International Journal of Thermophysics

Spontaneous polarization in bio-organic materials studied by Scanning PyroElectric Microscope (SPEM) and Second Harmonic Generation Microscope (SHGM)

--Manuscript Draft--

Manuscript Number:	IJOT-D-13-00513R1
Full Title:	Spontaneous polarization in bio-organic materials studied by Scanning PyroElectric Microscope (SPEM) and Second Harmonic Generation Microscope (SHGM)
Article Type:	S.I. : ICPPP17
Keywords:	imaging; keratin; molecular crystals; non-linear optical effect; polarization; pyroelectricity; second harmonic generation
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Abstract:	<p>Bio-organic materials such as bones, teeth and tendon generally show nonlinear optical, pyro- and piezoelectric properties, implying a permanent polarization, the presence of which can be rationalized by describing the growth of the sample and the creation of a polar axis according to Markov's theory of stochastic processes. Two proven, versatile techniques for probing spontaneous polarization distributions in solids are Scanning PyroElectric Microscopy (SPEM) and Second Harmonic Generation Microscopy (SHGM). We demonstrate the combination of pyroelectric scanning with SHG-microscopy in a single experimental setup leading to complementary pyroelectric and nonlinear optical data, providing us with a more complete image of the polarization in organic materials. Crystals consisting of the known polar and hyperpolarizable material, CNS (4-chloro-4'-nitrostilbene) are used as a reference sample, to verify the functionality of the setup, with both SPEM and SHGM images revealing the same polarization domain information. In contrast, feline and human nails exhibit a pyroelectric response, but a second harmonic response is absent for both keratin containing materials, implying that there may be symmetry-allowed SHG, but with very inefficient second harmonophores. This new approach to polarity detection provides additional information on the polar and hyperpolar nature in a variety of (bio) materials.</p>
Response to Reviewers:	<p>Q1: Can you measure the values of this (electrical polarization) vector through the SPEM and/or SHGM techniques? If so, the magnitude of this vector should be reported, and if not, the statements involving the word "measurement" should be rewritten in terms of "detection".</p> <p>A1: It is possible to estimate the polarization from the pyroelectric or SHG response, but due to uncertainties in thermal amplitude, laser spotsize and peak laser intensity, this was not attempted. All pyroelectric results are displayed in the current measured and the text is rewritten to 'detection of polarization' and 'measurement of pyroelectric current'.</p>

Q2: What is the difference between the polarization of feline and human nails? A discussion of how this polarization depends on the size and shape of the samples is required.

A2: There appears to be a factor 2 response difference between the samples. A small discussion has been added.

Q3: A clear discussion of the advantages and disadvantages of both the SPEM and SHGM techniques is highly required.

A3: A subsection "3.3 First conclusions on SPEM and SHGM combination microscope" has been added.

Q4: The current writing of the manuscript makes difficult to read and understand it. The English grammar of the whole manuscript needs to be deeply revised and improved. Furthermore, the revision of the references according to the journal format is required.

A4: The text has been edited to increase readability. References have been edited.

List of revisions to “Spontaneous polarization in bio-organic materials studied by Scanning PyroElectric Microscope (SPEM) and Second Harmonic Generation Microscope (SHGM)”

Page 2, Abstract:

Line 4: An introduction to polarity in organic matter is added

Page 4, 1 Introduction:

Line 10: Examples of SPEM and SHGM measurements are added

Page 4, 2.1 Scanning PyroElectric Microscopy (SPEM)

Line 61: Link between polarization and surface charges is included as introduction, as well as a reference to the secondary pyroelectric effect, which contributes a piezoelectric current.

Page 5, 2.1 Scanning PyroElectric Microscopy (SPEM)

Line 33: Text is added to explain that only one component of the three-dimensional pyroelectric current is measured with the setup and that all results are displayed in the current measured, rather than the pyroelectric coefficient.

Page 5, 2.2 Second Harmonic Generation Microscopy (SHGM)

Line 61: Text is added: “In this paper, though the magnitude of polarization via SHG was not determined, the SHG image was used to verify the polarization distribution.”

Page 7, 3.1 CNS

Line 17: After “An example for the latter can be seen in the lower half of the CNS crystal” following text is added “(Indicated by a red circle in both SHGM and SPEM image in figure 2.b and 2.c)” Figure 2 is adjusted correspondingly

Page 7, 3.2 Feline and Human nails

Line 44: After additional measurements on splinters of feline nail and pieces cut to a sharp edge/point, the large piezoelectric contribution could not be recreated (Images of measurement included).

The text was rewritten to include three plausible causes for the large increase in pyroelectric current:

- 1) A large macroscopic polarization, leading to a large pyroelectric coefficient
- 2) A change in thermal amplitude as the boundary conditions change near the tip
- 3) Increased mechanical stress: though not recreated, it cannot be excluded.

Line 53: The pyroelectric response is now displayed on a linear scale, capped at 150 fA and portrayed as a surface plot, to better visualize the result.

Page 8, 3.2 Feline and Human nails

Line 2: Reference to B.R. Masters et al is added, where the lack of SHG in keratin is also noted and found to be counterintuitive.

Line 10: Small discussion is added on the difference in pyroelectric response and a possible explanation based in the materials stiffness is included.

Line 10: An explanation is given for the lack of SHG, based on the optical properties of keratin.

Line 10: A new subsection is added “3.3 First conclusions on SPEM and SHGM combination microscope” where the advantages and disadvantages of the two techniques are described, based on experience gained with these measurements.

Page 8, Acknowledgements

Line 40: Financial support is added.

Page 9, References

Reference list was expanded.

Page 10, Figure Captions

Line 33: Caption is rewritten to match text.

Furthermore, spelling and grammar was checked, text was rewritten where necessary to increase readability.

**Spontaneous Polarization in Bio-organic Materials Studied by
Scanning PyroElectric Microscopy (SPEM) and Second
Harmonic Generation Microscopy (SHGM)**

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Abstract

Bio-organic materials such as bones, teeth, and tendon generally show nonlinear optical [1], pyro- and piezoelectric [2] properties, implying a permanent polarization, the presence of which can be rationalized by describing the growth of the sample and the creation of a polar axis according to Markovs theory of stochastic processes [3,4]. Two proven, versatile techniques for probing spontaneous polarization distributions in solids are scanning pyroelectric microscopy (SPEM) and second harmonic generation microscopy (SHGM). The combination of pyroelectric scanning with SHG-microscopy in a single experimental setup leading to complementary pyroelectric and nonlinear optical data is demonstrated, providing us with a more complete image of the polarization in organic materials. Crystals consisting of a known polar and hyperpolarizable material, CNS (4-chloro-4'-nitrostilbene) are used as a reference sample, to verify the functionality of the setup, with both SPEM and SHGM images revealing the same polarization domain information. In contrast, feline and human nails exhibit a pyroelectric response, but a second harmonic response is absent for both keratin containing materials, implying that there may be symmetry-allowed SHG, but with very inefficient second harmonophores. This new approach to polarity detection provides additional information on the polar and hyperpolar nature in a variety of (bio) materials.

Keywords: Imaging, Keratin, Molecular crystals, Nonlinear optical effect, Polarization, Pyroelectricity, Second harmonic generation

1 Introduction

Scanning pyroelectric microscopy (SPEM) and second harmonic generation microscopy (SHGM) are two versatile techniques for probing spontaneous polarization distributions in solids, either via the pyroelectric effect or second harmonic generation, respectively. Since both effects are sensitive to polarity on the molecular (in case of molecular crystals) and macroscopic scale, yet rely on very different mechanisms; they are complementary approaches that can be used to reveal spatial information about polarity as demonstrated for molecular crystals [5], organic inclusion compounds [6], zeolite-based host-guest materials [7], and ferroelectrics [8,9].

Pyroelectric imaging (SPEM) utilizes photothermal waves to locally perturb the sample temperature, resulting in changes in the (local) polarization and inducing a pyroelectric response proportional to the local polarization [10]. SHGM, on the other hand, applies femtosecond laser pulses to generate frequency doubled light due to the 2nd-order non-linear component of the dielectric susceptibility tensor [11]. However, since effective SHG generation requires a non-centrosymmetric arrangement of the hyperpolarizable units, the SHG response is a manifestation of the polarity as well [12]. Consequently, both techniques are deemed complementary as they provide information on the polarization distribution at different length- and timescales, by changing the macroscopic polarization and hyperpolarizing the individual molecules, respectively.

This paper deals with the implementation and verification of a new experimental approach that combines pyroelectric scanning with SHG microscopy in a single experimental setup. By sharing some optical components and the sample, this setup allows – to the best knowledge of the authors – for the first time a simultaneous characterization of polar materials by SPEM and SHGM. Validation on a known polar and hyperpolarizable molecular crystal (CNS) and first results on feline and human nails are presented and discussed in view of promising applications of the approach for studying complex and/or bio-organic systems.

2 Experimental Techniques

2.1 Scanning PyroElectric Microscopy (SPEM)

The polarization of a polar material can be described via an equivalent surface charge density, the spatial derivative of the local polarization. In equilibrium, the total electrical field of these surface charges is compensated by free, real charges in the sample. Assuming a non-zero pyroelectric coefficient, a temperature change of the sample will change the polarization, and thus change the surface charges, which can in turn be measured as a discharge current or surface potential. As most pyroelectric materials are also piezoelectric, the strain resulting from thermal expansion and contraction from the photothermal wave can induce a piezoelectric response. This secondary pyroelectric effect is generally small, typically not exceeding 10 percent of the magnitude of the primary effect [13].

In this experiment, a sinusoidal modulated laser diode was used as the heat source with partial absorption of the incident laser intensity causing periodical changes of temperature in the sample volume.

Translation of the laser beam over the sample surface allows for a two-dimensional image, with spatial resolution arising from the local character of the thermal wave, determined by the laser spot size and heat spread due to thermal diffusion, according to the expression for the thermal diffusion length μ [14,15], with α the thermal diffusivity and ω the laser intensity modulation frequency:

$$\mu = \left(\frac{2\alpha}{\omega} \right)^{1/2}$$

The wavelength of the laser is not critical as long as the laser light is well absorbed and the photons are not too energetic to induce a photoelectric current. Typically, a 100 mW red (650 nm) laser diode is used [16].

By virtue of a parallel-plate electrode model, any local change in the polarization results in a current response that can be related to a specific spatial region of the sample under investigation [10,17,18]. To increase the signal-to-noise-ratio for weakly polar samples, micrometer-spaced interdigitated electrodes (IDE) were used rather than parallel-plate electrodes, as recently proposed [16]. Since the local polarization is a three-dimensional vector, the pyroelectric current is also a three-component vector. Using the IDEs, only

one component of polarization is detected, namely, the projection perpendicular to the IDE electrodes.

Due to uncertainties in optical absorbance and laser spot size, it is difficult to obtain exact values for the local pyroelectric coefficient; therefore, all results are given in terms of the measured pyroelectric current.

The full measurement process is controlled by a MATLAB-based data acquisition system.

2.2 Second Harmonic Generation Microscopy (SHGM)

Second harmonic generation (SHG) is the nonlinear optical process of frequency doubling in materials lacking inversion symmetry. These materials exhibit a nonlinear polarization response to the electric field of a high-intensity, fundamental laser beam. This second-order nonlinearity gives rise to a polarization wave, which oscillates with twice the fundamental frequency, radiating an electromagnetic field [11].

Since nonlinear optical effects require very high electric fields, SHG experiments typically involve pulsed laser sources with pulse lengths on the order of 10 fs to 100 fs, at a repetition rate up to 80 MHz. A two-dimensional SHG intensity map is finally obtained either by scanning or using a wide-field setup. From the intensity of the SHG light, the magnitude and eventually the direction [19] of the polarization can be deduced [20]. In this paper, although the magnitude of polarization via SHG was not determined, the SHG image was used to verify the polarization distribution.

2.3 Simultaneous Pyroelectric and SHG-Imaging

By integrating the optically based pyroelectric microscope into an existing second harmonic microscope, complementary results on the nature of polarization can be obtained. The similarities and differences observed between the macroscopic alteration of the polarization and the microscopic hyperpolarization allows for a separation between contributions from pyroelectricity, photoelectricity, and third- or higher-order nonlinear optical effects for SHG.

A photograph of the integrated setup is included in Fig. 1: the 800 nm pulsed laser and accompanying pump laser are shown at the back of the breadboard and are guided to the sample holder, located inside the motorized rotary and translation stage. The 650 nm

continuous wave laser is located above the sample holder and is combined with the 800 nm laser beam through a dichroic mirror that reflects the 800 nm beam and transmits the 650 nm beam. In this setup the laser beams are kept stationary and the sample is translated to obtain a scanning image.

2.4 Sample Preparation

Prior to scanning, biological samples are, if needed, briefly rinsed in water and allowed to dry, while crystal samples are used as is. Samples are positioned onto a glass plate with aluminum interdigitated electrodes, which in turn is placed inside the translation stage.

3 Results and Discussion

3.1 CNS

CNS, or (Trans)-4-chloro-4'-nitrostilbene, forms needle-like crystals of which the average crystal structure belongs to the monoclinic space group symmetry $P2_1$, and thus are lacking an inversion center [20]. Crystals of CNS are built-up of macroscopic polar domains, the polarity of which is due to a subtle, microscopic imbalance of molecular orientation arising during crystal growth [10]. This polar, non-centrosymmetric crystal is used as a reference material for comparing the pyroelectric and second harmonic response. CNS has a well defined dipole moment that extends from the chlorine atom to the NO_2 group and is nearly in parallel to the molecule axis. Moreover, the dipole axis coincides very well with the major hyperpolarizability axis [5].

Representative pyroelectric and SHG results on a couple of differently oriented CNS single crystals are given in Fig. 2. Here, the aluminum electrodes of the IDE structure appear as black stripes in the SHGM results and as zero-current gaps in the SPEM results, since they are neither pyroelectric nor hyperpolarizable. Another feature of the IDEs is the artifact that the electrical field direction changes its sign periodically, leading to a corresponding alternation of the pyroelectric current in case of a unipolar sample. The true polarization distribution can be obtained by applying a two-dimensional (2D) Fourier transform [16] or by looking for deviations from the periodicity pattern. An example for the latter can be seen in the lower half of the CNS crystal (indicated by a red circle in both SHGM and SPEM images in Fig. 2b and c,

where two negative current regions follow each other, indicating a bipolar crystal. The same change in polarity can also be, indirectly, observed in the SHGM result, where there is a gap in the SHG intensity in the transversal crystal direction, indicating a region where the SHG photons interfere destructively due to local symmetry.

3.2 Feline and Human Nails

Feline and human nail samples, both composed of alpha-helices and beta-sheets of keratin [21], were scanned by the SPEM and SHGM setup in search of a possible polarity caused by macroscopic orientation of the keratin. Figure 3 displays the pyroelectric response of a domesticated housecat nail; due to the IDE orientation, only the longitudinal component of the response is measured. At the base of the feline nail, we observe a uniform pyroelectric response with a mean amplitude of 20 fA, implying a homogeneous polarization in the longitudinal (growth) direction. However, near the sharp tip of the claw, the current response increases by several orders of magnitude to a maximum of 15 pA.

There are three plausible contributions to this phenomenon. The most apparent is a strong polarization in the tip, caused by dense parallel packing of the keratin helices as a consequence of a cat's habit to sharpen its claws; the accompanying friction, heat, and shear force could align the polar keratin along the longitudinal axis, increasing the macroscopic polarization. A second contribution lies in the adiabatic heating of the sample. At the base of the nail, the sample is thermally thick, but at the tip, where the sample is thermally thin, the low thermal conductivity of air means the thermal wave has a larger amplitude, resulting in a larger current contribution. Lastly, it is prudent to consider a piezoelectric contribution of keratin [22] to the pyroelectric response since local thermal modulation will cause thermal expansion and contraction at the same frequency. At the base of the feline nail, this local volumetric change is constrained, while at the tip, the secondary pyroelectric effect is less constrained, allowing for a large piezoelectric contribution. Cutting a sharp edge at the base of the feline nail did not recreate this effect, nor was it observed in splinters of the base, leading the authors to believe that the large pyroelectric response is not an artifact of the sample geometry, but rather caused by a large, local spontaneous polarization at the tip.

1 The pyroelectric response implies a macroscopic, non-centrosymmetric orientation of
2 the polar, helical keratin proteins. Such a structure is also expected to be SHG-active;
3 however, no indication of any second harmonic response was found, in line with the
4 findings of Masters et al. [1], to quote “Since keratin fibers consists almost entirely of
5 alpha helices, it is not clear why it is not an effective second order harmonophore.”
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8 Similar results are found for human nails, of which a SPEM image is shown in Fig. 4,
9 suggesting a uniform polarization in the longitudinal (or growth) direction of the nail.
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11 The lack of a sharp tip in the human nail means there is no region with a large increase
12 of either polarization, thermal amplitude, or mechanical stress, and thus the current
13 response is more uniform than that of the feline nail, except for a few spots where wear
14 and tear are visible, characterized by a weaker response. Although with a mean response
15 of 50 fA, it is more than twice the response at the base of the feline nail. Similar to the
16 feline nail, again no SHG response was observed.
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19 During sample preparation, it was observed that the base of the feline sample was more
20 flexible than the human nail sample, alluding that the stiffer, human sample has a denser
21 packing or stronger macroscopic alignment of the keratin proteins than the feline base,
22 which translates to a difference in pyroelectric response.
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24
25 The presence of a pyroelectric response combined with the absence of SHG activity
26 seem counterintuitive, since many mechanisms can lead to a second harmonic
27 response [23] and only a non-centrosymmetric polar structure leads to a pyroelectric
28 response [14]. The answer most likely lies in the geometry and optical properties of the
29 sample. Both the feline and human nails are between 400 μm and 500 μm thick and
30 both keratin-based structures are good absorbers for 400 nm and shorter
31 wavelengths [24], the wavelength at which the SHG response occurs. This implies that
32 the chiral and non-centrosymmetric character of keratin can give rise to SHG activity;
33 however, the intensity generated is too little to pass through both the rest of the 500 μm
34 thick sample and wavelength filter and still be detected by the photomultiplier. In this
35 particular example, the SPEM setup has a higher sensitivity, as the detection of current
36 has little interference from optical properties.
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3.3 First Conclusions on SPEM and SHGM Combination Microscope

Based on the results presented, preliminary conclusions on the combination setup can be inferred. Since many mechanisms, besides the presence of polarity, can lead to SHG generation [23], the pyroelectric scan can help clarify the origin of the SHG response. If both the SHG- and pyroelectric distribution match, the sample is most likely non-centrosymmetric polar. However, if SHG is present, but a pyroelectric signal is absent, the SHG is likely to originate from third- or higher-order effects. In the interesting case where pyroelectricity is observed, but an SHG signal cannot be obtained, the sample geometry or optical properties inhibit the detection of frequency doubled light. Concerning these optical properties; a higher optical absorbance of the sample can result in a weaker SHG signal, but will actually increase the pyroelectric current detectable, due to the corresponding increase in the photothermal amplitude.

The main advantage of SHGM versus SPEM, for polarization domain mapping, is the time required to obtain a response map, where SHGM needs but a few minutes to obtain a 500-by-500 pixel image, SPEM requires nearly 8 h for a 100-by-100 pixel result.

The advantages of the SPEM setup are the higher flexibility in sample geometry and optical properties, and the ability to narrow current contributions to pyro- and piezoelectric effects and to directly detect the sign of polarization. The latter can also be achieved via phase-sensitive SHG, but requires a reference crystal and prefers thin samples [24].

4 Conclusions

By simultaneously measuring the pyroelectric and second harmonic activity, we are presenting a new technique to study the polarization of organic materials. In the case of CNS crystals, the two techniques reveal the same information. However, in the two keratin-based materials, the unfavorable geometry and optical properties of the samples inhibit SHG measurements and polarity is only detected with the photothermal SPEM setup. The complementary nature of these two different detection mechanisms leads to interesting insights and a better understanding of the polar and hyperpolar nature in a variety of (bio)materials.

Acknowledgments

The authors thank Jürg Hulliger (Department of Chemistry & Biochemistry at the University of Berne) and his research group for providing the CNS crystals.

M. W. and T. P. acknowledge financial support from the Research Council of the KU Leuven, Project OT/11/065.

References

1. B.R. Masters, P.T.C. So, *Handbook of Biomedical Nonlinear Optical Microscopy* (Oxford University Press, New York, 2008), pp. 385-386, ISBN 9780195162608
2. E. Fukada, I. Yasuda, J. Phys. Soc. Jap. **12**, 1158 (1957)
3. J. Hulliger, Biophys. J. **84**, 3501 (2003)
4. A. Batagiannis, M. Wübbenhorst, J. Hulliger, Curr. Opin. Solid State Mater. Sci. **17**, 107 (2010)
5. C. Gervais, T. Wust, N. Behrnd, M. Wübbenhorst, J. Hulliger. Chem. Mater. **17**, 85 (2005)
6. S.W. Roth, P.J. Langley, A. Quintel, M. Wübbenhorst, P. Rechsteiner, P. Rogin, O. König, J. Hulliger, Adv. Mater. **10**, 1543 (1998)
7. J. Caro, F. Marlow, M. Wübbenhorst, Adv. Mater. **6**, 413 (1994)
8. M. Wübbenhorst, T. Petzsche, C. Ruscher, Ferroelectrics **81**, 373 (1988)
9. T.V. Murzina, Yu. G. Fokin, T.V. Misuryaev, O.A. Aktispetrov, S.P. Palto, S.G. Yudin, "Two-dimensional Ferroelectricity Probed by SHG in Ferroelectric LB Monolayers," in *Conference on Lasers and Electro-Optics: CLEO* (Long Beach, CA, 2002), p. 317
10. A. Quintel, J. Hulliger, M. Wübbenhorst, J. Phys. Chem. B **102**, 4277 (1998)
11. P.A. Franken, A.E. Hill, C.W. Peters, G. Weinreich, Phys. Rev. Lett. **7**, 118 (1961)
12. M.A. Van Der Veen, B.F. Sels, D.E. De Vos, T. Verbiest, J. Am. Chem. Soc. **132**, 6630 (2010)
13. A. Batagiannis, Ph.D. Thesis (Universität Bern, Switzerland, 2009)
14. A. Batagiannis, M. Wübbenhorst, J. Hulliger, Curr. Opin. Solid State Mater. Sci. **14**, 107 (2010)
15. T. Putzeys, Master's Thesis (KU Leuven, Belgium, 2011)
16. T. Putzeys, M. Wübbenhorst, IEEE Trans. Dielectr. Electr. Insul. **19**, 1186 (2012)

17. A. Petre, D. Marty-Dessus, L. Berquez, J.L. Franceschi, S. Agnel, P. Notingher, J. Castellon, N. Didon, A. Toureille, "FLIMM and TSM: Two Thermal Methods for Space Charges Investigation," Solid Dielectrics, in *Proceedings of the 2004 IEEE International Conference on Solid Dielectrics*, Toulouse, France, vol. 1 (2004), pp. 213-216
18. G.J. Klap, M. Wübbenhorst, J.C. Jansen, H. van Koningsveld, H. van Bekkum, J. van Turnhout, Chem. Mater. **11**, 3497 (1999)
19. P. Rechsteiner, J. Hulliger, M. Florsheimer, Chem. Mater. **12**, 3296 (2000)
20. N.-R. Behrnd, G. Labat, P. Venugoplan, J. Hulliger, H.-B. Burgi, Crys. Growth Des. **10**, 52 (2010)
21. L. Kreplak, J. Doucet, P. Dumas, F. Briki, Biophys. J. **87**, 640 (2004)
22. H. Maeda, Biophys. J. **56**, 861 (1989)
23. R.W. Boyd, *Nonlinear Optics*, 2nd edition (Academic Press, Orlando, FL, 2003), ISBN 9780121216825
24. K.P. Nielsen, L. Zhao, J.J. Stamnes, K. Stamnes, J. Moan, "The Optics of Human Skin: Aspects for Human Health," in *Proceedings from the Symposium on Solar Radiation and Human Health*, ed. by E. Bjertness (The Norwegian Academy of Science and Letters, Oslo, 2008), pp. 35-46
25. S. Kluge, I. Dohnke, F. Buddea, J. Hulliger, Cryst. Eng. Comm. **5**, 67 (2003)

Figure Captions

Fig. 1 Photograph of the setup showing the pulsed 800 nm laser in the back, on the optical table, and the 650 nm continuous wave laser mounted directly above the sample translation stage

Fig. 2 Comparison: SPEM and SHGM measurement on CNS crystals, positioned on a system of interdigitated electrodes. Due to a slight microscopic imbalance in orientation, there is a macroscopic alignment of the polar and hyperpolarizable axis in the longitudinal direction of the crystals. (a) An optical photograph is given, to be compared with (b) the SHG response, (c) the real part of the pyroelectric response, in fA, and (d) the amplitude. The IDE causes an alternation in pyroelectric current sign, except where the polarity of the crystal changes sign, an example is the lower half of the lower left crystal, indicated by a red circle. This change in polarization is also visible as a gap in the SHG image

Fig. 3 Pyroelectric current response map of a small cat talon (in fA), with the corresponding optical photograph as an inset. The amplitude range is capped at 100 fA, to visualize both the 20 fA bulk response and up to 15 pA response at the tip. Plausible explanations are the presence of a large mechanical stress in the tip, contributing a significant piezoelectric effect, a large local polarization caused by dense parallel packing of keratin, or a change in thermal amplitude as the boundary conditions in the sample change near the tip

Fig. 4 Pyroelectric current response map of a human nail clipping (in fA), with the corresponding region and microscope picture as inset. A longitudinal polarization in the growth direction is detected. The pyroelectric response is uniform, except for a few spots where wear and tear of the nail has reduced the thickness of the sample, reducing the pyroelectric response

Fig. 1

Fig. 2

Fig. 3

Fig. 4