Development of an Intravessel Xylem Probe for Viniculture and Forest Ecology

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Abstract:

This research project presents the design, fabrication and testing of a single, hollow microneedle whose purpose was to be integrated with a water pressure sensor to form a microelectromechanical systems (MEMS) intravessel xylem probe (IVXP). The intent of this IVXP would be to continuously extract a fluid sample from plant xylem to monitor water content. Double polished silicon wafers were coated with photoresist, patterned via photolithography, and deep reactive ion-etched. The resultant matrix of various needle dimensions was diced to create individual needles, which then were characterized with grape vine xylem. Successful xylem insertion was observed for needles of 200 μm in length.

Introduction/Background:

Botanical research demands improved technology to measure water transport in the xylem tissue of plants. Initial methods were indirect as they measured, in surrogate, the water content of air and soil. Current lab-confined probes [1] yield a single measurement per puncture, are destructive to the plant tissue and suffer from a pressure limit of minus 10 atmospheres (atm).

As plant fluids function at relatively high negative pressures, this sap is metastable and defies easy extraction; this liquid readily changes phase from liquid to gas, which sabotages analysis. The design of this project's *in situ* probe, inserted into xylem, would continuously read water flow in a growing plant and would provide readings down to minus 80 atm. A micro-proportioned needle could traverse the short distance into the xylem but without causing plant mortality.

The smaller needles feature a 20 μm inner diameter and a 30 μm outer diameter. The largest needles feature a 40 μm inner diameter and a 100 μm outer diameter. Recent microneedle research and development [2] has etched needles as arrays, because, due to strength in numbers, these needles are less submissive to fracture. However, this project required a single needle strong enough to survive xylem probing. A successful microneedle should both penetrate grape leaf xylem without fracture and enable sufficient fluid transport of plant sap through its bore, without clogging. Nitrite sensors for plant nutrition logically could follow. Parallel fabrication and low device maintenance would ensure effective cost control.

Fabrication:

After furnace deposition of silicon nitride (Si_3N_4), the Si_3N_4 on the backside of the wafer was patterned using contact lithography and etched to create an etch mask. The exposed silicon was then etched using 33% potassium hydroxide at 90°C for 35 minutes. This created a backside trench of 68 μm deep (Figure 1). The presence of this trench reduced the time necessary to create a through hole in later inside-diameter etching. The trench also created a site for future pressure sensor placement. The next few steps in the process are shown in Figure 2. First, the annulus was patterned; 3 μm of oxide was first deposited on the front side of the wafer using plasma enhanced chemical vapor deposition (PECVD).

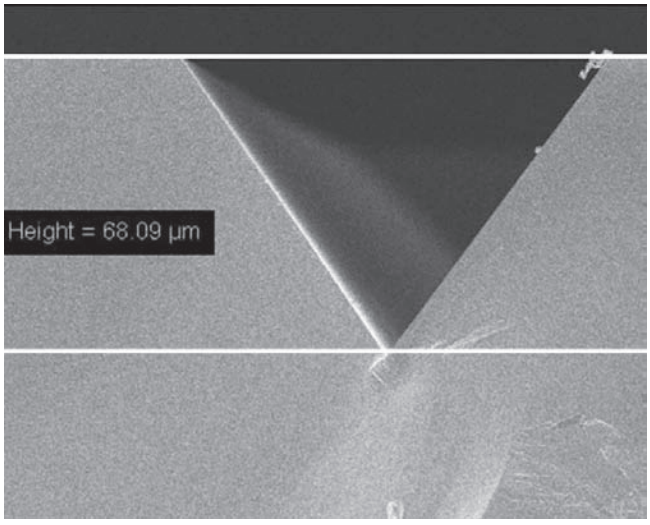

Figure 1: SEM of backside trench.

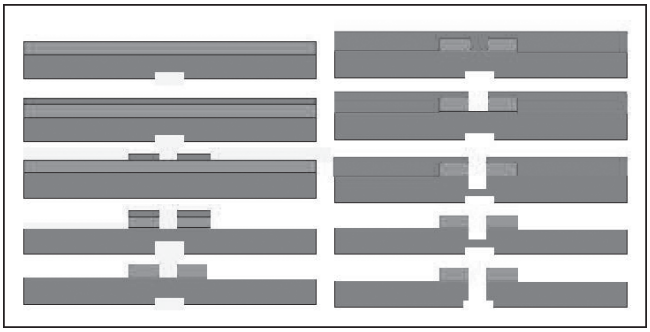

Figure 2: Process flow for needle fabrication.

This oxide was then patterned with contact lithography and etched using a 6:1 buffered oxide etch (BOE) for 35 minutes. The remaining oxide, after resist removal, served as silicon etch mask for the purpose of etching the annulus. Since only the inside diameter of the needle needed to be etched completely through the wafer (a longer etch time as opposed to the outside diameter etch duration), photoresist was applied and patterned in a manner such that only the inner diameter silicon was etched away. An initial 125 μm was etched using the Oerlikon deep silicon etcher. Then, after removing the resist, etching was continued so that the entire wafer (except the annulus, masked with oxide) was etched. This process created the sidewalls and allowed for further inner diameter etching. Upon etch completion, needle lengths of 150 to 200 μm were measured (Figure 3).

Results and Discussion:

Difficulties in boring completely through to the other side of the wafer were experienced. Further, in cases of sidewall thicknesses of less than 15 μm, the elongated episodes of linear etching down the length of the needle shaft partially eroded through these sidewalls (Figure 4). This resulted in needle breakage. Thicker sidewalls did survive etching and their strength was tested.

For initial testing of these needles, in lab methods were used. A grape vine leaf was removed from the plant and pressurized to mimic the vacuum existing in plants. Microneedle insertion into the xylem was monitored via microscope. Insertion was successful with needles of 200 μm length. Shorter needles drifted off location which resulted in breakage. The development of a single hollow microneedle requires further research and development. Longer etch times prior to resist removal could ensure through-hole success, and longer etches after resist removal (for needles with larger wall thicknesses) could generate longer needles. The fabrication of tapered versus parallel-walled needles should be explored. Ultimately, successful needle manufacture should be followed up with the installation of the water pressure sensor and nitrate sensor.

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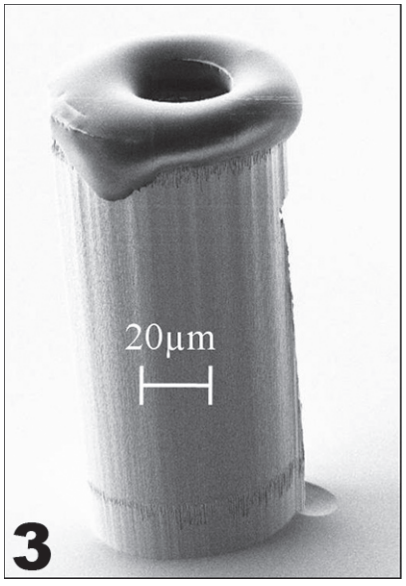
References:

[1] "Comparative measurements of xylem pressure in transpiring and non-transpiring leaves by means of the pressure chamber and the xylem pressure probe", P.J. Melcher et al., Journal of Experimental Botany, Vol.49, n°327, pp.1757-1760, October 1998.

[2] "Microneedle array for transdermal biological fluid extraction and *in situ* analysis", E.V. Mukerjee et al., Sensors and actuators A, Physical, 2004, vol. 114, no2-3, pp. 267-275.



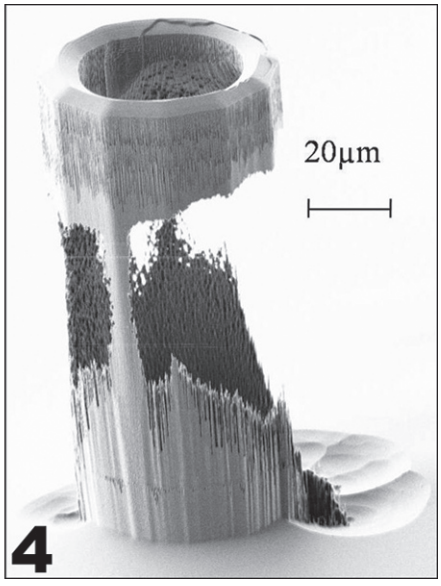


Figure 3: SEM of needle after etch completion.

Figure 4: Sidewall damage on needles with sidewall thicknesses of less than 15 µm.