STORM, first presented in 2006 by Xiaowei Zhuang and her group at Harvard University.[4] (For a similar, simultaneously developed method, described in PHYSICS TODAY, December 2014, page 18, and illustrated in figure 2 of that story, Eric Betzig received a share of the 2014 Nobel Prize in Chemistry.)

In STORM, as in conventional fluorescence microscopy, fluorescent molecules, or fluorophores, are attached to structures of interest. The fluorophores are optically excited with a laser, and the emitted light is captured with a camera. Each fluorescing molecule appears as a diffuse blob hundreds of nanometers wide, with that size governed by the wavelength of the emitted light, not the size of the molecule. But the centers of those blobs—the molecules' actual positions—can be located with much greater precision, provided the blobs don't overlap. Zhuang and company devised a system of fluorophores that could be switched repeatedly between fluorescent and nonfluorescent states. They could then switch off all but a few randomly chosen fluorophores, measure their positions with subwavelength precision, and repeat the process to build up a composite image with 20-nm resolution.

Although that's not quite good enough to resolve individual nucleosomes, it would, Cosma and Lakadamyali anticipated, allow a good look at their arrangement—including the 30-nm fiber, if it existed. But when they captured their first images of somatic cell nuclei, they didn't see continuous fibers at all. Instead, the images were blotchy and heterogeneous, with a lot of dark space, as shown in figure 2a. When they zoomed in as far as they could, they found the blotches to be composed of discrete bright spots tens of nanometers across.

At first, the researchers weren't sure what to make of their images. But sophisticated numerical analysis ultimately showed that the blotches could be further subdivided into nanodomains, shown in different colors in figure 2b, each representing a clutch of nucleosomes that were close in space. They couldn't directly count the number of nucleosomes in each clutch, but they could count the number of times a nucleosome from the clutch showed up in one of the component images that made up the STORM composite. Calibrating with images of structures containing known numbers of nucleosomes, they found that their somatic cells had a median of eight nucleosomes per clutch.

In stem cells, the researchers again saw evidence of clutches, but with median sizes between two and four. Somatic cells treated with TSA had similarly small clutches, as shown in figure 2c. And neural precursor cells—at an intermediate stage between stem cells and fully differentiated nerve cells—had clutches with a median of six nucleosomes.

Labeling the nucleosome proteins with fluorescent dyes required fixing the cells—that is, killing them—with an alcohol solution. To check whether their results were affected by that, or by any other aspect of their sample preparation, the researchers performed a series of control experiments, including one with living cells genetically modified to produce nucleosomes with fluorescent proteins already attached. None of the controls revealed anything different: The clutch structure showed up in all of them.

What's next

The biological function of the clutches is not yet known. But they do indicate—as Carlo Manzo, an author on the paper, puts it—that "heterogeneity is much more important than we previously thought," and the Barcelona researchers are just beginning to understand the full extent to which that's true. For example, the segments of nucleosome-free DNA that connect the clutches are invisible in the STORM images, so the researchers don't yet know just how the clutches arrange into larger structures. And they haven't yet attempted to find out whether the clutch size varies between genes or between chromosomes.

The researchers plan to further explore how chromatin structure changes as stem cells differentiate and somatic cells are reprogrammed—by getting images from key points during the processes, or even by watching them in real time. They're also interested in whether there's any structural difference between chromatin in healthy somatic cells and in cancer cells.

"Superresolution microscopy is still quite a new field," says Lakadamyali, "and the emphasis so far has been on technique development and proof-of-concept experiments. Only recently has there been a shift toward looking at real biological problems, and it's very exciting."

Johanna Miller

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A stellar source of lithium is caught in the act

To explain the observed abundance of the light metallic element, astrophysical modelers have concluded that much of it was produced in stars. But direct evidence has been lacking until now.

Of all the elements of the periodic table, lithium has perhaps the most complicated and mysterious origins, in part because it can be created in so many ways. Its dominant isotope, ^{7}Li, is one of the few species (along with deuterium, helium-3, and helium-4) to have been produced in Big Bang nucleosynthesis. Cosmic-ray spallation—nuclear fission initiated when energetic protons and other particles collide with interstellar carbon, nitrogen, and oxygen—is a significant source of Li, beryllium, and boron. And Li can be both produced and destroyed in stars, although questions remain about the nature of the stellar sources and how much Li they produce. In the effort to derive a clear picture of the chemical evolution of our galaxy by combining observations of elemental abundances with their known mechanisms of creation and destruction, the so-called "lithium problem" has been an especially tough one to crack.

Now Akito Tajitsu, of the National Astronomical Observatory of Japan, and his colleagues have found direct evidence of Li production in a stellar system—specifically, a classical nova: a thermonuclear explosion on a white dwarf that blows away the dwarf's outer

$$^3\mathrm{He} + {}^4\mathrm{He} \rightarrow {}^7\mathrm{Be} \rightarrow {}^7\mathrm{Li}$$
$$^7\mathrm{Li} + \mathrm{p} \rightarrow 2\,{}^4\mathrm{He}$$
$$^7\mathrm{Be} + \mathrm{p} \rightarrow {}^8\mathrm{B} \rightarrow \ldots$$

Figure 1. Lithium in stars is produced by the mechanism shown in red. The first step requires high temperature. But if the beryllium-7 and lithium-7 remain at high temperature, they're destroyed via the reactions shown in blue.

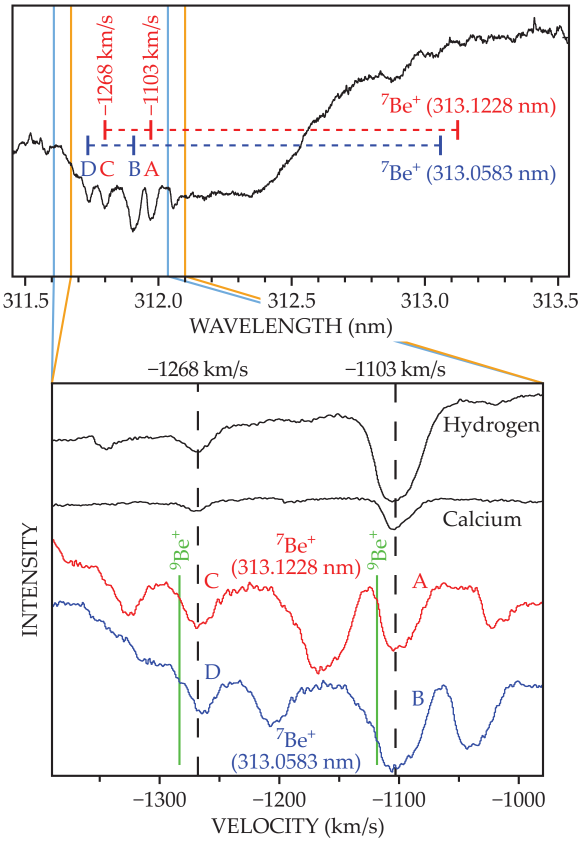

Figure 2. The spectrum of a 2013 nova contains four unusual absorption features in the near UV, labeled A, B, C, and D in the top panel. Nearby spectral lines of hydrogen and calcium show that the spectrum consists of two separate blueshifted components. Accounting for the blueshifts allows the four new features to be confidently assigned to two resonances of ^{7}Be^{+}. The green lines in the bottom panel show where the equivalent resonances of ^{9}Be^{+} would fall. (Adapted from ref. 1.)

layers.[1] The researchers used NAOJ's Subaru Telescope, located on Mauna Kea in Hawaii, to monitor the absorption spectrum of the cast-off layers. They found a set of absorption lines that pointed to the presence of ^{7}Be, a radioisotope and progenitor of ^{7}Li. The short-lived ^{7}Be must have been produced in the nova explosion.

"This is actually the first time that the formation of lithium has been caught 'in the act,'" says Francesca D'Antona of the Rome Observatory. If the observation can be repeated for other novae, it will help to determine how much of today's Li could have been produced in such explosions—a question that modelers of Li evolution would dearly like to see answered.

The lithium problem

The best data on Li abundances come from meteorites, which record the composition of our solar system just before the Sun started burning. Observations of stars of various ages provide information about other eras. Together, they suggest that Li abundance has been on the rise for most of our galaxy's history; the source of the increase lacks a clear explanation.[2] And of the solar system's Li, at most 30% is accounted for by Big Bang nucleosynthesis and cosmic-ray spallation together. Core-collapse supernovae can explain at most another 20%, which leaves at least 50% for all other stellar sources.[3]

The mechanism by which Li is synthesized in stars, shown in red in figure 1, seems almost contradictory. First, ^{3}He and ^{4}He combine to make ^{7}Be, which decays with a half-life of about 50 days into ^{7}Li. The He fusion step requires high temperatures—but if the nascent ^{7}Be and ^{7}Li remain at those temperatures, they're quickly destroyed via the reactions shown in blue.

A stellar Li factory therefore needs a way to create ^{7}Be and quickly cool it. Novae could easily qualify: The explosion itself is hot, and the cast-off layers are speedily ejected into interstellar space. A few classes of giant stars have also been identified as possible candidates. But models of each of them show that they collectively fall well short of producing enough Li to explain the solar system's present abundance.[3]

Blueshifted beryllium

Tajitsu and company have been using the Subaru Telescope and its High Dispersion Spectrograph (HDS) to study novae for several years. In mid-August 2013, when a nova appeared that was especially bright across a broad continuum of optical wavelengths, they hoped they could glean new information about the material expelled in the explosion. Light from the still-exploding star passes through the cast-off layers and thus captures their absorption spectra. Because the absorbing material is moving rapidly away from the star toward us, the spectrum is strongly blueshifted.

The researchers thought they might see absorption lines that they could attribute to Li itself, but they didn't—perhaps because all the Li produced in the nova was ionized. Instead, they saw a group of curious near-UV features, labeled A, B, C, and D in figure 2. The features were identifiable for only a few days, between six and seven weeks after the explosion reached its peak: Before that, they were too saturated for their exact wavelengths to be determined, and after that, they had faded away to nothing.

Nearby known spectral lines, from hydrogen and calcium, showed that the spectrum actually had two distinct velocity components, one at 1103 km/s and one at 1268 km/s. Undoing that double blueshift revealed that the four unusual lines exactly matched a pair of resonances in Be^{+}. Thanks to the HDS's high resolution, the researchers could conclusively assign the absorption features to radioactive ^{7}Be^{+} rather than stable ^{9}Be^{+}, even though their resonance wavelengths differ by less than 0.02 nm.

Comparing the Be^{+} lines to the analogous Ca^{+} lines allowed Tajitsu and colleagues to form a rough estimate of how much Be was expelled—and thus how much Li was created—in the explosion: perhaps 3 to 10 times as much as models predict. But that's just for one nova, whose Li production might be unusually high or low. Many more observations will be needed to determine the average Li yield.

Though our galaxy hosts about 40 novae per year, only about 10 of them are observed, and just a fraction of those are bright enough for observers to have a chance of seeing the Be^{+} lines. But at least now the researchers know what to look for, and they know they have the right instruments. "The beryllium-7 lines are located in the near-UV range," says Tajitsu. "To see them, we need a good location at high altitude, a large-aperture telescope, and a UV-sensitive spectrograph. The combination of the Subaru Telescope and the HDS is perfectly suitable."

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