MICROSCOPY TODAY

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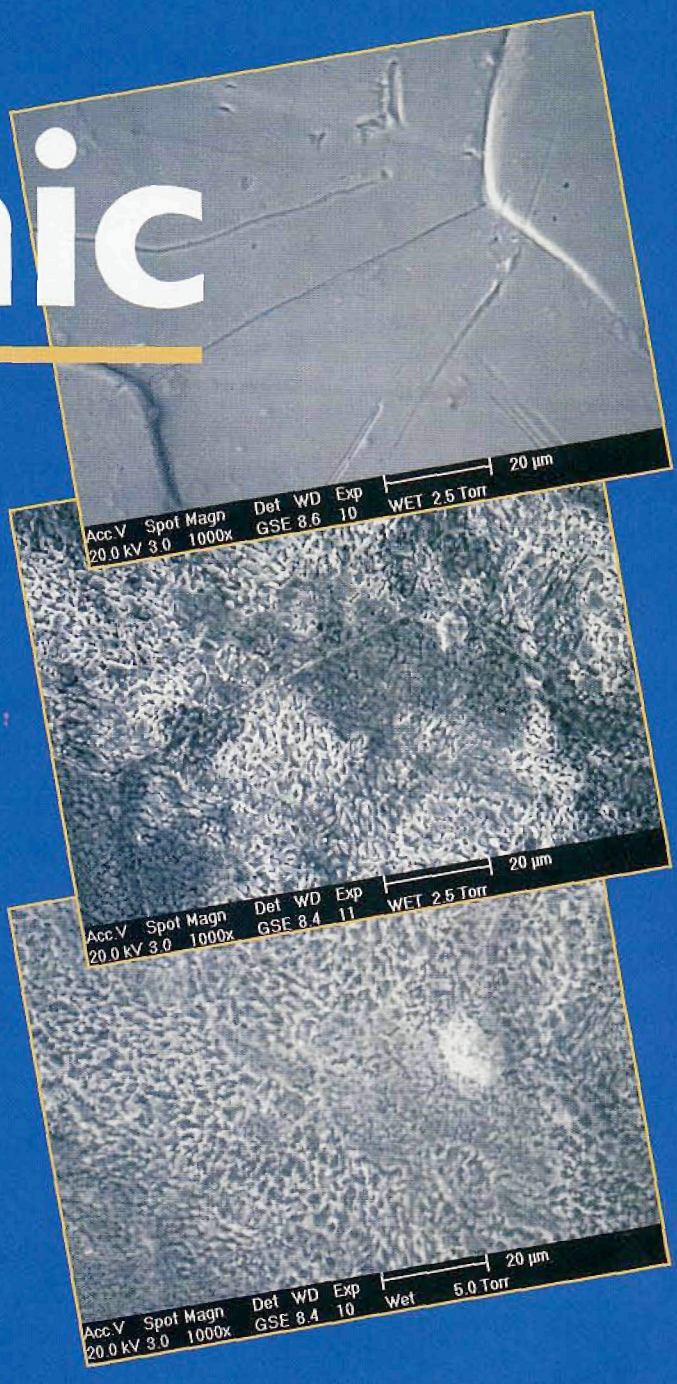

In-situ recrystallisation experiment performed in the ESEM-FEG using the optional heating stage. A titanium stabilised interstitial free steel galvanised with 0.15 wt% Al-Zn coating was heated to 500° C. Recrystallisation of the coating occurred, destroying the previously visible grain boundary structure.

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A TINY TOOLBOX

Stephen W. Carmichael,[1] Mayo Clinic

A recent article by Matthias Rief, Filipp Oesterhelt, Berthold Heymann, and Hermann Gaub[2] concluded with this sentence: "Single molecule force spectroscopy by AFM has proven to be a powerful addition to the nanoscopic piconewton toolbox." Everything about that conclusion is tiny. Clearly, the atomic force microscope (AFM) has given us a tool to examine structure at or near the atomic level. Earlier work from Gaub's laboratory, reviewed in this column[3], demonstrated that the AFM could directly measure the binding force between single molecules of biotin and avidin. This established that the AFM could be used as a tool to measure forces, not just observe structure. Their most recent experiments has added to this tiny toolbox.

Strands of dextran, a long polymer with an average molecular weight of 500,000, were glued to a gold substrate with epoxy-alkanethiols. The glucose units of the dextran were activated with a carboxymethyl group and reacted with streptavidin. It was determined that several streptavidin molecules were chemically bound to each dextran filament. The strands extended into a physiologic buffer so that they formed a "polymer brush." An AFM cantilever with biotin bound to its tip was carefully dipped into this brush until a binding event was registered. Using this "fly fishing mode," Rief *et al.* were able to snag single strands. In cases where multiple strands were bound, they would slowly pull back the tip, rupturing bonds until just one strand was left attached. With a single strand suspended between the tip and the gold substrate, they could manipulate the strand up to the point where the streptavidin-biotin "handle" broke. The characteristics of the tension changes at low forces were entirely consistent with a single dextran filament being stretched.

Molecular dynamics calculations showed that the elasticity at low forces is due to a twisting of C5-C6 bond that results in an elongation of the filament. At forces less than one nanoNewton, this elongation is proportional to the force and gives rise to a segment elasticity of 750 picoNewtons/Ångstrom. Experimental results and theoretical calculations were in agreement.

Measurements at higher forces were complicated by the fact that the streptavidin-biotin bond was not strong enough. A tighter bond, holding above one nanoNewton, was created by adsorbing the carboxymethylated dextran filaments (in the absence of streptavidin) onto an AFM tip that had been made hydrophobic by silanization. At higher tension forces the elasticity showed a discontinuity which molecular dynamics simulations attributed to a snap of the C5-C6 bond angle into a new, stiffer conformation.

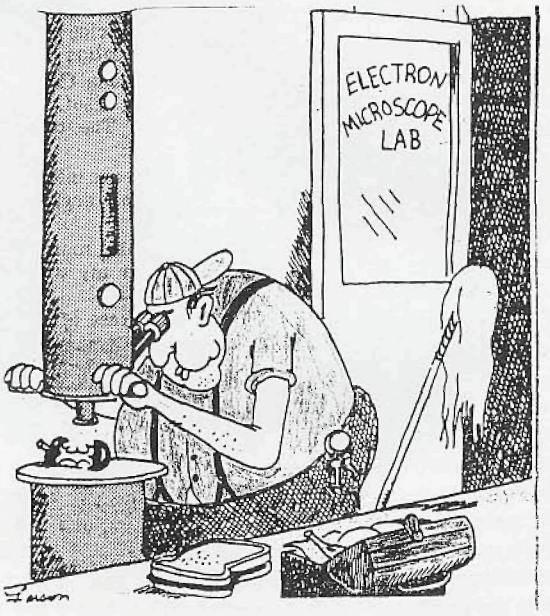
Individual filaments could be manipulated repeatedly, up to the rupture limit of its connection to the AFM tip. This experiment showed that the snap of the bond angle was reversible when the force was reduced again.

Rief *et al.* pointed out that this technique allows for the controlled manipulation of individual molecules can reveal details of the molecular basis of the mechanical properties of polymers that could not be obtained otherwise. The application of this tool to biologically significant properties of polymers remains to be performed, but clearly this is an important addition to a tiny toolbox!

1. The author gratefully acknowledges Matthias Rief for reviewing this article.

2. Rief, M., F. Oesterhelt, B. Heymann, and H.E. Gaub, Single molecule force spectroscopy on polysaccharides by atomic force microscopy, *Science* 275: 1295-1297, 1997.

3. Carmichael, S.W., Microscopy Isn't Just For Microscopists, Anymore. *Microscopy Today* 94-5, 28, 1994.



Front Page Image

GREEN CONE OPSIN EXPRESSION IN GOLDFISH RETINA DETECTED WITH NONRETROACTIVE *IN SITU* HYBRIDIZATION

Riboprobes to the goldfish green opsin visual pigment were hybridized to a tangential retinal cryosection and detected with an alkaline phosphatase color substrate (NBT/BCIP). The triangular purple profiles represent labeled green cones. The round profiles are another cone subtype, which expresses a different (ultraviolet) opsin gene.

For further information, review the article Nonreactive *In Situ* Hybridization on Cryosections on page 16 of this issue.

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Don Grimes, Editor