On the actual spatial resolution of Brillouin Imaging

S. Caponi\textsuperscript{1}, D. Fioretto\textsuperscript{2}, M. Mattarelli\textsuperscript{2*}

\textsuperscript{1}CNR-IOM - Istituto Officina dei Materiali - Research Unit in Perugia, c/o Dep. of Physics and Geology, University of Perugia, Via A. Pascoli, I-06123 Perugia, Italy

\textsuperscript{2}Dep. of Physics and Geology, University of Perugia, Via A. Pascoli, I-06123 Perugia, Italy

*corresponding author: maurizio.mattarelli@unipg.it

Abstract

The comprehension of the behavior of complex heterogeneous materials requires the adoption of imaging techniques to analyze spatially resolved properties. With plenty of prospects in biophysical and biomedical fields, Brillouin imaging is an emerging technique able to generate maps with mechanical contrast at microscales. As in any imaging technique a key parameter is the spatial resolution, which depends both on the control of the probe and on the physical mechanism of interaction with the sample. In particular, in Brillouin Spectroscopy the optical scattering volume is only one of the aspects that determine the spatial resolution, the other being the propagation of the detected vibrational modes, an aspect often overlooked in literature. Here, for the first time, by means of theoretical considerations and new experimental data, the importance of the propagating nature of the phonons is clearly disclosed. The unique features of our experimental set-up allow the simultaneous measure of Brillouin and Raman spectra, probing propagating and localized vibrations, respectively. Varying the optical conditions and testing systems with different phonon propagation length provides striking evidence that in Brillouin imaging the phonons contribution to the resolution, inversely related with the optical focusing, can be dominant. Surprisingly, the resolution can even deteriorate when decreasing the scattering volume.
In a peculiar manifestation of Heisenberg Uncertainty Principle, when focusing by a lens a collimated light beam, the increased spatial definition is obtained at the expense of the definition in the light wavevector. This is usually of no concern in many microscopy techniques where the signal is scarcely affected by the direction of the exciting light, and even near-field excitation, where the light wavevector is practically undefined, can be effectively exploited. However, this is not the case for Brillouin Spectroscopy (BS). As a matter of fact, in its standard configuration, BS is sensitive to the spontaneous thermally activated acoustic vibrations which are present in any material. Between all these vibrations, called “phonons”, the scattering configuration (wavevector and polarization of the exciting and collected light) selects by conservation laws, a subset of phonons whose properties are reflected on the measured spectrum. In particular, in the usual description of a visco-elastic medium, Brillouin spectra present a peak whose position, \( \omega_B \), and width, \( 2\Gamma \), are proportional to the sound velocity \( V \) and longitudinal viscosity \( \eta_L \), respectively: \( V=\omega_B/q; \eta_L/\rho = 2\Gamma/q^2 \), \( q \) being the exchanged wavevector and \( \rho \) the density of the medium (details can be found in recent reviews, e.g. 3–5).

The need for the mechanical characterization of elastically heterogeneous materials, such as the biological ones, has recently led to the development of confocal Brillouin microscopes, where the use of high NA objectives allows the reduction of the scattering volume down to the sub-micrometer scale. However, the wide range of involved scattering wave-vectors, which is associated with this optical configuration, has important implications on Brillouin measurements. In particular, for the first time in this letter, we provide evidence of the effect of the \( q \) angular spread on the spatial resolution of the Brillouin imaging, discussing the limit to which it can be enhanced in practice. The present study has a fundamental as well as a technological relevance giving insights for the correct data interpretation and for the future instrumentation design.

Figure 1 presents the calculated magnitude and angular distribution of the wavevectors collected by a micro-Brillouin experiment in two different “backscattering” configurations corresponding to the use of
air objectives with low (0.3) and high (0.9) NA. In the computation, a homogeneous incoming beam covering all of the objective aperture and an isotropic scattered radiation were considered. Figure 1 b), shows that the NA increase leads to a significant spread in $|\mathbf{q}|$: using the 0.9 NA objective in backscattering configuration, the average value of $|\mathbf{q}|$ is about 10% lower than the nominal one. Moreover, as reported in Fig.1 c), NA hugely affects the angular distribution of the collected wavevectors: the deviation $\theta$ from the optical axis reaches an average value of $8.5^\circ$ for the low NA objective and $32^\circ$ for the high NA one.

Figure 1. $\mathbf{q}$ distribution of the collected signal for a Brillouin scattering process in two different “backscattering” configurations exemplified in the upper cartoons for two NAs. The red and black curves represent the $\mathbf{q}$ distribution of the modulus, b), and of the angular deviation from the optical axis, c), for $\text{NA}=0.3$ and $\text{NA}=0.9$, respectively.
The $q$-distribution has two remarkable effects on the Brillouin spectra: on one hand, it lowers the spectral definition, due to the fact that both the frequency and the width of the peak depend on $|q|^{12-14}$. In this case, a straightforward, though delicate, deconvolution procedure is needed to extract the relevant material parameters $^{15}$. On the other hand, the $q$-distribution implies also a distribution in the investigated directions, which in NA=0.9 optics can reach up to $65^\circ$ from the optical axis. This is not relevant in homogeneous isotropic samples, but it becomes a fundamental issue when analyzing heterogeneous samples, i.e. just the ones where Brillouin imaging is of interest. In fact, given the propagating nature of the vibrations detected by BS, the $q$ angular distribution translates into a lateral broadening of the investigated region. The extent of this effect can be a priori estimated considering the mean free path of acoustic phonons, $L_2$. (see Figure 2)

*Figure 2. Schematic micro-Brillouin scattering diagram. $L_1$, $L_2$ and $L_3$ denote the relevant length scales for this interaction, given by phonon wavelength, mean free path and scattering volume, respectively. The acoustic volume, in red, takes into account the broadening induced by the phonon propagation.*
In principle, in a lossless homogeneous material such length is infinite. In real systems $L_2$ gets finite because of dissipative mechanisms, be they due to static reasons, such as elastic heterogeneities on the scale of the phonon wavelength ($L_1=2\pi/q\sim200$ nm for 532 nm lasers in backscattering) or dynamic ones such as relaxation processes$^{16-19}$. Note that both kind of mechanisms are present in biological materials and affect their Brillouin spectra$^{4,20-22}$. However, BS itself provides a quite effective way to measure $L_2$. In fact, the peak width is inversely proportional to the phonon life time, and knowing the acoustic velocity from the Brillouin peak position it is possible to obtain $L_2$ from the quality factor of the peak: $L_2 = \omega_B / 2\Gamma L_1$\textsuperscript{23}. Just as an example, in pure water the propagation length $L_2$ of the phonons detected in backscattering configuration using a 532 nm laser beam is $\sim2.1\ \mu m$.

A rough evaluation of the consequences of phonon propagation on micro-Brillouin spatial resolution can be obtained considering the broadening of the lateral resolution as arising from the average projection of $L_2$ by the off-axis angle, $<\sin(\theta)\ L_2>$. The actual Brillouin resolution will be then given by a spatial convolution of this contribution with the scattering volume individuated by the optical system. Always referring to water, the lateral broadening due to acoustic phonon propagation is 0.3 and 1.1 $\mu m$ for the two angular distributions considered in Figure 1 c). It is worth to notice that for the two considered NA, the Abbe diffraction limit is 0.7 and 0.2 $\mu m$, respectively, so that the acoustic term for high NA optics would be the dominant one.

In order to substantiate these considerations with experimental data, we measured the evolution of Brillouin spectra across a sharp interface between different materials. The change in intensity of the Brillouin peaks allows reconstructing the Edge Spread Function (ESF), from which we can obtain the spatial resolution of the technique by the width of the Point Spread Function (PSF)$^{15,24}$.

In particular, we analyzed two different interfaces: (i) between Polyethylene (PET) and Glycerol and (ii) between a sodium-silicate glass and water (Fig.3a). The choice of these two couples of materials was dictated by their relatively small refractive index mismatch and, more important, by their very
different acoustic mismatch, so providing a direct verification of the effect of the phonon propagation on the resolution. In fact, we can expect that acoustic propagation is stopped (reflected) at the glass-water interface, while it is scarcely hindered crossing the PET-Glycerol one, characterized by a lower acoustic mismatch. As the determination of the spatial resolution of the measurement can be affected by the sharpness of the interface, we carefully prepared the sample with optically polished interfaces, parallel to the optical axis. Moreover, in order to discriminate between optical and acoustic contributions to the ESF, we exploited the unique characteristics of our Raman-Brillouin combined set-up \(^{11}\) and simultaneously measured the ESF by both Brillouin and Raman Spectra (RS). Raman spectroscopy in fact, being related to the detection of non propagating optical phonons, can represent a good proxy for the optical contribution to the ESF as detected by the transition from one to the other material within the scattering volume. Two different objectives were used in order to change the excitation/collection configuration: a 20X NA 0.42 and a 60X water immersion objective with 1.2 NA. Note that the angular spreads of these objectives, when evaluated in the materials under consideration, match those of the objectives NA=0.3 and NA=0.9 in air, previously analyzed in Figure 1.

Several linear scans through the interface were probed for the two samples. As an example, in Figure 3 b) we show the evolution of Raman spectra acquired in the PET-Glycerol sample (Figure 3 a) and Figure 3 c) reports the Brillouin spectrum acquired at the interface together with its fitting curve. In detail, the Brillouin spectra were analyzed using the sum of two DHO peaks as fit function, keeping fixed the position and width of the peaks as determined from measurements on pure materials, and allowing only the intensity to vary. For the Raman spectra, few well-separated characteristic lines were used to estimate the materials presence.
Figure 3- a) Experimental configuration; b) Evolution of the Raman spectra thought the PET-Glycerol interface. The peaks located at ~500 cm\(^{-1}\) and ~3050 cm\(^{-1}\) are respectively selected to evaluate the glycerol and the PET presence in the scattering volume; c) Typical Brillouin spectrum at the PET-Glycerol interface. The experimental points were fitted by two DHO functions.

The intensity of the peaks, both Brillouin and Raman, changes abruptly across the interface. Figure 4 a) and Figure 4 b) report the ESF obtained for the two investigated interfaces with the 60X objective. The ESF were fitted by an error function, having 2\(\sigma\) as width of the corresponding Gaussian PSF (see the inset of the figures).
Figure 4 c) d) report the measured widths for the two couples of materials and for the two optical setups obtained from Brillouin, $2\sigma_B$, and Raman, $2\sigma_R$, measurements. The width of the laser spot, $2\sigma_L$, as measured when focusing on an optically polished silicon wafer, is also indicated$^{15}$. The Raman width $2\sigma_R$ appears equal or slightly larger than the spot width, taking also into account the small effect at these length scales due to the rugosity of the sample surfaces. What is striking is the different behavior when changing the numerical aperture of the objective: while $2\sigma_R$ follows strictly the laser spot size, the behavior of the Brillouin width $2\sigma_B$ strongly depends on the couple of interfaced materials, increasing for the PET and Glycerol interface and decreasing for the water glass one. This astonishing effect is the consequence of the propagating nature of the phonons. In fact, at the glass-water interface, no phonon present in one material can propagate into the other one, as the difference in the Brillouin peak frequency ~25GHz is much larger than peak linewidths. On the contrary, the Brillouin peaks of PET and Glycerol have a large region of superposition (Fig.2c) indicating that the phonons can propagate across the interface. Therefore, even when the scattering volume is completely inside one component, the collective vibrational modes living across the interface can still be detected. Only in this latter configuration, the width of the PSF takes also into account the effect of the phonon propagation and consequently it is able to provide the actual spatial resolution of Brillouin Imaging. A counterintuitive outcome of this analysis is the observation that, when reducing the scattering volume (passing from low to high NA), the lateral resolution in the measure of the elastic properties becomes worse (1.4 µm against 1.0 µm).
Figure 4 Rescaled ESF measured for Raman (red) e Brillouin (black) experiments through the glass-water interface a) and through the PET-Glycerol interface b). The dots are the experimental data and the lines are the fits. In the insets: Derivative of the fitting functions providing the PSF of the experimental setup. c) Width of the PSF (Brillouin, Raman, Si Reflectance indicated by black, red and blue dots, respectively) obtained from the two interfaces (Glass –Water and PET/Glycerol) in the two optical configurations (20X NA 0.3, 60X NA 1.2))

A further confirm can be obtained by the quantitative comparison between the experimental results and the previously reported calculations. In fact, the Brillouin resolution depends on the convolution of the scattering volume with the acoustic contribution, $2\sigma_E$, only due to the acoustic phonon propagation. Using $2\sigma_R$ as lateral dimension of the scattering volume and considering as a first approximation that the contributions to the spatial resolution add in quadrature (exact for Gaussian distributions), the lateral acoustic contribution can be found as $2\sigma_E = 2\sqrt{(\sigma_B)^2 - (\sigma_R)^2}$. While this value is always utterly
negligible for the glass-water interface, for the PET-Glycerol one studied by 1.2 NA objective $2\sigma_E$ results
$(1.2 \pm 0.4) \mu m$, indeed the dominating term in the final resolution. This value should be compared with
the average radial projection of the mean free path $L_2$ along the angle $\theta$. $L_2$ estimated from the Brillouin
spectra as $L_2=\omega_B/2\Gamma L_1$ is $\sim 2.5 \mu m$ for PET and $\sim 1.6 \mu m$ for Glycerol, while the average $\sin (\theta)$ from the
angular $q$ distribution reported in Figure 1 c) is $\sim 0.52$. The calculated value for $2\sigma_E$ thus falls in the range
$0.8 – 1.3 \mu m$, in good agreement with the experimental value.

The present analysis is of significant relevance for a critical assessment of a number of recent
works$^{15,21,24–27}$, where submicrometric features are discriminated on the basis of Brillouin signature. While
the comparison with different optical techniques indicates that the observed elastic modulation is indeed
related to structural changes, the measured values of the elastic constant should be considered as spatial
averages, whose extent depends on both NA of the optics and on the propagation length of phonons, in
turn depending on the investigated material. As a consequence, the optimization of the resolution could
even require the adoption of lower NA optics. For example, in a recent work$^{24}$, a reduction in the
mechanical contrast was observed passing from NA= 0.85 to NA=1.28. Our present findings suggest an
influence of the different lateral resolutions due to the acoustic contribution, which, for the two objectives
used in ref$^{24}$ is expected to be $\sim 0.3$ and $\sim 0.6 \mu m$, respectively.

Finally, as hinted by the measurements in the water-glass interface, it is important to highlight the
strong influence of interfaces on the acoustic contribution. Taking it to the limit, in the case of complete
phonon confinement, as occurs in nanoparticles, nanotubes or in films, the acoustic vibrations are standing
waves in the confined directions. In this condition, the spatial resolution in the measurements of the elastic
properties can be even higher than the optical resolution, also reaching the nanoscale$^{28–32}$.

In conclusion, we have shown that the ultimate resolution in the mechanical properties attainable by
Brillouin imaging is limited not by the optical system but by the propagation properties of the investigated
phonons. As a matter of fact, in Brillouin spectroscopy phonons and not light are the probe of the
mechanical properties of the material. The light (according to the scattering geometry) only allows selecting the range of phonons by which we interrogate the material. The selection is in frequency by the $|q|$ spread, in direction by the angle distribution of $q$, and spatially by the scattering volume. However, the spatial resolution depends not only on the dimension of the scattering volume, but also on the mean free path $L_2$ of the acoustic phonons, which can extend over a much larger space. As a consequence, the actual elastic resolution can indeed get worse when increasing the optical resolution (the NA). Note that this is an inescapable constraint arising from the very fundamental reason that the detected excitations are travelling acoustic waves.
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