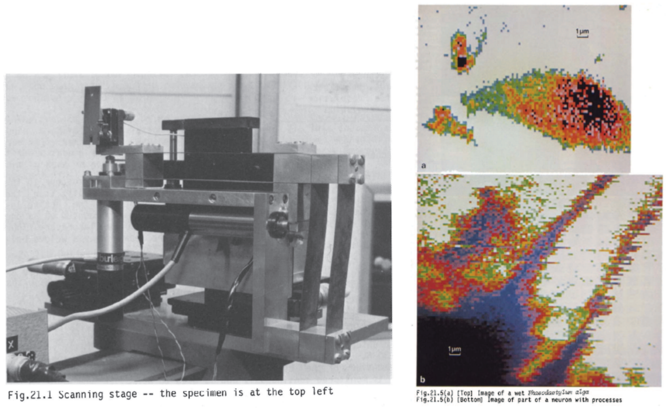

Figure 5. The first STXM (scanning transmission x-ray microscope) using zone plate optics was constructed by Rarback, Kirz, and Kenney at U-15 at the National Synchrotron Light Source at Brookhaven [27]. It used 300 nm zone plates and operated with $E/\Delta E \simeq 300$; the images shown took nearly an hour each to acquire.

a source with poor performance for coherent illumination), and so did the Göttingen group at BESSY [29]. Kunz's group at DESY built a microscope based on a commercial ellipsoid with terrible aberrations [30]. The Stony Brook group (Ade *et al.*) at NSLS built a scanning photoemission microscope two decades before the technology was ready for such an instrument [31]. MAXIMUM, a somewhat similar device at Wisconsin, was not much more successful [32]. Efforts at further development of holography at the NSLS achieved some successful demonstrations [33, 34, 35], but they also illustrated just how difficult it was to perform coherence-based experiments on second generation light sources. The first X-ray lasers were demonstrated at Livermore and Princeton [36, 37, 38], but at only a few pulses per day they were not very user-friendly for microscopy experiments.

The novelty of X-ray microscopy also led some people astray. Prof. Baldini from Harvard teamed up with scientists at IBM for flash-contact-microscopy of blood platelets [39]. One of the images even appeared on the front page of the Science section of *The New York Times* (Jan. 15, 1985). Unfortunately it was not an image, but rather the platelet stuck onto the photoresist "detector," as the authors later discovered. The euphoria over the development of x-ray lasers led to another cover article (April 2, 1985) in the Science section of *The New York Times* that gushed "But aside from its weapons applications, the X-ray laser has excited biologists, chemists and physicists because of its possible use in a super microscope, an instrument that will perhaps be capable of taking holographic three-dimensional movies of the genetic code of a living cell." The 1992 NASA Scientist of the Year Award was given to Richard Hoover for inventing (US Patent 5,107,526 on April 21, 1992), according to NASA, a "revolutionary new microscope [that] should enable researchers to see in great detail high contrast x-ray images of proteins, chromosomes and other tiny carbon structures inside living cells. Resolution of the microscope could be so high that it may produce detailed images of the building blocks of life—tiny DNA molecules." The instrument was to use a carbon K X-ray source and a multilayer-coated Schwartzschild objective, similar to a scanning microscope proposed by Eberhard Spiller some years before [40]. Several of us got together and sent a letter to the head of NASA, pointing out that radiation damage would make it impossible to do what was being claimed. We received an answer from the top lawyer at the agency, who assured us that no laws were violated. . .

It was also during this period that the present series of X-ray Microscopy Conferences started (see Fig. 6A), with the first one in Göttingen in 1983 hosted by Günter Schmahl and his group [41]. Major steps during this period included:

- The invention of Zernike phase contrast microscopy by Schmahl & Rudolph [42, 43].
- Following a proposal by Sayre [44], the development of e-beam fabricated zone plates at MIT [45] and more substantially at IBM [46]. IBM also introduced the community to silicon

nitride windows [47].

- The first undulator beamline for microscopy at the NSLS [48].
- The establishment of the Center for X-ray Optics in Berkeley by David Attwood.
- Spectromicroscopy using XANES [31, 49, 50, 51, 52].

Most of these conference proceedings are relatively easy to come by [53, 54, 55, 56, 57, 58]. The proceedings of the Sep. 20–24, 1993 conference held in Chernogolovka, Russia are a bit harder to find [59], but the conference was memorable: the Congress of People's Deputies was dissolved by President Boris Yeltsin on Sep. 21, and rumors were rampant during the meeting. Most foreign participants had returned home before street riots and battles took place over Sep. 28-Oct. 5.

1.6. The Age of Reason 1994–2002

X-ray microscopy started a major expansion during this period, with new instruments at new light sources, such as ALS, APS, ESRF, ELETTRA, NSRRC, Spring-8, Aarhus, Ritsumeikan, etc. Tomography [60, 61], cryo [62, 63], and cryo-tomography [64, 65, 66, 67] were demonstrated. The range of applications grew rapidly, including soil science, geochemistry, polymer science, magnetism, etc. Groups in Göttingen, Stockholm, London, Tsukuba and elsewhere were designing and building laboratory-based instruments. David Sayre's old dream of diffraction microscopy (recording the diffraction pattern of a non-crystal, and reconstructing it) had its first successful realization at the NSLS [68].

1.7. The Industrial Revolution 2003–2008

In the last five years X-ray microscopy has entered the mainstream. We are no longer working with an esoteric, new, unproven technique. What brought about this change is the rapidly growing list of successful and highly visible applications in environmental and soil science, geo- and cosmo-chemistry, polymer science, biology, magnetism, energy research, materials and surface science, among others. Without applications we are just a curiosity.

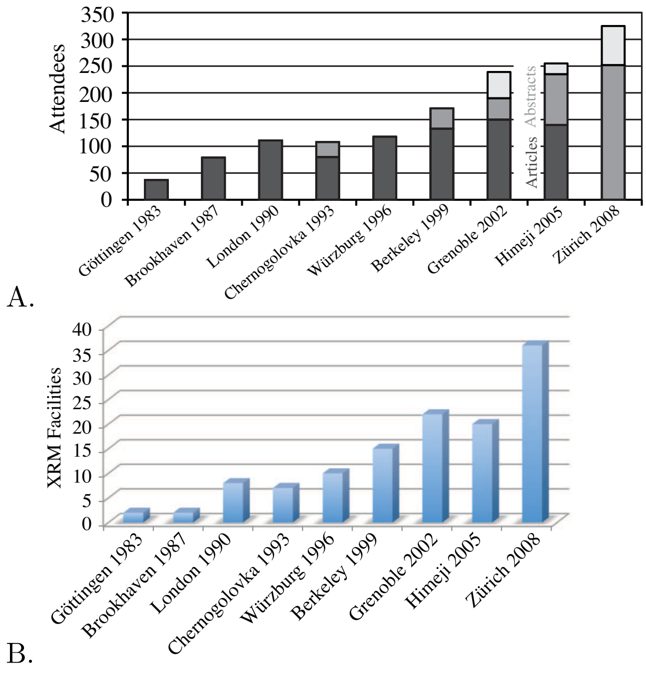

Figure 6. Growth in the x-ray microscopy community. A) Number of attendees, articles with abstracts, and abstracts only at the modern series of x-ray microscopy conferences. The number of articles at the 2008 meeting is not indicated in this figure. B) X-ray microscopy facilities at synchrotron light sources worldwide. The count is based on papers presented at each x-ray microscopy conference.

New microscopes are springing up everywhere, at new and old lightsources (CLS, SSRL, APS, SLS, ALS, BESSY, etc.; see Fig. 6B), and in laboratories without lightsources. The demand has resulted in the success of new commercial enterprises that sell microscopes (Xradia, ACCEL), zone plates (Xradia, zoneplates.com), detectors (DECTRIS), substrates (Silson), etc, and these enterprises have spurred the spread of microscopes. The growth has been particularly stunning in hard X-ray microscopy, where its early promise [21, 69] has been realized with new zone plates, Kirkpatrick-Baez optics, compound refractive lenses and multilayer Laue lenses [13, 70] getting to the point where they rival the resolution and efficiency of soft X-ray optics.

2. The future
It is foolish to try to prognosticate, yet here we go! What shapes the future are the new ideas. If we knew what they were, well ... So we are reduced to extrapolating from what we know, what is already in the pipeline, and especially what has been presented at this Conference. Among the areas that we feel are set for rapid growth is multi-dimensional microscopy, where the added dimension may be space, time, energy, or a combination of these. Since the added dimension generally requires multiple exposures, dealing with the issue of radiation damage becomes particularly important.

2.1. Time resolved microscopy
Until recently we have been largely restricted to the investigation of slowly varying phenomena. In many cases these are of tremendous technical importance, as with the destruction of interconnects in computer chips by electromigration [71, 72], or the curing of concrete [73]. More recently the very fast switching of magnetic vortices has been demonstrated by groups using both STXM and TXM based techniques. With the soft X-ray FEL source at Hamburg (FLASH) coming into operation, time resolved imaging has entered a new area. The time for an exposure is now on the order of 10 fs, and with clever arrangement the group of Chapman *et al.* have succeeded in observing phenomena with a time resolution that is comparable with this exposure time [74]. With the upcoming upgrade of FLASH and the completion of LCLS, XFEL and SCSS, we can confidently predict that very high time resolution and flash imaging will be a major growth area.

2.2. Tomography
Because one of the important advantages of X-rays is their penetrating power, much of the demand in applications is to examine samples too thick for electron microscopes [75, 76, 77]. Here one often encounters the problem of overlapping structures. 2D images are therefore often too complex and not informative, making 3D imaging particularly desirable. 3D microscopy with submicron resolution has been demonstrated over the past decade at several sources, with earlier results noted above and more recent results presented in talks at this conference by Brennan, Cloetens, Feser, Heim, and Larabell, among others. One major development of the past few years has been the success of the tomographic TXM instruments built by Xradia, which make hard X-ray tomography at < 60 nm resolution routine at lightsources [78, 79, 80] and in laboratory [81] instruments.

New, specialized tomographic instruments are also coming on line. At this Conference we saw the first stunning results from the National Center for X-ray Tomography, dedicated to cryo-tomography of biological specimens. The hard X-ray microtomography program at the ESRF has been evolving in the direction toward nanotomography based on projection geometry using a very intense nanoprobe [82].

Even higher resolution tomography has been demonstrated using diffraction microscopy [83, 84, 85, 86]. At this point, collecting and reconstructing the tomographic data set by this technique requires a great deal of effort, but as the technique matures, this should also become more routine.

We expect rapid growth in tomographic applications!

2.3. Elemental and chemical mapping

Spectromicroscopy, where a stack of images [52] is collected at closely spaced steps in energy near an absorption edge (especially near the carbon K edge) is now the main application of scanning microscopes operating at synchrotron light sources. The results provide spatially resolved information on the chemical state of the element in question. Here the incident X-ray energy is the third dimension in the data-set.

In a growing number of instruments it is the elemental constituents that are being mapped by X-ray fluorescence. This involves a hard-X-ray scanning microscope, where the third dimension of the data collected is the X-ray fluorescence spectrum (either in its entirety or sampled at key emission lines) for each pixel. Interest is growing rapidly in mapping trace metals in biological and environmental samples because of the importance of these in health, disease, nutrition and pharmacology. We expect a rapid growth in this area, and in a combination of X-ray fluorescence with spectromicroscopy to map the oxidation state of the elements found, to the extent allowed by radiation damage.

2.4. More than three dimensions

In samples that are less radiation sensitive, we can foresee four dimensional imaging. This may take the form of tomographic spectromicroscopy [87], tomographic movies, etc.

Other added dimensions may be in the form of correlative microscopy, be it visible, infrared, electron, or other. In fact this has always been a part of X-ray microscopy, but it is moving into a level of sophistication that has not been seen in the past. For example Larabell *et al.* developed a capability of looking at the same frozen hydrated specimen in their X-ray microscope, and in a visible light microscope equipped with a custom cryo-stage. This clearly points in a promising direction for the future!

2.5. The spatial resolution frontier

As the spatial resolution of x-ray optics improves (Fig. 7), an increasing number of scientifically important problems can be addressed. Taking this concept to the limit, the stated specification of the NSLS II facility is that it has to demonstrate 1 nm spatial resolution. This is an ambitious goal, though it raises interesting questions regarding radiation damage, depth of field, contrast, and the like. There is broader consensus among practicing x-ray microscopists that sub-10 nm resolution in practical applications is a worthy and ultimately reachable goal.

Zone plates are likely to demonstrate better than 10 nm resolution in the near future. They are most useful as microscope lenses, or to focus the radiation to form a nanoprobe as in a STXM. Multilayer Laue lenses are likely to get us to 5 nm in nanoprobe applications.

Diffraction microscopy is a different animal. It is an image forming technique that has already demonstrated very high resolution, though how exactly its resolution is determined is still a topic of some discussion. However, its lack of probe-forming optics rules out fluorescence mapping, and it does not give instant images. Plans for single molecule imaging [99] using X-ray lasers such as the LCLS call for high enough resolution to determine the structure of large protein molecules by diffraction microscopy, but this requires many identical copies of the molecule, each being exposed to the ultrashort and ultrastrong beam. Clearly if this, or something similar can be made to work, it will have tremendous impact!

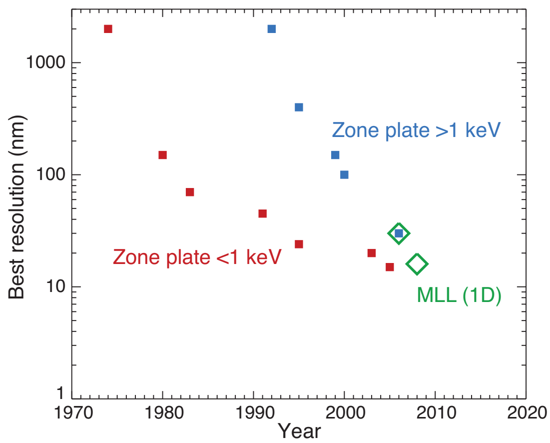

Figure 7. Best focusing values for diffractive focusing optics by year. Only those results that represent a new improvement from previous results are shown. Results are separated between those at ¡1 keV photon energies [23, 88, 25, 89, 90, 91, 92], and at ¿1 keV photon energies [93, 94, 95, 96, 97]. Also shown are 1D focusing results of multilayer Laue lenses (MLLs) [70, 98]. Results for refractive and reflective optics are not shown, though they have also experienced dramatic resolution improvements over time.

2.6. New ideas
A number of new ideas aim to extend the resolution that can be achieved in scanning microscopy, or in Fourier transform holography. The basic scheme is to record the diffraction pattern to high angles. In the approach by the Swiss Light Source investigators [100, 101], the detector in the STXM is the fast pixel-array detector PILATUS, and the full diffraction pattern is recorded at overlapping pixels of the scan. A four-fold resolution enhancement has been demonstrated. Williams *et al.* [102] use a beam diverging from a small focus to record the diffraction pattern. In this case the central part of the pattern is basically just the Fourier transform hologram of the object, and the resolution is set by the size of the small focus. But by detecting and reconstructing the large angle diffraction pattern, the resolution is extended substantially. The use of spherical, as opposed to planar, illumination facilitates the reconstruction.

3. Closing remarks
We have emphasized new developments. These often take heroic efforts, and take a while to mature, if they ever do. They are of great importance for the future of the field. It is equally, or may be even more important to have user-friendly workhorse microscopes that churn out results, and are available to the novice. We are entering a period where we have both. This guarantees the continued growth and health of X-ray microscopy.

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