

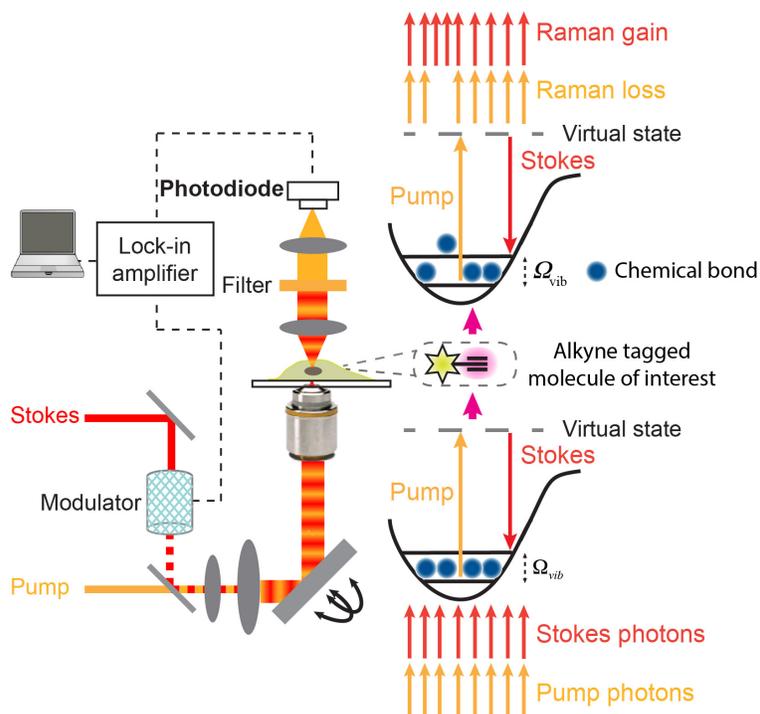
Live-cell imaging of alkyne-tagged small biomolecules by stimulated Raman scattering

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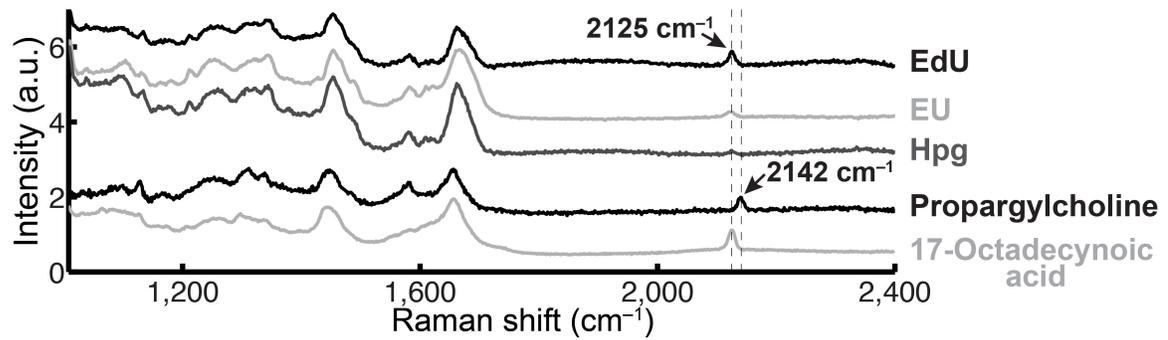
Supplementary Materials

Supplementary Figure 1: SRS microscope apparatus.



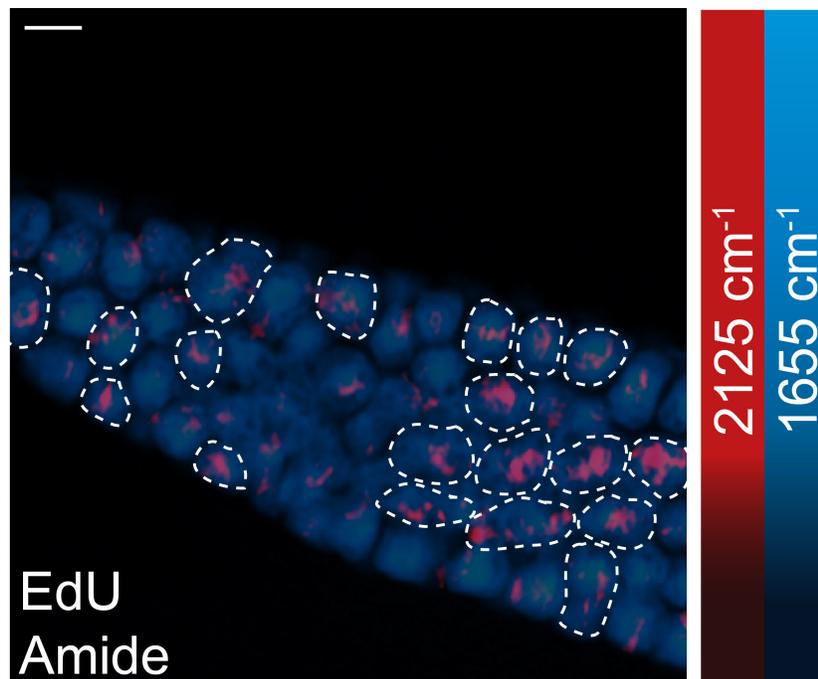
A pump beam (pulsed, pico-second) and an intensity-modulated Stokes beam (pulsed, pico-second) are both temporally and spatially synchronized before focused onto cells that have been metabolically labeled with alkyne-tagged small molecules of interest. When the energy difference between the pump photons and the Stokes photons matches the vibrational frequency (Ω_{vib}) of alkyne bonds, alkyne bonds are efficiently driven from their vibrational ground state to their vibrational excited state, passing through a virtual state. For each excited alkyne bond, a photon in the pump beam is annihilated (Raman loss) and a photon in the Stokes beam is created (Raman gain). The detected intensity changes of the pump laser through a lock-in amplifier targeted at the same frequency as the modulation of Stokes beam serve as the contrast for alkyne distributions.

Supplementary Figure 2: Spontaneous Raman spectra of cells incubated with EdU, EU, Hpg, propargylcholine and 17-octadecynoic acid (17-ODYA).



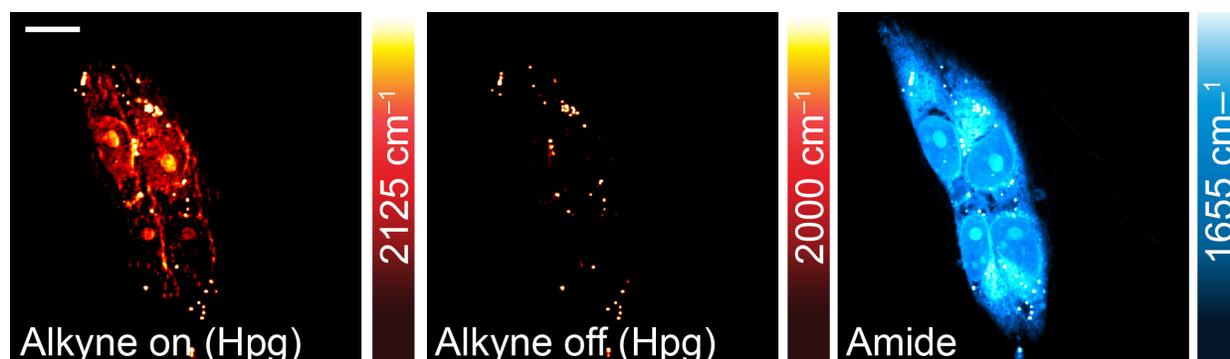
Spontaneous Raman spectra of cells incubated with EdU, EU, Hpg, and 17-octadecynoic acid show an alkyne peak at 2125 cm⁻¹; while spectrum with propargylcholine shows an alkyne peak at 2142 cm⁻¹.

Supplementary Figure 3: SRS imaging of distal mitotic region of *C. elegans* germline incorporated with EdU.



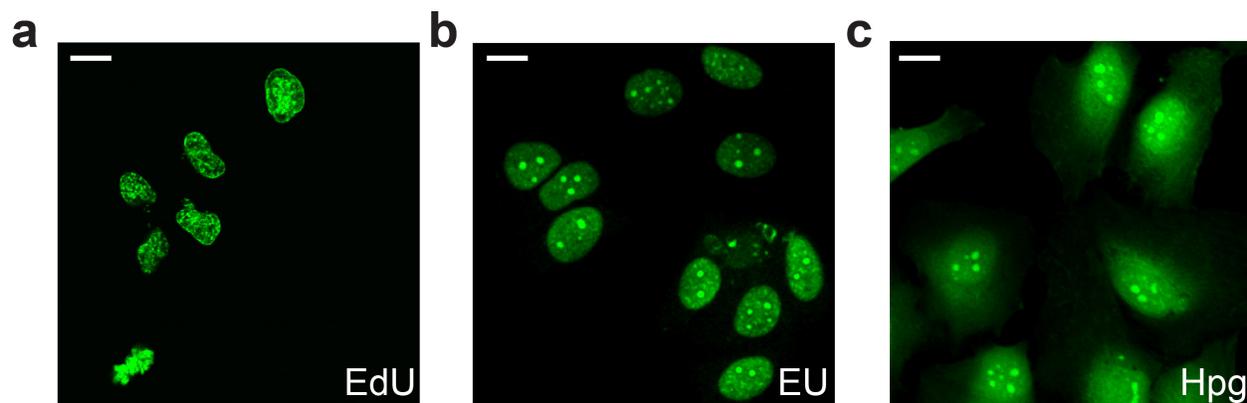
The composite image shows both the protein derived 1655 cm⁻¹ (amide) signal from all the germ cells, and the direct visualization of alkynes (2125 cm⁻¹ (EdU)) highlighting the proliferating germ cells. White circles show examples of EdU positive germ cells in the mitotic region of *C. elegans* germline. Scale bar, 5 μm.

Supplementary Figure 4: SRS imaging of fixed HeLa cells after incorporating with 2 mM Hpg.



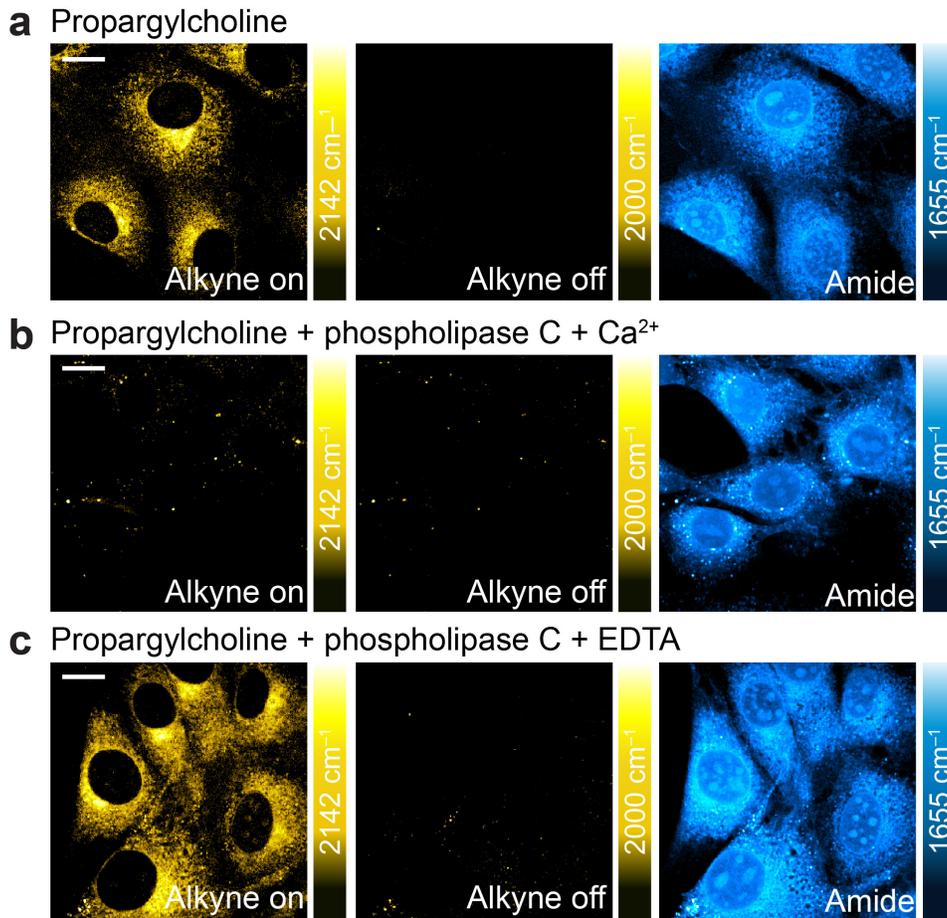
The alkyne-on image displays the Hpg distribution for the newly synthesized proteins. For the same set of cells, the off-resonant (alkyne-off) image shows vanishing signal, and the amide image shows total protein distribution. This result confirms that the detected signal is not from freely diffusive precursor Hpg itself (which is eliminated during the fixation process). Scale bar, 10 μm .

Supplementary Figure 5: Click-chemistry based fluorescence staining of fixed HeLa cells.



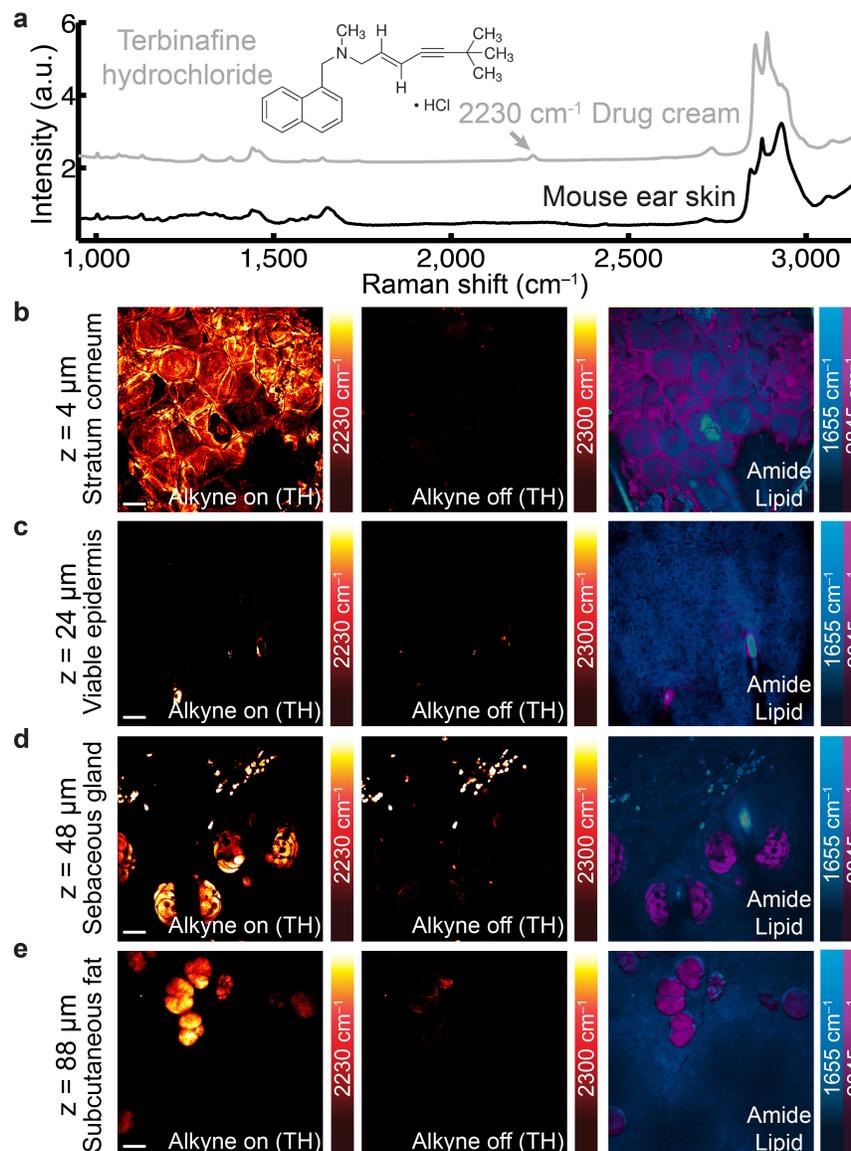
Fluorescence images of HeLa cells incorporated with **a**, EdU (for DNA); **b**, EU (for RNA); **c**, Hpg (for proteins). Scale bars, 10 μm .

Supplementary Figure 6: SRS imaging of propargylcholine incorporation in NIH3T3 cells and control experiments.



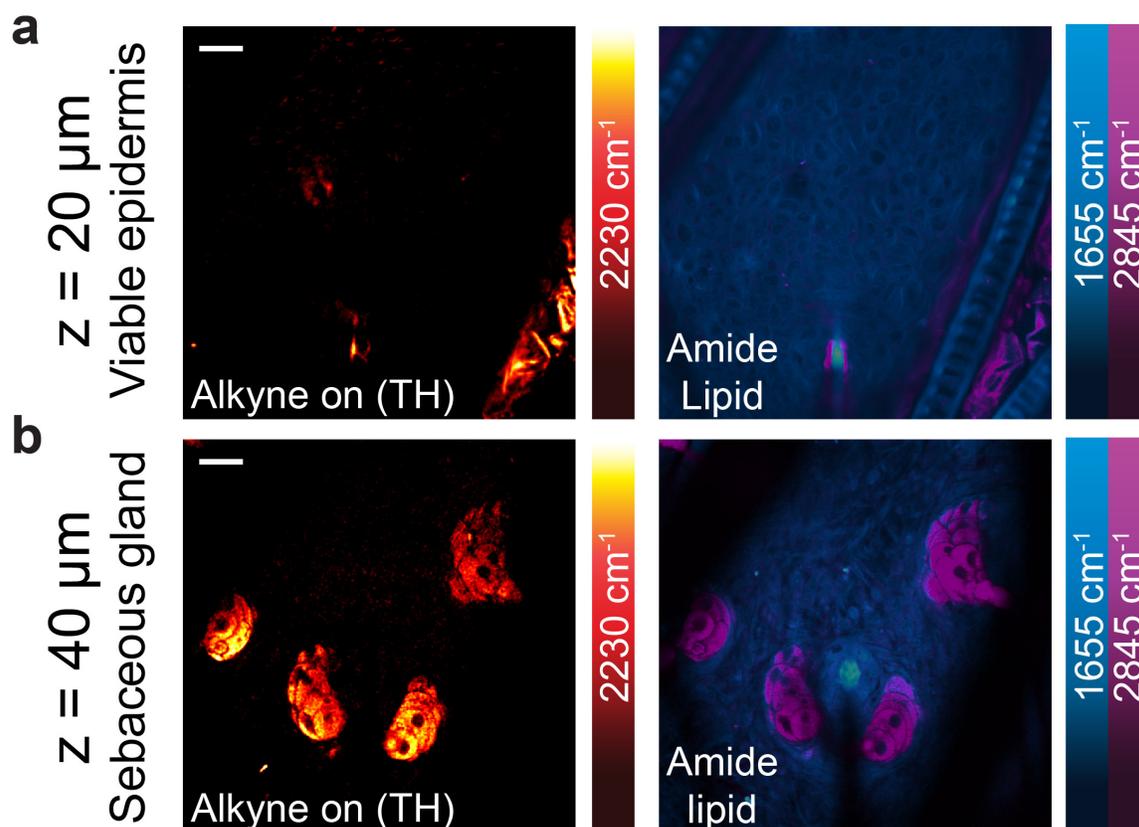
a, Fixed NIH3T3 cells after culturing with 0.5 mM propargylcholine for 48 hours. The alkyne-on image shows alkyne-tagged choline distribution. **b**, Treatment of fixed NIH3T3 cells with phospholipase C, which removes choline head groups of phospholipids only in the presence of calcium. The alkyne-on image shows the strong decrease of incorporated propargylcholine signal, supporting its main incorporation into membrane phospholipids. **c**, Treatment of fixed NIH3T3 cells with phospholipase C in the presence of EDTA (chelating calcium). Propargylcholine signal is retained in the alkyne-on image. For **a-c**: in the same set of cells as in alkyne-on images, the alkyne-off images show a clear background. The amide images display total protein distribution. Scale bars, 10 μm .

Supplementary Figure 7: *In vivo* delivery of an alkyne-bearing drug (TH in DMSO) into mouse ear.



a, Raman spectra of a drug cream, Lamisil, containing 1% TH and mouse ear skin tissue. **b-e**, SRS imaging of tissue layers from stratum corneum ($z = 4 \mu\text{m}$) to viable epidermis ($z = 24 \mu\text{m}$), sebaceous gland ($z = 48 \mu\text{m}$) and subcutaneous fat ($z = 88 \mu\text{m}$). To facilitate tissue penetration, DMSO solution containing 1% TH was applied onto the ears of an anesthetized live mouse for 30 min and the dissected ears are imaged afterwards. For all 4 layers: alkyne-on images display TH penetration; alkyne-off images show off-resonant background (The bright spots in **d** are due to two-photon absorption of red blood cells). The composite images show protein (1655 cm^{-1}) and lipid (2845 cm^{-1}) distributions. Scale bars, $20 \mu\text{m}$. a.u. arbitrary units.

Supplementary Figure 8: *In vivo* delivery of an alkyne-bearing drug (TH in Lamisil cream, a FDA approved drug cream) into mouse ear.



(a-b) SRS imaging of the viable epidermis layer ($z = 20 \mu\text{m}$) and the sebaceous gland layer ($z = 40 \mu\text{m}$). For both **a** and **b**: the alkyne-on images display the TH penetration into mouse ear tissues through lipid phase. The composite images show both protein (1655cm^{-1}) and lipid (2845cm^{-1}) distributions. Scale bars, $20 \mu\text{m}$.