

Introduction

The invention of Patch Clamp method and development of fluorescent Ca²⁺ indicators for imaging free calcium in living cell led to revolution in understanding of ionic channels' functionality and opened up the opportunities to ultra-local control of the cell membrane potential and intracellular calcium concentration both parameters being involved in the equation for electrochemical potential (EEP). The last uncovered enigma parameter in EEP that can dramatically influence the activity of ion channels' is temperature. However, its experimental use as an ultra-local variable for precise control in micro- and nano-volumes in a living cell is still limited by photobleaching, toxicity and sensitivity to the chemical environment of the current thermosensors.

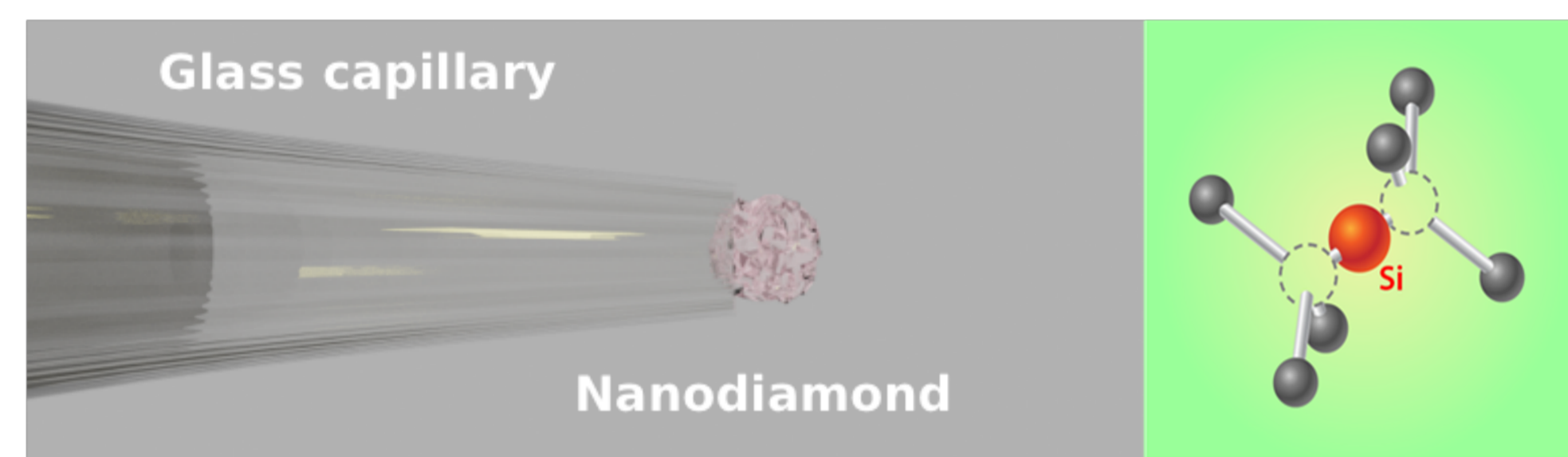


Fig.1. Principal of the instrument construction.

Here we describe a new practical implementation of ultra-local thermometry based on a specially designed nanodiamond containing luminescent "silicon-vacancy" (SiV) centers, which matches well nanoscale thermodynamic requirements in accuracy with no need for intracellular calibration, and allows perfect and easy nanoscale spatial targeting.

Materials

Temperature measurement with submicron spatial resolution is carried out with one luminescent diamond nanocrystal, which is fixed at the entrance of the inner channel of a micro- (nano-) pipette. In this work, we use a 500-nm SiV-luminescent diamond produced by chemical vapor deposition (CVD) technique.

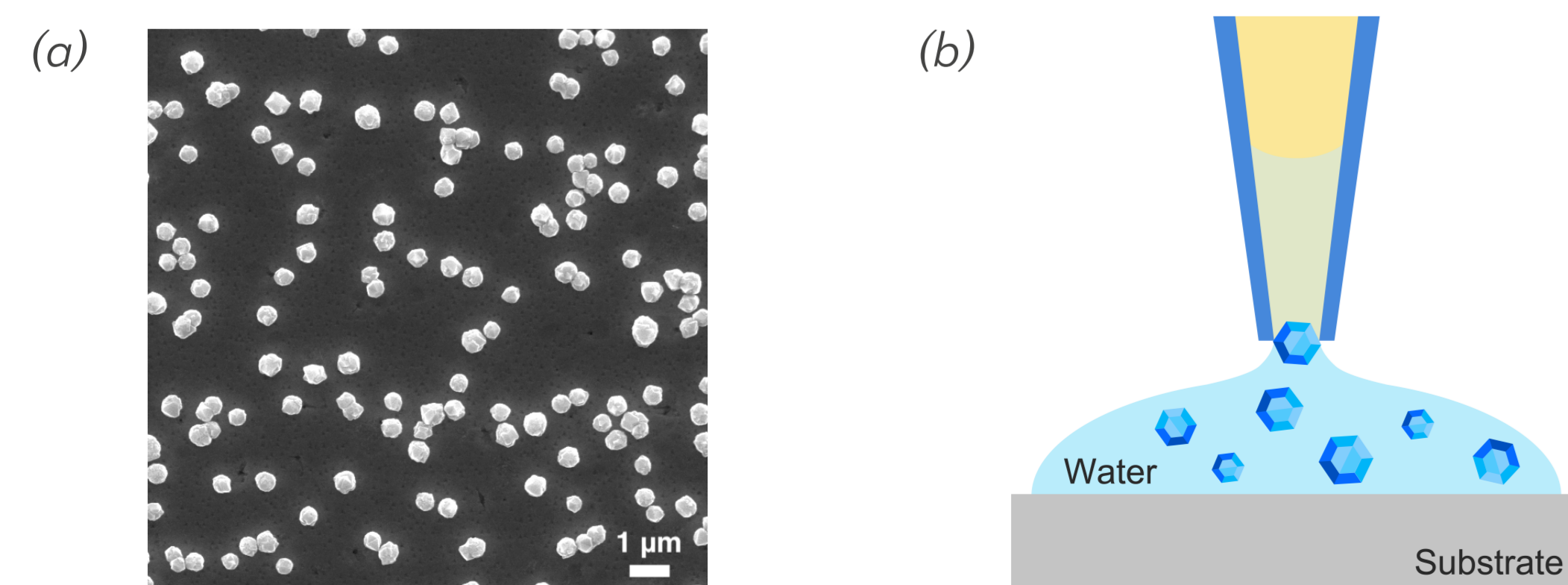


Fig.2. a) SEM-image of synthesized diamonds with SiV-centers, b) schematic representation of diamond drawing into the pipette channel from a water drop.

Methodology

To bind the diamond to the tip of the micropipette, a 5- μ L drop of distilled water is applied to the substrate with crystallites (Fig. 2a). At this time, some of the diamond crystallites pass into the aqueous medium. Then, the submicron pipette touches the surface of the drop, and a column of water is drawn into its inner channel under capillary forces (Fig. 2b).

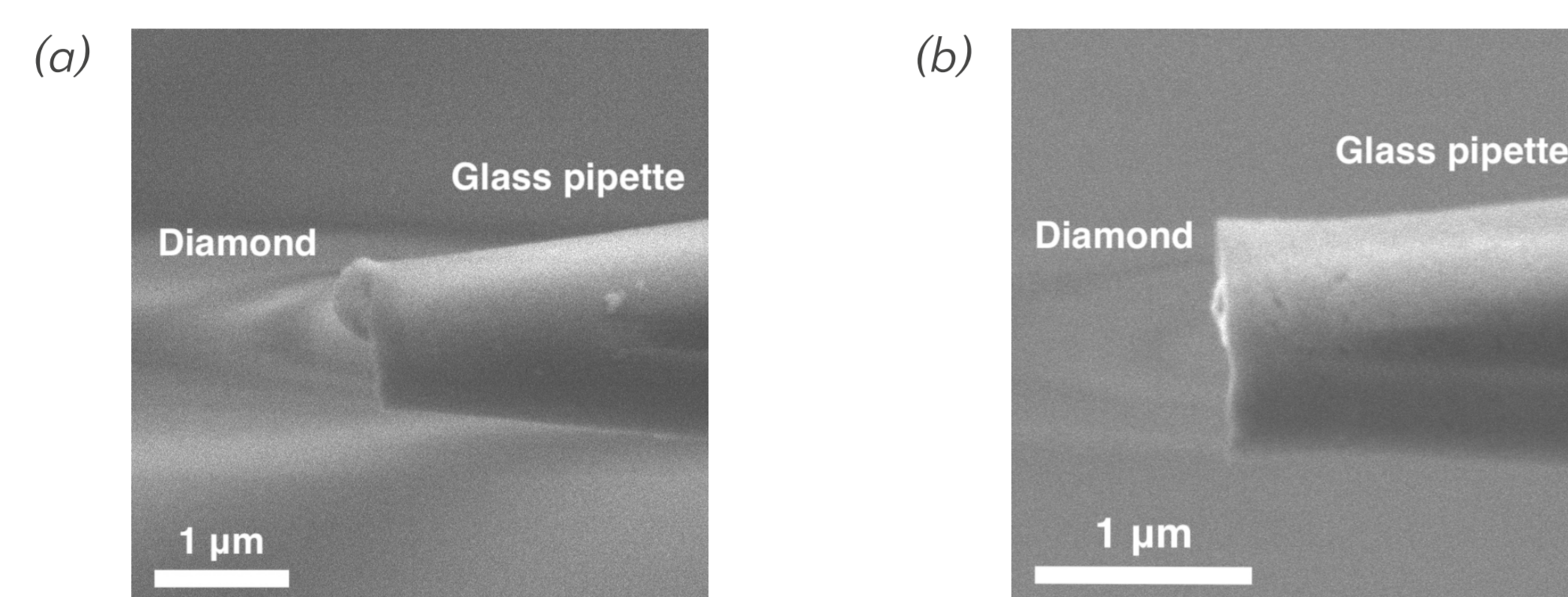


Fig.3. SEM images of a pipette with a diamond crystallite localized near the entrance (a), or at the inlet (b) into a capillary.

When the diamond particle approaches the pipette, it is likely to adhere either before entering the capillary (Fig. 3a) or at its inlet (Fig. 3b). To securely bind the diamond to the pipette, the glass tip is slightly melted with an electric heater. The temperature sensor is precisely (\sim 50 nm in accuracy) positioned in space using a micromanipulator identical to that used in the patch-clamp method.

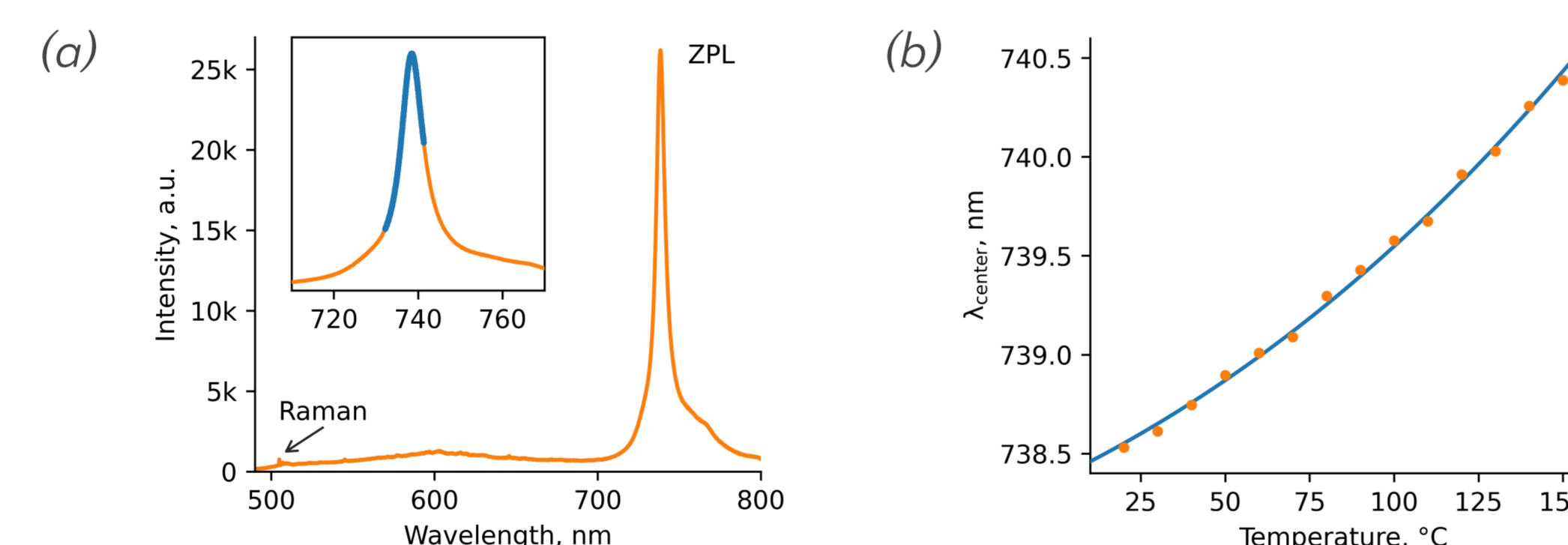


Fig.4. a) SiV-luminescence spectrum (orange curve). The blue part in the inset is approximated by the Lorentzian profile, b) calibration curve of SiV-luminescence from temperature.

All-optical detection of temperature based on the temperature dependence of the spectral position of the maximum of the zero-phonon line (ZPL) of SiV luminescence is used. The reading and processing of information about the temperature are carried out using a commercial (HORIBA) confocal luminescence spectrometer and a home-developed software algorithm for determining the temperature by the SiV line position. Diamond SiV-luminescence is pre-calibrated against the temperature in a thermostat (Fig. 4b). We demonstrate the submicron resolution of this thermometric instrument to the study of temperature distribution in an aqueous medium near a local heater.

which is a small aggregate of 100-nm aluminum nanoparticles at the tip of another micropipette. We use one laser beam at a wavelength of 473 nm to excite the SiV luminescence in a diamond (on the beam "tail") and heat the aluminum particles. The temperature dependence on the distance from the fixed heater is measured by moving the thermometer in steps of 0.2-1 μ m along the axis of heater pipette. At each step a PL-spectrum is recorded (Fig. 6a), the ZPL is approximated by a Lorentzian-curve, and its position is determined.

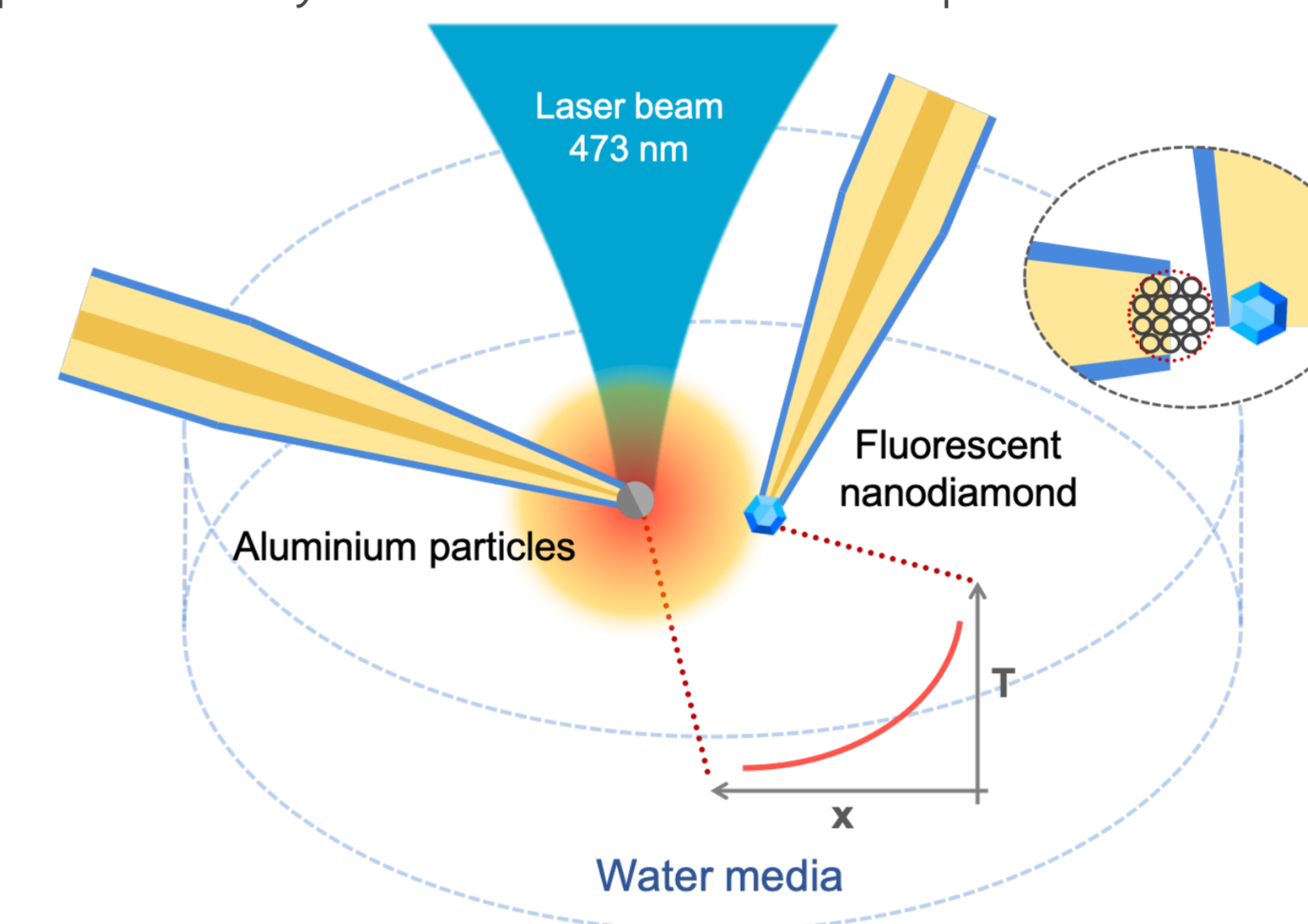


Fig.5. Schematics of temperature evaluation near the local heater: the relative position of the thermometer, heater and laser beam in a cuvette with water.

Results

Fig. 6b shows the dependence of the SiV ZPL position on the distance X between the heater and the thermometer. Nanodiamond thermometer reproducibly "senses" temperature changes of 2.1 $^{\circ}$ C over 200 nm and is capable of monitoring strong temperature gradients $\Delta T/\Delta X$ on the submicron scale (the drop $\Delta T \approx 15$ $^{\circ}$ C is detected within $\Delta X \approx 500$ nm near $X = 1$ μ m). When two pipettes come into contact, the temperature reaches a maximum value of

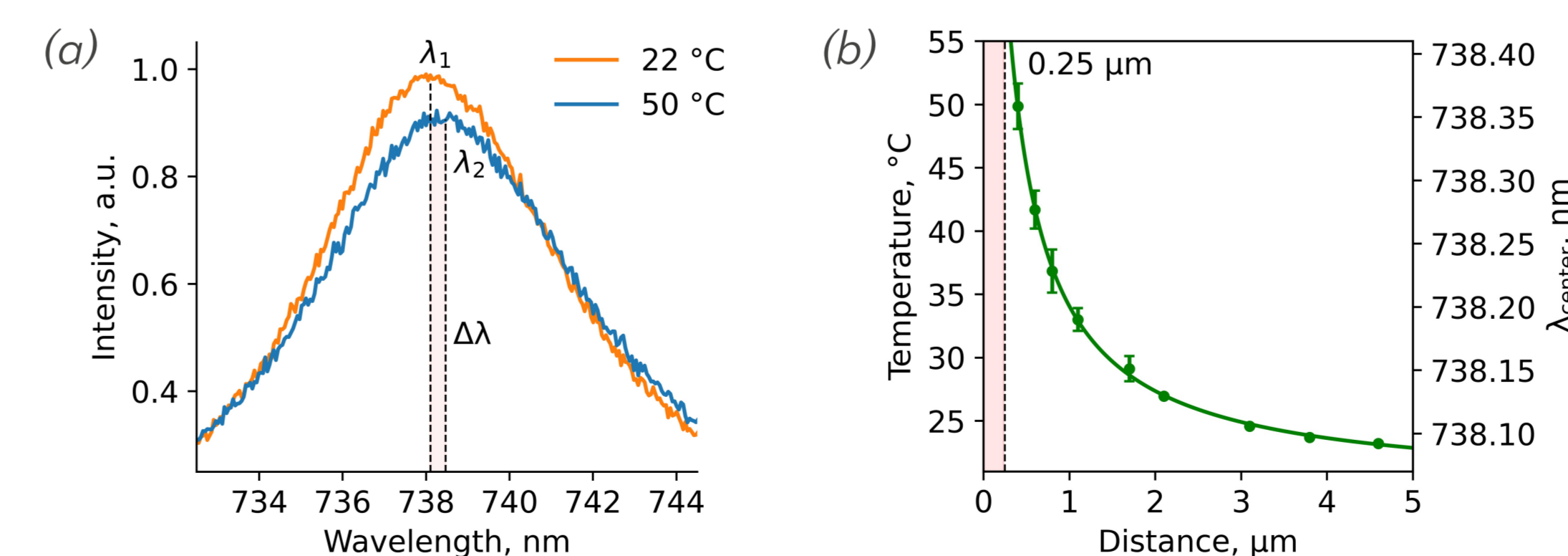


Fig.6. a) PL-spectra of the diamond thermometer, measured at different distances from the heater, the positions λ_1 and λ_2 correspond to 22 $^{\circ}$ C and 50 $^{\circ}$ C b) dependence of the SiV ZPL position and the temperature on the distance X.

Results

50.8 $^{\circ}$ C at the point $X = 0.41$ μ m which is a sum of the heater pipette wall thickness of \approx 0.16 μ m and a diamond crystal radius of \approx 0.25 μ m (inset of Fig. 5). The perturbation of temperature field caused by thermometer is analyzed using COMSOL Multiphysics with the simulated geometry closed to experimental one (Fig. 7a). Thermometer scans the temperature field of a spherical heat source $T(X)$ derived from heat equation

$$T(X) = T_0 + (T_h - T_0)R_1/(R_1 + X), \quad (1)$$

where $T_h = 65$ $^{\circ}$ C – surface heater temperature, $T_0 = 22$ $^{\circ}$ C – bath temperature.

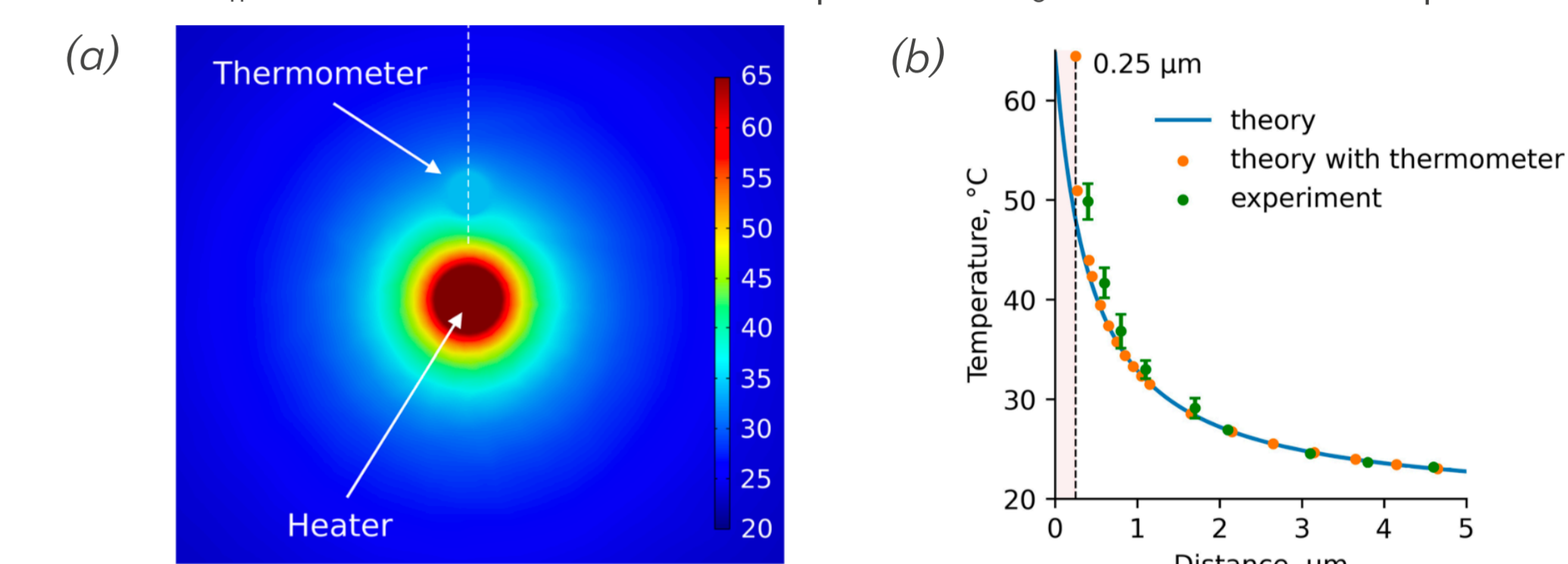


Fig.7. a) A temperature distribution map in the simulated "heater-thermometer" system, b) temperature dependence $T(x)$ without a thermometer (blue), calculated thermometer readings (orange) and experimental data (green).

In Fig. 7b the simulated temperature distribution $T(x)$ in the absence (blue) and in the presence (orange) of a diamond thermometer are shown. Near the heater a systematic error of temperature readings appears, and the gradient grows up. It is associated with the thermometer finite size and a strong temperature gradient (>20 $^{\circ}$ C/ μ m) and can be reduced by decreasing the thermometer size down to 100 nm. The difference between calculated and experimental thermometer readings (green) explains by some inaccuracy in the definition of the diamond size and the heater temperature.

Conclusion

We have developed a new design of a nanodiamond thermometer, which allows precise mapping of temperature fields near local heat sources in submicrometer watery volumes. Until now, temperature measurements at such high gradients (>20 $^{\circ}$ C/ μ m) have not been performed. The new thermometric tool opens up unique opportunities to answer the urgent paradigm-shifting questions of cell physiology thermodynamics about local intracellular temperature and temperature of open ionic channels.

Romshin Alexey, alex_31r@mail.ru

Head of the Carbon Nanophotonics Laboratory
Vlasov Igor, vlasov@nsc.gpi.ru

