Probing Italy: A Scanning Probe Microscopy Storyline

1. Early History of SPM in Italy

1.1. AFM and STM First Italian Prototypes

The history of scanning probe microscopy (SPM) in Italy starts in the late 1980s after the publication of the seminal works by Binnig and coworkers in 1982 on scanning tunneling microscopy (STM) [1] and 1986 on scanning force microscopy (AFM) [2]. Almost simultaneously, two research groups engaged in the construction of the first Italian prototypes: an STM in Rome, and an AFM in Pisa.

In Rome, Chiarotti from Tor Vergata University and Cricenti from Istituto di Struttura della Materia of the Consiglio Nazionale delle Ricerche (CNR) formed a group working on the construction of the first Italian STM operating in air [3]. Their efforts were recognized with two publications: a first paper published in 1988 [4] on atomic resolution imaging of graphite, followed by an interesting work on DNA imaging published in Science in 1989 [5].

In the following years, Chiarotti and coworkers mainly conducted research in the field of condensed matter, specifically the study of surfaces and their electronic properties.
In Pisa, the group led by Ascoli at the Istituto di Biofisica of the CNR realized the first AFM-STM setup in 1989 [6] and published its first account on AFM in 1992 [7]. This study investigated the mechanical and thermal effects on AFM cantilevers induced by laser absorption and reflection, including the measurement of radiation pressure. This achievement was possible thanks to the financial support of a European project (European Economic Community under Contract no. BREU 145) and by the CNR (as part of Progetto Strategico “Microscopia Tunneling”).

At some point, the two groups joined together for a common project which was aimed at realizing an instrument with interchangeable heads, i.e., AFM and STM, operating in vacuum. A prototype was eventually constructed that, however, did not lead to any publication attesting to this collaboration.

1.2. Earlier Experiments

In the 1960s, Gozzini and coworkers at the Scuola Normale Superiore in Pisa were studying a phenomenon known as the evanescent wave, an effect related to the so-called frustrated total internal reflection studied a long time before by Newton [8,9]. The experiments were first carried out approaching two parallel prisms, but the system was soon improved by introducing a curved one, since parallelism is difficult to achieve at a nanometer level. The distance between the prisms was scanned periodically at a low speed to explore the relevant range. These experiments were made possible by a thick Philips piezoelectric driven at high voltages (800 V) and piled upped in stacks to allow a total range of around a few microns. The controlling electronics were realized with high-voltage transistors connected in cascade.

The first report was published in 1971 [10]. It was shown that the decay of the intensity with distance was not exponential, as theoretically predicted, but sigmoidal. The relevance of this result within SPM history is the crucial role that piezoelectric and high voltage transistors have then played in the development of scanning probe microscopes. However, two details were missing thus preventing it from being considered an early example of scanning near-field optical microscopy (SNOM) [11]: there was no lateral scanning, and the laser spot had a diameter in the order of a micron.

1.3. Other Working Modes

Soon after the publication of the first AFM report, SPM spread in a wide range of diverse applications. For instance, the detection of friction force was implemented with the division in four quadrants of the photodiode to sense the cantilever rotation besides the deflection [12]. In Pisa, this mode was immediately implemented in the local homemade instrument [13]. Another interesting consequence from an experiment carried out in the 1960s was about the detection of paramagnetic resonance. The Bloch–Siegert effect and many-photon transitions were observed in a sample of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by Alzetta and coworkers with the method of angular-momentum detection, using a small quartz thread [14]. In 1996, Ascoli et al. successfully replicated the experiment measuring the torsion of an AFM cantilever loaded with DPPH and immersed in a magnetic field [15].

The idea to study biological samples with SPM was stimulated since the beginning with the construction of various liquid setups. Cappella et al., for instance, investigated the interaction between the tip and sample under liquid. This was obtained by means of the acquisition of force versus distance curves [16]. Nowadays, the same approach represents a standard operating mode of modern instruments, thanks to the technological developments of electronics and piezoelectric actuators.

In Cosenza, Samori’s group focused on the study of nanometric biological material using one of the first commercial instruments. He and his coworkers were very active contributors to the field of morphological analysis of DNA and nucleic acids [17]. Afterwards, in Bologna, the focus of his research gradually shifted towards single-molecule studies, employing the above-mentioned method of force versus distance techniques [18],
then upgraded to folding and unfolding maps by introducing velocity- and force-clamp modes \[19\].

In the biological field, a working mode was later employed that was capable of visualizing the topography in a non-invasive way and of measuring the mechanics of cells in vitro, i.e., scanning ion conductance microscopy (SICM) \[20\]. At the time, Pellegrino and coworkers were interested in studying the influence of external stimuli on growth cones of cells via the mechano-receptive channels. In collaboration with Ascoli and coworkers, they thus combined an inverted AFM with a SICM, in order to measure the distance dependence of ion current and solution pressure between a pipette and the back of a cantilever \[21\]. The first paper reporting the guidance of growth cones appeared in 2012, while measurements of membrane elasticity in 2013 \[22,23\].

A further drive to the diffusion of SPM was finally given by Scoles who, after many years spent at the Princeton University, in the early 2000s established a laboratory in Trieste devoted to the application of SPM to the nanomanipulation of organic and biological molecules \[24,25\].

2. Following Generations of Scanning Probe Microscopists

From its Italian debut in 1988, SPM has progressively diffused along the years. In particular, the so-called nanotechnology revolution that took place around the 2000s \[26\] assigned to SPM a key role for the investigation and manipulation of matter at the nanometric scale. This event promoted SPM diffusion in the whole country. This manuscript aims to provide a broad picture of the current distribution of the SPM groups in Italy. Accordingly, a survey consisting of six parts was prepared by LimeSurvey \[27\] and distributed via e-mail within the SPM community.

After obtaining informed consent, the survey sections ask for: (1) personal data; (2) institution data; (3) human resources of the research group; (4) microscopes available, techniques employed, and fields of interest; (5) research projects and educational/dissemination activities involving SPM in the last five years; and (6) two relevant publications of the group in the last five years. All the questions comprising the survey have been formulated in a simple and direct way, so the experts have not been required to rate the suitability of the method used to evaluate the answers. In total, approximately one-hundred surveys were analyzed.

2.1. Anatomy of an Italian SPM Research Group

Seventy-three groups declared themselves as pursuing SPM-based research in the survey, providing a lower limit to the number of currently active groups in the field throughout Italy. They are composed on average of 5 ± 2 members, divided between open-ended (3 ± 1) and fixed-term contracts, (2 ± 1). Small research groups are flanked by several large groups (≥14 members, ≈17% of the total, see Figure 1a), where the proportion between permanent and fixed-term positions is similar to that of small groups. Universities and research institutions host an almost equal number of SPM groups. Two of them are affiliated with private industries: Lyondellbasell in Ferrara \[28\] and Ricerca sul Sistema Energetico-RSE SpA in Piacenza (see Figure 1b).

Groups are present in most of the Italian regions (Figure 2a). Clustering the regions in three macro-areas (North, Centre, and South), 60% are in the North whereas 19% are in the Centre and the remaining 21% in the South. As shown in Figure 2b, the province distribution is more interesting. Large areas of Italy are completely devoid of results, whilst most SPM groups are concentrated around the largest universities (e.g., Roma, Bologna, Milano), large research institution areas (e.g., IIT and CNR), or large-scale facilities (e.g., Elettra Synchrotron).
Figure 1. (a) Plot of the percentage of groups versus the number of group members (from 1 to 20). Blue bars indicate groups composed of a maximum of 10 members, defined as “small”; green bars indicate groups with more than 10 members, defined as “large”. The distribution of blue bars has been fitted with a Gaussian distribution (dashed red line). (b) Plot of the percentage of groups versus the different institutions hosting them (Univ. stands for University, Res. for Research Institutions, and Ind. for Industries).

It is possible to trace back the current clustering observed in Figure 2b to those institutions and cities who hosted the pioneering groups that have become the de facto originators of local SPM schools: (1) Ascoli in Pisa; (2) Cricenti and Chiarotti in Roma; (3) Valdrè [29], Samori and Biscarini [30] in Bologna; (4) Valbusa [31] in Genova; and (5) Scoles and Modesti [32,33] in Trieste.

2.2. Microscopes and SPM Modes

The average number of microscopes available per group is one (≈38%) or two (≈27%). Only 17% of the groups have 5 or more (up to 8) microscopes, a number representing a large facility. Most of the microscopes are commercial setups (≈80% of the total) but some
home-built ones are still available ($\approx$20%). In 25% of the groups, the instruments are used daily, i.e., 5 days per week. The lowest datum is one day per week, 14%. They are used between 2 and 4 days per week in the rest of the groups.

In the Wikipedia page on “Scanning Probe Microscopy” [34], it is reported that new modes of operation are being continuously developed. These modes can be used to simultaneously investigate different tip-sample interactions. The list of modes reported in the Wikipedia webpage is frequently updated (the last update was on the 16 of February 2023). We believe that it can be considered a reliable source. This list is made of 42 items, divided in four blocks: (a) AFM-based; (b) STM-based; (c) scanning probe electrochemistry (SPE); and (d) other modes, e.g., FluidFM [35] and SNOM, scanning near-field optical microscopy [36].

Most of the Italian groups work with AFM-based modes ($\approx$69%), whereas the rest are divided in STM-based ($\approx$22%) and SPE modes ($\approx$9%, see Figure 3a). The modes included in the block “other modes” are available in laboratories (17) beside the basic modes. A wide array of basic and advanced AFM modes was declared as routinely employed by the respondents. In detail, 54% use electrical modes such as conductive-AFM (C-AFM) [37,38], electrostatic force microscopy (EFM) [39,40], Kelvin probe force microscopy (KPFM) [41,42], and scanning gate microscopy (SGM) [43], whereas an additional 30% use other modes such as piezoelectric force microscopy (PFM) [44] and magnetic force microscopy (MFM) [45,46] (see Figure 3b).

![Figure 3. Pie charts of blocks of SPM modes (a), relative distribution of advanced AFM-based (b) and SPM-based (c) modes.](image)

Groups working with STM-based modes mainly employ basic operating modes, i.e., STM imaging or scanning tunneling spectroscopy [47], mostly focusing on atomic and molecular resolution [48,49]. These experiments, often performed in an ultra-high vacuum and at a low temperature, aim at investigating small areas of the sample (typically, few tens of nm). The advanced STM modes are instead used in different environments and on a large scan size (few hundreds of nm). Two illustrative examples are the ballistic electron emission microscopy (BEEM) [50] and electrochemical scanning tunneling microscope (EC-STM) [51] performed in air and an electrochemical cell, respectively. These peculiarities make the advanced STM-based modes available only in 35% of the groups (see Figure 3c).
The electrochemical environment introduces the SPE block, in Italy represented by SICM (cp. to Section 1) and scanning electrochemical microscopy (SECM) [52]. The last block “Other modes” is dominated by optical applications such as SNOM [53] and tip-enhanced Raman spectroscopy (TERS) [54,55].

In summary, the Italian groups perform experiments with 70% of the 42 modes reported in Wikipedia, employing also other SPM modes not reported in the list: ultrasonic force microscopy (UFM) [56,57], bimodal AFM [58], and photoinduced force microscopy (PiFM) [59]. Occasionally, some chemical/physical properties of matter were investigated by using solely a combination of several SPM modes [58,60,61].

2.3. Scientific Research, Publications and Fundings of Italian SPM Groups

The declared main research areas of the respondents can be divided between material sciences for \( \approx 70\% \) and on life sciences for the remaining \( \approx 30\% \) (see Figure 4a). Specifically, the subject areas where Italian SPM groups work are material science, chemistry, physics, biochemistry, biophysics, and engineering, in this latter case with device applications.

Figure 4. (a) Plot of group percentage versus research fields. (b) Plot of publication percentage versus IF. Blue bars refer to review with an IF < 10 whereas green bars refer to IF \( \geq 10 \). The dashed red line is a Gaussian fit to calculate the average IF.

<table>
<thead>
<tr>
<th>Review</th>
<th>IF</th>
<th>Q</th>
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<tbody>
<tr>
<td>Microscopy and Microanalysis</td>
<td>4.099</td>
<td>Q1</td>
</tr>
<tr>
<td>Ultramicroscopy</td>
<td>2.994</td>
<td>Q1</td>
</tr>
<tr>
<td>Microscopy Research and Technique</td>
<td>2.893</td>
<td>Q2</td>
</tr>
<tr>
<td>Micron</td>
<td>2.381</td>
<td>Q3</td>
</tr>
<tr>
<td>Microscopy (Oxford, England)</td>
<td>2.072</td>
<td>Q3</td>
</tr>
<tr>
<td>Journal of Microscopy</td>
<td>1.952</td>
<td>Q4</td>
</tr>
<tr>
<td>Scanning</td>
<td>1.75</td>
<td>Q4</td>
</tr>
</tbody>
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The total average impact factor (IF) of journals in these subject areas is \( 2.9 \pm 0.6 \), as calculated by averaging the average IFs reported in Table 1 of Ref. [62].

In the last six years (from January 2017 to February 2023), the Italian SPM groups have published in journals with an average impact factor (IF) of \( 5 \pm 2 \) (see Figure 4b), fixing the Italian SPM publications well-above the total average IF. Specifically, articles are published in journals with an IF higher than the average IF for each subject area and within the top 20% journals class [63]. Notably, 25% of the SPM publications have been published in journals with an IF \( \geq 10 \). In these publications, SFM plays a fundamental role for \( \approx 45\% \) of
them, is important as well as other techniques in $\approx 20\%$, and has a supporting role in the remaining $\approx 35\%$.

The SPM papers, published both in Italy and worldwide, can be ideally split in two broad groups on the basis of the reported image scan sizes: (1) images smaller than $300 \times 300 \text{ nm}^2$ usually aim at achieving the highest possible structural detail, i.e., atomic or molecular resolution; (2) images larger than that usually aim at linking the physical/chemical properties of matter at the nanometric scale to the mesoscopic and macroscopic scales. The technique of choice for the former group is typically STM, while it is AFM for the latter.

In Italy, atomic and molecular resolution imaging has been obtained on reconstructed surfaces [64], 2D materials [65], and inorganic [66] and organic [48,49,67] films. Similar atomic resolution have been also obtained with friction in contact AFM [68]. On the other hand, morphology has been broadly investigated with both AFM and STM and findings have been useful to understand: (1) the evolution of growth processes in organic [69,70], inorganic [71,72], and biological [73–80] structures, for organic structures even in situ and in quasi real time [81,82]; (2) chemical/physical interactions between organic and inorganic matter [83,84]; (3) the aggregation phenomena [85–88]; (4) effects of chemical [89–93] and physical [94–102] processes to modify matter and/or its surface; (5) living organisms [103–105]. Morphology and electrical measurements may be also correlated to explain the performances of electronic devices [106–110].

The mechanical properties of nanometric objects have been extensively studied by force spectroscopy. AFM groups are interested in low-dimensional structures [111,112] in material science and cells [113–115], biological tissues [116], biological particles [117–120], and biological films [121,122] in life science. In singolo force spectroscopy (cp. to Section 1) is also used to study protein conformations [123,124].

Basic phenomena related to morphology [125,126], tribology [127], magnetism [128–130], electricity [131,132], topology [133], and optics [134] are also investigated by SPM. In addition, specific works on the basic SPM mechanisms are the contrast due to Van der Waals interactions [135] and metrological measurements [136,137]. Differently from the recent past [138], nanofabrication with SPM has been replaced by ion milling [139,140], whereas nanomanipulation has been abandoned, except for a recent example [141].

Several groups have been capable of extending the use of some SPM modes producing home-made tips, such as in the case of TERS [142], force spectroscopy [143,144], scanning impedance microscopy [145], and STM [146], or using additional electronics to apply mechanical/electrical stimuli [147,148]. The research works, performed in the last five years by $\approx 50\%$ of the SPM groups, have been produced within (or have been permitted to obtain) projects financed by both Europe and Italy (see Figure 5).

![Figure 5. Number of research projects financed versus the type of funding, obtained in the last five years from the European Commission (EU), Minister of University and Research (PRIN and FIRB), Italian government (PON, POR-FESR, PNNR), institutions (University and CNR), and other.](image-url)
2.4. Education and Dissemination

About 50% of the SPM groups are involved in education and dissemination activities. Currently, higher education does not propose any bachelor or degree course devoted specifically to SPM. It is usually integrated into general courses on nanoscience, nanotechnology, interfaces, low-dimensional systems, and nanobiotechnology. The situation is similar in PhD courses except for a course at the University of Bologna, “Probing material properties at the nano-scale with atomic force microscopy”, and one in Milano, “Measuring nanoscale interactions in biological systems and data analysis”.

Education for pupils of the primary and secondary schools consists of single lectures held at the pupils’ school or, sometimes, at the researcher’s institution in combination with laboratory visits and educational courses such as “Alternanza scuola-lavoro” and “Percorsi per le Competenze Trasversali e l’Orientamento”. Concerning these activities, CNR has realized two projects of science education for pupils, “Il linguaggio della Ricerca” [149] and SperimEstate [150], which were then extended into national and regional projects, respectively. They have successfully implemented science education courses in addition to educational institutions, thus extending training plans proposed to pupils.

Dissemination is mainly concentrated on big events such as “Researchers Night” (www.nottedeiricercatori.it), “Trieste NEXT” (www.triestenext.it), “Festival della Scienza” (www.festivalscienza.it), and “Pint of Science” (https://pintofscience.it/). Within these contexts, the art exhibition “Art at the nanoscale” [151] and the macro-AFM for hands-on activities (see Figure 6) are two unusual ways to perform dissemination.

![Figure 6. (a) Hands-on activity with macro-AFM. (b) A tip-sample system and (c) a detail of a 1 cent coin showing the door of Castel del Monte.](image)

3. History and Present of SPM SME in Italy

Besides a history of the early experiments followed by the realization of the first SPM prototypes in Italy, it is necessary now to narrate the history of the SMEs working with SPM. We shall concentrate on two enterprises that in the 1990s have extensively worked in this field. The first one was named Technobiochip that later evolved into Elbatech (www.elbatech.com). The second one, still active, is A.P.E. Research (www.aperesearch.com).

4. Technobiochip and Elbatech

During the 1990’s, the Italian Ministry of University and Research funded about twenty new realities throughout the less-industrialized regions of Italy. These were Consortia of Universities and private companies to unite researchers and industrial technicians. Among these Consortia, one was based on Elba Island, and it was called “Technobiochip”. One of its “industrial research topics” was SPM. At the time, the first commercial STM (1988, Omicron [152]) and AFM (1989, Digital Instruments—DI [153]) had just appeared on the market. The Technobiochip SPM Lab was founded in 1990 and headed by Marco Sartore, with the collaboration of the Pisa group led by Ascoli and Frediani.
One of the aims of the laboratory was the realization of an AFM for visualizing living cells in a liquid environment, a quite common feature in the majority of present AFMs, but a challenge at that time. The result was finally achieved by designing a smart head geometry with multiple laser beam reflections to allow top inspection of the sample with an optical microscope and a suitable cantilever holder for liquids. The Technobiochip SPM Lab was a multi-disciplinary place, as during those years nothing was yet existing off-the-shelf. A group was dedicated to the realization of the SPM controller electronics [154], another to the mechanics [155], a third to the acquisition software development, one to the image processing software [156], and the last to the experiments with the biology team [157–160].

In the same years, the general secretary Mikhail Gorbachev of the Soviet Union started his famous “glasnost” policy reform, and Perestroika was born, spreading Soviet scientists around the world. Because Technobiochip was directly dependent on the Italian Ministry, several of them were assigned there and started working in conjunction with the Italians. A Soviet company was moving its earlier steps, with the name “MDT”, and its founder Sergey Bykov came to Elba with their first STM prototype to develop it further together, starting a long-term collaboration. This productive effort was later exploited in a joint venture attempt between MDT and AsseZ, a Technobiochip company based in Padova, Italy. This joint venture was conceived to transform MDT from a group of researchers into a commercial company. Indeed, MDT changed to NT-MDT, and moved back to Russia where they had all the internal support and capabilities to become what they are today, with a wide range of multi-sensing systems on the market.

When Technobiochip ended, a group of scientists in 1999 established a new company called Elbatech. Elbatech started with enthusiasm from the earlier results and was able to produce and place on the market an “open” SPM controller named SPMagic, despite the company being supported neither by privates nor by the government. This controller was intended for use with any measuring head, the idea coming from the evidence that many universities and research centers in the world were designing and realizing their STM/AFM heads, customized and optimized to fit the experiments they were devoted to.

Today, Elbatech still supports AFM/STM laboratories designing custom electronics [161,162]. Recently, the IoT (Internet of Things) technology offered a chance to design a very compact SPM controller called NanoUp in conjunction with the National Council of Research of Genova [163]. However, Elbatech is mostly specialized in a strong by-product deriving from the SPM field (and still serving also that field): the high-voltage power amplifiers. Originally utilized to actuate XYZ motion of piezoelectric actuators in SPM heads, the HV amplifiers are today used in many fields, from optics to particle physics. Elbatech’s skill in this field is today acknowledged after the realization of multi-channel systems with extremely low-noise and stability performances.

5. A.P.E. Research

The company was founded in 1996 by five young researchers and one expert in finance. The skills in SPM were mainly brought by Stefano Prato who was introduced to STM during his period at RICE University Houston and then in the Tecnologie Avanzate di Superfici e Catalisi (TASC) laboratories of the Italian National Institute for Condensed Matter Physics (INFM) with Prof. Silvio Modesti. The company has been located in AREA Science Park of Trieste since 1997. In 1999, it became part of the spin-off program of INFM and is now on the list of spin-offs of the CNR.

In 1999, A.P.E. Research produced the first model of SNOM; in 2003, the A100-AFM was introduced, which then evolved our days into the A-100 PLUS AFM; in 2008, an STM for solid–liquid interface was produced; from 2018, the company produces scanning micro-Raman (also integrated with SPM systems).

In 1999, A.P.E. Research founded its own research laboratory named BioNanoLab (in the campus of Area Science Park), the first Italian private research center for the promotion of nanobiology and nanomedicine. Bionanolab is very attractive as a training facility and during recent years hosted and supported more than 20 students for Master and PhD theses.
During the last 20 years, the laboratory provided services and participated in R&D projects in collaboration with several Italian universities, public laboratories, and private laboratories. It has resulted in several publications, some of which are pioneering applications of SPM (especially SNOM) to biology and biomedicine [164–168].

In recent years, A.P.E. Research has focused its efforts mainly on the development and optimization of new tools for KPFM and PFM [169–172]. The company’s research group has twelve staff members, scientists (senior and young with PhD or degrees in physics, engineering, and biology), and technicians who are involved in R&D activities as well as in production, to share knowledge.

6. Conclusions and Perspectives

The Italian SPM community that results from our dedicated survey includes more than seventy groups and two SPM companies. It is active in many scientific fields and can produce high-quality research published in international journals. SPM groups are diffused all over Italy, although most of them are concentrated close to large academic and research institutions and, notably, in conjunction with locations where the Italian SPM pioneers have operated. All the SPM groups show a wide competence in the use of SPM (70% of the available modes), studying matter in original ways following state-of-the-art approaches. This approach allows them to publish research works in journals ranked within the top 20% class, as proved by an update bibliography of the last five years.

Nowadays, the diffusion of SPM in industry is sporadic, with only two groups involved. This is possibly due to extensive collaborations between the research institutions and industries themselves. This allows them to avoid purchasing an SPM microscope and dedicating their employees. Thus, most of the research performed with SPM is focused on the study of matter and few efforts have been dedicated to the development and understanding of SPM bases, such as contrast mechanisms, differentiation of tip-sample interactions, interaction control, etc. The reason for this might be twofold: (a) to date, SPM is a mature scientific field; (b) the Italian researchers, after an initial period, are inclined to combine instrumental developments and scientific research in a unique work.

This is different to what often happens in electron microscopy, both scanning and transmission, where the microscope bases and/or technical advancements are published prior in reviews specialized only in microscopy (see Table 1, such reviews are also appropriate for SPM), and then the developments are applied to studies of matter. A similar approach, however, might be useful to promote stronger collaborations between the Italian researchers and SPM companies, like what occurs in other countries such as Spain (e.g., Prof. Ricardo Garcia) and Austria (e.g., Prof. Peter Hinterdorfer).

To conclude, the authors hope that this manuscript might help the formation of a more structured SPM network in Italy, possibly through the Italian Society for Microscopical Sciences (S.I.S.M., www.sism.it). Moreover, since our survey could be partial, we are open to any “Addenda and Corrigenda” from other SPM pioneers or groups to broaden both the knowledge of historical events and update the number of SPM working groups.

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