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# Spatial Control of Crystal Nucleation in Agarose Gel**

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**Abstract**

Spatial and temporal control of crystal nucleation is demonstrated by nonphotochemical laser-induced nucleation of an aqueous agarose gel prepared with supersaturated potassium chloride. The location of nucleation was controlled by means of an optical mask; crystals were only observed in the area exposed to near-infrared laser radiation. The dependence of nucleation on laser power was measured, and the results suggest that the agarose gel reduces the effective supersaturation of the aqueous potassium chloride.

**Main text**

Crystallization has long been a primary tool used by chemists for the production of solid samples, e.g., as a means of purification or for analysis by diffraction methods.[1] By control of various chemical and physical parameters such as temperature, solvent, or co-solutes, chemists have been able to engineer the growth of crystals of many substances.[2,3] Unfortunately, crystal growing can still be considered a black art, since the initial stage of crystallization—nucleation—is a result of random fluctuations, and it is difficult to predict the effects of changing environment on the nucleation and subsequent growth.

Gels present many advantages as media for crystal growth: they are known to promote growth of larger, single crystals with fewer defects and different morphologies.[4,5,6] The suppression of convection currents and sedimentation in a gel produces an environment similar to microgravity, and so gels have become increasingly popular for growing crystals of proteins and other biological macromolecules.[7]

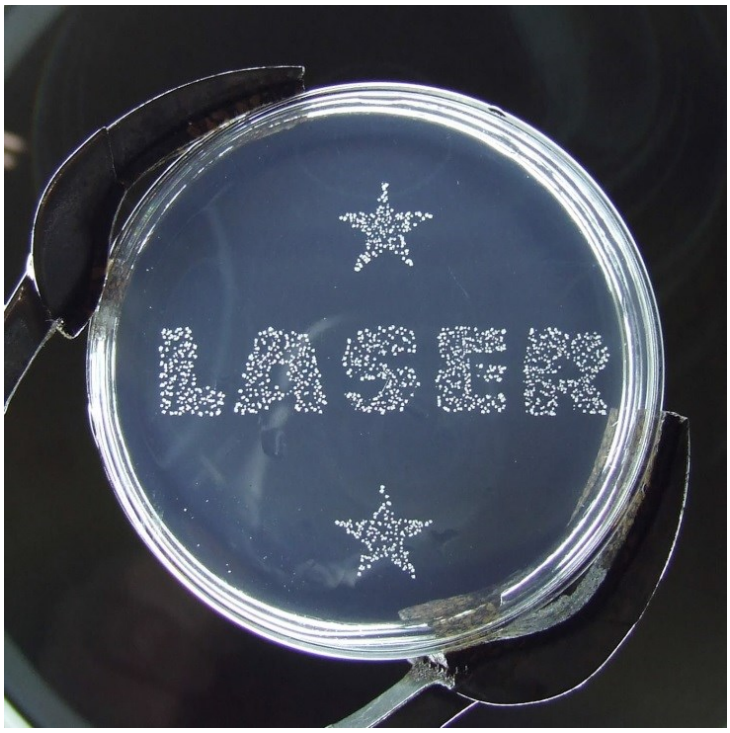
In this Communication, we demonstrate for the first time both spatial and temporal control of crystal nucleation in agarose gels using a recently discovered phenomenon of nonphotochemical laser-induced nucleation (NPLIN).[8] The method employs pulses of laser light at visible or near-infrared wavelengths and with relatively low powers to avoid photochemistry. The *peak* electric field of the light is sufficient to modify the free energy of pre-nucleating clusters, causing them to become supercritical and thereby subject to growth. The NPLIN effect was discovered by Garetz, Myerson, and co-workers,[8] and has been demonstrated so far in aqueous or ethanol solutions for a range of substances such as urea,[8] glycine,[9] hen egg-white lysozme,[10] and KCl.[11] We note that femtosecond laser light has also been used to induce nucleation, although the intensity of such light causes photochemical and photomechanical damage to solute and solvent.[12]

Supersaturated KCl–agarose gels were prepared by dissolving 0.12 – 0.75% w/w powdered agarose (Sigma–Aldrich, Type I, A6013) in supersaturated (106%) aqueous solutions of KCl at 95 °C. The hot

gel was then poured into vessels and allowed to cool to 23 °C and held at this temperature for about 30 minutes prior to shooting with a laser. A $Nd^{3+}$:YAG laser was used, producing pulses of near-infrared light (1064 nm, 6 ns pulse width) in a 5.5 mm diameter beam. The power of the laser pulses was varied by passing the polarized light through a Glan-laser polarizer.

As a qualitative demonstration of NPLIN in gel, we have used a simple optical lithography technique to control location of crystal nucleation. A thin (~2 mm) layer of gel (0.25% w/w) was prepared by pouring into a glass petri dish. After cooling, a cutout mask was placed over the gel which was then subjected to a series of laser pulses that were raster scanned across the area of the mask. The results are shown in Fig. 1, which shows that crystals are only observed where the light could pass through the mask. It is well known that mechanical shock can cause nucleation in supersaturated solutions.[1] By repeat experiments, we verified (to within ~100 μm) that no nucleation occurs beyond the edge of the mask, e.g., due to acoustic shockwaves.

The control we have demonstrated is limited by localization of the laser pulse. The nucleation within the illuminated region is stochastic, however, since it depends on the distribution of pre-nucleating (sub-critical) clusters. We have observed that simple focusing of the beam can lead to damage of the gel at higher laser powers. Two low-power beams can be combined to induce nucleation where they are crossed, opening the route to three-dimensional control of nucleation. The simple technique could be easily developed to a wide range of systems, such as nonaqueous gels or in droplets.[5,13] Recent developments in optical methods could be applied to improve localization within a solid matrix.[14]




← ***Figure 1.*** Photograph showing spatial control of laser-induced nucleation of KCl in an agarose gel. The pattern, showing the word "LASER" with stars above and below, was obtained using an optical mask. No crystals were observed in the regions that were masked. The diameter of the dish was 9 cm (see text for further details).

To measure the dependence of nucleation on laser pulse power, aliquots of gel were dispensed into small vials (~3 $cm^3$), each of which was shot with a single laser pulse. After approximately 10 minutes, the vials were photographed and crystals counted. The results (Fig. 2) show an apparent threshold power (~7 MW $cm^{-2}$) below which no crystals were nucleated, in agreement with previous results in solution.[11] At the present time, there is no clear explanation for a power threshold in solution or in gel, and further experiments are underway to investigate fully this phenomenon. At low agarose concentrations (≤ 0.25% w/w) the number of crystals nucleated increases approximately linearly with peak laser power. A similar dependence of fractions of aqueous samples nucleated versus laser power was reported; in those experiments, a single crystal per vial was produced, on average.[11] The relatively higher number of crystals nucleated in agarose can be explained by an effective lowering of the energetic barrier to nucleation due to the presence of agarose. For *homogeneous* nucleation of a sub-critical cluster of radius *r*, the classical free energy of formation can be written

$$\Delta G_{\text{hom}}(r,E) = 4\pi r^2\gamma - \tfrac{4}{3}\pi r^3(A\ln S + aE^2) \qquad (1)$$

where $E$ is the electric field due to the laser pulse, $\gamma$ is the cluster interfacial tension, $S$ is supersaturation ($S = C/C_{\text{sat}} = 1.06$) and $a = 1.7832 \times 10^{-12}$ F $M^{-1}$ depends on the dielectric permittivities of KCl and water (at 1064 nm).[11] The parameter $A = \rho RT/M = 6.553 \times 10^7$ J $m^{-3}$, where $\rho$ is the mass density and $M$ is the molar mass of KCl; $R$ and $T$ are the molar gas constant and temperature, respectively. We calculate the peak electric field to be $E = 1.787 \times 10^7$ V $m^{-1}$ at 42.38 MW $cm^{-2}$, taking into account slight focusing of the beam in the vial. The electric field interacts with the electronic polarizability of a dielectric cluster; the cluster experiences a reduction in free energy because its dielectric constant $\varepsilon_{\text{KCl}} = 2.1897$ is higher than the surrounding solvent $\varepsilon_{\text{water}} = 1.7535$.[11] For *heterogeneous* nucleation, Eq. (1) is modified to

$$\Delta G_{\text{het}}(r,E) = f(\theta)\Delta G_{\text{hom}}(r,E) \qquad (2)$$

where $f(\theta) = \tfrac{1}{2} - \tfrac{3}{4}\cos\theta + \tfrac{1}{4}\cos^3\theta$, and $\theta$ is the contact angle between cluster and substrate.[1] The barrier to nucleation can only be reduced by the crystal–agarose interaction, i.e., $f(\theta) \le 1$, resulting in more nucleating clusters.

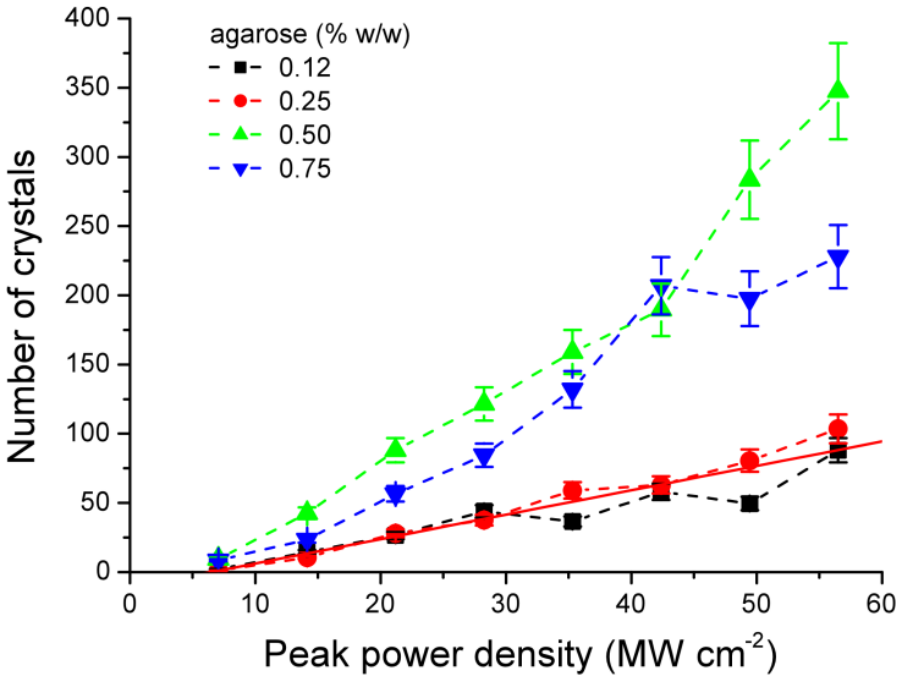



***Figure 2.*** Plot showing the number of crystals counted resulting from a single laser pulse as a function of the peak power of the pulse.

From previous experiments of NPLIN on aqueous KCl solutions we have determined $\gamma = 2.19$ mJ m$^{-2}$.[11] Taking averages over the classical Boltzmann distribution, $\exp(-\Delta G_{het}/k_B T)$, we calculated numbers of clusters that become supercritical as a function of laser power. Note that the calculations have been shifted along the power axis by 7 MW cm$^{-2}$ to reproduce the as-yet unexplained experimental threshold. The model predicts a linear dependence of numbers of nuclei versus peak power density. However, the calculated numbers of crystals are orders of magnitude too high even for the case of complete de-wetting ($\theta = 180°$, $f(\theta) = 1$; equivalent to homogeneous nucleation). This result suggests that our model underestimates $\gamma$ or overestimates $S$, or both. In the absence of independent estimates of $\theta$, we assume homogeneous nucleation ($\theta = 180°$) and explore possible effective parameters, $\gamma^{eff}$ and $S^{eff}$ that fit the data at 0.25% w/w agarose content. Assuming $S = 1.06$, we find that $\gamma^{eff} = 5.13$ mJ m$^{-2}$ gives a straight line fit. Alternatively, fixing $\gamma = 2.19$ mJ m$^{-2}$ the data can be fitted with $S^{eff} = 1.016$. These two different fits are indistinguishable and are shown as the straight line in Fig. 2.

There is no obvious explanation for why $\gamma_{eff}$ would be greater than $\gamma$ in solution. A reduction in the effective supersaturation, defined by $S^{eff} = C^{eff}/C_{sat}^{eff}$, could arise from one or both of the following effects. (i) The saturation concentration of KCl in gel may be higher than that in pure water (

$C_{sat}^{eff} > C_{sat}$ ), due to a stabilization of solvated ions through long-range coulombic interactions with charged groups (e.g., sulfate[15]) on the agarose-gel surface. (ii) The concentration of ions in solution and available for nucleation may be reduced ( $C^{eff} < C$ ), due to a sequestering of ions within the gel matrix.[16]

At higher laser powers, nonlinearity in the numbers of crystals nucleated may indicate the onset of different mechanisms. We also note that the 0.75% w/w agarose produced fewer crystals than 0.5% w/w agarose. This may be attributed to changes in the gel structure at higher agarose concentrations, including changes in pore size and connectivity.[17]

In summary, we have demonstrated remarkable temporal control (on a nanosecond timescale) and spatial control (to within ~100 μm) of crystal nucleation. Further work is currently underway to understand the mechanism for NPLIN and to extend the method to other solute and gel systems. The new method described here shows true potential for use as a routine tool in laboratory growth of crystals, e.g., for growth of single crystals of proteins or other materials for structure analysis.

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