A Rather Random Microscope

A new recipe from the microscopy world. Take a standard microscope, add a pinch of noise. Mix it all together and voilà: an image with improved resolution is served!

Recall, if you will, the static, and the resulting crackling noises of an old fashioned radio, when the dial was not tuned in properly. Would you call that a pleasant experience? When transmitting or receiving signals, noise is typically considered as a nuisance to be minimized — at least, this is one of the first lessons learned in engineering studies. However, more and more new research on noisy systems is shaking the ground on which this engineering pillar stands. This is also the case for the work by Anne Sentenac and colleagues at the Université Aix-Marseille in France, which shows how adding visual noise to the illumination of a standard microscope can significantly improve its resolution.

The resolution of a microscope mainly depends on how easy it is to see two close-by objects as separate. In the scientific parlance, this information is quantified by the *spatial frequency content* of an image: lower frequencies represent global information about shapes, orientations and proportions, while higher frequencies give information about the finer details of the sample. In a standard microscope, where the sample is uniformly illuminated and the resolution is typically around 300 nanometers, these details are lost: the frequency content of the final image, in fact, is limited by the characteristics of the detection objective and the color of the collected light.

How can the resolution of a microscope be improved? One way is to *force* the microscope image to record ever higher spatial frequencies, as, for example, is the case in Structured Illumination Microscopy. Imagine that the sample is a simple grid of lines separated by a distance — or *period* — that is barely visible with a microscope. When you superimpose an illumination grid, whose period is also barely visible, onto the sample grid, then you end up observing yet another grid, a coarse third one, with a now clearly visible period. At this point, as Sentenac points out, "if you know the period of the illumination grid, you are able to retrieve numerically (or analogically) the sample grid from this image," and this would constitute information to which you did not have access before. And she adds, "the resolution improvement stems from the frequency mixing between the sample and the inhomogeneous illumination," thus adding higher spatial frequencies — and more detail — to the recorded image. This phenomenon is known as the Moire effect, and it is at the very basis of Structured Illumination Microscopy.

"Our technique is related to Structured Illumination Microscopy," Sentenac says. "The interest of our algorithm lays in the fact that, now, even if you do not know the illumination grid, you are still able to reconstruct the sample grid." First, their approach records many images of the same sample under different random illumination patterns, such as the speckles obtained by moving a piece of paper through a laser beam. Then, thanks to the randomness of the illumination, a reconstruction algorithm is able to simultaneously retrieve an image of the sample together with the illumination patterns from the data stack, with a resolution that is twice as good as that of a standard microscope. Rainer Heinztmann, at the Friedrich-Schiller-Universität, Jena (Germany) agrees that the main novelty of this work is that Sentenac and colleagues' "reconstruction algorithm can simultaneously reconstruct the illumination field along with the unknown sample, and it requires only very few assumptions, such as the illumination patterns approximately summing to a flat illumination and the sample

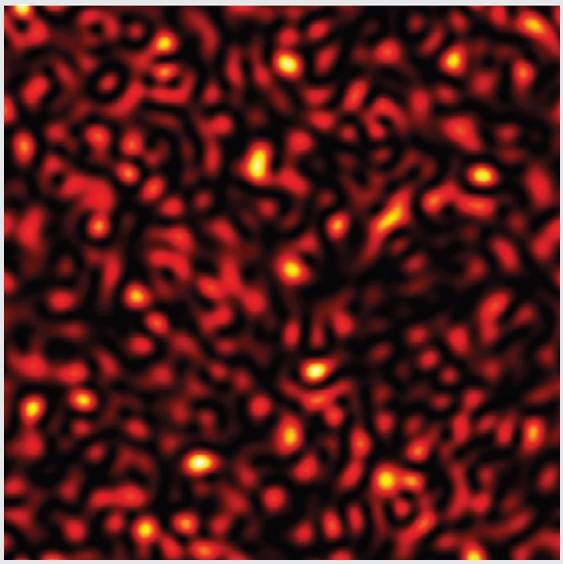

Figure 1: A random illumination pattern. The image shows a random intensity light pattern, or speckle pattern. A similar pattern can be generated by shining a laser through an opaque medium, such as a piece of paper or plastic.

remaining identical throughout the acquisition of the series of images."

"The interest of our technique," Sentenac adds, "as compared to other microscopy approaches using inhomogeneous illumination, such as confocal microscopy or structured illumination microscopy, is that it does not require any control of the illumination patterns. Hence, the experimental set-up is dramatically simplified. Our research," therefore, "stresses the interest of using sophisticated reconstruction algorithms in microscopy, and I hope that people from the signal processing community will consider this new challenge and try to ameliorate our reconstruction method."

What this study leaves clear is that noise is not always a drawback; the use scientists and engineers make of it can really make the difference between an unwanted nuisance and a propitious helping hand.

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E. Mudry, K. Belkebir, J. Girard, J. Savatier, E. Le Moal, C. Nicoletti, M. Allain & A. Sentenac, **Structured illumination microscopy using unknown speckle patterns**, Nature Photonics **6**, 312-315 (2012).